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Feeding Sites of Some Leaf- and Planthopper Insects (Homoptera: Auchenorrhyncha) Associated with Coconut Palms¹

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In the past three decades more than 50% of Florida's estimated one million coconut palms (Cocos nucifera L.) and over 80% of Jamaica's 4.3 million coconut palms have been killed by a pandemic disease called lethal yellowing (LY) (McCoy et al. 1983). Another economically important palm, the date (Phoenix dactylifera L.), has also been affected by LY. In all, more than 32 species of palms have been affected by LY (McCoy et al. 1983). These susceptible species represent 22 genera from eight tribes in three subfamilies of the palm family according to the classification of Uhl and Dransfield (1987). The disease is caused by mycoplasma-like organisms (MLO), microbes that cannot be cultured (McCoy et al. 1983). The MLO reside in the phloem, that part of the plant's vascular tissue which transports carbohydrates. The MLO are presumably transmitted by a planthopper insect, the American palm cixiid, Myndus crudus Van Duzee (Howard and Thomas 1980) which feeds on the phloem. However, unequivocal evidence that proves *M. crudus* is the sole vector of LY is lacking (Tsai 1980). Some leafhoppers and planthoppers (insects belonging to the order Homoptera: Suborder Auchenorrhyncha) feed on the MLO

harboring phloem. Other related species feed on the xylem, the water-conducting vascular tissue in the palm. Our study was designed to document the feeding sites of seven species of leafhoppers and planthoppers, which are associated with palm groves in south Florida, in order to further identify possible vectors of LY. We examined feeding on coconut and Veitchia palms [Veitchia merrillii (Becc.) H. E. Moore], which are susceptible to LY, and also periwinkle [Catharanthus roseus (L.) Nees] and St. Augustine grass [Stenotaphrum secundatum (Walt.) Kuntze], which are alternative food sources for the same insects.

Materials and Methods

Leafhoppers included in this study were:

- Spangbergiella vulnerata (Uhler) [Homoptera: Cicadellidae]
- Graminella sonorus (Ball) [Homoptera: Cicadellidae]
- Macrosteles fascifrons (Stal) [Homoptera: Cicadellidae]
- Oncometopia nigricans (Walker) [Homoptera: Cicadellidae]

Planthoppers tested in this study were:

- Myndus crudus (Van Duzee) [Homoptera: Cixiidae]
- Peregrinus maidis (Ashmead) [Homoptera: Delphacidae]

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Treehopper tested in this study was:

Idioderma virescens (Van Duzee) [Homoptera: Membracidae]

Spangbergiella vulnerata was collected from the St. Augustine grass. Both nymphs and adults of Myndus crudus (=Haplaxius crudus) were reared in the laboratory as described previously (Tsai et al. 1976); Peregrinus maidis adults were reared in the laboratory (Tsai 1975); Graminella sonorus adults were reared on rye (Secale cereale L.) (Bradfute et al. 1981); Macrosteles fascifrons adults were reared on rye and oats (Avena sativa L.) (Smith et al. 1981); Oncometopia nigricans adults were reared on leaf lettuce (Lactuca sativa L.) (Tsai and Anwar 1977); and Idioderma virescens adults were reared on the saw palmetto [Seronoa repens (Bartram) Small] (Kopp and Tsai 1983). Test insects were placed in cages containing the leaves and roots of St. Augustine grass, coconut palm, and leaves of Veitchia palm and periwinkle.

Plant parts fed on by the test insects were marked and excised. The excised tissues were fixed in FAA (formalin-alcoholacetic acid), embedded in paraffin, serially sectioned at 10 μ m increments, and stained with safranin and fast green (Sass 1958). The salivary sheaths secreted by the leafhoppers and planthoppers during feeding were examined under a light microscope and photographed using a green filter.

Results and Discussion

When leaf- and planthoppers feed on plant tissues, they puncture the leaf, stem, or root with their delicate sucking mouth parts. An elongated stylet is pushed into the plant, and the insect probes the internal tissues of the plant seeking the appropriate vascular tissue to feed on. During the process of probing and feeding, the insect secretes a proteinaceous gel, called the sheath, around the stylet. The sheath remains after feeding is terminated forming a visible feeding track (Houston et al. 1947, Day et al. 1952, Smith 1933). The surface of the feeding puncture is marked by a raised droplet of sheath material on the plant surface known as the labial imprint (see Figs. 6,9,12) (Waters 1977). The sheath stains with safranin so that the labial imprint and feeding track can easily be seen in stained sections of the plant tissue.

The insect can sense the proximity of the vascular tissue from which water and nutrients are sucked (Sogawa 1973). Xylem feeders tend to feed on the water conducting part of the plant's vascular system. Phloem feeders make use of the carbohydrate or food conducting region. In small veins of a leaf, the phloem is oriented toward the lower surface (abaxial), and the xylem is toward the upper surface (adaxial). All figures are shown with the upper leaf surface toward the top of the page. The midrib vein is more complex with xylem and phloem in several separate regions (see X and P in Fig. 13). In a root, xylem and phloem strands alternate around the periphery of the vascular core or stele, just inside a distinct, thick-walled layer, the endodermis (see X and P in Figs. 4,5).

By following the feeding track in serial sections, the pathway of the insect's stylet can be reconstructed, and the plant tissues that are affected can be distinguished by microscopic examination. However, the course of the feeding track only shows where the stylet was positioned and not necessarily where the insect fed. The actual site of feeding could not be distinguished from test probes (for example, Fig. 10). Feeding tracks often show that stylet probes never reach vascular tissues and, therefore, mark unsuccessful attempts by the insect to locate a feeding site. Insects were fed on the upper and lower surfaces of the leaf in these cage experiments. Such artificial environments do not necessarily reflect the natural, preferred feeding orientation of an insect. Brief descriptions of the feeding tracks for seven insects are given below.



1. Coconut leaf blade with feeding track of *Myndus crudus* ending in phloem of a small vein. 2. Coconut leaf with feeding track of *M. crudus* passing through xylem and ending in phloem of a large vein. 3. Coconut leaf with several feeding tracks of *M. crudus* from both surfaces ending in a large, longitudinally cut vein. 4,5. Adjacent sections of a coconut root with feeding track of *M. crudus* that branches after entering the vascular core; limits of the feeding track are shown by arrows. 6. Detail of same feeding track entering phloem region of root. E, endodermis layer; F, feeding track; L, labial imprint; P, phloem; V, vein; X, xylem. Scale bar = 40 μ m for Figs. 1-3, 6, 7; and 100 μ m for Figs. 4, 5. Scale bar = 30 μ m in Fig. 8.

Myndus crudus. On coconut leaves, many tracks ended in the smallest veins of the leaf which contained mostly or only phloem tissue (Fig. 1). The diameter of the feeding track often equaled that of the narrowest veins so that all vascular regions of those veins were included within the sheath boundary. In a few larger veins, the track clearly terminated in the phloem region, either bypassing or traversing the xylem (Fig. 2). There were often multiple feeding tracks originating from a single epidermal puncture marked by a lone labial imprint. Sometimes a track went all the way through the leaf to the opposite surface. In one case, a single vein was reached by separate tracks from either surface of the leaf (Fig. 3).

In coconut roots, feeding tracks went directly from the surface (Fig. 6), through the fleshy cortex, and either ended blindly or reached the lignified endodermis. In some cases several probes branched out from this point to follow the outer surface of the endodermal layer or, in one case, the pericycle layer immediately within the endodermis (Figs. 4,5), with one probe bent inward and terminated at one or more of the peripheral phloem sites (Fig. 8). In one case, the feeding track went in a straight line directly to a phloem site (Fig. 7).

In periwinkle leaves, the veins were about half as narrow as those of coconut so that the diameter of the feeding track was larger than the veins. Feeding tracks passed through veins, but it was not possible to determine which vascular tissue was at their termini.

In all *M. crudus* feeding tracks, the sheath material either filled the cell lumen or, in large parenchyma cells, formed a tube within the cell. The feeding track passed through cells (intracellular) and not between cells (intercellular).

Peregrinus maidis. In coconut leaves, many tracks ended without reaching vascular tissue. In the smallest veins, the sheath material completely filled the veins which consisted of mostly phloem and perhaps one or two narrow xylem elements. In larger veins, feeding tracks ended in the phloem and often passed through the xylem (Fig. 9). In one large vein, feeding tracks clearly terminated in defined phloem sites. In another vein, a feeding track had multiple short probes at its tip in which the sheath material filled some xylem vessels and xylem parenchyma and terminated in the phloem (Fig. 10). The sheath material was intracellular.

Graminella sonorus. In coconut leaves, feeding tracks reached small veins and often entered from the side of the vein. The sheath material ended either in the phloem of the smallest veins or in the phloem/ xylem boundary of larger veins. In this latter case, both xylem and phloem contained sheath material. Many feeding tracks ended in the intervascular leaf mesophyll. The sheath material was intracellular.

Macrosteles fascifrons. In coconut leaves, the feeding tracks followed the same pattern as in G. sonorus. One large vein had a branched track that terminated in three distinct phloem sites. Conversely, some tracks terminated in the xylem of smaller veins or at the xylem/phloem boundary in larger leaf veins (Fig. 11). The sheath material was intracellular.

Oncometopia nigricans. In coconut leaves, the feeding track was relatively massive and disrupted much of the mesophyll tissue. The sheath diameter was larger than the largest hypodermal parenchyma cells and the small veins. Many smaller veins were completely cut by probes that continued through the leaf (Fig. 12). There was often a cavity present in the center of the track. Feeding tracks commonly

^{9.} Coconut leaf blade with feeding track of *Peregrinus maidis* passing by xylem vessel and ending in phloem of a large vein. 10. Coconut leaf with multiple feeding tracks of *P. maidis*, one of which ends in phloem of small vein. 11. Coconut leaf with feeding track of *Macrosteles fascifrons* passing by xylem vessel and ending at the xylem/phloem boundary of a large vein. 12. Coconut leaf with feeding track of *Oncometopia nigricans* which passes through a small vein. 13. *Veitchia* leaf at midrib with feeding tracks of *Idioderma virescens*; one passing through blade and another (arrow) entering midrib. 14,15. Details of dark feeding track of *Spangbergiella vulnerata* entering the side of a vein at the xylem/phloem boundary. F, feeding track of *Spangbergiella vulnerata* entering the side of a vein at the xylem/phloem boundary. F, feeding track, I, labial imprint; P, phloem; V, vein; X, xylem. Scale bar = 10 μ m for Figs. 9-12, 14-16; and 100 μ m for Fig. 13.



went from one surface of the leaf to the other. In large veins, the track entered laterally at the xylem/phloem junction. Although this insect is known to be a xylem feeder, the tissue in which the sheath terminated was unclear. These large feeding tracks were clearly intracellular.

Idioderma virescens. In Veitchia leaves, there were many random feeding tracks in the mesophyll. Feeding tracks were relatively narrow, and many lacked stained sheath material and appeared as tunnels in the leaf (Fig. 13). One track approached the edge of the largest vein or midrib, penetrated the fibrous sheath, and ended in a phloem region (Figs. 14,15). The feeding track was intracellular.

Spangbergiella vulnerata. On coconut leaves, feeding tracks were similar to G. sonorus. Most tracks that reached a vein terminated either at xylem/phloem boundaries of larger veins or within the phloem core of the smallest veins. One track to a large vein ended in the phloem. Sheath material was intercellular.

In St. Augustine grass leaves, feeding tracks terminated in small veins which contained mostly phloem but also had one to several small tracheary elements in the xylem. Feeding tracks sometimes ended at the xylem/phloem boundary in large veins (Fig. 16). When the upper epidermis was punctured, the feeding track terminated in either the phloem and xylem, or only in the xylem alone. The sheath material was intercellular.

Conclusions

Our study has shown that the courses of salivary sheaths' passage into the phloem were mostly intracellular with the exception of intercellular feeding by *S. vulnerata*. In general, the Auchenorrhyncha insects (leafhoppers, planthoppers, and treehoppers) mostly probe intracellularly to phloem as compared to intercellular penetration by Sternorrhyncha insects (aphids, whiteflies and mealybugs) (Pollard 1973, Kennedy et al. 1978). In this study, we have noted that the points of entry of the stylet and the pathways through different tissues varied not only with test insects but also with plant species. Only O. nigricans was found to cause massive disruption of cells near the sheaths, a probable result of the extremely large size of this insect (15-18 mm long). We have observed the efficient feeding by M. fascifrons which fed on multiple phloem sites with only one stylet penetration. Test insects made many unsuccessful short probes as indicated by sheaths ending blindly in the mesophyll cells.

Of seven species of insects tested, only O. nigricans is known to be a xylem feeder and a vector of xylem limited pathogen (McCoy et al. 1978). Although phloem feeders can acquire LY MLO during the process of ingesting phloem sap, not all phloem feeders transmit the LY pathogen (Tsai 1979).

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