ASPECTS OF SEED RESISTANCE IN SELECTED VARIETIES OF COWPEA TO DAMAGE BY *CALLOSOBRUCHUS MACULATUS* (FABRICIUS) (COLEOPTERA: BRUCHIDAE)

BY

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ABSTRACT

Three experiments were used to assess the relative susceptibilities of three known resistant, one known susceptible and seven other varieties of cowpea available locally in Akure (Nigeria) markets under laboratory conditions (30 ± 1°C and 50 ± 10% r.h.). In the first experiment, a known susceptible and three resistant varieties of cowpea were subjected to three different levels of initial larval infestation (one, five and ten larvae per seed). A beetle colony derived from infested cowpea seeds in a local market in Akure was employed. In the second experiment, the susceptibility of seven varieties of cowpea commonly sold in local markets, to seed damage by *C. maculatus* were compared with that of three known resistant varieties and a well known susceptible variety. The development and survival of *C. maculatus* in two of the known resistant varieties over five successive generations of the beetle was further monitored in the third experiment. Speed of beetle development and larval survival to adult emergence in the cowpeas, were used to measure performance of the beetle in the cowpeas.

Results of the first experiment showed that in the resistant cowpeas, development time was not affected by level of larval infestation. Percentage adult emergence decreased significantly ($P < 0.05$) with increase in level of larval infestation in the resistant cowpeas.

In the second experiment, beetle development was generally slower and fewer individuals survived to adulthood in the known resistant cowpea compared to others. Development time was significantly longer and percentage adult emergence was significantly lower in three of the locally sold varieties, TVx 3236, White Drum and

Oloo than in the well known susceptible variety.

In the third experiment, there were no significant differences in the development time of *C. maculatus* in the resistant cowpeas (IT85F-2205 and IT90K-391) between five successive generations. The percentage of hatched beetle eggs which produced adults in the cowpea increased significantly from one generation to the next.

The significance of the observations in the experiments is discussed.

DEDICATION

This project is dedicated:

To God Almighty - My Father

Jesus Christ - My Redeemer

and

Holy spirit - My comforter.

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May God bless you all in Jesus name.

CERTIFICATION

I certify that this work was carried out by Mr. Adeduntan, Sunday Adeniyi under my supervision in the Department of Crop Production, The Federal University of Technology, Akure, Ondo State.

Dr. T. I. Ofuya
Supervisor

24/8/98

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CHAPTER ONE

1.0 GENERAL INTRODUCTION

Cowpea, *Vigna unguiculata* (L.) *Walp.* is an important food and trade item in tropical countries, especially in West Africa where it is a cheap principal source of protein (Oyenuga, 1968). As food, it is eaten in the form of dry seeds, green pods, green seeds and tender green leaves. It is also utilized for fodder and as a quick growing cover crop under a wide range of conditions on account of its ability to fix atmospheric nitrogen efficiently; up to 240 kg N/ha (Rachie and Roberts, 1974). However, insects are a major limiting factor to cowpea production and utilization in Africa (Singh and Jackai, 1985).

The major insect pests of cowpea in storage are bruchids, especially *Callosobruchus maculatus* (F.). The attack starts from the field when the pods are nearly mature. Eggs are laid on the pods but weevils prefer to slip inside the pods through holes made by the other pests and lay eggs directly on the seeds. After harvesting, bruchids multiply and do considerable damage to stored cowpea. *C. maculatus* is capable of causing hundred percent infestation within 3-6 months storage period in West Africa (Singh, 1977; Caswell and Akibu, 1980; Seck, 1993). The damaged seeds are riddled with adult emergence holes, defaced with egg covers and have reduced weight and poor germinating capacity. Loss in dry weight due to the cowpea bruchid can be as much as thirty percent in six months of storage (IITA, 1982). Heavy attack causes severe powdering (Kranz *et al.*, 1977) and the product is unfit for human consumption. The damage done by this bruchid is so substantial that many farmers in Africa, Asia and South America are compelled to dispose off their crop soon after harvest which not only reduces an important protein source for their families, but forces them to sell at lower prices (IITA, 1979; 1980; 1982).

Efforts have been made to find effective control measures for storage bruchids. One of these is the use of host plant resistance. The development and use of resistant cowpea varieties have been extensively studied because it offers a simple, cheap and attractive approach to reduction of *C. maculatus* damage in stored cowpea. Of about 15,000 accessions of cowpea in the germplasm collection at the International Institute of Tropical Agriculture (IITA), Ibadan, Nigeria, a few, especially TVu 625,TVu 2027, TVu 4200, TVu 11952, and TVu 11953 have been identified as having appreciable levels of seed resistance to *C. maculatus* damage (Singh, 1977; Adjadi *et al.*, 1985; Jackai and Singh, 1988). From these resistant progenitors, especially TVu 2027 many other *C. maculatus* resistant cowpeas have been developed at IITA (Singh, 1987; Singh and Singh, 1992). Some improved cowpea varieties combine bruchid resistance with resistance to one or two other major insects pests and many cowpea diseases, and have been recommended for release in Nigeria and other countries (Singh and Singh, 1992). This recommendation notwithstanding, many farmers do not grow these improved cowpeas for reasons which are not clear (Jackai and Adalla, 1997). Most of these improved varieties are hardly found in many Nigerian markets.

The level of resistance exhibited by the IITA varieties has, however, not been consistent amongst the different workers. It has been observed that *C. maculatus* from different geographical regions differ in their ability to damage seeds of resistant cowpea (Dick and Credland, 1986a; Ofuya and Credland, 1995a). Another factor that may cause discrepancy in observed level of seed resistance in cowpea by different workers is the experimental protocol, including the level of seed infestation (Ofuya and Reichmuth, 1994). Whilst Messina and Renwick (1985) found that seed resistance in some cowpea derived from TVu 2027 was not overcome by *C. maculatus* over six generations, Dick and Credland (1986b) observed that *C. maculatus* became more tolerant of seed resistant factors in TVu 2027 over three generations.

The objectives of this project were therefore to:

(i) determine the effect of level of initial larval infestation of *C. maculatus* on seed resistance in three known resistant and a susceptible cowpea.

(ii) compare susceptibility of seven commonly sold cowpea varieties with three known resistant varieties and a well known susceptible one and,

(iii) determine the ability of *C. maculatus* to overcome resistant factors in two known resistant cowpea over five successive generations.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 THE BIONOMICS OF *C. MACULATUS* AND DAMAGE TO COWPEA

The initial infestation occurs in the field when the pod are nearly mature. Adult female beetle can lay eggs on pods at various stages of maturation and their larvae are capable of developing on dry grains and on developing grains (Huignard *et al.*, 1985). During eclosion, the larvae burrow through the chorion of the egg directly into the pod wall, and then through the pod wall and seed coat into the seed, where the larvae develop and pupate (Prevett, 1961; Booker, 1967; Messina, 1984). In the field, beetles frequently enter inside the pods through holes made by other pests and lay eggs directly on the seeds [Singh *et al.*,1990]. Individuals which invade field crop may come from infested stores in the vicinity (Taylor and Agbaje, 1974) or from wild cowpea or other legumes [Booker, 1967]. Store infestation is frequently derived from harvested field infested pod or seeds (Booker, 1967; Caswell, 1976; Parr, 1994) but may also come from hidden infestation in the store. Irrespective of source of initial infestation, the founders consist of relatively few individuals that can breed and proliferate to pest proportions in a relatively short time (Prevett, 1961; Huignard *et al.*, 1985). The growth, development and reproduction of *C. maculatus* in the laboratory have been studied by different workers (Caswell, 1961; Howe and Curie, 1964; Osuji, 1982; Giga and Smith, 1987). The optimum conditions for these processes in this beetle are 30-35° C and 50-70% or more relative humidity which closely approximate to what obtains during a major part of the cowpea storage period in West Africa. Under optimal conditions, *C. maculatus* eggs hatch in 3-5 days and larval development takes 8-16 days. There is a 2-day prepupal and a 5-day pupal stage. After the pupal stage, adults emerge through exit [emergence] holes demarcated during the last larval instar. Males and females may mate shortly after emergence, and presumably several times during adult life (Ofuya, 1995). It has been reported that females release a pheromone that attracts males, (Qi and Burkholder, 1982). Oviposition starts soon after mating and majority of the eggs are laid within three days. Unmated females do not normally lay eggs. The adult life span is from 8-16 days but unmated individuals could live much longer.

The oviposition behaviour of mated females has been extensively studied and females prefer large (Mitchell, 1975), whole (Nwanze *et al.*, 975), smooth [Messina and Renwick, 1985a] seeds for egg-laying. The behavioural repertoire and mechanisms during oviposition have also been examined (Ofuya and Agele 1989a, b; Parr, 1994; Ofuya and Adenakan, 1997). Realized fecundity of females is variable and is influenced by kind of host infested (Ofuya, 1987a, b; Ofuya and Bamigbola, 1991), number of host available (Credland, 1986, Ofuya, 1987c), and the reproductive experience of males (Ofuya, 1995).

Adult population of *C. maculatus* is known to exhibit polymorphism (Taylor and Agbaje, 1974). Two primary morphs; called the secondary and active forms may be produced in infested stores. Active morphs appear when food quality is poor or when the temperature and humidity rise during severe infestation (Messina and Renwick, 1985b). Active morphs fly readily and are smaller, and have larger fat reserve, live longer and lay fewer eggs later in life. They are unquestionably important in dispersal of the species. It appears that *C. maculatus* produces intermediate morphs which exhibit variable takeoff ability (Pajni, 1986) and sedentary forms which may be able to fly well to invade field crops from nearby stores (Thanthianga and Mitchell, 1990).

Damage to cowpea seeds in storage by *C. maculatus*, is widespread in Africa and constitutes a major constraint to food availability and utilization. In Nigeria alone the estimated dry weight loss of cowpea due to bruchid infestation is about 30,000 tonnes (which represents about 4% of the total annual production) per annum with an estimated value in excess of 30 million U.S. dollars (Caswell 1961; 1980; 1981; Singh *et al.*, 1983). The infestation by this bruchid not only causes reduced seed weight but also affects the aesthetic and nutritional values of the grain and decreased seed germination potential (Booker, 1967; Caswell, 1981). Loss in seed germination due to bruchid attack may reach 100% for grains with four emergence holes (Santos, 1971).

2.2 CONTROL OF *C. MACULATUS* IN COWPEA

Over the years, various methods of controlling this bruchid with differing levels of effectiveness have been devised (Lale and Efeovbokhan, 1991). The methods include chemical control, modification of storage atmosphere, the use of plant-derived insecticides and the use of host plant resistance.

2.2.1 CHEMICAL CONTROL OF *C. MACULATUS*

Many insecticides have been demonstrated to be effective against *C.maculatus* either as dusts or fumigants. Insecticidal dust of malathion, pirimiphos-methyl or permethrin, have been reported to be effective against *C. maculatus* (Hill, 1983; Kranz *et al.*, 1977; Singh *et al.*, 1978; Caswell and Akibu, 1980; Taylor and Evans, 1980; Onolemhemhem and Oigiangbe, 1991; Ogunwolu and Idowu, 1994), and fenitrothion, carbaryl and deltamethrin (Rahman, 1990) can protect shelled cowpeas stored in bags or airtight containers from bruchid damage for several months. However, dusts of pirimiphos-methyl, tetrachlorvinphos and lindane have been observed to be ineffective against bruchid infestation in unthreshed cowpea (Caswell, 1976). Fumigation is especially useful for bruchid control in large-scale cowpea storage in air-tight facilities such as silos, warehouses and other enclosures. Aluminium phosphide which releases phosphine gas is commonly used (Caswell, 1976). Methyl bromide (Yoneda *et al.*, 1990) and dichlorvos (Rahman, 1990) are also effective fumigants against *C. maculatus*.

The role of insecticides in pest management systems of the future is now open to question especially in the developed economies, due to the decreasing tolerance of residues in foodstuffs (Longstaff, 1994). It is therefore, essential that stored products protection in the developing countries of West Africa is achieved through reliance on a range of alternative non-chemical pest control measures so as to reduce dependence on insecticides to a minimum.

2.2.2 MODIFICATION OF STORAGE ATMOSPHERE FOR *C.MACULATUS* CONTROL

An alteration of atmospheric gas concentration is an attractive method because insect deterioration can be controlled without use of pesticide. Different storage devices which provide hermetic conditions have been reported to be effective (Ripp, 1984; Shejbal, 1980). The consumers or households can hermetically store cowpea seeds in plastic bags with baft or cotton liners (Caswell, 1976). The atmosphere achieved depends on the degree of gas tightness, the temperature and moisture content of the grain and the initial abundance of pest and microflora (Evans, 1987). The storage insects eventually die from asphyxiation. In a hermetically sealed steel drum filled with cowpea seeds, the concentration of oxygen will drop to about 1% within 2 weeks because of normal infestation by *C. maculatus* which is deleterious to bruchids (Caswell, 1976). If the hermetic storage is efficiently carried out, the cowpea seeds will be preserved in a condition little worse than it was when storage began (Caswell, 1976). Insecticidal atmospheres can be produced artificially in storage for pest control (Banks, 1979; Banks and Annis, 1990). In 100% carbon dioxide or pure nitrogen atmosphere all stages of *C. maculatus* are killed in 5 days at 32° C (Ofuya and Reichmuth, 1992). Other carbon dioxide based atmospheres containing 30-88% CO_2 and nitrogen based atmospheres containing 1-4% O_2 have been demonstrated as effective against *C. maculatus* (Ofuya and Reichmuth, 1993b; 1994; Reichmuth and Ofuya, 1993). The adoption of the controlled atmosphere storage in developing countries will certainly be hampered by the cost of gas and sealing of storage structures.

2.2.3 BIOLOGICAL CONTROL OF *C. MACULATUS*

Many natural enemies attack bruchids, but before the populations build up to effective levels in storage, considerable damage has already been done to the cowpea seeds by the bruchids (Ofuya, unpublished observation). In West Africa, *C. maculatus* is mainly parasitized by *Dinarmus basalis (Rondani)* (Synonymous to *D. laticeptis* (Ashmead), *Eupelmus vuilleti Crawf.*, *Chaetospila elegans* Westwood and *Uscana lariophaga* steffan (Caswell, 1976; Owusu-Akyaw, 1987). Since some damage can be tolerated by cowpea consumers in West Africa, research on the use of these natural enemies for the control of bruchids may be valuable. Attempts have been made at classical biological control [the introduction of exotic agents for long-term suppression and regulation of pest populations] of bruchids in some parts of the world with varying degrees of success (Steffan, 1981; Arbogast, 1984). Some work has also been done on the bionomics of indigenous pasitoids of *C. maculatus* in West Africa (Leveque *et al.*, 1993; Alebeek, 1996). However, the adoption of this method by the farmer may be hindered by financial, technical and logistical problems. The method also requires a more thorough and detailed knowledge of the biology and ecology of the different natural enemies.

2.2.4 USE OF PLANT-DERIVED INSECTICIDES FOR *C. MACULATUS* CONTROL

The use of plant materials in the control of storage pests is an ancient measure in many parts of the world. The mixing of the plant parts (leaf, bark, seeds) and vegetable oils has been traditionally practised especially in Asia and Africa (Fujii *et al.*, 1989). There is an increase in the use of biocides from plants because insecticides derived from plants like pyrethrum and rotenone have been found to be non persistent in the environment, of low mammalian toxicity, and relatively safe to other non-target organisms. Neem, *Azadirachta indica* (A. Juss.)is one of the plants in the African landscape that are being investigated as a source of pest control on food crop (Olaifa *et al.*, 1987; Saxena 1989; Schmutterer; 1985). Black pepper, *Piper nigrum* (both the dried and brine-preserved unripe fruit) protects peas and bean seeds against *Callosobruchus* (Mylkado *et al.*, 1979; Su and Hovert, 1981); on contact it is highly toxic to adults and newly hatched larvae before they penetrate seeds. Similarly, Ofuya (1986) reported that dry chili pepper fruits, *Capsicum annum* and onion scale leaves, *Allium cepa* afforded some degree of protection against *C. maculatus* damage in stored cowpea. Lale [1992] has more recently demonstrated repellency of dry chilli pepper fruits to adults of *C. maculatus*. Citrus peels have also been shown to significantly reduce viability of *C. maculatus* eggs [Rajapakse, 1990].. Ofuya [1990] also reported that powders of *Nicotiana tabacum* L., *Erythrophleum suaveolens* and *Ocimum gratissimum L.* significantly reduced oviposition by the beetle.

Researchers have also been investigating the use of vegetable oils as surface protectants for cowpeas and beans against bruchids. Sangappa (1977) reported that neem oil applied at 0.75 to 1.0 (percent by weight) protected red gram seeds for 161 days from weevil infestation. Singh *et al.*, (1978) reported that mixing 5 to 10 ml groundnut oil with one kilogramme of cowpea seeds achieved an effective control of weevil for six months. Messina and Renwick (1983) reported that vegetable oils not only kill the eggs but also deter oviposition. The oil film also interferes with respiration of the insects. The oils prevented the hatching of the eggs which Tikka *et al.*, (1981) attributed to the interference of water balance resulting in undifferentiated and shrunken eggs.

However, many of these oils contain unsaturated fatty acids and fats that turn rancid due to oxidation. The result is a disagreeable odour and reduced protection against the insects (Su, 1984). Also high levels are needed for effective pest control, and at present small farmers cannot always afford to use oil on the bulk of their seeds for storage; there is the problem of cleaning the oil off the commodity before sale or use.

2.2.5 THE USE OF HOST PLANT RESISTANCE IN THE CONTROL OF *C. MACULATUS*

Plant resistance, has been defined by Fenemore (1984) as the ability of a plant species or variety to grow and produce economically despite the presence of the pest. The use of plant resistance offers a simple, cheap and attractive approach to reduction of *C. maculatus* damage in stored cowpea (Kitch *et al.*, 1991). Extensive and intensive screening work has been done at IITA to locate cowpea varieties that are less susceptible to seed damage by *C. maculatus*. Of about 15,000 accessions of cowpea in the germplasm collection at the IITA, Ibadan, Nigeria, a few, specifically TVu 625, TVu 2027, TVu 4200, TVu 11952 and TVu 11953 have been identified as having appreciable level of seed resistance to *C. maculatus* damage (Singh, 1977; Adjadi *et. al.*, 1985; Jackai and Singh, 1988). Many researchers have worked on inheritance of bruchid resistance in cowpea and reported that two recessive genes are required in the homozygous condition to confer resistance against *C. maculatus* infestation (IITA, 1982; IITA 1983; Redden and McGuire, 1983, and Adjadi *et al.*, 1985). These genes are digenically inherited (IITA, 1983; Adjadi *et al.*, 1985). Development of the bruchid on cowpea varieties that have the resistance gene is very poor and the build-up of *C. maculatus* population is therefore very slow (Singh, 1977; 1987; IITA, 1983). Seed resistance to *C. maculatus* in these IITA cowpea varieties has been confirmed by several other workers (Messina and Renwick, 1985; Ofuya, 1987; Kitch *et al.*, 1991; Mbata, 1993; Ofuya and Credland, 1995a). Cowpeas which exhibit moderate resistance to *C. maculatus* have also been identified among the wild and weedy species (Ofuya, 1987c). Some *Vigna* genera are immune to *C. maculatus* damage (Ofuya, 1987d).

Using the resistant parent lines especially Tvu 2027, IITA has developed several improved breeding lines which combine bruchid resistance with other desirable agronomic traits, such as high yield, and early maturity [Singh and Singh, 1992].

The level of resistance exhibited by the IITA varieties has however, not been consistent amongst the different workers. It has been observed that *C. maculatus* from different geographical regions differ in their ability to damage seeds of resistant cowpea (Dick and Credland, 1986a; Ofuya and Credland, 1995a). Another factor that may have caused discrepancy in observed level of seed resistance in cowpea by different workers is the experimental protocol, including the level of seed infestation. Level of larval infestation of seed has been shown to influence the degree of control exercised by controlled atmospheres (Ofuya and Reichmuth, 1994).

The mechanism of cowpea seed resistance to *C. maculatus* includes antibiosis (Singh, 1977; 1987; IITA, 1983; Ofuya, 1987a). The factors conferring resistance to cowpea varieties against bruchid infestation are morphological and biochemical in nature and both are inherited. The morphological factors especially affect feeding, oviposition and ingestion of the pest (Kumar, 1984) while the biochemical factors influence to a large extent the metabolic processes of the pest (Kogan, 1977).

2.2.5.1 MORPHOLOGICAL FACTORS IN COWPEA AND RESISTANCE TO *C. MACULATUS*

Physical characteristics of the cowpea pod and seed such as seed texture, size and thickness of the pod wall, have been linked to resistance of cowpea varieties to *C. maculatus* (Akingbohungbe 1976; Nwanze and Horber, 1976; Fatunla and Badaru, 1983a, b; Messina and Renwick, 1985; Khattak *et. al.*, 1987; Lale and Efeovbokhan, 1991; Mbata, 1992).

It has been reported that *C. maculatus* prefers to lay eggs on smooth-coated seeds than on rough-coated seeds (Howe and Curie, 1964; Nwanze and Horber, 1976; Messina and Renwick, 1985). Recent findings have shown that *C. maculatus* laid significantly more eggs on rough-coated seeds than on smooth-coated seeds of other varieties of cowpea (Lale and Efeovbokhan, 1991; Turaki, 1996). This latter finding lends support to the possible involvement of chemical factors as part of the oviposition cues of *C. maculatus*. Credland and Wright (1988) have also demonstrated that extracts obtained from the testae of different cowpea varieties either stimulated or deterred oviposition of *C. maculatus* without regard to whether the testae were rough or smooth.

Pod wall resistance is an alternative source for reducing seed damage by *C. maculatus* in cowpea and some less susceptible cowpeas have been identified (Akingbohungbe, 1976, Fitzner *et al.*, 1985; Kitch *et al.*, 1991; Ofuya and Awelewa, 1993). Cuthbert *et al.*, (1974), demonstrated that a pod factor inhibiting penetration through the pod wall by the adult, and antibiosis are under genetic control and can be transmitted to progeny. Fery and Cuthbert (1979) investigated the pod factor resistance and calculated the narrow and broad sense heritability estimates of 45 percent and 49 percent, respectively. Chambliss and Rymal (1982) investigated the genetic nature of the pod factor by analysing the pod-wall strength at dry pods in parental F_1, F_2 and backcross generations of a resistant X susceptible cross, and reported the narrow and broad-sense heritability estimates of 45 percent and 47 percent. These results suggested that pod-wall strength is closely associated with pod factor resistance. Dick and Credland (1986b) suggested the use of these physical characteristics to complement the biochemical resistance found in varieties derived from TVu 2027.

2.2.5.2 CHEMICAL FACTORS IN COWPEA AND RESISTANCE TO *C. MACULATUS*

Several chemical factors have been reported to be responsible for *C. maculatus* resistance to cowpea (Gatehouse *et al.*, 1979; Lale and Efeovbokhan, 1991). Gatehouse *et al.*, (1979) reported that a high level of trypsin inhibitor (about 2 fold increase) in TVu 2027 seeds compared to the susceptible varieties was responsible for resistance to *C. maculatus*. They also showed that trypsin inhibitor isolated from cowpea and mixed in ground cotyledons of a susceptible cowpea variety Tvu 57 reduced the survival of bruchid eggs. Baker *et al.*, (1989) analyzed trypsin inhibitor activity in ten TVu 2027 derived bruchid resistant breeding lines including TVu 2027 and 5 susceptible lines. Contrary to claims that elevated trypsin inhibitor levels may correlate with *C. maculatus* resistance (Gatehouse *et al.*, 1979; Janzen *et al.*, 1976; Gatehouse *et al.*, 1984) trypsin inhibitory activities in the seeds did not differ significantly between resistant and susceptible lines nor was the level of trypsin inhibitor correlated to *C. maculatus* developmental time or mortality. The trypsin inhibitor activity ranged from 32.2 T.I.U/mg to 50.0 T.I.U/mg with mean of 43.2 T.I.U/mg in resistant breeding lines compared to range of 32.8 T.I.U/mg to 44.5 T.I.U/mg with a mean of 38.9 T.I.U/mg in susceptible lines. The differences were non-significant. It has also been observed that artificial seeds made out of TVu 2027 or its derivatives are as susceptible to bruchids as susceptible lines (Murdock *et al*, 1988). Piergiovanni *et al.* (1990b) also found that the bruchid-susceptible line TVu 3000 had the highest value of trypsin inhibitor unit per milligram of proteins (66.2 T.I.U/mg protein) and one of the bruchid-resistant lines, IT82D-716, had the lowest (27.1 T.I.U/mg protein) of all the cowpea lines tested. These observations indicate that trypsin inhibitor alone may not account for bruchid resistance in cowpea.

A trypsin inhibitor isolated from *V. unguiculata* (Gatehouse *et al.*, 1979), and a phytohemagglutinin isolated from the dry bean *Phaseolus vulgaris* (Gatehouse *et al.*, 1984) were detrimental to artificial diets. However, a correlation analysis of 56 *Vigna* and 18 *Phaseolus* species failed to find any significant associations between levels of either phytohemagglutinins or trypsin inhibitors and seed resistance to *C. maculatus* (Birch *et al.*, 1985).

A previous study with cowpea examined levels of proteins which inhibit the activity of trypsin, chymotrypsin, leucine, aminopeptidase and carboxy peptidase to determine the relationship between such proteinase inhibitors and bruchid resistance (Gatehouse *et al.*, 1979). Recently, a cysteine proteinase with a mildly acidic pH optimum has been identified in the gut of *C. maculatus* (Gatehouse *et al.*, 1985; Murdock *et al.*, 1987). Addition of a cysteine proteinase inhibitor to artificial cowpea seeds prolonged developmental time and increased mortality for *C. maculatus* (Murdock *et al.*, 1988). Gatehouse *et al.* (1985) partially purified cysteine proteinase inhibitors from cowpea seeds and showed that they were effective inhibitors of *C. maculatus* larval proteinase. It seemed reasonable that the level of cysteine proteinase inhibitory activity in seeds might be related to *C. maculatus* resistance. Osborn *et al.*, (1988) have identified 'arcelin', a major seed protein in wild *Phaseolus vulgaris* as the factor responsible for resistance to bean bruchid *Zabrotes subfasciatus* (Boheman). Similarly, paraminophenyl alanine in several wild *Vigna* species was shown to be toxic to *Zabrotes subfasciatus* as well as to *Callosobruchus maculatus* (Birch *et al.*, 1986). Ishimoto and Kitamura (1988) showed that a water soluble substance present in kidney beans strongly inhibits the larval growth of *C. chinensis*.

Findings of a recent study suggest that considerable differences exist between bruchid-resistant and bruchid-susceptible cowpea lines in terms of their total unsaturated C^{18} fatty acids and some fatty acid ratios (Piergiovanni *et al.*, 1990a; 1990b). Lower contents of total unsaturated C^{18} fatty acids were typical of the resistant than the susceptible lines, whereas the ratios of the critical fatty acid were higher in the former than in the latter cowpea lines (Piergiovanni *et al.*, 1990b). This observation is in agreement with earlier findings of Boughdad *et al.*, (1987) in which they indicated that fatty acids may have significant adverse effects on the development of larval bruchids.

2.2.5.3 THE MERITS OF USING RESISTANT VARIETIES IN *C. MACULATUS* CONTROL.

The development of resistant varieties as a means of dealing with the problem of *C. maculatus* attack of cowpea may therefore be important in many different ways. First, it is an easy, economical and effective way of controlling the bruchid on stored cowpea (Fenemore, 1984; Kumar, 1984; Lale and Efeovbokhan, 1991; Lale, 1996). Secondly, it requires little technological inputs, creates no environmental hazards and is generally compatible with other methods of control (Pathak and Saxena, 1976). Thirdly, resistant cowpea varieties are known to effect a cumulative reduction in bruchid numbers when compared to susceptible varieties (Dick and Credland, 1984; 1986a, b). Fourthly, in an integrated bruchid control programme, it may improve the effectiveness of insecticides (Horbert, 1972, Fenemore, 1984). Fifthly, use of resistant varieties generally requires little or no cost to farmers, thus farmers can direct their resources to other desirable inputs (Kumar, 1984). Finally, it may be cheaper to develop resistant varieties than new pesticides.

These numerous advantages support the continued interest by researchers in vigorously pursuing investigations in the area of varietal control of crop pests and effective dissemination of findings.

CHAPTER THREE

3.0 EFFECTS OF COWPEA SEED RESISTANCE AND LARVAL COMPETITION ON THE DEVELOPMENT OF *CALLOSOBRUCHUS MACULATUS* (F.) (COLEOPTERA: BRUCHIDAE)

3.1 INTRODUCTION

The cowpea seed beetle, *Callosobruchus maculatus* (F.) is a cosmopolitan storage pest of cowpeas, *Vigna unguiculata* (L.) Walpers and related grain legumes (Jackai and Daoust, 1986; Singh *et al.*, 1990). Often, after six months in storage 100% infestation may be recorded (Singh, 1977; Seck, 1993). Damaged seeds are riddled with adult emergence holes and defaced with egg covers which leads to reduced weight and poor quality product. Beetle damage also causes significant reduction in seed viability. The development and use of resistant cowpea varieties have been extensively studied because it offers a simple, cheap and attractive approach to reduction of *C. maculatus* damage in stored cowpea. Of about 15,000 accessions of cowpea in the germplasm collection at the International Institute of Tropical Agriculture (IITA), Ibadan, Nigeria, a few, specifically TVu 625, TVu 2027, TVu 4200, TVu 11952 and TVu 11953 have been identified as having appreciable levels of seed resistance to *C. maculatus* damage (Singh, 1977; Adjadi *et al.*, 1985; Jackai and Singh, 1988). From these resistant progenitors, especially TVu 2027 many other *C. maculatus* resistant cowpeas have been developed at IITA (Singh, 1987; Singh and Singh, 1992). Many workers (Messina and Renwick, 1985; Dick and Credland, 1986a; Ofuya, 1987; Kitch *et al.*, 1991; Mbata, 1993; Ofuya and Credland, 1995a) have largely confirmed the lower susceptibility of these resistant IITA varieties to the seed beetle. The level of resistance exhibited by the IITA varieties has, however, not been consistent amongst the different workers. It has been observed that *C. maculatus* from different geographical regions differ in their ability to damage seeds of resistant cowpeas (Dick and Credland, 1986a; Ofuya and Credland, 1995a). Another factor that may cause discrepancy in observed level of seed resistance in cowpea by different workers is the experimental protocol, including the level of seed infestation (Ofuya and Reichmuth, 1994). This was examined in this experiment.

3.2 MATERIALS AND METHODS

3.2.1 *Beetle population*

C. maculatus originating from individuals infesting cowpea in a local market in Akure were used. They were used to infest Ife Brown cowpea in Kilner jars and the cultures were maintained and the study conducted at 30 ± 1° C and 50 ± 10% relative humidity in a cooled incubator.

3.2.2 *Cowpea varieties*

The varieties used were three known bruchid-resistant cowpeas initially developed at IITA, IT84S-2246-4, IT85F-2205, and IT90K-391, and a well-known susceptible variety, Ife Brown, and the seeds were obtained directly from IITA, Ibadan, Nigeria. All seeds were first stored in a deep-freeze for seven days prior to use and were thereafter conditioned in the cooled incubator for at least 7 days before use in the experiment to standardize their moisture content.

3.2.3 *Experimental protocol*

For each cowpea variety, 100 conditioned seeds were placed inside a glass Petri plate (9.0 cm in diameter) into which was introduced several freshly emerged males and females of *C. maculatus* for 24 hr so that females would oviposit on the seeds. The time of oviposition was recorded. Excess eggs were removed from seeds to leave either one, five or ten eggs per seed. Subsequently, each seed was placed in a separate test tube and closed with cotton wool such that emerging adults could not escape to the outside. Fourteen days after oviposition (after egg hatch but before adult emergence), seeds bearing eggs that failed to hatch (such eggs remain translucent) were discarded. For each level of seed infestation, 25 seeds with hatched eggs were retained. As from 20 days after oviposition, the seeds were examined at least twice daily and adults were removed as they emerged over a 70 day period (i.e. until 90 days after oviposition). This procedure was replicated three times. The time of emergence of each adult was recorded. Development period (time of egg laying to adult emergence from seed) and percentage adult emergence (number of emerged adults x 100/number of hatched eggs were calculated for each cowpea variety and each level of seed infestation. Data on developmental period (days) and percentage adult emergence were subjected to analyses of variance. Percentage adult emergence was arcsine transformed before analysis.

3.3 RESULTS

The development period of *C. maculatus* in seeds of the susceptible Ife Brown and the three resistant cowpeas was not significantly different ($P > 0.05$) whether one, five or ten larvae simultaneously infested each seed (Table 3.1). There were significant ($P < 0.05$) differences in the mean percentage adult emergence of the beetle from the cowpeas at the different levels of initial larval infestation, except in the susceptible variety (Table3. 2). For the resistant varieties, percentage adult emergence was significantly lower when ten larvae initially entered a seed than when one larva initially entered a seed. Percentage adult emergence was similarly significantly lower when ten larvae initially entered a seed than when five larvae initially entered a seed except for IT84S-2246-4. Percentage adult emergence when five larvae initially entered a seed was not significantly different from that observed when one larva initially entered a seed except for IT84S-2246-4. The interaction between larval infestation level and variety was significant ($P < 0.05$).

Table 3.1. The mean developmental period (days) of the local population of *C. maculatus* in seeds of selected varieties of cowpea at different levels of initial larval infestation

Cowpea variety	Resistance status	Mean developmental period (days) (± S.E.) of beetle in seeds initially bearing:		
		One larva	Five larvae	Ten larvae
Ife Brown	Susceptible	22.4 ± 0.22	22.2 ± 0.21	21.8 ± 0.18
IT84S-2246-4	Resistant	31.5 ± 1.35	31.4 ± 0.93	31.4 ± 0.85
IT85F-2205	Resistant	33.7 ± 0.93	34.1 ± 0.80	33.7 ± 1.28
IT90K-391	Resistant	33.2 ± 1.60	32.7 ± 0.63	32.9 ± 0.44

LSD (0.05) (for vertical comparison) = 0.55

LSD (for horizontal comparison) = 0.63

Table 3.2. Emergence of adults of the local population of *C. maculatus* from seeds of selected varieties of cowpea initially infested with different number of larvae per seed.

Cowpea variety	Resistance status	Percentage of adults emerging from seeds initially bearing:		
		One larva	Five larvae	Ten larvae
Ife Brown	Susceptible	93.0 ± 2.01	92.4 ± 1.88	90.8 ± 4.44
IT84S-2246-4	Resistant	71.7 ± 4.64	44.0 ± 2.89	41.3 ± 6.69
IT85F-2205	Resistant	68.3 ± 0.88	59.5 ± 5.48	32.7 ± 11.26
IT90K-391	Resistant	70.0 ± 11.54	61.3 ± 10.11	28.0 ± 5.03

LSD (0.05) (for vertical comparison) = 2.06

LSD (0.05) (for horizontal comparison) = 2.38

Table 3.3 Summary of ANOVA for the parameters taken during the experiment on effect of larval density on expression of seed resistance in cowpea to *C. maculatus*

Source of variation	df	Mean Squares	
		Development time	% adult emergence
Larval density (A)	2	0.95 NS	2270.68 *
Cowpea variety (B)	3	256.75 *	3498.86 *
A X B	6	0.50 NS	364.86 *
Error	24	0.42	6.00

NS = Not significant.

* = Significant at P = 0.05

3.4 DISCUSSION

Redden and McGuire (1983) determined that the most discriminating variable for measurement of seed resistance in cowpea to *C. maculatus* was mean emergence day, followed by percentage of adult emergence up to an optimum time. Irrespective of level of larval infestation, both parameters distinguished the resistant cowpeas from the susceptible check. This study has therefore further confirmed the lower susceptibility of these known resistant varieties to this Nigerian population of *C. maculatus*. These varieties have also been found to low susceptibilities to *C. Maculatus* populations from Burkina Faso and Campinans (Brazil) (Ofuya and Credland, 1995a).

Of practical significance is the observation that increase in level of larval infestation resulted in a decrease in the number of beetles that survived in the resistant cowpeas. This can enhance the value of the resistant varieties as much lesser damage will be inflicted on the cowpeas under severe infestation and if storage is for a long period. Credland *et al*. (1986), Credland and Dick (1987) and Mitchell, (990) have indicated that larval density in *C. maculatus* affects food availability, larval mortality, developmental time, adult size and egg production. Seed size can similarly modulate larval survival (Mitchell, 1990; Ofuya, 1997). However, for each variety, seeds of similar size (visually assessed) were used. Larval competition within the seed may have partly contributed to the decrease in progeny survival with increasing larval density. The significant interaction between cowpea variety and level of larval infestation indicates that larval competition modulated progeny survival differently in the varieties. For instance, due to increase in larval density from one to ten per seed, there was a 42% reduction in adult emergence in the resistant IT90k-391 compared to 3% in the susceptible Ife Brown. A vast array of antimetabolite/dosage level effects may be implicated in the expression of seed resistance in cowpea to bruchids (Gatehouse *et al.*, 1990; Xavier-Filho, 1991; Macedo *et al.*, 1993; Oigiangbe and Onigbinde, 1996). It may be postulated that larval competition in the resistant varieties interacted beneficially with the nutritional difficulty or antibiotic effects to produce higher larval mortality at the higher levels of larval infestation.

It has been demonstrated that individuals of two different populations of *C. maculatus* reared at one egg per seed on seeds of TVu 2027 for three generations showed a decrease in development time and an increase in survival from one generation to the next (Dick and Credland, 1986b). The higher mortality with increase in larval density observed for the three resistant cowpea varieties used in this study suggests that fewer tolerant *C. maculatus* individuals may evolve with their cultivation and storage.

It is too laborious to conduct screening over more than one generation of bruchids, especially with a large germplasm collection. The experimental protocol should nevertheless reveal the probable response of a resistant variety under severe infestation. Redden and McGuire (1983) suggested that the number of eggs per seed should not be excessively large, below 10-12, and preferably uniform over seeds. The stringent experimental protocol (one egg per seed) which has been adopted by some workers (Messina and Renwick, 1985; Dick and Credland, 1986b; Ofuya and Credland, 1995a, 1995b) is meant to eliminate the effect of competition on larval performance and survival. However, this stringent protocol may suggest very little about the durability of seed resistance except with very large replication or repetition over many generations. Booker (1967) indicated that in *C. maculatus*, within seed competition appears to be absent for up to six eggs per seed. However, the effect of larval competition on larval mortality has been shown here, to depend on the susceptibility of cowpea seeds to attack by the beetle. On the whole, the observations here support those of Redden and McGuire, (1983) and emphasize that moderate levels of infestation (5-10 larvae per seed) should be included in screening protocols for studying seed resistance to *C. maculatus* because it will still reveal the general trend in the relative susceptibilities of the cowpeas, and presumably, the durability of the most promising varieties under severe attack.

CHAPTER FOUR

4.0EVALUATION OF SEEDS OF SELECTED VARIETIES OF COWPEA, *VIGNA UNGUICULATA* (L.) WALP. FOR SUSCEPTIBILITY TO *CALLOSOBRUCHUS MACULATUS* (FABRICIUS) (COLEOPTERA: BRUCHIDAE).

4.1 INTRODUCTION

The cowpea seed beetle, *Callosobruchus maculatus* (F.) is the major storage pest of cowpeas, *Vigna unguiculata* (L.) Walpers in the tropics (Singh *et al.*, 1990). Often, after six months in storage 100% infestation may be recorded (Singh, 1977; Seck, 1993). Damaged seeds are riddled with adult emergence holes and defaced with egg covers which leads to reduced weight and poor quality product. Beetle damage also causes significant reduction in seed viability. The development and use of resistant cowpea varieties have been extensively studied because it offers a simple, cheap and attractive approach to reduction of *C. maculatus* damage in stored cowpea. Of about 15,000 accessions of cowpea in the germplasm collection at the International Institute of Tropical Agriculture (IITA), Ibadan, Nigeria, a few, specifically TVu 625, TVu 2027, TVu 4200, TVu 11952 and TVu 11953 have been identified as having appreciable level of seed resistance to *C. maculatus* damage (Singh, 1977; Adjadi *et al.*, 1985; Jackai and Singh, 1988). From these resistant progenitors, especially TVu 2027 many other *C. maculatus* resistant cowpeas have been developed at IITA (Singh, 1987; Singh and Singh, 1992). Many workers (Messina and Renwick, 1985; Dick and Credland,1986a; Ofuya, 1987; Kitch *et al.*, 1991; Mbata, 1993; Ofuya and Credland, 1995a) have largely confirmed the lower susceptibility of these resistant IITA varieties to the seed beetle. Some improved cowpea varieties combine bruchid resistance with resistance to one or two other major insect pests and many cowpea diseases, and have been recommended for release in Nigeria and other countries (Singh and Singh, 1992). This recommendation notwithstanding, many farmers do not grow these improved cowpeas, for reasons which are not clear (Jackai and Adalla, 1997). Most of these improved varities are hardly found in many Nigerian markets. This study compared the susceptibility of seven cowpea varieties commonly sold in local markets in Akure, in southern Nigeria with three improved known bruchid-resistant cowpeas and a well known susceptible variety, for susceptibility to a local population *of C. maculatus*. The ability of the beetle to survive in the known resistant varieties over five successive generations was also examined.

4.2. MATERIALS AND METHODS

4.2.1 *Beetle population*

C. maculatus originating from individuals infesting cowpea in a local market in Akure were used. They were used to infest Ife Brown cowpea in Kilner jars and the cultures were maintained and the study conducted at 30 ± 1° C and 50 ± 10% relative humidity in a cooled incubator.

4.2.2. *Cowpea varieties*

The varieties used were three known bruchid-resistant cowpeas initially developed at IITA: IT84S-2246-4, IT85F-2205, and IT90K-391, a well-known susceptible variety, Ife Brown, as well as seven varieties commonly sold in local markets in Akure, Nigeria: Sokoto, Oloka, Oloyin, Oloo, White Drum, Red Drum and TVx 3236. The seeds of the known resistant and the well known susceptible cowpeas were obtained directly from IITA, Ibadan, Nigeria. Other seeds were purchased from local markets in Akure. All seeds were first stored in a deep-freezer for seven days prior to use and were thereafter conditioned in the cooled incubator for at least 7 days before use in the experiment to standardize their moisture content. Some physical attributes of the various cowpeas are indicated in Table 4.1.

4.2.3. *Susceptibility of the different cowpeas*

For each cowpea variety, 100 conditioned seeds were placed inside a glass Petri plate (9.0 cm in diameter) into which was introduced several freshly emerged males and females of *C. maculatus* for 8 hr so that females would oviposit on the seeds. The time of oviposition was recorded. Excess eggs were removed from seeds to leave one egg per seed. Subsequently, each seed was placed in a separate test tube and closed with cotton wool such that emerging adults could not escape to the outside. Fourteen days after oviposition (after egg hatch but before adult emergence), seeds bearing eggs that failed to hatch were discarded. For each variety, 20 seeds with hatched eggs were retained and this was replicated three times.. As from 20 days after oviposition, the seeds were examined at least twice daily and adults were removed as they emerged over a 70 day

Table 4.1. Some characteristics of the selected varieties of cowpea.

Cowpea	Mean seed weight (g)	Colour of testa	Texture of testa*
IT84S-2246-4	1.67	Deep brown	1
IT85F-2205	1.50	Brown	1
IT90K-391	2.11	Golden brown	1
Sokoto	1.68	White	2
White Drum	2.86	White	1
Red Drum	2.65	Brown	1
Oloo	1.36	Light brown	1
Oloyin	1.98	Deep brown	1
Oloka	1.98	White with black specks	2
Tvx 3236	1.80	Mottled cream	1
Ife Brown	1.68	Brown	1

* 1 = mildly rough; 2 = rough.

period (i.e. until 90 days after oviposition). The time of emergence of each adult was recorded. Development period (time of egg laying to adult emergence from seed) and percentage adult emergence (number of emerged adults x 100/number of hatched eggs were calculated for each cowpea variety. Data on developmental period (days) and percentage adult emergence were subjected to analyses of variance. Percentage adult emergence was arcsine transformed before analysis.

4.2.4. *Ability of beetle to overcome seed resistance over many generations*

A selection experiment was carried out using two known resistant cowpeas, IT85F-2205 and IT90K-391. Fresh seeds of each of these cowpeas were separately infested in glass Petri plates (9.0 cm diameter) with *C. maculatus* obtained from the laboratory culture. The first three ovipositing couples of the beetle to emerge from the infested seeds were transferred to separate Petri plates containing 100 clean seeds of the host on which they had been reared. The beetles were removed from the seeds after 24 hr and eggs laid were allowed time for hatching. Subsequently, forty seeds, each bearing 1-3 hatched eggs were selected and placed separately in glass tubes (1.5 cm diameter; 15 cm long) which were kept in the cooled incubator. As from 20 days after oviposition, the seeds were examined at least twice daily and adults were removed as they emerged over a 70 day period (i.e. until 90 days after oviposition). The date of emergence of each adult was recorded. Adult emergence was calculated from the pooled data of cumulative total adults emerged from each tube and developmental period was calculated likewise. This procedure was repeated for five consecutive generations where the first three ovipositing couples of the beetle to emerge were used as parents for the next generation. Developmental periods of adults emerging in the different generations were compared using analysis of variance. Number of adults emerging from the different generations were compared using Chi square analysis.

4.3. RESULTS

4.3.1. *Susceptibility of the different cowpeas*

The results are summarized in Table 4.2. The mean developmental period of *C. maculatus* in the three known resistant cowpea varieties IT84S-2246-4, IT85F-2205 and IT90K-391 was 29.90, 30.10 and 29.00 days, respectively, and was significantly ($P < 0.05$) longer than in the well known variety (Ife Brown) as well as all the commonly sold varieties. Amongst the varieties sold in local markets, Tvx 3236, White drum and Oloo had developmental periods of 25.54, 24.92 and 24.54 days, respectively recorded for them and were significantly longer than the development period of the beetle on Ife Brown. The other locally sold varieties, Oloyin, Sokoto, Oloka and Red Drum allowed a development time similar to that on Ife Brown. Percentage adult emergence followed a similar trend as developmental period. Percentage adult emergence of *C. maculatus* in two of the known resistant cowpeas, IT90K-391 and IT85F-2205 was 24.00% and 38.67%, respectively and was significantly ($P < 0.05$) lower than that recorded for all other varieties. The other known resistant variety, IT84S-2246-4 showed a percentage adult emergence of 52.00% which was also significantly lower than that observed in Ife Brown. It was not significantly different from that observed in four of the locally sold

Table 4.2. Mean developmental period (days) and percentage adult emergence of *C. maculatus* reared on seeds of different varieties of cowpea.

Cowpea variety	Mean developmental period (days) ± S.E.	Mean % adult emergence ± S.E.
IT84S-2246-4	29.9 ± 0.95	52.0 ± 11.59
IT85F-2205	30.1 ± 0.83	38.7 ± 5.78
IT90K-391	29.0 ± 1.01	24.0 ± 4.51
Sokoto	23.0 ± 0.80	76.3 ± 8.17
Oloka	22.5 ± 0.79	51.7 ± 8.08
Oloyin	23.1 ± 0.80	71.7 ± 7.42
Oloo	24.5 ± 0.83	53.0 ± 6.66
White Drum	24.9 ± 0.83	50.0 ± 6.81
Red Drum	22.7 ± 0.80	69.7 ± 2.73
Tvx 3236	25.5 ± 0.84	47.0 ± 10.54
Ife Brown	22.5 ± 0.79	83.7 ± 5.41
LSD (0.05)	1.68	12.39

Table 4.3 Summary of ANOVA for the parameters taken during the experiment on susceptibilities of different cowpeas to *C. maculatus*

Source of variation	df	Mean Squares	
		Development time	% adult emergence
Cowpea variety	10	306.0291 *	679.0061 *
Error	22	13.15556	199.5455
Total	32		

* = Significant at P = 0.05

varieties, namely, TVx 3236, White Drum, Oloka and Oloo. All the locally sold varieties allowed significantly lower adult emergence than Ife Brown except Red Drum.

4.3.2 *Ability of beetle to overcome seed resistance over many generations*

The results are summarized in Table 4.4 and 4.6 There were no significant ($P > 0.05$) differences in development period of *C. maculatus* in the two resistant cowpeas tested (IT85F-2205 and IT90K-391) between five successive generations of the beetle (Table 4.5). The percentage of hatched beetle eggs which produced adults varied significantly between generations in each cowpea (Table 4.6). For IT85F-2205 $\chi^2 = 71.30$; df = 4, $P < 0.05$) and for IT90K-391 $\chi^2 = 118.54$, df = 4, $P < 0.05$). Percentage adult emergence increased with successive generations of the beetle on the cowpeas.

Table 4.4. Mean develomental period (days) of *C. maculatus* in two resistant cowpea varieties (IT85F-2205 and IT90K-391) over five successive generations.

Beetle generation	Mean developmental period (days) ± S.E.	
	IT85F-2205	IT90K-391
1st	32.5 ± 2.75	32.0 ± 1.72
2nd	27.5 ± 1.50	28.2 ± 1.31
3rd	28.0 ± 2.56	34.0 ± 2.55
4th	28.8 ± 0.88	28.0 ± 1.90
5th	28.2 ± 0.79	28.5 ± 0.62

ANOVA indicated that $P > 0.05$.

Table 4.5. Summary of ANOVA for development time taken during the experiment on ability of *C. maculatus* to overcome seed resistance in two resistant cowpeas (IT85F-2205 and IT90K-391) over five consecutive generations

Source of variation	df	Mean squares	df	Mean squares
	IT85F-2205		IT90K-391	
Generation	4	14.57578 NS	4	52.69828 NS
Error	40	9.701233	33	20.63805
Total	44		37	

NS = Not Significant at P = 0.05

Table 4.6. Percentage of hatched *C. maculatus* eggs which produced adults from two resistant cowpea varieties (IT85F-2205 and IT90K-391) over five successive generations.

	Mean percentage adult emergence :	
eetle generation	IT85F-2205	IT90K-391
t	13.0 (31)	20.0 (30)
nd	19.0 (21)	24.0 (33)
rd	52.0 (42)	39.0 (28)
th	42.0 (42)	44.0 (25)
th	58.0 (24)	57.0 (21)
^{2}	71.30; $P < 0.05$	118.54; $P < 0.05$

Figures in parenthesis are sample sizes.

4.4. DISCUSSION

This study has clearly shown that the locally sold varieties of cowpea were more susceptible to damage by *C. maculatus* than the known resistant cowpeas recast. The resistant varieties prolonged the development of the beetle and fewer adults emerged from them. Prolonged development would increase generation time in these resistant cowpeas and low percentage adult emergence would reduce the number of offspring contributing to population growth from generation to generation. These effects would culminate in lower damage to seeds of these resistant cowpeas over long period of storage. This work has therefore further confirmed the resistance status of IT84S-2246-4, IT85F-2205 and IT90K-391 to bruchid attack as has been reported by some earlier workers (Kitch *et al.*, 1991; Mbata, 1993; Ofuya and Credland, 1995a; 1995b). TVx 3236 ranked next to the three known resistant varieties in resistance status to *C. maculatus* damage. It is interesting that the variety is amongst the seven varieties obtainable in local markets because it was also developed in IITA and is specifically resistant to the flower bud thrips, *Megalurothrips sjostedti* (Trybom.) which is a major field insect pest of cowpea (Jackai and Singh, 1988). Lale and Efeovbokhan (1991) have similarly observed promising levels of resistance to *C. maculatus* in TVx 3236 seeds. It is probable that the Agricultural Development Project in Nigeria which is funded by the World Bank popularized the cultivation of this cowpea after its recommendation for release to farmers. It is hoped that bruchid resistant varieties like IT84S-2246-4, IT85F-2205 and IT90K-391 may soon become available in markets with better and more vigorous extension methods.

Factors influencing the susceptibility and resistance of cowpeas to *C. maculatus* have been reported by several authors. Messina and Renwick (1985) in their report of correlation between seed-coat texture and oviposition by the beetle stated that smooth seed-coat is preferred to rough. Seed-coat thickness and/or hardness also affects the ability of the newly hatched larva to penetrate the seed (Nwanze *et al.*, 1975). Baker *et al.* (1989) suggested that seed-coat thickness, seed density, moisture content, protein, fat, ash, carbohydrate and proteinase activity were not associated with the activity of *C. maculatus* on cowpea. While Gatehouse *et al.* (1979) reported that a high level of trypsin inhibitor in the cowpea variety TVu 2027 accounted for its resistance to *C. maculatus*, the outcome of the screening 56 varieties by Birch *et al.* (1985) did not demonstrate any association between the level of trypsin inhibitor and seed resistance. Xavier-Filho (1991), and Macedo *et al.* (1993) have reported that resistance in the seeds of IT81D-1045 to *C. maculatus* larval development is due to a variant form of vicilin storage protein which is resistant to digestion by midgut proteinases and thereby limits food supply to the larva. Contrary to the findings by Baker *et al.* (1989), Oigiangbe and Onigbinde (1996) have more recently reported that tannic acids in resistant cowpea seeds significantly reduced F_1 progeny and consequently the percentage adult emergence. This study did not attempt to investigate the basis for resistance in the cowpeas tested. However, the inconsistency in the reports of previous workers calls for further studies of the interactions or combined effects of different chemical and physical characteristics in the development of insect resistance in cowpea.

Dick and Credland (1986b) found that individuals of two different populations of *C. maculatus* cultured on seeds of TVu 2027 for three generations showed a decrease in development time and a significant increase in percentage survival from one generation to the next.. It was similarly observed in this study that percentage adult emergence increased significantly with successive generations of the beetle raised on seeds of IT85F-2205 and IT90K-391. Apparently, IT85F-2205 and IT90K-391 are descendants of TVu 2027. These studies, together with the paucity of seed resistance genes (Kitch *et al.*, 1991), raise concern over the durability of resistance expressed by TVu 2027 and its descendants. Bruchid resistance expressed in Tvu 2027 and its descendants may be ineffective for long term storage. In addition, many *C. maculatus* strains interbreed and produce fertile eggs (Credland, 1990) and the free movement of legume seeds in Africa (Taylor, 1981) presupposes hybridizations between *C. maculatus* populations. The selection pressure imposed by use of TVu 2027 and its descendants may presumably lead to evolution of beetle populations capable of surviving in large numbers and spreading their tolerant capacities through hybridization to other adjoining populations.

Messina and Renwick (1985) have however observed that individuals of *C. maculatus* cultured on seeds of some descendants of Tvu 2027 for six generations showed no significant decrease in development time and nor increase in percentage survival between the different generations. Shade *et al.* (1996) did not observe any development of resistant biotype of *C. maculatus* after 8 generations on TVu 2027. Developmental time of the beetle in IT85F-2205 and IT90K-391 was also similar between generations in this study. Variation in the responses of different populations of *C. maculatus* to seed resistance in cowpea (Dick and Credland, 1986a; Ofuya and Credland, 1995a) may cause some populations to develop observable tolerance within a few generations of selection than others.

Dick and Credland (1986b) assumed that the greater number of beetles surviving in the seeds of TVu 2027 after three generations may be due to their ability to overcome the adverse effects of the supposedly trypsin inhibitor responsible for expression of resistance. Macedo *et al.* (1993) suggested that the capacity of *C. maculatus* to overcome seed resistance may be associated with individuals possessing higher levels of midgut aspartic and cysteine proteinases. These require empirical verification. It emphasizes the necessity of plant breeders to maintain a broad base of resistant properties. Polygenic resistance is very difficult to overcome by pest populations (Pimentel *et al.*, 1984) and cowpea varieties with multiple component resistance can be expected to have much longer useful life than those based on a single resistant character. Pod resistance is an alternative source for reducing seed damage by *C. maculatus* in cowpea and some less susceptible cowpeas have been identified (Akingbohungbe, 1976; Fitzner *et al.* 1985; Kitch *et al.*, 1991; Ofuya and Awelewa, 1993). The combination of pod resistance with seed resistance in the same cowpea variety will form a broader and better basis for longer storage of cowpeas, and needs to be more vigorously pursued

CHAPTER FIVE

5.0. CONCLUSIONS AND RECOMMENDATIONS

5.1. CONCLUSIONS

1. Three descendants of TVu 2027 (IT85F-2205, IT90K-391 and IT84S-2246-4) show low susceptibility to *C. maculatus* attack.

2. Increase in level of larval infesstation resulted in a decrease in the number of beetles surviving in the resistant cowpeas. This would enhance the value of the resistant varieties as much lesser damage will be inflicted on the cowpea under severe infestation

3. The locally sold varieties of cowpea were more susceptible to damage by *C. maculatus* than the known resistant cowpeas (IT84S-2246-4, IT85F-2205 and IT90K-391) tested. However, some of them TVx 3236, White Drum and Oloo exhibited moderate susceptibility to *C. maculatus*.

4. The resistance of seeds of IT85F-2205 and IT90K-391 to *C. maculatus* may be ineffective for long term storage because an increasing number of adults emerged from them over five consecutive generations.

5.2. RECOMMENDATIONS

1. Moderate levels of larval infestation (5-10 larvae per seed) should be included in screening protocols for studying seed resistance to *C. maculatus* because it may reveal the general trend in the relative susceptibilities of the cowpeas, and the durability of the most promising varieties under severe attack.

2. Efforts should be made to popularize the improved bruchid resistant cowpeas amongst cowpea farmers.

3. If the assumption is that the increase in the number of *C. maculatus* surviving in the seed after five generations is due to their ability to overcome the harmful effects in seed, then it is necessary for plant breeders to maintain a broad base of resistant properties. Pod resistance, and seed coat resistance could be used to complement biochemical seed resistance for increased storage durability.

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