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PROTOCOL NOTE

Applications in Plant Sciences

Using disposable food packaging materials as printing, embedding, and sectioning media in the plant anatomy lab

Guillermo Angeles | **Carolina Madero-Vega**

Instituto de Ecología, A.C. Red de Ecología Funcional, Xalapa, Veracruz 91070, Mexico

Correspondence

Guillermo Angeles, Instituto de Ecología, A.C. Red de Ecología Funcional, Xalapa, Veracruz 91070, Mexico.
Email: guillermo.angeles@inecol.mx

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Abstract

Premise: During the COVID-19 pandemic lockdown, all laboratory work was suspended, and we were obliged to work from home, causing delays in our research. As the disruption to supply chains made it difficult to obtain our regular lab supplies, we were obliged to search for substitutes. We became familiar with a plastic material known as biaxially oriented polypropylene (BOPP) that is widely used in the food industry for wrapping or storing fruits, vegetables, and meat. BOPP is easily dissolved in organic solvents such as xylenes, acetone, or thinner, but these reagents are very toxic, flammable, and can cause nausea in some users. After testing several alternatives, we found a polyurethane remover that proved to be an effective and relatively harmless BOPP solvent.

Methods and Results: By dissolving thin slices of BOPP in a polyurethane solvent, we obtained a clean fluid that we used to obtain leaf surface prints that could be mounted on microscope slides with a coverslip. This fluid produced excellent bark and wood sections and can be used to obtain wood or charcoal surface prints. Our attempts to use it as a mounting medium were unsuccessful.

Conclusions: BOPP dissolved in a polyurethane remover is a handy, versatile resource for plant microtechniques. In addition to its economic advantages, it is useful in terms of reducing plastic pollution.

KEYWORDS

biaxially oriented polypropylene (BOPP), direct prints, mounting media, resin embedding

Resumen

Premisa: Durante el cierre por pandemia de COVID-19, se suspendió todo el trabajo de laboratorio y nos vimos obligados a trabajar desde casa, lo que provocó retrasos en nuestras investigaciones. Como la interrupción de las cadenas de suministro dificultó la obtención de nuestros suministros de laboratorio habituales, nos vimos obligados a buscar sustitutos. Nos familiarizamos con un material plástico empleado en la industria alimentaria, muy utilizado para envolver o almacenar frutas, verduras y carne. Este material se conoce como polipropileno orientado biaxialmente (BOPP, en inglés) y se disuelve fácilmente en disolventes orgánicos como xilenos, acetona o tíner. Sin embargo, estos reactivos son muy tóxicos e inflamables y pueden provocar náuseas a algunos usuarios. Tras probar varias alternativas, encontramos un removedor de poliuretano que demostró ser un disolvente eficaz para el BOPP.

Métodos y Resultados: Disolviendo tiras delgadas de BOPP en un disolvente de poliuretano, obtuvimos un fluido limpio que utilizamos para obtener impresiones de la superficie de las hojas que podían montarse en portaobjetos de microscopio con un cubreobjetos. Este fluido produce excelentes secciones de corteza y madera y se puede

usar para obtener impresiones de superficies de madera o carbón. Intentamos utilizarlo como medio de montaje, pero desistimos debido a los pobres resultados obtenidos.
Conclusión: El BOPP disuelto en removedor de poliuretano es un recurso práctico y versátil para las microtécnicas vegetales. Además de sus ventajas económicas, es útil para evitar que el plástico llegue a las corrientes de agua y a los océanos.

Laboratory work often requires improvisation, for example, in cases where a reagent is unavailable at a critical moment. During the COVID-19 pandemic lockdown, we experienced an unusual situation that prevented us from working in our laboratories. With no shortage of tasks to be completed, postponing this work was not an option and we were therefore obliged to improvise.

A number of common household products have been used in plant anatomy labs for purposes including leaf surface printing and tissue sectioning. For example, nail polish is a well-known medium for leaf surface printing, producing excellent images of structures including stomata, trichomes, and domatia (Hilu and Randall, 1984; Wu and Zhao, 2017). Other more sophisticated media such as collodion or polystyrene can be used for the same purpose, but these materials are much more expensive. Plastic embedding for tissue sectioning has long been in wide use. Examples of this material include JB-4, methyl-methacrylate, and epoxy resin (Bennett et al., 1976; O'Brien and McCully, 1981; Warmbrodt and Fritz, 1981), and these yield excellent results for producing semi-thin sections of primary or secondary tissues. Nanko and Côté (1980) experimented with ethyl cyanoacrylate (also referred to by trade names including Krazy Glue, Super Glue, and Permabond) as a superficial film for obtaining tree bark sections, with excellent results (which, surprisingly, has not been tried again, despite its simplicity).

We tested the use of biaxially oriented polypropylene (BOPP), which is widely used in the food industry to store fresh fruits and vegetables, eggs, or bread (Uejo and Hoshino, 1970). To our knowledge, the use of BOPP has never been reported for the microtechniques tested here, and we therefore propose its use based only on our own experience. In addition to its economic advantages, BOPP offers a viable means to achieve high-quality reproductions of leaf and wood surface impressions, as well as paradermal sections of leaves. Moreover, it serves as a suitable embedding medium for woody tissues.

METHODS AND RESULTS

BOPP is freely available in the form of laminates separating slices of cooked or cured meats in some delicatessen brands or can be ordered directly online from different suppliers (Figure 1A and B). BOPP laminates are easily dissolved in organic solvents such as xylenes, acetone, or thinner, but these reagents are very toxic, flammable, and can cause nausea in some users. To avoid these issues, we tested a number of alternative solvents, finally settling on a polyurethane remover (Polyform; PPG Industries, Pittsburgh, Pennsylvania, USA; https://www.polyform.mx/Home) that worked well, producing a clear syrup that could be smeared on leaf surfaces, yielding excellent prints.

After cleaning the BOPP laminates with a mild detergent and drying, we cut them into small pieces (approximately 1.5 × 1.5 cm), with the final size dictated by the size of the glass vial used to prepare the mix (syrup). Around 4.5–5 g of BOPP was placed in a tightly closed glass vial and 20 mL of polyurethane remover was added; this was stored at room temperature with occasional agitation. The BOPP dissolved within a couple of hours, producing a whitish syrup. At this point, it is advisable to filter the syrup by passing it through two or three layers of coffee filter paper in a glass funnel, after which the syrup should present a clearer appearance. These steps should be carried out under a fume hood or in a well-ventilated location. A detailed protocol to prepare BOPP syrup is provided in Appendix 1.

Applications

Here, we present examples of results obtained using the BOPP syrup applied to plant anatomy. Table 1 shows a comparison between the results we obtained using BOPP syrup and other classic microtechniques.

Leaf prints

The clear syrup is applied directly onto the dry, clean surface of the leaf of interest using a brush or glass rod. After 15 min, the film formed on the leaf surface can be lifted carefully with pointed tweezers and immediately placed on a clean microscope slide, to which a piece of double-sided adhesive tape has been attached. The side of the film that was in contact with the leaf surface must be placed toward the observer. A glass coverslip is placed on the print and secured with pieces of adhesive tape. Films of this type are very useful for obtaining images of stomata and trichomes with a very clear resolution, as shown in Figure 1C and D.

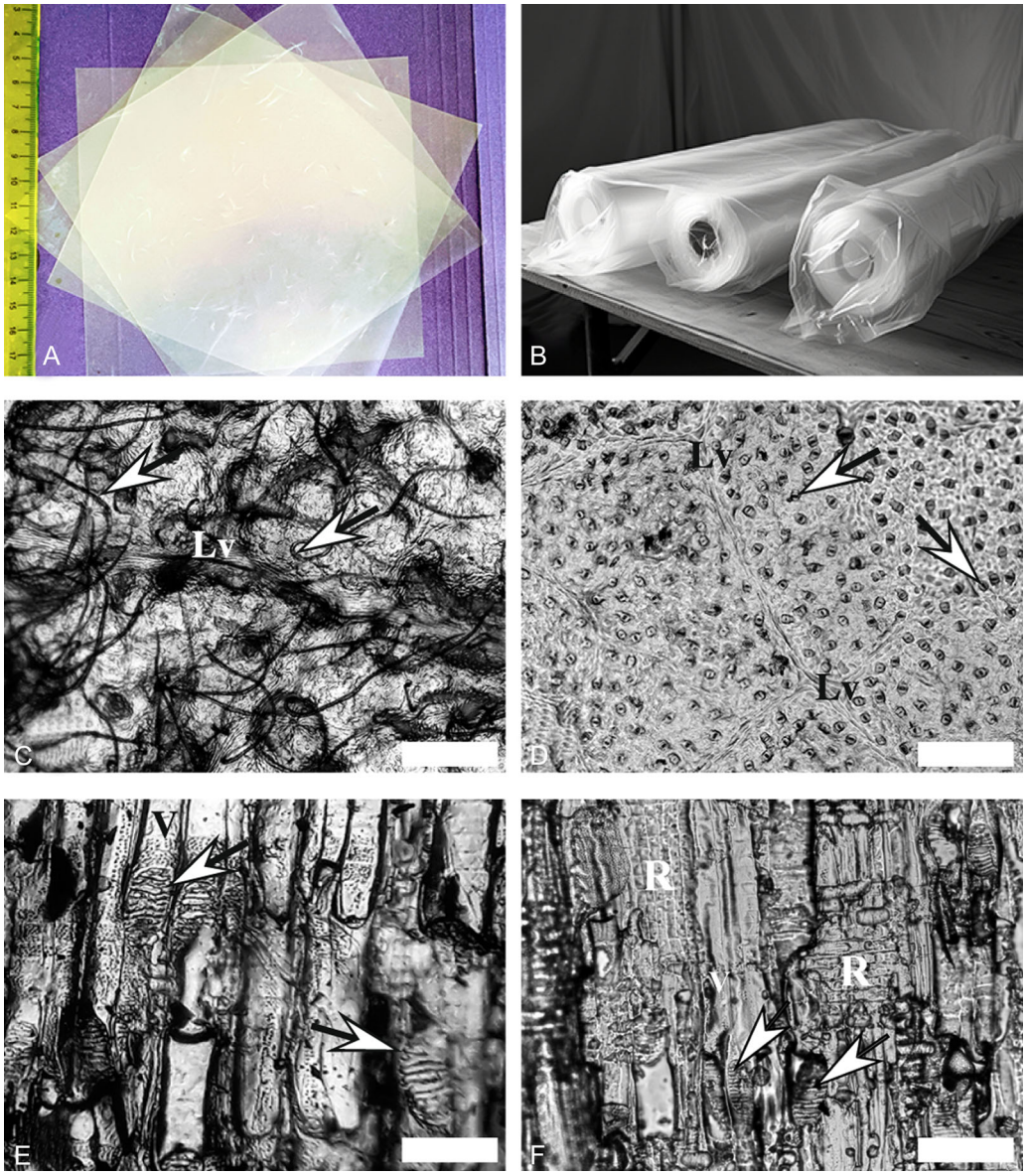

FIGURE 1 Materials used for this protocol and their suggested applications. (A) Laminates of BOPP, used in the food industry. (B) Commercial presentation of BOPP. (Image generated with artificial intelligence on Midjourney [https://www.midjourney.com].) (C) Indirect prints of the abaxial surface of a leaf of *Eriobotrya japonica* (Thunb.) Lindl. produced with BOPP syrup, showing the impression of long trichomes (arrows) and veins (Lv). Scale bar = 200 μm. (D) The same technique as described in (C) was applied on the abaxial surface of a *Quercus* sp. young leaf, showing numerous stomata (arrows) and veins (Lv). Scale bar = 200 μm. (E) Indirect print of a longitudinal radial surface of wood of *Alnus jorullensis* Kunth made by applying BOPP syrup to a direct replica of polysiloxane obtained from that surface, showing vessel elements (V) and scalariform perforation plates (arrows). Scale bar = 500 μm. (F) Indirect print obtained as described in (E), using nail polish in place of BOPP syrup, from the radial surface of wood of *A. jorullensis* showing rays (R), vessels (V), and scalariform perforation plates (arrows). Scale bar = 700 μm.

Wood and charcoal wood prints

High-resolution prints of woody structures can be obtained from tangential and radial surfaces of dry or charcoal wood using BOPP syrup. It is highly advisable to first obtain a direct print of these surfaces using a dental paste (Dental Speedex Light Body or Speedex; Coltene, Cuyahoga Falls, Ohio, USA), which is presented in two components, as described by Angeles (2001). After combining these two components of Speedex (Speedex Surface Activate and Speedex Universal Activator), the mixture is applied to the intended surface for printing and left to dry for 5–10 min to obtain a primary print from the wood or charcoal surface. A thin layer of the BOPP syrup is then applied to the primary print, as described by Kuraishi et al. (1990), to obtain a secondary print. The film that forms on the primary print surface should detach easily. After preparing a microscope glass slide as described above, the film is then placed directly onto the adhesive tape and covered with a glass coverslip. High-quality images can then be readily obtained with a dissecting or compound

TABLE 1 Comparison between the results obtained using biaxially oriented polypropylene (BOPP) syrup and other classic microtechniques.

	Method			
Species (technique)	**Paraffin**	**BOPP**	**Cyanoacrylate**	**Nail polish**
Ficus pumila L. (leaf print)	NT	Excellent	NT	Excellent
Ficus pumila (leaf paradermal section)	Excellent	Good	NT	NT
Alnus glutinosa (L.) Gaertn. (wood print)	NT	Excellent	NT	Excellent
Hedera helix L. (embedded stem)	Excellent	Excellent	NT	NT
Pinus sp. and *Ficus pumila* (film applied to stem surface for sectioning)	NT	Excellent	Excellent	NT

Note: NT = material not tried.

microscope (bright or dark fields) or using Nomarski optics. Figure 1E and F compare the results obtained using BOPP syrup and nail polish, respectively.

Embedding medium

To use the syrup as an embedding medium for plant tissues, the samples should first be fixed as if intended for paraffin embedding. After washing the fixative with tap water, samples should be dehydrated with an ethanol series reaching up to 100%. Finally, the samples are immersed in the polyurethane remover for 24 h, then transferred to a small glass container with the syrup such that the tissue samples are completely immersed. Sealing the container tightly and inverting it several times a day for two days ensures infiltration of the tissues by the syrup. The small container is then placed at the bottom of a larger glass container with its lid loosely closed; the lid of the large container should also be only loosely closed. It is advisable to mark the initial syrup level on the small container to visualize the decrease in volume and to place the containers under a ventilation hood with a gentle airflow. Once the volume has decreased to one-third of its initial level, one can assume that the tissues are embedded. At this point, the samples can be transferred to an uncovered, labeled aluminum dish of 5 cm in diameter and once again left under the ventilation hood. The samples are then left uncovered to allow the BOPP to harden. After 48 h, the excess hardened BOPP around the samples can be removed using scissors. Each BOPP-embedded sample is mounted on wooden or plastic blocks with strong glue at the desired orientation for sectioning. Sections of 8–20 µm can then be easily obtained using a rotary or sliding microtome. We present for comparison the results obtained from a stem of *Hedera helix* L. embedded in BOPP and paraffin (Figures 2A and B, respectively).

Paradermal leaf sectioning

Leaf samples measuring up to 2 cm per side, previously fixed and washed with tap water, can be stained with any water-soluble stain and gradually dehydrated with a series of increasing concentrations of ethanol, up to absolute alcohol. The samples are then transferred to BOPP syrup in a glass vial, as described in the "Embedding medium" section. After embedding the samples in BOPP syrup, they can be transferred to aluminum trays, lightly covered with another tray of the same size, and left to dry for a few days. The leaf pieces are then separated and mounted flat onto a wooden or plastic block for fitting into the microtome holder. When separating the leaf pieces, we recommend leaving sufficient BOPP around each leaf section to facilitate the handling of individual sections with tweezers during the staining and mounting process. Sections of 8–20 µm in thickness, obtained with a rotary microtome, are placed on a microscope slide and covered with distilled water. After drying on a warm plate, the leaf paradermal sections are mounted with synthetic resin and a coverslip. Figures 2C and D show examples of paradermal sections of *Ficus pumila* L. leaves embedded in BOPP syrup and paraffin, respectively.

Bark and wood sectioning

BOPP syrup can be used in the same way that Nanko and Côté (1980) used ethyl cyanoacrylate to section hardwood bark samples: pieces of branches (fresh or fixed) are firmly clamped in the sliding microtome sample holder and left to dry. After obtaining some thick (30–40 µm) sections, a layer of the syrup is applied to the exposed surface. After 15 min, once the solvent has evaporated, thinner sections (15–20 µm thick) can be obtained. Application of the syrup to the bark surface can be repeated, letting it dry before cutting again. The sections obtained are left on the knife surface to flatten, grasped with tweezers, and placed on a clean microscope slide for 2–5 min. Several slides with ordered sections are then placed on a warm plate to ensure that they remain flat. To avoid losing these sections, they should be tied in place using cotton or synthetic thread in case Coplin jars are to be used to conduct the staining and dehydrating processes. The sections can be stained with toluidine blue O or any other water- or ethanol-soluble dye. Once dry, the sections can be mounted with synthetic resin and a coverslip; light pressure must be applied to the

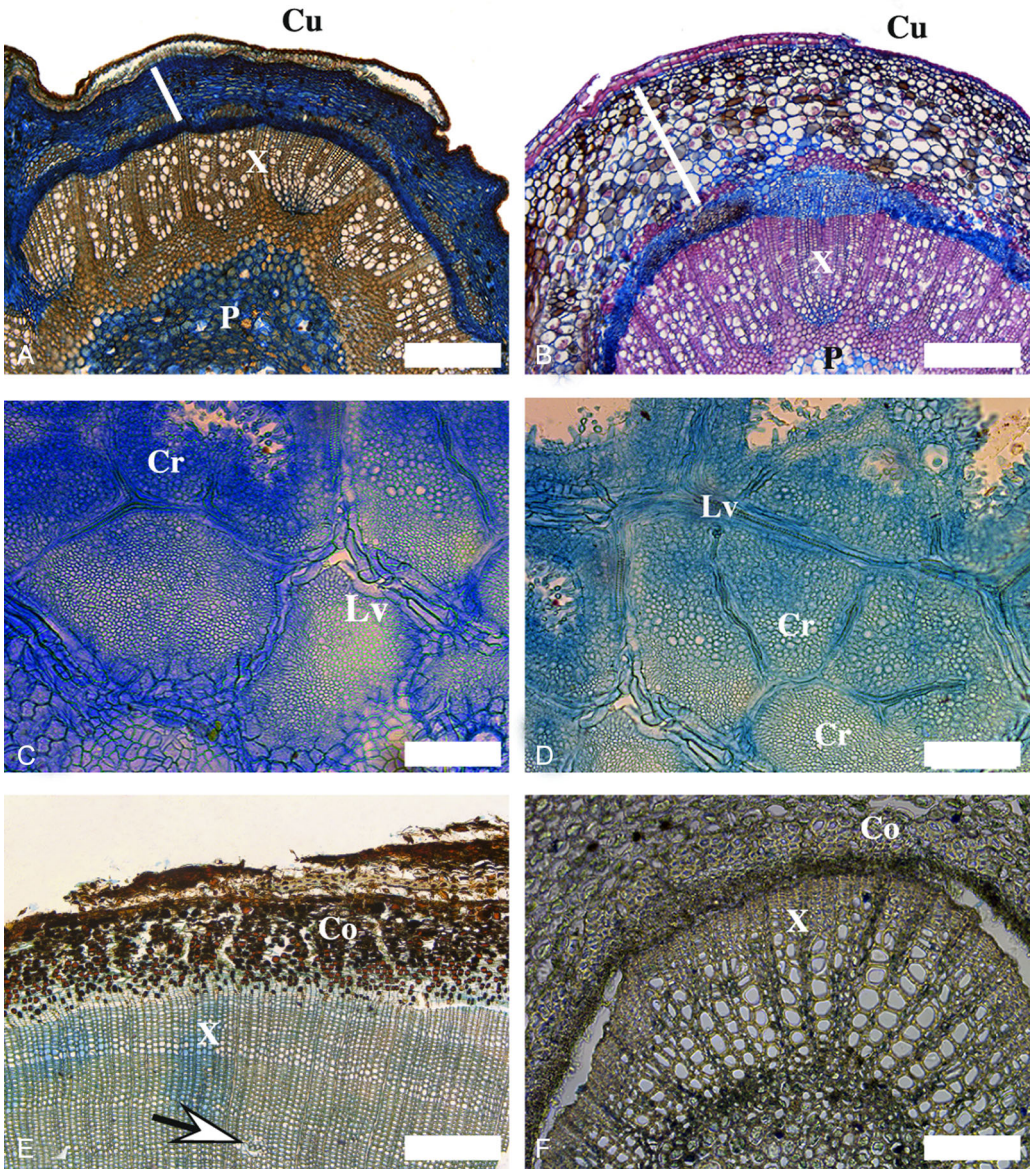

FIGURE 2 BOPP syrup used as an embedding material or film. (A) Cross sections of stems of *Hedera helix* embedded in BOPP and stained with safra blue. The cuticle (Cu), xylem (X), and pith (P) are well preserved. The cortex (white bar) is collapsed to some degree. Scale bar = 2.5 mm. (B) Same plant material as in (A), embedded in paraffin and stained with safra blue. The cuticle (Cu), xylem (X), pith (P), and cortex (white bar) are well preserved. Scale bar = 2.5 mm. (C) Leaf paradermal sections of *Ficus pumila* embedded in BOPP syrup, stained with crystal violet. Leaf veins (Lv) and crypts (Cr) are indicated. Scale bar = 270 μm. (D) Same plant material and same type of section as in (C), embedded in paraffin. Leaf veins (Lv) and crypts (Cr) are indicated. Scale bar = 300 μm. (E) A transverse section was obtained after applying a film of BOPP on the surface of an FAA-fixed branch of *Pinus* sp. stained with safra blue. The arrow points to a resin canal. Xylem (X) and cortex (Co) are well preserved. Scale bar = 200 μm. (F) A transverse section was obtained after applying a film of BOPP on the surface of an FAA-fixed stem of *F. pumila* stained with an aqueous 0.05% solution of toluidine blue O. Some collapse occurred at the vascular cambium and secondary phloem, which shows here as a dark area at the center of the image. Xylem (X) and cortex (Co) are well preserved. Scale bar = 400 μm.

top of the coverslip to expel any trapped air bubbles. Figures 2E and F present examples of this technique used to section a branch of *Pinus* sp. and a stem of *Ficus pumila*. In both cases, the bark remains attached to the wood and the structure can be appreciated in detail (e.g., a resin duct in the pine). Some collapse of the cortical parenchyma was observed in the plant material embedded in BOPP syrup.

Mounting medium

We tested BOPP syrup as a mounting medium, with apparent initial success; however, after reviewing several samples prepared some months earlier, we observed that the syrup had cracked, rendering the microscopic preparations useless.

CONCLUSIONS

BOPP is a freely available plastic that is easily dissolved in organic solvents such as the polyurethane remover used here. BOPP syrup proved to be excellent as a medium with which to obtain printings of plant surfaces such as leaves, wood, and charcoal wood, with or without an intermediate printing step using Speedex dental paste. It also produced excellent results when used in the sectioning of wood and bark tissues. It yielded moderate to poor results when used as an embedding medium for soft, fragile tissues, such as parts of flowers or fruits, but produced excellent results for paradermal sections of leaves and for embedding woody organs. However, its use cannot be recommended as a mounting medium.

BOPP syrup represents a cost-effective alternative to more expensive materials such as methyl-methacrylate, cyanoacrylate, or polystyrene. In addition to its economic benefits, it aids in circumventing the disposal of plastic materials in natural ecosystems, thus helping to reduce the levels of plastic reaching our water sources and oceans. The only antecedent of using waste material for plant tissue embedding is that of Barbosa et al. (2010), who reported a method for obtaining good-quality sections from bark tissue using a disposable material (anti-tear polystyrene foam solution).

AUTHOR CONTRIBUTIONS

G.A. conceived the research, acquired the funding, designed the protocols, and wrote the manuscript; C.M.V. performed all the laboratory work and prepared the images for the figures. All authors approved the final version of the manuscript.

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DATA AVAILABILITY STATEMENT

All supporting data is provided with the published article.

ORCID

Guillermo Angeles http://orcid.org/0000-0002-0455-4997
Carolina Madero-Vega http://orcid.org/0000-0002-2107-8355

REFERENCES

Angeles, G. 2001. New techniques for the anatomical study of charcoalified wood. *IAWA Journal* 22: 245–254.

Barbosa, A. C., M. R. Pace, L. Witovisk, and V. Angyalossy. 2010. A new method to obtain good anatomical slides of heterogeneous plant parts. *IAWA Journal* 31: 373–383.

Bennett, H. S., A. D. Wyrick, S. W. Lee, and J. H. McNeil. 1976. Science and art in preparing tissues embedded in plastic for light microscopy, with special reference to glycol methacrylate, glass knives and simple stains. *Stain Technology* 51: 71–97.

Hilu, K. W., and J. L. Randall. 1984. Convenient method for studying grass leaf epidermis. *Taxon* 33(3): 413–415.

Kuraishi, S., N. Sakurai, H. Miyauchi, and K. Supappibul. 1990. Measurements of the water economy of mangrove leaves. *In* Y. Hashimoto, P. J. Kramer, H. Nonami, and B. R. Strain [eds.], Measurement techniques in plant science, 151–163. Academic Press, New York, New York, USA.

Nanko, H., and W. A. Côté. 1980. Bark structure of hardwoods grown on southern pine sites, 2. Syracuse University Press, Syracuse, New York, USA.

O'Brien, T. P., and M. E. McCully. 1981. The study of plant structure: Principles and selected methods. Termarcarphi, Melbourne, Australia.

Uejo, H., and S. Hoshino. 1970. Structure of biaxially oriented polypropylene film. *Journal of Applied Polymer Science* 14: 317–328.

Warmbrodt, R. D., and E. Fritz. 1981. Embedding plant tissue with plastic using high pressure: A new method for light and electron microscopy. *Stain Technology* 56(5): 299–305.

Wu, S., and B. Zhao. 2017. Using clear nail polish to make *Arabidopsis* epidermal impressions for measuring the change of stomatal aperture size in immune response. *In* L. Shan and P. He [eds.], Plant pattern recognition receptors. Methods in Molecular Biology, Vol. 1578. Humana Press, New York, New York, USA.

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Appendix 1. Required materials and protocol for the preparation of biaxially oriented polypropylene (BOPP) syrup. Also available at: https://figshare.com/s/f7fefe05aed2d997b5e7

Equipment

Screw-cap glass vials (50 and 100 mL)
Screw-cap glass jar (1 L)
Glass beakers (200 mL)
Glass rod (0.5 mm diameter)
Scissors
Oven set at 60°C, with ventilation
Hot plate
Tweezers
Dissecting needles
Nitrile gloves
Goggles
Small metal spatula

Consumables

Aluminum foil
Aluminum dishes (5 cm diameter)
Disposable safety blades
Nail polish
Dental paste (Speedex; Coltene, Cuyahoga Falls, Ohio, USA)

Synthetic resin for mounting slides
Glass microscope slides (frosted)
Glass coverslips (24 × 2 mm and 24 × 40 mm)
Double-sided adhesive tape
Single-sided adhesive tape

Reagents
100% ethanol
96% ethanol
Polyurethane remover (e.g., 2-Minute Remover Advanced; Sunnyside Corporation, Wheeling, Illinois, USA)

Preparation of the BOPP syrup (preferably conducted under a fume hood)

1. Cut small pieces of BOPP laminate measuring 1–2 cm per side, depending on the volume of the glass vial to be used.
2. In a clean 50-mL glass vial, add 20 mL of polyurethane remover.
3. Weigh 4.5–5 g of BOPP laminate and add to the vial containing the polyurethane remover. Close the cap tightly.
4. Gently agitate the vial by periodically inverting it and agitating the contents. Repeat this process for approximately two days or until complete dissolution of the BOPP laminate is achieved.
5. If solids become visible during the agitation process, filter the resulting syrup-like solution through 2–3 coffee filters in a glass funnel.
6. Cover the upper end of the glass funnel with aluminum foil to prevent dust accumulation and rapid solvent evaporation.
7. Finally, label the glass vial containing the resulting syrup as "BOPP syrup" for identification purposes.

Applications

A. Printing leaf surfaces
1. Obtain the desired leaf specimen for examination.
2. Rinse the leaf under running water to remove any contaminants.
3. Pat the leaf dry using an absorbent paper tissue.
4. Place the leaf specimen between two or three layers of drying paper.
5. Insert the leaf and drying paper assembly between two pieces of cardboard.
6. Apply a suitable weight, such as a medium-sized book, to exert pressure on the assembly.
7. After a period of 4–6 h, remove the pressed leaf specimen from the assembly.
8. Use a glass rod to uniformly apply a thin film of syrup to the specific surface to be observed.
9. Allow the applied film of syrup to dry completely.
10. Carefully lift the dried film from the leaf surface using tweezers.
11. Place the dried film, with the print side facing upwards, onto the prepared microscope slide.
12. Place a coverslip of appropriate dimensions on top of the printed film.
13. Secure the coverslip to the slide using strips of adhesive tape.

B. Printing wood or charcoal wood surfaces

Follow the procedure with the inclusion of a preliminary step to obtain a positive print using dental paste:

1. On a clean glass surface, dispense the desired length of Speedex Surface Activate (depending on the surface to be covered).
2. On the same glass surface, in parallel form, extend an equal length of paste from the Speedex Universal Activator.
3. Apply the mixture onto the exposed tangential or radial surface of the wood or charcoal using a metal spatula.
4. Leave the paste on the wood surface for 10–15 min.
5. Remove the paste from the wood surface.
6. Apply a thin film of syrup onto the printed surface following the procedure described above for leaf surfaces.
7. From this point forward, follow the procedure starting from step 8, as described for leaf surfaces.

C. Bark and wood sectioning

1. Affix the fragment of the branch or stem containing both bark and wood tissue onto the sliding microtome sample holder.
2. Apply a drop of BOPP syrup onto the designated area for sectioning.
3. Allow it to desiccate for 30 min (or 15 min while directing a stream of air over the surface).
4. Obtain sections of thickness ranging from 12 to 20 μm.
5. Place the sections directly onto a microscope slide, securing them in place using thread, for staining.
6. After rinsing the stain with tap water, subject the sections to dehydration and clearing processes before mounting them using a synthetic resin and affixing a coverslip.

D. Embedding medium

1. Chemically fix the plant sample using formalin-acetic acid-alcohol (FAA), or any other fixative, ensuring that its dimensions do not exceed 0.5 cm per side.
2. Rinse the sample with tap water.
3. Gradually dehydrate the sample by subjecting it to a series of increasing ethanol concentrations, up to 100%.
4. Immerse the sample in a polyurethane remover for 24 h.
5. Place the sample in a glass vial and add a sufficient volume of BOPP syrup (approximately three times the volume of the sample). Seal the vial securely.
6. Invert the vial, allowing the syrup to flow down and return it to its original position. Repeat this process at least 10 times and allow the sample to settle overnight.
7. Loosen the cap of the vial and place it inside a large glass container. Leaving the container's cap slightly open, place the entire setup under a fume hood with moderate airflow.

8. Extract the plant sample from the vial once the syrup volume has decreased to one-third of its original volume. Transfer the sample to an aluminum tray.
9. Cover the sample with fresh syrup and keep it under the ventilation hood.
10. Once the syrup solidifies, isolate the sample and remove any excess surrounding BOPP.
11. Orient the sample according to the section you want to obtain and affix it to a wooden block.
12. Insert the wooden block and sample into the microtome holder.
13. Obtain sections of 12–20 μm in thickness, moistening the knife with a mixture of glycerin and water in a 1:1 ratio.
14. Apply an aqueous dye to stain the sections, followed by rinsing, dehydration, and mounting with synthetic resin using a coverslip.

E. Paradermal leaf sectioning

1. Select the leaf of interest and cut a 1.5 × 1.5 cm section from the desired area.
2. Fix the leaf sample in a fixative solution, such as FAA, for a period of 12 to 24 h.
3. After fixation, wash the leaf sample with tap water to remove any excess fixative.
4. Stain the sample in an aqueous dye solution, such as 0.01% safranin. Immerse the section in the dye solution for approximately 15 min and then wash with tap water to remove excess stain.
5. Dehydrate the leaf sample using increasing concentrations of ethanol, until reaching 100%.
6. From this point forward, follow the procedure starting from step 4 of the "Embedding medium" protocol.