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Detection of Embolism and Acoustic Emissions in Tracheids under a Microscope: Incidence in Diseased Trees Infected with Pine Wilt

by

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SUMMARY

During transpiration, xylem sap in water conduits is under tension. Water columns break under extreme tension and form bubbles (emboli). In healthy plants, water columns recover by rehydration of the conduits when the tension is reduced. However, in trees infected with wilting diseases, sap ascent stops because the xylem does not rehydrate. We observed embolism in light-microscope sections of pine (*Pinus densiflora*) inoculated with pine wood nematodes (*Bursaphelenchus xylophilus*). Embolism was recorded on videotape while acoustic emissions (AEs) were monitored with an AE transducer attached to the sections. We converted the ultrasonic AEs to audible signals and recorded them on the audio track of the videotape. Following the slow emptying of tracheids injured during sectioning, a bubble emerged within each intact tracheid, abruptly swelled, and filled the whole tracheid. The AEs coincided with almost all of the rapid bubble development. This result supports the idea that AEs detected in the trunks of living trees are produced by embolism in tracheids. In the second experiment, the time necessary for rehydration was measured after the addition of water to dehydrated xylem sections. Suggesting that certain substances that inhibit bubble dissolution may be released into the xylem after infection with pine wood nematode, the rehydration time is longer in tracheids of trees infected for one month than in healthy trees or trees infected for 10 days to two weeks.

Key words: Embolism, cavitation, xylem sap, acoustic emission, AE, *Pinus densiflora, Bursaphelenchus xylophilus,* Pine wilt disease, rehydration

INTRODUCTION

Xylem sap in water conduits is kept under tension during transpiration, but conduit water columns can break under high tension and form bubbles (Sperry & Tyree 1990). The bubbles expand rapidly and tracheids are dehydrated almost instantly (Lewis et al. 1994). This phenomenon is called embolism or cavitation and commonly occurs in healthy plants (Tyree & Sperry 1988). In healthy plants, xylem function recovers because dehydrated conduits are refilled with water (rehydration) when tension is released at night or during rain. In contrast, sap ascent completely stops in trees infected with wilting diseases like pine wilt disease. Kuroda (1989, 1991) found that water columns in tracheids break without refilling following infection with the

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pathogen. Ultrasonic acoustic emissions (AEs) can be detected during the cavitation of water conduits (Tyree & Sperry 1989). Using the AE technique of Ikeda & Ohtsu (1992), Kuroda (1995) clarified that AEs increase and are detectable even during the night 1 to 2 weeks after infection with the pine wood nematode. The area of nonconducting conduits rapidly enlarges during and after that period (Kuroda 1991; Kuroda *et al.* 1989).

In this study, the authors applied Lewis' method for viewing and recording embolism in xylem sections (Lewis 1987; Lewis et al. 1994) to elucidate the mechanism of xylem water disruption in diseased trees. We visually monitored embolism in light-microscope sections cut from trunks infected with pine wilt disease, and recorded AEs during embolism on the same sections. Rehydration after embolism in diseased trees was compared with rehydration in healthy trees. We discuss the relationship between bubble development and AEs in trees. Also, we discuss embolism without rehydration in pine wilt disease.

MATERIALS AND METHODS

Samples

Three-year-old Japanese red-pines (*Pinus densiflora* Sieb et Zucc.), 17 trees as a total, were inoculated with 7000/tree pine wood nematodes (*Bursaphelenchus xylophilus* (Steiner et Buhrer) Nickle) on Oct. 1, 1997 and harvested 2 weeks later, or inoculated with 10000/tree nematodes on Sept. 7, 1998 and harvested 10 days or 1 month after inoculation. At each sampling, one or two healthy, uninoculated trees were harvested as control. Trees were kept in the refrigerator until processing covering the cut ends of trunks with wetted tissue paper. The trees were classified by infection symptoms detected in stem transverse sections; areas of nonconducting conduits were visible as white patches (Kuroda 1991). Samples for monitoring embolism and AEs were obtained from lower stem at least two sites as 2 cm long stem segments (10 to 15 mm diameter). Radial sections of 60 or 70μm thick that contained an intact layer of tracheids were cut from the segments with a sliding microtome, soaked in water, and immediately used for experiments.

Observing embolism

Embolism in tracheids was recorded on videotape for observation by assembling an AE tester, AE transducer (140 kHz), data logger, color CCD camera, video tape recorder (VTR), and monitor with a light microscope as in Fig. 1. To monitor AEs as audible sound, the pulse terminal of the AE tester was connected to the audio terminal on the VTR, which resulted in conversion of the ultrasonic AEs to audible signals on the videotape audio track.

Xylem strips, 1mm wide by 5mm along the tracheids, were cut from the radial sections of infected and healthy trees. The strips from infected trees were classified by the visible symptoms of nematode infection : occlusion with oily substances or embolized, and areas without visible changes. A strip was set on the microscope slide without a cover glass. One end was secured with a loop of nylon thread, while the other end was held with the tip of the spring-metal clip that was attached to the AE transducer. Strips were left under the microscope during dehydration. Bubble formation in tracheids and AEs were recorded on videotape.

Collapse of bubbles following rehydration

To study the difference in the time required for rehydration in infected and healthy trees, the time for complete bubble collapse in the xylem strips was measured using the method described in Lewis et al. (1994). Three radial strips from each class were prepared from xylem areas with symptoms classified as pre-existing emboli, occlusion with oily substances, and

without change (normal) in infected trees, and from controls. Radial strips were dried for 3 minutes at room temperature, and then water drops were added to rehydrate the tissue. Bubble shrinkage was observed periodically at 15 minute intervals, until all bubbles collapsed. The experiment was conducted twice with all specimens. The time necessary for complete bubble collapse was measured, and healthy and infected pines were compared.

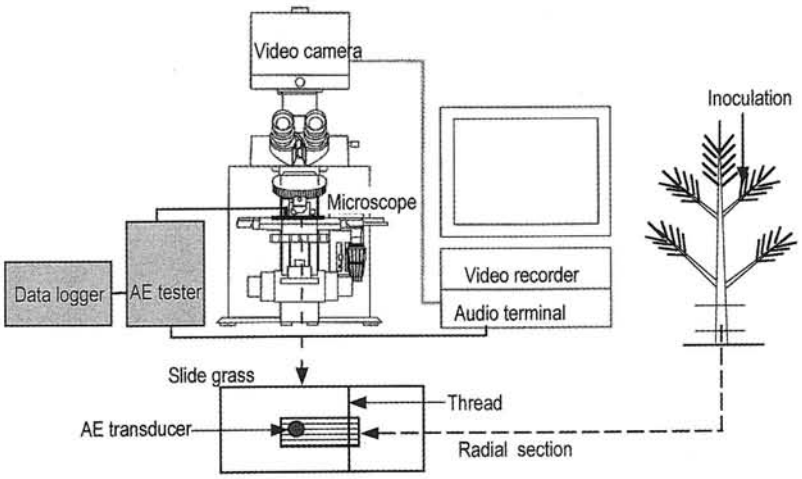

Fig. 1 How to detect embolism and AEs in xylem sections.

RESULTS

Embolism in tracheids and AEs

First, water in the xylem strips slowly withdrew from tracheids that were injured during sectioning. This was not abrupt embolism and no AEs were detected. Then, bubbles emerged within intact tracheids. In most cases, a bubble abruptly swelled and filled an entire tracheid within a second (Fig. 2, see Internet home page[4]). In rare cases, bubble expansion was slow and took a

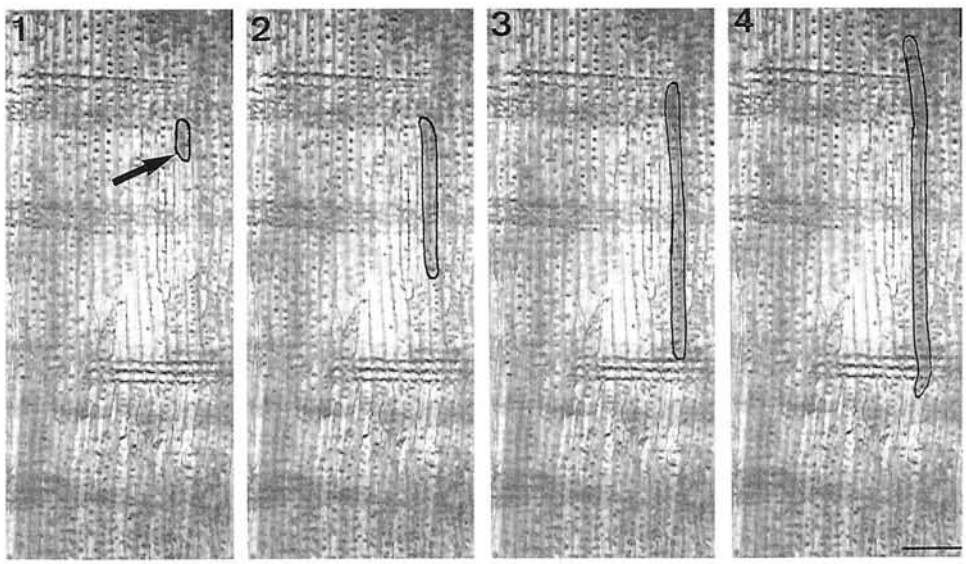

Fig. 2 Embolus formation (arrows) in xylem sections of *Pinus densiflora* (Bar:100㎛).

few seconds to fill the tracheid. During high-rate bubble formation, many AEs were produced (Fig. 3). On the videotape, the audible sound converted from ultrasonic AEs coincided with almost all of the rapid bubble development (see Internet home page[4]).

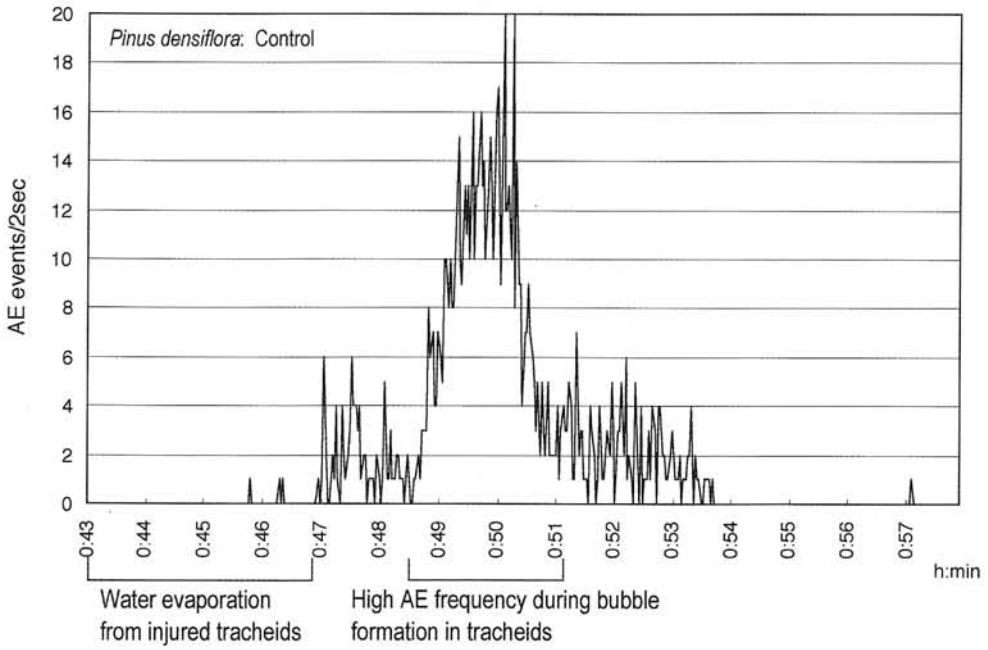

Fig. 3 AEs monitored during high-rates of embolism in *P. densiflora* xylem sections.

Time necessary for complete bubble collapse

In some specimens harvested 10 days to two weeks after inoculation with pathogenic nematodes, the nonconducting area was just visible as small white patches on transversely cut surfaces of the trunks (Kuroda 1991). In and around such areas, pre-existing emboli and leaks of oily substances were observed under the light-microscope (Table 1). In the specimen harvested one month after inoculation, there was more nonconducting xylem than in other specimens and oil occlusions were abundant.

Table 1. Disease development and visible features in sample trees.

Time after inoculation *	Tree	Stage of disease development
10 days	D	None
	C	Oil occlusions around resin canals
	A	Oil occlusions; some pre-existing emboli
	E	Oil occlusions; some pre-existing emboli
1 month	B	Pre-existing emboli
Control	1	None
	2	None
	3	Seasonal emboli in latewood

** Sample trees harvested 2 weeks after inoculation were omitted from the list because symptom development was slightly different between the year of experiments.*

In the rehydration experiments, each xylem strip was checked under the microscope for oil occlusions. After the addition of water, bubbles remained large for a while (Fig. 4a). Bubbles

shrank in a process that was much slower than embolus development (Fig. 4b). Time for complete bubble dissolution in xylem strips of the three symptom classes is indicated in Fig. 5. There were differences between inoculated and healthy trees. Areas with pre-existing emboli and those with oil occlusions took longer to rehydrate than xylem strips with no visible changes (Table 1; Fig. 5). The time for complete collapse in samples from healthy trees was about 120 min. In the specimens with pre-existing emboli or oil occlusions, some bubbles remained in tracheids even after 140 minutes after the addition of water (Fig. 5, Samples B, C). In some strips without pre-existing emboli areas ("Normal" in Fig. 5), the time for complete bubble collapse was less than in strips of healthy xylem (Fig. 5, Samples B, C).

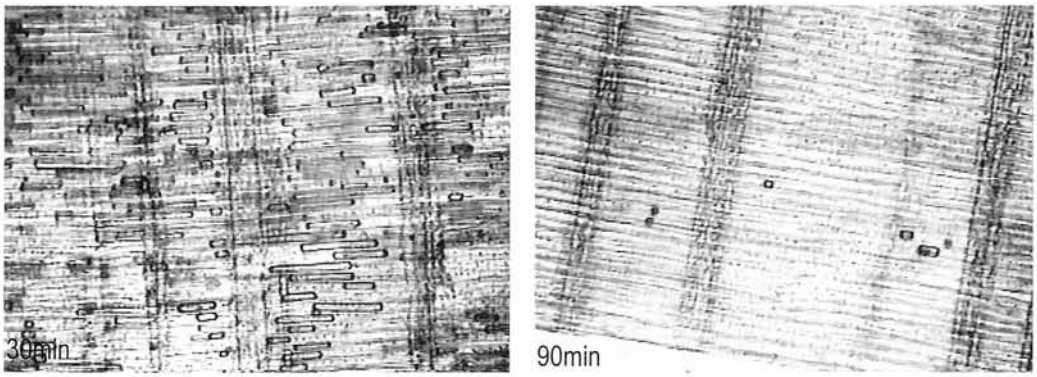

Fig. 4 Process of bubble collapse. 30 minutes (a) and 90 minutes (b) after adding water.

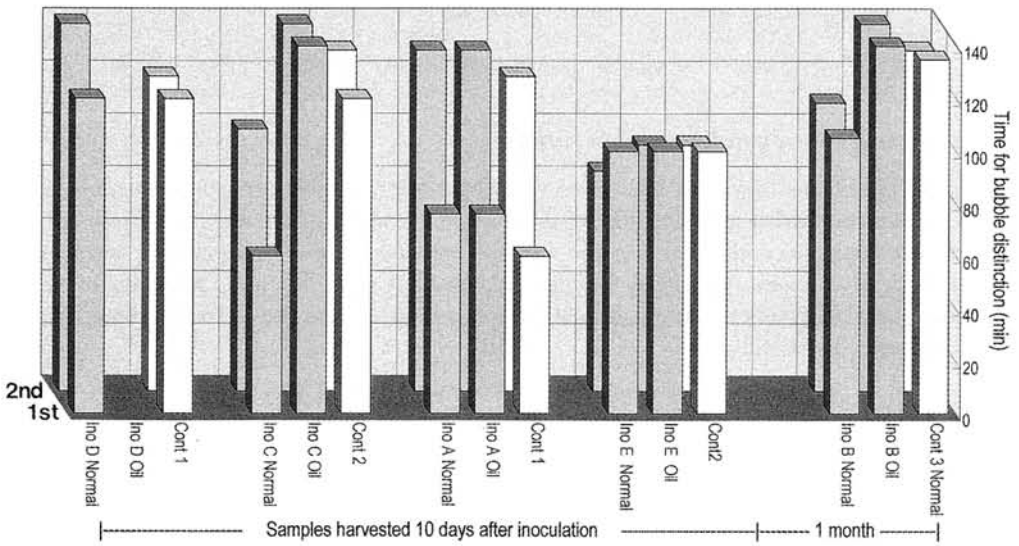

Fig. 5 Time for bubble collapse in *P. densiflora* xylem strips.

Ino: inoculated sample (5 trees); **Cont**: control (3 trees); **Oil**: oil occlusions or embolism; **Normal**: no visible changes.

DISCUSSION

Using videomicroscopy, we recorded dehydration-induced xylem embolism. Concurrently, we recorded ultrasonic AEs converted to audible signals on the audio track of the videotape.

The AEs were synchronized with almost all of the visible rapid bubble development. On the other hand, there were no AEs as water withdrew from injured tracheids at the start of dehydration. This result confirms Lewis' (1987) work in *Thuja*. The results in *Thuja* indicated that AEs detected in the trunks of living, transpiring trees are produced by embolism (cavitation) in tracheids. Bubble expansion in tracheids under tension is thought to initiate by the evaporation of water into a very tiny bubble (Zimmermann 1983). Such bubble expansion was shown by Lewis *et al.* (1994) to be very rapid, but slower expansion was observed on some cases in our experiment. This may be due to partial plugging of tracheid pits.

About 10 days to two weeks after infection with pine wilt disease, emboli that did not rehydrate had just started to develop. This means some physiological abnormality had just initiated. Xylem strips with pre-existing emboli or with oil occlusions required much more time for the rehydration of tracheids than did strips of xylem from healthy trees. In 1989, Kuroda reported that by 10 days to two weeks after infection by pine wood nematodes the presence of monoterpenes increased in infected trees. The release of hydrophobic substances such as monoterpenes into tracheids may prevent bubble dissolution. A larger sample is needed to determine whether the faster bubble collapse in apparently healthy xylem of infected trees is an artifact of the natural tree-to-tree variation in rehydration times or whether there is a physiological explanation.

Results of this type of laboratory experiment with xylem sections may include artifacts. However, the present experiments provide important information on the disruption of xylem water flow in diseased trees.

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