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Life Cycle and Biology of Mango Bark Beetle, *Hypocryphalus mangiferae* (Stebbing), A Possible Vector of Mango Sudden Death Disease in Pakistan

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Abstract.- The activity of *Hypocryphalus mangiferae*, Stebbing (Coleoptera: Scolytidae**)** was observed in mango orchards with the help of trap tree logs and its biology was studied in the laboratory. *H. mangiferae* usually preferred diseased and stressed mango trees and bored tiny holes (1.9-2.0mm in diameter) in the main tree trunk from which frass was emitted out. It remained active in terms of number of holes per trap log, from February to November to variable degrees. The maximum activity was observed in the month of May. The maturation feeding lasted for 6-8 days and the females laid 42.3±9.91 eggs. The five larval instars were distinguished from each other on the basis of the width of head capsule. The larvae completed their development in 16.90±2.12 days by constructing irregular galleries. The pupation period lasted for 3.85±1.41 days and finally the adult emerged that lived for 22.9±3.53 days.

Key words: Mango bark beetle, *Hypocryphalus mangiferae*, mango sudden death disease.

INTRODUCTION

Mango (*Mangifera indica* L., Anacardiaceae) is universally known as one of the finest fruit crops in tropical and subtropical areas of the world. The mango production has been declining due to various biotic and abiotic factors. Mango Sudden Death Syndrome (MSDS) is one of the major threats to mango industry. In Pakistan, yield of mango trees has been impaired by primary insect pests such as mango hopper, *Idioscopus clypealis* (Leth), midge, *Erosomya indica* (Grover and Prasad), mealybug, *Drosicha stebbingii* (Green), scale, *Aulacaspis tubercularis* (Newstead), and fruit fly, *Bactrocera dorsalis* (Hendel) (Mohyuddin and Mahmood, 1993; Pena *et al*., 1998). Besides these, the mango bark beetle, *Hypocryphalus mangiferae* (Stebbing) has frequently been observed on diseased mango trees (Saeed *et al*., 2007).

Bark beetles (Scolytidae: Coleoptera) are the most important forest pests. More than 6000 species of bark beetles have been described in the tropical and sub-tropical regions of the world (Lieutier *et al*., 2004). Bark beetles and pathogens interact to cause expensive losses in forest trees *e.g.* oak dieback in Japan (Ito *et al*., 1998), which is caused by ambrosia fungi, transmitted by an ambrosia beetle, *Platypus quercivorus*. Spruces (*Picea* sp.) are destroyed by the great spruce bark beetle, *Dendroctonus micans* (Kug), which is a primary pest in Euroupe (Storer *et al*., 1997).

The mango sudden death disease has been reported from Oman and Brazil and is consistently associated with a bark beetle, *Hypocryphalus mangiferae* (Ribiero, 1980; Al Adawi *et al*., 2006). The *H. mangifera* prefers diseased or dried portion of wood and makes tiny holes about 2mm in diameter from which sawdust is emitted. Upon peeling off the bark from the woody stem, irregular galleries can be observed having black appearance due to presence of a fungus in their galleries (Saeed *et al*., 2007).

The biology of *H. mangifera* is very important towards investigations of its role as a possible vector of MSDS. There are three phases of bark beetle life cycles *i.e.* reproduction, development and maturation leading to dispersal. After mating, they make a breeding gallery the form of which is fairly species specific. All the development stages feed under the inner bark. After maturation feeding, adult emerges from brood material and colonize healthy or stressed plants (Lieutier *et al.*, 2004). Most of the species of bark beetles breed in slash, broken, fallen, dying, or large

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limbs of trees but some are capable of primary attack on healthy trees when conditions are favorable (Wood, 1982). Therefore, bark beetles are attracted towards stressed timber, while healthy trees are normally defended by chemical or physical means (Speight *et al*., 1999). Placing dry tree logs near the vicinity of infected trees could be a very effective mechanism of monitoring bark beetle population (Stone and Simpson, 1990; Lawson, 1993).

There is no systematic information on the biology of *H. mangiferae* except few reports of its host plant associations (Beeson, 1941). Therefore, there was need to study the biology of bark beetle on mango plant so as to link its role with the etiology of MSDS in Pakistan. The main objectives of the current study were to: (i) monitor the activity of *H. mangiferae* throughout the year under natural field conditions and (ii) investigate the life cycle and biology of *H. mangiferae* under controlled laboratory conditions.

MATERIALS AND METHODS

Field studies

The study area was situated at a village "Faiz pur Bhuttia" near Khanewal Road, Multan at GPS position **(**30.270º N and 71.250º E). We selected two mango orchards suffering from bark beetle attack for the last three years. The one orchard comprised 2000 trees with total area of 25 acres and the other orchard of 3500 trees with total area of 15 acres. There were considered as high density population orchards. The most frequently planted commercial varieties were Chounsa, late Chounsa, White Chounsa, Ratol, Dosehari, Sindhari, Langra and occasionally Desi and Mehammad khan wale.

The visual damage pattern *i.e.* spatial distribution of holes and gallery pattern under the bark by *H. mangiferae* was recorded during the mango orchards survey in 2007. The activity of *H. mangiferae* was monitored by using tree logs during 2007-08 as described by Tavakilian *et al.* (1997). In 1st week of January, ten fresh tree logs (50-60 cm long and 30-35 cm diameter) were placed at a distance of 1 ft around the trunk of the infected alive tree with clear symptoms of bark beetle attack *i.e.* circular holes with frass. The activity of bark beetle in the logs was recorded by counting the number of holes at the end of each month, followed by installation of the new fresh logs in the same fashion around same or some other infected trees depending on tree conditions *i.e.* completely dried and dead trees were avoided for selection or reselection.

Laboratory studies

The biology of bark beetle was studied at Mango Research Laboratory, University College of Agriculture, Bahauddin Zakaryia University, Multan. The laboratory culture of bark beetles was maintained by placing infected logs (logs 50-60 cm long and 30-35 cm in diameter) in rearing cages with dimensions of 61x45x45 cm, made up of cast acrylic sheets fitted with a black mesh cloth (0.5 mm gauze) on its top end. A transparent collecting jar was fitted at the bottom end with an artificial light source. To maintain the culture, some fresh logs were placed with the infested logs inside the rearing cages with ten days of intervals.

The biological characteristics of bark beetle were studied by using disc method (Miller and Borden, 1985; Storrer *et al*., 1997). The discs (3 cm diameter and 1 cm in width) were cut from undamaged and healthy looking mango tree stems by using a sharp saw. The freshly emerged beetles were collected from tree logs and reared on modified mango discs at the rate of one pair (1:1) per disc. Each disc was then placed singly in a pressed thermopore glass having filter paper at the base to absorb excessive moisture, which prevented the development of fungal growth. The discs were then placed in the incubating chamber at 25°C and 60% relative humidity.

A total of 200 discs were prepared which were dissected with the help of sharp knife on daily basis to study the various stages of bark beetles. Characters of different developmental stages including egg, larva, pupa adult and maturation feeding were observed under stereomicroscope (Olympus, SZ 60X). Larval instars were distinguished from each others based on moulting and head capsule width by using microscope with ocular micrometer. Fernandez *et al.* (1999) used this head capsule measuring technique for describing various larval instars of *Tomicus minor* on pine tree.

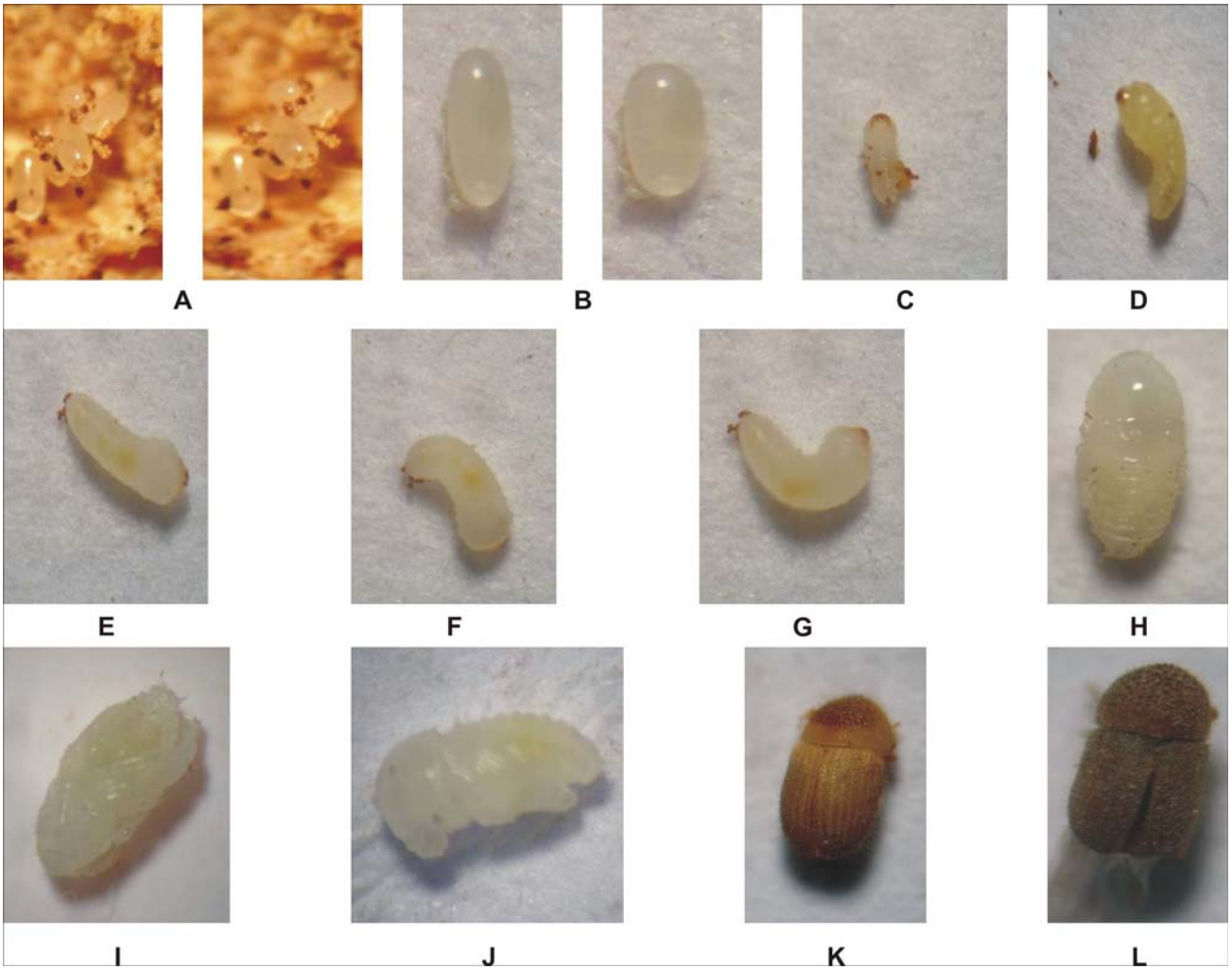

Fig. 1. Eggs and developmental stages of *Hypocryphalus mangiferae*; A, eggs in wood chamber; B, individual egg; C, 1st Instar; D, 2ndInstar; E, 3rd Instar, F, 4th Instar, G, 5th Instar; H, Pre-pupa; I, pupa with appendages; J, pupa near adult emergence; K, immature adults with yellowish body; L, Mature adult with dark brown body.

RESULTS AND DISCUSSION

Life history of H. mangiferae

Egg stage

The eggs (Fig. 1A, B) are cylindrical, shining and creamy white in color. Both the ends of the eggs are broadly rounded. The eggs hatched after 4.4±0.707 days with average width 0.25±0.025 mm and length of 0.53±0.033 mm (Table I). Each female laid 4.2±1.41 batches at the rate of 10.2±1.41 eggs per batch. Therefore, on an average, 42.3±9.90 eggs were laid per female (Fig. 2).

Larval stage

After hatching, larval duration was recorded on the basis of moulting process and five larval instars were observed (Fig. 1C-1G). The width and length of 1st and last full-grown larvae were distinguished on the basis of head capsule (0.21±0.039, 0.63±0.004 and 0.65±0.025, 2.06±0.090, respectively). The larval phase lasted for 16.90±2.121 days (Table I). The larvae, on hatching were legless, wrinkled, minute, cylindrical and creamy white in color. In typical larvae, head capsule is pale-tan to pale-rusty.

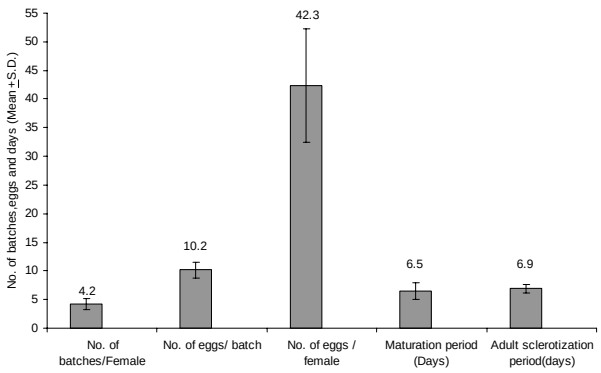

Fig. 2. Biological characteristics of *H. mangiferae* under laboratory conditions.

Table I.- Life sages of *H. mangiferae* and its body measurements.

Life stages	Duration (Days) (n=15)	Body measurement (mm) (n=10)	
		Width	Length
Egg	4.4±0.707	0.25 ±0.025	0.53±0.033
1st larval Instar	2.65±1.06	0.21±0.039	0.63±0.004
2nd Instar	3±0.707	-	-
3rd Instar	3.65±1.06	-	-
4th Instar	4±0.353	-	-
5th Instar/ Full grown	3.75±1.41	065±0.025	2.06±0.070
Overall larval period	16.90±2.121		
Pupa	3.85± 1.41	0.77 ±0.064	1.82±0.057
Adult	22.9±3.53	0.63±0.002	1.60±0.086

Pupal stage

The pupation took place near the exit hole. The pupae (Fig. 1H-1J) were soft and creamy white with 0.77±0.064 mm width and 1.82±0.057 length. Near maturity it turned pale tan. The pupal stage lasted for 3.85±1.41days. Immature adults after emergence from pupae were initially white. The process of adult sclerotization was completed in 6.9±0.73 days during which it changed from light brown to dark brown in color. Newly emerged adults started boring after 48 hours of their introduction on new healthy discs.

Adult stage

The adults (Fig. 1K, L) were cylindrical with average body length of 1.60±0.086 mm and width of 0.63±0.002 mm. It lasts for 22.9±3.9 days. The adults were cylindrical with shining dark brown pronotum had punctures on its surface (Bright and Skidmore, 2002). Eggs are laid along the margin of parental galleries and developing larvae mine into the inner bark tissue and complete their development in the pupal cells constructed at the end of larval feeding gallery of scolytid beetle (Raffa and Berryman, 1993).

Maturation feeding

After the emergence from pupae, immature bark beetles undergo maturation feeding for fully sclerotization. Maturation period was followed by sclerotization period *i.e.* 6.9±0.71 and 6.5±0.71 days, respectively (Fig. 2). In laboratory experiments, discs dissection (probing the entrance holes) revealed that maturation feeding lasted for five days during which immature adult (light brown in color) changed into mature adult (dark brown in color). Egg laying was started on 6th day in batches with average of 42 eggs laid by female and the maximum eggs were laid during 7th and 8th day *i.e.* 50 and 60 eggs laid, respectively. The maturation period of *Scolytus nitidus* and *S. mali* on apple was also found to be less than *H. mangiferae* on mango (Rudinsky *et al*., 1978; Buhroo and Lakatos, 2007). In *H. angustatus*, this activity of feeding was also observed under the bark of pine tree (Toit, 1975). The maturation feeding is very critical in the life cycle of the bark beetle as this acts as a carrier of disease spores for further disease transmission (Lieutier *et al*., 2004). Leech (1997) also observed that *Ips pini* and *I. grandicollis* are contaminated with fungal spores after emergence and searched for the suitable pine trees during their maturation period.

Characteristic damage pattern of H. mangiferae

The bark beetle, *H. mangiferae* was frequently observed on mango trees infected with sudden death disease. They made tiny holes (1.9-2.0 mm diameter) from which saw dust was emitted out (Fig. 3). It is a characteristic damage pattern of this beetle which is also found in other beetles of subfamily Scolytinae like pine bark beetle, *Hylastes angustatus* in Europe and different species of *Ips i.e.*

Fig. 3. Holes made by adults of *H. mangiferae.*

Fig. 4. Irregular gallery made by larvae of *H. mangiferae* under the bark.

Ips caligraphus and *I. gradicollis* on pine trees. Like *H. mangiferae*, these bark beetle species attack healthy as well as diseased and freshly cut logs of tree (Speight and Wylie, 2001). The attack of *H. mangiferae* was greater on the base of the main stem having thick bark as compared to upper branches (Roonwal, 1978). Similarly, *I. caligraphus* also prefers thick bark of trunk and large branches of pines in Jamaica (Speight and Wylie, 2001). On peeling off the mango bark from the woody stem, irregular galleries were obvious with black appearance. Such kind of damage pattern has also been reported in *Dendroctonus* sp. of family Scolytidae (Lieutier *et al.*, 2004). During the active period, frass was observed scattered on ground around the main trunk. This frass may act as a fungal (*Ceratocystis* sp. and *Lasiodiplodia theobromae*) inoculum of sudden death disease of mango, which is disseminated through the irrigation water (Al Adawi *et al*., 2006). This dissemination is additionally supported by air currents (Morales and Thomas, 2007).

Gallery pattern

In *H. mangiferae*, the pattern of the mother gallery was irregular (Fig. 4). Larval galleries were branched, originating from the mother galleries. Pupal chambers were present near the exit holes. The pattern of gallery is species specific which could be longitudinal (*Tomicus piniperda*, *Solytus scolytus*), transverse (*Tomicus minor*), circular (*Cryphalus piceae*) or irregular (*Dendroctonus micans*) (Lieutier *et al*., 2004). The female arrives first and excavates the entrance hole of a breeding gallery under the bark where a single male joins her. The female beetle lays eggs in the main gallery (Lieutier *et al*., 2004).

Seasonal activity pattern of H. mangiferae

During this study, freshly cut trap logs were used to observe the activity pattern of the beetle throughout the year. Speight *et al.* (1999) documented trap logs as a very efficient monitoring method. This method has also proven to be very effective in monitoring of tropical longhorn beetle (Cerambycidae: Coleoptera) in French Guiana (Tavakilian *et al.*, 1997). Lawson (1993) had already put his efforts in estimating the population of bark beetle; *Ips gradicollis* on Australian pine by using tree logs in the infested forest.

The visual observations by splitting the bark revealed that *H. mangiferae* overwintered under the phloem portion of mango as an immature adult having light yellowish appearance. This over wintering was done from late November to early February to avoid low temperature effects (15°C, R.H 83 %). Its activity was resumed in mid January and remained active up to November with variable activity pattern depending on temperature and humidity (Fig. 5). The maximum activity was recorded during May (386±171 holes) up to August (132±131 holes) (Fig. 6). Their emergence from infested tree was decreased due to summer rains

during June and July and increased in August (97±57, 73±68 and 133±131 holes respectively) per log. Similar type of seasonal activity has also been documented in the pine bark beetle, *Phloeotribus scarabaeoides* (Bernard) (Gonzalez and Campos, 1994).

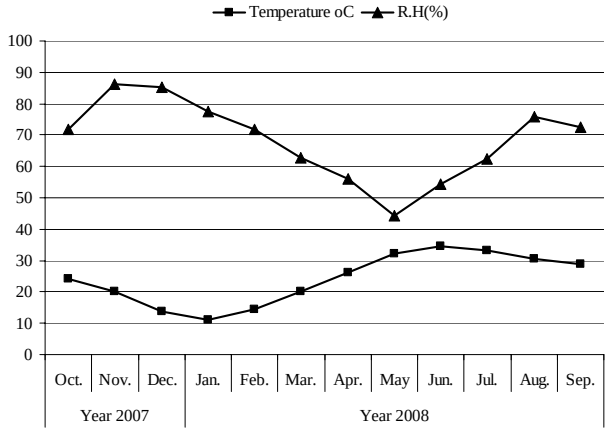

Fig. 5. Average monthly tempetature and relative humidity during year 2007-2008 in Multan.

The active period in the life cycle of bark beetle started from May to August, which is very much critical in the dissemination of sudden death disease spores in mango orchard. Due to its consistent association with diseased tree, *H. mangiferae* may be vector of fungal spores of mango sudden death disease (Al-Adawi *et al*., 2006). For management of bark beetle attack, cultural techniques like removal of branches pruned by farmers and destruction of trap logs after the infestation. An alternative method of control for *P. scarabaeoides* is chemical treatments with formathion, dimethoate and pyrathroid insecticides (Lozeno *et al*., 2001). For integrated control, ethylene is used for attraction of bark beetle and combined with insecticides for effective control (Pena *et al*., 1998a; Rodriguez *et al*., 2003). In Pakistan, fungicides and insecticides were used as a curative measures against diseases and insect pests irrespective of bark beetle (Saeed *et al*., 2007). Therefore, it is necessary that chemical treatments could be used before the emergence of *H. mangiferae* on infested tree so that healthy mango trees might be prevented from colonization of bark beetle and ultimately fungal spores' transmission.

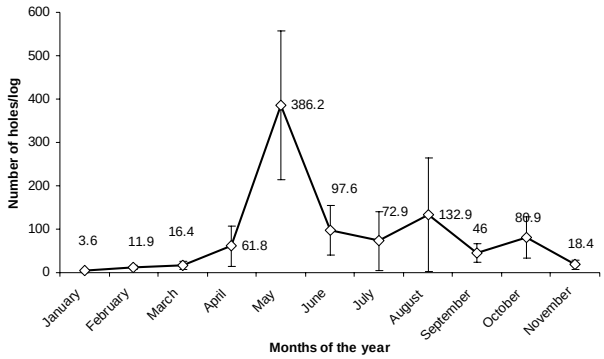

Fig. 6. Activity in term of holes of *H. mangiferae* during the year 2007-08 in Multan.

The minimum activity was observed during the overwintering period from November (18±10 holes) to early February (11.9±4.9 holes) per log. Like *H. mangiferae*, most other species of the bark beetles over winter in their breeding sites, waiting for the spring to disperse out (Lieutier *et al*., 2004).This behaviour has also been documented in other monogamous species like *Scolytus*, *Trypodendron* and *Tomicus* spp on pine and elm trees (Lieutier *et al*., 2004). The annual activity dynamics reveals that *H. mangiferae* may have 3-4 overlapping generations per year. The same number of generations was produced by *Scolytus nitidus* Schedl (Scolytidae) on apple and *S. amygdali* fruit trees of Kashmir and Balochistan (Jajua and Samuel, 1941; Buhroo and Lakates, 2007).

In Pakistan, mango survey has revealed that damage and feeding habit of the bark beetle *H. mangiferae* are mostly found on the diseased mango trees (Saeed *et al*., 2006). Reports from Oman and Brazil have also held this suspicion that it may be the possible vector of mango sudden death disease. The *H. mangiferae* also makes wounds in the trunk that might be the entry points of pathogens (Al Adawi *et al*., 2006). Similarly, other bark beetle species and ambrosia beetles are also studied as a possible vector of diseases. Association between *Ips pini*, *I. grandicollis* and blue stained fungi on pine trees was investigated which showed similar feeding and maturation behavior like *H. mangiferae* (Leach, 1997). The biology of *Scolytus multistriatus* also

revealed that it is the principal vector of Dutch elm disease and has similar feeding habit like *H. mangiferae*. This bark beetle species was also reported on apple, peach, elm and mountain ash tree as an effective vector of fungal spores (Leach, 1997). In Japan, oak decline is also associated with *Platypus quercivorus* through boring activity on the tree trunk and twigs (Ito *et al*., 1993). Therefore, biology of *H. mangiferae* is very important in relation to mango sudden death syndrome.

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