Pollen Fractionation

A method of increasing the viability of pollen samples

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The objects of pollen fractionation are: —

1) To clean and dry pollen in preparation for storage, or to re-humidify it before use.
2) To bring separate samples to equal viability for use in the Polycross Test.
3) To reduce the bulk, at the same time increasing the viability so that each batch of pollen can be given special care in fairly elaborate storage techniques, e.g. vacuum drying or deep freeze.

These requirements have been met to a certain extent by apparatus in which the pollen is winnowed or fractionated into "live" and "dead" portions by a gently moving vertical column of air passing upwards through the sample. If the speed of the air is finely adjusted a point is reached with each species when the dead grains, being lighter, float upwards and are carried away, the portion that is left being of higher viability than the original sample. The air-flow must not fluctuate and must be constant at any given pressure.

Two models of pollen fractionator have been built so far. The first was intended solely for pollen of *Pinus* species and quite low air-speeds were adequate to move the small grains. Figure 1 shows this model. Air was supplied from a small centrifugal compressor which was taken via a two-way tap and a high or low airspeed line to the column, a glass tube 80 cm. X 2.5 cm. diameter. A manometer containing water or ether was used to check the pressure of the air entering the column. The sample of about 10 ml. of pollen was introduced into the base of the column, being prevented from contaminating the lead-in lines by muslin and cotton-wool fiiters. Then the air pressure was gradually turned up until, under a hand lens used at the

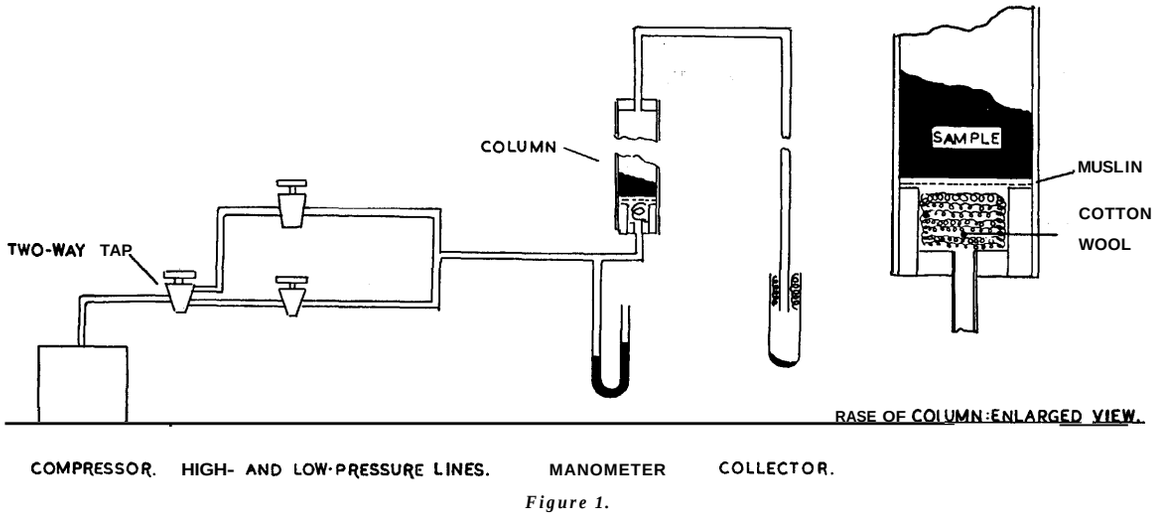

Figure 1.

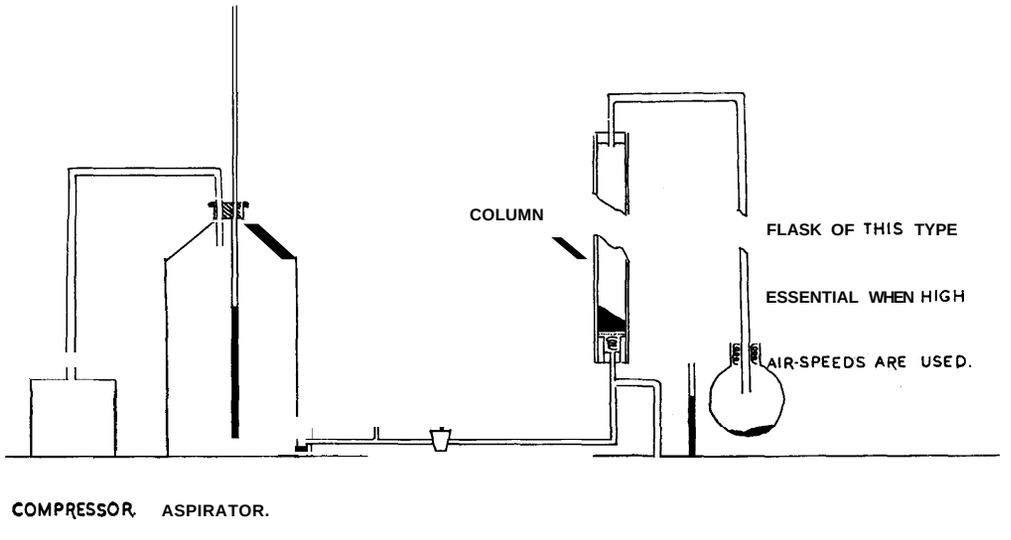

Figure 2.

column's top, some grains were seen still to be moving upwards. This pressure was maintained for about half an hour, when about half the sample had separated into a test-tube placed at the end of the 7 mm. bore tube used to carry away the effluent grains. The test-tube having been changed, the tap was turned to the high pressure air line and the flow adjusted so that the heavier grains were carried over, only clogged grains and dirt left in the pollen by the original filtering process being left in the column. Having once been adjusted as given above, the air lines can merely be switched from low to high pressure when further samples of the same species are being processed.

The second model, a diagram of which is given in figure 2, was built to handle various species of pollen of widely differing sizes and weights. The main difference from the first model is in the use of a reciprocating compressor, the airflow from which is stabilised in a 5 litre aspirator. This has its own manometer in addition to the one fitted later on in the line: this acts as a safety-valve should one of the lines become blocked and also provides a means of checking the pressure in two places along the line. For lower ranges of pressures the tap A is fully open allowing the majority of the air to escape, the tap B adjusted to give the correct working pressure on the second manometer. When higher pressures are required tap A is progressively closed and B opened, trying to keep the pressure on the first manometer constant. This keeps the *total* airspeed constant and there will be no changes in humidity over the experiment as a whole. The column used was the same as in the first model.

It was felt that the introduction of the aspirator into the equipment, with its rather large expanse of water at the bottom, might raise the humidity to an undesirable level. Table 1 shows humidity readings taken (1) in the laboratory, (2) in the column with no desiccant added in the airline, (3) with a calcium chloride tube 7 × 2 cm. and bottle 14 × 6 cm., and (4) with a Silica gel tube 7 × 2 cm. Sulphuric acid was found to be unsatisfactory as a desiccant in this instance, as the airspeeds used caused misting of the acid with consequent contamination of the equipment.

Table 1. — Humidity levels in Pollen fractionator, second model

Treatment of air-stream (Laboratory humidity 55%)	Humidity in column	Pressure in second manom. (cm Water)
Untreated: air line with no desiccant	64% 58%	6 22
Airline with 7×2 cm. $CaCl_2$ tube	47% 49%	6 22
Airline with 14×6 cm. $CaCl_2$ tube	42% 44%	6 22
Airline with 7×2 cm. Silica gel tube	50% 51%	6 22

The use of silica gel is recommended as it can be reconstituted and does not liquify, but it does not dry the pollen as effectively as calcium chloride. For most practical purposes no drying is necessary however, as the pollen seems to undergo no deterioration when stored for some time after fractionation.

The table shows differences in humidity for different *total* airspeeds through the apparatus. If the total airspeed remains constant, no difference was recorded in humidity between column pressures of 6 cm. and 22 cm.

The results of preliminary trials of this method of pollen fractionation are encouraging. Table 2 shows viability counts from slides made by the Methyl green/phloxin/glycerine jelly technique (Owczarzak, 1952), both before and after fractionating, with samples from high and low airspeed fractions from the latter. The pressures in cm. water or ether are also given.

Best fractionation has obtained from the sample of *Pseudotsuga taxifolia*. This is thought to be due to the small variation in grain size, coupled with the fact that they have not the large air-sacs seen in the pollen of *Pinus* or *Picea* species. The results given are for 20—60 minutes fractionating time at the low pressure stated. If greater concentration is required the samples can be subjected to a longer period of fractionation at a slightly lower pressure. As an example the result is given for Scots pine 'Hesse 1' pollen mixture, where insufficient gain in viability resulted in a second, longer run with another sample.

Picea breweriana pollen was chosen as the representative of this genus as there was plenty in store for testing purposes.

When pollen is of high viability but is contaminated with dirt, small pieces of the male strobile and clogged grains, these may be cleaned out quickly by fractionation at high pressure. Another use is to ensure the homogeneity of pollen mixtures: all the pollen samples are tipped into the column and high pressure used to clean and mix the pollen simultaneously.

Future developments of this apparatus with higher airspeeds might be used for winnowing seed, provided they had been previously dewinged.

Summary

A method of fractionating pollen samples to increase their effective viability is described. The apparatus consists of a compressor supplying a steady flow of air to a vertical glass tube into which the pollen is placed. The air-flow is varied until the dead, light gains float upwards and are carried away. Then the pressure is turned up further and all except the dirt and clogged grains is carried into a receptacle connected to the top of the glass tube. Results are given for *Pinus, Picea, Pseudotsuga* and *Larix* species, best results being obtained from the two latter which have no large air-sacs. In general, half an hour at the low pressure results in a doubling of the viability relative to an untreated sample. As well as increasing the viability of the samples, the apparatus may be used for conditioning the pollen to a wide range of humidities, and cleaning it prior to use or storage.

Zusammenfassung

Titel der Arbeit: *Pollenfraktionierung. — Eine Methode zur Steigerung der Lebensfähigkeit von Pollenproben.*

Eine Fraktionierungsmethode für Pollenproben zur Steigerung ihrer Lebensfähigkeit wird beschrieben. Die Apparatur besteht aus einem Kompressor, der einen gleichmäßigen Luftstrom erzeugt und in einen vertikalen Glastubus bläst, in dem sich der Pollen befindet. Der Luftstrom wird solange variiert, bis die toten und leichten Pollenkörner hochfliegen und weggeweht werden. Dann wird der Druck weiter erhöht, bis alle übrigen Pollenkörner mit Ausnahme der Schmutzteilchen und der verklumpten Pollen in ein Gefäß fliegen, das sich am Ende eines Glasrohres befindet. Es werden Ergebnisse für *Pinus-, Picea-, Pseudo-*

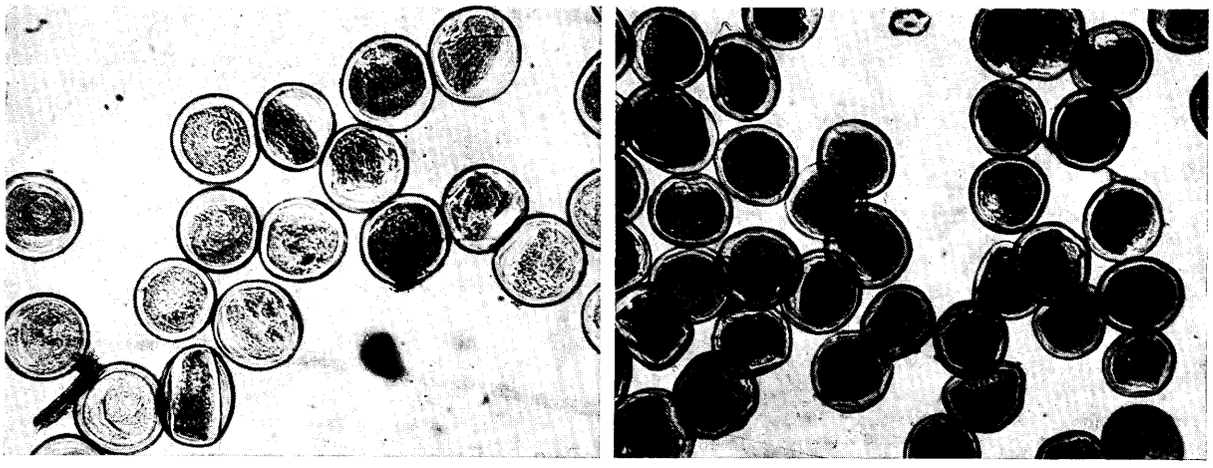

Figure 3. — Pollen of *Pseudotsuga taxifolia* after fractionation. — (a) Sample carried away from column, viability 1.8% (× 120). — (b) Sample retained in column, viability 98.4% (× 120). — In both pictures viable grains have dark centres. The original sample had a viability of 51.3%.

Table 2

Species and Origin	Mean size of grain (μ)	Amount fraction-ated	Low pressure (cm.)	High pressure (cm.)	Time at low press. (min.)	Time at high press. (min.)	*Viability %*		
							Untreated	Low pressure	High pressure
Pinus sylvestris (Trentino 1 pollen mixture)	67.5	10 ml.	4.5 Ether	7.0 Ether	30	5	12.3	7.7	54.0
Hesse 1 (pollen mixture)	66.3	10 ml.	7.0 Ether	11.0 Ether	20	5	3.8	2.5	5.7
	66.3	10 ml.	4.5 Ether	11.0 Ether	60	5	3.8	2.0	10.4
Pinus banksiana (pollen mixture)	55.0	5 ml.	1.6 Ether	5.8 Ether	20	5	50.0	32.4	86.6
Picea breweriana single tree	126.5	25 ml.	6.0 Ether	10.0 Ether	20	5	20.0	14.7	44.3
Pseudotsuga taxifolia plus tree no. 278.	101.2	10 ml.	16.0Water	28.0 Water	30	10	51.3	1.8	98.4
Larix leptolepis plus tree no. 7.	77.5	5 ml.	21.0Water	38.0 Water (maximum possible with the apparatus, but still not enough to move some of the grains)	20	15	47.6	38.4	77.2

tsuga- und *Larix*-Arten mitgeteilt. Die besten Resultate wurden bei den beiden letzten Gattungen erhalten, deren Pollen keine großen Luftsäcke besitzen. Im allgemeinen ergibt eine halbstündige Behandlung bei niederem Druck eine Verdoppelung der Lebensfähigkeit gegenüber unbehandeltem Pollen. Genauso wie zur Steigerung der Lebensfähigkeit der Proben kann der Apparat auch zur Pollenprüfung in weiten Feuchtigkeitsbereichen oder zur Pollenreinigung vor der Verwendung bzw. der Aufbewahrung benutzt werden.

Résumé

Titre de l'article: *Tri du pollen par fractionnement suivant la densité, en vue de l'amélioration de la viabilité des échantillons de pollen.*

Les échantillons de pollen sont fractionnés suivant la densité en vue d'améliorer leur viabilité réelle. L'appareil comprend un compresseur qui fournit un courant d'air rapide dans un tube de verre vertical qui contient le pollen. Le débit de l'air est réglé jusqu'à ce que les grains de pollen morts plus légers se rassemblent à la partie supérieure et sont emportés, puis la pression est augmentée et tous les bons grains de pollen, à l'exception des impuretés et des grains agglomérés, se rassemblent dans un récipient relié à la partie supérieure du tube. Des résultats sont donnés pour diverses espèces de *Pinus, Picea, Pseudotsuga* et *Larix*, les deux dernières donnant les meilleurs résultats (elles n'ont pas de grands sacs polliniques). En général, un traitement d'une demi-heure à basse pression donne un pollen dont la viabilité est double de celle du lot non traité. L'appareil peut également être utilisé pour obtenir du pollen à des taux d'humidité divers et pour le nettoyer avant l'emploi ou la conservation.

Reference

Owczarzak, A.: A rapid method for mounting pollen grains with special regard to sterility studies. Stain Technology **27**, 249—251 (1952). — Methyl green/phloxin method also mentioned from this reference in Medical and Biological Staining Techniques (E. Gurr), published Hill, London, 1956.