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Development of *Callosobruchus maculatus* Fabricius on Grain Legumes Used as Cover Crops

[1]B.N. Iloba and [2]S.B.A. Umoetok
[1]Department of Zoology, University of Benin, Benin, Edo State, Nigeria
[2]Department of Crop Science University of Calabar, Calabar, Cross River State, Nigeria

Abstract: The development of *Callosobruchus maculatus* F. on five grain legumes-*Calopogonium mucunoides* Desv., *Centrosema pubescens* Benth, *Desmodium intortium* (Mill) Urb., *Flemingo congesta* (Vestita Benth ex Barker) and *Pureria phaseoloides* (Roxb) Benth used as cover crops in Oil-Palm Plantations in Nigeria was studied under laboratory conditions. *Vigna unguiculata* L. Walp seed was used as control. Each of the six leguminous seeds was infested with five males and five females of *C. maculatus*. The treatments were arranged on a laboratory bench in Complete Randomised Design (CRD). *V. unguiculata* had the highest geometric mean diameter of 78.85 mm and 1000 seed mass of 283.33 g, followed by *C. pubscens* with 3.35 mm and 33.33±4.71 g seed mass. Eggs were laid on five of the species except *D. intortium* and the number increased with increase in seed size. The number of eggs laid was significant ($p = 0.05$) and ranged from 123.2 in *V. unguiculata*, *F. congesta* 63.5 and *C. mucunoides* 36.7, *C. pubesens* 18.2 and *P. phaseoloides* 17.2. Although development was complete in *F. congesta*, only miniature adults measuring 2.6 mm were produced while *C. maculatus* adults on *V. unguiculata* measured 3.9 mm. Development from egg to adult took 25±2 days in *V. unguiculata* and 33±1.5 days in *F. congesta* while it did not go beyond the third larval instar in *P. phaseoloides*. For management of *C. maculatus*, *C. mucunoides, C. pubescens*, *D. intortium* and *P. phaseoloides* are recommended for use as cover crops since they are not alternative host plants of *C. maculatus*.

Key words: Insect development, *C. maculatus*, oviposition, susceptible, cover crops, grain legumes

INTRODUCTION

Cowpea bruchid, *Callosobruchus maculatus* (F.) Walp is a cosmopolitan pest species of cowpea in the tropics and subtropics of the world and an important field-to-store pest of pulse crops in Africa and Asia[1-3]. Infestation usually occurs in the store, but the adult beetle can fly up to half a mile (0.8 kilometre) so that field crops within this distance are likely to be infested[2]. In this way, the bruchids can attack beans when they are still in the field[4,,5]. Murdock[6] reported that during traditional post harvest storage in Nigeria, cowpea grain stored in pods for 8 months had 50% of the grain damaged by bruchids but when stored as grain, 82% had one or more holes. The optimum conditions for development are a temperature of between 30° and 35°C and humidity of 90%. Development ceases below 22°C [7]. Host plants are primarily cowpeas, while soya bean and other legumes are secondary[2].

In the field, *C. maculatus* eggs are usually laid on the outside of pods whereas in storage, they are commonly laid directly on the grains and fastened in place with a secreted fluid. Adult *C. maculatus* mate on the first day of emergence and starts to lay eggs[8]. Oviposition period takes about three days and the eggs hatch after three days into scarabeiform larva[7]. There are four larval instars[8]. *C. maculatus* preference for legumes depends on factors such as roughness or wrinkling of the seed coat, seed size[9,10]. While[7,11] reported that *C. maculatus* has preference for seeds with smooth, well-filled coat[10] reported that under limited and multiple-choice conditions, preference was shown for seeds with wrinkled testa to smooth and rough ones and cowpea was preferred to other legumes and non-legumes[12-14] reported that many leguminous seeds contain inhibitors of proteolytic enzymes that are likely to affect the intake and digestibility of proteins in insects[14].

Leguminous crops are used as cover crops in rubber and oil-palm plantations in some countries such as Malaysia [15] and in Nigeria, where the legumes, apart from being used as cover crops, are also used for soil fertility enhancement as green manure and as forage for quality fodder production[16,17].

It was observed that legume seeds stored for planting as cover crops at the Nigerian Institute For Oil Palm Research were always attacked by the bean beetle

Corresponding Author: B.N. Iloba, Department of Zoology, University of Benin, Benin, Edo State, Nigeria

(Iloba, personal communication). This information prompted this study, to look at the development of *C. maculatus* (F.) Walp on five leguminous plants seeds used as cover crops in rubber or oil-palm plantations in Nigeria and to determine their status as alternative host plants, which are likely to serve as reservoirs for *C. maculatus*.

MATERIALS AND METHODS

The stock culture of *C. maculatus* was collected in cowpea stores from Uselu market and maintained on the local brown type of cowpea (ex Maiduguri). *C. maculatus* was reared at room temperature of 29±2°C and a relative humidity of 60±5%. The leguminous seeds i.e., *Calopogonium mucunoides* Desv. *Centrosema pubescens* Benth; *Desmodium intortium* (Mill) Urb.); *Flemingo congesta* (Vestita Benth ex Barker); *Pureria phaseoloides* (Roxb) Benth were obtained from the Nigerian Institute for Oil-Palm Research (NIFOR), Benin City, while *Vigna unguiculata* seed that was used as control was from the stock collected from Uselu market. All seeds used for the experiment were placed in an oven (Galenkamp Bs 160) at 60°C for one hour to disinfest of all stages of the insect present[18]. The seeds were then placed on the laboratory bench for 24 h to equilibrate with prevailing humidity.

Oviposition and adult emergence of *C. maculatus*: Five grams of each seed type were put separately in five glass jars (5 cm diameter and 7 cm in height) and covered with nylon mesh (1 mm^2) held in place with rubber bands. Five pairs (males and females) of newly emerged *C. maculatus* were placed in each of the jars. The sexes of *C. maculatus* were determined by examining the elytral pattern[19]. Females are usually dark coloured and possess four elytral spots, while males are pale brown and less distinctly spotted. Furthermore, males have comparatively shorter abdomen and the dorsal side of the terminal segment is sharply curved downwards and inwards. In contrast, females have comparatively longer abdomen and the dorsal side of the terminal segment is only slightly bent downwards[20]. Adult *C. maculatus* were removed after 24 h[8,21] to have progeny of uniform age. The treatments were replicated six times and arranged on a laboratory bench in a Complete Randomised Design (CRD).

Data collection: Physical assessment of the seed texture was done objectively by rubbing within the fingers and expressing the texture. Seeds mass was taken using a Top Loading Balance. Particle size was measured by calculating the seed geometric mean diameter thus: Micro Metre Screw Gauge was used to measure the length, breath and thickness of the seed. Particle seed size (Geometric mean diameter) was then calculated according to[21] using the formula:

$$\text{Geometric mean diameter} = (abc)^{1/3}$$

Where a = length of seed or major diameter
b = width of seed or intermediate diameter
c = thickness of seed or minor diameter

Oviposition: The number of eggs laid on the seeds in each glass jar was counted on the third day[8] using a dissecting microscope.

Emergence of adult *C. maculatus*: In another experiment 20 g of each variety of seeds were weighed out and placed in experimental jars (5 cm diameter and 7 cm in height). Five pairs of newly emerged *C. maculatus* were introduced into each jar covered with nylon mesh (1 mm^2) held in place with rubber bands and allowed to oviposit on the test seeds for 24 h. The treatments were replicated six times and arranged on a laboratory bench in a Complete Randomised Design (CRD). The insects were removed after 24 h to have progeny of uniform age. The seeds were then kept for three days for the eggs to hatch[7] and the seeds were dissected daily to observe the developmental stages and living conditions (life or death) of the insects. The cultures were examined for adult emergence from the first day of emergence until fourteen days when emergence ceased. The number of days taken from eggs to adult in each species was calculated. The experiment lasted 38 days when further emergence was not observed.

Statistical analysis: The recorded data were transformed using square root transformation (v X + 0.5) where applicable before subjecting them to analysis of variance and the Least Significant Difference (LSD) was used to compare the means[22].

RESULTS

The seed characteristics: The physical characteristics of seeds are shown in Table 1. There were significant ($p \leq 0.01$) differences in geometric mean diameter of the seed and 1000 seed mass. *V. unguiculata* had the highest geometric mean diameter and mass followed by *C. pubecens* and then *F. congesta*. There was no significant ($p \geq 0.05$) difference in geometric mean diameter and seed mass between *D. Intortium* and *F. congesta*. The number of eggs laid by *C. maculatus* on the different seed types was significant ($p \leq 0.05$) Table 2.

Table 1: Description of seed types

Seed type	Geometric mean diameter (mm)	1000 Seed mass (gm)	*Seed texture
V. unguiculata	7.85	283.33±4.71	Rough
C. pubescens	3.35	33.33±4.71	Smooth
C. mucunoides	2.56	21.33±1.89	Smooth
F. congesta	1.53	66.67± 2.47	Smooth
P. phaseoloides	2.56	41.67±1.25	Smooth
D. intortium	1.20	11.33±0.94	Smooth
LSD (5%)	0.34	0.02	

*Assessment was done physically, ± = Standard error of the mean

Table 2: Suvival of *C. maculatus* on six leguminous seed types

Seed type	Mean number of eggs laid	Developmental period from eggs to adult (days)	Progeny becoming adult (%)	Progeny size (mm)
V. unguiculata	123.2 (11.1)	25 (5.05)	81.4 (9.01)	3.9
F. congesta	63.5 (8.01)	32.5 (5.75)	50.7 (7.1)	2.6
C. mucunoides	36.7 (6.14)	0 (0.71)	0 (0.71)**	**
C. pubesens	18.2 (4.37)	0 (0.71)	0 (0.71) **	**
P. phaseoloides	17.2 (4.22)	17.5 (4. 24)	0 (0.71) ***	***
D. intortium	0 (0.71)*	0.0 (0.71)	0 (0.71)	*
L S. D. (5%)	0.22	0.14	0.45	

*No eggs were laid, **Eggs hatched but larva did not penetrate into the seed, ***Survived to third larval and died, Data within parenthesis are transformed using square root transformation vX + 0.5

The highest number of eggs was laid on *V. unguiculata*. This was followed by *F. congesta*, *C. mucunoides*, C. pubescens and *P. phaseoloides* in that order. No eggs were laid on *D. Intortium*. All the eggs hatched within three to four days and further development was observed only on three of the leguminous species-*V. unguiculata, F. congesta* and *P. phaseoloides*. The eggs that hatched into larvae on *C. mucunoides* and *C. pubescens* did not penetrate into the seeds but empty eggshells and dead larvae were observed on top of the seeds. Table 2 also shows the duration of eggs to adult on each species where applicable. *Vigna unguiculata* spent significantly less number of days from egg to adult than *F. congesta* while development of *P. phaseoloides* terminated at the third larval instars.

DISCUSSION

The inability of *C. maculatus* to lay eggs on *D. intortium* that had the smallest seed size (1.20 mm) and seed weight of 11.33±0.94 can be accounted for partially by Nwanze *et al*.[9] who reported that less eggs were laid on small seeds than on large ones. Under such condition the bruchid could not grip and sit properly on the seeds in order to deposit the eggs successfully on the surface. It is also possible that seed size could have contributed to the miniature adult obtained from *F. congesta*. On the other hand egg laying and further development did not completely relate to the geometric mean diameter but also to seed the mass. *P. phasioloides* that weighed 41.67 g had only 17.2 eggs and although the eggs hatched into larvae they did not proceed to adult progeny. This result has confirmed report by[23] that ovipositional preference does not necessarily imply suitability for development. The geometric mean diameter of *V. unguiculata* was 7.85 mm, while its mass was 283.33±4.71 g. In this study, the particle size (geometric mean diameter) could conveniently accommodate the insect whose size was 3.9±0.06 mm while the high mass had enough reserve food content to support the insect for growth from egg to adult stage. Logically it would be difficult for the seed of *F. congesta* that had 1.56 mm geometric mean diameter to conveniently accommodate a normal sized *C. maculatus*, which according to[7] is 2.5 mm-3.5 mm in length. The inability of *C. maculatus* to develop in the respective seeds cannot only be attributed to seed size (geometric mean diameter) or mass. Although the seed mass could have contributed immensely to the successful development of *C. maculatus* to adult other factors such as the type of antibiosis or growth inhibitors present, which according to[14], abound among the Leguminosae could have played major function and affected *F. congest*. These growth inhibitors can stop larval development or even inhibit the functioning of proteolytic enzymes in the insect[14]. Much of the resistance developed in the leguminous seeds to *C. maculatus* according to[12,13] could be due to the presence of a proteinaceous inhibitor of the digestive ∝- amylase of the bruchid. Such inhibitors may be present in *C. pubescens* and *C. mucunoides* and much reduced in *P. phaseoloides* but absent or if present, in an insignificant quantity in *V. unguiculata* and *F. congesta* where over 81.4% and 50.7% percent of the eggs laid on the two seed types developed into adults. This is also clearly illustrated by the poor performance of larvae in *P. phaseoloides* leading to total mortality at the third larval instar. This again is further elucidated by the less number of days taken by *V. unguiculata* from egg to adult stage, which was more in *F. congesta* and longest in *P. phaseoloides* to even get to the third larval stage.

CONCLUSION

C. maculatus developed into healthy adults only in *V. unguiculata and F. congesta*. *C. mucuniodes*, *C. pubescens* and *D. intortium* and *P. phaseoloides* are, therefore, recommended as cover crops if the population of *C. maculatus* in the ecosystem and in store is to be checked.

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