PREPARATION OF SAMPLES FOR LEAF ARCHITECTURE STUDIES, A METHOD FOR MOUNTING CLEARED LEAVES

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Leaf architecture, primarily the study of leaf venation patterns, has been pioneered by paleobotanists, whose primary study material consists of leaf impressions and compressions. However, a survey of the literature on leaf architecture shows several waves of interest in the subject and an expanding range of approaches and applications. These include the evolution of leaf form and function (e.g., Boyce and Knoll, 2002), genetic and other mechanisms in the ontogeny of leaf venation (e.g., Candela et al., 1999), applications in systematics and taxonomy (e.g., Hickey and Taylor, 1991; Fuller and Hickey, 2005), identification for floras and forest management (e.g., Procópio and Secco, 2008; Obermüller et al., 2011), quantitative analyses of leaf venation and other network systems (e.g., Katifori and Magnasco, 2012), and continuing work on paleofloras and paleoecology (e.g., Calvillo-Canadell and Cevallos-Ferriz, 2002; Wing et al., 2009; Gandolfo et al., 2011).

For all of the studies mentioned above, it is essential to have a way to observe in detail even the smallest veins. To this end, a number of clearing methods have been developed (Johansen, 1940; Foster, 1949, 1950; Morley, 1949; Fuchs, 1963; Larsten, 1967; Shobe and Larsten, 1967; Payne, 1969; Pérez-Harguindeguy and Díaz, 2013), but references for mounting and archiving cleared leaves are scarce (Christophel and Blackburn, 1974). For instance, Ellis et al. (2009) and Pérez-Harguindeguy and Díaz (2013) presented simple approaches to clearing and imaging, but mounting was not included.

The traditional mounting media present serious problems for archiving cleared leaves. Glass has desirable optical and archival qualities, but its rigidity usually leads to the formation of bubbles regardless of the sealant used (Fig. 1A, B). Canada balsam, a purified conifer resin, dries very slowly, leading to bubbles; furthermore, over time it shows serious problems with yellowing and crystallization (Fig. 1A). The resin cracking probably results from the loss of solvent from the medium, which oxidizes upon exposure to air or heat. Permount (Thermo Fisher Scientific, Waltham, Massachusetts, USA), a synthetic resin, causes loss of stain and has problems with expanding bubbles (Fig. 1B) as well as crystallization. Some synthetic epoxy resins, such as EPO-TEK 301 (Epoxy Technology, Billerica, Massachusetts, USA), produce bubbles and show problems with cell collapse/shrinkage and loss of stain. Unfortunately, efforts to restore damaged mounted leaves are time-consuming and expensive (Erika González, Smithsonian Institution, personal communication, 2011). Deteriorated mounted leaves, although useful, can affect interpretation of some features of the leaf architecture. This paper presents a method to permanently mount cleared leaves and provides a complete protocol that also details strategies and procedures for obtaining cleared leaves. Compared to traditional mounting methods, this method has the advantages of being relatively fast and inexpensive and of producing clear, thin samples that can easily be imaged, scanned, and stored (Fig. 1C–E). Moreover, after 37 years,