

Appendix D. Instructions for Clearing Leaves

Leaf clearing is the process of removing all pigment and then staining a leaf so that its vein architecture is clearly visible. This procedure can be used on leaves removed (with permission) from herbarium sheets or on live material. Many methods are used for clearing leaves; here we briefly describe one method. The following sources contain additional information on leaf clearing techniques: Foster (1953), de Strittmatter (1973), Hickey (1973), Shobe and Lersten (1967), Paine (1969), and Bohn et al. (2002). Note that this process must be performed in a well-ventilated area because some of the chemicals are harmful to humans.

Leaves are placed in glass containers, covered by a piece of fiberglass mesh to facilitate changing solutions, and submerged in 1–5% NaOH, the strength depending on the thickness of the material. The NaOH solution is changed every 1–2 days during the clearing process, which generally takes 2–10 days. The clearing process is finished by a wash in commercial Clorox® (typically 5–30 seconds), followed by a final wash in water to stop the bleaching process. Clorox removes any remaining pigment from the leaves in preparation for staining. This step requires caution because the leaves are typically fragile from the NaOH treatment and may disintegrate if bleached for too long.

Acid fuchsin is a particularly successful stain, although safranin dye can also be useful. Staining with acid fuchsin involves washing the leaves in 50% ethanol, staining them in 1% acid fuchsin for 3–8 minutes, and then putting the leaves through a dehydration series in 50%, 95%, and 100% ethanol. The first two dehydration steps destain the leaves because the water-soluble dye diffuses out of the leaf into the ethanol; the third step stops the process once there is proper contrast between leaf lamina and stained veins. The specimens can then be rinsed in clove oil, then xylene (a toxic solvent), and finally stored temporarily in a solution of 1:1 xylene:HemoDe®. Proceeding directly from dehydration to storage in HemoDe® also gives good results, but the leaves will eventually lose some pigment.

For photography, the leaves are floated in a glass dish placed on the backlit platform of a dissecting microscope with a digital camera attachment. The acid fuchsin dye fades over time, and limited restaining may be necessary in order to attain the necessary contrast for imaging. Leaves are then permanently mounted on glass slides using standard anatomical techniques. The leaves used in this publication were permanently mounted and then photographed using a light table or converted enlarger condenser as a source of transillumination.