Localization of Callose Deposits in Pollen Tubes of *Lilium longiflorum* Thunb. by Fluorescence Microscopy[1]

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Since pollen tubes elongate rapidly, identification of cell wall constituents in pollen tubes is of interest with regard to elucidating the mechanisms of cell growth in plants. Growth of pollen tubes is largely restricted to the tube tip (8, 9, 11) and thus represents a rapid, localized deposition of cell wall materials (11). Rates approaching 7.5 mm per hour are reported for tube elongation in the style (3) while pollen tubes cultured *in vitro* elongate at approximately one tenth this rate (3, 11).

Cell walls of pollen tubes are composed principally of glucose polymers (11), but the manner in which the glucose units are linked is not known. The existence of one type of glucoside polymer can be established through fluorescence microscopy. This is a β-1,3-linked glucan known as callose (5). Callose is synthesized rapidly in many types of plant cells (4, 6) and may be deposited in large quantities in localized cell wall regions (4). The enzymes necessary for this polymerization are widespread in plants (6). In this study, stains reported to be fluorospecific for callose were utilized to determine the extent of β-1,3-glucan deposition in pollen tube cell walls of *Lilium longiflorum*.

Materials and Methods

Mature lily (*Lilium longiflorum* Thunb. var. Ace) anthers were collected from greenhouse grown plants, dried and stored at —70° C. Pollen grains were germinated at 28° C in a 10% sucrose solution containing 100 ppm boric acid (9). Tubes were stained approximately 2 hours after the beginning of germination.

Living material was stained with aniline blue (a 0.01% solution of water soluble aniline blue prepared in M/15 dibasic phosphate for 10 min) for detection of small amounts of callose (5) or with acridine orange (0.01% acridine orange in 0.01 M phosphate-citrate buffer, pH 7.5 to 8.5 for 5 min) (2). After staining, material was viewed in a darkened room using a fluorescence microscope [American Optical; Model 645 Fluorolume (200 watt mercury arc lamp)]. Preparations were photographed with two exciter filters [Schott BG-12 (350-450 mμ) of 3 mm thickness and Corning 5840 (350-400 mμ) of 2 mm thickness] and a water barrier filter in the light path.

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Results

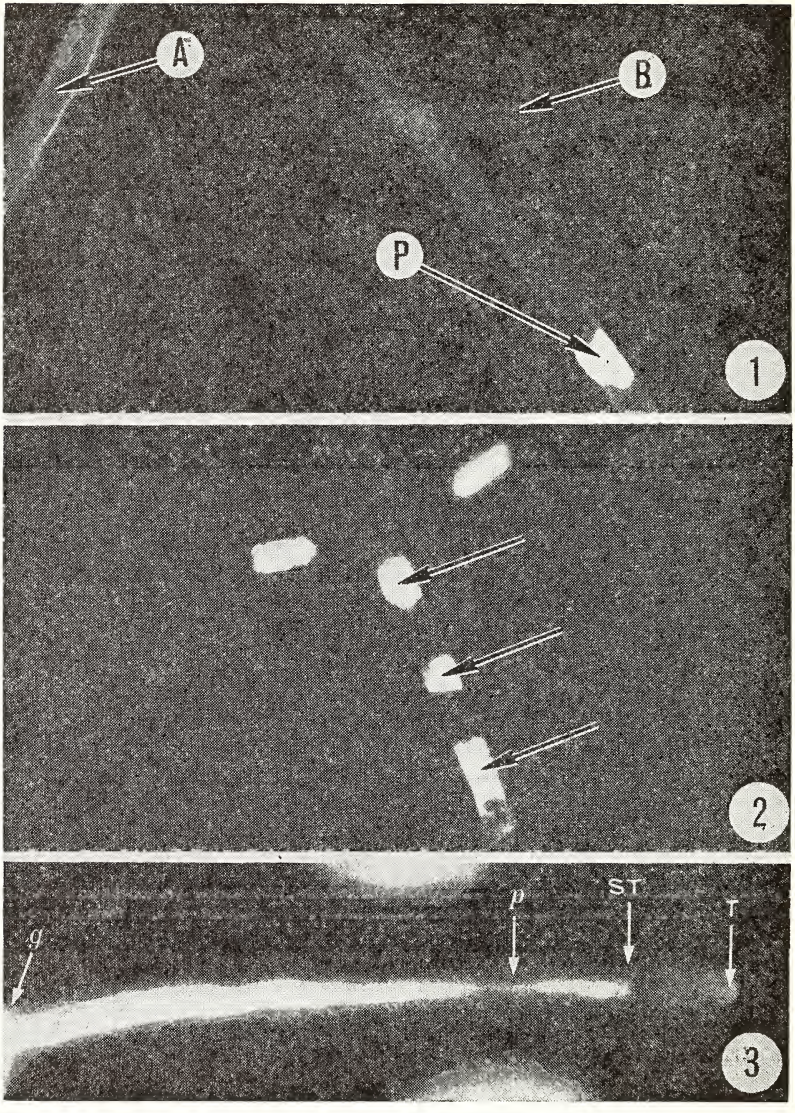
Some tubes which were stained with aniline blue fluorochrome showed a pale yellow fluorescence characteristic of callose (tube A, Fig. 1). In many tubes, however, this fluorescence appeared only as a yellow haze (tube B, Fig. 1) or was absent. The tube tip did not fluoresce with aniline blue. Heavy callose deposits were found in the tube distal to the tip in plug formations (Fig. 1, P). Aniline blue staining of tubes fixed with formalin-acetic acid or staining at a pH of 9 to 10 (5) gave similar results as did staining of pollen tubes of trumpet vine (*Campsis radicans* Seem.). In general, the plugs of callose were formed at regular intervals along the length of the tube as the tube elongated (arrows, Fig. 2). The continued plugging eventually subdivided the tubes into many small segments.

Acridine orange fluorochrome yielded an intense red fluorescence in a limited number of trials, a reaction reported to indicate callose deposition (2). The pH was critical with an optimum between pH 7 and 8 over the pH range of 5 to 10. The tip region proper (5 to 15 μ from the tube tip; T in Fig. 3) fluoresced a bright red to reddish orange while the subtip region (zone 20 to 30 μ in length from arrow at T to arrow at ST) fluoresced a deeper, more subdued, shade of red. The periphery of the tube (p) assumed the coloration of the subtip region. The pollen grain (g) and the central region of the tube were characterized by a yellow to yellowish green fluorescence.

Discussion

With aniline blue, dense callose deposits were restricted to plugs as reported previously by Currier (4) and Skvarla and Larson (10) for pollen tubes of other species. Callose plugs have been observed by fluorescence microscopy in pollen tubes growing in stylar tissues (7), and by phase microscopy in pollen tubes growing in culture. According to Brubaker and Kwack (3), the rapid tube elongation is a process of cell wall synthesis involving little or no net increase in cytoplasm. This they attribute to continued vacuolation of mature regions of the tube following callose plugging. In this manner, the callose plugs are thought to restrict the mass of cytoplasm to the growing tube tip.

In contrast to results with aniline blue, the pollen tube tip fluoresced for a length of 30 to 40 μ following acridine orange staining suggesting that callose was not the substance reacting. In pollen tubes, dictyosomes of the Golgi apparatus (8) produce secretory vesicles which migrate to the tube tip where they coalesce to pool wall precursor materials (9, 11). The tip region, where vesicle coalesence and fusion with the cell wall occur, corresponds to the region of bright red fluo:rescence with acridine orange fluorochrome. The subtip region which fluoresces a subdued shade of red may correspond to the region of vesicle accumulation, especially under the conditions of abnormal growth provided by the acridine orange solutions. These are the same regions that stain for polysaccharides following application of periodic acid-Schiff reagents or alcian blue (9). In the remainder of the tube, dictyosomes are distributed in random groups and secretory vesicles are fewer in number.



Figures 1 to 3. Living pollen tubes of lily (*Lilium longiflorum*) stained for polysaccharides and observed by fluorescence microscopy. Fig. 1. Aniline blue fluorochrome for callose showing variations in the intensity of fluorescence among different tubes. *P* denotes a callose plug formation. X 280. Fig. 2. Aniline blue fluorochrome showing a single tube with multiple plug formations of callose (arrows). X 280. Fig. 3. Acridine orange fluorochrome, pH 7.8, for tube tip matrix polysaccharides. Reddish fluorescence of tube tip (T) extends over subtip region to arrow (ST) and along the periphery (p). The grain (g) and the central portion of the tube were characterized by a yellow to yellow green fluorescence. This fluorochrome did not appear to be combining exclusively with callose. X 320.

Response to injury may contribute to the pattern of acridine orange fluorescence (1). Tube growth is easily disrupted by a variety of conditions including routine handling. Under these conditions, secretory vesicles often accumulate in the tip and subtip regions. Since acridine orange was used as a vital stain in these studies, such accumulations would be expected. Furthermore, they would account for the observation that the extent of the strongly fluorescent regions in the presence of acridine orange is somewhat greater than the region of vesicle accumulations observed in thin sections of pollen tubes fixed during steady state growth (11).

Summary

Using a fluorospecific stain, aniline blue, callose was found to be localized in plug deposits distal to the growing tip in pollen tubes of germinating lily (*Lilium longiflorum*) pollen. The results with acridine orange differed from those with aniline blue on identical material, thus casting doubt on the usefulness of acridine orange as a fluorospecific stain for callose. The dense reddish fluorescent deposits at the growing tips of pollen tubes which had been vitally stained with acridine orange corresponded to accumulations of wall precursors which were at least partially derived from the Golgi apparatus.

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