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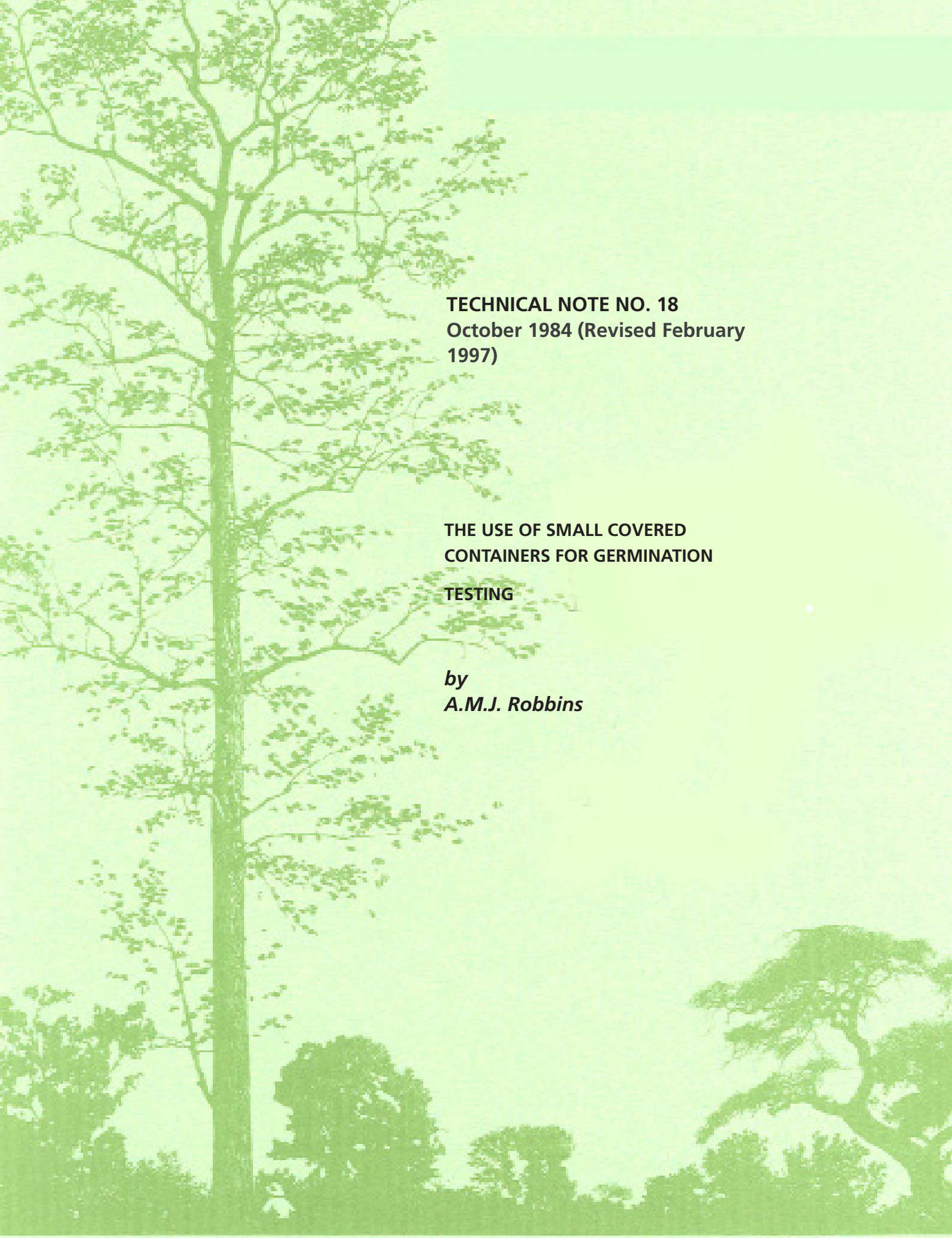

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THE USE OF SMALL COVERED CONTAINERS FOR GERMINATION TESTING

by
A.M.J. Robbins

DANIDA FOREST SEED CENTRE

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1. INTRODUCTION

Germination tests for tree seed can be carried out with a variety of media and systems for controlling moisture, temperature and light. One method that is simple and suitable for all species makes use of small, stackable, transparent containers with lids, one for each replicate of the test. Temperature and light can be controlled by placing the containers in an incubator, whereas moisture is maintained by keeping the container sealed using its lid. Thus the use of germinators with controlled relative humidity is unnecessary (Poulsen 1994).

2. CONTAINER TYPE AND SIZE

The ideal container should be:

(1) rectangular and stackable, for economy of space;

(2) large enough for adequate spacing of one replicate of seeds (100, 50 or 25 seeds, depending on seed size);

(3) sufficiently deep, to allow for the required depth of medium and to permit development of the seedling for proper assessment;

(4) provided with a well-fitting lid, to maintain a high moisture content of the media and surrounding air;

(5) easy to sterilise by heat or chemical treatment;

(6) transparent (at least the lid), to allow light if required for germination and for subsequent normal development of the seedling.

Plastic storage boxes. Rectangular, transparent polystyrene boxes with lids (used to store small hardware articles) are very suitable. They should preferably not have internal ridges or slots for partitions as these will make cleaning and sterilising difficult. A size of approx. 180 mm x 120 mm (base) x 70 mm (depth) is suitable for one replicate of 100 seeds of pine, leaving at least the width of one seed between the seeds. This type of box generally has a shallow lid and straight sides (see fig. 1).

Note that 'Tupperware' type polythene containers used commonly for food storage are generally not suitable, since they are not rigid, the lids often are difficult to remove, and the material is translucent, not transparent.

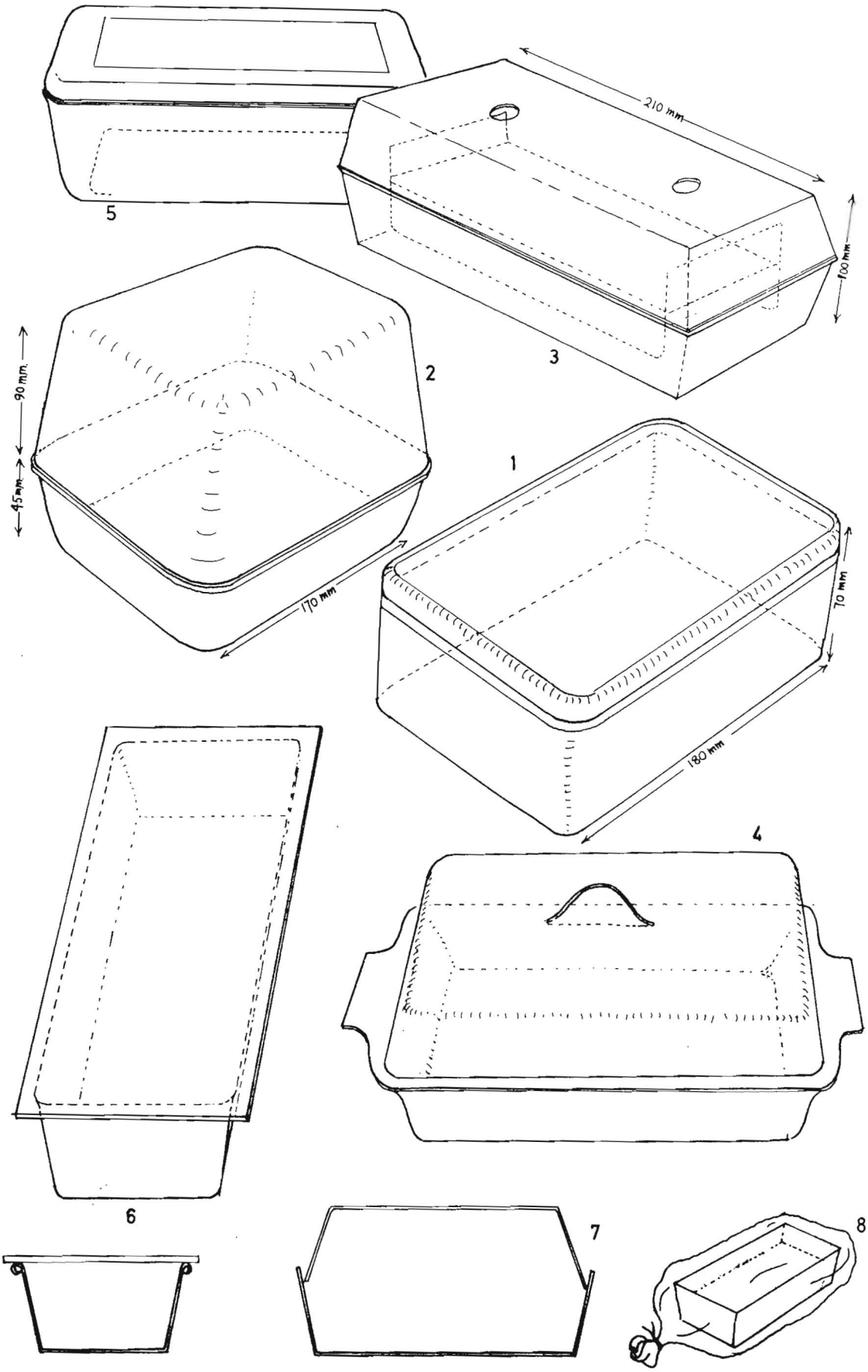

Figure 1-8. Containers for Germination Tests

Horticultural propagators. Several types of plastic container are available for the specific purpose of germinating seeds and propagating seedlings, but not all are suitable for seed testing. Most are too large and have drainage holes in the base and ventilation holes in the lid, which is not required unless the seedling is to be grown on for subsequent transplanting. A suitable small container of this type is shown in fig. 2. The base is sufficiently deep for the medium alone, covered by a high transparent lid which allows for seedling development. This type of container normally has sloping sides so that storage of base and lid when not used is less bulky.

A container specially designed for germinating seeds is shown in fig. 3, but it is principally for educational purposes and quite expensive.

Domestic containers. Locally available household containers can also be used. Plastic butter dishes (fig. 4) or larger boxes used for cutlery or cakes (fig. 5) may be suitable. Flat-bottomed metal containers, preferably rectangular, used for baking pies, bread or cakes could be used, covered with a sheet of glass (fig. 6). Ice-cube trays (without partitions) could be used in the same way. Alternatively, a combination of containers can be used, one for the base and an inverted one for the lid (fig. 7). If a suitable transparent well-fitting lid cannot be improvised, the container base can be placed in a thin polythene bag or covered with 'cling-film' (fig. 8).

The construction of boxes by glueing together acrylic sheet is not recommended. This has been tried by Wang and Ackerman (1983), but they found that the resultant containers tended to warp with changing temperature and humidity.

Petri dishes. These are often used for tests of small-seeded species (e.g. Eucalyptus), but they are not good containers for general testing as they are not large enough, particularly with respect to depth. Their main use is for culturing fungi or bacteria on thin substrates such as agar.

3. QUANTITY OF CONTAINERS REQIDRED

According to ISTA rules the standard germination test requires the use of 400 seeds, normally in 4 replicates of 100 seeds each. Therefore, each test will require 4 containers as a minimum one for each replicate. If the seeds are large, so that 50 or 25 are used for a replicate, the number of replicates (and containers) should be increased. Each seed lot will require testing, and therefore, if all are to be tested concurrently, the number of containers will be: number of replicates x number of seed lots. If research into media and temperature regimes etc. is to be carried out, then the total will have to be increased correspondingly, limited by the handling capacity of the laboratory technicians and the space available in the incubators.

4. MOISTURE CONTROL

A sufficiently high and constant moisture content of the medium and air surrounding the seed is obtained by adding a suitable quantity of water to the medium (see later) and then keeping the container covered with its lid at all times, except during assessment of the seedlings. If the volume of medium within the container is sufficiently large, and the lid well fitting, it will only be necessary to add water at the beginning of the test, thus avoiding the need to add water during the test.

Condensation will occur on the underside of the lid. To avoid its dripping onto the seeds and causing localised overwetting, the containers should be moved gently. The lids should be lifted at one comer and tilted when removed from the base. This will allow the condensate to run to one side and drip back into the box without touching the seeds.

Normally, ordinary tap-water is suitable and need not be sterilised unless it is known to be contaminated.

5. CONTROL OF TEMPERATURE AND LIGHT

Ambient. If an incubator is not available, the containers can be allowed to follow ambient conditions of light and temperature by placing them, stacked if necessary, on a table or bench next to a window, but out of direct sun-light.

Air-conditioned room. In tropical climates where the room temperature is likely to exceed 35°C, it will be advisable to use a room with air-condition and to let it run at least during the day. If testing is carried out at altitudes where night-time temperatures are very low, some form of heating may be necessary during the night as well as during the day.

Incubators. To germinate seed according to ISTA rules proper control of temperature and light is needed. Consequently the containers should be placed \within an incubator (fig. 14) with control of temperature, ideally at two levels so that alternating regimes can be imposed, and provided with fluorescent strip lighting. Note that many commercial incubators only control temperature at one level and may not have provision for lighting.

The main advantage of separate covered containers is that, even if an incubator should fail entirely, the tests can always be continued (albeit at a less precise level) by letting the containers follow ambient conditions.

When stacking the containers in an incubator, it is important to make sure that there IS adequate circulation of air around them so that temperature is uniform.

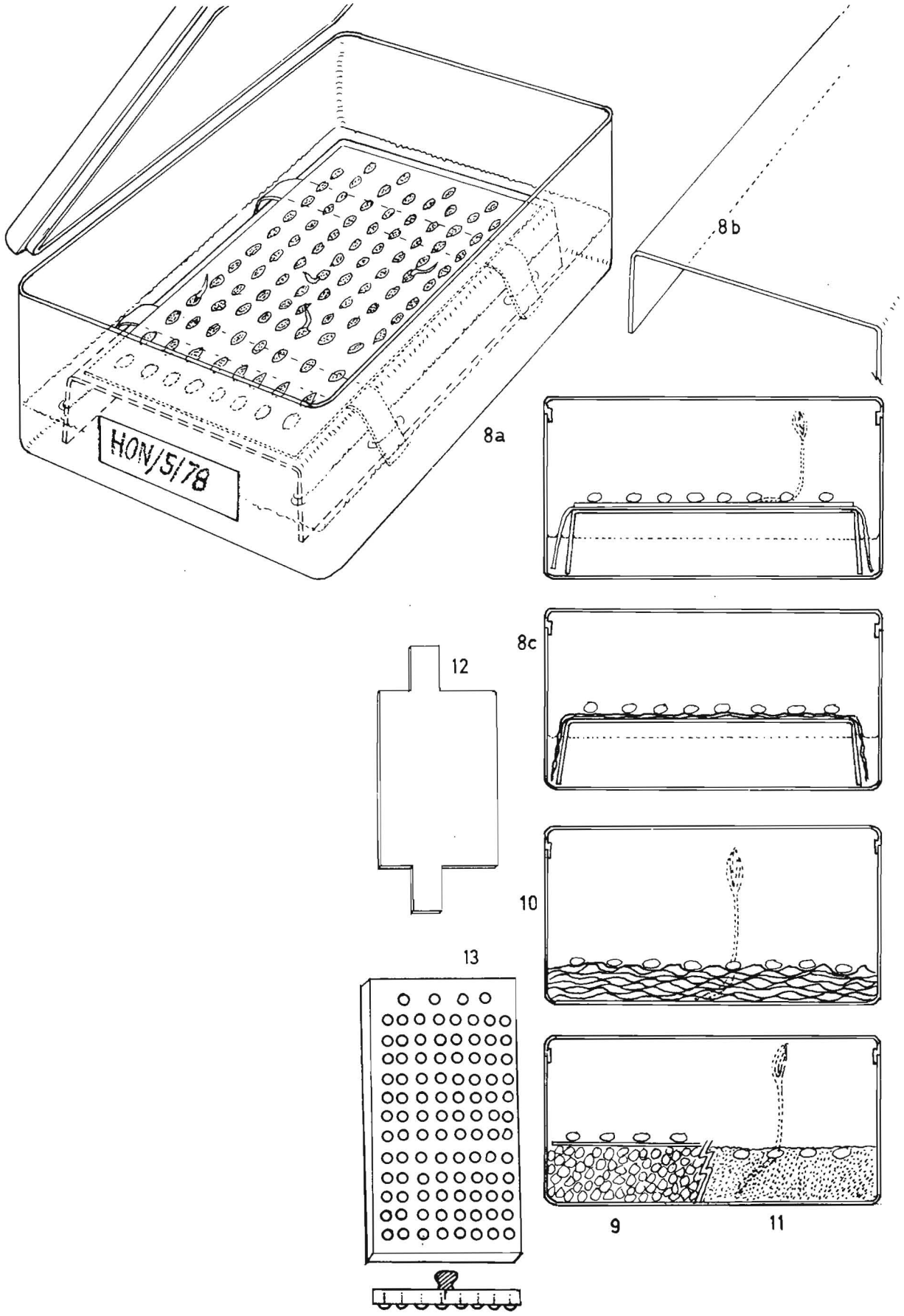

Figure 8a-13. Methods of Using Containers

6. GERMINATION MEDIA

Various media can be used in the containers. In principle there are two methods:

(l) on top of filter paper or between pleated filter paper, and

(2) in a medium like sand or vermiculite.

In the first method it is very easy to record germination, because the seed is not hidden in the material. If the system with a wick in a reservoir of water is used, it is also a very reliable system with a high repeatability. It is however not so easy to control fungi on paper as in e.g. sand.

Filter paper or blotter. The best method of using this medium is to place the paper on a raised support over a reservoir of water which is fed to the medium by wicks (fig. 8 a). This ensures a constant level of humidity (the principle of a Jacobsen germination table). The support can be made of thermoplastic sheet as shown in fig. 8 b or improvised from smaller, inverted containers.

The level of the water reservoir in relation to the surface of the medium is important and will depend on the absorbancy of the wicks and medium used. If the level is too high, the medium will become too wet, and conversely if too low. One method of checking if the right amount of water is getting to the medium is to press the surface gently with the finger; the depression that forms should show signs of free water, whereas the surrounding surface should not.

If the container shown in fig.l is used, the following specifications are suitable: filter paper, 0.5 mm thick, 150 mm x lOO mm; 2 wicks of same material, 105 mm x 10 mm each, placed across the width of the support under the paper, and with both ends bent downwards to touch the base of the container; sufficient water (about 150 ml) to give a water level 10 mm below the paper surface (see fig. 8 a).

If suitable commercial filter paper or blotter is not available, it may be possible to use several layers of absorbent toilet paper, tissue or kitchen paper towel. In this case, the material can be cut wide enough to allow it to be bent over the sides of the support, so that the material itself forms the wick (fig. 8 c). The level of water will be different for each material and must be determined by trial and error. It is important to make sure that the material is free from toxic additives and has a neutral pH (6.0-7.5).

An alternative arrangement for supporting the paper is to place it on a bed of absorbent material to which water has been added. In this way the paper absorbs moisture direct from the supporting material. Vermiculite is very suitable if obtainable. Cottonwool can be tried, but care must be taken to get the right balance of water and wool to keep the paper moist (see fig. 9).

The advantages of the paper methods are that they are fast, and it is easy to see the seeds, since they are not covered by the medium; this facilitates the final registration of types of ungenninated seeds. The disadvantages are that fungal attacks are easily spread, and that seed of many species will not be able to develop into nonnal seedlings without a proper rooting medium. Thus during the gennination test solely radicle protrusion is registered. However, the use of pleated (i.e. folded) paper (fig. 8d) will reduce the speed of fungal spreading since each seed is separated from other seeds. Seedling development may also be fine.

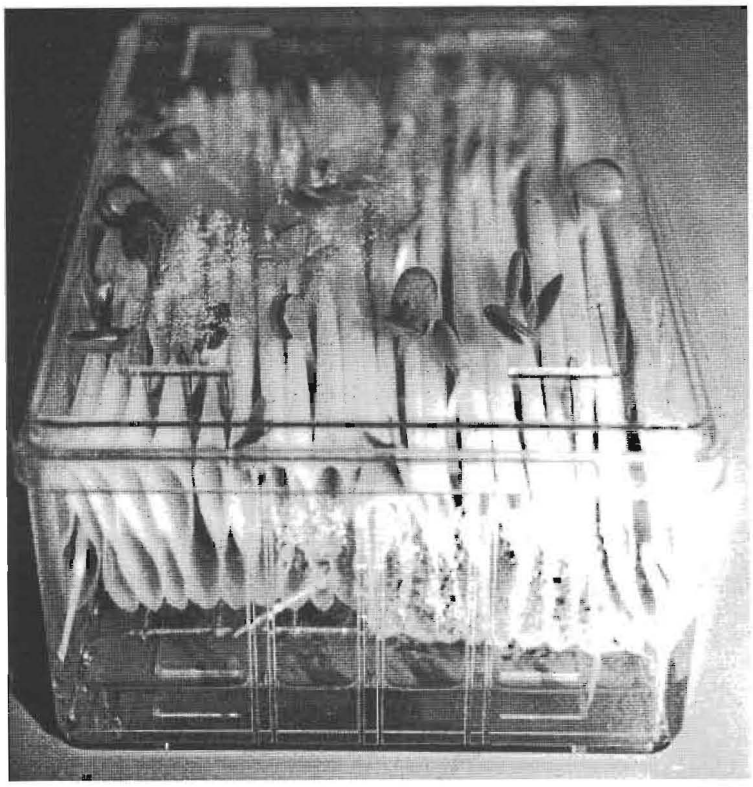

Figure 8.d. Germination of seed in pleated paper. A rectangular piece of filter paper is placed on a wire-netting with a wick into the water reservoir in the bottom of the box. Pleated paper is placed on top with 5 seeds in each fold, in total 100 seeds.

Germination towelling. Paper towelling or wadding specifically designed for gennination tests, such as 'Kimpak', makes a very convenient medium for use in the containers. In this case, two or three layers should be placed directly on the floor of the container (see fig . 10) so as to ensure sufficient water-holding capacity. If the container in fig. 1 is used, two layers of 'Kimpak' to which have been added about 50 ml of water are suitable. The paper will swell up to about 14 rnm's thickness. The seeds are gently pressed onto the surface of the towelling.

The main advantage of this medium is that it is very easy to prepare and the seedling radicle can penetrate the layers of the towelling, allowing it to develop upright. However, the material will generally have to be imported.

Sand. Sterilised and sieved sand can be used in the containers and forms a good medium for many tree species (fig. 11). It is particularly suitable for minimising fungal growth and spread. The sand should have a neutral pH (6.0-7.5) and should be free of toxic substances. It must be sterilised in open pans at 150°C for two hours in an oven, and then passed through 0.8 mm and 0.5 mm sieves, retaining the middle fraction, so that the particle size is within 0.5 and 0.8 mm in diameter. This particle size gives a good moisture and aeration environment for germination. If sieves are not available, fine sand from which the silt has been washed away can be used.

The amount of sand in the container should allow adequate rooting depth. The volume of water added should be about 50 -60% of the moisture-holding capacity of the sand. (This can be calculated as follows: add a known volume of water to the amount of sand to be used in each container, so that there is excess of free water. Drain off the excess, allowing time for it all to percolate out, and measure its volume. The moisture-holding capacity of the sand will be the original volume of water added minus the excess. Use 50-60% of this volume to moisten each container).

If the container in fig. 1 is used, about 250 ml of sand is needed, giving a depth of 18 mm, to which is added about 75 ml of water.

The sand should be levelled within the container. This can be facilitated by using a plywood scraper shown in fig. 12. The shoulders should be cut so that the scraper just levels a fixed amount of sand when resting upright on the sides of the container, and there is no excess sand pushed to one end.

To facilitate spacing of the seed and positioning, a board can be made just smaller than the inside dimensions of the container, with round-headed tacks nailed into one side at the correct spacing for the seeds. The board can then be pressed onto the surface of the wet sand, making small indentations into which the seed is placed (see fig . 13). The seed should be pressed gently level with the sand surface, and then just covered by sprinkling a thin layer of dry sand over the surface. Larger seeds should be sown by partially filling the container with sand, then positioning the seed before putting in the rest of the sand. In this case, a double-sided scraper will be useful -one side for partial filling and levelling, and the other side for final levelling so as just to cover the seed.

Other media may be used, but sand and paper are preferred.

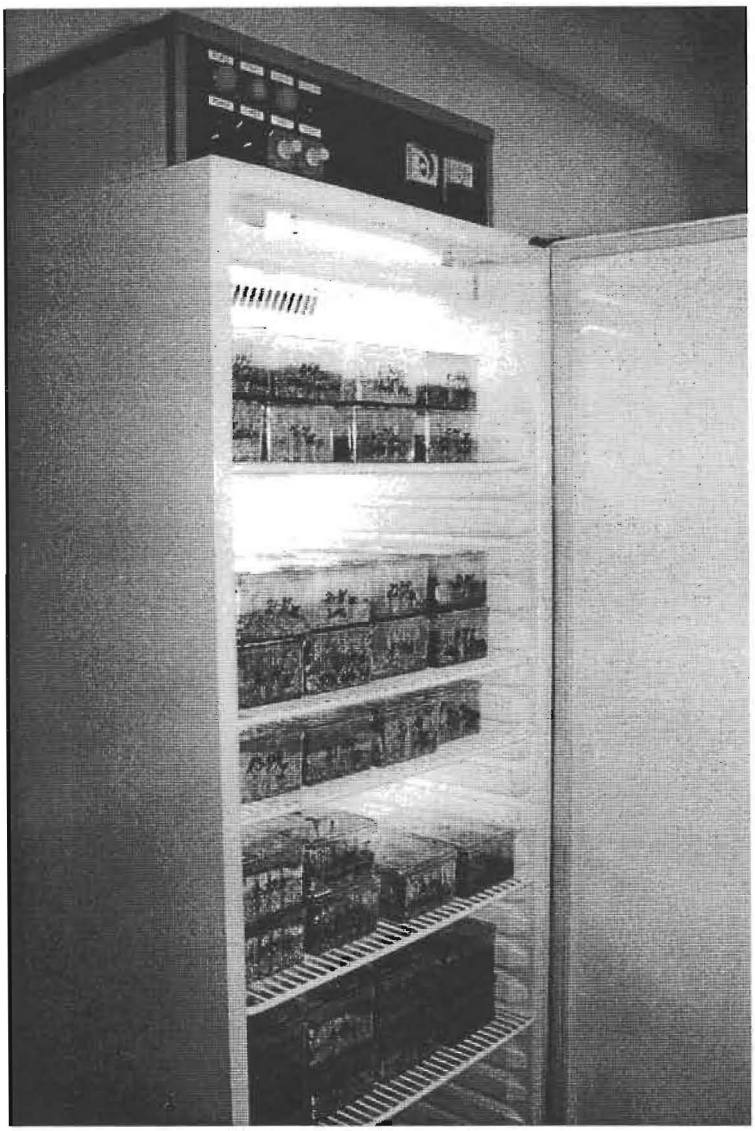

Figure 14. Germination cabinet (re-built refrigerator) with germination boxes.

7. STERILISING AND LABELLING THE CONTAINERS

Sterilising. It is very important to keep the containers clean and sterile between tests to avoid contamination from fungi and bacteria. Pyrex glass and metal containers can be heat sterilised using boiling water or an oven. Plastic containers must be sterilised using a suitable chemical. The container can be wiped carefully using a strong alcohol solution (min 70%) or soaked overnight in a dilute solution of bleach (sodium hypochloride, 12%, diluted 3 times), or formaldehyde. It is important to use a disinfectant that will not damage the plastic.

Labelling. The containers can be labelled using masking tape on which is written the identity number using an indelible pen. This type of tape is sufficiently sticky to remain on the container during the test, but can be removed easily afterwards. Label the base, not the lid, as the lids can be switched inadvertently.

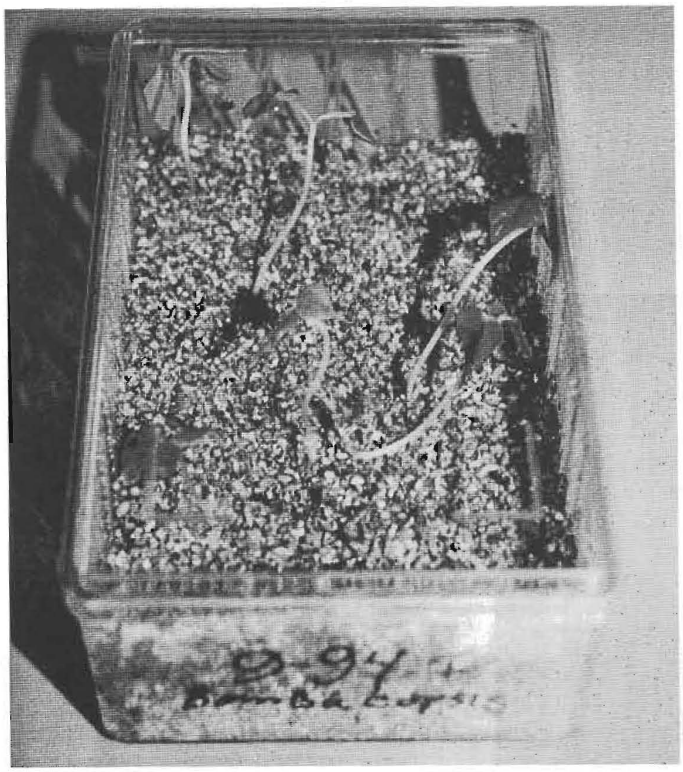

Figure 15. Germination in vermiculite. Covering lid is removed from the germination box.

8. ACKNOWLEDGEMENTS

The use of small covered containers for germination has been used extensively by the U.K. Forestry Commission Seed Testing Station at Alice Holt, and also at the Banco de Semillas, Escuela Nacional de Ciencias Forestales at Siguatepeque, Honduras. Part of the recommen-. dations given in this leaflet is based on the experience of these two stations. Other recommendations and certain equipment and suppliers are taken from Van der Burg et al. (1983).

Further details concerning 1STA recommendations for substrates and evaluation of tests can be found in the rules, annexes, and amendments of the Association. Copies may be obtained from: 1STA Secretariat -P.O. Box 412 -8046 Zurich -Switzerland.

9. REFERENCES

Van der Burg, W.J., Bekendam, J., van Geffen, A. and Heuver, M. 1983 — Project Seed Laboratory. Seed Science and Technology, 11, 157-227.

ISTA 1993 — International rules for seed testing 1976. Seed Science and Technology, Vol. 21, Supplement

Poulsen, Karen M. 1994 — Seed Testing. Danida Forest Seed Centre, Lecture Note C-8

Wang, B.S.P. and Ackerman, F. 1983 — A new germination box for tree seed testing. Information Report PI-X-27, Petawawa National Forestry Institute, Canada.

10. EQUIPMENT AND SUPPLIERS

In 1984, 1ST A Equipment Committee under the chairmanship of Dr. E. Madsen issued 'Survey of Equipment and Supplies for Seed Testing', third edition. A new survey has been completed and the fourth edition of the survey is in preparation.

We would like to draw your attention to this publication, which is available from ISTASecretariat, P.O. Box 412, CH-8046 ZUrich, Switzerland.

At the same time we would like you t~ make your own local survey. Much may be available, perhaps not originally intended for seed testing, but fully suitable for the purpose. Test what is locally available; local supply may need only minor adjustments.

DFSC laboratory buys filter paper from:

A.G. Frisenette & Sons
Godthabsvej 4
DK-8400 Ebeltoft Denmark
Tel. + 45 86 342244
Fax. + 45 86 34 5744

DFSC laboratory buys germination boxes of the make 'Ultra Plast' from:

Hoffstâtter & Ebbesen A/S
Bredgade 17
DK-1260 Copenhagen K.
Denmark
Tel. + 45 33 32 62 50
Fax. + 4533 152758