Detection of Wood Discoloration in a Canker Fungus-Inoculated Japanese Cedar by Neutron Radiography

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Dieback and canker disease of trees, such as Guignardia dieback / canker of Japanese cedar (*Cryptomeria japonica*) and Hinoki cypress (*Chamaecyparis obtusa*), is a serious problem for forestry, since it causes wood discoloration and decay, and sometimes results in mass mortality of these tree species. Outbreak of this disease is predisposed by stresses such as drought. We are studying the interaction among the host tree, the pathogenic fungus (*Guignardia cryptomeriae*), and the environmental stresses to find out the way to control the disease.

Since the attenuation coefficient of neutron is distinctively larger for light elements, such as H or B, neutron radiography NRG is a promising method to know the water distribution within living plants including woody plant species.

Moisture content generally decreases in discolored sapwood, which was induced by fungal invasion, and in surrounding transition zone (dry zone) of living coniferous trees, because of the blockage of sap ascent. In the present study, NRG was applied to trace the development of lesion in the wood of Japanese cedar after the canker fungus inoculation, to study the host-parasite interactions.

Because the area detected by NRG is thought to cover both discolored wood and dry zone, we considered the detected area the lesion in the present study.

Fig. 1 Bark lesion (upper) and wood discoloration (lower) of Japanese cedar inoculated with *G. cryptomeriae*. Inoculation test in 1990.

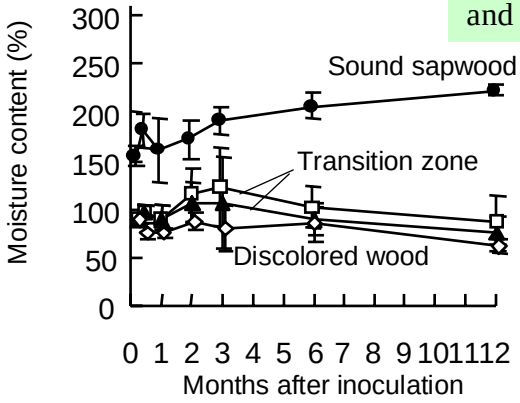

Fig. 2 Moisture content of discolored wood in Japanese cedar inoculated with *G. cryptomeriae*. Inoculation test in 1990.

Fig.4 Thermal neutron radiography facility of JRR-3M
Left, whole view; Center, inside the irradiation chamber; Right: seedling and Teflon standard fixed on an aluminum cassette, where an X-ray film (Kodak SR) and a gadolinium n / γ converter (25mm in thickness) were sealed in vacuum.

Effect of Neutron Beam Irradiation on the Fungal Growth

Experimental

Guignardia dieback fungus, *G. cryptomeriae* (virulent isolate MA14 and avirulent isolate MA21) was incubated on potato dextrose agar (PDA) in a plastic petri dish, and was irradiated with thermal neutron beam at a research reactor, JRR-3M, installed at the Japan Atomic Energy Research Institute (JAERI). The exposure time for neutron was 19 or 38s and the neutron flux was 1.5×10^8 n/cm^2/s. After the irradiation, fungus was incubated at 12, 20, and 25℃ in the darkness, and then hyphal growth rate was measured.

Results and Discussion

Most of neutron flux penetrated a plastic petri dish and PDA medium. No inhibitory effect, however, was observed on hyphal growth of the fungus in any case of this experiment, i.e., any isolate, exposure time, incubation temperature, and with or without the lid of petri dish (Figs. 4, 5). Neutron beam dose used in the experiment seemed to have no effect on the growth of the fungus inside the seedling, because of the smaller neutron dose compared with that for PDA.

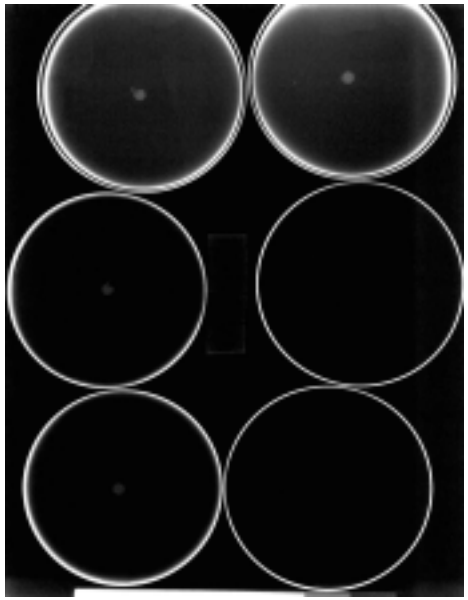

Fig. 4 NRG images of *Guignardia cryptomeriae* culture on PDA in plastic petri dishes.
Upper, with lid closed; Center & Lower, no lid.

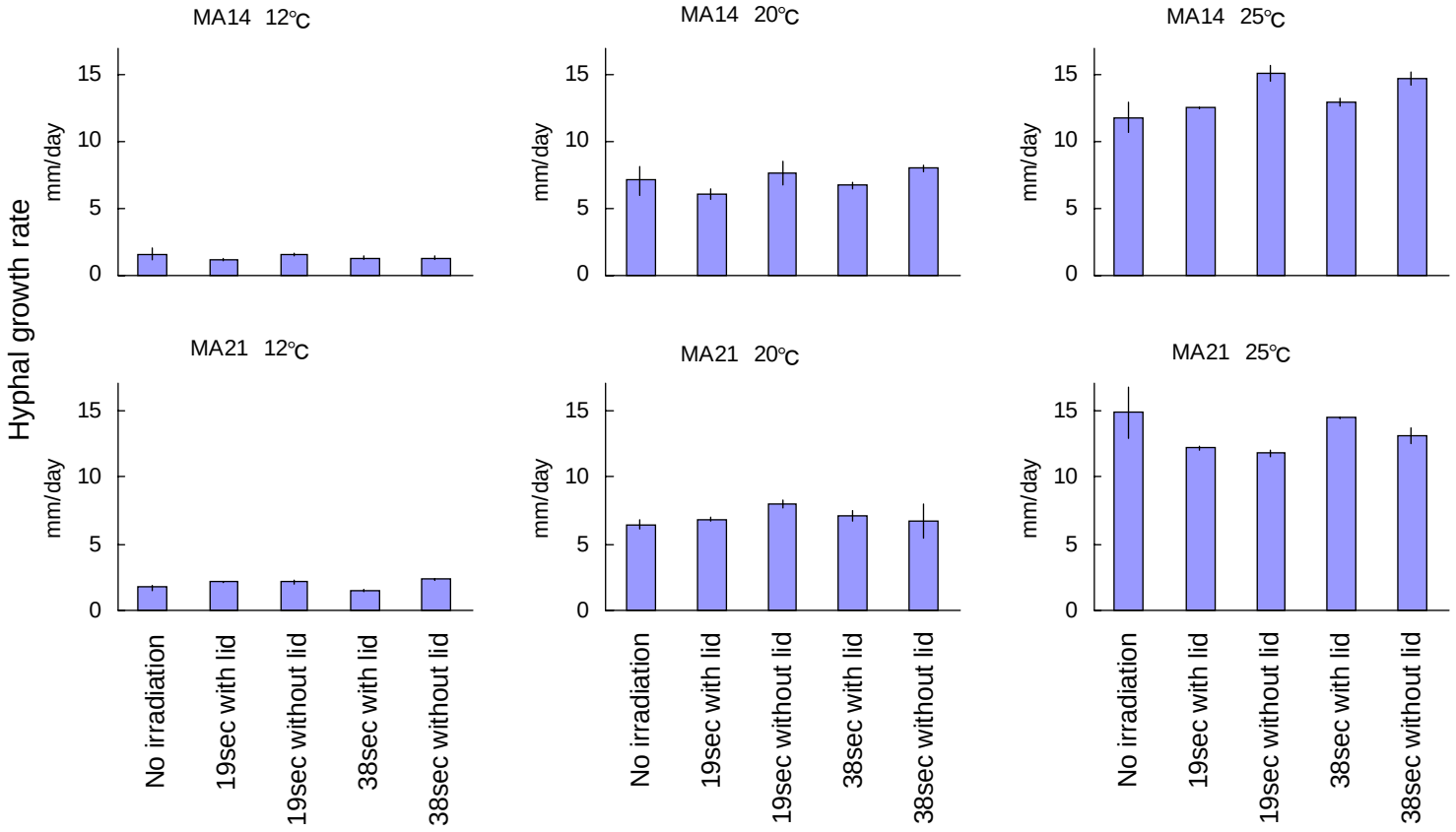

Fig. 5 Effect of neutron beam irradiation on the growth rate of *Guignardia cryptomeriae* isolate on PDA
Vertical bars indicate standard deviations.

Detection of Xylem Lesion

Experimental

Fungal inoculation The fungus was incubated on PDA with wooden toothpick at 25°C for 7 days. A potted 3-year-old Japanese cedar seedling (Fig. 6) was wounded or wound inoculated with an isolate MA14 (virulent) and MA21 (avirulent) of the fungus in June 2002. A toothpick containing fungal hyphae was inserted into the hole of ca. 3mm depth prepared with an awl. A sterilized toothpick was used for a wound control.

Irradiation of neutron and detection of xylem lesion Three, 7, 13, and 22 days after fungal inoculation or wounding, seedling was fixed on an aluminum cassette, where an X-ray film and a gadolinium n / γ converter were sealed. The cassette with the samples was irradiated for 19s with thermal neutrons in a neutron radiography facility at JRR-3M. A stairs-like Teflon® block or aluminum container filled with water was used as a standard. The seedling was kept at 22°C during the experiment.

Fig. 6 Inoculated seedling
Arrow indicates inoculation point.

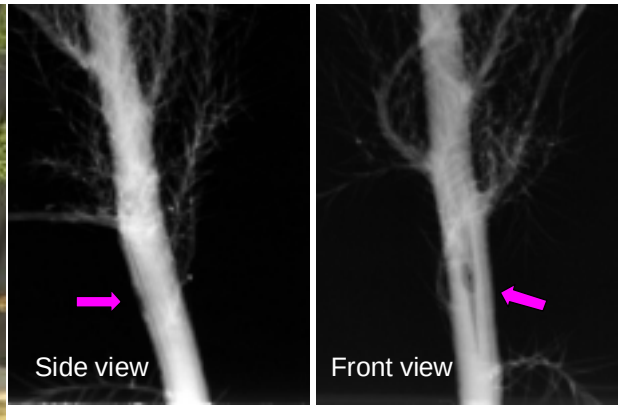

Fig. 7 Xylem lesion of seedling detected by NRG
22 days after MA14 inoculation. Arrows indicate inoculation points.

Results and discussion (1)

The whiteness in the NRG image corresponds to the content of water and the other H containing components in the sample. Discolored tissue and surrounding dry zone induced by the fungal inoculation was detected as dark area with high resolution (Fig. 7). Thus, it was indicated by NRG that xylem lesion of Japanese cedar was water deficient part, as well as destructive method.

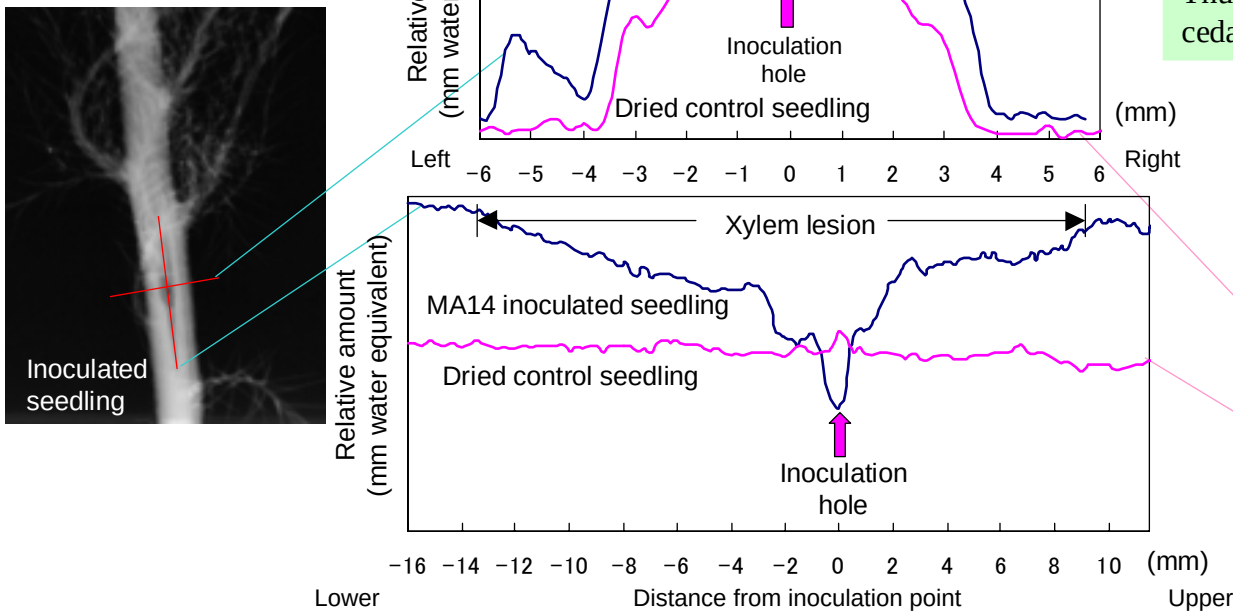

Fig. 8 Relative amount equivalent to water of inoculated seedling and dried control seedling

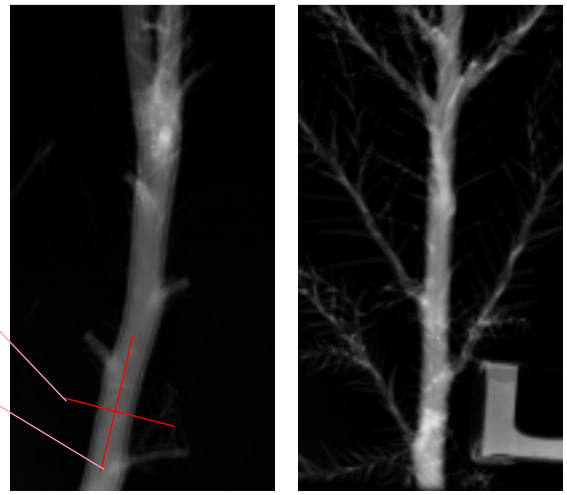

Fig. 9 NRG images of dried control seedling (left) and living control seedling (right)

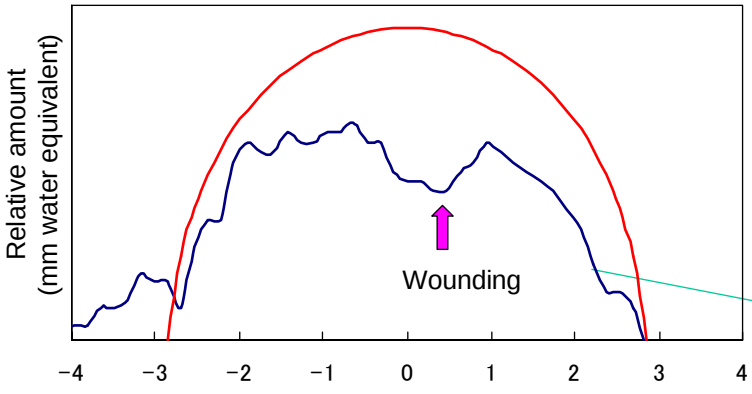

Fig. 10 Relative amount equivalent to water of wounded seedling (blue) and theoretical value of virtual water column (red).
Virtual water column: Stem of seedling was replaced with water.

Results and discussion (2)

Dry zone was detected as early as 3 days after inoculation or wounding (Fig. 12). Image of xylem lesion was very narrow and sharp 3 days after inoculation or wounding. It became broader and obscurer after 7th day of inoculation. Neutron images also showed the difference in the size of water deficient part due to the tissue damage among the treatment (Fig. 11).

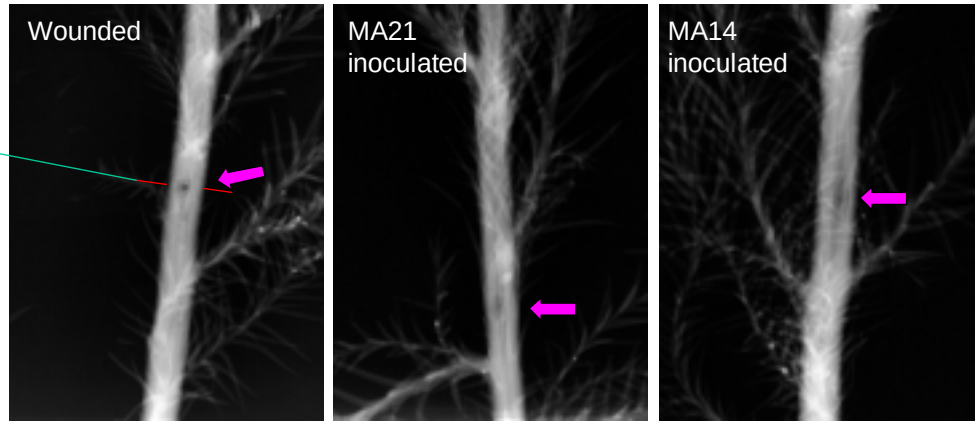

Fig. 11 Comparison of xylem lesion of seedling.
22 days after inoculation. Arrow indicates inoculation point.

Results and discussion (3)

Expansion of damaged tissue was most rapid in the seedlings inoculated with a virulent isolate (Fig. 13). Such a difference in lesion expansion rate appeared to reflect the difference in hyphal growth rate in the wood of living tree.

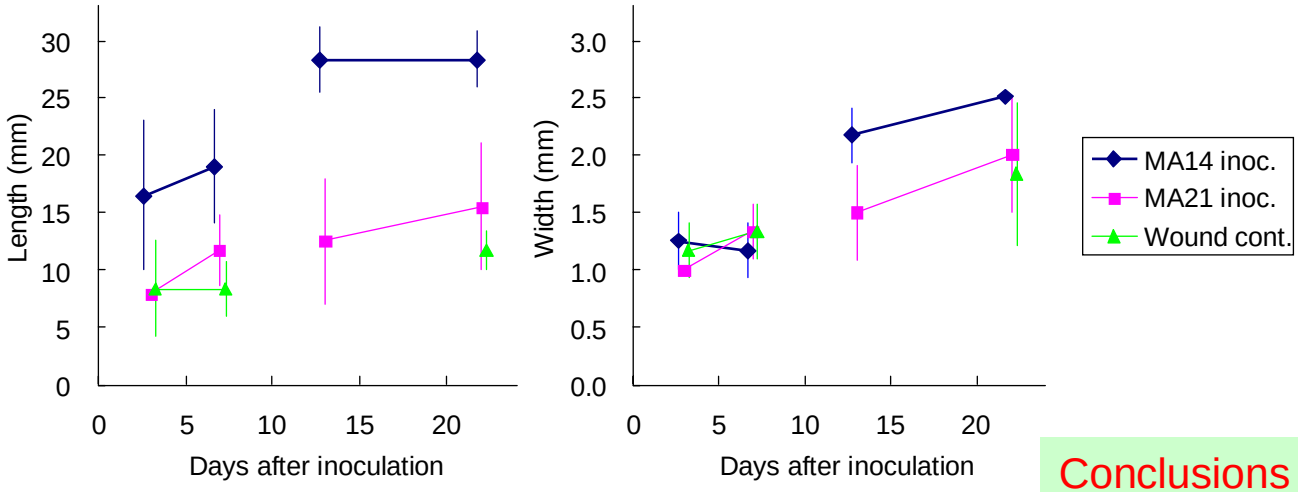

Fig. 13 Length (left) and width (right) of xylem lesion inoculated with *Guignardia cryptomeriae*
Seedlings measured at 3rd and 7th day, as well as those from 13th to 22nd day, were the same individuals. Bars indicate standard deviations.

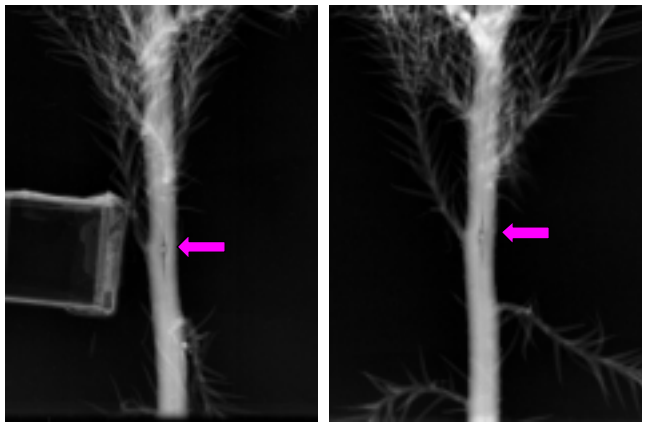

Fig. 12 Changes in xylem lesion of seedling.
3 days (left) and 7 days (right) after MA21 inoculation.
Arrows indicate inoculation points.

Conclusions

Neutron beam dose used in this experiment was found to have no effect on the growth rate of the fungus. We presented the performance of NRG method for nondestructive tracing of the lesion in Japanese cedar wood; therefore NRG is an effective tool for pathological research of trees.