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SUCCESSIVE FLUSHING OF STYLAR EXUDATE OF LILIUM LONGIFLORUM

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SUMMARY

A single flush with water or dimethylsulfoxide of pistils from two cultivars of Easter lily did not remove all the exudate present in the stylar canal. From the total amount of dry matter obtained after 4 successive flushes carried out at 1 h intervals, the first removed 30–40% from cv. Arai No. 5 and 60–70% from cv. Mount Everest pistils. The second flush removed 52–54% from "Arai No. 5" and 28–32% from "Mt. Everest" pistils. "Arai No. 5" pistils from non-senescent flowers (0–4 days old) contained much less dry wt of flushable exudate than did pistils from senescent flowers (5–10 days old), but the exudate was richer in carbohydrate (60% vs 48% of the dry wt) and protein (3.4% vs 2.1%). "Mt. Everest" pistils, in comparison with the same age "Arai No. 5" pistils (5–10 days old), contained more total dry matter (3.6 mg) of flushable exudate with about the same protein content (2.5% vs 2.1%), but with a higher carbohydrate content (61% vs 48%).

1. INTRODUCTION

Non-pollinated *Lilium longiflorum* pistils produce two types of exudates, stigmatic and stylar (ASCHER 1973; LABARCA & LOEWUS 1973). As the flower ages, the amounts of these exudates change.

Differences in the pattern of exudate production between stigma and style during aging could be based on different rates of synthesis and/or secretion, or they could be the result of inefficient removal of the exudate from the style by the flushing method employed, coupled with an increased releasability of the style exudate into the flushing agent as it rapidly flowed through the canal. If the flushing fluid were allowed to remain filling the canal for an extended time before it was flushed out, more substance may disperse into the flushing fluid and, eventually, on repeated flushing, all canal exudate would be removed. The pattern of total dry matter production of stylar exudate might then more closely match the pattern of stigmatic exudate production.

Experiments were therefore conducted to see if more than one flush was necessary to remove all the stylar exudate. Another objective was to see if the amount of dry matter, protein and carbohydrate present in the exudate changes with age of the style. Possibly this outcome will help to explain why compatible – and as the style ages, even incompatible – pollen tube growth is hampered, when apparently there are quantities of stylar canal exudate available that one would think would be able to support compatible pollen tube growth (ASCHER 1975).

2. MATERIALS AND METHODS

Open flowers of various ages from *Lilium longiflorum* Thunb. cultivars Arai No. 5 ("Arai", a clone) and Mount Everest ("ME", a seed strain) were cut from plants in the greenhouse.

Flowers of "Arai" were split into two age groups: Each of 6 styles from young (0–4 days old) and 29 styles from old (5–10 days old) flowers were flushed with 5 drops (~250 µl) of room temperature 5% DMSO (ASCHER 1975), 4 times at 1 h intervals, giving treatment times at 0, 1, 2 and 3 h after start of flushing. The fluid was injected by syringe and needle into the stigma and collected as it fell from the ovarian end of the style, the style having been snapped free of the ovary before flushing. The internal volume of the styles is 40–50 µl, which is thus the volume of fresh DMSO or water remaining in the canal between flushes.

Five to ten-day old flowers of "ME" were divided randomly into 4 groups, and the styles flushed 4 times at 1 h intervals; two groups of 7 and 8 styles were flushed with 5 drops of deionized, distilled water and 2 groups with 8 and 12 styles with 5 drops of 5% DMSO.

In both cultivars, the 4 flushes were separately collected in pre-weighed glass tubes. From each tube, an aliquot was removed for determination of carbohydrate (SCOTT & MELVIN 1953) and protein content (BRADFORD 1976), and the rest was dried at 105°C for 36 h. Data are presented on a per style basis as a percentage of style dry at as determined by drying the styles, after flushing, at 105°C for 36 h. Data are presented on a per style basis as a percentage of style dry wt as determined by drying the styles, after flushing, at 105°C for 24 h.

3. RESULT

3.1. Dry matter removed

For both cultivars, more than one flush was necessary to remove the exudate present in the style (*tables 1, 2*). Of the total dry matter removed during 4 successive flushes, the first removed 30–40% from "Arai" and 60–70% from "Me" styles, and the second 1 h later removed 52–54% from "Arai" and 28–32% from "ME" styles. Thus, the first two flushes removed about 90% of the flushable substance present in the stylar canal.

In "Arai", the second flush removed more than the first, 54.0% vs 29.6% of the dry matter released by all 4 flushes from young styles, and 52.1% vs 39.3% from old styles (*table 1*). In contrast, in "ME" (old styles), the first flush with water as well as with DMSO was more effective: water, 66.5% vs 27.6%; DMSO, 59.3% vs 32.2% (*table 2*). Water did not differ from DMSO in releasing exudate from the stylar canal. The third and fourth flush were progessively less effective, removing together in "Arai" an amount of 16.3% from young and 8.6% from old styles (*table 1*). The corresponding amounts for old "ME" styles were 5.9% for water and 8.6% for DMSO (*table 2*).

In young styles of "Arai", the total amount of substance obtained after four

Table 1. Dry matter content per style in four successive DMSO flushes at 1 h intervals of young and old "Arai No. 5" styles.

Stylar age	Flush number	Dry wt, µg	As % of dry wt of total flushes	As % of stylar dry wt*
young	1	391	29.6	0.68
(anthesis to 4 day old)	2	713	54.0	1.24
	3	126	9.5	0.22
	4	90	6.8	0.16
Total		1320	99.9	2.30
Old	1	1231	39.3	2.28
(5–10 days old, senescent)	2	1629	52.1	3.02
	3	206	6.6	0.38
	4	63	2.0	0.12
Total		3129	100.0	5.80

* Dry wt/style: young, 57.5 mg; old, 54.0 mg.

flushes had a dry wt of 1.32 mg/style. For old styles, the amount was 3.13 mg. Thus from younger styles less than half of the amount from older styles (42.4%) was released by the four flushes. However, younger styles after the fourth flush may yet contain stylar exudate, since the last flush still removed 6.8% from young, but only 2.0% from old styles (*table 1*).

In "ME" styles from 5 days old and older flowers, an average of 3.59 mg of dry matter per style was obtained after the four water or DMSO flushes (*table 2*). This amount is somewhat higher than that obtained from old "Arai" styles (compare *table 2* with *table 1*). Expressed as percentage of stylar dry wt, the

Table 2. Dry matter content per style in four successive water or DMSO flushes at 1 h intervals of old "Mt. Everest" styles, with standard deviation of the mean.

Flush agent	Flush number	Dry wt, µg	As % of dry wt of total flushes	As % of stylar dry wt*
Water	1	2266 ± 393	66.5	4.78
	2	942 ± 238	27.6	1.98
	3	164 ± 87	4.8	0.34
	4	37 ± 34	1.1	0.08
Total		3409	100.0	7.18
DMSO	1	2236 ± 479	59.3	4.00
	2	1214 ± 205	32.2	2.18
	3	245 ± 276	6.5	0.44
	4	78 ± 33	2.1	0.14
Total		3773	100.1	6.76

* Dry wt/style: approx. 47.8 mg.

Table 3. Carbohydrate and protein content per style in the first two successive flushes at 1 h intervals of young and old "Arai No. 5" styles with standard deviation of the mean. (For details see materials and methods.)

Stylar age	Flush number	Dry wt of flush, µg	Carbohydrate		Protein		Carbo/Prot ratio
			µg	%[a]	µg	%[a]	
Young (0-4 days old)	1	391	207.2±64.9	53.0	12.8±5.2	3.3	16.2:1
	2	713	449.4± 8.1	63.0	24.2±8.8	3.4	18.6:1
Total		1104	656.6	59.5	37.0	3.4	17.1:1
Old (5–10 days old, senescent)	1	1231	600.3± 1.9	48.8	26.3±2.6	2.1	22.8:1
	2	1629	757.2±95.2	46.5	32.5±5.2	2.0	23.3:1
Total		2860	1357.5	47.5	58.8	2.1	23.1:1

[a] Percentage of its respective dry wt of flush.

dry matter of the stylar exudate obtained from the four flushes makes up 2.3% in young and 5.8% in old "Arai" styles, and about 7.0% in old "ME" styles.

3.2. Amount of carbohydrate and protein

The stylar fluid from all four flushes of "Arai" and "ME" styles contained both carbohydrate and protein (*tables 3, 4*), but only the data from the first two flushes are reported because the low levels of carbohydrate and protein in the last two flushes made analysis difficult.

In "Arai" (*table 3*), the substance flushed from a single young style by two flushes contained 0.66 mg of carbohydrate (59.5% of the total dry matter in

Table 4. Carbohydrate and protein content per style in the first two successive water or DMSO flushes at 1 h intervals of 5–10 days old "Mt. Everest" styles, with standard deviation of the mean.

Agent of flush	Flush number	Dry wt of flush, µg	Carbohydrate		Protein		Carbo/Protein ratio
			µg	%[a]	µg	%[a]	
Water	1	2266±393	1717±375	75.8	49.6±11.8	2.2	34.6:1
	2	942±238	843±159	89.5	32.7± 7.9	3.5	25.8:1
Total		3208	2560	79.8	82.3	2.6	31.1:1
DMSO	1	2236±479	1272±268	56.9	45.4±11.1	2.0	28.0:1
	2	1214±205	829± 77	68.3	34.7± 7.1	2.9	23.9:1
Total		3450	2101	60.9	80.1	2.3	26.2:1

[a] Percentage of its respective dry wt of flush.

the first two flushes). In a single old "Arai" style, the value obtained was 1.36 mg (47.5%). Both these amounts and percentages are lower than those found in the stylar exudate of old "ME" styles (*table 4*), which contained about 2.33 mg carbohydrate per style, that is about 70% of the dry matter of the total flushes (average of water and DMSO flushes).

In comparison with the amount of carbohydrate, the protein content present in the stylar flushes of both cultivars was low. The first two flushes released from a single young "Arai" style 37.0 μg of protein (3.4% of the total dry matter of the first two flushes). In comparison with a young style, a single old "Arai" style contained more protein (58.8 μg) in the fluid released by the first two flushes; however, when expressed as percentage of total dry matter of the first two flushes less protein was present, only 2.1% as compared to 3.4% in the young style (*table 1*).

In "ME" the first two flushes of old styles contained 81.2 μg of protein (average of water and DMSO) or 2.4% of the dry matter of the first two flushes. Thus, the exudate found in old "ME" styles contained more protein than did old "Arai" styles (compare *table 4* with *table 3*), but calculated as a percentage of the dry wt of the flush was about the same.

Water flushes were more effective in releasing carbohydrate (but not protein) as could be shown by the comparison of water and DMSO flushes of old "ME" styles (*table 4*).

In "Arai", the ratio carbohydrate/protein differed between the flushes of young and old "Arai" styles (*table 3*). The ratio for the first two flushes in young styles was about 18:1, but in old styles was 23:1. In "ME" styles (old) there was a decline in the ratio carbohydrate/protein in the second flush, both for water as well as for DMSO (*table 4*). For water, the ratio for the first two flushes was 35:1 and 26:1; for DMSO, they were 28:1 and 24:1. The carbohydrate/protein ratio in the first DMSO flush of "ME" styles (old) was somewhat higher than in the first flush of old "Arai" styles (28:1 vs 23:1) (compare *table 4* with *table 3*).

4. DISCUSSION

In unpollinated styles of *Lilium longiflorum* of various ages, the accumulated stylar canal exudate cannot be completely removed by a single flushing with water or DMSO. This continued presence of exudate in the canal after an initial flushing might be due to new exudate being secreted into the stylar canal and made available to flushing, but this seems unlikely, especially in the case of cv. Arai No. 5 where the second flush removed almost twice as much as the first flush. Therefore it would seem that exudate is secreted into the hollow style in a form resistant to rapid and complete removal by water or DMSO, and that this resistance to flushing is cultivar-dependent since the exudate in cv. Mt. Everest styles was much more releasible.

This resistance to flushing would seem to be lowered by aging of the flowers, since both the first flush and the first two flushes together of old "Arai No.

5" styles removed more than in young styles.

The exudate in older styles would appear to be completely removed in four flushes, but in younger "Arai No. 5" styles the fourth flush still brought out exudate. The amounts brought out by the third and fourth flush might be a measure of the continued release of the exudate into the canal by the cells in both young and old styles, since styles from senescing flowers must continue to secrete exudate into the canal because the dry wt of exudate from these styles is greater than in styles from young, non-senescent flowers.

The stylar canal exudate of lily consists mostly of carbohydrate, especially in old styles of cv. Mt. Everest and in young styles of "Arai No. 5". This contrasts with the results of DICKINSON et al. (1982) who reported that stylar canal exudate of *Lilium longiflorum* obtained in one flush contained little carbohydrate. Also, the exudate of "Mt. Everest" contained a higher concentration of carbohydrate than "Arai No. 5", but the protein concentration was about the same. This difference in carbohydrate in stylar exudates between cultivars of *Lilium longiflorum* does not affect the ability of the exudate to provide a suitable substrate for cross-pollen tube growth. In fact, the other differences between the two different varieties, involving as they do releasibility and total amounts present per dry wt of style, indicate that other differences might exist, both between the stylar exudates of the two cvs and between their stigmatic exudates.

The much higher carbohydrate content of the exudate of old "Mt. Everest" styles when water was used as the flushing agent (80% vs 61% for DMSO) is difficult to explain, since interference by DMSO in the assay procedure was not a factor. Since DMSO causes increased permeability of cell membranes, a general leaching of the stylar cells might mask the true carbohydrate concentration, but this would occur only in the last three flushes. The carbohydrate concentration, however, was already higher (76% vs 58%) after the first flush. The protein content of the first flush was the same when the water and DMSO flushes are compared. DMSO, however, is the preferred flushing agent, for it alone (and not water) allows the recovery of a stylar canal substance still physiologically active in *in vivo* pollen tube growth studies (Ascher, personal communication).

The stylar canal exudate of young "Arai No. 5" styles was richer in protein and carbohydrate than that from senescing flowers. This change in the composition of the stylar canal exudate between young and old styles of "Arai No. 5" is in agreement with observations from other species in which the stylar canal secretion was found to differ at various flower ages (TILTON & HORNER 1980 on *Ornithogalum* and CRESTI et al. 1976 on *Lycopersicon*).

In an aging style no longer able to support normal pollen tube growth, one would expect the fluids through which the pollen tubes grow to be less available, either by loss or by lack of continued synthesis. That the stylar fluid in lily must change physiologically with aging is obvious because styles older than 5 days old are progressively less able to support compatible pollen tube growth (ASCHER 1975).

One change in the stylar canal fluid with age may be an increase in viscosity

due to drying, as happens on the stigma (CAMPBELL 1983), but this is difficult to establish and no attempt was made here to demonstrate the point. However, since air blown through the canal in 5-day and older styles produces bubbles at the ovarian end of the style, there is evidence for a physical change in the exudate, possibly accompanied by a decrease in viscosity.

Stylar fluid is used by both compatible and incompatible pollen tubes (CAMPBELL & ASCHER 1975; LABARRA et al. 1970). Yet, even after only a single flush (ASCHER 1973) where apparently a large amount of the material still remains in the canal, the once-flushed style can support only incompatible pollen tube growth no matter whether the pollen is compatible or incompatible; the stylar canal material left in the style cannot support compatible pollen tube growth. A delay between flushing and pollination does not see a return of compatibility (ASCHER 1975). It is possible that the easily removed material in the first flush contains compatibility substances which are not contained in the harder-to-remove material (released into the flushing fluid later and found deeper in the fluid layer). Thus there may be different layers of functionality in the canal. Since a second flush after 1 hr removes from 28% ("Arai No. 5") to 50% ("Mt. Everest") of the material, it is possible that pollen tubes growing in a style flushed twice before pollination may not be able to grow even to incompatible lengths.

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