CHAPTER II

Morphological analysis of Helopeltis theivora and infested tea leaves
2.1. Introduction

Plant bugs of the genus *Helopeltis*, sometimes called mosquito bugs, are serious pests of many cultivated crops worldwide, particularly major cash crops like tea, cocoa, cinchona, cashew and pepper (Stonedahl, 1991). There are more than 40 known species spread across Western Africa, Sri Lanka, Bangladesh, India, Papua New Guinea to Northern Australia. They have generally been regarded as only minor pests of forest trees, occasional damage have been reported from *Swietenia*, *Terminalia*, *Cinnamomum* and *Melia* but there are recent instances of severe damage being caused to young eucalypt and acacia plantations (Wylie et al., 1998). *H. theivora* has been reported to cause 80% infestation resulting in crop loss to the tune of 10-50% in tea from NorthEast India (Roy et al., 2010).

In order to study differential gene expression in tea due to insect feeding, as suggested by various authors (Felton et al., 1994; Stout et al., 1994, 1998; Alborn et al., 1997; McCloud and Baldwin, 1997; De Moraes et al., 1998; Felton and Korth, 2000; Kahl et al., 2000; Reymond et al., 2000; Musser et al., 2002a, b) it is desirable to begin the investigation by analyzing the pattern and extent of damage on the tissue. This chapter deals with studying the macroscopic and microscopic changes associated with initial and extended exposure to infestation, the tissue adjoining the infested area and observable changes affecting the whole plant-all at the morphological level. The chapter also discusses about the pest in detail-its taxonomic position, morphology (macro and micro level), mouthparts, habitat, life cycle and activity. SEM studies on pest mouthparts to unravel the arms race between herbivores and plants was reported earlier by Musser et al., (2002a).

2.1.1. Taxonomic position:

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Family          Miridae
Subfamily       Bryocorinae
Tribe           Dicyphini
Sub-tribe       Manaloniina
Genus           Helopeltis
Species         Helopeltis theivora waterhouse,1886
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2.1.2. *Helopeltis theivora*

Adults are black usually with greenish abdomen, characterized by very long antennae and black but with pale basal segment. Head and wings are black. A small drumstick like process can be seen standing vertically on the upper side. Eyes are prominent (Fig. 2.1). Appendages consist of three pairs of legs, one pair of antennae and one extended proboscis (Tea Board, India).

![Fig. 2.1 Adult (a) and instar(b) of *H. theivora*](image)

Adults are quick but not strong flyers. Females (7.5 mm) are bigger than males (6.3 mm) having curved ovipositor, males are lighter in colour than females.

2.1.3. Life cycle: Eggs, Nymphs and Adult

The life cycle generally spans 45-50 days, with nymph development period varying from 20-30 days with five nymph instar stages and one adult (Fig 2.2). Eggs are cylindrical, slightly curved with two filamentous structures at one end. Eggs remain inserted in young leaves and buds with filamentous structures projected from the surface. Eggs are 1.2 mm long and 0.2 mm wide. First instar nymph is tiny measuring about 1.5 mm with brownish head, legs and abdomen. Eyes are pink, antenna longer than body and labium extending to half the abdomen. Second instar is about 2 mm, have most of the body parts orange to brownish, drumstick-like process just appear. The third instar has reddish green body, about 3 mm long with just formed wing buds. In fourth instar, body turns greenish yellow measuring about 4 mm. Wing pad becomes bigger and darker.
than third instar. Finally, the fifth instar is reddish green having a green abdomen about 5 mm long. Drumstick process and wing pads well developed. At this stage sexes can be identified, males have blunt abdominal tip while females have a groove for a future ovipositor (Tea Board, http://www.teaboard.gov.in/NTRF_2009/NTRF_NESTP/page15.htm). One individual usually completes its life cycle on a single bush. The *H. theivora* occurs widely in the foothills and the plains mainly attacking tea and weeds specially *Mikania* growing in tea areas. Nymphs and adults are more visible in early and late hours of the day and take shelter under tea leaves specially in the lower frame during day time or when disturbed (Tea Board, http://www.teaboard.gov.in/NTRF_2009/NTRF_NESTP/page15.htm).

![Life cycle of Helopeltis theivora](image)

*Fig. 2.2. Life cycle of Helopeltis theivora*
2.2. Materials and methods

In order to have a greater understanding of underlying morphological changes in tea leaves due to infestation, microscopic examination of leaf samples and the insect was undertaken.

The experimental plot pertaining to the examination was identified (New clonal trial plot 2, Tocklai Experimental Station, TRA). Bushes were washed with water spray to remove residual pesticide (if any) present on the leaves before being pruned to allow new buds to develop. After 10-15 days, newly developed leaf shoots were found to be infested.

The insects were collected manually from infected field, by gently holding their antennae and transferring them into bell jars with nets at the top. A mixed population of instars and adults were collected.

**Compound Microscopy:** Infested Young Shoots and buds from the above plot were taken, dipped in water and mounted on the platform of the dissecting microscope (MEIJI-FMZ-TR). Sterile blades were used to prepare the sample for mounting onto the compound microscope (Olympus CH30) with the magnification of 10X. Puncture spots were visualized and images captured. No additives or preservatives were used for sample preparation.

Insects previously captured, were processed similarly except that none of the body parts were excised before mounting onto compound microscope.

**Scanning electron micrographs (SEM)** of the uninfested leaf, insect instars, mouth parts, infestation lesions on adaxial and abaxial regions of leaves were performed using JEOL JSM-6360 at Sophisticated Analytical Instrument Facility (SAIF), North Eastern Hill University, Shillong to ascertain the microscopic changes in the texture and shape of the leaf upon puncturing and infestation. Samples were prepared according to the protocol reported by Dey et al., 1989.
2.3 Results and discussion

The tea mosquito bug (TMB) primarily feeds on the apical buds, the first two leaves and young stems of the tea plant. Being a sap sucking insect, it is suitably adapted for effective penetration of its proboscis into the tissue, across the thick waxy epidermis of the leaf. Stylets transiently puncture epidermal, mesophyll, and parenchyma cells, and this mechanical damage may influence plant responses to infestation (Tjallingii, 1993).

During the puncture, the insect (nymphs and adults) sucks the sap out, while in the process injecting saliva containing a cocktail of unknown compounds that somehow triggers the breakdown of the leaf tissues. Initially post infestation, a thick exudate is seen oozing out from the site followed by browning of the tissues on the site of puncture. After 4-5 hours of infestation (Fig. 2.3), the area adjoining the punctured spot progressively turns dark brown. Careful examination of the puncture spots reveals that indentation and reduction of the thickness of the lamina is an associated feature of infestation. Such a progression may be attributed to hypersensitive reactions (Fernandes, 1990) and subsequent necrosis of adjoining tissues (Klingler et al., 2005; Gao et al., 2008) following herbivory. In young leaves, the occurrence of puncture spots around the leaf lamina (abaxial and abaxial surfaces) are seen in more numbers than elsewhere in the leaf. Puncture spots concentrated in a particular region of the leaf lamina is more lethal than spots evenly distributed over the leaf surface. Often, in such cases, leaves become blackened after 24 hours of infestation.

![Infestation spots after 30 min(a), 5 hrs(b), more than 24 hours(c) and curling and undulations after extensive puncturing (d).](image)

Fig. 2.3 Infestation spots after 30 min(a), 5 hrs(b), more than 24 hours(c) and curling and undulations after extensive puncturing (d).
In cases of extensive puncturing, leaf blades are also found to exhibit “curling and undulations’ (Fig 2.3d)

The adult pest is an agile feeder (sucker) characterized by lengthy antennae and a strong and pronounced stylet/proboscis (Fig 2.4). The compound eyes allow it to have a broader view of potential sites of feeding and its predators. Three pairs of legs enable it a better hold on the waxy leaf surface while the stylet helps in infestation and sucking the sap from the plant.

Fig.2.4. Head and upper thorax of *H.theivora*.

Scanning electron micrograph (SEM) was employed to study, in greater detail, herbivory induced submicroscopic changes to the leaf surface and also to visualize finer details of the mouthparts of the pest. A similar study was also done by Ledford and Richardsson in wheat (1994). SEM of abaxial and adaxial surface of the leaf lamina in control and infested samples (Fig.2.5) showed that structural morphological changes are more pronounced on the abaxial surface of the leaf blade due to *Helopeltis* infestation. There is an observable difference in the arrangement of trichomes between control and infested samples, to the effect that they are highly disoriented in the latter (Fig.2.5d). This may be attributed to hypersensitive reactions triggered in and around the infested region leading to hypertrophy. Moreover, the region of puncturing is clearly visible which have resulted in observable damage (rupture) to the surrounding tissue (Fig.2.5d). The resulting rapid browning of the injured tissue, characterized by chlorosis and necrosis (Miles 1999) can be due to plants own immune response to herbivory-a strategy that balances hypersensitive response with resource allocation to it Autophagy has been known to constitute a
'pro-survival' mechanism that controls the containment of host tissue-destructive microbial infections in plants (Lenz et al., 2011) Sap sucking aphids have been found to manipulate resource allocation within the plant (Goggin et al., 2007). The figures (Fig 2.6 a,b) show rupture and invagination at the site after 8 hrs. of infestation on the abaxial surface of leaf lamina.

Fig. 2.5 SEM of infested and control tea leaves. (a) Control abaxial (b) control adaxial (c) infested abaxial (d) infested adaxial

Fig2.6. SEM showing magnified images of puncture sites on leaf surface.
The SEM of the mouthparts show (Fig 2.7 b-d), the presence of hair like structures on the distal end and tip of the proboscis, which may have a significant role(s) to play in aiding/initiating the identification of potential tissues.

Fig. 2.7. Scanning electron micrograph of Helopeltis appendages and mouth parts. (a) Appendages of an adult (85X) (b) Location and position of stylet (40X) (c) Distal end of the stylet (550X) (d) Tip of the stylet (1900X).

In view of the above investigation, it is apparently conceivable that infestation by the TMB induces morphological changes in the tea plant, which is affected by both mechanical act of infestation and the resulting hypersensitive reactions, are induced as part of the plant's direct defence strategy. The reactions in the plant are highly pronounced and their manifestations appear after 30 minutes of infestation. The proboscis/stylet of the pest is, instrumental in not only penetrating the tissue and sucking in the cell sap, but also possibly identifying potential regions/leaves of infestation. These responses are rapid and constitutes part of plants strategy to minimise the extent of damage and economize on the resource allocation for such a
non productive activity. An investigation on the anatomical, biochemical and physiological changes associated with such a response is worth considering, given that necrosis is affected and triggered by them.

References:


