SCIENTIFIC APPARATUS AND LABORATORY METHODS

APPARATUS FOR THE PRODUCTION OF ARTIFICIAL FROST INJURY IN THE BRANCHES OF LIVING TREES[1]

NATURAL late frost injuries have been used by Glock in the past to determine the presence of multiple growth layers (or "rings") in one year in the branches of living trees. In connection with this work Studhalter suggested that, to serve the same purpose, an apparatus be devised for the production of artificial frost injury under controlled conditions.

The resulting apparatus has certain advantages over other types described by Sorauer[2] and by Mix[3] for application to a portion of a branch. These advantages include ease of application to branches, use of solid carbon dioxide (dry ice), which permits a wide range of low temperature, and the simulation of natural conditions in which the freezing element does not come into direct contact with the plant tissue.

The apparatus, square in cross section, is made of half-inch seasoned lumber and consists of three essential units (Fig. 1), namely, lid, dry-ice chamber and treatment chamber. Sheet cork gives insulation on the interior and aluminum paint on the exterior. Rubber gaskets are placed on all surfaces where the units come in contact.

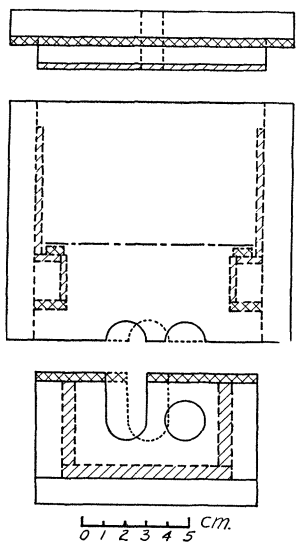

Fig 1. Diagram of apparatus

FIG. 1

A 1-cm hole through the center of the lid permits the escape of air at the beginning of an experiment and, later, of the carbon dioxide gas as it is pushed upward by the descending colder gas. In the dry-ice chamber a wire screen supports the solid carbon dioxide which is placed in the upper part of the unit. The wire screen rests on a rubber gasket made discontinuous in order to allow free passage of carbon dioxide gas into and out of the lower unit. The treatment chamber fits up into the bottom of the dry-ice chamber. In one side of the treatment chamber a hole receives a low-temperature thermometer held firmly by a cork. Into each of two sides, as shown in the figure, a channel is cut to receive the branch, the one on the same side as the thermometer hole being offset from the middle. Sponge rubber gaskets are cemented into the channels so as to fill them nearly to the top.

An apparatus with the dimensions shown in Fig. 1, which is drawn to scale, will receive branches up to 12 mm in diameter. When the apparatus is applied in the field, the treatment unit is brought up from below to the part of the branch to be frozen so that the branch sinks into the sponge rubber of the channels. Separate blocks of sponge rubber are inserted into the channels on top of the branch. Then the other units are lowered into place and the whole held together by strong rubber bands. If necessary for adequate support, a cord may be passed around the apparatus and over a superjacent limb.

It has been found by experiments during the past two years that the range of temperature obtainable extends from 0 to −45 degrees C. for an interval up to 7 hours. In order to obtain different temperatures, the following factors are varied as the experiments demand: absolute quantity of dry ice, size and number of pieces (to determine the amount of surface exposed), length of time of application and degree of pre-cooling. Extensive calibration studies prove that the dry-ice chamber should be above the treatment chamber for the most effective results.

Throughout two field seasons the apparatus has been used for anatomical and ecological field experiments and for the study of cambial activity especially during and after freezing. It has proved its efficacy in the duplication of natural frost injury and in the placement of an internal label whereby growth flushes are being timed and the number of growth layers determined.

R. A. STUDHALTER
WALDO S. GLOCK
TEXAS TECHNOLOGICAL COLLEGE,
LUBBOCK

[1] Presented at the Dallas, Texas, meetings of the American Association for the Advancement of Science, on December 29, 1941. Abstract in *Amer. Jour. Bot.*, 28 (10, Sup.): 6s, 1941.

[2] P. Sorauer, *Ber. deut. bot. Ges.*, 2: xxii–xxv, 1884.

[3] A. J. Mix, N. Y. (Cornell) *Agr. Exp. Sta. Bul.*, 382: 235–284, 1916.

THE USE OF TERTIARY BUTYL ALCOHOL IN MICROTECHNIQUE

TECHNICIANS, always interested in improving microtechnique by using new reagents, are especially anxious to conserve materials vital to war industry. Tertiary butyl alcohol (TBA) is used partially to replace dehydrating agents such as ethyl alcohol and clearing agents such as xylol and benzol, which are becoming increasingly expensive and difficult to obtain. TBA is obtainable without priority rating,[1] is cheaper than most laboratory reagents,[2] and safe to

[1] According to R. W. Greeff and Company, 10 Rockefeller Plaza, New York, N. Y.

[2] Based on list prices of the California Botanical Materials Company, 787 Melville Ave., Palo Alto, Calif.

use if prolonged exposure to a high concentration of vapors is avoided. TBA is miscible with most reagents in common laboratory use.

TBA was first introduced as a dehydrating agent for tissues by Johansen.[3] Although it has been recommended for plant microtechnique,[4,5] little has been written regarding its use for animal tissues.[6] During the past seven years I have found TBA unusually satisfactory for the dehydration of a large variety of normal and pathological mammalian tissues. A comparative study[7] of dehydrating agents showed that it caused less shrinkage of rabbit kidney than dioxan, xylol or chloroform. After TBA dehydration tissue hardening is comparatively slight and cytological details are well preserved.

Although techniques should be varied to suit the size and type of specimen, the following schedules have been found generally satisfactory. For dehydrating tissues a series of solutions of tertiary butyl-ethyl alcohol (TBEA) should be prepared as indicated in Table 1.

TABLE 1

PERCENTAGES OF TBEA SOLUTIONS

Constituents	50 per cent.	70 per cent.	85 per cent.	95 per cent.	100 per cent.
Distilled water	50 cc	30 cc	15 cc	....	....
95 per cent. ethyl alcohol	40 "	50 "	50 "	45 cc	....
Tertiary butyl alcohol ..	10 "	20 "	35 "	55 "	75 cc
Absolute ethyl alcohol ..	....	....	....	....	25 "

Fixed material dehydrated directly from water or through the lower percentages of ethyl alcohol is transferred to 50 per cent. TBEA for 1–2 hours and material washed in alcohol is placed in the corresponding concentration of the TBEA dehydrating mixture. Leave tissues in (1) 70 per cent. TBEA from 2 hours to several days; (2) 85 per cent. TBEA, 1–2 hours; (3) 95 per cent. TBEA, 1–2 hours; (4) 100 per cent. TBEA, 1–3 hours; (5) pure TBA, three changes in 4 hours to overnight; (6) equal parts of pure TBA and paraffin oil, 1–2 hours; and (7) infiltrate with paraffin. This infiltration is accomplished by filling shell vials three-fourths full of melted parowax or paraffin, allowing the paraffin to solidify and then placing the tissue just covered with TBA-paraffin oil mixture on top of the solid paraffin. The vials are then placed in a well-ventilated oven, the temperature of which is several degrees above the melting point of the paraffin. As the paraffin melts the tissue sinks and is gradually infiltrated with paraffin. Starting at least one hour after the tissue has sunk to the bottom of the vial, the melted paraffin should be changed at hourly intervals, at least until the odor of TBA is no longer detectable, usually 2–6 hours. The used paraffin is discarded. If a special paraffin is used for embedding, it should be used as the last change of melted paraffin in the oven. When necessary, very small pieces of tissue which are in 70 per cent. TBEA one morning may be dehydrated during the day, infiltrated with paraffin overnight and sectioned by noon the next day.

The two most important stages in the technique are the final dehydration with TBA and the infiltration with paraffin. It is essential that the free water be removed completely from the tissue before paraffin infiltration and that the TBA and paraffin oil have diffused from the tissues before they are embedded. Although it is better to discard all solutions after using once, if necessary the same solutions may be used several times.

When celloidin or paraffin-celloidin (double embedding) techniques are being used, after dehydration in 100 per cent. TBEA, tissues may be treated according to the usual schedules with ether-alcohol and infiltrated with celloidin or nitrocellulose. Johansen[4] has suggested the use of equal parts of tertiary butyl, ethyl alcohol and ether instead of the usual alcohol-ether as a solvent for celloidin or nitrocellulose.

Since many stains are less soluble in TBA than in ethyl alcohol, TBA is used in dehydrating stained sections, especially when one is anxious to reduce the extraction of ethyl alcohol soluble stains from the tissues.[8] Slides are mounted with balsam, damar or clarite directly from TBA or preferably after passage through xylol or toluol. Celloidin and nitrocellulose are only slightly soluble in TBA, and stained celloidin sections can be dehydrated directly through TBA or TBA with chloroform into xylol before mounting.

R. E. STOWELL

BARNARD FREE SKIN AND CANCER HOSPITAL,
ST. LOUIS, MISSOURI

[3] D. A. Johansen, SCIENCE, 82: 253, 1935.

[4] D. A. Johansen, "Plant Microtechnique," McGraw-Hill Book Company, New York, 1940.

[5] J. E. Sass, "Elements of Botanical Microtechnique," McGraw-Hill Book Company., New York, 1940.

[6] R. E. Stowell, *J. Techn. Methods*, 22 (in press).

[7] R. E. Stowell, *Stain Techn.*, 16: 67, 1941.

[8] N. D. Levine, *Stain Techn.*, 14: 29, 1939.

BOOKS RECEIVED

Biological Symposia: Vol. VIII: Levels of Integration in Biological and Social Systems. Edited by ROBERT REDFIELD. Pp. v + 240. The Jaques Cattell Press, Lancaster, Pa. $2.50.

BLANCHARD, W. O. *Exercises in Economic Geography.* Pp. ix + 64. McGraw-Hill. $1.00.

EGLOFF, GUSTAV, GEORGE HULLA and V. I. KOMAREWSKY. *Isomerization of Pure Hydrocarbons.* Pp. 499. American Chemical Society Monograph Series. Reinhold Publishing Corporation. $9.00.

HURLOCK, ELIZABETH B. *Child Development.* Illustrated. Pp. xiv + 478. McGraw-Hill. $3.50.

Procedure Handbook of Arc Welding Design and Practice. Seventh edition. Pp. xi + 1267. Lincoln Electric Company, Cleveland. Illustrated.