ACTA SOCIETATIS
BOTANICORUM POLONIAE
Vol. 50, nr 1—2: 151—160
1981

The haustorial synergids of *Cortaderia* (*Gramineae*) at maturity

MELVA N. PHILIPSON

Botany Division, Department of Scientific and Industrial Research,
Christchurch, New Zealand

Abstract

The ultrastructure of synergids which extend through the micropyle as haustoria and lie against the ovary wall are described in *Cortaderia selloana* and its F_1 hybrid with *C. araucana*. These haustoria bear typical transfer cell wall invaginations closely associated with the plasma membrane and with mitochondria. Their function seems to be one involved in the absorption and conduction of nutrients to the synergids which are atypical in their highly vacuolate structure, degenerate nuclei and few organelles. The synergids appear to act as repositories of nutrients which are readily accessible to the central cell by virtue of deep intrusions made into them by the central cell cytoplasm. Enzymatic secretion could also be a function of the distal end of the haustorial synergids, both in facilitating tissue peneration during its outward growth and in directing pollen tube growth. At anthesis, the haustorium — synergid complex appears to be past its peak of absorption and transport activity, and to be involved in a secretory or degenerative phase.

INTRODUCTION

Synergids with haustorial extensions are rare among flowering plants, having been described in four genera of the *Compositae* (*Ursinia, Calendula, Cotula* and *Mutisia*), in *Quinchamalium* (*Santalaceae*) (Agarwal, 1962; Davis, 1966) and in *Cortaderia* (*Gramineae*) (Philipson, 1977, 1978). However, the haustoria are fully developed before fertilization only in *Quinchamalium* and *Cortaderia*. This latter genus, which contains both apomictic and sexually reproducing species, is usually counted among the *Arundineae* and its species are commonly known as "pampas grasses". In my previous papers, haustorial synergids of *C. selloana* and *C. jubata* were described at the light microscope level. Here, features of their ultrastructure as they occur in two sexually reproducing forms are presented at the stage of anthesis just befor pollination, when the embryo sac may be said to be mature.

Fig. 1. Micropylar end of the embryo sac of *Cortaderia selloana*
The haustorium of one synergid extends through the micropyle and lies against the ovary wall. The haustorium of the second synergid (which is partially obscured by the egg cell) lies against the first in the micropyle before extending outwards in another plane. One polar nucleus with nucleolus is visible beyond the synergid (lower right). (The ovule has pulled away from the ovary wall in the course of specimen preparation). Nomarski optics. × 1,640

Abbreviations: CC — central cell, CCI — central cell intrusion, CW — common wall, D — dictyosome, DN — degenerating nucleus, E — egg cell, ER — endoplasmic reticulum, ESW — embryo sac wall, H — haustorium, II — inner integument, L — lipid body, M — mitochondrion, MM — membranous material, OI — outer integument, P — plastid, PN — polar nucleus, S — synergid, St — starch, V — vacuole, W — wall, WI — wall invagination, Ov — ovary.

Figurs are orientated with the micropyle to the left in 1, 2, 6, 7, 8.

Fig. 2. The wall between synergid and egg is well defined and without plasmodesmata
The vacuolate mass of the synergid, with lipid bodies and electron dense groundplasm, contrasts sharply with the structure of the egg cell. × 31,200
Abbreviations as in Fig. 1.

MATERIALS AND METHODS

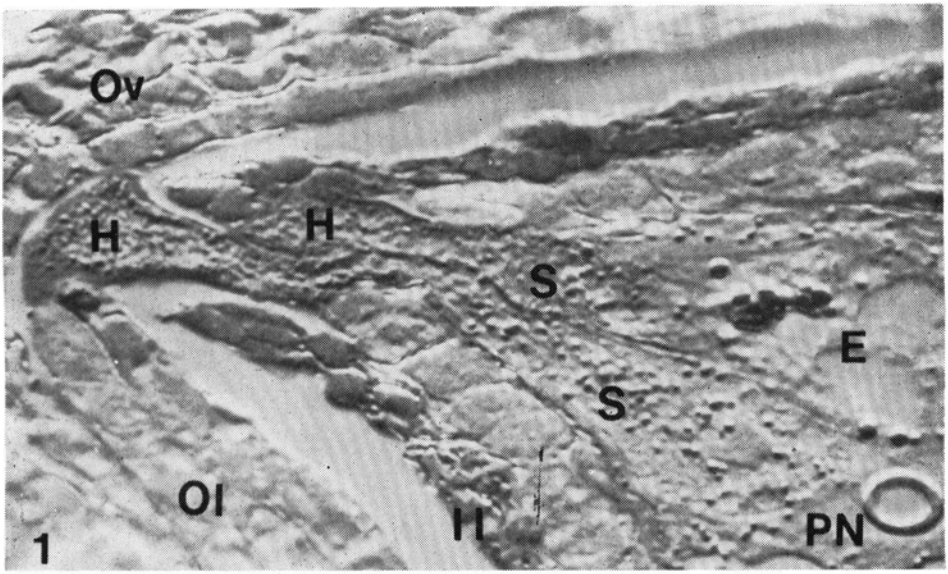
In a genus which strongly resists chemical fixation, this study has been restricted to *C. selloana*, and an F_1 hybrid between *C. selloana* and *C. araucana* which proved more amenable to specimen preparation. No differences were observed between the reproductive systems of these forms at either light or electron microscope level.

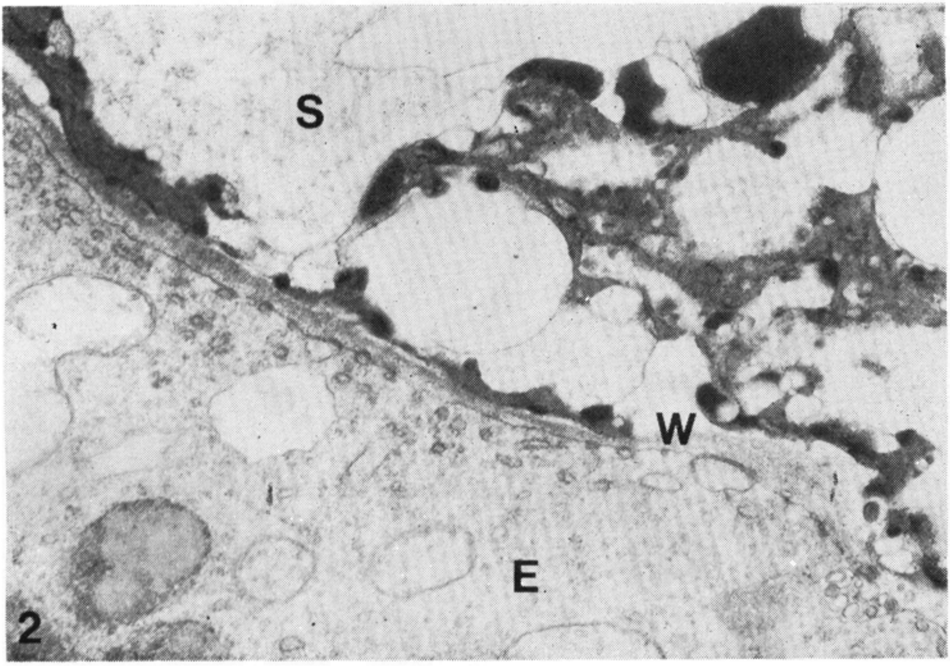
Ovaries from panicles at early anthesis which were free of pollen, were fixed in 6% glutaraldehyde in 0.1 M sodium phosphate buffer at pH 6.8 for 24 hours at room temperature, and post-fixed in 2% aqueous osmium tetroxide for a similar period. Because of the difficulty of chemical penetration, ovaries were first gently pressed onto double-sided sellotape and cut through to the ovule before being placed in the fixative. Dehydration was effected in a graded acetone series and specimens were embedded flat in Araldite. Thick, 4 μm sections were cut in the plane of the micropyle on an L.K.B. Pyramitome, photographed under Nomarski optics and selected for ultrastructural study. Selected sections were re-mounted according to the method of Woodcock and Bell (1967), thin-sectioned with glass knives on a Reichert OM U4 ultramicrotome, contrasted with 2% potassium permanganate and viewed under an Hitachi 11B electron microscope at 75 KV.

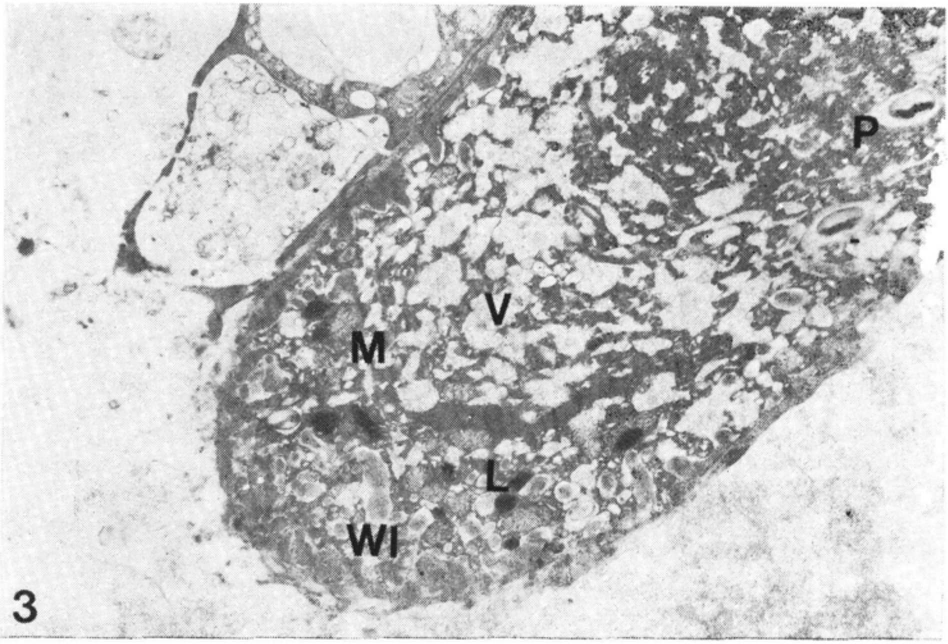
RESULTS

The sexually reproducing species of *Cortaderia* possess a monosporic, 8-nucleate, polygonum-type embryo sac, with 2 synergids and an egg cell lying in a triangular configuration at the apical end of the sac.

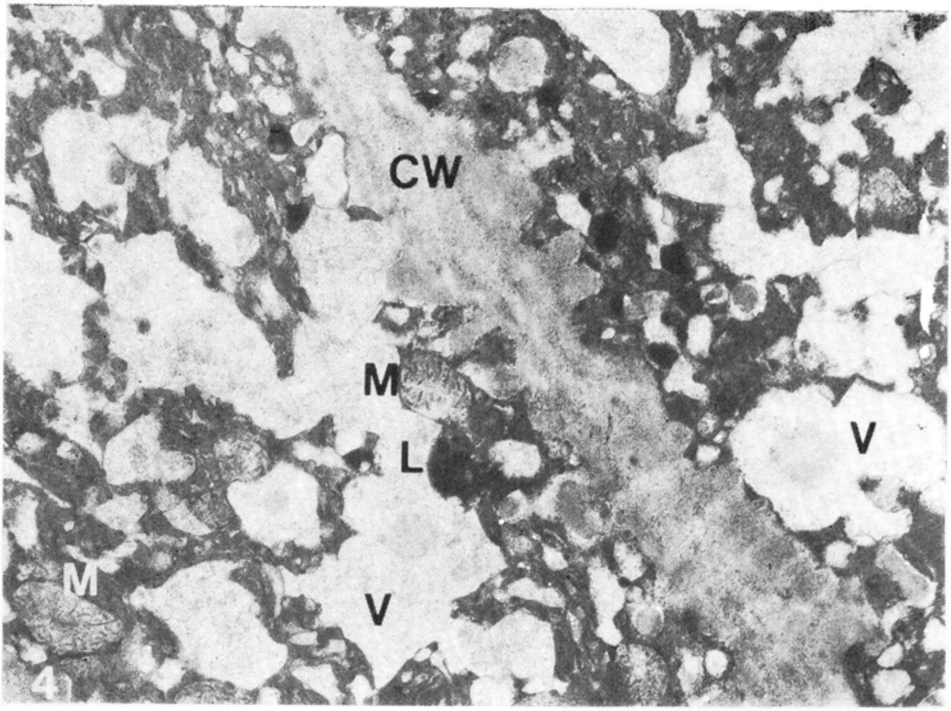
PLATE I







3



4

Fig. 3. Distal end of haustorium lying between ovule and ovary wall Invaginations occur on the inner wall surface. The heavy electron density of the groundplasm is apparent, also the many irregularly-shaped electron translucent areas containing fibrillar material. Mitochondria, plastids with starch, and lipid bodies also occur × 6,860
Abbreviations as in Fig. 1.

Fig. 4. The common wall between two contiguous haustoria in the micropyle bears transfer cell type invaginations on each side. × 18,000
Abbreviations as in Fig. 1.

In apomictic forms, the embryo sac is of nucellar origin and usually contains fewer nuclei (six being a common number) and only one synergid is usually present. The most striking feature of these synergids is the extension of their apices through the micropyle and the ultimate positioning of the haustoria between the ovule and the ovary wall, usually close to the entrance to the micropyle (Fig. 1). In some cases, one or both haustoria may become embedded between cells of the inner or outer integuments bordering the micropyle, or in others haustorial extensions from the synergids may not occur. Haustoria may penetrate the micropyle side by side, when viewed in the sagittal plane, or one may lie below the other when viewed in the same plane.

The two synergids are attached to the embryo sac wall apical to the hook region, and beyond it they extend into the central cell cytoplasm, where commonly, their chalazal ends are of irregular outline when viewed under the electron microscope.

A wall is discernible projecting from the embryo sac in the hook region, but usually after a short distance this becomes indistinguishable, so that the chalazal part of the synergids are bounded only by their plasma membranes. These, in many places, appear to break down, leaving only the plasma membrane of the central cell bordering the synergid. Occasionally, a thin, discontinuous and somewhat indistinct wall has been observed between synergid and central cell cytoplasm. It has not been possible to determine its full extent. Between synergid and egg cell a definite wall extends from their apical regions of contact (Fig. 2) but this becomes thin and disappears chalazally. No plasmodesmata have been observed in this wall. An irregular wall separates the two synergids apically and this also is without plasmodesmatal connections.

The apical region of synergids in general is occupied by the filiform apparatus, but in *Cortaderia* the synergid extensions, which may be called haustoria from this point, are developed in the place of these wall elaborations. All walls, i.e. those of the haustoria which lie free and separated and those which are shared when the haustoria are contiguous, are lined with transfer wall projections. At the tip of each haustorium

Fig. 5. Detail of haustorium
Wall invaginations are closely associated with amyloplasts and mitochondria. × 30,000
Abbreviations as in Fig. 1.

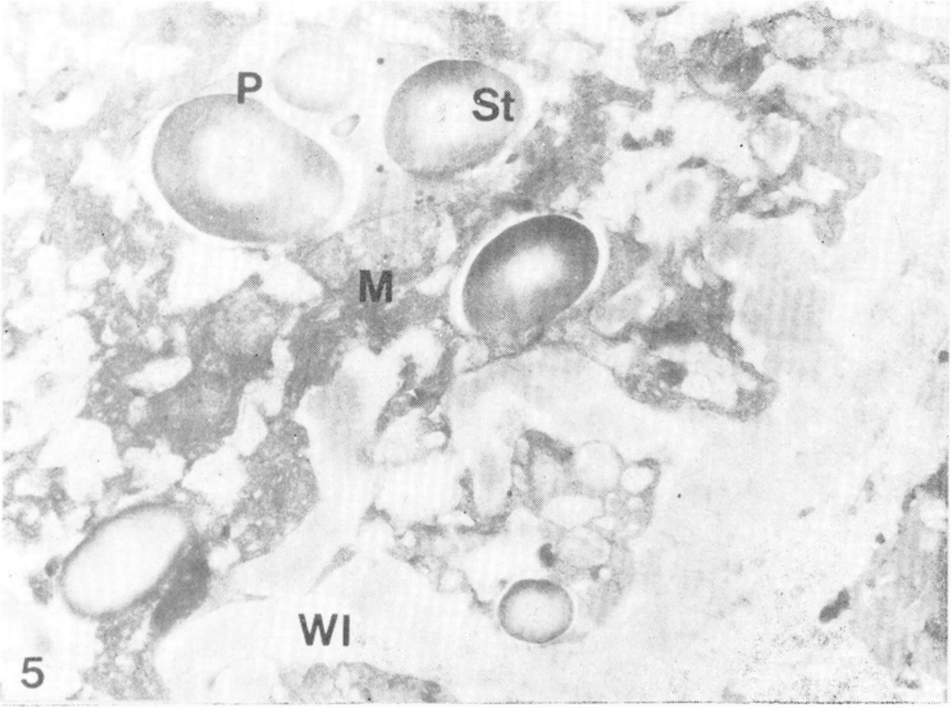
Fig. 6. Synergids in the apical region of the embryo sac
The haustorium of one synergid fills the micropyle, that of the second penetrates it at another level. Transfer cell invaginations do not extend along the synergid walls, and plasmodesmata do not occur on their common wall. × 10,400
Abbreviations as in Fig. 1.

---

there is a heavy elaboration of the wall (Fig. 3) but this may be variable. Nearer the embryo sac in the micropyle where the haustoria share a common wall for some distance, projections are well developed on each side of the common wall, so that they lie back to back (Fig. 4). These invaginations of the wall may be short and wide, or relatively narrow and project distance into the cytoplasm (Figs 3, 4, 5), and the plasma membrane is closely associated with their contours.

Owing to the heavy electron density of the groundplasm of the synergid-haustorium complex, the presence of endoplasmic reticulum is difficult to visualise, but it gives the impression at times of a complex system. It may be responsible for the fragmented appearance of the haustorial groundplasm and the formation of elongated dilatations of various shapes which enlarge by coalescence into irregularly shaped vacuoles with fibrillar contents. Many vacuoles contain a body of such fibrillar matter, of varying size and usually of a roughly circular shape, which may be the remnants of cellular protein undergoing degradation (Figs 3, 4). Mitochondria are plentiful in the haustoria, commonly of dense appearance, often of irregular and elongate shape, and usually possessing many long, narrow cristae of uniform width. Intramitochondrial granules may also be present, as well as tiny fibrils and possibly ribosomes. Dark, osmiophilic droplets are usually present at the site of the mitochondrial envelope. Plastids are obvious by virtue of their large starch granules which may occur singly or in clusters of several (Fig. 5). Their stroma rarely show membranes, but occasionally small electron dense bodies are present in the complex amyloplasts. Lipid bodies occur throughout the haustoria, of irregular shape and apparently associated with vacuoles or dilatations in the groundplasm.

Within the synergid, transfer wall projections rapidly disappear below the apical region, both on the synergid-embryo sac wall and especially on the wall common to the two synergids (Fig. 6). The synergids themselves consist almost entirely of a mass of vacuoles of many different shapes and sizes, some containing fibrillar matter, others translucent, and separated in places by groundplasm which is typically elec-


P
St
M
WI
5

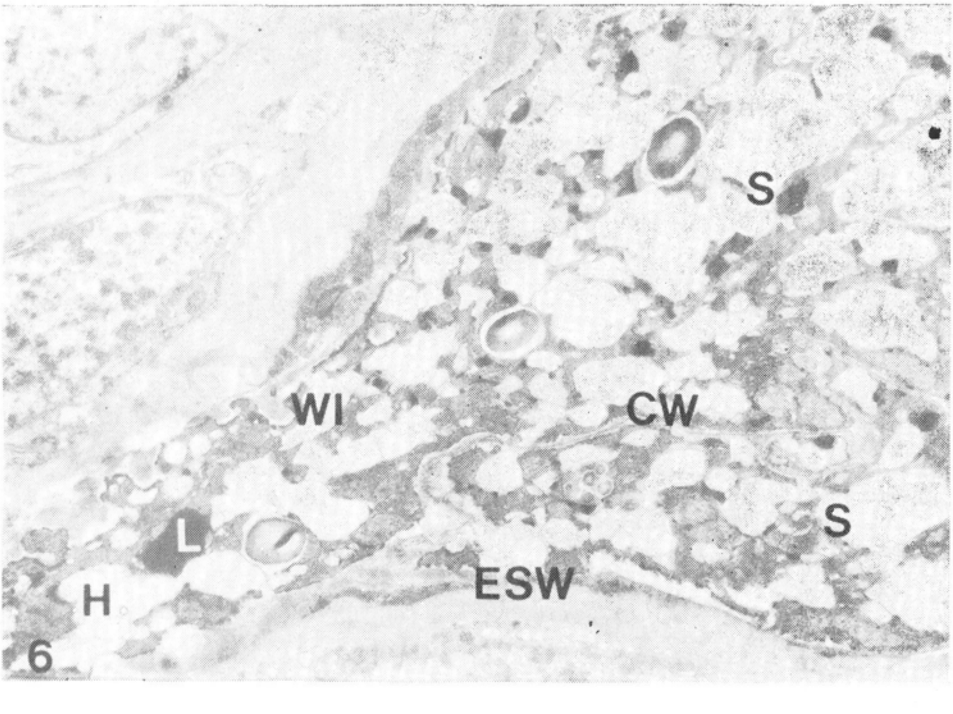
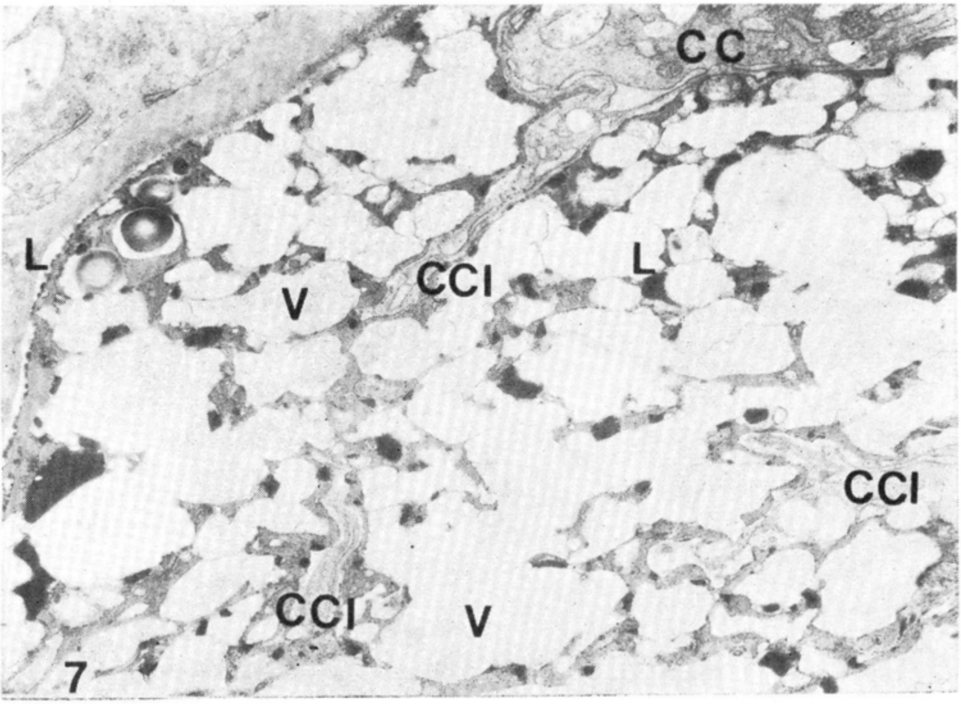

S
WI
CW
S
L
ESW
H
6

PLATE IV



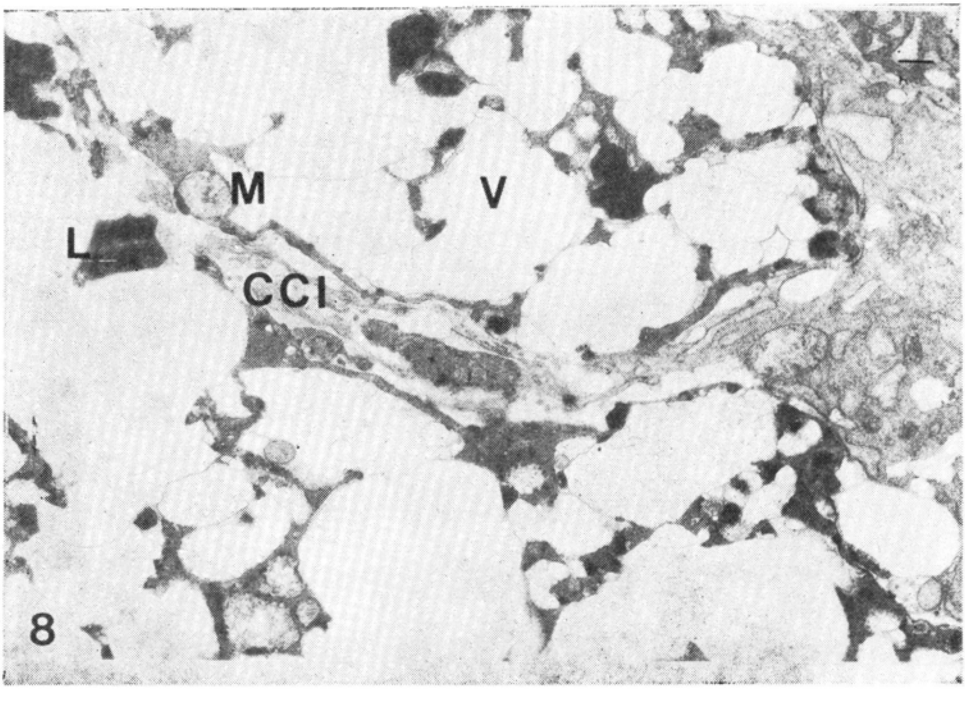


Fig. 7. Lateral aspect of a synergid in the hook region
Inroads of central cell cytoplasm lie among the vacuoles of the synergid. Lipid bodies occur in some abundance and also line the space bordering the embryo sac wall. × 5,800
Abbreviations as in Fig. 1.

Fig. 8. Dilated profiles of ER are present in the cytoplasm penetrating the synergid from the central cell
The bounding membranes of the two cells are indistinct. × 14,400
Abbreviations as in Fig. 1.

---

tron dense. This groundplasm may contain a few mitochondria, oval or round in shape, plastids with large starch granules, and lipid bodies. Osmiophilic droplets, resembling lipid bodies, lie on the outside of the plasma membrane between the synergid and the embryo sac wall, and between the synergids where they are separated by a common wall. In a few cases, when the groundplasm was not heavily electron dense, it has been possible to identify in it profiles of RER often with dilated ends and flocculent or fibrillar contents, dictyosomes producing electron dense vesicles, ribosomes and lipid bodies. Structures consisting of concentric rings of membranes may be found packed together just under the plasma membrane at the chalazal end of the synergid (Fig. 9).

The outline of the synergids is irregular, especially at the chalazal end (Fig. 9) and this irregularity is attributable in part to penetration by the central cell cytoplasm into the synergid at various points over its surface (Fig. 7). These intrusions from the central cell, contain mostly long profiles of tubular and dilated ER, usually with contents showing some electron density, and ribosomes (Fig. 8). The close proximity of membranes of tubular and vesicular elements of the ER of the central cell cytoplasm which both surrounds the synergids and penetrates into their interior, may give a false impression of a double membrane bounding parts of the synergids.

In contrast to the results of previous ultrastructural studies on synergids, the haustorial synergids of *Cortaderia* do not usually possess a functional nucleus. Occasionally, an irregularly shaped nucleus with nucleolus but very little chromatin may be detected, (Fig. 10) or only an irregular dense shape may occur with barely distinguishable nucleolus. Otherwise the nucleus is absent, presumably having degenerated, leaving very little trace.

DISCUSSION

The structure of the synergid complex in *Cortaderia,* consisting of two synergids within the embryo sac and their haustorial extensions beyond it, suggests that it is a highly efficient system for the collection

Fig. 9. Chalazal end of synergid where it is bounded mainly by the plasma membrane of the central cell
Whorls of membranous material are packed together at various sites along the boundary. × 5,800
Abbreviations as in Fig. 1.

Fig. 10. Degenerating synergid nucleus of amoeboid shape and with very little chromatin present
The surrounding cytoplasm is filled with vacuoles and profiles of tubular ER occur between some of them. Lipid bodies also occur. × 8,000
Abbreviations as in Fig. 1.

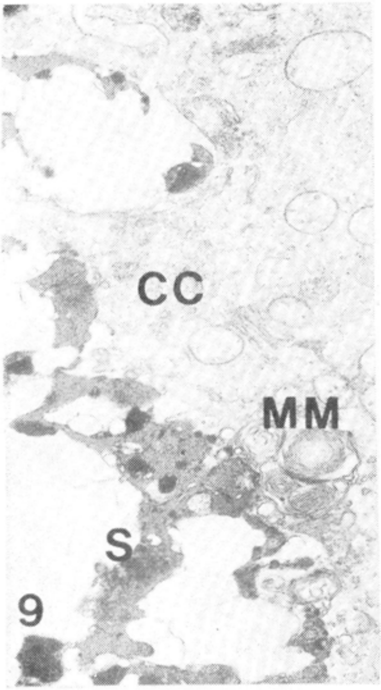
Fig. 11. The metabolically active central cytoplasm is traversed by long tubular RER containing flocculent material and showing irregular dilatations along their length
Dictyosomes, many with stacks of 6-7 cisternae release both small electron dense vesicles and larger translucent forms. Polyribosomes, mitochondria, lipids and plastids also occur, while vacuoles are often partially encircled by lengths of RER. × 19,800
Abbreviations as in Fig. 1.

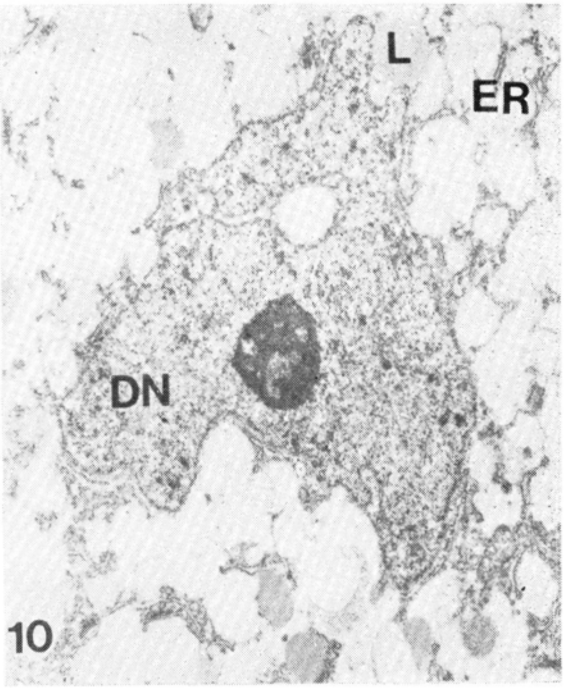
---

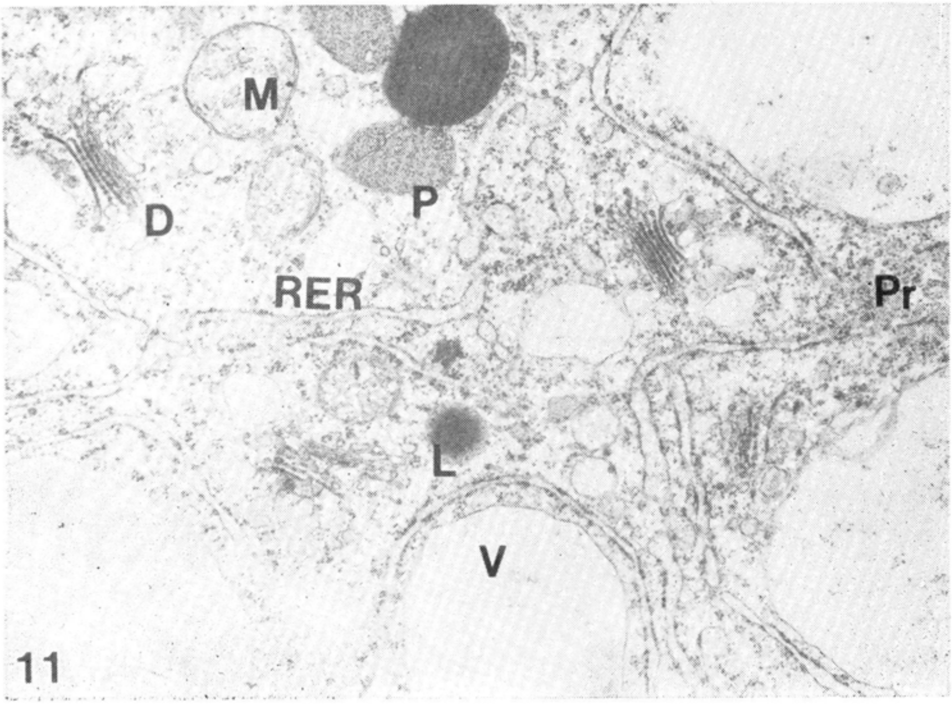
and transport of nutrients into the embryo sac. The extremely vacuolate nature of the synergids is contrary to all descriptions of synergids so far made. Deschamps (1973) records that synergids of *Linum catharticum* are alveolar in nature, but his micrographs do not show them to approach the almost totally vacuolate state with few organelles, which occurs in *Cortaderia*. However, in *Lilium regale* Mikulska and Rodkiewicz (1967) have described a state of advanced vacuolisation in synergids which bears certain resemblances to that in *Cortaderia,* especially in the presence of lipid bodies. The highly vacuolate nature of the synergids of *Cortaderia* may well indicate that they are a repository of nutrients, readily tapped, as shown by the deep penetrations made into them by the surrounding central cell cytoplasm — a phenomenon not previously known to occur in this form between cells of the embryo sac.

The absence of a wall or its partial absence in the chalazal region of the synergids is in line with observations of other authors (Diboll, Larsen, 1966; Schulz, Jensen, 1968; Vijayaraghavan et al., 1972; Maze, Lin, 1975; Ponzi, Pizzolongo, 1976). In *Cortaderia,* in the sexually reproducing forms described here, a wall may be quite absent in the chalazal region although a partial wall is occasionally present. In the apomictic *C. jubata* a wall, discontinuous in the chalazal region, encloses the synergid (pers. observation), and in addition, the synergid in this species possesses lateral plasmodesmatal connections with the central cell cytoplasm. It may be that small differences occur in the timing of wall deposition, accounting for its absence or inception at anthesis. Studies on the embryo sac at fertilization may clarify this point.

PLATE V.







The frailty of the synergid outer membrane is suggested by its absence in places, especially chalazally. While this could be due to breakdown during specimen preparation of a senescent or weakened membrane, there seems to be little obstacle, in any case, to the transfer of nutrients from synergid to the central cell. The central cell at maturity is in a state of high metabolic activity, containing long profiles of dilated RER with flocculent contents, dictyosomes producing two types of vesicles, and many polyribosomes, mitochondria, plastids (with few starch granules), enlarging vacuoles, and large lipid bodies (Fig. 11). In addition, the voluminous nucleoli of both polar nuclei (which do not fuse until fertilization or immediately before) indicate the high level of ribosomal RNA synthesis and a correspondingly high rate of protein synthesis, in the central cell cytoplasm.

The long haustorial outgrowths from the usual location of the filiform apparatus, with their internal wall elaborations, could be interpreted as extensions outward of the filiform apparatus itself. Except for the brief report of Torosian (1971), ultrastructural studies of haustoria originating from other cells of the embryo sac, e.g. micropylar haustoria of precocious endosperm cells of *Plantago lanceolata* (Vannereau, Mestre, 1975) have not shown the presence of similar wall projections which we know occur in transfer cells and to be associated with the short distance transport of solutes (Gunning, Pate, 1969). A high level of nutrient uptake by these extensions of the synergids is indicated by the close association of enlarged wall surface area and plasma membrane and the large mitochondrial population. The long tubular cristae of these haustorial mitochondria are unusual, and often occur here in mitochondria which themselves are elongated and of unusual shape.

Mitochondria with long narrow cristae were reported in the degenerate synergid and pollen tube of *Capsella bursa-pastoris* following pollination, but were not present in the synergid before the entry of the pollen tube (Schulz, Jensen, 1968). Mitochondria are present in the synergid of *Cortaderia* in much reduced numbers, in the usual configuration similar to those of the central cell cytoplasm, although long narrow cristae may be detected occasionally.

If the haustorium is interpreted as an outgrowth of the filiform apparatus, then it is probable that the filiform apparatus will also perform an absorptive function, such as has been suggested by Jensen (1965). It has been predicted by Mogensen (1978a) that the filiform apparatus will be found at the entrance to most angiosperm embryo sacs.

Some authors have also suggested a secretory function for the filiform apparatus — that of directing the growth of the pollen tube to the embryo sac (Vazart, Vazart, 1966; Godineau, 1969; van

Went, 1970; Mogensen, 1972; Fougère-Rifot, 1975), and further, of causing degeneration of the pollen tube cytoplasm prior to penetration of the synergid (Mogensen, 1978b). At embryo sac maturity the synergid-haustorium complex of *Cortaderia* does indeed appear to be involved also in secretory activity judged by the number of lipid bodies and the osmiophilic droplets, presumably lipid, which are present at the outer envelopes of mitochondria and associated with vacuoles, vesicular pieces of ER and the plasma membrane. Similar chemotropic activities have been reported in the synergid of *Aquilegia vulgaris* (Fougère-Rifot, 1975). While these features could be associated with pollen tube attraction and interaction, they may also be involved in the penetration of tissues by the haustoria. Crushing and degradation of bordering cells could be due, at least in part, to mechanical damage, but enzymatic dissolution may also occur. It is known that in the apomictic embryo sac of *C. jubata,* where 2 or more nucellar embryo sacs may develop, the haustorial synergid of only one will penetrate the micropyle. In such a situation the haustorium of another will turn and penetrate the embryo sac wall and ovular tissue laterally, until it comes to lie between the integumentary cells near the outer surface of the ovule (Philipson, 1978). The haustoria of the sexual species may posses a similar digestive ability.

The final position reached by the haustoria, which is most commonly between ovule and ovary wall not far from the micropyle, raises the question of the nutritive value of such a location. The ovule itself is encased in a thick cuticular layer which successfully resists chemical fixatives. On only very rare occasions has an haustorium been found to penetrate the inner surface of the ovarian tissue. An haustorium may become embedded in integumentary cells adjacent to the micropyle but usually the distal end lies outside the ovule. Is there differentiation of function here within the haustorium? Are chemotropic substances being secreted from the outwardly growing distal end, while absorption of nutrients occurs where the lateral walls are in contact with cells of the integuments? Or alternatively, does the distal end of the haustorium perform the secretory function of pollen tube attraction with the decline of nutritive uptake? The haustorium would be well placed for such a secretory role, in a position readily accessible to pollen tubes as they passed over the placental region towards the micropyle of the hemianatropous ovule.

Within the synergid-haustorium complex, wall projections are confined to the haustorium. They occur over the whole inner surface, including the proximal parts where they share a common dividing wall, and also the distal parts where the two haustoria lie independently in and beyond the micropyle. Projections do not occur on others parts of the synergid walls, although they are present on the outer walls of the central cell and antipodals from the time of organization of the embryo sac. Similar but more strongly developed wall ingrowths have been reported before fertilization in *Helianthus annuus* (Newcomb, 1973). The walls of the free-lying parts of the haustoria in *Cortaderia* have been derived from extension of the original outer wall of the embryo sac, whereas the common wall shared by the haustoria is formed between cells long after mitosis and without a phragmoplast. Invaginations on walls formed without a phragmoplast have been reported in *Plantago lanceolata* (Vannereau, 1978), *Proboscidea louisianica* (Mogensen, 1978) and *Nicotiana tabacum* (Mogensen, Suthar, 1979).

Most active haustoria from the embryo sac are reported to possess very large, conspicuous nuclei. In *Cortaderia,* synergid nuclei have been reported as degenerate or absnt (Philipson, 1978; Costas-Lippmann, 1979). This lends credence to the view that the whole system is one of senescence. However, the nature of this degeneration appears to be different from that described in angiosperm embryo sacs where one synergid degenerate shortly before fertilization, which is a slightly later stage than that at which this study was made. Degeneration of a synergid before fertilization is associated with reduction in cell size, heavy increase in cytoplasmic electron density making individual organelles difficult to distinguish, and nuclear degradation. It appears likely that in *Cortaderia* the peak of haustorial activity occurs before maturation of the embryo sac, so that by anthesis this function of the synergid complex is declining. However, the ultimate goal of fertilization has yet to be achieved in a system where the synergid and its haustorium, which occupies the micropyle, play a part. Details of the processes involved in this next stage will form the basis of a future publication.

REFERENCES

Agarwal S., 1962. In Plant Embryology — a Symposium. CSIR New Delhi pp. 162-169.

Costas-Lippmann M., 1979. Bot. Gaz. 140: 393-397.

Davis G. L., 1966. Systematic Embryology of the Angiosperms. Wiley, London pp. 1-528

Deschamps d'Alascio R., 1973. Bull. Soc. bot. Fr. 120: 189-200.

Diboll A. G., Larsen D. A., 1966. Amer. J. Bot. 53: 391-402.

Fougère-Rifot M., 1975. Comptes Rendus Acad. Sci. Paris, ser. D 280: 2445-2447.

Godineau J. C., 1969. Rev. Cytol. et Biol. vég. 32: 209-226.

Gunning B. E. S., Pate J. S., 1969. Protoplasma 68: 107-133.

Jensen W. A., 1965. Amer. J. Bot. 52: 238-256.

Maze J., Lin S. C., 1975. Can. J. Bot. 53: 2958-2977.

Mikulska E., Rodkiewicz B., 1967. Acta Soc. Bot. Pol. 36: 555-566.
Mogensen H. L., 1972. Amer. J. Bot. 59: 931-941.
Mogensen H. L., 1978a. Phytomorphol. 28: 114-122.
Mogensen H. L., 1978b. Amer. J. Bot. 65: 953-964.
Mogensen H. L., Suthar H. K., 1979. Bot. Gaz. 140: 168-179.
Newcomb W., 1973. Can. J. Bot. 51: 863-878.
Philipson M. N., 1977. New Zealand J. Bot. 15: 777-778.
Philipson M. N., 1978. New Zealand J. Bot. 16: 45-59.
Ponzi R., Pizzolongo P., 1976. J. Submicr. Cytol. 8: 327-336.
Schulz, Sister R., Jensen W. A., 1968. Amer. J. Bot. 55: 541-552.
Torosian C. D., 1971. Amer. J. Bot. 58: 456-457 (Abstract).
Vannereau A., 1978. Bull. Soc. bot. Fr. Actualités botaniques 125: 201-205.
Vannereau A., Mestre J. C., 1975. Comptes Rendus Acad. Sci. Paris, ser. D 281: 699-702.
Vazart B., Vazart J., 1966. Rev. Cytol. Biol. vég. 24: 251-226.
Vijayaraghavan M. R., Jensen W. A., Ashton M. E., 1972. Phytomorphol. 22: 144-159.
Went van J. L., 1970. Acta Bot. Neerl. 19: 312-322.
Woodcock C. L. F., Bell P. R., 1967. J. Roy. Micros. Soc. 87: 485-487.