安息香酸を吸収させてマツノザイセンチュウを接種したアカマツの木部仮道管壁孔の解剖学的観察

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短　報
Phytopathological Note

Anatomical Observation of Bordered Pits in Xylem of *Bursaphelenchus xylophilus*-Inoculated *Pinus densiflora* Shoot Cuttings Treated with Benzoic Acid

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Key words: pine wilt disease, pine wood nematode, bordered pit, pit aspiration, benzoic acid, scanning electron microscopy.

A positive correlation has been established between disease development in pines infected with pinewood nematode (PWN, *Bursaphelenchus xylophilus*) and accumulation of benzoic acid in the diseased tree[1]. When Mamiya *et al.*[2] treated one-year-old shoot cuttings of *Pinus densiflora* with 0, 50, 100, 300, or 500 ppm benzoic acid and inoculated them with PWN, a) shoot cuttings treated with 300 ppm benzoic acid solution did not become diseased and survived, and their water status as shown by base xylem pressure potential and moisture content of wood was very high, b) shoot cuttings treated with 0, 50, or 100 ppm benzoic acid solution became disease and died, c) although shoot cuttings treated with 500 ppm benzoic acid solution died by day 4 after treatment, wood moisture content was almost the same as that of shoot cuttings treated with 300 ppm benzoic acid solution, d) PWN populations did not increased in shoot cuttings treated with 300 ppm benzoic acid solution. Water movement between tracheid lumina in xylem of conifers occurs through bordered pits[3].

The objective of this study is to find differences in the anatomical features of tracheid lumen and bordered pits between shoot cuttings treated with various concentrations of benzoic acid solution at the scanning electron microscope (SEM) level and to discuss the relation between water status and the differences.

Samples, collected from shoot cuttings which treated with benzoic acid and inoculated with PWN[2] 3 to 4 weeks after inoculation, were immediately fixed in 3% glutaraldehyde solution, washed with phosphate buffer, dehydrated through an ethanol-series, and dried by applying the critical point method. The dried samples then were split, and exposed surfaces were evaporated with gold using an ion sputter. The tracheid lumen and bordered pits were observed using a scanning electron microscope (Hitachi S-510).

Major causes for loss of water conduction ability in the xylem of conifers are: (1) pit aspiration, *i.e.* closure of the pit aperture because of the torus being displaced and pushed against the pit border, (2) physical blockage in the lumen, (3) disruption of tracheids, and (4) plugging pit membranes with macromolecules[4]. We were unable to observe physical blockage of the lumen,

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disruption of tracheids and plugging of pit membranes, but there were distinctive differences in the anatomical features of bordered pits dependent on the concentration of benzoic acid solution used to treat the pines. Namely most of bordered pits were aspirated in dead shoot cuttings which were treated with 0, 50, or 100 ppm benzoic acid solution (Palte I-1). While in living shoot cuttings treated with 300 ppm benzoic acid solution and in dead shoot cuttings treated with 500 ppm benzoic acid solution bordered pits did not aspirate (Plate I-2) and several openings on the torus of some bordered pits were observed (Plate I-3). Aspirated bordered pits in dead pine trees inoculated with PWN were also shown by Ikeda and Suzaki[4].

It is thought that cavitation occurs in the tracheid lumen of xylem in PWN-inoculated pines[4,5] and that this may cause the increase in xylem resistance to water movement being due to the cavitation-induced aspiration of bordered pits[4]. Concentration of benzoic acid greater than 300 ppm seems to prevent cavitation so that bordered pits were not aspirate and to make openings on the torus. Therefore water movement in shoot cuttings treated with 300 ppm benzoic acid solution was not disturbed and water status remained high. This is apparently related that PWN populations in shoot cuttings treated with 300 ppm benzoic acid solution did not increase although living PWN were found in healthy cuttings.

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和 文 摘 要

池田武文・真宮靖治・庄司次男：安息香酸を吸収させてマツノザイセンチュウを接種したアカマツの木部仮道管壁孔の解剖学的観察

アカマツの1年生切り枝に各種濃度の安息香酸 (BA) 溶液を吸収させた後，マツノザイセンチュウを接種した。接種3～4週間後の切り枝の木部を走査電子顕微鏡で観察した。100 ppm 以下の溶液で処理した切り枝はすべて枯死し，木部仮道管の壁孔は閉塞していた。一方，300 ppm 溶液で処理した切り枝はほとんどが枯死せず，壁孔は閉塞していなかった。500 ppm 溶液で処理した切り枝は，BA 吸収による直接的影響で枯死したが，壁孔は閉塞していなかった。さらに 300, 500 ppm 溶液処理では，壁孔のトールスに穴が開いていた。これらのことから，300 ppm 濃度以上の安息香酸溶液は，壁孔の閉塞を防いだり，トールスに穴を開けることで木部水分通導性を高め，切り枝の水分状態を高く保っていることが考えられた。このことは，300 ppm 溶液で処理した罹病していない切り枝で，生きたマツノザイセンチュウの個体数が増加しないことと関連することが推察された。

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Explanation of plate

Plate I. Anatomical features of bordered pits.

1. Aspirated bordered pit of shoot cutting treated with 100 ppm benzoic acid solution. M: margo, T: torus.
2. Non-aspirated bordered pit of shoot cutting treated with 300 ppm benzoic acid solution. M: margo, T: torus.
3. Disrupted torus of bordered pit of shoot cutting treated with 300 ppm benzoic acid solution.

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Plate I