Size and Density of Resin Canals are not Factors Preventing Pathogen Activities in *Pinus densiflora* Cultivars Resistant to Pine Wilt

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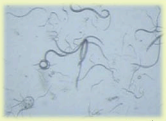
■ Introduction

Japanese red pine (*Pinus densiflora* Sieb. et Zucc.) and black pine (*P. thunbergii* Parl.) are highly susceptible to the pine wilt disease caused by the pine wood nematode (*Bursaphelenchus xylophilus*; PWN).

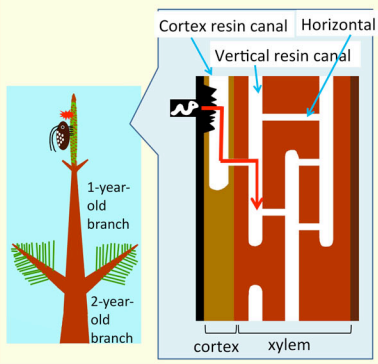


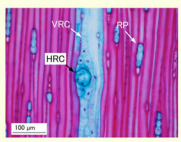
Pine wood nematode ↑

A Japanese red pine tree killed by the inoculation with PWN →

Resistant trees have been selected from the survived trees in the forests extensively damaged by this disease (Tajima, 1990). However, a certain proportion of the seedlings indicates low resistance and are killed after inoculation with PWN. We have to pick highly resistant trees based on the scientific criteria to produce seedlings that can survive from the PWN infection. Thus factors controlling resistance must be elucidated.

The migration and population growth of the PWN is suppressed in the resistant species and in some resistant cultivars of these susceptible species (Kuroda et al, 1991) However, the factors relating to the blockage of PWN in the resistant cultivars is still unknown. We hypothesized the structure or shape of resin canals that are the migration route of PWN from infection site (twig) to stem and roots may be contributing the retard of PWN migration. We investigated the size and the density of resin canals in xylem and checked whether those can be the factors preventing the migration of PWN.



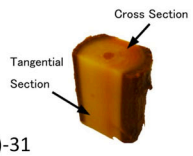


←Connection of vertical and horizontal resin canals in xylem. Tangential section, Stained with safranin and fast green, 20x

HRC: Horizontal resin canal

Pinus species have vertical and horizontal resin canals in xylem. PWN move through both resin canals and the connect points to migrate pine stems.

■ Materials and Methods



Plant materials

Pinus densiflora Sieb. et Zucc.

2-year-old branches of the following trees were used.

(1) Resistant cultivars*: Soja-(d)-39, Kibi-(d)-77, Kunimi-(d)-31
The resistance of these cultivars are graded as highest (grade5)

(2) Susceptible trees : Susceptible 1, Susceptible 2

Microscopy

Cross and tangential sections were made from specimens and stained with Nile blue. Anatomical characters of resistant cultivars were compared with those of susceptible trees under the light microscope.

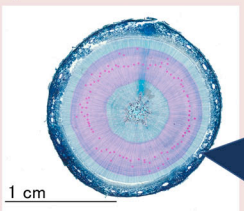
Measurement of size and density of resin canals

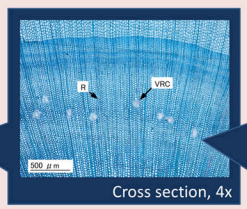
We measured the area inside of epithelial cells. Also we counted the number of resin canals in xylem and calculated the density per mm2.

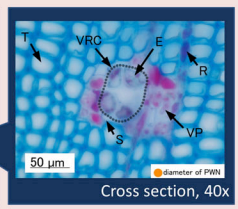
■ Results

1) Vertical resin canal

E: Epithelial cell
HRC: Horizontal resin canal
RP: Ray parenchyma
S: Sheath cell
VP: Vertical parenchyma
VRC: Vertical resin canal





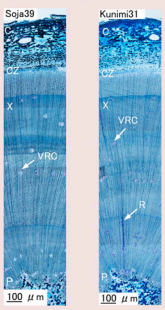


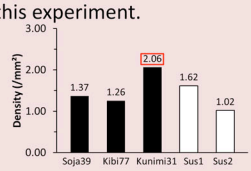
We counted vertical resin canals in 1-year-old xylem.

We measured inside of dotted line of a vertical resin canal.

➢ Density

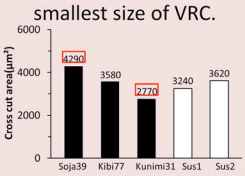
Kunimi-(d)-31 has the largest density of VRC among three resistant cultivars and susceptible two trees used for this experiment.

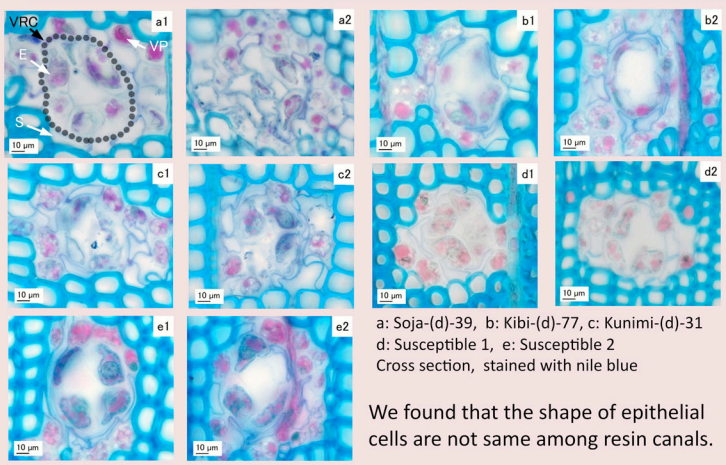




➢ Size

Soja-(d)-39 has the largrst size of VRC.
Kunimi-(d)-31 has the smallest size of VRC.

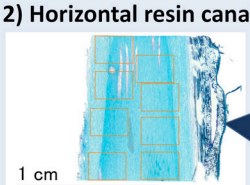


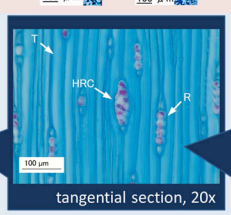


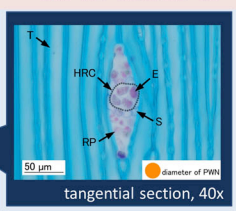
a: Soja-(d)-39, b: Kibi-(d)-77, c: Kunimi-(d)-31
d: Susceptible 1, e: Susceptible 2
Cross section, stained with nile blue

We found that the shape of epithelial cells are not same among resin canals.

2) Horizontal resin canal

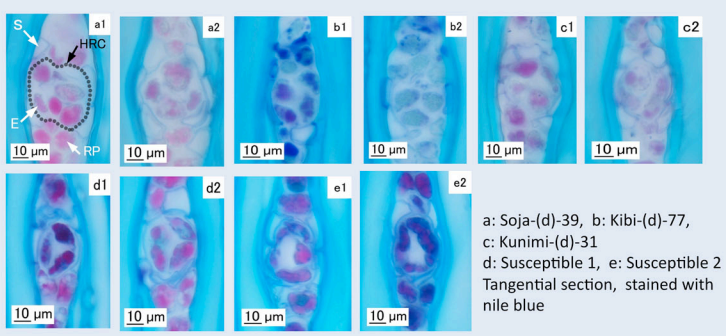






In tangential section, we counted horizontal resin canals more than 50% area of a section and averaged the number.

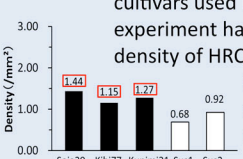
We measured inside of dotted line as a horizontal resin canal.



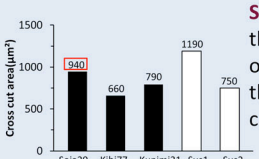
a: Soja-(d)-39, b: Kibi-(d)-77, c: Kunimi-(d)-31
d: Susceptible 1, e: Susceptible 2
Tangential section, stained with nile blue

➢ Density

Three resistant cultivars used in this experiment have larger density of HRC than that of susceptible trees.

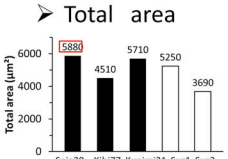


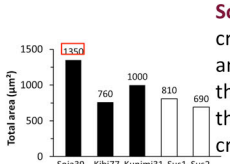
➢ Size



Soja-(d)-39 has the largest size of HRC among three resistant cultivars.

➢ Total area





Soja-(d)-39 has the largest total cross cut area of resin canals among resistant cultivars. Also in the cortex, Kuroda (2011) reported that Soja-(d)-39 has large total cross cut area of vertical resin canals in resistant cultivars.

■ Discussion

There was no clear tendency that the resistant cultivars have less or smaller vertical and horizontal resin canals in xylem than susceptible trees. This result suggests that resistant cultivars do not block pathogen's activity by the simple physical characters such as narrow resin canals.

The factors related to the resistance to PWN may be multiple. We will continue the investigation on the resin canal structure especially on the connection of resin canals at the knots (base of branches) that may affect the migration of PWN.