Acta Bot. Neerl. 40(4), December 1991, p. 339–356

Development of oil and mucilage cells in *Cinnamomum burmanni*. An ultrastructural study

M. E. BAKKER, A. F. GERRITSEN and P. J. VAN DER SCHAAF

Rijksherbarium/Hortus Botanicus, P.O. Box 9514, 2300 RA Leiden, The Netherlands

SUMMARY

The development of oil and mucilage cells in the shoot apex and young leaves in *Cinnamomum burmanni* is described. Three arbitrary developmental stages are distinguished. At stage 1, the cells differ from the surrounding cells by the absence of osmiophilic deposits in the vacuoles and the presence of distinct small plastids with reduced thylakoids. At stage 2, a suberized layer is deposited in the cells. At stage 3, the oil or mucilage secretion has started in the cells. For both oil and mucilage cells, three intergrading stages (3a, 3b, 3c) are recognized. In oil cells, the subdivision is based on the thickness of the inner-wall layer, the composition of the cytoplasm and the extent of the oil cavity. The thickness of the inner-wall layer increases with development. The oil cavity, enclosed by the plasmalemma and attached to the wall by a cupule, enlarges. Oil formation presumably takes place within the plastids and apparently results in the disintegration of the plastids. In mucilage cells, the substages are distinguished based on the amount of mucilage deposited between the suberized layer and the plasmalemma, and the composition of the cytoplasm which contains an increasing amount of dictyosome vesicles. For the first time, cupule-like wall structures are reported in mucilage cells. Therefore, the number of similarities in both oil and mucilage cells increases to four: the presence of a suberized layer, an extraplasmatic accumulation of the secretory product, and the presence of a cupule and characteristic plasmodesmata. These resemblances strongly support their hypothesized homology.

Key-words: Cinnamomum burmanni, cupule, extraplasmatic space, mucilage idioblast, oil idioblast, suberized layer.

INTRODUCTION

Cinnamomum burmanni Bl., like many other Lauraceae, has both oil and mucilage idioblasts in its vegetative organs. In an earlier paper (Bakker & Gerritsen 1989) we reported on certain aspects of mucilage cell development, especially on the deposition of a suberized-wall layer previously only convincingly demonstrated in oil idioblasts. Oil cell development was subsequently studied for comparison in *Annona muricata* L. (Annonaceae) (Bakker & Gerritsen 1990) a species which has exclusively this type of secretory idioblast. In the present paper, we will describe ultrastructurally, the development of oil and mucilage cells in *C. burmanni*. Such a comparison can shed light on the possible homology

and mutual replacement of these two types of secretory idioblasts proposed by Tschirch (1889, 1914) and Janssonius (1926, 1934).

MATERIALS AND METHODS

Young shoot apices with surrounding young developing leaves of a tree of *Cinnamomum burmanni* L., grown in the Hortus Botanicus in Leiden, were fixed in a modified Karnovsky fixative, rinsed and then postfixed in OsO_4. After dehydration in alcohol and propylene oxide, the material was embedded in Epon. The ultrathin sections were stained and examined with the transmission electron microscope (for more details see Bakker & Gerritsen 1989). For light microscopical examination, 1 μm sections were stained with toluidine blue 0 (see Bakker & Gerritsen 1990). The thicknesses of the suberized layer and the inner-wall layer were measured from electron micrographs. The measurements were correlated with other aspects of the oil cell development and statistically examined (see for details: Bakker & Gerritsen 1990).

RESULTS

General remarks

The oil and mucilage cells in *C. burmanni* mostly occurred in relatively large numbers in a zone underlying the epidermis, i.e. in the outer cortex of the shoot and in the mesophyll of developing leaves. Various developmental stages of both types of idioblasts were randomly situated among each other (Fig. 1a). Three arbitrary developmental stages could be distinguished in both types of idioblasts. The first two stages (stage 1 and 2) were the same for oil and mucilage cells. In developmental stage 3 the two types of idioblasts could be distinguished (see Fig. 9).

Common stages of development in oil and mucilage cells

Stage 1. Young idioblasts ($24 \pm 8 \times 16 \pm 7$ μm), possessing a large central vacuole, were recognized by the absence of osmiophilic deposits in the vacuoles, which always abounded in the vacuoles of the adjacent tissue (Fig. 1a and b). In these cells, no additional wall layers were deposited against the primary wall. The parietal layer of cytoplasm was slightly less electron dense than in the surrounding cells and contained identical organelles such as nucleus, plastids, rough endoplasmic reticulum, mitochondria, dictyosomes and small vacuoles (Fig. 1b and c). Part of these idioblasts however, showed small, typical plastids, containing reduced thylakoids and peripheral electron translucent globules (Fig. 1c).

Stage 2. The cells ($24 \pm 6 \times 17 \pm 9$ μm) showed a more or less angular outline and contained cytoplasm resembling that of cells in stage 1 (Fig. 2a). These cells possessed a suberized layer which was deposited against the initial cell wall (Fig. 2a, c and d) and had a mean thickness of 17 ± 5 nm (Fig. 8). This layer consisted mostly of 2–3 discontinuous lamellae (Fig. 2c); increasing up to approximately six lamellae (Fig. 2d). Sometimes plastids contained a lipid-like globule. In a few cells many cisternae of rough ER were seen aligned parallel to the cell wall (Fig. 2b).

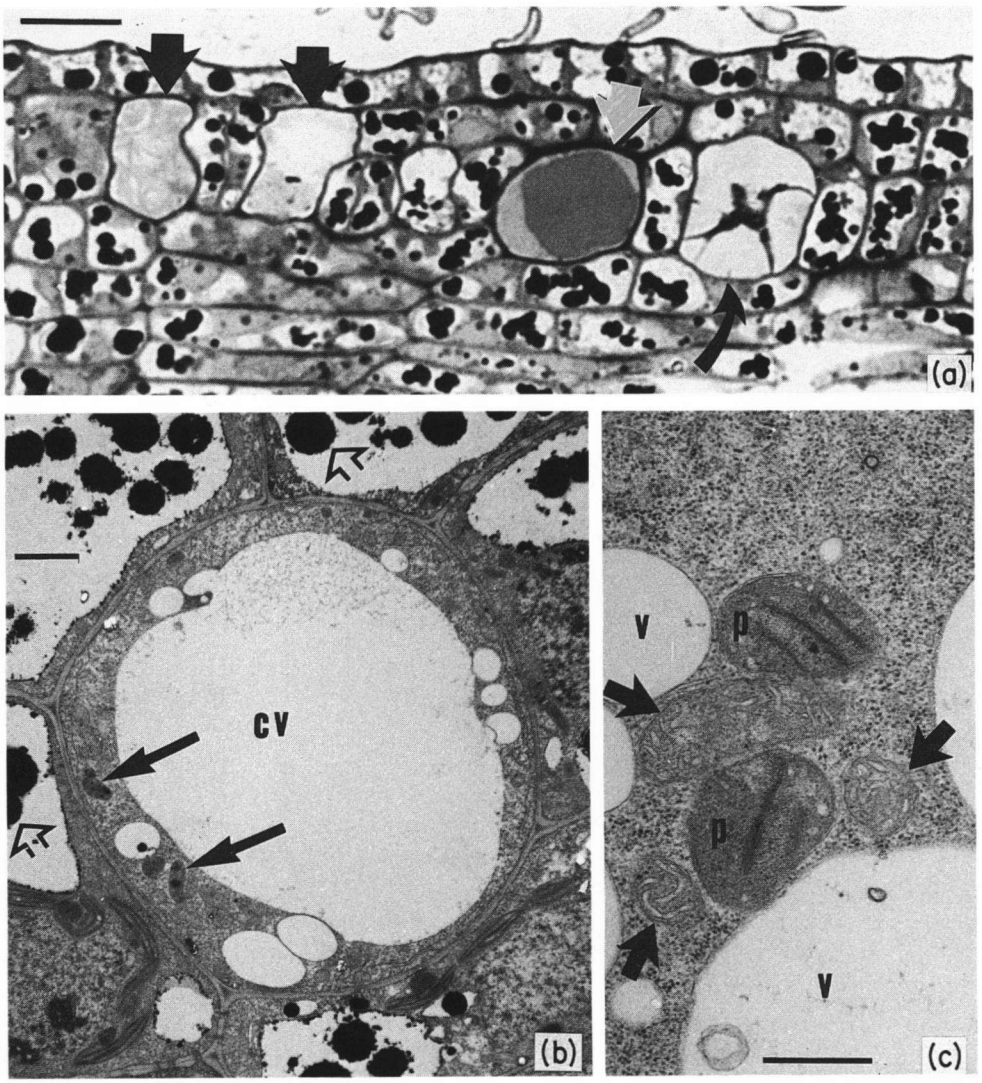

Fig. 1. *Cinnamomum burmanni.* Light micrograph of a longitudinal semi-thin section through a young leaf adjacent to the shoot apex (a) and electron micrographs of idioblasts in developmental stage 1 (b, c). (a) Light micrograph showing different stages of oil and mucilage cells present underneath the epidermis. One oil cell (white arrow, in the mid) contains dense oil in the oil cavity; the other oil cells (thick arrows) contain lightly stained oil. In the mucilage cell (curved arrow) cytoplasmic strands are present. Bar = 20 μm. (b) An idioblast at developmental stage 1. Note the central vacuole devoid of deposits and the presence of typical plastids (arrows). Adjacent cells show osmiophilic deposits in the vacuoles (open arrows). Bar = 2 μm. (c) Detail of the cytoplasm showing typical small plastids with reduced thylakoids and small peripheral translucent globules. Note the presence of mitochondria (arrows). Bar = 0·5 μm.

Subsequently to the formation of the suberized layer additional wall material was deposited. In one cell vesicles were present in the extraplasmatic space (Fig. 3a). In another cell a very thin layer of additionally deposited wall material (41 ± 12 nm thick) was present (Fig. 3b). The cytoplasmic composition of these cells, containing many mitochondria, small plastids and a slightly increased amount of dictyosomes did not yet indicate the type of idioblast.

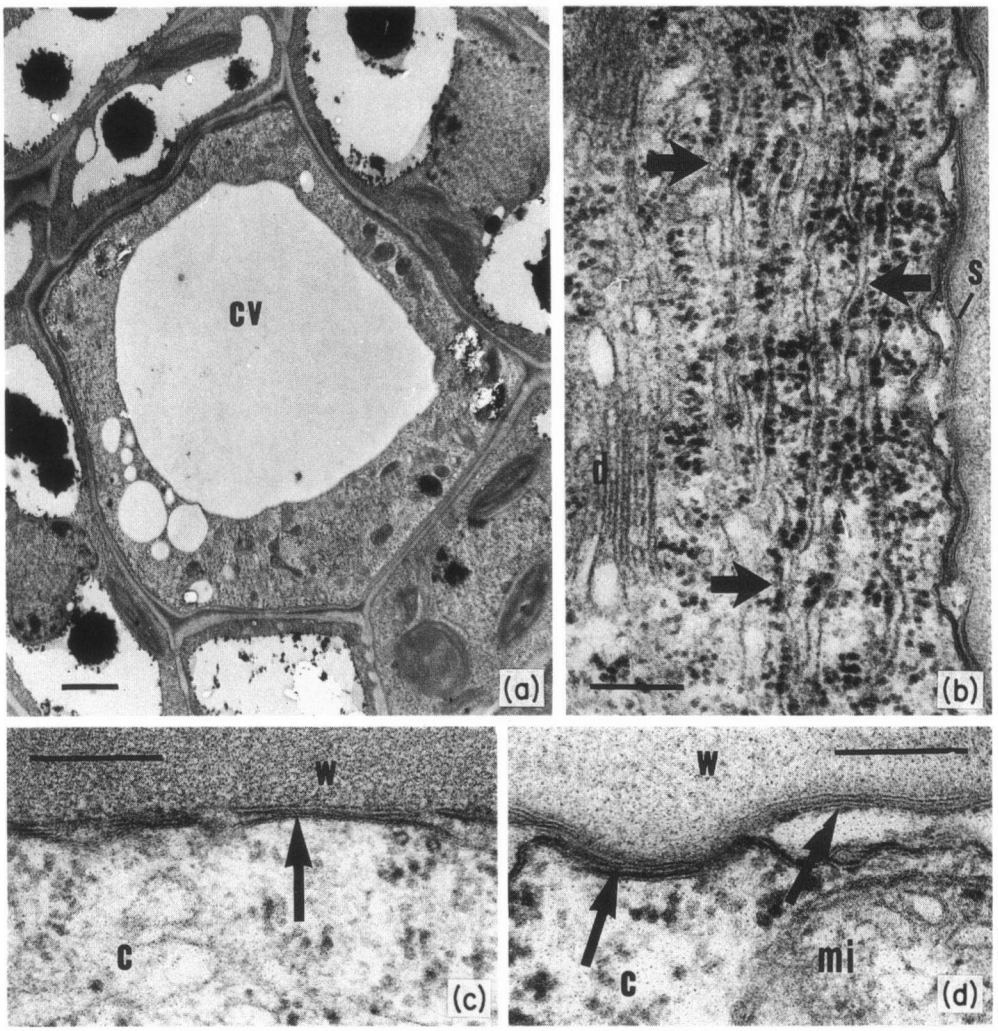

Fig. 2. *Cinnamomum burmanni.* Electron micrographs of idioblasts at developmental stage 2. (a) Idioblast resembling a cell at stage 1 (Fig. 1b) by containing a vacuole devoid of deposits and the typical plastids. However, a suberized layer has been deposited on the inner side of the cell wall. Bar = 2 μm. (b) Detail of the cytoplasm showing RER cisternae aligned parallel to the cell wall (arrows). Bar = 0·2 μm. (c) Detail of cell wall with three discontinuous lamellae of the suberized layer (arrow). Bar = 0·1 μm. (d) Detail of a suberized layer consisting of approximately six lamellae (arrows). Bar = 0·1 μm.

Stage 3

Oil cells

Idioblasts containing oil in the cytoplasm deposited a distinct inner-wall layer against the suberized layer. The thickness of the inner-wall layer increased significantly with progressing development from 44 nm to 162 nm ($P < 0{\cdot}05$; Fig. 8). In all developmental stages of the oil cells only a few characteristic plasmodesmata were present, showing a wall thickening on the oil cell side lined with the suberized layer and the inner-wall layer (Fig. 5d). Based on the thickness of the inner-wall layer and the composition of the cytoplasm, stage

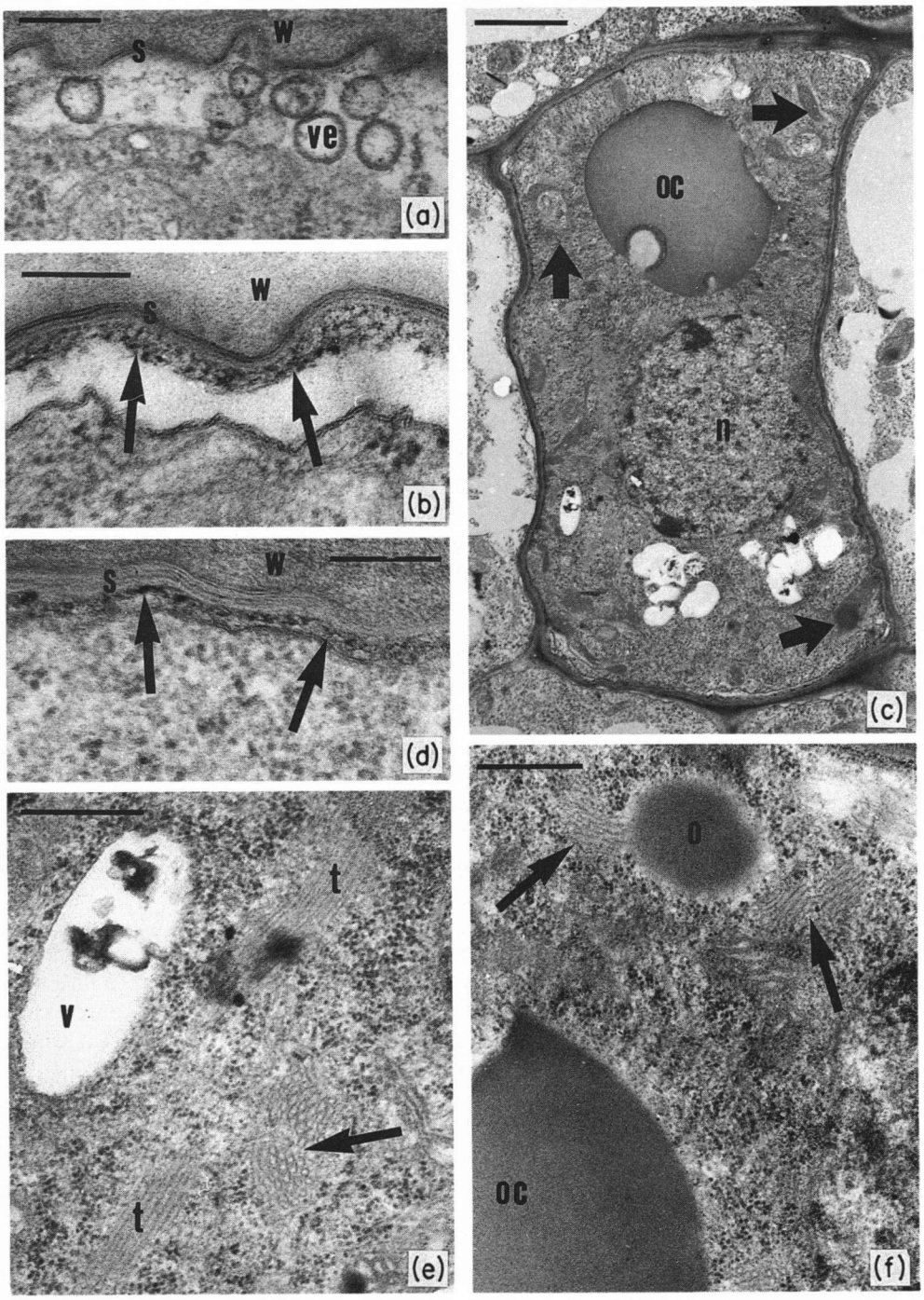

Fig. 3. *Cinnamomum burmanni.* Electron microscopical details of idioblasts at the final phase of developmental stage 2 (a–b). Electron microscopical overview and details of oil idioblasts at developmental stage 3a (c–f). (a) Detail showing the presence of vesicles in the extraplasmatic space. Bar = 0·2 μm. (b) Detail of an idioblast at the final phase of stage 2 in which a layer of additional wall material (arrows) has been deposited against the suberized layer. Bar = 0·2 μm. (c) An angular-shaped oil cell showing a small oil cavity, filled with electron dense oil. Note the absence of the central vacuole and the presence of typical plastids (arrows). Bar = 2 μm. (d) Detail of the wall of an oil cell at stage 3a showing a lamellated suberized layer and thin granular inner-wall layer (arrows). Bar = 0·2 μm. (e) Detail of the cytoplasm showing bundles of tubular ER sectioned both transversally (arrow) and longitudinally. Bar = 0·5 μm. (f) Detail of the cytoplasm adjacent to the oil cavity. Note small oil droplet (o) surrounded by bundles of tubular ER (arrows). Bar = 0·5 μm.

3 could be subdivided into three intergrading developmental stages: a, b and c. The main characteristics occurring during the oil cell development are summarized in Figure 9 and Table 1.

Stage 3a. The cells ($21 \pm 13 \times 11 \pm 9$ μm) were more or less angular in shape (Fig. 3c) and possessed a distinct suberized layer (approximately 36 ± 5 nm) and an inner-wall layer (approximately 44 ± 27 nm) (Fig. 3d). The large central vacuole had disappeared, but seemed to have been replaced by small vacuoles with wavy outlines (Fig. 3c). The cytoplasm was more compact than in the neighbouring cells (Fig. 3c) and contained a small oil cavity (Fig. 3c). Typical plastids, sometimes containing a large starch granule or an electron dense globule (Fig. 3c, lower right), were partly surrounded by appressed ER strands (not shown) and in some cases showed ring-like shapes (Fig. 3c, upper left). Other specific features were the presence of smooth tubular ER, appearing as bundles when sectioned longitudinally (Fig. 3e and f) or as groups when cut transversally (Fig. 3e) and of small oil droplets in the cytoplasm (Fig. 3f). Small cisternae of rough ER were present and ribosomes were grouped loosely in the cytoplasm as polysomes (Fig. 3e and f).

Stage 3b. The cells ($24 \pm 7 \times 17 \pm 4$ μm) were almost spherical (Fig. 4a). In the suberized layer (36 ± 6 nm; Fig. 8) the lamellation was not always distinct (Fig. 4c). Sometimes only the outermost lamellae could be detected. The inner-wall layer thickness had increased to 107 ± 54 nm (Fig. 4c). In most cases the groundplasm showed less structural definition than in the surrounding cells. The oil cavity had increased in volume and contained less electron dense oil (Fig. 4a). A cup-shaped cupule (Fig. 4a and b) was found in four cells out of the 31 cells assigned to this stage, implying that if entire cells had been serially sectioned, each oil cell would be expected to possess one cupule (see discussion). This structure is attached to a thickened part of the inner-wall layer: the cupule base (Fig. 4b), was *circa* 4·0 μm wide and 0·3 μm thick at the site of attachment. The cupule itself (*circa* 1·7 μm wide and 1·1 μm high) was variable in outline (Fig. 4b). The oil cavity was distinctly enclosed by the plasmalemma first (Fig. 3c), but later the plasmalemma partly disintegrated (Fig. 4a). In this stage the plastids disappeared and seemed to turn into vacuoles and to fuse with each other, thereby forming a mass of vacuoles, partly filled with thread-like contents in a reticulate pattern (Fig. 4a and d). Sometimes small amounts of oil were present within these vacuoles. The plastids (or their remnants) fused with the oil cavity presumably depositing oil into it; at these sites the plasmalemma often appeared interrupted. Individual oil droplets also fused with the plasmalemma surrounding the oil cavity. In the later phases of this developmental stage the vacuoles formed a zone lining the oil cavity, which was partly included within it. In addition in some cells were detected plastids, which gave the impression that their contents flowed out of the organelle into a vacuole surrounding the plastid (Fig. 4e). Bundles of tubular ER were present in the cytoplasm but lost their structural integrity. The other cytoplasmic organelles were poorly defined (Fig. 4a) and sometimes the cytoplasm had darkened by the abundance of loosely grouped ribosomes (e.g. Fig. 4b and d).

Stage 3c. The cells ($25 \pm 5 \times 18 \pm 3$ μm; Fig. 5a), nearing maturity, included cytoplasm that differed in electron density from the surrounding cells (Fig. 5a). The suberized layer was *circa* 36 ± 10 nm thick (Fig. 5c and d) and mostly showed up as a translucent layer. Sometimes the outermost lamella could be detected (Fig. 5c and d). The inner-wall layer (*circa* 162 ± 69 nm thick) was in most cases very electron dense (Fig. 5c and d). The

Table 1. Main similarities and differences in the developmental stages 3a–c of oil cells and mucilage cells in the shoot apex of *Cinnamomum burmanni*. For comparison, the characteristics of ground tissue cells are included

	Oil cells	Mucilage cells	Ground tissue cells
Cell size at maturity	Spherical 25 × 18 μm	Spherical 21 × 20 μm	Variable shapes mostly smaller
Cell wall at maturity	Typically 3-layered with suberized layer and inner-wall layer Cupule present Few specific plasmodesmata Local breakdown	Typically 2-layered with suberized layer First deposited mucilage resembles inner wall layer Sometimes a cupule present Few specific plasmodesmata Local breakdown	No additional wall layers Cupule absent Many normal plasmodesmata
Central vacuole	Absent at stage 3a; small vacuoles, devoid of deposits, disappear at stage 3c	Present at stage 3a; devoid of deposits. Disappears at stage 3b	Present with deposits
Plastids	Typical small plastids with reduced thylakoids disappear at stage 3b	Typical small plastids with reduced thylakoids loose their definition at stage 3c	Larger plastids with distinct thylakoids
Accumulation	Oil is stored in the oil cavity between cupule and plasmalemma	Mucilage is deposited between the wall and the plasmalemma	Osmiophilic granules in the central vacuole
Main organelles involved in secretion	Oil-formation in the plastids Migration guided by tubular ER Final fusion with oil cavity membrane: the plasmalemma	Mucilage secretion by hypertrophied dictyosomes Vesicles finally fuse with the plasmalemma	Does not apply
Cytoplasm	Formation of a parietal layer at stage 3c and final degeneration	The parietal layer is forced inward during development and finally degenerates	Includes distinct organelles and groundplasm

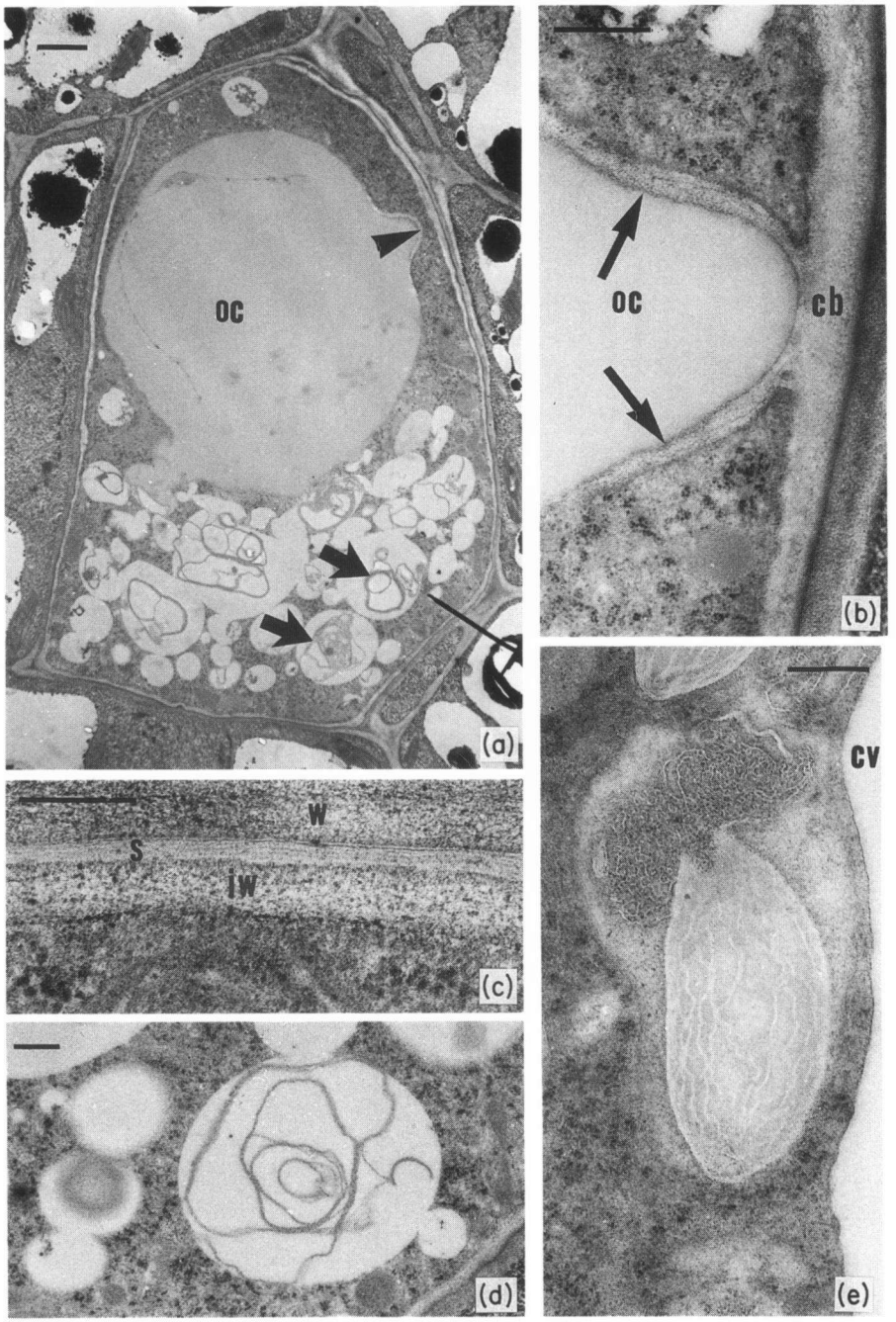

Fig. 4. *Cinnamomum burmanni*. Electron microscopical overview and details of oil idioblasts at developmental stage 3b. (a) An oil cell with an enlarged oil cavity, attached to the cupule (arrowhead), containing more or less electron-translucent oil. Note the cell shape and the vacuolar masses filled with thread-like contents (arrows). Bar = 2 μm. (b) Detail of the cupule depicted in Fig. 4a as seen a few sections from that plane. Note the cupule base and the cupule (arrows). The cytoplasm looses definition. Bar = 0·5 μm. (c) Detail of the thickened inner-wall layer. Note the suberized layer, in which the lamellae are mostly indistinct. Bar = 0·2 μm. (d) Detail of fused vacuoles containing thread-like contents possibly originating from degenerating plastids. Bar = 0·5 μm. (e) Detail of a plastid seemingly exuding its contents into a vacuole surrounding the plastid. The contents are visible as a granular mass. Bar = 0·5 μm.

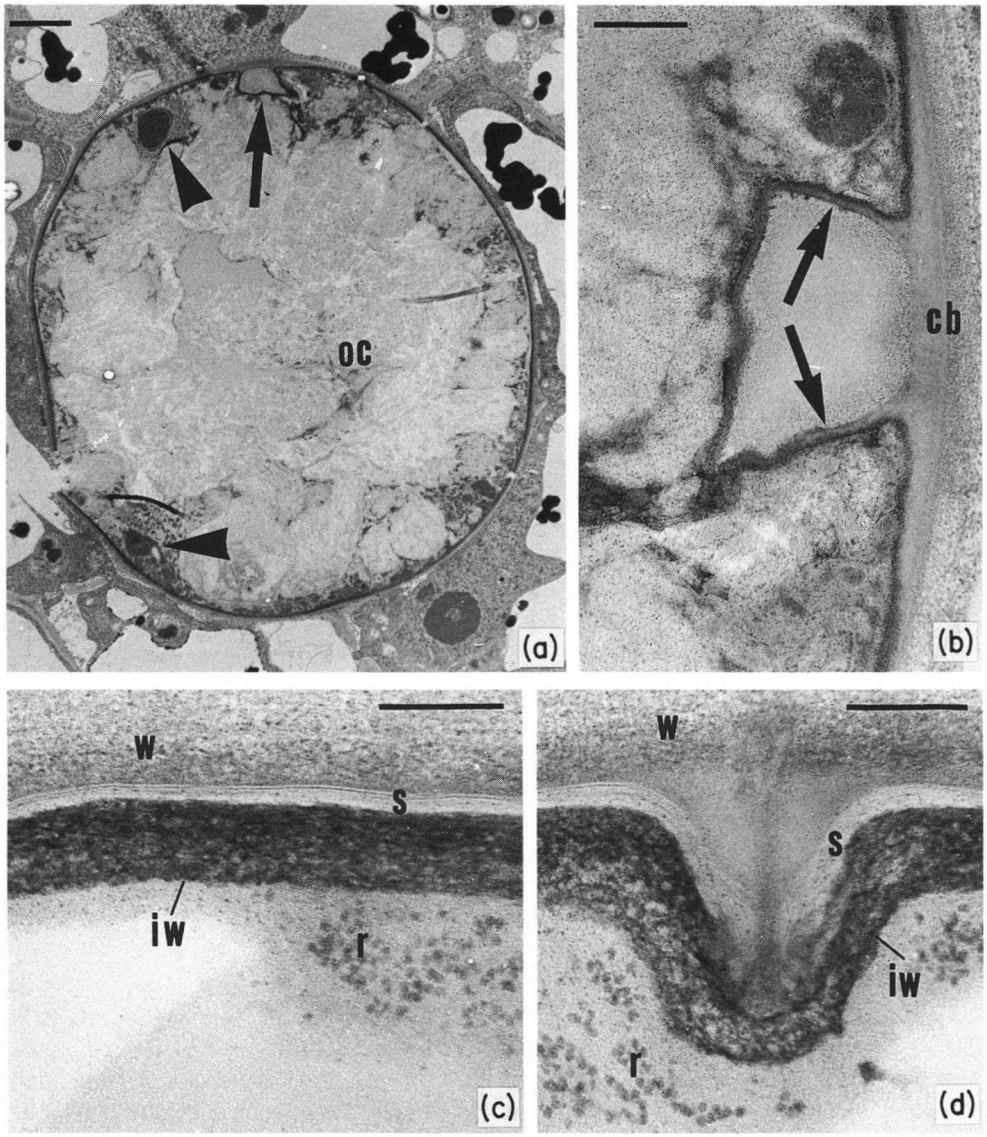

Fig. 5. *Cinnamomum burmanni.* Electron microscopical overview and details of oil idioblasts in developmental stage 3c. (a) Oil cell with an enlarged oil cavity, no longer enclosed by the plasmalemma and including cytoplasmic remnants. A cupule (arrow) and some degenerating organelles (arrowheads) are visible. Bar = 2 µm. (b) Detail of the cupule depicted in Fig. 5a. Note the cupule base and the obliquely sectioned cupule (arrows). Bar = 0·5 µm. (c) Detail of the cell wall containing an electron-translucent suberized layer with only the outer lamellae and the electron-dense inner-wall layer which has increased in thickness. Note the loosely grouped ribosomes. Bar = 0·2 µm. (d) A plasmodesma in the cell wall of a near mature oil cell. Note the specific bulge intruding into the cytoplasm. Bar = 0·2 µm.

cytoplasmic organelles further lost their structural integrity (Fig. 5a), but groups of ribosomes were discernable (Fig. 5c and d). The remnants of the plastids were included within the oil cavity (Fig. 5a), which was no longer delimited by the plasmalemma and was absorbed by the cytoplasm, leaving a small, parietal layer of discernable groundplasm

(Fig. 5a). Out of 32 cells in this stage, we detected three cells possessing a cupule (Fig. 5a and b). The cupule base was *circa* 2·9 μm wide and 0·3 μm thick; the cupule was *circa* 1·1 μm wide and 0·9 μm high (Fig. 5b). At full maturity the cells showed a complete degeneration of the cytoplasm. The oil filled the whole volume, mixed with indistinct remnants of cytoplasmic organelles. In some oil cells, a local breakdown of the cell wall was present (not shown) and oil had flowed into the adjacent cell, which certainly was not an idioblast, as was evident by the absence of a suberized-wall layer. In few cases the cell wall was broken at more than one site.

Mucilage cells

Some ultrastructural aspects of mucilage cell development have been described in a previous paper (Bakker & Gerritsen 1989). Although the present results confirm our earlier observations, we use a different numbering of the developmental stages for easy comparison with oil cells. Developmental stage 1 was not included in the earlier paper; stage 2 was described as stage 1 and the stages 3a, 3b and 3c replace the earlier described developmental stages 2, 3 and 4 respectively.

Stage 3. This stage comprised all cells in which mucilage was secreted and deposited in the extraplasmatic space between the suberized layer and the cytoplasm. In the cell wall few characteristic plasmodesmata were detected (Fig. 6d). With the onset of mucilage secretion, as seen by the abundance of hypertrophied dictyosomes, developmental stage 3 starts and can be subdivided into three intergrading stages: a, b and c. The main characteristics of the mucilage cells are summarized in Figure 9 and Table 1.

Stage 3a. The cells ($14 \pm 7 \times 10 \pm 4$ μm) had a collapsed appearance. The composition of the cytoplasm differed from the preceding stages by an abundance of hypertrophied dictyosomes budding off vesicles (Fig. 6a). The suberized layer (*circa* 43 ± 6 nm thick) showed its characteristic lamellae (Fig. 6b). Mucilage had just started to be deposited as a layer between the suberized layer and the cytoplasm (Fig. 6b). The latter was pushed inward, but the central vacuole was still present.

Stage 3b. The cells ($30 \pm 7 \times 21 \pm 8$ μm) had a more rounded outline. The main component in the cytoplasm was formed by dictyosome vesicles, filled with mucilage, which finally fused with the plasmalemma (Fig. 6c). Most other cytoplasmic organelles were indistinct. However, plastids could well be recognized by the presence of a distinct starch granule (Fig. 6c). In a few cases connections were detected between a cytoplasmatic strand and a plasmodesma in the cell wall (Fig. 6d). The suberized layer was *circa* 41 ± 9 nm thick (Fig. 6d and e). The first zone of deposited mucilage, appressed against the suberized layer was more compact that the later secreted mucilage (Fig. 6e). The cytoplasm had been forced to move inward by the prolonged mucilage deposition at the cost of the central vacuole.

Stage 3c. The cells ($21 \pm 8 \times 20 \pm 13$ μm) are round to oval in shape. The suberized layer is 37 ± 7 nm thick. The cells were completely filled with mucilage in which only a few cytoplasmic strands were present (Fig. 1a). The cytoplasm mainly contained dictyosome vesicles and finally degenerated. In mature mucilage cells, local breakdown of the cell wall was noted more than once. The mucilage penetrated the non-idioblastic neighbouring cell (not shown). Sometimes more than one opening was found in a mucilage cell wall.

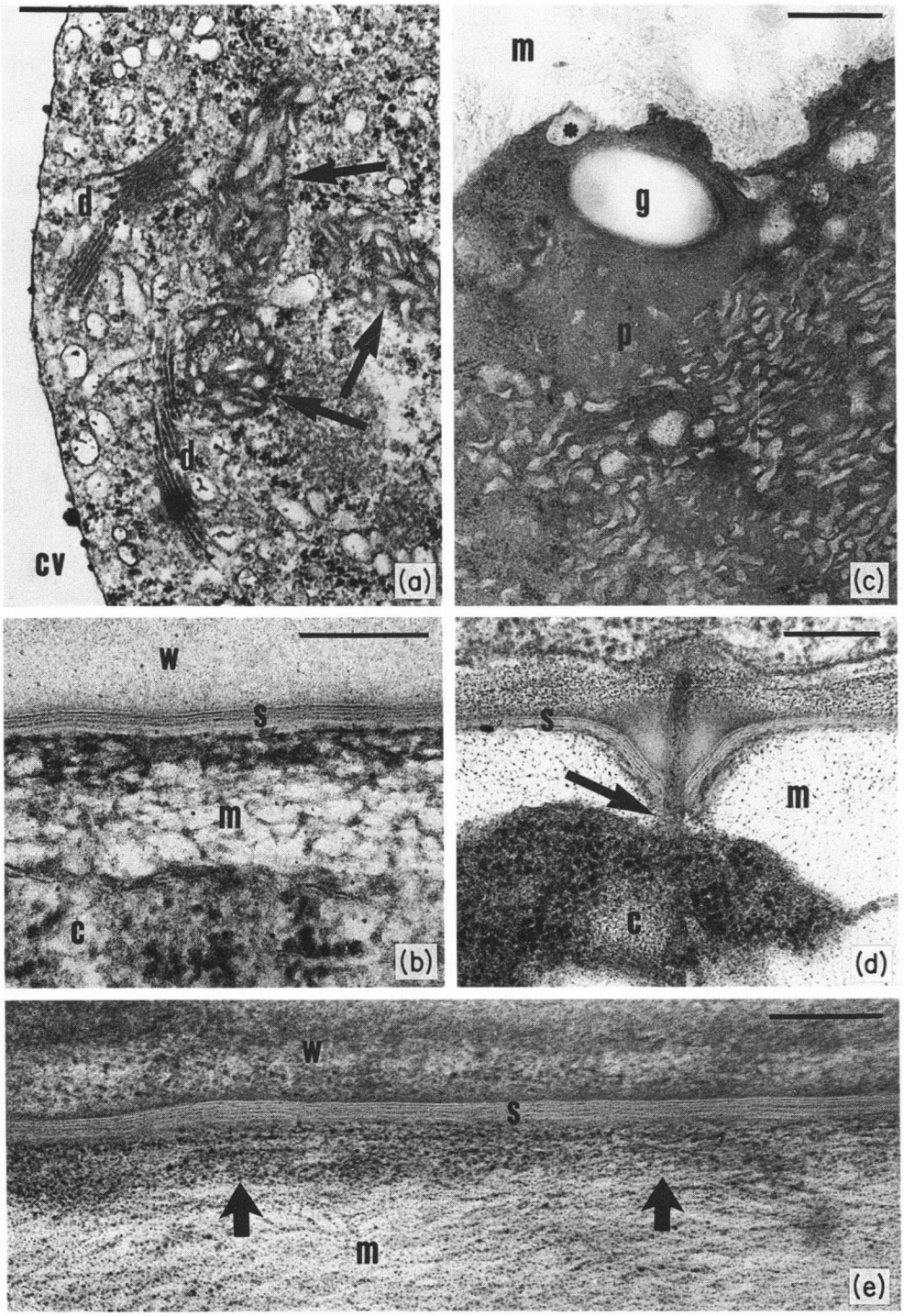

Fig. 6. *Cinnamomum burmanni.* Electron microscopical details of mucilage cells at different developmental stages. Fig. 6a–b = stage 3a, Fig. 6c–e = stage 3b. (a) Detail of the cytoplasm of a mucilage cell at stage 3a containing several dictyosomes budding off vesicles. Also note mitochondria (arrows). Bar = 0·5 μm. (b) Cell wall in a mucilage cell at stage 3a with mucilage deposited in the extraplasmatic space between the lamellated suberized layer and the plasmalemma. Bar = 0·2 μm. (c) Detail of the cytoplasm of a mucilage cell at stage 3b composed of dictyosome vesicles, which have lost their structural integrity. Note the vesicle fusing with the plasmalemma (*) and the indistinct plastid including a persistent starch granule. Bar = 0·5 μm. (d) Plasmodesma in a mucilage cell connected with the cytoplasmic strand embedded in the mucilage. Bar = 0·2 μm. (e) Cell wall of an older mucilage cell including the lamellated suberized layer. The first deposited mucilage appressed against the wall (arrows) is more compact than the later secreted mucilage. Bar = 0·2 μm.

Cupules in mucilage cells

In two mucilage cells structures were found more or less identical to the cupules in oil cells (Fig. 7a and b). Serial sectioning revealed that these structures showed a different shape but had a mean size comparable to that of cupules found in oil cells (compare Fig. 4b with Fig. 7b). The cupule base was approximately 3·9 μm wide and 0·3 μm thick and the cupule itself was *circa* 1·4 μm wide and 1·0 μm high. No oil was detected in these mucilage cells. In one mucilage cell only a cupule base was present.

Another young mucilage cell, containing a thin parietal layer of mucilage, possessed two very small cupules (0·7 μm wide and 0·5 μm high) on different places on the cell wall (Fig. 7c, d and e). However, the cupule bases were of the same dimensions as those found for oil cells (3·4 μm wide and 0·2 μm thick). One cupule surrounded an electron dense globule, perhaps oil (Fig. 7d). The cytoplasm in this cell resembled that of young oil cells by the absence of a central vacuole and presence of vacuoles with wavy outlines (Fig. 7c), but no oil droplets or bundles of tubular endoplasmic reticulum were found in the cytoplasm.

DISCUSSION

Characteristics of oil idioblasts during development

In the present study the absence of osmiophilic deposits in the vacuoles and the presence of typical plastids turned out to be useful criteria for recognition of future oil and mucilage cells (Fig. 1b and c). The absence of deposits was also used as a characteristic for future oil cells by Postek & Tucker (1983) and Bakker & Gerritsen (1990). The presence of typical plastids, possessing reduced thylakoids, is by now generally reported for future oil cells (Fahn 1979; Maron & Fahn 1979; Mariani *et al.* 1989; Bakker & Gerritsen 1990), but in the present study it also applies for future mucilage cells. As with *C. burmanni*, both types of idioblasts occur together, so we can recognize future oil or mucilage cells before the deposition of suberin but the presence of oil cells can only be ascertained after the oil secretion has started. Some authors, studying species which exclusively possess oil cells, could not distinguish young oil cells before the suberin deposition (Scott *et al.* 1963; Amelunxen & Gronau 1969; Platt-Aloia *et al.* 1983).

The cell wall of mature oil cells is typically three-layered (Amelunxen & Gronau 1969; Wattendorff 1974: Fahn 1979; Maron & Fahn 1979; Platt-Aloia *et al.* 1983; Postek & Tucker 1983; Baas & Gregory 1985; Mariani *et al.* 1989; Bakker & Gerritsen 1990 and the present study). The thickness of the suberized layer in different species varies from 36 nm in *C. burmanni* (this study) to 90 nm in *Acorus* (Amelunxen & Gronau 1969). The inner-wall layer in mature oil cells of *C. burmanni* (162 nm, Fig. 8) is twice as thick as in *Annona muricata* (88 nm; Bakker & Gerritsen 1990) and for *Acorus* even 180 nm was reported (Amelunxen & Gronau 1969) perhaps indicating taxon specific variation.

The presence of a cupule (contradicted by Amelunxen & Gronau 1969, Platt-Aloia *et al.* 1983 and Postek & Tucker 1983) is another characteristic which now is generally accepted to be typical for oil cells as corroborated by TEM studies (Scott *et al.* 1963; Fahn 1979; Maron & Fahn 1979; Mariani *et al.* 1989; Bakker & Gerritsen 1990 and the present study), but this structure was already known from older studies (e.g. Zacharias 1879; Müller 1905; Lehmann 1925; Leemann 1928; West 1969). The three-dimensional structure of the cupule of oil cells in *C. burmanni* closely resembles that reconstructed for oil cells in *A. muricata* (Bakker & Gerritsen 1990). It was calculated that one cupule was present per oil cell

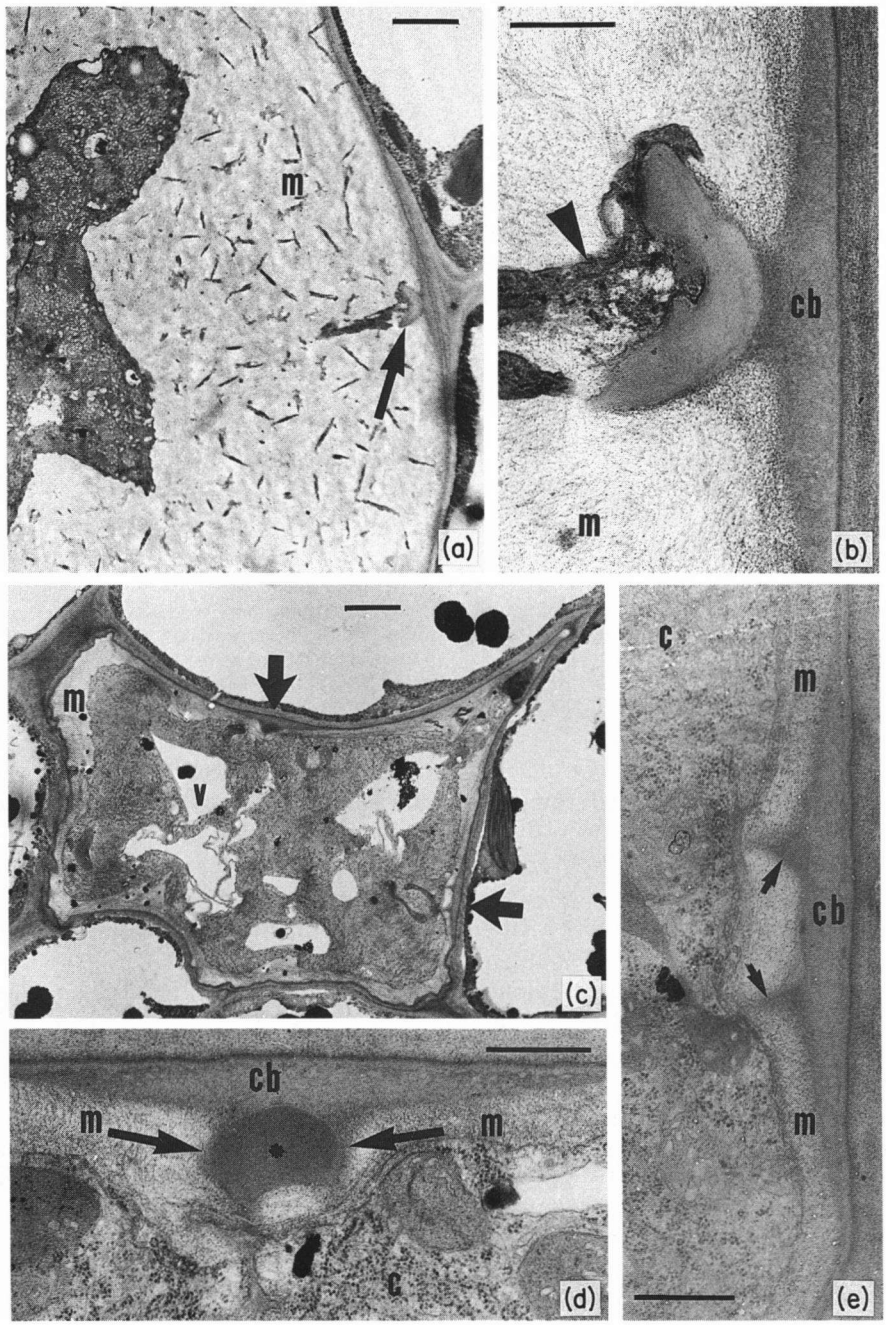

Fig. 7. *Cinnamomum burmanni*. Electron microscopical overview and details of mucilage cells containing cupules or cupule-like structures. (a) Part of a mucilage cell at stage 3b showing a cupule (arrow) in contact with a cytoplasmic strand, which is completely embedded in the mucilage. Bar = 2 μm. (b) Detail of the cupule in the consecutive section to the one depicted in Fig. 7a. Note the cupule base and the cupule itself in contact with the cytoplasmic strand. Bar = 0·5 μm. (c) A young mucilage cell possessing two small cupule-like structures (arrows). The cytoplasm resembles that of a young oil cell by the absence of a central vacuole. Bar = 2 μm. (d) Detail of the upper cupule-like structure of the cell depicted in Fig. 7c. Note the cupule base and the very reduced cupule (arrows) which surrounds an electron dense globule (*). Bar = 0·5 μm. (e) Detail of the second cupule-like structure on the right of the cell in Fig. 7a. Note the presence of a cupule base and reduced cupule (arrows). Bar = 0·5 μm.

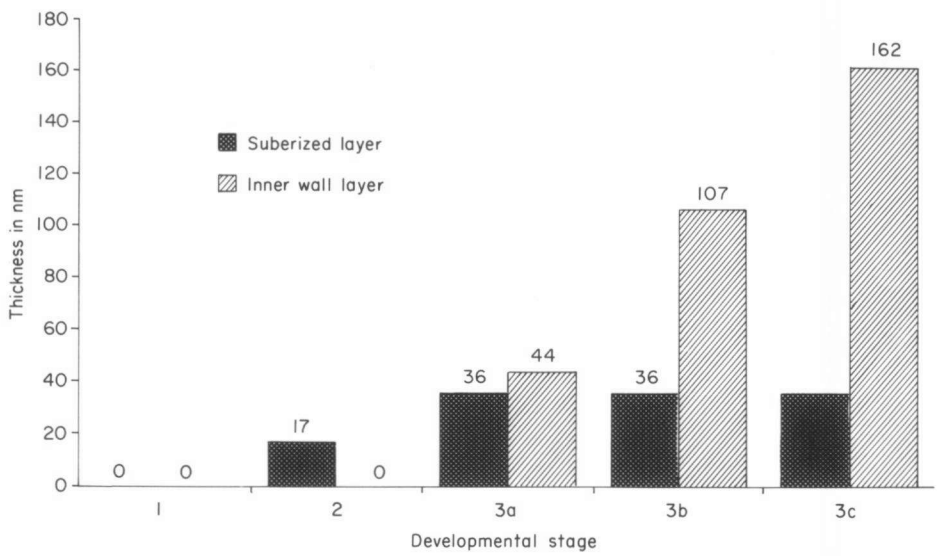

Fig. 8. *Cinnamomum burmanni*. Thickness of the suberized layer and the inner-wall layer at different stages in oil cell development (stage 1, 2, 3a, 3b and 3c). The mean value with the standard deviation at each developmental stage are: *Suberized layer*: stage 2: 17 ± 5 nm; stage 3a: 36 ± 5 nm; stage 3b: 36 ± 6 nm; stage 3c: 36 ± 10 nm. *Inner-wall layer*: stage 3a: 44 ± 27 nm; stage 3b: 107 ± 54 nm; stage 3c: 162 ± 69 nm. The numbers are average thickness-values of 10, 5, 7, 29 and 29 cells per stage respectively, each based on *circa* 25 measurements per cell.

(Bakker & Gerritsen 1990). An identical one to one occurrence can be estimated for cupules in *C. burmanni* oil cells, taking into consideration the cell proportions actually represented in the serial sections.

In the seven cell parts we observed of stage 3a of oil cell development no cupules were found. However, in analogy with the situation in *Annona muricata* (Bakker & Gerritsen 1990) we tentatively assume that a cupule also occurs in stage 3a in *Cinnamomum*. In Figure 9 it is indicated by a dotted line.

In some plastids an osmiophilic globule was present (Fig. 3c) in the younger developmental stages (stage 3a) and later, oil was detected within the vacuoles (Fig. 4a). Thus it is assumed that oil formation is more or less similar to that described for *A. muricata* (Bakker & Gerritsen 1990). As we found distinct degenerating plastids (Fig. 4e) it is supposed that the vacuolar assemblage is formed by plastidal remnants and that thus the disappearance of the plastids can be explained. Other studies on oil cell development (e.g. Amelunxen & Gronau 1969; Platt-Aloia *et al.* 1983; Postek & Tucker 1983; Mariani *et al.* 1989) do not describe specific plastidal degeneration, but only mention a general loss of definition for the cytoplasmic organelles.

The specific plasmodesmata described for oil (and mucilage) idioblasts in *C. burmanni* (Figs 5d and 6d) have also been observed in other oil cells (Maron & Fahn 1979; Platt-Aloia *et al.* 1983; Bakker & Gerritsen 1990).

Characteristics of mucilage idioblasts during development

Most features occurring in the development of mucilage cells in *C. burmanni*, such as the presence of a suberized layer, the mucilage secretion by hypertrophied dictyosomes, the inward movement of the cytoplasm forced by the prolonged mucilage deposition between the suberized layer and the plasmalemma, have been described in an earlier paper (Bakker & Gerritsen 1989). In the present study two characteristics are described for mucilage cells

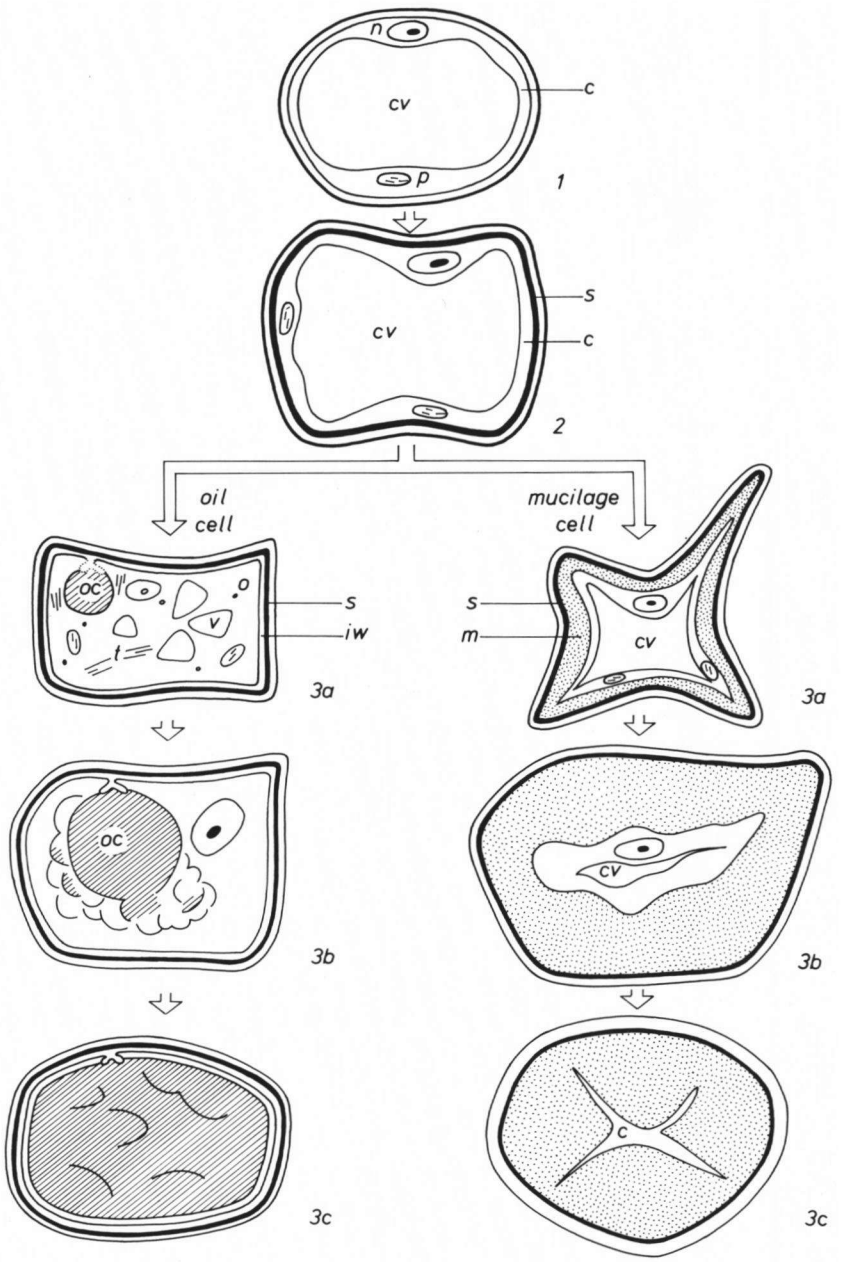

Fig. 9. Schematic representation showing the developmental stages of oil and mucilage cells in *Cinnamomum burmanni*. Stage 1: young cells with typical plastids and a central vacuole devoid of deposits. Stage 2: idioblasts with a suberized layer. *Oil cells*. Stage 3a: an inner-wall layer is present; a small oil cavity is attached to a cupule; oil droplets and bundles of tubular ER appear in the cytoplasm. Stage 3b: an enlarged oil cavity, enveloped by a partly disintegrated plasmalemma is in contact with vacuoles. Stage 3c: (near) mature cells with a very large oil cavity no longer enclosed by the plasmalemma. *Mucilage cells*. Stage 3a: Collapsed cells with mucilage deposited against the suberized layer. Stage 3b: cytoplasm moves inward due to prolonged mucilage deposition. The central vacuole disappears. Stage 3c: (near) mature cells, filled with mucilage surrounding degenerating cytoplasm.

Abbreviations used in all figures: c = cytoplasm; cb = cupule base; cv = central vacuole; d = dictyosome; g = starch granule; iw = inner-wall layer; m = mucilage; mi = mitochondrion; n = nucleus; o = oil droplet; oc = oil cavity; p = plastid; r = ribosomes; s = suberized layer; t = tubular endoplasmic reticulum; v = vacuole; ve = vesicle; w = initial cell wall.

for the first time: the presence of abnormal plasmodesmata (Fig. 6d), and (reduced) cupules (Fig. 7b, d and e). The plasmodesmata, containing a wall thickening on the idioblastic part of the cell wall, were not occluded in the mucilage cells and formed cytoplasmic connections with the adjacent cells (Fig. 6d). In oil cells this structure was occluded by the deposition of an inner-wall layer (Fig. 5d). The presence of cupules in mucilage cells is a remarkable feature (Fig. 7a–e). The presence of a cupule base is even more remarkable, because in oil cells the cupule base is formed by a thickened part of the inner-wall layer (Figs 4b and 5b). In mucilage cells there is no inner-wall layer and yet a cupule base was present (Fig. 7b, d and e), showing similarities to the cupule bases found in oil cells.

Common features during development of oil and mucilage idioblasts in C. burmanni

The suberized layer present in mucilage cells (Figs 6b, e and 9 and Table 1 in the present study; Bakker & Gerritsen 1989), resembles that in oil cells (Figs 3d, 4c and 5c) by showing the characteristic lamellation. Even the thickness of this layer at maturity was comparable: 37 ± 7 nm for mucilage cells and 36 ± 10 nm for oil cells (Fig. 8). In the past a suberized layer has been observed in mucilage cells in a few cases with light microscopy (Tschirch 1889; Scott & Bystrom 1970).

The oil and mucilage cells have strikingly angular shapes in developmental stage 2 and 3a which is possibly due to suberin deposition, which presumably blocks diffusion. Together with the low frequency of plasmodesmata this may induce a difference in osmotic pressure between the inside and outside of the cell resulting in the idioblasts becoming compressed by the turgescent surrounding cells. In young mucilage cells this effect is possibly most noticeable due to the presence of a large central vacuole. Young oil cells appear less compressed (Fig. 9). After the prolonged deposition of oil or mucilage the cell shape appears to recover.

After stage 2, but preceding developmental stage 3a, no distinction can be made yet between future oil and mucilage cells, because the first deposited mucilage or inner-wall layer material are similar in appearance. As the cytoplasmic composition in these cells did not reveal specific features of either oil or mucilage cells, it is assumed that they represent precursors of both types of idioblasts; initially developing a kind of inner-wall layer and thereafter starting the secretion of oil or mucilage respectively. Later in development the compact mucilage layer in (near) mature mucilage cells (Fig. 6b and e) still resembles the inner-wall layer in oil cells.

The presence of cupules in mucilage cells (Fig. 7b) has never been reported before. These structures more or less resemble the cupules in oil cells. The mucilage cell containing two very small cupules embedded in a layer of mucilage (Fig. 7c, d and e) possesses cytoplasm resembling that of a young oil cell. However, no oil droplets or bundles of tubular endoplasmic reticulum were found in the cytoplasm. On the other hand, the presence of a mucilage layer is an argument in favour of a mucilage cell. Therefore this cell represents an intermediate between an oil and mucilage cell. In older literature intermediate forms of oil and mucilage cells have been recorded at the light microscopical level (e.g. Tschirch 1889, 1914; Janssonius 1934; West 1969; see also discussion by Baas & Gregory 1985). Recently cupules accompanied by an oil droplet were also detected in starch accumulating cells of *Valeriana* (Lörcher 1990).

As the oil cavity is enclosed by the plasmalemma, this space forms an extraplasmatic space (Maron & Fahn 1979; Mariani *et al.* 1989; Bakker & Gerritsen 1990). In this topographical aspect mucilage deposition therefore resembles oil secretion.

The presence of typical plasmodesmata (Figs 5d and 6d) and locally destroyed cell walls in both oil and mucilage cells are other shared features.

Relation between oil and mucilage cells

The hypothesis of mutual replacement of oil and mucilage cells was proposed by Tschirch (1889, 1914) and Janssonius (1926, 1934) and was revived more recently by Baas and Gregory (1985) and Gregory and Baas (1989). Tschirch (1889, 1914) suggested that mucilage cells can develop into oil cells. This idea was refuted by Lehmann (1925) who demonstrated the absence of mucilage in oil cells. As both the inner-wall layer and mucilage are composed of polysaccharides, these structures stain in an identical way in light microscopical preparations. This explains why the inner-wall layer of oil cells was designated as a layer of mucilage by Tschirch (1914). After the initial deposition of the inner-wall layer, oil droplets are formed. This suggests a change from mucilage cell into an oil cell. At the ultrastructural level, cytoplasmic features concerning the production of oil and mucilage help to distinguish young oil and mucilage cells. In young oil cells, plastids contain oil globules and bundles of tubular ER are present, whilst mucilage cells show an abundance of hypertrophied dictyosomes. In the present study, the first developmental stages in both oil and mucilage cells (stages 1 and 2) are the same, but from stage 3a onwards both idioblast types differentiate independently from each other thereby developing specific structures for the production of either oil or mucilage.

Our detection of a cupule in mucilage cells and the ultrastructural similarities of the first deposited mucilage and inner-wall layer preceding developmental stage 3a in both idioblast types however, reduces the differences between oil and mucilage cells. Together with the shared presence of a suberized layer, an extraplasmatic space for accumulation of the secretory product, and of specific plasmodesmata, the case for homology of both idioblast types is further strengthened. Cell biological and biochemical studies are needed to elucidate by which triggers and at which stage the idioblasts start their differentiation as either oil or mucilage cells.

ACKNOWLEDGEMENTS

We thank Dr L. Goosen-de Roo (Botanical Laboratory, Leiden) for valuable suggestions and critical comments and Dr G.J.C.M. van Vliet (Hortus Botanicus, Leiden) for kindly providing samples of the cultivated *Cinnamomum burmanni*. This work was supported by the Foundation for Fundamental Biological Research (BION), which is subsidized by the Netherlands Organization for Scientific Research NWO (grant no. 811-440-023 to M.E. Bakker).

REFERENCES

Amelunxen, F. & Gronau, G. (1969): Elektronenmikroskopische Untersuchungen an den Ölzellen von *Acorus calamus* L. *Z. Pflanzenphysiol.* **60:** 156–168.

Baas, P. & Gregory, M. (1985): A survey of oil cells in the dicotyledons with comments on their replacement by and joint occurrence with mucilage cells. *Isr. J. Bot.* **34:** 167–186.

Bakker, M.E. & Gerritsen, A.F. (1989): A suberized layer in the cell wall of mucilage cells of *Cinnamomum*. *Ann. Bot.* **63:** 441–448.

— & — (1990): Ultrastructure and development of oil idioblasts in *Annona muricata* L. *Ann. Bot.* **66:** 673–686.

Fahn, A. (1979): *Secretory tissues and plants.* Academic Press, London, UK.

Gregory, M. & Baas, P. (1989): A survey of mucilage cells in the vegetative organs of the dicotyledons. *Isr. J. Bot.* **38:** 125–174.

Janssonius, H.H. (1926): Mucilage cells and oil cells in the woods of the Lauraceae. *Trop. Woods.* **6:** 3–4.

—(1934): *Mikrographie des Holzes der auf Java vorkommenden Baumarten.* 5er Band. Brill, Leiden.

Leeman, A. (1928): Das Problem der Sekretzellen. *Planta* **6:** 216–233.

Lehmann, C. (1925): Studien über den Bau und die Entwicklungsgeschichte von Ölzellen. *Planta* **1:** 343–373.

Lörcher, H. (1990): Achsenverdickung und Sprossanatomie bei Valerianaceae. *Tropische und subtropische Pflanzenwelt* **74:** 1–121.

Mariani, P., Cappelletti, E.M., Campoccia, D. & Baldan, B. (1989): Oil cell structure and development in *Liriodendron tulipifera* L. *Bot. Gaz.* **150:** 391–396.

Maron, R. & Fahn, A. (1979): Ultrastructure and development of oil cells in *Laaurus nobilis* L. leaves. *Bot. J. Linn. Soc.* **78:** 31–40.

Müller, R. (1905): Zur Anatomie und Entwicklungsgeschichte der Ölbehälter. Vorläufige Mitteilung. *Ber. der dtsch. bot. Ges.* **23:** 292–297.

Platt-Aloia, K.A., Oross, J.W. & Thomson, W.W. (1983): Ultrastructural study of the development of oil cells in the mesocarp of avocado fruit. *Bot. Gaz.* **144:** 49–55.

Postek, M.T. & Tucker, S.C. (1983): Ontogeny and ultrastructure of secretory oil cells in *Magnolia grandiflora* L. *Bot. Gaz.* **144:** 501–512.

Scott, F.M. & Bystrom, B.G. (1970): Mucilaginous idioblasts in okra, *Hibiscus esculentus* L. In: Robson, N.K.B., Cutler, D.F. and Gregory, M. (eds): *New Research in Plant Anatomy,* Academic Press, London. Supplement in bookform. *Bot. J. Linn. Soc.* **63:** Supplement 1 15–24.

—, — & Bowler, E. (1963): *Persea americana,* mesocarp cell structure, light and electron microscope study. *Bot. Gaz.* **124:** 423–428.

Tschirch, A. (1889): *Angewandte Pflanzenanatomie I.* Urban and Schwarzenberg, Vienna.

—(1914): Die membran als Sitz chemischer Arbeit. *Verhandl. der Schweiz. naturf. Gesellschaft.* II. Teil, Sektion für Botanik, 178–188.

Wattendorff, J.L. (1974): Ultrahistochemical reactions of the suberized cell walls in *Acoirus, Acacia* and *Larix. Z. Pflanzenphysiol.* **73:** 214–225.

West, W.C. (1969): Ontogeny of oil cells in the woody Ranales. *Bull. Torr. Bot. Club* **96:** 329–344.

Zacharias, E. (1879): Ueber Secret-Behälter mit verkorkten Membranen. *Bot. Zeit.* **37:** 617–645.