THE BOTANICAL REVIEW

VOL. 27 JULY-SEPTEMBER, 1961 NO. 3

PHYSIOLOGY OF POLLEN*

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*The survey of literature was completed in December, 1960.

STORAGE

INTRODUCTION

Horticulturists and plant breeders have long been interested in crossing varieties, species and even genera to produce new and improved types of plants better suited to human requirements. However, many of these attempts have failed, due to the barriers to crossability, e.g., flowering of the selected parents at different times or at distant places, failure of the pollen grains to germinate on the stigma, bursting of pollen tubes in the style, failure of tubes to grow through the style, and slow growth of the tubes so that they do not reach the ovules before abscission of the flower. Occasionally the pollen tubes no doubt enter the embryo sac, but sterility results either from failure of the male gamete to fuse with the egg nucleus or from subsequent failure or arrested development of the embryo and the endosperm (see Blakeslee, 1945; Maheshwari, 1950).

Nowadays it is possible to store viable pollen; therefore the difference in the time of flowering of the parents does not hold up breeding programmes. The ease with which stored pollen can be transported long distances without loss of viability has partly solved the problem of the parents occurring in different regions. "The maintenance of pollen viability in a light weight container, and in an otherwise uncontrolled environment is of particular advantage in the long-distance shipment of pollens. Such shipments today require costly packaging and special precautions for maintaining viability enroute. Loblolly pine (*Pinus taeda*) pollen was shipped to New Zealand and return and to Brazil and return, unopened. Each sample, packed in "Starfoam," weighed slightly over one ounce when shipped, and each had lain on a laboratory shelf for nearly two months before shipment. Both packages, and their enclosed samples, returned in excellent condition having been enroute 26 and 29 days respectively" (King, 1959). Moreover, transport of pollen by air, land or sea is not controlled by plant protection and quarantine regulations.

The earliest reference on the handling and storage of pollen concerns the date palm. Zirkle (1935) states that records of certain business contracts of the Hammurabi Period indicate that male inflorescences of the date palm were an important commodity of commerce as early as 2000 B.C. However, we have no information about the methods of storage, but it is unlikely that the stored pollen retained viability in the excessively low humidity and high temperature prevalent in those areas (Iran, Iraq, etc.).

Popenoe (1913) points out that "if date pollen is kept dry it preserved its value for a long time, and in some date-growing communities it is the custom to have a small supply from each year to the next . . . A pollination made in 1912 at the Mecca Experiment Station with pollen seven years old, sent from the Temple Garden, was entirely successful." Some other reports (see Nebel and Ruttle, 1937) also indicate that pollen is capable of bringing about fertilization even after long periods.

Stout (1924), on the other hand, writes: "It remains to be shown whether date pollen can ever be kept viable from one season until the next season of bloom. The evidence at hand rather decidedly indicates that it is not and perhaps cannot be thus kept." In this connection attention may be drawn to the conflicting reports of Albert (1930) and Crawford (1937). Whereas the former was able to obtain a moderate fruit set in the following season after using the pollen of the previous year stored at room temperature, Crawford (1937) failed to get any such result with similar pollen.

The longevity of stored pollen has been investigated mainly in relation to relative humidity, temperature, effect of diluents and nutrient media (see Holman and Brubaker, 1926; Nebel and Ruttle, 1937; Visser, 1955; Vasil, 1958a, 1961; Johri and Vasil, 1960). These and some other related topics will now be considered.

EFFECT OF HUMIDITY AND TEMPERATURE

A systematic study on the storage of pollen was initiated towards the end of the nineteenth century when Mangin (1886), Rittinghaus (1886) and Molisch (1893) investigated the longevity of pollen of more than 80 species stored under air-dry conditions (exposed to room atmosphere). Pollen of tomato survives for four days under laboratory conditions (?) and gives normal fruits; older pollen causes reduced fruit-size (Gorobec, 1958). The pollen of several species remains viable for a longer period at lower temperatures than at higher (see Goff, 1901; Sandsten, 1909; Roemer, 1915). Both Sandsten (1909) and Roemer (1915) observed a longer viability under dry conditions. In 1922 Knowlton stored the pollen of *Antirrhinum* at five temperatures and concluded that the lower the temperature the longer the viability.

Pfundt (1910) investigated the effect of 0, 30, 60, and 90 per cent

relative humidity (R.H.) on the viability of the pollen of 140 species at 17°–22°C. From his observations it is evident that the maximum longevity was obtained at low relative humidities (0–30 per cent). His results were later confirmed by Holman and Brubaker (1926) who tested the viability of stored pollen of another 52 species at 17° –22°C, and at 0, 27, 63 and 92 per cent R.H. They concluded that "storage at low humidities triples on the average the longevity of those pollens which it affects." These authors have also reviewed the pertinent literature on this subject and have compiled data on the pollen longevity of 231 species belonging to 175 genera and 23 families.

Horsford (1918) reported a longevity of about one year in lily, and Manaresi (1924) observed a similar behaviour in apple, pear, grape and plum. Since then the pollen of numerous species has been reported to retain longevity for one year or even longer when stored at 0°–10°C and 10–50 per cent R.H. In this connection attention is called to the works of Nebel and Ruttle (1937), King and Hesse (1938) and Nebel (1939) on the pollen of several fruit trees; Pfeiffer (1936, 1938, 1944) on *Amaryllis, Cinchona* and *Lilium*; Gollmick (1942) and Olmo (1942) on *Vitis;* and Stone et al. (1943) on *Pistacia.*

In contrast to the relatively long viability in the above plants, the pollen of the Gramineae is extremely short-lived and the range of humidity (0–40 per cent), which is so favourable to most other pollens, is decidedly harmful. As a rule, the pollen of grasses remains viable only for a few days, sometimes one to three weeks, when stored at 0°–10°C and 80–100 per cent R.H. This is evident from the work of Pfundt (1910) and Andronescu (1915) on *Zea mays* and several other grasses; Firbas (1922) on rye and wheat; Anthony and Harlan (1920) and Pope (1939) on barley; Knowlton (1922), Sartoris (1942), Jones and Newell (1948), Bergh (1952) and Liefstingh (1953) on maize; Sartoris (1942) on sugarcane; and Jones and Newell (1948) on buffalo-grass (*Buchloë dactyloides*). This short life-span may be due to desiccation during storage.

Pfundt (1910) reports that the pollen of *Prunus padus* remains viable for 15 days at 90 per cent, 22 days at 60 per cent and 181 days at 30 or 0 per cent R.H. Similarly, the pollen of *P. avium* retains viability for 102 days at 30 per cent R.H., 126 days at 0 per cent, but only 28 days when stored at laboratory temperature and uncontrolled humidity. According to Holman and Brubaker (1926), in none of their samples did the longevity of air-dry pollen exceed that of those stored over lower humidities. On an average, 27.2 per cent R.H. appeared to be slightly better than 0.005 per cent and much better than 94.2 and 63 per cent. The maximum longevity of 336 days in *Typha latifolia* was obtained when the pollen was stored at 17°–22°C over 0 per cent R.H. On the other hand, Knowlton (1922) mentions that the maximum longevity of maize is obtained at 50–80 per cent R.H. He concluded that desiccation appears to be one of the important factors that causes early death of pollen during storage. Nebel and Ruttle (1937) also state that "for the pollen of the species and varieties of *Prunus, Pyrus* and *Vitis* tested, a humidity of 50 per cent is possibly the optimum, the life curves becoming increasingly shorter and steeper as humidity is further increased and becoming shorter as humidity is further decreased." Again, according to Pfeiffer (1936), there is an intimate correlation between the longevity of pollen and moisture content of the air, with maxima and minima of different pollens at different humidities.

Visser (1955) points out that the longevity of pollen is, in general, negatively correlated with the relative humidity required for optimal storage. For instance, it is only at high humidity (80–100 per cent R.H.) that the pollen of the Gramineae retains its viability; and that, too, for only a short period. Daniel (1955) reports that the pollen of *Zea mays* remains viable for ten days at 7°C and 50–70 per cent R.H. Lower temperatures (–5° and –20°C) and less than 50 per cent humidity was detrimental. But the opposite holds true for the pollen of *Prunus* and *Pyrus* species which have a far greater viability at low humidities (30–50 per cent R.H.)

During the last seven years, investigations carried out at the University of Delhi indicate that the pollen of certain crop plants—*Arachis hypogaea, Brassica nigra, Pennisetum typhoideum, Solanum melongena* and *S. tuberosum*—shows maximum viability at 0 or 31–40 per cent R.H. Sometimes, especially at low temperatures, 50–60 per cent R.H. is also quite satisfactory (Vasil, 1958a, 1961).

Whereas over low humidities (0-40 per cent R.H.) the stored pollen remains viable for long periods, at high humidities (60 per cent R.H. and above) it is often attacked by bacteria and fungi which render the samples unfit for further use (Vasil, 1958a). Nebel and Ruttle (1937) also noticed the growth of mould in their pollen samples stored over 100 per cent R.H.

There is some recovery of the germinating capacity of *Cinchona* pollen upon transfer to favourable conditions of temperature and humidity after very dry conditions at room temperature (Pfeiffer, 1944; see also Nebel and Ruttle, 1937; Pfeiffer, 1955).

When the relative humidity is allowed to fluctuate frequently during storage, viability is lost quickly (Bullock and Snyder, 1946). It appears that pollen cannot withstand extreme variations in its environment.

As early as 1901, Goff (see also Sandstein, 1909) stated that in several fruit plants the longevity is not impaired after a short exposure to sub-zero temperatures. Griggs et al. (1953) have also shown that pollen can be stored for long periods in cotton-plugged vials in a home freezer (temperature close to 0°F) without any control of humidity. Under such conditions, the viability of hand-collected almond pollen was retained for 801 days, while bee-collected pollen remained viable up to 1130 days. Pollen of *Poncirus trifoliata* retained viability for 61 days over calcium chloride at 4°C (Soost and Cameron, 1954). Pollination with 36-day old stored pollen yielded fruits and viable seeds and the latter produced normal, healthy seedlings. In the same plant, Sawano (1954) reported a viability of 110 days over calcium chloride but nothing is known about the temperature at which pollen was stored. Pollen of eight species of *Nicotiana* stored at –5°C and 50 per cent R.H. for one year germinated almost as well as fresh pollen (Daniel, 1955). A temperature of 7°C with 18 per cent R.H. had a slightly adverse effect, while a humidity of 100 per cent proved definitely harmful. According to Knowlton (1922), the pollen of *Antirrhinum* remained viable longest at –18° to –30°C, and no reduction in its viability was noticed even when exposed for half an hour to a temperature of –190°C (liquid oxygen). The pollen of *Lilium* and *Amaryllis* (Pfeiffer, 1936, 1938), date (Crawford, 1937), grape (Olmo, 1942) and many other fruit plants (Antles, 1951; Griggs et al., 1953; Visser, 1955) can be stored for long periods at deep-freeze temperatures (ca –20°C) without being adversely affected. With similar conditions of storage, Ushirozawa and Shibukawa (1951) noticed a slight germination in the pollen of many fruit species even after nine years which is the longest viability so far recorded.

The pollen of apple and pear can be satisfactorily stored over dry ice (solid carbon dioxide) at –55° to –60°C (see Griggs et al., 1950; Antles, 1951). Bredemann et al. (1947) stored the pollen of *Lupinus* at –180°C (liquid air), and Visser (1951, 1955) stored the pollen of

apple, pear and tomato at –190°C. Pollen grains of apple stored for nine months in small, closed, glass vessels at –15°C showed 95 per cent germination which was as good as in fresh samples (Tupý, 1960). Visser (1955) reports that apple pollen, after being stored at –190°C, "germinated equally well and induced as good a fruit set after two years . . . as freshly collected pollen. Evidently the time 'stands still' for pollen when stored at –190°C." Bredemann et al. concluded that the viability of the pollen of *Lupinus* would remain unaffected for millions of years if stored over liquid air. Visser (1955) also points out that pollen stored at such low temperatures "can be given 'eternal life'," provided that it is resistant. However, contrary to general expectation, the pollen of maize shows a sharp decline in viabilty when stored at deep-freeze temperatures (Knowlton, 1922; Liefstingh, 1953).

INFLUENCE OF GASES AND PRESSURE

Besides temperature and humidity, certain other atmospheric conditions also affect the viability of pollen. According to Kellerman (1915), the pollen of *Citrus* retained a high percentage of germination when pre-dried and shipped under vacuum. In *Lilium* (Pfeiffer, 1936), apple and pear (Visser, 1955), reduced air pressure prolongs the viability. On the contrary the pollen grains of barley (Anthony and Harlan, 1920), *Antirrhinum* (Knowlton, 1922), sugarcane (Sartoris, 1942) and *Cinchona* (Pfeiffer, 1944) remain viable longer at normal than at reduced air pressure. High concentrations of carbon dioxide (this is automatically achieved when the pollens are stored over dry ice) also increase longevity (Knowlton, 1922; Antles, 1951), whereas storage in pure oxygen is less favourable (Knowlton, 1922). Pollen of *Pinus taeda* remains viable for 99 days when sealed in nitrogen gas following freeze-dry treatment and stored in an otherwise uncontrolled environment (King, 1959). Normal and fresh pollen of *Citrus* shows only 50 per cent germination. It loses viability more quickly in light than in dark. Longevity can, however, be increased by storing at high carbon dioxide concentrations (Resnik, 1958).

EFFECTS OF DILUENTS

The effects of various diluents (finely powdered dry materials mixed with the pollen) on the storage and viability of pollen has been studied by Pfeiffer (1948), Overley and Bullock (1947) and others. In re-

cent years, in order to minimise the deterioration, particularly due to desiccation, it has become usual practice (Antles, 1951) to use diluents during storage and transport of pollen. The useful effect of the diluent may be due to its regulating capacity on moisture and air around the pollen grains (Bullock and Overley, 1949).

There are over 30 different substances which have been tried as diluents; the more commonly used are lycopodium powder, powdered egg albumen, casein and talc (see Overley and Bullock, 1947; Pfeiffer, 1948; Bullock and Overley, 1949; Bullock and Snyder, 1946; Antles, 1951). The diluents can be used effectively only on dry and powdery pollen, since with sticky pollens they form large clumps and increase the possibility of their bursting. In high humidity (over 55 per cent) some of the diluents (eg., egg albumen) absorb moisture and become sticky, resulting in profuse bursting.

Diluents which adhere to the pollen reduce their viability and cause a significant decrease in fruit set (Bullock and Snyder, 1946). Antles (1951) states that when the electrostatic charge of the diluent material was opposite to that of the pollen grains, the diluting material gathered around the pollen grains, sometimes causing them to group. This resulted in less possibility for contact with the pistil of the flower during artificial pollination.

It may be of interest that at present diluents are widely used to increase the bulk of pollen in order to reduce wastage during artificial pollination programmes.

BURSTING OF POLLEN AS A TEST OF VIABILITY

Andronescu (1915) and Kearney and Harrison (1932) describe the bursting of pollen in nutrient solutions as 'pseudo-germination' and 'ejection.' They interpreted this behaviour for determining the viability. Kearney and Harrison (1932) report that the percentage of viability calculated on the basis of 'ejection' was confirmed after artificial pollination and the percentage of seed set. In *Gossypium herbaceum* and *Pennisetum typhoideum* the pollen fails to germinate even after short periods of storage. However, in the latter plant prominent protrusions are formed up to 186 days and the pollen grains burst when placed in the nutrient medium (Vasil, 1958a 1961). In both plants, the stored pollen showed gradual reduction in the percentage of bursting and protrusions. This is comparable to the gradual reduction in the percentage of germination of stored pollen in other plants.

The evidence presented above leads to the conclusion that the stored pollen which fails to germinate in vitro or only bursts and forms protrusions may not always be non-viable, and if used for pollination, it may give satisfactory fruit or seed set.

The viability of pollen is tested in different ways, but the commonest and most reliable are in the vitro and in vivo germination tests. Occasionally 2,3, 5-tetrazolium chloride (Vieitzev, 1952) is also used but this can be done only in pollen with a thin and colorless exine. Recently King (1960) determined viability on the basis of peroxidase reaction. Acetocarmine is sometimes utilised for this purpose. Bajpai and Lal (1958) considered pollens of several crop plants to be viable as long as they stained with acetocarmine. Unfortunately this criterion is not dependable, since it is well known that fresh as well as preserved pollens, irrespective of whether they are viable or non-viable, often stain alike with acetocarmine (Vasil, 1958c; see also King, 1955). The staining capacity depends not on the viability but on the contents of pollen grains. At times, even herbarium specimens (dead for many years!) have been used to study the nuclei in pollen grains (Leitner, 1938; Khosoo, 1956). Leitner (1938) states that the pollen of *Lolium perenne* taken from herbarium material stained normally, although it is known to lose viability after one day.

ERRATIC GERMINATION OF STORED POLLEN

A major source of difficulty to those engaged in the storage of pollen is the erratic germination of stored samples (see Nebel and Ruttle, 1937). Quite often even if pollen fails to germinate in one or two previous tests, it may give a high percentage of germination in subsequent tests. In some other cases, the percentage of germination after storage for a few days is higher than that of fresh pollen. Such irregularities are "generally found when the pollen is collected from apparently equally mature flowers on different days or in different, though neighbouring, localities, and even when it is taken from flowers at the same age in a given locality and at about the same time" (Holman and Brubaker, 1926). According to Nebel and Ruttle (1937), "these variations may be due to lack of uniformity in the sample, but other unknown factors may possibly be involved."

NUTRITIONAL REQUIREMENTS OF STORED POLLEN AND CAUSES OF LOSS OF VIABILITY

For optimum germination and maximum tube length, the fresh pollen of *Pennisetum typhoideum* vars. T.55, I.C. 1472 and T.25, and of *Arachis hypogaea* var. TMV.2, 115 requires 12.5, 25 and 10 per cent sucrose, respectively; stored pollen of these plants requires 15, 27.5 and 12.5 per cent sucrose (Vasil, 1958a, 1961). Other workers have also observed that, compared to fresh pollen, stored pollen requires higher concentrations of sugar for normal germination.

Kühlwein (1937) made similar observations in some gymnosperms. According to Kühlwein and Anhaeusser (1951), the fresh pollen of *Ceratozamia, Pinus* and *Picea* collected in June gave optimum germination in two per cent sucrose. In the middle of December, stored pollen required 20 per cent sucrose for optimum germination. With the ageing of pollen, from June to August and later to December, higher and higher concentrations of sugar were needed. This behaviour was attributed by the authors to decrease in the permeability of the pollen. Further, there also appears to be a correlation between high sugar concentration and intensity of respiration, which is usually lower in aged pollen. Aged pollen is like a resting cell; to stimulate it to germinate, higher concentrations of sugar are necessary. Visser (1955) has shown that even the boron-sensitivity of pollen increases with age.

As a rule, in most plants pollen loses viability soon after dehiscence of the anthers, especially where temperature and humidity are unusually high. Occasionally viability may be retained for some time even under natural conditions. In *Hordeum* and *Oryza,* fertilization cannot be secured with certainty unless the pollen is transferred directly from the anther to the stigma (Anthony and Harlan, 1920; Nagao and Takano, 1938). There is no seed formation in *Sorghum* when pollinated with five-hours-old pollen (Stephens and Quinby, 1934). Pollen of *Paspalum dilatatum* does not show any germination 30 minutes after dehiscence of the anthers (Bennett, 1959). Similarly the pollen of *Zea mays* stored in pollinating bags in direct sunlight, at 46°C, remained viable for only three hours. On the other hand, pollens of certain fruit trees, e.g., apple, pear and plum (also those of some gymnosperms), retain viability for several weeks or even months.

Naturally the question arises: What are the causes which lead to the loss of viability? It is well known that stored pollen usually becomes dry and shrivelled, and shows poor germination or none at all. However, it shows better results if exposed to higher humidities for some time before germination and is supplied with higher concentrations of sucrose than those required by fresh pollen. The probable causes of the loss of viability may, therefore, be desiccation, utilization of reserve food and inactivation of enzymes, causing failure of metabolic processes which may be responsible for germination, both in vivo and in vitro (see also Nebel and Ruttle, 1937). So far there is no conclusive proof to support these assumptions, but some indirect evidence has no doubt been put forward from time to time.

Nielsen (1956) reports that the pantothenic acid content of the pollen of *Zea mays, Alnus glutinosa, A. incana* and *Pinus montana* shows a substantial decrease after a year's storage. Pantothenic acid is a vital constituent of coenzyme A which is of paramount importance in respiration and general metabolism of plants. Thus it is likely that its loss during storage, along with the loss or inactivation of certain other vital systems, may be responsible for the failure of germination (Vasil, 1958a, 1961). Further, changes in the color of the oil sticking on the exine of *Lilium* pollen seem to be accompanied by the loss of viability (Pfeiffer, 1955). Knowlton (1922) has studied the correlation between ageing of pollen and loss of moisture, respiration, food depletion, and reduction in the quantity of certain enzymes.

The present state of our knowledge does not justify any definite conclusion, and extensive research work on the chemical composition of fresh and stored pollen grains may be helpful.

CORRELATION BETWEEN VIABILITY OF POLLEN AND FRUIT SET

Quite often stored pollen does not germinate in vitro but produces a satisfactory fruit set in vivo. Occasionally, there may be a poor fruit set or none at all, even if the pollen otherwise gives a moderate germination in vitro (see Sandsten, 1909; Knowlton, 1922; Gollmick, 1942). Failure of germination in vitro may probably be due to the deficiencies caused during storage which are later compensated by the stigmatic and stylar tissues at the time of germination. In support of this view can be cited the work on *Streptocarpus* (Roemer, 1915), date (Albert, 1930), *Pistacia* (Stone et al., 1943), grape (Olmo, 1942), tobacco (Hagiaya, 1949) and tomato (Visser, 1955), where stored pollen fails to germinate or the germination is very poor (sometimes as low as five

per cent) but the fruit set is fairly satisfactory. Pollen of potato stored at –30°F for 7, 12 and 13 months gave no germination or a very poor germination in culture media but when used for artificial pollinations it gave very satisfactory fruit and seed set. Thus, absence of germination in vitro does not necessarily imply that the stored pollen is dead or too weak for field use (King, 1955).

Holman and Brubaker (1926) also believed that pollen which is "incapable of germinating in artificial media may be stimulated by some substances in the stigma to form a tube, and perhaps under favourable conditions, bring about fertilization." Knowlton (1922) had earlier shown that *Antirrhinum* pollen, which remains viable for 670 days, will not germinate in artificial media after 180 days unless a piece of the stigma is placed in the medium. Stored apple pollen which failed to germinate otherwise was induced to give 40 per cent germination in vitro after being kept over 80 per cent R.H. for one week.

Stored pollen is of prime importance to plant breeders, horticulturists and others who use it for large-scale articial pollinations in the field. Whether or not the stored pollen will give a fruit set comparable with that of fresh pollen requires serious consideration. Overley and Bullock (1947) found that apple pollen, which gave 35 per cent germination in vitro produced satisfactory field results. On the basis of pollinations with stored pollen of apple and pear, Visser (1955) suggests the relationships in Table I between percentage of germination in vitro and fruit set:

TABLE I

Germination	Fruit set
Less than 20 per cent	Nil or poor
20 to 40 per cent	Poor to moderate
40 to 60 per cent	Moderate to normal
Over 60 per cent	Normal

However, Visser (1955) states that his "experiments with apple and pear have shown that pollen mixtures containing 98-99% dead pollen and 1-2% living pollen still gave moderate fruit set, provided comparatively large amounts were used for pollination."

In general, pollen grains showing 30–60 per cent germination in culture media produce a normal fruit set. The above investigations are of immense importance, and it would be worthwhile to extend our knowledge, particularly in relation to plants of economic importance.

LONGEVITY OF POLLEN AND TAXONOMIC RELATIONSHIP

By comparing the longevities of different pollens Pfundt (1910) attempted to conclude the taxonomic relationships of the various species studied by him. He pointed out that the more closely related the species, the greater the agreement in the longevity of their pollen. He further stated that "the longevity under the most favourable conditions is ordinarily uniformly long or short in the species of a given circle of relationship, short for example in the Helobiae, Gramineae, Polygonales and Opuntiales, and long in the Pinaceae, Pandanales, Salicaceae, Fagales, Rosales and Primulaceae." Holman and Brubaker (1926), who have dealt with this question in some detail, remarked that "with a few exceptions the conclusions drawn from his (Pfundt, 1910) data are not very convincing." On the basis of the mean longevity of air-dry pollen, they arranged the families as in Table II.

TABLE II

Rank	Family	Longevity in days
1	Amaryllidaceae	38
2	Primulaceae	34
3	Rosaceae	31
4	Leguminosae	28
5	Saxifragaceae	27
6	Ranunculaceae	25
7	Liliaceae	23
8	Salicaceae	21
9	Scrophulariaceae	19
10	Gramineae	1

On the other hand, on the basis of mean longevity of pollen depending on favourable humidity, the ranking was as in Table III.

TABLE III

Rank	Family	Longevity in days
1	Primulaceae	119
2	Leguminosae	115
3	Saxifragaceae	105
4	Rosaceae	101
5	Liliaceae	86
6	Amaryllidaceae	75
7	Salicaceae	71
8	Ranunculaceae	70
9	Scrophulariaceae	69
10	Gramineae	1

While the pollen grains of most of the Gramineae either show very poor germination or fail to germinate in artificial nutrient media, *Pennisetum typhoideum* is an exception (Vasil, 1958a, 1960b, 1961). In this plant (vars. T. 55,I.C.1472 and T.25) the pollen remained viable for 186 days over concentrated sulphuric acid (0 per cent R.H.) at 16°–35°C. Over other humidities, too, they were viable for similar periods, but at higher humidities (60 per cent and above) viability was limited to only five days. The percentage of in vitro germination (78 per cent) and tube lengths (4,320 microns) were also excellent.

From the foregoing account it is obvious that under low temperature and humidity, pollen usually retains viability for longer periods, although requirements vary from plant to plant. Generally, the near-freezing temperatures and 25–50 per cent R.H. are suitable for prolonging longevity. Temperatures as low as –180° or –190°C, obtained by using liquid air or liquid oxygen, respectively, appear to be ideal but are far too expensive and require a costly and elaborate set-up.

What factors are responsible for the loss of viability during storage are not fully understood at present.

CULTURE

INTRODUCTION

Giovanni Batista Amici (1824, 1830), an Italian astronomer and mathematician, while examining the papillate stigma of *Portulaca oleracea,* noticed that one of the 'hairs' terminated in a pollen grain. This was the discovery of the pollen tube. He also saw a pollen grain giving out a tube which entered the stigmatic tissues and gradually disappeared in the style. Ultimately, in 1830, he concluded that the pollen tubes elongate, bit by bit, and finally come in contact with the ovules (see also Maheshwari, 1950).

Horticulturists and plant breeders often fail to get fertile seeds in spite of all the care taken during artificial pollination. Unless sterility is the main cause, failure of seed setting may be due to slow growth of the pollen tube or its early degeneration in the style. By removing the stigma and part of the style and placing a drop of a sugar-agar-gelatin medium (suitable for the germination of the pollen of various species of *Solanum*) on the cut end of the style, Swaminathan (1955) obtained viable seeds of the cross *Solanum pinnatisectum* with *S. lanciforme* as well as with *S. bulbocastanum* which do not cross otherwise.

The earliest observations on pollen tube growth appear to be those of Von Mohl (1834) who noticed that in a saturated humid atmosphere pollen grains of some plants readily produced tubes. Later, Schleiden (1849), Van Tieghem (1869) and others studied the in vitro germination of pollen grains of several plants and concluded that the germination varied from species to species or even from variety to variety.

Van Tieghem (1869), Lidforss (1896, 1909) and Jost (1905, 1907) reported that the pollen grains of *Dactylis* and *Hippeastrum* germinated readily in water. Knight (1917), Martin and Yocum (1918), Schoch-Bodmer (1936) and Righter (1939) were able to germinate the pollen of *Corylus, Pinus* and *Pyrus* even in distilled water. Daniel (1952), Savelli and Caruso (1940) and Paton (1921) claim to have obtained good pollen tube growth in water alone (in *Impatiens, Nicotiana* and *Plantago*). On the other hand, Cooper (1939), Schwarzenbach (1953), Vasil (1958a, 1960a) and most other workers state that merely in water, germination is usually poor and the tubes are short.

Normally pollen grains do not germinate satisfactorily in water, but aqueous solutions of sucrose (occasionally other sugars also), with or without addition of accessory substances, produce good results (see Vasil, 1958a, 1960a). Pollens of some plants may germinate easily under a wide range of conditions, while in others the requirements may be very exacting, failing which there may be no germination. As a rule,

the length of the pollen tube obtained in vitro is significantly shorter than that in vivo. Consequently, one of the main problems is to obtain in vitro germination and tube length comparable to that in vivo. In a few cases the length of the tubes in cultures does equal that in nature, e.g., *Pyrus* (Knight, 1917), *Rumex* (Schoch-Bodmer, 1921), *Chionodoxa, Hippeastrum, Muscari, Puschkinia* and *Scilla* (Brink, 1924c), *Vitis* (Branscheidt, 1929, 1930), *Convallaria, Echeveria, Gagea, Genista, Impatiens, Pachyphyllum, Ribes, Scilla, Sedum, Tradescantia, Vicia, Vinca* and *Xanthosoma* (Ehlers, 1951) and *Pennisetum typhoideum* (Vasil, 1960b).

During recent years pollen grains have been increasingly used for physiological studies because they have a simple structure and lend themselves easily to metabolic investigations. As they are highly sensitive to external factors, particularly temperature and humidity, the effects of these factors on pollen germination and tube growth can be readily ascertained. However, the pollen grains show great variability when they are collected from different anthers of a flower or even from the same anther (see Brink, 1942a; Smith, 1942; Daniel, 1952; Kubo, 1954; King, 1955; Vasil, 1958a, 1960a; Kurtz and Liverman, 1958). It is not surprising that the diploid and tetraploid pollen grains (of *Cucumis melo*) also require different media for their optimal germination (Tanaka and Mukai, 1955). Therefore, apart from the other usual precautions, one requires uniform samples, collected under normal environmental conditions. Since the quantity and density also affect germination and tube length, approximately an equal number of pollen grains have to be spread evenly on the medium for obtaining comparable results (see Brink, 1924d; Visser, 1955).

It has been the experience of many workers that in spite of these and other precautions, there is always some variation. Even under apparently similar conditons, pollen grains germinated in the same nutrient medium on two successive days may fail to give identical results. These variations can, however, be largely attributed to the inherent variability of the pollen grains and the climatic conditions prevailing during the growth and flowering of the plant. With the change of season also, the germinability of pollen grains varies and they require higher concentrations of sucrose and boric acid. Sometimes marked differences are noticed if the pollen of the same species is cultured at two places, especially when one of them happens to be in a tropical country and the other in a temperate one.

ROLE OF SUGARS

Most pollen grains germinate successfully in sugar solutions. Sucrose has largely been used, but lactose (Bishop, 1949), dextrose (Faull, 1955) and several other sugars and sugar derivatives (O'Kelley, 1955; Raghavan and Baruah, 1956a; Vasil, 1958a, 1960a) have also been tried. According to Vasil (1958a) sucrose, dextrose, rhamnose, raffinose, lactose and galactose generally give good results, while fructose, mannose and mannitol are unsatisfactory. Sometimes galactose, dextrose and lactose serve as well as sucrose. The utilisation of lactose by germinating pollen grains is of considerable interest, since its presence in plants has not yet been conclusively demonstrated (Pigmann and Goepp, 1948). Kuhn and Löw (1949a, b) have recorded the presence of lactose in the pollen of *Forsythia;* this is perhaps the only record of its kind. In contrast, fructose—which is so common in plants—usually fails to produce satisfactory germination or tube length. It appears that the suitability of a particular sugar need not necessarily depend on its natural occurrence in plants.

It is well known that autotrophic plants or plant tissues which contain chlorophyll are able to manufacture carbohydrates on which their growth and development depend. In the absence of chlorophyll they become parasitic or saprophytic and derive their nutrition from other sources. As a rule, pollen grains do not contain chlorophyll (except in a few cases, e.g., in the Malvaceae; see Maheshwari, 1950) and are, therefore, dependent on external sources for the supply of essential nutrients. At the time of shedding, they do contain some reserve food materials which are utilised during the initial stages of germination but are not adequate for the enormous growth of the tubes which occurs in vivo or in vitro. As stated by Brink (1924a), the reserve food may be sufficient to support some amount of growth which could proceed until it is consumed. However, since the nutrients in the culture medium stimulate and improve the germination of pollen and elongation of the tubes, it indicates that this effect, partly if not completely, is due to the externally supplied nutrition.

In a recent personal communication to one of us (I.K.V.), Professor R. A. Brink (University of Wisconsin, Madison, Wis., U.S.A.) stated that "It is difficult to avoid the conclusion, it seems to me, that sugars serve as nutrients during pollen tube growth. The pollen tube formed in many species is a massive structure relative to the reserve

materials stored in the pollen grain, and the reserves often are quickly consumed. Where then are the materials for the pollen tube wall and for energy purposes to come if not from sugars of outside origin?"

Since the beginning of this century, the question whether the pollen tubes growing in vivo and in vitro utilize externally supplied nutrients has attracted much attention. The two main views on this question concern the endogenous or the exogenous utilisation of nutrients. The former school believes that the externally supplied sugars have only an osmotic role and are not utilized by the tube for any nutritional purposes (Jost, 1905, 1907; Martin, 1913; Anthony and Harlan, 1920; Visser, 1955). Adherents of exogenous nutrition point out that, apart from having an osmotic role, the externally supplied sugars in the medium (or in the style) definitely serve as a nutrient material for the growing tubes (Brink, 1924a; O'Kelley, 1955, 1957; Hellmers and Machlis, 1956; Vasil, 1958a, 1960a).

According to Visser (1955), the "presence of sugar is only essential for creating favourable osmotic conditions for germination" and "many pollen may germinate readily and produce tubes of considerable length in pure water or on substrata which do not contain sugar." In support, Visser (1955) quotes the work of Ehlers (1951) who cultivated pollen grains of different genera without any external source of nutrition in the medium and obtained tube lengths which were sufficient to effect fertilization. Visser concludes: "none of the observations with regard to exogenous nutrition of pollen tube can be regarded as valid proof" and believes that "the pollen tube is exclusively built up from the reserves of the pollen grain" and the "effect of sugar on germination is in all probability exclusively due to its osmotic properties in aqueous solutions."

Visser (1955) further mentions that "pollen germination depends among others on the rate at which water is released from the medium and taken up by the pollen." He calls this phenomenon the "diffusion rate of water" and points out that for "controlling the diffusion rate of water" the presence of sugars is essential in order "to create a certain osmotic value." He continues: "the growth of the tube of many different pollens, whether cultivated in vivo or in vitro, is independent of the presence of nutrients in the medium in the form of sugars." The supporters of endogenous nutrition of pollen tubes believe that, even in vivo, the tubes do not get any nourishment from the style (see Visser, 1955). However, Dr. Ir. T. Visser (Tea Research Institute, Talawakelle, Ceylon) recently wrote (to I.K.V.), after the publication of O'Kelley's work, that largely the externally supplied sugars do serve as a source of nutrition for the germination of pollen, though in some cases it may be a 'luxury' consumption, if taken up from external sources.

In *Hippeastrum aulicum,* Jost (1907) obtained 17,000 to 22,000-microns-long tubes in one per cent sucrose, but when the concentration was reduced to 0.25 or 0.5 per cent the length of the tubes was only 7,000 to 8,000 microns. In three per cent sucrose, Brink (1924a) obtained pollen tubes growing beyond 10,000 microns. In one to ten per cent sucrose the pollen grains of *Vinca minor* produced tubes 10,000 microns long (Bobilioff-Preisser, 1917; Brink, 1924a). Equally long tubes have been obtained with the pollen of *Nicotiana alata* and *Scilla* (Brink, 1924a), and with *Crotalaria juncea* and *Dolichos lablab* (Vasil, 1958a). In *Pennisetum typhoideum* the tube length (4320 microns) in vitro is sufficient to effect fertilization in vivo (Vasil, 1960b).

Further evidence concerning exogenous utilization of sugar, although indirect, comes from the experiments of several workers, e.g., Dodel-Port and Dodel-Port (1880), Mangin (1886), Green (1894a, b), Tanaka (1955, 1956) and Konar (1958), who observed that the pollen grains of different species (specially gymnosperms) show abundant starch when placed in sugar solutions. In *Typha latifolia,* pollen tubes grown on sucrose solutions gradually accumulate oil globules (Vasil, 1958a).

Brink (1924a) and Schoch-Bodmer and Huber (1947) noticed that when pollen was cultivated in sugar or sugar-agar media, the tubes were as long as, or even longer than, those formed in nature. In many plants germination is no doubt high but the tubes are considerably shorter than those formed in vivo, even when the medium is supplemented with different growth-promoting substances. However, this could be due to the shortage of certain essential growth-promoting substances in vitro. On the other hand, when the medium lacks sugar, there is profuse bursting of pollen grains and often no germination at all.

Although no attempts have been made to determine the dry matter of pollen before and after germination, it is obvious that it increases considerably during germination. This can be visualised from the massiveness of the tubes and the large number of callose plugs formed in them. Brink (1924a) states: "Tubes are formed with cellulose or

callose walls reaching a length in some cases equal to several hundred diameters of the pollen grain and containing in their frequently bulbous tips a considerably enhanced mass of protoplasm. These tubes, moreover, commonly form numerous callose plugs the total volume of which may alone exceed that of the grain. The inference is clear that the sugars in the medium have been drawn up in the development of these structures."

The studies of O'Kelley (1955) on the pollen tubes of *Tecoma radicans* gave the final blow to the theory of endogenous nutrition. He used C^{14}-labelled sugars and conclusively proved, for the first time, that pollen tubes respire during their growth and absorb externally supplied sucrose, fructose or glucose. To quote his own words: "The specific activity of the CO_2 produced showed that during pollen tube respiration of individual sugar 36 per cent of the CO_2 came from the glucose, or 66 per cent came from the fructose, or 72 per cent came from sucrose." Experimenting with the pollen grains of *Pinus ponderosa,* Hellmers and Machlis (1956) noticed that the pollen grains "absorb and metabolize a variety of mono-, di- and tri-saccharide sugars. These are synthesized into polysaccharides and are respired. Further, pollen germinated and supplied with sugar continues to be active and healthy when pollen dependent on endogenous reserves has obviously begun to die." In a more recent paper, Q'Kelley (1957) has provided further evidence to show that the externally supplied sugars are undoubtedly utilised.

Tupý (1960) has also demonstrated the utilization of exogenous sucrose by the growing pollen tubes of apple and *Nicotiana alata* and the enzymatic inversion of sucrose into glucose and fructose (see also Iwanami, 1959). The fact that inversion of sucrose stops when the pollen tubes are removed from the culture medium indicates that invertase is released by the tubes into the medium. The breakdown of sucrose takes place either within the tube or on its surface as in yeasts and somatic cells of higher plants. When boron is added to the medium, the inversion of sucrose is quicker. Another point which Tupý (1960) has emphasized is that growth of pollen tubes is not very satisfactory in the inversion products of sucrose, viz., glucose and fructose (this is in conformity with the findings of some earlier workers; see Vasil, 1960a). It may be assumed that pollen grains absorb sugars mainly in the form of sucrose.

According to Iwanami (1959), at the time of shedding, pollen grains usually contain sucrose, glucose or fructose as food reserves. If the pollen contains mainly glucose (e.g., *Impatiens*), it germinates earlier than those containing sucrose (e.g., *Lilium*). In the latter case sucrose is converted into glucose just before germination. Iwanami points out that at the time of germination in most pollen grains starch is converted into sugars, or vice versa. This regulates the osmotic pressure of the pollen grain in relation to the osmotic pressure of the medium or the tissues of the stigma and style. Whether it is a general phenomenon in pollen grains requires further investigation.

As early as 1889, Correns computed that the reserve food present in the pollen was not adequate to produce a pollen tube long enough to reach the ovules. It is well known that the conducting tissue of the style contains considerable quantities of starch, sugar and other carbohydrates (Brink, 1924a; Gessner, 1948). Straub (1947) concluded that in *Petunia* the pollen tubes are nourished by the conducting tissue during their growth through the style. Schoch-Bodmer (1945) and Schoch-Bodmer and Huber (1947) have also made similar observations. Linskens (1954; see also Bhattacharjya and Linskens, 1955) has recently demonstrated that during their growth down the style, pollen tubes not only absorb water but also take up quite an appreciable amount of sugar. After heavily pollinating the mature stigmas of *Petunia,* Linskens noticed that after the pollen tubes had reached the embryo sac, the sugar content of the styles was reduced to about half the amount that was present before pollination. During their growth towards the ovary pollen tubes of *Lilium* and *Petunia* also utilise exogenous substrates from the style (Linskens and Esser, 1959).

Concerning the role of sugar as an osmotic agent, Brink (1924a) writes: "A source of no little annoyance and perplexity to those who have attempted to cultivate pollen artificially has been the bursting of pollen and pollen tubes." There is profuse bursting in lower concentrations, and it would be interesting to know whether it is dependent on osmotic concentrations. According to Van Tieghem (1869), Molisch (1893) and Lidforss (1896), bursting can not be an osmotic phenomenon, since no distinct relationship is known between the number of pollen grains bursting and the concentration of sugar in the medium. Lloyd (1918) believed that bursting is entirely due to imbibition by the protoplast beyond the strength of the wall. Waddington (1929) holds that bursting of *Matthiola* pollen is in no way influenced by sugar concentration. However, his data clearly show that

the percentage of bursting was much lower in higher concentrations of sucrose than in lower. Bursting is inversely related to the osmotic concentration of the medium and sometimes it can be reduced by adding agar or gelatin to the sucrose medium (Anthony and Harlan, 1920; Walderdorf, 1924; Schoch-Bodmer, 1936; Kuhn, 1938; Bair and Loomis, 1941; Schwanitz, 1942). Vasil (1958a, b, 1960a) altered the osmotic concentration of the medium by adding different molar concentrations of mannitol (an inert substance which does not have any promoting effect on the germination of pollen) and showed that bursting of the pollen of *Cucumis melo* var. *utilissimus* and *Momordica charantia* invariably decreased with an increase in the osmotic concentration of the medium, and vice versa.

There is general unanimity of opinion on the role of sugars in controlling osmotic concentration during germination of pollen. That it does affect germination itself is also obvious, and probably an approximate similarity in the osmotic concentration of the medium with that of the pollen is a prerequisite for good germination. According to Schoch-Bodmer (1936), turgor pressure is very largely responsible for the germination of pollen and may also affect the length of pollen tubes. However, germination is not an osmotic or a turgor phenomenon alone, as shown conclusively by the work of O'Kelley (1955) and Hellmers and Machlis (1956), nor does sugar serve merely as a source of nutrition.

ROLE OF BORON

Since the very beginning most investigators have attempted to improve the germination of pollen grains and the length of tubes in vitro. During the years 1932, 1933 and 1935, Schmucker made an important discovery and published a series of papers from Göttingen on the stimulating effect of boric acid. He observed that pollen of the tropical species of *Nymphaea* hardly germinated in one per cent glucose, but that satisfactory germination resulted if stigmatic extract was added to it. After analyzing the stigmatic extract, he noticed that it contained appreciable quantities of boric acid. This led him successfully to germinate the pollen of *Nymphaea* in one per cent glucose supplemented with boric acid. On quantitative analysis it was found that the pollen grains required almost the same concentration of boric acid as was present in the stigmatic secretion. Therefore, he concluded that boron plays a strategic role in the germination of pollen. He further observed that 0.001 or 0.01 per cent boric acid promoted growth of the tubes not only of *Nymphaea* but also of a large number of other plants. Among the 40 species tested, ten showed marked sensitivity, and boric acid not only raised the percentage of germination but also accelerated the rate of growth of the tube. Schmucker was so much impressed by the stimulating action of boric acid that he designated it as an "anorganischer Wuchsstoff" (inorganic growth substance). He thought that boric acid was probably bound with hydroxyl-rich organic substances, like sugars, and formed boron-hydroxyl complexes which were related to the formation of cell wall material.

The stigmatic secretion of *Vitis* also contains a boric acid concentration equivalent to that required for optimal germination of pollen in vitro (Gärtel, 1952). Thomas (1952) also identified boron in the styles of *Lilium* and demonstrated its stimulating action on *Lilium* pollen in vitro.

Several workers have pointed out that ten ppm (0.001 per cent) or higher concentrations of boric acid are toxic to plant growth (see Schmucker, 1935; Visser, 1955). However, pollen grains can tolerate concentrations up to 1,200 ppm (0.12 per cent), although optimum results are obtained between ten and 150 ppm (0.001 to 0.015 per cent). Vasil (1958a, b, 1960a), who investigated the effect of various concentrations of boric acid (also borax) on the pollen of a number of crop plants, reports that 100 to 150 ppm (0.01 to 0.015 per cent) of boric acid is favourable for pollen germination, while higher concentrations are definitely inhibitory. The effect on elongation of the tube is much more marked than on the percentage of germination. In certain plants, e.g., *Capsicum annuum, Corchorus capsularis* and *Crotalaria juncea,* optimum germination and tube length were obtained in sucrose solutions alone, and addition of boric acid either had no effect or proved inhibitory. In these plants the natural level of borin appeared to be adequate and, therefore, any additional supply proved ineffective.

Pollen is said to be naturally deficient in boron (see O'Kelley, 1957) but the pollen grains and styles of at least some plants have a high boron content (Bertrand and Silberstein, 1938; Bobko and Zerling, 1938; Gärtel, 1952; Thomas, 1952). Even in boron-deficient pollen, the deficiency is likely to be made up by the boron from the style.

It may be argued that boric acid prevents the growth of microorganisms and also affects the pH of the medium. Vasil's (1958a) data do not confirm this view. Boric acid, in the concentrations generally

used for pollen culture, neither changes the pH of the medium nor is effective in checking the growth of the microorganisms which appear in the medium after six to eight hours of culture.

Another role of boron may be an interaction with certain substances which affect germination. Two views have been expressed, one by Gauch and Duggar in 1953 (see also Gauch and Duggar, 1954; Linskens, 1955; Sisler et al., 1956; Baker et al., 1956; O'Kelley, 1957) and the other by Visser in 1955.

While conducting experiments on the respiration of root tips of lima bean and pea, Gauch and Duggar (1953) made some interesting observations and proposed that "boron combines with sugar to form a sugar-borate complex (ionizable) which is translocated with greater facility than are non-borated, non-ionized sugar molecules." Linskens (1955) has now demonstrated experimentally that, in *Petunia,* sugar-borate complexes are formed during pollen germination. Gauch and Duggar (1953) pointed out two possibilities how boron affects the translocation of sucrose or its hydrolytic products. Firstly, "the borate ion could react with sucrose (or glucose or fructose), the sugar then passing through the cellular membranes as the ionized sugar-borate complex until such time as a cell utilizes this complex and liberates the boron ion." Secondly, it is also "possible that the borate ion is associated with the cellular membranes, that it there reacts chemically with the sugar molecule facilitating its passage through the membrane, and that the sugar is freed on the inside of the cell by a second reaction." They further state that the plants "absorb boron throughout their life cycle, indicating that, as new cell membranes are formed, more boron is required." Boron has also been shown to be relatively immobile in plants (Eaton, 1940; see also Kuhn, 1943). Thus the evidence is in favour of the second possibility, that the borate ion is associated with the cellular membranes.

In addition to boron, under certain conditions, some other factors may also limit the absorption and translocation of sucrose. According to Gauch and Duggar (1953) boron appears to be "the dominant factor in the movement of sucrose (or its hydrolytic products) from cell to cell in the plant"; and what holds good for plants in general, seems to be a pertinent explanation of the role of boron in pollen germination. However, as observed by Visser (1955) and Vasil (1958a, b, 1960a), boron shows some stimulating effect on the germination of pollen and elongation of tubes even when the pollen grains are germinated in water without any supply of sugars. So far, there does not seem to be any satisfactory explanation for this behaviour.

Schmucker's (1935) suggestion that the beneficial effect of boron is due to the fact that it regulates the hydration of colloids, is associated with polyhydroxyl compounds of the membrane, and is concerned in the formation of pectic substances, has been further substantiated by the experiments of Dennis (1937), Minarik and Shive (1939), Kuhn (1943), Baker et al. (1956), Münzer (1960) and others. Spurr (1957) has recently shown that in boron-deficient plants the cell walls are markedly thinner than in those where boron supply is adequate. His experiments indicate that boron is a "morphogenetic agent affecting the development of specific form of the cell wall of the plant."

The other view was put forward by Visser (1955), who made detailed investigations on the effect of boric acid on the germination of pear and apple pollen, and who suggested that boron plays an important role in its carbohydrate metabolism. He observed that the in vitro germination of pollen improved when boron (as aqueous solution of boric acid) was fed to the tree branches. Germination could also be stimulated by feeding sugar to the branches. However, optimum germination occurred in pollen collected from branches fed with sugar as well as boric acid. Therefore, Visser concluded that boron increases the vitality of pollen. It was also seen that the germinability of pollen is the same, "whether the 'extra' boron is taken up from the tree beforehand or taken up by the pollen from its germination medium afterwards" and "the degree of boron sensitivity of the pollen is inversely related to the boron level of the plant."

Bobko and Zerling (1938) observed higher viability in the pollen of *Trifolium pratense* when the plants were given boron-rich fertilizers. Antles (1951) reports that the pollen of pear which failed to germinate in sugar solutions without boric acid, gave 100 per cent germination in sugar alone when the plants were given boron-rich fertilizers for a period of four years. Batjer and Thompson (1949) obtained a higher fruit set by spraying boric acid on pear blossoms.

According to Visser (1955), the length of the tube is directly related to the concentration of boric acid, while the percentage of bursting is inversely related. The formation of protrusions through the germ pores is independent of boric acid supply, but their subsequent growth depends on its presence. In Visser's own words "boric acid is specifically required for the growth of the pollen tube." This is contra-

dicted by Vasil's (1958a, 1960) experiments where he has shown a greater increase in the percentage of germination in media containing boric acid than in those where boron had been omitted.

Visser (1955) also pointed out that the "necessity of a continuous and ample supply of boric acid implied that it is inactivated in the course of the elongation process." He emphasized that "On account of the favourable effect of boric acid on tube growth it can be assumed that a complex of boric acid and an unknown substance acts as a 'growth promoter' in tube elongation. . . . It is suggested, therefore, that the substance in question is partly present in a free form, termed X, and in a complex form with boric acid, termed XB; X has no germination properties, while XB has. The proportion of X and XB is determined by the boron level of the plant. On account of the above observations the following view can be held with respect to the increase of germination and tube growth upon addition of boric acid: Whenever the growth rate of the tube (as determined by sugar concentration and temperature) exceeds the supply of the complex compound from endogenous sources, this supply can be supplemented by addition of boric acid, thus leading to additional complex formation with the available free compound . . . Finally, it can be remarked that the substance X is possibly a glucoside." The higher percentage of germination seen when large groups of pollen grains are cultured in one drop, or by the addition of pollen extracts, or mutual stimulation of pollen, is also due to the presence of the substances X and XB. Loss of viability during storage of pollen and an increased boron-sensitivity of the stored pollen are due to the immobility of the substance XB.

Tupý (1960) has shown that "Boron encourages sucrose absorption proportionately to the stimulation of pollen tube growth. It is, therefore, possible that the stimulatory effect of boron on growth is connected with carbohydrate metabolism. In view of the fact that pollen tube growth is accelerated by boron at the time when the tubes are still drawing to a great extent or exclusively on the reserve substances in the pollen grain — and in view of the well known stimulatory effect of boron in distilled water — it follows that the main factor is not necessarily the translocation of carbohydrates into the pollen tubes, but that it is rather a question of the rate of their metabolism."

In a recent paper on the germination of pollen of *Tecoma radicans*, O'Kelley (1957) reports that addition of boron brought about a 24 per cent increase in sugar absorption over the controls grown without boron. According to him the effect of boron on pollen tube growth is "not very closely related" to borate effects on either respiration or sugar absorption, but "It appears that boron has a specific role in pollen tube growth, in addition to a stimulatory effect on oxygen uptake and on sugar absorption." The stimulation of tube growth may involve the synthesis of pectic materials required for wall formation of the elongating tube.

The effect of boron far surpasses the effect produced by any known hormone, vitamin or chemical. This is perhaps due to the fact that pollen grains initially contain adequate quantities of growth-promoting substances and that, therefore, any additional supply does not improve germination. On the other hand, pollen seems to be usually deficient in boron, and its addition to the nutrient medium improves not only the germination but also the elongation of the tubes.

On the basis of present evidence, the role of boron in pollen germination and pollen tube growth may be three-fold (Vasil, 1960a): (*a*) it promotes absorption and metabolism of sugars by forming sugar-borate complexes, (*b*) it increases oxygen uptake, and (c) it is involved in the synthesis of pectic materials for the wall of the actively elongating pollen tube.

Daniel and Váróczy (1957) believe that "boron is promoting the movement and availability of the reserve materials and stimulating the activity of the adenosine-triphosphate; however, the possibility of some pure physico-chemical effect of boron might also be taken in consideration."

Moewus (1950) claims that in *Forsythia intermedia* self-sterility could be overcome by the application of boric acid. Kuhn (1943) had pointed out earlier that boric acid plays the role of an inactivator for germination-inhibiting substances in tomato pollen. However, Moewus' (1950) work on the breaking of self-sterility in *Forsythia* has been shown to be incorrect by Esser and Straub (1954), Visser (1956) and Reznik (1957). It may be added that Moewus and Visser both worked on the same bushes of *Forsythia* but obtained entirely different results.

EFFECTS OF CHEMICALS, GROWTH SUBSTANCES, VITAMINS, ANTIBIOTICS AND CAROTENOIDS

Besides sugars and boric acid, several other substances are also known to have a stimulatory effect on the germination of pollen and

the growth of tubes. Lidforss (1896) observed that, even in small amounts, sodium chloride, potassium nitrate and calcium nitrate proved toxic to pollen grains, and Brink (1924b) has confirmed these results. However, according to several recent reports, it appears that calcium nitrate and manganese sulphate, as well as several other chemicals, improve the germination and elongation of pollen tubes (Loo and Hwang, 1944; Sen and Varma, 1955b; Raghavan and Baruah, 1956b, 1959; Takami, 1956; Vasil, 1958a, b, 1960a). Branscheidt (1930) and Gotoh (1931) traced the effect of these ions to the changes induced in the pH of the medium.

Hormones and vitamins have often been used to increase the percentage of germination and to accelerate the rate of growth of tubes (Dandliker et al., 1938; Smith, 1939, 1942; Addicott, 1943; Loo and Hwang, 1944; Antles, 1949; Anhaeusser, 1953; Dikshit, 1956; Sen and Varma, 1956b, 1958; Vasil, 1958a, b, 1960a). Contrary to the general belief, in many cases these substances do not produce a marked effect on the germination of pollen grains (see Vasil, 1958a; Rietsema, 1961). However, Brink (1924b) obtained a substantial increase in *Cucumis sativus, Lythrum salicaria* and *Primula obconica* by addition of 'sterile' yeast (which is a source of vitamins of the B group) to the nutrient medium. In this connection Cooper's (1939) observations are of interest. He states that the influence of several vitamins and amino acids on the pollen of *Carica papaya* appears to be due to their regulating effect on the pH.

Addition of aspartic acid, glutamic acid, histidine or cysteine to the sugar-agar medium causes germination of *Paris hexaphylla* pollen which otherwise fails to germinate (Sawada, 1958). Similarly, arginine, valine and alanine promote germination of *Oryza sativa* pollen (Sawada, 1958). The above amino acids also occur in the pollen grains and pistils of these plants.

Schwarzenbach (1953) investigated the effect of 25 carotenoids on the germination of *Cyclamen persicum* pollen and noticed that, while a few stimulated germination, three carotenoids (also present in the anther and pollen of *C. persicum*) inhibited germination. Therefore, he suggested that carotenoids of the anthers prevent a premature germination of pollen. When the pollen lands on the stigma, the inhibiting effect "is removed and transformed to one of stimulation by an unknown substance" from the unfertilized ovules. This unknown substance can be replaced in experiments by indoleacetic acid and vitamin K_1.

Curtis and Duncan (1947) point out that orchid pollen contains enough auxin for normal germination, while in *Antirrhinum* and *Bryophyllum* the auxin content is said to be a limiting factor for germination (Smith, 1942).

Occasionally addition of traces of penicillin and streptomycin (the latter is frequently contaminated with phenylacetic acid) to the culture medium improves the germination and growth of pollen tubes (Pulvertaft, 1946; Vasil, 1958a, b, 1960a; Sen, 1960). In some plants antibiotics are known to cause inhibition of growth (Rosen, 1957).

During the last decade, two new chemicals — kinetin and gibberellic acid or gibberellins—have been reported to show a marked effect on the growth of plants and plant tissues (Miller et al., 1955a, b, 1956; Brian 1957; Skoog and Miller, 1957; Stowe and Yamaki, 1957; Vasil, 1957; Stodola, 1958). In *Lilium*, gibberellic acid greatly accelerates the rate of elongation of the pollen tubes (Kato, 1955; see also Chandler, 1957; Stodola, 1958). Chandler (1957) observed that pollen, which did not germinate on a control medium and appeared non-viable, germinated satisfactorily with the addition of gibberellic acid. She presumed that, due to the stimulating effect of gibberellic acid on the pollen of *Lilium* and *Petunia*, it may be possible to "overcome some of the physiological incompatabilities characteristic of certain clones of these species." Whereas gibberellic acid promotes the germination of *Tulipa* pollen, IAA has no effect. However, in conjunction with gibberellic acid IAA has some beneficial effect (Laboureur, 1960; see also Bose, 1959, Ching and Ching, 1959).

So far, the effect of kinetin has not been tried on the pollen of angiosperms, but it is likely that its incorporation in nutrient media along with auxins may hasten the division of the generative cell. Konar (1958) reports that in *Pinus roxburghii*, kinetin alone (i.e., without any added auxin) promotes germination and increases the tube length, but nuclear divisions are not induced.

EFFECT OF pH

Although the influence of pH on the growth and metabolism of plants in nature as well as in cultures has been proved beyond doubt, there is not much work on the effect of pH on the germination of pollen. Opinions are so conflicting that it is difficult to decide whether pH at all influences the germination of pollen (Vasil and Bose, 1959).

As early as 1924, Gotoh pointed out that the dissolved alkali of the cover glass greatly affected the germination of pollen. A little later,

Brink (1925) observed that in *Lathyrus odoratus* optimum germination occurs at a pH of 7.0 and that the zone of hydrogen-ion concentration favourable for germination is rather narrow. He assumes that the pH may modify the growth of pollen tubes through a direct effect on the chemical reactions attending the digestion of reserve food materials. In *Petunia,* optimum germination occurred at a pH of 5.5 to 5.8. In several plants, Berg (1930) and Sisa (1930) obtained double-optima curves (two-peaked) expressing the percentage of germination at different pH (see also Branscheidt, 1930). Vasil and Bose (1959) observed that the pH of the medium remains almost unchanged, even after the pollen tubes have grown in it for one to two hours.

Satisfactory germination of pollen has been reported at pH 4–9 (Berg, 1930, Branscheidt, 1930; King and Johnston, 1958). In certain crop plants the percentage of germination and the length of the pollen tubes remain fairly constant at pH 3.5 to 9.0 (Vasil and Bose, 1959). The optimum pH range suitable for the germination of *Cosmos bipinnatus* pollen changes about every 20 days during the flowering season (Kubo, 1954). Pollen grains of several species of *Rhododendron* germinate only at pH 3 during the beginning and at the end of the flowering season, but during the middle of the season they germinate in a wide range (pH 1.8–7.8). Throughout the flowering season the optimum pH value gradually extended from the acidic to the alkaline range (Kubo, 1955a, b). The pH may not directly affect germination but whatever effect is produced may be entirely due to the cations and anions present in the medium in the form of buffer salts. Therefore, it has been suggested that instead of using different buffer mixtures, N/10 HCl or N/10 NaOH may be used for adjusting the pH of the medium from 3.5 to 9.0. Addition of buffer mixtures makes it almost impossible to avoid and estimate the effect of different salts present therein.

EFFECT OF TEMPERATURE

The correlation between temperature and pollen germination is usually represented by an optimum curve (Roberts and Struckmeyer, 1948; Visser, 1955), and maximum germination and tube length are obtained between 20° and 30°C (Winkler, 1926; Berg, 1930; King and Johnston, 1958). In tobasco variety of red peppers, Hirose (1957) reported optimum germination of pollen at 35°–40°C which is rather unusual since in other plants germination is inhibited at these temperatures. At low temperatures the percentage of germination and tube length are considerably reduced, and under such conditions in vivo the tubes may never reach the ovules (Smith and Cochran, 1935). At sub-optimal temperatures, satisfactory tube lengths can be obtained only if the period of growth is not a limiting factor. Adams (1916) reported 90–100 per cent germination in apple at 14°C after 24 hours. However, at 2°C, Östlind (1945) observed only 10–36 per cent germination after 69 hours, but it shot up to 90–100 per cent after 119 hours.

The diameter of the pollen tubes enhances with increase in the environmental temperature (Smith, 1942). For example, the pollen of *Antirrhinum* showed negligible growth at 15°C, optimum germination and tube length at 25°C, pronounced broadening and 'bloating' (swelling) of the distal portion of the tube at 30°C, and extensive bursting of the bloated tubes at 35°C. On the other hand, in *Bryophyllum* appreciable germination occurred at 15°C and optimum germination and tube length at 25°C. Smith mentions that "At 35°C a great diversity of tube lengths resulted with many short, broad tubes and some exremely long ones with broadened proximal portions." In *Medicago sativa* (Sexsmith and Fryer, 1943), for a half-hour growth period, a linear relationship was observed between the length of the pollen tube and rise in temperature. Thus the length increased as the temperature was raised from 70° to 100°F. There was no germination at 50°F but at 100°F the pollen tubes appeared to be normal. The Q_{10} is approximately two (Visser, 1955; Vasil, 1958a).

Sen and Varma (1956a) report that in *Pisum sativum,* "as compared to room temperature (10°–12°C) increased germination and elongation of pollen tubes was obtained at 15° to 21°C. At temperatures below 12°C the germination was comparatively poor, while at 24° the pollen tubes burst profusely." In *Areca catechu* optimum germination was noticed at 28°C, whereas at 15°, 20°, 30° and 35°C germination was usually poor (Raghavan and Baruah, 1956a). In *Dolichos lablab, Pisum sativum* and other crop plants, Vasil and Bose (1959) observed optimum germination and elongation of pollen tubes at 25°–30°C. At 5°C and below there was practically no germination, and it was inhibited above 35°C. At higher temperatures marked swelling or bursting of the tips was quite common. Irrespective of the temperature, the growth curve of the tube remains typically sigmoid (Vasil, 1958a; Vasil and Bose, 1959).

CHEMOTROPISM AND GROUPING OF POLLEN

The chemotropic reaction in vitro of pollen tubes to pistil has been widely described. Van Tieghem (1869) regarded that pollen tubes are attracted towards the ovary by hydrotropism, while Strasburger (1878) found no evidence of chemotropism towards ovules. Molisch (1893) noticed that the pollen tubes of *Narcissus tazetta* are negatively aerotropic; e.g., only the pollen grains near the periphery of the culture medium germinated and sent their tubes toward the centre. Miyoshi (1894) claims to have seen drops of fluid at the micropyle and believes that these secretions are responsible for attracting the pollen tubes in a number of plants. Chemotropism towards the stigma has since long been considered a common phenomenon (Miyoshi, 1894; Lidforss, 1896).

Recent studies indicate that, of a large number of species examined, only a few showed positive chemotropism towards their own pistils or other floral parts (Brink, 1924d; Gotoh, 1931; Savelli and Caruso, 1940; Tsao, 1949; Ustinova, 1954; Visser, 1955; Schneider, 1956; Iwanami, 1959). According to Miki (1955; see also Miki, 1954), "In *Camellia sinensis,* pollen tubes show positive tropism to the fresh style slices while they show negative tropism to slices of the styles which are steamed for 10 minutes at 60°C, 80°C or 99°C." and "both substances, which are responsible for the positive and the negative tropism, diffuse from styles to agar media." Slices of fresh, mature stigma and of the subjacent tissue stimulate pollen germination in *Lilium longiflorum* (Rosen, 1959). They exhibit chemotropism which is lost on heating, washing and exposure to air.

The aggregation of pollen grains in the culture medium (as well as on the stigma) has a marked stimulatory effect on the germination and the length of tubes. This can be compared with the 'bios effect' of yeasts and other microorganisms where the growth of the organism is stimulated when the inoculum is heavy. The stimulating[1] effect on pollen is presumably due to certain growth-stimulating substances which diffuse out of the pollen grains (Brink, 1924d; Kuhn, 1938; Savelli, 1940; Savelli and Caruso, 1940; Beck and Joly, 1941; Holubinsky, 1945; Rémy, 1953; Visser, 1955; Ariyasu, 1959; Iwanami, 1959). However, the effective concentration of these substances occurs only when the pollen grains are aggregated. Visser (1955) states that "The experiments with an increasing number of grains per drop and those with pollen extracts imply that a substance (or substances) with 'germination promoting' properties diffuses from the grains" and that this substance is neither boric acid nor an enzyme but perhaps a glucoside.

Our understanding of the stimulatory effects due to the grouping of pollen and chemotropism of pollen tubes must await further researches.

[1] In a recent conversation, Dr. W. R. Tulecke drew my (BMJ) attention to the forthcoming article by B. H. Kwack and J. L. Brewbaker (Amer. Jour. Bot.—In Press), pointing out that the stimulation, at least in the 15 species of plants tested by them, is due to calcium ion. Although calcium is not replaceable by ions such as strontium, other cations (Mg^{++}, K^{+} or Na^{+}) are required to permit the calcium activity.

EFFECT OF PLANT TISSUE EXTRACTS

The earlier reports on the chemotropic influence of pistil on pollen tubes initiated studies on the effect of extracts of various other floral parts. East and Park (1917, 1918) showed that the in vitro growth of pollen tubes of *Nicotiana* is promoted by stigmatic extracts. According to Knowlton (1922), even stored pollen, which otherwise failed to germinate in vitro, was stimulated to germinate with addition of small crushed pieces of the stigma to the culture medium. Tokugawa (1914) and Brink (1924d) obtained similar results in several other plants, e.g., *Narcissus*. In some cases even the crushed ovarian tissue and extracts of ovules and pollen proved stimulatory (see Brink, 1924d; Visser, 1955). Kuhn (1938) pointed out that anther extracts considerably raised the germination percentage and the length of tubes.

As early as 1926 Katz stated that the stigmatic secretion is indispensable for pollination but maintained that it merely protects the pollen from drying and probably does not act as a chemical stimulant. Kühlwein (1948) observed that the extracts of anther, pollen or stigma generally increased the percentage of germination and the length of the pollen tube. Addition of pollen extracts of *Tulipa,* and anther and stigmatic extracts of *Lilium,* greatly increased the percentage of germination as well as tube length in *L. candidum.* A few reports also indicate that the extracts of stigma, style or pollen either have no appreciable effect on germination or are inhibitory (Sasaki, 1919; Kaienberg, 1950; Sen and Varma, 1955a).

Takashima (1954) noticed that in the Cucurbitaceae "On the flowering day, a certain hormone generates in the stigma and that hormone promotes the growth of the pollen tube."

Kuhn (1938) mentions that in *Matthiola* a considerable increase in the percentage of germination and the length of tubes occurs by the addition of anther extract to the medium, and he assumes that the effective substances of the extract belong to the pollen. The promoting effect could not be replaced by yeast extract, small amount of sugar or diastase. High concentrations of the extract inhibited germination.

Visser (1955) also records some stimulation on the percentage of germination as well as on tube length, but generally it "is in no case as high as that in the sugar solution to which boron had been added."

GROWTH PHENOMENA

It has already been said that the growth of pollen tubes in vitro is characterised by a typical sigmoid curve. Even when pollen grains are germinated under different conditions, the pattern of growth remains unaltered. The maximum rate of elongation occurs some time before the middle of the growth period. Active elongation of the tube takes place only in the region of its tip (Schoch-Bodmer, 1945; Haeckel, 1951; O'Kelley and Carr, 1954; Mühlethaler and Linskens, 1956; Iwanami, 1959).

The recent work of Tulecke (1953, 1957, 1959) on the in vitro production of tissue masses from the pollen grains of *Ginkgo biloba* opens up a new field of study in the physiology of pollen. So far this is the only instance of such a behaviour but it is likely that angiosperm pollen would also respond similarly.

PROTOPLASMIC STREAMING. During the course of their growth, the pollen tubes of almost all angiosperms show rapid protoplasmic streaming. It begins within a few minutes after the pollen is sown in the culture medium or lands on a stigma and continues until the pollen tube has ceased to elongate. The rate of protoplasmic streaming varies from plant to plant and is directly proportional to the rate of elongation of the tube (see Vasil, 1958a, b, 1960a). The rate of movement (in vitro) is slow in the beginning, gradually increases and comes to a peak slightly before the middle of the growth period, and then gradually declines until the pollen tube stops elongating. Occasionally, however, the streaming movement may continue for some time even after the tube has ceased to elongate. In an actively growing pollen tube the rate of streaming is rather slow near the pollen-grain end, is fairly rapid in the rest of the tube, and there is practically no streaming in the extreme tip which has been termed as the 'cap block' by Iwanami (1956).

In gymnosperms, where growth of the pollen tube is extremely slow, the streaming of the cytoplasm has never been demonstrated (see Takeuchi, 1953). Even in some angiosperms, where the rate of elongation of the tubes is poor, the cytoplasmic streaming is either very slow or not at all visible, e.g., in *Citrus* and some species of *Solanum* (Vasil, 1958a).

Iwanami (1956, 1959) has described several types (e.g., rotation, circulation, fountain, contra-fountain etc.) of protoplasmic streaming in pollen tubes. In fact, such variations may occur at different places in the same tube or in different tubes in the same culture.

PATTERNS OF POLLEN TUBE. During in vitro growth under abnormal conditions, the tubes often exhibit characteristic patterns in shape which may be due to the pollen being out-of-season or old, or to high concentrations of the nutrient medium. The tube may be bloated—enormous swelling of the tip; blunt — abrupt and flattened tip; or spirally coiled like a cork-screw (see Hartmann-Dick and Müller-Stoll, 1955). Pollen collected during high atmospheric humidity and temperature shows similar behaviour.

Occasionally another interesting behaviour known as 'vesicle formation' has been described. Actively growing pollen tubes abstrict small vesicles at their tip. These vesicles have an independent existence for some time, show cytoplasmic streaming and finally degenerate (Bobilioff-Priesser, 1917; Vasil, 1958a). Sometimes the vesicle may contain one or both the sperm cells (Bobilioff-Preisser, 1917). Similar observations have recently been made by Iwanami (1959) in *Lilium longiflorum*. In *Brassica nigra* the formation of vesicles could be stimulated if the pollen was sown in a high concentration (35 per cent) of sucrose (Vasil, 1958a).

CALLOSE PLUGS. As the pollen tube elongates (in vitro), certain plug-like structures are formed at regular intervals all along its length and divide it into small segments. These plugs are made up of callose, a substance very similar to cellulose (Müller-Stoll and Lerch, 1957; Currier, 1957). Callose is a polyglucoside derived from glucose but its exact chemical nature is still uncertain (see Currier, 1957). Its formation depends on the limited capacity of pollen tubes to utilize the glucopyranose component of the metabolised sucrose during respiration (Tupý, 1960). This explains the increased accumulation of cal-

lose in incompatible and slow-growing tubes (Tupý, 1959; Vasil, 1960a).

Callose plugs also appear in tubes growing in vivo except in those cases where the tubes remain rather short. The pollen tubes of some plants may develop callose plugs in vivo but not in vitro, e.g., in cotton (Iyengar, 1938; Vasil, 1958a).

With the formation of successive callose plugs, the cytoplasm and nuclei move towards the tip of the tube. The latter is capable of independent existence, and it has been experimentally demonstrated in *Vinca minor* that the excised tip continues its growth normally (Brink, 1924d; see also Iwanami, 1959). The portion of the tube next to the pollen grain becomes isolated from the actively growing tip. It does not contain any nuclei, and the cytoplasm is also scanty. Gradually, as the tube elongates, the older portions continue to collapse. The formation of callose plugs not only gives mechanical strength to the tube but also restricts the protoplasmic streaming to the actively growing region. The cytoplasm between the two callose plugs may also show streaming movement for some time but finally it stops.

After the pollen tube has attained its maximum length, its wall becomes thickened and may sometimes appear like a fibre (Vasil, 1958a, b, 1960a).

Experiments with self-sterile *Nicotiana alata* and Lord Lambourne variety of apple showed that if pollen tubes failed to grow due to incompatibility, there was at least twice as much callose per unit length as in normally growing compatible tubes (Tupý, 1959). In apple this was primarily due to the greater length of the plugs, but in tobacco this was also due to their greater density. In incompatible tubes callose formation is not a primary result of incompatibility but is directly related to the inhibition of their growth. Vasil (1958a, 1960a) has demonstrated that in certain members of the Cucurbitaceae, after the pollen tubes cease to elongate, there is a heavy deposition of callose in the tubes both in vitro and in vivo.

CHEMISTRY

INTRODUCTION

As compared to our knowledge of the storage and culture of pollen, very little work has been done on the chemistry of pollen. The major drawback in this field is the difficulty in securing sufficient quantities

of uniform samples, and consequently studies have been restricted to plants producing relatively large amounts of pollen e.g., *Pinus* and *Zea mays*. Nevertheless, the chemistry of pollen is engaging attention partly because of its importance for physiological studies and partly due to its nutritional and medicinal value.

Pollen grains are of particular interest to the entomologist because they are the chief source of all nutrition, except water and perhaps carbohydrates, required by bees. The nutritional value depends on their chemical contents—inorganic elements, essential amino acids, vitamins, hormones and enzymes. A Russian botanist (Tsitsin; The Sunday Express, London, April 15, 1945) claimed that the longevity of most of the centenarians in Russia depended on their diet of honey or honey scrap (a thick layer of almost pure pollen at the base of the beehive).

Usually the pollen samples are obtained from pellets deposited by bees while passing through pollen traps (see Vivino and Palmer, 1944; Auclair and Jamieson, 1948). The pollen collected by bees is largely from a particular plant species, and there is very little mixture with foreign pollen. However, bee-collected pollen does show some difference as compared to hand-collected pollen from the same plant. This is partly due to certain changes occurring in the bee-pollen and also due to contamination with nectar and honey. Pollen stored in the beehive undergoes lactic acid fermentation and gives rise to the so-called bee-bread.

In 1954 Lundén published "A Short Introduction to the Literature on Pollen Chemistry" which summarises most of the important contributions on the subject. We have, therefore included only selected titles for review on this subject.

PROTEINS AND AMINO ACIDS

Medical men are interested in isolating pollen proteins which are probably the cause of pollen-allergy. Bee-keepers are also interested in this aspect with a view to develop a suitable food for rearing bees.

The technique of chromatography has proved very helpful in the qualitative and quantitative analysis of the amino acids of pollen proteins. Their amount varies from species to species and may be as high as 35 per cent of the total content (Todd and Bretherick, 1942). Almost all the common amino acids have been obtained from pollen grains, and they may occur either in a free state or bound to proteins (Auclair

and Jamieson, 1948; Sarkar et al., 1949; Nielsen et al., 1955; Takashima, 1955; Virtanen and Kari, 1955; Varma and Varma, 1956; Iwanami, 1959). However, the presence of phenylalanine, tryptophane, hydroxyproline, tyrosine and aminobutyric acid is not very common. The amino acid composition of pollen proteins and that of soybean flour, casein and whole egg powder does not show any marked differences (Weaver and Kuiken, 1951).

Considerable work has been done on the protein fractions isolated from pollen, and proteins of comparatively low molecular weight (about 5,000) containing carbohydrates have been obtained (see Lundén, 1954). Free proteins and those conjugated to carbohydrates or pigments, e.g., artefolin, trifidin and pratensin, have been isolated and purified. Nucleoproteins have also been reported in birch pollen (see Lundén, 1954).

CARBOHYDRATES

At various stages of development, pollen grains normally show considerable quantities of starch but invariably it disappears during cell divisions or at the time of shedding. In the first case it is due to the utilization of starch in the laying down of the new cell wall while its disappearance just before the dehiscence of anthers is said to be due to its conversion into sugars (Iwanami, 1959).

Carbohydrates have been frequently detected in protein fractions of pollen extracts, and the presence of starch and fat has been reported in innumerable embryological publications (see Tischler, 1917; Luxemburgowa, 1928; Maheshwari, 1950). Ribose and desoxyribose are invariably associated with nucleoproteins. Kuhn and Löw (1949a, b) reported lactose in the pollen of *Forsythia,* which happens to be the only authentic record of its presence in the plant kingdom. Some pollen also contains cellulose, pentosan and reducing sugars. The protein, carbohydrate and reducing sugar content of the pollen of *Pinus montana, Alnus glutinosa, A. incana* and *Zea mays* has been recently determined by Nielsen et al. (1955). In such studies the mode of collection of pollen is an important factor. For example, bee-collected pollen usually contains large quantities of reducing sugars partly because of the presence of honey or nectar in the cementing fluid, and partly because of the high humidity at which the pollen pellets are stored, which causes enzymatic breakdown resulting in a decreased content of non-reducing sugars. On the other hand mechanically collected and air-dried pollen is rich in non-reducing and poor in reducing sugars (Todd and Bretherick, 1942).

Quadrio (1928) and Mameli (1952) report that generally the anemophilous plants (wind-pollinated) have starchy pollen, while pollen of entomophilous plants (insect-pollinated) is rich in fat and sugar which serve as nutrients for the insects. According to Mameli (1952), there is an association between fatty pollen and porogamy (pollen tube entering through the micropyle), and between starchy pollen and aporogamy (pollen tube following any other path than entering through the micropyle). Such an association does not necessarily hold good since in some plants having starchy pollen the tube does enter through the micropyle (see Maheshwari, 1950).

VITAMINS AND GROWTH-PROMOTING AND GROWTH-INHIBITING SUBSTANCES

The pollen grains of gymnosperms usually have low vitamin content, whereas those of angiosperms are rich in vitamins of the B group but poor in fat-soluble vitamins (Lundén, 1954). Biotin, folic acid, inositol, nicotinic acid, pantothenic acid, pyridoxine, riboflavin, thiamin, ascorbic acid and vitamins A, D, E and K have been reported in the pollen of maize, date, plum and many other plants (see also Ridi and Aboul Wafa, 1950; Weygand and Hofmann, 1950; Nielsen et al., 1955). Pollen grains appear to be highly nutritive materials as far as water-soluble vitamins are concerned. The curative effect of corn pollen on avian polyneuritis was proved by Dutcher as far back as 1918.

Folic acid (also folic acid conjugates and folic acid conjugases) has been reported in the pollen of *Pinus montana, Phleum pratense, Secale cereale* and *Zea mays* (Nielsen and Holmström, 1957). The occurrence of folic acid is of considerable interest, since it "is believed to be an important component of the so-called vitamin T which is reported to be an important growth factor for insects" (Nielsen and Holmström, 1957).

Kakhidze and Medvedyeva (1956) have recently pointed out that the pollen of tobacco excretes considerable quantities of vitamins into the artificial germinating medium. Probably these are liberated even in vivo and are absorbed by the stylar tissue.

In 1956 Nielsen showed that while all other vitamins from the pollen of *Pinus montana, Alnus glutinosa, A. incana* and *Zea mays* did not

show any appreciable change after a year's storage (in a cool, dry place), the pantothenic acid content had substantially decreased in all the samples.

Oestrone and an oestrogenic substance have been detected in the pollen of *Salix* and date palm (see Lundén, 1954; Hassan and Aboul Wafa, 1947). Growth-promoting substances giving positive responses to *Avena*-test are known to occur in the pollen of a large number of plants: maize (Mitchell and Whitehead, 1941; Wittwer, 1943; Redemann, 1951), orchids (Müller, 1953), apple (Larsen and Tung, 1950) and *Pinus densiflora* (Tanaka, 1958). In the acid fraction of corn pollen, Fukui et al. (1958) have reported 3-indoleacetic acid and two other unidentified growth-promoting substances.

Besides the growth-promoting substances, growth inhibitors have also been occasionally reported (Larsen and Tung, 1950; Tanaka, 1958). Tanaka (1958) believes that the slow growth of the pollen tube of *Pinus densiflora* may be due to the presence of inhibitors in its own pollen.

ENZYMES

It is now generally accepted, especially after the publication of O'Kelley's (1955) work, that pollen tubes metabolise externally supplied sugars both in vitro and in vivo. It is also known that the pollen tubes digest the food reserve while passing through the tissues of the style, and this can be possible only if they secrete enzymes. As expected, several enzymes and coenzymes have been reported by different authors in the pollen of many plants, e.g., *Pinus, Digitalis, Fritillaria, Lilium, Nicotiana, Portulaca,* maize, rice, rye and apple (see Paton, 1921; Branscheidt, 1930; Kühlwein, 1937; Haeckel, 1951; Venkatasubramanian, 1953; Lundén, 1954).

Okunuki (1939, 1942, 1943) studied the repiration and coenzyme content of nine pollens, and concluded that the alcoholic fermentation brought about by pollen was identical with that caused by yeast. Haeckel (1951) made extensive studies on the phosphatase, amylase and saccharase activities of the pollen of *Digitalis, Fritillaria, Lilium,* etc. and found that the phosphatase activity was generally as high as in seeds. Palumbo (1953) recently investigated the content of succinic acid dehydrogenase, acid and alkali phosphatase and adenosinetriphosphate in the developing pollen of *Lilium longiflorum* and *Tradescantia paludosa.*

The more important conclusions of Haeckel (1951) are:

(*a*) There is a correlation between the stylar structure and the enzymatic activity. If a stylar canal is present, e.g., in many monocotyledons, the pollen tube does not come in intimate contact with the stylar tissue; therefore, pollen enzymes (amylase or invertase) act only extra-cellularly. When the styles are solid and possess a conducting tissue, e.g., in many dicotyledons, large quantities of phosphatase are found in the pollen. However, there is no correlation between the length of the style and the degree of enzymatic action.

(*b*) The enzymatic activity of the pollen diminishes with ageing and is sometimes as low as two-thirds to three-fourths of the original level. Enzymatic activity also drops from the influence of ultra-violet light, humidity and temperature. Under extremely low temperatures (–190°C) the enzymatic activity can be preserved without any loss for an "indefinite" period; therefore the pollen retains its viability for an equally long period (Bredemann et al., 1947).

(*c*) The amylase present in pollen not only hydrolyses starch but also synthesizes starch from the sugar present in the nutrient medium (Green, 1894a; Branscheidt, 1930; Kühlwein, 1937). According to Branscheidt (1930), it also promotes growth of the pollen tube.

(*d*) Invertase hydrolyses sucrose into monosaccharides which during further enzymatic breakdown liberate energy necessary for the growth of the pollen tube. It also helps in the osmo-regulation necessary for the elongation of the tube.

(*e*) Phosphatase probably plays a role in nuclear division. As a rule, the extreme tip of the pollen tube does not show any phosphatase activity. Although phosphatase is no doubt necessary for elongation of the pollen tube, it bears no relation to the rate of growth.

(*f*) During pollen germination, phosphatase, amylase and invertase activity rise considerably, sometimes as much as 600 per cent.

MISCELLANEOUS NOTES

Several ether-extractable materials, like saturated hydrocarbons, higher alcohols, sterols and fatty acids, also occur in pollen grains (see Lundén, 1954). A number of pigments, mainly flavonols and carotenoids, are responsible for their coloring. Insect-carried pollen is generally rich in carotenes but these are absent in wind-carried pollen.

Pollen grains invariably contain large quantities of pollenin, the

substance forming the thickened exine which makes the pollen 'resistant' to environmental and other factors.

The ash content of pollen usually varies from one to seven per cent and may reveal potassium, magnesium, calcium, copper, iron, silicon, phosphorus, sulphur, manganese and titanium.

Schwarzenbach (1955, 1956, 1957) has recently shown that the germination of pollen grains is often inhibited if the medium includes serum of human patients who have once suffered from jaundice, cancer, carcinoma and sarcoma. Sera of normal human beings or those suffering from other diseases (so far tested) does not affect the germination. In this field our knowledge is very much limited at present, but in due course it may be possible to utilize the inhibitory action of the sera of diseased persons for the detection of various diseases.

Further studies on the chemistry of pollen will undoubtedly prove useful in understanding the causes of loss of viability during storage, for controlling diseases due to pollen-allergy, for detecting some serious diseases, and above all for developing a highly concentrated and nutritious food for human beings.

SUMMARY AND CONCLUSIONS

The present review largely deals with the storage and culture of pollen. A few pages have also been devoted to the chemistry of pollen. Further, attention has been drawn to the utilization of such investigations in plant breeding and horticulture.

In order to retain viability for prolonged periods, a near-freezing temperature and a relative humidity of 25–35 per cent are ideal. The longest period of viability (3287 days at −17° to −37°C) is reported for the pollen of *Pyrus communis* and *P. malus* (Ushirozawa and Shibukawa, 1951). Loss of viability during storage may be due to utilization of stored food materials, desiccation and inactivation of certain vital systems like the enzymes. Stored pollen requires higher concentrations of sugar for proper germination, and if germination is about 35 per cent in vitro, it may result in normal fruit set in the field. In some cases, stored pollen, which may apparently be dead, revives if kept for a few days over comparatively high humidities.

In the U.S.A. and some countries in Europe it is now a standard practice to use stored pollen for artificial pollinations. It would be worthwhile to establish 'pollen banks' in every country so that the pollen of economically important plants could be available throughout the year.

Sugars act as nutritive materials for the germination of pollen as well as for the growth of the tube. However, for proper germination a near-similarity in the osmotic concentration of the nutrient medium and that of the pollen is a prerequisite. The percentage of germination and the length of tubes are directly proportional to osmotic concentration, while bursting is inversely proportional.

The effect of boron on pollen germination and on elongation of tubes is much more marked than the effect of any known hormone, vitamin or chemical. It promotes absorption of sugars and their metabolism by forming sugar-borate complexes, increases oxygen uptake and is involved in the synthesis of pectic materials required for the wall of the actively elongating pollen tube. Pollen of most species seems to be naturally deficient in boron.

Hormones, vitamins, carotenoids, antibiotics and several other inorganic salts help to improve germination in many cases. Recently, a new chemical, gibberellic acid, has also been shown to have a marked effect on the elongation of pollen tube. It is likely that pollen contains adequate quantities of vitamins and hormones; therefore, their addition to the nutrient medium generally does not markedly improve germination and tube length.

Pollen grains germinate in a wide range of pH, but best results are obtained in pH 5.5 to 6.5, and the pH of the medium remains almost unchanged even after the tubes have grown in it for one to two hours. Under normal conditions the pollen of most plants shows optimum germination between 20°–30°C, and the Q_{10} is approximately 2. Higher temperatures cause bursting of pollen and the pollen tubes show abnormal forms.

Pollen tubes of several plants exhibit positive chemotropism towards their own floral parts and sometimes to floral organs of other plants, too. Grouping of pollen grains causes higher percentage of germination and better elongation of the tubes. This seems to be due to the excretion of certain 'growth-promoting' substances from the pollen grains themselves. Extracts of pollen, ovules, ovaries, styles and stigma also stimulate germination.

The growth curves of pollen tubes in vitro are typically sigmoid and do not undergo any alteration, even if the temperature or the nutrients are changed. The pollen tubes of angiosperms usually show rapid pro-

toplasmic streaming which is directly proportional to the rate of elongation of the tube. Callose plugs are commonly formed in the tubes. Under abnormal conditions pollen tubes exhibit various types of deformities.

Pollen grains contain several inorganic substances, carbohydrates, proteins, vitamins, growth-promoting substances and enzymes. Studies on the chemistry of pollen are of vital importance for bee-keeping, treatment of allergies and the development of a nutritious and concentrated food.

In conclusion, it may be pointed out that studies on the physiology of pollen offer great possibilities for overcoming barriers to crossability; understanding the effect of pollination on the growth of fruit; inducing parthenogenesis; obtaining parthenocarpic fruits; increasing fruit set; treatment of pollen allergies and in the detection of some serious human diseases.

ACKNOWLEDGEMENTS

We are greatly indebted to Professor P. Maheshwari for suggesting this field of study, for stimulating discussions, and for the benefit we derived from his vast collection of reprints in the preparation of this review. Thanks are also due to Professor K. V. Thimann (Harvard University, U.S.A.), Professor R. A. Brink (University of Wisconsin, U.S.A.), Dr. Ir T. Visser, (Tea Research Institute of Ceylon, Ceylon) and Professor H. E. Street (University College of Swansea, U.K.) for giving valuable suggestions, and to Dr. (Mrs.) Manasi Ram, who in initial stages assisted in the collection of some material for this review. Our colleagues, Dr. B. D. Sanwal and Dr. S. C. Maheshwari, very kindly helped in the translation of some German literature. The financial assistance received from the Indian Council of Agricultural Research, Ministry of Food and Agriculture, Government of India, New Delhi, in granting a scheme of research on the "storage and viability of pollen grains and physiology of pollen tube growth" is gratefully acknowledged.

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