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Effect of grain splitting on biology and development of *Callosobruchus Maculatus* (Coleoptera: Bruchidae) in storage

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Abstract

Bruchids belonging to *Callosobruchus* spp. Order Coleoptera are the main storage loss causative pests on cowpea grains in East Africa. Losses have been estimated to be as high as 5-15 % within a few months of storage at farmers' level. In this study the effect of grain splitting on the biology of *C. maculatus* was investigated. The cowpea grain treatments compared included split grains + no testa, split grains with testa, compared with whole un-split cowpea grains. Gravid female 4-day old *C. maculatus* were allowed to oviposit on the cowpea grain which had been pre-conditioned to 12% moisture content, then removed. Data on oviposition, adult eclosion and weights of emergent adults were collected over 3 weevil development generations. Mean separations were done using Analysis of Variance. There was significant reduction ($P < 0.05$) in oviposition and emergent adults for *C. maculatus* at F1, F2 and F3 generations on spilt compared to whole grains. Mean oviposition levels on split grain without testa was with only 1.3 ± 1 eggs compared to 258 ± 14 eggs in the controls at F3. Oviposition levels reduced with generation time in all the treatments. The control had the highest adult weevil emergence and mean adult weevil weights (78.8 ± 6.9) with no emergent adults from completely split grains at F3. Mean weevils were highest in the controls and reduced with generation time with least adult weights from the split grain. In this study we conclude that grain splitting reduces development of *C. maculatus* spp. and could be a viable option in the integrated management of this bruchid pest during storage at farmer level.

Key words: Biology, *Callosobruchus maculatus*, grain splitting

Introduction

Cowpea (*Vigna unguiculata* (L.) is an important source of protein and staple food in Uganda. It also plays an important role in ensuring food security, soil improvement through nitrogen fixation and as green manure. Cowpea is particularly known to contain 24.8% protein and as much as 63.6% carbohydrates (Davis *et al.*, 1991). FAO estimates that 3.3 million tonnes of cowpea dry grains were produced worldwide by 2000. Total area grown to cowpea was 9.8 million hectares, about 9.3 million hectares of these in West Africa.

Traditionally in East Africa, cowpea is grown on small farms, often intercropped with cereals such as millet and sorghum. In Uganda, the highest cowpea producers found in Eastern Uganda, farmers depend on the legume as food and green vegetable. Cowpea production is mainly done at small holder level and is usually on small holdings of less than an acre. It is consumed with other staple foods as food grain and as a vegetable especially at small holder level in Uganda.

Insect pests however, cause extensive damage on cowpea both in the field and during storage. Among these are pod sucking pests including pod borers, aphids, midges and thrips, and mealy bug damage, which results in extensive damage on the developing grain in the pods, thus affecting quality of the resultant grain (Davis *et al*., 1991).

In addition to these, cowpea is damaged by *Callossobruchus maculatus* (Coleoptera bruchidae). The pest is a primary storage pest on cowpea which usually starts infestation of the grain prior to harvest during field drying. The female weevil usually oviposits eggs on mature ripening cowpea pods. The developing larvae then bore into the seed prior to harvest. The larvae are the destructive stages inside the grain, causing reduction in quantity and quality of the infested seed. Then after pupation the adult emerge leaving characteristic emergence holes on the grain. The complete cycle is 20-30 days based on environmental factors.

Losses attributed to the bruchids are conservatively estimated at 5 to 15% within a storage duration of 3 to 6 months (Agona and Muyinza, 2003). Due to the unsure seed quality as a result of bruchid damage, especially, for farmers' home-saved seed, most farmers in Uganda plant up to 6 seeds per hole to safeguard against poor germination (Kabeere *et al*., 2003). High seeding rates curtails the amount of grain available for consumption.

At small holder level in Uganda, the available integrated pest management options against the pest include drying, grain solarisation and grain admixture with botanicals such as tobacco and *Tagetes minuta* leaf powders. Farmers also use synthetic pesticides including malathion 2 % dust for grain protection (Agona and Muyinza, 2003). This however, is prone to user abuse due to farmers' inability to read and apply needed dosage rates. Also most farmers lack the funds to purchase the relatively expensive pesticides and due to lack of funds to purchase the pesticides.

Previous research has shown potential in use of physical methods such as sieving and winnowing to effectively reduce grain pest infestation and enhance the value of small seeded legumes such as cowpea and pigeonpea (Agona and Muyinza, 2005). Others have shown that dehulling of cowpea enhanced its market value and shelf life significantly even at small holder level compared to the whole grain. It was postulated by some studies on cowpea that the reduced level of damage on dehulled cowpea may be due to an effect on the suitability of the grain for the developing bruchids and could thus be developed as a control measure against the bruchids. There was however, inadequate research to validate these claims and also their potential use in developing an option for cowpea weevil management a management option for cowpea weevil loss reduction.

Thus, this study aimed at evaluating the effect of altering cowpea grain size by splitting and seed coat removal by dehulling on the biology of *C. maculatus* on cowpea grain. This would provide information on the potential use of grain splitting and dehulling as a pest management option against *C. maculatus.* Thus the objective of the study was to evaluate the effect of grain splitting and de-coating on the biology and development of *C. maculatus* in cowpea

Methodology

Culture insects

Experimental insects were obtained from cultures at National Agricultural Research Laboratories (NARL), Kawanda in Uganda. Four day old insects were obtained by sieving off the adults and allowing new insects to emerge over two days. These were then allowed to mate over 2 days then used in the experiment.

Experimental grain

Freshly harvested cowpea grain was obtained from farmers. It was dried to 12% moisture content, fumigated using Phosphine in a gas chamber for 6 days and aired for 3 days.

Grain splitting

The bioassays described below were carried out to determine the effect of grain splitting on levels of oviposition and emerged adult weights at F1 *C. maculatus* generation. Two kilograms of the grain were randomly collected, from which, 200 grains were obtained and analysed to determine initial damage and initial viability of the whole grain.

Three (3) kg of clean pest free grain was soaked in cold water overnight and then dried to 12 % moisture content. It was de-hulled using a stone 'chakki', as used by small holder farmers in cowpea processing to get dhal. Another lot of grain from the split grain was also split and the testa carefully removed. The third lot of grain was left whole (un-split) but was soaked and the testa removed, grain was re-dried to 12% moisture content; and the control was the un-dehulled and un-split grain. Fifty (50) g replicates of each of these grain lots were introduced into 150ml polyethylene jars with perforated lids which allowed air in but did not allow insect escape.

Thus, the treatments included whole grain (control) whole grain without seed coat, split grain with seed coat and split grain without seed coat (dhal). Then to 50 g lots of each of the grain lots, 2 pairs of adult bruchids were introduced and the containers placed on the shelf in the laboratory in a completely randomised design. The adults were allowed to oviposit for 3 days and removed.

The number of oviposited eggs were counted on the fourth day. The grain was then allowed to stand until F1 adult emergence started. When weevils started emerging, the emerged adults were carefully removed every after 2 days and weighed until adult emergence ceased.

Data were collected on the following parameters; number of oviposited eggs at F1, number of emerged adults weevils, weight of emergent adults, number of insects per generation time and initial and final moisture content of grain.

Grain splitting and de-coating

Following the end of F1 *C. maculatus* emergence, the levels of oviposition on the grain were revalidated and then the grain re-incubated until adult emergence of F2 and F3 in the different treatments. These were similarly removed, counted and weighed until F3 generation weevils emerged and were similarly evaluated. When all the F3 had emerged the bio-assay was terminated.

Results

Grain splitting on oviposition of C. *maculatus*

There was significant variation in the mean numbers of oviposited eggs with treatment. Whole un-split grains had the highest and split grains with no testa, the least number of oviposited eggs across all the three weevil generations (Table 1).

There were however, no significant differences in mean oviposited eggs of whole grain and split grain which were without testa. Whole and undehulled grain had the largest number of oviposited eggs (Table 1). The mean number of oviposited eggs on whole grains was however not significantly different from those oviposited all the grain treatments where the testa remained for both F1 and F2 generations. At F3 more oviposited eggs were oviposited on the grain treatments with the testa compared to testa free grain (Table 1).

Grain splitting on *C. maculatus* emergence

There was significantly higher numbers of emerged adults from whole grain compared to the split and testa free

Table 1. Variation in mean oviposited eggs of *C. maculatus* with treatment

Treatment	Number of eggs		
	F1	F2	F3
Split no seed coat	9 ± 3.2 a	8.0 ± 3.0 a	1.3 ± 1a
Split with seed coat	66 ± 9.9 c	160 ± 36 ab	161 ± 5 b
Whole seeds no sc	92 ± 2 c	265 ± 65 ab	108 ± 12 b
Whole	140 ± 14 c	318 ± 81 b	258 ± 14 c
SED	2.4	8.4	0.9

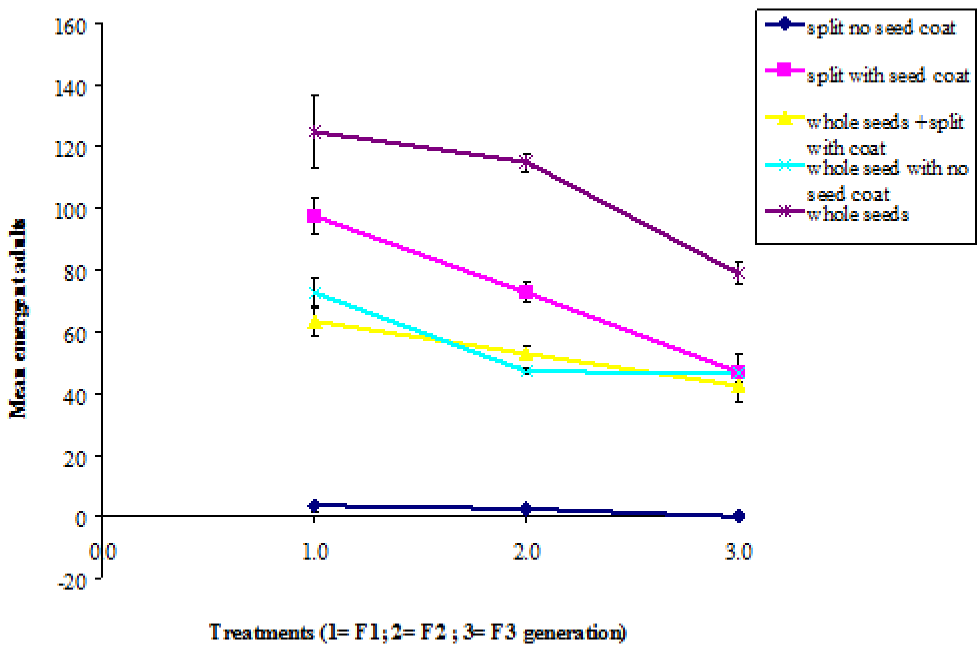

Figure 1. Variation in mean emergent *C. maculatus* adults with time among treatments.

treatments (Fig. 1). No emergent adults emerged from the split grain without testa (dhal).

Significantly (P< 0.05) fewer weevils emerged from the split and testa free grain compared to those of the other treatments. The least number of emerged adults were from the split and testa free grain at all the generations of weevil emergence and the largest number of emergencies were from the control grain (Fig. 1). For all treatments the numbers of emergent adults decreased with generation time with highest number occurring at F1, followed by F2 with the least emergence in F3 generation (Table 2).

Grain splitting and tests removal on emergent adult weevil weights

There were significant (P<0.05) differences between the mean weights of the emergent adults among treatments at F1 and F2 (Fig. 2). At F3 the least weight of weevils were observed from split grain although this was not significantly different

Table 2. Variation in mean weight of *C. maculatus* at F3 generation

Treatment	Weight of insects (mg)	Mean no of adults
Split no seed coat	0.0 + 0 a	0.0 + 0 a
Split with seed coat	0.28 ± 0 b	46.7 ± 12 b
Whole /no seed coat	0.35 ± 0 b	46.3 ± 5 b
Whole grain	0.38 ± 0.2 b	78.9 ± 7 c
SED	0.02	7.1

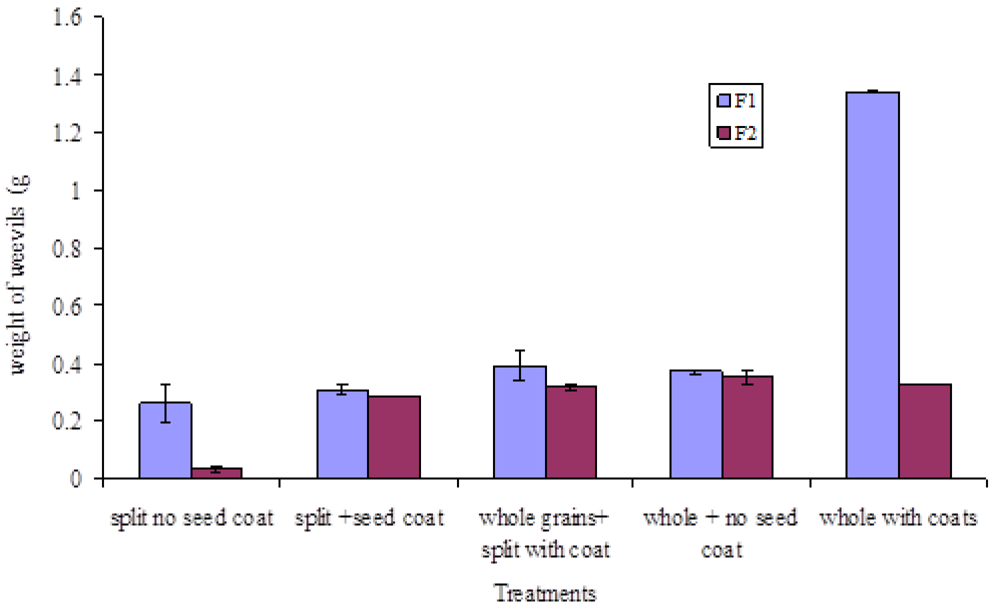

Figure 2. Variation in weight of emergent *C. maculatus* adults at F1 and F2 generation among treatments.

from those of the control (Table 2). However, whole grain without testa had adult weight higher than those of the control at F2 generation (Fig. 2), but at all generations more weevils were recovered from the whole grain compared to whole and testa free grain (Table 2).

Discussion

From the results of this study it is clear that grain splitting and testa removal affects different aspects of the biology of *C. maculatus*. From the significantly lower ($P< 0.05$) number of oviposited eggs on the split and testa free grain compared to the whole grain it is possible that the adult female weevils may have lowered preference to oviposit on grain lacking the rough characteristics of the grain testa.

Since the female weevil usually has to get a good grip of the seed during oviposition, it could be that the dehulled and testa free grain is too smooth for this purpose thus resulting in lower number of oviposited eggs. This is in agreement with previous studies which reported surface properties of grain as being important in ensuring acceptability for oviposition by herbivorous insects (Muyinza, 1999).

In addition, the low number of emerged adults from the split grain compared to the whole seeds could imply that the developing weevil stages may find less space for the developing larvae and pupa to adequately develop in the grain compared to that developing in the control.

This is further supported by the reduced weight of the emerging adults which could indicate reduced feeding and development of the pests in the reduced particles of grain compared to those developing in whole grain.

The significant reduction in adult emergencies with generation time across all the treatments observed during this study, further gives some indication that with time the developing insects obtain less favourable conditions to develop inside infested grain. This may indicate that grain physical factors play an important role in ensuring the rate of development and level of damage of the pests in cowpea. This could mean that control measures may be designed to utilise size reduction and testa removal as a method of rendering the grain less acceptable to pest attack.

Some previous studies have shown that dehulled grain has better taste and longer storability and value than unprocessed grain (Agona and Muyinza, 2005). The findings of this study showed split and testa free cowpea grain to support significantly les oviposited eggs and less emergent adults across two generations and to result in no weevil emergence at F3. This therefore could imply that by dehulling and removing the testa on cowpea, the conditions for bruchid development are made less favourable for pest presence and multiplication, which is key in insect pest management. Thus grain splitting could be easily combined with other non-chemical methods to reduce damage by *C. maculatus*.

Other studies involving combinations of pest management options have found increased efficacy when methods such as grain vegetable oiling and solarisation is used for maize weevil management (Muyinza *et al.,* 2012). In this study oiling resulted in lowered oviposition and at higher dosage zero oviposition on grain by this weevil. It is possible that grain splitting could also be combined with oiling to reduce initial oviposition and since the smaller size and absence of testa significantly lowered oviposition and number of emerged adults and adult weights. This could provide a viable combined storage pest management strategy against *C. maculatus*. Indeed grain splitting could also be used to reduce damage of other bruchids in the storage of legumes.

This study provides evidence that grain splitting and testa removal affects *C. maculatus* grain biology and could be harnessed as one of the options in management of the pest in cowpea. This would provide an affordable and user and environmentally friendly management option against this pest which can be easily used at small scale farmer level.

Further studies however, could investigate its combination potential with other non chemical weevil management options and evaluate cheaper, user-friendly and faster options of grain dehulling to ensure that larger grain volumes can be processed and protected using this technique. However, other technologies for protection of whole grain, which can be used as seed, could also be developed.

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