# THE POTENTIAL OF USING HYDROTHERMAL TREATMENT FOR PROTECTION OF STORED COWPEAS.AGAINST CALLOSOBRUCHUS MACULATUS (F.)

BY

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# DECLARATION

I certify that the work described in this thesis was carried out by me at the Departments of Zoology and Nutrition and Food Science, University of Ghana, Legon under the supervision of Dr. David Wilson, Zoology Department and Dr. Daniel Obeng-Ofori, Department of Crop Science.

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## ABSTRACT

Laboratory experiments were carried out to evaluate the effect of steaming cowpeas on the oviposition and larval development of the cowpea beetle, *Callosobruchus maculatus* (F.). The possibility of oven and solar drying as well as the effect of the initial temperature of the cowpeas on the steaming effect were also investigated.

Samples of four cowpea varieties (Soronko, Asontem, Amantin and US Blackeye) were steamed for 10 and 15 minutes and solar or oven dried. Some initially stored at $4^0$C or at room temperature were steamed for ten minutes while other samples were oven dried, solar dried, treated with Actellic super dust or untreated.

The results showed that when Soronko, Asontem and Amantin cowpeas were steamed for 15 minutes and oven dried at 70 $^0$C for 8 hours, oviposition by *C. maculatus* was not significantly different from unsteamed beans. Of the four cowpea varieties tested, US blackeye cowpeas were most preferred as oviposition substrate and also for larval development followed by Soronko, Asontem and Amantin. The drying method (solar or oven drying) did not affect oviposition of the bruchids. Significantly fewer eggs were laid on steamed beans compared with unsteamed beans. When US blackeye were steamed for 10 or

15 minutes and solar dried, the grains were protected against the development of bruchid larvae comparable to Actellic Super Dust insecticide. In contrast, steaming blackeye cowpeas for 10 or 15 minutes and oven drying delayed adult bruchid emergence for about 4-5 days due to starch gelatinization and protein denaturation. Furthermore, steaming cowpeas initially at a lower temperature of 4$^{0}$C significantly reduced oviposition in gravid *C. maculatus* compared to beans at room temperature.

Fifteen or ten minutes steaming did not affect damage to the beans so long as the seeds were solar dried to attain safe shelf moisture levels before storage. Microscopic examination of hatched eggs in steamed cowpeas which failed to develop into adults showed death of larvae due to non-utilization of the normal nutrition.

All seeds of the four varieties of cowpeas that were steamed lost the ability to germinate.

# DEDICATION

THIS THESIS IS DEDICATED TO MY FAMILY FOR ALWAYS BEING THERE FOR ME.

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# CHAPTER ONE

## 1.0 INTRODUCTION

The most widely consumed legume in Africa is the cowpea (*Vigna unguiculata*) (FAO, 1991). Cowpea is an important staple crop in Ghana with an annual production of about 30,000 tons (PPME, 1989). It constitutes an important protein source in the diet of Ghanaians (Biney, 1978) and is usually stored to provide a food reserve as well as seeds for planting. The dry seed is a valuable source of vitamin B and protein and the green leaves and pods are eaten as vegetables, while the haulms are fed to livestock.

The principal agents causing loss of food grains during storage are insects, microorganisms and vertebrate pests. Insects are a major cause of post-harvest grain losses and insect infestation is a major constraint to cowpea production because the cowpea is highly susceptible to insect pest attack. There are about 80 insect species which attack cowpeas in Ghana with at least 9 species being of major pest status including *Callosobruchus*. Several species of *Callosobruchus* (Coleoptera: Bruchidae) cause damage in storage but the two principal pests of cowpea in storage are *C. maculatus* (Fabricius) and *C. chinensis* (Linnaeus) (Anon, 1991).

Estimates of post-harvest food losses of grains in the developing world from mishandling, spoilage and pest infestation are put at 25% of the total food grain production. Thus a quarter of what is produced never reaches the consumer (FAO, 1989). In Ghana, for instance, Prempeh (1971), Youdeowei & Service (1981) and Tyler & Boxall (1984) estimate food losses due to insect attack to be 25-45%.

There are many different technologies currently available to control cowpea beetles. They include treatment of grain with edible oils, ash, botanicals, plastic-bagging and solar disinfestation (Murdock *et al.*, 1995). Most of these treatments are effective if properly used and are affordable at least to some in the community who store cowpeas.

The hydrothermal process involves the exposure of the seeds to steam from a steam generator for a specified period of time followed by drying to remove excess water before storage.

Steaming of cowpeas followed by drying has been found to effectively control the bruchid weevil infestation. In the search for cheap, safe and readily available methods of controlling bruchid beetle infestation, the hydrothermal treatment becomes a promising alternative to synthetic insecticides. The efficacy of the method to the various varieties of cowpea available in the country must be investigated.

The project is being sponsored under the Collaborative Research Support Program (CRSP) on Beans and Cowpea with the following objectives:

1. To determine the effect of steaming of different cowpea varieties on the oviposition and developmental biology of *C. maculatus*.

2. To determine whether oven drying instead of solar drying could be used after steaming in order to reduce the drying time.

3. To determine the effect of steaming cowpeas initially held at a lower temperature (4 $^{0}$C) on the oviposition and larval development of *C maculatus.*

4. To determine the effect (if any), of 15- and 10-minute steamed and solar dried cowpeas on the oviposition and larval development of *C. maculatus*.

5. To assess the viability of hydrothermally treated cowpeas.

# 2.0 LITERATURE REVIEW

## 2.1 GRAIN LOSS

Despite the remarkable progress made in increasing production at the regional level approximately half of the population in developing countries do not have access to adequate food supplies because of high post-production losses (Boxall, 1986). Various estimates of the magnitude of these losses have been done in different parts of the tropics. In Sierra Leone, about 30% of stored rice is lost to insect pests; 25-45% of stored maize in Ghana is lost to insect pests while farmers in Nigeria expect 50-60% of their stored cowpeas to be attacked by insects after only 6 months of storage (Hill and Waller, 1988). Similar levels of losses to various stored food and other products occur in east and southern Africa and in other parts of the tropical world (Boxall, 1986).

Quantitative and qualitative food losses of variable magnitude occur at all stages in the post-production system from harvesting, through handling, storage, processing and marketing to final delivery to the consumer. Bruchid infestations may lead to nutritional and economic losses and may also significantly affect seed germination. According to Wolfson (1989) and Lowenberg-DeBoer (1995), it is probably for this reason that many farmers make special efforts to treat their seed grain with insectides.

In Africa, many subsistence farmers sometimes remove produce for food or for sale shortly after treating with insecticides, during which time the residue levels of insecticides in the grain might still be dangerously high. Ak'habuhaya and Lodenius (1988) estimated 1,000 reported deaths per year in Tanzania to be due to various pesticide poisoning. In Ghana, the use of stored grain protectants is rather costly and less than 1% of small scale farmers use insecticide on their produce (Nyanteng, 1972). Much attention has been given of late to other methods of insect control including the use of locally available plant material that have the ability to protect stored grain from attack by insect pests.

## 2.2 PHYSICAL AND CHEMICAL CHARACTERISTICS OF COWPEAS

Cowpea seeds can vary considerably in size, color, shape and eye patterns (the pigmented area around the hilum). Marconi *et al.*, (1990) reported that the seed coat colour of wild species ranges from light brown to black and white to black. The bulk of cowpeas produced in Ghana are made up of the white, red and brown varieties. The black, cream and mottled varieties are of less commercial importance. There is widespread preference for the large, white seeded, quick-cooking varieties in Africa. The coat may be smooth, rough or wrinkled and can vary in color from white through various shades of green, brown, red and purple to black sometimes mottled, blotched or speckled patterns (Osei, 1994). Laghetti (1990) gave the lengths to be in the range 5-13 mm.

The major components of cowpea are protein and carbohydrate. Cowpea seed protein consists of two main protein classes- albumin and globulin. The former which makes up 90 percent of the proteins is water insoluble while the water-soluble globulin makes up 10 percent (Pedalino *et al.*, 1990). Starch content has been reported to range from 50.6 to 67.0% with 20.9 to 48.7 percent amylose and 11.4 to 36.6% amylopectin (Tolmasquim *et al.*, 1971). Usually, the raw legume seeds are not eaten because of the presence of several anti-nutritional and other toxic factors, which reduce efficient utilization of legume nutrients. These include trypsin inhibitors, lectins, goitrogens, cyanogenic glucosides, lathyric factors and other compounds that cause favism (Sinniah *et al.*, 1983).

## 2.3 BIOLOGY OF *C. maculatus*

Bruchids are recognized with a pair of parallel ridges on the ventral side of each hind femur, each of which bears a tooth near the apical end. In bruchids the teeth are roughly equal in length (Haines, 1989). Females often have strong markings on the elytra, which consist of two large marginal dark patches mid-way along the elytra, and smaller patches at the anterior and posterior ends, leaving a slight grey-brown cross-shaped area covering the rest. The males are much less distinctly marked (Anon, 1991). There are two known phases of the cowpea weevil, a sedentary morph and an actively dispersing morph. The dispersing morph accumulates high levels of lipid reserves that can supply energy for dispersal (Nwanze *et al*, 1976)

The cowpea beetle, *Callosobruchus maculatus* (F.) follows a common bruchid life history pattern (Johnson, 1981). Gravid females oviposit on the testa of seeds. When confined in the presence of pods or seeds, they lay 3-4 times as many eggs on seeds as on pods (Murdock *et al*, 1995). The eggs are glued on top of the seed in storage; they are glossy and oval when fresh. On hatching, the larvae use their mouthparts to bore through the chorion and through the seed testa. Many species of Bruchidae are able to avoid toxins present in the pod walls and seed coat by not ingesting them as they bore into the seed, so that all feeding takes place in the cotyledon. Within the seed the larvae moult three times and pass through four instars, the longest of which is the fourth (Shade *et al*, 1990). The eggs take about three to five days to hatch and the minimum development period on cowpea is about 21 days at 30 $^0$C and 80% R.H (Anon, 1991). Emerging females can lay viable eggs on the first day of emergence (Murdock *et al*, 1995). The adult beetles do not feed under normal culture conditions on stored produce for lack of functional mouthparts and are very short-lived. The average adult life span is usually not more than 12 days (Anon, 1991). The life cycle is completed in about 30 days at 30 $^{0}$C and 80% R.H.

Alternative host plants include numerous cultivated and wild cowpea (*Vigna*) species as well as other legume seeds (Johnson, 1981). In addition to cowpea, *C. maculatus* has been bred in the Samaru laboratory, Zaria ,on the mung bean, (*Phaseolus aureus* Rox), the pigeon pea (*Cajanus cajan* (L) Druce), *Voandzeia subterranea* (L) D.C., *Sphenostylis stenocarpus* Harms, and *Kerstingiella geocarpa* Harms. However, in experiments to compare the development of *C. maculatus* on different hosts, only *P. aureus* consistently proved satisfactory as a food source (Booker, 1965). The knowledge of the range of host species is not complete and it is not clear whether known wild hosts harbour a large or inconsequential reservoir population of bruchids (Murdock *et al*, 1995).

## 2.4 VARIETAL SUSCEBTIBILITY TO *C. MACULATUS*

Experiments on varietal susceptibility of cowpeas to *C. maculatus* attack by Booker (1965) showed that cowpea varieties differ in susceptibility to attack. It was also shown that *C. maculatus*, when offered a choice, has a preference for seeds with a smooth testa even when the smooth seeds are smaller than the rough. Several factors contribute to the determination of the total number of eggs laid during the lifetime of a female. These depend on the food in which the adult beetles are bred, humidity and temperature (El-Sewaf, 1956), the density of the adult beetles in a culture (Utida, 1941), the plumpness or wrinkling of the seed coat and perhaps the size and hardness of the seed as well as odor (Nwanze *et al*, 1975). Nwanze *et al*., (1976) reported that differences in the seed coat of cowpeas affect oviposition and larval development of *C. maculatus*. According to Ramzam *et al*., (1990), *C. maculatus* prefers few varieties including blackeye for oviposition. Blackeye cowpea suffered the maximum loss in terms of exit holes (69.2%) and loss in weight (34.5%). Moreover, wrinkled and infested seeds are less preferred than sound and healthy seeds by *C. maculatus* and *C. chinensis* (Bais, 1986). However, the role of physical (curvature), and chemical (aqueous and/ or methanol fractions of cowpea seed coat) stimuli assessed in a quantitative and on a comparative basis for ovipositional preference of *C. maculatus* and *C. chinensis* showed that the ovipositional responses of gravid *C. maculatus* was not affected by the curvature of the oviposition substrate, and that gravid females are solely guided by a chemical stimulus perceived from the oviposition substrate (Gokhale *et al.*, 1990).

## 2.5 CONTROL OF *C. MACULATUS*

### 2.5.1 CHEMICAL CONTROL

Synthetic insecticides have been and are still the most widely used control agents recommended to farmers in many developing countries against insect pests of stored grain (Baba, 1994). Many types of chemicals from many chemical classes developed by industry have proliferated the chemical markets of the developing countries with alarming consequences. These toxic materials are broad-spectrum and have profound effect on non-target species in the agricultural ecosystem (Baba, 1994). Actellic super dust (0.6% permethrin and 1.6% pirimiphos methyl) applied at 100 g dust per 90-100 kg bag of grain is the recommended insecticide in Tanzania (Golob, 1988; Uronu, 1988). The insecticide is admixed with shelled grain before loading into traditional silos. This produces residues amounting to 3.3 mg/kg of permethrin and 17.7 mg/kg of pirimiphos methyl, which are far in excess of the FAO/WHO recommended residue levels of 2 mg/kg of permethrin and 10 mg/kg of pirimiphos methyl. However, these high levels are expected to reduce as a result of loss of active ingredient during mixing of the grain and decomposition after long storage (Golob, 1988). The control of cowpea weevils rely heavily on the use of insecticides. The indiscriminate use of these insecticides result in such problems as posing a health hazard to the applicators, toxic residues in food, environmental pollution and development of resistant strains of insects. With the removal of subsidies on all agricultural inputs, these insecticides are now too expensive to resource-poor farmers (Baba, 1994).

### 2.5 2 USE OF VEGETABLE OILS AND BOTANICALS

An effective way to preserve dry beans such as cowpeas is to mix the grain thoroughly with small amounts of vegetable oil (Schoonhoven, 1978; Shukla *et al*, 1988). The oil coats the testa of the grain and acts primarily as an ovicide by plugging the egg micropyle, thus hindering oxygen supply to the developing embryo. In some cases the oil may deter oviposition or cause mortality of the adult bruchids (Murdock *et al*, 1995). In addition, oil products have been found by several workers to be toxic or to cause osmotic tension and consequently coagulation of protoplasm and mortality of eggs (Su *et al.*, 1972; Singh *et al*, 1978). Oil treatment, while effective, has some disadvantages. It is messy to use and is easy to pick up dirt and debris while applying the oil. The oils should also be thoroughly and evenly applied to the surfaces of the seeds, so that any eggs already present or subsequently placed on the seeds come into contact with it. If large amounts of grain (e.g. tens of kilograms and larger) are to be treated, the task of a thorough application becomes quite difficult. Some oils may also become rancid with time and promote the development of harmful fungi when the storage environment is humid and badly aerated. This situation is aggravated by high temperatures. This micro-climatic situation is what is encountered in silos in the humid parts of the tropics, especially at the resource-poor farmers' level (Baba, 1994). Thus, while effective, the practical value and acceptability of oil treatment appear to be limited.

Traditional control procedures for the bruchid weevil involve the plant products possessing insecticidal, antifeedant and repellent properties. Various mints, aromatic or pungent plant materials have been used. Black pepper, *Piper nigrum*, has been found to be highly toxic to many stored products pests including *C. maculatus* and *Sitophilus oryzae* (Su *et. al.*, 1972; Gunathilagaraj and Kumaraswani, 1987). Powdered dry orange peel was found to be toxic to *C. maculatus* and significantly reduced oviposition and progeny emergence (Taylor, 1975). The insecticidal and antifeedant potencies of neem (*Azadiracta* *indica* A. Juss.) have been reported by many workers, (Ivbijaro, 1983 ; Sowumi and Akinnusi 1983 ; Allotey and Dankwa, 1994). Ivbijaro (1983) found that neem seed powder significantly reduced fecundity, prolonged developmental period and decreased adult emergence but did not affect survival of adult *C. maculatus*.

Ash is widely used to protect stored cowpea in sub-Saharan Africa (Golob and Webley, 1980; Ofuya, 1986; Zewar, 1986; Shukla *et al*, 1988; Lowenberg-DeBoer, 1995). A survey of post-harvest storage methods in northern Cameroon showed that the most common method was mixing cowpea grain with sieved ash (Wolfson *et al.*, 1989). Systematic studies at Purdue University (Wolfson *et al.*, 1991) showed that the method can work extremely well except for some shortcomings. The most important of which relates to the ash:grain proportion. Ash does not provide complete protection against a build-up of cowpea weevils unless the ratio of ash to grain is three or more parts ash to four parts grain. In short, when properly used, ash arrests cowpea bruchid population development within the store but does not kill the generation already within the seed (Kitch and Ntuokam, 1991). The consequence of this latent infestation is that, farmers sometimes mix apparently undamaged grains with ash and discover later that they have emergence holes, evoking doubts about the effectiveness of the method in their minds. (Murdock *et al.*, 1995).

## 2.7 SOLAR AND OTHER HEAT DISINFESTATION TECHNIQUES

Insects have very little capacity to thermoregulate and therefore die when exposed to high temperatures. The eggs, larvae and pupae of *C. maculatus* are practically immobile and so unable to escape from a hot environment. In sub-Saharan Africa, cowpeas are heated on iron plates over wood fires to disinfest them (Murdock *et al*, 1995. Radiation sterilization by gamma rays has been used to reduce the fecundity of female *C maculatus* (Ahmed *et al*, 1979). Food preservation by irradiation is considered a potential hazard by some consumers (Wolf, 1992) and in developing countries where a lot of the processors of food are small scale industrialists, it is only cost effective when the radiation plant is put to large scale use. The high capital initial investment often prevents its effective use.

Post-harvest treatment given to foods, especially the use of heat as in drying, may cause physical, chemical and protein functional changes. Post-harvest handling and storage conditions further induce changes in physico-chemical properties of legumes e.g. when seeds are stored at high temperatures and high humidity conditions

(Aguilera and Ballivan, 1987). In grain legumes, the hard-to-cook defect associated with storage is widely known in cowpeas (Sefa-Dedeh *et al.*, 1979).

The physical treatment of seeds influence their storage and processing properties. The use of heat (steaming or parboiling) on cereals and pulses and even macaroni and pasta results in a case hardening effect that make them highly resistant to insect infestation (Osei, 1994). Majumder (1982) explains that enzymes of the digestive tract of insects seem to have specific roles in assimilating constituents of cereals and pulses. Thus, case hardening does not only induce mechanical resistance to mandibular chewing but also prevent utilization of the gelatinized starch by gut enzymes in stored product insects.

It has been reported that steaming followed by drying of cowpea seeds prior to storage effectively controls the bruchid weevil infestation (Osei, 1994). Steaming may cause physical and structural modifications on the surface of the cotyledon leading to a case hardening effect (Sefa-Dedeh and Demuyakor, 1994). Steaming also changes the physico-chemical properties of the seed coat and some part of the cotyledon. According to Akinyele and Akinlosotu, (1986), physico-chemical characteristics may also vary with seed coat thickness. Steaming can also result in the gelatinization of starch molecules and denaturation of the protein molecules, which further affect some functional properties of the cowpea during processing. Cockfield (1992) compared steaming of cowpeas with groundnut oil and insecticide (pirimiphos-methyl) application for the control of *C. maculatus*. He reported that while steaming offered no permanent protection, groundnut oil appeared to be nearly as effective as the pirimiphos-methyl treatment. He did not specify any time period for the steaming effect. However, Sefa-Dedeh *et al* (1994) found that steaming of dry cowpeas for ten minutes or more before drying to acceptable moisture levels effectively controlled the bruchid beetle infestation.

According to Osei (1994), cowpea seeds steamed for 10 minutes were not significantly different from those steamed for 15 minutes in terms of the presence of emergence holes and both times were better than when steaming was done for 5 minutes. This result was confirmed by Sefa-Dedeh and Saalia (1997), who reported that the length of time of steaming did not seem to have much influence on moisture gain by the seeds, and the final moisture levels of all the test samples after solar drying were low enough for safe storage of the grains.

It was concluded that steaming was an effective means of preventing beetle infestation in cowpea and that steaming for 10 minutes provides complete protection from the beetles. When steamed cowpeas were evaluated for boiling and eating quality, even though some discolorations appeared after steaming, these disappeared after soaking and the final cooked texture and flour were not adversely affected.

The resistance of *Phaseolus vulgaris* (haricot bean) to infestation by *C. maculatus* was investigated by Seifeinasr (1991) and the roles contributed by its physical or chemical characteristics were examined. Haricot bean seed coat was not the thickest and its seeds were not the least preferred for oviposition by the bruchid beetles. It was concluded that the tiny shallow depressions underneath the dead larvae indicated that the larvae had initiated feeding after completion of embryonic development though none of them had successfully developed or survived to adulthood. Thus, the physical characteristics of the haricot bean were probably not the major factors causing the observed premature death of the bruchid larvae. Alanine, an amino acid usually existing in association with its toxic analogue of Beta - cyanoalanine and Alpha-diaminobutyric acid, were found only in haricot bean among the tested grain legumes. Insect susceptibility test conducted on a US blackeye cowpea variety indicated that steaming was effective in enhancing the storage property of the cowpea with thin seed.

Many protease inhibitors have been found in a number of legumes (Liener, 1975) but much of our knowledge on trypsin inhibitors has come from studies on soybeans (Liener, 1972). Various processing techniques have direct influence on the amount of trypsin inhibitors found in legumes. Heat processes such as cooking, steaming and sterilization can inactivate trypsin inhibitors in legumes. Liener (1975) reported that, autoclaving legumes in an atmosphere of steam at about 1 kg/cm for 15-20 minutes inactivates trypsin inhibitors. Heating at 100 degrees Celsius for 30 minutes at a pH of 1 inactivates soybean trypsin inhibitors (Boonvisut and Whitaker, 1976).

Osei (1994) noted that trypsin inhibitor levels decreased in the cowpea varieties in the order Asontem > Soronko > Amantin and that at high temperatures small increases in temperature result in increased denaturation rate. The potency of the trypsin inhibitors is related to the $K_i$ of the inhibitors or the ability to compete with the substrate for the enzyme. Protease inhibitions usually form reversible inhibitor-enzyme complexes. Thus, the protective effect in steamed cowpea may be due to the enzyme-substrate complex formation being partially or completely blocked (Osei *et al* 1997).

However, when the physical and chemical characteristics of fifteen lines of cowpea were compared in relationship with their resistance to *C. maculatus* (F.), it was found that contrary to claims that elevated trypsin inhibitor levels may correlate with *C. maculatus* resistance, trypsin inhibitor 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 (Baker *et al*, 1989).

According to work done by Sefa-Dedeh *et al* (1994), steaming of cowpeas for ten or more minutes can cause structural and functional modifications in the seeds. It induces micro-structural changes in the surface cells of the cotyledons and this may contribute to the reduction in the seed water absorption rate and capacity. Preliminary field trials using 10 minutes steam treatment followed by drying of the US blackeye cowpea variety showed effective protection comparable to that afforded by conventional synthetic insecticides.

## 2.8 DAMAGE ASSESSMENT

The methods generally used to assess losses caused by insects focus upon a loss in weight. The methods have arisen largely from a basic research study of farm-level storage losses carried out by Adams and Harman in 1977 in Zambia, and relate specifically to losses in weight caused by grain-boring insects rather than by surface feeding insects (Boxall, 1989). Adams and Schulten (1978) suggest three methods of determining losses in a sample of grain:

1. Determination of the weight of a measured volume of grain. In this method, the loss in weight of grain samples over a period of time is taken as a reflection of loss caused not only by insects but also by micro-organisms and other factors. This method is referred to as the volumetric, bulk density or Standard Volume Weight (SVW) method. The principle of this method is to establish the condition of the grain at the beginning of the storage season and to compare the condition of the grain samples collected throughout the season with this baseline condition. The weight of grain occupying a standard volume container, determined from a sample collected at the time of storing, represents the baseline and losses are recorded by following changes in the

weight of grain occupying the same standard volume on subsequent occasions.

2. Separation of damaged and apparently healthy or sound grains and a comparison of their weights calculated as percentage of the whole sample. This is referred to as the gravimetric method or the count and weigh method.

3. Determination of percentage insect-damaged grain in a sample and its conversion to a weight loss using a predetermined factor sometimes referred to as the converted percentage damage method. In this method, a laboratory experiment should first be conducted to determine the relationship between the percentage damage and weight loss. The results of the experiments are then applied to field samples of the same variety infested with the same insect pest.

In this experiment, the assessment of damage was based on the gravimetric method (count and weigh method). The count and weigh method is applied to a single sample and thus uses a smaller sample. It provides an estimate of loss where a baseline cannot be determined at the beginning of the storage period and requires the calculation of:

i. the proportion by weight of grains damaged by insects,

ii. the percentage of damaged grains

The proportion of damaged grains is calculated from the mean grain weights of undamaged and damaged grains as:

$$\frac{\text{Mean weight of undamaged grain - Mean weight of damaged grain}}{\text{Mean weight of undamaged grain}}$$

$$= \frac{U/Nu - D/Nd}{U/Nu}$$

This proportion, i.e., the averaged weight loss per damaged grain, is then multiplied by the percentage of damaged grains in the sample to obtain the weight loss. This can be expressed as follows:

$$\%\text{ weight loss} = \frac{U/Nu - D/Nd}{U/Nu} \times \frac{Nd}{Nu+Nd} \times 100$$

$$= \frac{Und - Dnu}{U(Nd+Nu)} \times 100$$

where,
U = weight of undamaged grains

D = weight of damaged grains

Nu = Number of undamaged grains

Nd = Number of damaged grains

Since the method involves a single sample it is considered unnecessary to determine the moisture content of the separate fractions, since the differences are likely to be small. This method may be less accurate because;

i. It assumes that insects attack the grains at random, which is often not the case

ii. It does not account for hidden infestation, which may be counted as undamaged, and

iii. At high levels of damage there may be multiple infestations.

Adams and Harman (1977) used the method in Zambia and noted the problems of variation in grain size, variation in average grain weight for damaged grain at high levels of infestation and of counting grains with internal infestation as undamaged (Boxall, 1989). Despite the disadvantages of this method, it is considered as the standard technique for situations where no baseline could be obtained. Moreover, no allowance might be made for non-random insect attack by modifying the procedure if the problem of insects selectively attacking the larger (or smaller) grains in a sample is not considered serious. However, if it is considered serious, then for example, before separating the damaged and undamaged fractions, grains could be divided into large and small categories (or as many size categories as necessary) using a suitable set of sieves. After counting and weighing the grains in each fraction size category, the weight loss can be calculated as follows:

Weight of "undamaged" reference sample (weight UN) =

$$\text{weight UN} = \frac{\text{Weight undamaged Large Grains}}{\text{Number undamaged Large Grains}} \times \text{Total Large Grains} + \frac{\text{Weight undamaged Small Grains}}{\text{Number undamaged Small Grains}} \times \text{Total Small Grains}$$

$$\%\text{weight loss in sample} = \frac{\text{weight UN} - \text{weight sample} \times 100}{\text{Weight UN}}$$

This is expected to improve the figure for weight loss obtained by this method (Boxall, 1989).

# CHAPTER THREE
## 3.1 MATERIALS AND METHODS

The experiments were conducted at the laboratories of the Zoology Department of the University of Ghana. Four varieties of cowpea (soronko, asontem, amantin and black-eye) obtained directly from a supplier, in order to ensure they were free from chemical insecticide treatment, were used for the work. Asontem is dark brown with a blackeye. It literally means early maturing (60-70 days). It has a thick seed coat and is resistant to insects. Soronko obtains its name from its unique light red color. It is a medium maturing variety (75 - 80 days) and has a characteristic seed coat which is resistant to insects. It tastes better than most cowpea varieties. The characterization of the various varieties of cowpea used in the experiments is shown below in Table 1.

Table 1. Characteristic features of the four cowpea varieties used.

| Variety | Length (cm) | Width (cm) | Thickness (cm) | Seed coat thickness (cm) |
|---|---|---|---|---|
| Asontem | 0.676± 0.040 | 0.630± 0.033 | 0.465± 0.040 | $7.626x10^{-3}$ |
| Soronko | 0.685± 0.054 | 0.568± 0.036 | 0.460± 0.031 | $7.626x10^{-3}$ |
| Amantin | 0.650± 0.056 | 0.541± 0.028 | 0.392± 0.028 | $5.212x10^{-3}$ |
| Blackeye | 0.823± 0.040 | 0.546± 0.031 | 0.433± 0.028 | $5.084x10^{-3}$ |

(Source: Osei, 1994).

The varieties with thin seed coat may be more susceptible to insect attack than the Asontem and Soronko varieties with thicker seed coat. The bruchid weevils used for the experiment were collected from the local Madina market using a pooter and brought to the laboratory for breeding.

### 3.1 REARING OF *C. maculatus*

Blackeye cowpeas were purchased at the local Madina market and sterilized by heat disinfestation for two hours at 70 $^{0}$C. About 500 g each of the cowpeas was put into three insect breeding cages of height 4m X 2m and infested with 200 unsexed *C. maculatus* adults per cage. Adults that subsequently emerged from the eggs that were laid were used for the various experiments. All cultures and experimental units were maintained at ambient laboratory conditions of 28 $^{0}$C and 70 - 80% RH.

### 3.2 STEAMING AND DRYING PROCEDURES

Steaming was done with thinly spread layers of the various varieties in shallow drying trays at a steam pressure of 50 bars. The steamed cowpeas were then either solar or oven dried.

Steaming of samples was done using a rectangular steam jacket of dimensions; Length, 244 meters; Width, 52 meters; and an inner depth of 25 meters. The whole jacket was attached to vertical stands that keep it 82 meters off the floor. The jacket is connected to an Electric Steam Generator (General Electric), 50Hz, 150A and 108KW that is capable of producing steam at maximum pressures of 300 bars.

Oven drying was done using a Compenstat Air Oven at 70$^0$C for eight hours, while solar drying was done in dryers of dimensions; Length, 122 meters; Width, 60 meters; and an inner depth of 14 meters. The dryers were covered by a lid of plain polythene sheet and kept on stands in the open sun and overnight until well dried.

Chemical treatment was done using Actellic super dust (0.6% permethrin and 1.6% pirimiphos methyl) as the standard insecticide at the recommended application rate of 0.2 g of the insecticide per 400 g of cowpeas.

### 3.3. EFFECT OF STEAMING ON OVIPOSITION AND BIOLOGY OF *C. maculatus*

To determine the effect of steaming the different cowpea varieties on the oviposition response and developmental biology of *C. maculatus*, batches of 100 g of Soronko, Asontem and Amantin seeds steamed for 15 minutes and oven dried were put into 500 ml storage bottles and 10 pairs of newly emerged adults (0-1 day old) from the laboratory cultures were introduced into each jar. The jars were covered with muslin cloth secured by rubber bands to allow adequate ventilation while preventing escape and cross infestation. The setup was repeated for the seeds that were oven dried only, Actellic Super Dust treated seeds and untreated (control) seeds of each of the three varieties of cowpeas. Each treatment was replicated four times. Two weeks after the experimental set up, all dead adults were removed and the total number of eggs laid per sample was recorded for each replicate and kept in a petri dish. Adults that emerged from eggs on cowpeas kept in the petri dish were carefully removed with an aspirator and counted and the number of days taken to emerge noted. Damage caused at the end of two months was assessed based on the count and weigh method of Adams and Schulten (1978) and data obtained was analyzed using analysis of variance (ANOVA) and Duncan's Multiple Range Test for the separation of means.

### 3.4 COMPARISON OF OVEN AND SOLAR DRYING

To determine whether oven drying can be substituted for solar drying in order to reduce drying time, batches of 100 g of 15 minutes steamed, oven and solar dried US blackeye cowpeas were put into 500 ml storage bottles and 10 pairs of newly emerged adults (0-1 day old) were introduced into each jar. The jars were covered with muslin cloth secured by rubber bands. The setup was repeated for oven dried only seeds, Actellic Super Dust treated and untreated (control) seeds. There were four replicates for each treatment. Two weeks after the set up, all dead adults were removed and the total number of eggs laid per sample was recorded for each replicate and kept in a petri dish. Adults that emerged from eggs on cowpeas kept in the petri dish were carefully removed with an aspirator and counted and the number of days taken to emerge noted. Damage caused at the end of two months was assessed using the count and weigh method of Adams and Schulten (1978) and data obtained was analyzed using analysis of variance (ANOVA). Duncan's Multiple Range Test was used to separate the means.

**3.5 EFFECT OF INITIAL TEMPERATURE**

In order to determine if the initial temperature of the cowpeas before steaming has any effect on the oviposition response and larval development of *C. maculatus*, two 100g batches of US blackeye cowpeas, one steamed for 10 minutes at an initial temperature of $4^{0}C$ and solar dried and another steamed for 10 minutes after allowing to stand overnight to attain room temperature of about 28 $^{0}C$ and solar dried were put into 500 ml storage bottles and 10 pairs of newly emerged adults (0-1 day old) were introduced into each jar. The jars were covered with muslin cloth secured by rubber bands. The experiment was repeated for solar dried seeds only, Actellic Super Dust treated seeds and untreated seeds. Two weeks after the introduction of insects, all dead adults were removed and the total number of eggs laid per sample was recorded for each replicate and kept in a petri dish. Adults that emerged from eggs in the petri dish were carefully removed with a pooter and counted and the number of days taken to emerge noted. Damage caused at the end of two months was assessed based on the count and weigh method of Adams and Schulten (1978) and data obtained was analyzed using analysis of variance (ANOVA) and Duncan's Multiple Range Test was used to separate the means.

### 3.6 EFFECT OF DURATION OF STEAMING TIME ON OVIPOSITION AND ADULT EMERGENCE

To determine if 15 minutes steamed cowpeas would be statistically different compared to 10 minutes steamed cowpeas, 15 minutes steamed and solar dried US blackeye cowpeas was compared to 10 minutes steamed and solar dried US blackeye cowpeas that had been allowed to attain room temperature before steaming. Damage caused at the end of two months was assessed based on the count and weigh method of Adams and Schulten (1978) and data obtained was analyzed using analysis of variance (ANOVA) and Duncan's Multiple Range Test was used to separate the means.

**3.7 SEED VIABILITY BIOASSAY:**

To assess the viability of the cowpea seeds after hydrothermal treatment, 25 seeds of each treatment and variety in three replicates were placed on moist filter paper in petri dishes and the number of germinated seeds were recorded after one week. Data obtained was analyzed using ANOVA and Duncan's Multiple Range Test was used to separate the means.

**STATISTICAL ANALYSIS**

Percentages were transformed to Arcsine values and insect counts were transformed using logarithm transformation [$Log_{10}$ (X+1)] to meet ANOVA assumptions of normality and homogeneity of variances. The results were then analyzed using STATGRAPHICS version 4.3.

# CHAPTER THREE
# RESULTS

## 4.1. Effect of steaming on oviposition and biology of *C. maculatus*.

The oviposition and adult emergence and damage by *C. maculatus* (F.) to Soronko, Asontem and Amantin cowpea varieties under the four treatments: 15 minutes steaming and oven drying; oven drying only; actellic super dust; and no treatment (control) are shown in Tables 2 - 4. The mean number of eggs laid after steaming each of soronko and asontem cowpeas for 15 minutes and oven drying at 70$^0$C for eight hours was not significantly different from that recorded in unsteamed cowpeas. Mean number of eggs in unsteamed amantin was significantly less than in steamed cowpeas. The mean number of eggs recorded on steamed soronko, asontem and amantin were not significantly different. Actellic super dust was significantly more effective than steaming and oven drying in suppressing oviposition by *C. maculatus* in all four varieties of cowpeas.

The mean number of emerging adults in soronko and asontem varieties was not significantly different between steamed and oven dried, and unsteamed cowpeas. There was no significant difference in emergence between soronko and amantin. Actellic super was significantly more effective than steaming and oven drying in suppressing adult emergence in all four varieties of cowpeas. Percent loss in weight in steamed soronko cowpeas was not significantly different from thse teated with Actellic super dust. Non-steamed asontem seeds were also not significantly different from Actellic super treated cowpeas. The least percent weight loss in amantin cowpeas was recorded in Actellic super dust treated seeds.

Among the treatments tested, only steamed and oven dried beans delayed adult *C. maculatus* emergence by about 4-5 days. All other treatments recorded adult emergence after about 23 days of incubation. However, there was no significant difference between treatments with respect to days of adult emergence.

Table 2. Oviposition of *Callosobruchus maculatus* (F.) in soronko, asontem and amantin cowpeas.

| TREATMENTS | Number of eggs laid (mean ± S.E.) | | |
|---|---|---|---|
| | SORONKO | ASONTEM | AMANTIN |
| STEAM+OVEN DRY | 69.3 ± 31.54b(a) | 83.8 ± 10.9c(a) | 74.3 ± 12.1c(a) |
| OVEN DRY ONLY | 82.0 ± 16.1b | 52.5 ± 8.7b | 19.8 ± 6.7b |
| ACTELLIC SUPER DUST | 7.8 ± 2.2a | 9.3 ± 2.1a | 8.5 ± 4.7a |
| CONTROL | 93.8 ± 38.8b(b) | 126.3 ± 8.8c(b) | 25.3 ± 4.2b(b) |

Table 3. Mean percent adult emergence of *Callosobruchus maculatus* (F.) in soronko, asontem and amantin cowpeas

| TREATMENTS | Percent adult emergence (%) | | |
|---|---|---|---|
| | SORONKO | ASONTEM | AMANTIN |
| STEAM+OVEN DRY | 70.7 ± 24.5b(a) | 76.7 ± 4.7c(a) | 90.6 ± 10.3c(a) |
| OVEN DRY ONLY | 54.9 ± 7.0b | 84.8± 6.1b | 80.8 ± 6.2b |
| ACTELLIC SUPER DUST | 19.2 ± 1.3a | 43.0± 2.9a | 56.5 ± 2.1a |
| CONTROL | 69.1 ± 28.8b(b) | 83.9 ± 10.6c(b) | 90.1 ± 2.5b(a) |

Table 4. Percent loss in weight in soronko, asontem and amantin cowpeas.

| TREATMENTS | percent loss in weight (%) | | |
|---|---|---|---|
| | SORONKO | ASONTEM | AMANTIN |
| STEAM+OVEN DRY | 20.55c | 10.68c | 19.78c |
| OVEN DRY ONLY | 28.14c | 18.10c | 32.45cd |
| ACTELLIC SUPER DUST | 0.63a | 3.17a | 0.08a |
| CONTROL | 29.6c | 21.69c | 35.19d |

Mean of four replicates. Values for each variety followed by the same letter (s) are not significantly different at the 0.05 level.New Duncan's Multiple Test.

() = varietal comparason.

## 4.2. Comparison of oven and solar drying

The oviposition and adult emergence of *C. maculatus* (F.) on US blackeye type cowpeas that had been steamed for 15 minutes and solar or oven dried is shown in Table 5. The mean number of eggs laid when 15 minutes steamed blackeye cowpeas were solar dried or oven dried was significantly lower compared to unsteamed beans. The mean number of eggs laid on solar dried or oven dried seeds were not significantly different. Thus, unsteamed seeds recorded the highest number of eggs followed by oven dried only or solar dried only seeds. Among the treatments tested, Actellic Super Dust was the best deterrent to oviposition by *C. maculatus.*

The percentage of adults emerging from 15 minutes steamed and oven dried seeds was significantly higher in seeds steamed for 15 minutes and oven dried, compared to solar dried seeds. There was no significant difference in percent adult emergence between solar or oven dried cowpeas and untreated cowpeas. Steamed and solar dried beans afforded better protection against population build-up of bruchids compared to Actellic Super dust treated beans.

Table 5. Oviposition and adult emergence of *Callosobruchus maculatus*(F.) on 15 minutes steamed, oven and solar dried US blackeye cowpea

| TREATMENT | | | No. of eggs laid (m±se) | Percent adult emergence (%) |
|---|---|---|---|---|
| Steaming (min) | Drying | Actellic | | |
| 15 | Solar | - | 119±6.7b | 0.0a |
| 15 | Oven | - | 118±9.4b | 82.5c |
| - | Solar | - | 124.5±7.3b | 83.5c |
| - | Oven | - | 128.3±8.3b | 83.0c |
| - | - | + | 12.0±2.8a | 55.7b |
| - | - | - | 163.3±12.4c | 85.9c |

Mean of four replicates. Values for each followed by the same letter (s) are not significantly different at the 0.05 level. New Duncan's Multiple Test.

## 4.3. Effect of initial temperature

The oviposition and adult emergence of *C. maculatus* on two batches of US blackeye cowpeas that were steamed at initial temperatures of 4 $^0$C (cold) and 28 $^0$C (warm), respectively are shown in Tables 6. The mean number of eggs laid on beans that were steamed from an initial lower temperature of 4 $^0$C was significantly lower than was recorded on beans that were steamed at an initial temperature of 28 $^0$C. But the mean number of eggs laid on beans steamed with a temperature of 28 $^0$C was not significantly different from untreated beans. Within each cowpea treatment, Actellic Super Dust was the best protectant against oviposition by *C. maculatus*.

The mean number of emerging adults was not significantly different between beans steamed at the two initial temperatures ($p>0.05$) and both were comparable to adult emergence in Actellic Super Dust treated beans but significantly lower than in untreated beans.

Table 6. The oviposition and adult emergence of *C. maculatus*(F.) on US blackeye cowpeas that were steamed at initial temperatures of 4°C and 28°C.

| TREATMENT | | | No. of eggs laid (m±se) | Percent adult emergence (%) |
|---|---|---|---|---|
| Steaming (min) | Drying method | Actellic | | |
| 10 min at 4°C | Solar | - | 142.5±15.1b | 2.5 |
| 10 min at 28°C | Solar | - | 206.3±2.7c | 0.7 |
| - | Solar | - | 194.9±13.6b | 59.6 |
| - | - | + | 3.0±0.8a | 35.0 |
| - | - | - | 207.0±22.0c | 62.4 |

Mean of four replicates. Values for each followed by the same letter (s) are not significantly different at the 0.05 level. New Duncan's Multiple Test.

**4.4. Effect of duration of steaming time on oviposition and adult emergence.**

The response of *Callosobruchus maculatus* (F.) adults to blackeye cowpea seeds under the following treatments: 15 minutes steaming and solar drying; 10 minutes steaming at an initial temperature of 4 $^{0}$C and solar drying; and 10 minutes steaming at an initial temperature of 28 $^{0}$C and solar drying, is shown in Tables7. A significantly higher number of eggs was recorded in ten minute steamed and solar dried beans compared to 15 minutes steamed and solar dried beans. Mean number of eggs laid in ten minutes steamed and solar dried beans was not significantly different in unsteamed beans. The least mean number of eggs was recorded on Actellic Super dust treated beans.

The highest mean number of emerging adults was recorded on solar dried only beans, which was not significantly different from unsteamed beans. There was no significant difference in the percent mean number of emerging adults between either 15 minutes or 10 minutes steamed and solar dried seeds. Both were significantly less than in Actellic Super dust treated seeds.

Percent weight loss was not significantly different between 15 minutes and ten minutes steamed and solar dried cowpeas but was significantly less than in solar dried only and untreated cowpeas.

Table 7. The oviposition of *Callosobruchus maculatus*(F.) to US Blackeye cowpeas to three different treatments.

| TREATMENT | | | No. of eggs laid (m±s) | Percent adult emergence (%) |
|---|---|---|---|---|
| Steaming (min) | Drying | Actellic | | |
| 15 | Solar | - | 119±6.7b | 0.0 |
| 10 | Solar | - | 206.3±2.7c | 0.7 |
| - | Solar | - | 150±11.4b | 76.5 |
| - | - | + | 8.2±1.7a | 41.5 |
| - | - | - | 185.7±14c | 73.5 |

Mean of four replicates. Values for each followed by the same letter (s) are not significantly different at the 0.05 level. New Duncan's Multiple Test.

Table 8. Percent loss in weight of blackeye cowpeas after two months in storage.

| TREATMENT | | | Percent loss in weight (%) |
|---|---|---|---|
| Steaming | Drying | Actellic | |
| 15 | Solar | - | 0.44a |
| 10 | Solar | - | 0.02a |
| - | Solar | - | 22.9b |
| - | - | + | 21.6b |

Mean of four replicates. Values for each followed by the same letter (s) are not significantly different at the 0.05 level. New Duncan's Multiple Test.

## 4.5 Seed viability

The germination of the three varieties of cowpeas under the various treatments are shown in Tables 12 and 13. All the steamed and oven dried seeds and steamed and solar dried seeds did not germinate but there was 100% germination in the other treatments.

Table 9. Germination in Soronko Asontem, Amantin and Blackeye cowpea seeds that have been steam-treated for 15 minutes.

| | PERCENT GERMINATION (%) | | | |
|---|---|---|---|---|
| **TREATMENT** | **SORONKO** | **ASONTEM** | **AMANTIN** | **BLACKEYE** |
| STEAM+OVEN DRY | 0a | 0a | 0a | 0a |
| OVEN DRY ONLY | 100b | 100b | 100b | 100b |
| ACTELLIC SUPER DUST | 100b | 100b | 100b | 100b |
| CONTROL | 100b | 100b | 100b | 100b |

Mean of three replicates of 25 seeds. Values for each variety followed by the same letter (s) are not significantly different at the 0.05 level. New Duncan's Multiple Test.

Table 10. Germination in US blackeye cowpeas that have been steamed for 10 minutes and solar dried, from initial temperatures of 4 °C and 28 °C.

| | PERCENT GERMINATION (%) | |
|---|---|---|
| **TREATMENT** | **4°C Initial Temp.** | **28°C Initial Temp.** |
| STEAM+SOLAR DRY | 0a | 0a |
| SOLAR DRY ONLY | 100b | 100b |
| ACTELLIC SUPER DUST | 100b | 100b |
| CONTROL | 100b | 100b |

Mean of three replicates of 25 seeds. Values for each variety followed by the same letter (s) are not significantly different at the 0.05 level. New Duncan's Multiple Test.

# CHAPTER FOUR

## DISCUSSION AND CONCLUSION

The physical treatments given to seeds influence their storage and processing properties. It has been reported that steaming followed by drying of cowpea seeds prior to storage effectively controls the bruchid weevil infestation (Sefa-Dedeh *et al*, 1994).

### 4.1 Effect of steam treatment on oviposition of *Callosobruchus maculatus*

The results indicate that when soronko, asontem, and amantin cowpeas were steamed for 15 minutes and oven dried at 70 $^0$C for eight hours, no significant difference was shown in the mean number of eggs laid on soronko and asontem cowpeas, and these were significantly higher than the mean number of eggs recorded in amantin cowpeas. But the percentage of adults that emerged was highest in amantin (90.6%) than in soronko (70.7%) and asontem (76.7%). Actellic super was significantly more effective than steaming and oven drying in suppressing oviposotion and adult emergence in *C. maculatus* in all the varieties of cowpeas tested. However, unsteamed soronko and asontem were preferred to amantin as oviposition substrate. Soronko and asontem cowpeas are similar in seed coat thickness (7.626 X $10^{-3}$ cm) and size, soronko (0.46cm) and asontem (0.47cm), and have smoother seed coats than amantin seeds. Nwanze and Hober (1976) reported that differences in the seed coat of cowpeas affect oviposition and larval development of *C. maculatus*. Booker (1961) showed that *C. maculatus* prefers smooth testa seeds even when the smooth seeds are smaller than the rough. Sefa-Dedeh and Stanley (1979) also noted that cowpeas with thick coats showed a relatively smooth surface while those with thin seed coats have rough and convoluted surface. Asontem was also significantly more preferred to soronko and amantin for larval development.

Since steamed cowpea of the three varieties recorded comparable mean number of eggs laid in spite of the small differences in seed coat thickness and surface area, compared to their control samples, the steaming process may have altered the chemical responses perceived by gravid *C. maculatus*. Ghokale *et al* (1990) reported that gravid females are solely guided by a chemical stimulus perceived from the oviposition substrate. Thus, the physical characteristics of the steamed cowpeas may have played a major role in the preference as oviposition substrates in the absence of these chemical stimuli. Sefa-Dedeh and Demuyakor (1994) had reported that steaming changes the physico-chemical properties of the seed coat and some part of the cotyledon. Among the varieties of cowpea tested, unsteamed black-eye cowpea was most preferred as oviposition substrate followed by asontem, soronko and amantin in that order.

The comparable preference of steamed amantin cowpea as oviposition substrate in spite of its rough surface (as a result of its smaller seed coat thickness) to soronko and asontem may be attributed to the absence of such perceived chemicals by *C. maculatus* as a result of the steaming process. Osei *et al* (1997) had noted that varieties with thin seed coat might be prone to insect attack. This result agrees with Nwanze *et al*, (1975) who reported that the total number of eggs laid during the lifetime of female *C. maculatus* depends perhaps on the size and hardness of the seed as well as odour. Thus, in the absence of chemical stimuli (Ghokale, 1990), oviposition by gravid *C. maculatus* may be determined by purely physical factors in which seed size and smoothness may not be important. Unsteamed black-eye cowpeas was most preferred as oviposition substrate among the four cowpea varieties tested while amantin was least preferred. This may have been due to the differences in seed surface area; asontem (0.198cm$^3$), soronko (0.179cm$^3$), amantin (0.138cm$^3$), and black-eye (0.195cm$^3$).

When black-eye cowpea was steamed for 15 minutes and solar dried, the mean number of eggs laid was not significantly different from when the seeds were oven dried but compared to untreated seeds, steamed cowpeas recorded significantly lower mean number of eggs. When black-eye cowpeas were steamed for 10 minutes at an initial temperature of 4 $^0$C, a significantly lower number of eggs was recorded compared to beans that were steamed at room temperature. Since steaming time and varietal type were the same, the major factor causing the difference may be the initial steaming temperatures of the cowpeas. Steaming may affect the chemical response perceived by gravid females, so the factor(s) causing the difference in preference may be a reduced perception of the chemical stimulant by *C. maculatus* or a diverse change in the physical characteristics of the seeds steamed from the cold.

Thus, steaming black-eye cowpeas at an initial temperature of 4 $^0$C may have resulted in micro-structural changes that diminished its preference by *C. maculatus* as an oviposition substrate. However, a thorough explanation of the factors leading to the loss in preference of beans steamed at an initial low temperature may require further work. According to Paurie *et al* (1960), reliable information on the chemical composition of the specific tissues, structure of cells and localization of chemical constituents in cells of legume seeds is a prerequisite for the understanding of physical and chemical changes taking place in the seed during mechanical, thermal and enzymatic processes.

## 4.2 Effect of steaming on larval development

Even though a comparable mean number of eggs was recorded in blackeye cowpeas that were steamed for 15 minutes and solar dried and those that were oven dried, the mean number of emerging adults were significantly higher in the oven dried samples than in solar dried samples. Since the steaming time was the same for both batches of cowpeas, the cause of the difference occurring will be the method of drying. Sefa-Dedeh *et al.* (1994) noted that steaming cowpeas for 10 minutes or more might cause structural and functional modifications in the seeds. Although seeds of soronko, asontem and amantin varieties that were steamed for 15 minutes and oven dried did not ultimately protect the beans against bruchid damage, adult *C. maculatus* emergence was delayed for 4-5 days compared to other treatments. However, there was no significant difference between treatments with respect to days of emergence. When 10- or 15-minutes steamed cowpeas were solar dried, adult *C. maculatus* emergence was comparable to that in conventional insecticides.

Microscopic examination of hatched eggs in some steamed and solar dried US black-eye cowpeas that failed to emerge into adults showed that, indeed hatched larvae had initiated feeding and penetration of the seed cotyledon after embryonic development but only few successfully penetrated the seed and developed into adults. Seifelnasr (1991) observed that the premature death of bruchid larvae in haricot beans may be due to alanine, an amino acid that usually exists in association with its toxic analogues of Beta-cyanoalanine and Alpha-diaminobutyric acid. Steamed cowpeas could possibly contain altered amino acids that could be toxic and therefore not conducive for the development of the larvae of *C. maculatus*. However, the fact that very few larvae survived to adulthood may suggest that even though steaming may have altered the nature of proteins in the majority of the cowpeas, a few cowpeas which were not well exposed to the steam probably did not undergo major protein changes. Thus, steaming may have started these protein functional changes in the oven-dried seeds but these were short of completion as a result of the quick drying effect of the oven method. However, just enough protein changes may have occurred to increase the length of time required by *C. maculatus* larvae to complete development to the adult stage.

Thus, microstructural changes of the seed coat resulting from the steaming process may not have been the major factor causing larval death of *C. maculatus*. Oven drying at 70°C for eight hours was drastic, and removed a lot of water in a relatively short time, so as to arrest the functional changes resulting from the steaming process (which require water), whereas solar drying (which may take about three days to a week depending on day maximum temperatures) was more gradual and allowed such functional changes to go to completion. Possible changes could have been gelatinization of starch molecules and denaturation of protein molecules.

Among the four varieties of cowpeas tested, steamed and oven dried black-eye cowpea suffered the most damage in terms of percent loss of food material after two months in storage, followed by soronko and asontem and then amantin. This confirms the findings of Ramzan *et al* (1990) who noted that black-eye cowpea suffered the most damage in terms of loss in weight (34.58%).

Cowpeas steamed for 15 minutes were not significantly different from those steamed for 10 minutes when the seeds were solar dried to attain safe moisture content levels in terms of percent loss in weight. Contrary to claims by Osei (1994) that cowpeas become mouldy in storage when steamed for 15 minutes, such steamed cowpeas were found to be in comparably good condition to 10 minutes steamed cowpeas after two months in storage.

When US black-eye cowpea was steamed for either 10 or 15 minutes, the mean number of emerging adults was significantly lower ($p<0.05$) than in untreated cowpea only when they were solar dried, and these were comparable to adult emergence in Actellic Super dust treated seeds. Thus steaming of cowpeas and solar drying that resulted in the completion of the functional changes caused the death of the bruchid larvae as a result of non-utilization of the normal nutrition, which is derived from unsteamed cowpeas. Majumder (1982) explains that enzymes of the digestive tract of insects seem to have specific roles in assimilating constituents of cereals and pulses. Sefa-Dedeh and Demuyakor (1994) also point out that steaming may lead to a case hardening effect. Thus, steaming of cowpeas may induce mechanical resistance to mandibular chewing and prevent utilization of the gelatinized starch by gut enzymes in *C. maculatus*.

### 4.3 Contribution of drying to the steaming effect

Oven dried or solar dried cowpeas of all varieties of cowpea tested showed no significant difference from untreated beans in terms of number of eggs laid and emerging adults. This means, oven drying or solar drying may not offer any permanent physical or chemical change in contribution to the steaming effect. But the effect of the steaming process on larval development may be affected by the method of drying. Thus, the effect on oviposition and development of the larvae may be largely due to the steaming process. However, the length of time used in oven drying and the temperature at which the seeds are dried in the oven after steaming may play a significant role in determining whether ongoing functional changes due to steaming proceed to completion or not.

### 4.4 Effect of steaming on the viability of exposed seeds

In all the varieties tested and in the three sets of experiments conducted, steaming completely inhibited germination. This method is therefore not applicable to seed stock.

## CONCLUSION

The steaming process has proved very effective against cowpea bruchids and afforded levels of protection comparable to conventional standard insecticides. The fact that larvae had initiated feeding after embryonic development before death shows that the steaming process may have altered the nature of surface proteins which is unsuitable for larval development. Solar drying for about a week or generally to attain safe moisture content levels of seeds allows the effects of steaming to proceed to completion. Steaming cowpeas initially at lower temperature also significantly reduces the mean number of eggs laid on seeds but the effect on larval development was not significantly different.

The steaming process results in the death of the germ and hence seed viability. Steaming is safer and simple to use and can be applied on seeds destined for consumption. The nature of the protein changes is not clear and needs to be studied in detail in order to offer a better explanation for factors causing larval death.

## RECOMMENDATION

1. Furhter research need to be done on the nature of the functional changes which take place after the steam treatment.

2. Further experiments should be done to determine if reducing the temperature of the oven during drying and increasing the oven drying time would give the same effect as sun drying.

3. The treatment should be applied to other legumes and grains to test the universal applicability of the method.

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# APPENDICES

Tables of ANOVA for the various analyses.

## APPENDIX I

SORONKO (EGGS)

| | DF | SS | MS | F-val | P-val |
|---|---|---|---|---|---|
| EEFECTS | 6 | 19400.875 | 3233.4792 | 4.275 | .0258 |
| Replicates | 3 | 1330.188 | 443.3958 | .586 | .6391 |
| Treatment | 3 | 18070.687 | 6023.5625 | 7.963 | 7.0067 |
| Residual | 9 | 6808.0625 | 756.45139 | | |
| TOTAL | 15 | 26208.938 | | | |

| | |
|---|---|
| Steam + drying | ab |
| Drying only | b |
| Actellic super | a |
| Control | b |

## APPENDIX II

SORONKO (ADULTS)

| | DF | SS | MS | F-val | P-val |
|---|---|---|---|---|---|
| EFFECTS | 6 | 9983.8750 | 1663.9792 | 4.589 | .0209 |
| Replicates | 3 | 1180.6875 | 393.5625 | 1.086 | .4036 |
| Treatment | 3 | 8803.1875 | 2934.3958 | 8.093 | .0063 |
| Residual | 9 | 3263.0.625 | 362.56250 | | |
| TOTAL | 15 | 13246.938 | | | |

| | |
|---|---|
| Steam + drying | b |
| Drying only | b |
| Actellic super | a |
| Control | b |

## APPENDIX III

### ASONTEM (EGGS)

| | DF | SS | MS | F-val | P-val |
|---|---|---|---|---|---|
| EFFECTS | 6 | 29575.375 | 4929.2292 | 76.546 | .0000 |
| Replicates | 3 | 243.688 | 81.2292 | 1.261 | .3446 |
| Treatment | 3 | 21589.687 | 7196.5625 | 145.528 | .0000 |
| Residual | 9 | 445.06250 | 49.451389 | | |
| TOTAL | 15 | 22131.438 | | | |

| | |
|---|---|
| Steam + drying | c |
| Drying only | b |
| Actellic super | a |
| Control | d |

## APPENDIX IV

### ASONTEM (ADULTS)

| | DF | SS | MS | F-val | P-val |
|---|---|---|---|---|---|
| EFFECTS | 6 | 21686.375 | 3614.3958 | 73.090 | .0000 |
| Replicates | 3 | 96.688 | 32.2292 | .652 | .6016 |
| Treatment | 3 | 21589.687 | 7196.5625 | 145.528 | .0000 |
| Residual | 9 | 445.06250 | 49.451389 | | |
| TOTAL | 15 | 22131.438 | | | |

| | |
|---|---|
| Steam + drying | c |
| Drying only | b |
| Actellic super | a |
| Control | d |

## APPENDIX V

### AMANTIN (EGGS)

| | DF | SS | MS | F-val | P-val |
|---|---|---|---|---|---|
| EFFECTS | 6 | 10300.375 | 1716.7292 | 29.342 | .0000 |
| Replicates | 3 | 168.688 | 56.2292 | .961 | .4521 |
| Treatment | 3 | 10131.688 | 3377.2292 | 57.724 | .0000 |
| Residual | 9 | 526.56250 | 58.506944 | | |
| TOTAL | 15 | 10826.938 | | | |

| | |
|---|---|
| Steam + drying | b |
| Drying only | a |
| Actellic super | a |
| Control | a |

## APPENDIX VI

### AMANTIN (ADULTS)

| | DF | SS | MS | F-val | P-val |
|---|---|---|---|---|---|
| EFFECTS | 6 | 9129.3750 | 1521.5625 | 39.801 | .0000 |
| Replicates | 3 | 120.1875 | 40.0625 | 1.048 | .4176 |
| Treatment | 3 | 9009.1875 | 3003.0625 | 78.554 | .0000 |
| Residual | 9 | 344.06250 | 38.229167 | | |
| TOTAL | 15 | 9473.4375 | | | |

| | |
|---|---|
| Steam + drying | c |
| Drying only | ab |
| Actellic super | a |
| Control | b |

**APPENDIX VII**

**Soronko/Asontem/Amantin (EGGS)**

| SOURCE | DF | SS | MS | F-val | P-val |
|---|---|---|---|---|---|
| EFFECTS | 5 | 52231.729 | 10446.346 | 38.944 | .0000 |
| Treatment | 3 | 39773.063 | 13257.688 | 49.424 | .0000 |
| Variety | 2 | 12458.667 | 6229.333 | 23.223 | .0000 |
| INTERACTIONS | 6 | 17761.000 | 2960.1667 | 11.035 | .0000 |
| T'ment/Var | 6 | 17761.000 | 2960.1667 | 11.035 | .0000 |
| RESIDUAL | 36 | 9656.7500 | 268.24306 | | |
| TOTAL(CORR.) | 47 | 79649.479 | | | |

Variety (Eggs) by Treatment

| | |
|---|---|
| Steam + drying | c |
| Drying only | b |
| Actellic super | a |
| Control | c |

Variety (Eggs) by Variety

| | |
|---|---|
| Soronko | b |
| Asontem | b |
| Amantin | a |

APPENDIX VIII

Soronko/Asontem/Amantin (ADULTS)

| | DF | SS | MS | F-val | P-val |
|---|---|---|---|---|---|
| EFFECTS | 5 | 34237.563 | 6847.5125 | 45.233 | .0000 |
| Treatment | 3 | 28392.063 | 9464.0208 | 62.518 | .0000 |
| Variety | 2 | 5845.500 | 2922.7500 | 19.307 | .0000 |
| INTERACTIONS | 6 | 11010.000 | 1835.0000 | 12.122 | .0000 |
| T'ment/Var | 6 | 11010.000 | 1835.0000 | 12.122 | .0000 |
| RESIDUAL | 36 | 5449.7500 | 151.38194 | | |
| TOTAL(CORR.) | 47 | 50697.313 | | | |

<u>Variety (Eggs) by Treatment</u>

| | |
|---|---|
| Steam + drying | c |
| Drying only | b |
| Actellic super | a |
| Control | c |

<u>Variety (Eggs) by Variety</u>

| | |
|---|---|
| Soronko | a |
| Asontem | b |
| Amantin | a |

## APPENDIX IX

**Storage temp/Heat treatment (EGGS)**

| | DF | SS | MS | F-val | P-val |
|---|---|---|---|---|---|
| EFFECTS | 4 | 219719.37 | 54929.844 | 64.799 | .0000 |
| StorTemp | 1 | 569.53 | 569.531 | .672 | .4291 |
| HeatTrmt | 3 | 219149.84 | 73049.948 | 86.175 | .0000 |
| INTERACTIONS | 3 | 10643.344 | 3547.7813 | 4.185 | .0162 |
| StorTem/Heat | 3 | 10643.344 | 3547.7813 | 4.185 | .0162 |
| RESIDUAL | 24 | 20344.750 | 847.69792 | | |
| TOTAL(CORR.) | 31 | 250707.47 | | | |

| | |
|---|---|
| Steam + drying | b |
| Drying only | b |
| Actellic super | a |
| Control | b |

## APPENDIX X

**Storage temp/Heat treatment (ADULTS)**

| | DF | SS | MS | F-val | P-val |
|---|---|---|---|---|---|
| EFFECTS | 4 | 117756.87 | 29439.219 | 78.071 | .0000 |
| StorTemp | 1 | 171.13 | 171.125 | .454 | .5141 |
| HeatTrmt | 3 | 117585.75 | 39195.250 | 103.943 | .0000 |
| INTERACTIONS | 3 | 604.62500 | 201.54167 | .534 | .6631 |
| StorTem/Heat | 3 | 604.62500 | 201.54167 | .534 | .6631 |
| RESIDUAL | 24 | 20344.750 | 847.69792 | | |
| TOTAL (CORR.) | 31 | 127411.50 | | | |

| | |
|---|---|
| Steam + drying | a |
| Drying only | b |
| Actellic super | a |
| Control | b |

Some raw data on oviposition and adult emergence of *C. maculatus* on the different cowpea varieties.

**SORONKO**

| Treatments | Rep 1 | | Rep 2 | | Rep 3 | | Rep 4 | |
|---|---|---|---|---|---|---|---|---|
| | Eggs | Adults | Eggs | Adults | Eggs | Adults | Eggs | Adults |
| Steam + oven dry | 46 | 36 | 39 | 21 | 89 | 67 | 103 | 72 |
| Oven dry only | 87 | 51 | 95 | 46 | 64 | 35 | 94 | 48 |
| Actellic dust | 5 | 2 | 7 | 0 | 10 | 1 | 9 | 3 |
| Control | 110 | 63 | 62 | 46 | 141 | 106 | 62 | 44 |

**ASONTEM**

| Treatments | Rep 1 | | Rep 2 | | Rep 3 | | Rep 4 | |
|---|---|---|---|---|---|---|---|---|
| | Eggs | Adults | Eggs | Adults | Eggs | Adults | Eggs | Adults |
| Steam + oven dry | 73 | 58 | 88 | 66 | 77 | 64 | 97 | 69 |
| Oven dry only | 50 | 40 | 45 | 40 | 50 | 45 | 65 | 53 |
| Actellic dust | 7 | 1 | 8 | 3 | 11 | 8 | 11 | 4 |
| Control | 139 | 119 | 120 | 94 | 121 | 109 | 125 | 102 |

**AMANTIN**

| Treatments | Rep 1 | | Rep 2 | | Rep 3 | | Rep 4 | |
|---|---|---|---|---|---|---|---|---|
| | Eggs | Adults | Eggs | Adults | Eggs | Adults | Eggs | Adults |
| Steam + oven dry | 62 | 54 | 91 | 78 | 72 | 65 | 72 | 72 |
| Oven dry only | 21 | 18 | 25 | 18 | 10 | 7 | 23 | 21 |
| Actellic dust | 2 | 2 | 8 | 5 | 12 | 5 | 12 | 7 |
| Control | 24 | 23 | 21 | 20 | 31 | 26 | 25 | 22 |

Black-eye steamed at an initial temperature of $4^0C$

| Treatments | Rep 1 | | Rep 2 | | Rep 3 | | Rep 4 | |
|---|---|---|---|---|---|---|---|---|
| | Eggs | Adults | Eggs | Adults | Eggs | Adults | Eggs | Adults |
| Steam + solar dry | 182 | 2 | 115 | 0 | 123 | 2 | 150 | 1 |
| Solar dry only | 244 | 155 | 197 | 121 | 194 | 105 | 222 | 122 |
| Actellic dust | 0 | 0 | 3 | 1 | 2 | 2 | 1 | 0 |
| Control | 233 | 142 | 266 | 178 | 189 | 117 | 129 | 83 |

Black-eye steamed at an initial temperature of 28°C

| Treatments | Rep 1 | | Rep 2 | | Rep 3 | | Rep 4 | |
|---|---|---|---|---|---|---|---|---|
| | Eggs | Adults | Eggs | Adults | Eggs | Adults | Eggs | Adults |
| Steam + solar dry | 207 | 2 | 213 | 2 | 200 | 4 | 205 | 6 |
| Solar dry only | 214 | 122 | 186 | 105 | 156 | 89 | 146 | 109 |
| Actellic dust | 4 | 2 | 4 | 1 | 7 | 1 | 3 | 1 |
| Control | 171 | 89 | 216 | 128 | 212 | 150 | 240 | 146 |