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Development and damage assessment of the storage beetle, *Callosobruchus maculatus* (Thanjavur and Coimbatore strain) under normal and controlled conditions

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ABSTRACT

The cowpea weevil (*Callosobruchus maculatus* Fabricius) is one of the most important storage insect pests of grain legumes and comprises geographically distinct strains. The biological parameters of South Indian *C. maculatus* strains originated from two different agro-ecological areas of Tamil Nadu, India, were examined on commercially grown Indian variety of greengram [*Vigna radiata* (L.) R. Wilczek] and blackgram [*V. mungo* (L.) Hepper] seeds under two distinct laboratory conditions. The number of eggs oviposited by *C. maculatus* differed significantly with strain, seed species and temperature. The oviposition per female by Thanjavur strain recorded 113.6 and 64.2 eggs/50 greengram seeds under controlled and normal conditions compared to 51.2 and 43.4 eggs/50 blackgram seeds. On the other hand, Coimbatore strain showed reduced fecundity on both the seed species irrespective of the studied temperatures. Assessment of damage was based on per cent seed damage with 1–9 score (one exit hole per seed for South Indian strain) rather than the generally followed Howe's Index of Suitability (IS). Based on the comparative performance of two strains of bruchid populations, Thanjavur strain showed superiority to Coimbatore strain in terms of all the developmental parameters and damage potential with significant variations. The effect of host change on bruchid development was also observed. The present study revealed that seed loss due to bruchid damage during grain storage may differ with respect to seed crop, insect population source and prevailing climatic and (or) storage conditions. Hence this research will give a brief idea on the choice of storage conditions at different regions which will serve as one of the alternatives to reduce bruchid damage at least to some extent.

Key words: *Callosobruchus maculatus,* Coimbatore strain, Index of suitability, Oviposition, Seed damage, Thanjavur strain

The storage beetle, *Callosobruchus maculatus* (Fabricius), which is one of the most serious stored insect pests (Southgate, 1979), causes substantial damage in different regions of the world and threatens the safety of stored legumes (Credland, 1994). The adults start laying eggs on the pods in field and secondary infestation continues in the storehouse after the seeds are harvested. Eggs are laid continuously on the stored seed lot and several generations can be completed during storage within a year (Southgate, 1979). Damage is done on the seeds causing weight loss, decreased germination potential and reduction in commercial value thus hindering consumption (Caswell, 1976). Rapid build up of the insect population occurs in storage and seed damage (seeds with emergence holes) can rise up to 99% within 6 months (Seck et al., 1991). Bruchid larvae bore into the seeds and feed on the cotyledons where they pupate and emerges reproductively matured. Insect feeding also reduces the quality and quantity of the seeds. Sometimes, the seeds may be almost completely

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hollowed out by feeding activities of the larvae, and characteristic emergence holes with round flap of the seed coat are evident after the adult leave the seeds.

The family Bruchidae consists of several genera of seed beetles. Of the seed feeding genus *Callosobruchus*, *C. maculatus* is the most widespread species (Southgate, 1979; Howe et al., 1964). Several researchers have studied its biology, development on various legume seeds and got different results due to geographical and climatic differences. The development of a sound pest management programme must be based on a thorough understanding of the biology and ecology of the pest and will require such population studies as developmental rates, fecundity, per cent survivability and survivorship as well as temperature regime during storage period (Armitage et al., 1994). Understanding the effect of host, temperature and relative humidity on the reproduction and developmental biology of *C. maculatus* is of paramount importance for determining its geographical distribution, number of generations per year both in field and storage conditions, and also to make inferences on the possible effects of climate change on its population growth (Soares et al., 2014). Hence considering these above points, some biological research were carried out on the life parameters of *C. maculatus,* which is the most dominant species in South India (Raina, 1970) including Tamil Nadu. Also, the present study was carried out with the objective to revise the information regarding realized reproductive potential and development of *C. maculatus* populations from Thanjavur and Coimbatore district of Tamil Nadu on two important pulse crops of India, viz. mungbean or greengram [*Vigna radiata* (L.) R. Wilczek] and urdbean or blackgram [*V. mungo* (L.) Hepper] under normal and controlled laboratory conditions.The study also investigated the feasibility of using mungbean as a more suitable host for rapid mass-culturing and long-term maintenance of bruchid generations for any behavioural, physiological, management and resistance studies under laboratory conditions.

MATERIALS AND METHODS

The two insect populations used in the present experiment differed in their geographical locations. One population was initially procured from Indian Institute of Crop Processing Technology (IICPT), Thanjavur (T-strain), which was being reared on hybrid greengram seeds and the other from Department of Seed Science and Technology, Tamil Nadu Agricultural University (TNAU), Coimbatore (C-strain), reared on blackgram. Prior to the study, both the insect strains were cultured on susceptible greengram cv. ‘Co 6’ for one generation each (inside incubator at 30ºC and 70% r.h.) following Strong et al. (1968), so as to eliminate any short-term changes in behaviour associated with the change of host variety from that used for culturing to that being tested (Dobie, 1974). Pesticide-free greengram and blackgram seeds (cv. ‘Co 6’ and ‘T 9’)

Table 1 Reproductive potential and development of two bruchid strains on *Vigna radiata* and *V. mungo* under two laboratory conditions

	Crop	Eggs laid/50 seeds	Eggs hatched	% hatching	Hollowed seed	% damage	MDP (days)	% survival	Howe’s index
				Thanjavur strain					
UC	GG	64.2± 5.60^{d}	53.2^{d}	82.87^{cd}	40.2^{e}	80.4^{e}	26.96^{b}	64.31^{b}	0.067^{c}
	BG	43.4± 0.75^{bc}	33.2^{b}	76.67^{abc}	31.8^{c}	63.6^{c}	31.19^{e}	73.33^{b}	0.060^{b}
CC	GG	113.6± 3.93^{e}	106^{f}	93.39^{e}	45.6^{f}	91.2^{f}	24.88^{a}	40.34^{a}	0.064^{c}
	BG	51.2± 0.66^{c}	40.8^{c}	79.80^{bcd}	36.2^{d}	72.4^{d}	30.73^{d}	70.77^{b}	0.060^{b}
				Coimbatore strain					
UC	GG	52.2 ±5.01^{c}	41.4^{c}	80.29^{bcd}	34.6^{d}	69.2^{d}	30.74^{d}	68.26^{b}	0.059^{b}
	BG	29.6 ± 0.68^{a}	21.2^{a}	71.73^{a}	26.2^{a}	52.4^{a}	35.38^{g}	88.77^{c}	0.055^{a}
CC	GG	108.2 ± 2.65^{e}	90.8^{e}	83.99^{d}	39.2^{e}	78.4^{e}	28.88^{c}	36.32^{a}	0.054^{a}
	BG	35.4 ± 1.33^{a}	26.2^{a}	74.44^{ab}	29.4^{b}	58.8^{b}	32.96^{f}	83.29^{c}	0.058^{b}
	Mean	62.23 ± 2.58	51.6	80.39	35.4	70.8	30.22	65.7	0.059
	SEd	4.53	3.25	3.28	1.17	2.34	0.19	4.66	0.001
	CD (*P*=0.05)	9.23	6.62	6.68	2.38	4.77	0.39	9.49	0.002

GG, Greengram; BG, blackgram; CC, controlled condition (incubator –30ºC, 70% r.h.); UCC, uncontrolled condition (lab. - room temperature); MDP, mean developmental period; per cent survival = per cent adult emergence; Mean of 5 replications (mean±SE); Means with different letters in the same column are significantly different at 5% level by LSD were used for the present study. One pair each of the two insect strains were released per 50 seeds of both the crops, laid out in a completely randomized design (CRD), replicated five times. One set was kept inside the incubator (controlled condition; 30ºC, 70%) and the other was kept at room temperature (uncontrolled). Data on the prevailing room temperature and relative humidity during the study period were obtained from the Department of Agricultural Meteorology, TNAU. Observations on the number of eggs laid, eggs hatched (successful larval entry indicated by the presence of white frass and a small dot when eggs are scrapped off), number of adults emerged or hollowed seeds (one adult per seed for South Indian strain) were made to calculate various life parameters like hatching per cent, seed damage per cent, mean developmental period (MDP in days), per cent survivability, index of suitability (Howe's index) and seed weight loss per cent (%) according to Soumia et al. (2015). Scoring (1–0%, completely resistant; 3–1 to 9%, resistant; 5–10 to 69%, moderately susceptible; 7–70 to 99%, susceptible and 9–100%, completely susceptible) based on seed damage per cent was done following the criteria given by Weigand and Tahan (1997). The number of adults emerged were recorded daily to determine the duration of development from egg to adult. Newly emerged adults were separated by gender and sex ratio was determined as: RS = number of females/(number of males + females).

Data pertaining to various parameters were subjected to analysis of variance (ANOVA) to determine any significant differences between the means at 5% by LSD. Correlation study was performed to describe the relationship between all the biological

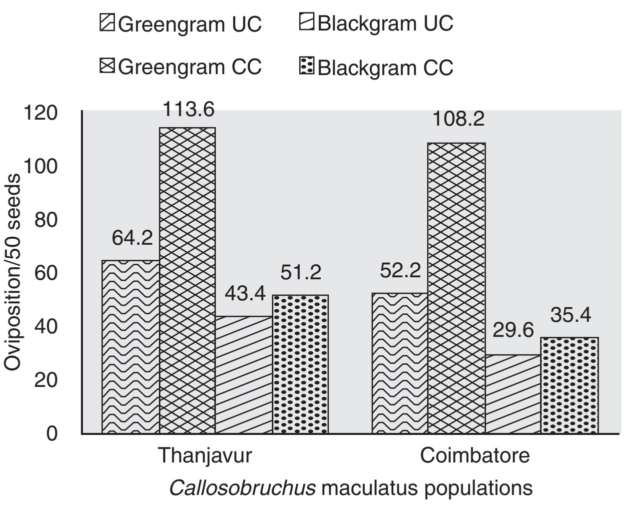

Fig. 1. Variation in the number of eggs laid by two South Indian populations of *Callosobruchus maculatus* (UC, Uncontrolled; CC, Controlled condition)

Table 2 Adult emergence (number of males and females) and sex ratio (RS)

	Crop	Adults#/ 50 seeds	Males	Females	Sex ratio
		Thanjavur strain			
UC	GG	40.2 ± 0.58^{e}	19.6^{d}	20.6^{d}	0.51^{ns}
	BG	31.8 ± 0.58^{c}	15.2^{a}	16.2^{b}	0.52^{ns}
CC	GG	45.6 ± 0.93^{f}	23.0^{e}	22.4^{e}	0.49^{ns}
	BG	36.2 ± 0.73^{d}	17.4^{c}	18.8^{c}	0.52^{ns}
		Coimbatore strain			
UC	GG	34.6 ± 1.47^{d}	16.0^{c}	18.6^{c}	0.54^{ns}
	BG	26.2 ± 0.73^{a}	13.2^{a}	13.0^{a}	0.50^{ns}
CC	GG	39.2 ± 0.49^{e}	19.4^{d}	19.8^{cd}	0.50^{ns}
	BG	29.4 ± 0.68^{b}	14.6^{ab}	14.8^{b}	0.50^{ns}
	Mean	35.4 ± 0.77	17.3	18.03	0.51^{ns}
	SEd	1.17	0.88	0.86	0.02
	CD (P =0.05)	2.38	1.79	1.75	0.03

One adult emergence per seed for South Indian strain of *C. maculatus* (Mitchell, 1991) unlike few holes for *C. chinensis*; mean of 5 replications (mean ± SE); GG, greengram; BG, blackgram; CC, controlled; UCC, uncontrolled; Different letters in the same column are significantly different at 5% level by LSD; ns, non-significant

parameters recorded and measured for *C. maculatus* development using SPSS software.

RESULTS AND DISCUSSION

The reproductive activity of two *C. maculatus* strains (Thanjavur, T-strain and Coimbatore, C-strain), mean developmental period (in days) of their offsprings and their damage potential on two important pulse crops were studied (Table 1). The number of egg oviposition significantly varied with seed species and strain under two distinct conditions. Mookherjee and Chawla (1964) reported that adult females produced an average of 25–61 eggs throughout their life span, and the highest number of eggs/day/female was achieved at 30ºC considering the oviposition period. However, *C. maculatus* can lay up to 115 eggs/female in its life span compared to 65 eggs by *C. chinensis* female (CABI, 2014). In the present study, fecundity per female was also recorded highest at 30ºC (controlled) for T-strain with 113.6 ± 3.93 eggs/50 greengram seeds, whereas lowest oviposition was observed for C-strain 29.6 ± 0.68 eggs/50 blackgram seeds under normal condition (Table 1 and Fig.1). These findings are also in corroboration with the conclusion of Giga and Smith (1983) suggesting similar pattern in oviposition where these variations reflect differences in insect geographical populations. *C. maculatus* can be easily raised in laboratories and has been used as a model organism in a number of ecological studies. Nevertheless, its development is strongly influenced by temperature and humidity (Xu et al., 1999), host substrate and population source (strain or biotype; Messina and Slade, 1999) which supported the present findings. Moreover, the results of the current experiment also correspond to an interesting extreme case of the differences in biological parameters noted among three *C. maculatus* populations studied by Dick and Credland (1984).

The number of adults emerging from the two host seeds were estimated for both the strains studied (Table 2). Cumulative daily emergence of *C. maculatus* adults was faster at the controlled temperature with a maximum mean of 45.6 ± 0.93 exit holes from 50 seeds; one adult emergence hole per seed for South Indian *C. maculatus* strain as described by Mitchell (1991) and most adults emerging before day 25 from greengram seeds. The larvae of South Indian populations of *C. macultaus* are highly competitive and it is rare for more than a single adult to emerge from any seed (Mitchell, 1991). In the present experiment, the mean emergence of females was slightly higher than males (18.03♀ over 17.3♂), but was significantly at par in all the experiments conducted which is also supported by the non-significant results for sex ratio (Table 2). Moreover, Fox et al. (2006) studied the temperature and host species effects on the nuptial gift size in *C. maculatus* and reported that although the sex ratio of emerging adults are significantly female biased, the effect of temperature on sex ratio is very small which holds true for the current results. This biased emergence trend may be explained with the assumption that female sex determination might have taken place during larval development inside the seed, since females play a more important role (egg laying/offspring production) for their continuous perpetuation in store. Earlier studies reported different results for other bruchid species such as *C. chinensis* and *C. analis* (Yadav and Pant, 1974; Soumia et al., 2015). Moreover, Howe (1971) showed that *C. maculatus* populations showed fastest development around 31°C, which approximates our findings for the Thanjavur population of *C. maculatus*. On the other hand, Deng et al. (2000) reported that extremely high temperature of about 35°C caused a significant decrease in development rate of combined immature stages in *C. chinensis*. With regard to uncontrolled conditions (< 30°C, room temperature), a lower developmental threshold was observed for both T-strain and C-strain. The genetic cause of this difference in the natural populations from Thanjavur and Coimbatore is unknown but may partly be attributed to adaptation on a particular host, greengram in this case. The range of room temperature and relative humidity during the study period was 25.4–31.6°C and 83.20–100% r.h. (*Source*: Department of Agricultural Meteorology, TNAU during Oct. – Dec. 2014).

Table 3 Percentage seed weight loss per cent (%) due to bruchid larval development

	Crop	Initial weight (g) 50 seeds	Final weight 50 seeds	Weight loss (%)
		Thanjavur strain		
UC	GG	4.06	2.37^{c}	41.77 ± 1.08^{c}
	BG	4.43	2.71^{de}	38.81 ± 0.11^{b}
CC	GG	4.04	1.96^{a}	51.51 ± 1.08^{e}
	BG	4.34	2.61^{d}	39.85 ± 0.30^{c}
		Coimbatore strain		
UC	GG	4.08	2.46^{c}	39.61 ± 1.45^{bc}
	BG	4.43	2.82^{e}	36.30 ± 0.38^{a}
CC	GG	4.06	2.17^{b}	46.58 ± 0.84^{d}
	BG	4.34	2.70^{d}	37.82 ± 0.50^{ab}
	Mean	4.22	2.48	41.53 ± 0.72
	SEd	0.05	0.05	1.19
	CD	0.10	0.11	2.42

Mean of 5 replications (mean ± SE); GG, greengram; BG, blackgram; CC, controlled; UCC, uncontrolled; Different letters in the same column are significantly different at 5% level by LSD

The cosmopolitan distribution of *C. maculatus* and its successful establishment in different regions of the world was facilitated by increased anthropogenic domestication and diffusion of grain legumes (Oliveira et al., 2013). Information regarding the developmental biology and feeding behaviour of *C. maculatus* is needed for the design of better control strategies and predictive models of population dynamics. Since bruchids possess a short generation time and high reproductive potential in warm climates, it can produce several generations per year under favourable conditions (Soares et al., 2014). The controlled condition (30°C and 70% r.h.) of the incubator in this study might have served more favourable for

Table 4 Overall correlation between different developmental parameters of two *C. maculatus* strains on *V. radiata* and *V. mungo* under controlled and uncontrolled conditions

	Eggs	Eggs hatched	Hatch %	Adults emerged	Damage %	% Seed wt. loss	MDP (days)	% survival	Howe's index
Eggs	-	0.99**	0.66**	0.83**	0.83**	0.94**	-0.81**	-0.96**	**0.11**
Hatched		-	0.74**	0.85**	0.85**	0.95**	-0.83**	-0.93**	**0.18**
Hatch %			-	0.75**	0.75**	0.69**	-0.76**	-0.56**	**0.49****
Adults				-	1.00**	**0.83****	**-0.93****	**-0.75***	**0.57****
Damage %					-	0.83**	-0.93**	-0.75**	**0.57****
% wt. loss						-	-0.82**	-0.88**	**0.23**
MDP							-	0.78**	**-0.65****
% Survival								-	**-0.04**
Index									-

** Correlation is significant at 0.01 level of significance, Bold values indicate correlation comparisons of bruchid biological parameters with damage per cent and Index of suitability (IS)

the bruchid developmental parameters than the room temperature. Seed weight loss (%) [(initial weight-final weight) × 100/initial weight] was also calculated for 50 seeds at the end of the experiment and greengram seeds kept inside the incubator recorded a maximum of 51.51 ± 1.08% weight loss (Table 3). Host quality is another significant factor affecting bruchid development. Since many seed beetles (including *C. maculatus*) do not feed as adults, their body size, fecundity, and longevity depend on resources obtained from the seed cotyledon during the larval stages (Timms,1998). In this study, the mean developmental period was extended to a maximum of 35.38 days (C-strain) followed by 31.19 days (T-strain; Table 1) in blackgram under uncontrolled condition. This may be due to change in the host seed used for culturing (greengram variety) to that being tested (blackgram). Moreover, the fact that greengram is more nutritious than blackgram in terms of vitamins and minerals content corroborated the present results. The food influence in this study may indicate an ancestral cause or fitness cost depending on the species (Gbaye et al., 2011). Some reports have indicated differences when adults being tested on a particular variety, were previously conditioned on that variety for successive generations prior to the screening test (Swella and Mushobozy, 2009; Yadav and Pant, 1974) which are in terms with the current findings. Although the use of cowpea [*Vigna unguiculata* (L.) Walp.] seeds has been suggested as the most favourable host for rapid bruchid mass-culturing by Strong et al. (1968), Jackai and Asante (2003), Swella and Mushobozy (2009), this study demonstrated the feasibility of using mungbean seeds as equally suitable bruchid rearing host taking into account of the comparatively shorter developmental period (24 days; Table 1). Hence both cowpea and greengram can be used for rapid mass-culturing and long-term maintenance of bruchid generations for any behavioural, physiological, management and resistance studies under laboratory conditions. Xu et al. (1999) suggested that storage temperatures above 12ºC can reduce the germination percentage, nutritional quality and protein availability in beans due to ageing with possible negative effects on the development of bruchid beetles. Hence lower temperature (e. g. cold storage) with appropriate relative humidity may be recommended during seed storage as far as bruchid infestation is concerned.

Several workers conducted studies on the damage potential of *C. maculatus* on different legume seeds (Yadav and Pant, 1974; Swella and Mushobozy, 2009). In general, parameters like number of eggs laid, per cent survival rate, growth index (Howe's Index) and seed weight loss (%) are considered for determining the damage potential and bruchid resistance or susceptibility against various legumes (Howe, 1971; Dobie, 1974; Nwanze et al., 1975; Jackai and Asante, 2003; Ponnusamy *et al*. 2014; Soumia et al., 2015; Dasbak et al., 2009). Seed damage per cent is seldom taken into account for the assessment of bruchid infestation except for few workers like Tomooka et al. (2000) and Sun Lei (2008) where bruchid resistance evaluation was based on per cent damage score for QTL mapping and identification of molecular markers linked with bruchid resistance in greengram, adzuki bean, etc. Both the crop species in the present investigation were damaged by the bruchid but the damage potential was higher on greengram with 91.2% seed damage compared to 72.4% in blackgram under favourable conditions (Table 1). Therefore, a damage score of 9 was assigned to the two crops which fall under highly susceptible (HS) category. The correlation study (Table 4) between all the bruchid developmental parameters revealed that seed damage per cent is significant and highly correlated (r = 0.83, 0.85, 0.75, 1.00, 0.83, -0.93, -0.75, 0.57) with all the other bruchid parameters measured, whereas few correlations were significant (r = 0.49, 0.57, 0.57, -0.65) with respect to Howe's growth index. Moreover, Howe's Index depends on per cent survivability and mean developmental period (MDP) where MDP considers only 50% emergence of the bruchid progeny, which ultimately shows the number of exit holes on the seeds, i.e. the number of damaged seeds in which calculation of damage per cent is based. Therefore, damage per cent may be considered as more important criterion than other parameters in laboratory method for evaluating bruchid resistance or susceptibility in host plant resistance studies.

CONCLUSION

Seed loss due to bruchid damage during storage may differ with respect to the seed crop stored, insect geographical location and the prevailing climatic and (or) storage conditions. This study undoubtedly confirmed the effect of temperature and relative humidity on bruchid development. The findings clearly revealed that subsequent host changes in bruchid rearing affect the development and reproductive potential which is evident in the case of *C. maculatus* growth on blackgram. Hence appropriate selection of host choice may be considered according to the objectives and the type of crop to be studied. For instance, selection of greengram as host seed for mass-culturing may be recommended for bruchid resistance evaluation in greengram for host plant resistance studies and likewise for other pulse crops. With regard to mass-culturing and long-term maintenance of bruchid generations for any behavioural, physiological, management and resistance studies, mungbean is a more suitable host for *C. maculatus* than urdbean. Further comparisons between different populations/strains originating from other districts where legume seeds are grown may be undertaken. *Callosobruchus maculatus* has the potential for rapid, even exponential increase to a devastating level at the optimal temperature on its preferred host seeds. Hence bulk storage of seeds at higher temperature should be avoided. Moreover, this study provided a better understanding of the development of this pest and as a result it may lead to better predictions of outbreak and improvements in the timing of pest management practices for stored seeds which will serve as one of the options for reducing bruchid damage at least to some extent.

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