Botanical Journal of the Linnean Society, 2004, **146**, 181–190. With 6 figures

On the presence of extracellular calcium oxalate crystals on the inflorescences of Araceae

DENIS BARABÉ[1]*, CHRISTIAN LACROIX[2], MATHIEU CHOUTEAU[1] and MARC GIBERNAU[3]

[1]*Institut de recherche en biologie végétale, Jardin botanique de Montréal, Université de Montréal, 4101 Sherbrooke Est, Montréal, Canada, H1X 2B2*
[2]*Department of Biology, University of Prince Edward Island, 550 University Avenue, Charlottetown, PEI, Canada, C1A 4P3*
[3]*Laboratoire d'Evolution & Diversité Biologique, UMR 5174, Université de Toulouse III, 118 Route de Narbonne, Bât. IV R 3–B 2, 31062 Toulouse Cedex 4, France*

Received August 2003; accepted for publication April 2004

This study presents a survey of the species of the Araceae where extracellular production of calcium oxalate crystals has been observed and discusses the patterns of production of the crystals in different genera. For all Araceae studied using SEM, the oxalate crystals exuding on the epidermal surface correspond to extended aggregate/druses or crystal sand and the oxalate crystals mixed with pollen correspond to raphides or styloids (prismatic crystals). The type of crystals associated with pollen varies among genera. However, the presence of crystals associated with pollen is a specific rather than a generic characteristic. Our results show that the presence of raphides mixed with pollen seems to be a widespread phenomenon in the aroid family. © 2004 The Linnean Society of London, *Botanical Journal of the Linnean Society*, 2004, **146**, 181–190.

ADDITIONAL KEYWORDS: anther – dehiscence – flower development – pollination.

INTRODUCTION

The presence of extracellular calcium oxalate crystals is a phenomenon that appears to be more or less widespread in plants. Extracellular crystal deposition on the epidermal surface is a characteristic feature of many lichens (Garty *et al*., 2002) and gymnospermous species (Oladele, 1982; Fink, 1991a,b; Pennisi *et al*., 2001). In angiosperms this phenomenon has been reported for Casuarinaceae (Berg, 1994), *Draceana* (Fink, 1991a; Pennisi *et al*., 2001), *Gleditsia* (Borchert, 1984), *Nymphaea* (Franceschi & Horner, 1980; Kuo-Huang, 1992), *Sempervivum* (Fink, 1991a; Vladimirova, 1996), and *Stelis* (Chase & Peacor, 1987). Tapetal raphides are formed in Commelinaceae (Hardy & Stevenson, 2000; Prychid, Furness & Rudall, 2003), Haemodoraceae, Philydraceae and Pontederiaceae (Prychid *et al*., 2003). Although these raphides are mixed with developing pollen grains or present in the locules of the anther, it is not clear whether or not they are mixed with pollen at dehiscence. In recent surveys of the distribution of calcium oxalate crystals in monocotyledons (Prychid & Rudall, 1999, 2000), there is no mention of extracellular crystals in this class of plants. In his survey of the anatomy of Araceae, Keating (2002) does not report the presence of extracellular crystals whereas intracellular crystals (e.g. raphides, styloids and druses) are common in this family. Based on this information, the presence of extracellular crystal exudates appears to be uncommon in monocotyledons in general and Araceae in particular.

D'Arcy, Keating & Buchmann (1996) published a review on the presence of oxalate packages (a mass of crystals or cluster of raphides) in the anthers of some angiosperms. The location of the crystals and their mode of production are well documented in Solanaceae (D'Arcy *et al*., 1996). The presence of packages of extracellular calcium oxalate crystals mixed with pol-

*Corresponding author. E-mail: denis.barabe@umontreal.ca

len are also reported in the aroid genus *Anthurium* by the same authors. Extracellular calcium oxalate crystals mixed with pollen were also documented a long time ago in *Arum* (Hegelmaier, 1871), *Calla* (Hegelmaier, 1871; Pohl, 1941), *Pinellia* (Hegelmaier, 1871) and *Zantedeschia* (Hegelmaier, 1871). However, there is neither a description in these reports of where the crystals are produced nor any indication of the mode of crystal liberation.

Recent developmental studies have shown that extracellular calcium oxalate crystals are visible on the surface of the apical portion of nearly mature stamens of many species of *Philodendron* (Barabé & Lacroix, 2001b; Barabé, Lacroix & Jeune, 2002a). These exudates are also visible on stamens, staminodes, or bristles in the genera *Arum* (Barabé, Lacroix & Gibernau, 2003) and *Schismatoglottis* (Barabé *et al*., 2004), where they form a more or less globular mass on the epidermal surface and appear to correspond to an oxalate package of crystals, *sensu* D'Arcy *et al*. (1996).

Although the presence of extracellular calcium oxalate crystals has been reported in some aroid genera, there is no morpho-functional analysis of this phenomenon in this plant family. It is also important to note that the function of extracellular crystals remains unclear in angiosperms. For example, is there a link between the production of extracellular crystals and pollination mechanisms? At what stage of development are the crystals liberated? In the present paper we address these questions by looking at the pattern of production of extracellular crystals in different genera of Araceae.

This study is intended to update the status of our knowledge on the presence of oxalate packages or free extracellular oxalate crystals in the Araceae family. More specifically its aims are: (1) to present a survey of the species where extracellular production of calcium oxalate crystals was reported; (2) to further document the liberation of calcium oxalate crystals by floral organs; and (3) to discuss the possible role(s) of these exudates in the context of the floral biology of the Araceae.

MATERIAL AND METHODS

Plant material

The material used in this study was collected in the field (French Guiana and Corsica) or in the living collections of the Montreal Botanical Garden and Biôdome as indicated in Tables 1 and 2. In the case of published studies, the provenance and preparation of samples are described in the indicated references. Voucher specimens have been deposited at the Herbier Marie-Victorin (MT).

Microscopy

Inflorescences from 23 species at different stages of development (Table 1) were observed using scanning electron microscopy (SEM). Specimens were partially dissected, fixed in formalin-acetic acid-alcohol (1:1:9 by volume), and later stored in 70% ethanol. Specimens were dehydrated in a graded ethanol series to absolute ethanol. They were then dried in a LADD model 28000 critical point dryer using CO_2 as a transitional medium, mounted on metal stubs, and grounded with conductive silver paint. Specimens were sputter-coated with gold/palladium to approximately 30 nm using a Denton Vacuum Desk II sputter coater, and viewed with a Cambridge Instruments S604 SEM with digital imaging capabilities (SEMICAPS).

Pollen and crystals

We slightly modified the method of D'Arcy *et al*. (1996) to collect pollen and associated crystals. The surface of recently dehisced anthers was touched gently with a microscope slide and the collected pollen then covered with a drop of diluted glycerine and a lamella (22 mm × 22 mm). In one case (*Arum pictum*) we also used the pollen that was accumulated at the bottom of the spathe. The abundance of oxalate crystals was assigned using the following density grades:

Rare = at least one crystal was observed on the surface area of the slide covered by the lamella.
*= Low density: 10–20 crystals were counted on the surface area of the slide covered by the lamella.
** = Moderate density: 20–100 crystals were counted on the surface area of the slide covered by the lamella.
*** = High density: > 100 crystals were present on the surface area of the slide covered by the lamella.

RESULTS

The presence or absence of crystals in 63 species of Araceae is summarized in Tables 1 and 2. The data are a combination of references from the literature (several from our studies on floral development in Araceae) and new observations made during this study. Based on our visual observations, it is not possible to determine precisely the type of crystals in each species studied. However, in nearly all members of Araceae observed using SEM, the oxalate crystals exuding on the epidermal surface of the stamens correspond to extended aggregate/druses (Figs 2–4) *sensu* Metcalfe & Chalk (1983; fig. 4.2). In a few cases (Fig. 6) they resemble crystal sand as defined by Franceschi & Horner (1980, fig. 12). The oxalate crystals mixed with pollen (Table 2) at dehiscence corre-

Table 1. Presence (+) or absence (–) of crystals on floral structures observed in selected species of Araceae using SEM. *n*, number of inflorescences observed; ?, stage of development not observed

Species	Stamens	Staminodes	Stigma	Stages		Origin and voucher
				Early	Mature	
Anaphyllopsis americana (Engl.) A. Hay (*n* = 12)	–	No staminode	–	–	–	French Guiana (*Barabé 83*)
Ambrosina bassii L. (*n* = 17)	–	No staminode	–	?	–	Corsica (Barabé & Gibernau 180)
Arisarum vulgare Targ.-Tozz. (*n* = 22)	+ (mixed with pollen)	No staminode	+	?	+	Corsica (*Barabé & Gibernau 178*)
Arum italicum Mill.	+	+	–	+	–	Barabé *et al*. (2003)
A. pictum L. f. (*n* = 5)	–	–	–	?	–	Corsica (Barabé & Gibernau 179)
Caladium bicolor (Ait.) Vent.	–	–	–	–	–	Barabé & Lacroix (2002)
Culcasia saxatilis A. Chev.	–	–	–	–	–	Barabé & Bertrand (1996)
Cercestis stigmaticus N. E. Br.	–	–	–	–	–	Barabé & Bertrand (1996)
Montrichardia arborescens (L.) Schott	–	–	–	–	–	Barabé & Lacroix (2001a), Boubes & Barabé (1997)
Philodendron callosum K. Krause (*n* = 18)	–	–	–	–	?	French Guiana (*Barabé 68*)
P. acutatum Schott	–	–	–	–	–	Boubes & Barabé (1996)
P. fragrantissimum (Hooker) Kunth	–	–	–	–	–	Barabé, Lacroix & Jeune (2000)
P. grandifolium (N. J. Jacq.) Schott	–	–	–	–	–	Barabé & Lacroix (2001b)
P. insigne Schott	+	–	+	+	+ (Stigma)	Barabé, Lacroix & Jeune (2002a)
P. megalophyllum Schott	+	–	–	+	–	Barabé & Lacroix (2001b)
P. melinonii Brong. ex Regel	–	–	+	–	+ (Stigma)	Barabé & Lacroix (2000)
P. ornatum Schott (*n* = 8)	?	?	+	?	+	French Guiana (*Barabé 30*)
P. pedatum (Hooker) Kunth (*n* = 36)	+	–	–	+	–	French Guiana (*Barabé 35*)
P. solimoesense A.C. Smith	–	–	–	–	–	Barabé & Lacroix (1999)
P. squamiferum Poepping	–	–	–	–	–	Barabé, Lacroix & Jeune (2002b)
Schismatoglottis calyptrata (Roxb.) Zoll. & Moritzi (*n* = 16)	+	+	–	–	+	JBM (3152–59) (*Barabé & Archambault 194*)
S. americana Jonker & Jonker (*n* = 14)	+ (mixed with pollen)	–	–	–	–	French Guiana (*Barabé 82*)
Zamioculcas zamiifolia (Lodd.) Engl	–	–	–	–	–	Barabé *et al*. (2002c)

spond to raphides or styloids (prismatic crystals) *sensu* Prychid & Rudall (1999).

In all species of *Philodendron* examined to date, the accumulation of extracellular calcium oxalate crystals on the surface of stamens takes place at nearly mature stages of development, before the formation of the stigma on female flowers and before the release of pollen. These exudates form globular masses on the surface of the epidermis (Figs 1–4). During early stages of their formation, the calcium oxalate packages appear to be covered by the cuticle (Fig. 3). This accumulation of oxalate crystals eventually breaks through the cuticular cover prior to anther dehiscence (Fig. 4). In *Philodendron*, the presence of oxalate crystals was not observed on the surface of mature stamens when the spathe opened up. However, raphides and prismatic crystals were observed with pollen at anthesis in *P. megalophyllum* and *P. moonenii* (Table 2). It is not possible to determine conclusively if styloids mixed with pollen at

Table 2. List of types and density of oxalate crystals mixed with pollen based on the method of D'Arcy et al. (1996)

Species	Crystals	Origin	Voucher
Anthurium acaule (Jacq.) Schott	Raphides (*)	JBM (2240–57)	*Barabé et al. 223*
	Raphides (Rare)	JBM (2240–57)	*Barabé et al. 223*
Anthurium amoenum Kunth	No	Biodôme (7012–00)	*Barabé et al. 252*
Anthurium clavigerum Poepp. & Endl.	No	JBM (2150–52)	*Barabé et al. 221*
Anthurium consobrinum Schott	No	Biodôme (7226–92)	*Barabé et al. 254*
Anthurium crystallinum Linden & André	Raphides (***)	JBM (1645–42)	*Barabé et al. 241*
Anthurium cubense Engler	Raphides (*)	JBM (539–39)	*Barabé et al. 225*
	No	JBM (539–39)	*Barabé et al. 225*
Anthurium fendleri Schott	No	JBM (2317–53)	*Barabé et al. 220, 222*
	No	JBM (2727–51)	*Barabé et al. 220, 222*
	Raphides (*)	JBM (2727–51)	*Barabé et al. 220, 222*
Anthurium guildingii Schott	No	JBM (2436–92)	*Barabé et al. 229*
Anthurium halmoorei Croat	Raphides (*)	JBM (3393–88)	*Barabé et al. 224, 242*
	No	JBM (3393–88)	*Barabé et al. 224, 242*
Anthurium harrisi (Grah.) Endl.	Raphides (Rare)	Biodôme (7241–92)	*Barabé et al. 228, 253*
	Raphides (*)	JBM (635–42)	*Barabé et al. 228, 253*
	No	JBM (635–42)	*Barabé et al. 228, 253*
Anthurium jenmanii Engler	Raphides (*)	JBM (1538–98)	*Barabé et al. 248*
	Raphides (*)	JBM (1585–94)	*Barabé et al. 248*
Anthurium longistamineum Engler	No	JBM (3038–59)	*Barabé et al. 231, 233, 250*
	Raphides (*)	JBM (3038–59)	*Barabé et al. 231, 233, 250*
	Raphides (Rare)	JBM (3038–59)	*Barabé et al. 231, 233, 250*
Anthurium longistamineum Engler	Raphides (*)	JBM (3038–59)	*Barabé et al. 231, 233, 250*
Anthurium magnificum Linden	Raphides (*)	Biodôme (7845–39)	*Barabé et al. 256*
	Raphides (**)	Biodôme (7845–39)	*Barabé et al. 232, 256*
Anthurium ornatum Schott	No	JBM (1776–56)	*Barabé et al. 237*
Anthurium pallidiflorum Engler	Raphides (*)	D'Arcy *et al*. (1996)	
Anthurium pedato-radiatum Schott	Raphides (**)	Biodôme (7074–98)	*Barabé et al. 240*
Anthurium polyrrhizum C. Koch & Augustin	Raphides (***)	JBM (2288–51)	*Barabé et al. 226*
	No	JBM (2288–51)	*Barabé et al. 226*
Anthurium schlechtendalii Kunth	Raphides (*)	JBM (2463–54)	*Barabé et al. 219*
Anthurium signatum C. Koch & Mathieu	Raphides (**)	Biodôme (7016–00)	*Barabé et al. 255*
Anthurium sp. 53	No	JBM (2751–93)	*Barabé et al. 247*
Anthurium sp. 63	No	JBM (2389–92)	*Barabé et al. 246*
Anthurium spectabile Schott	Raphides (**)	JBM (2044–68)	*Barabé et al. 234*
Anthurium torresianum Engler	No	JBM (1413–58)	*Barabé et al. 230*
Anthurium trinerve Miq.	No	JBM (3380–88)	*Barabé et al. 243*
Anthurium upalaense Croat & Baker	No	JBM (2061–68)	*Barabé et al. 227, 245*
	No	JBM (2061–68)	*Barabé et al. 227, 245*
Anubias heterophylla Engler	Raphides (**)	JBM (1909–99)	*Barabé et al. 235*
Anubias sp. 2	Raphides (***)	JBM (1649–86)	*Barabé et al. 236*
Arisarum vulgare Targ.-Tozz.	No	Corsica	*Barabé 178*
	No	Corsica	*Barabé 178*
	Raphides (*)	Corsica	*Barabé 178*
	Raphides (*)	Corsica	*Barabé 178*
	Raphides (***)	Corsica	*Barabé 178*
	Raphides (***)	Corsica	*Barabé 178*
Arum pictum L. f.	Raphides (*)	Corsica	*Barabé 179*
	Raphides (*)	Corsica	*Barabé 179*
Arum sp.	Raphides	Hegelmaier (1871: 648)	
Calla palustris	Raphides	Hegelmaier (1871; Fig. 9) Pohl (1941: 85)	
Cercestis stigmaticus N. E. Br.	No	Biodôme (7078–98)	*Barabé et al. 239*
Cercestis stigmaticus N. E. Br.	No	Biodôme (7078–98)	*Barabé et al. 239*

Table 2. *Continued*

Species	Crystals	Origin	Voucher
Homalomena rubescens (Roxb.) Kunth	No	JBM 1721–55	*Barabé et al. 238*
Monstera delisiosa Liebm.	No	JBM (1636–53)	*Barabé et al. 249*
Pinellia sp.	Raphides	Hegelmaier (1871: 648)	
Philodendron grandifolium (N. J. Jacquin) Schott	No	French Guiana	*Barabé 210*
Philodendron megalophyllum Schott	Raphides (*) and prismatic crystals (**)	French Guiana	*Barabé 214*
Philodendron moonenii Croat	Raphides (*) and prismatic crystals (**)	French Guiana	*Barabé 204*
Philodendron solimoesense A. C. Smith	No	French Guiana	*Barabé 203*
Philodendron tripartitum (N. J. Jacquin) Schott	No	JBM 2803–50	*Barabé et al. 244*
Stenospermation longipetiolata Engler	Raphides (****) and prismatic crystals (*)	Biodôme (7057–98)	*Barabé et al. 251*
Syngonium auritum (L.) Schott	No	Biodôme (7342–92)	*Barabé & Chouteau 216*
Syngonium shottianum H. Wendl. ex Schott	Raphides (***) and prismatic crystals (*)	Biodôme (7013–98)	*Barabé & Chouteau 212*
Zantedeschia aethiopia (L.) Sprengel	Raphides	Hegelmaier (1871: 649)	

JBM, Montreal Botanical Garden; Biodôme, Living collections of the Biodôme de Montréal.
*low density; **moderate density; ***high density (for a quantitative description of the density levels, see Material and Methods).

dehiscence correspond to oxalate packages observed on the surface of stamens during the early stages of development. Free oxalate crystals not mixed with pollen were also present on the stigmatic surface in *P. insigne*, *P. melinonii*, *P. ornatum* and in *Arisarum vulgare* (Table 1, Fig. 5).

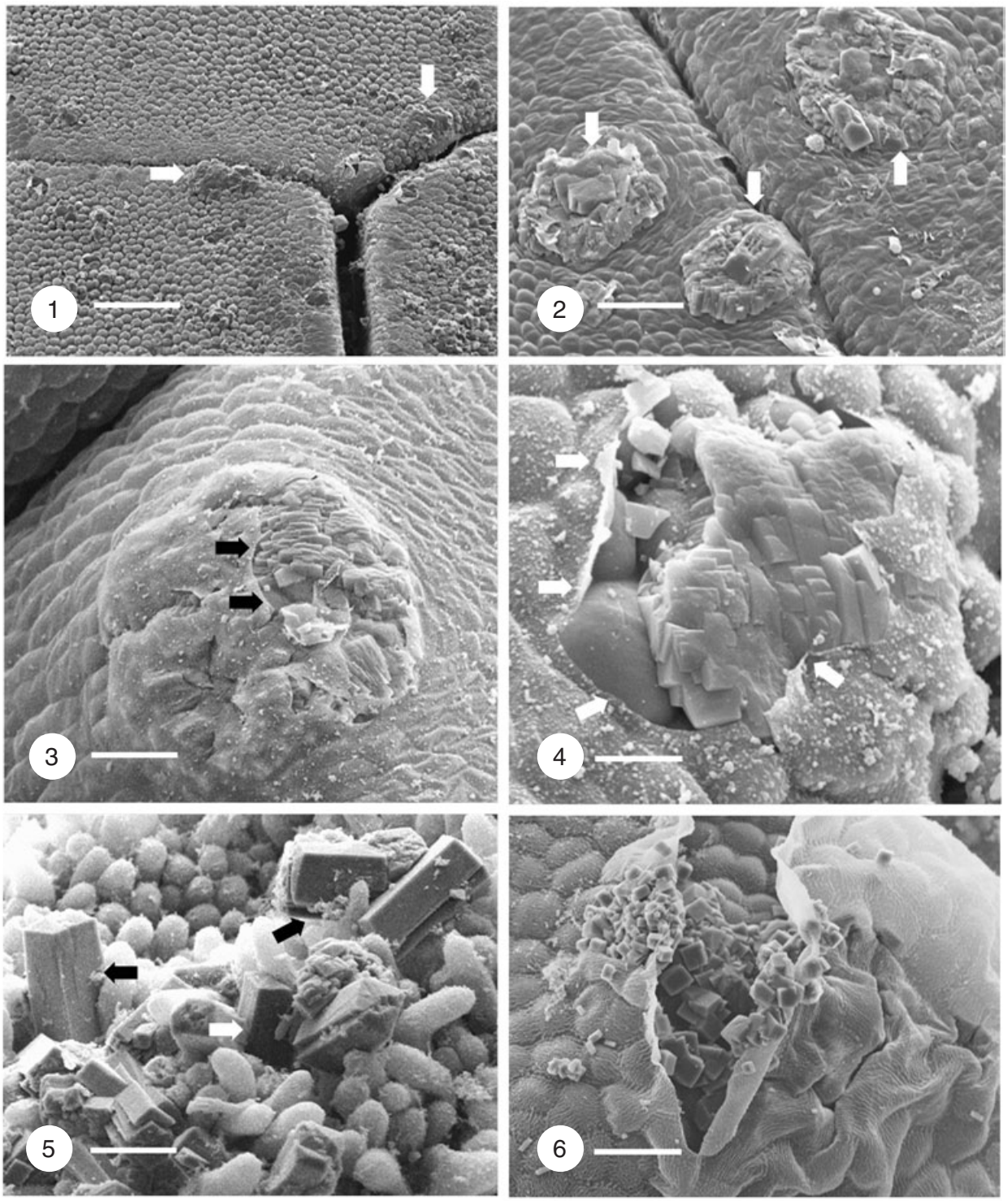
In *Arum italicum*, the accumulation of extracellular crystals occurs during the early stages of development of stamens, staminodes and the sterile appendix, when an inflorescence reaches approximately 6% of its final size (Barabé *et al*., 2003). As in *Philodendron*, the oxalate crystals also accumulate under the cuticular surface forming a flattened globular mass. When the cuticle splits, crystals are liberated and become widespread on the surface of the male and sterile portions of the inflorescence. In the female zone, few crystals were observed. However, prismatic crystals found on stigmatic surfaces are too large to have originated from the surface of the anthers.

Globular masses were not observed on the surface of the flowers of *Arum italicum* or *A. pictum* at maturity (D. Barabé, unpubl. data). Unfortunately, early stages of development of *A. pictum* were not available to confirm the presence of crystals at that stage as in *A. italicum*.

In *Arum* and *Philodendron*, the oxalate packages are produced by the male and/or sterile organs before the formation of the stigma. In *Schismatoglottis*, the oxalate packages are produced in the centre of the top portion of the stamen near a stomate (Fig. 5). The release of oxalate crystals occurs when the stamen and the stigma are mature (Barabé *et al*., 2004). In *Arisarum vulgare* and *Schismatoglottis americana* few prismatic crystals mixed with pollen were observed before dehiscence (Table 1).

No raphides were observed on the staminal epidermal surface of stamens in any of the 23 species listed in Table 1.

Crystals associated with pollen at dehiscence were reported in *Anthurium*, *Anubias*, *Arisarum*, *Arum*, *Philodendron*, *Stenospermation* and *Syngonium*. However, in the genera *Anthurium*, *Philodendron* and *Syngonium* the presence of this character varied among species. For example, raphides mixed with pollen were observed in *Anthurium acaule*, whereas they were absent in the pollen of *Anthurium upalaense* (Table 2). This indicates that the presence of crystals associated with pollen is a specific rather than a generic characteristic.



Figures 1–6. Occurrence of extra-cellular crystals in species of Araceae. Figs 1–4. *Philodendron pedatum*. 1, Overall view of surface features of nearly mature stamens producing calcium oxalate crystals (arrows). Scale bar = 75 μm. 2, close-up of calcium oxalate crystal packages (arrows). Scale bar = 300 μm. 3, early stage of formation of crystal packages. Note the partial cuticular cover on the crystals (arrows). Scale bar = 150 μm. 4, high magnification of calcium oxalate crystals showing the torn cuticular cover (arrows). Scale bar = 75 μm. Fig. 5, *Philodendron ornatum*, crystals (arrows) between papillae on the stigmatic surface. Scale bar = 300 μm. Fig. 6, *Schismatoglottis calyptrata*, crystal package partially covered by the cuticle on the top portion of the stamen. Scale bar = 300 μm.

The type of crystals associated with pollen also varies among genera. For example, in the genus *Anthurium* only raphides mixed with pollen were found. However, in the genera *Philodendron* (e.g. *P. megalophyllum*) and *Syngonium*, the pollen is associated with raphides and other types of crystals (Table 2). Variations can even occur within the same species. For example, in *Arisarum vulgare*, the prismatic crystals observed using the SEM were not visible in pollen collected on the slide at the time of dehiscence. Also, two out of six specimens of this species had pollen that was not associated with raphides. There was also some variation in the quantity of crystals observed among pollen, ranging from low (*) to high densities (***) of crystals (Table 2).

DISCUSSION

Crystals mixed with pollen

In the Araceae, D'Arcy *et al*. (1996) noted that the stamens of *Anthurium pallidiflorum* delivered raphides and pollen when touched with a moistened microscope slide. Hegelmaier (1871) reported the presence of crystals mixed with pollen in the genera *Pinellia* and *Arum*. Crystals mixed with pollen were also observed in *Zantedeschia* (Hegelmaier, 1871 citing Van Tieghem, 1866) and *Calla* (Hegelmaier, 1871; Pohl, 1941). Unfortunately, Van Thiegmen's original publication (1866) does not contain any information on the presence of oxalate packages in the flower of *Zantedeschia*. Our results on 63 species show that the presence of raphides mixed with pollen seems to be a widespread phenomenon in the aroid family.

The quantity of crystals associated with pollen varies among individuals or within the same inflorescence. In the case of *Arisarum vulgare*, it ranges from completely absent to very dense (Table 2). This variation may be a consequence of the method used to collect pollen or to an intrinsic variability associated with the biology of the species. However, given the small size of the inflorescence and the reduced number of stamens in *Arisarum*, it is plausible to think that the method used to collect pollen can produce an artefact. In *Anthurium*, we observed that the quantity of raphides mixed with pollen varies among inflorescences of the same species. Therefore, future studies should repeat the collection of pollen on different inflorescences to confirm the presence or absence of crystals in a given species.

Oxalate packages

In *Arum italicum*, the accumulation of extracellular calcium oxalate crystals also occurs during early stages of development of stamens, staminodes and the sterile appendix. As in *Philodendron*, the oxalate packages accumulate under the cuticular surface and form a flattened globular mass. The extracellular calcium oxalate crystals observed in these genera are similar to those reported for *Welwitschia* (Scurfield, Michell & Silva, 1973) and *Tsuga* (Gambles & Dengler, 1974). However, in the two latter cases the crystals do not seem to be released in clusters.

As noted by D'Arcy *et al*. (1996: 179) '. . . Oxalate packages bearing anthers are found in plants living under a wide range of environments . . . displaying a variety of forms . . . and facing a wide range of interactions . . .' This is also true for plants of the Araceae family where extracellular oxalate crystals occur in epiphytic (e.g. *Anthurium*), hemi-epiphytic (e.g. *Philodendron*) or terrestrial plants (*Arum*, *Schismatoglottis*). Moreover such crystals are present in tropical and temperate aroids and also in beetle-, bee- and fly-pollinated taxa. Additionally, the production of extracellular calcium oxalate crystals in Araceae is not restricted to stamens. It also occurs, depending on the genera, on staminodes (e.g. *Schismatoglottis*) or sterile appendices (*Arum*).

In contrast to *Philodendron insigne* and *P. megalophyllum*, no extracellular oxalate crystals were observed on the surfaces of stamens of *P. acutatum, P. fragrantissimum, P. melinonii,* and *P. solimoesense*. These observations indicate that the potential to produce oxalate substances on stamens is not common to all *Philodendron*. To date, no explanation for the presence (or absence) of oxalate packages within a genus with a uniform floral biology (24 h anthesis, bi-phasic thermogenetic cycle, beetle pollination) exists. On the other hand, it seems that closely related species have the same patterns of crystal liberation. For example, in *Philodendron megalophyllum* and *P. moonenii*, two species that have the same habit and inflorescence morphology (resiniferous canals in the spathe and not in spadices), styloids and raphides mixed with pollen are found.

The presence of extracellular crystals on developing anthers occurs in species of *Philodendron* producing resin on the spadix (e.g. *P. pedatum*) or on the spathe (*P. megalophyllum*, *P. moonenii*). Even though resin is also produced by the spathe of *P. solimoesense* (subgroup *Meconostigma*), no crystals (raphides or styloids) were observed during the development of anthers or were mixed with pollen at dehiscence. This seems to indicate that the production of crystals is not linked with that of resin. However, more species of *Philodendron* have to be analysed in order to confirm this. It would be interesting to determine in this context, if there is a correlation between the phylogeny of the different species and the presence of oxalate packages. Unfortunately, there is presently no phylogenetic study of the genus *Philodendron* on which we could base such an analysis.

Crystals and anther dehiscence

Crystals mixed with pollen may play a defensive role against pollinator consumption or in pollen liberation (D'Arcy *et al*., 1996). In the case of *Arum*, *Philodendron* and *Schismatoglottis* the presence of calcium oxalate crystals on the epidermal surface does not appear to be related to a dehiscence mechanism. The crystals are exuded on the surface of the epidermis, and in a few genera (e.g. *Arum*, *Philodendron*) they are released during early stages of development, long before anther dehiscence occurs. In *Schismatoglottis*, although the crystals are released during dehiscence, their location on the stamen is not related to that of the opening of the anther. The production of crystal therefore appears to be morphologically independent from anther dehiscence (Barabé & Lacroix, 2001b). In *P. megalophyllum*, the presence of styloids was observed on stamens during early stages of development of the inflorescences. These styloids were mixed with pollen at the time of dehiscence (Table 2). This indicates that the dynamics of release of extracellular crystals varies according to their location. They are exuded on the epidermal surface before the complete maturity of stamens, but they remain on the stamen surface until pollen liberation. The situation is different with raphides. Raphides are mixed with pollen at dehiscence and are not present on the surface of stamens during development. Their mixing with pollen is probably the result of the breakdown of the wall of the theca. It remains unclear whether the release of raphides plays a role or not in dehiscence mechanisms. The absence of raphides in many species belonging to different genera seems to suggest that their release is not directly involved in dehiscence mechanisms. The release of raphides may therefore be a consequence of the dehiscence mechanism, instead of an essential step in the process of pollen sacs opening as it is the case for example in *Solanum* (D'Arcy *et al*., 1996). The quantity of raphides mixed with pollen in a given species would therefore depend on the quantity of raphides present in the anther wall before dehiscence occurs.

Formation of crystals

In the genus *Stelis* (Orchidaceae), extracellular oxalate crystals have been interpreted as the product of pseudo-nectaries (Chase & Peacor, 1987). Dauman (1930, 1970) reported the production of nectariferous fluid droplets by the inflorescence in the genera *Anthurium, Arisaema* and *Arum.* However, we have not been able to relate the presence of extracellular crystals to pseudo-nectaries or stomata in *Arum, Philodendron* or *Schismatoglottis.* The modes of crystal deposition in epidermal subcuticular areas still remain unknown in the Araceae. On the other hand, they might be similar to those observed in *Dracaena*, another monocotyledon (Pennisi *et al*., 2001), where crystals are also found between the primary cell wall and the cuticle. Although the function of the extracellular crystals remains unclear, a variety of uses have been proposed. For example, the formation of crystals on the thallial surface of *Ramalia* is a response to environmental conditions (Garty *et al*., 2002). In *Pistia stratiotes*, druse and raphide crystals inside the leaf play a role in calcium regulation in tissues (Keates *et al*., 2000; Volk *et al*., 2002). It is plausible that in Araceae, as in other families, the oxalate crystals play a physiological role by removing the oxalate which may otherwise accumulate in toxic quantities (Franceschi & Horner, 1980).

Free crystals were also observed on the stigmatic surface in *Philodendron melinonii* (Barabé & Lacroix, 2000) and *P. ornatum* (Fig. 5). As far as the presence of oxalate crystals on stigmatic surfaces is concerned, Miaja *et al*. (1998–99) reported exudates of calcium oxalate crystals on the stigmatic surface of *Vitis vinifera* L. cv. Barabera, but they did not discuss the possible significance of this observation. For many species, boron and calcium are required for pollen tube growth (Richards, 1986). Calcium is found on the surface of some pollen grains and is often required for germination. It is also involved in pectin synthesis and control of osmotic conditions in pollen (Richards, 1986). Therefore, it is possible that the presence of oxalate crystals on the stigma could improve the success of pollen germination and ultimately reproduction efficiency when they are mixed with pollen (Stephenson *et al*., 1992; Iwano *et al*., 2004).

Extracellular crystals may serve purely as a protective device against foraging animals, as it has been suggested for druses or raphides present in different tissues (Franceschi & Horner, 1980). On the other hand, we know that Araceae tissues contain large amounts of druses or raphides. In that context, the advantage of producing extracellular crystals is not immediately evident. What could be the selective advantage of producing a calcium oxalate exudate in addition to intracellular crystals? It has been postulated that extracellular oxalate crystals may also play a biotic role in enhancing pollination. Oxalate crystals could facilitate pollination by providing a visual signal or a scent interesting to insect (Chase & Peacor, 1987; D'Arcy *et al*., 1996).

The connection between the presence of extracellular calcium oxalate crystals and pollination mechanisms in Araceae is difficult to establish at this point. For example, *Schismatoglottis* and *Arum* are two genera pollinated by flies (Drosophilidae and Psychodidae/Sphaeroceridae, respectively) (Mayo, Bogner & Boyce, 1997). In *Schismatoglottis*, crystals are exuded at the time of the dehiscence, and in *Arum* the

crystals are released during early stages of development. Furthermore, the crystals seem to be mixed with pollen in both cases.

Finally, we cannot exclude the possibility that the presence of extracellular calcium oxalate crystals is a nonadaptative feature in the Araceae family.

The results of this study suggest that a careful anatomical and developmental study of the different types of flowers in the family Araceae could reveal new cases where extracellular oxalate crystals are present. We believe that the study of the developmental morphology of a greater number of species will show that the release of oxalate packages before anthesis is more frequent than it appears and that the presence of extracellular calcium oxalate crystals is a widespread feature in Araceae. Further studies are needed to assess the biological/physiological role(s) of the oxalate calcium crystals in plants.

ACKNOWLEDGEMENTS

We thank the staff of the Laboratoire Environnement de Petit Saut (French Guiana) for technical support and Dr Jean-Jacques de Granville for permission to work at the Herbier de Guyane (Cayenne). This research was supported in part by individual operating grants from the Natural Sciences and Engineering Research Council of Canada to D.B and C.L.

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