UPWASH AND DOWNWASH OF POLLEN AND SPORES IN THE UNSATURATED SURFACE LAYER OF *SPHAGNUM*-DOMINATED PEAT

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SUMMARY

The vertical movement of *Corylus* and *Cedrus* pollen and of *Lycopodium* spores in *Sphagnum* and peat was studied in a factorial experiment with four flow regimes (no flow, downward, upward, alternate down and up), three injection depths (1, 5 and 9 cm below the surface) and three forms of packing (natural, hand-packed at natural density, haphazard). The experiment lasted for 36 d.

The results were corrected for compaction and differential recovery of pollen and spores, but the main results were unaffected by these corrections.

Downward, upward and alternating flows moved the median pollen position by about 1·5 cm during the experiment. The flows also increased the interquartile span by 2 cm: about 25 % of grains moved at least 3 cm.

The velocity of upward flow is probably more than sufficient to overcome the rate of sinking of pollen and spores in the unsaturated zone. Reversals of flow may be of importance in dislodging grains into the main channels again.

Key words: Pollen, peat, movement.

INTRODUCTION

It is a possibility, frequently acknowledged but rarely tested, that solutes and particles deposited on the surface of a peat bog may subsequently move vertically and horizontally. Pollen diagrams with samples at 5-cm or 10-cm intervals often have several samples with approximately the same value, followed by a sharp change to higher or lower values in the next series of levels. Because the change is sharp rather than spread over a sequence of levels, it is assumed that movement of pollen after it is deposited is unimportant. In this particular case – discrete point samples at 5-cm or 10-cm intervals showing a sharp change – the assumption is reasonable. During the initial stages of peat formation, however, decay of the peat matrix may be relatively rapid, and the dry bulk density may increase by a factor of about 10 (Clymo, 1983), so a 5 cm vertical separation in the water-saturated, largely anaerobic, catotelm represents at least 50 cm in the unsaturated, largely aerobic, surface acrotelm. What happens in this zone has become of greater significance as acrotelm peat is sampled at closer intervals (Aaby & Jacobsen, (1979), used 1-cm intervals, for example) and particularly as interest has developed in the record of industrial activity preserved in peat, much of it in the acrotelm. The time scales of interest are in the range 0 to 300 years, Pollen 'events' may be used for dating (Livett, Lee & Tallis, 1979; Tallis, 1985); ^{210}Pb and ^{137}Cs dating

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depend on the assumption of negligible movement; so do the interpretations of profiles of, for example, Zn and Cu concentrations. The interpretation of profiles of the increasing number of magnetic variables (Oldfield, Brown & Thompson, 1979) is also subject to qualification if iron-containing particles have moved.

Early work by Rowley & Rowley (1956) suggested that pollen movement might occur, but that it might be restricted to the acrotelm. But now that fine resolution sampling is being used in catotelm peat, an initial differential movement might be important, even if no further movement occurs. For example, Garbett (1981) used 2 mm intervals, and Sturludottir & Turner (1985) used 1 mm intervals in the catotelm. These would possibly correspond to one to six seasons' growth and to a 1 to 2 cm depth when the incipient peat was in the acrotelm.

We began work on the movement of particles and solutes in peat in 1976. Here, we report the results of a laboratory experiment to discover the pattern and extent of movement of pollen and spores in the conditions found in the unsaturated zone of *Sphagum* and peat in the acrotelm (Ingram, 1978) of a peat bog.

MATERIALS AND METHODS

Apparatus used and experimental design

The experimental unit was a cylinder of *Sphagnum papillosum* and, in some cases, underlying peat. The cylinder was 83 mm in diameter and 100 mm tall. It was contained in a plastic bottle of the same diameter from which the top had been cut off at a height of 175 mm. The cut-off top could be pushed inside the bottle if need be, either as a support at the bottom of the bottle or as a cap to prevent evaporation at the top (Fig. 1). The *Sphagnum* and peat cylinder was supported on a circle of 2-mm mesh plastic screen.

The experimental design was $4 \times 3 \times 3$ factorial. The factors were: flow regime; depth at which pollen and spores were injected; and type of packing of the core. In addition, there were three types of pollen or spore. These were originally considered as separate variates, but analyses of variance showed little difference between them, and they may arguably be considered as a fourth factor.

Flow regime

The first and most important factor was the type of water flow. There were four treatments: no flow; downwards flow only; upwards (evaporation) only; and

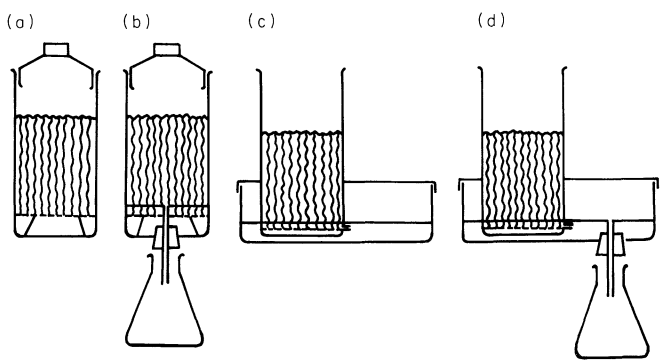

Fig. 1. Apparatus used to produce the four flow regimes. (a) No flow; (b) downflow; (c) upflow; (d) alternate downflow and upflow.

alternating downwards and upwards flow. In all treatments, the water table was kept at 1 cm above the base of the *Sphagnum* and peat cylinder, i.e. at a nominal −9 cm.

(1) In the no-flow treatment, water was added to establish the water table at the start of the experiment. The cut-off top of the bottle was pushed inside the top of the rest of the bottle as a cap to prevent evaporation (Fig. 1).

(2) The downflow treatment was applied once a day for the 36 d of the experiment. The open-topped cut-off plastic bottle was temporarily extended upwards by another inverted one which acted as a shield. Through a hole in the top, 50 ml of water (equivalent to 10 mm of rain) was sprayed with the atomizer from an EEL flame photometer. Air and a small amount of water escaped through slots. The distribution of this simulated rain was not quite even: central areas received about 30% more than peripheral ones except for the perimeter itself which received drainage down the walls. This wall drainage comprised about 10% of the total. In the downflow pots, the plastic grid on which the plant cylinder rested was raised 2 cm from the bottom of the bottle by the cut-off top of an expanded polystyrene drinking cup. This allowed a rubber bung to be pushed up through a hole in the centre of the base of the cut-off bottle (Fig. 1). Through this, a vertical glass tube reached up 1 cm into the plant material and acted as an overflow during and just after spraying. The overflowing liquid was collected in a conical flask, and this device kept the water table at the nominal −9 cm. Immediately after spraying water, the extension shield was removed, and a cut-off top was used to cap the bottle and prevent evaporation. Pollen and spores in the overflow were centrifuged down and added to those extracted (at the end of the experiment) from the basal slice of the core.

(3) For the upflow treatment, each bottle was pushed through a tightly fitting hole at one end of the lid of a polyethylene lunch box 12 × 19 × 6 cm deep, so that the bottle rested on the base of the box (Fig. 1). Six small holes had previously been drilled around the perimeter at the bottom of the bottle to allow water to pass from the box to the plant material inside the bottle. The top of the bottle was left open. The water level in the box fell by about 1 mm for 4 mm evaporated from the *Sphagnum*. The lost water was replenished to constant weight each day. Evaporation was encouraged by putting the boxes in a rudimentary wind tunnel (0·5 × 0·5 m in section × 2 m long). A large fan produced a fairly constant wind of about 1 m s^{-1} at a temperature of about 25 °C. The box positions were re-randomized daily, as described below. Under these conditions, the rate of evaporation was about 10 mm d^{-1}. The upward flux of water was thus about the same as the downward flux in the downflow treatment.

(4) The alternating upflow and downflow treatment was applied to box-and-bottle containers similar to those used in the upflow treatment, but with the addition of a rubber bung and overflow tube similar to that used in the downflow treatment except that it was placed in the base of the box (Fig. 1). Water was applied once a day at the same rate as it was applied to the downflow treatments. This took about 5 min. For the rest of the time, the water evaporated in the wind tunnel. Overflowing pollen and spores were added to the basal slice.

Depth of injection

Pollen or spores were placed at one of three depths relative to the surface, from which later measurements were made: −1 cm; −5 cm; and −9 cm. The pollen and spore suspension in water was injected through a 15 cm needle at 10 positions

defined by equidistant holes in a circular template of the same diameter as the bottles. The needle end had been blocked, and four holes had been drilled just above the block to ensure, as far as possible, that the suspension spread sideways. A total of 2 ml of suspension was injected ($10 \times 0{\cdot}2$ ml).

Type of packing

There were three types of core. The first (natural) was removed from an *S. papillosum* lawn in the field with the help of a sharp-ended tube of 85 mm internal diameter. Cores were cut to 100 mm length with a sharp knife and pushed gently into the cut-off polyethylene bottles. The second type of core was of *S. papillosum* plants cut individually to 10 cm long and hand-packed at natural density – about 100 dm^{-2} – in the cut-off polyethylene bottles. The third type contained the same fresh weight – about 100 g – of *S. papillosum* cut to about 2-cm lengths and packed haphazardly in an unstructured way in the cut-off bottle and gently jolted down to form a cylinder 10 cm tall. This third type of core was intended to show the extent to which the intact structure of the plants was important.

Pollen and spores

The 2 ml of injected pollen or spore suspension contained $3{\cdot}1 \times 10^5$ grains of *Corylus avellana*, $2{\cdot}8 \times 10^5$ grains of *Cedrus deodara* and $2{\cdot}5 \times 10^5$ spores of *Lycopodium*. The suspension density was determined by the method described below, with a standard deviation in the totals added of about $0{\cdot}3 \times 10^5$. These taxa were chosen because they differ in size and structure, and because they could be obtained easily in large amounts. *C. avellana* is fairly smooth, approximately spherical, and about 25 μm in diameter. *C. deodara* is similar to *Pinus* in having a central body and two lateral bladders. The body is about 65 μm across, and the whole grain is about 110 μm across the bladders. The bladders have a clearly patterned surface. *Lycopodium* – a commercial sample of undetermined species – had tetragonal spores about 35 μm across and was, therefore, of intermediate size. The spore surface was the roughest of the three taxa. (We planned but were unable to include an echinate type.) The pollen of *C. deodara* was distinguishable from all the naturally occurring pollen in the *Sphagnum* and peat – even from that of *Pinus*. The background concentration of *Lycopodium* was vanishingly small as well. The added *C. avellana* swamped the natural background by a factor of 20 or more. The *Sphagnum* and peat were collected from Moore House NNR at about 680 m altitude and several kilometres from the nearest hazel trees. The total concentration of pollen and spores added was about 8000 cm^{-2}. The present day total influx of pollen and spores to bogs is about 1000 cm^{-2} per annum (Beckett 1979).

Conduct of the experiment

The cylinders and containers were all placed in the wind tunnel, but in two groups. Those with caps (no flow and downflow) were arranged in two close-packed rows of nine pots at the sides of the tunnel. They were thus subject to a similar thermal regime as the physically larger boxes of the upflow and alternating treatments. These boxes were arranged in six rows of three in the centre of the tunnel. Within each group of 18, the position of each pot or box was at random and was re-randomized every second day for 36 d using an 18 position $\times$ 18 d Latin square generated by a computer program which used random choices. Thus, each physical container occupied every location within its group exactly once and in random order.

After 36 d treatment, the cut-off bottles with *Sphagnum* and peat were deep-frozen. The plant material was then removed as a cylindrical block and its height and weight recorded. The core was put into a device which allowed the core to be pushed up, by turning a bolt, through a hole in a flat plate. The projecting disc was then cut off with a sharp knife. In this way, the cores were cut into 1 cm thick discs which were stored individually in a freezer at −16 °C. The lowest 2 to 3 cm of the natural cores could not be cut in this way. They were sliced instead with a fine-bladed saw. The amount of plant material lost to the saw was about 5 % of that in a complete slice.

Extraction of pollen and spores

Pollen and spores were then extracted from the discs. The whole disc was allowed to melt and then dispersed into a sludge by a high-speed liquidizer applied for 15 s. Some water was added to the drier discs. The sludge was sieved (40 mesh), then resuspended in water and resieved several times. The material retained on the sieve was compressed, and expressed liquid was added to the material which had already passed through the sieve. The compacted plant material was dried and weighed. The accumulated washings containing most of the pollen and spores were passed through a 12-mm diameter 100-mesh filter held in a screw cap at the narrow end of a funnel. The deposit on the filter was washed twice. The accumulated liquid in a 250 ml beaker was left undisturbed at a constant temperature for at least 24 h to allow pollen and spores to sediment. All but the bottom 5 mm of liquid was then removed by very gentle suction through a **J**-shaped tube of 6 mm bore. The **J** shape ensured that the flow lines into the tube were from above. Pollen and spores were resuspended in the remaining liquid, and the suspension was transferred to a Sterilin bottle, the inside base of which tapers down to a point. Pollen and spores were again allowed to settle for at least 12 h, and most of the supernatant was removed with another **J** tube with a 1·5 mm bore. Often there was more suspension than the Sterilin tube could hold and the procedure had to be repeated. The final volume in the Sterilin tube was calculated from the height of liquid in it – accuracy of about 2 % is easily possible – and the sediment was resuspended. A 5 μl aliquot was put on a slide in the centre of an area defined, for convenience, by two parallel strips of PVC tape 18 mm apart. When a cover slip was placed on the drop it rested on the tape, and the drop formed a circle about 10 mm in diameter. The exact diameter and thickness did not matter, as the volume was measured accurately, and pollen and spores in the whole drop were counted. If the concentration was too high for counting, an aliquot was diluted; if too low, then several 5 μl drops were scanned. These procedures gave a mean recovery of 55 %, which is tolerably satisfactory.

Derived variables and treatment of data

The number of pollen grains and spores in each disc and the total recovery for each cylinder were calculated. The pollen and spore distribution with depth was often strongly skewed. We treated it as a frequency distribution and calculated mean depth and standard deviation, but we also calculated the median depth and, as a more useful measure of dispersion, the interquartile range. We also used other similar ranges – those encompassing 30 and 80 % of the grains. They gave very similar results to the interquartile (50 %) range and are not considered further. We also calculated two dimensionless measures of skewness. The first is the conventional $g_1 = k_3/k_2^{1.5}$, where $k_3 = n\,S_3/(n-1)\,(n-2)$ and $k_2 = S_2/(n-1)$, with

S_2 and S_3 the sum of squares and cubes of deviations of n observations. For the second, based on the median, m, and quartile values, b and t, several measures were considered. The measure $(b-m)/(m-t)$ is not satisfactory, as it is not symmetrical for skewness in different directions. Nor does it have an upper limit, with the result that a few positive values would dominate any analysis. These defects can be corrected but only rather messily. Instead we used $A = [(b-m)-(m-t)]/(b-t)$. This is the difference between the length of the quartiles as a proportion of the interquartile range. It ranges from -1 to $+1$. Various transformations might be applied to increase or decrease the weight of values near the extremes, but we could see no good reasons for doing so and used A as the non-parametric counterpart of g_1.

For each species, an analysis of variance was made of each variable, using the second-order interaction between flow regime × depth × packing to estimate error. The results were so similar that it seemed justifiable to treat 'species' as a factor, even though pollen or spores of all three were together in the same pot. In the four-factor analyses, second- and third-order interactions were aggregated to estimate error.

RESULTS

Only those results with $P < 0{\cdot}01$ are mentioned: in most cases the results discussed have $P < 0{\cdot}001$. An initial analysis of the extent of compaction during the experiment showed non-significant ($P > 0{\cdot}05$) reductions from 10 cm (initial) to 9·2 (natural cores), 9·0 (hand-packed) and a significant reduction to 7·7 cm (haphazard). As all but one of the final slices were always 1·0 cm thick, there was a serious bias in the estimate of apparent movement, particularly in the 5 cm and even more in the 9 cm injection treatments. A subsidiary experiment with coloured plastic markers in haphazard cores – those most affected in the main experiments – showed that the compaction was almost linear. For each core, the values in as many slices as were measured were therefore reallocated, assuming linear compaction, to exactly 10 × 1 cm notional slices.

The next analysis was of the recovery of added pollen and spores. The overall mean was 55%. It differed among species: the recovery of *Corylus* and *Cedrus* (60, 63%) was significantly greater than that of *Lycopodium* (43%). More important were differences depending on the depth of injection: 76, 52 and 39% at 1, 5 and 9 cm depth. This indicates a systematic difference of recovery according to depth which would bias the results. The cause of this difference is not clear, but it was similar for all three species and injection depths and was not obviously affected by packing or flow treatments. The species × depth interaction was tiny so, to correct for this recovery bias, a gently curved quadratic function of depth was used for each species to multiply the pollen and spore counts and give a fixed recovery at all depths. All subsequent analyses were made both with the values uncorrected for differences in recovery and with corrected values. In all but one trivial case, the effect of this correction was to reduce the significance of effects. The changes in mean values were $< 10\%$. The results reported are for corrected values.

The results of the separate analyses of the two measurements of movement from the depth of injection (mean, median) of the two measures of spread (standard deviation, interquartile range) and of the two measures of skewness (g_1, A) were remarkably similar. None showed any significant effect of species or packing, and

all showed a highly significant interaction between flow regime and depth. Most showed effects of these two factors individually too. This makes it easy to summarize the results in a single diagram (Fig. 2).

There was no indication of movement when there was no flow and pollen or spores had been injected 1 cm and 5 cm below the surface. The interquartile range

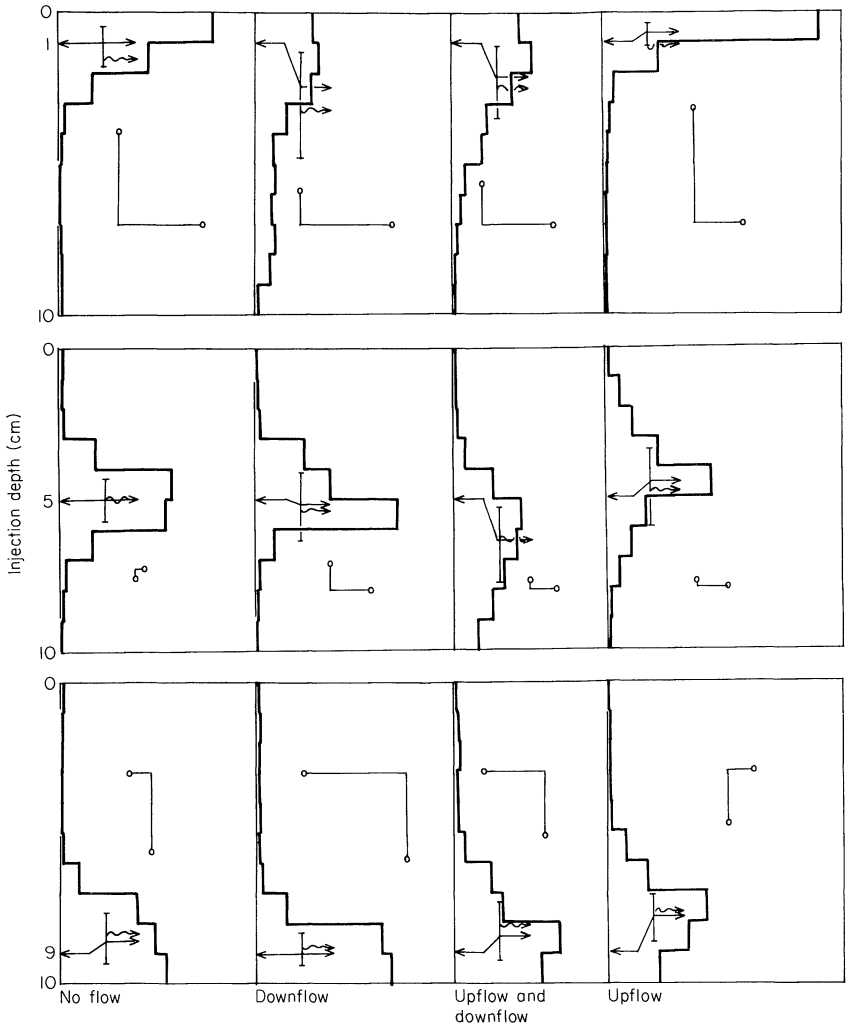

Fig. 2. Profiles of distribution of pollen and spores for four flow regimes and with pollen injected initially at one of the three depths (1, 5, 9 cm), shown by the left-facing arrow, arranged down the page. Flow regimes, arranged across the page: no flow; downflow (sprayed with the equivalent of 10 mm of rain once each day for 36 d); alternate (continuous evaporation interrupted by spraying of water, 10 mm d^{-1}, for 5 min each day); continuous evaporation (at about 10 mm d^{-1} for 36 d). The values are proportions (i.e. the area under each histogram is the same) and are the mean for nine cores (three types of packing × three species). The proportions have been corrected for shrinkage and for differences in recovery with species and depth. See text for more details. The mean is shown by a right-facing wavy arrow, the median by a right-facing straight arrow. The line joining median and injection depth – usually an oblique line – shows the median movement. The, often unequal, vertical cross bars show the interquartile range. The two lines at right angles with circles at the tips show the skewness: g_1 vertically and A horizontally. One might expect them to bound the first and third quadrants, i.e. both positive or both negative.

was about 2 cm, and the 5 cm injection was distributed symmetrically. The skewness at 1 cm and 9 cm injections may be partly an artifact of the relative coarseness of slicing, but it could be a genuine indication of the spread of pollen and spores during injection.

The downflow and upflow treatments show that the median is displaced in the expected direction, and by the greatest amount (1·5 cm) in downflow of the 1 cm injection but in upflow of the 9 cm injection. This could be summarized by the statement that the movement is greatest closest to where the water started. Part of the explanation must be that the opportunities for movement, say upward from 1 cm, are limited. But there is no such limit for the 5 cm injection which nevertheless shows less movement. This particular case shows that the pollen and spores have become more widely dispersed than in the equivalent no-flow treatment.

The alternate upflow and downflow treatment has produced spreads which are wider, on average, than either upflow or downflow alone. Downward movement predominates for material injected at 1 cm and 5 cm depth; upward movement dominates at 9 cm depth.

The skewness measures are, as might be expected, generally large and in the first quadrant for 1 cm injections, large and in the third quadrant for 9 cm injections, and smaller (and not restricted to first and third quadrants) for the 5 cm injections.

DISCUSSION

These experiments have shown that pollen and spores do move in the same direction as water flowing through the unsaturated upper layer of *Sphagnum* and peat. The median is displaced, and a long 'snout' develops, which is most clearly seen in downflow of material injected near the surface. About 25 % of the grains moved 3 cm or more further than they would have done if there had been no flow. There are equally clear indications of upward flow, though on a more limited scale. Although the total flux of water upward was about the same as that downward, the upward flux was continuous, whilst that downward was in short pulses. This pulsed movement probably had two effects. Firstly, it filled reservoirs between leaves to overflowing for a short time. Secondly, the maximum rate of flow was probably greater.

When Rowley & Rowley (1956) did their field experiment, they used the pollen of *Dodonaea viscosa* Jacq. and of *Pinus resinosa* Ait. Their account is ambiguous, but they seem to have dispersed the pollens in the air above the *Sphagnum* bog surface. They give no numerical results but state that 'as would be expected the smaller spheroidal *Dodonaea* pollen penetrated deeper than the larger aspherical-vesiculate grains of [*Pinus resinosa*]'. Our very different experiments showed that differences among *Corylus*, *Cedrus* and *Lycopodium* were small and, in the statistical sense, of no significance. It is possible that the difference between the two sets of results lies in the differing ability of pollen grains to penetrate the dense mat of *Sphagnum* capitula. In our experiments, the pollen and spores were injected below the surface. We had the same expectation as Rowley & Rowley and were surprised to find so little systematic size- and surface-related differences.

The extent of upward movement is, at first sight, rather surprising. The dry bulk density of the material in the cylinders was about 0·01 to 0·02 g cm^{-3}. Much of this material was in horizontally spreading structures (in all but the haphazardly packed cores) and about 20 to 30 % in vertically-oriented structures – the main

channels of vertical movement – such as the pendant branches around the stems. The assumption that 0·1 % of the cross-sectional area is occupied by moving capillary films of water is therefore probably on the high side. An evaporation rate of 10 mm d^{-1} thus requires an average velocity of $10/(0{\cdot}001 \times 24 \times 60) = 7$ mm min^{-1}. Stokes' Law can be applied to an assumed spherical pollen grain of 40 μm diameter. The bulk density of the whole grain may be about 1·1 g cm^{-3}, and the velocity of sinking in water at 25 °C is then 1·3 mm min^{-1}, which is about that (0·99 mm min^{-1}) observed directly with a travelling microscope. Stokes' Law is probably not strictly valid for the moving capillary films considered here, but these simple calculations do show that the upward rate of water movement may be expected to exceed the rate of sinking of pollen. This is a necessary, though not a sufficient, condition for upward dispersal of pollen and spores. In practice, many pollen grains must lodge in backwaters and are unavailable for movement in any direction. It may be that it is the alternation of upflow and downflow which is important in dislodging some of these particles and making them available for movement. The alternating flow treatment did not simply move grains up and back down, with no net movement: it spread them out according to whichever was the more effective, upflow or downflow.

It is difficult to extrapolate to movement in the field. A simple multiple of the results over 36 d would indicate median movements of 15 cm in a year. After decay and compaction this would represent about 1·5 cm per annum in catotelm peat. Extrapolation to a precipitation of 1000 mm per annum would give values of approximately one-third of these. Perhaps more important is the spreading of the distributions, with consequent loss of sharpness at genuine boundaries. Only a field experiment can answer the questions, 'How much movement and spread occur in the field?', and it is with this problem that our next experiments are concerned.

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References

Aaby, B. & Jacobsen, J. (1979). Changes in biotic conditions in the last millennium as reflected in ombrotrophic peat in Draved Mose, Denmark. *Danmarks Geologiske Undersogelse. Årbog 1978*, 5–43.

Beckett, S. C. (1979). Pollen influx in peat deposits: values from raised bogs in the Somerset Levels, south-western England. *New Phytologist*, **83**, 839–847.

Clymo, R. S. (1983). Peat. In: *Ecosystems of the World, Vol. 4A. Mires: Swamp, Bog, Fen and Moor*. (Ed. by A. J. P. Gore), pp. 159–224. Elsevier, Amsterdam.

Garbett, G. C. (1981). The elm decline: the depletion of a resource. *New Phytologist*, **88**, 573–585.

Ingram, H. A. P. (1978). Soil layers in mires: function and terminology. *Journal of Soil Science*, **29**, 224–227.

Livett, E. A., Lee, J. A. & Tallis, J. H. (1979). Lead, zinc and copper analyses of British blanket peats. *Journal of Ecology*, **67**, 865–891.

Oldfield, F., Brown, A. & Thompson, R. (1979). The effect of microtopography and vegetation on the catchment of airborne particles measured by remanent magnetism. *Quaternary Research*, **12**, 326–332.

Rowley, J. R. & Rowley, J. (1956). Vertical migration of spherical and aspherical pollen in a *Sphagnum* bog. *Proceedings of the Minnesota Academy of Sciences*, **24**, 29–30.

Sturludottir, S. R. & Turner, J. (1985). The elm decline at Pawlaw Mire: an anthropogenic interpretation. *New Phytologist*, **99**, 323–329.

Tallis, J. H. (1985). Mass movement and erosion of a southern Pennine blanket peat. *Jounal of Ecology*, **73**, 283–315.