

A comparison of scanning electron microscopy preparation methods to observe anatomical features and microbial changes during the vase life of *Acacia* and *Rosa*

Virginia G. Williamson

Scientia Horticulturae 282: 110039. (2021)

Abstract

There are several sample preparation methods used to observe plant material in scanning electron microscopy (SEM) and choosing the most appropriate method can be difficult. This choice is especially critical when investigating bacterial colonisation of cut stem ends during vase life, in case some preparation methods remove microbial material. When critical point drying (CPD) was used to compare bacterial colonisation at days 0, 1, 3 and 5 of vase life in *Acacia amoena* (Wendl.), few bacteria were observed by 3 d. Colonisation only became evident after 5 d. Because our previous study's enumeration of bacteria revealed high numbers (10^3 cfu mL⁻¹) by 3 d, it was hypothesised that the numerous changes of solution during CPD had removed bacteria. To test this, three SEM preparation methods: air drying (AD); CPD; and freeze-drying (FD), were compared, based on their ability to detect microbial changes and observe anatomical features in *Rosa hybrida* L. 'Sonia', a well-studied plant. The choice of CPD, AD or FD did not markedly affect bacterial colonisation in *Rosa*, implying that CPD was not the cause of lower bacterial numbers in *Acacia*. Amorphous deposits found in both plant genera were likely to be the bacterial biofilms seen by others. Vestures and warts were observed on inner xylem vessel walls and pits in *Acacia*, but not definitively in *Rosa*. All SEM preparation methods provided clear images of plant anatomy and microbial colonisation in *Rosa*, suggesting that they can also be used validly in the study of other genera like *Acacia*, although AD preserved bacterial biofilms more clearly. The easiest preparation method in terms of equipment required and labour intensity was AD but, overall, CPD provided the highest quality images with no surface distortion. When choosing the most appropriate SEM preparation method, it is also important to consider the purpose of the observations.