

Effects of Exogenous Materials on Pollen Tube Growth in *Lilium longiflorum* Pistils

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With the use of stigmatic exudate or distilled water as carriers, various antimetabolites, inhibitors, and miscellaneous materials were injected into the hollow styles of detached *Lilium longiflorum* pistils before, at, or after compatible or incompatible pollination. Pollen tube lengths were measured 48 hr after pollination with pollinated styles incubated at 22-23°C. Substances considered inhibitors of protein synthesis in microbial systems significantly retarded both compatible and incompatible pollen tube growth while inhibitors of RNA synthesis tended to significantly inhibit compatible pollen tube growth with less or no effect on incompatible pollen tubes. Application of the inhibitors in stigmatic exudate at or after compatible pollination produced significant results at the lowest concentrations. Significant retardation of pollen tube growth also occurred after injection of 2,4-dinitrophenol, mercaptoethanol, indoleacetic acid, naphthaleneacetic acid, benzyladenine, dimethyl sulfoxide, or potassium or sodium iodide. Pollen tube growth in detached pistils of *L. longiflorum* may be useful as a bioassay *in situ* for screening biologically active materials.

Introduction

Lilium longiflorum Thunb., the Easter lily, is a major floricultural crop in the United States with unique value as a research tool. Easter lily bulbs are asexually propagated (cloned) from bulb scales by specialized growers. Clones available in the United States include Ace, Nellie White, and less frequently Croft, used commercially for production of potted plants; Georgia Belle for cut flowers; and Arai, which is imported from Japan and used as a cut flower. Since the market demands a limited number of cloned genotypes, the researcher is assured of a large supply of a particular genotype from year to year.

For research purposes, the Easter lily can be brought into flower on a year-round schedule. *L. longiflorum* bulbs dug in the autumn require a cool period for rapid floral initiation. Commercially, this is supplied by 6 weeks at 4°C applied to bulbs either in the packing case or after potting. Bulbs can be cooled longer to delay flowering, although excessive cold storage reduces flower number and

maximizes expression of virus symptoms. With care and a glasshouse or other suitable high-light facility, *L. longiflorum* can be flowered a second time in the same container. To do this, bulbs should be potted originally in standard containers at least 15 cm in diameter in a well drained medium such as equal parts soil, sphagnum peat moss, and perlite, and given a complete fertilizer regularly. After flowering the plants should be provided full light and the fertilization continued for 2-3 months. At this time plant tops should be cut at the soil level and the potted bulbs returned to cold storage to repeat the cycle.

Large chromosomes, good correlation between anther length and meiotic stage, and synchrony of meiotic stage among anthers in a single bud make *L. longiflorum* an excellent subject for cytogenetic studies (1-6). The Easter lily is also uniquely suited for studies of pollen-pistil interactions. Lily styles are hollow, lined with specialized secretory cells (7) on which pollen tubes grow from the stigma to the ovary. This hollow style facilitates pollen tube staining in that aqueous aniline blue (or other stain) can be injected into the style through the stigma with a needle and syringe (8). Refrigeration of aniline blue-stained pistils for 1 hr or more in-

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creases pollen tube staining and reduces background. In addition, stained pistils can be stored at 4°C for several weeks without loss of freshness, so long as they are not allowed to dry. To determine pollen tube length, the style is slit with a triangular needle and the stylar canal held open with another needle under a dissecting microscope, beginning at the ovarian end and progressing toward the stigma. When pollen tube tips are visible, the distance to the stigmatic surface is measured with a ruler. Data may be taken as the length of the longest one or more pollen tubes, depending on statistical design.

Because the Easter lily is self-incompatible, two types of pollen tube growth occur from intraspecific pollination. Self or intracclone pollination yields incompatible pollen tubes which reach 40-50% of the length of the style in 48 hr at 22-23°C, while interclonal pollination yields compatible tubes which penetrate 80-90% of the length of the style under the same condition (9). Of the clones commonly grown in the United States, Georgia Belle and Arai are cross-incompatible (bear the same *S* genes for incompatibility) but both are reciprocally compatible with Ace, Nellie White, and Croft which are, in turn, reciprocally cross-compatible in all combinations (10). Pollen tube growth rate in *L. longiflorum* pistils is sensitive to temperature since elevated temperatures (30°C or higher) destroy the self-incompatibility reaction (11). Therefore, incubation temperature following pollination must be standardized. Pollen tube growth in pistils detached from the plant and incubated on moist filter paper in petri dishes does not deviate from growth on the plant, so long as temperatures are equal. The use of detached pistils and controlled temperature incubation after pollination provides reproducibility.

In addition to facilitating pollen tube staining and measurement, the hollow lily style provides an opportunity to introduce exogenous materials into the pollen tube environment *in vivo*. Mature Easter lily flowers secrete exudate from the stigma. This exudate serves as a postpollination carrier of exogenous substances into the stylar canal (12). Repeated freezing and thawing and prolonged storage at -29°C has no measurable effect on the biological activity of stigmatic exudate as a carrier. Both compatible and incompatible pollen tube growth is slightly enhanced when styles are filled with stigmatic exudate at or 6 hr after pollination, although the incompatibility reaction is not obscured (13). Exudate injection 12 hr after pollination produces pollen tube lengths not significantly different from noninjected controls while injection 24 hr after pollination significantly retards the growth of both compatible and incompatible pollen tubes. Stigmatic

exudate remains in the stylar canal throughout the pollen tube growth period and appears as bubbles from the ovarian end of the style when stain is injected (10). Therefore, using stigmatic exudate as a carrier constitutes a combined stylar-canal, pollen-tubes treatment.

Prepollination treatment of the stylar canal alone can be accomplished using distilled water as a carrier (14). Soluble materials injected into the lily style in water appear to be taken up by the cells lining the canal 4-6 hr after injection (15). However, if there is a question of material remaining free in the stylar canal, the pistil may be flushed with distilled water prior to pollination without adverse affect on pollen tube growth (14).

Using various inhibitors of RNA or protein synthesis and either stigmatic exudate or distilled water as carriers, we found that both compatible and incompatible pollen tubes require protein synthesis for normal growth, that only compatible tubes require RNA synthesis, and that the style requires RNA synthesis to produce the self-incompatibility reaction in *L. longiflorum* (14, 16). The purpose of this presentation is to report the effects on pollen tube growth of a number of exogenous materials injected into *L. longiflorum* styles. Although the screening of biologically active materials was not the purpose of the experiments, these data should provide a background from which to draw conclusions as to the sensitivity and suitability of the system.

Material and Methods

Lilium longiflorum cultivars Ace, Nellie White, and Arai provided pistils and pollen for all of the experiments. The plants were glasshouse grown and flowered throughout the year. To minimize contaminating pollinations during harvest without having to emasculate each flower before anther dehiscence, flowers were cut individually early on the day of opening and placed in water in containers in the laboratory. Pistils were harvested one day later by cutting through the ovary with a scalpel, leaving the stamens and the rest of the flower intact. These flowers served as sources of pollen.

Exudate to be used as carrier was collected twice daily with a syringe from the stigmas of flowers cut early on the day of anthesis and maintained in containers of water in the laboratory (22°C) or cold room (4°C). After collection, the exudate was frozen (-29°C) until used. Stock solutions were made fresh for each experiment. Stocks for experiments using stigmatic exudate as a carrier were made 10 × final concentration in distilled water and the test solution made by adding 1 part stock solution

to 9 parts thawed stigmatic exudate. Control for these experiments was 1 part distilled water in 9 parts stigmatic exudate. Material injected into the style at or before pollination was carried in distilled water. In some experiments styles were filled with exogenous material in water 6 or 12 hr before pollination and flushed with 10 drops distilled water just before pollination. Material insoluble in water but soluble in ethanol or alkali (KOH or NaOH) were dissolved in the appropriate solution and diluted in distilled water. Exudate or water controls were made with the same dilution of ethanol or alkali.

Pistils were prepared for injection by snapping the remaining ovary tissue from the style. However, the ovary of *L. longiflorum* is also hollow so that materials and stain can be injected without removal of the ovary. Disposable hypodermic syringes with 22 gauge needles were used to administer the treatments. The Easter lily stigma is triangular, with 3 sutures which converge in the center. Inserting the needle into a suture often caused cracking of the suture and the material to be injected spilled out of the damaged stigma rather than filling the stylar canal. Therefore, care should be taken to place the needle between sutures. Solutions were injected until a drop appeared at the ovarian end of the style. In experiments involving water as carrier, the filter paper in the petri dishes was folded into ridges to prevent contact between the ovarian end of the style and the moist filter paper. Such contact caused the style to drain.

Incubation of all experiments was in the dark at 22-23°C in controlled temperature cabinets. Various prepollination incubation times were used but postpollination incubation lasted 48 hr for all experiments. At this time aqueous aniline blue was injected into the styles and the styles were refrigerated for pollen tube measurement as reported previously (8).

Two experimental designs were employed, both completely random (17). One design involved compatible versus incompatible pollination as one variable and carrier versus three concentrations of exogenous material as the other. Concentrations usually differed by a factor of 10. These treatments were replicated four times giving a total of 32 lily styles per experiment. The other design involved compatible versus incompatible pollination as one variable and carrier versus one concentration of material to be tested as the other. These treatments were replicated 8 times, again resulting in 32 styles per experiment. Both designs incorporated two observations per style, treated as subsamples. The two observations were the length of each of the two longest pollen tubes. Data were subjected to

standard analysis of variance and F tests and means were separated with Duncan's new multiple range test (17).

Experiments were performed over a 7-year period and involved a large number of metabolites, antimetabolites, inhibitors and miscellaneous materials. For data presentation, these have been grouped into somewhat arbitrary classifications. The substances tested are listed in Tables 1-9.

Results and Discussion

Data from examples which typified the 76 experiments carried out to determine the effect on pollen tube growth in Easter lily of materials considered inhibitors of protein synthesis in microorganisms appear in Table 1. Compatible pollen tube growth in *L. longiflorum* was most sensitive to cycloheximide administered in stigmatic exudate at pollination; $1 \times 10^{-6} M$ caused retardation. Combined pollen tube and stylar canal treatments, that is injection at or after pollination using stigmatic exudate as carrier, tended to retard both types of pollen tube growth, suggesting that protein synthesis is necessary for development of the male gametophyte in *L. longiflorum* (16). Interestingly, prepollination injection of these inhibitors in water decreased compatible pollen tube lengths at concentrations which had no effect on incompatible tube growth.

Table 2 summarizes all experiments conducted with these inhibitors. Tetracycline did not appear stable in water since precipitation usually occurred during injection. On the other hand, none of the four experiments with puromycin at $1 \times 10^{-3} M$ in exudate exhibited significant change in pollen tube growth although this concentration significantly retarded both types of pollen tubes when carried in water. Chloramphenicol could not be tested in that the KOH used to dissolve it affected the control sufficiently to prevent all pollen tube growth (Table 2).

The results of selected experiments involving materials considered to be inhibitors of RNA synthesis are shown in Table 3. When carried in stigmatic exudate and injected at or after pollination, these substances tended to significantly retard compatible pollen tube growth at concentrations which did not affect incompatible tube lengths. However, injection in water before pollination often significantly increased the length of incompatible pollen tubes. In the 26 experiments conducted with these materials (Table 4), compatible pollen tube growth was more sensitive than incompatible to RNA synthesis inhibition, suggesting that compatible pollen tube development requires RNA synthesis unnecessary for normal incompatible pollen

Table 1. Representative experiments involving injection of protein-synthesis inhibitors into *L. longiflorum* styles before, at, or after compatible or incompatible pollination.

Inhibitor, carrier and time of injection relative to pollination	No. of replications	Concentration, <i>M</i>	Mean pollen tube length 48 hr after pollination and incubation at 22-23°C, nm ^a	
			Incompatible	Compatible
Cycloheximide in stigmatic exudate at pollination	4	0	44.8 ^b	96.4
		1 × 10 ⁻⁶	53.8	71.2
		1 × 10 ⁻⁵	21.2	24.6
		1 × 10 ⁻⁴	4.2	6.2
Cycloheximide in water 12 hr prepollination, flushed with 10 drops distilled water at pollination	4	0	41.4 ^b	100.1
		1 × 10 ⁻⁵	53.1	54.5
		1 × 10 ⁻⁴	7.3	6.7
		1 × 10 ⁻³	0	0
Tetracycline in exudate 6 hr after pollination	8	0	71.0 b	83.5 a
		4.5 × 10 ⁻⁴	39.6 d	48.2 c
Streptomycin in exudate 6 hr after pollination	8	0	63.2 b	82.1 a
		2 × 10 ⁻³	39.4 d	51.7 c
Streptomycin in water 6 hr before pollination, flushed at pollination	4	0	52.2 bc	81.1 a
		1 × 10 ⁻⁴	49.1 bc	82.1 a
		1 × 10 ⁻³	64.2 ab	62.8 ab
		1 × 10 ⁻²	42.4 c	42.5 c
5-Fluorouracil in exudate 12 hr after pollination	8	0	69.2 b	80.8 a
		1 × 10 ⁻³	48.0 c	65.1 b
5-Fluorouracil in water 6 hr before pollination, flushed at pollination	4	0	47.5 bc	82.8 a
		1 × 10 ⁻⁴	61.1 b	82.2 a
		1 × 10 ⁻³	50.5 bc	61.1 b
		1 × 10 ⁻²	39.1 c	49.9 bc
Puromycin in water, 12 hr before pollination, flushed at pollination	4	0	56.0 bc	83.0 a
		1 × 10 ⁻⁵	42.2 cd	80.8 a
		1 × 10 ⁻⁴	48.2 c	67.0 b
		1 × 10 ⁻³	31.0 d	45.2 cd

^aMeans within experiments followed by the same letter are not significantly different at the 1% level, Duncan's new multiple range test.

^bExperiments with treatments reducing pollen tube growth to near zero cannot be subjected to standard analysis of variance since the variance is correlated to treatment.

tube growth (16). The stimulation of incompatible pollen tubes after prepollination treatment with water as a carrier suggests that stylar RNA synthesis is necessary for the elaboration of the self-incompatibility reaction (14).

Mercaptoethanol and 2,4-dinitrophenol significantly retarded both compatible and incompatible pollen tube growth, while dimethyl sulfoxide (DMSO) was the only substance tested which significantly retarded incompatible pollen tubes at a concentration that had no significant effect on compatible tube growth (Tables 5 and 6). Cyclic AMP up to 1 × 10⁻² M produced no significant change in pollen tube growth. DMSO at a concentration of 5% in an *in vitro* culture medium has been reported to reversibly inhibit pollen tube growth of *L. longiflorum* (18). The significant retardation of only incompatible pollen tube growth by 5% DMSO injected into the lily style (Table 6) points to metabolic parallels between incompatible pollen tube growth *in vivo* and *L. longiflorum* pollen tube growth *in vitro*

which are not shared with compatible tube growth *in vivo* (19).

The plant hormone indoleacetic acid (IAA) and growth regulator naphthaleneacetic acid (NAA) significantly retarded compatible pollen tube growth when injected into the lily style at relatively high concentrations in water while the cytokinin 6-furfurylaminopurine (kinetin) stimulated incompatible pollen tube growth without affecting compatible, and the cytokinin benzyladenine retarded both types of pollen tubes (Tables 7 and 8). Gibberellic acid (GA), succinic acid 2,2-dimethylhydrazide (SADH), and (2-chloroethyl)phosphonic acid (Ethaphon), the first a plant hormone and the others growth regulators, had no significant effect. All of these plant hormones and growth regulators were tested at concentrations considerably higher than those considered physiologically active in the plant. Stimulation of incompatible pollen tube growth without effect on compatible tubes by kinetin (Table 7) resembles the data from experiments with

Table 2. Summary of experiments involving injection of protein-synthesis inhibitors into *L. longiflorum* styles before, at, or after compatible or incompatible pollination; measurements 48 hr after pollination, incubation at 22-23°C.

Inhibitor	Number of experiments	Concentrations tested, <i>M</i>	Lowest concentration causing significant change in pollen tube growth— (type of change)		Carrier (stigmatic exudate or water)	
			Incompatible	Compatible		
Cycloheximide	6	1×10^{-3}	1×10^{-5} (retardation)	1×10^{-6} (retardation)	Exudate	
		1×10^{-4}				
		1×10^{-5}				
		1×10^{-6}				
Tetracycline	7	4.5×10^{-4}	4.5×10^{-4} (retardation)	4.5×10^{-4} (retardation)	Exudate	
		4.5×10^{-5}				
		4.5×10^{-6}				
		4.5×10^{-7}				
Streptomycin	18	1×10^{-2}	2×10^{-3} (retardation)	2×10^{-3} (retardation)	Exudate	
		1×10^{-3}				
		2×10^{-3}				Water
		1×10^{-4}				
5-Fluorouracil	22	1×10^{-2}	1×10^{-3} (retardation)	1×10^{-3} (retardation)	Exudate	
		1×10^{-3}				
		1×10^{-4}				
		1×10^{-5}				
Puromycin	19	1×10^{-3}	1×10^{-3} (retardation)	1×10^{-4} (retardation)	Water	
		1×10^{-4}				
		1×10^{-5}				
		1×10^{-6}				
Chloramphenicol	4	1×10^{-2}	None ^a	None ^a	Either	
		1×10^{-3}				
		1×10^{-4}				
		1×10^{-5}				
		1×10^{-6}				

^aChloramphenicol was dissolved in 0.8 ml 1*N* KOH and diluted to 10 ml for the 1×10^{-2} *M* solution. Control containing same dilution of KOH prevented pollen tube growth.

Table 3. Representative experiments involving injection of RNA-synthesis inhibitors into *L. longiflorum* styles before, at, or after compatible or incompatible pollination.

Inhibitor, carrier and time of injection relative to pollination	No. of replications	Concentration	Mean pollen tube length 48 hr after pollination (incubation at 22-23°C), nm ^a	
			Incompatible	Compatible
Actinomycin-D in water, 12 hr before pollination	4	0	50.5 b	100.5 a
		1×10^{-7} g/ml	61.2 b	99.0 a
		1×10^{-6} g/ml	58.0 b	95.0 a
		1×10^{-5} g/ml	50.0 b	63.2 b
Actinomycin-D in exudate, 6 hr after pollination	8	0	63.2 b	82.1 a
		2×10^{-5} g/ml	51.6 c	49.4 c
6-Methylpurine in water at pollination	4	0	50.9 b	94.2 a
		1×10^{-4} <i>M</i>	59.3 b	95.1 a
		1×10^{-3} <i>M</i>	88.2 a	89.5 a
6-Methylpurine in exudate at pollination	8	1×10^{-2} <i>M</i>	21.4 c	27.6 c
		0	77.1 b	96.5 a
		1×10^{-1} <i>M</i>	74.1 b	75.5 b
Acridine orange in exudate at pollination	4	0	73.1 bc	86.6 a
		3×10^{-6} g/ml	68.5 c	87.2 a
		3×10^{-5} g/ml	78.4 bc	85.5 a
8-Azaguanine in exudate at pollination	4	3×10^{-4} g/ml	74.9 bc	75.0 bc
		0	71.6 b	89.2 a
		1×10^{-5} <i>M</i>	71.4 b	70.5 b
Rifampin in water, 12 hr before pollination, flushed at pollination	4	1×10^{-4} <i>M</i>	68.8 b	67.8 b
		1×10^{-3} <i>M</i>	66.8 b	65.6 b
		0	48.4 c	88.6 a
		2×10^{-5} g/ml	48.8 c	87.8 a
		2×10^{-4} g/ml	36.6 c	61.0 b
		1×10^{-3} g/ml	18.2 d	21.6 d

^aMeans within experiments followed by the same letter are not significantly different at the 1% level, Duncan's new multiple range test.

Table 4. Summary of experiments involving injection of RNA-synthesis inhibitors into *L. longiflorum* styles before, at, or after compatible or incompatible pollination; incubation 48 at 22-23°C after pollination.

Inhibitor	Number of experiments	Concentrations tested	Lowest concentration causing significant change in pollen tube growth (type of change)		Carrier (stigmatic exudate or distilled water)
			Incompatible	Compatible	
Actinomycin-D	14	$1 \times 10^{-4}M$ $2 \times 10^{-5}M$ $1 \times 10^{-5}M$ $2 \times 10^{-6}M$ $1 \times 10^{-6}M$ $1 \times 10^{-7}M$	$2 \times 10^{-5}M$ (retardation)	$1 \times 10^{-5}M$ (retardation)	Either
6-Methylpurine	17	$1 \times 10^{-2}M$ $1 \times 10^{-3}M$ $1 \times 10^{-4}M$	$1 \times 10^{-2}M$ (retardation) $1 \times 10^{-3}M$ (stimulation)	$1 \times 10^{-4}M$ (retardation) $1 \times 10^{-2}M$ (retardation)	Exudate Water
Acridine orange	17	1×10^{-3} g/ml 1×10^{-4} g/ml 1×10^{-5} g/ml 1×10^{-6} g/ml	1×10^{-1} g/ml (retardation) 1×10^{-3} g/ml (retardation)	1×10^{-4} g/ml (retardation) 1×10^{-4} g/ml (retardation)	Exudate Water
8-Azaguanine	5	$1 \times 10^{-3}M$ $1 \times 10^{-4}M$ $1 \times 10^{-5}M$	None	$1 \times 10^{-5}M$ (retardation)	Exudate
Rifampin	3	1×10^{-3} g/ml 2×10^{-4} g/ml 1×10^{-5} g/ml ²	1×10^{-3} g/ml (retardation)	2×10^{-4} g/ml (retardation)	Water

Table 5. Representative experiments involving injection of 2,4-dinitrophenol, mercaptoethanol, or dimethyl sulfoxide (DMSO) into *L. longiflorum* styles before, or at the time of compatible or incompatible pollination.

Material, carrier and time of injection relative to pollination	No. of replications	Concentrations tested	Mean pollen tube length 48 hr after pollination (incubation at 22-23°C), nm ^a	
			Incompatible	Compatible
2,4-Dinitrophenol in exudate at pollination	4	0	75.9 b	89.8 a
		$1 \times 10^{-5} M$	71.1 b	86.4 a
		$1 \times 10^{-1} M$	74.0 b	82.9 a
		$1 \times 10^{-3} M$	23.9 d	53.9 c
Mercaptoethanol in water at pollination	4	0	60.0 b	84.8 a
		0.01%	59.1 b	89.0 a
		0.1%	58.1 b	88.4 a
		1.0%	32.5 c	23.2 d
DMSO in exudate 12 hr after pollination	8	0	59.2 b	77.7 a
		5%	50.4 c	75.1 a

^aMeans within experiments followed by the same letter are not significantly different at the 1% level. Duncan's new multiple range test.

6-methylpurine (Table 3). Since both are substituted purines, the kinetin effect is probably one of interfering with RNA synthesis rather than one of hormonal activity.

As a prelude to radioactive labeling experiments, 1-amino acids were tested for activity in the Easter lily pollen tube growth system. Amino acid analogs were tested as potential inhibitors of protein function. Table 9 lists the amino acids and amino acid

analogs used, all without significant effect. Sodium bicarbonate, potassium bicarbonate, and magnesium bicarbonate, carried in water in 15 experiments and in exudate in four experiments with concentrations up to 8000 ppm, failed significantly to affect pollen tube growth. However, 3000 ppm KI or NaI administered either in water or in exudate significantly retarded compatible pollen tube growth without changing incompatible tube

Table 6. Summary of experiments involving injection of 2,4-dinitrophenol, cyclic AMP, mercaptoethanol, or dimethyl sulfoxide (DMSO) into *L. longiflorum* styles before, at, or after compatible or incompatible pollination; measurements made 48 hr after pollination, incubation at 22-23° C.

Material	Number of experiments	Concentrations tested	Lowest concentration causing significant change in pollen tube growth (type of change)		Carrier (stigmatic exudate or water)
			Incompatible	Compatible	
2,4-Dinitrophenol	2	$1 \times 10^{-2}M$	$1 \times 10^{-2}M$ (retardation)	$1 \times 10^{-3}M$ (retardation)	Water
		$1 \times 10^{-3}M$			
		$1 \times 10^{-4}M$			Exudate
		$1 \times 10^{-5}M$			
Cyclic AMP	5	$1 \times 10^{-2}M$	None	None	
		$1 \times 10^{-3}M$			
		$1 \times 10^{-4}M$			
		$1 \times 10^{-5}M$			
Mercaptoethanol	2	1.0%	1% (retardation)	1% (retardation)	Water
		0.1%			
		0.01%			
Dimethyl sulfoxide (DMSO)	4	5%	5% (retardation)	None	Exudate
		1%			

Table 7. Representative experiments involving injection of plant hormones or growth regulators into *L. longiflorum* styles before, or at time of compatible or incompatible pollination.

Material, carrier and time of injection relative to pollination	Replications	Concentrations tested, ppm	Mean pollen tube length 48 hr after pollination (incubation at 22-23°C), nm ^a	
			Incompatible	Compatible
Indoleacetic acid in water at pollination	4	0	49.9 c	76.9 a
		100	50.6 c	78.2 a
		500	43.6 c	64.6 b
		1000	41.6 c	46.6 c
Kinetin (6-furfurylaminopurine) in water at pollination	4	0	54.4 c	86.1 a
		1	47.9 c	81.1 a
		10	53.1 c	85.9 a
		100	70.4 b	82.8 a
Kinetin in water 6 hr before pollination	8	0	44.5 b	80.6 a
		200	73.5 a	75.2 a

^aMeans within experiments followed by the same letter are not significantly different at the 1% level. Duncan's new multiple range test.

Table 8. Summary of experiments involving injection of plant hormones or plant growth regulators into *L. longiflorum* styles before, at, or after compatible or incompatible pollination; measurements made 48 hr after pollination, incubation at 22-23°C.

Material	Number of experiments	Concentrations tested, ppm	Lowest concentration causing significant change in pollen tube growth, ppm (type of change)		Carrier (stigmatic exudate or water)
			Incompatible	Compatible	
Indoleacetic acid (IAA)	7	1, 10, 100,	None	500 (retardation)	Water
		250, 500, 1000			
Naphthaleneacetic acid (NAA)	2	250, 500, 1000	None	1000 (retardation)	Water
Gibberellic acid (GA)	3	10, 100, 250,	None	None	Either
		500, 1000			
Kinetin	20	1, 10, 50,	50 (stimulation)	None	Water
		100, 200			
Benzyladenine	3	10, 50, 100, 200, 400	1000 retardation	500 (retardation)	Water
		500, 1000, 2000			
Succinic acid 2,2-dimethylhydrazide (SADH)	4	10, 100, 1000, 1250,	None	None	Either
		2500, 5000			
Ethrel (2-chloroethyl)-phosphonic acid	1	500, 1000, 2000, 3000	None	None	Exudate

Table 9. *l*-Amino acids, *d*-amino acids, and amino acid analogs having no consistently significant effect on pollen tube growth when injected singly at 1×10^{-2} , 1×10^{-3} or 1×10^{-4} M in stigmatic exudate or water into *L. longiflorum* styles before or at the time of compatible or incompatible pollination.^a

<i>l</i> -Amino acid	Number of experiments	Amino acid analog	Number of experiments
Alanine	2	<i>d</i> -Alanine	3
Arginine	1	<i>d</i> -Asparagine	7
Asparagine	2	<i>d</i> -Aspartic acid	7
Cysteine	1	<i>d</i> -Dopa	6
Glutamic acid	1	<i>d</i> -l-Ethionine	2
Glutamine	1	<i>d</i> -Glutamic acid	5
Glycine	1	<i>d</i> -Histidine HCl	3
Histidine HCl	1	<i>d</i> -Isoleucine	3
Isoleucine	1	<i>d</i> -Leucine	4
Leucine	1	<i>d</i> -Methionine	8
Lysine	1	Norleucine	4
Methionine	2	<i>d</i> -Phenylalanine	4
Phenylalanine	1	<i>d</i> -Threonine	8
Proline	1	<i>d</i> -Serine	4
Serine	1	<i>d</i> -Tryptophan	4
Threonine	2	<i>d</i> -Valine	8
Tryptophan	1		
Tyrosine	2		
Valine	1		

^aPollinated styles were incubated 48 hr at 22-23°C; treatments were replicated 4 times.

growth. A total of 13 experiments were performed. Treatment with KCl had no effect in the seven experiments completed.

Conclusions

The hollow style of *Lilium longiflorum* which facilitates the application of exogenous materials into the pollen tube environment and the fact that pollen tube growth in detached pistils reflects growth *in situ* make this system intriguing as a potential bioassay for biologically active materials. Materials injected into the lily style can be screened against two metabolisms. Prepollination injection of the material carried in water with a stylar flush immediately before pollination offers a test of the exogenous substance on the metabolism of the cells lining the stylar canal. The function of these cells is secretory; they nourish the growing pollen tubes. Postpollination injection of the material carried in stigmatic exudate exposes both the growing pollen tubes and the cells lining the stylar canal to the exogenous substance. Our data suggest the greatest sensitivity occurs when exogenous materials are carried in stigmatic exudate and injected into detached pistils at or after compatible pollination.

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