

THE INHERITANCE OF RESISTANCE IN COWPEA  
(VIGNA UNGUICULATA (L.) WALP) TO POD  
BUGS (CLAVIGRALLA TOMENTOSICOLLIS  
STAL)

By

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II

Diploma

National Agricultural College Deventer

The Netherlands

1975

Submitted to the Faculty of the Graduate College  
of the Oklahoma State University  
in partial fulfillment of the requirements  
for the Degree of  
MASTER OF SCIENCE  
July, 1981

Thesis  
1981  
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## ACKNOWLEDGMENTS

The author owes deep gratitude to Dr. Maurice J. Lukefahr, Grain Legume Entomologist at the International Institute of Tropical Agriculture (I.I.T.A.), Ibadan, Nigeria, for his moral and inspiring guidance throughout this study and whose critical review has helped produce this manuscript.

My devout appreciation goes to my major adviser, Dr. Don C. Peters, Head of Department of Entomology, for arranging for this study at I.I.T.A., and for his constructive criticism, innovative suggestions and in depth review of this manuscript. I am also thankful to Dr. William A. Drew and Dr. Richard C. Berberet, Department of Entomology who served as members of my committee, for their valuable suggestions and review of this manuscript.

Thanks are also extended to the Leadership of the Grain Legume Improvement Program (GLIP) of I.I.T.A., notably to Dr. Peter Goldsworthy, Dr. Redden, the Grain Legume Breeder, my friend, Dr. Louis E. Jackai, and Ta'ama Moffi for their facilities and helpful suggestions. Special thanks are expressed to Livinus and the other GLIP technicians for their assistance in collecting the data.

Immense thanks are expressed to Dr. Judson McGuire, the Biometrician, and his technician, Sam Amoa, of the I.I.T.A. Computer Center for their painstaking assistance in analyzing the data for this study. Dr. Ron McNew also helped in some of the final statistical efforts.

Dr. S. Lawani and the library staff of I.I.T.A. deserve special mention for the exceptional assistance given me in my literature search. The author is grateful to Dr. Wade Reeves, Director of Training at I.I.T.A., for his compassionate and helpful counselling.

My deep appreciation is expressed to the U.S. Agency for International Development (U.S./A.I.D.) for its financial support for my graduate studies in crop protection. In this connection, special mention is made of Messrs. John Franklin, Rudolf Thomas (AID - Cameroon) and David Mateyka who efficiently collaborated each in his own way in preparing my study program.

In the Office of International Programs at Oklahoma State University, Mr. Hugh Rouk, ably assisted by Mr. Conrad Evans and the staff offered every possible assistance and for this I am thankful.

I am also thankful to the Government of the United Republic of Cameroon (Ministry of Agriculture) for granting me the permission to pursue this study.

A special word of appreciation goes to Mrs. Anne Hunt for her patience and comprehension in typing this manuscript.

This work is dedicated to my mother, Martha Bisi, for her encouragement and enduring support.

Finally, I am grateful and deeply indebted to my wife, Doreen, for her patience, moral support and above all, for the good care she took of our sons, Jerry and Ngwangwen; this was a source of great encouragement that sustained this study.

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## NOMENCLATURE

ANOVA	Analysis of Variance
B.t.	Bacillus thuringiensis
BHC	Benzene Hexachloride
cm	centimeter
DAF	Days After Fertilization
DAP	Days After Planting
DS	Damaged Seeds
EAA	Essential Amino Acids
GDP	Gross Domestic Product
ha	hectare
HPR	Host Plant Resistance
IPM	Integrated Pest Management
IITA	International Institute of Tropical Agriculture
IRAT	Institut Recherche Agricole Tropicale
Kg	kilogram
m	meter
mm	millimeter
NPK	Nitrogen Phosphorus Potassium (Fertilizer Mixture)
PC	Puncture Counts
TVU	Tropical Vigna Unguiculata
TVX	Tropical Vigna cross
ULV	Ultra Low Volume

## CHAPTER I

### INTRODUCTION

#### 1.1. Background and Justifications

Malnutrition is widespread in most of tropical Africa, especially in West Africa where diets are principally cereals and root-tubers and the people rarely receive half of their daily protein requirements. The Tse-tse fly and other factors greatly hinder the production of animal proteins and this protein deficiency has resulted in an estimated five deaths per 1,000 population, primarily in post-weaning children. Pulse legume proteins have been used in India and elsewhere to treat kwashiorkor-children (Rachie, 1973; Patwardhan, 1975).

The production of grain legume crops such as lima beans, pigeon peas, chickpeas and cowpeas which have a protein content of 2.9 to 34.6% (Boulter et al., 1973), offers an exceptional, immediate potential source of improving the dietary protein needs. The cowpeas, by their many inherent advantages of quick growth under a wide range of environments and on poor soils without supplementary nitrogen fertilizers, are particularly suitable for subsistence agriculture (Rachie, 1973; Albrecht, 1975).

#### 1.2. The Objective and Value of the Study

The success of a host plant resistance (HPR) program is based on

having the ability to recover resistant plants from a segregating progeny. Without this capability, it is not possible to make progress in incorporating resistance into agronomically acceptable cultivars.

The use of insect resistant crop varieties is playing an increasing role in integrated pest management (IPM). Plant resistance, the "built-in" protection which can be effective throughout a plant's life, offers a unique and compelling advantage for protecting crops against insects. The utilization of genetic resistance could suppress the build-up of insect pests and reduce damage caused by them.

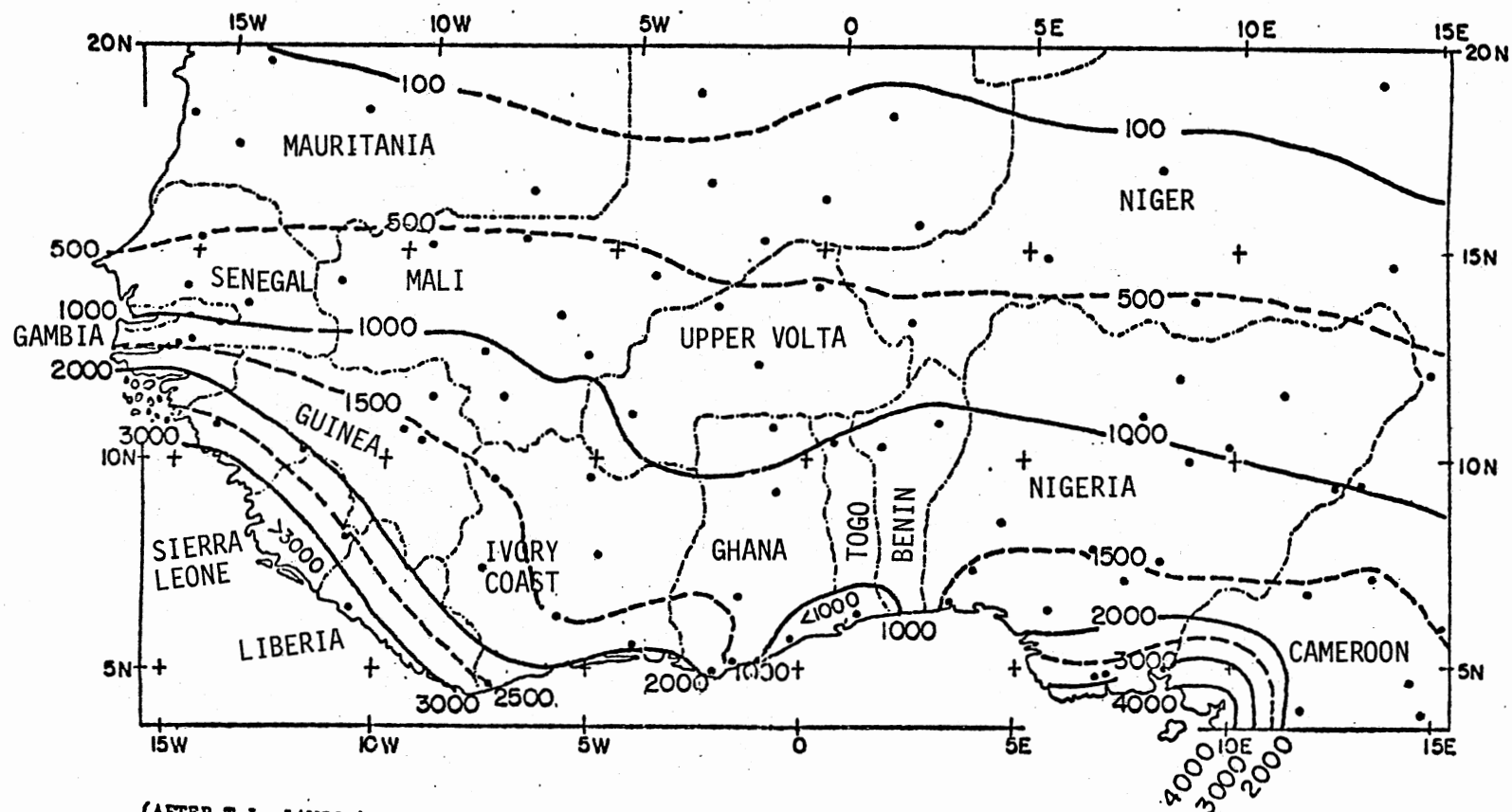
The value of the study of resistance in cowpea is manifold. Besides providing economical crop protection without causing any environmental problems, host plant resistance is suitable for any level of farming, requiring no new technology in utilization as would be required for pesticide application.

Nutritionally, cowpea is a highly acceptable food in many forms. It is rich in proteins, vitamins, minerals, with a low crude fiber and oil content. It is also of high biological value, calorific, having no metabolic inhibitors and flatus. The other distinct advantages of cowpea include: simplicity in food preparation and multiplicity of edible forms. The tender green shoots, leaves, unripened whole pods, are eaten as vegetables. The green peas, dried and ground into powder, are used for soups or baked into "Akara" balls or "Moin-moin". Cowpea seeds provide the only unprocessed, storable, and transportable protein food concentrate for both the rural and urban populations in the tropics (Oyenuga, 1968; Rachie, 1973).

Baby food industries are increasing demand for grain legume proteins for blending with milk products (Sosulski et al., 1978).

Cowpea seeds are a valuable feed concentrate for livestock and can be grown mixed with either corn or guinea corn for silage or hay.

In Cameroon, agriculture is one of the most important economic activities, accounting for over 40% of the Gross Domestic Product (GDP), and providing a source of income for about 80% of the population. The soil and climatic conditions allow the production of over 20 kinds of food crops including cowpeas and other grain legumes. Rachie (1973), quoting a FAO 1966 report, stated that nutrition deteriorates with increasing rainfall from the semi-arid north with a daily 80 gram protein consumption to the humid south with a 33 gram protein intake per day. Lower protein consumption in the south is attributable in part to a higher consumption of starchy foods principally plantains, roots and tubers (Figure 1). Unfortunately the same situation exists in Cameroon where more than two-thirds of the population are inhabitants of the humid zone. Therefore, the production of high yielding quality cowpea cultivars which can be grown under the peasant multi-cropping system will make a significant improvement in the dietary deficiency of the people.



(AFTER T.L. LAWSON)

Figure 1. Mean Annual Rainfall in West Africa in Millimeters

## CHAPTER II

### LITERATURE REVIEW

#### 2.1. Origin and Nomenclature of Cowpea

Vigna unguiculata (L) Walp, is most frequently called the cowpea, but other common names encountered in literature are: Southern pea and blackeye pea (in the U. S.), beans (in Anglophone West Africa), niébé (in Francophone Africa), lubia or lobia, coupé frijolé, asparagus beans, yard-long beans, and sitao (the last three generally refer to the sub-species sesquipedalis) (Lawani, 1979).

Cowpeas are indigenous to West Africa. Many subspecies of the cultivated, weedy and wild forms are found in both the savannah and forest zones of West Africa. The extent of introgression among the cultivated, weedy and wild forms and other ethnobotanical evidence suggests West Africa as the center of cowpea domestication with Nigeria and other savannah zone countries as genetic centers (Rawal, 1975).

Rawal (1975), states that the weedy forms are well distributed all over the African continent and Malagasy. These weedy forms thrive in disturbed habitats such as fields and roadsides while the wild types grow in undisturbed habitats such as secondary forests, woodland savannahs and near swamps where they grow throughout the year. Flowers are aromatic with strong pleasant fragrance which attract insects. The seeds of the semi-wild types are not eaten but used by some tribesmen of Northeastern Maradi in the Republic of Niger as an aphrodisiac in

male puberty and initiation rites (Rawal, 1975). Artificial hybrids between races, subraces and cultivars are fully fertile and hybrids between weedy forms and cultivars produced intermediate-type plants which when backcrossed, produced a pattern of variation typical of interbreeding populations. Of the five recognized subspecies of V. unguiculata, only sesquipedalis, cylindrica and unguiculata are cultivated whereas dekindtiana and memsensis are spontaneous (Rawal, 1975; Rachie and Rawal, 1976).

## 2.2. The Morphology of the Cowpea

The growth habits of cultivated forms of V. unguiculata range from determinate, erect, non-branching types to indeterminate, prostrate or climbing and profusely branching types. The cowpea is an annual plant with cylindrical to slightly ribbed, twisted and hollow stems bearing alternate trifoliate leaves. Flower-buds start emerging from about the fifth internode upwards. There are usually 5 to 7 fruiting positions from which peduncles grow bearing several flowers (see Figure 2).

For breeding programs, cowpeas have a broad range of genetic diversity with a high degree of compatibility. There is a reasonable level of natural crossing and a convenient controlled crossing to facilitate making of large numbers of handcrosses. Moreover, cowpeas have out-crossing mechanisms such as simple inherited genetic male sterility (Rachie et al., 1975).

Under short photoperiods (11.5-12.00 hrs) at the International Institute for Tropical Agricultural (IITA), Ibadan, Nigeria (located at 07° 34'N, 03° 54'E with average elevation of 220m), flowering occurs 33 to 90 days after planting (DAP) and pod filling takes 17 to 24 days

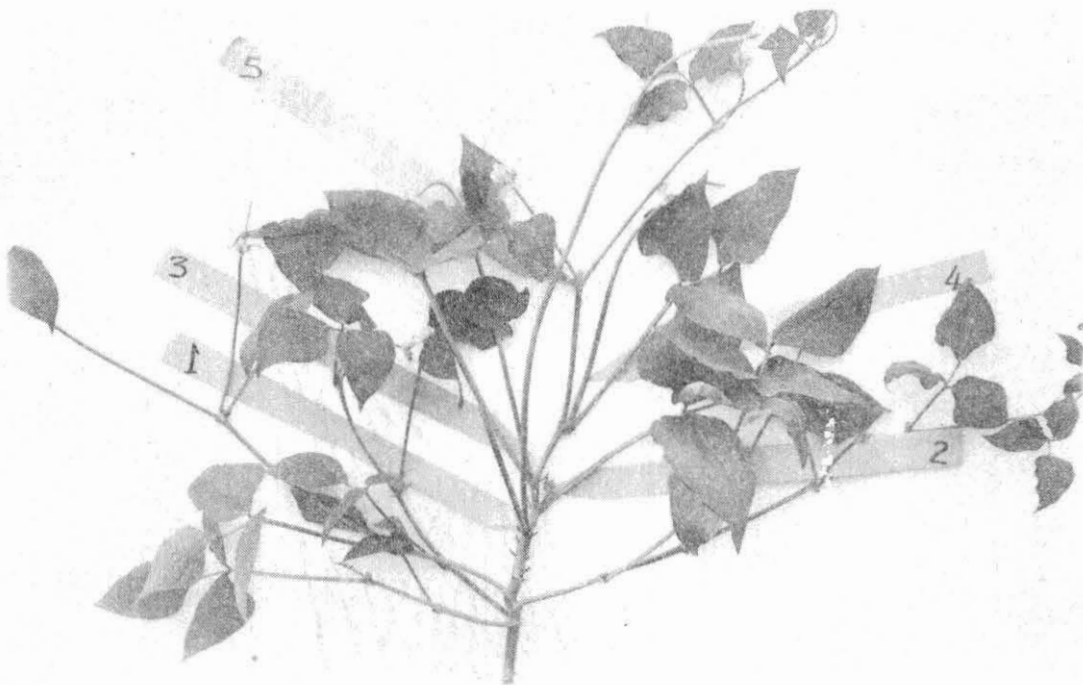


Figure 2. The First Five Main Fruiting Positions on a Determinate Cowpea Plant

after fertilization (DAF). The flowers are large, of varying colors and have attractive nectral glands that exude sweet liquid that attracts both pollinators and insect-pests like Maruca testulalis (Geyer). A peduncle may carry 1 to 5 pods but generally 3 pods are most common. The pod length varies from 10 to more than 100 cm. Pods may be borne pendant, vertical or curved. The color of immature pods vary from light-green to dark-green with some purple. The texture of both the green and dried pods is usually smooth with no trichomes or pubescence although coarse textures occur. Seed shape varies from square to kidney-shaped and the seed coat (testa) color varies considerably. Seeds are classified according to eye pattern and eye colors. The weight of 100 viable seeds varies from 2 to 33 grams. The cowpea cultivars grown in Nigeria and Niger Republic are predominately indeterminate, prostrate and photosensitive types that flower sparsely under long-day conditions (Rawal, 1975; Rachie and Rawal, 1976).

### 2.3. Ecological Distribution of Cowpea

The general distribution of the cowpea crop in Africa is along the Southern fringes of the Sahara desert from the West Coast Islands to East Africa and southwards. In West Africa, the cowpea growing region extends from latitudes 5° to 15°N covering the humid tropical regions with a heavy rainfall of 2,000 mm to the Semiarid zone with only 500 mm of rainfall per year. However, the greatest area of cowpea production occurs in the hot subhumid Savannah belt (9° to 14°) with a rainfall range of 500 to 1,200 mm, tending towards a monomodal distribution (Figures 1 and 3). Although cowpeas are grown on a wide range of soils without any supplementary nitrogen fertilizers, they grow better on well

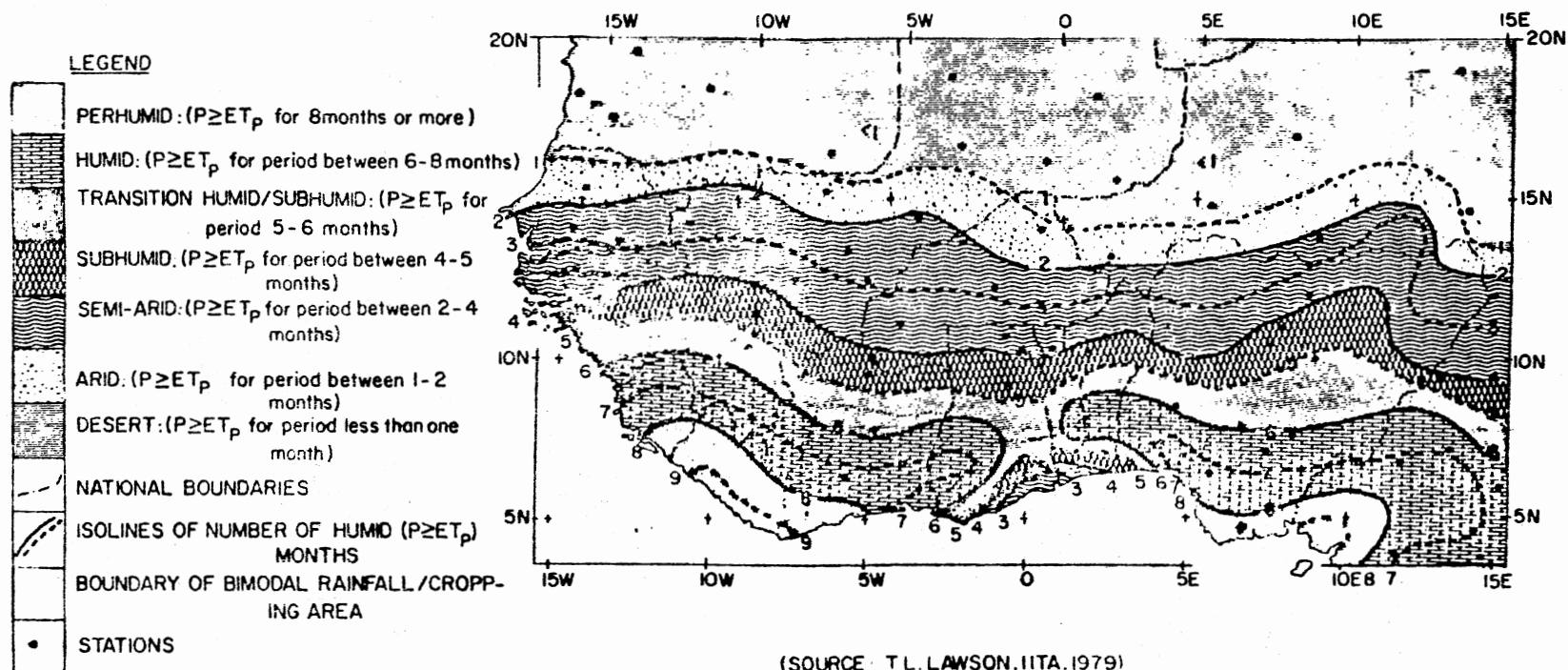


Figure 3. The Generalized Agroclimatic Map of West Africa

drained soils at low to intermediate altitudes as either sole or mixed crop (Okigbo, 1978; Rachie, 1973; I.I.T.A; 1977).

#### 2.4. The Classification of Clavigralla Spp.

The brown bug or pod bug species, Clavigralla tomentosicollis, formerly known as Acanthomia tomentosicollis was originally described by Stal in 1855 from specimens collected from the Cape colony in South Africa. Stal erected it as a division of Clavigrallaria, of the sub-family Pseudophloeinae of the family Coreidae (Hemiptera). Clavigralla [= Acanthomia] tomentosicollis Stal belongs to the genus Clavigralla, described by Spinola in 1837 (Dolling 1978, 1979). A checklist of the described species of Clavigralla Spinola is presented according to their morphological classification groups (Table I).

#### 2.5. The Morphological Characteristics of Clavigralla

There had been a lot of confusion in the taxonomy of the tribe Clavigrallini due to different definitions of morphological characteristics, lack of standardized nomenclature and modern instruments for detailed morphological studies. The present brief descriptive characteristics of the Tomentosicollis group is adopted from the recent revisions by Dolling in 1978 and 1979.

1. The tongue of the male genital capsule is trifid.
2. Pronotal disc with a pair of large, blunt, sublateral tubercles;
3. The membrane of the hemelytron suffused fairly evenly with brown pigments.

Although some morphometric variations exist between the males and

TABLE I  
CHECK LIST OF DESCRIBED SPECIES OF THE GENUS  
CLAVIGRALLA SPINOLA

Species Group	Species Name	Authority/Year	Location Specimen Collected	Geographical Distribution
<u>Tuberculicollis</u> Group				
1.	<u>C. tuberculicollis</u>	Reuter, 1887	Malagasy Rep. (Madagascar)	Malagasy and adjacent Islands
2.	<u>C. leroyi</u>	Schouteden, 1938	Zaire	Tropical Africa; from Guinea to Malawi
3.	<u>C. zambiae</u>	- 1943	Zambia	Southern Central Africa
4.	<u>C. uelensis</u>	Schouteden, 1938	Zaire	Central Africa
<u>Elongata Group</u>				
5.	<u>C. hystrix</u>	Dallas, 1852	Sierra Leone	Equatorial Africa; from Sierra Leone to Uganda
6.	<u>C. hystericodes</u>	Stal, 1866	Sierra Leone	Tropical Africa; from Sierra Leone to Tanzania and Transvaal
7.	<u>C. elongata</u>	Signoret, 1860	Tanzania, Madagascar	Cape Verde, Central, Eastern, Southern Africa, Madascar and Yemen.

TABLE I (Continued)

Species Group	Species Name	Authority/Year	Location Specimen Collected	Geographical Distribution
<u>Elongata Group</u> <u>Continued</u>				
8.	<u>C. shadabi</u>	Dolling, 1972	Nigeria, Cameroon, Sudan	West to Central Africa and S. Sudan
9.	<u>C. breviceps</u>	--	Nigeria, Zaire	West and Central Africa
10.	<u>C. ankatoensis</u>	Dolling, 1972	Madagascar	Madagascar
11.	<u>C. madagascariensis</u>	--	Madagascar	Madagascar
12.	<u>C. asterix</u>	ForsythMajor, 1894	Madagascar	Madagascar
13.	<u>C. schnelli</u>	Villier, 1950		
14.	<u>C. mira</u>	--	Ivory Coast, Zaire, Tanzania	West, Central and Eastern Africa
15.	<u>C. annectans</u>	--	Zaire	Zaire
16.	<u>C. insignis</u>	Distant, 1908	Uganda	East African Highlands
17.	<u>C. minor</u>	Schouteden, 1938	Burundi	Burundi
18.	<u>C. andersoni</u>	Anderson, --	British East Africa	East Africa

TABLE I (Continued)

Species Group	Species Name	Authority/Year	Location Specimen Collected	Geographical Distribution
<u>Elongata Group</u> <u>Continued</u>				
19.	<u>C. angolensis</u>	-- --	Angola	Angola
20.	<u>C. egregia</u>	-- --	Malawi	Malawi
21.	<u>C. longispina</u>	-- --	Zambia, Zaire	Southern Zaire and Northern Zambia
22.	<u>C. aculeata</u>	-- --	Natal-South Africa	South Africa
23.	<u>C. horrida</u>	Germar, 1840	Rhodesia, Cape Colony	Rhodesia and south Africa
24.	<u>C. natalensis</u>	Stal, 1855	Transvaal	Southern Africa
<u>Tomentosicollis Group</u>				
25.	<u>C. leontjeri</u>	Gergroth, 1908	Ethiopia, Senegal, Zambia	Africa: Between Sahara desert and Zambesi river
26.	<u>C. griseola</u>	Linnavouri, 1978	Eritrea and S. Yemen	Ethiopia and S. Yemen

TABLE I (Continued)

Species Group	Species Name	Authority/Year	Location Specimen Collected	Geographical Distribution
27.	<u>C. ruandana</u>	Schouteden, 1957	Rwanda	Central African Highlands and Rfit Valley
28.	<u>C. biston</u>	-- --	Tanzania, Malawi	Highlands of Central Africa
29.	<u>C. oxonis</u>	-- --	Zambia	Northern Zambia
30.	<u>C. bivolla</u>	-- --	Zambia	Northern Zambia
31.	<u>C. pusilla</u>	-- --	Madagascar	Southern Madagascar
32.	<u>C. strabo</u>	-- --	Botswana, Natal, Rhodesia	Southern Africa: between latitudes 17° and 30°S
33.	<u>C. pabo</u>	-- --	Mozambique, Natal	East Coast of Southern Africa
34.	<u>C. spiniscutis</u>	Bergroth, 1913	Senegal, Nigeria, Tanzania	Widespread in Africa
35.	<u>C. marmorata</u>	-- --	Rhodesia, Transvaal	Southern Africa
36.	<u>C. tomentosicollis</u>	Stal, 1855	Senegal, Nigeria, Zaire, Cape province, SW Africa	Africa south of the Sahara except Comoro Islands

TABLE I (Continued)

Species Group	Species Name	Authority/Year	Location Specimen Collected	Geographical Distribution
37.	<u>C. scutellaris</u>	Westwood, 1842	India, S. Yemen, Sudan	Kenya through Arabia, Pakistan to Western India
38.	<u>C. curvipes</u>	Stal, 1873	Guinea-Bissau, Nigeria, Uganda	Occupies between latitudes 15°N and 3°S from Guinea-Bissau to Uganda
39.	<u>C. simillima</u>	-- --	Zaire, Tanzania, S. Africa	Africa south of latitude 6°S
40.	<u>C. wittei</u>	Schouteden, 1938	Ivory Coast, Zaire, Kenya	Widespread in tropical Africa except Transvaal
41.	<u>C. neavei</u>	-- --	Zaire, Uganda	Central Africa
42.	<u>C. alpica</u>	Bergroth, 1927	Zaire, Ethiopia, Kenya	Highlands of Northeast and Central Africa
43.	<u>C. montana</u>	-- --	Cameroon Mountain	Cameroon Highlands
44.	<u>C. gibbosa</u>	Spinola, 1837	India, Sri Lanka	Ceylon and Indian Penisular
45.	<u>C. orientalis</u>	Dolling, --	China	Southern China
46.	<u>C. orientalis</u> <u>orientalis</u>	Dolling, --	India	Northern India, Burma to Indo China

females of C. tomentosicollis, the adult insects are generally robust with lengths varying from 8.3 to 11.5 mm. The heads are anteriorly declivent at about 45° to the vertical. The antennae and rostrum are 4-segmented with the basal segment of the rostrum directed posteriad at rest. The posterior femur has 2 major subapical spines beneath with the more distal being 1.5 times longer. The posterior tibia is straight except for a slight basal curvature.

## 2.6. The Distribution of Clavigralla

The general distribution pattern of the economically important species of Clavigralla is wide-spread across tropical regions, stretching from Cape Verde Islands across Africa to the Indo-Burmis peninsulars, together with the South-eastern Asian countries. The "Tur pod bug", Clavigralla gibbosa Spinola, and other oriental species are predominately spread in the Indo-Pakistani region attacking leguminous crops (Bindra, 1965). The "African pod bug", "Spiny brown bug" or "bean bug", C. tomentosicollis which is also known as "ysterbek" in Afrikaans, is the most widespread pest species throughout the African mainland from Senegal and Sudan in the north, to the Cape Province in South Africa. Aina (1975), in a survey of 53 local farms growing cowpeas mixed with other crops in Southwestern Nigeria, found 9 different species of coreid bugs including C. tomentosicollis and Clavigralla horrida (Germar), infesting the cowpea. He observed that C. tomentosicollis was present in 42% of all the farms surveyed and was abundantly distributed in all 4 ecological zones: Rain Forest, Derived Savannah, South Guinea and North Guinea Savannahs. C. horrida, probably either Clavigralla shadabi Dolling or C. elongata Signoret was restricted to the Derived Savannah

and South Guinea zones. C. horrida was conspicuously absent in the North Guinea zone where Booker (1965) had reported it as a major pest on cowpea in Zaria, Northern Nigeria. Libby (1968) also implicated an unidentified Acanthomia sp. together with Miperus jaculus (Thnb) as coreid bugs on Guinea corn and millet in Northern Nigeria. C. shadabi was abundantly found at Ibadan, Nigeria located at 07° 34'N, 03° 54'E at 220 m elevation, in the first cowpea growing season (May-August) and thereafter the population declined. The same pattern of appearance was observed in the second growing season (September-December) during which the population of C. shadabi though comparatively lower than that of C. tomentosicollis, was present in late October to November and declined in December.

Dolling (1979) stated that C. horrida often cited by many workers (Aina, 1972, 1975; Booker, 1965; and Egwuatu 1975) as pest in West Africa, was misapplied to C. shadabi. C. horrida is a pest restricted to Rhodesia and Southern Africa while C. shadabi and C. elongata were widespread in West Africa and Zaire.

## 2.7. The Host Range and Pest Status

The production of grain legumes, particularly V. unguiculata, is severely limited by a number of factors the most serious of these being insect pest depredation. Without insecticidal protection, yields may be decreased from 1,534 kg/ha to as little as 160 kg/ha. This represents a 190% reduction in yields if the crop is not protected (Bliss, 1973; Raheja and Hays, 1975).

The insect pests attack the cowpea crop in all stages from seedling to harvest. These pests can be classified into pre-flowering and

post-flowering pests. The pod sucking bugs are the most damaging among the post-flowering pests, often reducing yields to zero (Aina, 1972, 1975; Akingbohunge, 1977; Ayen-Sampong, 1978; Booker, 1964, 1965; Egwuatu, 1975; Egwuatu and Taylor, 1976; Jones, 1953; Kayumbo, 1977; Materu, 1968, 1970; Rachie and Rawal, 1976; Singh et al., 1978; Singh and van Emden, 1979; Swaine, 1968; and Taylor, 1969).

Kayumbo (1977) listed a total of 43 species of insect pests found feeding on the cowpea crop in the Morogoro area of Tanzania, belonging to the following orders: Thysanoptera, 2 spp., Diptera, 3 spp., Lepidoptera, 5 spp., coleoptera, 14 spp., and Hemiptera, 19 species of which 9 were the following coreid pod sucking bugs:

Acanthomia tomentosicollis Stal

Acanthomia horrida (Germar)

Acanthomia hystricodes Stal

Anoplocnemis curvipes (F)

Mirperns jaculus (Thnb)

Riptortus dentipes (F)

Riptortus flavorittatus Stal

Sjostedtina robustus (Distant)

Homoecerus Spp.

Similar finding by many workers are described elsewhere in this study. C. tomentosicollis lives, feeds and reproduces on the cowpea plant, completing several generations in a growing season especially on cowpea varieties that flower continuously. Materu (1968), reported that C. tomentosicollis takes 3 to 4 weeks to complete development from egg to adult while Egwuatu and Taylor (1977a) noted a mean development period of 23 days in both insectary and field conditions at Ibadan.

Although the coreid bugs, probably Clavigralla Spp. were recognized as pests almost 200 years ago, no extensive study has been undertaken on the host plant range. However, from the few works reported, the host plant range pattern of the genus Clavigralla seems to indicate that these insects feed almost exclusively on plants of the leguminous and related families. The exact factors influencing this preferential choice of leguminous plants as food has not yet been identified. However, evidence points to the effect of a glucoside of the nature of a triterpenoid saponine as the attractive factor (Fraenkel, 1959). The preferential acceptance of a certain family or species of plants as a food source by a genus or species of insects, seems to further strengthen the theory on insect-host plant long time co-evolution (Jermy, 1976).

It appears that increased cultivation of grain legume crops particularly cowpeas in recent years in West Africa and other tropical zones, has increased the recognition of C. tomentosicollis and its related species as major economic pests of grain legume crops. Fuller (1922) reported a "bean bug" (Acanthomia tomentosicollis) as a destructive insect on beans (Phaseolus spp.) in the fields in the Coastal districts of S. Africa. Golding (1972) listed C. gibbosa as a pest of a haycynth beans, Dolichos spp., damaging the shoots and pods. It is suspected that C. gibbosa cited by Golding might have been either C. tomentosicollis or C. shadabi since C. gibbosa is more restricted to Ceylon and the Indo-Pakistani region.

Jones (1953) reported that coreid bugs, C. tomentosicollis and C. horrida previously recorded only once as attacking beans (Phaseolus spp.) in the Italian Somaliland (now Somalia), were appearing in larger

numbers and causing serious damage and deterioration of crop. He listed the symptoms as:

- Dimpling of the seed coat,
- Browning and shrivelling of seed, and
- Wrinkling of the seed coat.

He further reported that many Hemiptera species were implicated in transmitting the fungus Nematospora coryli (Pegl.) which produced yeast-spot symptoms on the cotyledons of the bean seeds. From the symptoms of attacked seeds it would appear that seed quality and viability were deteriorated by the fungal infection.

Booker (1964, 1965) listed the major pests of cowpea and classified them into pre-flowering and post-flowering. Among the post-flowering he listed were: Maruca testulalis (Geyer), Piezotrachelus varium (Wagn.), Acanthomia brevirostris Stal now [Clavigralla scutellaris (Westwood)], C. horrida, A. curvipes and M. jacobus as main pests feeding on green cowpea pods.

Swaine (1968) ascribed crop losses of beans [Phaseolus vulgaris (L)] grown for seeds in Northern Tanzania to infestation by penatomid bug [Nezara viridula (L)], beanfly [Melangromyza phaseoli (Tryon)] and coreid bugs, notably C. tomentosicollis and C. horrida. N. viridula, was singled out as the agent transmitting the fungus N. coryli. Both the adults and nymphs of Nezara spp. and Clavigralla sp. attacked young developing bean pods, sucking out juice from them. Severely attacked pods shrivelled up, turned yellow and aborted. Pod damage by Clavigralla was assessed to range from 0.6 to 8.7% of the seeds from 50 plants. On heavily infested farms, damage was 26.8 to 30.8% from 0.1 hectare plots. This represented a net yield loss of 896 kg/ha.

Hill (1975) discusses C. tomentosicollis as vector of N. coryli which was introduced during piercing and sucking of cowpea pods. Besides P. vulgaris, pigeon pea [Cajanus cajan (L) Millsp] and Dolichos lablab (L), Solanum incanum (L), served as an alternate host.

In his account of cowpea pests prevalent in Nigeria, Libby (1968) listed A. brevisrostris (C. scutellaris), A. curvipes, Riptortus dentipes, and C. horrida, as the main cowpea pod bugs causing premature drying and shrivelling. The feeding punctures on pods are marked by brown darkened circular wet spots.

Materu (1968, 1970) studied the biology and the damage caused by C. tomentosicollis and C. horrida in the Arusha area of Tanzania on cultivated grain legumes (beans, pigeon pea and cowpea) found that over 50% of the damaged seeds of beans and peas were infected by the fungus N. coryli and that the germination ability was greatly reduced. Clavigralla spp. density ranged from 2.5 bugs per plant (10.5%) to 11.6 bugs per plant (44.4%). In the Mwanza area, C. horrida was recorded as a pest on Centrosema spp. and other alternative host plants such as: Triumfetta dikindticana (Engl.), Triumfetta rhomboidea (Jacq.) Tiliaceae) and S. incanum. C. tomentosicollis is labelled as a pest of Mangoes, [Mangifera indica (L)] in the National Museum in Nairobi, Kenya.

Materu and Makusi (1972) described the chemical control of these 2 species with DDT and endosulfan and observed that C. horrida was more tolerant to endosulfan doses fatal to C. tomentosicollis.

Kayumbo (1977) reported of large numbers of both C. tomentosicollis and C. horrida causing severe damage on green cowpea pods in the Morogoro area of Tanzania. Ayen-Sampong (1978) listed over 150 pest species attacking cowpea in Ghana, among them C. tomentosicollis and

other unspecified Clavigralla spp., that sometimes caused a total crop loss but when the crop was protected with insecticides, yields increased from 300 to 800%.

From the above cited cases of Clavigralla spp. damage, especially on cowpea and other grain legume crops, their pestilent activity requires concerted efforts to suppress them.

## 2.8. The Biology of C. tomentosicollis Stal

The biology of C. tomentosicollis, notably the life cycle, has been described by Materu (1968, 1970), Egwuatu and Taylor (1976, 1977 a, b). Aina (1972) described the biology of C. horrida while Bindra (1965) studied that of C. gibbosa. The pod bug, C. tomentosicollis, shares the general morphological and developmental characteristics of the Hemiptera. They have piercing and sucking mouth parts (stylet-form) and a paurometabolous development in which there is no marked development change between stages but a gradual resemblance change from one instar to the other, and usually in the same habitat. C. tomentosicollis has 5 nymphal instars before attaining adult stage.

The rate of development and fecundity of adult insects is influenced by several factors, notably the food source, water and climatic conditions. Egwuatu and Taylor (1977 a,b) studied the biology and the effects of nymphal density on the development of C. tomentosicollis. They observed that under insectary and field conditions, C. tomentosicollis completed its life cycle from egg to adult in 17 to 21 days.

Egwuatu (1975) studied the bionomics of C. tomentosicollis and C. horrida and their egg parasite, Gryon gnidus (Nixon) in cowpea fields at

Ibadan. Egwuatua and Taylor (1976) studied the effects of different leguminous hosts and water needs on the development and fecundity of C. tomentosicollis using the following leguminous plants: Centrosema pubescens (Benth), Puereria phaseoloides (Roxb), Calapogonium mucunoides (Desv.), Crotolaria juncea (L), Vigna aureus (L), Sphenostylis stenocarpa (Hochst), Glycine max (L) Mert., and Cajanus cajan (L) Millsp. Their findings revealed that the type of legume food source affected both the rate of nymphal development and the live weights of the ensuing adults. C. pubescens, C. mucunoides, C. juncea and C. stenocarpa could not support nymphal development beyond the 1st instar but sustained adult bugs. G. max could only support nymphal development till the 4th instar and was a suitable host for adults. Apart from V. unguiculata and C. cajan, the most cultivated grain legume crops in southern Nigeria, P. phaseoloides, V. aureus and P. vulgaris were suitable alternate hosts. Since C. tomentosicollis does not diapause at any stage of its development, these alternate host plants served as available source of food to ensure its survival between cowpea growing seasons. According to their finding, nymphal development was faster on pigeon pea, C. cajan, (13 days) with a 92% adult emergence, than on common beans, P. vulgaris, (21 days). Only 10% adult emergence was recorded on P. phaseloides.

Female bugs reared during the nymphal stage on pigeon pea and later on cowpea in the adult stage, laid an average of 648 eggs while females reared during both nymphal and adult stages on cowpea laid an average of 514 eggs. Dissected newly emerged female adults from both treatments revealed that eggs were already mature at adult emergence and mating could occur.

Materu (1968) observed from field collected eggs that the duration of different nymphal instars of C. tomentosicollis was not the same. The egg stage was the longest (7 days), the 1st instar was the shortest (2 days) while the 2nd, 3rd and 4th instars were 3 days each. The 5th instar was twice longer than the 1st instar and the bugs complete their life-cycle in about 3 to 4 weeks. Egwuatu and Taylor (1977a) found that C. tomentosicollis laid eggs in batches ranging from 2 to 99 and the mean pre-oviposition period in the insectary for mated females was 7.7 days and 17.3 days for unmated female bugs. The unmated females lived for 174 days while mated female bugs lived only for 127 days. The duration of egg laying was 13 weeks for the mated females and 16 weeks for the unmated. This prolonged oviposition period seemed to be an adaptation to secure a male partner where there was a scarcity of males. The life cycle under field conditions were identical to that in the insectary.

Egwuatu and Taylor (1976) found that the availability of water had a profound influence on the development and fecundity. Water was essential for egg maturation because eggs contained 30 to 80% water. This fact probably explains why pod bug populations decline markedly during the dry season when free rainwater or dew on the host plants was scarce or absent. Ample water supplied to mated females influenced fecundity and longevity. Female bugs given water and food, laid an average of 213 eggs and lived for 87 days while those denied water could lay only 57 eggs and lived only 31 days, a 65% reduction in longevity.

## 2.9. The Role and Value of Host-Plant Resistance

Man suffers from the effects of pesticide contamination in other

animals and through the accumulated residues he consumes in foods. All these threaten his immediate health and that of his offspring. Even more threatening is the menace of human hunger. Therefore, more safe and effective insect pest control measures are required if agriculture is to feed the ever increasing human population and at the same time preserve and protect the quality of the environment by reducing the excessive use of insecticides (Dahm, 1972; Duggan and Duggan, 1973). Host plant resistance (HPR), the use of crop varieties resistant to attack or damage by insect pests without causing any environmental problems, provides an alternative method of control. It has been used alone or in combination with other methods to provide satisfactory suppression of serious pests without pesticides (van den Bosch and Messenger, 1973).

Past experiences with other crops, notably cotton in the United States and elsewhere have shown that the use and extensive reliance on chemical insecticides as the only means of suppressing pests has resulted in many environment problems such as:

- Selective development of insects resistant to insecticides.
- Insurgence of secondary pest to major pest status.
- Destruction of beneficial insects: pollinators, predators and parasites.
- Environmental contamination of soil, water, fish and wildlife.
- Hazardous effects on man: carcinogenic, tumorigenic, and teratogenic effects (van den Bosch, 1978; Metcalf and Luckmann, 1975; Matsumura, 1975).

Snelling (1941) defined resistance in the widest sense to include those characteristics which enable a plant to avoid, tolerate or recover

from infestation under conditions that would cause greater injury to other plants of the same species. The most widely accepted definition is that of Painter (1951); he defined plant resistance as the relative amount of heritable qualities in a plant that influence the ultimate degree of damage done by the insect. Painter continued that in practical agriculture, resistance represented the ability of certain varieties to produce a larger crop of good quality than do ordinary varieties at the same level of insect population. The degree of resistance exhibited by specific hosts to specific insects was recognized to vary from immunity, indicating no consumption or injury under any given condition, to high susceptibility, indicating the potential for much greater than average damage by the insect to the plant.

Beck (1965) redefined resistance to exclude tolerance. "Resistance is the collective heritable characteristics by which a plant species, race, clone, or individual may reduce the probability of a successful utilization of that plant as a host by insect species, race, biotype or individual." He felt tolerance implied a biological relationship substantially different from the other 2 components of resistance (Coppel and Mertins, 1977). Painter (1951) recognized 3 main mechanisms of resistance as: (a) Nonpreference or antixenosis (Kogan and Ortman, 1978) denoting a plant's character(s) which adversely affects the insect's behavioral response towards the plant for use in oviposition, as food or shelter or both. (b) Antibiosis: exerts an adverse influence on growth and survival of the pest by preventing, injuring or destroying the insect's normal life, often but not always by chemical means. (c) Tolerance: includes all plant responses resulting in the ability to withstand infestation and to support insect populations that would

severely damage susceptible plants. As Painter (1951) emphasized, resistance was definable in relative terms and may be additive, in time and place, as well as influenced by several factors (Horber, 1979; Coppel and Mertins, 1977). The values of utilizing resistant varieties are manifold. First resistance provides protection and insurance against insect damage at no extra cost in material or labor to the farmer and with no risks to the environment. It is valuable in crops of low economic value per hectare, or in situations where yields vary greatly due to uncertainty of weather (like the West African Sahelian zone). It is valuable in developing countries or in situations where individual farm holding are too small and the use of insecticides is not well known, unavailable or too costly. An insect resistant variety of one crop may have a beneficial effect on other crops attacked by the same pest in that area. For example the use of a corn earworm resistant corn variety in the Southern United States, also greatly reduced damage on cotton caused by same insect pest (Coppel and Mertins, 1977). Resistance is compatible with biological and other cultural methods of control since the actions of predators and parasites are not obstructed as they would be with chemical control (van den Bosch and Messenger, 1973).

Resistance has a cumulative persistent effect on pest populations in contrast to chemical controls which are often dangerously unselective, and decreasing in effectiveness unless reapplied. The leading resistant Hessian fly wheat variety in Kansas was only 50% resistant but was effective and nearly exterminated the pest. In this connection, screening for high resistance may lead to the aggressive development of biotypes. The defensive nature of tolerance has so far not been challenged by most insects in contrast to antibiosis and nonpreference

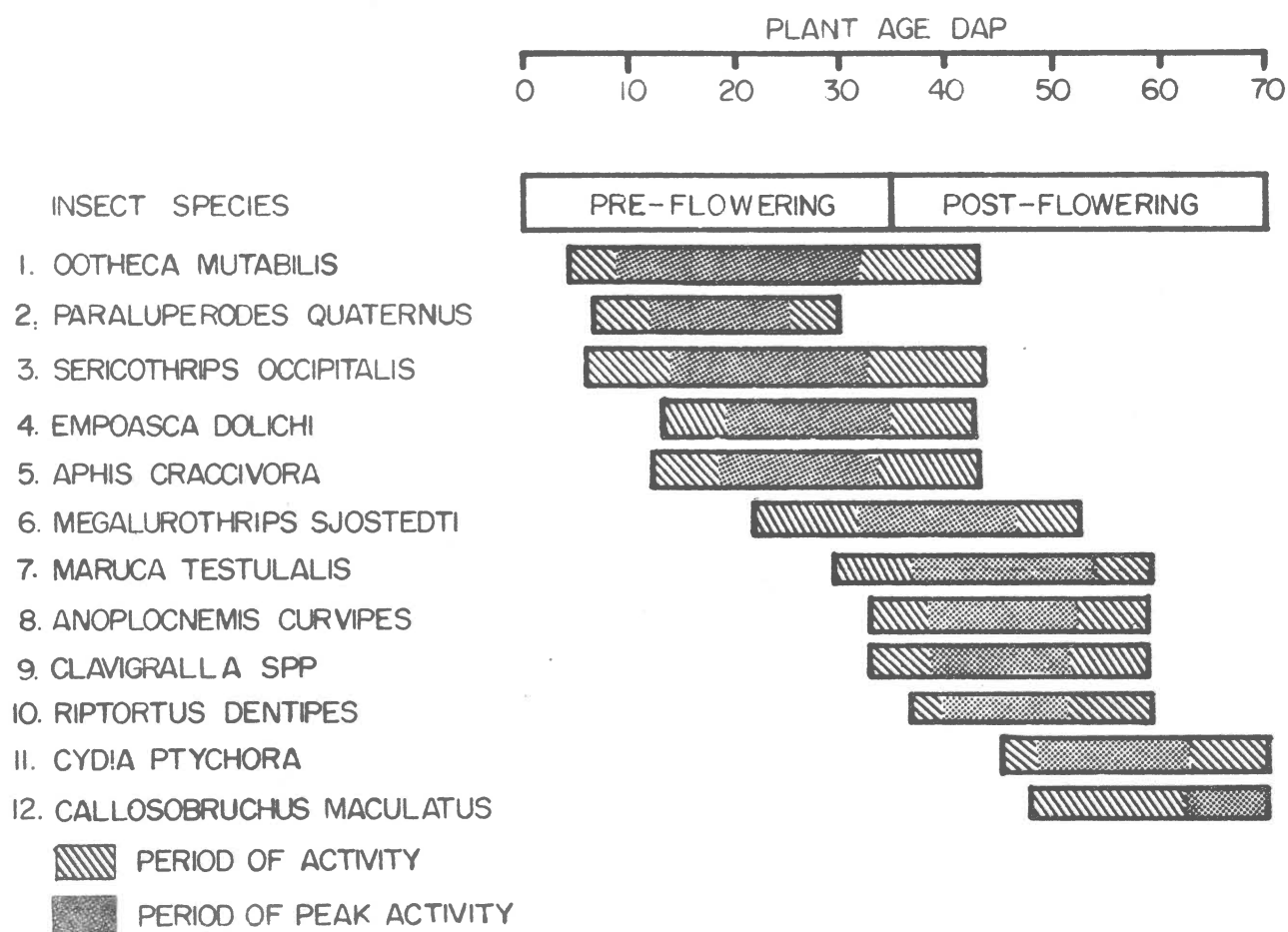
(Coppel and Mertins, 1977).

Host plant resistance yields higher returns. McKelvey (1972), stated that HPR accounted for only 10 to 20% in pest population reduction as against 80 to 90% reduction by other control methods. However, when it comes to analyzing the cost-benefits, HPR returns outweigh those of other methods. It is estimated that host plant resistance yielded 100:1 return on dollars invested for the development of spotted alfalfa aphid varieties in the U.S.A. The use of Hessian fly resistant wheat varieties in Kansas, saved 5 million bushels of grain per year. The U.S.A. Council on Environmental Quality estimated that the total cost of developing resistant varieties to the Hessian fly, wheat stem sawfly, European corn borer and spotted alfalfa aphid by the federal government, state and private agencies was about 9.3 million dollars. The estimated annual savings in reduced losses to farmers was 308 million dollars and for a 10 year period, research on HPR would save 3 billion dollars, a net gain of 300 dollars for every dollar invested (Coppel and Mertins, 1977).

However, host plant resistance has its limitations. In most cases it takes a relatively long time (10 to 20 years) to develop resistant varieties especially for perennial crops. With cowpea, it takes some 60 to 80 days to mature a crop and so it may be possible to develop cowpea resistant varieties in a relatively shorter time. As mentioned earlier, host plant resistance is compatible with many other methods of control. There are numerous examples of such well coordinated, harmonized techniques which are blended into a multifaceted, flexible system called, integrated pest management (IPM), the ultimate goal of which is not pest annihilation as with pesticides but the reduction of pest populations

below economic levels and compatible with ecological acceptance. The development of an integrated pest control system in the San Joaquin Valley cotton in California is an example of this new approach (van den Bosch and Messenger, 1973; van den Bosch, 1978).

In cowpea production, Singh (1977) has made some useful proposals for integrated control of certain major cowpea pests, utilizing a combination of cultural, host resistance, and limited chemical control (See Figure 4). Taylor (1969) studied the integrated control approach of the pest complex of cowpea in Nigeria using bacterial preparations of Bacillus thuringiensis (Berliner) (B.t) on the lepidopteran pest, Maruca testulalis (Geyer) and gamma BHC on the pod sucking bugs. The results of his finding showed that the removal of old crop debris from the fields greatly increased the efficacy of either treatment. The mortality of M. testulalis larvae was significantly higher in plots treated with B.t. preparations than on those treated with gamma BHC. Gamma BHC-treated plots had a high mortality of pod sucking bugs. From these findings Taylor concluded that a combination of microbials, chemicals and removal of crop residues offered a promising trend in integrated control of cowpea pest complex. What remained unresolved was the question of availability and storability of microbials and other chemicals at the peasant farmer's level.



After SINGH, S. R., (1977 IITA)

Figure 4. Diagrammatic Presentation of Cowpea Insect Pest Complex, Time of Occurrence and Peak Activity on Prima Cowpea

## CHAPTER III

### MATERIALS AND METHODS

#### 3.1. Laboratory Bioassays

Parental lines were selected based on preliminary laboratory tests in which TVX 2940-01D, IRAT 146, and TVU 2870 showed the greatest reductions in feeding punctures when compared to Ife Brown. VITA 5 was found to be intermediate while TVU 7133 was as susceptible as Ife Brown, the susceptible standard (Lukefahr, Personal communication). Therefore these parental lines were crossed to give all the possible combinations and formed the basis of the genetic study described herein.

The basis of the bioassay procedure for screening for resistance was to evaluate the lines for the number of feeding punctures made in pod walls. The pod bugs must puncture in order to exhibit preference for a certain line as a good source of food and the lines with fewer feeding punctures are considered to be less preferred.

Therefore the objective of this experiment was to evaluate in the laboratory 5 parental lines to establish the level of resistance of each line in relation to each other and to the susceptible standard. To achieve this objective, a few adjunct tests were needed to determine the following:

1. Pod Location: To determine if pod bugs have any preference for pods from any fruiting positions on the cowpea plant. This

TABLE II  
ORIGIN AND PRELIMINARY REACTION OF PARENTAL LINES TO  
CLAVIGRALLA TOMENTOSCILLIS

Parental Lines	Origin	Preliminary Reaction
IRAT 146	West Africa	Reduced punctures
TVX 2940-01D	IITA (Cross)	Reduced punctures
TVU 2870	India	Reduced punctures
VITA 5	Nigeria	Intermediate
TVU 7133	India	High puncture counts
Ife Brown (Check)	Nigeria	Higher puncture counts

might also detect any nutritional differences due to plant maturity.

2. Optimum Number: To determine the number of pods (6-10) per replicate, and the optimum number of replicates needed for an analysis of variance (ANOVA) significant at 5% probability level.

#### 3.1.1. Test on Fruiting Positions

Seeds of Ife Brown, the susceptible standard, were grown outdoors in almost natural field conditions and were protected from insect pests and fungal attacks with insecticides and fungicides when necessary until plants started flowering and were moved into a mesh house to prevent infestation when pods were formed. The seeds were sown in 10 liter plastic buckets (bottom perforated), usually referred to as "pots", containing non-sterilized field collected top soil. About 3 to 4 seeds were sown in a pot and later thinned to 2 plants per pot at the trifoliate stage. A supplementary fertilizer dose of 3-5 grams NPK (15:15:15) per pot was applied 14 days after planting (DAP). At about 35-40 DAP, most plants had formed flower buds and about 25% flowered. The plants were sprayed with 0.5% chlorpyrifos, an organophosphate insecticide, to control Maruca larvae. For this test, 10 plants were moved into a quarantined mesh house where pods were formed free of insect infestation. Four to 6-day old pods in a succulent, pliable stage that could be easily bent into a ring-form from the different fruiting positions were harvested with long peduncles (15-25 cm), tagged, and put into 500 ml flasks containing ordinary tap water. The pods, 8 in number, were circularly, but randomly, arranged and firmly corked with cotton

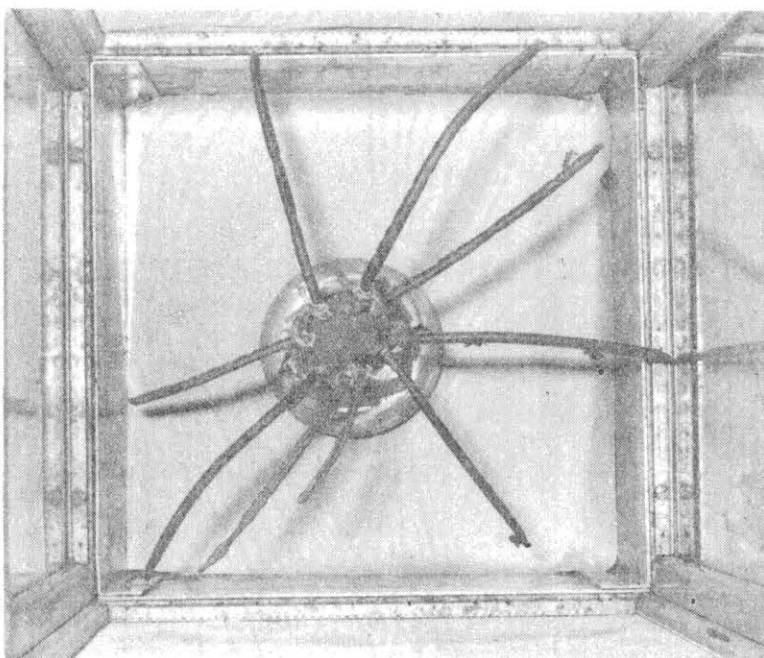


Figure 5. Test-Cage Containing Cowpea Pods Infested With C. tomentosicollis, Used in Laboratory Bioassays

wool to hold the pods in their spaced positions and to prevent the pod bugs from drowning in the flasks. The flasks containing the pods were put into prefabricated aluminum cages 28 x 28 x 40 cm, with the sides covered with 1 mm wire mesh (Figure 5). The pods were infested at the rate of one bug per pod and left for 72 hours under laboratory conditions of 21-23°C and 80-90% relative humidity.

At the end of the test period, pods were examined and puncture counts (PC) recorded. The pod examination consisted of splitting open with a razor-blade longitudinally and removing the seeds and pulpy contents. Using a magnifying lamp with 6X magnification, punctures made on the pod walls were visible and were counted. The ANOVA was computed.

### 3.1.2. Evaluation of Parental Lines

#### (Preference Test)

The seeds of the parental lines were grown outdoors in almost natural field conditions in field collected top soil and cultured until flowering as described in experiment 3.1.1.

To control aphids and whiteflies, the 3-week old plants were sprayed with a 0.1% solution of monocrotophos, a systemic organophosphate insecticide. Pythium stem rot and Anthracnose diseases were treated with captafol (Difolatan) 0.4% a.i. bi-weekly application and benomyl 0.2% a.i. respectively. At about 35-40 DAP, when most plants had formed flower-buds, chlproprifos was sprayed at 0.1% a.i. to control Maruca larvae and transferred into a mesh house so that pods were formed free of infestation. A minimum of 12-14 plants per variety was labelled and used for this bioassay.

A flask (replicate) consisted of 6 pods, one each from the 5 parent

lines and the susceptible standard. The stage of test pods, infestation rate, and pod examination procedure was the same as described in experiment 3.1.1. Damage rating was done and an ANOVA was computed. The mean puncture (MP) per variety were compared to that of the susceptible standard.

The advantage of using uniform age pods conspicuously exposed to the pod bugs excludes any bias factors such as plant growth habits (prostrate and profusely branching types) which under the field conditions may hide some pods from pod bug attack and such escaped plants might be mistaken for resistant plants. The method employed in this bioassay eliminates such bias (Figure 5).

### 3.2. Evaluation of Parents, $F_1$ and $F_2$ Lines

The aim of this series of experiments was to determine if the genetic basis for resistance is recoverable from a segregating progeny and if the resistance was governed by: (1) Dominant or recessive gene(s) (Intra-allelic) or (2) Complementary or additive action (Inter-allelic).

The general management of the test plants in these experiments up to laboratory bioassay and pod examination was the same as those described under sections 3.1.1. and 3.1.2. However, there were some additions. Besides evaluating the 5 parental lines, the  $F_1$  and  $F_2$  progenies from 5 diallelic crosses and 3 top-cross series were tested. There was no reason to expect differences from these two crossing patterns.

Diallelic Crosses

VITA 5 X IRAT 146

TVU 7133 X IRAT 146

TVX 2940-01D X VITA 5

TVX 2940-01D X TVU 7133

TVX 2940-01D X IRAT 146

Top-Cross Series

TVU 2870 X TVX 2940-01D

TVU 2870 X TVU 7133

TVU 2870 X VITA 5

The composition of the test materials for each of these series was as follows:

8-12 plants from Parent<sub>A</sub>, 48-72 pods.

8-12 plants from Parent<sub>B</sub>, 48-72 pods.

8-12 plants from the check variety, 48-72 pods.

8-12 plants from the F<sub>1</sub> plants, 48-72 pods.

40-50 plants from the F<sub>2</sub> plants, 200-300 pods.

Each individual plant was to be tested 4 to 6 times and to ensure this, the plants were numbered. When the test-pods were harvested, they were tagged at the time and the number of pods harvested from the plant was recorded on the tag hanging on the plant.

A replicate consisted of 10 pods harvested as follows:

1 pod from Parent<sub>A</sub>

1 pod from Parent<sub>B</sub>

1 pod from the check

1 pod from the F<sub>1</sub>

6 pods from the F<sub>2</sub> plants

The rate of infestation was still 1 bug per pod (10 bugs per cage) and the exposure time was 72 hours.

### 3.3. Evaluation of Parents and Backcrosses

The aim in this series of experiments was to gather more information on the genetic basis of resistance and determine if the gene(s) for resistance have a high heritability and can readily be transferred to agronomically accepted cultivars.

All 8 combinations and their backcrosses: designated as Backcross<sub>A</sub> and Backcross<sub>B</sub>, were evaluated. The general management of the test plants in these experiments, up to the laboratory bioassay and including puncture count examination, was the same as described under sections 3.1.1. and 3.1.2. There were some slight changes in the composition of each series. Each of the 8 series consisted of:

- 8-10 plants from Parent<sub>A</sub>, 40-60 pods.
- 8-10 plants from Parent<sub>B</sub>, 40-60 pods.
- 8-10 plants from the check, 40-60 pods.
- 15-20 plants from Backcross<sub>A</sub>, 80-120 pods.
- 15-20 plants from Backcross<sub>B</sub>, 80-120 pods.

The backcrosses were made in the greenhouse where the F<sub>1</sub>s were backcrossed with Parents A and B. Parent seeds were obtained by selfing the parent materials in the greenhouse. A replicate consisted of 9 pods harvested as follows:

- 1 pod from Parent<sub>A</sub>
- 1 pod from Parent<sub>B</sub>
- 1 pod from the check
- 3 pods from Backcross<sub>A</sub>
- 3 pods from Backcross<sub>B</sub>

Infestation rate was 1 bug/pod and exposure time was 72 hours. Insects used for these tests were for the most part field collected or from the outdoor reserve mesh cages.

### 3.4. Field Plot Trials

The objective of this trial was to test the performance of the 5 parental lines under field conditions so as to assess the pest population and crop damage. The seeds of these lines were planted in a randomized block design field in the second growing season (early September at I.I.T.A.). The seeds were treated with chloroneb (Demosan) at the rate of 2g/kg of seed against soil borne fungi. Each line was replicated 5 times in plots that measured 3.5 x 3.0m. A one meter alley separated the treatments. The row spacing was 75 cm and 20 cm planting space within row. A plot consisted of 5 rows with at most 17 plants per row, making a theoretical 85 plant populations per plot. The plots were sprayed with permethrin, a pyrethroid insecticide (Ambush 5 ULV) against pre-flowering pests since this insecticide is known to have little impact on pod bug populations. The field plot was three-quarters surrounded by pigeon pea, a reservoir crop that maintained a pod bug population till the cowpea had pods. The entire trial suffered a severe depredation by lizards and bush-fowls that ate the emerging seedlings and it had to be replanted twice.

Sampling started at the 7th week, a week after the last insecticide application. The sampling was done by using a one-meter long green cloth (drop-cloth) laid between 2 rows at 2 different locations per plot. The cowpea plants were shaken over the cloth. The number of each pest species was recorded per plot. The pest populations were low and



Figure 6. Pod Bugs Confined With Pods in Nylon Mesh Bags for 72 Hours in No-Choice Test on Live-Plants

the field was sampled 4 times at weekly intervals until the crop was harvested at the 12th week.

### 3.5. No-Choice Test on Live-Plants

The objective of this experiment was to assess the reaction of the pod bugs in a no-choice situation in order to verify the results of the laboratory bioassays conducted with excised pods. The management of the test plants for this experiment up till the mesh house stage is the same as in the previous tests. About 60-70 plants per parent line were kept in the mesh house and pods of 4-6 days old were bagged with small white nylon mesh bags (25 x 15 cm), containing adult pod bugs (Figure 6). The pods were infested at two levels: 1 bug per pod, and 2 bugs per pod and confined for 72 hours. At the end of the test period, the tagged pods were harvested and examined in the laboratory in the same method as those in the previous experiments.

The following parameters were investigated:

The number of punctures made/line,

The number of seeds per pod/line,

The number of seeds damaged/line and,

The number of insects dead after feeding on a line.

The mesh bags were usually washed and oven-dried at 50°-70°C to kill any eggs laid before reuse.

### 3.6. Biological Studies of C. tomentosicollis

#### 3.6.1. Longevity and Puncture Study

The purpose of this experiment was to investigate the average life span and average feeding capability of both adult male and female bugs.

It was also intended to obtain additional information on the reproductive potential of this pest.

Field collected nymphs of C. tomentosicollis were reared in a prefabricated aluminum cage (28 x 28 x 40 cm) with the sides covered with fine wire mesh under laboratory conditions of 21°-23°C and 80-90% relative humidity. The nymphs were fed with fresh cowpea pods which were replaced when necessary.

Newly emerging adult bugs were paired (male and female) and transferred into small cylindrical mesh cages about 17 cm high. These cages were then fitted to a bottom part, a plastic pot (7.5 x 7.5 cm), containing a hollowed cork disc 6.2 cm in diameter into which was inserted a 1 dram glass vial. The glass vial was half-filled with tap water and corked with a foam cork through which was inserted a cowpea peduncle while the pod was left exposed to the bugs.

Pods were replaced daily and the water changed. Pods fed upon were examined and puncture counts recorded. The dates of deaths were also recorded. Oviposition was greatly reduced probably because of the close confinement of the adults in the small cages. Twenty-four pairs were involved in this test and about 12% of the insects were still alive after 102 days when the experiment was terminated.

### 3.6.2. Nymphal Development and Damage Study

The aims of this experiment were:

1. To investigate the duration of each nymphal stadium in order to determine the approximate number of generations in a growing season.
2. To investigate the extent of damage caused by nymphal feeding.

It was previously thought that feeding by nymphs of pod bugs was negligible. However, during the laboratory bioassays, it was noticed that nymphal feeding influenced the number of puncture counts. It was decided to investigate the extent of damage caused by nymphal feeding.

Newly hatched first instar nymphs from field collected eggs were put singly into 8-dram perforated plastic vials, fitted onto 10-dram glass vials containing tap water. Ife Brown pods inserted through a foam cork were fed on by the nymphs. Pods were changed every two days and at the end of each molt. The test pods were examined in the same way as those described under sections 3.1.1. and 3.1.2. The following data was recorded:

1. The number of punctures per instar.
2. The number of seeds damaged per instar.
3. The duration of each instar.
4. The number of punctures and seeds damaged during the first 5 days of adult stage.

About 80 nymphs were studied in this test.

## CHAPTER IV

### RESULTS

#### 4.1. Results of Adjunct Test

##### 4.1.1. Results of Pod Positions - Variability Test

The results of analysis of variance (ANOVA) did not reveal any significant feeding preference by the bugs for pods from any fruiting position, thus confirming that there were no detectable nutritional differences due to plant maturity (Appendix Table XII).

##### 4.1.2. Optimum Numbers

The optimum numbers of pods per replicate was found to range from 6 to 10 pods and depending on the size of the test-plant population, the optimum number of replications needed for analysis of variance significant at 5% probability level was from 30 replicates and above.

#### 4.2. Parental Lines Evaluation (Preference Results)

As shown in Table III, C. tomentosicollis showed a distinct preference between the 5 parental lines as food source. Ife Brown, the susceptible standard, had a mean puncture count of 15.39, was rated highly accepted (100%). VITA 5 and TVU 7133 had 4.89 and 4.61 mean puncture counts, respectively, and were rated moderately accepted (31.8% and

TABLE III

MEAN NUMBER OF PUNCTURES PER POD ON FIVE COWPEA LINES IN THE  
STUDY OF INHERITANCE OF RESISTANCE TO C. TOMENTOSICOLLIS  
STAL IN PREFERENCE TEST

Mean for Lines			
Parent Lines	Mean (PC) <sup>1</sup>	Mean Separation <sup>2</sup>	Rated % Damaged
(Ife Brown, Susceptible Check)	15.39	a	100.0%
(B (VITA 5)	4.89	b	31.8%
(TV <sub>U</sub> 7133)	4.61	bc	29.9%
(TV <sub>X</sub> 2940-01D	3.08	cd	20.0%
(TV <sub>U</sub> 2870)	2.08	d	13.5%
(IRAT 146)	1.89	d	12.3%
F-Value	51.20**		
Fisher's Protected LSD	1.97 at 5% probability level		

<sup>1</sup>Each number is a mean value of 36 replicates.

<sup>2</sup>Means followed by the same letter do not differ significantly at 5% probability level.

29.9% of the standard, Ife Brown). TVX 2940-01D, with a mean puncture count of 3.08, was rated lowly accepted (20.0% of Ife Brown), while TVU 2870 and IRAT 146 had 2.08 and 1.89 mean puncture counts respectively, were rated as poorly accepted (13.5% and 12.3% of Ife Brown). The ANOVA and LSD tests at 5% (Appendix Table XIII) showed 3 statistically different groups viz:

Ife Brown, the susceptible standard;

VITA 5 and TVU 7133; and

TVX 2940-01D, TVU 2870, and IRAT 146 (all of which had shown reduced feeding punctures in preliminary laboratory tests).

#### 4.3. Results of F<sub>2</sub> Generations

The results from the F<sub>1</sub> and F<sub>2</sub> progenies were not as clear cut as expected due to early instar nymphal feeding that might have confounded them. Literature citations implied or stated that early instar nymphs of C. tomentosicollis were unable to cause damage to cowpeas. Therefore the exclusion of these early instar nymphs from the test-cages used for evaluating the F<sub>1</sub> and F<sub>2</sub> populations was overlooked until evidence from nymphal development studies clearly indicated that these early instars were capable of causing damage. Therefore, the results obtained might have been confounded by the presence of these nymphs. The puncture counts for the parental lines, F<sub>1</sub> and F<sub>2</sub> progenies were variable. The bar graphs illustrate the relative distribution of pod counts in the respective categories while the "X"s show the means for pods from each plant. Several transformations and heritability tests were attempted but with negative results insofar as establishing the gene action involved. We could reject the simple dominant hypothesis. The

distribution pattern (Figures 7a through h) would seem to suggest that pod punctures are quantitatively determined.

However, the bar graphs of the  $F_2$  populations (Figure 7) clearly indicates that in all cases, the segregating progenies had a range that would appear desirable to select the first 2 categories and make further screening for the resistance characters.

The crosses between the resistant line TVU 2870 and the two susceptible lines VITA 5 and TVU 7133 had relatively fewer  $F_2$  individual pods (18 and 23%) in the first 2 categories. These crosses present little prospect for further screening for resistant characters (Figure 7b, h). By comparison, larger numbers of individual  $F_2$  pods (25 to 34% in the first category and 48 to 59% in the first 2 categories) with desirable resistance characters were obtained from crosses between the resistant lines (TVX 2940-01D, and IRAT 146) and the susceptible lines VITA 5 and TVU 7133 (Figure 7a, d, e, g). Still higher numbers of prospective resistant individual pods (22 to 34% in the first category and 49 to 62% in the first 2 categories) were obtained in  $F_2$  progenies from crosses between resistant X resistant lines (TVU 2870, TVX 290-01D and IRAT 146 (Figure 7c, f). Doubling the infestation level or other means of intensifying the selection pressure would be necessary to determine the contribution of each parent and their complimentary potential.

Evidence obtained from crosses between the resistant lines seems to suggest that the resistance factor(s) causing reduced feeding by adult bugs contributed by resistant lines appears to be uniquely independent and heritable. The cross TVU 2870 x TVX 2940-01D produced 34% would-be resistant pods in the first category while the cross TVX 2940-01D x IRAT 146 produced only 22% promising resistant pods in the same category.

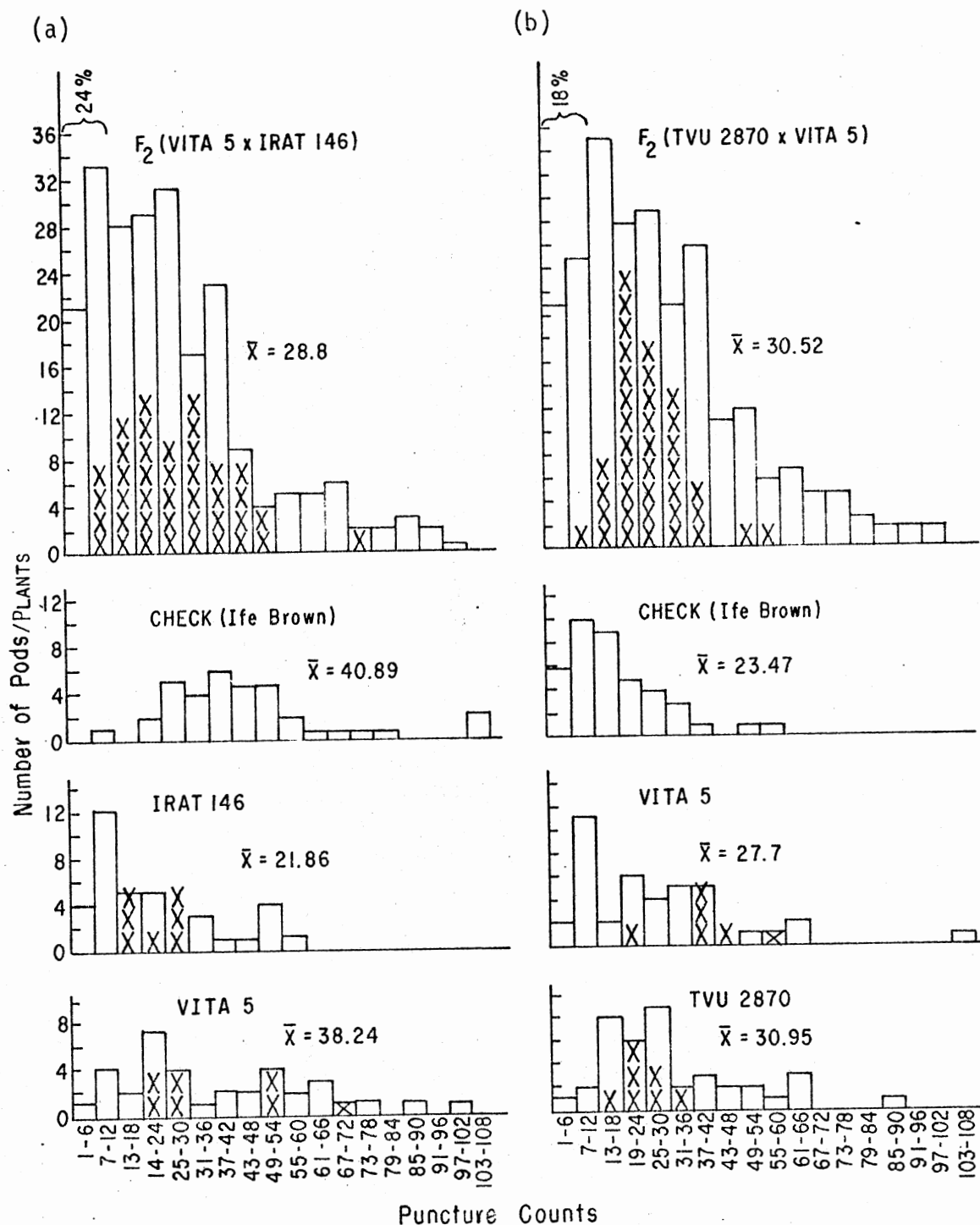
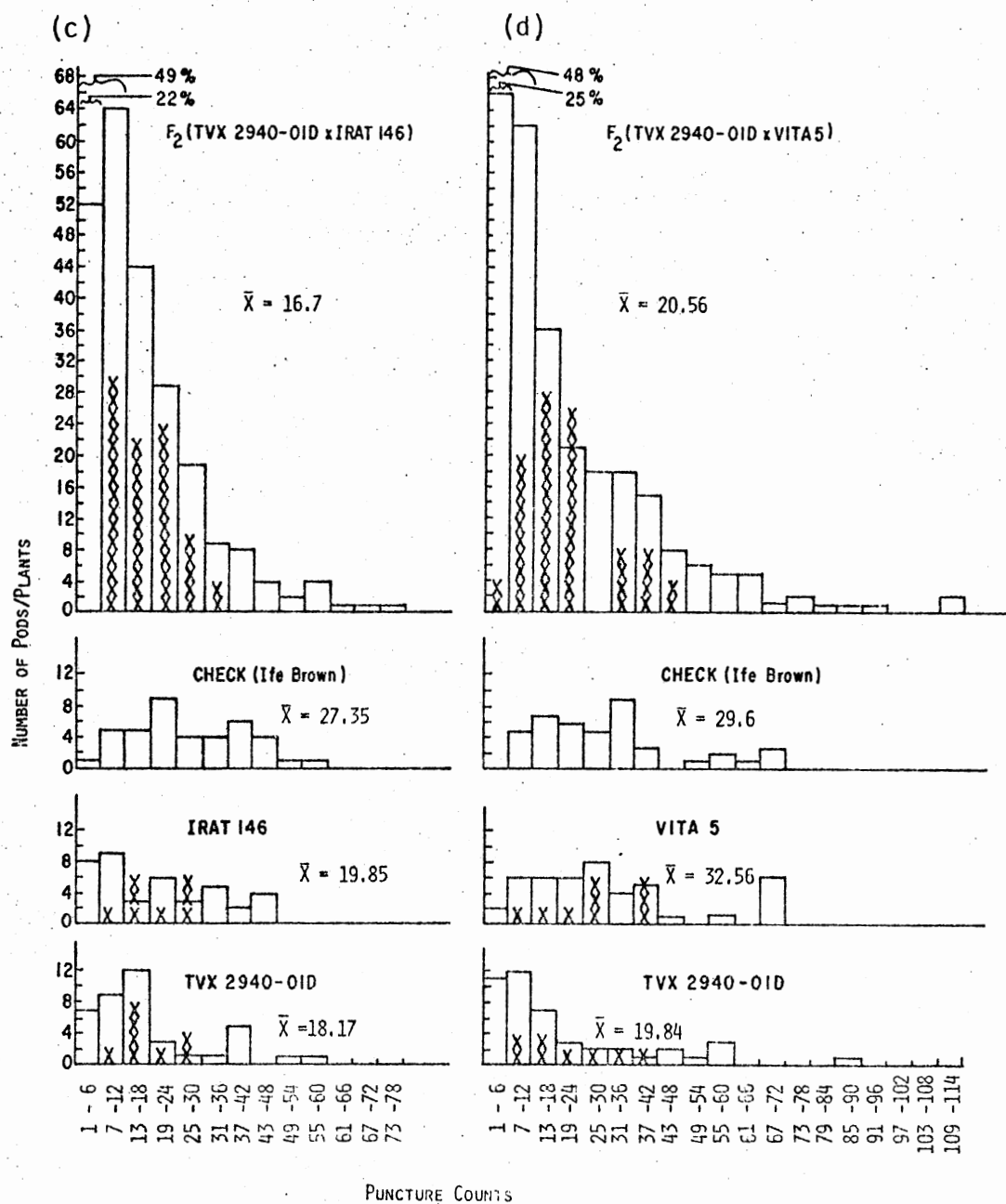


Figure 7. Bar Graphs of Punctures and Mean Punctures/Pod of Parents and F<sub>2</sub> Progenies (X's Represent the Number of Plants.)



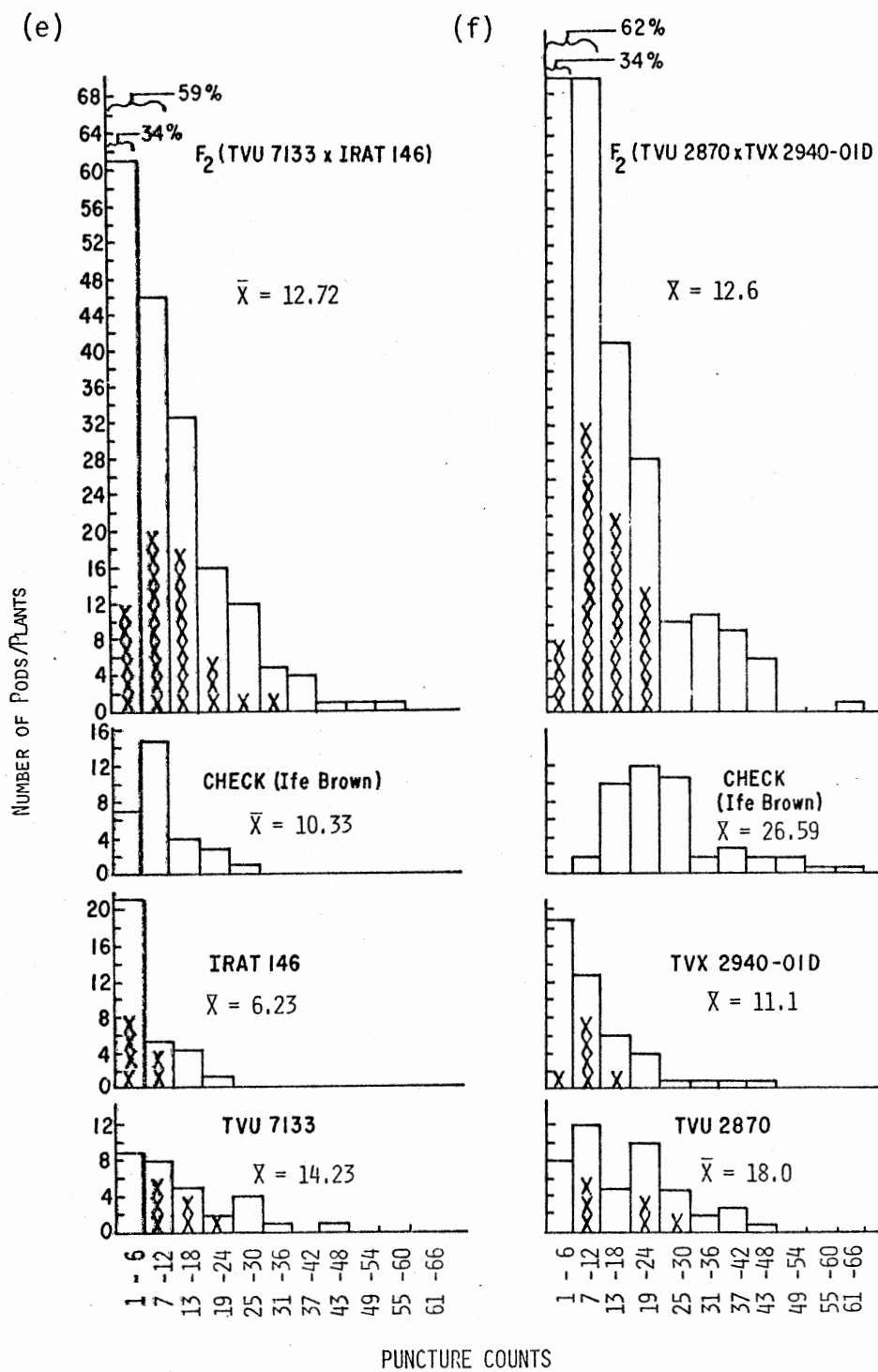


Figure 7. (Continued)

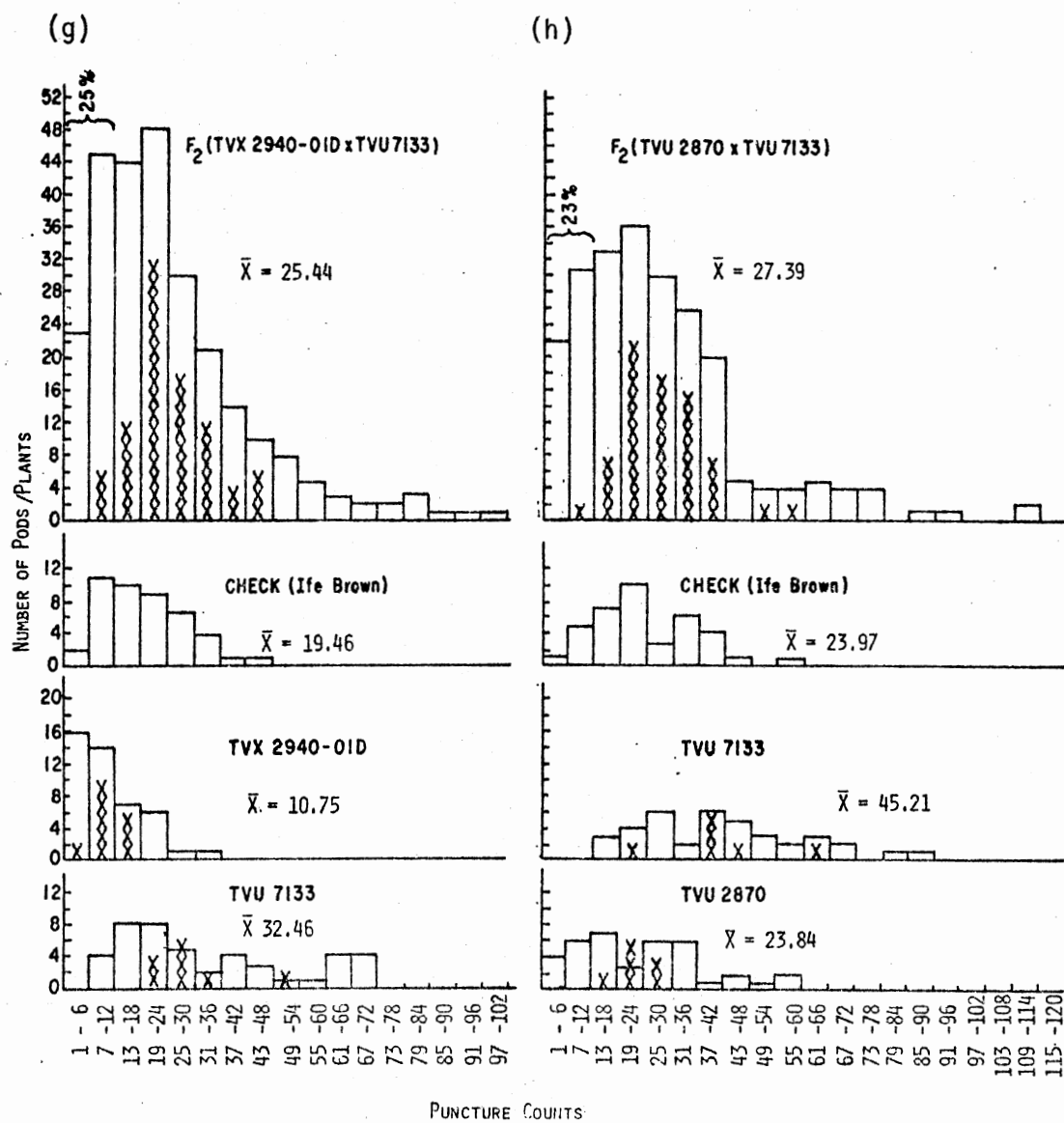


Figure 7. (Continued)

Data from this study also indicates that the resistant lines exhibited heterogeneity and that the  $F_1$  progenies did not show any evidence that the factor(s) reducing or inhibiting feeding in pod walls behaved as a simple dominant.

Despite the influencing factor of the nymphal feeding, the general trend of the  $F_2$  data is supportive of the results obtained from the backcross populations evaluated free of nymphal interference.

In most cases, the general distribution pattern of the  $F_2$  plant population more closely approached normality than that of the pods. Between 43 to 50% of the plants produced pods which had fewer punctures than the mean puncture count in each cross (Figure 7). Only the crosses TVX 290-01D X TVU 7133 and TVU 2870 X VITA 5 had 55% and 65% of the  $F_2$  plants that produced pods with fewer number of punctures than the mean puncture counts.

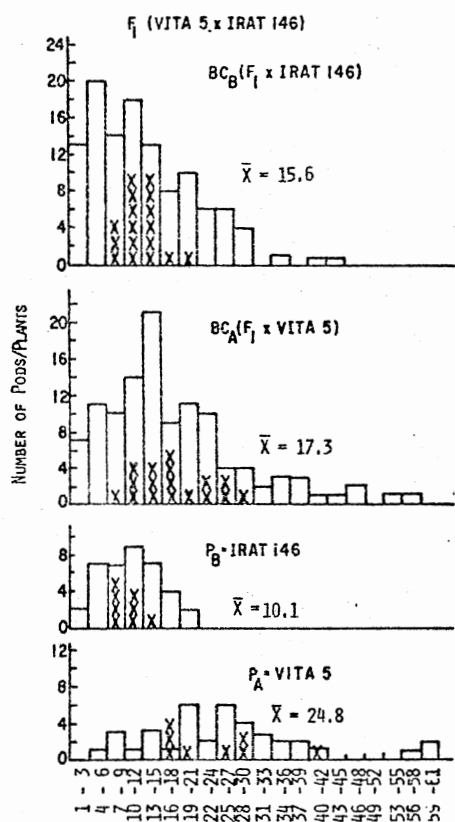
Selection of resistant plants consistent with categories identified with the above discussion on desirable levels of resistance for pods appears feasible.

#### 4.4. Results of Backcross Progenies

The general trend indicates that backcrossing to resistant donor-lines produces many plants with pods having reduced feeding punctures while backcrossing to susceptible donor-lines produces plants with pods having higher puncture counts (Figure 8a to e, h).

These results suggest that the resistant parental lines are heterozygous for the factor(s) that reduced feeding in pod walls by C. tomentosicollis. Backcrosses of (TVU 2870 X TVX 2940-01D; TVX 2940-01D X IRAT 146) R X R did not appear homozygous (Figure 8f, g). Since no

(a)



(b)

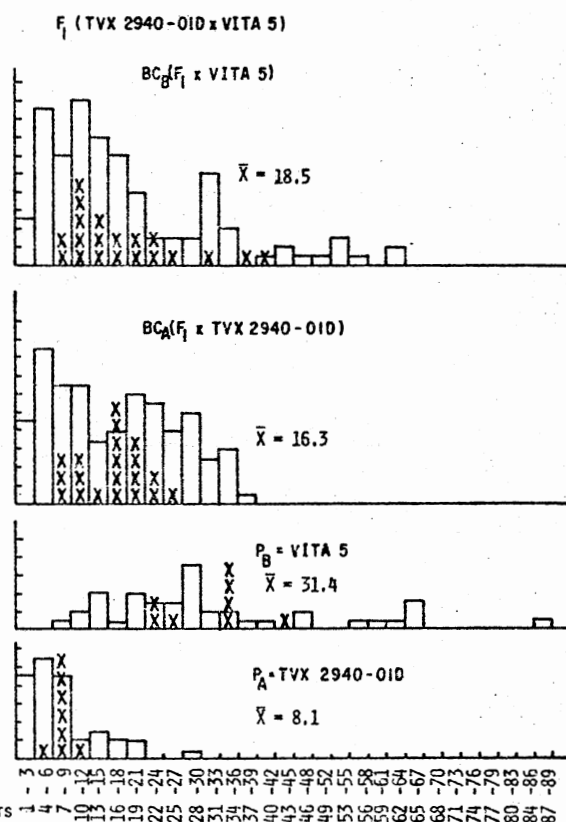
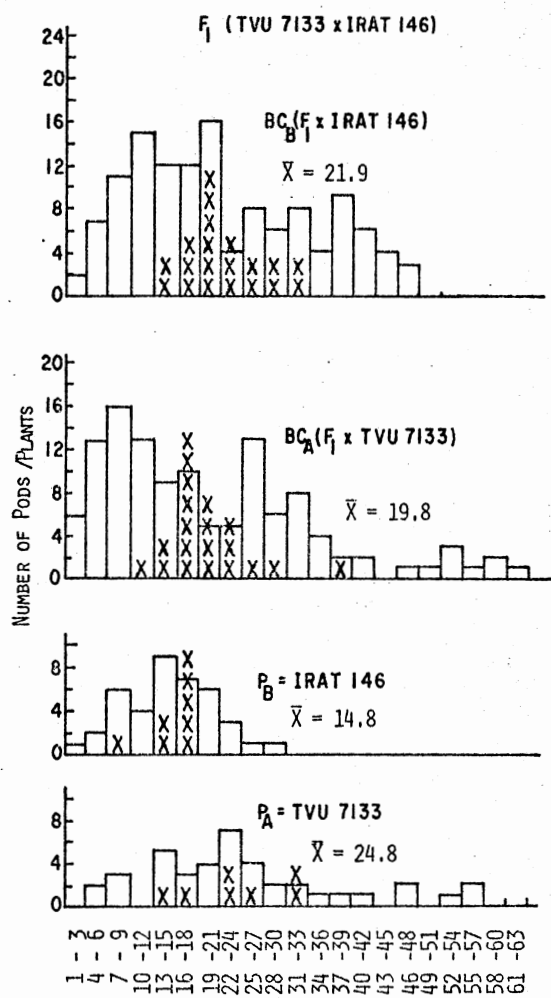


Figure 8. Bar Graphs of Punctures and Mean Punctures/Pod of Parents and Backcrosses (X's Represent the Number of Plants.)

(c)



(d)

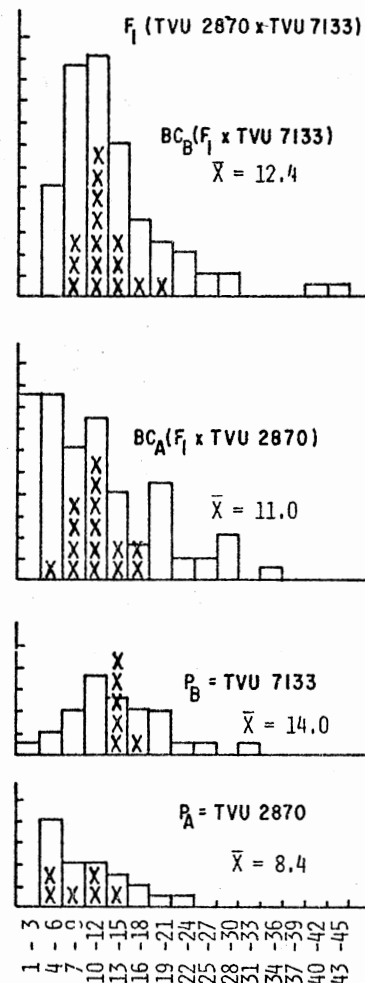


Figure 8. (Continued)

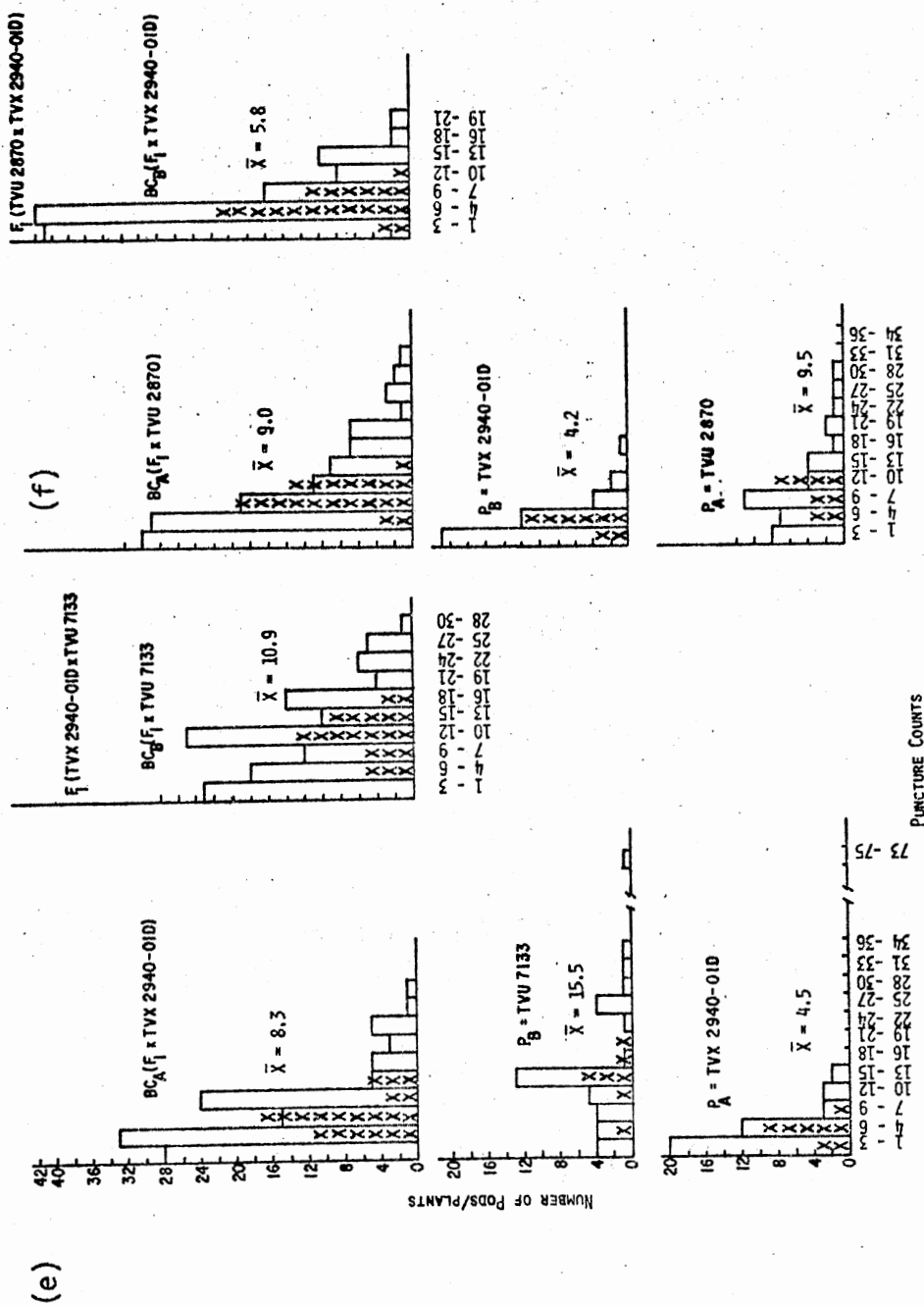


Figure 8. (Continued)

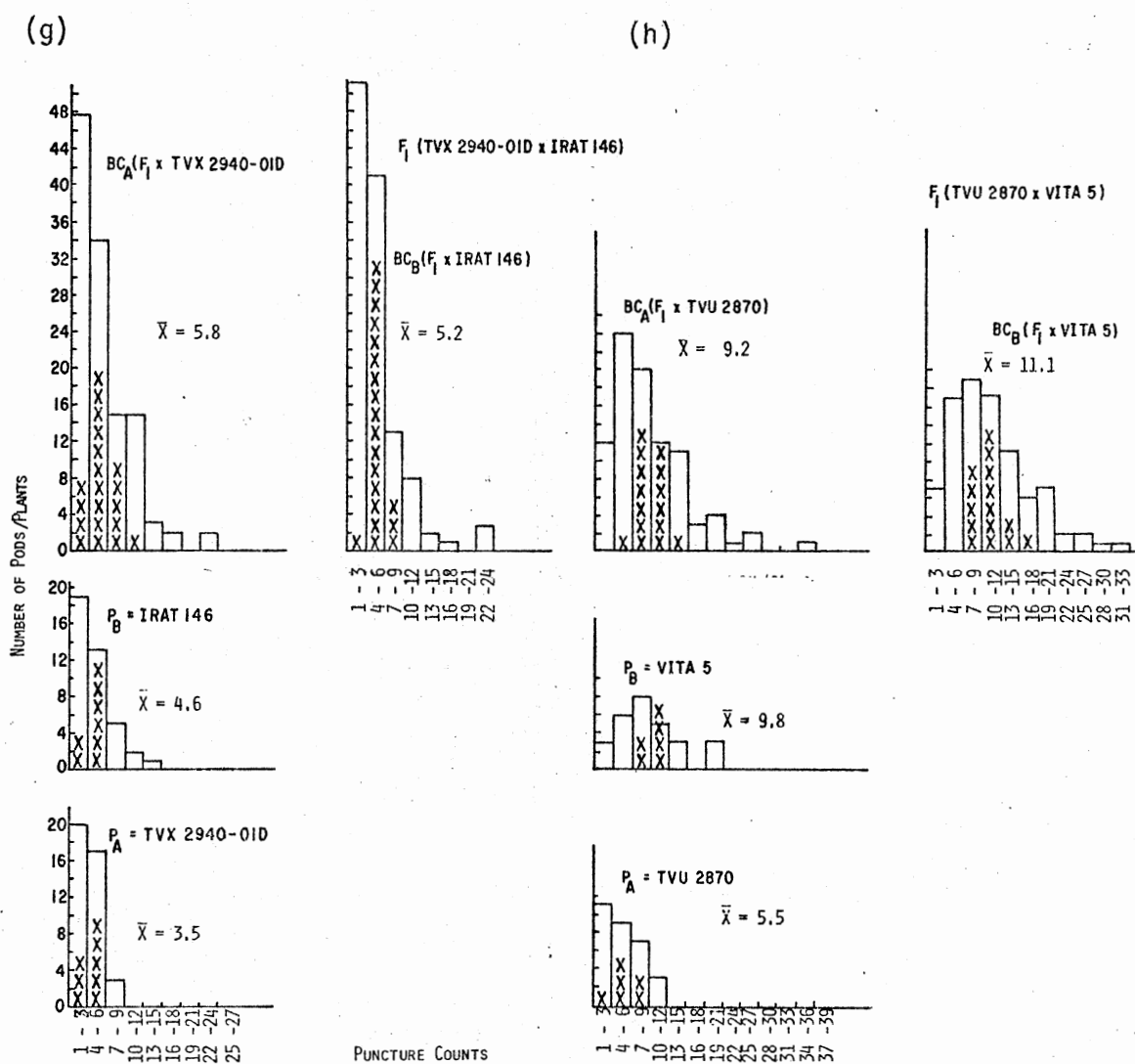


Figure 8. (Continued)

prior selection was made for this character but unselected material collected from the germ plasm collection, it is therefore not an unexpected event that these lines appear to be heterozygous.

From the bar graphs (Figure 8e, f, g) the backcross of TVX 2940-01D and IRAT 146 appears to be more promising with a less heterogenous population. In the backcrosses to TVX 2940-01D and TVU 2870, the backcross to TVU 2870 is more heterogenous than that of TVX 2940-01D. On the whole, TVX 2940-01D appears to be less heterogenous than the other resistant lines.

Backcrossing to the susceptible lines generally produces some promising segregating plants from which further selections can be made. In backcrossing, the aim is to improve cultivars deficient in a few desirable characters by transferring these traits from a donor parental line through repeated backcrossing with the recurrent parent. In an isogenic line development, it will require at least 6 backcrosses with selfing to achieve 99% homozygosity (Allard, 1960). In the present study, the data obtained is only from the first backcross of unselected segregating material in which the genetic expression of the resistance factor could be masked or partially masked. However, from the results of the present study, it would seem that the resistance factor(s) is multigenic. The genes controlling these factor(s) are heritable and can be recovered from a segregating progeny.

It is suggested that further studies should include a rigid selection of parents so as to obtain lines that are homozygous for the character(s) that reduce or inhibit feeding in pod walls by C. tomentosicollis.

Backcrossing R X R, did produce a higher number of resistant plants

suggesting that in the next cycle of screening, more selection pressure must be exerted in order to eliminate escaped individuals and increase the level of resistance.

#### 4.5. Results of Field Plot Trials

The pest populations were generally low during the season, however, the trials were attacked by an array of insect pests from seedling to harvest (Table IV). The ANOVA (Appendix Table XIV) for the total seasonal pest distribution indicated no significant differences at 5% probability level between the treatments. However there was a trend for some parental lines to have many more pod sucking bugs than the other (Figure 9). Nonsignificant results were also obtained in ANOVA of individual pod bug species distribution among the treatments (Table IV). The analysis for % damaged seeds (Appendix Table XV) also showed no significant differences between the parental lines tested. The lines IRAT 146 and TVU 2870 had 40.4% and 45.7% mean damaged seeds when compared to Ife Brown the standard susceptible with 55.8% (Appendix Table XV).

However, when the adjusted weights of good seed per plot based on the average number of pods/plant for 85 plants/plot were analyzed, the results indicated that TVU 2870 had a significantly higher yield (897 gms) than the other parental lines with a 190% increase when compared to Ife Brown (Figure 10, Tables V, XV and XVI).

Some points are worth noting from the various results obtained from the field plot trials attacked by an array of pod sucking bugs each with possible differences in food preference. C. tomentosicollis accounted for 41% of the pod bug population while the other species accounted for nearly 59% (Table IV). In such a situation, nonsignificant results are

TABLE IV  
FIELD PLOT PESTS SAMPLING DATA TREATMENTS

Pest Species	Total Per Species	% of Total Bug Population	VITA 5	IRAT-146	TVX 2940-01D	TVU 7133	TVU 2870	Ife Brown
<u>Clavigralla</u> <u>tomentosicollis</u>	290	41.1%	19 2.7%	45 6.4%	45 6.4%	75 10.6%	16 2.3%	90 12.7%
<u>Clavigralla</u> <u>shadabi</u>	112	15.9%	14 2%	15 2.1%	31 4.4%	18 2.6%	15 2.1%	19 2.7%
<u>Aspavia armiga</u>	135	19.1%	28 4%	24 3.4%	14 2%	26 3.7%	11 1.5%	32 4.5%
<u>Nezara viridula</u>	157	22.2%	21 3%	23 3.2%	34 4.8%	33 4.7%	27 3.8%	19 2.7%
Other Pests	12	1.7%	1 .1%	0 0%	5 .7%	3 .4%	2 .3%	1 .1%
TOTAL	706	100%	83 bugs	107 bugs	129 bugs	155 bugs	71 bugs	161 bugs

Field plots were sampled weekly when pods had been formed and infestation observed. The pest populations were low because the bugs were late in appearing and sampling was only done four times.

Riptortus dentipes, a prevalent pod sucking bug appeared rather late in the season and being fast fliers, they usually took off before the sampling cloth was laid down.

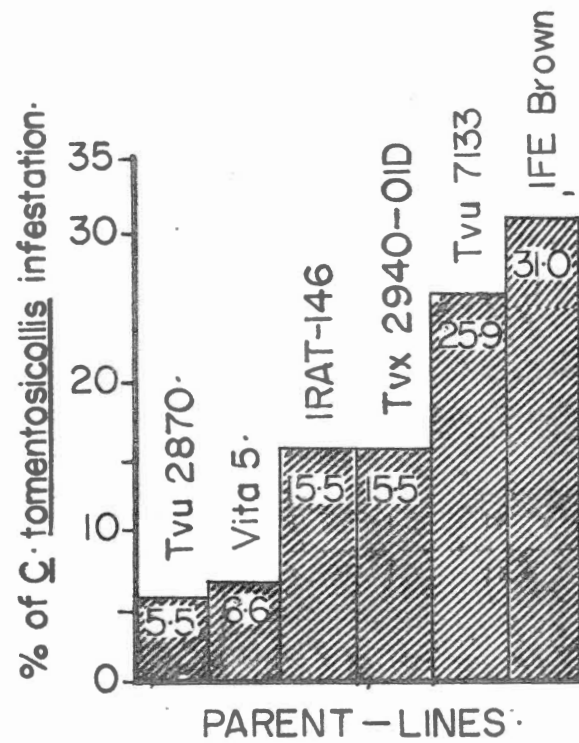


Figure 9. Preferential Infestation of Parental Lines by *C. tomentosicollis* on Field Plot Trials

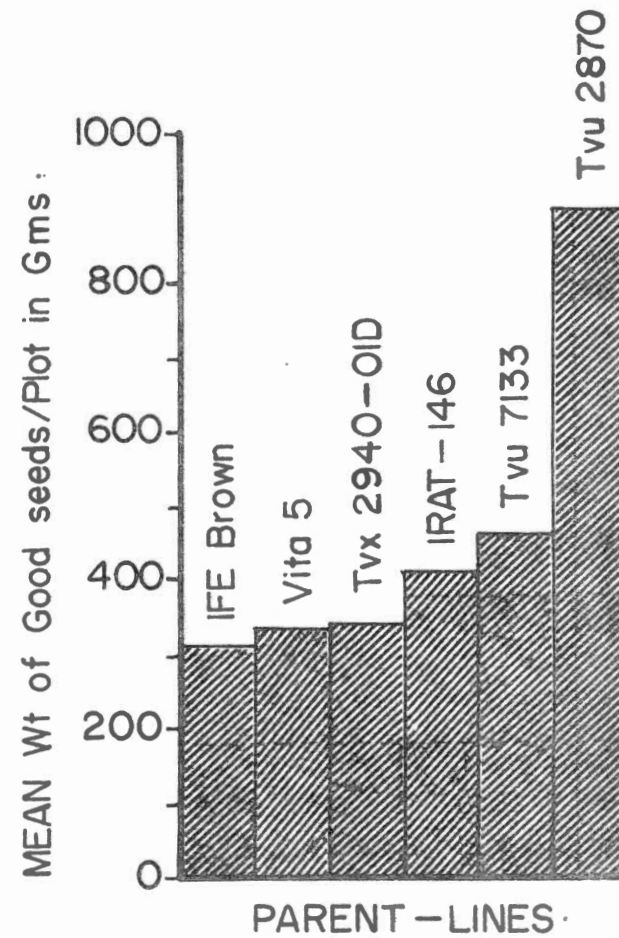


Figure 10. Histogram of Adjusted Mean Weights Good Seeds Based on the Average Number of Pods for 85 Plants/Plot

TABLE V  
ADJUSTED MEAN WTS. OF GOOD SEEDS BASED ON THE AVERAGE  
NUMBER OF PODS/PLANT FOR 85 PLANTS/PLOT

Parent Lines	Mean Wt. <sup>1</sup> Grams	% Increase
TV <sub>U</sub> 2870	897 b <sup>2</sup>	190%
TV <sub>U</sub> 7133	466 a	50%
IRAT-146	412 a	30%
TV <sub>X</sub> 2940-01D	339 a	10%
VITA 5	336 a	10%
Ife Brown (Check)	312 a	--
F-Value	7.97**	
LSD	232	

<sup>1</sup>Each number is a mean value of 5 replicates.

<sup>2</sup>Means followed by the same letter do not differ significantly at 5% probability level.

not unexpected. The significant yield from the line TVU 2870 cannot be attributed to resistance (or low preference) by C. tomentosicollis alone (Figures 9 and 10). From these data, it would seem that while C. tomentosicollis showed less preference for TVU 2870 and VITA 5, the other pod sucking bugs apparently fed more on VITA 5 and other lines except TVU 2870, hence its significant higher yield (Figure 9). Although the food preferences for these other pod sucking bugs is not known, it would seem that TVU 2870 was less preferred by them or TVU 2870 is an escaped line (Appendix Table XVI and Figure 10).

#### 4.6. Results of No-Choice Test on Live Plants

Data on the following parameters were recorded in order to determine if significant biological differences occurred when C. tomentosicollis did not have an opportunity to choose its food source:

Mean number of punctures per line

Mean number of damaged seeds per pod per line

Mean number of seeds per pod per line and the

Number of dead insects after feeding on a parental line

##### 4.6.1. Results of Puncture Counts

The results of ANOVA for both levels of infestation (1 bug/pod and 2 bugs/pod) were highly significant at 5% probability level (Appendix Tables XVII and XVIII).

From these data (Table VI), 3 distinct levels of damage are distinguished. The lines TVU 2870 and IRAT 146 consistently show the least number of puncture counts at both levels of infestation. TVX 2940-01D, TVU 7133 and Ife Brown are intermediates between these and VITA 5 with

TABLE VI  
 MEAN NUMBER OF PUNCTURES ON 75 PODS/PARENTAL LINE  
 EXPOSED TO TWO LEVELS OF INFESTATION BY POD  
 BUGS IN A NO-CHOICE TEST

Parental Lines	1 Bug/Pod/72 Hours	2 Bugs/Pod/72 Hours
VITA 5	22.19 c <sup>1</sup>	30.59 c <sup>1</sup>
Ife Brown (Standard)	19.36 c	24.17 b
TVU 7133	16.40 b	28.23 c
TVX 2940-01D	15.36 b	20.07 a
IRAT 146	10.96 a	18.23 a
TVU 2870	10.60 a	19.63 a
F-Value	18.61**	12.61**
LSD at 5% Level	2.95	4.00

<sup>1</sup>Numbers followed by the same letter do not differ significantly at 5% probability level.

the largest number of punctures. These data from no-choice tests are generally in agreement with the results obtained in parental lines preference tests in which TVU 2870 and IRAT 146 had the lowest numbers of feeding punctures, TVU 7133 and TVX 2940-01D were intermediates, while VITA 5 and Ife Brown the Susceptible Check had the greatest numbers of feeding punctures.

#### 4.6.2. Number of Damaged Seeds/Pod

The results of ANOVA for both levels of infestations (1 bug/pod and 2 bugs/pod) indicated significant differences at 5% probability level between the parental lines tested (Appendix Tables XIX and XX).

Here again, 2 distinct levels of damage are distinguished from these data (Table VII). The parental lines of IRAT 146 and TVU 2870 appear to be constant with the smaller mean numbers of damaged seed per pod. The lines Ife Brown and VITA 5 are constant with larger mean numbers of damaged seeds per pod, while the lines TVU 7133 and TVX 2940-01D are intermediates at both levels of infestation. Once more, these data are supportive of the results obtained in puncture counts of parental lines preference tests (Table III).

#### 4.6.3. Mean Number of Seed/Pod/Parental Line

As shown in Table VII, the results of the ANOVA of the mean number of seeds per pod/parental line (Appendix Tables XXI and XXII) were highly significant indicating different yield potentials for individual lines. In order to quantify and determine the actual damage caused by C. tomentosicollis feeding in terms of seed losses, the mean % of damaged seeds for each line was computed.

TABLE VII

PERCENTAGE DAMAGED SEED BASED ON THE MEAN NUMBER OF SEED/POD/PARENTAL LINES  
WHEN EXPOSED TO TWO LEVELS OF INFESTATION BY POD BUGS IN NO-CHOICE TEST

Parental Lines	1 Bug Per Pod for 72 Hours				2 Bugs Per Pod for 72 Hours			
	Mean Number of Seed/Pod				Mean Number of Seed/Pod			
	Total Seed	No Damaged	% Damage		Total Seed	No Damaged	% Damage	
Ife Brown (Standard)	12.97 <sup>1</sup> b <sup>2</sup>	6.83 <sup>1</sup>	56.6%		12.52 <sup>1</sup> b <sup>2</sup>	9.11 <sup>1</sup>	72.9%	
VITA 5	11.51 a	6.12	53.2%		12.21 b	8.41	68.9%	
TVU 7133	13.35 bc	4.24	31.8%		14.17 c	8.24	58.2%	
TVX 2940-01D	14.21 c	6.05	42.6%		13.79 c	7.95	57.6%	
TVU 2870	11.23 a	4.73	42.2%		10.85 a	7.09	65.4%	
IRAT 146	14.07 <sup>**</sup> c	4.12	29.3%		15.03 cd	8.40	55.9%	
F-Values	16.36**				24.13**			
LSD at .05 level	0.90				0.90			

<sup>1</sup>Each number is a mean of 75 replicates.

<sup>2</sup>Numbers followed by the same letter do not differ significantly at 5% probability level.

$$\% \text{ Damaged Seed} = \frac{\text{Mean Damaged Seed/pod/line}}{\text{Mean Seed/pod/line}} \times 100.$$

As shown in Tables VI and VII, the relationship between punctures in the pod wall and damage seed is not clear cut. It should be noted that the lines with the greatest number of seeds had the lowest percentage of damaged seeds. This trend was evident even with the susceptible line TVU 7133. However, it should be noted that the number of punctures in seed was not recorded but only whether the seed was damaged or undamaged. A damage seed could have 1 or more punctures. Furthermore, experience had shown that it is more difficult to detect punctures in seeds than in pod walls. Therefore the data on seed puncture is not as precise as the pod wall data. The importance of pod wall punctures as compared to seed punctures will be discussed in the discussion section of this study.

#### 4.6.4. Results: Number of Dead Bugs

The results of ANOVA for this parameter were nonsignificant, indicating that there were no apparent toxic factors(s) which resulted in a high rate of mortality when the bugs fed on pods of the parental lines tested. This data suggests that the pod walls of the most resistant lines (Table VI) had factor(s) which inhibited feeding and bugs fed at a reduced rate but sufficient to sustain the bugs for the duration of the test (72 hours).

#### 4.7. Results of Biological Studies of

##### C. tomentosicollis

The results of the laboratory studies of adult longevity and the nymphal development period of C. tomentosicollis are presented below.

#### 4.7.1. Longevity and Puncture Studies of Adult Bugs

The results of this investigation (Table VIII) revealed that the males of C. tomentosicollis lived for 46.7 days ( $\pm 6.2$ ), ranging from 16 to 102 days while the female bugs had a lifespan of 61.5 days ( $\pm 6.2$ ) with the same range.

The results of the analysis of punctures made during feeding showed that the female bugs feeding averaged 3.82 punctures a day and 3.72 for male bugs.

Although the mean puncture counts for adult bugs in this study are rather low when compared with other puncture results, the following reasons seem to account for this inconsistency. In the no-choice experiments in which bugs were confined with pods for 72 hours, it was frequently observed that whenever the bugs succeeded to penetrate into the seeds in the first 2 to 4 attempts they did not make anymore punctures, indicating that their nourishment needs had been satisfied. Only Ife Brown, the susceptible standard, was used in this study and it is very probable that the bugs easily succeeded in puncturing into the seeds and obtained their needed nourishment and did not make many probes.

Another probable reason is limited space that reduced the mobility of the bugs. The bugs were reared in small mesh cages (17 cm high by 7.5 cm wide) for more than 60 days. These bugs are strong fliers in their natural habitat. In larger test cages (28 x 28 x 40 cm) in which the bugs enjoyed greater mobility, high puncture counts were recorded, indicating greater energy needs. It becomes evident that the amount of feeding is dependent on the energy needs of the insects. A high correlation (.895) was shown to exist between the total feeding punctures and

TABLE VIII  
 LONGEVITY AND FEEDING PUNCTURES OF 24 PAIRS OF ADULT  
C. TOMENTOSICOLLIS WHEN CONFINED IN  
 LABORATORY CAGES

Variables	Males	Females
Number of Individuals Tested	24	24
Mean Longevity (in Days)	46.7	61.5
Standard Deviation (Days)	31.7	30.4
C. V. %	67.9%	49.4%
Range (Days)	16-102	16-102
Average Number of Punctures	159.8	210.3
Mean Puncture Counts/Day	3.82	3.72
Standard Deviation (Punctures)	93.1	88.2
Range % (Punctures)	57-468	80-364
C. V. %	58.2%	41.9%

longevity for the male and female bugs.

During this study, it was also observed that the egg laying ability of the female bugs was very reduced and it was speculated that perhaps the narrow mesh cages in which the bugs were reared, or some other factor(s) affected the oviposition ability of the bugs. This probability became particularly high when it was noticed at a later period that the same species of bugs were ovipositing freely in larger test cages (28 x 28 x 40 cm) under the same laboratory conditions. This phenomenon seemed to lead to the tempting conclusion that cage size affected or influenced oviposition and energy needs in this species.

#### 4.7.2. Results: Nymphal Development and Damage Study

The results of this study showed that: the 1st to 3rd nymphal instars lasted for 3 days each; the 4th instars lasted for 4 days and the 5th instar nymphs lasted for 6 days (Table IX and Figure 11). The mean nymphal development period was 17.8 days ( $\pm 0.08$ ). About 55% of the nymphs took 18 days to complete development from nymph to adult while 32.5% and 12.5% took 17 to 19 days respectively. Considering the duration of the majority of the nymphs in each stage, it is concluded that the total nymphal development period of C. tomentosicollis in the laboratory was 18 days. The results of ANOVA for the puncture counts (PC) and damaged seeds (DS) by each nymphal instar (Table X and Figure 12) indicated an increasing trend from one instar to the next. During the first instar (3 days) the mean puncture counts (PC) was  $5.0 \pm .23$  and  $1.4 \pm .16$  for damaged seed (DS). This rate of damage increased progressively till the 5th instar when the mean puncture count was 24.3

TABLE IX  
DURATIONS OF DIFFERENT NYMPHAL INSTARS OF  
C. TOMENTOSICOLLIS STAL REARED IN  
THE LABORATORY

Duration of Instars in Days	1st Instar		2nd Instar		3rd Instar		4th Instar		5th Instar	
	No. of Bugs	%	No. of Bugs	%	No. of Bugs	%	No. of Bugs	%	No. of Bugs	%
2	12	15%	20	25%	8	10%	-	-	-	-
3	63	78.75%	57	71.25%	68	85%	51	63.75%	-	-
4	5	6.25%	3	3.75%	2	2.5%	29	36.25%	-	-
5	-	-	-	-	2	2.5%	-	-	29	36.25%
6	-	-	-	-	-	-	-	-	42	52.50%
7	-	-	-	-	-	-	-	-	9	11.25%
TOTAL	80	100%	80	100%	80	100%	80	100%	80	100%
<sup>1</sup> Mean $\pm$ S.E Per Instar	2.9 $\pm$ .54 Days		2.79 $\pm$ .55 Days		2.99 $\pm$ .60 Days		3.36 $\pm$ .54 Days		5.75 $\pm$ .72 Days	

Mean total nymphal development period = 17.83  $\pm$  .08 Days

<sup>1</sup>Each number is a mean value of 80 replicates.

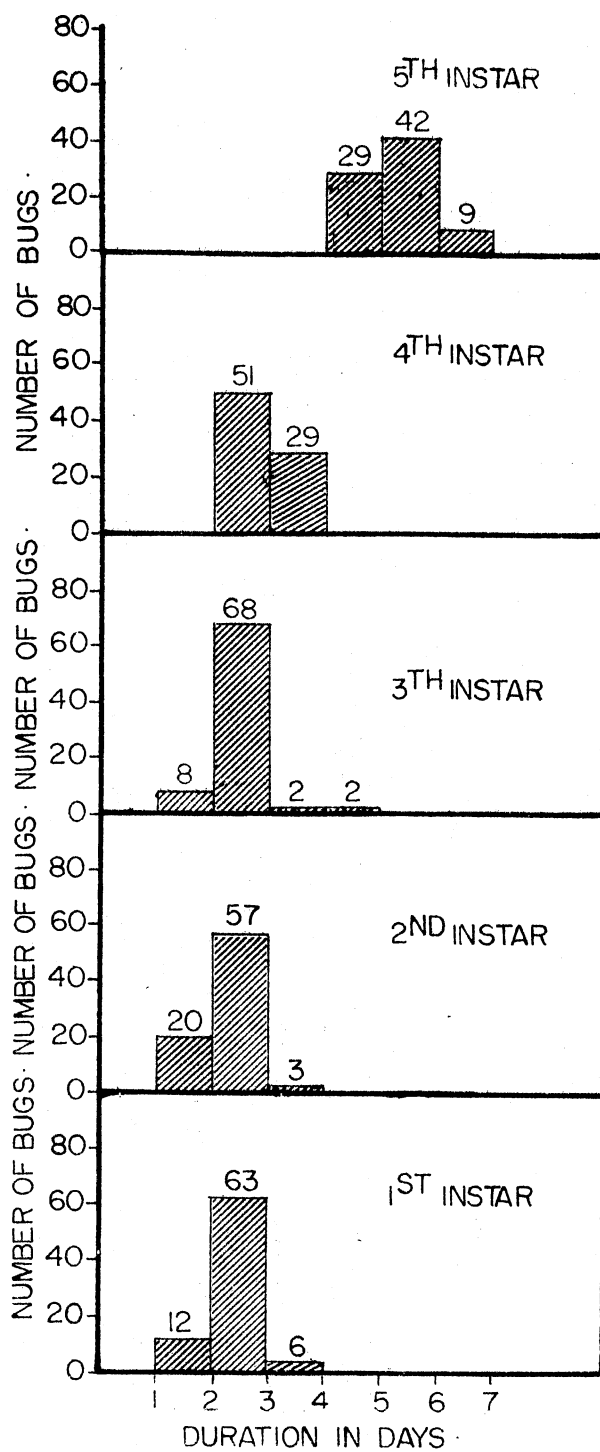


Figure 11. Duration of Different Nymphal Instars of *C. tomentosicollis* Reared in the Laboratory

TABLE X

MEAN + S.E. OF PUNCTURES AND DAMAGED SEEDS PER INSTAR  
OF C. TOMENTOSICOLLIS STAL IN THE LABORATORY

Stage	Puncture Counts		Damaged Seeds	
	Mean <sup>1</sup> $\pm$ S.E.	Range	Mean <sup>1</sup> $\pm$ S.E.	Range
1st INSTAR	5.0 $\pm$ .23	10	1.4 $\pm$ .16	0-5
2nd INSTAR	7.9 $\pm$ .32	17	2.6 $\pm$ .15	0-7
3rd INSTAR	10.7 $\pm$ .37	16	3.6 $\pm$ .17	1-8
4th INSTAR	14.2 $\pm$ .45	19	3.6 $\pm$ .19	0-10
5th INSTAR	24.3 $\pm$ .54	35	5.6 $\pm$ .20	2-11
5-Day Old Adult	53.0 $\pm$ 1.50	57	12.2 $\pm$ .30	5-18

<sup>1</sup>Each number is a mean value of 80 replicates.

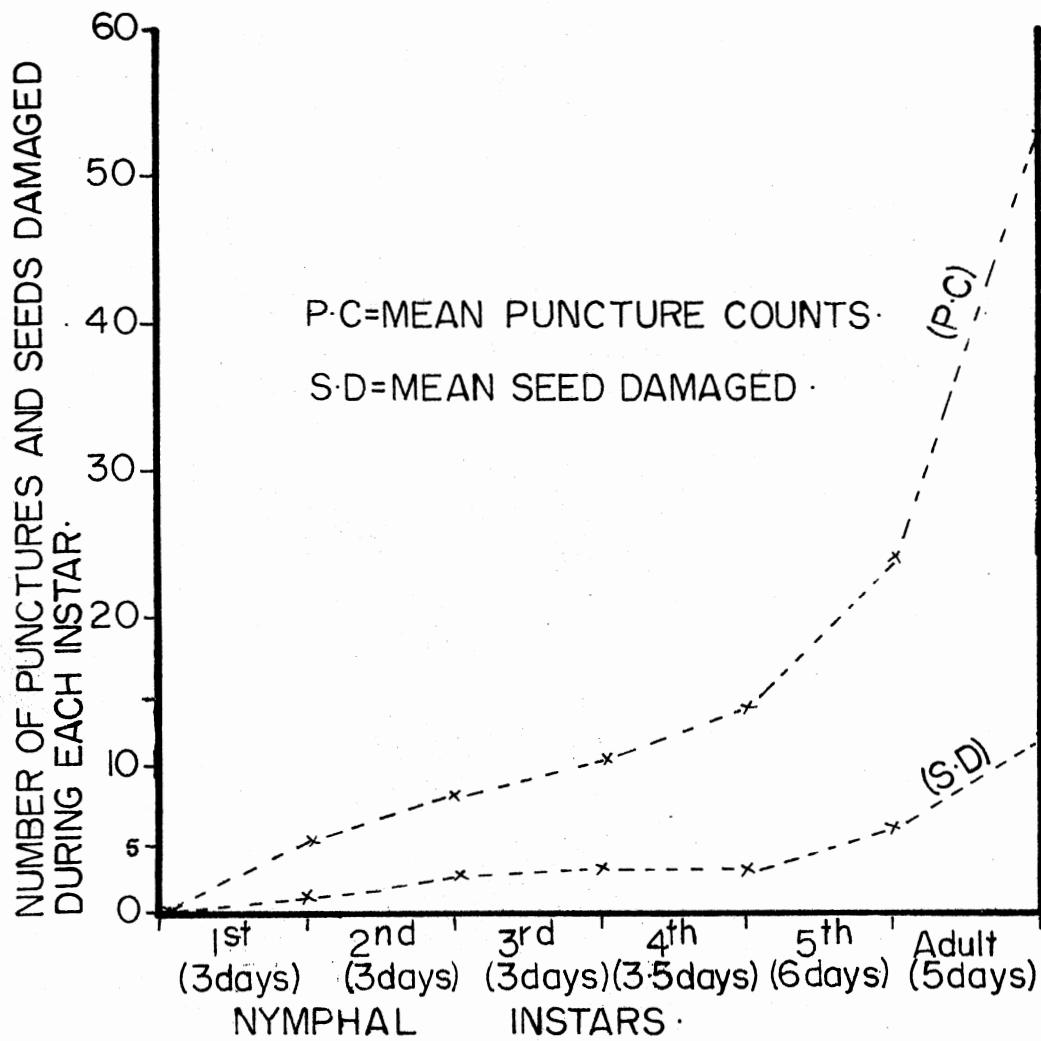
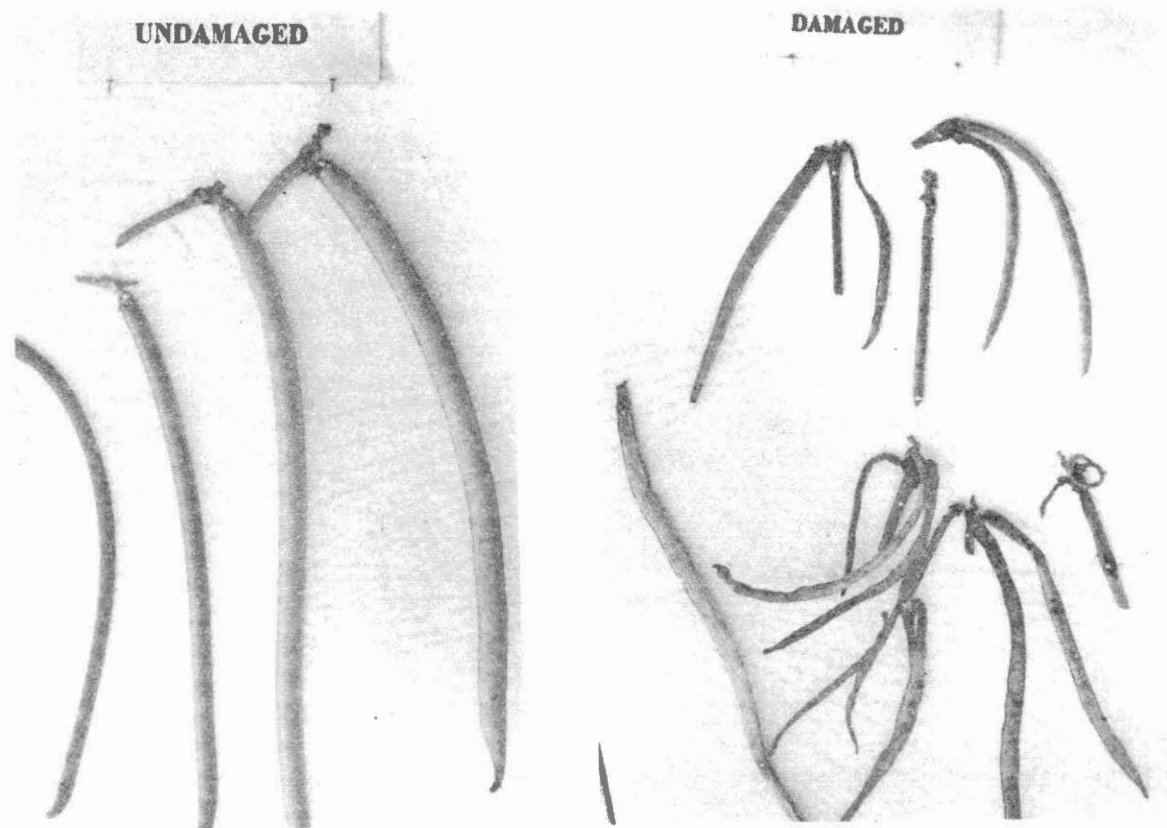


Figure 12. Nymphal Development, Feeding Punctures and Seeds Damaged During Each Instar



(a)

(b)

Figure 13. Contrast Between Undamaged (a) and Damaged (b) Cowpea Pods by C. tomentosicollis

It was frequently observed in the field and rearing cages that the 1st to 3rd instar nymphs tended to aggregate at the food source or beneath the leaves (Figure 14). However, at the later 3rd instar stage these nymphs started dispersing and 5th instar nymphs did not aggregate. The reason(s) for this behavior was not investigated, but Hodjat (1967) suggested a number of reasons why individuals of a species may aggregate. These individuals may be:

1. Attracted towards a favorable food source or habitat
2. For mutual protection and
3. To conserve either energy or water.

Egwuatu and Taylor (1976) encountered the same aggregation tendencies in C. tomentosicollis and observed that the crowding seemed to lead to a type of color dimorphism. From their findings, nymphs reared singly had no dark color while nymphs reared in groups of tens and twenties per cage, about 39% and 61%, respectively, of the 5th instar nymphs were black. Ommochromes are a group of pigments derived from tryptophan amino acids, and are widely distributed in masking pigments such as yellow, red, brown and pink body pigmentation. Ommochromes are produced during protein breakdown in animal systems (Chapman, 1969). Cowpea has a high protein content between 22.9 and 34.6 % Boulter et al., 1973) and this protein contains 24 mg tryptophan per gram of essential amino acids (EAA) (Oyenuga, 1968). In locusts, darkening occurs progressively at 26°C and increases the sclerotization of the cuticle (Chapman, 1969). It could be assumed that the crowding of the 1st to 3rd instars of C. tomentosicollis serve to conserve warmth which speeds up the maturation process; thus the 4th and 5th instar nymphs that have acquired stronger cuticles are dispersed. When nymphs are newly hatched



Figure 14. Aggregation Behavior of 1st to 3rd  
Instar Nymphs of C. tomentosicollis

during protein breakdown in animal systems (Chapman, 1969). Cowpea has a high protein content between 22.9 and 34.6 percent (Boulter et al., 1973) and this protein contains 24 mg tryptophan per gram of essential amino acids (EAA) (Oyenuga, 1968). In locusts, darkening occurs progressively at 26°C and increases the sclerotization of the cuticle (Chapman, 1969). It could be assumed that the crowding of the 1st to 3rd instars of C. tomentosicollis serve to conserve warmth which speeds up the maturation process; thus the 4th and 5th instar nymphs that have acquired stronger cuticles are dispersed. When nymphs are newly hatched or molted they are soft bodied and generally reddish-pink in color. They gradually become greyish-brown and then darken with a stronger cuticle. The dark coloration is believed to be a protective camouflage mechanism against predators.

The importance of the nymphal aggregation tendency is related to the damage caused by heavy debilitating group feeding on pods (Figure 14). The other problem posed by this behavior is the clumped distribution which is a hinderance for any scouting or sampling program intended to monitor and establish economic threshold for this pest.

## CHAPTER V

### DISCUSSION AND CONCLUSIONS

In a breeding program that is designed to incorporate insect resistance into agronomically improved cultivars, techniques must be available to recover individual plants from segregating progenies. It is also important to have as much information as possible on the inheritance of the resistant characters in order to facilitate the recovery of resistant plants.

This study represents the first attempt to design a laboratory screening procedure to accomplish these objectives for pod bug resistance in cowpeas.

#### 5.1. Discussion

The importance of using pod wall feeding punctures as a selection criterion for cowpea resistance is based on the nature of damage caused by the pod bug feeding activity. The bugs feed by inserting their stylets into the succulent pod walls of the cowpeas. The juice (essential nutrients for pod and seed development) is sucked out, and depending on the feeding intensity, the pods may recover, or are debilitated, shrivel up and dry (Figure 13b). The stylet may penetrate into the developing seed and the juice sucked out. In this case, the development and future viability of the seed is reduced. Furthermore, during the feeding, the bugs may introduce pathogenic fungi or feeding punctures may offer easy

entrance for these organisms.

The fungus, N. coryli, which causes pod rot is transmitted by pod bugs. Therefore it is important to select cowpea lines which have factor(s) that inhibit or reduce feeding on the pod walls.

Based on previous preliminary laboratory tests, the resistant and susceptible lines were selected and crosses made for this genetic study, but no data was available on the segregation ratio, mode of inheritance or on the number of plants required to obtain this information. The parental lines used in this study appeared to be genotypically stable lines which had not been evaluated to determine if there was segregation for factor(s) which reduced the feeding activity of C. tomentosicollis.

As shown in Tables III, VI, and VII, TVU 2870, IRAT 146 and TVX 2940-01D did exhibit significant differences from the susceptible lines (TVU 7133 and VITA 5). This was demonstrated in preference and in no-choice tests. Data obtained from replicated field plots in which a natural population of pod-sucking bugs to which these lines had not been evaluated, also showed this trend. In the laboratory bioassay, TVU 2870, IRAT 146 and TVX 2940-01D, designated as resistant lines had significantly fewer punctures in pod walls and in the developing seeds. Furthermore, when these resistant parental lines were compared with the susceptible lines in the evaluation of the  $F_1$  and  $F_2$  progenies, and in the evaluation of the first backcross generation, reduction of 48% in feeding punctures were found (Table XI).

The  $F_2$  evaluation results may have been confounded by early instar nymphal feeding. However, when the backcross progenies were being evaluated, the nymphs were not a factor and the resistant lines had over 65% fewer punctures than the susceptible lines indicating that clear cut

TABLE XI

SUMMARY OF PARENTAL LINES REACTION WITH *C. TOMENTOSICOLLIS* IN NO-CHOICE,  
PARENTS PREFERENCE, BACKCROSS AND F<sub>2</sub> PROGENY TESTS

Mean Puncture Counts Per Test					
Parental Lines	Resistance Reaction	No-Choice Test	Parents Preference Test	Backcross Test	F <sub>2</sub> Test
IRAT-146	Resistant	10.96	1.89	10.00	16.03
TVU 2870	Resistant	10.60	2.08	8.95	20.53
TVX 2940-01D	Resistant	15.63	3.80	5.25	14.97
		$\bar{x} = 12.40$	$\bar{x} = 2.38$	$\bar{x} = 8.07$	$\bar{x} = 17.18$
TVU 7133	Susceptible	16.40	4.61	18.10	30.35
VITA 5	Susceptible	22.19	4.89	28.10	36.13
		$\bar{x} = 19.30$	$\bar{x} = 4.75$	$\bar{x} = 23.10$	$\bar{x} = 33.24$
Ife Brown	Standard Susceptible	19.36	15.39	15.44	26.40
% Puncture Counts Reductions		36%	51%	65%	48%

The % Puncture Counts (Damage) reduction is computed as follows:

$$100 - \left( \frac{\text{Mean Puncture counts resistant lines}}{\text{Mean puncture counts susceptible lines}} \right)$$

differences occurred (Figure 8 and Table XI).

It will, however, be noted that all of the resistant lines produced pods that the adult bugs found acceptable for feeding. One possible explanation is that these resistant parental lines were heterozygous for the character(s) that caused inhibition of feeding by adult bugs. These lines had not undergone any selection for this character before being used as parents. There was no evidence to suggest that segregation was occurring until this study was completed. Phenotypically, they appeared to be uniform.

When the lines TVX 2940-01D and IRAT 146; TVU 2870 and TVX 2940-01D (Resistants X Resistants) were evaluated, this heterozygosity was evident. It was more magnified in crosses with susceptible lines. However, as shown in the bar graphs (Figure 8, f, g) the resistance was heritable, evidenced by the recovery of a large number of progeny that inhibited adult feeding.

It is not possible from this data to estimate the number of genes involved but evidence clearly indicates that the resistance is multigenic.

From the data shown in Tables VIII, IX and X, it becomes evident why this insect is such a serious pest on cowpea. Only 18 days are required for development and adults live longer than 60 days. During the entire life cycle, all stages are causing damage to the crop. This insect is among the major limiting factors in the production of cowpea and other grain legumes in Africa. This study addresses the great need and importance of developing insect resistant crops for areas where insecticide utilization is not well known, unavailable or too costly.

## 5.2. Conclusions

Resistant parent lines were tested in numerous experiments and the resultant levels of resistance were identified. The resistant parental lines tested exhibited heterogeneity. It is strongly suggested that these lines should be subjected to a rigorous selection pressure in order to ensure that homozygous material is used for future crosses.

Data from the F<sub>2</sub> generations indicated that the factors that caused reduced feeding on the pod walls was heritable but not as a simple dominant factor. The distribution pattern of pod punctures on parental lines and progenies studied suggests that the resistance is multigenic.

Resistant progeny recovered in the backcross generations included plants with a level of resistance equal to that of the parent lines.

The laboratory bioassay techniques used in this study were able to recover resistant progenies, but were not precise enough to measure the number of genes involved.

The information obtained from the biological studies explains why C. tomentosicollis is such a serious pest (short developmental period of nymphs), permitting several generations during a growing season, and long adult longevity makes this insect a pest of the first order of importance in cowpea production.

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APPENDIX

TABLES

TABLE XII  
ANOVA FOR PUNCTURE COUNTS OF POD POSITIONS

Source of Variation	DF	SS	MS	F-Value
Mean	1	2563.28		
Positions	4	131.32	32.83	1.01 NS*
Plant Positions	45	1465.40	32.56	
Total	50	4160.00		

\*NS - F-Value is not significant at 5% probability level.

TABLE XIII  
ANOVA OF PUNCTURE COUNTS OF PARENTAL LINES EVALUATION

Source of Variation	DF	SS	MS	F-Value
Correction for Mean	1	6122.69		
Replication	35	771.32	22.04	
Lines	5	4655.59	931.12	51.20**
Experimental Error	175	3182.41	18.19	
Uncorrected Total	216	14732.00		

Fisher's Protected LSD at 5% Probability Level = 1.97

TABLE XIV  
ANOVA FOR TOTAL SEASONAL PEST DISTRIBUTION ON  
COWPEA LINES IN FIELD TRIALS

Source of Variation	DF	SS	MS	F-Value
Replications	4	1827.1	456.8	
Treatments	5	1132.8	226.6	1.13NS
Error	20	4020.9	201.0	
Total	29	6980.8	240.7	

LSD at 5% Probability Level = 18.7

NS = F-value is not significant at 5% probability level. Duncan's Multiple Range Test: Ife Brown, 30.4; TVu 7133, 30.2; TVx 2940-01D, 25.4; IRAT 146, 22.0; VITA 5, 17.0; TVu 2870, 14.2.

TABLE XV  
ANOVA FOR PERCENTAGE DAMAGED SEED/LINE IN FIELD TRIALS

Source of Variation	DF	SS	MS	F-Value
Replications	4	385.12	96.28	
Lines	5	1041.11	208.22	.759NS
Error	20	5479.67	273.98	
Total	29	6905.90	238.13	

LSD at 5% Probability Level = 21.87

NS = F-value is not significant at 5% probability level. Duncan's Multiple Range Test: TVu 7133, 56.15; Ife Brown, 55.76; TVx 2940-01D, 53.79; VITA 5, 46.27; TVu 2870, 45.75; IRAT 146, 40.44.

TABLE XVI

ANOVA FOR YIELD OF FIELD PLOT TRIALS: ADJUSTED WEIGHT OF  
GOOD SEEDS BASED ON THE AVERAGE NUMBER OF PODS/PLANT  
FOR 85 PLANTS/PLOT

Source of Variation	DF	SS	MS	F-Value
Replications	4	83374.88	20843.72	
Lines	5	1228644.16	245728.83	7.97**
Error	20	616616.39	66504.67	

LSD at 5% Probability Level = 232.05

TABLE XVII

ANOVA OF PUNCTURE COUNTS IN NO-CHOICE TEST ON  
LIVE-PLANTS, 1 BUG/POD/72 HOURS

Source of Variation	DF	SS	MS	F-Value
Replications	74	11586.44	156.57	
Lines	5	7822.52	1564.50	18.61**
Error	370	31110.65	84.08	
Total	449	50519.61	112.52	

Fisher's Protected LSD at 5% Probability Level = 2.95

TABLE XVIII

ANOVA OF PUNCTURE COUNTS IN NO-CHOICE TEST ON  
LIVE-PLANTS, 2 BUGS/POD/72 HOURS

Source of Variation	DF	SS	MS	F-Value
Replications	74	23741.72	320.83	
Lines	5	9570.95	1914.19	12.61**
Error	370	56181.72	151.84	
Total	449	89494.39	199.32	
Fisher's Protected LSD at 5% Probability Level = 3.96				

TABLE XIX

ANOVA OF DAMAGED SEEDS IN NO-CHOICE TEST ON  
LIVE-PLANTS, 1 BUG/POD/72 HOURS

Source of Variation	DF	SS	MS	F-Value
Replications	74	1447.39	19.56	
Lines	5	479.50	95.90	9.60**
Error	370	3697.33	9.99	
Total	449	5624.22	12.53	
Fisher's Protected LSD at 5% Probability Level = 1.02				

TABLE XX  
ANOVA OF DAMAGED SEEDS IN NO-CHOICE TEST ON  
LIVE-PLANTS, 2 BUGS/POD/72 HOURS

Source of Variation	DF	SS	MS	F-Value
Replications	74	1673.76	22.62	
Lines	5	164.94	32.98	2.48*
Error	370	4921.89	13.30	
Total	449	6760.59	15.06	

Fisher's Protected LSD at 5% Probability Level = 1.17

TABLE XXI  
ANOVA OF TOTAL SEEDS/POD IN 75 PODS PER LINES IN  
NO-CHOICE TEST ON LIVE-PLANTS,  
1 BUG/POD/72 HOURS

Source of Variation	DF	SS	MS	F-Value
Replications	74	612.11	8.27	
Lines	5	602.36	120.47	16.36**
Error	370	2723.97	7.36	
Total	449	3938.44	8.77	

LSD at 5% Level = 0.87

TABLE XXII

ANOVA OF TOTAL SEEDS/POD IN 75 PODS PER LINES IN  
NO-CHOICE TEST ON LIVE-PLANTS,  
2 BUGS/POD/72 HOURS

Source of Variation	DF	SS	MS	F-Value
Replications	74	557.72	7.54	
Lines	5	862.92	172.58	24.13**
Error	370	2646.25	7.15	
Total	449	4066.89	9.06	
LSD at 5% Level = 0.86				

VITA<sup>2</sup>

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Master of Science

Thesis: THE INHERITANCE OF RESISTANCE IN COWPEA (VIGNA UNGUICULATA (L) WALP) TO POD BUGS CLAVIGRALLA TOMENTOSICOLLIS STAL)

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