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Response of Cowpea Beetle to Food Quantity and Methods of Sterilization and Effects of Age and Storage Methods on Maize Weevil

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ABSTRACT

Cowpea beetle (Callosobruchus maculatus (F.) and maize weevil (Sitophilus zeamais Mots.) are two serious insect pests of stored grains in the tropics. Experiments were conducted at the Department of Crop, Soil and Pest Management, the Federal University of Technology, Akure, Nigeria to determine the influence of food quantities and methods of sterilization on the development of C. maculatus and to evaluate the effects of age and storage method on the performance of S. zeamais under laboratory conditions. Results revealed significant differences ($p<0.05$) in the mean number of seeds with eggs, seeds without eggs, seed weight loss and number of seeds with holes. However, no significant differences existed in mean number of eggs laid and number of emerged adults though highest number of eggs was recorded for C. maculatus provided with 20g of cowpea seeds. Investigations also revealed that methods of sterilization did not have significant effect on number of eggs laid, number of emerged adults and number of seeds with eggs. However, methods of sterilization had significant effect on number of seeds with eggs. Highest number of eggs was laid on and the lowest number of adults emerged from seeds heat sterilized at 60°C and acclimatized for 3hrs. It was however observed that the weight of infested cowpea seed reduced substantially with damage by the cowpea beetle. Results from the study also revealed that despite non-significant effect ($p>0.05$) of insect age on the number of emerged adults at first month of maize storage, insect age had significant effect on seed weight loss, with the highest weight loss value was obtained from grains infested with 3 days old insects. Insect age did not have significant effect ($p>0.05$) on the number of adults that emerged and seed weight loss when the seeds were stored for 2 months. Generally, 3 days old insects performed better than insects of other ages. Storage materials did not have significant effect on the number of emerged adults and weight loss at both 1 and 2 months of maize storage period. However, lowest number of adults emerged from grains stored in polythene bag while the lowest weight was lost on grains stored in glass jar at 1st month of storage. Based on the results from this study, it was evident that food quantity and method of sterilization could be among the factors that determine performance of C. maculatus in the laboratory while age of infesting adults and storage material could influence the depredative capacity of the maize weevil on maize during storage.

Key words: *Callosobruchus maculatus, Sitophilus zeamais,* quantity, sterilization

INTRODUCTION

Several insect species attack cowpea while on the field and in storage (Adebayo *et al*., 2013). The most fearsome primary insect pests that attack cowpea during storage belong to different species of bruchids (Atanda *et al*., 2012). Among these species, *Callosobruchus maculatus* (F.) is considerably important as a cosmopolitan pest of stored cowpea (and to a lesser extent of other stored legumes) (Ofuya, 2001; Atanda *et al.*, 2012). *Callosobruchus maculatus* causes particular problem for cowpea production across many regions of West Africa (Appleby ad Credland, 2002). *Callosobruchus maculatus* has drawn attention because it is widely distributed throughout the tropical and sub-tropical regions, thus it has become tropicopolitans (Jackai and Daoust, 1986; Ofuya, 2001; Lale, 2002). The adults typically lay their eggs on the surface of pods or dehisced seeds in the field before or around the time of harvest (Germain *et al*., 1987, Adebayo *et al.,* 2013). These are then brought unnoticed into seed stores where the protected environment, buffered from major external environmental fluctuations, results in rapid insect development and population growth. If unchecked, storage populations of *C. maculatus* can grow exponentially causing significant losses in seed weight, germination, viability and marketability (Southgate, 1979; Ofuya, 2003; Adebayo and Eyo, 2014). Though lots of work have been done on the seed beetle, continuous research to understand their behavior, ecology, physiology and factors that will

enhance their performance is imperative. This is because of the important roles that cowpea play in the diet of millions of people worldwide. Cowpea is a good substitute for the expensive sources of protein such as egg, meat and fish (Ileke *et al*., 2013). A substantial part of the world cowpea production comes from Nigeria with about 4 million hectares and approximately 1.7 million tons of beans annually (Ofuya, 2003). Production of cowpea has however been adversely affected as a result of attack from by *C. maculatus*.

Maize (*Zea mays* L.) is one of the most important and most widely distributed staple crops in the world (CIMMYT, 2004). It is the most important crop in the United State of America which produces half of the world's total (Purseglove, 1992). Maize was introduced into Africa from its native Mesoamerica in the 16th century (CIMMYT, 2004). Worldwide, more than 200 species of insect pests have been recorded on maize (Purseglove, 1992). The most common among these are *Sitophilus zeamais* Mots. *Sitophilus oryzae* (L.), *Rhysopertadominica* (L.) and *Prostephanus truncatus* Horn (NRI, 1996).

Postharvest storage of maize is greatly constrained by the pest, *Sitophilus zeamais* (Markham *et al*., 1994). Damage to maize grain begins from the field just before harvest and the insects are carried into the store where the population builds up rapidly (Appert 1987; Adedire and Lajide, 2001; Kim and Kossou, 2003). Ayertey (2002) affirmed that losses to food grains caused by storage insects are serious threat to food security thus postharvest production management is critical to ensuring food security (Sefa-Dedeh and Senanu, 1995; Idoko and Adebayo, 2011). Therefore, this present study seeks to determine the influence of food quantities and methods of sterilization on the development of *Callosobruchus maculatus* (F.) as well to evaluate the effects of age and storage materials on the performance of *Sitophilus zeamais* Mots in the laboratory.

MATERIALS AND METHODS

Study site

The experiment was conducted at the analytical and research laboratory of the Department of Crop, Soil and Pest Management, Federal University of Technology, Akure, Ondo State, Nigeria. All the investigations were carried out under ambient laboratory conditions of 28±3^{o}C temperature and 65±10% relative humidity.

Collection of Materials

Clean uninfested Ife brown cowpea variety (3kg) were purchased from Agricultural Development Project Ondo State while uninfested whole grains of local white maize (about 3kg) was sourced from Isikan market, Akure, Ondo State. The grains were kept in cold storage at a temperature of 100^{o}C to disinfest any possible attacking insects. Adults of *C. maculatus* and S. zeamais used for the study were obtained from already infested cowpea seeds and maize grains maintained in the entomology section of the laboratory.

Culturing of insects

Cowpea seeds infested by the *C. maculatus* were collected and maintained in the laboratory. The infested seeds were set aside in a plastic container and covered with muslin cloth until the adults emerged. Freshly emerged adults from the container were sieved into another plastic container and provided with clean cowpea seeds for oviposition. The container was undisturbed until the emergence of new adults. Subsequent freshly emerged adults were used for the experiments. The same procedure was follow for the culturing of *S. zeamais* to obtain pure culture.

Response of cowpea beetle to food quantity and methods of sterilization

Prior to the setting up of experiment, portions of clean uninfested Ife brown cowpea seeds were heat sterilized for three hours in an oven at 60° C and for two hours at 70°C, they were also cold stored and acclimatized for two and three hours. 5g, 10g, 15g and 20g each of the sterilized cowpea seeds were infested with six pairs of 24-48 hours old *C. maculatus* adults in petri dishes. Adult insects were sieved out after a week and eggs laid counted and recorded. The setups were left untouched on the shelf for the emergence of adults of C. maculatus. Emerged adults were counted and recorded. Weight of the cowpea seeds was taken before and after adult emergence to determine weight loss due to *C. maculatus* infestation. Number of seeds with eggs, seeds without eggs as well as seeds with and without emergent holes were also counted and recorded. Experimental design used was Completely Randomize Design.

Effects of age and storage methods on the performance of maize weevil

Adults of *Sitophilus zeamais* were derived from a stock culture maintained on susceptible variety of maize in the laboratory. The storage methods investigated in this study were plastic container, polythene bag, bottle and paper bag. Samples of clean uninfested maize were heat-sterilized prior to setting up the experiment as was done by Allotey and Azalekor (2000). After cooling, 250g of the grains were put into each storage material (plastic container, polythene bag, bottle and paper bag). Twenty

unsexed, 3, 7 and 14 day-old adults of S. zeamais were introduced into the four storage materials with an aspirator and replicated three times. Each container was covered tightly to prevent contamination of the culture. The cultures were left undisturbed in the laboratory while adult insects were sieved out after 14 days of eggs laying. The setups were monitored for 8 weeks. Number of emerged adults was counted and weight loss of the maize grains was recorded.

Data Analysis

Data obtained by counts were square root transformed and one way analysis of variance performed the two experiments using Statistical Package for Social Sciences (SPSS) version 15. Significant means were separated using DMRT at 5% level of significance.

RESULTS AND DISCUSSION

Results

The highest number of eggs was laid by *C. maculatus* on 20 gram seeds while lowest number of eggs was laid on 5g seeds. However, results from the experiment on the response of *C. maculatus* to food quantity (Table 1) did not reveal any significant difference ($p>0.05$) in the mean number of eggs laid by the beetles. Similarly, more *C. maculatus* adults emerged from 20g seeds followed by 15g seeds while the lowest adults emerged from the 5g seeds but there were no significant differences ($p>0.05$ in the number of adults that emerged from the different seed quantities (Table 1). However, significant differences ($p<0.05$) were observed in the number of seeds with eggs, number of seeds without eggs, number of seeds with holes and weight loss of seeds across the various seed quantity treatments (Table 1). Twenty gram seeds contained statistically similar number of seeds with eggs with 15g seeds but were significantly higher than the number of seeds with eggs in the other seed quantities that were investigated (Table 1). The numbers of seeds with eggs in 5g were significantly lower than all the other variables. The number of seeds without eggs under 20g seeds was significantly higher than the other seed quantities. Ten, 15 and 20 grams seeds had statistically similar number of seeds with holes, but were statistically higher ($p<0.05$) than the number of seeds with holes in 5 g seeds. Five gram seeds had least weight loss, which was not statistically different from the weight loss of 10g and 15g seeds but significantly different from the weight loss recorded in 20 g seeds. From table 2, methods of sterilization seem not to affect the performance of the cowpea seed beetle in this study as most of the parameters measured were not statistically significant ($p>0.05$), except for seeds heat-sterilized at 60°C for 3hrs. Heat sterilization of cowpea seeds at 60°C for 3hrs had higher values in some of the measured parameters as shown in Table 2. For example, more eggs were laid, highest number of seeds with eggs and highest weight loss were obtained when seeds were heat-sterilized at 60°C for 3hrs while highest number of adults emerged, highest number of seeds without eggs and holed seeds were recorded on seeds heat-sterilized at 70°C for 2hrs. For number of seeds with eggs, the results of mean separation showed that significant difference ($p<0.05$) was only observed between seeds heat-sterilized at 60°C for 3hrs and heat-sterilized at 70°C for 2hrs (Table 2).

Table1: Mean number of eggs laid, emerged adults, seeds with eggs, seeds without eggs, seeds with holes and weight loss of different food quantities

Food quantity (g)	No. of eggs laid	No. of emerged adults	No. of seeds with eggs	No. of seeds without eggs	No. of seeds with holes	Mean weight loss (g)
5	11.73a	3.43a	5.50c	1.72c	5.69b	1.23b
10	12.08a	3.48a	8.46b	2.81bc	7.50a	1.53ab
15	12.56a	3.68a	9.40ab	4.18b	7.65a	1.55ab
20	13.08a	3.84a	10.64a	7.50a	7.78a	1.91a

Means followed by the same letter along the column are not significant different at 5%

Table 2: The effect of sterilization methods on number eggs laid, emerged adults, seeds with eggs, seeds without eggs, seeds with holes and weight loss.

Sterilization methods	No. of eggs laid	No. of emerged adults	No. of seeds with eggs	No. of seeds without eggs	No. of seeds with holes	Weight loss
HS 60°C, 3hrs	13.49a	3.57a	9.24a	3.90a	7.20a	1.92a
HS 70°C, 2hrs	11.41a	3.63a	7.63b	4.24a	7.27a	1.45a
CSAC 2hrs	11.96a	3.59a	8.48ab	4.02a	6.80a	1.49a
CSAC 3hrs	12.58a	3.62a	8.63ab	4.00a	6.85a	1.34a

HS = Heat sterilization; CSAC = Cold sterilization, Acclamatize. Means followed by the same letter along the column are not significantly different at 5%.

Table 3: Mean number of emerged adults and weight loss after a month and 2 months of infestation with *S. zeamais* of different age

Age (days)	Storage period (month (s))			
	1		2	
	No. of emerged adults	Weight loss (g)	No. of emerged adults	Weight loss(g)
3	3.30a	1.32a	11.05a	3.60a
7	3.02a	1.03b	9.57a	3.37a
14	3.16a	1.15b	10.47a	3.48a

Means followed by the same letter along the column are not significantly different at 5%.

Table 4: Mean number of emerged adults and weight loss after a month and 2 months of storage using different storage materials

Storage methods	Storage period (month (s))			
	1		2	
	No. of emerged adults	Weight loss (g)	No. of emerged adults	Weight loss(g)
Paper bag	3.44a	1.27a	10.22a	3.48a
Polythene bag	2.99a	1.17a	10.27a	3.48a
Glass jar	3.03a	0.99b	10.45a	3.48a
Plastic container	3.18a	1.24a	10.51a	3.49a

Means followed by the same letter along the column are not significantly different at 5%.

From Table 3, there was no significant effect ($p>0.05$) of age of *S. zeamais* on the number of number of emerged adults. However, the age of insect had significant effect on the weight loss of the seeds, with younger insects (3 days old) having significantly higher seed weight loss that older insects (7 and 14 days).. Similar pattern was observed for number of adult emergence at the end of 2 months of storage where no significant differences ($p>0.05$) were observed for insects of different ages. However, unlike one month storage, there were no significant differences in the weight of seeds subjected to insects of different ages (Table 3). Both number of emerged adults and seed weight loss were higher at two months of storage than one moth of storage.

Results on type of storage material followed similar pattern as studies on varying age. No significant differences were observed for number of emerged adults after a month of storage but weight loss was significantly different ($p<0.05$), with glass jar having significantly lower weight loss than other storage material used (Table 4). There were no significant differences for the number of emerged adult and weight loss after 2 months of storage. Both the number of emerged adults and weight loss increased in value as period of storage increased.

DISCUSSION

Several factors have been reported to influence the performance of storage insect pests. In this present study quantity of food and methods of sterilization affected the performance of *Callososbruchus maculatus* (F.) on stored Ife-brown cowpea while insect age and storage materials did not significantly influenced the performance of *Sitophilus zeamais* Mots on white maize in the laboratory. Food availability also has been indicated to influence the oviposition decision making in female *C. maculatus*. The quantity of food determined the number of eggs that were laid in this study. Highest number of eggs was laid on 20g of cowpea seeds which also had the highest number of emerged adults. Hong (1996) reported that deposition of eggs by the female beetles was observed to be influenced by the availability of food. A study to determine the effects of the food quantity on the performance of the cowpea seed beetle, *C. maculatus* was conducted by Igbalajobi in 2012, who observed that when small quantity of food was provided, the beetle tend to crowd the available seeds with eggs while they scattered the eggs in the presence of plenty seeds. Thus, highest number of emerged adults from the 20g seeds of cowpea might be due to provision of sufficient food for the developing offspring. This agrees with the reports of other researchers (Adebayo and Idoko, 2012; Oke, 2014).

In entomological procedures, sterilization is done to eliminate any infesting insects and contaminating microorganisms so as to obtain a pure culture of the test insect. In this study methods of sterilization seem not to affect the performance of the cowpea seed beetle as most of the parameters measured were not significantly different. However, the performance of the *Callosobruchus maculatus* in the study was best on the seeds heat-sterilized at 60^{o}C for 3hrs. Contrary to the result of this study, Azalekor (2000) suggested sterilization of substrates at 700C for 3hrs. Similar results to those obtained for the measured parameter such as number of eggs laid, emerged adults, seed with eggs, seeds without eggs, seeds with hole, seeds without holes and weight loss in this study have been reported by several researchers (Idoko and Adebayo, 2011, Adebayo and Idoko, 2012, Adebayo and Eyo, 2014). Cold sterilization and acclimatization for 2 hours and 3 hours was observed to have negative effects on the development of the beetles. This might be due to its effects on the texture of the seeds (Stoll, 1988).

Age of an organism is an important factor determining its life functions. In this study, 3 days old *Sitophilus zeamais* had the highest number of emerged adults and caused highest mean weight loss after 1 and 2 months of storage, which were not significantly different from seeds subjected to older insects (7 and 14 days old), except for seed weight loss during one month storage. It has been reported that most female insects lay most of their viable eggs early during oviposition time (Ofuya and Agele, 1990; Adebayo, 2015). Adebayo (2007) also reported similar results while working with *Sitophilus linearis* Herbst on the seeds of tamarinds (*Tamarindus indica*) during which more than 70% of the eggs were laid within 3 days of oviposition. This observation could be as a result of vigor and stored energy in the body of insects which depletes as oviposition progresses. Deposition of eggs has also been reported to depend on several factors such as oviposition marker, a chemical to deter other insects from laying in the same position in stored product insects (Ofuya and Agele, 1990). Therefore, it is possible that the chemical affects the insect after oviposition or the insect has a mechanism to prevent larval competition when eggs are crowded (Dick and Credland, 1986; Ofuya and Agele, 1990; Wilson, 1994).

Storage is as important as production of crop itself. It has been widely reported that storage and storage structure form important part of crop production at various farm level. In this study paper bag, polythene bag, glass jar and plastic container were evaluated for the storage performance of *Sitophilus zeamais*. Though paper bag and plastic container storage produced the number of the weevils at 1st they were not significantly different from the performance of the other storage materials while after second months of storage glass jar and plastic container produced highest number of weevils. However, none of the storage methods actually prevented the damage of the maize when infested as varying losses were observed.

In several maize-producing countries especially in Africa, indigenous seed storage to control *S. zeamais* has been practiced at the farmer level, particularly in marginal communities (Pierrard, 1986; Saayman, 1995). Even with the advent of synthetic pesticides, most resource poor farmers hardly adopted advanced seed protection technologies for various reasons (Ngobeni, 2004; Ngobeni and Mashela, 2005). For instance, use of synthetic pesticides for seed protection did not provide the much desired flexibility for consuming stored seeds (Ngobeni and Mashela; 2005). However, results of indigenous seed storage vary from region to region, probably due to

climatic variations (Ngobeni, 2004). The results observed for the storage materials showed their suitability for the development of the maize weevils thus were not good enough for the storage of maize against *Sitophilus zeamais*.

CONCLUSION AND RECOMMENDATION

Based on the observations from the study the quantity of food and methods of sterilization affected the performance of *Callososbruchus maculatus* (F.) on stored Ife-brown cowpea. However, insect age and storage materials did not significantly affect the performance of the *Sitophilus zeamais* Mots on white maize in the laboratory. Twenty gramme (20g) food and heat sterilization at 60^{o}C for 3hrs enhanced the performance of *Callososbruchus maculatus* with resultant high number of emerged adults while maize infested by weevils of age 3 days and stored in the paper bag and plastic container produced highest number the weevils in the 1st month of storage. Therefore, no matter the age of insect, number of emerged insect was approximately the same. The materials used in storing the maize seeds did not significantly affect the number of emerged insect for both month(s) of storage. It is recommended that other indigenous storage materials used at farm level should be evaluated for the protection of maize against *Sitophilus zeamais* Mots.

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