

COLONIZING RESPONSES OF MARUCA TESTULALIS (GEYER)
(LEPIDOPTERA: PYRALIDAE) TO DIFFERENT COWPEA
CULTIVARS IN RELATION TO THEIR
RESISTANCE/SUSCEPTIBILITY

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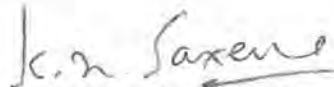
CERTIFICATION

This work has not been submitted to any other University. It is my work and all help has been duly acknowledged.



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A B S T R A C T

A comparison of the resistance/susceptibility levels of ten cowpea cultivars to the pod borer Maruca testulalis revealed that VITA 1 was the most susceptible. Tvu 946 most resistant and VITA 5, chola local and kamboinse local moderately resistant. In all the cultivars which were compared, flowers and pods suffered the heaviest damage compared to the stems.

Colonizing responses of M. testulalis namely oviposition, larval orientation, feeding, utilization of ingested food, larval development and adult fecundity were studied on three cultivars namely VITA 1 (susceptible), VITA 5 (moderately resistant) and Tvu 946 (resistant). The adults showed ovipositional nonpreference for Tvu 946 and VITA 5 when these cultivars were available to the moths as a choice against VITA 1. All the cultivars were equally accepted for oviposition under no choice situation. Majority of the eggs were laid on the leaves.

Settling sites for 1st instar larvae on the cowpea plant in a decreasing order of preference were: flowers > terminal shoots = flower buds. The first instar larvae were capable of reaching any part of the cowpea plant either by walking or by means of silken thread which they produced and used for swinging from one plant part to the other. However, the 4th instar larvae were not very efficient in climbing vertical objects since a majority of them lost grip and dropped down when climbing up the peduncles of the cowpea plant, especially those of Tvu 946, which were very long and erect.

As a result, pods of the cowpea plant (Tvu 946 cultivar) which were raised above the canopy were less infested by M. testulalis larvae.

Attraction and arrest/stay of 1st instar larvae by the resistant Tvu 946 and VITA 5 was lower than those by VITA 1. The volatiles serving as olfactory stimuli from the leaves and flowers and the chloroform and n-hexane extracts of these parts were attractive to M. testulalis larvae. However, the attractancy of the volatile and extracts of VITA 1 to the larvae was higher than that from Tvu 946 and VITA 5. The larvae were also attracted by high humidity and their speed and rate of arrival on the cowpea plant were enhanced on a moist ground than on a dry surface.

Nonpreference for larval feeding coupled with a shorter feeding duration was also observed on Tvu 946 plant parts as compared to VITA 1. Growth and development of the larvae on stems of Tvu 946 and VITA 5 were lower than those on VITA 1 because Tvu 946 was consumed in very small quantities while food from VITA 5 was poorly converted into body tissues. Although quantity of food ingested from pods was similar for VITA 1 and Tvu 946 and even higher on VITA 5, digestibility of the food from Tvu 946 was very low compared to VITA 1. This led to a higher larval mortality on Tvu 946 than on VITA 1. Conversion of the food VITA 5 into body tissue was very low and this led to a lower pupal weight on VITA 5 pods. Antibiosis was therefore considered to be partly involved in the resistance of Tvu 946 and VITA 5 pods and stems.

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GENERAL INTRODUCTION

Cowpea Vigna unguiculata L. Walp is an important pod and fodder crop in Africa and a number of other tropical countries (Steel and Mehra, 1980). As a food crop it is used as a vegetable, particularly in eastern Africa (Singh and van Emden, 1979; Okeyo-Owuor and Ochieng, 1981). In many places, its grains form part of the diet of the people and serve as the source of protein (IITA, 1975, Singh and van Emden, 1979).

However, a number of factors are known to reduce the yield of cowpea, one of them being its attack by insect pests. Booker (1965) reported at least 85 species of these pests in the various cowpea growing regions of the world, the number varying from region to region. Some of the important pests include coreid bugs and seed weevil. Maximum damage is caused by thrips, the pod borer Maruca testulalis and pod sucking bugs (Singh, 1978 and Singh et al., 1984).

M. testulalis (Geyer) (Lepidoptera:Pyralidae) is a major pest in Africa and is also widely distributed in the tropics and sub-tropics (Singh and Allen, 1979). Yield losses due to M. testulalis vary from region to region but in severe cases up to one hundred percent damage has been reported (Singh et al., 1984). In Kenya, cowpea damage by M. testulalis is variable. The data at the Mbita Point indicate yield losses of between 10 and 80 percent in terms of cowpea seed (Okeyo-Owuor and Ochieng, 1981).

The M. testulalis larva which is the destructive stage feeds on terminal shoots, tender parts of the stem, flower buds, flowers and green pods (Taylor, 1967, 1978; IITA, 1975; Singh, 1978 and Jackai, 1981b). The flowering stage in cowpea is the most favourable stage for M. testulalis oviposition and it is the principal stage at which young larvae cause substantial damage (Taylor 1978; Okeyo-Owuor and Ochieng, 1981). Although it is mainly a post-flowering pest (Booker, 1963), it comes into the cowpea field in the pre-flowering stage (Singh, 1978; Jackai, 1981b). Usua and Singh (1975) found that stem attack was an important form of damage in the highly susceptible varieties of cowpea.

At present, use of chemical insecticides is the only available control measure for M. testulalis in cowpea (Singh, 1978; Singh et al., 1984; Karel, 1985). However, they are not widely used (Singh et al., 1983). In East Africa, the use of the cowpea leaf and seed as human food does not favour the option of controlling this pest by chemicals. In West Africa, chemical control measures have been developed but their use by the farmers is limited mainly due to the high cost of insecticides and sprayers (Singh et al., 1984). These limitations, coupled with many more problems resulting from the use of chemical pesticides in general, make it necessary to evolve non-chemical methods of control.

The use of resistant cultivars offers a promising alternative control method for M. testulalis. Several examples have been cited where resistant cultivars have been successfully used as a major

insect control method (Painter, 1951, 1958; Panda, 1979; Maxwell and Jennings 1980). The resistant cultivars are particularly advantageous because: (a) they reduce the need for insecticide inputs, thus preventing environmental pollution; (b) they reduce the chances for the development of insecticide-resistant insect strains; (c) they do not pose any threat to parasites and predators and, (d) their use involves no extra cost to the farmer (Singh, 1978). However, resistant cultivars also have their limitations: (a) availability of resistant germplasm for development of resistant cultivars may pose a problem; (b) the pests may develop biotypes which can overcome varieties previously known to be resistant; and (c) resistance to one insect pest in a cultivar may be accompanied by its susceptibility to another insect pest of the same crop. Despite all these limitations, resistant cultivars can still combine very well with other control methods in an integrated pest management programme.

With the emphasis on the use of resistant cultivars, scientists at IITA started collecting and screening germplasm resistant to insect pests of cowpea in 1970 (Singh, et al. 1983). Some lines with a low to a moderate level of resistance to M. testulalis have been identified (IITA, 1982; Jackai, 1982; Singh et al., 1984). The cultivars Tvu 946, VITA 5 and Kamboinse local with moderate level of resistance have been identified by the above authors as promising donors for resistance in breeding programme for cowpea resistance to M. testulalis.

Although the above mentioned lines have been identified to be good sources of resistance, it is important to develop cultivars combining high resistance with other desirable agronomic characters. In order to achieve this, it is important to obtain information on the principles governing resistance or susceptibility so that resistance - imparting characters may be incorporated and the susceptibility - imparting characters may be eliminated in the cultivars to be developed.

At present our knowledge of the principles imparting resistance or susceptibility to cowpea against M. testulalis is meagre and lacks sufficient experimental evidence. The study of these principles has therefore been taken up in the present work as spelt out in the objectives given below.

Objectives

1. To evaluate the levels of susceptibility or resistance in some selected cowpea cultivars to M. testulalis.
2. To elucidate the factors determining differences in the susceptibility or resistance in three selected cowpea cultivars to M. testulalis.

These factors, under identical environmental conditions, would involve an interaction between (1) the insects' colonizing responses involved in the establishment of its population on a

plant, and (2) the plant characters determining these responses (Saxena, 1969, 1985; Southwood, 1973).

The responses of an insect which favour or hamper its colonization of a plant according to Saxena (1969, 1985), belong to the following main categories:

- (1) Orientation responses, determining the insects arrival/arrest on or avoidance of a plant;
- (2) feeding responses determining quantity of the food ingested;
- (3) metabolic utilization of food by the insect determining its nutrition;
- (4) growth and development of the larva, determined by both the food intake and nutritive value;
- (5) survival and egg production in the adult stage determined by both intake and nutritive value
- (6) oviposition.

The plant factors influencing the above mentioned insect responses may be broadly categorized as biophysical or biochemical in nature. The biophysical plant characters would include those perceivable at a distance e.g. visual (colour, pattern and shape) or those perceivable by contact (hairiness, toughness). The biochemical characters of the plants would include distance-perceivable olfactory stimuli (non-aqueous volatiles), hygro-stimuli (aqueous-vapour), contact-perceivable chemical stimuli (nutritional chemicals and toxic chemicals interfering with metabolism or other chemicals influencing phagostimulation and palatability of the food).

All the above six insect responses of M. testulalis in relation to its host plant cowpea have been studied. In this work some of the characters determining the above mentioned responses of M. testulalis to different cowpea cultivars have also been examined and include:

- (1) Plant architecture in relation to the accessibility of the flowers and pods to the larvae
- (2) Plant chemical extracts in relation to larval orientation (attraction or repulsion);
- (3) Plant extracts in relation to larval feeding responses.

CHAPTER 1

REVIEW OF LITERATURE

1.1 Distribution of Maruca testulalis

M. testulalis popularly known as the legume pod borer occurs throughout the tropics and sub-tropics (Singh and Allen, 1979) Singh and van Emden (1979) gave a review of literature on the distribution, damage, life cycle and control of this pest. As cited in their review only Europe is left out by this pest. M. testulalis has been reported in East, West and South Africa (Booker, 1965; Halteren, 1971; Nyiira, 1973; Rhelms and Oosthuizen, 1958; Singh, 1977; and Taylor, 1964). In Asia, the pest has been recorded in the Philippines (Barroga, 1969; Djamin, 1961), India (Saxena, 1974; Srivastava, 1964), Fiji Islands and Indonesia (Oei-Dharma, 1969), Sri Lanka (Subasinghe and Fellowes, 1978), Papua New Guinea (Lamb, 1978), Taiwan (Lee, 1965). In the Americas the pest is found in Puerto Rico (Scott, 1940; Wolcott, 1933), Brazil (Smith, 1978) and Southern USA and Australia (Wilson and Genung, 1957; Passlow, 1968).

1.2 Behaviour and Biology of Maruca testulalis

Both adult and larvae of M. testulalis exhibit nocturnal activity (Taylor, 1978; Singh and van Emden; 1979, Okeyo-Owuor and Ochieng, 1981). Moths have dark greyish with white and brown

patterned wings. M. testulalis moths rest with their wings spread horizontally and when in the cowpea field tends to rest under crop canopy especially on the lower leaf surfaces thereby exhibiting a kind of camouflage behaviour (Okeyo-Owuor and Ochieng, 1981). According to the above authors, at the ICIPE - Mbita Point Field Station the moth activity starts at around 1800 h and ends at about 0630 h. The moths emerge between 2000 and 2300 h. Both natural and artificially induced darkness initiate adult activity. The moths also respond to light and can be caught by light traps at night (Taylor, 1967, 1978).

Without mating M. testulalis lays few infertile eggs which never hatch (Ochieng, unpublished). Being a nocturnal, insect mating occurs at night with a peak at 0100-0200 h. The female produces a pheromone which normally lures the male into mating (Okeyo-Owuor and Agwaro, 1982). Mating takes place within 1-4 days after adult emergence.

Studies at the ICIPE in East Africa, shows that oviposition takes place at night and occurs on all the aerial parts of the cowpea plant, although the leaves are the most preferred (Okeyo-Owuor and Ochieng, 1981). In West Africa (Nigeria) Jackai (1981a) and Taylor, (1978) reported that majority of egg laying takes place on the flowers and flower buds. Such differences in the preferred oviposition sites in the two regions could be due to

differences in experimental procedures or could be an indication of the existence of biotypes in the populations of M. testulalis in East and West Africa. According to Okeyo-Owuor and Ochieng (1981) temperatures below 22°C impares oviposition. Singh (1978) reported that in captivity the moths oviposit liberally all over the host plant and even on the walls of cages.

The moths are known to show oviposition preference when offered a resistant and a susceptible cowpea, cultivar simultaneously (Macfoy et al., 1983). However, there is no information on the oviposition responses under no choice situation. The present study has covered both oviposition under free-choice and no-choice situation.

Taylor (1978) observed that eggs are usually laid in small batches (2-16). While laying, the female elongates the abdomen to form a slender telescopic tube used to deposit the eggs between the whorls of flower buds. However, it is not clear whether the insects use the ovipositor for assessing the substrate before oviposition.

Since most of the eggs are not deposited on the preferred parts of the plant by the adult, the emerging larvae have to disperse into the suitable parts of the plant. M. testulais larvae are highly mobile and are capable of reaching any part of the plant within a very limited time (Okeyo-Owuor et al., 1983). Such rapid movements are facilitated by production of silken threads on which they can hang or swing to other parts of the plant (Taylor,1978).

There is no information relating to the glands which produce the threads. Like the moths, the larvae also like hiding and are usually found feeding inside the food substratum during most of their life time (Taylor, 1978; Okeyo-Owuor and Ochieng, 1981; Okeyo-Owuor et al., 1983). The young larvae are usually found in the young growing plant tips, flower buds and flowers. In the flowers they concentrate their feeding on the reproductive parts and first consume the anthers, filaments, style, stigma and ovaries before feeding on the internal components of the corolla which is usually limited (Taylor, 1967). Usually more than one larvae may be found in each flower although M. testulalis larvae are not gregarious. The hardy third, fourth and fifth instars are capable of boring into and damaging the fresh pods and occasionally the peduncles and stem (Taylor, 1967; Usua and Singh 1975).

Literature on the range of larval dispersal, orientation responses as is influenced by plant and non plant stimuli is not available. This was covered in the present study

M. testulalis eggs hatch in 2-3 days, the emerging larvae undergo 5 larval instars in 8-14 days (Taylor, 1967; Okeyo-Owuor and Ochieng 1981; Jackai, 1981b; Singh and Jackai, 1985). Pre-pupal period lasts for 1-2 days during which time the larva does not feed. Pupa is greenish or pale yellow initially but darkens to a greyish-brown colour. Pupal period lasts for 6-9 days (Taylor, 1967). In Nigeria whole life cycle lasts for 18-25 days but may be

longer (Taylor, 1967). At Mbita Point life cycle lasts for 20-57 days (Okeyo-Owuor and Ochieng, 1981). Differences in climatic conditions e.g. temperature and humidity between Mbita and Nigeria may account for the differences in the length of the life cycle of this pest in the two regions.

1.3 Damage by Maruca testulalis

According to Singh and van Emden (1979) and Singh and Allen (1979), damage is caused by the larva and the greatest damage occurs on flower buds and flowers. Extensive damage also occurs on pods. The tender parts of the peduncles and stems are also attacked by older instars. The common symptoms of M. testulalis infestation are webbing of flowers, pods and leaves with frass often on pods and shoot tips.

Tayo (1986, unpublished) observed that at the pre-flowering stage, stem damage normally occurs on nodes 2-6 which is about 2-4cm. from the plant tip. Larval feeding is restricted to the pith which is often completely depleted from the attack by M. testulalis. Attack on the peduncles also leads to complete hollowing and feeding occurs on the pith and part of the vascular bundles. Attack on pods often starts at the pod base near the point of attachment to the peduncle where infested flowers touch the young pods and tunnelling proceeds inwards leading to a weakening of the attachment to the peduncle, thus restricting nutrient supply to pod and seeds.

According to Tayo (1986, unpublished), in the susceptible cultivar (VITA 1) pods of all ages are attacked by M. testulalis. In the young pods the seeds are completely eaten but as the pods become bigger, feeding becomes restricted to the inner lining of the pericarp and the tissues between the seeds, although some parts of the developing seeds are also consumed.

1.4 Host plants of M. testulalis

Although M. testulalis is generally referred to as the cowpea pod borer, it is known to thrive on a number of leguminous plants (Taylor, 1964). Taylor (1978) gave an annotated list of the host plants which fell under five families namely Papilionaceae, Caesalpinaceae, Pedaliaceae, Malvaceae and Mimosaceae. Majority of the plants belong to the family Papilionaceae which include the following: Vigna unguiculata (L. Walp), Vigna mungo L., Vigna radiata L. Wlczek, Cajanus cajan L. Millsp, Crotalaria incana L., Arachis hypogea L., Dolichos lablab, Phaseolus vulgaris L., Phaseolus lunatus L., Peschocarpus tetragonolobus L., Gliricidia sepium Steud., Vicia faba L., Vigna triloba L.

Larval feeding on all the above listed families is confined to flowers and pods and in majority of them only flowers are suitable except in Vigna unguiculata (L. Walp) and Vigna mungo L. where feeding also occurs on stem, flowers and pods. Esclerana dolabriformis L. is unique in that feeding occurs on leaves only

(Taylor, 1978). M. testulalis has also been found in soyabeans (Smith, 1978). Vigna unguiculata (L. Walp) and Vigna mungo L. can be considered as the principal hosts of M. testulalis because the larvae can thrive on the stem, flowers and pods unlike in other host species where the pest can only thrive on the flowers.

Jackai and Singh (1983) studied the suitability of flowers of eight different plant species, Crotalaria retusa L., C. juncea L., C. saltiana L., C. miserensis, C. amazonas, Cajanus cajan L. Millsp and Vigna unguiculata (L.Walp), and found that there was a variability in the suitability of these plants as hosts of M. testulalis. His studies confirmed that Vigna unguiculata (L. Walp) was the most suitable host plant. Some of the plants (Crotalaria juncea L.) attracted heavy oviposition but could not support development of the larvae beyond 3rd instar.

1.5 Varietal Resistance of cowpea to M. testulalis

1.5.1 Screening for resistance. With the realization of the importance of insect resistant cultivars, the current programme on cowpea improvement at IITA has placed maximum emphasis on breeding for insect resistance (Singh et al. 1984). However, there has been a handicap in development of a suitable screening method and mass rearing technique for M. testulalis which now seems to have been solved (Singh et al., 1983).

The first reported attempt at developing a screening technique for M. testulalis under field conditions was by Wolley and Evans (1979) at IITA. This techniques had many flaws and has not been adapted. The technique placed more emphasis on flower damage than on pod damage and the parameter for assessing flower damage was by the ratio of number of pods under unsprayed conditions to the number of pods under sprayed conditions. The resistance to overall post flowering damage was assessed by ratio of seed yield under unsprayed conditions to seed yield under sprayed conditions. One major flaw in this method is that by spraying, all the insects pests are controlled and under unsprayed conditions all the insects pests are available. The differences in pod production and seed yield cannot be attributed entirely to damage by M. testulalis.

A standardized method of field screening was developed and reported by Jackai (1982) using natural insect populations. According to Jackai (1982) most important parameters in the assessment of cowpea resistance to M. testulalis are: (i) larval infestation which is determined by larval population in flowers, (ii) pod infestation which is determined by percent infestation obtained from a sample of pods and (iii) seed damage measurement. Jackai (1982) suggests that measurement of stem damage should also be included in the assessment and recommends that any material selected under field natural infestation should ultimately go through artificial infestation to eliminate any risk of some varieties escaping infestation due to low insect population .

Due to common problems encountered by relying on natural insect population, Ochieng et al. (1981) developed a mass rearing technique for M. testulalis at the ICIPE for artificial infestation. This enabled Dabrowski et al. (1983) to develop a screening method using artificial infestation with eggs. They found that 5-7 shoots stage was suitable for screening for resistance in the preflowering period. Ten eggs per plant were sufficient to show the differences. At flowering stage 10-20 eggs per plant is recommended. Dabrowski et al. (1983) also recommended percent infestation of stems, flowers and pods as suitable parameters for comparison of resistance of cowpea to M. testulalis.

1.5.2 Cultivars with sources of resistance. By 1983 the cowpea germplasm collection had reached 11,500 accessions (Singh et al., 1983). A total of 6000 germplasm lines have been screened and sources of resistance to M. testulalis identified (Singh et al., 1984). Tvu 946 is the only cultivar which has been identified to have an acceptable level of resistance which can be used in a breeding programme since it has a moderate level of resistance (Singh, 1978 ; Jackai 1982; Dabrowski et al., 1983 and Singh et al., 1984). Other cultivars reported to have some resistance (although lower than Tvu 946) include VITA 5 (Singh, 1978, Jackai, 1982), Kamboinse local and Tvu 1 (Jackai, 1982; Singh et al., 1983).

1.5.3 Mechanisms and causes of resistance to *M. testulalis*. As was stated by Simmonds (1984). "many mistakes have been made in the past and many more will no doubt be made in the future in deciding which procedure or strategy to adopt in breeding for resistance due to our shaky knowledge of host-pathogen systems", status of knowledge on relationship of *M. testulalis* and its cowpea host plant is still limited. Existing literature (Singh, 1978; Jackai, 1981b, 1982; Dabrowski et al., 1983; and Macfoy et al., 1983) shows that flowers and pods of Tvu 946 cowpea cultivar are significantly less damaged than other varieties.

Attempts to elucidate the mechanisms and causes of resistance of Tvu 946 cultivar to *M. testulalis* by Macfoy et al (1983) showed non-preference for oviposition by the females to Tvu 946 under a choice situation against VITA 1 in a greenhouse. A low level of antibiosis to the larvae fed on stems of Tvu 946 was also reported. Allelochemicals (Methanol extracts) were suggested as causes of resistance to stem feeding (Macfoy et al., 1983). A detailed bioassay of methanol extracts from stems of Tvu 946 by Otieno et al (1985) revealed that there is a feeding deterrent in methanol extract. The above work concentrated more on stems. The stem, however, is not the preferred part of cowpea plant by *M. testulalis*. Existing literature, (Taylor, 1967; 1978; Singh 1978; Okeyo-Owuor and Ochieng, 1981; Jackai, 1981) show that preferred parts are flowers, terminal shoots and pods. Stems are only attacked by 3rd-5th instar larvae in the absence of pods and flowers.

Causes for low damage to pods and flowers in the field have not been established. Singh (1978) reported that the narrow angle between two pods or pods touching each other enables a significantly higher infestation to occur. Also varieties that have short peduncles with pods touching the leaf or other parts of the plant have M. testulalis entry holes at the point of contact. He therefore, concluded that such plant characteristics do enhance susceptibility to pods damage. This explanation by Singh (1978) did not account for factors responsible for the lower infestation on flowers by M. testulalis.

Jackai's (1981b and 1982) reports simply showed the differences among the cowpea cultivars in relation to damage and larval population of M. testulalis. There were no explanations relating to larval development on the resistant and susceptible cultivars. Dabrowski et al. (1983) attempted to show differences among the cultivars in terms of larval populations in different parts of the plant. They too did not explain whether low larval populations on the resistant Tvu 946 cultivar was due to larval mortality or non-preference. There is no explanation for the low larval populations on the flowers of the resistant Tvu 946 cultivar. Plant architecture of Tvu 946 is simply suggested as one of the factors responsible for low damage to Tvu 946 by M. testulalis but there are no substantive data to support the suggestion. In this study an attempt has been made to fill the above mentioned gaps on the mechanisms and causes of resistance of cowpea to M. testulalis

Anatomical examination of plant parts of the resistant Tvu 946 and the susceptible VITA 1 cultivars are being made by Tayo (1986, unpublished) at the ICIPE. Tayo's (1986, unpublished) results show that although the transverse section of the stem of the uninfested plants in the two cultivars have similar patterns of tissue arrangements, the relative thickness of various tissues in the stem diameter are different. Tvu 946 has the least stem girth (1.4 cm) while VITA 1 has the greatest girth (2.6 cm). According to Tayo (1986) feeding is concentrated on the soft parenchymatous tissue in the pith and in the two cultivars, VITA 1 has a greater proportion of the pith compared to Tvu 946 which suggests that biophysical factors are likely to play a role in the resistance of stems of Tvu 946 to M. testulalis.

Jackai and Daoust (1986) have given a complete review of literature on M. testulalis and other cowpea pests.

1.6 Colonising Responses of Insects to Plants

Since the host plant of a phytophagous insect is the universe in which it finds its nourishment and shelter (Kogan, 1975), resistance of plants to insects may be better understood by studying the processes involved in the successful or unsuccessful colonization by the insect pests. With this approach, plant resistance to insect pests is studied in two dimensions (Saxena, 1969; Ortman and Peters, 1980).

- (i) the first dimension involves the understanding of the insect populations on different cultivars or plant species as determined by the responses of insect to plants.

- (ii) the second dimension involves variations in the host plant as determined by the host plants characteristics of the plants influencing the insect responses.

Six responses in the establishment of an insect population on different plants are widely accepted (Saxena, 1969, 1985). These include (1) orientation, (2) feeding, (3) metabolism of ingested food, (5) egg production in the adult, (6) oviposition.

The first step during the establishment of an insect on a plant is orientation which may be involved in two ways:

(i) insects which are away from plants may move to the plants or avoid the plants (ii) insects already on the plants like larvae emerging from eggs may move to stay at an appropriate site within the plant or they may move out of the plant. If the plant is avoided in the orientation process, there will be no insect's establishment on it and subsequent responses would not follow and hence the plant will be resistant.

Whenever the insect arrives on the plant, the next response may be feeding or oviposition, depending on the insect's need. If feeding takes place, the subsequent steps are metabolism of ingested food, development and egg production.

By measuring the above responses on different cultivars it is possible to categorize the cultivars as resistant or susceptible depending on the intensity of the positive responses. As explained by Saxena (1969; 1985), the lower the positive responses of each category, the lower would be the population of the insect establishment on a plant and the greater would be the resistance.

1.7 Plant characters determining insect's responses.

Plant factors influencing the above mentioned insect responses may be broadly categorized as biophysical or biochemical (Painter, 1951; Saxena, 1969; Southwood, 1973; Kogan, 1975; Norris and Kogan; 1980 Kumar, 1984).

The biophysical factors generally include succulence, hardness of tissues, presence of thorns or spines, hairiness, incrustation of minerals in the cuticle and surface waxes which facilitate or hamper normal feeding or oviposition. Colour and shape of plants have also been reported to influence behaviour of insects. Kennedy (1958) reported that colour may allow aphids to find a host in a more adequate physiological stage.

Although many structural differences in plant varieties have been reported to be involved in the resistance, there are usually inadequate correlative data, to show the relationship of these plant structural differences to the behaviour of the insect.

As reported by Kogan (1975), the external environment surrounding the plant is surrounded by chemical compounds of secondary metabolism which exude from the outer layers of tissues. These compounds generate olfactory stimuli which are important in host finding and recognition (Kogan, 1975; Chapman and Berneys, 1977). The insects feeding on a plant perceive a complex of compounds which forms the internal environment of a plant. Some of these chemicals have nutritional value while some have no effect on the insect. The nutrients are converted into insect tissue or utilized to produce energy. The non-nutritional chemicals quite often act as token stimuli and have no nutritional value (Fraenkel, 1959; Kogan, 1975; Norris and Kogan, 1980). These non-nutritional chemicals in the plant are referred to as allelochemicals. The term allelochemicals was introduced and defined by Whittaker (1970) as non-nutritional chemicals produced by an individual of one species that affect growth, health, behaviour or population biology of another species. Some of these allelochemicals have been found to be associated with adverse effects (repulsion, toxicity, feeding inhibition) on the insects (Beck, 1965; Reese and Beck, 1976; Saxena and Okech, 1985). Allelochemicals with such negative effects on the receiving organism (insect) are termed allomones (Whittaker and

Feeny, 1971). Other allelochemicals may serve as feeding stimulants or attractant and sometimes may interact with nutrients to increase their metabolic utilization by the insect (Reese, 1977; Waiss et al, 1977). Allelochemicals which give adaptive advantage to the receiving organisms are termed Kairomones (Whittaker and Feeny 1971).

1.8 Influence of plants on behavioural processes of insects.

Plants may influence the behavioural responses of insects in two ways (Kumar, 1984 Saxena, 1985):

- (1) by providing sensory stimuli; and
- (2) by providing mechanical features which facilitate or hamper behavioural responses.

The sensory stimuli from plants may be perceived at a distance and included visual stimuli (colour or shape), hygro stimuli (water vapour) and olfactory stimuli (non-aqueous volatiles). The sensory stimuli may also be perceived by contact. The contact perceivable characters may provide gustatory and tactile stimuli. Gustatory stimuli are mostly biochemicals while tactile stimuli are mostly physical plant characters.

As stated by Saxena (1985), distance-perceivable characters may determine the insects' orientation, eliciting its repulsion, attraction or non response. Insects which are already on the plant would perceive various contact characters. Both contact and certain distance characters may inhibit or stimulate feeding or oviposition in the insect or elicit no response from it. Therefore, as applied to plant resistance, non preference of insects to plants is due to: (1) the absence of arrestant or attractant, (2) presence of a repellent or (3) an unfavourable balance between arrestant and/or repellent (Panda, 1979; Dethier, 1982; Miller and Strickler, 1984).

1.9 Influence of plants on antibiosis (metabolic processes).

The general explanation of the symptoms of antibiosis may be one or more of the following (Kogan, 1975; Norris and Kogan, 1980). (1) Presence of toxic metabolites, (2) absence or sub optimal amounts of some essential nutrients, (3) unbalanced proportions of nutrients, (4) presence of antimetabolites that render some nutrients unavailable to the insects, (5) presence of enzymes that inhibit normal process of food digestion and consequently utilization of nutrients.

1.10 Distinction between non preference and antibiosis.

Procedures for determining non preference and antibiosis are explained by Painter (1951), Beck (1965), Saxena (1969,1985), Dahms (1972), Kogan (1975), and Panda (1979).

In most cases, non preference is divided into two behavioural responses involving orientation:

- (i) Oviposition preference of adult females usually measured by counting the number of eggs oviposited on susceptible or on resistant plants offered simultaneously.
- (ii) Feeding preference, usually measured by:
 - (1) Offering a choice of resistant and susceptible plants and observing aggregation of insects on plants after a period of time.
 - (2) Measuring the quantity of feeding on the resistant and susceptible plants.
 - (3) Measuring feeding duration in given time.

Antibiosis type of mechanism of resistance involves metabolic responses of insects and can be measured as follows:

- a) Observation of comparative effects of forced feeding (confinement) on plants or cultivars by measuring length of life cycle, mortality, weight of insect after definite feeding period on different cultivars.

- b) Determination of number of eggs laid (fecundity)

- c) Measurement of the amount of food utilized by the insect.

However, Saxena (1985) stated that mere failure of development, survival or egg-production in an insect on a plant cannot be taken to suggest its resistance due to antibiosis since both parameters are determined by quantity of food ingested and its nutritional value. The roles of feeding and nutrition have to be separately determined before classifying mechanism of resistance as antibiosis.

CHAPTER 2

GENERAL MATERIALS AND METHODS

2.1 Insects

The insects for this study were obtained from a laboratory culture which was maintained on the flowers of the susceptible cowpea cultivar, Tvx 66-2H, using a standard rearing method developed by Ochieng et al. (1981). Rearing the insects on a resistant variety may alter their behaviour (Saxena and Schoonhoven, 1978; De Boer and Hanson, 1984), so it was important to use the susceptible cultivar. The adults taken from the culture were mated and were four days old when they show maximum oviposition (Ochieng et al., 1981). The larvae used were in the 1st instar for most aspects and in the 4th instar for a few aspects. Details of the rearing procedure are as follows:

Mated females were confined for oviposition on one week old potted cowpea plants in oviposition boxes 50 cm high, 35 cm wide and 45 cm long covered with fine nylon mesh. Cowpea leaflets containing M. testulalis eggs were plucked off the main leaf and left for incubation in a petri-dish for 3 days. The emerging larvae were reared on flowers in transparent polyvinyl sandwich boxes 6 x 12.5 x 17.5 cm in size.

The sandwich box had its floor lined with filter paper on which a piece of nylon netting (15 mesh/linear inch) was placed with several holes (5 mm in diameter) opened at regular intervals. Each box contained 50 flowers in which 50 1st-instar larvae were introduced. The top of the box was securely covered with black cotton cloth.

Fresh food was introduced into the box at 2-day intervals. Changing of the food was done by lifting the sides of the nylon mesh containing the larvae and placing them on top of the fresh food without touching the larvae individually.

When the larvae were about to pupate they were provided with sand placed on the floor of the box. The larvae moved from the food into the sand for pupation. The pupae were then collected and kept in adult emergence cages similar to the oviposition cages.

Whenever the larvae to be used were needed at the 4th instar stage they were reared individually in glass test tubes where their growth was monitored daily to ascertain their correct age.

2.2 Plant Materials

Seven cowpea cultivars from the International germplasm at IITA showing varying levels of resistance to M. testulalis (Jackai, 1982) and three cultivars from the Kenya germplasm were used in the comparison of resistance-susceptibility to M. testulalis. Cultivars

from IITA were VITA 1, VITA 3, VITA 5, Tvu 1, Tvu 946, Tvx 3890-10F and Kamboinse local. Cultivars from Kenya included Machakos 68, Chola local and Ex-Luanda.

VITA 1 was used as a susceptible check while Tvu 946 was the resistant check. VITA 1 is an indeterminate late maturing cultivar with thick succulent stems and broad leaves which form a thick canopy. Its pods are big with large red seeds. Its pods and flowers are usually within the leaf canopy. Tvu 946 is a determinate early maturing cultivar with small seeded pods. It has thin hard, stems and narrow leaves (wild-type). The peduncles are very long and carry the pods above the leaf canopy.

The local Kenyan cultivars are landraces which lack uniformity. Machakos 68 resembles VITA 1 in its growth habit. Chola local resembles Tvu 946 while Ex-Luanda is an indeterminate leafy cultivar with medium size stems and leaves.

Potted plants were used in some of the experiments. All such plants were grown from seed in 9-litre pots filled with garden soil.

2.3 Experimental site

All the experiments were conducted at the International Centre of Insect Physiology and Ecology (ICIPE), Mbita Point Field Station on the shores of Lake Victoria (3000 ft above sea level).

Mbita has two rainy seasons: long rains lasts for 4 months (March-June). The short rains lasts for 2 months (October and November). The amount of rainfall varies from year to year. Due to unreliability of rainfall, irrigation was used.

The soil type in Mbita field station largely varies from sandy clay to clay and the colour of the top soil varies from dark brown to very dark greyish brown (Rachilo and Wataka, 1980). Temperature during long rains ranges from 21-30°C and in the dry season the temperature rises up to 35°C.

2.4 Plant Extracts

The cultivars tested were Tvu 946 (resistant), VITA 5 (resistant) and VITA 1 (susceptible). Plants were grown at 50 x 30 cm on 3x7 m plots in insect-proof mosquito net cages in the field. Leaves, stems, flowers and pods were harvested and divided into 100 gm (fresh weight) samples.

Each sample was extracted sequentially by first dipping the sample in chloroform for 5 minutes after which the sample was removed and kept in the open for 6 h to allow traces of chloroform to evaporate. After extraction by chloroform the sample was dipped in n-hexane for 24 h after which the extract was transferred into a refrigerator and the sample dipped in a second jar of hexane for a further 24 h. The hexane extracts were then pooled together in one jar and the sample was kept in the open for 6 h to allow traces of hexane to evaporate. The same sample was finally extracted by methanol using the same procedure as for hexane.

To obtain pure extracts the bulk of the solvents were evaporated separately to approximately 10 ml using a rotavapor and decanted into preweighed glass vials. The extracts were then dried under nitrogen. The vials were re-weighed to obtain the actual amount of extracts in the vials. The residues were then re-dissolved in chloroform, Hexane or methanol to desired concentrations for bioassays.

2.5 Analysis of Data

All the data which were collected in percentages were transformed into arcsine $\sqrt{\frac{x}{n}}$ or square-root before statistical analyses as and when necessary (Gomez and Gomez, 1984). The significance between the means were determined by "F" test following the analysis of variance (Steel and Torrie, 1960). If F test was significant, the means were compared by Duncan's Multiple Range Test (DMRT) or the Least Significant Difference (LSD) according to Steel and Torrie (1960). Other statistical tests used for certain specific experiments are stated in the appropriate chapters.

CHAPTER 3

COMPARISON OF RELATIVE SUSCEPTIBILITY-RESISTANCE LEVELS IN THE SELECTED COWPEA CULTIVARS

3.1 Introduction

Both magnitude and the expression of genetic resistance of plants to insect and pathogens are influenced by environmental factors. Some of these environmental factors include climatic, edaphic and cultural factors of the crop environment (Tingey and Singh, 1980). There are innumerable examples of resistance that are adequate in one place being inadequate elsewhere and vice versa (Simmonds, 1984). Therefore, it was considered necessary to re-determine the relative susceptibility levels of the above cultivars to M. testulalis under Mbita Point ecological conditions. The experiment was also expected to provide information about the plant growth characteristics of the cultivars to enable selection from among them for more detailed studies.

3.2 Materials and Methods

Planting. Each of the 10 cultivars was planted in field plots of 2.2 m x 3 m in a completely randomized block design with four replications. The experiment was conducted during the short

rains (October-December, 1983). Tvu 946 and Chola local are early maturing cultivars, so they were planted seven days later than the rest of the cultivars to synchronize their flowering times. Spacing was 50 cm between rows and 30 cm within the rows.

Infestation of plants with larvae. Twenty-eight days after planting (preflowering phase), 20 plants in the middle rows in each plot were infested with M. testulalis by fixing 20 eggs at black head stage on the stem of each plant following a method by Dabrowski et al. (1983) for assessment of damage to the stem branches. At peak flowering time twenty plants in each plot were again infested with eggs as above for assessment of damage to flowers and pods.

Parameters and their measurements. The damage to the stems was measured 10 days after infestation. Two parameters were used: (i) percentage of the stem branches per plant harbouring the larvae as indicated by entry holes and (ii) percentage of stem branch tunnelled. For the first of these, the twenty plants which were infested were uprooted. The total number of stem branches per plant were counted and dissected to check for the larvae or entry holes. Those branches which had the larvae or entry holes were counted and recorded. The damage was expressed in percentage as follows:

$$\% \text{ damage to stem branches} = \frac{\text{No damaged stem branches}}{\text{Total No. stem branches}} \times 100$$

For the second parameter, the damaged stem branches were sorted out. The total length of each branch was measured. The length of the tunnel in the branch was also measured. The damage was computed as:

$$\text{Percentage of stem branch tunnelled} = \frac{\text{Length of tunnel}}{\text{Length of stem branch}} \times 100$$

For damage to flowers and pods, the percentage of those damaged by the larvae per plot was recorded. Fifty flowers were randomly sampled from all the 20 plants, five days after infestation. The sampled flowers were dissected to count the number of flowers with M. testulalis larvae. The damage was computed as:

$$\% \text{ damaged flowers} = \frac{\text{No. of flowers with larvae}}{\text{total No. of sampled flowers}} \times 100$$

Damage to the pods was assessed ten days after infestation following the same procedure as from damage to the flowers.

The data (percentage) were transformed to arcsine $\sqrt{\%}$ before statistical analysis. The significance between the means was determined as explained in chapter 2.

3.3 Results

With reference to the stem damage, the percentage of damaged stem branches was highest for the susceptible check VITA 1 and VITA 3 ($P = 0.01$). In Tvu 946 and Ex-Luanda the damage was lowest, being about one-sixth of that for the susceptible check (Table 1 and appendix 1). Tvu 1, Tvx 3890-10F and Machakos 68 were statistically identical to the susceptible check whereas VITA 5, Kamboinse local and chola local were statistically identical to Tvu 946 (Table 1).

In terms of stem tunnelling as well, the damage was highest ($P = 0.05$) in VITA 1 and VITA 3 and lowest (about one-third of the susceptible check) in Tvu 946 (Table 1). However, the stem tunnelling in Ex-Luanda unlike the percent damage to the branches was as high as that in VITA 1. Tvu 1, Tvx 3890-10F, Machakos 68, Chola local and Kamboinse local were not significantly different from VITA 1 in stem tunneling. VITA 5 was not significantly different from Tvu 946 in stem tunneling as reported above for percent damage in stem branches.

In most cases the damage started at the tip of the shoots. However, in some cases the larvae bored into the stem at about 1-4 internodes from the tip of the shoot. In such cases the branch broke at the point of the larval entry hole and the portion of the branch above the larval entry hole withered. Some cultivars reacted to the stem damage by producing new shoots below the damaged point.

With reference to flowers, the percent damage was high and not significantly different for VITA 1, VITA 3 Tvx 3890-10 F and Ex-Luanda ($P = 0.5$). For the remaining cultivars the percent damage was significantly lower (being about two-thirds) than that for the susceptible check VITA 1 (Table 1).

With reference to pods, the percent damage was highest for VITA 1 as well as Ex-Luanda and Machakos 68 ($P = 0.01$). For the remaining cultivars it was significantly less being about one-third to one-half to that for VITA 1 (Table 1).

In view of the above, three cultivars namely the highly susceptible VITA 1, the moderately resistant VITA 5 and the most resistant Tvu 946 were selected for the subsequent studies on the insect plant relationships.

3.4 Discussion

As was stated by Painter (1951), Panda (1979) and Kumar (1984) the term resistance is relative and is definable only in terms of other varieties of a species. Based on the parameters which were measured in this study, there were statistical differences among the cultivars which can assist in differentiating the resistant from the susceptible.

Table 1. Comparison of susceptibility-resistance levels of certain cowpea cultivars to Maruca testulalis

CULTIVAR	% (+ S.E.) DAMAGE ^{1/}			
	Stem**	Stem tunnelling*	Flower*	Pod**
VITA 1	20.03 + 3.1a	47.66 + 7.8a	49.00 + 8.1a	60.00 + 7.3a
VITA 3	20.49 + 3.1a	48.32 + 9.4a	43.50 + 9.1a	-
VITA 5	6.89 + 2.8cd	25.83 + 2.8bc	30.00 + 5.7b	20.00 + 0.8b
Tvul	10.24 + 1.6abc	42.75 + 6.7ab	30.00 + 6.0b	29.00 + 9b
Tvu 946	3.27 + 1.2d	18.71 + 0.8c	21.00 + 3.7b	25.50 + 2.6b
Tvx 3890-10F	13.67 + 1.7abc	35.78 + 7.0ab	48.00 + 4.9a	24.50 + 4.9b
Kamboinse local	9.55 + 3.3bcd	34.74 + 6.9ab	27.00 + 5.1b	21.00 + 3.1b
Chola local	5.89 + 3.4cd	29.11 + 5.0ab	24.00 + 2.4b	22.50 + 3.8b
Machakos 68	10.69 + 3.6abc	43.61 + 4.8ab	36.75 + 3.5ab	49.00 + 7.5a
Ex-Luanda	3.15 + 2.8d	47.48 + 2.1a	43.00 + 5.2a	58.50 + 9.6a

^{1/} Mean of 4 replications (detransformed data)

* In a column, means followed by a common letter are not significantly different at P = 0.05 by DMRT.

** In a column, means followed by a common letter are not significantly different at P = 0.01 by DMRT.

Considering the overall resistance or susceptibility levels of the plant, the tested cultivars can be categorized into four broad groups:

- (1) Those cultivars in which the values for all the 4 damage parameters were high and therefore can be regarded as the most susceptible among the tested cultivars. These include VITA 1 and VITA 3.
- (2) Those cultivars in which all the values for the damage parameters were low and therefore can be regarded as the most resistant. Only Tvu 946 can be included in this group.
- (3) The third group includes those cultivars which were similar to VITA 1 in one of the damage parameters and to Tvu 946 in the remaining parameters. These cultivars were considered as moderately resistant and included VITA 5, Kamboinse local, Chola local and Tvu 1.
- (4) The fourth group includes those cultivars which were similar to VITA 1 in most of the damage parameters (3 out of 4). These cultivars were considered as moderately susceptible and included Ex-Luanda (ICV 6), Machakos 68 and Tvx 3890-10F.

Tvu 946 has consistently remained the only cowpea cultivar with the highest level of resistance to M. testulalis (Singh 1978; Jackai, 1982; Dabrowski et al., 1983 and Singh et al., 1984). Kamboinse local Tvu 1 and Tvx 3890-10F were also reported by Jackai (1982) as resistant. In this study the overall resistance of these cultivars to M. testulalis was also lower than that of Tvu 946.

The levels of resistance of the tested cowpea cultivars as reported above are somewhat different from those reported by Jackai (1982). Taking pod damage parameter as an example, in Jackai's (1982) data Tvu 946 had a significantly lower infestation compared to Kamboinse local, VITA 5 and Tvx 3890-10F. In the present study the pod damage in these cultivars as well as in Tvu 946 was statistically equal. These differences would be due to changes in environmental conditions (Painter, 1951; Tingey and Singh, 1980; Kumar, 1984) or differences in the evaluation methodology. While screening, Jackai (1982) planted the test cultivars in single row plots side by side, each 5 m long. He also depended on natural insect populations for infestation of the test cultivars. This arrangement may have given the moths a free choice so that if Tvu 946 was planted next to a preferred cultivar, it had more chances of escaping the attack compared to when it was planted in multi-row plots and infested artificially with uniform insect population.

Saxena and Khan (1984) observed that planting the test varieties in single rows side by side in a screening exercise leads to some

less resistant rice varieties escaping the attack by whitebacked plant hopper due to emigration of the insect into the more preferred susceptible varieties.

Of the cultivars which have been classified here as resistant, Chola local is a local Kenyan selection and is being reported for the first time as resistant, relative to the susceptible check (VITA 1).

CHAPTER 4

EGG PRODUCTION (FECUNDITY) AND OVIPOSITION

4.1 Introduction

The first step in the selection of a plant by a lepidopteran insect like M. testulalis involves egg-laying. Differences in the number of eggs laid on different cowpea cultivars would therefore contribute to those in their colonization by this pest. The number of eggs laid on different plants by the insect may differ due to;

- (1) Egg-production, i.e., the actual number of oocytes developed in the ovaries reflecting the insects fecundity, and,
- (2) oviposition i.e., the number of eggs actually deposited on the plant.

As mentioned in chapter 1 on the review of literature, hardly any information is available on the egg production (fecundity) in M. testulalis in relation to different cowpea parts and cultivars. Also, there are conflicting reports on the cowpea plant part(s) on which the eggs are laid. According to some reports, flowers, flower buds and abscission scars are the most preferred sites for oviposition (Jackai, 1981a) in Nigeria. Taylor (1978) reported that eggs are normally deposited on flower buds and flowers although oviposition also occurs on leaves, leaf axils, terminal shoots and pods. According to Okeyo-Owuor and Ochieng (1981) in Kenya, most of the eggs (86%) are laid on leaves. Some of these reports were based on field

observations (Taylor, 1978) where factors other than plant characters (for example differences in temperature, light intensity and humidity) can also influence ovipositional differences. But, there is hardly any information available on oviposition on different plant parts and cowpea cultivars due to influence of plant characters.

These gaps in our knowledge have been filled in the present work with reference to three selected cultivars i.e., VITA 1 (susceptible), VITA 5 (resistant), Tvu 946 (resistant), and the results are given in this chapter.

4.2 Materials and Methods

4.2.1 Egg production (fecundity)

One hundred 1st-instar M. testulalis larvae were reared to pupation separately on stem, leaves, flowers and pods of the three cultivars in the laboratory. The emerging adults were sexed and paired for mating. Each pair was caged in nylon mesh cages (25 x 25 x 40 cm) (Fig. 1). Each cage contained 10-day old potted plants of the susceptible VITA 1 cultivar for oviposition. The moths were fed on 10% sucrose solution soaked in cotton wool in a glass petridish. The plants were examined everyday for eggs and replaced with new ones.



Fig.1. A nylon mesh cage used for enclosing Maruca testulalis females on cowpea plants for studying egg production (fecundity).

Number of eggs laid by each female was recorded daily until it died. Dead insects were dissected immediately to count the mature eggs in the ovary. Total number of eggs per female was recorded. Number of insects tested was variable because of differences in development of insects on different plant parts.

4.2.2 Ovipositional Responses to Different Cowpea Cultivars.

Oviposition response of the female to the test cultivars was compared under choice and no-choice situation. Four-day old mated females from the laboratory culture were used.

In no-choice tests, 4 potted plants of each cultivar at peak flowering stage were caged in a wire net (8 meshes/cm) test chamber (1m x 1m x 1m). The experimental design was completely randomized block with eight replications. Three females in the standardized ovipositional stage mentioned above were introduced into each cage for 24 h for oviposition. The total number of eggs laid on each cultivar was counted at the end of 24 h period.

A choice test was conducted in a 3 sector chamber, 2.4 x 0.8 x 0.8 m (Kumar and Saxena, 1985) (Fig. 2). The top of the cage and its doors were made of glass. The remaining parts were made of wire net. Potted plants were buried in the soil at 2m apart. The plants were then covered with the cage so that VITA 1 was in one end sector while Tvu 946 was in the opposite end sector of the chamber. The tests were conducted using flowering and

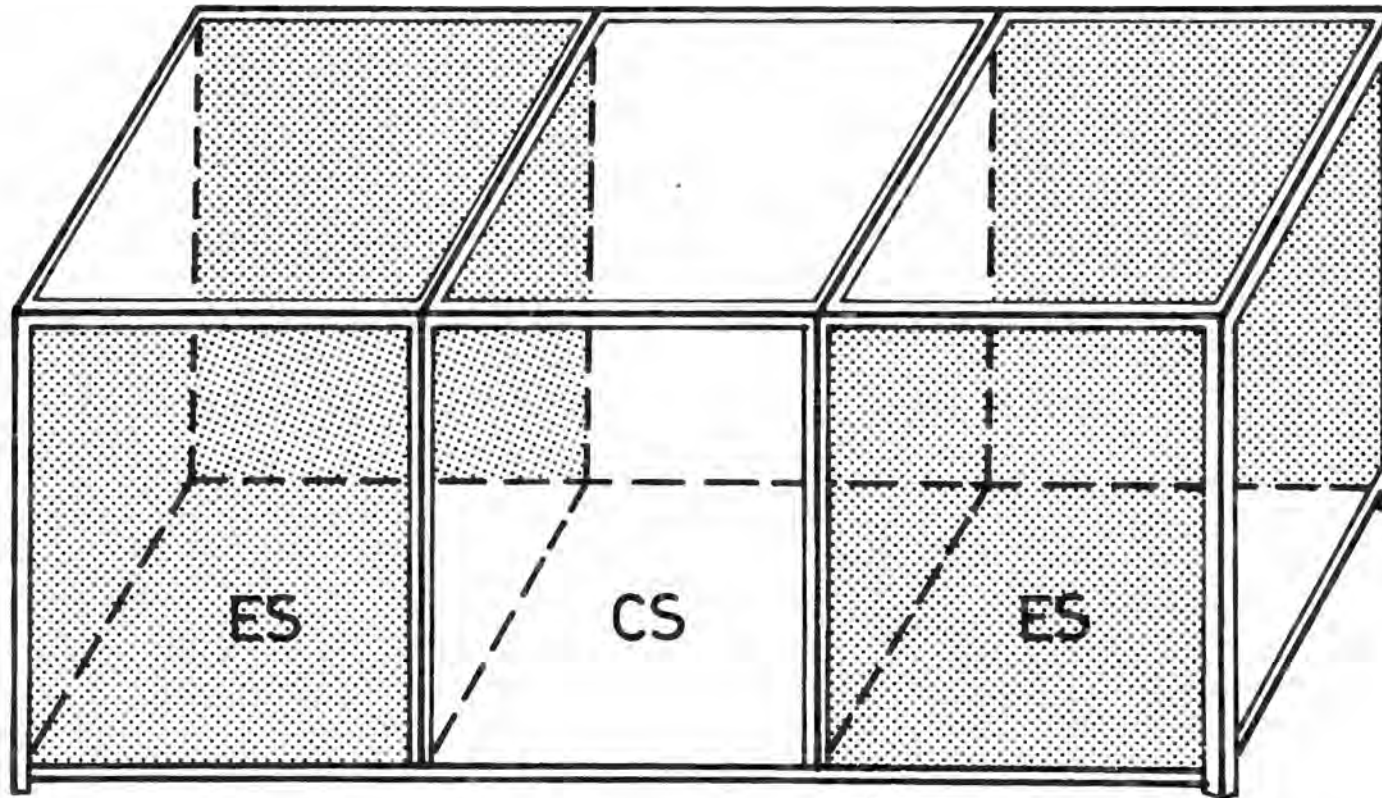


Fig:2. A 3-sector chamber for studying oviposition responses of *M. testulalis* females to different cowpea cultivars under a two choice situation. (Kumar & Saxena,1985).
ES = End sectors of the chamber CS = central sector of the chamber

preflowering plants. Five moths were introduced into the chamber at 16.30h and removed the following day at 14.30 h (after 20 h). The experiment was replicated seven times. Number of eggs laid on each cultivar was counted and the data was compared by Chi-square test.

4.2.3 Ovipositional Responses to Different Parts of Cowpea Plants

Oviposition responses to different parts of the cowpea plants were studied using whole plants and freshly excised plant parts. Experiments using whole plants were conducted in single compartment wire net chamber described above. Whole potted cowpea plants which were at peak flowering stage with their different parts being available in natural proportions were offered to the females in the chamber for 24 h for oviposition. Each chamber contained 4 cowpea plants and 3 M. testulalis females. Each cultivar occupied a single cage. The experimental design was 3 x 6 factorial with 8 replications.

Factor "A" comprised the three cultivars and factor "B" comprised the six different parts of a cowpea plant namely stem, leaves, peduncles, flower buds, flowers and pods. The plants were removed and checked for eggs at the end of the 24 h period. Egg numbers and distribution within different parts of the plants were recorded. The percentage distribution was calculated for each plant part.

The experiment, using excised parts was conducted in the laboratory at 25-28°C room temperature. Twelve small holes (7mm diameter) were perforated along the edge of the cap of a plastic jar (8 cm diameter) forming a circle removed 3 mm from the edge.

The jar was filled with tap water and then wrapped with aluminium foil. Two of each of the six parts of the cowpea plants, stems, leaves, peduncles, flower buds, flowers and pods were randomly fixed in the circle with their cut ends dipping in water to keep them fresh (Fig. 3). The jars containing the excised plant parts were kept singly inside circular nylon mesh cages (28 cm diameter, 40 cm high). (Fig. 3). One female was introduced into each cage over night (24 h) for oviposition.

A total of 24 females were tested (8 females for each cultivar). The number of eggs laid on each plant part was recorded at the end of the 24 h period and converted into percentage of the total oviposition per cage.

4.3 Results

4.3.1 Egg production (Fecundity)

Egg production in F₁ females emerging from the larvae reared on stems and leaves was very low (about one-third) compared to eggs produced by those emerging from flowers and pods (Fig. 4 and



A



B

Fig.3. Arrangement for studying oviposition responses of Maruca testulalis females to excised parts of cowpea plant in the laboratory.

A- Freshly excised plant twigs with their ends immersed in water in a plastic jar to keep them fresh.

B- Cages containing the above twigs and one M. testulalis female.

Appendix 2). Egg production by females emerging from flowers and pods were more or less similar. On an average, there were no significant differences among the three cultivars in this regard.

4.3.2 Ovipositional Responses to Different Cowpea Cultivars.

These responses were studied for the preflowering as well as peak-flowering stages of the 3 test cultivars under no-choice and two-choice situations. When each test cultivar was offered alone (no-choice test) in a single compartment chamber, the number of eggs laid on the three cultivars were not statistically different in pre-flowering as well as peak-flowering stages (Fig. 5 and Appendix 3).

However, on presenting the most susceptible VITA 1 as a choice against the resistant Tvu 946 at preflowering stage in the two end-sectors of the 3 sector chamber for oviposition, the number of eggs laid on VITA 1 was three times higher than that on the resistant Tvu 946 which was significant at $P = 0.01$ by Chi-square test (Fig. 6 and Appendix 4). Similar trend was observed at flowering stage.

Similarly, when comparing oviposition on VITA 1 and the resistant VITA 5 in a 2-choice situation, at preflowering stage the egg laying on VITA 1 was twice as much as on VITA 5 which were statistically significant at $P = 0.01$ by Chi-square test. At flowering stage egg laying on VITA 1 was one and a half times higher than on VITA 5 (Fig. 6 and Appendix 4). Thus M. testulalis shows

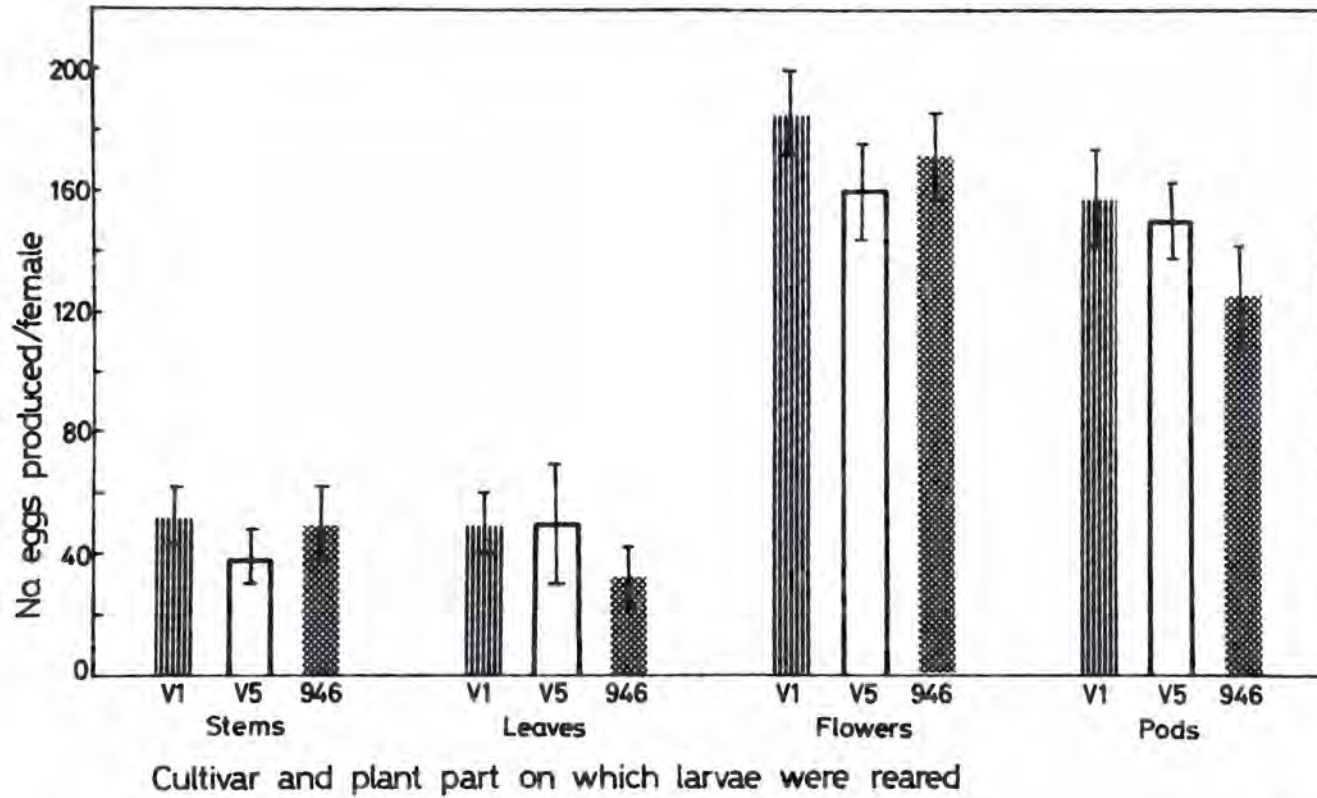


Fig:4. Egg production (fecundity) in *Maruca testulalis* females (FI) reared on different parts of cowpea plant.
VI = VITA I; V5 = VITA 5; 946 = Tvu 946

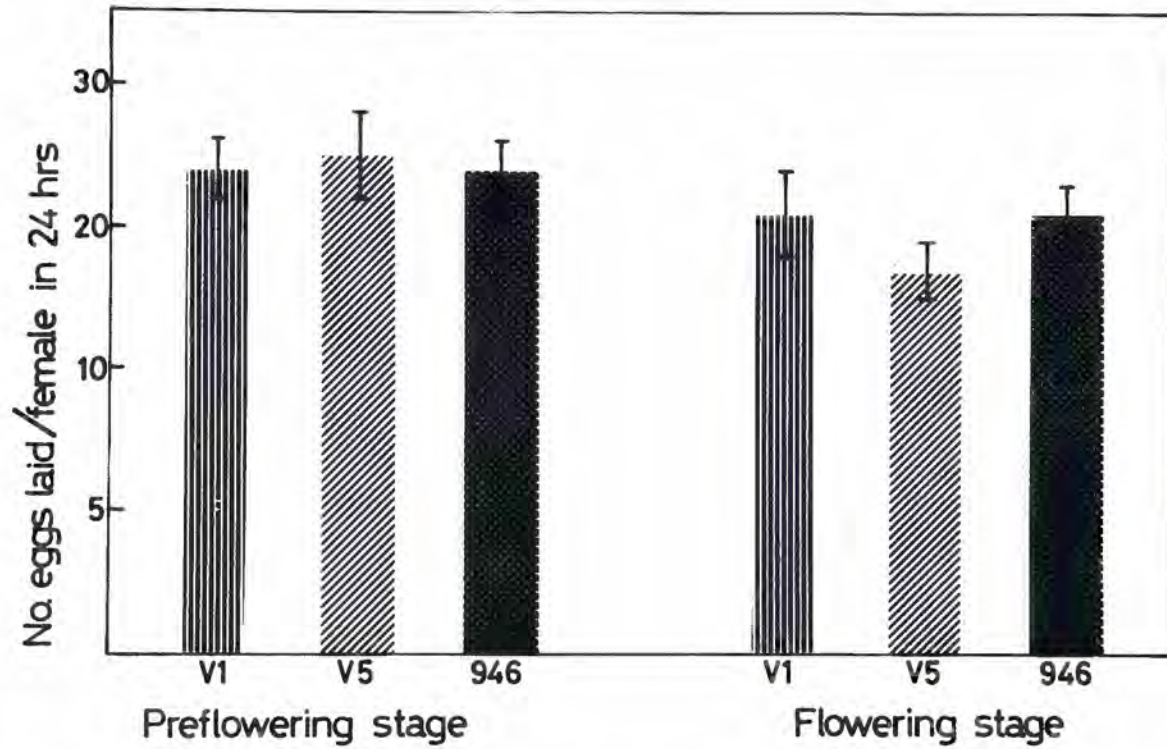


Fig:5 . Ovipositional responses of Maruca testulalis females to different cowpea cultivars under no choice situation.
V1 = VITA I; V5 = VITA 5; 946 = Tvu 946

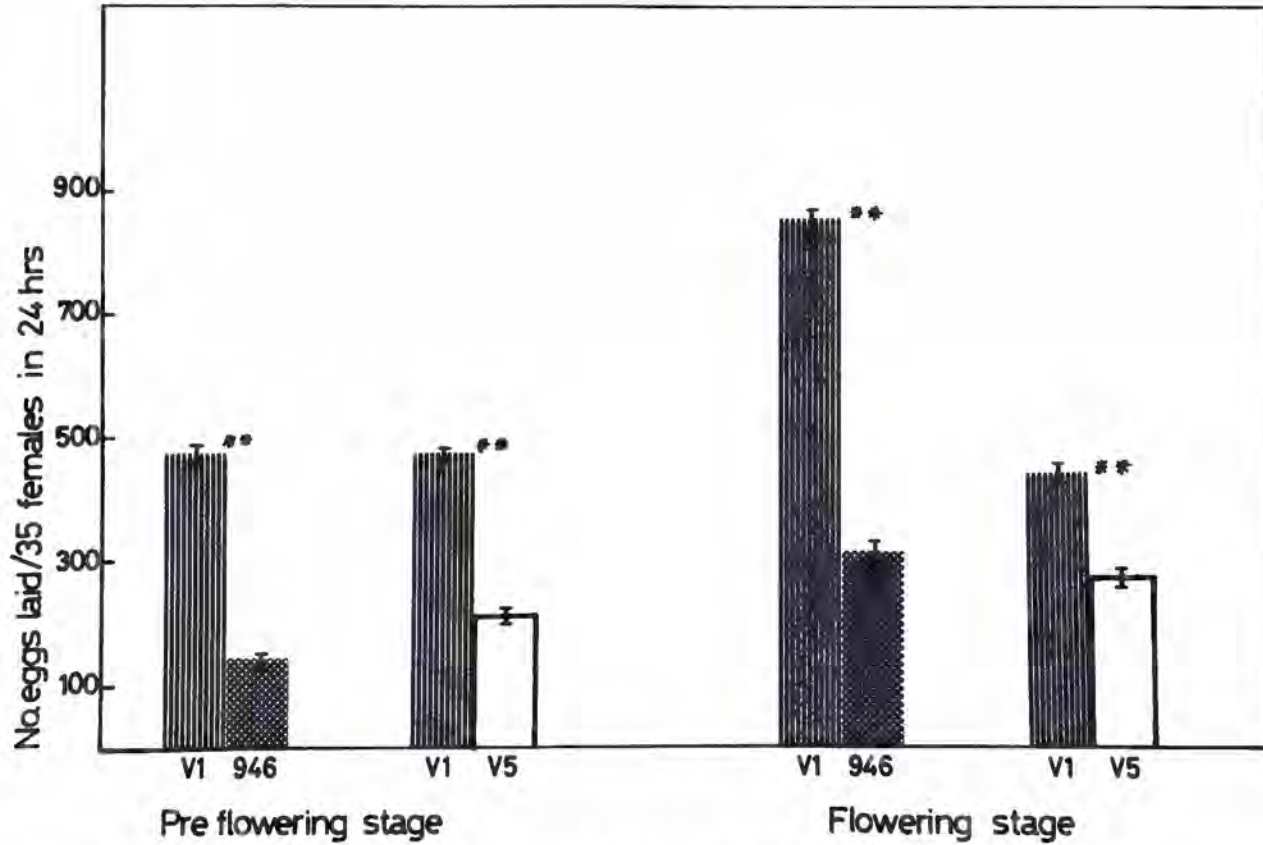


Fig. 6. Ovipositional responses of *Maruca testulalis* females to different cowpea cultivars under a 2-choice situation. V 1 = VITA 1; V5 = VITA 5; 946 = TVu 946. The column marked with ** is significantly different from the unmarked column at $P = 0.01$ by χ^2 .

ovipositional preference for VITA 1 against Tvu 946 or VITA 5 under free choice situations both at flowering and preflowering stage.

4.3.3 Ovipositional Responses to Different Parts of cowpea Plant.

When the females were allowed access to a flowering cowpea plant on which all its parts were available, the percentage of eggs laid on the leaves was the highest (Fig. 7 and Appendix 5). The percentage of eggs laid on the remaining parts of the plant was low and not statistically different. There was a tendency to concentrate the eggs along the base of the veins of the lower surface of the leaf, grooves and scars along the stem, peduncles and other parts of the plant.

Even when different parts of each cultivar were excised and offered together in almost equal proportions so that their surface area was almost identical, the oviposition on the leaves of the susceptible VITA 1 was much greater than that on the remaining parts ($P = 0.05$) (Fig. 8 and Appendix 6). For VITA 5; the egg-laying on both leaves and peduncles was equally higher than on the other parts, whereas on Tvu 946, the percentage of eggs laid on all the parts was identical.

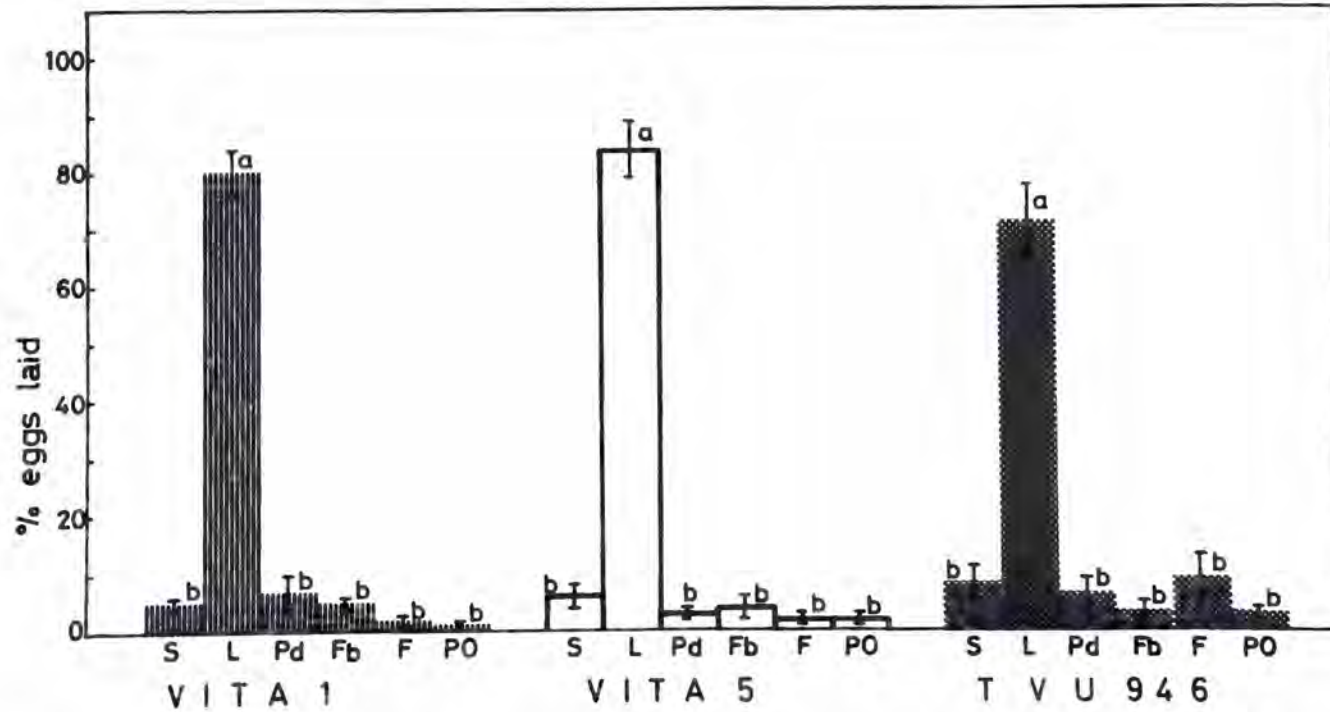


Fig:7. Percentage of *M. testulalis* eggs laid on different parts of potted cowpea plants S= Stem; L= Leaves; Pd= Peduncles; Fb= Flower buds; F= Flowers; PO= Pods. Within each cultivar, columns with a common letter are not significantly different at P= 0.01 by DMRT.

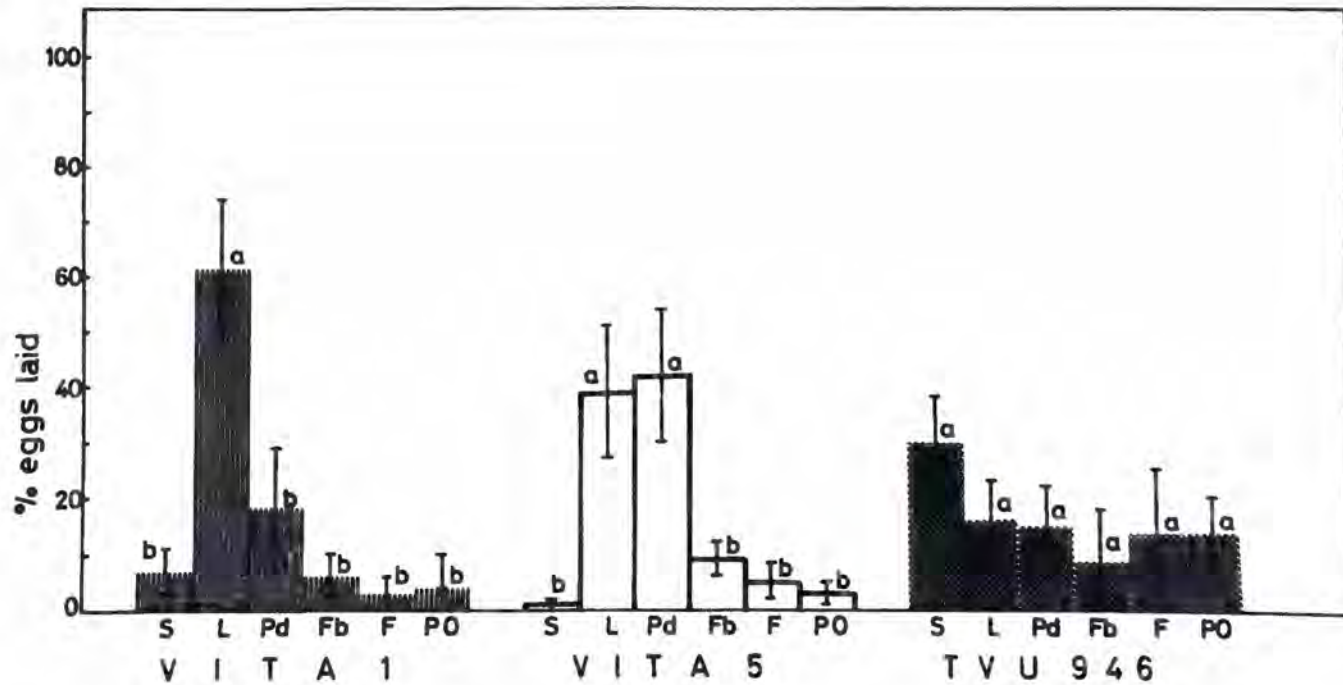


Fig:8. Oviposition responses of *M. testulalis* to freshly excised parts of certain cowpea cultivars under a free choice situation in the laboratory.
S = Stem; L = Leaves; Pd = Peduncles; Fb = Flower buds; Po = pods.
Within each cultivar column with a common letter are not significantly different at $P = 0.01$ by DMRT.

4.4 Discussion

4.4.1 Egg production (Fecundity)

Determination of egg production is one of the parameters for determining suitability of a host plant of an insect. It is also an indicator of antibiosis (Painter, 1951; Kogan, 1975; Saxena 1969, 1985).

The above results reveal two points which are important in the colonization process of the cowpea plant by M. testulalis

- (i) The part of the cowpea plant on which the M. testulalis larvae developed did not affect the fertility of the emerging moths since all the moths were able to mate and lay fertile eggs.

- (ii) Although M. testulalis larvae fed and developed, though at a low percentage, on the leaves and stems of a cowpea plant, the fecundity of the moths was drastically reduced on these plant parts than when the larvae developed on flowers and pods. This shows that the leaves and stems are unsuitable not only for the larvae but also for production of eggs in the adults. But in Kenya it should be noted that leaves are preferred for oviposition.

4.4.2 Ovipositional responses to different cowpea cultivars

The data presented above on the oviposition by M. testulalis shows that both the resistant and the susceptible cultivars were equally accepted for oviposition under no choice conditions. However, under free choice situation the susceptible VITA 1 was preferred to Tvu 946 and VITA 5. Macfoy et al (1983) also reported that VITA 1 was more preferred for oviposition than Tvu 946 when the two were planted side by side in a greenhouse. They did not report on oviposition responses under no choice conditions. This relationship provides an indication that the resistant cultivars (Tvu 946 and VITA 5) do not have an ovipositional repellent or deterrent. On the other hand, both the susceptible and the resistant cultivars evidently have stimuli which elicit oviposition, those from the susceptible VITA 1 being more effective than from the resistant cultivars.

Normally in the farmers fields one cultivar is grown in the plot. Such a situation does not give the insect an opportunity to choose the most preferred cultivar. Under such conditions, the observed non preference for oviposition by M. testulalis may be rendered ineffective as a defence mechanism by the cowpea (Tvu 946 and VITA 5 cultivars).

4.4.3 Ovipositional responses to different parts of cowpea plant

Preference for cowpea leaves to other plant parts for oviposition by M. testulalis was first reported by Okeyo-Owuor and

Ochieng (1981). This was however contradicted by Jackai (1981a) who reported that flowers and flower buds were the most preferred sites for oviposition. The data obtained in this study confirms the observations of Okeyo-Owuor and Ochieng (1981) that leaves are the most preferred oviposition sites although M. testulalis oviposits on all the aerial parts of the cowpea plant. M. testulalis is known, to invade cowpea fields as early as 21 days after germination. Jackai (1981b). It is therefore not surprising to find majority of the eggs on the leaves since at this stage flowers and flower buds are not available on the cowpea plant. This also provides a further indication that oviposition stimulant(s) for M. testulalis seems to exist in all the aerial parts of the cowpea plant

Studies by Okeyo-Owuor and Ochieng (1981) were conducted in East Africa while studies by Jackai (1981a) were conducted in West Africa. Since these two regions are geographically different, there is a possibility that the insects in the two regions could represent different biotypes, of the same species.

CHAPTER 5

LARVAL ORIENTATION

5.1 Introduction

The larvae emerging from the eggs laid on a plant need to orientate appropriately in order to reach a suitable site for feeding on the same or another plant. The continued stay of the larvae on the plant on which they emerge reflects their 'arrest' by the plant. On the other hand, the larvae that move out of that plant may sooner or later reach other plants in the vicinity. The arrival of these larvae on some plants may be accidental or due to their attraction. On the other hand the failure of the larvae to move to some other plants may be due to their repulsion or lack of attraction.

In view of the above, differences among cultivars in influencing larval movements and in eliciting their arrest/stay, attraction or repulsion can contribute to the susceptibility or resistance of these cultivars. These responses of M. testulalis for the target cultivars have, therefore, been studied in this work.

5.2. Materials and methods

5.2.1 Arrest/stay of larvae on plants

The suitability or otherwise of the test cultivars for larval arrest was compared on the basis of two parameters: (i) The rate of departure of the larvae from the plants; (ii) The percentage of the larvae retained by the plants 24 hours after release.

For studying the rate of departure of the larvae from the plants, a piece of polythene sheet was spread around the base of a 30-day-old potted cowpea plant, covering the whole top surface of the pot. Ten first instar larvae were gently picked singly with a camel hair brush and placed randomly on the leaves of the plant. The movements of the larvae were observed and the number of larvae dropping or walking away from the plant was recorded every 10 minutes. The observations were continued for 75 minutes after which no more larvae were seen to move away from the plant. The rate of departure of the larvae was determined by the number of larvae moving away from the plants at successive 10-min. intervals. The faster the rate of the larval departure from the cultivar, the lower would be the larval arrest on it. The experiment was replicated 5 times with different batches of larvae at the room temperature (25-29°C).

The larval retention on a cultivar was studied with reference to pre-flowering as well as flowering plants in the field

and in the laboratory. In the field, the three test cultivars (VITA 1, VITA 5 and Tvu 946) were planted in 2m x 2.4 m plots, each in a completely randomized block design with 4-6 replications. The spacing was 60 cm between rows and 30 cm within rows. Preflowering plants were infested when they were 28 days old. Five plants in the middle row were covered with nylon mesh cages to protect them from infestation by natural populations of M. testulalis. When these plants were 28 days old (pre-flowering stage or 42 days old (flowering stage), the cages were removed and 20 newly hatched (1st instar) larvae were released randomly on the leaves of each plant at 18.00 h, making a total of 100 larvae for 5 plants. The surrounding uninfested plants were removed. After 20hrs, the percentages of the released larvae remaining on the plants was recorded and reflected the larval arrest. Since Tvu 946 is an early maturing cultivar, so it was planted one week later than VITA 1 and VITA 5 to synchronize their flowering time.

For the laboratory studies on the larval arrest/stay on the test cultivars, potted plants at peak flowering stage were used. Twenty eggs at the black head stage were glued randomly on the leaves. The pots were randomly arranged on a table in the laboratory at room temperature 23-28°C. Twenty hours after infestation, the eggs were examined under a microscope for egg hatch. Number of hatched eggs was recorded and the plants were also dissected to count the number of larvae in them. The data on number of larvae on the plant was expressed as percentage of the hatched eggs.

5.2.2 Sites for larval arrests in relation to their site of release on the cowpea plant.

Sites for the arrest of 1st instar larvae on the test cultivars were studied on the potted plants at peak flowering stage in a 3x4x6 factorial experiment with 4 replications. Factor 'A' treatment consisted of the three cultivars. Factor 'B' consisted of sites of egg placement and factor 'C' consisted of six settling sites namely stem, leaves, terminal shoots, flower buds, flowers and pods. The pots were randomly arranged on a table in the laboratory at room temperature 23-28°C. Twenty M. testulalis eggs at the black head stage were fixed on the stems, leaves, flowers or pods. Forty eight hours after the infestation, the plants were dissected to recover the larvae. The percentage of larvae recovered from each part of the plant was recorded.

5.2.3 Attraction of the larvae by the plants

Both no choice and free choice experiments were conducted in the field and in the laboratory.

In the no choice situation under field conditions, preflowering plants (30-day-old) and flowering potted plants (42-day-old) of each cultivar were randomly buried in the ground so that only the pot was under the ground while the whole plant shoots remained above the ground. Spacing was 60 cm between and within the rows. There were 5 replications for each cultivar. The ground was

watered 6 h before the release of the larvae. Fifty first instar larvae were placed on the ground uniformly distributed around each plant (15 cm from the stem). In the laboratory; potted plants were randomly placed in rows on a table at room temperature 25-29°C. Fifty first instar larvae were placed on the soil surface along the edge of the wall of the pot. The wall of the pot was smeared with tangle foot glue to trap the larvae which were moving out.

Free choice experiments were conducted by offering the three cultivars simultaneously to the larvae. In the field, potted plants were arranged in an equilateral triangle 15 cm on all sides and buried in the ground. In the laboratory, the pots were kept side by side in a triangular manner (15 cm apart). The surface was covered with polythene sheet with plants emerging through the sheet. Both in the field and in the laboratory, fifty first instar larvae were released in the centre of the plants so that they could choose where to move to. The experiments were replicated 5 times in a completely randomized block design.

In both no choice and free choice experiments, the plants were dissected after 24 h and the number of larvae arriving on each cultivar was recorded. The data were expressed as percentage of larvae which were released.

In order to determine the attraction of the larvae to the plants in relation to their distance from the plant, an experiment was conducted in a 2 x 3 factorial design with 5 replications.

Factor 'A' consisted of two cowpea cultivars (VITA 1 and Tvu 946). Factor 'B' was the distance of larval placement from the plant which were 10, 20 and 30 cm.

Thirty-day-old potted plants were buried 2 m apart in the ground in the screenhouse. There were 5 plants for each cultivar and each plant formed a replicate. The ground was watered 4 h before the larvae were released. Twenty 1st instar larvae were released uniformly around each plant at 10, 20 or 30 cm from the plant. Twenty four hours after larval release the plants were dissected to count the larvae in them. Number of larvae found in each plant was converted and expressed as a percentage of the total number which was released.

5.2.4 Larval movement from one cultivar to another grown in alternate rows in a plot.

The three test cultivars, VITA 1, VITA 5 and Tvu 946 were planted in 2m x 2.4m plots in the field. There were a total of 9 combinations including pure stands of each cultivar. The combinations were as follows:

- | | |
|---|---|
| 1. VITA 1 pure stand | 6. VITA 1 middle row
VITA 5 side rows |
| 2. Tvu 946 pure stand | 7. VITA 1 side rows
VITA 5 middle row |
| 3. VITA 5 pure stand | 8. Tvu 946 middle row
VITA 5 side rows |
| 4. VITA 1 middle row
Tvu 946 side rows | 9. Tvu 946 side rows
VITA 5 middle row |
| 5. VITA 1 side rows
Tvu 946 middle row | |

Each combination was replicated 4 times. There were 5 rows in each plot, the middle row being a cultivar different from the one planted in the remaining rows except in the pure stand plots. Spacing between rows was 50 cm while spacing between plants was 30 cm. Plots were laid 3 m apart. Planting was staggered to synchronize the flowering time of the test cultivars. VITA 5 was planted four days later and Tvu 946 seven days later than VITA 1.

At peak flowering time, five plants from the middle row in each plot were infested with 100 first instar larvae. Forty eight hours after the infestation, 15 plants (the five plants in the middle row which had been infested and the neighbouring 5 plants from

each of the next adjacent row on each side) were uprooted and dissected to count the larvae in them. The larvae recovered from the middle row and the adjacent rows were recorded separately. The significance of the difference between the larval distribution in the two cultivars in each plot was determined by Student's 't' test.

5.2.5 Role of soil moisture in determining
arrival of first instar larvae on plants.

Two 30-day old potted plants of VITA 1 were buried 2m apart on the ground. The soil surface (30cm radius) around one plant was watered before larval release. The soil surface around the other plant was kept dry. A single larva was placed on the ground 10 cm from the plant. Its movement was observed and time taken (min) to reach the plant was recorded. A total of five observations were made using five different insects. All the observations were made from 8.00 am - 12.00 noon when the air was relatively still.

The experiment was also conducted in the laboratory using a filter paper to determine the speed of the larvae on the moist and dry surfaces in the absence of plants. The experiments were conducted in 14 cm glass petridish lined with filter paper. The filter paper was divided into two equal halves. One half was moistened with distilled water and the other half was left dry. A single first instar larva was placed in the centre of either dry or wet filter paper and its movement was traced for 10 minutes. The

distance covered by the insect was measured in cm and the speed (cm/min.) calculated. Five observations were made on each of the surfaces using different insects. However, sketch pattern was not drawn.

Under field conditions, 1m² ground which had been ploughed was flattened and large stones removed. Half of the prepared ground was watered before larval release. Five larvae were released, one at a time in the centre of the moist or dry surface. The movement was tracked for 10 min. and distance measured in the same way as for the laboratory test.

5.2.6 Role of plant volatiles on the larval attraction/arrest

The study of this aspect was undertaken to examine the role of the volatiles, which might serve as olfactory stimuli, from the cultivars in attracting the larvae. For this, larval responses were measured towards the leaves the flowers of the plants as well as their raw juice, chloroform and n-hexane extracts. These extracts were prepared as mentioned in chapter 2.

The method used was such as would allow the larvae to perceive the volatiles without getting into contact with the stimuli from the above test materials. This method involved the use of a glass tunnel which was similar to the type used by Saxena and Prabha (1975) except for slight modifications. The tunnel consisted of 3 glass tube segments (2.5cm inner diameter). One central and two

terminal joined end-to-end with one another (Fig.9). The central segment was 16cm long and was marked off into three equal sectors (left, centre, right) along its length using a rubber band. An entry hole (6mm dia.) was located in the middle of the central segment. Each of the ends of this central segment was formed by a female socket of ground glass joint (size 24/29). Each terminal segment was 15cm long and was joined to the central segment through a matching male ground glass socket. The other end of this segment was open. A piece of fine meshed thin muslin cloth was stretched across the inner end of the male joint of the terminal glass tube so as to provide a porous barrier between the interior of the terminal and central segments.

Each test material was introduced into one terminal segment (labelled 'A') through its open end which was then closed with a parafilm membrane . The other terminal segment was similarly provided with muslin barrier at its inner end and closed at the opposite end with a parafilm but was left empty to serve as a blank control. The volatiles from the test material in one terminal segment could pass through the muslin cloth into the central tunnel. Ten first instar larvae were released in the central segment through the entry hole. Their movement was observed for 10 minutes. The number of larvae present in each sector was recorded every minute. The tunnel was rotated by 180° at the end of each minute. The number of the larvae reaching the ends 'A' and 'B' of the tunnel were recorded at the end of the 10-minute observation period.

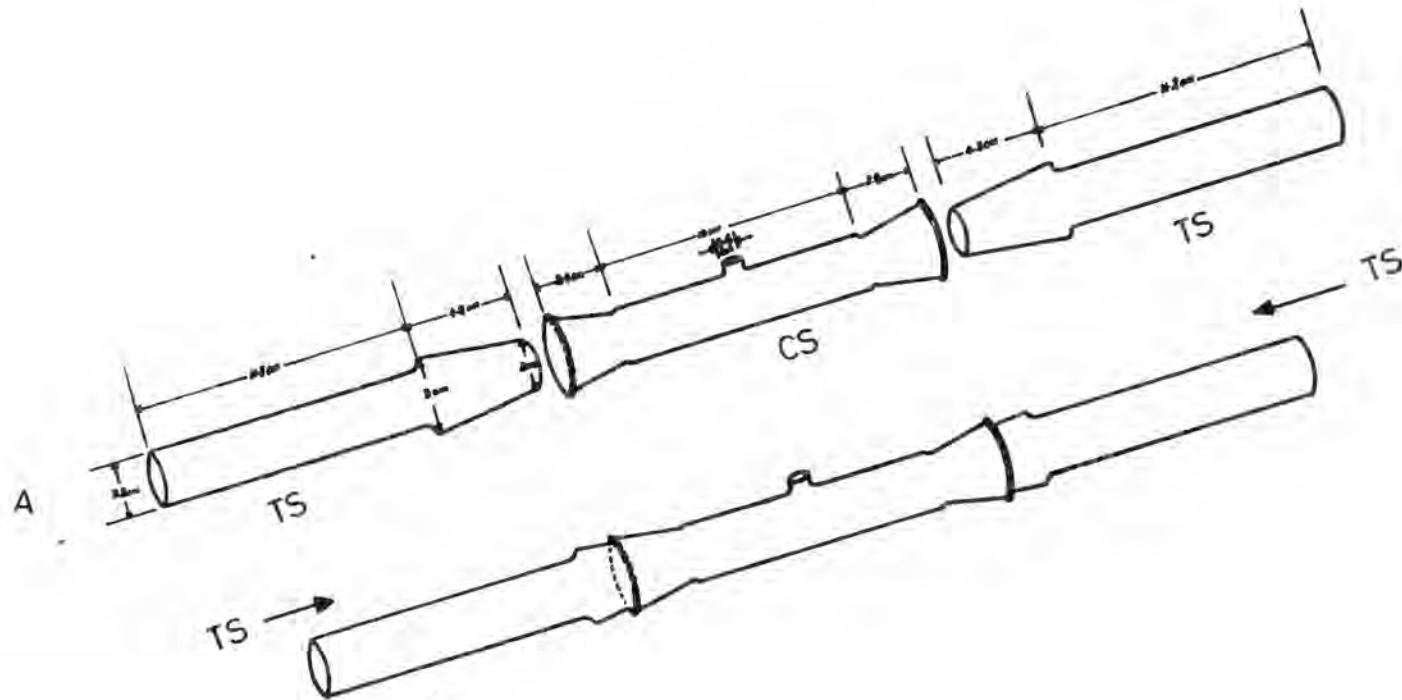


Fig.9. A glass tunnel olfactometer used for testing orientational response of *M. testulalis* larvae to volatiles from cowpea leaves and flowers. TS= Terminal segments of the olfactometer; CS= Central segment of the olfactometer.

A- The olfactometer dismantled to show its three components.
B- The three components of the olfactometer assembled.

A greater percentage of larvae moving towards the end 'A' than towards 'B' would reflect the larval attraction by 'A'. On the contrary, a lower percentage of larvae moving towards 'A' than towards 'B' would reflect larval repulsion by 'A' relative to 'B'. (Saxena and Prabha, 1975; Saxena and Khattar, 1977; Saxena and Rembold, 1984).

The table on which all the observations were made was uniformly illuminated by 4 fluorescent tubes (40 w each) suspended 130 cm above.

Sources of stimuli included freshly excised leaves, flowers, dry leaves, raw juice extract from leaves and flowers, chloroform and n-hexane extracts from leaves and flowers and moist and dry cloth.

Dry leaves were obtained by collecting fresh leaves from the field and drying them under room temperature in the laboratory at 25-28°C for 10 days.

Raw juice of the leaves or flowers of each cultivar was obtained by crushing them in a mortar and squeezing through muslin cloth with a pestle. The residues were removed and the same cloth in which the leaves were crashed was soaked in the juice extract, the cloth was folded and placed in the tube which was then covered with a dry piece of muslin cloth.

Choloroform and n-hexane extracts of the test cultivars were obtained as explained in chapter 2 and were tested at following concentrations, 0.10, 0.20 and 0.50 mg/ml. The extracts were impregnated on a 2 cm² piece of cheese cloth which was then introduced into the tunnel. The respective solvents served as control (Blank end).

5.2.7 Role of humidity and visual stimuli on larval orientation

To test the role of humidity, a 5 cm² piece of white cotton cloth was folded and soaked in 5 ml distilled water and placed in one of the terminal segments (side 'A') of the glass tunnel. A similar dry piece of cloth was placed in the terminal segment 'B'.

To eliminate possible differences in larval orientation due to the role of humidity when comparing leaves of two different cultivars; freshly excised leaves of the test cultivars were prepared and placed at the two opposite ends of the tunnel and covered with wet cheese cloth which had been soaked in distilled water.

Role of visual stimuli was tested by placing three leaves in the terminal segment 'A' whose inner opening was covered with a glass cover slip before joining it with the central segment of the tunnel. The visual stimuli from the leaves could then pass through the cover slip into the central segment of the tunnel.

5.2.8. Role of plant architecture on the larval arrival and arrest on flowers and pods

The resistant cultivar, Tvu 946, was used in these experiments because it has long peduncles which bear flowers and pods above the leaf canopy (see Chapter 2 paragraph 2.2). However, flowers are normally formed while the peduncle is still within the canopy. The peduncle grows very fast and in 2-3 days, the flowers are pushed above the leaf canopy. Therefore it is possible to have flowers at different positions within the plant. Pods are usually 15-25 cm above leaf canopy.

Plants were grown in 2.4 x 1.8m plots in the field. There were four rows per plot. Spacing between rows was 50 cm and spacing between plants in a row was 30 cm. The experimental design was completely randomized block.

Tests on the position of flowers were conducted using the 1st instar larvae. There were 2 treatments and a control with four replications. Treatments were as follows:

1. All flowers that were located about 15-20 cm above the leaf canopy were retained and all other flowers which were within or at the same level of the leaf canopy were removed.
2. All flowers were maintained within the canopy by removing all those flowers which were above the canopy.

3. Control: The flowers were maintained in their natural distribution within the plant.

Treatments were applied at peak flowering time. Ten plants in the middle two rows in each plot were inspected for wild infestation and then covered with nylon mesh cages 4 days before infestation. Each plant had 25 flowers, giving a total of 250 flowers per plot. Infestation was achieved by randomly releasing first instar larvae on the leaves of the plant at the rate of 200 larvae per plot. Twenty four hours after release of the larvae, all flowers in the infested plants were picked and dissected to look for the larvae. Number of flowers with the larvae were recorded and expressed as a percentage of total number of flowers. The data was transformed into arcsine before analysis of variance.

Tests on position of pods were conducted using 4th instar larvae. There were 2 treatments; (1) all pods were maintained at 20-25 cm above the leaf canopy (2) all pods were folded into the leaf canopy. There were 8 replications of 5 plants each. Infestation was effected by randomly releasing one hundred 4th instar larvae per replicate. Ten larvae were released on leaves of each plant at the rate of one larva per leaf. Forty-eight hours after infestation, all the pods in the infested plants were picked and examined for infestation. Number of attacked pods was recorded and expressed as percentage of total number of pods per plot. The data was transformed into arcsine before analysis of variance.

5.3 Results

5.3.1 Arrest/stay of 1st instar larvae on plants

Larval dispersal behaviour was also noted while studying the rate of departure. All the larvae made exploratory movements by walking round the leaf initially at least once or more. After such exploratory movements, two forms of dispersal were observed. The first form of dispersal included those larvae which actively walked down the leaf petiole almost in a straight line but occasionally stopped to feel the surrounding by raising the head upwards and sideways. From time to time, they would also go round the leaf petiole. A few larvae changed their course and walked back upwards the leaf lamina. However, majority of the larvae moved straight until they reached the stem or peduncle or got in contact with another leaf blade, petiole peduncle or flower. Once they reached the stem, majority of the larvae (>70%) tended to move upwards along the stem and encountered other parts of the plant.

All the larvae had a common tendency of pushing their head into crevices or cracks they came across as they moved. Some of the larvae stayed permanently in such cracks throughout the observation time, but some of them stayed for only 1-3 minutes and walked out. Most of those larvae that stayed longer inside such crevices tended to feed while those that walked out almost immediately never fed.

The second form of dispersal from the initial point of release within the plant involved the use of a thread. The larvae having moved round the leaf, produced a thread on which they hang on after dropping from the leaf. In some cases the larvae could drop until they land on another leaf or any other part of the plant. In case there was wind, they swung with the wind and got blown off the plant. Whenever there was no wind and the larvae swung out of the plant and failed to land on any other part of the plant or on the soil, they remained hanging on the thread for sometime (approximately 10-25 minutes) then they moved back to the leaf by swallowing the thread.

When the rate of emigration of larvae from the three cultivars was observed for 75 minutes in the laboratory, there was a higher emigration from the plants within the first ten minutes after larval release (Table 2 and Appendix 7). The rate of emigration declined with time in all the three cultivars. However, by the end of the 75 minutes observation the number of larvae which had emigrated from Tvu 946 was significantly higher than emigration from VITA I and VITA 5.

When the larvae were allowed to stay on the plant for 24 hours the data show that under field conditions, the percentage which stayed on VITA I cultivar was significantly higher than on VITA 5 and Tvu 946 ($P = 0.05$). The trend was similar both at preflowering and flowering stage (Fig. 10 and Appendix 8, 9 and 10).

Table 2. Rate of emigration of 1st instar M. testulalis larvae from certain cowpea cultivars

Cultivar	% (+S.E.) larval departure from the plants at different time intervals (min.) after their release on leaves $\frac{1}{\text{leaf}}$							Average*
	10	20	30	40	50	75	Total	
VITA 1(S)	16 \pm 6	6 \pm 4	0	2 \pm 2	2 \pm 2	0	26	4.3 \pm 2.5 a
VITA 5(R)	20 \pm 5	8 \pm 3	4 \pm 2	6 \pm 2	0	0	38	6.3 \pm 3.1ab
Tvu 946(R)	36 \pm 5	8 \pm 5	10 \pm 3	2 \pm 2	6 \pm 6	2 \pm 2	64	10.7 \pm 5.2 b

$\frac{1}{\text{leaf}}$ /Ten 1st instar larvae per plant per test released on the leaves. Each cultivar replicated 5 times.

* In a column, means followed by a common letter are not significantly different from each other by DMRT at P=0.05

(S) Susceptible, (R) resistant.

When the experiment was repeated in the laboratory using M. testulalis eggs fixed on plants at flowering stage, results were similar to those from the field experiments with VITA 1 and Tvu 946. Percent larvae which stayed on Tvu 946 plants after egg hatch were significantly lower than that on VITA 1 ($P = 0.05$). VITA 5 was intermediate in this regard and was not significantly different from VITA 1 or Tvu 946 (Fig. 10).

Although the trend of results was similar for both laboratory and field experiments, under field conditions the arrest/stay of the larvae on the resistant plants was lower especially at flowering stage compared to laboratory conditions.

5.3.2 Sites for larval arrest in relation to their site of release on the plants

When the eggs were distributed all over the cowpea plant, the data show that on emergence larvae settled according to the following order of preference: flowers > terminal shoots = flower buds > pods = stems = leaves (Fig. 11 and appendix 11). There were no first instar larvae on open leaves, stems and pods. Preference order of settling site was identical for all the three cultivars and starting site of the larvae did not influence their preference for settling site. There was an interaction between the starting site of the larvae and the settling site indicating that starting site may be facilitating the arrival of the larvae at the settling site without altering their preference for the settling site.

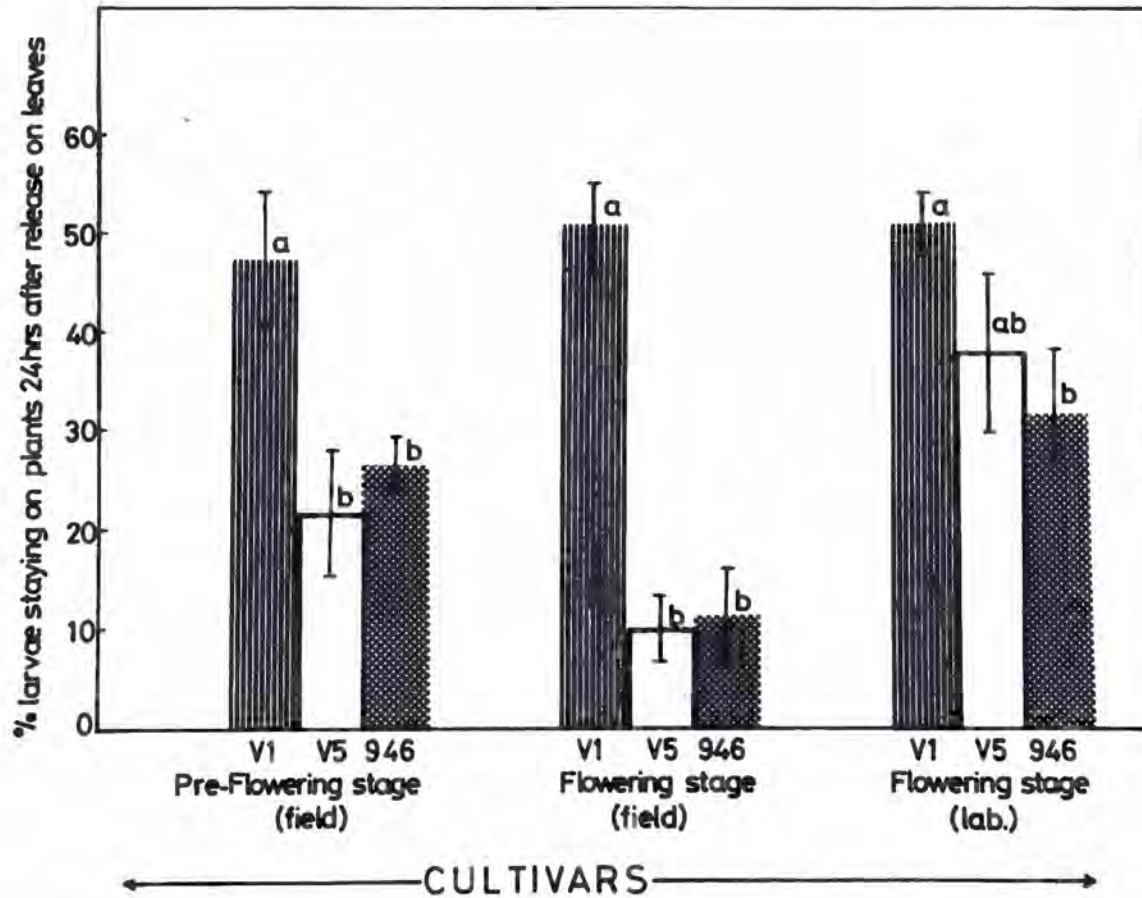


Fig. 10. Arrest/stay of 1st instar *Maruca testulalis* larvae on certain cowpea cultivars. Within each plant growth stage, columns with a common letter are not significantly different at $P = 0.05$ by DMRT.

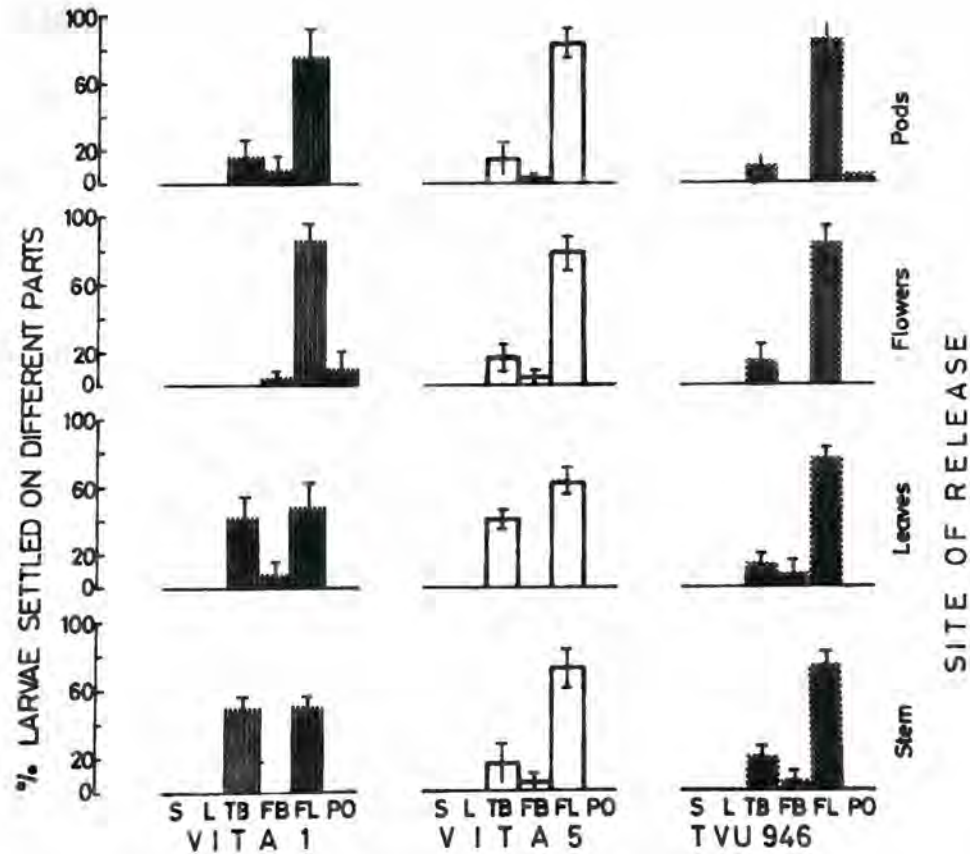


Fig. 11. Settling sites for first instar larvae released on different parts of certain cowpea cultivars in flowering stage during 48 hr. period. S = stem; L = leaves; TB = terminal buds, FB = flower buds; FL = flowers; PO = pods.

5.3.3. Attraction of larvae by the plants

Both laboratory and field experiments showed that at preflowering stage, when the three cultivars were offered simultaneously to the first instar larvae in a free choice situation, significantly ($P=0.05$) more larvae moved into VITA I than to VITA 5 and Tvu 946. However, under no choice situation, percent arrival of the larvae on the three cultivars was statistically similar (Fig. 12 and Appendix 12, 13, 14 and 15). Although the laboratory and field tests showed a similar trend, the number of larvae recovered under field conditions were fewer than the number of larvae recovered under laboratory conditions.

However as shown in Figure 13 and Appendix 16 and 17, at flowering stage in the field, significantly more larvae moved into VITA I than to VITA 5 and Tvu 946 in both no choice and free choice tests ($p = 0.05$).

In both VITA I and Tvu 946 cultivars, the number of larvae which arrived on the plants was higher when they were released 10cm away from the plants compared to 20 and 30cm (Fig.14 and Appendix 18). At 30 cm the larvae did not arrive on the plants. At 20 cm the number which reached the plants was half the number which reached the plants at 10 cm. However more larvae arrived on VITA I compared to Tvu 946.

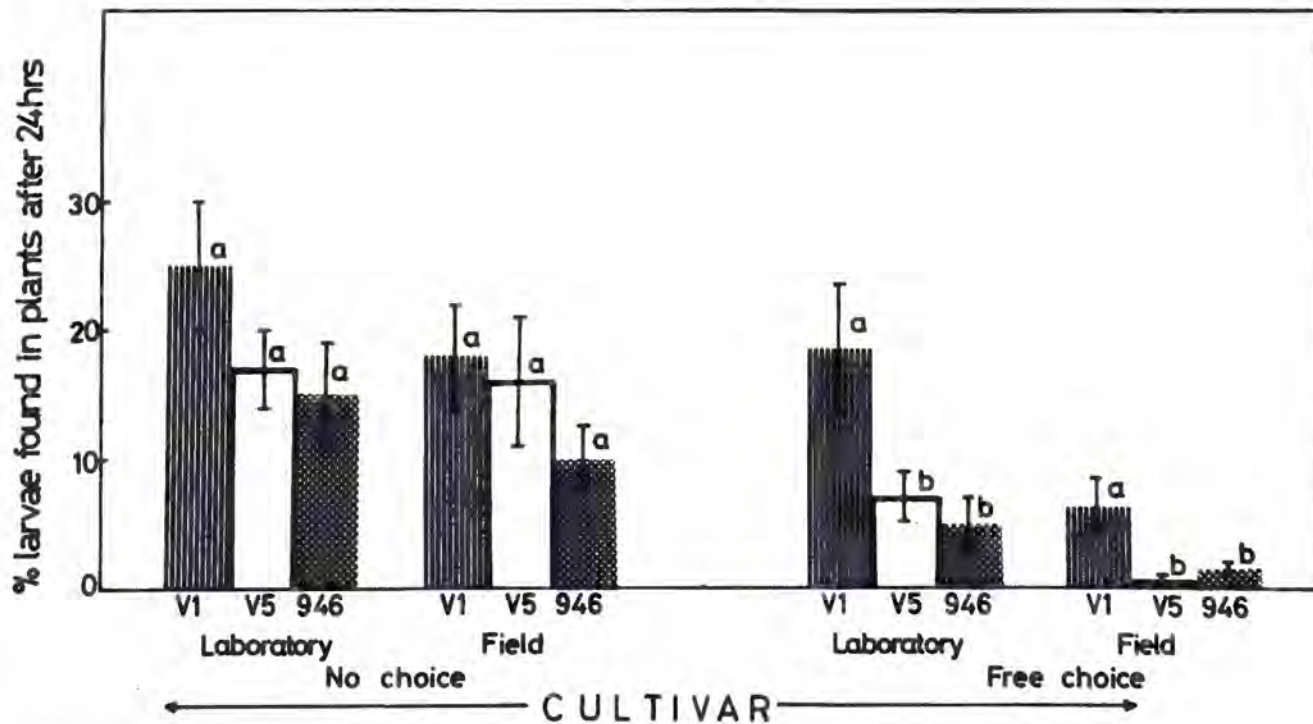


Fig. 12. Attraction of 1st instar *Maruca testulalis* larvae to certain cowpea cultivars in preflowering stage. V 1 = VITA 1; V5 = VITA 5 ; 946 = TVu 946. Within each test, columns with a common letter are not significantly different at $P = 0.05$ by D.M.T.

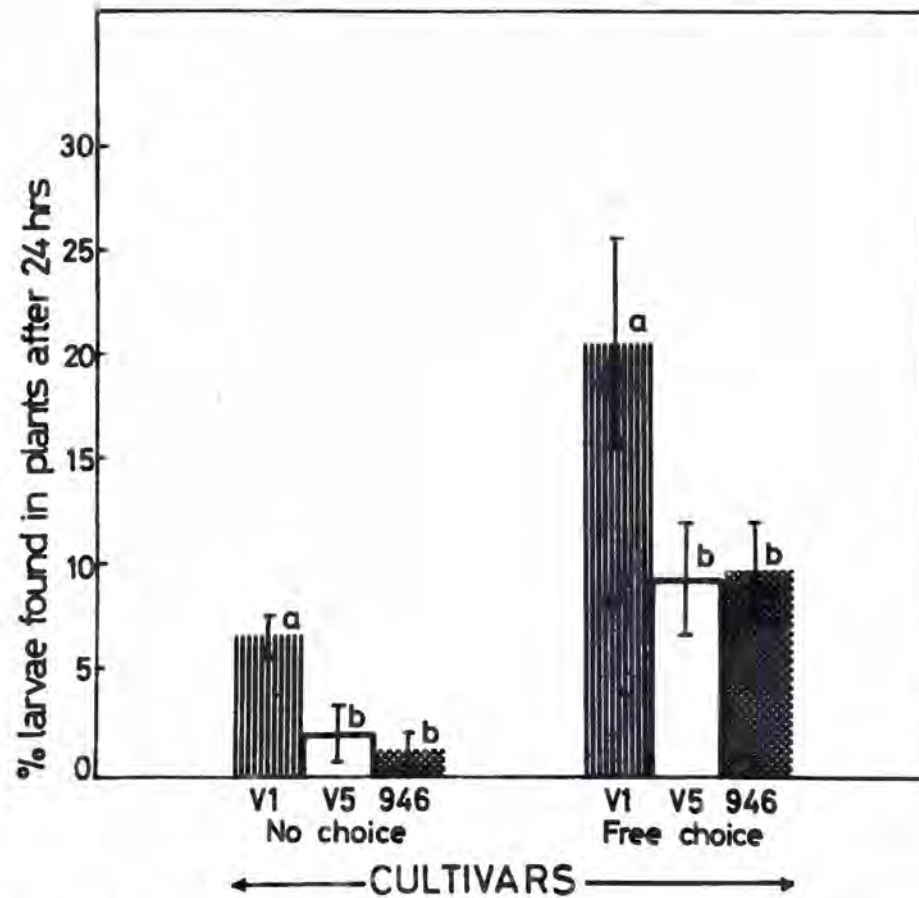


Fig. 13. Attraction of 1st instar *Maruca testulalis* larvae to certain cowpea cultivars in flowering stage in the field. V1 = VITA 1; V5 = VITA 5; 946 = TVu 946. Within each test, columns with a common letter are not significantly different at $P = 0.05$ by DMRT.

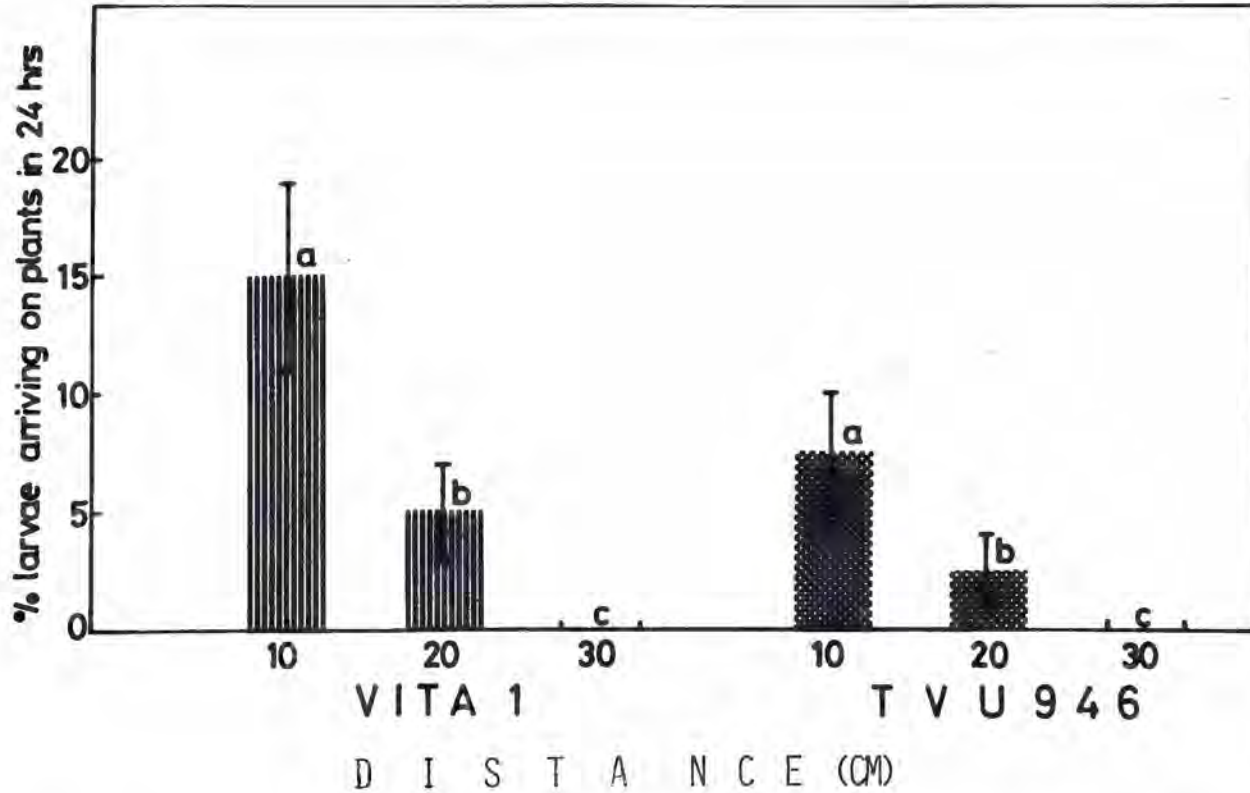


Fig. 14. Attraction of the *M. testulalis* larvae to plants in relation to their distance. Within each cultivar, columns with a common letter are not significantly different at $P = 0.05$ by D.M.T.

5.3.4. Larval movement from one cowpea cultivar to another grown in alternate rows in a plot

Pure stand plots (infested plants surrounded by plants of the same cultivar) In all the three cultivars, VITA I, VITA 5 and Tvu 946 there was some emigration of the larvae from the release point (middle row) into the surrounding plants which resulted into a fairly uniform distribution of the larvae on all the rows (Table 3 and Appendix 19). However, the total percent larvae recovered was significantly low ($P=0.01$) on plots of Tvu 946 and VITA 5 than on VITA I.

Mixture (infested plants surrounded by plants of different cultivars). When larvae were released on a row of Tvu 946 surrounded by rows of VITA I plants, there was a very high emigration from Tvu 946 into VITA I. As shown in table 3 in a combination of one row of Tvu 946 in the middle surrounded by two rows of VITA I, 37.53% of the larvae which were released (on Tvu 946) was recovered. Out of the 37.53%, only 5.75% were in the middle row (Tvu 946) on which they were released and 31.75% were on the surrounding rows (VITA I). Similar trends in results were observed when the larvae were released on VITA 5 surrounded by VITA I.

When the order was reversed and larvae were released on VITA I surrounded by Tvu 946 or VITA 5, there was very little emigration from VITA I into the surrounding cultivar. In a

combination where one row of VITA I in the middle was surrounded by two rows of Tvu 946, 22% of the larvae which were released (on VITA I) was recovered. Out of the 22%, only 4.75% emigrated into the surrounding rows of Tvu 946 while 17.25% were on VITA I (where they were released). (Table 3 and Appendix 19).

The total percent larvae recovered in mixtures of Tvu 946 and VITA 5 cultivars were significantly lower than in mixtures of VITA I and Tvu 946 or VITA 5.

5.3.5 Role of moisture in determining arrival of 1st instar larvae on plants

The larvae took twice as long time (36.8 ± 4.8 min) to arrive on plants when the ground was dry as when the ground was moist (18.6 ± 3.31 min) (Fig.15 and Appendix 20). The larvae were also easily blown off by wind when they were on a dry soil surface.

When the larvae were released on moist and dry surface, their speed was faster on moist surface than on the dry surface (Fig.15 and Appendix 21). Generally the speed was 6-8 times faster on a filter paper (under laboratory conditions compared to soil surface (field conditions).

Table 3. Movement of 1st instar M. testulalis larvae from one cowpea cultivar to another grown in alternate rows in a plot

Combination of the cultivars in a plot		No. larvae released/replicate	% (\pm s.e.) larvae recovered from each cultivar after 48 h <u>1/</u>			Total % larvae recovered in each combination <u>2/</u>
A	B		A	B	"t" value	
VITA 1	VITA 1	100	28.25 + 3.4	22.50 + 1.5	2.62ns	50.75 + 4.9 a
Tvu 946	Tvu 946	100	7.50 + 2.7	4.00 + 2.3	2.25ns	11.50 + 4.7 c
VITA 5	VITA 5	100	7.25 + 1.4	3.50 + 1.2	1.54ns	10.75 + 0.9 c
Tvu 946	VITA 1	100	5.75 + 1.3	31.75 + 1.9	8.44**	37.50 + 1.5 ab
VITA 5	VITA 1	100	3.00 + 1.0	35.25 <u>±</u> 8.7	3.98*	38.25 <u>±</u> 9.5 ab
VITA 1	Tvu 946	100	17.25 <u>±</u> 1.1	4.75 + 2.5	3.59*	22.00 <u>±</u> 1.7 b
VITA 1	VITA 5	100	26.25 + 6.1	4.75 + 1.0	2.80*	31.00 + 5.8 ab
Tvu 946	VITA 5	100	7.00 + 1.7	3.25 + 1.9	2.61ns	10.25 + 3.4 c
VITA 5	Tvu 946	100	4.75 + 1.6	6.50 + 4.6	0.51ns	11.25 + 6.0 c

1/ cultivar on which the larvae were released

1/ Average of 4 replications; 100 larvae released on 5 plants in each plot

2/ In a column, means followed by a common letter are not significantly different at P=0.05 by DMRT

* = "A" is significantly different from "B" at P=0.05; **="A" is significantly different from "B" at P=0.01; ns="A" is not significantly different from "B".

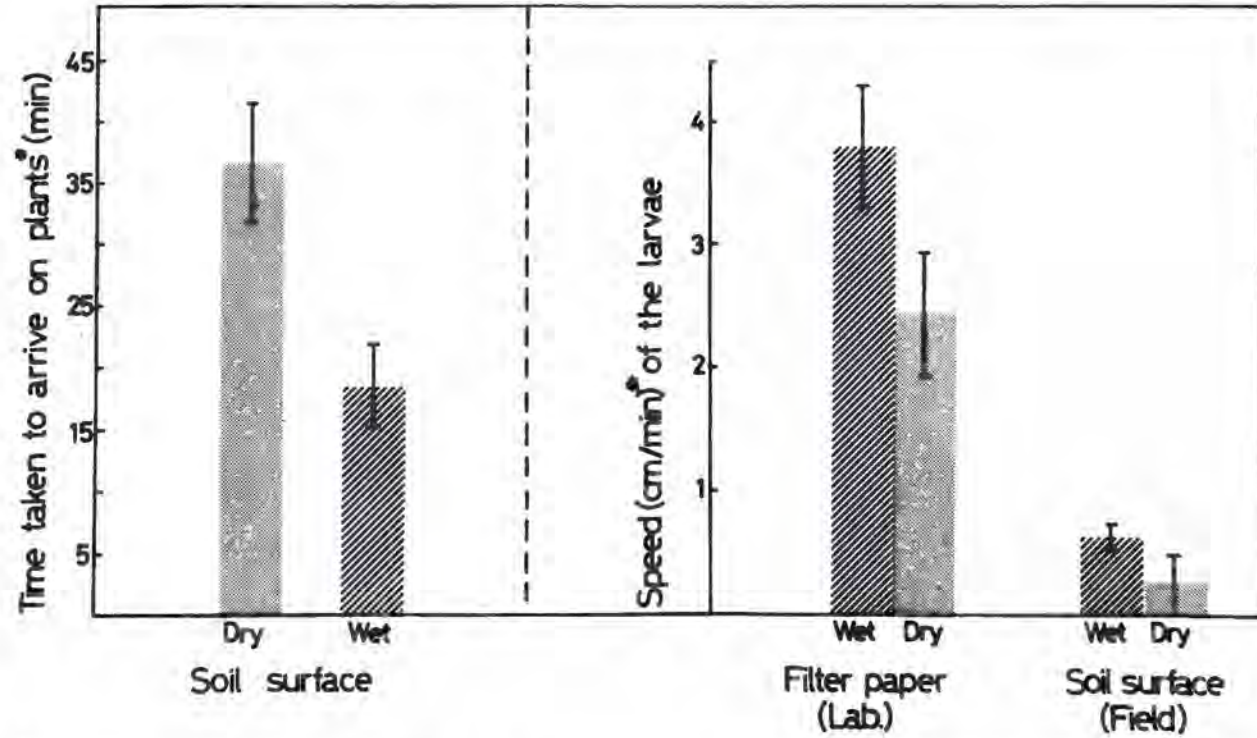


Fig. 15. Role of moisture in determining arrival of 1st instar *M. testulalis* larvae on plants.

5.3.6. Role of plant volatiles on the larval attraction/arrest

No choice- In the no-choice experiments when the plant parts were offered against a blank, the odour from all the freshly excised plant parts of all the three cultivars were attractive to the larvae (Table 4). Dry leaves did not elicit any response.

Raw leaf and flower juice extracts also attracted the larvae (Table 5).

When the degree of attraction (% orientation preference) of the three cultivars were compared, odour of VITA I leaves was more attractive to the larvae compared to VITA 5 and Tvu 946 (Fig.16 and Appendix 22). In flowers VITA I was again more attractive compared to Tvu 946 and VITA 5. Generally leaves and flowers were equally attractive.

Two-choice. The above results on percent orientation preference were amplified when the excised tissues were offered to the insects in a two choice situation. The odour emanating from leaves and flowers of VITA I was more attractive to the larvae than when they were offered against VITA 5 or Tvu 946 (Table 6). Raw juice extracts from leaves of VITA I were also more attractive than those of VITA 5 and Tvu 946. However, raw juice extract from flowers of the three cultivars was similar.

When the leaf and flower surface chemicals were extracted with chloroform and n-hexane and tested for their attractancy, both extracts elicited positive orientational response by the larvae (Fig.17, 18 and 19; and Appendix 23, 24 and 25). A drop of chloroform extract on a filter paper placed on a table attracted and arrested a 4th instar larva for over 5 minutes.

When offered chloroform extract, especially from the leaves, larval attraction increased with the concentration of the extract and reached a peak in between 0.2 and 0.5 mg/ml chloroform (Fig. 17 and Appendix 23). However, the pattern differed with the cultivars. Between 0.10 and 0.20 mg, VITA I and VITA 5 elicited a sharp increase in larval attraction compared to Tvu 946. Between 0.20 and 0.50 mg, VITA I and VITA 5 elicited further increase in larval attraction but to a very low extent. In Tvu 946 there was a small drop in the attraction. Statistically, the rise in attraction between 0.10 and 0.20 mg was significant. The rise or drop in attraction between 0.20 and 0.50 mg was not significant.

When chloroform extract from the leaves of the three cultivars were compared at 0.10 mg extract/ml chloroform, level of attraction of VITA I and Tvu 946 were similar and were higher when compared to VITA 5. At 0.20 and 0.50 mg concentrations, level of attraction was statistically lower in Tvu 946 compared to VITA I ($P=0.05$) VITA 5 was similar to VITA I at 0.20 mg but significantly lower than VITA I at 0.50 mg concentration in this regard.

Table 4. Orientation responses of 1st instar *M. testulalis* larvae to odour from freshly excised leaves and flowers of certain cowpea cultivars

Source of stimuli		No. larvae tested	% + s.e. larvae moving to		"t" value
Side A	Side B		Side A	Side B	
NIL	NIL	80	30.00 \pm 6.0	27.00 \pm 3.0	0.33 ^{ns}
VITA 1 Leaves	NIL	80	70.00 \pm 6.6	5.00 \pm 2.7	9.50**
VITA 5 Leaves	NIL	80	40.00 \pm 5.0	20.00 \pm 3.3	2.83*
Tvu 946 Leaves	NIL	80	52.50 \pm 4.0	7.50 \pm 2.5	9.00**
VITA 1 flowers	NIL	80	68.75 \pm 3.0	15.00 \pm 4.2	8.60**
VITA 5 flowers	NIL	80	56.25 \pm 3.2	15.00 \pm 3.3	8.01**
Tvu 946 flowers	NIL	80	51.25 \pm 4.0	23.75 \pm 3.2	4.66**
VITA 1 dry leaves	NIL	80	30.25 \pm 4.4	23.75 \pm 3.2	1.03 ^{ns}

* "A" is significantly different from "B" at p=0.05

** "A" is significantly different from B at p=0.01

ns "A" is not significantly different from "B"

Table 5. Orientation responses of 1st instar M. testulalis larvae to raw juice extract from leaves and flowers of some cowpea cultivars

Source of stimuli (Juice extract)		No. larvae tested	% (+ s.e.) larvae moving to		"t" value
Side A	Side B		side A	Side B	
VITA 1 Leaves	NIL	80	75.00 \pm 4.63	11.25 \pm 4.41	7.20 **
VITA 5 leaves	NIL	80	70.00 \pm 3.78	8.75 \pm 2.95	10.02**
Tvu 946 leaves	NIL	80	61.25 \pm 5.81	10.00 \pm 3.27	8.02**
VITA 1 flowers	NIL	80	73.75 \pm 5.65	15.00 \pm 5.0	7.04**
VITA 5 flowers	NIL	80	88.80 \pm 4.00	3.80 \pm 3.0	18.50**
Tvu 946 flowers	NIL	80	76.30 \pm 7.0	10.00 \pm 3.0	8.29**

** "A" is significantly different from "B" at p=0.01

Table 6. Orientation responses of 1st instar Maruca testulalis larvae to odour of certain cowpea cultivars in a 2-choice situation

Source of stimuli		No. larvae tested	% (+ s.e.) larvae moving to		"t" value
Side A	Side B		Side A	Side B	
VITA 1 (L)	Tvu 946(L)	80	60.00 \pm 3.27	16.22 \pm 3.75	11.67**
VITA 1 (L)	VITA 5 (L)	80	52.50 \pm 4.53	17.50 \pm 5.90	3.78**
VITA 1 (F)	Tvu 946 (F)	80	57.50 \pm 5.26	7.50 \pm 2.50	8.82**
VITA 1 (F)	VITA 5 (F)	80	40.00 \pm 4.63	16.25 \pm 3.24	3.64**
VITA 1 (LJ)	Tvu 946 (LJ)	80	60.00 \pm 3.27	22.50 \pm 3.13	6.36**
VITA 1 (LJ)	VITA 5 (LJ)	80	71.25 \pm 4.41	18.75 \pm 2.23	8.90**
VITA 1 (FJ)	Tvu 946 (FJ)	80	46.30 \pm 3.00	48.80 \pm 4.0	0.42 ^{ns}
VITA 1 (FJ)	VITA 5 (FJ)	80	47.50 \pm 6.00	46.30 \pm 5.0	0.80 ^{ns}

(L) = Leaves; (F) = Flowers; (LJ) = Leaf juice; (FJ) = Flower juice

** "A" is significantly different from "B" at p=0.01

ns "A" is not significantly different from "B"

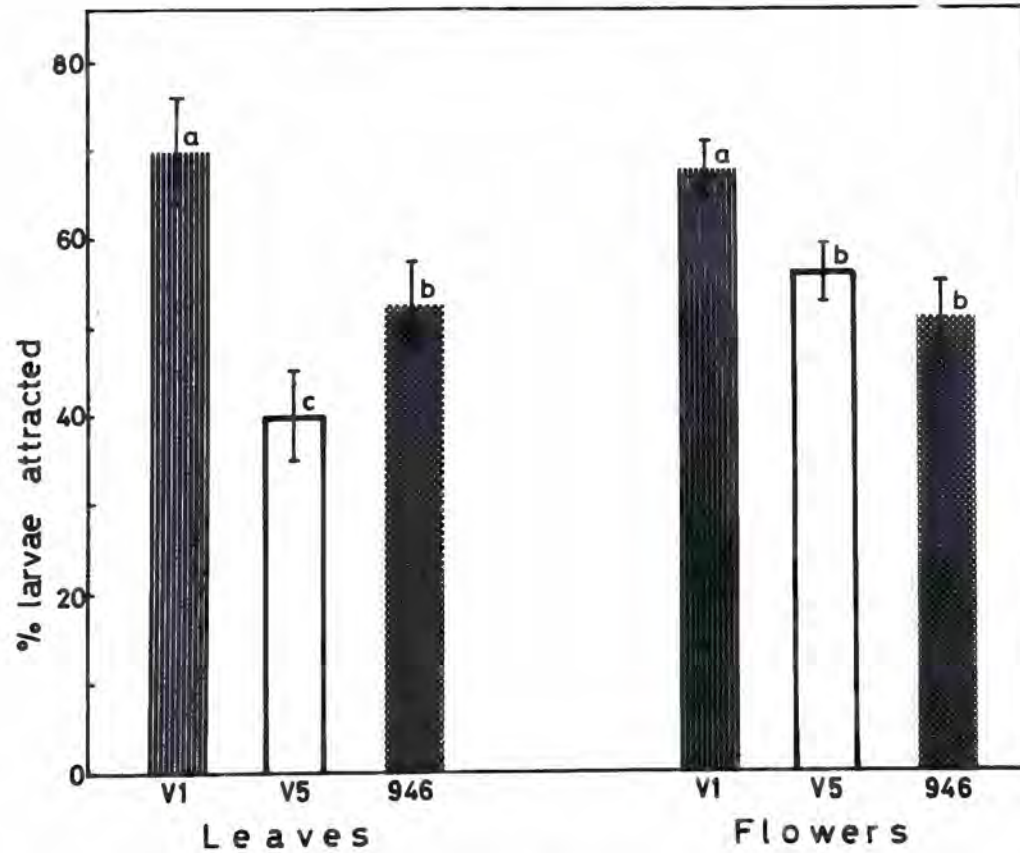


Fig. 16. Attraction of 1st instar *Maruca testulalis* larvae by volatiles from excised leaves and flowers of certain cowpea cultivars under no choice situation. V 1 = VITA 1; V5 = VITA 5; 946 = TVu 946. Within each plant part, columns with a common letter and not significantly different at $P = 0.05$ by D.M.T.

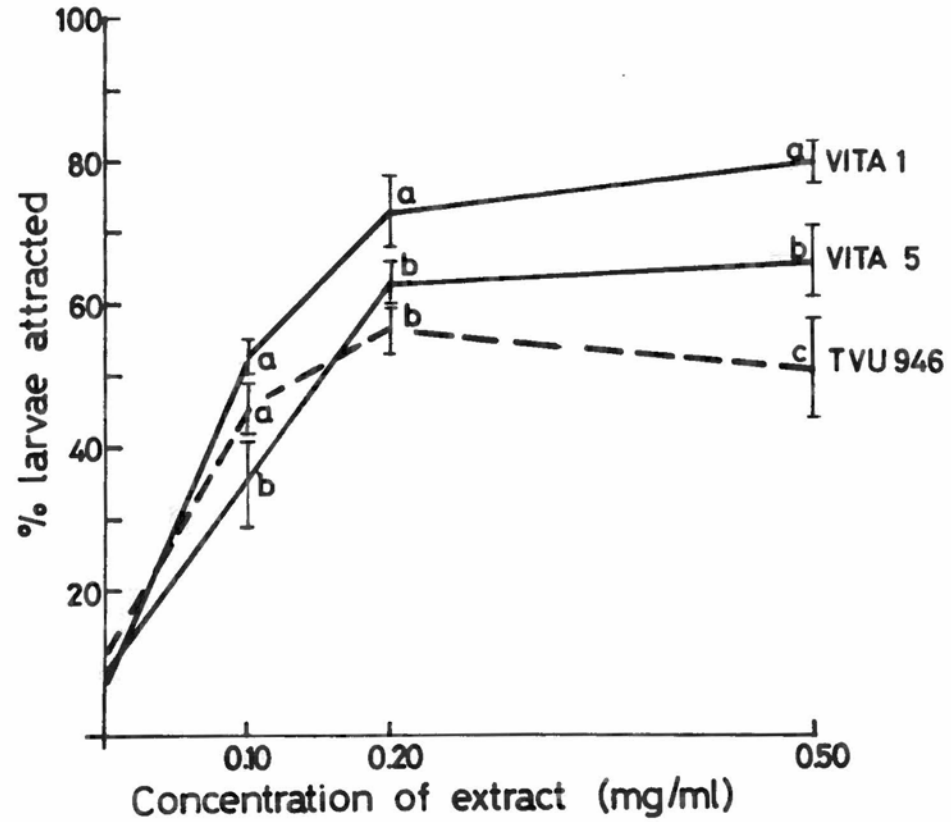


Fig. 17. Attraction of 1st instar *Maruca testulalis* larvae by chloroform extract of leaves of certain cowpea cultivars. Means with a common letter are not significantly different at $P = 0.01$ by DMRT.

VITA I hexane extract from leaves also had a higher attractancy than that of VITA 5 and Tvu 946 (Fig.18 and Appendix 24).

Orientational responses to chloroform extracts from flowers showed a similar trend with those from leaf extracts. Chloroform extract from VITA I flowers were significantly more attractive than that of Tvu 946 at lower doses (0.10 and 0.20 mg/ml chloroform). However at a higher dose, 0.50 mg/ml, there were no significant differences between the two cultivars (Fig.19 and Appendix25).

5.3.7 Role of humidity and visual stimuli on orientation

Results on the role of humidity and visual stimuli on orientation of the larvae are shown in Table 7. More larvae were attracted to the wet cloth than to the dry cloth. However, fresh leaf attracted more larvae than the wet cloth. There were no differences in larval orientational responses to fresh leaves hidden behind a glass slide barrier and a blank source of stimuli.

5.3.8 Role of Plant architecture on the larval arrival and arrest on flowers and pods

There were no significant differences in percent infestation between the flowers which were raised above the canopy and flowers which were inside the canopy when plants were infested with 1st instar larvae (Fig.20 and Appendix 26).

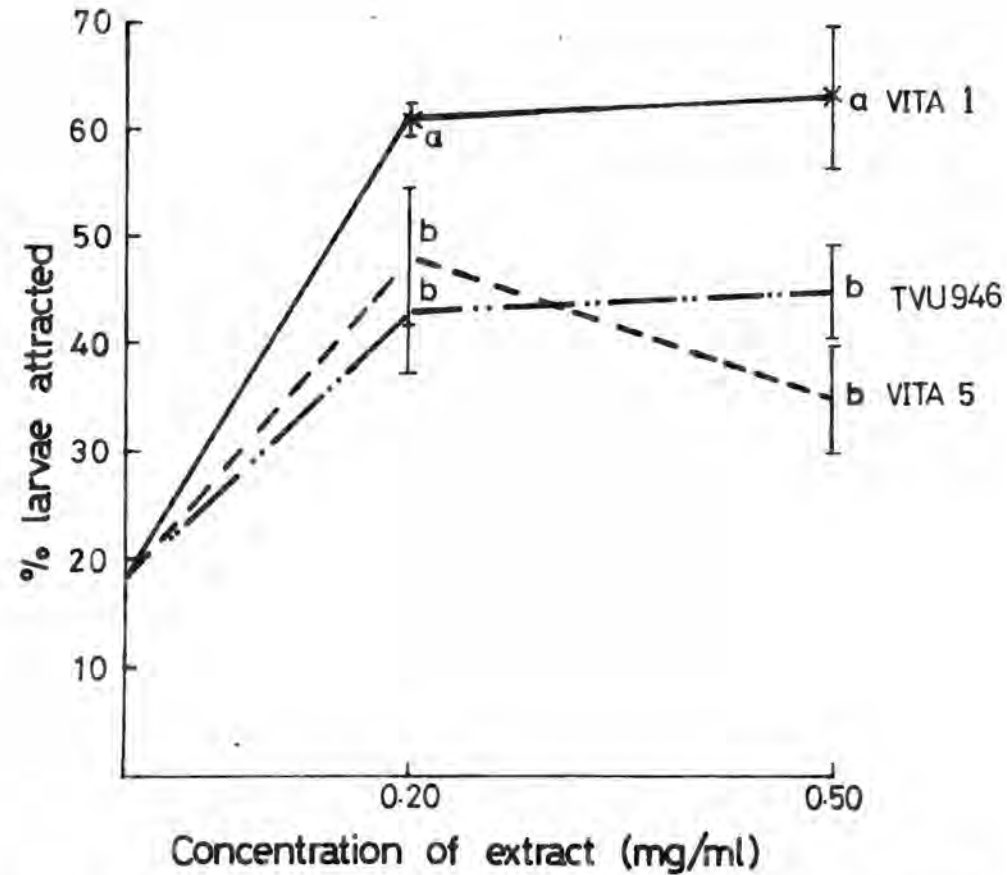


Fig. 18. Attraction of 1st instar *Maruca testulalis* larvae by n-hexane extract of leaves of certain cowpea cultivars. Means with a common letter are not significantly different at $P = 0.01$ by D.M.T.

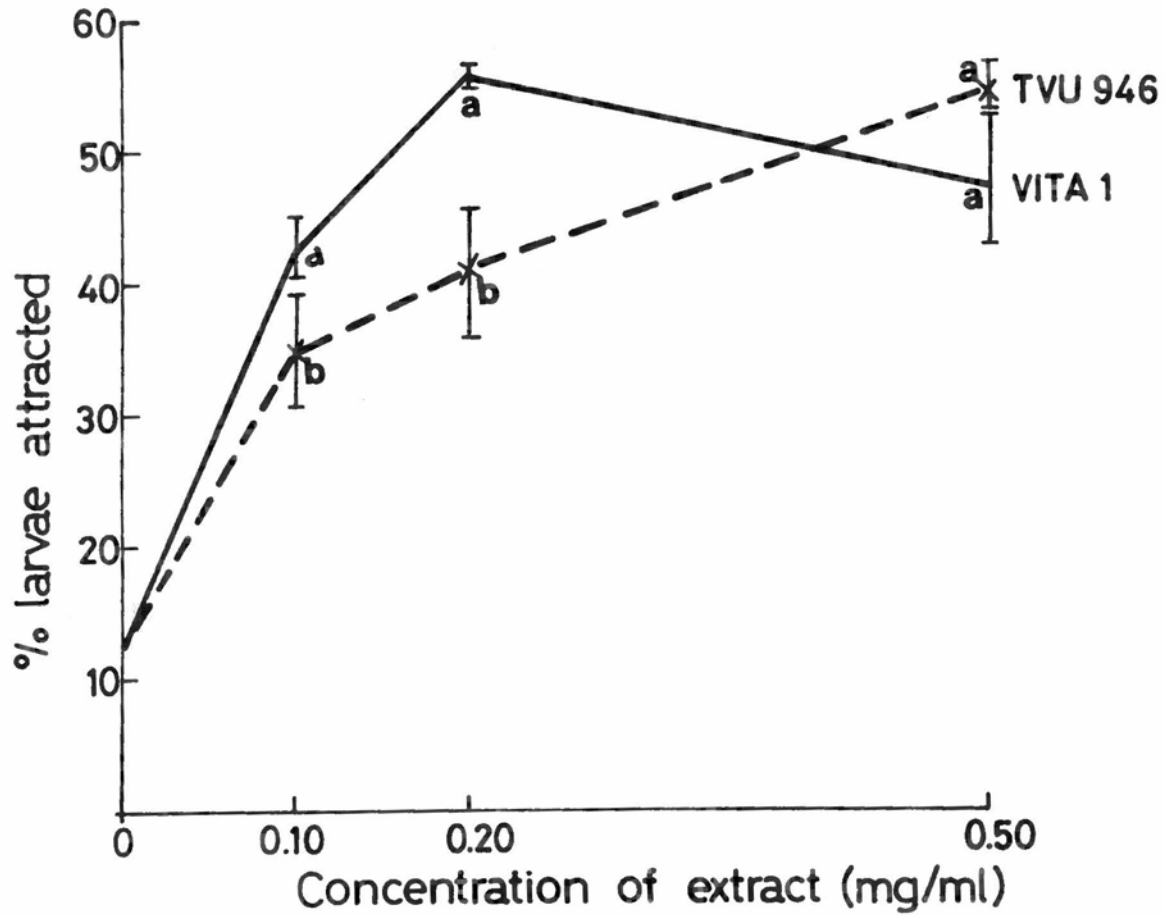


Fig. 19. Attraction of 1st instar *Maruca testulalis* larvae by chloroform extract of flowers of certain cowpea cultivars. Means with a common letter are not significantly different at $P = 0.01$ by DMRT.

Table 7. Orientation of first instar Maruca testulalis larvae to humidity and visual stimuli.

<u>Source of Stimuli</u>		No. larvae tested	<u>%(\pms.e.) larvae moving to</u>		"t" value
Side A	Side B		Side A	Side B	
I Wet cloth	Dry cloth	80	35.00 \pm 4.23	13.75 \pm 3.75	3.23*
II Fresh leaf	Blank	80	33.75 \pm 5.32	30.00 \pm 2.67	0.53 ^{ns}
	behind glass				
III Fresh leaf	Wet cloth	80	60.00 \pm 5.00	10.00 \pm 5.00	6.67**
	(VITA 1)				

I = role of humidity

II = role of visual stimuli

III = role of odour against humidity

* "A" is significantly different from "B" at p=0.05

** "A" is significantly different from "B" at p=0.01

ns "A" is not significantly different from "B"

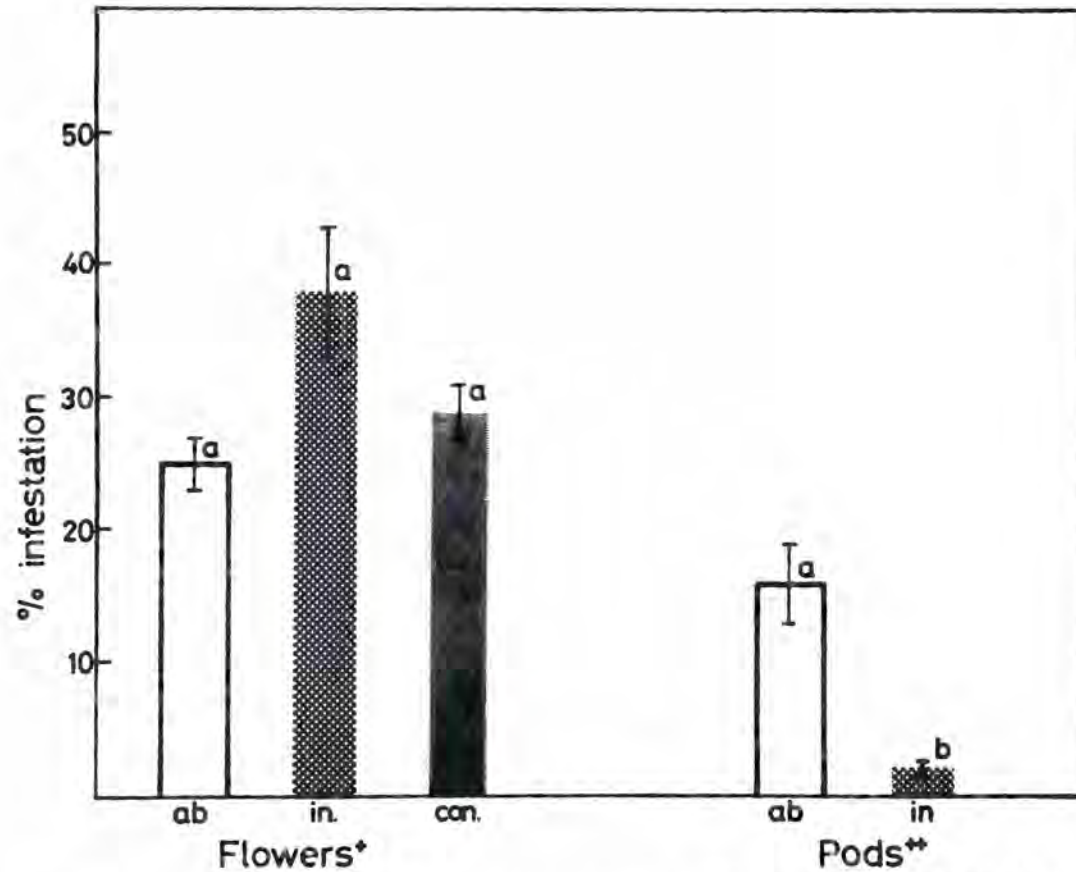


Fig. 20. Effect of plant architecture on the arrival/arrest of *M. testulalis* larvae on flowers and pods of cowpea TVu 946. ab = flowers or pods restricted above the leaf canopy; in = flowers or pods restricted inside the canopy; Con = control (flowers maintained in their natural distribution). Within each plant part, columns with a common letter are not significantly different at $P = 0.05$ by D'ERT.

Damage on pods which were restricted inside the leaf canopy was significantly higher (about eight times) than damage on pods which were above the canopy ($P=0.05$), (Fig.20 and Appendix 27).

Observations in the laboratory and field showed that majority (>50%) of the older larvae (4th instar) hardly moved up along the peduncles of Tvu 946 cultivar. Most of those larvae which moved upwards along the peduncle dropped down just before reaching the pods.

5.4 Discussion

5.4.1 Arrest/stay of first instar larvae on the plants

As was stated by Saxena (1985) usually after the eggs have hatched, the emerging larvae have to choose whether to stay on the host plant on which the eggs were oviposited or to move out and look for another host or to move and stay at the appropriate site within the plant. In most cases the decision to stay is governed by the suitable factors in the plant. However, there are also some inherent characteristics of the larvae to disperse immediately after egg hatch. The major difference between the resistant and the susceptible cowpea cultivars during M. testulalis larval orientation stage was observed in the arrest/stay of the 1st instar larvae on the plants. This was demonstrated both in the field and in the

laboratory that after the larvae had been introduced into the plant or the larvae emerged from eggs which were in the plants some of them emigrated. Emigration took place from both the susceptible and the resistant cultivars. However, those which emigrated from the resistant cultivars were more than those from the susceptible cultivar ($P=0.05$). The observed marginal arrest/stay of larvae on Tvu 946 and VITA 5 is a very important factor of resistance in the two cultivars because even if optimum oviposition takes place on all the three cultivars, the eventual larval population on VITA 5 and Tvu 946 will be lower than than on VITA 1. As a result the chances of VITA 1 suffering a heavier damage than Tvu 946 and VITA 5 will be higher especially under field conditions.

Arrest/stay of larvae on VITA 5 was lower than that of VITA 1 under field conditions. But in the laboratory, the two cultivars were statistically identical. This indicates that the arrest/stay of larvae on the resistant VITA 5 was enhanced by the environmental conditions. As was stated by Painter (1951), greenhouse and cage tests may make conditions so severe that valuable sources of resistance, particularly that resulting from nonpreference may be discarded. It was therefore, important to have the tests on arrest/stay repeated under field conditions.

Two forms of larval dispersal were observed, the first being those larvae which actively walked on the plant. The second

form involved the use of thread for swinging from one plant part to the other or to another plant. Both these two forms of dispersal by M. testulalis larvae were also reported by Taylor (1978). It was also observed that whenever the larvae were walking around on the plant they had a tendency of pushing their heads into the crevices or cracks and sometimes even stayed in such cracks. Such a behaviour suggests that after egg hatch the larval dispersal which takes place is aimed at finding a shelter first then feeding. Painter (1951) stated that presence or absence of shelter for some insects is also important in resistance. Whereas negative photokinesis may be concerned in the reaction, insects also tend to find shelter by bringing all possible parts of the body into contact with nearby surfaces.

Based on the above observation, the (larvae seem to engage the use of threads for dispersal only after walking around the plant without successfully encountering a proper shelter and a feeding site.

The data showed that emigration took place from both the resistant (VITA 5 and Tvu 946) and the susceptible (VITA 1) cultivars. But the number which emigrated from Tvu 946 was higher ($P=0.05$) than that from VITA 1. Emigration from VITA 5 was intermediate. This observation further confirms that arrest/stay of larvae on Tvu 946 is lower than that of VITA 1.

5.4.2 Site for first instar larval arrest after their release on different parts of the cowpea plant

Although majority of the eggs were laid on the leaves (Chapter 4), the emerging first instar larvae did not settle on the leaves. They emigrated and settled on flowers, terminal buds and flower buds. This preference order for the cowpea plant parts was also reported by Taylor (1978), Jackai (1981b), Okeyo-Owuor and Ochieng (1981). The preference order for the larval settling sites was similar for both the resistant and the susceptible cultivars. Flowers and terminal shoots are known to possess more meristematic tender cells which may be suitable for feeding by the very young larvae which have not yet developed stronger mandibles. Morphologically, flowers and terminal shoots also offer more crevices and foldings in which the larvae can find shelter. Southwood (1973) stated that food and shelter are the major links for the insect and its host plant. Dethier (1970) noted that plants serve not only as food but also as a microhabitat, shelter and protection for phytophagous insects. Flower, terminal shoots and flower buds of the cowpea plant has all these requirements which are needed by the very young larvae.

5.4.3 Attraction of larvae by the plants

The data on the arrival of the larvae on the cowpea plants showed that all the three cultivars attracted the larvae but VITA 1 was more attractive than VITA 5 and Tvu 946. This indicates

that although the arrest/stay of larvae was low on Tvu 946 and VITA 5, repellence is not likely to be involved in the resistance of the two cultivars.

Olfactory stimulus is known to play a major role in food detection and acceptance (Kennedy, 1977; Dethier, 1982; Miller and Strickler, 1984). Rejection of a potential food source by an insect as a result of olfactory stimulus may be due to (i) repellence (Feeny, 1976; Reese and Beck, 1976) or (ii) due to a diluted undetectable attractant signals (Baliddawa, 1983, 1985; Miller and Strickler, 1984). The second reason seems to be the most likely cause for such low arrival and arrest/stay of M. testulalis larvae on Tvu 946 and VITA 5 cowpea cultivars.

It was reported above that flowers are the most preferred part of the cowpea plant by M. testulalis larvae. Cowpea plant is also known to produce a large number of flowers some of which are aborted. When a flower which is infested by M. testulalis larvae drops from the plant the larvae have to walk out and find their way back into the plant to continue their development. Since larval attraction leading to their arrival on Tvu 946 and VITA 5 is lower compared to VITA 1, the larvae which have dropped from Tvu 946 and VITA 5 have a very low probability of getting their way back into the plant.

5.4.4 Larval movement from one cowpea cultivar to another grown in alternate rows in a plot

When the resistant Tvu 946 or VITA 5 cultivar was interplanted with the susceptible VITA 1 there was a tendency for the larvae to congregate on VITA 1. This is a further proof of a low arrest/stay of larvae on Tvu 946 and VITA 5 and a higher attractancy of VITA 1. The data also confirms nonpreference for Tvu 946 and VITA 5 and amplifies Knipling's (1979) concept that nonpreference is mostly expressed in the presence of a more preferred variety.

When infested plants were surrounded by plants of the same cultivar there was a uniform distribution of the larvae within the plot. This indicates that there is a natural tendency for M. testulalis larvae to disperse from one plant to the other. The observed dispersal and redistribution of the larvae within the plant may have been aimed at optimal foraging (Zimmerman, 1979).

Larval dispersal and redistribution within the plants in the plots planted with the resistant cultivars resulted into lower larval populations in such plots compared to that in plots planted with susceptible cultivars. This suggests that larval dispersal from the resistant cultivars was due to nonpreference instead of being aimed at achieving optimal foraging as in the case of the susceptible cultivar.

5.4.5 Role of moisture in determining arrival of first instar larvae on plants.

It was observed that the speed of the larvae was faster on moist surfaces. Their rate of arrival on plants was also higher when the soil surface was moist. Conversely when the soil surface was dry, speed of the larvae was very low, they were also easily blown away by wind and the number that arrived on the plant was also low. This suggests that availability of moisture in the cowpea environment is one of the factors which enhance the establishment of M. testulalis on its host.

The very young larvae were capable of moving into the plants from a distance of 20cm as was confirmed by the data above. Thus whenever, the larvae drop off the plant or are blown off by the wind and they fail to land on another plant, they would still be capable of surviving if the plants are not very far apart and the soil surface is moist.

5.4.6 Role of plant volatiles on the larval attraction/arrest

The data on the attraction of M. testulalis larvae by the odour emanating from the freshly excised cowpea plant parts corroborates the observations made on the arrest/stay and arrival of larvae on the plants. The odour from both the resistant and the susceptible cultivars were attractive to the larvae but the degree of attraction of VITA 1 was higher than that of Tvu 946 and VITA 5.

Dry leaves did not elicit any larval response. This suggests that the attractants are volatile in nature.

The data further showed that the attractants can be obtained as chloroform and n-hexane extracts. The most important point which was observed in the bioassays is that the larvae which were attracted to the odour stayed around the stimuli source for a long time. Thus suggesting that these extracts serve both as attractants and arrestants. Plant odours are known to generate oriented movement towards plants (Kennedy, 1977; Schoonhoven, 1972). Attractant odours may also serve as arrestants (Thorsteinson, 1960) and even induce examining of the food (Miller and Strickler, 1984). Feeding stimulants and volatile attractants evoke responses at certain concentration (Schoonhoven, 1972). In this study, larval orientational response to the odour from chloroform and hexane extracts increased with the concentration of the extracts. The optimum concentration was between 0.2 and 0.5 mg/ml solvent. It was clear from the data that larval response to the extract from VITA 1 was higher than that of Tvu 946 and VITA 5. This could be one of the causes for the lower arrest/stay of the larvae on these two cultivars. Two reasons can be suggested as an explanation for higher attractancy of VITA 1 extracts compared to that of Tvu 946 and VITA 5:

- 1) Although the active compound(s) influencing positive responses is present in all the three cultivars, its concentration may be lower in Tvu 946 and VITA 5 than in VITA 1.

- 2) The extract contain a combination of stimulants, repellents and or deterrents but VITA 1 stimulants may be much stronger than the repellents or deterrents. Dethier (1982) stated that the palatability of any compound depends on other compounds with which it is mixed. Schoonhoven (1972) gave an example of alfalfa seed chalcid Bruchophagus rodii which is attracted by odour of 38 chemicals out of the 95 compounds known to occur in its food plant, 9 out of these compounds are repellent and the remainder are non stimulating.

5.4.7 Role of humidity and visual stimuli on larval orientation

The above results showed that the larvae were attracted to the humidity source in an olfactometer. However, fresh leaf was more attractive than a moist cloth. This suggests that humidity also influences orientation of 1st instar M. testulalis larvae but odour of the plant has a stronger influence in generating oriented movement.

There were no differences in larval orientation responses to fresh leaves hidden behind a glass slide barrier and a blank source of stimuli indicating that visual stimuli may not play an important role in the orientation of 1st instar M. testulalis larvae compared to odour and humidity.

5.4.8 Role of plant architecture on the larval arrival and arrest on flowers and pods

Differences in growth habits and architecture of Tvu 946 and VITA 1 are described in chapter 2. The very long erect peduncles which carry the flowers and pods above the leaf canopy in Tvu 946 have been suggested to be responsible for their low infestation compared to other cultivars in which these parts remain inside the canopy (Singh, 1978). The data obtained in this study confirms that this suggestion is true in the case of pods only and not for flowers. The flowers are mostly infested by first, second and third instar larvae (Taylor, 1978). The observations in the laboratory revealed that the 1st instar larvae were very mobile and were capable of reaching any part of the cowpea plant without any hindrance . Therefore the flowers could not escape infestation by 1st instar larvae due to their position. It was also observed that many flowers of Tvu 946 are produced when the peduncle is still within the leaf canopy. As the peduncle grows, the flowers are pushed out of the canopy. It is possible for the larvae to attack the flowers at this stage.

Pods are usually attacked by older instars (Taylor, 1978). It was observed that the pods which were raised above the canopy were less infested than those which were inside the canopy. It was also observed that 4th instar larvae were not very efficient in climbing vertical objects since majority of them dropped down when climbing up the peduncles of the cowpea plant, especially those of Tvu 946 which are very long and erect . The pods of

Tvu 946 could have partly escaped infestation because they could not easily be reached by the larvae. Hence, Tvu 946 pods could be considered to be less apparent than those of VITA 1 which are normally inside the canopy. As was suggested by Feeny (1976) any plant which cannot be easily found by an insect which normally feeds on it, is regarded as unapparent. Apparency does not limit itself to a whole plant only. A specific plant part on which the predator feeds also has to be apparent. Kumar (1984) stated that certain hosts evasion tactics are heritable. Architecture of Tvu 946 is a heritable character which can be exploited. However, it is important to point out that for the cowpea pods to escape the attack by M. testulalis larvae through exploitation of the plant architecture, the peduncles of the plant should be very long, strong and erect. A merely long peduncle which easily lodges down or droops to bring down the pods back inside the canopy may not be useful. This cause of resistance also needs to be complimented either by nonpreference or antibiosis or both to keep down the larval population in the plant down.

Based on the above observations and discussion, three points have been made clear:

- (1) At the larval orientation stage, the colonization of Tvu 946 and VITA 5 by M. testulalis larvae is not as optimal as compared to the susceptible VITA 1. This is because the attractancy of Tvu 946 and VITA 5 and the arrest/stay of the larvae in them is lower than that of VITA 1 due to biochemical differences.

- (2) Majority of the pods of Tvu 946 evade infestation by M. testulalis because the older (4th instar) larvae which normally enters the pods cannot easily reach them hence they are less apparent compared to those of VITA 1.

Thus, both nonpreference and escape mechanisms of resistance are implicated in the resistance of Tvu 946 cowpea cultivar to M. testulalis.

- (3) Availability of moisture in the cowpea environment facilitates the movement and arrival of M. testulalis larvae on the plants. The newly hatch 1st instar larvae are capable of walking on the ground into the cowpea plant from a distance of upto 20cm.

CHAPTER 6

LARVAL FEEDING AND UTILIZATION OF INGESTED FOOD

6.1 Introduction

After the larvae of M. testulalis arrive and settle on appropriate feeding sites on a plant, their feeding responses, utilisation of ingested food and its nutritive value determine the subsequent establishment of the insect on the plant (Scriber, 1984).

Feeding activity may differ from one plant cultivar to another in terms of percentage of insects feeding, duration of feeding and quantity of food ingested. These differences may be determined by physical and/or chemical characters of the plants. Therefore it is important to compare the feeding activity in respect to the three parameters.

After the food is ingested by the larvae, it has to be properly utilised within the body and it has to provide the required nutrients in order to enable the larvae to grow and complete their development. The utilisation of food involves the following main steps: (i) digestion and absorption of the food constituents; (ii) assimilation i.e. conversion, of the absorbed food constituents into tissues. The percentage of the ingested food that is absorbed has been referred to by Waldbauer (1968) as Approximate Digestibility (AD). The percentage of absorbed food material that gets converted

into body tissues has been termed by Waldbauer (1968) as Efficiency of Conversion of Digested food (ECD). The overall percentage of ingested food which is incorporated into tissues has been referred to as Efficiency of Conversion of Ingested food (ECI) by Waldbauer (1968) and according to Saxena (1969) it reflects the overall nutritive value of the food. A comparison of these parameters of utilisation of food ingested by M. testulalis larvae from different parts of the cowpea cultivars is necessary.

There is hardly any information available on the differences in the suitability of different cowpea cultivars in the above respects. Hence, feeding responses and utilisation of food by the M. testulalis larvae to the test cowpea cultivars were compared in this work.

6.2 Materials and Methods

6.2.1 Larval feeding responses to different cowpea cultivars

Feeding responses of the 1st instar larvae in a 2-choice situation to young tender leaves or flowers of the susceptible VITA 1 and the resistant Tvu 946 were measured by the methods followed by Kogan (1972). Ten first instar larvae were offered a choice of leaves or flowers from VITA 1 and Tvu 946 cultivars in a glass petridish (14 cm diameter) lined with a moist filter paper disc.

The petridishes were placed on a table at room temperature 25-28°C and were covered with a black piece of cloth. Each petridish formed a replicate and there were five replications. The cloth was removed and percentage of stationary larvae on each leaf or flower was recorded at intervals of 1, 3, 6, 12, 24, and 36 hours. It was already established in an experiment on larval feeding duration, as presented in the next section, that the larvae which were stationary on a plant part were actually feeding on it. Hence, percentage of larvae stationary on each test plant part reflected the percentage of those feeding on it. The data was transformed into percentage and analysed as a 2 factor factorial in a randomized complete block design.

Feeding responses of the larvae to pods were studied using ten day-old pods. Ten pods each of the susceptible VITA 1 and the resistant Tvu 946 were randomly mixed up and kept in a sandwich box (14 x 8.5 x 5.5 cm) (Fig.21). Twenty fourth instar larvae, starved for six hours, were enclosed in the sandwich box containing pods for 24 hours. The top surface of the box was covered with a black piece of cloth. Each sandwich box formed a replicate and there were ten replications. The two cultivars were considered as treatments. At the end of the 24 hour period, the insects were removed. The pods were then examined for feeding. In each cultivar, pods with feeding holes were separated from uneaten pods and counted. The data was expressed in percentages and was compared by 't' test.

The experiment was repeated using VITA 1 and VITA 5.



Fig.21. Arrangement of cowpea pods in a sandwich box used for studying feeding responses of Maruca testulalis larvae to pods of resistant Tvu 946 and susceptible VITA 1 cowpea cultivars in a free choice situation.

6.2.2 Larval feeding duration

The feeding duration of the larvae on the leaves, flowers and pods of VITA 1 and Tvu 946 was compared by the following methods: For measuring the larval feeding duration on leaves or flowers, one young tender leaflet or a flower was sandwiched between two glass slides where in one 1st instar larva was introduced. For measuring the larval feeding duration on the pods, a single pod was placed within a glass tube (1.2 cm diameter, 7 cm long), where in one 4th instar larva was introduced.

The larvae were continuously observed under a microscope. The periods of larval feeding and pauses between feedings were recorded on a strip chart recorder (Biosciences model PR100, Brrd and Tatlock, U.K.). When the insect started feeding as indicated in the movements of its mouth parts, the recorder pen was manually switched on resulting in its full-scale deflection. When the larva stopped feeding, the pen was switched off so that it returned to its base line. The recording was continued over 60-min observation period, the chart paper speed being 0.5mm per sec. The plateaus in the chart represented feeding time while the valleys represented resting time or time spent moving. Feeding duration was determined by measuring the total length of the plateaus on the chart and converted to minutes using the formula below:

$$\text{Feeding duration (min)} = \frac{x}{60y}$$

where x = total length of the plateaus in the chart

y = Speed of the chart (mm/sec).

Each experiment was repeated with five different insects at a time on each of the plant part.

6.2.3 Quantity of food ingested from plants

Leaves: Young tender leaves were collected from plants in the field. Two leaves of the same plant were placed on top of each other, forming two layers of leaves. The leaves were kept in glass petridishes (8.5 cm diameter) lined with moist filter paper. Ten first instar larvae were introduced in between the layers of leaves in each petridish. The dish which was then covered with black cloth to keep it dark, was placed on a table in the laboratory at room temperature 25 - 29°C. Each treatment was replicated 10 times.

After allowing the larvae access to the leaves, the area consumed was measured using a sheet of Letratone paper with 21.5 dots per cm or 462.25 dots per cm². For this, each leaf was placed on the Letratone paper illuminated from underneath. The number of dots visible through the lesions resulting from larval feeding on the leaf were counted. The area consumed (mm²) was calculated as:

$$100x/462$$

where x = the number of dots visible
through the eaten area of the
leaf placed on top of the
letratone paper.

Flowers: Newly opened flowers were picked and kept in small petridishes (8cm diameter). Twenty first instar larvae were introduced into each flower and the petridish was closed tightly. Ten flowers kept one each in a dish comprised a replicate and each treatment was replicated four times. After feeding for 24 hours, the larvae were removed. The degree of feeding was scored visually using the scale below:

Proportion of <u>petals consumed</u>	Score
0	0
>0 ≤ 1/4	1
>1/4 ≤ 1/2	2
>1/2 ≤ 3/4	4

<u>Stamen feeding</u>	<u>Score</u>
No feeding (all anthers present)	0
Low feeding (1-2 anthers eaten)	1
Medium feeding (3-4 anthers eaten)	2
Extensive feeding (all anthers eaten)	3

<u>Pistil feeding</u>	<u>Score</u>
No feeding	0
feeding	1

Overall feeding on the flowers was then assessed by summing up the feeding scores for petals, stamens and pistil.

Stems: Freshly excised pieces of stems were prepared from top young three internodes of 30-day-old plants. Consumption was measured according to Waldbauer (1968). Each piece was trimmed to 6cm long and was weighed to get its fresh weight (F_1) before being kept singly in a test tube (14.5cm long and 2cm diameter). Each tube was lined with aluminium foil to facilitate removal of faeces after feeding. Newly emerged fourth instar larvae which had been starved for 6 hours were weighed individually and the initial weight before feeding (W_1) was recorded. Each larva was kept individually in test tubes and allowed to feed for 2 days. The control for food offered was set up without the insect in the tube and its weight was recorded as FC_1 . To estimate the dry weight of each insect before feeding, the mean dry weight was obtained by weighing a sample of 50 larvae (WC_1) which were then dried to a constant weight (WC_2) in an oven at 60°C. The experiment was replicated 10 times with 10 larvae per replicate. The tubes were mounted in a randomized complete block design in the test tube holders in the laboratory at 24-29°C. At the end of feeding duration (2days) the faeces were separated from the uneaten food. Insects, faeces and control (food) were transferred into an oven where they were dried to a constant weight at 60°C.

Final weights after drying were recorded as follows:

F_2 = uneaten food

FC_2 = Control (food)

W_2 = Weight of insect

Quantity of food ingested was calculated on dry weight basis as:

$$F_1(FC_2/FC_1) - F_2$$

Weight gained by the insect on dry weight basis was calculated as:

$$W_2 - W_1 (WC_2/WC_1)$$

Pods: Ten-day-old pods were used. Each pod was cut into two equal halves and weighed. One half of each was offered to a 4th instar larva and the second half was used as a control for estimating the initial dry weight of the pod. The rest of the procedure was similar to that for stem feeding. The experiment was replicated four times, using ten larvae per replicate.

Since M. testulalis larvae feed on green seed as well as the inner lining of the pericarp of the pod, it was decided to further split the pod into its two components (green seed and the

pericarp) and to measure the amount of food consumed on these two parts separately.

For this, each pericarp was split into two equal halves and weighed before offering one piece to the insect for feeding. The second half was used as the control. With regard to the seeds, ten of them were placed in a test tube. The rest of the procedure was similar to that for stem and pod feeding described above. Each experiment was replicated four times, using ten larvae per replicate.

6.2.4 Feeding on artificial diet containing stem powder or pod powder of the cowpea plant

Preparation of the powder. Fresh stems and pods were harvested from VITA 1, VITA 5 and Tvu 946 plants grown in the field. Stems were harvested from preflowering plants (28 days old) whereas pods were taken when they were 10 days old. The harvested plant parts were chopped into small pieces and dried in the laboratory at room temperature (22 - 26°C). The dry stems or pods were ground into fine powder using an electric grinder.

Composition and preparation of the diet. The fine powder obtained as described above was incorporated in an artificial diet developed by Ochieng and Bungu (1983). Composition of the diet is shown in Table 8. Incorporation of the powder was done by substituting cowpea flower powder with a similar quantity of stem or pod powder of the test cultivars (VITA 1, VITA 5 and Tvu 946). The

ingredients in fraction A (Table 8) were mixed in a blender for 3 min. Agar agar in fraction B was heated in 200 ml. distilled water to boiling point. Boiling agar agar was then cooled down to about 60°C and added to fraction A in a blender and mixed thoroughly for 3 min. Formaldehyde was then added and blended for 1 1/2 min. The diet was then dispensed in glass petridishes.

Measurement of feeding. Consumption was measured following Waldbauers' (1968) method as described above. Uniform discs of the diet were cut out from the petridishes using a cork borer and weighed before offering to 4th instar larvae for feeding in glass tubes as described above. The three cultivars were considered as treatments and each treatment was replicated 4 times using 10 larvae per replicate. The original diet by Ochieng and Bungu (1983) containing flower powder was used as the control.

6.2.5. Feeding responses to the plant extracts

Preparation of plant extracts. Raw juice of fresh leaves, stems and flowers of VITA 1, VITA 5 and Tvu 946 was extracted by crushing 500 gm of each separately with a mortar and pestle. The juice was then squeezed out and collected in a beaker and incorporated into agar-cellulose gel as required. Methanol extract was obtained as described in Chapter 2.

Table 8. Composition of a Kabuligram based diet for rearing M. testulalis (Ochieng and Bungu,1983)

Ingredients	Amount
Fraction A	
1. Water	120.0 ml.
2. Yeast	4.g.
3. Ascorbic acid	1.11 g.
4. Methyl-p-benzoet	0.80 g.
5. Sorbic acid	0.40 g.
6. Kabuligram (Chick pea)	35.00 g.
7. Cowpea flower powder	5.00 g.
8. Euromycin	0.14 g.
9. Vitamin E	0.40 g.
Fraction B	
10. Agar agar	5.10 g.
11. Water	200.0 ml.
12. Formaldehyde 1%	1.00 ml.

Composition of the gel. The composition of the gel was as follows:

For raw juice:

Agar	4g
Cellulose	1g
raw juice	50ml
Distilled water	45ml

Control:	Agar	4g
	Cellulose	1g
	Distilled water	95ml

For methanol:

Agar	4g
Cellulose	1g
Methanol extract	0.5g
Distilled water	94.5ml

Preparation of the gel. The gel was prepared by adding a mixture of agar and cellulose to the required amount of water in a beaker and stirred thoroughly with a stirring rod. Boiling agar cellulose was cooled down to about 60°C and the plant extract was then added and stirred thoroughly before dispensing in clean glass petridishes.

Measurement of quantity of the gel ingested. To measure the quantity of the gel consumed by the larvae, uniform discs of the gel were cut out from the petridishes using a cork borer and weighed on an analytical balance (Oertling HC2z). The initial weight was recorded as F_1 . The disc was then kept in a small glass tube (1 cm diameter, 7cm long). A single 4th instar larva was introduced into the glass tube and covered with cotton wool. After feeding for 24 hours, the insect and the frass were removed and the remaining uneaten food was reweighed and recorded as F_2 . Loss of weight due to evaporation during the feeding period was measured as the difference ($C_1 - C_2$) between the initial (C_1) and final (C_2) weights of the control disc kept without the insect.

Quantity of the gel eaten by the insect was obtained as follows: $F_1 - F_2 + (C_1 - C_2)$.

Where F_1 = weight of food before eating
 F_2 = weight of uneaten food
 C_1 = weight of control at the beginning
of the experiment
 C_2 = weight of control at the end of the
experiment.

The experiment was replicated four times with 10 insects per replicate and was analysed as a completely randomized block.

6.2.6 Feeding responses to sugars

The sugars towards which the larval feeding responses were tested included sucrose, glucose and fructose. Each sugar was tested at 0.1, 0.05, 0.01 and 0.05 M. concentrations. Preparation of the gel and other experimental procedures were as described above.

6.2.7 Utilization of Ingested Food

Utilization of food ingested by the larvae from stems, pods, pericarp and seeds of the cowpea test cultivars was studied by methods basically similar to those adapted by Waldbauer (1968). Flowers were not included in this study because they wilt very quickly under dry conditions, and decay under moist conditions. The larvae in the 4th instar were taken for the study because it is in this stage that they bore into stems and pods.

The different parameters of food utilisation (Waldbauer, 1968) were calculated from the data obtained from measurements of quantitative food intake by the larva from the cowpea stems and pods, as described in the preceding sections:

$$\text{Approximate digestibility (AD)} = 100 (F-E)/F;$$

Efficiency of conversion of

Digested food into body tissues

$$\text{(ECD)} = 100 G/(F-E);$$

Efficiency of conversion of
Ingested food into body
substances (ECI) = 100 G/F;

(F being the dry weight of food ingested by the larvae, E the dry weight of the excreta, and G the dry weight gain of the larvae).

6.3 Results

6.3.1 Larval feeding responses to different cowpea cultivars

When 1st instar larvae were offered a choice between tender leaves of VITA 1 and Tvu 946 cultivars, significantly more larvae were observed feeding on VITA 1 leaves than on resistant Tvu 946 leaves. Such differences in such larval aggregation were noticed within the first one hour after the release of the larvae and upto 12 hours. After 24 and 36 hours there were no significant differences in the percentage of larvae feeding on the two cultivars' leaves (Fig.22 and Appendix 28). In fact, 24 and 36 hours after the larval release the leaves were wilted and some of the larvae were dead.

The flowers of the two cultivars, offered as a choice, also differed in their suitability for feeding by the larvae.

Significantly, more larvae were found feeding on the flowers of VITA 1 than on those of Tvu 946 (Fig. 23 and Appendix 29). At 24 and 36 hours after the larval release, the petals started wilting and the experiment was concluded.

With reference to the pods, when those of VITA 1 and Tvu 946 were given together in equal numbers to the 4th instar larvae in a clean plastic sandwich box, significantly more pods of VITA 1 were fed upon than Tvu 946 pods (Fig. 24 and Appendix 30). Similarly, VITA 1 pods were fed upon four times more than VITA 5 pods when the two were offered in a mixture (Fig. 24 and Appendix 31).

6.3.2 Larval feeding duration on different cowpea cultivars

When 1st instar larvae were offered leaves of the two cultivars (VITA 1 and Tvu 946), the time taken by the larvae to commence feeding i.e., pre-feeding duration, was longer (7.3 ± 3.3 min) on Tvu 946 leaves than on VITA 1 leaves on which the larvae started feeding within 0.6 ± 0.3 min. After the commencement of feeding, the total duration for which the larvae fed in on hour period on VITA 1 was almost twice that on Tvu 946 leaves (Fig. 25 and Appendix 32).

On flowers, prefeeding duration was also longer for Tvu 946 than for VITA 1. However, the feeding duration per 1-hour observation period was almost identical for the two cultivars (Fig. 25 and Appendix 32).

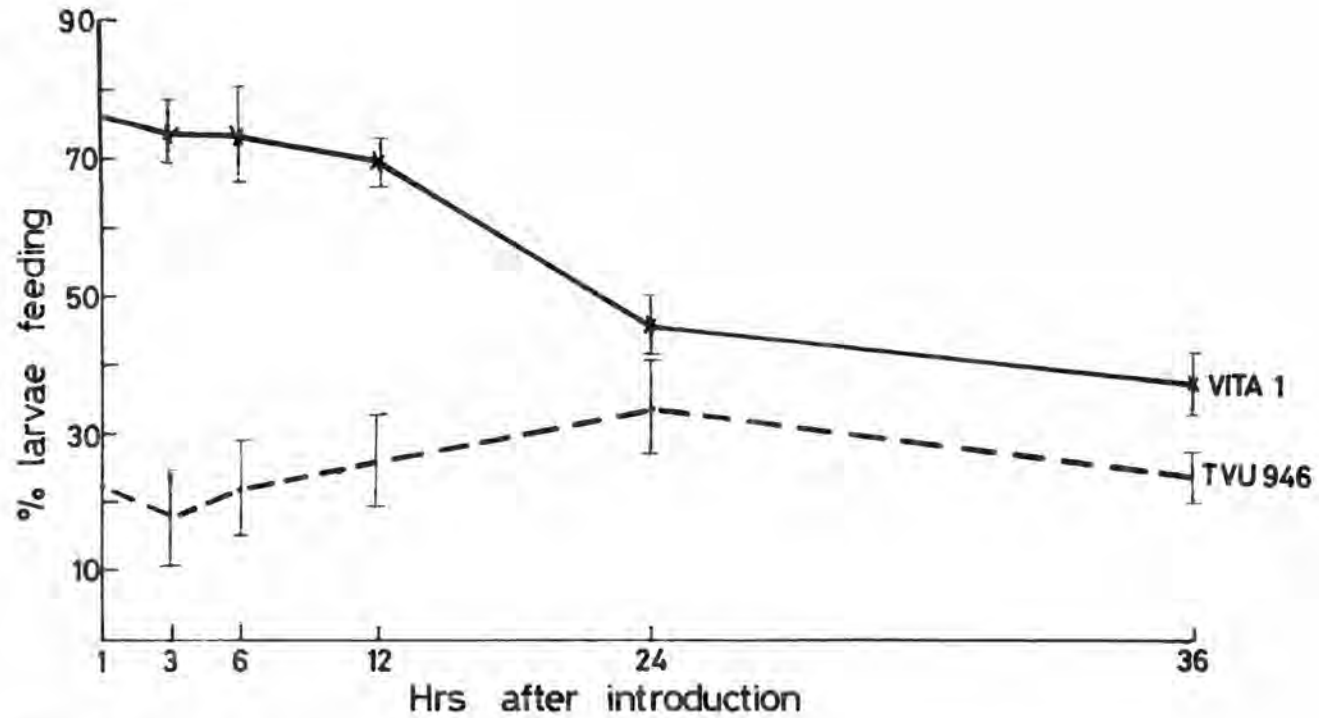


Fig. 22. Feeding responses of 1st instar *M. testulalis* larvae to leaves of the susceptible VITA 1 and the resistant TVu 946 cowpea cultivars in a 2-choice situation.

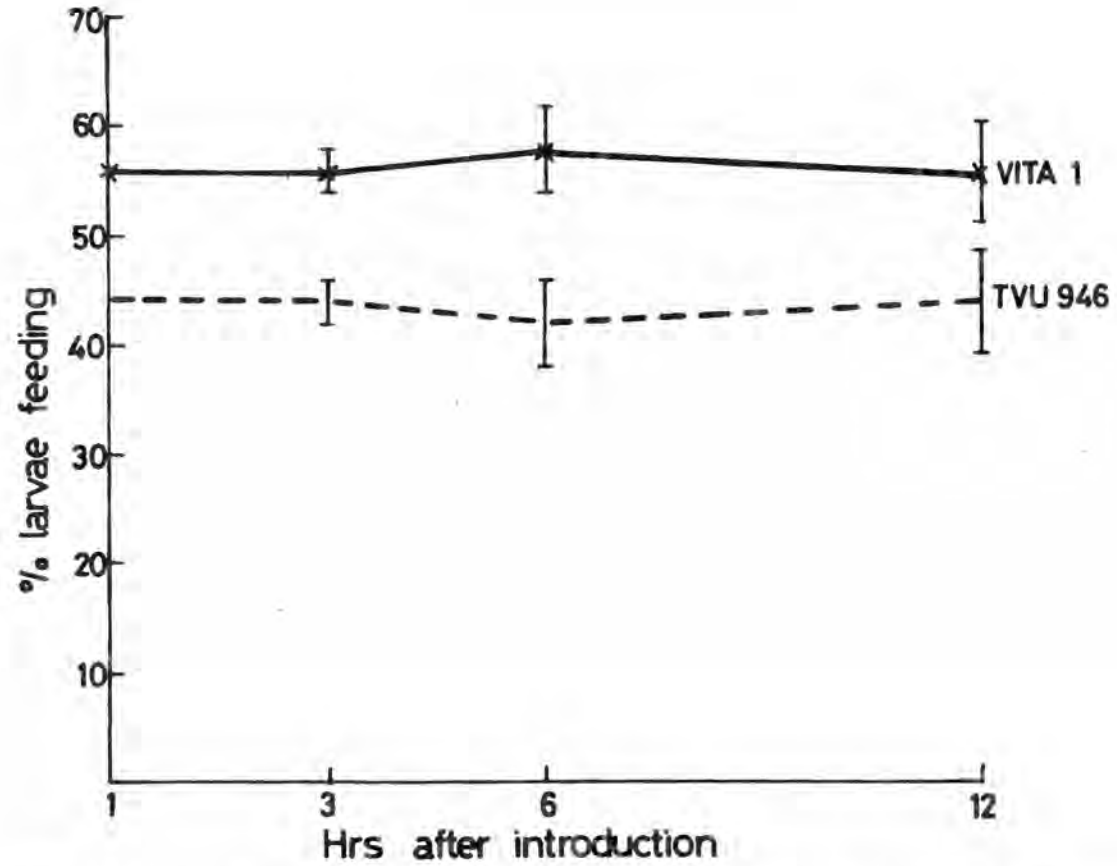


Fig:23 Feeding responses of 1st. instar *M. testulalis* larvae to flowers (Petals) of the susceptible VITA 1 and the resistant TvU 946 cowpea cultivars in a 2- choice situation.

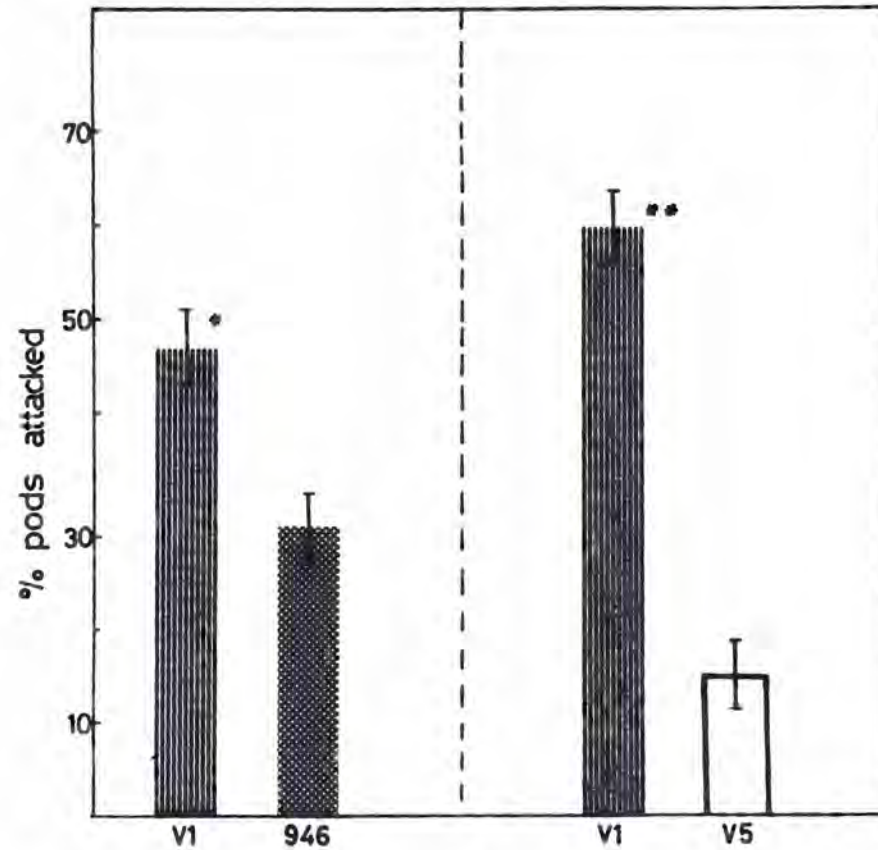


Fig. 24. Feeding responses of 4th instar *Maruca testulalis* larvae to pods of certain cowpea cultivars in a 2-choice situation.
VI = VITA 1; 946 = TVu 946; V5 = VITA 5
The columns marked with * and ** are significantly different from the unmarked columns at $P = 0.05$ and $P = 0.01$ by χ^2 test respectively.

With regard to the pods offered to the 4th instar larvae, the prefeeding duration was almost the same for VITA 1 and Tvu 946. But, the feeding duration on VITA 1 was twice that on Tvu 946 (Fig. 25 and Appendix 32).

6.3.3 Quantity of food ingested from plants

Leaves: When 1st instar larvae were caged for feeding on tender leaves of VITA 1, VITA 5 and Tvu 946 for 48 hours, there were no significant differences among the three cultivars in respect of leaf area consumed (Fig. 26 and Appendix 33). However, VITA 1 leaves are almost twice as thick as Tvu 946 leaves and, therefore, it can be considered that Tvu 946 leaves were less consumed than VITA 1 leaves. While feeding, the larvae tended to avoid the leaf veins and concentrated their feeding on the leaf blade.

Flowers: The quantitative food intake by the larvae from the flowers, as reflected in the feeding scores, was almost same for all the three cultivars (Fig 26 and Appendix 34). The first instar larvae tended to concentrate their feeding on the petals causing lesions much more than on the stamens or pistil.

Stems: When the 4th instar larvae were offered tender stems of the three cultivars for feeding, the quantity (mg. dry weight) ingested from Tvu 946 was significantly lower than that from VITA 1 or VITA 5 ($P = 0.01$). Consumption of the stems of the resistant VITA 5 cultivar was not significantly different from the susceptible VITA 1 (Fig. 27 and Appendix 35).

Pods: The quantitative food intake by the 4th instar larvae from the pods of the resistant VITA 5 was significantly higher than from VITA 1 or Tvu 946 ($P=0.05$) (Fig.27 and Appendix 36). The consumption of the pods of VITA 1 and Tvu 946 were statistically identical.

An examination of the pods fed upon by the larvae revealed that the inner lining of the pericarp as well as the seeds were eaten. Hence the pods were split open to separate their pericarp from the seeds which were then offered to the 4th instar larvae for feeding. Both, the pericarp and seeds of the susceptible VITA 1 pods were equally fed upon. But, with regard to the VITA 5 and Tvu 946, the seeds were consumed more than the pericarp (Fig. 28 and Appendix 37). When offered the pericarp alone, that from VITA 1 was ingested significantly more than from VITA 5 or Tvu 946 ($P=0.01$) (Fig. 28 and Appendix 37). On the other hand, when the larvae were offered the seeds alone, those of VITA 5 were fed upon two to three times more than of VITA 1 or Tvu 946, differences being significant at $P=0.01$. (Fig. 28 and Appendix 37). VITA 1 and Tvu 946 did not differ significantly from each other in this regard.

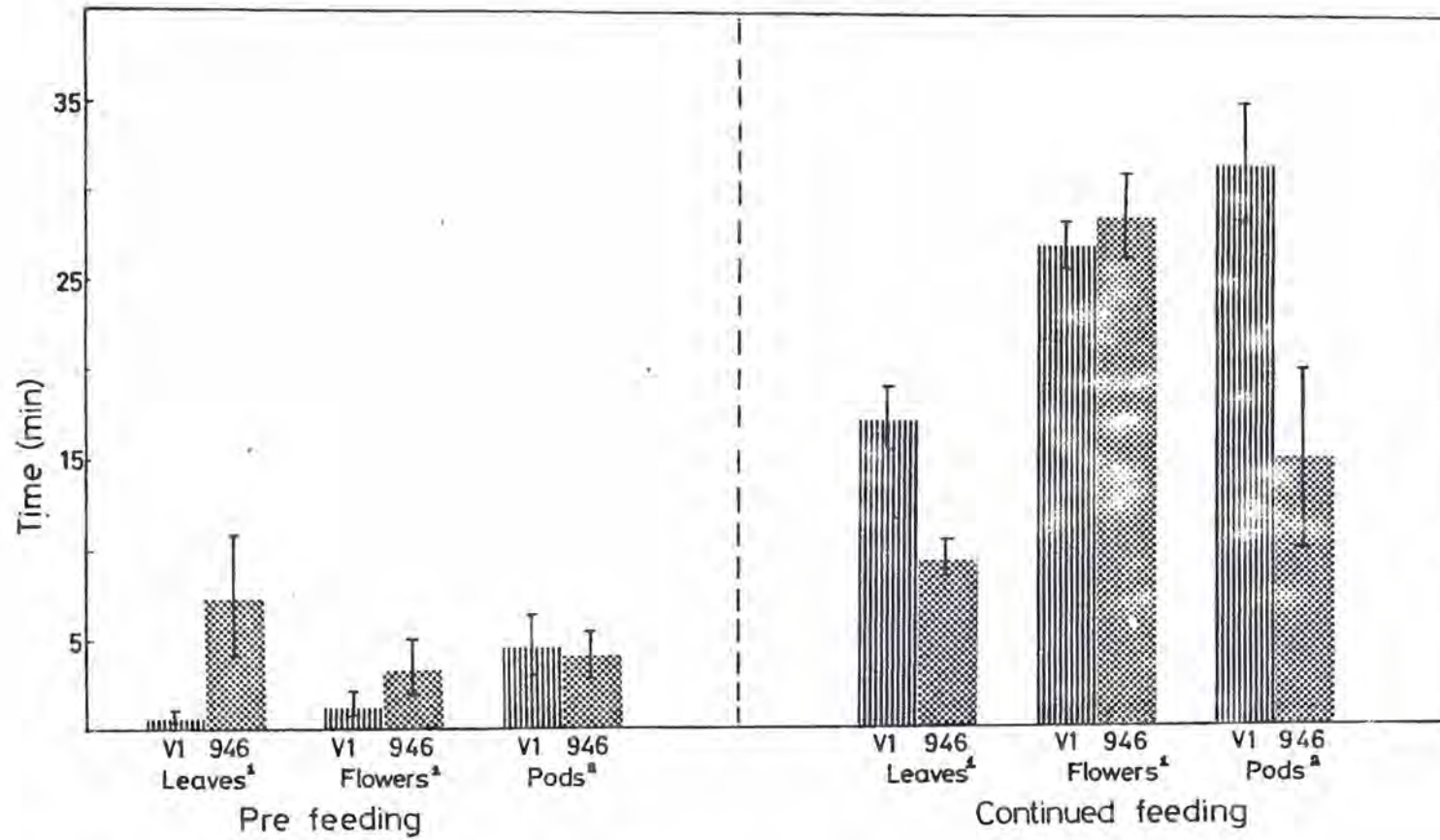


Fig. 25. Feeding duration (min/h) of *M. testulalis* larvae on excised parts of the susceptible VITA 1 and the resistant TVu 946 cultivars under no choice conditions. V1 = VITA 1; 946 = TVu 946.

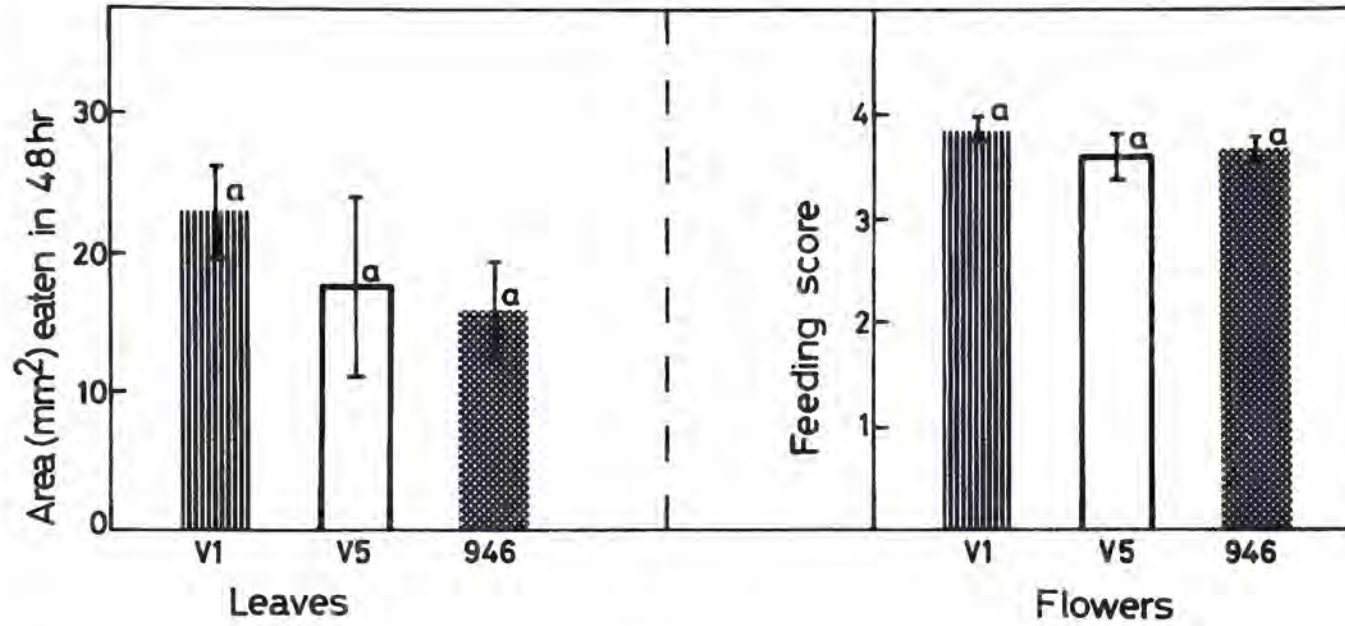


Fig. 26. Quantity of leaves and flowers of certain cowpea cultivars ingested by 1st instar *M. testulalis* larvae.

Within each plant part, columns with a common letter not significantly different.

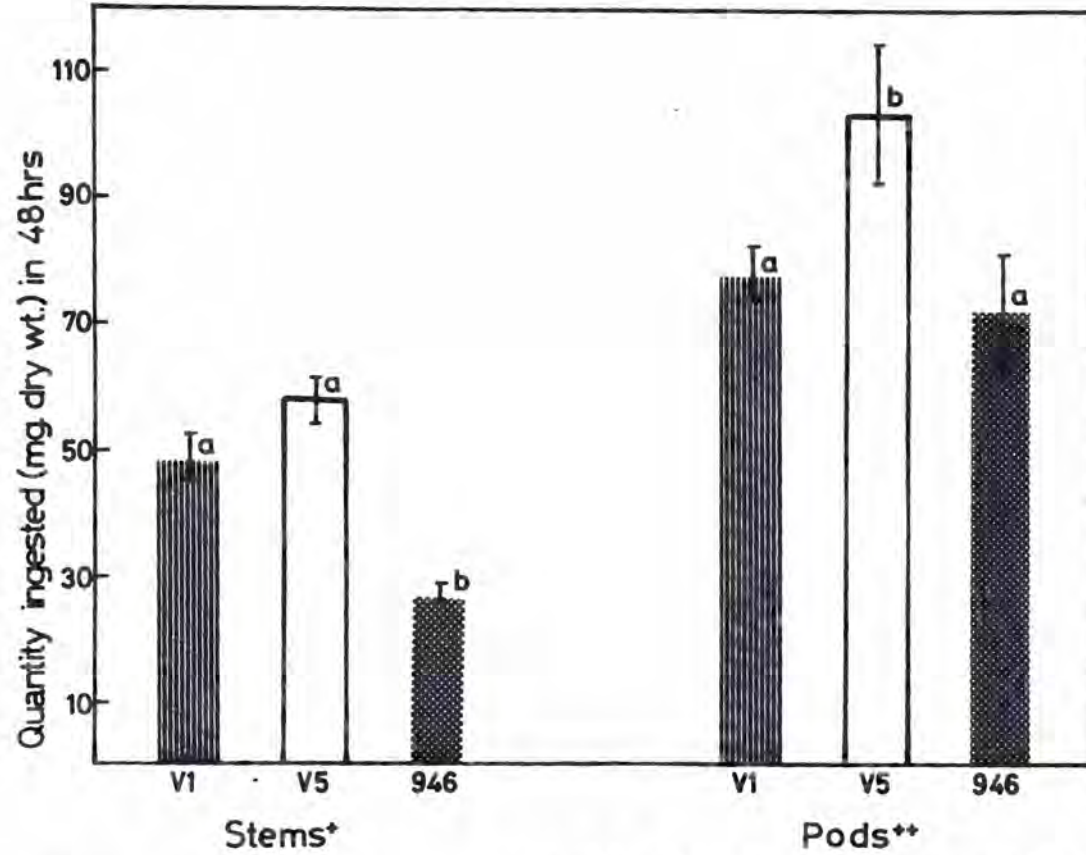


Fig. 27. Quantity of food ingested by 4th instar *Maruca testulalis* larvae from stems and pods of certain cultivars. V 1 = VITA 1; V5 = VITA 5; 946 1 TVu 946. Within each plant part, columns with a common letter are not significantly different at $P = 0.05$ by DMRT.

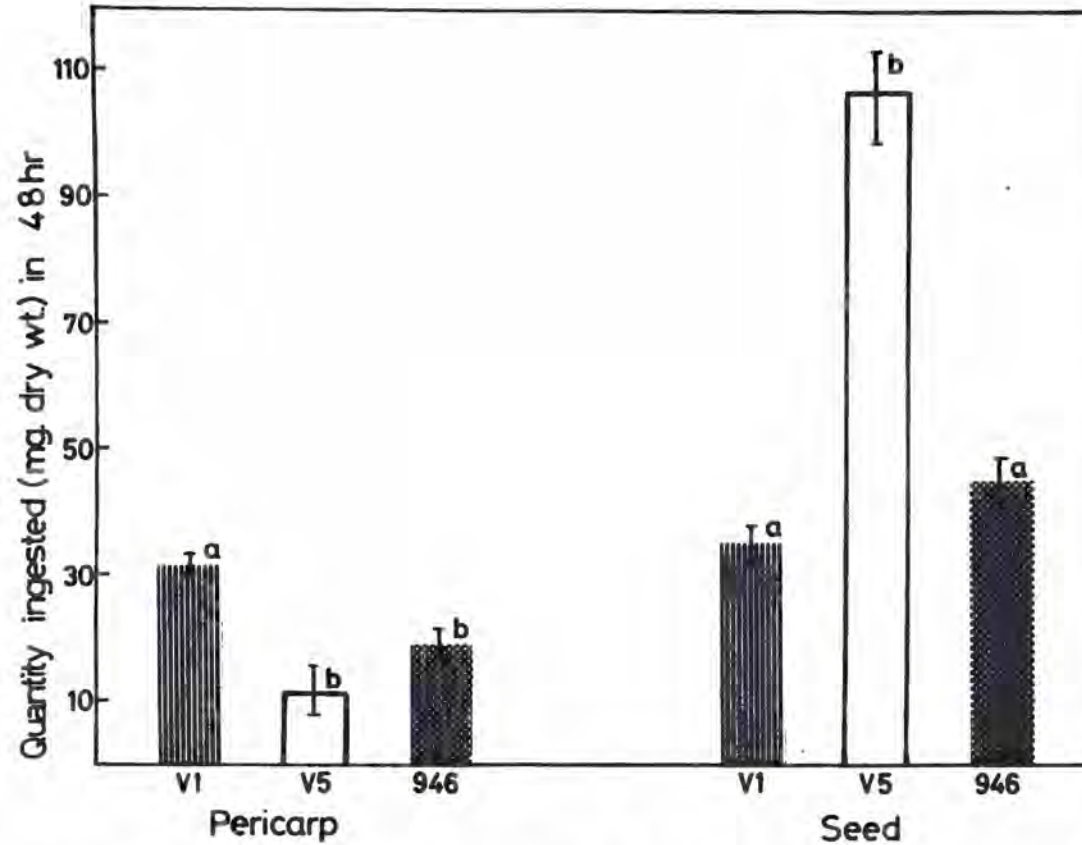


Fig. 28. Quantity of food ingested by 4th instar *Maruca testulalis* larvae from pericarp and seed of certain cowpea cultivars. V 1 = VIA 1; V 5 = VITA 5; 946 = TVu 946. Within each plant part, columns with a common letter are not significantly different at $P = 0.01$ by DMRT.

6.3.4 Feeding on artificial diet
containing stem or pod powder

Under no choice situations, the amount of food consumed (mg dry wt) by 4th instar larvae given the artificial diet containing stem powder from the susceptible VITA 1 was significantly more than that containing the resistant VITA 5 or Tvu 946 powder (Table 9 and Appendix 38). With regard to the diet containing the pod powder, there were no significant differences among the cultivars in respect of the quantity of food ingested (Table 9 and Appendix 39).

6.3.5. Feeding responses to plant extracts

Raw plant juice: Incorporation of raw juice extracted from any of the cowpea cultivar in the agar-cellulose gel resulted in a significantly ($P = 0.01$) greater intake of the gel by 4th instar larvae than that of the plain gel (control) under no choice situation (Table 10). This observation suggests that certain chemicals of the cowpea plants extracted in the juice serve as phagostimulants from M. testulalis larvae. A comparison among different parts of cowpea plants (VITA 1, VITA 5 and Tvu 946, cultivars) revealed that the juice from the flowers was the most phagostimulatory, followed by that from the leaves. The juice from the stems was the least phagostimulatory. There were no significant differences among the cultivars in phagostimulation by juice of those plant parts (Table 10 and Appendix 40, 41, and 42).

Table 9. Quantity of food ingested by 4th instar M. testulalis larvae on artificial diet containing stem and pod powder of certain cowpea cultivars

Diet	Consumption (\pm s.e.) (Mg. dry wt.) in 24h $\frac{1}{2}$	
	Stem powder	Pod powder
VITA 1	33.75 \pm 1.9 a	34.60 \pm 3.6 a
VITA 5	27.24 \pm 2.3 ab	27.50 \pm 2.1 a
Tvu 946	26.70 \pm 1.9 b	27.25 \pm 1.9 a
Control	31.25 \pm 3.1 a	

$\frac{1}{2}$ Mean of 40 larvae in 4 replicates of 10 larvae each

In a column, means followed by a common letter are not significantly different at P=0.05 by DMRT

Table 10. Feeding response of 4th instar M. testulalis larvae to raw juice extract from different parts of certain cowpea cultivars incorporated in agar-cellulose gel

Cultivar	Quantity of Gel ingested (mg) in 24h <u>1/</u>		
	Stem	Leaves	Flower
VITA 1	84.5 \pm 11.4 a	110.4 \pm 9.4 b	207.6 \pm 38.9 a
VITA 5	99.7 \pm 9.4 a	156.4 \pm 17.5 a	200.2 \pm 29.1 a
Tvu 946	85.8 \pm 13.5 a	147.4 \pm 13.1 ab	232.4 \pm 18.7 a
Sucrose 0.1M	42.0 \pm 4.1 b	52.0 \pm 12.3 c	52.4 \pm 2.7 b
Control <u>2/</u>	12.2 \pm 1.2 c	19.6 \pm 2.0 d	16.5 \pm 1.6 c

1/Mean of 40 larvae in 4 replicates of 10 larvae each. in a column, means followed by a common letter are not significantly different at p=0.01 by DMRT

2/Plain agar-cellulose gel

Methanol extracts. Under no choice conditions, incorporation of the methanol extract from the pods of any of the three cultivars (VITA 1, VITA 5 and Tvu 946) in the agar-cellulose gel stimulated significantly greater intake ($P=0.01$) of the gel than of plain agar-cellulose gel (Table 11 and Appendix 43). No significant differences were observed among the three cultivars in this respect.

6.3.6 Feeding responses to sugars.

As sugars have been reported to stimulate feeding in several species of insects, it was considered important to examine the role of certain sugars in determining feeding in M. testulalis as well. The results show that the quantity of the agar-cellulose gel ingested by 4th instar larvae increased with the concentration of each of the 3 sugars i.e., sucrose, glucose, fructose in the agar-cellulose gel. (Fig. 29 and Appendix 44,45,46). Thus all these sugars serve as phagostimulants for the larvae.

6.3.7. Utilization of ingested food

Stem. The approximate digestibility (AD) of the food ingested by the larvae from the stems of the susceptible VITA 1 and the resistant VITA 5 as well as Tvu 946 were statistically identical (Fig. 30 and Appendix 47). The efficiency of conversion of the digested food (ECD) as well as of ingested food (ECI) into body

Table 11. Feeding response of 4th instar M. testulalis larvae to methanol extract from pods of certain cowpea cultivars incorporated in agar-cellulose gel

Cultivar	Quantity of the gel ^{1/} ingested in 24h. (mg/insect)
VITA 1	43.00 ± 7.2 a
VITA 5	32.67 ± 4.0 a
Tvu 946	40.67 ± 6.0 a
Control ^{2/}	7.00 ± 1.7 b

^{1/}Average of 30 larvae in 3 replicates of 10 larvae each.

In a column, means followed by a common letter are not significantly different at p=0.05 by DMRT.

^{2/}Plain agar-cellulose gel.

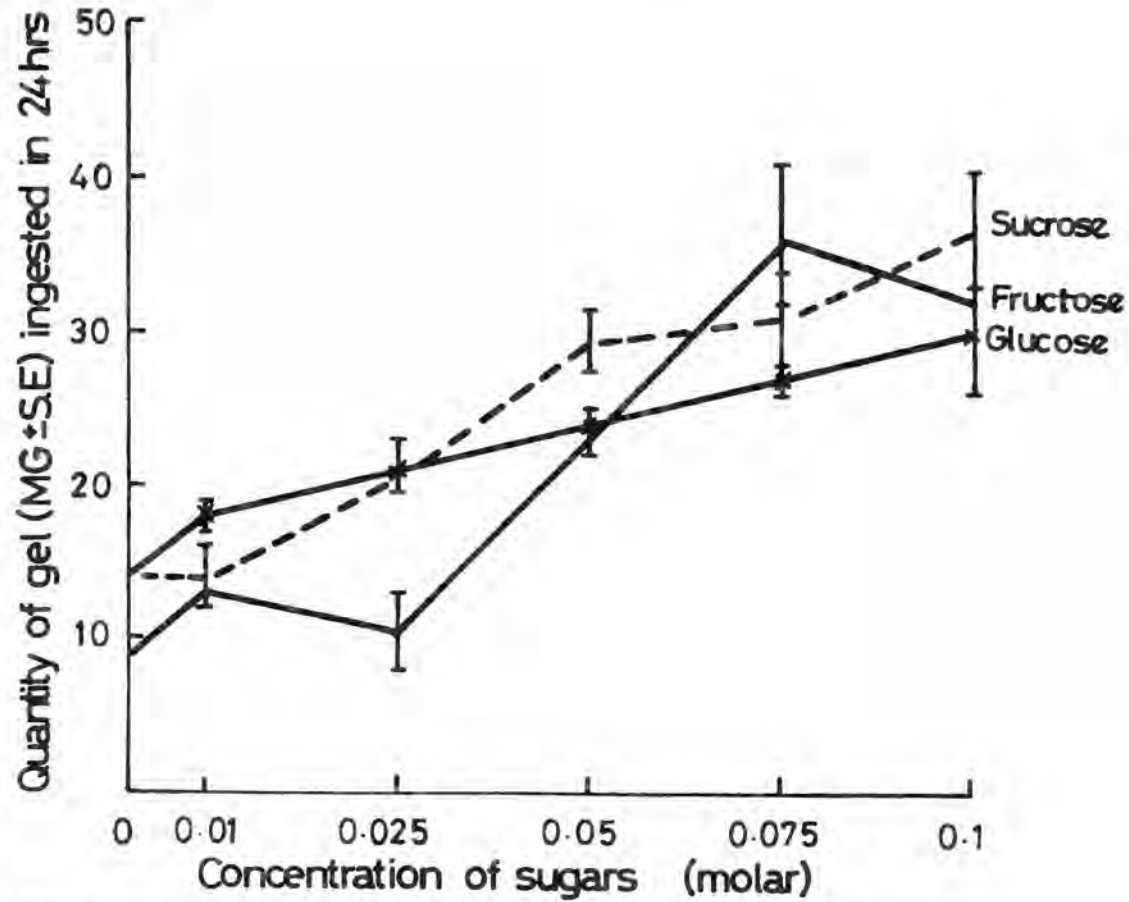


Fig. 29. Feeding responses of 4th instar *Maruca testulalis* larvae to certain sugars incorporated in agar-cellulose gel.

tissues were identical for the susceptible VITA 1 and the resistant Tvu 946. But VITA 5 was unique in as much as both ECD and ECI were significantly lower than those for the other two cultivars at $P = 0.05$ (Fig. 30 and Appendix 48, 49).

Pods. The AD of pods of all the cultivars was higher than that of stems. However, AD of Tvu 946 was lower ($P = 0.05$) than that of VITA 1 and VITA 5 pods. This shows that Tvu 946 pods have a lower digestibility than the other two cultivars (Fig. 30 and Appendix 50). The pods of VITA 1 and VITA 5 were statistically identical in this respect. On the other hand, the ECD and ECI of the food ingested from the pods of VITA 5, like those for its stem, were significantly lower than those for VITA 1 and Tvu 946 (Fig. 30 and Appendix 51 and 52).

When the pericarp and seeds of the pods were given separately to the larvae, AD of VITA 1 pericarp was highest and significantly different from that of VITA 5 and Tvu 946 at $P = 0.05$ (Table 12 and Appendix 53). ECD of the pericarp was higher for VITA 5 than for VITA 1 and Tvu 946. But, ECI of the pericarp of all the three cultivars was statistically identical (Table 12 and Appendix 54, 55).

As regards the seeds, AD of the resistant VITA 5 was twice as high as that of the susceptible VITA 1 and the resistant Tvu 946, the difference being significant at $P = 0.05$. But ECD and ECI of VITA 5 seeds were lower than those of VITA 1 and Tvu 946.

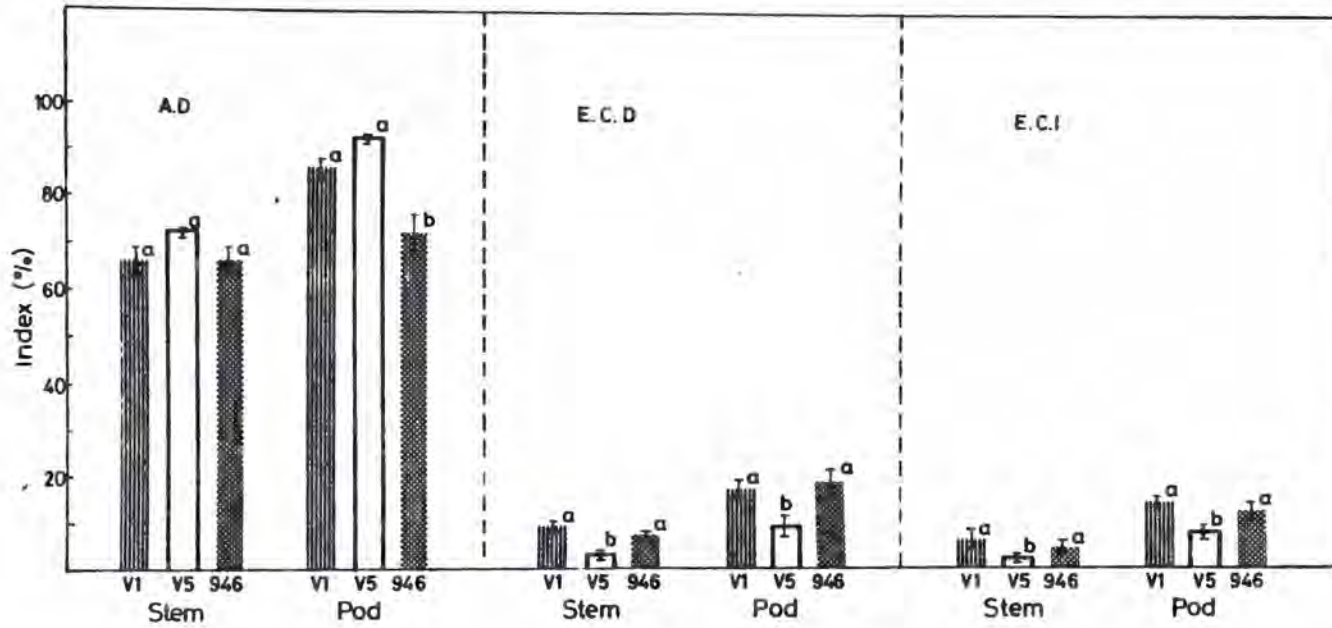


Fig.30. Utilization of food ingested from stem and pods of certain cowpea cultivars by 4th instar *M. testulalis* larvae. V1= VITA 1; V5= VITA 5; 946= TVU 946. Within each part of the plant, columns with a common letter are not significantly different at P=0.05.

Table 12. Utilization of ingested food from pericarp and seed of certain cowpea cultivars by 4th instar *M. testulalis* larvae $\frac{1}{\checkmark}$

Indices	Cultivar	Part of the pod		Difference
		Pericarp	Seed	
AD	VITA 1	94.37 \pm 1.5 a	36.74 \pm 2.1 a	57.63*
	VITA 5	62.14 \pm 11.8 b	83.10 \pm 1.1 b	20.96*
	Tvu 946	69.93 \pm 3.6 b	42.02 \pm 2.4 a	27.91*
ECD	VITA 1	29.13 \pm 2.8 a	51.63 \pm 9.1 a	22.50*
	VITA 5	44.76 \pm 11.4 b	8.26 \pm 0.6 c	36.50*
	Tvu 946	33.19 \pm 3.7 a	39.48 \pm 2.2 b	6.29 ^{ns}
EC 1	VITA 1	27.38 \pm 2.5 a	18.38 \pm 1.9 a	9.00*
	VITA 5	29.06 \pm 4.7 a	6.85 \pm 0.4 b	22.21*
	Tvu 946	22.89 \pm 1.7 a	16.45 \pm 0.8 a	6.44 ^{ns}

$\frac{1}{\checkmark}$ Average of 40 larvae tested in 4 replications of 10 larvae each.
 In a column, means followed by a common letter are not significantly different at $p=0.05$ by DMRT.

* Utilization of pericarp is significantly different from seed at $p=0.05$ by LSD; ^{ns} = not significant

AD = Approximate digestibility

ECD = Efficiency with which digested food is converted to body substances

EC1 = Efficiency of conversion of ingested food to body substances.

ECI of Tvu 946 was also lower than that of VITA 1 although their ECDs were identical.

The fact that the AD, ECD and ECI of VITA 1 were higher than those of its seeds suggests that the seeds were poorly utilised compared to the pericarp. For VITA 5, AD of the seeds was higher and their ECD as well as ECI lower than those of the pericarp, indicating that, despite a higher digestibility of the food from the seeds, its conversion into body substances was poorer than that of the pericarp. For Tvu 946, AD of the pericarp was higher than that of the seeds while ECD and ECI of the two were similar.

6.4 Discussion

6.4.1 Larval feeding responses to different cowpea cultivars

Miller and Strickler (1984) used the term preference to mean to put before something else in one's liking or positive responsiveness. They further suggested that preference or non preference should apply whenever multi items come into an insect's sensory field simultaneously and a given item is consistently taken. The opinion of the above two authors was accepted when studying the feeding responses of M. testulalis larvae in this work. In Chapter 5 it was observed that of M. testulalis larvae preferred VITA 1 over Tvu 946 and VITA 5 for orientation from a

distance when the plants of all the three cultivars were available together. Thus, the larvae showed orientational non preference for the resistant Tvu 946 and VITA 5.

The results presented in this chapter show that the larvae show non preference for for VITA 5 and Tvu 946 for feeding as well. The parameter used for judging feeding preference on leaves and flowers was based on the aggregation of the larvae on the food. Although initially (the first one hour) aggregation could have been due to differences in the larval attraction by the cultivars as was reported in chapter 5, the larvae were expected to move out after some time if they did not like the food. It was thus assumed that aggregation of the larvae on a particular food for more than 12 hours was accompanied by feeding. Dethier (1982) stated that recognition and preference of a host plant involve the integration of a complex of neural and metabolic events. These include: sensing and encoding characteristics of the sense organs, decoding mechanisms in the central nervous system, assessment of cross fibre patterns and deterrent/stimulant ratios, pre-and post-ingestion factors such as satiety and nutritional factors.

The time of exposure of the insects to the food was long enough to allow for the above interaction mentioned by Dethier (1982). The trend of larval distribution on the plants was such that there was a consistent aggregation of the larvae on VITA 1 for more than 12 hours. Thus VITA 1 leaves and flowers were more preferred over those of Tvu 946 and VITA 5. The method which was

followed in determining larval preference to leaves and flowers has been used by other workers as well (Kogan, 1975).

When determining feeding preference on pods, arrangements were such that the pods of Tvu 946 and VITA 1 were touching each other so that the question of larval attraction from a distance did not arise. Furthermore the parameter for judging the preference was based on the actual feeding rather than larval aggregation.

The observed feeding nonpreference, when combined with low attraction of larvae (orientational non preference) under field conditions are very important mechanisms of resistance in Tvu 946 and VITA 5. This is mainly because the larvae would not be expected to settle down in these two cultivars within a short time since they would spend more time in search of a more preferred cultivar. In the process of the search, there would be more chances of encountering pathogens, and other natural enemies. The larvae also exhaust their energy and become weak and may die before finding a suitable host.

6.4.2 Larval feeding duration on different cowpea cultivars

Usually after the insect has landed on a plant, the next step to follow is either feeding or egg laying depending on its need (Saxena, 1969, 1985). However, these would only take place if the plant is accepted by the insect. Therefore, a measure of the degree of consumption can also be an indicator of the degree of

acceptance. A measure of feeding duration is one of the ways of determining the degree of consumption (Miller and Strickler, 1984). In this chapter the results show that the larvae took a very long time to start feeding on leaves of Tvu 946. But after initiation of feeding, the continued feeding duration was very short compared to that on VITA 1 leaves. Thus, the larvae remained unsettled for a very long time on Tvu 946 leaves while on VITA 1 they settled down to feed much quicker. This suggests that VITA 1 was more accepted than Tvu 946. Also if the insect had not been confined it would have moved out of Tvu 946 faster than it would have on VITA 1. The observed larval response while measuring its feeding duration also suggests that (i) since prefeeding duration was shorter on VITA 1 than on Tvu 946, the susceptible VITA 1 may be having more feeding stimulant(s) (ii) longer prefeeding duration coupled with a shorter feeding duration on Tvu 946 than that on VITA 1 may be an indication of the presence of feeding deterrent or less feeding stimulants.

When feeding on flowers, prefeeding duration was longer on Tvu 946 than on VITA 1 but the continued feeding duration was similar. This may mean that the rate and amount of food intake from flowers of the two cultivars are the same although VITA 1 flower may be having a stronger feeding stimulant than that of Tvu 946 flowers.

When feeding on pods, prefeeding duration was similar but feeding duration was shorter. Thus the insects spent more time

moving about within the one hour it was exposed to the food. It can therefore be considered that Tvu 946 pod was less accepted than that of VITA 1.

6.4.3. Quantity of food ingested

It was earlier shown that Tvu 946 and VITA 5 pods were less preferred than to those of VITA 1 (paragraph 6.3.1). At the same time when the quantity of food ingested was measured it was found that consumption of Tvu 946 and VITA 1 were similar and that of VITA 5 was even higher (paragraph 6.3.3).

According to Knipling (1979), insects avoid attacking resistant varieties only if a preferred variety is available. Without an alternate preferred variety, the degree of attack on the nonpreferred variety might be near normal. When measuring the amount of food consumed, the insects were starved for 6 hours before confining them on different cultivars. According to Dethier (1982) starvation temporarily alters the behaviour of an insect so that foods which would otherwise be rejected are consumed.

The data on the amount of food consumed when the pod was split into pericarp and seed showed that the pericarp of VITA 5 and Tvu 946 pods was less consumed compared to that of VITA 1. It can therefore be suggested from the data that nonpreference of Tvu 946 and VITA 5 was manifested mainly in the pericarp. Equal and even a higher amount of feeding on the pods of Tvu 946 and VITA 5 was concentrated mainly on the seed.

The stems of Tvu 946 were less consumed than those of VITA 1. This confirms the observation by Macfoy et.al. (1983). Otieno et.al. (1985) reported that there is a feeding deterrent in the stems of Tvu 946 cultivars. This may be an explanation for low consumption of Tvu 946 stems.

6.4.4. Larval feeding responses to plant extracts and sugars

The larvae were phagostimulated by sucrose, glucose and fructose. However, whole juice extracts and methanol extracts from cowpea leaves, flowers and pods were more phagostimulating than sucrose. This suggests that cowpea plants have feeding stimulant(s) in addition to the sugars. Phagostimulation by whole juice and methanol extracts from the resistant and the susceptible cultivars were similar. This suggests that if the plant factors responsible for nonpreference to larval feeding on Tvu 946 and VITA 5 are biochemical, then they may not be obtained from plant juice or the methanol extract.

Feeding deterrent (from methanol extract) was reported by Otieno et al. (1985). Phagostimulation by methanol extract from the pods does not indicate existence of any feeding deterrent compound. It may thus be assumed that the feeding deterrent compound obtainable as methanol extract from the cowpea plant may be present in stems only.

6.4.5 Utilization of ingested food

Nutritional index data are extremely useful in preliminary investigations on physiological processes influenced by plant allelochemicals (Reese, 1977). Several workers have used such data in their studies. The most notable examples are studies by Soo Hoo and Fraenkel (1966) on Prodenia eridania and by Reese and Beck (1976) on black cutworm.

According to Soo Hoo and Fraenkel (1966), several conditions may render a food plant less nutritious: (i) plants can be well digested but are eaten in such small quantities that low utilization results. For example an insect may be digesting 60% or more of the ingested material but it cannot efficiently utilize what it consumes because the rate of ingestion is so low that most of the material must be used for maintenance and not growth; (ii) the rate of consumption can be high while digestibility is low resulting in low utilization.

When M. testulalis larvae were reared on stems and pods of the three cowpea cultivars, VITA 1 (susceptible) was regarded as the standard optimal food. Feeding and utilization of food from Tvu 946 and VITA 5 were therefore compared with those of VITA 1 to see whether there were any metabolic deficiencies.

When feeding on stems of VITA 5, the larvae consumed as much food as on VITA 1. But, the ingested food was less efficiently converted into body material compared to VITA 1. As a result, the rate of growth would be lower in VITA 5 than in VITA 1. This could have been due to inadequate nutrients in VITA 5 stems or VITA 5 stems may be having some conversion inhibitor so that the insect requires to eat much more in order to achieve the same growth rate as when it is feeding on VITA 1.

When the larvae were given Tvu 946 stems, consumption was very little compared to VITA 1. However, the efficiency of conversion of the ingested food (Tvu 946) was statistically equivalent to that of VITA 1. As a result the larvae required to eat more in order to compensate for maintenance and growth as well. This gives an indication that factor or conditions which make Tvu 946 stem a marginal food could be due to one or more of the following: (1) lack of feeding stimuli, (2) a feeding deterrent, hardness or high fibre content. When the larvae were given the pods for feeding, in VITA 1 feeding was uniformly spread on the inner lining of the pericarp as well as on the seed. But in Tvu 946 and VITA 5 feeding was concentrated on the seeds. The data show that the pericarp of Tvu 946 and VITA 5 were eaten in very small quantities and also were poorly utilized compared to that of VITA 1. Poor utilization of Tvu 946 pericarp was mainly due to low digestibility while pericarp of VITA 5 was poorly converted into body tissues.

Consumption and utilization of Tvu 946 and VITA 1 seeds were thus similar. Poor utilization of VITA 5 seed was compensated for by very high consumption. Thus, antibiosis is also partly involved in the resistance of Tvu 946 and VITA 5 pods to M. testulalis and may be mainly due to less consumption and poor utilization of the pericarp.

CHAPTER 7

LARVAL GROWTH AND DEVELOPMENT

7.1 Introduction

Studying growth and development of an insect on its host plant is usually one of the means used to determine the mechanisms of resistance. Also data on growth and development provides further evidence for antibiosis (Painter, 1951). The existing literature provides very limited comparative data on M. testulalis larval development on the susceptible and resistant cowpea cultivars. Singh (1978) simply reported differences in resistance among some cowpea varieties to M. testulalis. Jackai (1981b) only reported on the differences in larval populations on some susceptible and resistant varieties. Macfoy et al. (1983) did some comparative tests on larval growth and development on selected susceptible and resistant cultivars but the tests were limited to stems only. Leaves, flowers and pods were excluded.

A comprehensive study on larval growth and development on stem, leaves, flowers and pods of VITA 1, VITA 5 and Tvu 946 as representatives of susceptible, moderately resistant and resistant cultivars was therefore considered necessary so as to observe whether some differences exist among the cowpea cultivars in this regard. The parameters which were measured included: (i) percentage of larvae surviving and developing from 1st instar to 3rd

instar, pupal and adult stages (ii) larval weight gain and pupal weight and, (iii) periods of larval and pupal development.

7.2 Materials and Methods

Leaves intact with the live plants, freshly excised stems, flowers and pods of the three cultivars VITA 1, VITA 5 and Tvu 946 were used.

7.2.1 Growth and development on leaves

Larval development on leaves was studied using small plastic cages (3.5 cm diameter; 75 cm long) having nylon mesh windows. (Fig.31). The bottom of the cage was perforated and provided with tightly fitting cork. The cork was split longitudinally into two symmetrical halves with a small groove in the centre. The open end of the cage through which the larvae were introduced had a nylon mesh sleeve which was tied with a rubber band after introducing the larvae into the cage. A single leaf of a potted cowpea plant was pushed inside the cage through the bottom hole. The two symmetrical halves of the cork were then placed together with the petiole of the leaf passing through the groove. The cage was anchored with a wire clamp stand. Pots were arranged in a completely randomized design in a screen house. Tests with each cultivar were replicated 5 times with different batches of 10 larvae per replicate. The temperature ranged between 26-30°C. The larvae were transferred to a new leaf every second day.

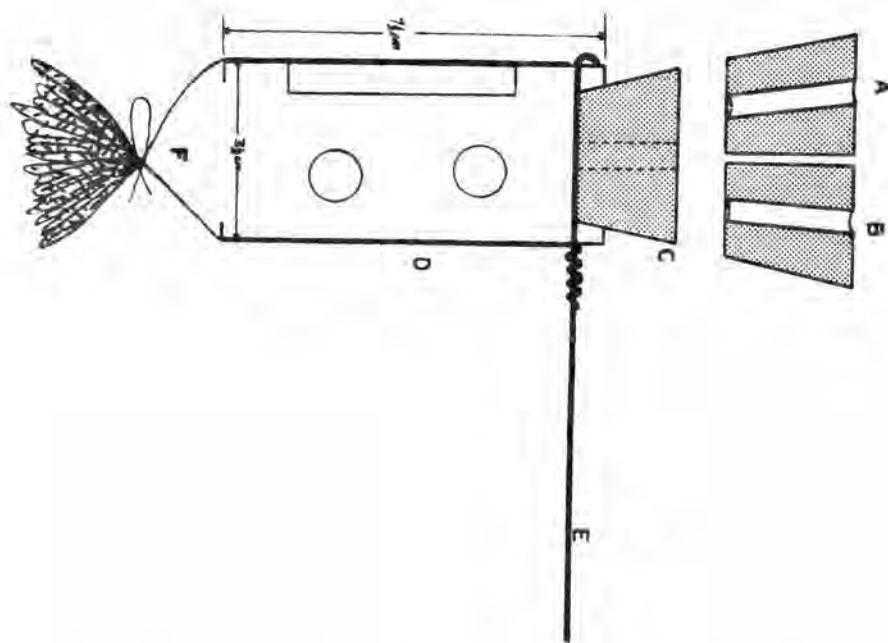


Fig. 31. A cage used for confining *M. testulalis* larvae on leaves of cowpea plant when studying their growth and development. A, B = Cork; D = Cage; E = a wire clamp stand; F = sleeve of the cage.

7.2.2. Growth and development on freshly excised stems,
flowers and pods

Development on stems, flowers and pods were studied separately in test tubes (2cm diameter; 14.5cm long).

Freshly excised segments (6cm long) were collected from the top youngest 3 internodes of each cultivar and kept singly in a test tube.

Newly opened flowers were similarly kept in the test tube. Initially one tube contained one flower but as the larvae grew the number was increased to two flowers per tube.

Eight to ten-day-old pods were trimmed to 6cm and also kept singly in the test tubes.

In all cases each test tube contained one larva and was covered with dry cotton wool. The experiment was replicated 5 times in a completely randomized block design with 10 larvae per replicate. The test tubes which were mounted on test tube holders were kept in the shelf under darkness by covering with black cloth. Food was changed every second day.

7.2.3 Growth and development on pericarp and seed

The green pods were further split into pericarp and seeds which were given separately to the larvae. A split plot

experimental design was used. The pericarp and seeds served as the sub-plots while the three cultivars served as the main plots. There were five replications for each treatment with ten larvae per replicate. The parameters measured were the same as described before.

7.2.4. Growth and development on artificial diet
containing stem and pod powder

The stem and pod powder of the three cultivars and the diet were prepared as described in Chapter 6, (paragraphs 6.2.7.1 and 6.2.7.2). The standard artificial diet for M. testulalis (Ochieng and Bungu, 1983) presented in Table 8 served as the control.

The diet was cut into discs using a cork borer size No.5 and placed in small corning glass test tubes (1 cm inner diameter and 7 cm long). Newly hatched 1st instar larvae were singly introduced into the tubes containing the diet and immediately closed tightly with a sterile dry cotton wool. A total of 100 larvae in four replicates of 25 larvae each for each cultivar (treatment) were tested in a completely randomized block design. The tubes were kept under darkness in shelves covered with black cloth in the laboratory. Room temperature was 26-29°C. The parameters measured were the same as for growth and development on leaves, stems, flowers and pods described above.

7.3. Results

7.3.1 Growth and development on different parts of cowpea plant

Percent larval survival and development. For all the three cultivars (VITA 1, VITA 5 and Tvu 946) flowers were most suitable for larval development, followed by pods and stems (Table 14). Development on leaves was the lowest.

When larvae were reared on stems, there was a very high mortality within the first two larval instars (> 60%) leading to a very low survival to 3rd instar in all the three cultivars. However, mortality on Tvu 946 was the highest ($P=0.01$) compared to that on VITA 1 and VITA 5 (Table 14). As a result of high mortality, the number of larvae which developed to 3rd instar on Tvu 946 stems was only about one half that on VITA 1 and VITA 5. From 3rd instar to pupation, mortality was low on VITA 1 and Tvu 946 but very high on VITA 5, which led to a significantly lower percentage of larvae developing to pupal stage than on VITA 1. Despite the lower larval mortality between 3rd instar and pupal stage on Tvu 946 stems, the high mortality in the 1st and 2nd instar larval stages resulted in a significantly lower percentage of pupation on Tvu 946 stems than on VITA 1 (Table 13). On all the three cultivars, mortality between pupal and adult stage was very low. However, adult emergence was significantly

lower on VITA 5 and Tvu 946 stems than on VITA 1 stems (Table 14).

When the larvae were reared on leaves, mortality was very high within the first two larval instars (74-84%) in all the three cultivars. The same trend continued between 3rd instar and pupation. This led to a very low survival on leaves and only less than 10% pupation occurred on leaves. Adult emergence was less than 5%. There were no significant differences among the three cultivars at 3rd instar stage and both pupa and adult emergence (Table 13).

There were no significant differences among the cultivars when the larvae were reared on flowers (Table 14 and Appendix 51). Although mortality was high in the first two instars (38-48%) percent survival to third instar was very high compared to that on other plant parts. In flowers mortality between 3rd instar and adult emergence was very low in all the tested cultivars.

On pods also mortality was very high within the first two instars, but it was highest ($P=0.01$) on Tvu 946 compared to VITA 1 and VITA 5. As a result the number of larvae which survived on Tvu 946 upto 3rd instar and pupation was less than half that on VITA 1 and VITA 5 (Table 13). In all the cultivars mortality between 3rd larval instar stage upto adult emergence was low. However, survival on Tvu 946 pods remained lowest ($P=0.05$) compared to VITA 1 and VITA 5. There were no significant differences between VITA 1 and VITA 5.

Table 13. Growth and Development of *M. testulalis*
Larvae on certain cowpea cultivars

Plant part	Cultivar	% (S.E.) Larvae developing to various stages ^{1/}		
		3rd Instar	Pupa	Adult
Stem	VITA 1	34.29 + 3.7 a	23.76 + 2.4 a	16.00 + 2.4 a
	VITA 5	36.82 + 2.5 a	7.13 + 2.0 b	6.00 + 2.0 b
	Tvu 946	19.80 + 4.0 b	6.00 + 4.0 b	6.00 + 4.0 b
Leaves	VITA 1	16.00 + 2.5 a	4.00 + 2.4 a	2.00 + 2.0 a
	VITA 5	16.00 + 6.0 a	6.00 + 2.4 a	4.00 + 2.4 a
	Tvu 946	26.00 + 6.8 a	2.00 + 2.0 a	0.00 + 0 a
Flowers	VITA 1	62.50 + 3.7 a	50.00 + 4.5 a	42.00 + 5.0 a
	VITA 5	62.33 + 3.4 a	54.00 + 5.1 a	42.00 + 4.0 a
	Tvu 946	52.00 + 2.0 a	48.00 + 3.7 a	44.00 + 2.0 a
Pods	VITA 1	40.37 + 3.2 a	28.46 + 3.0 a	18.40 + 3.3 a
	VITA 5	41.42 + 5.8 a	25.48 + 5.7 a	13.38 + 5.1 a
	Tvu 946	14.39 + 8.3 b	12.29 + 3.0 b	4.75 + 2.4 b

^{1/}Average of 50 larvae tested in 5 replications of 10 larvae each
In a column, means followed by a common letter are not significantly different at p=0.05 by Duncan's Multiple Range Test

When the pods were split into pericarp and seeds and the larvae reared on them from 3rd instar to pupation, there were no significant differences among the cultivars as such. However, there was an interaction between the cultivar and the part of the pod (pericarp or seed) on which the larvae were reared ($P=0.05$). This indicated that the effect of cultivar on larval development depended on whether the larvae were reared on pericarp or seed. Thus, when the larvae were reared on pericarp, VITA 1 was more suitable for larval development than VITA 5 and Tvu 946. But, when the larvae were reared on seeds, VITA 5 was better for larval development than VITA 1 and Tvu 946 (Fig. 32).

When comparison was done between larval development on pericarp and seeds of the same cultivar, significantly more larvae developed to pupa on pericarp than on seed in VITA 1. There were no significant differences in this respect on Tvu 946 and VITA 5.

Pupal weights. The pupae which emerged from flowers and pods were heavier than those from stems and leaves (Table 14). However, the number of pupae which emerged from leaves was very low (3) and therefore may not offer a very reliable basis for comparison with other parts of the plant.

On stems and pods, the pupae which emerged from VITA 1 were significantly heavier than those which emerged from VITA 5 and Tvu 946 (Table 14).

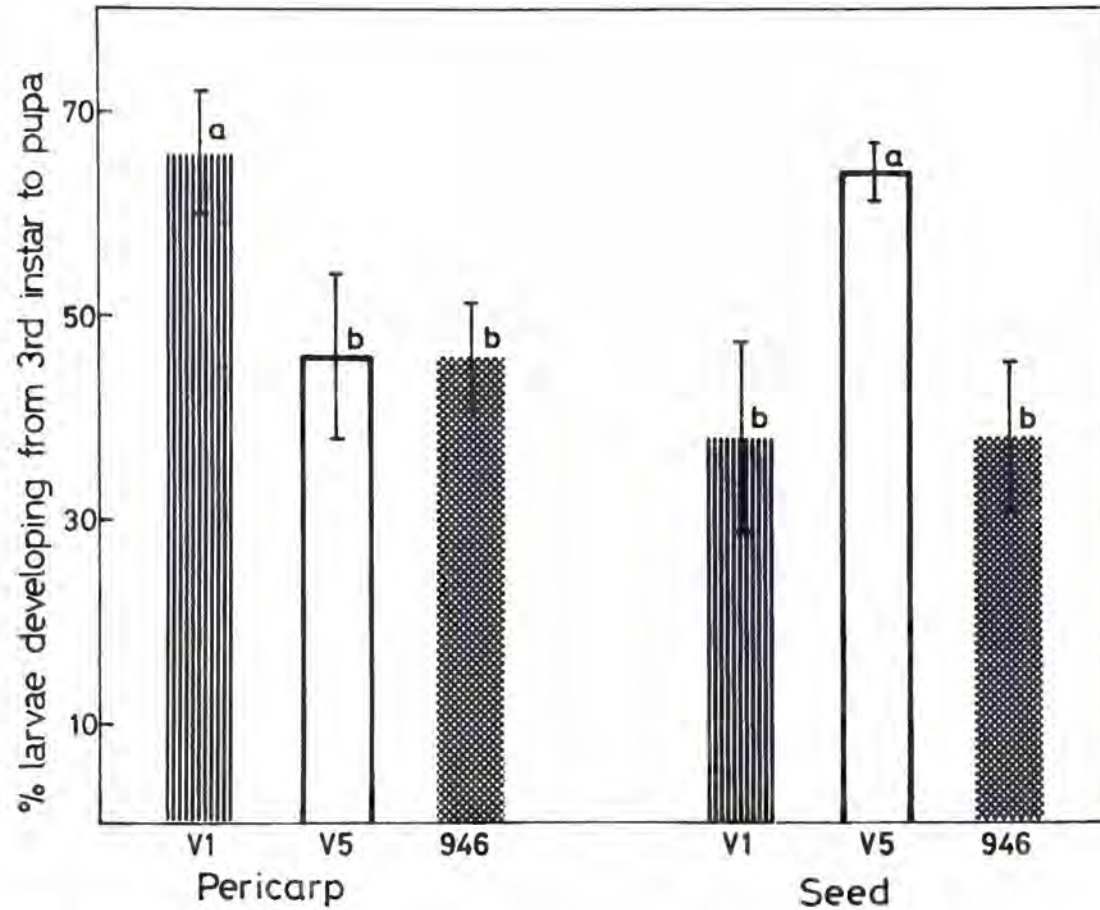


Fig. 32. Growth and development of *Maruca testulalis* larvae from 3rd instar to pupation on pericarp and seeds of certain cowpea cultivars. V1 = VITA 1; V5 = VITA 5; 946 = TVu 946. Within each plant part, columns within a common letter are not significantly different at $P = 0.05$ by DMRT.

Table 14. Mean pupal weights (mg) of M. testulalis when larvae are reared on different parts of certain cowpea cultivars in the Laboratory

Cultivar	Mean pupal wt. (mg. \pm s.e.) on different parts of cowpea plant <u>1/</u>			
	Stem	Leaves	Flowers	Pods
VITA 1	36.60 \pm 2.4a (n = 12)	35.00 \pm 0.3 (n = 2)	47.4 \pm 2.0a (n = 25)	51.73 \pm 2.7a (n = 14)
VITA 5	24.00 \pm 2.1b (n = 4)	35.00 \pm 0.1 (n = 3)	43.6 \pm 0.8a (n = 27)	42.55 \pm 2.2b (n = 13)
Tvu 946	19.52 \pm 0.6b (n = 3)	34.00 (n = 1)	43.8 \pm 1.6a (n = 24)	42/69 \pm 1.6b (n = 6)

(n=) is the number of pupae obtained from 50 larvae reared on the specified plant part and tested.

1/ In a column, means followed by a common letter are not significantly different at $p = 0.05$ by Duncan's Multiple Range Test (DMRT)

There were no significant differences in weights of the pupae from flowers of the three cultivars (Table 14).

Mean larval period (days). On VITA 1, larval period upto pupation and adult emergence increased in the order: flower pods leaves stems. On VITA 5 and Tvu 946, the larval period on flowers and pods was more or less identical but shorter than on leaves and stems. However, the number which developed to pupa and adult on leaves was very low, and, therefore reliable comparisons were only made between the stems, flowers and pods (Table 15).

When the larvae were reared on stems, larval period upto adult emergence was shorter on VITA 1 than on VITA 5 and Tvu 946 (Table 15).

On flowers, also larval period from 1st instar upto adult stage on VITA 1, VITA 5 and Tvu 946 was more or less similar.

When the larvae were reared on pods, larval period was similar on the three cultivars (Table 15).

7.3.2 Growth and development on artificial diet containing stem and pod powder

Percent larval survival and development. When stem powder was incorporated into the artificial diet of M. testulalis as

Table 15. Mean Larval period (days) of Maruca testulalis on different parts of certain cowpea cultivars in the Laboratory

Parts of Cowpea Plant	Mean larval period (days) \pm s.e.					
	Pupa			Adult		
	VITA 1	VITA 5	Tvu 946	VITA 1	VITA 5	Tvu 946
Stem	18.00 \pm 1 (n=12)	21.00 \pm 7.1 (n=4)	19.30 \pm 1.3 (n=8)	28.42 \pm 1.1 (n=3)	30.75 \pm 1.8 (n=3)	30.67 \pm 1.2
Leaves	16.67 \pm 0.7 (n=2)	21.33 \pm 0.7 (2=3)	18.00 (n=1)	27.33 (n=1)	27/67 \pm 0.9 (n=2)	-
Flowers	11.60 \pm 0.7 (n=25)	13.7 \pm 0.5 (n=27)	13.00 \pm 0.8 (n=24)	19.82 \pm 0.9 (n=21)	21.35 \pm 0.5 (n=21)	20.46 \pm 0.7 (N=22)
Pods	13.00 \pm 0.4 (n=14)	12.75 \pm 0.4 (n=13)	13.20 \pm 0.8 (n=6)	21.00 \pm 0.9 (n=9)	21.71 \pm 0.9 (n=7)	21.40 \pm 1.2 (n=3)

(n=) is the number of insects obtained from larvae reared on the specified plant part and tested.

a substitute for flower powder, percentage of larvae developing to 3rd instar and pupation on the diets containing VITA 1 and Tvu 946 was statistically as much as that on the standard diet (control). However, between pupation and adult emergence, mortality was higher on diets containing VITA 1 or Tvu 946 powder leading to a significantly lower ($P=0.05$) adult emergence on these diets, than on the standard diet (Table 16).

On incorporating pod powder in the diet, percentage of larvae completing development was as high as that on the control regardless of whether the pods were from the resistant cultivars VITA 5 and Tvu 946 or from the susceptible cultivar VITA 1 (Table 16).

Pupal weights. On the diets containing stem powder or pod powder, there were no significant differences among the cultivars including the control (flower powder diet) when the weights of the emerging pupae were compared (Table 17).

Larval period. The larvae took a shorter time to develop from 1st instar to pupa (about 14 days) on the diet containing stem powder than that with pod powder of VITA 1 or Tvu 946 (16 days). But, development from pupation to adult emergence was longer on the stem powder diet (about 10 days) than to that on the pod powder diet (6 days) (Table 18). As a result, the developmental period from 1st instar to adult emergence was shorter by 1-2 days on the pod powder diets than on stem powder diets.

Table 16. Growth and development of *M. testulalis* larvae on artificial diet containing stem and pod powder of certain cowpea cultivars

Cultivar	% (\pm s.e.) larvae surviving to ^{1/}		
	3rd instar	Pupa	Adult
Stem powder diet			
VITA 1	82.0 \pm 2.6 a	57.0 \pm 5.3 a	33.0 \pm 3.4 b
VITA 5	25.0 \pm 3.4 b	23.0 \pm 3.0 b	13.0 \pm 3.4 c
Tvu 946	82.0 \pm 7.4 a	52.0 \pm 3.7 a	22.0 \pm 3.5bc
Control*	81.5 \pm 5.6 a	67.0 \pm 4.8 a	54.0 \pm 5.0 a
Pod powder diet			
VITA 1	83.0 \pm 5.6ab	70.0 \pm 9.6 a	43.5 \pm 3.9 a
VITA 5	74.0 \pm 3.5 b	61.0 \pm 3.9 b	43.0 \pm 3.8 a
Tvu 946	91.0 \pm 1.9 a	72.0 \pm 4.9 a	40.0 \pm 8.0 a
Control*	72.0 \pm 4.3 b	68.0 \pm 4.9 a	48.0 \pm 1.1 a

^{1/}Mean of 100 larvae in 4 replications of 25 larvae each.

In a column, means followed by a common letter are not significantly different at $p=0.05$ by DMRT.

* Original diet by Ochieng and Bungu (1983) containing cowpea flower powder.

Table 17. Weight of M. testulalis larvae emerging from artificial diet containing stem and pod powder of certain cowpea cultivars

Diet	Mean pupal weight (mg) (+ s.e.) ^{1/}	
	Stem powder diet	pod powder diet
VITA 1	51.7 ± 1.0 a (n=57)	49.1 ± 1.7 a (n=70)
VITA 5	48.7 ± 1.3 a (n=23)	47.8 ± 0.9 a (n=61)
Tvu 946	51.2 ± 1.7 a (n=52)	47.8 ± 2.3 a (n=72)
Control	49.7 ± 3.1 a (n=67)	48.9 ± 1.1 a (n=68)

^{1/}In a column, means followed by a common letter are not significantly different at p=0.05 by DMRT.

(n=) is the number of pupae which were weighed

Table 18. Mean larval period (days) of Maruca testulalis reared on artificial diet containing stem and pod powder of certain cowpea cultivars.

Diet	Mean larval period (Days) to		
	3rd instar	Pupa	Adult
<hr/>			
Stem powder			
VITA 1	7.8 ± 0.2	13.7 ± 0.3	23.8 ± 0.4
VITA 5	12.2 ± 0.6	18.1 ± 0.6	27.6 ± 0.6
Tvu 946	7.4 ± 0.3	13.5 ± 0.3	23.8 ± 0.2
Control	9.8 ± 0.5	15.4 ± 0.6	22.3 ± 3.3
Pod powder			
VITA 1	9.9 ± 0.4	16.4 ± 0.5	22.6 ± 0.3
VITA 5	9.3 ± 0.4	15.0 ± 0.5	21.2 ± 0.1
Tvu 946	9.5 ± 0.3	15.6 ± 0.3	21.7 ± 0.1
Control	9.0 ± 0.4	15.2 ± 0.2	21.7 ± 0.3

On VITA 5 the differences in the developmental period on stem and pod powder diets were even wider (Table 18). The period upto pupation and adult emergence was shorter on pod powder diet than on the stem powder diet by 3 and 6 days respectively.

7.4 Discussion

7.4.1 Larval growth and development on different plant parts

Larval survival. The above results show that on all the cultivars and in all the parts of the cowpea plant, highest mortality was recorded within the first two larval instars. However, during these stages, larval survival was highest when they were fed on flowers and lowest when they were fed on leaves. This suggests that the flowers besides being highly suitable for feeding by the larvae, are also nutritionally suitable for the larvae at the beginning of their establishment on the cowpea plant. If the larvae cannot find flowers after their emergence, the population of M. testulalis will be low on the cowpea plant due to a higher larval mortality on the vegetative parts. According to literature (Taylor, 1967, 1978; Singh and Allen, 1979; Jackai, 1981b, 1982 and Okeyo-Owuor and Ochieng, 1981) peak M. testulalis larval population on the cowpea plant is recorded at peak flowering time. However, the insect infests cowpea as early as 20 days after germination (Jackai, 1981b). Very high larval mortality on the leaves may partly be one of the reasons for the very low larval population on the cowpea at preflowering stage.

The cowpea leaves can attract M. testulalis larvae (chapter 5) but can only marginally support their growth and development. The very young larvae (1st instar) were unable to settle and feed on stems and pods unless these parts were cut and opened or macerated. When the larvae were mechanically aided to settle on these parts as stated above, they fed, grew and developed better than when they were confined on the leaves. In the natural state, the epidermis of the stem and pods are usually intact and the 1st instar larvae cannot bore into the softer tissues. Their feeding is therefore usually restricted to the terminal shoots which is composed mainly of very young tender leaves.

In the absence of flowers, the survival growth and development of M. testulalis were affected by the cultivar on which they were fed. VITA 1 was more suitable than VITA 5 and Tvu 946 when the larvae were confined on the stems. Also, when confined to pods, survival of the larvae on Tvu 946 was lower than on VITA 1. The larvae will not find flowers in the field at preflowering stage so that their population will be lower on Tvu 946 at this stage. Flowering duration of Tvu 946 is usually very short (about 12 days) (Tayo, 1986, unpublished) compared to that of VITA 1 (19 days). Thus, the larvae have a very limited duration within which they can find flowers on Tvu 946. Hence, the population of M. testulalis on Tvu 946 will remain lower than than on VITA 1.

Mean pupal weights. Apart from higher survival rate of the larvae on flowers and pods of the cowpea plant, the weight of

the pupae emerging from these plant parts was also higher than those from stems and leaves. This indicates that growth of the larvae was better on flowers and pods than on stems and leaves. The cultivar on which the larvae were reared also affected their growth (as indicated by their weights) when they were confined to stems and pods. The weight of pupae on stems and pods was lower on Tvu 946 and VITA 5 than that on VITA 1. The observations provide an indication that antibiosis is one of the mechanisms of resistance in stems and pods of Tvu 946 and VITA 5.

It seems that the larvae survive and grow better when they feed on both the pericarp and the seed. As can be observed in VITA 1, feeding was evenly spread on both the pericarp and the seeds. Larval survival was also better on the pericarp than on seeds when they were reared on pods of VITA 1. In Tvu 946 and VITA 5, the larvae concentrated their feeding on seeds. Therefore, they may have missed any additional nutrients which may be available in the pericarp but are not present in the seed.

The data obtained in chapter 6 may be used to provide some of the explanations for the conditions which may have led to a poorer larval growth on the stems and pods of Tvu 946 and VITA 5 than that on VITA 1:

- (i) When the larvae were confined to stems of Tvu 946, consumption of the food was very little. As a result, most of the ingested food may have been

converted for energy use rather than for growth. (Waldbauer (1968) and SooHoo and Fraenkel (1966) stated that there is a minimum amount of nutrient required by the insects to provide for energy. The insect therefore has to eat food in excess of this requirement before growth can take place. Although consumption of VITA 5 stems was high, conversion of the ingested food material into body tissue was less efficient compared to VITA 1. Therefore the rate of weight gain on VITA 5 was low.

- (ii) When the larvae were confined to pods of Tvu 946, consumption was similar to that on VITA 1, but digestibility of Tvu 946 pods was lower than that of VITA 1. Therefore the larvae may have been unable to obtain the maximum nutritional requirements from Tvu 946 pods as they had from VITA 1. When the larvae were confined on pods of VITA 5, they ate more than they did on VITA 1 but the conversion of the ingested food was very low.

Larval period. The larvae took longer to develop into pupa and adult when they were fed on leaves and stems than when they were fed on flowers and pods. This indicated that the rate of development was faster on flowers and pods than on stems and leaves. There are two main disadvantages when the larvae take too long to become adults:

- (i) The number of generations in a given season or year will be few.

- (ii) The chances of encountering the natural enemies are increased. Although M. testulalis larvae bore into stem or pods normally they do not confine their feeding to one hole. They do move out to feed on other pods also. Although M. testulalis can grow and complete their life cycle on stems and leaves of the cowpea plant, high larval mortality coupled with longer larval period and very low adult fecundity on these parts are an indication of their unsuitability for M. testulalis.

7.4.2 Larval growth and development on artificial diet containing stem and pod powder

The data (paragraph 7.3.1) revealed that all the aerial parts of the cowpea plant can support growth and development of M. testulalis larvae. However, suitability of these plant parts for larval growth was not similar. Flowers were the best followed by pods. Stems and leaves were the poorest. There were also differences among the cultivars when the larvae were reared on excised stems and pods of the resistant Tvu 946, and the susceptible VITA 1.

Incorporation of plant powder or extracts in an artificial diet is one of the methods of studying feeding behaviour (Thorsteison, 1958; Nayar and Faenke, 1966, SooHoo and Fraenkel, 1966). Reese and Beck (1976) used the same method for testing the effect of some allelochemicals on larval growth, development and utilisation of food by black cutworm, Agrotis ipsilon.

In this study it was observed that when cowpea stem powder was incorporated in the artificial diet for M. testulalis, larval survival growth and development upto pupation was similar with the control which contained cowpea flower. However, adult emergence from the diet containing stem powder was statistically lower ($P=0.05$) than that from the diet containing flower powder. This suggests that cowpea stems in general may not be nutritionally suitable for M. testulalis food compared to flowers or there may be a toxin in the stem which has a chronic effect on M. testulalis larvae.

There were no differences in growth and development of larvae in the diets containing stem powder of VITA 1 and Tvu 946 yet larval development was poorer on fresh Tvu 946 stems than on VITA 1. This could be due to one or both of the following reasons:

- (1) The toxic or antifeedant effect of the freshly excised Tvu 946 stems may have been destroyed by drying.

- (2) A chemical feeding deterrent in Tvu 946 reported by Otieno et al. (1985) may have become diluted in the diet.

Results in Chapter 6 indicated that digestibility of Tvu 946 pods was lower than that of VITA 1. This may have contributed to the lower development of larvae on Tvu 946 pods than on VITA 1 as shown above. However, data in this chapter show that when the pods of VITA 1 and Tvu 946 were ground into powder and incorporated in an artificial diet, the larvae fed and developed equally on both the diets. This suggests that grinding Tvu 946 pods improved its digestibility. It can also be argued that if grinding of a food improves its digestibility then the poor digestibility of that particular food is due to biophysical or mechanical rather than chemical.

Generally when the larvae were reared on freshly excised pods mortality was higher in the 1st and 2nd larval instars. This led to a lower larval development on pods compared to flowers. However, when the pods were ground into powder and incorporated in the diet larval development was as good as that in the diet containing flower powder. Normally it is the older instars (3rd - 5th) larvae which bores into the cowpea pods (Taylor, 1967, 1978) because they have strong mandibles. When studying development on freshly excised pods, the larvae were confined on the pods immediately after egg hatch. At this stage they had not developed stronger mandibles to enable them to feed effectively on the pods.

Therefore when the pods were ground into powder and incorporated in the diet, the larvae may have been able to feed more effectively immediately after egg hatch. Their growth was therefore improved as a result of improved feeding.

CHAPTER 8

S U M M A R Y

1. Resistance/susceptibility levels of ten cowpea cultivars to M. testulalis were compared. These included VITA 1, VITA 3, VITA 5, Tvu 1, Tvu 946, Kamboinse local, Chola local, Tvx 3890-10F, Machakos 68 and Ex-Luanda (ICV6). Comparison was based on percent damage to (i) stem branches, (ii) stem tunnelling, (III) flowers and (iv) pods.
2. The results showed that flowers and pods had much more damage than stems.
3. The tested cultivars could be categorized into four groups:
 - i) Those cultivars in which the values for all the 4 damage parameters were high ($p=0.05$) and therefore can be regarded as the most susceptible among the tested cultivars. These include VITA 1 and VITA 3.
 - ii) Those cultivars in which all the values for all the damage parameters were low ($p=0.05$) and therefore can be regarded as the most resistant. Only Tvu 946 can be included in this group.
 - iii) The third group would include those cultivars which were similar to VITA 1 in one of the damage parameters

and to Tvu 946 in the remaining parameters. These cultivars were considered as moderately resistant and included VITA 5, Kamboinse local, Chola local and Tvu 1.

iv) The fourth group would include those cultivars which were similar to VITA 1 in most of the damage parameters (3 out of 4). Those cultivars were considered as moderately susceptible and included Ex-Luanda (ICV6), Machakos 68 and TVX 3890-10F.

4. Chola local has been reported for the first time to be resistant, though moderately so. It is a wild line which closely resembles Tvu 946 in its growth habit. It has thin hard stems and narrow leaves. The peduncles are very long and carry the pods above the canopy. However, Chola local is a landrace cultivar which still lacks uniformity in its growth habits.
5. Stem tunnelling may not be a very important parameter for evaluating cowpea for resistance to M. testulalis since in a majority of cases the upper section of the stem above the larval entry hole was found to wither off regardless of the depth of the tunnel.
6. Colonizing responses of M. testulalis namely oviposition, larval orientation, feeding, utilization of ingested food, larval development and adult fecundity were studied in

three cultivars. The cultivars were VITA 1 (susceptible), VITA 5 (moderately resistant) and Tvu 946 (resistant). Selection of these cultivars was based on the consistency of their resistance/susceptibility.

7. M. testulalis females were found to show ovipositional nonpreference for Tvu 946 and VITA 5 when these cultivars were available as a choice against VITA 1. But when each cultivar was available alone, both the resistant (Tvu 946 and VITA 5) and the susceptible (VITA 1) cultivars were equally oviposited upon.
8. A majority of eggs were deposited on the leaves. Settling site for first instar larvae on the plant was in the order: flowers > terminal shoots = flower buds.
9. First instar larvae were very mobile and were capable of reaching any part of the cowpea plant either by walking or by means of threads which they produced and used for swinging from one plant part to another. However, 4th instar larvae were not very efficient in climbing vertical objects since a majority of them lost grip and dropped down when climbing up the peduncles of the cowpea plant, especially those of Tvu 946, which were very long and erect.
10. Attraction, and arrest/stay of first instar M. testulalis larvae leading to their settling on the resistant Tvu 946

and VITA 5 was lower than on VITA 1 ($P=0.05$). As a result their emigration from these resistant cultivars was higher than that from the susceptible VITA 1.

11. The volatiles, serving as olfactory stimuli, from the susceptible VITA 1 leaves and flowers were more attractive to M. testulalis larvae than those from Tvu 946 and VITA 5.
12. Chloroform and n-hexane extracts from the cowpea leaves, flowers and pods were attractive to M. testulalis larvae when impregnated on a piece of muslin cloth. However, the attractancy of the extracts from VITA 1 was higher than that from the resistant Tvu 946 and VITA 5.
13. The tests on the role of green colour (cowpea leaf kept behind a glass barrier) and humidity (muslin cloth soaked in water) in larval orientation revealed that the larvae were attracted to the humidity source, but showed no response to the green colour.
14. The speed of the larvae during their locomotor movement and the rate of their arrival on cowpea plants was higher on a moist ground surface than on a dry surface.
15. Pods of the cowpea plant (Tvu 946 cultivar) which were raised above the canopy were less infested by M. testulalis larvae than those which were inside the canopy when the plants were artificially infested with 4th instar larvae.

16. The larvae preferred to feed on VITA 1 plant parts when they were offered simultaneously for feeding in a free choice situation with those of Tvu 946 and VITA 5. Feeding duration on leaves, stems and pods of Tvu 946 was also shorter than that on VITA 1. However, quantity of ingested food and feeding duration on flowers was similar for all the three cultivars. This indicates that apart from orientational nonpreference for Tvu 946, there is also nonpreference for feeding which is manifested in the stems, leaves and pods.

17. The sugars glucose, fructose and sucrose were phagostimulatory to M. testulalis larvae. However, whole juice and methanol extracts from leaves, flowers and pods of the cowpea were more phagostimulatory than the sugars ($p=0.05$). Phagostimulatory response of the larvae to the extracts was similar for all the three cultivars.

18. In VITA 5, utilization of ingested food from both stems and pods was lower ($p=0.05$) than that of food from VITA 1 despite a higher consumption of VITA 5. This indicated that antibiosis was also involved in the resistance of VITA 5 to M. testulalis. In Tvu 946, utilization efficiency of food from stem was similar to that of VITA 1 but Tvu 946 was consumed in very low quantities. Consumption of Tvu 946 and VITA 1 pods was similar but digestibility of Tvu 946 was very low. This also suggested that antibiosis was partly involved in the resistance of Tvu 946 pods.

19. On VITA 1 pods, larval feeding was found to be evenly spread on both the inner lining of the pod as well as part of the seed. On Tvu 946 and VITA 5, on the other hand feeding was concentrated on the seeds.

20. When pods were split into pericarp and seeds consumption and digestibility of Tvu 946 pericarp was lower ($p=0.05$) than that of VITA 1. Digestibility of VITA 5 pericarp was similar to that of VITA 1 but it was consumed in very low quantities. Consumption of seeds was similar in VITA 1 and Tvu 946 and was even higher in VITA 5. The data therefore tend to suggest that pods of Tvu 946 and VITA 5 are marginal foods for M. testulalis as compared to VITA 1 because: (i) in Tvu 946 and VITA 5, feeding is concentrated on the seeds instead of being evenly spread on both the inner lining of the pericarp and the seed as in the case of the susceptible VITA 1. (ii) the digestibility of Tvu 946 pericarp is lower ($p=0.05$) than that of VITA 1. This may largely influence the overall digestibility of Tvu 946 pod.

21. Larval growth and development was higher on flowers and pods than on leaves and stems of the cowpea plant. However, stems and pods of Tvu 946 and VITA 5 were less suitable for larval growth and development because larval mortality was higher and larval period was also longer in them than in VITA 1 stems and pods. Weight of the pupae were also lower ($p=0.05$) than those from VITA 1.

22. Larval growth and development on flowers was similar for all the three cultivars which is an indication of the lack of antibiosis compound in flowers.
23. Incorporation of Tvu 946 stem powder in the artificial diet slightly decreased the consumption of the diet and larval survival in it ($p=0.05$) compared with that of the standard artificial diet for M. testulalis. However, incorporation of pod powder from Tvu 946 did not affect consumption, larval survival and development on the diet. This suggested that, by grinding the Tvu 946 pod into powder, the factors which were reducing its digestibility when it was fresh were eliminated.
24. Fecundity of M. testulalis moths emerging from larvae which were reared on stems and leaves was lower than that of moths emerging from larvae which were reared on flowers and pods. There were no differences among the three cultivars in this regard.
25. Since there was no evidence of resistance in the flowers of both the resistant and susceptible cultivars, the lower damage of Tvu 946 flowers by M. testulalis in the field could be associated with larval orientational nonpreference for Tvu 946 leading to lower population on it at the time of colonization.

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APPENDICES

APPENDIX 1 Comparison of susceptibility/resistance levels of some cowpea cultivars to M. testulalis

A % stem damage

ANOVA				
Source of variation	Degrees of freedom	Sum of squares	Mean squar	F value
Cultivars	9	1674.866	186.096	3.98*
Replicates	3	218.167	73.722	1.58
Error	27	1261.846	46.735	
Total	39	3154.878		

Coefficient of variation = 40.68%

B % stem tunneling

ANOVA				
Source of variation	Degrees of freedom	Sum of squares	Mean squar	F value
Cultivars	9	1448.210	160.912	2.96*
Replicates	3	24.865	8.288	0.15
Error	27	1469.119	54.411	
Total	39	2942.193		

Coefficient of variation = 19.72%

Appendix 1 (Cont'd)

C % Flower damage

ANOVA				
Source of variation	Degrees of freedom	Sum of squares	Mean squar	F value
Cultivars	9	1178.138	130.904	2.90*
Replicates	3	302.015	100.672	2.23*
Error	27	1219.577	45.169	
Total	39	2699.729		
Coefficient of variation = 19.31%				

D % Pod damage

ANOVA				
Source of variation	Degrees of freedom	Sum of squares	Mean squar	F value
Cultivars	8	3242.560	405.320	8.45*
Replicates	3	77.814	25.938	0.54
Error	24	1151.802	47.991	
Total	35	4472.176		
Coefficient of variation = 19.56%				

APPENDIX 2. Fecundity of *M. Testulalis* reared on different parts of cowpea cultivars

NO. INSECTS TESTED	NO OF EGGS											
	VITA 1				VITA 5				Tvu 946			
	l	St	Fl	Po	l	St	Fl	Po	l	St	Fl	Po
1	43	12	128	276	12	5	173	80	57	45	221	63
2	52	49	176	98	85	27	102	101	78	60	127	130
3	12	68	105	94	53	17	123	117	14	30	141	157
4	67	51	257	122		42	95	157	11	79	204	183
5	78	53	203	184			278	184	12	101	176	94
6		52	302	133			329	102	24	21	111	145
7		112	189	143			222	293		11	94	107
8		35	183	207			107	175			72	52
9		44	184	126			112	223			87	128
10		22	182	75			124	124			134	93
11		96	112	114			109	155			307	123
12			224	301			89	153			183	
13			123	129			78	95			197	
14			324	141			321				321	
15			221	99			243				174	
16			117	79			121				128	
17			130	273			133				204	
18			291	225			126				206	
19			95				127				178	
20			174				183					
TOTAL	252	588	3703	2819	150	91	3195	1959	196	347	3265	1375
MEAN ±SE	50.4 ±11	53 ±9	186 ±15	157 ±17	50 ±21	23 ±9	160 ±17	150 ±16	35 ±11	50 ±12	172 ±15	125 ±17

l = Leaves; St = Stems; Fl = Flowers; Po = Pods

Appendix 3.

Ovipositional responses of *Maruca testulalis* females to different cowpea cultivars under no choice situation.

A. Flowering stage

Cultivar	No. of eggs laid/female in 24 hours at flowering stage								
	RI	RII	RIII	RIV	RV	RVI	RVII	RVIII	X + S.E.
VITA 1	16	13	33	18	15	18	24	30	21 + 3
VITA 5	18	17	22	15	13	16	12	25	17 + 2
Tvu 946	21	14	24	24	16	36	16	20	21 + 2

B. Preflowering stage

Cultivar	No. of eggs laid/female in 24 hours at preflowering stage								
	RI	RII	RIII	RIV	RV	RVI	RVII	RVIII	X + S.E.
VITA 1	23	32	20	29	17	25	16	27	24 + 2
VITA 5	37	31	19	18	22	19	34	23	25 + 3
VITA 946	30	33	20	24	25	25	24	12	24 + 2

ANOVA A (Flowering stage)

Source of variance	df	SS	MS	F	tabular F 0.05
Cultivar	2	476	237.79	1.64 ns	
Replicates	7	3683	526.14	3.62*	3.80
Error	13	1888	145.26		
TOTAL	22	6047	274.86		

CV = 29.65%

Appendix 4.

Ovipositional responses of Maruca testulalis to different cowpea cultivars under two choice situation

A. Preflowering stage.

Rep.	No. of eggs laid per 5 females	
	VITA 1	Tvu 946
1	32	4
2	98	3
3	53	6
4	96	53
5	58	18
6	30	26
7	110	28
Total \pm SE	477 \pm 10.34	138 \pm 10.34

$$x^2 = 185.76^{**}$$

Rep.	No. eggs laid per 5 females	
	VITA 1	VITA 5
1	54	42
2	36	27
3	142	71
4	47	18
5	57	8
6	75	23
7	61	31
Total \pm S.E.	472 \pm 12.25	220 \pm 12.25

$$x^2 = 91.04^{**}$$

Appendix 4 cont.

B. Flowering stage

Rep	No. eggs laid per 5 females	
	VITA 1	Tvu 946
1	35	9
2	80	21
3	69	11
4	267	0
5	190	103
6	42	151
7	167	20

Total \pm S.E. 850 \pm 15.6 315 \pm 15.6

$$\chi^2 = 244.8^{**}$$

Rep	No. eggs laid per 5 females	
	VITA 1	VITA 5
1	40	10
2	20	20
3	60	0
4	70	110
5	80	30
6	100	60
7	62	39

Total \pm S.E. 432 \pm 12.88 269 \pm 12.88

$$\chi^2 = \qquad\qquad\qquad 37.438^{**}$$

Appendix 5 . Percentage of Maruca testulalis eggs laid on different parts of potted cowpea plants at peak flowering stage.

Cultivar	Part of plant	Eggs (%) on various plant parts								X + S.E.
		RI	RII	RIII	RIV	RV	RVI	RVII	RVIII	
VITA 1	Stem	4.26	2.63	3.03	10.91	9.09	0.00	4.23	7.69	5.23 + 1.29
	Leaves	59.57	86.84	81.81	80.00	65.91	92.73	80.28	92.31	79.93 + 4
	Peduncles	23.40	2.63	6.06	0.00	15.91	5.45	0.00	0.00	6.68 + 3.0
	Fl. buds	10.64	7.89	1.01	3.64	9.09	1.82	5.63	0.00	4.96 + 1.4
	Flowers	2.13	0.00	7.07	1.82	0.00	0.00	8.45	0.00	2.43 + 1.2
	Pods	0.00	0.00	1.01	3.64	0.00	0.00	1.41	0.30	0.70 + 4
VITA 5	Stem	0.00	12.00	1.54	0.00	10.53	14.29	8.11	2.67	6.14 + 2
	Leaves	90.57	88.00	95.38	91.30	81.58	55.10	70.27	96.00	83.53 + 5
	Peduncles	9.43	0.00	1.54	0.00	5.26	6.12	0.00	0.00	2.79 + 1
	Fl. buds	0.00	0.00	1.54	4.35	2.63	10.20	13.51	0.00	4.03 + 1.8
	Flowers	0.00	0.00	0.00	4.35	0.00	6.12	2.70	0.00	1.65 + 9
	Pods	0.00	0.00	0.00	0.00	0.00	8.16	5.41	1.33	1.80 + 1.1
Tvu 946	Stems	3.13	0.00	21.92	13.89	14.29	5.61	0.00	6.67	8.19 + 2.8
	Leaves	51.56	88.37	43.83	62.25	65.31	81.31	91.67	80.00	70.54 + 6
	Peduncles	26.56	4.65	0.00	11.11	0.00	0.90	0.00	3.33	5.82 + 3
	Fl. buds	4.69	0.00	0.00	0.00	18.37	2.80	0.00	0.00	3.23 + 2.3
	Flowers	6.25	0.00	34.25	12.50	2.04	0.00	8.33	8.33	8.96 + 4
	Pods	7.81	6.98	0.00	0.00	0.00	9.35	0.00	1.67	3.23 + 1.4

Appendix 5 cont.

ANOVA

Source of variance	d.f.	SS	MS	f
Replicates	7	124.83	17.83	0.24 ^{ns}
Cultivars	2	11.18	5.59	0.07 ^{ns}
Plant parts	5	60713.70	12142.74	161.56 ^{**}
Cultivars X plant parts	10	1222.43	122.24	1.63 ^{ns}
Error	119	8943.85	75.16	

Appendix 6: Oviposition responses of *Maruca testulalis* female to excised different parts of cowpea in the laboratory.

Cultivar	Part of plant	Eggs (%) on various plant parts								X ± S.E.
		RI	RII	RIII	RIV	RV	RVI	RVII	RVIII	
VITA 1	Stem	10.53	33.33	0.00	0.00	0.00	3.57	0.00	8.10	6.94 ± 4
	Leaves	63.15	33.33	0.00	100.00	100.00	96.43	40.91	50.43	60.53 ± 13
	Peduncles	10.53	0.00	90.00	0.00	0.00	0.00	18.18	22.27	17.68 ± 11
	Fl. buds	0.00	11.11	2.50	0.00	0.00	0.00	27.27	6.49	5.92 ± 3
	Flowers	0.00	11.11	2.50	0.00	0.00	0.00	9.09	4.86	3.44 ± 4
	Pods	15.79	11.11	5.00	0.00	0.00	0.00	4.54	5.40	5.23 ± 6
VITA 5	Stem	0.00	0.00	4.88	0.00	0.00	1.60	1.00	0.00	0.94 ± 0.6
	Leaves	45.45	16.13	68.29	100.00	0.00	42.40	45.97	0.00	39.78 ± 12
	Peduncles	21.21	58.06	12.19	0.00	100.00	35.20	38.29	75.00	42.49 ± 12
	Fl. buds	0.00	19.35	9.76	0.00	0.00	8.80	5.82	25.00	8.59 ± 3.3
	Flowers	21.21	6.45	0.00	0.00	0.00	7.20	5.53	0.00	5.05 ± 3
	Pods	12.12	0.00	4.88	0.00	0.00	4.80	3.40	0.00	3.15 ± 1.5
Tvu 946	Stem	70.59	12.12	23.40	0.00	14.29	50.00	24.24	42.31	29.62 ± 8
	Leaves	14.71	45.45	0.00	0.00	21.42	50.00	0.00	0.00	16.45 ± 7
	Peduncles	5.89	6.06	0.00	7.69	14.29	0.00	45.45	42.31	15.21 ± 6.5
	Fl buds	0.00	3.03	72.34	0.00	0.00	0.00	0.00	0.00	9.42 ± 9.0
	Flowers	0.00	0.00	0.00	92.31	7.14	0.00	15.15	0.00	14.33 ± 11
	Pods	8.81	3.33	4.25	0.00	42.86	0.00	15.15	15.38	14.97 ± 5.54

Appendix 6 cont.

ANOVA

Source of variance	d.f.	SS	MS	f
Replicate	7	380.27	54.32	0.15 ^{ns}
Cultivars	2	13.52	6.76	0.02 ^{ns}
Plant parts	5	12183.04	2436.61	6.70*
Cultivar X Plant part	10	11961.79	1196.18	3.29*
Error	119	43273.13	363.64	

Appendix 7 Rate of emigration of first instar M. testulalis larvae from certain cowpea cultivars

Cultivar	Time interval (min)	% larval departure					X + s.e.
		RI	RII	RIII	RIV	RV	
VITA 1	10	10	0	10	30	30	16 + 6.6
	20	10	20	0	0	0	6 + 4.0
	30	0	0	0	0	0	0
	40	0	10	0	0	0	2 + 2
	50	10	0	0	0	0	2 + 2
	75	0	0	0	0	0	0
VITA 5	10	40	20	10	20	10	20 + 5.5
	20	10	10	0	0	20	8 + 3.7
	30	0	0	0	10	10	4 + 2.4
	40	10	10	0	0	10	6 + 2.4
	50	0	0	0	0	0	0
	75	0	0	0	0	0	0
Twi 946	10	40	30	20	50	40	36 + 5.1
	20	10	30	0	0	0	8 + 5.8
	30	20	0	10	10	10	10 + 3.2
	40	0	0	0	0	10	2 + 2
	50	30	0	0	0	0	6 + 6
	75	0	0	10	0	0	2 + 2

ANOVA

Source of variation	Degrees of freedom	Sum of squares	Mean square	Computed F	Tabular F 5%	F 1%
Replicate	4	482.22	120.556	1.99 ns	2.50	3.60
A cultivars	2	628.89	314.444	5.19**	3.13	4.92
B time interval	5	5502.22	1100.444	18.17**	2.35	3.29
A X B	10	917.78	91.778	1.52 ^{ns}	1.97	2.59
Error	68	4117.78	60.556			

Coefficient of variation = 59.43%

** = significant at P = 0.01

ns = not significant

2

Appendix 8 . Arrest/stay of 1st instar *M. testulalis* larvae on certain cowpea cultivars at preflowering stage in the field.

Cultivar	% larvae recovered after 24 h						x <u>±</u> s.e.
	RI	RII	RIII	RIV	RV	RVI	
VITA 1	20	50	60	60	35	60	47.50 <u>±</u> 6.8
VITA 5	40	25	5	40	15	5	21.66 <u>±</u> 6.5
Tvu 946	20	35	30	20	25	30	26.67 <u>±</u> 2.5

ANOVA

Source of variation	Degrees of freedom	Sum of squares	Mean square	Computed F	Tabular 5%	F 1%
Cultivars	2	942.73	471.365	4.30*	4.10	7.56
Error	10	1097.19	109.719			
Total	17	2238.96	131.703			

Coefficient of variation = 30.80%

Analysis conducted using arcsine $\sqrt{\%}$ transformed data.

ns = not significant, * = significant at 5% level

Appendix 9 Arrest/stay of 1st instar M. testulalis larvae on certain cowpea cultivars at flowering stage in the field.

Cultivar	% larvae recovered after 24 h				
	RI	RII	RIII	RIV	$\bar{x} \pm \text{s.e.}$
VITA 1	44	54	63	42	50.75 \pm 4.85
VITA 5	10	9	13	11	10.75 \pm 0.85
Tvu 946	13	6	3	24	11.50 \pm 4.66

ANOVA

Source of variation	Degrees of freedom	Sum of squares	Mean square	Computed F	Tabular F 5%	F 1%
Replicates	3	19.19	6.396	0.13 ^{ns}	5.99	10.92
Cultivars	2	1882.26	941.130	18.97 ^{**}		
Error	6	297.72	49.620			
Total	11	2199.17				

Coefficient of variation = 25.40%

Analysis conducted using arcsine $\sqrt{\%}$ transformed data

ns = not significant, ** Significant at 1% level

* Significant at 5% level

Appendix 10 Arrest/stay of 1st instar *M. testulalis* larvae on certain cowpea cultivars at flowering stage in the laboratory

Cultivar	% Larvae recovered after 24 h				
	RI	RII	RIII	RIV	X \pm s.e.
VITA 1	59	44	50	50	50.75 \pm 3.1
VITA 5	35	45	45	25	37.50 \pm 4.8
Tvu 946	35	15	30	40	30.00 \pm 6.6

ANOVA

Source of variation	Degree of freedom	Sum of squares	Mean square	Computed F	Tabular F 5%	F 1%
Cultivars	2	883.1167	441.58	5.37*	4.26	8.02
Error	9	739.7500	82.19			
Total	11	1622.9167				

Coefficient of variation = 23.00%

* = Significant at P = 0.05

Appendix 11. Sites for 1st instar M. testulalis larval settling after their release on different parts of the cowpea plant

ANOVA

Source of variation	Degrees of freedom	Sum of squares	Mean square	Computed F	Tabular F 5%	F 1%
Replicates	3	185.70	61.90	0.43 ^{ns}		
A cultivars	2	23.35	11.67	0.08 ^{ns}		
B starting site	3	327.45	109.15	0.76 ^{ns}		
A X B	6	310.89	51.81	0.36 ^{ns}		
C settling site	5	137194.07	27438.81	190.24**	2.26	3.11
A X C	10	1691.11	169.11	1.17 ^{ns}		
B X C	15	7548.10	503.21	3.49**	1.74	2.17
Error	243	35047.66	144.23			

Coefficient of variation = 26.21%

ns = not significant; ** = significant at 1% level

Appendix 12 Attraction of first instar M. testulalis larvae by certain cowpea cultivars at preflowering stage in the laboratory under no choice situation

Cultivar	% larvae found in plant after 24 h										X ± s.e.	
	RI	RII	RIII	RIV	RV	RVI	RVII	RVIII	RIX	RX		
VITA 1	20	10	40	13	30	53	23	33	30	0	25.20	+ 4.9
VITA 5	33	10	13	10	7	10	37	20	13	16	16.90	+ 3.2
Tvu 946	7	3	20	17	3	37	33	20	10	0	15.00	+ 4.0

ANOVA

Source of variation	Degrees of freedom	Sum of squares	Mean squares	Computed F	Tabular 5%	F 1%
Replicates	9	1715.138	190.571	2.54*	2.22	3.08
Cultivars	2	237.887	118.943	1.59 ^{ns}	3.33	5.42
Error	18	1350.591	75.031			
Total	29	3303.616	113.918			

Coefficient of variation = 44.62%
 Analysis conducted using arcsine $\sqrt{\%}$ transformed data
 ns = not significant; * = significant at 5% level

Appendix 13 Attraction of 1st instar *M. testulalis* larvae by certain cowpea cultivars at preflowering stage in the field under no choice situation.

Cultivar	% larvae found on the plants after 24 h					X \pm s.e.
	RI	RII	RIII	RIV	RV	
VITA 1	15	30	25	15	5	18 \pm 4.36
VITA 5	25	0	30	15	10	16 \pm 5.34
Tvu 946	5	10	20	5	10	10 \pm 2.74

ANOVA

Source of variation	Degrees of freedom	Sum of squares	Mean squares	Computed F	Tabular 5%	F 1%
Replicates	4	8.387	2.097	1.09 ^{ns}	3.11	5.56
Cultivars	2	2.708	1.354	0.71 ^{ns}	3.74	6.51
Error	8	15.336	1.917			
Total	14	26.430	1.888			

Coefficient of variation = 38.27%
 analysis conducted using transformed data (\sqrt{v})
 ns = not significant

Appendix 14. Attraction of 1st instar *M. testulalis* larvae by certain cowpea cultivars at preflowering stage in the laboratory under free-choice situation

Cultivar	% larvae found on the plants after 24 h.					X \pm s.e.
	RI	RII	RIII	RIV	RV	
VITA 1	8	18	8	24	34	18.4 \pm 4.96
VITA 5	6	0	4	18	8	7.2 \pm 3.01
Tvu 946	0	8	4	0	8	5.0 \pm 1.91

ANOVA

Source of variation	Degrees of freedom	Sum of squares	Mean squares	Computed F	Tabular 5%	F 1%
Replicates	4	6.759	1.690	1.21 ^{ns}	3.11	3.56
Cultivars	2	14.232	7.116	5.08*	3.74	6.51
Error	8	11.215	1.402			
Total	14	32.207	2.300			

Coefficient of variation = 42.2%

Analysis conducted using transformed data ($\sqrt{\quad}$)

ns = not significant; * = significantly different at 5% level

Appendix 15 Attraction of 1st instar *M. testulalis* larvae by certain cowpea cultivars at preflowering stage in the field under free-choice situation

Cultivar	% larvae found on the plants after 24 h				
	RI	RII	RIII	RIV	X \pm s.e.
VITA 1	12	6	4	4	6.5 \pm 2.0
VITA 5	0	2	0	0	0.5 \pm 0.5
Tvu 946	2	2	2	0	1.5 \pm 0.5

ANOVA

Source of variation	Degrees of freedom	Sum of squares	Mean squares	computed F	Tabular 5%	F 1%
Replicates	3	1.011	0.337	1.76 ^{ns}	4.76	9.78
Cultivars	2	5.603	2.802	14.66 ^{**}	5.14	10.92
Error	6	1.146	0.191			
Total	11	7.760	0.705			

Coefficient of variation = 28.56%

Analysis conducted using $\sqrt{\quad}$ -transformed data

ns = not significant; ** = significantly different at 1% level.

Appendix 16. Attraction of first instar *M. testulalis* larvae by certain cowpea cultivars at flowering stage in the field under no-choice situation

Cultivar	% larvae found on plants after 24 h					
	RI	RII	RIII	RIV	RV	X + s.e.
VITA 1	10.00	3.33	6.67	6.67	6.67	6.67 ± 1.05
VITA 5	3.33	0.00	0.00	6.67	0.00	2.00 ± 1.33
Tvu 946	0.00	0.00	0.00	3.33	3.33	1.33 ± 0.82

ANOVA

Source of variation	Degrees of freedom	Sum of squares	Mean squares	Computed F	Tabular 5%	F 1%
Replicates	4	3.047	0.762	2.15 ^{ns}	3.84	7.01
Cultivars	2	6.004	3.002	8.48*	4.46	8.65
Error	8	2.832	0.354			
Total	14	11.883	0.849			

Coefficient of variation = 34.62%

Analysis conducted using $\sqrt{\quad}$ transformed data

ns = not significant, * = significant at 5% level

Appendix 17. Attraction of 1st instar *M. testulalis* larvae by certain cowpea cultivars at flowering stage in the field under free choice situation

Cultivar	% larvae found on plant after 24 h					X \pm s.e.
	RI	RII	RIII	RIV	RV	
VITA 1	12	20	35	8	28	20.6 \pm 5.0
VITA 5	6	5	17	5	15	9.2 \pm 2.5
Tvu 946	8	18	10	6	7	9.8 \pm 2.2

ANOVA

Source of variation	Degrees of freedom	Sum of squares	Mean squares	Computed F	Tabular 5%	F 1%
Replicates	4	6.780	1.695	2.91 ^{ns}	3.84	7.01
Cultivars	2	6.580	3.290	5.66*	4.46	8.65
Error	8	4.652	0.582			
Total	14	18.012	1.287			

Coefficient of variation = 21.97%

Analysis conducted using $\sqrt{\quad}$ transformed data

ns = not significant; * = significant at 5% level.

Appendix 18. Attraction of first instar M.testulalis larvae to certain cowpea cultivars in relation to distance.

Cultivar	Distance (cm)	% larvae arriving on plants					X \pm s.e.
		RI	RII	RIII	RIV		
VITA 1	10	15	25	5	15	15 \pm 4.1	
	20	5	10	0	5	5 \pm 1.6	
	30	0	0	0	0	0	
Tvu 946	10	10	10	10	0	7.5 \pm 2.5	
	20	5	0	5	0	2.5 \pm 1.4	
	30	0	0	0	0	0	

Appendix 19. Movement of 1st instar *M. testulalis* larvae from one cowpea cultivar to another grown in alternate rows in a plot.

Combination	% larvae recovered in each row after 48 h					Computed "t" value	Tabular "t"	
	RI	RII	RIII	RIV	$\bar{X} \pm \text{s.e.}$		5%	1%
VITA 1 middle row	24	30	37	22	28.25 ± 3.4	2.62 ^{ns}	2.78	4.60
VITA 1 side rows	20	24	26	20	22.50 ± 1.5			
VITA 1 middle row	20	18	16	15	17.25 ± 1.1	3.59*		
946 side rows	1	2	4	12	4.75 ± 2.5			
VITA 1 middle row	42	17	20	16	26.25 ± 6.1	2.80*		
VITA 5 side rows	3	3	6	7	4.75 ± 1.0			
946 middle row	6	3	5	9	5.75 ± 1.3	8.44**		
VITA 1 side rows	32	37	30	28	31.75 ± 1.9			
946 middle row	8	2	8	10	7.00 ± 1.7	2.61 ^{ns}		
VITA 5 side rows	0	0	5	8	3.25 ± 1.9			
946 middle row	11	4	2	13	7.50 ± 2.7	2.25 ^{ns}		
946 side rows	2	2	1	11	4.00 ± 2.3			
VITA 5 middle row	10	4	9	6	7.25 ± 1.4	1.54 ^{ns}		
VITA 5 Side rows	2	5	4	5	3.50 ± 1.2			
VITA 5 middle row	4	0	4	4	3.00 ± 1.0	3.98*		
VITA 1 side rows	53	17	47	24	35.25 ± 8.7			
VITA 5 middle row	7	2	2	8	4.75 ± 1.6	0.51 ^{ns}		
VITA 1 side rows	5	0	1	20	6.50 ± 4.6			

ANOVA

Total % larvae recovered in each combination of the cultivars.

Source of variation	Degrees of freedom	Sum of squares	Mean square	Computed F value	Tabular 5%	F 1%
Replicates	3	297.800	99.267	2.12 ^{ns}	3.01	4.72%
Combinations	8	3617.163	452.145	9.68**	2.36	3.36
Error	24	1121.318	46.722			
Total	35	5036.281	143.894			

Coefficient of variation = 24.07%

Analysis conducted using arcsine transformed data

Appendix 20 Role of moisture in determining arrival of first instar M. testulalis larvae on cowpea plants under field conditions

Observation	Distance from plant (cm)	Time taken to arrive on the plants (min)	
		Dry surface	wet surface
1	10	37	20
2	10	54	18
3	10	33	30
4	10	25	15
5	10	35	10
Average	10	36.8 \pm 4.8	18.6 \pm 3.31

Appendix 21. Speed of 1st instar M. testulalis
larvae on wet and dry surfaces

Observation	Speed (cm/min)	
	Dry surface	Wet surface
1	3.0	2.4
2	2.1	3.4
3	2.1	5.0
4	4.0	4.5
5	1.0	4.0
Average	2.44 \pm 0.50	3.86 \pm 0.45

Appendix 22 Attraction of 1st instar *M. testulalis* larvae by odour from excised leaves and flowers of certain cowpea cultivars under no choice situation

Plant Part	Cultivar	% Larvae attracted								X ± s.e.
		RI	RII	RIII	RIV	RV	RVI	RVII	RVIII	
Leaves	VITA 1	80	90	80	90	60	40	50	70	70.00 ± 6.55
	VITA 5	40	50	30	30	30	40	30	70	40.00 ± 5.00
	Tvu 946	40	60	70	40	50	40	60	60	52.50 ± 4.10
Flowers	VITA 1	80	70	70	60	60	80	70	60	68.75 ± 3.00
	VITA 5	70	60	40	60	50	60	60	50	56.25 ± 3.20
	Tvu 946	70	60	60	40	40	50	40	50	57.25 ± 4.00

ANOVA

Source of variation	Degrees of freedom	Sum of squares	Mean squares	Computed F	Tabular F 5%	F 1%
Replicates	7	566.15	80.879	1.46 ^{ns}	2.28	3.18
A. Plant parts	1	70.11	70.108	1.27 ^{ns}	4.11	7.39
B. Cultivars	2	1565.27	782.634	14.13**	3.26	5.25
A X B	2	302.42	151.208	2.73 ^{ns}	3.26	5.25
Error	35	1938.04	55.372			

Coefficient of variation = 15.19%

Analysis conducted using arcsine $\sqrt{\frac{x}{n}}$ transformed data

Appendix 23 Attraction of 1st instar *M. bestulalis* larvae by chloroform extract of leaves of certain cowpea cultivars.

Cultivar	Conc. of extract (mg/ml)	% larvae attracted						X \pm s.e.
		RI	RII	RIII	RIV	RV	RVI	
VITA 1	0	10	10	0	10	10	0	6.67 \pm 2.1
	0.10	40	50	50	60	60	50	51.67 \pm 3.1
	0.20	90	60	80	80	60	70	73.33 \pm 4.9
	0.50	70	90	80	80	80	80	80.00 \pm 2.6
VITA 5	0	10	10	10	10	10	0	8.33 \pm 1.6
	0.10	40	40	40	30	30	30	35.00 \pm 2.2
	0.20	70	60	70	50	60	70	63.33 \pm 3.3
	0.50	50	80	70	80	60	60	66.67 \pm 4.9
Tvu 946	0	10	10	10	20	10	10	11.67 \pm 1.7
	0.10	60	50	50	60	30	30	46.67 \pm 5.6
	0.20	60	60	70	60	50	40	56.67 \pm 4.2
	0.50	30	70	60	40	50	60	51.67 \pm 6.00

ANOVA

Source of variation	Degrees of freedom	Sum of squares	Mean square	Computed F value	Tabular 5%	F 1%
Replicates	5	385.06	77.013	1.92 ^{ns}	2.38	3.37
A. Cultivars	2	536.36	268.180	6.69**	3.17	5.01
B. Concentration	3	17651.32	5883.773	146.87**	2.78	4.16
A X B	6	1205.64	200.940	5.02**	2.27	3.15
Error	55	2203.35	40.061			

Coefficient of variation = 15.22%

Analysis conducted using arcsine, $\sqrt{\%}$, transformed data

Appendix 24: Attraction of 1st instar *M. testulalis* larvae by n-hexane extract from leaves of certain cowpea cultivars at varying concentrations

Cultivar	Conc. of extract mg/ml	% larvae attracted						X + s.e.
		RI	RII	RIII	RIV	RV	RVI	
VITA 1	0.00	20	20	20	20	20	30	21.67 + 1.7
	0.20	60	70	60	60	60	60	61.67 + 1.7
	0.50	40	80	80	60	70	50	63.33 + 6.7
VITA 5	0.00	30	20	10	20	20	20	20.00 + 2.6
	0.20	50	60	20	50	50	60	48.33 + 6.0
	0.50	40	30	40	20	30	50	35.00 + 4.3
Tvu 946	0.00	10	10	10	10	10	20	11.67 + 1.7
	0.20	40	40	60	60	30	30	43.33 + 5.6
	0.50	30	40	40	60	60	40	45.00 + 5.0

ANOVA

Source of variation	Degrees of freedom	Sum of square	Mean square	Computed F value	Tabular F 5%	F 1%
Replicates	5	108.92	21.784	0.49 ^{ns}	2.45	3.51
A cultivars	2	1026.10	513.052	11.63**	3.23	5.18
B concentration	2	5219.68	2609.840	59.15**	3.23	5.18
A X B	4	401.76	100.441	2.28 ^{ns}	2.61	3.83
Error	40	1764.91	44.123			

Coefficient of variation = 17.43%

Analysis conducted using arcsine $\sqrt{\%}$ transformed data.

Appendix 25. Attraction of 1st instar *M. testulalis* larvae by chloroform extract from flowers of VITA 1 and Tvu 946 cowpea cultivars at varying rates of concentration

Cultivar	Conc. of extract (mg/ml)	% larvae attracted						X \pm s.e.
		RI	RII	RIII	RIV	RV	RVI	
VITA 1	0.00	20	10	10	10	20	20	15.00 \pm 2.2
	0.10	40	40	40	40	50	50	43.33 \pm 2.1
	0.20	60	60	50	50	70	50	56.67 \pm 0.4
	0.50	40	70	40	50	50	40	48.33 \pm 4.8
Tvu 946	0.00	10	10	10	0	10	10	8.33 \pm 1.7
	0.10	30	40	20	50	40	30	35.00 \pm 4.3
	0.20	50	30	60	30	40	40	41.67 \pm 4.8
	0.50	60	50	50	50	60	60	55.00 \pm 2.2

ANOVA

Source of variation	Degrees of freedom	Sum of squares	Mean squares	Computed F value	Tabular 5%	F 1%
Replicate	5	183.72	36.743	1.22 ^{ns}	2.48	3.58
A cultivar	1	217.69	217.686	7.22**	4.11	7.39
B concentration	3	5558.17	1852.722	61.46**	2.86	4.38
A X B	3	280.66	93.555	3.10*	2.86	4.38
Error	35	1055.11	30.146			

Coefficient of variation = 14.83%

Analysis conducted using arcsine $\sqrt{\%}$ transformed data

Appendix 26. Effect of plant architecture on the arrival/arrest of first instar M. testulalis larvae on flowers of Tvu 946 cowpea cultivar.

Treatment	% infested flowers				
	RI	RII	RIII	RIV	$\bar{X} \pm \text{s.e.}$
All flowers inside the canopy	31.24	31.24	52.53	39.23	39.56 \pm 5.02
All flowers above the canopy	28.79	25.48	23.11	22.46	24.96 \pm 1.43
Control-flowers in their natural distribution in plants	29.47	26.35	33.40	28.73	29.49 \pm 1.47

ANOVA

Source of variation	Degrees of freedom	Sum of squares	Mean square	Computed F value	Tabular F 5%	F 1%
Replicate	3	124.931	41.644	1.10		
Treatments	2	283.691	191.846	5.04 ^{ns}	5.14	
Error	6	228.182	38.030			
Total	11	736.804	66.982			

Coefficient of variation = 19.89%

Appendix 27. Effect of plant architecture on the arrival/arrest of 4th instar M.testulalis larvae on pods of Tvu 946 cowpea cultivar

Treatment	% infested pods								X <u>±</u> S.E.
	RI	RII	RIII	RIV	RV	RVI	RVII	RVIII	
Pods above canopy	0	8.33	0	0	0	9.46	0	0	2.20 <u>±</u> 1.46
Pods inside canopy	19.64	13.18	11.09	18.24	16.64	16.63	19.73	13.44	16.05 <u>±</u> 1.13

ANOVA

Source of variation	Degrees of freedom	Sum of squares	Mean square	Computed F value	Tabular F 5%	F 1%
Replicates	7	76.931	10.990	0.68		
Position of pods	1	763.693	763.693	47.10**	5.59	12.25
Error	7	113.492	16.213			
Total	15	954.116	63.608			

Coefficient of variation = 44.13%

Appendix 28. Feeding responses of 1st instar *M. testulalis* larvae to leaves of the susceptible VITA 1 and the resistant Tvu 946 cowpea cultivars in a 2-choice situation

Cultivar	Time interval (h)	% larvae feeding					X ± s.e.
		RI	RII	RIII	RIV	RV	
VITA	1	60	90	80	80	70	76.00 ± 5.1
	3	60	80	80	80	70	74.00 ± 4.0
	6	60	80	80	70	80	74.00 ± 4.0
	12	60	70	70	70	80	70.00 ± 3
	24	40	50	40	60	40	46.00 ± 4
	36	40	30	50	40	30	38.00 ± 4
Tvu 946	1	40	10	20	20	20	22.00 ± 5.0
	3	40	10	10	10	20	18.00 ± 6.0
	6	40	20	10	20	20	22.00 ± 5.0
	12	40	30	10	20	30	26.00 ± 5.0
	24	40	10	50	20	50	34.00 ± 8.0
	36	20	20	20	30	30	24.00 ± 2.0

ANOVA

Source of variation	Degrees of freedom	Sum of squares	Mean square	Computed F	Tabular F	
					5%	1%
Replicates	4	82.68	20.670	0.38		
A. cultivars	1	8781.45	8781.454	162.25**	4.06	7.24
B. Time interval	5	850.47	170.095	3.14*	2.43	3.46
A X B	5	2010.30	402.060	7.43**	2.43	3.46
Error	44	2381.48	54.124			

Coefficient of variation = 17.95%

Analysis conducted using arcsine $\sqrt{\%}$ transformed data.

Appendix 29. Feeding responses of 1st instar *M. testulalis* larvae to flowers of the susceptible VITA 1 and the resistant Tvu 946 cowpea cultivars in a 2-choice situation.

Cultivar	Time interval (h)	% larvae feeding					X \pm s.e.
		RI	RII	RIII	RIV	RV	
VITA 1	1	80	40	50	50	50	56.00 \pm 7
	3	60	50	60	60	50	56.00 \pm 2
	6	50	60	60	50	70	58.00 \pm 4
	12	60	50	40	70	60	56.00 \pm 5
Tvu 946	1	20	60	50	50	40	44.00 \pm 7
	3	40	50	40	40	50	44.00 \pm 2
	6	50	40	40	50	30	42.00 \pm 4
	12	40	50	60	30	40	44.00 \pm 5

ANOVA

Source of variation	Degrees of freedom	Sum of squares	Mean square	Computed F	Tabular F 5%	F 1%
Replicates	4	0.00	0.000	0.00		
A. Cultivars	1	1690.00	1690.000	12.86**	4.20	7.60
B. Time interval	3	0.00	0.000	0.00 ^{ns}	2.95	4.57
A X B	3	30.00	10.000	0.08 ^{ns}	2.95	4.57
Error	28	3680.00	131.429			

Coefficient of variation = 22.93%

ns = not significant

Appendix 30. Feeding responses of 4th instar M. testulalis larvae to pods of VITA 1 and Tvu 946 cowpea cultivars in a 2-choice situation.

Replicate	% pods attacked		
	VITA 1	Tvu 946	Difference
1	50	30	20
2	60	30	30
3	60	30	30
4	60	10	50
5	20	30	-10
6	40	50	-10
7	40	40	0
8	50	20	30
9	40	30	10
10	50	40	10
X \pm s.e.	47.00 \pm 4.0	31.00 \pm 3.5	16.00 \pm 6.18
Tabular "t"	Computed "t" value	=	2.589*
	p = 0.05	=	2.228
	p = 0.01	=	3.169

Appendix 31. Feeding responses of 4th instar M. testulalis larvae to pods of VITA 1 and VITA 5 cowpea cultivars in a 2-choice situation.

Replicate	% pods attacked		
	VITA 1	VITA 5	Difference
1	50	40	10
2	50	20	30
3	60	20	40
4	70	20	50
5	80	0	80
6	50	10	40
7	70	10	60
8	50	20	30
9	70	10	60
10	50	0	50
$\bar{x} \pm \text{s.e.}$	60.00 ± 3.65	15.00 ± 3.73	45 ± 6.19
	Computed "t" value		= 7.270**
	Tabular "t" value P = 0.05		= 2.228
	P = 0.01		= 3.169

Appendix 32. Feeding duration of *M. testulalis* larvae (min/h) on different parts of the susceptible VITA 1 and the resistant Tvu 946 cowpea cultivar under no choice conditions.

PRE FEEDING DURATION (MIN.)

Observation	Leaves		Flowers		Pods	
	VITA 1	Tvu 946	VITA 1	Tvu 946	VITA 1	Tvu 946
1	0.2	2.8	0.4	0.3	3.2	7.3
2	1.6	16.9	0.4	1.4	9.5	1.0
3	0.3	3.8	0.3	4.3	1.5	4.7
4	0.4	5.5	3.8	8.6	4.7	2.8
5	7.3	1.4	1.4	4.7	4.0	4.0
$\bar{X} \pm \text{s.e.}$	0.6 ± 0.3	7.3 ± 3.3	1.3 ± 0.7	3.2 ± 1.5	4.7 ± 1.7	4.0 ± 1.3

CONTINUED FEEDING DURATION (MIN.)

Observation	Leaves		Flowers		Pods	
	VITA 1	Tvu 946	VITA 1	Tvu 946	VITA 1	Tvu 946
1	17.5	7.3	23.6	30.0	31.9	27.5
2	16.4	10.1	30.7	26.4	40.1	2.4
3	21.5	11.7	26.0	37.2	22.8	17.7
4	13.3	8.4	29.5	25.3	31.6	12.6
5	16.9	9.6	25.3	24.4	32.1	16.2
$\bar{X} \pm \text{s.e.}$	17.12 ± 1.3	9.42 ± 0.7	27.0 ± 1.3	28.7 ± 2.3	31.7 ± 2.7	15.28 ± 4.1

Appendix 33. Quantity of food ingested by 1st instar *M. testulalis* larvae from leaves of certain cowpea cultivars

Cultivar	Area consumed in 48 h (mm ²)										X ± s.e.
	R1	R2	R3	R4	R5	R6	R7	R8	R9	R10	
VITA 1	23.58	32.02	30.07	12.11	22.06	21.20	17.52	46.73	12.76	15.14	23.32 ± 3
VITA 5	14.93	24.10	27.04	15.36	26.18	12.33	15.54	10.60	21.85	10.16	17.51 ± 7
Tvu 946	7.57	38.94	16.66	6.27	9.73	27.91	6.92	11.46	18.82	14.71	15.90 ± 3

ANOVA

Source of variation	Degrees of freedom	Sum of squares	Mean square	Computed F	Tabular F 5%	F 1%
Replicates	9	1083.730	120.414	1.66		
Cultivars	2	304.682	152.341	2.10 ^{ns}	4.41	8.28
Error	18	1308.418	72.689			
Total	29	2696.829	92.994			

Coefficient of variation = 45.10%

Appendix 34 Quantity of food ingested by 1st instar M. testulalis larvae from flowers of certain cowpea cultivars.

Cultivar	Feeding score				X \pm s.e.
	RI	RII	RIII	RIV	
VITA 1	4.1	3.7	4.0	3.7	3.88 \pm 0.1
VITA 5	3.3	3.4	4.0	3.8	3.63 \pm 0.2
Tvu 946	3.5	3.7	3.8	3.7	3.68 \pm 0.1

Appendix 35 Quantity of food ingested by 4th instar *M. testulalis* larvae from stems of certain cowpea cultivars.

Cultivar	Quantity (mg. dry wt.) ingested										X \pm s.e.
	RI	RII	RIII	RIV	RV	RVI	RVII	RVIII	RIX	RX	
VITA 1	56	47	53	34	34	34	44	61	65	58	48.6 \pm 3.7
VITA 5	51	50	53	69	81	53	52	58	41	67	57.5 \pm 3.6
Tvu 946	28	29	21	20	24	23	31	27	33	34	27.0 \pm 1.5

ANOVA

Source of variation	Degrees of freedom	Sum of squares	Mean square	Computed F	Tabular F	
					5%	1%
Replicates	9	556.967	61.885	0.52		
Cultivars	2	4920.066	2460.033	20.85**	4.26	8.65
Error	18	2123.934	117.996			
Total	29	7600.967	262.102			

Coefficient of variation = 24.48%

Appendix 36 Quantity of food ingested by 4th instar M. testulalis larvae on pods of certain cowpea cultivars

Cultivar	Quantity (mg. dry wt.) ingested				X \pm s.e.
	RI	RII	RIII	RIV	
VITA 1	70.00	89.00	81.00	73.00	78.25 \pm 4.27
VITA 5	119.60	124.10	89.30	82.00	103.75 \pm 10.6
Tvu 946	86.00	75.00	45.00	83.00	72.25 \pm 9.4

ANOVA

Source of variation	Degrees of freedom	Sum of squares	Mean square	Computed F	Tabular 5%	F 1%
Cultivars	2	2634.667	1317.333	5.244*	5.12	8.02
Error	9	2260.709	251.190			
Total	11	4895.3776				

Coefficient of variation = 18.52%

Appendix 37. Quantity of food ingested by 4th instar M. testulalis larvae from pericarp and seed of certain cultivars

Part of pod	Cultivar	Quantity of food ingested (mg. dry wt.)				
		RI	RII	RIII	RIV	$\bar{X} \pm \text{s.e.}$
Pericarp	VITA 1	34.00	30.00	30.00	32.00	31.50 \pm 0.96
	VITA 5	7.10	7.90	5.89	25.40	11.57 \pm 4.60
	Tvu 946	15.00	15.00	23.00	23.00	19.00 \pm 2.3
Seed	VITA 1	30.14	30.00	42.00	37.00	34.79 \pm 2.9
	VITA 5	12.75	97.00	112.48	98.50	107.63 \pm 8.2
	Tvu 946	54.00	37.00	45.00	45.00	45.25 \pm 3.5

ANOVA

Source of variation	Degrees of freedom	Sum of squares	Mean square	Computed F	Tabular F 5%	F 1%
Replicate	3	263.99	87.998			
A. plant part	1	10558.81	10558.815	77.44**	4.75	9.33
Error	3	409.05	136.351			
B. cultivar	2	3866.99	1933.493	33.00**	3.88	6.93
A X B	2	9301.27	4650.635	79.38**	3.88	6.93
Error	12	703.09	58.59			

Coefficient of variation = 18.37%

Appendix 38. Quantity of food ingested by 4th instar M.testulalis larvae from artificial diet containing stem powder of certain cowpea cultivars

Type of diet	Quantity ingested (mg. dry wt.)				
	RI	RII	RIII	RIV	$\bar{X} \pm s.e.$
VITA 1	38.00	35.00	33.00	29.00	33.75 \pm 1.9
VITA 5	28.42	24.20	33.33	23.00	27.24 \pm 2.3
Tvu 946	24.00	29.60	30.20	23.00	26.70 \pm 1.9

ANOVA

Source of variance	Degrees of freedom	Sum of squares	Mean square	Computed F	Tabular F 5%	F 1%
Treatment	2	123.205	61.603	5.48*	5.14	10.92
Replicate	3	82.622	27.541	2.45		
Error	6	67.468	11.245			
Total	11	273.295	24.845			

Coefficient of variation = 11.47%

Appendix 39. Quantity of food ingested by 4th instar *M. testulalis* larvae from artificial diet containing pod powder of certain cowpea cultivars

Type of diet	Quantity ingested (Mg. dry wt.)				X \pm s.e.
	RI	RII	RIII	RIV	
VITA 1	29.40	45.00	34.00	30.00	34.60 \pm 3.6
VITA 5	24.00	32.00	24.00	30.00	27.50 \pm 2.1
Tvu 946	22.00	31.00	29.00	27.00	27.25 \pm 1.9
Control	29.00	39.00	40.00	17.00	31.25 \pm 5.4

ANOVA

Source of variance	Degrees of freedom	Sum of squares	Mean square	Computed F	Tabular F 5%	F 1%
Treatment	3	145.78	48.59	1.57 ^{ns}	3.86	6.99
Replicate	3	318.98	106.33	3.44		
Error	9	278.24	30.92			
Total	15	743.00	49.53			

Coefficient of variation = 18.44%

Appendix 40 Feeding response of 4th instar M. testulalis larvae to raw juice extract from stem.

Cultivar juice	Quantity of gel ingested (mg) in 24 h				
	RI	R2	R3	R4	X \pm s.e.
VITA 1	116.6	84.6	65.6	71.2	84.50 \pm 11.4
VITA 5	110.6	111.6	104.6	71.9	99.68 \pm 9.4
Tvu 946	106.1	83.5	105.2	48.4	85.80 \pm 13.5
Sucrose 0.1M	48.1	40.7	30.8	48.2	41.95 \pm 4.1
Control	15.1	10.3	13.3	10.0	12.17 \pm 1.2

ANOVA

Source of variance	Degrees of freedom	Sum of squares	Mean square	Computed F	Tabular F 5%	F 1%
Treatment	4	213.47	53.37	15.90**	3.06	4.89
Error	15	50.35	3.36			
Total	19	263.82				

Coefficient of variation = 10.95%

Appendix 41 Feeding response of 4th instar M. testulalis larvae to raw juice extract from leaves.

Cultivar juice	Quantity of gel ingested (mg) in 24 h				
	RI	RII	RIII	RIV	X \pm s.e.
VITA 1	125.7	95.9	92.4	127.4	110.35 \pm 9.4
VITA 5	160.0	178.9	180.9	105.7	156.38 \pm 17.5
Tvu 946	144.9	176.1	155.2	113.2	147.35 \pm 13.1
Sucrose 0.1M	79.1	66.6	31.0	31.0	52.03 \pm 12.3
Control	15.1	18.8	24.6	19.8	19.58 \pm 2.0

ANOVA

Source of variation	Degrees of freedom	Sum of squares	Mean square	Computed F	Tabular F 5%	F 1%
Treatment	4	570.24	142.56	24.67**	3.06	4.89
Error	15	86.67	5.78			
Total	19	656.91				

Coefficient of variation = 9.58%

Appendix 42. Feeding response of 4th instar *M. testulalis* larvae to raw juice extract from flowers

Cultivar juice	Quantity of gel ingested (mg) in 24 h				
	RI	RII	RIII	RIV	$\bar{X} \pm \text{s.e.}$
VITA 1	291.4	237.3	194.8	107.0	207.63 \pm 38.9
VITA 1	231.9	266.1	154.7	148.0	200.18 \pm 29.1
Tvu 946	266.5	262.8	201.2	198.9	232.35 \pm 18.7
Sucrose 0.1 M	55.3	53.6	44.6	56.1	52.40 \pm 2.65
Control	16.3	12.8	16.2	20.5	16.45 \pm 1.58

ANOVA

Source of variance	Degrees of freedom	Sum of squares	Mean square	Computed F	Tabular F 5%	F 1%
Treatment	4	1585.79	396.45	18.33**	3.06	4.89
Error	15	326.28	21.75			
Total	19	1912.07				

Coefficient of variation = 3.3%

Appendix 43. Feeding response of 4th instar M. testulalis larvae to methanol extract from pods of certain cowpea cultivars incorporated in agar-cellulose gel under no choice conditions.

Cultivar	Quantity ingested (mg/o) in 24 h			
	RI	RII	RIII	$\bar{x} \pm \text{s.e.}$
VITA 1	47	29	53	43.00 \pm 7.2
VITA 5	26	32	40	32.67 \pm 4.0
Tvu 946	29	44	49	40.67 \pm 6.0
Control	10	7	4	7.00 \pm 1.7

Appendix 44 Feeding responses of 4th instar M. testulalis larvae to sucrose incorporated in agar-cellulose gel.

Concentration (Molar)	Quantity ingested (mg. fresh wt.)				
	RI	RII	RIII	RIV	$\bar{X} \pm \text{s.e.}$
0.1	40	29	32	48	37.25 \pm 4.3
0.075	35	38	20	31	31.00 \pm 3.9
0.050	40	27	27	24	29.50 \pm 3.6
0.025	19	25	20	18	20.50 \pm 1.6
0.010	15	11	15	17	14.50 \pm 1.3
Control	15	18	13	12	14.50 \pm 1.3

ANOVA

Source of variance	Degrees of freedom	Sum of squares	Mean square	Computed F	Tabular F 5%	F 1%
Treatment	5	1783	356.64	10.48**	2.9	4.56
Replicate	3	116	38.82	1.14		
Error	15	510	34.02			
Total	23	2410	104.78			

Coefficient of variation = 23.77%

Appendix 45 Feeding response of 4th instar *M. testulalis* larvae to glucose incorporated in agar-cellulose gel

Concentration (molar)	Quantity ingested (Mg. fresh wt.)				
	I	II	III	IV	$\bar{x} \pm \text{s.e.}$
0.1	23	42	29	28	30.50 \pm 4.05
0.075	28	26	26	30	27.50 \pm 0.96
0.050	31	21	23	34	24.75 \pm 2.17
0.025	17	24	22	22	21.25 \pm 1.49
0.010	16	15	19	22	18.00 \pm 1.58
Control	15	14	17	13	14.75 \pm 0.85

ANOVA

Source of variance	Degrees of freedom	Sum of squares	Mean square	Computed F	Tabular F 5%	F 1%
Treatment	5	702	140.34	6.64**	2.90	4.56
Replicate	3	13	4.38	0.21		
Error	15	317	21.14			
Total	23	1032	44.87			

Coefficient of variation = 20.17%

Appendix 46 Feeding responses of 4th instar M.testulalis larvae to fructose incorporated in agar-cellulose gel

Concentration Molar	Quantity ingested (mg fresh wt.)				
	RI	RII	RIII	RIV	$\bar{X} \pm s.e.$
0.1	39	28	26	37	32.50 \pm 3.23
0.075	55	27	34	29	36.25 \pm 6.42
0.050	25	27	21	19	23.00 \pm 1.83
0.025	12	11	6	12	10.25 \pm 1.44
0.010	21	11	11	10	13.25 \pm 2.59
Control	14	4	11	7	9.00 \pm 2.20

ANOVA

Source of variation	Degrees of freedom	Sum of squares	Mean square	Computed F	Tabular F 5%	F 1%
Treatment	5	2752	550.34	19.09**	2.90	4.56
Replicate	3	391	130.26	4.52		
Error	15	432	28.83			
Total	23	3575	155.43			

Coefficient of variation = 25.93%

Appendix 47 Approximate digestibility (AD) of stems of certain cowpea cultivars by 4th instar M. testulalis larvae.

Replicate	AD		
	VITA 1	VITA 5	Tvu 946
1	57.14	68.82	71.43
2	62.10	77.40	68.27
3	52.83	66.60	50.95
4	65.59	73.18	50.00
5	72.94	78.02	63.75
6	74.70	76.03	78.26
7	72.73	74.42	59.03
8	83.60	71.38	74.57
9	72.31	65.12	75.57
10	50.34	72.39	68.53
X + s.e.	66.40 ± 3.4	72.23 ± 1.0	66.04 ± 3.0

ANOVA

Source of variation	Degrees of freedom	Sum of squares	Mean square	Computed F	Tabular 5%	F 1%
Replicated	9	1018.078	113.1198	1.89		
Cultivar	2	250.698	125.3491	2.09 ^{ns}	3.55	6.01
Error	18	1079.743	59.9857			
Total	29	2348.519	80.9834			

Coefficient of variation = 11.34%

Appendix 48 Efficiency of conversion of digest food (ECD) from stems of certain cowpea cultivars into body tissues by 4th instar M. testulalis larvae

Replicate	ECD		
	VITA 1	VITA 5	Tvu 946
I	7.50	2.60	4.50
II	9.66	3.10	2.02
III	11.07	2.83	13.08
IV	8.97	0.99	9.00
V	8.10	1.74	13.07
VI	8.66	2.23	6.67
VII	8.44	4.91	2.73
VIII	6.47	4.76	7.42
IX	7.87	6.36	6.00
X	14.54	2.47	9.01
$\bar{x} \pm \text{s.e.}$	9.28 ± 0.4	3.20 ± 0.5	7.35 ± 1.0

ANOVA

Source of variation	Degrees of freedom	Sum of squares	Mean square	Computed F	Tabular 5%	F 1%
Replicate	9	45.1974	5.0219	0.70		
Cultivar	2	177.8572	88.9286	12.37**	3.55	6.01
Error	18	129.3960	7.1886			
Total	29	352.4506	12.1535			

Coefficient of variation = 40.6%

Appendix 49 Efficiency of Conversion of Ingested Food (ECI) from stem of certain cowpea cultivars into body tissues by 4th instar M. testulalis larvae

Replicate	ECI		
	VITA	VITA 5	Tvu 946
I	4.30	1.8	3.2
II	6.00	2.4	1.4
III	5.60	1.9	6.7
IV	5.90	0.7	4.5
V	5.90	1.4	8.3
VI	6.50	1.7	5.2
VII	6.10	3.7	1.6
VIII	5.40	3.5	5.6
IX	5.70	4.2	4.5
X	7.10	1.8	6.2
$\bar{X} \pm s.e$	5.9 ± 2.0	2.3 ± 0.4	4.7 ± 0.7

ANOVA

Source of variation	Degrees of freedom	Sum of squares	Mean square	Computed F	Tabular 5%	F 1%
Replicate	9	15.971	1.775	0.74		
Cultivar	2	66.779	33.389	14.01**	3.55	6.01
Error	18	12.898	2.383			
Total	29	125.647				

Coefficient of variation = 36.00%

Appendix 50 Approximate digestibility (AD) of pods of certain cowpea cultivars by 4th instar M. testulalis larvae

Cultivar	AD				
	RI	RII	RIII	RIV	$\bar{x} \pm \text{s.e.}$
VITA 1	85.71	86.52	90.12	80.82	85.79 \pm 1.9
VITA 5	94.30	94.20	91.04	89.15	92.17 \pm 1.3
Tvu	78.56	73.33	61.11	77.51	72.63 \pm 4.0

ANOVA

Source of variation	Degrees of freedom	Sum of squares	Mean square	Computed F	Tabular 5%	F 1%
Cultivars	2	776.00	388	13.18**	4.26	8.02
Error	9	265.00	29.44			
Total	11	1041.00				

Coefficient of variation = 6.49%

Appendix 51 Efficiency of conversion of Digested Food (ECD) from pods of certain cowpea cultivars into body tissues by 4th instar M. testulalis larvae

Cultivar	ECD				
	RI	RII	RIII	RIV	$\bar{x} \pm s.e.$
VITA 1	15.00	16.14	13.70	22.03	16.72 \pm 1.8
VITA 5	6.81	6.36	10.22	12.91	9.08 \pm 1.5
Tvu 946	13.32	17.73	27.93	13.83	18.20 \pm 3.0

ANOVA

Source of variation	Degrees of freedom	Sum of squares	Mean square	Computed F	Tabular F 5%	F 1%
Cultivars	2	198	99.0	4.26*	4.26	8.02
Error	9	209	23.22			
Total	11	407				

Coefficient of variation = 32.80%

Appendix 52 Efficiency of conversion of ingested food (ECI) from pods of certain cowpea cultivars into body tissues by 4th instar M. testulalis larvae

Cultivar	ECI				
	RI	RII	RIII	RIV	$\bar{x} \pm \text{s.e.}$
VITA 1	12.86	13.97	12.35	17.81	14.25 \pm 1.2
VITA 5	6.40	6.00	9.20	11.51	8.28 \pm 1.3
Tvu 946	10.47	13.00	17.22	10.72	12.85 \pm 1.6

ANOVA

Source of variation	Degrees of freedom	Sum of squares	Mean square	Computed F	Tabular 5%	F 1%
Cultivar	2	78	39.00	4.74*	4.26	8.02
Error	9	74	8.22			
Total	11	152				

Coefficient of variation = 24.31%

APPENDIX 53

Approximate digestibility (AD) of pericarp and seed of certain cowpea cultivars by 4th instar Maruca testulalis larvae

Part of pod	Cultivar	AD				X	s.e
		RI	RII	RIII	RIV		
Pericarp	VITA 1	97.06	90.33	96.00	94.10	94.37	± 1.5
	VITA 5	81.69	41.77	38.10	87.00	62.14	± 118
	Tvu 946	69.60	60.00	74.35	75.78	69.93	± 3.56
Seed	VITA 1	30.33	39.00	38.10	39.51	36.74	± 2.1
	VITA 5	82.38	86.60	82.11	81.30	83.10	± 1.1
	Tvu 946	47.72	44.14	36.87	39.33	42.02	± 2.43

ANOVA

Source of variation	Degrees of freedom	Sum of squares	Mean square	Computed F	Tabular F	
					5%	0%
Replication	3	410.48	136.826	0.70		
A part of pod	1	2781.89	2781.891	14.16**	4.75	9.33
Error	3	589.34	196.447			
B Cultivar	2	1116.66	568.329	5.09*	3.88	6.93
A X B	2	6299.48	3149.739	28.73**		
Error	12	1315.47	109.622			

Coefficient of variation = 16.18%

APPENDIX 54

Efficiency of conversion of digested food (E.C.D.) from pericarp and seed of certain cowpea cultivars into body tissues by 4th instar Maruca testulalis

Part of pod	Cultivar	ECD				X	s.e
		RI	RII	RIII	RIV		
Pericarp	VITA 1	25.39	30.26	24.31	36.54	29.13	± 2.79
	VITA 5	43.79	73.64	43.50	18.11	44.76	± 11.40
	Tvu 946	30.84	42.22	35.09	24.61	33.19	± 3.70
Seed	VITA 1	78.99	42.74	43.75	41.04	51.63	± 9.13
	VITA 5	7.94	6.83	8.80	9.49	8.26	± 0.6
	Tvu 946	33.33	42.87	42.17	39.54	39.48	± 2.17

ANOVA

Source of variation	Degrees of freedom	Sum of squares	Mean square	Computed F	Tabular F	
					5%	0%
Replication	3	440.82	146.941	0.81		
A part of pod	1	38.41	38.405	0.21 ^{ns}	4.75	9.33
Error	3	542.41	180.804			
B Cultivar	2	806.37	403.185	2.57 ^{ns}	3.88	6.93
A X B	2	3700.98	1850.491	11.78 ^{**}		
Error	12	1884.87	157.073			

Coefficient of variation = 36.40%

APPENDIX 55

Efficiency of conversion of digested food (E.C.I.) from pericarp and seed of certain cowpea cultivars into body tissues by 4th instar Maruca testulalis larvae.

Part of pod	Cultivar	ECI				X	s.e
		RI	RII	RIII	RIV		
Pericarp	VITA 1	24.46	27.33	23.33	34.38	27.38	± 2.5
	VITA 5	35.77	30.38	34.63	15.47	29.06	± 4.7
	Tvu 946	21.47	25.33	26.09	18.65	33.19	± 3.70
Seed	VITA 1	23.95	16.67	16.67	16.22	18.38	± 1.9
	VITA 5	6.51	5.92	7.23	7.72	6.85	± 0.4
	Tvu 946	15.74	18.92	15.56	15.56	16.45	± 0.8

ANOVA

Source of variation	Degrees of freedom	Sum of squares	Mean square	Computed F	Tabular F	
					5%	0%
Replication	3	39.25	13.084	1.65		
A part of pod	1	954.27	945.266	119.21**	4.75	9.33
Error	3	23.78	7.926			
B Cultivar	2	99.92	49.962	1.66 ^{ns}	3.88	6.93
A X B	2	286.83	143.413	4.76**		
Error	12	361.18	30.098			

Coefficient of variation = 27.21%

Appendix 61

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

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

Regression equation (3rd Order)

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

P = 0.0004

Spikes mean = 73.55