

Proceedings of the 7th International Working Conference on Stored-product Protection
—Volume 1

the oviposition and development of a Pakistani biotype of *Callosobruchus maculatus* (F.)
(Coleoptera: Bruchidae) on different host legumes

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Abstract

The study was conducted to observe the preferential response of a Pakistani biotype of *Callosobruchus maculatus* (F.) to two different Pakistani varieties each of *Vigna radiata* (NM92 & NCM209) (green gram) and *Vigna mungo* (NARC1 & NARC3) (black gram). The number of eggs, development period, percentage adult emergence, weight of newly emerged adults and growth index were studied. Number of eggs was recorded for four

consecutive generations. Oviposition occurred on all varieties. For the first two generations, Hopkin's host varieties selection principle could be applied, but in third and fourth generations, the larval host effect on ovipositing male did not occur, suggesting that oviposition decisions by the pulse beetle are not only determined by Hopkin's principle.

In general, the maximum number of eggs was laid on NM92, while on NARC1 a minimum number of eggs was laid. Preference for oviposition was also an indication of suitability for larval development. There was significant difference between the development pattern in seeds of green gram and that in seeds of black gram. The slowest [development] was recorded in NARC1 (43.6 days), while the fastest was recorded in NM92 (27.2 days). On the basis of growth index, NM92 proved to be of maximum food value for the insects while NARC1 proved to be of minimum food value. The heaviest insects were recorded from cowpea which were especially used in this bioassay to observe the response of *C. maculatus* on a standard host).

Introduction

By 1st January 1997, the total population of Pakistan was estimated to be 135.28 million (Economic Survey of Pakistan 1996—97). Per capita income at constant prices of 1980—81 indicated a decrease of 0.4 percent per year (Economic Survey of Pakistan 1996—97). As a result of accelerating population increase and declining per capita income, the majority of the people in Pakistan are malnourished. Most of them are unable to afford animal proteins as a food source.

Pulses contain 20—30 per cent proteins and can provide a comparatively cheaper alternative to animal proteins. They are short duration plants and the high lysine level in the protein makes them ideal supplements to cereals (Fernandez and Talker, 1990). Among the important pulses consumed in Pakistan, *Vigna mungo* (black gram) and *Vigna radiata* (green gram) are the most widely grown. Owing to small land holding, poor farming techniques, scarcity of inputs and low literacy rate among the farming community, yields of pulses are low. The situation is further exacerbated by insect pests, which damage pulses during storage (Dhepe et al., 1993). Birch et al. (1985) and Reciden et al. (1983) described bruchids as one of the most important insect groups attacking both grain and legume plants in the arid tropics.

Among the bruchids, *Callosobruchus maculatus* (F.) is one of the most destructive pests of stored pulses in India and Pakistan. The damage in some pulses can be so extensive that the whole of the seed material is eaten and only seed coats with empty cavities are left behind (Vir and Jindal, 1981).

It has been established that *C. maculatus* can occur in geographically distinct populations (biotypes) which exhibit different biological characteristics (Credland, 1990). Earlier studies have revealed that occurrence of insect biotypes, able to successfully utilise a plant supposedly resistant, can hinder pest management programmes (Gould, 1978; Futuyama and Peterson, 1985). The response of three populations of *C. maculatus* to seed resistance in selected varieties of *Vigna unguiculata* (cowpea) was studied by Ofuya and Credland, (1995). Kitamura et al. (1990) undertook their investigations to characterise bruchid resistance factors present in *Phaseolus vulgaris* (kidney bean) and *Vigna sublobata* (the wild mung bean). Credland (1990) studied the biotypic variation and host change in bruchids, using ten biotypes of *C. maculatus* against

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Vigna unguiculata (cowpea) and *Lens culinaris* (lentils) as host pulses. Vir and Jindal (1981), studied oviposition response and development of *C. maculatus* on four pulses viz.; *Vigna radiata* (mung), *Vigna acuticulata* (moth), *Vigna unguiculata* (cowpea) and *Co. Janus cajan* (pigeon pea).

Very little work has been done on Pakistani biotypes of *C. maculatus*. Personal communication with workers from Pakistan pointed at a possible preference of *C. maculatus* for green gram against black gram. This preference was confirmed in a preliminary experiment where adults of *C. maculatus* were free to choose their host for oviposition from black gram (NARC1, NARC3), green gram (NM92, NCM209), control bean and cowpea (*Vigna unguiculata*) (Sulehrie, Unpublished data). In most storage conditions, the opportunities for insects to choose their preferred host from a variety of hosts are rare. Therefore the present investigation was undertaken in a 'no choice situation'. The results presented here are also analysed in the context of Hopkins's Host Selection Principle (Hopkins 1916 cited in Wasserman, 1981), which could explain the preference of *C. maculatus* for green gram. The Hopkins principle states that adult female insects will prefer to oviposit on the host upon which they had fed as immatures.

The first stage in the insect's life where preference for a particular host could be manifested is at oviposition. Mitchell (1975) observed that *C. maculatus* are selective in the kind and size of beans on which they lay eggs so the first set of experiments was conducted to check the ovipositional response of females on different hosts.

The second bioassay was undertaken to assess differences during the remaining stages of the insect's life cycle, i. e. larval development, adult weight at emergence and fecundity of the newly emerged adults. Redden & McGuire (1983) determined the most discriminating measurements of resistance within the seed accessions to *C. maculatus* to be mean emergence day (the development period), followed in decreasing order by percentage adult emergence, percentage of undamaged seeds, mean number of holes per seed and percentage loss in seed weight. The effect on larval development was determined using Redden & McGuire's parameters, mean emergence day and percentage adult

emergence.

Material and Method

Origin and routine maintenance of strain of *C. Maculatus*

All insects used in this study were obtained from a stock culture of *C. maculatus* collected from National Agricultural Research Council, (Islamabad) Pakistan. The insects had been in culture since October 1995 at NRI, Chatham, Kent. The insects in the stock culture were

reared on a commercially available Australian cultivar of green gram. All the experiments were undertaken in a constant temperature and humidity (CTH) room (27 ± 1 and $70 \pm 10\%$ rh).

Standardisation of seeds

Prior to experimentation, all seeds investigated were frozen at -20°C for one week and then stored at 4°C to prevent infestation. Small samples of each legume variety were equilibrated in a CTH room for a minimum of three weeks before use. This stabilised the moisture contents of seeds at about 11% (Parr et al. , 1996). All seeds were visually examined and those having hard, rough, cracked or damaged testa were excluded as these factors are known to influence a seed's acceptability, (Nwanze & Horber, 1976; Messina, 1984).

Ovipositional bioassay

Two hundred seeds each of green gram; *Vigna radiata* (varieties: NM92 & NCM 209), and black gram; *Vigna mungo* (varieties: NARC 1 & NARC 3) were placed separately in a 70 mm \times 40 mm crystallising basin, respectively, with one pair of newly emerged male and female insects that had been allowed to mate for two hours. Commercially available Australian cultivar of green gram was used as a control. Twenty replicates were undertaken for each legume variety. Two hundred seeds were allocated to each replicate, because a preliminary study of ovipositional pattern revealed that the maximum number of eggs laid by a single female was 156.

The number of eggs laid in each replicate was recorded when the female reached 10 days old, following Dick and Credland (1984) who reported that oviposition is completed in about 8 days and females die about 10—12 days after their emergence. Seeds with one egg were selected and placed in microtitre plates in the CTH room until adults emerged. Newly emerged females were crossed with standard males (reared in control seeds) of known age. The oviposition bioassay was repeated using these insects as parents of the next generation. Following Credland (1987), generations were numbered in relation to the habitat of the larval stage. Thus, the first generation (F₁) of NM92 are those larvae in NM92 produced by adults, which were themselves reared on control beans (FO).

Development Bioassay of *C. maculatus*

For each of six varieties of host legume (NM92, NCM209, NARC3, NARC1, cowpea and control), 250 seeds were placed in six 70 mm \times 40 mm crystallising basins. *C. maculatus* is commonly known as the cowpea beetle and most of the workers have used cowpea in bioassays. Therefore, commercially available cowpea from California was included in this bioassay to compare the test varieties

with a standard host. Eight pairs of newly emerged male and female adults that had been allowed to mate for two hours were introduced into each crystallising basin and were kept in the CTH room for six hours. The insects were removed, and 150 seeds with one egg were isolated from each of six legume varieties. The weight of each individual seed was recorded on a microbalance (Sartorius) weighing to ± 0.001 mg. The weighed seeds were placed in Sterilin 100mm X 100mm 'repli dishes' with 25 compartments (Merck Ltd), so that each seed occupied a separate compartment. The dishes were placed in the CTH room for rearing.

The seeds were examined and adult emergence recorded twice daily, beginning 21 days after the oviposition occurred, which is the minimum development period of *C. maculatus*, as recorded by Dobie et al. (1991) until there was no emergence for three consecutive days (Credland, 1987).

All the newly emerged insects were sexed and weighed soon after emergence on a microbalance (Mettler AE160) weighing to ± 0.0001 gram. The emerged insects were removed after each observation to prevent any chances of mating and oviposition of newly emerged insects. The suitability of food was assessed on the basis of a growth index defined as log of percentage emergence of adults/ mean development period in days (Vir and Jindal, 1981).

Calculations and data analysis

The statistical package used for all the calculations was SPSS for Windows (release 6.1.3., 5 Dec. 1995). Applying 'GENERAL FACTORIAL ANOVA', customised model, carried out analysis of variance. Residual plots, means of dependent variable/s, within \pm residual error term and unique sum of squares were displayed in SPSS output. Data were subjected to appropriate transformation where required to reduce the residual errors.

Oviposition Bioassay

The total number of eggs laid on each variety was recorded and subjected to general factorial analysis of variance, as described above. In case of the parent generation (FO), data was transformed into square roots for number of eggs laid to reduce the residual error.

Developmental Bioassay

The following parameters were measured for each variety:

Hatching percentage ($\text{Number of eggs hatched} / \text{total number of eggs} \times 100$)

- The developmental period (time of egg laying to adult emergence from seed);
- Percentage adult emergence ($\text{number of emerged adults} \times 100 / \text{total number of seeds}$);
- Growth index ($\text{Log of percentage emergence of adults} /$

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mean development period in days);

Sex ratio (total number of males/ total number of

females);

Average weight of males and females (mg).

Data were subjected to general factorial analysis of variance. Data on growth index were first multiplied by 1000 and then subjected to ANOVA.

The correlation coefficient was calculated between the weight of seeds and insect weight, between the weight of seeds and development period, and between the insect weight and development period.

Results

Egg laying on different hosts

Adults of *C. nuiculatu.s*, which had developed in control green gram for more than 15 consecutive generations, laid eggs on a!! the test varieties of black gram and green gram. The maximum and minimum number of eggs was laid on control beans and on NARC1, respectively (Table 1). With the exception of NARC1, there were no significant differences among all other host legumes (LSD at 5% level = 2.08 when square roots of the total number of eggs laid were taken).

Table 1. Means of egg count (\pm SE) for different host legumes, laid by FO females.

Host Legumes

Mean total number of eggs laid/female \pm SE

Control mung

57.80 \pm 6.60

NM92

46.15 \pm 9.72

NCM2O9

52.30 \pm 9.92

NARC1

21.00 \pm 5.02

NARC3

49.05 \pm 9.97

For the F1 generation, the maximum number of eggs was laid by females reared from seeds of NM92 and placed on seeds of NM92, while the minimum were laid by females reared on seeds of NCM2O9 and placed on control seeds (Table 2). With the exception of females reared from seeds of NARC3, where there was a non significant difference, a!! the other females laid significantly higher numbers of eggs on their respective larval hosts than on control beans (LSD at 5% level = 16.96).

For F2 generation females, there were no significant differences among the number of eggs laid on control beans and those laid on seeds of their respective larval hosts, with the

exception of females reared in seeds of NARCI where the number of eggs laid on the control were significantly

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higher than those laid on its larval host. (LSD at 5% level = 18.23) (Table 3).

There were significantly greater numbers of eggs laid by F3 females reared in seeds of NARC1, on control seeds compared with seeds of the larval hosts. However, the opposite was observed for females reared in seeds of NM92. For F3 females reared in seeds of NCM209 and NARC3, there was no significant difference between the total number of eggs laid on their larval hosts as compared with the control mung (LSD at 5% level = 12.21) (Table 4).

Table 2. Means of egg count (\pm SE) for different host legumes, laid by F1 females.

Legume varieties

Host Test

Mean total number of eggs laid/female \pm SE.

N1v Control

80.00 \pm 3.21

NM92 NM92

114.10 \pm 9.72

NCM209 Control

59.45 \pm 5.70

NCM209 NCM209

87.15 \pm 2.55

NARC1 Control

67.75 \pm 8.77

NARC1 NARC1

92.20 \pm 4.73

NARC3 Control

72.00 \pm 6.38

NARC3 NARC3

80.00 \pm 9.19

Table 3. Means of egg count (\pm SE) for different host legumes, laid by F2 females.

Legum Host

e varieties Test

Mean total number eggs laid/female \pm

of

SE

M92

Control

58.20 \pm 8.21

NM92

NM92

72.25 \pm 8.39

NCM209

Control

60.70 \pm 6.40

NCM209

NCM209

75.65 \pm 4.02

NARC1

Control

94.35 \pm 6.09

NARC1

NARC1

73.75 \pm 5.00

NARC3

Control

82.70 \pm 7.75

NARC3

NARC3

80.85 \pm 5.58

Host: parental developmental variety. Test: Pulses on which number of eggs was recorded.

Table 4. Means of egg count (\pm SE) for different host legumes, laid by F3 females.

Legume varieties

Host Test

Mean total number of eggs laid/female \pm SE

NM92 Control

42.60 \pm 5.29

NM92 NM92

55.80 \pm 3.94

NCM2O9 Control

72.25 \pm 3.03

NCM2O9 NCM2O9

83.15 \pm 4.25

NARCI Control

87.85 \pm 4.72

NARCI NARCI

67.75 \pm 4.99

NARC3 Control

64.70 \pm 2.87

NARC3 NARC3

58.10 \pm 4.77

Host: parental developmental variety. Test: Pulses on which number of eggs was recorded.

Table 5. Means (\pm SE) of the larval development period for different host legumes.

Host Legumes

Mean Development

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Period (days) \pm SE

Control mung

27.85 \pm 0.12

Cowpea

29.51 \pm 0.23

NM92

27.23 \pm 0.77

NCM2O9

28.55 \pm 0.86

NARCI

Discussion

The study was undertaken to assess the ovipositional and developmental responses of a Pakistani biotype of *C. maculatus* against different host legumes. It is apparent from the results that this biotype is well adapted to survive on the commercially available Australian cultivar of green gram on which it had been reared for many generations (control). It has not, however, become so specialised that it has lost the capacity to survive on other hosts. Females were therefore, able to lay eggs on seeds of all the pulses investigated (a phenomenon shared with the Turkish strain of *C. maculatus* which was adapted to survive on lentils but also developed in cowpea (Credland, 1987).

Hopkins host selection principle states that adult female insects prefer to oviposit on the host upon which they had fed as immature larvae (Hopkins, 1916, cited in Wasserman, (1981)). Insects used for this experiment were obtained from a population cultured on control beans for 15 generations. The maximum number of eggs was laid on beans from the larval host while minimum number of eggs were laid on seeds of NARC1 by F0 generation (parental) females.

Second generation females (F1) also laid more eggs on their larval hosts than on control beans. However females reared in seeds of NARC3 showed no significant difference in number of eggs on the larval host and on the control.

Wasserman (1981) suggested that insects marking utilised hosts are more likely to switch onto less preferred hosts, when the preferred hosts are scarce, than non-marking species. His main emphasis was on impact of ovipositional markers on female behaviour resulting in oviposition patterns mimicking a Hopkins effect. Yamamoto (1990) described the effect of BCS (biological conditioning substances, a mixture of lipids consisting of fatty acids, hydrocarbons and triglycerides and mono- and di-glycerides as minor components) on female ovipositional behaviour and quoted Yoshida ((1961) cited in Yamamoto, (1990)) that *C. maculatus* females prefer non- or less-BCS conditioned beans for further oviposition.

In setting up these experiments, attempts were made to minimise the effect of ovipositional markers through providing more seed than the maximum number of eggs a female could lay.

The third and fourth generation females from seeds of NARC1 laid significantly more eggs on control seeds than on their larval host. The fourth generation females from NM92 also laid more eggs on control beans than on their larval hosts, while there was no significant difference for the rest of the treatments. The ovipositional behaviour of first two generations would be consistent with Hopkins Host Selection principle but no such evidence was found from the third and

fourth generations. Earlier research has tested for the existence of Hopkins effect. Dethier (1954), Ishii ((1952) cited in Wasserman 1981) and Wasserman (1981) could not find any experimental evidence for its existence while Craighead (1921), Phillips and Barends (1975), and Smith and Cornell (1979) indicated existence of a positive Hopkins effect.

Redden and McGuire (1983) determined that percentage adult emergence and mean development period were the two most important indicators of seed resistance to bruchid damage in cowpea. In this study, the development period was shortest in seeds of NM92 and longest in seeds of NARCI. Among green gram varieties, there was a significant difference for seed size. Although NCM 209 was the smallest, the development period was similar for all green gram varieties. Although all varieties differed significantly with each other for development period, black gram and green gram could be easily categorised into two separate groups. Development was much slower in black gram varieties. This may be due to the chemical composition of the seed, which could slow down the metabolic activities of the developing insects, the non-adaptability of larvae to utilise the seed proteins or the presence of other deterrent effects in the host seed.

Vir and Jindal (1981) assessed suitability of food on the basis of a growth index. We observed a maximum growth index in seeds of NM92 indicating that this variety was the most suitable food for larvae of the biotype under study. The minimum growth index was observed in seeds of NARCI indicating that according to Vir and Jindal's criteria, it was less suitable as a food source. Keeping in view Redden and McGuire's parameters, Vir and Jindal's criteria and our preliminary work on free choice tests (Sulehrie, unpublished data), it is evident that seeds of NM92, in this context, are the preferred host for oviposition as well as for development of pulse beetles, while seeds of NARCI were least preferred.

In the present study, oviposition preference concurs with suitability for larval development. This is in contrast to the findings of Vir and Jindal (1981) who quoted Singh et al. (1977) in their support, and found that oviposition preference is not an indication of suitability for development. One of the reasons for this contrast may be a result of the fact that Vir and Jindal studied the oviposition behaviour for a single generation while we studied it for four consecutive generations. The use of different biotypes of *C. maculatus* or use of different host varieties may be other factors responsible for this difference as Vir and Jindal (1981) observed the oviposition pattern of an Indian biotype of *C. maculatus* on different varieties of host legumes. Furthermore, they laid out free choice tests while we conducted free choice as well as no choice tests. Regarding Singh et al., they worked on a different species, *C.*