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# Bionomics of *Batocera rufomaculata* De Geer (Coleoptera: Cerambycidae) in mulberry farms of Jammu and Kashmir (India)

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Abstract: Investigations on the bionomics of *Batocera rufomaculata* De Geer (Coleoptera: Cerambycidae) in mulberry farms of Jammu and Kashmir state (India) demonstrated an annual life cycle with an adult emergence period of over four months from May to September; adult emergence started in May, peaked in June and ended in August. Adults required maturation feeding period of  $14.00\pm0.561$  days. Sexually mature beetles mated promiscuously and copulation period averaged  $60.50\pm6.23$  seconds. Females deposited eggs singly into the bark of host plants with an average daily and life time fecundity of 1.27 and 148.66 eggs respectively. Eggs hatched in  $10.20\pm1.25$  days and larvae developed through 9 instars in  $253.35\pm4.37$  days. Ultimate larval instar pupated in the pupal cells, made by the ultimate larvae; adults emerged from the pupal cells after  $27.5\pm1.35$  days through circular emergence holes. Larvae as well as adults caused damage to the host plants; the latter compensated the damage caused by adults to some extent by sprouting lateral buds while as grubs caused irreparable damage and lead the host plants to death. The extent of damage caused to the host plants is also reported in the paper.

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# 1. Introduction

The Cerambycid beetle. Batocera rufomaculata De Geer commonly called red spotted longicorn beetle, is a polyphagous pest attacking more than 50 deciduous tree species in Oriental region (Duffy, 1968). It has been reported from Burma. Ceylon, Israel, Pakistan, West Indies and East Africa (Hussain and Khan, 1940; Beeson, 1941). Hussain et. al. (2009) reported it as a serious pest of mulberry plants in Jammu and Kashmir (India). Cerambycid borers cause wide spread mortality among deciduous broadleaf tree species in China (Yang et. al. 1995). Borers are known as chronically damaging pests (Nielson, 1981). Characteristically B. rufomaculata populations repeatedly attacking mulberry plants can cause serious damage to host plants affecting leaf vield both qualitatively and quantitatively as well as the vigour of host plants. The inaccessibility of grubs in woody hosts made it difficult to study their biology and population dynamics in field conditions and least control programs are available to check their population (Nielson, 1981).

The repeated infestation of the lamiine species to the host plants (mulberry plants) posses a great threat to the sericulture industry in the region. Keeping in view the problems associated with the increasing rate of infestation of *B. rufomaculata* among mulberry plants, an attempt was made to study the bio-ecology of the pest species as a preliminary investigation for the development of effective management strategies.

#### 2. Materials and Methods

#### 2.1 Field Study Sites

Current studies were carried out in 2005-2008 in mulberry farms of Jammu and Kashmir (India); eight districts, four each from Kashmir and Jammu divisions were selected and in each district two locations/sites were marked. The marked sites were Achabal and Yuour-Khushi-Pora in Anantnag, Mansbal and Uri in Baramulla, Pampore and Shopian in Pulwama, Noonur and Tulsibagh in Srinagar, Banihal and Ramban in Doda, Miransahib and Talab-Tillo in Jammu, Barnoti and Basoli in Kathua and Cherry and Tikri in Udhampur.

2.2 Host Plant

Different genotypes of *Morus* spp. (Moraceae); the genotypes were *Goshoerami*, *Chinesewhite*, *KNG*, *Tr-10*, *S-146*, *Mandalay*, and *V-1*.

## 2.3 Incidence and Distribution

Monthly survey was conducted from March 2005 to March 2008 and incidence of the pest species was recorded in earmarked plots. Frass indexing method was adopted to assess the borer infestation and rate of incidence of borers was calculated by the following formula:

% infestation = 
$$\frac{\text{Number of infested plants}}{\text{Total number of plants observed}} \times 100$$

#### 2.4 Laboratory Culture

The borer infested logs of mulberry plants were cut into pieces of 12 inches in length, sealed at both the ends with melted paraffin wax to avoid the moisture loss and transported to the laboratory. These logs were put in jars covered with muslin cloth till emerge of adults. The adults were removed from the jars and were used for further biological studies.

## 2.5 Biology

Adults emerged from infested logs in the laboratory were used to study adult longevity. maturation feeding period, and reproductive behavior. Soon after emergence, the beetles were assigned to glass/rearing jars (one pair; one female and two males; two females and one male to each jar) and 15 replications were maintained to study these parameters. Adult longevity was determined by computing adult life span of individual beetles. Sex specific adult longevity was also recorded. Plant tissues were supplied to the beetles assigned to the rearing jars to study the feeding behaviour and maturation feeding period. In sexual behaviour, impact of body size of males and females, visual stimuli and body odour were studied. One pair of matured beetles was assigned to each glass jars, containing oviposition logs (mulberry logs/twigs of 12 inches in length) and host plant tissues for feeding of beetles to study their reproductive potential. Feeding materials were supplied to the beetles daily and the oviposition logs were replaced weekly. Old age males were replaced by young stout males to maintain the mate availability. Upon death, female body length and width was measured and body size was calculated by  $\pi r^2 L$  (beetle considered as a cylinder) to study the relationship between body size and reproductive potential.

# 2.6 Population Dynamics

Population dynamics of the pest species was studied by capture recapture method in mulberry farms and population parameters were estimated by Jolly-Seber method. Beetles were sampled from the ethanol baited mulberry trees, sexed, marked with nail polish and released to mix with rest of the population. After specific time intervals, beetles were sampled again and total population was determined by:

$$N = n \frac{M}{m}$$

Where

N= Total population

- M=Total number of individuals captured in first sampling, marked and released
- n=Total number of individuals captured in 2<sup>nd</sup> sampling
- m=Number of marked individuals recaptured in 2<sup>nd</sup> sampling

## 2.7 Damage Assessment

Damage caused by Cerambycid borers to mulberry plants were assessed by

- **a.** Leaf yield per plant (weight)
- **b.** Leaf moisture content
- c. Bioassay test

Leaf of infested and un-infested trees was harvested and weighed separately. Ten replications were maintained for each mulberry genotype. Mean leaf yield of the infested and un-infested trees were compared and the reduction in leaf yield in infested trees was ascribed to the borer infestation.

Leaf moisture content was calculated by:

% leaf moisture = 
$$\frac{\text{Fresh weight} - \text{Dry weight}}{\text{Fresh weight}}$$
  
× 100

Bioassay was carried out using a popular silk worm hybrid SH6×NB4D2. Silkworm rearing was carried out on leaf of infested and un-infested trees separately and following parameters were studied:

- a. Cocoon weight
- **b.** Shell weight
- **c.** Shell ratio
- d. Single cocoon filament length

#### 2.8 Data analysis

The observations were tabulated and graphically presented. The data is presented as Arithmetic mean $\pm$ SE (Standard error of mean). Student's t-test and Chi square ( $\Box^2$ ) tests were employed to analyze the data and conclusions were made at P $\leq$  0.05.

# 3. Results

## 3.1 Distribution

In the study area, Jammu and Kashmir state (India), *B. rufomaculata* is widely distributed in mulberry farms of Jammu province and is restricted to Uri belt of Kashmir valley; the beetle has been recorded from all locations/study sites of Jammu province viz. Banihal, Ramban, and Batote (Doda), Talab-Tillo, and Miransahib (Jammu), Cherry, and Tikri (Udhampur) and Barnoti and Samba (Kathua) while as in Kashmir valley, it was only found at Uri of Baramulla district. The distribution pattern of *B. rufomaculata* in Jammu and Kashmir state indicate that the pest species is of tropical and subtropical habitat.

# 3.2 Description

The description of various developmental stages of *B. rufomaculata* is discussed under following subheads.

Egg: Eggs are oval,  $5.93\pm0.11$  mm (mean $\pm$ SE; N=10) in length and  $1.96\pm0.06$  mm (mean $\pm$ SE; N=10) in diameter; micropylar end slightly thicker; dirty white when laid, but changes to brown before eclosion; chorion thick and leathery (Fig. 1a).

Larva: Newly hatched larva creamy white in colour with dark brown head; soft; cylindrical, thickest at the thoracic region, gradually tapering towards the anal end; 7.98±0.099 mm (mean±SE; N=10) in length and 2.69±0.082 mm (mean±SE; N=10) broad at the thorax; mandibles strong and dark in colour; body covered with numerous minute spines (Fig. 1b). Mature larva (9<sup>th</sup> instar larva) is light yellow in colour, elongate, 72.20±1.22 mm (mean±SE; N=10) long and 15.55±0.425 mm (mean±SE; N=10) broad across the thorax, tapering behind the 8th abdominal segment. Head prognathous, dark brown, stout and articulate with head by a single condyle; maxillae lie behind mandibles, maxillary palpi three segmented; galea clothed with large number of sensory setae. Pronotum rectangular with margins covered with setae; lateral margins of mesonotum and metanotum have thick setae; minute tubercles and denticles arranged on the thorax and abdominal segments. Thoracic legs rudimentary, extremely small, encircled with sharp minute bristles. Nine pairs of spiracles, just like oval pits, situated on prothoracic and  $1^{\text{st}}$  to  $8^{\text{th}}$  abdominal segments (Fig. 1c).

Pupa: Pupa 47.70 $\pm$ 0.817 mm (mean $\pm$ SE; N=10) long and 21.40 $\pm$ 0.686 mm (mean $\pm$ SE; N=10) broad across the thorax; newly transformed pupa light yellow, but latter change to pale brown; head slightly deflected, antenna very long, pass along the thorax on each side and then make a spiral over the respective meta-leg; first two pairs of legs folded over the wing pads and the meta legs folded below the tips of wing pads; pronotum shield shaped and bears one protuberance on each side. Abdominal tip is tapering and curved upwards (Fig.1d).

Adult: Large, elongate, robust, dark brown longicorn beetle; ventro-lateral sides with a white strip running length wise (Fig. 1e); scutellum white; males 45.20 ±1.30mm (mean±SE; N=10) in length and 14.75±0.38 mm (mean±SE; N=10) in breadth and females 48.70±0.99 mm (mean±SE; N=10) and 15.83±0.44 mm (mean±SE; N=10) in length and breadth respectively. Well developed hypognathous head with prominent mouth parts, powerful mandibles black in colour, labium and labrum reddish brown. Antenna long, measuring 63.20±1.10 mm (mean±SE; N=10) and 48.00±0.92 mm (mean±SE; N=10) in length in males and females respectively; dark brown in colour: 11 jointed, first joint swollen, embedded in a socket,  $2^{nd}$  thickest,  $3^{rd}$  joint longest with a row of small teeth on inner edge, remaining segments of equal size, apical segment laterally compressed. Antenna in males is 39.82% longer than the body; however in females it is shorter than the body (Fig. 1f). Pronotum with two kidney shaped orange-yellow spots, projecting into a sharp stout tooth/spine on either side in the middle; elytra with a variable number (usually 3-6 on each elytrum) of light yellow spots and its cephalic region has numerous black tubercles and one small, sharp tooth on each shoulder; lateral margins of elvtra dark. Elvtra cover the whole abdomen in males while as in females last abdominal segment project behind elvtra (Fig. 1f). All the tree pairs of legs are more or less equal in shape and size; coxae swollen; trochanter small, triangular in shape; femur thick clothed with pubescence; tibia slightly longer and thinner than femur; tarsi four segmented, first three bilobed, dorso-ventrally flattened, fourth segment a bit longer, project into a pair of bluish black claws.



Fig. 1. Developmental stages of *B. rufomaculata* (a) Eggs (b) First instar larva (c) 9<sup>th</sup> instar larva (d) Pupa in pupal cell (e) Ventral side of adult beetle showing white strip along ventro-lateral side of the body (f) Adult beetles, left: male-antennae longer than the body and abdomen covered by elytra; right: female, antennae shorter than body and last abdominal segment project behind elytra

#### 3.3 Bioecology

3.3.1 Adult emergence and maturation feeding period Adult beetles appeared in May through September in mulberry farms of Jammu and Kashmir State (India). In Jammu division adult emergence started in early May, while in Kashmir division adults first appeared in the middle of the month. The beetles are relatively long lived and have been observed and subsequently collected till early September in mulberry farms of both regions of the study area. (Fig. 2). Beetles of B. rufomaculata are nocturnal and remain active from dusk to dawn. During the day time, beetles were found hidden in the dense foliage and on basal part of limbs of host trees. However, occasionally beetles were found feeding on the apical shoots. The adults emerged over a period of 4 months. The development of insects in any life stage can be subdivided into starting period, peak period and ending period when 16%, 50% and 84% of the population reaches this stage respectively (Xiao, 1992). Accordingly the starting period of adult emergence of fig tropical borer fell in between May 10<sup>th</sup> & 12<sup>th</sup> and May 23th and 25<sup>th</sup> in Jammu and Kashmir divisions respectively, when 16% individuals became adults in each division. The peak period in Jammu province and Kashmir valley were observed in between 7-9 June and 23-25 June (50% adults emerged). The ending period in both the regions were observed in between 8-11 August (Fig. 2). Newly emerged beetles fed on the growing tips and tender bark of primary branches, caused damage to apical buds of host plants (Fig. 3). Beetles appeared to require 14.00±0.561 days (mean±SE; N=30) of feeding to attain sexual maturity.

3.3.2 Copulatory Behaviour

Beetles matted promiscuously; no precopulatory courtship behaviour were observed; males directly approached females and copulated for an average of 60.50±6.23 seconds (mean±SE; N=10). Visual and olfactory cues guided males towards females. Females preferred larger males for mating and refused smaller ones much more frequently; however, no such behaviour were observed in males. 3.3.3 Host Selection and Oviposition

Larval host selection by females of B. rufomaculata is very critical as the grubs are legless and incapable of locating the host tree. Females located larval hosts by olfaction. Role of olfaction in host selection were studied by supplying mulberry (host) and elm (tentative non-host, afterwards nonhost) oviposition logs to rearing cages containing gravid females. Oviposition logs of host and non-host were removed after 7-days from the cages; scars on the oviposition logs were dissected and categorized into oviposited sites and nicks. It was observed that 13 ovipositing females made 109 scars and deposited 82 eggs in host (mulberry) logs while only 39 scars were made on non-host (elm) logs, all without eggs. Observations revealed that females oviposited only on mulberry logs and not on elm logs suggesting odour of host trees is perceived by females. Females gnawed egg niches in the bark with their mandibles and deposited eggs singly in the egg sites/niches during the night. After placing eggs in the niches, females

rubbed the oviposited sites and sealed them with a colourless liquid from the ovipositor which hardened soon after secretion.

Daily and life time fecundity of B. rufomaculata were studied in the laboratory. Fifteen pairs of beetles were caged (one pair per cage), supplied with tender mulberry twigs daily as food and mulberry logs/oviposition logs (5-32 mm in diameter and 30cm in length) for oviposition. Oviposition logs, categorized on the basis of diameter into four groups viz. I ( $\leq 8$  mm), II (9 mm to16 mm), III (17 mm to 24 mm) and IV (25 to 32 mm), were replaced weekly till death of females. Once removed, oviposition logs were held for 15 days after which scares were dissected and categorized as nicks, aborted eggs, non viable eggs (unhatched) and viable eggs (presence of grubs and/or frass). Caged females laid a total of 2230 eggs with an average life time and daily fecundity of 148.66 and 1.27 eggs respectively. Fecundity rate declined with age of females, but advanced age females laid more viable eggs (Fig. 4). Females laid 52.06% eggs in group IV oviposition logs, 43.94% and 3.99% on group III and group II oviposition logs respectively. However beetles did not laid eggs in group I logs (Table 1).

## 3.3.4 Incubation Period and Hatching

The grubs of *B. rufomaculata* hatched out from eggs after an incubation period of  $10.20\pm1.25$  days (mean±SE; N=10). Soon after hatching, grubs started boring without exposing themselves and made

their way into the heart wood of host plant. It has been observed that host tree moisture is necessary for hatching of grubs. Eggs removed from egg niches, when placed in glass tubes shrinked and failed to hatch, however, eggs placed in moist cotton (cotton soaked in distilled water) hatched normally.

Feeding and Tunneling Behaviour of Grubs 3.3.5 Newly hatched grubs fed on phloem and cambium while as latter instars made their way into the heart wood through sap wood, excavating a zigzag round-oval feeding tunnel down wards (Fig. 5a & b). Grubs chewed several sub-tunnels from the main feeding tunnel to expel the excreta and to aerate it for physiological processes (respiration). These subtunnels open to the exterior through a round circular hole, often covered with extruded frass (Fig. 5c). The cell sap is always oozing out through circular holes (openings of sub-tunnels). Grubs excavate large number of sub-tunnels in the main stem as compared to limbs of infested trees.Grubs chewed more wood than it could eat which is distinguishable from the round grains of excreta. The feeding tunnel is filled with a large amount of fibrous matter/chewed wood which has not been passed through the alimentary canal of the grubs. The excreta extruding through the sub-tunnels and frass packed in the main feeding tunnel was examined and has been found to be different from each other. The chewed wood packed in main feeding tunnel is fibrous while as excreta is fine to coarse in nature.



Month of observation

Fig. 2. Cumulative adult population of B. rufomaculata in mulberry farms of Jammu and Kashmir State



Fig. 3 Growing tips/apical buds of mulberry plants chewed by B. rufomaculata adults

| racie il e ilposition piere | Two i i o ripoblion prototono or Britigoniae analisioni ji togo or antiotoni alameteri. |                  |             |  |  |  |  |  |
|-----------------------------|---|------------------|-------------|--|--|--|--|--|
| Oviposition log group       | Log diameter (mm)   | No. of eggs laid | % eggs laid |  |  |  |  |  |
| Ι                           | $\leq 8$  | 0                | 0           |  |  |  |  |  |
| II                          | 9-16  | 89               | 3.99        |  |  |  |  |  |
| III                         | 17-24   | 980              | 43.94       |  |  |  |  |  |
| IV                          | 25-32   | 1161             | 52.06       |  |  |  |  |  |
|                             |   |                  |             |  |  |  |  |  |

Table 1. Oviposition preference of *B. rufomaculata* on mulberry logs of different diameter.

3.3.6 Duration and Number of Larval Instars

The duration of grub stage of B. rufomaculata, Cerambycid beetle under investigation, were observed to be  $253.35\pm4.37$  days (mean  $\pm$  SE; N=20). Larval stage duration was studied in the laboratory by rearing it from the egg through pupal stage. The data were recorded on day to day basis right from hatching of grubs to the onset of pupation. The grubs on hatching bored in the bark of host tree logs and started chewing the soft tissues, filling the bore with fine powder. Larvae of B. rufomaculata pass their life in the stem of infested trees which made it difficult to observe the number of larval instars directly. Therefore, the number of larval instars of B. rufomaculata were established by Dyar's rule (Dyar, 1890), which asserts that the growth ratio does not vary between moults, by measuring the width of head capsule of mature larvae sampled in the field. The head capsule width of first instar larvae were measured and reared through 2<sup>nd</sup> instar in the

laboratory to calculate the growth ratio (Dyar's ratio). The average head capsule width of 1st instar larvae was 1.27 mm (N=10) and that of  $2^{nd}$  instar was 1.64 mm (N=10). Thus the ratio of increase in head capsule width is 1.29. This ratio (Dyar's ratio) when multiplied with observed head capsule width of 1st instar larvae gives the expected head capsule width of 2<sup>nd</sup> instar which when multiplied again by Dyar's ratio gives the expected head capsule width of 3<sup>rd</sup> instar and so on (Table 2). A thorough look on table 2 reveals that the larval instars of *B. rufomaculata* are 9 as the expected head capsule width of 9<sup>th</sup> instar larvae is having insignificant difference (P>0.05) from that of the observed head capsule width of mature/last instar larvae. Expected values of head capsule width of all instars other than 9<sup>th</sup> instar are highly significantly different (P<0.05) from the observed head capsule width of last instar larvae of red spotted longicorn beetle, thus confirming its larval instars to be 9.



Age (weeks)

Fig. 4 Proportion of weekly oviposition of total, viable, nonviable, and aborted eggs by *B. rufomaculata* on mulberry logs.



Fig. 5 (a) Larva of *B. rufomaculata* boring down wards through sap wood (b) Larva in main feeding tunnel, preparing for pupation (c) Frass accumulated at the mouth of sub-tunnel arising from the main feeding tunnel

# 3.3.7 Overwintering of Grubs

Grubs overwintered from November to March in the host trees. In late November, grubs widen the feeding tunnel, excavated an elliptical chamber in heart wood for overwintering period. Onset of overwintering is marked by the presence of dry coarse fibrous frass at the mouth of sub-tunnels with subsequent stoppage of any fresh frass extrusion through sub-tunnels. Entrance holes to elliptical diapausing chambers were plugged with coarse fibers. Overwintering grubs did not fed, as no signs of feeding (fresh frass at the opening of sub-tunnels) have been observed during overwintering period. However, in rearing cages some grubs has been observed to feed meagerly during the overwintering period, as indicated by the accumulation of oval pellets of excreta in rearing jars. Grubs resumed feeding in March after an overwintering period of about 4 months until pupation which occured in the pupal chamber, prepared by the grubs in woody tissues at a distance of 18-25 mm from bark surface (outer surface of host trees). Grubs which were about to pupate extruded brick coloured excreta, which mark the onset of pre-pupal stage, stop feeding and pupated in the pupal chamber. Galleries which open in the pupal chamber were plugged with coarse wood fibers and the lining of the chamber was made by fine wood fibers. The pupal chamber is somewhat oval in shape measuring 60-73 mm in length and 21-29 mm in breadth.

# 3.3.8 Symptoms of attack

The first sign of attack is the wilting of growing tips and only the careful observer could reveal the signs caused by the feeding of adults (Fig. 3). These signs indicated the presence of adults and much careful observer could find out the beetles among thickets of mulberry trees. Later the gravid females made oviposition slits on the thick limbs or main stem (in case of host plants are below 6-7 years old) of mulberry plants. It is difficult to discover these sites, but cell sap oozing out through these silts is helpful to locate the oviposited sites. On opening up the slits one can discover the egg or newly hatched larvae. The newly hatched grubs extrude excreta mixed with fibrous matter through the holes, which could be revealed on close examination of the trees.

Latter, when grubs made their way into the heart wood of infested trees, piles of fresh frass could be

Growth ratio (Dvar's ratio) = 1.64/1.27 = 1.29.

seen on the opening of sub-tunnels and on ground below the infested stem (Fig. 5c).

Table 2: Comparison of expected and observed values of head capsule width (mm) of *B. rufomaculata* grubs.

| Mean observed head | capsule width | of 1 <sup>st</sup> | instar (N | √=10) = 1.27mi | m. |
|--------------------|---------------|--------------------|-----------|----------------|----|
| Mean observed head | capsule width | of 2 <sup>nd</sup> | instar (N | V=10) =1.64mn  | n. |

| Mean observed head capsule width of 9th instar (mature larvae) $(n=10) = 9.63$ mm |          |       |         |                                     |        |  |
|---|----------|-------|---------|-------------------------------------|--------|--|
| Larval Instar   | Expected | Head* | Capsule | Observed head capsule width of last | t-test |  |
|   | Width    |       |         | instar <sup>#</sup>                 |        |  |
| Ι   | 1.27     |       |         | 9.63±0.259                          | 33.44  |  |
| II  | 1.63     |       |         | 9.63±0.259                          | 32.00  |  |
| III   | 2.11     |       |         | 9.63±0.259                          | 30.08  |  |
| IV  | 2.72     |       |         | 9.63±0.259                          | 27.64  |  |
| V   | 3.51     |       |         | 9.63±0.259                          | 24.48  |  |
| VI  | 4.53     |       |         | 9.63±0.259                          | 20.40  |  |
| VII   | 5.85     |       |         | 9.63±0.259                          | 15.12  |  |
| VIII  | 7.54     |       |         | 9.63±0.259                          | 8.36   |  |
| IX  | 9.73     |       |         | 9.63±0.259                          | 0.40   |  |

\* Calculated from the mean of observed Dyar's ratio (r=1.29) # mean  $\pm$  SE

## 3.3.9 Larval infestation

The borer infestation was recorded in all the mulberry genotypes screened against the attack of *B. rufomaculata*. Infestation rate varied significantly among different age groups of mulberry plants (P<0.05). Red spotted longicorn beetle pestered least number,  $6.564\pm0.37\%$  (mean ±SE), of mulberry plants in the age group of 1-5 years, where as 12.69±0.66% (mean±SE), and 17.56±0.82% (mean ±SE) trees in the age groups of 6-10 years and above 10 years were attacked respectively. The infestation rate of age group 1-5 years is significantly different from that of 6-10 years (t=8.15; P<0.05; df=12); which in turn is again different from the above 10 year old mulberry plants (t=4.68; P<0.05; df=12). The difference in infestation rate between 1-5 year and above 10 year

old plants is highly significant (t=12.31; P<0.05; df=12). Among the genotypes Chakmajra variety showed slight resistance to the attack of longicorn beetle under study. Results revealed that infestation rate is significantly lower (P>0.05) in Chakmajra as compared to the other genotypes studied, where as it is highest in Goshoerami. The beetle pestered  $4.71\pm0.52\%$  (mean±SE),  $9.71\pm0.68\%$  (mean±SE) and  $13.00\pm0.61\%$  (mean±SE) plants of Chakmajra variety and  $7.5\pm0.5\%$  (mean±SE) plants of Goshoerami in age groups of 1-5 year, 6-10 year and above 10 years respectively. Infestation rate among other genotypes of different age groups ranged between 6.00-18.87% (Table 3).

| Table 3: Infestation rate of <i>B</i> . | rufomaculata | among different age | groups of  | mulberry plants. |
|---|--------------|---------------------|------------|------------------|
|   | ~            | 0 0                 | <b>U</b> 1 | 21               |

| Genotype      | Infestation rate (mean $\pm$ SE | Infestation rate (mean $\pm$ SE) in different age groups* |                          |  |  |  |
|---------------|---------------------------------|---|--------------------------|--|--|--|
|               | 1-5 years                       | 6-10 years  | Above 10 years           |  |  |  |
| Goshoerami    | $7.50^{a}\pm0.50$               | $15.50^{b} \pm 1.50$                                      | 19.50 <sup>c</sup> ±1.50 |  |  |  |
| Chinese white | $7.12^{a}\pm0.55$               | $13.50^{b} \pm 0.32$                                      | 18.87 <sup>c</sup> ±0.69 |  |  |  |
| KNG           | $6.00^{a}\pm1.00$               | $12.50^{b}\pm0.50$  | 17.50 <sup>c</sup> ±0.50 |  |  |  |
| Tr10          | $6.65^{a}\pm042$                | $12.37^{b}\pm0.46$  | $17.87^{c} \pm 0.63$     |  |  |  |
| Chakmajra     | 4.71±0.52                       | 09.71±0.68  | 13.00±0.61               |  |  |  |
| Mandalay      | $6.57^{a}\pm0.57$               | $12.14^{b}\pm 0.59$                                       | $17.57^{c} \pm 0.84$     |  |  |  |
| V-1           | $7.42^{a}\pm0.48$               | $13.14^{b} \pm 0.82$                                      | 18.85 <sup>c</sup> ±0.98 |  |  |  |

\*Means followed by the same superscripts are not significantly different (P>0.05)

3.3.10 Nature and extent of damage

Adults caused damage by chewing the growing tips and debarked the tender twigs (Fig. 3). The attacked twigs wither and break off easily. Growing tips of 5.6% twigs were chewed by the

beetles in the infested mulberry plots, resulted in their stunted growth, thus reduction in leaf yield. However, the damage caused by adults is compensated to some extent by sprouting/growing of lateral buds. Grubs damage plants by tunneling through the bark into wood which transports nutrients and water to the leaves. Their infestation, so the damage, often go unnoticed until plants or parts of plants die. Grubs made oval round tunnels in the stem (Fig. 6), reducing the yield and vigour of trees and ultimately lead the plants to die retrogressively. The irreparable damage caused by grubs has been assessed by studying the growth/yield parameters of infested and non-infested (control) mulberry plants.



Fig. 6. Cross section of mulberry plant showing larval tunnels of *B. rufomaculata*.

Leaf yield studies revealed that *B. rufomaculata* caused significant damage to mulberry plants (P<0.05). The borer infestation reduced the leaf yield by 6.952% to 12.193% in different genotypes (Table 4). The maximum loss in leaf yield was cased in Chinese white (12.193%) and the least in Goshoerami (6.952), while as in Chakmajra, Tr-10 and V-1 varieties, 10.454%, 11.153 and 10.244% loss were caused by the borer infestation respectively. Though the damage caused in the given genotypes is different, but it is statistically insignificant among the varieties (P>0.05).

*B. rufomaculata* caused considerable reduction in leaf moisture content of mulberry (Table 5). The maximum moisture content loss was recorded in Chinese white variety (7.75%) followed by Goshoerami (5.47%), Tr-10 (4.87%) and V-1 (4.70%) while as least was recorded in Chakmajra (4.66%). However, the difference in moisture content reduction among different varieties of mulberry is statistically insignificant (P>0.05).

Bioassay revealed that the borer infestation in mulberry plants degraded leaf quality. The reduction in economic characters like cocoon weight, shell weight and filament length is significant (P<0.05), however, loss in shell ratio due to the borer infestation is insignificant (Table 6).

| Table 4. Lear yield of maloerry varieties (mested and non mested). |                                      |            |                       |         |  |  |  |
|--|--------------------------------------|------------|-----------------------|---------|--|--|--|
| Genotype   | Leaf yield (Kg/plant; mean $\pm$ SE) |            | Leaf yield* reduction | t-ratio |  |  |  |
|  | Non-infested                         | Infested   | (%)                   |         |  |  |  |
| Goshoerami   | 7.753±0.13                           | 7.214±0.11 | 6.952                 | 3.17    |  |  |  |
| Chinese white  | 7.504±0.20                           | 6.589±0.17 | 12.193                | 3.53    |  |  |  |
| Chak majra   | 7.241±0.19                           | 6.484±0.16 | 10.454                | 3.06    |  |  |  |
| Tr10   | 7.289±0.19                           | 6.476±0.19 | 11.153                | 3.08    |  |  |  |
| V-1  | 7.409±0.26                           | 6.650±0.02 | 10.244                | 2.73    |  |  |  |

Table 4: Leaf yield of mulberry varieties (infested and non-infested).

| J = 1.517 |
|-----------|
|-----------|

Table 5: Leaf moisture content of mulberry.

| Genotype      | Moisture content % (mean $\pm$ SE) |                     | Moisture reduction | t-ratio |
|---------------|------------------------------------|---------------------|--------------------|---------|
|               | Non-infested                       | Infested            | (%)                |         |
| Goshoerami    | 74.94 <u>+</u> 0.25                | 70.84±0.48          | 5.47               | 7.56    |
| Chinese white | 75.16 <u>+</u> 0.25                | 69.33±0.36.         | 7.75               | 13.37   |
| Chakmajra     | 73.48 <u>+</u> 0.22                | 70.05 <u>±</u> 0.55 | 4.66               | 5.78    |
| Tr10          | 72.94±0.43                         | 69.40±0.27          | 4.87               | 6.90    |
| V-1           | 74.36±0.31                         | 70.86±0.41          | 4.70               | 6.82    |

| Table 6: | Bioassay | studies | of infested | and | non-infested | mulberry | plants |
|----------|----------|---------|-------------|-----|--------------|----------|--------|
|          | 2        |         |             |     |              |          |        |

| Parameter           | Non-infested (mean±SE) | Infested (mean±SE) | % loss | t-ratio |
|---------------------|------------------------|--------------------|--------|---------|
| Cocoon weight (g)   | 1.704±0.030            | $1.460\pm0.024$    | 14.31  | 6.43    |
| Shell weight (g)    | 0.319±0.011            | 0.253±0.007        | 20.68  | 5.00    |
| Shell ratio (%)     | 18.71±0.51             | 17.30±0.33         | 7.53   | 1.34    |
| Filament length (m) | 871±37                 | 692.7±17           | 20.47  | 4.28    |

#### 4. Discussion

*B. rufomaculata* is a polyphagous pest attacking more than 50 tree species in Oriental region and has been earlier reported from Burma, Ceylon, Israel, Pakistan and West Indies (Beeson, 1941; Duffy, 1968). In India, it has been reported from Assam, Bihar, Himachal Pradesh, Punjab, Maharashtra, Utter-Pradesh, Tamil Nadu, West-Bengal and Jammu and Kashmir (Stebbing, 1914; Beeson and Bhatia, 1939; Beeson1941; Sharma and Tara, 1985; Hussain *et. al.* 2009). Husain and Khan (1940) reported that *B. rufomaculata* infest a large number of tree species in tropical and sub-tropical regions and present investigator made similar observations regarding its distribution.

Beetles emerged from May through September; the observation is in conformation with that of Husain and Khan (1940) and Beeson (1941) who reported the emergence of the beetle in North India from May to August and March to August respectively. Lamiines are known to have a longer life span than species of other Cerambycid subfamilies (Linsley, 1959; Hanks, 1999), so their presence in the field for a month or so after the emergence period is not a matter of debate.

Adult feeding is common in Lamiine species and almost all species of the subfamily require maturation feeding period and Lamiine species feed for an average of 6.7±1.2 (mean±SE) days to attain sexual maturity (Linsley, 1959; Hanks, 1999). B. rufomaculata, Cerambycid beetle under study, being a member of Lamiinae required a maturation feeding period of 14±0.561 days (mean±SE) which seems to be in contradiction with that of Hanks 1999, but, this is not the case, because, the author reviewed the entire subfamily (Lamiinae) and the difference is due to the species specificity. Present findings are also in conformation with that of Dijia and Yining (1997) who studied the biology including the maturation feeding of another longicorn beelte, Anoplophora glabripennis Motschulsky and reported that adult feeding is essential for the maturation of germ cells and improvement of copulation.

In laboratory conditions the beetles mated repeatedly, which is a common observation among Lamiine species (Beeson, 1941; Adachi *et al.*, 1992; Fukaya, 2004). The beetle did not display any courtship behaviour and males directly approached females and mated successfully. The observation of Wang *et. al.* (1996) on anther longicorn, *Phytoecia rufiventris* confirms our finding strongly. Besides, the present observation is at par with that of Hanks (1999) in that most of the Lamiines does not display any precopulatory courtship behaviour. Smaller males of *B. rufomaculata* got least chance to secure their mate in the laboratory and hence inferior to larger males in

acquiring females. In insects mating behaviour and reproductive success are related to the body size; larger males attain higher reproductive success through sexual selection (female choice) and malemale aggression over female (Thornhill and Alcock, 1983; Lawrence, 1986; Goldsmith *et al.*, 1996).

Husain and Khan (1940) reported that B. rufomaculata laid eggs singly in transverse slits, cut by mandibles in the limbs and stem of fig trees; in the present study it was observed that the beetles chewed the oviposition niches in the bark and deposited their eggs singly under the bark of oviposition logs, more than 9mm in diameter, however, only 3.92% eggs were deposited in oviposition logs ranging between 9-16mm in diameter. Daily and life time fecundity of the Cerambycid beetle under study averaged 1.27 eggs and 148.66 eggs respectively and it is positively correlated with the body size of females. Smith et al. (2002) reported the same relation between the fecundity rate and body size of females in another beetle, Anoplophora longicorn glabripennis Motschulsky. Longicorn beetles locate their hosts by olfaction (Linsley, 1961) and the same behaviour was observed in the lonicorn beetle under study to locate their host plants in the present study.

The observation on the feeding behaviour of grubs of the beetle revealed that the newly hatched larvae feed on the inner bark and latter made their way in the heart wood via sap wood. The galleries excavated in the bark were zigzag, but in the heart wood they were more or less straight. Husain and Khan (1940) observed that larvae feed in the inner portion of bark for a considerable period, making zigzag tunnels, however, the authors reported that if the eggs were laid in small branches, then the larvae enters into the wood very soon and its path is not zigzag, but it enters into the wood of the branch and makes a straight tunnel, thus confirming the present study. Grubs chewed several sub tunnels from the main feeding tunnel to expel excreta and chewed wood fibers, however, a small proportion of the latter were retained in the main feeing tunnel just below the openings of sub-tunnels to plug the tunnel and bypass the sap flow to the exterior through these sub-tunnels.

The present observations revealed that *B. rufomaculata* is a univoltine (one generation per year) species with an average larval stage of 253.35 days; this period includes the over wintering period of about 4 months. Husain and Khan (1940) reported the larval stage of the beetle varies from 3-6 months; Butani (1978) and Palaniswamy *et al.* (1979) observed the larval stage of the longicorn beetle to be 6 months and about 5 months respectively.

The concealing nature of grubs of the longicorn beetle made it difficult to observe the number of larval

instars directly and the same problem has been faced by Duffy (1946), Palaniswamy *et al.* (1979), and Logarzo *et al.* (2002) to ascertain the number of larval instars of different longicorn beetles. These workers employed Dyar's ratio (Dyar, 1890) to determine the number of larval instars. In the present study expected head capsule width of grubs was calculated by Dyar's ratio (table 22) and measured the head capsule width of mature larvae. Student's t-test revealed that head capsule width of mature larvae more or less coincide (difference between observed and expected head capsule width insignificant) the expected head capsule width of 9<sup>th</sup> instar, thus the number of larval instars of the longicorn beetle are 9.

The pupae of the longicorn beetle were naked in the pupal cells, prepared by the mature larvae in the stem. Mature overwintering larvae pupated in the month of April while immature overwintering larvae resumed feeding and pupated latter. Mean pupal period of 27.5 days in B. rufomaculata was recorded in the present study which is in confirmation with Palaniswamy et al. (1979) observed the pupal period of 29.1 days in the longicorn beetle under question. However, current observation is in contradiction with that of Husain and Khan (1940). The variation in the developmental period is insects is a natural phenomenon and has been observed by Solomon (1977), in Goes debilis LeConte; Jolles (1932) in Cerambyx dux Fald.; Ahmad et al. (1977) in Aeolesthes sarta; all belong to Cerambycidae.

B. rufomaculata is a polyphagous pest attacking more than 50 species including mulberry plants (Husain and Khan, 1940; Beeson, 1941; Duffy, 1968; Sharma and Tara, 1995). Preliminary work carried out by Sharma and Tara (1995) on the infestation rate revealed that Chakmaira variety is relatively resistant to the attack of B. rufomaculata and in the present study Chakmajra variety continued to be relatively resistant as compared to the other genotypes (table 23). Among the rest of the screened genotypes, the difference in the rate of infestation was insignificant by Student's t-test (p=0.05). Infestation rate was significantly different among different age groups, least (6.564±0.368%; mean±SE) in 1-5 year age group and highest (17.594±0.817%; mean±SE) in above 10 year old plants.

Adult longicorn beetles relatively cause negligible damage to the host plants (1941; Beeson and Bhatia, 1939; Husain and Khan, 1940; Craighead, 1950; Hussain, 1972; Hay, 1969); however, Linsley (1959, 1961) and Hanks *et al.* (1990) reported that the nature and extent of damage is species specific. The adults of *B. rufomaculata* chewed the growing tips of mulberry plants which resulted in their withering followed by loss of meristematic activity (Fig. 25). Husain and Khan (1940) reported that the shoots of fig trees attacked by adult beetles (*B. rufomaculata*) remain stunted and the leaves of which the petioles are damaged fall off. The foregoing discussion reveals that damage caused by adult Cerambycids is species specific and depends on population size.

The grubs of Lamiine species caused wide spread mortality among many deciduous trees in China (Yang et al., 1995) and the damage caused to the infested trees is irreparable. Husain and Khan (1940) reported that grubs of B. rufomaculata does not kill the infested tree immediately, but the tree may continue to live for a considerable time, even years, but finally it dries up; indicating that the borer infestation causes irreparable damage. The current observation revealed that the damage caused by grubs of B. rufomaculata to mulberry plants in terms of quality and quantity of leaf is significant. The extent of damage caused is irrespective of the mulberry variety, though all screened genotypes were equally susceptible to it, however, Chakmajra variety offered slight resistance to *B. rufomaculata*, but of no avail.

Wood borers not only reduce the growth and vigour of host trees, but also reduce the value of the products made from attacked trees (Hay and Wootten, 1955; Bryan, 1960; Moris, 1964; Donley, 1974). Solomon (1968, 1972, 1974, 1995) and Yang et al. (1995) reported that intensity of damage caused by an insect species varies among host trees. Cerambycid borers cause considerable damage to their hosts and extent of damage is host species specific (Linsley, 1959; Donley, 1974; Donley and Terry, 1977; Goodwin et al., 1994; Goodwin, 2005). The present observations revealed that the intensity of damage caused by B. rufomaculata varied among different varieties of the host tree. The foregoing discussion depicts that the Lamiine species cause considerable damage to their hosts and the damage intensity is a dynamic phenomenon.

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