- Prescott, J. A., and G. B. Stirk. 1951. Studies of the Piche evaporimeter. *Aust. J. Appl. Sci.* 2: 243-256.
- Van Bavel, C. H. M., F. S. Nakayama, and W. L. Ehler. 1965. Measuring transpiration resistance of leaves. *Plant Physiol.* 40: 535-540.
- Wallihan, E. F. 1964. Modification and use of an electric hygrometer for estimating relative stomatal apertures. *Plant Physiol.* 39: 86-90.

DETERMINATION OF ABSOLUTE POLLEN FREQUENCY¹

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Abstract. Changes in technique have simplified and made more reliable a method for determining the absolute numbers of pollen grains in sediment samples. The prepared sample is suspended in its entirety in the volume of fluid from which measured portions are taken for counting; tertiary butyl alcohol, instead of benzene with dissolved silicone fluid, is used as the suspending fluid.

The absolute numbers of pollen grains in sediment samples may yield valuable information about the environment at the time the sediments were deposited. Traditionally pollen analyses have involved only the proportions of different pollen types within the total counted, but recently absolute numbers as well have been included. The increased interest stems from the availability of sediment age determinations by the radiocarbon method. A number of age determinations from different levels within a core permits estimation of the time interval represented by the thickness of a single sample. Correction by this means for the variable lengths of time represented by different types of sediment allows meaningful comparison of the absolute pollen content of samples of different ages (Davis and Deevey 1964, Whitehead 1966). Assays of absolute numbers of pollen grains are also essential to the use of sediment traps, which measure modern rates of pollen deposition in lakes (Davis 1965*b*). A simple and accurate means for determining numbers of pollen grains presents new possibilities for laboratory experimentation on problems that have long concerned palynologists, such as the effects of preparation procedures on the pollen content of samples. The assay method can also be used for direct measurement of pollen productivity through measurement of the numbers of pollen grains per flower. It may be supposed, too, that a method perfected for pollen may also be useful for assaying other kinds of particles that are similar in size and weight to pollen grains.

In a recent paper (Davis 1965*a*) I described a method for determining the absolute numbers of pollen grains in sediment samples. Subsequently I simplified this method and improved its reliability. The modifications, reported here for those who wish to study absolute as well as relative pollen frequencies, are two: the entire sample, rather than a subsample, is now suspended in the volume of fluid from which measured portions are taken; and tertiary butyl alcohol, rather than benzene with dissolved silicone fluid, is used as the suspending fluid.

After quantitative sediment samples have been prepared in the laboratory, the pollen-containing residue is dehydrated by successive washes in absolute ethanol. The residue is washed once with tertiary butyl alcohol and is then transferred, with additional tertiary butyl alcohol,

to a graduate. The residue and its suspending alcohol, now of known volume, are poured into an Erlenmeyer flask, to which are added additional measured rinses of the graduate until the desired total volume (from 200 to 1,000 ml, depending on pollen frequency) has been attained. The alcohol is viscous, so care must be taken that the graduate drains well each time it is emptied. The temperature of the alcohol should also be kept constant.

A stirring bar is then placed in the flask, which is corked and placed on a magnetic stirrer. The sample must be stirred at high speed for at least 5 min to obtain even suspension of pollen; in the case of large volumes, 10 or 15 min is recommended. Stirring continues while measured portions of the suspension are removed by pipette. Pipetting is done with short-style pipettes with orifice diameter greater than 1.5 mm (Bellco 12-318 spl). The volume within the pipette is controlled with a syringe attachment; this can be made easily from a sawed-off tuberculin syringe with stopcock grease on the plunger, using a short length of rubber tubing as an adapter.

A drop of silicone fluid sufficient to spread out under a coverglass of the desired size is placed on a microscope slide and warmed on a hotplate or warming table at 170°C. Measured portions (usually 0.2 ml) of the pollen-tertiary butyl alcohol suspension are then drawn up into the pipette. The outside of the pipette is wiped off, and the pipette is emptied slowly, drop by drop, onto the silicone fluid on the slide. The silicone fluid dissolves in the alcohol, which continually evaporates during this process, so that only a small volume of fluid is on the glass slide at a time. Consequently after all the alcohol in the measured portion has evaporated the pollen that was contained therein remains in the central area of the slide, dispersed in the drop of silicone fluid. Fresh tertiary butyl alcohol is then drawn up into the pipette as a rinse; the rinse is added in the same manner, one drop at a time, to the pollen and silicone fluid on the slide. Two rinses are recommended. The pipette should then be inspected for remaining pollen under a stereomicroscope; no more than 1% of the total should remain within the pipette. A coverglass is placed over the silicone fluid and its contained pollen; the completed slide is left on the warming table until the mixture spreads to the edges of the coverglass.

The entire area under the coverglass is scanned under

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the microscope and all pollen is identified and tabulated (Davis 1965a). For counts of fossil material, for example, a microscope can be used with specially constructed ocular diaphragms that block off a portion of the field of view; the remaining field should be an integral fraction of a millimeter (e.g., 0.5 mm) in diameter at the magnification used for counting. The slide is then advanced the width of the field (e.g., 0.5 mm) after each sweep across the slide by means of the calibrated mechanical stage. Several slides are prepared using the same size measured volume, and each is counted. The total number of grains in the original suspension is extrapolated from the mean number of grains counted per slide, and a confidence interval for the estimate is calculated using the variance of the counts. Results of experiments testing the reliability of estimates made in this way are given in the earlier paper (Davis 1965a). Separate estimates of absolute frequency can be made for each pollen type, as they have been tabulated separately, and the same counts can be used also for calculation of percentage values within the total pollen count.

After sufficient material for quantitative slides has been subtracted from the tertiary butyl alcohol suspension, the remainder can be concentrated for storage by centrifugation or filtration. Silicone fluid added to the concentrate will protect the pollen from desiccation when all the alcohol evaporates.

In my earlier description of this technique I advocated splitting the prepared sample into several fractions which were differentially diluted for storage, on the one hand, and counting, on the other. Although this can be done accurately, there is no control provided to assure that it is done accurately every time. I now prefer to use a single suspension of the sample, with the variance of counts providing an estimate of the thoroughness with which the sample was mixed in the suspending fluid and

the accuracy with which pipetting was done. The more important modification, however, is the use of tertiary butyl alcohol instead of benzene. Benzene is non-polar, and pollen grains suspended in it, which are apparently charged, stick to glass and to one another, particularly in the presence of even very small amounts of water. In alcohol, however, the pollen disperses readily and rinses easily from glass surfaces. It is therefore no longer necessary to use extreme care in drying glassware, or in dehydrating the pollen sample. Further, the relatively high viscosity of the alcohol obviates the need for adding a measured quantity of silicone fluid to the suspending fluid before subtracting measured portions. This saves time and permits greater variation in the volumes that can be removed from the suspension and placed on a single microscope slide. Tertiary butyl alcohol is far less volatile and somewhat less toxic than benzene, but considerable care must still be taken to avoid breathing fumes. Both sample dilution and slide preparation should be done using a fume hood.

LITERATURE CITED

- Davis, M. B. 1965a. A method for determination of absolute pollen frequency, p. 674-686. In B. Kummel and D. Raup [ed.], *Handbook of paleontological techniques*. W. H. Freeman and Co., San Francisco.
- . 1965b. Sediment traps for measuring pollen deposition. *Proc. 8th Conference on Great Lakes Res.*, p. 248.
- Davis, M. B., and E. S. Deevey, Jr. 1964. Pollen accumulation rates: estimates from late-glacial sediment of Rogers Lake. *Science* 145: 1293-1295.
- Whitehead, D. R. 1966. Full-glacial vegetation and climate in southeastern United States. In E. J. Cushing and H. E. Wright, Jr. [ed.] *VII Int. Quaternary Congress* (Boulder, Colo., 1965), *Proc. (in press)*.

AN IMPROVED METHOD OF OBTAINING DENSITY FROM LINE-TRANSECT DATA

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Abstract. Measurements are taken perpendicular to the transect across the greatest extent of each intercepted plant. The sum of the reciprocals of these measurements is multiplied by the desired unit-area and divided by the length of the transect to obtain the density statistic. The relationship of this method to that of McIntyre (1953) is discussed, and the stratified random sampling pattern is suggested in preference to a single long transect.

INTRODUCTION

The speed and convenience of the line-transect method of vegetational measurement make it a desirable tool for determining the density statistic. The method proposed here is a variation of that of McIntyre (1953), differing from his in the measurement employed. Two reasons for presenting this method are: the derivation of McIntyre's technique is inaccessible to anyone lacking knowledge of integral calculus and statistics, whereas the present method's derivation can be apprehended visually and logically; and this method obviates the necessity of the correction factors employed by McIntyre.

METHOD

Consider an area, $A = L W$ (L = length, W = width), such as that shown in Fig. 1A, the contained plants being

arranged edge to edge, without overlap, so as to exactly stretch across W . It is clear that any number of sub-transects of length L , parallel to L , must intercept just one plant per transect. Conversely, the total transect length (the sum of the sub-transects) divided by the number of interceptions must be equal to L . Note that L can intercept one plant per transect only if there are no gaps or overlaps between the plants. The number of plants contained in A can be determined by dividing W by the average of the width measurements, M (measured as shown in Fig. 1C) of the intercepted plants. Now, if these plants were re-arranged, without rotation, as in Fig. 1B, so that they no longer lay in a chain across W , and if a large number of sub-transects were again laid down parallel to L , the total transect length divided by the number of interceptions, N , must approximate L .