Nematode distribution and occurrence of xylem embolism in pine stem inoculated with pine wood nematodes

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Pine wilt disease, caused by pinewood nematode (*Bursaphelenchus xylophilus*) introduced from North America, is one of the most serious tree diseases in the north temperate zone of Eurasia. This pathogenic nematode is known to induce xylem embolism by migration and feeding activity in an infected pine stem. Previous studies have shown that a number of nematodes stay around the infection site with few nematodes migrating through resin canal in the early stage, and then rapidly multiply through the tree in the later stage of this disease symptom. It was unclear whether the distribution of nematode corresponds to the occurrence of xylem embolism. In this study, development of xylem embolism was monitored nondestructively and 3-dimensionally with multi-cross-sectional slices taken by a compact MRI in Japanese black pine seedlings, and compared to the distribution of nematodes destructively observed by fluorescent microscopy with F-WGA staining. In a seedling in which mass embolism occurred in slices from +2 cm to -2 cm from the inoculation point, there was a few nematode in cortex resin canal of +1 cm from the inoculation point. In a seedling in which mass embolism occurred from +1 cm to -8 cm, there were few nematodes beyond ±3 cm although there were many nematodes in xylem- and cortex resin canals and cambium around the inoculation point. When the total length of the observed xylem was embolized, nematodes were firstly observed at the opposite side of the inoculation point throughout the stem. These result suggested that nematode distribution was restricted to near the inoculation wound and patchy embolisms around xylem resin canals are induced by few nematodes which were moving through resin canals. After mass embolism occurred in total length of observed xylem, nematodes could colonize the opposite side of the stem from the inoculation point.

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