Pollen Germination, Pollen Tube Growth, Fruit Set, and Seed Development in *Schlumbergera truncata* and *S. ×buckleyi* (Cactaceae)

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Additional index words. electron microscopy, flowering potted plants, ornamental plants

Abstract. The reproductive biology of *Schlumbergera truncata* (Haworth) Moran and *S. xbuckleyi* (T. Moore) Tjaden was examined in a series of experiments. At anthesis, pollen grains are spherical, 54 to 62 µm in diameter, and tricellular. The receptive surface of the stigma is densely covered with elongated papillae and is devoid of exudate during the period of flower opening. When compatible pollen was applied to mature stigmas, germination occurred between 20 and 30 minutes after pollination and pollen tubes penetrated the stigma surface between 30 and 40 minutes after pollination. Pollen tubes exhibited a nonlinear pattern of growth in the upper two-thirds of the style, and the maximum rate of growth (>1.9 mm·h⁻¹) occurred between 12 and 18 hours after pollination. Full seed set was attained between 32 and 48 hours after pollination. Genotypic variation in the time required to achieve full seed set was partly attributable to differences in stylar length. Seeds were fully mature 6 months after pollination, but delaying fruit harvest until 8 months after pollination did not affect seed germination.

The genus *Schlumbergera* Lemaire (Cactaceae, subtribe Rhipsalidinae) is comprised of about five species of epiphytic shrubs that are indigenous to southeastern Brazil (Barthlott, 1987; Hunt, 1969). *Schlumbergera truncata*, also known as zygocactus or crab cactus, is the most commonly cultivated species and is an economically important floricultural crop in northern Europe and North America (Cobia, 1992). The other four *Schlumbergera* species are rare in cultivation but have been utilized for breeding. Hybridization between *S. truncata* and *S. russelliana* (Hooker) Britton & Rose has yielded many cultivars, including the well-known Christmas cactus (Hunt, 1981). The collective name for hybrids of *S. truncata* × *S. russelliana* parentage is *S. ×buckleyi* (T. Moore) Tjaden (Tjaden, 1966). Cultivars of *S. truncata* and *S. ×buckleyi* are distinguished primarily by the morphology of their flowers and phylloclades (stem segments) and their flowering time under natural photoperiods (Barthlott and Rauh, 1977; Hunt, 1969, 1981). *Schlumbergera truncata* has also been crossed with *S. opuntioides* (Loefgren & Dusen) Hunt (Barthlott and Rauh, 1977) and *S. orssichiana* Barthlott & McMillan (Horobin and McMillan, 1985), but few cultivars have been produced from the resultant progeny. Hence, nearly all of today's commercial cultivars remain either *S. truncata* or *S. ×buckleyi* (Cobia, 1992; McMillan, 1985).

The reproductive biology of *Schlumbergia* is not well characterized. All *Schlumbergia* species except for *S. obtusangula* (Schumann) Hunt are reported to be self-incompatible (Ganders, 1976; McMillan, 1991). Flowers of *Schlumbergia* are hermaphroditic and exhibit many features that are clearly adoptive to bird pollination, i.e., a long flower tube, a staminal nectar chamber, exerted stamens and style, and tepals in varying shades of red (Barthlott and Rauh, 1975; Buxbaum, 1953; Hunt, 1969). Abendroth (1969) observed hummingbirds (Trochilidae) visiting flowers of *S. truncata*. Several reports suggest that fruit of *S. truncata*

requires 12 months or longer to reach maturity (Abendroth, 1969; Barthlott and Rauh, 1975; Horobin, 1981). although Kölli (1988) found that fruit matured 6 months after pollination. The mature fruit is an indehiscent, fleshy berry (Barthlott and Rauh, 1975; Buxbaum, 1955).

To optimize breeding efforts, it would be desirable to gain more information about the reproductive biology of *Schlumbergia*. In the present article we report on pollen germination, tube growth, fruit set, and seed development in *S. truncata* and *S. ×buckleyi*.

Materials and Methods

General procedures. The cultivars that were used for study are listed in Table 1. All cultivars were propagated vegetatively from stock plants maintained at the Univ. of Massachusetts, Amherst (lat. 42°22.5'N). Plants were grown in glasshouses with temperature setpoints of 18/22C (heat/vent). Actual glasshouse temperatures ranged from 16C minimum to a maximum of >40 C for short durations in the summer. Shading compound (Kool Ray, Continental Products Co., Euclid, Ohio) was applied to the glass to maintain photosynthetic photon flux (PPF) below ≈ 650 µmol·m⁻²·s⁻¹.

Pollination was performed by rubbing recently dehiscent anthers on the stigmatic lobes. Each flower was pollinated once using pollen collected from two or more flowers. Four experiments were conducted.

Effect of timing of harvest on seed maturity (Expt. 1). Seventy-five flowers of '88-50' were pollinated on the day of

Table 1. The *Schlumbergera* cultivars used for study and their taxonomic status and source.

Cultivar	Binomial	Source
Buckleyi	<i>S. xbuckleyi</i>	Rainbow Gardens, Vista, Calif.
Dark Marie	<i>S. truncata</i>	P. Madsen, Odense, Denmark
Eva	<i>S. truncata</i>	J. de Vries, Aalsmeer, The Netherlands
Linda	<i>S. truncata</i>	P. Madsen, Odense, Denmark
88-29	<i>S. xbuckleyi</i>	Univ. of Mass. breeding program
88-50	<i>S. xbuckleyi</i>	Univ. of Mass. breeding program
88-68	<i>S. xbuckleyi</i>	Univ. of Mass. breeding program

anthesis with compatible ('Dark Marie') pollen. All pollinations were performed within a 10-day interval. Fruit were harvested at 4,5,6,7, and 8 months after Pollination. Seeds that were shriveled or collapsed were discarded, and the remaining seeds were counted and germinated in covered petri dishes on top of blotter paper that was moistened with deionized water. Seeds were germinated in a controlled-environment chamber (model I-35LVL; Percival Scientific, Boone, Iowa) kept at 20 ± 1 C and providing $52 \pm 8 \mu\text{mol}\cdot\text{s}^{-1}\cdot\text{m}^{-2}$ PPF for 12 h daily from cool-white fluorescent lamps (Bachtaler, 1989).

Data were collected on seed set (number of seeds at harvest minus shriveled or collapsed seeds) and number of seeds germinated at 10, 20, and 30 days after sowing. The number of seeds germinated at 40 days after sowing was also recorded for fruit harvested 4 months after pollination. A seed was considered germinated upon emergence of the radicle. The experiment consisted of 10 replications (fruit) per harvest period. Percent seed germination was calculated for each treatment and the data was arcsin-transformed before analysis. All variables were analyzed by SAS Institute's (1985) General Linear Model (GLM) procedure.

Morphology of the stigmatic surface and mature pollen grain (Expt. 2). Five styles of 'Dark Marie' were collected at anthesis, fixed in 2% (w/v) glutaraldehyde in 0.1 M phosphate buffer (pH 7), dehydrated through an ethanol series, critical-point dried in CO_2 , and coated with 200 to 300 Å of gold-palladium. Stigmas were viewed on a scanning electron microscope (JSM-25S; JEOL, Peabody, Mass.).

Fresh stigmas of 'Dark Marie' were examined under a dissecting microscope ($\times 20$ to $\times 50$) to determine the presence or absence of stigmatic exudate. Styles were collected at four developmental stages between anthesis and senescence (Scott et al., 1994). Five stigmas were examined per developmental stage.

Pollen of 'Buckleyi' and 'Dark Marie' was collected from >10 flowers on the day of anthesis and was fixed in 3 ethanol: 1 glacial acetic acid (v/v) for >24 h. Fixed pollen was dispersed on a microscope slide and stained for >24 h with the DNA-specific fluorochrome mithramycin A [$0.50 \mu\text{g}\cdot\text{ml}^{-1}$ in McIlvaine-Lillie buffer at pH 7.0 (Coleman and Goff, 1985)]. Slides were examined with a Zeiss epifluorescence microscope equipped with a 100-W high-pressure Hg lamp and a filter set for ultraviolet-violet waveband excitation (exciter filter BP 395-425, dichromatic beam splitter FT 425, and barrier filter LP 450). A minimum of 500 grains were observed per cultivar.

Kinetics of pollen adhesion and germination (Expt. 3). Whole flowers of 'Eva' and '88-29' were collected on the day of anthesis and placed in covered petri dishes containing blotter paper moistened with deionized water. Flowers were transferred to the laboratory, and compatible pollen ('Dark Marie') was applied to the stigmatic surface of each flower with a fine artist's brush. A brush was used for pollinations in order to maximize the number of pollen grains in direct contact with the stigmatic surface and minimize the variation in germination time due to pollen clumping (Thomson, 1989). The covers were replaced on the dishes after pollination, and the flowers were transferred to an incubator with the same temperature, irradiance, and photoperiod conditions described in Expt. 1. About 7 min elapsed between the flower collection and placement in the incubator.

At 10, 20, 30, 40, 50, and 60 min after pollination, stigmas were excised and placed in scintillation vials containing ≈ 5 ml of 3 ethanol : 1 glacial acetic acid (v/v). Immediately after placing the stigmas in fixative, the vials were shaken twice with a vortexer set at high speed (3 sec on/3 sec off/3 sec on) in order to dislodge grains

Table 2. Influence of timing of fruit harvest on seed set and the germination percentage for the cross female *Schlumbergera* $\times$ *buckleyi* '88-50' $\times$ male *S. truncata* 'Dark Marie'.

Harvest time (months after Pollination)	Seed set (no. seeds/ fruit)	Germination (%)			
		Days after sowing			
		10	20	30	40
4	215.1	1	5	9	12
5	254.1	53	68	73	---
6	229.9	73	82	85	---
7	237.5	72	81	84	---
8	238.5	73	81	83	---
F test	NS	***	***	***	---
Contrasts					
4 vs. 8 months	---	***	***	***	---
5 vs. 8 months	---	***	***	***	---
6 vs. 8 months	---	NS	NS	NS	---
7 vs. 8 months	---	NS	NS	NS	---

NS*, **, ***Nonsignificant or significant at $0.05 \geq \alpha > 0.01$, $0.01 \geq \alpha > 0.001$, or $\alpha \leq 0.001$, respectively, according to F test of contrast between treatments.

that were not strongly adherent. Stigmas were fixed for ≥ 24 h, stained for ≈ 30 min with 0.05% (w/v) aniline blue in lactophenol (Arlington and La Cour, 1942), and mounted in pure lactic acid. Dislodged pollen grains settled to the bottom of the vials and were taken up in a drop of fixative with a Pasteur pipette and placed on a microscope slide. Pollen was stained with aniline blue in lactophenol and viewed 24 h later. Stigmas and pollen were examined using visible light microscopy. A minimum of five stigmas were examined for each time interval.

Kinetics of pollen tube growth and fruit set (Expt. 4). Twelve plants of '88-50' with mature flower buds (≈ 1 to 2 days before anthesis) were transferred from the glasshouse to a controlled-environment chamber set at 20 ± 1 C and $65\% \pm 5\%$ relative humidity, and providing $52 \pm 8 \mu\text{mol}\cdot\text{s}^{-1}\cdot\text{m}^{-2}$ PPF for 12 h daily. Flowers were pollinated on the day of anthesis with compatible ('88-68') pollen. Entire styles were excised at the point of attachment to ovaries at either 6, 12, 18, 24, 28, 32, 36, 40, 44, 48, 60, or 72 h after pollination. Styles remained intact on control flowers. Ten flowers were used for each treatment, and the treatments were randomized over all plants. Plants were returned to the glasshouse following upon completion of the last stylar excision (72 h).

Excised styles were fixed in 3 ethanol : 1 glacial acetic acid (v/v) >24 h, washed in deionized water, softened for 20 h in 4 N NaOH, stained for ≈ 24 h in 0.1 % (w/v) decolonized aniline blue in 0.1 M $\text{K}_3\text{P}_2\text{O}_4$ (Martin, 1959), and examined with an epifluorescence microscope fitted with the same lamp and filter set described in Expt. 2. The longest pollen tube in each style was measured to the nearest millimeter.

Six months after pollination, the number of set fruit was counted and fruit were harvested. The procedures used for extracting and germinating seeds were identical to those described previously. The experiment consisted of five replications (fruit) per harvest period. The number of germinated seed was recorded 30 days after sowing, and the data was analyzed by SAS Institute's (1985) GLM procedure. Percent fruit set was calculated for each treatment [(no. fruit set $\div$ no. flowers pollinated) $\times$ 100] and the data was evaluated by chi-square analysis.

A second study was performed for the cross female 'Dark Marie' $\times$ male 'Linda'. Eight plants of 'Dark Marie' were transferred from the glasshouse to a controlled-environment chamber providing the same temperature, relative humidity, PPF, and

Fig. 1. (A) A scanning electron micrograph of the stigmatic surface of *Schlumbergera truncata* 'Dark Marie'. A portion of a stylar lobe is shown, illustrating the density and arrangement of stigmatic papillae. Bar = 20 μm . (B) Ungerminated pollen of *Schlumbergera x buckleyi* 'Buckleyi'. Fixed pollen was stained with mithramycin and viewed using epifluorescence microscopy. The vegetative nucleus is flanked by two smaller sperm nuclei (s). Bar = 50 μm .

photoperiod described previously. Flowers were pollinated on the day of anthesis using freshly dehiscent pollen. Entire styles were excised at the point of attachment to ovaries at either 12, 24, 36, 48, 60, or 72 h after pollination, or the styles were left intact (controls). The experiment consisted of seven treatments with five replications (fruit) per harvest period. The experimental protocol and methods used for data collection and statistical analysis were identical to those described for the cross female '88-50' x male '88-68'.

Experiment 1. There were no significant differences between fruit harvest dates with regard to the number of seeds per fruit (Table 2). Date of fruit harvest, however, markedly influenced the percentage of seeds germinated 10, 20, and 30 days after sowing. Percent seed germination increased as fruit maturity increased from 4 to 6 months after pollination. No increase or decrease in percent seed germination was obtained by delaying fruit harvest from 6 to 8 months after pollination.

Fig. 2. In vivo pollen tube growth for the cross female *Schlumbergera x buckleyi* '88-50' x male *S. x buckleyi* '88-68'. Each data point is the mean of 10 styles $\pm$ 1 SE.

Experiment 2. The mature stigma of *S. truncata* and *S. x buckleyi* consists of five or six stigmatic lobes that are erect, connivent, and $\approx$ 3 mm in length. The receptive surface of the stigma is densely covered with elongate papillae that are $\approx$ 25 μm wide and -120 to 150 μm long (Fig. 1A). No exudate was present on the papillate surface during the period from anthesis to senescence. Pollen grains collected at anthesis were spherical, 54 to 62 μm in diameter, and tricellular (Fig. 1B). Each grain contained a large vegetative nucleus and two smaller sperm nuclei.

Experiment 3. Pollen adhesion commenced between 10 and 20 min after pollination, and the numbers of grains per style increased substantially during the first hour after pollination. Pollen loads averaged only 22 ± 10 grains per style at 20 min after pollination, but increased to 148 ± 28 grains per style at 30 min, 300 ± 103 grains per style at 40 min, and -500 grains per style at 50 min.

Most of the pollen germinated between 20 and 30 min after pollination, with stigmatic penetration occurring between 30 and 40 min after pollination. By 60 min after pollination, pollen tubes had reached the transmitting tissue in the stigmatic lobes, turned -90° and started growing towards the ovary.

Experiment 4. Pollen tubes exhibited a nonlinear pattern of growth in vivo (Fig. 2). The rate of pollen tube growth was -0.8 $\text{mm}\cdot\text{h}^{-1}$ between 6 and 12 h after pollination but increased to -1.9 $\text{mm}\cdot\text{h}^{-1}$ between 12 and 18 h after pollination and then decreased to $\approx$ 1.1 $\text{mm}\cdot\text{h}^{-1}$ between 18 and 24 h after pollination. The average stylar length for '88-50' (from the tip of the stigma to the entrance of the ovary cavity) was 49.3 ± 0.4 mm ($n = 10$). Based on these measurements, pollen tubes traversed -17% of the style within 6 h, 27% of the style within 12 h, 50% of the style within 18 h, and 63% of the style within 24 h after pollination (Fig. 2).

For the cross female '88-50' x male '88-68', fruit set did not occur when styles were excised at or before 28 h after pollination (Table 3). Excision of styles at 32 h resulted in only 20% fruit set and seed yields were lower than for flowers with intact styles (controls). When styles were removed at 36 h or later, fruit set was 100% and seed yields were similar to the controls. For the cross female 'Dark Marie' x male 'Linda', fruit set was prevented when styles were excised at or before 36 h after pollination (Table 4). However, removal of styles 48 h after pollination or later resulted in >80% fruit set and seed yields that were comparable to the controls.

Table 3. Effect of timing of stylar excision after pollination on fruit set and number of viable seeds per fruit for the cross female *Schlumbergera × buckleyi* '88-50' x male *S. × buckleyi* '88-68'.

Timing of stylar excision (h after pollination)	Fruit set (%)	Viable seeds/fruit (no.)
6	0 ^z	---
12	0 ^z	---
18	0 ^z	---
24	0 ^z	---
28	0 ^z	---
32	20 ^z	45.0
36	100	180.6
40	100	174.8
44	100	167.8
48	100	185.8
60	100	192.8
72	100	185.2
Control (intact style)	100	195.2
F test ^y	---	***
Contrasts		
32 h vs. control	---	***
36 h vs. control	---	NS

^zTreatment significantly different from control by chi-square test, $P = 0.05$; chi-square = 3.841 for 1 degree of freedom.

^yF test performed on data from 32, 36, 40, 44, 48, 60, and 72 h and control treatments.

NS,***Nonsignificant or significant at $\alpha \leq 0.001$, respectively.

Discussion

The timing of seed maturation is of significant interest to plant breeders who desire to minimize the time from pollination to seed collection and maximize the yield of viable seed. Our results indicate that *Schlumbergera* seed attains physiological maturity as early as 6 months after pollination, thus corroborating the findings of Kölli (1988). Kölli (1988) suggested that seed maturation periods longer than 6 months probably result from growing plants at suboptimal temperatures. Relatively low temperatures, as well as high soil moisture levels and high relative humidity, can delay seed ripening (George, 1985). It cannot be concluded with certainty that suboptimal temperatures were responsible for longer seed maturation periods in the studies of Abendroth (1969), Barthlott and Rauh (1975), and Horobin (1981) because the authors did not report the temperature regimes under which fruiting plants were grown.

Seed that is harvested prematurely typically exhibits poor germination and is short-lived (Austin, 1972; Cochran, 1943; Kerr, 1963). Harvest and extraction of seed before the attainment of physiological maturity was also detrimental to germination in *Schlumbergera*. This is readily apparent from comparing the germination percentages for seeds from 4-month-old fruit at 40 days after sowing (12%) vs. seeds from 5-month-old fruit at 10 days after sowing (53%) (Table 2). These two groups of seeds were harvested and extracted on different dates but were similar in physiological age, i.e., the interval from pollination until data collection.

The Cactaceae belongs to the centrosperous order (Chenopodiales), and all taxa within this order have tricellular pollen (Gibson and Nobel, 1986). Generally, tricellular pollen tends to lose its viability quickly, exhibits poor germination in vitro, and is difficult to store for extended periods (Brewbaker, 1967; Hoekstra, 1973). These attributes of tricellular pollen are

Table 4. Effect of timing of stylar excision after pollination on fruit set and number of viable seeds per fruit for the cross female *Schlumbergera truncata* 'Dark Marie' x male *S. truncata* 'Linda'.

Timing of stylar excision (h after pollination)	Fruit set (%)	Viable seeds/fruit (no.)
12	0 ^z	---
24	0 ^z	---
36	0 ^z	---
48	80	104.3
60	100	130.6
72	100	98.5
Control (intact style)	100	119.8
F test ^y	---	NS

^zTreatment significantly different from control by chi-square test, $P = 0.05$; chi-square = 3.841 for 1 degree of freedom.

^yF test performed on data from 48, 60, and 72 h and control treatments.

^{NS}Nonsignificant ($P > 0.05$).

important to plant breeders, who need to maintain and assess pollen viability and maximize seed set. Investigations are needed to determine the optimum storage conditions and maximum longevity for *Schlumbergera* pollen. *Schlumbergera* species vary considerably in their natural flowering time (Barthlott and Rauh, 1977), and the development of reliable pollen storage techniques would aid in broadening the genetic base by interspecific hybridization.

In vivo pollen tube growth typically proceeds at a linear rate in species with tricellular pollen and at a nonlinear rate in species with bicellular pollen (Mulcahy and Mulcahy, 1983; Willemse and Franssen-Verheijen, 1988). Nonlinear pollen tube growth was observed in the tricellular species *Plumbago zeylanica* L. and was correlated with changes in the architecture of the transmitting tissue (Russell, 1986). Our results demonstrate the existence of another species with tricellular pollen and nonlinear pollen tube growth in vivo. Additional research is needed to identify the causes of nonlinear pollen tube growth in *Schlumbergera*.

Excision of styles at 36 h after pollination resulted in 100% fruit set and high seed yields for '88-50', but 0% fruit set for 'Dark Marie' (Tables 3 and 4). The lack of conformity in fruit set for these two clones may be due to differences in stylar length. The average length of 'Dark Marie' styles (from the tip of the stigma to the entrance of the ovary cavity) was 67.7 ± 0.6 mm ($n = 10$), i.e., $\approx 40\%$ longer than '88-50' styles (49.3 ± 0.4 mm). Thus, genotypic variation in the time required to achieve full seed set is partly attributable to differences in stylar length.

Self-incompatibility (SI) occurs in *Schlumbergera* (Ganders, 1976; McMillan, 1991) and in many other genera of the Cactaceae (Boyle et al., 1994; Breckenridge and Miller, 1982; Ganders, 1976; Ross, 1981; Taylor, 1976). However, little is known about the physiology or genetic control of SI in this family. Several traits that occur in *Schlumbergera* are typically associated with sporophytic SI systems, i.e., tricellular pollen and dry, papillate stigmas (Brewbaker, 1957; Heslop-Harrison et al., 1975). The related genus *Rhipsalidopsis* also exhibits these traits, along with two others that are associated with gametophytic SI systems: inhibition of incompatible pollen tubes in the style and absence of reciprocal differences in outcrosses (Boyle et al., 1994).

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