

COCONUT POLLEN

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SUMMARY

Coconut pollen grains are monocolpate. Those of the variety *typica* measured 0.065 to 0.069 mm. in length and 0.028 to 0.030 mm. in diameter. There were no significant inter-varietal differences in size or shape of pollen grains.

The pollen output per anther in a flower of a healthy tree has been estimated to be between 1,11,000 to 2,21,000 grains. In this respect hybrid palms have been found to contain generally more pollen than the variety *typica*. Among the *nana* varieties, dwarf green was intermediate between dwarf orange, (highest) and dwarf yellow (lowest). All samples contain a small percentage of "dummy" pollen and this fraction is reported to increase in diseased palms.

Studies conducted in India have revealed a diurnal variation in pollen catches, with pollen catches during the night significantly lower than during the day. A seasonal variation was also observed in production of pollen. Atmospheric pollen catches in March and April were found to be significantly higher than during other months. There was an increase of yield of nuts during periods when pollen catches were heavy and vice versa.

Pollen studies conducted at this Institute were made necessary to implement a comprehensive breeding programme, making the maximum use of a few palms identified as prepotent, through progeny tests. Consequently our trials were limited to a study of collection and storage of pollen and related topics. Though each male flower is reported to shed an excessive amount of pollen, particular periods are more favourable for collection of pollen. The male phase of the variety *typica* is estimated to last 18 to 22 days, with the optimum period for collection of pollen generally between the third to the eighth day after opening of the spathe. A large quantity of viable pollen could be collected by leaving anthers in large desiccators maintained at 10-25 per cent relative humidity. Coconut pollen is short-lived under atmospheric conditions, but is amenable to storage under artificial conditions. When stored in desiccators at about 50 per cent relative humidity, viability could be maintained for about 14 days and this is sufficient for ordinary work. With limitation of collection of pollen to a few palms, and particularly in carrying out programmes of in-breeding, or when the pollen parent is located at a great distance from the pollination stations, it is necessary to store pollen for very much longer periods. A new technique of pollen storage is described, where with the combined action of low temperatures [0°C] and 50 to 65 per cent relative humidity, pollen remains viable for a year or more. Gelatine powder and lycopodium were tried as diluents to minimise wastage during pollination, and the latter proved to be very useful for this purpose.

Stored pollen has to be tested before issue for pollination work, to ensure that at least 50 per cent of the grains are viable. 10 per cent sucrose and 2 per cent gelatine is a satisfactory culture medium for pollen viability tests. The incorporation of traces of borax in this medium reduces the occurrence of burst pollen tubes and also promotes rapid growth of pollen tubes, thus facilitating viability counts under the microscope.

Our pollen dispersal studies were largely limited to determine the extent of pollen transport in the atmosphere, and it was observed in the course of a few simple trials that coconut pollen is not carried through a thick jungle belt of about 350 yards even when winds are favourable. This fact was made use of in determining the degree of spatial isolation necessary in the establishment of an isolated Coconut Seed Garden.

INTRODUCTION

Pollen grains differ greatly in size and in configuration from one species to another, but their physiological role is similar in all species. Some plants produce pollen only sparingly, others with great prodigality. Tremendous over-production of pollen is typical in general of the more primitive seed plants. Although wasteful in the extreme, production of pollen in numbers over and above that required for pollination increases the possibility of a few grains being blown by wind to the stigmas of flowers thus effecting pollination. A single corn plant, for example, is estimated to release 50,000,000 pollen grains of which only about a thousand grains are required for fertilization of all the ovules in one plant. However, with specialisation of flowers for insect pollination by developing such features as colour, scent and nectar, the organised conveyance of pollen from anthers to stigmas of flowers proved to be more efficient than reliance on the wind, and much less pollen is produced in entomophilous flowers.

Pollen studies are undertaken primarily for two purposes. Palynology or the study of pollen and spore science deals chiefly with the walls of pollen grains and spores and not with their live interior. Such morphological studies are of taxonomic value and often of use in the classification of plants where other characters may be of doubtful value. Even in fossilized plants impressions of pollen grains and spores are often obtained. Pollen grains of a species are characteristic in size and shape, and number and position of furrows and such morphological features. The other aspect of pollen study is more concerned with the live interior of pollen—the physiology of pollen, its nutrition *in vivo* and *in vitro*, pollen longevity, viability and related topics. Such a study is a necessary pre-requisite in implementing any large-scale breeding programme.

POLLEN MORPHOLOGY

At the time of shedding, coconut pollen grains are more or less spherical with a smooth exine. On exposure to the air they assume an ellipsoidal shape with a prominent furrow running along the length of the grain. This one-furrowed or monocolpate type of grain besides occurring throughout the Cycadales is characteristic of many monocotyledons and primitive dicotyledons. The presence of a groove or grooves does not necessarily mean that the grains are primarily adapted to be carried away by wind.

Pollen grains of the variety *typica* measured 0.065 to 0.069 mm. in length and 0.028 to 0.030 mm. in diameter. There was no appreciable difference in size between pollen from pre-potent palms and that from ordinary *typica* palms. On the addition of water, sucrose solution or 0.5 per cent acetocarmine, pollen grains rapidly absorb moisture and assume a very even shape with an average diameter of about 0.05 mm. It is reported that the number of pollen grains varies from 1,11,000 to 2,21,000 per anther in a flower of a healthy tree, (Varkey and Davis, 1960). Gangolly *et. al.* (1961) report that hybrid palms have been observed to contain generally more pollen than the Tall varieties. They further report that among the Dwarf palms examined, Dwarf green was observed to be intermediate between the Dwarf orange (highest) and Dwarf yellow (lowest), in respect of pollen production.

In the air dry condition the majority of pollen grains conform to one size and shape. However each sample carries a small fraction, usually below 10 per cent, of shrivelled grains which are non-viable and often appear to be devoid of cytoplasm. This is referred to as "dummy pollen" by certain workers who further state that the percentage of these dummy grains increases in diseased palms, (Varkey and Davis, 1960).

POLLEN DENSITY AND DISPERSAL STUDIES

This aspect has been very extensively studied in India, by Gangolly, Kamalakaran, Balakrishnan and Pandalai, (1961). They report "that there is a significant seasonal influence on production of pollen in individual male flowers as well as total production of pollen in the inflorescences. Pollen production in individual flowers has been observed to be significantly higher in summer and cold seasons than in the rainy season. Studies on atmospheric coconut pollen catches made by the use of an aeroscope have indicated the influence of climatic features in the different months on pollen content of the atmosphere. Pollen catches during March and April have been observed to be significantly higher than those recorded in the other months of the year. There appears to be a possible relationship between pollen catches in certain months of the year and the corresponding yield of nuts. During periods when pollen catches are heavy there is a corresponding increase in yield of nuts and *vice versa*. A diurnal variation in coconut pollen catches has been observed, with coconut pollen catches during the night significantly lower than that during the day."

Our trials conducted in 1954-1955 were largely limited to determine the distance to which coconut pollen is wind-borne through a thick jungle barrier. This was a necessary step to determine the extent of spatial isolation necessary in the establishment of an isolated Coconut Seed Garden, (Annual Report of the Botanist 1955). Preliminary trials indicated that pollen catches were greater at a height of about 20 feet above ground than at either higher or lower levels, and similar results have been obtained by other workers, Gangolly, *et al.* (1961). Slides (area of each 8.75 square inches)-smearred with neutral glycerine jelly were mounted on aeroscopes and fixed on trees at four points within a thick jungle. The points were 10,100,200 and 350 yards from the nearest coconut plantation. The slides were removed every four hours and the pollen grains on each slide were scored. The results indicated that coconut pollen is not wind-borne through a jungle belt of about 350 yards even when the winds are favourable, although a fair percentage of pollen was found up to about 200 yards.

COLLECTION OF POLLEN

The structure of the coconut inflorescence has been reviewed earlier, (Liyanage, 1949, 1950). The period between the opening of the first male flower, which generally coincides with the bursting of the spathe, and the abscission of the last male flower is termed the male phase. This varies according to the age and physiological condition of the tree and possibly the nature of the environment too. Patel (1938) observed that most of the male flowers had opened by the fifteenth day after opening of the spathe. In Ceylon, Liyanage (1950) estimated the male phase of the variety *typica* to last 18 to 22 days. Whitehead (1964) reports that in the "Jamaica tall" and "San Blas" varieties, the male cycle lasts 22.1 and 23.1 days respectively.

Though pollen is available in immense quantities, collection of pollen and its storage is beset with many difficulties. Earlier methods of pollen collection were based on gathering the pollen that fell off opened male flowers on spikelets whose cut ends were placed in water. (Liyanage 1954; Manthirratna and Liyanage 1961). It was found that the optimum period for cutting of spikelets for collection of pollen

is generally between the third to the eighth day after opening of the spathe. Very little pollen could be obtained before the third day and after about the eighth day due to fewer opened male flowers on the spikelets. It is observed that after the peak is reached a large number of male flowers drop off the spikelets without opening and shedding pollen.

With pollen collection being limited to prepotent palms a large quantity of pollen has to be collected to make the maximum use of these proved palms for breeding purposes. Recent attempts to increase the quantity of pollen that could be collected from each inflorescence are reported and these methods are based on the induced shedding of pollen by the use of pressure, suitable temperature or a combination of both on unopened male flowers (Whitehead, 1963). A modification of this method was used to obtain large quantities of viable pollen by drying anthers dissected from unopened male flowers (Annual Report of the Botanist, 1963). It was possible to obtain 9.0 grammes of pollen from a single inflorescence of a prepotent palm whereas using opened male flowers on cut spikelets not more than a gramme could be collected from the same source.

During the course of extensive trials it was observed that the viability of pollen collected in the above manner decreased rapidly with prolonged storage although the initial viability was about 60 per cent. The method which is currently in use is to dissect anthers from un-opened male flowers and leave them in petridishes placed in desiccators maintained at 10-25 per cent relative humidity at laboratory temperatures. The humidity is controlled by the use of appropriate concentrations of sulphuric acid. After about 20 hours the anthers dehisce and the pollen can be separated from the debris by sieving through a BS/410 100-mesh sieve. The pollen collected by this technique gives an initial viability of 60-70 per cent, and there is no rapid fall in viability with storage at deep freeze conditions.

POLLEN STORAGE

Under atmospheric conditions pollen grains gradually lose their capacity for germination and probably also for fertilization. In the coconut, viability of pollen is lost in about 48 hours at temperatures and humidities prevailing in the laboratory. As early as the end of nineteenth century it was realised that longevity of pollen was closely related to temperature and humidity. Both Sandsten (1908) and Roemer (1915) observed a longer viability of pollen under dry conditions and at lower than at higher temperatures. While the pollen of most species is amenable to storage for varying periods that of the Gramineae is reported to be extremely short lived. This is attributed to desiccation of pollen in storage and according to Knowlton (1922) early death of pollen in storage is largely caused by this factor.

Several methods of storage of coconut pollen have been reported. According to one method pollen is stored in desiccators at about 50 per cent relative humidity obtained with the use of 43.4 per cent sulphuric acid. About 50 per cent of the grains remain viable up to about 14 days of storage, (Annual Report of the Botanist, 1957). Similar results have been quoted by Whitehead (1963), who further states that the initial condition of the pollen is probably more important than the humidity during storage.

With limitation of collection of pollen to prepotent palms it is necessary to store pollen for very much longer periods and with this object in view a series of trials were undertaken. Earlier work indicated that pollen grains remain viable for about 14 days at 50 per cent relative humidity at room temperature. Subsequent trials were carried out combining different relative humidities with low temperatures. Different concentrations of sulphuric acid were used in desiccators to give relative humidities of 10, 25, 35, 50, 60, 75 and 90 per cent. Pollen collected from several palms was bulked together and small quantities introduced into narrow glass tubes sealed at one end. The tubes were left in desiccators at the above

humidities for about two hours after which they were taken out and the open ends sealed by heating with the least delay. The sealed samples were left in a refrigerator (0°-4°C) and viability tests carried out at 15 day intervals. It was observed that viability fell rapidly at lower humidities, 10 to 25 per cent, and less rapidly at the higher humidities with best results in the range 50 to 65 per cent relative humidity. Pollen storage in the range 50-65 per cent relative humidity at 0°C was given an extended trial. The majority of samples stored in this manner have maintained over 50 per cent viability for over six months (Annual Report of the Botanist, 1957), with some samples retaining adequate viability for over a year or more. Whitehead (1962) reports that freeze dried coconut pollen could be stored for prolonged periods at room temperature, and that the viability after prolonged room temperature storage is in excess of that normally required for pollination. It is reported that further experiments are in progress to determine the optimum period of freeze drying. There appears to be no uniformity in the viabilities of stored samples and this may be largely due to imperfect sealing techniques or to inherent differences in the pollen source. These factors have not been investigated so far. Similar observations have been made by Whitehead (1963), who states that for reasons not yet understood, results of storage for longer periods were again inconsistent. It is reported that the pollen of many species could maintain viabilities for long periods at deep freeze temperatures. Ushirozawa and Shibukawa (1951) noticed a slight germination in the pollen of many fruit trees even after nine years which is the longest viability so far recorded. Visser (1955) also believes "that pollen stored at such low temperatures can be given eternal life, provided that it is resistant".

DILUENTS FOR COCONUT POLLEN

The Institute has identified a few prepotent palms through progeny tests and the maximum use has to be made from the little pollen available from these in carrying out programmes of controlled pollination. To minimise wastage during pollination it is necessary to 'adulterate' pollen with foreign materials preferably in powder form. According to Johri and Vasil (1961) over thirty substances have been tried as diluents, some of the more common ones being lycopodium powder, powdered egg albumen, casein and talc. It is suggested that in addition to their principal role, most diluents form an effective 'packing material' around the pollen grains, thus buffering the effect of external factors. Two substances were tried with coconut pollen, namely finely powdered gelatine and lycopodium powder. The former used in the ratio 1 of pollen : 8 of gelatine had no adverse effects on pollen viability. However, gelatine often aggregates the pollen into lumps and does have the disadvantage of absorbing moisture from the stigmatic surface at time of pollination making pollination brushes sticky and subsequent pollinations more difficult. Although Bullock and Snyder (1946) have reported that such diluents reduce pollen viability and cause a significant decrease in fruit set, quite contrary results were obtained with coconut pollen with viabilities being maintained for long periods.

Lycopodium as a diluent was tried on a large scale since October 1958. The dry powdery nature of this material is advantageous in many ways. The pollen mixes well with no tendency of clumping and pollinations are made easy as the pollen lycopodium mixture readily falls off the brush during dusting of pollen. Lycopodium confers a further indirect advantage as the spores remain unstained in acetocarmine solution in sharp contrast to the stained pollen grains and pollen tubes thus facilitating viability counts. Mixtures consisting of pollen/lycopodium in the ratio 1 : 10 have maintained viability for long periods as judged by both germination tests as well as percentage set of female flowers when used in subsequent pollinations. (Table 1). However, it should be borne in mind that the initial condition of the pollen at the time of collection and sealing will greatly influence the viability of pollen with prolonged storage.

TABLE 1

Setting of ♀ flowers at Marandawila Estate (Jan.-Dec. 1962) using Pollen/lycopodium
(1 : 8) mixture stored at 0°-4°C

Month	No. of Palms	No. of ♀ fls. pollinated	No. of buttons developing at 8 weeks	% set at 8 weeks	No. of nuts harvested	% set at harvest	Pollen sample No.	Age of pollen (days)	Pollen viability (per cent)
January ..	33	574	304	53.0	201	35.0	21/1257	124	62.8
February ..	41	654	317	48.4	197	30.1	21/1257	152	62.3
March ..	17	327	146	44.6	85	26.0	21/1257	183	not tested
April ..	51	831	469	56.4	325	39.1	21/1344	74	not tested
May ..	40	719	377	52.4	319	44.4	21/1344	105	not tested
June ..	55	921	570	61.8	548	59.5	21/1267	288	63.0
July ..	34	625	379	60.6	324	51.8	21/1267	320	not tested
August ..	50	942	567	60.1	520	55.2	21/1267	351	not tested
September	22	423	236	55.7	174	41.1	21/1267	381	not tested
October ..	14	270	147	54.4	100	37.0	21/1267	396	not tested
November	42	758	425	56.0	256	33.8	25/1404	107	not tested
December ..	35	600	331	55.2	165	27.5	25/1404	138	not tested

The difference in percentage set 'at 8 weeks' and 'at harvest' could be attributed to shedding of nuts during the growth cycle following fertilization.

POLLEN VIABILITY

The establishment of a 'pollen bank', with collection and storage of pollen from pre-potent palms, (Annual Report of the Botanist, 1959) and the subsequent issue of pollen to the Institute's pollination stations as well as to private estates implementing their programmes of controlled pollination, necessitates quick and reliable methods of testing pollen viability, (Annual Report of the Botanist, 1961).

Pollen viability could be tested in different ways. Aceto-carmin is often used for this purpose. It is generally recognized that aceto-carmin stains both nucleus and cytoplasm irrespective of whether they are living or not. It is safe to conclude that pollen grains that do not stain in aceto-carmin are non-viable although the reverse may not always be true. Vieitzev (1952) determined the viability of maize pollen by the use of a 2 per cent solution of 2, 3, 5—triphenyltetrazolium chloride. The tetrazolium salt was reduced to red triphenyl formazan by viable pollen, while dead pollen produced no red colour. This test is reported to be possible only in pollen with a thin and colourless exine. The reduction of nitro-blue tetrazolium as an index of pollen viability is reported by Hauser and Morrison (1964). The peroxidase reaction as an indicator of pollen viability, (King, 1960) was tried with coconut pollen. Although the colour reaction was characteristic of the test, there was no possibility of assessing percentage viability with any degree of reliability.

The *in vitro* germination of pollen has been found to be a reliable test of pollen viability in a number of crop plants. Pollen of some species may germinate over a wide range of conditions, while certain other types may require a set of specific conditions failing which there may be no germination, (Johri

and Vasil 1961). The pollen grains of a few species are reported to germinate in water, but even if they do, germination is poor and the tubes are short. Coconut pollen does not germinate in water and germination is poor in sucrose alone in the range 5 to 30 per cent, with a few stunted pollen tubes which usually burst. It is reported that water from tender nuts provides a good germinating medium, and germination has been also observed in a weak solution (1/8000) of hydrogen peroxide, (Menon and Pandalai, 1958). Whitehead (1963) has germinated coconut pollen using 5, 10, 15 and 20 per cent sucrose, combined with 10, 20 and 30 per cent gelatine with each concentration of sucrose, and according to him there is no difference between germination in the different sucrose concentrations. In most instances the highest gelatine concentrations (30 per cent) appeared to be the most suitable.

10 per cent cane sugar and 2 per cent gelatine was found to be a satisfactory medium for pollen germination, (Annual Report of the Botanist, 1948), and all subsequent work connected with pollen viability carried out at this Institute is based on this medium. Pollen grains are dusted onto the medium contained in a covered watch glass and the percentage germination is counted under the microscope after a lapse of about two hours. The use of 0.5 per cent aceto-carmin on the germinated pollen greatly facilitates viability counts as the germinated grains with pollen tubes are deeply stained in sharp contrast to the unstained lycopodium spores as well as "dummy grains". One drawback in this technique was the occurrence of burst pollen tubes which made counting difficult and at times inaccurate. Varkey and Davis (1960) report that "the natural medium *i.e.* the stigmatic secretion gave the maximum germination with 5 per cent cane sugar and 2 per cent gelatine as the next best". They further state that "in the course of these studies for germination several pollen grains were observed to have burst open and the inner contents to have purged out. Such pollen grains were considered to be ungerminated". This phenomenon was also observed by earlier workers. Andronescu (1915) and Kearney and Harrison (1932), describe the bursting of pollen in nutrient solutions as 'pseudo-germination' and 'ejection'. The bursting could occur either without the formation of a pollen tube, or if a pollen tube is formed, anywhere along its length, but more often at the base of the pollen tube. Bursting liberates the bulk of the cytoplasm *en masse*. However, it is likely that pollen which bursts *in vitro* may germinate to produce perfectly normal pollen tubes on the stigma under natural conditions, so that in germination tests it may be incorrect to write off burst pollen grains as non-viable. Thus if bursting could be reduced or altogether eliminated it would greatly facilitate and quicken pollen viability counts and make such determinations more accurate.

BORON AND POLLEN TUBE GROWTH

The element boron is believed to play an important part in the reproductive cycle of flowering plants and this was confirmed from Schmucker's discovery of the stimulating effect of boric acid on pollen physiology. He found that in water lilies pollen tubes failed to develop *in vitro*, but germinated readily in stigmatic extract. Analysis of stigmatic extract confirmed the presence of appreciable amounts of boric acid, which if added to the culture medium in the same concentrations promoted pollen tube growth. This proved conclusively that boron plays an important role in the reproductive cycle particularly in pollen germination and post-pollination processes. Similar results were obtained by other workers in *Vitis* and *Lilium*, O'Kelley (1959). Vasil (1960), who investigated the effect of various concentrations of boric acid on the pollen of a number of crop plants reports that 100 to 150 p.p.m. (0.01-0.015 per cent) of boric acid is favourable to pollen germination while higher concentrations are definitely inhibitory.

Pollen is said to be naturally deficient in boron, (O'Kelley, 1957) and in nature this deficiency is probably made up by the boron from the stigma, style and related tissues. Two contrasting views have been expressed on the role of boron in pollen germination and tube growth. Gauch and Duggar (1953) proposed that "boron combines with sugar to form a sugar-boric acid complex which is translocated with greater facility than are non-borated non-ionized sugar molecules". Visser (1955) expressed the view that "boron plays an important part in carbohydrate metabolism" and suggested that "the degree of boron sensitivity of the pollen is inversely related to the boron level of the plant".

Boron in the form of an aqueous solution of borax was experimented with in the *in vitro* germination of coconut pollen. Lower concentrations of borax did not control the bursting of pollen tubes, whereas higher concentrations inhibited germination of pollen completely. The use of an aqueous solution of 0.1 per cent borax in the ratio 1 part of borax: 10 parts of a mixture of 10 per cent sucrose and 2 per cent gelatine gave very good results with a minimum of bursting with long well defined pollen tubes. Nambiar (1960), too reports that germination of pollen in the standard medium is improved by the addition of boric acid. It was observed that the speed of germination was greatly enhanced and as reported by other workers "the effect on the elongation of the tube is much more marked than on percentage germination of pollen". According to Visser 'boric acid is specifically required for the growth of the pollen tube'.

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