

I A W A
B U L L E T I N
1974 / 3

OUR COVER

This photomicrograph is a species of *Brownea* (Leguminosae) according to information from B. F. Kukachka of the Center for Wood Anatomy Research, Madison, Wisconsin. Magnification: 50X.

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The International Association of Wood Anatomists was organized in 1931 to advance the knowledge of wood anatomy in all its aspects. It does this in part by attempting to promote and facilitate cooperation among the relatively small number of specialists in wood anatomy.

Prospective members are invited to write to the Office of the Executive Secretary for a copy of the Constitution, an application form, and information about IAWA. Membership dues, which includes a subscription to the IAWA Bulletin, are currently \$5.00 (U. S.) per year.

EDITORIAL

As this issue of the IAWA Bulletin goes to press, members of the Council are considering and reporting back on the financial future of the Association. Since the Bulletin represents the single greatest expense the editors are naturally concerned about the future.

The most straightforward way of solving the financial dilemma is to ask for a substantial increase in annual dues, as was mentioned in our last editorial. We know that some Council members are opposed to this for various reasons, and undoubtedly some members-at-large would also prefer to see the dues remain at \$5.00 per year.

What are the alternatives? There are several, actually, and it is only a question of deciding whether the Association is worth preserving with its current standards or whether our members would prefer to compromise on quality and quantity.

For example, two issues of the Bulletin per year would reduce costs of paper, printing, postage, as well as reducing the burden on the volunteer staff. Another would be to make the Bulletin strictly a newsletter. Lower paper quality and reduction in printing quality for the plates could save some dollars. These and other options are being considered by the Council. You are urged to express your views through any member of the Council or directly to the Office of the Executive Secretary. Since this is your Association, let us know how you would like to have it run on your behalf.

W. A. Côté

C. H. de Zeeuw

Wood Structure in Relation to Latitudinal
and Altitudinal Distribution

By

P. Baas and N. A. Van der Graaff¹

In "classical" comparative wood anatomy the implications of correlations between wood structure and environmental factors are usually fully neglected. As a matter of fact, there is still a great gap in our knowledge about such correlations, and the little information available is not always in mutual agreement. Recently we analyzed the wood anatomical variation in a number of genera and species, with reference to latitudinal and altitudinal distribution. Latitude and altitude are generally accepted as being rough indicators of the environmental factor temperature. Part of the results have been published in the journal of the Rijksherbarium BLUMEA (Baas, 1974), and more data will be published in the same journal (Van der Graaff & Baas, 1974). We are presenting an abstracted version here, because BLUMEA is, as yet, not widely circulated amongst wood anatomists.

The most extensive study of wood anatomical variation was carried out on the cosmopolitan genus *Ilex* (Aquifoliaceae), 81 species of which were examined (Baas, 1974). The very considerable wood anatomical variation was found not to be related with taxonomical subdivision of the genus, but with latitudinal and altitudinal distribution of the individual species. In both the northern and southern hemisphere, and in both the Old and New World, temperate to subtropical species are characterized by conspicuous growth rings, numerous narrow vessels, relatively short vessel members, few bars per perforation plate, and conspicuous helical thickenings on both vessel and fibre walls. In the tropical lowland species growth rings are absent or less marked, the vessels are scanty and wide, the vessel members are long, and the number of bars per scalariform perforation

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plate is high; helical thickenings are absent or faint, or occur only in a minor part of the axial elements. Tropical montane species (from altitudes over 2000 m) resemble the temperate ones to a great extent, but this does not apply to growth rings and helical thickenings, which are as in the tropical lowland species.

Further studies were carried out in a limited number of species belonging to 25 miscellaneous eurytherm genera (24 dicotyledonous genera and 1 coniferous genus (Baas, 1974; Van der Graaff & Baas, 1974). In combination with the data on *Ilex*, and some data from the literature, the following latitudinal trends could be established as generally valid:

With increasing latitude a miniaturization of secondary xylem elements occurs: shorter vessel members, narrower vessels, shorter and narrower fibres, lower rays. In addition vessel frequency, and frequency and expression of helical thickenings increases. Increasing altitude shows similar but much weaker effects, but none for helical thickenings. The number of bars per scalariform perforation is in general not correlated with latitude or altitude.

Within the limited number of species with a wide ecological range studied, the infraspecific variability did not show any correlation with latitude or altitude. This suggests that environmental factors, other than temperature may have played a dominant role, or that characters related to latitudinal and altitudinal distribution within genera have become genetically fixed in the individual species.

The strong shortening of vessel members with increasing latitude and altitude is of some significance for the interpretation of phylogenetic wood anatomy as established by Bailey and his students. The presumably very important climatic influence on vessel member length has led us to believe that the general phylogenetic trend from long to short vessel members must be reversible to a considerable extent. Our results may also indicate that climatic changes have had some directing influence on wood specialization.

Finally it is obvious that all characters shown to be strongly related with altitude and latitude of provenance are of very restricted value for systematic anatomical studies.

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Note: Reprints of the above papers are or will be available on request from P. Baas, Rijksherbarium, Schelpenkade 6, Leiden, The Netherlands.

SEM/EDXA as a Diagnostic Tool for Wood and Its Inclusions

By

Richard L Gray¹ and Wilfred A. Côté²Introduction

The scanning electron microscope (SEM) is proving to be very useful as a tool in wood research. In recent years almost all scientific publications concerned with wood research have had one or more articles demonstrating how the SEM can be employed for investigating such areas as wood structure and wood failure. When the SEM is interfaced with an energy dispersive X-ray analyzer (EDXA), the system can also become an effective analytical tool.

Instrumentation

In the SEM the primary beam of electrons impinges on the sample and excites the emission of secondary electrons which can be utilized in creating a three-dimensional image on a cathode ray tube (CRT). Simultaneously, X-rays whose energies are characteristic for each element, are emitted from the sample excited by the electron beam. If a solid state detector, such as one of lithium-drifted silicon, is used to capture these X-rays, the characteristic energy of each photon of X-ray radiation can be measured. These pulses can be processed (sorted and stored) with the EDXA and a spectrum of the various energy peaks displayed on a CRT. One can collect information and identify elements with an EDXA system far faster and more easily than with wave length dispersive X-ray analyzers. Detection is limited to elements above the atomic number of sodium in most units. In wood research this limitation can be advantageous since the carbon and oxygen so commonly present in wood do not create interference. For a more detailed explanation of the EDXA system, the reader is referred to the following recent papers.

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Practical utilization of this SEM/EDXA system in the paper field for the study of printing inks, identification of specks or deposits, and corrosion analysis was recently discussed and illustrated by Parham (1973). The evaluation of pulping liquor penetration using this approach was recently investigated by Côté *et al.*, 1974. The use of this system to detect and locate concentrations of silica in rice husks (Côté, 1974) showed the great potential SEM and EDXA will have in applications where the topography or configuration of a material and the chemical nature of its surfaces may be important.

Specimen Preparation

Relative ease of specimen preparation is another advantage of using SEM/EDXA as an analytical tool. Specimens are mounted on pyrolytic carbon plachets and carbon coated by evaporation under high vacuum if they are to undergo X-ray analysis. Metal specimen supports and/or metal coatings complicate interpretation due to interference of their peaks with some elements found in wood. Relatively large specimens can be accommodated in the SEM chamber and they may be in the form of microtomed sections, small cubes or split pieces. Moisture content of samples cannot be above air dry condition without creating vacuum problems. Normally the use of low moisture contents in the samples is preferred.

Wood Studies

The purpose of this paper is to demonstrate the potential of the SEM/EDXA system as a diagnostic tool for wood and its inclusions. The occurrence of crystal formations in wood cells is well known. Elemental analysis of these crystals has been a slow and tedious operation using conventional laboratory techniques. With the use of the SEM/EDXA system, elemental analysis can now be completed in a relatively short time. Once a crystal is located in a wood cell and displayed on the CRT, a point analysis can be used to determine which elements are present. Knowing this, there are two possible techniques that can be employed to show the distribution of individual elements in a specimen. These are element mapping and line scan analysis.

Figure 1 shows a line scan across a tangential surface of wood. In each instance that a crystal is encountered, a peak indicating the presence of the particular element in the crystal rises from the base line as a function of concentration. In this particular case (Fig. 1), the crystal was a calcium compound. Figure 2 represents an element map for the same sample as in Figure 1. Knowing that the crystals are composed mainly of calcium, for example, the element map will reveal the distribution or localization of this element throughout the wood section. These same procedures were repeated for Figures 3 and 4 which represent a radial surface of wood. In this particular case the crystals have a high concentration of silica. In Figure 4 there is evidence for a crystal in the upper left corner of the section which does not appear in Figure 3. This illustrates the fact that SEM provides only a surface view, whereas with EDXA the electron beam penetration can generate X-rays from materials below the surface. Depth of penetration is a function of energy of the beam and density of the specimen.

The advantages of three-dimensional viewing of crystals or inclusions provided by the SEM are also apparent from Figures 1, 3, 5, 6. The common cubical form of crystal is illustrated in Figure 1, while the more globular form of silica or "silica sand" is apparent in Figure 3. Figure 5 reveals excellent detail of the "druse" crystalline form. The unusual crystalline forms found in ray parenchyma cells (Figure 6) proved to be sodium chloride after a point analysis was performed.

It becomes quite apparent from this brief discussion and a few examples that the potential of SEM/EDXA in wood research, especially as a diagnostic tool, has hardly been realized. This approach obviously brings an added dimension and higher resolution to the study of wood inclusions. The ease of identifying elements and illustrating their distribution in a specimen could make SEM/EDXA a standard tool of the wood anatomist in the future.

ACKNOWLEDGMENTS

The scanning electron micrographs prepared for this study were made with an ETEC Autoscan which was purchased through the generosity of the New York State College of Forestry Foundation, Inc. Without this thoughtful support, studies of this nature could not be undertaken at this Center.

Helpful discussion with Dr. C. H. de Zeeuw and the helpful contributions of staff members A. C. Day and J. J. McKeon to this study are gratefully recognized.

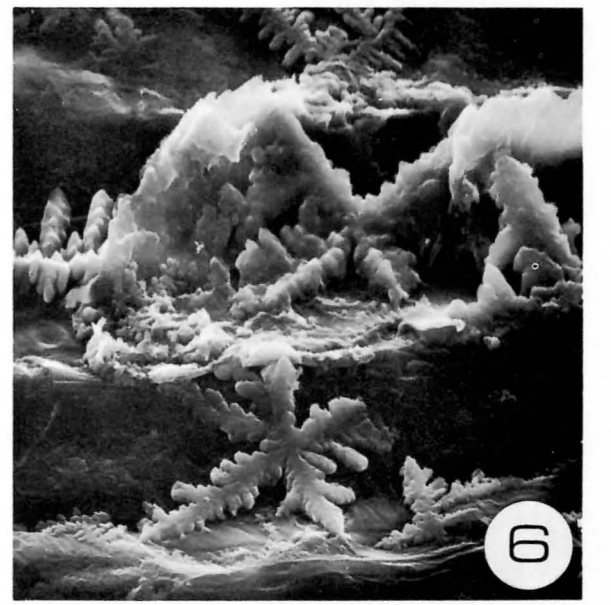
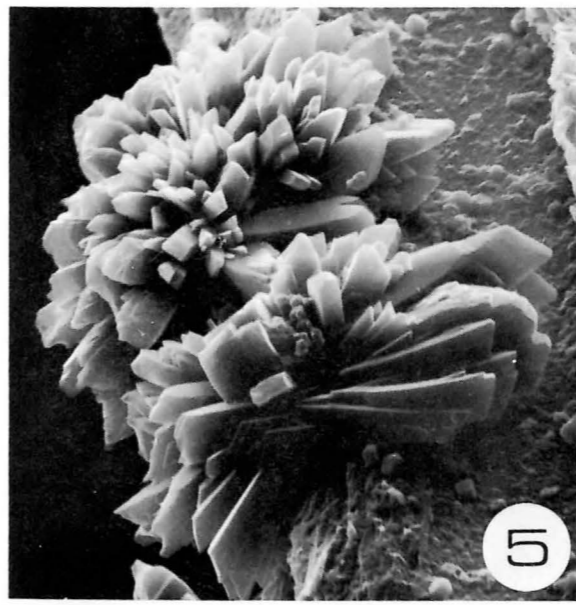
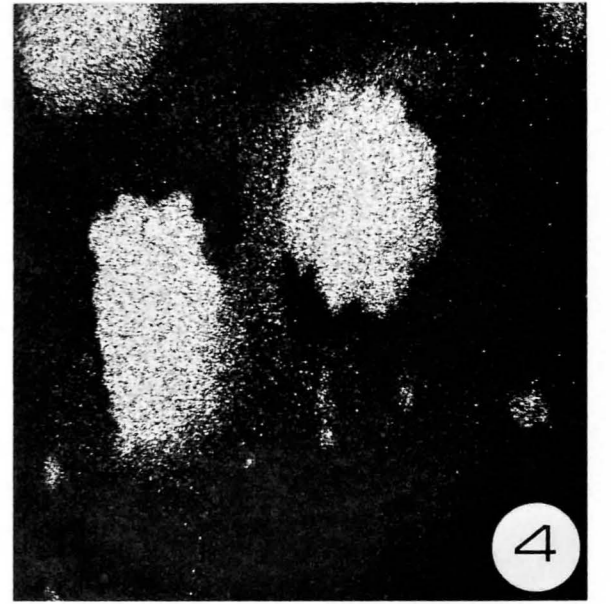
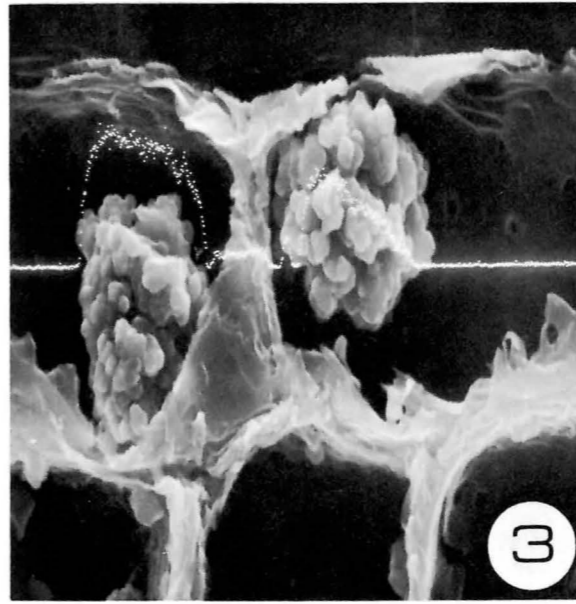
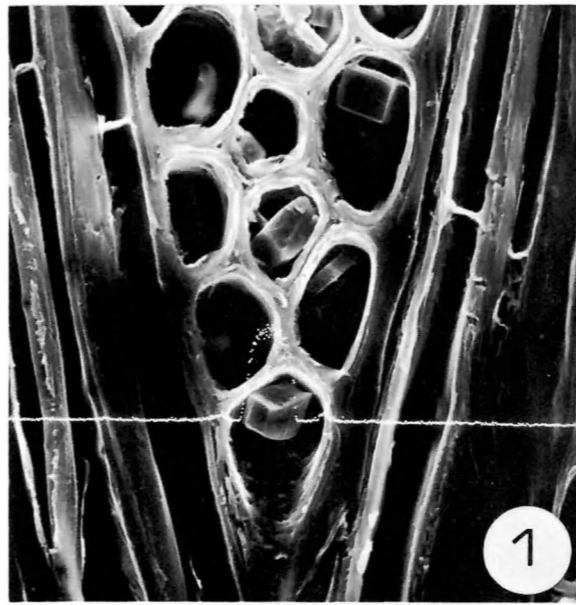
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FIGURES

- Figure 1. Scanning electron micrograph showing a line scan across a tangential surface of *Vitex payson* (Lour.) Merr. (TEVw 18733). Note the peak rising from the base line as a crystal is encountered indicating the presence of calcium in this particular case. Carbon coated. 1000X.
- Figure 2. Element map showing distribution of calcium in the same specimen as in Figure 1. Note high concentration of this element where crystals are located. Carbon coated.
- Figure 3. Scanning electron micrograph showing a line scan across a radial surface of *Vitex berteroana* Pittier (SJRw 33755). Note two peaks rising from the base line as the crystals are encountered, indicating in this case the presence of silica or "silica sand". Carbon coated. 1800X.

- Figure 4. Element map showing distribution of silica in the same specimen as in Figure 3. Note high concentrations of this element where "silica sand" is located and presence of silica not visible in Figure 3. Carbon coated.
- Figure 5. Scanning electron micrograph showing detail of a druse type crystal in *Schoutenia ovata* Korth (BWCw 15884). Carbon and gold/palladium coated. 2500X.
- Figure 6. Scanning electron micrograph showing unusual crystal formation in ray parenchyma cells of *Vitex masoniana* Pittier (BWCw 18757). A point analysis of these crystals identified them as sodium chloride. Carbon and gold/palladium coated. 1700X.



An Improved Solvent-Extraction Apparatus for Preparing
Direct Carbon Replicas

By

R. W. Meyer¹

Synopsis: A useful apparatus for removing backing material during preparation of replicas for transmission electron microscopy.

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One critical stage in preparation of direct carbon replicas, using the method of Côté *et al.* (1964), is the removal of the paraffin backing layer previously placed on a replica to protect it during dissolution of the substrate being replicated. The paraffin-removal operation is most efficiently done using a continuously refluxing solvent-extraction apparatus.

Solvent extractors previously used by the author, and interim extractors constructed prior to the model described here, were generally unsatisfactory in design. Undesirable features were considered when the following design criteria for the improved extractor were formulated.

- a) The grid-carrying basket should not be an integral part of the stopper because strain from the heavy cooling-water rubber tubing may break the glass tubing, or the joint where the glass tubing enters the stopper, should the rubber tubing catch on something during handling of the stopper. Also, replicas may be jarred from the basket, since the comparatively long stopper assembly is not easily placed in the extractor body when replacing the stopper.

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- b) An all-glass construction is desirable. Where metal is used to provide a cooling surface, its different thermal-expansion characteristics may break joints through which the metal enters the glass extractor, or the joint caulking material can be attacked by the solvents employed in the extractor.
- c) The cooled surface on which the grids are placed should be located in the region of greatest concentration of solvent vapors. This region appeared to be located just beneath the coldest surface in the extractor.
- d) The surface on which grids are placed should be slanted so condensed solvent flows away from the grids, carrying with it dissolved paraffin.
- e) The base of the mouth of the port providing access to the condensing surface should be about as high or higher than the actual condensing surface. A region of highly concentrated solvent vapors normally extends horizontally outward from this surface to the walls of the chamber. It was felt that solvent vapors flooding out of the extractor, when grids are being inserted or removed, would constitute an unnecessary safety hazard.

With these design criteria in mind, plans for an all-glass solvent-extraction apparatus were prepared and given to a local glass blower for construction.* The apparatus shown in the accompanying figures was then prepared and has been in use in the author's laboratory for several years with excellent results.

The body is formed of 45-mm-diameter glass tubing through which glass tubing for cooling water passes. Inside the body is a cooling-water

*The efforts of Arpad Takacs, owner of Vancouver Scientific Glassblowing, are greatly appreciated.

loop formed of 6-mm tubing which is connected to 8-mm tubing nipples outside the body. A basket formed below the cooling loop, made of a 3-mm-diameter glass rod, is constructed at a slight angle to permit easy access of a filter paper on which grids supporting paraffin-backed replicas are placed. The basket measures approximately 23 by 25 mm. The 34/28 standard-taper ground-glass joint stopper is large enough to permit easy access of the grid-carrying filter paper. The access tubing is placed at an angle, so that condensed solvent flows away from the boiler body when the stopper is removed. This angle also raises the mouth of the access port, so that the region of greatest concentration of vapors generated by the cold surfaces on which they condense is not so high that vapors flood out of the boiler when the stopper is removed. The basket is tilted to ensure that condensed solvent runs down the filter paper, carrying dissolved paraffin away from the grids. The top condenser is placed at an angle, using an adapter bent 45°, to prevent dust from settling into the system. Water circulation is through the cold finger and then through the condenser (Fig. 1).

The several views of the extraction apparatus illustrate construction details sufficiently well so a similar apparatus can be constructed. More information and additional dimensions are available from the author.

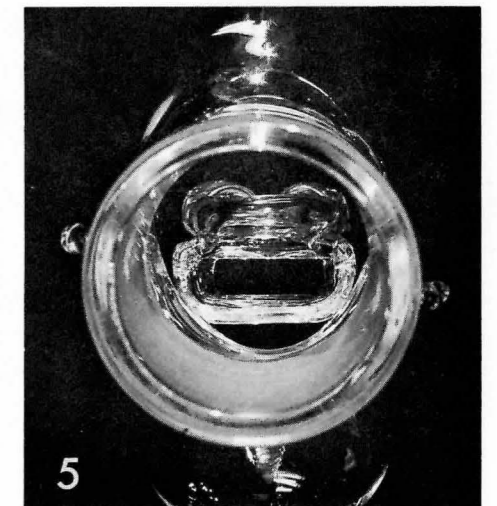
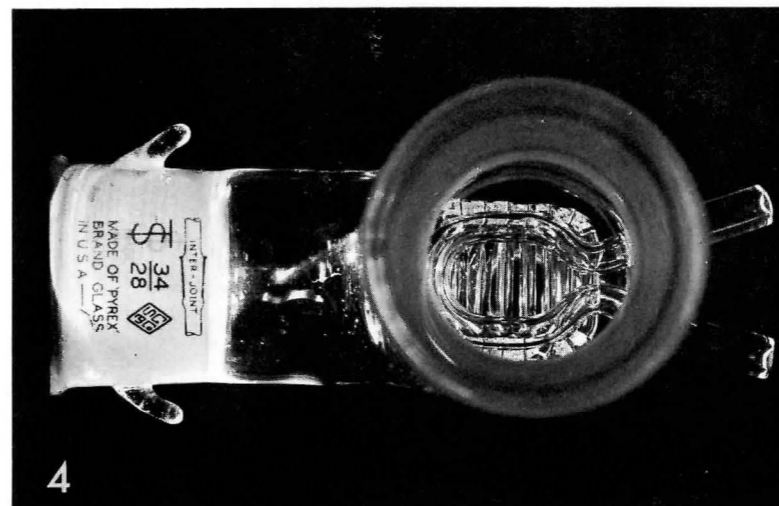
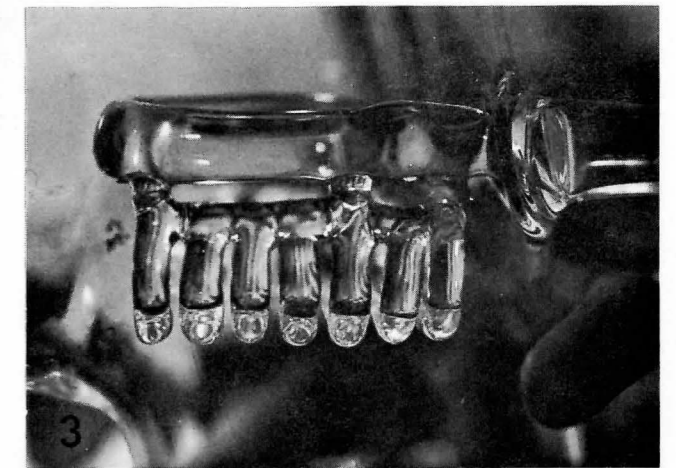
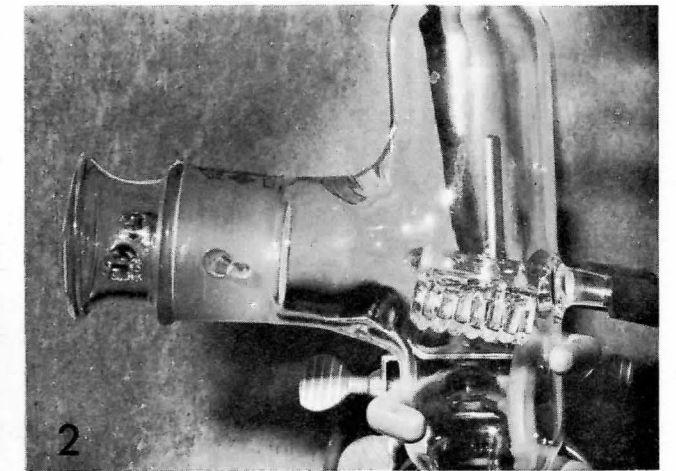
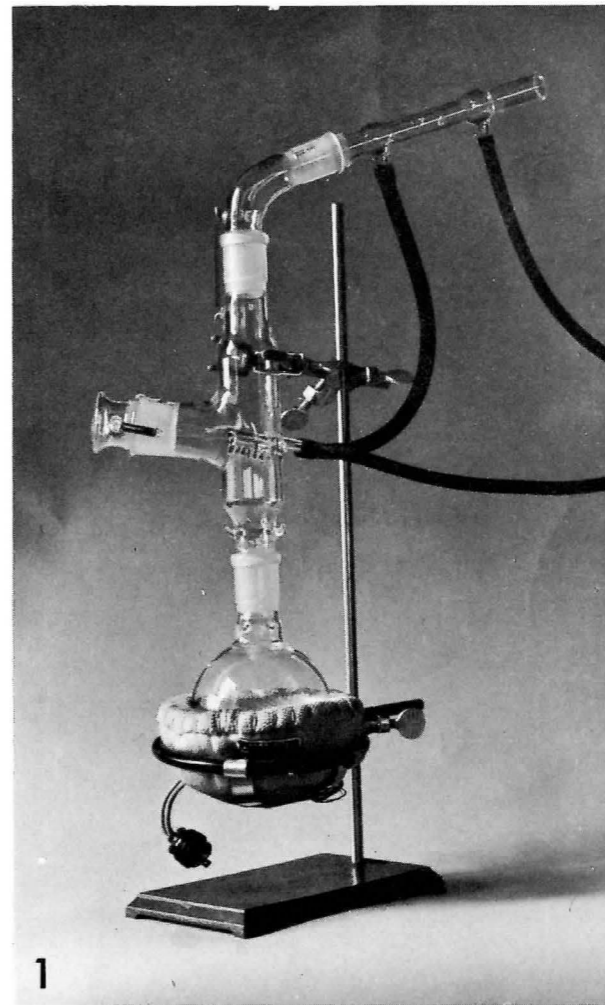
Operation of the solvent extractor is simple. Grids are placed on a piece of filter paper and inserted into the basket. There are no tubing connections to handle or awkward cold fingers to maneuver. The stopper is held in place with springs to prevent its being dislodged should the solvent bump during operation. Bumping is greatly reduced by using boiling stones. Fast reflux rates are possible, washing the grids in a minimum of time. Replicas are placed in the extractor while solvent is condensing on the cold basket. Actual time for washing is determined by cooling water temperature, wax composition and thickness of the wax layer being removed. Our experience, using Ladd microcrystalline wax and fairly cold (7°C) water, is that no more than 15 minutes are required to dissolve all wax with benzene.

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FIGURES

- Figure 1. General side view of apparatus.
- Figure 2. Side view, showing angle of basket and access port in relation to body of apparatus.
- Figure 3. Side view of basket showing detail.
- Figure 4. Top view through ground-glass joint, showing configuration of cooling loop.
- Figure 5. Front view through access port, showing basket suspended beneath cooling-water loop.



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WOOD ANATOMY ACTIVITIES AROUND THE WORLD

The Netherlands -- Request for Wood Samples and Cooperation

The wood anatomy of the Rhizophoraceae (including Anisophyllaceae) will be the subject of an extensive study by Mr. G. J. C. M. Van Vliet in the near future. Reliably identified wood samples, preferably with herbarium

vouchers, of as many genera and species as can be obtained are needed to make the effort a worthwhile one, because a general survey of the wood anatomy of the family is already available (cf. Metcalfe and Chalk's *Anatomy of the Dicotyledons*). Special attention will be paid to ecological aspects (mangrove - inland representative, etc.) and accompanying collecting data will therefore be highly appreciated. The availability of several specimens per species will also greatly increase the value of any taxonomic or ecological conclusion. Curators of wood collections are therefore kindly requested to supply us with sectioning blocks of their Rhizophoraceous wood samples. Duplicate slides will of course be sent after completion of this study.

The wood anatomical study of the Rhizophoraceae follows a detailed anatomical analysis of the Crypteroniaceae, and it is intended to cover more families of the Myrtales in the future, in order to arrive at a synthesis on the anatomical affinities within this interesting order of Dicots. No doubt other colleagues will be involved in scattered members belonging to this order, or even intend to cover a whole family (cf. Dr. de Zeeuw's current work on Lecythidaceae). We would like to be aware of any wood anatomical activities in the families Lythraceae, Combretaceae, Sonneratiaceae, Punicaceae, Myrtaceae, Melastomaceae, Oliniaceae, Penaeaceae and Onagraceae in order to avoid unnecessary duplication and if possible to arrive at some form of collaboration in the future.

Wood samples and any reactions to this intended project should be addressed to: P. Baas, Rijksherbarium, Schelpenkade 6, Leiden, Holland.

England -- IAWA/Linnean Society of London Joint Meeting

A joint meeting has been arranged between the Plant Anatomy Group of the Linnean Society of London and the International Association of Wood Anatomists for 26-27 June 1975.

There will be a number of invited papers but offered papers will be considered on anatomical/taxonomic subjects and other technological aspects of plant anatomy. About half of the program will be devoted to aspects of

secondary xylem. Suitable papers will be selected to form a balanced program. The main contributions will be of 30 minutes with time allocated for brief 10 minute notes.

The Linnean Society, like IAWA, is unable to provide money for travel, but it is hoped that if people are already going to the Botanical Congress at Leningrad, they will be able to break their journey to attend this symposium.

The meeting will be held at the rooms of the Linnean Society, Burlington House, Piccadilly, London. Fuller information will be given later.

If you have a paper or note to offer, or would like further details, please write to Dr. David F. Cutler, Jodrell Laboratory, Royal Botanic Gardens, Kew, Richmond, Surrey, England.

U.S.S.R. -- XII International Botanical Congress (1975)

As this issue of the IAWA Bulletin goes into the mails, your committee for the XII International Botanical Congress is actively exchanging correspondence in an attempt to finalize the program for the technical sessions. Dr. W. C. Dickison, Chairman, has indicated that five sessions can be planned for with the following tentative titles:

- Session 1. Wood anatomy and the environment
- Session 2. Taxonomic and evolutionary value of wood anatomy
- Session 3. Cambium and differentiation
- Session 4. Ultrastructure of woody tissues
- Session 5. Living elements in wood

Chairmen for the sessions are being selected and will be announced with a tentative listing of papers to be presented. It is hoped that the 1974/4 issue of the Bulletin will carry this information. Most of the titles offered by members and non-members will be accommodated in these sessions, but there are a few which do not fit the subject matter titles very appropriately. The committee is attempting to justify these points before the program is published.

In addition to the five technical sessions, there will likely be a sixth session which could be used for an invited lecture, for informal presentations, or for brief reports. This session would terminate following a business meeting of the Association.

Professor W. Liese and Professor Yatsenko-Khmelevsky, the other two members of the Congress committee, have offered some of the above suggestions. Members wishing to participate in the program or desiring to offer ideas for the program are requested to contact any one of the three committee members directly. Their mailing addresses are listed in the Membership Directory.