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Calcium Oxalate Crystals in Monocotyledons: A Review of their Structure and Systematics

CHRISTINA J. PRYCHID and PAULA J. RUDALL
Royal Botanic Gardens, Kew, Richmond, Surrey, TW9 3DS, UK

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Three main types of calcium oxalate crystal occur in monocotyledons: raphides, styloids and druses, although intermediates are sometimes recorded. The presence or absence of the different crystal types may represent 'useful' taxonomic characters. For instance, styloids are characteristic of some families of Asparagales, notably Iridaceae, where raphides are entirely absent. The presence of styloids is therefore a synapomorphy for some families (e.g. Iridaceae) or groups of families (e.g. Philydraceae, Pontederiaceae and Haemodoraceae). This paper reviews and presents new data on the occurrence of these crystal types, with respect to current systematic investigations on the monocotyledons.

Key words: Calcium oxalate, crystals, raphides, styloids, druses, monocotyledons, systematics, development.

INTRODUCTION

Most plants have non-cytoplasmic inclusions, such as starch, tannins, silica bodies and calcium oxalate crystals, in some of their cells. Calcium oxalate crystals are widespread in flowering plants, including both dicotyledons and monocotyledons. They were first discovered by Leeuwenhoek in the 17th century (see Frey, 1929, for a review of early literature). They have been documented using light microscopy (LM) and polarisation microscopy, and more recently using X-ray diffraction (Frey-Wyssling, 1935, 1981; Pobeguin, 1943; Arnott, Pautard and Steinfink, 1965), infra-red spectroscopy (Scurfield, Michell and Silva, 1973) and electron microscopy, both scanning (SEM) and transmission (TEM) (e.g. Arnott and Pautard, 1970; Arnott, 1976; Franceschi and Horner, 1980*a*, *b*; Horner and Franceschi, 1981).

The distinct shapes and birefringence of calcium oxalate crystals, especially raphides and styloids, make them readily observable, particularly in young, actively growing tissues, although smaller, rounded druses are more easily missed. Crystals normally form intracellularly, but extracellular crystals have also been reported, e.g. on the outside surfaces of palisade parenchyma in *Tsuga* leaves (Gambles and Dengler, 1974). However, since these crystals have a covering of fibrous material (Horner and Franceschi, 1978) they are probably initiated intracellularly. Similarly, in *Welwitschia bainesii* the crystal-encrusted spiculate cells are covered by a sheet-like layer (Scurfield *et al.*, 1973). Some tissues may contain other crystal types in addition to calcium oxalate crystals; in particular silicate crystals are characteristic of the monocot superorder Commelinanae, many of which have calcium oxalate crystals (see below).

The value of calcium oxalate crystals to normal plant growth and development is largely unknown and probably variable (Frey, 1929; Arnott, 1976; Franceschi and Horner, 1980*b*). They may represent storage forms of calcium and oxalic acid, and there has been some evidence of calcium oxalate resorption in times of calcium depletion (Arnott and Pautard, 1970; Sunell and Healey, 1979). They could also act as simple depositories for metabolic wastes which would otherwise be toxic to the cell or tissue. In some plants they have more specialist functions, such as to promote air space formation in aquatic plants (see below), or help prevent herbivory, although many plants containing calcium oxalate crystals are eaten by birds and animals. The barbed and grooved raphides of some Araceae (e.g. *Xanthosoma sagittifolium*) are particularly irritating to mouth and throat tissues when eaten. Grooves in crystals which have embedded themselves in animal tissues may allow the entrance of a chemical irritant such as a toxic proteolytic enzyme (Walter and Khanna, 1972) or a glucoside (Saha and Hussain, 1983) into the wound (Sakai, Hanson and Jones, 1972).

Character homologies of different types of calcium oxalate crystals in monocotyledons require further assessment and clarification. Since there appears to be some significance in their systematic distribution in monocotyledons, this paper reviews the current knowledge of the structure and systematics of these crystal types and also incorporates new data on the occurrence of these crystals throughout the monocotyledon families. Only calcium oxalate crystals are considered here; the distribution of silicate crystals in monocotyledons (present in Orchidaceae and Commelinanae) will be considered separately in a later paper.

Samples for light microscopy were fixed in FAA (Johansen, 1940) and conventionally embedded in Paraplast before being sectioned, stained with Alcian blue and safranin and examined on a Leitz Diaplan photomicroscope. Scanning electron microscope samples were fixed in FAA, dehydrated, critically point dried, and sputter-coated with platinum before observation with a Cambridge Stereoscan

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S240. Samples for transmission electron microscopy were preserved using Karnovsky's fixative (Glauert, 1975) and 1% osmium tetroxide, dehydrated, embedded in LR White resin, sectioned and stained with uranyl acetate and lead citrate (Reynolds, 1963) before observation with a JEOL JEM-1210.

STRUCTURE AND SYSTEMATIC DISTRIBUTION

Calcium oxalate crystals appear in a variety of shapes which are consistent and repeatable from one generation to the next, demonstrating that the physiological and genetic parameters controlling them are consistent. There are three main types of calcium oxalate crystal in monocotyledons: raphides, styloids and druses, and these may be treated as three separate characters in cladistic analyses. The different types are sometimes, but not always, mutually exclusive. Raphides are by far the most common type in monocotyledons, and are often present at the same time as either druses or styloids (Table 1). Druses and styloids rarely occur together in the same plant, except occasionally in *Acorus* (Fig. 1A) and Araceae. Araceae is the only family in which all three main crystal types are recorded. Most monocotyledons have calcium oxalate crystals of some type, but they are entirely absent from some taxa, such as some families of Liliales, Poales and all Alismatidae (Table 1). Presence or absence of different crystal types in plants may therefore represent 'useful' taxonomic characters in some groups. For example, on the basis of both morphological and molecular characters, Rudall and Chase (1996) demonstrated that the genera formerly included in Xanthorrhoeaceae *sensu lato* probably represent three distinct families: Xanthorrhoeaceae *sensu stricto*, Lomandraceae and Dasypogonaceae. The distribution of calcium oxalate crystals in these taxa reflects this taxonomy (Table 1): raphides are common in Lomandraceae, absent from Xanthorrhoeaceae, where they are replaced by styloids, and rare or absent in Dasypogonaceae, where silicate crystals (characteristic of Commelinanae) are present in the leaf epidermal cells.

Calcium oxalate crystals occur either in a monohydrate (whewellite) form or a di- (or tri-) hydrate (wheddellite) form (Arnott, 1981). Raphides normally belong to the monoclinic system with the whewellite or monohydrate form (Kohl, 1899), although, due to the narrowness of the needles, their large angles, and the curvature of the crystal face, this is often impossible to define without the aid of X-ray diffraction (Frey-Wyssling, 1935, 1981; Pobeguin, 1943; Arnott *et al.*, 1965).

Crystals may be present in almost every part of both vegetative and reproductive organs, often in crystal idioblasts near veins, possibly due to calcium being transported through the xylem, although experiments to demonstrate this have failed (Frank, 1967). Environmental conditions such as seasonal changes may influence the amount of oxalate produced and the number of crystals formed. Aquatic plants often have calcium oxalate crystals associated with aerenchyma tissue, sometimes projecting into air spaces: raphides (and sometimes druses) in Araceae (Fig. 1B) and *Typha*, styloids in *Eichhornia* (Fig. 1C, D) and druses in *Acorus* (Fig. 1E, F). Indeed, crystals may be associated with air space formation: in young leaves of *Typha angustifolia*, raphide crystal idioblasts circumscribe parenchymatous tissues which break down to form air space compartments. Cell wall plasticity may be increased around air spaces, as calcium is sequestered within crystal idioblasts (Kausch and Horner, 1981). Seubert (1997) found that, in Araceae, raphides and sclereids seem to perform the same role and are present in inverse relative proportions: where many sclereids are present, raphides are few, and *vice versa*. Mayo (1989) found that, in infloresences of *Philodendron* (Aracaeae), where both raphides and druses occur, druses are more common in the styles, whereas raphides are more common in aerenchymatous tissues.

Raphides

Raphides are bundles of narrow, elongated needle-shaped crystals, usually of similar orientation, with pointed ends at maturity. Often (e.g. in Araceae) one end is abruptly pointed whereas the other tapers to a point or is wedge shaped. There are varying numbers of crystals in each bundle. They are usually found in crystal idioblasts in parenchymatous tissues (Fig. 2A, B), although there are examples of raphides occurring in specialized structures, such as aerenchyma (see above), or plant hairs. In *Conanthera campanulata* (Tecophilaeaceae) the uniseriate hairs fringing the tepals contain a bundle of raphides in each cell (Fig. 2C) and, similarly, raphides have been recorded in trichomes of *Cocos* (Arecaceae) (Frey, 1929). In some species of *Curculigo* (Hypoxidaceae) numerous small, loosely arranged crystals are present in leaf epidermal cells, in addition to 'normal' bundles of raphides in mesophyll cells (Rudall *et al.*, 1998*a*).

Although most raphide crystals are four-sided (Fig. 3A), those of some taxa are at least six-sided, appearing almost elliptical in cross section, for example in *Yucca* (Eilert, 1974), *Agave*, *Cordyline* (Wattendorff, 1976*b*, 1979) and *Ornithogalum* (Tilton and Horner, 1980). In these taxa, raphides are initially four-sided with wedge shaped ends but later develop into six- and eight-sided crystals with pointed ends (Fig. 3B). In *Typha angustifolia* (Kausch and Horner, 1981), mature crystals are hexagonal towards their ends and octagonal in their central region. During development, each raphide becomes ensheathed in lamellae and surrounded by mucilage (see below). Addition of material to the ends of the raphides extends them from wedge-shaped to pointed, and overlap of lamellae at these pointed ends results in characteristic wing formations (Eilert, 1974; Wattendorff, 1976*b*; Tilton and Horner, 1980; Horner, Kausch and Wagner, 1981).

Grooved raphides occur in Araceae (Fig. 3C, D). Apparently all raphides in Araceae are grooved, including those of *Spirodella* (Ledbetter and Porter, 1970) and *Lemna* (Arnott, 1966; Arnott and Pautard, 1970), which are sometimes placed in a separate family, Lemnaceae. Grooved raphides therefore represent a significant apomorphy for Araceae. At initiation, the crystals have a rounded appearance but are grooved at maturity. Ledbetter and Porter

TABLE 1. *Distribution of crystal types in monocotyledons* (*classification of Angiosperm Phylogeny Group 1998*)

Family	Crystal type
ACORALES	
Acoraceae	Raphides absent (e.g. Gulliver, 1863–1865); small druses present in stems and flowers of *A. gramineus* and *A. calamus* (this paper: Fig. 1 A, F), recorded as 'crystalline granules' by Gulliver (1865). Small rhomboidal styloids sometimes present in bundle sheath cells (Fig. 1 A)
ARALES	
Alismatidae (Alismataceae, Aponogetonaceae, Butomaceae, Cymodoceaceae, Hydrocharitaceae, Juncaginaceae, Limnocharitaceae, Posidoniaceae, Potamogetonaceae, Ruppiaceae, Scheuchzeriaceae, Zosteraceae	Crystals absent (e.g. Gulliver, 1863–1865; Singh, 1965; Tomlinson, 1982), except small rods or styloids recorded in *Butomus* (Stant, 1967), and crystals sometimes present in Hydrocharitaceae (Shaffer-Fehre, 1987)
Araceae	Both raphides and druses commonly present (e.g. Gulliver, 1863–1865; Johow, 1880; Wakker, 1888; Sakai *et al.*, 1972; Gaiser, 1923; Genua and Hillson, 1985; Grayum, 1990). Crystal sand also reported (see Frey, 1929; Grayum, 1990). Styloids present in a few taxa, both elongated (in Zamioculcadeae) and rhomboidal (in Potheae and Monsteroideae) (Grayum, 1990; Seubert, 1997)
Tofieldiaceae	Raphides absent; small druses present (e.g. Gulliver, 1863–1865; this paper)
UNPLACED (NON COMMELINOID) TAXA	
Corsiaceae	Raphides absent (Rübsamen, 1986; this paper)
Japanoliriaceae	Crystals absent (this paper)
Petrosaviaceae	Raphides reported by Groom (1895), but not confirmed by Tomlinson (1982) or this paper, although druse-like crystals observed here in *Petrosavia borneensis* and *P. sakuraii*
Triuridaceae	Crystals absent (Tomlinson, 1982; and this paper: *Sciaphila* spp.)
ASPARAGALES	
Agavaceae (including *Hosta*)	Raphides and styloids present (Gulliver, 1863–1865; Arnott, 1966, 1981; Sakai and Hanson, 1974; Wattendorff, 1976*a*, *b*; Dahlgren and Clifford, 1982; McDougall *et al.*, 1993)
Alliaceae	Raphides and styloids present (Ricci, 1963; Arnott, 1981; Kausch and Horner, 1982); crystals absent in a few species of *Allium* (Gulliver, 1864); occasional druse-like crystals recorded in *Allium* (Ricci, 1963)
Amaryllidaceae	Raphides present (Dahlgren and Clifford, 1982); occasional druses reported by Johow (1880)
Anemarrhenaceae	Raphides present (Conran and Rudall, 1998)
Anthericaceae	Raphides present (e.g. Dahlgren and Clifford, 1982). In *Chlorophytum comosum*, druses reported by Kenda (1961), but styloids found in material examined here (this paper)
Aphyllanthaceae	Raphides present (Dahlgren and Clifford, 1982)
Asparagaceae (incl. *Hemiphylacus*)	Raphides present (e.g. Gulliver, 1863–1865; Rudall *et al.*, 1998*b*)
Asphodelaceae	Both raphides and styloids present (e.g. Rudall and Cutler, 1995)
Asteliaceae	Raphides present (Rudall *et al.*, 1998*a*)
Behniaceae	Raphides present (Conran, 1998*a*)
Blandfordiaceae	Crystals absent (Rudall *et al.*, 1998*a*)
Boryaceae	Raphides present (Conran, 1998*b*)
Convallariaceae *s.l.* (including Dracaenaceae, Eriospermaceae Nolinaceae, Ruscaceae)	Both raphides and styloids and intermediate forms present (Gulliver, 1863–1865; Rothert and Zalenski, 1899; Dahlgren and Clifford, 1982; Cutler, 1992; this paper)
Doryanthaceae	Raphides absent; styloids present (Dahlgren and Clifford, 1982)
Hemerocallidaceae *s.l.* (incl. Phormiaceae and Johnsoniaceae)	Crystals absent in *Simethis*, recorded as present or absent in *Hemerocallis*; in *Phormium* raphides absent and styloids present (e.g. Gulliver, 1863–1865; Dahlgren and Clifford, 1982; Rudall and Cutler, 1995)
Herreriaceae	Raphides present, styloids absent (Rudall and Cutler, 1995)
Hyacinthaceae	Raphides present (Gulliver, 1863, 1864; Tilton, 1978; Tilton and Horner, 1980; Kausch and Horner, 1982; Svoma and Greilhuber, 1988)
Hypoxidaceae	Raphides present, styloids absent (Rudall *et al.*, 1998*a*)
Iridaceae	Raphides absent, styloids present in most genera (e.g. Gulliver, 1863–1865; Goldblatt *et al.*, 1984; Wu and Cutler, 1985; Wolter, 1990; Rudall, 1994, 1995), but absent from *Sisyrinchium* and its close allies (Rudall, Kenton and Lawrence, 1986) Occasional druses reported in *Nivenia concinna* (Rudall and Burns, 1989)

TABLE 1. (*cont.*)

Family	Crystal type
Ixioliriaceae	Raphides present, styloids absent (Rudall and Cutler, 1995)
Lanariaceae	Raphides absent, styloids sometimes present (Rudall, 1998)
Laxmanniaceae (omandraceae)	Raphides present; styloids sometimes present (Rudall and Chase, 1996)
Orchidaceae	Raphides present, styloids absent (e.g. Gulliver, 1863–1865; Smith, 1923; Sandoval, 1993; Pridgeon, 1994; Stern, 1997). Druse-like structures also present in *Dendrobium aloifolium* (Carlsward *et al*., 1997). Rhomboids present in stem ground tissue of *Platythelys vaginata* (Stern *et al*., 1993)
Tecophilaeaceae	Raphides present in most genera (Simpson and Rudall, 1998)
Themidaceae	Raphides present (this paper)
Xanthorrhoeaceae	Raphides absent, styloids present (Rudall and Chase, 1996)
Xeronemataceae	Raphides present (this paper)
LILIALES	
Alstroemeriaceae	Raphides present; small crystals also present in *Bomarea hirtella* (this paper)
Calochortaceae	Crystals absent (e.g. Dahlgren and Clifford, 1982; Goldblatt *et al*., 1984)
Campynemataceae	Raphides present (Goldblatt *et al*., 1984)
Colchicaceae (including some former Uvulariaceae)	Raphides mainly absent (Gulliver, 1863–1865; Dahlgren and Clifford, 1982) although crystal sand recorded by Goldblatt *et al*. (1984) in some taxa (e.g. *Uvularia*), and 'raphide bodies' in *Streptopus amplexifolius*
Liliaceae (including some former Uvulariaceae)	Raphides absent (Gulliver, 1863–1865; Dahlgren and Clifford, 1982; Goldblatt *et al*., 1984; this paper). Druse-like crystals sometimes present in *Tricyrtis latifolia* (this paper)
Luzuriagaceae (only *Drymophila* and *Luzuriaga*)	Raphides present but rare in leaf mesophyll; styloids absent in *Luzuriaga* (Arroyo and Leuenberger, 1988). Raphides absent but small crystals present in *Luzuriaga radicans* (this paper)
Melanthiaceae (including Trilliaceae, but excluding Tofieldiaceae and Nartheciaceae)	Raphides present (Gulliver, 1863–1865; Dahlgren and Clifford, 1982)
Philesiaceae	Raphides present (Dahlgren and Clifford, 1982; this paper)
Smilacaceae (including Ropogonaceae)	Raphides present (Dahlgren and Clifford, 1982; this paper); small crystals also present in *Smilax china* (this paper) and styloids and crystal sand (Guaglianone and Gattuso, 1991)
DIOSCOREALES	
Burmanniaceae	Raphides absent (Rübsamen, 1986)
Dioscoreaceae (including *Dioscorea*, *Rajania* and *Tamus*)	Raphides normally present (Gulliver, 1863–1865; Ayensu, 1972); styloids normally absent but rarely present (Ayensu, 1972); unusual tiny calcium oxalate crystals associated with starch grains recorded by Okoli and Green (1987) in seven species of *Dioscorea*. Solitary crystals present in *Dioscorea alata* (Al-Rais *et al*., 1971); calcium oxalate present in stem sheath in *Dioscorea* (Xifreda, per. comm.); small crystals adjacent to vascular bundle in *Dioscorea minutiflora* (this paper)
Nartheciaceae	Raphides absent (pers. obs.) or rarely present, reported by Gulliver (1863–1865) in *Narthecium*
Stenomeridaceae (*Stenomeris*)	Raphides present (Ayensu, 1972)
Thismiaceae	Raphides present (Rübsamen, 1986)
Trichopodaceae (*Trichopus*, *Avetra*)	Raphides present (Ayensu, 1972)
PANDANALES	
Cyclanthaceae	Raphides and styloids present (Dahlgren and Clifford, 1982; Wilder, 1985); also small crystals present in *Asplundia insignis* (this paper)
Pandanaceae	Raphides present; also styloids crystals in *Pandanus gasicus* (Gulliver, 1863–1865; Dahlgren and Clifford, 1982; Huynh, 1989; this paper)
Stemonaceae	Raphides present; styloids present in *Stemona* (Ayensu, 1972)
Velloziaceae	Raphides possibly present (Dahlgren and Clifford, 1982); but reported as absent by Gulliver (1863–1865)
COMMELINOIDS: UNPLACED TAXA	
Abolbodaceae	Calcium oxalate crystals absent
Bromeliaceae	Raphides present (Tomlinson, 1969; Dahlgren and Clifford, 1982; this paper)
Dasypogonaceae	Calcium oxalate crystals rare or absent (Rudall and Chase, 1996)
Hanguanaceae	Calcium oxalate crystals absent or raphides rarely present (this paper)
Mayacaceae	Calcium oxalate crystals absent (Tomlinson, 1969)

TABLE 1. (*cont.*)

Family	Crystal type
Rapateaceae	Calcium oxalate crystals absent (Tomlinson, 1969), although small crystals seen in *Cephalostemon rupestris* (this paper)
COMMELINOIDS: ARECALES	
Arecaceae	Raphides present in all palms (Tomlinson, 1961; Weiner and Liese, 1995)
COMMELINOIDS: COMMELINALES	
Commelinaceae	Raphides present (Tomlinson, 1969; Kausch and Horner, 1982; this paper)
Haemodoraceae	Raphides present in some taxa (Gulliver, 1863–1865; Dahlgren and Clifford, 1982), but rare or absent in others; styloids present in some taxa, e.g. in flowers of *Wachendorfia* (this paper)
Philydraceae	Raphides and styloids present (Gulliver, 1863–1865; Dahlgren and Clifford, 1982)
Pontederiaceae	Raphides present (Sakai and Hanson, 1974; Kausch and Horner, 1981); also styloids (Gulliver, 1864; Dahlgren and Clifford, 1982)
COMMELINOIDS: POALES	
Anarthriaceae	Calcium oxalate crystals rare or absent (Cutler, 1969; Linder and Rudall, 1993)
Centrolepidaceae	Calcium oxalate crystals absent (Cutler, 1969)
Cyperaceae	Calcium oxalate crystals rare or absent (Metcalfe, 1971)
Ecdeiocoleaceae	Calcium oxalate crystals absent (Linder and Rudall, 1993)
Eriocaulaceae	Calcium oxalate crystals present (Tomlinson, 1969)
Flagellariaceae	Raphides absent (Linder and Rudall, 1993); calcium oxalate crystals present as small druse-like bodies (Tomlinson, 1969)
Joinvilleaceae	Calcium oxalate crystals present (Tomlinson, 1969)
Juncaceae	Calcium oxalate crystals absent (Gulliver, 1863–1865; Cutler, 1969)
Poaceae	Calcium oxalate crystals normally absent (e.g. Metcalfe, 1960; Linder and Rudall, 1993), but occasionally present in *Panicum* species (Ellis, 1988), and druses present in *Phyllostachys viridi-glaucescens* (this paper)
Restionaceae	Calcium oxalate crystals absent (Cutler, 1969)
Sparganiaceae	Raphides present (Cook and Nicholls, 1986); occasional druses also recorded in *Sparganium americanum* (Kausch and Horner, 1981)
Thurniaceae	Calcium oxalate crystals absent (Cutler, 1969)
Typhaceae	Raphides present (Horner *et al.*, 1981; Kausch and Horner, 1981; Rowlatt and Morshead, 1992)
Xyridaceae	Calcium oxalate crystals absent (Gulliver, 1863–1865; Tomlinson, 1969)
COMMELINOIDS: ZINGIBERALES	
Cannaceae	Calcium oxalate crystals absent (Tomlinson, 1969)
Costaceae	Calcium oxalate crystals absent (Tomlinson, 1969)
Heliconiaceae	Raphides present (Tomlinson, 1969); small crystals also present in mesophyll of *Heliconia rostrata*
Lowiaceae	Raphides present (Tomlinson, 1969)
Marantaceae	Calcium oxalate crystals absent (Tomlinson, 1969)
Musaceae	Raphides present (Tomlinson, 1969; Lott, 1976; McDougall *et al.*, 1993)
Strelitziaceae	Raphides present (Tomlinson, 1969)
Zingiberaceae	Calcium oxalate crystals normally absent (Tomlinson, 1969), but raphides and styloids recorded in *Aframomum melegueta*, *Amomum cardamomum*, *A. globosum*, *A. villosum* (Berger, 1958)

(1970) found that, in *Spirodella oligorrhiza*, the crystal chambers (raphidosomes) have an hour-glass outline in transverse section, and crystal formation is initiated between the two constrictions in the membrane profile. A calcium pump at these points concentrates Ca^{2+} within the chamber which then combines with free oxalate that has diffused into the compartment. However, from our own work, grooved raphides in Araceae form within rounded membrane chambers, suggesting that some of the calcium gates/channels have shut down, resulting in no crystal growth at these points (the grooves of the crystals). Many cellular modifications occur during the genesis of crystals, which is a highly complex process (Kausch and Horner, 1983).

Raphides in Araceae may also be barbed, such as those of *Alocasia*, *Colocasia*, *Dieffenbachia* and *Xanthosoma*, in which the tips of the barbs are slightly hooked and oriented away from the tapering end and towards the abruptly pointed end of the raphide. The barbs alternate along

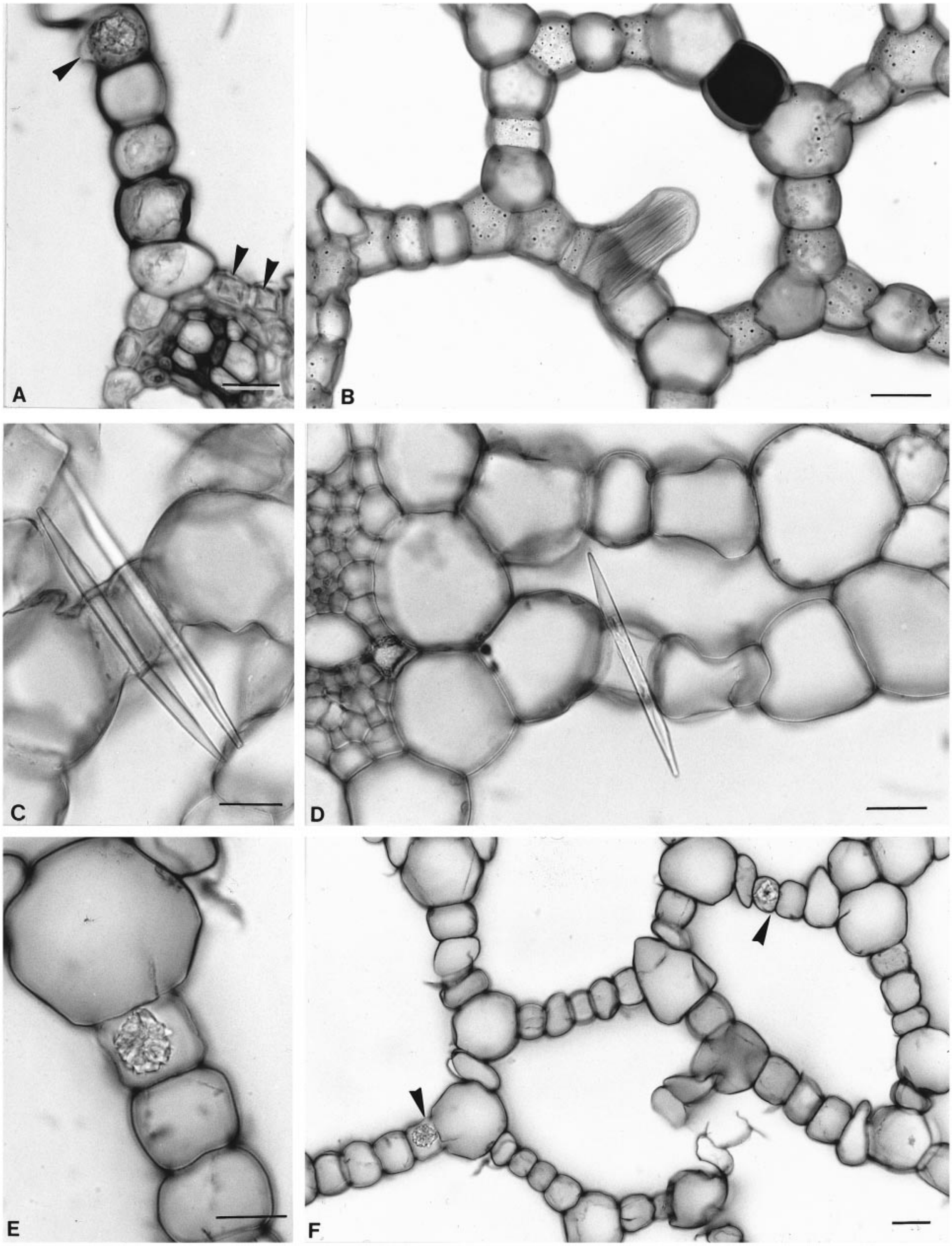

FIG. 1. Photomicrographs of the three main calcium oxalate crystal types present in monocotyledons. Bars = 20 µm. A, *Acorus calamus* (Acoraceae) transverse section of rhizome showing druses and styloids (arrowheads); B, idioblast in the stem of *Peltandra virginica* (Araceae) with raphides projecting into an air space; C, D, styloids projecting into intercellular spaces in transverse sections of rhizome of *Eichhornia crassipes* (Pontederiaceae); E, F, idioblasts containing druses found around intercellular spaces in *Acorus calamus*.

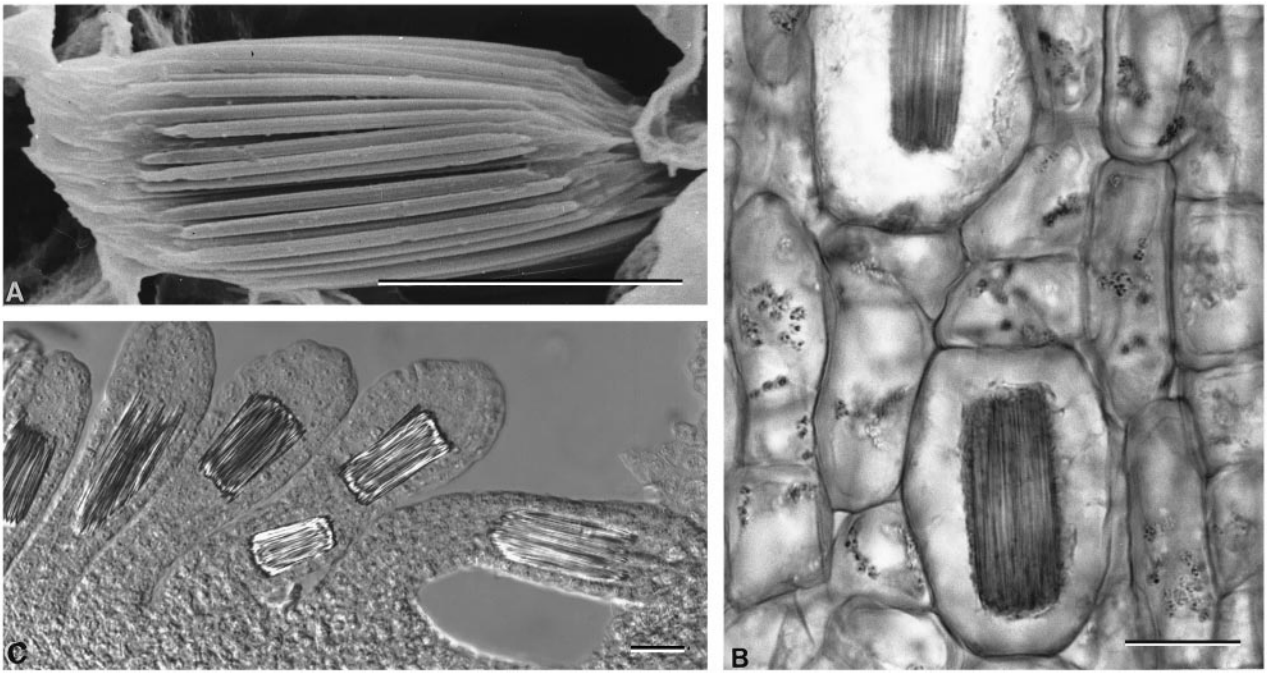

FIG. 2. Raphides may be present in parenchymatous tissues (A, B) and in specialized structures (C). Bars = 20 μm. A, SEM of a raphide bundle found in the ovary of *Lachenalia bulbifera* (Hyacinthaceae). Note that all raphides within a bundle are all orientated in the same direction; B, raphides found in *Liriope platyphylla*; C, DIC photomicrograph showing raphide bundles present in the uniserate hairs fringing the tepals of *Conanthera campanulata* (Tecophileaceae).

opposite sides of a crystal giving a pseudo-helical appearance (Sakai, Hanson and Jones, 1972; Sakai and Hanson, 1974; Sakai and Nagao, 1980; Cody and Horner, 1983). Monoclinic crystal structure partly determines the general direction of barb growth, although calcium concentration also plays a part.

Absence of raphides represents a synapomorphy for some groups, such as some families of Liliales (Rudall *et al.* unpubl. res.), Poales and all Alismatidae (Table 1). Raphides are also absent from *Acorus* (Table 1), which was formerly included in Araceae, but now placed in a separate family, Acoraceae, sister to the rest of the monocotyledons. In this respect it resembles some 'primitive' dicotyledons, such as Piperales (Grayum, 1987); further review of this character among monocot outgroups is required.

Styloids

Styloids, also called prismatic crystals or 'pseudoraphides', are thicker than raphides and usually solitary within a cell (Fig. 4A–D), although intermediate forms are recorded. The term styloid encompasses a broad morphological range. Styloids may have pointed ends (Fig. 1C, D), or squared ends (Fig. 4B), and may be elongated or cuboidal. Some styloids are 'twinned' crystals, e.g. the arrow-shaped styloids of *Iris pseudacorus* (Kollbeck, Goldschmidt and Schröder, 1914). In *Allium*, Arnott (1981) recorded interpenetrant twin calcium oxalate crystals (styloids) and Frey (1929) and Ricci (1963) reported a morphological range of styloids in different species, including 'normal' rhomboidal crystals, pyramidal crystals with irregular faces, small crystalline granules, and long prisms, sometimes isolated, and sometimes gathered together to form a druse. Large crystals may have adhering small crystals.

In monocot leaves, styloids are usually found either in parenchymatous bundle sheath cells around vascular strands or in crystal idioblasts in adjacent mesophyll tissues, although, in *Xanthorrhoea*, the styloids in the leaf are frequently epidermal (Rudall and Chase, 1996). In Iridaceae, elongated styloids occur in axially elongated mesophyll cells, and short cuboidal crystals in the shorter cells of the parenchymatous outer bundle sheaths; both types commonly occur in the same leaf (see below).

Styloids are characteristic of some families of Asparagales (Lilianae) (Table 1), including some 'higher' asparagoids (Agavaceae, Alliaceae, some Convallariaceae *sensu lato* and Lomandraceae) and some 'lower' asparagoids (Asphodelaceae, Doryanthaceae, some Hemerocallidaceae *sensu lato*, Lanariaceae, Iridaceae and Xanthorrhoeaceae). Sometimes raphides and styloids are mutually exclusive (in Doryanthaceae, Iridaceae, Xanthorrhoeaceae, *Phormium*), but in cases where both types occur (e.g. in Convallariaceae) intermediate forms can be present, with occasionally two or three crystals per cell. Styloids also represent a synapomorphy for some Commelinanae, especially Philydraceae and Pontederiaceae, and possibly also Haemodoraceae (Table 1), although more work is needed in this area.

Styloids are a diagnostic feature of certain families, notably Iridaceae. In Iridaceae, raphides are invariably absent but almost all taxa have styloids (Goldblatt, Henrich and Rudall, 1984; Rudall, 1994, 1995), with the exception of *Sisyrinchium* and its close allies, which lack crystals altogether (Rudall and Burns, 1989; Goldblatt, Rudall and

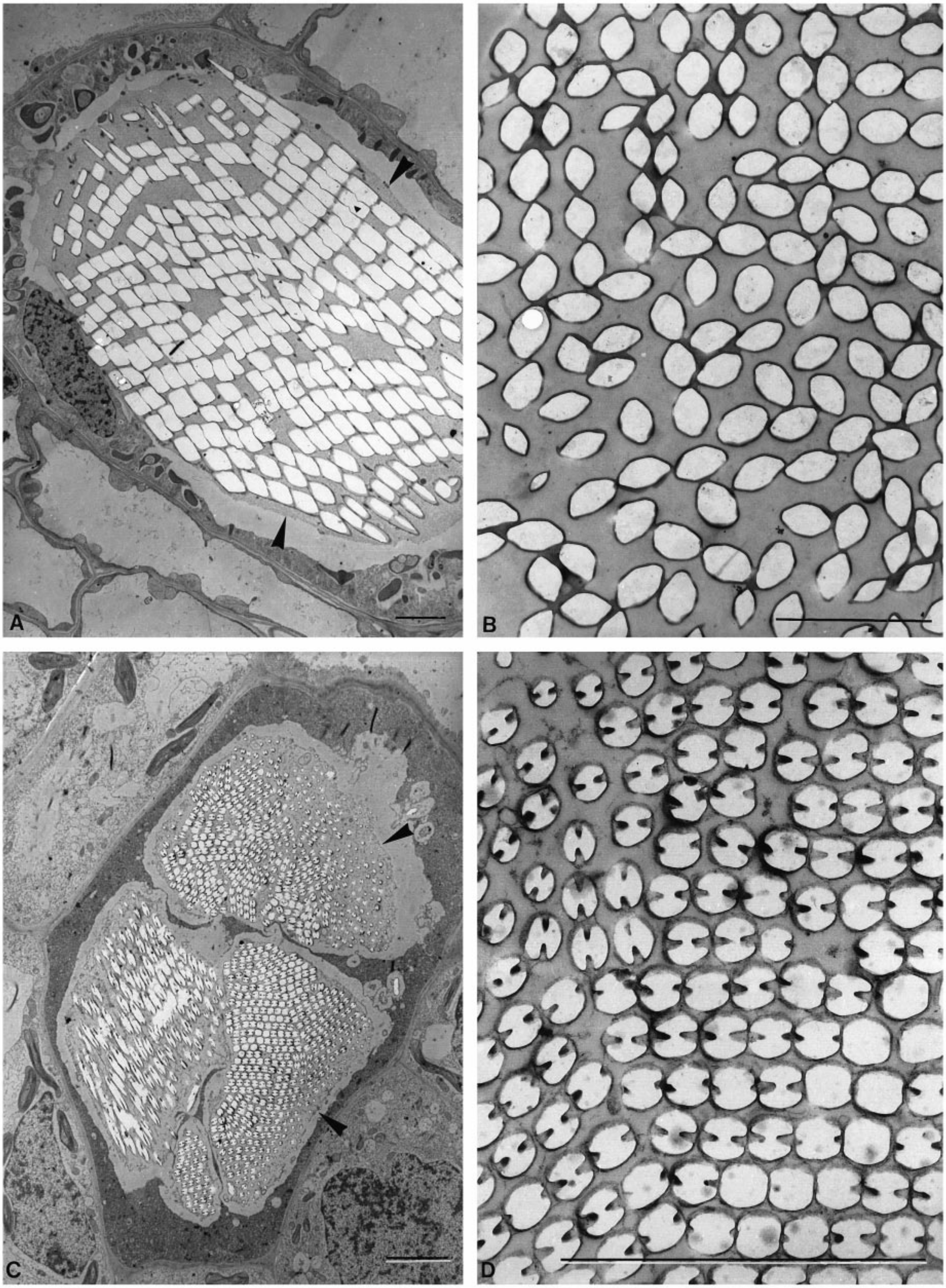

FIG. 3. TEMs of cross sections of raphide idioblasts from a leaf of *Aspidistra lurida* (Convallariaceae) (A, B) and the ovary of *Arisarum vulgare* (Araceae) (C, D). Note the mucilage surrounding the immature raphides in A and C (arrows). Bars = 5 µm. A, The immature crystals are mainly four-sided; B, the crystals have mainly six to eight sides in this mature raphide idioblast; C, D, grooved raphides occur in members of the Araceae.

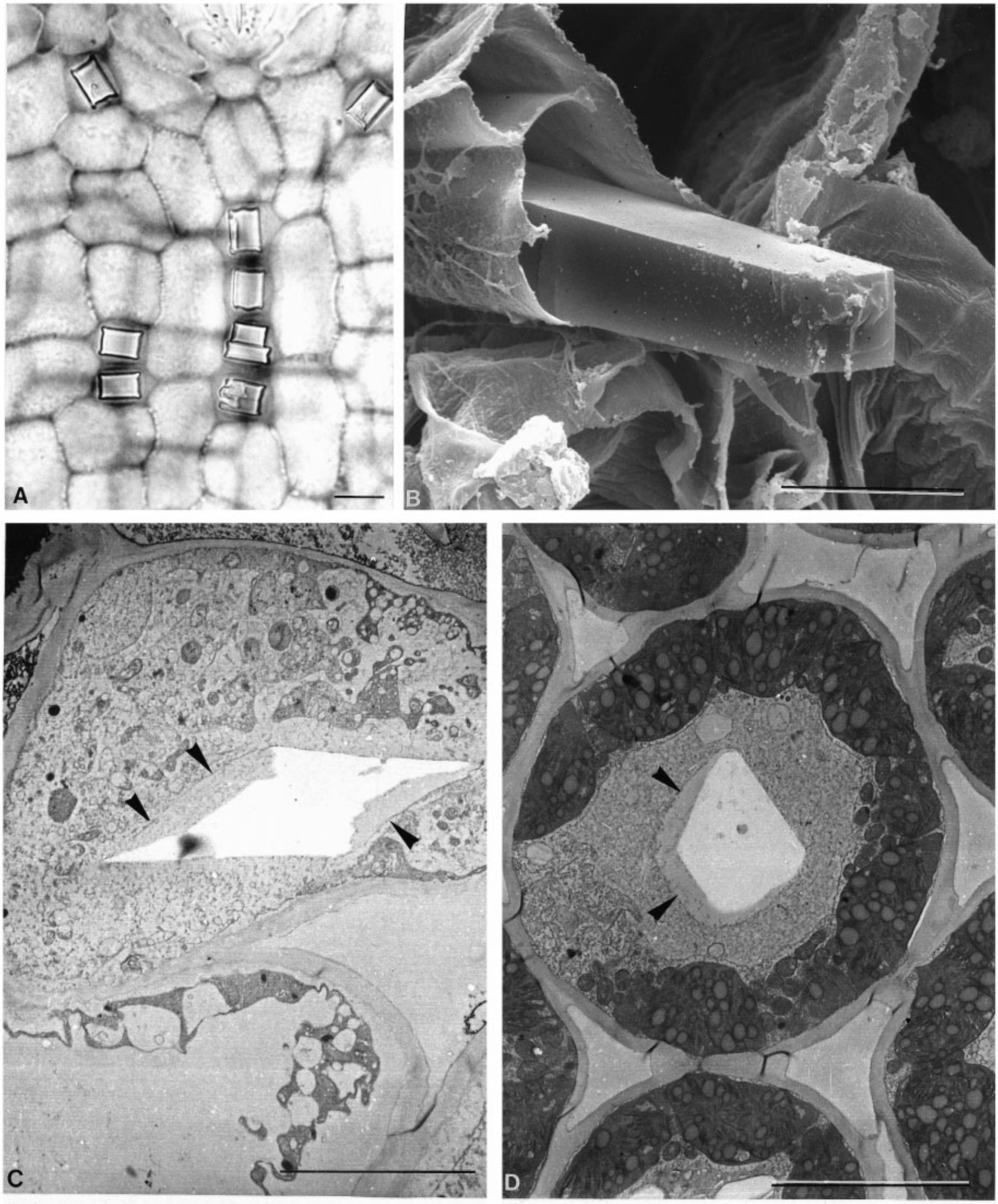

FIG. 4. Styloids tend to be solitary within a cell. Bars = 10 μm (A, C, D) or 100 μm (B). A, Styloids present in *Freycinetia javanica* (Pandanaceae). Note presence of extensions around edges of crystal; B, SEM of a large styloid in *Chlorophytum comosum* (Liliaceae). Such crystals could possibly have a structural function; C, D, TEMs of developing styloids in *Iris versicolor* (Iridaceae) and *Ruscus aculeatus* (Ruscaceae) respectively. Note presence of membraneous chambers around the crystals (arrows).

Henrich, 1990). Styloids vary considerably in size and shape; in cross section they may be square or rectangular, occasionally with the longer walls convex; in longitudinal section typically longer and slender (100–300 μm or longer), with pointed, forked or sometimes square ends. In some Iridaceae, such as *Bobartia* (Strid, 1974), *Dietes* (Rudall, 1983), *Hexaglottis*, *Patersonia* and *Romulea* (De Vos, 1970), short (16–25 μm long) more or less cuboidal crystals have been observed, usually in cells surrounding the sclerenchymatous bundle sheaths (Rudall, 1994). Wu and Cutler

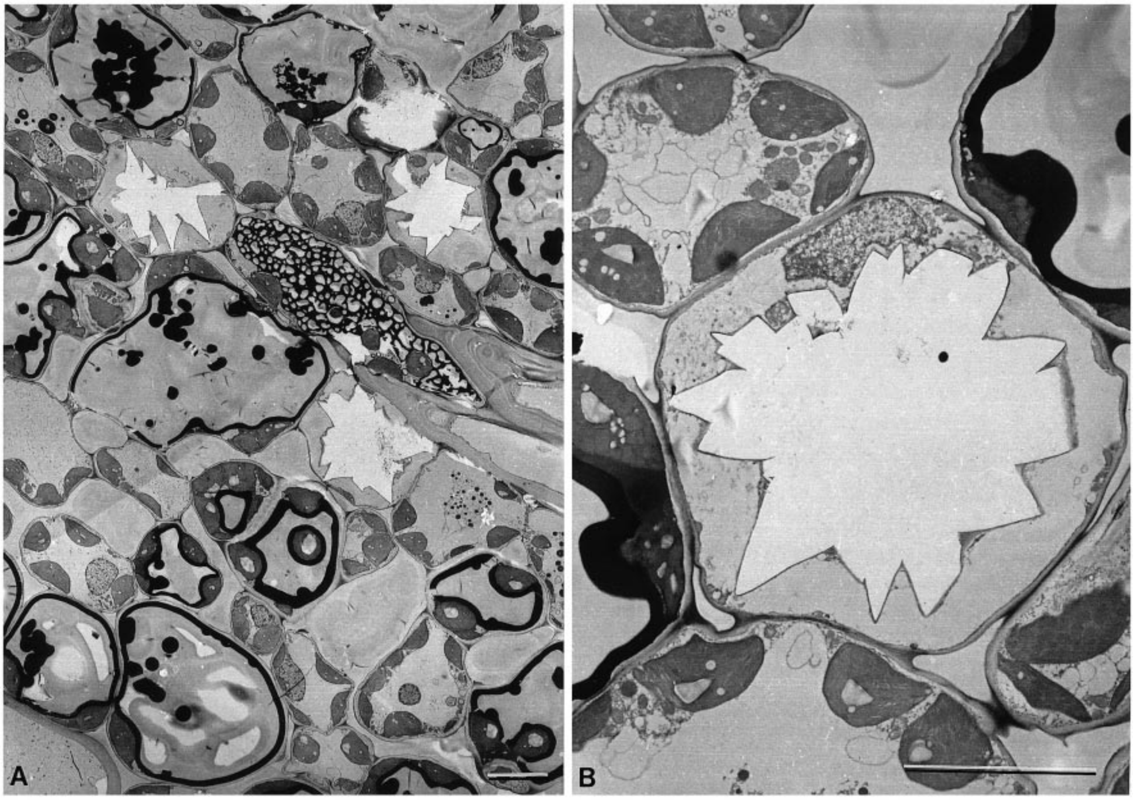

FIG. 5. TEMs of druses in *Monstera dubia* (Araceae). The crystals themselves are lost during the processing leaving an outline of their shape within the idioblast. Bars = 10 μm.

(1985) found that variation in styloid size and shape has some taxonomic application among species of *Iris*. Wolter (1990), in a comprehensive survey of corm tunics of *Crocus* species, found 'typical' elongated pointed styloids in 90% of species, and square-ended or cuboidal (prismatic) crystals in the rest, the latter type occurring irregularly among the different sections of the genus. Styloids also occur in the Tasmanian genus *Isophysis*, which is probably the sister taxon to all other Iridaceae, and in the Madagascan saprophyte *Geosiris*, which was originally placed in its own family, but has now been shown to belong within Iridaceae (Goldblatt *et al*., 1987). Styloids are also present (and raphides absent) in *Doryanthes* (Doryanthaceae), one of two monogeneric families which are now considered most closely related to Iridaceae, based on analysis of molecular data (Chase *et al*., 1995): in the other family, Ixioliriaceae (*Ixiolirion*), only raphides are recorded.

In other families of Asparagales, both raphides and styloids and intermediate forms may be present, particularly in a group of closely related 'higher' asparagoid families: Agavaceae and Convallariaceae *sensu lato* (including Dracaenaceae, Nolinaceae and Ruscaceae) (Rothert and Zalenski, 1899; Cutler, 1992). Cutler (1992) recorded a range of crystal types in leaves of *Liriope*, *Peliosanthes* and *Ophiopogon* (Convallariaceae), from 'normal' raphide bundles, axially oriented in a cell, to variously oriented crystal 'plates', fractured prisms and paired or grouped coarse styloids. At the species level, differences in calcium oxalate crystals were characters used to discriminate between *Polygonatum cirrhifolium* and *Polygonatum verticillatum* (Namba *et al*., 1991).

Druses

Druses (formerly called 'sphæraphides') are multiple crystals that are thought to have precipitated around a nucleation site to form a crystal conglomerate (Horner, Kausch and Wagner, 1981). The individual component crystals of a druse can also include contact twins. Both the monohydrate and dihydrate forms of calcium oxalate can form druses (Al-Rais, Myers and Watson, 1971; Franceschi and Horner, 1979). Druses may have a similar defensive function to that of raphides, as they also have sharp points (Fig. 5A, B) resulting in considerable irritation if eaten.

Druses are common in dicotyledons (see Frey, 1929) but relatively rare in monocotyledons, where they are almost entirely restricted to the first-branching taxa, *Acorus*, some Araceae and *Tofieldia* (Table 1). Although there are very few records of druses in the literature, in fact they are quite common in *Acorus*, especially in aerenchymatous tissues. In Araceae they may occur in conjunction with raphides (Table 1), in *Aglaonema*, *Alocasia*, *Anthurium*, *Colocasia*, *Dieffenbachia*, *Hydrosme*, *Philodendron*, *Pistia*, *Scindapsus*, *Spathiphyllum*, *Symplocarpus*, *Syngonium* and *Zantedechia* (Gaiser, 1923; Sakai *et al*., 1972; Kausch and Horner, 1981; Sunell and Healey, 1981; Genua and Hillson, 1985). There are also a few other records of druses in monocotyledons, e.g. in *Chlorophytum*, *Dendrobium*, *Flagellaria*, *Phyllostachys* and *Sparganium* (Table 1).

DEVELOPMENT

Crystals form within vacuoles of actively growing cells and are usually associated with membrane chambers, complexes, lamellae, mucilage and fibrillar material (Fig. 6A–D). This material appears before the crystals are initiated, indicating that the development of crystal idioblasts is equivalent to other processes of normal differentiation going on in the plant body rather than a pathological event forced upon the cell by a crystal (Zindler-Frank, 1980). Druses, which are multiple crystals (see above) are also associated with paracrystalline bodies in their early developmental stages. These bodies, which are possibly proteinaceous, are the nucleation sites around which single crystals grow to produce an aggregate druse crystal (Horner and Wagner, 1980).

Cells containing mature crystals often have a thin, peripheral layer of cytoplasm surrounding the central crystal-containing vacuole. Sheaths have been found to develop around some mature crystals; for example suberized sheaths occur around styloid crystals in *Agave* (Wattendorf, 1976*a*) and cellulosic sheaths around those of *Rhynchosia caribaea*, a dicot legume (Horner and Zindler-Frank, 1982). The production of wall material may be a result of a change in the metabolism of the cell that is similar to a wound response.

The shape and growth of crystals is controlled by membranous crystal chambers (Figs 4C, D, 6A–C) which occur in the vacuole and within which the calcium oxalate crystallizes (Arnott and Pautard, 1970). Developing crystals are coated with hydration layers (Nancollas and Gardner, 1974) which may be repelled by hydrophobic proteins and/or lipids of the chamber membrane (Cody and Horner, 1983). The chambers expand as the crystals within them grow. In the case of the needle-like raphide crystals (see below) these membrane-limited chambers seem to link together to become part of an extensive intravacuolar membrane system which orientates the raphide needles (Fig. 6D).

Cody and Horner (1983) proposed a model for crystal growth. They postulated an initial random distribution of dissolved calcium and oxalate ions in the crystal chamber, which may come together to form a crystalline configuration or 'cluster'. These clusters break up and reform easily. As the concentration of the dissolved ions increases, the crystal clusters may grow in size, rather than break up, such that crystal nuclei form. This increase in concentration to the point of saturation is controlled by plant metabolism. The crystal nuclei redissolve unless they reach a stable 'critical size', when their free energy is lower than that of the solution. As one crystal nucleus grows, it lowers the concentration of the dissolved ions in solution thus causing other precritical nuclei in the vicinity to dissolve. This tends to restrict crystal growth to one crystal per chamber.

During the growth of a single crystal, successive layers of ions are assembled on the lowest energy positions available on its surface. An ion may occupy an alternative, slightly higher, energy position, giving rise to a stacking error. The structure is still stable and when successive layers of ions are laid in the new most stable configuration, a contact twin will have been formed. The probability of twin crystal formation increases as the concentration of the dissolved ions in the crystal chamber increases. Since the concentrations of calcium and oxalate are governed by the plant's own metabolism, which in turn is governed by its genetic makeup, a particular taxon can have a characteristic crystal shape (Franceschi and Horner, 1980*b*), although some species have different crystal types in adjacent cells.

Crystal cell induction and development have also been studied using cultured vegetative tissues which produce calcium oxalate crystals. Tissue-cultured crystal idioblasts that are adjacent or in close proximity contain crystal bundles which bear no apparent orientation to each other, in contrast to intact tissues (Franceschi and Horner, 1980*a*). Callus tissue grown in the dark generally produces more idioblasts than light-grown tissue, except in leaf primordia and young expanding leaves where crystal idioblast production is high (Horner and Franceschi, 1981). Indeed, one of the major pathways suggested for oxalate synthesis is *via* glycollate and photosynthesis (Hodgkinson, 1977; Franceschi and Horner, 1980*b*).

Grooved raphides, which occur in Araceae (see above) are twinned crystals. Twin raphides are thought (Cody and Horner, 1983) to have begun growth at one end of a developing raphide bundle. It is possible that the inner surfaces of the membrane chambers in a vacuole have specific nucleation sites at which crystal growth is initiated. It could be that paracrystalline bodies, such as those that occur during druse development, are associated with the crystal chambers. It is not known whether calcium 'gates' or 'channels' are positioned throughout the crystal chamber membrane or are located around the periphery of the chamber. In the former case, the 'gates' are closed successively to maintain growth at the raphide tip, rather than throughout the developing crystal (Cody and Horner, 1983).

SUMMARY

Three main types of calcium oxalate crystal occur in monocotyledons: raphides, styloids and druses, although intermediates are sometimes recorded and more than one type may be present in a species. The absence of raphides, the most common crystal type in monocots, represents a synapomorphy in some groups, such as Alismatales, Poales and some Liliales. Raphides are bundles of narrow, elongated needle-shaped crystals usually found in crystal idioblasts in parenchymatous tissues. Most raphide crystals are four-sided but in some taxa they develop further into at least six-sided crystals with pointed ends. Grooved raphides are characteristic of Araceae.

Styloids ('pseudoraphides') are thicker than raphides and usually solitary within a cell. They may have pointed or squared ends, and may be elongated or cuboidal. Styloids are characteristic of some families of Asparagales, notably Iridaceae, where raphides are entirely absent. The presence of styloids is therefore a synapomorphy for some families (e.g. Iridaceae) or groups of families (e.g. Philydraceae, Pontederiaceae and Haemodoraceae), and more detailed studies on this aspect may well prove worthwhile for systematic studies on these groups. In some other families

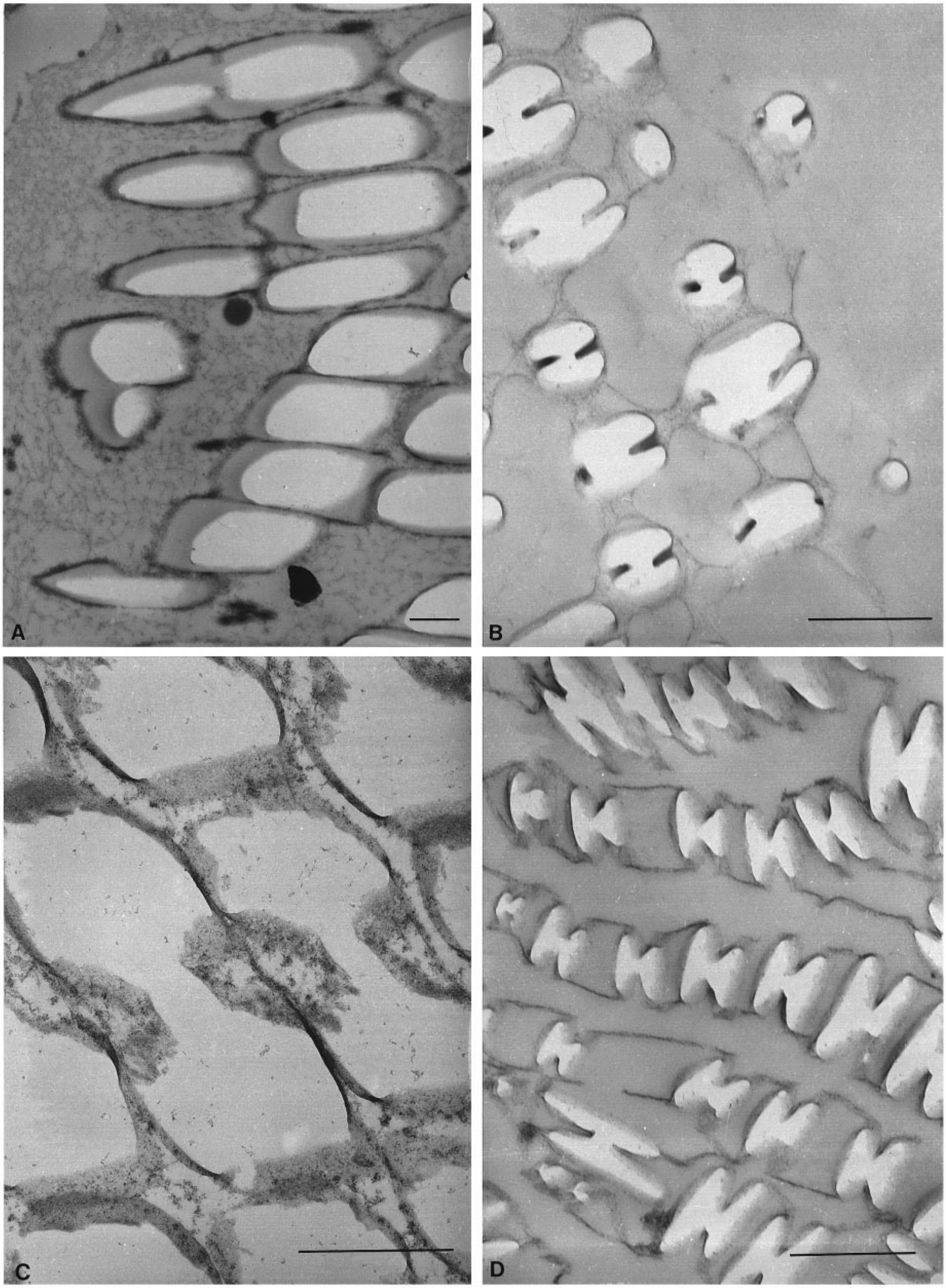

FIG. 6. TEMs of cross sections of raphide idioblasts of *Aspidistra lurida* (Convallariaceae) (A) and *Alocasia micholitziana* (Araceae) (B, C, D). Bars = 1 μm (A, C, D) or 100 nm (B). A–C, Raphides form within membraneous crystal chambers which control growth. Mucilage is also associated with the developing crystals; D, the membranes seem to link together to form 3-D networks which serve to orientate the crystals.

both raphides, styloids and intermediate forms may be present, particularly in a group of closely related 'higher' asparagoid families, such as Convallariaceae *sensu lato* and Agavaceae.

Druses ('sphæraphides') are crystal conglomerates possibly formed around a nucleation site. They are common in dicotyledons, relatively rare in monocotyledons, where they are almost restricted to some early-branching taxa such as *Acorus*, Araceae and *Tofieldia*.

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