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SEM Observations on the Citrus Green Aphid, *Aphis citricola* van der Goot (Homoptera: Aphididae)

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Abstract: *Aphis citricola* van der Goot was observed under scanning electron microscope. The head, prothorax, mesothorax, metathorax and smoothly surfaced abdomen were clearly recognized. A pair of eyes having several facets occupied the base of the antennae. The posterior part of the eye possessed a projected ocular tubercle. Clypeus and labrum positioned to anterior lower part of the head. The clypeus joint with the labrum at its lower portion as a separated organ. The rostrum originates bellow the labrum and had a length of 310-480 µm. The stylets were projected from the underside of labrum and were inserted into the basal and middle portion of the rostrum. Rostrum had three segments and a suture in the middle region extended up to the apex. A tube-like canal was found at the middle part of the rostrum. The inserted stylets were projected from the apex and diverted in three directions. The middle portion of the stylets was thick and laterals were thin having sharp tips. The stylets were 460-630 µm long and having a diameter of 2.5-3 µm.

Key words: *Aphis citricola*, clypeus, labrum, rostrum, stylets

Introduction

Aphids are the most important pest insects in the agriculture of the temperate climate zones. Citrus green aphid, *Aphis citricola* van der Goot attack the citrus plants in autumn. This aphid parasites on the new leaf and shoots. The infection of this pest caused tremendous damage by polluting the leaves and fruits (Moritsu, 1983; Kfoury and El-Amil, 1998).

Most aphids are autocious living on one or few species of a particular genus of plants (Eastop, 1972). About ten percent are heterocious spending autumn, winter and spring on a primary host and classified as polyphagous. It is noteworthy that most of them live only on single species of plant at a time. They are sequentially monophagous (Takada, 1979; Weber, 1982). Gall forming aphids have evolved a particularly close association with host plants. They do not synchronize development with their host plant but they also modify the development and metabolism (Dixon, 1983). Several species of aphids attack citrus in Japan (Furuhashi and Nishino, 1968; Komazaki, 1981). Some of them transmit citrus tristeza virus (Raccah *et al*., 1976) and their feeding damage is serious (Kato, 1974). *Toxoptera citricidus*, *Aphis citricola* and *Aphis gossypii* attack citrus during the entire summer season and their population rapidly increased. Because of the feeding damage, new shoots become curled and do not fully extend (Norman and Grant, 1956; Sasaki, 1974; Raccah *et al*., 1976). The aphid population increase on citrus plants due to the over wintering eggs which hatched in next spring (Komazaki *et al*., 1979; Escudero *et al*., 1994).

Forbes (1969) has discussed the mouthparts of aphids in a review of morphology of Homoptera that emphasized virus vectors. Reviews by Miles (1968, 1972) reported saliva and salivary functions in the Hemiptera-Homoptere. A review by Pollard (1973) emphasized on the structure and function of stylets but the morphology of mouthparts and mechanism of stylets in relation to rostrum have not been described in case of *A. citricola* and other relevant aphids.

Considering the importance of the family Aphididae from the point of view of economic entomology of citrus. We thought to investigate the morphology and mechanism of the mouthparts and associated organs in a member of this family. There have been many questions regarding the mechanism of feeding in aphids about which our knowledge was obscure. The method of the stylets protraction and retraction in the rostrum and in plant tissues was not clearly understood. There are several reports on damage and physiology of citrus caused by aphids but lacks in comprehensive morphological studies. Therefore, the present experiments were conducted to observe the mouthparts and their mechanism of food accusation under scanning electron microscope.

Materials and Methods

The experiment was conducted in the Citriculture Laboratory, Faculty of Agriculture, Ehime University. The citrus leaves infested by *A. citricola* were collected from the campus vicinity. The aphids were isolated from the collected leaves under stereoscope. Two kinds of aphid samples were selected, protruded and none protracted stylets from the rostral apex. The rostrum was sectioned by using common razor for the observation of food channel.

Samples were pre-fixed in 4% glutaraldehyde in 0.1 M phosphate buffer in pH 7.2 followed by rising in the same buffer and were post-fixed in 1% osmium tetroxide. Dehydration was carried out with a graded ethanol series and were dried in a Vacuum Device Inc., VDF-20 freeze dryer. Dried samples were mounted on the specimen stubs, coated with gold using Eiko Engineering Co., LTD., 1B-2 ion sputter and finally were viewed under Hitachi S-2250N Scanning Electron Microscope at 20kV and photographed.

Results

Scanning electron micrographs showed the body of the *A. citricola* consisted of head (H), prothorax (Pr), mesothorax (MS), metathorax (Mt) and abdomen (Ab). Head possessed a pair of segmented antenna (An) and a pair of siphunculus (Si) at the posterior part of the abdomen. The head and the thoracic region was segmented while the dorsal portion of the abdomen was smoothly surfaced (Fig. 1). A pair of compound eyes (CE) was present behind the bases of antenna. They were glabrous and had several facets on its surface. The eye possessed another projection (arrow) on their top portion. Clypeus (Cl) and labrum (L) were on the anteriorly lower part of the head (Fig. 2).

Clypeus (Cl) had triangular shape where the labrum (L) attached in down word style. The facets of the compound eye (CE) exposed granular shape and the ocular tubercle (arrow) was globular-shaped (Fig. 3). Anterior view revealed the clypeus (Cl) occupied middle of the head and the labrum (L) joint at the lower portion as a separate organ. The rostrum (Ro) extended below the labrum and had a length of 310-480 µm (Fig. 4). The surface of the clypeus (Cl) was lobed and possessing five setae on its frontal region. Clypeus and labrum were fixed together in vertical position. Labrum exposed uneven upper and smooth conical lower surfaces directed ventrally over the rostrum (Ro) (Fig. 5).

The stylets were extruded from the underside of labrum (L) beneath the clypeus (Cl) area and were inserted into the basal segment of rostrum (Ro) (Fig. 6). The shoot-like stylets (ST) were penetrated into distal portion of the rostral segment one (R1) and encased up to two (R2) and three (R3) (Fig. 7). The inserted stylets (St) passed through the rostral segments (R1, R2, R3) and projected from the apex (Fig. 8). The narrow tip of the rostral

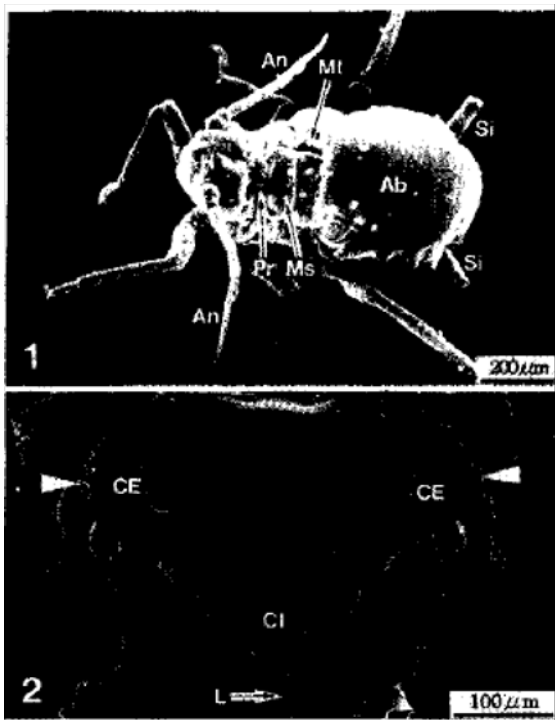

Fig. 1-2: SEM micrographs on the external features of the citrus green aphid, *Aphis citricola* van der Goot. 1: Dorsal view of head (H), antenna (An), prothorax (Pr), mesothorax (MS), metathorax (Mt), abdomen (Ab) and siphunculus (Si). 2: Anterior view of head showing compound eyes (CE), clypeus (Cl) and labrum (L)

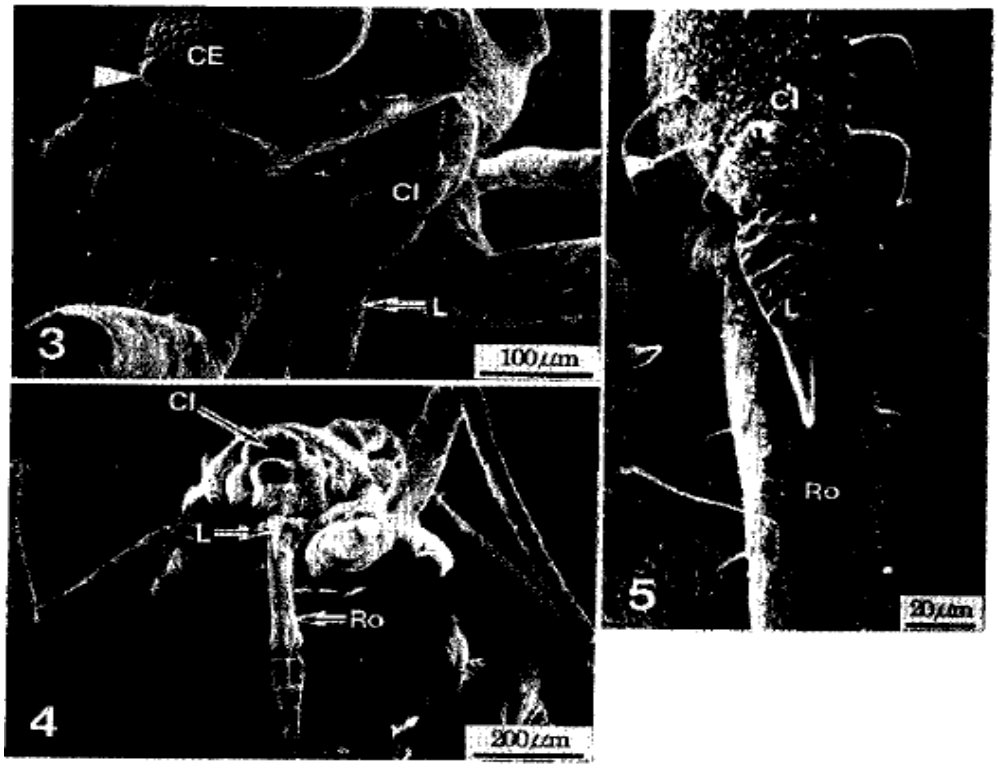

Fig. 3-5: SEM micrographs on the related organs with head in *Aphis citricola* van der Goot. 3: Lateral view of clypeus (Cl), labrum (L) and compound eyes (CE) having ocular tubercle (arrow) on top. 4: Anterior view of clypeus (Cl), labrum (L) and rostrum (Ro). 5: Connection of the clypeus (Cl) and labrum (L) appearing above the rostrum (RO)

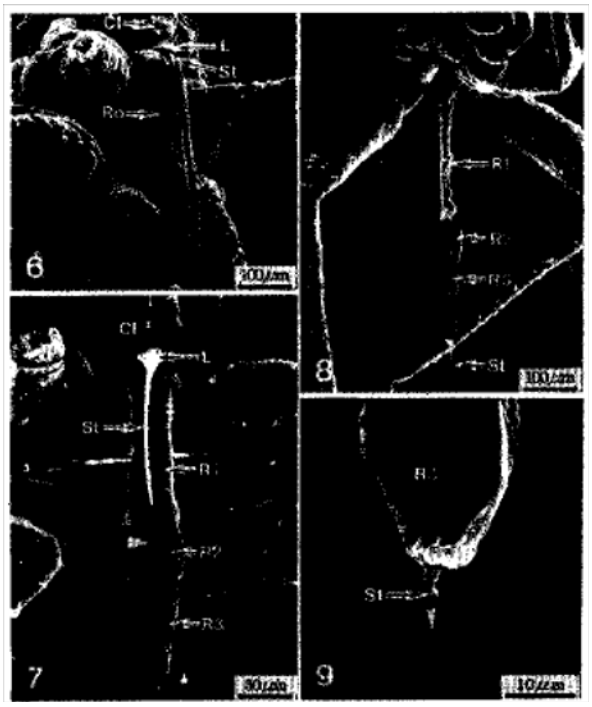

Fig. 6-9: SEM micrographs on the relationship of stylets and rostrum in citrus green aphid. 6: Stylets (St) projected from underside the labrum (L) below the clypeus (Cl) and inserted into the base of the rostrum (Ro). 7: Stylets (St) protected from the ventar of labrum (L) below the clypeus and inserted into the first segment of the rostrum (R1) encased in second (R2) and third (R3). 8. Protruded stylets (St) from the apex of the three segmented rostrum (R1, R2, R3). 9: Third segment of rostrum (R3) showing several setae and the stylets (St) projected from the apex

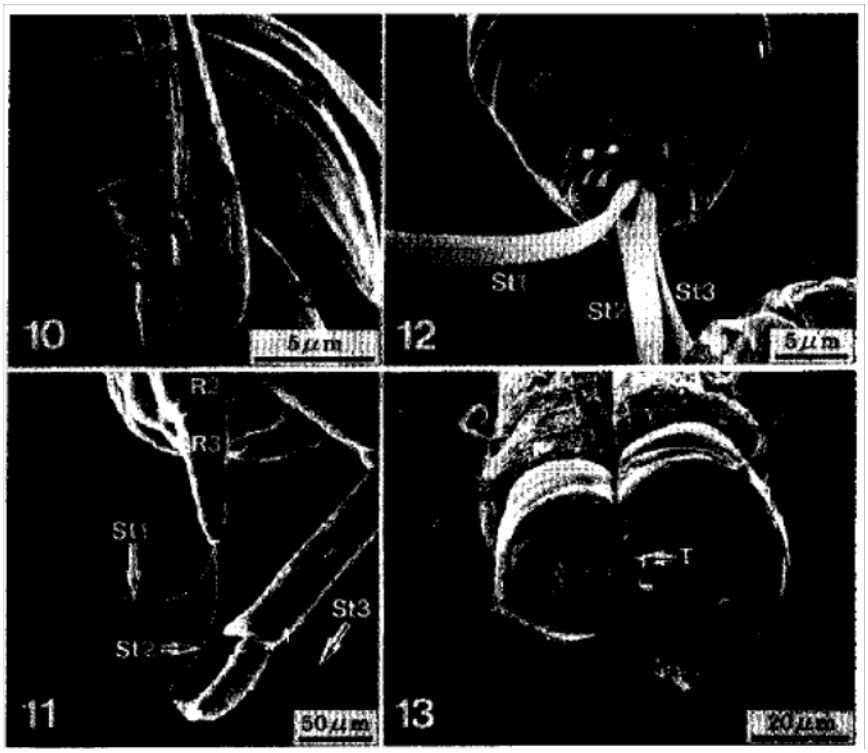

Fig. 10-13: SEM micrographs on the structure of rostrum and stylets *Aphis citricola* van der Goot. 10: The stylets bundle packed in layers at the apical portion and divided into parts. 11: Extruded stylets (St) embedded in the second and third rostral segments (R1, R2, R3) diverted in different directions. 12: Magnified few of three stylets (St1, St2, St3) possessing thin lateral and thicker middle portion. 13: Transverse section of rostrum showing tube-like (T) canal in the upper middle portion

segment three (R3) exposed ejected stylets, which had thicker base and sharp apex. This segment of the rostrum occupied by several hairs-like setae on the apical area (Fig. 9).

High-magnified view of distal portion revealed roughly surfaced and blunt tipped stylets. They were fixed in sheath-like layers towards bases and separated to the apical region (Fig. 10). The stylets had a diameter of 2.5-3 µm and the length of 460-630 µm. The protruded stylets from the apex of the rostral segments (R2, R3) divided into three shoots which were diverted in different directions (St1, St2, St3) (Fig. 11). The magnified view of the rostral tip showed the base of the protracted stylets. These were segregated from the projecting point and had the thicker middle than lateral parts (Fig. 12). The transverse section of the second rostral segment exposed a tube-like (T) canal in the middle which provide the path for the stylets bundle while the relative lateral regions revealed irregular cavities (Fig. 13).

Discussion

The body of the aphid consisted of head, thorax and abdomen. Head composed of antenna, a clypeus, a pair of compound eyes, rostrum and stylets. The head and prothorex may be fused together. In Hormoaphidae often have head and the thoracic segments fused together. A pair of tube-like structure on posterior parts of the abdomen is present which termed as siphunculi or cornicles (Miyazaki, 1987). Similarly, we found that the body of *A. citricola* consisted of head, prothorex, mesothorex, metathorex and abdomen. A pair of segmented antennae was present at the dorsal portion while the posterior region contained a pair of siphunculus. Head and thorax was segmented and the dorsum of the abdomen was smoothly surfaced.

The rostrum is the modified labium. It constitutes a sheath to hold stylets in groove formed on its dorsal surface and the ultimate segment firmly grips the stylets bundle and fixes the point of stylets insertion. Rostrum consist of five segments, the first is membranous and more or less sclerotized. Second segment was telescoped and showed narrow sclerotization and certain genera of Drepanosiphidae the sclerotization developed into a wishbone-shaped arch. The forth segment bears a pair of minute setae and apically have three pairs of ordinary ones which are seen as the dorsal, lateral and ventral pairs. The fifth segment is a small triangular bearing some minute spine-like setae. This segment is usually fused with the forth segment (Forbes, 1977; Miyazaki, 1987). In our observation the rostrum was hanging below the labrum in citrus green aphid had a length of 310-480 µm. Rostrum of *A. citricola* comprised of three segments in contrast with other aphids. The tip of the rostrum possessed several thin setae and a suture run on the dorsal portion from base up to the apex. The transverse section of rostrum revealed a tube-like canal, which provided a passage for the stylets movement and might support in the protection of tilting.

The stylets bundle consists of two pairs of needle-like stylets. The inner pair is maxillary stylets and the outer pair of mandibular stylets. The maxillary stylets are tightly fixed together by interlocking grooves and ridges on their inner surfaces with two pair of grooves opposed to form a food canal and a salivary canal between them (Forbes, 1969). The mandibular stylets fitted to each side of the bundle of maxillary stylets. They can move independently when piercing into plant tissues. Apically they bear a row of ridges on the outer surface. In each mandibular stylets runs a central duct containing nerve fibers (Forbes, 1977; Miyazaki, 1987). In our study, the stylets were projected from the ventral part of the labrum in a single shoot-like organ and were inserted into the rostral segment one. When the stylets protracts from the apex of rostrum they separate into three parts in various directions. The middle portion was thick which favors the existence of food canal embedded in it. The distal portion of the stylets was made up of sheath layers and was separated near the tip. The buckling mechanism regarding the stylets and rostrum connection can well be refereed to the Morley (1935). The theory of buckling provides well understanding the mechanism of stylets insertion in rostrum and than penetration into the leaves of the citrus plants. If the stylet shaft is long and thin their elasticity depends on the dimension. Due to the long length of the stylets and small diameter, it could be assumed that their penetration in the leave tissues and diversion could be made possible. This phenomenon is accomplished with the provision of rostrum tube, which provide a passage and strength to protect from bending.

In conclusion, the citrus green aphid consisted of segmented rostrum which provide a tube-like canal for the movement of the stylets. The stylets were inserted in the rostrum and projected from its apex. They had three parts, which were diverted in different directions. Their middle portion was thick and the laterals were thin. Stylets were fixed in compact layers and were roughly surfaced.

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