Pollens Development of *Rondeletia odorata* (Rubiaceae)\(^1\)

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Pollin wall ontogeny of *Rondeletia odorata* was studied with transmission electron microscopy (TEM) and scanning electron microscopy (SEM) from tetrad stage until maturity. The ontogenetic sequence of wall development in *Rondeletia* follows, to some extent, the basic scheme in the angiosperms, i.e., development starts centripetally with the pro-columellae in a plasmalemma surface coating (primexine) at the early tetrad stage when the microspores are still enveloped by callose, until intine formation in young pollen grains. The main ontogenetical features of *Rondeletia odorata* pollen are (1) the very thin irregular foot layer, (2) development of a continuous layer of radially oriented membranous granular material under the thick endexine, (3) initiation of intine before first mitosis with characteristic radial plasmalemma invaginations, and (4) a strong stretching force upon engorgement just prior to dehiscence, which leads to reduction in thickness of all wall layers. The possible function of Golgi vesicles in the considerable increase in surface area of the plasmalemma at intine initiation is discussed. The endocingulum observed on acetolysed and sectioned mature grains is explained ultrastructurally.

**Key words:** endocingulum; Gentianales; membranous granular layer; pollen wall development; *Rondeletia*; Rubiaceae; ultrastructure.

The cosmopolitan family Rubiaceae is essentially tropical and comprises \(~11\,000\) species (Robbrecht, 1988, 1994). It is regarded as a key family for understanding the phylogeny of the Gentianales. Palynological interest in Rubiaceae has recently resulted in several mostly morphological-systematic papers (e.g., Andersson, 1993; Pire, 1997; Stoffelen, Robbrecht, and Smets, 1997; De Block, 1998).

*Rondeletia odorata* Jacq. is a small tree with bright orange-red blossoms native to Cuba and Panama. The genus *Rondeletia* Linn. (\(~250\) species) belongs to the tribe Rondeletieae in the subfamily Cinchonoideae, where tribal relationships are blurred by recent fundamental changes in the systematics, e.g., by works dealing with the delimitation of the Cinchoneae (Andersson and Persson, 1991), the Isertieae (Bremer and Thulin, 1998), and the Rondeletieae (Delprete, 1996). The pollen morphology of *Rondeletia* has been studied previously with light microscopy (LM) and scanning electron microscopy (SEM) (Igersheim, 1993).

Our knowledge of pollen ultrastructure and development is surprisingly poor for such a vast family as Rubiaceae. Transmission electron microscope (TEM) images of mature pollen exist have been published for a few genera, mainly in systematic papers (Johansson, 1987; Igersheim and Weber, 1993; Weber and Igersheim, 1994; Endress et al., 1996; Tilney and van Wyk, 1997). Abadie and Keddam-Malplanche (1975) illustrated briefly two rubiaceous species with TEM.

Available data on pollen wall development are even more scarce. Andronova (1984) investigated pollen development in several species with special attention to the tapetum. In their series of light microscopy studies on the floral morphology and embryology of *Pavetta gardenifolia*, von Teichman, Robertse, and van der Merwe (1982) briefly described microsporogenesis.

There is only one study of pollen wall development of Rubiaceae, namely on *Mitriostigma axillare*, a species with permanent tetrads (Hansson and El-Ghazaly, in press). As for the order Gentianales, we know of two similar studies, one on *Catharanthus roseus* in the Apocynaceae (El-Ghazaly, 1990) and the other on the *Asclepiadaceae* (Dannenbaum and Schill, 1991).

In our work on the development of pollen in *Rondeletia odorata* we aimed at documenting the main developmental features of the exine and intine from tetrad stage until pollen maturity. Special attention was paid to relate cell organelle content of the microspores with the ontogenetic sequence of wall formation. Our data on tapetum and orbicule development in the same species will be published later.

**MATERIALS AND METHODS**

This study is based on fresh flower buds of cultivated *Rondeletia odorata* Jacq. collected in the National Botanic Garden Belgium (specimen number 39-2109) on 10 and 24 October 1996, the greenhouses of Ghent University (Belgium) on 2 December 1996, and the Botanical Institute of Stockholm University (specimen SU-c-88.24) on 2 June 1997.

The approximate stage of development can be determined by the dimensions of the anthers, but light microscopic squashes give a more accurate indication of the developmental stage. For different flower bud sizes, one anther was crushed on a slide, stained with toluidine blue, and examined with LM. Anthers with microspores in tetrads were \(~1.5\) mm long, and mature anthers were \(\geq 2.7\) mm. In *Rondeletia* the early stages of pollen development, and hence the most significant events, proceed very rapidly; this has also been observed in *Mitriostigma* (Rubiaceae; T. Hansson, personal communication, Palynological Laboratory, Stockholm).

**TEM/LM**—Decapitated anthers were fixed in 2% glutaraldehyde in 0.05
mol/L Na-cacodylate buffer, pH 7.4 for ±24 h and postfixed in 1% OsO₂ for 1 h. Anthers were blockstained with uranyl acetate for 10 min, dehydrated in a series of acetone and propylene oxide, and embedded in araldite. Ultrathin sections were stained with uranyl acetate and lead citrate. Electron micrographs were taken with a Zeiss EM906 electron microscope at 80 kV.

**SEM**—Fresh decapitated anthers were fixed as for TEM and dehydrated in an acetone series. Anthers were transferred to gelatine capsules filled with acetone 100% and frozen in liquid nitrogen and fractured on a TF-2 chamber. After critical point drying, the anther fragments were fixed on a stub by silver paint. Pollen in Figs. 28 and 29 was acetolyzed and sectioned using a Jeol lab Cryostat freeze microscope. Pollen sections were transferred to a stub. SEM observations were made with a Jeol SM6400 and a Jeol JSM-5800 LV microscope.

**RESULTS**

*Rondeletia odorata* has tetrasporangiate anthers. The anther wall typically consists of epidermis, endothecium, one, two or rarely three middle layers and tapetum. The tapetum is uninucleate and one or two layered. In rare cases we observed tapetal cells with two nuclei. The microspore mother cells (MMCs) are angular in shape with a large nucleus and a dark-staining nucleolus, which often shows one or sometimes two small vacuoles. The cell wall of the MMCs and the tapetum appears undulating.

Although the development of the pollen wall is a continuous process in *Rondeletia*, we have organized the descriptions of developmental events and cytological changes in four stages: tetrads, free microspores, young pollen grains (after mitosis), and pollen grains at dehiscence.

**Tetrad stage**—After meiosis, tetrads of haploid microspores randomly filled the entire space of the anther locules. The tetrads were arranged in tetrahedral and decussate units. Each tetrad was surrounded by a thick, asymmetrical callose envelope. The shape of tetrads in the callosic envelope was multangular (Fig. 1). Callose was thickest on tetrads in the center of the locules. The thickness of the callosic partitions between the microspore units of a tetrad was variable. The edges of the callosic layer appeared porous in early tetrads (Figs. 1, 2).

**Early tetrads**—The plasmalemma of the microspores was initially straight and in direct contact with the callose (Fig. 3). The cytoplasm was rich in vacuoles, ribosomes, and Golgi vesicles; mitochondria were not observed. Exocytosis was apparent through the fusion of vacuoles with the plasmalemma (Fig. 3). Between the callose and the plasmalemma a fibrillar surface coat developed (Fig. 3) that, upon further development, increased in thickness. This plasmalemma surface coat (primexine matrix) had a loose, irregular fibrillar texture. Its thickness was uneven on the circumference of microspores. Pro-orbicules were nestled in cup-like depressions of the plasmalemma of tapetum cells (Figs. 2–4).

In slightly older tetrads, rod-shaped electron-dense units were radially oriented in the distal part of the plasmalemma surface coat. These rods formed the columellae (Fig. 5). Sometimes more than one developmental stage was observed in the same anther. This suggests that the early events in microspore development occur rapidly.

**Late tetrads**—By end of tetrad stages, all layers of the exine were obvious (Figs. 6–9). The columellae were not solid and contained distinct cavities (Figs. 6, 7). The foot layer was thin and the endexine started to develop on a white line centered lamella (Figs. 7–9). The tectum was thin and interrupted by perforation, and the apertures were distinguished by development of numerous lamellae and thick granular material (Fig. 9).

**Free microspores**—Upon dissolution of the callosic envelope and release of the microspores from the tetrads, their walls increased considerably in thickness (Figs. 10–16). The tapetum appeared hyperactive and some cells seemed to protrude between the microspores. Tiny orbicules (~0.20 µm) with a thin continuous coat lined the surface of the tapetal cells, including the inner tangential side, the radial and the outer tangential surfaces (Fig. 10). At the end of this stage the tapetum started to mature, tapetal organelles were found in the locule (Fig. 16), and initiation of the characteristic endothecium thickenings took place. In cases where the septum between the locules was continuous, microspores in one locule showed a different stage of development from the other locule. When the septum was interrupted, all microspores in the connected locules were synchronized in development.

**Early free microspores**—The pro-columellae increased in thickness particularly at their distal ends (Figs. 10–12). On oblique tangential sections through the columellae, they appeared as darkly stained circular units with an electron-lucent, hollow center (Fig. 10). The pro-tectum was developed while additional sporopollenin-like material was deposited between the distal portions of the columellae (Figs. 11, 12). The foot layer appeared as a thin, hardly distinguishable layer at the bases of the columellae (Figs. 11–13). There were indications of a white-line-centered lamella, which separated the foot layer from the endexine during early development. The endexine continued to develop on the proximal surface of this white line and consisted of several trilamellated structures at later stages. On some sections the white lines of the endexine appeared to extend into the base of the columellae (Fig. 11). At the apertural sites, the endexine dilated into several white-line-centered lamellae with a thin coating of electron-dense material, apparently sporopollenin. These lamellae were separated from each other by fibrillar material, i.e., remains of the primexine matrix (Figs. 12, 13). Beneath the endexine, at apertural sites, a thick granular body was differentiated (Figs. 13–16). Upon further development, this granular material extended into the interapertural regions forming a continuous granular layer with radially oriented elements (Figs. 14–16). Ribosomes, mitochondria, rough endoplasmic reticulum, small vacuoles, and vesicles were common in the cytoplasm of microspores. Few dictyosomes were observed. Storage products were lacking in microscopes at this stage (Fig. 16). A prominent feature of this stage was the abundance of ribosomes (Figs. 14, 15) and endoplasmic reticulum (Fig. 15, arrowheads). The layer beneath the endexine consisted mainly of granular material and tubular units similar to those of the endoplasmic reticulum (Fig. 15). We refer to this layer as membranous granular layer (MGL). The tubular units of the MGL had direct continuities with the electron-dense endexine layer (Fig. 15). As maturation continued, the MGL became compressed and differentiated into a mixture of granules and membranous components (see Fig. 22).
Figs. 1–5. Microspores in tetrad stage. 1. Tetrads in a callosic envelope. Note the more porous edges of the callose (arrows) and the remnants of the cell walls of the MMCs in between the tetrads (SEM). 2. A tetrahedral tetrad of similar developmental stage as in 1. At bottom left, intact tapetal cells with cell wall and extracellular pro-orbicules (arrowheads). 3. Detail of one microspore in callosic envelope (C) with thin plasmalemma surface coat (arrowheads). Cytoplasm around large central nucleus rich in small vacuoles (V), free ribosomes and poorly differentiated mitochondria (M). 4. Detail of a tapetal cell and pro-orbicules (arrowheads). 5. Detail of the plasmalemma surface coat in a slightly late tetrad stage; note radial, darker stained rods (pro-columellae, arrows).
Late free microspores—As development proceeded, the microspores expanded and increased both in volume and in wall thickness. The columellae were numerous, and radially oriented, and the intercolumellar void contained fibrillar material (Fig. 17).

The sporopollenin coat on the white lines in the apertural region became thicker. In several sections we observed that the plasmalemma receded in the interapertural regions, creating a large pericytoplasmic space under the radially oriented membranous granular material. We assume that this event might indicate the very start of intine development. At the end of this stage the plasmalemma was highly undulated and protruded at several sites (Fig. 17). Several vacuoles were prominent in the cytoplasm of the microspores.

Young pollen grains—The main developmental event during this stage was the formation of the intine, the last wall layer. The young pollen grains had punctate exine and three distinct apertures (Fig. 18). The tapetum appeared to be degenerating, and tapetal organelles and lipid droplets were observed in the locale between the pollen grains (Figs. 19, 20).

The intine started to develop on the radial protrusions of the plasmalemma as a continuous, evenly thick layer between the apertures (Fig. 19). Under the apertures, however, the intine was thicker and bulged out together with part of the protoplasm (Fig. 20). The distal part of the intine, close to the radially oriented MGL, had more fibrillar structure and was more electron dense than the rest of the intine (Figs. 19, 20). None of the sections with an early intine showed two nuclei. The vegetative nucleus, however, was often excentric, but this might also be due to vacuolation. We therefore assume that initiation of the intine took place prior to first mitosis. The cytoplasm contained long profiles of rough endoplasmic reticulum parallel with the plasmalemma, many mitochondria, vacuoles of different size, and numerous dictyosomes (Figs. 19, 20). The cytoplasm was dense with vesicles that were budding from dictyosomes. These vesicles were directed toward the plasmalemma and fused with it (Figs. 21, 22). The MGL appeared slightly compact and irregular in shape (Fig. 22). The white line separating the foot layer from the endexine was still visible. The foot layer was readily distinguishable and had the same stainability as the sexine (Fig. 22). Additional sporopollenin accumulated on the exine of young pollen as well as on orbicules. The tectum appeared solid, and microchannels were presumably obscured by filling material. The columellae arcade contained fibrillar material, probably remains of the primexine (Figs. 21, 22).

The next step in the ontogenetic sequence clearly showed the spindle-shaped generative cell adjacent to the intine and surrounded with an intine-like wall (Fig. 23). Later in development the generative cell migrated toward the central vegetative nucleus. The generative nucleus was characteristically surrounded by lipid droplets (Fig. 24) during its association with the vegetative nucleus. The intine-like wall around the generative nucleus remained intact. The intine became compact and appeared more electron dense. In several sites the intine protruded between the MGL and reached the endexine (Fig. 25). Later on the MGL compressed and appeared more compact than before (Fig. 25). The cytoplasm was characterized by abundance of compound starch grains and some lipid droplets (Figs. 23, 24). The increase in lipid and starch content of the young pollen grains was concurrent with abundance of lipid droplets in tapetal cells. Ribosomes, rER, and small mitochondria were abundant near the undululating plasmalemma. Dictyosomes and Golgi vesicles, common in early pollen grains, were absent in this stage.

The volume of the pollen grains progressively increased, resulting in a stretching force on the pollen wall (compare Figs. 19 and 24). The effect of this stretching was more pronounced in pollen grains at dehiscence. The intine considerably thickened under the apertures. The aperture was coated with the fibrillar layer of the intine (Fig. 26) and the colpus membrane appeared of endexinous nature (Fig. 27).

Pollen grains at dehiscence—The pollen grains were fully engorged and appeared more or less circular in outline. Mature pollen grains taken from dehisced anthers were covered by a considerable amount of lipidic material (positive staining with Sudan III), which may be classed as pollenkitt (Fig. 28). This material was released by the degenerating tapetal cells, which were compressed into a tapetal membrane, densely covered by orbicules (Fig. 28).

The pollen wall was extremely stretched due to an increase in volume compared to the previous stage. This stretching affects all layers of the wall, i.e., all layers decreased in thickness. At the final stage of pollen development the intine was very thin. On the equator the apertures were covered with darkly stained tangentially oriented tubules or lamellations with a hollow, electron-transparent center (Fig. 29 and insertion). The MGL became more widely spaced and appeared discontinuous due to outstretching of the sporoderm (Fig. 30). This is obvious on full outlines of mature pollen grains (Fig. 31).

Lipid droplets were observed, often in association with the apertures (Fig. 31). The lipid bodies were much more numerous than starch grains and they were mostly enfolded in a single rER strand (Figs. 29, 31). In some sections lipid globules were conspicuously associated with the generative nucleus/sperm cells. Moreover, we observed numerous dictyosomes, elongated mitochondria, and a large number of free ribosomes (Fig. 31). The generative cell was enclosed by the intine-like wall (Fig. 31). Several pollen grains showed elongated, spindle-shaped sperm cells; thus some grains were trinucleate at dehiscence.

Mature pollen grains (LM and SEM)—The mature pollen grains were monads, small [P (polar axis) 16–(17.7)–19 μm, E (equatorial diameter) 18–(19.3)–21 μm, oblate spheroidal (P/E 0.92 on average) in equatorial view and subtriangular in polar view. The grains were planaperturate generally with three, rarely four, compound apertures. The short and narrow ectocolp was acute ends. There was a lololate pore in the

and protectum elements in distal part of surface coat. Scale bar = 0.5 μm.

Figure Abbreviations: A, apertural region; C, callose; CM, colpus membrane; Ec, endocingulum; G, granular material; GN, generative nucleus; I, intine; L, lipid droplet; M, mitochondria; MGL, membranous granular layer; N, nucleus; O, orbicules; S, starch; T, tapetum; V, vacuole; VN, vegetative nucleus. Scale bars = 1 μm, unless stated otherwise.
Figs. 6–9. Microspores in late tetrad stage. 6. The callosic envelope (C) starts to disintegrate. The pro-tectum, pro-columellae, foot layer, and endexine are distinguishable. Note the start of aperture development and presence of several lamellae at aperture sites. 7. Magnified part of the wall showing pro-columellae with cavities (arrowhead), very thin foot layer, and endexine. The plasmalemma surface coating is obvious between the pro-columellae. 8. Magnified part of a microspore wall and an aperture, indicating the separation of lamellae towards the aperture and the presence of coarse granular material at aperture site. Arrowhead indicates the remnant of the callosic envelope. 9. Magnified part of the wall to emphasize the thin foot layer (arrowhead) and endexine with white line centered lamellae (arrow). Scale bar of Figs. 7–9 = 0.1 μm.
Figs. 10–12. Early free microspores. 10. Overview of several free microspores with large central nucleus (N). Tectum, columellae, foot layer, and endexine are recognizable. In apertural regions granular material is deposited (arrowheads). On oblique sections, at bottom left, columellae in cross section appear hollow (arrows). At bottom right, part of a tapetal cell (T) and extracellular orbicules with thin sporopollenin coat are shown. 11. Sporoderm in cross section not far from aperture at the right. Note parallel white-line-centered lamellae (in distal part of the endexine) tending to go up into base of columellae; the foot layer is very thin and hardly distinguishable. The plasmalemma retracts to give space to loosely arranged granular material under the endexine, initially only in apertural regions. Scale bar = 0.5 μm. 12. Sporoderm in apertural region showing dilated endexine with separated white-line-centered lamellae covered with sporopollenin and thick granular body (G) underneath.
Figs. 13–16. Free microspores. 13. Detail of microspore wall in apertural region (aperture to the right); tops of columellae are stained darkest and are connected to form the tectum. The white lines in the endexine are only visible in the distal portion; the foot layer is extremely thin. Close to the apertures, the endexine consists of several white-line-centred lamellae that are coated with sporopollenin (arrows) and separated by fibrillar material. Underneath this structure deposition of the granular material (G) takes place. Scale bar = 0.25 μm. 14. Slightly later in development than in Fig. 13. The granular material is now a continuous layer (MGL) under the endexine. Scale bar = 0.5 μm. 15. Slightly oblique section showing condensation of the granular material into radially oriented elements. Note tubules in the peripheral cytoplasm, perpendicular on plasmalemma (arrowheads), which continue through the exine up to the columellar arcade (arrows). Scale bar = 0.5 μm. 16. Overview of full microspore showing a continuous membranous granular layer under the endexine.
Figs. 17–20. Initiation of intine. 17. Late free microspore with excentric vegetative nucleus (N) and well-developed exine, including continuous granular layer under endexine; plasmalemma is undulating (arrowheads), indicating start of intine formation. Note fibrillar deposits between columellae. 18. SEM of pollen grains (in same stage as Fig. 19). Exine punctuation and apertures are well developed. Note imprints of microspores in tapetum (T). 19. Slightly later in development than in Fig. 17. Vegetative nucleus (VN) with prominent nucleolus and start of generative cell (GN). Initiation of intine on distal face of the undulating plasmalemma (arrowheads). Maturing tapetal cells (T) with orbicules in upper right corner (arrows). Note tapetal organelles and lipid droplets in locule between the young pollen grains. 20. Detail of aperture in same stage as Fig. 19. Note highly undulating plasmalemma producing membrane fragments (arrowheads). Active cytoplasm with rER-strands, mitochondria, small vacuoles, and many vesicles. At the left, remnants of maturing secretory tapetal cells with many lipid droplets (L) in contact with the exine.
DISCUSSION

The discussion will focus on the characteristic features of the development of *Rondeletia* pollen and attempt to relate cytological events with the ontogenetic sequence of sporoderm formation.

**Pattern initiation of early exine**—During the callose period the exine is initiated as rods extending from the plasmalemma. These rods are exine units that become columellae as well as part of the tectum and foot layer. Oblique sections of the early exine show that the tectum consists of the distal portions of close-packed exine units. The pro-columellae are tubular in structure, and they subsequently develop into mature wall elements by the accumulation of sporopollenin. We assume that units of pre-exine are continuous with structures in the cytoplasm, which are cytoskeletal.

In early tetrad stages of *Rondeletia* microspores, when the tectum becomes evident, the exine units show a honeycomb pattern resulting from close packing and interdigitation of the units. Dickinson (1970) showed the occurrence of such a honeycomb arrangement in *Lilium*. El-Ghazaly and Jensen (1985 and 1986) presented a similar pattern in *Triticum* in early stages of microspore development. Wodehouse (1935) pointed out that a reticulate pattern is a common theme in nature and is formed where even shrinkage occurs within a uniform matrix.

**Wall layers that deserve special attention**—Columellae—The columellae in *Rondeletia* pollen are the first wall component to be synthesized in the fibrillar plasmalemma coat in early tetrad stage. In oblique (tangential) sections from the early free microspore stage, the columellae appear circular with an electron-lucent center. Thus in three dimensions they are hollow cylindrical and radial supportive elements. Later in development the centers become obscured by material of the same electron density as sporopollenin. Nowicke, Bittner, and Skvarla (1986) used plasma-ashing on many species of *Paeonia* and found that rod-shaped substructures were evident in pollen that had not been observed before ashing. Blackmore and Claugher (1984, 1987) used fast atom bombardment in studying the exines of *Fagus* and *Scorzonera* and found that the exines were composed of hollow tubes. Blackmore (1990) in a developmental study of pollen of *Echinops* found that exine processes appeared to be hollow during early stages. In

Figs. 21–22. Maturation of intine. 21. Detail of early intine (I) at aperture; plasmalemma protrusions are particularly visible; cytoplasm is bulging out at aperture and covered by darkly staining fibrillar material. Note structural differences within early intine. Dictyosome vesicles are abundant in hyperactive cytoplasm and fusing with undulating plasmalemma (arrows). Scale bar = 0.5 μm. 22. Same developmental stage as Fig. 21, but cross section of inter-apertural region. Early intine with structural differences distal from undulating plasmalemma; membranous granular layer under solid endexine is more compressed; foot layer is easily distinguishable; active cytoplasm is rich in mitochondria, rER, vesicles, and free ribosomes.
Figs. 23–27. Young pollen grains. 23. Pollen grain after first mitosis showing generative cell adjacent to intine and surrounded by intine-like wall (arrowheads); remnants of vacuole (V) in cytoplasm, and initiation of storage products, lipids (L) and compound starch grains (S). 24. Later in development, the generative cell (GN) associates with vegetative nucleus (VN) and is characteristically surrounded by lipid droplets; starch grains and lipid droplets are abundant in cytoplasm. 25. Slightly oblique cross section of sporoderm in interapertural region. Granular material compressed into thin layer under solid endexine; white line separating endexine from foot layer remains visible (arrowhead); mature intine (I) with a distal fibrillar sublayer and a proximal part with the characteristic radial membrane fragments. The distal part of the intine protrudes into the granular material, touching the endexine (arrow). 26. Cross section of sporoderm at aperture. The proximal part of the intine (I) is considerably thicker under the aperture. 27. Cross section of sporoderm at aperture, showing thick intine (I), colpus membrane (CM) is shown to be of endexinous nature.
Figs. 28–31. Mature pollen grains at dehiscence. 28. Pollen grains in locule covered with lipidic droplets (L) of tapetal origin; tapetal cells completely degenerated, orbicules (O) lying on endothecium (SEM). Scale bar = 10 μm. 29. Cross section through aperture. Cytoplasm under aperture with many free ribosomes and lipid droplets (L) characteristically surrounded by a single strand of rER, starch granules (S) less abundant than before; note the thin stretched intine (I) even under the aperture. The pore is covered with tubular endexine components with a low contrast core (arrows). Insertion shows detail of the endexine low contrast cores in cross section (small arrows). 30. Detail of sporoderm in interapertural region; all wall layers stretched and reduced in thickness, especially the membranous granular layer (MGL) and the intine (I). Scale bar = 0.5 μm. 31. Pollen grain prior to anther dehiscence with lipid droplets on its surface (L); storage products in cytoplasm are mainly lipids with fewer starch grains; the cytoplasm appears grey because of the abundance of free ribosomes and dictyosomes. The generative nucleus (GN) is located towards the periphery of the vegetative cell.
Echinodorus, El-Ghazaly and Rowley (1999) also observed probaculae with an unstaining, hollow-appearing core zone.

Foot layer—The foot layer was hardly visible in early stages. It became more pronounced and had a similar stainability as the sexine, i.e., darker than endexine, from the vacuolate stage on. In Rondeletia the junction between the endexine and the foot layer appears as a white line. Similar structure was observed in other species and described as “junction plane” (Xi and Wang, 1989; Rowley and Rowley, 1996) or “commissural line” (Simpson, 1983).

Endexine—Formation of the endexine clearly involves white-line-centered lamellae. The “lamellations” can be rodlets, but there are several reports of actually sheet-like lamellations (e.g., Stone, Sellars, and Kress, 1979). Rowley (1996) suggested that a tubular organization with reversible lateral cross-linking offers versatility for growth and internal trans-
port. Researches should consider the possibility of extensive lateral cross-linking of the rodlets or tubules into sheets. At the apertures endexine components separate from each other (most conspicuously in free microspore stage) contrary to the solid appearing endexine in interapertural regions. In these apertural regions the endexine shows lamellations with whiteline centers in longitudinal profile and tubular components with a low-contrast core in cross sections. In Poinciana (Leguminosae) Rowley and Skvarla (1987) also showed that endexine components in apertural regions were tubular with a low-contrast central core. In interapertural regions, where white-lines in the endexine are commonly believed to represent lamellae, an alternate interpretation of their morphology might be fascicles of the tubular form.

Our results show that ER units at the periphery of the cytoplasm extend radially toward the developing wall and form the membranous part of the MGL beneath the endexine. We have also observed these units further extended to the endexine and even to the arcade between columellae. Because our observations were consistent in several sections and the ER units were quite obvious, we resist the idea that ER might have a role in transfer of material between microspores or pollen grains and tapetal cells.

Membranous granular layer (MGL)—Echlin and Godwin (1969) described in Helleborus that after formation of the endexine on lamellae, deposition of sporopollenin appeared as small granules that gradually coalesce. A similar phenomenon was observed in Rondeletia where membranous tubular units intermingled with granular material accumulated on the proximal side of the endexine. A similar layer was observed in Nelumbo (Kreunen and Osborn, 1999), Nymphaea (Gabarayeva and El-Ghazaly, 1997), and Catharanthus (El-Ghazaly, 1990). The question of whether this layer is mainly developed from extensions of the ER or whether it belongs to the endexine, e.g., endexine II, or a layer not homologous with the endexine remains open. It is definitely not part of the intine since its granular part can resist acetolysis. The granular ornamentation of the inside of mature acetylated granules corresponds most likely to this granular material. A histochemical study of this layer in different species will provide useful information on the chemical nature of this layer. Such a study is in progress by the authors of this paper.

Intine—The intine, like other primary plant cell walls, develops generally after mitosis (Robards, 1970) and thus in pollen grain stage. In Rondeletia, however, intine formation might be initiated before mitosis as was also observed by Heslop-Harrison (1968) in Lilium and Silene, and in Dioscorea dumetorum (P. Schols, personal communication, Laboratory of Plant Systematics, K. U. Leuven). Although the exact timing of intine initiation might be doubtful, the mode of deposition is not. The Golgi vesicles are clearly deposited onto the plasmalemma and are spilled out by exocytosis into the periplasmic space to form the intine, which is found pressed against the exine. This is common in other families and has been observed by Echlin and Godwin (1969) in Helleborus foetidus, in other Ranunculaceae by Roland (1971), in Platanus by Suarez-Cervera and Seoane-Camba (1986), etc. In Rondeletia the irregular, sometimes branched ingrowths of the plasmalemma into the developing intine are very pronounced in late free microspore stage and immediately after first mitosis (Figs. 17, 19–22).

Apertures—We did not observe parallel endoplasmic reticulum profiles in future aperture sites as was reported for Lilium (Dickinson, 1970), Helleborus (Echlin and Godwin, 1968) and Securidaca (Coetzee and Robbertse, 1985). From early tetrad stage, however, the plasmalemma surface coat is much thiner on three equally distributed areas believed to be the destined apertures. The intine is characteristically thicker under the apertures before engorgement of the grains. Prior to dehiscence the intine becomes very much stretched and of equal thickness all around the profile of the grain (see below). In mature pollen grains we showed that the colpus membrane is of endexinous nature (Fig. 27) and that in cross sections the pore is covered with tubular endexine components with a low-contrast core (Fig. 29).

Cytology—The dynamics of the cytoplasm components during pollen ontogeny is astonishing. Cell organelles are formed according to the demands of the developmental processes. Next to this variation in time there is also variation in space. Comparison of sections of different angles in the same developmental stage showed that the cell organelles are not evenly distributed in the cytosol. The peripheral cytoplasm at the late vacuolate stage is dense with Golgi vesicles and dictyosomes, and the vesicles fuse with the plasmalemma (Figs. 21, 22). As stated above, the distal face of the intine is formed by material transported in Golgi vesicles that through exocytosis is released into the periplasmic space. We believe that there is also membrane flow from the dictyosomes to the plasmalemma. During the exocytosis process the membranes of the vesicles are incorporated into the plasmalemma, increasing considerably its surface area. This membrane flow explains how the plasmalemma retains its surface area during formation of the radial membrane fragments in the proximal part of the intine. Membrane flow and differentiation from the ER through the Golgi apparatus to the plasmalemma have been long known (e.g., Möré et al., 1971; Gabarayeva, 1987; see also reviews by Steer, 1991 and Sitte, 1998).

In Rondeletia, the vegetative cell of the maturing pollen grain synthesizes the two intracellular lipid structures found in pollen: oil bodies and intracellular membranes (Fig. 24).
mature pollen grains of *Rondeletia*, both starch grains and lipid bodies are found as storage products. Compound starch grains appear first, i.e., at the late free microspore stage. After the first pollen mitosis, oil body accumulation begins and starch contents of the cytoplasm decrease. At anther dehiscence lipids are the main storage product. The lipid body accumulation in *Rondeletia* is preceded by a transient accumulation of starch as demonstrated in many other plant species (e.g., Reznickova, 1978; Wetzal and Jensen, 1992; Clément et al., 1994; Hess, 1995).

The ER is considered to be a site of lipid body formation in both animal (Zaczek and Keenan, 1990; Bozhkov and Dlubovska, 1992) and plant cells (Grabski, de Feijter, and Schindler, 1993; Lacey and Hills, 1996). In late free microspore stages and young pollen grains, we observed a clear proliferation of an extensive network of ER membranes. These ER units are generally associated with maturation and expansion of the cytoplasm of the pollen vegetative cell (Jensen, Fisher, and Ashton, 1968; Charzynska, Murgia, and Cresti, 1989). An extensive network of ER during the young pollen grain stage was also observed in *Ledebouria* (Hess, 1995) and in *Brassica* (Piffanelli, Ross, and Murphy, 1998). In mature pollen grains of *Rondeletia*, the storage oil bodies are characteristically enfolded in a single rER strand. Similar pockets of ER have been described in the pollen of *Gossypium hirsutum* (Jensen, Fisher, and Ashton, 1968), *Impatiens walleriana* (Fisher, Jensen, and Ashton, 1968), and *Tradescantia reflexa* (Noguchi, 1990). It has been suggested that the extensive network of ER membranes might protect the oil bodies from coalescence during de- and rehydration (e.g., Piffanelli, Ross, and Murphy, 1997). Secondly, the intracellular membrane system can also provide lipid precursors for the increase of surface area of the plasmalemma which follows germination and pollen tube growth (Piffanelli, Ross, and Murphy, 1997). In addition to the prominent ER, there are numerous vesicles, apparently developed from the dictyosomes, that are laying beneath the surface of the plasmalemma. These membranous bodies undoubtedly contribute to the dramatic increase in surface area of the plasmalemma during maturation of the microspores and formation of the pollen grains (Piffanelli, Ross, and Murphy, 1998).

We observed several grains with three nuclei at maturity; thus the second mitosis of the generative nucleus takes place before release of the pollen grains. This observation contradicts Wunderlich (1971) and Puff (1994) who stated that pollen of *Rondeletia* is bicellular when shed.

**Volume increase at dehiscence**—The pollen wall of *Rondeletia* becomes abnormally stretched just before dehiscence. This stretching affects the spacing between columellae and the thickness of all distinguished layers of the wall. The effect was particularly obvious on the radially oriented granular material. This material appeared irregular in shape and in its distribution at pollen maturity. The intine was no longer thick in apertural regions at dehiscence as is generally the case.

**Endocingulum**—On the inside of mature grains, a broad endocingulum occurs with an irregular rod-like surface structure in contrast to the granular ornamentation of the remainder of the nexine. The ultrastructural explanation of this structure is clear from Fig. 34. Next to the aperture there is a zone free of radially oriented granular material, endexine, and foot layer. The columellae are branched and interconnected in this area.

To us, the inside view on the endocingulum shows the proximal ends of the columellae. The most plausible functional interpretation of the endocingulum is a role in harmomegathic (accommodate variations in the volume of the cytoplasm) processes but dehydrated grains observed in SEM contradict this hypothesis (Fig. 35).

**Summary of major ontogenetic events**—The main ontogenetic events in pollen wall development of *Rondeletia odorata* are presented schematically in Figs. 36–43. Our drawings are mainly based on TEM micrographs included in this work or on unpublished material. Our present study shows some interesting features that can be related to the dynamic processes of pollen development. We show ER cisternae extending between plasmalemma protrusions, and material of the membranous granular layer beneath the endexine (Fig. 15). The ER cisternae are also observed within the arcades between columellae (Figs. 15, 22). Our interpretation is that the early columellae with hollow cylindrical center, as well as other layers of the exine, may function as a dynamic transfer system between the cytoplasm of developing microspores and the tapetum. Heslop-Harrison (1963) showed the presence of cisternae of the endoplasmic reticulum under the plasmalemma in connection with the conduits of columellae. Similar observations were reported by Skvarla and Larson (1966) as part of their ontogenetic study of *Zea mays*. The interpretation then was that there might be a connection between sites of columellar initiation and ER cisternae. To understand the origins of this communication or transport system it would be helpful to study and understand the processes involved in the localization of initiation sites of exine layers on the plasmalemma.

The development of a very thin foot layer and relatively thick endexine is one of the characteristic features of pollen of *Rondeletia*. Another feature is the membranous granular layer initiated at early free microspore stage. The membranous part of this layer consists of ER units extending from the cytoplasm, which are clearly presented in our study. Furthermore, we have observed several cytological structures that are pronounced in *Rondeletia*: first, the abundant occurrence of Golgi vesicles and their demonstrated role in initiating the intine before mitosis, and second, the numerous lipid droplets surrounding the generative nucleus.

**LITERATURE CITED**


