



*Alternative
Leaf Clearing
and Mounting
Procedures*

Walter K. Buechler
2004

Introduction and update on "Alternative Leaf Clearing and Mounting Procedures"

The brochure "Alternative Leaf Clearing and Mounting Procedures" was written in 2004 and used as a hand out at a talk given at the 2004 BSA meeting in Snowbird, Utah. It has since been available on request for free. As I am now running out of stock I am taking the opportunity to post the brochure in its original form on the INTERNET. The text is now more than six years old and some references to web pages, companies, and products may not be valid anymore. The reader will have to check these references and in some cases find alternative solutions.

Most of my early specimens are still in good condition. Probably due to insufficient pressing before framing, I did have some leakage problems with Cargill B immersion oil specimens. To overcome the problem I designed an aluminum frame with a polyurethane foam gasket that can be used with the low viscosity Cargille A immersion oil. Specimens made with the new frame have now been tight for the past two years. Because of the relatively high cost of these frames I usually reserve them for very precious, organically preserved fossil leaf remains which are too fragile to be mounted in high viscosity Cargille B or cedar wood oil. Because of the thin foil and the optical properties of the oil, specimens can be examined under the microscope at magnifications up to 400 x. If necessary the frame can easily be opened and the specimen removed or re-arranged. The design of the new frame is shown in three attachments following Annex J of the original brochure.

Over the past few years I have done most of my preparations in cedar wood oil and acetate foil because it is the least time consuming procedure and the specimens will last for at least 6-10 years. As most of the leaves are used for a very specific purpose, I take high resolution pictures with a camera or scanner and, if necessary, with the microscope and store them as TIFF files. These files can be used for publications and be easily transformed into other file formats.

8/20/2010, Walter Buechler

1192 E. Braemere Rd., Boise, Idaho 83702
e-mail: buecdoug@msn.com

Alternative Leaf Clearing and Mounting Procedures

(Summary of a talk given at the 2004 BSA meeting in Snowbird, Utah)
Walter K. Buechler *

Abstract

Chemically cleared leaves are an important tool for identifying fossil leaf remains. Collections with thousands of specimens are at the Yale Peabody Museum, the Smithsonian Institution and the University of California, Museum of Paleontology. Some of the older specimens are degrading due to oxidation of the mounting medium Permout. Traditional clearing methods use potentially dangerous chemicals (e.g. toluene) and a controlled substance (chloral hydrate). The purpose of this study was to find alternative clearing and mounting procedures that would result in long-lasting, archival-quality specimens. Transparency of the final specimen depends on closely similar refractive indexes of cell wall cellulose ($r = 1.47$) and the mounting medium. Toluene-based Permout can be replaced by cedar oil or synthetic immersion oil, making the use of toluene for clearing and staining unnecessary. To prevent the oil from leaking, glass plates are replaced by thin, flexible acetate or Teflon foils. Cedar oil mounted specimens are self-sealing, because the highly volatile components of the oil evaporate from the margin area and the residual fraction glues the foils together. Because synthetic immersion oil does not evaporate, such specimens have to be sealed with a pressboard frame. Cedar oil mounted acetate foil specimens show no signs of degradation after four years. Immersion oil mounted Teflon foil specimens have been used for about one year and have not leaked or otherwise changed during that time. Based on general experience with synthetic immersion oil and Teflon, and from specifications by manufacturers, these specimens may have archival quality and a lifetime of potentially more than 30 years. Degrading Permout specimens can be dismantled in xylene and re-mounted in immersion oil between Teflon foils. The immersion oil mounting method can also be used for well-preserved fossil leaf compressions.

Introduction

When I started clearing leaves in August 2000, I ran into several problems. I realized that some of the chemicals are not easily available. Some are potentially dangerous or otherwise unpleasant to use. Until 20 to 30 years ago, glass plates for mounting were taken from stocks of used lantern slides and photographic plates. Today those stocks are long gone, and thin, pre-cut glass plates are difficult to find. Some of the older specimens are now degrading due to yellowing or oxidation of the mounting medium Permout. In U.S. museums, hundreds of specimens are probably already in a critical state and thousands may follow in the future. These problems were reason enough to search for alternative clearing and mounting procedures that minimize the use of obnoxious chemicals, substitute for the mounting medium Permout, and hopefully result in long-lasting, archival-quality specimens.

Dilcher (1974) reviewed the history of leaf preparation methods and listed many of the clearing and mounting procedures used in the past. I started my own work with the standard techniques used at the University of California Museum of Paleontology (Annex A) and at the Smithsonian Institution (Annex B), and modified them step by step. Some of the potentially dangerous chemicals were substituted for by less obnoxious ones and the problematic Permout replaced by cedar oil or immersion oil. Using a liquid for mounting instead of a solid introduced a new problem, leakage. Glass plates could no longer be used, because breaking glass and leakage would have disastrous consequences in a museum collection. The leakage problem was solved by using flexible plastic foils made of cellulose acetate or transparent Teflon. Most of my work was done with leaves of the genus *Salix*, a taxon that may be easier to process than others. Leaves of evergreen species and succulent plants may require longer processing times than

* 1192 E. Braemere Rd., Boise, Idaho 83702; Tel. + fax: 208-345-0933;
E-mail: buecdoug@msn.com

those given here. The life span of archival specimens is hard to predict. If possible I am using materials that have been known for many decades and of which detailed specifications are available from manufacturers. I also prefer procedures that allow easy restoration of specimens if detrimental effects should become obvious at a later time.

Leaf Processing Procedures

Introduction

I started my work with standard procedures as described in Annex A and B. Table 1 compares in a schematic way chemicals used in traditional methods with their less obnoxious or more easily available replacements. Highlighted are substances that I consider most questionable in one way or the other. Detailed descriptions of alternative clearing, staining and mounting procedures are given in the respective chapters below. Material specification and safety information are given in chapter **Materials**.

Two alternative mounting methods are described:

1) Mounting in **cedar oil** is more convenient and avoids the use of xylene. Specimens do not need to be framed because the alcohol-like fractions of the oil evaporate from the margins and the residual fraction sticks the foils together. My first cedar oil specimen is now 4 years old and shows no signs of change or degradation. Specimens are lightweight, unbreakable and may be viewed under a light microscope at magnifications of up to 400. The foils may be separated with a razor blade and the leaf used for other examinations.

2) Mounting in **immersion oil** produces potentially more durable specimens. Because immersion oil does not evaporate, specimens have to be framed to keep the foils from slipping apart. Its honey-like viscosity prevents it from leaking. Because immersion oil does not mix with alcohol, xylene has to be used as an intermediary.

Both acetate foil and Teflon foil may be used with cedar oil or immersion oil. Because Teflon is mechanically less stable, I recommend using it only with a frame.

Table 1: Comparison of chemicals used in traditional and alternative procedures (highlighted are potentially dangerous substances).

	Traditional	Alternative	
Clearing	Sodium hydroxide Sodium hypochlorite Chloral hydrate Alcohol Toluene	Household lye (contains sodium hydroxide) Household bleach (contains sodium hypochlorite) not used Alcohol not used	
Staining	Safranin O (ethanol / toluene) Alcohol Toluene	Safranin O (ethanol) Alcohol not used	Safranin O (ethanol) Alcohol Xylene
Mounting	Permout (toluene)	Cedar oil	Immersion oil

Clearing (for all staining and mounting procedures)

Both, fresh or dried plant materials may be used for clearing. Leaves taken in late fall often have fungal spots that stain bright red and may obscure the venation.

- 1) Epidermal wax is removed in denatured alcohol (shellac thinner) for 1 – 2 hours. Adding 10% acetone speeds the process and helps prepare the leaf for the next step. Leaves may be stored in alcohol for weeks or month without degradation.
- 2) Soak leaf at room temperature in a solution of 5 gram Red Devil Lye in 100 cm³ water for 3 – 6 hours or until translucent. Succulent leaves and leaves from evergreen species may take significantly longer.
- 3) Rinse several times in H₂O and neutralize in 10% acetic acid for 15 – 30 min.
- 4) Clear in household bleach (3% or 5.25% sodium hypochlorite) at room temperature for 30 – 60 min. or until leaf is sufficiently cleared. Wash several times in H₂O.
- 5) Dehydrate in 70% isopropanol (rubbing alcohol) and then in 100% ethanol. Leaves may be stored in 100% ethanol indefinitely.

Staining for cedar oil mounting

- 1) Leaves are stained in a solution of 0.5 gram Safranin O in 100 cm³ ethanol (3 –10 minutes; do not over-stain!).
- 2) Wash several times in 70% isopropanol (rubbing alcohol) to remove unbound Safranin from the tissue. The last rinse should be in 100% ethanol.
- 3) Put leaf in cedar oil until absolutely clear. This may take several hours or a day. Final clearing is due to the similar refractive indexes of cedar oil and cell wall material (cellulose, $n = \text{approx. } 1.47$, see table of refractive indexes in Annex C). Leaves may be stored indefinitely in cedar oil. Because of evaporation, cedar oil should always be stored in a closed container. Evaporation would alter the chemical composition of the oil.

Staining for immersion oil mounting

- 1) Leaves are stained in a solution of 0.5 gram Safranin O in 100 cm³ ethanol (3 –10 minutes; do not over-stain!).
- 2) Wash several times in 70% isopropanol to remove unbound Safranin from the tissue. The last rinse should be in 100% ethanol.
- 3) Leaves are soaked in xylene until absolutely clear. This may take 4 –12 hours. Transfer quickly into immersion oil Cargille Type B. Cover leaf immediately with about 5 mm of oil so that no air enters the tissue. Set the oil bath on a slide warmer at 40 - 50°C. Let stand open for 3 days, so that xylene can evaporate (synthetic immersion oil does not evaporate!). Residues of xylene would lower oil viscosity which could potentially lead to oil leakage. Leaves may be stored indefinitely in immersion oil. At room temperature (20°C) 8 days should be allowed for xylene to evaporate.

Mounting in cedar oil (sample specimen in Annex D)

- 1) Specimens are sandwiched between two sheets of precut acetate or Teflon foil.
- 2) Place 4-5 pieces of white paper on a hard, flat surface. Lay the sandwiched foils on top. Fold soft paper towels (10 - 12 layers) to a size slightly larger than the foils and lay it on top of the foils. Cover with a flat board and weigh it down with 12 - 15 kg of books. This will squeeze out surplus oil within one or two hours without crushing the leaf.
- 3) Cut a cardboard frame that covers the margin area and relieves the leaf completely from pressure. Set it on the sandwich and weigh it down with 20 –25 kg of books. This will squeeze out more oil from the margin area.
- 4) Clean both sides of the sandwich with an alcohol-wetted cotton ball (or cotton “square”). Any stronger solvent (e.g. acetone) might attack the acetate foils. The two foils stick together without further attachment. The margins may leak small amounts of oil for a few days but this

can be wiped off with a paper towel. The alcohol-like fractions of the cedar oil evaporate from the edges, and the remaining fraction glues the foils together. I am storing my specimens between sheets of printer paper until leaking has stopped.

For operations 2) and 3) (squeezing), I am using a press made of two wooden boards. Four guiding rods near the four corners prevent the boards (and the foils) from sliding apart.

Scanning cedar oil specimens (see Annex E)

If no permanent specimen is needed, non-mounted cedar oil specimens can be scanned directly on a flatbed scanner. For preparation of specimens, follow steps under section **Staining for cedar oil mounting**.

- 1) Put some cedar oil directly on the glass plate of the scanner.
- 2) Lay the cleared leaf in the puddle of oil and cover with acetate foil. Squeeze out air bubbles and surplus oil.
- 3) Install a white, glossy piece of paper about 5 mm above the specimen (this softens shadows from leaf venation).
- 4) After scanning, cedar oil is wiped off and the glass cleaned with an alcohol wetted paper towel.

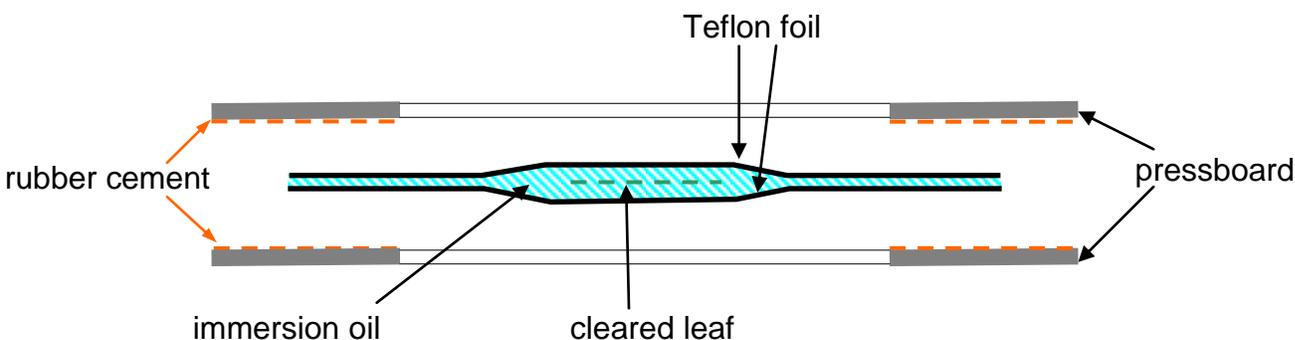
Putting the specimen directly on the scanner glass, results in an optimal resolution. Magnifications of up to 10x are possible. Instead of a white paper, an electroluminiscent light table can be used. Best results are achieved with a through-light flatbed scanner.

Mounting in immersion oil (sample specimen in Annex F)

- 1) Cut two identical frames out of pressboard or illustration board or use precut frames (see section **Materials**). The frame opening should be at least 40 mm wider and longer than the cleared leaf. The frame width should be about 25 mm (30 mm for frames approaching letter size). It is important to choose pressed and highly glued material because leaking oil would penetrate ordinary cardboard. Leaves with a thick blade or a thick petiole should get a larger frame to allow a gradual transition from the thick part of the specimen to the margin. A 1.2 mm thick object should be placed not closer than 35 mm to the foil margin.
- 2) Cut two pieces of Teflon foil so that they cover half of the frame width on all four sides. Lift the leaf out of the oil and let surplus oil drip off. Lay and position the leaf on one of the foils. Add fresh, uncontaminated oil (with no xylene residues) with an eyedropper and cover with the second foil. Previously xylene-contaminated oil can be stored in a wide-open cup and reused for step 3 of **Staining for immersion oil mounting**. To avoid fingerprints on the Teflon foils (they are the only objects that stick well on Teflon) I recommend wearing disposable rubber or plastic gloves. Wiping the Teflon with a xylene wetted cotton square before applying the oil reduces the formation of air bubbles in the sandwich.
- 3) Place 4-5 pieces of white paper on a hard, flat surface. Lay the sandwiched foils on top. Fold soft paper towels (10 - 12 layers) to a size slightly larger than the foils and lay it on top of the foils. Cover with a flat board and weigh it down with books. I am using 25 – 30 kg of weight for an area of 20 x 20 cm. For very delicate leaves the weight may have to be applied gradually. This will squeeze out surplus oil within one or two hours without crushing the leaf.
- 4) Cut a cardboard frame that covers the margin area and relieves the leaf completely from pressure. Set it on the sandwich and weigh it down with 20 –25 kg of books. This will squeeze out more oil from the margin area.
- 5) Clean both sides of the sandwich with an alcohol wetted soft tissue (avoid too much pressure, because Teflon may easily be scratched).
- 6) Paint the inner sides of both frames (the whole width) with rubber cement and let it dry. Assemble all parts as shown below and press the two frames hard together. Leaking oil (if

there is any) will be caught between the frames and not leak outside. Immersion oil does not weaken the cement bond between the frames.

Steps 3) and 4) are very important. They determine whether the specimen will have the tendency to leak or draw air bubbles in. If done correctly, the top Teflon foil is slightly deformed and superficially adapted to the profile of the leaf, thus minimizing the amount of oil and the distance between the foils. In the margin area the oil film is probably less than 1/100 mm thick. The high viscosity of the oil and the small cross section prevent the oil from moving in or out of the sandwich. Even without a frame, such a specimen would be stable for months or possibly years and not leak or draw air bubbles. For steps 3) and 4) (squeezing), I am using a press made of two wooden boards. Four guiding rods near the four corners prevent the boards (and the foils) from sliding apart.



Remounting procedure for Permout / glass mounted specimens (Annex Ga and Gb)

- 1) Degrading Permout / glass mounted specimens are soaked in xylene until the top glass can be taken off without force. This may take between one to several days. I have been using aluminum "loaf pans" (that come in different sizes) as containers.
- 2) After the top glass has been removed, the leaves are scooped out with curved-tip forceps or a table fork and put on a new (clean) glass plate. It is advisable not to squeeze the leaves with the forceps, but to use the forceps as a support only. The leaves are then placed on a piece of aluminum netting (screen door netting) and covered with immersion oil using an eyedropper. Proceed as indicated in section **Mounting fossil plant material**, steps 5 and 6.

Sealing Permout specimens with aluminum tape (Annex H)

- 1) Cut Nashua 322 self sticking aluminum tape to a width of about 12.5 mm (1/2").
- 2) Clean edges of specimen with alcohol. Let dry.
- 3) Apply the tape to the edges, so that it covers about 4.5 mm on each side of the specimen. Press on firmly, but be careful not to let the glass edges cut the tape. The tapes should overlap in the four corners of the specimen.

Sealing with aluminum tape makes probably a more effective gas barrier than shellac (see below). The tape is easy to apply and produces, at least at the beginning, a very strong and tight bond with the glass. The life span of this bond is hard to predict (no archival property specifications are available). The method would, however, allow easy restoring of specimens if detrimental effects should become obvious at a later time. Mildly degraded Permout specimens may be washed with xylene to remove oxidized Permout and replenished with fresh Permout prior to sealing.

Sealing Permout specimens with shellac (Annex H)

- 1) Dissolve Hock Dewaxed Shellac Flakes in Hock Water Free Shellac Solvent until the solution has the viscosity of thin honey. Pour about 4 mm of the solution in a leveled flat-bottom container.
- 2) Clean edges of specimen with alcohol. Let dry.
- 3) Dip the specimen with one side vertically into the solution, withdraw, let surplus liquid drip off, and hang the specimen (vertically) to dry.
- 4) Repeat procedure with all four sides.
- 5) Repeat steps 3) and 4) one to two times.

Sealing with shellac provides an effective gas barrier of archival quality. The method is, however, more complicated and more prone to “accidents” than the aluminum tape method.

Mounting fossil plant material (Annex I)

Both cedar oil and immersion oil can be used to mount well-preserved fossil leaf compressions. Fossil material used during this study is from the Middle Miocene *Clarkia* deposit in northern Idaho and was generously provided by William C. Rember, University of Idaho, Dept. of Geological Sciences, Moscow, Idaho. It had previously been “lifted” from the matrix with hydrofluoric acid, washed in water, partially bleached with hydrogen peroxide and sodium bicarbonate, and temporarily stored in glycerin between acetate foils. For staining and mounting I have used the following procedure:

- 1) Specimens are placed in water until the acetate foils can be separated.
- 2) The leaves are transferred onto a piece of fine aluminum netting (screen door netting) and dehydrated in 70% isopropanol and 100% ethanol. Leaves stay on the same piece of netting until the first part of step 5 is completed.
- 3) If necessary, stain in Safranin O (see section **Staining for immersion oil mounting**, steps 1 and 2)
- 4) Leaves are soaked in xylene until clear. This may take 4 –12 hours.
- 5) Lift netting and leaf out of xylene and cover the upper side of the leaf with immersion oil Cargille Type B (with eyedropper). Flip (invert) netting and leaf, and place them on one of the precut Teflon foils. With an eyedropper, add immersion oil to the leaf through the netting. Remove netting. Remove surplus oil (that is not in contact with the leaf). Let stand open for 3 days at room temperature (or 1 day at about +50°C), so that xylene can evaporate (synthetic immersion oil does not evaporate!). Residues of xylene would lower oil viscosity, which could potentially lead to oil leakage.
- 6) Proceed as indicated in section **Mounting in immersion oil**. To prevent specimens from being torn apart by the flowing oil during pressing, the weight (step 3) should be applied gradually, beginning with a few 100 g every 15 minutes and proceeding to steps of about 1 kg up to a total weight of about 8 kg.

Cedar oil versus immersion oil mounting

Both mounting methods have advantages and disadvantages. Because of its simplicity, I recommend the cedar oil / acetate foil method for projects that require only up to about 5 years of storage time or projects where specimens need to be temporarily mounted until they can be scanned or otherwise further processed. The lifetime of cedar oil / acetate foil specimens may potentially be limited by: 1) evaporation of the volatile fractions of the oil from the edges or their diffusion through the foil, and 2) detrimental effects of UV light and oxygen on the foil material. Using Teflon instead of acetate foil would solve the UV problem at the cost of a reduced

mechanical stability of the specimen. Using thicker (Teflon) foils, which would reduce evaporation rate, could extend the lifetime of a cedar oil specimen, but would further reduce mechanical stability.

I recommend the immersion oil / Teflon method for all projects aiming at archival quality specimens. The disadvantages (time consuming processing, use of xylene) can partially be overcome by having the frames prefabricated (see section **Precut Mat Frames** in chapter **Materials**) in a few standard sizes, having Teflon foils precut to standard sizes, investing in tools like a press for squeezing surplus oil out of the sandwich, and installing a hood for the xylene work.

Lifetime of all foil type specimens can be optimized by avoiding high storage temperatures and by storing them in a horizontal position.

Materials

Household lye

Instead of chemically pure sodium hydroxide [NaOH], I am using "Lewis Red Devil Lye", a household drain opener. According to the manufacturer, it "contains sodium hydroxide" (probably in a high concentration). Lye is strongly alkaline and potentially dangerous if not handled properly. Eyes should be protected against lye dust and splashes of the solution. I am dissolving 5 grams Red Devil Lye in 100 ml water, a solution that can be stored indefinitely in a glass or polyethylene bottle. Both sodium hydroxide and household lye are equally suitable for clearing purposes.

Household bleach

Household bleach is available as a 3% or 5.25% solution of sodium hypochlorite [NaHClO₂] in water. It doesn't pose a real safety problem because everybody is used to handling it with appropriate care. I tried to replace it with 5% hydrogen peroxide but found that clearing was not sufficient.

Chloral hydrate [CCl₃CH(OH)₂]

Dilcher (1974) listed several clearing methods using a super saturated (250%) solution of chloral hydrate in water. Clearing probably depends on similar refractive indexes of the chloral hydrate solution and cell wall cellulose. This effect is, however, lost after dehydration. I did not notice a lasting positive effect of the chloral hydrate treatment on the final specimen. Chloral hydrate is a controlled substance and only available on prescription (it is used as sedative and for short-term treatment of insomnia).

Toluene and Xylene

Toluene and Xylene are non-polar, aromatic hydrocarbon solvents. They mix with and dissolve oily substances (including synthetic immersion oil) but do not mix with water or alcohol. Vapors have an unpleasant smell and may induce headaches or dizziness. There is inadequate data available to evaluate the risk of developing cancer from exposure to toluene or xylene. Both solvents, however, contain trace amounts of benzene, a chemical known to cause cancer. Because of its lower evaporation rate, exposure to xylene may be less dangerous than exposure to toluene. I am using paint-thinner or brush-cleaner quality xylene from Home Depot for clearing and mounting purposes.

Material safety information on toluene and xylene can be found at:

<http://www.sunnysidecorp.com/MSDS/pdf/msds821.pdf> (toluene)

<http://www.sunnysidecorp.com/MSDS/pdf/msds822.pdf> (xylene)

Safranin O

Safranin O is a histological stain that stains lignified cells red. In cleared leaves the venation appears darker red than the surrounding tissue. Safranin is soluble in water and alcohol. It is not soluble in 100% toluene or xylene. Staining of Permout specimens is traditionally done in an alcohol-toluene solution (50 / 50%). I was able to eliminate the toluene part because I am using toluene-free oils as mounting media instead of toluene-based Permout. I dissolve 0.5 gram Safranin O (dry) in 100 cm³ ethanol.

Material safety information can be found at:

<http://www.proscitech.com.au/catalogue/msds/c138.pdf>

Safranin O is available at Fisher Science Education (see address under cedar oil).

Permout

Permout is a mounting medium for microscopy. It contains a pinene resin, toluene as a solvent and 2,6 di-tert-butyl-p-creso as a plasticiser. Older versions of Permout tended to yellow with age. It is not clear whether the newer product is more archival. If not sealed properly, oxidation of Permout specimens will start at the edges and at slowly proceed inward. The leaf will be bleached and will finally deteriorate. Permout takes a long time to dry and it shrinks significantly. Specimens have to be inspected and replenished for several weeks to prevent the formation of air bubbles (Annex J).

Material safety information can be found at:

<http://www.proscitech.com.au/catalogue/msds/ia019.pdf>

Cedar oil

Cedar(wood) oil is extracted by steam distillation from wood of several members of the family Cupressaceae. Its composition is complex and varies depending on the source material. Alcohol-like fractions evaporate relatively fast, leaving behind a highly viscose residue. The advantage of cedar oil over synthetic immersion oil for cleared leaf mounting is that specimens are self-sealing and require no frame. Cedar oil is soluble in ethanol and other alcohols. No standards or guidelines have been set by NIOSH or the Occupational Safety and Health Administration (OSHA) for occupational exposure to or workplace allowable levels of cedar wood oil.

Information about chemical, physical and health related properties of cedarwood oil can be found at:

http://ntp-server.niehs.nih.gov/htdocs/Chem_Background/ExSumPdf/cedarwood_oil.pdf

Refractive indexes of several mounting media including cedarwood oil are given in Annex C.

Cedarwood oil can be ordered from:

Fisher Science Education
485 S. Frontage Road
Burr Ridge, IL 60521
Tel: 1-800-955-1177
Fax: 1-800-955-0740
<http://www.fisheredu.com>

Cedar wood oil can also be bought in health food stores. Eucalyptus oil seems to be equally suitable as a mounting medium.

Immersion oil

The first synthetic immersion oil was developed by Cargille Laboratories Inc. in 1940. Cargille Type B immersion oil does not evaporate and it is compatible with most solid materials (including cellulose acetate and Teflon). It is soluble in toluene and xylene but does not mix with alcohol. Despite its honey-like viscosity of 1250 centistokes, Type B immersion oil penetrates xylene-soaked plant tissue easily. Detailed specifications can be found at:

<http://www.cargille.com/type-b.html>

I ordered my immersion oil from:

Cargille Laboratories Inc.
55 Commerce Rd.
Cedar Grove, NJ 07009
Tel: 973-239-6633
Fax: 973-239-6096

<http://www.cargille.com/index.html>

Ordering information:

Immersion oil Cargille Type B (1250 centistokes (4 oz. \$10.00 plus shipping and handling)

Acetate foil

Acetate foil is available as overhead foil or laminating foil (I bought my material in an art supply store). Unfortunately, manufacturers do usually not specify the chemical identity of their products. The label “acetate” does not mean that something is really made of cellulose acetate. Before using so-called “acetate foil”, the material should be tested for compatibility with the mounting oil and alcohol. Acetate foil is mechanically stiffer than Teflon film of the same thickness and more scratch resistant.

Oil – foil compatibility test: Put a drop of mounting oil on a foil sample. For cedar oil, cover the oil drop with a 1 x 1 cm patch of foil to slow evaporation. After one day, one week and one month, blot the oil off with a soft tissue (don't rub!). Check for change in optical reflectivity of the foil surface at the place where the drop was (if necessary wipe the surface with an alcohol wetted, soft tissue beforehand). Check also for change in surface topography (if oil had diffused into the foil surface, the foil would have been slightly extended and a “bump” would be visible. This test is useful for all oil – foil combinations.

Teflon (Polytetrafluoroethylene, PTFE)

“Teflon” is the DuPont trademark for Polytetrafluoroethylene. It was discovered in 1938 at a DuPont Laboratory in New Jersey. The first products were sold commercially in 1946. The family of Teflon fluoropolymers from DuPont consists of PTFE (the original resin), FEP (introduced in 1960), Tefzel® ETFE (1970), and PFA (1972). Teflon is inert against most chemicals, UV light, and oxygen. After discussions with DuPont I used Teflon FEP and Teflon PFA film (both 0.005” thick) for mounting experiments. Both materials seem to be well suited for that purpose. To avoid fingerprints (they are the only thing that really sticks to Teflon!), I recommend wearing disposable rubber or plastic gloves.

Detailed specifications for transparent Teflon films can be found on web sites of CS Hyde Company, a retailer for DuPont products:

<http://www.cshyde.com/FEPspec.htm> (Teflon FEP)

<http://www.cshyde.com/PFAspec.htm> (Teflon PFA)

I ordered my Teflon films from:

American Durafilm Co. Inc.
55 Boynton Road, P.O. Box 6770
Holliston, MA 01746
Tel: 508-429-8000
Fax: 508-429-8500
Email: durafilm@aol.com
<http://www.americandurafilm.com/contact.html>

Ordering information:

Teflon FEP film type 500A, .005" thick x 24" wide (\$6.70/ft plus shipping and handling)
Teflon PFA film type 500LP, .005" thick x 24" wide (\$11.20/ft plus shipping and handling)

Pressboard

Pressboards and illustration boards described here are used as frame material for immersion oil specimens. They provide mechanical stability to the specimen and prevent the cover foils from slipping apart. Because immersion oil specimens can not be tightly sealed, the frame needs (for esthetical reasons) to be impermeable for small amounts of immersion oil that could potentially leak from the specimen. A drop of immersion oil put on a pressboard sample may creep along and into the surface, but should not appear on the other side after 2 –3 weeks.

I bought my pressboard (0.5 mm thick) more than 20 years ago at a herbarium supply store in Switzerland. I therefore assume that it is of archival quality but I have no record of it.

A wide variety of acid-free board materials can be found on the INTERNET. I did not attempt a thorough search for the best-suited material. Instead I choose two boards from the same manufacturer (Strathmore) and tested them:

Strathmore 500 Series Cold Press Illustration Board (0.064" thick) is made of 100% cotton fiber, acid-free paper. It is designed for artistic painting and drawing and is available in an off-white color. Because the material is softer and its surface more absorbent than heavy pressboard, I recommend using the 0.064" version for better stability and applying two layers of rubber cement instead of one. Frame halves made of illustration board can be sprayed with two to three layers of Bulls Eye Shellac (available in spray cans) on the outer side to harden their surface and to avoid fingerprints through handling.

Strathmore Museum Mounting Board is made of 100% cotton fibers, is acid-free and is buffered with calcium carbonate to guarantee longevity. The material is even softer than the Illustration Board mentioned above and its surface more absorbent, which makes the use of rubber cement difficult or impossible. Unless the gluing problem can somehow be solved, I do not recommend this material for frames.

Strathmore Boards (and other art supplies) can be bought from:

Dick Blick Art Materials
P.O. Box 1267
Galesburg, IL 61402-1267
Phone (800) 828-4548
Fax (800) 621-8293

http://www.dickblick.com/zz133/05/products.asp?param=0&ig_id=959

Precut Mat Frames

Precut, archival quality (cotton rag material) frames can be ordered in standard sizes or cut to customer requirements at:

Matcutter.com:

<http://www.matcutter.com/catalog.php?catid=14&styleid=1>

Ten standard size 8" x 10" frames (outside dimensions) are sold for \$18.00. Minimum quantity for custom sizes is 25 frames (per size). I coated the outer surfaces of the frame with two coats of Bulls Eye Shellac spray to harden the surface and to avoid soiling by fingerprints when handling the frame.

Rubber cement

Rubber cement is used to glue the two pressboard frame parts of immersion oil specimens together. Gluing has to be done with a dry glue because a liquid glue could be pressed between the two foils and appear as bubbles within the mounting medium. I am using Elmer's acid-free rubber cement, available at office supply stores. The Material Safety Data Sheet is at:

http://www.elmers.com/msds/me425_a.htm

Information about archival properties of rubber cement were not available from the manufacturer.

Aluminum tape

Adhesive-coated aluminum foil tape may be used to seal the edges of Permout-mounted glass-plate specimens against the influence of oxygen. General purpose aluminum tapes are usually 2 mils (0.05 mm) thick and are single coated with synthetic rubber adhesive. Two mils of aluminum provide a much better gas barrier than any non-metallic material (including shellac) of comparable thickness. General purpose aluminum foil tape is "specially designed for taping joints and seams against moisture and vapor on foil jacketing insulation and are also used in the automotive industry for general repair" (application note for Nashua foil tape). Nashua 322 tape is available at Home Depot. Adhesion to glass is excellent and the tape can easily be bent around sharp edges (glass) without breaking. The synthetic rubber adhesive is specified for a maximum temperature of 200°F, which suggests a relatively high life expectancy for the bond. Archival quality specifications are not available. Specifications for Nashua 322 foil tape are available at:

http://www.tycoadhesives.com/site/content/tape_search_item.asp?id=77

<http://www.tycoadhesives.com/site/pdf/DT322.pdf>

Shellac

Shellac is another possible sealant for Permout specimens. Shellac is a natural product secreted by *Laccifer lacca*, an insect living on some tree species in India and Thailand. The lac is scraped from the trees, crushed, sieved, washed and finally melted. Shellac has been used for several thousand years for dyeing and protecting wood. Today its main application is in wood finishing and wood sealer products. The first gramophone records were made from shellac. Shellac is durable, non-toxic, resistant to UV, and bonds strongly with glass and other smooth surfaces. It dissolves easily in alcohol. More information about historical and modern uses of shellac can be found at:

<http://www.vegansociety.com/html/animals/exploitation/shellac.php>

I got my shellac from:

Woodcraft Supply Corp. at:

<http://shop.woodcraft.com/Woodcraft/assets/html/homepage.asp?URLCheck=1>

Ordering information:

HOCK Dewaxed Shellac Flakes

HOCK Water Free Shellac Solvent (99% denatured alcohol)

A ready-made shellac solution (Zinsser Bulls Eye Shellac Sealer & Finish, clear) is available in spray cans at Home Depot and Woodcraft Supply Corp.

Acknowledgments

I wish to thank the following people for their help:

Amanda Ash, Smithsonian Institution, Washington DC, for information on clearing and mounting procedures used at the Smithsonian Institution.

Diane Erwin, University of California Museum of Paleontology, Berkeley, CA, for information on UCMP cleared leaf collections and mounting procedures, for providing Permout specimens for remounting experiments, and for numerous discussions.

Elizabeth Hermsen, Plant Biology Dept., Cornell University, for feedback on the cedar oil clearing and mounting method and for numerous discussions.

William C. Rember, University of Idaho, Dept. of Geological Sciences, Moscow, ID for fossil leaf specimens from the Middle Miocene Clarkia fossil site, and for numerous discussions and encouragement.

Bibliography

Dilcher, D. L. 1974. Approaches to the identification of angiosperm leaf remains. *The Botanical Review* 40: 157 pp.

Basic clearing, staining and mounting procedure used at the University of California Museum of Paleontology (transmitted by Diane Erwin, collection curator).

1. If possible, use pressed, dry leaves, these clear better and faster than green leaves
2. Mix a solution of 5% sodium hydroxide:
 - a) Place nylon screen in bottom of Petri dish or container used (leaf gets very soft when cleared, screen used to lift it out)
 - b) Put in leaf and cover with sodium hydroxide; depending on the type of leaf it will generally take 4-7 days to clear in cold sodium hydroxide. This step can be speeded up by, 1) warm sodium hydroxide, do not boil as the leaf will fall apart, or 2) add about 1 tsp. of common household bleach, but watch closely and remove in 1-3 hours or the leaf will be completely digested
3. When leaf is cleared, 1) wash twice in water, and then cover with chloral hydrate (leave in chloral hydrate at least overnight, the leaf will become almost transparent)
4. Wash twice, cover with 50% water / 50% alcohol for 15 min., pour off and save for next leaf
5. Cover with absolute alcohol (indefinitely if wished)
6. Transfer leaf to Petri dish of 50% toluene / 50% alcohol for 2-5 min.
7. Stain in Safranin O, usually about 5-10 min. Depending on the texture of the leaf of desired color (Stain solution is made by 100 cc alcohol, 1 gram Safranin O dry, 100 cc toluene)
8. Transfer leaf to solution of 50% toluene / 50% alcohol to bleed out excess stain
9. Transfer to absolute toluene until ready to mount on slides
10. Mount leaves using Permount (tends to yellow, but newer form may be more archival) or equivalent mounting medium. This is done by pouring just enough mounting medium on the glass slide to cover it, then place the leaf into this medium, then pour half the amount of medium on the other slide, flip it over and gently lower the upper slide starting at one end, lowering the slide down so it pushes the excess medium out over the leaf and lower slide while pushing out any air bubbles that may have formed.
11. Let stand for 2-5 days, then place on a slide warmer (40-45°C) for about a week
12. Excess dried mounting medium can be removed by razor blade and cleaned with toluene

Standard clearing, and staining procedure used at the Smithsonian Institution (transmitted by Amanda Ash, Leaf Architecture Working Group).

1. Soak the leaf in a 10% NaOH solution for a few days
2. Wash in water and then in a weak solution of acetic acid
3. Put in standard household bleach.
4. Wash in water
5. Soak in a supersaturated solution of chloral hydrate
6. Use 50-70-90- 100% solutions of ethanol for dehydration
7. Stain in a toluene-based solution of Safranin O
8. Put the leaf in pure toluene prior to mounting in a synthetic resin. You may find that it is not until the final staining and soaking in toluene that the leaf becomes totally cleared.

Refractive Indexes

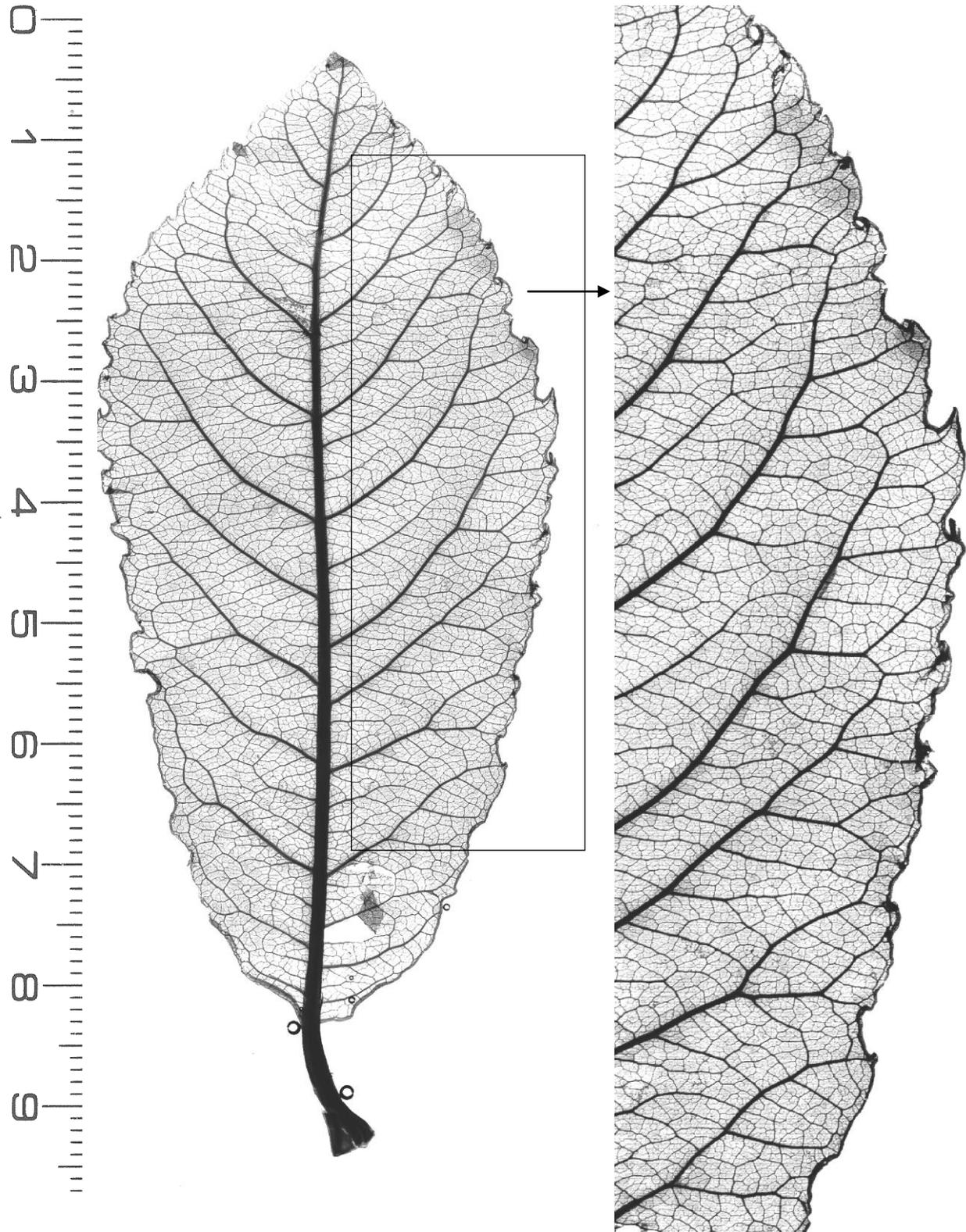
Vacuum	1.00000 (by definition)
Air	1.00029
Water	1.333
Teflon	1.345
Cedar oil	1.455 to 1.461
Eucalyptus oil	1.4580 to 1.4700
Cellulose	1.47
Toluene	1.4940
Plexiglas	1.491
Immersion oil	1.517 (Cargille Type B)
Glass	1.52 to 1.65
Permout	1.525

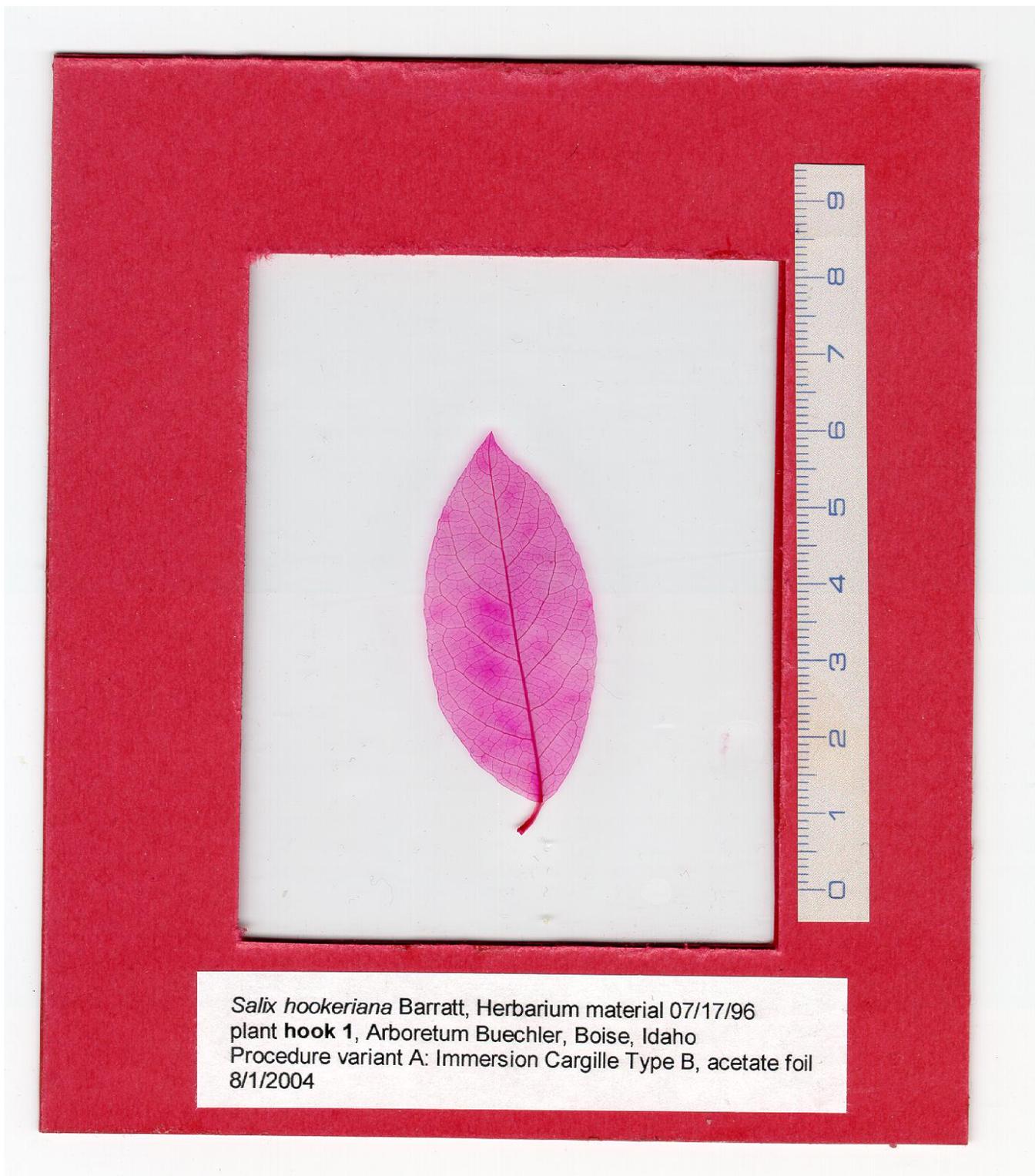


Cedar oil mount between acetate foils

Salix appendiculata Villars (subg. *Salix*; sect. *Cinerella*)

(09/30/2001; cultivated in Boise, Idaho; orig. from Wattwil, Switzerland; fresh material)





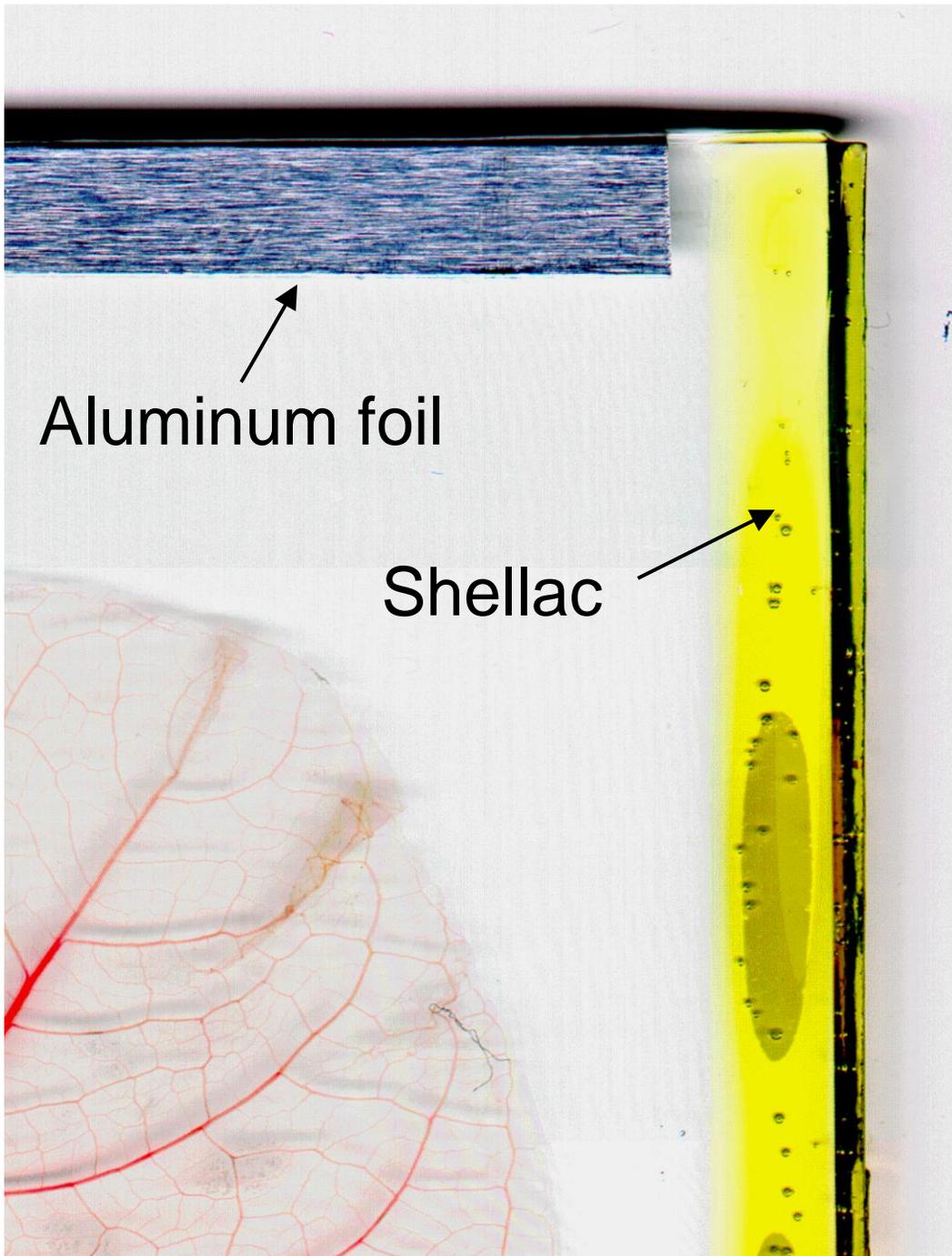
Immersion oil mount, between acetate foils,
with frame



UCMP specimen Permout between glass



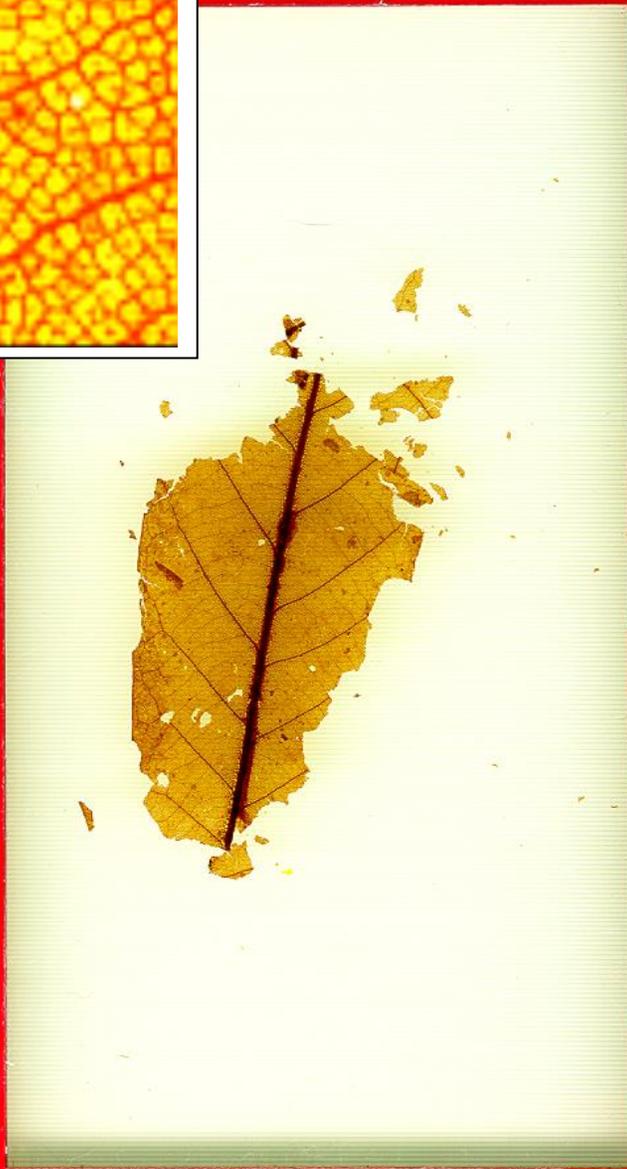
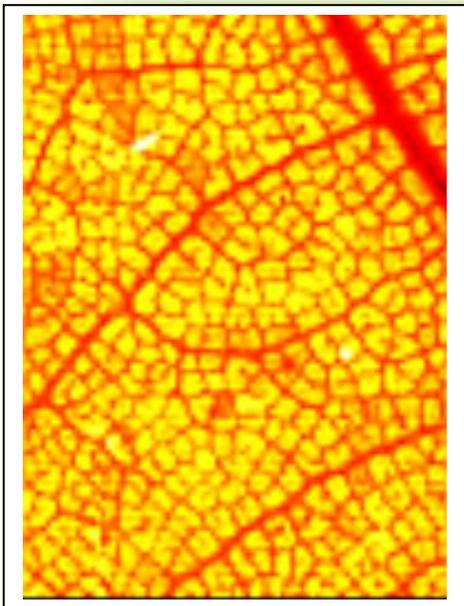
UCMP specimen remounted in immersion oil between acetate foils



Aluminum foil

Shellac

Sealing of Permout / glass specimens with aluminum foil or shellac



Lithocarpus species
Middle Miocene (~15 my)
Clarkia, Idaho

[2]

4/6/04



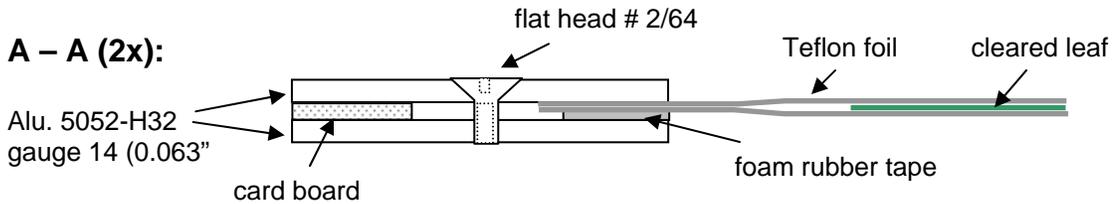
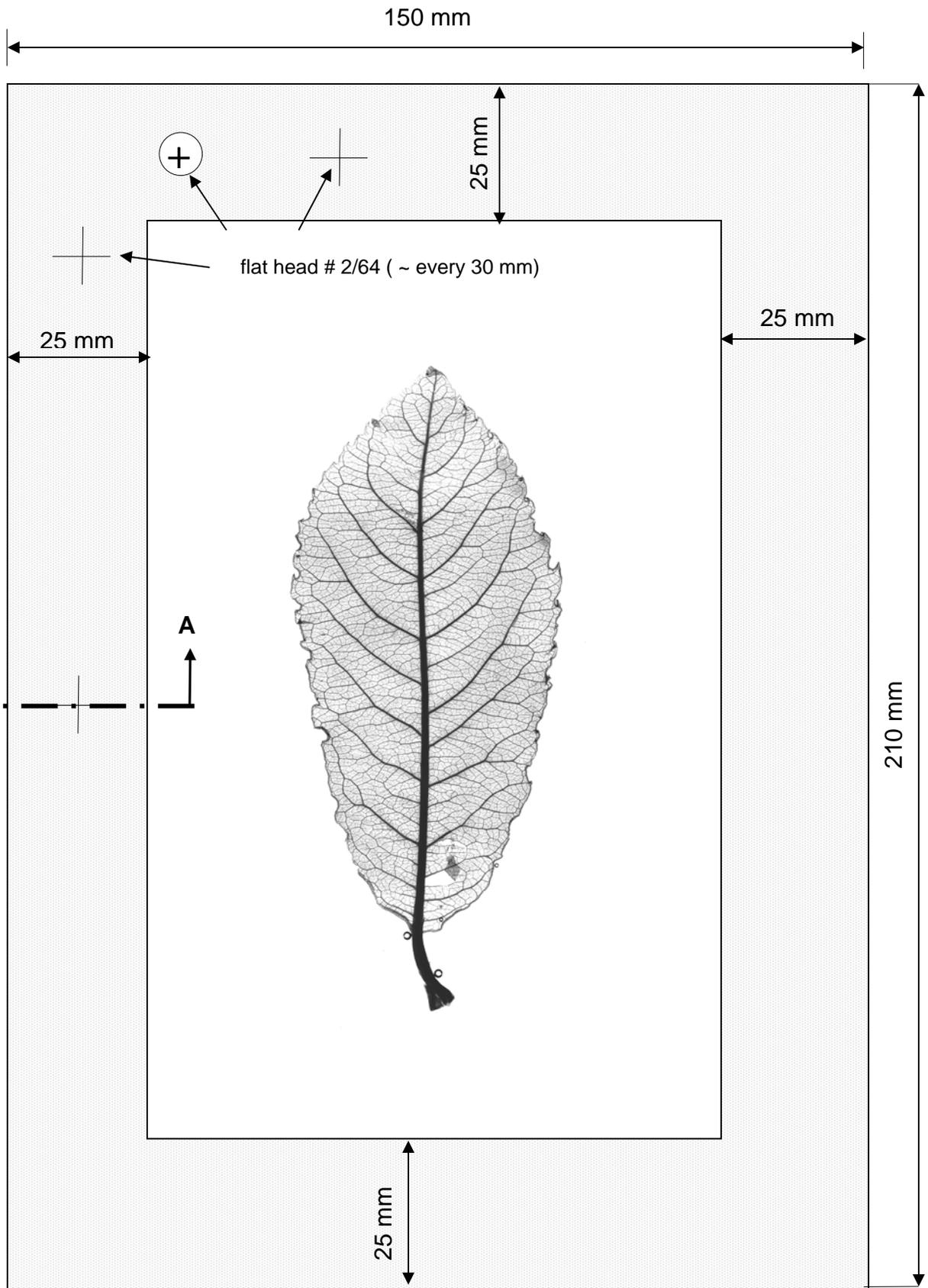
Cleared leaf specimen, Permount[®]
between glass.



Salix appendiculata Villars (female)

Arboretum Buechler, Boise, ID (appe 2), originally from Switzerland
Teflon PFA, immersion oil Cargill Type B 08/30/2008 001

Frame for cleared leaves



Material for aluminum – Teflon foil – immersion oil frame

Frame

Aluminum 5052-H32, gauge 14 (0.063")

I had the prototype frames water jet-cut at Boise Metal Works. They charged me \$ 69.00 for 6 pieces (3 frames), including programming of the machine and material (\$ 15.50 for the six pieces). At larger quantities the price should come down by about 30%. The relatively high cost seems to be due to the low cutting speed. Laser cutting or punching might be cheaper at large quantities.

Screws

Pan Head (or Flat Head), Phillips SS Machine Screw # 3-56 x 3/16" long, Stainless Steel.

The screws on the prototype frame were originally ¼" long and had to be shortened. I had planned using Flat Head (countersunk) screws but they were not on stock at the time.

Tacoma Screw Products, Inc.
2001 Center Street
Tacoma, WA 98409-7895
Phone: 800-562-8192

Teflon foil

For the prototype frame I have used PFA film but FEP could be used as well.

Teflon PFA film type 500LP, .005" thick x 24" wide (\$11.20/ft plus shipping and handling)

Teflon FEP film type 500A, .005" thick x 24" wide (\$6.70/ft plus shipping and handling).

American Durafilm Co. Inc.
55 Boynton Road, P.O. Box 6770
Holliston, MA 01746
Tel: 508-429-8000
Fax: 508-429-8500
Email: durafilm@aol.com
http://www.americandurafilm.com/0201_teflon_tefzel_films_products.cfm

Immersion oil

Immersion oil Cargille Type B (1250 centistokes (prize in 2001: 4 oz. \$10.00 plus shipping and handling). Cargille Type A has a lower viscosity (150 centistokes). The two oils may be mixed in any ratio to adjust for a desired viscosity. A frame made with Cargille Type A oil has so far lasted for two years without leaking.

Cargille Laboratories Inc.
55 Commerce Rd.
Cedar Grove, NJ 07009
Phone: 973-239-6633
Fax: 973-239-6096
<http://www.2spi.com/catalog/ltmic/cargille.shtml>

Foam rubber

FDA-Compliant Silicone Foam Rubber Sheet, Adhesive Back, 1/16" thick, 12" x 12". Catalog number 5025T111 (\$ 17.10 each).

McMaster – Carr
9630 Norwalk Blvd.
Santa Fe Springs, CA 90670-2932
Phone 562-692-5911
e-mail: la.sales@mcmaster.com
Web page : <http://www.mcmaster.com/>

Press board

13001-2516 Museum Mounting Board, Gray, 32" x 40", 4 Ply (~1.54 mm).
The manufacturer doesn't specify a tolerance for the thickness of this board. The difference in thickness between this mounting board (~1.54 mm) and the thickness of the uncompressed foam rubber sheet (~2.35 mm) determines the force by which the two Teflon foils are pressed together.

Strathmore Museum Mounting Board is made of 100% cotton fibers, is acid-free and is buffered with calcium carbonate to guarantee longevity.

Dick Blick Art
Materials P.O. Box
1267
Galesburg, IL 61402-1267
Phone (800) 828-4548
Fax (800) 621-8293
<http://www.dickblick.com/zz133/05/products.asp?param=0&iq id=959>

W. Buechler, 09/02/2008