Water Relations of Quercus monogolica var. grosseserrata Seedlings Inoculated with Raffaelea quercivora : Ambrosia Fungi Related with Mass Mortality of Oaks in Japan

Water Relations of *Quercus mongolica* var. *grosseserrata* Seedlings Inoculated with *Raffaelea quercivora*: Ambrosia Fungi Related with Mass Mortality of Oaks in Japan

Mariko YAMATO, Toshihiro YAMADA, Kazuo SUZUKI

Graduate School of Agricultural and Life Sciences, The University of Tokyo, Tokyo, 113-8657, Japan

Abstract – Mass mortality of oaks (*Quercus serrata* and *Q. mongolica* var. *grosseserrata*) has appeared along the Japan Sea since the late 1980's. The oak mortality may be associated with the blockage of sap ascent that is induced by infection with *Raffaelea quercivora*, an ambrosia fungus vectored by the pinhole borer *Platypus quercivorus*,. We inoculated *Q. mongolica* seedlings with *R. quercivora* and investigated the water relations of the seedlings. Hydraulic conductivity of the inoculated seedlings was significantly lower than that of control seedlings near the inoculation point. We concluded that the blockage of sap flow occurring around the inoculation point causes the tree's death.

I. Introduction

Mass mortality of deciduous oaks has been prominent, along the coast of the Japan Sea since the 1980's,. The whole crown of mature oaks wilts and turns brown within a few weeks in mid summer, resulting in high levels of mortality of *Quercus mongolica* var. *grosseserrata* and *Q. serrata* [1, 2].

The dead oaks were mass-attacked by the pinhole borer *Platypus quercivorus*, an ambrosia beetle that makes intricate gallery inside the wood. Dark discoloration expands around the beetle's gallery. Previous research revealed that the sap conduction of this discolored sapwood is disturbed [4], and the recently described ambrosia fungus *Raffaelea quercivora* was isolated from the discolored sapwood and *P. quercivorus* [3]. *R. quercivora* is hypothesized to be closely associated with the mortality of oaks.

The current hypothesis of the mechanism of oak mortality is as follows: The *R. quercivora* ambrosia fungus is transmitted by mass attacks of *P. quercivorus* pinhole borers; the fungus expands in the oak sapwood and simultaneously disturbs the flow of sap in many places, thus causing the death of the tree by widespread blockage of sap conduction.

The objective of this study was to clarify the mechanism of tree mortality induced by *R. quercivora* infection. We investigated the water relations of oak seedlings inoculated with *R. quercivora* to understand the relations among *R. quercivora* infection, disturbance of sap conductance, and tree mortality.

II. Materials and Methods

All experiments were conducted at the Tanashi Experiment Station, Tanashi, Tokyo. Four year old *Q. mongolica* var. *grosseserrata* seedlings, 0.6-1.0 m tall, with 15-20 mm basal diameters were planted in the Tanashi nursery field. Forty-eight seedlings were inoculated with *R. quercivora* on July 1st, 2002. The inoculum was prepared by growing *R. quercivora* on wheat and rice bran media for 2 weeks under dark conditions at 23 ℃. At approximately 20 cm above the soil line, the bark of each seedling was peeled in a 5 mm wide semicircle on both sides of the stem, 5 mm vertically apart, and two small notches were added on the opposite side of each wound (Fig. 1). *R. quercivora* incubated bran media was placed on the wounds and plastic film was wrapped around it. Sixteen control seedlings were inoculated similarly with sterile media.
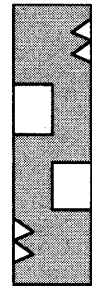

Fig.1 Wound for inoculation

A. Physiological measurements

Transpiration rate and chlorophyll fluorescence were measured using a LI-1600 steady state porometer (Li-Cor, Inc. Lincoln, NE, USA) and a MINI-PAM photosynthesis yield analyzer (Heinz Walz GmbH, Eichenring, Effeltrich, Germany), respectively. Transpiration rate was measured on one leaf of each seedling during the midday hours (11.00-15.00). Chlorophyll fluorescence was measured on three leaves of each seedling at night (22.00-24.00) and maximal quantum yield of photosystem II (Fv/Fm) was calculated [6].

B. Hydraulic conductivity

Hydraulic conductivity of the stem was measured using a High-pressure Flow Meter (HPFM) (Dynamax, Inc. USA) [9]. Seedlings were cut at their base when they showed wilt symptoms, and the stem was divided into 5-20 cm lengths under water. The stem segment was connected to HPFM with a watertight seal. Conductivity was measured using a "transient measurement". Water flow into the stem (F, in m^3) and applied pressure (P in Mpa) were measured every 3 s while increasing the pressure at a constant rate of 3-7 kPa S^{-1},

and dF/dP was calculated as the slope of the plot of F versus P. Segment length (L in m) and cross sectional area of the xylem (A in m^2) were also measured. Hydraulic conductivity was calculated as:

K = (dF/dP) L/A

Fourteen seedlings were measured as wilt seedlings. Seedlings inoculated with *R. quercivora* that appeared to be healthy were sampled 6, 17, 21, 45 days after inoculation, and control seedlings inoculated with sterile media were sampled at 5, 10, 15, 20, 40 days after inoculation. We measured the hydraulic conductivity of one seedling at a time, using the methods described above.

C. Re-isolation of R. quercivora

Seedlings were checked for *R. quercivora* expanded length after we measured hydraulic conductivity. Five pieces of 3 mm^3 xylem were cut from 20 cm above and 8 cm below the inoculation point, at 2 cm intervals.. The xylem cubes were put on Potato Dextrose agar media, incubated for 1 week under dark at 23℃ and then checked for the presence of *R. quercivora* fungi.

III. Results

External symptoms of wilt appeared 5-60 days after inoculation with *R. quercivora* fungi. Whole foliage above the inoculation point turned brown within 1-3 days following slight drying of the foliage (Fig. 2). Foliage below the inoculation point showed no apparent change even when the part of the tree above the inoculation point had wilted. By 60 days after the inoculation, 61% of the inoculated seedlings had wilted. Light gray discoloration was observed on the sapwood near the inoculation point. Bark tissue around the inoculation point showed necrosis.

A. Physiological measurements

The average transpiration rate of *R. quercivora* inoculated seedlings became significantly lower (P<0.01) than the control seedlings at 11 days after inoculation and it did not recover until the end of the experiment (Fig. 3). Transpiration rates of seedlings that eventually wilted had dropped to less than half the value of the controls from 3-17 days after inoculation (Fig. 4). The duration between the decline of transpiration rates and the appearance of external symptoms varied among individual seedlings. A few seedlings survived for 40 days with low transpiration rates of around 0.5μg cm^{-2} s^{-1}; transpiration rates this low indicate that most of the stomata on the leaf measured were closed.

For the *R. quercivora* inoculated seedlings, the maximum quantum yield of phyotosystem II dropped rapidly with the appearance of wilt symptoms (Fig. 5). Before this rapid drop, no significant difference was detected between *R. quercivora* inoculated seedlings and controls.

B. Hydraulic conductivity

The hydraulic conductivity of both the *R. quercivora* inoculated seedlings and the controls was reduced near the inoculation point. The hydraulic conductivity of wilted seedlings was significantly lower than that of controls, within a 15 cm distance from the inoculation point (Student's *t*-test, P<0.01) (Fig. 6). However, there was no significant difference between inoculated and control stems at distances more than 15 cm from the inoculation point. Hydraulic conductivity of intact seedlings ranged between 0.40 and 0.97 (Kg s^{-1} Mpa^{-1} m^{-1}). Values for *R. quercivora* inoculated seedlings that looked healthy ranged between the values of wilted and control seedlings.

C. Re-isolation of R. quercivora

The maximum expanded length of *R. quercivora* was 20 cm at 45 days after inoculation (Fig. 7). *R. quercivora* was recovered only from wilted seedlings.
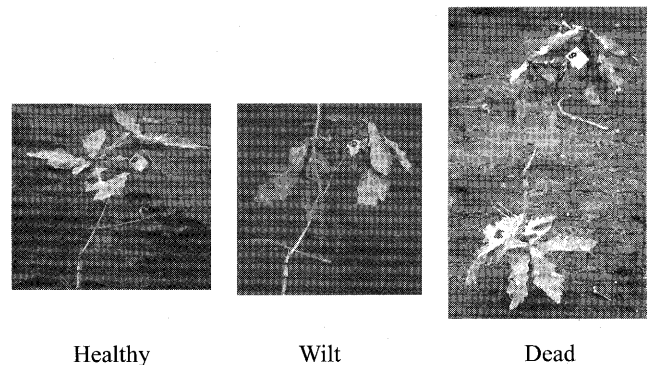

Healthy   Wilt   Dead

Fig. 2. Wilt process of *R. quercivora* inoculated seedlings. Foliage below the inoculation point showed no change even when the part of the tree above the inoculation point was wilted.
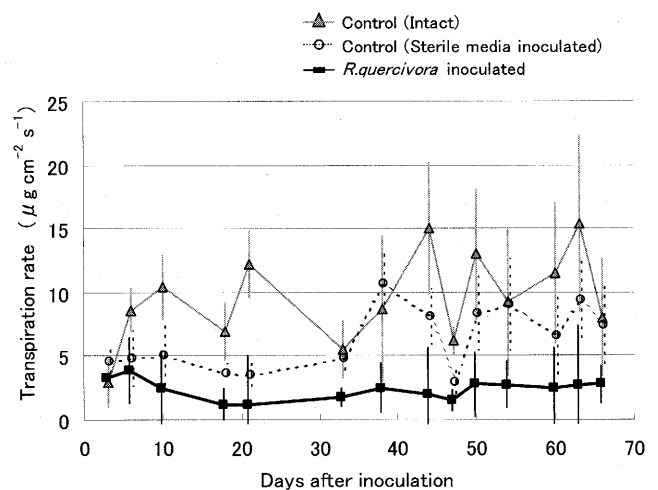

Fig. 3. Average transpiration rate of *Q. mongolica* var. *grosseserrata* seedlings inoculated with *R. quercivora*. Error bars are standard deviations.
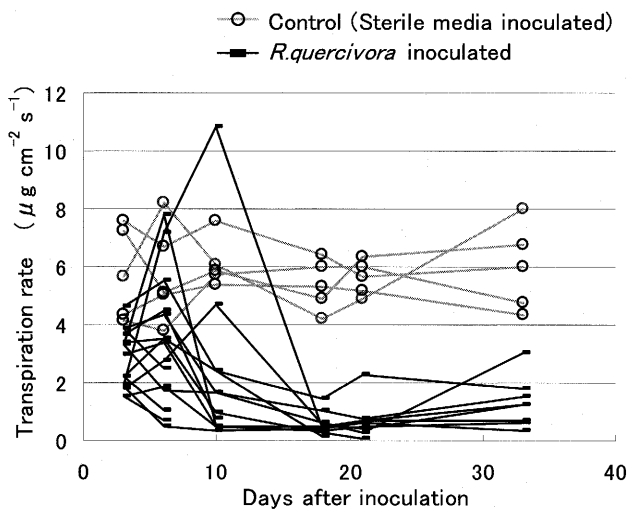
Fig. 4. Transpiration rate of *R. quercivora* inoculated seedlings that eventually wilted.
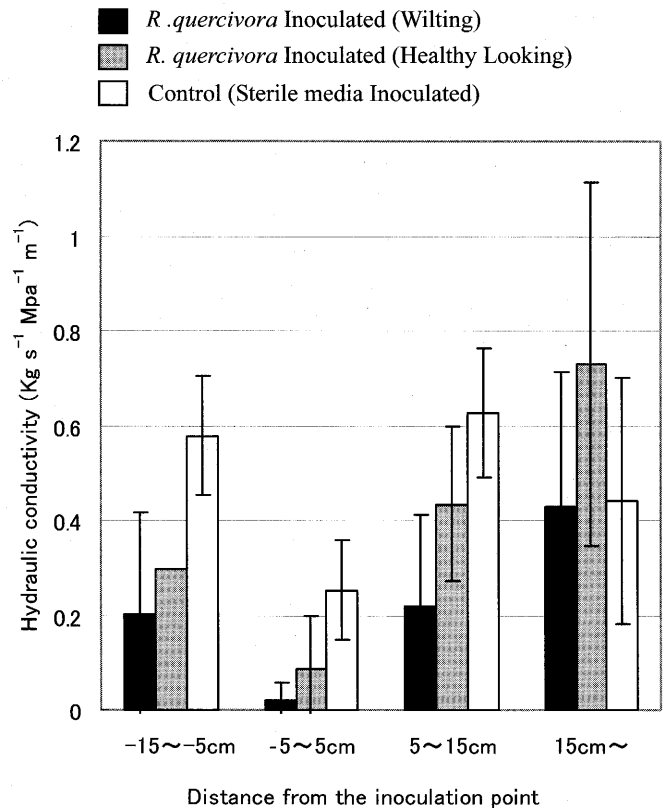

Fig. 6. Hydraulic conductivity of the seedlings inoculated with *R. quercivora*. Error bars are standard deviations.
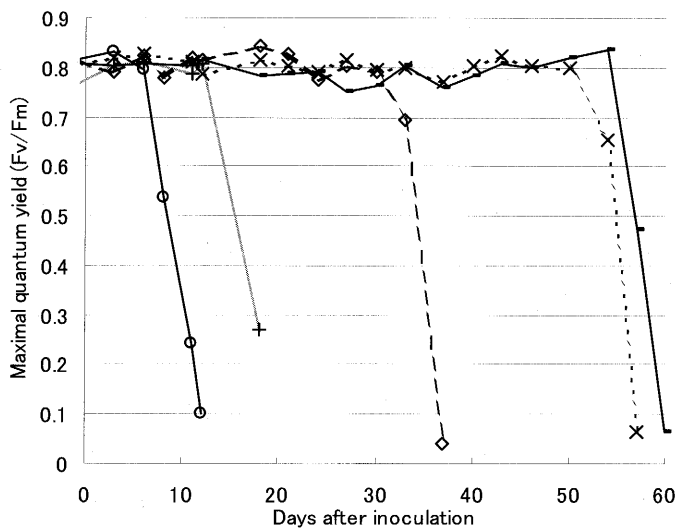

Fig. 5. Maximum quantum yield of *R.quercivora* inoculated seedlings that eventually wilted within 60 days after inoculation.
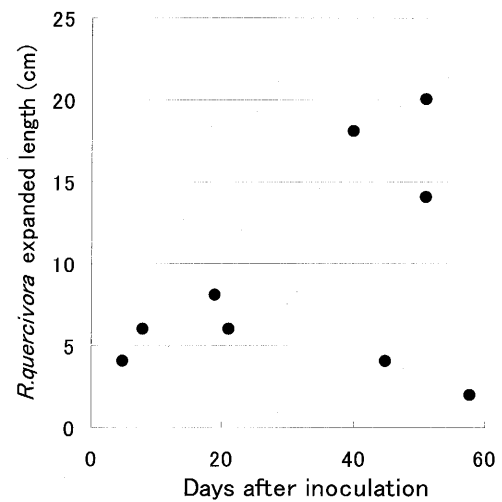

Fig. 7. Maximum expanded length of *R quercivora*. Each point represents one seedling.

IV. Discussion

Hydraulic conductivity was significantly reduced within a 15 cm distance from the inoculation point, which was similar to the maximum expanded length of *R. quercivora*. This demonstrates that *R. quercivora* distributes locally around the inoculation point and affects the hydraulic pathway around the infected xylem. Hydraulic conductivity can change as a result of interruption of the water column in the vessels. The possible causes of interruptions are vapor blockage (cavitaion) [5], high molecular substances that clog the pit membrane, and occluding components such as fungal hyphae, tyloses, and gums. There are reports that tyloses and gums are abundant around the xylem infected with *R. quercivora*. Further research is necessary to clarify what factors are responsible for the reduction of hydraulic conductivity.

Transpiration rates dropped and remained low for *R. quercivora* inoculated seedlings. This suggests that stomata closed in order to maintain turgor pressure under reduced hydraulic conductivity [8]. Even with closed stomata, evaporation from the cuticle and bark would have continued. With severe reduction of hydraulic conductivity, this evaporation would be enough to cause the seedlings to wilt .

Maximum quantum yield of phyotosystem II (Fv/Fm) dropped rapidly with the appearance of wilt symptoms. According to previous research, this rapid drop was delayed by supplying water to the wilting part of the tree [10]. This suggests that the change of Fv/Fm was caused by water deficits.

R. quercivora distributes locally around the inoculation point, causing significant reduction of hydraulic conductivity. We conclude that this blockage of sap flow is the cause of tree death. The unique part of this wilt mechanism is the localization of the pathogen. With other wilt diseases such as Oak wilt or Dutch elm disease, the pathogen induces leaf wilt after it invades the whole tree [7]. Further research is necessary to confirm whether blockage of sap flow alone is sufficient to induce wilt.

References

[1]   S. Ito, T. Yamada, "Distribution and spread of the mass mortality of oak trees," *Journal of Japanese Forestry Society*, Vol. 80, pp. 229-232, 1998. (In Japanese)

[2]   S. Ito, T. Kubono, N. Sahashi, T. Yamada, "Associated fungi with the mass mortality of oak trees," *Journal of Japanese Forestry Society*, Vol. 5, pp. 170-175, 1998. (In Japanese with English summary)

[3]   T. Kubono, S. Ito, "*Raffaelea quercivora* sp. nov. associated with mass mortality of Japanese oak, and the ambrosia beetle (*Platypus quercivorus*)." Mycoscience, Vol. 43, pp. 255-260, 2002.

[4]   K. Kuroda, T. Yamada, "Discoloration of sapwood and blockage of xylem sap ascent in the trunks of wilting *Quercus* spp. Following attack by *Platypus quercivorus*," *Journal of Japanese Forestry Society*, Vol. 78, pp. 84-88, 1996. (in Japanese with English summary)

[5]   D. Newbanks, A. Bosch, M. H. Zimmermann, "Evidence for xylem dysfunction by embolization in Dutch elm disease,"

Phytopathology, Vol. 73, pp. 1060-1063, 1983.

[6]   K. Rohacek, M. Batak, "Technique of the modulated chlorophyll fluorescence: basic concepts, useful parameters, and some applications. *Phyotosynthetica,* Vol. 37, pp. 339-363.

[7]   W. Sinclair, H. H. Lyon, W. T. Johnson, *Disease of Trees and Shrubs*, Cornell University Press, 1987.

[8]   J. S. Sperry, N. N. Alder, S. E. Eastlack, "The effect of reduced hydraulic conductance and xylem cavitation," *Journal of Experimental Botany*, Vol. 44, pp. 1075-1082, 1993.

[9]   M. T. Tyree, S. Patino, J. Bennink, J. Alexander, "Dynamic measurements of root hydraulic conductance using a high-pressure flowmeter in the laboratory and field," *Journal of Experimental Botany*, Vol. 46, pp. 83-94, 1995.

[10]   M. Yamato, T. Yamada, K. Suzuki, "Wilt of oaks – blockage of xylem sap ascent," Bulletin of the Tokyo University Forests, Vol. 106, pp. 69-76, 2002. (in Japanese with English summary)