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Influence of Borate and Pentaerythritol Concentrations on Germination and Tube Growth of *Lilium longiflorum* Pollen[1]

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Abstract. **Rapid tube growth occurred when lily (*Lilium longiflorum* Thunb. cv. Ace) pollen germinated in a liquid medium containing 0.29 M pentaerythritol. Growth was reduced by increasing or decreasing the pentaerythritol concn. There was a hyperbolic relationship between concn of H_3BO_3 in the medium and stimulation in rate of pollen tube growth, with 30 μM H_3BO_3 causing one-half maximal stimulation of growth. Neither early pollen tube growth nor percent germination was stimulated by the hormones indoleacetic acid and gibberellic acid, nor was there any effect by 2'-isopropyl-4'-(trimethylammonium chloride)-5'-methylphenyl piperidine-1-carboxylate (Amo 1618), an inhibitor of gibberellin biosynthesis.**

The requirements for pollen germinating in a defined medium include calcium, borate, and an osmoticum (4, 10, 15, 24, 25). There is information concerning the role of calcium (10), but the role of borate is unknown (20, 22) and the relation between pollen tube growth and osmotic concn of the culture medium is not well understood. Earlier work in this area utilized, as osmotic agents, sugars which were readily absorbed and metabolized (11, 16, 23). Gibberellin and auxins are also reported to stimulate germinating pollen (3, 5, 17, 21, 26).

The present work was undertaken to determine how germination of 'Ace' lily pollen is affected by the addition to the culture medium of boric acid, GA_3, IAA, Amo 1618, and pentaerythritol. Pentaerythritol is an osmoticum which is absorbed slowly and not metabolized by germinating lily pollen (11).

Materials and Methods

Glass distilled water was used for preparation of all reagents. GA_3 (grade III) was from Sigma Chemical Company, St. Louis, Missouri. IAA was from the Eastman Co., Rochester, N.Y. and Amo 1618 (B grade) from Calbiochem (San Diego, California).

Pollen of 'Ace' lily was used in all experiments. Procedures for collecting, handling, and germinating the pollen were reported earlier (10, 11). Each pollen sample (4.0 mg) was incubated with 1.0 ml culture medium in a 10 ml Erlenmeyer flask. Flasks were incubated on a metabolic shaker at 30°C. The standard culture medium contained 0.29 M pentaerythritol or 0.29 M glucose and the following salts: 300 μg/ml (1.27 mM) $Ca(NO_3)_2 \cdot 4\ H_2O$, 100 μg/ml (0.99 mM KNO_3, 10 μg/ml (0.162 mM) H_3BO_3. The pH was 5.2. In several experiments the concn of pentaerythritol and borate were altered as described under Results. There were duplicate pollen samples per treatment for each experiment. Small portions of germinating pollen were removed at intervals from each flask. Germination percentages and tube lengths were determined photographically on duplicate microscope fields as reported earlier (9), and average values were calculated for each flask. The difference between duplicate flasks is indicated by the vertical lines which accompany each point and bar in the figures. The number of pollen grains counted per flask is given in Fig. 4, and these values are typical for the experiments reported here.

Leakage of pollen carbohydrates was determined on samples of pentaerythritol medium after removal of pollen. At the times indicated, samples were withdrawn and placed on chilled microscope slides. These samples were used to determine percentage germination and tube length. The microscope slides were stored briefly on ice and photographed as soon as possible. The remainder of each pollen sample was immediately poured into a chilled 15-ml glass centrifuge tube (round bottom, Corex brand) and pollen was sedimented by 5 min centrifugation at 8,000 *g* and 0°C. The clarified culture medium was withdrawn carefully using Pasteur pipets with orifices which were reduced by flaming so that pollen grains could not enter. Total carbohydrate was determined in duplicate on each sample using the anthrone procedure described earlier (10).

Calculations for the reciprocal plot of the pollen growth data (Fig. 1B) are similar to calculations done earlier for the activator saturation curve of an enzyme (14). In both cases the basal value was subtracted from the activated values before calculation of reciprocal values for a double reciprocal plot.

Results

The effects of various borate concn on lily pollen germination and tube growth in glucose medium are presented in Fig. 1. Pollen tubes were quite short (100 μm) after 3 hr incubation without added borate, and progressive increases in tube length occurred when borate was increased from 0.5 to 20 μg/ml (Fig. 1A). The data of Fig. 1A gave a straight line in a double reciprocal plot (Fig. 1B), indicating a hyperbolic relation between borate concn and enhancement of tube length at 3 hr. Only the lowest borate concn did not fit the line. The asymptote for the curve in Fig. 1A was 1,330 μm as calculated

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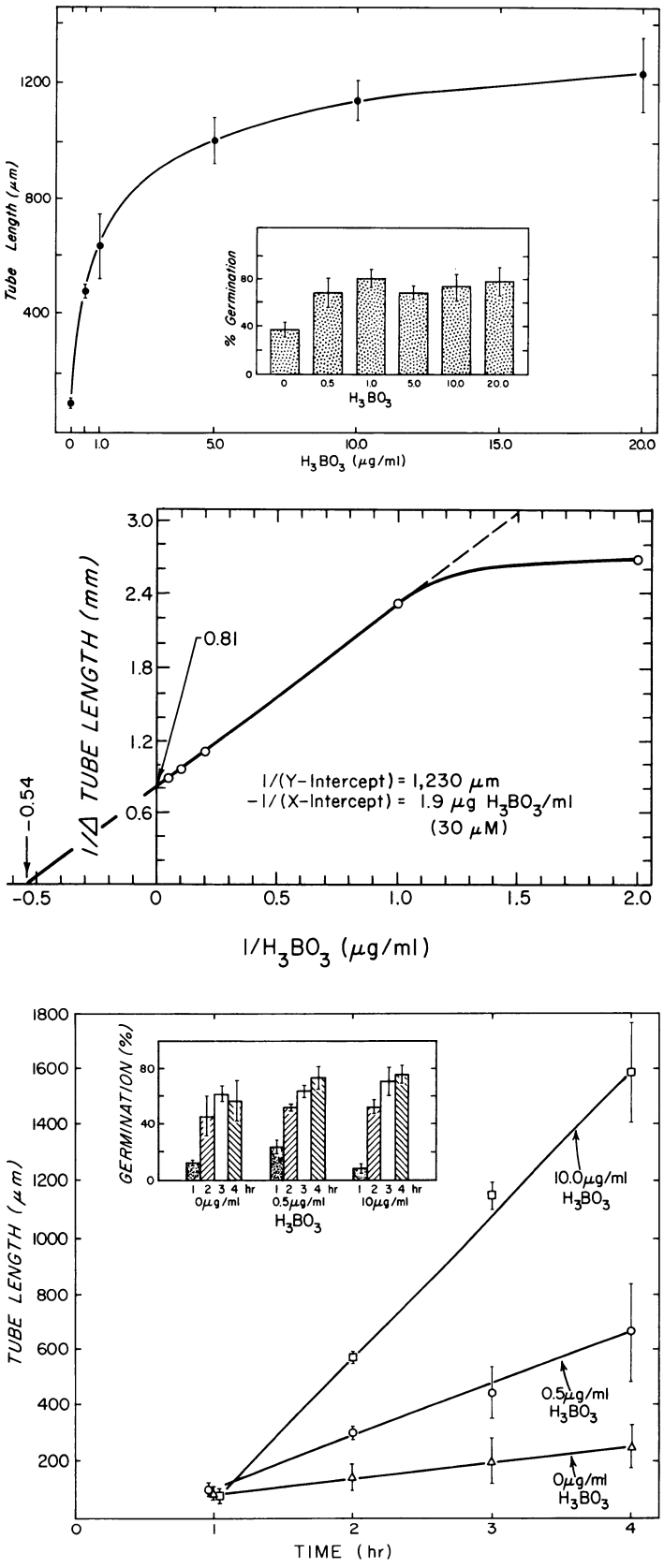

Fig. 1. Lily pollen tube growth and % germination (insets) in glucose medium containing various concn of borate. Each point or bar is the average of duplicate flasks, and the vertical lines indicate differences between duplicates. (Top) 3 hr incubation. (Middle) Double reciprocal plot of data from Top. (Bottom) Incubation for various lengths of time. The first time 3 samples were withdrawn at exactly 1 hr, but 2 points are slightly displaced so that the vertical lines can be seen.

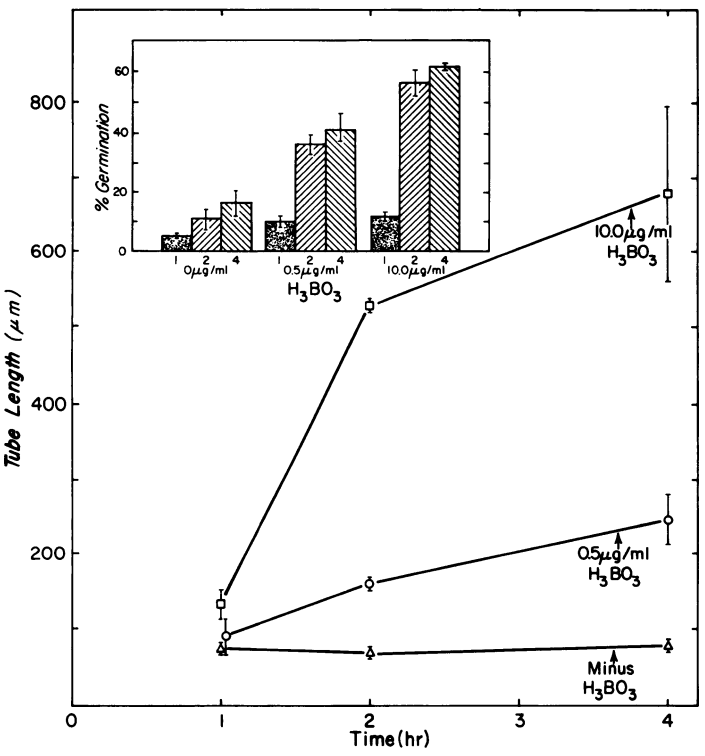

Fig. 2. Lily pollen tube growth and % germination (inset) in pentaerythritol medium containing various concn of borate. Each point or bar is the average of duplicate flasks, and vertical lines indicate differences between duplicates.

from the reciprocal plot and then adding the basal growth in absence of borate. This is the maximal pollen tube length that would be expected at high borate concn. Tube lengths attained in the standard culture medium (10 μg/ml H_3BO_3) are about 86% of this maximal value. Thirty μM added borate gave one-half of the maximal growth response (Fig. 1B).

The data of Fig. 1A did not reveal whether boron enhancement of the tube growth resulted from 1) progressively earlier appearance of pollen tubes, 2) transient enhancement of growth rate, or 3) a continuous effect on growth rate. The data of Fig. 1C revealed that added borate affected only the rate of pollen tube growth and not the time of tube initiation. Percent germination was low at 1 hr, and pollen tubes in the several treatments (0, 0.5, and 10 μg borate/ml) were all equally short. By 2 hr, there were differences in tube lengths among the 3 treatments. These differences persisted, with rates of tube growth being the expected 500 μm/hr in 10 μg borate/ml, about 10% of this rate in the absence of added borate, and 37% of this rate in 0.5 μg borate/ml.

The dependence of tube growth on added borate was not diminished when pentaerythritol was substituted for glucose in the culture medium (Fig. 2), although some differences in growth pattern were observed. After 2 hr incubation in pentaerythritol, tubes growing in 0.5 μg borate/ml were 31% as long as tubes in 10 μg borate/ml with a similar relationship (36%) at 4 hr. These values are quite similar to the value of 37% cited above for the glucose medium. No growth occurred after 1 hr in pentaerythritol medium which lacked borate whereas slight growth did occur in glucose medium which lacked borate.

The effect of less than optimum borate on pollen permeability was determined by measuring the loss of pollen carbohydrates into the medium. Carbohydrate released from pollen germinating at 0, 0.5, and 10 μg borate/ml (Fig. 3) was quite low (225 μg carbohydrate/ml or less) compared to the 800-1,000 μg carbohydrate/ml released from similar pollen sample

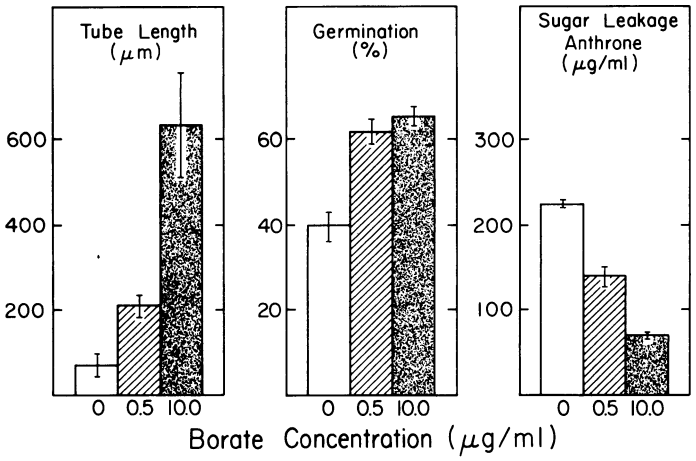

Fig. 3. Effect of various borate concn on lily pollen germination, tube growth, and loss of sugars to the medium. Pollen was incubated 3 hr in pentaerythritol medium.

by addition of EDTA to the medium (10). Cell fragments, evidence of cell breakage, were seen in the flasks lacking borate, although many pollen tubes appeared to be intact. This breakage was undoubtedly responsible for part of the 225 μg carbohydrate/ml which appeared in the medium. The 140 and 70 μg carbohydrate/ml that appeared in the low and high borate treatments may have leached from the pollen walls because little or no cell rupture was observed.

The effects of various concn of pentaerythritol on pollen tube growth, % germination, and carbohydrate leakage were determined (Fig. 4). Pollen tube growth was reduced when pentaerythritol concn were < 0.29 M, but bursting did not occur as evidenced by the lack of carbohydrate leakage. Raising the pentaerythritol concn above 0.29 M also had an adverse effect.

IAA and GA_3 were added to standard glucose media in attempts to stimulate lily pollen tube growth. Amo-1618, an inhibitor of gibberellic acid biosynthesis (8) was added to determine whether germinating lily pollen must synthesize gibberellin to sustain rapid tube growth. After 2 hr of incubation, there was no stimulation by 1 or 10 μM IAA, and 100 μM IAA was inhibitory. Average percentage germination values for the control and the lower 2 IAA concn were 82-92% and average tube lengths were 670-720 μm. Germination and tube length in 100 μM IAA were only 14% and 170 μm, respectively. Amo-1618 and GA_3 were incubated 2 hr with pollen at concn of 1, 10, 100, and 1,000 μM. Control values were 76% germination and 611 μm tube length. None of the levels of Amo-1618 was inhibitory; germination was 75-93% and tube lengths 559-791 μm in the various concn of this compound. The only pronounced effect of GA_3 was inhibition at 1,000 μM; germination was 38% and tube length 27 μm.

Discussion

The apparent hyperbolic relation observed here between borate concn and tube growth indicates that borate binds in a reversible way to growth-related sites of the pollen tubes and that the binding sites were one-half saturated at 30 μM added borate. The data did not reveal whether such binding sites were a growth-related process within the cells or a transport process at the cell surface. An effect of borate on enzymes of cell wall biogenesis was postulated for cotton fibers (2), and the relevant pollen enzymes (12, 13, 18) might be similarly affected. Boron deficiency does not cause an early effect on root cell elongation in contrast to the present results with germinating pollen. The first processes affected in roots are cell division and nucleic acid metabolism (6).

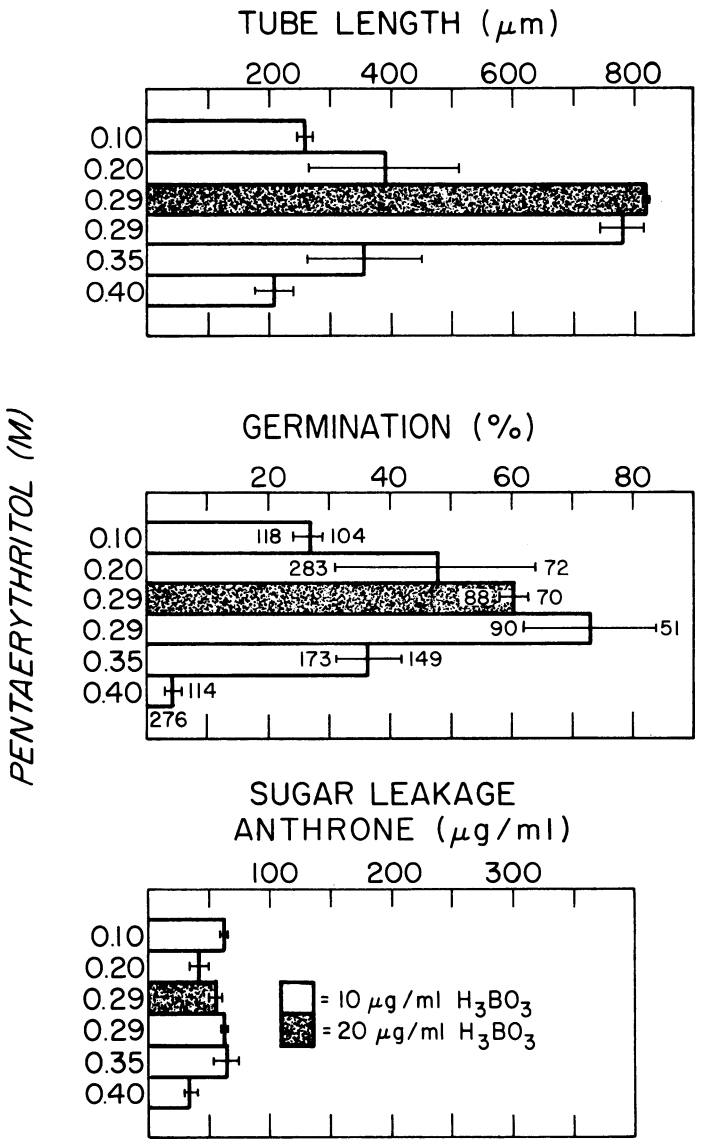

Fig. 4. Pollen growth and loss of sugars to the medium during germination in various concn of pentaerythritol. Pollen (4 mg) was germinated 3 hr in 1 ml of medium. Carbohydrate was determined after removal of pollen from the medium. The results are expressed as μg glucose/ml culture medium. Each bar is the average of duplicate pollen samples, and the horizontal lines indicate the differences between duplicates. The numbers of pollen grains in the samples withdrawn for determining % germination and tube length are given in the middle graph and are typical of all experiments.

Deficiencies of borate and calcium both drastically reduce lily pollen tube growth. These deficiencies differ in that lack of calcium causes extensive bursting and almost complete release of pollen sugars into the medium (10), but relatively little bursting or sugar leakage resulted from lack of borate. Nor was there estensive sugar leakage into the medium when pentaerythritol was lowered sufficiently to drastically reduce tube growth. The reduction of tube growth by altered pentaerythritol levels is quite different from the broad optimum reported for lily pollen germinating in various sucrose concn (16).

The present work indicates that lily pollen tube initiation and early tube growth are not stimulated by auxin or gibberellin, and in this respect lily resembles *Setaria* pollen (7). Such results are in agreement with the ability of germinating

pollen to produce IAA (19) and the report that mature *Lilium* pollen contains gibberellin (1). Although endogenous gibberellin levels decrease during lily pollen germination and early tube growth (1), the results with Amo-1618 indicate that there is no need for gibberellin biosynthesis during that time. Chandler (5) reported that gibberellic acid stimulated germination and tube growth of *Lilium speciosum* pollen, and Kato (17) reported a similar stimulation of *L. longiflorum* pollen with gibberellic acid. Both of these workers germinated pollen on an agar culture medium without borate, so their experimental conditions were quite different from the conditions of the present study.

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