TECHNIQUES FOR SCREENING COWPEAS, *VIGNA UNGUICULATA* (L.) WALP. IN THE LABORATORY FOR RESISTANCE TO *CALLOSOBRUCHUS MACULATUS* FAB. (COLEOPTERA, BRUCHIDAE)

by

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A MASTER'S THESIS

submitted in partial fulfillment of the

requirements for the degree

MASTER OF SCIENCE

Department of Entomology

KANSAS STATE UNIVERSITY
Manhattan, Kansas

1973

Approved by:

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INTRODUCTION

The cowpea, *Vigna unguiculata* (L.) Walp, is an important tropical and sub-tropical annual legume crop grown chiefly for its seed (green and dry) and for fodder. In West Africa, where the crop is a major protein source, various forms of bean foods are consumed (Oyenuga, 1955; Stanton, 1966). In the drier parts of East Africa the leaves are eaten as a substitute for spinach (Mehta, 1970). In the United States of America several varieties bear different local names. Cowpeas are also extensively grown in China, the West Indies and South America.

There was disagreement among scientists as to the center of origin of this crop, both India and Africa having been suggested. Faris (1963, 1965) after detailed biosystematic analysis involving extensive cytological and morphological studies of some 13 wild *Vigna* species and approximately 700 cultivated cowpeas concluded that *V. unguiculata* originated in West Africa. Nwanze (1971), in a pilot program using computer aided analysis of morphological variation in wild and cultivated cowpeas, suggested Nigeria as the most probable center of origin.

The wide range of growth habits among varieties has been classified as erect, vining, semi-vining and bushy types. Growth habits change with season within a single variety (Ligon, 1958). Some varieties produce erect, bush-type plants one year and more or less prostrate, vining plants another year or vary during the same year with different planting dates or localities.

Early varieties mature within 50-60 days and late varieties within 90 days. Flowers are self-pollinated but many are shed unfertilized with only one to four pods developing on each peduncle.

Seeds are highly variable in shape, color, size and texture of seed coat. Seed shape varies from globular to kidney with intermediate forms of ovate, ovoid or rhomboid. Color may be white, cream, red, brown, yellow, or black and sometimes variously mottled or speckled. Three categories of seed coat texture are recognized, namely, smooth, rough (sometimes wrinkled) and cracked. Size varies from 0.4 to 1.2 cm in length and from 0.3 to 1.0 cm in width.

During the evolution of insect-plant relationship it is believed that both have developed adaptive patterns. Applebaum (1964) theorized that while legumes have evolved protease inhibitors as defense mechanisms against insects, bruchids have overcome this by fulfilling protein requirements through metabolic pathways insensitive to protease inhibition. A similar theory was arrived at by Bell and Janzen (1971) regarding the ability of the larvae of *Caryedes brasiliensis*, one of the only two known bruchids that can feed successfully on *Mucuna* seeds. These authors also theorize that during the initial stages of this co-evolution, there must have been a strong selection favoring the evolution of a bruchid genotype capable of metabolizing or detoxifying L-dopa.

Severe losses occur to the cowpea crop due to heavy insect attack in the field and in storage. The following insects have been reported associated with the crop: Callosobruchus maculatus (Fab.) which is by far the most notorious, C. chinensis (Lin.), C. rhodesianus (Pic.), Bruchidius atrolineatus (Pic.), Maruca testulalis (Geyer), Laspeyresia ptychora (Meyer), Acanthoscelides obtectus (Say), and Chalcodemus aeneus (Boheman).

The cowpea weevil, Callosobruchus maculatus, is an important field-to-store pest of the cowpea. The weevil has been known to spread along with the crop due to the favorable climatic conditions in the regions where the crop grows abundantly. Being a field-to-store pest, the weevil is able to survive unfavorable seasons in the storehouse causing tremendous damage to the harvests of the previous season. Weevil damage to seeds causes a critical reduction in seed germination when three or more emergence holes are made per seed. Booker (1967) recorded 82% germination in undamaged seeds after 3 days of planting as against 66% in seeds with 3 emergence holes. Whereas Jotwani et al. (1967) recorded 4% germination in seeds with 4 emergence holes.

For a time most of the studies associated with the crop were centered on field selections of varieties least susceptible to the weevil. Field studies do not reveal the finer nuances of resistance and it should be understood that while man selects along a particular line thus leading into a single optimum, nature selects in such a disruptive way leading into many maxima of different characters. Consequently, resistance to insects in this crop, as in many others, has been "scattered" over the hundreds of varieties which have evolved. Studies in relation to resistance of the cowpea to the cowpea weevil are carried on at the Botany Department of the University of Durham, the International Institute of Tropical Agriculture in Nigeria and the University of Ife, Nigeria.

This study was to develop techniques for screening cowpea varieties in the laboratory with the hope of revealing varieties which possess some degree of resistance to the cowpea weevil. It is hoped that in the course of these studies it would eventually become feasible to interbreed different varieties with varying degrees and kinds of resistance.

LITERATURE REVIEW

Origin and Taxonomy of Insect

The origin of the cowpea weevil is uncertain though it undoubtedly originated from the Old World where it has long been associated with its host plant, the cowpea. Collected specimens now spread through the tropics over almost every museum from Brazil to the East Indies, several West African countries, India and southern United States of America. However, since the weevil would breed on many legumes it is apt to spread along with many hosts into regions formerly uninfested.

Callosobruchus maculatus (Fabricius) (Bridwell, 1929a:40) belongs to the family Bruchidae, order Coleoptera. The insect was first described by Fabricius in 1775 as *Bruchus maculatus*. Subsequent synonyms namely, *Bruchus quadrimaculatus*, Fabricius (1792); *Bruchus ornatus*, Boheman (1829); *Bruchus vicinus*, Gyllenhal (1833); *Bruchus vicinus*, Gyllenhal (1833); *Bruchus ambiguus*, Gyllenhal (1839); *Bruchus* sinuatus, Fahraeus (1839) have been reported (Southgate et al., 1957). C. maculatus is quite variable and often confused with C. analis, the former having forms which have the rufous ground color of C. analis as against a usually dark brown or black ground color (Southgate et al., 1957).

Another species, C. chinensis, is also a serious pest of the cowpea but due to the direct competition with the more aggressive cowpea weevil which is predominant on cowpeas, studies with C. chinensis center mainly on other pulses.

The sexual dimorphism in the cowpea weevil is expressed by the male elytra showing a black ground color along the lateral margins and apices, whereas elsewhere being testaceous (Plate I). Pubescence is chiefly golden with scale-like hairs. The pygidium of the male also demonstrates a wholly black ground color or may be testaceous with margins. Sometimes a median black line bearing a fawn or grey pubescence is present. In the female, the elytra are black along the sutural and lateral margins with a median black bar across the center. The ground color of the pygidium is testaceous with a median line bearing a white pubescence.

Under certain unfavorable conditions such as crowding, high temperature, food of low water content or very short or long photoperiods, a phase dimorphism in the adult stage appears as an abnormal form of the weevil. This has been described as a "flight" form (Utida, 1954) or "active" form (Caswell, 1960) and possesses distinct external characteristics. This form has been regarded as a migratory form similar to the phase polymorphism in locusts (Utida, 1972). Caswell (1960) suggested some evidence of a hereditary factor which is

EXPLANATION OF PLATE I

Upper: Normal male and female cowpea weevils.

Lower: Male and female of the active form.

PLATE I

independent of external conditions. Arora et al. (1967) suggested a possible hybridization between C. maculatus and C. analis as responsible for the occurrence of this form, but Bawa et al. (1972) reported reproductive incompatibility between both species.

The flight form is ellipsoidal in shape at the time of emergence, with the last abdominal segments exposed beyond the end of the elytra. The female is characterized by a ground color that is black thus giving a prominent appearance to the median black spots on the outer margins of the elytra whereas the white and golden pubescence appears grey. The pygidium has a white appearance and is not as long as in the normal female. These characteristics are also apparent, though less distinctly, in the male of the flight form (Plate I).

Further morphological differences have been shown by Yasui, according to Utida (1972). Statistical differences between the forms exist in both sexes for body weight, body length, pygidial length and wing dimensions, the latter being biologically most significant and accounts for the difference in ability to fly.

General Biology

Infestation of the crop usually begins in the field soon after pods are mature and dry. Eggs are cemented onto the dry surfaces of the pods and the larvae bore through into the seed where development continues in the storehouse. Thus it is possible for a shipment of tons of threshed cowpeas to be accepted as "free of infestation" and arrive at its destination completely ruined. Store infestation is easier to detect. Females glue the glossy oval eggs on seed surfaces. Eggs vary from 0.409 mm to 0.756 mm in length and 0.294 to 0.462 mm in width (Larson and Fisher, 1938) and are viscid in appearance when freshly laid but the egg shell becomes milky white as soon as the embryo develops and the first instar larvae penetrates into the seed. Unfertilized or unhatched eggs remain transparent (Larson and Fisher, 1938).

The first instar larvae hatches in about 3 days under favorable conditions. After molting 3 times and reaching the fourth instar, the larva constructs a cell in which it pupates (Larson and Fisher, 1938). Duration of the larval stage is determined by temperature and relative humidity. Under optimum conditions of 80°F and 60% RH there is a peak of adult emergence between 23 and 25 days after oviposition; this increases to 30-31 days when temperature drops to 75°F (Mookherjee et al., 1964). Caswell (1960) reported a mean developmental period of 25 days in March in Nigeria and 30 days in June. Active forms have a longer developmental period ranging from 32 days in March to 36 in June (Caswell, 1960). At both extremes of temperature, 15°C and 39°C, larvae normally do not hatch, but when they do, they do not develop into adults. Contrary to Scoof (1941), at 90% relative humidity the growth of fungal bodies reduce emergence of adults but little effect on the developmental period is noticed at low humidities if temperature is constant (Mookherjee et al., 1964; Booker, 1967). However, the combined egg, larval and pupal periods are decreased with higher humidities, being most pronounced at 91% RH.

At lower relative humidities and high temperatures the incidence of the active form increases (Utida, 1954; Caswell, 1960). Gill (1971) noticed an abundance of the abnormal "sterile" forms in the summer months of April to August, whereas during the winter there was a decline in incidence. Caswell (1960) in Nigeria reported a peak of appearance of the active form after February and March when the high temperature continues and from April to October with a peak in July (Prevett, 1961; Booker, 1967). Sano (1967) and Utida (1972) postulate that the incidence of the active form increases with the number of larvae developing in each bean. When the density of larval population is high, the temperature in heaps of beans rises beyond the air temperature, and should this coincide with the predominance of the middle instars (2nd and 3rd), then development is slowed down and more active forms develop. Furthermore, the absolute water content of beans may not be directly important but the change in water content at a certain intermediate stage of larval development, which is most susceptible for the induction of the flight form (Utida, 1972).

After casting off the pupal skin, adults remain quietly within the seed for one or more days during which development is completed. The newly developed adult cuts a tiny groove in the seed coat along the edge of the larval burrow, sometimes arriving at a complete disk. Emergence is aided by the adult pushing out this disk with its head and front legs, after which a round exit hole is visible.

The adult life span varies with temperature. Low winter temperatures considerably increases longevity of both the normal and abnormal adults from 5-8 and 12-18 days, respectively, in summer to 30-35 and 60-75 days, respectively in winter (Gill et al., 1971). Mookherjee et al. (1964) reported an adult lifespan of 2-3 days at 38-40°C as against 31 days at 10°C. Males and unmated females live longer than mated females. Utida (1972) observed a lifespan of 2 months for the active form at 17 or 18°C, and half as long for the normal form. Generally, the active form is more able to withstand lower temperature than the normal form.

Adults die at humidities of 0-3% and 21% after 1 1/2-2 days but they live longer (2-7 days) at higher humidities (Schoof, 1941). Larson and Fisher (1938) have reported a shorter lifespan for weevils that have no seeds on which to oviposit.

Mating in the normal form starts almost immediately after emergence. Eggs may be laid within a few minutes or hours after emergence. Oviposition occurs freely on seeds stored either in dark or in light. But a situation of continuous light or darkness would result in a high percentage of the active form (Utida, 1969). Each female may lay 60-70 eggs during its lifespan with a peak on the first or second day after emergence from the seed. By the third day more than 50% of the total number of eggs are laid and viability is maintained up to the sixth day (Booker, 1967).

Contrarily, the active form demonstrates a well defined pre-oviposition period during which neither sex is active. On emergence the abdomen of both sexes of the active form possesses a sizable fat body as against mature reproductive organs of the normal form; these organs do not develop in the active form until later in life. A pre-mating period of 2-3 days occurs and the active weevils do not make any effort to mate with either their abnormal counterparts or the normal ones (Gill et al., 1971) and would even reject attempts of the normal males (Utida, 1972). Peak of oviposition has been reported on the fifth day by Utida (1972) with a lifetime oviposition of 15 eggs per female. The active form needs a high humid environment for high fecundity and fertility.

Normal adults of the cowpea weevil do not fly, whereas active forms take to flight very readily when dropped from a height, usually flying to windows nearby while normal forms would drop to the ground feigning death. Sometimes normal forms consistently make fruitless attempts to fly by extending their wings. Development of active forms in storage has been suggested as an adaptation of the insect whereby a migratory form develops which flies out from storage conditions into the field in order to maintain the species (Sano, 1967; Utida, 1972).

Adult weevils normally do not feed but live longer with access to sugar water. When water is obtained egg development is slowed down, pre-oviposition period lengthened, and females spend more time in search of material on which to oviposit (Larson and Fisher, 1938).

Host Preference During Oviposition

Callosobruchus maculatus discriminates in its choice of seeds for oviposition though it may oviposit randomly on any smooth surface ranging from glass boxes to castor beans on which larvae die soon after hatching from the egg. There is a preference for seeds with smooth, well-filled coats whereas cowpeas with cracked or loosened seed coats are rejected. Even on seeds of the same variety but different in seed coat texture, the weevils would oviposit on the smooth ones. They may even preferentially oviposit on the smooth areas of a partly wrinkled seed (Larson and Fisher, 1938). More eggs are laid on larger seeds which provide more nutrients for the larvae; seed color has no effect on oviposition preference or damage caused by the insect (Booker, 1967; Bates, 1969).

Successful breeding of the cowpea weevil has also been reported in Nigeria on _Phaseolus aureus_ Rox, _Cajanus cajan_ (L.) Druce, _Voandzeia subterranea_ (L.) D.C., _Spenostylis stenocarpus_ Harms and _Kerstingiella geocarpa_ Harms. Elsewhere it has also been bred on _P. mungo_ L., _Glycine max_ (L.) Merril, _Cicer arietinum_ L. and _Dolichos lab-lab_ L. (Booker, 1967). Except on _P. aureus_ developmental period was extended and high mortality reported.

Field and Laboratory Studies of Resistance

Cowpeas

Painter (1951) classified resistance as seen in the field under a threefold basis, namely, non-preference, antibiosis and tolerance. The interrelation of these three components, one or more of which is frequently present in resistant varieties, combined with environmental factors determine to what degree a plant variety is resistant to insect attack. In any given variety genes for one or more of these characteristics may be present. Additional genes may be found in others, thus presenting the possibility of accumulating a higher degree of resistance into one variety by genetic recombination.

While antibiosis and non-preference are essentially insect-plant relationships, tolerance involves a predominant role played by the plant as expressed in regeneration of tissue, regrowth and recovery. Under storage conditions tolerance may not be expressed except when damage is tolerable to a certain degree without interfering with viability or germination in a particular variety. One advantage of storage over field conditions is that resistance due to antibiosis and/or non-preference is less exposed to variable ecological conditions, the problem only arising when a variety resistant in storage turns out to be susceptible in the field.

The finding of plants by insects for food or oviposition may be a response to the same stimuli or to different ones. Responses to color and intensity of light, roughness or smoothness of plant or seed surface and to chemical constituents of the plant are paramount factors to host-selection in insects (Painter, 1951). _C. maculatus_ oviposits freely on beans and peas of several colors, shapes and sizes, but they show a marked preference for larger and smooth seeds (Larson and Fisher, 1938). Raina (1971) reported the presence of a rough spiny seed coat in variety G109-1 of chickpea as the factor to which he attributed resistance to infestation by _C. analis_, _C. maculatus_ and _C. chinensis_.

In spite of smoothness of seed coat, _C. maculatus_ avoids tepary beans. Though the surface seems suitable for oviposition the seed coat, when moistened, soon wrinkles, a phenomenon which the insect seems aware of.

An attraction to sacks of beans and mature pods and seeds in the field have been attributed to an odor emitted by the crop (Larson and Fisher, 1938), but no evidence has been reported of any odor differential existing between varieties. It may well be that non-preference for some varieties may lie in their ability to emit strong repulsive odors. Oviposition apparently is not affected by light since it proceeds under equal conditions just as readily in the dark (Larson and Fisher, 1938). However, these authors did not show whether the same number of eggs were laid.

Jotwani et al. (1967) tested the repellent property of Neem seed (*Azadirachta indica*) against *C. maculatus* on four leguminous seeds. Decorticated dried neem fruits were crushed into a fine powder and used as a seed protectant. Effective control of the weevil on mung bean, Bengal gram, peas and cowpeas for various lengths of time was reported. However, they did not specify whether this treatment prevented oviposition by the weevil or whether it affected its lifespan. Su et al. (1972), using citrus oils, also showed a high degree of effectiveness as surface protectants for cowpeas against attack by the weevil. Oviposition on lemon oil treated seeds was considerably reduced. These two tests indicate that naturally occurring substances exist in plants to some degree which can serve as deterrents to insect oviposition. Effectiveness of odors emitted by the beans as a possible non-preference factor in resistance should be investigated. Such factors may be linked with seed coat texture but as yet are unknown. Teotia et al. (1966) stated that though C. chinensis preferred to oviposit on smooth seeds of maize it does not lay eggs on smooth seeds of wheat, unhusked barley and rice, thereby postulating that "some factor or factors other than smoothness of the seeds also influences the oviposition of the beetle." This agrees with a previous study by Srivastava and Bhatia (1959) who showed that the weevil did not lay eggs on smooth cucurbit seeds while preferring okra seeds.

These *studies* so far do not indicate the ability of any one cowpea variety to withstand damage by the weevils yet maintaining its ability to germinate and produce effectively. Jotwani et al. (1967) tested the extent of damage done by grubs of C. maculatus on germination of leguminous seeds. Germination of gram, cowpeas and mung bean seeds were reduced by 82%, 96%, and 100%, respectively. Unfortunately only a single variety of cowpeas was used.

Gundurao and Majumder (1964) indicated that intergranular space was a limiting factor for the growth of pulse beetles. Using C. chinensis on different pulses other than cowpeas, they observed that the depth of infestation increased with grain size and resulting intergranular space. Smaller grains restricted this insect to the top region of grain bulk. Such physical attributes of the grain mass due to seed size may also influence progeny population.

Larvae of C. maculatus failed to develop in bean flour made from cowpeas nor did they develop successfully on oily seeds (Larson and Fisher, 1938); the few adults that emerged were not vigorous and produced few eggs. Ishi (1952), after studying the host preference of the cowpea weevil, concluded that saponins may be a growth-hindering substance in non-favorable hosts. However, growth hindering of non-host plants may not depend on just one single component alone but may require the interaction of complementary fractions of several substances in adequate combination.

Borchers et al. (1947) extracted a trypsin inhibitor from seeds of leguminosae; 43.7 units/ml extract was found in Vigna sinensis exceeded only by Phaseolus vulgaris with 44.1. According to Ham and Sandstedt (1944) quoted by Borchers et al. (1947), the soybean fraction has been shown to have growth-inhibiting properties. Though inhibition was shown on chicken and rat the chances are that such fractions may also inhibit bruchid development.

Subsequent studies on varietal resistance to Bruchus sp. in cowpeas under storage conditions were done by Chandola et al. (1969). Of 40 varieties studied only one, "T_2", showed resistance which was measured by the damage caused by these pests. No indication as to factors determining damage was given. Green (1970) screened 150 lines of cowpeas to the cowpea curculio, Chalcodermus aeneus; all lines showed some degree of infestation. In a further trial with 60 new varieties, "Mississippi silver" was more resistant than others and he concluded that cowpeas of Eastern Hemisphere origin were more infested than those of the Western Hemisphere. These studies indicate that somewhere in the germplasm of the cowpea, varieties exist that may show high degrees of resistance.

Gokhale and Srivastava (1969) found that C. maculatus did not develop on French beans but live 2nd and 3rd instar larvae were found in autoclaved beans. Previously, Todd and Canerday (1968) had evaluated 51 varieties of Vigna for resistance to Chalcodermus aeneus. Differences in adult preference for oviposition were significant with Fla. 453-01 being least damaged. Antibiosis was also noted in impairment of larval development especially in Ala. 963.8 and Va. 59-119. Subsequently, Canerday and Chalfant (1969) found an arrestant and feeding stimulant for the cowpea curculio in several parts of the Vigna sinensis plant. Further investigations by Cuthbert and Davis (1972) confirmed that the factors of adult non-preference and failure of adults to penetrate through the pod wall account for differences between resistant and susceptible lines. They also showed conclusively that the amount of feeding stimulant reported by Canerday and Chalfant (1969) was a factor in the non-preference exhibited by the adults. Though further screening of seedlings for the non-preference factor is suggested, a consequence for the seeds in storage was not foreseen.

Cuthbert and Chambliss (1972) also surveyed plant introduction accessions, varieties and breeding lines of cowpeas revealing potentially valuable sources of curculio resistance. The relatively poor success of the curculio to penetrate pods of certain varieties may be overcome on exposed seeds in storage. When resistance in the field is accounted for by amount of damage done to pods it must be remembered that differences in dates of maturity and fruiting introduce an escape mechanism which may be misconstrued as a non-preference factor. Some varieties may fruit poorly or profusely depending on dates of planting and site.

Soybean Inhibitors

One drawback in resistance studies of cowpeas and its associated bruchids is the lack of better understanding of the resistance mechanisms involved. This would be helpful in efforts to develop resistance either to Curculio or C. maculatus. Most of the studies on tropical grain legumes so far have used C. chinensis as the insect pest and soybeans or chickpeas as host plants. A survey of these studies may reveal similar resistance mechanisms in cowpeas.

Following the studies of Ishi (1952) on the role of saponins as a growth hindering substance, Lipke et al. (1954) examined soybean for possible unidentified toxins which may be responsible for the crop's freedom from insect damage. The following inhibitors, soyin, crystalline trypsin inhibitor, alkaline, acid or ethanolic extracts of the seeds, have varying effects on vertebrates but showed no significant decrease in the growth rate of Tribolium larvae. But at the 2.5% level a material precipitable from a pH 4.6 extract of raw soybean by 0.475 saturation with ammonium sulfate was toxic to all the larvae. The material could be the factor earlier described by Borchers et al. (1947), Kunitz (1947) and subsequently studied by Birk and Applebaum (1960). The latter authors concluded that the purified acetone insoluble factor and the factors designated C_1 and C_2 strongly inhibit growth of T. castaneum larvae by inhibiting proteolytic activity of larval midgut enzyme solution.

Applebaum (1964) examined the effects of soybean trypsin inhibiting activity on C. chinensis and Acanthoscelides obtectus. The trypsin inhibitor designated as crystalline soybean trypsin inhibitor (CSBTI) had negligible effect on the proteolytic activities of both insects and had no effect on the overall development of Callosobruchus. Thus it appeared that the bruchids had evolved a pathway insensitive to protease inhibitors of legumes. Applebaum et al. (1965) later revealed that developmental incompatibility of soybeans for C. chinensis was partly due to the presence of soybean saponins. Larvae did not complete development and this is attributed to soybean "saponin fraction C". Fraction C and urease are also ovipositional attractants for Callosobruchus whereas these authors concluded that the constituents of soy-saponins, i.e., their sapogenins and sugars, had no apparent effect on the development of the weevil. The insensitivity of Callosobruchus to trypsin inhibitor was further confirmed (Applebaum et al. 1965). Saponins of seeds of chickpeas, garden peas, broad beans, haricot beans, lentils and groundnuts have been fractionated into saponin Preparation I (CaO-precipitable) and Preparation II (CaO-nonprecipitable) (Applebaum et al., 1969). Preparation I but not II, was shown to have effect on development of C. chinensis whereas a mixture of both fractions extracted from chickpeas or lentils (normally attacked by the weevil) had no adverse effect. But a similar mixture from a variety of garden peas (resistant to this insect) inhibited development. In haricot beans saponins are regarded as an ancillary factor of resistance in contrast to groundnut saponins which completely prevent larval development. Thus as suggested by Ishi (1952) the resistant factor may be expressed in the presence and/or absence of complementary chemical components.

Varieties of *Vicia faba* L. are relatively resistant to *C. chinensis* but low levels of saponin content in the seeds is not sufficient to affect the development of the bruchid (Podolar and Applebaum, 1968). The relative resistance of some varieties is due to difficulties encountered by the larvae to penetrate through the seed coat.

The possible development of a strain of *C. chinensis* which would hydrolyse soysaponins has been suggested by Applebaum et al. (1968). Strains of this species from Israel and Japan were compared and though attempts failed to establish populations of both strains on a number of soybean varieties, the Israeli strain showed partial larval development.

Su et al. (1971) examined the possibility for use of soybean saponin as a protectant against stored grain insects. A high degree of protection of wheat dusted with soysaponin and its calcium salt was reported on the rice weevil.

Other Legume Seed Inhibitors

Apart from saponins other seed chemical fractions have been suggested as factors conferring resistance to legumes. Ishi (1952) attributed the presence/absence or low content of pentosans in legume seeds as responsible for enhancing or inhibiting development of *C. chinensis* on haricot beans. In a later study, Applebaum et al. (1970) suggested that the heteropolysaccharide and starch fractions of *Phaseolus vulgaris* are a part of a complex of natural components of this plant which confer resistance to the beans against *C. chinensis*.

Heteropolysaccharides are a distinctive feature of legume seeds and contain large amounts of pentoses. Larval development and mortality, in addition to reduced adult fecundity, were reported when both fractions were incorporated into artificial beans. Subsequently Applebaum and Guez (1972) examined the comparative resistance of _P. vulgaris_ beans to _C. chinensis_ and _A. obtectus_. The ability of the latter pest to thrive on haricot beans has been attributed to its better ability to effectively digest a large portion of the heteropolysaccharide.

Six species of the genus _Mucuna_ have been found to possess high concentrations of free L-dopa (3,4-dihydroxyphenylalanine), a drug used for the treatment of Parkinson's disease in man (Bell and Janzen, 1971). L-dopa was also suggested by these authors as responsible, at least in part, for the immunity of the seeds to insect attack. Further studies by Rehr et al. (1973) confirmed the toxic effects to the caterpillar of the southern armyworm, _Prodenia eridania_ Cramer, which is widely polyphagous.

Therefore, it is obvious that the mere presence of one chemical component does not necessarily confer resistance to a plant against an insect. It may require the presence or additional components or a minimal concentration threshold in order for resistance to be expressed. Finally, the modality of the insect-plant inter-relationship would determine whether a variety would be resistant or not, depending on further modifications by ecological conditions of the environment.

MATERIALS AND METHODS

Since no previous laboratory study detailing the techniques and procedures developed for screening small samples of cowpeas or related grain legumes was available, it became necessary to run a series of preliminary tests on biology and behavior of Callosobruchus maculatus under laboratory conditions to determine optimum conditions to obtain maximum oviposition and homogeneous infestation of samples to be screened.

Cowpea Seed Samples

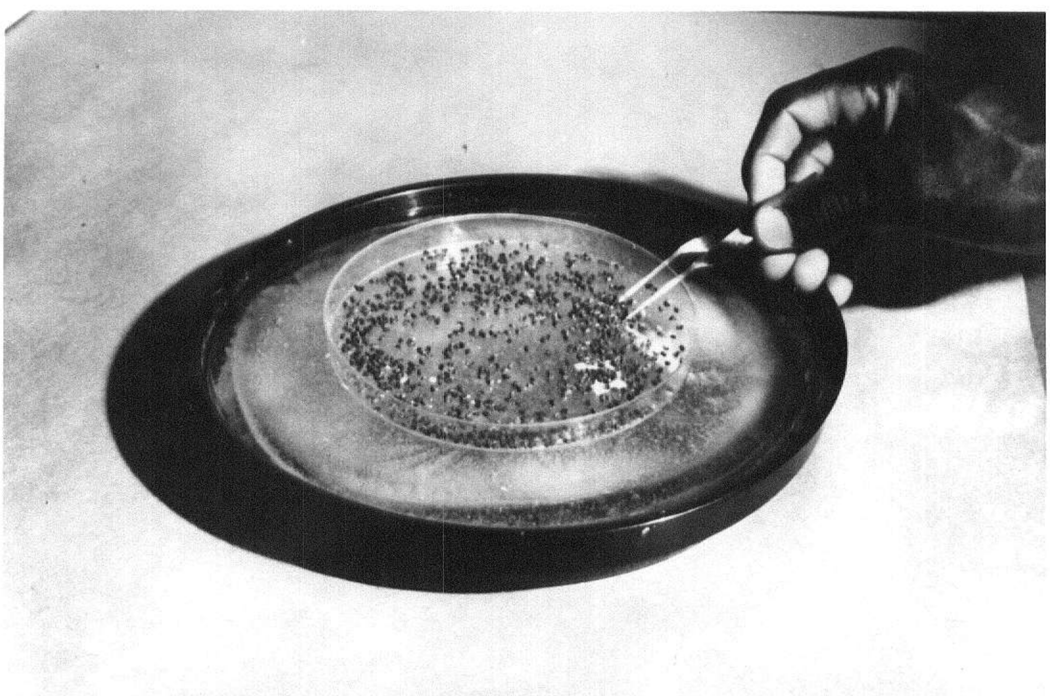
In June, 1972, about 1100 entries of cowpeas were supplied by Dr. H. B. Green of Mississippi State College, State College, Mississippi. Since these had already been treated with DDT, a random sample of 60 varieties was grown in the greenhouse in August, 1972, in 6-inch clay pots in two replicates (Plate II). Growth habit and maturity dates were recorded. Only 26 varieties produced sufficient seed for screening tests. In September, 1972, 78 varieties arrived from the International Institute of Tropical Agriculture (I.I.T.A.), Ibadan, Nigeria. These also were contaminated with "Aldrex T". In April, 1973, 13 varieties arrived from the same source, untreated. A commercial variety popularly called "Black-eyed pea" which was used for the preliminary tests, brought the total to 40. In this study all samples, lines or introductions are referred to as "varieties" regardless of source. The variety number, source of supply, pedigree, growth habit and maturity of each are listed in Appendix II.

EXPLANATION OF PLATE II

Upper: Cowpea seed multiplication in the greenhouse.

Lower: 25 cm diameter plastic dish containing ice for temporarily knocking down weevils.

PLATE II



Rearing Procedure for Weevils

The source of the original stock culture is unknown. Cultures are started weekly in the Stored Products Insects Laboratory of the Department of Entomology, Kansas State University. Every week, three wide-mouthed quart jars with one pint of the commercial variety of cowpeas available are infested with 300 newly emerged, 1-2 days old, unsexed adults. This yields an average of 6400 progeny (Strong et al., 1968). The jars are covered with lids made of Mason wide-mouth jar rings into which a kelthane treated filter paper (to prevent mite infestation) is placed under a 40-mesh wire screen. The weevils remain exposed in the jars in the rearing room at 80 $\pm$ 2^{o}F and 67 $\pm$ 3% RH for 24 days by which time the dead weevils are screened off. The room is lighted during a 12-hour photoperiod.

Between the 27th and 30th day after starting the culture, peak of adult progeny emergence occurs. On the 35th day, by which time there is an increase in number of the active form, cultures are sieved and adults used to set up new ones.

When weevils were needed for screening cowpea varieties, a carbon dioxide air mixture was passed into the culture jar at a pressure of 50 lb/sq in. for 5 to 10 seconds which temporarily knocked the insects down. Weevils were immediately sieved through an 8-mesh screen and discarded and the culture jar(s) kept in the rearing room. The procedure was repeated after 24 hr to collect 0-24 hr old adults for subsequent tests.

Handling Procedure

The effect of carbon dioxide on the biology and behavior of insects is well documented (Willis et al., 1954; Press et al., 1967; Lum and Phillips, 1972; Lum and Flaherty, 1972). Consequently a chilling procedure suggested by Dr. Horber of the Department of Entomology, Kansas State University, was employed for this study (Plate II). A 14 1/2 cm diameter petri dish was placed on ice in a plastic dish, 25 cm in diameter and 4 cm deep. The petri dish served as a receptacle for the weevils to be chilled and inactivated for subsequent handling. Temperature on the surface of the petri dish averaged 39°F. Weevils were sieved through an 8-mesh screen when needed, usually about a dozen at a time, onto the petri dish. They were left on the petri dish for 5-10 seconds while being exposed to chilling. This duration was found adequate for a temporary knock-down. The petri dish was removed from the ice dish, weevils were sexed and introduced into the test boxes before they became active again.

Sexing was based on elytra characteristics and weevils were picked up with a pair of feather-weight forceps or a "Schuco" vacuum tweezer.

Equilibration and Measurement of Moisture Content of Seeds

Screening experiments require seeds of the same moisture content. Since no standard method for the determination of moisture content of cowpeas was available, the air oven method was employed for weighed samples at four different temperatures and durations. Samples were returned to the rearing room to equilibrate and moisture content was redetermined after 5 weeks. Four randomly selected varieties were then compared with the commercial variety.

To determine the period necessary to equilibrate seeds in the rearing room, moisture content of samples was periodically determined at the end of 0, 3, 6, 12, 18 and 30 days after introduction into the rearing room. Three grams of each sample were oven dried at 103°C for 5 hr and replicated 5 times.

Ovipositional Studies

Test 1. Influence of Age of Parent Insects on Oviposition and Progeny Development

This test had two major objectives:

(a) To determine the length of time required after emergence to insure mating of all the adults.

(b) To determine any biological differences in progeny emerging from eggs laid earlier or later in life.

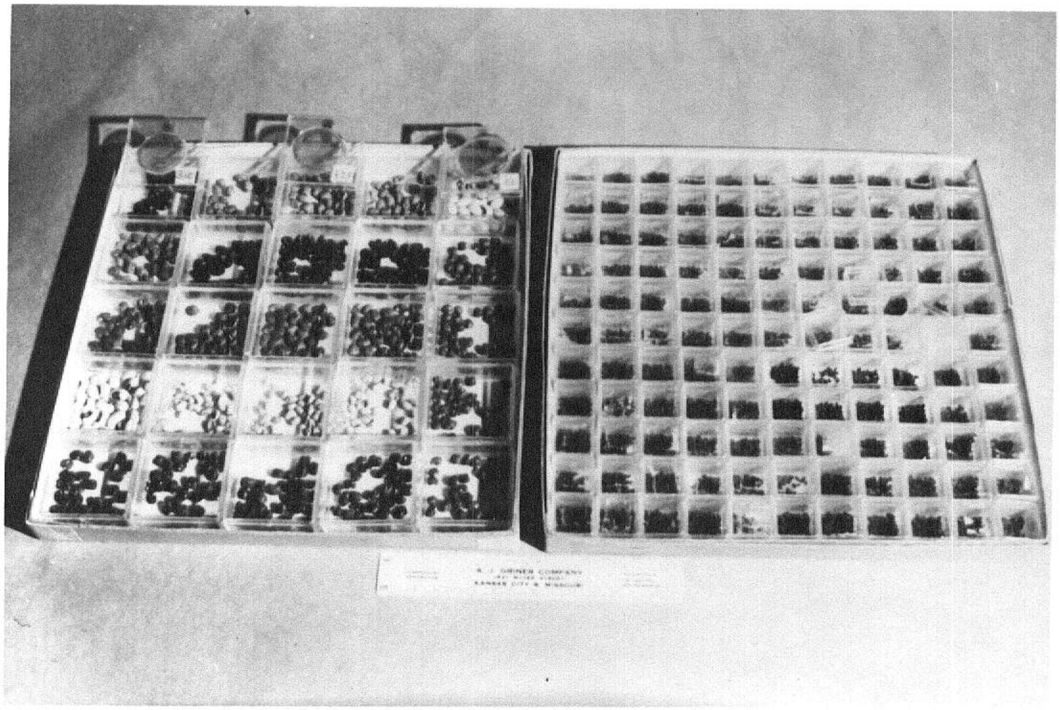
Insects used were 1-day-old females, 1-day-old females plus 1 male, 2- and 3-day-old females. Plastic boxes (48 mm x 48 mm x 18 mm) with screened lids (Swoyer, 1969) (Plate III), each containing 30 seeds, were infested with 3 females and 3 females plus one male accordingly and replicated 4 times. After 10 days the weevils were removed, by which time most were dead. Eggs laid were counted two weeks after infestation and daily observations for adult progeny in each box commenced as from the 25th day after infestation. Adults were removed and placed in 20 mm x 20 mm x 18 mm plastic boxes with lids (Plate III) and stored in a deep freezer. Observations were stopped on the 50th

EXPLANATION OF PLATE III

Upper left and lower: Plastic screening boxes with screened lids, 48 mm x 48 mm x 18 mm containing cowpea samples.

Upper right: Plastic boxes 20 mm x 20 mm x 18 mm with lids for holding adult progeny weevils.

PLATE III



day after infestation to avoid a second generation but allow for emergence of all active forms. Progeny weevils were then oven dried at 60°C for 6 days and weighed on a Mettler Model H15 electrobalance.

The procedure from infestation to measurement of dry weight of weevils was the same in all tests except where otherwise specified.

Test 2.
Influence of Seed Number and Parent Insect Number on Oviposition and Progeny Development

Since it is not known whether the active form of the weevil may be associated with resistance, it was necessary to determine the number of insects needed to obtain maximum normal progeny from an optimum seed number and at the same time producing a minimal percentage of the active form.

Four lots of 20 seeds each were infested with 2, 3, 4, and 5 females (24-48 hr old), respectively. The same stepwise increased infestation was repeated for lots of 30, 40, 50, and 60 seeds. The test was replicated 4 times. Number of eggs/female, total adult progeny and number of active forms were recorded.

Test 3.
Effect of Handling Procedure (Carbon dioxide and Chilling) on Parent Insect Longevity, Fecundity and Progeny Development

To compare the effects of the chilling and treatment with carbon dioxide, respectively, as alternative methods of inactivating them, weevils were subjected to 10, 20, 40, 60, and 90 sec exposure to each treatment. For each of the 4 replicates in each treatment about 10 insects were carefully removed from the culture jar by suction. Carbon dioxide treatment consisted of passing the gas at 50 lb/sq in. for the period desired into a test tube containing the insects and fitted with a glass tube into which the gas outlet rubber tubing was connected.

The chilling was done as previously described except insects were exposed on the petri dish for the different periods indicated. Untreated weevils served as controls. After treatment three females were introduced into each box containing 30 seeds. Treatments and control were replicated 4 times. Observations are listed in Table 6.

Test 4.
Duration of Exposure of Parent Weevils to Seeds for Oviposition

This test was designed to determine the period necessary to expose the seeds to the weevils for oviposition to produce maximal adult progeny at the same time as a minimal percentage of the active form. Each of ten batches or lots of 30 seeds in four replicates were infested with 3 mated females. Each day, at approximately the same time, all weevils were removed from a batch and discarded. This procedure was continued for 10 days. Total eggs, adult progeny and active forms were recorded for this purpose.

Test 5.
Influence of Previous Oviposition on the Attractiveness of Beans

Since cowpea weevils prefer smooth seeds and they usually are exposed to the seed samples for periods longer than one day, a test was carried out to determine seed attractiveness after previous oviposition.

In conjunction with Test 4, another set of 30 seed samples in 4 replicates were each infested with three females on the first day. Next day all weevils from each box were transferred to another set of samples with fresh seeds. This was repeated daily for 10 days; each day all weevils were transferred onto fresh seeds. Number of eggs, adult progeny and active forms were recorded.

Screening by Artificial Infestation

Prior to infestation of the 40 varieties listed in Appendix I, seed coat color and seed coat texture were scored with arbitrary scales, sufficient units being used to include every distinct type or class (Appendices II and III).

Due to seed shortage among the Mississippi varieties these samples consisted of 20 seeds only in 3 replicates, while the I.I.T.A. seed samples were screened with 30 seeds in 4 replicates. All procedures from infestation to weighing of adult progeny were the same as described in previous tests.

Statistical Analysis

Data were analyzed on an IBM 360 computer, for analysis of variances, LSD values for multiple comparisons and correlation coefficients.

RESULTS AND DISCUSSION

Moisture Equilibration of Seed Samples

It is commonly known that seeds which are moisture-equilibrated in a constant RH, then oven-dried, will not re-equilibrate in the same RH to the original moisture content. This phenomenon of hysteresis probably explains the results presented in Table 1. Since seeds apparently lose other volatile components other than moisture at higher oven temperatures and longer exposures, a temperature of 103°C was used and seeds were dried for 5 hr.

Table 1. Equilibration moisture content (at 27°C and 67 ± 3% RH) of cowpeas before and after drying as measured by air-oven using different temperatures and exposure periods.

Sample wt. (g)	Oven temp. °C	Duration (hours)	Moisture content[1] (%)		Mean[2] loss in equilibration m.c.
			Initial	Final	
3.0	103	5	12.6	12.2	0.4a[3]
3.0	103	48	13.1	12.4	0.7b
3.0	103	72	13.5	12.6	0.9b
10.0	130	3	13.3	12.1	1.2b

LSD (5%) = 0.65**

[1] Average of five determinations.

[2] Probably due to hysteresis.

[3] Means followed by the same letter are not significantly different.

Figure 1 clearly shows that the seeds gain most of their moisture within the first 3 days in the rearing room. A constant seed moisture content is attained within 2 weeks. Thus, all seed samples were kept for 2-3 weeks in the rearing room prior to use. The 4 randomly selected varieties exhibited similar patterns of moisture equilibration.

Ovipositional Studies

Test 1. Influence of Age of Parent Insects on Oviposition and Progeny Development

Table 2 shows that most eggs are laid on the first two days after adult females have emerged from the seeds. The results agree with previous studies by Larson and Fisher (1938) and Utida (1972). When cowpea weevils are randomly selected from a culture jar, chances are that some may not be mated though *C. maculatus* adults would mate soon after emergence (Larson and Fisher, 1938). This fact is aptly demonstrated in the treatment where one male was added to the 3 females (Table 2). Though there was a significant effect of age of parent weevils on oviposition, this did not significantly influence progeny development. A difference might exist with older weevils. Though there was a higher percentage of the active forms in the second treatment, i.e., 1 (+1 male), this was not significant. This test indicates therefore to use 24-48 hr old weevils. To accomplish this, freshly emerged adults (0-24 hr old) were transferred to culture jars containing fresh seeds for 24 hr. This ensured proper mating of insects and less individuals of the active form among progeny.

EXPLANATION OF FIG. 1

Days required for moisture equilibration of 3 g samples of cowpea seeds (commercial U.S.A. variety) in 27°C and 67 ± 3% RH. Moisture determination was by the air-oven method (103°C for 5 hr).

THIS BOOK CONTAINS NUMEROUS PAGES WITH DIAGRAMS THAT ARE CROOKED COMPARED TO THE REST OF THE INFORMATION ON THE PAGE.

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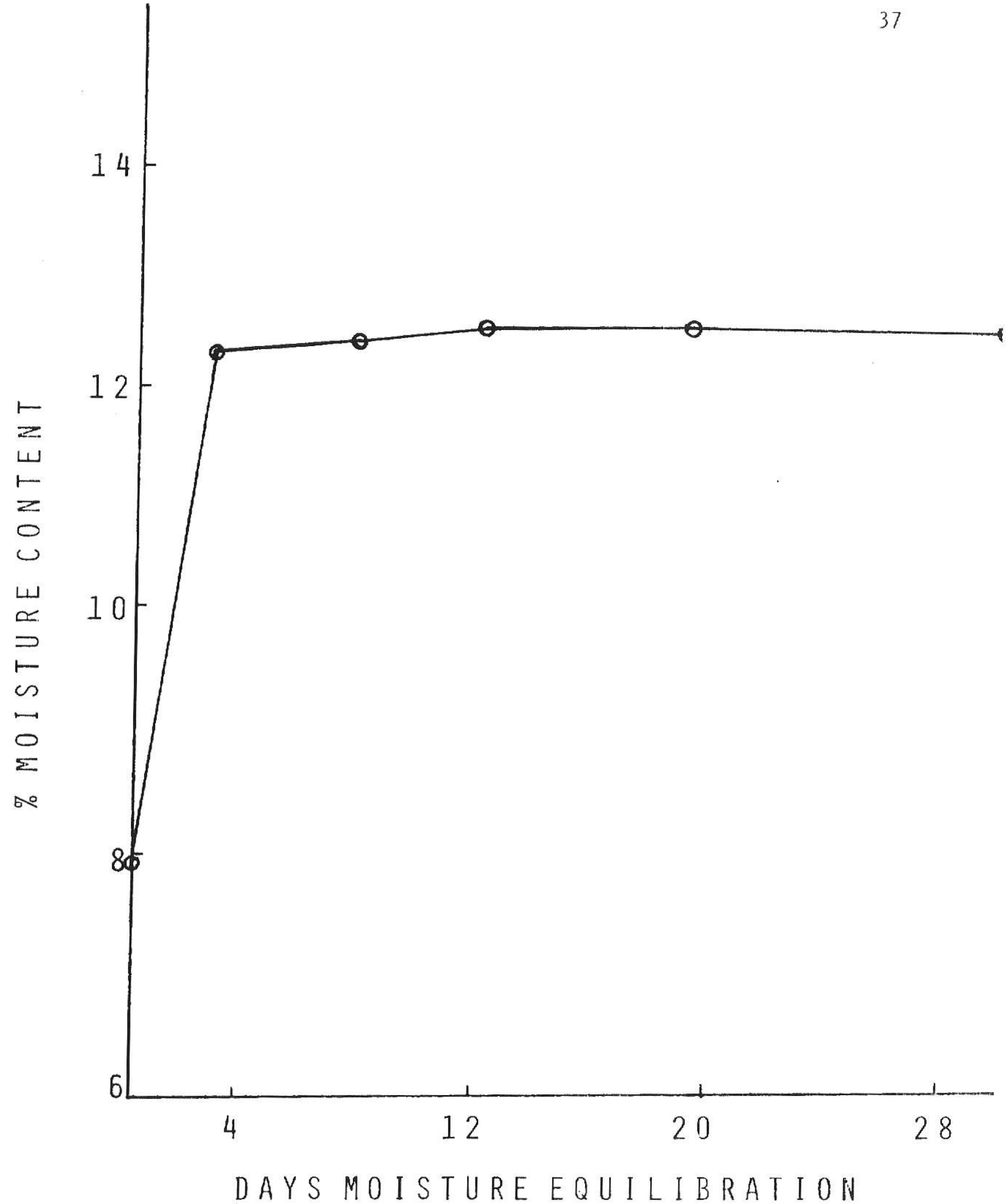
14
12
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% MOISTURE CONTENT
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DAYS MOISTURE EQUILIBRATION

Fig. 1

Table 2. Age influence on parent oviposition and progeny development.[1]

Age of weevils (days)	Mean no. of eggs laid/ female	Mean developmental period (days)	% Adult progeny emergence[2]	% active form[3]
1	36.0a[4]	31.5	76.1	4.1
1 (+ 1 male)	46.3b	31.6	75.0	9.9
2	32.0a	31.7	75.0	5.0
3	13.4c	32.0	72.8	6.4
LSD (5%)	7.78*	n.s.	n.s.	n.s.

* Significant difference between means ($P < .05$).

1 Data on this table are averages of 4 replicates.

2 From total number of eggs laid.

3 Calculated from total adult emergence.

4 Means followed by the same letter are not significantly different.

Test 2.
Influence of Seed Number and Parent Insect Number on Oviposition and Progeny Development

Strong et al. (1968) in rearing cowpea weevil cultures for laboratory studies indicated that development was affected when higher numbers of parent stock were used. Results in Tables 3, 4 and 5 support this phenomenon. As female parent number increased there was a decrease in the number of eggs laid per female. This may be due to interference between insects. Furthermore, it may be postulated that eggs laid early in the weevil's lifespan would act as deterrent to oviposition making the seed surface less attractive,

Table 3. Influence of seed number and parent weevil number on oviposition of the cowpea weevil.[1]

Number of females	Mean number of eggs laid per female						
	Number of seeds						
	20	30	40	50	60	Mean	LSD (5%)
2	37.6	40.9	48.0	50.8	45.8	44.6a[2]	
3	32.8	39.2	44.7	34.4	41.7	38.5b	4.70*
4	32.2	32.6	31.9	31.1	47.0	37.9b	
5	33.7	31.4	36.6	41.7	46.3	35.0b	
Mean	34.1a	36.0ab	40.3bc	39.5b	45.2c		
LSD (5%)		5.26*					

* Significant difference between means ($P < .05$).

1 Mean of 4 replicates.

2 Means followed by the same letter are not significantly different.

Table 4. Influence of seed number and parent weevil number on development of the cowpea weevil.[1]

Number of females	Percentage adult emergence[2]						
	Number of seeds						
	20	30	40	50	60	Mean	LSD (5%)
2	68.2	64.1	76.6	61.6	78.7	69.8ab[3]	
3	67.4	72.1	83.3	72.6	78.3	74.7a	6.27*
4	60.3	60.9	59.1	67.4	75.9	64.7b	
5	58.8	71.8	64.1	71.7	73.0	67.9b	
Mean	63.7a	67.2ab	68.3ab	70.8bc	76.4c		
LSD (5%)			7.00*				

* Significant difference between means ($P < .05$).

1 Mean of 4 replicates.

2 From total number of eggs laid.

3 Means followed by the same letter are not significantly different.

Table 5. Influence of seed number and parent weevil number on the occurrence of the active form of the cowpea weevil.[1]

Number of females	Percentage active form emergence[2]						
	Number of seeds						
	20	30	40	50	60	Mean	LSD (5%)
2	10.2	11.1	6.7	7.3	15.3	10.1a[3]	
3	9.6	14.1	13.8	13.7	11.8	12.6ab	5.85*
4	18.2	20.2	17.3	13.6	16.4	17.1b	
5	15.8	15.8	10.5	17.9	17.0	15.4b	
Mean	13.4a	15.3a	12.0a	13.1a	15.2a		
LSD (5%)		n.s.					

* Significant difference between means ($P < .05$).

1 Mean of 4 replicates.

2 From total adult emergence.

3 Means followed by the same letter are not significantly different.

i.e., by roughening it or otherwise. However, as seed number was increased we found this interference effect was cancelled. Thus, there was a threshold or point of interaction between seed number and female parent number. To exclude undesirable interference either seed number must be increased or number of females reduced. However, the chore of counting eggs is geometrically increased with an increasing number of females and seeds. It is thus not feasible to use 50 or 60 seeds nor 4 or 5 females where one desires to screen a large germplasm collection.

As shown in Table 3, 30 or 40 seeds appear as the optimum seed number required. It was decided to use 40 seeds when seed supply is in abundance and 30 when limited. Since the difference in oviposition on 20 or 30 seeds was not significant it is reasonable to use 20 or 25 seeds in the event of very limited seed supply. Besides, it reduces labor in counting eggs. Since weevils lay more eggs when not crowded, the results in Table 3 indicate that 2 females should be used, this being significantly different from other weevil numbers. But with 3 females optimum percentage adult emergence was attained (Table 4). This was most obvious with 40 seeds (83.3%). Percentage adult progeny emergence also shows the same trend as number of eggs laid/female. This was probably due to crowding. Fewer larvae develop to maturity when female number is increased but this phenomenon is compensated for as seed number increased.

The data on the active forms do not follow the same pattern (Table 5). Supposedly, a larger proportion of active forms would be produced as seed number is reduced and female parent weevils increased. This would be the case when optimum conditions are exceeded (Utida, 1972; Sano, 1966). But in our tests conditions favoring crowding, temperatures or photoperiod were controlled and production of active forms was held to a minimum. Data in Table 5 confirm the results presented in Tables 3 and 4. There was no significant difference in percentage of active forms due to seed number but with an increase in female weevil number, leading to more eggs/seed, the percentage of active forms increased. There was no significant difference in number of active forms produced by 2 or 3 females.

It can be concluded that for samples of 30 or 40 seeds, 3 females per replicate would be most desirable.

Test 3.
Effect of Handling Procedure (Carbon dioxide and Chilling) on Parent Insect Longevity, Fecundity and Progeny Development

The analysis of variance for each of the observations taken showed no significant influence of either cold or carbon dioxide (as used in this test) on the biology of the insect (Table 6). This does not prove that subjecting the insect to these treatments has no overall effect but rather that a short exposure of the weevils to either treatment does not have a deleterious effect on the weevils. Previous studies involved the use of longer periods of exposure or of lethal doses of the gas for control measures (Willis et al., 1954; Press et al., 1967; Lum et al., 1972).

Since it was more convenient, cheaper and faster to work with the ice dish, this procedure was used in all tests.

Table 6. Effect of carbon dioxide and ice-chilling on parent cowpea weevil oviposition and progeny development.[1]

Treat-ment	Mean no. of eggs/ female	Longevity of parents	Mean develop-mental period	% adult progeny emergence[2]	% active form[3]	Mean wt. of adult progeny (mg)
Control	42.7	8.5	32.6	85.6	2.8	1.9
			Ice			
10 sec.	43.3	10.3	33.1	89.5	1.2	2.0
20 "	37.3	9.8	33.0	87.8	4.9	1.9
40 "	40.7	8.8	32.5	81.6	2.4	1.9
60 "	39.5	9.2	32.9	80.3	1.8	1.9
90 "	43.8	10.3	33.2	79.1	5.5	1.9
			Carbon dioxide			
10 sec.	41.8	10.3	33.1	88.7	1.8	1.9
20 "	41.5	9.2	32.7	84.4	5.0	1.9
40 "	42.7	10.4	33.0	84.8	5.1	2.0
60 "	39.2	9.8	33.0	84.0	4.0	1.9
90 "	42.3	10.8	33.4	75.8	6.2	2.0
LSD (5%)	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.

[1] Mean of 4 replicates.

[2] From total number of eggs.

[3] From total adult emergence.

Test 4.
Duration of Exposure of Parent Weevils to Seeds for Oviposition

Obvious differences existed in the mean total number of eggs laid per female, mean number of eggs laid per female per day and the percentage adult emergence (Table 7). Weevils continue to lay eggs until the 5th day, then egg production declines. Reduction in eggs laid after the 5th day could be attributed to age of parent, exhaustion of egg supply or the deterrent effect of previously laid eggs.

Percentage adult emergence also exhibits the same pattern as the number of eggs laid. After the 5th day adult emergence declines. This may be due to less viability of eggs laid later in the parent's life (Booker, 1967; Utida, 1972) or due to crowding effect. No difference was found in Test 1 (Table 2) because weevils used in that test were no older than 3 days. There is no consistency in the percentage of the active forms produced. As previously explained in Test 2, this may be attributed to the fact that experimental conditions did not exceed the threshold.

From the results of this test it was concluded that weevils need not be exposed to the seeds for more than 5 days after which they should be removed and discarded. Egg counting should be delayed until about the 10th day when larvae should have hatched and bored into the seed, thus avoiding egg mortality.

Table 7. Duration of exposure of parent weevils to seed samples for oviposition.

Days of oviposition	Cumulative mean no. of eggs/ female	Mean no. of eggs/ female/ day	% adult progeny emergence	% active form
1	10.4	10.4	87.0	4.9
2	24.5	14.1	80.1	6.2
3	33.5	9.0	83.3	7.1
4	39.1	5.6	81.5	7.6
5	42.6	3.5	84.3	12.9
6	45.4	2.8	76.1	4.8
7	48.0	2.6	70.7	4.8
8	48.8	0.8	76.6	6.9
9	49.6	0.8	78.7	5.8
10	49.9	0.3	69.1	9.8
LSD (5%)	5.9*	2.7*	8.8*	n.s.

* Significant difference between means ($P < .05$).

Test 5.
Influence of Previous Oviposition on the Attractiveness of Beans

Oviposition followed the same pattern as in Test 4 (Table 8). However, it terminated on the 8th day as opposed to the 10th day in Test 4. It appeared that in the absence of any deterrent factor, all eggs are oviposited in a short time. Furthermore, more eggs were laid in this test (58.7) than in Test 4 (49.9). This more or less supports the idea that due to previous exposure to oviposition, the cowpea seeds became less attractive to the weevils.

Table 8. Influence of previous oviposition on the attractiveness of beans.

Days of oviposition	Cumulative mean no. of eggs/ female	Mean no. of eggs/ female/ day	% adult progeny emergence	% active form	Mean develop-mental period
1	15.6	15.6	88.3	6.1	30.9
2	27.5	11.9	87.2	0.0	30.8
3	39.5	12.1	89.9	1.4	31.3
4	49.3	9.9	86.3	2.8	31.9
5	54.6	5.3	83.1	3.1	32.5
6	57.5	2.9	77.5	0.0	33.4
7	58.4	0.9	71.7	5.0	33.3
8	58.7	0.6	77.5	10.0	33.0
LSD (5%)	1.6*	5.7*	n.s.	n.s.	n.s.

A comparison of the two tests is shown in Table 9, with the results of an analysis of variance. There were no differences in eggs laid in the first 2 days of oviposition, but from the 3rd day as larvae began to hatch and egg shells hardened, seed surface probably became rough and this may have inhibited oviposition of weevils. Thus, further support for the postulation made earlier in Test 2 is provided.

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2	27.5	11.9	87.2	0.0	30.8
3	39.5	12.1	89.9	1.4	31.3
4	49.3	9.9	86.3	2.8	31.9
5	54.6	5.3	83.1	3.1	32.5
6	57.5	2.9	77.5	0.0	33.4
7	58.4	0.9	71.7	5.0	33.3
8	58.7	0.6	77.5	10.0	33.0
LSD (5%)	1.6*	5.7*	n.s.	n.s.	n.s.

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Table 9. Influence of previous oviposition on attractiveness of beans. Comparison of Tests 4 and 5.[1]

Days of oviposition	Cumulative mean number of eggs/female			Percentage adult progeny emergence			Percentage active form		
	Test 4	Test 5	P(5%)	Test 4	Test 5	P(5%)	Test 4	Test 5	P(5%)
1	10.4	15.6	n.s.	87.0	88.3	n.s.	4.9	6.1	n.s.
2	24.5	27.4	n.s.	80.1	87.2	n.s.	6.2	0.0	*
3	33.5	39.5	*	83.3	89.9	n.s.	7.1	1.4	n.s.
4	39.1	49.3	*	77.5	86.3	n.s.	7.6	2.8	n.s.
5	42.6	54.6	*	84.3	83.1	n.s.	12.9	3.3	n.s.
6	45.4	57.5	*	76.1	77.5	n.s.	4.8	0.0	*
7	48.0	58.4	*	70.7	71.7	n.s.	4.8	5.0	n.s.
8	48.8	58.7	*	76.6	77.5	n.s.	6.9	10.0	n.s.

* Significant difference between the two tests ($P < .05$).

[1] In Test 4 female weevils were confined to same samples of seeds.

In Test 5 female weevils were transferred to new samples of seeds each day.

It also indicates the need to use at least 20 seeds per 3 parent female weevils since the inhibitory effect of eggs on further oviposition may mask other non-preference characteristics of seeds present in any one variety.

Both tests also confirm that eggs laid later in parent life were less viable though no statistically significant difference occurred in adult emergence in Test 5. It was also indicated that progeny from eggs laid later in parent life developed slower (Table 8). The absence of significant difference in percentage adult progeny emergence in Test 5 as compared to Test 4 may be due to reduced oviposition per seed since test insects were offered fresh seeds daily, thus avoiding crowding effect which probably had an effect in Test 4. Though weevils develop within secluded cells in the seeds, the more developing within the same seed, the more limited food would be available to progeny weevil which in turn would reduce the number of progeny developing into adults.

Differences occurred also in mean developmental period in Test 5 (Table 8) although they are not statistically significant.

Screening by Artificial Infestation

Results presented in Table 10 are more or less a measure of the diversity existing within the two groups of seed samples. Greater variation existed among the Mississippi varieties as reflected by the significant differences among our observations on the biology of the insect, perhaps because they were 27 varieties as against 13 from Nigeria and since the cowpea has long been a stable food in Nigeria, varieties have been cultivated year after year and selections made

Table 10. Summary of results obtained from analysis of variance of mean seed weight and nine observations relating to biology of the cowpea weevil.[1]

Variety source	Mean seed wt. (mg)	No. of eggs per female	Mean develop-mental period	Duration of adult emergence	% adult progeny emergence[2]	No. of weevils per g of seed	Mean wt. of progeny weevils	% small form[3]	% adult female progeny[3]	% active form[3]
Mississippi (27 varieties)	*	n.s.	*	*	*	*	*	*	n.s.	n.s.
Nigeria (13 varieties)	*	n.s.	n.s.	n.s.	*	n.s.	n.s.	n.s.	*	n.s.

* Significant differences between varieties ($P < .05$).

1 Analysis was not run on discrete seed characteristics.

2 From total number of eggs laid.

3 From total number of adult emergence.

under similar environmental conditions, thus tending to bring the different varieties to a competitive level. In contrast, the Mississippi varieties are collections still maintaining most of their diversity originating from widely different proveniences.

Although differences exist in seed coat texture these were not significant enough to affect oviposition of parent weevils which oviposited freely on all varieties tested. Due to the limited number of varieties examined, it was not possible to draw definite conclusions. Resistance as expressed by any mode of action may be found only in one out of several thousand varieties. This may then change a whole set of results.

Teotia and Singh (1966) recorded a higher ratio of females to males of C. chinensis on more nutritious host seeds than on inferior diets. Whereas no statistical differences were observed in the percentage of adult female progeny emerging from the Mississippi group, the contrary was observed in the Nigerian varieties. The only explanation that can be offered for this discrepancy is the limited seed supply.

Percentage of active forms was not significantly different between varieties; this parameter is considered unrelated to resistance.

A form of the weevil much smaller than the normal form was recorded on some varieties (Plate IV). This form weighed 0.69 mg (average of 100 insects) and was quite variable in size as compared with the normal form (1.8 to 2.0 mg). This form has also been reported on soybeans (Partida, personal communication) which is a

EXPLANATION OF PLATE IV

Upper: Normal male and female cowpea weevils.

Lower: Small forms of weevils from some cowpea varieties.

PLATE IV

poor host plant for the cowpea weevil. Though no significant differences existed in the number of the small form emerging from varieties collected from Nigeria, it is considered an important parameter for measuring resistance.

Due to the different seed numbers used in the two seed groups from Nigeria and Mississippi, flaws would be introduced into the results. Though it was pointed out in Test 2 under ovipositional studies that 20, 30 or 40 seeds per box could be used in case of limited seed supply, differences would nonetheless still exist. It is of paramount necessity, therefore, to employ the same number of seeds throughout the screening procedure. Inability to do this may explain the discrepancies in results between seed samples from Nigeria and Mississippi.

The results of the correlation analyses between the different observations studied are presented in Tables 11 and 12.

Seed color did not show any significant correlation with any observation. There is therefore no color effect on oviposition or damage caused by the insect. This agrees with previous observations of Booker (1967) and Bates (1969).

Occurrence of the active form prolongs the mean developmental period and the duration of adult progeny emergence. It also increases the mean weight of the progeny weevils since this form weighs more than the normal form. No significant correlation existed between percentage of active forms and mean seed weight or number of eggs laid per female. In a previous test it was shown that the number of the active forms increased as more eggs were laid per seed, due to increased number of parent weevils. But in our screening tests

Table 11. Correlation coefficients matrix for seed characteristics and observations relating to the biology of the cowpea weevil.[1] (Mississippi varieties.)

Observations	Mean seed weight	Mean developmental period	Duration of adult progeny emergence	% adult progeny emergence[2]	No. of weevils/g of seed	Mean wt. of progeny weevils
Mean developmental period	0.38**					
Duration of adult progeny emergence	0.52**	0.44**				
% adult progeny emergence[2]	0.72**	0.33*	0.45**			
No. of weevils/g of seed	-0.75**	-0.28*	-0.31*	-0.48**		
Mean wt. of progeny weevils	0.73**	0.46**	0.43**	0.58**	-0.76**	
% small form[3]	-0.57**	-0.27*	-0.27*	-0.57	0.62**	-0.62**
% adult female progeny[3]	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
% active form[3]	n.s.	0.36**	0.39**	n.s.	n.s.	0.30*

* Significant correlation at 5%.

** Significant correlation at 1%.

n.s. = No significant correlation.

[1] Correlation coefficients between observations not listed are not significant.

[2] From total number of eggs.

[3] From total adult emergence.

Table 12. Correlation coefficients matrix for seed characteristics and observations relating to the biology of the cowpea weevil.[1] (Nigerian varieties.)

Observations	Mean seed weight	No. of eggs/ female	Mean developmental period	% adult progeny emergence[2]	No. of weevils/g of seed	Mean wt. of progeny weevils
Mean developmental period	n.s.	n.s.				
Duration of adult progeny emergence	n.s.	n.s.	0.38**			
% adult progeny emergence[2]	-0.37**	-0.30*	n.s.			
No. of weevils/g of seed	0.38**	0.78**	n.s.	n.s.		
Mean wt. of progeny weevils	0.29*	-0.35*	n.s.	n.s.	-0.51**	
% small form[3]	-0.45**	0.60**	n.s.	-0.41**	0.60**	-0.36**
% adult female progeny[3]	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
% active form[3]	n.s.	n.s.	0.34*	n.s.	n.s.	0.31*

* Significant correlation at 5%.

** Significant correlation at 1%.

n.s. = No significant correlation.

[1] Correlation coefficients between observations not listed are not significant.

[2] From total number of eggs.

[3] From total adult emergence.

conditions favoring crowding, high temperatures and extremes of photoperiod are being controlled, thus production of the active form was reduced to a minimum.

The development of insects in seeds depends not only on the size of the seeds but also on seed compactness and quality. Though a variety may afford enough quantity of food, the quality may be such as to delay development. Thus the occurrence of small sized weevils may be an expression of antibiosis or may be just due to insufficient food supply due to small seeds. Hence, when more weevils develop within seeds of such a variety individuals emerging tend to be small whereas in a highly susceptible variety that affords adequate supply of food a high percentage of normal adults emerge and few small forms, if any, occur (Tables 11 and 12). Resistance in a variety may be expressed in the production of more small forms as well as in a reduction in the total adult progeny that emerge and/or in an extension of the developmental period of the insect which in turn may extend the duration of adult emergence.

But apparently results in this study do not agree with the above deductions (Table 11). It appears that when weevils were offered more suitable varieties in terms of quantity or quality of seeds, they completed their development in longer time and the duration of adult progeny emergence is prolonged. This observation is further supported by the fact that with a higher percentage of small forms the mean developmental period and duration of adult progeny emergence were reduced (Table 11).

With the above understanding in mind a comparison was made between 6 varieties as to outstanding seed weight, seed damage as measured by the percentage weight loss after infestation, percentage adult progeny emergence, percentage small forms and mean weight of progeny weevils (Table 13, Fig. 2). Varieties IVu. 201, PI. 292871 and the commercial variety afford ample food for progeny weevils to develop in, due to a high seed weight. This is most evident in the commercial variety where the highest percentage adult progeny emergence occurred but percentage seed damage was lowest. Thus it appears that this variety possesses enough nutrient reserves in its seeds to maintain a high degree of infestation. The high level and susceptibility of these 3 varieties is further indicated by the near absence of small progeny weevils.

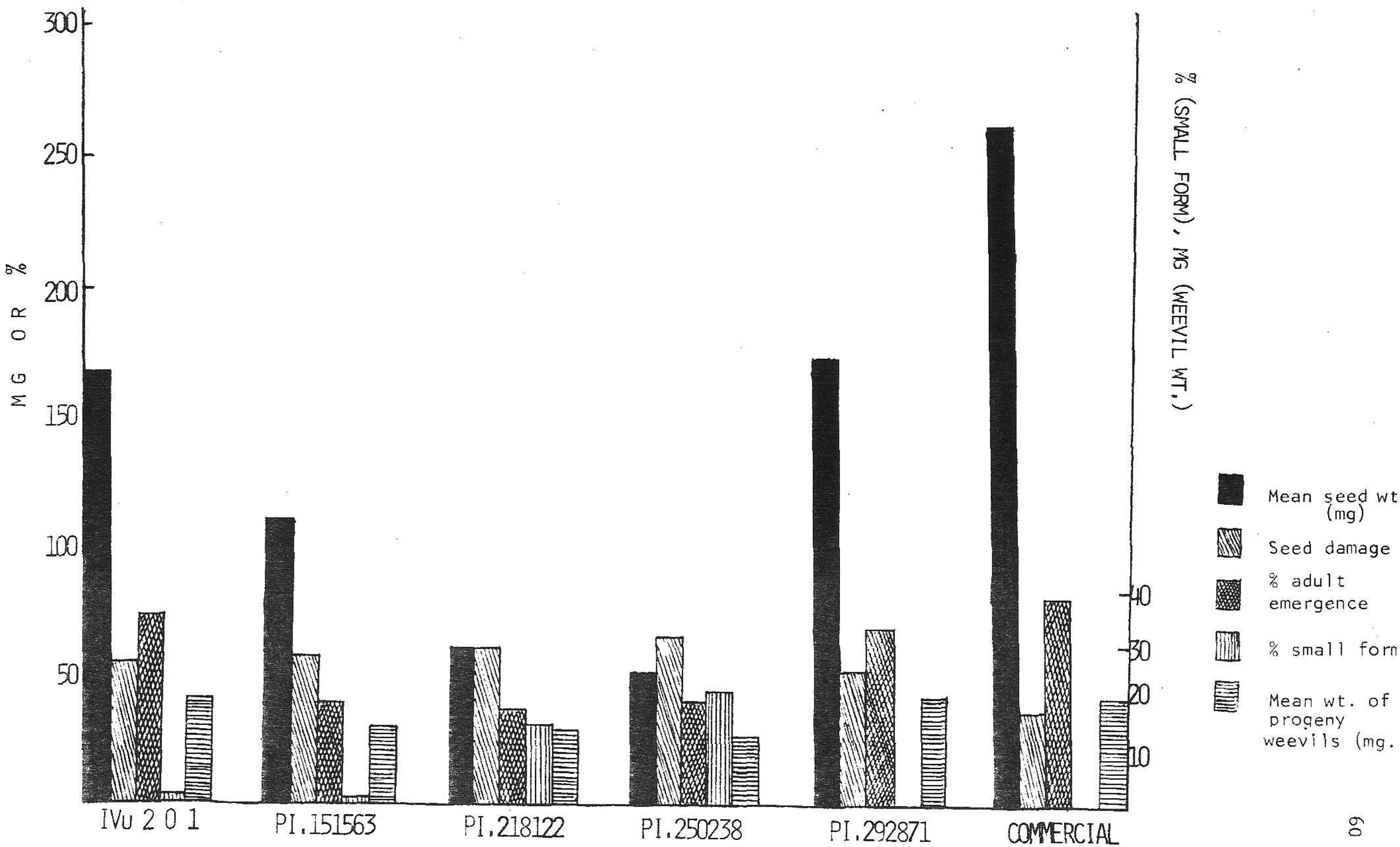
Table 13. Comparison of 6 cowpea varieties in relation to their susceptibility of attack by the cowpea weevil.

Variety	Mean seed wt. (mg)	Seed damage[1]	% adult progeny emergence	% small form	Mean wt. of progeny weevils (mg)
IVu. 201	162.7	53.0	72.3	0.4	2.0
PI. 151563	110.6	56.5	40.5	1.2	1.7
" 218122	59.0	59.3	36.1	15.4	1.5
" 250238	49.9	61.4	38.9	11.4	1.5
" 292871	174.1	54.1	69.0	0.0	2.1
Local	259.7	35.5	78.8	0.0	2.0

[1] Measured by percentage loss in seed weight after infestation.

EXPLANATION OF FIG. 2

Comparison of 6 cowpea varieties in relation to their susceptibility of attack by the cowpea weevil.



MG OR %
300
250
200
150
100
50
% (SMALL FORM), MG (WEEVIL WT.)
40
30
20
10
IVu 2 0 1
PI.151563
PI.218122
PI.250238
PI.292871
COMMERCIAL
Mean seed wt (mg)
Seed damage
% adult emergence
% small form
Mean wt. of progeny weevils (mg.

Fig. 2

While it may be argued that the low percentage adult progeny emergence in varieties PI. 218122 and PI. 250238 was due to the small size of seeds (59.9 and 49.9 mg, respectively), this evidently was not the case in variety PI. 151563 with seeds averaging 110.6 mg. Seed damage, though not significantly different in these 3 varieties, it may be suggested that the higher number of small forms in varieties PI. 218122 and PI. 250238 is due to insufficient food quantity as a result of small seeds, the latter factor being also reflected in a low percentage adult progeny emergence. From the almost complete absence of small forms in variety PI. 151563 it may be concluded that the low percentage of adult progeny emergence may be due to some intrinsic seed factor(s).

In support of the above argument, the mean weights of progeny weevils were considerably higher in the more susceptible varieties IVu. 201, PI. 292871 and commercial (2.0, 2.1, and 2.0 mg, respectively). Whereas in varieties PI. 218122 and PI. 250238 (1.5 mg each) low progeny weight may be due to small forms as a result of smaller seeds. The smaller weevils from variety PI. 151563 (1.7 mg) is unexplained. It follows therefore that variety PI. 151563 may possess a degree of resistance due to antibiosis.

Following up the observed pattern of correlation between seed size and percentage of small forms, mean developmental period and duration of adult emergence, respectively, these latter two parameters as well as percentage active form were considered for six varieties (Table 14).

Table 14. Comparison of mean developmental period, duration of adult progeny emergence and percentage of active forms in 6 cowpea varieties.

Variety	Mean developmental period (days)	Duration of adult progeny emergence (days)	% active form
IVu. 201	31.4	17.5	6.7
PI. 151563	32.4	12.3	0.8
PI. 218122	31.4	12.7	0.8
" 250238	30.7	8.3	0.0
" 292871	32.9	15.7	3.3
Commercial	32.7	17.0	3.2

No other explanation than crowding effect can be given for this pattern, confirming the observation that weevils take longer time to develop and that duration of progeny emergence is prolonged in varieties that offer more nutrient supply to the insects (IVu. 201, PI. 292871 and Commercial) than in those that offer less either quantitatively or qualitatively (PI. 151563, PI. 218122 and PI. 250238).

SUMMARY AND CONCLUSIONS

Vigna unguiculata commonly known as "cowpeas" is seriously attacked both in the field and storage by the weevil, Callosobruchus maculatus (Fab.). This study was designed at developing a laboratory technique for screening cowpea varieties for resistance to the weevil with the hope of detecting varieties with some degree of resistance.

Preliminary investigations were carried out to determine the optimum laboratory environment possible for weevil development at the same time eliminating conditions which might influence the expression of varietal resistance. Seeds were equilibrated to a constant moisture content before infestation and all tests were conducted in a rearing room kept at $80 \pm 2^{o}F$ and $67 \pm 3\%$ RH. The room is lighted during a 12-hr photoperiod.

A chilling procedure for easy handling of weevils was found to be most satisfactory to inactivate them. Neither this method nor the use of CO_2 produced observable after-effects on the weevils.

Ovipositional studies revealed best results when three 24-48 hr old female parent weevils were introduced into plastic boxes 48 mm x 48 mm x 18 mm, containing 40 cowpea seeds. Twenty or 30 seeds per box were used. With an increase in parent number, percentage adult progeny emergence decreased whereas percentage of active form increased. As a result of a test on the optimal duration of oviposition, a 5-day period was used; this period minimized the production of the active form and produced a high enough percentage of adult progeny. Eggs laid later in life were less viable.

Adult progeny emergence was observed until the 50th day after start of infestation to allow for emergence of all active forms but short of the appearance of a second generation of weevils. Progeny weevils were oven-dried at $60^{o}C$ for 6 days before weighing.

It was also shown that previous oviposition of weevils renders the seed coat less attractive for further oviposition. This may be explained by the behavior characteristics of cowpea weevils in preferring smooth seed coats to rough ones for oviposition. Thus non-preference may play a role in varietal resistance.

Thirteen cowpea varieties obtained from Nigeria and 26 from Mississippi and one commercial variety were subsequently screened by artificial infestation. Three seed characteristics, mean seed weight, seed coat color, and seed coat texture, and nine observations relating to the insect's biology were studied. Data were analyzed on the IBM 360 computer by analysis of variance to obtain LSD values for multiple comparisons and correlation coefficients with tests for significance.

Seed coat color had no influence on oviposition. The percentage of active forms was unreliable as a measure of resistance. Besides, it did not show any significant correlation with the seed characteristics studied. The percentage adult female progeny did not show any significant variation.

The other observations, mean seed weight; number of eggs per female; mean developmental period; duration of adult progeny emergence; percentage adult progeny emergence; number of weevils/g of seed; mean weight of progeny weevils and percentage of "small forms" were significantly different between varieties.

A form of the weevil designated as the "small form" in this study was observed in certain varieties. This form weighed less than half the normal form of the weevil (0.69 mg as compared to 1.9 mg, respectively). It occurred more in smaller than in larger seeds and correlation between seed size and the proportion of small forms was significantly negative. While a resistant variety may prolong or arrest the development of an insect, antibiosis may also be expressed in the production of individuals of reduced weight or of malformed types. This phenomenon may be due to either insufficient food quantity or quality. The correlation between percentage adult progeny and percentage of small forms was also significantly negative.

A comparison was made between 3 varieties with the highest percentage adult progeny but the lowest percentage small form and vice versa. It was suggested that the low percentage adult emergence and high percentage of the small forms in varieties PI. 218122 and PI. 250238 was due to small seeds. For variety PI. 151563, 40.5% adult progeny emergence did not differ significantly from 31.1% and 38.9% of the former two, respectively, but it produced only 1.2% of the small forms as against 15.4% and 11.4%. It is suggested that variety PI. 151563 possesses characteristics which confer some degree of resistance. Varieties PI. 292871, IVu. 201 and the local variety were highly susceptible.

However, since only a limited number of varieties were screened to test the suitability of the methods rather than to find a whole range of different types of resistant cowpeas, results obtained so far are by no means conclusive. Although statistical analyses offer generalized conclusions, it is possible that one desirable variety may be "hidden" among several thousands. It is necessary to examine a more representative sample of the world germplasm for conclusive deductions about the different combinations of resistance modalities and extreme expressions of resistance occurring naturally. It would be challenging to further analyze the contributions of several seed characteristics to resistance by crossing of varieties and studying the segregating offspring as to correlation between characters and expression of resistance.

ACKNOWLEDGMENTS

The author wishes to express sincere thanks and deep gratitude to the following:

Professor T. Ajibola Taylor, Head, Department of Agricultural Biology, University of Ibadan, Ibadan, Nigeria for providing the opportunity enabling him to pursue postgraduate studies;

Dr. Herbert Knutson, Head of the Department of Entomology, Kansas State University, for providing facilities;

Dr. E. Horber, Professor of Entomology, Kansas State University, for his guidance during the course of this study and his valuable advice and criticism as major advisor;

Dr. R. B. Mills, Associate Professor of Entomology, and Dr. G. J. Partida, Assistant Professor of Entomology, for their kind assistance and suggestions;

Dr. R. J. Elzinga, Professor of Entomology, for his photographic assistance;

Dr. Ken Rachie and Mr. P. N. Mehta of the International Institute of Tropical Agriculture, Ibadan, Nigeria and Dr. H. D. Green of Mississippi State College, State College, Mississippi for providing cowpea seed samples.

Mr. John L. Krchma for his statistical assistance; and finally to Mrs. Loretta Charlton for maintaining the weevil cultures.

LITERATURE CITED

Applebaum, S. W. 1964. Physiological aspects of host specificity in the Bruchidae. I. General considerations of developmental compatibility. J. Ins. Physiol. 10:783-8.

Applebaum, S. W., B. Gestetner and Y. Birk. 1965. Physiological aspects of host specificity in the Bruchidae. IV. Developmental incompatibility of soybeans for Callosobruchus. J. Ins. Physiol. 2:611-16.

Applebaum, S.W., B. J. Southgate and H. Podolar. 1968. The comparative morphology, specific status and host compatibility of two geographic strains of C. chinensis L. (Coleoptera, Bruchidae). J. Stored Prod. Res. 4:135-46.

Applebaum, S. W., S. Marco, and Y. Birk. 1969. Saponins as possible factors of resistance of legume seeds to the attack of insects. J. Agr. Food Chem. 17(3):618-22.

Applebaum, S. W., V. Tadmor and H. Podolar. 1970. The effect of starch and of a heteropolysaccharide fraction from P. vulgaris on development and fecundity of C. chinensis (Coleoptera: Bruchidae). Entomologia exp. appl. 13(1):61-70.

Arora, G. L., H. R. Pajni, and T. Singh. 1967. The ambiguity of the abnormal male of Callosobruchus maculatus (F.). Res. Bull. Punjab Univ. Sci. 18:501-5.

Bates, J. A. M. 1969. Influencia da cor do feijao de corda, V. sinensis Endl, no ataque dogorgulho, Callosobruchus analis. (The influence of the color of cowpeas (V. unguiculata) on attack by the Bruchid C. analis. Turrialba 19(2):296-7.

Bawa, S. R., K. C. Kanwar and R. K. Gupta. 1972. Interspecific hybridization and the abnormal strain of Callosobruchus maculatus (Coleoptera:Bruchidae). Ann. Entomol. Soc. Am. 65(5):1241-3.

Bell, E. A. and D. H. Janzen. 1971. Medical and ecological considerations of L-dopa and 5HTP in seeds. Nature 229:136-7.

Birk, Y., and S. W. Applebaum. 1960. Effect of soybean trypsin inhibitors on the development and midgut proteolytic activity of Tribolium castaneum larvae. Enzymologia 22:318-26.

Booker, R. H. 1967. Observations on three Bruchids associated with cowpeas in Northern Nigeria. J. Stored Prod. Res. 3(1):1-15.

Borchers, R., C. W. Ackerson and L. Kimmet. 1937. Trypsin inhibitor - IV. Occurrence in seeds of the leguminosae and other seeds. Arch. Biochem. 13:291-3.

Canerday, D. J. and R. B. Chalfant. 1969. An arrestant and feeding stimulant for the cowpea curculio, Chalcodermus aeneus. (Coleoptera:Curculionidae). J. Georgia Entomol. Soc. 4(2):59-64.

Caswell, G. H. 1960. Observations on an abnormal form of Callosobruchus maculatus. Bull. Entomol. Res. 50:671-80.

Chandola, R. P., K. B. Trehan and L. R. Bagrecha. 1969. Varietal resistance to Bruchus sp. in cowpea (V. sinensis) under storage conditions. Curr. Sci. 38:370-1.

Cuthbert, F. P. and O. L. Chamblis. 1972. Sources of resistance to cowpea curculio in Vigna sinensis and related species. J. Econ. Entomol. 65(2):542-5.

Cuthbert, F. P. and B. W. Davis. 1972. Factors contributing to curculio resistance in southern peas. J. Econ. Entomol. 65(3):778-81.

Faris, D. G. 1963. Evidence for the West African origin of Vigna sinensis (L.) Savr. (Ph.D. Thesis). Library, Univ. of Calif., Davis.

Faris, D. G. 1965. Origin and evolution of the cultivated forms of V. sinensis. Canad. J. Gen. Cytol. 7:433-52.

Gill, J., K. C. Kanwar and S. R. Bawa. 1971. Abnormal "sterile" strains in Callosobruchus maculatus. Ann. Entomol. Soc. Am. 64:1186-8.

Gokhale, V. G., and B. K. Srivastava. 1969. Development of C. maculatus F. on autoclaved French beans. Ind. J. Entomol. 31(1):71-2.

Green, H. B. 1970. Resistance of selected lines of southern peas (Vigna unguiculata) to the cowpea curculio, Chalcodermus aeneus Boheman, with notes on resistance to the cowpea weevil, Callosobruchus spp. Doctoral dissertation, Mississippi State Univ. 99pp.

Gundurao, H. R. and S. K. Majumder. 1964. Intergranular space as a limiting factor for the growth of pulse beetles. J. Econ. Entomol. 57(6):1013-14.

Ishi, S. 1952. Studies on the host preference of the cowpea weevil. Japan: Bull. Nat. Inst. Agr. Sci. 1(c):185-256.

Jotwani, M. G. and P. Sircar. 1967. Neem seed as a protectant against the Bruchid Callosobruchus maculatus (Fab.) infesting some leguminous seeds. Indian J. Entomol. 29(1):21-4.

Jotwani, M. G., P. Sircar and T. D. Yadar. 1967. Studies on the extent of insect damage and germination of seeds. II. Germination of some leguminous seeds damaged by the developing grubs of C. maculatus (Fab.). Indian J. Entomol. 29(3):309-11.

Kunitz, M. 1947. Crystalline soybean trypsin inhibitor. II. General properties. J. Gen. Physiol. 30:291-310.

Larson, A. O. and C. K. Fisher. 1938. The bean weevil and the southern cowpea weevil in California. U. S. Dept. Ag. Tech. Bull. No. 593.

Ligon, L. L. 1958. Characteristics of cowpea varieties (V. sinensis). Okla. State Univ. Expt. Sta. Bull. B-518.

Lipke, H., and G. S. Fraenkel. 1954. Growth inhibitors on the growth Tribolium confusum. J. Agric. Food Chem. 2(8):410-4.

Lum, P. T. M. and B. R. Flaherty. 1972. Effect of carbon dioxide on production and hatchability of eggs of Plodia interpunctella (Lepidoptera:Phycitidae). Ann. Entomol. Soc. Am. 65(4):976-7.

Lum, P. T. M. and R. H. Phillips. 1972. Combined effects of light and carbon dioxide on egg production of Indian meal moths. J. Econ. Entomol. 65(5):1316-7.

Mehta, P. N. 1970. The effect of defoliation on seed yield of cowpeas (Vigna unguiculata (L.) Walp) and analysis of leaf harvest for dry matter and nitrogen content. Tech. Comm. Int. Soc. Hort. Sci. No. 21:167-71.

Mookherjee, P. B. and M. L. Chawla. 1964. Effect of temperature and humidity on the development of Callosobruchus maculatus Fab., a serious pest of stored pulses. Indian J. Ent. 26(3):345-51.

Nwanze, K. F. 1971. Computer aided analysis of the morphological variation in wild and cultivated Vigna unguiculata (L.) Walp. from Africa. B.Sc. research project. Univ. Ibadan, Nig. Dept. of Ag. Bio.

Oyenuga, V. A. 1955. Nigeria's foods and feeding stuffs. Ibadan Univ. Press. 1968.

Painter, R. H. 1951. *Insect resistance in crop plants.* Macmillan, N. Y. p. 521.

Podoler, H. and S. W. Applebaum. 1968. Physiological aspects of host specificity in the Bruchidae-IV. Varietal differences in the resistance of Vicia faba L. to C. chinensis L. J. Stored Prod. Res. 4:9-11.

Press, A. F. and P. K. Harein. 1967. Mortality of Tribolium castaneum (Herbst) (Coleoptera, Tenebrionidae) in simulated peanut storages purged with carbon dioxide and nitrogen. J. Stored Prod. Res. 3:91-6.

Prevett, P. F. 1961. Field infestation of cowpeas (Vigna unguiculata) pods by beetles of the families Bruchidae and Curculionidae in Northern Nigeria. Bull. Entomol. Res. 52:635-45.

Raina, A. K. 1971. Comparative resistance to three species of Callosobruchus in a strain of chickpea (Cicer arietinum). J. Stored Prod. Res. 7(3):213-6.

Rehr, S. S., and D. H. Janzen, and P. P. Feeny. 1973. L-dopa in legume seeds: A chemical barrier to insect attack. Science 181:81-2.

Sano, I. 1967. Density effect and environmental temperature as the factors producing the active form of Callosobruchus maculatus. J. Stored Prod. Res. 2(3):187-95.

Schoof, H. 1941. The effect of various relative humidities on the life processes of the southern cowpea weevil, Callosobruchus maculatus (Fab.). at 30 ± 0.8°C. Ecology 22(3):297-307.

Southgate, B. J., R. W. Howe and G. A. Brett. 1957. The specific status of Callosobruchus maculatus (F.) and Callosobruchus analis (F.). Bull. Entomol. Res. 52:79-89.

Srivastava, B. K. and S. K. Bhatia. 1959. The effect of host species on the oviposition of Callosobruchus chinensis Lin. (Coleoptera: Bruchidae). Ann. Zool. 3(3):37-42.

Stanton, W. R. 1966. Grain legumes in Africa. F.A.O., Rome.

Strong, R. G., G. J. Partida and D. N. Warner. 1968. Rearing stored-product insects for laboratory studies: bean and cowpea weevils. J. Econ. Entomol. 61(3):747-51.

Su, H. C. F., R. D. Speirs and P. G. Mahany. 1971. Toxic effects of soybean saponins and its calcium salt on the rice weevil. J. Econ. Entomol. 65(3):844-7.

Su, H. C. F., R. D. Speirs and P. G. Mahany. 1972. Citrus oils as protectants of blackeyed peas against cowpea weevils: Laboratory evaluation. J. Econ. Entomol. 65(5):1433-6.

Swoyer, G. F. 1969. A simple technique for making insect testing cages. J. Econ. Entomol. 63(2):679-80.

Teotia, T. P. S. and V. S. Singh. 1966. The effect of host species on the oviposition, fecundity and development of C. chinensis Linn. Bell. Grain Technol. 4(1):3-10.

Todd, J. W. and T. D. Canerday. 1968. Resistance of southern peas cowpea curculio. J. Econ. Entomol. 61(5):1327-9.

Utida, S. 1954. Phase dimorphism observed in the laboratory population of the cowpea weevil, Callosobruchus maculatus. Jap. J. Zool. 18:161-8.

Utida, S. 1969. Photoperiod as a factor inducing the flight form in the population of the southern cowpea weevil, C. maculatus. Jap. J. appl. Entomol. Zool. 13(3):129-34.

Utida, S. 1972. Density dependent polymorphism in the adult of Callosobruchus maculatus (Coleoptera, Bruchidae). J. Stored Prod. Res. 8:111-26.

Willis, E. R. and L. M. Roth. 1954. Reactions of flour beetles of the genus Tribolium to carbon dioxide and dry air. J. Exp. Zool. 127:117-52.

VITA

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Mr. K. F. Nwanze was admitted into the Department of Entomology at Kansas State University in January 1972 to commence graduate studies under the advisorship of Dr. E. Horber.

A P P E N D I C E S

APPENDIX I

List of 40 cowpea varieties screened for resistance to attack by Callosobruchus maculatus

Identification Number	Source	Pedigree	Growth habit	Maturity[1]
IVu[2] 32	I.I.T.A. (Nigeria)	Iran Grey	su	63
" 37	"	Pale Green	su	62
" 57	"	New Era (169)	E	57
" 72	"	59-25	su	65
" 74	"	Kwara Exkwai-C, 68003	su	64
" 76	"	Prima, Improved West-bred-68006	-	-
" 176	"	C 5723-11, 64021	su-E	58
" 201	"	California Blackeye 5a, 64056	su	64
" 256	"	58-115-3, 64141	su	67
" 335	"	Jebba Pea B, 64243	su	57
" 352	"	C 239, 64272	su	57
" 354	"	Hanbru-58-123	su	58
" 1283	"	-	-	-
Pl. No.[3] 115674	Mississippi (U.S.A.)	Hen Me	E	87
" 151563	"	Frijolitos Cabe Ita Negra	Hb	82
" 162924	"	Tape	Hb	87
" 162925	"	-	Lb	87
" 186458	"	-	Lb	82
" 188704	"	-	Hb	102

APPENDIX I (concluded).

Identification Number	Source	Pedigree	Growth habit	Maturity
PI. No. 208845	Mississippi (U.S.A.)	-	E	82
" 211111	"	-	Lb	102
" 214069	"	-	Hb	82
" 218122	"	Lubia	E	71
" 218123	"	Lubia	Hb	71
" 220849	"	Lobia-I-Surkh	Hb	82
" 225921	"	-	Hb	74
" 250238	"	-	E	74
" 255811	"	-	Lb	82
" 270065	"	Rambo	Hb	82
" 292871	"	-	Hb	136
" 292895	"	-	Hb	82
" 292908	"	-	E	62
" 293448	"	Alabama Browney	Hb	87
" 293464	"	Blacks	E	87
" 294531	"	Large Black	Hb	87
" 300171	"	New Era	Lb	120
" 302457	"	-	E	82
" 312203	"	Fijol Del Casti La	Lb	87
" 315750	"	Combine Pea	E	-
Commercial	-	-	-	-

[1] From date of planting to first mature/ripe pod.

[2] IVu = International *Vigna unguiculata*.

[3] PI. No. = Plant Introduction number.

E = Erect; su = Semi-upright; Hb = High bushy; Lb = Low bushy.

APPENDIX II

Scoring system for seed coat color

Color	Score
White	1.0
Cream	2.0
Speckled white or cream	3.0
Tan	4.0
Brown	5.0
Mottled brown or tan	6.0
Black	7.0
Mixture of more than two colors	8.0

APPENDIX III

Scoring system for seed coat texture

Seed coat texture	Score
Smooth	1.0
Slightly smooth	1.5
Rough	2.0
Slightly wrinkled	2.5
Wrinkled	3.0
Slightly depressed	3.5
Depressed	4.0
Cracked	5.0
Mixture of smooth and wrinkled	6.0
Others	7.0

TECHNIQUES FOR SCREENING COWPEAS, *VIGNA UNGUICULATA* (L.) WALP. IN THE LABORATORY FOR RESISTANCE TO *CALLOSOBRUCHUS MACULATUS* FAB. (COLEOPTERA, BRUCHIDAE)

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AN ABSTRACT OF A MASTER'S THESIS

submitted in partial fulfillment of the

requirements for the degree

MASTER OF SCIENCE

Department of Entomology

KANSAS STATE UNIVERSITY
Manhattan, Kansas

1973

This study was to develop a laboratory technique for screening cowpea varieties for resistance to the cowpea weevil, Callosobruchus maculatus (L.) Walp.

In preliminary investigations the best environmental conditions for weevil development were tested to eliminate conditions which might influence and alter the expression of varietal resistance. Seeds were equilibrated to a constant moisture content before artificial infestation.

Ovipositional studies revealed best results when three female parent weevils 24-48 hours old were introduced into plastic boxes 48 mm x 48 mm x 18 mm containing 40 cowpea seeds. However, 20 or 30 seeds may also be used in case of seed shortage. All tests were conducted in a rearing room at 80 $\pm$ 2^{O}F and 67 $\pm$ 3% RH.

Due to previous oviposition the seed coat became less attractive for further oviposition.

Forty cowpea varieties were screened by artificial infestation. Three seed characteristics and nine parameters relating to the biology of the weevil were studied. Seed coat color did not influence oviposition and the occurrence of the active form was unpredictable. Percentage adult female progeny did not show any significant variation. Other characters studied differed significantly between varieties.

In our study we observed in certain varieties a "small form" which weighed less than half the normal form.

Results obtained indicated that varieties PI. 292871, IVu. 201 and the commercial variety were highly susceptible while variety PI. 151563 showed some degree of resistance.