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Full Length Research Paper

Variation in reproductive and developmental parameters of *Callosobruchus maculatus* (F) reared on ten Botswana cowpea landraces

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A study was conducted on ten Botswana cowpea landraces to evaluate their resistance to the cowpea weevil *(Callosobruchus maculatus (F)* (Coleoptera: Bruchidae). The variations in reproductive and developmental parameters were used to evaluate the landraces for resistance against C*. maculatus* under laboratory conditions. The landraces B339, B383, B013-F and B261-B had significant lower percentage adult emergence and oviposition than the landraces B001-B, B055, B109-C and B154. The landraces B339, B383, B013-F and B261-B also significantly prolonged developmental period of beetles. The reduction in oviposition, beetle emergence and prolonged developmental period by these landraces indicate inherent level of resistance against *C. maculatus*.

Key words: Landrace, *Callosobruchus maculatus,* resistance, developmental period, Botswana.

INTRODUCTION

Cowpea (*Vigna unguiculata* (L.) Walp.) has been cultivated in many countries for many centuries. It is one of the important food legume crops in the tropical and subtropical regions covering Asia, Africa, Southern Europe, Central and South America (Singh and Jackai, 1985; Diouf, 2011). Cowpea is an essential component of cropping system in the drier regions and marginal areas of the tropics and sub tropics (Duke, 1990). It is a drought tolerant and warm weather crop well adapted to the drier regions where other food legumes do not perform well (Singh, 1987). It fixes atmospheric nitrogen through its root nodules and grows well in poor soils with more than 85% sand low organic matter and levels of phosphorus (Caswell, 1981). In addition, it is shade tolerant and therefore compatible as an intercrop with maize, millet, sorghum, sugarcane and cotton. In Botswana, cowpea is the most important crop after maize (Zea *mays*) and grain sorghum (*Sorghum bicolor*). Cowpea is consumed in many forms; young leaves, green pods and green seeds are used in various food preparations. With high protein content, cowpea is a natural supplement to cereal; root and tuber staples in the diets of may Africans (Giga, 2001).

Despite its importance in tropical regions, cowpea yield potential and seed quality is often reduced by insect pest damage. In Botswana, yield loss of more than 50% due to pests had been reported when control measures were not applied (Obopile, 2006). One of the major destructive post harvest pest of cowpea worldwide is the cowpea weevil (*Callosobruchus maculatus* (F) (Coleoptera: Bruchidae). In stored seeds, cowpea weevil causes irreparable damage to the tissue, one that can reduce nutritive value and quality seeds for planting in many areas of developing countries. The damage is caused by larvae feeding and development inside the seed, and when adults emerge they leave circular exit holes (Davidson and Lyson, 1979). The damage reduces the weight and may render the seeds to be unfit for human consumption due to fungal growth associated with increased temperatures in storage. Many pest control options are available to the cowpea producers to reduce losses from cowpea weevil in Africa. The treatments with synthetic insecticides have been recommended for the control of the cowpea weevil for many years (Pierrard, 1986). The application of insecticides however, is not

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sustainable due to long term negative impact on the environment and the safety of food and livestock feed (Zettler and Cuperus, 1990; Silver, 1994). In order to reduce over-dependence on pesticides for management of cowpea weevil, research on host plant resistance has been developed (Ofuya, and Credland, 1995; Appleby and Credland, 2004). The use of resistance varieties is sustainable and does not have long term undesirable impact on the environment. In Botswana, the recommended cowpea varieties are susceptible to the cowpea weevil. Studies conducted elsewhere have shown that different varieties of cowpea differ in susceptibility to *C. maculatus* (Edde and Amatobi, 2000) and provide genetic sources for cowpea breeding programmes.

Besides Javaid (1993), no other research has been done on screening cowpea landraces for resistance against *C. maculatus* in Botswana, although there are over 800 landraces of cowpeas with genetic variability stored at the National Plant Genetic Resource Center. These landraces are commonly grown side by side with recommended varieties by small scale farmers who keep their own seeds. In this study, we used variation in reproductive and developmental parameters to assess ten Botswana landraces for resistance against *C. maculatus.* Our predictions were that landraces with inherent resistance to cowpea weevil would have lower oviposition and adult emergence, as well as prolong developmental period of *C. maculatus.*

MATERIALS AND METHODS

The cowpea landraces used in this study were provided by National Plant Genetic Resource Center located in the Department of Agricultural Research at Gaborone, Botswana (Table 1). The 10 landraces were not tested before for bruchid resistance. The landrace seeds were placed in a cold room (0 to 5°C) for 14 days to eliminate any possible contamination with insects. Before the beginning of the experiment, seeds from each landrace were stored in sealed plastic bags in the laboratory at room temperature for 30 days to condition them.

Insect cultures and rearing

Experiment was performed in a laboratory using reared population of *C. maculatus* that was originally obtained from a susceptible seeds of cowpea (cv. Tswana) collected from infested seeds provided by the Botswana Seed Multiplication Unit of the Department of Agricultural Research at Sebele in Gaborone. The stock culture of *C. maculatus* was reared by placing about 100 unsexed adults on 4 jars measuring 500 ml with three quarter full of disinfected Tswana, a commonly grown cowpea variety in Botswana. The jars were covered with open screw caps having muslin cloth to prevent bruchids from escaping. The beetles were allowed to mate for seven days at 27 ± 1°C and 60 to 90% relative humidity to mate and lay eggs after which they were removed.

Experimental procedure

The first generation of adults emerging from stock culture was used in this study. 40 seeds from the ten landraces were placed in glass jar covered with muslin cloth. The experiment was laid out in a completely randomized design with four replications per landrace making a total of 40 experimental units. Two pairs (two females and two males) of newly emerged (0 to 24 h) adults from the stock culture were introduced into 40 seeds of each landrace for oviposition. The insects were allowed to mate and lay eggs for 36 h and removed thereafter to prevent further breeding.

Data collection

The total number of eggs laid and number of eggs not hatched were counted under dissecting microscope. After seven days, unhatched eggs remained transparent and those hatched and from which larvae entered seed became opaque, thus helping in differentiating them when recording. The percentage of unhatched eggs was calculated by dividing the number of eggs not hatched by total number of eggs laid. After observation of the first beetle emergence, F_1 adults were removed daily and their number recorded over a 90 day period after oviposition (Ofuya and Credland, 1995). Percentage adult emergence was calculated by dividing the number of adults that emerged by the number of hatched eggs (not reported here). The developmental period (time to adult emergence) was calculated based on the total number of bruchid that emerged on a given day. The mean time to adult emergence (developmental period) was calculated using the following formula (Edde and Amatobi, 2000):

$$\text{Time to adult emergence (days)} = \frac{x_1 y_1 + x_2 y_2 + \ldots x_n y_n}{Total\, x\, emerged}$$

Where, x is beetle numbers emerging on a given day and y is the number of days from initial infestation: 1, 2 and *n* are the first second and last days of observation respectively. The growth index (G. I) of bruchids reared on each landrace was determined using the formula: G. T = log S/T, where, S is the percentage of emerged adults and T = mean developmental time (Jackai and Singh, 1988). Damage to seeds was scored by counting the number of bruchid exit holes per replication.

Data on percentages of unhatched eggs and adult emergence were transformed using the arcsine square root [arcsine $\sqrt{}$ (percent x / 100)] to stabilize variance. Counts of eggs and bruchid exit holes were transformed using log (x + 1) transformation. Data was analyzed using mixed model procedures (PROC MIXED in SAS (SAS Institute, 2003). Multiple comparisons were made on least square means. All the comparisons were based on LSD and considered significant when P≤ 0.05.

RESULTS

The landrace significantly affected number of eggs laid ($F_{9, 27}$ = 3.58; P = 0.0048), percentage of eggs not hatched ($F_{9, 27}$ = 3.10; P = 0.011) and percentage adult emergence ($F_{9, 27}$ = 3.68; P = 0.0041) (Table 1). The mean number of eggs laid ranged from 7.5 to 51 eggs, while percentage adult emergence ranged from 22 to 65% across the landraces. The landrace B339, B013-F, B383 and B261-B had the lowest mean number of eggs laid and percentage adult emergence than the rest of landraces (Table 1). The percentage of eggs not hatched was significantly higher on landrace B339, B383 and

Table 1. Reproductive and developmental parameters of the cowpea weevil, *Callosobruchus maculatus* in ten Botswana landraces [§].

Landrace	% Adult emergence (±SE)	Mean number of egg (± SE)	% Eggs not hatched (± SE)	Mean developmental period	Growth index
B109-C	64.82 ± 9.64[a]	48.75 ± 17.02[a]	32.79 ± 8.09[c]	29.50 ± 0.29[e]	0.061 ± 0.002[a]
B044	60.22 ± 4.60[ab]	30.50 ± 13.19[abcd]	41.70 ± 3.66[c]	39.75 ± 1.25[ab]	0.045 ± 0.002[a]
B001-B	58.73 ± 0.65[abc]	51.00 ± 14.53[a]	35.71 ± 5.76[c]	28.50 ± 7.84[dc]	0.057 ± 0.004[ab]
B187	58.20 ± 3.42[abc]	15.50 ± 4.29[bcd]	44.11 ± 2.93[bc]	37.75 ± 1.25[bc]	0.046 ± 0.002[dc]
B055	57.14 ± 6.30[abc]	37.25 ± 8.61[abc]	44.71 ± 6.71[bc]	28.25 ± 0.75[e]	0.062 ± 0.002[a]
B154	48.83 ± 5.72[abcd]	39.50 ± 7.35[ab]	51.17 ± 5.72[bc]	34.28 ± 3.13[dc]	0.050 ± 0.004[bc]
B383	44.05 ± 6.82[bcd]	10.25 ± 1.10[d]	53.68 ± 9.02[bc]	40.98 ± 1.07[ab]	0.040 ± 0.002[de]
B013-F	38.75±9.46[cde]	8.00 ± 2.80[d]	48.75 ± 13.13[bc]	43.30 ± 1.67[a]	0.036 ± 0.000[ef]
B339	34.44 ± 9.13[de]	7.50 ± 0.65[d]	79.56 ± 8.84[a]	37.75 ± 1.25[bc]	0.04 ± 0.004[de]
B261-B	21.93 ± 7.58[e]	10.75 ± 1.65[cd]	66.39 ± 13.99[ab]	41.09 ± 2.35[ab]	0.031 ± 0.005[f]

[§]The numbers are based on 40 seeds per replication. Means with the same letter in a column are not significantly different. LSD ($P \leq 0.05$)

B261-B compared to other eight landraces indicating a reduction in hatchability of eggs laid on these landraces. The percentage of unhatched eggs ranged from 32 to 80%.

Landrace also significantly affected the mean time to adult emergence ($F_{9, 27} = 10.85$; $P < 0.0001$) and growth index ($F_{9, 27} = 14.07$; $P < 0.0001$). The mean time to adult emergence (developmental period) which ranged from 28 to 43 days, was significantly extended on landraces B013-F, B383 and B261-B compared to the other seven landraces (Table 1). The landraces that prolonged developmental period of *C. maculatus* had significantly lower growth index indicating their unsuitability as host for optimal growth and development of the beetles (Table 1). The number of adult exit holes was significantly lower ($F_{9, 27} = 6.52$; $P < 0.0001$) on landraces B339, B013-F, B383, and B261-B than other landraces tested (Figure 1). The mean number of bruchid exit holes ranged from two to 30 eggs.

DISCUSSION

The reproductive and developmental parameters usually associated with fitness of *C. maculatus* were adversely affected when reared on landraces B339, B013-F, B383 and B261-B than on landraces B044, B001-B, B055, B154, B187 and B109-C. The varieties that adversely affect parameters associated with fitness of insects, compared with values in susceptible varieties, are considered resistant (Ofuya and Credland, 1995). Oviposition of *C. maculatus* was significantly reduced on landraces B339, B013-F, B383 and B261-B, indicating some level of resistance. Other researchers have also reported reduced oviposition on resistant varieties. Ofuya and Credland (1995) observed significant reduction in fecundity on varieties with known level of resistance in different populations of *C. maculatus*. Edde and Amatobi (2000) also reported lower number of eggs laid on varieties resistant to *C. maculatus* in Nigeria. The mechanistic explanation for reduced oviposition in Botswana landraces was not studied in this work. Jackai and Asante (2003) showed that egg deposition by *C. maculatus* increased with increase in seed density but the number of eggs per seed was inversely related to the number of seeds. The number of seed exposed to *C. maculatus* in this study was fixed (40 seeds per replication) to reduce the variation in oviposition associated with seed density. Since *Callosobruchus* species distribute their eggs evenly on seeds (Credland and Wright, 1990; Mbata, 1992) the number per seed is unlikely to cause significant variation in egg deposition. Studies carried out elsewhere showed that surface area (Avidov et al., 1965; Sullehrie et al., 2003) and seed size (Cope and Fox, 2003) were significant oviposition stimuli for *C. maculatus*.

Jackai and Asante (2003) suggested that generally, counts of eggs alone would not be sufficient to predictive resistance in host pant resistance studies of *C. maculatus*. More also, Redden and McGuire (1983) and Jackai and Asante (2003) found that percentage adult emergence, developmental period, growth index and weight loss were the most reliable indicators for resistance to bruchid damage in cowpea seeds. In our study, we also used percentage adult emergence, developmental period and growth index parameters to distinguish resistant varieties from susceptible ones. There was a significant reduction in adult emergence on resistant landraces B339, B013-F, B383 and B261-B compared to susceptible B044, B001-B, B055, B154, B187 and B109-C landraces. Similar results were previously reported by other authors (Edde and Amatobi, 2000; Ofuya and Credland, 1995; Appleby and Credland, 2003) who observed reduced adult emergence and delayed developmental period.

The reduction in percentage of adult emergence would lower the number of progeny, contributing to population growth from generation to generation. We also observed

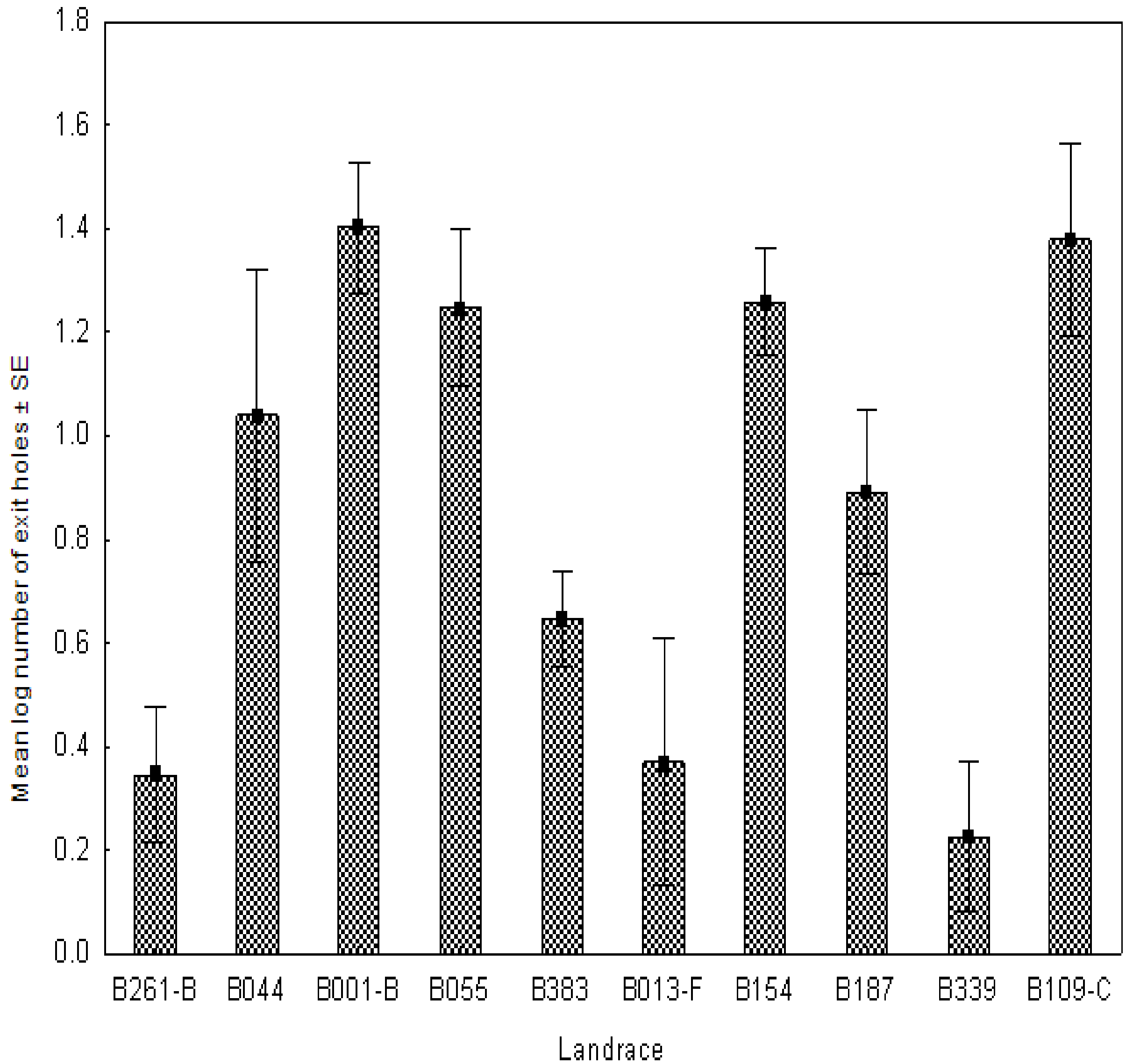

Figure 1. Mean log number of exit holes made by adult during emergence.

significant delay in development of *C. maculatus* on resistant landraces, indicating that the rate of population growth in natural population would be lower due to prolonged generation time. The extended period of development may lead to considerable reduction in yield loss during storage, due to low rate of multiplication and lower rate of population growth. The delay in development of resistant landraces was confirmed by lower growth index values observed on resistant landraces compared to susceptible ones. Significant reduction in adult emergence which was also evidenced by increased percentage of eggs that failed to hatch, and delay in development may suggest that antibiosis (Painter, 1951) is the modality of resistance against *C. maculatus*. Variation in oviposition and subsequent number of eggs laid may also suggest the presence of antixenosis. However more detailed work on physico-chemical characteristics of Botswana landraces will determine the mechanisms of resistance.

Therefore, the landraces B339, B013-F, B383 and B261-B constitute useful genetic resources for cowpea production system. These landraces can be used in breeding programmes aimed at developing integrated pest management strategies for *C. maculatus*. All the landraces were collected originally from small scale farmers and are still grown by some farmers in rural areas. There is a need to encourage farmers to grow the resistant landraces especially when rainfall is sufficient, because they are long season plants. The resistant landraces are also suitable for low input farmers because they store and select their own seeds for planting. Because of their indeterminate growth, the landraces provide leaves that are harvested and processed for preparation of traditional dishes. The processed leaves provide roughage in diets and have become popular in towns where they are sold, thus providing income for rural dwellers.

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