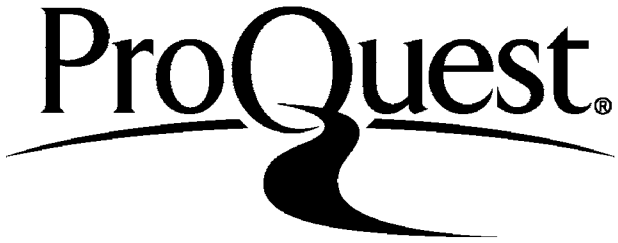
Some aspects of the biology of *Coccus hesperidum* L., with particular reference to the feeding behaviour.

William James Knight



Abstract

Past work on the morphology and biology of C.hesperidum is reviewed.

Examination of the feeding sites, by means of sections and whole mounts, showed that penetration by the stylets is due to mechanical pressure, the stylets taking an intracellular path to the phloem with no visible effects on the cell contents. A stylet sheath surrounds the stylets and remains in position following their retraction. The origin of this sheath is discussed and attributed to the saliva. A tactile stimulus is suggested as the initial stimulus directing the stylets to the phloem.

A study of stylet retraction indicated that retraction is aided by the labium and the presence of part of the stylets within the crumena. The time taken for retraction varied from 6 to 70 minutes, whilst the speed increased during retraction.

Separation of the stylets revealed an intrinsic tension in the mandibles which causes them to coil up. This tension is indicated as being overcome by the surface tension of the saliva existing between the maxillae and mandibles. This is suggested as a basic function of the saliva, responsible for the presence of the stylet sheath.

Growth continues throughout each respective instar although increase in the length of the stylets during the life-cycle is small.

A method of studying the feeding behaviour on the leaves, throughout the insect's life-cycle, is described. Most individuals remain in the same position throughout the life-cycle, a single penetration by the stylets lasting for the duration of each respective instar. At ecdysis, the shedding of the old stylets appears to aid the initial extension of the new stylets.

Observations were made throughout the life-cycle which lasted for 69 days at 27°C. Activity was restricted mainly to the first few hours although the ability to move is retained until parturition.

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Systematics

Hemiptera.

Coccoidea.

Family Lecaniinae.

Coccus hesperidum Linn.

Morphology

The females of Coccus hesperidum, with which this paper is concerned, have an incomplete metamorphosis. The life-cycle consists of 3 stages which are termed respectively the larval, nymphal and adult stage (Quayle, 1932; Balachowsky, 1939; Fonseca, 1953). The possibility of more than 3 stages was suggested by Berlese (1894) and Dingler (1923) whilst Bodenheimer (1951) suggests the probability of 2 nymphal stages.

A detailed account of the morphology has been given by Berlese (1894) and Fonseca (1953). Briefly, this is as follows.

Larva

The body is oval in shape and compressed dorso-ventrally (Figs. 1 and 2). There is no distinction between the head and thorax whilst that between the thorax and abdomen is visible only in the young larva. The legs are situated on the ventral surface, in a line approximately parallel to the edge of the body and about midway between the latter and the medial line. The antennae consist of 6 segments and are situated on the ventral surface in the antero-lateral region,

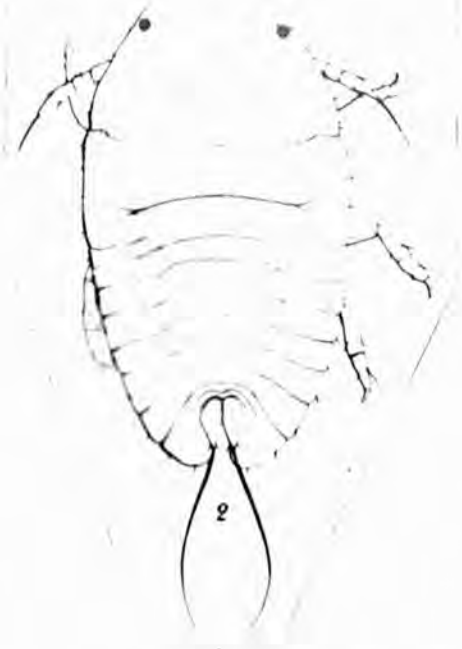


Fig. 1. Dorsal view of larva of C.hesperidum. (Reproduced from Berlese, 1894)

Fig. 2. Ventral view of larva of C.hesperidum. (Reproduced from Berlese, 1894.)

slightly removed from the edge of the body. The 3rd, 4th and 5th segments of the antennae possess a characteristic cross striation (Fonseca, 1953), although Berlese (1894) records it on the 3rd segment only. In the active larva, the antennae and legs project beyond the edge of the body, but when the larva settles, they are applied against the ventral surface. In the latter position, the antennae are directed posteriorly, the first pair of legs are directed anteriorly and bent at the femur-tibial joint, whilst the 2nd and 3rd pair of legs are fully extended and directed towards the posterior end in a line approximately parallel with the edge of the body. This orientation of the appendages is also seen in the later instars. Two simple eyes are situated on the dorsal surface at the antero-lateral edge and the mouth-parts are situated between the anterior pair of legs. There are 2 depressions at the lateral margin on either side of the body, one situated opposite the 1st pair of legs and the other midway between the 2nd and 3rd pair. These are termed the spiracular depressions and each corresponds to a spiracle situated on the ventral surface midway between the line of the legs and the edge of the body. Within each depression there are three setae, the spiracular setae, the middle one being the largest, whilst a transverse suture connects the two posterior depressions. Posterior to this suture, the body is divided up into 8 segments which are visible only in the young larva. The first segment is the

metathoracic and the remainder are abdominal. The posterior segment contains a wide indentation in the medial line, the region of the segment on either side of this indentation being termed the anal lobes. The anus, situated at the base of this indentation, in the furrow of the 8th dorsal arc, opens onto the dorsal surface. Guarding the anus dorsally, there are two triangular plates, the anal plates, which articulate with the body and can be separated from each other during excretion. At the tip of each plate there is a long seta which in length equals the width of the body. In the young larva, the dorsal surface of the body is flat and the larva is ochreous in colour although when feeding commences, the larva changes to a greenish-yellow and the dorsal surface becomes slightly convex (Fonseca, 1954). There is considerable variation in the sizes recorded for the larvae, which Dingler (1923) relates to the habitat. Berlese (1894) records an average length of 500μ and an average breadth of 300μ, whilst Fonseca (1953) gives average values of 381μ and 211μ for the length and breadth respectively and states that the size increases after the larva has settled.

Nymph

The morphology of the nymph is essentially the same as in the larva. The antennae consist of 6 segments, although the cross striations present in the larva are absent. The indentation at the posterior end of the body is narrower than in the larva and the anal lobes lie closer together. The long setae, present on the anal plates of the larva, are lost

at the first moult (Berlese, 1894; Dingler, 1923) although Fonseca (1953) states that they are present in the young nymph but are discarded soon after the moult. The dorsal surface is slightly convex and the colour is greenish yellow although the presence of diffuse or definite tracts of brown pigment are seen on the dorsal surface in some of the older individuals. The sizes, as stated by Fonseca (1953), show a variation in length from 857μ to 1333μ and a variation in width from 500μ to 833μ, the average size being recorded as 1125μ x 676μ.

Adult

The shape of the body in the adult varies between a symmetrical oval shape and an asymmetrical form, found in some of the older individuals, in which one side of the body is approximately straight whilst the other is curved. The antennae consist of 7 segments and the anal lobes are close together and overlap each other slightly. The young adult is slightly more convex on the dorsal surface than the nymph, although the periphery of the body is depressed and scale-like. The colour is still greenish-yellow although the dorsal surface is often darkly pigmented, the intensity and disposition of the pigmentation varying in the different individuals. The ventral surface is pale yellow. In the older adult, towards the time of parturition, the dorsal surface increases in height and becomes progressively pigmented in the abdominal region, in which the maximum width is now situated, as opposed

to its previous position in the thoracic region. At the same time, the ventral surface of the abdomen also becomes pigmented and retracts towards the dorsal surface whilst the periphery of the abdomen and the whole of the thorax remain in contact with the plant. This retraction forms a brood space, between the adult and the surface of the plant, within which the young are deposited. In these sexually mature adults, the segmentation of the abdomen, visible in the young larva, is again visible on the ventral surface of the abdomen. Fonseca (1953) gives the size of the immature adult as 1050 x 900μ and that of the sexually mature adult as 3950 x 2450μ. Berlese (1894) gives the length as about 3800μ and the width as about 2700μ whilst a length of from 3-4 mm. is recorded by Hubbard (1885), Atkinson (1886), Marlatt (1903) and Hume (1926).

The structure of the mouth parts

The structure of the mouth parts of coccids in general has been dealt with in great detail by Pesson (1944) whilst Berlese (1894) and Fonseca (1953) have both given a detailed account of the mouth parts of C.hesperidum.

Briefly, the mouth parts, situated between the anterior pair of legs, consist of the clypeus, which appears externally as a pentagonal protruberance, the single-segmented labium, which is conical in shape and situated posterior to the clypeus, and the stylets themselves. The general structure is shown in Fig. 3.

The stylets consist of the elongated maxillae and mandibles which lie adjacent to each other to form the single

penetrating organ. The maxillae are connected together along their entire length, distal to the hypopharynx, by means of interlocking longitudinal grooves and ridges, whilst the mandibles are applied against their external surface, one on either side (Fig. 4). Two longitudinal canals are present between the maxillae and are produced by the presence of coincident grooves present in the latter. At their proximal end, the dorsal canal is in continuity with the pharynx and is the channel along which liquid food passes, whilst the ventral canal receives the salivary duct. Both canals open to the exterior at the distal end of the stylets. The bases of the stylets diverge from each other at the tip of the hypopharynx and their enlarged bases rest upon inwardly directed outgrowths of the tentorium (Fig. 3). The stylets as a whole pass to the exterior through the tip of the labium via the enclosed labial gutter situated along the length of the latter. In the retracted position the stylets are lodged as a loop in an ectodermal invagination at the base of the labial gutter known as the crumena.

Movement of the stylets

Muscles attached to the basal region of the stylets, and connected also to the inner wall of the clypeus, are responsible for the movement of the stylets in a longitudinal direction. Such movement is limited in extent and occurs independently for each stylet. The method of protraction and retraction of the stylets has not as yet been fully

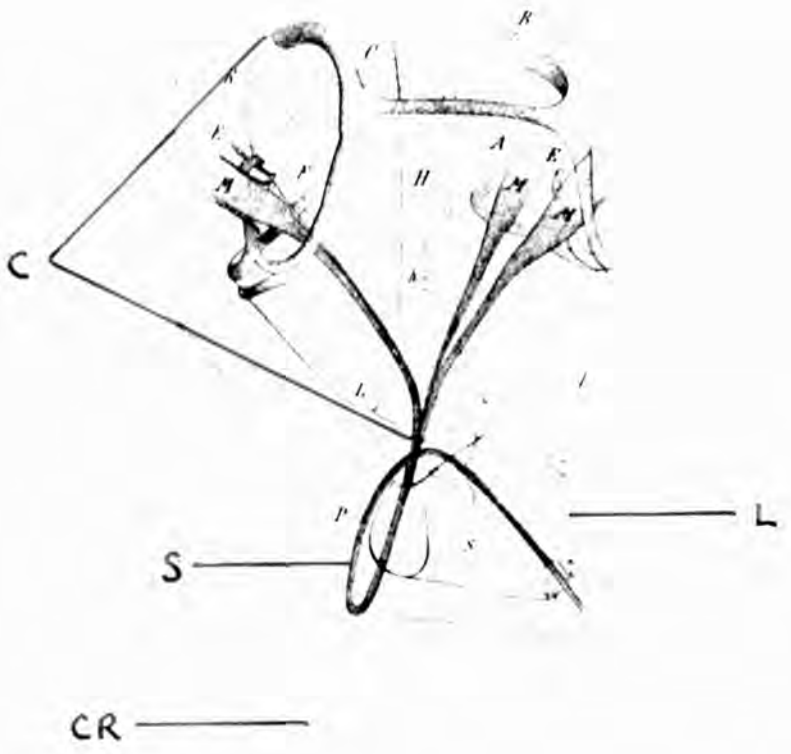


Fig. 3. Ventral view of mouth parts of C.hesperidum. (Reproduced from Berlese, 1894.) C, clypeus; L, labium; S, stylets; CR, crumena

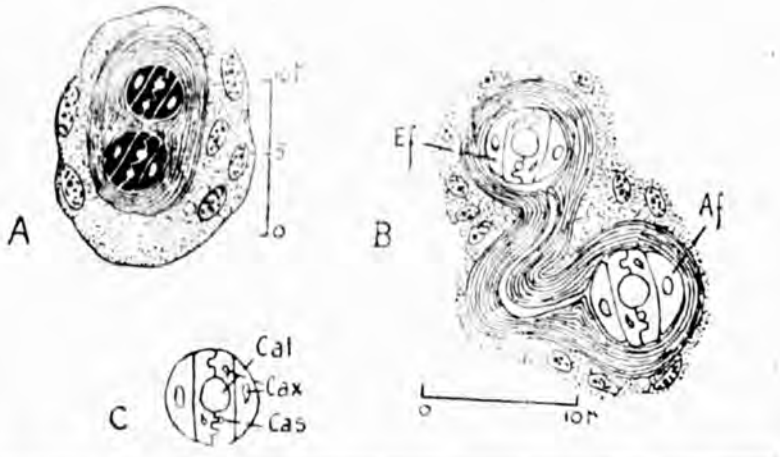


Fig. 4. Transverse sections of the crumena of Aulacaspis rosae, A, and Pseudococcus adoniidum, B, and a transverse section of the stylets of Pulvinaria mesembryanthemii, C. (Reproduced from Pesson, 1944.) Af, afferent portion of stylet loop; Ef, efferent portion of stylet loop; Cal, food canal; Cas, saliva canal; Cax, lumen of stylets.

explained, but is discussed theoretically at great length by Pesson (1944). He considers that the most essential feature of both these processes is the fact that the stylets are united into a single unit and that the movement of any one component at a time is guided by a rigidity imposed by the rest. The sequence in protraction is that one mandible is protracted a short distance, followed by the other mandible and finally the two maxillae together. The region of the stylets extended beyond the labium is then held in this new position by a gripping action of the labial gutter whilst the bases of the stylets are returned, by means of the appropriate muscles, to their initial position thereby withdrawing the stylets slightly from the crumena. The repetition of this process results in the gradual extension of the stylets. The reverse process results in their retraction.

Formation of the stylets

A bulbous cellular structure, known as the retort-shaped organ or styliform organ, is situated at the base of each stylet (Fig. 5). This organ is responsible for the production of the new stylet at the time of moulting. The method of formation of the stylet is essentially the same in the embryo, the stylet arising from a retort-shaped invagination of the embryonic cuticle.

The method of formation of the new stylet has been considered in detail by Pesson (1944 and 1951) and his diagrammatic representation of this process in the embryo is shown in Fig. 6. The resting condition of the styliform organ

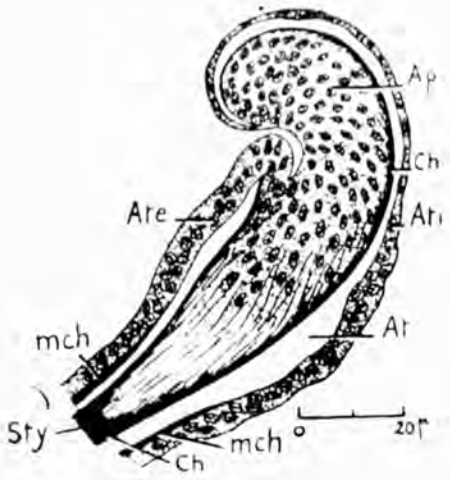


Fig. 5. Section of base of stylet of Pulvinaria mesembryanthemii adult. (Reproduced from Pesson, 1944.)

Ap, cellular structure responsible for production of new stylet; At, atrial cavity; Ate, Ati, walls of atrium which surrounds base of stylet; Ch, cuticle; mch, cuticle of atrium; Sty, stylet.

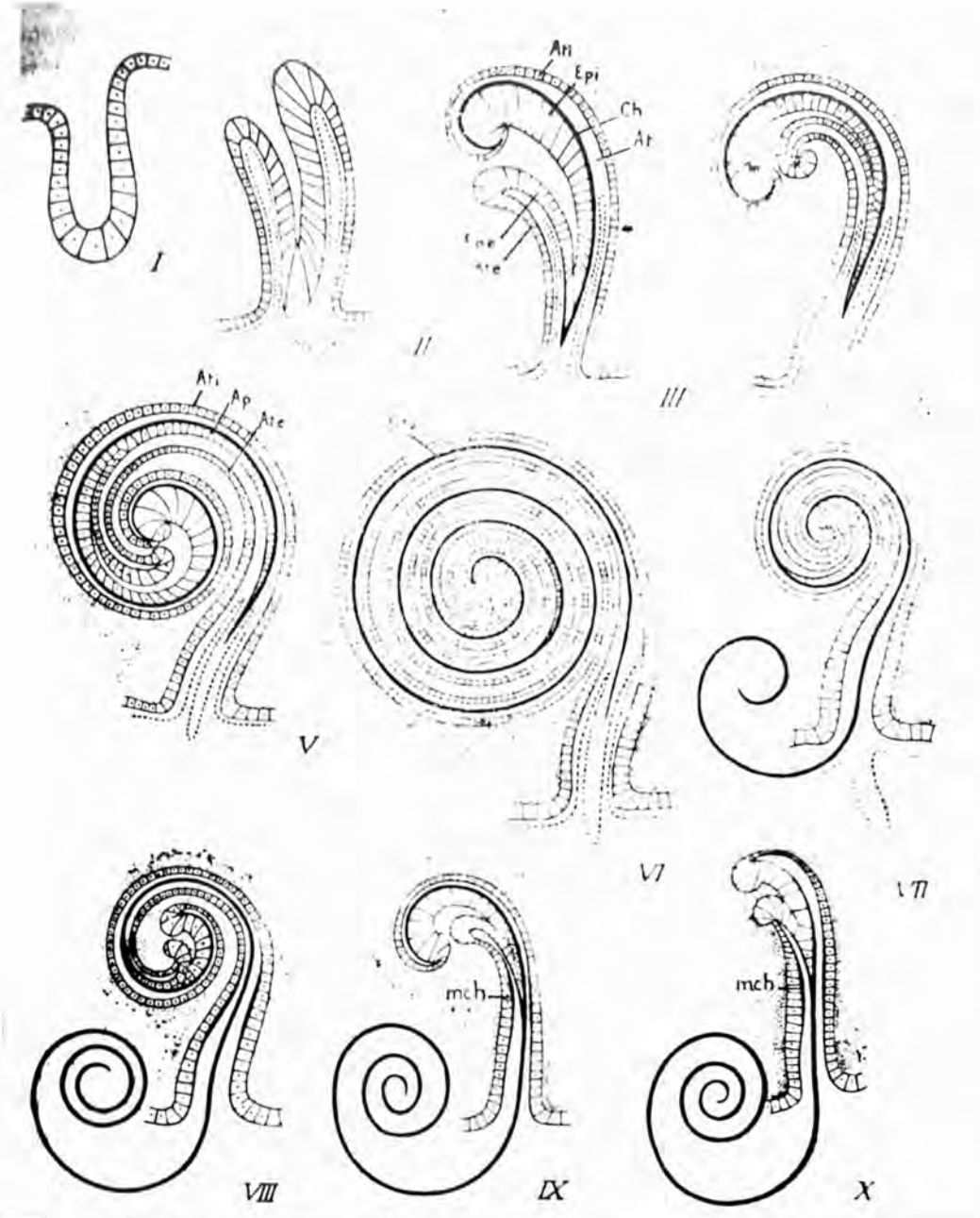


Fig. 6. Diagrams showing the stages in the production of a stylet in the embryo of Coccides. (Reproduced from Pesson, 1944.) At, atrial cavity; Ap, appendage producing new stylet; Ate, Ati, walls of atrium; Ch, cuticle; Epe, Epi, walls of appendage; mch, cuticle of sheath which surrounds base of stylet; Sty, stylet.

(Fig. 5) is comparable to stage II in Fig. 6. The organ, in effect, is an appendage which becomes long and slender, pushing its base into the interior of the head as it grows, its tip remaining stationary within the base of the present stylet or the embryonic cuticle (stages II-VI). As the base of the organ sinks deeper into the head, accommodating its length by means of a spiral formation, it takes with it the adjacent teguments which form a spiral atrial pocket with the appendage implanted at its base. The medial surface of the appendage is responsible for secreting the chitin for the new stylet, whilst the other surfaces of the organ, though ectodermal and covered with chitin, do not produce an important layer. Following the complete growth of the organ, the appendage itself gradually retracts to the base of the atrial pocket (stage VI). During this process, it continues to secrete viscous chitin which it leaves in position. Thus the tip of this chitin resides in the base of the old stylet or the embryonic cuticle and the new stylet is produced as the secreted chitin progressively replaces the retracting appendage. When the latter is fully retracted, it exists as a cellular mass at the base of the spiral atrium and is surrounded by the enlarged base of the new stylet. The latter later becomes hardened and pigmented and acquires its final shape. The emergence of the stylet is brought about by the rapid shortening of the atrial pocket (stage VII-X).

Historical survey of the biology of Coccus hesperidum

Coccus hesperidum is a common pest found in many parts of the world. It exists mainly within the tropics and subtropics, its range extending between latitudes 40°S and 40-50°N (Fonseca, 1955), and is very common in greenhouses (Leydig, 1854; Hubbard, 1885; Marlatt, 1903; Berlese, 1894; Theobald, 1909; Pettit and McDaniel, 1920; Metcalf and Flint, 1939; Rivnay, 1944).

In its feeding habits, C.hesperidum is polyphagous, a list of the actual host plants being given by Fonseca (1955 and 1956). It does however prefer plants with smooth bark and thick glossy leaves (Hubbard, 1885) whilst Bodenheimer (1934) separates the hosts into 4 categories. These are "genuine hosts", upon which the insect develops normally, "tolerated hosts", on which development is normal but the mortality is increased, "partial hosts" upon which development past the larval stage does not occur, and "unsuitable hosts" on which no development at all takes place. The majority are "tolerated" or "partial hosts" although Citrus spp., Laurus nobilis and Nerium oleander are "genuine hosts" (Fonseca, 1955). It shows a strong preference for Citrus spp. on which it is very common (Batchelor and Webber, 1948) and which is the most important host plant economically (Rivnay, 1944; Fonseca, 1955). Amongst Citrus spp., it is found mostly on tangerine and less frequently on the orange, whilst on the lemon it is rare (Fonseca, 1955).

Within a citrus plantation, it is usually restricted to a few trees, and only part of the tree is actually infected (Hubbard, 1885; Quayle, 1932; Batchelor and Webber, 1948). It is mainly the young trees and nurseries that are attacked (Hubbard, 1885; Cook and Horne, 1908; Delcurto, 1925), usually trees less than 4-6 years old (Essig, 1915). On the older trees it is rare (Essig, 1915; Bodenheimer, 1951), but where it does occur on these it is found only on the young shoots (Hubbard, 1885; Marlatt, 1903; Green, 1904; Essig, 1915; Cook and Horne, 1908; Quayle, 1932; Andison, 1940; Batchelor and Webber, 1948; Bodenheimer, 1951). This is in contrast to the holly in which 15 year old trees are more attacked (Andison, 1940) and also the Laurel on which the older leaves are attacked in preference to the young (Dingler, 1923). It prefers shady, humid places (Berlese, 1894; Rivnay, 1944) whilst Bodenheimer (1951) records it mostly in the half shade between the centre and the periphery of the tree. It is never (Berlese, 1894) or else rarely (Fonseca, 1955) found on the fruit.

Its actual distribution on the leaves is variable. Fonseca (1955) records its presence mainly on the upper surface in tangerines, a distribution recorded also by Berlese (1894), Teodoro (1916) and Silvestri (1939). Balachowsky (1932) however, records it on the lower surface whilst a similar preference for this surface is recorded for lemons (Fonseca, 1955) and Laurels (Dingler, 1923). In the orange

however, there is no discrimination (Fonseca, 1955). Balachowsky and Mesnil (1935) also note this lack of discrimination although the name of the host is not recorded.

On the leaves it is situated along the mid-rib (Berlese, 1894; Marlatt, 1903; Essig, 1915; Dingler, 1923; Delcurto, 1925; Fonseca, 1953) and also along the smaller veins (Dingler, 1923; Delcurto, 1925; Balachowsky and Mesnil, 1935) although it is more frequent along the former (Bodenheimer, 1951). The fact that the insect actually feeds from the vein was stated by Berlese (1894).

Rivnay (1944) records three types of infestation within a citrus plantation,

(a) Single females dispersed on the leaves and young twigs, evenly distributed in the grove or part of the grove,

(b) Larvae attacking the young fruit beneath the sepals and causing the fruit to wither and fall prematurely,

(c) Branches and twigs encrusted on only a few trees within the grove.

Damage to the host may be caused by feeding (Hollinger, 1923; Quayle, 1932; Batchelor and Webber, 1948) although Bodenheimer (1951) states that it is never in sufficient numbers to cause damage in this way. It also causes damage however, due to the secretion of honeydew, upon which a sooty fungus grows (Berlese, 1894; Hollinger, 1923; Delcurto, 1925; Quayle, 1932; Batchelor and Webber, 1948; Bodenheimer, 1951). Such fungus coats the leaves and fruits upon which the honeydew accumulates, and consequently reduces photosynthesis and

in the case of the fruit renders the latter unsaleable. Although it is rated as a serious pest by Hollinger (1923), and recorded as doing heavy damage in Sicily (Berlese, 1894), Bodenheimer (1951) states that it only causes damage to young trees whilst Batchelor and Webber (1948) consider it as a major pest of citrus in South Africa alone. Balachowsky and Mesnil (1935), Quayle (1938) and Rivnay (1944) rate it as a non-serious pest of only secondary economic importance.

Reproduction in C.hesperidum is usually parthenogenetic and males are rare (Fonseca, 1954). The duration of the life-cycle varies with the temperature and at 30°C it lasts for 24 days, whilst at 15°C it takes 210 (Bodenheimer, 1951). Silvestri (1939) and Gomez-Clemente (1943) record it lasting a little over a month in favourable conditions. Several generations are produced per year, depending on the temperature. There are 2-3 in Argentina (Blanchard, 1930), 3-4 in hothouses in Germany (Dingler, 1923), 3-5 in Southern California (Quayle, 1932), 6 in Palestine (Bodenheimer, 1951) and 10 in the tropics (Silvestri, 1939). The various generations are not restricted to a particular season and all stages are found together (Bodenheimer, 1951). It is ovoviviparous (Green, 1904; Quayle, 1932; Metcalf and Flint, 1939) and the young are deposited within the brood-space beneath the adult. The period of oviposition varies from 10-20 days in Palestine to 2 or more months in moderate climates (Bodenheimer, 1951), only a few eggs being laid per

day (Metcalf and Flint, 1939; Bodenheimer, 1951). The young larvae shed their embryonic membranes 15-30 minutes after birth according to Fonseca (1953), although Bodenheimer (1951) records it as a few hours. The larvae then undergo a period of torpor lasting from 24 to 48 hours (Fonseca, 1954), which Balachowsky (1939) refers to as a true diapause. The length of time spent beneath the female is stated by Berlese (1894) as lasting for some days. The larvae then emerge from beneath the adult and wander over the plant prior to settling, such activity lasting a few hours (Fonseca, 1954). They are gregarious in habit (Marlatt, 1903; Green, 1904; Cook and Horne, 1908; Andison, 1940) and usually settle near the female (Metcalf and Flint, 1939; Bodenheimer, 1951) in a compact colony (Quayle, 1938) on the young and tender growth penetrable by the stylets (Hubbard, 1885; Fonseca, 1955). Although they remain in one spot if not disturbed (Bodenheimer, 1951), the larvae may move about for a time after settling (Metcalf and Flint, 1939; Fonseca, 1954). This mobility is shown also by the later instars although Bodenheimer (1951) and Fonseca (1954) state that it is usually restricted to the period after a moult. Dingler (1923) records that the nymphs alternate repeatedly between moving and feeding but settle prior to moulting and insert their new stylets into the plant and feed. Although Quayle (1938) states that this mobility is retained until the individual is about half grown, Balachowsky and Mesnil (1935) state that it is mobile up to

an advanced stage. Berlese (1894) notes that the mature female can move even when the brood-space is present although Hubbard (1885), Bodenheimer (1951) and Fonseca (1953) state that it can move only until it is formed and Dingler (1923) records that movement is no longer possible as the brood-space increases in height. Although the larvae settle on the leaves, the nymphs and adults are seen to migrate to the branches at the approach of parturition (Balachowsky and Mesnil, 1935) a fact also noted by Fonseca (1954) who states in addition that the young adults usually seek the young branches as a more convenient position for egg-laying. The fact that some individuals mature on the leaves however, has been noted by Quayle (1932), Batchelor and Webber (1948) and Fonseca (1953).

SECTION II

Feeding

Feeding

Techniques

The host plants used throughout this investigation were seedlings of the Rough Lemon which had been grown in small pots inside a greenhouse.

The feeding sites on the leaves and stems were studied by sections of fixed and unfixed material. In the case of the fixed material, Fleming's No. 1 was used as fixative, and the sections were cut at 10 microns. The sections were stained with safranin and light green, which have been recorded as producing successful results in the study of the stylet tracks of both aphids (Davidson, 1923; K.M. Smith, 1926) and coccids (K.M. Smith, 1926). The unfixed material was cut at 25 microns with a freezing microtome, using frozen normal saline as the supporting medium.

The feeding sites on the leaves were also studied by means of whole mounts prepared in the following way. The whole leaf was plunged into hot (70°C) 95% alcohol to kill the insects instantaneously and prevent any retraction of the stylets. The leaf was then cut up into small pieces approximately 1 cm. square, each containing one individual, and treated with 70%, 90% and 95% alcohol for one hour respectively. They were then placed in absolute alcohol, until all the chlorophyll had been removed, and then into clove oil till clear. The use of this technique made it possible to see the stylets within the leaf.

In the examination for the possible breakdown of starch, whole mounts of leaves, sections of stems and petioles, and artificial substrates were all used. For the whole mounts, the leaves were plunged into boiling water for 1 minute and then cut up into pieces approximately 1 cm. square, each containing one individual. These pieces were then placed into 70% alcohol, until the chlorophyll had been removed, and then into a solution of iodine in potassium iodide. The blackened leaves were rinsed in distilled water, the insect dislodged, and the feeding site examined. Since Aonidiella aurantii Maskell is known to break down the starch in Citrus leaves (Baranyovits, 1953), leaves containing this species were treated in a similar manner and used as a control in this technique.

Hand sections of unfixed stems and petioles were taken through the feeding site, the sections treated with iodine solution, and the stylet tracks examined for the breakdown of starch in their vicinity. Since the starch of citrus plants is strongly retained by the chloroplasts and is difficult to breakdown (Batchelor and Webber, 1948), additional starch substrates were used.

The use of potato tubers was carried out as follows. Tubers with smooth skins were selected, washed and dried. Small pieces of leaf, containing the insect, were placed on their surface and held in position by pins. The tubers were then placed in closed dishes within the greenhouse and examined daily. As the leaves dried up, the insects moved

onto the tubers and commenced to feed. Portions of tubers, approximately ½ cm. square and ½ cm. in depth, containing the insect, were removed and fixed in Fleming's No. 1. They were then sectioned at 15 microns and stained with iodine.

An agar substrate, containing starch, was also used. Various concentrations of starch and thickness of agar films were tested in order to obtain an intensity of colouration with iodine which would not obscure any possible breakdown of starch within the interior of the film. The concentration which was finally used was a 0.25% solution of starch in a 5% solution of agar, this being plated out in petri dishes to a depth of approximately 2 mm. The agar was allowed to set and then cut up into squares approximately 2 cm. in length. The squares were lifted with a section lifter, placed on slides and each one covered with a cover-slip so that the latter protruded beyond the edge of the agar in each case. In this way the surface of the agar available to the insect was restricted, so that the examination of the stylet tracks was not obscured by the body of the insect. Small pieces of leaf, containing the insect, were then placed on the slides around the agar and the slides placed in covered petri dishes in the green-house and examined daily.

In addition, salivary glands were dissected out and squashed onto agar films, containing starch of the above concentration. The preparation was left to stand for several minutes and then treated with iodine.

RESULTS

Distribution on the plant

The insects were present on the stems and the leaves. On the latter they were found on both the upper and lower surface.

On the stems they were present on the smooth green surfaces and absent on the rough surfaces towards the base. In all cases they were situated with their longitudinal axis parallel with that of the stem itself.

On the lower surface of the leaves, they were settled alongside the smaller veins as well as the mid-rib with no marked preference for the latter. Although present on the upper surface, they were not as common as on the lower and were restricted mainly to the mid-rib with only occasional individuals on the veins.

The individual settles alongside the vein with the longitudinal axis of the body parallel to it, so that the edge of the body nearest the vein overlies the latter in the case of the small veins, or one side of it in the case of the larger ones. In 246 individuals examined (100 larvae, 46 nymphs, 100 adults), all showed this same orientation.

Food source and method of penetration

The stylet track of C.hesperidum is of the third type mentioned in Busgen's classification (Busgen, 1891), the stylets taking an intracellular path to the phloem (Figs. 7, 8, 9 and 12). In the case of individuals feeding on the stems and mid-ribs, the tracks were seen in a few cases to pass beyond the phloem and enter the vessels of the xylem. (Fig. 7).

The stomata are not used in penetrating the epidermis in spite of the fact that in some cases they lay adjacent to the point of entry (Fig. 10). Penetration of the thick-walled

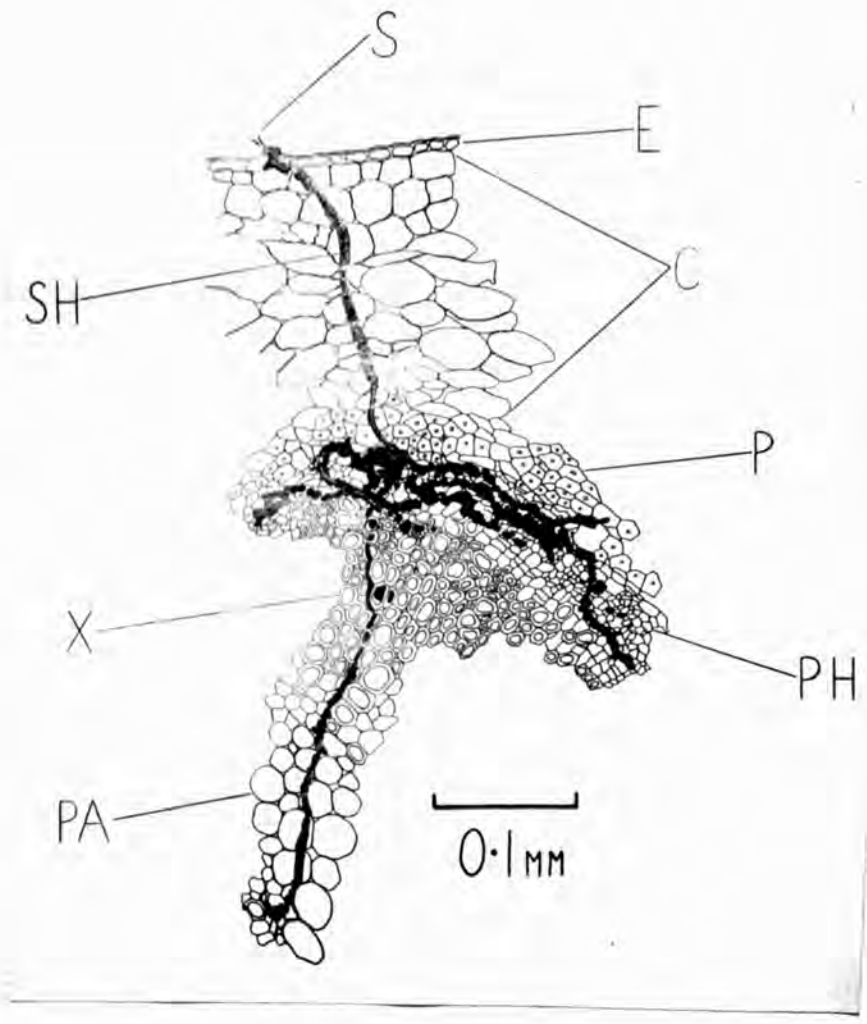


Fig. 7. Transverse section of the mid-rib of a leaf of the Rough Lemon showing penetration by the stylets of an adult of C.hesperidum. (Material fixed and stained.)

N.B. The intracellular method of penetration, the branching of the stylet track within the phloem and the change in direction of the distal end of the stylets.

C, cortex; E, epidermis; P, pericyclic fibres; PA, parenchyma; PH, phloem; S, stylets; SH, stylet sheath; X, xylem.

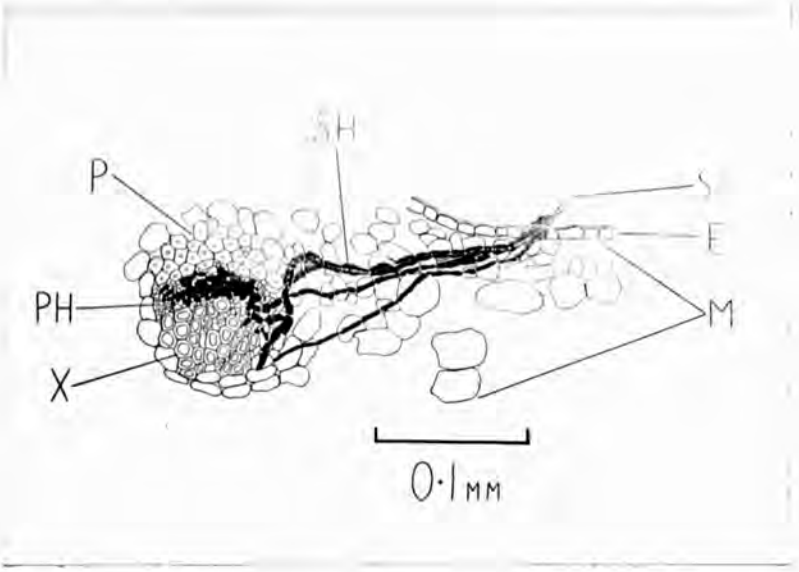


Fig. 8. Transverse section of a side vein of a leaf of the Rough Lemon, showing penetration by the stylets of an adult of C.hesperidum. (Material fixed and stained).

N.B. The oblique penetration of the epidermis. The two tracks below the stylets have been made during previous penetrations and show the presence of the residual sheath within the air-spaces.

M, mesophyll. For other abbreviations see Fig. 7.

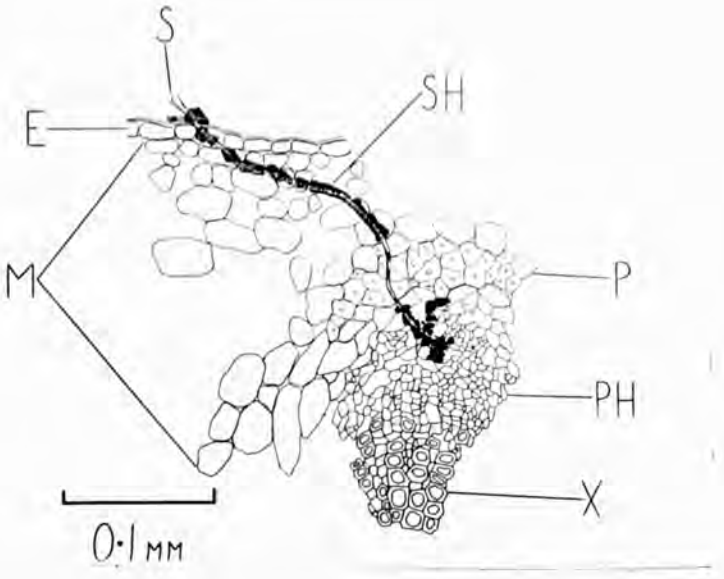


Fig. 9. Transverse section of a side vein of a leaf of the Rough Lemon, showing penetration by the stylets of an adult of C.hesperidum. (Material fixed and stained.)

N.B. The oblique penetration of the epidermis and the intercellular path through the pericyclic fibres with a reduction in the thickness of the stylet sheath.

For abbreviations see Figs. 7 and 8.

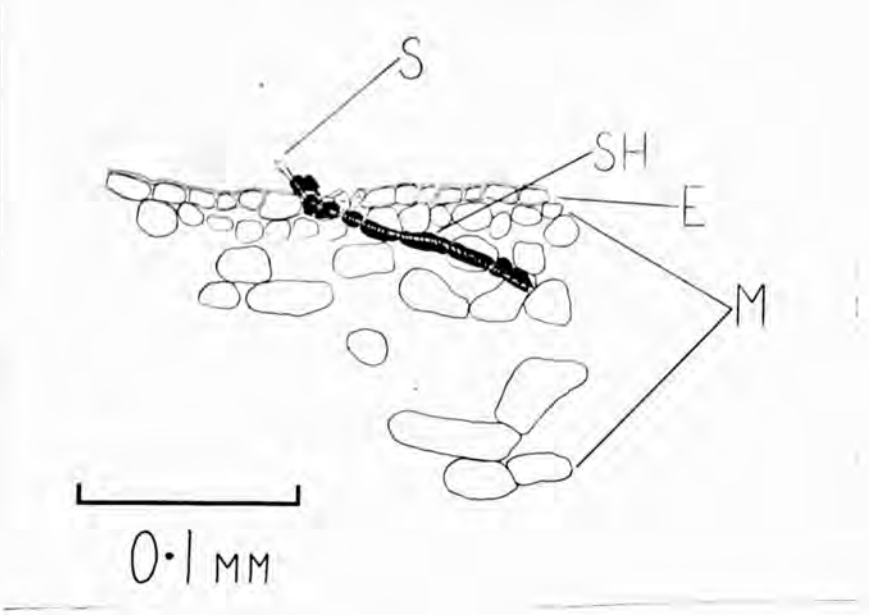


Fig. 10. Transverse section of the mesophyll of a leaf of the Rough Lemon, showing penetration by the stylets of an adult of C.hesperidum. (Material fixed and stained.)

N.B. The oblique penetration of the epidermis and the constriction of the stylet sheath at the cell walls.

For abbreviations see Figs. 7 and 8.

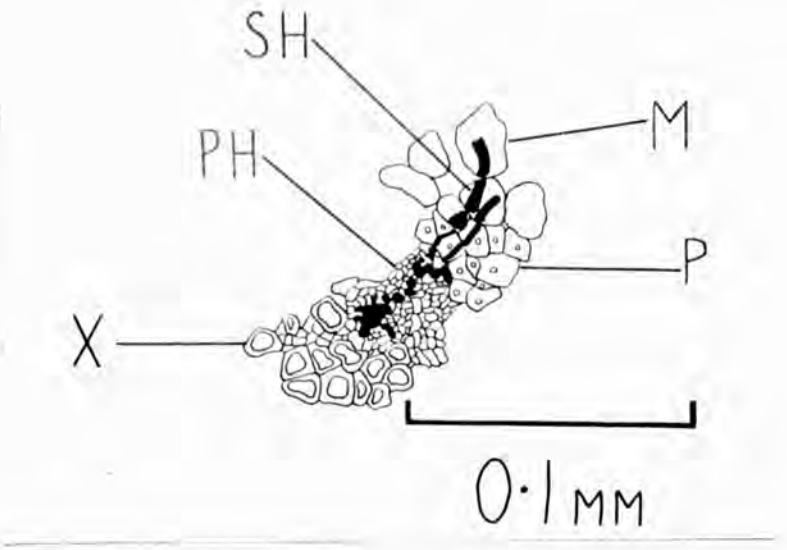


Fig. 11. Transverse section of a side vein of a leaf of the Rough Lemon, showing the residual sheath present within the tissues after the retraction of the stylets of C.hesperidum. (Material fixed and stained.)

N.B. The intercellular and intracellular path through the pericyclic fibres and the constriction of the stylet sheath at the cell walls.

For abbreviations see Figs. 7 and 8.

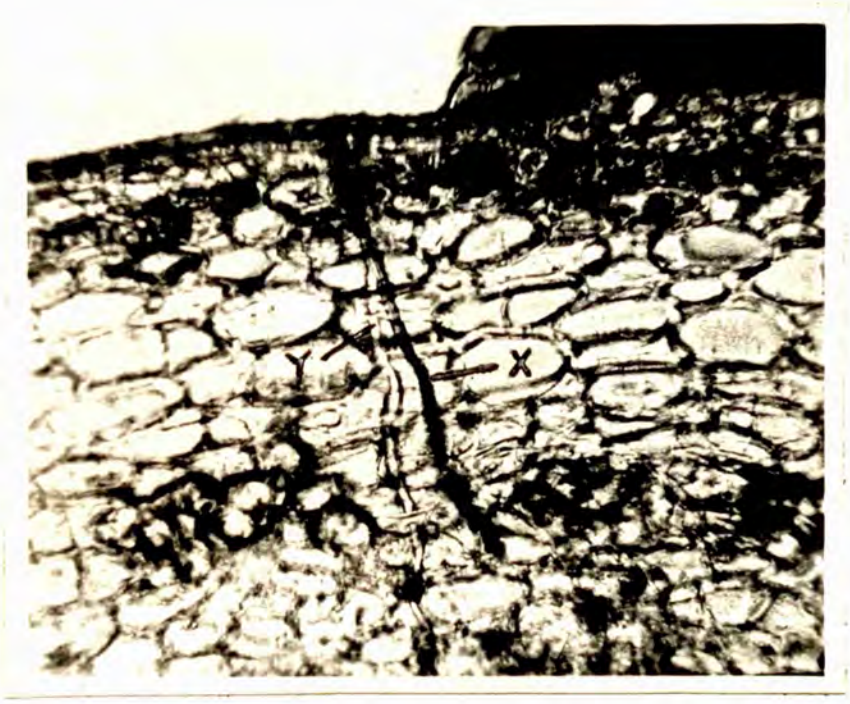


Fig. 12. Transverse section of the outer cortex of a stem of the Rough Lemon, showing penetration by the stylets (X) of an adult of C.hesperidum. (Unfixed material, x 100.)

The adjacent track (Y) is the residual sheath of an earlier penetration.

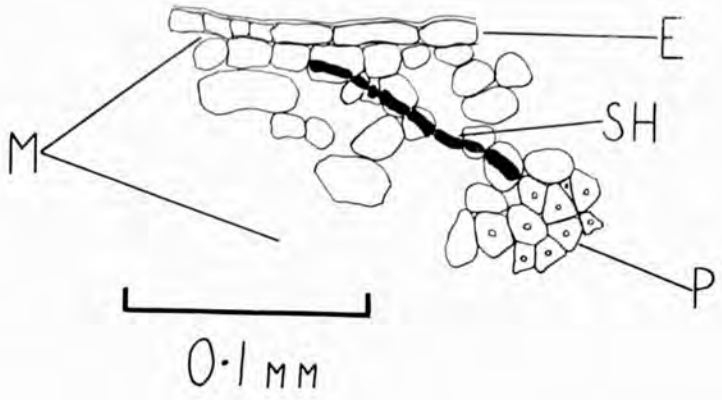


Fig. 13. Transverse section of the mesophyll of a leaf of the Rough Lemon, showing the residual stylet sheath present within the tissues after retraction of the stylets of an adult of C.hesperidum. (Material fixed and stained.)

N.B. The constriction of the sheath at the cell walls and its presence within the air space.

For abbreviations see Figs. 7 and 8.

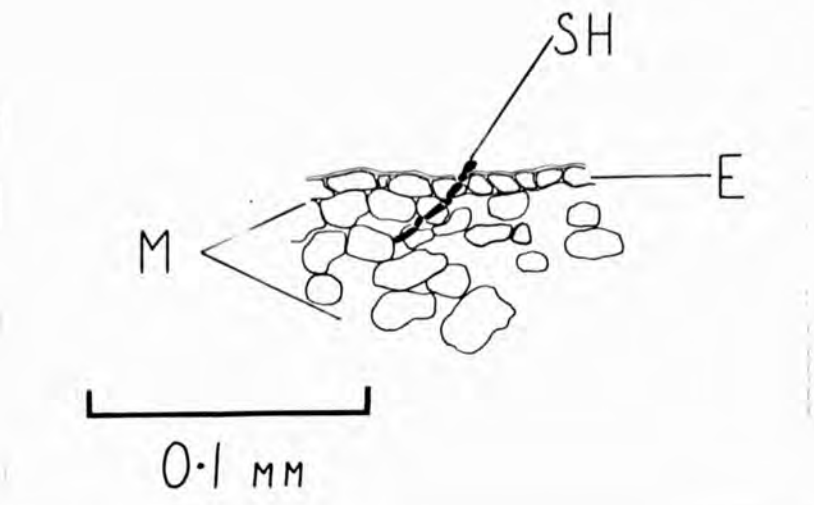


Fig. 14. Transverse section of the mesophyll of a leaf of the Rough Lemon, showing the residual stylet sheath present within the tissues after the retraction of the stylets of a larva of C.hesperidum. (Material fixed and stained.)

N.B. The oblique penetration of the epidermis and the intracellular course.

For abbreviations see Figs. 7 and 8.

pericyclic fibres is either inter- or intra-cellular (Figs. 7, 9 and 11). Passage through the cell walls appears to be entirely by mechanical pressure exerted by the stylets, since the diameter of the aperture in the cell wall does not exceed the diameter of the stylets themselves to indicate a dissolving action by the saliva (Figs. 7-14).

The stylets penetrate the epidermis directly beneath the labium, and in both the leaves and the stems they pass to the phloem, either by means of a straight path or else a slightly meandering one (Figs. 12, 25, 30, 34 and 35). (Further details regarding this variation in the behaviour of the stylets, in the case of individuals feeding on the leaves, is given in Section V). In those individuals feeding on the stems, the stylets penetrate the tissues at approximately right-angles to the surface of the epidermis (Figs. 12 and 17). On the leaves however, the stylets pass obliquely towards the vein with respect to the surface of the epidermis (Figs. 8, 9, 10, 13 and 14). In these individuals, the significant fact, as discussed below, is that the penetration of both the cuticle and the epidermis is also obliquely towards the vein. Within the leaves, owing to the orientation of the insect with respect to the vein, and the fact that the stylets pass towards the vein along an approximately straight path, the distance travelled by the stylets is approximately half the width of the body. The entire length of the stylets is never inserted into the leaf during

feeding, the excess being retained looped up within the crumena. In 246 individuals examined, feeding on the leaves, all showed this residual loop, the size of the latter depending however on the path taken by the stylets within the leaf.

The stylet sheath

Surrounding the stylets within the plant there is a distinct sheath which stains brightly with safranin (Figs. 7-15). This sheath is present in both the cells and the intercellular air-spaces, although where the stylets penetrate the wall of a cell it is reduced to a thin film surrounding the stylets (Figs. 9, 10 and 13). Owing to this, the stylet track has the appearance of a string of beads, the length of each bead depending on the distance between one cell wall and the next. The diameter of the sheath also varies throughout the length of each individual bead. This variation is so pronounced in places as to give rise to distinct nodules on the sheath (Figs. 9, 10 and 15). Where the stylets penetrate between the cells of the pericyclic fibres, the sheath is reduced to a thin film surrounding the stylets, whilst penetration through the wall of a fibre causes a similar reduction in the diameter of the sheath (Figs. 7, 9 and 11). The sheath is also present to a small extent on the surface of the epidermis. At the distal end of the stylets, the sheath completely encloses the tips.

The sheath is restricted to the stylets themselves, and

the staining reaction of the sections offered no indication that it diffuses away from the stylets. Even within the cells themselves it is closely applied to the stylets and does not occupy any other part of the cell.

When the stylets are retracted, this sheath remains in position and even the internal lumen, previously occupied by the stylets, remains free of sheath material. Sections of plant material in which this residual sheath had been sectioned within an air-space, showed that the free end of the sheath retained the shape and position of its cut surface within the air-space. These facts were observed in both fixed and unfixed material (Figs. 8, 12, 15 and 16). This residual sheath is known to occur in other phytophagous Hemiptera, a fact which was noted for the Homoptera by Arnaud (1918). Due to this residual sheath, it is possible to see the path taken by the stylets in previous movements within the plant and an accurate recording of this past behaviour, as well as the position of the stylets themselves, on that particular point on the plant is thereby obtained. This was very clear in the whole mounts of the leaves from which it was possible to obtain a complete picture of this past behaviour (Figs. 30 and 34). Thus, in addition to the stylet track itself, side-branches, composed of the sheath alone, are found in some individuals. These arise in either the mesophyll (Figs. 15 and 16) or the vein (Figs. 7-9) indicating that the insect withdraws its stylets a short distance and then inserts them

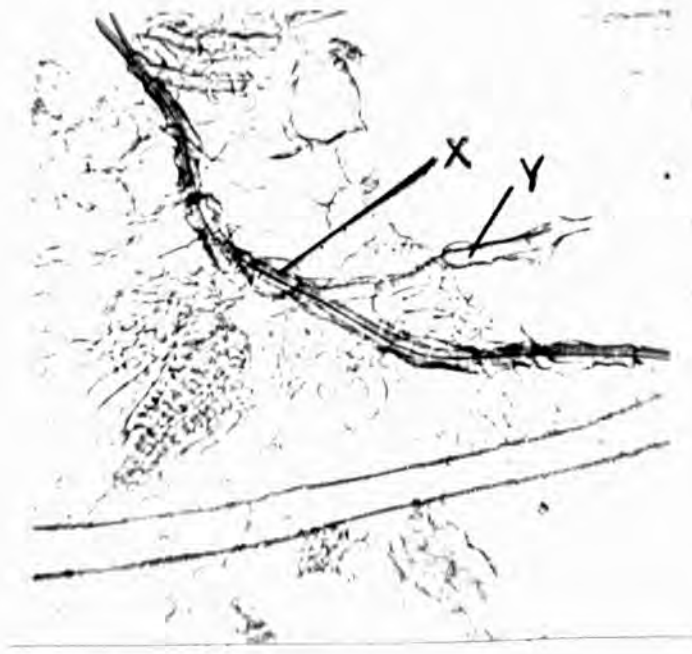


Fig. 15. Transverse section of the mesophyll of a leaf of the Rough Lemon, showing the passage of the stylets (X) of an adult of C.hesperidum. (Unfixed material x 400.)

N.B. The presence of the internal lumen in the side-branch (Y).

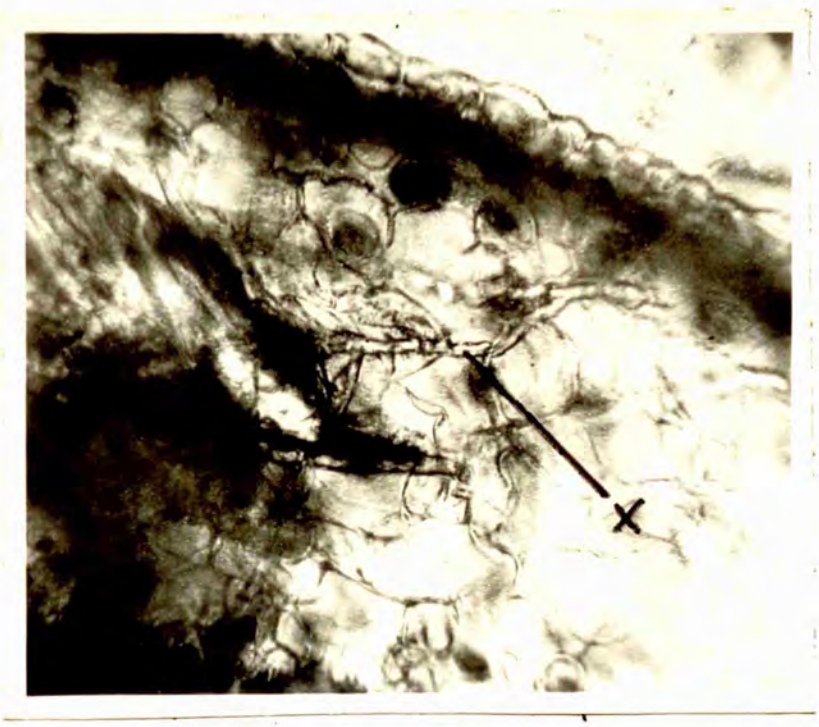


Fig. 16. Transverse section of a leaf of the Rough Lemon, showing the presence within the mesophyll of the residual sheath (X) after the retraction of the stylets.

in another direction. This branching is often more intense within the vein than the mesophyll although many individuals show no sign of branching at all. The presence of this residual sheath in a large number of the phloem cells, which, due to their small size, are completely filled by the sheath, shows that the stylets probe about within the phloem itself (Figs. 7-9). Within the phloem, the stylets travel in a plane at right angles to the longitudinal axis of the cells so that the maximum number of cells are penetrated. This habit is very conspicuous in those individuals feeding on the stems and midribs (Fig. 7). Branching of the stylet track is seen to occur in other phytophagous Hemiptera (Arnaud, 1918; Davidson, 1923; K.M. Smith, 1926; Pussard, 1932; Tate, 1936-37; Leonhardt, 1940) and its occurrence in C.hesperidum will be considered in greater detail in Section V in which the feeding behaviour throughout the life-cycle is studied.

Effect of Penetration

The external appearance of the stem or leaf is not affected by the penetration of the stylets. Within the plant tissue, there is likewise no visible effect upon the plant cells. The actual penetration of the cell walls appears to be by mechanical pressure alone, and there are no signs of dissolution or breakdown of the cell walls along the path of the stylets. The cells through which the stylets pass, and those adjacent to the stylets, show no sign of

plasmolysis.

Within the chloroplast-containing cells of the leaves and stems, the stylets, surrounded by their sheath, pass alongside the chloroplasts without affecting their shape or colour (Fig. 17).

The saliva has likewise no effect upon the starch. The whole mounts of the leaves showed a uniform black colouration with no variation in the intensity of the stain at the feeding site. The mounts of Aonidiella aurantii however, used as a control, gave clear indication of the breakdown of the starch along the path of the stylets. The passage of the stylets through the starch zone of the outer cortex of the stems and petioles likewise has no effect upon this starch (Fig. 18). Similar results were obtained with those individuals fed on potato tubers. In the case of the latter, starch grains situated adjacent to the stylet track showed no variation from those seen elsewhere in the sections.

No conclusive results regarding the action of saliva on starch were obtained with the use of the agar substrates. The salivary glands proved to be too small to remove completely free of surrounding tissue, and this made efficient squashing on the agar plates difficult. The absence of starch breakdown within the agar was consequently not accepted as a conclusive result.

The feeding experiments with the agar squares were also unsuccessful due to the failure of the individuals to settle. Movement from the pieces of leaves commenced within a few

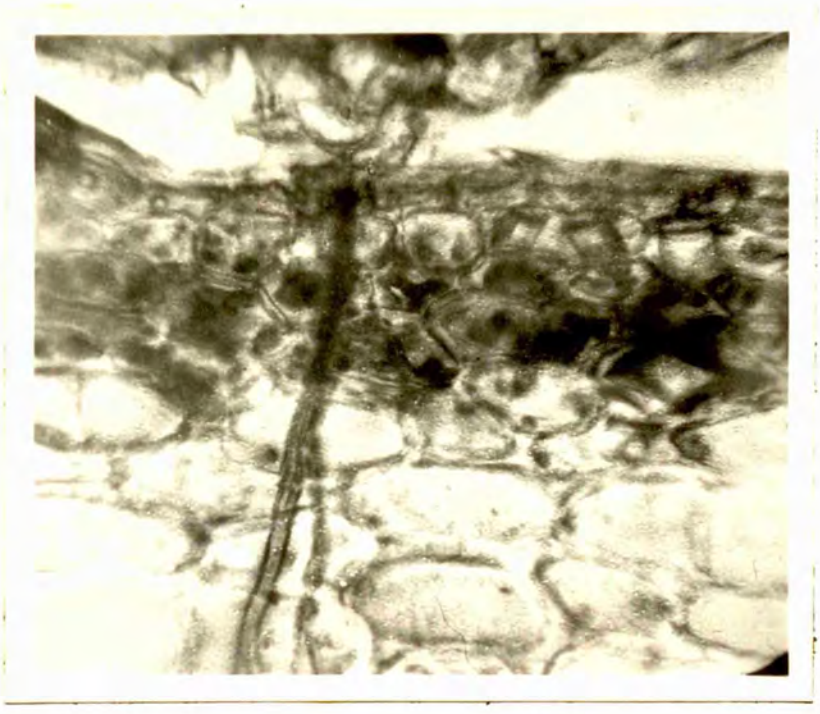


Fig. 17. Transverse section of the outer cortex of a stem of the Rough Lemon, showing the passage of the stylets of an adult of C.hesperidum through the chlorophyll zone. (Unfixed material; x 400.)

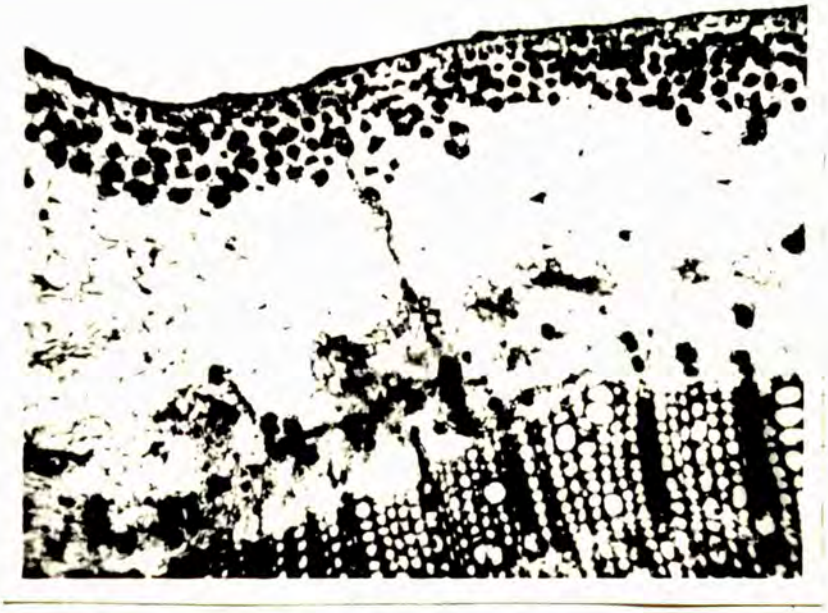


Fig. 18. Transverse section of a stem of the Rough Lemon, showing the passage of the stylets of an adult of C.hesperidum, through the outer starch zone of the cortex. (Unfixed material, stained with iodine; x 60.)

hours, and all pieces were vacated within 2 days, but no individuals were found on the agar.

Ability of the stylets to change direction

An interesting feature in the behaviour of the stylets seen in the whole mounts, and one that is shown by all instars, is their ability to change direction. In addition to the meandering course of the stylets in some individuals, and the branching of the stylet track connected with the search with a new food source, some individuals showed a change in direction which has no apparent connection with feeding itself.

An extreme example of this behaviour was shown by individuals whose stylets travelled in a straight line towards the vein, then doubled back through approximately 180°, changing direction again almost immediately by another 180° to continue their course towards the vein ("Hair-pin bend"). Less extreme behaviour showed the stylet track with a sharp right-angle turn to either left or right followed almost immediately by another right-angle turn towards the vein ("Right-angle bend"). This change in direction of the stylets occurs at any point along their path between the epidermis and the phloem. Table 1 shows the frequency of this behaviour in the individuals examined. It has no significant effect on the course towards the vein and in many cases was not apparent until the stylets were examined under high power. In no case was there any material object in the path of the stylets which could have deflected them off their course. The possibility

that they are deflected by the cell walls is unlikely owing to the method of penetration. In addition, this change of direction in some cases occurs in the middle of a cell or an air-space.

Table 1. The frequency of extreme changes of direction of the stylets in individuals of C.hesperidum feeding on the leaves.

Instar	Total examined	"Hair-pin bend"		"Right-angled bend"	
		Within mesophyll	Within vein	Within mesophyll	Within vein
Larva	100	7	10	5	3
Nymph	46	0	2	2	1
Adult	100	3	0	2	3

DISCUSSION

The origin of the stylet sheath

The presence of a stylet sheath has been noted in Coccidae by Brown (1916), Arnaud (1918), K.M. Smith (1926), Carter (1945), and Guennelon-Aubanel (1951). It is also seen in aphids (Zweigelt, 1914; Arnaud, 1918; Brandes, 1923; Davidson, 1923; Horsfall, 1923; Chrystal, 1926; K.M. Smith, 1926; Tate, 1936-37; Leonhardt, 1940), whiteflies (K.M. Smith, 1926), jassids, (K.M. Smith, 1926; F.F. Smith, 1933), psyllids (Pussard, 1932) and Lygaedae (Painter, 1928), although it is absent in capsids (K.M. Smith, 1926).

The origin of this sheath in phytophagous Hemiptera has been discussed by previous workers and ascribed to the plant, the insect, or an interaction of substances from both sources. Although Millardet, working on Phylloxera vastatrix Planch., considered it to be entirely of plant origin and composed of cellulose, the majority of workers consider the sheath to be either the product of an interaction between the cell-sap and the insect's saliva (Zweigelt, 1914; Horsfall, 1923; Davidson, 1923; King and Cook, 1932; Pussard, 1932; Tate, 1936-37; Balachowsky, 1937; Baranyovits, 1953), or else entirely of insect origin (Woods, 1900; Busgen, 1891; Petri, 1908-11; Arnaud, 1918; F.F. Smith, 1933).

Brown (1916), working on Chionaspis pinifoliae Fitch. and K.M. Smith (1926), working on Aspidiotus hederae Vallot and Aleyrodes vaporariorum Westd., both consider that the sheath is composed of saliva, whilst Zweigelt (1914), Brandes (1923), Withycombe (1926) and K.M. Smith (1926) have actually observed the secretion of this saliva in various species during the penetration of the plant tissue, and likewise consider that this is the origin of the sheath. Bennett (1934) observed the formation of the sheath by the jassid Eutettix tenellus Baker in a synthetic food substrate and Carter (1945), working on Pseudococcus brevipes Ckl., observed the formation of the sheath in inert agar by the successive secretion of drops of saliva from the tips of the stylets, the latter penetrating the drops as they were produced. A

similar formation of the sheath has been observed in Pseudococcus citri Risso which extended its stylets in air (Guennelon-Aubanel, 1951).

In C.hesperidum it has been shown above that the penetration of the cell wall is equal to the diameter of the stylets alone. It is therefore difficult to conceive that the material composing the sheath could have flowed from within the cells to surround the region of the stylets in the intercellular spaces, and yet be of equal development in both regions and show similar nodular formations. These facts, together with the occurrence of the sheath on the surface of the epidermis, do not support the theory of plant origin in C.hesperidum.

Such a distribution of the sheath material does however suggest that it is of insect origin. It has been mentioned above that saliva is secreted during the penetration of the plant tissues by other species of Hemiptera and it has also been shown that, in two species of coccids, the sheath is composed of these secretions which are penetrated by the stylets. Such an origin could account for the appearance of the sheath in C.hesperidum. The presence of the nodules upon the sheath can be explained by variations in either the rate of secretion or rate of penetration, thereby producing unequal accumulation of saliva along the path of the stylets. The presence of the sheath on the surface of the epidermis indicates that the insect commences to secrete saliva as the

tip of the stylets leave the labium and before the epidermis is actually penetrated. K.M. Smith (1920-21) observed this occurring in the capsid Psallus ambiguus, which secreted a drop of saliva onto the surface of the leaf and then penetrated the leaf through the saliva, whilst a similar habit was noted in Aphis maidis by Brandes (1923). K.M. Smith (1926) also refers to this surface nodule in Myzus circumflexus Buckt. as saliva, although Davidson (1923) refers to it in Aphis rumicus L. as the contents of the sheath which have oozed out onto the surface of the epidermis. Heriot (1937) however, considers this surface nodule in aphids as the basal membranes of the epidermal invaginations producing the stylets, which are brought down with the latter and are sloughed off during penetration. This latter explanation however, does not conform to the account of the formation of the stylets given by Pesson (1944).

The viscosity of the saliva when it is first secreted is indicated by the fact that the sheath is restricted to the stylets and that the slight variations in thickness along its length are retained. The additional fact that its shape, position and internal lumen are retained following the retraction of the stylets, giving it the appearance of a solid structure, suggests that the viscosity is increased within the plant since the saliva must have been less viscous during its actual secretion. Whether this hardening of the saliva in C.hesperidum, noted also by Zweigelt (3) (1914) and

Withycombe (1926) in aphids, is due to substances from the plant or intrinsic in the saliva itself, is not known. But the fact that the salivary track of Pseudococcus brevipes in agar hardened sufficiently to enable it to be dissected out (Carter, 1945), and that the salivary track of Pseudococcus citri in air also hardened sufficiently to retain its shape and internal lumen (Guennelon-Aubanel, 1951), shows that the presence of plant substances is not essential to cause such hardening to occur.

Function of the saliva and stylet sheath

In many species of phytophagous Hemiptera, the penetration of the plant tissues has a marked effect upon the external appearance of the plant in the vicinity of the feeding site. An extreme example is seen in the aphid Schizoneura lanigera Hausm. which causes tumours on apples (Arnaud, 1918). In some species there is a discolouration of the plant tissue over a wide area around the point of penetration. This is seen in the aphid Lachnus piniradiatae Davidson on pine-needles (Brown, 1916) and K.M. Smith (1926) has noted the destruction of the chlorophyll around the point of penetration of the aphids Myzus persicae Sulzer and M.circumflexus and the jassids Eupteryx auratus and Zygina palludifrons Edw., due to the diffusion of saliva, which also causes plasmolysis and cell disorganisation by the two aphids. A similar destruction of the chlorophyll around the point of penetration due to the diffusion of the saliva is seen in the Californian

Red Scale, Aonidiella aurantii (Baranyovits, 1953). This wide-spread discolouration is at times followed by the actual death of the affected area as in the case of the coccids Aspidiotus abietus Schr. and Chionaspis pinifoliae on pine-needles, the entire needle being killed (Brown, 1916). Herbert (1920) notes that the Cypress Bark Scale, Ehrhornia cupressi, actually causes the eventual death of the entire tree. Such discolouration, and eventual killing of the tissue, has been found to occur also in the capsids, Calocoris bipunctatus Fab. and Lygus pabulinus Linn. (K.M. Smith, 1926). A spreading effect of the injury produced by penetration is seen also in the aphid Myzus cerasi on peach (Davidson, 1923), whilst Carter (1945) actually traced the diffusion of the saliva of Pseudococcus brevipes in agar with radio-active phosphorus, similar work being done later by Nuorteva and Reinius (1953) on Lygus rugulipennis, fed on wheat kernels, using C^{14}-labelled saliva. In addition to the ability of the saliva in some species to diffuse through intact cell walls and thereby cause this spreading of the injury, some species are capable of actually dissolving the cell walls. This property of the saliva, which appears to facilitate penetration, causes only slight dissolution along the path of the stylets themselves in Aphis rumicus (Davidson, 1923), Aphis maidis (Brandes, 1923) and Dactylopius longispinus Targ. Tozz (K.M. Smith, 1926). In Chionaspis pinifoliae (Brown, 1916) and the capsids Calocoris bipunctatus and Lygus pabulinus (K.M. Smith, 1926) the dissolution of the cell walls

is widespread. The saliva of Chionaspis pinifoliae, in addition, is also able to break down the starch within the cells (Brown, 1916), a property shown also by the saliva of Aphis rumicus (Davidson, 1923), Phylloxera vastatrix (Petri, 2), Aonidiella aurantii (Baranyovits, 1953) and psyllids of the genus Psyllus (Pussard, 1938).

In the case of C.hesperidum, none of these functions can be assigned to the saliva. It has no visible effect on the cell walls, thereby aiding penetration, or on the cell contents, thereby aiding the uptake of food, and the saliva, together with the sheath that it produces, appears to be functionless. Although it is possible that the saliva has an enzymatic effect upon the sap within the phloem itself, the reason for the secretion of the sheath along the entire path of the stylets, and even prior to the actual penetration of the epidermis, appears to be rather problematical.

Various theories as to the function of the sheath in phytophagous Hemiptera have been suggested, in addition to the digestive action of the saliva aiding penetration or feeding or both. Busgen (1891) suggests that in aphids the salivary track hardens and acts as a support for the stylets, whilst Arnaud (1918) considers that the sheath is produced as a water-tight tube round the stylets to provide a canal for the uptake of sap. However, since such a canal is formed by the maxillae themselves, this latter function of the sheath appears unnecessary. Sukhov (1944) has suggested that the sheath filters off particles in the sap which might otherwise

obstruct the food canal of the maxillae. This would assume that the sheath is a semi-permanent structure surrounding the tip of the stylets during feeding since it is the tip alone which actually takes in the food. Thus, since the saliva is essentially a fluid when it is first secreted, the tip of the stylets would have to rest in the food until it had hardened sufficiently to prevent it being taken up with the food, this resting period occurring at each subsequent movement of the stylets to a new food source.

It is possible however, that the sheath is present as the result of the saliva functioning in a capacity other than digestive, which would explain its presence in C.hesperidum. Lester and Lloyd (1928) suggest that the saliva serves to keep the stylets moist and clean in the intervals between feeding, and lubrication of the stylets would appear to be a necessary function during penetration and retraction. A study of the stylets of C.hesperidum has revealed a further essential function of the saliva which accounts for the presence of the stylet sheath. The results of this study, and the conclusions which arise from it, are presented in Section III of this paper.

The stimulus guiding the stylets to the phloem

The fact that the stylets of C.hesperidum pass directly towards the vein and enter the phloem, indicates the presence of a factor which guides the stylets during penetration.

Fife and Frampton (1936) have suggested that the presence

of a pH gradient between the phloem and the parenchyma is responsible for directing the stylets to the phloem in the case of Eutettix tenellus. The origin of the stylets in Coccidae precludes the possibility of a sense organ at their tips, although the presence of a sense organ in the roof of the cibarium in all phytophagous Hemiptera (Pesson, 1951, 2) suggests that the insect is capable of tasting the sap during penetration.

However, in the case of C.hesperidum, those individuals feeding on the leaves have been shown to penetrate the epidermis itself in an oblique manner towards the vein. Thus it appears that a stimulus, guiding the stylets to the food source, is acting upon the individual prior to the actual commencement of penetration. Such behaviour does not support the theory of a pH gradient alone guiding the stylets, since pH can only act upon the stylets within the plant so that the initial penetration would be without guidance and consequently would only take place towards the vein by chance.

It has been stated above that the insect settles alongside a vein with its longitudinal axis parallel to it, and that the edge of the body nearest the vein overlies the latter in the case of the small veins, or one side of it in the case of the larger ones. Since the ventral surface of the body is closely applied against the surface of the leaf, this side of the body is distorted slightly as it fits into

the angle between the vein and blade. Such distortion, might act as a stimulus and direct the stylets towards this side of the body and hence towards the vein itself. In the case of the individuals feeding on the stems, this unequal stimulation would be absent, and the insect would not be induced to direct its stylets towards one side more than the other and would therefore insert them directly down into the plant i.e. towards the phloem. Although this explanation could account for the initial directing of the stylets, it is difficult to see how it would enable the insect to discriminate between the cells of the vascular bundle. It is possible that this final location of the phloem is due to the presence of a chemical stimulus, possibly a pH gradient, in the close vicinity of the vein. Thus it would appear that there are two factors guiding the stylets, an initial one directing them towards the vein and a final one guiding them into the phloem itself. (See also Section V page 137.)

It has also been seen that, in the case of the individuals feeding on the leaves, some show extreme changes in the direction of the stylets during the course towards the vein. A possible reason for this change in direction is to afford anchorage to the plant, a function performed by the stylets in the last 2 larval stages of species of the Margarodidae (Balachowsky, 1939). The actual method by which the insect initiates this behaviour however, is difficult to understand,

since such behaviour is only possible if the tips of the stylets themselves are capable of being deflected by the insect during penetration.

SECTION III

The Cohesion and Retraction of the Stylets

The Cohesion and Retraction of the Stylets

Introduction

The method of penetration of the long and flexible stylets of Coccidae has been studied by Pesson (1944) and, as stated in the General Introduction, the efficiency of the process attributed essentially to the fact that the stylets are united in a single bundle.

In their functional position, the maxillae lie side by side, connected together by longitudinal grooves and ridges, whilst the mandibles are applied against them, one on either side (Fig. 4). There are no visible means of attachment between the maxillae and mandibles to maintain the latter in position, such as the interlocking arrangement present in the capsid Lygus pabulinus (Awati, 1914). Pesson (1944) has explained this visible lack of attachment by stating that the cuticle of the upper and lower edges of the mandibles is elastic in nature, which enables them to apply themselves tightly against the maxillae. However, since the mandibles are relatively flat on their inner surface and lack intrinsic muscles (Pesson, 1944), it is difficult to see how such a grip could be maintained unless it was permanent. The fact that the mandibles also slide longitudinally alongside the maxillae makes it difficult to see how both a gripping action and a sliding action can occur at the same time, since the friction imposed by the former would oppose the latter. It is difficult to conceive how the insect could relax such a

grip during movement, and it must also be remembered that it is actually during movement that it is most essential for the mandible to be closely applied to the maxillae and therefore for any such gripping device to be in operation.

The most efficient method of attachment would hold the mandibles against the maxillae but at the same time would not oppose longitudinal movement. It was thought possible that the method was by surface tension since this would fulfill all the necessary requirements. The presence of a liquid film of sufficiently high surface tension between the maxillae and mandibles would prevent separation but would not oppose movement and would in fact facilitate the latter due to its lubricating effect. If surface tension is the operative force, then it should be possible to break it down and thereby cause the mandibles to separate from the maxillae. The following study was carried out in order to obtain further information regarding this problem.

TECHNIQUES

The insect with its stylets, but devoid of the stylet sheath, was removed from its feeding position on the plant by placing the point of a fine scalpel beneath it.

Retraction of the stylets into the crumena was observed under a microscope. The insect was placed on a slide, with its ventral surface uppermost, and covered with a cover-slip in order to hold the stylets in a horizontal plane and facilitate their observation. The stylets could then be

clearly observed during the entire process of retraction. Observations on the speed of retraction were made with the use of a calibrated eyepiece and a stop watch.

The separation of the stylets was performed with a fine mounted needle. The insect was placed on a slide with its dorsal surface uppermost and the stylets arranged so that they extended beyond the edge of the body on one side. The moist nature of the stylets served to make them adhere to the surface of the slide along their entire length and maintain them in position during the separation of the individual components. Separation was achieved by commencing at their tips and working along the stylets towards the labium. Each mandible was in turn separated from the maxillae and then the 2 maxillae themselves were separated. Separation was also carried out in the same way on stylets that had been cut off close to the body. This severing of the stylets, prior to their separation, was achieved simply by pressing the stylets against the surface of the slide from above with a needle, when the stylets were easily broken.

The action of soap solution on the stylets was examined in the following way. The insect was placed on a slide as for the separation of the stylets, with the latter projecting beyond the edge of the body on one side. A cover-slip was placed over the insect and the stylets observed under the low power of the microscope. Soap solution was applied to the slide in small drops with a looped needle and run slowly under

the cover-slip till it was observed that the stylets were fully immersed. It was so placed on the slide that it approached the insect from the side opposite to the stylets themselves and immersed the latter starting at their base. This procedure was carried out in all cases so that any response by the stylets was due entirely to immersion and not to a physical effect exerted by the surface film of the approaching solution on the distal ends of the stylets. Control experiments were also carried out using distilled water in place of the soap solution.

Experiments to show the action of soap solution on the stylets after they were cut off were carried out in a similar manner. The insect was placed on the slide as before and the stylets severed close to the body. The latter was then removed and the stylets covered with a cover-slip.

An alternative method for examining the action of soap solution on the severed stylets was as follows. The stylets were cut off close to the labium and picked up, at their base, with the aid of fine forceps. A drop of soap solution was placed on the slide and the stylets inserted into it, tip first. Control experiments, using a drop of distilled water in place of the soap solution, were also carried out.

An additional experiment, using soap solution, necessitated by the results and conclusions obtained from the above, was performed as follows. The insect was removed from the plant and placed on a slide as before, so that the stylets

projected beyond the edge of the body on one side. A small piece of wax was placed on the slide alongside the stylets, just behind the tip and then melted with the point of a hot needle so that it covered and enclosed the stylets in this region. The insect was then covered with a cover-slip and treated with soap solution as before. Extreme difficulty was encountered in this technique owing to the small amount of wax required. Application of the needle of too high a temperature caused the wax to melt rapidly onto the needle instead of surrounding the stylets.

RESULTS

Retraction of the stylets

31 individuals were studied comprising 8 larvae, 13 nymphs and 10 adults. All the larvae and 6 of the nymphs showed no movement of the stylets at all, whilst complete retraction occurred in only 8 adults and 4 nymphs.

When the insect is removed from the plant and placed on the slide, the stylets remain united in a single bundle and project beyond the labium free from contact with the body. In this position, the stylets as a whole were seen in some individuals to move slowly from side to side, such movement either being from the base or else restricted to the distal extremity.

During the actual retraction of the stylets, both mandibles were seen to move independently in a longitudinal direction whilst the two maxillae moved either together or

else independently, a variation noted in coccids by Pesson (1944). Retraction was not a one way movement only. Each stylet was seen to be retracted a short distance followed by its protraction once more. Such movement was not restricted to any one stylet at a time, since at least two were seen to be in motion together, although in different stages of this retraction and protraction rhythm. The immobile stylets were either at the end of the protraction stage or the retraction stage. A regular sequence of movement in which the mandibles move in succession followed by the two maxillae together, as recorded for coccids during penetration (Balachowsky, 1937; Weber) and retraction (Hovasse; Pesson, 1944) could not be detected however. A similar failure to detect a regular order of movement in some cases in coccids during retraction, has also been recorded by Pesson (1944). During these movements, the stylets as a whole were seen to be progressively retracted into the crumena. (As explained below, however, such movement by the stylets may occur without the actual retraction of the stylets taking place.)

In addition to the repeated contraction and relaxation of muscles at the base of the stylets, visible through the cuticle of the clypeus, there was intense activity within the labium, which suggests that the latter plays a significant role in retraction. Although there was no external movement of the labium, a chitinous region within the labium, visible through its cuticle, was seen to move rapidly towards the

base of the labium followed by a gradual return to its initial position, this apparent tugging motion being repeated continually throughout retraction. A chitinous region of the labial gutter in Homoptera, surrounding the stylets like a pincer and capable of being relaxed by the appropriate muscles, has been stated by Weber and Pesson (1944) to grip the stylets and hold them in their successive positions during retraction or penetration. Pesson (1944) also states that in Icerya sp., although this pincer can be pulled backwards and forwards and is therefore capable of pushing the stylets into the plant or withdrawing them, such activity had not been observed in the living insect. The apparent tugging action observed to occur within the labium of C.hesperidum, suggests that it does play an active role in retraction. Throughout retraction, the stylets were seen to be moist, especially at their tips where the presence of a fluid was noticeable between the stylets. In all cases the stylets left a trail of this fluid behind them where they touched the glass.

The actual time taken for the retraction of the stylets varied from 6.5-70 minutes in the case of the four nymphs and 10-38 minutes in the eight adults, depending to some extent on the length of the stylets outside the body, a time of 37 minutes occurring in one adult whose stylets were fully extended.

Three distinct phases could be distinguished in nymphs

and adults during retraction although all three may not be seen in one individual. There was an initial period of complete immobility of the stylets when the insect was first removed from the plant. This was followed by the second phase, during which the stylets themselves moved, as described above, but no retraction occurred, and then the final phase of retraction itself.

In Table 2, the time recorded for each individual, with respect to the length of these 3 phases, is shown, together with the total time taken for the stylets to be fully retracted.

From these figures it can be seen that retraction of the stylets occurred more readily in the adults than in the preceding instars and that in all the adults, except two (1 and 10), movement of the stylets commenced at once, whilst three adults (4, 7 and 8) actually started to retract their stylets as soon as they were removed from the plant. In contrast to the adults, the larvae and the nymphs, except one (11), showed an initial period of immobility of the stylets when first removed from the plant, whilst complete retraction only occurred in four nymphs and in no larvae. In addition to this variation between adults, regarding the behaviour of the stylets, it is seen that the times recorded for any one phase also shows considerable variation. Thus, even individuals showing the same sequence of behaviour, vary in the actual duration of each phase.

Table 2. The time, in minutes, recorded for the retraction of the stylets of C.hesperidum.

	Presence of stylets in crumena	Phases of Retraction			
		Phase 1 Initial period of immobility	Phase 2 Movement of stylets no retraction	Phase 3 Movement of stylets retraction	Total time
Larva 1	+	60			
" 2	+	15			
" 3	-	15			
" 4	-	15			
" 5	-	15			
" 6	-	17			
" 7	-	18			
" 8	-	20			
Nymph 1	+	15			
" 2	+	15			
" 3	+	15			
" 4	-	15			
" 5	-	15			
" 6	-	17			
" 7	-	5	15		
" 8	-	13	87		
" 9	-	3.5	21.5		
" 10	+	8.5	0	16.5	25
" 11	+	0	0	6.5	6.5
" 12	+	15	0	45	60
" 13	+	15	0	55	70
Adult 1	-	18	12		
" 2	-	0	50		
" 3	+	0	12	23	35
" 4	+	0	0	10	10
" 5	+	0	15	16	31
" 6	+	0	19	13	32
" 7	+	0	0	18	18
" 8	+	0	0	38	38
" 9	+	0	4	18	22
" 10	-	4	4	29	37

Footnote: In the larvae and nymphs 1-6, the time recorded for the initial period is that during which the stylets were kept under observation. These individuals were subsequently left overnight and re-examined the following day when, although the insect was still alive, the stylets were found to be still in their original position. In nymphs 7-9 and adults 1-2, the time recorded for phases 1 and 2 is the full duration of these phases. Movement of the stylets then ceased. These individuals were also left overnight and re-examined the following day when the stylets were found to be still in their original position although the insect was still alive. In the remaining individuals, the full duration of each phase is recorded.

Movement of the stylets with the complete absence of subsequent retraction is possibly related to the absence of the stylets from the crumena (see Table 2). Although the proximal ends of the stylets are retained as a loop within the crumena during feeding, these are sometimes pulled out on removing the insect from the plant. In all those individuals in which movement of the stylets was seen although no retraction occurred (nymphs 7-9 and adults 1-2), the stylets had been completely removed from the crumena (see Discussion).

The actual speed of retraction was not the same in all individuals, and was seen to vary even within one individual. Seven adults were timed over consecutive distances of 0.23 mm., the length of the stylets outside the body permitting only two such readings to be taken for 6 of the adults whilst in the remaining adult three readings were possible. The results are shown in Table 3.

Table 3. Time, in minutes, taken for the stylets of seven adults of C.hesperidum to be retracted over consecutive distances of 0.23 mm.

	Adult 1	Adult 2	Adult 3	Adult 4	Adult 5	Adult 6	Adult 7
1st reading	4.5	7	13	9.5	11.5	23	19
2nd reading	3.5	5	5	4.5	6.5	11	7
3rd reading	5						

It can be seen that there is a marked increase in the speed of retraction towards the final stages.

A further point, arising from the observations made upon the retraction of the stylets, is related to the presence of a bend along the path of the stylets. In some individuals, the stylets are seen to be bent when they are first removed from the plant. These cannot be straightened out and appear to be the result of bending imposed upon the stylets within the plant. Others, imposed upon them when the cover-slip is placed in position, can be straightened out. The interesting point is that any bend in the stylets, regardless of origin, remains the same distance from the labium during retraction, so that the bend, in effect, moves towards the tip of the stylets until the latter are eventually retracted round it and the bend itself is no longer present (see Discussion).

Separation of the stylets

Experiment 1

With 10 individuals, the stylets were separated from each other whilst they were still attached to the insect.

When one of the mandibles was separated from the maxillae, and the stylets then lifted free of the slide, this mandible was seen to coil up towards its base. The other mandible, still in contact with the maxillae along its entire length, curved in the opposite direction taking the maxillae with it but did not coil up (as in Fig. 19). This behaviour of the separated stylets did not occur until they were lifted free of the slide, since their moist nature, which caused them to

adhere to the slide during separation, consequently prevented any coiling action taking place. It was possible to straighten the detached mandible by inserting the needle through the centre of the coil and moving the needle away from the labium. When this was done and the mandible finally released, the latter immediately sprung back into a coil. It was possible however to return the mandible to its normal position alongside the maxillae by straightening the coil out and at the same time applying it against the side of the maxillae. This process of separation and replacement was repeated several times with the same mandible. When the other mandible was likewise separated from the maxillae, it also coiled up in the same manner (as in Fig. 20).

The separation of the two maxillae from each other was more difficult than the separation of the mandibles. Awati (1914) records a similar difficulty in the separation of the maxillae of Lygus pabulinus, whilst Pesson (1944) states that the separation of the maxillae of coccids is only possible if it is begun at their tips and the maxillae are spread apart at an angle, similar to that at which they meet at the tip of the hypopharynx. The two maxillae in C.hesperidum showed no sign of bending or coiling either before or after separation.

Similar results to those described above were obtained with each of the 10 individuals examined.

Experiment 2

In 5 individuals, the stylets were separated from each other after they had been cut off close to the labium.

The mandibles, after separation from the maxillae, were picked up at one end with fine forceps, and in all cases were seen to coil up. As explained above, this coiling behaviour did not occur until after they had been picked up. The maxillae, when separated from each other and picked up by one of their ends, also showed a slight tendency to coil up although not to the same extent as the mandibles. Both maxillae showed this tendency to coil up in 3 individuals whilst in the other 2 individuals it was present in only one of the maxillae.

In the case of 2 of these 5 individuals, the coiled mandibles were picked up separately on the top of a needle, and placed vertically on the surface of the slide so that the coil was in contact with the slide at only one point of its circumference. The mandible remained in this position when the needle was first removed. It then slowly uncoiled until its entire length, except for the extreme tip at both ends, had straightened out and was in contact with the slide. When the mandible was removed from the slide once more with the needle, it again coiled up. This process was repeated several times with each mandible.

In the remaining 3 individuals, a human hair was used in place of the slide. The hair was stretched horizontally

just above the surface of the slide, between two pieces of glass, and fastened to the latter by means of balsam. The surface of the hair was moistened with soap solution and the coiled mandible picked up at one of its ends with fine forceps, and this end placed in contact with the side of the hair. When this was done, the mandible immediately straightened out alongside the hair, although it again coiled up when it was removed. This process was repeated several times for each mandible in all 3 individuals and identical results obtained in all cases.

Action of soap solution on the stylets

Experiment 1

The action of soap solution on the stylets whilst they were still attached to the insect was carried out on 11 adults and 6 nymphs. Immediately the stylets were covered by the soap solution, they were observed to separate from each other.

In 5 of the adults and one of the nymphs, both mandibles separated from the maxillae, bent away from the latter and finally coiled up towards their base (Fig. 20). The maxillae did not separate, except for a slight divergence at their extreme tips, and remained outstretched in their initial position.

In the remaining individuals only one mandible separated completely from the maxillae and coiled up towards its base. The other mandible bent in the opposite direction taking the maxillae with it but did not coil up (Fig. 19). It separated

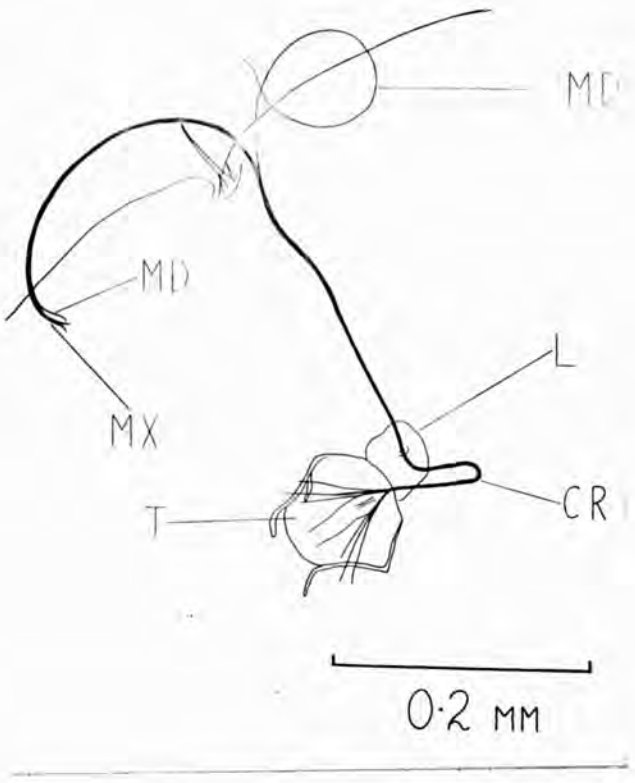


Fig. 19. Behaviour of the stylets of C.hesperidum, following the application of soap solution.

CR, crumena loop of stylets;
L, labium; MD, mandible;
MX, maxillae; T, tentorium.

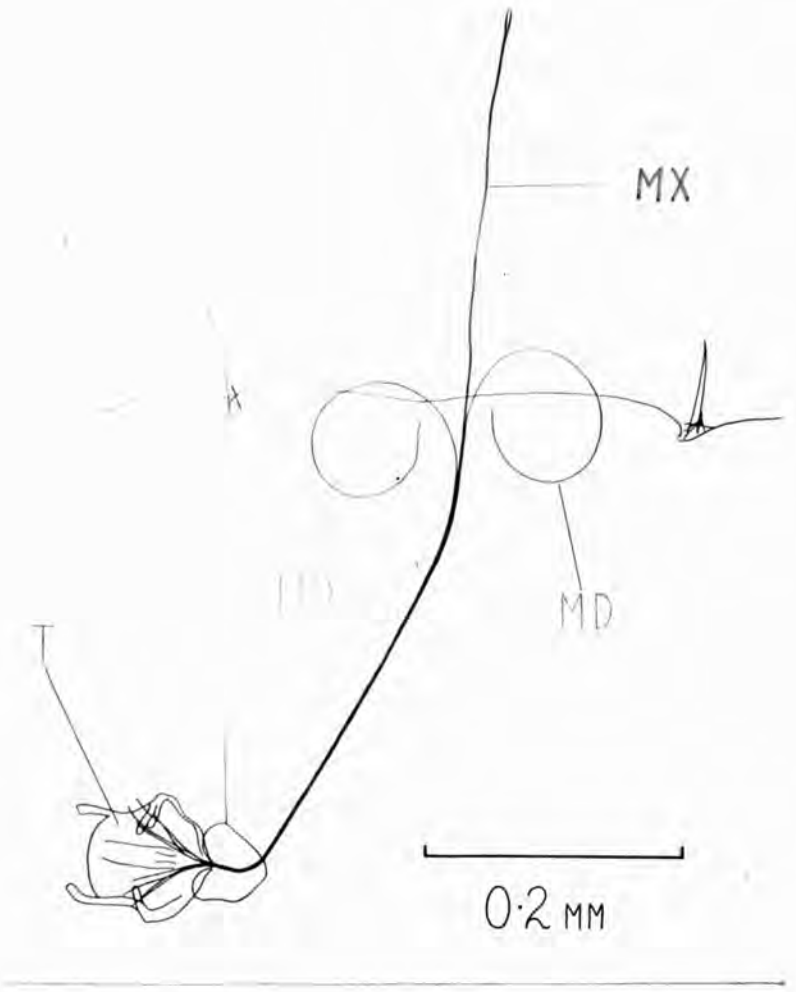


Fig. 20. Behaviour of the stylets of C.hesperidum, following the application of soap solution.

SC, spiracular setae.
For other abbreviations see Fig. 19.

from the maxillae only at its tip. The maxillae showed no separation except at their extreme tip.

The control experiments, using distilled water in place of the soap solution, was carried out on 3 adults and 3 nymphs. In all cases there was no separation of the mandibles or the maxillae.

Experiment 2

In 6 adults the soap solution was added to the stylets after they had been detached from the body close to the labium. The soap solution approached the stylets from their base and separation started from this end immediately the soap solution reached them.

In 3 of the adults both mandibles separated from the maxillae, coiled up and floated away from the latter (Fig. 21). The maxillae did not separate from each other except at their extreme tips where they diverged slightly.

The remaining 3 adults showed only one of the mandibles separating completely and coiling up. The other mandible also coiled up, from its base towards its tip, but remained in contact with the maxillae just behind the tip. The maxillae themselves showed only a slight divergence at their extreme tips.

Experiment 3

In 10 individuals the stylets were cut off close to the labium and picked up at their base with the aid of fine forceps. The tip of the stylets were then slowly inserted

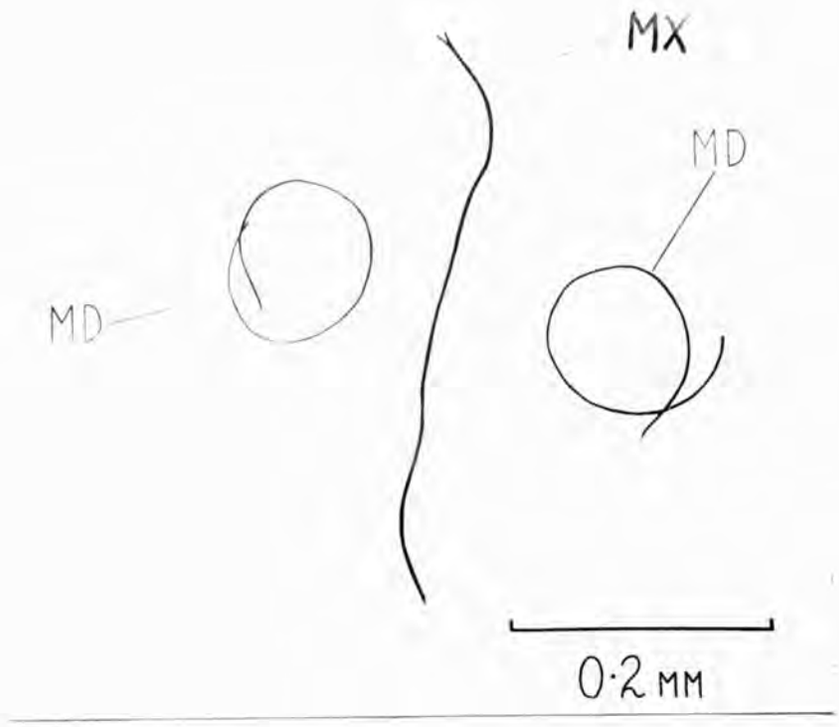


Fig. 21. Behaviour of the stylets of C.hesperidum, detached from the body, following the application of soap solution.

For abbreviations see Fig. 19.

into a drop of soap solution on the slide. Following the penetration of the drop, both mandibles immediately separated from the maxillae and bent outwards in opposite directions away from the latter. The maxillae themselves did not separate. As the stylets were withdrawn from the drop, the mandibles regained their normal position alongside the maxillae. This process of penetration and withdrawal was repeated several time and the above results obtained in all cases.

The tips of the stylets were then inserted several times into a drop of distilled water. The mandibles did not separate from the maxillae except during the first insertion.

The stylets were then inserted alternately into the soap solution and the water, several insertions being carried out on the same drop each time. During this procedure the mandibles were seen to separate from the maxillae in the soap solution but not in the water, except during the first insertion each time into the latter. This initial separation in water is doubtless due to the soap solution adhering to the stylets and exerting an effect during the first penetration into the water.

Similar results were obtained with the stylets of all 10 individuals.

The penetration of the water was possible only by inserting the stylets at right angles to the surface. If

Table 4. Variations in the behaviour shown by the tips of the stylets of C.hesperidum, following the application of soap solution. *

	Both mandibles separated from maxillae			One mandible only separated from maxillae	
Behaviour	Mandibles diverged away from maxillae	One mandible only diverged away from maxillae	Mandibles did not diverge away from maxillae	Mandible diverged away from maxillae	Mandible diverged away from maxillae, other mandible bent in opposite direction taking maxillae with it.
Number of Individuals	5	5	1	5	1

* Footnote: The maxillae remained outstretched in all cases, except where otherwise stated, and diverged from each other only at their extreme tips.

the latter was approached obliquely, the stylets failed to penetrate. This is in contrast to the penetration of the soap solution which offered no resistance.

Experiment 4

The action of soap solution on the tips of the stylets was tested on 17 individuals.

The wax proved effective in holding the stylets together and separation occurred only at their tips beyond the wax. The results are given in Table 4.

Of the 17 individuals examined, 11 showed both mandibles separating from the maxillae along the entire length of the exposed tip. Five of these individuals showed both mandibles diverging outwards away from the maxillae, 5 individuals showed only one of the mandibles diverging outwards, the other mandible remaining alongside the maxillae, whilst in the remaining individual, both mandibles remained alongside the maxillae.

The remaining 6 individuals showed only one of the mandibles separating away from the maxillae and bending backwards, the other mandible showing no sign of separation although in one individual it bent backwards taking the maxillae with it.

The examination of the stylets of other species

Lecanium hemisphaericum.

Specimens of Lecanium hemisphaericum were found on Caffea arabica. It was possible to remove the insect from

the plant with its stylets extended.

The stylets of 5 individuals, whilst still attached to the insect, were immersed in soap solution. In all cases both mandibles separated from the maxillae, bent outwards in opposite directions away from the latter, and finally coiled up towards the base. This response was obtained immediately the soap solution covered the stylets. The maxillae did not separate.

Distilled water was used in place of the soap solution in 5 individuals and no reaction obtained.

Aonidiella aurantii

Specimens of Aonidiella aurantii were obtained from seedlings of the Rough Lemon. The insect was first exposed by removing the covering scale, and then lifted from the leaf with the point of a scalpel. The stylets were found to be more fragile than in C.hesperidum and Lecanium hemisphaericum and were easily broken.

The separation of the stylets whilst still attached to the insect was carried out on 5 individuals. When the mandibles were separated from the maxillae and lifted free of the slide, they coiled up towards their base. The separation of the two maxillae was more difficult, as in the case of C.hesperidum, and as in this species the isolated maxillae showed no sign of coiling up. The coiled mandible was then detached from the insect and stood vertically on the slide, as described for C.hesperidum. It slowly uncoiled

and straightened out until its entire length was in contact with the slide. When removed from the slide it again coiled up. These results were obtained with all 5 individuals.

The stylets of 5 individuals were treated with soap solution whilst still attached to the body. In all cases, separation of the mandibles occurred as soon as the stylets were covered by the soap solution. Commencing at their tip both mandibles, in all 5 individuals, bent outwards away from the maxillae and coiled up towards their base. The maxillae did not separate. A control experiment, using distilled water, was carried out with 5 individuals. In no case did separation occur. When soap solution was added to the water under the cover-slip both mandibles gradually separated from the maxillae and coiled up towards their base.

Dactylopius longispinus

Specimens of Dactylopius longispinus were found on the ventral surface of the leaves of Cycas revoluta. As soon as they were touched, prior to removal from the plant, they became active and moved away from their feeding site, dragging their stylets out of the plant and trailing them behind. Retraction of the stylets was too rapid to enable them to be separated or treated with soap solution. The method employed therefore was to cut off the labium together with the extended stylets as soon as possible. 10 individuals were treated in this way.

The labium was picked up with fine forceps and the stylets slowly inserted, tip first, into a drop of soap solution. The mandibles separated from the maxillae at once and bent outwards and backwards. The maxillae did not separate. When withdrawn from the soap solution, the mandibles regained their original position alongside the maxillae. This process of insertion and withdrawal was repeated several times with the same results. The stylets were then inserted and withdrawn several times into a drop of water. As in the case of C.hesperidum, it was found difficult to make the stylets penetrate the surface of the water unless they entered at right angles to the surface. Except during the first insertion, when the mandibles separated, there was no separation in water. Following this insertion in water, separation of the mandibles was again obtained in soap solution. Identical results were obtained in all 10 individuals.

DISCUSSION

During the observations on the retraction of the stylets, it was seen that in all those individuals in which movement of the stylets was seen, although no retraction occurred, the stylets had been completely removed from the crumena. This indicates either that the crumena itself plays an active role in retraction, or that the presence of the proximal ends of the stylets within the crumena aids in directing the stylets into the latter as they are withdrawn

into the body. Since one adult, whose stylets were completely withdrawn from the crumena, showed normal retraction (Table 2, adult 10), the second suggestion appears to be more likely. Therefore it is probable that when no part of the stylets are within the crumena, retraction is impeded rather than prevented. This might explain the retention of part of the stylets within the crumena during feeding, as it would enable retraction to occur more readily.

It is not possible to tell whether the observations recorded during the retraction of the stylets represent the true picture of normal retraction from the plant. It is possible that the removal of the insect from the plant and the consequent dislodging of all or part of the stylets from the crumena has an effect upon behaviour. It would appear that the individuals showing the complete absence of retraction are most certainly affected by the complete removal of their stylets from the crumena. It is perhaps significant in this respect that the least activity is shown by the larvae and nymphs, since any effect on behaviour, due to removal from the plant, would be expected to be shown by the younger stages rather than the adults.

It has also been shown that, during the retraction of the stylets, any bend in the stylets remains the same distance from the labium during retraction. The significance of this fact is apparent when a bend is present along the course of the

stylets within the plant (see Section II, p.40). If it moved progressively towards the labium during retraction, it would impede retraction. The fact that it remains in the same position with respect to the labium, shows that such an obstacle is not imposed upon the stylets during retraction from the plant. The method by which this process is achieved is clearly seen by reference to the method of movement of the stylets given by Pesson (1944). He states, in effect, that the movement of any one stylet is guided by the rigidity of the rest. Such a process ensures that in the vicinity of the bend, sufficient support is present to guide the stylets round it. Thus, a bend would be expected to remain in the same position with respect to the labium and the surrounding tissues by virtue of this guidance and the tips of the stylets themselves eventually move round it.

The fact that the stylets remain in close proximity to each other and move efficiently when removed from the plant, shows that the method of attachment between the maxillae and mandibles resides in the stylets themselves and does not depend on the plant tissues to hold them together.

It has been shown how the mandibles coil up when separated from the maxillae. This behaviour shows quite clearly that the mandibles, when straightened out, are in a state of tension. The fact that this coiling up occurs also in the stylets which have been detached from the insect shows that the tension is intrinsic and that the coiling is not

brought about by the insect itself. The reason for this intrinsic tension on the part of the mandible may be its mode of origin within the coiled invaginations of the head. Due to this method of formation, one side of the mandible, that on the inner side of the coiled invagination, must of necessity be shorter than that on the outer side. Therefore, when the mandible is straightened out alongside the maxillae to assume its functional position, a tension is imposed upon it. The maxillae however, show no such tension, although they are formed in exactly the same way as the mandibles. The reason for this might quite possibly be due to their possession of longitudinal grooves and ridges as opposed to the flat sided structure of the mandibles.

The presence of this tension within the mandibles has apparently escaped the notice of previous workers since no mention is made of it, although its presence is seen in their illustrations in some cases. Photographs showing penetration by the aphid Anaecia querci (Tate, 1936-37), (Fig. 22), show the base of the stylets detached from the insect together with the separation and curling of the mandibles. The same behaviour is seen in photographs showing penetration by Lachnus tomentosus, L.hyalinus and L.pichtae (Leonhardt, 1940). Arnaud (1918) has actually figured this separation and curling of the mandibles in the case of Lecanium oleae (Fig. 23) although no reference is made to it. It is of interest however, that Davidson (1914) states that the stylets of

Fig. 22. Cross section of leaf of dogweed, showing stylets of Anoecia querci penetrating vascular bundle.

(Reproduced from Tate, 1936-37.)

N.B. The separation and coiling of the mandibles near the epidermis.

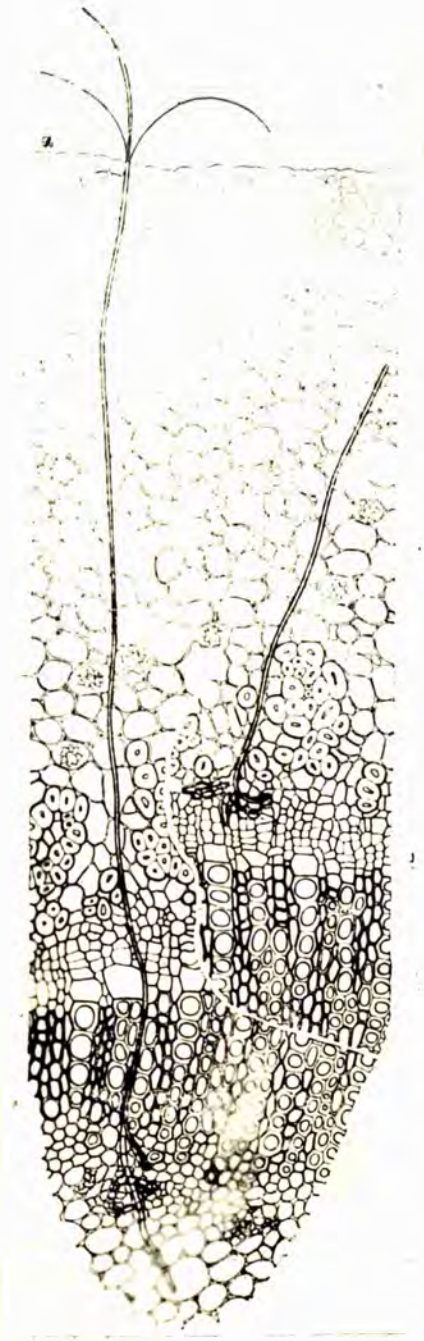


Fig. 23. Transverse section of stem of Nerium oleander, L., showing penetration by the stylets of Lecanium oleae.

(Reproduced from Arnaud, 1918.)

N.B. The separation and coiling of the mandibles above the epidermis.

Schizoneura lanigera lie loose from each other and also (1923) that the mandibles of aphids sometimes separate from the maxillae outside the plant although no mention is made of any coiling behaviour of the mandibles. Actual separation of the stylets of coccids has been performed by Pesson (1944) with a similar lack of information on any coiling behaviour of the mandibles. It is conceivable however, that in the latter case the stylets were in contact with a flat surface, such as a slide, during separation, which would prevent any coiling action taking place, as explained above for C.hesperidum.

The fact that the mandibles retain their position alongside the maxillae indicates that whatever the force that holds them in position, it must of necessity be strong enough to counteract the tension within the mandibles. Therefore, in the light of this discovery of the tension within the mandibles, it seems more unlikely that Pesson's explanation of the method of attachment of the mandibles to the maxillae is correct. Although Pesson depicts the coiling of a stylet diagrammatically, at the time of moulting, when it emerges from the coiled invagination (Pesson, 1944; and Pesson, 1951 (3), see Fig. 6, section I), he makes no mention of the intrinsic tension within the mandibles. It is now clear that a gripping of the maxillae by the mandibles as suggested by Pesson, would not only have to prevent them drifting slightly away from the maxillae but actually to

overcome a strong tendency to separate. Thus the grip itself would have to be quite strong and of a permanent nature, thereby imposing constant friction between the maxillae and mandibles. Finally, the fact that the tension can be overcome, and the mandible remain in position, simply by applying it to the side of the maxillae, hardly supports the theory of a gripping action. The ability of the mandible to remain in position when applied against the side of the maxillae would be possible if there was a surface film of liquid on the maxillae which held it there by surface tension. The fact that the coiled mandible can be straightened out by a surface film of liquid on the side of a hair shows quite clearly that the tension within the mandible can be counteracted by the forces of surface tension. The additional fact that the coiled mandible can straighten out on the dry surface of a slide shows that there is a sufficient surface film of liquid on the mandible, when separated from the maxillae, to enable surface tension to operate.

The results of the experiments with the soap solution show that the force which maintains the mandibles alongside the maxillae is broken down by the presence of a liquid which is able to lower the surface tension of other liquids. The control experiments with distilled water show that the presence of a liquid alone is not in itself sufficient. It was seen however in the case of Aonidiella aurantii, that

when soap solution is added to the water then separation of the mandibles occurs. The evidence points therefore to the presence of a liquid film existing between the mandibles and maxillae, keeping them together by surface tension.

The source of the liquid which holds the mandibles and maxillae together must now be considered. The most likely liquid is the saliva itself. Secretion of saliva throughout penetration has been found to occur in aphids (Zweigelt, 1914 (3); Brandes, 1923; Davidson, 1923; Withycombe, 1926; Tate, 1936-37) and coccids (K.M. Smith, 1926; Balachowsky, 1937; Baranyovits, 1953), whilst Carter (1945) and Guennelon-Aubenal (1951) have, in coccids, observed the stylets penetrate the drops of saliva as they are secreted. Such behaviour would not only produce a stylet sheath, as mentioned in Section II, but would ensure that the saliva penetrated between the stylets. When the stylets are forcibly removed from the plant for examination, or during their normal retraction by the insect, the sheath is left behind, but the saliva between the stylets would remain in position and thereby hold them together.

The fact that penetration depends on the stylets being united in a single bundle, has been described by Pesson (1944). Since the mandibles function as the penetrating organs and the maxillae enter the hole that they produce, it is clear that if the two mandibles were to diverge slightly away from the maxillae at their tips alone they would give

rise to two independent penetrations of the cell wall so that the maxillae themselves would then have to penetrate the intervening wall. It has been shown that the tension within the mandibles is sufficient to cause them to diverge from the maxillae at their tips when the force of attraction is broken down in this small region alone. The conditions within the plant are similar to those obtained by the use of the wax in that the cell wall just penetrated would hold the stylets together behind the tip and allow only the tips of the mandibles, free in the cell, to diverge away from the maxillae.

From the above observations made upon the stylets as a whole, a possible reason for the presence of the stylet sheath can now be put forward. The evidence suggests that the presence of saliva is essential to maintain the mandibles in position by means of surface tension, and also that this surface tension must operate along the entire length including the tip to enable penetration to occur. The presence of saliva between the stylets is achieved by the continuous secretion of drops of saliva from the tips of the stylets, and their subsequent penetration, which ensures that the stylets are always in contact with saliva. The stylets therefore appear to move continually within a medium of saliva and the cell sap itself does not come in contact with them. The possible need for this lack of contact is clear when the penetration of a single cell is considered. Following the

entry through the cell wall into the cytoplasm, it is conceivable that the surface tension of the sap would be such as to lower the surface tension of the saliva and bring about the divergence of the tips of the mandibles. Such behaviour would prevent the penetration of the opposite cell wall. If however a drop of saliva was secreted into the cell prior to the stylets actually entering the cytoplasm, this would insulate them from the sap and prevent any possible breakdown of the surface tension. As they penetrated further into the plant, the stylets would automatically become surrounded by these successive drops of saliva and the stylet sheath thereby formed. Such a completed sheath could continue to protect the saliva between the maxillae and mandibles and prevent any possible breakdown of the surface tension by the cell sap.

It would appear therefore, that owing to the mode of formation of the mandibles, and the resultant tension within them, a primary function of the saliva in all phytophagous Homoptera is to maintain the mandibles alongside the maxillae and that the injection of the saliva into the plant, to enable it to perform this function, results in the formation of the stylet sheath. The presence of enzymes within the saliva causing the dissolution of the cell walls and the breakdown of chlorophyll and starch etc., is not found in all species and possibly depends mainly on the food source of the species in question. Therefore some species, as in the case

of C.hesperidum, although showing no visible signs of external digestion by the saliva still possess a stylet sheath.

The presence of the stylet sheath on the surface of the epidermis in C.hesperidum, as well as other phytophagous Hemiptera, and the actual secretion of saliva observed to occur prior to penetration in aphids (Zweigelt, 1914 (3); Withycombe, 1926; K.M. Smith, 1926), can be explained on the basis of this primary function. K.M. Smith (1920-21) actually refers to the difficulty in accounting for the secretion of saliva outside the leaf in the case of Psallus ambiguus, but this behaviour by the insect is now understandable. It is of interest to note also that Carter (1945) observed that individuals of Pseudococcus brevipes repeatedly produced a salivary track in plain agar and consequently remarked that it was clear that the presence of the saliva was in some respect independent of nutrition.

There is a possibility that the tension within the mandibles, and the function of the saliva in counteracting it, might bear some relation to host selection and alternation in aphids. If the effect of the sap was sufficient to overcome the surface tension of the saliva, then, as explained above, penetration of the plant could not occur. It is therefore possible that the surface tension of the sap acts in this way as a factor in host selection.

SECTION IV

The Length of the Stylets

THE LENGTH OF THE STYLETS

Technique

Measurements of the length of the stylets were taken in the following way. The insect was removed from the plant, as described in Section III, and the stylets thereby obtained in the extended position. The individual was then placed on a cover-slip with its dorsal surface uppermost and the stylets projecting beyond the edge of the body on one side. The tips of the stylets were held firmly to the cover-slip with a drop of Canada balsam and the body of the insect pushed gently away from the latter with a mounted needle, until the tips of the stylets were observed to move within the balsam. This ensured that the stylets were fully withdrawn from the crumena and also straightened out to facilitate their measurement. The cover-slip was then reversed, with the insect adhering to it, onto a slide so that the ventral surface of the body was uppermost and covered with the cover-slip. Measurements were taken with a micrometer eyepiece and the stylets measured from their tips to the tip of the labium.

Results

The length of the stylets was recorded for 15 individuals in each instar. The results are given in Table 5 together with the maximum and minimum length of the body recorded for each instar in Section V.

Table 5. Length, in mm., of the stylets, in 45 individuals of C.hesperidum.

	Larvae	Nymphs	Adults
	0.50	0.54	0.62
	0.50	0.55	0.63
	0.50	0.55	0.63
	0.51	0.56	0.65
	0.52	0.57	0.65
	0.52	0.58	0.66
	0.52	0.59	0.67
	0.52	0.60	0.68
	0.52	0.61	0.69
	0.52	0.61	0.70
	0.52	0.61	0.72
	0.53	0.62	0.73
	0.53	0.62	0.76
	0.54	0.62	0.83
	0.55	0.63	0.83
Average	0.52	0.58	0.69
Minimum body length	0.29 mm.	0.73 mm.	1.27 mm.
Maximum body length	0.84 mm.	1.61 mm.	3.03 mm.

Discussion

Although there is an increase in the length of the stylets at each moult, this increase is not very great in comparison with the increase in size of the body itself. The length of the body varies from a minimum of 0.29 mm. in the larvae to a maximum of 3.03 mm. in the adults, whereas the stylets vary from a minimum length of 0.50 mm. in the larvae to a maximum length of 0.83 mm. in the adults. The stylets are therefore proportionately better developed in the larvae than the adults, a fact recorded for coccids in general by Balachowsky (1937).

In contrast to C.hesperidum, the stylets of Pulvinaria immunerabilis "increase materially in size" with each moult (Putnam, 1876-78), a similar fact noted also for Aonidiella aurantii, in which the length of the stylets increases from 0.33 mm. in the first instar to approximately 3 mm. in the third instar (Baranyovits, 1953). This considerable variation between C.hesperidum and Aonidiella aurantii is possibly related to the feeding habits of these 2 species. Aonidiella aurantii is completely sessile after the first moult and since it feeds on the parenchyma it is essential that the stylets increase in length at each moult to enable them to obtain food beyond the range of the cells, utilized by the preceeding instars. C.hesperidum on the other hand, is mobile throughout its life cycle and in addition feeds on the phloem. Therefore, quite apart from the advantage

of mobility, it is possible for the individual to remain in the same position on the plant without any increase in the length of the stylets since, as will be shown later (Section V) the distance travelled by the stylets within the plant is approximately the same in all instars.

SECTION V

Feeding Habits throughout the Life-cycle, and their Relationship to Moulting

FEEDING HABITS THROUGHOUT THE LIFE-CYCLE AND THEIR RELATIONSHIP TO MOULTING

Introduction

In Section II of this paper it was shown that the stylet sheath remains in position following the retraction of the stylets themselves. It was stated at the time that such a fact makes it possible, not only to see the present course taken by the stylets, but also the previous course or courses which they had followed. It was thought possible that a detailed study of these tracks might throw some light on the biology of the insect itself. For the purposes of this study it was essential to know the particular instar of the insect and also the relative ages of the individuals within a particular instar, in order that any differences between the tracks of individuals might, if possible, be related to age. It was also desirable that individuals about to moult should be studied so that the relation between this process and feeding might be established. Finally, it was hoped that the study of the tracks might provide some information concerning the wandering habits of the various instars.

Technique

The host plants used in this study were seedlings of the Rough Lemon, approximately 18 inches high. They were infected with the insect and kept under conditions of 70°F and 70-75% relative humidity until the insect had severely

infested both the stems and the leaves. Only those on the leaves were studied, since the technique used was not applicable to the stems owing to their thickness. Insects of all ages were present on the leaves so that individuals in different stages of the life-cycle could be examined. Five leaves were taken at random from each of four plants and plunged into hot (70°C) 95% alcohol in order to kill the insects instantaneously. The leaves were then treated as described for the whole mounts in Section II (see p. 20).

This technique rendered both insect and leaf transparent and proved satisfactory for this work. It was possible to see the stylets within the leaf tissue whilst the adjacent salivary tracks of previous penetrations were also visible. In the insect itself, it was possible to trace the stylets back into the crumena, whilst some aspects of the internal anatomy of the insect were visible. Thus it was possible to examine the head region and follow the development of the new stylets as they were formed. The approach of a moult could also be determined due to this transparency which gave a picture of the formation of the new cuticular structures beneath the existing ones. In the case of the adults, the presence or absence of eggs and embryos within the body could be seen.

The piece of leaf to be examined was placed in a drop of clove oil on a slide and viewed under the low power without a cover-slip. Keeping the insect in view, it was possible

to dislodge it from its feeding site with the aid of mounted needles, thus breaking the stylets either at their origin within the insect, close against the leaf surface or else midway between these two points. The removal of the insect itself allowed a clearer view of the actual feeding tracks. In this way it was possible to relate a particular set of tracks to the insect responsible. A cover-slip was then placed over both insect and leaf and a detailed examination was made. A drawing of both the stylet tracks and salivary tracks was made for each individual and the size and instar of the latter recorded. The occurrence of moulting was noted in the case of the individuals concerned. In the adults the presence or absence of eggs and young within the body was also recorded.

The number of segments in the antennae were used as the basis of identification of the various instars. The presence of long setae on the anal plates of the larvae was noted but was not used by itself as a means of identification.

The method of determining the relative ages of the individuals within a particular instar was only approximate and was based on the fact that the individuals were found to increase in size during an instar. This increase in size during the larval and adult stage has been noted previously by Fonseca (1953). A similar increase in size during instars occurs also in Physokermes insignicola (Moulton, 1907), the larval stage of Pulvinaria innumerabilis (Putnam,

1876-78) and the 4th instar of Aleyrodes vaporariorum (Hargreaves, 1914-15). By recording the size of the individual and relating it to the total size range of the instar, the approximate age difference between individuals was obtained. In the case of the adults, the presence of eggs and embryos within the body towards the end of the instar gave an additional indication regarding their relative ages. It must be remembered that owing to the range of variation of the maximum size of individuals within each instar, the accuracy of this method is small. However, since only an indication of the relative ages was required, the method was adequate for this investigation.

The sizes recorded were measurements of the maximum length and breadth of the insect taken with the aid of a micrometer eyepiece. In the larvae, nymphs and young adults, the maximum breadth was between the anterior and posterior spiracular depressions. In the older adults, the maximum breadth was towards the posterior half of the body, especially in those individuals in which the brood space was fully developed.

RESULTS

The size-range of the larvae

A total of 132 larvae were examined. To cover the entire larval instar it was necessary to measure those actually beneath the female, and those which had just emerged

and were found wandering on the plant, as well as the individuals which were feeding. The size of each individual is given in Table 6 and the size range is presented in the form of a histogram in Fig. 24.

Amongst those found beneath the female, some were seen to have the embryonic membranes still attached to the posterior end of the body. Six of these individuals were measured and their sizes found to vary from 0.29 x 0.17 mm. to 0.31 x 0.17 mm. (Table 6, BB). Other larvae were found within the brood space, devoid of any attachment to their embryonic membranes. Eight of these larvae were measured and their sizes found to vary from 0.30 x 0.19 mm. to 0.41 x 0.19 mm. (Table 6, B).

Those found wandering on the leaf itself, of which 18 were measured, varied in size from 0.34 x 0.19 mm. to 0.40 x 0.20 mm. (Table 6, W).

All the above larvae were ochreous in colour and had the abdominal segments distinct.

The measurements of 100 larvae found settled on the leaf with their stylets inserted into the leaf, ranged in size from 0.39 x 0.20 mm. to 0.84 x 0.42 mm. It can be seen from these figures that there is a considerable increase in size once feeding has commenced. They approximately double their size whilst feeding. They also lose their ochreous colour and become yellowish-green so that it is often difficult to discern them with the naked eye. The abdominal segments

Table 6. Length and breadth in mm. of larvae of C.hesperidum.

Ref. no. of individual*	Size L	B	Col. A	Col. B	Ref. no. of individual*	Size L	B	Col. A	Col. B
	0.29	0.17	BB		35	0.48	0.25		
	0.30	0.18	BB		36	0.48	0.25		
	0.30	0.18	BB		37	0.49	0.24		
	0.30	0.18	BB		38	0.49	0.26		
	0.30	0.19	BB		39	0.49	0.26		
	0.30	0.19	B		40	0.50	0.26		
	0.31	0.17	BB		41	0.51	0.26		
	0.32	0.20	B		42	0.51	0.26		
	0.32	0.20	B		43	0.51	0.27		
	0.33	0.21	B		44	0.51	0.27		
	0.34	0.19	W		45	0.52	0.27		
	0.35	0.19	W		46	0.52	0.29		
	0.35	0.19	W		47	0.53	0.27		
1	0.35	0.19			48	0.53	0.27		
	0.35	0.20	W		49	0.54	0.28		
	0.36	0.19	B		50	0.54	0.30		
	0.36	0.20	W		51	0.55	0.27		
	0.36	0.20	B		52	0.55	0.29		
	0.38	0.19	W		53	0.55	0.29		
	0.38	0.19	W		54	0.55	0.29		
	0.38	0.20	W		55	0.55	0.30		
	0.38	0.20	W		56	0.56	0.31		
	0.38	0.21	W		57	0.57	0.30		
	0.38	0.21	W		58	0.57	0.31		
	0.39	0.19	B		59	0.57	0.34		
	0.39	0.20	W		60	0.58	0.32		
	0.39	0.20	W		61	0.58	0.32		
	0.39	0.20	W		62	0.59	0.34		
	0.39	0.20	W		63	0.60	0.33		
2	0.39	0.20			64	0.61	0.31		
	0.40	0.19	W		65	0.61	0.33		
	0.40	0.19	W		66	0.62	0.35		
	0.40	0.20	W		67	0.63	0.33		
3	0.40	0.20			68	0.64	0.34		
4	0.40	0.20			69	0.64	0.34		
5	0.40	0.22			70	0.65	0.35		
6	0.40	0.24			71	0.65	0.36		
	0.41	0.19	B		72	0.65	0.37		
7	0.41	0.22			73	0.66	0.36		
8	0.42	0.22			74	0.68	0.33		
9	0.43	0.21			75	0.72	0.36		C
10	0.43	0.23			76	0.72	0.38		C
11	0.43	0.23			77	0.72	0.39		C
12	0.43	0.23			78	0.72	0.41		
13	0.43	0.23			79	0.73	0.34		C
14	0.43	0.23			80	0.73	0.36		
15	0.43	0.24			81	0.74	0.35		MM
16	0.44	0.21			82	0.74	0.38		MM
17	0.44	0.21			83	0.74	0.39		C
18	0.44	0.22			84	0.74	0.40		C
19	0.44	0.22			85	0.74	0.41		M
20	0.44	0.23			86	0.75	0.42		
21	0.44	0.23			87	0.78	0.41		C
22	0.44	0.24			88	0.79	0.38		C
23	0.45	0.22			89	0.79	0.41		C
24	0.45	0.22			90	0.79	0.42		C
25	0.45	0.24			91	0.79	0.42		C
26	0.45	0.24			92	0.79			MM
27	0.45	0.27			93	0.80	0.42		C
28	0.46	0.24			94	0.81	0.41		
29	0.46	0.25			95	0.82	0.40		MM
30	0.46	0.25			96	0.82	0.40		MM
31	0.47	0.24			97	0.82	0.44		MM
32	0.47	0.25			98	0.83	0.42		C
33	0.47	0.25			99	0.83	0.46		M
34	0.48	0.22			100	0.84	0.42		M

* See Table 7. Abbreviations (For details see text.)

L, length; B breadth.

Column A: BB Beneath female, embryonic membrane attached at posterior end of body.
B Beneath female, devoid of attachment with embryonic membrane.
W Wandering on leaf.
Other individuals feeding, except where indicated in Column B.

Column B: C Feeding, styliform organ coiled.
M Stylets retracted, new cuticle present, old cuticle still intact.
MM Old cuticle being shed.

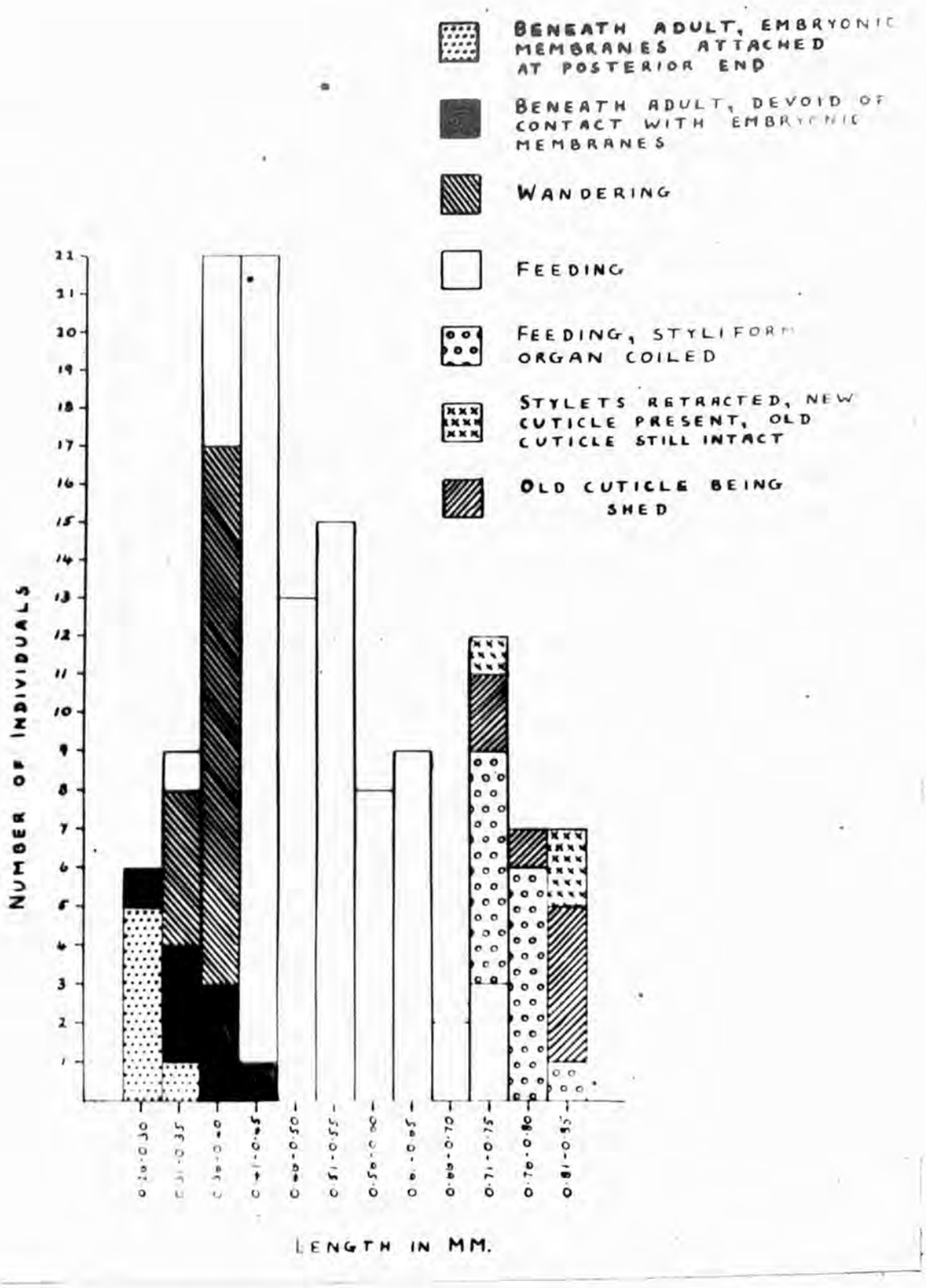


Fig. 24. Histogram showing the size range of the length in mm. of larvae of C.hesperidum, together with an indication of the behaviour of these larvae. (For details, see text.)

at the same time become indistinct and in shape and general appearance the larvae resemble nymphs.

Although there is a slight difference between the size range of those found beneath the adult and those found wandering on the leaf, there are not sufficient data to determine whether this is significant. It would appear however, that a slight increase in size does occur between the shedding of the embryonic membranes and the end of the wandering phase.

The size range of those individuals in which moulting was taking place varied from 0.74 x 0.35 mm. to 0.84 x 0.42 mm. The size of each individual is given in Table 6, whilst their relationship to the rest of the size range is shown in Fig. 24. The actual description of moulting itself is dealt with later.

The feeding tracks of the larvae

The 100 individuals which were found to be feeding were, in all cases, settled alongside a vein as previously described. 90% showed a single penetration through the epidermis whilst the remaining 10% showed tracks too complex for an analysis to be made.

Amongst the individuals showing a single penetration, there was a wide variation in the behaviour of the stylets within the leaf itself, 7 distinct types of behaviour being distinguished.

Type 1: In 31 individuals the stylets passed direct to the vein, the stylet track being completely devoid of any side-branches (Fig. 25).

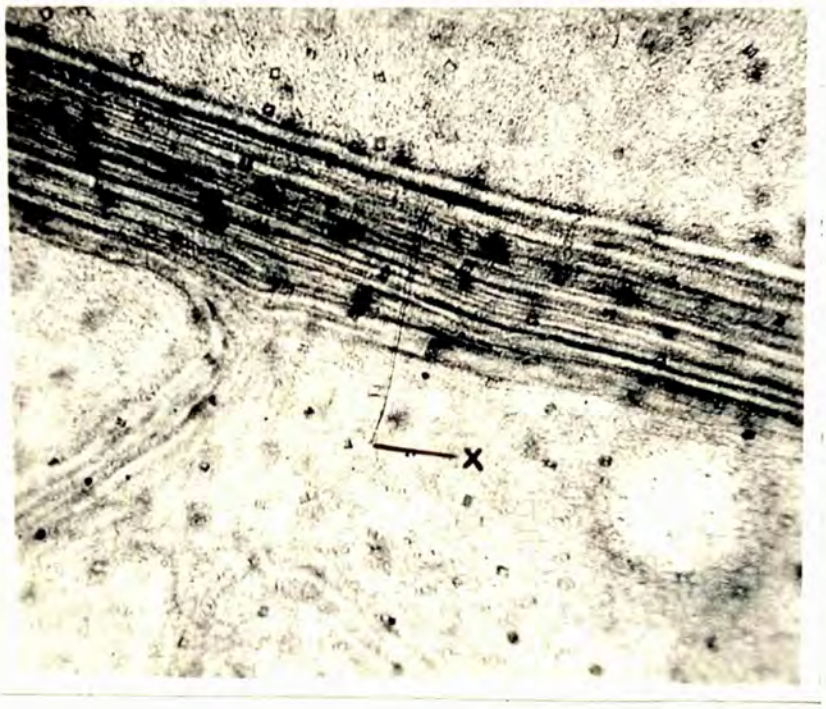


Fig. 25. Photomicrograph of a cleared leaf of the Rough Lemon showing penetration of a vein by the stylets of a larva of C.hesperidum (x 120).

Penetration of the epidermis occurs at the point X. The dark patches are cells within the leaf containing crystals of calcium oxalate whilst the light circular patch is an oil gland.

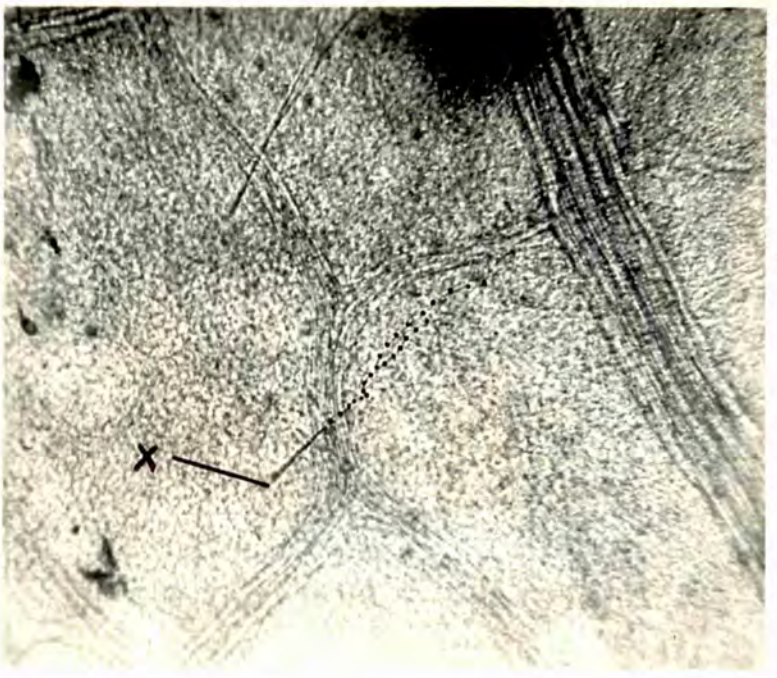


Fig. 26. Photomicrograph of a cleared leaf of the Rough Lemon showing penetration by the stylets of a larva of C.hesperidum (x 120).

The stylets penetrate the epidermis at the point X and pass into the vein whilst a side-branch (indicated by dotted lines) passes beyond the vein and branches into two.

Type 2: In 16 individuals, although the stylets themselves passed direct into the vein, side-branches of the sheath were present. In some cases these side-branches originated from the part of the stylet track within the vein, the branches either being confined to the vein itself or else passing beyond it (Fig. 26). Other individuals showed the side-branches arising from that part of the stylet track outside the vein, some specimens showing these side-branches entering the vein whilst in others they were confined to the mesophyll. This type of behaviour shows that the larvae, following the initial penetration of the epidermis, has from time to time partially withdrawn its stylets and then inserted them elsewhere within the leaf, although it has never withdrawn them completely whilst in this position on the leaf.

This direct approach into the vein by the stylets was not seen in all larvae. Some of them showed the stylets passing from the epidermis into the vein along a meandering path. In some cases this meandering was intense whilst in others it consisted of a kink in an otherwise straight track. There were 2 kinds of behaviour in the larvae showing this meandering path into the vein, referred to as Types 3 and 4.

Type 3: In these larvae the stylet track was devoid of side-branches. This behaviour was seen in 10 individuals.

Type 4: In 15 individuals, side-branches were present arising from the stylet track either within the vein or else within

the mesophyll (Fig. 27). The paths taken by these side-branches showed the same variation between individuals as in the larvae showing a direct penetration into the vein. Of those arising in the vein, some individuals showed them restricted to the vein, whilst others showed them passing beyond it. Those arising in the mesophyll in some cases entered the vein whilst in others they did not.

The stylet tracks so far described all had one feature in common in that they penetrated the vein. This was not the case in all larvae.

Type 5: In 6 larvae, the stylets followed a straight path through the mesophyll but did not enter the vein and were completely devoid of side-branches.

Type 6: In 3 larvae the behaviour was similar to that of type 5 except that the stylets meandered through the mesophyll.

In both type 5 and 6, the stylets in some cases had penetrated the mesophyll for only a short distance, indicating that penetration was only just commencing.

Type 7: The remaining 9 larvae also showed the stylets meandering through the mesophyll without actually penetrating the vein, but in these individuals the stylet track showed side-branches, although none of these entered the vein.

Table 7 shows the analysis of the tracks in accordance with the behaviour patterns described above. In Fig. 28, the individuals are grouped on the basis of this behaviour and the sizes of the individuals within each group are shown

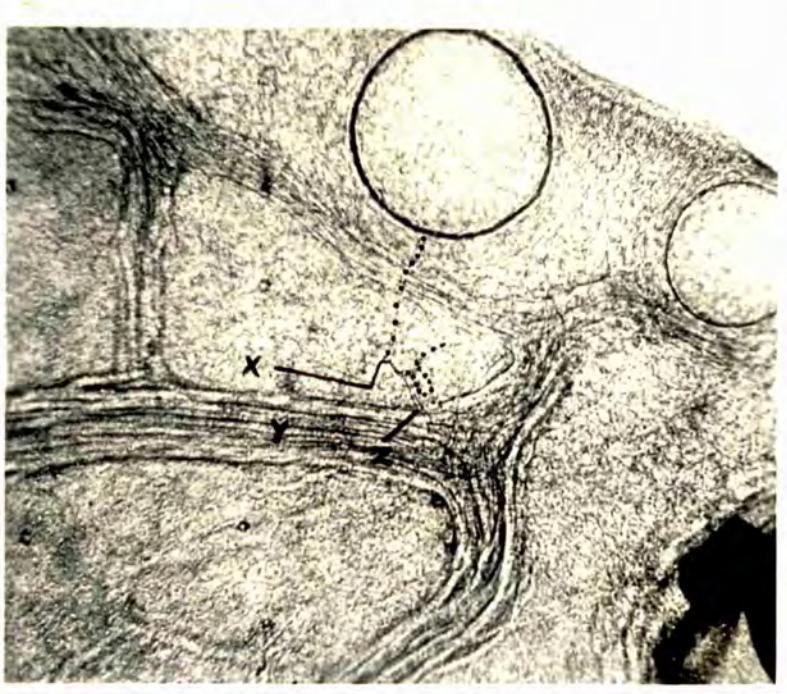


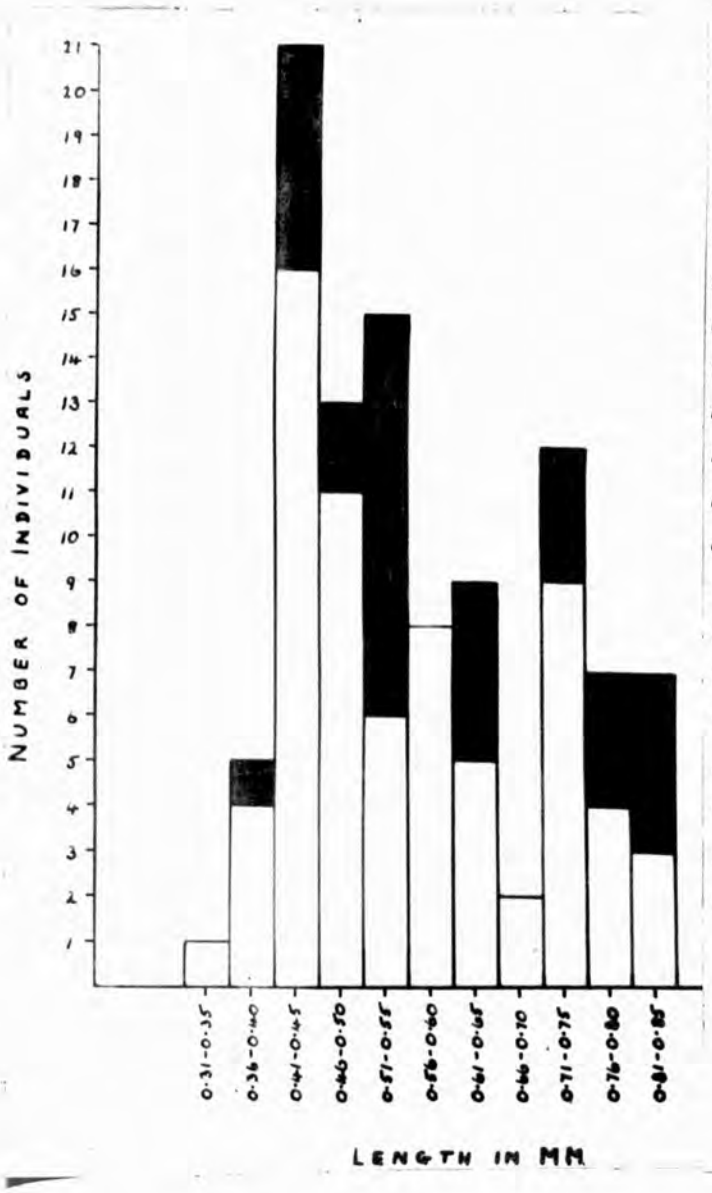
Fig. 27. Photomicrograph of a cleared leaf of the Rough Lemon showing penetration by the stylets of a larva of C. hesperidum (x 120).

The stylets penetrate the epidermis at the point X, pass away from vein Y and then turn and penetrate this vein at the point Z. Side-branches are indicated by dotted lines. The light circular patches are oil glands in the leaf.

Table 7. Variations in the behaviour of the stylet tracks of larvae of C.hesperidum.

Number of penetrations of epidermis	Track entering vein	Track taking direct course	Track branching	Number of individuals	Reference number * of individuals
1	+	+	+	16	7, 8, 24, 35, 40, 46, 51, 58, 61, 74, 75, 80, 83, 88, 90, 100.
1	+	+	-	31	4, 10, 15, 17, 20, 27, 28, 31, 41, 42, 43, 44, 45, 48, 49, 52, 54, 64, 65, 67, 69, 77, 79, 86, 89, 91, 93, 94, 97, 98, 99.
1	+	-	-	10	6, 13, 18, 25, 32, 37, 56, 71, 81, 87.
1	+	-	+	15	14, 21, 33, 38, 47, 50, 53, 59, 60, 62, 63, 66, 72, 76, 92.
1	-	-	+	9	1, 2, 5, 11, 16, 19, 30, 39, 85.
1	-	+	-	6	9, 26, 36, 57, 73, 82.
1	-	-	-	3	3, 68, 96.
Tracks too complex for analysis				10	12, 22, 23, 29, 34, 55, 70, 78, 84, 95.

* See Table 6.


NUMBER OF INDIVIDUALS
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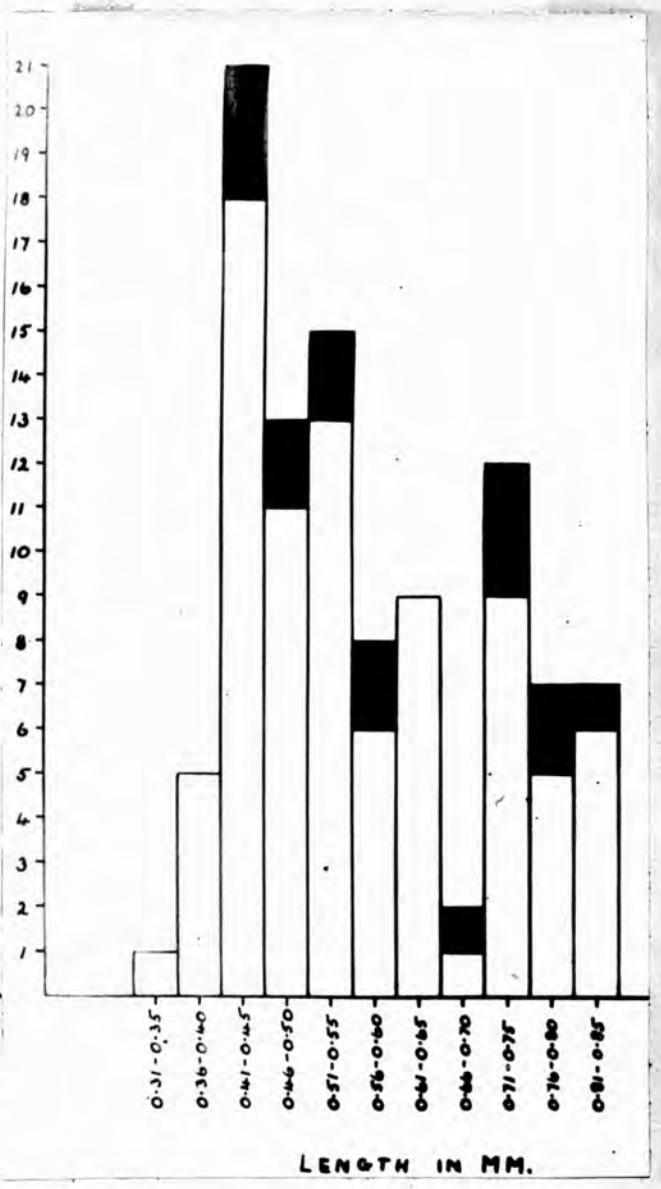
0·31-0·35
0·36-0·40
0·41-0·45
0·46-0·50
0·51-0·55
0·56-0·60
0·61-0·65
0·66-0·70
0·71-0·75
0·76-0·80
0·81-0·85
LENGTH IN MM

Type 1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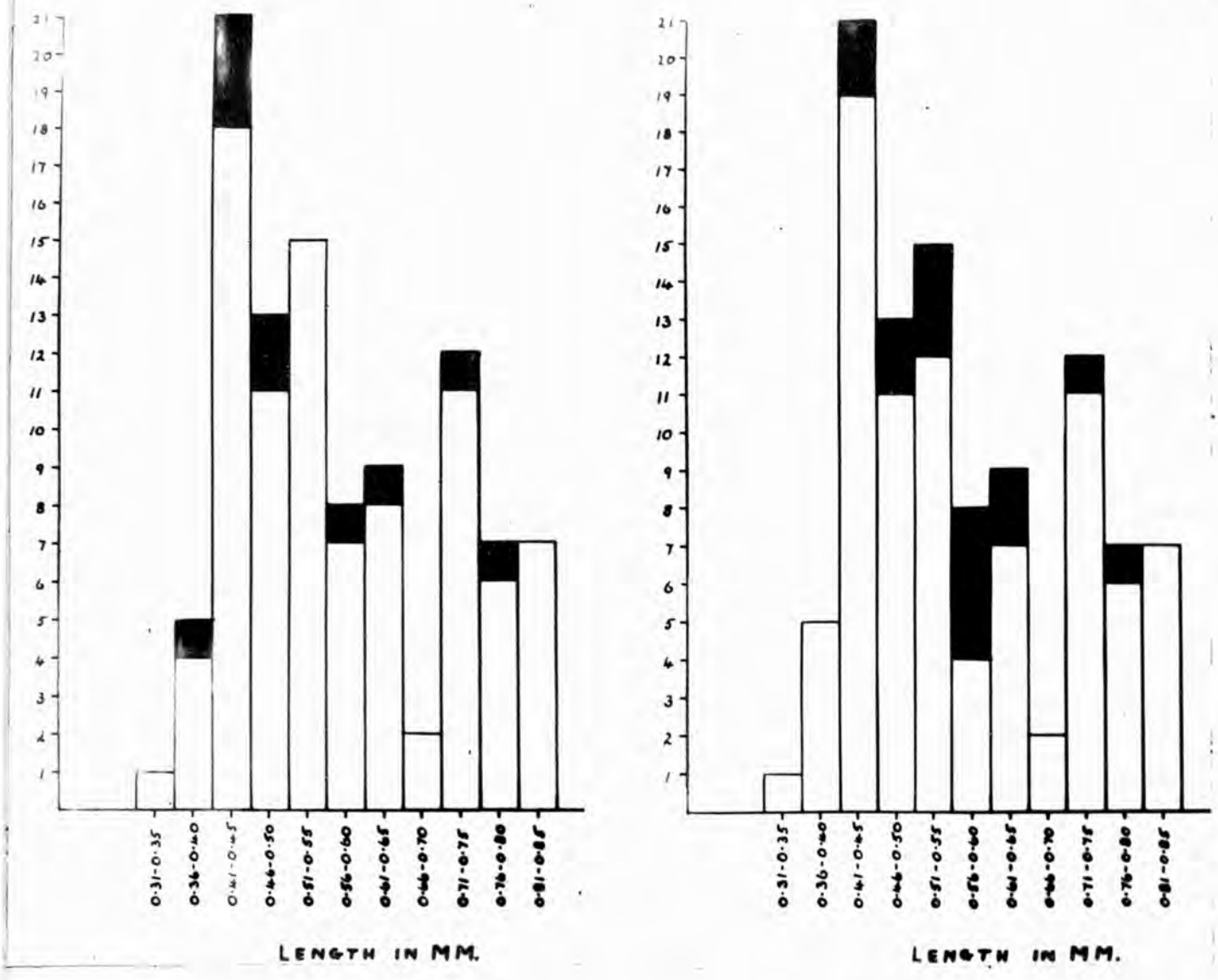
LENGTH IN MM.

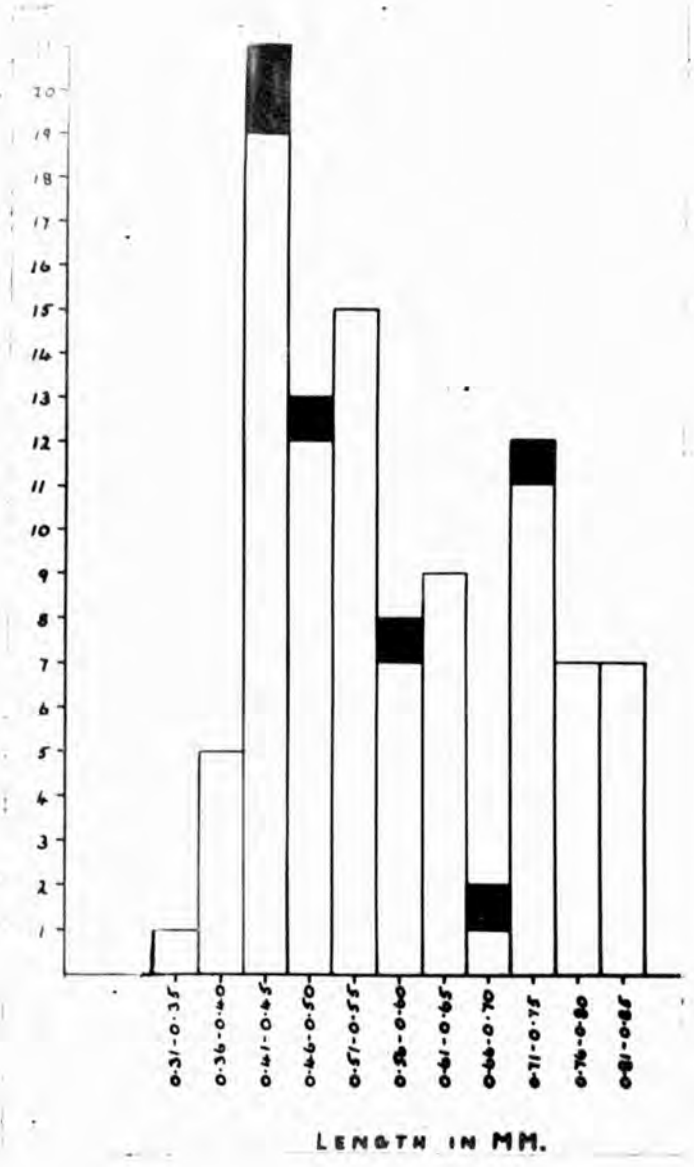
Type 2



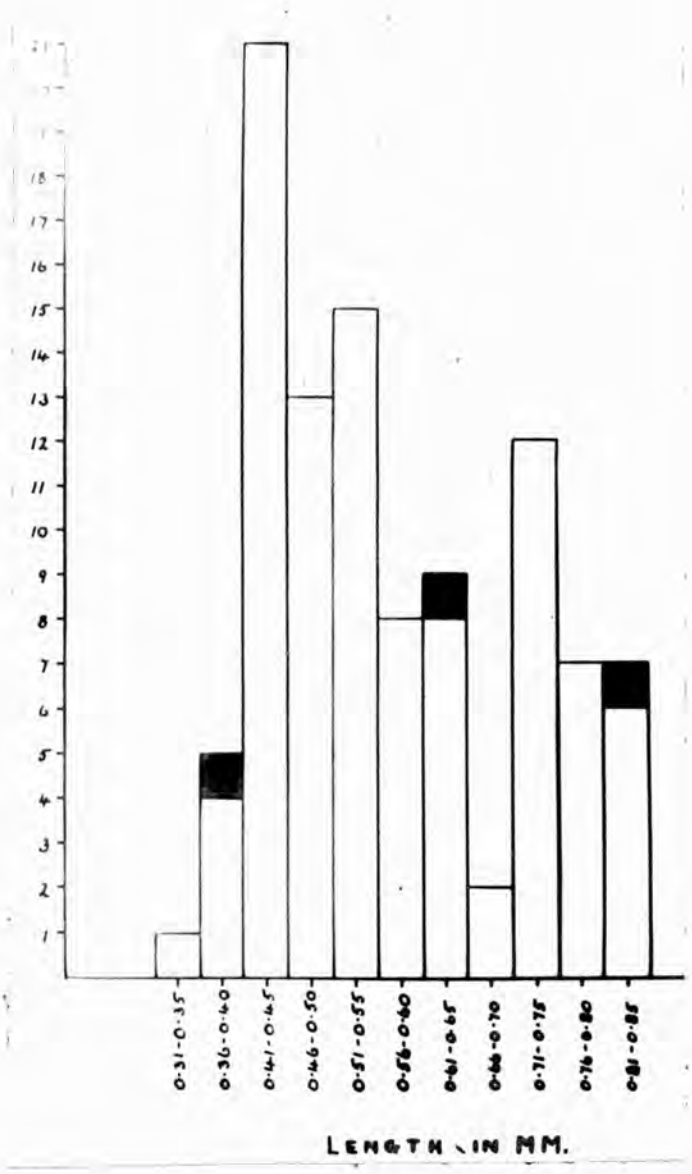
Type 3

Type 4

Fig. 28. The distribution, throughout the size range, of larvae of C.hesperidum showing different types of behaviour of the stylets (See pp. 98 to 105).


0·31-0·35
0·36-0·40
0·41-0·45
0·46-0·50
0·51-0·55
0·56-0·60
0·61-0·65
0·66-0·70
0·71-0·75
0·76-0·80
0·81-0·85
LENGTH IN MM.

Type 5


0·31-0·35
0·36-0·40
0·41-0·45
0·46-0·50
0·51-0·55
0·56-0·60
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0·66-0·70
0·71-0·75
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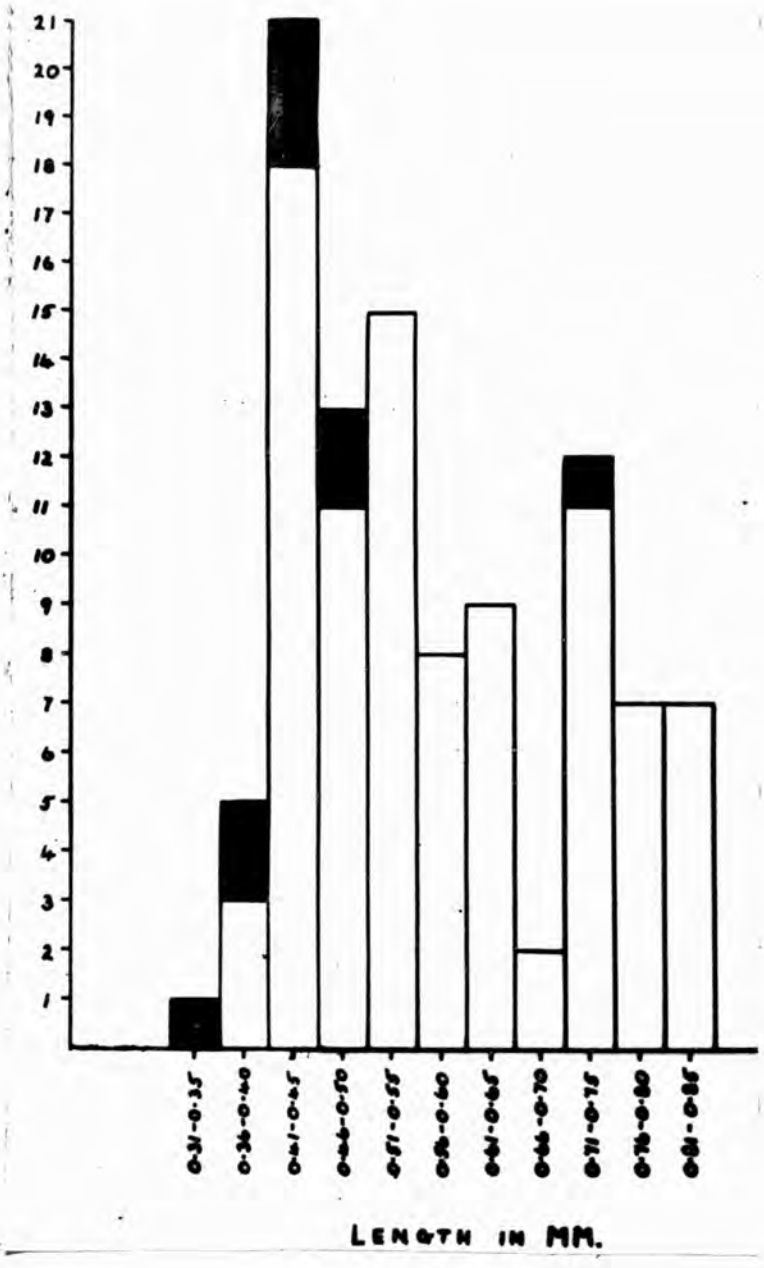
LENGTH IN MM.

Type 6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0·41-0·45
0·46-0·50
0·51-0·55
0·56-0·60
0·61-0·65
0·66-0·70
0·71-0·75
0·76-0·80
0·81-0·85
LENGTH IN MM.

Type 7

in relation to the total size range of the larvae. From Fig. 28 it can be seen that each type of behaviour pattern is found to be distributed throughout the size range with no marked restriction to any particular part of this range. Thus there is no indication that the behaviour varies with the age of the larvae, but appears to be a variation between individuals.

The size range of the nymphs

46 individuals were examined, all of which were feeding and were settled alongside a vein as previously described.

Their sizes ranged from 0.73 x 0.37 mm. to 1.61 x 0.83 mm. the size of each individual being shown in Table 8, whilst in Fig. 29 the data are presented in the form of a histogram.

There is a slight overlap with the upper limits of the larval range, covering the size range of the larvae which were found to be moulting. Although the size range of the nymphs is greater than that of the larvae, it does not form such a continuous series, this being very marked at the upper limits. This lack of continuity appears to be due mainly to the small number of nymphs examined which were rare in comparison with the larvae and adults.

Moulting was found to be taking place in only 3 individuals of the following sizes 1.27 x 0.68 mm., 1.34 x 0.73 mm. and 1.56 x 0.89 mm.

The presence of long setae on the anal plates of the young nymphs mentioned by Fonseca (1953) was not verified.

Table 8. Length and breadth in mm. of nymphs of C.hesperidum.

Ref. no. of individual *	Size L	Size B	Col. B	Ref. no. of individual *	Size L	Size B	Col. B
1	0.73	0.37		24	1.07	0.56	
2	0.75	0.38		25	1.07	0.58	
3	0.78	0.38		26	1.07	0.63	
4	0.78	0.38		27	1.12	0.58	
5	0.78	0.43		28	1.12	0.63	
6	0.79	0.42		29	1.12	0.66	
7	0.79	0.42		30	1.15	0.63	
8	0.81	0.43		31	1.17	0.63	
9	0.82	0.43		32	1.17	0.63	
10	0.82	0.44		33	1.17	0.68	
11	0.83	0.44		34	1.22	0.63	C
12	0.85	0.45		35	1.22	0.68	C
13	0.87	0.49		36	1.22	0.73	
14	0.88	0.48		37	1.24	0.63	C
15	0.88	0.48		38	1.27	0.68	M
16	0.88	0.49		39	1.27	0.68	
17	0.89	0.45		40	1.27	0.73	C
18	0.90	0.49		41	1.29	0.68	
19	0.93	0.44		42	1.34	0.73	M
20	1.02	0.49		43	1.37	0.73	
21	1.02	0.53		44	1.37	0.78	C
22	1.02	0.53		45	1.56	0.89	M
23	1.02	0.58		46	1.61	0.83	C

* See Table 9. For abbreviations see Table 6.

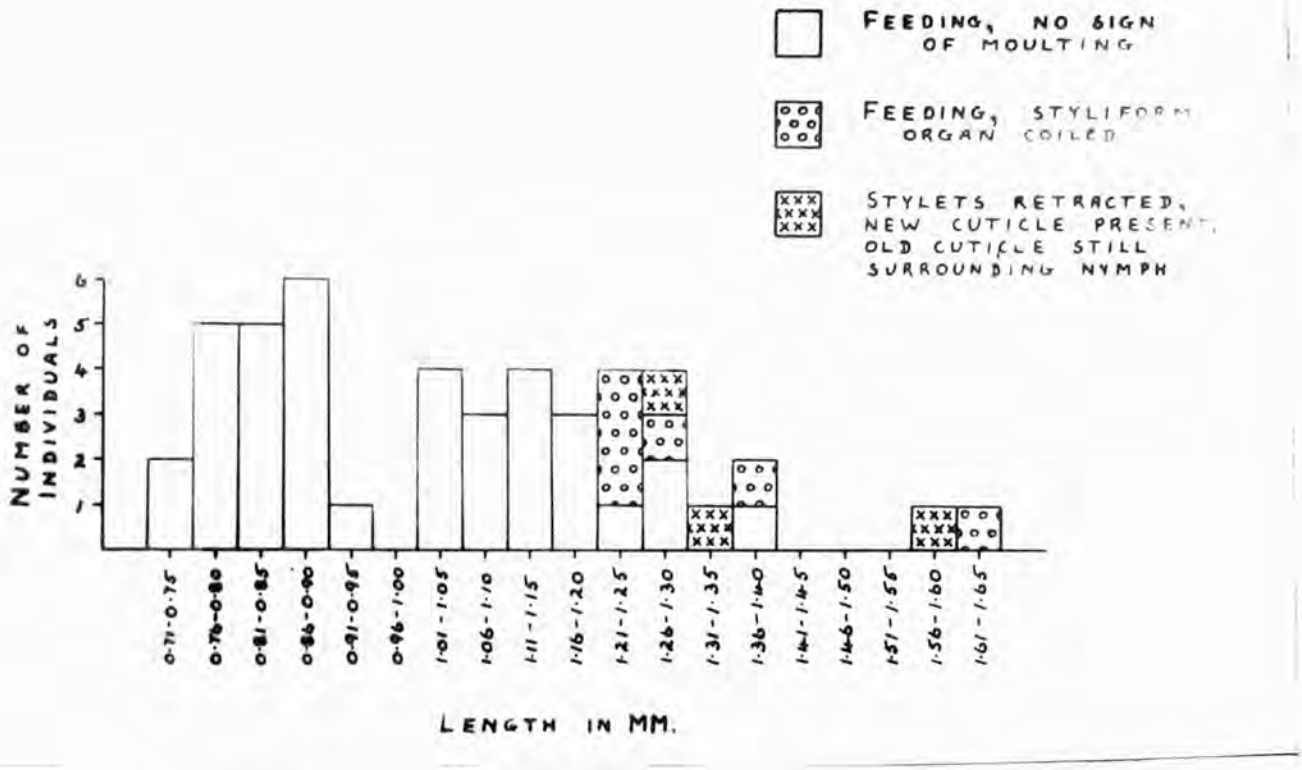

Fig. 29. Histogram showing the size range of the length in mm. of nymphs of C. hesperidum, together with an indication of the sizes at which moulting occurs. (For details see text.)

Nymphs in the process of shedding the larval skin were examined and found to lack these setae. Also, larvae which were about to moult, and in which the new anal plates could be seen beneath the cuticle of the larval ones, showed no setae on these plates.

The feeding tracks of the nymphs

The 46 nymphs examined all showed the stylets penetrating the leaf near a vein, and in most cases the stylets entered this vein.

The most obvious point that arose from the study of the nymphal feeding tracks was the presence of 2 independent tracks lying side by side, one containing the stylets and the other consisting only of saliva (Fig. 30). These were found in 37 (85.7%) of the nymphs, the rest, as described below, showing only a single track of the stylets themselves. The feeding tracks of the nymphs will be considered further under the headings of those individuals with two tracks and those with one.

Individuals with 2 tracks:

In all cases the salivary track was narrower than the one possessing the stylets. These two independent tracks showed independent penetrations of the epidermis in 80.5% of the individuals, the two points of penetration occurring side by side on the leaf surface. The remaining 19.5% showed the two tracks entering the epidermis at the same point. In Table 9, the two tracks of the individual are

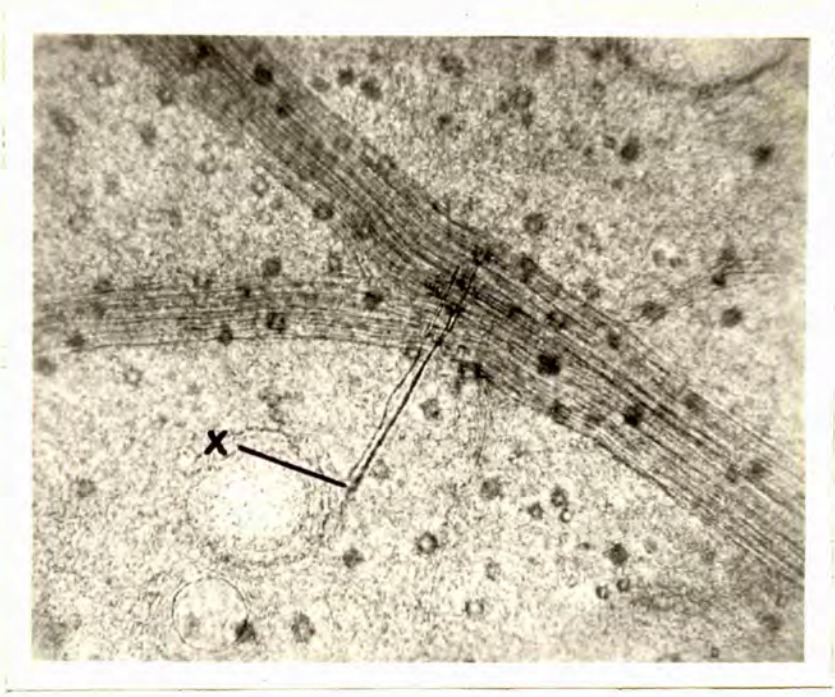

Fig. 30. Photomicrograph of a cleared leaf of the Rough Lemon showing penetration of a vein by the stylets of a nymph of C.hesperidum (x 120).

The stylets penetrate the epidermis at the point X. The adjacent lighter track is the residual sheath of an earlier penetration.

Table 9. Variations in the behaviour of the stylet tracks of nymphs of C.hesperidum

Number of independent tracks from epidermis	Number of independent penetrations of epidermis	Track entering vein		Track taking direct course		Track branching		Number of individuals	Reference number * of individuals
		Stylet track	2nd track	Stylet track	2nd track	Stylet track	2nd track		
2	1	+	+	+	+	+	+	1	13
2	2	+	+	+	+	+	+	3	20, 22, 41
2	1	+	+	+	+	+	-	1	35
2	2	+	+	+	+	+	-	7	6, 7, 17, 25, 26, 31, 33.
2	1	+	+	+	+	-	+	1	14
2	2	+	+	+	+	-	+	4	2, 8, 11, 15
2	1	+	+	+	+	-	-	4	19, 21, 36, 38
2	2	+	+	+	+	-	-	9	3, 10, 12, 24, 37, 40, 42, 45, 46
2	2	+	+	+	-	+	-	1	4
2	2	+	+	-	+	+	-	1	1
2	2	-	+	+	+	-	+	1	28
2	2	-	+	+	+	-	-	1	5
2	2	-	+	-	-	+	+	1	9
2	2	+	-	-	-	+	-	1	32
2	2	-	-	-	-	+	+	1	16
1	1	+		+		+		3	27, 30, 34
1	1	+		+		-		2	39, 44
1	1	-		-		+		1	23
Tracks too complex for analysis								3	18, 29, 43

* See Table 8.

analysed separately on the same basis as for the larvae.

The presence of 2 independent tracks, each with their own point of penetration through the epidermis, shows conclusively that the individuals concerned have withdrawn their stylets from the leaf and re-inserted them. This re-insertion appears to take place usually alongside the old one, but in some cases it occurs at the same point thus giving individuals that show only the one point of penetration. In most cases it was seen that both these tracks took a direct course into the vein, although a few individuals lacked this direct approach and in some cases even failed to enter the vein. When the presence of these two independent tracks is related to the actual size of the individual (Fig. 31), it is found that they occur in nymphs of all sizes.

In some cases the stylet track was seen to possess side branches composed of the stylet sheath alone. This showed that the stylets had from time to time been withdrawn from the vein itself and then re-inserted into it, along a path more or less parallel to the previous one. In a few instances it was seen that this branching was restricted to within the vein itself, showing that the individual had withdrawn its stylets only a very short distance prior to re-inserting them. The adjacent salivary track also showed similar branching in some cases,

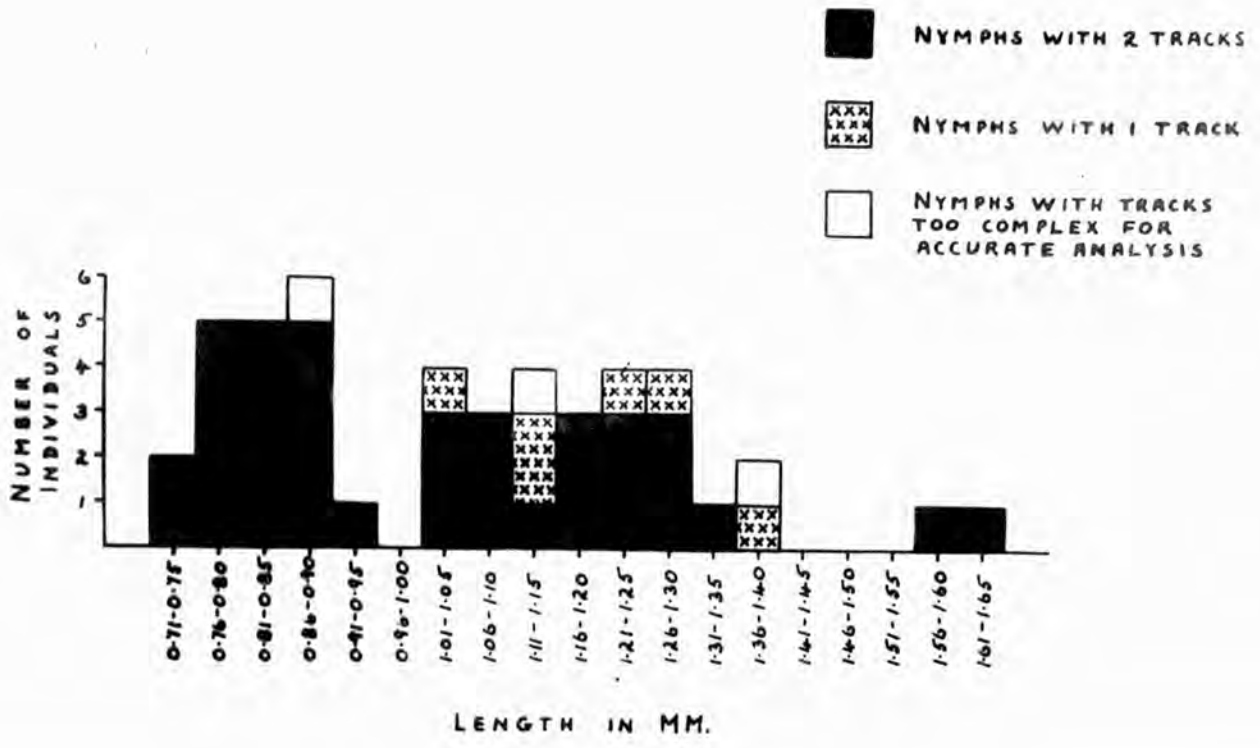

Fig. 31. The distribution, throughout the size range, of nymphs of C.hesperidum showing one or two stylet tracks.

indicating that after the stylets had been inserted in that initial position, they had also been partially withdrawn from time to time and re-inserted into the vein. Branching very seldom occurred in both tracks of any one individual, whilst in some cases neither track showed it. The occurrence of this branching in the various individuals can be seen in Table 9. In Fig. 32 the presence or absence of branching of the track containing the stylets is related to the actual size of the individual concerned and it can be seen that some of the older nymphs as well as some of the young ones show no branching at all. The significance of this fact is discussed below.

3 nymohs were found that were actually about to undergo a moult and in which the stylets themselves had been withdrawn from the leaf and looped up in the crumena. In all 3 cases there were two independent tracks of saliva passing from beneath the labium directly into the vein, without branching.

Individuals with 1 track:

As mentioned above, two independent tracks were not shown by all the nymphs. Nine (14.3%) of them showed only a single track, that of the stylets themselves. In all these cases this single track took a direct course to the vein, and entered at approximately right angles to it (as

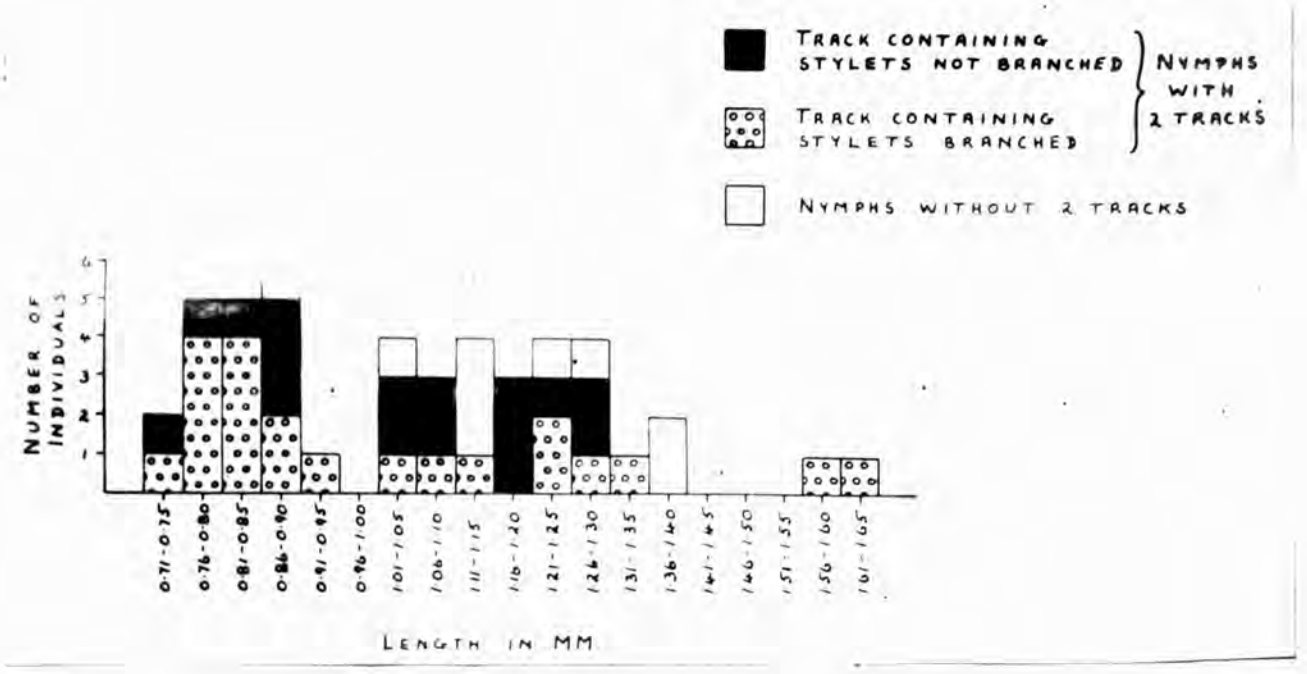

Fig. 32. The distribution, throughout the size-range, of nymphs of C.hesperidum possessing two tracks, showing the occurrence of branching in the track containing the stylets.

in Figs. 25 and 35). Once again, the presence of side-branches of saliva in some of the individuals showed that the stylets had been withdrawn slightly and re-inserted. In one case however the stylets did not enter the vein but took a branching meandering course. Individuals possessing this single track are shown in Table 9 and their distribution throughout the size range is shown in Fig. 31.

The size range of the adults

100 individuals were examined, all of which were found to be feeding. They varied in size from 1.27 x 0.73 mm. to 3.03 x 2.05 mm., this range being approximately twice that of the nymphs. The individual sizes, together with an indication of their sexual maturity, is shown in Table 10, and are presented in the form of a histogram in Fig. 33. They overlap the nymphal range between individuals 1.27 mm. in length and 1.61 mm. in length, thus covering the size of the nymphs that were about to moult. One adult (1.42 x 0.68 mm.) was found which had just emerged from the nymphal skin, and is seen to occur within the size range of the nymphal moults.

Table 10, column A, shows the state of each individual as regards the presence of eggs or embryos within the body. This is just an approximate indication of the reproductive state of the adults and is based simply on what could be seen within the

Table 10. Length and breadth in mm. of adults of C.hesperidum.

Ref. no. of individual *	Size L	B	Col. A	Ref. no. of individual *	Size L	B	Col. A
1	1.27	0.73		51	2.15	1.47	E
2	1.32	0.73		52	2.20	1.32	E
3	1.34	0.83		53	2.20	1.47	E
4	1.37	0.78		54	2.20	1.56	Y
5	1.37	0.80		55	2.25	1.27	E
6	1.42	0.78		56	2.25	1.27	E
7	1.44	0.78		57	2.25	1.37	Y
8	1.47	0.83		58	2.25	1.47	
9	1.47	0.93		59	2.25	1.56	Y
10	1.51	0.83		60	2.30	1.42	Y
11	1.51	0.88		61	2.30	1.51	Y
12	1.51	0.93		62	2.30	1.51	Y
13	1.54	0.78		63	2.30	1.61	E
14	1.56	0.88		64	2.35	1.42	E
15	1.56	1.02		65	2.35	1.42	Y
16	1.61	0.88		66	2.35	1.47	E
17	1.61	0.92		67	2.35	1.51	E
18	1.66	0.93		68	2.39	1.41	Y
19	1.66	0.98		69	2.40	1.32	E
20	1.66	1.07		70	2.40	1.42	Y
21	1.66	1.07		71	2.40	1.47	E
22	1.71	0.78		72	2.40	1.47	E
23	1.71	1.02		73	2.40	1.47	E
24	1.76	0.88		74	2.40	1.71	E
25	1.76	1.07		75	2.45	1.37	Y
26	1.76	1.12		76	2.45	1.47	Y
27	1.81	1.27	E	77	2.45	1.61	Y
28	1.86	1.07		78	2.45	1.61	Y
29	1.91	1.17	E	79	2.45	1.76	E
30	1.91	1.17		80	2.49	1.42	E
31	1.91	1.17		81	2.49	1.47	Y
32	1.96	1.12	E	82	2.49	1.61	Y
33	1.96	1.17	Y	83	2.54	1.42	Y
34	1.96	1.27		84	2.54	1.56	Y
35	2.00	1.12	Y	85	2.54	1.61	E
36	2.00	1.17	E	86	2.54	1.71	E
37	2.05	1.22	E	87	2.59	1.71	Y
38	2.05	1.27	Y	88	2.64	1.61	E
39	2.05	1.27	Y	89	2.64	1.71	E
40	2.05	1.27	Y	90	2.64	1.86	E
41	2.05	1.27		91	2.69	1.61	Y
42	2.10	1.22	E	92	2.69	1.96	Y
43	2.10	1.27	E	93	2.71	1.96	E
44	2.10	1.32	E	94	2.74	1.76	Y
45	2.10	1.32	E	95	2.74	1.91	Y
46	2.10	1.32	E	96	2.74	1.91	Y
47	2.13	1.37	E	97	2.74	2.00	Y
48	2.15	1.27	E	98	2.79	1.86	E
49	2.15	1.32	E	99	2.84	2.00	E
50	2.15	1.37	Y	100	3.03	2.05	Y

* See Table 11.

Abbreviations:

L, length; B, breadth

Col. A E - eggs present within body
Y - embryos and eggs present within body

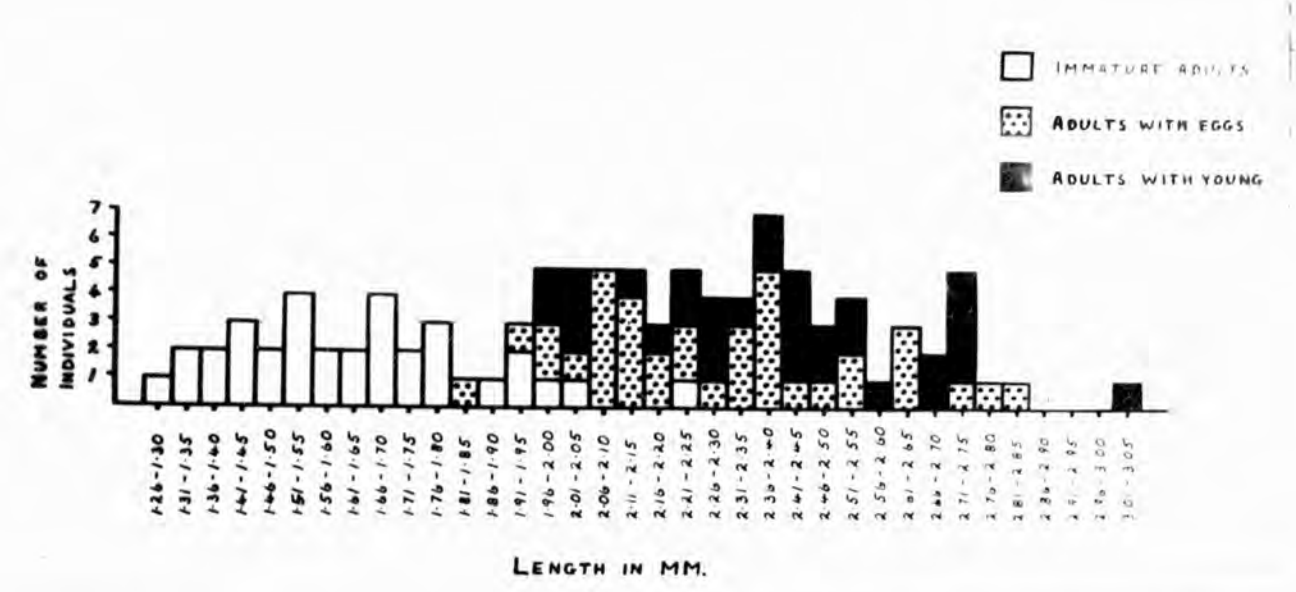

Fig. 33. Histogram showing the size range of the length in mm. of adults of C. hesperidum, together with the size range of the sexually mature adults. (For details, see text.)

body through the transparent cuticle. No attempt was made to study the reproductive organs or maturation of the egg in detail. The adults at the lower end of the size range, from 1.27 x 0.73 mm. to approximately 1.76 x 1.12 mm., did not possess any visible sign of eggs and resembled nymphs in colour and shape. Eggs were clearly visible for the first time in individuals of 1.81 x 1.27 mm. although there is no sharp distinction in size between individuals with or without eggs since adults as large as 2.05 x 1.27 mm. and 2.25 x 1.47 mm. were found to be without eggs.

Following the appearance of the eggs within the body, the adults show the change in colour and shape which is characteristic of this phase in their life. The abdominal region increases in height and width and becomes the widest part of the body, which was previously situated between the anterior and posterior spiracular depressions. The abdomen at the same time becomes deeply pigmented making it more difficult to observe the contents of the body.

Fully formed larvae were seen within the body of an adult of size 1.96 x 1.17 mm. and from this size up to the maximum size of 3.03 x 2.05 mm., the individuals, with three exceptions, contain either eggs or fully developed larvae with no distinction as regards size between those adults containing eggs and those containing larvae. This variation in size illustrates the considerable size variation existing between the mature females.

The feeding tracks of the adults

Of the 100 individuals examined, 67 showed 3 independent tracks passing from the epidermis into the vein (Fig. 34). One of these tracks was that of the stylets themselves, whilst the other two were composed of the residual stylet sheaths of previous penetrations.

10 individuals showed only 2 independent tracks, one being that of the stylets and the other the residual sheath of an earlier penetration.

15 individuals showed only the stylets themselves passing into the vein with no additional tracks alongside (Fig. 35).

The remaining individuals did not conform to any of the above groups. 2 of them showed the presence of 3 tracks in addition to the stylets, whilst 2 showed 5 tracks in addition to the stylets. The tracks of the remaining 4 individuals were too confusing to allow an analysis to be made.

The tracks will be considered in more detail under the headings of those individuals with three tracks, those with two and those with one.

Individuals with 3 tracks:

In those individuals possessing 3 tracks, the latter were seen in all cases to pass through the epidermis in the region directly beneath the labium and from here to pass towards the vein, either lying parallel to each other or else radiating slightly as seen in Fig. 34. The actual penetrations of the epidermis by the 3 tracks were seen in 29 cases to lie side by side. In 30 individuals however, one of the 3 penetrations

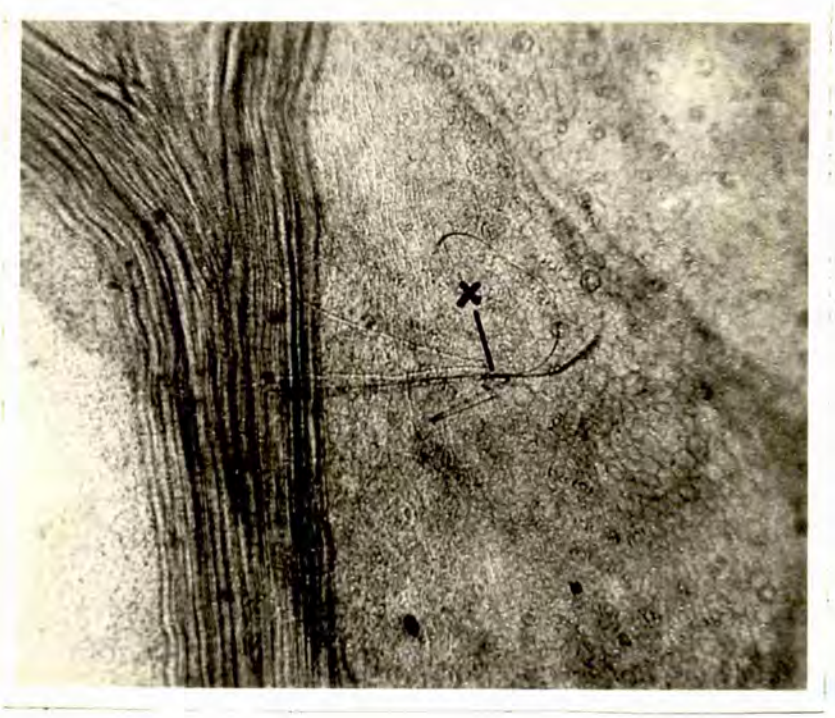

Fig. 34. Photomicrograph of a cleared leaf of the Rough Lemon, showing penetration of a vein by the stylets of an adult of C.hesperidum (x 120).

The stylets penetrate the epidermis at the point (X) and pass direct to the vein whilst the two adjacent lighter tracks are the residual sheaths of earlier penetrations. The curled proximal ends of the stylets can be seen protruding above the epidermis.

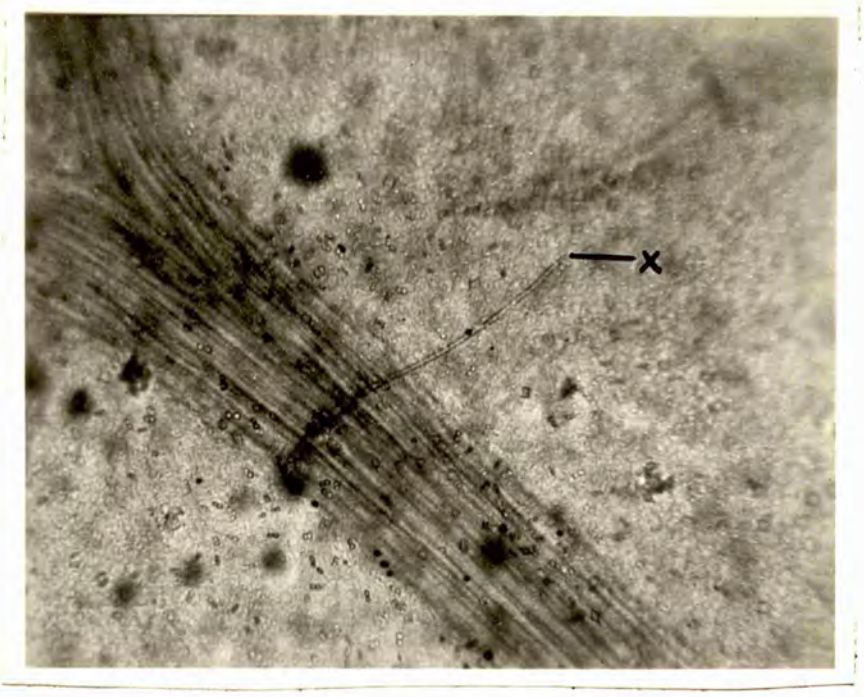

Fig. 35. Photomicrograph of a cleared leaf of the Rough Lemon showing penetration of a vein by the stylets of an adult of C.hesperidum (x 120).

The stylets penetrate the epidermis at the point (X).

actually coincided with one made previously so that only two distinct penetrations of the epidermis were visible, whilst in the remaining 8 cases all 3 coincided. Thus it can be seen that whilst remaining in one position on the leaf, the insect has made 3 distinct penetrations into the leaf, retracting its stylets completely from the leaf between each one. The fact that these tracks enter the epidermis so close together, and are actually coincident in some cases, shows that there has been no significant change in position of the labium itself.

There was a distinct difference in the width of the 3 respective tracks. Of the 2 tracks composed of a residual stylet sheath, one was much narrower than the other, whilst the track containing the stylets themselves was wider than both of them.

In Table 11 the 3 tracks are analysed separately, as for the larvae and the nymphs, those formed by the sheath alone being referred to as the 2nd and 3rd tracks, the latter being the narrowest.

All individuals, except 2, showed the 3 tracks entering the vein. In the 2 exceptions the stylets themselves did not enter it, although since they were in a straight path towards the vein, this was apparently due to penetration only just commencing. The path taken by the 3 tracks from the epidermis into the vein was, in most cases, a straight one, although in a few, one or other of the tracks was seen to

Table 11. Variations in the behaviour of the stylet tracks of adults of C.hesperidum.

Number of independent tracks from epidermis	Number of independent penetrations of epidermis	Track entering vein			Track taking direct course			Track branching			Number of individuals	Reference Number* of individuals
		Stylet track	2nd track	3rd track	Stylet track	2nd track	3rd track	Stylet track	2nd track	3rd track		
1	1	+			+			+			7	27, 33, 38, 60, 61, 62, 90
1	1	+			+			0			5	8, 16, 22, 43, 56
1	1	0			+			+			1	37
1	1	0			+			0			2	3, 9
2	1	+	+		+	+		+	+		2	17, 53
2	2	+	+		+	+		+	+		2	12, 39
2	1	+	+		+	+		+	0		1	92
2	2	+	+		+	+		+	0		2	13, 36
2	2	+	+		+	+		0	+		1	99
2	2	+	+		+	0		0	+		1	6
2	1	+	0		+	+		0	0		1	19
3	2	+	+	+	+	+	+	+	+	+	1	15
3	3	+	+	+	+	+	+	+	+	+	2	65, 73
3	1	+	+	+	+	+	+	+	+	0	2	52, 86
3	2	+	+	+	+	+	+	+	+	0	2	2, 97
3	3	+	+	+	+	+	+	+	+	0	4	51, 63, 76, 80
3	1	+	+	+	+	+	+	+	0	+	1	68
3	2	+	+	+	+	+	+	+	0	+	4	48, 74, 81, 98
3	3	+	+	+	+	+	+	+	0	+	2	25, 55
3	2	+	+	+	+	+	+	0	+	0	3	31, 66, 71
3	2	+	+	+	+	+	+	0	0	+	2	49, 82
3	2	+	+	+	+	+	+	+	0	0	7	26, 35, 41, 50, 54, 75, 83
3	3	+	+	+	+	+	+	+	0	0	7	11, 32, 57, 72, 79, 84, 89
3	1	+	+	+	+	+	+	0	0	0	3	5, 42, 96
3	2	+	+	+	+	+	+	0	0	0	3	28, 58, 94
3	3	+	+	+	+	+	+	0	0	0	7	10, 20, 45, 47, 69, 87, 91
3	2	+	+	+	+	+	0	+	+	+	1	40
3	3	+	+	+	+	+	0	+	+	+	1	34
3	3	+	+	+	+	+	0	+	0	+	1	21
3	2	+	+	+	+	+	0	0	+	0	1	23
3	1	+	+	+	+	+	0	+	0	0	1	85
3	2	+	+	+	+	+	0	+	0	0	2	77, 95
3	3	+	+	+	+	+	0	+	0	0	1	93
3	2	+	+	+	+	+	0	0	0	0	1	88
3	3	+	+	+	+	+	0	0	0	0	1	64
3	2	+	+	+	+	0	+	+	+	0	1	59
3	3	+	+	+	0	+	+	+	0	0	1	67
3	2	+	+	+	0	+	0	+	+	0	1	78
3	3	+	+	+	+	0	0	0	0	0	1	24
3	2	+	+	+	0	0	+	0	0	0	1	100
3	3	0	+	+	+	+	+	+	+	0	1	1
3	1	0	+	+	+	+	+	0	+	0	1	4
Tracks too complex for analysis											8	7, 14, 18, 29, 30, 44, 46, 70

* See Table 10

meander slightly.

In many cases the tracks themselves branched, the branches usually entering the vein, although not always. Branching sometimes occurred outside the vein whilst in other cases it was confined to the part of the track within the vein. The actual occurrence of branching in the individual tracks of each specimen is shown in Table 11. In only a few individuals did all 3 of the tracks show branching. Usually it was confined to one or two of the tracks, mostly the track containing the stylets, while some individuals show a complete lack of branching in any of the tracks.

Individuals with 2 tracks:

In the individuals in which only 2 tracks were visible, one was that of the stylets and the other an adjacent track of the stylet sheath (as in Fig. 30). It was noticeable that the narrowest stylet sheath track which had been present in the "3-track adults" was the one that was missing in these "2-track adults", and in all cases it was seen that the track consisting of the stylet sheath alone was slightly narrower than the track containing the stylets. Both tracks passed through the epidermis directly beneath the labium and ran alongside each other in a direct course towards the vein. Only in one instance did one of these tracks, the stylet sheath track, take a meandering course towards the vein. In all cases, except one, both tracks entered the vein, the one exception showing the track of the sheath actually passing

above the vein without entering it.

In 6 individuals it was seen that the 2 tracks penetrated the epidermis alongside each other, whilst in the remaining 4 individuals the stylets had been re-inserted at the same point as previously thus giving only a single point of penetration.

Branching of the tracks was present as before, showing the various positions in which the stylets had been inserted during each penetration. In some individuals only one of the tracks showed branching, either the stylet track or the track of the sheath, whilst some showed it in both tracks. One individual lacked branching in either track.

Individuals with 1 track:

These individuals showed only the track containing the stylets with no additional tracks alongside. In all individuals, the stylets passed directly towards the vein and, except for 3 individuals, entered the vein. The 3 exceptions were possibly due to the fact that penetration was only just beginning, as explained above for the "3-track" adults. In some instances the track of the stylets possessed side-branches which also entered the vein.

The actual sizes of the adults possessing 1, 2 or 3 tracks respectively, are related to the size range of the adults in Fig. 36, and an approximate idea of their ages is thereby obtained. It can be seen that individuals possessing either 1, 2 or 3 tracks are distributed throughout the size

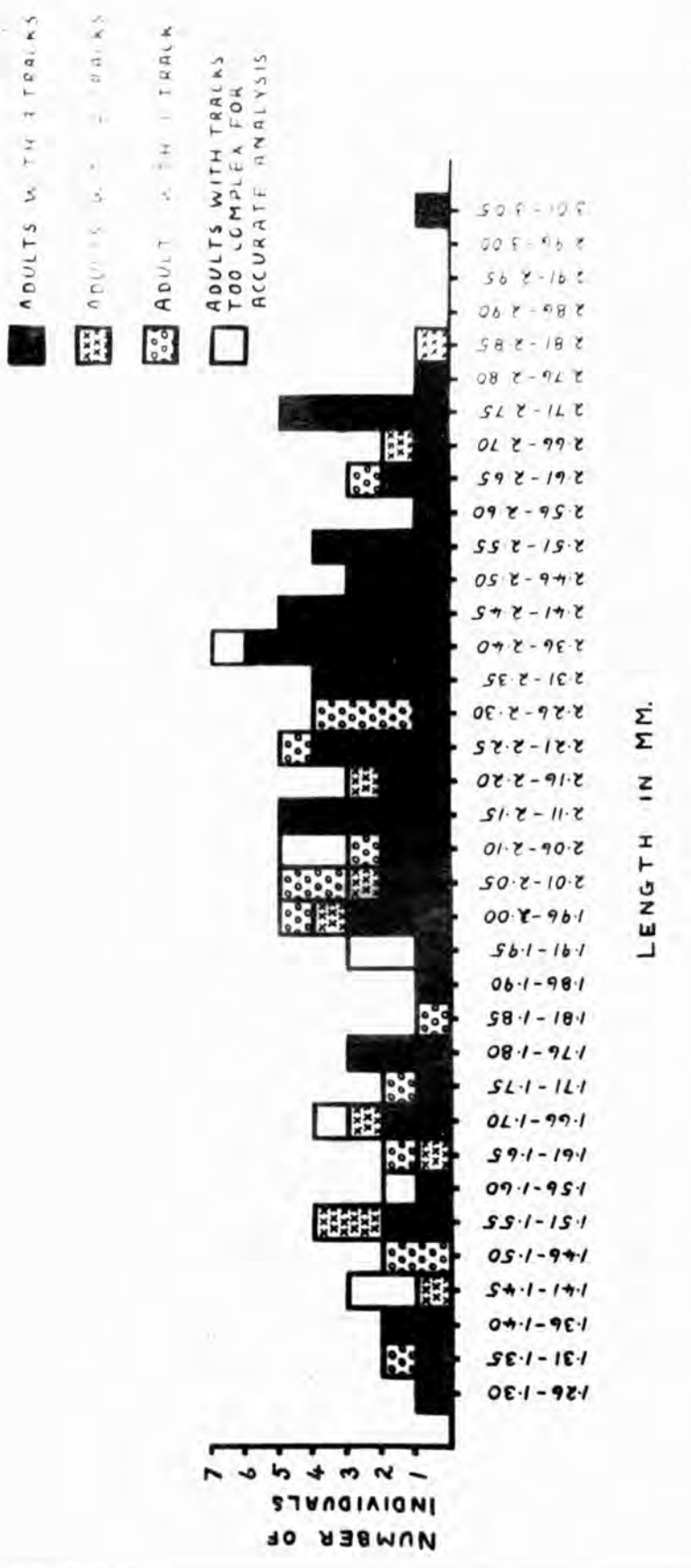

Fig. 36. The distribution, throughout the size range, of adults of C.hesperidum showing one, two or three stylet tracks.

range whilst the most significant fact, as discussed below (pages 132 to 134), is the presence of 2 and 3 tracks in individuals at the lower end of this size range.

It is of interest that the stylets of all the adults were found to be inserted in the plant, showing that they remain inserted during gestation and parturition, a fact noted also in Pulvinaria innumerabilis by Putnam (1876-78). Balachowsky (1937) states however, that the stylets of many species cease to function at the time of gestation due to the compression of the gut by the ovaries. It is possible therefore, that the fact that the stylets remain inserted in these mature adults is no proof that feeding is still taking place.

Moulting

The sizes at which moulting occurs within the larval and nymphal stages have been indicated.

The first visible sign that the individual is preparing to moult is the coiling of the styliform organs. This was taking place in 13 larvae varying in size from 0.72 x 0.36 mm. to 0.83 x 0.42 mm., and in nymphs varying in size from 1.22 x 0.63 mm. to 1.61 x 0.83 mm., the individuals being indicated by "C" in Tables 6 and 8 respectively. During this period the stylets are still inserted into the plant tissue.

The next stage in the moulting process that was seen was that in which the cuticle, still intact, was separated slightly from the body, the structure of the new cuticle being clearly

distinguishable beneath it (Tables 6 and 8, E). In both larvae and nymphs the general pattern was the same. The antennae and legs could be seen lying within their old cuticle which still surrounded each of these appendages. The new anal ring, with its accompanying setae, was seen to be anterior to the old one (Fig. 37), the tips of its longer setae being level with the old anal ring. Around the margin of the body, the new marginal setae could be seen beneath the old cuticle, an increase in the number of these setae at the time of moulting being noted. In each spiracular depression the three new spiracular setae were also visible, their number remaining constant in each respective instar. The new spiracular setae were colourless and considerably larger than the old ones which were a dark brown colour. The new stylets were seen coiled up in the head region (Figs. 37 and 38), the two on each side being separate along their entire length. It is possible that the new stylets are fully developed before the cuticle separates from the body, since one individual was found showing the new stylets complete although the new cuticular structures were not visible except for one of the new spiracular setae. In all these individuals, the old stylets were withdrawn from the plant and looped up in the crumena (Fig. 37). Thus when the moulting process has reached this stage, feeding has temporarily ceased. The degree of development of the new cuticle when the individual withdraws its old stylets is not known and is possibly not

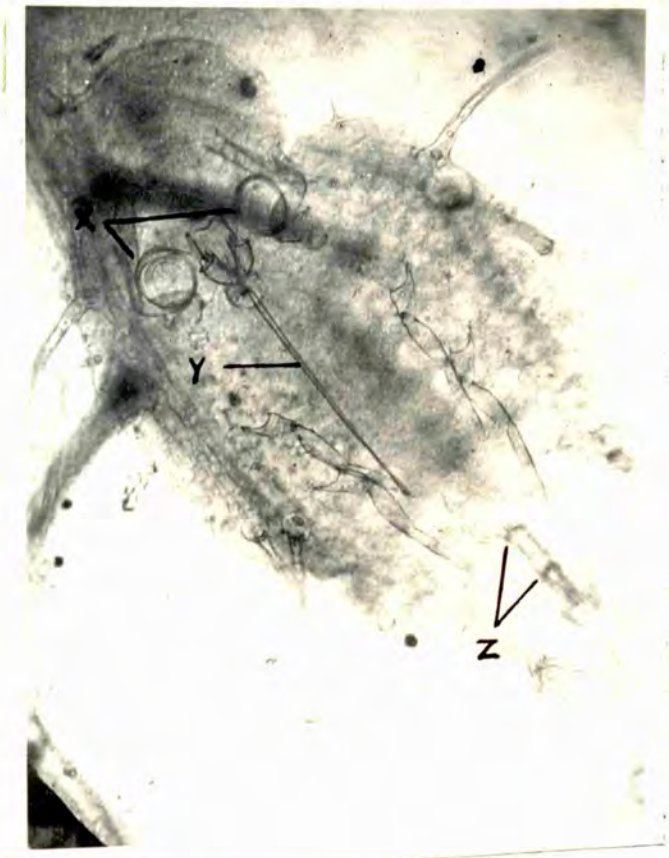

Fig. 37. Photomicrograph of a cleared specimen of a nymph of C.hesperidum prior to the shedding of the old cuticle (x 120).

The new stylets (X) can be seen coiled up in the head whilst the old stylets (Y) are looped up in the crumena. The old and new anal rings (Z) can also be seen.

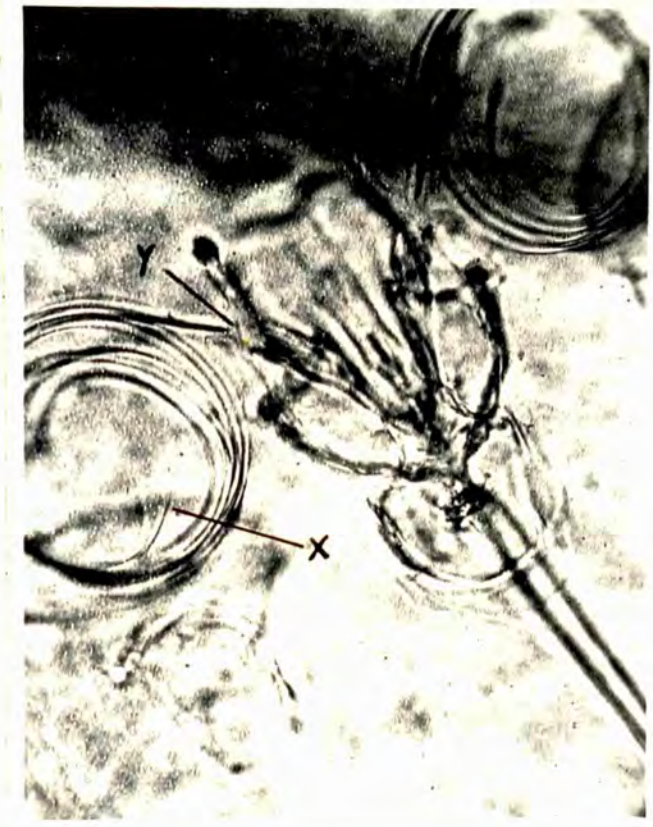

Fig. 38. Photomicrograph of the mouth parts enlarged from Fig. 37.

Note the enlarged base of a new stylet (X) situated in the centre of the coil whilst its tip (Y) is situated within the enlarged base of an old stylet.

the same for all individuals. One individual had withdrawn its old stylets although the new cuticle was not visible except for the new stylets and one spiracular seta. On the other hand, one individual in which all the new structures were visible as described above, had its old stylets still inserted into the plant. Therefore, although the old stylets appear to remain functional up to the time of moulting, a fact recorded for coccids by Pesson (1944), evidence regarding the actual time of their withdrawal from the plant was not given by the individuals examined.

The remaining individuals in which moulting was taking place showed the old cuticle in the process of being shed towards the posterior end of the body (Tables 6 and 8, MM), various stages in this process being found. The important feature of this process is centred round the stylets themselves. Prior to the cuticle being shed, the old stylets, as mentioned above, are withdrawn into the body and looped up within the crumena, whilst the new stylets lie coiled up in the head region. The bases of the new stylets are situated in the centre of the coil, whilst their tips are situated within the bases of the old stylets present in the tentorium (Fig. 38). As the old cuticle moves towards the posterior end of the body, the bases of the old stylets (Figs. 39 and 40, OS) retain their position within the old cuticle of the tentorium (OC) whilst the tips retain their position within the old cuticle of the labium (CL). Thus, the old

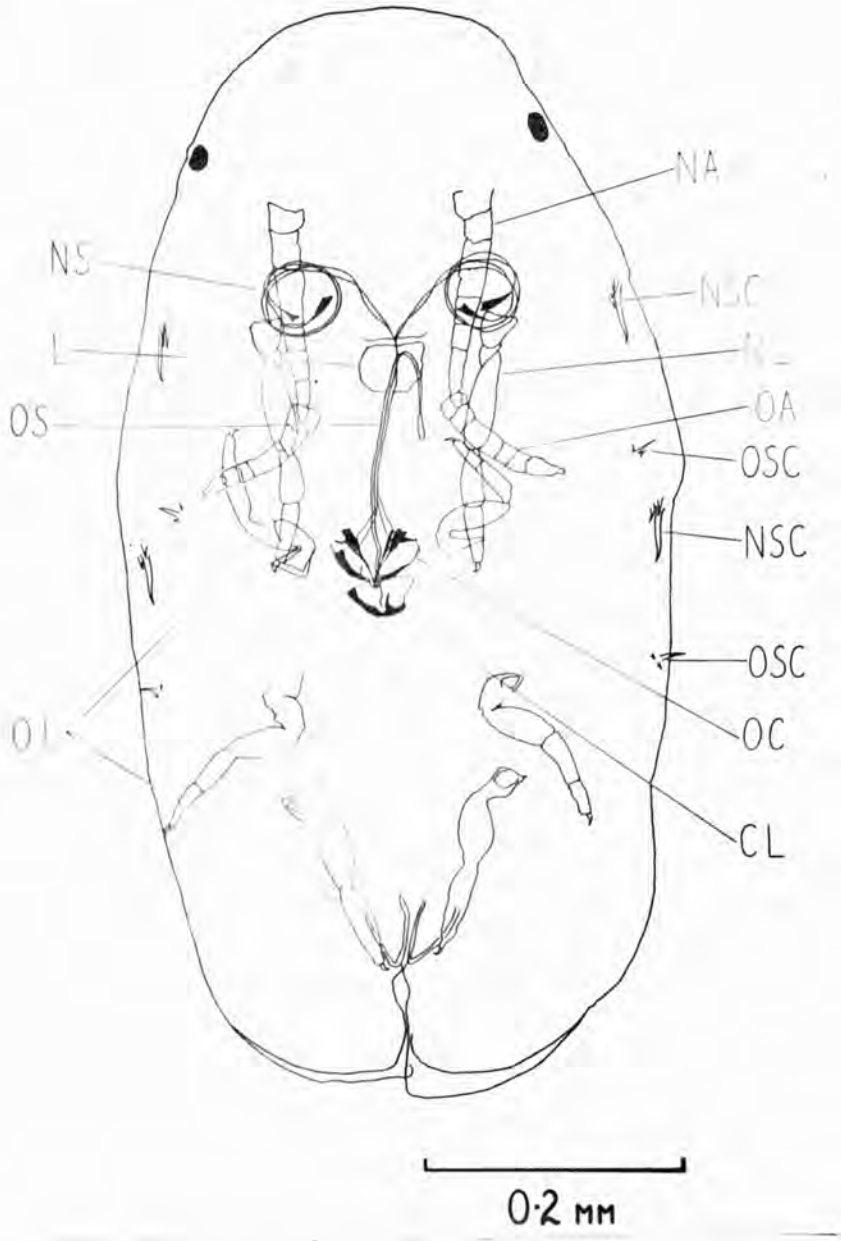

Fig. 39. Nymph of C.hesperidum showing an initial stage in the shedding of the old cuticle towards the posterior end of the body.

Note the old stylets (OS) being withdrawn in the looped condition through the labium (L) together with the new stylets (NS) and that the tips of the latter are still in contact with the bases of the old stylets situated in the old cuticle (OC) of the tentorium.

Other abbreviations:
Old cuticle - CL, labium; OA, antennae; OL, legs; OSC, spiracular setae.
New cuticle - NA, antennae; NL, legs; NSC, spiracular setae.

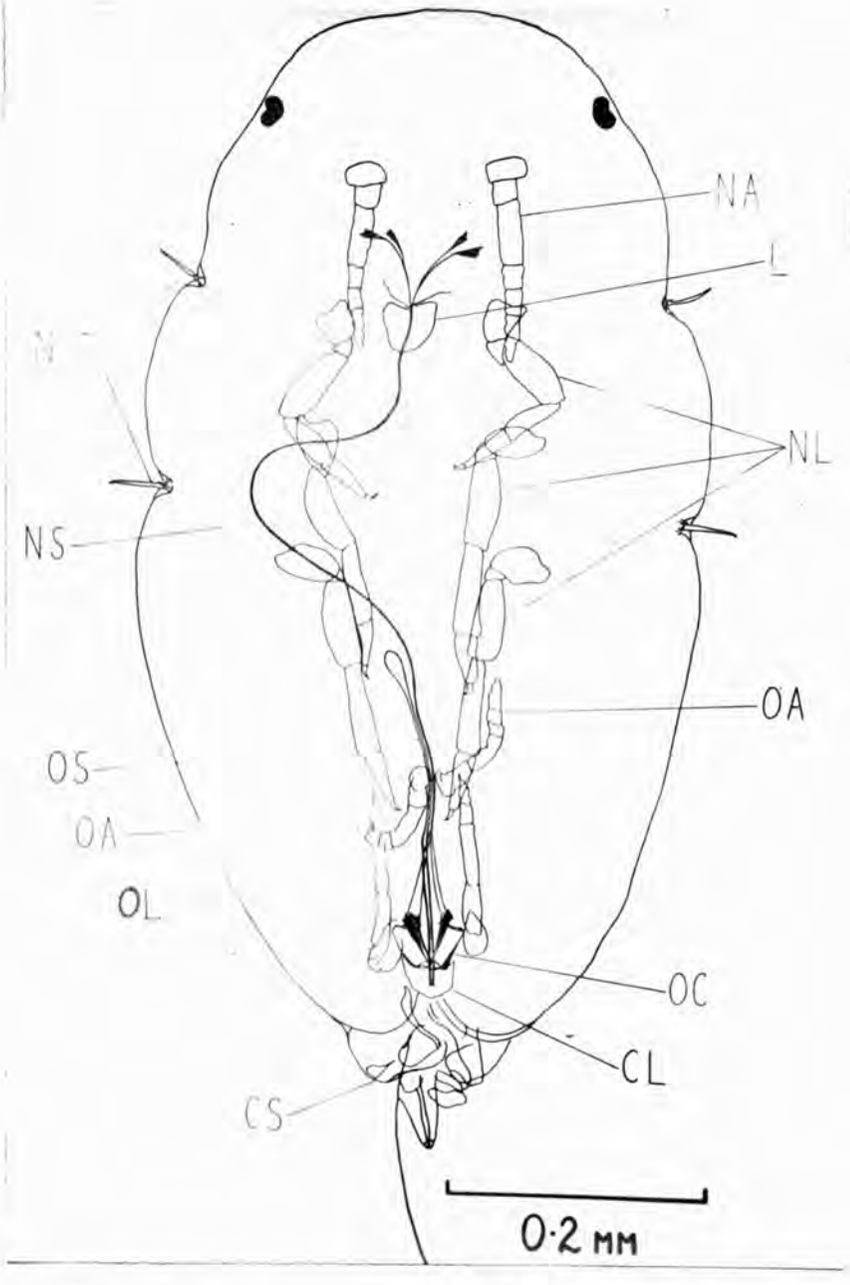

Fig. 40. Nymph of C.hesperidum showing a final stage in the shedding of the old cuticle.

Note the old stylets (OS) still in the looped condition and the new stylets (NS) extended outside the body with their tips still in contact with the base of the old stylets.

CS, cast skin. For other abbreviations see Fig. 39.

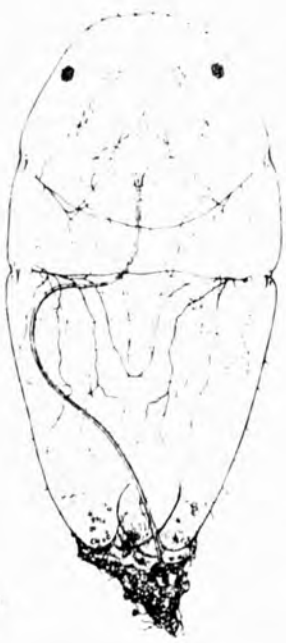

Fig. 41. Larva of C.hesperidum following the shedding of the embryonic membrane. (Reproduced from Dingler, 1923).

Note the stylets extended outside the body. (cf. Fig. 40.)

stylets, still looped up, are gradually withdrawn from within the body through the tip of the labium and trail behind the old cuticle of the tentorium and labium (Figs. 39 and 40). As the old cuticle moves towards the posterior end of the body, the new stylets (NS) uncoil from within the head region and are extended through the tip of the labium. Throughout this process, the tips of the new stylets remain within the bases of the old ones. Stages in this process were seen in the various individuals, although none were found showing the new stylets completely uncoiled and with their bases in their functional position within the tentorium. Outside the body, the new stylets could be seen lying alongside the loop of the old stylets (Fig. 40). All four components of the stylets were lying adjacent to each other, diverging at their tips to meet the bases of the old stylets.

No further stages in the moulting process were found, although an examination of the cast skins found on the plant revealed the old stylets still looped up and attached at their base and tips to the cuticle of the tentorium and labium respectively.

After moulting, the cast skins may sometimes fall free of the plant at once, although in most cases they remain in contact with the insect for several days and in some cases until the next moult. The presence of these cast skins attached to the posterior end of the body has been recorded by Dingler (1923).

DISCUSSION

The feeding tracks

It has been shown that within each instar, most individuals show a specific number of independent feeding tracks, the actual number being characteristic of that particular stage. In the larval stage it is one, in the nymphal stage two, whilst in the adult stage it is three.

Now, in both the nymphal and adult stage, it is seen that the individuals possessing the characteristic number of feeding tracks are not restricted to a particular part of the size range of that instar, but that young as well as old individuals show them (Figs. 31 and 36). The fact that young nymphs show two tracks and young adults show three tracks suggests that in both cases they were not all produced during the respective instar in which the individual was found.

From the study of the moulting behaviour it was seen that at the time of moulting the stylets are withdrawn from the plant and looped up in the crumena prior to the cuticle being shed. In the case of nymphs about to moult, and which had withdrawn their stylets from the plant, it was seen that two tracks of the stylet sheath passed from beneath the labium into the vein, whilst in the case of larvae about to moult a single track was present.

When the presence of two tracks in the young nymphs is considered in the light of these facts, it suggests that the track adjacent to the stylets themselves was produced during

the larval stage. This conclusion is supported by the fact that this adjacent track is narrower than the one containing the stylets. This difference in width of the tracks is seen to occur in all nymphs possessing two tracks and it is logical to assume that in all these individuals, the salivary sheath track was produced during the larval stage.

In the case of the young adults showing three tracks it follows that the two adjacent to the stylets were present during the nymphal stage and that one of them was in fact produced by the larva. This is again supported by the difference in width of the three tracks. Since all the adults with three tracks show the same difference in width of the respective tracks, it indicates that in all cases one of them was produced by the larva, one by the nymph and the third by the adult itself.

These facts show that amongst the individuals examined there was a marked tendency to remain in the same position on the leaf from the larval to the adult stage. At the end of the larval stage the individual ceases to feed, withdraws its stylets from the plant and undergoes a moult. The nymph then inserts the new stylets into the plant alongside the path made by the old ones. From the study of the tracks in the adults it can be seen that during the nymphal stage the stylets may be partially withdrawn from time to time, and then inserted elsewhere in the vein, but they are never completely removed from the plant. At the end of the nymphal stage

however, feeding again ceases and the stylets are withdrawn into the crumena. The individual moults and the young adult inserts the new stylets into the plant alongside the tracks made by the larva and nymph respectively. The close proximity of the epidermal penetrations of each respective instar shows that the movement of the labium with respect to the epidermis at the time of a moult is negligible.

Balachowsky and Mesnil (1935) and Fonseca (1954) record that although the larvae settle on the leaves, the nymphs and adults migrate to the twigs at the approach of parturition, and a similar type of behaviour has also been recorded for Eulecanium corni and E.persicae (Balachowsky, 1932 (2)) and E.nigrofasciatum (Simanton, 1916). The fact that some individuals of C.hesperidum mature on the leaves has been noted however by Quayle (1932), Batchelor and Webber (1948) and Fonseca (1953). With the aid of the technique used in the present study, it is possible to show that not only is there a tendency to mature on the leaf but actually in the position chosen by the larva. It is essential to stress however, that this technique allows only those individuals that were on the leaves at the time of the investigation, to be studied and that this might in fact be only a small proportion of the initial larval population of the leaf.

Although the tracks produced by the respective instars show branching in some cases, indicating the change in position

of the stylets within the vein, there are other individuals in which one or other of the tracks, and in some cases all the tracks, show no sign of branching. In the case of the nymphs, many of the older individuals show the stylet track itself devoid of branches (Fig. 32). This indicates that the initial penetration, following the larval moult, contacted a source of food which was adequate to provide the nymph with nourishment during the major part of the instar. It is further seen that in many of the adults, the track produced by the individual during the nymphal stage is devoid of branching (Table 11), showing that this food source can suffice for the entire nymphal stage without the stylets being moved elsewhere within the vein. The stylet track itself in many of the older adults, also shows no sign of branching (Table 11), indicating that the adults can also contact a continuous food source, following the nymphal moult, so that there is no need for the stylets to be placed elsewhere. This behaviour of the stylets to remain in one position during an entire instar, suggests in itself, apart from the confirmation given in Section II, that the food source is the phloem since this would provide a continuous supply. The fact that some tracks show branching suggests either that the individual failed to penetrate the phloem at its first insertion, or else that the part of the phloem providing the food became blocked for some reason, thereby cutting off the supply of food.

It has been seen that some of the nymphs possess only a

single track of the stylets themselves and therefore must have shown rather different behaviour. It is clear that such nymphs have, for some reason, moved from the position at which moulting occurred so that the track produced by the larval stage is no longer present alongside the stylets. Amongst the adults, there are two groups which show less than three tracks. Those individuals showing only one track alongside the stylets, instead of two, clearly indicate that, during the nymphal stage, they have changed their position on the plant so that the track produced by the larva is not present. The adults with only the stylet track itself present, show that a change of position has taken place during the adult stage itself, since the tracks produced by both larva and nymph are absent. This ability of the adults to change their position on the plant has been recorded by Hubbard (1885), Berlese (1894), Bodenheimer (1951) and Fonseca (1953). Further details concerning the movement of the adults, together with experimental support for the origin of the tracks present in both the nymphs and adults, is given in Section VI.

The behaviour of the stylets in the larvae

In the study of the larval tracks there was seen to be considerable individual variation in the behaviour of the stylets within the leaf tissue. Unlike the nymphs and adults, there is no indication as to how long the larvae have remained in one position on the leaf, so it is not possible

to say whether these tracks have served to provide the larvae with food since the time they left the broodspace. It would be of interest to know this in the case of those individuals in which the tracks do not penetrate the vein, especially the older larvae showing this behaviour, since it would indicate that the latter had utilised the mesophyll as a food source. There is also no indication as to whether the individual larva showed the same behaviour in each feeding position as it shows in its last one, so it is possible that those larvae not showing penetration of the vein when examined, had in fact penetrated it elsewhere on the leaf.

The fact that some larvae direct their stylets straight into the vein, whilst others show a branching or meandering path indicative of trial and error, and others even fail to penetrate the vein at all, suggests an individual variation in the perception of the stimulus guiding the stylets to the phloem. Whether this is due to a variation in the strength of the stimulus itself or a variation in the ability of the larvae to perceive the stimulus is not known. It was suggested in the discussion at the end of Section II that the stimulus might be a tactile one, originating from the vein alongside which the insect settles, and which is in contact with only one side of the body. Since the width of a larva is small in comparison with the width of a vein, the intensity of such a stimulus would be less than in the case of the nymphs and adults and an undirected path by the stylets

would be expected. Now it has been seen that the paths of the stylets in the larvae are more erratic in the majority of cases than they are in the larger individuals (nymphs and adults). However, a direct path into the vein is shown by 31% of the larvae, some of which are at the lower end of the size range (Fig. 28, 1). The behaviour of these larvae showing a direct path into the vein, therefore shows that the stimulus guiding the stylets to the vein can be detected by the larvae in some cases, regardless of their size, and therefore throws doubt upon the theory of a tactile stimulus.

Moulting

It has been shown that when ecdysis occurs, the old cuticle is shed towards the posterior end of the body, the actual movement of the cuticle being attributed to the action of the legs by Dingler (1923). No personal observations were made on this point, although it is of interest to note that in Aleyrodes vaporariorum, in which the cuticle is also shed towards the posterior end of the body, the movement of the cuticle is attributed mainly to the alternate contraction and expansion of the abdomen, whilst the legs play only a minor role (Hargreaves, 1914-15).

One of the most interesting features of moulting concerns the stylets, owing to their relationship to feeding and also to the great length of the stylets themselves. It has been shown that the old stylets remain inserted in the plant up to the formation of the new cuticle beneath the old one; the

fact that the stylets of coccids remain functional up till the time of moulting has been recorded previously by Pesson (1944). Prior to the shedding of the cuticle, the old stylets are withdrawn from the plant and looped up in the crumena. They are then shed, in the looped condition, along with the rest of the cuticle. The fact that the stylets are retracted prior to moulting was suggested by Dingler (1923) who observed that connection with the plant by means of the stylets would obstruct moulting.

Pesson (1951 (4)), in his general account of the formation of the new stylets in coccids, states that as the old cuticle is shed, the styliform organs shorten and the bases of the new stylets are thereby brought into position within the tentorium, the stylets passing directly to the exterior and being looped in the crumena only after their retraction. It has been shown that in C.hesperidum, as the old cuticle moves towards the posterior end of the body, the new stylets uncoil from within the head and are extended through the tip of the labium, the tips of the new stylets remaining within the bases of the old stylets. It would appear therefore, that in the case of C.hesperidum, the process described by Pesson is aided by the old cuticle which, as it is pushed towards the posterior end of the body by the legs, withdraws the new stylets from the head. This function of the old cuticle appears, at the time of hatching, to be carried out by the embryonic membrane which is likewise shed towards the

posterior end of the body. Personal observations made upon the larvae, following the shedding of these membranes, have revealed the new stylets fully extended and attached to the membrane situated at the posterior end of the body. The similarity between moulting and hatching, with regard to the extension of the new stylets, is seen by comparing Figs. 40 and 41. Heriot (1937) has recorded a similar behaviour in Adelges abietis at the time of hatching, the new stylets being fully extended and attached to the old egg shell. The presence of a loop along the length of the fully extended stylets during both moulting and hatching in C.hesperidum, suggests however that the extension of the new stylets is not wholly dependent, at least during the latter stages of their extension, upon a "pulling" effect exerted by the old cuticle or embryonic membrane.

Heriot (1937) states that, unlike aphids, coccids do not extend their stylets during hatching. He maintains that the stylets pass direct from the rolled condition in the head into the labium and are then doubled back into the crumena. In Pulvinaria innumerabilis, the fact that the stylets are not extended during hatching has also been recorded (Putnam, 1876-78). This behaviour is certainly not the case in C.hesperidum, whilst Pesson (1944) states that the stylets of coccids in general are extended at the time of hatching. Pesson also states however (1944), that the extension of the stylets occurs prior to hatching, the stylets passing between

the body and the embryonic cuticle. This behaviour certainly precludes the possibility that the shedding of the embryonic membrane aids the extension of the stylets. Although it appears that in C.hesperidum the shedding of the embryonic membrane does aid the extension of the stylets, there is no conclusive proof that the two processes occur together as in moulting.

In moulting it is clear that, in the case of C.hesperidum at least, the tension within the mandibles would necessitate the attachment and guidance of their tips during their initial extension, since it is difficult to see how saliva could be present between the maxillae and mandibles at this stage. Apart from the tension within the mandibles, some form of guidance during their initial extension would appear to be necessary to direct them through the labial gutter and also to prevent them being damaged outside the body.

Although the shedding of the old cuticle towards the posterior end of the body occurs also in Ehrhornia cuppressi (Herbert, 1920), Eulecanium nigrofasciatum Pergonde (Simanton, 1916) and Aleyrodes vaporariorum (Hargreaves, 1914-15), it is difficult, in view of the above considerations in C.hesperidum, to account for the extension of the stylets in those species in which the moulting behaviour precludes the possibility of guidance by the old cuticle. In Physokermes insignicola for example, the skin splits along the medial dorsal line and the two halves remain attached to the sides of the body (Moulton,

1907) whilst in the female lac insect, Laccifer lacca Kerr., the cast skin of the first stage larva is found at the anterior end of the body (Misra, 1930). In the diaspids the old stylets remain in the plant (Pesson, 1944) whilst Baranyovits (1953) states that in Aonidiella aurantii the new stylets are first inserted into the plant and then the old skin is shed, the dorsal surface being incorporated into the scale, the ventral surface remaining beneath the individual and the old stylets remaining within the plant.

A further point of interest, in connection with the moulting of C.hesperidum, is the fact that since the new stylets are first extended prior to being looped into the crumena, they must be retracted into the latter before they can be inserted into the plant. Therefore their tips must become detached from the bases of the old stylets to enable this to occur and at this stage the mandibles would be free to coil up. Such coiling by the mandibles would not only make retraction difficult but would possibly damage the stylets. It is difficult to understand how the insect prevents the coiling. It is possible that saliva is secreted via the tips of the maxillae prior to retraction and that this saliva diffuses between the maxillae and mandibles. Heriot (1937) has recorded normal retraction in the case of Adelges abietis following the extension of the stylets during hatching. It is possible however, that in the case of C.hesperidum, the ventral surface of the body, closely applied against the

surface of the plant, holds the mandibles in position and prevents them coiling backwards.

SECTION VI

Experiments on the movements of the adults and the origin of their feeding tracks

INTRODUCTION

From the study of the feeding tracks and the moulting behaviour, described in Section V, it was possible to obtain some information concerning the movement of the different instars. The origin of the 3 independent tracks in the adults was attributed to the larval, nymphal and adult stage respectively, indicating that the individual had remained in the same position on the leaf from the larval to the adult stage. To verify these conclusions, it was necessary to make direct observations on the movement of the insect throughout its life-cycle, and follow it by an examination of the feeding tracks at the end of the life-cycle (Experiment 1). The tracks could then be related to the time which the individual was known to have remained in its final position.

The fact that the ability to move is retained by the adults has been shown by the presence of a single track in some individuals. Hubbard (1885), Bodenheimer (1951) and Fonseca (1953) however, state that movement is only possible until the broodspace is formed, a fact noted also by Dingler (1923). Since the body of the adult eventually provides a covering, in the form of the broodspace, for the larvae deposited on to the surface of the plant, it would appear that movement at this stage would no longer occur. Berlese (1894) however, notes that the mature female can move even when the broodspace is actually present. Since the presence of a single track in the mature adults gives no information

on this point, the following observations on the movement of the adults were made (Experiment 2).

TECHNIQUES

Experiment 1:

Two seedlings of the Rough Lemon, approximately 12 inches high, were used as the host plant. Six leaves on each plant were labelled by means of small tags attached to the petioles. Active larvae were removed from the broodspace of mature females with the aid of a fine camel hair brush, and one larva placed on each of the selected leaves. The petioles of the latter had previously been covered with vaseline to restrict the insect to the leaf itself. The plants were kept in a greenhouse during the experiment and the temperature and relative humidity recorded automatically by means of a Casella thermohygrograph. The average temperature throughout the experiment was 27°C. On some days there was an increase in temperature in the mornings lasting approximately 2 hours. Although this increase usually varied between 28° and 32°C, on two days it rose as high as 34° and 35°C. The average relative humidity was 64, with a range of 54 to 74. There was an additional reduction, to as low as 50, coinciding with the sudden rise in temperature in the mornings. Observations were made every hour during the first 9 hours although they were subsequently restricted to one per day.

Throughout the experiment, the position in which the insect settled on the leaf was marked on the leaf by means of 2 small

spots of Indian ink placed one on either side of the insect, slightly away from the latter. The presence of the ink was found to have no effect on the subsequent movement of the insect. Changes in position were noted and the appearance of a cast skin at the posterior end of the body, indicating a moult, was also recorded.

The experiment was concluded on the 69th day when the insects were fully mature and larvae present within their broodspace. The piece of leaf containing the adult was then cut out of the leaf, treated as for the whole mounts in Section V, and the tracks examined.

Experiment 2:

Healthy uninfected leaves of the Rough Lemon were detached from the plant. A small wad of cotton wool was wrapped round each petiole, tied in position, and soaked in saline solution to keep the detached leaves in a fresh condition. Leaves, on which were adults in various stages of development, were cut up into small pieces approximately 1 cm. square, each containing one adult. This ensured that the part of the leaf on which the insect was feeding would dry up quickly and induce the insect to move, if possible, to a new feeding position. The detached leaves were then placed in the bottom of a dish, the pieces of leaves containing the adults placed on their surface, and the dish placed in the greenhouse. At hourly intervals, those adults which had moved and re-settled on the healthy leaves were removed and examined.

The dorsal and ventral surface of the abdomen was examined for pigmentation and signs of a broodspace. The adult was then placed on a slide with its ventral surface uppermost, a cover-slip placed over it, and the contents of the body examined under a microscope for the presence of eggs and embryos. The extension of the stylets was taken as an indication that feeding had re-commenced or was about to re-commence.

The experiment was continued until the pieces of leaves containing adults were hard and brittle and a further two days had elapsed with no further movement of adults from them. The adults remaining on the pieces of leaves were then examined as described above.

RESULTS

Experiment 1:

The observations recorded on each insect are shown in Table 12.

The larvae are quite active during the first day, frequently changing their position on the leaf. Although they were all found to be settled one hour after being placed on the leaf, subsequent observations revealed them in a new position, either settled in this new position or else actually wandering on the leaf surface. The duration of activity of the larvae varied with the individual and ranged from 4 to 9 hours. In the case of 5 individuals (on leaves 3, 6, 9, 10 and 11), the position on the leaf at

Table 12. Observations on the behaviour of C.hesperidum throughout its life cycle.

Time	Plant I					Plant II						
	Leaf 1	Leaf 2	Leaf 3	Leaf 4	Leaf 5	Leaf 6	Leaf 7	Leaf 8	Leaf 9	Leaf 10	Leaf 11	Leaf 12
1st day 10.00a.m.						Larvae placed on leaves						
11.15a.m.	Settled	Settled	Settled	Settled	Settled	Settled	Settled	Settled	Settled	Settled	Settled	Missing
1.15p.m.	Settled+			Wandering			Missing		Wandering			
2.15p.m.	Wandering		Settled+	Wandering		Settled+		Settled+	Settled		Settled+	
3.15p.m.	Wandering	Wandering		Missing				Wandering		Wandering		
4.15p.m.	Missing	Wandering	Wandering		Wandering			Wandering	Wandering	Settled	Settled+	
5.15p.m.		Wandering	Settled		Wandering			Settled	Wandering			
7.30a.m.		Wandering			Settled				Settled			
8.30a.m.		Wandering										
9.00a.m.		Settled										
2nd day		Settled+										
3th day		Settled+			Settled+							
10th day								*				
11th day								Settled+				
13th day											*	
18th day					*	*						
23rd day										*		
25th day								*				
28th day					*							
32nd day			*			*						
50th day		Settled+										
69th day												
Number of tracks from surface of leaf, in final position	-	1	5	-	3	3	-	2	3	3	3	-

*: Moulting
Settled+; settled in new position
Changes in position only are noted.
Lack of entry indicates position of insect is as previously recorded.

the end of this period was kept for the remainder of the life-cycle. The larva on leaf 5 however, moved to a new position on the 5th day but did not move again after this. The individual on leaf 8, changed its position on the 11th day whilst in the nymphal stage. The individual on leaf 2 was more active than the others, changing its position on the 2nd, 5th and 50th day. The moults of this individual were not recorded, owing to cast skins not being observed, and therefore it is difficult to relate these changes in position to a definite stage in the life-cycle. By comparison with the moulting of the other individuals however, the change in position on the 5th day occurred during the larval stage, whilst that on the 50th day occurred during the adult stage.

The number of independent tracks, shown by each individual at the end of the experiment, is also shown in Table 12. Those individuals which remained in the same position on the leaf since the early part of the larval stage (on leaves 3, 5, 6, 9, 10 and 11) show three tracks. The individual which changed its position during the nymphal stage (on leaf 8) shows only two tracks, whilst the one that moved during the adult stage (on leaf 2) shows only one track.

Subsequent attempts to repeat this experiment failed owing to the insects dying or being lost during the course of the experiment.

Experiment 2:

A total of 36 adults moved onto the detached leaves

Table 13. The stage of sexual maturity of adults of C.hesperidum and their tendency to move.

Eggs within body	Embryos within body	Dorsal pigmentation present	Brood-space present	Larvae present in brood-space	Number of adults		
					Moved		No movement
					Feeding	Not feeding	
-	-	-	-	-	8	2	
+	-	-	-	-	14	9	
+	+	-	-	-	1		
+	-	+*	+*	-	2		
+	+	+	+	+			10

* The degree of development of the brood-space and dorsal pigmentation in both individuals was very slight.

while 10 adults remained on the dried pieces of leaf. The stage of maturity of these 46 adults is shown in Table 13.

Most of those which had moved resembled nymphs in shape and colour, no sign of pigmentation on the dorsal or ventral surface of the abdomen being visible. Ten of these adults showed no sign of eggs or embryos within the body, 23 showed eggs alone, whilst only one showed both eggs and embryos. Only 2 adults found on the leaves showed slight pigmentation on the dorsal and ventral surface of the abdomen, indicating the beginning of the development of the broodspace. These individuals contained eggs although no sign of embryos was present.

The 10 adults remaining on the dried pieces of leaf at the end of the experiment were all found to possess a well developed brood-space with larvae in it. The dorsal surface of the abdomen was also darkly pigmented. All these individuals were found to be still alive.

DISCUSSION

The results of experiment 1 show that after the initial period of wandering, which occurs during the first few hours after leaving the brood-space, 6 specimens settled in a position which was retained for the remainder of the life-cycle. In this one position these individuals underwent 2 moults during each of which they are known (Section V) to withdraw their stylets completely from the plant. Such individuals, at the end of the life-cycle, show 3 independent tracks within

the plant tissue. Therefore one of these tracks was made by the larva, another by the nymph and the third, containing the stylets themselves, by the adult.

The individual (on leaf 8) which changed its position during the nymphal stage underwent a single moult in its final position. This specimen showed only 2 tracks at the end of the life-cycle, one of which must have been made by the nymph and the other by the adult.

Finally, the individual on leaf 1 changed its position in the adult stage and showed only one track.

Therefore, the study of the feeding tracks of the above individuals, based upon a knowledge of the time spent in the position of these feeding tracks by the individual and the behaviour of the stylets at the time of moulting, confirms the suggested origin of the respective tracks. Although the number of specimens is small, the results indicate that the conclusions in Section V, based upon the study of the tracks alone, are correct.

The results of experiment 2 show that the adults are still capable of moving up to the time when the development of the broodspace begins, even though eggs and embryos may be present within the body, a fact recorded also for Pulvinaria innumerabilis (Putnam, 1876-78). It would appear however, that movement ceases, or is no longer possible, when the broodspace is fully developed and young larvae are present within it.

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* Footnote: References marked with an asterisk could not be traced in the "World List of Scientific Periodicals", and are consequently given their full or an abbreviated title.