Anatomical and Histochemical Effects of Feeding by *Citrus* Leafminer Larvae (*Phyllocnistis citrella* Stainton) in *Citrus* Leaves

D.S. Achor,¹ H. Browning,² and L.G. Albrigo³

Citrus Research and Education Center, IFAS, University of Florida, Lake Alfred, FL 33850

ADDITIONAL INDEX WORDS. callus cells, wound periderm, suberin, cutin, lignin

ABSTRACT. Young expanding leaves of 'Ambersweet' [*Citrus reticulata* Blanco x *C. paradisi* Macf. x *C. reticulata*] x *C. sinensis* (L.) Osb.] with feeding injury by third larval stage of citrus leafminer (*Phyllocnistis citrella*) were examined by light and electron microscopy for extent of injury and tissue recovery over time. Results confirmed that injury is confined to the epidermal layer, leaving a thin covering over the mine tunnel that consisted of the cuticle and outer cell wall. Wound recovery consisted of two possible responses: the production of callus tissue or the formation of wound periderm. The production of callus tissue developed within 3 days of injury when the uninjured palisade or spongy parenchyma below the injured epidermis produced callus tissue through periclinal or diagonal cell divisions. After 1 month, the entire epidermis was replaced by callus tissue. In the absence of secondary microbial invasion, this callus tissue developed a thick cuticle, followed by development of a covering of platelet wax after 4 months. Alternatively, wound periderm formed if the outer cuticular covering was torn before the cuticle had developed sufficiently to prevent the exposed cells from being desiccated or invaded by fungi, bacteria, or other insects. The wound periderm consisted of a lignified layer of collapsed callus cells, a suberized phellem layer, and a multilayered phelloderm-phellogen. Since there were always cellular collapse or fungi and bacteria associated with wound periderm formation, it was determined to be a secondary effect, not a direct effect of leafminer feeding.

Citrus leafminer (*Phyllocnistis citrella*) feeds primarily on young expanding leaves, although during heavy infestations or lack of suitable flush it will feed on young fruit (Hering, 1951). Moths oviposit on newly emerged leaves, and, depending on the site of oviposition, larvae can be found on both leaf surfaces, although lower surfaces are most often infested. The larval developmental period is short, generally spanning 3 to 5 d from egg hatch to pupation (Knapp, 1995). Thus, each of the three larval instars complete feeding within a period of 24 to 36 h. In citrus, feeding is limited to the epidermis only, leaving the outer epidermal wall and cuticle as a remnant to form the outer wall of the mine (Sohi and Verma, 1965). The mine originates near the midvein on the lower surface of the leaf where the egg is generally laid. The mine is serpentine in form and gets broader with each subsequent instar. Most of the damage is caused by the third instar. The mature larva spins a foundation of silk in the mine at the leaf's edge, creating a leaf fold in which the leafminer pupates. The young leaf continues to expand somewhat and harden in spite of the epidermal injury. This can result in distortion of leaf form and tearing of the cuticular covering. Wind and rain can also tear the cuticular covering of the mine, creating openings for invasion by microbial and insect pests and causing desiccation of the underlying tissues (Hering, 1951).

Hering (1951) reported that the most common plant reaction to mine formation is the development of callus tissue. The cells of this tissue are typically large and turgid with thin cell walls. The moist, protected environment of the mine is conducive to the formation of these fragile cells. He further reported that callus development was more often associated with injury to young than mature tissue. He

described callus development when feeding was in the mesophyll of leaves in species other than citrus but did not mention feeding or recovery in epidermal tissue of these other plants.

Wound periderm formation is a common response to injury in most plants, including citrus (Bloch, 1941). It is an organized meristematic tissue consisting of a phellogen (the meristematic layer), the phellem (a suberized cork layer of cells exterior to the phellogen), and the phelloderm (a parenchymatous layer interior to the phellogen) (Fahn, 1982). In contrast to callus tissue, wound periderm usually forms when the underlying tissue is no longer protected from the external environment. Formation of wound periderm has not been described as a typical response to leafminer feeding in other leafminer x plant interactions (Hering, 1951) or in citrus (Sohi and Verma, 1965). In fact, Hering (1951) states that mines "practically never contain a genuine cork tissue." He attributes this to the protective nature of the mine walls.

Our objective was to characterize the histology of feeding injury and recovery in *Phyllocnistis* mines in citrus leaves. Initial leafminer injury and subsequent recovery were observed over a 6-month period using light microscopy (LM), histochemistry, transmission electron microscopy (TEM), and scanning electron microscopy (SEM). To examine Hering's (1951) conjecture that wound periderm does not usually form because of the protected internalized nature of leafminer feeding, we removed mine coverings immediately after injury and monitored recovery and secondary microbial or insect invasion of these leaves.

Materials and Methods

MICROSCOPY. Ninety leaves with larvae in the third instar stage of development were tagged in October 1995 on new flush of 'Ambersweet' [*Citrus reticulata* Blanco x (*C. paradisi* Macf. x *C. reticulata*) x *C. sinensis* (L.) Osb.] orange trees growing in Lake Alfred, Fla. Third instar mines, with the larvae still present and feeding, were chosen because they were the largest and most conspicuous. All tagged leaves were examined during each collection period for general leaf maturity (degree of expansion and

Received for publication 13 Feb. 1997. Accepted for publication 26 June 1997. Florida Agricultural Experiment Station journal series no. R-05589. The cost of publishing this paper was defrayed in part by the payment of page charges. Under postal regulations, this paper therefore must be hereby marked *advertisement* solely to indicate this fact.

¹Electron microscope specialist.

²Professor.

³Professor.

hardness of the leaf) and degree and type of injury. Five leaves showing representative injury were collected for LM, TEM, and SEM on each sampling day: on the day of tagging, after 3 d, 7 d, 2 weeks, and monthly from 1 to 6 months. In later collecting times, leaves showing scarring were separated from leaves that had regreened and were processed in separate vials. To determine the effect of cuticle removal on the anatomy of mine recovery, the cuticle was removed with forceps from 30 third-instar mines on the same trees, and subsequent tissue development was observed in the same manner as undisturbed mines. Four to eight 3-mm square samples were cut from each leaf in areas injured by the larvae. These were fixed for microscopy in 3% glutaraldehyde in 0.1 M potassium phosphate buffer, pH 7.2, for 4 to 8 h at 26 °C. The samples were then washed in buffer and postfixed in 2% osmium tetroxide in the same buffer for 4 h at 26 °C or overnight at 4 °C. For LM and TEM, the samples were dehydrated in acetone and embedded in Spurr's resin (Spurr, 1969). For SEM, the samples were dehydrated in ethanol and critical point dried using a critical-point dryer (Ladd Research Industries, Burlington, Vt.) and liquid CO₂.

For LM, 1-mm sections from 10 tissue pieces from each time period were made on an ultramicrotome (Huxley; LKB Instruments Inc., Rockville, Md.) with glass knives and stained with methylene blue-azure A and basic fuchsin (Schneider, 1981). Light micrographs were made using a standard research LM (Carl Zeiss, West Germany) with an attached camera. For TEM, thin (90- to 100-nm) sections were made from five tissue blocks from each time period using a diamond knife on the same microtome, mounted on copper grids, and stained with uranyl acetate (Stempack and Ward, 1964) and lead citrate (Reynolds, 1963). Micrographs were made on a TEM (model 201; Philips Scientific and Analytical Equipment, Eindhoven, The Netherlands). SEM samples of six leaf pieces from each time period were mounted on stubs, sputter coated with gold/palladium (80/20) using a sputter coater (Ladd Research Industries) and viewed with an SEM (model S530; Hitachi, Ltd., Tokyo).

HISTOCHEMISTRY. At the time we were preparing samples for histochemical staining of fresh tissue, the early stages of leafminer feeding were not available in the grove. Therefore, 3-month-old feeding injury was taken from 'Ambersweet' in the grove and 1- to 4-week-old injury was taken from 'Swingle' plants grown in a greenhouse. It was observed that the injury on 'Swingle' leaves grown in the greenhouse in high humidity and a somewhat constant temperature were almost 1 month behind callus cell development in leaves collected in the grove at a comparable time. Therefore, for purposes of comparison, the following identifying features were observed and defined as the same between the greenhouse and grove samples: initial injury, beginning callus formation, dividing callus cells, thickening of callus cell walls, beginning wound periderm, regreening, wax formation, and late wound periderm formation.

To prepare the tissue for histochemistry initial injury, beginning callus formation and dividing callus cell phases were taken from greenhouse grown 'Swingle' leaves. The remaining phases were taken from 'Ambersweet' leaves grown in the grove. Each sampling consisted of five leaves. From each leaf, 4-mm squares were taken from third-instar feeding injury and frozen on a microtome chuck in Tissue-Tek using liquid nitrogen. Sections 10 µm thick were made on a rotary microtome (model 815; American Optical, Buffalo, N.Y.) and placed in the following staining solutions:

1. For lignin staining, saturated, aqueous phloroglucinol in 20% HCl for 1 h, then mounted in glycerin (Jensen, 1962).
2. For cutin and suberin staining, saturated solution of Sudan IV in 70% ethanol for 1 h, then mounted in glycerin (O'Brien and McCully, 1981).
3. For suberin and lignin staining, 0.01% fluorol yellow in polyethylene glycol and glycerin 50:50 1 h, then mounted in glycerin (Brundrett et al., 1991).
4. For cutin staining, 0.01% in 0.05 M tris HCL buffer, pH 7.2 auramine-O 1 h, then mounted in glycerin (Heslop-Harrison, 1977).
5. For suberin staining, 0.1% neutral red in 0.1 M potassium phosphate buffer, pH 6.5, 1 h, poststained in 0.05% toluidine blue in the same buffer 5 min (to extinguish autofluorescence), then mounted in water (Lulai and Morgan, 1992).

The fluorescent stains were viewed and photographed with a microscope (Laborlux S; Ernst Leitz Wetzlar GMBH, Germany) equipped with 50-W mercury lamp, blue excitation filter BP 450-490, dichromatic mirror RKP510, and suppression filter LP515 using Tmax ASA 400 and 3200 black and white film. The samples stained with phloroglucinol and Sudan IV were photographed on a LM (Vanox T; Olympus Optical Co., LTD., Tokyo) using Royal Gold Kodachrome colored film, ASA 100, and a blue filter.

INITIAL FEEDING OBSERVATIONS. Larval feeding was observed through the transparent cuticular covering of mines on freshly collected leaf tissue using a time-lapse video camera (Hitachi Ltd., Tokyo, Japan) attached to a dissecting microscope.

Results

To correlate histochemical data with LM, TEM, and SEM observations, the description of injury and recovery has been divided into major distinguishing features. Two processes are described: the formation and maturation of callus cells and the formation and maturation of typical wound periderm. Callus cell formation was observed to be a direct response to leafminer feeding, while wound periderm formed only after the tissue in the mine was exposed to desiccation by rupture of the mine covering or by microbial invasion.

INITIAL INJURY. Visual observations on citrus leafminer feeding behavior in Florida confirmed that leafminer larvae are well adapted to feeding on epidermal cells on both citrus leaf surfaces. Forward movement of larvae located just beneath the leaf cuticle results in rupture of anticlinal cell walls (Fig. 1A). The head capsule shape of leafminer larvae (wedge-shaped in lateral profile) produces pressure against the cell walls within two to three cells from the larva, causing the cells to rupture and the cell fluid to pool in front of the leafminer's head. As the larva progresses through the epidermis, mechanical disruption of cells is easily viewed with a dissecting microscope; many cells are ruptured per minute of continuous forward and lateral movement of the larval head capsule. Suction then is used to ingest the fluid cell contents as the larva moves forward via abdominal peristalsis. That larvae actively imbibe cell fluid contents was readily observed using video microscopy on infested leaves. An additional element of feeding behavior is that larvae display lateral "waving" movements of the head capsule as they move through epidermal tissue, effectively disrupting cells over a broader area than would otherwise be affected. The resulting mine is considerably broader than the maximum width of the larvae contained within, especially in third instars. To determine the tissues injured during initial feeding, young expanding leaves, ≈0.33% of their final size, were sampled. Feeding affected only the epidermal cells, leaving the outer cell

Fig. 1. (A) Initial injury. Light micrograph (LM) of initial injury to abaxial epidermis of young citrus leaf. Only the epidermal cells have been injured, leaving the outer cell wall and cuticle as the upper wall of the mine; cmc = cuticularized mine covering, gc = guard cell, le = lower epidermal cells. (B) Beginning callus cell formation. Surface view scanning electron micrograph (SEM) of injury after 3 d, with outer wall and cuticle removed. Most of the micrograph is showing remains of lower epidermal wall and part of the side wall that delimited injured cells; a small group of emerging callus cells (cc) is also shown. (C) Callus cells dividing. LM showing swollen callus cells and callus cells undergoing periclinal division (pd), anticlinal division (ad), and oblique division (dd). (D) Beginning wound periderm formation. LM of a group of cells in the lower leaf surface undergoing multiple periclinal divisions (arrows)—the beginnings of multi-layered wound periderm. Notice the collapsed subepidermal cells above the dividing cells. (E and F) Thickening of cell walls of callus cells. (E) Transmission electron micrograph (TEM) of thickened walls of callus cells. fh = fungal hyphae, se = subepidermal cells, cw = cuticularized wall. (F) TEM of normal leaf epidermis showing cuticularized wall (cw) and epidermal cells (ec).

Fig. 2. Regeneration of epidermal cells (histochemistry) (A) Positive staining by Sudan IV in thick-walled callus cells indicating the presence of cutin or suberin. (B) Negative results of staining by phloroglucinol in thick-walled callus cells, indicating absence of lignin. (C) Autofluorescence of thickened callus cell walls (white arrow) and positive staining by fluoro yellow of the phellem layer (sl) of wound periderm, indicating the presence of suberin in the phellem layer, but not in thickened callus cell walls.

wall plus the cuticle to form the outer wall of the mine (Fig. 1A).

BEGINNING CALLUS FORMATION AND DIVIDING CALLUS CELLS. Samples taken 3 d after feeding revealed that cells from the subepidermal layer were swelling and protruding into the mine cavity (Fig. 1 B and C). In four of the ten leaf pieces sectioned for LM, there was evidence of periclinal, anticlinal, and oblique cell divisions in these swollen cells (Fig. 1C). Limited reaction to histochemical stains was observed at this early stage. However, the cuticle of the upper and lower epidermis stained positively with Sudan IV and auramine-O (not shown), indicating the presence of cutin.

BEGINNING WOUND PERIDERM FORMATION. Leaves were $\approx 50\%$ expanded 7 d after injury. The cuticular coverings of the mines showed evidence of tearing from leaf expansion. This represented early tearing of the cuticular covering because the cells below had not yet hardened to the effects of atmospheric drying. In a few areas

Fig. 3. Fully developed wound periderm. (A) Light micrograph of fully developed wound periderm; sc = sclerotized cells, sl = suberized phellem layer, pl = phellogen layer. (B) Transmission electron micrograph of suberized phellem layer showing typical ultrastructure of suberin (arrows).

(three out of ten samples), there was evidence of groups of cells undergoing multiple periclinal divisions (Fig. 1D), which marked the beginning of wound periderm formation. In these areas of multiple periclinal divisions there was also evidence of collapse of overlying cells (Fig. 1D), indicating that injury was occurring as a result of exposure to the air. In addition, there were more callus cells evident than at the 3-d collection period and more of these cells were dividing periclinally, anticlinally, or diagonally. At this stage, there was still limited reaction of the subepidermal cells and new callus cells to any of the histochemical stains (data not shown).

THICKENING OF CALLUS CELL WALLS. After 2 weeks, the cuticular coverings of all tagged injured leaves had developed tears, which likely increased desiccation of the injured areas. In response, callus cells had developed very thick cell walls. TEMs of these cells revealed that an irregular thick cuticular covering was over most of the callus cells (Fig. 1E). This feature was in contrast with the evenly cuticularized wall of normal epidermal cells (Fig. 1F). Slightly more developed thickened cell walls of injured leaves stained with Sudan IV (Fig. 2A) and auramine-O but not with phloroglucinol (Fig. 2B) or fluorol yellow (Fig. 2C), indicating the

presence of cutin but the absence of lignin or suberin.

FULLY DEVELOPED WOUND PERIDERM. After 1 month, the mined leaves had fully expanded and hardened but were smaller than unaffected leaves (data not shown). By this time scarred areas were easily identified compared to areas that had developed hardened callus tissue as described above. Areas that typically showed multiple periclinal divisions earlier (Fig. 1D) had fully developed wound periderm. The wound periderm consisted of a collapsed multilayered corky phellem and an underlying phellogen (Fig. 3A). Phelloderm had not developed in these areas. Above the phellem there was a collapsed sclerotized layer of dead callus cells that had developed in response to tissue drying and from being cut off from deeper tissue by the wound periderm. TEM images revealed the typical ultrastructure of suberin found in the walls of phellem cells (Fig. 3B). The phellem layer stained well with Sudan IV, further confirming the presence of suberin or cutin (Fig. 4A), but not well with phloroglucinol, indicating the general absence of lignin (Fig. 4B). Figure 4B shows a small area of sclerotized tissue that stained well with phloroglucinol, indicating the presence of lignin in these cells. Even at this stage, there was a lack of staining of thick-walled callus cells with phloroglucinol.

REGREENING. Second and third month field observations of remaining tagged leaves showed either typical light brown crustiness of scar tissue in areas where the cuticular covering had prematurely torn or leaves that had a regreened surface where there was now sloughing of the thin cuticular covering. LMs of regreened areas looked similar to those in Fig. 2A. LMs of scarred areas looked similar to those in Fig. 3A. SEMs of the regreened area showed that the entire surface was covered by bulbous callus cells with thick cell walls (Fig. 5A). This new regenerated covering was not as smooth as normal epidermis (Fig. 5B) and did not contain guard cells. The regreened areas revealed few to no bacteria or fungi on the surface. In contrast, SEMs of the scarred areas showed collapsed callus cells and microbial invasion of the wound periderm outer cells (Fig. 5C).

WAX FORMATION. By the fourth month, the callus cells in the regreened areas showed a heavily cuticularized outer wall and a layer of platelet wax (Fig. 5D and E), which was much heavier than the wax covering on an unmined leaf of the same age (Fig. 5F). In fact, only minimal amounts of wax platelets were observed on unmined leaf tissue.

LATE WOUND PERIDERM. Concurrent with regreening in the fourth month, wound periderm continued to develop in other leaves (Fig. 6A). These areas exhibited a sclerotized zone consisting of one to four layers of callus and underlying former subepidermal cells that were collapsed and dead. This zone was created by cell death and lignification after being walled off from healthy tissue by the development of periderm. Beneath this was wound periderm with multilayered suberized phellem layer above a phellogen layer. A few cells in the outer layer consisted of thick-walled callus cells stained with Sudan IV, as did the phellem layer (Fig. 6A), indicating the presence of suberin or cutin. The remainder of the sclerotized zone, between the outer callus cells and the suberized layer, stained strongly with phloroglucinol (Fig. 6B), confirming the presence of lignin. The phellem layer, but not the outer sclerotized cells or the inner phellogen layer, fluoresced strongly with neutral red (Fig. 6C), indicating the presence of suberin, and not cutin, in the phellem layer.

RECOVERY AFTER CUTICLE REMOVAL. All samples taken from regions where the cuticular mine covering was mechanically removed within a day of initial injury developed wound periderm (data not shown). Desiccation and invasion by microbes was so severe that small holes sometimes developed in the mine areas in

Fig. 4. Fully developed wound periderm (histochemistry). (A) Positive staining of phellem layer (arrows) with Sudan IV, indicating the presence of suberin or cutin. (B) Negative staining of the phellem layer (arrows) and callus cells (cc) with phloroglucinol, indicating the absence of lignin. Positive staining in a small island of sclerotized tissue (sc).

Fig. 5. Recuperated epidermis. (A) Scanning electron micrograph (SEM) of recovered epidermis showing layer of callus cells covering the surface of a mine that has lost its outer covering; ccc = collapsed callus cells. (B) SEM of normal leaf epidermal cells showing distribution of guard cells (gc) in a young leaf lower epidermis. Wax layer (wl) not heavy. (C) SEM showing scarred area covered with fungal hyphae (fh) and bacteria (b). (D–F) Formation of wax covering. (D) SEM of wax platelets covering a callus cell. (E) Transmission electron micrograph (TEM) of thick cuticularized wall (cw) of callus cells and wax layer (wl). (F) TEM of a control leaf epidermis showing normal cuticle (cw) thickness and no visible wax layer.

these leaves. These areas, where the cuticular covering was mechanically removed, never regreened. The conclusion was that premature removal of the cuticular covering led to periderm formation and prevented regreening.

Discussion

This study confirmed, as reported by Sohi and Verma (1965), that initial injury to citrus tissue by leafminer larvae is limited to the epidermal cells. The response of the remaining tissue was 2-fold depending on whether the mine covering remained intact. The initial response to injury was to produce large, thin-walled callus cells (Fig. 1 B and C). This has been reported to be the most common response of noncitrus plants to other leafminer larvae (Hering, 1951). As mines age, production of callus tissue continues through swelling, protrusion, and division of the subepidermal layer until the whole surface of the leaf is covered with these thin-walled cells. If the cuticular mine cover remains intact and microbial invasion or desiccation does not occur, the callus cells develop a thick layer of cutin and later a layer of wax platelets. The entire former mine is thus covered by a new regreened epidermal layer formed from the callus tissue. However, there was no differentiation of stomata.

If the mine covering becomes torn by environmental causes or by normal leaf expansion, wound periderm is likely to form. This was observed early in mine recovery as multiple layers of periclinal divisions. As the scarred areas developed over time, a typical wound periderm developed consisting of a multilayered, suberized phellem layer and a phellogen layer. Above the periderm was a lignified collapsed outer layer of dead callus and subepidermal cells caused by exposure to environmental drying. Although the formation of wound periderm or scarring is a normal response in most citrus tissues to feeding injury by citrus rust mite [*Phyllocoptruta oleivora* (Ashmead)] (Achor et al., 1991; Albrigo and McCoy, 1974), wind scar (Broderick, 1970; Freeman, 1976), flower thrips [*Frankliniella bispinosa* (Morgan)] (Achor and Childers, 1995), and some bacterial and fungal diseases (Arimoto et al., 1982; Baba and Asada, 1970), it was not found to be a direct response to leafminer feeding in this study. Rather, wound periderm formed only in injured areas that were exposed to desiccation or invasion by fungi or bacteria after premature removal of the cuticular covering and before the development of thick walls on the callus tissue. The importance of damage to the cuticular covering in the development of wound periderm formation was supported by our removal of the mine covering and having only wound periderm form as a response. Bloch (1941) stated that wound periderm is the usual form of activity after injury, while callus formation is most often formed under excessively humid conditions when transpiration is reduced to a minimum, as is found in early mine formation.

This study suggests that the fragility of the mine covering, which consists of cuticle and outer cell wall, may be an ideal infection court for secondary microbial invaders and possibly disease organisms (Hill, 1918; Graham et al., 1996), if entry is made before the new protective thick cuticle has formed over the

underlying cells. In addition, we also found, as Bloch (1941) reported, that callus cell development may depend on environmental conditions. This was incidentally observed by comparing callus

Fig. 6. Late wound periderm formation (histochemistry). (A) Positive staining with Sudan IV of the phellem layer (sl), and slight staining of the sclerotized layer (sc), indicating the presence of cutin or suberin. (B) Positive staining with phloroglucinol of the sclerotized area (sc) of collapsed callus cells and subepidermal tissue, indicating the presence of lignin in the walls. The outer cuticularized layer (cw) and inner phellem layer (sl) do not stain with phloroglucinol. (C) Positive fluorescence with neutral red staining of the phellem layer (arrows), but not the exterior cuticularized layer (cw), indicating the presence of suberin in the phellem layer.

cell development in greenhouse-grown leaves submitted to milder growing conditions (including higher humidity) with those from grove trees. Host recovery in the greenhouse plants was as much as 1 month behind that in the grove trees. The effects of environmental conditions, plant nutrition, and variety on host recovery from leafminer injury need to be investigated further. Since humidity and temperature play a large role in the speed of differentiation of the regenerated epidermis and is a factor of the rate of leaf maturity, protection of injured leaves from disease infection is somewhat subjective.

Literature Cited

- Achor, D.S., L.G. Albrigo, and C.W. McCoy. 1991. Developmental anatomy of lesions on 'Sunburst' mandarin leaves initiated by citrus rust mite feeding. *J. Amer. Soc. Hort. Sci.* 116:663-668.
- Achor, D.S. and C.C. Childers. 1995. Fixation techniques for observing thrips morphology and injury with electron microscopy, p. 595-600. In: B.L. Parker (ed.). *Thrips biology and management*. Plenum Press, New York.
- Albrigo, L.G. and C.W. McCoy. 1974. Characteristic injury by citrus rust mite to orange leaves and fruit. *Proc. Fla. State Hort. Soc.* 87:48-55.
- Arimoto, Y., Y. Homma, and T. Misato. 1982. Studies of citrus melanose and citrus stem-end rot by *Diaporthe citri* (Faw.) Wolf. Part 3. Mode of reaction in citrus fruit and leaf against infection of *D. citri*. *Ann. Phytopathol. Soc. Jpn.* 48:559-569.
- Baba, H. and Y. Asada. 1970. Comparative anatomy and ultrastructure of some diseases appearing on peels of citrus fruits. *Ehime Univ. Mem. Sec. VI Agr.* 15:1-18.
- Bloch, R. 1941. Wound healing in higher plants. *Bot. Rev.* 7:110-146.
- Broderick, H.T. 1970. Investigations into blemishes on citrus fruits. II. The effects of wind at various stages of development. *S. Afr. Citrus J.* 44:13, 15, 17, 25.
- Brundrett, M.C., B. Kendrick, and C.A. Peterson. 1991. Efficient lipid staining in plant material with Sudan Red 7B or Fluorol Yellow 088 in polyethylene glycol-glycerol. *Biotechnic Histochem.* 66:111-116.
- Fahn, A. 1982. *Plant anatomy*. Pergamon Press, New York.
- Freeman, B. 1976. Rind blemish of citrus. II. Structure and ultrastructure. *Scientia Hort.* 4:329-336.
- Graham, J.H., T.R. Gottwald, H.W. Browning, and D.S. Achor. 1996. Citrus leafminer exacerbated the outbreak of Asiatic citrus canker in South Florida, p. 83. In: M.A. Hoy (ed.). *Managing the citrus leafminer*. Proc. Intl. Conf., 23-25 Apr., Orlando, Fla.
- Hering, E.M. 1951. *Biology of the leaf miners*. Uitgeverij Dr. W. Junk, 's-Gravenhage.
- Heslop-Harrison, Y. 1977. The Pollen-stigma interaction: Pollen-tube penetration in crocus. *Ann. Bot.* 41:913-922.
- Hill, G.F. 1918. History of citrus canker in the northern territory (with notes of its occurrence elsewhere). *N. Terr. Austral. Bul. (Darwin)* 18:1-8.
- Jensen, W.A. 1962. *Botanical histochemistry*. Freeman and Co., San Francisco. p. 55-59.
- Knapp, J.L. 1995. Citrus leafminer workshop. 8-9 Feb., Orlando, Fla. p. 4-5.
- Lulai, E.C. and W.C. Morgan. 1992. Histochemical probing of potato periderm with neutral red: a sensitive cytofluorochrome for the hydrophobic domain of suberin. *Biotechnic Histochem.* 67:185-195.
- O'Brien, T.P. and M.E. McCully. 1981. *The study of plant structure—Principles and selected methods*. Termarcaphi Pty., Melbourne.
- Reynolds, E.S. 1963. The use of lead citrate at high pH as an electron dense stain for electron microscopy. *J. Cell Biol.* 17:208-212.
- Schneider, H. 1981. *Plant anatomy and general botany*, p. 315-373. In: G. Clark (ed.). *Staining procedures for biological stain commission*. 4th ed. Wms. and Wilkins, Baltimore.
- Sohi, G.S. and G.C. Verma. 1965. Feeding habits of *Phyllocnistis citrella* Stn in relation to the anatomical structure of the leaf. *Indian J. Entomol.* 27:483-485.
- Spurr, A.R. 1969. A low-viscosity epoxy resin embedding medium for electron microscopy. *J. Ultrastructure Res.* 26:31-43.
- Stempak, J.G. and R.T. Ward. 1964. An improved staining method for electron microscopy. *J. Cell Biol.* 22:697-701.