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THE EFFECT OF LECTINS ON GERMINATING POLLEN OF LILIUM LONGIFLORUM I. EFFECT ON POLLEN GERMINATION, POLLEN TUBE GROWTH AND ORGANIZATION OF MICROFILAMENTS

E. S. PIERSON, P. G. M. WILLEKENS, M. MAESSEN and J. P. F. G. HELSPER*

Department of Botany, University of Nijmegen, Toernooiveld, 6525 ED Nijmegen, The Netherlands

SUMMARY

Five lectins were tested for their binding capacity to pollen tubes of *Lilium longiflorum* and for their effect on pollen germination and pollen tube growth. Only Concanavalin A (Con A) and *Helix pomatia* agglutinin (HpA) showed strong binding to the cell surface, in particular to the colpus of the pollen grain and to the tip of the pollen tube. Wheat germ agglutinin, phytohaemagglutinin and *Ulex europeus* agglutinin showed hardly any binding. No noticeable differences in binding capacity were observed between pollen tubes grown 'in vitro' or 'in vivo' and between pollen tubes regained after compatible or incompatible pollination. 'In vitro' germination of pollen increased at low Con A concentrations (up to 50 μg/ml), but it was inhibited at higher concentrations. Pollen tube growth was inhibited both at low and high Con A concentrations. HpA caused a slight increase in pollen germination, but did not influence pollen tube growth. The other lectins had no effect. When germinated pollen was supplied with Con A, a disorganization of the microfilamental strands was observed in the tip region of the pollen tubes.

1. INTRODUCTION

Lectins occur in a wide variety of plant species. Although their function is not fully understood, they are assumed to play a role in intercellular communication processes (KAUSS 1981), including the self-incompatibility response (HESLOP-HARRISON & HESLOP-HARRISON 1982, ANDERSON et al. 1983). Components with lectin-like properties have been isolated from pistils of several plant species (GOLYNSKAIA et al. 1976, PETTIT 1977, GLEESON 1980). Application of Con A** and PHA** to the stigmatic surface can overcome incompatibility in *Petunia hybrida* and *Eruca sativa* (SHARMA et al. 1985). These two lectins were also reported to stimulate 'in vitro' germination of pollen of *Lilium longiflorum* (SOUTHWORTH 1975). Moreover, Con A treatment affects phospholipid turnover and the biosynthesis of pectic polysaccharides (HELSPER & PIERSON, this issue).

* Address for reprint requests.

** Abbreviations: Con A = Concanavalin A, HpA / *Helix pomatia* agglutinin, PHA = phytohaemagglutinin, UeA = *Ulex europeus* agglutinin, WGA = wheat germ agglutinin, FITC = fluoresceine isothiocyanate, rh-ph = rhodamine-phalloidin

Pollen tube extension, which only occurs at the tip, is assumed to be mediated by a filamentous network, presumably consisting of F-actin (PICTON & STEER 1982). Recently a dense system of microfilaments has been visualized at the tip of pollen tubes (PIERSON et al. 1986). Therefore Con A may influence pollen tube growth via an effect on the structural organization of microfilaments.

This paper describes the binding capacity of several lectins to pollen tubes of *Lilium longiflorum*, grown either 'in vitro' or 'in vivo', and their effect on pollen germination and pollen tube growth 'in vitro'. The effect of Con A on the organization of microfilaments was also investigated.

2. MATERIALS AND METHODS

2.1. Plant material

Pistils, used for pollen tube growth 'in vivo', were excised from plants of *Lilium longiflorum* Thunb., cv. Arai no. 5 or cv. Mount Everest, which were grown under greenhouse conditions. Pollen was collected from dehisced anthers of *Lilium longiflorum* cv. Arai no. 5 and stored below −20 °C.

2.2. Pollen germination and pollen tube growth

For pollen tube growth 'in vivo' pistils of *Lilium longiflorum* cv. Arai no. 5 or cv. Mount Everest were pollinated with pollen of *Lilium longiflorum* cv. Arai no. 5, resulting in incompatible or compatible pollination, respectively. After 48 hours of incubation in the dark at 25 °C pollen tubes were collected from the stylar canal according to LI & LINSKENS (1983).

For growth 'in vitro' pollen was incubated at 25 °C in liquid medium, containing 0.29 M pentaerythritol as the osmotic component (DICKINSON 1968) (5 mg pollen/ml). In experiments on the effect of Con A on pollen germination and pollen tube growth, lectins (final concentration 0-500 μg/ml) were given for the first 4 hours of incubation. In other experiments pollen was incubated for 4 hours in pentaerythritol medium containing no lectin. In studies on the effect of Con A on the cytoskeleton, pollen tubes were incubated for one more hour in the presence or absence of Con A (200 μg/ml).

Incubations were stopped by addition of formaldehyde to a final concentration of 3 mg/ml, except in preparations used to determine the binding capacity of FITC-lectins. In these experiments the germinated pollen remained unfixed.

2.3. Labeling with fluoresceine isothiocyanate-lectin conjugates

Incubations of pollen tubes with FITC**-lectins (0.25 mg/ml 2 mM Tris·HCl, pH 7.0) were carried out at room temperature for 1 hour immediately after culture. Unbound FITC-lectins were removed by washing. Fluorescence of bound FITC-lectins was measured under a Leitz fluorescence microscope.

In investigations on the carbohydrate specificity of lectin binding the following complementary sugars were added in 1000-fold molar excess for 1 hour prior to incubation with pollen tubes: D-α-methylmannoside to FITC-Con A, D-glucosamine to FITC-HpA**, N-acetyl-D-galactosamine to FITC-PHA,

L-fucose to FITC-UeA** and N-acetyl-D-glucosamine to WGA** (BROWN & HUNT 1978).

2.4. Staining of microfilaments

Microfilaments were visualized in pollen tubes after Con A treatment using rh-ph** as described earlier (PERDUE & PARTHASARATHY 1985, PIERSON et al. 1986, in press).

2.5. Chemicals

All chemicals were reagent grade. Lectins were purchased from Stigma (St. Louis, USA) and FITC-lectins from LKB (Bromma, Sweden). Rh-ph was a gift from Prof. Wieland (Max-Planck-Institute, Heidelberg).

3. RESULTS

3.1. Binding capacity of FITC-lectins

From the five FITC-conjugates tested those of the lectins HpA and Con A showed considerable affinity to the surface of pollen tubes of *Lilium longiflorum* (*table* 1). In particular the colpus of the pollen grain (*fig. 1a*) and the tip of the pollen tube (*fig. 1b, table 1*) displayed a bright fluorescence. The FITC-conjugates of PHA, UeA, and WGA were hardly bound. Intact pollen tubes, recovered from pistils, showed fluorescence of similar intensity as pollen tubes grown 'in vitro' (*fig. 1c, table 1*). No noticeable difference was observed between pollen tubes from compatible or incompatible pollination. Treatment of FITC-lectins with an excess of their complementary sugar before incubation with pollen tubes resulted in only a slight decrease in their binding capacity.

Table 1. Fluorescence of pollen tubes of *Lilium longiflorum*, grown 'in vitro' or 'in vivo', after incubation with fluoresceine isothiocyanate (FITC)-labeled lectins. Pollen tubes, grown 'in vivo', were obtained after compatible or incompatible pollination. –A– shows the fluorescence at the tip and –B– at the remaining part of the pollen tube. In the "control" only buffer was present during the labeling period.

FITC-labeled lectin	'In vitro'		'In vivo'			
			Compatible		Incompatible	
	A	B	A	B	A	B
Concanavalin A	++	++	++	++	++	++
Helix pomatia agglutinin	+++	+++	+++	++	++	++
Phytohaemagglutinin	+O	O	+	+	+	+
Ulex europeus agglutinin	+	+	+	+	+	+
Wheat germ agglutinin	O	+O	O	+O	+O	+
Control	O	O	O	O	O	O

O = no fluorescence; +O = very weak fluorescence; + = weak fluorescence; ++ = moderate fluorescence; +++ = bright fluorescence.

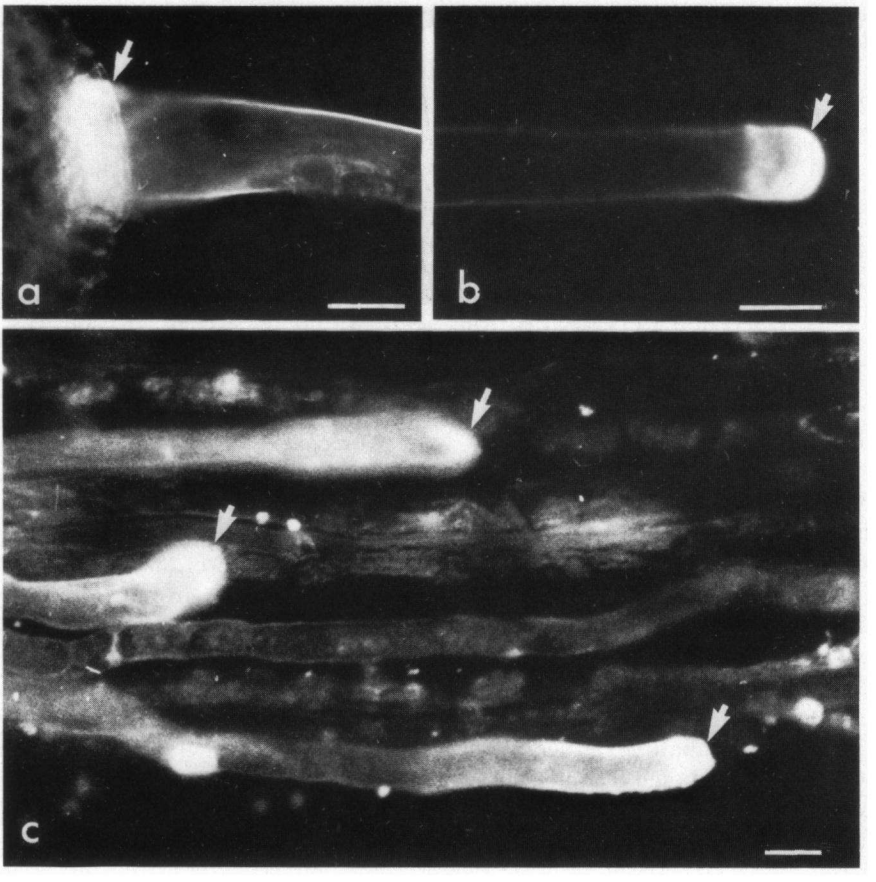

Fig. 1. Micrographs of pollen tubes of *Lilium longiflorum*, labeled with fluoresceine isothiocyanate conjugate (FITC) of Concanavalin A. *Fig. 1a* shows the tip region of a pollen tube and *fig. 1b* the colpus region of a pollen grain after germination 'in vitro'. *Fig. 1c* shows several pollen tubes, recovered at 48 hours after compatible pollination. Bars correspond to 20 μm. Arrows indicate sites with bright fluorescence.

3.2. Effect of lectins on pollen germination and pollen tube growth 'in vitro'

At low concentrations, up to 50 μg/ml, Con A stimulated pollen germination 'in vitro', while it had an inhibitory effect at concentrations of 100 μg/ml and higher (*fig. 2*). Pollen tube growth was inhibited by Con A, also at low concentrations, in a dose-dependent manner (*fig. 2*). HpA had no effect on pollen tube growth but it slightly stimulated pollen germination up to at least 500 μg/ml. At this concentration the stimulation was about 30%. The other lectins had no effect on pollen germination or pollen tube growth.

Effects of Con A on pollen germination and pollen tube growth were only slightly diminished by preincubation of Con A with a specific complementary sugar.

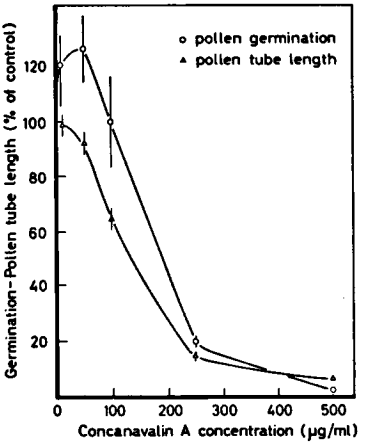

Fig. 2. Effect of Concanavalin A (Con A) concentration on pollen germination (○) and pollen tube length (△) in *Lilium longiflorum*. Results are expressed in percentage of the control value (no Con A added). The average germination percentage in the control was 43% and the average tube length 1.33 mm. Vertical bars indicate the standard error of means.

3.3. Effect of Concanavalin A on the organization of microfilaments

In the absence of Con A distinct strands of microfilaments were observed over the entire length of the pollen tube, including the tip region. These strands were oriented predominantly parallel to each other and to the axis of the pollen tube (*fig. 3a*), which is consistent with previous observations (PERDUE & PARTHASARATHY 1985, PIERSON et al. 1986). The transfer of growing pollen tubes to medium containing Con A had no effect on the organization of the microfilaments in the part of the pollen tube proximal to the grain, but in the tip region only spots of fluorescence could be distinguished (*fig. 3b*). There was an abrupt transition between the two zones (*fig. 3c*).

4. DISCUSSION

The cell surface of germinating pollen of *Lilium longiflorum* has binding capacity for Con A and HpA, but it shows hardly any affinity for PHA, UeA and WGA. Con A induces a physiological response on pollen germination and pollen tube growth. Since no FITC-conjugated Con A was observed in the cytoplasm, it is likely that the physiological response is mediated by interaction with binding sites at the cell surface. These are relatively abundant at the pollen tube tip, which is the site of cell extension and probably also of growth control (PICTON & STEER 1982, HESLOP-HARRISON & HESLOP-HARRISON 1982). An excess of complementary sugars had hardly any effect on the binding capacity of FITC-lectins and on the physiological response to Con A. Therefore interaction with the cell surface might not only occur via the sugar binding site of the lectins. Such 'aspecific' interaction for lectins has been described before (KAUSS 1981).

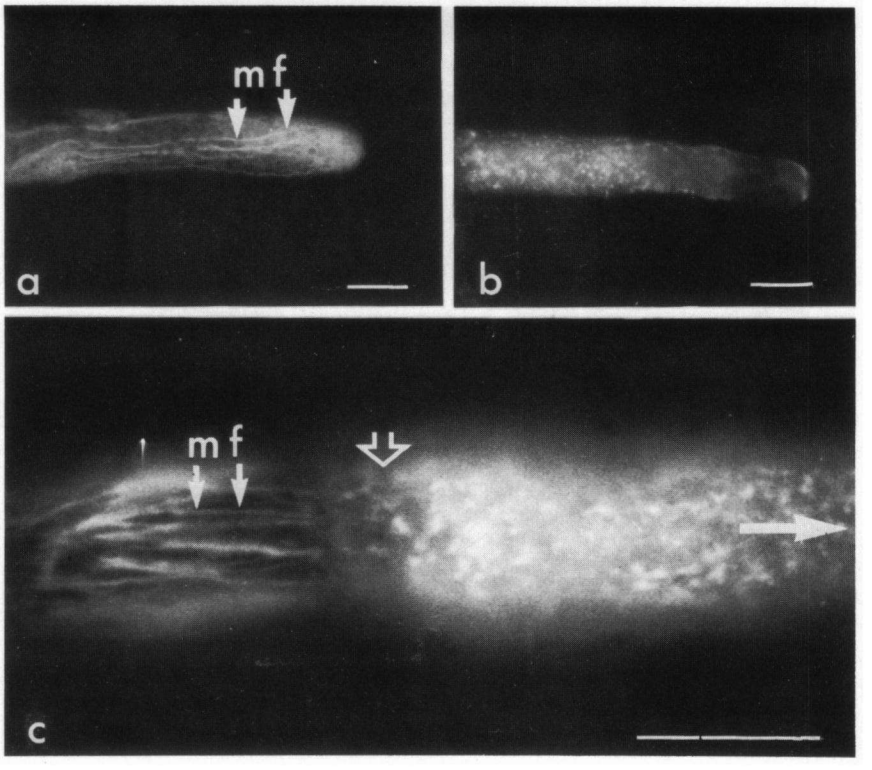

Fig. 3. Micrographs of pollen tubes of *Lilium longiflorum* stained with rhodamine-phalloidin to visualize F-actin microfilaments (mf). *Fig. 3a* shows strands of microfilaments in a pollen tube grown in pentaerythritol medium. *Fig. 3b* shows the tip of a pollen tube incubated for 1 hour in medium containing 200 μg Con A/ml after 4 hours of preincubation in the absence of lectin; only spots of fluorescence can be distinguished. *Fig. 3c* shows the zone proximal to the tip in a pollen tube treated as described in *fig. 3b*; a distinct transition (open arrow) is visible between the zone with parallel strands of microfilaments and that with spotted fluorescence. The closed arrow points towards the pollen tube tip. Bars correspond to 20 μm.

At low Con A concentrations pollen germination is stimulated in our study, which is consistent with the effect reported by SOUTHWORTH (1975). In contrast to Southworth's results we observed an inhibition of pollen germination beyond 50 μg Con A/ml. This discrepancy may be due to the fact that Southworth used sucrose as an osmotic component in the culture medium, while we use pentaerythritol. Since Con A has a high specific affinity for the glucose moiety of sucrose (KANEKO et al., 1972; GOLDSTEIN, 1976), we think that pentaerythritol is a more suitable component for studies with this lectin.

Unlike WHEELER et al. (1985), who found that Con A stimulates the polymerization of actin in human platelets, our results indicate that the presence of Con A (200 μg/ml) disturbs the integrity of the actin cytoskeleton in *Lilium* pollen tubes. Disturbance of the structural organization of microfilaments, as was

observed after Con A treatment, has probably a negative effect on the rate of extension of pollen tubes. This conclusion fits well with the results about pollen tube length after treatment with Con A.

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