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MORPHOLOGICAL AND CYTOLOGICAL STUDIES ON *ARCEUTHOBium*
(VISCACEAE) IN RELATIONSHIP TO HOST PHLOEM
WITH STUDIES ON THE HEALTHY PHLOEM IN
PINUS SABINIANA (PINACEAE).

by
MARGARET CAROL ALOSI

A dissertation submitted in partial fulfillment of the
requirements for the degree of

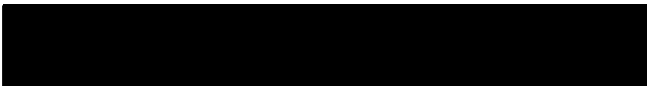
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in
ENVIRONMENTAL SCIENCES AND RESOURCES---BIOLOGY


Portland State University

1980


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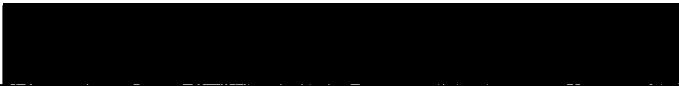

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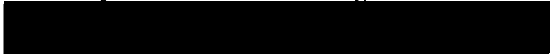

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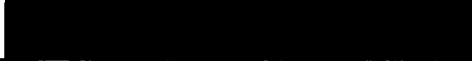

Alfred Levinson


Robert O. Tinnin

APPROVED:


W. Herman Taylor, Chairman, Department of Biology


Gary L. Gard, Acting Dean of Science

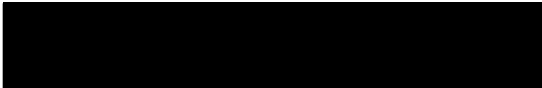

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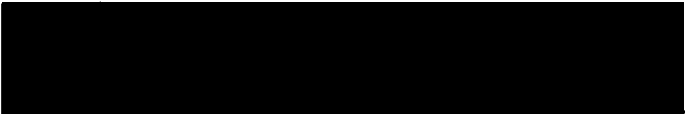
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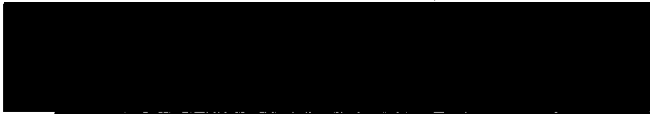
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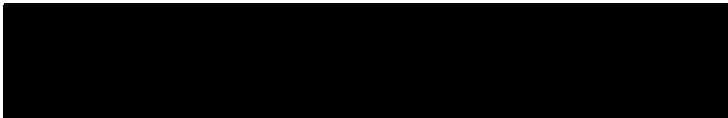
APPROVED BY MEMBERS OF THE DISSERTATION COMMITTEE:


Clyde L. Calvin, Chairman


Kwan Hsu


Donald Knutson


Alfred Levinson


Robert O. Tinnin

Anatomical and developmental tissue relationships between *Arceuthobium* spp. endophytic tissues and host vascular tissues were examined by light and transmission electron microscopes. The host-parasite pairs studied were *Pseudotsuga menziesii*/*A. douglasii*, *Tsuga heterophylla*/*A. tsugense*, *Pinus sabiniana*/*A. occidentale*, and *P. lambertiana*/*A. californicum*.

The morphological form and growth characteristics of *A. douglasii* in different aged host tissues was found to be coordinated with growth and maturation of the host. It is proposed that morphological forms of *Arceuthobium* endophytic tissue be categorized as 1) primary, 2) diffuse-secondary, or 3) localized-secondary in order to semantically clarify the relationship of endophytic morphology with primary or secondary growth stages of host tissue.

In localized-secondary endophytic forms, the parasite integrates with host rays to form multiseriate infected rays. At both the light and electron microscope level, parasite cells can usually be identified by their distinctive chromocentric nuclei and abundant lipid bodies or lipid ghosts. Sinker cells of *Arceuthobium* have unusual plastids which resemble etioplasts and which do not store starch. Sinker cells have distinctive mitochondria with unusually large nucleoid areas. They have abundant endoplasmic reticulum. Wall/plasmalemma specializations

increase the membrane surface area in relation to cell volume in sinker cells.

The walls of host and parasite are fused at the middle lamella common to both organisms and the organisms share a common apoplast. Pit-like regions in the fused walls of the host/parasite interface were commonly seen in light microscope studies, although such interspecific pitting is seen less than intraspecific pitting. It was determined that, whereas intraspecific pits are traversed by complete plasmodesmata, interspecific pits had no plasmatic channels, or, only half plasmodesmata on the host side of the pit. In one case a half plasmodesmata was seen on the *Arceuthobium* side of the host/parasite interface.

On the basis of the electron microscope studies of the host/parasite interfaces it appears that plasmatic connections between host and parasite do not normally occur. Because of this plasmatic isolation it can be concluded that nutrient acquisition does not involve direct flow of nutrients via interspecific symplastic bridges. Therefore, photosynthate, normally housed within phloem cells, must be leaked into the common apoplast of both host and parasite before becoming available for absorption into the parasite symplast. Since host and parasite lack symplastic continuity but share a common apoplast, apoplastically-mobile herbicides should be tested for their ability to accumulate in parasite tissues.

Cytopathological effects on the host cells were relatively mild although a significant increase in the ratio of radial to axillary vasculature was noted in infected tissue. Other modifications included a tendency for increased numbers of specialized phloem parenchyma (Strasburger cells) in infected rays. No apparent anomalies were observed in conjunction with host sieve cell structure and development.

The structure and ontogeny of healthy *P. sabiniana* phloem was also studied. It was found that the sieve element reticulum (SER) of mature sieve cells is derived from plastids during the maturation of the sieve cell. After maturation some of the SER membranes seem to disassociate into individual 60 Å fibrils. These fibrils then re-associate and appear to be condensed into paracrystalline bodies which, in turn, participate in the generation of new membranes in the mature sieve cell. Because of their plastid origin, the SER membranes may have energy transducing and ionic pump capabilities that commonly are associated with plastid membranes. Such specialized functions of SER may contribute to the translocating capacities of sieve cells.

PREFACE

The importance of the forest environment and its ecological complexities are now widely appreciated in our culture. The multiple use concept recognizes the value of the forest not only for lumber and pulpwood, but also for the role of the forest in erosion control, water resources, and national scenic quality. Recreation, fish, game, and wilderness preservation are also valued components of the multiple use concept. Because of the diversity of interaction between humans and the forest environment, management becomes politically, economically, and scientifically complex. The task is made even greater by the longevity of the dominate organisms (the trees) and the geographical extent of the national forests. Since powerful political and economic factors are entangled with control of forested lands, priorities have often been set "under political pressure for immediate answers, with too much regard for specific short-term problems and too little consideration for broader management objectives", according to a study by the National Research Council (NRC) (1975, p. 419).

Forest pest control management is particularly vulnerable to pressure for action-oriented policies. The NRC (pest control) study group recommended that these historically results-oriented trends be countered by new United States Forest Service (USFS) policies promoting research and management of a broader philosophical

base. The nature of the specific recommendations presented by the study group indicated a need to incorporate basic biological research along with the applied research. Such a research shift may be actuated by increasing research freedoms within government laboratories (Lyon, no date) and/or by cooperation with universities and industries having programs related to forest biology (Re: Waterman, 1959). In view of the recommendations by the NRC and others (Lyon, no date; Dasmann, 1972) concerning the need for broad-based management and research goals when dealing with the national forests, it is clear that university research can make substantial contributions to forest resource management by supplementing the more applied research of the government laboratories with basic research on a wide range of forestry-related subjects.

This spirit of cooperation between university and government is evident in the collaborative efforts on dwarf mistletoe related research between investigators at Portland State University and scientists in USFS laboratories. Investigations into anatomical, physiological, and ecological aspects of dwarf mistletoes and their hosts at the University supplement the monitoring of wood production loss, silviculture manipulations, integrated control studies and other stand management strategies carried out by the Forest Service personnel.

It is in this spirit of collaboration in a larger effort that the following studies on dwarf mistletoes and their hosts are presented.

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Part of my research was conducted at the University of California, Berkeley, through an informal agreement with Dr. Karl Dittmer, Dean of the College of Science, Portland State University, and Dr. David Schlegel, then Chairman of the Department of Plant Pathology,

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CHAPTER I

A MORPHOLOGICAL BASIS FOR ENDOPHYTIC FORM

VARIATION IN *ARCEUTHOBIMUM DOUGLASII*

INTRODUCTION

The single most important disease causing agent in many western forests is the parasitic angiosperm *Arceuthobium* (Hawksworth and Wiens, 1972, pg. 28). This pathogen directly contributes to the loss of great amounts of economically important softwoods because of disease-induced growth anomalies in infected trees. Additionally, heavy infections in individual trees may either kill the trees outright, or contribute to increased susceptibility to secondary infection by other pathogens or insects (Scharpf, 1978). Decayed stems and greater size and weight of infected branches also create a windfall and breakage hazard in recreational areas (Parmeter and Scharpf, 1963) and a fire hazard in slash (Boyce, 1938). The decline of large forest regions is presently occurring, or threatened due to the *Arceuthobium* disease syndrome (Scharpf, 1978). Unfortunately, there is no known method of eradication of dwarf mistletoe without destroying all or part of the host tree. Management practices at the present level of sophistication are tedious and are limited to selective pruning and removal of infected trees. In some cases, clear-cutting with reforestation or conversion of entire stands to

a different resistant species is recommended (Hawksworth, 1961, pg. 36; Parmeter and Scharpf, 1963). Furthermore, dwarf mistletoe disease may become a chronic condition in some forested areas which cannot be clear-cut and/or reforested; and costly, complex, long-term management is required to control the disease (Myers, *et al.*, 1972).

Because of the complexities of the forest ecosystem and the drastic measures (such as clear-cutting and stand conversion) which often must be employed to control the parasites, management of dwarf mistletoe may affect not only timber quality, yield and economics, but also water quality, fish and game, recreational and scenic aspects of vast regions of our national coniferous forests.

Many researchers feel that more sophisticated methods of dwarf mistletoe control will come from increasing our understanding of the biology of *Arceuthobium*, its relationship with host trees, and to the forest ecosystem as a whole (Hawksworth and Wiens, 1970; Tainter, 1971). Such an approach has been adopted for the present study. Specifically, information is sought on cytological features of the parasite, and the unique morphological relationship it enjoys with its host. Because structure in biological systems is indicative of functions (*e.g.*, Haberlandt, 1884, p. 1; Morrison, 1966, p. 1), knowledge of these features should lend insight into methods of control.

The dwarf mistletoe infects its host when a seed, adhering to a young host stem, germinates and produces a radicle. A holdfast develops from the radicular tissue and produces a primary haustorium

that penetrates the bark and grows toward the host cambium (Boyce, 1938, p. 357). Peripheral haustoria (Schmidt and Lindemann, 1979) (= cortical haustoria) develop from the primary haustoria and extend through inner bark mainly in longitudinal directions. Periodically, radially-oriented growths, that are termed *sinkers* (Solms-Laubach, 1867), may be initiated from strands of the peripheral haustoria. Sinkers eventually become embedded in host secondary vascular tissue.

Aerial shoots arise from buds in the haustorial system after the latter is well established. Flowering and fruiting is delayed at least one more season (Boyce, 1938, p. 359).

The entirety of the dwarf mistletoe plant that develops within the host has also been termed the *endophytic system* (Thoday and Johnson, 1930). Two fundamentally different endophytic forms have been recognized among *Arceuthobium* species. These forms produce either *localized* or *diffuse* infections in the host plant (Kuijt, 1960, pg. 360).

Localized infections, as the term implies, are contained within a limited section of a host branch. Fusiform swellings, due to hypertrophy and hyperplasia of host tissues and to the presence of the endophyte, mark the extent of the invasion of the parasite into the host stem. Aerial shoots are limited to the swollen regions and occur in random tufts. As far as is known, all *Arceuthobium* species are capable of developing localized endophytic infections on appropriate host stems (Kuijt, 1960, pg. 360).

In contrast to the localized condition, diffuse infections are produced only in the case of a few specific combinations of *Arceuthobium* sp. and hosts (Kuijt, 1960). The ability to produce diffuse infections is the more evolutionarily advanced condition (Hawksworth and Wiens, 1972). Instead of being contained within a relatively limited portion of host stem, diffuse infections involve entire branches of the affected host, extending into the youngest portions of the shoot and even into the apical and lateral buds (Thoday and Johnson, 1930; Parke, 1951; Kuijt, 1960). Aerial shoots of diffuse infections usually show a pattern of emergence related to the age of the host tissue (Kuijt, 1960). They are scattered more evenly along the branch than is typical for localized infections (Boyce, 1938) and are particularly concentrated at the branch girdles (Kuijt, 1960).

Although it is well established that these two endophytic forms represent stable, morphological variations in the genus *Arceuthobium*, the causative factor(s) in determining form have not been fully recognized. The following report details anatomical features and tissue relationships between stems of Douglas-fir and endophytes of *A. douglasii*. During the process of studying anatomical aspects of the host/parasite relationship, the principal factor in morphological form determination became clear. New terminology based on this broadened perspective of endophytic morphology is introduced in the Discussion section.

In host/parasite anatomical studies, it is important to accurately distinguish host from parasite cells. Therefore, in addition to morphological considerations, cytological characteristics which allow identification of host and parasite cells are reported.

THE VEGETATIVE SHOOT OF DOUGLAS-FIR

In order to more fully appreciate the relationship of the primary endophytic form of the Douglas-fir dwarf mistletoe to the host primary tissue, it is helpful to review aspects of normal shoot development in Douglas-fir.

The development of the shoot of Douglas-fir is governed by an annual growth cycle which is divided into two phases, separated by a dormant period (Sterling, 1946). During the first phase, a telescoped apical bud is formed (Fig. 1.1). Phase II begins, after winter dormancy with cell division and elongation. Differentiation proceeds acropetally (Sterling, 1947). Procambial cells in the individual vascular bundles divide tangentially, increasing the radial seriation of the primary vasculature. Vascular bundles also enlarge laterally by conversion of contiguous cells to procambial cells, a process which eventually results in bundle coalescence and the formation of a procambial cylinder (Sterling, 1947). Just outside the procambial cylinder, certain cortex cells enlarge in diameter, elongate tremendously; then undergo cytoplasmic disintegration. The empty lumens of these cells form a system of lysigenous

ducts of unknown function around the primary vascular cylinder. Frequently these ducts are bordered by smaller, living cells (Sterling, 1946).

Douglas-fir shoots may continue elongation over two seasons before secondary growth becomes fully established (Sterling, 1947). Rays form when shoot elongation ends (Sterling, 1947).

Yearly growth increments are well delineated externally by bud scales or scars, and by slight swellings subtending the annual shoot portion (Fig. 1.1). Bud scale regions are termed *segment girdles*; regions of elongation of the yearly increments are called *intergirdle areas*, or *segments* (Kuijt, 1960, p. 339-340) (Fig. 1.1).

MATERIALS AND METHODS

Stems, including shoot tips, were collected from Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco) branches showing dwarf mistletoe (*Arceuthobium douglasii* Englemann) diffuse infections. Collection was made in Clackamas County, Oregon, in early spring. Year old shoot portions above the second set of bud scales were selected for examination (Fig. 1). This region was flexible and non-woody. No aerial portions of dwarf mistletoe were present. Samples were also taken further down the young stem in an area above the fourth set of bud scales where dwarf mistletoe aerial shoots were present (Fig. 1). Tissue was fixed in Karnovsky's (1965) solution and postfixed in unbuffered 2% osmium tetroxide.

Specimens were dehydrated through a graduated acetone series to propylene oxide and embedded in Epon. One micron sections were cut onto water with glass knives on a Porter-Blum ultramicrotome. Sections were transferred to distilled water drops on very clean glass slides; then warmed to promote evaporation of water, section flattening, and section adherence. Slides were cooled to room temperature before staining with 1% aqueous solution of toluidine blue-0 for 15 mins. Slides were gently water rinsed; then mounted in glycerol or dried and mounted with Permount. Photography was through Zeiss optics using Kodak Panatomic X film.

Branch segments showing localized infections of *A. douglasii* were obtained from Dr. Donald Knutson of the U. S. Forest Service Northwest Range and Experiment Station in Corvallis, Oregon. These specimens were from a fall collection in southern Oregon. Infected stem pieces were soaked briefly in water; then sectioned about 25 microns thick with a sliding microtome. Sections were stained with cotton blue-lactophenol, water rinsed; then stained with phloroglucinol-HCl. Sections were mounted directly in glycerol (no water rinse) for study and photography. This staining combination turns lignified tissues red; other tissues stain blue and purple. Endophytic tissue frequently stains more densely than surrounding host tissue, which facilitates identification of parasite tissue at low magnification.

OBSERVATIONS

Host/Parasite Relationships in Host Primary Tissues

Host Tissues. Figures 1.2-1.15 show sections of Douglas-fir shoots that are infected with *Arceuthobium*. It is believed that these tissues were still capable of primary growth, or were in an early transitional period between primary and secondary growth. The criteria for deciding that the tissues were still primary are:

- a) There is no significant crushing of primary phloem (Figs. 1.2, 1.3, 1.4, 1.7).
- b) The ducts that surround the primary vasculature are still expanded (d, Figs. 1.2, 1.4, 1.7). These ducts are lost after secondary growth is established (Fig. 1.15).
- c) The most recently produced tracheids are the metaxylem type--mostly with scalariform wall sculpturings (Figs. 1.4, 1.7). Some tracheids have circular bordered pits (x, Fig. 1.2) but the elements lack the tertiary spiraled lignification pattern that is characteristic of secondary xylem elements of Douglas-fir (Sterling, 1947).
- d) The elongated appearance of pycnotic nuclei in the differentiated sieve elements (p, Fig. 1.3) is characteristic of nuclei in primary phloem sieve cells (Sterling, 1946; Alosi, 1971, p. 31). Pycnotic nuclei in secondary sieve elements are not extensively elongated.

Vascular cells of Douglas-fir are short and more densely staining in the bud scale (girdle) regions (Fig. 1.10). Away from the bud scale region, the procambial tissue and xylem and phloem

derivatives become very long and slender.

The very slender primary sieve cells in the intergirdle region of the shoot develop thick, nacreous walls over the entire wall except at sieve areas. The lumens of sieve cells are consequently restricted to a thin central channel (Figs. 1.3, n; 1.11, S). Mature sieve elements retain pycnotic nuclei. These nuclei were very densely staining and were always greatly elongated in the intergirdle region (p, Figs. 1.2, 1.3).

Some phloem derivatives undergo pseudotransverse, anticlinal division and develop into Strasburger cells (= albuminous cells, Sauter, *et al.*, 1976) (st, Fig. 1.3). Other cells appear to arise in the same manner as Strasburger cells but do not develop sieve area connections. They become storage cells for dense, birefringent ergastic materials (Figs. 1.3, 1.11). Cortical cells that line ducts and surround the vascular cylinder may also store this birefringent product (Figs. 1.4-1.6). Their relatively low length to width ratio in comparison to the vascular tissue, procambium, and ducts indicates their continued ability to divide and keep pace with stem elongation.

Cuboidal crystals* are commonly found in both uninfected (Sterling, 1947) and infected primary phloem tissue of

*Intercellular crystals are considered to be calcium oxalate (Strasburger, 1891; Frey-Wyssling, 1935; Holdheide, 1951). They are thought to form whenever a source of free calcium ions meets a source of free oxalate ions. Thus, crystals may be (*continued on next page*)

Douglas-fir (c., Figs. 1.3, 1.12). Sterling thought the crystals were located within the sieve cells; but they appear to be inter-cellular in the tissue I have examined.

Endophytic Tissues. Kuijt (1960) reported the presence of *A. douglasii* endophytic tissue in Douglas-fir dormant buds. He found that the distribution of parasite tissue in the buds appears to be related to procambial strands that develop in relationship to leaf primordia and to a procambial plexus at the base of the bud.

Kuijt's investigations of infected Douglas-fir buds indicates an early and very close relationship between host and parasite. Detailing aspects of this relationship in the differentiating, elongating shoot requires accurate identification of various cell types within the infected shoot.

Parasite cells have certain cytological features in common with some host cell types, particularly with Strasburger cells and the secretory cells that line the ducts (compare "A" with "s", Fig. 1.7). These similarities can make identification difficult (Kuijt, 1960, p. 371-2). Fortunately, nuclear differences aid in distinguishing host cells from parasite cells (Kuijt, 1960). *Arceuthobium* has *chromocentric* nuclei, while Douglas-fir has the *reticulate* type (see Lafontaine, 1974, for review of plant nuclei substructure).

(continued from page 9) expected to be formed within walls where a tissue which carries oxalic acid, such as ray and cortex tissue (Frey-Wyssling, 1935), meets another tissue which extrudes calcium ions, such as sieve elements of phloem (MacRobbie, 1975b).

The chromocentric nuclei of *A. douglasii* have a delicate meshwork of euchromatin throughout the nucleoplasm (e, Figs. 1.8, 1.9). Dense, birefringent, heterochromatin masses are associated with the nuclear envelope or with the single, sometimes enlarged and vacuolated nucleolus (h, Figs. 1.5, 1.8, 1.9). The nucleus of Douglas-fir cells have characteristics typical of the reticulate nuclear type--a conspicuous reticulum of chromatin and lack of heterochromatin masses in the interphasic condition (rN, Figs. 1.7, 1.12).

Another cytological characteristic that distinguishes *A. douglasii* cells from host cells is the method of lipid storage. *Arceuthobium* cells store lipids in relatively large lipid droplets (l, Figs. 1.8-1.11) (Tainter, 1971). Douglas-fir cells store lipids in small spherosomes (sp, Fig. 1.12).

Plastids of *A. douglasii* are small and inconspicuous at the light microscope level. In contrast, many Douglas-fir cells have larger, recognizable plastids. Most *A. douglasii* cells lack a large, central vacuole, but have many small vacuoles with clear contents in resin-embedded preparations (Figs. 1.8-1.10).

The endophytic tissue of *A. douglasii* present within the relatively mature primary shoot of Douglas-fir is composed of a complex of mainly longitudinally-oriented, uniseriate, hyphal-like strands (A, Figs. 1.2, 1.3, 1.7). The concentration of parasite tissue is greatest in the girdle or bud scale regions. Both host vasculature and parasite cells are shorter in this region (Fig. 1.10). The host

vasculature in the intergirdle region elements are very long and slender (Figs. 1.2, 1.3); but parasite cells in this area are less elongated having apparently continued to undergo transverse division. Longitudinal rows of mistletoe cells are often seen in association with host phloem (A, Fig. 1.7).

It is likely that some of the intergirdle *A. douglasii* tissue is derived from tissue originally associated with the host's procambial strands (as described by Kuijt, 1960) during phase I of host shoot development; but much of the intergirdle endophytic tissue in a mature primary shoot comes from the reinvasion of host tissue during phase II of host shoot growth. During this phase, the procambium of the Douglas-fir shoot is enlarged by lateral addition to existing procambial strands. *A. douglasii* becomes associated with this additional vasculature by hyphal-like extension of endophytic tissue through the host cortex (Fig. 1.2). Subsequently, some of these strands turn back towards the cambial cylinder and reinvade the phloem by intrusive growth (Figs. 1.4, 1.5).

Occasionally a strand of mistletoe cells is found to cross one of the duct-like empty cells, *en route* to the host vascular cylinder (Fig. 1.4). Interference microscopy shows host cell wall formed around the parasite cells that have grown through the duct (compare Figs. 1.4 with 1.5). Interspecific wall specializations in the form of pit-like wall thinnings are developed between the duct cells and the invading dwarf mistletoe cells at some sites (arrows, Figs. 1.5, 1.9).

Only the outer procambium, the phloem, and the inner cortex were infected with strands of the mistletoe endophyte. I saw no parasite tissue in primary xylem or in the pith. The parasite cells within primary host tissues all appear to be the same meristematic cell type: No specialized cells differentiate in relationship to host phloem and no tracheary elements are formed.

The parasite cells exhibit abundant pit field connections with each other (Fig. 1.8). Wall thinnings resembling pit field connections were seen fairly frequently between various host parenchymal cell types and contiguous parasite cells (unlabelled arrow, Fig. 1.12), but were rarely seen between host sieve cells and adjacent parasite cells.

Host/Parasite Relationships During the Transition of Douglas-Fir to Secondary Growth

The conversion from primary growth to secondary growth in plants is usually considered to be a gradual process involving subtle morphological changes at the cellular level (Philipson, *et al.*, 1971). In Douglas-fir, as in other plants, the beginning of secondary growth is marked by formation of the vascular cambium and a shift from predominately axillary development to lateral growth and differentiation. Figures 1.13-1.15 illustrate tissue relationships between host and parasite in a young woody stem after secondary growth has occurred and the cambium is at the onset of reactivation following winter dormancy. Dead sieve cells and Strasburger cells

of primary phloem have been crushed by the first increments of secondary growth (cp, Fig. 1.13). Crystals, absent in secondary phloem, remain as markers of primary phloem positions (c, Fig. 1.13). Endophytic strands of *A. douglasii* (A) and Douglas-fir storage parenchyma present in the region of primary phloem remain viable and their radial expansion probably contributes to the crushing of primary sieve cells (Fig. 1.13).

The predominately uniseriate strands of *A. douglasii* established during development in the host primary shoot, begin a transition to the more complex, multiseriate, longitudinal strands found in secondary host tissues (as described by Parke, 1951) by ceasing elongation, expanding radially, and then undergoing periclinal cell division (unlabelled arrow, Fig. 1.13).

The host vascular cambium appears to have a growth polarizing effect on endophytic tissue: Apical cells of hyphal-like strands respond by intrusively growing towards the cambium. Other cells, in intercalary positions of longitudinal strands remaining from the primary endophytic stage, develop outgrowths that extend toward the vascular cambium by cellular elongation and cell division. Growth is intrusive among declining tiers of secondary phloem derivatives and among newly formed ray cells (A, Fig. 1.14). These radially oriented strands of cells will become the sinkers within the secondary host vasculature (see Fig. 1.16).

Only a few sinkers become established in the secondary xylem during the first period of secondary growth. Occasionally, a very large sinker may be formed during the first period of secondary growth. These large sinkers develop in relationship to medullary rays (Fig. 1.15).

Host/Parasite Tissue Relations in Established Secondary Host Tissues

The basic structure of *A. douglasii* endophytic tissue in stems with established host secondary vasculature is the same in both diffuse and localized infections. It consists of an integrated system of mainly longitudinally oriented strands housed within the host cortex and phloem with radially oriented sinkers that extend from the strands through the host secondary phloem and cambium and into the xylem (Fig. 1.16). In contrast to endophytic tissue found in host primary growth, both the strands and the sinkers are capable of developing into multiseriate structures made up of two or more cell types.

Despite the similarity in basic structure, the origins of endophytic tissue in woody host stems differ in diffuse and localized infections. I believe this difference in origin largely accounts for the development of gross differences seen in branches displaying diffuse or localized infections.

The Diffuse Condition. The endophytic tissue found in secondary host tissue showing diffuse infection symptoms develops, as described above, from pre-existing hyphal-like strands distributed

throughout the primary phloem. Morphological conversion of endophytic tissue occurs more or less simultaneously throughout the stem segment relative to activation of the host's vascular cambium and the consequential formation of secondary xylem and phloem. Because the entire segment is involved at once, there are no localized swellings to delimit positions of the endophytic system.

In diffuse infections an interaction of parasite and host has persisted throughout the entire development of certain portions of the host plant body. Apparently, a consequence of the continual association of parasite and host tissues in diffuse infections is a less anomalous wood structure than is found in localized infections. This is illustrated by comparison of Fig. 1.17 (localized infection) with Fig. 1.20 (diffuse infection). In the diffuse infection, annual growth rings have a consistent size and sinkers extend to the inner growth rings. Whereas, in the localized infection (Fig. 1.17) sinkers extend only part way into the wood and growth rings are uneven. In the samples I examined, traumatic resin ducts were uncommon in diffuse infections, while they were abundant in the enlarged growth rings of the localized infections (*c.f.* 1.17, 1.20).

Douglas-fir trees having diffuse infections of *A. douglasii* often have infected stems with unusually long yearly stem segments. This condition may reflect prolonged or intensified seasons of primary growth.

The Localized Condition. The endophytic systems of localized infections of *A. douglasii* do not originate from parasite tissue present in the primary stage of the stem, but instead originate directly

from infection from an externally located mistletoe seed on a woody host stem. This infection process and the formation of longitudinal strands and sinkers may be generally the same as in other *Arceuthobium* species (Scharpf and Parmeter, 1967) and as outlined in the Introduction of this chapter. However, Boyce (1938) suggests that after the initial infection event, species capable of forming diffuse infections (such as Douglas-fir) only grow acropetally in the host stem.

Localized infections, as diffuse infections, must eventually establish sinker continuity with the host's vascular cambium. Then, cell division of the parasite cells within the cambial zone becomes coordinated with host cambial activity and sinker cells are incorporated into host rays. In general I have observed that infected rays in both diffuse and localized infections of *A. douglasii* do not become as enlarged as is commonly seen in species capable of forming only localized infections.

Formation of infected rays occurs concomitantly with hyperplasia of the xylem. Wide growth rings may be produced annually in the immediate vicinity of sinkers or infected rays (Fig. 1.17). A tangential section taken from a narrow, inner growth ring (such as those seen in Figure 1.17) is shown in Figure 1.18. Only normal, uninfected rays are present. But a section taken from an outer, enlarged growth ring from the same block of wood shows many bi- and triseriate infected rays (Fig. 1.19).

Over the years a localized swelling of the branch is produced at the original site of infection due to an increase in numbers of

xylary elements. In addition there is hypertrophy of the individual tracheids (*cf.* Figs. 1.18 and 1.19).

In addition to xylem hyperplasia, tracheid hypertrophy, and enlarged rays, localized infections may have many traumatic resin ducts throughout the xylem (Fig. 1.17).

Xylary elements may develop within the sinker tissue and within the longitudinal strands, but, according to Parke (1951, p. 30), a continuum between strand xylem and sinker xylem does not form in *A. douglasii*. Other *Arceuthobium* species may have more extensive xylem development with continuities between sinker xylem and longitudinal strand xylem (Srivastava and Esau, 1961) (Chapter II). Phloem, absent in most *Arceuthobium* species, may exist only in certain primitive species (C. L. Calvin, unpublished results).

DISCUSSION

This study has described the morphological forms of *A. douglasii* in diffuse and localized infections. The main factor that contributes to morphological form variation in this species appears to be whether the parasite proliferates in the primary or secondary host tissue. New terms are herewith proposed which take into account the influence of the host's growth pattern on endophytic morphology:

Primary endophytic form refers to endophytic tissue specialized for growth in host primary tissues.

Secondary endophytic form refers to endophytic tissue specialized for growth in host secondary tissues. There are two subtypes of

the secondary endophytic form--diffuse and localized.

- 1) *Diffuse-secondary endophytic forms* result from the conversion of primary endophytic tissue to secondary endophytic tissue in coordination with the transition of host tissues from primary to secondary growth. Diffuse infections (as described above) are produced in the host tissues over a period of years.
- 2) *Localized-secondary endophytic forms* result from the direct development of the secondary endophytic form of *Arceuthobium* within established host secondary tissues. This type of endophytic form is not preceded by a primary endophytic stage. Localized infections result.

These proposed terms are tentatively considered to be applicable to other *Arceuthobium* species and may be useful in providing a morphological basis for classifying endophytic types within the genus. The relationships between the proposed terms, other terms that have been used in reference to *Arceuthobium* endophytic systems (see Baranyay, Hawksworth, and Smith, 1971), and morphological features are shown in Table I.

TABLE I
TERMINOLOGY RELATING TO *ARCEUTHOBIUM* ENDOPHYTIC FORM

Proposed terms:	Primary Endophytic Form	Secondary Endophytic Form	
		Diffuse	Localized
Alternate terms:	isophasic	isophasic diffuse systemic	anisophasic localized non-systemic
External morphological features:	Sometimes abnormal host shoot elongation.	Regular pattern of aerial shoot emergence; aerial shoots spread along branch.	Localized aerial shoot eruptions. Swelling of host stem.
Location within host plants:	Primary plant body	Secondary plant body	Secondary plant body
Parasite specialization:	Can convert to secondary form.	Preceded by primary form.	Secondary form only.
Frequency of occurrence:	5 <i>Arceuthobium</i> species ¹ on appropriate hosts ^{2,3} .	5 <i>Arceuthobium</i> species ¹ on appropriate hosts ² .	All <i>Arceuthobium</i> species (approximately 30) ³ .

(1) *A. americanum*, *A. douglasii*, *A. pusillum*, *A. minutissimum*, *A. campylopodium*

(2) Kuijt, 1960.

(3) Hawksworth and Wiens, 1972.

The conversion of the primary endophytic form to the secondary endophytic form is coincident with the transition of host tissue from primary to secondary growth.

The transition from primary to secondary growth in plants involves a fundamental cytoplasmic change that is expressed by conversion from predominately longitudinal growth to lateral or radial growth. While the major driving force for cell extension (growth) is internal hydrostatic pressure (turgor pressure) (*e.g.*, Green, 1964), the anisotropic growth forms of plant cells are due to localized variations in an individual cell wall's resistance to deformation. This deformation resistance is affected by anisotropic wall textures from patterns of cellulose microfibril deposition (Green, 1964), and transmitted pressures from surrounding, expanding cells.

Longitudinal extension occurs because primary tissues are frequently reinforced by circumferentially-oriented cellulose microfibrils, with less reinforcement in a longitudinal direction. Consequently, wall deformation or cell extension occurs in relationship to the weaker, longitudinal plane in the cell wall. The coordinated extension of host and parasite cells in the intergirdle part of the primary stem segment is probably the result of this type of wall anisotropy.

Primary tissues may also expand longitudinally by tip growth. In tip growth, the walls' resistance to deformation progressively

increases from the tips towards the middle of the longitudinal cell walls. Localized variations in wall resistance other than at a cell tip may allow eccentric longitudinal extension from other sites in the wall. The hyphal-like, unidirectional growth of intrusive parasite tissue is probably the result of a tip-growth mode of extension.

Secondary tissues extend relatively little in the longitudinal direction but instead, expand radially. This is because cellulose microfibrils in secondary walls of secondary tissues are laid down either in an approximately longitudinal direction (in relationship to stem length) (Preston, 1974), or their cross-striation patterns produce a vector sum along the longitudinal axis. Such longitudinal reinforcement creates greater resistance to deformation along the longitudinal plane than exists in the transverse or radial direction.

When plant tissue converts from a primary growth form to the secondary growth form, an entire region of cells is affected and coordinated growth of the plant body in a new direction occurs. Although the mechanical factors involved in growth are fairly well understood (as discussed above), the biological factors that control and coordinate growth in the plant body are incompletely understood.

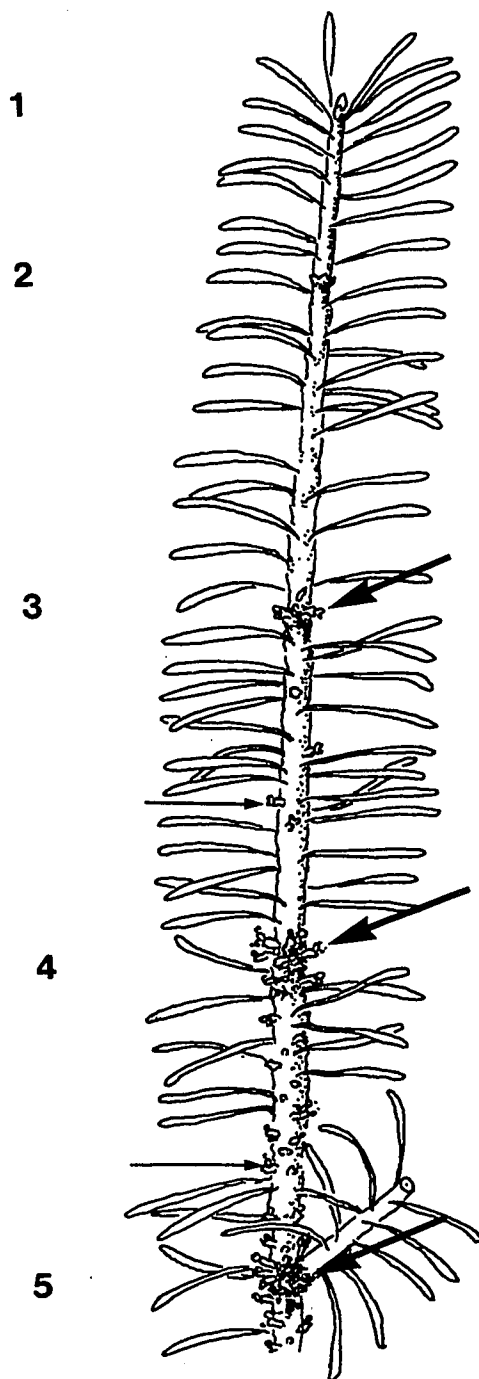
Endophytic tissue of dwarf mistletoe appears to be sensitive to the same unknown growth factors that influence the direction of cell extension and govern the predominating planes of cytokinesis in the host plant body. Because of this sensitivity to host growth stimuli, *Arceuthobium* integrates with host tissues in a manner that is

unequalled by most other obligate parasites. In *A. douglasii*, sensitivity to host growth patterns reaches a highly advanced level since the endophyte is able to assume different morphological forms and to make form interconversions.

Sinkers are not found in primary endophytic systems of *A. douglasii*. Like host rays, they are products of radial growth that is characteristic of the development of the secondary plant body. Sinkers originate from longitudinal strands in host phloem and grow toward the cambium in relationship to medullary rays, recently formed rays, or within declining tiers of phloem cells. According to Parke (1951) *A. douglasii* sinkers may also be formed directly from longitudinal strands that contact host cambium, but this was not observed in the present study.

Besides *A. douglasii*, at least four other *Arceuthobium* species are capable of forming diffuse infections. From the description of their growth habits and some anatomical details given by Kuijt (1960), it would appear that the terms introduced in this paper (primary endophytic form, secondary endophytic form--diffuse and localized), could be appropriately applied to other *Arceuthobium* species showing complex morphological form variation. However, the specific anatomical features of primary endophytic systems of *A. douglasii* and certain host/parasite tissue relationships may not be shared by all dwarf mistletoes having complex morphological forms. For example, Kuijt (1960) describes a peculiar centrifugal sinker origin in *A. americanum* forming diffuse infections in

Pinus contorta tissue. His report implies that, in addition to longitudinal strands, sinkers are present in the primary endophytic stage (pg. 364-365). However, Kuijt's interpretations appear to be based on extrapolations from isolated examples of diffuse secondary endophytic tissue rather than on actual studies on primary and transitional endophytic tissue. Because of the disparity of anatomical details between what is presented here for *A. douglasii* and what is given for other *Arceuthobium* species exhibiting primary endophytic and diffuse-secondary forms, it is clear that no generalizations on primary endophytic anatomy are appropriate until additional anatomical details on species exhibiting complex morphological forms are available.



1.1

FIGURE 1.1. *Pseudotsuga menziesii* shoot infected with *Arceuthobium douglasii*. Bud scale regions (nos. 1-5) delimit annual growth increments. Arrows indicate aerial shoots of *Arceuthobium* which are particularly concentrated at bud scale regions. 1.5 X.



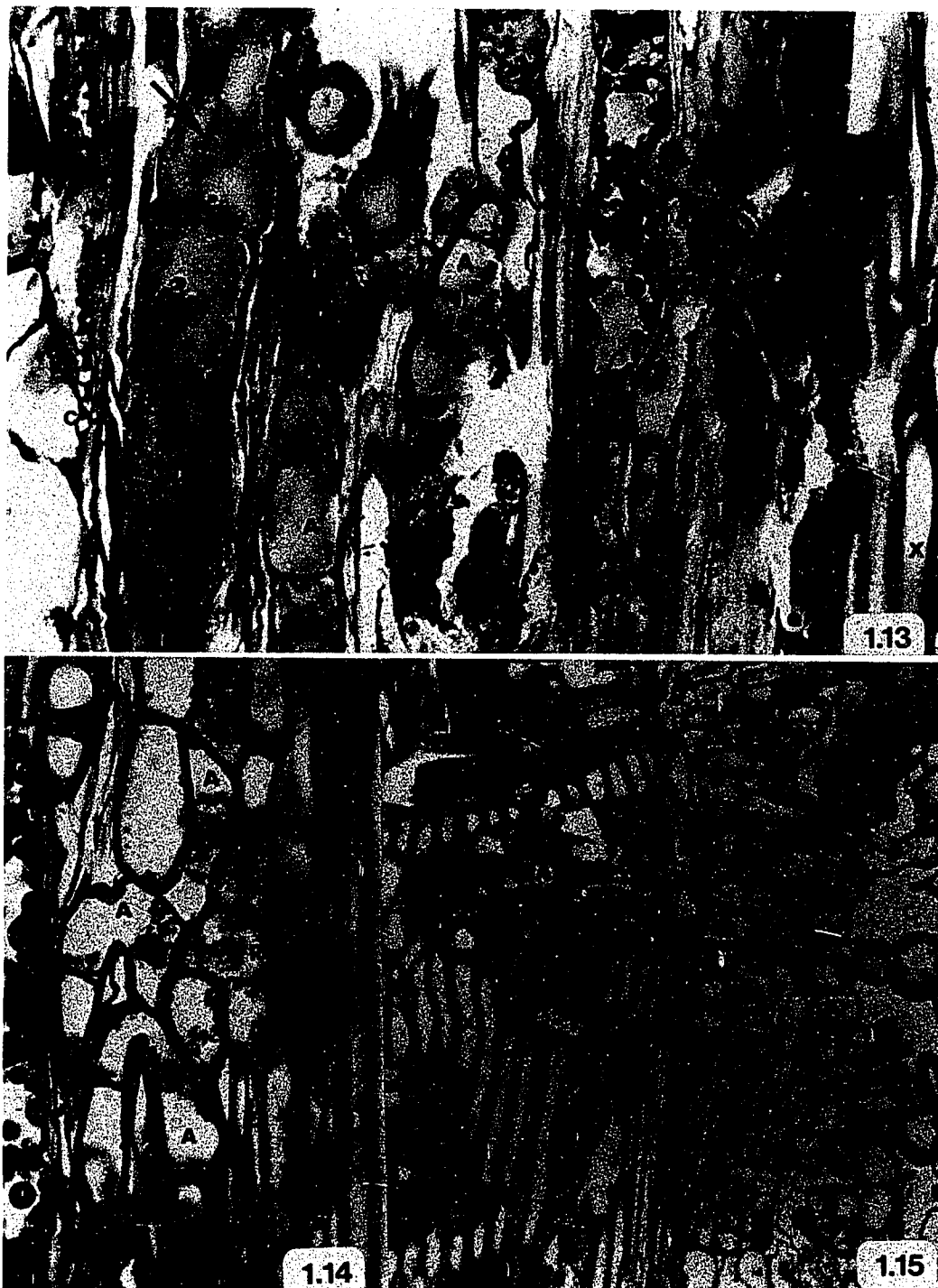
FIGURES 1.2-1.3--Tangential sections of infected *P. menziesii*.
 FIG. 1.2--Strands of the primary endophytic form of *A. douglasii* (A) surround the host's primary vascular cylinder but do not extend into the xylem (x); some strands are found in the duct system of the inner cortex (d). 50X. Fig. 1.3--The primary endophyte is integrated into the primary phloem of the host. The slender host sieve cells have thick nacreous walls (n) and pycnotic nuclei (p). Strasburger cells (st) are contiguous to sieve cells. Intercellular crystals (c) are abundant in the phloem. 170X. e, epidermis; s, storage cells.



FIGURES 1.4-1.8. Primary endophytic cells of *A. douglasii* in association with host primary tissue. Long. sections. Figs. 1.4-1.5-- An endophytic strand within the host duct system (d) appears to be invading host phloem (P) by intrusive growth. Fig. 1.5 demonstrates birefringent walls (interference microscopy). A pit-like area is at the arrow. 150X. Fig. 1.6--An *Arceuthobium* cell with an eccentric outgrowth (arrow) found among host birefringent storage cells. 150X. Fig. 1.7--Note similarity of parasite cells (A) and host cells (s). 150X. Fig. 1.8--Parasite cells in host phloem; phase contrast. 500X.



FIGURES 1.9-1.12. Primary endophytic cells of *A. douglasii* in association with host primary tissue. Compare chromocentric nuclei (cN) of parasite cells (A) with reticulate nuclei of host cells (rN). Unlabelled arrows=pit-like regions between host and parasite. All 450X. Fig. 1.9--Parasite cells associated with duct cells. Fig. 1.10--Parasite cells in the girdle region. Fig. 1.11--Parasite cells in the intergirdle region. Fig. 1.12--Parasite cells (A) juxtaposed to host phloem parenchyma. c, crystal; e, euchromatin; h, heterochromatin; l, lipid; S, sieve cell; sa, sieve area; sp, spherosome.



FIGURES 1.13-1.15. The transition of the primary endophytic form to the secondary endophytic form. Radial sections. Fig. 1.13--Radial expansion of strand cells (A) and periclinal divisions (arrow) are characteristic of transition. 200X. Fig. 1.14--Intrusive growth of endophytic strand cells (A) between ray cells (R). 200X. Fig. 1.15--Infected medullary ray. 100X. c, crystal; co, cortex; cp, crushed phloem; P, phloem; S, sieve cell; X, xylem.

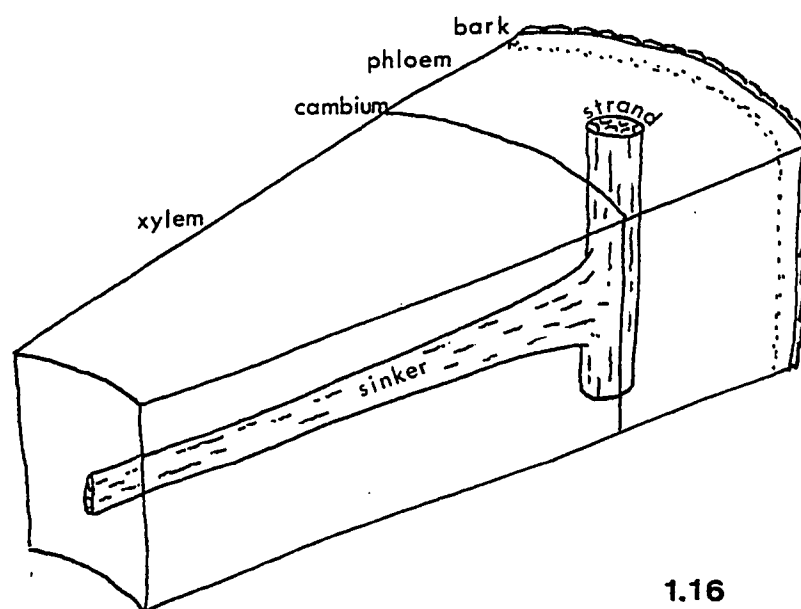
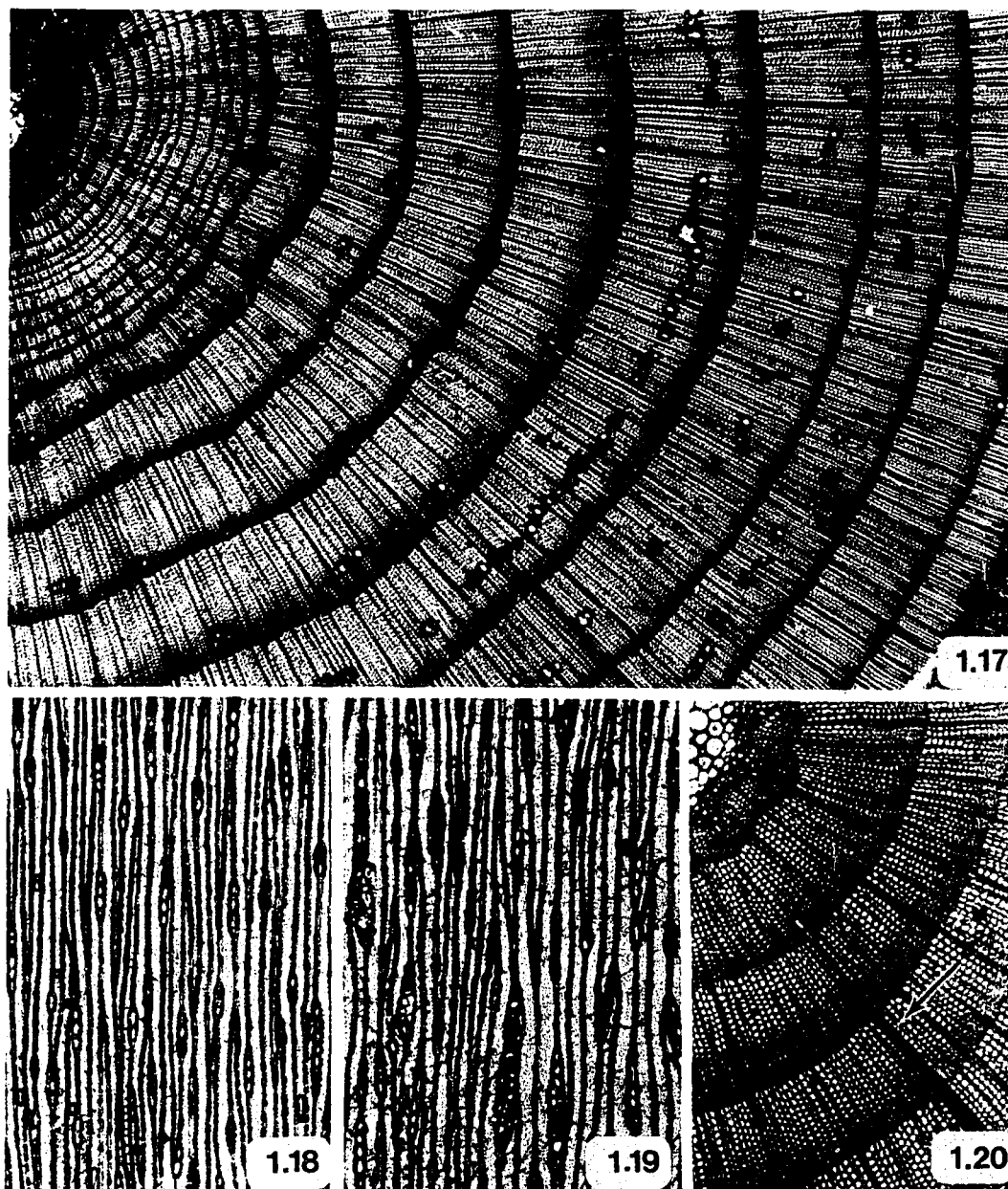


FIGURE 1.16. A diagrammatical representation of the secondary endophytic form of *Arceuthobium douglasii* in host secondary tissue.



FIGURES 1.17-1.20. Infected and uninfected wood of *P. menziesii*. Fig. 1.17--Cross sec; stem with localized infection of *A. douglasii*. The outer 9 rings show hypertrophy which is characteristic of localized infections. The smaller, inner rings were produced prior to infection. 20X. Fig. 1.18--Tang. sec.; an inner, uninfected growth ring, as in Fig. 1.17. Small, uniseriate rays are normal. 50X. Fig. 1.19--Tang. sec. through an outer, hypertrophied ring such as in Fig. 1.17. Rays are abnormally wide due to the presence of *Arceuthobium* cells within the rays. 50X. Fig. 1.20--Cross sec. of stem having diffuse infection symptoms. Uniform growth rings are present despite the presence of the parasite. Sinkers (s) extend to the medullary rays. 35X.

CHAPTER II

TISSUE RELATIONSHIPS BETWEEN DWARF MISTLETOE (*ARCEUTHOBIMUM*)

HAUSTORIA AND HOST CAMBIUM AND PHLOEM

INTRODUCTION

Haustorial structures--specialized organs of absorption of parasitic plants (Kuijt, 1977)--have been of major interest to investigators of parasitic angiosperm morphology as well as to botanists in general. These structures are key participants in host specificity and host resistance reactions, they may be directly involved in induction of abnormal host growth forms, and they facilitate interspecific nutrient translocation. By increasing our understanding of the unique problem of interspecific nutrient translocation, we also gain valuable insights into the more general problem of nutrient distribution in plants. Knowledge of host/parasite tissue relationships and interspecific translocation mechanisms may be of practical value in dealing with the problem of control of parasites, such as *Arceuthobium*, that affect economically important host plants.

The haustorial system of *Arceuthobium* is commonly called the endophytic system in the literature (Kuijt, 1960). There is variation in endophytic form within the genus, depending on particular host/parasite combinations and the site of infection on the host

plant (Kuijt, 1960, p. 360). The basis for endophytic morphological variation in the genus lies with whether the endophyte is able to proliferate in the host's primary plant body, in addition to developing within the host's secondary plant body (Chapter II). The *Arceuthobium* species considered in this chapter develop in host secondary tissues. Morphologically, they exhibit the localized-secondary endophytic form as defined in Chapter I.

Secondary endophytic forms of *Arceuthobium* originate from primary haustoria at the original infection site. The primary haustoria give rise to peripheral haustoria. The latter are composed of strands that extend longitudinally and circumferentially through the host cortex and outer phloem. Radially oriented growths termed *sinkers** extend from the longitudinal strands into the host secondary vasculature (Fig. 2.1)(Schmidt & Lindemann, 1979).

*The term "sinker" was first used by Solms-Laubach in 1867 (Srivastava and Esau, 1961). The grammatical usage in the literature implies reference to a discrete structure. In the green mistletoe *Phorodendron* for example, sinkers do appear to be distinct organs of the haustorial system, as the sinkers fit like wedges into the host secondary tissue without intermingling with host cells (Calvin, 1967). But, *Arceuthobium* sinkers lose much of their identity as individual structures during maturation. Therefore, "sinker" is a non-specific term in this report, used in reference either to the radially oriented portions of the endophytic system or to a tissue type.

The ontogenetic development of the endophytic system of *Arceuthobium* is difficult to determine in sectioned material due to the ramifying nature of the *Arceuthobium* haustorium. Erroneous conclusions about sinker development may have been made by investigators who concentrated primarily on the mature endophytic structure (Kuijt, 1960, pg. 354). Also, sinkers may arise somewhat differently in localized endophytic forms than in diffuse forms (Chapter I). These problems of anatomical interpretation have led to conflicting reports on the origin of sinkers. For example, Kuijt reports that sinkers originate from individual cellular protuberances from longitudinal strands housed within the host cortex or inner bark. According to Kuijt, a radially-oriented strand of cells is formed which grows intrusively towards the host vascular cambium. After reaching the cambium and becoming "anchored" in the host xylem, the intrusive growth phase of the sinker stops (Kuijt, 1960, pg. 354-355). Another version of sinker origin has been given by Gill (1935) and Cohen (1954). These authors reported that intrusively growing portions of longitudinal strands contact the cambium directly, and there, sinkers are initiated. After a series of cell divisions a radial file of sinker cells is produced which maintains its connection with the longitudinal strand from which it was derived.

An established sinker extends through host phloem and cambium and is embedded in host xylem (Fig. 2.1). There are differing views on how the radial file of sinker cells comes to be embedded in host xylem while an association with the host phloem

is also maintained. Several authors believe that an intercalary meristem is formed near the host cambial zone which provides cells that subsequently become embedded in the host xylem (Thoday and Johnson, 1930; Srivastava and Esau, 1961). Others have suggested that the intercalary meristem is found at the "neck" of the sinker (where the sinker fuses with the longitudinal strand) (Cohen, 1954; Kuijt, 1960, pg. 356). Finally, at least one author suggests that two meristems exist--one at the neck, the other at the host cambial zone (Parke, 1951).

Because of the juxtaposition of sinkers to host xylem and phloem, the sinkers are considered important nutrient absorbing structures. Direct contact between host tracheids and sinker xylem is evidence for a water and mineral extracting function of sinkers (Gill and Hawksworth, 1961). Hull and Leonard (1964a) demonstrated that *Arceuthobium* obtained substantial amounts of host-originating photosynthate. Investigators have speculated that transfer of organics occurs in the region where the sinkers cross host phloem (Pierce, 1905; Weir, 1916; Gill, 1935; Leonard and Hull, 1965), but the actual cellular relationship of the parenchymatous sinker cells with host secondary phloem has not been heretofore determined.

The purpose of this Chapter is to elucidate the morphological relationship between sinker tissue and host secondary vasculature (in particular, the host phloem). A major problem in morphological studies involving higher parasitic plants is the exact determination

of host and parasite cells (Dörr and Kollmann, 1974; Kuijt, 1960). Therefore, a substantial portion of the observation section is devoted to the anatomical characterization of cell types in the tissues under consideration. Certain aspects of the functional significance of *Arceuthobium*-infected phloem, inferred from the anatomical results, are discussed in relationship to host/parasite nutrition.

MATERIALS AND METHODS

Branches of *Tsuga heterophylla* (Raf.) Sarg. (western hemlock) with localized infections of *Arceuthobium tsugense* (Rosendahl) were collected in the Polk County forests of the Oregon Coast Range. Branches of *Pinus sabiniana* Dougl. with localized infections of *A. occidentale* Englemann were collected in Contra Costa County, California. Specimens were fixed in FPA (Sass, 1958) under vacuum, dehydrated in *tert*-butyl alcohol and embedded in paraffin. Serial sections, 12 microns thick, were obtained and stained with either ferric chloride/tannic acid/lacmoid for phloem detail (Cheadle *et al.*, 1953), the aniline blue callose stain for fluorescence microscopy (Currier, 1957), or with rose bengal/methyl green. Fresh material was sectioned on a sliding microtome (20-30 μ thick), or by hand with razor blades. Sudan IV, $I_2 \cdot KI$ (Jensen, 1962) and aniline blue were used to check for lipids, starch, and callose respectively in the fresh material. In other fresh sections a staining combination employing cotton blue and phloroglucinol (see Chapter I) was used to differentiate *Arceuthobium* tissue within the host tissue.

Plastic-embedded material of infected hemlock stems were fixed, embedded, sectioned and stained as described previously (Chapter I).

Tissue ratios in infected and uninfected vasculature were compared by a technique similar to that used by Mitchell (1967) who studied cell volume ratios in healthy and aphid-infested fir trees. Serial tangential sections (12 μ thick) of three different hemlock stems infected with *A. tsugense* were used. A typical tangential view of the uninfected wood from an inner growth ring of each stem was photographed (Fig. 2.20). The depth of field extended through the thickness of the section. Likewise, a typical view of an outer infected growth ring of each stem was photographed at the same magnification (Fig. 2.21). Pictures were enlarged equally, printed on medium weight paper; then cropped to the same size. Tissue types (host ray cells, host tracheids, and parasite cells) were cut out of each photograph and weighed to obtain a measure of proportionate volume for each tissue, a value which was, in turn, considered to be proportionate to the tissue type volume in the actual 12 μ thick sections. Estimates of volumetric percentages and volumetric ratios of the tissue types *in situ* were thus obtained. The significance of the data was analyzed by the Wilcoxon two-sample test for the unpaired case (Alder and Roessler, 1964, pg. 141).

OBSERVATIONS

The Origin of Sinkers

Problems arising from the ramifying nature of the *Arceuthobium* haustorium have resulted in inconclusive accounts of the origins of

dwarf mistletoe sinkers. However, a section of a strand of the peripheral haustorial system which partially circumscribes a host stem helps to illustrate sinker origin in *A. occidentale*. A series of sinkers all from the same circumscribing endophytic strand is seen in Fig. 2.2A, B, C. A diagram of the actual section given in series in Fig. 2.2 is shown in Fig. 2.3. The age of individual sinkers can be estimated by the extent of the sinker through host growth rings (Scharpf and Parmeter, 1967). Therefore, the most mature regions of the included endophytic system are seen in Fig. 2.2A and 2.3 (at A) where sinkers extended into 1 and 2 year-old wood. Sinkers in region B of the figures were extended into year-old and the current year's early wood, while those in region C were associated only with the current year's late wood. The short sinkers shown in C are very young. Continuities with the peripheral haustorial strands are not visible. However, because of their positional relationship, it is likely that they have originated from outgrowths from the main circumscribing strand seen in A and B. The tiny strands have made contact with the host vascular cambium and have initiated sinkers, that, for the most part, lie within the host cambium and xylem (unlabelled arrows, Fig. 2.2). This type of sinker origin is similar to the process described by Gill (1935) and Cohen (1954).

Sinkers do not always originate within the vascular cambium, however. Fig. 2.4 shows a young sinker that extends through collapsed phloem and the dormant vascular cambium into late wood. The

laterally-crushed phloem cells (*) and the distorted rows of xylem suggests that the sinker originated outside of the cambium and grew intrusively through the phloem and into the newly-initiated, soft-walled xylem. This early intrusive phase of sinker origin is similar to the process described by Kuijt (1960). The difference between sinker origin in Figs. 2.2 and 2.4 is likely due to the relationship to the strands from which the sinkers are derived. In Fig. 2.2 the distal part of the strand directly contacts the cambium. In Fig. 2.4 the sinker is derived from a longitudinally invading strand that does not appear to dip down to the cambium. Although young sinkers may grow intrusively into unligified host xylem, eventually all sinkers stop intrusive growth and coordinate their radial growth with host secondary growth by establishing sinker initials in the host cambial zone (si, Fig. 2.5, 2.12).

Sinker tissue in established regions of the endophytic system is almost always seen in association with host rays (Thoday and Johnson, 1930; Cohen, 1954; Kuijt, 1955, 1960; Srivastava and Esau, 1961), (Figs. 2.6, 2.8). Sometimes the sinker develops in relationship to a pre-existing ray, but in other cases, sinkers are initiated without involvement of host rays (Fig. 2.4). Only young sinkers (in their 1st year of growth) were found to be independent of host rays (Fig. 2.4); therefore, it is likely that ray tissue is quickly formed in relationship to newly initiated sinkers. Formation of new ray initials appears to proceed *via* the process of fusiform initial segmentation and formation of a declining tier of cells, like that which has been described for normal ray development

(Srivastava, 1963) (dt, Fig. 2.7). Decline of the tier proceeds in relationship to the position of the parasite cell(s). Conversion of fusiform initials to ray initials in positions adjacent to parasite cells results in the formation of a relatively tall, multiseriate structure consisting of radially-oriented sinker cells more or less surrounded (as seen in sectional view) by host ray cells (Figs. 2.7, 2.10).

Coordinated development of sinker tissue and ray tissue perpetuates this chimera-like morphological unit. The structure has been termed an "infected ray" by Srivastava and Esau (1961). Their terminology is appropriate since at this stage the sinker no longer exists as a distinct morphological unit of the *Arceuthobium* endophyte. Because of the integration of host and parasite cells, the term sinker can now be used only in reference to a tissue type of the infected rays and endophytic system.

Anatomy of Infected Rays

Examination of infected rays reveals that as many as five cell forms can be identified: host ray parenchyma, host Strasburger cells (=albuminous cells, Sauter et al., 1976), sinker ground parenchyma, sinker xylem, and sinker sheath cells. Certain characteristics of these cells are described below.

Host Ray Parenchyma. These large cells (RC in Figs. 2.10, 2.11) have brown-staining peripheral cytoplasm and usually a large, clear, central vacuole when stained with the phloem stain of Cheadle, et al., (1953).

Histological stains show starch grains and lipid bodies within the cytoplasm of the ray parenchyma. The amounts of each of these storage products depended on the time of year that the collection was made and the severity of the infection. A distinct pattern of pit callose distribution is characteristic of phloem ray parenchyma. Dense arrays of plasmodesmata with associated pit callose deposits cause the ray-cell end walls to appear remarkably like sieve plates in radial section and aniline blue fluorescence microscopy (Fig. 2.15). These wall characteristics may be indicative of the rays' active role in radial symplastic translocation (Calvin, 1966). Paired callose pads--indicative of pits--are sparse in the lateral walls of phloem ray cells (Fig. 2.15), but lateral pits are abundant in the lignified walls of xylem ray parenchyma (Fig. 2.17).

Strasburger Cells. Host cells which have Strasburger cell characteristics (Sauter and Braun, 1968; Srivastava, 1963, pg. 13). are quite abundant in infected rays in the region of conducting host phloem. Unlike other ray cells, Strasburger cells have connections with lateral sieve areas of adjacent sieve cells (Fig. 2.11). However, when sieve area connections are not included in a section, Strasburger cells can be distinguished from ordinary ray parenchyma by a dense granular cytoplasm that frequently stains grey-purple with the phloem stain (AC in Fig. 2.10). Strasburger cells lack a large, central vacuole, and the prominent nucleus is usually centrally located. Strasburger cells of the Pinaceae are usually at the top and bottom of the uniseriate rays; or, they may be found in the parenchyma

strands of the axillary system (Srivastava, 1963, pg. 13). But in the multiseriate infected rays, Strasburger cells may be found in any position that is adjacent to a sieve element (Figs. 2.10, 2.11). They are frequently more upright in orientation than the procumbent ray parenchyma.

Sinker Ground Parenchyma. Sinker cells generally have a smaller diameter than host cells in the infected ray, when viewed in tangential section (*e.g.*, Fig. 2.10). However, sinker cells in newly infected rays or within the cambial zone, may be similar in size and shape to contiguous host ray cells (Figs. 2.6, 2.8, 2.9). Cell walls of sinker ground parenchyma are thick (Fig. 2.10), except near the cambial zone (Figs. 2.8, 2.9). Numerous pits are found on all walls contiguous with other sinker ground tissue cells (Fig. 2.10). Fluorescence microscopy shows abundant pit callose and a degree of pitting not as apparent with conventional microscopy (Figs. 2.15, 2.17). Fresh sections stained with $I_2 \cdot KI$ for starch are negative. However, fresh material stained with Sudan IV shows that sinker cells are packed with large lipid bodies. Lipids are removed during processing for paraffin embedding, giving the cytoplasm of sinker cells a bubbly appearance. These vacuole-like spaces are termed lipid ghosts (*g*, Figs. 2.10, 2.11). Lipids are retained in the resin embedding process, and the droplets are conspicuous in the one micron resin sections when using phase contrast optics (1, Fig. 2.9).

Nuclei are rather small in the sinker ground parenchyma and stain very densely with the ferric chloride/tannic acid stain. There

is typically one nucleolus per nucleus (Fig. 2.10). One micron sections of resin-embedded material stained with toluidine blue differentiates the nuclear structure: The nucleus of *Arceuthobium* is the chromocentric type (Chapter I). The chromatin is distributed in a very fine network throughout the nucleoplasm. Heterochromatin masses are usually associated with the nuclear membrane or with the nucleolus, but they may be absent in initial cells (n, Fig. 2.6).

Sheath Cells of the Sinker. These cells, of parasite origin, are usually found only in wide, infected rays in the outer, non-conducting host phloem regions. They are continuous with a layer of sheath cells around an associated longitudinal strand (lss, Figs. 2.5, 2.12). Sheath cells surround the more centrally located sinker ground tissue, helping to delimit sinker tissue from host ray tissue within the outer portions of infected rays (Ss, Fig. 2.14).

Sheath cells have large, central vacuoles with stainable contents (Figs. 2.5, 2.12, 2.14). In paraffin preparations lipid ghosts are abundant in the peripheral cytoplasm and are silhouetted against the stained vacuolar contents (g, Fig. 2.12). Host cortex parenchyma are often very similar in size and stain affinity (both cytoplasmic and vacuolar) to sheath cells, but the host cells lack the distinctive lipid ghosts of the *Arceuthobium* cells. Small amounts of starch are sometimes found in the sheathing cells of the longitudinal strands, particularly near aerial shoot eruptions, but starch was not detected in sinker sheath cells.

Sinker Xylem. Strands of tracheary elements usually are found only in the larger infected rays (x, Fig. 2.7; AX, 2.15-2.17). When xylem is present in sinker tissue of *A. tsugense*, the overlapping strands form a continuous system through the center of the sinker (SX, Fig. 2.16). Sinker xylem may connect outwardly with the xylem of an associated longitudinal strand, and inwardly with the host xylem (at arrows in Fig. 2.16). The parasite xylem has helical thickenings which allow extension in relationship to radial stresses. Ultimately, radial extension of surrounding tissue may destroy some of the xylary elements (Figs. 2.7, 2.17) resulting in the formation of protoxylem lacunae in the center of some infected rays (1a, Fig. 2.7).

Some Morphological Consequences of the Host/Parasite Association

Compared to normal rays (nr, Fig. 2.19), infected rays sometimes become very large (Figs. 2.7, 2.13, 2.18). This is due to three factors: presence of parasite tissue within the rays, lack of normal decline in the tiers of cells producing new ray initials, and fusion of infected rays.

Infected rays are larger in the nonconducting phloem due to an increase in volumes of both host and parasite cells and additional cell divisions by parasite cells. The increased size of infected rays is most noticeable during spring in relationship to the new phloem increment. The infected ray may appear constricted within the host xylem, cambium, and new phloem increment, but expands

immediately in the most recently senescent phloem increment (arrows, Fig. 2.13). Expansion presumably is allowed by the increased availability of space within this region and the loss of turgor pressure in adjacent, nonfunctional sieve elements.

The sinker tissue has a more distinct boundary in the outer phloem because expansion of parasite tissue in this region results in a rounder, more symmetrical grouping of parasite cells in sectional view (Fig. 2.14). Differentiation of sheath cells in some sinkers further delimits parasite tissue from host tissue (Ss, Fig. 2.14).

Uninfected rays within an infected area remain uniseriate. Srivastava and Esau (1961) found more rays (both infected and uninfected) per unit area in some *Arceuthobium*-infected species when compared to uninfected host tissues. Their findings suggest that the normal, species-specific ratios of tissues in radial and axillary systems (Philipson, *et al.*, 1971), may be altered in *Arceuthobium*-infected stems. This hypothesis was tested by a photographic process which gave an estimate of proportionate volume of tissue types in 12 μ thick sections (see methods section).

The sampling process compared proportionate tissue volume ratios in the inner (uninfected) and outer (infected) growth rings of single stems (*cf.* 2.18, 2.19). The results are shown in Table II. In all three of the different stem samples, the ratio of tracheary tissue volume (axillary system) to ray tissue volume (radial system) is higher in uninfected wood than in wood of the same stem produced after infection ($p=95\%$).

TABLE II

ESTIMATES OF VOLUMETRIC PERCENTAGES AND VOLUMETRIC RATIOS OF TISSUE
TYPES IN SAMPLES OF INFECTED AND UNINFECTED HEMLOCK WOOD

	Sample	Tracheid % (host)	Ray % (host)	Sinker % (parasite)	Ratio Tracheid/ray
I	Uninfected	96%	4%	—	24.0
I	Infected	80%	12%	8%	6.6
II	Uninfected	95%	5%	—	19.0
II	Infected	81%	12%	7%	6.8
III	Uninfected	94%	6%	—	15.7
III	Infected	78%	16%	6%	4.8

Parasite: *Arceuthobium tsugense*
Host: *Tsuga heterophylla*

DISCUSSION

Origin of Sinkers and Development of Infected Rays

A fundamental event in the establishment of endophytic tissue of *Arceuthobium* within host secondary tissue is the invasion of the host cambium. This invasion can occur by initiation of sinkers from strands of the peripheral haustoria located external to the vascular cambium. Alternately, the cambium may be reached directly by the growing tips of strands of the peripheral haustoria. Once parasite tissue has established a position in the cambial cylinder, meristematic sinker activity proceeds in relationship to phases of derivative production by the host cambium.

Thus, an intrusively growing portion of the dwarf mistletoe endophyte reaching the vascular cambium during host xylem production will produce a sinker initial that, like the host initials, generates mainly centripetal derivatives. Presumably, most of the phloic portions of sinkers, derived from initials, will not be produced until the following spring when the host phloem increment is produced.

Intrusion into the host vascular cambium and early stages of sinker growth can proceed independently of host rays; but ultimately sinkers form an association with pre-existing host rays or induce the formation of additional ray initials. Increased production of host ray tissue (at the expense of host axillary cells) and proliferation of the parasite tissue leads to the formation of an infected ray which resembles a multiseriate ray, but consists of a unique admixture of host and parasite cells (Fig. 2.10).

The Physiological Implications of the Anatomical Studies

Because sinker tissue tends to become surrounded by host ray cells there is little opportunity for host-originating photosynthate to pass directly from host sieve elements to parasite cells. However, a close physical association of parasite with host ray cells, an ontogeny coordinated with that of ray cells, and the development of pit-like wall thinnings between juxtaposed ray cells and parasite cells (Fig. 2.10) are indications that organic nutrient acquisition by the parasite is linked to the sinker cell/ray cell association.

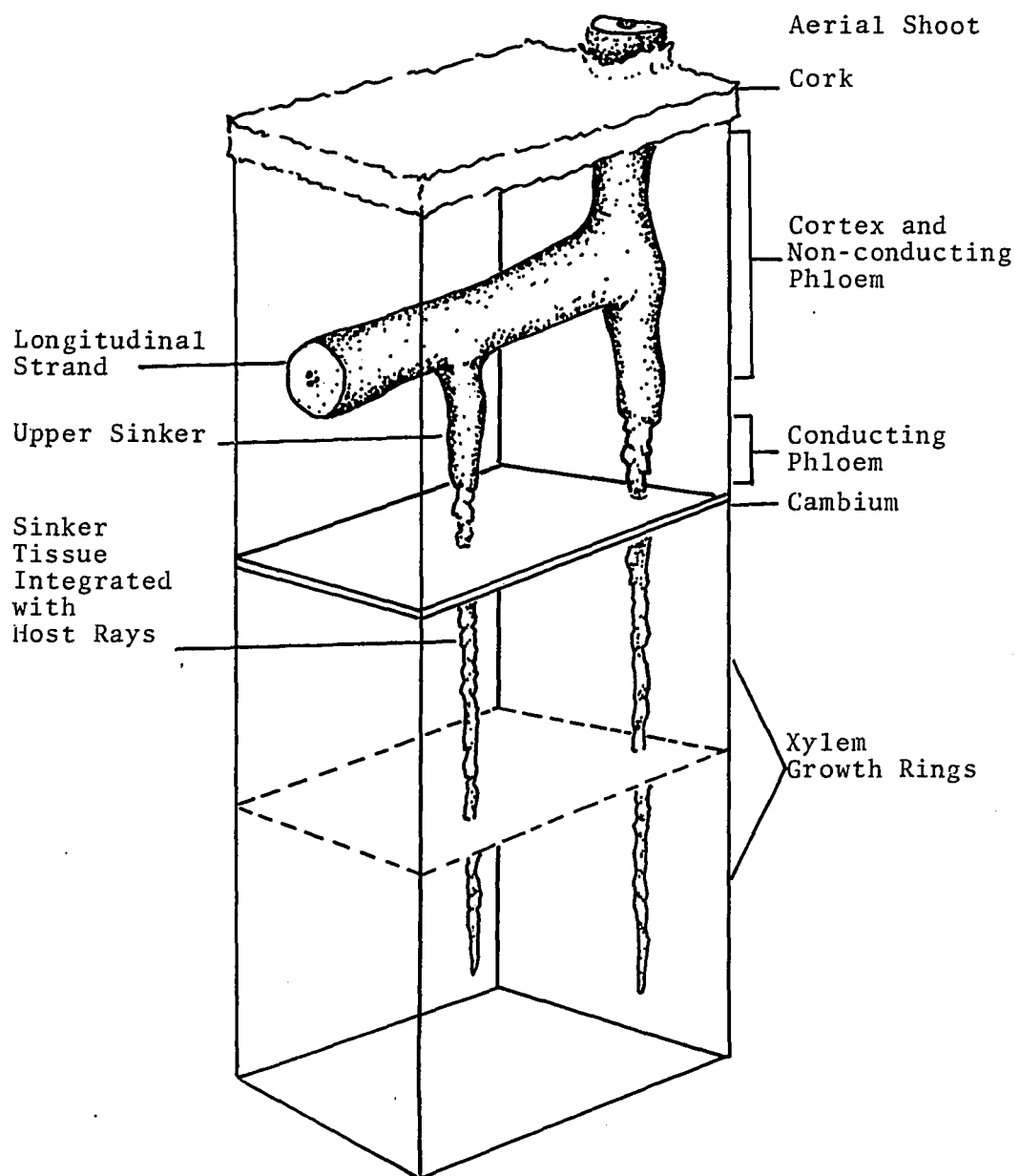
Knowledge of the substructure of the pit-like regions is crucial in interpretation of *Arceuthobium* nutrient acquisition. It is important to know whether the protoplasts of the two organisms are linked through tiny channels within these pits or if they remain separated by cell wall material. However, resolution limitations of the light microscope precludes the determination of the substructural detail; therefore, electron microscope examination of the host/parasite interface is necessary (see Chapter III).

Sinker cells do not appear to store starch although the surrounding host ray cells do. Starch is, however, found within the cells of the strands of the peripheral endophytic system and is abundant in the aerial shoots. Lack of starch stores indicates a peculiar metabolic specialization. Whether or not sinker cells are *completely* without starch cannot be determined at the light microscope level, for tiny grains may be located in plastids that are not visible with the light microscope. Electron microscope examination is necessary to resolve plastid substructure (see Chapter III).

Copious lipid storage is a dominant feature of *Arceuthobium* sinker cells, but the role of lipids and lipid precursors in dwarf mistletoe nutrition and translocation is unclear at this time. Recently initiated work on lipid chemistry in *Arceuthobium* by other investigators (Drs. Donald Knutson and Richard Tocher, independently) represents an important beginning in understanding the significance of the massive lipid stores in *Arceuthobium*.

An increase in the volume of ray tissue per unit volume of host tissue is an outstanding anomaly of *Arceuthobium*-infected tissue. Altered volumetric ratios between the axillary and radial system of infected host xylem may be considered to reflect corresponding alterations in the vascular cambium. This assumption is based on the fact that conifer xylem derivatives are found in rank with the initials from which they were derived (Esau, 1965). Although xylem derivatives may divide before they differentiate, virtually all derivative divisions are tangential and maintain the original pattern of the cambium (Philipson, *et al.*, 1971, pg. 11).

For any given species a more or less constant ratio of ray tissue to axillary tissue is maintained in normal, healthy stems (Philipson, *et al.*, 1971, pg. 42). Ray cells are the nutrient sources for cambial cells and internal tissues (Ziegler, 1964, pg. 319). The siphoning-off of nutrients from host tissue by a parasite such as dwarf mistletoe, reduces the ability of existing ray tissue to nourish internal tissues. Decreased sugar levels reaching the cambium may have the effect of inducing morphological changes in the cambial cylinder as existing cell ratios no longer maintain an adequate physiological balance between the axillary and radial systems. Thus, the increased volume of ray tissue in *Arceuthobium*-infected tissue may be a manifestation of compensation for nutrient loss to the parasite.



2.1

FIGURE 2.1. A diagrammatical representation of *Arceuthobium tsugense* in host tissue.

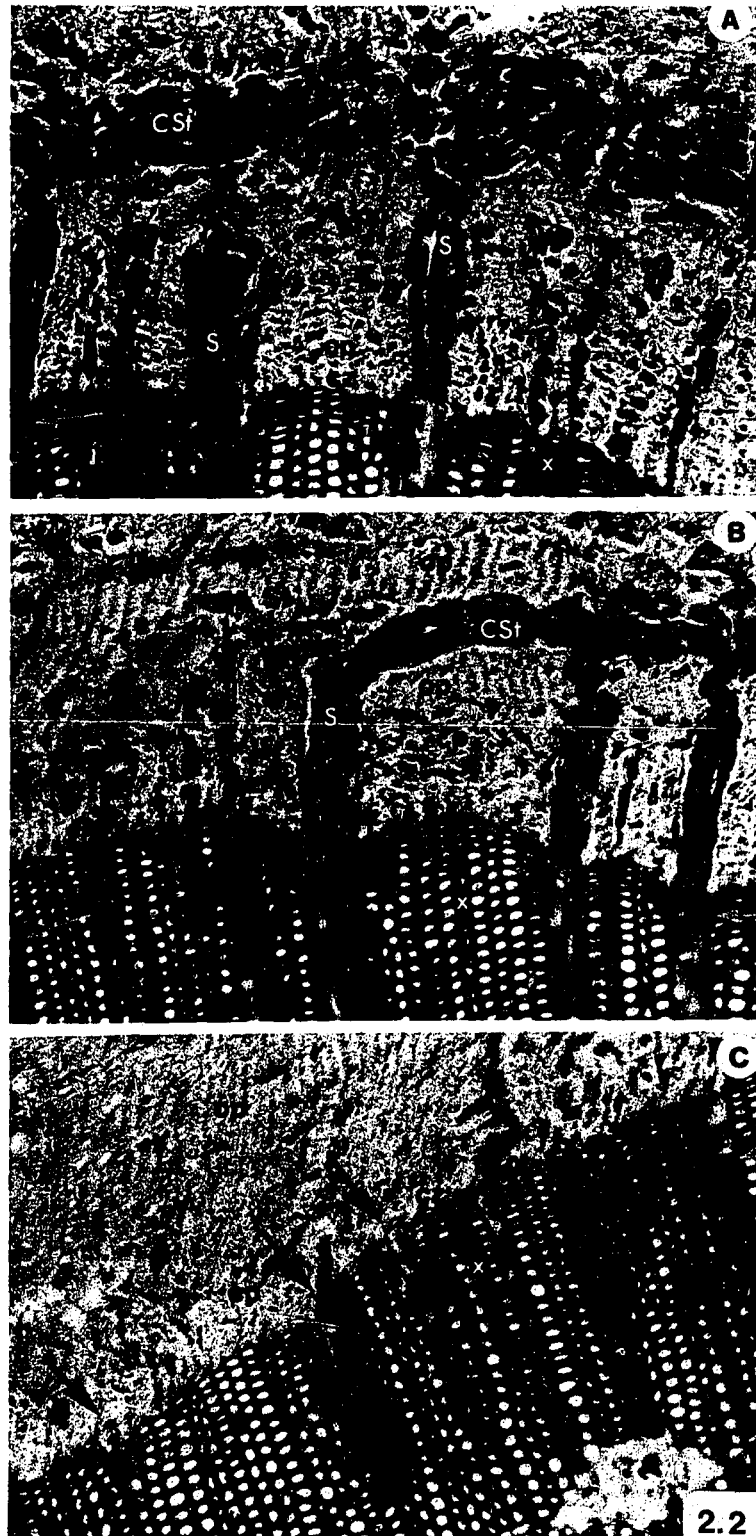
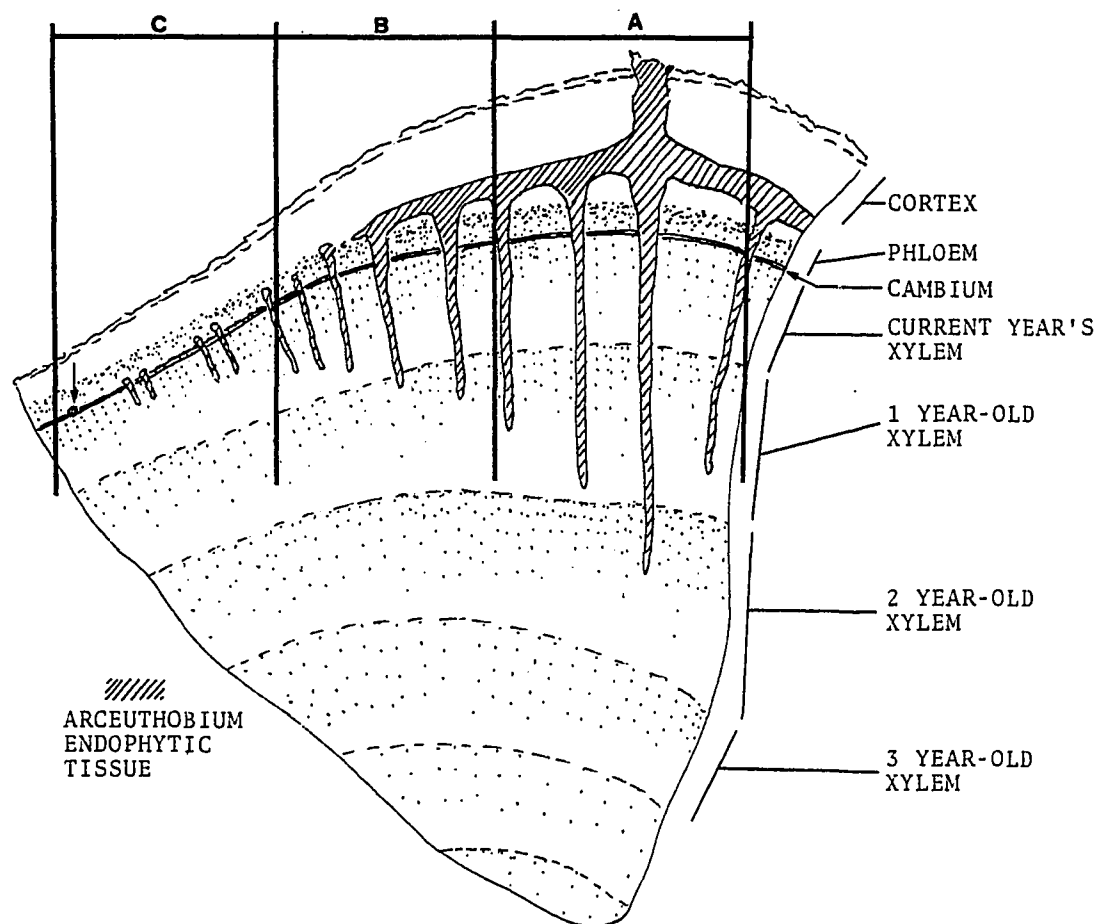


FIGURE 2.2 A,B,C. A circumscribing endophytic strand of *A. occidentalis* in *P. sabiniana*. A,B, and C are serial photographs. See the diagram on the next page for orientation. 65X. cp, current phloem; CSt, circumscribing strand; cz, cambial zone; op, old phloem; S, sinker; St, strand; x, xylem.

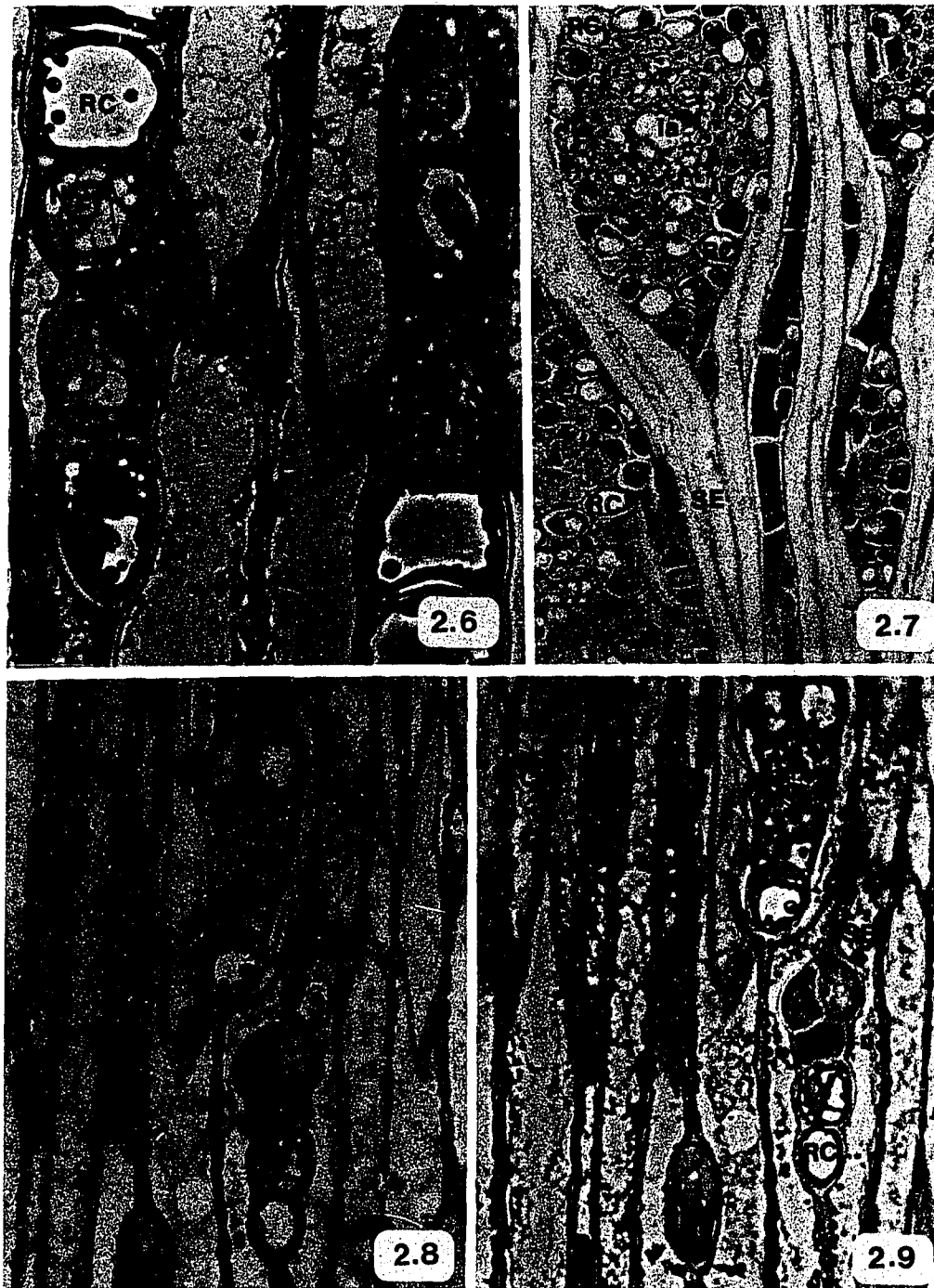


2.3

Figure 2.3. Diagram of circumscribing endophytic strand partially shown in Fig. 2.2A, B, C. The depth of the individual sinkers in host growth rings is indicative of the age of the infection in that region of the host stem. The oldest portion of the endophytic system shown is in section A, where sinkers extend into two year-old xylem. The youngest portion is in section C, where the endophyte lies entirely within the cambial and xylary regions of the host. Sinkers are initiated within the cambial zone from strand tissue that contacted the host cambium (arrow).



FIGURES 2.4-2.5. *Arceuthobium* sinkers in *Tsuga heterophylla*. Cross sections; paraffin embedded. Fig. 2.4-- A young sinker (S) with the innermost cell differentiated into a tracheary element. The sinker appears to have established itself in the host vasculature by intrusive growth through the phloem (P) independently of established host rays. Starred cells (*) are sieve elements anticlinally crushed from the intruding sinker. 500X. Fig. 2.5--An older, established sinker (S) confluent with a differentiated longitudinal strand. Sinker initials (si) correspond to the plane of the host cambial zone (cz). 115X. lss, longitudinal strand sheath cells; sx, strand xylem; X, host xylem.



FIGURES 2.6-2.9. Tangential sections of infected rays of *T. heterophylla*. Fig. 2.7 is paraffin embedded; others are plastic embedded, 1μ sections. Fig. 2.6--Infected rays among differentiating sieve cells. 450X. Fig. 2.7--Large infected rays; declining tiers (dt) are associated with infected rays. 170X. Fig. 2.8--Infected rays within cambial zone (C). 340X. Fig. 2.9--Phase contrast of Fig. 2.8. Lipid droplets (l) fill parasite cells. A, *Arceuthobium* cell(s); la, protoxylem lacunae; N, host nucleus; n, parasite nucleus; RC, ray cell; SE, sieve element; x, parasite xylem.

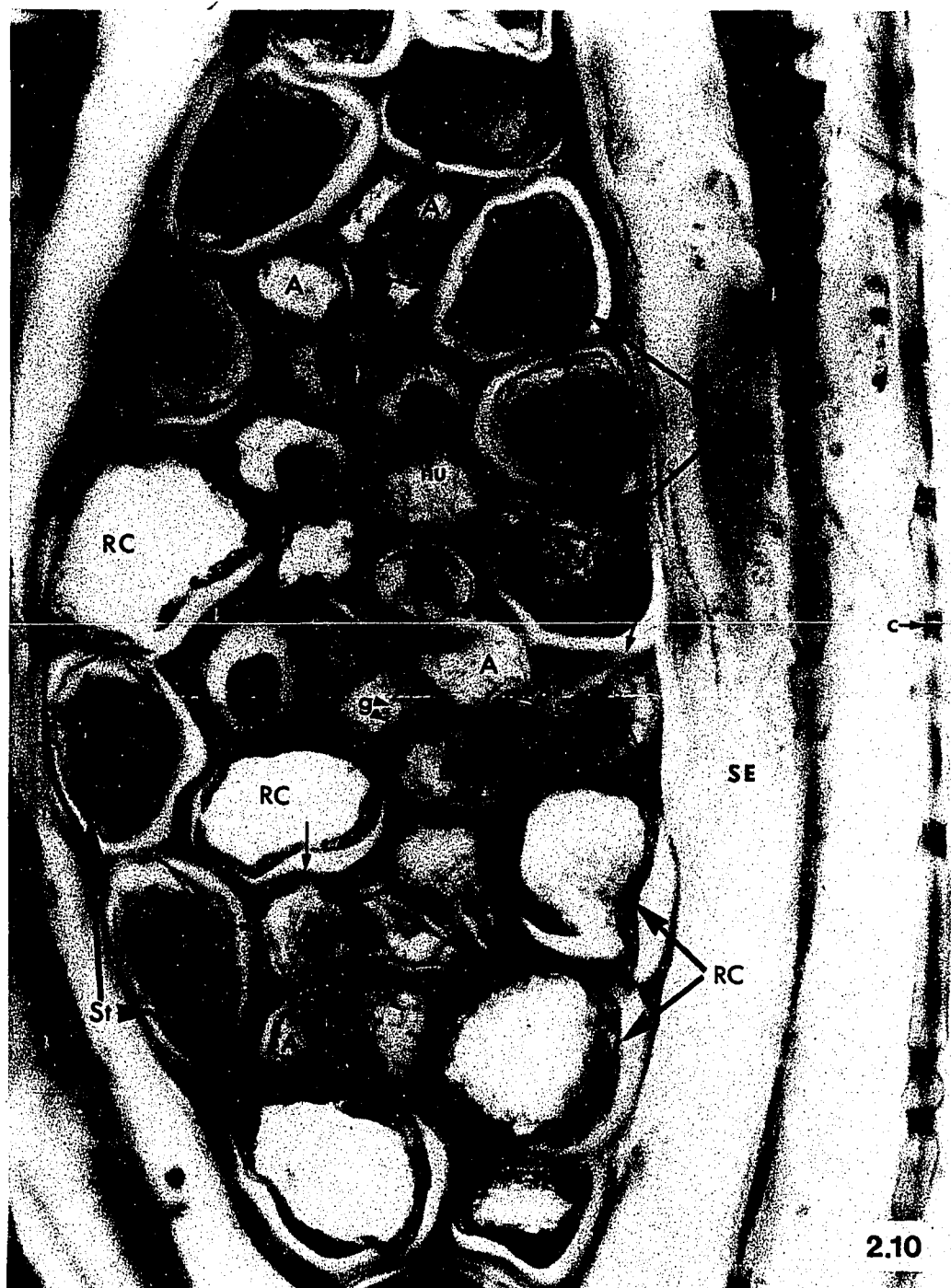
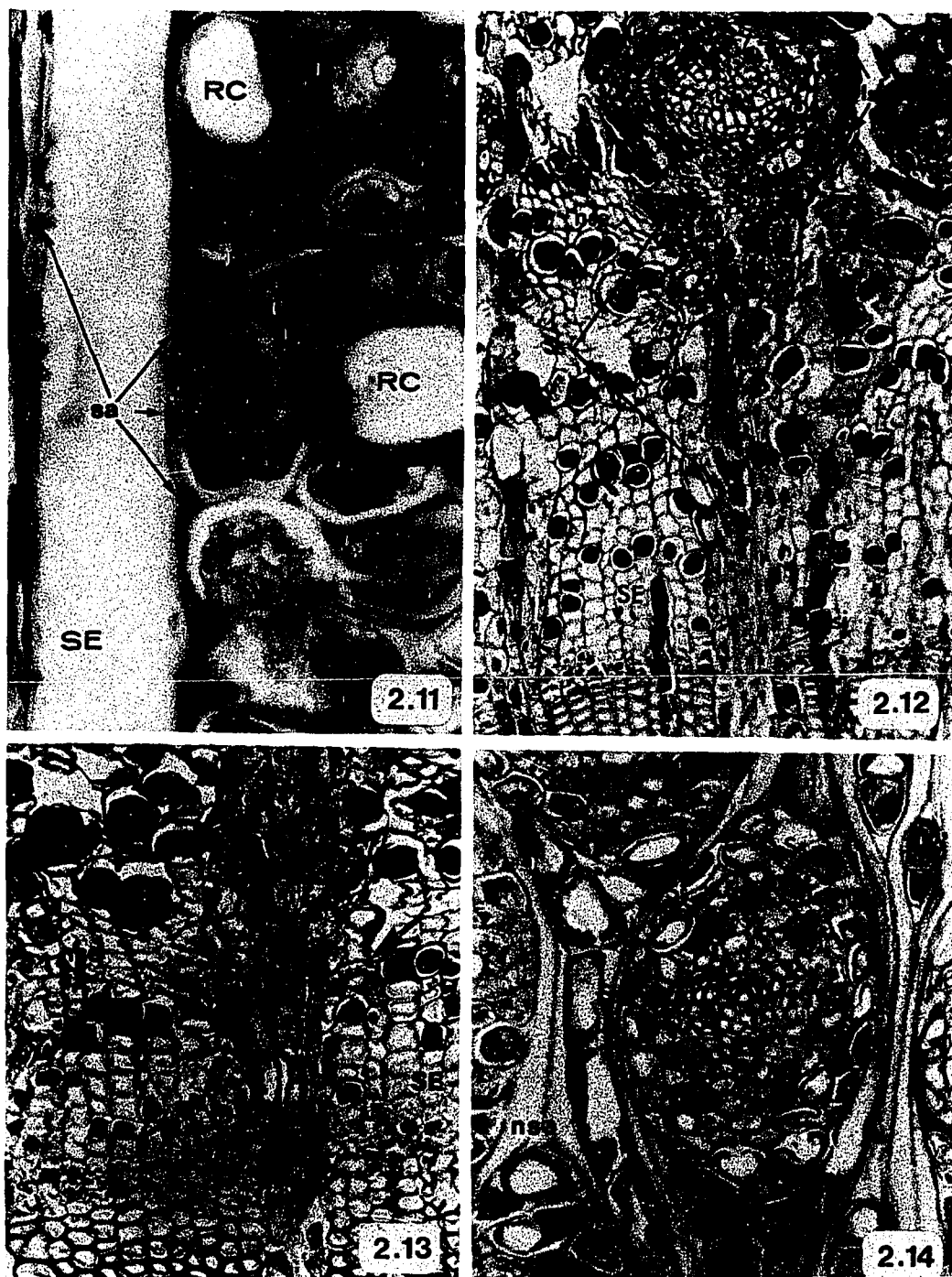
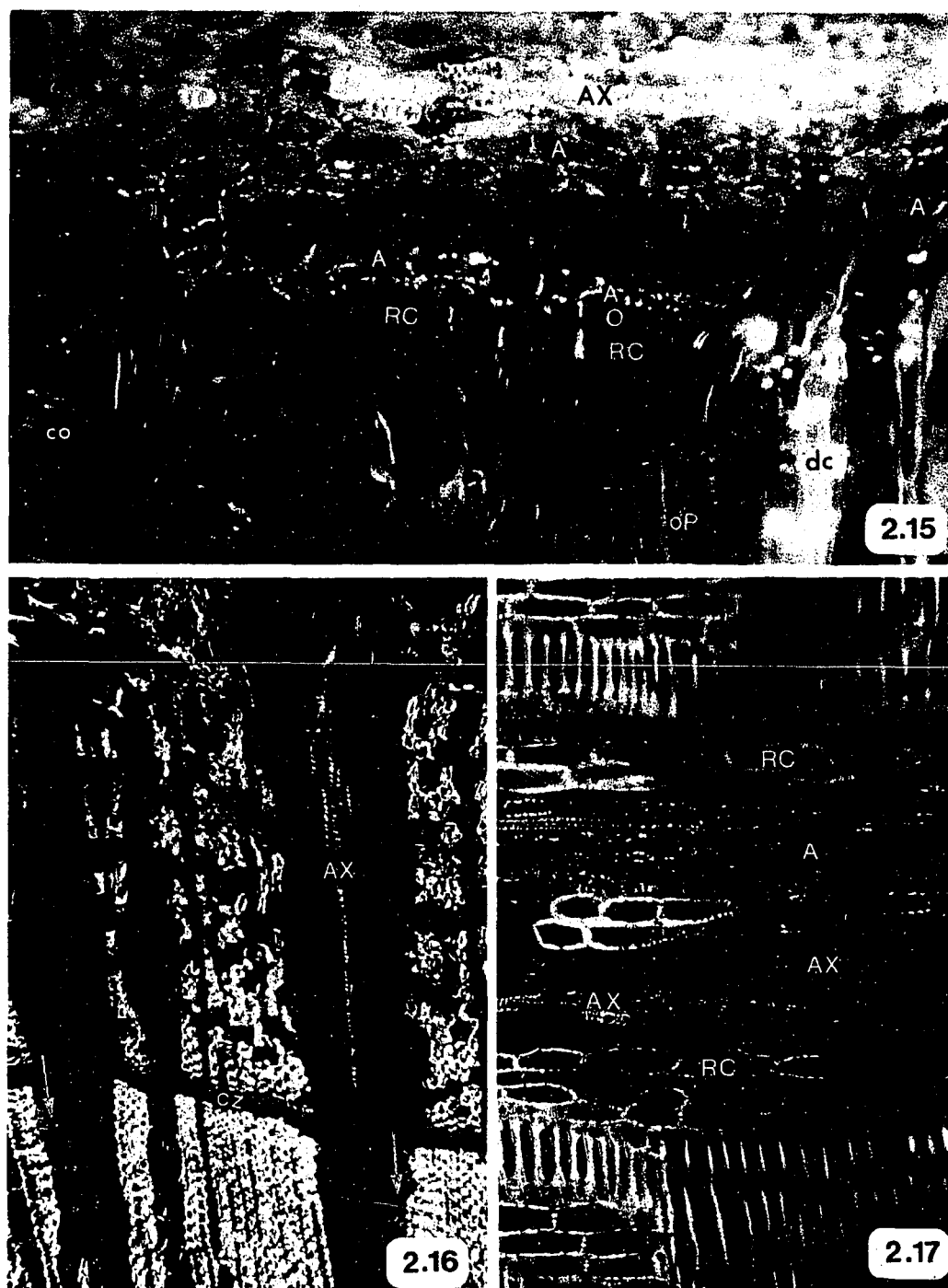


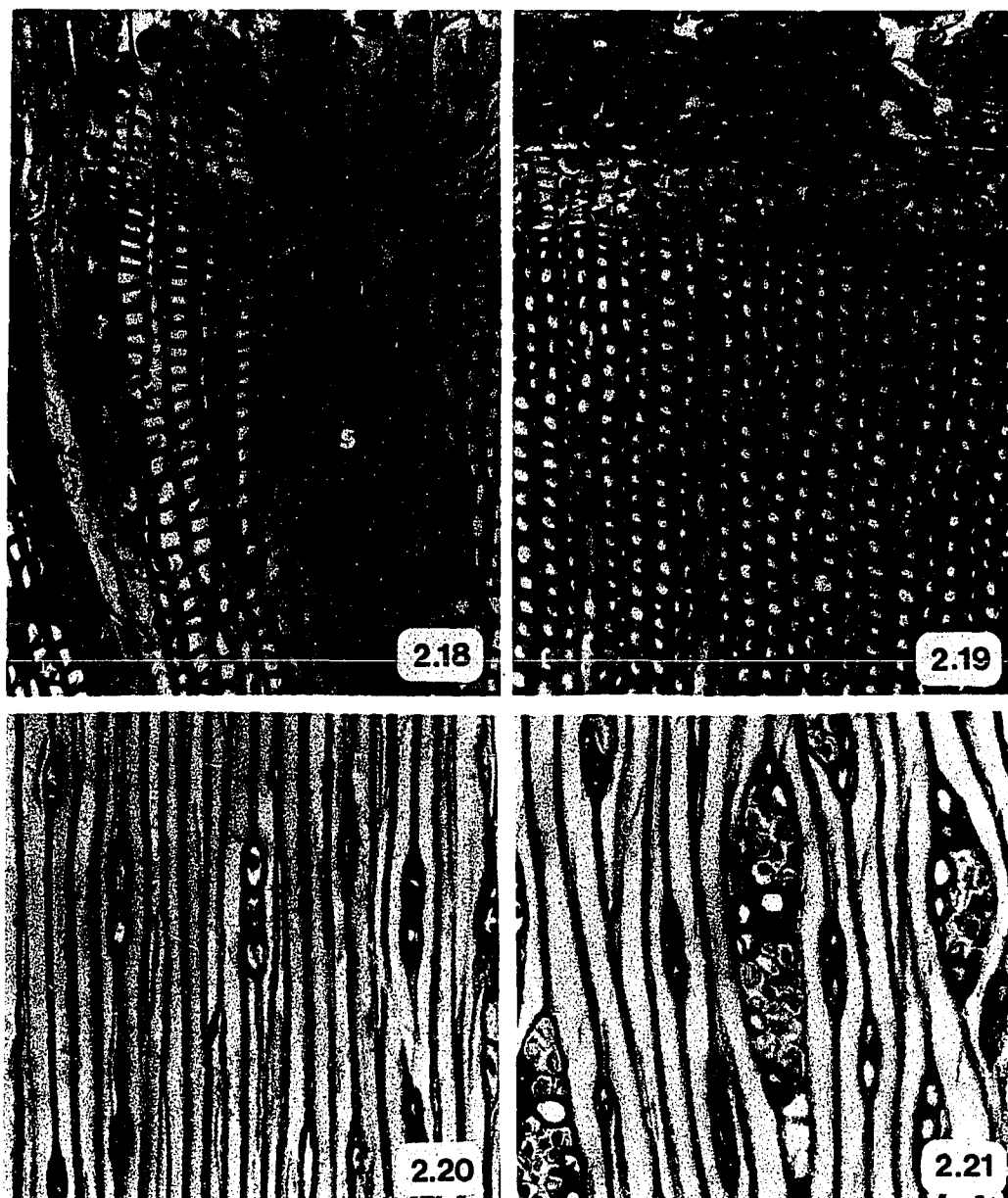
FIGURE 2.10. An infected ray of *T. heterophylla* in the region of functional sieve elements (SE). The smaller *Arceuthobium* cells (A) occupy the central region; they are surrounded by ordinary ray cells (RC) and Strasburger cells (St). Interspecific, pit-like regions are at unlabelled arrows. Lipid ghosts (g) are abundant in parasite cells. 800X. c, callose; nu, nucleolus; p, intraspecific pit.



FIGURES 2.11-2.14. Infected *T. heterophylla* tissue. Fig. 2.11--Tang. sec.; an infected ray and a host sieve element (SE). 700X. Fig. 2.12--Cross sec.; sinker (S) and longitudinal strand (LS). Sinker sheath cells (Ss) are confluent with long. strand sheath cells (lss). 110X. Fig. 2.13--Cross sec.; sinker tissue is constricted within recently produced phloem and cambium (vc) (below arrows). 125X. Fig. 2.14--Infected rays in old phloem. 170X. A, *Arceuthobium* cells; g, lipid ghost; nse, non-conducting sieve elements; RC, ray cells; sa, sieve area; St, Strasburger cells; sx, sinker xylem.



Figures 2.15-2.17. Large, infected rays of *T. heterophylla* viewed with u.v. fluorescence and polarized light. Fig. 2.15--Radial sec., fluorescence; phloem and cortex (co) regions. Callosed pit fields are abundant in the sinker cell (A) walls. Pit callose is seen between ray cell (RC) and sinker cell at the "O". 150X. Fig. 2.16-- Cross sec., polarized; sinker xylem (AX) is continuous through the sinker and joins with host xylem (arrows). 100X. Fig. 2.17--Radial sec., fluorescence; host xylem region. Sinker has primary xylem strands (AX). 150X. cz, cambial zone; dc, definitive callose; oP, old phloem.



FIGURES 2.18-2.21. Infected and uninfected wood of *T. heterophylla*. Fig. 2.18--Cross sec.; infected hemlock vascular tissue during winter dormancy. 140X. Fig. 2.19--Cross sec.; uninfected hemlock vascular tissue during winter dormancy. 140X. Fig. 2.20--Tang. sec.; uninfected wood from an inner growth ring of an infected hemlock stem. 200X. Fig. 2.21--Tang. sec.; infected wood from an outer growth ring of an infected hemlock stem. Dwarf mistletoe is present in the rays. 200X. nr, normal ray; P, phloem; S, sinker; vc, vascular cambium; x, xylem.

CHAPTER III

THE ULTRASTRUCTURE OF DWARF MISTLETOES AND
ASSOCIATED HOST CELLS IN THE REGION OF
THE HOST CAMBIUM AND PHLOEM.

INTRODUCTION

The anatomy of the dwarf mistletoes has been rather extensively studied at the light microscope level (Kuijt, 1955, 1960; Chapters I, II), and a general understanding of tissue organization in infected stems now exists. The endophyte of *Arceuthobium* stands apart from many other phanerogamic parasites by its lack of a centralized haustorial system, and relatively simple morphology. The primary endophytic form of *Arceuthobium* (found in host primary tissues) is most reduced. In *A. douglasii* it consists of a system of mostly uniseriate, hyphal-like strands of (apparently) a single cell type (Chapter I). The secondary endophyte (found in host secondary tissue) is differentiated into two major portions: longitudinal (or circumscribing) strands of the peripheral haustoria and radially-oriented sinkers (Chapter I, II). Three different cell types have been identified in the secondary endophytic tissue (Chapter II).

Despite prodigious investigation of dwarf mistletoe biology over many years, aspects of the physiological (functional) relationships between dwarf mistletoes and their hosts remain obscure.

The obscurity is heightened by the parasites' morphologically-reduced, but pervasive endophytic systems. It is known that the dwarf mistletoes receive large portions of host-originating photosynthate (Hull and Leonard, 1964a), yet sieve elements or recognizable phloem cells—which typically function in uptake and transport of carbon-based nutrients—are only rarely detected in *Arceuthobium* cells (C. L. Calvin, unpublished results).

Physiological implications of intra- and intercellular organization frequently becomes more readily inferable with increased resolution of structural detail. Subtleties of cell structure reflecting metabolic specializations may be undetectable at the light microscope level, but are apparent at higher resolution. The present study seeks to extend the knowledge of the functional relationships between dwarf mistletoes and their hosts by electron microscopic examination of infected tissue.

There has been one previous ultrastructural study of *Arceuthobium*. Tainter (1971) surveyed the ultrastructure of *A. pusillum* aerial shoots, radicles (=penetration structures), and endophytic tissues in association with larch and spruce hosts. His study included some details of parasite endophytic cell structure in the region of host needle-trace phloem. The walls of the two organisms appeared fused. Tainter described what he felt were plasmodesmata in small, localized areas of the common wall between cells of host needle-trace phloem parenchyma and "cortical bundle cells"

(=strands of the peripheral haustoria) of the parasite. The presence of interspecific plasmodesmata would have important consequences in the interpretation of cell structure in relationship to nutrient uptake, since plasmodesmata are considered channels for symplastic nutrient flow (Tyree, 1970). However, the published micrograph (Tainter, 1971, Fig. 6b) shows only what appear to be half-plasmodesmata in the common wall. Half-plasmodesmata are structures distinct from plasmodesmata (Burgess, 1972); and unlike true plasmodesmata, half-plasmodesmata cannot be used as evidence for plasmatic continuity between cells.

In situ, sinker tissue is almost completely embedded in host vasculature. Nutrient transfer probably occurs in relationship to this juxtaposition of parasite with host vasculature (Leonard and Hull, 1965, p. 142). It would be of value to know the fine structural features of sinker cells and associated host cells--particularly in relationship to the little-understood process of parasite nutrient acquisition. Tainter's study of endophytic tissue did not include examination of sinker tissue, but was limited to the outer "cortical bundles" (=longitudinal strands). Because of the complete lack of information on sinker fine structure, a primary goal of this study was to investigate the sinker cell ultrastructure within the region of the host's functional phloem and cambium, with a view towards contributing information dealing with the nutrition of *Arceuthobium*.

Light microscope studies have revealed pit-like wall specializations between endophytic cells and host tissues (Chapter I, II). Ordinary primary pit fields are perforated by plasmodesmata (Esau, 1965, pg. 38). But in *Arceuthobium*-infected tissues, I could not resolve whether perforations (such as plasmodesmata) penetrated the pits. Because of the inconclusive nature of the interspecific plasmatic connections reported by Tainter, and the unresolved nature of the pit-like wall depressions reported in Chapters I and II, another goal of this study was to clarify the nature of the interspecific union within infected rays by transmission electron microscopy.

This study also describes aspects of the fine structure of the host cells associated with sinker cells. When the structure of the diseased tissue is compared with that of healthy pine phloem (Chapter IV), the cytopathological impact of dwarf mistletoes on host phloem tissue is apparent.

MATERIALS AND METHODS

Western Hemlock Infected with Hemlock Dwarf Mistletoe

Branches of *Tsuga heterophylla* (Raf.) Sarg. with localized infections of *Arceuthobium tsugense* (Rosend.) Gill were collected in the Polk County forests of the Oregon Coast Range in early February, 1972. Samples of host phloem and cambium were obtained from regions that showed severe invasion by parasite endophytic

tissue. Stem segments were kept bathed in fixative of 5% glutaraldehyde in 0.1M potassium phosphate buffer (pH 7.4) during the trimming and dicing. Tissue pieces 2 mm in width were placed in vials containing fresh fixative and allowed to stand for 45 minutes at room temperature before being placed in a 45°C water bath for 20 minutes. Samples were then removed from the water bath and remained at room temperature for an additional 30 minutes. (The above fixation method is adapted from Peracchia's and Mittler's (1972) glutaraldehyde procedures). Tissue was rinsed briefly in three changes of buffer, then allowed to soak in buffer for four hours with frequent buffer changes, before being placed in 2% unbuffered OsO_4 , overnight, at 4°C. The following day samples were subjected to gradual dehydration to 100% acetone; then to acetone/propylene oxide (1:1, v/v), and to 100% propylene oxide. The samples were placed in a 1:1 (v/v) mixture of Epon (epoxy resin) and propylene oxide and left loosely covered at room temperature for two days to allow the propylene oxide to evaporate. Individual samples were transferred to capsules containing 100% Epon and placed in a 60°C oven for 2 days to harden. Sections were cut from blocks with a diamond knife on a Porter-Blum MT-2 ultra microtome, and mounted on bare copper grids (300 mesh and 75x300 mesh). Sections were stained in drops of uranyl acetate (saturated aqueous solution) for 10 minutes, rinsed and post stained in lead citrate (Reynolds, 1963). The sections were examined with a Zeiss 9S electron microscope operated at 60 kV.

Digger Pine Infected with Digger Pine Dwarf Mistletoe

Branches of *Pinus sabiniana* Dougl. with localized infections of *Arceuthobium occidentale* Englemann were obtained from container specimens maintained in a lath house at U.C. Berkeley. Samples were obtained in July and September, 1976. Four to five year-old stem regions which showed localized dwarf mistletoe infections were cut in cross sections while bathed in fixative. Two-mm pieces were taken from the cambium and phloem regions. The fixatives employed were 5% glutaraldehyde in 0.1M potassium phosphate buffer (pH 7.0) for the July collection and 4% glutaraldehyde + 3% paraformaldehyde in 0.04M potassium phosphate buffer (pH 7.0) for the September collection. Samples were aspirated moderately off and on for 15 minutes followed by continuous mild aspiration for an additional 30 minutes. (Tissue was kept submerged by vinyl netting). The September samples were left for an additional one hour period without aspiration. Samples were rinsed with buffer and placed in unbuffered 2% OsO_4 at room temperature for five hours. Fixed tissue was rinsed briefly then dehydrated gradually through an acetone series followed by infiltration with a low-viscosity, acetone-soluble epoxy resin (E. Fullam, Inc.) which proceeded from an acetone/resin series to 100% resin. Embedded tissue was hardened in pans at 70°C for two days. Sections were cut and mounted as above and stained for two hours in 2% aqueous uranyl acetate; rinsed, and post stained for 3-10 minutes with lead citrate (Reynolds, 1963). Specimens were examined with a RCA III electron microscope at 50kV and a Philips 300 electron

microscope operated at 60 or 80kV.

Sugar Pine Infected with Sugar Pine Dwarf Mistletoe

Pinus lambertiana Dougl. with localized infections of *Arceuthobium californicum* Hawksw. & Wiens were collected by Dr. John Parmeter on the western slopes of the Sierra-Nevada foothills in late October, 1976. Branches were kept in water for four days before fixation. The fixation method employed was adapted from techniques used for phloem fixation by Neuberger and Evert (1974) and Parthasarathy (1974b). The primary fixative was in 6% w/v paraformaldehyde and 6% v/v glutaraldehyde in 0.08M sodium cacodylate buffer (pH7.0). Infected branch segments about five years old were cut into 3 cm pieces. While bathed in fixative, the outer bark was cut off and pieces were split longitudinally in quarters or less and placed in fresh fixative. After one hour the middle 1cm of each stem piece was removed and the ends discarded. Retained pieces were placed in fresh fixative for 1½ hours. The samples were trimmed again (discarding ends, sides and xylem portions) to obtain pieces of infected phloem and cambium that were about 2mm per side. These small pieces were placed in fresh fixative for 16 hours during which time the fixative was replaced with fresh fixative 3 times. Following aldehyde fixation samples were soaked for four hours in cacodylate buffer (with three changes to fresh buffer). Tissue was post fixed in cacodylate-buffered 2% OsO₄ for four hours at room temperature, rinsed briefly in water and dehydrated, embedded, polymerized,

sectioned and stained as described above for *P. sabiniana*/*A. occidentale* tissue. Sections were examined with a Philips 300 electron microscope operated at 60 or 80 kV.

OBSERVATIONS

Cytological features of sinker tissue will be considered first. There was a commonality of sinker cell structure in all three species of *Arceuthobium* despite fixation differences; therefore all observations are generally applicable to *A. tsugense*, *A. occidentale*, and *A. californicum*, but exceptions are indicated. Following the report of cytological features of *Arceuthobium* are observations on cell wall characteristics at the host/parasite interface. Lastly, certain features of host cells, contiguous to parasite cells are presented.

Please refer to the Glossary (pp. 224-238) for explanation of terms.

Dwarf Mistletoe Tissue

Plastids. The plastid structure of *Arceuthobium* sinker cells are highly characteristic and may be used to help identify parasite cells among host cells. The plastids are generally ovoid or round (PL, Figs. 3.1, 3.2). Only rarely were they cupped around portions of cytoplasm (PL, cy, Fig. 3.3). Plastids are surrounded by two outer membranes; the internal membranes contained a few thylakoids (th) occasionally organized into small grana (gr) Figs. 3.1). Latticed or tubular prolamellar bodies are common in

Arceuthobium plastids (Fig. 2.1 (P), 3.2) and a few membraneous sheets extend out from the central paracrystalline structure (unlabelled arrows Fig. 2.1). Numerous, small, extremely electron-dense, plastoglobuli are contained within the plastid stroma (Figs. 3.1-3.4, 3.10, 3.15, 3.22). Ferritin granules, sometimes in large deposits, are seen in some plastids (f, Figs. 3.1, 3.3). DNA regions were not detected in the plastids. Most sinker cell plastids had no starch. However, a few starch grains were seen in plastids of meristematic cells in the center of a large sinker (s, Fig. 3.22).

Mitochondria. Most sinker cells have abundant mitochondria. *Arceuthobium* mitochondria are unusual in appearance and like the plastids, may serve as a reliable identifying factor for parasite cells. The mitochondria are round or ovoid and are about the same size as the plastids (M, Fig. 3.2, 3.3, 3.5, 3.7). The most outstanding feature is the central electron-lucent nucleic acid region containing coalesced DNA strands (M, dna, Figs. 3.1, 3.2, 3.20). Cristae are rather indistinct in the cacodylate-buffered material as compared to the heat-treated, phosphate-buffered material (cf. 3.1 and 3.12 at M). It appears that the internal membranes are numerous and unordered.

Nuclei. Plant nuclei can be divided into two basic nuclear types: *chromocentric* and *reticulate*. An extensive description of these nuclear types has been prepared by Lafontaine (1974). *Arceuthobium* spp. have chromocentric nuclei while their coniferous

hosts have the reticulate type (Chapters I, II). In keeping with the classic chromocentric structure, the nuclei of *Arceuthobium* have dense heterochromatin masses (chromocenters) associated with the internal nuclear membrane (h, Fig. 3.4) and/or around the nucleolus (cc, Figs. 3.3, 3.5, 3.34). Heterochromatin is composed of condensed DNA coated with densely-staining histones. Smaller heterochromatin clumps are distributed evenly throughout the nucleoplasm (Figs. 3.2, 3.3). Euchromatin, or uncondensed DNA, occupies the lucent regions of the nucleus and lends a finely-fibrillar texture to the nucleoplasm (eu, Fig. 3.4). Micropuffs are common in *Arceuthobium* nuclei within euchromatin regions (mp, Figs. 3.3, 3.5). Micropuffs are not well studied in plant nuclei. Reports are limited to observations on reticulate nuclear types. Micropuffs are said to be associated with the heterochromatin reticulum. It is theorized that they represent de-repressed sites of an associated portion of a condensed chromosome. This hypothesis is supported by histochemical results which indicate that the micropuffs are comprised of DNA, RNA, and protein (Lafontaine, 1974 and lit. cited therein). However, in *Arceuthobium*, micropuffs are *not* associated with heterochromatin; therefore the reported association of micropuffs with heterochromatin in species having reticulate nuclei is apparently not a fundamental micropuff morphological feature.

Nuclei of *Arceuthobium* may have small lobes that frequently correspond to sites of chromocenter attachment to the nuclear membrane

(Figs. 3.2, 3.3, 3.5, 3.34).

The interphase nucleus has one conspicuous nucleolus that exhibits different zones of electron density and a few electron lucent lacunae (Figs. 3.5, 3.7, 3.34 at n).

Since host and parasite cells have morphologically distinct nuclei (see Fig. 3.5) nuclear structural features usually are helpful in distinguishing between the two organisms (Chapter I and II). However, nuclear structure is dynamic--as transcription, replication and mitotic activities vary throughout the cell cycle. Clear-cut chromocentric and reticulate nuclear structure is not found in all cells, particularly those positioned near the cambium or meristematic regions of the sinker. For example, Figs. 3.6, 3.7 show parasite nuclei (Nu) without any predominate heterochromatin details, and the host nuclei in adjoining cells have variable amounts of heterochromatin.

Lipid Bodies. Most *Arceuthobium* cells have an abundant supply of very electron-dense lipid bodies (droplets) in contrast to nearby host cells without such dense bodies. The droplets are of various sizes and are distributed randomly through the cytoplasm. The extreme osmium-reducing nature of the droplets is indicative of a highly unsaturated type of lipid (Dawes, 1971). Although most droplets have distinct boundaries, they are not membrane bound. Occasionally a droplet will have a fuzzy border (unlabelled arrow, Figs. 3.10, 3.11) a feature which may be indicative of enzymatic activity at the lipid/cytoplasm interface (L, Figs. 3.2, 3.6, 3.7, 3.14, 3.15, 3.26).

The presence of dense lipid bodies cannot always be relied upon for accurate identification of parasite cells. Sectional views of some sinker cells show little or no osmiophilic lipid droplets free in the cytoplasm (Figs. 3.3, 3.5, 3.22). Meristematic cells in the center of large sinkers or near the host vascular cambium often have relatively minor lipid stores.

Homogeneously-textured, light-grey bodies 0.1 to 0.3 μ in diameter sometimes are found in direct contact with the black lipid droplets (Figs. 3.11, 3.19, 3.21 at b). In most cases these bodies did not appear to be membrane bound, but in a few cases there was a suggestion of a thin boundary membrane (Fig. 3.11 at arrowhead). The rounded or ovoid bodies assumed a lenticular shape when sandwiched between a vacuole and a large, dense lipid body. Similar lenticular structures, associated with lipid bodies and having an unknown function, were seen in *A. pusillum* sinker cells (Tainter, 1971).

Endoplasmic Reticulum. Sinker cells have a large amount of endoplasmic reticulum (ER) (Figs. 3.8, 3.13-3.15).

Smooth surfaced ER is more abundant, but since ribosomes were not particularly well preserved or stained in most samples, the apparent scarcity of rough ER may not reflect the *in vivo* condition. Peripheral regions of the cells often have lamellar arrays of ER parallel to the cell wall (Figs. 3.8, 3.14, 3.26). These arrays frequently are present parallel to juxtaposed host cells (Figs. 3.8, 3.15). Tubular, smooth ER is also abundant at both interspecific

(Fig. 3.32) and intraspecific (Fig. 3.25) junctions. Tubules of ER and extensions from ER sheets fuse with invaginations of the plasmalemma (Figs. 3.1, 3.4, 3.9, 3.25 at EN) in a manner consistent with the endomembrane concept presented by Morré and Mollenhauer (1974). The endomembrane concept hypothesizes a developmental continuum of membranous cell components from the cell surface to the internal regions of the cell. These apparent membrane continuities provide minute openings between the cell wall and the ER cisternate, and in a sense, extend the apoplastic space of the cell wall into the cell interior.

Golgi Bodies. Golgi bodies are common organelles in parasite cells, but they show no unusual features (g or gb, Figs. 3.13, 3.19).

Vacuoles. Most sinker cells have small vacuoles (v, Figs. 3.3, 3.13, 3.14, 3.22). However, sinker sheath cells in the region of host non-conducting phloem develop a large central vacuole with electron-dense vacuolar material (v, Fig. 3.30) (Chapter II). Cell vacuoles of *Arceuthobium tsugense* contained amorphous material and were expanded (v, Fig. 3.13-3.15). The dwarf mistletoe tissue in the pine samples, on the other hand, usually had partially collapsed vacuoles (v, Fig. 3.2, 3.3). Sometimes the tonoplast had completely collapsed to a flattened shape (vm, Fig. 3.24). These fixation images suggest that many "vacuoles" in sinker cells are not individual, closed osmotic bags; but rather, the vesicles may be portions of an open-ended ER system from which water, during fixation, exits laterally to produce a flattened membrane profile.

Plasmalemma. All sinker cell plasma membranes were very irregular with many small invaginated pockets (Figs. 3.1 (pm), 3.4, 3.8, 3.9, (in), 3.13). Between the plasmalemma and the dense cell wall material there is an electron lucent region containing a network of randomly arranged microfibrils (fi, Figs. 3.1, 3.9).

Saccules. A complex specialization of membranes and wall material is seen in some parasite cells. From a relatively narrow orifice at the plasmalemma in the region of a primary pit field (o, Fig. 3.20, 3.21) saccules invaginate into the cytoplasm (sa, Figs. 3.19-3.23). These saccules are filled with a fibrillar component that is identical to and confluent with the randomly arranged cell wall fibrils in primary pit fields. The saccules usually cluster together in a rounded mass that may extend deep into the cell interior. Certain section planes may give views of saccules isolated from their wall origin (sa, Figs. 3.10, 3.15, 3.22). The membrane bound saccules are subject to shrinkage during fixation, leaving more or less collapsed units surrounded by an empty space (sa, Figs. 3.10, 3.15, 3.20, 3.22). The phosphate-buffered tissue of *A. tsugense* had more severely shrunken saccules (cf. 3.16 and 3.20). Saccule development appears to be a specialization of certain cells since only a minority of parasite sinker cells had the structures. Furthermore, saccules seemed to be developed more extensively on one side of the common pit field (Figs. 3.19-3.21).

It was not determined if these saccules end blindly or if, in keeping with the endomembrane concept (Morré and Mollenhauer, 1974),

they have continuities with other membraneous structures such as vacuoles and ER. A possible relationship between the saccules and smooth ER is seen in Fig. 3.10, which shows a system of meandering, compressed sheets of smooth ER (ser) that is particularly concentrated near a collection of shrunken saccules (sac). Osmiophilic materials (lipids?) are sequestered within these membranes.

Ribosomes. Ribosomes were found associated with portions of ER (Fig. 3.26), clustered in small groups or chains as polyribosomes (Fig. 3.1 (r), 3.21), or individually distributed in the cytoplasm (r, Fig. 3.9). Ribosomes were not particularly abundant in the parasite tissue examined for this study nor were they well preserved and stained. A granular background stain artifact often obscured the ribosomal population.

Microtubules. Profiles of microtubules (MT) in cross and longitudinal section were occasionally found in sinker cells (mt, Fig. 3.1, 3.4). Since MT are labile structures--having, for example, sensitivities to ion concentrations and osmium (Pickett-Heaps, 1974)--their scarcity in this study may be due to disassociation during osmium fixation.

Microfilaments. Microfilaments were occasionally seen in sinker cells. (mf), Fig. 3.24 shows a large bundle of microfilaments.

Plasmodesmata. Symplastic continuities in the form of plasmodesmata are usually concentrated in thin-walled primary pit field in the cell walls between adjoining parasite cells (pd, Figs. 3.3, 3.12). On the end walls of radially-oriented, uniseriate sinkers

there may be no pits and plasmodesmata are distributed across the rather thin periclinal wall (pd, Fig. 3.8). Most plasmodesmata are the branched type, typified by several plasmodesmata converging to a common median cavity in the middle lamella. Sometimes the middle lamella region of a pit is extensively eroded (pd, Fig. 3.23). This feature, combined with irregular wall surfaces, saccule invaginations, and ER continuities (as discussed above) makes many intraspecific junctions very complex with tremendous membrane surface area associated with the site (Figs. 3.4, 3.22, 3.23, 3.25).

Cell Walls. Meristematic sinker cells--those located at the host cambial zone (Figs. 3.6, 3.8) or in the center of large infected rays (dW, Fig. 3.22)--have thin, primary walls. As the parasite cells age, they may acquire thick, dense walls (Figs. 3.3, 3.14). The parasite walls in the infected rays of hemlock were denser in appearance and 2-4 times as thick as contiguous host walls. Infected pine material did not show this same relationship: Contiguous host and parasite walls often had walls similar in thickness and stain affinity (Figs. 3.2, 3.5, 3.26). Sometimes the common walls between adjacent parasite cells were thicker than at interspecific junctions. This intraspecific wall thickening appears to be the result of swelling of matrix material in the outer regions of the primary walls and middle lamella regions (Figs. 3.3 at eCW). An electron-dense stain precipitate was selectively deposited in the expanded regions. This selective deposition indicates the presence of negatively charged

sites in the wall that trap and nucleate lead ions. Similar lead precipitates are selectively deposited in certain regions of healthy pine sieve cell walls (Chapter IV).

Interspecific Wall Specializations

Cell walls of juxtaposed host and parasite cells are fused and a normal-appearing middle lamella region develops between the two organisms (*e.g.* Fig. 3.2, 3.26, 3.31).

Localized thin regions in the common cell walls at host/parasite interfaces were reported in previous light microscope studies (Chapter II); at lower magnifications these regions resemble primary pit fields. However, electron microscopy shows that they differ from primary pit fields in that complete plasmodesmata do not penetrate the thick, cell walls (Fig. 3.16, 3.26). One exception to this was found: Figs. 3.29 and 3.30 show a single plasmodesmata-like channel (apparently originating from the parasite cell) which joins with a cluster of host half-plasmodesmata. In the infected pine samples, the *Arceuthobium* wall was very thin at the sites while the pine wall was not reduced in thickness (wt, Figs. 3.26, 3.28, 3.31). Half-plasmodesmata initiated from the host protoplast penetrated the interspecific wall region to the middle lamella and abruptly stopped upon meeting the very thin parasite wall portion (hpd, Figs. 3.26, 3.28, 3.31).

These interspecific wall specializations were fairly common in infected pine tissue at the interspecific junctions of rays (Figs. 3.5, 3.26) and cambial cells (Figs. 3.6). Wall speciali-

zations between pine sieve elements and *Arceuthobium* cells were not common, in one case an extremely thin, imperforate parasite wall opposed a one-sided sieve area (Fig. 3.32). The sieve area in Fig. 3.32 is heavily callosed (ca), as were all sieve areas. The callose deposit ended slightly away from the actual interspecific wall junction.

Where a dwarf mistletoe cell is directly contiguous with a pine xylem element it is not uncommon for a half-bordered pit to be formed next to the parasite cell (bp, Fig. 3.7). The parasite cell does not develop thin wall regions opposite the host's bordered pit (Fig. 3.8). But the parasite's plasmalemma and wall show extensive invaginations and laminar ER arrays opposite the half-bordered pit (Fig. 3.8, 3.9, in).

Only a few pit-like regions were seen in ultra thin sections of infected hemlock samples. In the example shown in Fig. 3.16, both host and parasite walls are thin at the pit, but the parasite wall is thinner (wt, Fig. 3.16). The plasmalemma of *A. tsugense* maintains a close, crenulated association with the surface of the cell wall at the thinned-out wall region (Fig. 3.16, arrows); while, on the opposite side of the wall thinning, the host membrane has retracted from the wall during fixation. No half-plasmodesmata were found between *A. tsugense* and *T. heterophylla* cells. But because few pit-like regions were actually seen, no generalized conclusions can be made about the existence of plasmodesmata between *A. tsugense* and *T. heterophylla* cells.

Hemlock Ray Tissue

The Protoplast. Hemlock tissue infected with dwarf mistletoe was subjected to an unusual fixation method that had been claimed to improve fixation quality in animal tissues (Peracchia and Mittler, 1972) but had not been previously tested on plant tissue. The major divergence of the Peracchia and Mittler technique over conventional fixation is a brief soaking in glutaraldehyde heated to 45°C. In my opinion, the hemlock tissue did not show any improvement in fixation quality when compared to pine material fixed in a more conventional manner. However, some interesting modifications directly attributable to heat were produced. The heating apparently hydrolyzed the starch in plastids to soluble carbohydrates which were subsequently lost. The electron lucent starch grains normally present in ray plastids (*e.g.*, see Fig. 3.5, at s) are completely gone and the space is occupied by a filamentous substance that has condensed into vermiform tubes (F, Figs. 3.15, 3.17). Sometimes the membrane integrity of the plastids was lost and the filamentous substance became confluent with the cytoplasm (arrow, Figs. 3.18). The rest of the plastid appeared well-preserved and consisted of plastid stroma housing an extensive, but faint, internal membrane system which only rarely was organized into laminar configurations or tubular arrays (Fig. 3.17). Small, dense osmiophilic plastoglobuli (pl) are found in the plastid stroma. Plastids in hemlock usually were ovoid in apparent accommodation to the once inflexible starch grain. Occasionally plastids had tail-like extensions from an ovoid,

starch-bearing "head" (Fig. 3.17).

In contrast to the severely altered plastids, hemlock mitochondria were typical in structure and comparatively well preserved (M, Figs. 3.14, 3.16, 3.18). Sometimes mitochondria were shown to be quite elongate (Fig. 3.14). The hemlock ray tissue also had moderate amounts of irregularly organized endoplasmic reticulum with loosely associated ribosomes (ER, Fig. 3.17). Golgi bodies were infrequently seen. Hemlock nuclei are the reticulate type with a prominent, single nucleolus.

The hemlock cells associated with parasite cells had numerous small vacuoles, many of which appeared extensively shrunken (v, Fig. 3.14). In general, the more highly vacuolate hemlock material suffered poorer fixation than the adjacent dwarf mistletoe tissue, which in comparison displays relatively minor shrinkage (Figs. 3.14, 3.15). This may indicate that the parasite tissue was more osmotically balanced to the rather high solute fixative media.

The hemlock cells had many lipid-containing bodies which I consider to be typical, membrane-bound spherosomes. Spherosomes originate by pinching off from ER. As lipids are produced they accumulate lipid between the protein layers of the single, surrounding unit membrane (Matile, 1974); thus the outer membranes of spherosomes are often indistinct because the formerly trilaminate structure of the original ER membrane has been bloated by lipid accumulation in the middle lipid layer. When intermediate stages can be found, the spherosome origin of lipid-containing bodies is easier to access

(re: Matile, 1974) (Fig. 3.6, 3.34, i), but in hemlock no membranes, and no intermediate stages were seen. However, the relatively small, uniform size of the lipid bodies, and the fact that they are not randomly dispersed but lined up in single file around the cell's periphery (Figs. 3.14, 3.15) suggests they are true sphaerosomes. The lipid in hemlock appears to be more saturated than the lipid stores in the parasite, since the former has less affinity for osmium ions than the latter.

Cells Walls and Plasmodesmata. In the material examined, the hemlock ray cell walls were thin in comparison to walls of *Arceuthobium* and fairly electron lucent (Fig. 3.14). Typically, there is a thin, dark, innermost layer from which the plasmalemma has retracted somewhat (Fig. 3.14, 3.16, 3.17). Plasmodesmata between contiguous hemlock ray cells occurred in clusters, but not necessarily in wall depressions or primary pit fields. Typically several plasmodesmata converged to a median nodule or cavity in the middle lamella (pd, Fig. 3.18).

Pine Ray Tissue

The following observations pertain to pine cells in infected rays only. Uninfected rays in infected stems were not studied.

The Protoplast. Pine ray cells in infected rays are readily distinguishable from parasite cells by their characteristic plastids. Plastids of *P. sabiniana* are polymorphic, usually with long, flattened tails and a more expanded head that houses inclusions consisting of small lipid droplets, small starch grains, and/or polygonal crystals

(PL, Fig. 3.33). Often the flattened plastid portion cups around portions of cytoplasm (Fig. 3.26, 3.33). The internal membrane system of the plastids of *P. sabiniana* are extensive and cristae-like, with some organization into thylakoid-like sheets (PL, Fig. 3.26, 3.31, 3.33). The plastids of *P. lambertiana* were not found to be as polymorphic as those of *P. sabiniana*, and tail-like extensions were not common. In general, the shape of the plastids in *P. lambertiana* appeared to be dictated by the relatively large, lenticular starch grains (s, Fig. 3.5). Polymorphic crystals, abundant in the *P. sabiniana* ray plastids were not seen in *P. lambertiana*.

Mitochondria in *P. sabiniana* infected ray cells appear so similar to cross sections of plastid tails that it was often not possible to distinguish with certainty between the two organelles (Figs. 3.26). Mitochondria of *P. lambertiana* were subtly distinctive from plastids however. They appear slightly swollen with an electron lucent background containing tubular cristae (compare PL and M in Fig. 3.5). The differences in mitochondrial structure in the two pines may be related to the fact that *P. sabiniana* was fixed with phosphate-buffered aldehyde, while *P. lambertiana* was fixed with cacodylate-buffered aldehyde. DNA regions typical of all parasite mitochondria were not detected in pine ray cell mitochondria.

Like the hemlock tissue, both of the pine species had abundant lipid stores in what may be spherosomes. The spherosome nature of the lipid bodies in phloem ray cells may be deduced from the occur-

rence of similar bodies in cambial cells which demonstrate the characteristic structure of young spherosomes. Lipid bodies in cambial cells have a central core surrounded by remnants of the inner leaflet of a unit membrane (Fig. 3.6 arrow). Unlike the hemlock spherosomes which were frequently lined along the periphery, pine spherosomes were randomly distributed through the ray cell (sp, Figs. 3.26, 3.30, 3.33).

A fairly abundant ribosome population was found in the infected pine ray cells. (This is in contrast to a sparser, indistinct ribosome population in adjacent dwarf mistletoe cells and is perhaps due to fixation differences). Ribosomes in pine cells of infected rays were distributed in loose clumps or vague strings (Fig. 3.31); this pattern differs both from the tighter polyribosome clustering in healthy albuminous cells and the free, randomly distributed ribosomal population in other healthy ray cells (Chapter IV; Sauter, *et al.*, 1976).

Host ray cells had small amounts of ER that showed no particular organization in the cell (Fig. 3.31); healthy pine ray cells also have small amounts of ER (Chapter IV).

Pine cells have nuclei that may be classified as "reticulate" according to the description of nuclear types given in a recent review of plant nuclei (Lafontaine, 1974). Nuclei of infected ray cells have (as is typical of reticulate nuclear types) a loose reticulum of heterochromatin throughout the nucleoplasm, which appears disconnected in thin section (Fig. 3.5, 3.33). This

heterochromatin reticulum is heavier than the more delicate and disjointed-appearing reticulum found in the nuclei of *Arceuthobium*. Also, there are few chromocenter masses in the pine cell nuclei, while sinker cell nuclei have several. In general, nuclei of infected ray cells are similar in appearance to the nuclei in healthy albuminous cells of rays: Both usually have less condensed chromatin than other ray cells (compare Figs. 3.33 and 4.58 with 4.62).

Cell Walls and Plasmodesmata. Pine cell walls in infected rays were similar in stain affinity, thickness, and texture to the walls of adjacent parasite cells (Figs. 3.2, 3.26). In the instances where intraspecific plasmodesmata were found between adjacent pine ray cells, the plasmodesmata were in groups, but not in wall depressions or primary pit fields (hpd, Figs. 3.26-3.31). The plasmodesmata from juxtaposed host cells often converged to chambers in eroded regions of the middle lamella. Similar half-plasmodesmata groups that converge to eroded regions of middle lamella are seen opposite thin walled regions of juxtaposed parasite cells (hpd, Figs. 3.31).

DISCUSSION

Nutrient Transfer and the Structure of the Host/Parasite Interface

A major goal of my anatomical studies of *Arceuthobium* was to gain an understanding of host/parasite tissue relationships and cytological features of both host and parasite cells. It is hoped that knowledge of the structure of dwarf mistletoe infected tissue

would contribute to comprehension of the functional interactions of host and parasite, particularly in regards to interspecific translocation of nutrients. I believe this goal has been largely achieved through coupling information on cell and tissue organization revealed in optical studies (Chapters I, II) with observations of cell substructure derived from the present electron microscope study.

It became apparent from the light microscope studies that sinker cells of *Arceuthobium* thoroughly integrate with host ray cells in the region of host cambium and functional phloem, but actual contact with the hosts' functional sieve cells is limited (Chapter II). Important questions left unresolved after the light microscope studies are: (1) What cytological features contribute to the parasite's perennial success as an obligate parasite? (2) What is the nature of pit-like regions that are found between contiguous host and parasite cells? (3) What specific cytological features of the host/parasite interface may facilitate the acquisition of host-originating nutrients?

The electron microscope study confirmed that sinkers are integrated into host rays in an intimate fashion with little disruption or damage to adjacent host vascular tissues. Sinker tissue is composed predominately of parenchymatous cells with some centrally located xylem. The parenchyma cells of sinkers demonstrate a high volume of cytoplasm relative to the vacuolar volume; they have abundant

ER systems and they have large plastid and mitochondria populations. However, the ribosomal population was small (which may or may not be an artifact of fixation). Most sinker cells had copious lipid stores, but only rarely were small starch grains observed. The plasma membrane area is large relative to cell volume in most sinker cells due to the irregular wall surface and correspondingly irregular plasmalemma. These cytological features are similar to those of metabolically active plant cells such as transfer cells (Gunning and Pate, 1974) and gland cells (Schnepf, 1974, pg. 332-333). Thus, in reference to question one above, the success of *Arceuthobium* as an obligate parasite may be due to its ability to integrate into the host stem without major disruption of host tissues. Furthermore, the morphologically simple, yet pervasive endophytic form of the parasite, and the apparently metabolically active cellular nature of the sinkers may contribute to the adaptability of the parasite to host tissue and provide a competitive advantage for space and nutrients within the host stem.

In the present investigation, attention has been given to the ultrastructure of the host/parasite interface in an attempt to obtain information concerning the structural basis for nutrient transfer (re: questions two and three above). Particular attention was given to areas having pit-like depressions in cell walls. The host/parasite interfaces of three species of *Arceuthobium* and their respective host species were examined. Some variation in the

substructure of the pit-like regions was found in different species. Although in all cases, the parasite wall was thinner than the host wall at the sites. Plasmodesmatal channels were not found in the thin-walled regions of *A. tsugense* and *Tsuga heterophylla*. But it is possible that more extensive electron microscopy may reveal variation in the substructure of the thin-walled regions. In contrast, groups of plasmodesmatal-like channels forming half-plasmodesmata were found occasionally on the host side of contiguous walls of *A. occidentalis* with *Pinus sabiniana* and *A. californicum* with *Tsuga heterophylla*. In one instance a single half-plasmodesmatal-like channel, apparently from the *A. occidentalis* protoplasm, was found in the parasite's thin-walled region (Figs. 3.29, 3.30). This parasite plasmodesma matched up with a single half-plasmodesma of a set of half-plasmodesmata on the host side. Continuity of plasmodesmata through the middle lamella regions is possible, but lack of membrane resolution in the micrograph forestalls conclusions.

Plasmatic connection has been suggested (Tainter, 1971) or implied (Hull and Leonard, 1964a, p. 1006) as the method by which *Arceuthobium* obtains host-originating photosynthate. However, the evidence from this study affords no sound basis for presuming that complete plasmatic channels occur between dwarf mistletoes and their hosts. If complete channels do occur (as in Fig. 3.29), they are rare events.

Formation of interspecific plasmatic channels would necessitate interspecific membrane fusion. Fusions of diverse organisms have been achieved *in vitro* with certain animal cell combinations and naked plant protoplast combinations. The event requires the involvement of viruses, lysolecithin, or other substances that alter membrane charge distribution (Wallach, 1972, p. 27-28; Kao, *et al.*, 1974). *In situ* vegetative fusion of protoplasts of diverse plant organisms has been frequently presumed, particularly in regard to symplastic (cytoplasmic) unity of stock and scion in graft unions (Neilson-Jones, 1969). But these presumptions are not supported with sound cytological evidence (see Carr, 1976, p. 254-5). Pits, with what appear to be connecting threads, seen at interspecific junctions with the light microscope do not necessarily imply symplastic continuity between two organisms. For example, pit-like regions are found in the cell walls at the interspecific junction of various periclinal chimeras (Buder, 1911; Hume, 1913; Burgess, 1972). Plasmatic connections appeared to the early investigators, to penetrate the pits. However, an ultrastructure study of one of the same chimeras studied by both Buder and Hume, showed that the pits seen at the interspecific junction are usually unperforated (Burgess, 1972). (By way of comparison, they are thus similar to those described herein, between *A. tsugense* and *Tsuga heterophylla*). Occasionally, the chimera pits were eroded by half-plasmodesmatal channels. (In this case, they are similar to those seen in this study and to those reported by Tainter (1971) between *A. pusillum* and *Picea*

mariana). On rare occasions, half-plasmodesmata at a chimera interface were found to match-up, forming what might be loosely interpreted as a complete plasmodesmata (as in Figs. 3.29, 3.30 of this study). However, because of fixation problems, and the inherent difficulty in resolving plasmodesmatal substructure, the presence or absence of interspecific membrane continuity through the matched half-plasmodesmata could not be determined (Burgess, 1972).

Substantial interspecific translocation of photosynthate does not appear to necessitate symplastic continuity. In my opinion and also that of Kuijt (1977) there is, at this time, no convincing ultrastructural evidence that plasmatic connections form between hosts and their phanerogamic parasites. But there is good evidence that plasmatic connections may form in certain mycoparasitic associations of fungi (Hoch, 1977). The question of plasmatic connections between higher plant parasites and hosts is not resolved, however. For example, there is conflicting evidence pertaining to the nature of the union between *Cuscuta* and hosts. Dörr (1968, 1972, 1974) has published electron micrographic studies which show that cells of *Cuscuta* make elaborate modifications at the point of contact between haustoria and host sieve elements. However, she found no evidence of plasmatic connections between host phloem cells and the parasite contact cells. But indirect evidence from other sources suggests that *Cuscuta* does make plasmatic connections with host phloem: *Cuscuta* has been found to act as a very efficient vector

for mycoplasma-like organisms (MLOs) (Nienhaus and Sikora, 1979). When allowed to form dual infections on a MLO-diseased host and a MLO-free host, the MLOs are readily transferred to the originally MLO-free host. MLOs are found almost exclusively within living phloem cells of their hosts. Thus, it would seem that protoplasmic continuities would have to exist between *Cuscuta* haustoria and host phloem for transfer to occur.

Even though matching of half-plasmodesmata may sometimes occur at the interface of *Arceuthobium* cells and host cells, it is, in my opinion, highly unlikely that such rare plasmatic continuities are important channels for nutrient transfer. Furthermore, the thin-walled, pit-like regions that are found between *Arceuthobium* spp. and hosts may not be major sites for nutrient transfer, since (although they are not uncommon) the pit-like regions are not plentiful.

In absence of plasmatic continuity, other mechanisms involving symplast/apoplast (*i.e.*, cytoplasm/cell wall) interchanges must be operating in tissue infected with dwarf mistletoe. It may be that host cells become abnormally leaky, and release nutrients into the apoplast common to both organisms. In this manner nutrients are made available for absorption around the entire surface of parasite tissue.

The low water potential values* known to be generated by aerial shoots of *Arceuthobium* (Fisher, 1975) would tend to channel apoplastic

* Please see the footnote on the following page for an explanation of water potential.

nutrients to regions surrounding parasite cells and allow nutrients to move eventually into the aerial shoots (Alosi, 1979).

Leakage of nutrients out of host cells and into the common apoplast could be caused by elevated hormone levels in the diseased tissue. Externally applied indole acetic acid (IAA) is known to increase glucose leakage out of plant cells (Simon, 1974). Hormone imbalances in trees bearing dwarf mistletoe infections has long been suspected (Kuijt, 1960, p. 385; Agrios, 1969, p. 394) because of the abnormal growth forms of the infected branches. Recently, elevated cytokinin levels have been found to be associated with branches having *Arceuthobium* infections (Paquet, 1979). Cytokinins often act in concert with IAA, and it is possible that increased hormone levels at infection sites may cause host phloem to leak sugars.

In both the apoplast and the symplast the water potential (ψ) may be estimated by the formula $\psi = P - \pi$ (White, 1974), where P is usually thought of as a mechanical pressure and π is the solute concentration expressed as osmotic pressure. In the apoplast, P often has a negative value due to tension on the water column generated by transpiration. In the symplast, P usually is positive due to the rigidity of the cell wall relative to turgor pressure. Water flows in the direction of lower water potential. Thus, an increase in solute concentration (π) and/or a decrease in pressure (P) at one site in the system reduces the water potential value and allows flow towards the region of lower water potential.

Modifications for acquisition of nutrients from the apoplast seem to be present in sinker tissue. The sinker cells have very irregular inner cell wall surfaces with closely associated plasma-lemma (Fig. 3.9). This modification increases the absorbing surface of the parasite cells in relationship to the surrounding apoplast. Sinker cell walls are often thicker than host cell walls (Figs. 3.3, 3.14). Thick cell walls increase the apoplastic holding capacity surrounding the *Arceuthobium* protoplast, a modification which is probably important for maintenance of flux across the membrane actuated by a large membrane surface area.

These features of *Arceuthobium* sinker cells are reminiscent of wall/membrane modifications of transfer cells (Pate and Gunning, 1969) although in typical transfer cells, the wall ingrowths are much larger than the irregular wall projections of sinker cells.

It is a widely accepted hypothesis that the wall/membrane modifications of transfer cells function to intensify solute flux between the apoplast/symplast systems (Gunning and Pate, 1974). This concensus has developed as a result of extensive anatomical studies which consistently link transfer cells with regions of the plant body where absorption or secretion is known to occur or is presumed to occur (*e.g.*, Pate and Gunning, 1969; Dörr, 1974; Gunning and Pate, 1974; Jones and Gunning, 1976). However, quantitative accessment of solute flux augmentation is not yet available.

There has been relatively little speculation about the actual mechanism of flux intensification. But some discussion on mechanisms

has been presented in a review article by Gunning and Pate (1974). The most attractive hypothesis they present has been linked with the theory of a "standing-gradient osmotic flow" first applied to animal tissues (Diamond and Bossert, 1967). The hypothetical mechanism as applied to absorbing transfer cells, involves active transport and osmosis *at the membrane site*, and mass flow *on either side of the membrane*. According to the theory, solute pumps at the membrane withdraw solutes from the apoplast and transfer them to the symplast during absorption. (For secretory functions, the solutes are pumped from the symplast to the apoplast). The change in water potential* actuated by solute pumping causes water to follow in the direction of the pumped-solute flux. This spontaneous movement of water (*i.e.*, without added energy) allows a *mass flow* of all free substances dissolved in the water in the direction of the membrane. The mass movement, consequently, greatly increases rates of solute movement toward the pumps in comparison to rates attainable by diffusion alone.

In order for a *dynamic* steady state situation to develop, the continual intake of water into a receptacle (either symplast or apoplast, depending on whether absorption or secretion is occurring) must be balanced by export of water at another site of

*For an explanation of the components of water potential see the footnote on pg. 89 .

the receptacle. If the receptacle is the symplast, water may exit *via* the plasmodesmata, especially if the adjoining cell has a lower water potential. If the receptacle is the apoplast, water ultimately exits through the free space of the cell wall towards evaporative surfaces (such as those along intercellular spaces in leaves or at lenticle sites on the stem).

Sinker cell membrane surface area is augmented at primary pit field regions between some contiguous parasite cells by peculiar structural modifications. Membrane-bound saccules with contents confluent with the cell wall, originate at primary pit fields and invaginate into the cell interior. These structures are unique, as far as I know, but they are reminiscent of the wall/membrane apparatus (Gunning and Pate, 1974) of transfer cells in their confluence with boundary cell wall material, their randomized microfibrillar substructure, and their ability to increase membrane surface area (Gunning and Pate, 1969). They are also similar to transfer cell wall modifications in that they increase the apoplastic volume in a localized manner. The matrix material in which microfibrils are embedded in both saccules and transfer cell wall ingrowths share a common feature--that of susceptibility to shrinkage during processing. Shrinkage is thought to create the electron lucent "space" which is consistently found between the plasmalemma and denser, more external portions of transfer cell wall ingrowths (Gunning and Pate, 1974, pg. 443).. Similarly, I have interpreted the electron lucent space surrounding each saccule

as an artifact of shrinkage. It is noteworthy, however, that, while the transfer cell wall pulls *away from* the plasmalemma, leaving an intervening space, the saccule membrane retracts *with* the shrinking saccule, leaving the space behind.

Amplification of membrane surface area in relationship to a contiguous cell is taken to imply a preferential loading or secreting activity of the surface-amplified cell in relationship to the juxtaposed, non-modified cell (*e.g.*, Gunning and Pate, 1974). The development of a polarized membrane amplification in relationship to a primary pit field with plasmodesmata, such as that demonstrated by *Arceuthobium* sinker cells, is a complexity that, to my knowledge, has not been described heretofore. In fact, plasmodesmatal sites are mentioned specifically in relationship to their lack of ingrowths in transfer cell studies (Gunning and Pate, 1974, pg. 463). Regardless of their apparent uniqueness, the function of the surface-amplified pit fields of certain *Arceuthobium* cells can be surmised in light of the hypothetical dynamic steady state condition described above for functioning transfer cells. As stated above, the movement of solutes and water across the surface-amplified membrane into the cell lumen must be balanced by movement of the substances out of the cell through plasmodesmata. The *unilateral* amplification of membrane in relationship to the primary pit field may allow a steep water potential gradient to develop across plasmodesmata that traverse such specialized pit

fields. This supposition is based on the premise that increased membrane surface area reflects a capacity for increased flux . A pressure differential across the plasmodesmata will drive the water across the plasmodesmata, following the path of least resistance (Tyree, 1970). Consequently, non-restricted solutes (*i.e.*, glucose) will be flushed through the plasmodesmata. Obviously, if both sides of the primary pit field were to develop equally effective saccules and solute pumps, exchange between the two cells would be limited, although they both may export to other surrounding cells.

This proposal for the function of the relatively large plasma membrane surface area in sinker cells and the saccules that are found at some primary pit fields between adjacent sinker cells, helps to explain how *Arceuthobium* sinker tissue can acquire substantial amounts of host-originating photosynthate (Leonard and Hull, 1965) without direct plasmatic connections with host phloem tissue; and how absorbed nutrients can be transported at least for short distances in the sinker. However, ultimately, nutrients must be translocated out of sinkers to other regions of the endophytic tissue and into the aerial shoots.

In most vascular plants, rapid translocation of concentrated amounts of sugars is accomplished by specialized cells in the phloem. However, with only rare exceptions (C.L. Calvin, personal communication) *Arceuthobium* does not have phloem tissue. (It is a great botanical puzzlement that *Arceuthobium* and other parasitic

angiosperms have lost, through evolutionary processes, this important tissue.) Unfortunately, no information is available at this time on the actual rates of translocation of host-originating nutrients within *Arceuthobium*. Knowledge about the rate of transfer is important in order to evaluate whether nutrients move in a strictly diffusional manner, or whether, because of demonstratively rapid rates, additional processes are operating which allow more rapid rates of flow. Although the crucial quantitative data on translocation rates is lacking, there are indications, based on anatomical features, that suggest translocation of nutrients is facilitated by differential carbohydrate metabolism in the parasite plant body.

For many years it has been known that carbohydrates in plants move in a source-to-sink pattern. That is, the soluble substances move out of areas of high concentration (where they were produced and/or accumulated) to areas where events of growth, storage, or metabolism continually reduce the available assimilate.

Mature, photosynthesizing leaves are the usual carbohydrate sources in higher plants; whereas, growing tips, fruits, storage roots are typical carbohydrate sinks. In parasitic plants this general relationship between carbohydrate sources and sinks, and organs of the plant body may be in part, reversed, since the photosynthetic apparatus often represents only a minor assimilate source relative to the host/haustoria interfacial region.

The present electron microscope study confirms earlier observations (Chapter II: Kuijt, 1960, p. 356) that (except for minute

grains in meristematic sinker cells) the parasite cells in regions of host conducting phloem are devoid of starch. In the more external portions of the endophytic system (the longitudinal or circumscribing strands) Tainter (1970) found a reduced amount of starch in comparison to amounts detected in aerial shoots. Thus, from the available evidence, there appears to be a graduated distribution of starch from sinkers to aerial shoots. Differential starch synthesis through the *Arceuthobium* plant body provides a mechanism for maintaining not only a concentration gradient of solutes through the parasite plant body, but also a hydrostatic pressure gradient from source (sinkers) to sink (aerial shoots). In the sinkers, where nutrients may be actively accumulated without starch formation, maximum turgor (hydrostatic) pressures will result as water flows into the cell in response to the lower chemical potential of water in the solute-rich cells (re: Nobel, 1974, pg. 64). Whereas, in more external regions of the endophytic system and in the aerial shoots, removal of solutes by starch formation will allow a decrease in turgor pressure if water is allowed to leave the cells and eventually be transpired.

Aerial shoots of *Arceuthobium* are capable of photosynthesis, but investigators have determined that the rate of CO₂ fixation is far below that necessary for autotrophic nutrition (Hull and Leonard, 1964a; Miller and Tocher, 1975). Therefore,

vegetative growth processes and formation of reproductive structures would also tend to deplete organic solutes and would further reduce the solute concentration of the aerial shoot cells.

The maintenance of dual negative gradients of pressure and solutes as described above, is dependent on steady state conditions and irreversible thermodynamic principles. That is, the continual mass flow of water and solutes through the parasite symplast towards the aerial shoot sinks is dependent on the "openness" of the system, with water moving into the cells at the source and moving out of the cells at the sinks. Likewise, solute must be continually supplied at the source and removed at the sinks. Such a pressure differential established between the endophytic and aerial portions of the *Arceuthobium* plant body assures directionality to symplastic translocation and encourages the passive but facilitated movement of nutrients out of the sinker tissue. Theoretically, facilitated translocation of nutrients may occur in such a system because "the flux of the solute is not only dependent on the negative gradient of its own chemical potential...but it may also be influenced by the gradient in the chemical potential of water" (Nobel, 1974). The above described system of coupled solute and water flux is proposed as a mechanism by which nutrients may be readily translocated through the *Arceuthobium* symplast, particularly at the rate-limiting sites of plasmodesmata passage.

Tyree (1970) has mathematically described a similar theoretical system of facilitated movement through plasmodesmata and has termed it "trans-symplastic water flow". The system is also similar to the Münch model of translocation through sieve elements, which explains phloem translocation based on steady state models of pressure flow (re: Tyree, 1975). It has been previously explained that an indication of a negative solute gradient is found in the differential distribution of starch. It can also be noted that an indication of low aerial shoot pressures (turgor pressure) is found in the marked tendency for *Arceuthobium* shoots to wilt shortly after being picked (D. Knutson, personal communication).

Mature sinker cells have unusual plastids. They have prominent paracrystalline prolamellar bodies, few thylakoid membranes, and occasional grana. They characteristically have many plastoglobuli, and do not store starch. Often the plastids have significant ferritin deposits.

As described above, the plastids of *Arceuthobium* sinker cells closely resemble etioplasts. An etioplast is a type of plastid found in angiosperms. Etioplasts form in potentially photosynthetic tissue (e.g., leaf tissue) that has developed entirely in the dark. Etioplasts lack grana and have few thylakoid membranes. Their most outstanding feature is the presence of one or more prolamellar bodies. Prolamellar bodies are

composed of a collection of tubular membranes that branch and interconnect in a variety of patterns forming a three dimensional membrane lattice. In the most highly ordered state they form paracrystalline arrays. If etiolated (dark grown) plants are exposed to sufficient light their potentially chlorophyllous plastids will undergo irreversible structural changes leading to the development of typical chloroplast structure. (For additional review literature on etioplasts see Gunning and Steer, 1975).

The etiolated condition in nature is transitory and to prolong it requires (usually) artificially-induced dark conditions. Eventually, even under continuous dark, the highly organized prolamellar bodies become disordered. Under weak light, plastids may have mixed characters of etioplasts and chloroplasts, or etioplasts and amyloplasts with only small regions of the prolamellar body persisting (Gunning and Steer, 1975).

The function of the prolamellar body is unknown. It has been suggested that its major function is to store membranes for rapid reorganization into chloroplast grana and thylakoids upon illumination, but, it is suspected that other functions are carried out in the etioplast stage (Gunning and Steer, 1975, pg. 114). Physio-chemical aspects of development are incompletely understood; however, it has been shown that phytochrome has an influence on prolamellar bodies (Mohr and Kasemir, 1975). Also, it is clear that the occurrence of prolamellar bodies is associated with lack of chlorophyll due either

to a physiological block brought on by the absence of light during proplastid differentiation (Mohr and Kasemir, 1975), or a mutational block in chlorophyll synthesis (Röbbelen, 1966).

Plastids of sinker cells differ from typical etioplasts in that their prolamellar bodies are persistent and maintained in the paracrystalline state. Also some single grana may be present (Fig. 3.1). The etioplast-like structure is apparently not a transitory condition of differentiation in mature sinker cells. The morphogenesis of etiolated sinker plastids may be influenced by the low light levels within the host stem and (probably) reduced capacity of the dwarf mistletoe tissue to form chlorophyll (re: Miller and Tocher, 1975). But the relative abundance of etioplast-like plastids in sinker cells and their persistent complexity are suggestive of function beyond that of a vestigial organelle. I believe the etioplast-like plastid population in sinker cells of *Arceuthobium* reflects the biochemical properties of the cells -- specifically in relationship to the synthesis of the large amounts of unsaturated fatty acids found in *Arceuthobium* tissue. The following information supports this hypothesis: For several years plastids have been thought to be involved in lipid synthesis (Yang and Stumpf, 1965) and at the present time there is much evidence that links certain types of lipid synthesis to light-dependent reactions in the chloroplasts (Stumpf, 1976). However, synthesis of polyunsaturated lipids from ^{14}C -acetate is very poor in mature leaves,

but actively occurs during the greening process of etiolated tissue, in immature leaf tissue, or during the maturation of some seeds (Mazlik, 1973, p. 293-294). Zilkey and Canvin (1972) identified proplastids in cell fractions of developing castor bean endosperm as the site of synthesis of the unsaturated fatty acid, oleic acid--the molecules of which are metabolized and accumulate as oil droplets in the cytoplasm. The Zilkey and Canvin study has been accepted as demonstrating that proplastids have more than just a precursory function (Gunning and Steer, 1975). However, I have examined Zilkey and Canvin's published micrographs of the subcellular particles associated with oleic acid synthesis and have concluded that the plastids they identify as proplastids are, in fact, etioplasts since they have prolamellar bodies. This re-evaluation of Zilkey and Canvin's study and other reports which link polyunsaturated fatty acid metabolism with etiolated (Trémolières and Lepage, 1971) or naturally shaded tissue (*e.g.* developing castor bean seeds) supports the hypothesis that naturally occurring etioplasts, such as those found in the endophytic system of *Arceuthobium*, are involved with the synthesis of unsaturated fatty acids. After synthesis, the fatty acids are elongated and accumulate as unsaturated lipid droplets in the cytoplasm.

Cytopathological Effects on Host Cells

The light microscope study of dwarf mistletoe infected hemlock determined that the volumetric ratio between host ray tissue and axillary tissue increased in sites of dwarf mistletoe localized infections in comparison to uninfected portions (Chapter II). Furthermore, the numbers of Strasburger (albuminous) cells increased in infected rays. However, in terms of cytological modification, few anomalies in cell structure could be discerned in diseased tissues in comparison to healthy phloem cells as described in Chapter IV. Only occasionally did host cells adjacent to parasite cells appear decadent. An example of a damaged host cell is seen in Fig. 3.34 where a dense, partially crushed, host ray cell lies adjacent to an expanded, normal-appearing parasite cell.

Sieve cell ontogeny and mature sieve cell structure was similar in both healthy and diseased tissues. Many of the ray cells of infected rays of pine had features intermediate between ordinary ray cells and the more specialized albuminous cells. Actual plasmatic connections with sieve cells are important for precise determination of albuminous cells (Alfieri and Evert, 1968) but in this study connections were not seen. Host ray cells associated with *Arceuthobium* cells have a high proportion of cytoplasm to vacuolar volume. Their vacuoles do not have tanniniferous contents. Ray cells contiguous with parasite cells usually have an electron lucent background cytoplasm and polymorphic plastids with limited starch

stores. In healthy tissue, albuminous cells have these same cytological features (Chapter IV) and are associated with the movement of compounds into and out of sieve cells (Sauter, *et al.*, 1976). The significance of the ray cells surrounding parasite cells may be that they contribute to the unloading of nutrients from sieve cells in the diseased stem.

Suggestions for Chemical Control of Dwarf Mistletoes

Studies on systemically active substances have shown that there are chemical differences between compounds that move apoplastically versus compounds that are absorbed into the symplast of the plant. For example, a characteristic of symplastically-mobile substances appears to be the presence of a weak acid moiety (Crisp, 1972). In order to select systemic pesticides for specific needs, it is necessary to understand both the translocation characteristics of a compound, and whether the target pest is associated with the host's symplast, apoplast, or both. Furthermore, the ability of the plant to take up and then translocate a substance is strongly affected by whether the initial application method is compatible with the systemic characteristics of the substance. Apoplast mobility is dependent on water potential gradients (in particular, transpiration) for distribution. Therefore, substances generally must be applied below the target area so that they can be pulled upward in the xylem and within cell walls, toward the target. On the other hand, symplastically-

mobile substances often move in a source-to-sink pattern and have phloem mobility; therefore foliage spray may be a suitable application method.

This study has shown that *Arceuthobium* does not have plasmatic continuity with host cells. Because of this, many symplastically-mobile substances may be ineffectual for chemical control if administered through host tissues. However, since the host and parasite share a common apoplast, apoplastically-mobile substances may gain access to parasite tissue if stem-injected below the infection site. Other research has shown that dwarf mistletoes have, in comparison to their hosts, low water potential values within aerial shoots (Fisher, 1975). Given lower water potentials in aerial shoots than the surrounding needle-bearing branches of the host, it is probable that apoplastically-held substances will be mobilized towards the dwarf mistletoe tissue to ultimately become concentrated in the parasite aerial shoots. Selective killing of *Arceuthobium* may then be achieved. It is therefore suggested that future research on chemical control of *Arceuthobium* investigate apoplastically-mobile substances for control of dwarf mistletoes.

An example of a potentially selective herbicide against dwarf mistletoes is a substituted urea compound called monuron. Monuron has been shown to be a strongly apoplastically mobile substance, and non-mobile in the symplast (Crafts, 1967). Because the substance moves strictly in relationship to a pathway of decreasing water

potential, it may become particularly concentrated around endophytic tissues and subsequently travel into the aerial shoots, where, if the substance is effective against the parasite, the aerial shoots may die and drop off, thus preventing reabsorption of the herbicide into the host.

Another herbicide that should be investigated for a possible selective killing action against *Arceuthobium* is a benzoic acid herbicide called dinoben. Its potential usefulness lies with the fact that it is a selective herbicide which inhibits fatty acid and lipid synthesis in fat-storing plants (Muslih and Linscott, 1977). It is possible that selective inhibition of *Arceuthobium* sinker cell metabolism can be achieved with this herbicide, despite the close physical relationship of endophytic cells with host vascular tissue. This is because sinker cells store lipids almost exclusively, while conifers store mainly starch in warm months (Ziegler, 1964). Application of the herbicide should correspond with the starch-storing phase of host tissues. I have not found information on the systemic characteristics of dinoben. Therefore, I would suggest that various application methods be tried to determine the most suitable method of concentrating the herbicide in *Arceuthobium* tissues.



FIGURE 3.1. Sinker cell structure of *A. occidentale*. Plastids (PL) may have prolamellar bodies (P), plastoglobuli (pl), and ferritin deposits (f). Thylakoid membranes (th) associate with the prolamellar body (unlabelled arrows). Mitochondria (M) have DNA centers (dna). Endoplasmic reticulum (ER) and plasmalemma (pm) form endomembrane continuities (EN). An electron lucent region with microfibrils (fi) occurs between the plasmalemma and denser regions of the cell wall (CW). 42,500X. gr, grana; mt, microtubule; r, ribosomes.

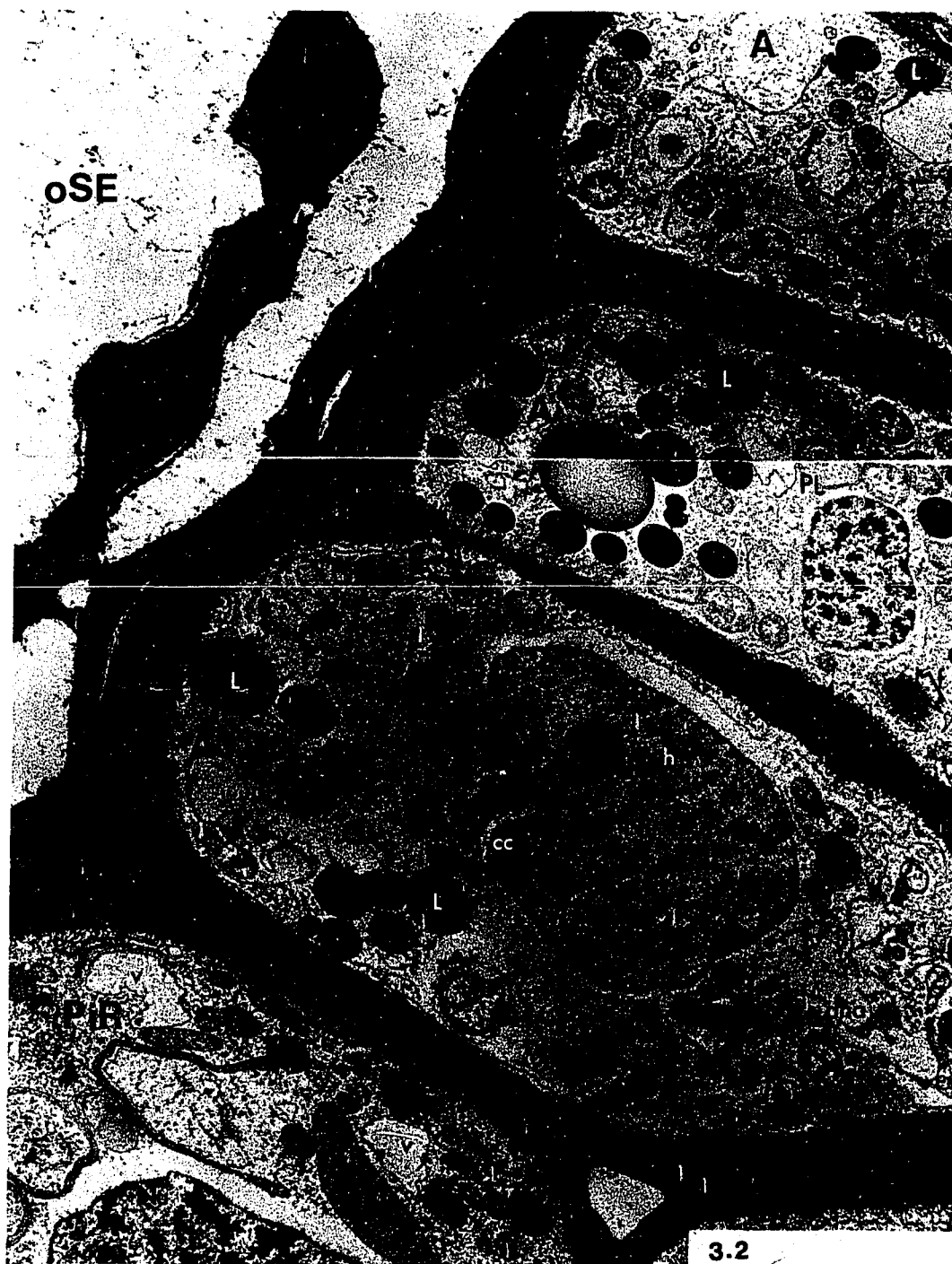


FIGURE 3.2. Sinker cells (A) of *A. occidentale* with ray cells (PiR) and old, non-functional sieve cells (oSE). 6700X. cc, chromocenter; dna, DNA; f, ferritin; g, golgi body; h, heterochromatin; L, lipid droplet; M, mitochondria; Nu, nucleus; PL, plastid; sp, spherosome; v, vacuole.

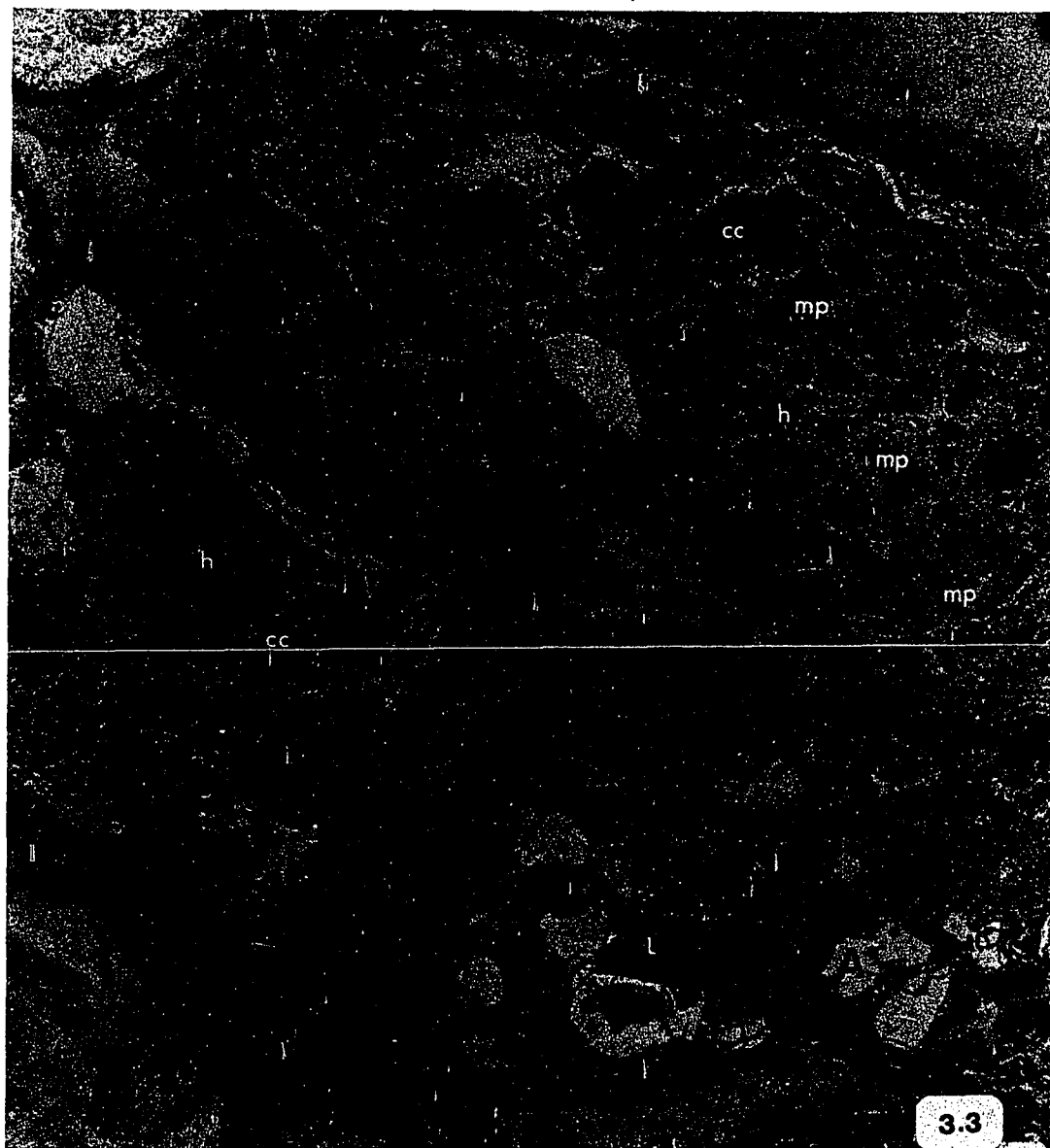


FIGURE 3.3. Sinker cells of *A. occidentale* (A). These cells are from the center of a large sinker in the region of the host phloem. Expanded cell walls (eCW) sometime occur between parasite cells. Typical chromocentric nuclear structure is evident with a disjointed appearing reticulum of heterochromatin (h) and chromocenter masses (cc) associated with the nuclear membrane. Micropuffs (mp) are very common. Mitochondria (M) are abundant. Plastids (PL) may be cupped around portions of cytoplasm (cy) or have large ferritin deposits (f) but do not store starch. 8700X. ER, endoplasmic reticulum; L, lipid droplet; pd, plasmodesmata; v, vacuole.



FIGURE 3.4. Plasmodesmata in *A. occidentale* sinker tissue. Intra-specific plasmatic connections *via* plasmodesmata (pd) at primary pit fields are abundant in parasite tissue. Endomembrane continuities (EN) occur between the plasma membrane (pm) and internal membranes. 38,000X. CW, cell wall; eu, euchromatin; h, heterochromatin; mt, microtubules.



FIGURE 3.5. *Arceuthobium californicum* (A) and functional phloem cells of *Pinus lambertiana*. 5700X. cc, chromocenter; mp, micropuff; M, mitochondria; n, nucleolus; PiR, pine ray cell; PL, plastid; s, starch; SE, sieve element; sp, spherosome.



FIGURE 3.6 *Arceuthobium occidentale* (A) and cambial tissue (CZ) of *P. sabiniana* during winter dormancy. A partially differentiated sieve element (dSE) and tracheary element (X) are seen. A sieve area (sa) has been formed between a sieve element (SE) and an *Arceuthobium* cell. A thin wall region (wt) occurs between a cambial cell and an *Arceuthobium* cell. 5500X. bp, bordered pit; ca, callose; ER, endoplasmic reticulum; L, lipid droplet; na, nacreous wall; Nu, nucleus; Pl, plastid; sp, spherosome. Arrow indicates spherosome inner membrane leaflet.

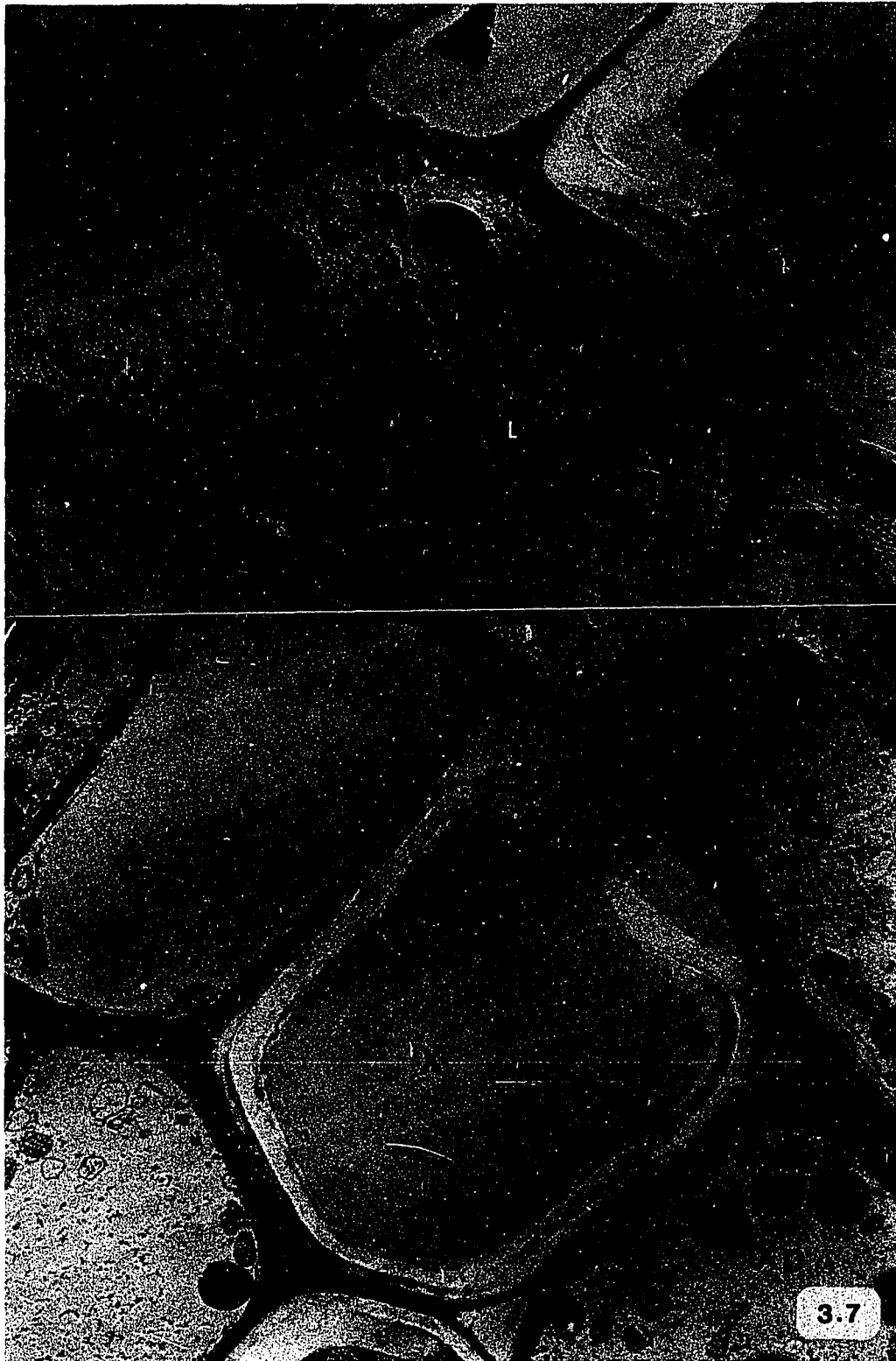


FIGURE 3.7. *Arceuthobium occidentale* (A) and xylem (X) of *Pinus sabiniana*. 5500X. bp, bordered pit; L, lipid droplet; n, nucleolus; Nu, nucleus; Xp, xylem parenchyma.



FIGURE 3.8. Sinker cells (A) of *A. occidentale* and tracheids (X) of *P. sabiniana*. Extensive endoplasmic reticulum (ER) is found in the parasite cells. The pit membrane of the tracheids are normal in appearance and are fused with the cell walls of the parasite. 16,500X. pd, plasmodesmata. Box region is enlarged in Fig. 3.9.

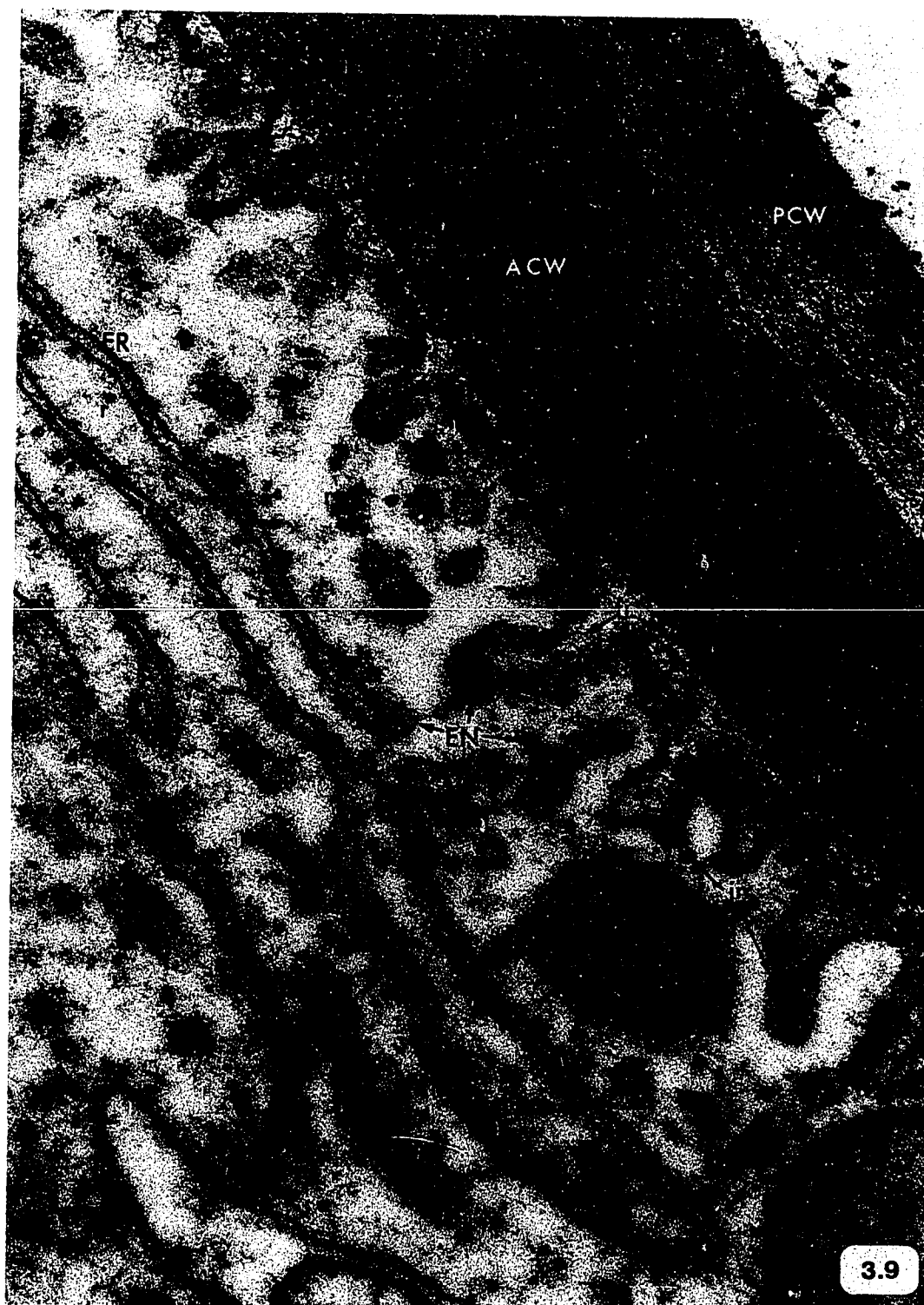
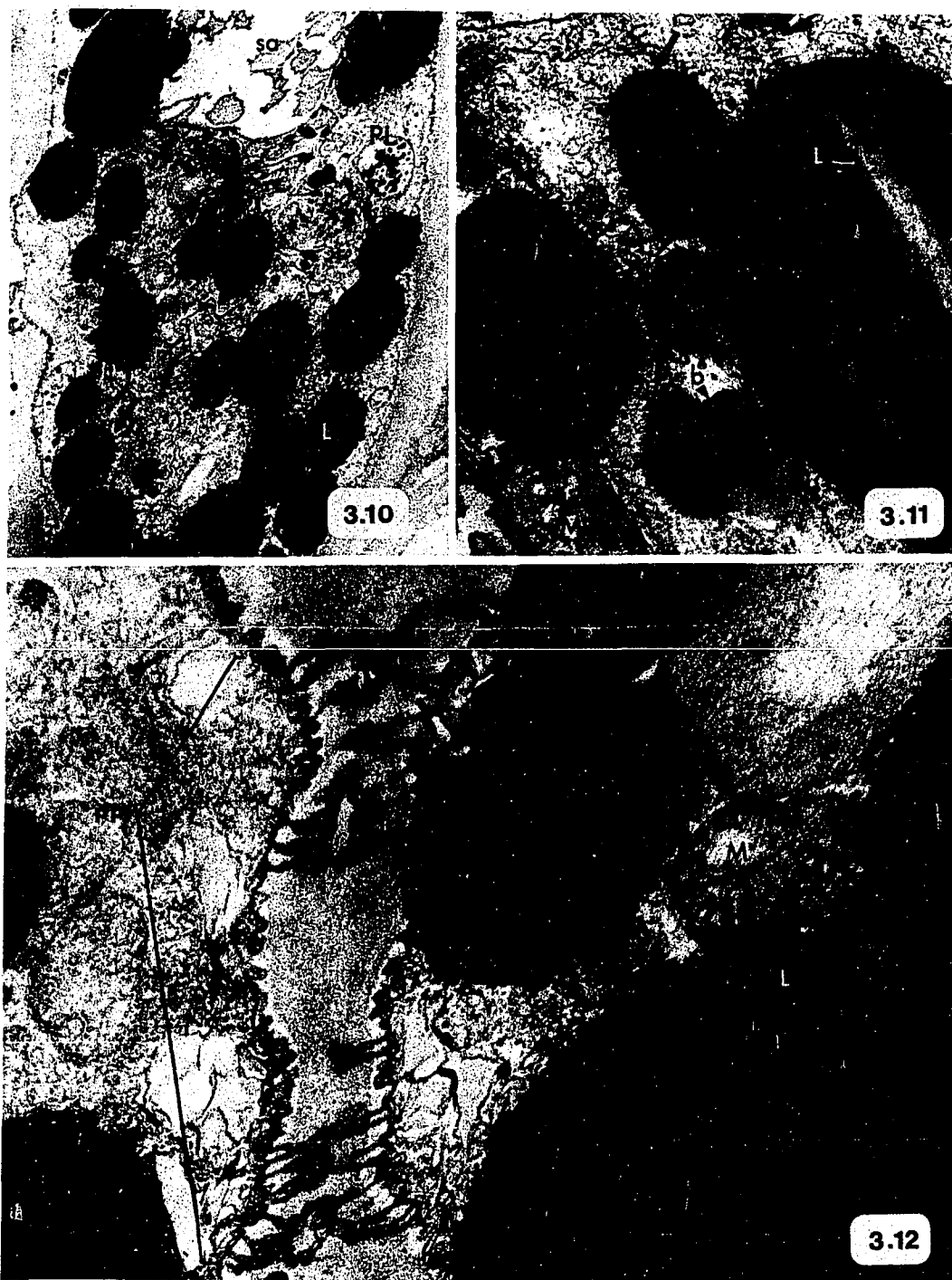


FIGURE 3.9. Detail of Fig. 3.8. Small wall ingrowths (in) of the *Areceuthobium* cell wall (ACW) contain microfibrils (fi) and may make endomembrane confluence (EN) with endoplasmic reticulum (ER). 75,400X. PCW, *Pinus* cell wall; r, ribosomes.



FIGURES 3.10-3.12. Sinker cell structure of *A. tsugense*. Fig. 3.10--Lipids (L) are found as large bodies with distinct or fuzzy (arrow) margins and are also sequestered within smooth ER (ser). 5700X. Fig. 3.11--Bodies (b), perhaps membrane-bound (light arrow) are associated with lipid droplets. 30,000X. Fig. 3.12--Plasmodesmata connections (pd) between *Arceuthobium* cells. 30,000X. M, mitochondria; PL, plastid; ppf, primary pit field; sa, saccules.

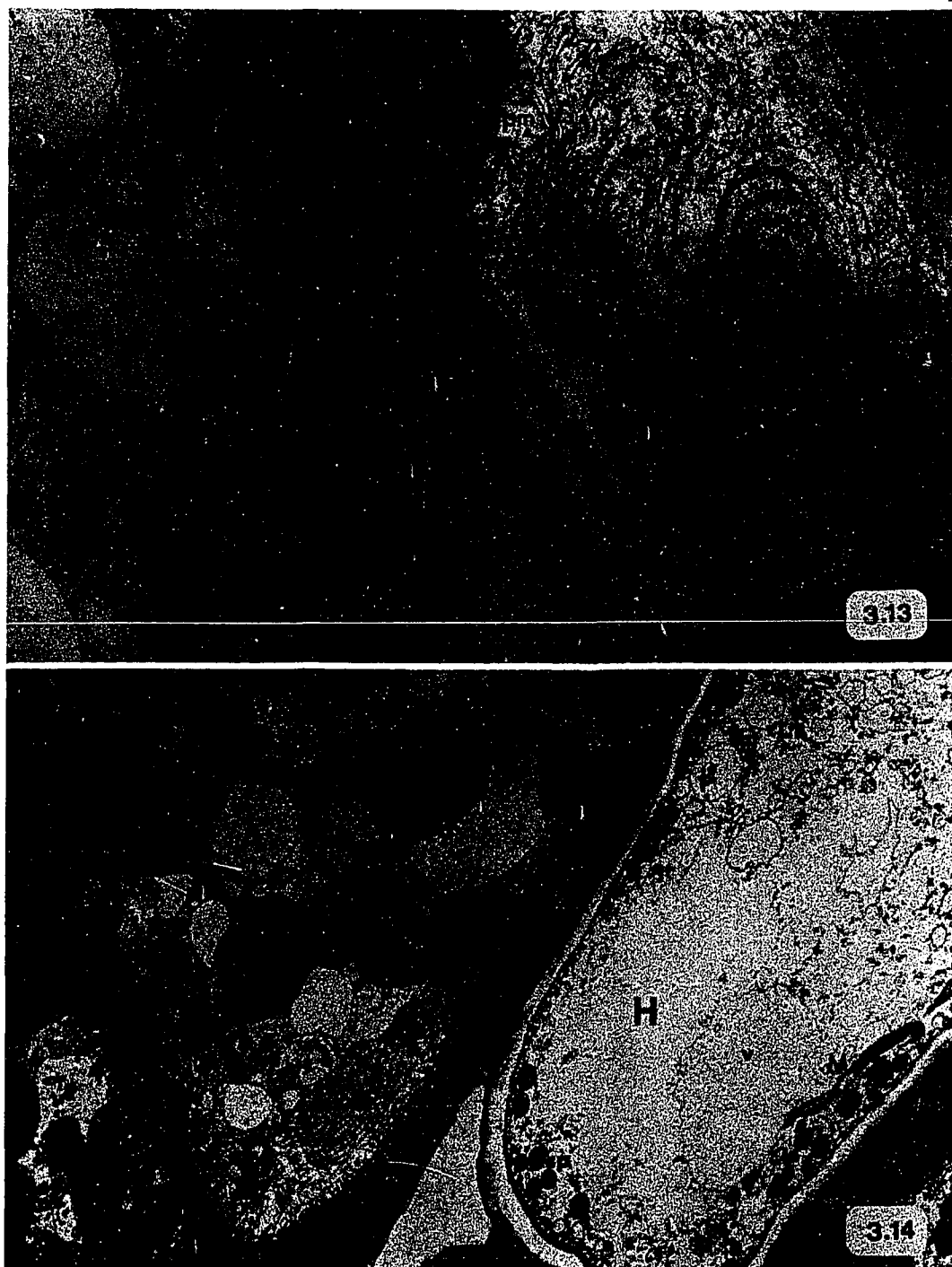
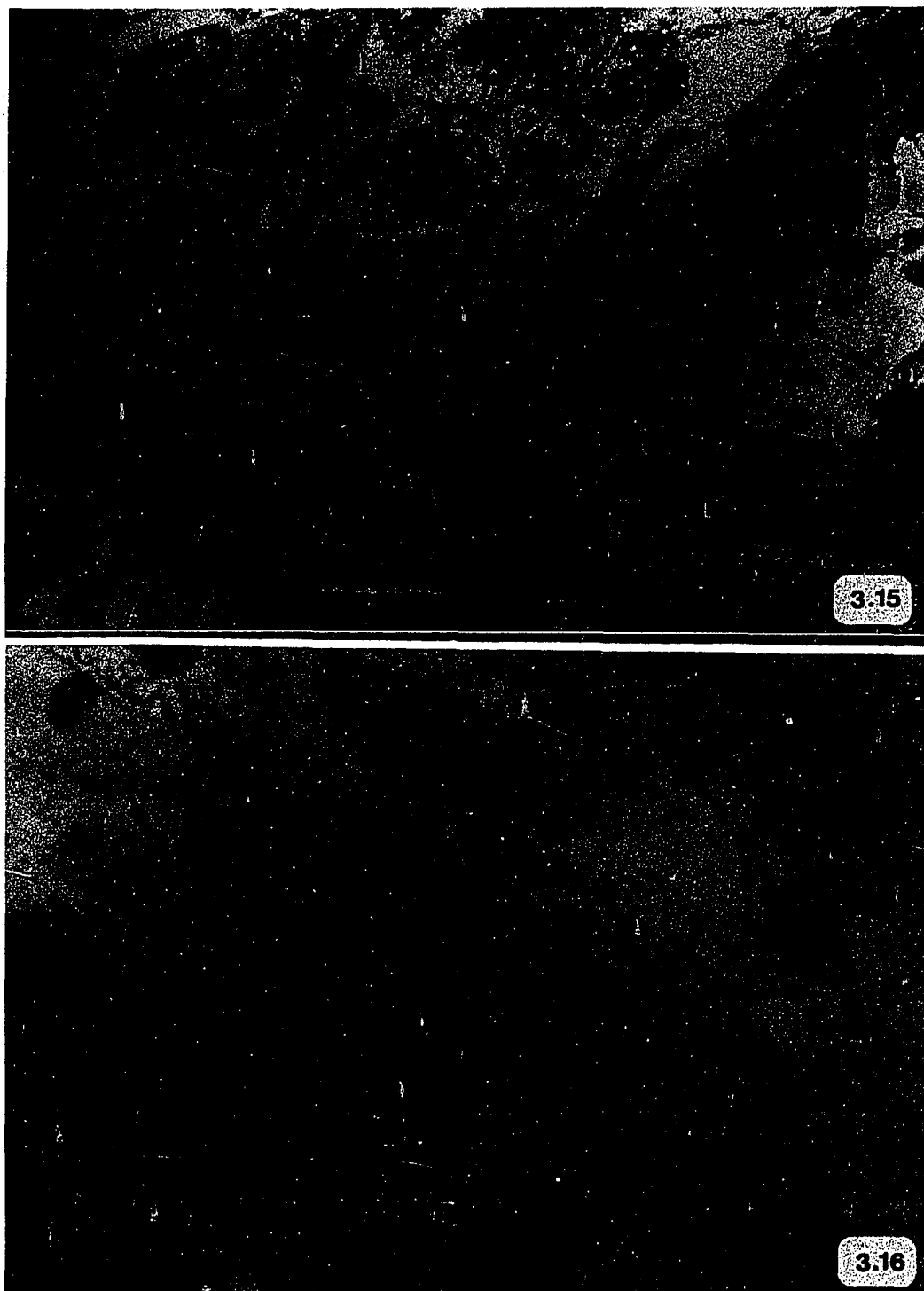
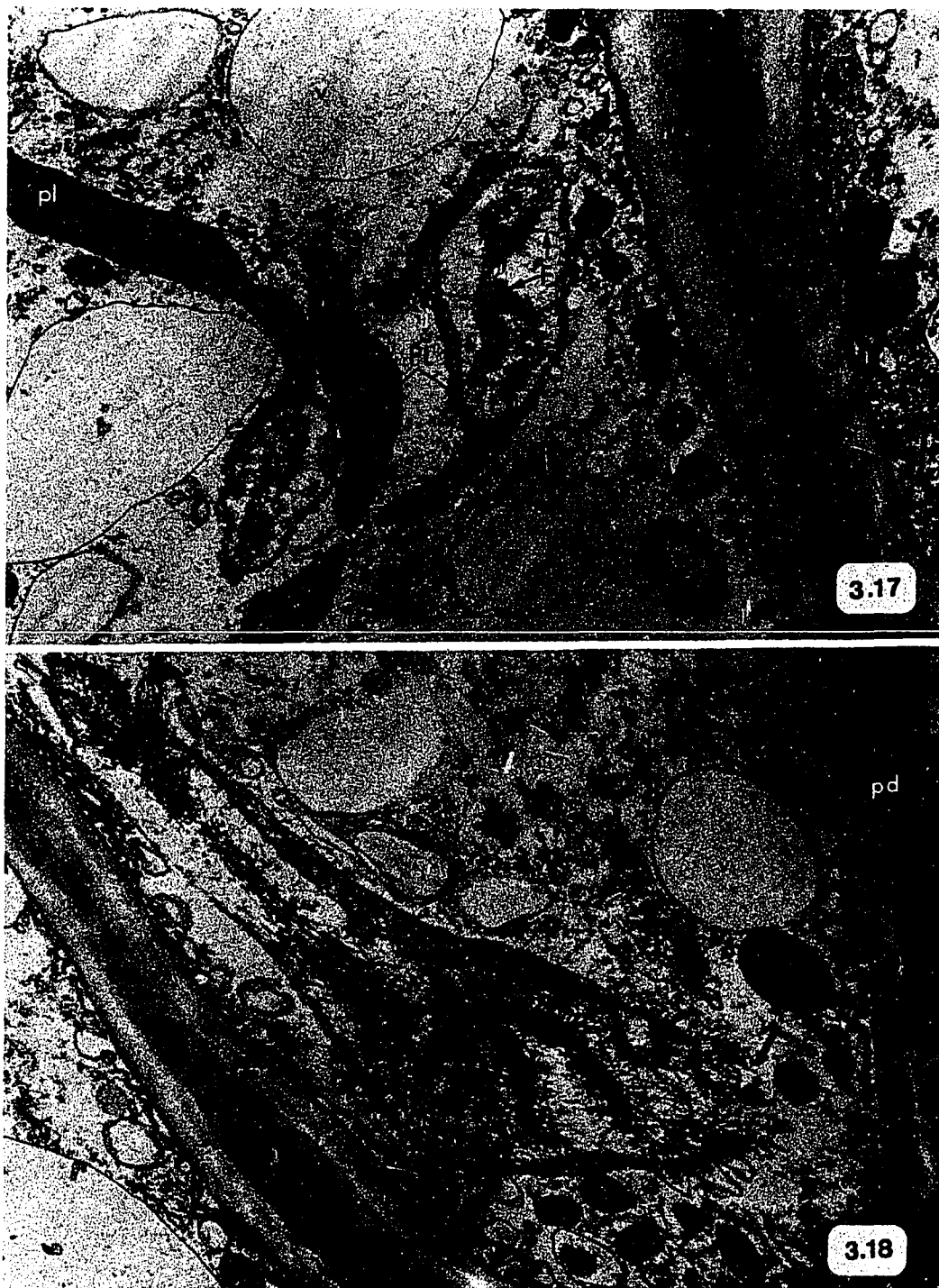


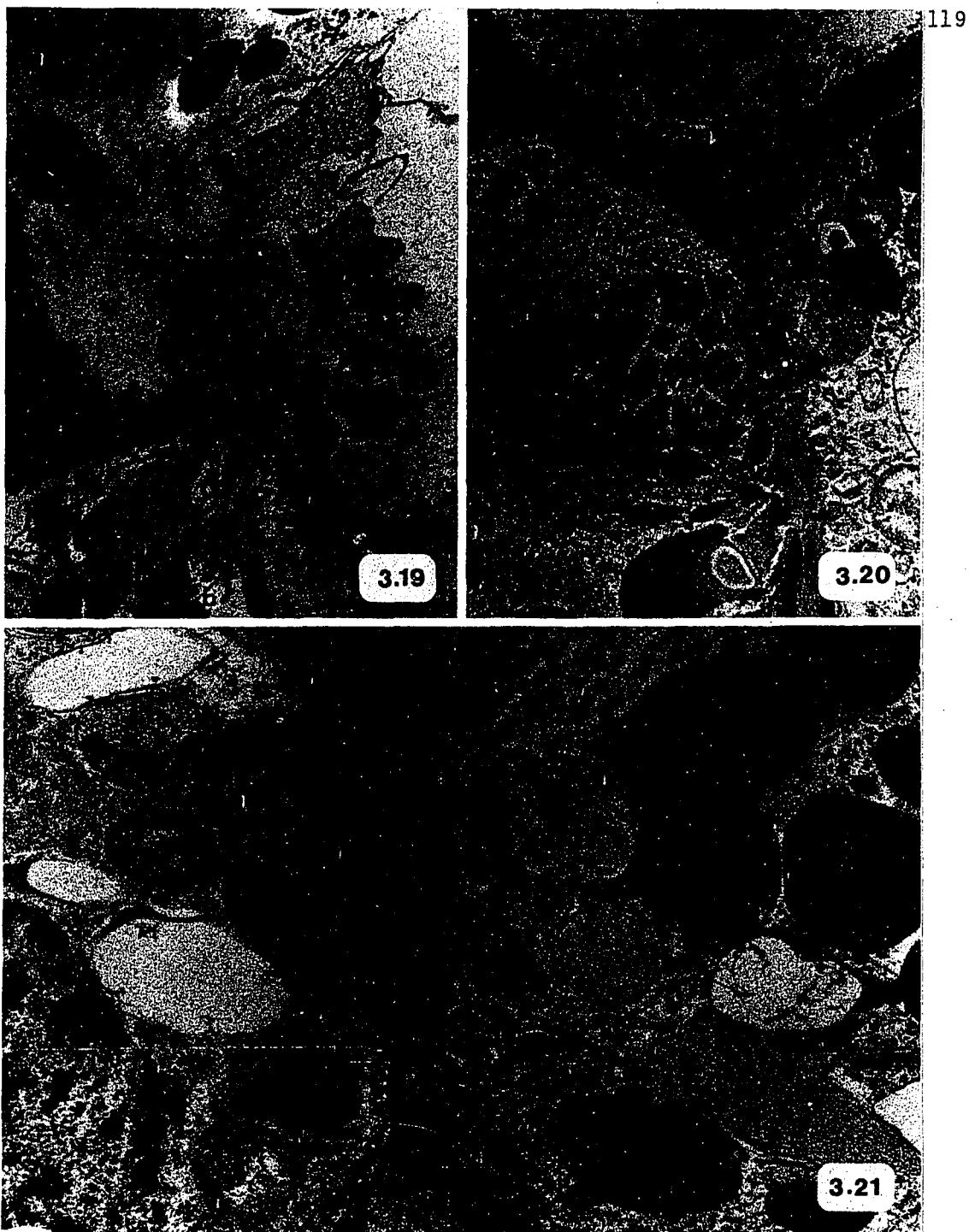
FIGURE 3.13-3.14. Cells of *A. tsugense* and *T. heterophylla*. Fig. 3.13-- Sinker tissue with lamellar arrays of endoplasmic reticulum (ER) and golgi bodies (gb). 11,000X. Fig. 3.14--Host (H)/ parasite (A) interface. Note distinct differences in cell wall appearance in the two organisms. 5100X. M, mitochondria; pm, plasma membrane, sp, sphaerosomes, v, vacuole.



FIGURES 3.15-3.16. Cellular interfaces of *A. tsugense* and *T. heterophylla*. Fig. 3.15--Plastids (PL) of *T. heterophylla* have condensed fibrillar component (F). Walls of host and parasite are fused; the parasite wall is thick and densely staining. 6000X. Fig. 3.16--A thin wall region (wt) (ca. 0.2 μ) is shown at host (H)/parasite (A) interface. Note irregular wall/membrane surface at arrows. 27,000X. ER, endoplasmic reticulum; L, lipid droplets; M, mitochondria; v, vacuole.



FIGURES 3.17-3.18. Cells of *T. heterophylla*. Tissue was subjected to heat during fixation. Heat digested starch leaving behind condensed fibrils (F). Fig. 3.17--13,000X. Fig. 3.18--A plastid is shown which has disrupted limiting membranes with fibrous component confluent with cytoplasm. 13,400X. ER, endoplasmic reticulum; M, mitochondria; pd, plasmodesmata; pl, plastoglobuli; v, vacuole.



FIGURES 3.19-3.21. Saccules and other cytological details of sinker cells of *A. occidentale*. Saccules originate from membrane invaginations at primary pit fields (o), are confluent with the cell wall, and are filled with microfibrils. Fig. 3.19--17,00X. Fig. 3.20--9000X. Fig. 3.21--29,400X. b, body associated with lipid; dna, DNA; g, golgi body; M, mitochondria; Nu, nucleus; sa, saccule.

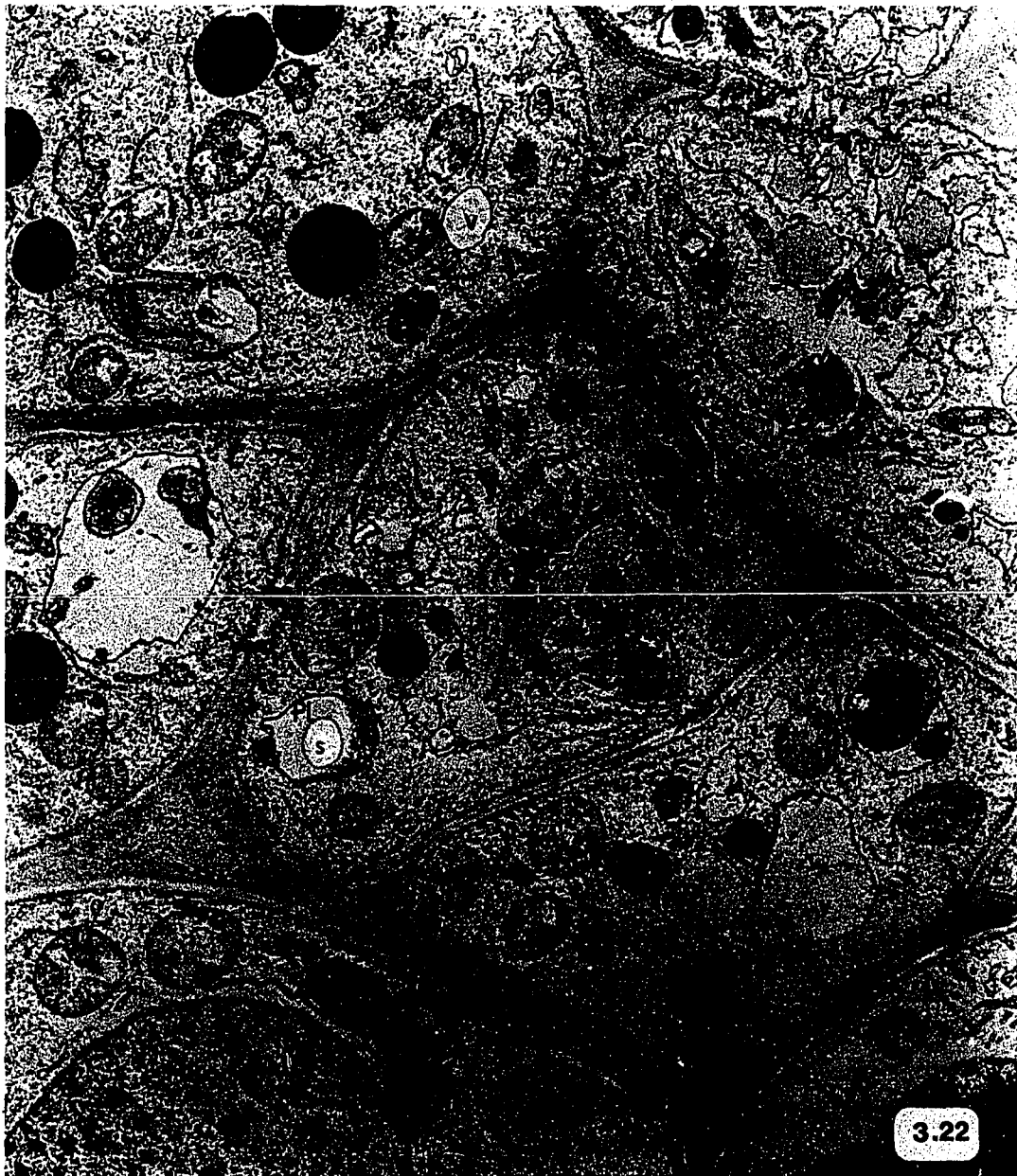


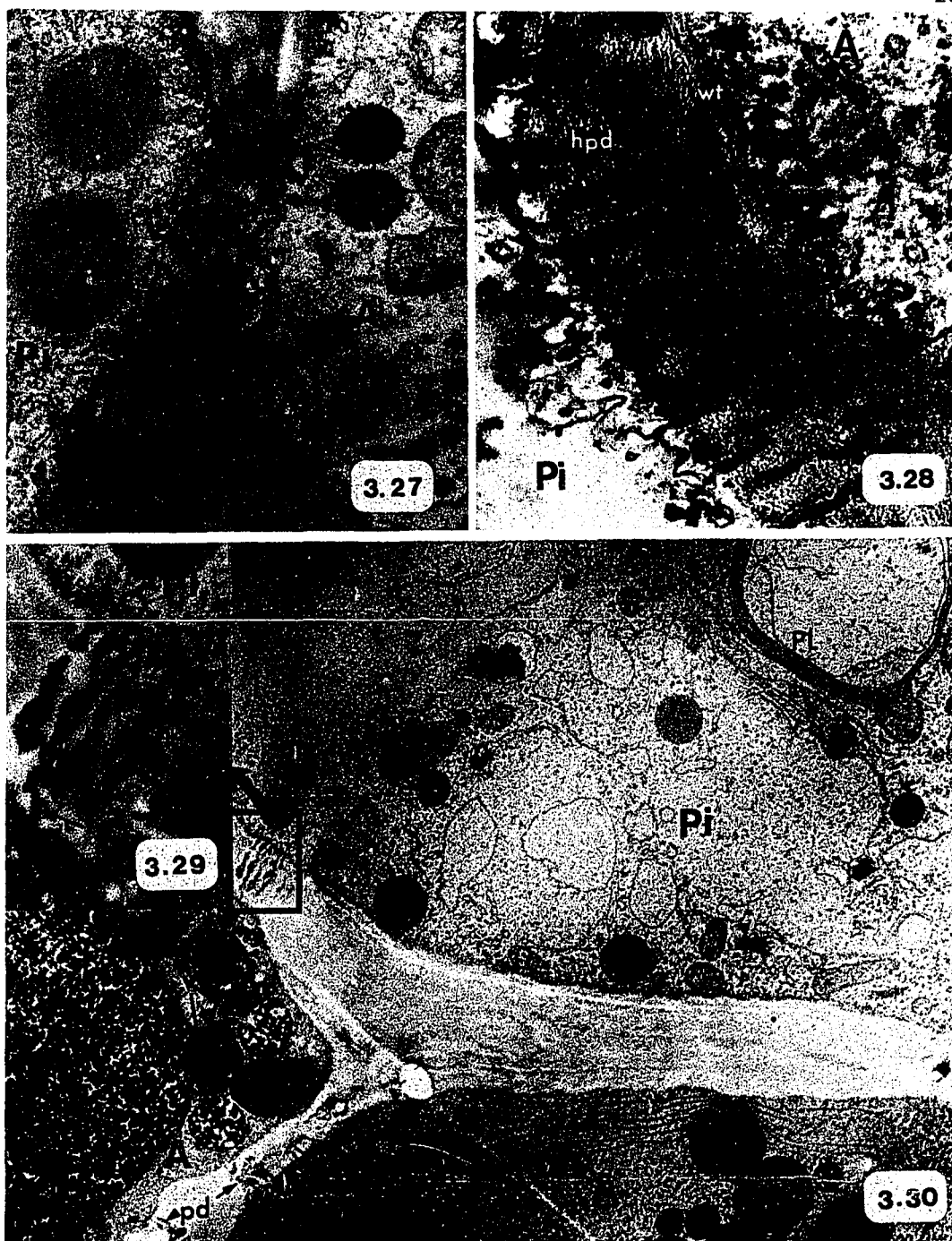
FIGURE 3.22. Meristematic cells in *A. occidentale*. These cells were located in the center of a large sinker. In contrast to most sinker cells, meristematic sinker cells have little lipid stores but may have small starch grains (s) in some plastids (PL). Extensive wall sacculles (sa) are evident and primary walls (pw) and division walls (dw) are thin. 9500X. M, mitochondria; Nu, nucleus; pd, plasmodesmata; v, vacuole.



FIGURES 3.23-3.25. Cell structure of *A. occidentale*. Fig. 3.23--Detail of Fig. 3.22 showing large membrane surface area associated with plasmodesmata (pd) and saccules (sa). 14,900X. Fig. 3.24--Microfilaments (mf) in a sinker cell. 23,500X. Fig. 3.25--Endomembrane continuities (EN) between endoplasmic reticulum (ER) and the plasma membrane. 33,100X. CW, cell wall; vm, vacuole membrane.



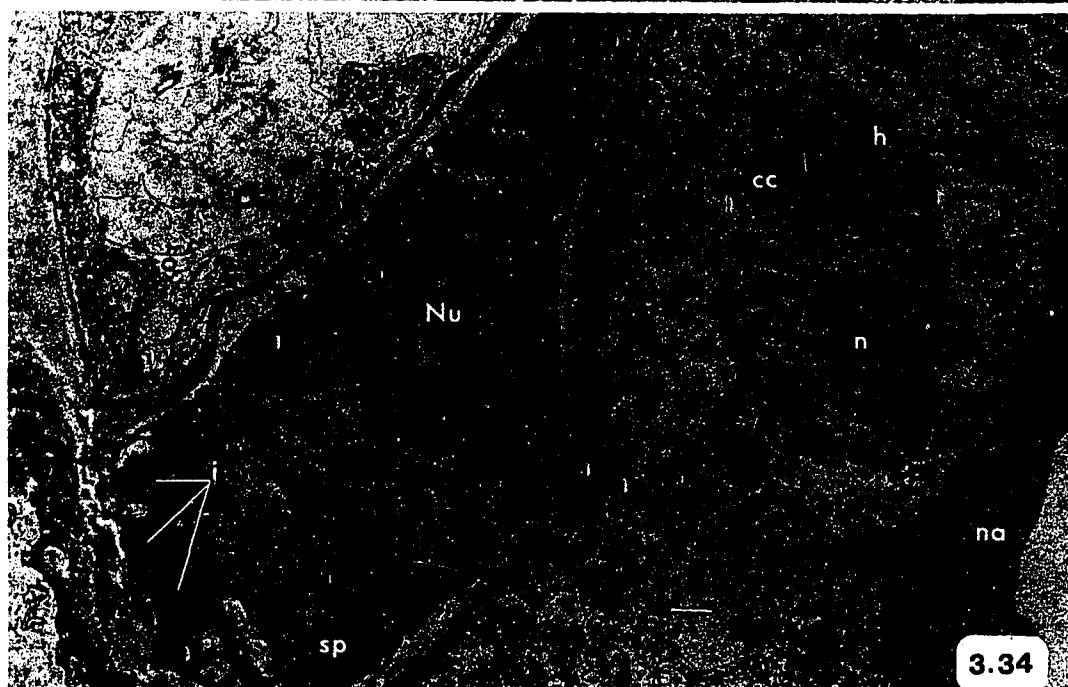
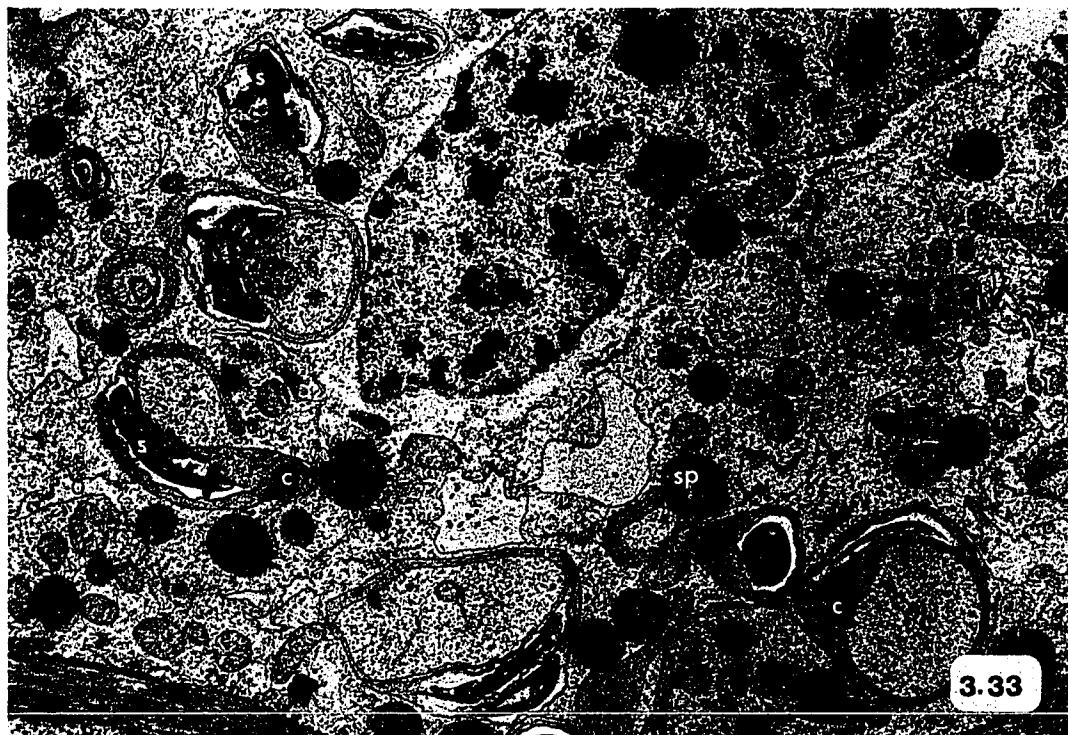
FIGURE 3.26. Wall thinning between host and parasite. The interface of *A. occidentale* and *P. sabiniana* has fused walls with regions of wall thinning (wt) on the parasite side (A) adjoining half plasmodesmata (hpd) on the host side (Pi). 9200X. cy, cytoplasm; ER, endoplasmic reticulum; L, lipid droplet; M, mitochondria; ml, middle lamella; Pl, plastid; s, starch; sp, spherosome.



FIGURES 3.27-3.30. Wall thinning (wt) between host (Pi) and parasite (A) cells. Fig. 3.27--Walls of both organisms are thin; half plasmodesmata (hpd) occur on the host side. 16,000X. Fig. 3.28--A wall thinning shown opposite a collection of half plasmodesmata from the pine cell. 41,400X. Fig. 3.29--Detail of Fig. 3.30 showing a collection of half plasmodesmata from the pine cell connecting with a single half plasmodesma from the parasite cell. 15,200X. Fig. 3.30--5600X. ER, endoplasmic reticulum; pd, complete plasmodesmata; PL, plastid; v, vacuoles with ergastic contents.



FIGURES 3.31-3.32. Wall thinning between host (Pi) and parasite (A) cells. Fig. 3.31--half plasmodesmata (hpd) from host cells end at middle lamella cavities (c). 24,000X. Fig. 3.32--Parasite cell with wall thinning joins with a callosed (ca) sieve area of a pine sieve element (SE). 20,700X. ER, endoplasmic reticulum; PL, plastid; r, ribosomes.



FIGURES 3.33-3.34. Cytopathological features of host cells. Fig. 3.33--Polymorphic plastids (PL) have cytoplasmic inclusions. 4100X. Fig. 3.34--A dense, collapsed host cell is adjacent to expanded *Arceuthobium* cells (A). 6600X. c, crystal; cc, chromocenter; h, heterochromatin; i, inner membrane of spherosome; n, nucleolus, na, nacreous wall; Nu, nucleus; Pi, pine cell; s, starch; sp, spherosome.

CHAPTER IV
STRUCTURE AND DEVELOPMENT OF *PINUS SABINIANA* PHLOEM

INTRODUCTION

The Relationship of Phloem Studies to Dwarf Mistletoe Research and
Other Environmental and Resource Needs

The preceding chapters of this dissertation have emphasized host/parasite anatomical relationships pertaining to the growth and differentiation of the endophytic systems of *Arceuthobium* tissue. Also, an examination of the fine structure of the host/parasite interface has allowed insight into the process of interspecific transfer of host-originating, phloem-mobile nutrients.

However, an understanding of the full significance of the host/parasite relationship and possible cytopathological effects on the host tissue requires an adequate comprehension of the structure and cytology of healthy tissue. There has been no previous ultrastructural study of the healthy phloem of any of the three mistletoe-infected species examined and reported on in Chapter III; therefore, it is clear that a study of healthy phloem of at least one of the same species studied in the infected condition is necessary. *Pinus sabiniana* was chosen because I had studied its diseased state extensively.

Knowledge about the structure and function of phloem is valuable, regardless of a relationship to a specific diseased condition however. New information on phloem may contribute to a broader concern related to the need to understand more completely translocation processes in plants. In a recent publication on the subject of transport the editors state:

"A knowledge of the manner in which organic materials and nutrients are distributed in plants is important for an understanding of the limits to harvestable yield, how environmental factors regulate growth, how nutrients may be used more efficiently, and how diseases spread in plants. To this list can also be added the effectiveness of substances applied to plants such as growth regulators, herbicides, and insecticides."(Wardlaw and Passioura, 1976, p. xv).

Historical Aspects of Phloem Studies and Review of the Structure of Conifer Phloem

For over a century, botanists have recognized that phloem has a primary role in the translocation of elaborated food materials (Esau, 1950). The rate of transfer through the tissue is much faster than can be accounted for by simple diffusion and protoplasmic streaming. Calculated speeds ranging from 20 cm hr^{-1} to over 300 cm hr^{-1} have been reported (for review see Crafts and Crisp, 1971, p. 392). Two major, unanswered problems have puzzled botanists for years and have stimulated much of the phloem research: Firstly, what processes generate such relatively rapid rates? Secondly, how are translocation processes reconciled with the unique sieve element structure?

The persistence of these problems is due to certain inherent obstacles that invariably arise in studies of phloem tissue: Living,

conducting sieve elements are thoroughly integrated with all parts of the plant body, and as such are difficult to study independently of influences from other cell types. Furthermore, a system of connecting sieve elements forms a pressurized conduit that, when disturbed chemically or physically, is subject to disruption. In recent years various killing and fixing techniques have been used to minimize cell damage, but much controversy still exists over the extent to which disruption is controlled by fixation modifications. Subjective conflicts concerning phloem anatomy have made it difficult for phloem physiologists, biophysicists, and cytologists to prepare a unified concept of phloem translocation that integrates structure and function (Weatherley, 1975 b).

Many investigators have taken the approach of studying the ontogenetic processes that lead to the development of mature, conducting sieve elements. This method is technically useful since the immature sieve element is less vulnerable to pressure disruption because the sieve pores (which connect elements of the conduit) are not fully opened, and the young cell remains somewhat isolated from the rest of the phloem tissue. Also, study of the process of differentiation, in itself, is enlightening since the dramatic changes in cell structure can be interpreted as preparations that will facilitate translocation upon maturity.

It is well known that all differentiating sieve elements of higher plants undergo radical cytological changes from the original, undifferentiated cambial derivatives. The most fundamental change

is the complete breakdown and disappearance of the nucleus, or an alteration of the nucleus to a pycnotic form that appears to be nonfunctional (Srivastava, 1975).

Other changes that occur are loss of most of the typical, eucaryotic cell components, such as ribosomes, dictyosomes, microtubules, and microfilaments. In most studies the vacuolar membrane has been found to disappear before maturation; but the plasmalemma is maintained and remains differentially permeable throughout differentiation and the life of the mature sieve element (see Srivastava, 1975, for review literature). Despite the drastic catabolic processes that result in the loss of most cellular constituents, certain cell components, curiously, escape destruction and may even be part of anabolic processes during sieve element differentiation. Many studies (see Srivastava, 1975) confirm that mitochondria are retained through differentiation and are found in a relatively normal-appearing state in the mature sieve element. Plastids, while unusual in substructure, are also retained in mature sieve elements (Behnke, 1974). Some plants (notably dicotyledonous types) develop a proteinaceous material (termed P-protein) during differentiation that persists throughout the conducting life of the cell (Cronshaw, 1975b). Also, certain plants develop extensive membrane systems during differentiation which are retained in the mature sieve element (*e.g.*, Parthasarathy, 1974a, b; Neuberger and Evert, 1974, 1975). The relationship between these extraordinary cellular modifications as outlined above, and translocation functions has not been understood.

Historically, dicotyledonous phloem has served as a basis for conceptualization of the structure and physiology of phanerogamic sieve elements. However, within the last decade, advances in our understanding phloem in a wide variety of vascular plants has been achieved. These studies have shown that sieve elements of angiosperms and gymnosperms have many common characteristics.

However, there are fundamental differences between angiospermous phloem and phloem of coniferous species. The significance of these differences in relationship to evolutionary trends is not well understood (Esau, *et al.*, 1953; Esau, 1965, p. 270).

The term *sieve element* is generally applied to the conducting cells of both angiosperms and gymnosperms. More specifically, *sieve tube member* is usually the term of choice for angiospermous sieve elements. One of the differences between angiosperm and gymnosperm phloem tissue lies in the ontogenetic relationships between the cell types in the phloem. In most angiosperms, a specialized parenchymatous cell type, the *companion cell*, arises directly from divisions of a sieve tube mother cell. The companion cells are not only ontogenetically related but also are functionally related to sieve elements since they die when their associated sieve cell dies. Companion cells are thought to serve as reservoirs for structural and energetic substances for the functionally enucleate sieve element. They also may actively participate in loading and unloading substances into and out of the sieve element (Sauter, *et al.*, 1976).

An exact counterpart for the companion cell is missing in conifer phloem. But the so-called *Strasburger cells* (=albuminous cells) unique to gymnosperms, are thought to carry out the same functions as the companion cells (Sauter, *et al.*, 1976). Strasburger cells do not originate from the same initial derivative as their associated sieve elements. However, Strasburger cells, like companion cells, are the only cells (besides adjoining sieve elements) to have direct plasmodesmatal connections with sieve elements; and, like companion cells, they die when their associated sieve element succumbs (Sauter, *et al.*, 1976).

Another way sieve cells are distinguished from sieve tube members is by the size and position of the *sieve pores*--wall pores that connect the lumens of adjoining sieve elements. Conifer sieve pores are characteristically rather small in comparison to the pore diameter of sieve tube members. Pores tend to be distributed more evenly on radial walls of the sieve cell while the larger pores of sieve tube members are heavily concentrated on end walls, and are often arranged in patterns to form what are known as *sieve plates* (Esau, 1965, p. 278).

The application of ultra-thin sectioning to electron microscopic studies of biological material initiated a new era of phloem study which, besides developing a deeper appreciation of the complexities of the tissue, also has revealed additional cytological details including subtle, sub-cellular differences between angiosperms and gymnosperms.

Kollmann and Schumacher published the first ultrastructural study of gymnosperm phloem in 1961. Over the next few years these investigators contributed, through a series of papers on *Metasequoia glyptostroboides*, new information on the cytology and dynamic nature of the phloem of this gymnosperm. Kollmann and Schumacher (1963, 1964) found that an extensive membrane system, which they identified as endoplasmic reticulum (ER)*, developed in the sieve cell during differentiation while other membranes, such as the tonoplast, break down. These "ER" membranes persisted in vesicular, tubular, or lamellar form in the mature sieve cell. The "ER" was found to penetrate sieve pores, ramble within cavities formed in the middle lamella, continue through adjoining pores, and appear to become continuous with "ER" membranes of contiguous sieve cells. Massive "ER" systems and membrane-filled pores had, at the time, no known parallel in angiospermous sieve elements. Unfortunately, Kollmann and Schumacher's efforts to elucidate phloem cell structure were limited by their fixation technique which employed OsO_4 , K_2CrO_4 , plus NaCl . The high salt concentration of the fixative (employed as an osmotic balance for the highly turgid sieve cells) probably induced extensive artifacts in the differentiating cells and caused the vesication of

*I do not agree that they are endoplasmic reticulum membranes, however.

membranes apparent in the mature sieve cells. Furthermore, many proteinaceous compounds could not be fixed by the osmium/dichromate fixation method and must have been lost or destroyed in processing.

In 1963 Sabatini and Jacobs introduced new, improved methods of fixation using glutaraldehyde. Shortly thereafter, Ledbetter and Porter (1963) demonstrated the advantages of aldehyde/osmium fixation in plant tissue and the technique was immediately adopted. Three papers on conifer phloem appeared in 1966, utilizing the new aldehyde/osmium fixation methods (Murmanis and Evert, 1966; Srivastava and O'Brien, 1966 a,b; Wooding, 1966).

Since that time several other papers on gymnosperm phloem ultrastructure have appeared, all using the now standard double-fixation with aldehydes and osmium (Wooding, 1968; Murmanis and Evert, 1967; Harris, 1972; Campbell, 1972; Parameswaran, 1971; Evert, *et al.*, 1973; Behnke and Palival, 1973; Neuberger and Evert, 1974; 1975, 1976; Sauter, 1977; Sauter, *et al.*, 1976). These studies have shown that the various gymnosperm species have many common phloem characteristics. At maturity the sieve cells of all species studied are, like angiospermous sieve elements, drastically modified: Most constituents of typical plant cells are lost; this includes ribosomes, microtubules, dictyosomes, rough endoplasmic reticulum, and tonoplast membranes (*ibid.*). The nucleus of mature sieve cells is condensed to a pycnotic mass. Mitochondria and the plasmalemma remain in more or less normal appearing states (Srivastava and O'Brien, 1966). There are some ultrastructural features that differ

from typical angiosperm elements, however. In all gymnosperms studied so far an extensive membrane system is formed and retained in the mature sieve cell. These membranes are comparable to the "ER" membranes described by Kollmann and Schumacher (1963, 1964). They are aligned in parallel layers around the periphery and form great masses over sieve pores. The membranes in the mature sieve cell are sometimes referred to as the sieve element reticulum (SER) (Srivastava and O'Brien, 1966b). Similar membranes may be found in sieve tube members but the quantity of membrane is usually much less (Esau and Gill, 1971).

The origin and significance of the massive aggregates of membranes has not been resolved in the literature. Many investigators imply that rough endoplasmic reticulum is converted to the smooth membranes of the SER by loss of ribosomes and form modification (Wooding, 1966; Parameswaran, 1971; Behnke and Paliwal, 1973; Neuberger and Evert, 1974). Actual documentation of conversion of rough ER to the smooth membrane system of the mature sieve cell is lacking, however. Srivastava and O'Brien (1966b), in harmony with the tenet that the SER is a form of endoplasmic reticulum, suggested a *de novo* origin of the SER stating that coincident with the breakdown and loss of the rough ER, "a new reticulum is formed".

Peculiar plastids are also found in the mature sieve cell, and components of plastids (fine fibrils, starch and crystalline bodies) are found free in the sieve cell interior. There is disagreement in

the interpretation of the fate of the plastids during differentiation. Some authors contend that plastid materials are released as a result of fixation-induced lysis (Wooding, 1968; Behnke, 1974; Neuberger and Evert, 1974, 1976). However, it has also been suggested that some plastids lose membrane integrity during differentiation and plastid contents become dispersed naturally (Srivastava and O'Brien, 1966b; Harris, 1972; Parameswaran, 1971; Gamaley, 1973; Sauter, 1977). Some investigators have related the fine fibrils seen in mature sieve cells to the fibrillar P-protein found in dicots (Murmanis and Evert, 1966; Campbell, 1972; Sauter, 1977) without recognizing their possible plastid origin. One of the early investigators of sieve cell ultrastructure (Wooding, 1966) suggested that the membranes are discrete masses of a degenerate ER system which may have little to do with facilitating translocation. Wooding attempted to reconcile the popular mass flow theory of translocation with the sieve cell structure by speculating that the membrane clumps were swept aside during actual translocation, only to fall back to the pores upon cessation of flow. Since Wooding's study it has become clear that massive membrane systems represent a fundamental cytological specialization in gymnospermous sieve cells, and that the individual masses seen in sections of sieve cells are interconnected within the sieve cell and between sieve cells (Neuberger and Evert, 1974) forming one vast, complex system. Clearly, the structure of the sieve cell, as it is now known, is not reconcilable with mass flow (Spanner, 1978) and the sieve element

reticulum cannot be conceived simply as collections of degenerate membranes.

The results of these cytological investigations, while certainly not allowing reconciliation of structure and function of gymnosperm phloem, have at least allowed us to formulate more specific questions in relationship to the physiological state of the tissue. One might ask, for example: *What is the significance of simultaneous degenerative and generative processes which occur in the differentiating sieve cell? How are these highly selective processes maintained or controlled when the sieve cell nucleus is undergoing chromatin condensation and ribosomal loss? (These events would seem to preclude all gene expression!) How can the massive membrane systems, present in all investigated gymnospermous sieve cells, be reconciled with translocation processes?*

The following report addresses these fundamental problems with information gained from a reexamination of the ultrastructural dynamics of sieve cell differentiation. From my preliminary studies I came to believe that part of the reason why events of sieve cell differentiation and the structure of the mature sieve cell seemed so enigmatic was because key ontogenetic events had been missed or had been erroneously interpreted by prior investigators. In the present study considerable effort was made to detail with many examples the significant stages in *P. sabiniana* sieve cell ontogeny, in order to try to avoid misinterpretation because of inadequate ontogenetic

representation. As a result of my investigations, previously unreported ontogenetic events are exposed and a fundamentally new interpretation of sieve cell structure is made. These findings are used in the discussion to address the problems posed above and to clarify the significance of the structure of the differentiating and mature sieve cell. A major contribution of the present study may be that the results and the implications discussed allow even more specific questions to be posed for future investigations.

MATERIALS AND METHODS

Pinus sabiniana stems were collected on the University of California, Berkeley, campus in March when the emerging needles were about 2 cm long. A fixation method adapted from Parthasarathy's (1974a,b) study on palm phloem was used. Branch portions about 1.5 cm in diameter were sawed into approximately 4 cm pieces. During the subsequent trimming, stem pieces were bathed with glutaraldehyde fixative. The aldehyde fixative chosen for this study was 4% glutaraldehyde and 4% formaldehyde in 0.08 M cacodylate buffer (pH 7.0) used at $\pm 25^{\circ}\text{C}$. The phelloderm was removed with a sharp razor blade and the rough ends cleanly cut off leaving each piece about 3.5 cm long. The stems were then quartered longitudinally. Much of the xylem was removed by a clean tangential cut through the length of each piece. Trimmed samples were submerged in fresh fixative for 1 hour; and then cut along the sides and ends to produce thinner

pieces about 2 cm in length. Samples were submerged in fresh fixative for another hour; then trimmed to a 1 cm length. After an additional hour in fresh fixative, the tissue pieces were trimmed on the sides and ends to give thin, approximately 2 mm lengths of tissue. Each of the final, small samples was thus derived from the middle of one of the longitudinal quarters of the original 4 cm stem segments. The tissue was soaked for another hour in fresh fixative before washing 3 times (15 minutes each) with cacodylate buffer. The tissue was transferred to 1.5% OsO_4 in 0.08M cacodylate buffer and kept overnight at 4°C. Post-fixed tissue was rinsed twice with distilled water (15 minutes total) to remove traces of cacodylate, then dehydrated through an acetone series (15,30,45,60,70,75,85,95, and 100%) in 15 minute intervals. After a second change in 100% acetone, the tissue was transferred to acetone mixtures of 25%, then 50% low viscosity resin (type RD-4, E. Fullam Co.) for 2 hours each. The samples were placed in 75% resin/acetone overnight at room temperature. The following day the samples were transferred to 100% resin for 3 hours (uncovered). Finally, samples were placed in fresh resin for an hour then arranged in cross, radial, or tangential orientations in rubber molds. Resin, which embedded the samples, was polymerized in a 65°C oven for 2 days. Gray and silver sections (ca. 800Å thick) were cut with a diamond knife onto deionized water, using a Porter-Blum MT-2 ultramicrotome; sections were picked up on bare, acid-cleaned, 400-mesh grids and stained with aqueous, 1% uranyl-acetate for 2 hours. Grids and sections were rinsed in deionized water and

stained for an additional 15 minutes in Reynold's (1963) lead citrate followed by deionized water rinse. Some plant material was collected during the preceding October but was not subjected to the gradual trimming process described above for the spring collection. These samples were cut directly into small pieces and were fixed in 3 hours in an aldehyde-cacodylate mixture, as described above. Subsequent fixation, embedment, sectioning, and staining procedures were as above, except deionized water was not used during sectioning and staining--a factor which often increased staining precipitation problems. A Philips 300 electron microscope operated at 60 or 80 kV was used to study tissue and obtain micrographs.

OBSERVATIONS

Part of the difficulty in reconciling controversies such as the origin of the SER and plastid developmental sequences lies with the apparent difficulty in locating certain intermediate stages during sieve cell differentiation. The previously cited investigators of gymnosperm phloem ultrastructure have given their interpretations of the ontogenetic process of sieve cells, but actual documentation of the processes they describe with published micrographs is incomplete.

Srivastava and O'Brien (1966b) state that "exact temporal sequence (in sieve cell ontogeny) is not clear--probably many simultaneous changes occur quickly as intermediate stages are hard to find." In the present study the temporal events of cell maturation have been divided into four stages in order to facilitate the report

of the many complex cytological changes that occur. These stages are apparently not all the same duration, however. As Srivastava and O'Brien suggest, probably some events occur more quickly than others, and are thus more rarely visualized. Early stages were particularly hard to find.

Stage 1 of Sieve Cell Differentiation

Cells in the cambial zone have very thin tangential walls and thicker radial walls. Cambial initials (CA, Fig. 4.1) have many small vacuoles. The first evidence of differentiation is the formation of a large, central vacuole and the loss of many of the small vacuoles (cv, Fig. 4.1). The cytoplasm becomes very dense (Fig. 4.1, 4.3). Numerous ribosomes are found dispersed individually in the cytoplasm (cR, Fig. 4.3). Profiles of ER cannot be distinctly resolved in the dense cytoplasm.

Amyloplasts are the dominant organelles in the cytoplasm. They conform to generalized plastid morphology in that they are surrounded by two mutually distinct membranes--the "inner" and "outer" membranes (*e.g.*, Thomson, 1974). The inner membrane contributes to an ill-defined tubular internal plastid membrane system with some electron lucent cisternae (Fig. 4.3, arrows). One or more lens-shaped starch grains are found in each plastid. The grains have a typical ultrastructural image: an electron lucent center surrounded by a halo of slightly denser material (s, Fig. 4.3). The staining differences between core and halo may reflect differences in hydration states--

the grey halo being more hydrated (Thomson, 1974, p. 147). Plastid stroma is very dense and rich with plastid-type ribosomes (pR, Fig. 4.3). One end of the plastid is often elongated forming a tail that extends for some distance through the parietal cytoplasm to become obscured in the similarly-textured cytoplasm (pl, Fig. 4.6). Within the plastid stroma, groups of straight filaments similar to cytoplasmic microfilaments can sometimes be seen in both longitudinal and cross sectional views (mf, Fig. 4.3).

A peculiar type of thickened wall is a common characteristic of many kinds of phloem. This characteristic wall has been termed the nacreous wall (Esau, 1939). In pine, the nacreous wall is distinctly a secondary wall deposited on the internal surface of the primary wall. It is first evidenced in late Stage 1 by loose deposits of microfibrils that are more or less layered (nw, Figs. 4.1 - 4.3). Throughout differentiation the wall becomes thicker and more complex. Details of the development are discussed separately, later in this report. The mostly parietal position of the abundant golgi bodies perhaps represents an association with the developing nacreous wall and future sieve areas (g, Fig. 4.4).

The nucleus, late in Stage 1, has coarse granular heterochromatin with moderate amounts of electron lucent euchromatin (Fig. 4.4).

There is apparently an increase in membrane fluidity during early stages of sieve cell differentiation. One aspect of this

fluidity is reflected ultrastructurally by increased susceptibility to plasmalemma invagination and possible vesication during killing and fixing (Fig. 4.7). Fig. 4.7 shows a radial file of three differentiating sieve cells in Stages 1, 2, and 3, bounded on the upper side by phloem ray cells. Cell 1 in Fig. 4.7 is a developing sieve cell in Stage 1 with plasmalemma invaginations, flocculent vacuolar material, and a large starch-containing plastid. Magnification of cell 1 in the region of the plastid shows that the tonoplast has become discontinuous allowing vacuolar material and cytoplasm to become confluent (arrow, Fig. 4.8).

Stage 2 of Sieve Cell Differentiation

At the beginning of Stage 2 boundaries between plastids and cytoplasm become less distinct because in some regions the limiting membranes of the plastids seem to pull away from the plastid (unlabelled arrows, Figs. 4.5, 4.6, 4.7, 4.9). Notice that this usually occurs on the side of the plastid facing the central vacuolar regions.

Vacuole-like vesicles fill the space once occupied by the single, large, central vacuole (Figs. 4.6, 4.7). The mixed origin of these vacuole-like vesicles is indicated by subtle fixation image differences between the vesicles. One type of vacuole may be derived from the tonoplast. These vacuoles are often larger and collapsed slightly (v^1 , Fig. 4.6). A second distinctive vacuole type usually is expanded. When seen in appropriate sections, type-2 vacuoles

may each contain a dense, fibrillar inclusion (i) that often touches the internal side of the membrane (Fig. 4.6, (v^2), 4.7, 4.11, 4.14). The origin of this type of vacuole seems to be from separation and ballooning of membranes from the plastid body, and the fibrillar inclusion appears to be derived from a fibrous component of the plastid stroma (i, Fig. 4.9). These vacuoles increase in number throughout Stage 2; while most of the other vacuoles disappear (Fig. 4.15).

During Stage 2, the starch-containing plastid "heads" lose their association with the stroma and membrane tails (Fig. 4.4). Perhaps the discontinuity arises because a disappearance of limiting membranes allows plastid tail regions to expand through the cell lumen and to integrate with the cytoplasm. The plastid heads then round up, encased only partially by membranes. One to several osmiophilic crystalline granules appear near the starch grain (c, Figs. 4.10 - 4.12, 4.14) and an increasing volume of flexuous filaments curve around the centrally placed starch grain (fi, Fig. 4.11, 4.14). Similar groups of filaments have been identified as fibrillar protein in other gymnosperms (Behnke, 1974).

As differentiation progresses, the nucleus acquires a coarse, granular texture due to condensation of the chromatin (Fig. 4.5).

A system of anastomosing tubules is seen in the central portion of the differentiating sieve cell towards the end of Stage 2 (T, Fig. 4.10 - 4.12). The origin of the tubule mass is unknown.

There is no ontogenetic evidence in this study that would justify calling these tubules a form of endoplasmic reticulum, even though similar tubular masses have been called endoplasmic reticulum in studies of differentiating sieve cells (*e.g.*, Behnke and Paliwal, 1973; Evert, *et al.*, 1973, Neuberger and Evert, 1975). The tubules bear a resemblance to the internal tubular membrane system of plastid tails seen in earlier periods of differentiation (Figs. 4.1, 4.13). Since the appearance of the tubules coincides with the partial loss of plastid envelopes and the complete loss of recognizable plastid tails, it is possible that the tubular mass is an aggregation of pre-existing plastid tubule systems that had been released into the cytoplasm. Other circumstantial evidence that links the tubules with a plastid origin is the clustering arrangement of plastid heads that is sometimes seen around the periphery of a tubule mass (T, Fig. 4.11). Densely staining polyribosomes are present within the tubule mass (pr, Fig. 4.12) as well as another less distinct, granular component, which corresponds in size and stain affinity to plastid ribosomes. Other cell components are excluded from the central tubule mass region. Bundles of longitudinally-oriented microfilaments (mf) are often found around the periphery of the tubule mass (Fig. 4.12).

The exclusive nature of the central tubule mass and the increasing population of small (type 2) vacuoles, displaces other cytoplasmic components to the cell borders (Figs. 4.10, 4.11, 4.15).

At the borders of the differentiating sieve cell are found abundant, normal golgi bodies and mitochondria (Figs. 4.10, 4.12). Lamellae of rough endoplasmic reticulum (ER) are found (Fig. 4.11) and coated vesicles (re: Hoefert, 1979) are sometimes seen in the cytoplasm near the plasmalemma (cv, Fig. 4.11).

Stage 3 of Sieve Cell Differentiation

Stage 3 of sieve cell ontogeny is signaled by structural changes in the once definitively-shaped starch grains housed in plastid heads. The starch material becomes swollen and amorphous with small, irregular, clear regions among larger, grey, (presumably) hydrated regions (hs, Fig. 4.7 (cell 3), 4.11, 4.17, 4.18, 4.20, 4.21). I believe these morphological changes in sieve cell starch represent visual evidence of amylopectin (a component of starch) gelatination in sieve elements. (Histochemical tests have shown that sieve elements have a high proportion of amylopectin starch (Srivastava, 1975). Amylopectin is a high molecular weight branching carbohydrate and will form a gel when hydrated.) After starch gelatination, the filamentous protein may be found within the carbohydrate masses (fi, Fig. 4.17).

Plastid regions associated with gelatinated starch may retain partial encasement by double membranes during Stage 3 (PLm, Fig. 4.14). But usually only one membrane remains associated with the starch and fibrillar material; and sometimes starch and fibrils are directly adjacent to the cytoplasm (Figs 4.11, 4.14, 4.16, 4.18).

The loosely organized central tubule mass characteristic of Stage 2 disappears during Stage 3. It was not determined whether

the tubule system breaks down or is reorganized in some fashion. However, an elaborate membrane system, distinct from the central tubule mass, develops. As outlined below, this membrane system originates as emanations from plastid heads and becomes the sieve element reticulum (SER) of the mature sieve cell.

Coincident with the gelatination of starch, plastidial membrane elaborations develop at the periphery of the plastid heads usually in proximity to a collection of filaments. In many sections the filaments gradually blend into the membranes allowing the interpretation that the filaments are incorporated directly into membranes (arrowhead, Fig. 4.17). In early stages of SER formation, parallel membranes, that are adjacent to plastid heads, may anastomose regularly with each other by short tubules (SER, Fig. 4.18; t, Fig. 4.20). When sectioned parallel to the plane of membranes, the membranes appear as perforated sheets (PS, Figs. 4.18 - 4.20). The perforations are the orifices of short, interconnecting tubules, similar to those seen in longitudinal section in Fig. 4.20. A granular substance occupies the interior of the short tubules. These collections of parallel membranes interconnected by short, substance-containing tubules are quite similar to membrane configurations typically found in etiolated plastids undergoing the greening process (Gunning and Jagoe, 1967; Weier and Brown, 1970).

During the first part of Stage 3 when the SER is being produced, ribosomes occur in clumps and there are many small vesicles

throughout the cytoplasm. Mitochondria are generally located along the sides of the cell, away from pore sites. Some microfilaments are still seen during this time as well as a few short lengths of rough ER (Fig. 4.19). Golgi bodies were not found in early Stage 3 cells. As Stage 3 progresses, the SER membranes become more and more extensive, especially around the future sieve pores where plastid heads have tended to congregate. By the end of Stage 3, the SER completely engulfs plastid heads and mitochondria, to form a system of anastomosed, flattened sacs and sheets with a paired-membrane spacing of about 100 \AA (Fig. 4.21). Coincidentally with the extensive SER development, most other cytoplasmic components disappear. The nucleus become condensed to an electron opaque mass. The plasmalemma is, in contrast to the condition in Stage 1 cells, tightly oppressed to the cell wall following the extensive irregularities of the inner surface of the sieve cell wall (*cf.*, Fig. 4.21 with 4.7). Mitochondria remain as do the plastids and their highly developed membrane derivatives. In sum, *all cytoplasmic components (with the exception of the plasmalemma) exclusively dependent on the nuclear genome are lost, while those components with independent genomes (see Tewari, 1971) survive.*

Stage 4. The Mature Sieve Cell

The mature sieve cell may be identified by the presence of sieve pores and median nodule regions completely lined with callose. (Compare the sieve pores of the immature cell in Fig. 4.21

with the callose-lined pores in Fig. 4.23). In this study, heavy wound callose has been redeposited around sieve pores of mature sieve cells, constricting the pores back to nearly plasmodesmata size (Fig. 4.23). The development of sieve pores will be discussed later in this report in relationship to sieve cell wall development.

The lumen of a sieve cell at the onset of maturity is completely filled with anastomosing vesicles and channels of SER (Fig. 4.24). Plastids (Fig. 4.24, arrowheads) within the lumen are greatly swollen with hydrated starch. Vesicles of SER tend to define the boundaries of the plastid forming a discontinuous membrane perimeter. In places the interior of the plastid is confluent with the luminal space of the sieve cell (unlabelled arrows, Fig. 4.24). The most elaborate membrane configurations are found near the sieve pores and are arranged in pairs with approximately 100\AA between associated membranes (Fig. 4.23, 4.24). As the sieve cell continues to age--and presumably becomes a conducting element--the lumen becomes more open with fewer SER vesicles (Fig. 4.24). The contents of enlarged, open plastids (such as those seen in Fig. 4.24) disperse with the clearing of the lumen. Large membrane masses remain in the region of the sieve pores. Plastids trapped in the dense, paired membrane do not disperse. Their borders are completely defined by the SER (Figs. 4.46, 4.47). Continuities of the inner portion of the plastid and the intermembrane space of the SER is sometimes found

(Fig. 4.47 unlabelled arrow).

The nuclear material in the mature sieve cell has condensed to an extremely osmiophilic, often angular, mass (Fig. 4.26). It is enveloped with SER. Pycnotic nuclear material may migrate between what appears to be SER membranes for a short distance (m, Fig. 4.27).

Attempts to resolve the SER membrane substructure gave variable results due in part to the section plane of the membrane. Portions of membranes sectioned perpendicularly had a trilamellate, somewhat beaded structure (Fig. 4.22). In other cases, the membrane seemed to have a fibrillar component and is interpreted to be more obliquely sectioned (Fig. 4.29, small arrows); while sometimes no distinct substructure could be determined.

At about the time the lumen of the recently mature sieve cell begins to clear of vesicles of SER, unusual structures begin to form (Figs. 4.25, 4.30, at arrows). For reasons to be outlined below, these structures are assumed to be membrane sequestering bodies (MSB) that store membrane components released by the disassociation of "excess" SER and dispersed filamentous protein from plastids.

The bodies appear to arise in two different manners. One less common way, is by a process within a membrane-enclosed body. Large, three-dimensional polyhedra with a paracrystalline structure having several lattice dislocations are characteristic of membrane-enclosed MSB (Figs. 4.31, 4.32). The substructure of these and other paracrystalline MSB consists of hexagonally close packed tubules. The tubule walls appear to be formed by stacks of rings (re, Fig.

4.31). The developmental process of the membrane-enclosed MSB was not determined.

Most MSB originate in a more exogenous fashion, but in relationship to another membrane surface--usually the plasmalemma (Fig. 4.30, 4.33 - 4.36). The ontogeny of these MSB can be more readily surmised than the rarer, membrane-bound type, since numerous generation sites with MSB in various stages of complexity can be found in a single longitudinal section of a recently matured sieve cell (Fig. 4.25; 4.30, arrows). Dispersed, 60Å filaments, (f, Fig. 4.30, 4.34) (which resemble the proteinaceous plastid filaments that are involved in SER production during Stage 3), move from random to clumped orientation (f, Fig. 4.30, 4.34) to parallel association (fs, Fig. 4.34, 4.36); then to fusion into an undulated sheet (unlabelled arrows, Fig. 4.30). After the framework of the membranes is established by the protein fibrils, dense, amorphous material is incorporated with the protein (Figs. 4.34 - 4.36). This matrix-like material may be lipoidal in nature as suggested by its osmiophilic character. As fibrillar sheets (fs) form, and matrix material is incorporated, the sheets fuse at intervals with established undulating sheets creating a three-dimensional mesh (Figs. 4.33 - 4.34).

The highly-ordered paracrystalline MSB appear to be derived from the fibrous sheets which form as described above. It is my hypothesis that the paracrystalline substructure is formed by the fusion of contracted, very regular, corrugated sheets along opposing

peaks and valleys. Such condensations will produce a tubular, hexagonally close-packed array such as seen in Figs. 4.30, 4.33, 4.35, 4.36. Figure 4.37 diagrammatically shows the proposed origin of the MSB. According to the model the tubule walls should be single-layered on all but two "sides" of each hexagonally close packed tubule. But sections of MSB which are perpendicular to the longitudinal axis of the tubules show what appear to be multilayered, ringed walls. It is possible that the walls are made of stacks of discrete rings rather than stacks of sinuously arranged filaments, which are continuous from tubule to tubule. However, the layered appearance of the walls as seen in Fig. 4.38 could be the result of viewing stacks of curved 60\AA fibrils through a section several times as thick. Also, the occasional suggestion of fibril continuity from one tubule to the next lends credulity to the model (Fig. 4.38, arrow).

The disunion of MSB's appears to contribute directly to the development of the SER. Fine structural images of SER linked to a MSB often include regions of transition from the extremely ordered MSB to the loosely arranged SER. By studying MSB/SER transition zones and utilizing the model of the development of MSB's (Fig. 4.37), it is possible to formulate an interpretation of the ontogenetic relationship between MSB's and SER, as follows:

The disassembly of the MSB's to the more loosely ordered SER may begin when the regularly arranged tubule conformations of the MSB's lose their coherency by the collapse of the corrugations to

form fused, paired, membraneous sheets. This hypothetical event is diagrammatically shown in Fig. 4.37 (right side of Fig.) and is illustrated in association with an actual MSB in Fig. 4.31 (at x). Fig. 4.39 shows a well resolved portion of a transitional MSB. In section, the fused membrane complex consists of 8 layers to form an approximately 260 Å unit. These 8 layers can be thought of as 2, 80 Å, beaded, "tri-lamellate" membrane portions enclosing an electron lucent region with a central, 40 Å, electron-dense core layer. Eventually the core layer is removed and the membrane complex parts into 2 separate membranes (unlabelled arrows, Fig. 4.31). Close pairing of the originally fused sheet may persist (Fig. 4.31) with an approximately 100 Å space between membranes.

Fig. 4.29 demonstrates the relationship between plasmalemma-associated MSB and the SER. Single fibrillar sheets (fs) integrate with fused membrane complexes (MSB) in a loosely organized MSB. The adjacent SER is not shown to connect with the MSB in the micrograph but a relationship between SER and MSB can be inferred by the presence of small, fused increments of SER (large arrows) exactly like fused portions of the MSB. The SER in Fig. 4.29 has a very distinct fibrillar membrane (small arrows) which is perhaps a feature that persists from the contribution of filaments in the origins of SER from plastids and MSB.

The SER in sieve cells several months old tends to become more sparse but usually more ordered. The lumen becomes clear, but extensive arrays of SER are still found associated with the

cell periphery and sieve areas. The paired sheets maintain a spacing of 100 - 125Å and may be perpendicular or parallel to the sieve cell wall (Fig. 4.42, 4.43). In the region of sieve pores a massive membrane labyrinth remains throughout the life of the sieve cell (Fig. 4.45, 4.46). The even spacing of paired membranes is found here also, with abundant, random anastomosing between membranes. More rarely, lattice configurations of membranes, similar to prolamellar body lattices (Gunning and Steer, 1976) are seen (L, Fig. 4.45). Even in this older tissue, the plastid origin of the SER may still be inferred: Sometimes plastid heads, consisting of hydrated starch (hs), fibrous protein inclusions (fi) and crystalline bodies (c), are found in the middle of a membrane mass (Fig. 4.46, 4.47). These plastid heads are defined by surrounding membranes, and in some places confluence of the internal regions of the plastid and the SER cisternae are apparent (unlabelled arrows Fig. 4.47). In other cases, plastids may have direct connections with lamellar SER arrays (unlabelled arrowheads, Fig. 4.43, 4.44).

Sometimes another membrane lies on top of the plasmalemma (dm, Fig. 4.41), the significance of this is not known.

The Sieve Cell Wall

The walls of sieve elements are often termed "nacreous" (Esau, 1939) walls because of their characteristic lustrous appearance in fresh sections. The physical and chemical nature of the walls as well as their physiological relationship to the

translocation process are not well understood (Esau, 1969). Nacreous walls are more highly developed in some taxa than in others (Esau, 1969). Pine nacreous walls are among the most highly developed as far as thickness and complexity are concerned.

The tangential walls of the initials from which *P. sabiniana* sieve cells are derived are extremely thin (Fig. 4.1). During the process of differentiation, the primary tangential walls thicken slightly, while the thicker, radial walls sometimes become thinner due to radial extension of the cell. Secondary wall deposition begins early in relationship to cytological differentiation of the pine sieve elements. For example, in Stage 1, when plastids are not yet discernibly altered, loose, nacreous wall material is evident (nw, Fig. 4.3). Then, by the time that the outer plastid membrane begins to pull away from the plastid body, the nacreous wall is conspicuous, although not yet fully developed (na, Fig. 4.7).

In this study the secondary (nacreous) wall of pine had a distinct lamellate appearance, made more obvious by separation into layers of microfibrils and electron lucent matrix regions (nw, Fig. 4.1). Throughout Stages 1 through 3 of differentiation the secondary cell wall thickens rather evenly around the cell with alternating layers of microfibrils and matrix (Fig. 4.7). But at the end of the differentiation process, as the sieve element becomes mature, bulges of electron lucid material develop between the primary and secondary walls (wb, Fig. 4.26). Wall bulges usually develop only on the cambial side of the periclinal walls, extending

sometimes around the corners into the anticlinal walls. But, they are not found on the anticlinal walls near sieve areas. Adjacent sieve cells in a radial series usually do not both develop wall bulges in their common tangential walls; but the one bulge distorts both sets of walls (wb, Fig. 4.26, 4.48).

Microfibrils are randomly dispersed within the electron lucent bulges and electron-dense, channel-like areas develop throughout the region (Fig. 4.27, 4.28, 4.51). The complexity of the wall bulges seems to be related to an association with a living, functional sieve element. Figs. 4.49 - 4.52 demonstrate differences in substructure of wall bulges around functional sieve cells, cells that recently died, and those that have been dead for a longer period of time. The bulging walls of contiguous, living sieve cells are seen to contain many electron-dense channels (ch, Figs. 4.49, 4.51). Fig. 4.50 is a portion of wall associated with a dead cell but only a few cells away from the functional sieve cells. The electron-dense, channel-like regions (ch) are gone from the center of the bulge (wb), existing only around the periphery of the expanded, electron lucent portion. The micrograph of Fig. 4.52 was taken several cells external to Fig. 4.50. Here, electron-dense channels are completely gone, and the microfibrillar component (mfi) of the electron lucid region of the wall bulge (wb) is quite sparse. In still older cell walls, the microfibrillar component may completely disappear, and the bulge will close due to the crushing of old sieve cells.

The walls of a mature sieve cell and an adjacent Strasburger cell may be compared in Fig. 4.53. The section is through radial walls and does not include a wall bulge. A middle lamella (ml) delimits the sieve cell wall from the Strasburger cell wall. Primary walls (pw) of the two cell types are almost identical in stain affinity, substructure, and thickness. The thick secondary (na-creous) wall deposited over the inner surface of the primary wall of the sieve cell is very distinctive, however. It is 5 or 6 times thicker than the primary wall and has the typical alternating layers described previously. A particulate stain reaction product (rp) is abruptly restricted to the secondary wall. Furthermore, the reaction product is specifically associated with the grey layers of the secondary wall. This particulate component is usually absent, however, from the electron-dense band immediately adjacent to the sieve cell plasmalemma. The reaction product is also heavily associated with protein fibrils and other components in the sieve cell, but not associated with cytological structures in the albuminous cell. In regions of the wall bulges (e.g. Fig. 4.51) reaction product (rp) is associated specifically and heavily with the channels (ch), and less heavily with the microfibrils (mfi) found in the electron lucent regions. The localized reduction of metal ions to form a particulate reaction product reflects chemical peculiarities of the sieve element, the nature of which is unknown. When deionized water was used for rinses, deposition seemed to be reduced.

Sieve Pore Development

Sieve cells get their name from the sieve-like pores that perforate their walls. Sieve pores develop from enlarged plasmodesmata in primary pit fields that connect adjoining differentiating sieve cells (pd, Fig. 4.10) (Wooding, 1966; Neuberger and Evert, 1975).

A complex carbohydrate called *callose* is almost always seen in preparations of phloem tissue in association with developing or mature sieve pores (Esau, 1969, pg. 57-58). The substance is found external to the plasmalemma--between the membrane and the more electron-dense, cellulosic portion of the cell wall (*e.g.*, ca, Fig. 4.16). Callose is a β -(1,3)-linked glucan which is synthesized independently of cellulose and, unlike cellulose, it may be deposited and reabsorbed quickly (Frey-Wyssling, 1976, pg. 117-120). The function of callose may be related to its ready synthesis and transitory character. For example, it may serve to temporarily isolate a protoplast from surrounding tissues, or produce localized variations in cellulose wall depositions by acting as a temporary, restrictive template (Esau 1969, pg. 57-58; Frey-Wyssling, 1976, pg. 120).

Callose deposition in connection with sieve pore development shows "finely graded variability" (Esau, 1969, pg. 58) following a characteristic pattern of deposition related to the developmental stage of the sieve element. Although the presence of callose in sieve elements prepared for microscopy may be partially or completely due to an extremely sensitive wounding response, the stability of the pattern of deposition serves as a useful diagnostic feature of

the developmental and physiological states of sieve elements (Esau, 1969, pg. 58).

The future sieve area (fsa) of a Stage 1 differentiating cell of *P. sabiniana* is recognizable as a thin-walled primary pit field surrounded by the thickening nacreous wall. Plasmodesmata traverse the depression. The first evidence of pore differentiation is seen in Stage 2 when small, bulbous, callose rings (ca) surround the plasmodesmatal channels where they open into the luminal cytoplasm (fsa, Fig. 4.15). The site of callose deposition is interpreted as marking regions of cellulosic wall removal. On the basis of the pattern of callose deposition it appears that cellulosic wall material is progressively removed from around the plasmodesmata--proceeding from the callose ring (first seen in Stage 2) toward the middle lamella. Concomitant to the progressive deposition of callose, cavities develop in the middle lamella regions during Stage 3. These cavities are called *median nodules* (mn). Throughout Stage 3 the median nodule cavities remain free of callose (mn, Fig. 4.18, 4.20, 4.21); but callose deposition eventually reaches the middle lamella region, and the cavities become lined with callose (Stage 4) (mn, Fig. 4.23).

Whether or not any of the callose seen in the micrographs is an integral part of the process of pore widening *in vivo* could not be determined. This is because a relatively slow killing and fixing process was used in tissue preparation, which allowed significant amounts of wound callose to be deposited around developing or mature sieve pores. The presence of wound callose would mask the smaller

amounts of structural callose, if the latter were present.

However, the slow fixation technique involving gradual trimming of an initially rather long piece of stem, seems to help avoid the blow-out effect which usually occurs when the pressurized, mature sieve element conduit is suddenly opened up to atmospheric pressure (Parthasarathy, 1974b). The protection from blow-out is probably due to the opportunity for large amounts of wound callose to be built up (effectively sealing off individual sieve cells) before the cells were subjected to final trimming and fixing. Blow-out is more likely to occur when samples are obtained by immediate dicing of tissue at the onset of the fixation.

The above contention is supported by comparison of Figures of mature cells killed gently and slowly (Figs. 4.23 - 4.25, 4.30, 4.42) with samples diced immediately in fixative upon removal of a length of stem from the branch (Fig. 4.54). It is felt that Figs. 4.23 - 4.25 and 4.30 represent mature sieve elements with cell contents fixed in relatively normal positions, although displacement was still apparent in some cells. Whereas, Fig. 4.54 shows a typical blow-out effect of a abruptly severed phloem conduit. Mats of SER are collapsed on one side of the sieve area connection between adjoining sieve cells. The SER in the lower cell of Fig. 4.54 appears to be blown away from the sieve pores. Callose deposition in these cells is not as heavy as the deposits seen in tissue killed more slowly (*cf.* Fig. 4.23, 4.54). Since the collapse of the SER from pressure release occurs immediately, but callose deposition

occurs over a period of minutes, the callose in Fig. 4.54 must have been deposited after the collapse of the SER.

Strasburger Cells

Strasburger cells have an indirect ontogenetic relationship with sieve cells in that they arise from initials that have previously produced sieve cells (Srivastava, 1963). They are the only cells with direct plasmodesmatal connections with sieve cells. The connections are typically branched on the Strasburger cell side, leading to a median nodule. Single sieve pores from the contiguous sieve cell connect to the median nodule cavity (Figs. 4.55, sp; 4.58, pd).

The existence of plasmodesmatal connections with an adjoining sieve cell is the feature that most accurately allows identification of Strasburger cells (Alfieri and Evert, 1968), and some investigators have limited identification of Strasburger cells to those cells displaying such connections (Neuberger and Evert, 1975). In the present study I found Strasburger cells to have such a distinctive fine structure that, whether or not the section plane included a sieve cell connection, they could be easily distinguished from other phloem parenchyma cells. One of the most obvious features is the electron lucent ground cytoplasm rich with organelles and generally lacking a large central vacuole (St, Figs. 4.55, 4.62). Ribosomes are either grouped in polyribosomes or absorbed to the rather sparse ER. In general, no individual ribosomes are free in the cytoplasm (Figs. 4.56, 4.61).

The dominant storage product of *P. sabiniana* Strasburger cells in the spring is lipid--as witnessed by the numerous, small spherosomes (sp, Fig. 4.55). Starch grains are found only in a small percentage of the plastids (s, Fig. 4.62).

Plastids are considerably different from those found in other ray parenchyma but similar to plastids in Stage 1 of differentiating sieve cells. The small starch grains, if present, are housed at one end of the plastid--the other end often extends into a long, curved tail (unlabelled arrowheads, Fig. 4.62). Strasburger cell plastids are similar to mitochondria in that they possess an extensive inner cisternal system (*cf.* PL and M of Fig. 4.55). However, the enveloping membranes are less distinct than those of mitochondria (*cf.* PL and m in Figs. 4.55, 4.58, 4.59, 4.61). Sometimes the plastid membranes seem to be discontinuous or absent in certain regions (PL, Fig. 4.61). The internal membranes are even less distinct than the outer limiting membranes. But, despite membrane vagueness, the plastid stroma is clearly compartmentalized with the inner cisternal spaces remaining electron lucent (Fig. 4.61).

A medianly-located bundle of microfilament-like fibrils was observed in a few instances within albuminous cell plastids (Figs. 4.59, 4.60). The presence of microfilaments or microfilament-like bundles of fibrils has not been previously reported in plastids as far as I am aware.

Collections of tubules are seen in Strasburger cells, sometimes in association with plastids (T, arrowhead, Fig. 4.55). These tubules are similar to the tubules that form the central tubule mass in Stage 2 of sieve cell differentiation.

The inner surfaces of Strasburger cell walls are very irregular in tissue obtained from spring samples (Fig. 4.59). Likewise, the plasmalemma was irregular in contour. Some vesication of the plasmalemma is seen (Fig. 4.61). Bundles of cytoplasmic microfilaments are readily found in Strasburger cell cytoplasm (mf, Figs. 4.55, 4.56, 4.58). Vacuoles are generally small (Fig. 4.55).

Nuclear pores were well preserved in the nuclear membrane of Strasburger cells (Fig. 4.57). Pore diameter ranges from 200 Å to 300 Å, indicative of either a conical or hourglass shape through the depth of the pore. At the levels of the smaller diameter, a centrally located particle is found (cpc, Fig. 4.57). Eight particle units surround the aperture forming the walls of the pores. In the larger diameter portion of the pore, there is a less distinct pore component. Heterochromatin, normally associated with the inner nuclear membrane, is absent from a radius of about 500 Å from the center of the pores (h, Fig. 4.57).

DISCUSSION

Theories of Translocation

Several theories have been suggested to account for the phloem translocation process in vascular plants. Among the theories,

the Münch hypothesis has become the most widely accepted explanation of translocation (Keeton, 1972, p. 211). The Münch theory explains phloem translocation on the basis of mass flow. A build-up of osmotic pressure at assimilate sources is the driving force for mass (or volume) flow of solution through the sieve element conduit to regions of lower pressure (sinks). The Münch model does not require energy augmentation along the translocation path, although additional energy may be required for loading or unloading the conduit (Crafts and Crisp, 1971). Although more experimental evidence has accumulated which supports mass flow than has accumulated against the mass flow hypothesis (see Weatherly, 1975a) many phloem investigators and transport physiologists are not convinced that the Münch model provides sufficient explanation of phloem transport phenomena (Wardlaw, 1974; Spanner, 1979; Lüttge and Higinbotham, 1979, pg. 316).

There is no dispute as to whether the Münch model works. Mass flow simply obeys equilibrium principles of the second law of thermodynamics for the energetics of the system, and recognizes the interdependence of fluxes (*e.g.*, water, solutes, ions) for bulk transfer in the system (Tyree, 1975). The applicability of the model lies with whether an entirely "passive" motive force such as volume-flow can account for observed high rates of flow under conditions inherent in living sieve element conduits.

In order to evaluate whether a volume flow system, alone, could account for translocation *in vivo*, scientists must define the factors that interact in translocation to determine the fit of the observed conditions with the model predictions. Detailed biophysical analysis of translocation requires elaborate computations (Tyree, 1975). However, for the purpose of demonstrating general relationships between factors involved in passively-driven volume flow, the following simple equation is useful:

$$v = L (P_{\text{source}} - P_{\text{sink}})$$

In the above equation (adapted from Tyree, 1975, pg. 526) v = volume flux, L = hydraulic conductivity of the conduit, and P = hydrostatic pressure.

Values for v are readily obtained by radioactive tracer techniques (Wardlaw, 1974, and literature cited therein). Pressure differentials from source to sink are more difficult to obtain but various techniques have allowed reasonable estimates (Weatherley, 1975a). By far the most difficult parameter to estimate is the hydraulic conductivity (L) (Tyree, 1975). This factor depends on the viscosity of the cell sap, the coupling of individual fluxes and metabolic events, and the ultrastructure of living, conducting sieve elements. Biophysicists have calculated that if mass flow, alone, is to account for the observed rapid translocation rates over long distances, the hydraulic conductivity must remain high so that there is relatively little impediment to flow.

Acceptance or rejection of mass flow as a tenable theory of translocation currently rests on whether sieve element ultrastructural images are interpreted as flow-restrictive, or non-restrictive. A substantial number of investigators feel that *in vivo*, sieve elements have a clear lumen with open pores. Occluded pores, as well as membraneous and filamentous material within the sieve element lumen are thought to be artifacts of a disrupted cell (*e.g.*, Giaquinta and Geiger, 1973; Cronshaw, 1975b; Fisher, 1975). But many workers have published electron micrographs illustrating lumenal structures which are not considered to be artifacts. Their results demonstrate that many sieve elements are not virtually empty conduits but contain filamentous materials (P-protein) (Johnson, 1975) and/or membraneous structures (*e.g.*, Srivastava and O'Brien, 1966b; Wooding, 1968; Parameswaran, 1971; Behnke and Paliwal, 1973; Evert, *et al.*, 1973; Parthasarathy, 1974a,b; Schmitz and Srivastava, 1974; Walsh and Evert, 1975; Melaragno and Walsh, 1976; Sauter, 1977) which would obstruct purely passive flow through the sieve element, and reduce the hydraulic conductivity of the sieve cell conduit. Reduced conductivity may, in turn, reduce flow rates to the point that a passive mass flow system becomes inadequate to account for the observed translocation rates despite substantial pressure differences from source to sink.

The electron micrograph evidence presented here confirms previous claims (*e.g.*, Srivastava and O'Brien, 1966b; Evert, *et al.*, 1973) that an extensive membrane system covers the sieve pores of gymnosperms.

Since these membranes are present before the sieve cell is mature, their pore-occluding position in the mature cell cannot be interpreted as a result of displacement toward pores upon severance of the conduit. From the ontogenetic study, the SER has been shown to be a proliferation of plastid-derived membranes. These findings immediately suggest that the sieve element reticulum has important functional significance. The plastid origin of the SER may allow reconciliation of structure with function in sieve cells: Ordinary plastid membranes have unique energy transducing capabilities and are thought to be capable of ionic pump activities (Junge, 1977, and literature therein). These same capabilities, applied to the translocation system of plants, would provide a mechanism for generating "activated flow" (MacRobbie, 1975a) or a type of electro-osmosis (Spanner, 1979), and thus overcome hydraulic conductivity restrictions of membrane-occluded sieve cells. In addition, a theory of translocation based on the active participation of membranes may explain the apparent directional and rate versatility of sieve elements that has been difficult to reconcile with a passive flow mechanism of phloem translocation (Canny, 1971). Furthermore, the unexplained enhancement effects of light on translocation (Reinhold, 1975) may be attributable to a photon-sensitive character of plastid membranes.

Recently Sauter (1977) has demonstrated through ultrastructural histochemistry that certain specific sites (SER, plasmalemma, and free protein filaments) in *P. nigra* sieve cells appear to have

substantial enzymatic capabilities for splitting energy-rich compounds such as nucleotides. These interpretations are based on a lead phosphate reaction product obtained from lead staining after incubation of tissue with ATP or other high-energy phosphate substrates. High phosphatase activity associated with SER and the plastid-derived filaments is consistent with a theory of an "active" plastidial membrane contribution to the rate of flow. Sauter's results are equivocal, however, since a similar reaction product has been found associated specifically with the nacreous wall layers of pine sieve cells (Chapter III), and with SER and the free protein filaments in some of the tissue used in this study (Figs. 4.49-4.53). The reaction product was specifically-located and conspicuous even though the tissue was not incubated with high-energy substrate (as a source of phosphate). In the present investigation, a poor quality lead stain and/or contamination in the water rinses may have provided ions for lead complexes which became bound to certain charged sites in the sieve cell. More histochemical work is needed before conclusion can be made regarding the localization of high energy compounds within sieve cells and the significance of lead complex binding in sieve cells.

Membrane Biogenesis of SER

The biogenesis of SER membranes is an unusual and dynamic process. The components of this membrane system include filamentous, 60 Å units derived from proteinaceous fibrillar plastid inclusions,

and an amorphous matrix component that, because of its affinity for osmium, may include unsaturated lipids (Dawes, 1971, p. 27).

The results of this study have allowed the following conclusions concerning the SER of *P. sabiniana*: 1) The membranes are initially the direct product of plastid synthetic processes involving incorporation of preformed, fibrillar, plastid proteins into membranous arrays; 2) some membranes formed during ontogeny may disassociate into component parts when translocation begins; 3) dispersed components may later reassociate by self assembly into membrane sequestering bodies; 4) the SER may be secondarily generated from MSB, throughout the life of the sieve cell.

Although the above proposal concerning SER biogenesis is original, the concept of reversible transformations from discrete filaments to membranous structures, by self assembly mechanisms, is not new. W.W. Franke (1971), for example, in his study of the apparent relationship of filamentous "beards" with Hela cell nuclear membranes, visualized that certain components were capable of participating in "transformation from one self-assembly system to another." He further envisioned that "membrane derived protein, after being freed from the phospholipid 'glue' might be imagined to assemble into tubular and filamentous aggregates."

Franke attempted to apply the membrane/filament transformation hypothesis to ontogenetic events in sieve tube development. He proposed that filamentous protein components and membranes of sieve elements, like the filaments and membranes of Hela cells of his study,

participate in interrelated disintegration and self assembly transformations. Franke speculated that "progressive disintegration of ER-derived membrane material" resulted in the developmentally linked appearance of P-protein tubules and filaments during ontogeny of sieve elements. The phloem literature available at the time actually gave very little support to Franke's hypothesis despite his claim that the hypothesis was "consistent with the results hiterto obtained in this field [phloem studies]". However, the present work strongly supports Franke's concept of self assembly transformations in sieve elements, showing (by ultrastructural imagery) intricate, reversible transformation of a fibrillar protein component to morphologically distinct structures in pine sieve cells.

There is evidence that membrane filament transformations occur in sieve elements of other taxonomic groups also, although these events have been largely unappreciated. For example, Parthasarathy (1974 a,b) found filaments in parallel configuration associated with the SER of mature palm sieve elements. Loose fibrils similar to those associated with the membranes were also seen. The amount of loose fibrils/to amount of membranes in a given sieve element appeared to be inversely proportional, suggesting that membrane break-down had released the fibrils into the sieve element lumen.

More recently D.B. Fisher (1975) studied the structure of mature, functional soybean phloem prepared by freeze substitution to avoid chemically-induced artifacts during killing and fixing. (Lipids were lost by the process, however). Fisher found that "the

'ER membranes', seemed to consist entirely of parallel arrays of 100 Å protein fibrils." Even in this rapidly frozen and carefully processed tissue, some of the membranes were disorganized, with free and sometimes aligned 100 Å fibrils nearby.

Both Fisher and Parthasarathy tended to interpret the fibrous membranes as ER with filaments adsorbed to the cisternal side of the membranes; *however, the investigators recognized that an alternative interpretation is that the filaments are actually an integral part of the membranes.* But this latter interpretation was unattractive to Fisher since a filamentous membrane substructure was not consistent with "that expected for a lipoprotein membrane" which others (*e.g.*, Esau and Gill, 1971) have "shown...to originate from normal appearing ER."

In another recent ultrastructural study of phloem, Sauter (1977) observed apparently plastid-derived filaments (*ca.* 55 Å) within mature *P. nigra* sieve cell lumens. He observed "electron dense small complexes with a tubular substructure...in mature sieve cells" which correspond to the MSB of this study. Sauter noted continuities between these complexes and "convoluted 'membranes'...which in turn show close spatial and structural relationship to filaments." Sauter suggested that both the tubular complexes and the peculiar convoluted 'membranes' were stages in the generation of filaments; or, in reverse, were stages in the reaggregation of filaments. But unlike the present study, Sauter did not relate these elements to the SER (which he terms the "vesicular membrane system").

Finally, of interest in interpreting the significance and origin of fibrous membranes, is a study on ultrastructural features of plastids in an achlorophyllous plastid mutant of *Arabidopsis* (Röbbelen, 1966). One of the features of the mutant plastids was that the internal membranes were associated with, or included a uni-layer of 75 Å filaments in parallel array. *The filaments were also associated with tubule-like conformations and these tubule/fibril arrays blended into single thylakoids.* Röbbelen concluded: "From this striking aggregation the possibility of a laminal fusion of tubules as a process of normal thylakoid growth is deduced."

Röbbelen's study supports my proposal of a plastid origin of the SER by demonstrating similarities in achlorophyllous plastid membrane development and the (presumably) achlorophyllous, plastid-derived SER membrane. Both of the developing plastid membrane systems involve fibril/tubule/membrane transformation.

In the present study some of the micrographs depict the SER membrane substructure as vaguely fibrillar when sectioned obliquely (Fig. 4.29) or having a beaded appearance when sectioned perpendicularly to the plane of the membrane (Fig. 4.22, 4.39). This substructural image is consistent with membranes having fibrillar components. Corresponding to these results, a beaded membrane substructure has been reported for chemically-fixed chloroplast membranes (Gunning and Steer, 1976, pg. 104).

P-Protein and the Plastid Fibrillar Component

In the palm, soybean, and pine studies discussed above, the investigators were reluctant to identify the filamentous elements (either in the loose, disassociated form, or in uni-layered sheets) as P-protein, even though the filaments correspond ultrastructurally to certain types of P-protein. Their reluctance to relate free and membrane-associated filaments with P-protein stems from a prevalent (*e.g.*, Parathasarathy, 1974b, Kruatrachue and Evert, 1974; Fisher, 1975) but restrictive concept that *true* P-protein is defined on the basis of its origin from discrete cytoplasmic bodies during sieve element differentiation.

However, the original application of the term *P-protein* to replace the less appropriate term *slime* was not dependent on involvement of a P-protein developmental process such as that characteristic of many dicotyledonous plants (Cronshaw and Esau, 1967). Since the time of the original coining of the term, the heterogeneity of P-protein has become well known, and Cronshaw (1975b) now defines the substance in even broader terms than in his earlier papers. He states: "P-protein then, is the proteinaceous material in the phloem which is sufficiently characteristic when observed with the electron microscope to warrant a special term." While a more exact definition based on function, morphology, and chemistry would be desirable, there is not enough known about the substance to warrant a more limiting description.

The most elemental morphological form of P-protein may be a granular form such as found in some plants during differentiation (Cronshaw, 1975b). But most commonly, plants have a flexuous filamentous form that has a diameter of 20-200 Å, depending on the plant investigated (Cronshaw, 1975). In some plants (such as strawberry) fibrils can be aggregated in larger, tubular forms. Sheet-like aggregation of P-protein *in situ* have not been reported (to my knowledge), although some published micrographs suggest such a configuration (see Cronshaw, 1975b, Fig. 12). Also, I have observed sheet-like fragments of aligned, banded P-protein in strawberry phloem (unpublished observation). It is known that purified P-protein can be induced to form membranous sheets *in vitro* (Kleinig, *et al.*, 1971). Thus, in angiosperms, a P-protein membranous conformation may exist in the mature sieve element; but such conformations may be very labile and are well preserved only in relatively undisturbed sieve elements, such as those processed by freeze-substitution. A sheet or membranous configuration of P-protein in angiosperms may correspond in functional significance to the filamentous protein component incorporated in the SER in the mature pine sieve cell.

Future Research

If a plastid origin of SER is substantiated by future investigations, the field of phloem research may be fundamentally altered. This is because phloem transport research will necessarily fuse

with the already extensive and rapidly expanding research effort on plastid structure and function. Although phloem transport investigations may require more extensive interdisciplinary cooperation, the application of already established research methods used in plastid studies and membrane characterization investigations may rapidly advance our understanding of phloem translocation, once the technical problems inherent in phloem studies are overcome. Furthermore, the fundamental morphological and evolutionary nature of phloem tissue investigations may be elucidated by interdisciplinary teamwork between phloem investigators and geneticists interested in what may be the most advanced expression of the morphological and physiological potential of semi-autonomous organelles. This outstanding potential is attested to by the events of sieve cell differentiation outlined in this report, which demonstrate an example of a higher plant cell, that after deactivation of the nuclear material, is converted to a chamber which houses only those extra-nuclear organelles with genetic autonomy. Furthermore, in the case of the plastid population prolific synthetic processes result in extraorganelle membrane arrays, which may prove to be actively involved in the translocation of carbon-based nutrients.

Comparative Aspects of the Cytology of Healthy Phloem and the Phloem of Stems Infected with Dwarf Mistletoes.

This study has served as a basis for accessing the cytological impact of dwarf mistletoes on host phloem tissues. As stated in Chapter III, the cytopathological effects on *P. sabiniana* phloem

appear mild. No anomalous cytological features of mature sieve elements were apparent in the diseased tissue when compared to healthy, mature sieve elements (as represented in this study). However, it is not possible to judge whether sieve cell *ontogeny* is affected in tissues infected with dwarf mistletoes, since ontogenetic studies were not undertaken in the studies of diseased tissues.

There were some cytological differences between phloem ray cells in infected and healthy tissue. These differences have been noted in Chapter III and discussed in relationship to the cytology of the host/parasite interface.

In my opinion, it is not surprising that *Arceuthobium* produces only mild cytopathological effects on host phloem at infection sites, even though, commonly, the pathological effect on the total tree is significant. This may be a general feature of obligate parasites, such as *Arceuthobium*, which do not kill associated host cells [except perhaps during the initial penetration process], but develop in such a manner so as to obtain host-originating nutrients either by direct penetration of living cells, or, [as in dwarf mistletoe infections] by establishing close contiguity with them (Agrios, 1969). In the particularly long-lived associations characteristic of dwarf mistletoes and their hosts, the stability of the association may depend, in part, on the continued health and efficiency of host phloem (at least in relationship to infected branches) in order to maintain the parasitic mode of existence and to provide at least a subsistence-level

of nutrition for associated host cells.



FIGURE 4.1. Cells in the cambial zone (CA) of *P. sabiniana*. Sample taken during spring displays differentiating sieve elements (dSE). First evidence of differentiation is the formation of a large, central vacuole (cv) surrounded by dense cytoplasm containing numerous mitochondria and starch-storing plastids (PL). Nacreous walls (nw) then form. 6800X. s, starch; v, vacuole.



FIGURES 4.2-4.3. Stage 1 of sieve cell differentiation. Fig. 4.2-- Long. sec., starch-storing plastids in dense cytoplasm. Cytoplasm is withdrawn from loose, layered nacreous wall (nw). 9200X. Fig. 4.3-- Detail of Fig. 4.2. Double membrane surrounds plastids; the inner membrane invaginates to form an internal membrane system (between arrows). Large, lenticular starch grains dominate plastids. 57,000X. cR, cytoplasmic ribosomes; mf, microfilament-like elements; pR, plastid ribosomes; s, starch.



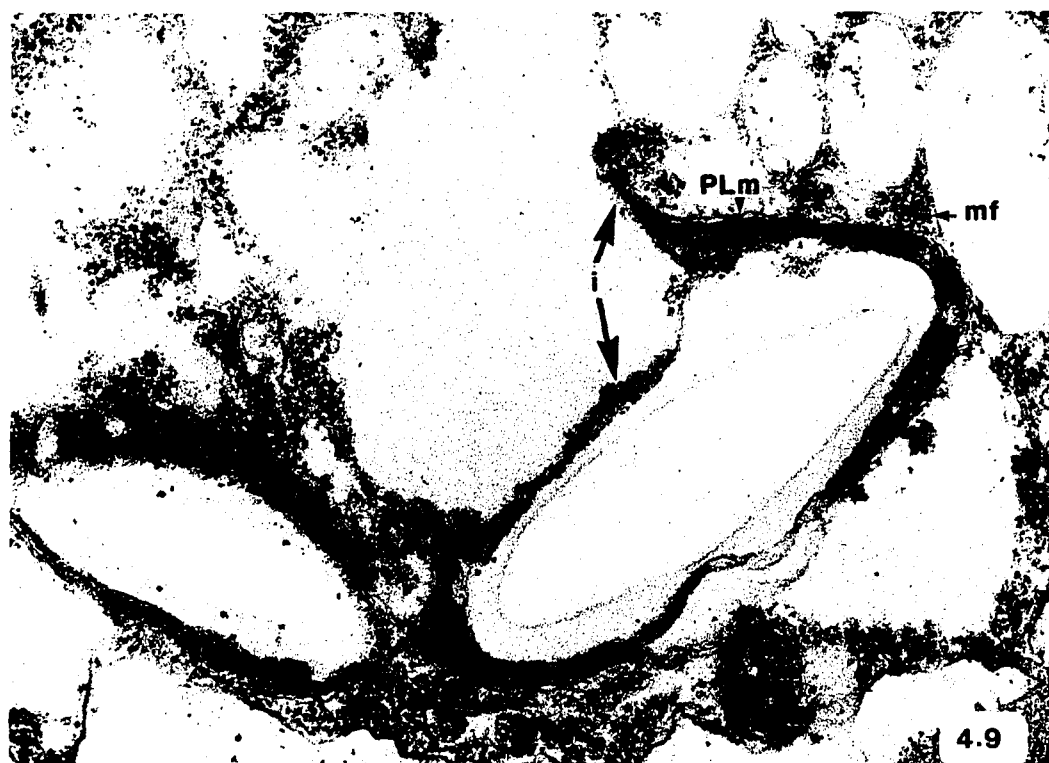
Figures 4.4-4.5. Nuclei in differentiating sieve cells. Fig. 4.4--Nucleus (Nu) in stage 1 has about equal proportions of heterochromatin (hc) and euchromatin (electron lucent area). Golgi bodies (g) are associated with the future sieve area (fsa). 19,000X. Fig. 4.5--Early stage 2. Condensed chromatin (Cc) dominates nucleus. Nuclear and plastid (PL) membranes are indistinct in places (unlabelled arrow). 20,000X. nw, nacreous wall; PLm, plastid membrane; sp, spherosome.



FIGURE 4.6. Stage 2 of sieve cell differentiation. Tang. section. Two types of vacuoles occupy cell center (v^1 , v^2). v^1 is collapsed; v^2 is expanded and has a fibrous inclusion (i). Plastid (PL) has an elongated tail and a starch-bearing head. Limiting membranes are absent around head (arrows). 21,600X. fsa, future sieve area; s, starch.



FIGURE 4.7. Differentiating sieve cells. Cross sec. Immature sieve cells are shown in stages 1, 2, and 3 (1,2,3). See text for details. 10,500X. cv, central vacuole; hs, hydrated starch; i, fibrous inclusion; m, mitochondria; na, nacreous wall; pl, plasmalemma; RC, ray cell; s, starch; unlabelled arrow=plastid without distinct membranes.



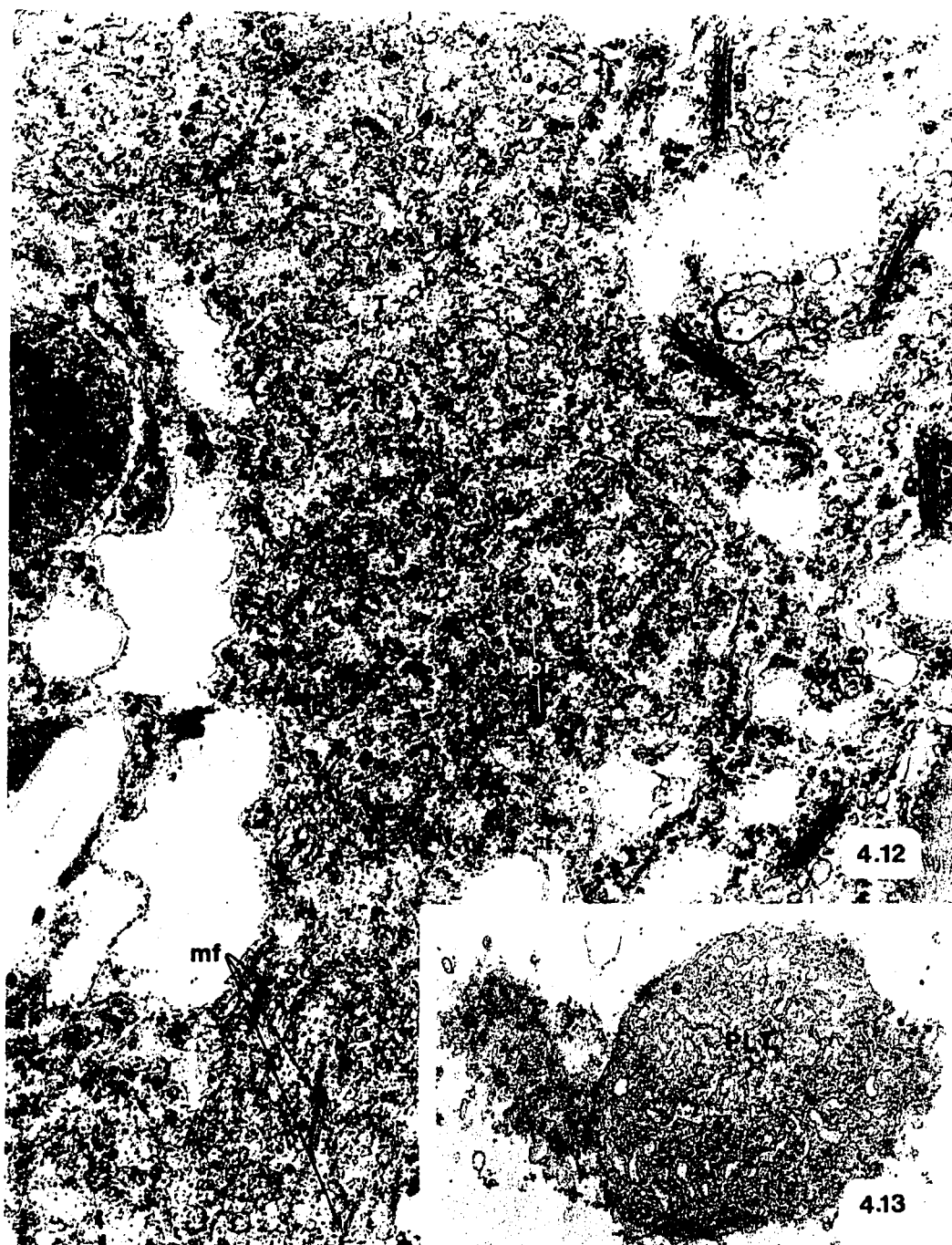
FIGURES 4.8-4.9. Details of Fig. 4.7. Fig. 4.8--Plastid in cell 1, Fig. 4.7. Plastid has distinct limiting membranes (PLM) but the tonoplast (to) is discontinuous, allowing the contents of the central vacuole (cv) to become confluent with the cytoplasm (arrow). 41,000X. Fig. 4.9--Detail of Fig. 4.7, cell 2. Plastid limiting membranes (PLM) have pulled away from plastid body. Fibrous inclusion material (i) is associated with plastid stroma. 40,700X. mf, microfilaments in cross sec. (?); s, starch.



FIGURE 4.10. Late stage 2 of sieve cell differentiation. Long. sec. Center of cell is occupied by a tubule mass (T) and small vacuoles (v) with inclusion material (i). Plastids (PL) have rounded up. Callose (ca) is on the ends of plasmodesmata (pd) at future sieve areas (fsa). 11,000X. c, crystal; g, golgi; s, starch.



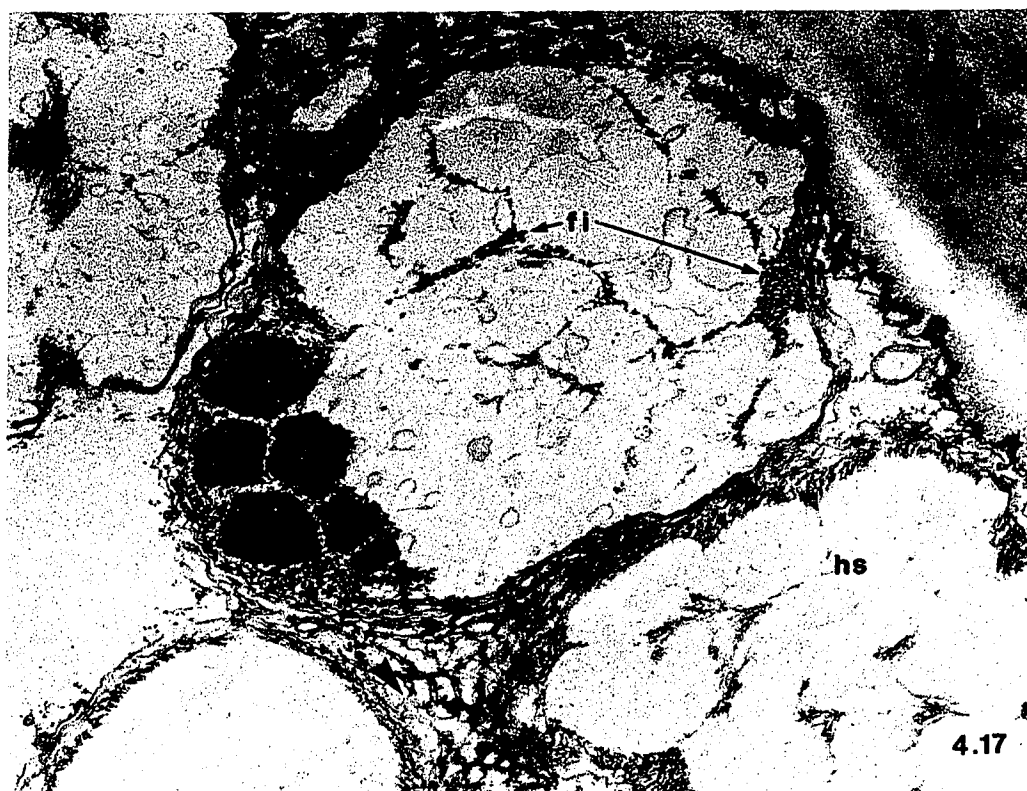
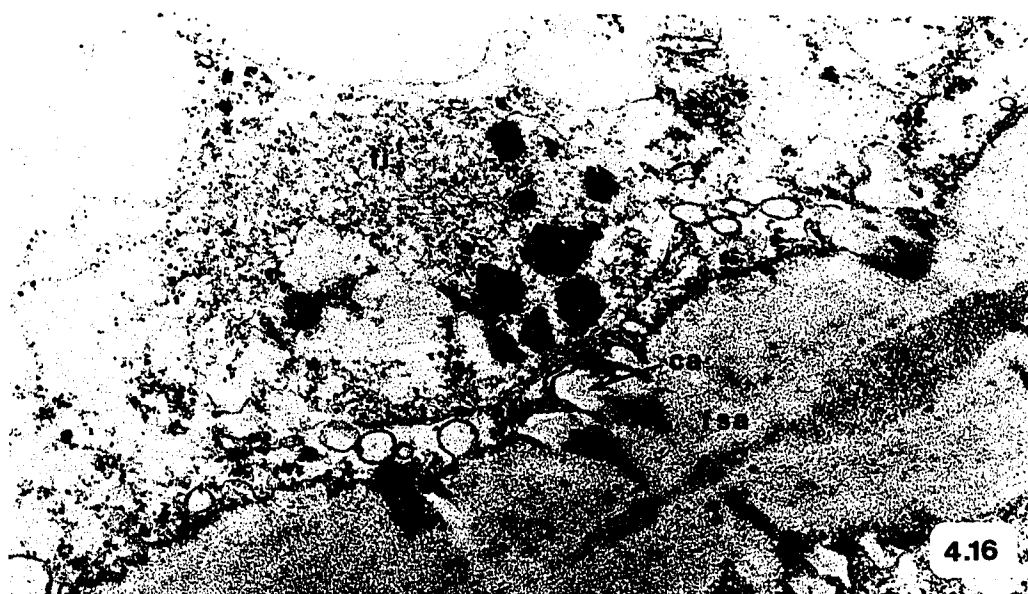
FIGURE 4.11. Early stage 3 of sieve cell differentiation. Starch grains become hydrated to produce a rounded starch mass (hs). Plastids do not have distinct limiting membranes. They contain a large amount of fibrous or filamentous protein (fi) and densely staining crystals (c). The plastids are clustered around the periphery of a central tubule mass (T). 17,300X. ER, endoplasmic reticulum; i, fibrous inclusion in vacuole; M, mitochondria; r, ribosomes; sv, coated vesicle.



FIGURES 4.12-4.13. Cytoplasmic details of differentiating sieve cells. Fig. 4.12--Tubule mass (T) in a stage 2 (late) sieve cell. Polyribosomes (pr) are among the tubules, as well as smaller independent granules. Other cell structures are excluded from the tubule mass. 28,900X. Fig. 4.13--Section through a plastid tail region in early stage 2. A complex tubule system (PLT) is present in the plastid as well as small ribosomes. The tubule mass and small granules as in Fig. 4.12 may be derived from the tubules and ribosomes of plastids. 28,700X. g, golgi body; mf, microfilaments.



FIGURES 4.14-4.15. Cytoplasmic details of differentiating sieve cells. Fig. 4.14--Detail of Fig. 4.15; plastid outer membranes (PLm) are sporadically preserved in early stage 3. Vacuoles (v) are associated with plastid stroma (arrow). Plastids contain filamentous protein (fi), crystals (c), and starch (s). 10,100X. Fig. 4.15--Tang. sec.; inclusion (i)-containing vacuoles (v^2) fill cell center. Future sieve area (fsa) has small callose (ca) deposits on plasmodesmata. 23,300X. M, mitochondria; mf, microfilaments; T, tubules.



FIGURES 4.16-4.17. Stage 3 in sieve cell differentiation. Fig. 4.16--Sieve pore development. Plasmodesmata at future sieve area (fsa) have callose deposits (ca). Plastid contains no distinct membranes. 62,000X. Fig. 4.17--Late stage 3. Filamentous protein (fi) blends into tubules of forming SER (arrowhead). Hydrated starch (hs) is swollen extensively. 64,800X.

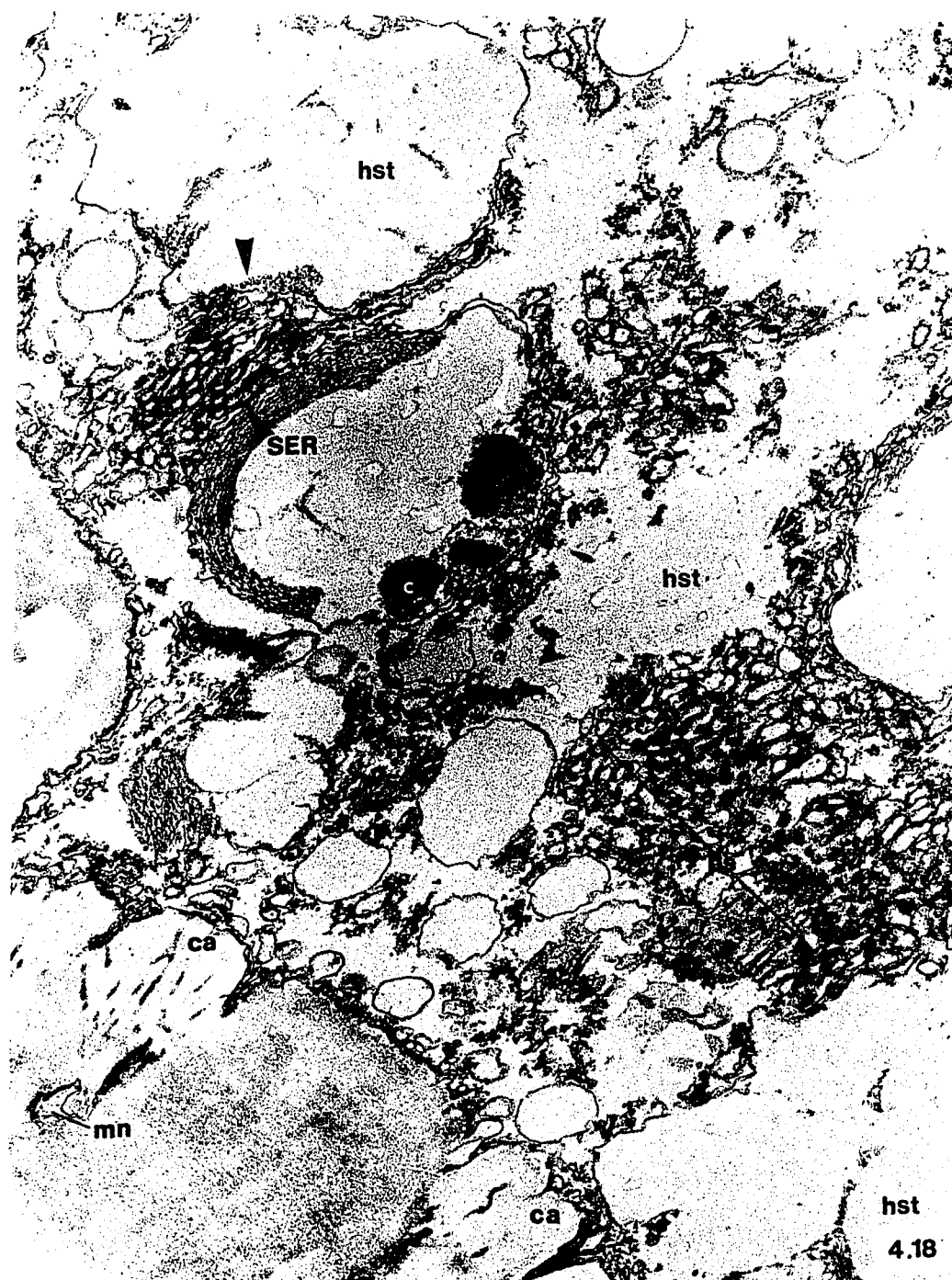
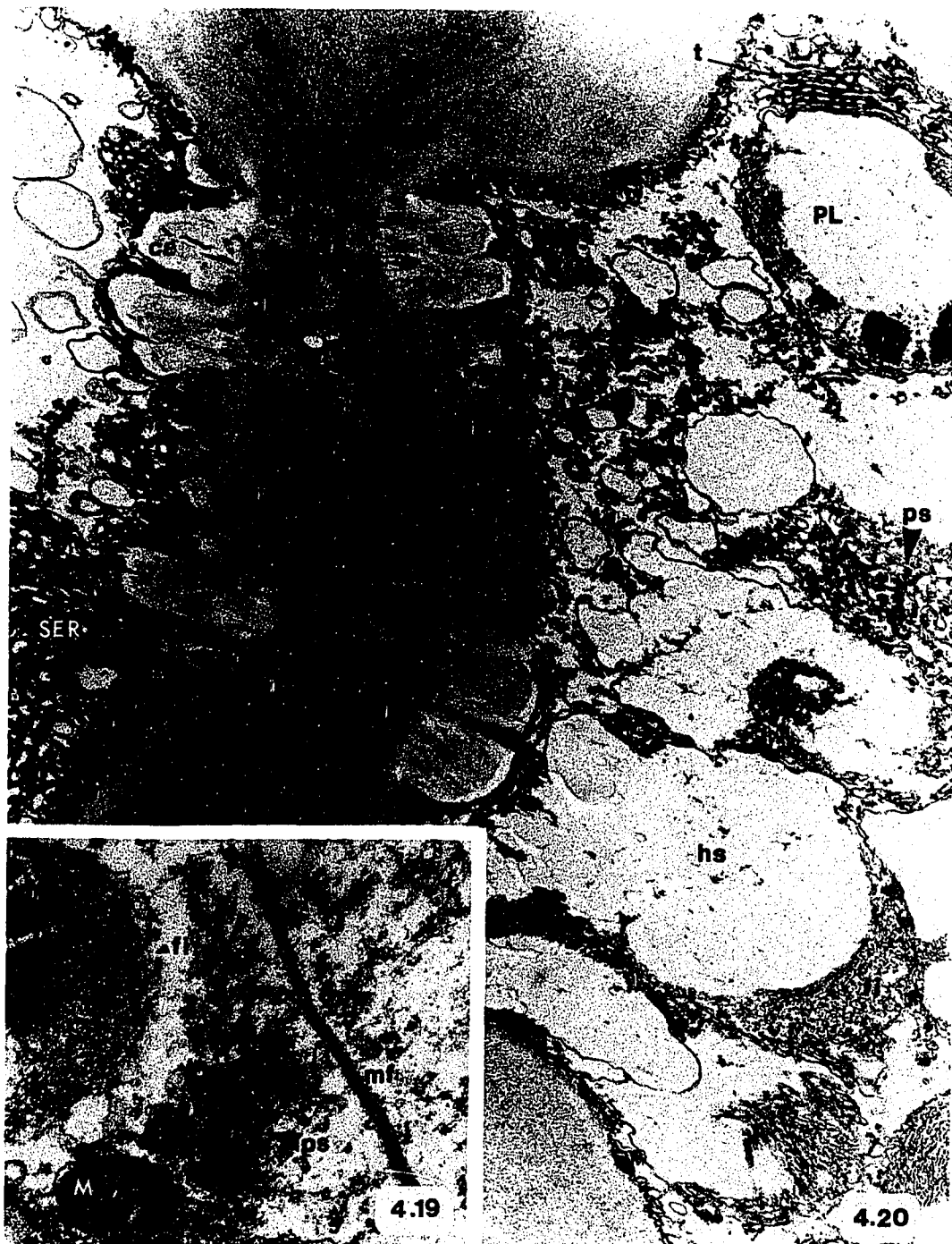


FIGURE 4.18. Stage 3 in sieve cell differentiation—formation of SER. SER emerges from the filamentous protein (fi) of the plastids (arrowheads). In face view, the young SER membranes are seen to be perforated sheets (ps) with granular material within the perforations. 58,000X. c, crystal; ca, callose; hst, hydrated starch; mn, median nodule.



FIGURES 4.19-4.20. Cytoplasmic details of sieve cells in stage 3. Fig. 4.19--Perforated sheets (ps) are seen in association with filamentous protein (fi) of plastid. Dark material (ribosomes?) occupy center of perforations. 23,600X. Fig. 4.20--Stacks of perforated sheets joined by short tubules (t) are seen in association with a plastid (PL). Callose (ca) deposition near the median nodules (mn). 26,600X. mf, microfilaments; r, ribosomes.



FIGURE 4.21. Cytoplasmic details of a sieve cell in late stage 3. Plastids (PL) have formed extensive SER membranes. Median nodules (mn) are very eroded but callose (ca) has not yet reached the nodules. 27,900X. fi, filamentous or fibrous protein of the plastid; fsa, future sieve area; hs, hydrated starch.



FIGURES 4.22-4.23. Early stage 4 in sieve cell differentiation--the SER. Fig. 4.22--Detail of boxed area in Fig. 4.23. Trilamellate, but somewhat beaded substructure of SER is seen. 132,000X. Fig. 4.23--Stage 4 in differentiation. Callose (ca) has completely lined the sieve pores and the median nodule cavity (mn); formation of sieve area (SA) is complete. SER and related vesicles (v) completely fill cell lumen. 13,200X. hs, hydrated starch; sp, sieve pore.

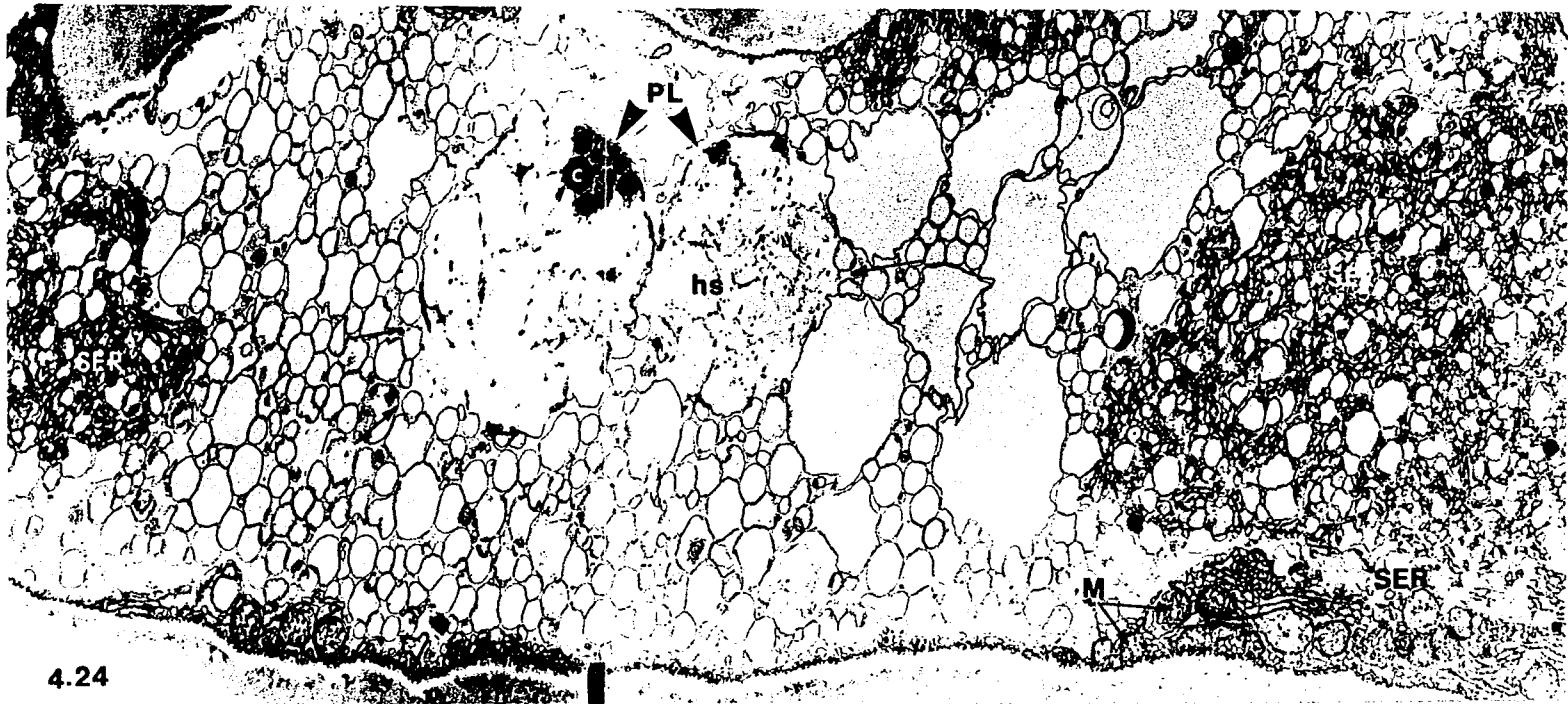


FIGURE 4.24. Early stage 4 in sieve cell differentiation. Vesicles and SER membranes fill the cell lumen. Large masses of membranes are found near sieve areas. Two plastids (PL), greatly enlarged by hydrated starch (hs) are open into spaces between vesicles (arrows); plastid and vesicular membranes are confluent. 10,100X. M, mitochondria; SER, sieve element reticulum.

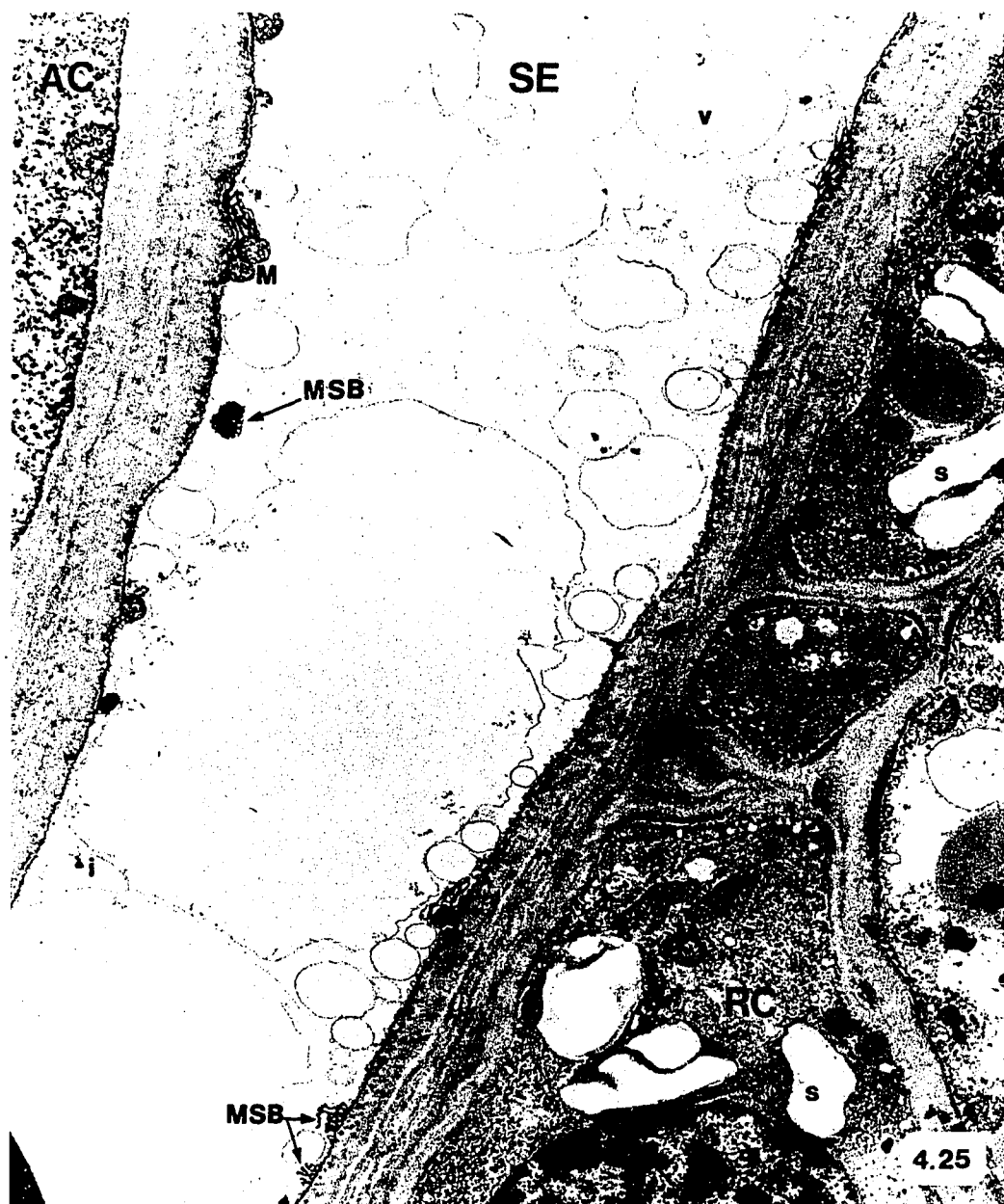
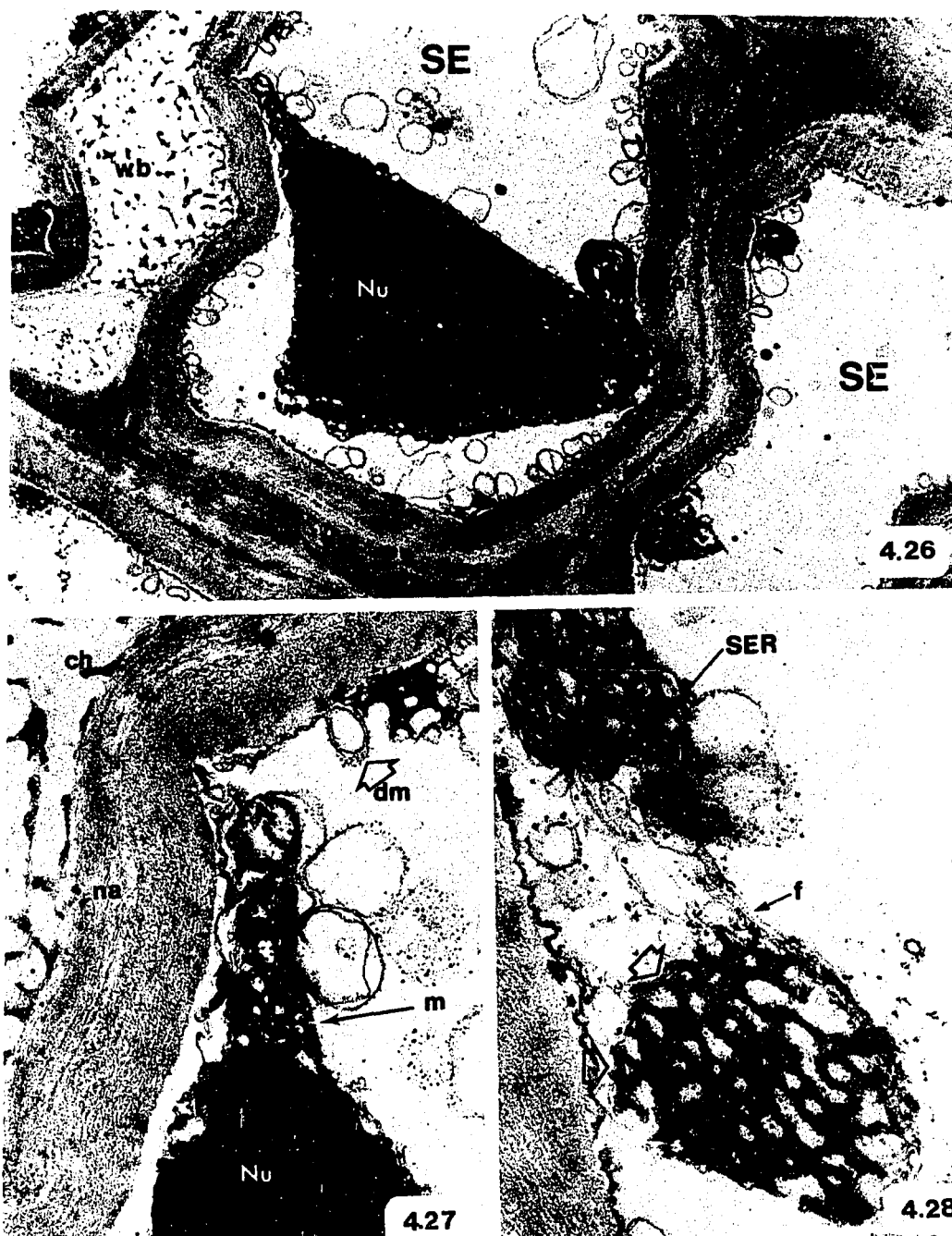


FIGURE 4.25. A recently-matured sieve cell. Long. section. The lumen has cleared somewhat of the vast number of vesicles present in stage 4 (see Fig. 4.24). Membrane sequestering bodies (MSB) have appeared. At this stage the sieve cell is thought to be a conducting cell. This cell is flanked by an albuminous cell (AC) and ordinary ray cells (RC). 8300X. i, fibrous inclusion in vesicles; M, mitochondria; s, starch; v, vesicle.



FIGURES 4.26-4.28. Cytoplasmic details of mature sieve cells. Cross sections. Fig. 4.26--Pycnotic nucleus (Nu) occupies center of cell. A wall bulge (wb) is present in tangential wall. 7800X. Fig. 4.27--Pycnotic nucleus is continuous with a membrane system (m). Channels (ch) are present in the wall bulge. 24,700X. Fig. 4.28--Individual filaments (f) form parallel arrays (open arrows) which condense to convoluted body. 41,200X. dm, double membrane associated with plasmalemma; na, nacreous wall; SE, sieve element; SER, sieve element reticulum.

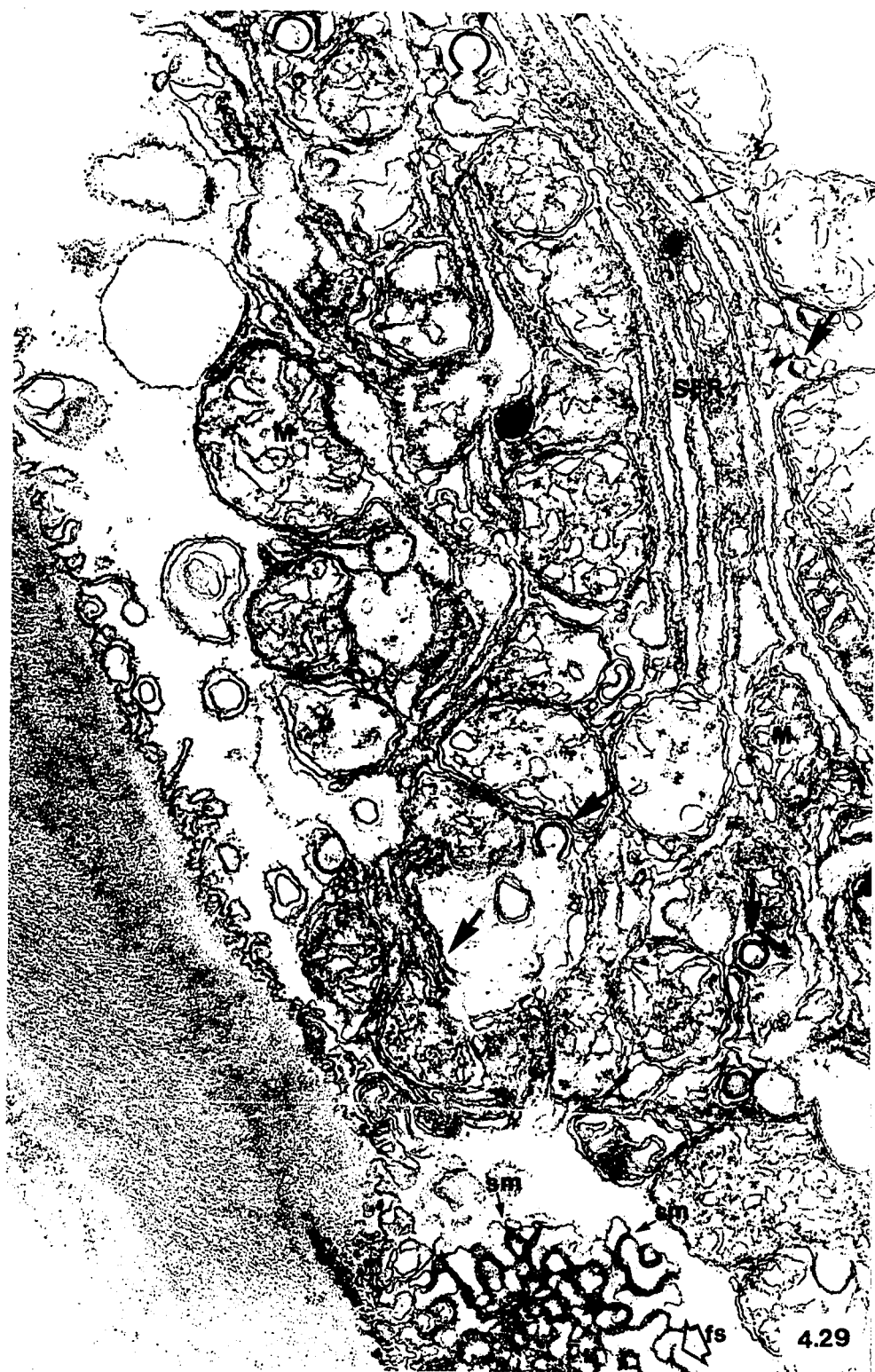
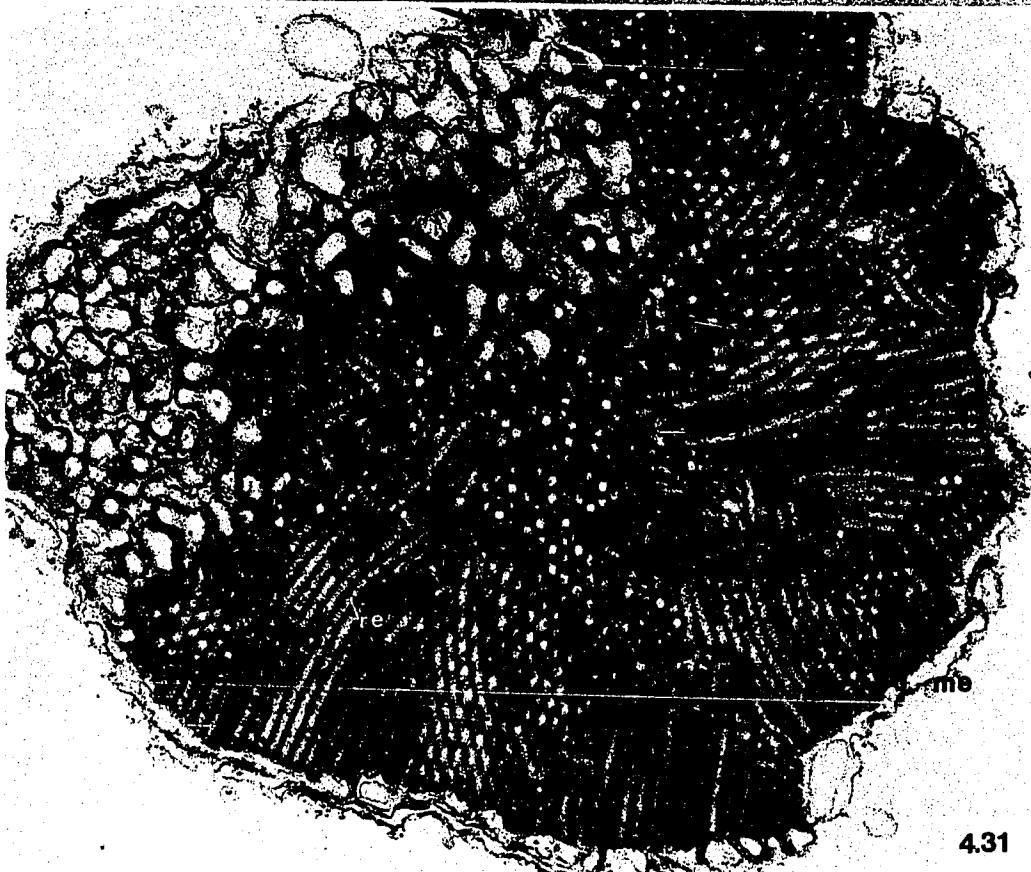
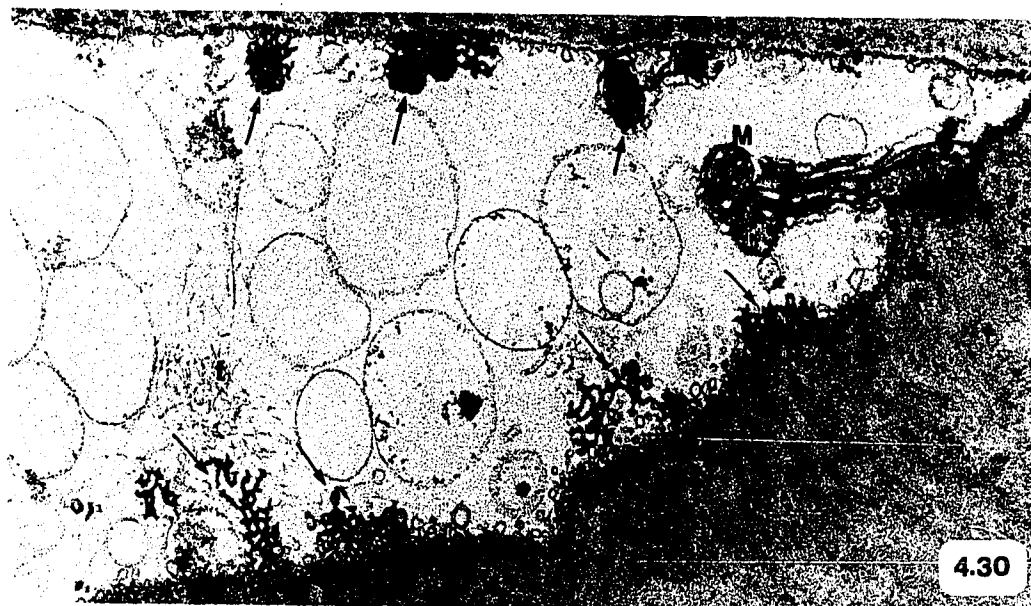
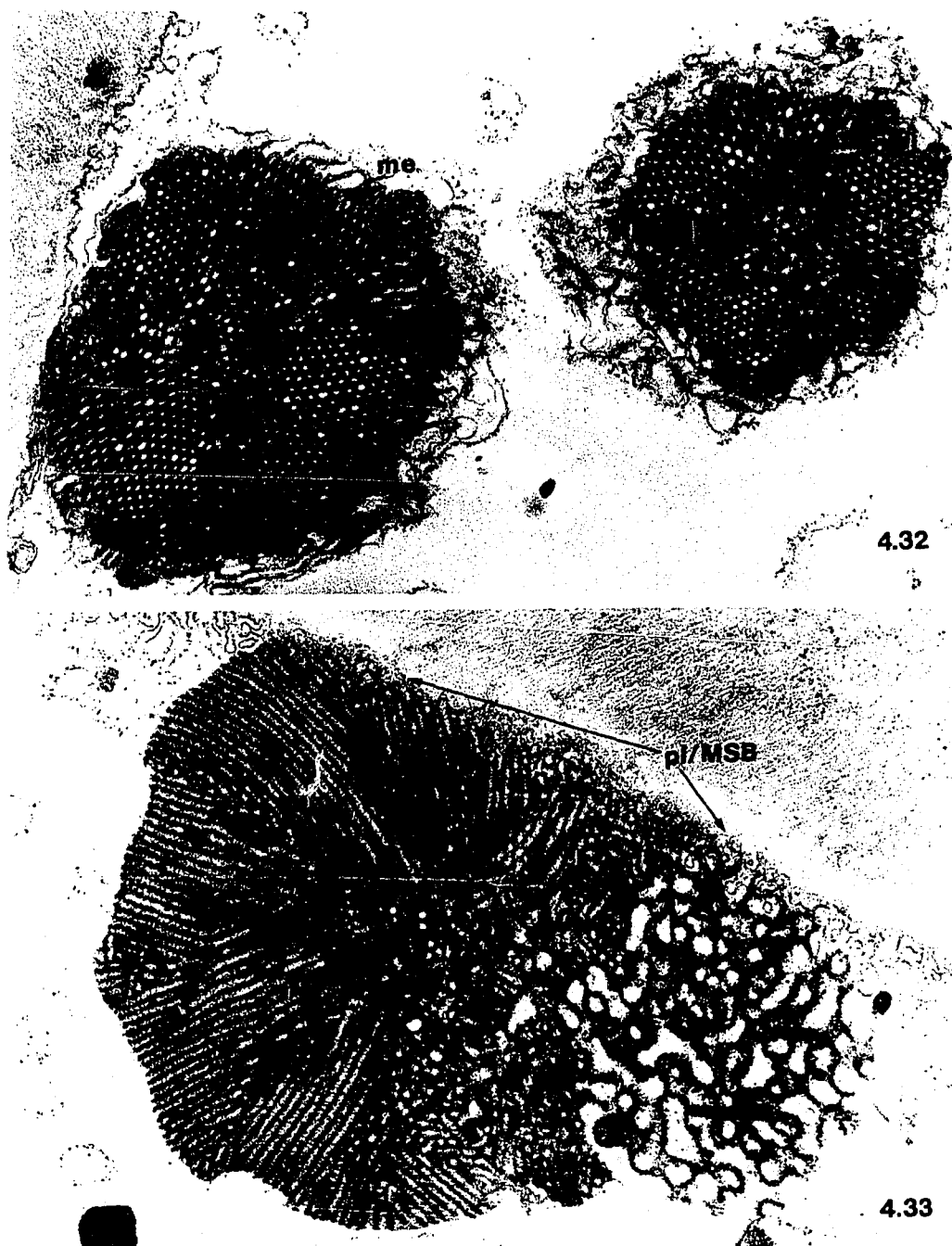


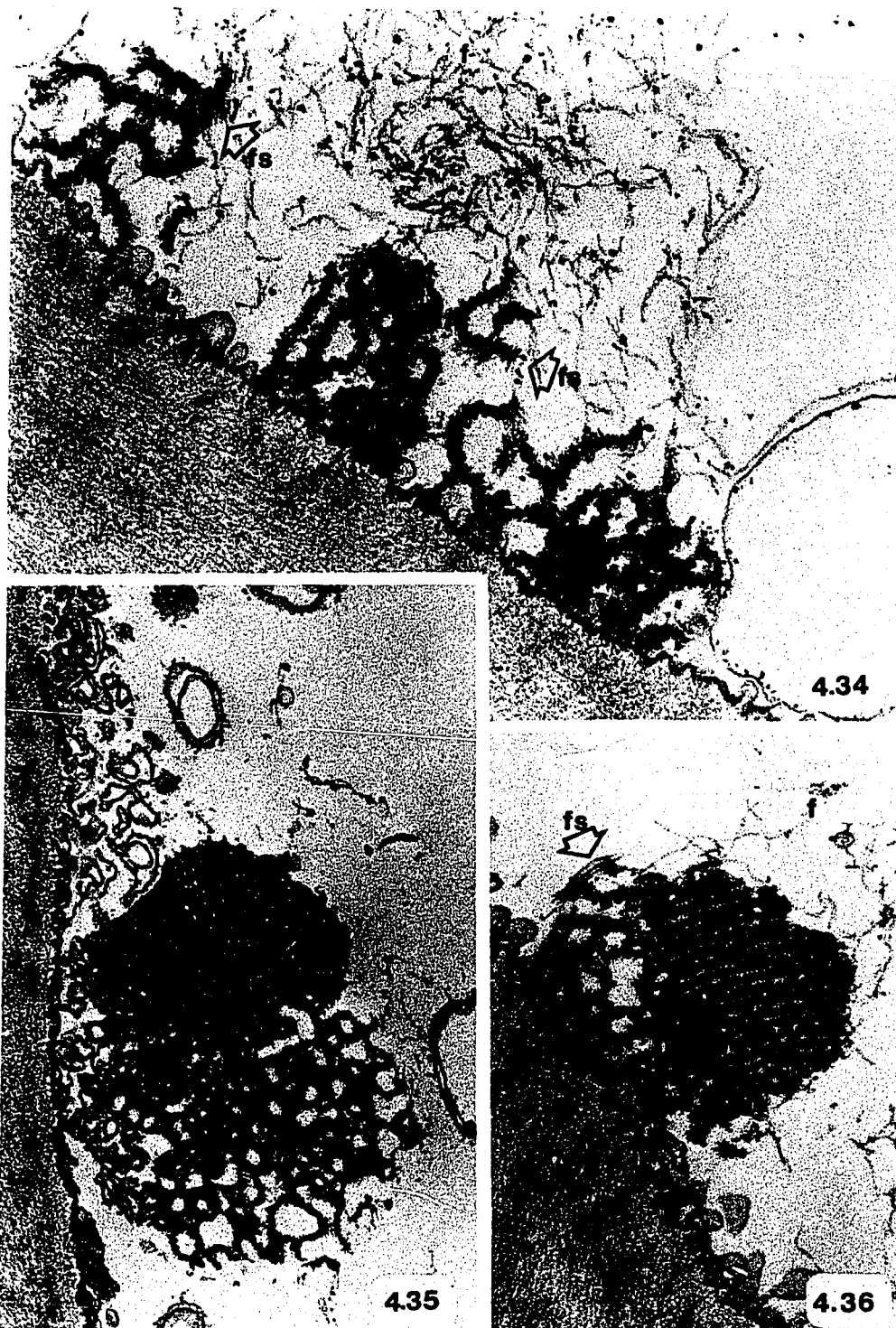
FIGURE 4.29. Sieve element reticulum (SER) and mitochondria (M) in a mature sieve cell. 42,000X. fs, fibrous sheets; sm, separating membranes; large arrows=fused membranes; small arrows=fibrous membranes.



FIGURES 4.30-4.31. Membrane sequestering bodies (MSB) in recently matured sieve cells. Fig. 4.30--Long. sec. MSBs at arrows. 15,500X. Fig. 4.31--Membrane bound (me) MSB with tubules formed of ringed elements (re). Fused membranes associated with tubules separate to form paired membranes (arrows). 42,600X. f, filaments; M, mitochondria.



FIGURES 4.32-4.33. Large, complex membrane sequestering bodies (MSB). Fig. 4.32--Membrane bound (me) MSBs with many lattice dislocations. 20,100X. Fig. 4.33--MSB associated with the sieve cell plasmalemma (pl/MSB). 28,700X.



FIGURES 4.34-4.36. Formation of membrane-sequestering bodies (MSB). Fig. 4.34--Loose 50 Å filaments (f) form fibrous sheets (fs) and dense convoluted forms. 59,300X. Fig. 4.35--Convoluted fibrous sheets associate with tubular arrays. 21,000X. Fig. 4.36--Filaments, fibrous sheets, and tubules with ringed walls are in association. 57,100X.

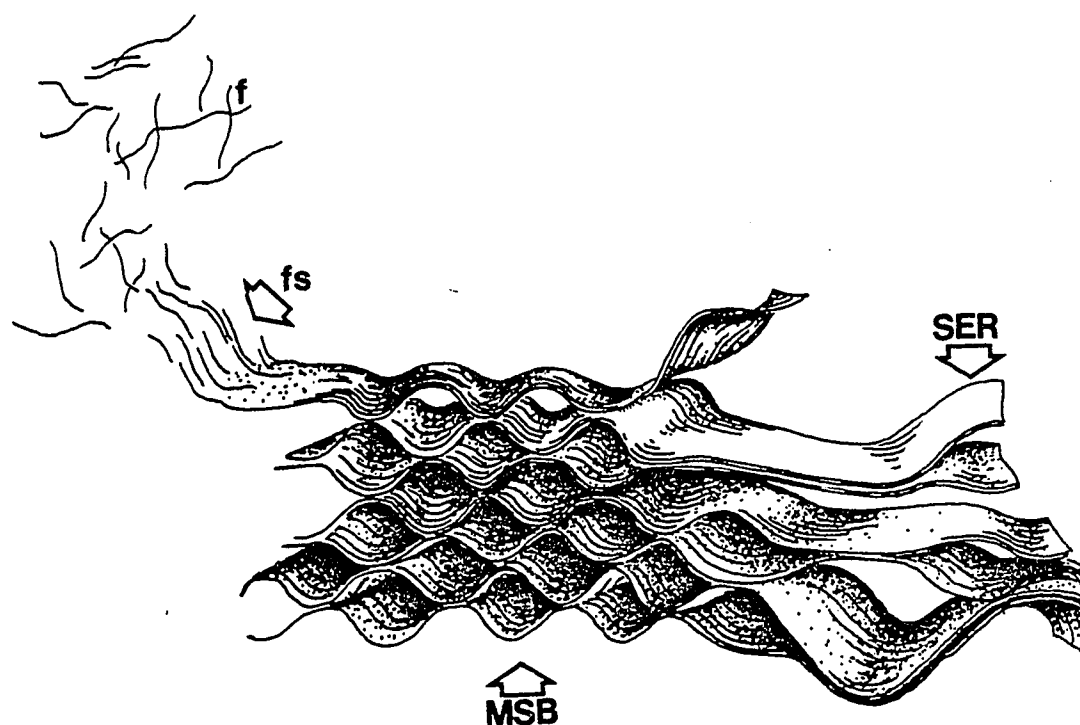
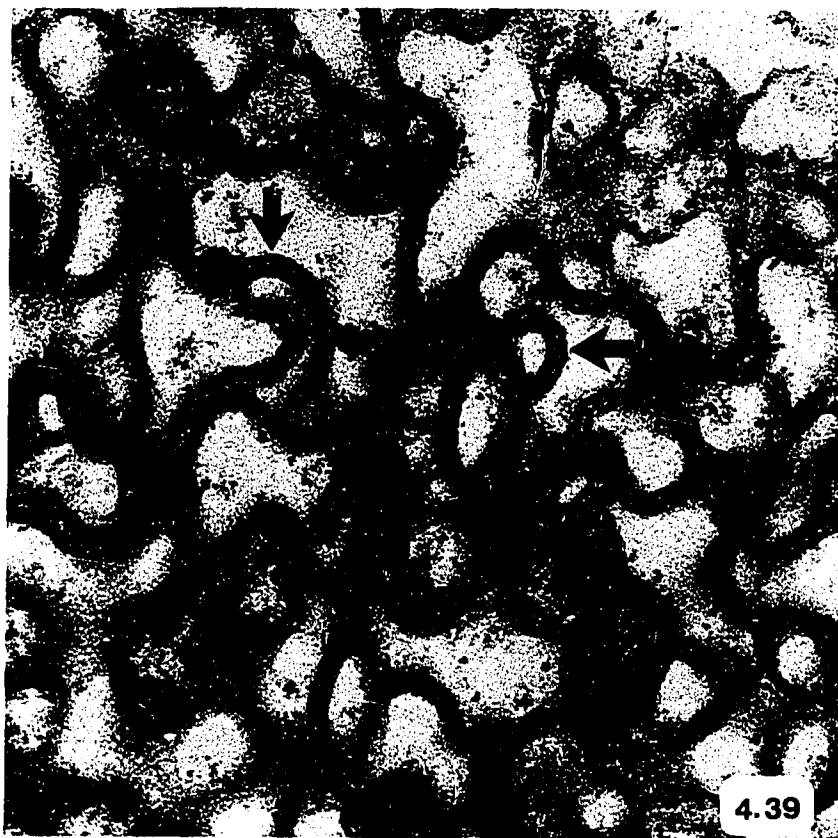
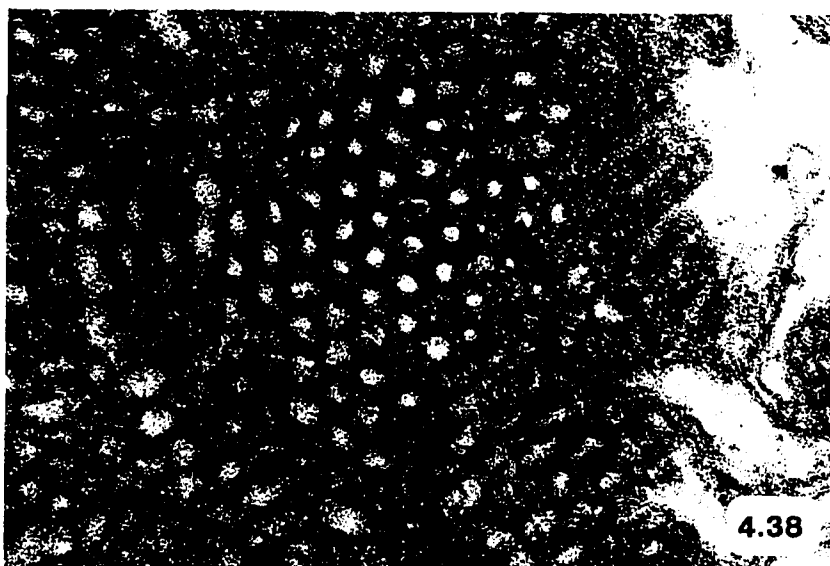
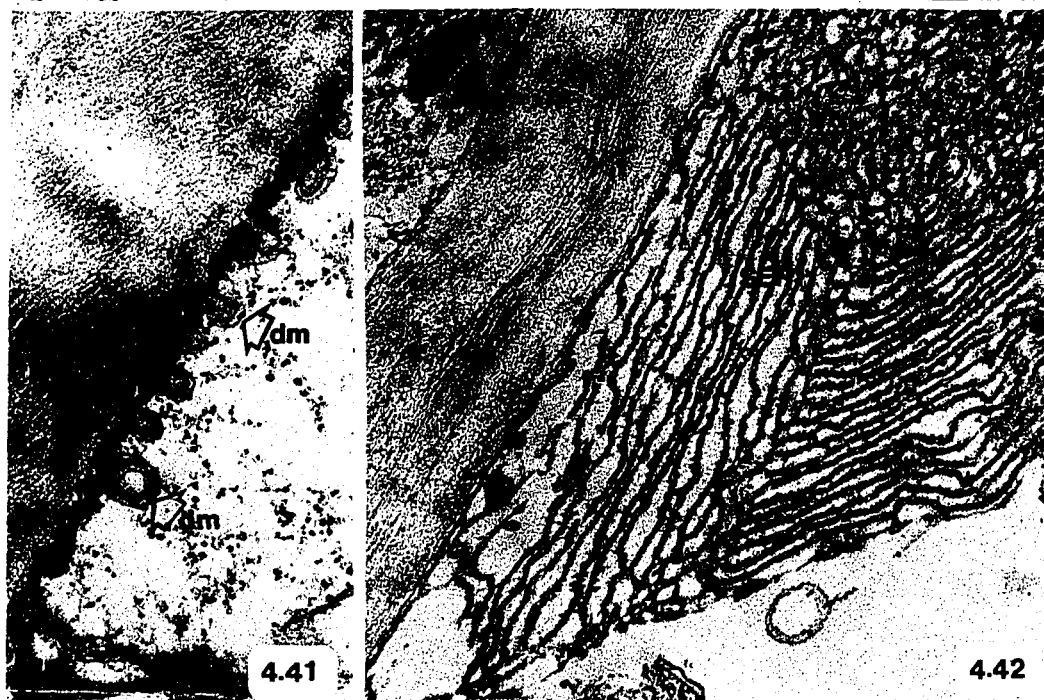
**4.37**

Figure 4.37. Hypothetical model of the relationship between free filaments (f) in the mature sieve cell lumen, fibrous sheets (fs), paracrystalline membrane sequestering bodies (MSB) and the sieve element reticulum (SER). See text for details.



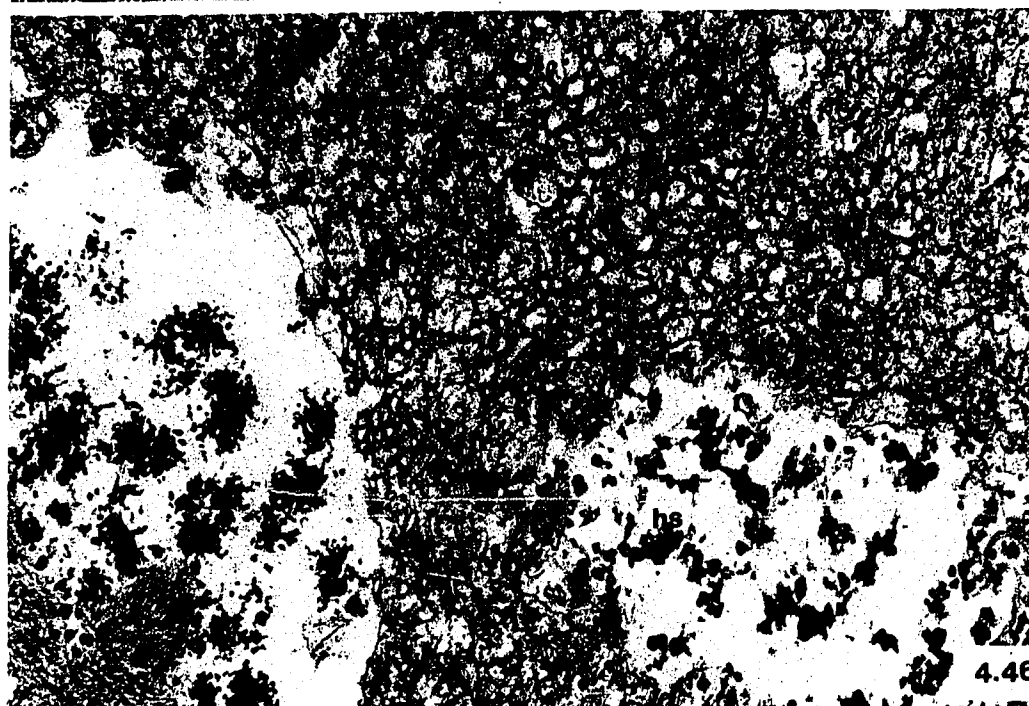
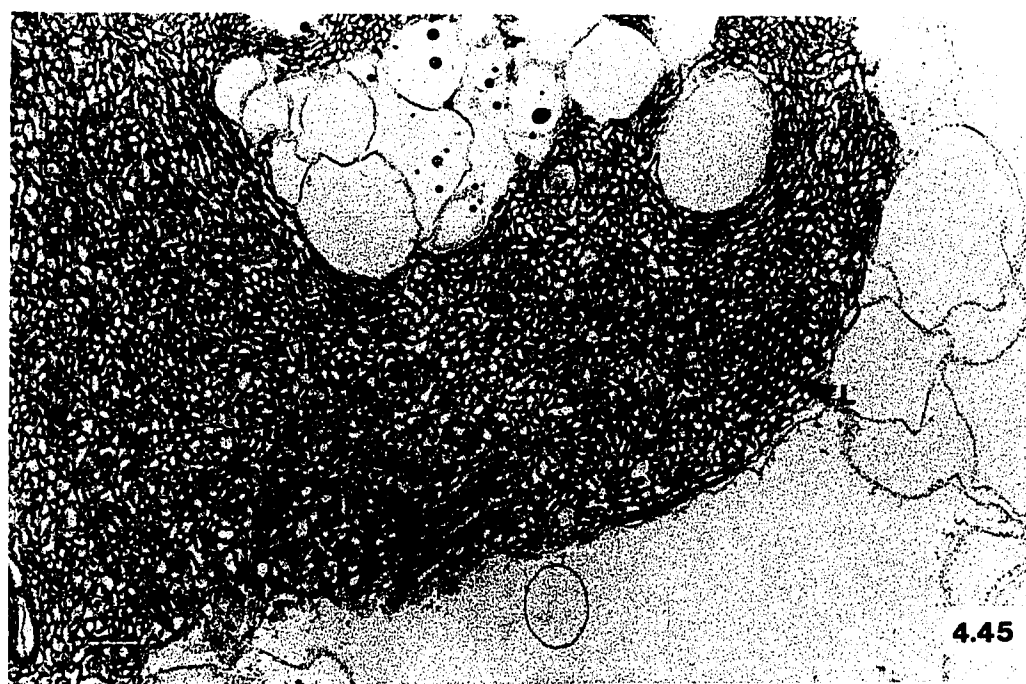
FIGURES 4.38-4.39. Details of membrane sequestering bodies (MSB). Fig. 4.38--Ringed elements of the tubule walls of MSBs appear to be continuous between tubules (arrow). 100,000X. Fig. 4.39--Substructure of convoluted membranes associated with MSBs prior to their separation into paired, trilamellate membranes. Note vague, beaded substructure of outer trilamellate components, as in Fig. 4.22. 100,000X.



FIGURES 4.40-4.42. Membranes in older, mature sieve elements. Fig. 4.40--Parallel arrays of sieve element reticulum (SER) originate perpendicular to another membrane lying parallel to the cell wall (CW). The paired membrane sheets maintain a 100 Å spacing. 91,300X. Fig. 4.41--Here, the plasmalemma is overlaid with another membrane, to form a double membrane (dm). Fig. 4.42--Different SER orientations are seen in this cell, but the 100 Å spacing remains consistent. 41,800X.



FIGURES 4.43-4.44. Sieve element reticulum (SER) shown in relationship to a plastid in an older sieve element. Fig. 4.43--Extensive, paired parallel SER arrays are seen in a sieve element (SE). Plastid (PL) connects with SER (arrowheads). 13,000X. Fig. 4.44--Detail of plastid/SER connection. 50,000X. hs, hydrated starch.



FIGURES 4.45-4.46. SER membranes in older sieve elements. Fig. 4.45--Highly ordered, latticed membranes are sometimes found within masses of randomly anastomosing membranes. 13,300X. Fig. 4.46--Paired membranes of SER surround regions of hydrated starch (hs) and filamentous protein (fi) of "spent" plastids. Note that the SER membranes are paired. 41,000X.

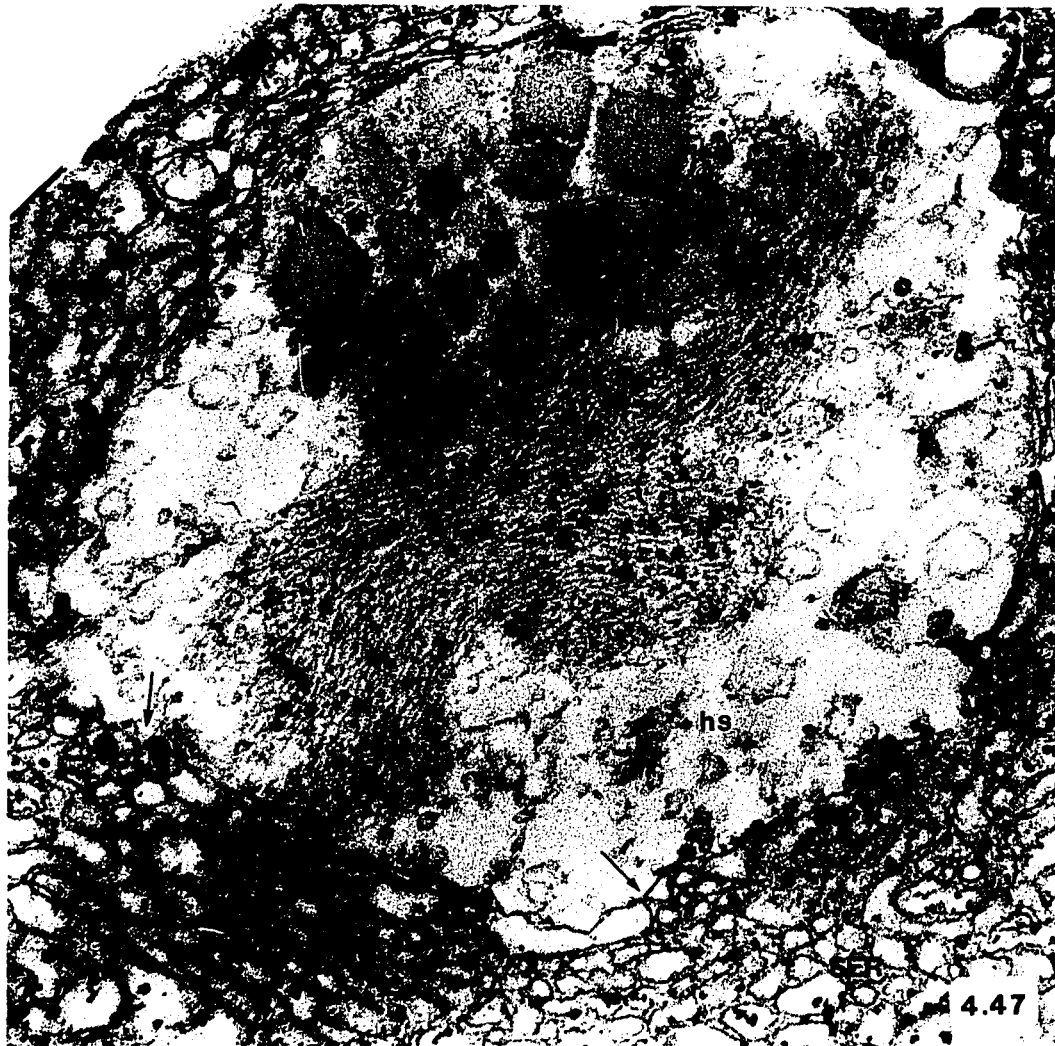


FIGURE 4.47. A plastid enmeshed in SER. The boundary membrane of the plastid is continuous with the sieve element reticulum (SER) (arrows). 90,000X. c, crystal; hs, hydrated starch; fi, filamentous protein.

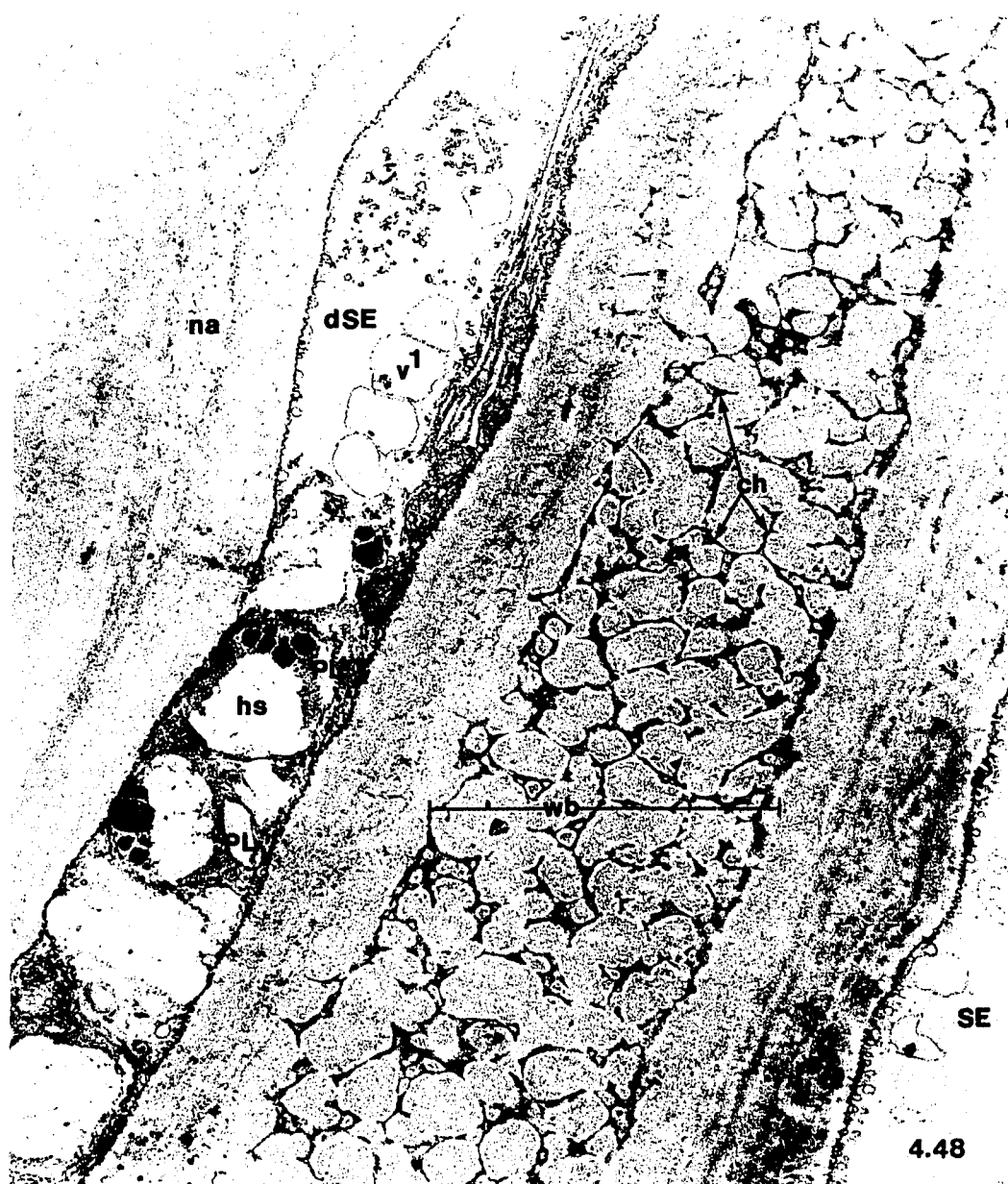
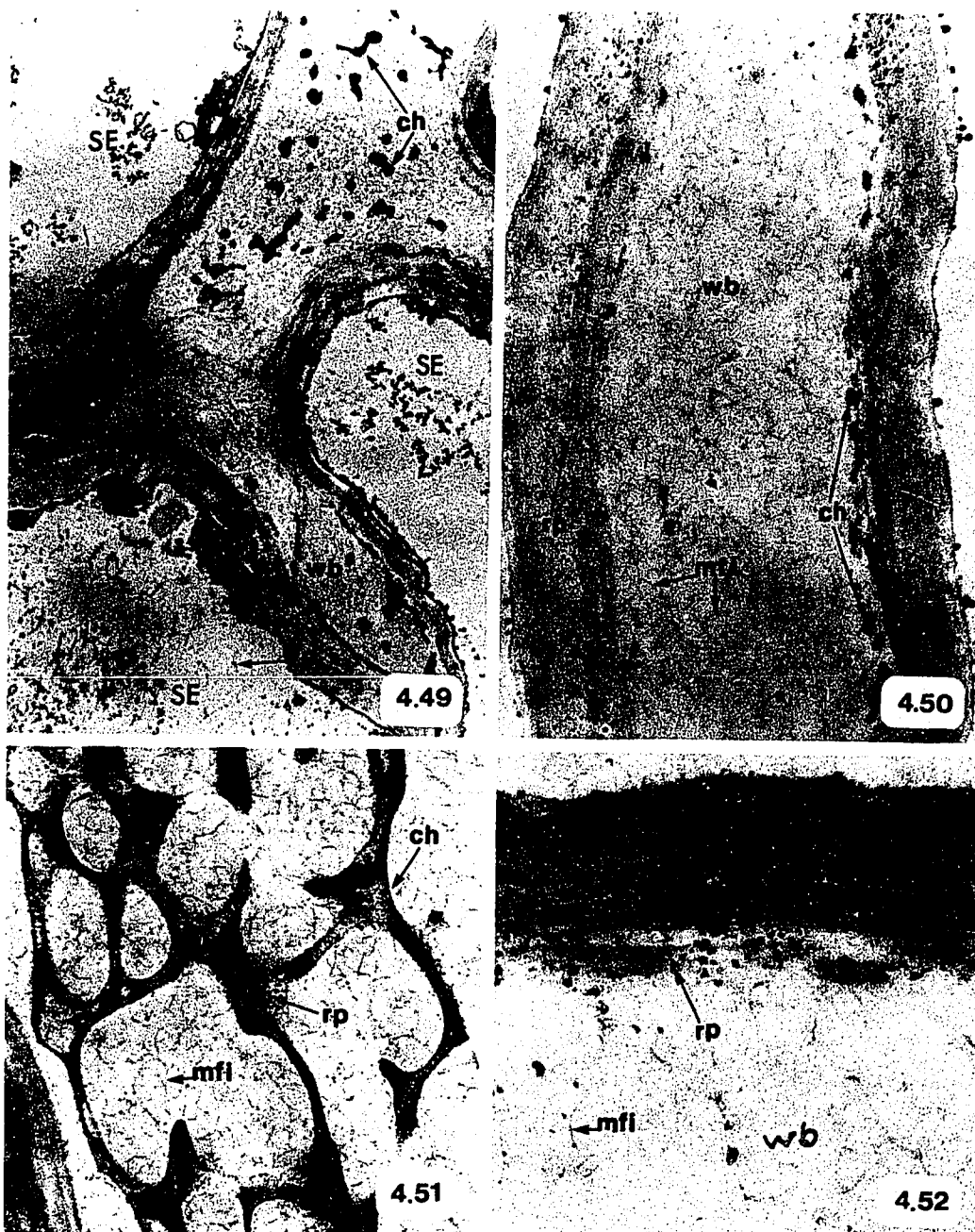


FIGURE 4.48. A wall bulge (wb) which has developed between a differentiating sieve element (dSE) and a mature sieve element (SE). Channels (ch) of electron dense material permeate the wall bulge. 70,100X. hs, hydrated starch; na, nacreous wall; PL, plastid; v², vesicle.



FIGURES 4.49-4.52. Details of wall bulges. Fig. 4.49--Cross sec. Wall bulges (wb) between living sieve elements (SE) contain electron dense channels (ch) within a lucent, fibrillar matrix. 13,000X. Fig. 4.50--Wall bulge between sieve cells that have recently died. Channels are only on the border of the bulge. 33,100X. Fig. 4.51--Bulges associated with a living sieve cell. Reaction product (rp) deposited at channel sites. 20,000X. Fig. 4.52--Wall bulge associated with an old, dead sieve cell. Channels are no longer present; microfibrils (mfi) are sparse. 40,000X.

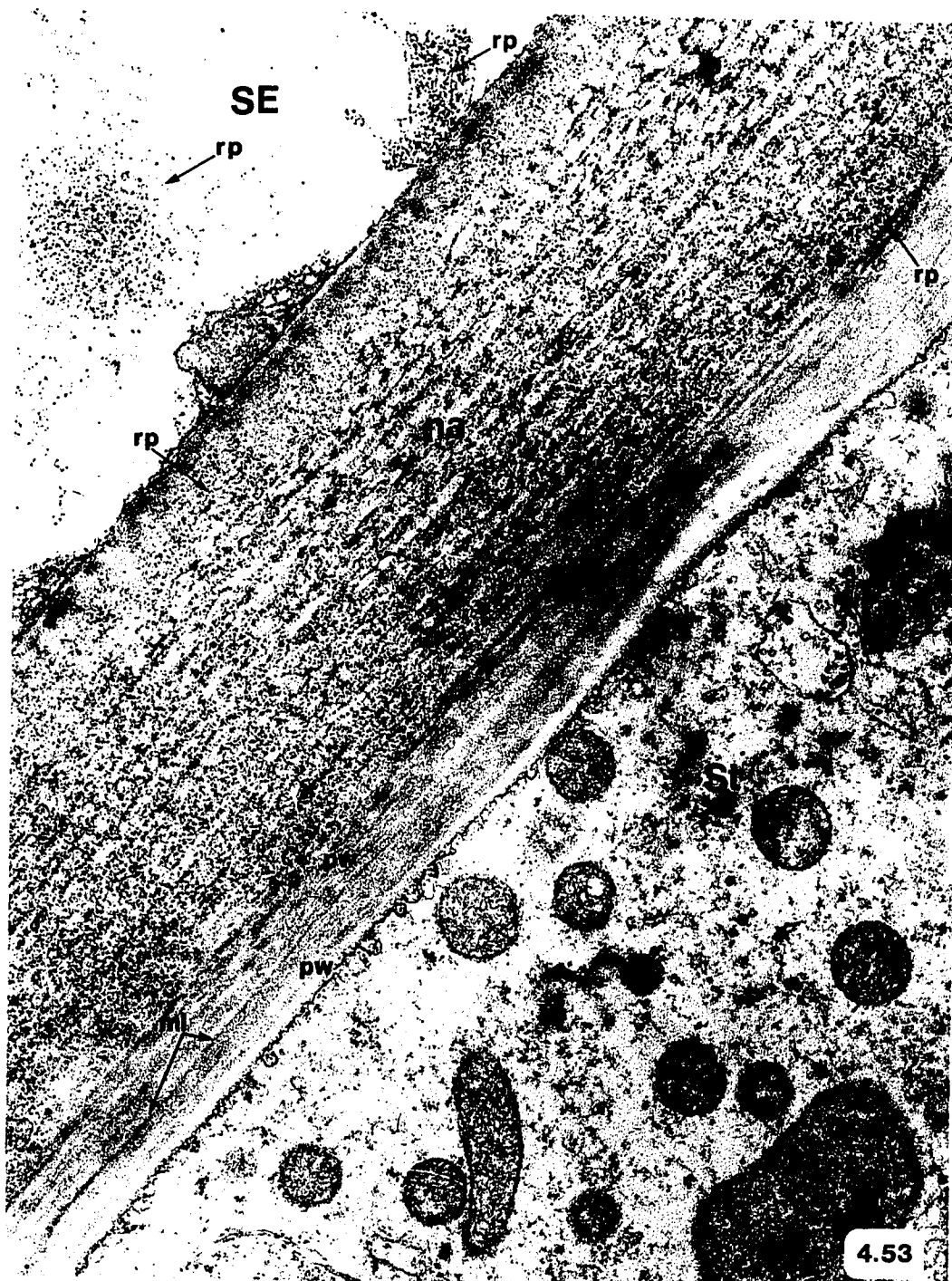


FIGURE 4.53. Juxtaposed walls of a sieve element (SE) and a Strasburger cell (St). The middle lamella (ml) delineates the two cells' walls. Primary walls (pw) of the cells are identical. An electron-dense reaction product (rp) is selectively deposited on the nacreous wall (na) of the sieve cell wall and also on some internal components of the sieve cell. 17,000X.



FIGURE 4.54. The cytological effects of sudden pressure release in sieve cells during fixation. This tissue was diced directly in fixative instead of being gradually trimmed down. The effects of sudden pressure release is apparent: The sieve element reticulum (SER) in the upper cell has collapsed onto the sieve pores (sp), while the SER in the cell below has been blown away from the pores. The SER has become an unordered mass; the lumen is cleared of contents. 17,000X. ca, callose.

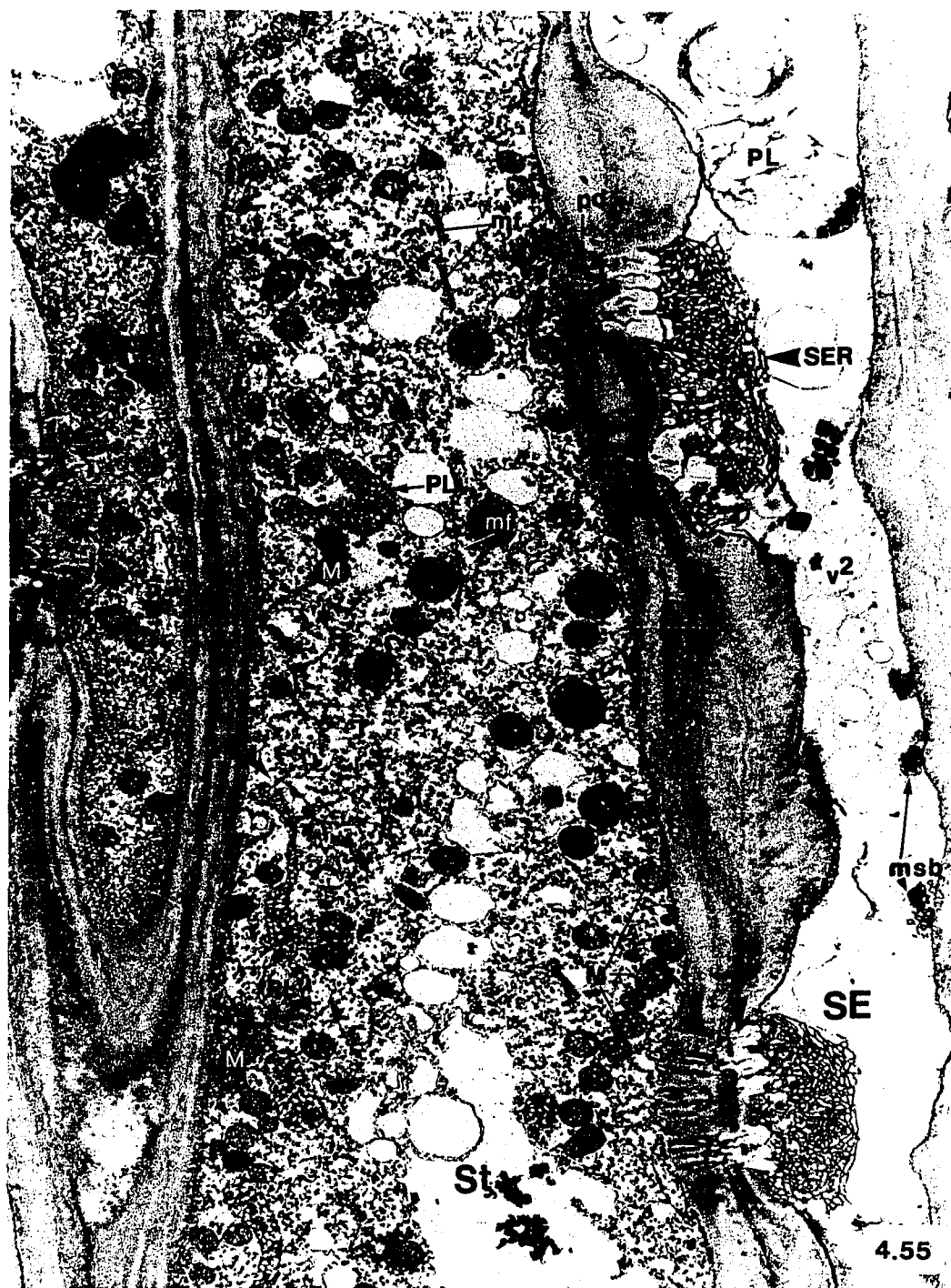


FIGURE 4.55. A Strasburger cell (St) joined to a sieve element (SE) by sieve pores (spo) and plasmodesmata (pd). 7500X. Mi, mitochondria; mf, microfilaments; msb, membrane sequestering body; Pl, plastid; SER, sieve element reticulum; sp, spherosome; T, tubules of unknown origin; v², inclusion-containing vacuole; arrowhead = plastid/tubule association.



FIGURES 4.56-4.58. Details of Strasburger cells (St). Fig. 4.56--Microfilaments (mf) are commonly seen in the cytoplasm; ribosomes (r) associate with endoplasmic reticulum (ER) or in polyribosomes. 31,600X. Fig. 4.57--Nuclear pores (np) have a central pore component (cpc). Fig. 4.58--Typically, branched plasmodesmata (pd) connect with sieve pores of adjoining sieve element (SE). 8400X. ca, callose; g, golgi body; h, heterochromatin; hs, hydrated starch; M, mitochondria; Nu, nucleus; PL, plastid; SER, sieve element reticulum; sp, spherosome; v, vacuole.



FIGURES 4.59-4.61. Details of Strasburger cells--plastids (PL) and mitochondria (M). Most plastids are larger than mitochondria and have indistinct limiting membranes. Fig. 4.59--A plastid-like organelle (PL?) has a large, centrally located bundle of microfilaments. 11,700X. Fig. 4.60--Detail of Fig. 4.59 showing microfilament (mf) bundle. 50,200X. Fig. 4.61--A polymorphic plastid with indistinct membranes. 55,400X. ER, endoplasmic reticulum; r, ribosomes.



FIGURE 4.62. A Strasburger cell (St) and an ordinary ray cell (RC). The Strasburger cell demonstrates distinctive cytoplasmic features as compared to an ordinary ray cell. See text for details. 7400X. Nu, nucleus; PL, plastid; s, starch; sa, sieve area; arrowheads indicate elongated, polymorphic plastids of the Strasburger cell.

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GLOSSARY

aerial shoot--The part of the mistletoe plant body that develops outside the host stem.

acropetal--Development of a plant body which precedes upward toward the apex.

amylopectin--One of two types of starch present in plastids. It consists of branching, α -(1,4)-linked glucose units.

amyloplast--A plastid specialized for storing starch; not for photosynthesis.

angiosperms--Flowering plants which have seeds that develop in enclosed ovules.

anticlinal--Perpendicular to the surface.

apoplast--The counterpart of the symplast. It is made up of non-living material and spaces in the plant body such as the cell walls, tracheary lumens, and intercellular spaces. Like the symplast, the apoplast forms a free space continuum in the plant body through which solutions can flow in response to chemical potential gradients and transpiration tensions.

bordered pit--A pit in which the pit is overarched by secondary wall material.

branch girdles--Areas of a coniferous stem bearing bud scales and delimiting yearly growth in lengths of vegetative branches.

callose--A polysaccharide with β -(1,3)-linked glucose units which occurs in cell walls, often in association with plasmodesmata or sieve pores.

cambial initials--Refers to cells of the vascular or cork cambium which form derivatives by periclinal division in two directions.

cellulose microfibril--A thread like component of a cell wall composed of cellulose molecules.

cell wall--A more or less rigid envelope surrounding the protoplast of a plant cell. In higher plants it is composed largely of cellulose and matrix material.

chlorophyll--The green pigment in plants necessary for photosynthesis.

chromatin--The name given to nuclear DNA and associated stainable material.

chromocenter--A mass of heterochromatin found in chromocentric nuclei.

chromocentric nuclei--The morphological form of plant nuclei characterized by dense, heterochromatin clumps within a generally light-staining nucleoplasm.

cisternae--The volume within membranous tubules or flattened saccules found in cytoplasmic structures and plastids.

coated vesicles--Small, membrane-bound vesicles which are coated on the outside with bumps, ridges, or bristles. They may be associated with the conveyance of protein through the cells.

companion cells--A specialized parenchyma cell in the phloem of an angiosperm. It is associated with a sieve tube member and arises from the same mother cell.

conifer--A cone-bearing gymnosperm.

coniferous--In reference to conifers. Members of class Gymnospermae, order Coniferales--the cone-bearing plants.

cortex--The primary tissue between the vascular system and the epidermis.

cristae--Membranous pockets formed by invaginations of the internal membranes of mitochondria.

cytokinin--A plant hormone.

cytokinesis--Division of the cytoplasm.

cytology--The study of cells.

cytopathology (n), cytopathological (adj.)--Terms relating to disease expression at the cellular level.

cytoplasm--The visibly structureless, colloidal portion of the protoplasm which encloses all other components of the protoplasm.

declining tiers--An associated group of secondary vascular cells in radial and longitudinal series. The series show a declining height due to loss of cambial initials and changes in cell shape from longitudinally elongate to more isodiametric forms.

de novo--Referring to a new origin or arising again.

dicotyledonous--In reference to dicots. A taxonomic group of flowering plants which have two embryonic cotyledons or seed leaves.

electro-osmosis--Coupled movement of water, neutral solutes, and ions through pores in response to changes in electrical potentials.

endomembrane concept--The hypothetical developmental continuum of membranous cell components from the cell surface to the internal regions of the cell.

endoplasmic reticulum (ER)--A system of membrane-bound channels in the cytoplasm associated with many different synthetic activities. Rough ER has ribosomes adsorbed to the membrane; smooth ER does not.

endophyte--The part of the parasitic plant enclosed within host tissues.

endophytic system--The portions of the dwarf mistletoe plant that develops within host tissue.

ergastic material--Passive storage products of protoplasm.

euchromatin--Uncondensed DNA probably without a histone coat.

eucaryotic--In reference to cells containing distinct, membrane-bound nuclei. Pertains to all organisms except microorganisms such as bacteria and blue-green algae.

exogenous--Growing from or on the outside. External.

ferritin--Deposits of iron-containing protein frequently found in achlorophyllous plastids.

fine structure--See ultrastructure.

free space--The volume open to free diffusion.

fusiform initial--In the vascular cambium an elongated cell with wedge-shaped ends which gives rise to elements of the axillary system in secondary xylem and phloem.

genome--One haploid set of chromosomes and the genes they contain.

golgi bodies--Membranous subcellular structures that may play a role in synthetic and secretory processes.

grana--Disk-like stacks of membranes in the internal portion of a plastid associated with photosynthetic pigments.

gymnosperms--Seed bearing plants which have naked ovules.

half-plasmodesmata--Plasmodesmata which end blindly at the middle lamella.

haustorium--A specialized organ of absorption of a parasitic plant.

Hela cells--Cells of a tissue culture line that has been maintained and studied for many years. Originally the tissue came from a cancer tumor of a woman called Helen Lane.

heterochromatin--condensed DNA coated with densely-staining histones.

histones--Nuclear proteins which have an affinity for stains. They coat coiled DNA and RNA, and as such they may have a regulatory action in gene action.

holdfast--A portion of a plant which serves to anchor.

hyperplasia--A plant overgrowth due to increased cell division.

hydraulic conductivity--The physical factors which influence the movement of water through a conduit.

hypertrophy --A plant overgrowth due to abnormal cell enlargement.

indolacetate (indolacetic acid)--A plant hormone.

in situ--In the original or natural position.

intraspecific--Between the same species.

- intercalary--A term which refers to tissues positioned between meristematic centers which exist in a longitudinal series.
- interspecific--Between different species.
- in vitro*--Removed from the living organism.
- in vivo*--In the living organism.
- lenticle--Special cellular formation in the periderm which has intercellular spaces that allow gas and water diffusion through the bark.
- lignification--Impregnation with lignin.
- lignin--A complex organic substance associated with cell walls and impermeable to water.
- lysigenous ducts--Ducts that form by the dissolution of cells.
- median nodule--A membrane lined cavity in the middle lamella which is continuous with plasmodesmata.
- medullary rays--Tissue regions between vascular bundles in primary shoots.
- meristematic--Capable of cell division.
- metaxylem--Primary xylem that differentiates after the elongation of associated primary tissue is complete.
- microfilaments--Filaments, 50-80 Å wide found in the cytoplasm, usually in bundles; they are associated with initiating movement.
- micropuff--A bulbous body in the interphase nucleus thought to be a morphological modification of a chromosome. The function is unknown, but may relate to sites of genes which have special physiological activities.

microtubules--Stiff tubules found in the cytoplasm or nucleus. They are about 250 Å in diameter and of long, usually indeterminate, length. They are associated with support, motility and morphogenesis, but their specific functions are not known.

middle lamella--A wall layer usually of pectic substances which is common to two adjoining cells and serves to cement them together. It is usually permeable to water and solutes.

mitochondria--Cytoplasmic organelles which have their own DNA component. They participate in respiratory functions.

mitosis--The morphologically consistent process by which replicated chromosomes separate by virtue of chromosome movements.

Münch, E.--A German scientist who in the 1930's proposed a model system to account for movement of the assimilates in plants.

nacreous walls--Peculiar walls of many sieve elements which are thickened and in fresh section have a pearly luster.

needle trace--A small vascular bundle which leads from branch vascular tissue towards a needle insertion site.

nucleolus--An RNA containing mass in a nucleus.

nucleoplasm--The colloidal substance which fills the nuclear cavity and surrounds the other components of the nucleus.

nucleotides--A chemical entity consisting of a five carbon sugar with a phosphate group and a purine or pyrimidine attached. These important molecules are present in nuclei acids as well as in substances which function as energy carriers.

nucleus--The large, membrane-bound, protoplasmic body housing the genetic material of a cell.

ontogeny--The course of development of an organism or its parts.

organelle--A morphologically well defined, sub-cellular structure.

parenchyma--A tissue composed of cells which, in general, are morphologically unspecialized, relatively thin-walled, and retain meristematic capabilities.

periclinal chimera--A hybrid which develops from a graft or natural mutation of the superficial layers of an apical meristem. The plant which develops has layers of genetically different cells which have been derived from the grafted or mutated apical meristem tissue.

peripheral haustoria--The portion of a haustorial system of mistletoe that invades the host peripheral stem tissue.

pit field--A thin place in the cell wall.

phanerogamic--A term pertaining to seed plants.

phelloderm--A tissue formed by the cork cambium in the opposite direction to the cork and which resembles cortex cells.

phloem-- The main photosynthate-conducting tissue in vascular plants.

photosynthate--Substances produced from photosynthesis that may be translocated.

phytochrome--A pigment system which, in response to specific wave lengths of light, triggers various morphogenetic responses.

plasmalemma--The outer membrane of the cell.

plasma membrane--The outer membrane of the cell.

plasmodesma (pl. - plasmodesmata)--A plasmalemma-lined canal through a pore in the cell wall which joins the protoplasts of two adjacent cells.

plastids--Cytoplasmic bodies concerned with assimilatory metabolism.

plastoglobuli--Round, osmiophilic bodies in the stroma of plastids which are composed of acetyl-CoA derivatives such as lipids and carotenoid pigments.

polyribosomes--Clusters of ribosomes.

P-protein--A proteinaceous substance found in some sieve elements which persists throughout the functional life of a sieve element.

pressure flow--The translocation of substances resulting from pressure differentials.

primary growth--Growth of roots and shoots by expansion and division of cells initiated by the shoot and root apical meristems.

primary haustorium--The original penetrating haustorium.

primary phloem--Phloem differentiated from the procambium.

primary tissue--Tissue that arises from apical meristems and their derivative tissues.

primary wall--Cell wall formed chiefly while the cell is increasing its size. At the ultrastructural level it is defined by random or transversely-parallel orientations of cellulose microfibrils.

primordia--Cells or rudimentary organs in their earliest stage of development.

procambial--Referring to the procambium.

procambium--Primary meristematic tissues which differentiates into primary vascular tissues.

procumbent ray cells--A ray cell elongated in the radial direction.

prolamellar body--An ordered, sometimes paracrystalline array of membranes composed of anastomosed short tubules; found in etiolated plastids and other achlorophyllous plastids.

proplastid--An undifferentiated plastid which is the precursor for other plastid types. It is capable of division.

protoxylem lacunae--Spaces in the protoxylem surrounded by parenchyma. These spaces appear when protoxylem tracheary elements are destroyed after extension of the primary vasculature.

pseudo-transverse division--A cell division plane that may appear to be a transverse, but is actually an oblique longitudinal division.

pycnotic nuclei--Degenerative nuclei marked by shrinkage and increase in density and stain affinity.

radicle--The embryonic root.

ray--A panel of tissue formed by ray initials of the vascular cambium and extending radially in a secondary xylem and phloem.

ray initial--A cambial ray cell which gives rise to ray cells of the secondary xylem and phloem.

replication--The process by which existing DNA or RNA molecules produce copies of themselves during interphase of the mitotic cycle.

reticulate nuclei--Nuclei which in interphase have a distinct chromatin reticulum throughout the nuclear cavity.

ribosomes--A small, granular cytoplasmic organelle that functions in protein synthesis.

sacculles--A term used in this dissertation in reference to a complex specialization of membranes and wall material found at primary pit fields of some sinker cells.

scalariform--A ladder-like pattern of secondary wall material formed on tracheary elements.

secondary cell wall--Cell wall deposited in some cells over the primary wall after the primary wall ceased to increase in surface. At the electron microscope level secondary cell walls are identified by the parallel and layered pattern of cellulose microfibril deposition.

secondary growth--In gymnosperms and many angiosperms, a type of growth characterized by an increase in thickness of stem and roots from derivative production by the vascular cambium.

secondary tissue--Tissue that arises from vascular cambium and cork cambium and contributes to increasing girth of the plant.

self assembly--The assembly of subcellular structures from monomers to polymers by an equilibrium process. Synthesis or de-

gradation is usually coordinated with changes in the micro-environment surrounding the units.

spherosomes--Cytoplasmic bodies specialized for lipid storage in which lipids accumulate in the lipid layer of the limiting trilaminar membrane of precursor bodies.

sieve area--A pit-like area of a sieve element wall which has membrane-lined pores.

sieve cells--A sieve element typical of gymnosperms and lower vascular plants characterized by small, uniformly-distributed sieve pores.

sieve element--A phloem cell specialized for longitudinal conduction of food materials.

sieve element reticulum--A system of membranes in sieve elements.

sieve plates--A collection of sieve pores often arranged in a pattern. Found in sieve tube members.

sieve tube--A series of sieve elements arranged end-to-end and interconnected through sieve plates.

sieve tube member--One of the cellular components of a sieve tube.

sink--A site in the plant body which has greater demand (consumption) than supply (production) in terms of nutritional balances.

Therefore, nutrients are imported.

sinkers--Radially oriented portions of the haustorial system of mistletoes.

source--A site in the plant body which has greater nutrient availability than it metabolizes. Nutrients are exported.

Strasburger cell--Specialized cell type in gymnosperm phloem which is closely associated (morphologically and physically) with a sieve cell. Also termed albuminous cell.

stroma--The proteinaceous, colloidal material surrounding all other components of the internal regions of a plastid.

symplast--The totality of living, membrane-bound, protoplasm-filled cells in the plant body. The symplast is considered a continuum because the individual cells are not isolated but are interconnected by protoplasmic channels. The non-living cell wall material is not part of the symplast.

systemically active substance--A substance that is capable of being translocated through the plant body.

tanniniferous--Pertaining to tannin.

tannin--An ergastic substance stored in vacuoles. A phenol derivative.

tertiary spiraled lignification--Pertaining to the lignification of the three layers of the secondary walls of tracheary elements. These layers are deposited independently of each other in spiral patterns around the longitudinal axis of a tracheary element.

thylakoid--Internal membranous sheets in plastids.

tonoplast--The cytoplasmic membrane surrounding a vacuole.

tracheary elements--A general term for a water conducting cell (tracheid or vessel).

tracheid--A cell type of xylem that has varying patterns of cell wall lignification and no wall perforations.

transcription--The process by which the portions of the genetic code of nuclear DNA is coded in RNA in preparation for the transport of genetic information to the cytoplasm.

transfer cell--A cell characterized by irregular wall ingrowths.

The function is thought to be related to the transfer of substances between the apoplast and symplast.

translocation--The movement of organics and ions from one place to another in plants.

traumatic resin ducts--A resin duct formed in response to injury.

turgor pressure--Pressure exerted by the contents of a cell against the cell membrane or wall.

ultrastructure--Plant structure which is beyond the resolution of light microscope.

unsaturated lipids--A lipid molecule with double carbon to carbon bonds, and a correspondingly reduced number of hydrogen-carbon bonds.

vacuole--A cavity within the cytoplasm containing various solutions and delimited by a membrane.

vascular--Refers to the specialized longitudinal conducting tissue of the plant (the xylem and phloem).

vascular cambium--A lateral meristem that forms the secondary vascular tissue.

vector (n), vectorial (adj.)--A quantity with magnitude and direction. It is commonly represented by a directed line segment

(\longrightarrow) whose length represents the magnitude and whose orientation in space represents the direction.

vector sum--The sum of a number of vectors that for the sum of two vectors is geometrically represented by the diagonal of a parallelogram whose sides represent the two vectors being added.

water potential--The chemical potential of water which is often considered in terms of pressure.

xylem--The principal water-conducting tissue in vascular plants.