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# The Influence of RNA-Synthesis Inhibitors on *in vivo* Pollen Tube Growth and the Self-Incompatibility Reaction in *Lilium longiflorum* Thunb.*

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**Summary.** Injection of $3 \times 10^{-4}$ g/ml acridine orange, $2 \times 10^{-5}$ g/ml actinomycin-D, or $1.34 \times 10^{-3}$ g/ml 6-methylpurine in stigmatic exudate into the hollow styles of *Lilium longiflorum* immediately before, or 6 or 12 hr after pollination restricted compatible pollen tube growth to lengths typical of incompatible tubes. Treatment 24 hr after pollination resulted either in no effect on compatible tube growth or in a significant retardation of compatible tube growth but not to the length of tubes in incompatibly pollinated styles. Aqueous solutions of these inhibitors at the same concentrations injected into lily styles 6 or 12 hr before pollination significantly stimulated incompatible pollen tube growth. These results suggest that RNA synthesis in the style is necessary for the self-incompatibility reaction and RNA synthesis in the pollen tube is required for compatible tube growth. The RNA synthesis in the pollen tube may be completed by 24 hr after pollination.

## Introduction

The hollow style of *Lilium longiflorum*, through which pollen tubes grow to the ovary, makes the Easter lily an excellent tool for *in vivo* studies of pollen tube growth and the self-incompatibility reaction. Utilizing stigmatic exudate as a carrier, metabolic inhibitors can be injected into the style to elucidate the pathways involved in pollen tube growth. The results of such an experiment with 6-methylpurine, an inhibitor of RNA synthesis, suggested that compatible pollen tube growth in the lily requires RNA synthesis but incompatible tube growth does not (Ascher and Drewlow, 1970b). However, the interpretation of these *in vivo* experiments is complicated, for injection places the inhibitor in contact with both the style and the pollen tubes growing in the stylar canal. Modification of pollen tube growth by an altered stylar metabolism is confounded with the direct effects of the inhibitor on pollen tube metabolism.

Treatment of pollen tubes outside of the style is hindered by the necessity of *in vivo* pollen tube growth for the self-incompatibility reaction. However, treatment of styles without pollen tubes could be accomplished by injection of an inhibitor in a suitable carrier into the style some time before pollination. While lily styles filled with stigmatic exudate contain exudate up to 48 hr after injection, styles filled with distilled water appear to absorb the water within 2 hr of injection. In addition, the presence of distilled water in the style at the time of pollination, whether due to injection or flushing, has no significant effect on pollen tube growth or the self-incompatibility reaction (Ascher, unpublished). A comparison of the results of pre-pollination stylar treatment involving RNA inhibitors in water with those of pollination or post-pollination treatment of pollen tubes and style involving inhibitors in stigmatic exudate should clarify the role of RNA synthesis in the metabolisms of *in vivo* grown compatible and incompatible pollen tubes.

## Materials and Methods

Pistils from flowers of greenhouse-grown *Lilium longiflorum* 'Ace' and 'Nellie White' were harvested for experimental use on the day after anthesis by cutting through the ovary. Cut flowers held in water in the laboratory served as pollen donors. Incompatible pollen tubes result from intracultivar pollination while compatible tubes result from intercultivar pollination. Styles were incubated in petri dishes containing moist filter paper at 24.5 °C for 48 hr after pollination. After incubation, pollen tube length was determined by bissecting the styles longitudinally with a razor blade, staining (Ascher and Drewlow, 1970b), and measuring the longest pollen tube to the nearest mm in each half style with the aid of a dissecting microscope. Half-style values formed subsamples in completely random experimental designs; each inhibitor treatment and application time were separate experiments with 4 or 8 replications.

The stigmatic exudate used as a carrier for pollen tube treatments was gathered in the laboratory from cut flowers and stored frozen (Ascher and Drewlow, 1970a). Glass redistilled water served as the carrier for stylar treatments. Injection of the styles was accomplished by snapping the ovary from the style, inserting a hypodermic needle on a syringe into the stigma, and injecting until a drop of the injected material appeared at the ovarian end of the style. Acridine orange at $3 \times 10^{-4}$ g/ml, actinomycin-D at $2 \times 10^{-5}$ g/ml, and 6-methylpurine at $1.34 \times 10^{-3}$ g/ml in stigmatic exudate were individually injec-

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ted into styles immediately before, 6, 12, or 24 hr after pollination for pollen tube treatment. Solutions were made by dissolving the inhibitor in glass redistilled water at a concentration ten-fold higher than the final concentration and diluting the stock solution with 9 parts thawed stigmatic exudate. Injections for control styles consisted of stigmatic exudate containing 10% redistilled water (v/v).

Stylar treatments involved injecting styles 6 or 12 hr before pollination with aqueous solutions of the inhibitors at the same concentrations used for pollen tube treatment, with the exception of actinomycin-D, which was also used at $1 \times 10^{-4}$ g/ml. Styles were flushed with glass redistilled water at the time of pollination to remove unabsorbed inhibitor. Control styles were injected with water 6 or 12 hr before pollination and flushed with water at pollination.

## Results and Discussion

Acridine orange at $3 \times 10^{-4}$ g/ml in stigmatic exudate injected into styles of *L. longiflorum* immediately before, or 6 or 12 hr after pollination restricted compatible pollen tube growth to lengths not significantly different from incompatible tubes, without affecting incompatible pollen tube growth (Table 1).

Table 1. *The effect of $3 \times 10^{-4}$ g/ml acridine orange in stigmatic exudate injected before, 6, or 12 hr after pollination on pollen tube growth in L. longiflorum styles*

| Treatment | Length of pollen tubes (mm) 48 hr after pollination | |
|---|---|---|
| | Incompatible | Compatible |
| Injection before pollination | | |
| Acridine orange | 74.9 a[1] | 75.0 a |
| Control (exudate only) | 73. 1 a | 86.6 b |
| Injection 6 hr after pollination | | |
| Acridine orange | 78.3 a | 79.5 a |
| Control (exudate only) | 70.2 a | 98.2 b |
| Injection 12 hr after pollination | | |
| Acridine orange | 67.2 a | 64.6 a |
| Control (exudate only) | 65.6 a | 80.8 b |

[1] Means within treatment times followed by different letters are significantly different at the 1% level (Duncan's New Multiple Range Test).

The results of acridine orange treatment duplicated the results observed with 6-methylpurine treatment. Since the response of pollen tube growth to 6-methylpurine in this study did not differ from that reported earlier (Ascher and Drewlow, 1970b), the data from pre-pollination, and 6 and 12 hr post-pollination treatments with 6-methylpurine in stigmatic exudate will not be repeated here. Injection of actinomycin-D at $2 \times 10^{-5}$ g/ml in exudate before, 6, or 12 hr after pollination retarded compatible pollen tube growth to lengths typical of incompatible tubes (Table 2). However, post-pollination applications of actinomycin-D resulted in somewhat less tube growth in treated styles than in incompatibly-pollinated control styles.

Table 2. *Pollen tube growth in styles of L. longiflorum filled with $2 \times 10^{-5}$ g/ml actinomycin-D in stigmatic exudate before, 6, or 12 hr after pollination*

| Treatment | Length of pollen tubes (mm) 48 hr after pollination[1] | |
|---|---|---|
| | Incompatible | Compatible |
| Injection before pollination | | |
| Actinomycin-D | 50.5 a[1] | 58.8 a |
| Control (exudate only) | 59.4 a | 95.6 b |
| Injection 6 hr after pollination | | |
| Actinomycin-D | 54.9 a | 57.6 ab |
| Control (exudate only) | 61.7 b | 83.8 c |
| Injection 12 hr after pollination | | |
| Actinomycin-D | 58.3 a | 58.8 a |
| Control (exudate only) | 69.2 b | 80.8 c |

[1] Means within treatment times followed by different letters are significantly different at the 1% level (Duncan's New Multiple Range Test).

All three inhibitors affect RNA synthesis (Goldberg, Reich and Rabinowitz, 1963; Miller and Kempner, 1963; Nicholson and Peacocke, 1966). When applied in exudate to the Easter lily style, they restrict compatible pollen tube growth to lengths typical of incompatible tubes, suggesting that lily pollen carries sufficient RNA for growth as an incompatible tube but requires the synthesis of new RNA for compatible growth.

The effect of these inhibitors on pollen tube growth is less clear when application occurs 24 hr after pollination (Table 3). While 6-methylpurine had no effect on compatible or incompatible tube growth, actinomycin-D significantly retarded compatible pollen tube growth, but not to the level of incompatible tu-

Table 3. *The effect on pollen tube growth of three RNA synthesis inhibitors injected in exudate into the style of L. longiflorum 24 hr after pollination*

| Treatment | Length of pollen tubes (mm) 48 hr after pollination | |
|---|---|---|
| | Incompatible | Compatible |
| 6-methylpurine at $1.34 \times 10^{-3}$ g/ml | 43.3 a[1] | 50.2 b |
| Control (exudate only) | 44.1 a | 54.7 b |
| Acridine orange at $3 \times 10^{-4}$ g/ml | 55.9 c | 51.2 b |
| Control (exudate only) | 43.9 a | 58.5 c |
| Actinomycin-D at $2 \times 10^{-5}$g/ml | 45.9 a | 52.4 b |
| Control (exudate only) | 43.9 a | 58.5 c |

[1] Means within each inhibitor treatment followed by different letters are significantly different at the 1% level(Duncan's New Multiple Range Test).

bes. Acridine orange significantly retarded compatible tube growth and significantly stimulated incompatible tube growth. The relatively shortened pollen tubes in these experiments arise from the adverse effect of injections 24 hr after pollination (Ascher and Drewlow, 1970a). However, a comparison of pollen tube lengths in treated compatibly-pollinated styles with tube lengths in non-treated incompatibly-pollinated styles suggests that the RNA synthesis necessary for compatible pollen tube growth is well underway, if not nearly completed, by 24 hr after pollination.

In contrast to its inhibitory effect on compatible pollen tube growth when applied in stigmatic exudate at or after pollination, 6-methylpurine administered in water to styles 6 hr before pollination stimulated incompatible pollen tube growth to lengths not significantly different from those of compatible tubes (Table 4). Treatment 12 hr before pollination yielded the same results. Injection of acridine orange in water into the style 6 hr before pollination also stimulated incompatible pollen tube growth but, in addition, significantly retarded compatible tube growth (Table 5). Treatment of styles 12 hr before pollination with aqueous acridine orange had no effect on incompatible tube growth although compatible tube growth was decreased to the same degree observed in 6 hr pre-pollination treatment. There was no significant difference in the lengths of compatible and incompatible pollen tubes grown in styles injected with actinomycin-D in water 12 hr before pollination (Table 6). Pollen tube growth in treated styles was significantly longer than in incompatibly-pollinated control styles but significantly shorter than in compatibly-pollinated controls.

Stylar treatment with each of the RNA inhibitors significantly increased the length of incompatible pollen tube growth, suggesting that RNA synthesis in the style is necessary for the self-incompatibility reaction. Since the effect of stylar treatment with inhibitors of RNA synthesis appears to be one of weakening the self-incompatibility reaction thereby allowing longer incompatible tube growth, the effect of combined pollen tube-stylar treatment at or after pollination with inhibitors in exudate must result from the influence of the inhibitor on pollen tube metabolism preventing compatible pollen tube growth. Therefore, compatible pollen tube growth in *L. longiflorum* must depend on RNA synthesis in the pollen tube after pollen germination. The significantly shortened pollen tube length observed in styles treated before pollination with acridine orange or actinomycin-D might be explained by the effect of residual inhibitor in the style on the nucleic acid metabolism of the pollen tubes. Although pollen tubes receiving no stylar contribution to the incompatibility reaction from an inhibitor-blocked stylar metabolism should be capable of compatible growth, inhibitor present in or on the surfaces of the cells lining the stylar canal may prevent the RNA synthesis in the pollen tube necessary for compatible tube growth.

Table 4. *The effect on pollen tube growth of 1.34 × $10^{-3}$ g/ml 6-methylpurine in water injected into L. longiflorum styles 6 hr before pollination (styles flushed with distilled water immediately before pollination)*

| Treatment | Length of pollen tubes (mm) 48 hr after pollination | |
|---|---|---|
| | Incompatible | Compatible |
| 6-methylpurine | 84.9 a[1] | 88.1 a |
| Control (water only) | 49.3 b | 98.9 a |

[1] Means followed by different letters are significantly different at the 1% level (Duncan's New Multiple Range Test).

Table 5. *The effect on pollen tube growth of 3 × $10^{-4}$ g/ml of acridine orange in water injected into L. longiflorum styles 6 hr before pollination (styles flushed with distilled water immediately before pollination)*

| Treatment | Length of pollen tubes (mm) 48 hr after pollination | |
|---|---|---|
| | Incompatible | Compatible |
| Acridine orange | 74.1 b[1] | 79.8 b |
| Control (water only) | 46.8 c | 97.4 a |

[1] Means followed by different letters are significantly different at the 1% level (Duncan's New Multiple Range Test).

Table 6. *The effect on pollen tube growth of actinomycin-D in water injected into L. longiflorum styles 12 hr before pollination (styles flushed with distilled water immediately before pollination)*

| Treatment | Length of pollen tubes (mm) 48 hr after pollination | |
|---|---|---|
| | Incompatible | Compatible |
| Actinomycin-D at 2 × $10^{-5}$ g/ml | 52.8 b[1] | 54.8 b |
| Actinomycin-D at 1 × $10^{-4}$ g/ml | 58.7 b | 57.2 b |
| Control (water only) | 41.4 c | 100.1 a |

[1] Means followed by different letters are significantly different at the 1% level (Duncan's New Multiple Range Test).

The role of RNA synthesis in pollen tube growth and the self-incompatibility reaction in *L. longiflorum* appears to consist of at least two steps: a stylar synthesis involved with manufacturing the stylar contribution to the incompatibility reaction, and synthesis

in the pollen tube requisite for compatible tube growth. Experiments in progress utilizing radioactive labeled precursors to RNA and protein synthesis should further clarify the mechanism of the self-incompatibility reaction.

### References

1. Ascher, P. D., Drewlow, L. W.: Stigmatic exudate: A potential carrier of exogenous substances into the style of *Lilium longiflorum* Thunb. Jour. Amer. Soc. Hort. Sci. (1970a, in press). — 2. Ascher, P. D., Drewlow, L. W.: The effect of cycloheximide and 6-methylpurine on *in vivo* compatible and incompatible pollen tube growth in *Lilium longiflorum*. Theoret. Appl. Genetics **40**, 173—175 (1970b). — 3. Goldberg, I. H., Reich, E., Rabinowitz, M.: Inhibition of ribonucleic acid-polymerase reactions by actinomycin and proflavine. Nature **199**, 44—46 (1963). — 4. Miller, J. H., Kempner, E. S.: Effects of an adenine analog on yeast metabolism. Biochim. Biophys. Acta **76**, 333—340 (1963). — 5. Nicholson, B. H., Peacocke, A. R.: The inhibition of ribonucleic acid polymerase by acridines. Biochem. J. **100**, 50—58 (1966).

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