

The Nutritional Role of Pistil Exudate in Pollen Tube Wall Formation in *Lilium longiflorum*

II. PRODUCTION AND UTILIZATION OF EXUDATE FROM STIGMA AND STYLAR CANAL¹

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ABSTRACT

Detached pistils of *Lilium longiflorum* were labeled with D-glucose-U-¹⁴C 24 hours after anthesis and then sampled for the next 6 days to determine the appearance of label into exudate from the stylar canal and the stigmatic surface of the pistil. Results were obtained with unpollinated cv. Ace pistils and pollinated cv. Ace pistils, selfed or crossed (cv. Croft pollen). Limited data were also obtained on cv. Croft pistils, selfed or crossed (cv. Ace pollen).

Exudate appeared in the canal and on the stigmatic surface soon after anthesis. In unpollinated pistils it continued to accumulate for about 5 days in the canal and for the full term of the experiment, 7 days, on the stigmatic surface. Canal exudate eventually mixed with stigmatic exudate in unpollinated pistils. Pollination interrupted the flow of exudate, and a portion of the pistil secretion product was diverted toward the synthetic requirements of the developing pollen tubes. Two days after pollination, the specific radioactivity of pollen tube cytoplasm had reached a level comparable to canal exudate. Twenty-four hours later, the specific radioactivity of pollen tube wall substance was about 80% of the value found in canal exudate. Similar patterns of ¹⁴C incorporation and similar carbohydrate contents were obtained from self- and cross-pollinated pistils, although the former contained pollen tubes of less than one-half the length of the latter.

tors such as rate of exudatic secretion by stigma or canal, relative contributions of stigmatic and canal exudate to pollen tube wall formation, composition of canal exudate, and temporal relationship of exudatic secretion to utilization of exudate by growing pollen tubes were not examined.

The present study explores these additional factors and provides new information on the nutritional role of pistil secretion product during pollen tube development which supports the view that pistil exudate is the major source of carbohydrate during pollen tube wall biosynthesis in *L. longiflorum*.

MATERIALS AND METHODS

Procedure for Labeling and Sampling Detached Pistils. At anthesis, flowers were detached from plants, stripped of tepals and stamens, and stored in a growth chamber at 27 C and 70% relative humidity. A regime of 8 hr of light and 16 hr of darkness was used. One day after anthesis, each pistil was given 0.1 ml of 0.01 M D-glucose-U-¹⁴C by placing the cut pedicel in a small vial containing the labeled solution. Most of the ¹⁴C was taken up by the pistil within 2 hr. Two 0.1-ml portions of distilled water were added to the vial to make sure that all of the radioactivity was removed by the pistil. At this point, pistils were transferred to larger vials containing distilled water. Water was replaced daily. One day after labeling, the stigma of each pistil that was scheduled for pollination was covered with pollen (about 5 mg) from a single, freshly harvested anther.

All experiments were initiated at the same time to minimize variations created by growing conditions. The flowers were obtained from a commercial greenhouse.

Experiment 1 provided data on production of stigmatic and canal exudate during 7 days following anthesis. Onset of senescence as determined by visible deterioration of detached pistils usually appeared about the 6th or 7th day. This experiment included 135 pistils, cv. Ace. Ten pistils were analyzed for exudate at anthesis. Again on the 1st and 2nd days after anthesis, times of labeling and pollinating, respectively, sets of 10 pistils were analyzed. Remaining pistils were divided into three sets of 35 pistils. One set was self-pollinated with fresh Ace pollen, another cross-pollinated with fresh Croft pollen, and the final set left unpollinated. From each set, five pistils were taken at 0.5, 1, 1.5, 2, 3, 4, and 5 days after pollination for analysis of stigmatic and canal exudates, and recovery of pollen tubes. D-Glucose-U-¹⁴C with a specific radioactivity of 3 mc/mole was used.

Experiment 2 provided exudates and pollen tube tissue for further studies of carbohydrate composition. A group of 60 Ace pistils was divided into sets of 20. One set was left un-

Evidence for a nutritional dependence of pollen tube wall formation on pistil secretion product has been obtained by injecting polysaccharide-rich, high molecular weight component, G-100-I, of stigmatic exudate from *Lilium longiflorum* into the stylar canal of freshly detached lily pistils and then cross-pollinating the injected pistils (5). About one-fourth of the tube wall carbohydrate was traced to oligomeric and monomeric residues of injected G-100-I. In that study, many fac-

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pollinated. The others were pollinated with Ace (selfed) or Croft pollen. Exudates and pollen tubes were recovered from pistils 2 days after pollination. In this experiment, D-glucose-U-¹⁴C with a specific radioactivity of 10 mc/mmole was used.

Experiment 3 provided information on exudates and pollen tubes when cv. Croft furnished the pistils. Only 10 detached pistils were available for this experiment making it necessary to forego data from unpollinated pistils. These pistils were divided into two sets, five cross-pollinated with Ace pollen and five self-pollinated. D-Glucose-U-¹⁴C with a specific radioactivity of 3 mc/mmole was used. As in experiment 2, exudates and pollen tubes were recovered 2 days after pollination.

Recovery of Exudates. Only unpollinated pistils secreted sufficient stigmatic exudate as droplets to warrant collection by pipette during the period from labeling to dissection of the pistil. Immediately prior to dissection, each stigma was gently rinsed with 1 ml of distilled water to remove adhering exudate. This wash was combined with prior collections of exudate. Pistils stored under conditions of low humidity or high temperature failed to secrete droplets of exudate, possibly a consequence of exudate drying on the stigma shortly after secretion.

To recover canal exudate, stigma-style portions were detached from the ovaries and their canals rinsed with 0.3 M pentaerythritol by injecting the rinse through the opening in the stigma formed by fusion of carpels. The rinse was collected as it flowed from the lower end of the detached style. Only 1 ml of pentaerythritol solution was needed to recover exudate from unpollinated pistils, but pollinated pistils required at least three successive 1 ml rinses to remove soluble carbohydrate from the canal due to occluding pollen tubes. Exudates were analyzed for carbohydrates (1) and ¹⁴C.

Gel Filtration of Exudates. Freeze-dried exudates from experiment 2 were redissolved in 3 ml of distilled water. Most of the pentaerythritol present in samples of canal exudate remained insoluble and was removed by centrifugation at 5 C. About 75% of the canal exudate in a sample was recovered, and the procedure offered the practical advantage of removing most of the pentaerythritol prior to gel filtration.

Each exudate was fractionated on a freshly prepared column of Sephadex G-100 (1.2 × 80 cm) with 0.1 M acetic acid as eluant. Fractions were analyzed for carbohydrate (1) and ¹⁴C. Fractions corresponding to G-100-I were pooled, hydrolyzed with acid, and examined by ion exchange and paper chromatography (5).

Pollen Tubes. Tubes were removed from labeled pistils as described earlier (5). Excised tubes were washed with five portions of 0.3 M pentaerythritol to remove adhering exudate. The final wash contained less than 2% of the radioactivity detected in the first wash. The contribution of radioactive exudate adhering to washed pollen tube surfaces was negligible in subsequent ¹⁴C assays of pollen tube cytoplasm and wall. Washed tubes were resuspended in cold distilled water and ground in a motor-driven glass homogenizer until large particles were no longer visible by eye. Centrifugation at 1,000g for 10 min produced a turbid supernatant and a pellet. The latter was twice resuspended in cold distilled water and spun at 1,000g for 5 min. Washes were combined with supernatant, and this fraction is referred to hereafter as "pollen tube cytoplasm." The washed residue is referred to as "pollen tube wall." Some starch grains might have been carried down in the wall fraction, but even if all the glucose recovered from this fraction were starch, a most unlikely possibility in view of the β-glucan content of pollen tubes walls, it would amount to less than 20% of the labeled carbohydrate (5). Tube wall substance was hydrolyzed with 2 M trifluoroacetic acid.

Aliquots of pollen tube cytoplasm and soluble, acid-hydrolyzed tube wall were analyzed for carbohydrate and ¹⁴C.

Tube wall hydrolyzates from experiment 2 provided sufficient material for gas-liquid chromatography of neutral monosaccharide components as their alditol acetates (5). Acid-resistant residues of pollen tube walls from experiment 2 were treated with cellulase to obtain an estimate of the ¹⁴C present as wall-bound glycan that was solubilized with this enzyme.

Analytical Procedures. Procedures not described in this paper will be found in earlier publications (4, 5). Portions of the analytical data given below are calculated on a "per pistil" basis from averages obtained with sets of 5 or 10 pistils.

RESULTS

Exudate Production. The appearance of stigmatic and canal exudate, in mg of carbohydrate/pistil, is plotted in Figure 1. Exudate from five pistils was pooled for each point. To estimate exudate production on the stigma, the carbohydrate content of the exudate recovered by pipet was added to that recovered by rinsing the stigma. In pollinated pistils, recovery of canal exudate became increasingly difficult with time as pollen tubes plugged the canal, especially in the case of cross-pollinated pistils.

Production of stigmatic exudate in unpollinated pistils rose slowly during the first 2 days following anthesis, then almost in linear fashion for 3 days reaching an accumulation of 4 mg of carbohydrate/pistil. Production of canal exudate, though less pronounced, followed a similar pattern.

Pollination altered the course of exudate accumulation. In the canal, this occurred about 1 day after pollination, and in the stigma, about 1 day after self-pollination or 2 days after cross-pollination. The gross appearance of the stigma surface was also affected by pollination. When Ace pistils were self-pollinated, the surface developed a dry appearance, but when cross-pollinated with Croft pollen, retained the moist appearance of unpollinated pistils. Quite the reverse occurred when Croft pistils were used. Here, it was the cross-pollinated pistils that developed the dry-looking stigma, while the self-pollinated pistils retained the moist look. These observations suggest that, in addition to the compatible or incompatible relationship between pollen and pistil, there is a factor associated with the pollen which influences exudate production. In the present ex-

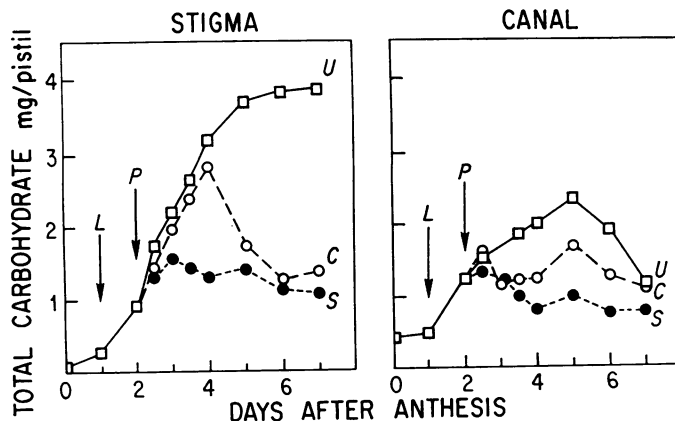


Fig. 1. Carbohydrate content of stigmatic and canal exudates of Ace pistils. At arrow, L, all pistils were labeled with D-glucose-U-¹⁴C. At arrow, P, pistils were divided into three sets: unpollinated, U; cross-pollinated, C; and self-pollinated, S. Data are from experiment 1.

Table I. Comparison of Carbon-14 Recovered in Secreted Droplets of Stigmatic Exudate and in Rinsings from the Stigmatic Surface of Unpollinated Pistils

Period after Anthesis days	Specific Radioactivity	
	In droplets	In rinsings
0-2	26,700	10,800
2-2.5	77,600	54,200
2.5-3	120,200	71,300
3-3.5	134,200	83,300
3.5-4	118,600	75,000
4-5	102,200	61,800
5-6	Not collected	50,800
6-7	Not collected	44,300

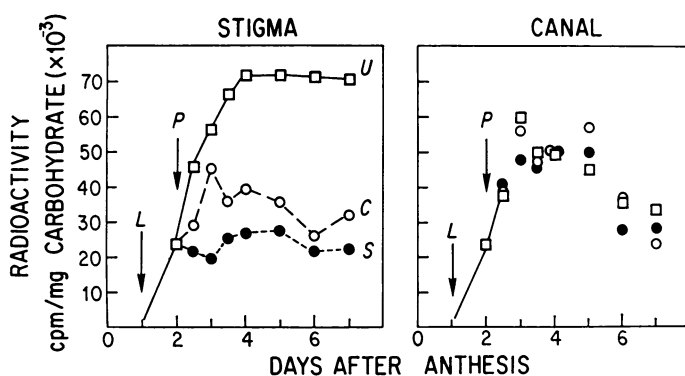


FIG. 2. Appearance of ^{14}C in stigmatic and canal exudates of Ace pistils. Data were taken from experiment 1. See Fig. 1 for explanation of symbols. In the absence of significant differences, lines linking successive points of U, C, and S canal exudates have been deleted from the plot.

periments, it was the Croft pollen that elicited greatest exudate production.

Incorporation of Label into Exudate. In experiment 1, about 5% of the ^{14}C appeared in stigmatic exudate of unpollinated pistils, a value similar to results obtained in previous studies (4). At the height of canal exudate production, 5 days after anthesis, 2% of the ^{14}C was present in that exudate. In experiment 2, 4 days after anthesis, 3.4% of the ^{14}C was in stigmatic exudate and 1.6% in canal exudate.

Stigmatic exudate secreted from unpollinated pistils as droplets had a specific radioactivity much greater than exudate recovered by rinsing stigma surfaces with water (Table I). Washing the stigma apparently removed other soluble carbohydrates that were deposited prior to secretion of labeled product. Following pollination, stigmatic exudate was obtained only by washing the stigma. The lower values for radioactivity in stigmatic exudate from pollinated pistils (Fig. 2) probably reflect this reduction in secretion at the stigma.

Pollination did not significantly alter the pattern of incorporation of ^{14}C into canal exudate (Fig. 2). A maximum of about 50,000 cpm/mg of carbohydrate was reached 2 days after label was introduced, and the canal exudate remained close to this value until onset of senescence. It is recognized that the method of introducing labeled precursor, the concentration of glucose used in the labeling, the timing of pollination, and many other factors will influence this pattern of incorporation of label into secretion product.

Gel Filtration of Exudates. Stigmatic exudate from *L. longi-*

florum consists of a high molecular weight component, G-100-I, that accounts for the bulk of the exudate and a low molecular weight mixture of carbohydrate and unidentified compounds, G-100-II. Nomenclature used here is that adopted in earlier studies (4, 5). The present work compares gel filtration profiles based on carbohydrate content and ^{14}C in recovered fractions from stigmatic and canal exudates of labeled unpollinated, cross-pollinated, and self-pollinated Ace pistils. Exudates from experiment 2 were used. Results are given in Figure 3. A quantity of exudate containing 2×10^6 cpm was applied to the gel column in each case. This permitted direct comparison among the six profiles, since areas under the curves were equivalent. Canal exudates gave profiles of greater polydispersity in the G-100-I fraction and contained very little G-100-II as compared to stigmatic exudates. Pollination followed by 2 days of pollen tube development altered profiles of canal exudate only slightly but caused appearance of labeled carbohydrate in the region between G-100-I and G-100-II in profiles of stigmatic exudate. Stigmatic exudate from self-pollinated pistils showed the greatest change, notably a large increase in labeled carbohydrate in G-100-II.

Samples of G-100-I from each profile were hydrolyzed with 2 M trifluoroacetic acid, chromatographed on ion exchange resins to remove acidic components, and converted to alditol acetates in order to resolve the neutral sugar residues by gas-liquid chromatography. Figure 4 compares stigmatic and canal exudate samples from unpollinated pistils in this regard. Major sugar residues were galactose, arabinose, and rhamnose. In stigmatic exudate, these sugar residues were accompanied by traces of xylose and mannose. Fucose may have been present as a slight shoulder on the trailing edge of the rhamnose peak. G-100-I hydrolyzates of exudates from pollinated pistils gave similar chromatograms, but a quantitative difference was detected in galactose. In stigmatic G-100-I from unpollinated or cross-pollinated pistils, the neutral sugar fraction contained about 18% galactose whereas that from self-pollinated pistils contained 34%. Analysis of G-100-I from droplets of stigmatic exudate that had been collected from unpollinated pistils and was not mixed with stigma rinsings revealed a value of 27.5% galactose in the neutral sugar residue. The corresponding galactose contents of canal-derived G-100-I were 25% for unpollinated and cross-pollinated pistils and 49% for self-pollinated pistils.

Pollen Tubes. Tube growth in self- and cross-pollinated Ace pistils is recorded in Figure 5. Cross-pollinated tubes (cv. Croft) reached the ovary, a distance of 10 cm, within 3 days. Further tube elongation within the ovary during the 4th and 5th days was not measured, since styles were detached from the ovaries for recovery of canal exudate and only that portion of the pollen tubes present in the stigma-styles was recovered. Incompatible (self-pollinated) pollen tubes grew only 4.2 cm during the first 3 days, then ceased to elongate.

Within the stylar canal, pollen tubes occupy a superficial position with respect to cells bordering the canal, the so-called "canal cells" (7), and are embedded in exudate produced by these cells. When pistils are labeled with D-glucose- ^{14}C , transfer of ^{14}C from pistil to pollen tube must proceed via this exudate. The course of this uptake of ^{14}C is plotted in Figure 5. During the first 2 days following pollination, uptake by incompatible tubes exceeded that of compatible tubes. Presumably uptake continued to increase in compatible tubes as they penetrated the ovary but that portion was not included in pollen tube tissues assayed on the fourth and fifth days.

Excised pollen tubes were rinsed with pentaerythritol to remove adhering exudate and then fractionated into cytoplasm and tube wall. The latter was hydrolyzed with acid, a

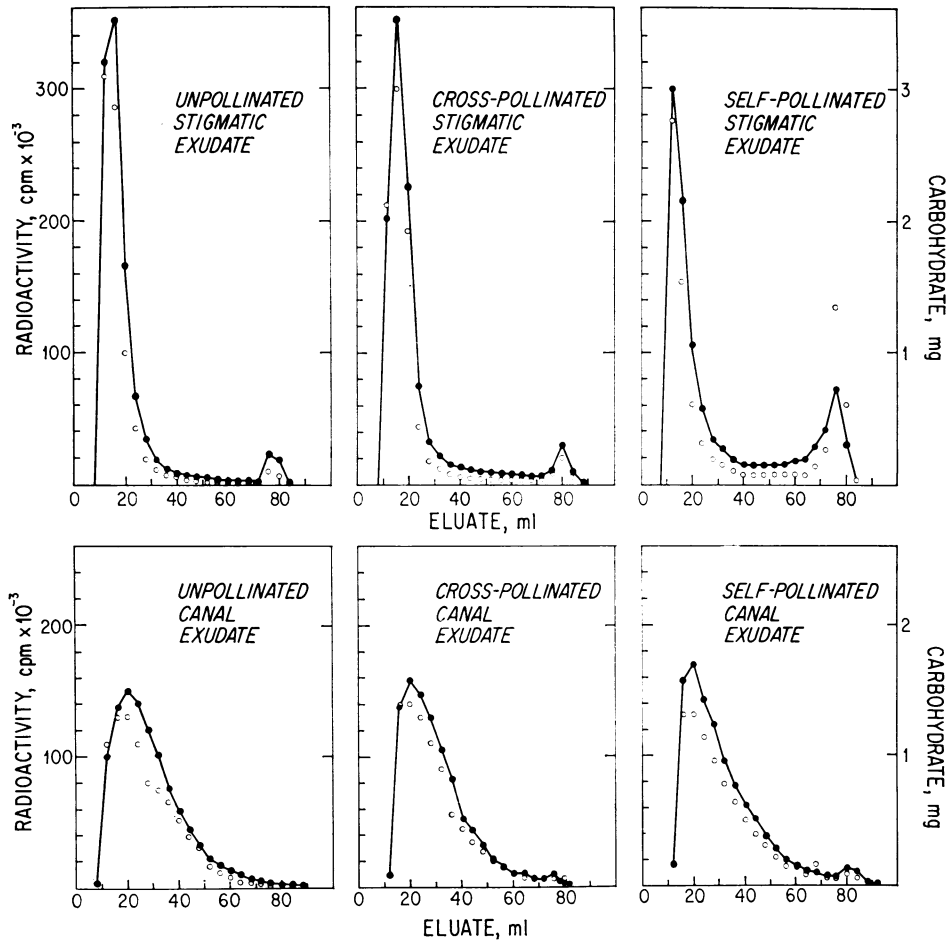


FIG. 3. Fractionation of stigmatic and canal exudates from unpollinated, cross-pollinated, and self-pollinated pistils, cv. Ace, on Sephadex G-100. Fractions were assayed for ^{14}C (●) carbohydrate (○).

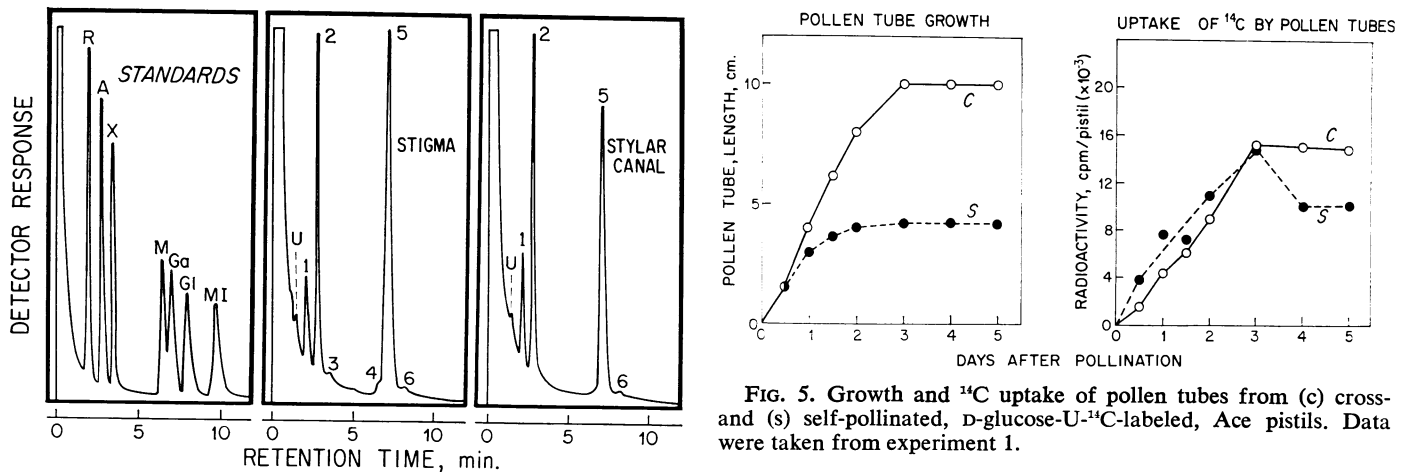


FIG. 4. Gas-liquid chromatograms of alditol acetates of neutral sugars in hydrolyzates of G-100-I component from stigmatic and canal exudates. Standard contained rhamnitol, R; arabinitol, A; xylitol, X; mannitol, M; galactitol, Ga; glucitol, Gl; and myo-inositol, MI. Numbered peaks correspond to alditol acetates derived from rhamnose, 1; arabinose, 2; xylose, 3; mannose, 4; galactose, 5; and glucose, 6. The small peak, U, is unknown. Retention times are given in the preceding paper by Labarca and Loewus (5).

FIG. 5. Growth and ^{14}C uptake of pollen tubes from (c) cross- and (s) self-pollinated, D-glucose- ^{14}C -labeled, Ace pistils. Data were taken from experiment 1.

treatment that released 60 to 80% of the ^{14}C . Most of the remaining wall-bound label was solubilized by treatment of

the acid-resistant residue with cellulase. Cytoplasm and acid-hydrolyzed wall fraction from pollen tubes obtained in experiment 1 were assayed for carbohydrate (Table II). These values correspond to pooled pollen tissue from five pistils. Incompatible tubes contained as much or more carbohydrate during the first 3 days when compared to compatible tubes even though the latter were longer.

Incorporation of ^{14}C into pollen tube cytoplasm and acid-hydrolyzed tube wall, reported as cpm/mg of carbohydrate, for the 5 days following pollination is given in Figure 6. Carbon-14 appeared more rapidly in cytoplasm than wall, and the

Table II. Carbohydrate Content of Pollen Tube Cytoplasm and Wall from Cross-Pollinated and Self-Pollinated *Ace* Pistils

Period after Pollination <i>days</i>	Cytoplasm		Acid-hydrolyzed Wall (soluble fraction)	
	Cross	Self	Cross	Self
	<i>mg¹</i>			
0.5	0.22	0.23	0.32	0.32
1.0	0.25	0.27	0.50	0.68
1.5	0.29	0.31	0.65	0.62
2.0	0.32	0.35	0.78	0.82
3.0	0.56	0.54	1.05	1.00
4.0	0.65	0.61	1.10	0.85
5.0	0.78	0.58	1.10	0.70

¹ Tubes from five pistils were pooled for each determination.

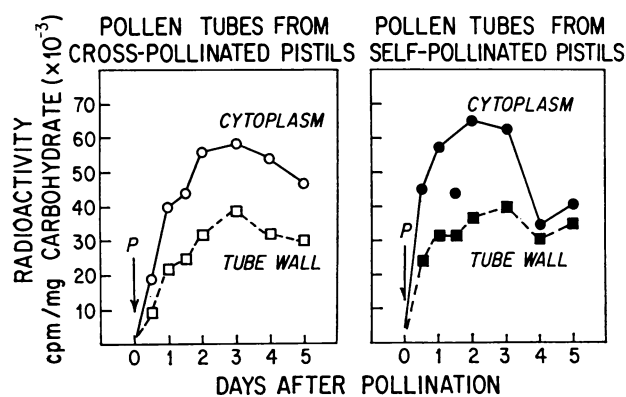


Fig. 6. Incorporation of ¹⁴C into cytoplasm and wall of pollen tubes from cross-pollinated and self-pollinated *Ace* pistils which were labeled with D-glucose-U-¹⁴C. The ¹⁴C value for cytoplasm from self-pollinated pistils at 1.5 days was unusually low and was not used to prepare the curve.

cytoplasm contained more ¹⁴C/mg of carbohydrate than the wall throughout the postpollination period. It is worth noting that the specific radioactivity of pollen tube cytoplasm approximated that of canal exudate (compare Figs. 2 and 6) and exceeded that of stigmatic exudate. Cytoplasm in incompatible tubes contained higher levels of ¹⁴C/mg of carbohydrate than cytoplasm in compatible tubes during the first 24 hr following pollination, and this was reflected in wall label recovered during the same interval.

DISCUSSION

Carbohydrate is the major component of stigmatic exudate of *L. longiflorum* (4). Results obtained in the present study show that carbohydrate in stigmatic exudate of detached unpollinated pistils continued to accumulate until senescence interrupted its production. Carbohydrate in canal exudate also accumulated for 5 days following anthesis but at a rate less than stigmatic exudate. Cross-pollination of *Ace* pistils with Croft pollen did not alter the rate of carbohydrate accumulation during the first 2 days following pollination. An apparent reduction in carbohydrate observed in canal exudate during this period reflected technical difficulties during recovery rather than a decrease in carbohydrate. Self-pollination of *Ace* pistils resulted in an abrupt decrease in carbohydrate accumulation in stigmatic and canal exudates. When pollinating cultivars were reversed, *i.e.* Croft pistils pollinated with *Ace* or Croft pollen, cross-pollinated pistils produced less carbohydrate

in stigmatic and canal exudate than self-pollinated pistils during the 2 days following pollination. In the absence of information regarding production of carbohydrate in exudate from unpollinated Croft pistils, further discussion must be postponed but this observation does emphasize the role of the genetic character of pollen which, in the case of *Ace* pollen, reduced carbohydrate production in stigmatic exudate in both *Ace* and Croft pistils.

The suggestion that pollination triggers release of secretion product from stigmatic tissues (10) or, more specifically, from cells lining the stylar canal (9) was not justified by results obtained in the present study. Pollination altered carbohydrate production, but this change led to less, not more, secretion of exudate. Moreover, secretion of carbohydrate-rich exudate in unpollinated pistils was observed soon after anthesis in stigma and stylar canal, and it continued until senescence.

There was evidence that accumulation of pistil secretion product on the surface of the stigma in unpollinated pistils included contributions of exudate from the canal. Most of the D-glucose-U-¹⁴C administered during the 2-hr labeling period was rapidly translocated to sites of utilization. In canal exudate, specific radioactivity reached a maximum about 3 days after labeling. As canal exudate of lower activity was produced at later times, it displaced material of higher specific activity secreted earlier, diverting the latter toward the stigma where it mixed with stigma-produced exudate. The high specific radioactivity of droplets collected from the stigma surface as compared to the much lower value of stigma rinses at 5 days after anthesis (Table I) fits this interpretation. Further support was found in comparison of galactose content of neutral sugar residues from hydrolyzates of G-100-I fractions of exudates. Highest values were in canal material, intermediate in pure droplets of stigmatic exudate, and lowest in stigmatic exudate containing stigma rinsings. Apparently, the galactose content of these droplets was enriched by contributions from the canal. The observation that stigmatic exudate reached a high level of specific radioactivity about 3 days after label was given to the pistils and continued to maintain that level as the rate of carbohydrate production slowed down (Figs. 1 and 2) also supports the mixing hypothesis.

Pollination did not affect the course of ¹⁴C incorporation into canal exudate. This can be seen in the time course study (Fig. 2) and in gel filtration studies (Fig. 3). In stigmatic exudate, it did alter carbohydrate content, appearance of ¹⁴C, and composition. With the experimental conditions used in this study, pollen tubes in cross-pollinated pistils required between 2 to 3 days to reach the ovary. At 2 days, some tubes had grown over 75% of this distance. In pollen tubes, growth is highly restricted to the tip region (8) and metabolism of exudate seems to be most intense at the tip (3). These conditions would lead to rapid consumption of canal exudate by invading pollen tubes about 2 days after pollination, depriving stigmatic exudate of supplementary canal-derived exudate and leading to the drop in carbohydrate content and ¹⁴C observed. Continued tip growth of cross-pollinated (compatible) tubes toward the ovary would provide a fresh source of carbohydrate in secretion product from canal cells lying in the path of growing tubes. Self-pollinated (incompatible) tubes which are restricted in growth to a portion of the canal only 2 to 4 cm below the stigma would have to rely to a large extent on stigmatic exudate as an exogenous source of carbohydrate. A gel filtration profile of stigmatic exudate taken from self-pollinated pistils at 2 days after pollination revealed depletion of the G-100-I component but no significant change in the corresponding component of canal exudate (Fig. 3). The high galactose content found in neutral sugar residues of the G-

100-I component of both stigmatic and canal exudates from self-pollinated pistils may reflect selective utilization by incompatible tubes of carbohydrate components other than those components rich in galactose. To assess fully changes in molecular weight distribution profiles and changes in composition of the polysaccharides, it will be necessary to analyze G-100-I fractions of exudates from pollinated pistils over the entire course of pollen tube development, from germination to fertilization, a project that could not be undertaken at this time.

Gas-liquid chromatograms of neutral sugar residues from G-100-I components of stigmatic and canal exudates also contributed to the view that canal exudate was produced independently but eventually mixed with stigmatic exudate as pistil secretion product filled the stylar canal. Only stigmatic exudate contained traces of xylose and mannose, sugar residues already characterized as components of a very high molecular weight fraction of stigmatic G-100-I which was referred to as G-200-I in the earlier study (5).

Uptake of ^{14}C by self-incompatible pollen tubes exceeded that of compatible tubes for 3 days following pollination. There were only minor differences in the carbohydrate content of tube cytoplasm and tube wall from these two set of data (Table II). These results indicate that the self-incompatibility reaction places no restraint upon utilization of exudate by growing pollen tubes. Temperature pretreatment of *L. longiflorum* styles leads to inactivation of self-incompatibility (2). The effect of such treatment on uptake and utilization of labeled exudate, *in vivo*, should be included in future studies. Present results reveal no significant differences in incorporation of ^{14}C into cytoplasm and tube wall of compatible and incompatible pollen tubes (Fig. 6).

Comparison of data in Figures 2 and 6 provides a means of estimating the extent of the nutritional dependence of pollen tubes on exudate utilization. Two days after pollination, canal exudate had a specific radioactivity in the range of 50,000 cpm/mg of carbohydrate, a minimal value considering the method used to flush this secretion product from the stylar canal. Pollen tubes from these pistils, carefully rinsed to remove all traces of labeled exudate, yielded cytoplasm with about 60,000 cpm/mg of carbohydrate. Incorporation of ^{14}C

into tube wall polysaccharide lagged behind appearance of label in the cytoplasm as might be expected of a wall assembly process dependent on transfer of carbohydrate precursor from cytoplasm. Three days after pollination, acid-hydrolyzed wall fractions contained nearly 40,000 cpm/mg of carbohydrate. Clearly, most of the carbohydrate utilized by the pollen tube for wall formation was derived from pistil secretion product. Although the mechanism whereby polysaccharide fragments of exudate secreted by tissues remote to the growing tube wall are incorporated into pollen tube cytoplasm and subsequently re-utilized for wall polysaccharide biosynthesis remains obscure, results presented here and earlier (5) strongly suggest such a mechanism does exist.

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