Positional relationship between hyphae and embolism in *Raffaelea quercivora*-inoculated saplings

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Mass mortality of oak trees in Japan due to Japanese oak wilt (JOW) caused by a pathogenic fungus *Raffaelea quercivora* has been tremendous since the late 1980s, especially in areas along the Japan Sea. The fungus is vectored by an ambrosia beetle *Platypus quercivorus* which reiterate local invasion and explosion. The fungus introduced into the gallery by the beetle and proliferates on the gallery wall, and causes dysfunction of water conduction of vessels. Takahashi et al. (2010) investigated to the distribution of the fungus and water conduction loss in xylem in artificially inoculated oak saplings, and suggested that wilting of *Quercus* trees may be induced by dysfunction of many vessels and it requires that the fungal hyphae spread from many galleries bored by beetles during mass attacks. The mechanism of the dysfunction of xylem, especially the fungal role, is not known. We compared detailed distribution of water in vessels in the xylem by cryo-scanning electron microscopy (cryo-SEM) and distribution of *R. quercivora* hyphae in the same specimen by F-WGA (fluorescein-conjugated wheat germ agglutinin) staining, and examined their spatial relationships in *Q. crispula* saplings four days after inoculation. The cryo-SEM observation showed that current-year vessels were filled with water, and the previous-year vessels were vacant in both inoculated and control saplings. Although the previous vessels were vacant, vasicentric tracheids around those vessels were filled with water in both saplings. In inoculated saplings, hyphae have invaded xylem 2 cm above the inoculation site. Tyloses were distributed in vessels 0.2 to 1.2 mm outer the areas invaded by the hyphae, that means no water conduction in the xylem. Water conductivity was lost in the xylem area around the hyphal invasion; however, vessels were filled with water. Therefore, the dysfunction of water conduction in these seedlings could not be attributed to cavitation in vessels.

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