THE ESSENTIAL ROLE OF CALCIUM ION IN POLLEN GERMINATION AND POLLEN TUBE GROWTH[1,2]

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ABSTRACT

BREWBAKER, JAMES L., and BEYOUNG H. KWACK. (U. Hawaii, Honolulu.) The essential role of calcium ion in pollen germination and pollen tube growth. Amer. Jour. Bot. 50(9): 859–865. Illus. 1963.—A pollen population effect occurs whenever pollen grains are grown in vitro. Small pollen populations germinate and grow poorly if at all, under conditions which support excellent growth of large pollen populations. The pollen population effect is overcome completely by a growth factor obtained in water extracts of many plant tissues. This factor is shown to be the calcium ion, and its action confirmed in 86 species representing 39 plant families. Other ions (K^+, Mg^{++}, Na^+) serve in supporting roles to the uptake or binding of calcium. The high requirement of calcium (300–5000 ppm, as $Ca(NO_3)_2 \cdot 4H_2O$, for optimum growth) and low calcium content of most pollen may conspire to give calcium a governing role in the growth of pollen tubes both in vitro and in situ. It is suspected that ramifications of this role extend to the self-incompatibilities of plants and to the curious types of arrested tube growth distinguishing, for example, the orchids. A culture medium which proved its merit in a wide variety of pollen growth studies included, in distilled water, 10% sucrose, 100 ppm H_3BO_3, 300 ppm $Ca(NO_3)_2 \cdot 4H_2O$, 200 ppm $MgSO_4 \cdot 7H_2O$ and 100 ppm KNO_3.

THE ELONGATION of the pollen tube in flowering plants is exceedingly rapid and its requirements, in general, seem quite unimpressive, i.e., water, oxygen and a suitable osmotic milieu. Despite extensive attempts to hasten this growth process with the conventional host of growth factors, few have met with convincing success. Boron, as borate, is the only factor previously shown to enhance growth markedly in a wide range of species. Its role is completely enigmatic. It is the purpose of this report to show that calcium ion plays a similarly prominent role in pollen germination and pollen-tube growth. The role of calcium, like that of boron, remains an intriguing enigma. A preliminary report of these findings has been made (Kwack and Brewbaker, 1961).

MATERIALS AND METHODS—Several hundred flowering plant species were used in these tests. Most intensive studies were made with 2 self-incompatible species, *Petunia inflata* and *Ornithogalum virens*, which were brought to flower in controlled growth chambers. *Ornithogalum virens* ($n = 3$) was used in all studies; like many of its liliaceous relatives, it has large pollen grains, rapid pollen germination and growth, and clear-cut responses to growth constituents.

The basal medium for most studies included 10% sucrose and 100 ppm boric acid in deionized distilled water; this is referred to as the 10:100 solution. Pollen grains were sown in standing drops of 1/50 ml on cover slips. The cover slips were then placed on moist filter paper in Petri dishes, and normally left in room temperature during growth (up to 24 hr). Extensive studies were made of refinements of this technique (sterilization, humidity control, etc.), and of other techniques (Van Tieghem cells, agar smears, etc.), without exceeding its pure simplicity and consistency of success.

Most studies were made with small populations of pollen grains, *rarely exceeding 20 grains in the 1/50-ml drops.* We emphasize the importance of this procedure, for it is a sine qua non for the effects to be described. Average germination and growth data were obtained by sowing all pollen cells in at least 10 drops of each medium tested.

Population effect and the pollen growth factor—A population effect occurs whenever angiosperm pollen is grown in vitro (Brewbaker and Majumder, 1961). Small pollen populations rarely germinate well in standard sugar or sugar-agar media. However, as the numbers of pollen grains are increased in a constant amount of medium, the germination percentages and pollen tube lengths increase proportionately. The occurrence of the population effect was suggested by Brink (1924) and first demonstrated convincingly by Savelli and Caruso (1940). Subsequent confirmation has been provided by a number of investigators (see Brewbaker and Majumder, 1961, for references). Most of these studies indicated simply that a few pollen grains germinated badly, whereas, in cultures of comparable volume, large populations of grains germinated well.

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TABLE 1. *Genera in which population effect and the response to calcium have been demonstrated. Numbers of species tested, when greater than one, are given in parentheses. Pollen marked[a] were trinucleate; all others binucleate*

Family	Genera
AMARYLLIDACEAE	*Clivia, Crinum (2), Hippeastrum, Hymenocallis, Narcissus, Zephyranthes*
APOCYNACEAE	*Allamanda, Catharanthus, Nerium, Plumeria*[a]
ARACEAE	*Anthurium, Monstera, Spathiphyllum, Symplocarpus*
ASCLEPIADACEAE	*Cryptostegia*
BALSAMINACEAE	*Impatiens*
BEGONIACEAE	*Begonia*
BIGNONIACEAE	*Cydista, Tabebuia*
BOMBACAEAE	*Bombax*
BROMELIACEAE	*Ananas, Billbergia*
CAMPANULACEAE	*Campanula*
CAPRIFOLIACEAE	*Lonicera*
CARICACEAE	*Carica, Jacaratia*
COMMELINACEAE	*Tradescantia*
CRUCIFERAE	*Brassica*[a], *Matthiola*[a]
CUCURBITACEAE	*Cucumis, Lagenaria, Momordica*
EUPHORBIACEAE	*Euphorbia, Maranga, Ricinus*
GESNERIACEAE	*Saintpaulia*
GRAMINEAE	*Pennisetum*[a], *Zea*[a]
JUNCACEAE	*Luzula*[a]
LEGUMINOSAE	*Bauhinia, Crotalaria (3), Mimosa*
LILIACEAE	*Aloe, Gasteria, Haworthia, Hemerocallis, Lilium, Ornithogalum (2), Polianthes, Sansevieria, Trillium*
MALPIGHIACEAE	*Byrsonima*
MARCGRAVIACEAE	*Norantea*
MYRTACEAE	*Melaleuca (2), Psidium*
NYCTAGINACEAE	*Bougainvillea*
OLEACEAE	*Jasminium, Ligustrum*
ONAGRACEAE	*Oenothera*
PALMAE	*Cocos*
PLANTAGINACEAE	*Plantago*[a]
PRIMULACEAE	*Cyclamen*
PROTEACEAE	*Macadamia*
PUNICACEAE	*Punica*
RUBIACEAE	*Coffea, Gardenia, Paederia*[a], *Rondeletia*
RUTACEAE	*Citrus, Murraya*
SCROPHULARIACEAE	*Antirrhinum*
SOLANACEAE	*Brunfelsia, Capsicum (2), Lycopersicon, Nicotiana, Petunia, Solanum*
STERCULIACEAE	*Waltheria*
TROPAEOLACEAE	*Tropaeolum*
VERBENACEAE	*Holmskioldia*

A survey of 86 flowering plants, including 79 genera representing 39 families (Table 1), showed the pollen population effect to be essentially universal in its occurrence. Pollen germination percentages decreased markedly as the size of pollen populations was decreased. Similarly, tube growth in vitro was arrested much earlier in small than in large populations. Grains sown singly rarely germinated. While binucleate pollen germinates with ease, trinucleate pollen grains rarely germinate satisfactorily in vitro (Brewbaker and Majumder, 1959). However, the germination of trinucleate pollen grains from several species was consistent enough to permit confirmation of the population effect (Table 1).

Pollen from different species differed greatly in its response to increasing population density. The data from 4 species which more or less embrace the range encountered are summarized in Fig. 1. Germination was impaired when the population dropped below 2500 grains in some species, while with others it was not impaired until population dropped below 150 grains in the 1/50-ml drops. These differences did not correlate with differences in pollen volume, which ranged from 11,500 μ^3 (*Saintpaulia ionantha*) to 828,000 μ^3 (*Oenothera organensis*). Different responses to population density were observed for *Ornithogalum* plants grown in 2 different locations, and in tomato and other species grown under diverse field conditions. The following studies indicate that cation nutrition could play a prominent role in these differences.

Response to tissue extracts—It was suggested in studies by Visser (1955) and Brewbaker and Majumder (1961) that the pollen population effect resulted from the action of a stable, water-soluble growth factor in the pollen grains (referred to as PGF by Brewbaker and Majumder, 1961). PGF was postulated to be a highly diffusible

constituent of pollen which, by leaching into external media, could deprive the pollen of a concentration necessary for germination and growth. These suspicions were confirmed by the demonstration that cell-free extracts from pollen could overcome the population effect (Fig. 2). Extracts of petunia anthers in 10:100 solution were obtained by maceration and centrifugation, and the germination of small pollen populations recorded. Germination percentages were increased in proportion to the number of anthers extracted, attaining optimum levels with the use of 100 anthers in the 2-ml sample.

Samples of approximately 10 mg (fresh weight) from several plant tissues were macerated and centrifuged in 2 ml of the 10:100 solution. Leaf and stem extracts from a variety of plants showed full PGF activity, as did a diverse assortment of tissues, including roots, pistils and petals. These exploratory studies made it clear that PGF occurred not only in pollen grains, but essentially universally in plant tissues.

The addition of large pollen samples from one species to a small sample (ca. 10 grains) from another also enhanced the germination of the smaller population. This was first observed by Savelli and Caruso (1940) who referred to it as a "mutual stimulation effect," an effect confirmed in the present study for a random sample of 10 species. Borriss and Krolop (1955) reported inhibitory effects of extracts from composite pollen, a report we were able to confirm with certain species of *Crepis* and *Chrysanthemum*. The trinucleate pollen grains of composites have not been germinated in vitro with any consistent measure of success. It is inferred that trinucleate grains incorporate a germination inhibitor that must be overcome or removed by the stigma, a view that would complement nicely the observation that self-incompatibility occurs at the stigma surface in most species with trinucleate pollen (Brewbaker and Majumder, 1959).

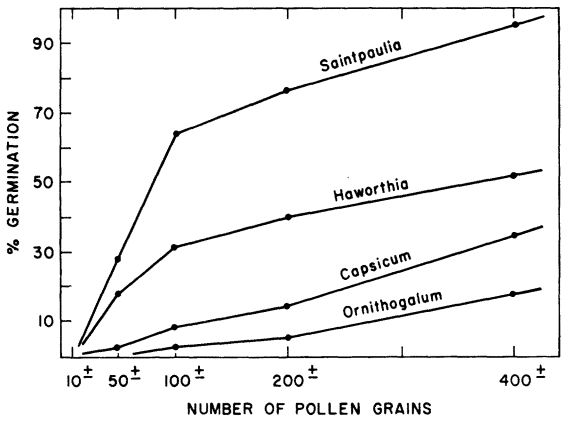

Fig. 1. Relationship of pollen germination percentages to pollen population size when cultured in 1/50-ml drops of 10:100 medium.

Increased growth of pollen tubes was reported by Brink (1924) as the result of mixing crushed stigmas, ovaries, or the extract of raw potato with culture media. The growth-enhancing effects of crushed stigmas and stigma extracts were reported for many other species in the early 1900's. It must be stressed that most of these studies involved large populations of pollen. It is debatable whether or not PGF activity, defined as the ability to stimulate germination under the limiting conditions somehow imposed by a small pollen population on its own growth, can be inferred from most of these early studies.

Response to growth constituents—A series of organic growth substances was tested on small populations of *Petunia* and *Ornithogalum* pollen. Coconut milk (optimum, 2–10%) and yeast extract (optimum, 100 ppm) showed full PGF activity (Table 2). It is noteworthy that yeast extract was among the earliest growth promoters recommended for pollen (Doroshenko, 1928). Other growth factors, including kinetin, IAA, 2,4-D, NAA, and gibberellic acid, were tested alone and in various concentrations and combinations. None of these stimulated pollen germination or elongation, nor did the following substances: casein hydrolysate, glycine, asparagine, glutamic acid, tyrosine (all tested at 100 ppm), pectin (0.1–1%), urea (10 ppm), purine- and pyrimidine-analogs (including hypoxanthine, orotic acid, cytosine, guanine, cytidylic acid and uracil [100 ppm]), and vitamins (including ascorbic acid [5 ppm], pyridoxine [2 ppm], nicotinic acid [10 ppm], thiamine [2 ppm], and biotin [2 ppm]).

The salutary effect of growth-promoting chemicals on pollen has been suggested periodically

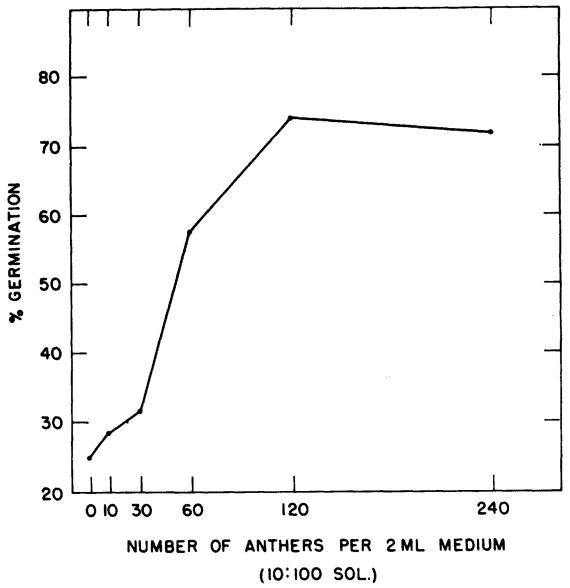

Fig. 2. Increasing germination of petunia pollen (small populations) with increasing levels of anther extract. Each value represents 33 replications.

TABLE 2. *Germination of small pollen populations (5 or more tests) of Ornithogalum virens pollen in 1/50-ml drops*

Culture medium	Percent germination
10% sucrose + 100 ppm boric acid ("10:100")	0
Pollen extract in 10:100	42.9
Plant-tissue extracts[a] in 10:100	50.1
Coconut milk in 10:100	60.2
Yeast extract in 10:100	36.5
Amino acids in 10:100	0
Vitamins in 10:100	0
Purines and pyrimidines in 10:100	0
Pollen-ash extract in 10:100	48.5
Pollen-ash extract run through cation-exchange resin, in 10:100	0

[a] Tissues included petals, pistils, stems, leaves, ovaries, and roots.

with limited supporting data. These growth substances include auxins and vitamins, amino acids, purines and gibberellin. No significant increases in germination and tube growth were observed in the present studies with any of these constituents, even though some were tested on many different species, at varying concentrations, and in combination with other growth factors. It seems likely that some of the stimulatory effects suggested in other studies occurred either as the consequence of suboptimal population size or of suboptimal borate or auxin concentrations in the pollen. Stimulation by these substances in the presence of optimal borate and calcium has not been reported.

Properties of PGF—Petunia pollen was used for the initial physico-chemical studies of PGF (Brewbaker and Majumder, 1961). These tests were extended in the present study to include various species and solvents. The solutes were added to 10:100 solution, following solvent evaporation, to make a concentration equivalent to 120 petunia anthers per 2 ml. Extracts with ethyl ether, petroleum ether, and chloroform showed no effect on the germination of small pollen populations. However, extracts with ethanol, butanol and acetone showed measurable PGF activity, although these were consistently less than water extracts. Differences in the activity of the solvents appeared to correlate directly with the miscibility of solvent with water. Paper chromatographic methods (using 4 butanol:1 acetic:3 water) were applied for various tests of organic constituents; no characteristic spots distinguished the water

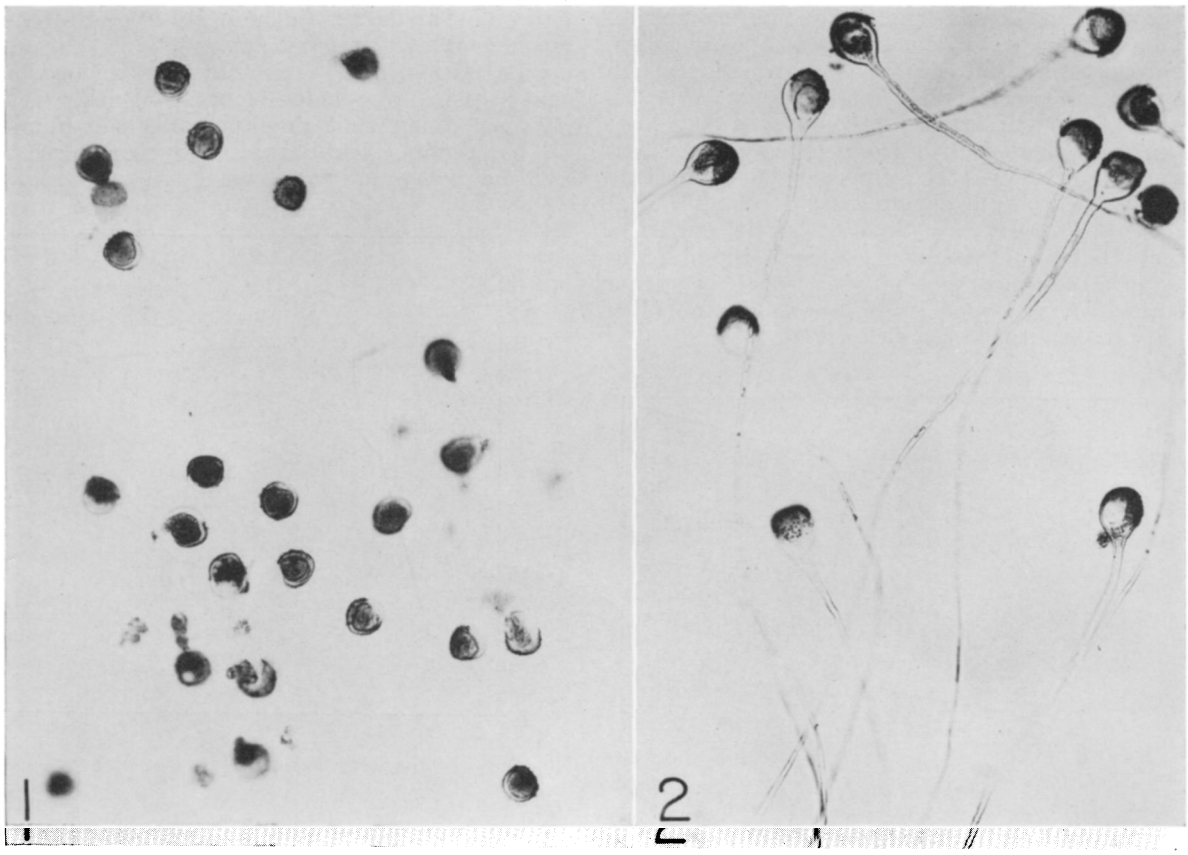

Fig. 3. The response of small populations of pollen from *Ornithogalum virens* to calcium ion. Entire pollen populations in 1/50-ml drop are shown. Medium 1 was a basic 10:100 solution supplemented with White's basic salts excluding calcium; medium 2 included calcium. Magnification × 1300.

extracts from extracts in other solvents. Elution from paper strips with water showed PGF activity to be highest in areas near the original spot, although measurable diffusion occurred.

The pollen growth factor was then tested for heat stability. Pollen extracts retained activity when maintained at 100 C for 10 min, while autoclaving for 15 min at 120 C (15 lb pressure) significantly impaired the activity. In all tests, the pH was readjusted to 5.5. The full activity of PGF was maintained upon passage through conventional dialysis membranes.

The developing contention that PGF was inorganic in nature was confirmed by tests of the ash from flamed pollen. The ash was placed in HCl, excess acid evaporated, and the pH adjusted with 5% NaOH. When this ash extract was added to the 10:100 medium, germinations of small pollen populations were increased significantly in all tests (Table 2). The PGF activity of both *Petunia* and *Ornithogalum* pollen-ash extracts disappeared completely upon passage through the cation exchange resin, Dowex 50W–X8. It was apparent at this stage that, in all probability, PGF was an inorganic cation.

PGF identified: The calcium ion—The preceding studies indicated that PGF was heat stable and water soluble and that its activity was retained in pollen ash but lost when extracts were filtered through cation-exchange resin columns. Successive tests of the common salts in 10:100 solution were made, and none of the salts alone showed PGF activity. When, however, the combined salts of White's inorganic medium were provided en masse, the population effect was overcome. PGF thus appeared to be not one, but several, inorganic salts or ions. At optimum concentration, these salts included $Ca(NO_3)_2 \cdot 4H_2O$ (288 ppm), Na_2SO_4 (200 ppm), KCl (80 ppm), $NaH_2PO_4 \cdot H_2O$ (19 ppm), $MnSO_4 \cdot H_2O$ (5 ppm), KI (0.75 ppm) and $MgSO_4 \cdot 7H_2O$ (737 ppm).

Tests of the deletion type were then made. Single salts were successively omitted from the basic inorganic medium. Germination was excellent in each case except one; *growth was inhibited fully in the absence of calcium.* Pollen germination and growth were excellent whenever almost any soluble calcium salt was used, over a wide range from 50 to 5000 ppm (Fig. 3). These tests included samples of calcium chloride and nitrate, acetate and pantothenate, as well as calcium EDTA. No differences could be shown for any of the common anions tested.

Although calcium could not be omitted from any medium without impairing germination of the small pollen samples, it was evident that other ions needed to be present to permit calcium activity. By successive deletion of the basic salts from White's formula, it was shown that the ions K, Na or Mg, singly or together, could act to enhance calcium activity (Table 3). In the absence of all 3 ions, calcium was not active in overcoming the population effect. Optimum germination occurred in the presence of K^+ and Mg^{++} as supporting ions for the calcium effect.

TABLE 3. *Germination of small populations of Ornithogalum virens pollen in cation-supplemented solutions (see Table 2)*

Culture medium[a]	Percent germination
10% sucrose + 100 ppm boric ("10:100")	0
Ca alone in 10:100	0
K alone in 10:100	0
Mg alone in 10:100	0
Ca + K in 10:100	33.3
Ca + Mg in 10:100	34.0
Ca + K + Mg in 10:100	50.5
Ca + K + Mg + Na + Mn + Zn in 10:100	60.8
K + Mg + Na + Zn in 10:100	0
Ca + K + Mg in glycerol:boric	60.0
Ca + K + Mg in lactose:boric	54.7
Ca + K + Mg in *l*-sorbose:boric	0
Ca + K + Mg in sucrose but no boric	0
Ca + K + Mg in boric but no sucrose	0
Ca + K + Mg through cation-exchange resin in 10:100	0
Sr + K + Mg in 10:100	0
Ba + K + Mg in 10:100	0

[a] Ca commonly added as 300 ppm $Ca(NO_3)_2 \cdot 4H_2O$, K as 100 ppm KNO_3 and Mg as 200 ppm $MgSO_4 \cdot 7H_2O$.

A series of studies was undertaken to test the interaction of calcium with the cations, Mg^{++} and K^+. A basal medium was prepared, comprising 300 ppm Ca $(NO_3)_2 \cdot 4H_2O$, 200 ppm $MgSO_4 \cdot 7H_2O$ and 100 KNO_3 in 10:100 solution. The concentration of each salt was varied independently of the others. The salts showed a broad threshold in action, depressing germination (in the presence of 300 ppm calcium nitrate) below 10 ppm and above 1000 ppm, depending upon the species. Increasing calcium concentration resulted in essentially logarithmic increase in germination for the 2 plants, *Petunia* and *Ornithogalum*, on which extensive data were taken. Some depression in germination could be observed at the very high level of 5000 ppm Ca^{++}, although growth remained surprisingly good at 10,000 ppm (in contrast, ca. 0.01 *M* Ca^{++} inhibits stem apex elongation). In these, as in other studies, tube growth was equally if not more sensitive than germination. The effects of increasing calcium were also apparent in the rigidity and straightness of pollen tubes, with little or no coiling observed at high levels of calcium.

A basal medium for all subsequent research was chosen as the result of these studies. This included, in distilled water:

10% sucrose
100 ppm H_3BO_3
300 ppm $Ca(NO_3)_2 \cdot 4H_2O$
200 ppm $MgSO_4 \cdot 7H_2O$
100 ppm KNO_3

The addition of agar to this medium did not increase germination in any species tested, although it permitted longer tube growth with some pollen types. Many cultural methods were tried, and the simplest proved to be the best, i.e., placing medium in a standing drop on a cover slip on moist filter paper in a Petri dish. Except for the uncooperative trinucleate pollen types, pollen germination under these circumstances rarely fell below 75%. It is intriguing that the concentration of sugar is most often altered in attempts to improve pollen germination, while such changes least often were found to be of value in these studies when calcium was present. Lactose, reducing sugars and glycerin substituted adequately for sucrose over a wide range of concentrations. Generally, sugar concentrations above 40% impaired growth measurably, while those below 2% led to increased bursting of pollen and tubes (cf. Brewbaker and Majumder, 1961).

Strontium and barium were unable to replace calcium as a pollen growth factor. $Sr(NO_3)_2$ and $Ba(NO_3)_2$ were added independently to 10:100 solutions containing the Mg^{++} and K^+ ions in standard amounts, and no germination was obtained unless Ca^{++} ion was added (Table 3). The calcium ion was not able to promote germination when boric acid was withheld, and while sucrose activity could be replaced by non-nutrient lactose, glycerol, and reduced sugars, the boron activity could not be replaced, for example, by GeO_2 or by $Sr(NO_3)_2$.

The universality of the calcium effect was confirmed by testing the growth in small pollen populations of all 86 species (Table 1). Pollen was cultured in 10:100 solutions supplemented with Mg^{++} and K^+ ions, with or without added Ca^{++} ion. The dramatic effect of calcium ion on germination and tube lengths in the test species, *Ornithogalum virens* (Fig. 3), was duplicated in many species (Table 1). Tests to be reported in detail elsewhere were made with Ca^{45}-labeled salts. Radioautographs showed calcium to be distributed uniformly along the pollen tube walls, and essentially no label appeared in the cytoplasm from burst tubes.

Discussion—Calcium is widespread and abundant in most flowering plant tissues, averaging around 1.5% of the dry weights of leaves, 1.2% in shoots and 0.2% in seeds. Pollen grains are also low in calcium content, averaging about 0.03% (Todd and Bretherick, 1942). It is interesting to note that gymnosperm pollen, with its unhurried growth and an ability to produce tissue cultures (Tulecke, 1959), appears to have lower calcium contents than angiosperm pollen, ranging between 0.03% and 0.04% for the 3 species studied by Todd and Bretherick. Many pollen grains (e.g., *Impatiens, Ornithogalum, Anthurium* spp.) are shed in the company of abundant, needlelike crystals of calcium oxalate. Although these crystals are readily dissolved below pH 3, this calcium is essentially unavailable in situ. Tests of the relative amounts of bound and exchangeable calcium (cf. Thimann and Takahashi, 1961, p. 363–380) in pollen have not been made.

Many important roles of calcium in plant growth have been demonstrated. Among these, the role of calcium in relation to pectin seems the most pertinent to studies of both pollen and root growth. Burström (1952) and others have shown calcium ion to be a major regulator of root and root hair growth. There is a general similarity in growth of root hairs and pollen tubes. Both develop unidimensionally, elongating rapidly at the tip and bursting at the tip under osmotic stress. Both respond markedly to exogenous calcium and borate, and give no convincing response to auxins. In each system, the secondary cell wall and middle lamellae are absent, while callose is present; lignin is also absent, or present in only minor amounts, in pollen and root hairs. In both pollen and root activities, borate plays an important but puzzling role. Although definite quantitative relationships occur between calcium and borate in plant tissues (Marsh and Shive, 1941), borate does not simply increase the rate of calcium uptake by either root hairs or pollen tubes. In direct contrast to elongating root or pollen cells, the multidimensional enlargement of stem apex cells is inhibited by calcium or borate concentrations of but a fraction of those optimal for pollen tube elongation. Calcium binding by pectate molecules in the middle lamella has been held to account for the inhibition of stem growth by high levels of calcium (Cooil and Bonner, 1957 .

It seems probable that the improvement in germination and growth of pollen due to calcium relates primarily to the binding of calcium to pectate carboxyl groups along the pollen wall. Ca^{45}-radioautographic studies (Kwack, unpublished) suggest that calcium is incorporated nonmetabolically, in exchangeable form. The interactions observed here between Ca^{++} and other ions is reminiscent of those detailed by Epstein (1961) and by Higinbotham, Pratt, and Foster (1962) for monovalent ion absorption by root and stem tissues, as well as by Couey and Smith (1961) and others working with rusts and other fungal spores. In the pollen studies Ca^{++} could not be replaced by strontium or barium, and ions such as K^+, Mg^{++}, Na^+ could be used interchangeably to enhance the calcium effect. Cationic equilibria involving calcium are requisite to the normal permeability and selectivity properties of roots. Although these properties must not be excluded in considerations of pollen growth, yet the tubes do not appear to require exogenous cations such as K^+ or Mg^{++} for growth. Rather, these cations may be viewed as maximizing the association of Ca^{++} in the cell wall, which in turn lends structural rigidity and physiological properties such as permeability and ion selectivity to the wall. In any speculations concerning the roles of calcium,

however, the metabolism of carbohydrates such as cellulose, callose and pectin must be considered (Ekdahl, 1957). A role of Ca^{++} in metallic enzyme activity is also probable, perhaps even in a type of incompatibility enzyme as that proposed by Mäkinen and Lewis (1962).

The calcium effect presents new approaches to questions raised by several fertilization and incompatibility phenomena in plants (cf. Brewbaker and Majumder, 1961). Incompatibility (S) alleles appear to act via immunochemical reactions which impair pollen germination or tube growth. The interaction of S allelic protein and antiprotein in pollen and style, respectively, occurs in all probability at the surface of pollen intine or tube. Self- and cross-incompatibility reactions could inhibit growth by impairing calcium binding to pectic substances in these walls. Studies of calcium's interaction with pectinase and other enzymes, metallic poisons, and a variety of growth inhibitors, to be reported in a subsequent paper, support this contention. The temporary arrest of orchid and other pollen tube growth during maturation of the embryo sac, often for periods of months, with subsequent resumption of elongation leading to fertilization is a botanical enigma. Arrested tube elongation in these and other instances might be considered the possible result of arrested calcium supply to pollen tubes during ovular development. The probability must also be asserted that calcium gradients encourage pollen tubes "chemotropically" toward the embryo sac (Mascarenhas and Machlis, 1962). Similarly, calcium deficiency may play an important role in natural pollen mutation rates, as well as in the increasing rates of mutation during prolonged pollen storage (Brewbaker and Emery, 1961).

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