

DEVELOPMENT OF CALLOSOBRUCHUS MACULATUS (F.) ON SOME PULSES IN BOTSWANA

J. ALLOTEY¹, M. SEAGO, N. MAKATE AND M. MPHU

Department of Biological Sciences, University of Botswana, Private Bag 0022, Gaborone, Botswana.

ABSTRACT: Some aspects of the biology of *Callosobruchus maculatus* (F.) were studied on seven pulses viz., Cowpea (5 varieties), Bambara groundnut, Mung bean, Pigeonpea, Lablab, Soya bean and Kidney bean, under ambient laboratory conditions (temperature range: 24 - 28°C and 61 - 77% R.H.). Oviposition by *C. maculatus* on the above pulses ranged from a mean of 41.10 ± 1.98 to 66.50 ± 4.85 eggs per female. The differences in the number of eggs laid per female were significant ($F = 4.2$, $P < 0.001$). With the exception of Lablab, the number of eggs hatched from oviposition by *C. maculatus* on the pulses was more than 90%. Emergence of *C. maculatus* from the pulses ranged from 18.83% on Lablab to 76.44% on Black eye cowpea. The differences in emergence numbers were found to be significant ($F = 8.7$, $P < 0.001$). The mean developmental period of *C. maculatus* on the pulses ranged from 30.17 ± 0.01 to 36.86 ± 0.18 days, while the longevity of the adults ranged from 1.0 - 14.0 days. Sex ratios of emerged *C. maculatus* were observed to be approximately 1♀:1♂ on all the pulses.

Key words: *Callosobruchus maculatus*, pulses, development, oviposition, longevity

INTRODUCTION

Pulses, such as cowpeas *Vigna unguiculata* (L.) Walp, beans *Phaseolus vulgaris* (L.), soya beans *Glycine max* (L.) Merr, bambara groundnut *Voandzeia subterranea* (L.) Thou, mung bean *Vigna radiata*, lablab *Dolichos lablab* and pigeonpea *Cajanus cajan* (L.) Millsp; serve as important sources of dietary protein for many people in the tropics, especially Africa including Botswana (ALLOTEY, 2003; MISHILI *et al.*, 2009; ALLOTEY *et al.*, 2010). Due to their high protein contents, pulses are of great dietary importance (FAO, 1989).

Pulses suffer heavy quantitative and qualitative losses from the attack of *Callosobruchus* species during storage. Beginning from the field and during storage, pulses are attacked by *Callosobruchus maculatus* (F.), which is a major storage pest of legumes in the tropics and subtropics (OGUNWOLU and IDOWU, 1994; OKONKWO and OKOYE, 1996; RAJA *et al.*, 2000; AJAYI and LALE, 2001; GIGA, 2001; TAPONDJOU *et al.*, 2002; PARK *et al.*, 2003; ALLOTEY and OYEWO, 2004). The larvae bore into the pulse grains which become unsuitable for human consumption. Severe infestation of cowpeas by *C. maculatus* can result in losses in storage, ranging from 50 to 90% annually throughout Africa (IITA, 1989). These losses can be reduced or prevented by using appropriate control measures (LALE and MUSTAPHA, 2000; SUBRAMANYAM and HAGSTRUM, 2000; ARTHUR and PHILLIPS, 2002; MAINA and LALE, 2004). However this can only be achieved by sound knowledge of the biology of this important pest species under prevailing environmental conditions.

In Botswana, there has been growing awareness of the damage caused by *C. maculatus* to many of the pulses that are utilised locally. It is envisaged that a thorough understanding of the biology of this important pest under prevailing conditions in Botswana, could lead to effective control of this destructive pest. The objective of the present study was to provide scientific information on the the biology of *C. maculatus* on some pulses under ambient laboratory conditions in Botswana.

MATERIALS AND METHODS

Ten cultures of *C. maculatus* were established in Kilner jars (16 cm deep × 9 cm diam.). Each culture contained 400 g black-eyed cowpeas with 50 randomly selected adults of *C. maculatus*. The cowpeas were obtained from the main mall of Gaborone, while the insects were obtained from laboratory cultures of the Insectary of the Department of Biological Sciences, University of Botswana, Gaborone.

All equipment used in handling insects was dry-heat treated at 100°C for at least 3 h as a routine measure to prevent disease or cross infestation. The pulses were dry-heat treated for 2 hours in an oven at 80°C, except for soya bean that was treated at 60°C to prevent oil extraction from the seeds; before experimentation. The procedures for maintaining cultures were similar to those described by ALLOTEY and GOSWAMI (1992). All cultures and experimental jars were maintained at room temperature (range 24 - 28°C) and 61 - 77% R.H. with alternating 12-h light and 12-h dark cycle.

The experimental pulses utilized were 5 local varieties of cowpeas (*Vigna* spp.): Black eye, BOO5-C, Tswana, Local landrace B and B319; Bambara groundnut (*Voandzeia subterranea*); Soya bean (*Glycine max*); Pigeonpea (*Cajanus cajan*); Kidney bean (*Phaseolus vulgaris*); Lablab (*Dolichos lablab*) and Mung bean (*Vigna radiata*). The pulses were obtained from the Agriculture Research Centre, Sebele, and locally from the market.

Ten replicates were set up per pulse in glass jars (7.5 cm. × 3.0 cm diam.), and newly emerged adults (less than 24 hrs old) of *C. maculatus* were introduced at a ratio of 1male: 1female per 30 seeds per jar. Each jar was covered with a muslin cloth secured in place by a rubber band to allow for aeration. After 14 days, dead adults were removed with forceps and the eggs laid on the seeds in each jar were counted. The number of hatched eggs was also recorded. Adult weevils emerging at the end of the developmental period were counted on a daily basis until there was no further emergence. The sex ratio of emerged adults was also determined.

To determine the longevity of the weevils for each pulse variety, 30 adults were randomly selected from those emerging each day and kept in jars (7.5cm × 3.0cm diam.). Five replicates were set per pulse. The jars were covered with muslin cloths held in place with rubber bands and mortality of adults was recorded on a daily basis; and the longevity determined when all the weevils were dead.

RESULTS AND DISCUSSION

The results from the experimental studies were analysed using the analysis of variance (1 way ANOVA). Oviposition and egg hatchability of *C. maculatus* on the six pulses, including five varieties of cowpeas have been given in Table 1. There was a significant difference ($F = 4.2$, $P < 0.001$, Table-4a) between the different pulses; the highest oviposition was recorded on the cowpea varieties, Local landrace B and B319 ($x = 66.50$) and lowest on cowpea variety Tswana ($x = 41.10$). Oviposition by *C. maculatus* on seeds has been reported to be affected by plumpness, wrinkling of seed coat and perhaps by the size and hardness as well as their odours (NWANZE and HORBER, 1976; ALLOTEY and DANKWAH, 1995). Many factors affect the number of eggs laid by females. For example, populations from different areas vary in their fecundity on the same number, species and cultivar host species (AJAYI and LALE, 2001). The pulse seeds used in the present study all had smooth coats but their sizes differed relatively. Size apparently had no effect on oviposition by *C. maculatus* and so the observed differences could have been due to surface odours of the seeds, which might attract or repel the weevils (NWANZE and HORBER, 1976; ALLOTEY and OYEWO, 2004). Egg hatchability of *C. maculatus* was more than 90% on all the pulses except on Lablab, where the hatchability was 89.2%. There was a significant difference ($F = 4.4$, $P < 0.001$, Table-4b) in the number of eggs hatched on the different legumes.

Table-1: Oviposition and hatchability of *C. maculatus* reared (1♀:1♂ / 30 seeds/replicate) on different pulses

Pulse variety (n = 10)	Oviposition(x± SE)	Hatchability(x ± SE)	Hatchability (%)
Cowpea variety			
Local landrace B	66.50 ± 4.85d (36 – 96)*	65.10 ± 4.81c (35 – 94)	97.9
B005-C	56.30 ± 7.05c (19 – 90)	55.80 ± 6.95b (19 – 89)	99.1
Black eye	44.10 ± 3.31a (28 – 60)	43.30 ± 3.41a (26 – 56)	98.2
B319	66.50 ± 4.18d (37 – 91)	64.50 ± 4.05c (37 – 88)	96.9
Tswana	41.10 ± 1.98a (31 – 50)	40.00 ± 1.94a (30 – 49)	97.3
Bambara groundnut	47.50 ± 3.42b (32 – 64)	47.40 ± 3.37a (32 – 63)	94.9
Mung bean	47.00 ± 4.19b (28 – 69)	45.30 ± 4.40a (28 – 63)	96.4
Lablab	50.00 ± 3.57 b (37 – 69)	44.60 ± 3.37a (31 – 60)	89.2
Pigeon pea	46.20 ± 3.16b (26 – 61)	44.20 ± 2.86a (25 – 56)	95.7
Soya bean	46.10 ± 2.85b (35 – 58)	45.60 ± 2.79a (35 – 58)	98.9
Kidney bean	46.60 ± 4.70 b (25 – 63)	45.80 ± 4.53a (24 – 62)	98.3

n = number of replicates, * Ranges are given in parentheses. Means within data column followed by the same superscripts are not significantly different ($p > 0.005$, by the Student-Newman-keuls method for multiple comparison).

Emergence of *C. maculatus* from the legumes was highest on cowpea variety Black eye (76.44%) and lowest on Lablab (18.83%) (Table-2). Emergence of *C. maculatus* from the different pulses can be summarised in decreasing order as follows: Black eye (76.44%) > Mung bean (70.64%) > Tswana (67.75%) > B005-C (63.44%) > Bambara groundnut (61.42%) > Pigeonpea (55.56%) > B319 (48.68%) > Local landrace B (48.16%) > Lablab (18.83%) (Table-2). There was no emergence from Soya bean and Kidney bean. This failure

to emerge was also recorded by GOKHALE (1973). In general, there was a significant difference ($F = 8.7$, $P < 0.001$, Table-4c) in the emergence of adult weevils on the different pulses. Peak emergence of *C. maculatus* was reached on day two on most pulses. The peak of daily emergence of *C. maculatus* on each pulse is characteristic of the insects' ability to utilise the seeds (ALLOTEY and OYEWO, 2004), and early peak indicates that particular variety is more susceptible to bruchid attack than others. Many pulses contain certain inhibitors that affect the digestibility of proteins by the bruchids, and it is the concentration of these inhibitors that determine the susceptibility or resistance of the legume (DONGRE *et al.*, 1996; IGNACIMUTHU *et al.*, 2000; SRINIVASAN and DURAIRAJ, 2007).

The shortest developmental period of *C. maculatus* was recorded on Black eye as 30.17 ± 0.01 days (Table-2). Longer developmental periods of *C. maculatus* on Pigeon pea and Lablab indicate that either the seeds were not suitable for larval development or the insects could not utilise food material efficiently (MORENO *et al.*, 2000). Hence, longer developmental periods can be associated with seed resistance while shorter developmental periods can be linked with susceptibility. On the basis of this observation, the order of susceptibility of the legumes to *C. maculatus* is as follows: Black eye (34.25) > Mung bean (32.53) > Tswana (33.44) > B319 (34.20) > Local landrace B (34.25) > B005-C (34.51) > Bambara groundnut (35.59) > Lablab (36.33) > Pigeonpea (36.86). GOKHALE (1973) recorded a developmental period of *C. maculatus* on Pigeonpea as 25.7 days at $30^{\circ}\text{C} \pm 1^{\circ}\text{C}$ and 55 - 65% R.H. The differences observed in these developmental periods of *C. maculatus* could be attributed to differences in environmental conditions.

Adult *C. maculatus* does not feed but rely on food reserves to prolong its life span (HAINES, 1991). In the present study, the longevities of the adults reared on the different pulses were approximately the same, ranging from one to fourteen days. The sex ratios of emerged *C. maculatus* from paired adults (1♀:1♂) reared on the different legumes were approximately 1:1 in all the pulses, with the exception of the sex ratio from Tswana which was 1:1.2 (Table-3).

From the present study on the development of *C. maculatus* on the various pulses, it can be concluded that; there were significant differences ($F = 4.2$, $P < 0.001$) in the oviposition by *C. maculatus* on the various pulse varieties. Egg hatchability was more than 90% on all the pulses except lablab (89.2%). The mean developmental periods were: 34.25 days on local landrace B; 34.51 days on B005-C; 30.17 days on black eye; 34.20 days on B319; 33.44 days on Tswana; 35.59 days on bambara groundnut; 32.53 days on mung bean; 36.33 days on lablab and 36.86 days on pigeonpea. There were significant differences in the emergence of *C. maculatus* on the pulses ($F = 8.7$, $P < 0.001$). However, there was no emergence on soya bean and kidney bean. Emergence ranged from 18.83% on lablab to 76.44% on black eye. Longevity of adult *C. maculatus* from all the legumes ranged from one to fourteen days. The sex ratios of emerged adults reared on the legumes were approximately 1:1 in all the legumes.

Table- 2: Emergence and development of *C. maculatus* reared (1♀:1♂ / 30 seeds / replicate) on selected pulses.

Pulse variety n = 10	Adult emergence (x ± SE)	% Emergence (x ± SE)	Dev. Period (days)
Cowpea variety			
Local landrace B	32.00 ± 3.11c(17 - 52)	48.16	34.25 ± 0.11 (31 - 40)
B005-C	35.40 ± 3.86 c (18 - 55)	63.44	34.51 ± 0.13 (30 - 41)
Black eye	33.10 ± 2.91c (17 - 49)	76.44	30.17 ± 0.01 (28 - 37)
B319	31.40 ± 2.97c (12 - 45)	48.68	34.20 ± 0.17 (29 - 42)
Tswana	27.10 ± 4.30b(14 - 34)	67.75	33.44 ± 0.14 (30 - 39)
Bambara groundnut	27.70 ± 2.55b (11 - 39)	61.42	35.59 ± 0.11 (34 - 41)
Mung bean	32.00 ± 2.68c (19 - 45)	70.64	32.53 ± 0.13 (30 - 39)
Lablab	8.40 ± 1.18a (5 - 13)	18.83	36.33 ± 0.32 (31 - 41)
Pigeon pea	24.6 ± 2.00 b (16 - 32)	55.66	36.86 ± 0.18 (33 - 44)
Soya bean	0.00	0.00	0.00
Kidney bean	0.00	0.00	0.00

n = number of replicates; * Ranges are given in parentheses. Means within data column followed by the same superscripts are not significantly different (p>0.005, by the Student-Newman-keuls method for multiple comparison).

Table- 3: Sex ratios of emerged *C. maculatus* reared (1♀:1 ♂ / 30 seeds / replicate) on different pulses

Sex	Pigeon pea	Local land race	Black Beye	B005C	B319	Tswana	Bambara	Mung	Labl ab
(♂)	156	158	178	158	123	141	158	42	124
(♀)	160	173	176	156	148	136	162	42	122
Sex- Ratio (♂/♀)	1.0:1.0	1.0:1.1	1.0:1.0	1.0:1.0	1.0: 1.2	1.0:1.0	1.0:1.0	1.0: 1.0	1.0: 1.0

* = Total number of emerged adults, (♂) = male, (♀) =female

Table-4: Analysis of variance (ANOVA - 1 way) for the various experiments (p < 0.001)

Table- 4a:ANOVA for Oviposition

Sources of variation	df	ss	ms	F	Comments
Between treatment	10	7489.964	748.996	4.2	S
Error	90	15967.309	177.415		
Total	109	24368.264			

The differences in the mean values among the treatment groups are greater than would be expected by chance; there is a statistically significant difference, S = significant.

Table- 4b: ANOVA for Hatchability

Sources of variation	df	ss	ms	F	Comments
Between treatment	10	7522.818	752.282	4.4	S
Error	90	15219.000	169.100		
Total	109	23756.918			

The differences in the mean values among the treatment groups are greater than would be expected by chance; there is a statistically significant difference. S = significant

Table- 4c: ANOVA for Emergence

Sources of variation	df	ss	ms	F	Comments
Between treatment	8	5178.556	647.319	8.7	S
Error	72	5362.778	74.483		
Total	89	10984.456			

The differences in the mean values among the treatment groups are greater than would be expected by chance; there is a statistically significant difference.

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