ULTRASTRUCTURAL DETAILS ON THE
MORPHOLOGICAL ADAPTATIONS OF
SOME OF THE TEA PESTS
4.1 INTRODUCTION

Tea plantation requires hot and humid climatic conditions. From climatic point of view, tea is essentially a crop of the tropical region, but also grows well in subtropical regions too. There seems to be no upper limit of rainfall as the growth of tea is concerned. However, the lower limit to be 127 cm. provided other environmental conditions exert mitigating circumstances (Rustagi, 1995).

Since the growth of tea requires unique climatic conditions, the insets and other arthropod pests attacking different parts of the tea plant must thrive in these specific environmental conditions. Therefore, it is quite certain that the pest would exhibit some specialisation in their morphological features. Although significant modifications in gross morphology may not be expected. Detailed studies on the fine structure of various organs of the pest may exhibit some important modifications.

Keeping the above in view, a Scanning Electron Microscopic study on mouth parts, antennae, general body cuticle, thoracic appendages, head and its appendages etc. of some of the major/minor tea pests of Barak Valley was undertaken. Emphasis was laid on the ultrastructural studies on cuticle and its associated sensilla, since, success as terrestrial animals in resisting desiccation and in breathing atmospheric air etc. are directly related to the properties of their integument (Smith, 1968). Once the structural specializations of the pests in relation to the host plant and prevailing environmental conditions are established, a well planned strategy can be adopted for management of the pests, which cause major economic loss to the tea industry.
4.2 REVIEW OF LITERATURE

Gross morphology and ultra structural features are known to exhibit remarkable modifications in different species of insect pests (Calvert and Hanson, 1974; Miller and Stricler, 1984) in relation to their association with various plants under varied environmental conditions. The mouth parts of Alfalfa weevil were reported to show morphological adaptations in terms of gross as well as fine structure. The shape and dimensions of mandibles were found to be different in different species of weevil feeding on various host plant species (Bland, 1984). Fine structure of antenna (Itoh et al., 1991) of drosophila morphology and distribution of sensilla on the cephalic appendages, tarsi and oviposition of some lepidopteran pests (Fancheux, 1991) and antennal sensory organs of some coleopteran pests (Cronan and Cronan-Roy, 1991) were found to be modified in relation to the host plant species and environmental conditions.

It is well known that the adaptational diversity of an insect is best expressed by the chemical as well as structural specialization of the chitinous cuticle covering the body, which is different in different species as well as in different body parts of the same individual (Neville, 1975). Scanning Electron Microscopy, because of its large depth of field and high resolving power has been used to reveal the surface ultrastructural features of insect cuticle with specialised functions (Neville, 1978). Extensive Scanning Electron Microscopic studies have been carried out on specialized cuticular structures, known as sensilla from different parts of insect body in various groups, which has been shown to perform a variety of functions (Fancheux, 1991; Dey, 1995; Dey et al., 1995; Dey et al., 1998). It appears that the first step in understanding the complexity of behaviour of an insect requires a thorough
examination of its sensory structures. Till recently, excepting the light microscopic studies, no in depth/elaborate study was made on the morphology of the tea pests in relation to their behaviour, infestation pattern, severity of damage etc. The present study was, therefore, aimed at a search for the morphological peculiarities that might relate to the ecophysiology, behaviour and damaging pattern of some of the major/minor tea pests of Barak Valley.

4.3 MATERIALS AND METHODS

The major pests of Barak Valley taken for the present study include i) Live-wood eating termite (*Microtermes obesi*, Holmgreen), ii) Scavenging termite (*Odontotermes* sp.), iii) Red spider mite (*Oligonychus coffeae*, Nietner) and iv) Thrips (*Scirtothrips dorsalis*, Hood) while the minor pest studied was the Aphids (*Toxoptera aurantii*, Boyer).

The parts used for ultra-structural studies were mouthparts, antennae, legs, head, dorsal and ventral body surface, cuticular folds and sensillae associated with them.

The parts excised from body following exposure to benzene vapour were fixed in 2.5-3% glutaraldehyde prepared in 0.1M sodium cacodylate buffer for 4 hours at 4°C. The primary fixation in glutaraldehyde was followed by buffer wash of the samples for 20-30 minutes, and secondary fixation in 1% buffered osmium tetroxide for 30 minutes to one hour. The fixed samples were dehydrated through increasing concentrations of acetone and were dried either in critical point drier (CPD) using acetone as the intermediate fluid and carbon-di-oxide as the transitional fluid, or in tetramethyl silane drying technique of Dey et al. (1989) as follows:
The dehydrated samples were transferred from dehydrating fluid to tetramethyl silane and were kept at 4°C for 10 minutes. The samples along with tetramethyl silane were then transferred to a coverslip to provide large surface area for evaporation, and were dried at 26°C. The dry samples were secured horizontally to brass stub (10 mm x 10 mm) with double sided adhesive tape. Care was taken to avoid trapping of air inside the tape. A thin conductive coating of gold was applied to the sample using a fine coat ion Sputter, JFC-1100 (Jeol), maintaining a low vacuum (10⁻³ torr).

Observations were made with a Scanning Electron Microscope [JSM 35 CF (Jeol)] using the secondary electron emission mode. The accelerating voltage applied was 20KV. The working distance (WD) selector was set at 15 mm, and the tilt control was fixed at zero degrees for setting the specimen stage in a horizontal position.

4.4 RESULTS

The Scanning Electron Microscopy of different body parts of the tea pests shows certain interesting ultra structural features that are relevant to the ecophysiology of the pests.

4.4.1 Aphids (*Toxoptera aurantii*, Boyer)

The most significant observations on ultra-structural features of aphids is the modification in hypopharynx and labium. The hypopharynx is characterised by the presence of swollen base and pointed tip. Beside these, it has some small sensilla with swollen base, probably sensilla basiconica with gustatory or chemoreceptive role. The labium, as expected is long (270 μm) and is modified for sucking the sap. However,
PLATE X: Scanning Electron Microscopy of Aphids (Toxoptera auranti, Boyer)

1. Entire pest (dorsal view)
2. Anterior part of head showing sensilla and compound eye
3. Tip of leg
4. Mouth parts (ventral view) showing sucking organ
5. Sucking organ (enlarged)
unlike piercing sucking type of mouth parts, its tip is blunt. The labium shows three major segments among 70-120 μm long and 23-42 μm broad of these segments the lowest one is the largest (120 μm) (Plate X - 4). The labium contains a few sensilla basiconica around 20-25 μm long and 2-3μm broad with well developed sockets at its dorsal surface. In addition, it contains some long (105-110 μm) sensilla trichodea at the tip, dorsal and dorso-lateral surface of the labium (Plate X - 4). The lateral position of the head contains two well developed compound eyes with compact ommatidia (Plate X - 2).

The legs of aphids are covered with sensilla chaetica, which probably acts as contact chemoreceptors. As far as the gross morphology is concerned, the claws on the tarsus of pro, meso and metathoracic legs are bent inwards and are pointed and sharp, suitable for damaging/cutting plant parts and clinging to it (Plate X - 3). Besides sensilla chaetica, the dorsal and dorsolateral surface of tarsus contains some short sensilla basiconica (3-5 μm) with well developed sockets (Plate X - 3). The general body cuticle contains a few sensilla basiconica (20-40 μm long) with characteristic swollen base (Plate X - 1).

4.4.2 Thrips (*Scirtothrips dorsalis*, Hood)

In thrips, antennae show some interesting features. The number and type of sensilla in antennae are few. The general morphology of the antennae, with a large number of cuticular folds suggests a highly flexible nature of the structure. The sensillae present on the antennae are mostly basiconic sensilla with characteristic features (i.e., swollen base) of chemoreceptors (Plate XI - 3). The most significant modification, however, is observed in the piercing-sucking mouth parts. The tip of the
PLATE XI : Scanning Electron Microscopy of Thrips (*Scirtothrips dorsalis*, Hood)

1. Mouth parts showing antennae and sucking organ
2. The tip and swollen base of sucking organ
3. Tip of one antenna showing sensilla
4. Tip of leg (showing anchor like structure)
sucking organ is highly elongated (96 μm) and the base is swollen (10 μm) (Plate XI - 2).

The tip of antennae show a number of sensilla trichodea (6.5-7 μm long and 1 μm broad) with striated surface. The base of the sensilla was found to be non flexible (Plate XI - 3). The tip of leg shows some anchor like structure (Plate XI - 4), being broad at the centre and pointed at the lateral part.

4.4.3 Red spider mite (Oligonychus coffeae, Nietner)

Scanning Electron Microscopy revealed detailed ultrastructural features of the red spider mite. The length and breadth or the body has been found to be around 350 μm and 170 μm respectively (Plate XII A - 1 & 2). The appendages are well developed and found to be equipped with a large number of sensilla chaetica and sensilla trichodea (Plate XII A - 6). The general body cuticle shows the characteristic foldings observed in most of the mite species (Plate XII A - 3 & 4). Besides there, wavy striations of alternate electron dense and electroluscent bands are evident (Plate XII A - 5). The electroluscent bands are conical in appearance and are raised from the general cuticular plane. The height and periodicity of these conical structure are more or less uniform (0.5 μm and 1.5-2.2 μm respectively). The appendages also contain striations but they do not contain any conical structure (Plate XII A - 7). The sensilla present on the surface of the appendages comprise mostly of sensilla chaetica about 70 μm in length and 2 μm breadth. Besides, sensilla trichodea also occur in few numbers. The surface of the sensilla trichodea shows longitudinal striations and secondary ridges while sensilla chaetica shows lateral spiny outgrowth.
PLATE XII A: Scanning Electron Microscopy of Red spider mite (*Oligonychus coffea*, Nietner)

1 & 2. Entire organism

3 & 4. Cuticular folds and sensilla on dorsal body surface

5. Cuticular folds on the body

6. Cuticular folds in the legs showing alternate Electron dense & Electroluscent bands

7. Cuticular folds in leg (enlarged)
1. One of the sensilla (*Sensilla trichodea*) showing the socketted base

2 & 3. Middle part & tip of sensilla chaetica

4. Mouth parts (ventral view) also showing sensilla chaetica

5 & 6. Rammer shaped sensilla chaetica at the apical part of leg
(Plate XII B - 1 & 2 respectively). The tip of the sensilla chaetica is about 0.1 μm in breadth and is blunt (Plate XII B - 3).

Sensilla trichodea present on the surface of appendages are found to be characterised by the presence of a well developed socket (2.8-3 μm in diameter) and longitudinal striations, indicating their porous nature. The base of the sensilla has been found to be non flexible (Plate XII B - 1).

The apical portion of the appendages has been found to contain about four highly specialised sensilla chaetica with some unique features. All these specialized sensilla appears to have a common origin at the apex of the appendages. The base of the sensilla is swollen, which gradually tapers, but again broadens at the tip. The broad tip has been found to be rammer shaped. All these sensilla has been found to be more or less of the same length (about 10.5 -12.5 μm). The width of the swollen base, narrow central part and the flattened tip has been observed to be about 1.4 μm, 0.4 μm and 7 μm respectively (Plate XII B - 5 & 6).

4.4.4 Live-wood eating termite (Microtermes obest, Holmgren)

4.4.4.1 Soldier: The dorsal surface of the head, thorax, abdomen and the thoracic appendages are found to be covered with cuticular spines ranging from 20 μm to 100 μm in length. These spines do not show the presence of a well developed socket and also do not exhibit the characteristic features of the chemoreceptors (Plate XIII - 1). The ventral surface of the same region exhibits the similar type of sensory structure.

The significant feature of the mouth parts is the presence of serrated mandibles. The serrations has been observed at the inner edge (Plate XIII- 2 & 3). This particular device in live wood eating termite is perhaps meant for cutting the heartwood of the
PLATE XIII: Scanning Electron Microscopy of Live-wood eating termite
(Microtermes obesi, Holmgreen) soldier

1. Entire body in dorsal view
2. Mouth parts (dorso-lateral view) showing serrated inner edge of mandibles
3. Serrated inner edge of mandibles
4. Mouth parts (ventral view)
5. Antenna showing different types of sensilla
tea bushes. The maxillary palp shows the presence of a large number of sensilla, consisting of simple cuticular spines, sensilla trichodea and sensilla basiconica (Plate XII - 4).

All the portions of the mouth parts, i.e., hypopharynx, maxillary palp, labial palp and labrum shows almost similar types of sensilla. Three different types of sensilla can be recognised. These are sensilla trichodea, about 35-60 μm long and 3-5 μm broad with well developed socket (about 7 μm in diameter), sensilla basiconica, about 10 μm long as 3 μm broad, and sensilla chaetica, about 45-50 μm long and 2-3 μm broad. The sensilla trichodea and sensilla basiconica have been observed to be more or less of the same width, but the sensilla trichodea is longer. Sensilla chaetica, on the other hand is more or less of the same length as that of sensilla trichodea, but its width is much less (Plate XIII - 4).

The antennae are twelve segmented. Excepting the terminal segment, all the segments are short and condensed. All the segments except the scape has been found to contain sensilla trichodea, sensilla chaetica and sensilla basiconica. While sensilla trichodea and sensilla basiconica are localised in the lateral portion of each segment, the basiconic sensilla are present on the central portion. The sensilla trichodea are thicker (3 μm) than sensilla chaetica (1.8 μm), and both them are comparatively longer (i.e., sensilla trichodea 27 μm in length and sensilla chaetica 48 μm in length) than sensilla basiconica (9μm in length). The width of the sensilla basiconica is about 1.8 μm (Plate XIII - 5).
4.4.4.2 Worker. General morphology of the body surface, appendages, antennae, mouth parts of the worker of live wood eating termite is more or less same as that of soldiers. The only significant difference has been observed in the head cuticles which are 3-25 \( \mu \text{m} \) long and the cuticular spines are sharp and pointed (Plate XV - 6).

4.4.5 Scavenging termite (\textit{Odontotermes} sp.)

4.4.5.1 Soldier. Scanning Electron Microscopy of the ventral surface of the mouth revealed all the parts distinctly. The mandibles are well developed and are pointed at the tip. However, unlike the live wood eating termite, the inner edge of the mandible is not serrated. The smooth, non-serrated inner edge in these termite is one of the examples of morphological adaptations, since, unlike live-wood eating termite, they are concerned with cutting dead/decomposed wood which are comparatively soft (Plate XIV - 7). Hypopharynx and labrum shows fewer sensilla as compared to those or live wood eating termite. However, the maxillary palpi show the presence of a large number of sensilla trichodea and sensilla chaetica; sensilla basiconica, however, could not be detected (Plate XIV - 3).

The general organisation of antennal morphology is roughly similar to that of live-wood eating termite. However, the antennal segments are fifteen in number (Plate XIV - 3) and are more long and elliptical in comparison to those of live wood eating termite which are much short and condensed. The tip of the antenna showed the presence of a large number of sensilla trichodea, sensilla basiconica and sensilla chaetica. The length of the three types of sensilla has been found to be about 71, 3-4 and 28-30 \( \mu \text{m} \) respectively and the breadth is about 3, 2 and 2 \( \mu \text{m} \) respectively.
PLATE XIV: Scanning Electron Microscopy of Scavenging termite (*Odontotermes* sp.)
Soldier

1. Entire body (dorsal view)

2. Entire body (ventral body)

3. Mouth parts (ventral view) showing non-serrated inner edge & antenna

4. Tip of antenna showing different types of sensilla

5. Enlarged view of few sensilla chaetica and sensilla basiconica

6. Tip of one leg

7. Mandible showing the non-serrated inner edge
PLATE XV : Scanning Electron Microscopy of worker of Scavenging termite (Odontotermites sp.) and Live-wood eating termite (Microteremus obesi, Holmgreen)

Scavenging termite :
1. Entire body (ventral views)
2. Mouth parts in ventral view
3. Dorsal surface of head showing cuticular spines

Live-wood eating termite :
4. Entire body (ventral view)
5. Dorsal surface of head showing large number of cuticular spines
6. Some of the cuticular spines (on head surface) under high magnification
The basiconic sensilla with swollen non-flexible base and blunt tip suggests their chemoreception and/or olfactory role (Plate XIV - 4 & 5).

The thoracic appendages of scavenging termite are more or less similar to those as observed in live wood eating termite. However, the tarsal segments are characterised by the presence of only one type of sensilla i.e., sensilla trichodea. These sensilla show well developed socket, about 7 μm in diameter. The surface of the sensilla show striations, indicative of porous nature. The base of the sensilla are flexible. The tip of the tarsus shows the presence of a pair of well developed claws. Cuticular foldings and ventral companiform sensilla are present on the joint regions of the claw (Plate XIV - 6).

The general body cuticle, particularly, the ventral region is marked by the presence of very few sensilla (Plate XIV - 2). However, the dorsal region shows a few sensilla (mostly cuticular spines), although their numbers are much less than that of live wood eating termite (Plate XIV - 1).

4.4.5.2 Worker: Gross morphology of the entire body, organisation of mouth parts and sensory system of worker of scavenging termite has been found to be similar to those as described in the context with the live wood eating termite. However, some differences exist in the finer ultrastructural details. Hypopharynx contain some long (about 200 μm) sensilla trichodea, and a few very long (1750-2400 μm) cuticular spines on the dorsal surface of the head (Plate XV - 3). The labial palpi are shorter (330-340 μm) but thicker (100-160 μm) and contain a few sensilla basiconica. Further, cuticular folds are distinct on them (Plate XV - 3). Labium contain few
sensilla trichodea (125 μm in length) with well developed sockets (12 μm in diameter) on a few sensila basiconica (10-12 μm in length) (Plate XV - 2).

4.5 DISCUSSION

The Scanning Electron Microscopic studies on various structures of the tea pests throw some light on the relation between the morphological features and ecophysiology of the insects. The ultrastructural findings, in fact, confirms the ecophysiological observations on the relationship among pest incidence/severity, infestation of the different plant parts, humidity, temperature and soil conditions etc., since structural modifications are important for interacting with plant parts and perception of different types of stimuli.

In aphids, the labium is long and has attained the required modifications for sucking sap from the shoot. The bluntness of the tip suggest that piercing of the shoot is not needed for sucking sap, but in fact, laceration of the tender shoots by the tip is enough for liberation of sap. The presence of sensilla trichodea and sensilla basiconica on mouth parts suggest the presence of chemo and gustatoreceptors. Sensilla chaetica present on the thoracic appendages are likely to be contact chemoreceptors. The compact ommatidia (Plate X - 2) on the compound eye indicates a very good vision which helps in the general behaviour of the insect.

In thrips, flexibility of antennae and presence of sensilla basiconica suggests chemoreceptive and gustatory role performed by it in response to stimuli received from different directions of the substrate (Slifer, 1970). The anchor like structure at the tip of the leg is also an important morphological adaptation which helps the insect in adhering itself to the plant parts. In fact, these structures cause laceration on the
rolled young tea leaves, which is retained as two to four permanent sand papery lines on the ventral surface of matured leaves (Plate VI - 3 & 4).

The sensory system in the red spider mite also shows significant correlation with their ecology and behaviour. The well developed sensilla chaetica and sensilla basiconica present in various parts of the body suggests that contact chemoreception and reception of volatile chemicals from the tea plants are carried out with sufficient precision. This is supported by their behavioural studies, where, it has been observed that, after being washed down to the lower zones of the bushes during the heavy rain, the pest again migrate to their original position (dorsal surface of the leaf) on subsequent withdrawal of rain. This migration would not have taken place, had the mites were not equipped with a well developed chemical sense, for responding to the stimuli received from phytochemicals of the tea leaves. The presence of pits and some poreless sensilla with inflexible sockets may be related to hygro and thermoreception (Lacher, 1964; Altner et al., 1983). The functional significance of these sensilla is confirmed by their characteristic behaviour of occurring in large number on dorsal surface of the tea leaves when the ambient temperature is high (i.e., during April-May, July-September) since high temperature is related to high intensity of light, therefore, they occur in large number/cause major damage during the summer months when the intensity of light is maximum. The high light intensity, again, is associated with a problem for the mite species, since there is a likelihood of their visual prominence to the predators. It is highly interesting to note that some morphological adaptations of the general body cuticle might help the mite species to overcome this problem. The wavy striations of alternate electron dense and elecroluscent bands raised from the general cuticular plane, with more or less uniform periodicity and height may act as
anti-reflection device, similar to corneal nipple present on the lens cuticle (Miller et al., 1966; Dey, 1988; Dey, 1991; Dey and Dkhar, 1992).

Ecological and behavioural studies revealed that live-wood eating termite devours the heart wood of living tea plants. Since the pest has a preference for the heartwood, it appears that they have some specific sensory mechanism to respond to the stimuli produced by phytochemicals from the tea plants. A large number of sensilla trichodea and sensilla chaetica with characteristic features of contact chemoreceptors suggests that these sensilla help the termite species to identify the specific phytochemicals from the plant and also to differentiate between live and dead wood. The innumerable cuticular spines on the surface of head, thorax, abdomen and thoracic appendages are likely to perform protective role as and when the pest penetrates deep inside the tunnels within heartwood.

The serrated mandible of live-wood eating termite (soldiers) appears to be an important adaptation for cutting the bark/wood of the living tea plant. The presence of quite a number of sensilla trichodea and sensilla basiconica on the maxillary palp suggests that chemoreception of volatile chemicals and contact chemoreception of various types of stimuli are used by the mouth parts before feeding activity starts (Chapman, 1982). The shape, length and width of basiconic sensilla suggests their role in chemoreception and olfaction. The sensilla trichodea on the other hand shows the characteristic features of chemoreception (Bland, 1983). The dimension, localised distribution and shape of the basiconic sensilla detected in the antenna has the characteristic features of sensory peg responsible for reception of moisture. These hygroreceptors were reported in many social insects, but there is no report on termite (Dietz and Humphreys, 1971). The presence of the hygroreceptors (Altner et al.,
1983) is related to the specific behaviour of the pest where the population abundance shows an inverse relationship with the soil moisture content. Although the reasons for less incidence/damage caused by termite at high soil moisture is not known, it is certain that the insect is well equipped with sensory mechanism to detect any change in the soil moisture content.

An interesting correlation between morphology of mandible and feeding habit of termite species, has been found. The presence/absence of serrated margins at the inner edge of mandible is directly related to the feeding behaviour of the pest. Since the scavenging termite does not basically devour the heartwood of living tea plants, therefore, unlike live-wood eating termite, it does not require serrated edge in the mandible. However, besides acting as defensive organ, the pointed tip of the mandible is important for the pest to penetrate into the dead and rotten wood. The presence of fewer sensilla in the mouthparts of the scavenging termite suggests that it does not require the various stimulus from phytochemicals to be sensed. However, the presence of sensilla chaetica and sensilla trichodea suggests that contact chemoreception and mechanoreception may be well developed. The presence of sensilla basiconica, similar to hygroreceptor in the antenna, signifies the inverse relationship between the occurrence/severity of the termite sp. and the moisture content of the soil, similar to that observed in the live-wood eating termite. In thoracic appendages, the presence of only one kind of sensilla (i.e., sensilla trichodea) with well developed socket and surface striations suggest that the termite has a well developed contact chemoreceptor to detect the dead and rotten wood of the tea plant. The flexible base of the sensilla further supports this view (Slifer, 1970). The presence of a pair of well developed claw on the tarsal tip, along with the occurrence of cuticular foldings and ventral
campaniform sensilla on the joint region between claws and tarsus suggests that the claws are used for holding the substrates and can be moved at different angles (Schmidt and Smith, 1987).
4.6 BIBLIOGRAPHY


