THESIS

"Some effects of humidity on sexual and asexual reproduction in Phascum cuspidatum Hedw."

Submitted for the degree

of M.Sc. at

The University of Aston in

Birmingham.

July 1966.

by

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ACKNOWLEDGEMENTS.

I would like to thank Prof. A.J.Matty, B.Sc., Ph.D., F.I.Biol., and Mr. C.G.Gardener B.Sc., M.I.Biol., for making available facilities and equipment in the Department of Biological Sciences.

My thanks are also due to Mr. P.N.Nield B.Sc., C.Eng., A.M.I.E.E., of the Department of Electrical Engineering for help with the choice of electronic equipment, and to Mr. S.W.Lawrence and Mr. P.Whittle for their help with plumbing, electrical supplies and photocopying.

CONTENTS.

TITLE OF THESIS.

Some effects of humidity on sexual and asexual reproduction in Phascum cuspidatum Hedw.

Summary.

An apparatus has been designed and constructed, to supply streams of air to mosses growing under controlled conditions. Each air stream could be adjusted to a known humidity, and the humidities could be measured continuously.

The relevant literature has been reviewed in two parts. The first part includes an account of atmospheric humidity together with the various hygrometers now available, and the second part contains an account of the water relations of the bryophytes.

Details of the design and construction of the apparatus are set out, together with notes on its use and a discussion of its performance.

Two quite separate aspects of the effects of humidity have been investigated. Haploid sexual plants have been used to determine the effect of humidity on antheridial dehiscence, and diploid apogamous plants,

to investigate the effects of humidity on their leafless axes.

Past experience has shown that it is difficult to initiate the sporophyte generation in Phascum cuspidatum growing in pure culture. This has been referred to the failure of antheridial dehiscence and two explanations of this suggest themselves. The conditions in culture vessels are very unlike field conditions in two respects, the humidity is always at or near 100% r.h., and the air is still. Both of these explanations have been investigated and it is shown that the necessary stimulus is mechanical rather than a change in humidity, Conclusions, and a discussion of these findings are presented before the account of the other investigation.

It has been suggested by several investigators that the development of the leafless axes of the diploid apogamous plants can be controlled by the "dryness" of the culture medium, and that these structures may function as propagules if sporangia do not form.

The investigations showed that the plants are extremely sensitive to desiccation. From this, and other considerations, it has been possible to deny both of these earlier suggestions.

INTRODUCTION.

The moss Phascum cuspidatum Hedw., has been maintained in sterile culture for several years. It has been shown however, that it does not readily complete its life cycle under these conditions (Hughes 1958). The sporophyte generation is rarely produced, and part of the investigations described here, were designed to examine some aspects of this situation. The usual gametophyte plants were used for this part of the work, but to prevent confusion they are refered to as haploid sexual plants.

The necessary control of humidity has been achieved by designing and building an apparatus specifically for this investigation. After development it has provided up to three air streams, each having a controlled dew-point temperature. Continuous monitoring of the air streams was built into the apparatus. Thus it has been possible to investigate the moss plants growing under controlled conditions, including controlled atmospheric humidity.

The other part of the investigation has involved the use of the same apparatus for controlling humidity,

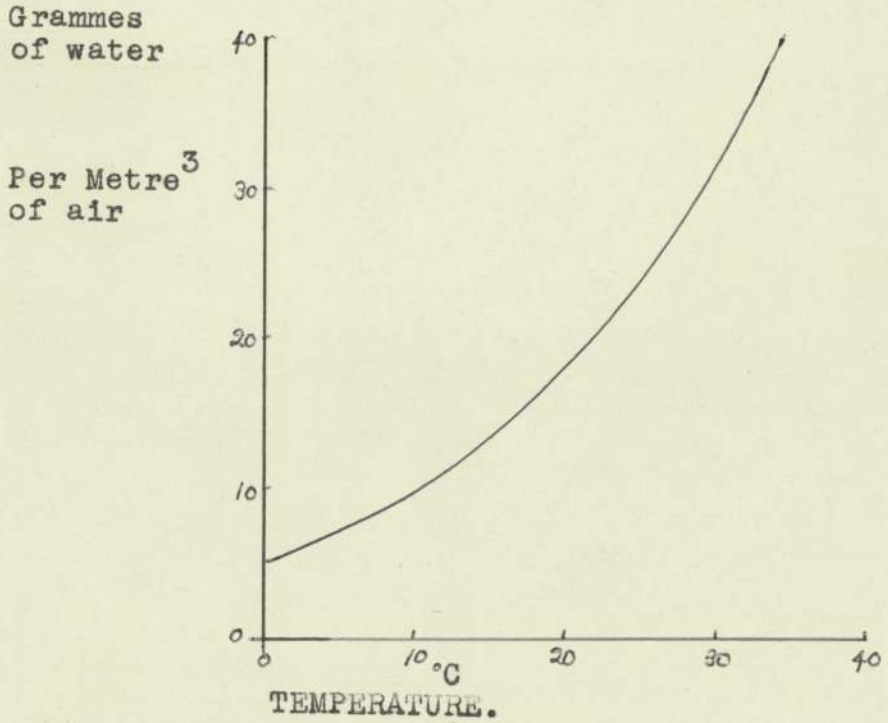
but diploid apogamous plant material. This has made it possible to investigate the response of haploid and diploid plants. The diploid apogamous plants have been raised from seta-cuttings (Hughes 1958), and the effect of humidity on the development of their leafless axes, has been investigated.

Phascum cuspidatum Hedw., has previously been propagated from seta-cuttings by E & Em. Marchal (1911), and by Wettstein (1923). Many morphological peculiarities of their clones were described. Springer (1935) was able to reveal that these plants were capable of producing sporangia and could therefore be apogamous. The seta-cutting produces protonema which bears leafy shoots in the same way as haploid protonema. However the adult plants are unusual in that their leaves may bear club-shaped organs at the nerve endings. Alternatively very long cylindrical stalks (leafless axes) may be produced, and these may be branched. E & Em.Marchal suggested that these various organs could break away from the parent plant and function as propagules. Springer however found that fragments of these appendages showed limited powers of regeneration when compared with the rest of the plant.

ATMOSPHERIC HUMIDITY.

Water is in many ways one of the most important constituents of the atmosphere. It can occur as an invisible vapour, as a liquid (forming fog, cloud and rain drops), and as a solid (ice crystals, hail and snowflakes). There is an upper limit to the amount of water that air can contain, and this limit varies with temperature. The presence of other gases in the Atmosphere has very little effect on this value.

Fig.1. Absolute Humidity of Saturated Air at 760mm.Hg.



(From figures given by Kaye and Laby.)

The water vapour content of the air can be expressed in several ways.

(1) VAPOUR PRESSURE. (e mb.)

Vapour pressure can be defined as the pressure excerted by the water vapour in the air. It can be expressed in any units of pressure, and does not vary with total pressure.

(2) ABSOLUTE HUMIDITY. (X g/m^3.)

The absolute unit for this measure of density is grammes per cubic centimetre, but for convenience the larger unit is used. Conversion to Absolute Humidity from Vapour Pressure can easily be made using the following equation.

$$X = \frac{217e}{T}$$ (If e is in millibars, and T in K.)

This unit is used in diffusion studies.

(3) SPECIFIC HUMIDITY.

This is defined as the mass of water vapour per unit mass of the air, including the water vapour it contained.

(4) MIXING RATIO. (x g/kg.)

This unit is similar to number (3) except that it is given per unit mass of Dry air.

(5) SATURATION DEFICIT.

This unit is merely the difference between the actual vapour pressure, and the saturated vapour pressure, at the temperature of the sample. It is usually stated in millibars.

(6) RELATIVE HUMIDITY. (h).

$$h = \frac{\text{Vapour pressure of the sample.}}{\text{Saturated vapour pressure at the temperature of the sample.}}$$

Or as it is usually expressed:-

$$r.h = \frac{\text{Vapour pressure x 100}}{\text{Saturated vapour pressure.}}$$

Therefore there are no units for relative humidity, and it is usually expressed as a percentage.

The saturation deficit can now usefully be stated in terms of h.

Saturation Deficit = S.V.P.(1-h).

Both saturation deficit and relative humidity vary either with the actual vapour pressure, or with the saturated vapour pressure, and therefore any statement of either unit must properly include the temperature at which it was measured.

(7) DEW-POINT TEMPERATURE. (t_d).

This is usually and rather misleadingly shortened to DEW-POINT.

Therefore the Dew-point is that temperature at which a given sample of air will just produce a film of dew on a suitable surface, if suitable precautions have been taken. For values below 0°C the temperature is usually called the Frost-point temperature.

(8) WET-BULB TEMPERATURE. (t_w).

Is the temperature indicated by a thermometer with its bulb kept wet by a suitable wick and shielded from radiation. Unlike the dew-point temperature the wet-bulb temperature can only be measured by increasing the water vapour of air.
(Definitions taken from Penman 1958).

Any measurement of the water vapour present in air at a given time is of very little use, unless the temperature of the sample is also known. The mass of water vapour which unit volume of air can absorb varies widely with temperature. It can be seen from Fig 1. that a sample of air containing, say, 5gms. of water per cubic metre would be saturated at 0 C,

but could absorb another 45gms per cubic metre at about 37 C. This difficulty can be largely overcome, by careful selection of the most suitable units for the work in hand. In almost all situations involving living material, it is the water balance between the organisms and their environment, which matters. So that some indication of the ability of the air to absorb more water is needed, and can be supplied by expressing the humidity as Relative Humidity, or perhaps by giving its Saturation Deficit.

THE MEASUREMENT OF HUMIDITY.

Any instrument which can actually measure humidity is called an hygrometer, and crude non-measuring instruments are sometimes called hygroscopes.

That incredible man Leonardo da Vinci seems to have been the first to design and build an hygrometer (about A.D. 1500). He weighed a ball of wool and showed that its weight changed with changes in atmospheric humidity. In 1783, H.B. de Saussure described an hygrometer which depended on the change in length of a degreased human hair. He was able to show that its length increased as humidity rose and decreased as

humidity fell. C.W.B. Boeckman used a form of "Wet and Dry" hygrometer in 1802. J.W. Dobereiner in 1822 and H.V. Regnault in 1845 both measured dew-point. Regnaults apparatus was a highly polished silver cylinder, which was cooled until dew just formed on its surface. The temperature at which this occurred was taken as the dew-point. In 1938 Dunmore introduced the Lithium chloride electric hygrometer.

These basic methods, together with gravimetric chemical methods involving the weighing of water collected in drying tubes, have steadily been improved and refined to cope with the increasing demand for humidity control, and therefore, humidity measurement. All the methods mentioned so far are still used, except for the increasing number of research fields, where it is essential to involve transmission of the information over considerable distances. It often happens in these cases that accuracy need not be high, and other factors such as robustness are much more important. Despite the recent proliferation of hygrometers one can still summarise them:-

(1) THE PSYCHROMETER.

(2) THE DEW-POINT HYGROMETER.

(3) "ELECTRIC" HYGROMETERS.

(4) HYGROMETERS based on ABSORPTION SPECTRA.

(5) MISCELLANEOUS.

(1) THE PSYCHROMETER.

In all its various forms the Psychrometer measures the temperatures of a dry surface and of a similar wetted surface. The humidity can then be obtained from prepared tables using the two measured temperatures. The psychrometer is not a primary instrument, but despite this and despite its inherent errors, it has proved to be the most useful, and most widely used type of hygrometer. In any accurate work it is always recommended that the psychrometer be shielded from any heat exchange by radiation, and that a current of air be maintained over the wet and dry surfaces. For each application a so-called psychrometric "constant" must be employed when using the tables, and it has been shown by many authors that this "constant" is dependent upon many factors, but Bindon (1965) states that ventilation is by far the most important parameter, and that it is necessary to choose the appropiate constant for each application.

For example, the following values of a (the psychrometer constant) are quoted by Penman (1955), from the Austrian meteorological tables.

Calm (wind 0-0.5 m/s) $a = 0.667 mb/^{o}F.$

Light air (wind 1-1.5 m/s) $a = 0.444 mb/^{o}F.$

Strong wind (wind greater than 25 m/s) $a = 0.364 mb/^{o}F.$

These values being used in the equation:-

$e = e_w - a (T_a - T_w)$ Where e = Vapour pressure (m.b.)
e_w = Saturation vapour pressure (m.b.)
T_a = "Dry" temp - temp of air.
T_w = "Wet" temp.

although in practice either tables or slide rules are used, to avoid the solving of equations. One big disadvantage of this type of instrument is that after measurement the water content of the air has been increased by a variable amount, so that the sample of air would normally have to be discarded. One of the many difficulties in designing and using any Psychrometer is the shielding from radiation. The more efficient this is, the more difficult it becomes to achieve satisfactory ventilation of the thermometers.

Non Ventilated Psychrometers.

This type of psychrometer can only be satisfactory in very special circumstances, such as those obtaining

in a Stevenson screen. Here the screening and ventilation are both adequate and reliable readings (not more accurate than ± 5% r.h.!) can be expected, provided that a close-fitting muslin wick is used with a few threads of darning cotton dipping into a resevoir of distilled or rain water. The muslin must be replaced regularly because of contamination from atmosphere and resevoir. The thermometer must be sensitive, and easily read to 0.1°C. If they are to be used in a Stevenson screen for long they should be "sheathed" thermometers. That is, the thermometer proper, with its engraved scale is enclosed in a further glass tube.

The measurement of humidities in soil poses a special problem, and one very interesting attempt to solve the supply of water to the wet junction is that of Box (1965). He uses the Peltier effect to cool the appropriate region so that a layer of water condenses on to his instrument.

Ventilated Psychrometers.

(a) Sling psychrometer.

This is the simplest type of ventilated psychrometer, and provided that there is room to swing the

thermometers around ones head it can be quite satisfactory. Enough water is put on to the muslin sleave to last for one reading.

(b) Assman psychrometer.

Here the thermometer bulbs are mounted inside elaborate ducts of highly polished metal, to avoid radiation problems. An air stream is maintained past each thermometer by a motor and fan. The motor may be clockwork so that the instrument is portable. This instrument cannot be used to measure the humidity of small volumes of air, nor where its air stream with disturb experimental conditions.

(c) Shiba and Tozawa (1965) have approached this aspirated type of psychrometer from a different aspect. They show that the temperature difference shown by a psychrometer is proportional to the difference between the wet bulb temperature (t_w) and the dew-point temperature t_D, within a small temperature range near to the dew-point. So that, provided the pressure is kept constant a straight-line graph can be drawn for ($t-t_w$) against t, and the point of intersection between the line and the t axis will be the dew-point. Thus by using a "wet and dry" thermopile and by precooling

the air, dew-point may be found. It proved to be possible to indicate dew-point directly by controlling the precooling to maintain a constant depression of temperature ($t-t_w$).

The recent developments in psychrometry show that any type of thermometer can be used. The choice however may be difficult in practice, and will depend on the sensitivity required as well as robustness. The following is a necessarily brief summary of the main types of thermometer, and their particular attributes in psychrometry.

(1) Liquid in glass. These instruments are completely self contained and properly made and tested are accurate. Human errors will apply every time they are used, and they are not easily converted for distant reading. However it must be said that simple unventilated psychrometers with their pair of mercury in glass thermometers outnumber all other types put together.

(2) Liquid in steel. These thermometers are necessarily more sluggish that other types, but are extremely robust, and are distant reading. Generally these are used where robust instruments of low accuracy are required.

(3) Resistance thermometers, thermocouples and thermistors. Although these are really very different, they are considered together because all three can provide information which can be made available anywhere. Their output can be used simply to indicate humidity or tc control machines capable of changing humidity. Whole air conditioning plants are dependent on the electrical output of a hygrometer. In particular thermocouples can be very small, and can respond quickly to changes in temperature. Thermistors show a very large negative co-efficient of resistance so that relatively insensitive, and hence robust, bridge circuitry can be used.

(2) DEW-POINT HYGROMETERS.

In principle all Dew-point Hygrometers are simple instruments giving a direct indication of dew-point. In practice the great difficulty is that of recognising the initial deposition of dew. A thorough review of the process of dew formation is given by Wylie, Davies and Caw (1965) and they conclude that an accuracy better than 0.2°C can probably only be obtained using automatic temperature control, photoelectric detection and heavy dew deposits. However as Brewer

(1965) points out a single dew-point hygrometer, provided that it can deal with deposits of ice as well as water, can cope with the tremendous range of concentration of water vapour found in the atmosphere. Brewer goes on to give a critical account of technique and operation, pointing out the extreme difficulties experienced at very low frost points, and saying how glad he is that he has never encountered one lower than -86 C! He states that wherever extremes of water content or relative humidity are encountered no precautions are too pedantic. Use clean metal pipes or glass, avoid rubber, and suspect plastic. Cold spots must be carefully avoided and even greater care is needed to avoid leaks. The deposition surface must be hydrophobic, and not hydrophilic since in the latter case a thin continuous film of water forms and this cannot be detected. It is essential that the deposition should be in the form of discrete particles. He gives a careful account of methods used for the visual hygrometer, and then considers the automatic hygrometers, pointing out the very considerable difficulties to be overcome.

Whatever method is used for cooling the mirror, some form of thermal damping is used. Even the servo mechanism used to maintain Peltier elements at the dew-point tend to be oscillatory, and must be damped. Further, when a cooled surface is held at the required temperature for long, temperature differences are bound to occur over the mirror, and either pools of water, or large ice crystals develop. Either of these conditions will affect the scattering of the light, and the only answer would appear to be the frequent clearing of the deposit. A further unfortunate complication is the fact that dust and dirt particles tend to be deposited on cool surfaces, so that a large proportion of the particles in the air stream will be deposited on the mirror. It is usual now to avoid both of these difficulties by using a scratched mirror, so that water droplets form in the scratches before the dew-point is reached, because of the reduction in vapour pressure produced by the curvature of the scratches, and thus the instrument can be made to stabilise at a temperature very slightly higher than the dew-point.

Paine and Farrah (1965) give details of modern

materials which can be employed to prevent condensation of water, in places other than on the mirror surface.

(3) "ELECTRIC" HYGROMETERS.

Mathews (1965) gives a brief account of Dunmore's early work from 1938 onwards, and gives an exhausitive account of the history of the Lithium chloride hygrometer. This type of hygrometer has been almost exclusively employed for radiosondes. In an extension of his paper Mathews describes methods of making and investigating this type of hygrometer. This hygrometer is not a primary instrument, the elements must be calibrated, and ageing must be taken into account. (Kobayashi and Toyawia (1965)). A careful study of the accuracy of Lithium chloride sensors is given by Handegord and Hedlin (1965). Rogers (1965) describes a very small Dunmore type of sensor, and explains how it can be used for studies of humidity in microclimates and specifically to investigate relative humidities near a leaf surface.

Carbon humidity elements are reviewed by Stine (1965). There are many forms of these, but in priniciple the resistance of a thin plastic sheet containing

finely divided carbon changes with relative humidity.

An account of polyelectrolyte electrical resistance hygrometer elements is given by Musa and Schnable (1965), and a comparison of these elements with Carbon humidity elements is given. Cerium titanate elements are used by Johnson and Duggan (1965), lead iodide films, by Jones (1965), and Aluminium oxide by many workers.

The aluminium oxide hygrometers appear to have great possibilities, especially in view of the "hygroelectric effect" reported by Jason (1965). The elements can be needle-like, and the use of a probe in checking the humidity in stored bags of rice is described by Miyata and Watari (1965).

COULOMETRIC HYDROMETER

This method is reviewed by Jones and Petersen (1965), and as they point out it dates from the paper of Keidel (1956), and depends on the measurement of the cell current rather than resistance as in those systems just described. The cell is described as the electrolytic moisture cell, and reference is made to the review by Czuha (1962). The reaction involved shows unique regenerative behaviour, and provides an

accurate measure of absolute humidity in gases. The current passes as a result of the hydrolyis of a thin film of polyphosphoric anhydride to phosphoric acid, which is further electrically dissociated into hydrogen, oxygen, and the original anhydride. The net effect is the breakdown of water and the passage of a current. King (1965) describes yet another electrical route to humidity measurement. By depositing a hygroscopic film on a radio frequency quartz crystal he was able to measure humidity as a frequency change. Nelson and Amdur (1965) measure change in capacitance of a plastic film condenser.

A most interesting hygrometer was introduced by Wylie (1955) and its development brought up to date in 1965, again by Wylie (1965). A water soluble crystal such as potassium chloride will take up water from the surrounding air provided that the vapour pressure of the water in the air is higher then the vapour pressure of a saturated solution of the salt at that temperature. This thin layer of salt solution is maintained at the same thickness, that is between 100 A and several 1000 A thick, by controlling temperature

and therefore maintaining the same resistance of the thin layer of solution. A somewhat similar method based on the use of the actual phase transition temperature is explained by Nelson and Amdur.

(4) HYGROMETERS based on ABSORPTION SPECTRA.

A most helpful introduction is given by Johns (1965) who provides most necessary guidance through the complexity of this subject. All of the hygrometers based on these phenomena are essentially for specific, and very often difficult applications, and serve to underline the fact that there is no one "standard" hygrometer. The best one for the particular situation must be chosen. Some of the possible applications are listed by Randall, Hanley and Larison (1965), and for the specifically meteorological applications, by Foster, Volz and Foskett (1965) and Staats, Foskett and Jensen (1965). Wood (1965) gives details of the use of an infra-red Hygrometer to control humidity in a small test chamber.

It would seem to be quite possible that hygrometers of the utmost importance to Biologists will be developed from these instruments based on absorption spectra.

MISCELLANEOUS.

The humidity of a gas stream affects the thermal conductivity of the gas, and this fact has been used in industry since about 1910. It can be simple and most satisfactory in certain circumstances. The present position is reviewed by Cherry (1965). Wildhack, Perls, Kissinger and Hayes (1965) describe an hygrometer which continuously absorbs the water from an air stream by using a dessicant, and operates by comparing the pressure of two nozzles, one carrying the gas before, and the other after, dessication. Natural materials as the basis of hygrometers are brought up to date by Davey (1965) for hair, and Muller (1965) for other materials. The well known colour change of Cobalt Chloride solution has been developed and exploited, mainly for packaging [Blinn (1965)]. Wylie, Caw and Bryant (1965) describe a method which can be used for low dew-points. The water vapour is converted into combustible gasses which on burning give temperature changes which are easy to measure. Potentially this is a most useful method.

Finally of tremendous interest to biologists is

the Hygrophotographic method of Sivadjian (1965). He explains how humidity can reverse a blackish-violet colour produced on a special emulsion, by exposure to light. This leads to the use of such a sensitised plate for "Hygrophotographing" the water droplets in butter, margarine and sausage, for examining perspiration, and for other applications such as a study of soil water. The emulsion has to be calibrated, and Sivadjian describes the methods he used.

THE WATER RELATIONS OF BRYOPHYTES.

Introduction.

The modern bryophytes may be said to represent an early, and only partially successful attempt, to colonise the land. Wherever bryophytes are successful in dealing with dry conditions, they seem to owe their success to an ability to survive desiccation, and not to any method of preventing water loss. This unusual physiological achievement seems to favour the bryophytes as land plants. However their method of fertilization using a free swimming male gamete would seem to be a most primitive, and limiting possession. In fact generalisations about water relations in bryophytes are difficult, because of the great range in their form and apparently in their physiology. This results in frequent contradictions and uncertainties.

CONTENTS.

(1) Water Content and Respiration.

Early measurements of the effect of water content on respiration are quoted by Stiles (1956 and 1960). The earliest work quoted is that of Bastit (1891), who compared the intensity of respiration of leaves of Polytrichum juniperinum Hedw, when in the "open" position in damp air, and when in the "closed" position in drying air.

In ten experiments the ratio of respirations measured under these two conditions, varied between 0.15 and 0.58 (all except two were between 0.42 and 0.58). The respiratory quotient was always near to unity, so that the respiratory substrate was probably carbohydrate, at least under the conditions of the experiment.

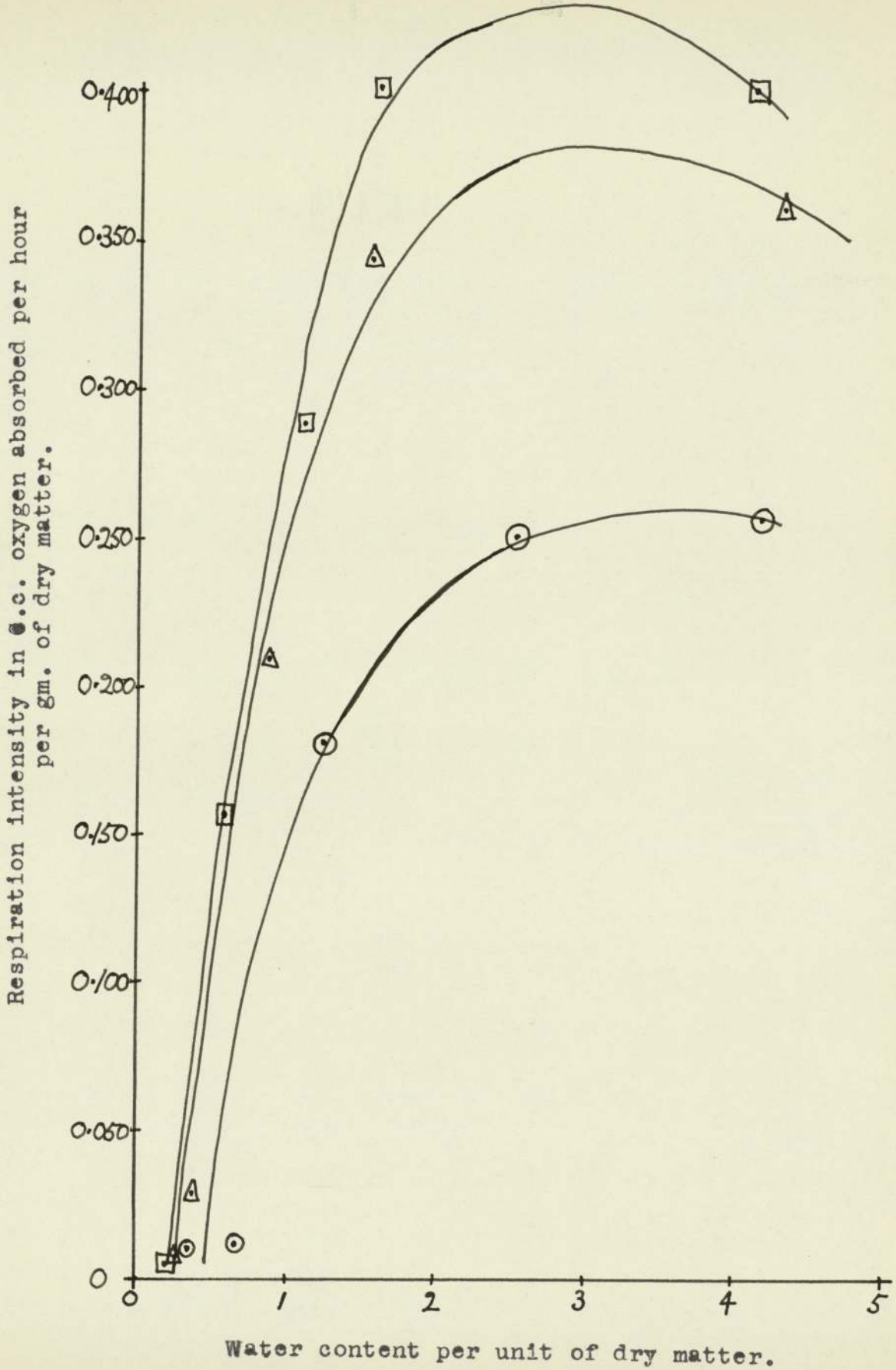
Fig 1. Relation between water content and intensity of respiration in the moss Mnium undulatum Hedw from Jonsson (1894) after Stiles (1956).

Water content in per cent.	Carbon dioxide in c.c. evolved per g. dry weight per 10 hrs.
40	0.75
59	1.35
65	3.90
84	9.68

Fig.2 Relationship between respiration intensity and water content of Rhytidiadelphus triquetrus Hedw. Warnst. from Plantefol (1927), after Stiles. (1960).

Water content per unit of dry matter.	Respiration intensity in cc. oxygen absorbed per hour, per gm. of dry matter.
0.317	0.0115
0.672	0.113
1.22	0.180
2.52	0.250
4.18	0.257
0.22	0.008
0.354	0.028
0.88	0.208
1.59	0.344
4.32	0.360
0.19	0.005
0.667	0.157
1.10	0.289
1.63	0.401
4.13	0.400

Fig.3. Graphs drawn from Fig 2.



This relationship between water content and respiration was confirmed by Jonsson (1894), for Mnium undulatum Hedw (Fig.1.), and by Plantefol (1927) for Rhytidiadelphus triquetus (Hedw) Warnst. Plantefol's results are shown in fig.2. and fig.3. Plantefol (1927) also measured respiratory quotient in Rhytidiadelphus triquetrus (Hedw) Warnst, and although he agreed with Bastit (1891) up to a point, he found that further desiccation increased the respiratory quotient to 6.9, when the water content approached 27% of the dry weight. This suggests that a change to anaerobic respiration occurs during severe desiccation.

However Romose (1940) found that although there was a similar increase in respiration in Camptothecium sericeum (Hedw) Kindb. until the water content was equal the wt. of dry matter, little further increase occured up to 700% of the dry matter.

Since respiration reflects the state of metabolism of the whole cell, it follows from these findings that the water content and hence the water relationships of bryophytes is very important.

Perhaps even more important is the implication that the water content can easily be varied, and has an enormous effect on respiration.

(2) Water Uptake in Bryophytes.

Before considering water uptake it is helpful to introduce the physiological classification of the Bryophytes given by Buch (1945-1946). Richards (1959) points out that much of the earlier studies on conduction in bryophytes suffered from faults in technique, but the resulting contradictions have been largely resolved by the more recent studies of Magdefrau (1935), Buch (1945), Zacherl (1956), and in a small paper by Bopp and Stehle (1957). In particular Buch (1945) suggests that bryophytes can be assigned to two major physiological groups, with the exception of some intermediate forms that he terms MYXOHYDRIC. The characteristics of Buch's groups are as follows.

a) ENDOHYDRIC BRYOPHYTES.

This group includes mosses with a well developed central conducting strand. They take up water by the basal rhizoids and pass it internally via the stem to the leaves. A transpiration stream exists, and is capable of supplying water to the leaf cells, and of maintaining their turgor from supplies of soil water, even if the plant is surrounded by air having a considerable saturation deficit, and when no external water conduction can take place. Buch gives the following species, As examples: Mnium undulatum Hedw., Bryum capillare Hedw., and Polytrichum juniperinum Hedw. Buch (1945) showed that the leaves in these mosses appear to have a cuticle-like covering, but Richards (1959) states that there is some evidence that this "cuticle" has thin spots through which water loss or uptake can occur more easily than over the rest of the surface. He further states that these "spots" can function as hyd athodes in a saturated atmosphere. Buch (1945) states that endohydric bryophytes can be recognised by using the following features.

(1) They show a well developed basal rhizoid system.

(2) Their young fully expanded leaves do not stain readily with basic dyes.

(3) Their external cell walls are relatively impermeable to electrolytes. (This would seem to be a result of the "cuticle").

(4) After air drying the leaves will only slowly become turgid again when placed in water.

b) ECTOHYDRIC BRYOPHYTES.

This group which probably includes the majority of mosses and all the leafy liverworts, shows no obvious, differentiated conducting strand. Water, and dissolved substances can be absorbed through virtually any part of their surface, and they show no regular internal movement of water. Watson (1964) gives the genera Trichostomum, Tortella, Orthotrichum and Ulota, as good examples of this type of bryophyte. In general this type of bryophyte does not show the features listed above for Endohydric types, and in particular the leaves of this type can recover from air drying very quickly indeed.

Richards (1959), states that in addition to the Endohydric, Ectohydric and Myxohydric groups of Buch, the terrestrial Marchantiales form a separate group, except that, by absorbing water and solutes entirely through the rhizoids and lower surface of the thallus, they resemble the endohydric group.

Stocker (1956) reviews the experimental work which has been carried out on water relations in bryophytes, and to some extent reviews the whole of the Thallophytes. He gives two illustrations from the work of Romose (1940) which involved the measurement, (by weighing) of the water absorbed by Homalothecium Sericeum B & S (now Camptothecium sericeum (Hedw) Kindb) when in equilibrium with water vapour over a known solution (Fig. 4. & 5). This method enables very precise measurements to be made, provided that time is given for the solution, water vapour and plant to reach a dynamic equilibrium, and provided that no temperature change is allowed. Fig. 6. shows the uptake of water from water vapour by the moss Bryum argentum Hedw. at three different humidities as measured by Magdefrau (1931).

Both Fig.5. and Fig.6. seem to show that at least in these mosses only relative humidities very near to 100% r.h. are going to allow the mosses to remain turgid. In fact it seems reasonable to state that these two species have virtually no control over their equilibrium position with the water vapour around them.

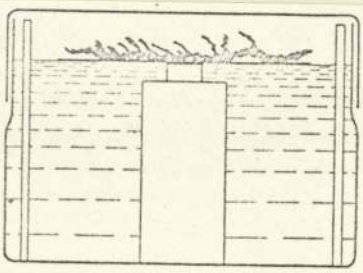


Abb. 9. Apparatur zur Messung des Wassergehaltes von Moosen im Dampfdruckgleichgewicht mit NaCl-Lösungen bekannten Dampfdruckes. Die Moosprobe liegt auf einem Deckglas dicht über der Lösung, die Wände des Luftraumes sind mit Filtrierpapier ausgekleidet. (Aus ROMOSE 1940.)

Fig 4. (Apparatus to measure the water content of mosses at "Vapour pressure equilibrium" with Na Cl solutions with known vapour pressures. The moss sample is lying on a cover slip, slightly above the solution in a space lined with filter paper. (from Stocker (1956) after Romose (1940).

Water content
(% of dry substance)

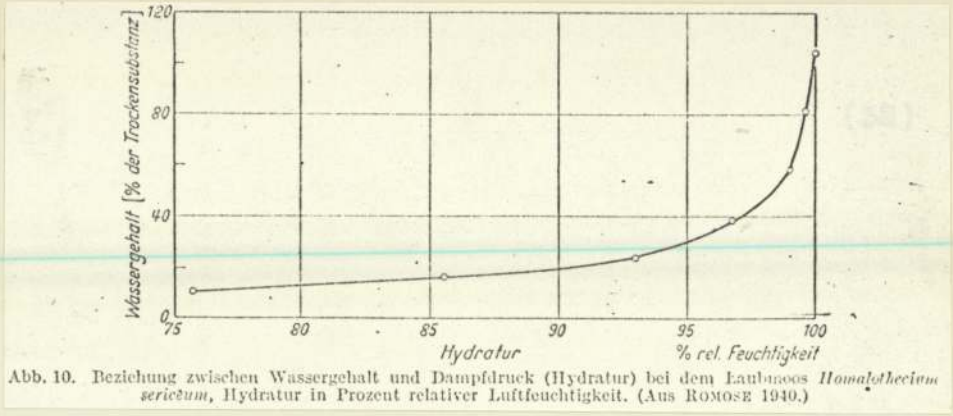


Abb. 10. Beziehung zwischen Wassergehalt und Dampfdruck (Hydratur) bei dem Laubmoos *Homalothecium sericeum*, Hydratur in Prozent relativer Luftfeuchtigkeit. (Aus ROMOSE 1940.)

Fig 5. (The relation between water content and vapour pressure (hydratur) of the moss Homalothecium sericeum. Hydratur in percentage relative humidity, (from Stocker (1956) after Romose (1940).

Weight
(% air dry weight)

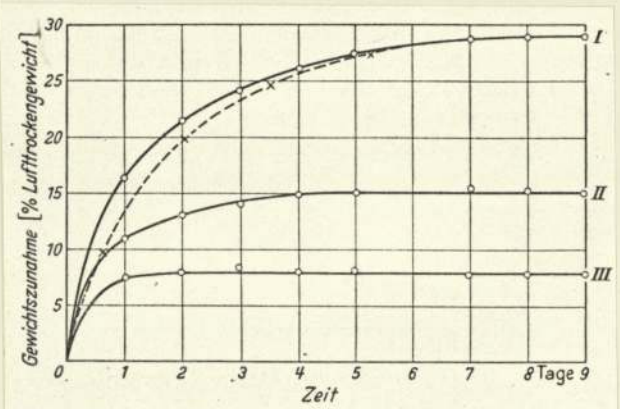


Abb. 11. Wasserdampfaufnahme eines Laubmosses *(Bryum argenteum)* I über Wasser (100% relative Feuchtigkeit); punktierte Kurve berechnet nach der Formel (FREUNDLICH, Kapillarchemie, Leipzig 1923) $k = \frac{1}{t} \cdot \ln \frac{a_\infty}{a_\infty - a}$ (k = Geschwindigkeitskonstante, a = zur Zeit t, a_∞ = im Quellungsmaximum aufgenommene Wassermenge). II über 0,4 mol NaCl-Lösung (98,8% r. F.), III über 1,0 mol NaCl-Lösung (96,9% r. F.). (Aus MÄGDEFRAU 1931.)

Fig 6. Water vapour uptake by the moss Bryum argentum Hedw.
I over water (100% relative humidity); the dotted curve is calculated according to the formula (Freundlich, Kapillar-chemie, Leipzig 1923)

$$K = \ln \frac{a}{a - a}$$

K - velocity constant, a - at the time t, and a - swelling with the quantity of water.
II over 0.4 mol. Na Cl solution (98.8% relative humidity).
III over 1.0 mol. Na Cl solution (96.9% relative humidity).
(from Stocker (1956) after Magdefrau (1931).

In their investigations into the transport between gametophyte and sporophyte in Funaria hygrometrica Hedw.; Bopp and Stehle (1957) found that the transport of fluorescent dyes within the rhizoids was very slow. It took forty hours for the dye to traverse 10 cells, and in fact they state that the main function of the rhizoids in Funaria hygrometrica really consists of a capillary movement of water. However the functions of the rhizoids and in fact the whole question of water absorption is by no means clear. For example Zacherl (1956), found that of the fluorescent dyes he was using only one was taken up by rhizoids, and in this they were similar to roots of higher plants such as Avena and Tradescantia. He found that the moss leaves behaved in the same way.

Water uptake in the thalloid liverwort Pellia epiphylla (L) has been investigated by Clee (1939). He set up the apparatus shown in Fig.7., so that the plants could be exposed to known conditions, and also, have their water supplied from a solution of the selected stains. He found that the stain reached

the tip of a thallus very quickly indeed. The average time taken under the conditions of the experiment was 61 seconds.

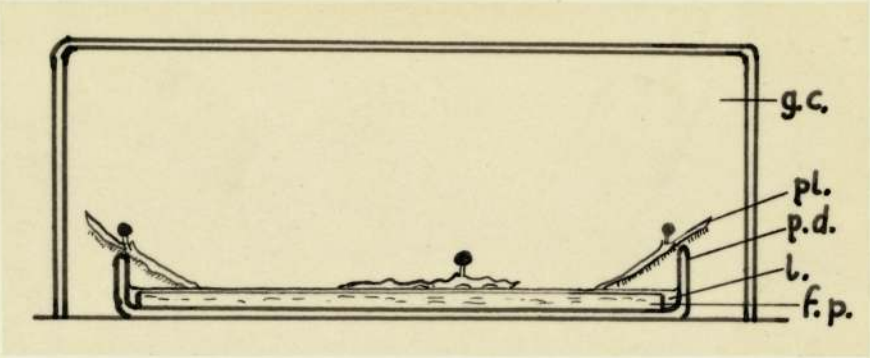


Fig.7. Diagram of apparatus used to investigate the mechanism of absorption and conduction of water in Pellia epiphylla (L) g.c. glass chamber; pl plant; p.d. petri dish; f.p. filter paper; l. liquid. (from Clee 1939)

Washed, and surface dry, plants were placed on the filter paper so that they were either flat or 30°or 40° to the horizontal. If the plants were inclined to the horizontal only the end was in contact with the filter paper. The filter papers were saturated with 0.5% solutions of a variety of "vital" stains, and then finally the petri dish was enclosed in a glass chamber to maintain a high humidity.

No attempt was made to control humidity. Clee (1939) found that the water travelled in a capillary film, between the rhizoids, and over the under surface of the thallus. The water was partly absorbed by the under surface of the thallus, and partly retained in the antheridial regions after passing over the surface of the thallus. Very little internal conduction seems to take place.

According to Prat and Minassian (1928) and Stocker (1956) bryophytes require liquid water if they are to live properly, and that gaseous water is absorbed with difficulty. However Ochi (1952) did state that hygrophilous mosses are capable of imbibing water from air which is not saturated with water vapour.

(3) CONDUCTION and STORAGE.

a) Conduction:

Magdefrau (1935) made precise measurements of rates of conduction in mosses, using the technique illustrated in Fig.8.

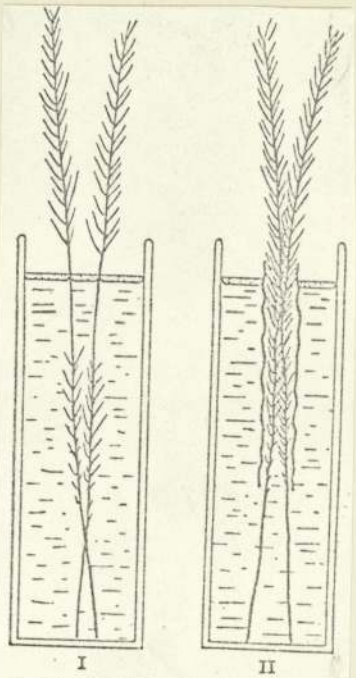


Abb. 2. Versuchsanordnung zur Bestimmung der inneren (I) und der Gesamtleitung (II) von Wasser bei *Polytrichum*. Das Wasser ist mit Paraffinöl (punktiert) überschichtet. Dieses hindert in I das Wasser am äußeren capillaren Aufstieg, wobei das Moosstämmchen selbst gegen Infiltration durch einen nicht gezeichneten Kakaobutterring geschützt ist; in II wird das Paraffinöl durch eine Stanniolhülle vom Stämmchen ferngehalten, so daß auch äußere Leitung stattfinden kann. (Aus MÄGDEFRAU 1935/36.)

Fig 8. The experimental arrangement for estimating the internal (1) and the total conduction (II) of water in Polytrichum. The water is covered with paraffin oil (dotted). This prevents in NoI, the ascent of water in the outer capillary. The moss-stem itself being protected against infiltration by a ring of cocoa-butter (not shown). In NoII the paraffin oil is kept away from the stem by a tin foil cover so that an outer conduction can take place.
From Magdefrau (1935/36) after Stocker (1956).

As a result of this type of experiment Magdefrau (1935), concluded that the earlier work by Bowen (1931 & 1933) was based on unreliable methods, and that in fact both internal and external conduction of water can be important in mosses. He found that internal conduction is carried out largely in the conducting strand, and that in certain mosses this internal conduction is sufficient to maintain turgidity in leafy shoots exposed to a relative humidity of 90%. In most mosses both types of conduction are essential in atmospheres having a relative humidity below 90%. He lists his measurements of total and internal conduction for 21 species, and has drawn up a table showing presence or absence of central strand, importance of rhizoids, and comments on both structures for 189 species. The external conduction is within a surface film of water so that its actual rate in a moss living under normal conditions will depend on many factors. Among these would be size and geometry of attachment of leaves, the distance apart of these leaf bases, the presence and frequency of rhizoids or paraphyllia, the amount of branching, and all the

environmental factors which affect humidity since this will also control the thickness of the water film.

Zacherl (1956) further investigated internal conduction in mosses using fluorescent dyes. His techniques enables him to examine internal conduction in great detail, and the results of this work are most important. He found that in mosses of the "Mnium type" the transport of water was confined to the central strand of the stem, and the midrib in the leaf. The rest of the stem tissue ("ground tissue") and the leaf lamina are only secondarily supplied from these two sources. Zacherl states that the differentiated tissues of the midrib only extend into the ground tissue of the stem, and do not reach the central strand. He considers that water from the central strand must cross the "ground tissue" to reach the differentiated tissue or "false leaf traces" which then act as wicks to pass the water on to the leaves.

In mosses of the "Polytrichum type" however there is direct connection between the water conducting

tissues of leaf and stem, and here he found that the transfer of water to the leaves is carried out by the leaf traces of the central strand. The supply of water is more rapid and direct to the upper leaves so that the lower leaves are usually supplied with water later than the tips of the shoot. He also showed that within the leaf midrib, the central cells form the main conducting tissue. These central cells of the midrib, and the cells of the central strand were both shown to conduct the water in their lumina.

Zacherl found no inner conduction in those mosses with no central strand, nor in those with a very small central strand.

Values for the speed of the transpiration stream in Polytrichum commune Hedw. are given as 140c.m./hr. at a relative humidity of 70%, and 200cm/hr. lower in the shoot where the central strand has a smaller diameter. The value for Mnium undulatum Hedw. is 120cm/hr. at a relative humidity of 65%. These values were shown to be comparable with those to be expected under natural conditions. Zacherl goes on to compare these values with those quoted by earlier text

books for ring porous and diffuse porous woods of spermatophytes. The values quoted are the highest speeds found at noon. Ring porous wood shows values between 43.6 m/h. (Quercus Pedunculata) and 3.9 m/h (Cytisus Laburnum).

Values for diffuse porous wood are:-

Populus balsamifera	6.25 m/h.
Tilia tormentosa	3.43 m/h.
Salix viridis	3.00 m/h.
Acer pseudoplatanus	2.40 m/h.
Almus glutinosa	2.00 m/h.
Betula verrucosa	1.60 m/h.
Carpinus betulus	1.25 m/h.
Pirus communis	1.11 m/h.
Fagus sylvatica	1.07 m/h.
Aesculus hippocastanum	0.96 m/h.

So that the speed attained by Mnium undulation Hedw., and Polytrichum commune Hedw., is not worse than that of wood. In fact the speed of their streaming is better than that of Aesculus, Fagus, Pirus, Carpinus

and Betula. Zacherl makes his point very firmly.

"This fact may be the more surprising since many an Author is inclined to think little of the inner conduction in mosses having a well developed central strand. They think little of this compared to capillary conduction. Indeed the opposite is the case. Investigations trying to prove capillary conduction in Mnium and Polytrichum showed that such a capillary conduction does not exist for great parts (of the stem)."

Bopp and Stehle (1957), refering to the earlier work by von Oltmanns (1884), Haberlandt (1884), Magdefrau (1935), Buch (1945) and Zacherl (1956), state that without doubt the conduction of water and other materials has been largely worked out in mosses. However they consider that the supply of the sporophyte by the gametophyte and conduction within the sporophyte, have been more or less overlooked in these earlier studies, except for a few studies of a few species. As far as conduction in the gametophyte is concerned they consider that one can distinguish between those which transport their water mainly or

solely by external capillary conduction, and those where inner conduction plays the main part in the transport of water. Bopp and Stehle (1957) concentrate on transport to, and conduction in, the sporophyte and further confine themselves to Funaria hygrometrica Hedw. Fluorescent dyes were again used in this study. The authors found that Funaria is a myxohydric moss, since conduction in the gametophyte is a combination of internal and external flow. They found that the flow in the central strand was important, but that it did not seem to supply the leaves to any great extent, and perhaps even more surprising they found a very slow flow in the rhizoids. This can be interpreted by supposing that supply to the base of the stem is via external channels.

The explanation of the gametophyte supply to the sporophyte, given by these two authors depends on their concept of a sucker-like or haustorial base of the foot, plunged into the tissue of the gametophyte central strand, and therefore becoming sheathed by the gametophyte ("beaker-wise"). They found that a narrow intercellular space separates the tissues of the two generations. Their experiments using fluores-

cent dyes showed that this intercellular space was coloured before the top of the central strand, and before the tip of the haustorium. They called this space an "internal capillary channel" and showed that it was effective as a pathway. Conduction within the seta was shown to be largely via the central strand. Most interesting observations were made on the calyptra, which was shown to act as a "transpiration shield" the removal of which led to an increase in upwards flow of the experimental fluid. The histology of the central strand of the stem has been studied in Polytrichum and Pallavicinia. Watson (1964) states that the relatively large cells of the central strands form the hadrom of many authors, while the surrounding tissue made up of small cells has usually been called the leptom. Some authors have described the outer layers of the leptom as forming a separate sheath of starch-filled cells. Outside this layer can be seen a cortical layer of cells which are normally given in young stems are themselves bounded by the thick walled cells of the peripheral layers. Patches of colourless hadrom forming "leaf

traces" can usually be seen in the cortical layer. Watson stresses that the leptom and hadrom must not be equated with xylem and phloem of vascular plants. He points out particularly that hadrom cells show neither the lignification nor the pitting of tracheids, and stresses that leptom cells are not seive elements. However Smith (1964) reporting briefly on the gametophytes of Pallavicinia and Symphyogyna, states that they "contain a unique water-conducting system strikingly analogous to the xylem of the vascular plant sporophyte". Watson also points out that many of the apparent anomalies in the work of Lorch (1931), can now be explained in the light of the more recent physiological findings. For instance the lower parts of ectohydric species may often be dead. Since they are ectohydric then it now seems reasonable to assume that as far as conduction is concerned the dead parts are just as effective as the living, provided that they keep their shape. Watson also refers to the most interesting findings of Lorch that many pleurocarpous mosses have the conducting strand better developed in erect stems than in prostrate stems, and that in dend-

roid mosses the hadrom cells were often collapsed in creeping stems, but healthy in erect stems. These now seem perfectly reasonable in the light of Zacherl's (1956) work.

b) Storage:

It is convenient to consider storage here, even though it is such a specialised topic. Before considering storage tissues it should be mentioned that the external film of water so important to bryophytes and especially to ectohydric species, is itself a supply of stored water.

Watson (1964), suggests that the descending branches of Sphagnum are wick-like and therefore an efficient device for external capillary conduction. He suggests that the lack of a central strand could be almost expected.

The extent of cell specialisation is outlined and Watson points out that this is still difficult to explain. However he takes care to stress that Lorch (1931) implied that the chief function of thick cell walls is that of water storage.

In liverworts water storage tissue does not seem

to be developed, but leaves may be specialised to form "water sacs", or water may be held as a film over the upper surface as shown by the work of Clee (1939) with Pellia epiphylla. (L).

However Clausen (1952) found in her experiments with hepatics that the "water bags" of species of Frullania did not enable them to resist desiccation better than species of Lophocolea which do not have them. However she did find that the greatly divided leaves of Ptilidium species really did seem to delay desiccation.

(4) Water Loss from Isolated Plants.

The work done on water loss from isolated plants is summarised by Stocker (1956). He gives two figures from the publications of Prat and Minassian (1928) (fig.10 & 11).

It is most interesting that there is only a negligable difference between the behaviour of living and dead plants, although the living plants behave differently from filter-paper. Thus there is early evidence for the general view that the drought resistence

of mosses is very real, but largely passive. The experiments do not help to decide which part of the plant is responsible for this behaviour. However the results of Plantefol (1927) (fig.13) do seem to show that the rate of loss of water by Polytrichum formosum Hedw. is higher than that in more "normally" constructed mosses such as Hypnum cupressiforme Hedw. so that the plate-like outgrowths of the leaves do not seem to delay water loss, in this case at least.

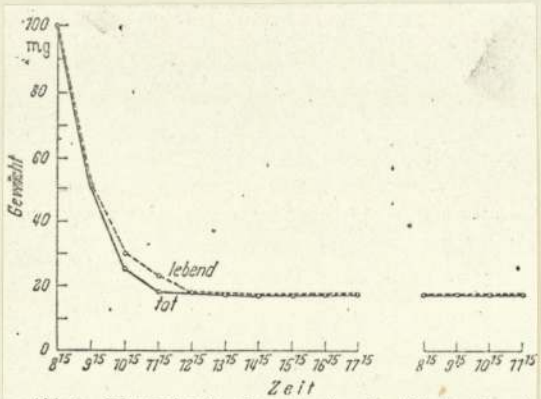


Abb. 1. Wasserabgabe eines lebenden (gestrichelte Kurve) und toten (ausgezeichnete Kurve) Laubmooses (*Polytrichum commune*) beim Austrocknen. Abszisse Frischgewichte, beginnend mit dem wassergesättigten Zustand. Abtötung mit heißem Wasser. (Aus PRÁT und MINASSIAN.)

Fig.10. The water loss from living (dotted curve) and dead (continuous curve) mosses (Polytrichum commune Hedw.) during drying out. Abscissa fresh-weight, beginning with (the plants) in a water-saturated condition. Killing off with hot water. From Prat and Minassian (1928) after Stocker (1956).

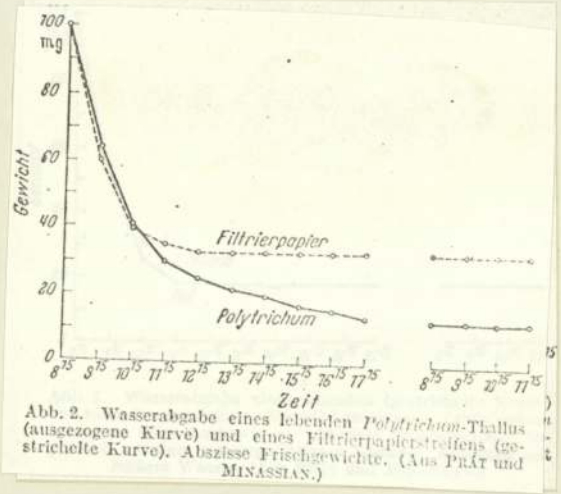


Abb. 2. Wasserabgabe eines lebenden *Polytrichum*-Thallus (ausgezogene Kurve) und eines Filtrierpapierstreifens (gestrichelte Kurve). Abszisse Frischgewichte. (Aus PRÁT und MINASSIAN.)

Fig.11. Water loss from a living Polytrichum thallus (continuous curve) and a piece of filter paper (dotted curve). Abscissa freshweight. From Prat and Minassian (1928) after Stocker (1956).

Tennant has investigated the water loss from cut shoots (1954) using Thamnium alopecurum (Hedw) B & S., in a potometer enclosed in a chamber. The chamber was provided with dishes of acid solution to control humidity, but no reference is made to temperature control. Even so this investigation represents a real step forward since water loss (by weight) was followed accurately under different environmental conditions.

In summing up Tennant (1954) feels able to state

that, "it may be concluded that, in the species investigated at least, there is little or practically no physiological control of transpiration, and a net water-loss from the plant occurs in atmospheres which have relatively high humidition".

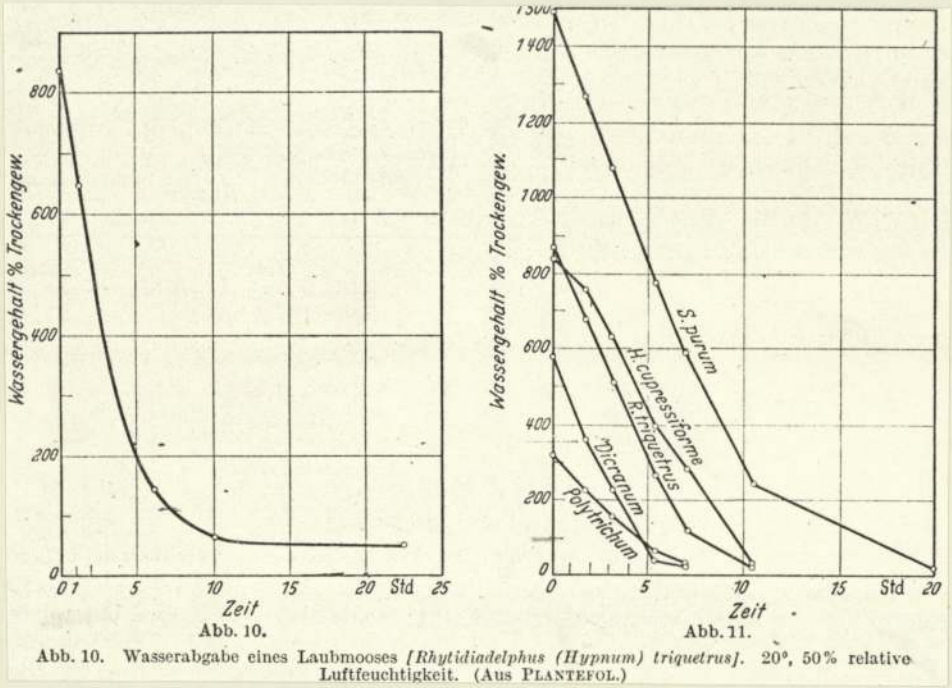


Abb. 10. Wasserabgabe eines Laubmooses *[Rhytidiadelphus (Hypnum) triquetrus]*. 20°, 50% relative Luftfeuchtigkeit. (Aus PLANTEFOL.)

Fig.12. Water loss by moss plants (Rhytidiadelphus Triquetrus (Hedw) (Warnst) 20°C and 50% relative humidity. From Plantefol (1927) after Stocker (1956).

Abb. 11. Wasserabgabe von Laubmoosen *[Scleropodium (Hypnum) purum, Hypnum cupressiforme, Rhytidiadelphus (Hypnum) triquetrus, Dicranum scoparium, Polytrichum formosum]*. 20°, 50% relative Feuchtigkeit. Isolierte, nicht Wasser saugende Pflanzen. (Aus PLANTEFOL.)

Fig.13. Water loss from the mosses Hypnum cupressiforme Hedw., Rhytidiadelphus triquetrus Hedw., Dicranum scoparium Hedw., and Polytrichum formosum Hedw. 20 C and 50% relative humidity. Isolated plants not taking in water. From Plantefol (1927) after Stocker (1956).

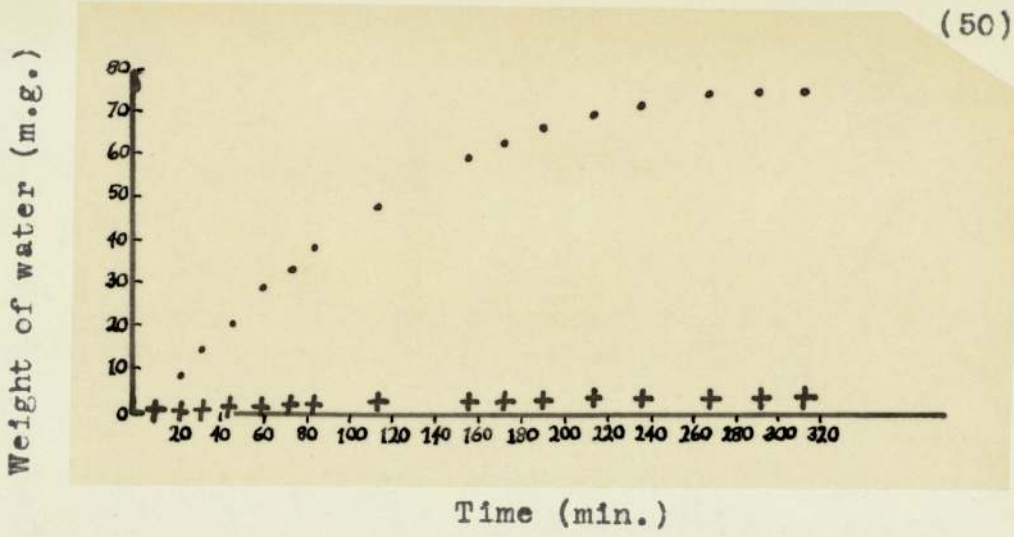


Fig.14. Graph of the actual figures for transpiration (dotted curve) and water uptake (crossed) of a cut shoot of Thamnium alopecurum (Hedw) B & S in an atmosphere of 75% relative humidity. Drawn from Tennant (1954).

5a) Distribution in the field in relation to resistance to dessication.

Hosokawa and Kubota (1956), as a result of their work on osmotic pressure in relation to desiccation of epiphytic mosses, concluded that when kept under constant conditions, the higher the relative humidity, the longer the mosses survive, and the higher the osmotic value of a species the greater is its resistance to desiccation. They controlled humidity by put-

ting their mosses in sealed petri dishes with a "small open dish" of dilute sulphuric acid. No mention is made of temperature control, nor do they apparently make any correction for the severe dilution of their acid solutions which must have taken place. One drawback to these results, even if the relative humidity estimations is correct, is the failure to measure actual conditions in the field. This makes it difficult to apply these findings.

A very comprehensive investigation was carried out by Clausen (1952) in a most careful and detailed study of hepatics, relating their ability to withstand desiccation to actual conditions in the field. Miss Clausen demonstrated quite clearly that there is close agreement between the ability to withstand desiccation and distribution of the plants in terms of the humidity conditions of the habitat.

The microclimates of the habitats were found using apparatus based on the well-known bimetallic strip and hair hygrometer, using the usual precautions to avoid incident radiation and abnormal effects from the hair. It became evident that these instruments would

not be adequate to measure the humidity near the hepatic thalli, and much more sensitive methods were used in all cases where the thalli did not reach 1.5cm. above the ground. Sulphuric acid tubes were used in the manner described by Nielsen and Thamdrup (1939), and thermo-electrical instruments for temperature. The Sulphuric acid tubes were prepared by filling capillary tubes with known solutions of sulphuric acid, and then attaching them or suspending them just above a thallus. Only a solution which is in equilibrium with the atmosphere will remain as it is, the others either withdraw into the tube, or overflow. Great care was taken to ensure that no insect or other "foreign body" touched the tube and made solution flow out.

Miss Clausen's findings are of great interest. In a preliminary examination of the turgescence of the species in the field she found that although a large number of species grow in places, so moist, that they are always turgescent, there were others which dry slightly now and then. There were transitions from these to species which in summer are dried up for

long periods, and only keep turgescent for a few hours after rain or dew-fall. Under heather this type often recieves no dew in the morning, but keeps moist for a long time after rain, whereas in open places there is a greater dew-fall, but the plants only remain turgescent for a short time. Even after heavy showers of rain they dry up fast when the weather in summer is bright and windy. She found that although epiphytes are exposed to longer periods of drought the relative humidity in the wood never fell below 55% so that the desiccation of the epiphytes is never as great as that found in a sandy field facing south. In the experiments investigating resistance to desiccation Clausen was able to show that plants which experienced drought were appreciably more resistant after experience of the drought than they were before. She summarized her findings in terms of resistance. Some species show very little resistance to desiccation, but are normally found in moist places. In contrast to this group are the epiphytes which are always very resistant. Even in the wettest parts of a damp season they can withstand 15% r.h. or less. Finally there is a heterogeneous

group with varying resistance, and with a resistance which varies with exposure. A good example of the success of the measuring techniques used, is the record quoted from a lane, where an r.h. of 85% was recorded above Nardia scalaris, but on a small prominence only 5cms away, 55% was measured above Frullania tamarisci.

5b) Morphology and resistance to dessication.

In his discussion of the histology of bryophytes, Watson (1964) points out that their resistance to desiccation, although so widely examined is still imperfectly understood. It would certainly seem to follow from the findings of Zacherl (1956) that ectohydric bryophytes could only survive drying conditions by resisting water loss, since they have no method of obtaining water from their substratum once their surface film has evaporated. Watson (1964) mentions that the early investigations of such workers as Irmscher (1912) and Malta (1921) showed clearly that different species possess very different

abilities to resist desiccation. The examples given are Fontinalis squamosa Hedw. which could only withstand one week of air drying, presumably because it is aquatic, Philonotis fontana Hedw. Brid. and Grimmia Pulvinata Hedw. Sm. The Philonotis fontana plants died after fifteen to twenty weeks of air drying, but the xerophytic Grimmia pulvinata plants withstood sixty weeks in a desiccator at 20°C.

Ochi (1952) uses the quantity hydrability to express his results quantitatively. Hydrability $H = \frac{h_o - h_i}{h_o}$ where h_o = maximum hydration when the sample is immersed in water. h_i = actual hydration of the sample.

The results enabled him to make certain generalisations. The maximum hydration (%) of xerophytes is less than that of mesophytes which in turn is less than that of hygrophytes. However he found that the minimum hydrability of xerophytes is approximately the same as that of mesophytes, but less than that of hyprophytes. He states that, it seems that xerophilous mosses do not easily reduce their water below the quantity of their own minimum hydrability when air dried, that they are capable of imbibing vapour from

air which is not saturated with water vapour. Hygrophilous species do not show these peculiarities, and he supposes that the differences are caused mainly by peculiarities in their protoplast. He also finds that air dried samples of dead xerophilous mosses show almost the same water relationships as air dried living samples, and considers that this property is important in their ecology.

Gimingham and Birse (1956) in their studies of growth-form in Bryophytes, draw up a classification of life-forms in terms of cushions, turfs, canopy formers, mats and wefts. The system was based on morphology only, and was free from assumptions about the adaptive significance of structural characteristics. They found that growth form distribution clearly reflects gradients, in the moisture and light factors. They also found that as relative humidity decreases in conjunction with increasing light intensity the following sequence in zonation is observed: Dendroid forms and thalloid mats; Rough mats; Smooth mats; Short turfs, and small cushions. While their general conclusions about relative humidities may well be correct

they do not appear to have measured them close to the plants.

Tagawa (1961) examines the ecological significance of growth form in the single species Ulota crispula Brid. from an experimental standpoint. The equipment used was a modified form of that used by Tennant (1954) for investigations using a potometer. In this case however, whole moss "cushions" were suspended from the balance, and exposed to air which had been passed through saturated salt solutions to give a suitable humidity. Unfortunately no mention is made of temperature control, and once again relative humidity values must be suspect. It was found that although the weight of water which could be held by a cushion increased as the number of shoots increased, the water holding capacity per shoot decreased. By experimenting with dead plants Tagawa showed that this was not due to a regulatory mechanism. There was one finding however which could have an important ecological role. The greater the mass of water held in a cushion, the longer the time needed to reach equilibrium to a given atmosphere. Thus the growth form of a moss cushion must be effective in reducing water loss from the plants despite its inefficiency for water holding.

5c) Distribution in the field interpreted in the light of growth under controlled conditions.

This type of investigation was carried even further by Forman (1964) in his investigation of the distribution of the moss Tetraphis pellucida Hedw. He set up a complex "microphytotron" to investigate the effects of many factors on the growth of his moss.

Forman (1964) was particularly interested in those factors which limit the range of his species. He found that neither p.H. nor light intensity were normally limiting in the field, and that moisture and temperature range in terms of Phototemperature and Nictotemperature were the most important factors in interpreting continental distribution. He expressed the interrelationships of these factors as a complex graph drawn from the results of an experiment (Fig 15).

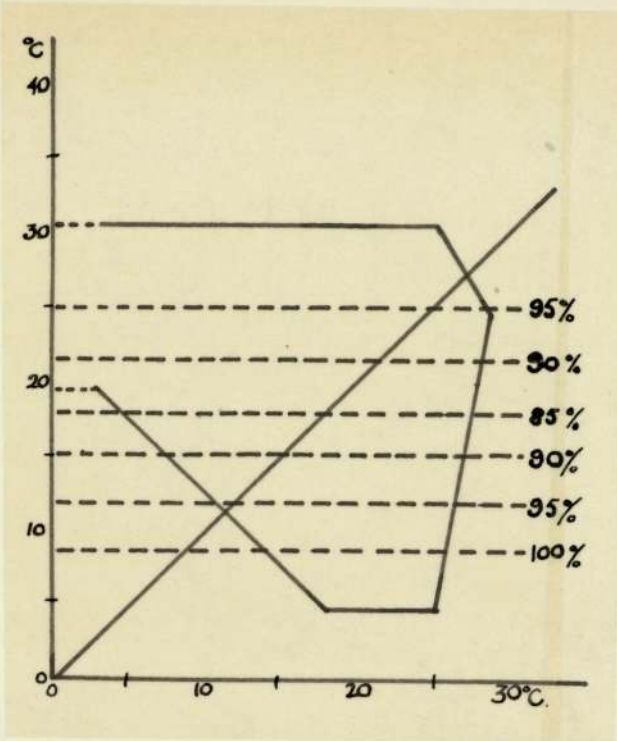


Fig.15. The effect of photo- and nictotemperatures on shoot growth. The enclosed area represents the temperature regimes under which the species will grow. The long diagonal line represents constant temperatures. The percentages refer to relative humidity. This graph was used throughout, to determine whether the species will grow at any spot in North America. Growth from young shoots on inocculated mature plants; length of experiment 21 days; light intensity 100 f.c., relative humidity 100%; p.H.5.1. (from Forman 1964).

In his summary of this work Forman states that the transplant experiments he carried out, and the work with the microphytotron, indicate that the specific

experimental limits for growth of the leafy shoot are directly applicable to those climatic conditions measured in the field. Therefore the distribution pattern at each level in the hierarchy is explained by applying the experimental limits to known field conditions. The explanation at the continental distribution level is worked out in greatest detail based on correlating combinations of environmental factors with diurnal and seasonal climatic fluctuations in nature. He compared this method with several others, and found it to be the only satisfactory one. Forman's (1964) results show that Tetraphis pellucida Hedw. is very intol erant to low humidity, and in fact he does not seem to have had specimens growing at humidities as low as 85% r.h. Forman was well aware of the problems of humidity, and devotes a paragraph to explaining this aspect of his experiments. He admits that to a large extent the humidity could not be measured, and that it was necessary to estimate the actual conditions under which the mosses were growing.

6) Reproduction and the water relationships of bryophytes.

The reproduction of bryophytes can normally occur in four different ways, three of which are asexual and one is sexual. The three asexual methods as listed by Watson (1964) are: by growth and branching, followed by the death and decay of the older parts; by the separation of whole organs, and the regeneration of new plants from them; and thirdly, by means of specialised units of propagation called gemmae. Watson (1964) points out that the first method is widespread, and gives as an example the genus Riccia where the vegetative spread of the plant may be by this method. However this is strictly vegetational spread rather than vegetative propagation. The second method on the other hand is a very effective method in mosses is by whole shoots or shoot tips which become detached. However Watson (1964) states that there is little precise information about the actual spread of species by this method, although Myurium hebridarum Schp. must be spread by means of deciduous branching since no sporophyte has ever

been found in Britain.

Propagative organs of definite form, unlike the parent, and usually originating from one cell are usually called gemmae. These extremely effective propagules are widespread in occurence in the bryophytes. According to Watson (1964) the occurence is uneven in British bryophytes, and gemmae are found in less than 10% of the Marchantiales, in just less than 20% of Metzgeriales whereas almost 40% of the British leafy liverworts (Jungermanniales) are known to produce gemmae. Gemma production seems to be particularly associated with colonisation of tree stems and bare soil. Water has always been assumed to be the most important factor in the dispersal of gemmae, and in particular heavy rain drops are said to be effective in dispersing gemmae from gemma cups.

It could be said then that water has little or no more effect on asexual reproduction than it does on the normal life of the gametophytes.

In sexual reproduction however the complex problem of transfer of antherozoids is involved, and here liquid water is of paramount importance.

It appears that most bryophytes are dioecious so that their antherozoids must normally travel from male to female plants, and this may involve distances of several feet. Moreover they are shed from the antheridium while they are still inside the mother cell, although release does occur eventually. When liberated the antherozoid is usually naked, elongate, biflagellate, and in fact consists mainly of its one nucleus. Its structure and in particular the relatively large size of its nucleus prevents the antherozoid from having any extensive food store. Active "swimming" must therefore only be possible for a short distance. Since many terrestrial bryophytes form separate "cushions" of male and female plants it becomes very difficult to imagine how fertilization is ever effected. Cavers (1903) and Pierce (1902) observed explosive discharge of antherozoids in Fegatella conica and Asterella californica respectively, and after carefull examination of the discharges they decided that it was adequate to explain antherozoid dispersal in those two species. The sprays sometimes reached a height of several centimetres, and

lasted for several seconds. However further work by Showalter (1926), Anderson (1931), and Muggoch and Walton (1942) showed that explosive dehiscence was not of general occurence, and that some other mechanism must be found to explain antherozoid dispersal. In fact Muggoch and Walton (1942) showed that in Mnium hornum Hedw., there was a passive dispersal of antheridial content as soon as that content reached the water-air interface. Before this method of transport was demonstrated they had failed to understand the success of the sexual processes, if it was to be dependent upon such external factors as insect transport. For example, they pointed out that a hair or even an insect when dipped into the perichaetial cups of male plants carry away very few antherozoids. Muggoch and Walton (1942) observed the discharge of antherozoids from a large number of bryophytes, and their observations support those of Goebel (1898), in that dehiscence of antheridia under water is normally relatively slow, and the subsequent dispersal is also slow. Their great contribution however was to observe that as soon as the dehiscent mass reaches the

water-air surface the antherozoids within their mother cells (spermatocytes), are spread very rapidly, and evenly over the surface of the water. They quote Showalter (1926) as stating that if a large number of antherozoids of Riccardia pinguis (L) Gray. were placed at one end of a small pool of water 1cm x 0.5cm nearly all of them remained crowded at that end of the pool one hour later. Thus it would seem that the motile powers of antherozoids are not very great, and that the surface tension spreading effect, which Muggock and Walton (1942) showed to be probably caused by a fat, is a most important mechanism.

The most doubtful part of a system based on this process is the escape of the antherozoids from the surface film. However the shape and nature of the open end of the archegonium may overcome this.

The initiation of dehiscence of the antheridium is not yet satisfactorily explained. Muggock and Walton (1942) state that about 4 mins. after placing water on mature antheridial cups of Mnium hornum Hedw. antheridial dehiscence occurs. It would certainly seem reasonable to suppose that some sort of mechanism ensures that dehiscence only takes place when there is a surface film of water to allow the antherozoids to

disperse. Goebel (1898) states that he investigated the emptying of the antheridium in Funaria, Mnium, Splachnum, Catharinea and Polytrichum. He found that on the whole they were the same. In contrast to the liverwort antheridia, the moss antheridia have an "opening lid", sharply divided from the other parts of the antheridium wall. This may consist of one apical cell (as in Funaria, but in which two or ocassionally more "sheath cells"may be found) or of more as in Mnium and Polytrichum.

The cells of the "opening lid" possess a strong mucilaginous deposit on the cell membrane. In water this layer swells up, so that the lid with its domed cuticle looks like a clear vesicle. Next the cap cells of the opening lid tear in either of two ways. If the inner walls tear the contents of the cap cells pass into the antheridium until they finally pass out with the spermatocytes. If however the outer wall and cuticle tears first then their contents pass out immediately. This account by Goebel (1898) cleared up many earlier misconceptions based on the apparent

membranes produced at a water interface. In fact Goebel decided that the slow streaming out of the antheridial contents was due to the elastic properties of the antheridial wall which had been previously stretched by the developing contents. However as Goebel (1898) and later Muggock and Walton (1942) have recorded, when the contents of the antheridium pass out a fluid appears at the proximal end of the antheridium, and slowly fills it. Presumably the hydrostatic pressure exerted by this fluid also helps to expel the spermatocyte mass. Unfortunately the nature of this fluid has not yet been investigated. The fluid can be observed to flow out after the spermatocyte mass has all passed out, and may push the mass further away. In fact Muggock and Walton (1942) were able to give in their conclusion, a careful account of dehiscence, which they said applied to a large number of species of mosses. Dehiscence in liverworts seem to be much simplier than in mosses except for the explosive mechanism already mentioned.

The subsequent passage of the sperm down the neck of the archegonium probably presents little

problem, since water must be readily available if the antherozoids are to reach the archegonia at all.

Finally, a most interesting problem in the field of water relations is mentioned by Richards (1959), in his discussion of factors determining alternation of generations. He states that Wettstein (1942), showed that some diploid gametophytes of Phascum cuspidatum Hedw. have a tendency to produce swellings at the end of the costa which forms the tip of the leaf. These swellings may eventually give rise to protonemal filaments from which further diploid gametophytes may develop or, under other conditions they may develop into fairly normal sporogonia producing functional haploid spores. These sporogonia arise directly from a gametophyte, and are therefore apogamous. Wettstein showed that these two possible fates of the swellings can be influenced by their environment. Protonema and leafy shoots were produced on dilute culture media, and in the presence of abundant water, whereas sporogonia are produced on more concentrated media, and in drier conditions.

Section 3 Materials and Methods.

CONTENTS.

3a) The plant material.

The plant material was obtained from the cultures of Dr. J.G.Hughes of the Biology Department, University of Aston in Birmingham. The species used was Phascum Cuspidatum. Hedw., and two forms of this species were available.

One form consisted of haploid sexual plants which were originally propagated from fragments of a single plant. The other plants were diploid apogamous plants raised from seta cuttings taken from the haploid sexual plants. All of these plants have been maintained in aseptic culture.

3b) Cultivation. - The agar medium and the aseptic propagating technique.

(1) Details of the culture medium

CULTURE MEDIUM. (Modified from Hutner 1953).

Solution I.

K_2HPO_4	= 0.04 gms.
E.D.T.A.	= 0.05 gms.
$MgSO_4.7H_2O$	= 0.05 gms.
NH_4NO_3	= 0.02 gms.

Made up to 100 ml. of stock solution.

E.D.T.A. is ETHYLENEDIAMINE TETRAACETIC ACID, which serves as a non-metabolisable metal-buffer.

Solution 2.

Ca - 6.0 mgs. (as $CaCO_3$ gravimetric factor = 2.50)
Dissolve the $CaCO_3$ in as little HNO_3 as possible and make up to 100 ml of stock solution.

Solution 3.

Fe - 0.5 mgs. (as $FeSO_4.7H_2O$ gravimetric factor = 4.98)
Make up to 100 mls for stock and acidify with H_2SO_4.

Solution 4.

Zn - 1.5 mgs	(as $ZnSO_4.7H_2O$	gravimetric	factor	= 4.39)
Mn - 0.5 "	(as $MnSO_4.H_2O$	"	"	= 3.08)
B - 1.0 "	(as H_3BO_3.	"	"	= 1.42)
Mo - 1.0 "	(as $Na_2MoO_4.H_2O$	"	"	= 2.52)
Cu - 0.1 "	(as $CuSO_4.5H_2O$	"	"	= 3.94)
Co - 0.04 "	(as $CoSO_4.7H_2O$	"	"	= 2.38)

Make up to 100 ml of stock solution and slightly acidify with H_2SO_4.

The Stock solutions are made up for use as follows:-

Solution I.	- dilute 10 times and then dispense	50 ml.
Solution 2.	- dilute 1000 " " " "	1 ml.
Solution 3.	- dilute 1000 " " " "	1 ml.
Solution 4.	- dilute 1000 " " " "	2 ml.
	Make up to	1000 ml.

Then adjust the p.H. to 5.6 - 6.0.

Finally this medium was made into a 1½% Agar gel in the usual way. The gel was not "sloped", because of the requirements of the apparatus.

2) Propagation.

Protonemal fragments were always used for sub-culturing, and gave very good results. Any part of the plant can be used if necessary.

3) Innoculation.

All innoculations of fresh cultures, were carried out under a large hood, but not under aseptic conditions. Before use, the interior of the hood was sprayed with a mixture of 50% industrial spirit, and 50% ethylene glycol. This fine spray tends to bring spores out of the air on to the floor of the chamber. This proceedure together with normal microbiological practice such as flaming of the innoculation needle, gave very satisfactory results.

C. The Apparatus for Humidity Control.

1) The principles of the humidity control.

2) The general lay-out of the apparatus.

3) The moss culture vessels.

4) The air supply to the mosses.

5) Air flow control.

6) Details of control equipment.

7) Details of measuring equipment.

1) The principles of the humidity control.

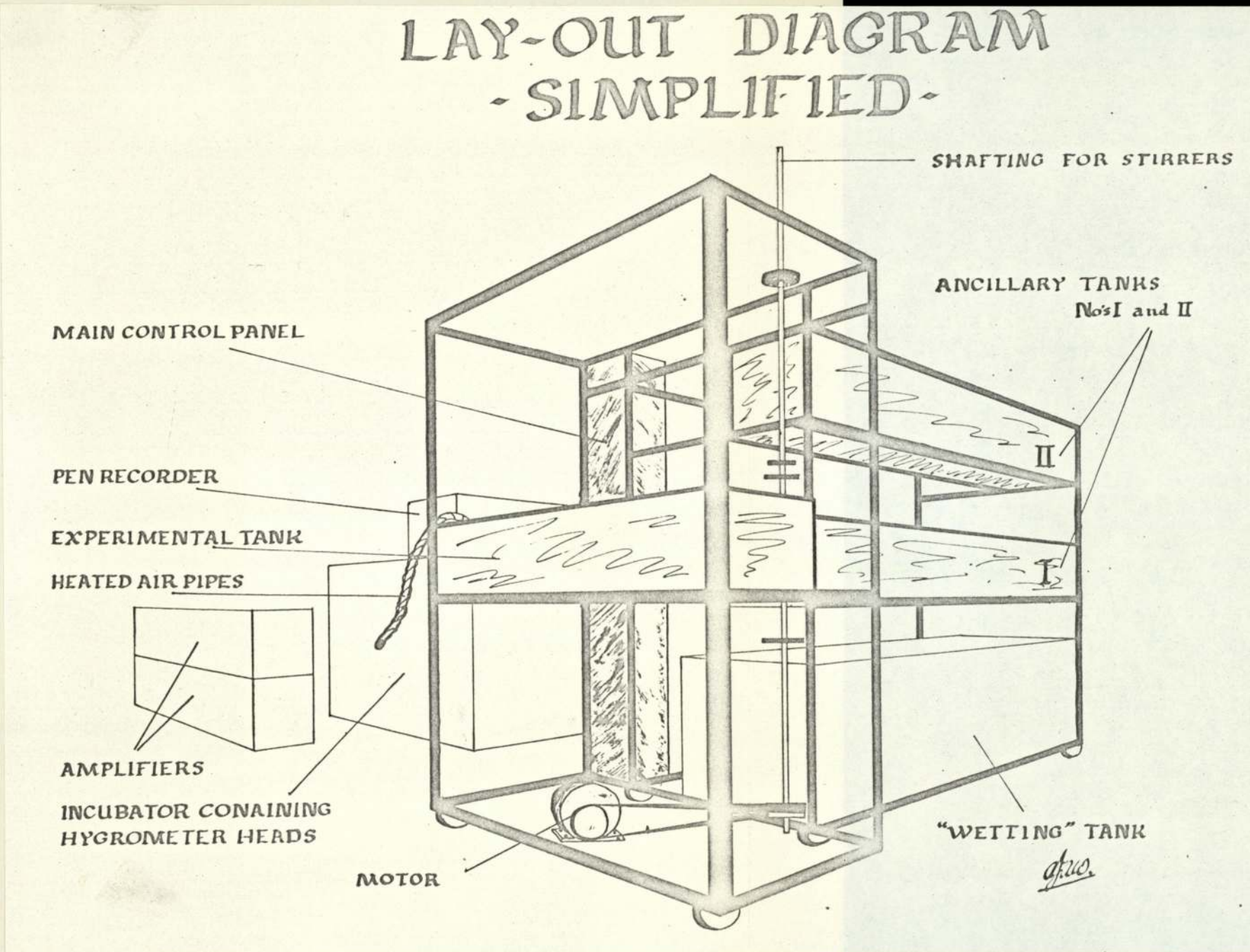
Untreated air from the laboratory was pumped through a series of gas washing bottles, each containing water. This process was carried out under conditions which ensured that the emergent air contained excess water vapour. This "wet" air was then cooled to a predetermined temperature and held at that temperature until an equilibrium was reached between liquid water, and the water vapour of the air. Thus the dew-point temperature of the air was now fixed. If a relative humidity of 100% was required, then the air was used at its dew-point temperature. If however a relative humidity of less than 100% was needed, then the air was used at a suitable temperature, higher than its dew-point temperature.

Thus humidity control was obtained by controlling the temperature of the appropriate parts of the apparatus.

2) Description of the apparatus.

Lay-out. The general relationships of the different

Fig.16. Simplified general view of the apparatus.



units can be seen in the simplified lay-out diagram (Fig.16). Each unit is largely independent except for its stirrer which is driven from one main shaft, and the air lines which connect the units together. Most of the units of the apparatus were mounted in a framework of heavy "Handy Angle" and mounted on eight heavy duty casters so that the whole equipment could be moved. All measuring equipment was grouped in and around a constant temperature cabinet or incubator. The amplifiers serving the thermoelectric hygrometers were isolated as far as possible, to avoid heating problems.

3) Moss Culture Vessels.

The moss Phascum cuspidatum Hedw. was grown for the purposes of these experiments in 25mm x 150mm rimless test tubes. 10ccs. of agar culture medium was used in each tube so that the depth of agar gel in each tube was approximately 25mm. Air was circulated over the plants by attaching the tubes to rubber bungs which were part of specially designed manifolds. Two types of manifold were designed (Figs. 17 & 18), one

for use at constant humidities, and the second for those experiments involving intermittent treatment. Both types of manifold were made from annealed copper tubing, and were designed to act as a support for the test tubes as well as to supply air.

Air supply to mosses.

Four air lines were built into the "wetting" tank, each consisting of a row of seven gas washing bottles. These bottles were made from copper tubing, rubber bungs and aluminium cans, and were connected by copper tubing as shown in Fig.19. Four heated copper tubes then led the "wet" air to the experimental tank, where all the air connections which needed regular attention, or selection, were concentrated. Further heated tubes lead to the ancillary tanks as shown in Fig.19. as well as heated tubes connected to the Hygrometers. The temperatures of these connecting tubes were controlled by energy regulators. The "X" units also shown in Fig.19. are made up from coils of copper tubing which ensure that the air is brought to the temperature of the appropriate tank, and a flask which was used as a water trap.

Fig.17. Manifold for use in continuous treatments.

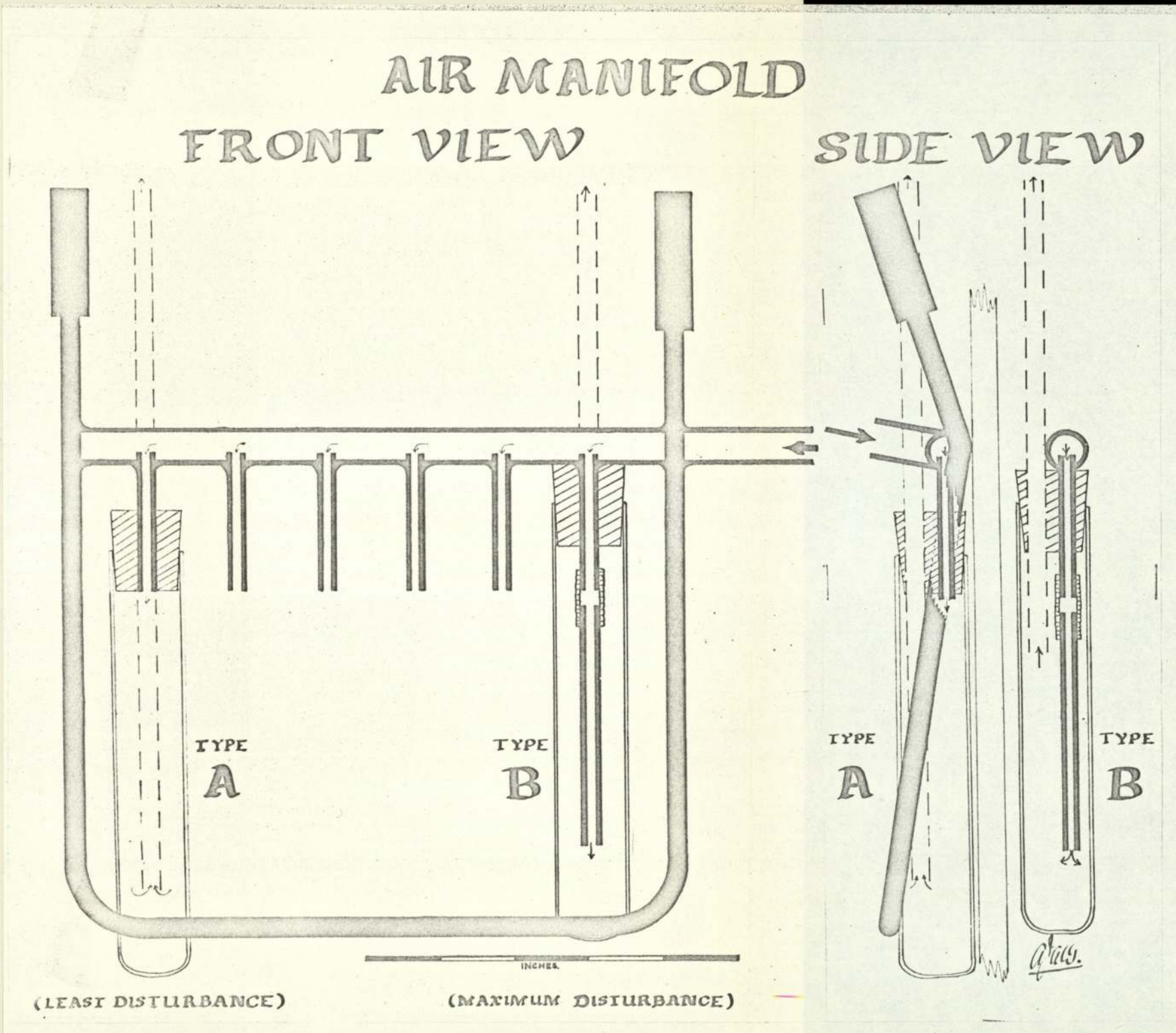
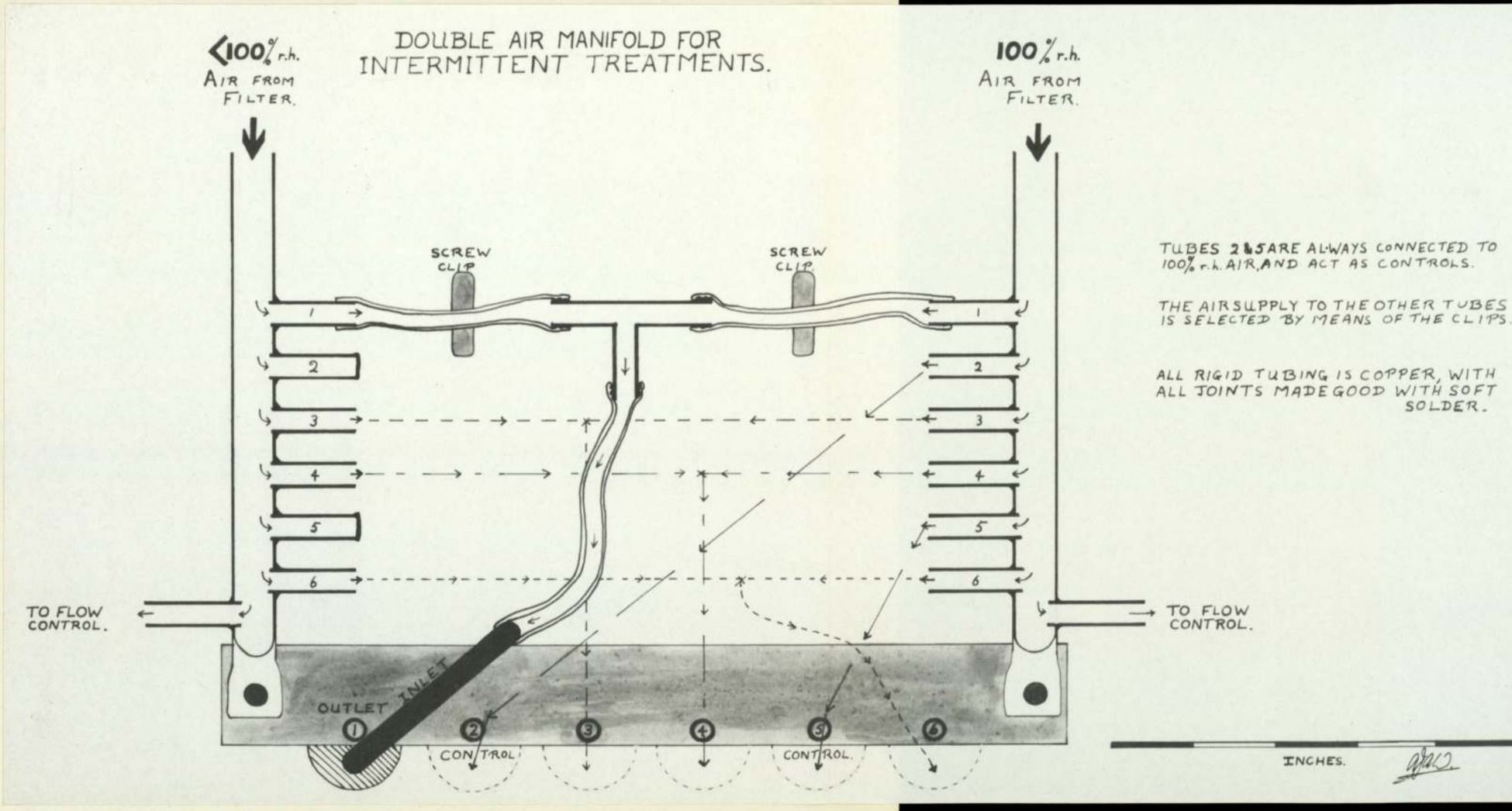


Fig.18. Manifold for use with intermittent treatments.



Trapped water could be drained off whenever necessary. Standard copper tube of 8mm. outside diameter and 6mm. inside diameter was used throughout except in the moss manifolds.

Air flow control.

The air passing out of each culture tube was led to a gas jar fitted with a bung and seven copper tubes. Six of these tubes acted as inlet tubes for the air from a manifold of six moss tubes, and the seventh was an outlet tube. Each gas jar contained water, so that the flow of air bubbles from the bottom of each inlet tube could be compared and if necessary adjusted by the use of taps in each air line. The air flow from each of these gas jars was compared in a similar way in a flask, and finally the outlet tube from the flask went to a gas meter for measurement. Three of these gas jars together with the flask can be seen in operation in Fig.20.

Fig.19. Diagram to illustrate the air supplies within the apparatus.

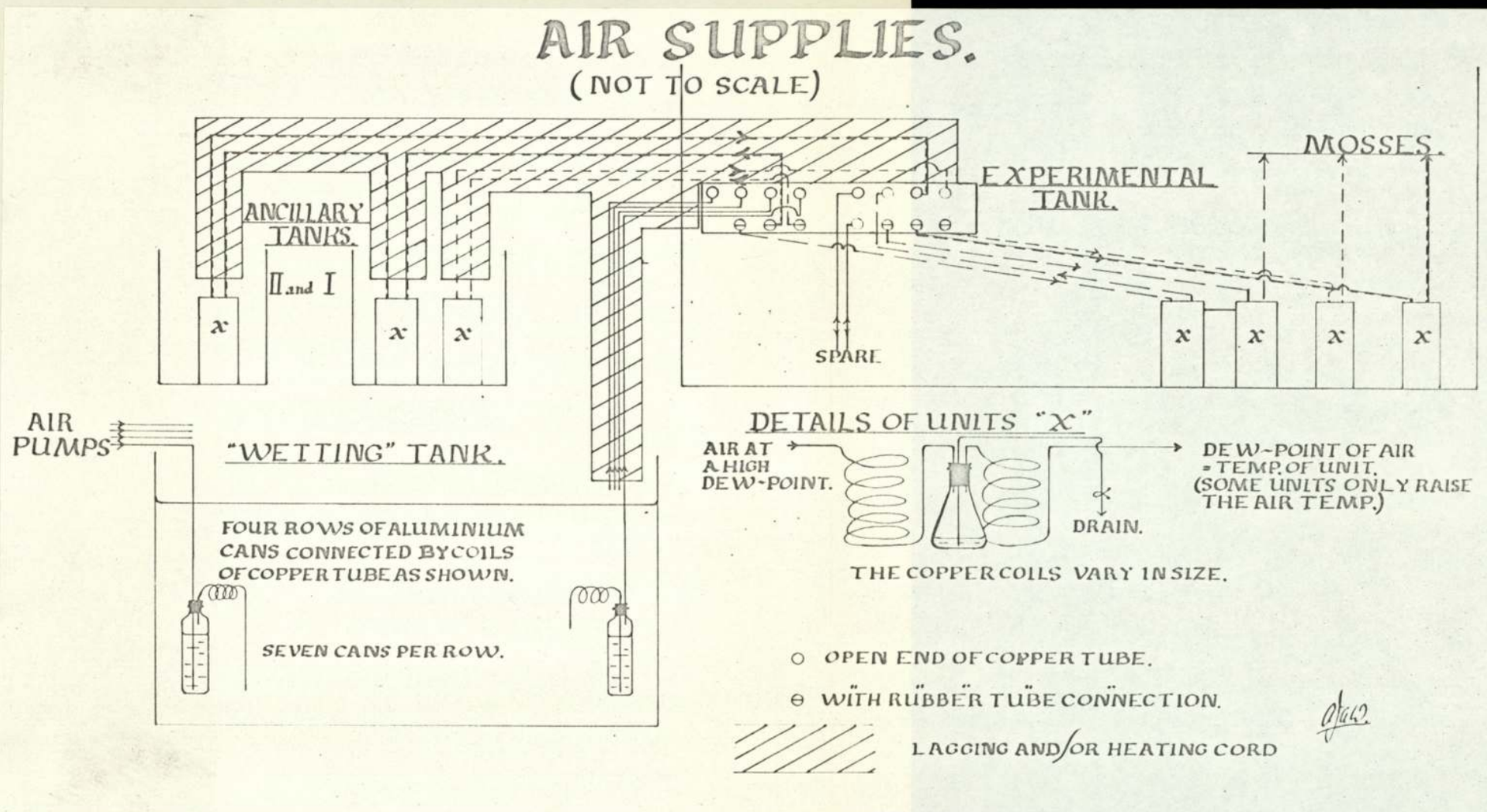
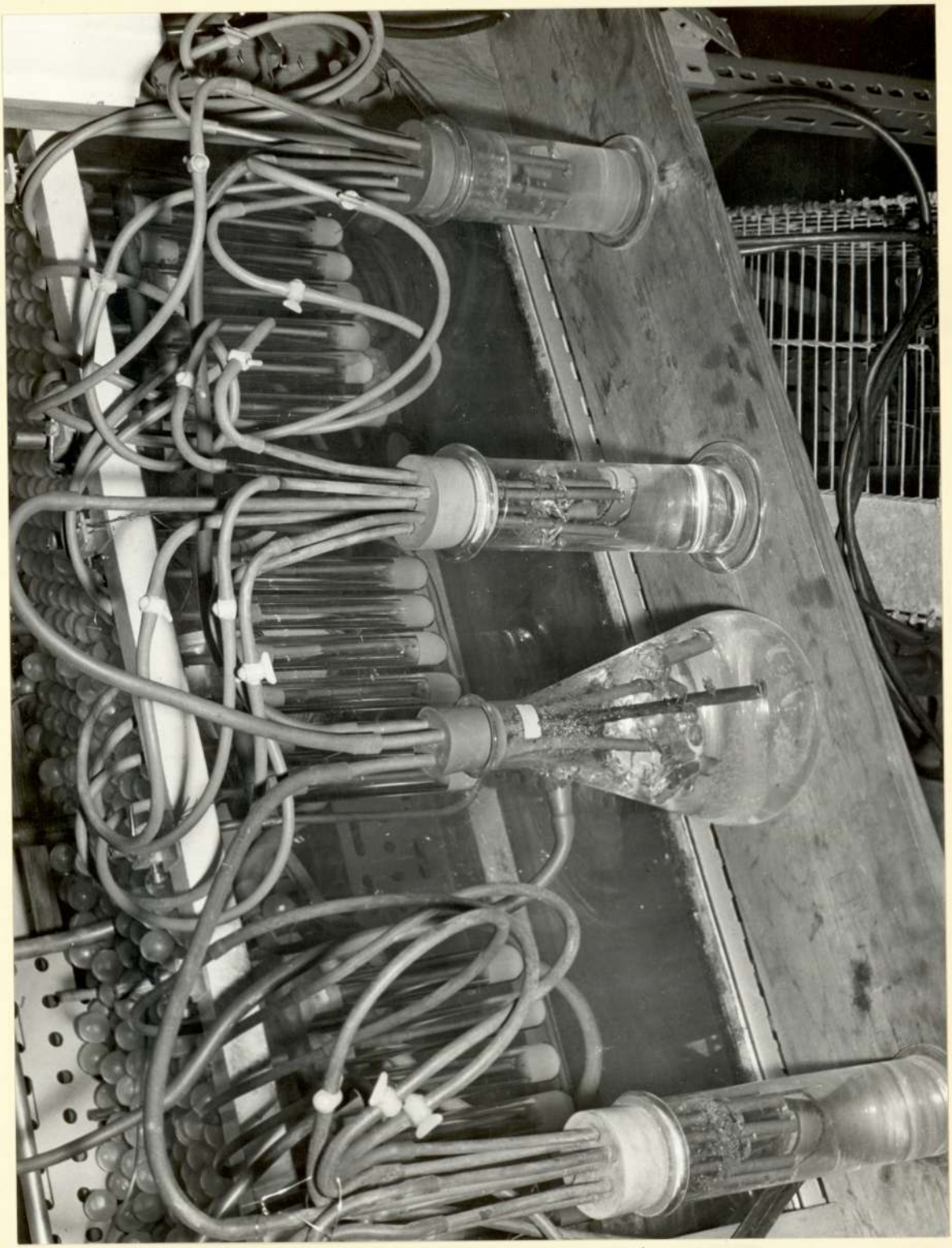


Fig.20. Three "continuous" manifolds in use.

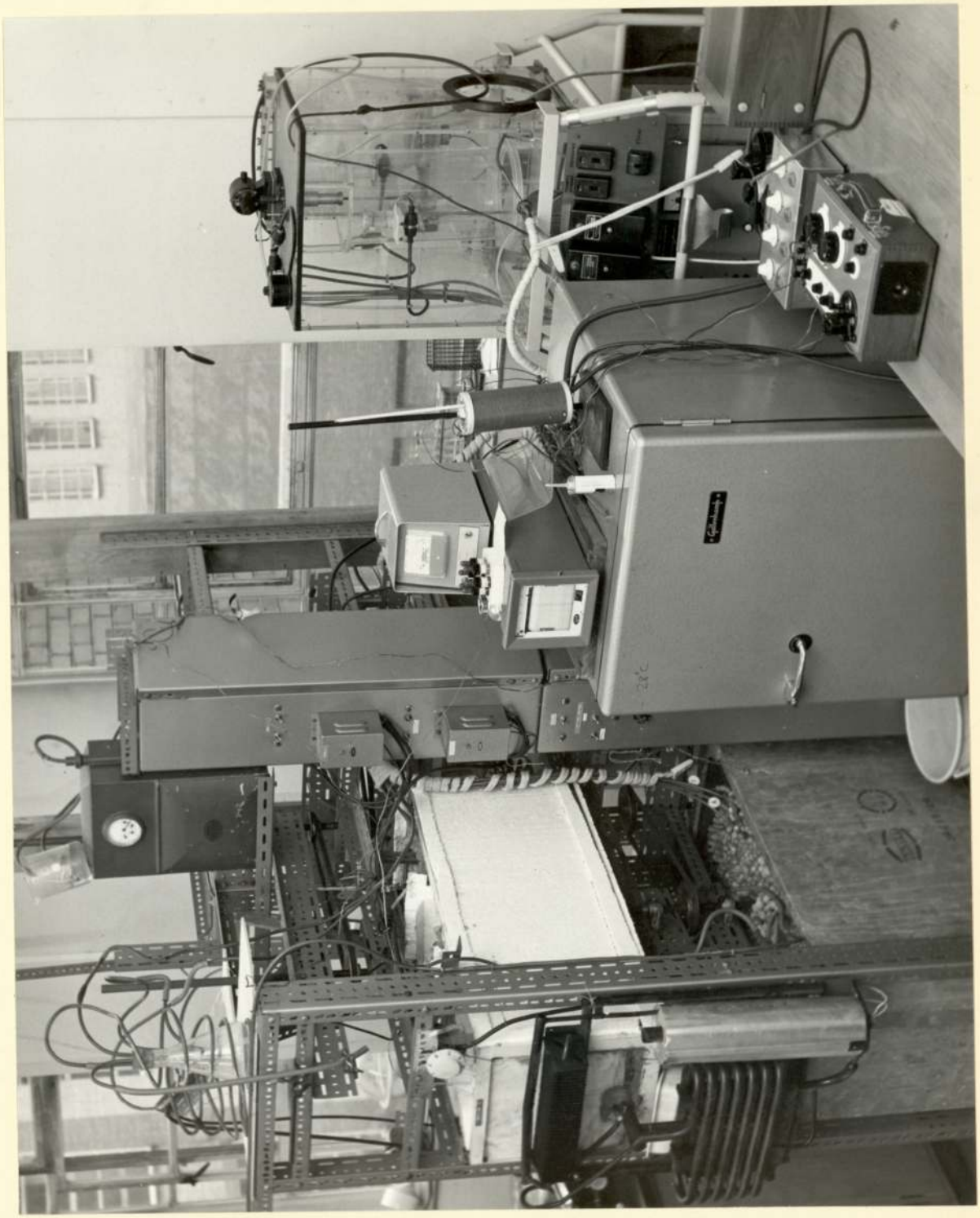


6) Details of the Control equipment.

a) "Wetting" tank. (36ins x 18ins x 24ins high. cap. 55galls). Heating in this tank was supplied by domestic type immersion heaters controlled by a "Gallenkamp Compenstat" thermostat (T.M.505). The load was usually less than 3 Kilowatts, and no relay was needed. A six inch diameter stirrer was made from "Dexion" duralumin angle, and was driven at 120 r.p.m. from the main shafting. Evaporation from the surface of the tank was reduced by using floating wooden lids and "Alplas" balls. Control of the air supply was by built in rheostat in the Austen D.Y.M.K.1. pumps or by high pressure hand valves if the big Edwards vane type compressor (R.B.5) was used.

b) Ancillary Tank No.1. (36ins x 15ins x 15ins cap. 29galls). Temperature control in this tank was critical, and was maintained by a Jackson type (T.C.) thermoregulator capable of giving a temperature control better than $\pm 0.01^{\circ}C$. The thermoregulator controlled normal 60watt or 100watt aquarium heaters via a "Sun-vic" electronic relay type E.A.4. A 140watt "electrolux"

Fig.21. GENERAL VIEW (Fig.16. was from the opposite side)



refrigerator unit was also fitted, (Fig.21.), and for temperatures at or below ambient this was used in opposition to the thermoregulator, so that the fine temperature control was retained. Expanded polystyrene was used inside, and outside the tank to provide thermal insulation (Fig.21). Alplas balls formed a lid. The stirrer was made from small size "Dexion" and driven at 72 r.p.m. from the main shafting.

c) Ancillary Tank No.2. Many different pieces of equipment were used here, and the tank shown in the layout diagram (Fig.16.) was never fitted. However it is very convenient to retain the name.
A commercial deep-freeze and a "Tompson and Mercer" water bath were employed. In Fig.21. the laboratory air is being used to maintain the temperature of the big "X" unit in the position of Ancillary tank No.2.

d) Experimental Tank. (36ins x 15ins x 15ins. Cap. 39galls). Heat control was provided by a Jackson type (T.C) thermoregulator, a "Sunvic" electronic relay type E.A.4., and two 100 watt aquarium heaters.

No thermal insulation was used, but evaporation was limited by alplas balls. Lighting was controlled by a "Sangamo" time switch and up to four 30 watt fluorescent tubes.

e) Stirring. The main shafting was driven at 120 by a ½ H.P. three phase motor (720 r.p.m.) driving via a flat meteor belt.

7) Details of Measuring equipment.

All measurements were taken as temperatures, including humidity measurements which were dew-point temperatures. Distant-reading thermometers were all made up as copper/constantan thermocouples using a common cold junction unit. This unit, an "A.E.I" cold junction thermostat type C.J.1 maintained a temperature of approximately 45°C which was in fact higher than most of the temperatures measured. An "A.E.I" potentiometric multipoint recorder was calibrated with this cold junction unit so that the chart gave direct temperature measurement. (Fig.21). Six coloured traces

were available to record the output from two hygrometers, and from four other thermocouples anywhere in the system. The multipoint recorder was monitored when necessary by a "Pye" slide wire potentiometer, so that any zero-error could be detected and corrected. (Fig.21).

Two similar thermo-electric dew-point hygrometers were used. Dew was electronically maintained on a surface-silvered mirror, and the output from a copper/constant thermocouple soldered to the mirror was fed into the multipoint recorder. A ventilated Assman hygrometer, a hair hygrometer and a "Shaw" portable hygrometer were available when required.

D. NOTES ON THE USE OF THE APPARATUS.

(1) Pumps.

(2) "Wetting" tank.

(3) Heated pipe lines.

(4) Ancillary tank No 1.

(5) Ancillary tank No 2.

(6) Experimental tank.

(7) Stirrers.

(8) Hygrometers.

(9) Multipoint pen recorder.

(10) Cold junction and incubator.

(11) General procedure for using the apparatus.

(1) Pumps.

The small "Austen" pumps were extremely good, and ran continnously for up to 4 months at a time. However if any appreciable resistance was introduced, air was taken from the "Edwards" pump. A setting of 9-10 lbs. per.sq.ins. was usually adequate, and this low pressure reduced oil leakage from the automatic cut-out dampers.

(2) "Wetting" Tank.

Early experiments showed that glassware would not conduct heat fast enough, and in fact evaporation of water in glass vessels resulted in very considerable cooling of the air. Practical tests showed that aluminium and copper used in the place of glassware would overcome the problem, provided that coils of copper tubing were used to increase the time allowed for heat transfer. The very coarse temperature control of $\pm$ 2°C proved to be quite acceptable. Evaporation was approximately 4 galls per day in spite of the lids.

(3) Heated pipe lines.

The use of copper tubes wound with heating tape worked well. The energy regulator only gave coarse control of a whole tape, but finer adjustment was made by winding the tape closer in some places than in others. The main problem here was the high dew-point temperature produced if liquid water reached a heated pipe.

(4) Ancillary Tank No I.

Lagging proved to be essential here. The latent heat given up as the excess water vapour condensed, proved to be a problem, and all other sources of heat had to be reduced as far as possible when low temperatures were needed.

(5) Ancillary Tank No 2.

Whenever any equipment was needed at this point its temperature was always to be at or below, ambient. Thus no heated tubes were needed, and long air lines could often be used since their temperature, which was that of the laboratory, was well above the dew-point temperature of the air inside them.

(6) Experimental Tank.

Temperature control in this tank proved to be extremely good, and was better than $\pm 0.10^{o}C$. This was obtained by taking measurements under the worst possible conditions, and at opposite ends of the tank. When 100% r.h. air was being passed through the experimental tank there was never any sign of condensation unless the tube accidently rose above the surface of the water, or unless lights were used. The method of making all air line connections in this tank with the water level low, and then raising the water level above the connections proved to be a good one. Trials showed

that unwanted condensation could take place even in thick walled rubber tubing if this broke the surface of the water bath. The growth of algae was kept down by introducing detergents whenever there were no moss cultures in the tank.

(7) Stirrers.

The very crude paddles made from punched angle proved to be extremely good. They gave a most turbulent flow, which penetrated to all corners of the tank, and yet caused little surface disturbance. Trouble was encountered in driving the main shafting at 120 r.p.m. Originally a reduction gear was used on a single phase electric motor. This arrangement was mechanically sound, but very noisy, so a 720 r.p.m. 3 phase motor had to be used instead. This involved a flat belt drive from a $1\frac{1}{2}$ inch diameter pulley to a 9 inch diameter pulley. The smaller pulley was on a horizontal shaft, and the larger one on a vertical shaft. This arrangement proved to be effective and very quiet, but the belts wore out quickly. This could probably be overcome by using 3" and 18" pulleys, but this was impossible in the space available.

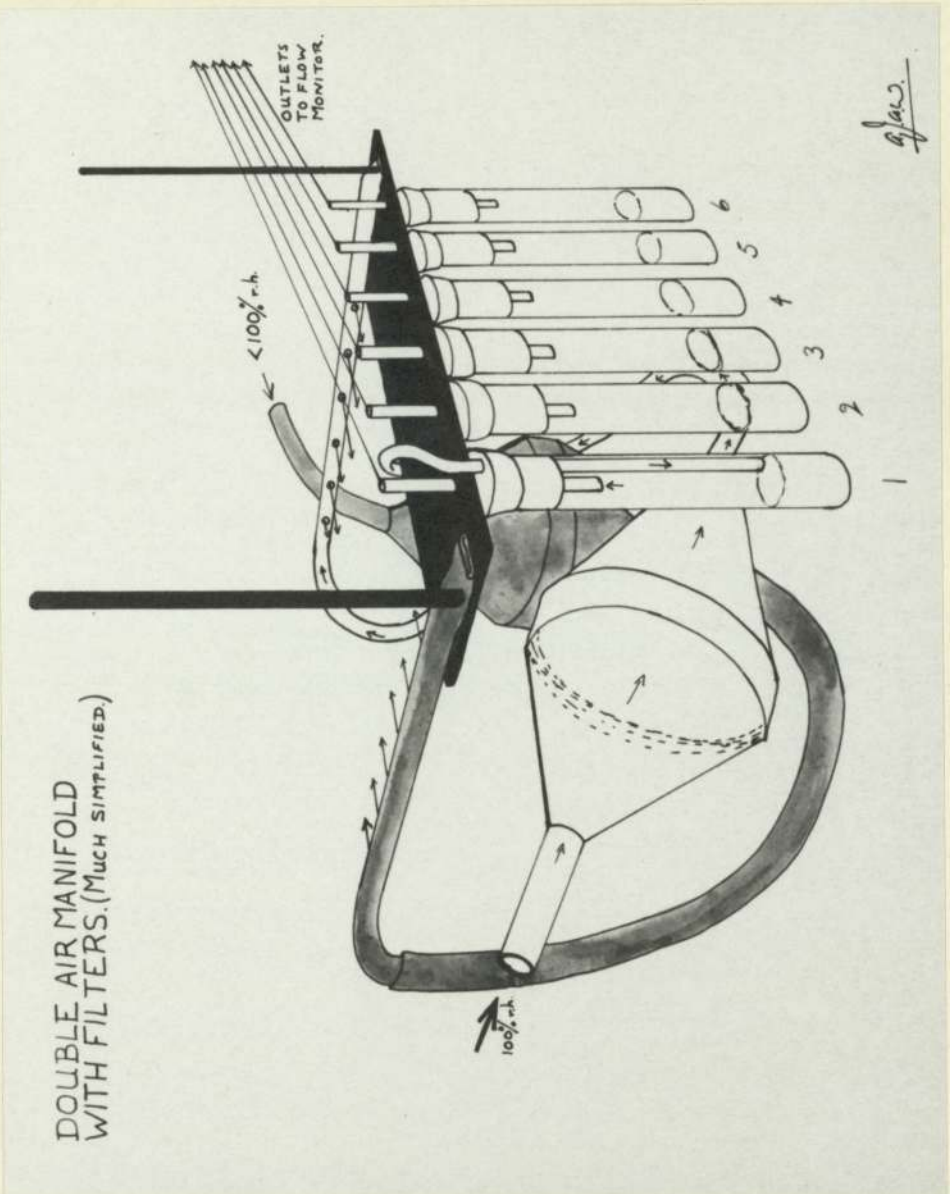
(8) Hygrometers.

These instruments proved to be very reliable and easy to maintain, after initial teething troubles. The cool surface of the mirror attracts all small particles in the air streams, and frequent cleaning is essential. Their amplifiers give little trouble, but the copper and constantan thermocouple leads are very thin and fragile. This was overcome by shortening them and soldering on more substantial wires.

(9) Multipoint pen recorder.

This piece of equipment needed frequent attention but gave no trouble if this was given. Constant oiling of all moving parts was essential. The most troublesome part was the drum carrying the ink pads. Water-based ink was used and produced a hard deposit all over the still and the carriage. This deposit caused one mechanical failure. The makers say that oil based inks are being developed.

Fig.22. Simplified diagram to show the spacial relationships of an intermittent manifold unit prepared for use.



(10) Cold junction and incubator.

These gave no trouble at all.

(11) General procedure for using the apparatus.

The following routine was established to avoid excessive condensation as far as possible, and to enable measurements to begin very early in the procedure.

1) Switch on the automatic cold junction, and the incubator containing the hygrometer "heads". Leave for at least one hour.

2) Switch on all air-pipe heating tapes, and tank heaters. Switch on the stirrers taking great care that the propellors are not fouled.

3) Empty all water traps, and fill the gas washing bottles in the "wetting" tank with water.

4) Make the appropriate air connections, so that the air circuits and their connections with the hygrometers have been established. Switch the air pumps on.

5) Switch on the hygrometers and the multipoint recorder so that all the thermostats and the dew-points are monitored. Leave the apparatus at this stage until the required dew-points have been reached. This usually took 48 hours.

6) The appropriate manifolds were prepared as follows. All connections were made with rubber tubing with the exception of the inlet to the bacterial filter which was not connected at this stage, and the outlets from the test tubes. Empty test tubes were placed in position on the bungs, with a layer of glazed paper between the rubber and the glass. The outlets were plugged with cotton wool, and the whole assembly autoclaved at 15lbs/sq.in. for 30 minutes.

7) Tubes containing plant material were attached to the bungs instead of the empty test tubes. This operation was carried out under the hood, taking suitable precautions.

8) The air supply was connected, together with the rubber outlet tubes to the flow controllers. It was usually possible to leave the cotton wool plugs in

position. The assembly was then clamped into the experimental tank using the appropriate supports on the manifold.

9) The flow through the tubes was adjusted so that the flows were equal. Then the flow rate was adjusted at the pump, using the gas meter for measurements.

(e) The performance of the apparatus.

The apparatus as finally assembled and used, was most reliable, and successfully supplied air streams of the required temperature, and dew-point temperature. The whole system relied on temperature control, and particularly on the temperature control in the experimental tank and in ancillary 1. Measurements in the experimental tank showed variations of up to 0.04°C throughout the tank. This was achieved in spite of its exposed position and the absence of lagging. If the door and windows of the laboratory were opened together on a cold day, it was possible to produce a larger variation, but this situation was avoided during experiments. It is claimed that the temperature

of the experimental tank was maintained within an error of ±0.1°C. The temperature of the ancillary tank varied much less than 0.1°C. The treatment of the air streams ensured that the final dew-point temperature and relative humidity, depended on the temperature of the experimental and ancillary tanks. Therefore the variation of the dew-point temperature and relative humidity as supplied to the mosses was that of the temperatures in the tanks. This could only break down in two ways. First the air from the "wetting" tank may not contain sufficient water. This situation was avoided by refilling the gas washing bottles every seven days. Secondly the excess water normally present in the air entering either the experimental or ancillary tanks may not be completely removed. However if this happened condensation was always visible, and situations leading to this were all prevented during the experiments. If it had occured during the experiments it would have shown on the monitoring trace as a rise in dew-point when droplets of liquid water reached the heated copper pipes. It is

probable that the dew-point temperatures of the air supplied to the mosses was accurate to at least 0.1°C, but it was impossible to measure this. The stability of the reference cold junction was 0.1°C itself, and the hygrometers were only capable of an accuracy of 0.25°C. However it is certainly possible to claim an accuracy greater than the ±1.0°C of currently available commercial growth chambers with controlled humidity, these rely on air thermostats.

Flow control was comparative and although it was quite adequate for all normal purposes, it proved to be quite inadequate for the highly sensitive diploid apogamous plants. All attempts to find suitable commercial flow meters failed.

The apparatus was used continuously for up to four months at a time, it was monitored continually, and proved to be most reliable. Whenever anything went wrong in the trials the potentiometer record always showed the failure. It proved to be quite easy to change dew-point temperatures because of the

adjustment available on the Jackson Thermoregulators, but it was essential to allow at least 24 hours for stable conditions to be re-established.

The design could easily be adapted to any scale. Fluorescent lighting controlled by a time-switch was fitted outside the experimental tank. Unfortunately the radiant energy from even one of the four 30watt tubes was sufficient to interfere with humidity control. This could usually be seen because of condensation formed in the culture tubes.

Section 4. EXPERIMENTAL WORK.

Introduction.

Two quite separate aspects of the moss Phascum cuspidatum Hedw., have been investigated. In both cases air streams of known humidity have been directed onto the moss plants, and the results of varying the humidity has been examined. The effects of this type of treatment on antheridial dehiscence has been recorded as section 4.A., and the effect on the leafless stems of the diploid apogamous plants has been recorded as section 4.B.

4.A. The effects of humidity on antheridial dehiscence in Phascum cuspidatum Hedw.

CONTENTS.

Introduction.

This investigation has been carried out, using the haploid sexual plants which have been maintained in culture since 1954. During this peroid difficulty has been experienced in obtaining fertilisation and thus, in initiating the sporophyte generation. (Hughes 1958). It will be shown that this may be refered to a low frequency of antheridial dehiscence. Two explanations suggest themselves, the high humidity in the culture vessels, and the stillness of the plants when in culture. Both of these possibilities have been investigated.

Section (1) DEHISCENCE.

a) An account of the Antheridium.

Phascum cuspidatum Hedw., is paroecious, and secondary male branches can arise. Thus antheridia may be found in three positions:-

1) If the plant is young, then there may be a terminal bunch of antheridia.

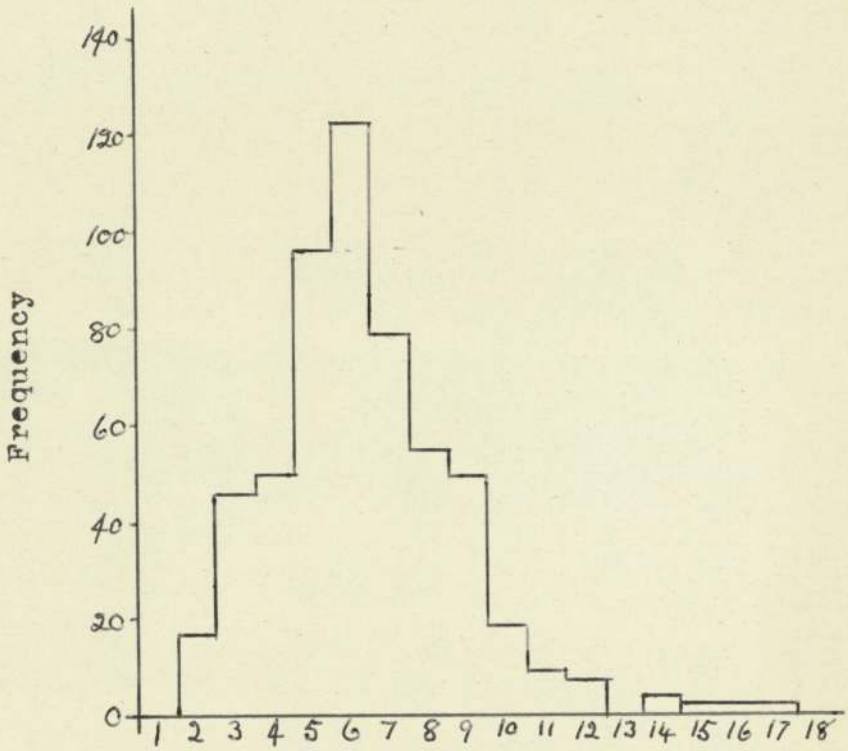
2) If the plant is older then the "female branch" may be well developed, and the antheridium appear to be bourne on a small lateral branch.

3) In a well developed plant there may be a succession of "lateral" branches bearing antheridia as well as younger branches bearing terminal clusters of antheridia. The antheridia have never been seen as solitary structures, and are usually in groups of 6. (Fig.23).

The antheridium developes as an elongated green sac, with walls one cell thick. As it grows the apical cells remain more or less isodiametric, whereas the other wall cells become very much elongated. (Fig 24. Nos. 1 & 2).

At maturity the apical cells have a distinctive appearance. In Fig 24. Nos.3., they are becoming much rounder, and their contents begin to change. The contents become more granular (Fig.25), can have a more or less prominent vacuole (Fig.26), and generally appear to develop a reddish colouration at maturity. Finally the apical cells may either become browner as

Fig.23. Diagram to show the number of antheridia per plant.



the red-green particles change to a brown colour, or may become colourless. The antheridium itself is always well rounded and turgid, until after maturity when it steadily becomes browner and more shrivelled. In very old antheridia the whole structure is transparent and almost flat. The cell contents are now only tiny brown granules, but all the cell walls may be seen clearly. (Figs 27 & 28). In all the "bunches" of antheridia examined there was never any evidence of the loss of antheridia, so that to this extent at least, the whole history of the bunch was evident.

The length of the antheridium in Phascum cuspidatum Hedw., is 0.17 mm. (average), of Mnium hornum 0.6 mm. (average), and of Polytrichum piliferum 1.1 mm. (average).

b) The process of dehiscence.

At dehiscence the antheridium has a well-rounded, green appearance with a well differentiated group of apical cells. Dehiscence is effected when these apical cells rupture and liberate the contents of the antheridium. Fig 29., shows such contents, which on dehisc-

Drawings of antheridia to illustrate points arising in the text.

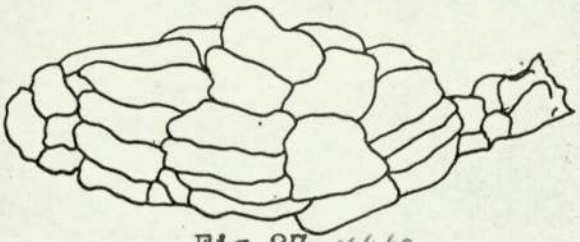


Fig.27. ×460

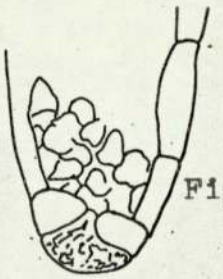


Fig.25. ×563

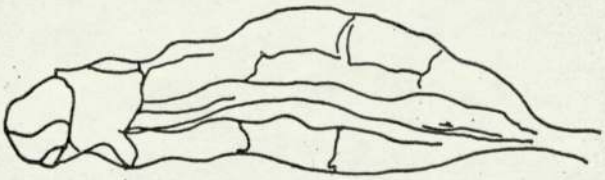


Fig.28. ×460

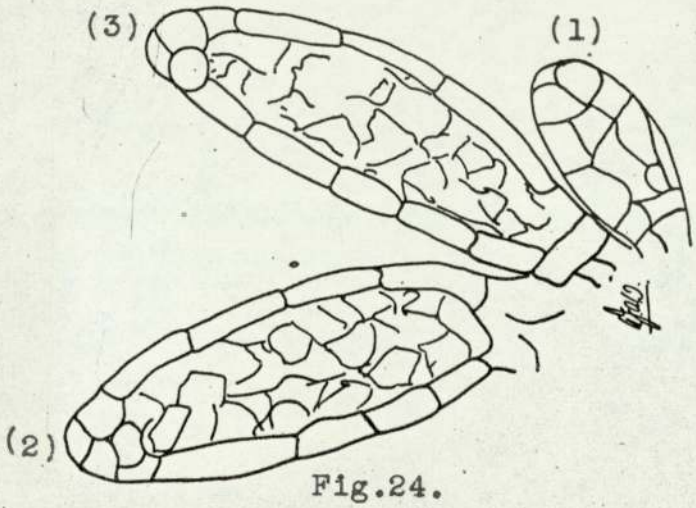


Fig.24.
×563

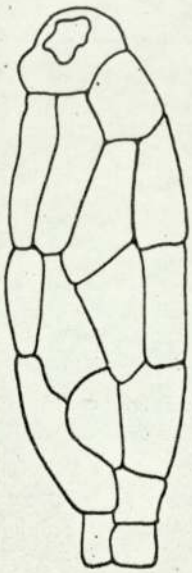


Fig.26. ×460

ence contain antherozoids still inside their mother cells. The antherozoids are quite active inside the cells, but are usually still trapped there several hours later.

Muggock and Walton (1942) summarise dehiscence in the mosses they have examined, and state that dehiscence is preceeded by a bursting inwards of the "cap-cell" (Goebel's terminology), and in some instances the adjoining cells at the apex of the antheridium. This condition has been observed in Phascum cuspidatum Hedw., but has not been seen to dehisce. Fig. 30 No.2., shows an antheridium in this condition. The cells of the antheridium wall were still green, although the apex no longer contains chloroplasts, and the antheridial contents appear to be confined to the lower half of the antheridium. Despite these changes the antheridium appears to have lost some of its turgor, and would seem to be unlikely to dehisce. Of the other two antheridia (Fig.30) No.1 has brown contents in the apical cells, but green wall cells, and No.3 has green apical cells.

Fig.29. Part of the extruded content of a mature antheridium showing the spermatozoids still coiled in the spermatocytes. approx X3000

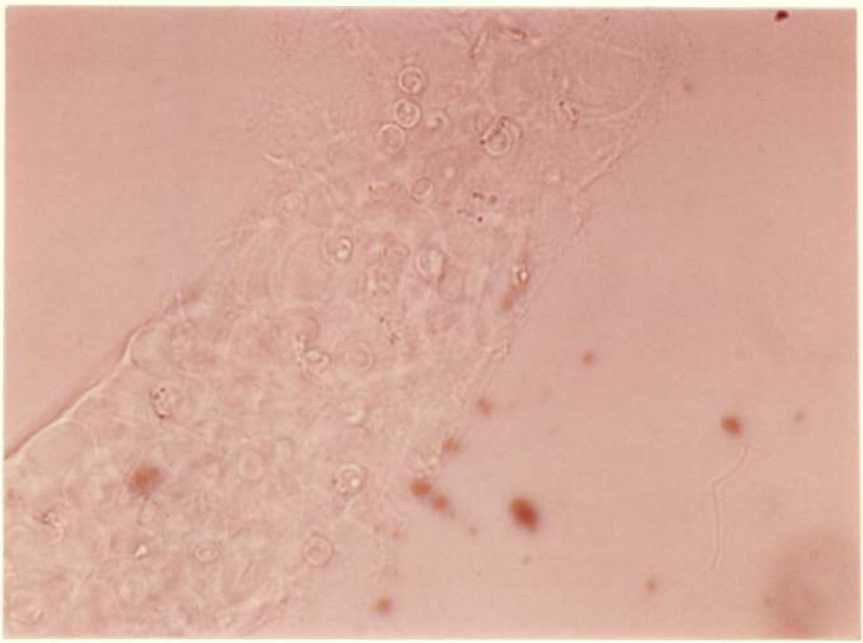
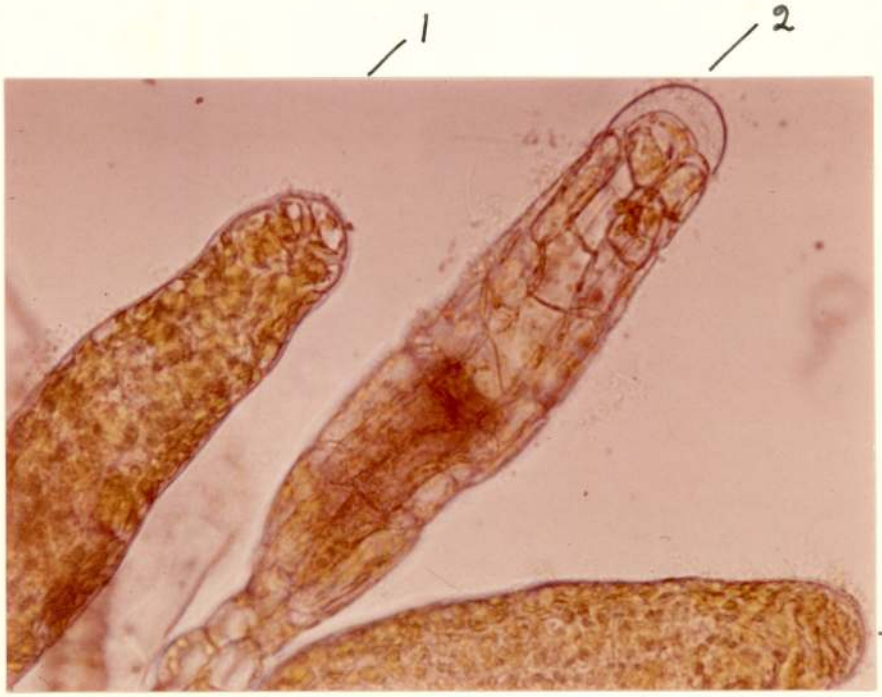


Fig.30. Three stages in the development of the apical cells of the antheridia. approx x900



The mechanism which forces the contents out of the antheridium is not clear. Muggock and Walton (1942) consider that the colourless fluid which can be seen filling the space behind the moving spermatocyte mass, is largely responsible. However Goebel (1898) suggests that early explanations, in terms of the swelling of mucilaginous walls were partly correct, and that part of the force came from the elasticity of the previously stretched antheridial walls. Certainly the diameter of the antheridium is reduced after dehiscence. (Fig.31). Whatever the expulsion mechanism, it is rarely sufficient to force the spermatocyte mass clear of the antheridium, and the mass usually remains in that position for long periods, unless it touches a water-air interface, and is dispersed by the surface tension effects as described by Muggock and Walton (1942).

c) Scoring Dehiscence.

An antheridium was scored as "denisced" if its apex was ruptured. Thus many different ages of antheridia might be involved. In Fig.31., the scoring

was straightforward. Numbers 1,3 & 5 show ruptured apices and were scored as "dehisced" whereas Nos. 2, 4,6and 7 had intact apices, and were scored as indehiscent.

In those cases where the apex was neither obviously ruptured, nor well rounded and intact, pressure was applied to the antheridium using miniature needles. If the apex was already ruptured the remaining contents of the antheridium passed out easily, if not, then the busting of the antheridium could be seen.

Thus the scoring of dehiscence did not take into account the date at which dehiscence took place. If dehiscence took place at all regularly, then the dehiscence as scored would be cumulative as the culture aged.

Fig.31. A typical bunch of antheridia showing the wide age range. approx ×200

Section 2. Experimental Procedures.

Procedure 1.

In an experiment lasting for 6 hours, plants still growing on agar under aseptic conditions, were exposed to air streams of known dew-point temperatures.

The apparatus was set up to supply three air streams, each having a different dew-point temperature, and therefore providing a different relative humidity. A single control culture was supplied with air at 100% r.h., one manifold of cultures was supplied with air at 78.14% r.h., and another manifold of cultures was supplied with air varying between 55.9% r.h., and 47.8% r.h. At intervals one culture was removed from each manifold, and was replaced by an empty tube. Both cultures were removed from their culture tubes, and antheridia were dissected out from the plants. The antheridia were then examined individually for dehiscence. The period of treatment for each culture, and its general appearance after treatment is shown in Fig.32.

Procedure 2.

In experiments lasting approximately 6 hours, plants plucked from pure cultures and placed in clean dry tubes, were treated with air streams of different dew-point temperatures.

Batches of 20 isolated moss plants were selected. The choice of plants was governed by the readiness with which antheridia could be seen. As a result the choice included, (1) plants in which antheridia formed a terminal cluster, and the female branch was hardly formed, and (2) plants which were approaching maturity and had terminal clusters of archegonia as well as a lateral bunch of antheridia, although perichaetial leaves were not quite fully grown, and lateral branching had not begun.

As the mosses were removed from the surface of the agar they were already covered with a film of water. The chosen plants were lifted with forceps, taking every care to ensure that smaller plants were not taken as well. Often the plant was quite intact and included its "wick" of protonema. The plant was

then placed in a microbiological loop, which contained a drop of water and therefore retained the plant. The drop would in fact pull the plant off the forceps. Within 5-10 minutes one batch of 20 plants was accumulated in the loop, and by this time much of the water had evaporated, although the plants were still wet. As soon as the surface of the plants was dry they were transfered into a weighed test tube, and were weighed. These weighed batches were then exposed to air streams of different relative humidities for six hours. They were reweighed after treatment. The final weighing was carried out as quickly as possible. The test tube was then returned to the experimental tank, and the mosses were covered with water which was already at the temperature of the experimental tank. Finally the antheridia of all the plants were dissected out and examined for dehiscence. Further experiments were carried out using this same technique, but with the "rewetting" carried out with colder water. Finally the whole experiment was repeated at lower temperatures. The results of all these experiments are set out in Fig.35.

Procedure 3.

Four types of severe stimuli were applied.

a) Whole cultures of plants were shaken in an electric shaker, either with added water or with no pretreatment at all.

b) Undisturbed cultures were placed in an autoclave at room temperature, and were exposed to a pressure of 10 lbs.per.sq.in., by connecting the outlet cock of the autoclave to an air compressor.

c) Undisturbed cultures were connected to a "Speedivac" high vacuum pump. The vacuum was applied progressively until bubbling ceased, when a pressure of a few millimetres of mercury was maintained. The whole culture was very much disturbed by this treatment, so that the stimulus could be the vacuum, or the disturbance produced by the vacuum, or both.

d) Cultures in situ on their agar were left exposed to wind and rain for 48 hours.

The results of all these treatments are set out in Fig.36.

Procedure 4. "Fertilisation Tests".

This test has been used to demonstrate that the induced dehiscence does lead to fertilisation of the archegonium, and hence to the development of sporophytes. Sterile distilled water is added to the culture for 24-48 hours, and is then decanted off. After four weeks any resulting sporophytes are counted, and the result is expressed as the percentage of the mature plants which bear sporophytes.

Section 3. RESULTS.

Experiment (1).

The effects of treating plants in culture with air of different humidities, as described under procedure (1), is recorded in Figs. 32,33 and 34.

Fig.32. The appearance of the cultures.

Material	Period of treatment.			
	1hour	2hours	3hours	6hours
One tube at 96.04% r.h.	No change	No change	No change	Some wilting
4 tubes at 78.14% r.h.	No change	looked dryer	Severe wilting	Severe wilting
4 tubes at 55.9-47.8% r.h.	Severe wilting	Severe wilting	Severe wilting	Severe wilting

Fig.33. The dehiscence "scores" of experiment 1.

Experiment No.	Number of plants examined.	Temp.of experiment.°C.	Dew-point temperature °C.	r.h. %	Total No. of Antheridia.	No of indehiscent Antheridia.	No. of dehiscent Antheridia.	No.ofplants having dehiscent Antheridia.
1	38	29.2	25	78.14	179	177	2	2
2	31	29.2	17	56-48	176	169	7	5
3	20	29.2	28.5	96.04	125	121	4	2

Fig.34. The dehiscence "scores" for each culture.

Period of treatment	Antheridia	Relative Humidity 96% Control	78%	56-48%
1 hour	No.dehiscent / No.examined	Not examined	0/24	0/21
2 hours	No.dehiscent / No.examined	Not examined	0/40	2/34
3 hours	No.dehiscent / No.examined	Not examined	1/49	4/57
6 hours	Nodehiscent / No.examined	4/125	1/66	1/64
Total dehiscent / Total examined		4/125	2/179	7/176

The results set out in Figs. 33 and 34 appear to show that the treatment had no significant effect on dehiscence. This can be tested conveniently by using 2 x 2 contingency tables, to compare the behaviour of treated plants with that of the control plants.

Comparison of 78% r.h. treatment with the 96% control.

Treatment	Dehisced	Indehiscent	Totals.
96%	4	121	125
78%	2	177	179
Totals	6	298	304

Applying Yates' correction:-

$$\chi^2_{c.} = 0.7492$$

Giving a probability of between 0.5 and 0.3.

Comparison of 56-48% r.h. treatment with 96% control.

Treatment	Dehisced	Indehiscent	Totals
96%	4	121	125
56-48%	7	169	176
Totals	11	290	301

Applying Yates' correction:-

$$\chi^2_c = 0.0018$$

Giving a probability of approximately 0.9

Thus it can be stated that the treatments had no significant effect on dehiscence.

Wilting was produced in all cultures, but the control tube at 96% r.h. only showed wilting after 6 hours.

Experiment (2).

The effects of treating isolated plants with air streams of different humidity as described under procedure 2., are recorded in Fig.35.

Fig.35. The dehiscence "scores" obtained from procedure 2.

Expt. No.	No.of Plants	Total Fresh weight gms.	Loss in wt.(per Cent. fresh wt)	Temp.of experimental Tank. °C	Temp.of "rewetting". °C	Total No. of Antheridia.	No.of antheridia dehiscent.	Percentage dehiscent. %	Age of cultures (days)	No.of plants with dehiscence.
1	20	0.005	Nil	29.2	29.2	114	3	2.6	47	3
2	20	0.012	50%	29.2	29.2	113	9	8.0	47	7
3	20	0.010	80%	29.2	29.2	116	4	3.5	47	3
4	20	0.0056	Nil	29.2	8.0	104	8	7.7	50	6
5	20	0.0122	32%	29.2	8.0	117	22	18.8	50	13
6	20	0.0104	52%	29.2	8.0	127	18	14.2	50	11
7	20	0.0167	Nil	19.5	19.5	132	14	10.6	54	11
8	20	0.0148	79.05%	19.5	19.5	131	11	8.4	54	10
9	20	0.0040	Nil	15.6	15.6	144	14	9.7	56	11
10	20	0.0062	79.0%	15.6	15.6	133	28	21.1	56	16
11	20			15.6	15.6	137	21	15.3	56	11

All of the experimental results set out in table 35., are accompanied by the results of appropriate controls, so that any statistical test of their significance should be applied to the experiment in relation to its control. The greatest differences are between experiment 9 & 10 and 4 & 5. These have been tested using the 2 x 2 contingency table.

	Dehisced	Not dehisced	Totals
Expt. 4	8	96	104
Expt. 5	22	95	117
Totals	30	191	221

Applying Yates' correction:-

$$\chi^2_c = 4.89$$

giving a probability between 0.05 and 0.02.

	Dehisced	Not dehisced	Totals
Expt. 9	14	130	144
Expt. 10	28	105	133
Totals	42	235	277

Applying Yates' correction:-

$$\chi^2_c = 6.05$$

which gives a probability between 0.02 and 0.01.

If the table is examined in detail it can be seen that although dehiscence in Expt. 2., is greater than its control (Expt.1), the plants in Expt.3 which were even more desiccated, show less dehiscence. Similarly Expt. 6 shows less dehiscence than Expt. 5. In the pair of experiments 7 and 8 there is less dehiscence after drying, whereas in Expts. 9 & 10, where the results are apparently significant, the drying has produced about 3 times the dehiscence. This is despite the fact that the treatments were almost the same.

Thus the probabilities examined are significant although they fail to reach the 1% level. However

when they are considered in the light of the rest of the findings it must be concluded that drying is not a stimulus to dehiscence.

Experiment 3.

The effects of the "mechanical" stimuli described in procedure (3) are given in Fig. 36.

Fig.36. The results obtained using the "Mechanical" stimuli.

Expt. No.	No.of plants.	Type of treatment.	Period of treatment.	Total No. of antheridia.	Dehiscent Antheridia.	% of Antheridia dehiscent.	Age of cultures.	Number of plants with dehiscent antheridia
A	20	Shaking with water	18hrs	131	49	37.4	57days	16
B	20	Shaking no water	18hrs	125	16	12.8	57days	9
C	20	Vacuum	15mins	148	55	37.2	58days	16
D	20	Nil (control)	Nil	142	4	2.8	58days	4
E	20	Pressure (10lbs/sq in).	5mins	146	3	2.1	58days	3
F	20	Wind and rain.	48hrs	127	28	22	75days	16

Once again the individual results can be compared with their control (D) using a 2 x 2 contingency table.

Comparison of the results of experiment A with the experimental control D.

Experiment	Dehisced	Indehiscent	Totals
A	49	82	131
D	4	138	142
Totals	53	220	273

Applying Yates' correction:-

$$\chi^2_c = 49.92$$

giving a probability of less than 0.001.

Comparison of the results of experiment B with the experimental control D.

Experiment	Dehisced	Indehiscent	Totals
B	16	109	125
D	4	138	142
Totals	20	247	267

Applying Yates' correction:-

$$\chi^2_c = 8.175$$

giving a probability between 0.01 and 0.001.

Comparison of the results of experiment C with the experimental control D.

Experiment	Dehisced	Indehiscent	Totals
C	55	93	148
D	4	138	142
Totals	59	231	290

Applying Yates' correction:-

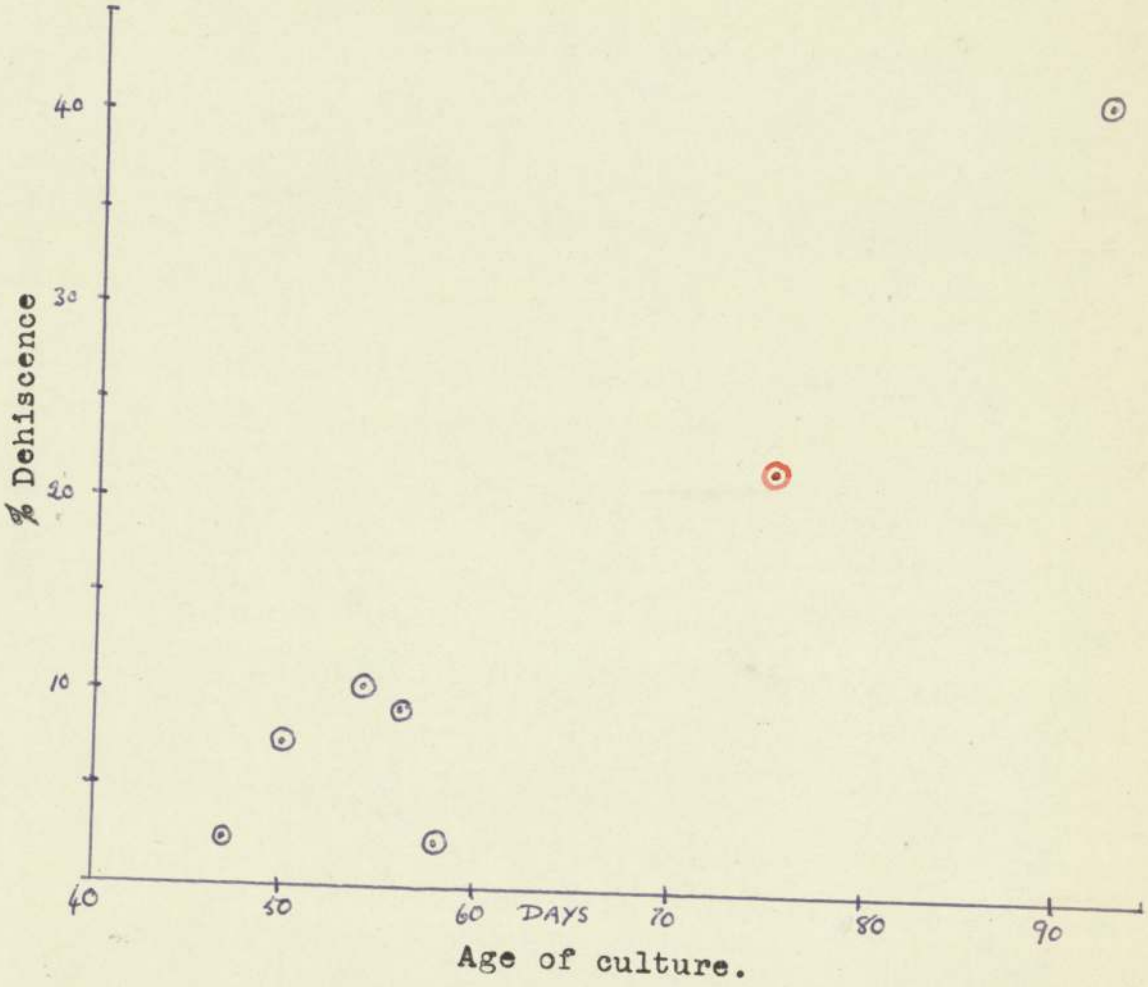
$$\chi^2_c = 50.65$$

giving a probability of less than 0.001.

Experiment E showed lower percentage dehiscent than the control so that treatment E had no significant effect. However the other treatments tested so far (A,B and C) certainly caused dehiscence to take place. Thus violent disturbance of the plants does make the antheridia dehisce. The difference between shaking with water and without water, can be refered to the physical effect of the waves and droplets produced by shaking the water. Vacuum treatment also produced violent disturbance of the culture and its agar.

Experiment F gives a result between that of A and B, but the culture was older. An age effect has been noticed in the results, and has been interpreted as the effect of a continuous dehiscence at a very low rate. The result of experiment F is compared with the results from all the controls in Fig.37.

Fig.37. The antheridial "score" of all controls with the "score" of experiment F in red.



It would appear that the treatment with wind and rain was ineffective.

Experiment 4.

Plants, shaken in experiment (3) were given a fertilisation test, and were scored for sporophytes after 4 weeks. The results of these tests, and of others carried out on unshaken cultures are presented in Fig. 38.

Fig. 38. Results of "Fertilisation Tests".

Conditions	Month of sowing	Month of Fertilisation.	% of mature plants with sporophytes.	No.of plants with sporophytes.	Total No. of plants examined.
Field Temperature.	April	July	6.01	22	366
	May	August	3.4	10	293
	June	August	1.43	5	350
	July	Sept.	26.1	97	372
25°C.	April	July	2.0	9	453
	May	August	0	0	300approx
	June	August	6.9	20	289
	July	Sept.	15.64	56	358
35°C.	April	July	0	0	489
	May	August	0	0	300approx
	June	August	0	0	274
	July	Sept.	3.0	8	271
Exper. 3.	August	Oct.	8.74	32	334

This method does work in the sense that it enables Phascum cuspidatum Hedw., to complete its life cycle while still in aseptic culture. However there is a striking fluctuation in the success achieved. When compared with the previous results the results shown by the shaken plants would seem to be quite good. However they are not as good as one would expect from the percentage of dehiscence produced by shaking.

It may be concluded that the increased dehiscence produced by shaking does lead to some increase in the number of sporophytes produced.

CONCLUSIONS.

1) The drying of plants, either in position on agar, or when plucked and dried with no external source of water does not stimulate dehiscence.

2) Placing moss plants in water after drying does not stimulate dehiscence.

3) The plants are easily dried, even when still in position on agar.

4) Some violent mechanical stimuli can stimulate dehiscence. Exposure to high vacuum, and shaking with added water are effective. The apparently similar stimuli provided by high winds and heavy rain do not seem to have stimulated dehiscence.

5) The sporophyte generation can be produced in test-tube cultures, by adding sterile distilled water, and then decanting it off after 24-48 hours. However the effect is very variable.

6) The increased dehiscence produced by shaking or vacuum treatment does lead to some increase in the production of sporophytes. The increase is not as great as the antheridial dehiscence would suggest.

7) The frequency of successful fertilisation, and the frequency of antheridial dehiscence are both low in culture populations.

DISCUSSION.

The experience of previous workers suggests that whenever mature antheridia are covered with water, they dehisce. In their account of dehiscence of the antheridium of Mnium hornum L., Muggock and Walton (1942) state that "if a drop of water is placed in an antheridial cup in which there are mature antheridia, dehiscence takes place in about 4 min". Similarly it is regular practice in studying the genetics of Marchantia to make the antheridia dehisce by placing one drop of water on the male receptacle just as it is illuminated by the morning sun. The drop is then withdrawn, and contains the antheridial contents.

However these experimental findings with Phascum cuspidatum Hedw., show quite conclusively that under the experimental conditions, the wetting of antheridia does not stimulate dehiscence. It has been further shown that noneof the drying produced by air streams led to increased dehiscence on rewetting. Despite these findings, past experience with cultures of Phascum cuspidatum Hedw., in test tubes has shown that

adding water to mature plants for a day or two and then decanting it off so that the plants do not drown, sometimes leads to the development of sporophytes. (Hughes 1958). However the result of such a fertilisation experiment is quite unpredictable. In view of these experimental findings it is difficult to understand the mechanism by which this added water stimulates the production of sporophytes. It is very unlikely that it is just an effect of wetting the cultures, since Phascum cuspidatum Hedw., is an ectohydric moss, and in cultures, a water film can always be seen on mosses which are not actually wilting. The stimulus provided by adding water could thus be merely mechanical, and would only be applied when pouring water into or out of the tube. If this were the case then the unpredictable results of the fertilisation experiments can be understood in terms of how and where the water was poured on to the culture. Even the amount of water added to the culture may be critical if dispersal of the antherozoids is a surface tension phenomenon as suggested by Muggock

and Walton (1942). Presumably if too much water is added the antherozoids could be carried to the surface, and the distance from there to the archegonia could easily be too great for any fertilisation to take place.

In view of these difficulties it can only be suggested that any further fertilisation experiments should be carried out with measured volumes of water and the test-tubes should be shaken.

It is possible that the liberation of spermatozoa in the field is a different process from that seen in the laboratory. However there is some evidence to show that it is normally an "explosive" mechanism.

1) Dried or flaccid antheridia have not been seen to dehisce.

2) All of the antheridia examined (3,355) were either intact, or had the "cap-cells" ruptured. No other opening was seen.

3) Antheridia are turgid until well past maturity, unless they have been dried.

4) If the apex is ruptured with a needle, the antheridial contents are forcibly ejected.

5) If the side wall of a mature antheridium is perforated, the antheridial contents pass out forcibly. At the same time the inner walls of the "cap-cells" rupture, allowing the contents of the "cap-cells" to pass into the interior of the antheridium. Thus the inner wall of the "cap-cells" must normally be subjected to a pressure on both sides.

6) When an antheridium has dehisced its volume can be seen to have diminished.

Thus it must be assumed that in the field, dehiscence of antheridia is forcible. From the experimental findings it would seem that the antheridia are very insensitive, but can probably best be stimulated by violent mechanical shocks. Strong winds and heavy rain do not seem to be sufficient, and some other agent must be found.

The small invertebrate animals associated with Polytrichum, were investigated by Harvy-Gibson and Miller-Brown (1927), and spermatocytes were found entangled in their hairs. Thus it seems reasonable to propose that the effective stimulus in the field may be provided by an animal. There is no direct evidence of this, and the proposal has been arrived at mainly by a process of elimination. Springtails would seem to be a suitable size, and certainly produce large mechanical shocks when they jump. The effect of springtails is being investigated. Alternatively the antherozoids may be set free in the field by the activities of some herbivore, which regularly feeds on the antheridia.

b) The investigation into the effects of humidity on the leafless axes of the diploid apogamous plants.

Introduction.

Cultures of apogamous diploid plants were grown until most of the individuals in each culture bore well developed leafless axes. Fig.39., shows an apogamous diploid plant in a suitable condition, although axes longer and shorter than this were necessarily involved. Whole cultures were then exposed to air-streams of 100% r.h., and some of them were further treated with drier air. After this prolonged treatment the plants were removed from the apparatus, but were kept at the same temperature and grown for a further period. This ensured that any modification of the cells produced by the treatment would become evident.

CONTENTS.

Fig.39. A diploid apogamous plant with a well developed leafless axis.

(1) PROCEDURE.

The plants were exposed to a moving air stream having the low relative humidity of 82% for a few hours each day. For the rest of the time the plants were supplied with air having a relative humidity of 100%. Further cultures were exposed to an air stream having a relative humidity of 100% all the time, and finally one culture was not exposed to moving air at all.

a) DETAILS OF TREATMENT.

Temperature.

All plants were kept at 29.2°C.

Humidity.

The dew-point temperature of one air stream was 29.2°C. Therefore the relative humidity of that stream when passed to the plants at 29.2°C was 100%.

The dew-point temperature of the other air stream was 26.0°C. Therefore its relative humidity when passed to the plants at 29.2°C

$$= \frac{\text{Vapour pressure of water at } 26^{\circ}C \times 100}{\text{Vapour pressure of water at } 29.2^{\circ}C} \quad (\% \text{ r.h.})$$

$$= \frac{25.181}{30.368} \times 100$$

$$= 82.92$$

Therefore the relative humidity of this air was 82.92%. The rate of flow per tube was 150ccs.per.min.

DAILY TREATMENT.

Day No	Duration (hrs)	Day No	Duration (hrs.)
1	6½	10	4
2	11	11	5
3	0	12	2½
4	11½	13	5
5	7½	14	0
6	5	15	3
7	4	16	3
8	4	17	2
9	4		

b) DETAILTS OF MEASUREMENTS AND SCORING.

At the end of the seventeen day period of treatment with air streams, those plants which had leafless axes were placed in order, on filter paper.

The length and maximum diameter of every leafless axes was measured, together with the length, and length

of any branches, of the leafy shoot. When each set of plants had been measured and set out on filter paper soaked in culture medium, they were kept at the experimental temperature for a further fourteen days. Each plant was then remeasured and also examined and "scored" in terms of appearance. Two separate effects were recorded.

a) The leafless axes were divided into categories, which were effectively white and green, although these conditions were masked to some extent by brown pigmentation of the epidermis. The two categories for the leafless axes were scored as green, and not green.

b) The appearance of the leafy shoots was examined quite separately, and was initially scored in three categories. These were, green, regenerating and dead. The green and dead categories were straight forward. For instance, if a plant showed no green pigmentation at all, it was called dead. However those plants scored as regenerating were of two grades.

First those plants with only their extremities killed, and which showed regeneration as leafy buds developing from the green parts of the leafy stem. The second grade was shown by those plants which had leaves, stem and extremities affected, and where only a mosaic of green and white cells remained. In these plants regeneration was in the form of protonema, which grew out from the green patches. In fact it appeared that only a very few green cells was needed for regeneration to take place.

Regeneration from leafless axes was never observed.

(2) RESULTS.

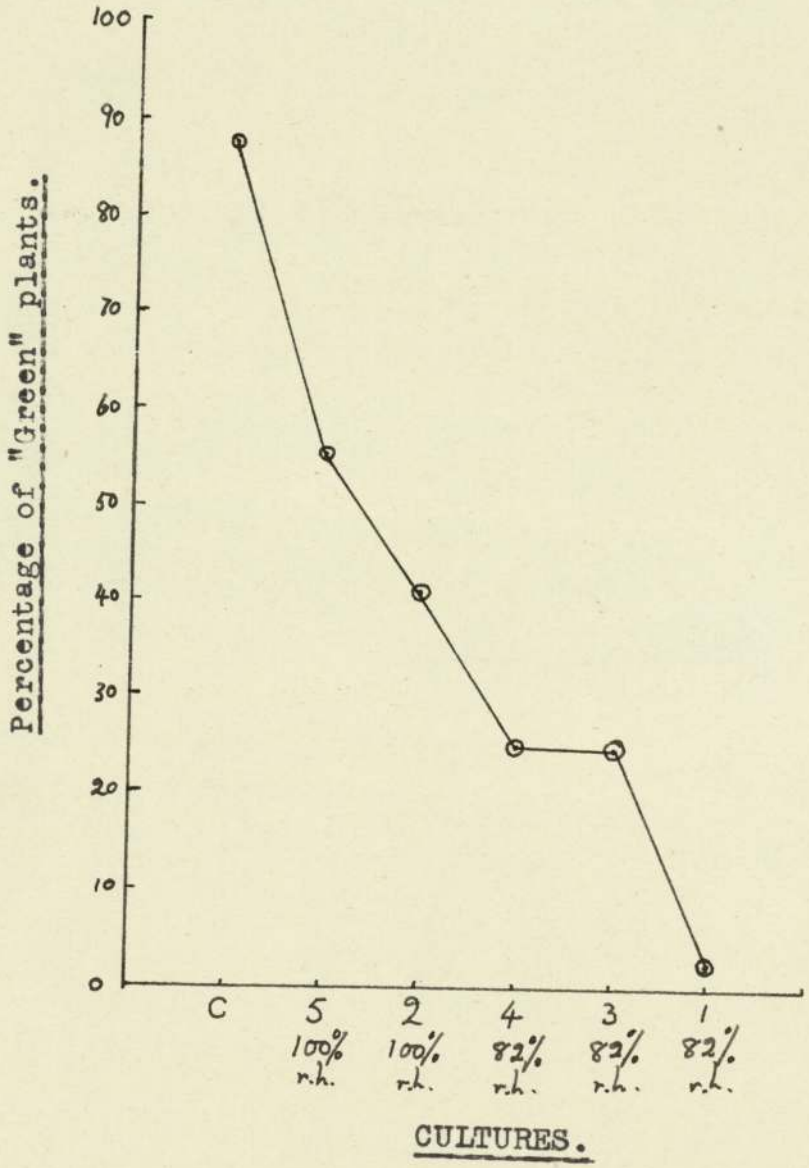
POINT I

All of the cultures in a moving air stream showed harmful effects. Figs.40 and 41 show this clearly.

Fig 40.
Summary of the state of the plants after treatment, and a further fourteen day period without treatment.

State of the longest leafless axis.	Green	Non-Green	Green	Non-Green	Green	Non-Green
State of the leafy shoot.	Green		Regenerating		Dead	
Culture No.						
1	1	4	0	24	0	15
2	37	31	9	12	0	1
3	19	45	3	11	0	0
4	12	1	9	24	0	2
5	26	8	6	4	1	2
Control	70	9	0	0	0	1

Fig.41. A comparison of the percentage of unaffected plants in each culture. (Derived from Fig.40.)



POINT 2.

The leafless axes are more readily damaged than the leafy parts.

Fig 42. The response to dessication. Expressed as totals and as percentages.

Longest leafless axis	Green	Not Green	Green	Not Green	
State of leafy parts	Green	Green	Not Green	Not Green	Totals.
1	1 2.3%	4 9.1%	0 0%	39 88.6%	44
2	37 41.1%	31 34.4%	9 10%	13 14.4%	90
3	19 24.4%	45 57.7%	3 3.8%	11 14.1%	78
4	12 25%	1 2.1%	9 18.8%	26 54.2%	48
5	26 55.3%	8 17%	7 14.9%	6 12.8%	47
Control	70 87.5%	9 11.3%	0 0%	1 1.3%	80

The two parts of the treated plants, can be shown statistically, to have behaved very differently. The information given for the treated plants in Fig 42., can be presented as a 2 x 2 contingency table:-

	Green	Non-green	
leafless	123	184	307
leafy	184	123	307
	307	307	614

Therefore if Yates' correction is applied:-

$$\chi^2_c = 23.45$$

Therefore the probability is less than 0.001.

Fig 43., shows the state of all leafless axes and all leafy shoots. Once again Point 2 can be checked by the use of a 2 x 2 contingency table using the totals of all treated plants scored in Fig 43.

	Green	Non-green	
leafy	183	126	309
leafless	226	321	547
	409	447	856

Using Yates' correction as before:-

= 24.66.

This again indicates a probability of less than 0.001.

Thus it can be said quite confidently, that a difference in behaviour between the leafy shoot, and the leafless axis has been demonstrated. The difference has further been shown to be due to a greater response of the leafless axis.

Fig.43.

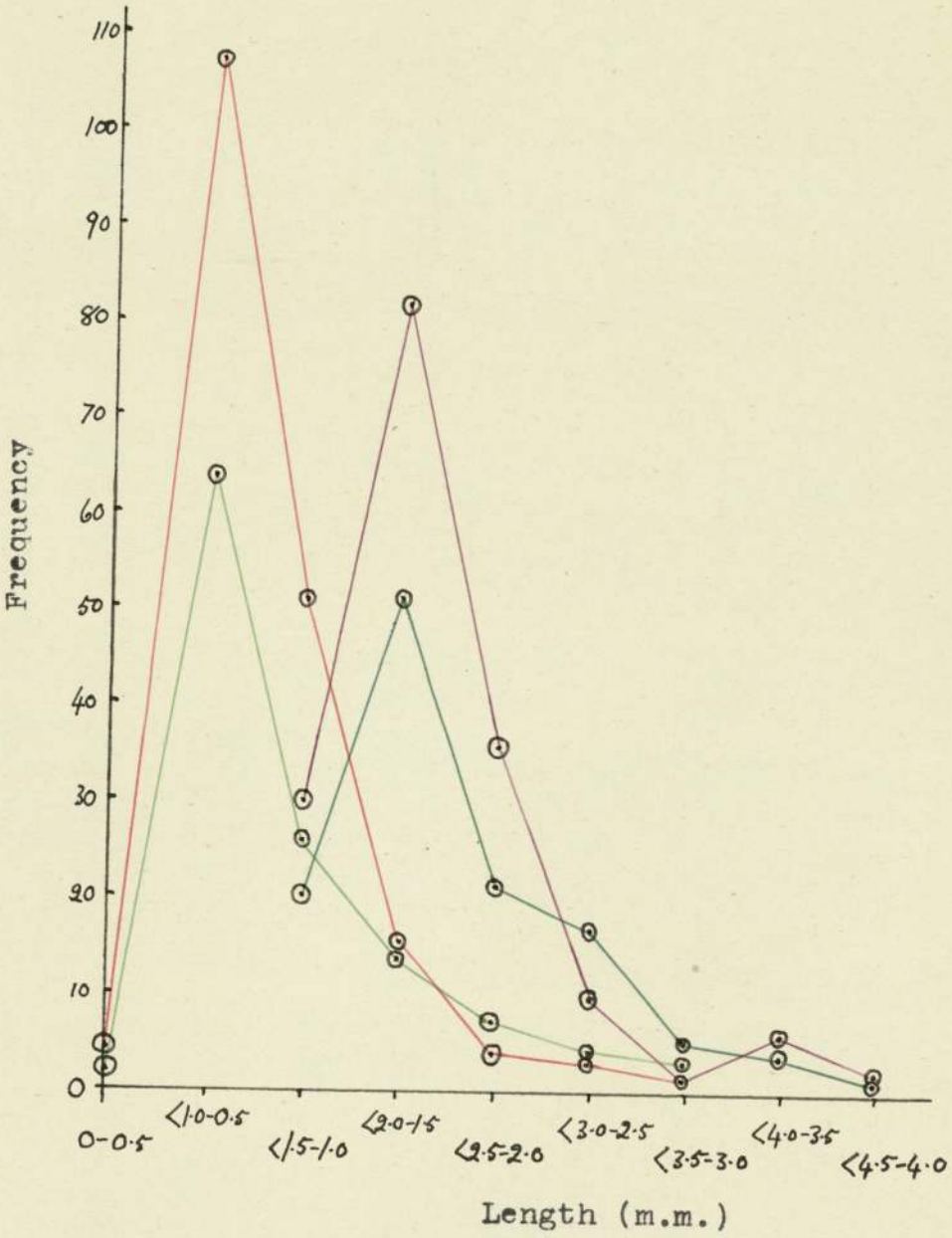
Culture No.	LEAFY SHOOTS			LEAFLESS AXES		
	Green	Not Green	Total	Green	Not Green	Total
1	5 11.4%	39 88.6%	44	1 1.4%	71 98.6%	72
2	68 75.6%	22 24.4%	90	87 48.1%	94 51.9%	181
3	63 78.8%	17 21.3%	80	43 33.33%	86 66.66%	129
4	13 27.1%	35 72.9%	48	35 43.2%	46 56.8%	81
5	34 72.3%	13 27.7%	47	60 71.4%	24 28.6%	84
Control	78 97.5%	2 0.25%	80	106 88.33%	14 11.66%	120

Point 3.

The longest leafless axis is more readily damaged than the shortest one.

This is a matter of relative length, not of absolute length. When the frequency of damage to the leafless axes, is plotted against length of axis (Figs. 44 & 45) it would not appear that the longest shoots are more readily damaged than the shorter ones. Even the inclusion of the length of the leafy shoot makes no significant difference to these graphs (Figs. 44 & 45). However the difference becomes evident when in individual plants, the frequency of damage to the longest axis of a plant, is recorded relative to the frequency of damage to the shortest axis, of the same plant. This has been done in Fig 46. The contingency table and the subsequent calculation shows that the difference between the response of the longest and shortest axes is highly significant.

Fig.44. Diagram to show the state of the longest leafless axes.



Purple - non green axes.
Dark green - green axes.
Brown - non green axes plus length of leafy shoot
Light green - green axes plus length of leafy shoot.

Fig.45. Diagram to show the state of the shortest leafless axes.

Purple - non green axes.
Dark green - green axes.
Brown - non green axes plus leafy shoot.
Light green - green axes plus leafy shoot.

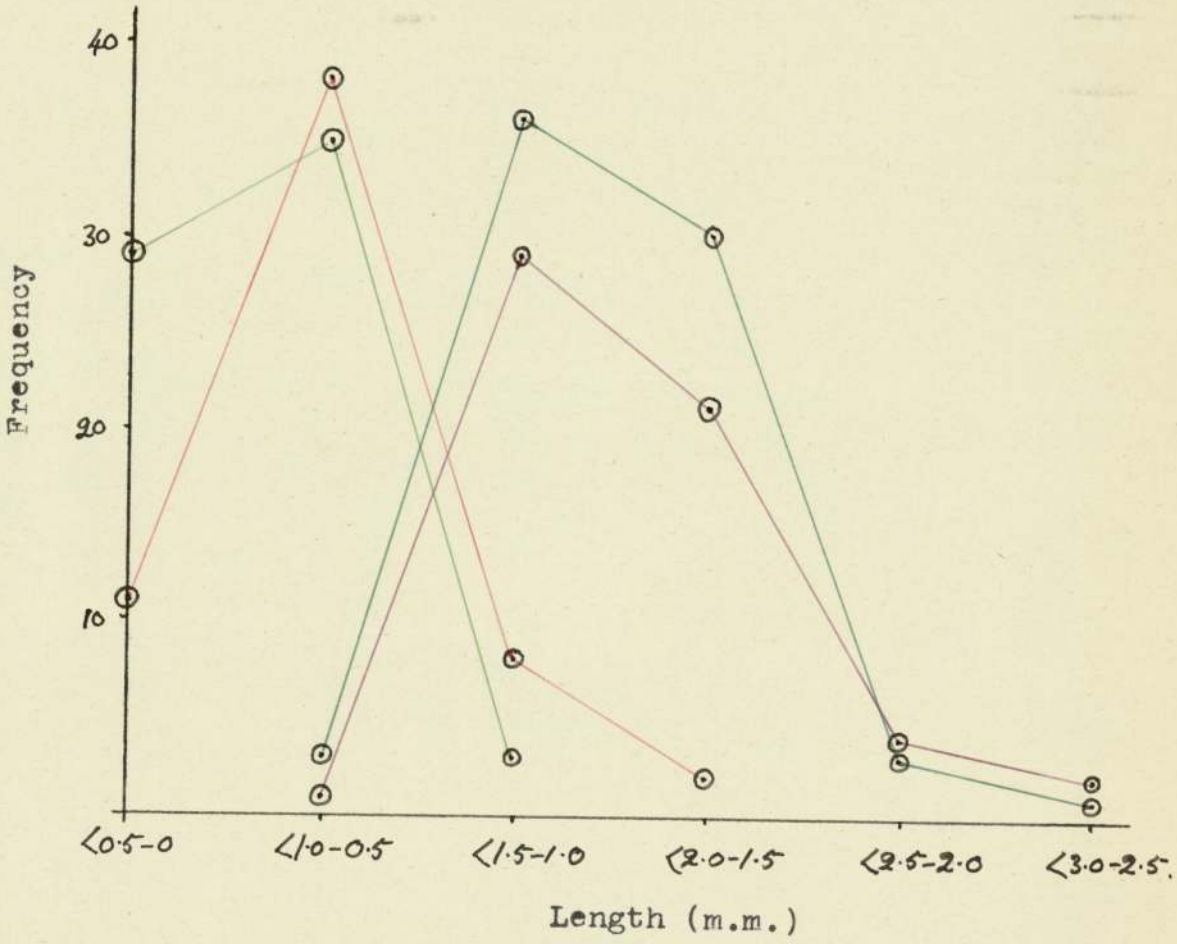


Fig.46a. To show the frequency of plants in each length category.

Culture No.		← LENGTHS IN MILLIMETRES →																	
		4.5-4.0		<4.0-3.5		<3.5-3.0		<3.0-2.5		<2.5-2.0		<2.0-1.5		<1.5-1.0		<1.0-0.5		<0.5-0	
		N	G	N	G	N	G	N	G	N	G	N	G	N	G	N	G	N	G
1	A									1	0	10	0	17	0	15	1		
	B			1	0			5	0	10	0	25	1	2	0				
	C													3	0	13	0	2	1
	D					1	0			1	0	7	0	9	1				
2	A									0	5	1	11	12	12	29	18	1	0
	B			1	0	0	2	1	11	7	9	28	20	6	5				
	C											1	0	2	0	12	18	2	1
	D							1	1	1	1	4	14	9	5				
3	A									2	0	1	0	13	3	42	16	0	1
	B	1	0	1	0			1	0	6	2	31	8	17	11				
	C													1	1	6	9	4	6
	D							1	0	0	1	4	5	7	9				
4	A									1	0	1	0	6	4	17	14	3	1
	B			1	0					8	3	16	12	5	2	0	1		
	C													1	1	4	3	3	11
	D									0	1	4	2	3	9	1	3		
5	A					1	3	3	4	0	2	2	3	3	7	4	15		
	B	1	1	2	4	1	3	3	6	5	7	2	10	0	2				
	C											1	0	1	1	3	5	0	10
	D							0	1	2	1	2	9	1	5				

N - not green
G - green
A - length of the longest axis
B - length of the longest axis & the leafy shoot.
C - length of the shortest axis
D - length of the shortest axis & the leafy shoot

Fig.46.

Longest / Shortest / Tube No.	Green / Green	Green / Not Green	Not Green / Green	Not Green / Not Green	Total
1	0	0	1 5.6%	17 94.4%	18
2	15 44.1%	5 14.7%	2 5.9%	12 35.3%	34
3	1 3.4%	0	15 51.7%	13 44.8%	29
4	3 13.0%	0	12 52.2%	8 34.8%	23
5	10 47.6%	1 4.76%	6 28.6%	4 19.0%	21
Control	25 80.6%	2 6.5%	2 6.5%	2 6.5%	31

Contingency table drawn up from Fig 46., using all the data on the treated cultures.

Axes	Green	Not Green	Totals
Longest	35	90	125
Shortest	65	60	125
Totals	100	150	250

Applying Yates' correction:-

$\chi^2_c = 14.017$

Therefore the probability is less than 0.001, and the statement that the longest axis is more readily damaged than the shortest can be made confidently.

Point 4.

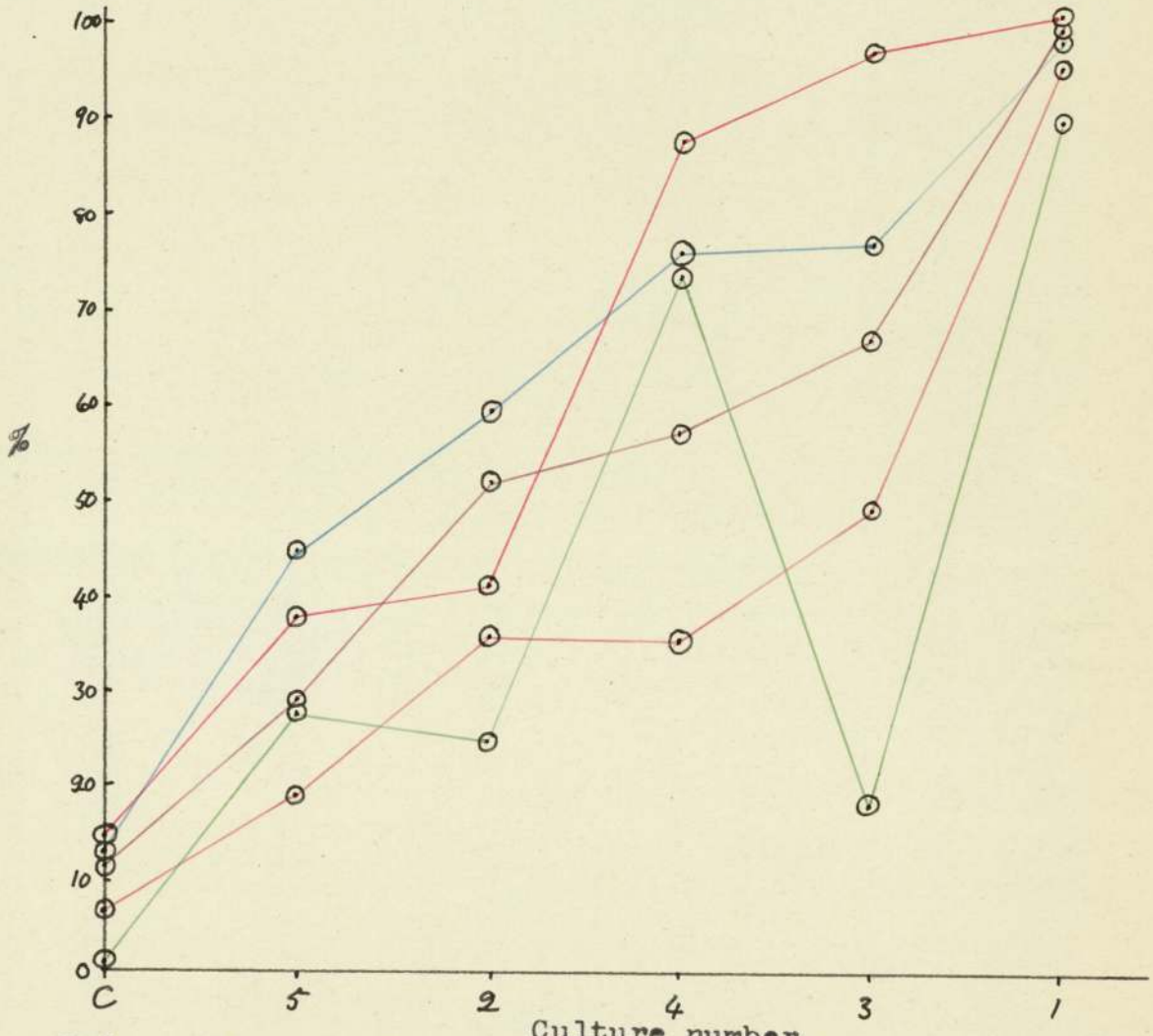
The frequency of damage increases with the drying effect of the air stream.

The effects of moving air streams have been presented together in Fig 47. The data included in this figure has already been summed and presented in earlier comparisons. However in Fig.47., it is made

apparent that there are variations between cultures which have received similar treatment. The most consistent results are given by the graphs for the percentage of plants affected, the percentage of all leafless axes affected and the percentage of longest leafless axes affected, in those plants with two or more leafless axes. These graphs show that the control culture, the cultures supplied at a relative humidity of 100% and the cultures supplied at 82% r.h., have behaved differently from one another. However the two graphs for those plants with the longest and shortest axes both affected, and the percentage of leafy shoots affected do not behave in quite the same way.

The scoring for the state of the longest and shortest leafless axis on the same plant was made after growth had occured for two weeks subsequent to the experimental treatment. Many of the green shortest axes had developed during that period and this may well have invalidated this particular comparison.

Fig.47. Diagram to summarise the measured effects of the air streams.



Red - longest of 2 or more leafless axes responded.
Blue - plants responded.
Grey - all leafless shoots responded.
Green - leafy shoots responded.
Brown - longest and shortest leafless axes responded.

The very low percentage of leafy shoots affected in culture three cannot easily be explained. However Fig.48., does show that culture 3 had the smallest percentage of plants with two or more leafless axes, with the exception of the control culture. This could indicate that the leafy shoots of culture 3 are younger than those of other cultures and therefore possibly more resistant. But the differences between the values in Fig.48., are very small.

Fig.48.

Culture number.	% of plants with two or more leafless axes.
1	40.9
2	37.8
3	36.3
4	47.9
5	44.7
C	33.8

The fact that it has been possible to place the cultures in the order C, 5,2,4,3, and 1 shows that there were significant differences in treatment between each tube.

These differences between cultures 5 and 2, which were both supplied with air at 100% r.h., and between 1, 3 and 4 which were supplied with air at 82.9%, can largely be refered to differences in flow rate. The drying effect of each air stream depends on flow rate as well as relative humidity. It is supposed therefore that the three tubes 1, 3 and 4 were exposed to different drying conditions.

Culture 1 being dried more than culture 3 which was dried more than culture 4. This supposition can be supported by observations on the shrinkage of agar made during the course of the experiment. Similarly it might be said that culture 2 was dried more than culture 5, but more important than this is the difference between the two cultures 2 and 5 and the control culture. It was intended that culture 2 and 5 should provide controls for comparison with the effects

produced by treatment with air at 82.9% r.h. Once again agar shrinkage during the experiment showed that cultures 2 and 5 were exposed to slight drying despite the fact that the air was supplied at 100% r.h.

This drying can be refered to the effect of incident radiant energy. Care was taken when setting up the apparatus that the incident energy should be only just above the minimum required by the plant, and only "north light" was used. Even with these precautions it was possible to show on a very dull day a temperature rise of 0.04°C in the region of the mosses using a normal mercury in glass thermometer. Following these readings a copper constantan thermocouple was made, and used with the reference "cold" junction screened by rubber tubing and held alongside the culture tube. In this way any fluctuation of temperature in the experimental bath would be automatically compensated. Using this very small thermocouple a difference of 0.5°C was demonstrated between the back of a moss "clump" and the front and also between

the experimental bath and the air flowing out of the tube. These measurements were also obtained on a very dull day. If a total temperature difference of 0.1°C is assumed to exist between the air supplied at 100% r.h., and the air around the moss then the relative humidity could be 99.4% for this increase in temperature, but would tend to fall because of water evaporating from the moss and agar surfaces.

This effect can only occur during the day, and was far beyond the measuring ability of the hygrometers. However it is possible to state that the drying effect produced by it was very small. Approximately 2ccs., of agar evaporated from each of tubes 2 and 5 in the 17days of treatment.

Taking all of these factors into account it does seem that the frequency of damage does increase with the drying effect of the air stream.

POINT 5

Low humidities do not stimulate sporangial development. The harmful effects shown by the plants in cultures 2 and 5, to the very slight drying produced, shows that the only response which can be made by these diploid apogamous mosses is regeneration. At least this is true under the conditions of the experiment.

DISCUSSION.

The response of the diploid apogamous plants exposed to air streams of 100% r.h., was quite unexpected. Certainly no such response has been seen in the haploid sexual plants. The damage caused by the treatment increased with the drying effect of the air streams, and can only be interpreted as a response to desiccation. Thus the main problem raised by this part of the investigation, is that of explaining how drying can be produced by an air stream, which is saturated with water vapour.

There are two purely physical explanations, which could account for the phenomenon.

Despite the very good temperature control in the experimental tank, the temperature certainly does fluctuate, and it is possible that an appreciable reduction of relative humidity could occur if the air was heated after reaching equilibrium with free water in the "catcher" flasks. However it is claimed that the temperature control is better than $\pm$ 0.05°C, so that, under the worst possible conditions this would

only give a reduction from 100% to 99.4% r.h. at 29.2°C.

The other explanation is that the plants themselves modified the air stream. This can be explained in terms of some of the incident solar energy, heating the green moss plants after transmission through the several layers of glass and about half an inch of water. The measurements carried out with a special thermocouple arrangement seem to confirm this theory. Under very "dull" conditions no temperature difference could be detected between the water bath and the agar or the mosses on the side of a "moss-turf" away from the window. There was however a temperature difference of about 0.5°C between the bath, and the "front" mosses, and between the bath and the air leaving the culture tube. Since the agar in these culture vessels did lose some of its volume during the experiment, water was lost to the air. Presumably this rise in temperature provides the explanation of such water loss, and therefore it is useful to make some assessment of the conditions to

which the plants were exposed. If one assumes that the difference between the temperature of air leaving the culture vessel, and its dew-point temperature is 0.35°C., then a calculation can be made. This figure is not arbitrary, since it also represents the limits of measurement of hygrometers, as claimed by their manufacturers. Such a temperature difference at 29.2°C., leads to a relative humidity of 96.04%.

Since all the treated cultures showed harmful responses it can be claimed that diploid apogamous plants of Phascum cuspidatum Hedw., can only grow satisfactorily at 100% r.h. They do grow well in the culture vessels, and it must be assumed that the humidity under these conditions is maintained at 100% r.h., since the results of even mild desiccation are not seen. Moreover a humidity of 100% r.h., must be maintained by a big range of concentrations of agar, since the results of desiccation are not evident in culture growing on agar which has lost much of its water. Wettstein (1942), in his work on diploid apogamous plants of Phascum cuspidatum Hedw., found

that the developmental pathways of the swellings on leaf tips could be changed by using different concentrations of agar. Wettstein (1942) and later workers such as Näf (1962) attribute this result in part to the "dryness" of the medium. However it would now seem that if such a control does exist, it can only operate in a very narrow humidity range of approximately 98-100% r.h. Also any attempt to reconcile these findings with the suggestions of Wettstein, must explain in what sense 3% agar is dry, when compared with (say) 1% agar. Moreover if such a control exists one would expect the behaviour of cultures to change with the volume of agar, since such changes must be accompanied by changes in agar concentrations.

Marchal E & Em. (1911), and Wettstein (1923), suggested that the swellings on the tips of leafs and the leafless axes can act as propagules. In view of the extreme sensitivity to desiccation shown by these plants, and since regeneration was never seen to take place from leafless axes, this function would now

seem to be impossible.

Diploid apogamous plants of Phascum cuspidatum Hedw., have been found growing in the field (Hughes 1958). In view of their extreme sensitivity to desiccation, it must be assumed that these plants survived because of a copious supply of water. The plants are ectohydric, and it would seem that water lost to drying air, (or even to saturated air, if the plants are being heated by incident energy), is lost from the surface film. If the surface film is used up it can only be assumed that harmful effects are produced. The usual resistance to damage caused by desiccation, (pollacauophytic properties) as measured by such workers as Ochi (1952) does not seem to be present. However their test for survival has been plasmolysis in suitable solutions, and their results cannot therefore be compared directly with this work. In this work, where the plants were actually grown after treatment it was possible to pick out individual living cells and it was not necessary to rely on averages. Thus these findings are not strictly comparable with those relying on the less sensitive

plasmolytic methods. Even so the distinction between these diploid apogamous plants and most other bryophytes must still stand.

The exact way in which these diploid apogamous plants avoid desiccation in the field had not yet been determined.

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