Morphology, seasonal variation, fine structure and function of resin glands on buds and leaves of *Populus deltoides* (*Salicaceae*)

by

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INTRODUCTION

It is commonly known that overwintering buds of several *Populus* species contain abundant resin, yet little is known about where and how it is produced or what its function might be. The first published reference to resin secretion in *Populus* was probably by Hanstein (1868), who described briefly the adaxial secretory surface of bud scales on an unnamed species. Secretion from leaf teeth was described briefly but accurately in *P. balsamifera* and *P. laurifolia* by Reinke (1874). Tschirch (1906; cited by Feher, 1923) mentioned secretion in *P. balsamifera* and *P. nigra*. Feher (1923) studied resin secreting structures in several European poplars: *P. balsamifera*, *P. nigra*, *P. canadensis* (*deltoides*), *P. tremula*, *P. alba* and *P. canescens*. He described the glandular tips of leaf teeth and the secretory epidermis of stipules and bud scales, but he did not consider seasonal variation of these structures. His work is the most comprehensive to date.

Trelease (1881) observed individuals of *P. tremuloides*, *P. balsamifera*, *P. candicans*, *P.* grandidentata, *P. monilifera* and *P. tremula* in the field and reported nectar secretion from some of the same structures that Feher later observed secreting resin. Trelease seemed completely unaware of resin secretion in these structures, and Feher was equally

unaware of nectar secretion and appears to have overlooked Trelease's work. Edelstein (1902) induced guttation from leaf teeth of P. laurifolia and called the area where this occurred a hydathode, even though it did not have an epithem. He also mentioned similar hydathodes in P. balsamifera and P. tremula. Lippmann (1925) questioned this interpretation. She thought the area that Edelstein called a "hydathode" was anatomically too unspecialized and suggested that guttation occurred instead from the glandular tips of the teeth. Lepeschkin (1921) attempted to induce guttation from leaf teeth of P. nigra but succeeded only in observing resin secretion. Uphof (1962) reviewed much of the work on resin secretory structures and hydathodes on Populus leaves but did not explain the discrepancy.

The anatomy of many plant structures has been described with little or no consideration given to seasonal variation. In some areas of plant anatomy, however, workers have begun to realize that seasonal variation is important. For instance, it is now known that shoot apices of many plants show seasonal variation in anatomy and histochemistry.

I undertook a study of secretory structures on Populus deltoides (cottonwood), a common North American species with abundant resin, to learn more about their

morphology, anatomy, fine structure and seasonal variation. I also hoped to settle the controversy raised by previous workers concerning resin secretion and guttation of water and nectar. In addition, I made field observations and did a laboratory experiment related to the possible function of the resin.

A comparative study of secretory glands of Populus and Salix was originally proposed to include a survey of the two genera using herbarium material and a detailed study of two representative species, Salix interior and Populus deltoides. This was not done for several reasons. The proposed study was too large an undertaking to complete in a reasonable time. Salix interior proved to be a poor choice to represent that genus. Glands on herbarium specimens of Salix and Populus were often poorly preserved and broke off easily, making it difficult to tell whether leaves were glandular or eglandular. These specimens had been collected at different times of the year. Considering the range of seasonal variations found on Populus deltoides, I felt that few valid conclusions could be made from such specimens.

Preliminary observations of one Salix species, Salix pentandra, are included because of some special characteristics displayed by its secretory glands.

MATERIALS AND METHODS

Field Observations and Collections

Field observations were made from March, 1970 to August, 1973 on gross morphology of buds, leaves and stipules of Populus deltoides growing in the Ames, Iowa area. Trees of all ages were examined, but most observations were made on younger trees upon which the vigorously growing upper branches were accessible. Field notes were made and a photographic record made using a Pentax 35 mm single lens reflex camera with extension tubes. Leaves, stipules and buds were removed from the trees, sealed in plastic bags and brought into the laboratory for more detailed observation with dissecting microscopes and for processing for electron microscopy.

Representative leaves were judiciously collected from herbarium specimens at herbaria of Iowa State University, Missouri Botanical Gardens and Field Museum , Chicago. Most of the 35 to 40 Populus species and over half of the 300 to 400 Salix species were collected.

Microscopy

FAA-fixed leaves and stipules of Populus deltoides and Salix and Populus leaves from herbarium collections were cleared using a technique modified from Shobe and Lersten (1967) and stained with safranin and chlorazol black E.

Some FAA-fixed material, especially buds or sclerified bud parts, was placed in 10% aqueous hydrofluoric acid overnight to soften, then dehydrated and infiltrated with paraffin. Eight µm serial sections were cut on a rotary microtome and stained with chlorazol black E and safranin.

Material for plastic sections for light and electron microscopy was fixed in 3% glutaraldehyde with 3 drops of 3% hydrogen peroxide per 10 ml of 3% glutaraldehyde (Peracchia and Mittler, 1972) in a 0.1 M phosphate buffer (pH 7.2) for 16 hr at 4° C, postfixed in 1% osmium tetroxide for 1 hr (same buffer), dehydrated with acetone and embedded in Epon 812. Several dehydrating agents and embedding media were tried in an attempt to retain the Populus resin intact in the bud. However, this resin was soluble in one or more components of each series tested.

Plastic sections 1-4 µm thick were cut with glass knives on an LKB Ultratome III, floated on water on a standard microslide over a 55° C heating plate and left for several hours to dry. The sections were then covered with Paragon multiple stain (Spurlock, Skinner and Kattine, 1966) for 30 sec while still on the plate, rinsed with double distilled water, allowed to dry and mounted in Piccolyte.

For transmission electron microscopy, plastic embedded material was sectioned 60-70 nm thick with a diamond knife on an LKB Ultratome III and double stained on 200 mesh grids with lead citrate (15 min; Reynolds, 1963) and 20% methanolic uranyl

acetate (20 min; Stempak and Ward, 1964). Material was observed with a Hitachi HS-8-2 microscope.

Material collected for scanning electron microscopy was fixed in osmium tetroxide fumes for 20 min, dehydrated in acetone and dried using the critical point apparatus technique of Anderson (1956) with liquid carbon dioxide. The material was gold coated using a Ladd Tilting Variable Speed-rotary shadower and a Varian VE-30 M vacuum evaporator and viewed on a JEOL JSM-S1 scanning electron microscope with the assistance of James Amrine, Department of Zoology and Entomology, Iowa State University.

Thick sections and clearings were photographed with bright field (BF) or phase contrast (Phaco) optics on a Leitz Ortholux microscope. For low magnification micrographs of whole structures, a Leitz Macrodia photomicrography apparatus was used.

Resin and resin secreting tissues were subjected to histochemical tests for lipids. Resin was spread on a clean glass slide and placed in osmium tetroxide fumes for 30 min (Cain, 1950; in Jensen, 1962). The resin turned black, especially in areas where it was spread thinly. A negative reaction (lack of stain) would indicate the absence of lipid in the resin. Identically prepared slides were placed in 1% aqueous Nile blue for 30 sec, rinsed in 1% acetic acid and washed in distilled water (Cain, 1947; in Jensen, 1962). The resin stained blue. A negative reaction (lack of stain)

would indicate the absence of lipid. Fresh free-hand cross sections of stipules were placed in a saturated solution of Sudan III in 70% ethyl alcohol for 20 min then rinsed in 50% ethyl alcohol for 1 min (Gomori, 1952; in Jensen, 1962). Outer mesophyll cells stained orange indicating the presence of lipids. Because resin is soluble in ethyl alcohol, I was unable to test it for lipid content using Sudan dyes.

OBSERVATIONS

Terminology

I adopted the terminology of Critchfield (1960). Leaves initiated the previous year which overwinter in buds and complete their development the next spring are called "early leaves"; those that are initiated and reach maturity during one growing season are called "late leaves." Leaves within overwintering buds are either "embryonic leaves" or "leaf primordia"; the former have some vascular tissue and a recognizable lamina.

Buds are designated "dormant" or "active." Two phases of active buds are: (1) buds in spring and summer from which leaves are emerging, internodes are elongating and in which new leaf primordia are being initiated; (2) buds in late summer or early fall in which leaf primordia are still being initiated, but leaf emergence and internode elongation have ceased.

Although stipules are considered parts of the leaf, for convenience I use "leaf" to include only the petiole and lamina. Leaves have glands at the tips of marginal teeth (marginal glands) and at the tips of small stalks arising at the juncture of the lamina and petiole (basal glands). The latter term simplifies Hickey's (1973) "basi-laminar glands."

Hydathodes guttate water containing varying concentrations of sugar. If the solution is sweet to the taste, I call it nectar.

The material produced by secretory glands is a fragrant,

yellow, sticky substance that becomes clear and brittle when dry. Most of its components are soluble in organic solvents such as acetone, xylene and ethyl alcohol, but insoluble in water. Because of these chemical characteristics I call it resin, as have all previous investigators of Populus.

I collected large numbers of dormant buds which were analyzed by Dr. William Wildman, Chemistry Department, Iowa State University. Results of the analysis were inconclusive but indicated the resin is composed of a complex mixture of compounds. Wollenweber and Egger (1971) and Wollenweber and Weber (1973) report that resin of Populus nigra is also a complex mixture.

Seasonal Bud Cycle

The dormant overwintering vegetative bud contains leaf primordia, embryonic leaves and stipules. Spaces in the bud are filled with a clear, yellow resin. One or more leaf scars occur at the base of terminal buds. The stipules associated with these leaf scars do not abscise; instead, they form the outer series of squat bud scales (Fig. 1B-C). The next two to five bud scales are stipules associated with tiny leaves that are shriveled and black (Fig. 1D-G). All of the stipules that act as bud scales (Fig. 1B-H) are thick, sclerified (Fig. 3) and arranged in a series with increasing length from outside to inside.

Axillary buds, in contrast, have two to five bud scales (Fig. 2B-E) that are true cataphylls. In both terminal and axillary buds, stipules interior to the bud scales (Fig. 1I-T,

2F-L) become progressively smaller.

During late April most terminal buds and many axillary buds break dormancy and leaves begin to emerge. Initiation of new leaves resumes. As internodes elongate, stipular bud scales of terminal buds and cataphylls of axillary buds abscise. Internode elongation and leaf enlargement continue acropetally. Soon after each leaf unrolls, its stipules abscise.

New buds begin to form when internodes stop elongating. Leaves stop emerging and enlarging soon afterward but not before one or more leaves have emerged with unexpanded internodes. These leaves abscise, but their stipules remain, forming the squat outermost bud scales of the overwintering bud (Fig. 1B-C). Leaves continue to be initiated within the bud for several weeks, but their development is arrested quite early. Stipules of the outer embryonic leaves continue to develop and form bud scales.

Stipular bud scales and a few adjacent inner stipules produce resin which fills the spaces of the bud and often exudes onto the bud surface. In small axillary buds, the outer one or two cataphylls nearly surround the rest of the bud. These cataphylls are often split longitudinally by continued bud growth before dormancy (Fig. 2B-C).

Over 100 overwintering terminal buds and 100 axillary buds were dissected and studied in detail. Terminal buds contain 2-5 dead leaves (Fig. 1D-G), 5-15 living embryonic leaves (Fig. 1H-T), and a few leaf primordia. Thus the

largest axillary buds equal the largest terminal buds in size, but the smallest axillary buds are much smaller than the smallest terminal buds.

For each terminal bud studied, leaf scars were counted on the previous year's growth. The number of leaf scars represents the total number (T) of leaves that emerged from the bud during the previous growing season. I also counted the number of early leaves (E) in this year's overwintering bud. I assumed that last year's overwintering bud contained the same number of early leaves and calculated that total leaves (T) minus early leaves (E) equals late leaves (L). In small buds the total number of leaves that emerge (T) is usually the same or a few more than the number of living embryonic leaves (E) in the previous year's overwintering bud, i.e. small buds usually have only early leaves. L is nearly zero. Large buds produce early and late leaves. Late leaves usually continue to emerge throughout the summer, long after leaves have ceased emerging from smaller buds. As many as sixty leaves may emerge from an active bud during one year's growth, yet I never found more than fifteen leaves in any overwintering bud (Fig. 4). Therefore as many as 45 late leaves (L) may form from one bud.

In P. deltoides there is no clear distinction between "short shoots" (those bearing only early

leaves) and "heterophyllous shoots" (those bearing both early and late leaves), as reported for P. trichocarpa (Critchfield, 1960). Instead, there seems to be a gradient from small axillary and terminal shoots which have only early leaves to long shoots which have a high proportion of late leaves. One year's growth in terms of length of stem from a terminal bud ranged from less than 2 cm to over 3 m.

Stipules

Stipular bud scales associated with leaf scars (Fig. 1B-C) have longitudinal adaxial ridges whereas the remaining stipules are not ridged. The adaxial surface of all bud scales contains a longitudinal strip or strips of collapsed cells (Fig. 3). Only the exposed part of the abaxial surface has a thick cuticle and a periderm (Fig. 6) giving it a brown color. The covered part of the abaxial surface is usually green, with a thinner cuticle and no cork or cork cambium (Fig. 7, 8). The mesophyll is composed of isodiametric parenchyma cells with conspicuous intercellular spaces, relatively small vascular bundles and large parallel bundles of fibers (Fig. 5) not directly associated with the vascular bundles.

The next series of stipules (Fig. 1I-K) are entirely enclosed, have a very thin cuticle, fewer and smaller bundles of fibers and a collapsed adaxial epidermis (Fig. 3, 8). The innermost stipules (Fig. 1L-T) form a gradually

diminishing series toward the interior, until they became unrecognizable primordia close to the apex. These stipules lack bundles of fibers and have an adaxial epidermis composed of living isodiametric parenchyma cells (Fig. 5, 6, 8).

In nearly all stipules and leaves of overwintering buds, subepidermal cells and mesophyll cells were separated by an extensive schizogenous space (Fig. 8). Wiegand (1906), who examined freehand bud cross sections outside in winter, showed ice crystals in similar spaces in Populus candicans and P. dilatata. He suggested that ice crystal formation caused the spaces. I did not observe such spaces in late leaves and stipules.

As buds break dormancy in spring, the internode above the last-formed dead embryonic leaf (Fig. 1G) elongates slightly. Stipules in this region often remain together and abscise as a unit, leaving stipular scars that form the terminal bud scale scars. Stipules of the first leaves to emerge from the bud have an adaxial epidermis of collapsed secretory cells. These stipules abscise soon after internodes begin elongating.

As the first leaves emerge, the inner stipules of the bud resume growth. The adaxial epidermal cells elongate perpendicular to the surface, forming a secretory layer which becomes ridged or corrugated at the base (Fig. 9-11). Before a leaf emerges from the bud its

stipules have begun producing resin (Fig. 12) that covers the next inner leaves and stipules. Stipules continue to secrete resin as petioles and internodes above them elongate (Fig. 15). Secretion from a pair of stipules ceases soon after its associated petiole stops elongating, and the stipules abscise shortly after.

On some late leaf stipules, a secretory epidermis covers the entire adaxial surface (Fig. 12-14), and may even extend around onto the abaxial surface (Fig. 19). On most late leaf stipules, however, marginal cells are non-secretory and some trichomes are often present. The center one-half to two-thirds of the adaxial secretory surface is ridged (Fig. 12-14, 17-19, 28, 29). The non-secretory abaxial surface has numerous raised stomata (Fig. 18, 30) scattered below the same area covered by secretory ridges. One, two or occasionally three vascular bundles enter the stipule and form an open dichotomous system which parallels the adaxial ridges.

Within mature stipules most cells, except for those of the secretory epidermis and the vascular tissue, are isodiametric, vacuolate and contain chloroplasts. Occasional druse crystals also occur. Cells of the abaxial epidermis tend to be slightly elongated perpendicular to the surface. Small vascular bundles come within five or six cells of the secretory epidermis. No cells with wall invaginations (transfer cells) were found between the

vascular tissue and the secretory cells (Fig. 17-19, 26, 27).

Mesophyll cells immediately below the secretory epidermis often contain osmiophilic material which appears black when viewed with bright field optics but light when viewed with phase contrast optics (Fig. 24, 25). Histochemical tests (see Materials and Methods) indicate that this material is lipid.

Cells of the secretory epidermis, except those at the base of troughs, are 20-35 µm long and 5-10 µm wide. They usually lack large vacuoles and have dense, darkly staining cytoplasm and a single, relatively large nucleus (Fig. 16, 20-23). Cells on the crest of a ridge are often narrow at the base and wide at the surface. The cuticle is separated from the cell wall and appears broken in some places. It gives a faint positive reaction to Sudan III in 95% ethanol. Debris, which may be residue from resin, was often seen within the troughs but usually outside the cuticle (Fig. 26, 27).

Stipules begin secreting resin while still within the active bud, but it is difficult to determine exactly when, because considerable resin is already present from activity of older stipules (Fig. 15). If one assumes that adaxial cuticle disruption is the result of resin production, then secretion starts at least three days before the

internode above a stipule begins to elongate. Secretion therefore continues in an individual stipule for a minimum of six days, probably for at least ten days.

The cycle of stipule enlargement, resin production, elongation of adjacent internodes, stipule senescence and abscission, recurs continually throughout the growing season.

Leaves

Within overwintering cottonwood buds there is a continuum from immature leaves with a recognizable petiole and lamina to primordia adjacent to the shoot apex. The largest leaves have a well developed midvein (Fig. 3, 8); phloem of secondary veins is well developed and tracheary elements are just beginning to differentiate. Each lamina margin is inrolled toward the adaxial surface (Fig. 3, 8, 33, 45, 47, 48). Leaf teeth have formed, but no secretory cells have differentiated.

Leaves emerge from the bud by petiole elongation, lamina expansion and internode elongation. Emerging leaves are covered with resin produced the previous year by stipular bud scales. As leaves emerge they rapidly unroll and enlarge.

Teeth of the first leaves to emerge (Fig. 34) lack glands, have relatively small vascular bundle endings and an eglandular margin densely covered by trichomes.

Several large stomata occur abaxially near the margin. Nectar was observed guttating from some of the non-glandular teeth of such leaves emerging from buds forced in the laboratory. Leaves emerging a few days later have a few glandular teeth with relatively small vascular bundle endings and a margin covered by trichomes, except for the glandular tip (Fig. 35, 36). On subsequent leaves all teeth are glandular with more secretory cells and larger vascular bundle endings. Eventually the secretory gland comprises much of each late leaf tooth surface (Fig. 31-33, 38-40).

The leaf tip is elongate and conspicuous, particularly in the young leaf (Fig. 45-48). It reaches anatomical and physiological maturity soon after emerging from the bud, according to Isebrands and Larson (1973), and contains the large ending of the midvein (Fig. 9 of Isebrands and Larson). The tip is covered by many raised stomata (Fig. 46), but it lacks resin-secreting structures. Nectar secretion was never observed from the leaf tip.

Non-glandular teeth of early leaves usually have a median vascular bundle that ends inconspicuously. Stomatal position is not correlated with location of the bundle ending. Large stomata occur above bundles near the margin of the tooth and proximal from its tip. The vascular bundle ends near the leaf margin and is often obscured by trichomes that cover the tip of the tooth (Fig. 34).

Early leaf teeth with small glands have nearly identical venation (Fig. 36), but subsequent leaves have teeth with larger glands and two veins. The smaller outer vein parallels the leaf margin, whereas the larger inner vein is closer to the sinus. The two veins usually remain separate, the inner forming a large vein ending within the gland and the outer ending near, but usually not inside, the gland (Fig. 38-40). Often, especially in leaves emerging later in the summer and early fall, there are several stomata above the ending of the outer bundle (Fig. 40, 43, 44). In some teeth the two veins merge into one large ending (Fig. 37); the inner portion extends into the secretory gland and the outer portion terminates below the stomata from which guttation presumably occurs.

Vein endings are composed mainly of tracheary elements. I was unable to positively identify sieve tube members or companion cells but found what could be interpreted as phloem parenchyma, or perhaps procambium, extending beyond the tracheary elements into the secretory glands (Fig. 40).

Marginal teeth secrete resin and nectar. In laboratory conditions both early and late leaves occasionally guttate a clear, sweet nectar (Fig. 42) that covers the tooth. Nectar accumulated until it fell off as drops or ran down the leaf surface. Nectar drops were never observed on

plants outdoors, yet wet areas were seen on leaves, probably because the nectar was dispersed by leaves shaking in the breeze. It seems likely that guttation occurs from the large stomata proximal to the secretory gland. Although this portion of the leaf tooth is an extra-floral nectary by definition, it lacks the specialized internal structure usually associated with nectaries or hydathodes (Fig. 43, 44).

Resin is secreted from glandular teeth (Fig. 41) of both early and late leaves, first appearing as a milky, yellow-white liquid, but later turning clear and yellow. In both conditions it is somewhat bitter.

Resin secretion begins about the time of petiole elongation, when the glandular teeth are still within the rolled-up lamina. The glands continue to produce resin which exudes from either end of the young tubular lamina as the leaf unrolls (Fig. 48). Secretory marginal teeth of leaves as large as 6 cm long and 4 cm wide remain curved over the adaxial epidermis (Fig. 33). Secretion ceases at about the time the leaf finishes unrolling and the teeth are no longer arched over the adaxial surface (Fig. 32). Within a few weeks the glandular tip of each tooth turns brown. The duration of secretion on an individual leaf probably occurs for 8-10 days, about the same length of time as secretion on the stipules.

The secretory epidermis of glandular leaf teeth is composed of cells similar to those of the secretory layer of the stipules. The secretory epidermis, however, extends farther down the tooth on the adaxial side. This is especially prominent in large glands that form on late summer leaves. The cuticle of the secretory gland, like that of stipules, separates from the cell wall.

There are usually two basal glands present on the first leaves to emerge from the bud. They are generally the only resin secreting structures present and are about 1 mm long at maturity (Fig. 56A). Those of Figures 49-53 are not fully enlarged.

The tip has a secretory epidermis. On the side closest to the petiole are one or two stomata. Trichomes cover the flank nearest the leaf margin and occasionally also the opposite flank. One or two vascular bundles extend from the marginal vascular bundle to about the level of the stomata, but seldom all the way to the secretory tip (Fig. 51, 52, 56A). There seems to be little variation in structure among early leaf basal glands which were the only ones observed to guttate.

On leaves emerging later, basal glands show more diversity, but a pattern can be seen. They become progressively narrower and usually shorter, although a few may elongate to 1.5 mm. Stomata do not develop and the area of secretory epidermis is reduced (Fig. 54, 56B)

or sometimes absent (Fig. 56C, center two glands). Basal glands of Figure 55 are exceptions. As in the leaf teeth, however, vascular bundles tend to become larger and more numerous and end near the secretory layer. On some leaves no basal glands were seen, whereas on others there were as many as five, with outer glands usually longer than inner ones (Fig. 56B). In some leaves, glands near the attachment of the petiole to the lamina appeared intermediate in structure between marginal teeth and basal glands.

The first leaves to emerge from buds (collected April 24-30), with few exceptions, had two basal glands each. An abrupt change was observed about a week later (May 3, 1972), when few of the expanding young leaves had any basal glands at all. Within another 7-10 days, most of the emerging leaves had two basal glands; some had three or four; a small number had none. Throughout the rest of growing season, the percentage of emerging leaves with three, four or five glands increased greatly. In August, for example, emerging leaves averaged about four basal glands apiece.

Basal glands perform the same secretory functions as marginal teeth, but are more easily studied because they are exposed for a longer period of time. As young leaves begin to unroll, their basal glands are visible almost immediately, and one can see a small drop of yellow-

white resin at the tip immediately above the secretory epidermis. Within a short time the resin becomes clear and yellow (Fig. 45). On leaves that emerge later it seems likely that these structures also secrete resin, but this is difficult to verify because the whole adaxial surface of the leaf is covered by newly secreted resin from the marginal glands.

Secretory Cell Fine Structure

The study of fine structure of stipule resin secreting cells has been divided into five chronological stages. Enough leaf teeth glands were studied to indicate that their fine structure follows the same chronological stages. Stages 1 and 2 occur during rapid stipule enlargement and cell differentiation; resin secretion occurs during stages 3 and 4; senescence occurs at stage 5.

Plastic sections one µm thick were made of each stipule at the time thin sections were made. Representative thick sections of stipules at all stages are shown in Figures 16 through 23 and in Figure 27. Representative low magnification electron micrographs are shown in Figures 57 through 59, 62 through 64, 73 and 85. Cells at or near the top of ridges were often at a later stage than those near the base of the trough. Therefore, in many stipules, especially older ones, several stages can be seen (Fig. 16, 23).

Stage 1

Figures 17 and 20 are cross sections of stipules at stage 1. Ridges are just beginning to form. The epidermal cells of the secretory gland are generally elongated perpendicular to the stipular surface. These cells usually have a large central nucleus, a large, dark-staining nucleolus, several relatively large vacuoles, endoplasmic reticulum (ER), ribosomes, dictyosomes, mitochondria and plastids (Fig. 57). The plastids lack organized grana and stroma but usually contain osmiophilic inclusions. Dictyosomes appear to be actively producing vesicles which are electron translucent (Fig. 58). The cuticle is the dark external boundary of the outer cell wall (Fig. 57, 58).

Cells of the mesophyll are usually isodiametric and contain a large central vacuole which appears empty except for small clumps of osmiophilic material on its periphery (Fig. 57). Their plastids have grana and stroma (Fig. 61). Cytoplasm in these cells stains more darkly than that of epidermal cells, but otherwise they differ little from the epidermal cells.

Stage 2

Figure 21 is a cross section of a stipule at stage 2. Cells in this stage also occur in Figures 16 and 23. During the time between stages 1 and 2 cells of the whole

stipule continue to enlarge. Within the epidermal cells there is a parallel increase in the numbers of mitochondria, plastids, ribosomes and dictyosomes. Vacuole number increases but average size decreases greatly (Fig. 62).

In outer mesophyll cells just below the elongate epidermis of non-ridged areas and in most mesophyll cells of ridged areas, osmiophilic material has begun to accumulate in the vacuoles (Fig. 59-62). Along the periphery of the vacuole, dark clumps of myelin-like material often occur adjacent to plastids (Fig. 61, arrow).

Stage 3

Figures 16, 23 and 27 are cross sections of stipules with epidermal cells at stage 3. From stage 2 to 3 the epidermal cells continue to enlarge to about 35 µm long and 10 µm wide (Fig. 63, 64). The numbers of plastids, mitochondria and dictyosomes within this layer increase greatly and occasional osmiophilic bodies occur. The outer cell wall becomes much thicker, up to 1.5 µm and appears fibrillar(Fig. 64). Walls between epidermal cells become up to 0.4 µm thick but walls adjacent to mesophyll cells enlarge little, remaining about 0.1 µm thick (Fig. 63). The cuticle becomes detached (Fig. 64). The most striking characteristic of the stage is the large numbers of invaginations of the plasmalemma (Fig. 63-67). These are due, in part, to fusion of vesicles

with the plasmalemma (Fig. 65, arrow). The vesicles are produced in large numbers by dictyosomes. Invaginations may also be formed by fusion of vesicles and vacuoles of unknown origin. Invaginations contain material that appears structurally different from cell wall materials (Fig. 67). In some stipules at this stage, plasmalemma invaginations contained a dark-staining substance. The same material also occurred within regions of the cell wall (Fig. 68).

More osmiophilic material accumulates in mesophyll cells just below non-ridged secretory epidermis and within the ridges (Fig. 69), but little or none appears in the rest of the mesophyll (Fig. 70).

Stage 4

Figures 16, 23 and 27 are cross sections of stipules with some cells at stage 4. Between stages 3 and 4 invaginations of the epidermal cell plasmalemma disappear. Myelin-like lamellar material accumulates between the plasmalemma and cell wall (Fig. 71, 73), most prominently next to the outer wall (Fig. 73).

Cell walls become even thicker than before but this seems due at least in part to a loosening of wall components. Large numbers of spaces or channels, which often appear to be formed by a splitting of the middle lamella, occur in all walls of the cell (Fig. 72-77). Within this region, spaces appear that form "channels." These are

particularly pronounced in anticlinal walls at the juncture of three or four cells and are easily distinguished in paradermal sections (cross section of epidermal cell) (Fig. 76, 77), but also in stipule cross sections (longi-section of epidermal cells) (Fig. 72, 73, 75). These intercellular channels extend from the outer mesophyll cells to the cuticle.

Organelles within the epidermal layer change in structure and number from stage 3 to 4. In most cells there is a great increase in the amount of ER (Fig. 73, 75). There are fewer dictyosomes, but those present still produce large numbers of vesicles. Plastids are still present in large numbers but their contents are of about the same density as the surrounding cytoplasm.

There is an increase in numbers of osmiophilic bodies within the cells (Fig. 73). In many of these bodies the contents seem to have been partially or completely removed, leaving behind only a lamellar network (Fig. 78, 79), which eventually migrates to the plasmalemma (Fig. 79) and contributes to the dark mass of material that accumulates next to the cell wall.

Mitochondria have begun to degenerate, a process that will continue more rapidly in stage 5. Figures 80-84 indicate a possible series of chronological stages of mitochondrial breakdown, resulting finally in a vacuole-like structure usually without a double membrane.

The amount of osmiophilic material within the vacuoles

of mesophyll cells decreases greatly. This material continues to decrease into stage 5.

Stage 5

Figures 16 and 23 are cross sections of stipules with epidermal cells at stage 5, shortly before stipule abscission. Epidermal cells contain many large osmiophilic bodies (Fig. 16, 23, 85). All stages of mitochondrial degeneration occur and mitochondrial derivitives seem to fuse and form larger vacuole-like structures (Fig. 86). Plastids change little from stage 4.

Organelles within the outer mesophyll cells, in contrast, do not undergo noticeable changes. The central vacuoles now contain little or none of the osmiophilic material. All that usually remains is material that may be a remnant of the osmiophilic substance and some lamellar material. Figures 87-90 indicate a possible series of chronological stages of movement of osmiophilic material out of the vacuoles of the cells. The material appears to move en masse out of the vacuoles into the cytoplasm. I saw no indication of how the material then moves out of the cells.

Table 1 outlines the activities of cell components in all five stages.

Insect Relationship

During late summer and early fall several insect species were observed feeding on young leaves. Two of

the most devastating were the cottonwood leaf beetle, Chrysomela scripta, and an Aphis species herded by an unidentified species of large black ant. Leaves damaged by the aphids became wrinkled, then curled and died. Leaves attacked by the beetles were eaten down to the midrib and larger lateral bundles. Both species favored newly expanded leaves on which the resin had dried.

To determine the importance of resin, I collected about a hundred larvae of Chrysomela scripta and divided them between two transparent plastic containers. Cottonwood leaves on which only the margin was still resinous were put into one container; young leaves still covered with sticky resin were put into the other.

Leaves were replaced at regular intervals. In the first container, larvae left the midribs (Fig. 91, 92) and often the still resinous leaf margin. They pupated and adult beetles emerged (Fig. 93) which eventually laid eggs. In the other container larvae avoided the resin-covered young leaves, and most died. Some pupated, but no adults emerged.

Salix

As stated previously, this study was originally proposed to include a detailed study of Salix. This seemed desirable because Salix and Populus are the only genera in the family Salicaceae. I hoped to compare structure and function of their secretory glands. Because of time limitations this was not done. However, observations of glands on one

Salix species are worth mentioning because of some of the peculiar characteristics they seem to exhibit.

This species, growing in Beeds Lake State Park, Hampton, Iowa was identified as *S. pentandra* but had some characteristics of *S. lucida*. A cursory study was made on glands of leaves and stipules from this species. When cuttings were grown under conditions of low relative humidity the material secreted appears to be forced out of the end of the gland forming a thread sometimes several centimeters long. The threads vary considerably in thickness and length; finer threads are usually much longer than thick ones (Fig. 97). Basal glands of leaves seem to remain meristematic long after leaves have unfolded (Fig. 94-96). Some seem to have active intercalary meristems below their tips and many appear to branch and form smaller lateral glands. Although of little importance in the study of secretion in *Populus deltoides*, these glands are important because they have characteristics previously undescribed in secretory glands: presence of intercalary meristems, ability to branch and formation of a filamentous secretory product.

DISCUSSION

Resin covers shoot apices of Populus deltoides and their young leaves throughout the year. Stipules produce the resin found in buds during late fall, winter and spring. During the summer, resin is produced by both stipules and leaf teeth. From field observations and a simple laboratory experiment with insects, it appears that the resin repels insects. As a consequence the shoot tip seems immune to insect attack, and the shoot apex can continue to produce new leaves even when fully expanded leaves are subject to insect damage. Levin (1973), in a review of trichomes and glands, emphasizes their role in plant defense, particularly against insects, and cites several examples of defense mechnanisms similar to that in cottonwood.

As young leaves emerge from buds they are covered by fresh resin which dries at about the time the leaf is completely unrolled and nearly fully expanded. The resin forms a thin coat over the entire leaf and may have an effect similar to the cuticle in retarding transpiration from epidermal cells.

Leaf teeth can secrete more than one substance. The glandular tips produce resin, and nectar or water guttation occurs from stomata just below the tip. Previous workers were aware of one secretory product or the other, usually assuming that the fluid they observed came from the glandular

tip of the tooth. Only Edelstein (1902) has suggested that a hydathode occurs below each gland. Since I observed nectar guttation on only a few occasions, and even then only in the laboratory, it is not surprising that it has often been overlooked. Populus seems to have an unspecialized hydathode that can also be a nectary under certain conditions.

When Trelease (1881) studied Populus basal glands, which he called extra-floral nectaries, he did not report resin. He was convinced that nectar secretion occurred from the elongate epidermal cells at the tip of the gland and even described the process of nectar accumulation resulting in a distention and eventual disruption of the cuticle. Resin secretion is a common occurrence in most of the Populus species studied by Trelease. It seems unlikely that he would confuse resin with nectar since it is not sweet and has few of the properties associated with nectar, but this seems to have been the case. It is unfortunate that no indication is given in his paper of where and when his observations were made.

Trelease's description of the secretory epidermis of basal glands of P. tremuloides as a "double layer of thin walled elongated cells" also seems questionable. In P. deltoides and in a cursory examination of P. tremuloides, I found the secretory epidermis composed of only a single layer of elongate cells. In a few instances (Fig. 51, arrow),

secretory epidermal cells had undergone periclinal divisions, but this seems to be an unusual event.

Marcet (1961) studied leaf morphology of Populus deltoides clones from all parts of the species range, growing in the Populetum of the University of Wisconsin, Madison during May 13-15, 1959. She concluded that leaves of northern clones have few or no basal glands whereas southern clones usually average four or more basal glands per leaf. Such broad generalizations, however, should not be made from so few observations. Marcet may have made an unfortunate choice of collection dates. Her collections, considering the more northern latitude of Madison, are probably equivalent to mine of May 3. This would explain how she got an average of 0.1 basal glands per leaf on northern clones. Buds of southern clones may have broken dormancy earlier than those of northern clones and thereby have been at a later stage of seasonal development at the time Marcet made her collections. At later seasonal stages, emerging leaves have several basal glands. Marcet's hypothesis should be re-examined with these problems and questions in mind.

From the study of chronological changes in leaf and stipule secretory cell fine structure, I have derived a hypothesis on the mechanism of resin production. Pre-secretory epidermal cells (stages 1 and 2) contain relatively large numbers of active dictyosomes which are probably involved in wall formation since the cells are enlarging

during this period. When the cuticle becomes detached from the cell wall (the first sign of resin secretion), dictyosomes become more numerous and more active, producing many vesicles that are probably filled with a carbohydrate precursor of resin.

These vesicles migrate to the plasmalemma and fuse with it, releasing their contents to the outside, causing the plasmalemma to appear invaginated. This activity would seem to be related to cell wall synthesis. However, the material in the invaginations appears structurally different from wall material. Thickening of cell walls at this stage may represent only a loosening of wall components, which would also help to account for the spaces and channels that form in the wall. Eventually, during stage 4, fusion of vesicles stops and the plasmalemma regains its smooth outline. This is accomplished by the deposition of excess plasmalemma membrane in myelin-like piles.

The outer mesophyll cells also play an important role in resin production. Before resin secretion begins, lipids accumulate in these cells. As the lipids or their derivatives migrate from the subepidermal mesophyll cells, they cause the middle lamella to separate. The material migrates through the resulting intercellular spaces and channels to the cuticle, below which it accumulates. Some lipids from mesophyll cells may also migrate into secretory epidermal

cells, causing the increased number of osmiophilic (lipid) bodies found in stage 5. Mixing of resin precursors, including lipids, occurs below the cuticle and a milky, yellow-white resin results. The fact that the resin clears and eventually hardens upon exposure to air may indicate that it is a combination of several different compounds.

Mitochondrial degeneration in stages 4 and 5 suggests that the energy-consuming metabolic process involved in the production and secretion of resin precursors is over. I feel this hypothesis gives reasonable explanation for most of the phenomena observed at the fine structure level.

Indirect evidence that lipids are resin precursors in Populus deltoides is furnished by Wollenweber and Egger (1971) and Wollenweber and Weber (1973), who analyzed Populus nigra resin and found that it contains many substances, including lipid derivatives.

The only previous fine structure study of resin-secreting cells is on the resin ducts of Pinus pinea (Wooding and Northcote, 1965). They reported that lipid synthesis occurs in plastids with little internal structure that are enveloped by endoplasmic reticulum. The lipids are speculated to be resin precursors. Dictyosomes, according to them, seem to play no role in resin synthesis.

Because resin production in Populus and Pinus seems dependent upon secretion of lipids or lipid derivitives, it seemed likely that oil secreting structures might have similar

systems. In several rather cursory studies of the fine structure of oil glands of several species, Schnepf has described lipid-producing leucoplasts enveloped by endoplasmic reticulum in secretory cells: Arctium lappa (1969a), Calceolaria (1969b), Solidago canadensis (1969c), and Heracleum sphondylium and Dorema ammoniacum (1969d). In the latter two species he suggested that dictyosomes also play an active role in producing the secretory product.

Amelunxen (1965) studied fine structure of Mentha piperita glands and suggested that dictyosomes were active in secretion. Because of the poor quality of his micrographs, however, I am unconvinced that dictyosomes are involved.

The fine structural changes seen in Populus deltoides secretory cells and my interpretation of the pathway of resin secretion appear to be unlike any previously described plant secretory system.

My findings on the gross morphology of secretory structures confirm what little has been previously reported. However, much greater seasonal variation occurs than previously suggested. I find that guttation and resin secretion both occur from leaf teeth as previously reported. However, resin secretion occurs from the glandular tips of teeth whereas guttation occurs from unspecialized hydathodes proximal to the glands. I have described the fine structure

of the resin secreting system and have hypothesized a mechanism of resin production. Resin in Populus deltoides seems to act as an insect repellent and probably also lowers transpiration rates of young, expanding leaves.

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APPENDIX A: TABLE

Table 1. Structural changes in secretory cells

Structure	Stage 1	Stage 2	Stage 3	Stage 4	Stage 5
Epidermal cells					
Endoplasmic reticulum	some, rough	*		increases	
Ribosomes	many				
Plastids	lack grana and stroma contain osmiophilic bodies stain darkly up to 1 µm long		large increase in number some contain starch many elongate up to 2.5 µm contain osmiophilic bodies	no starch still elongate stain lighter contain osmiophilic bodies	
Mitochondria	up to 1 µm long		large increase in number up to 4 µm long	some begin to degenerate	most are 1 µm long most are in some stage of degeneration
Dictyosomes	a few present actively producing vesicles		increased number corresponding to cell enlargement large numbers of vesicles in cytoplasm		

*Blanks indicate no change from previous stage.

Table 1. (continued)

Structure	Stage 1	Stage 2	Stage 3	Stage 4	Stage 5
Epidermal cells					
Osmiophilic bodies	few present		some present	many present osmiophilic material removed from some	
Plasmalemma	smooth		has many invaginations	smooth with black clumps of membranes	
Vacuoles	many per cell up to 3 µm in diameter	more, but only 1 µm in diameter			(late) many up to 3.5 µm in diameter
Nucleus	about 4 µm in diameter				
Nucleolus	about 2 µm in diameter darkly stained			absent or lightly stained	
Cuticle	attached to cell wall		detached from cell wall		
Cell wall	outer 0.25 µm thick others 0.1 µm thick		outer 0.3-1.2 µm thick others 0.25 µm thick spaces and channels form		

Table 1. (continued)

Structure	Stage 1	Stage 2	Stage 3	Stage 4	Stage 5
Outer Mesophyll	small amounts of osmiophilic material on margin of central vacuole	increase in osmiophilic material	some vacuoles filled with osmiophilic material	decrease in osmiophilic material	vacuoles often lack osmiophilic material but contain myelin-like material

APPENDIX B: FIGURES

Figure Label Abbreviations

Light microscopy

C	cuticle	Phaco	phase contrast
E	secretory epidermis	BF	bright field
F	fibers		
P	periderm		
R	resin		
SS	schizogenous space		

Electron microscopy

C	cuticle
D	dictyosome
ER	endoplasmic reticulum
I	plasmalemma invagination
M	mitochondria
N	nucleus
Nu	nucleolus
O	osmiophilic material or body
P	plastid
S	space or channel in cell wall
V	vacuole
Ve	vesicle
W	cell wall

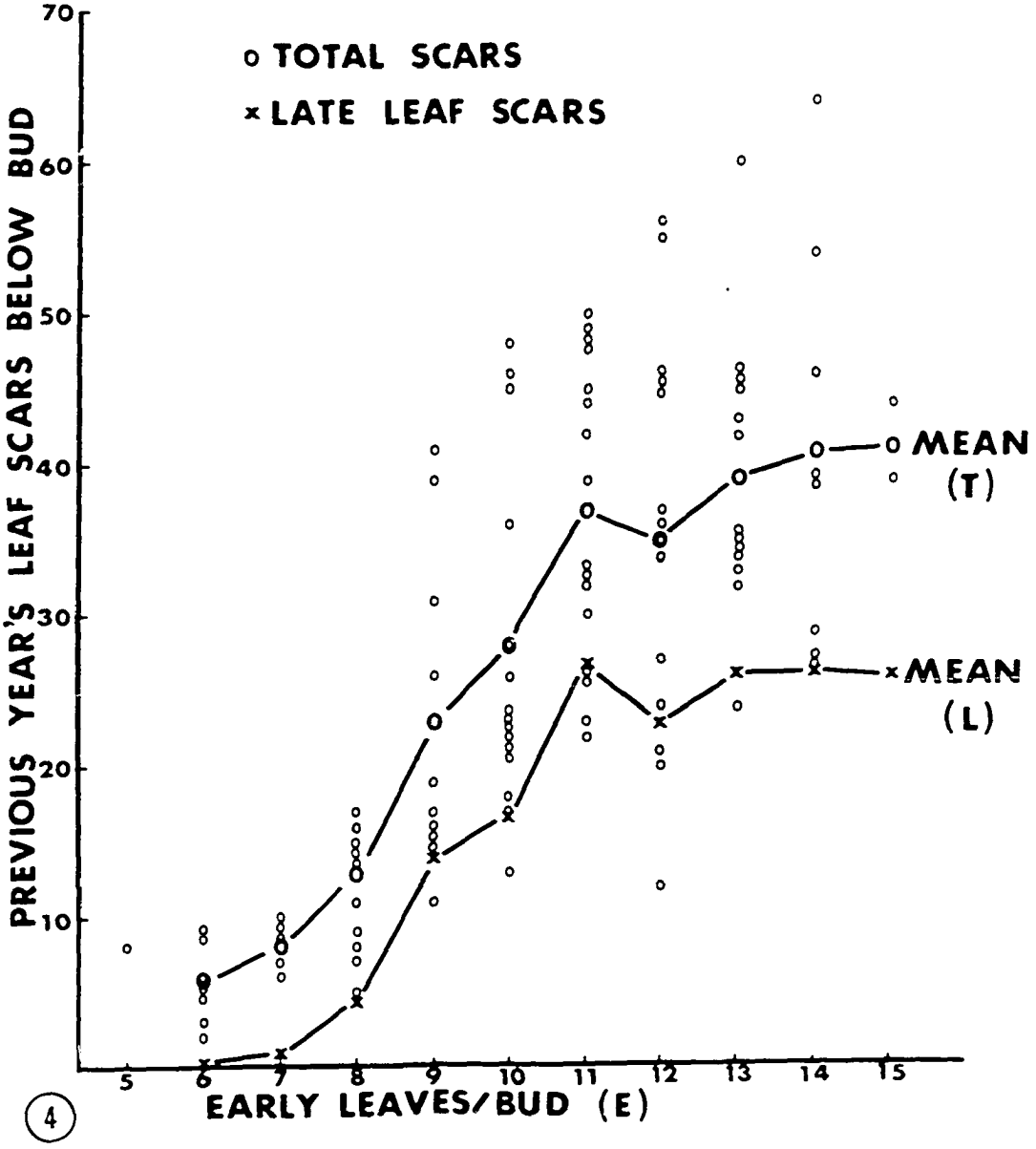
Fig. 1. Overwintering terminal bud (A) with dissected components (B-T) in order from outside to center. Line represents 5 mm.

Fig. 2. Overwintering axillary bud (A) with dissected components (B-L) in order from outside to center. Line represents 5 mm.

Fig. 3. Cross section of overwintering terminal bud at level of dashed line across Fig. 1A with leaves (I-O) labeled corresponding to leaves of Fig. 1. Heavy black lines represent collapsed adaxial epidermis of stipular bud scales. Line represents 0.5 mm.

A
B
C
D
E
F
G
H
I
J
K
L
PRIMORDIA
1
2
3
PRIMORDIA
M
J
L
N
O
K
I

Fig. 4. Relation between numbers of living leaves in overwintering buds (early leaves) (E) and the number of leaf scars on the previous year's growth below each bud (T). Late leaf scar numbers (L) were estimated by subtracting early leaf number (E) (counted in overwintering buds) from the average number of leaf scars per bud (T).


o TOTAL SCARS
× LATE LEAF SCARS
PREVIOUS YEAR'S LEAF SCARS BELOW BUD
70
60
50
40
30
20
10
5
6
7
8
9
10
11
12
13
14
15
EARLY LEAVES/BUD (E)
o MEAN (T)
× MEAN (L)

(4)

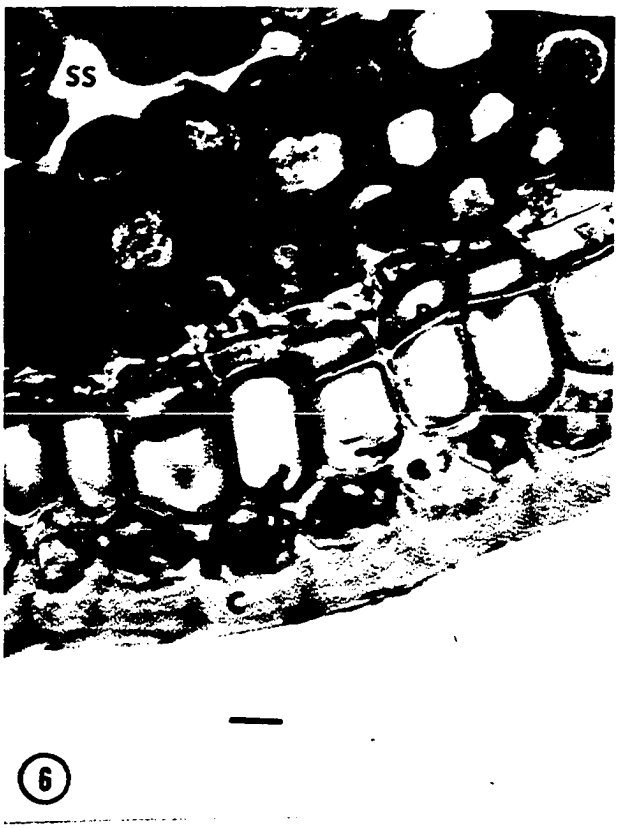
Fig. 5-7. Cross sections of stipular bud scales.

Fig. 5. BF. Winter bud scale with thick abaxial cuticle (C), schizogenous spaces (SS) and fibers (F). Line represents 10 µm.

Fig. 6. BF. Winter bud scale with thick abaxial cuticle (C), schizogenous space (SS) and periderm (P). Line represents 10 µm.

Fig. 7. Phaco. Fall bud stipule before collapse of adaxial secretory epidermis (E) equivalent to J. of Fig. 1. Line represents 10 µm.

5



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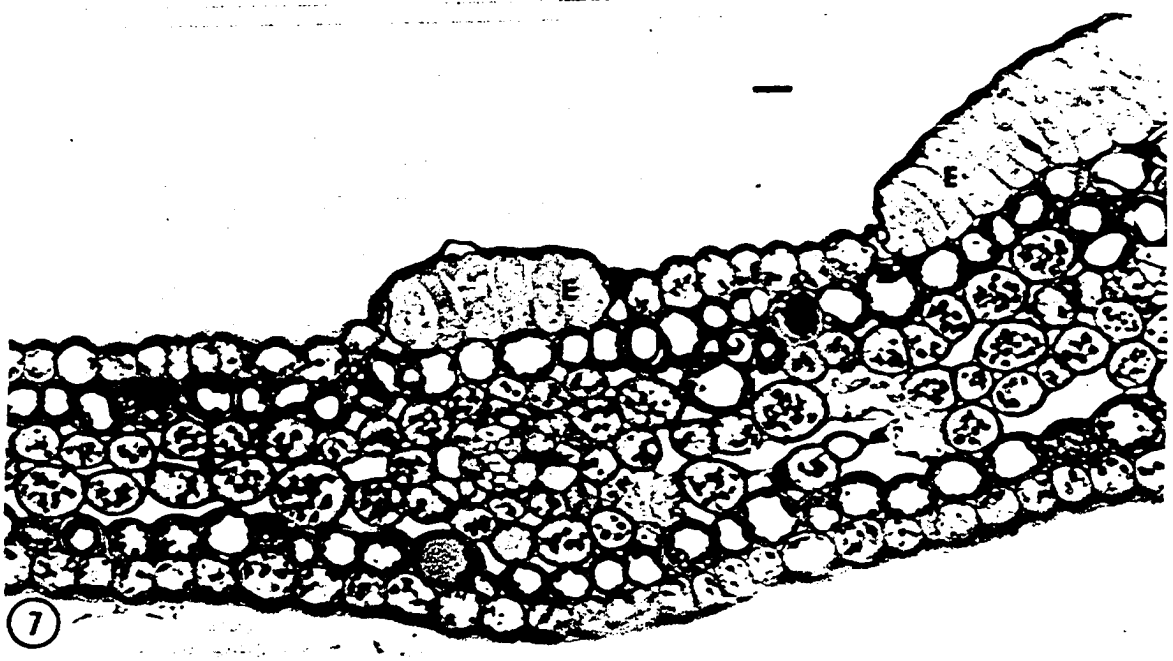


7

Fig. 8. BF. Cross section of a portion of an overwintering bud equivalent to the area around leaf M in Fig. 3. Schizogenous spaces (SS) and collapsed adaxial secretory epidermis (E). Line represents 50 μm.

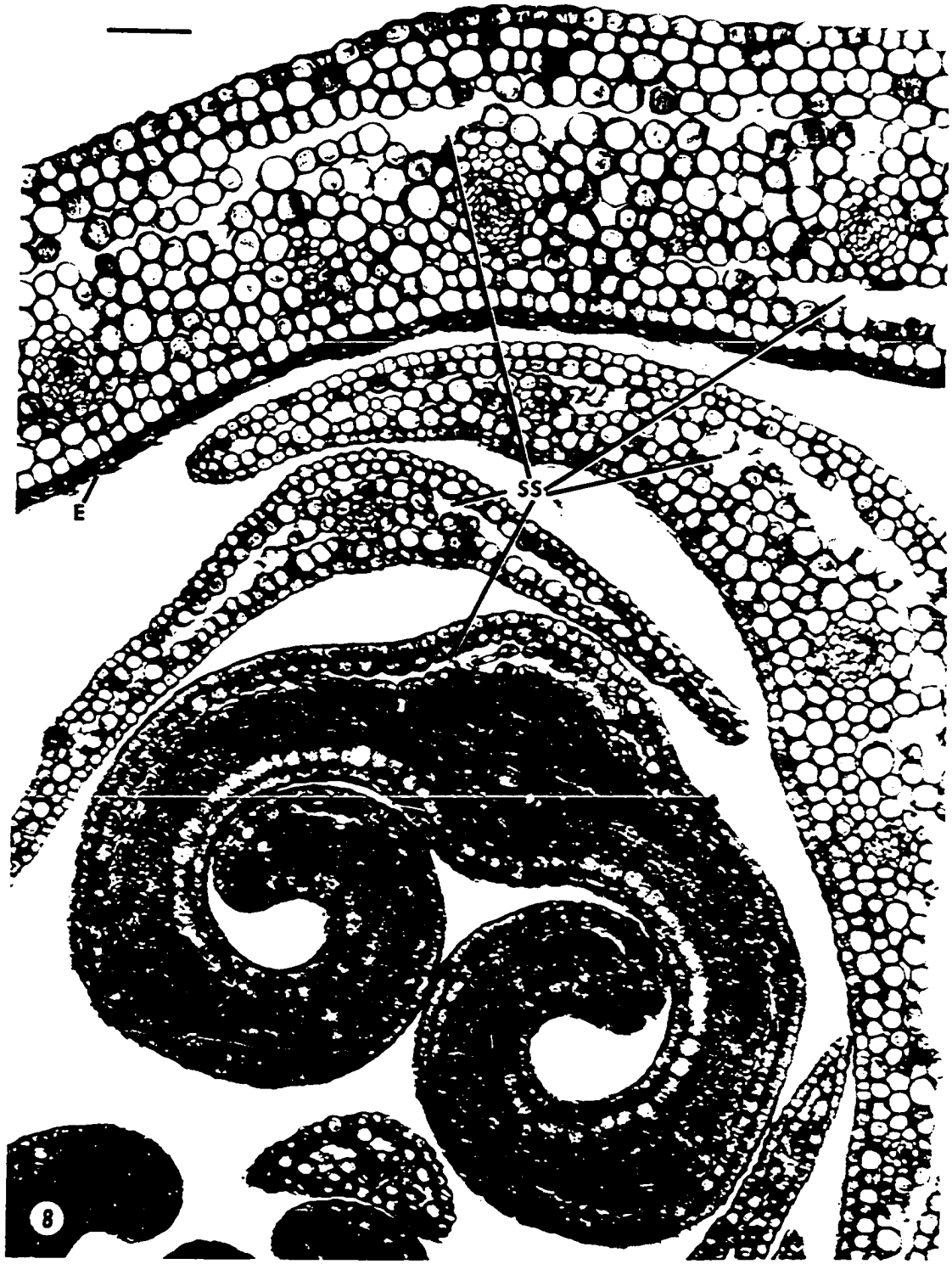

E
SS
8

Fig. 9-14. BF. Adaxial surfaces of secretory stipules.

Fig. 9. Stipule of early spring bud just after bud breaks dormancy, equivalent to I in Fig. 1. Note small ridges of secretory epidermis at base. Line represents 1 mm.

Fig. 10. Stipule of early spring bud just after bud breaks dormancy, equivalent to K in Fig. 1. Line represents 1 mm.

Fig. 11. Early leaf stipule several weeks after overwintering buds break dormancy, equivalent to later developmental stages of M-Q of Fig. 1. Line represents 1 mm.

Fig. 12. One of last early leaf stipules to emerge, equivalent to R, S or T of Fig. 1. Note resin (R). Line represents 1 mm.

Fig. 13. Same stipule as Fig. 12 with resin removed to expose secretory surface. Line represents 1 mm.

Fig. 14. Late leaf stipules with pronounced ridges, some of which end in finger-like projections (arrows). Line represents 1 mm.

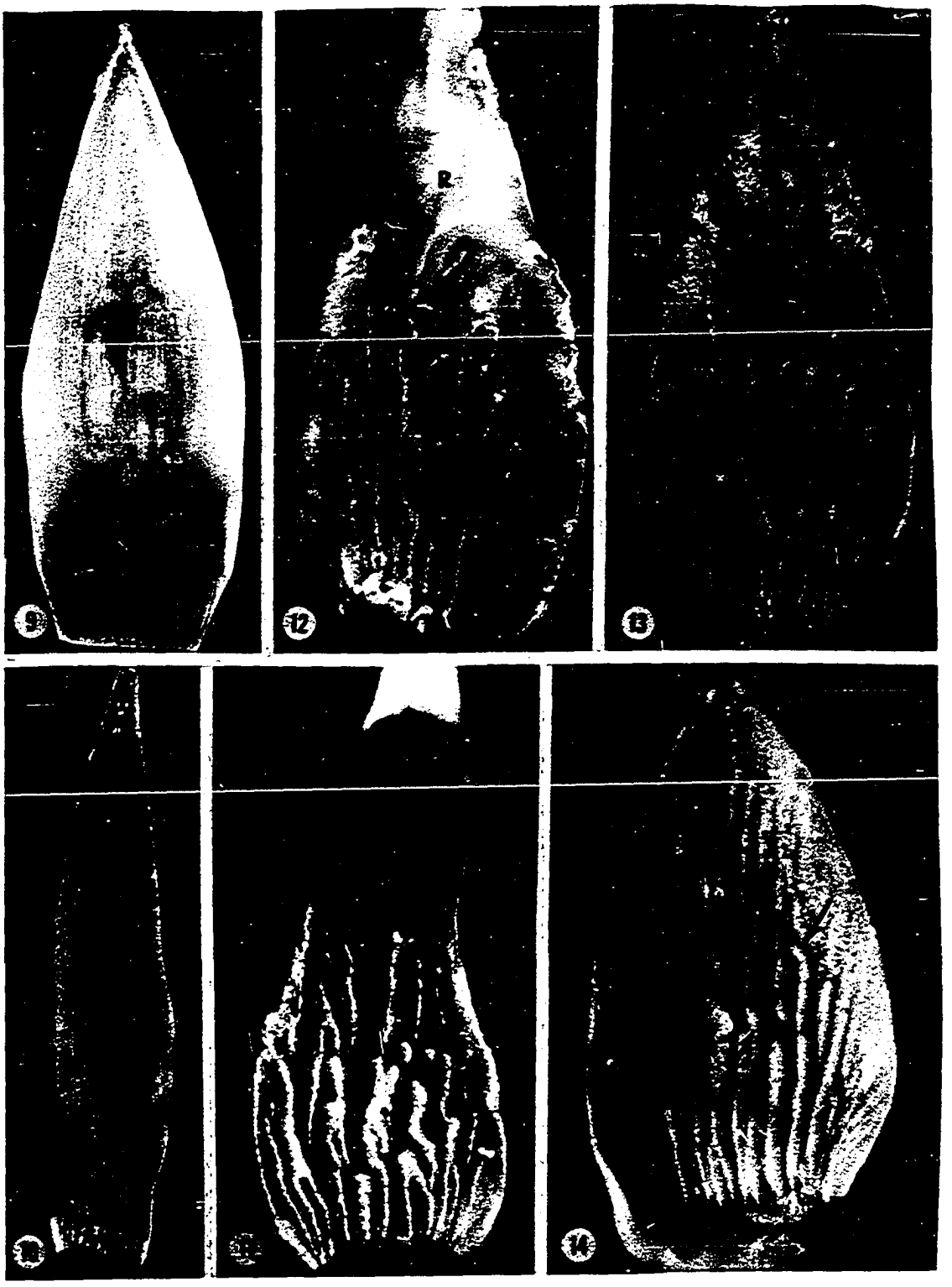

R
9
12
13
10
11
14

Fig. 15. Resin (R) covered active bud of late summer.

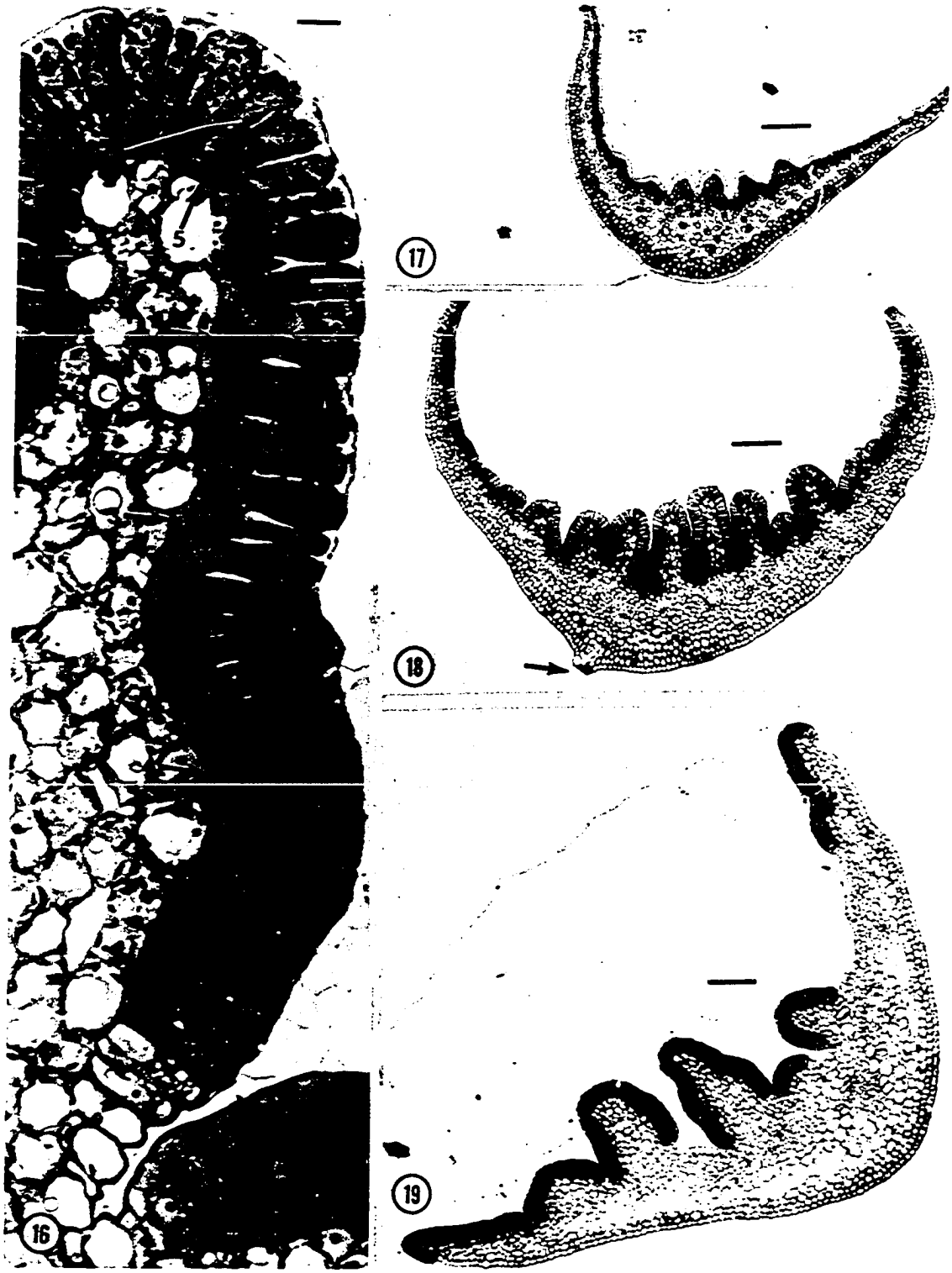

R
15

Fig. 16. Phaco. Cross section of adaxial ridge of stipule in Fig. 19. Areas of adaxial epidermis are numbered 3-5 according to their stage of development (see electron microscope observations). Line represents 10 µm.

Fig. 17. BF. Cross section of young late leaf stipule. Line represents 100 µm.

Fig. 18. BF. Cross section of late leaf stipules with secretory epidermis. Arrow indicates raised abaxial stomate. Line represents 100 µm.

Fig. 19. BF. Cross section of large stipule, equivalent to Fig. 11. Note secretory epidermis extends around stipule margin. Line represents 100 µm.


5
16
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18
19

Fig. 20. Phaco. Cross section near margin of stipules in Fig. 17. Cells of adaxial secretory epidermal layer (arrow) are at stage 1 of development. Line represents 10 µm.

Fig. 21. Phaco. Cross section of young late leaf stipule. Cells of secretory epidermis are at developmental stages 1 and 2 (arrows). Line represents 20 µm.

Fig. 22. Phaco. Cross section of old stipule. Cells of secretory epidermis are at developmental stage 5 (arrow). Line represents 10 µm.

Fig. 23. Phaco. Cross section of stipules in Fig. 18. Cells of secretory epidermis at developmental stages 1 through 3 (arrows). Line represents 10 µm.

Fig. 24. BF. Stipule cross section showing mesophyll in ridge. Osmiophilic material appears black. Line represents 10 µm.

Fig. 25. Phaco. Same as Fig. 24. Osmiophilic material appears clear.

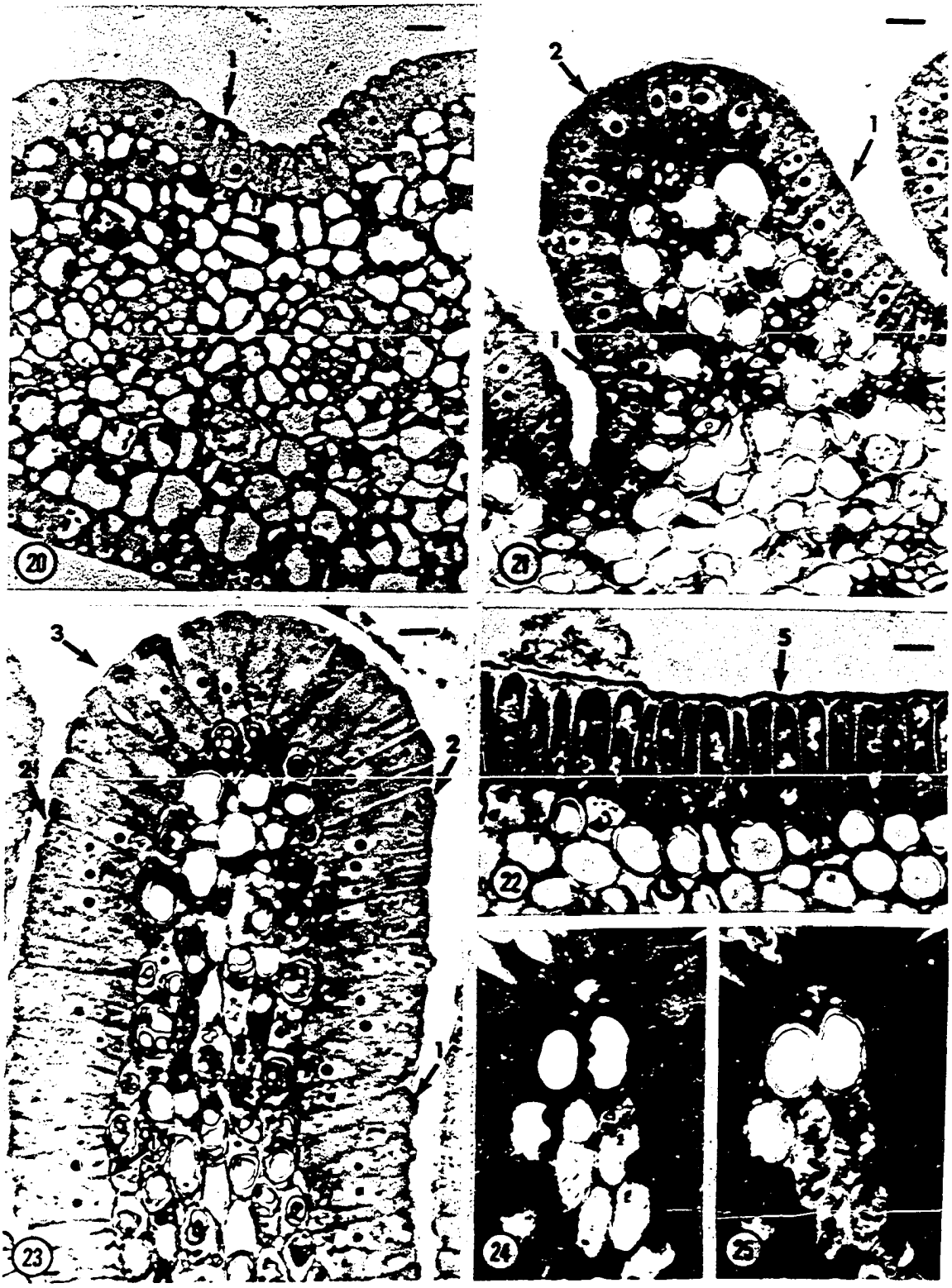
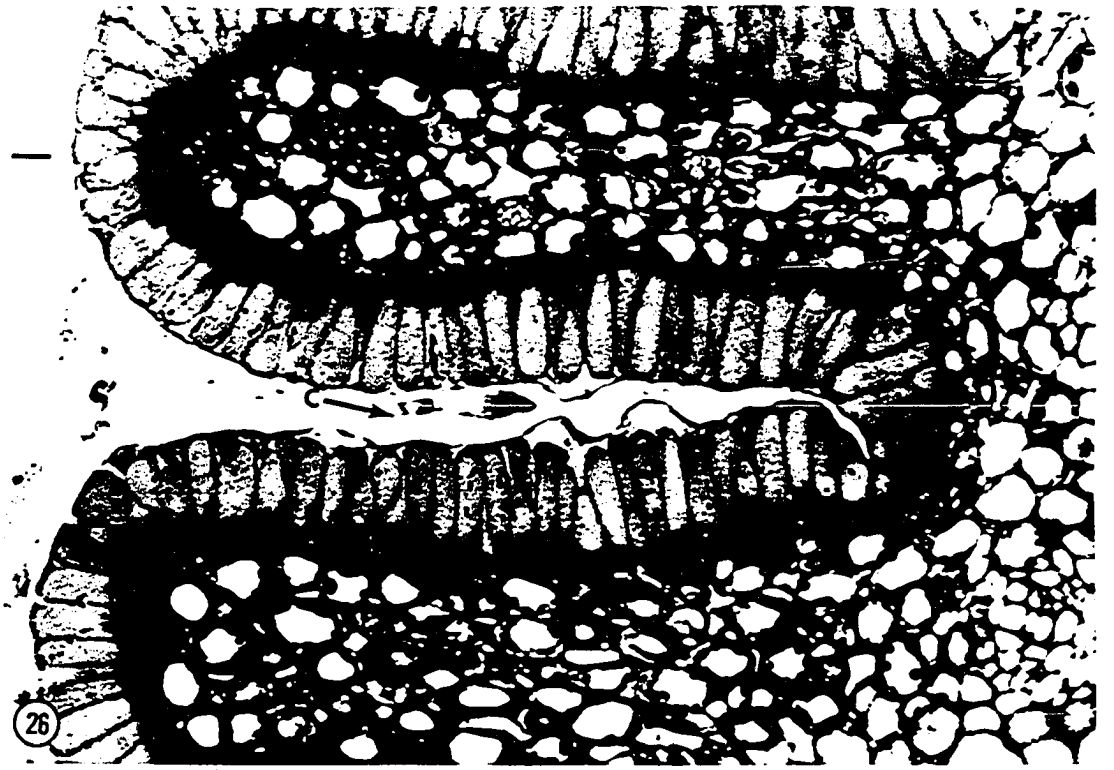

1
20
2
1
1
21
3
2
2
1
23
5
22
24
25

Fig. 26. BF. Stipule cross section with cuticle (C) detached from cell wall. Line represents 10 µm.

Fig. 27. Phaco. Stipule cross section with cuticle (C) detached from cell wall. Most epidermal cells appear to be at stage 5 even though few osmiophilic bodies are present. Line represents 10 µm.



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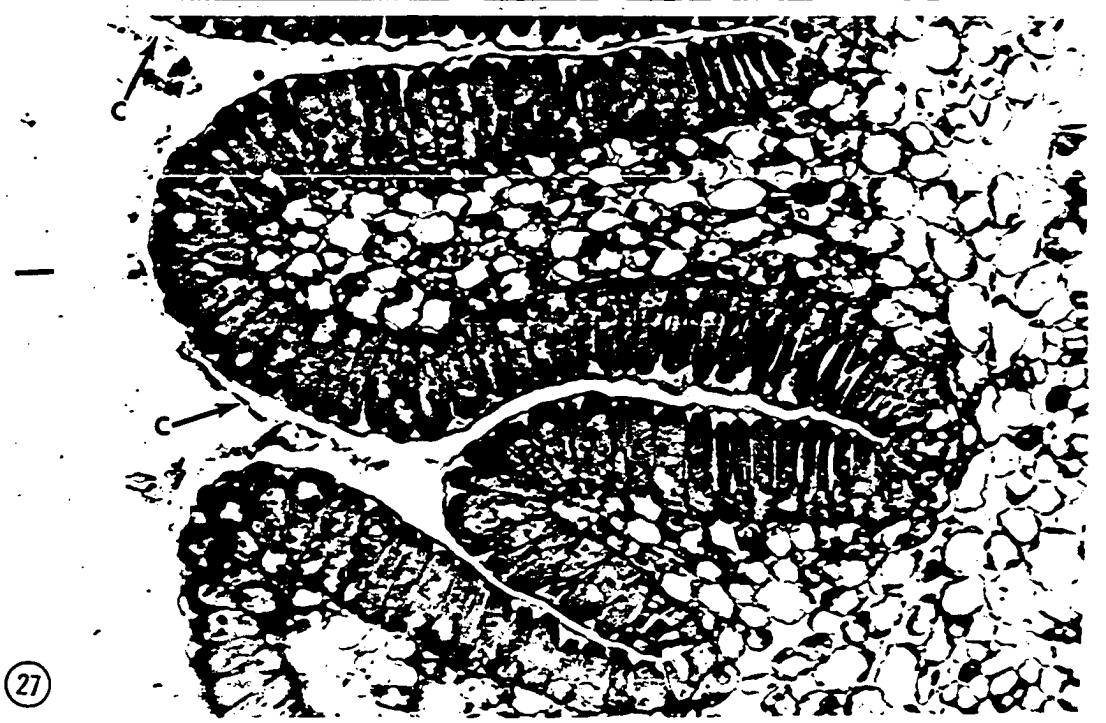


27

Fig. 28. Scanning electron micrograph of adaxial surface of late leaf stipule with pronounced ridges. Line represents 0.5 mm.


28

Fig. 29. Scanning electron micrograph of adaxial surface of late leaf stipule with pronounced ridges. Line represents 0.5 mm.

29

Fig. 30. Scanning electron micrograph of abaxial surface of late leaf stipule showing position of raised stomata. Line represents 0.5 mm.

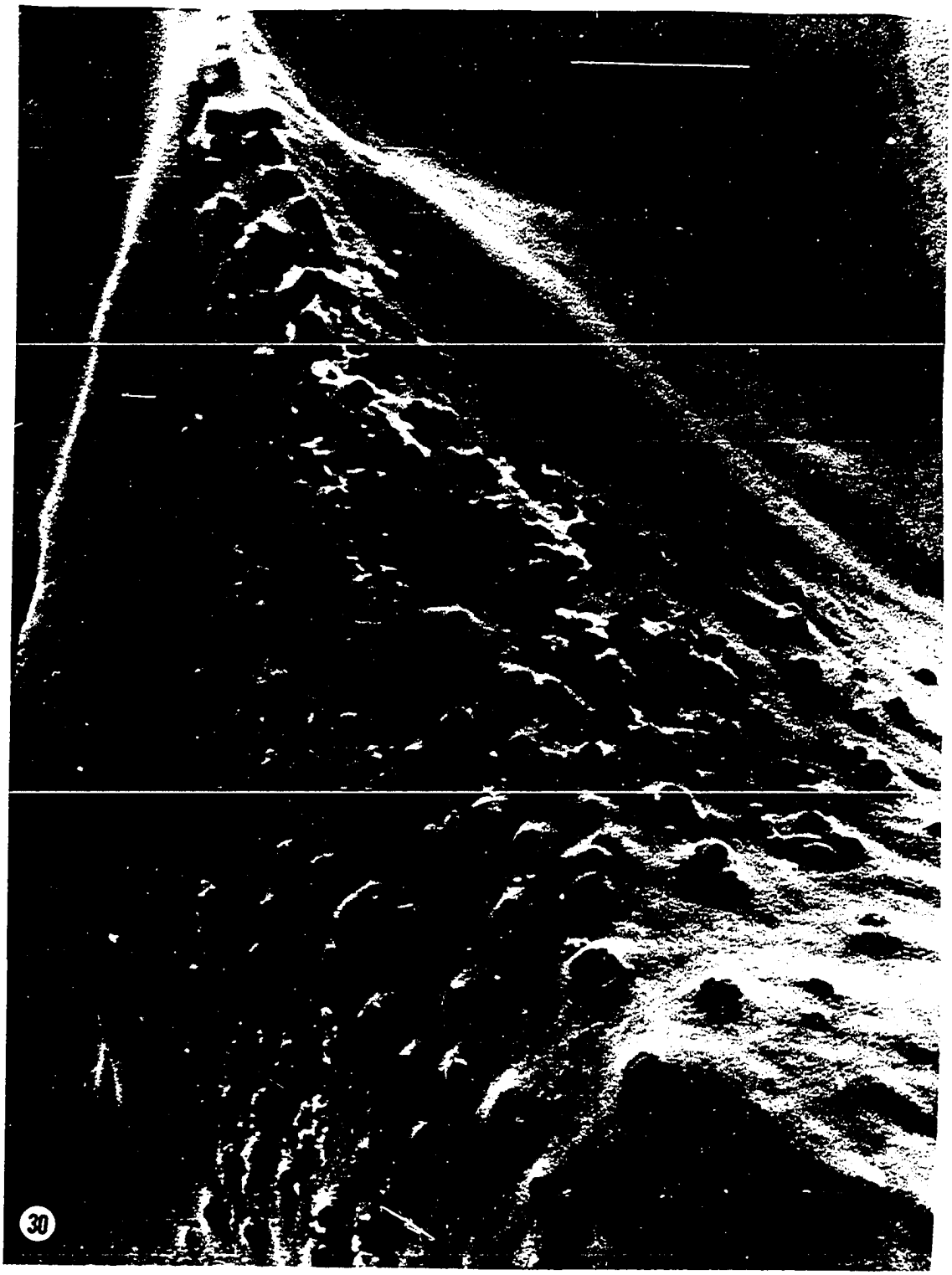

30

Fig. 31. Clearing of early leaf with large marginal glands. Line represents 1 mm.

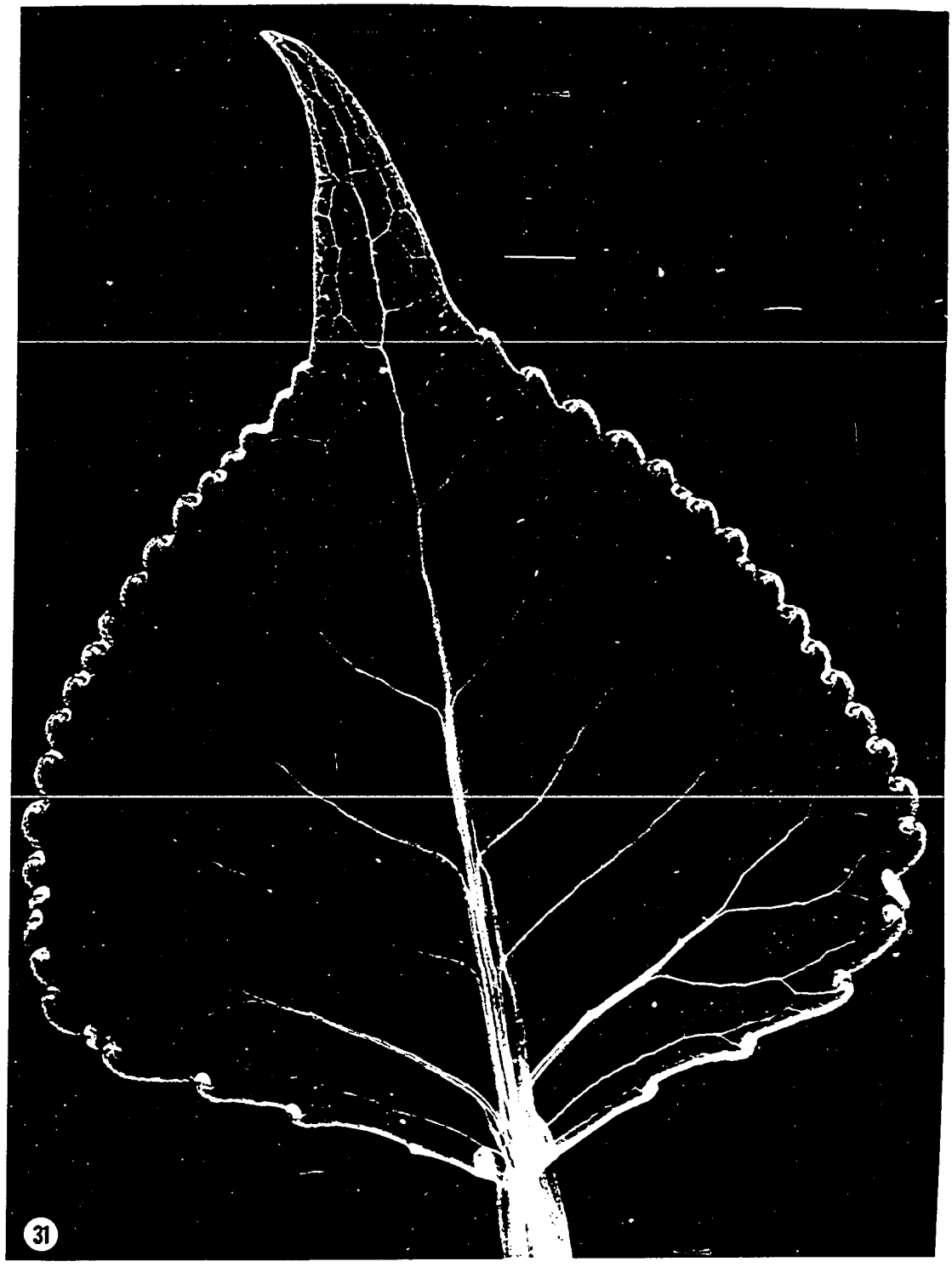

31

Fig. 32. Enlarged portion of leaf similar to that in Fig. 31. Line represents 0.5 mm.

Fig. 33. Margin of young, cleared late leaf. Note that teeth are usually two veined and marginal trichomes are fewer than in early leaf (Fig. 32). Line represents 0.5 mm.

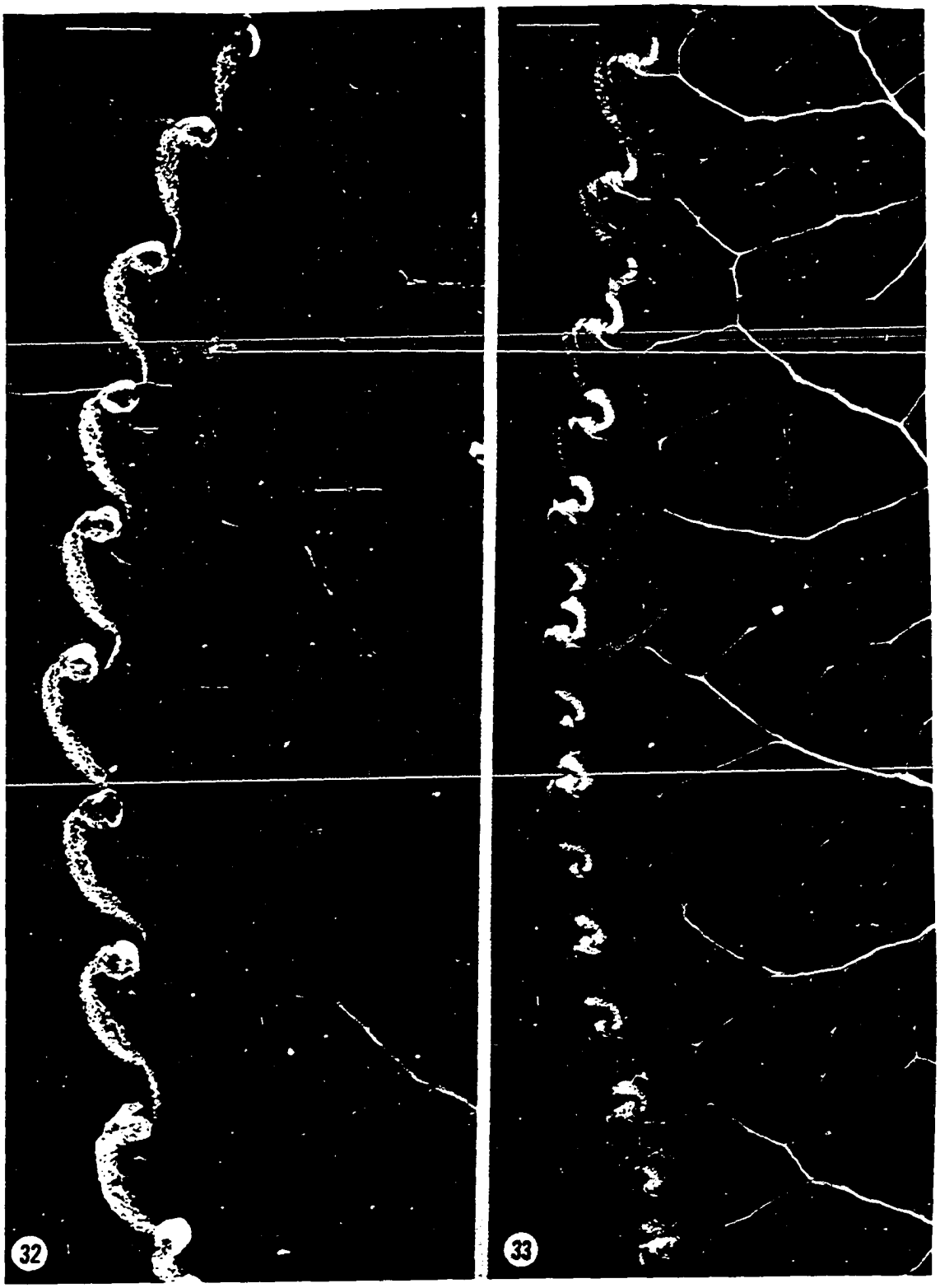

32
33

Fig. 34. Cleared eglandular tooth of first emerging early leaf. Line represents 100 µm.

Fig. 35. Cleared early leaf tooth with small secretory gland. Line represents 100 µm.

Fig. 36. Paradermal 1 µm thick section of leaf tooth similar to that in Fig. 35. Line represents 100 µm.

Fig. 37. Cleared leaf tooth with fused vascular bundle endings. On one of last early leaves or first late leaves to emerge from bud. Line represents 100 µm.

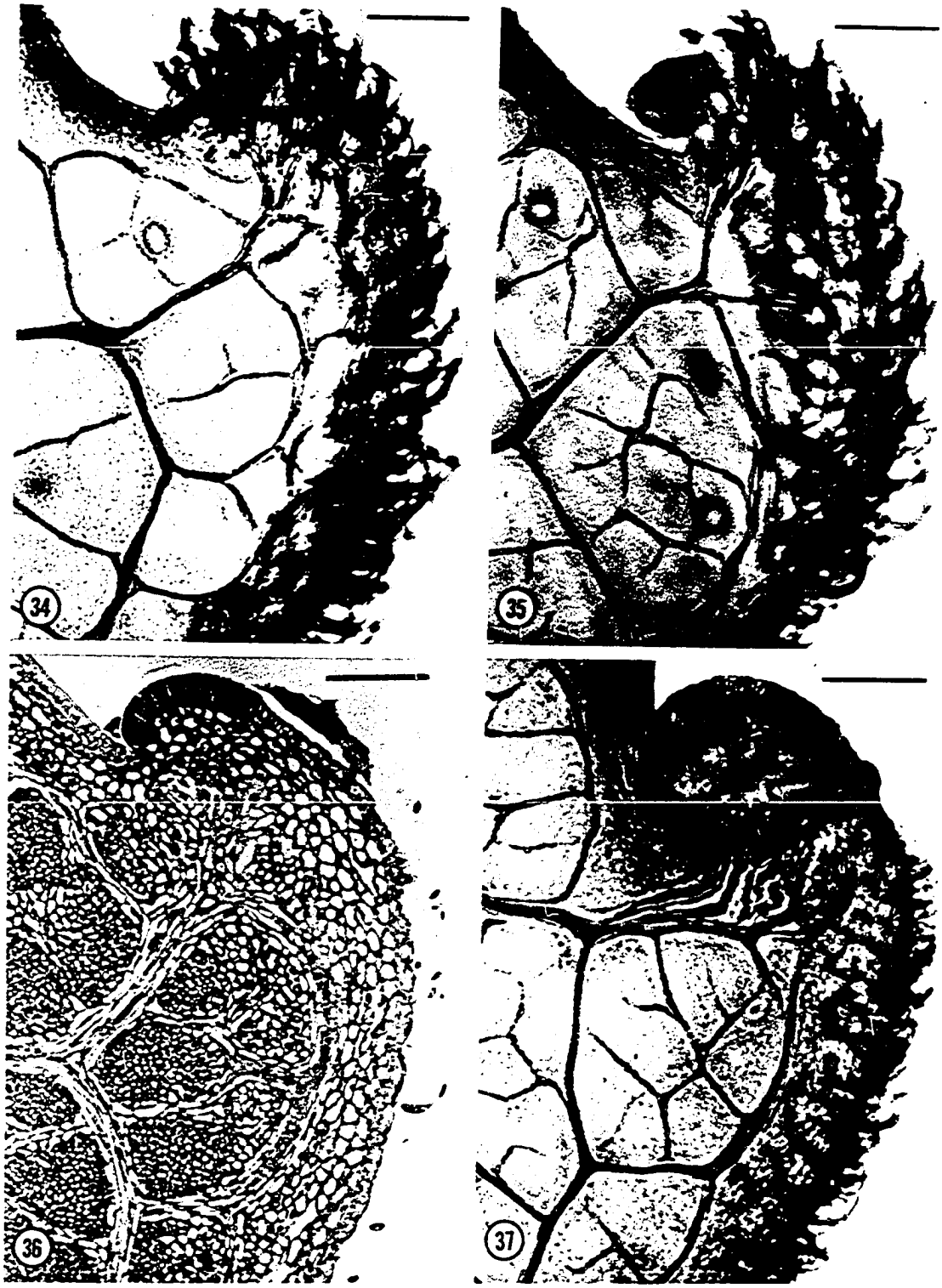

34
35
36
37

Fig. 38. Optical median plane in clearing of late leaf tooth with separate bundle endings. Arrow indicates same position as arrow on Fig. 39. Line represents 100 µm.

Fig. 39. Same tooth as Fig. 38 but focused on abaxial epidermis. Arrow indicates hydathode area above outer vascular bundle ending--same position as arrowhead in Fig. 38. Diagonal line represents plane of section in Fig. 43. Line represents 100 µm.

Fig. 40. Paradermal section of late leaf tooth. Arrow indicates vascular bundle ending below hydathode. Line represents 100 µm.

Fig. 41. Leaf tooth with newly secreted resin.

Fig. 42. Leaf tooth covered by drop of nectar.

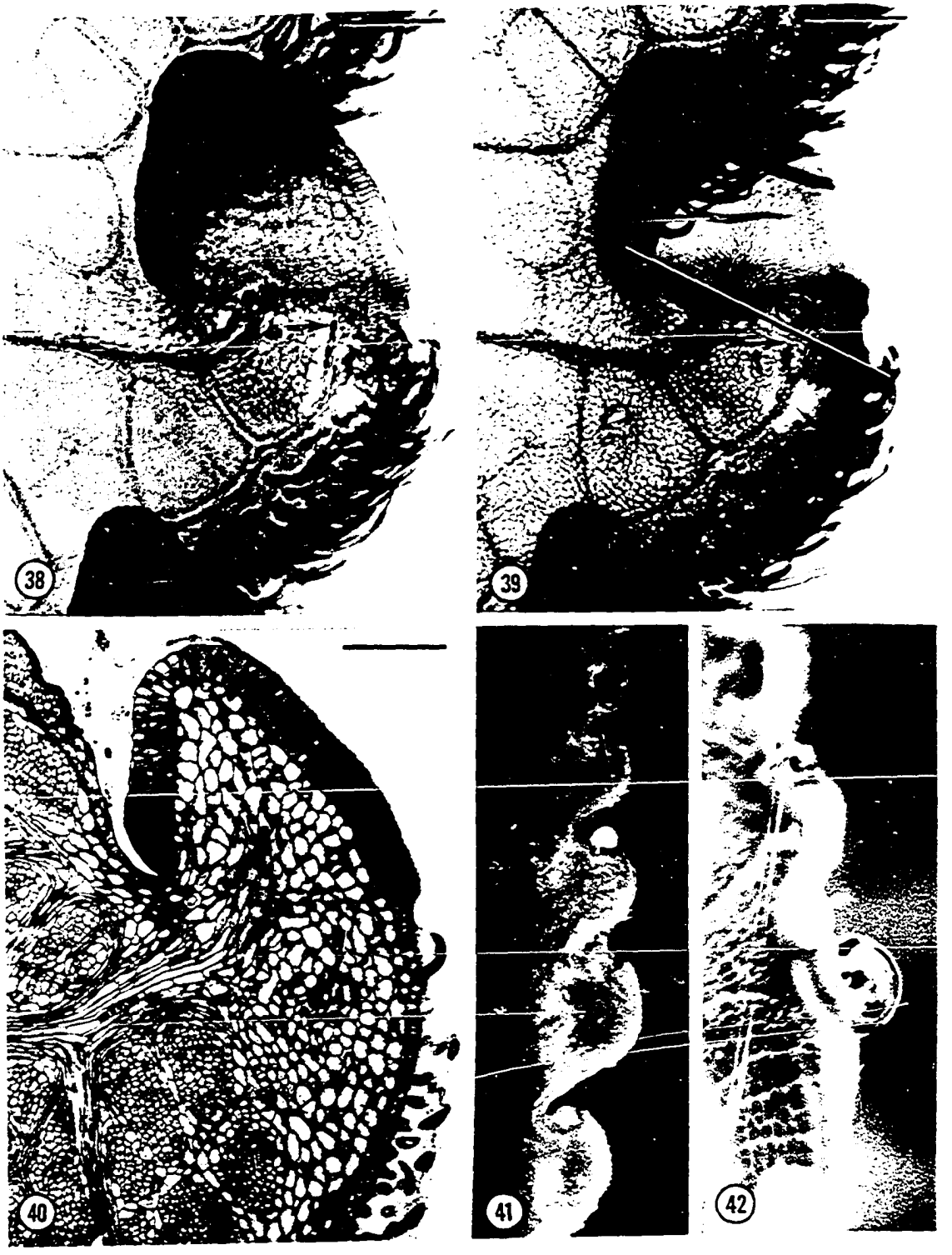

38
39
40
41
42

Fig. 43. Leaf cross section through hydathode area in plane indicated by line in Fig. 39. Line represents 100 µm.

Fig. 44. Leaf cross section through hydathode area in plane parallel to that of Fig. 43 but further from gland. Line represents 100 µm.

Fig. 45. Early leaf recently emerged from bud. Top arrow indicates elongated leaf tip; bottom arrow indicates resin secreting basal glands.

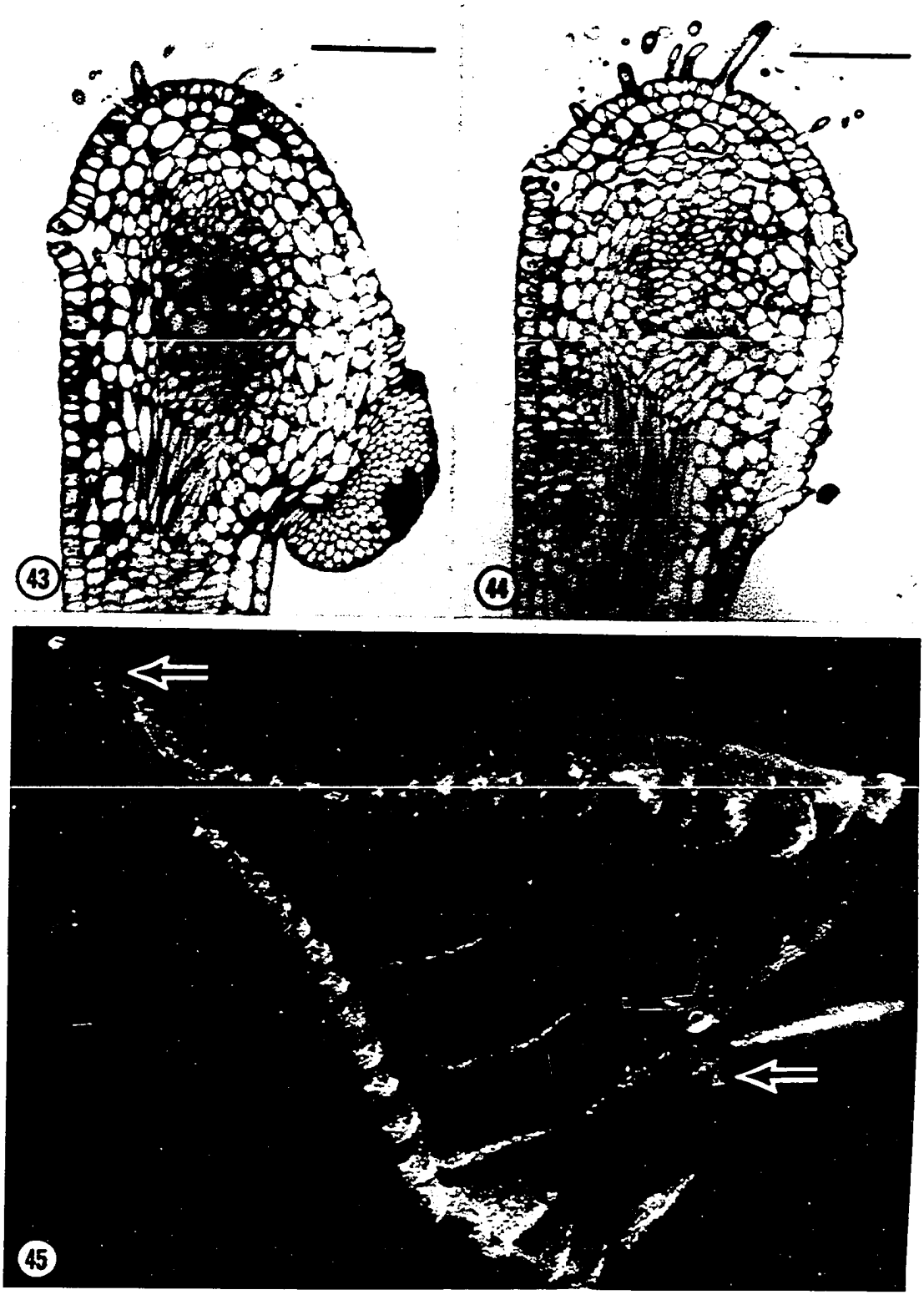

43
44
45

Fig. 46. Clearing of leaf tip showing many raised stomata. Line represents 100 µm.

Fig. 47. Cleared young leaf with large tip. Line represents 1 mm.

Fig. 48. Same leaf as Fig. 47 before clearing. Note resin (arrow).

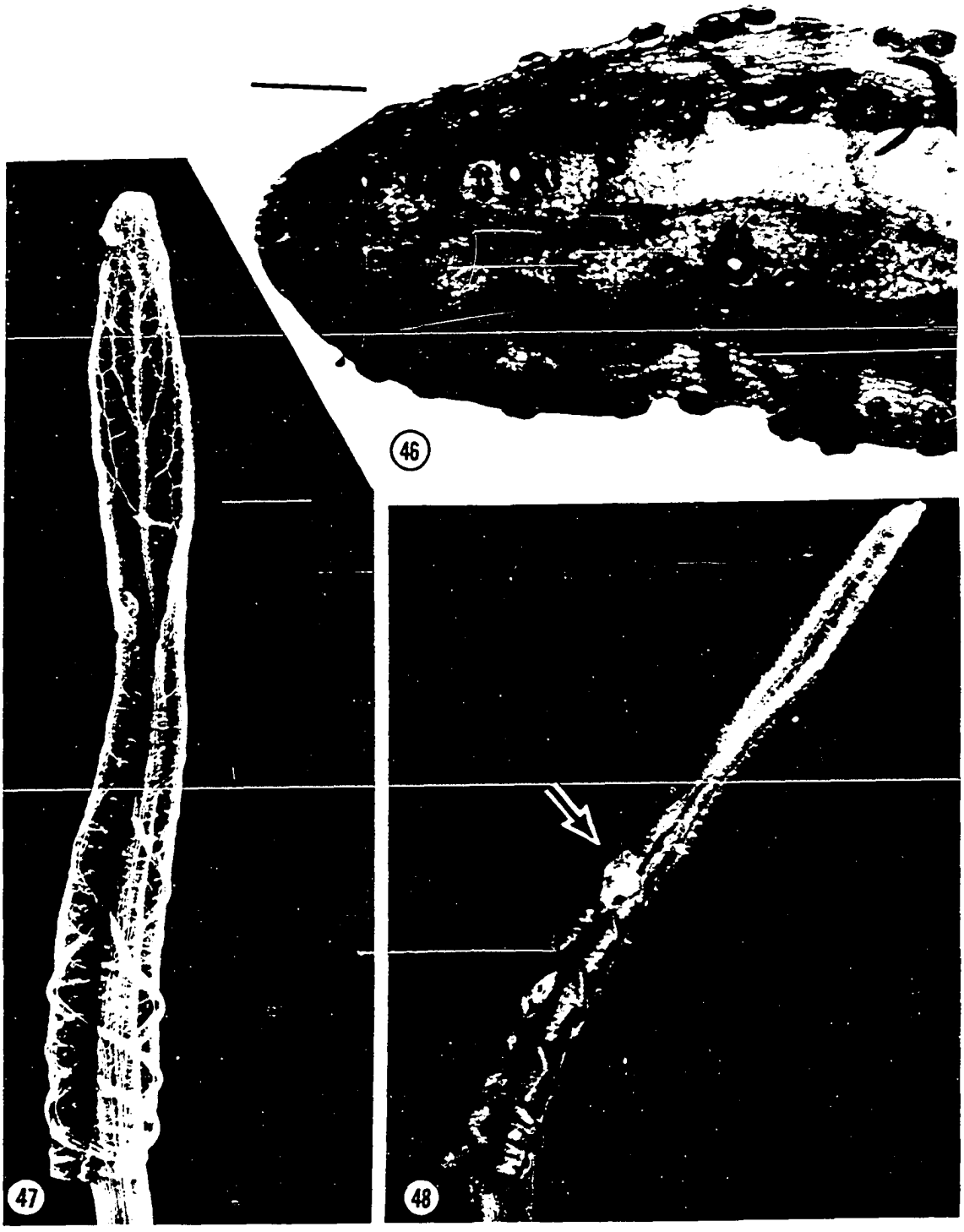

46
47
48

Fig. 49. Cleared basal gland of young early leaf. White line represents plane of section of Fig. 51 and 52. Line scale represents 100 µm.

Fig. 50. Cleared basal gland of young early leaf showing two stomata. Line represents 100 µm.

Fig. 51. Longisection of basal gland of young early leaf in plane indicated by white line of Fig. 49. Arrows indicate epidermal cells with periclinal walls. Line represents 100 µm.

Fig. 52. Same as Fig. 51.

Fig. 53. Resin secreting basal glands of an early leaf.


49
50

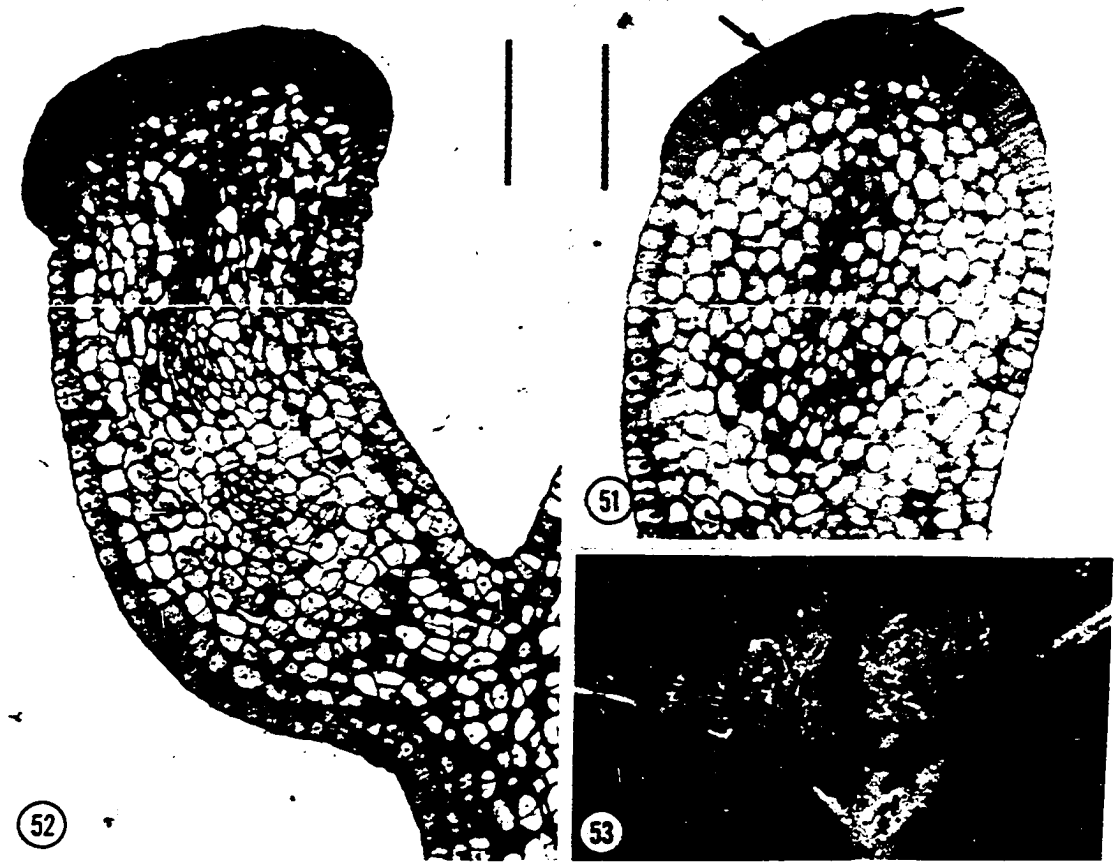

51
52
53

Fig. 54. Cleared basal glands of young late leaf, equivalent to B, Fig. 56. Line represents 100 µm.

Fig. 55. Scanning electron micrograph of late leaf fully enlarged basal glands--typical of late spring. Line represents 100 µm.


54

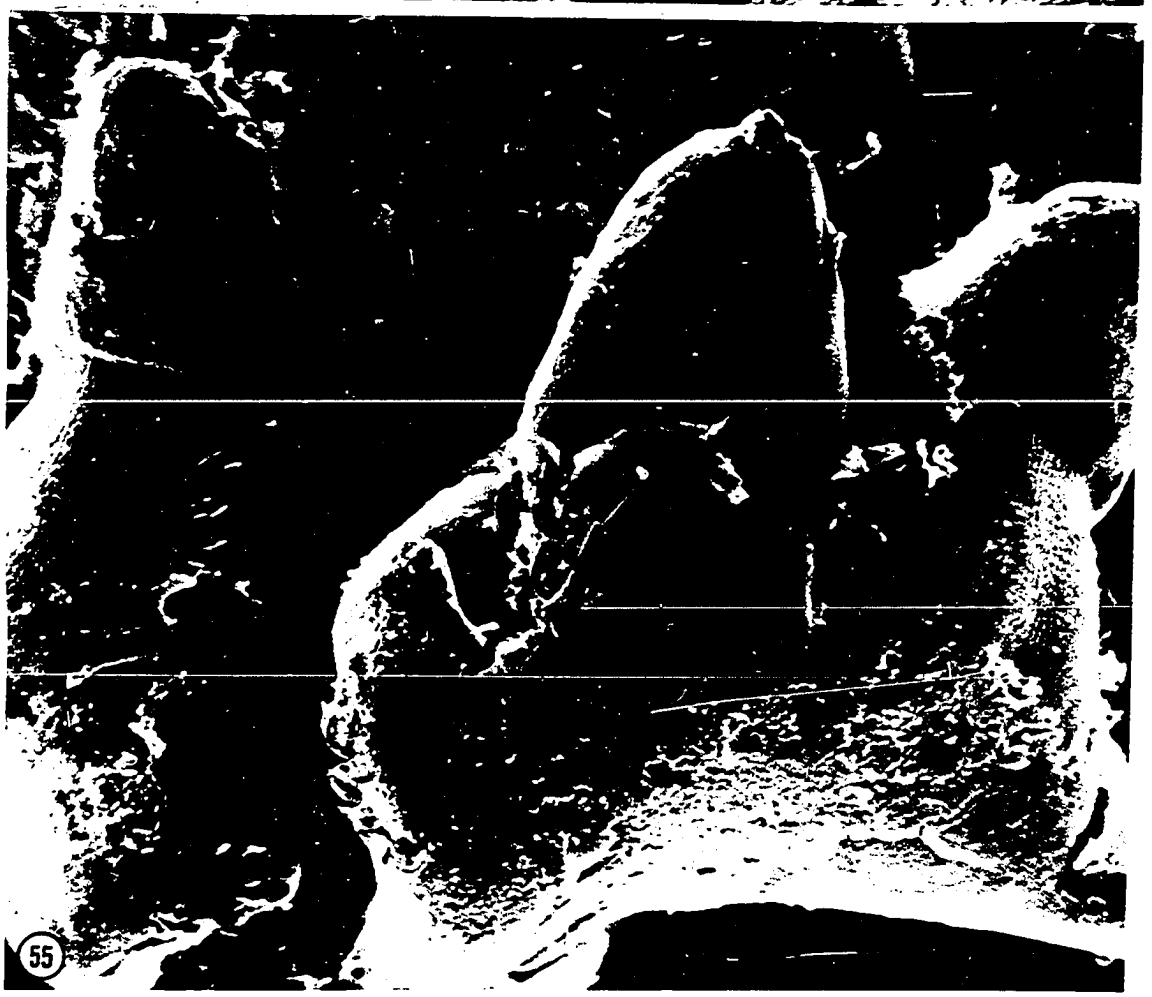
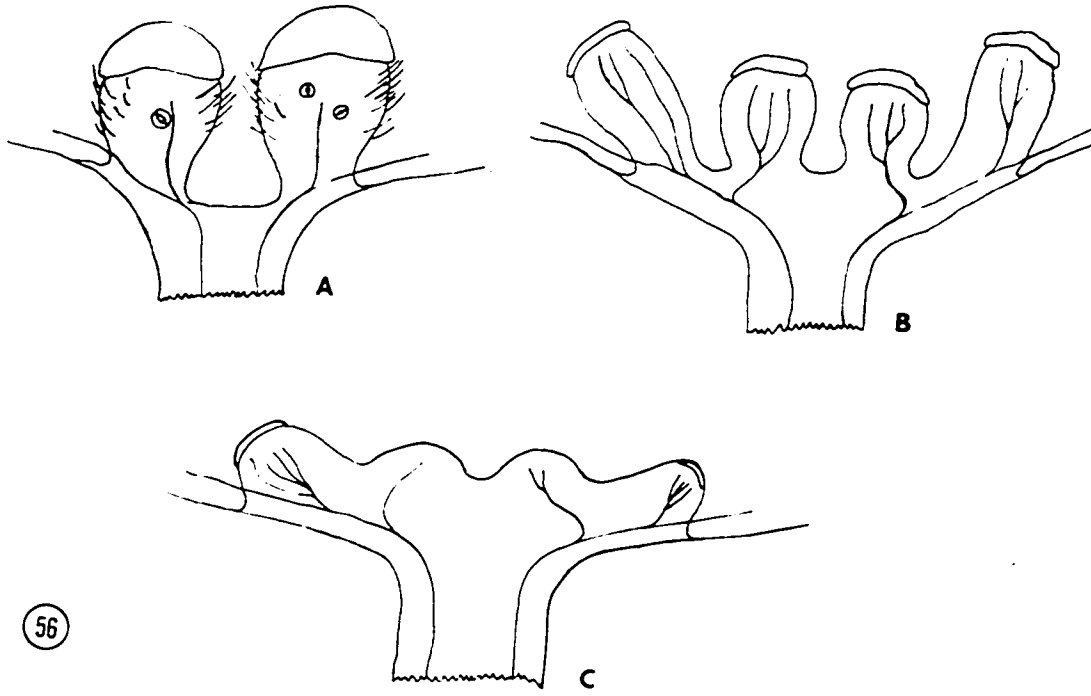

55

Fig. 56. Diagrammatic representation of trends in basal gland seasonal variation on leaves emerging in early spring (A), late spring and summer (B) and late summer (C).



56

Fig. 57. Adaxial secretory epidermis at stage 1. Line represents 1 µm.

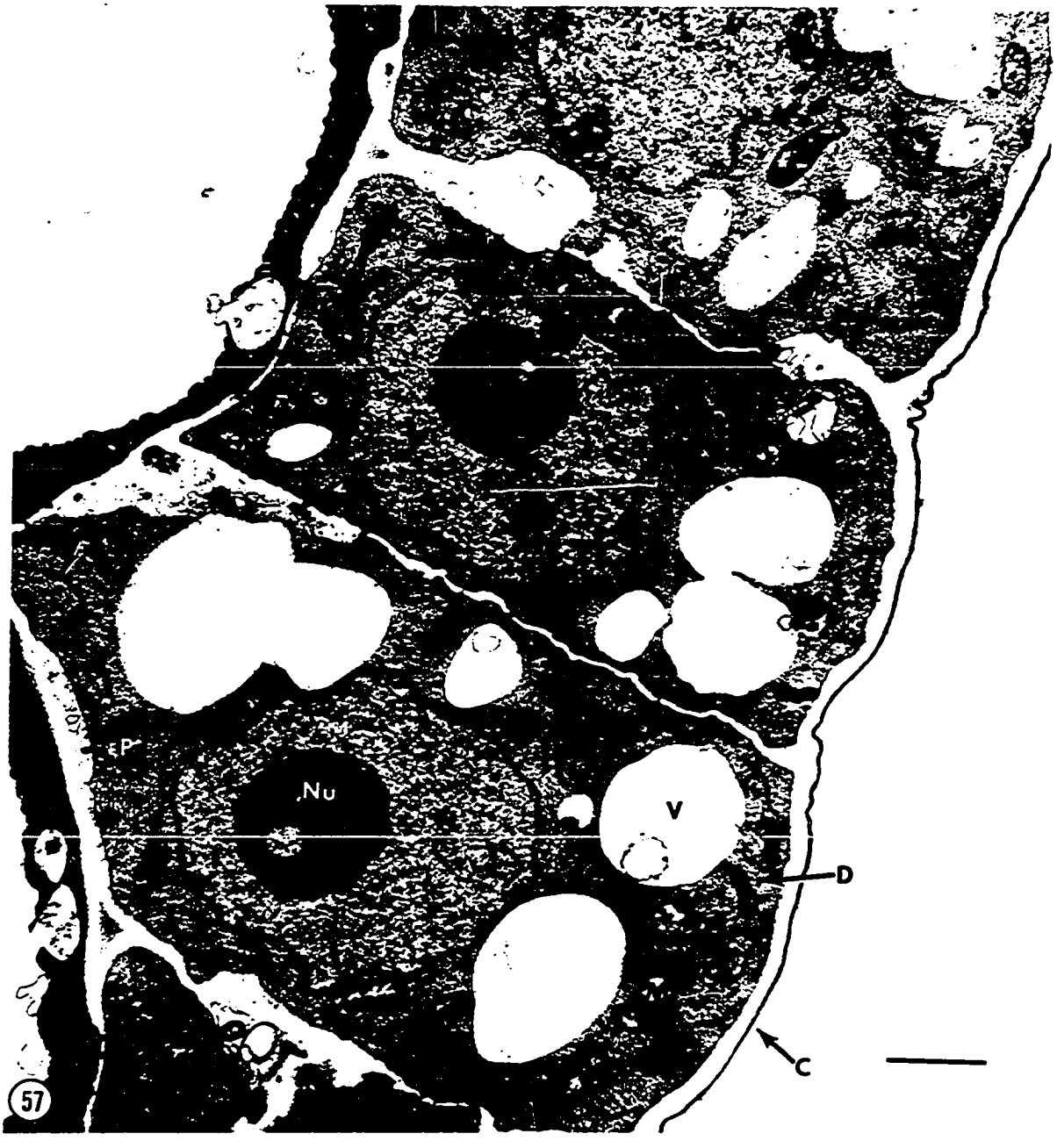

EP
Nu
V
D
C
57

Fig. 58. Adaxial secretory epidermal cell at stage 1. Line represents 0.5 µm.

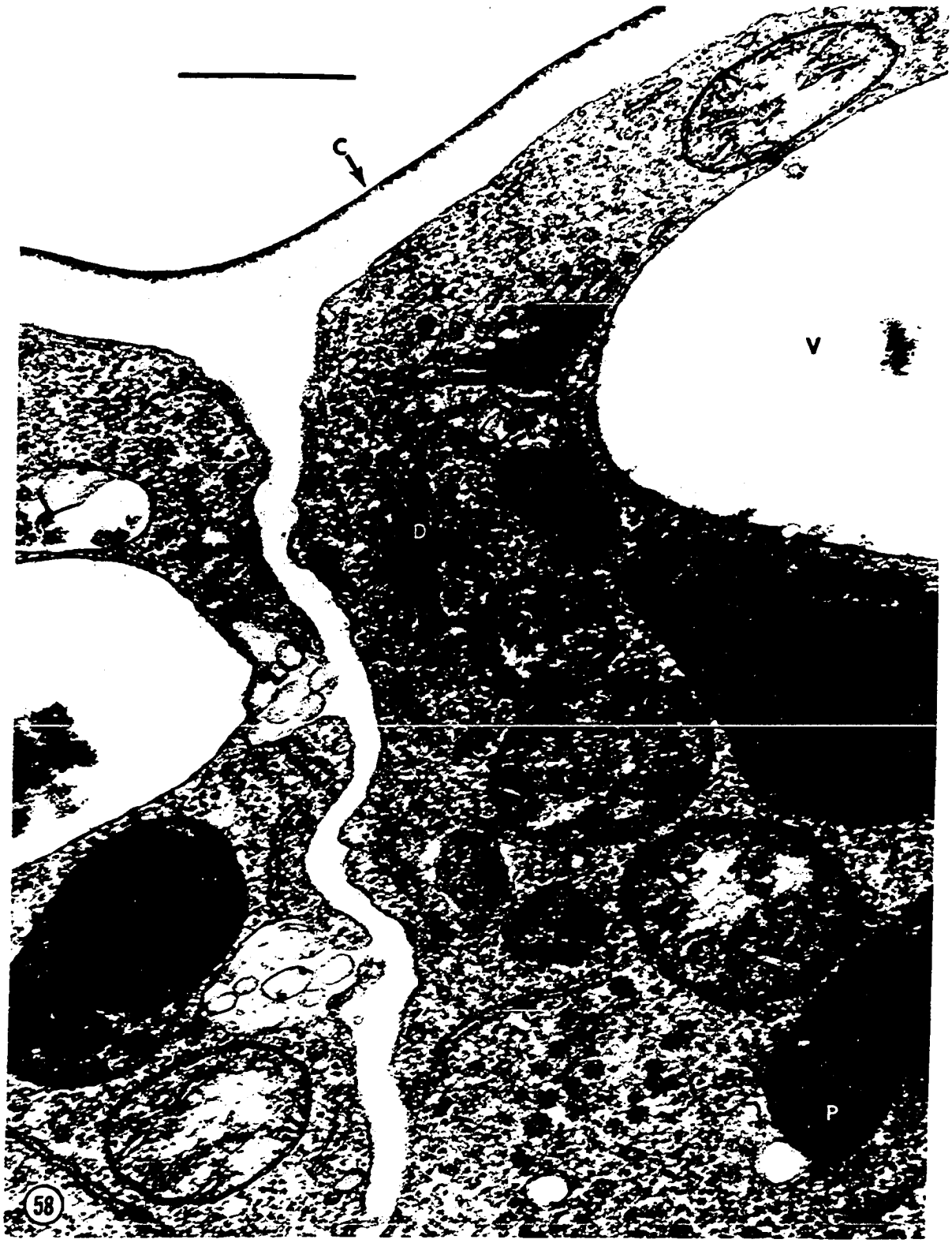
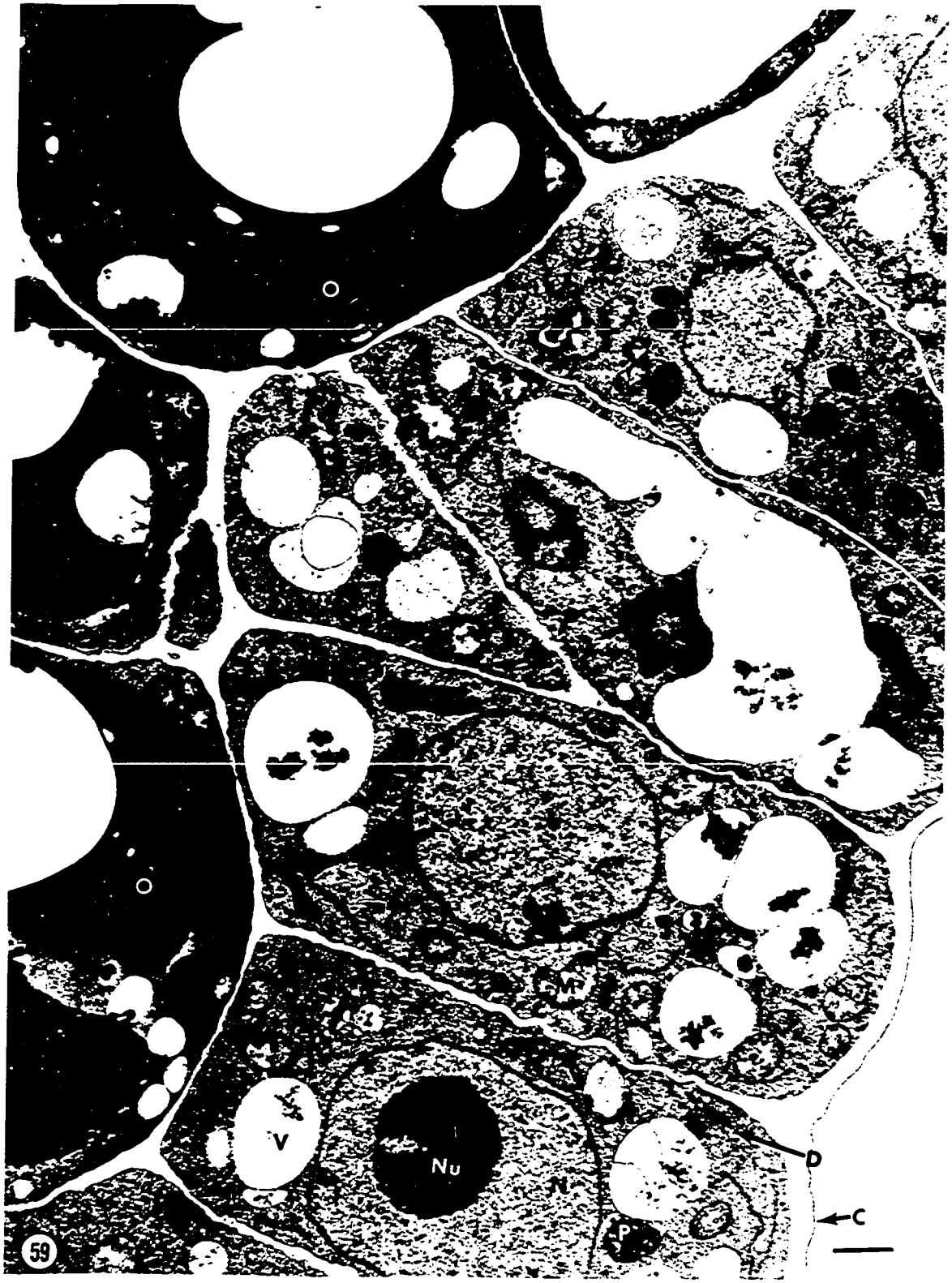

C
V
D
P
58

Fig. 59. Adaxial secretory epidermis at stage 2. Note osmiophilic material (O) in outer mesophyll cells. Line represents 1 µm.


V
Nu
N
P
D
C
59

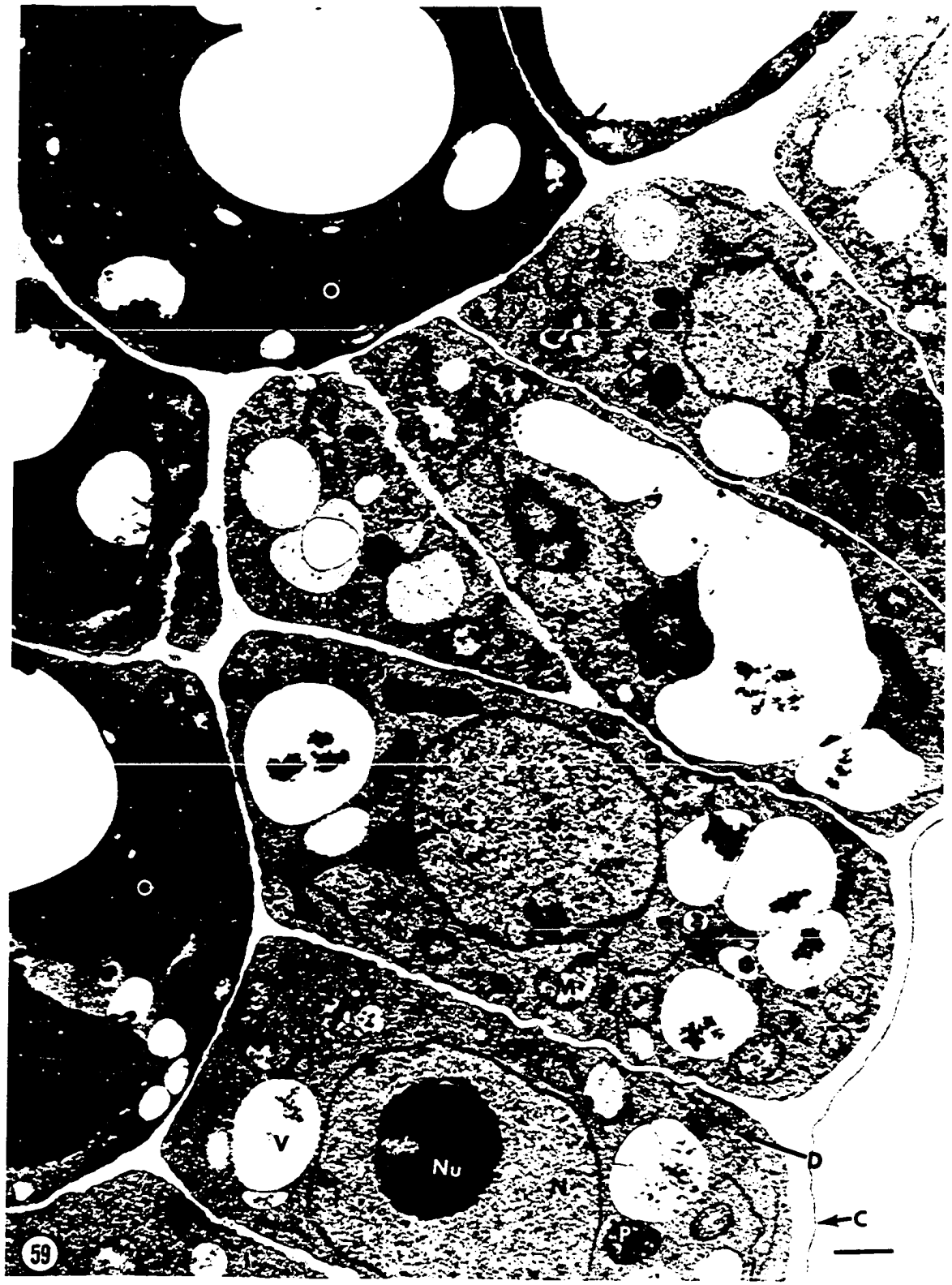

V
Nu
N
P
D
C
59

Fig. 60. Adaxial secretory epidermis at stage 2 and outer mesophyll cells. Line represents 1 µm.

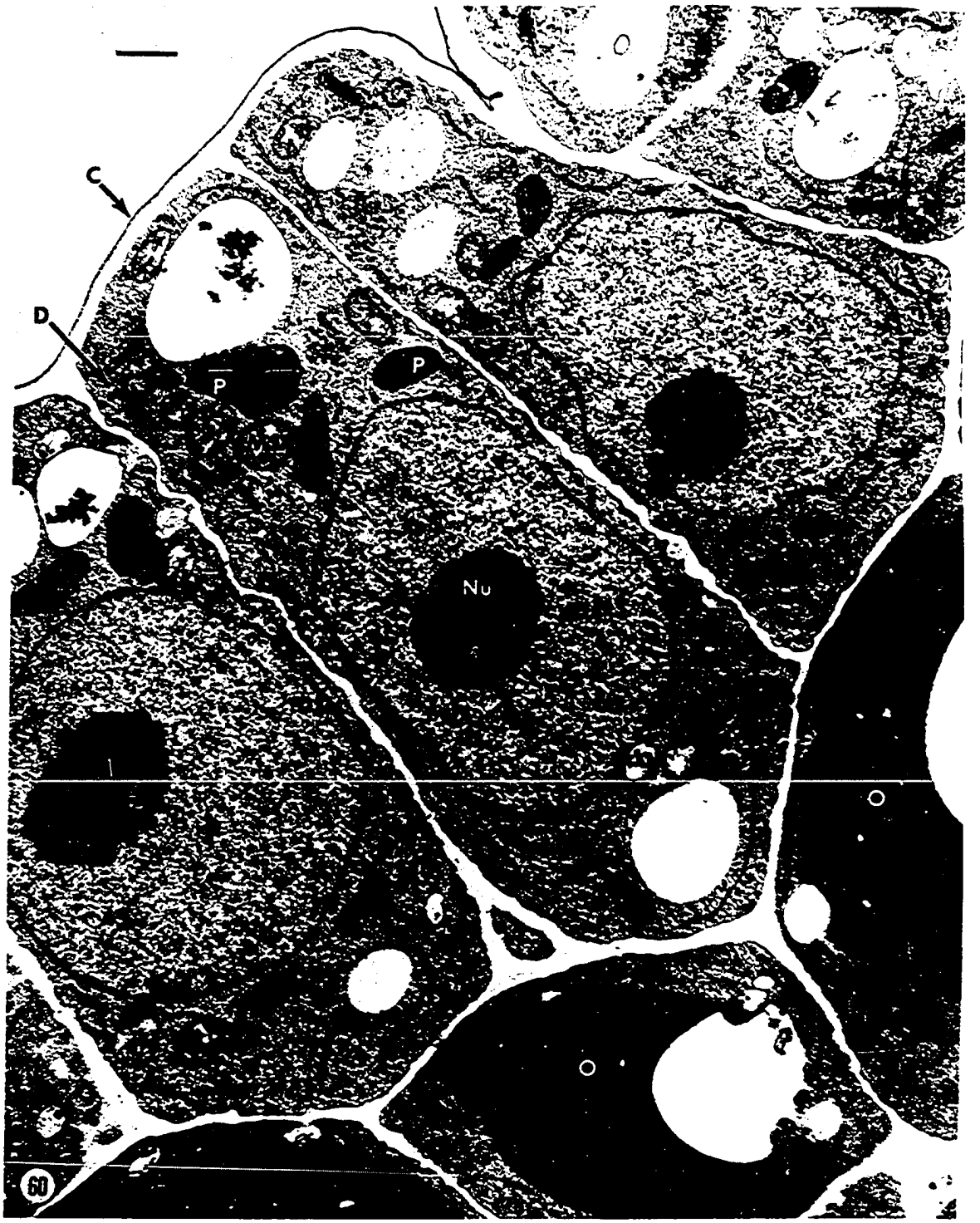

C
D
P
P
Nu
60

Fig. 61. Outer mesophyll cell below secretory epidermis with osmiophilic material (O), myelin-like material (arrow) and plastid (P). Line represents 0.5 μm.


61

Fig. 62. Secretory epidermis at late stage 2; vacuoles (V) are more numerous and smaller. Line represents 1 µm.

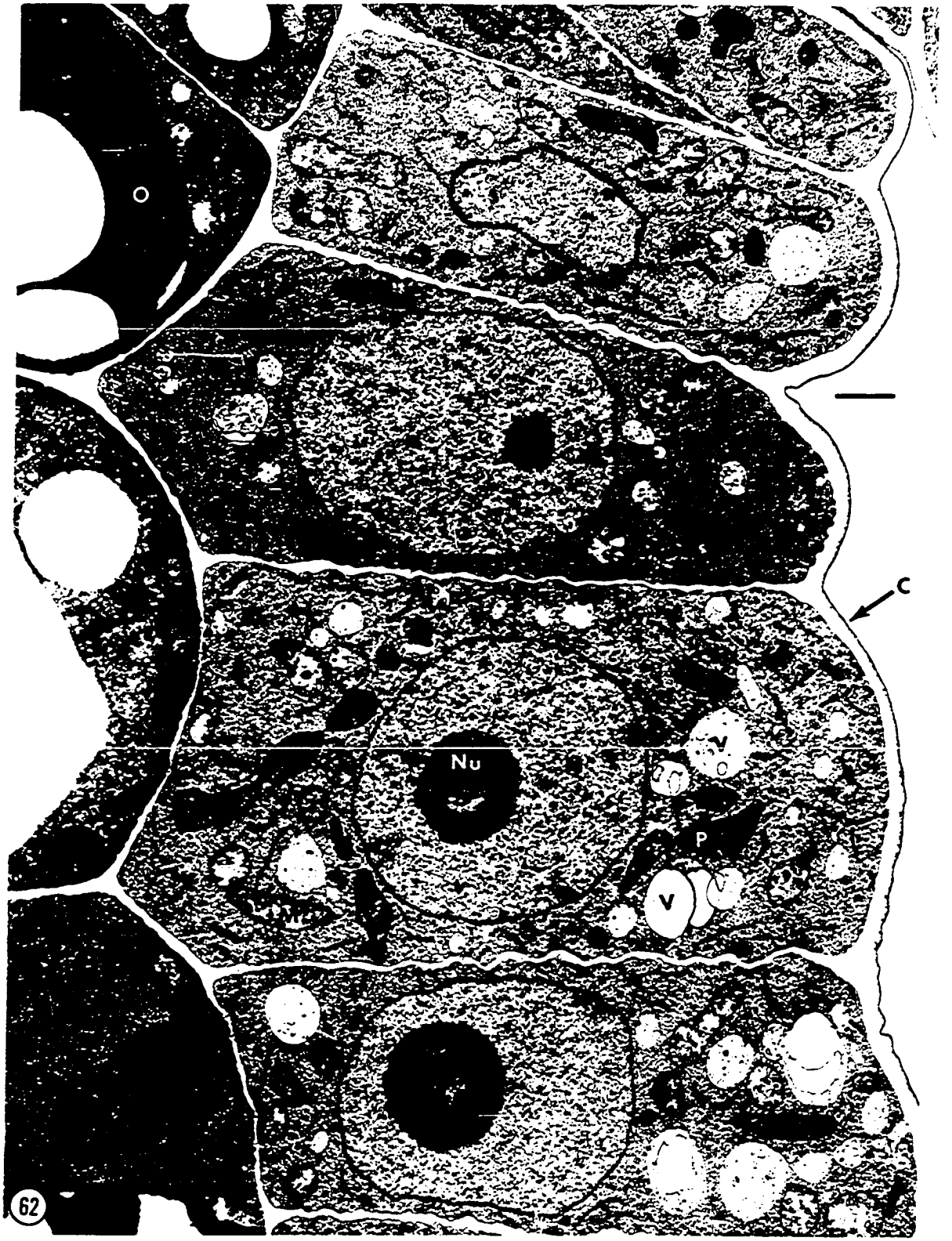

O
C
Nu
V
P
V
62

Fig. 63. Secretory epidermis at stage 3. Note numerous invaginations (I) of plasmalemma--same cells as Fig. 64. Line represents 1 µm.

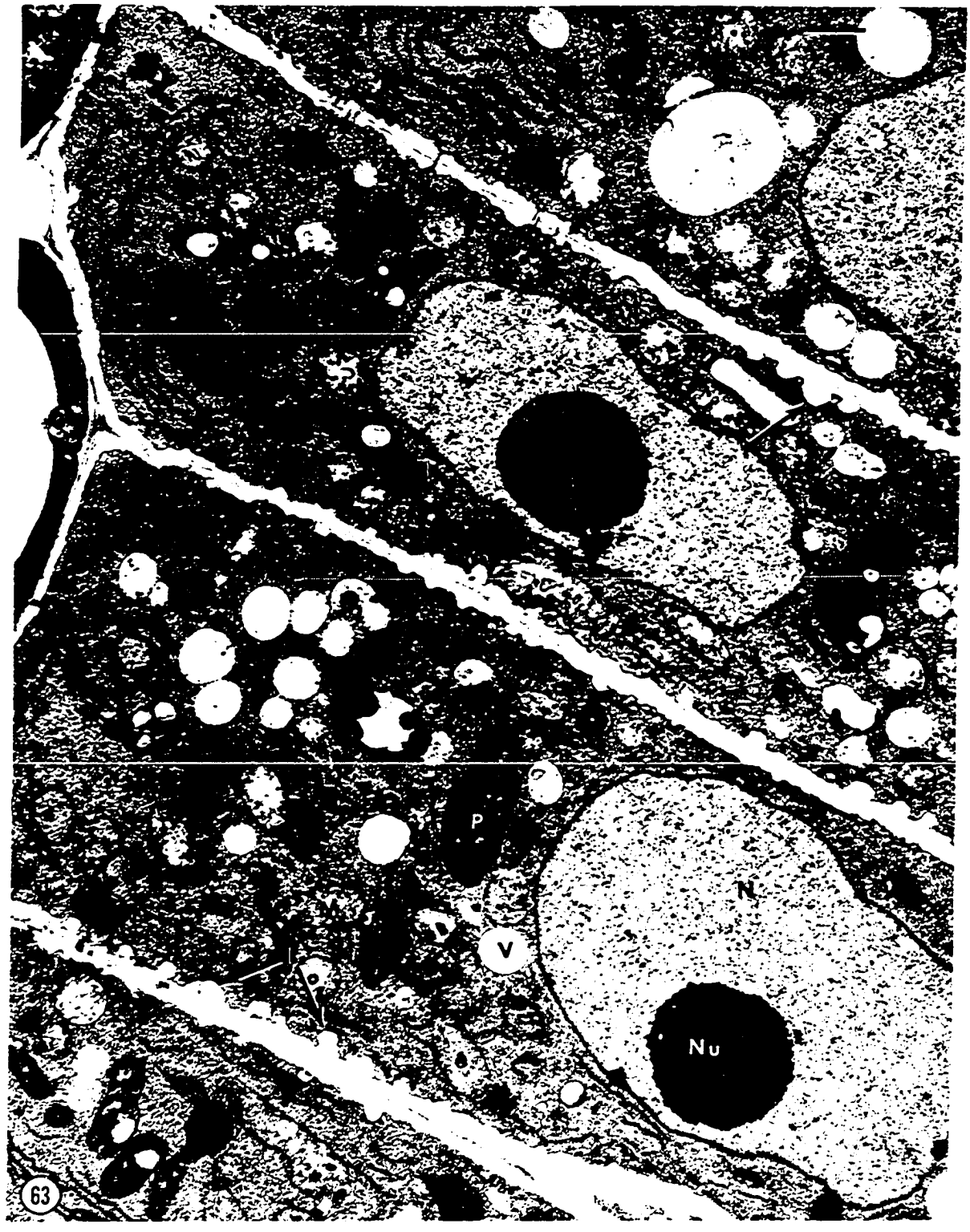

P
N
V
Nu
63

Fig. 64. Secretory epidermis at stage 3. Note invaginations of plasmalemma (I), thickened outer wall (W) and lack a cuticle--same cells as Fig. 63. Line represents 1 µm.

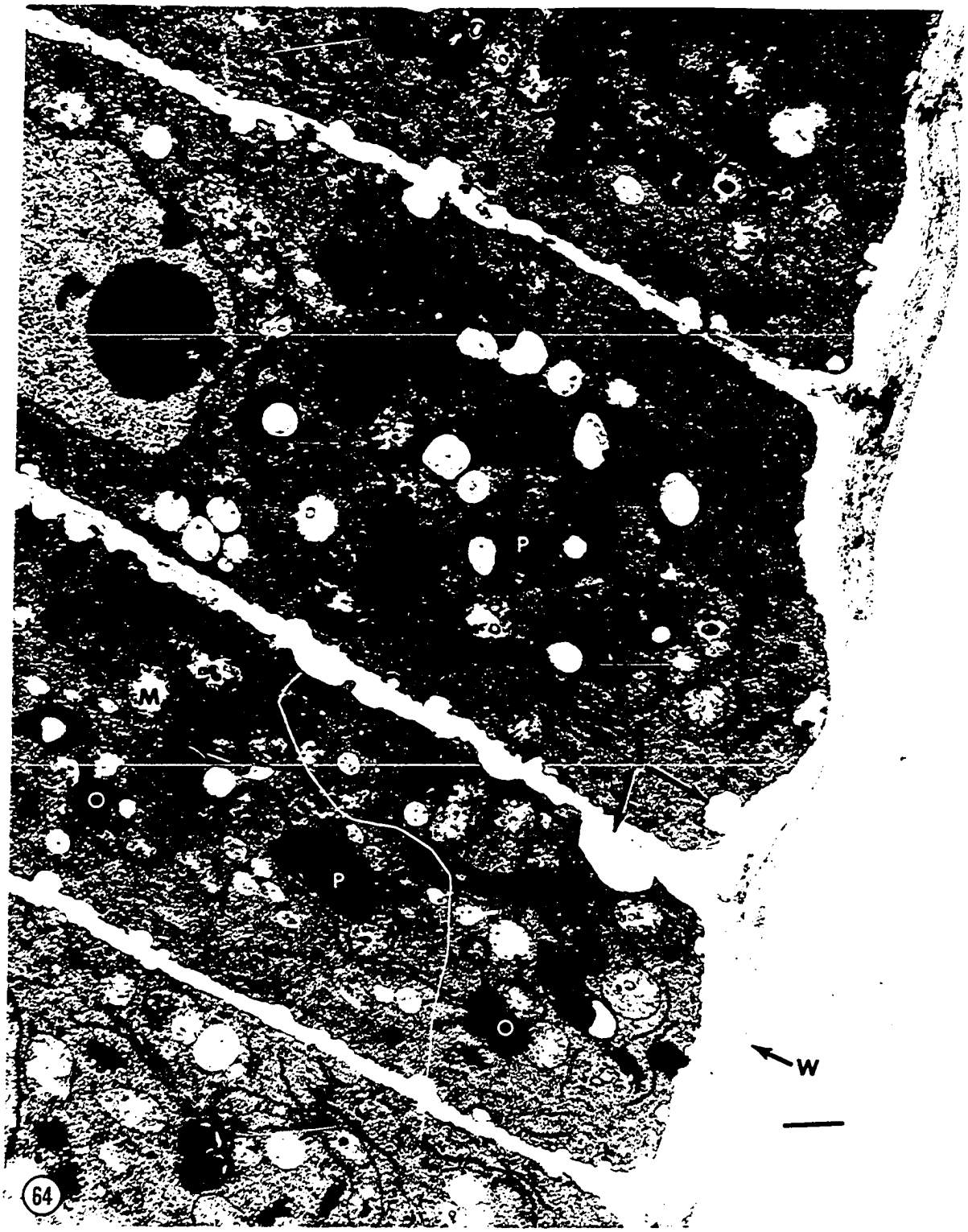

P
M
P
W
64

Fig. 65. Enlarged portion of secretory epidermal cell at stage 3 with numerous invaginations of the plasmalemma (I). Note vesicle (Ve) fusing with plasmalemma and large numbers of vesicles produced by dictyosomes (D). Line represents 0.5 µm.


I
I
I
V
D
I
I
O
65

Fig. 66. Enlarged portion of secretory epidermal cell at stage 3 with numerous plasmalemma invaginations (I). Note dictyosomes (D) and numerous vesicles (Ve). Line represents 0.5 µm.

Fig. 67. Enlarged portion of secretory epidermal cell at stage 3 with numerous plasmalemma invaginations (I). Developed to show structure within invaginations. Line represents 0.5 µm.


I
I
I
D
D
Ve
66

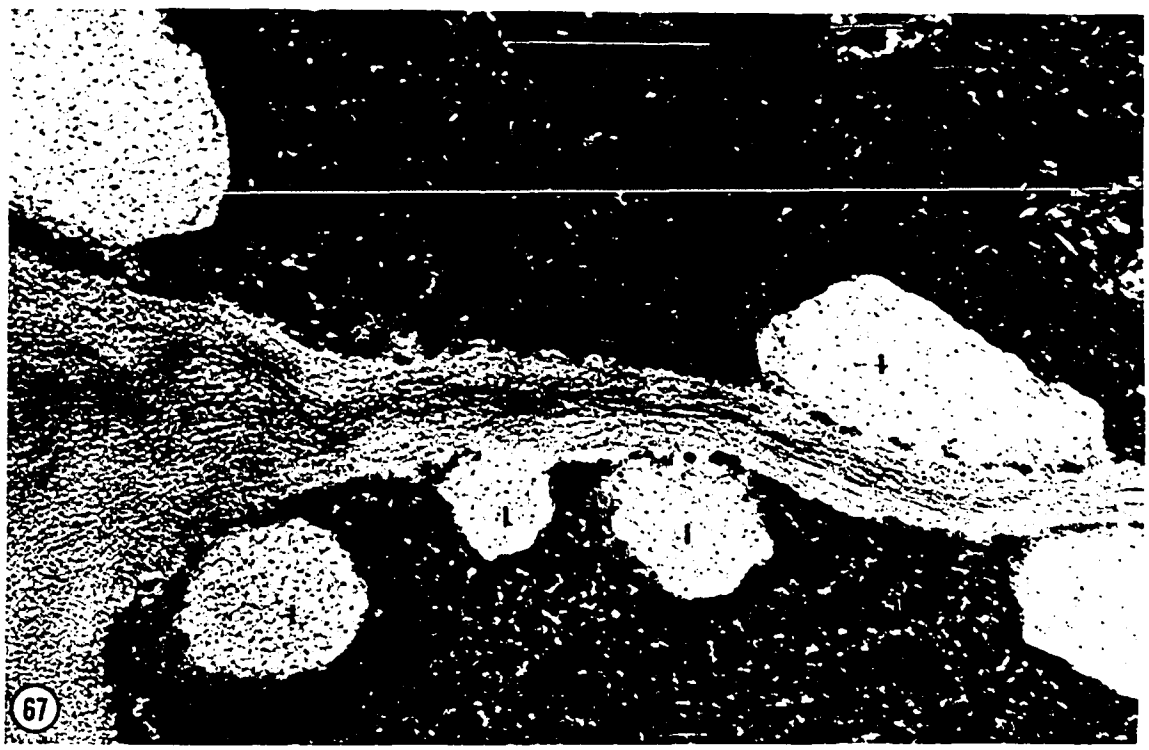

I
I
I
67

Fig. 68. Enlarged portion of secretory epidermal cell at stage 3 with osmiophilic material in plasmalemma invaginations and in cell wall. Line represents 0.5 µm.

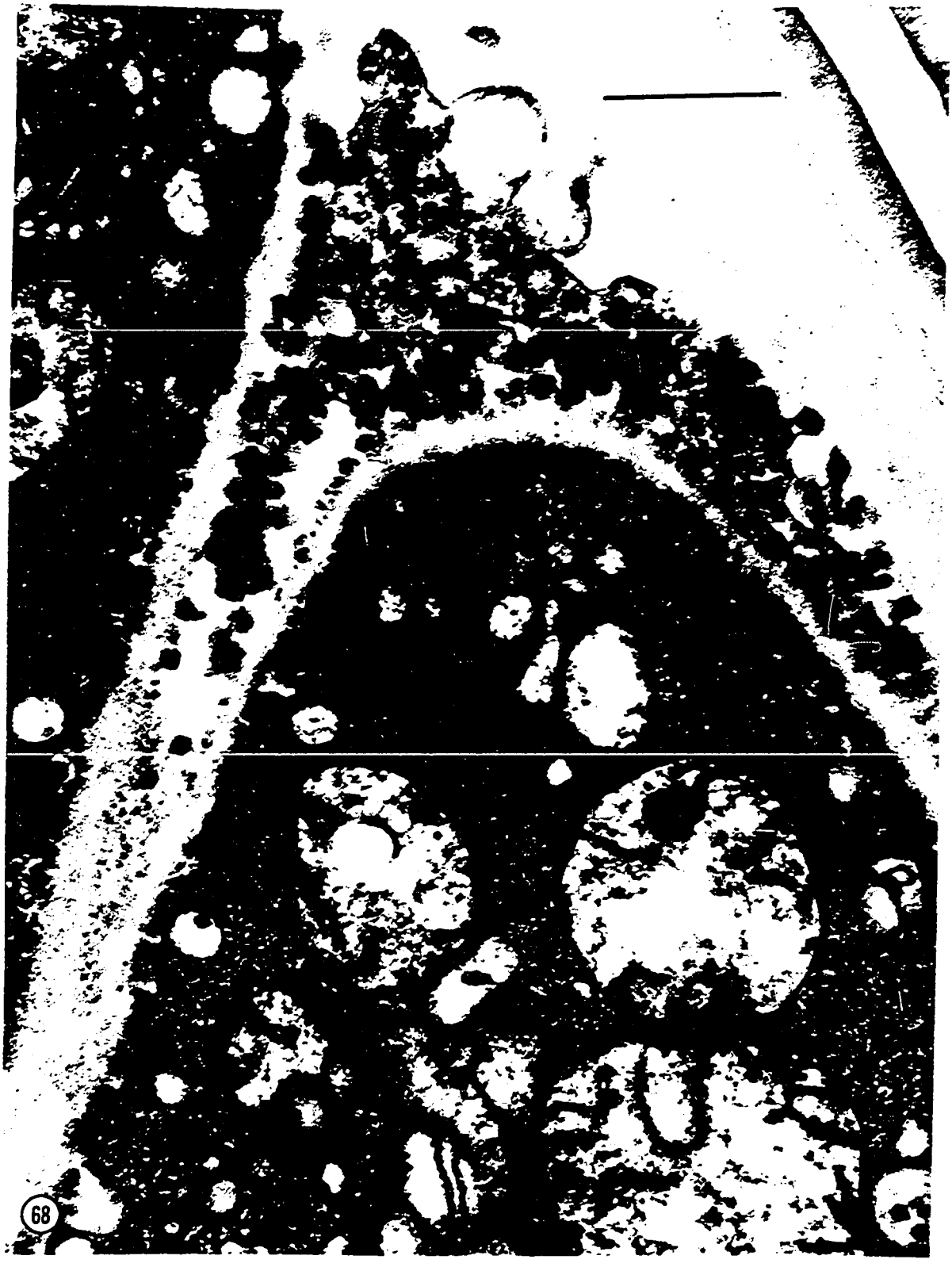
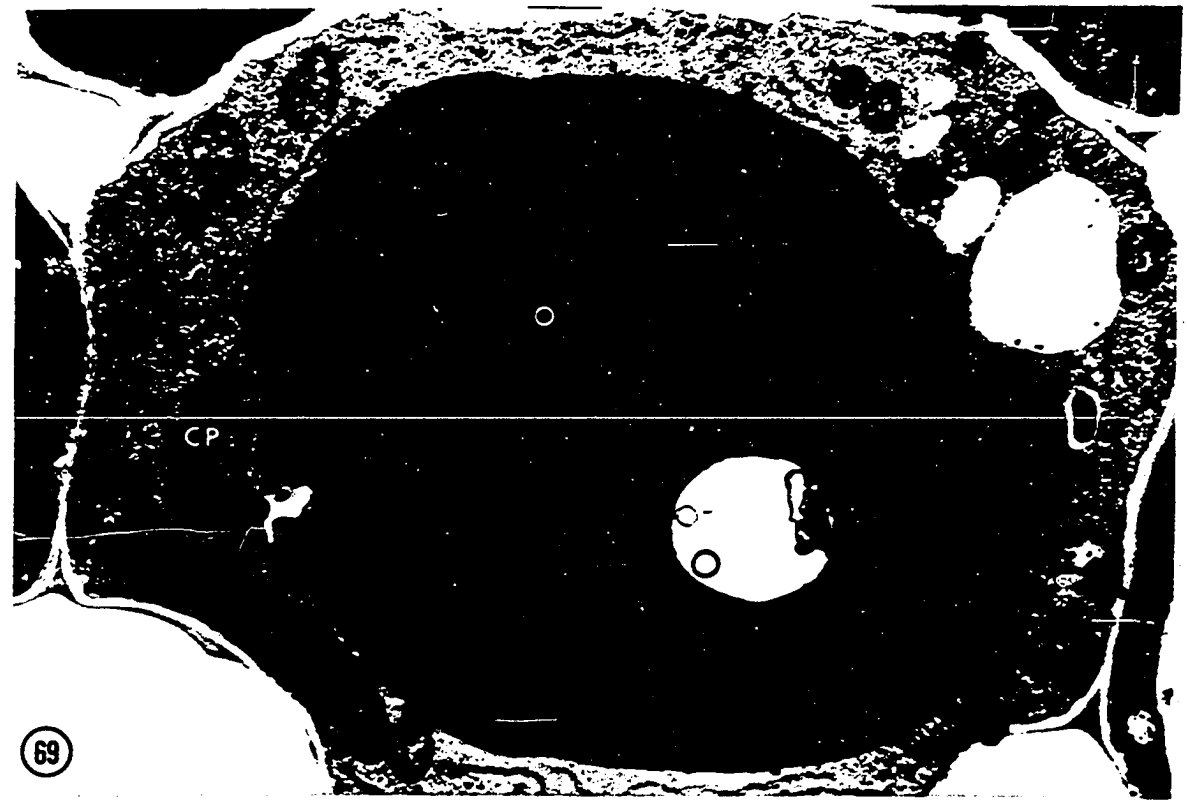

68

Fig. 69. Outer mesophyll cell just below epidermal cells at stage 3. Note chloroplasts (CP) and osmiophilic material (O) in central vacuole. Line represents 1 mm.

Fig. 70. Inner mesophyll cell about two cell layers below secretory epidermis at stage 3. Note chloroplasts (CP) and small quantities of osmiophilic material (O) in vacuoles. Line represents 1 µm.

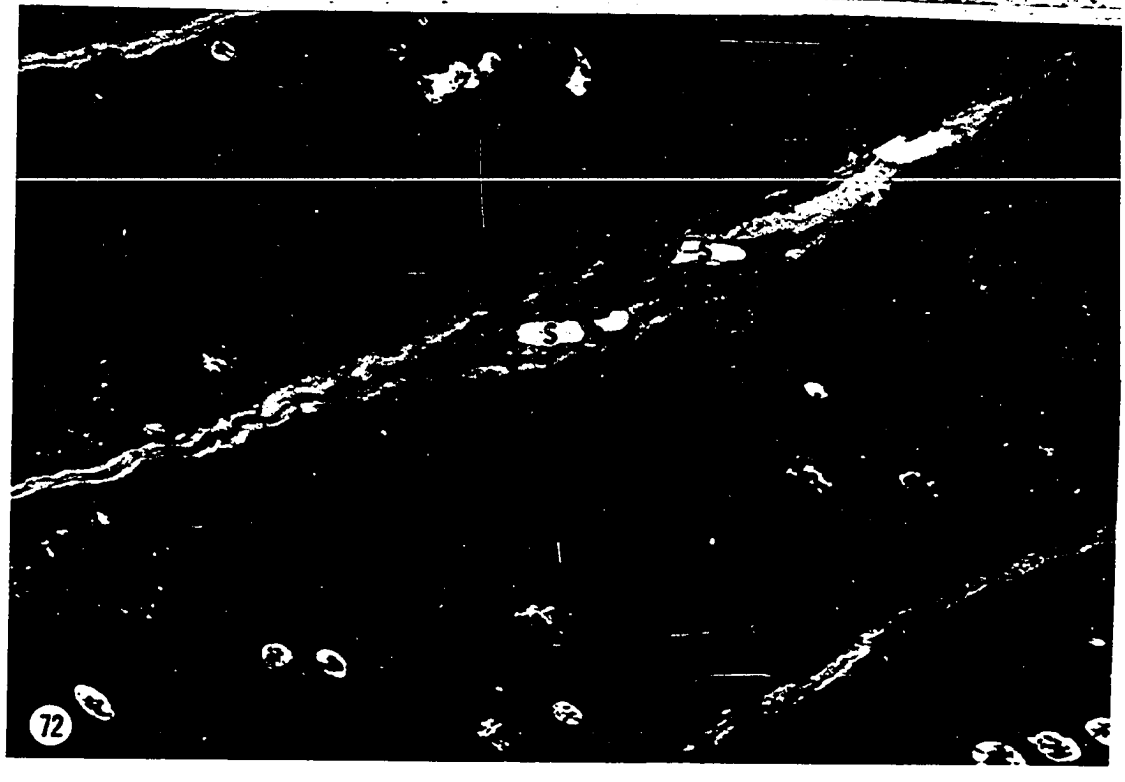

CP
69


CP
70

Fig. 71. Portion of secretory epidermal cell at stage 4 with myelin-like material (arrows) next to cell wall. Line represents 0.5 µm.

Fig. 72. Portion of secretory epidermal cells at early stage 4 showing cell wall spaces (S). Line represents 1 µm.

71



72

Fig. 73. Secretory epidermis at stage 4. Note absence of cuticle, increased numbers of osmiophilic bodies (O), lack of plasmalemma invaginations, increased endoplasmic reticulum (ER), light staining plastids (P), increased numbers of vacuoles or vacuole-like structures (V), build-up of myelin-like material next to cell wall, and cell wall spaces (S). Line represents 1 μm.

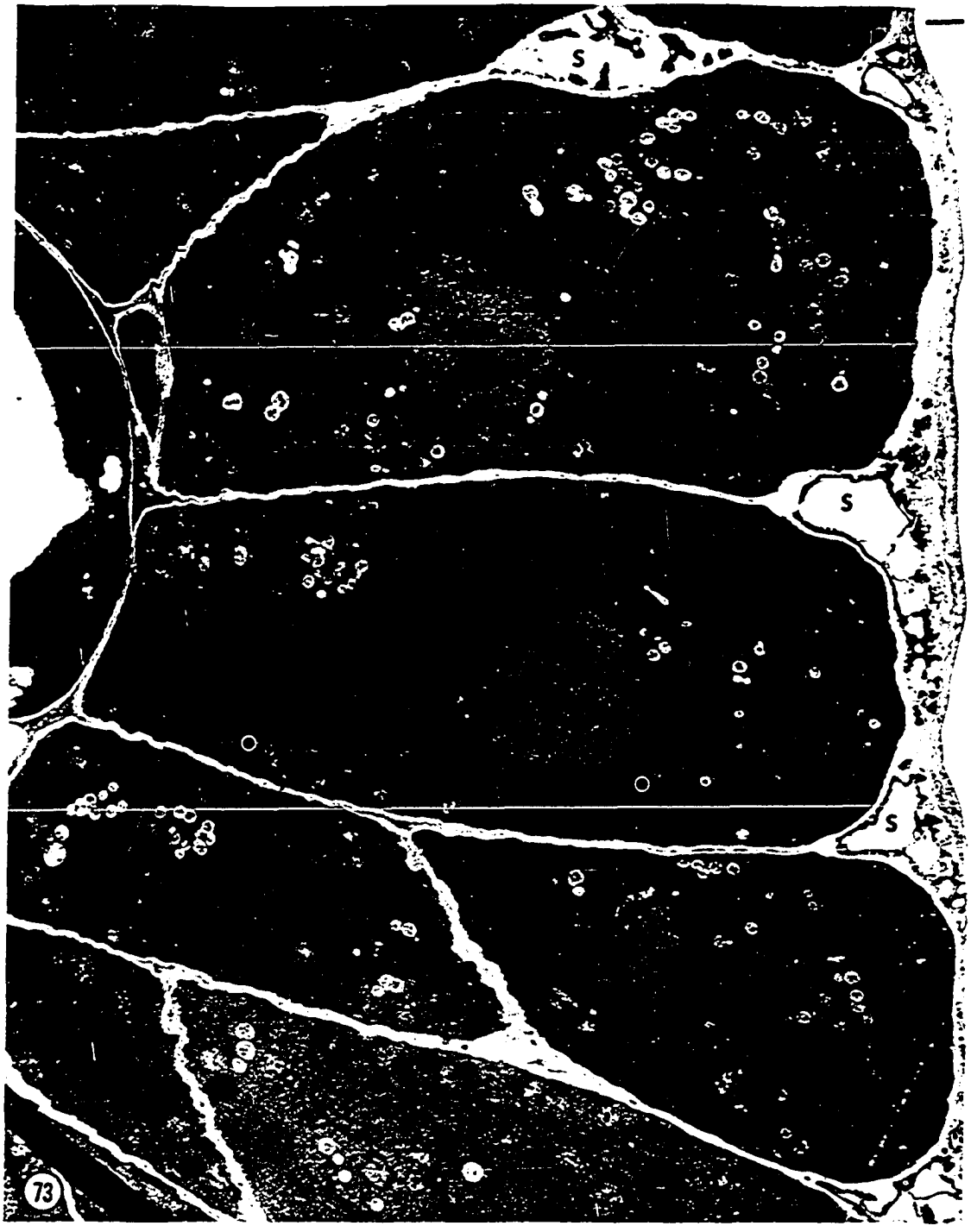

S
S
S
73

Fig. 74. Secretory epidermal cell wall showing spaces (S) and raised cuticle (C). Line represents 0.5 µm.

Fig. 75. Longitudinal section of secretory epidermal cells with cell wall spaces (S). Line represents 1 µm.

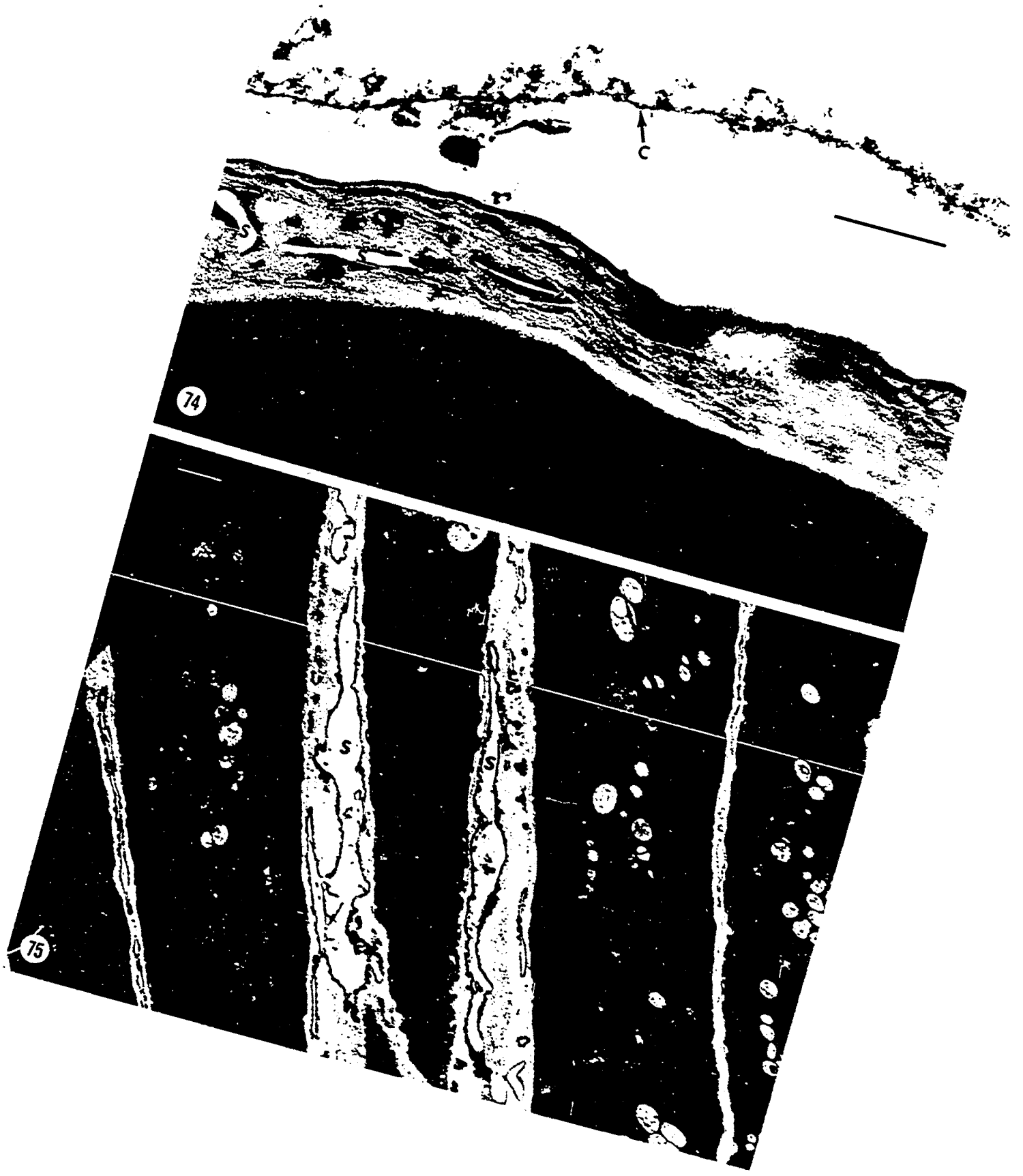
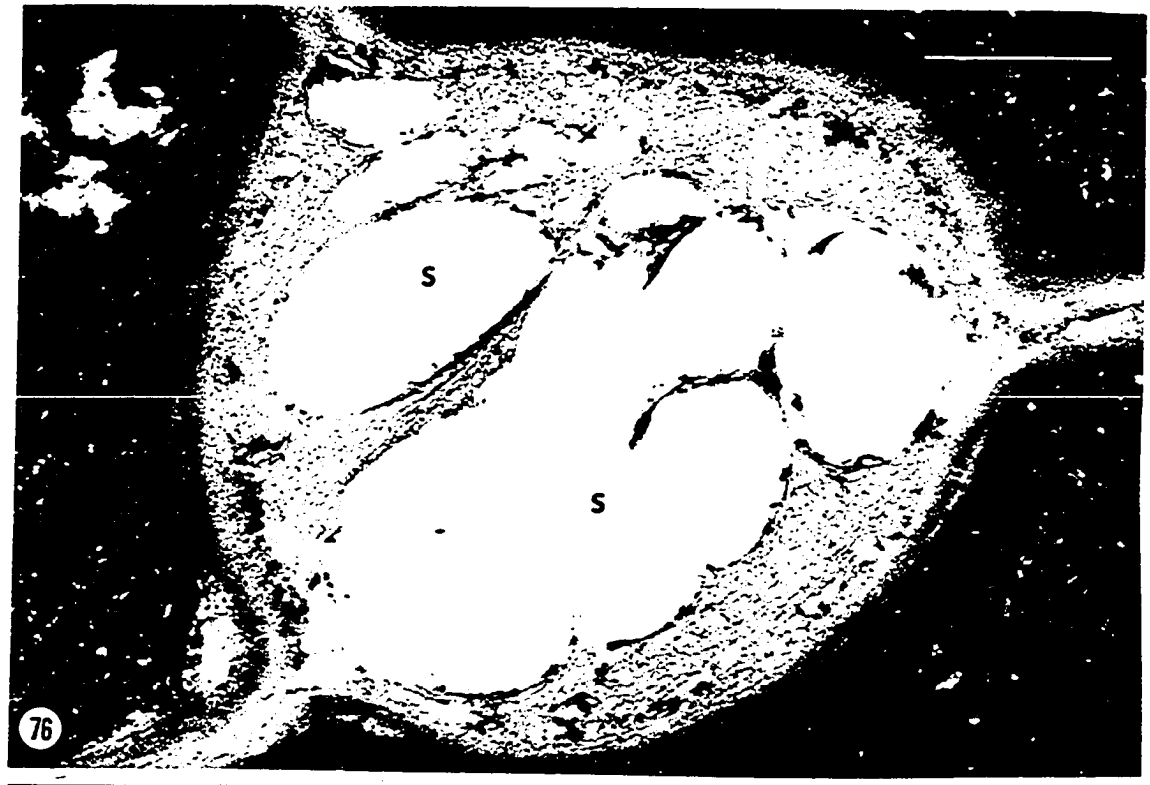

C
S
74
S
S
75

Fig. 76. Cross section of anticlinal wall between three secretory epidermal cells (paradermal section of stipule) showing intercellular channel (S). Line represents 0.5 µm.

Fig. 77. Same as Fig. 76. Line represents 1 µm.


S
S
76

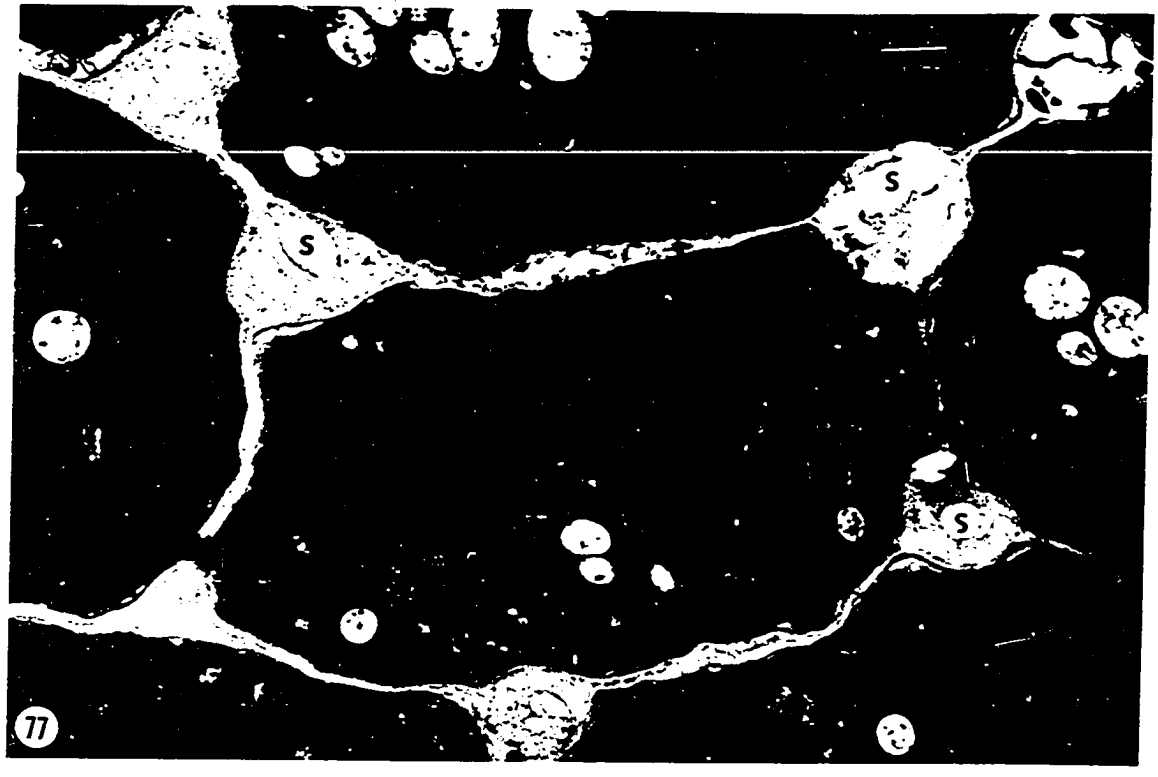
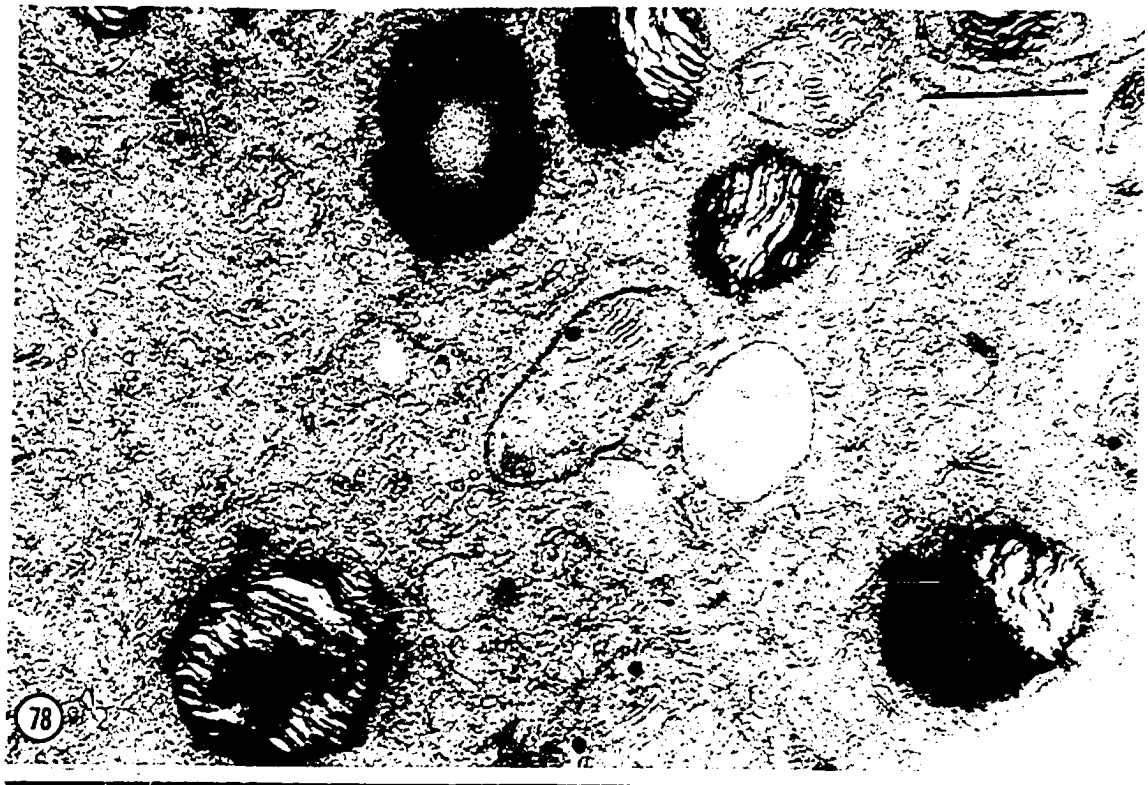
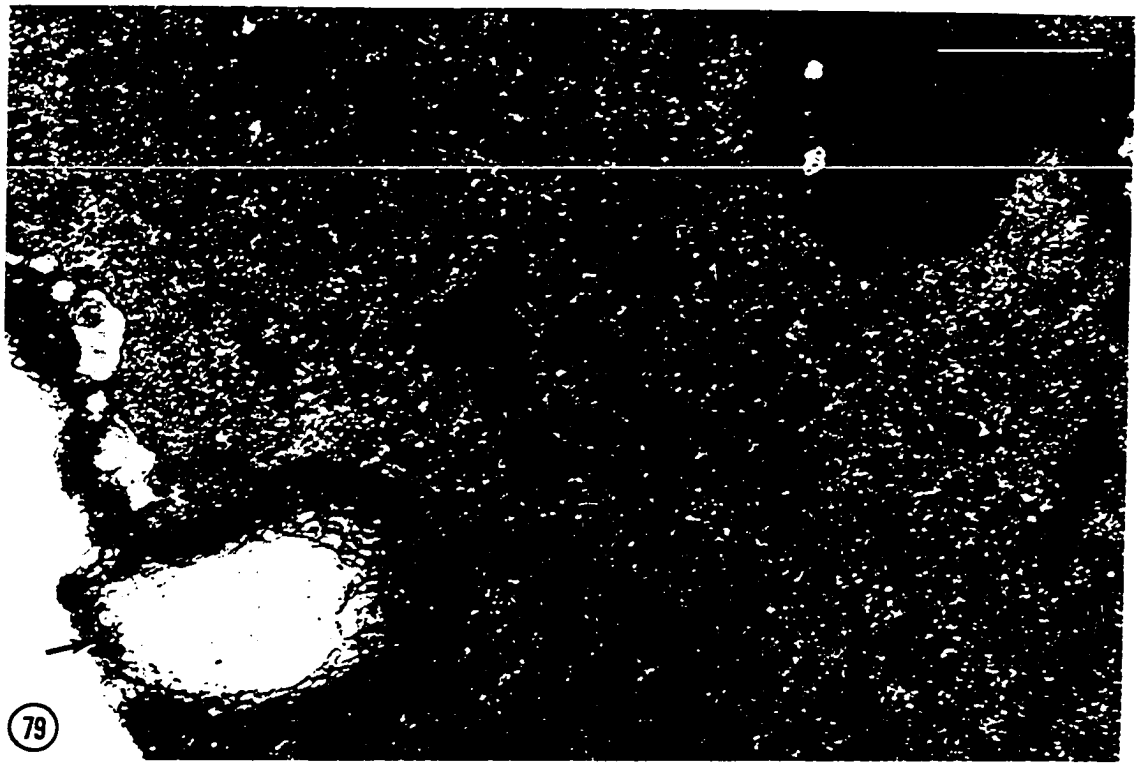

S
S
S
S
77

Fig. 78. Osmiophilic bodies intact and with contents removed leaving skeletal lamellar network. Line represents 0.5 µm.

Fig. 79. Lamellar network of osmiophilic body adjacent to cell wall (arrow). Line represents 0.5 µm.



78



79

Fig. 80-82. Different stages of mitochondrial degeneration in secretory epidermal cells at stages 4 and 5. Numbers 1-6 of Fig. 80-84 indicate possible chronological stages in degeneration. Continued Fig. 83-84. Lines represent 0.5.um.

Fig. 80. Mitochondria before degeneration begins.

Fig. 81. Early stage of degeneration.

Fig. 82. Double-membraned vacuole-like structures.

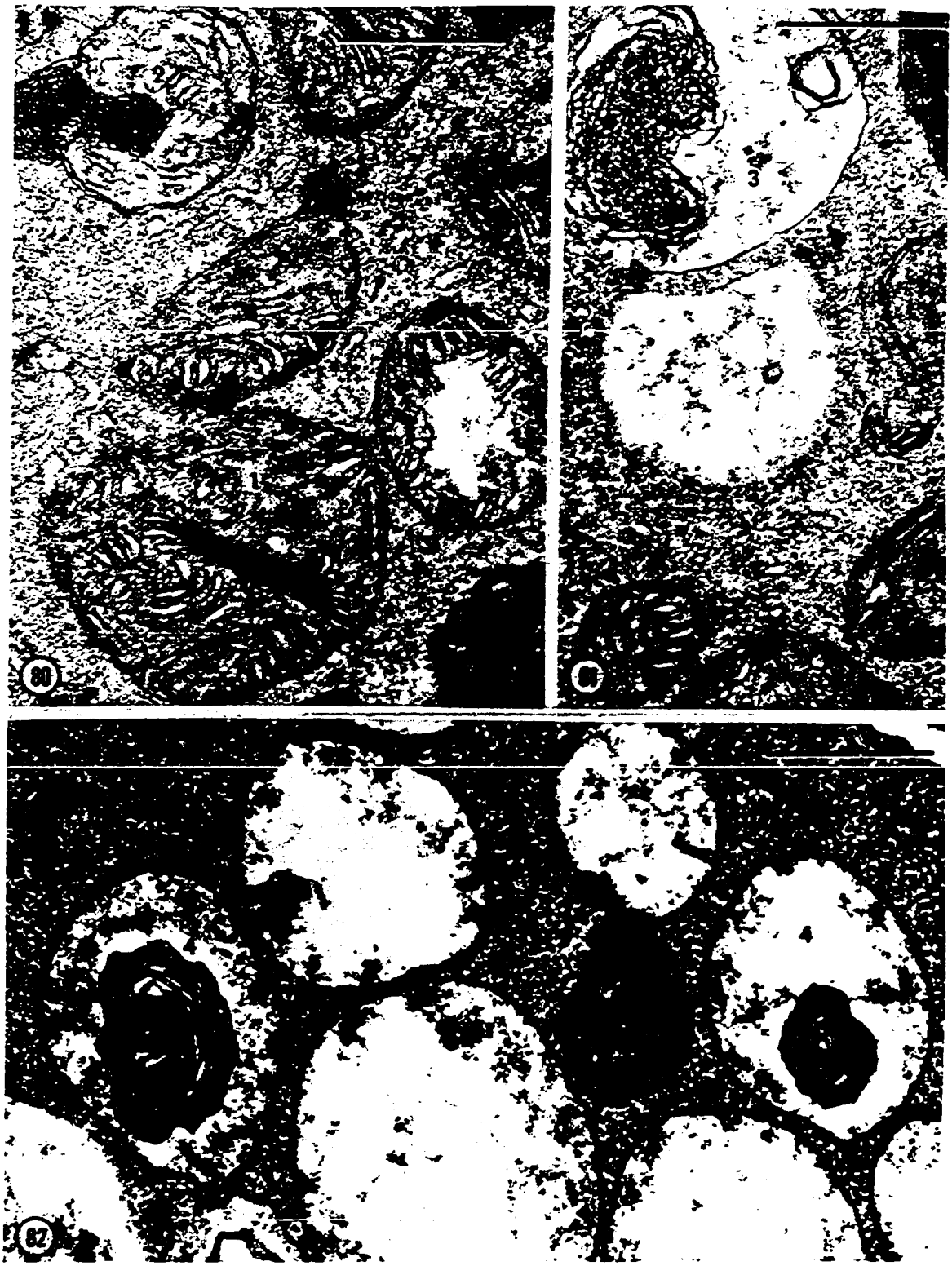
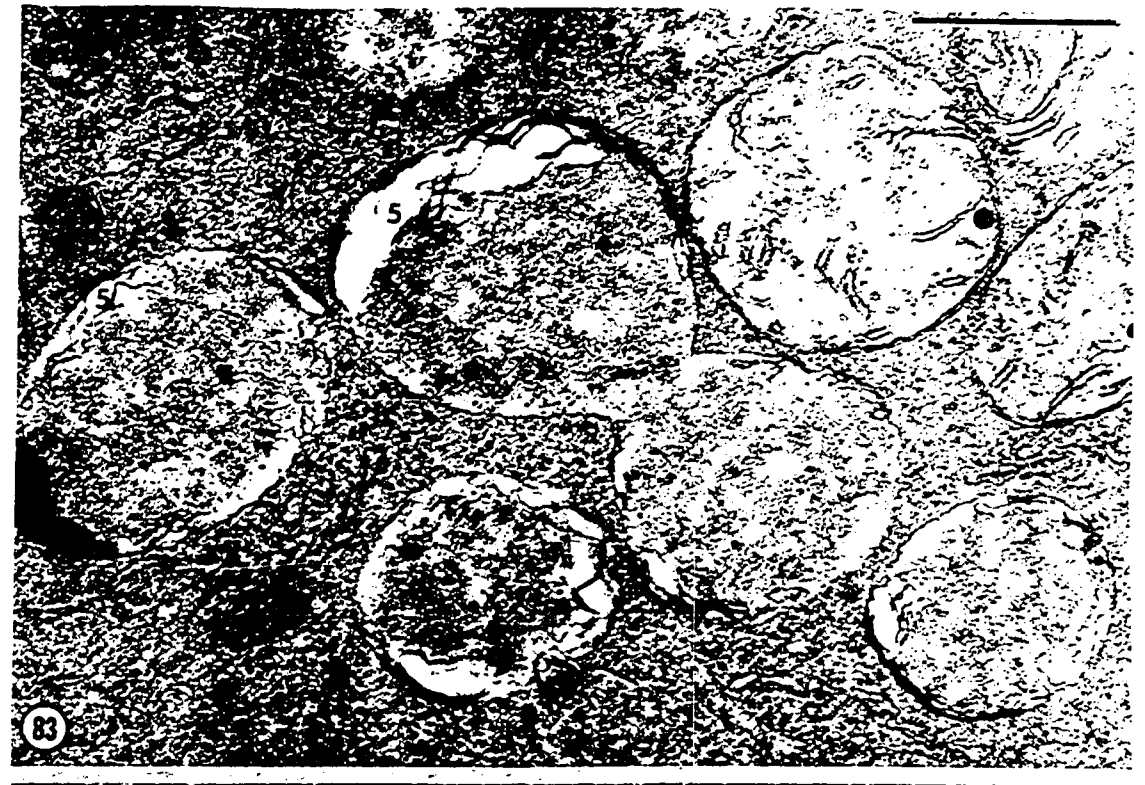

80
3
82

Fig. 83-84. Continued from Fig. 80-82. Lines represent 0.5 µm.

Fig. 83. Late stage of degeneration. Some membrane structures remain

Fig. 84. Vacuole-like mitochondrial derivitives. Only outer membrane remains.


5
5
83

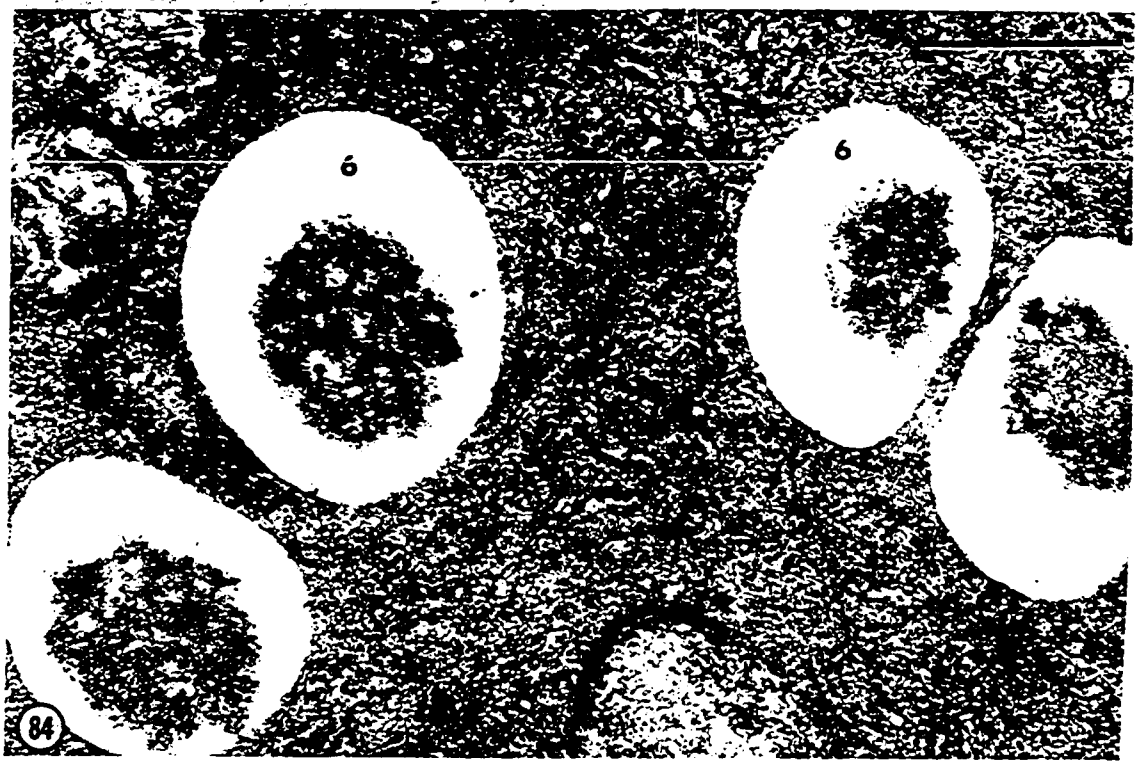

6
6
84

Fig. 85. Secretory epidermal cells at stage 5. Note large numbers of osmiophilic bodies (O) and large numbers of vacuoles or vacuole-like structures (V). Line represents 1 µm.

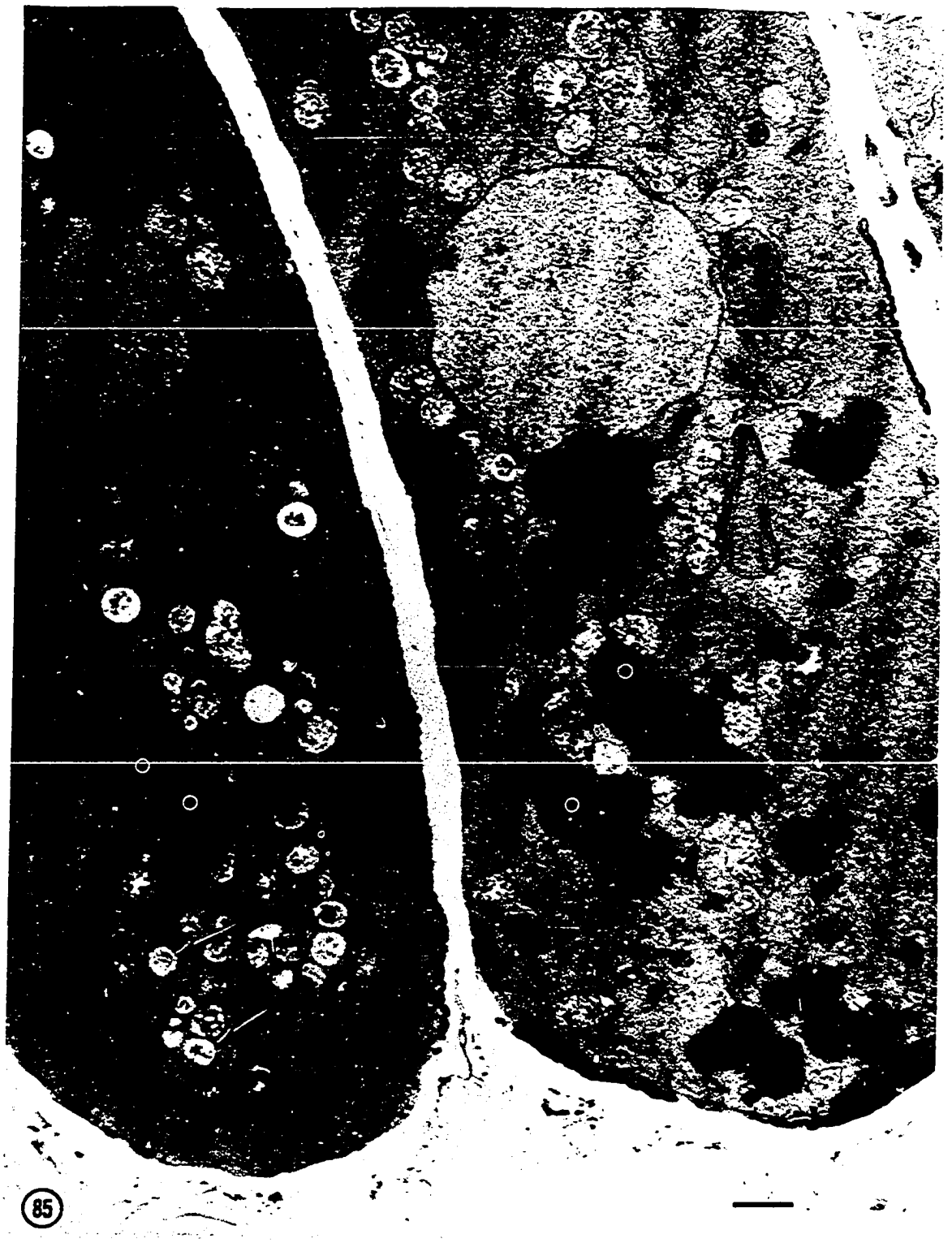

85

Fig. 86. Secretory epidermis and outer mesophyll of senescing stipule at late stage 5. Note large numbers of large vacuoles (V) in epidermal cells and relatively small quantities of osmiophilic material (O) in mesophyll cells. Line represents 1 μm.

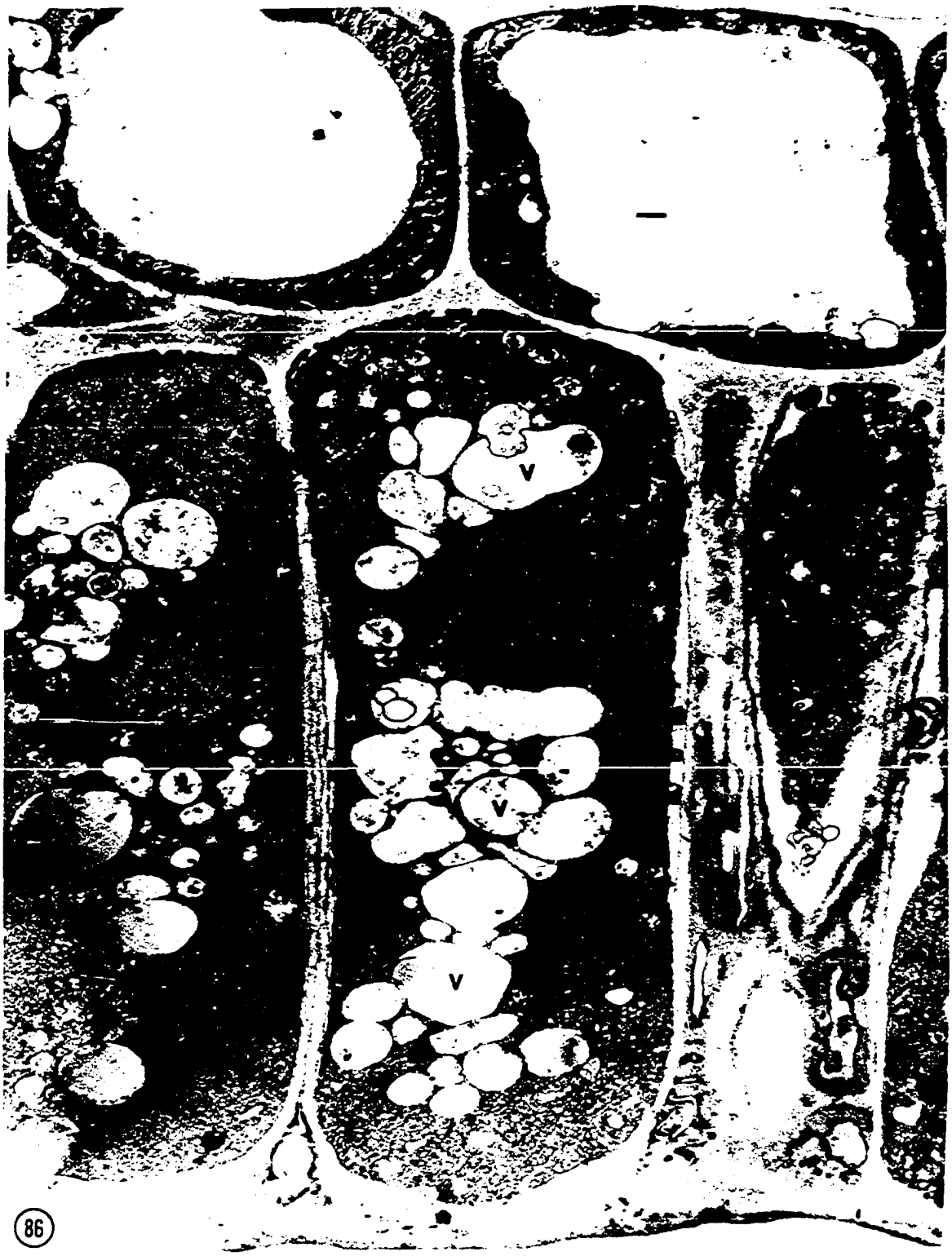

V
V
V
86

Fig. 87-90. Vacuoles of outer mesophyll cells below secretory epidermal cells at stage 5. Series indicates possible pathway of movement of osmiophilic material (O) out of the vacuoles (V). Lines represent 0.5 µm.

Fig. 87. Osmiophilic material intact in vacuole.

Fig. 88. Osmiophilic material just after migrating into cytoplasm.

Fig. 89. Osmiophilic material in cytoplasm.

Fig. 90. Central vacuole and surrounding cytoplasm without osmiophilic material.

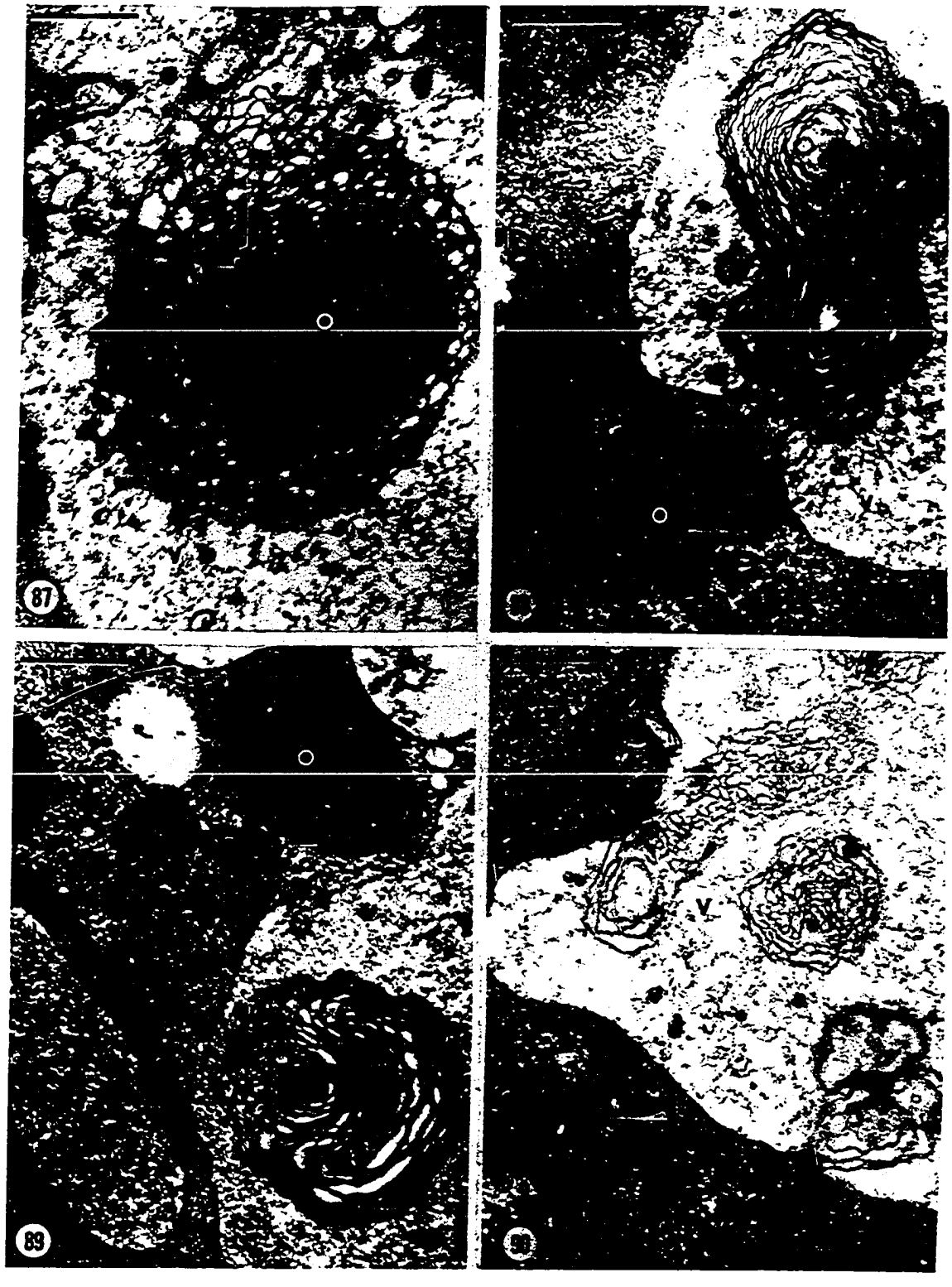
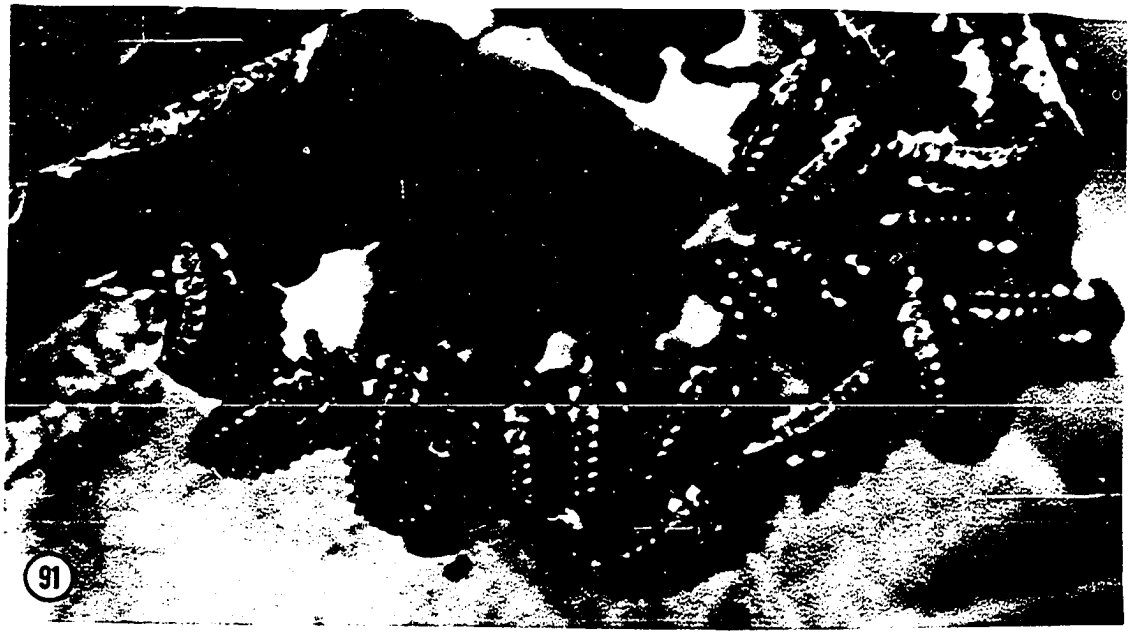

87
V
89
V

Fig. 91. Larvae of Chrysomela scripta feeding on fully expanded cottonwood leaf.

Fig. 92. Same as Fig. 91.

Fig. 93. Adult Chrysomela scripta on partially eaten leaf. Arrow indicates intact resinous margin.


91


93


92

Fig. 94. Scanning electron micrograph of basal glands on fully expanded leaf of Salix pentandra. Line represents 0.5 mm.

Fig. 95. Scanning electron micrograph of basal gland on young S. pentandra leaf which is not yet fully expanded. Line represents 0.5 mm.

Fig. 96. Light micrograph of basal glands and marginal glands of young S. pentandra leaf. Line represents 1 mm.

Fig. 97. Light micrograph of marginal glands of young S. pentandra leaf growing in conditions of low relative humidity. A thread of secretory product is formed by each gland. Line represents 1 mm.


94
95
96
97