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caused by pine wood nematode

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# Acoustic Emission Technique for the Detection of Abnormal Cavitation in Pine Trees Infected with Pine Wilt Disease

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## Introduction

I have been investigating early physiological changes of pine trees following infection with the pine wood nematode (*Bursaphelenchus xylophilus* (Stainer and Buhrer) Nickle) to know the mechanisms of symptom development in pine wilt disease. I found an interesting phenomenon, the abnormal cavitation of tracheids in those pine trees (Kuroda et al. 1988, 1991a,b).

Process of disease development and the significant meaning of cavitation in pine wilt disease is explained as follows (Kuroda 1991). Usually, xylem sap is kept under tension during the period of active transpiration. In healthy plants, water columns in the stem are cut and cavitate during sunshine period as a common event (Tyree and Sperry 1988). Cavitated tracheids or vessels are refilled during nights again for weakened tension. Therefore, sap ascent does not blocked in healthy plants.

In the pine trunks susceptible to pine wilt, inoculated nematodes rapidly migrate through resin canals (Kuroda and Ito 1992), and stimulate terpene synthesis in parenchyma cells from several days after their infection (Kuroda 1991). When accumulated monoterpenes are exuded from parenchyma cells into tracheids, those volatile substances will induce or accelerate cavitation in many tracheids under tension. The xylem-sap ascent is blocked at such air filled sites. Initially, cavitated and air filled tracheids are found as small white patches on trunk cross section, usually 1 or 2 weeks after the nematode inoculation (Kuroda 1991, Kuroda et al. 1991a). As the cavitated areas enlarge, such trees suffer from water deficit, and wilt at last (Kuroda et al. 1988). Therefore, blockage of xylem sap ascent is the determinant of pine-tree mortality.

The development of cavitation could not be traced on the same tree so far, unfortunately, because sample trees are cut one by one for the observation of trunk cross-section. Now, there is an useful technique to monitor cavitation events from outside of trees without cutting down, the acoustic emission (AE) technique (Sperry and Tyree 1990). When cavitation occurs in xylem, AE, a kind of ultrasonic from 100 to 300kHz is produced. To correlate the cavitation events with blockage of xylem sap ascent and symptom development, I used this technique.

## Materials and Methods

Japanese black pine (*Pinus thunbergii* Parl.), 4 and 5 years old, was selected for inoculation. Prior to the inoculation, 6 AE sensors (140kHz) were attached on the pine trunks ca 20 cm above ground, slightly peeled the outer bark. Simple type AE-testers (NF Electronic Instruments) were connected with each sensor.

Trees were inoculated with 10000/tree of pine wood nematodes from an upper branch, on August 4, 1994. On 1995, new sample trees were inoculated on July 31, or August 24. AE events were continuously monitored every 10 minutes on 15 inoculated pines and 2 controls in total during ca. two months. The data in the data-loggers were read with several days intervals. For most inoculated samples, AE was monitored until they were killed around the end of September to October.

Symptom developments were checked once a week at least. A few trees were cut down when AE production became abnormal, when symptoms were visible, and when the AE production decreased again. On the disks of those harvested trees cut at intervals of 40 to 50 cm, enlargement of cavitated areas and blockage of xylem sap ascent were observed.

## Results and Discussion

In healthy pine trees, AE production started around 7 or 8 o'clock, when transpiration becomes active for strong sunshine on the fine day during summer (Fig. 1). It stopped during 16 to 18 o'clock, before sunset. The peak of AE production is around 10 to 14 o'clock, and AE event rate was 300 to 500 times per 10 minutes. When it became cloudy, AE production immediately decreased. AE production also varied depend on soil moisture.

During night, there is no AE production, because transpiration stops.

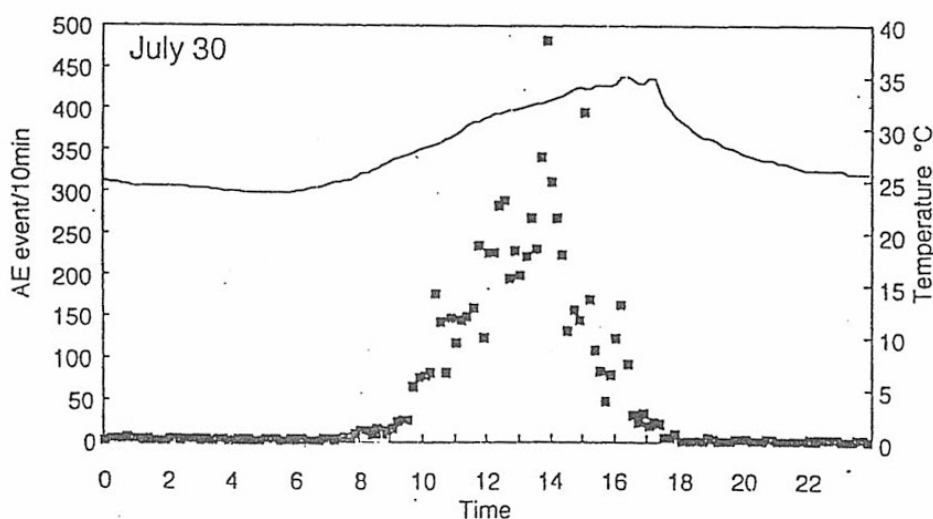


Fig. 1 AE production in the trunk of healthy *Pinus thunbergii* on a fine day July 30, 1995.

During the first week of nematode inoculation, AE production was normal (Fig. 2). AE signals abruptly increased about 1 to 2 weeks after the nematode inoculation (Fig. 2) in most sample trees. For instance, AE production is normal during daytime on August 8, but AE events began to increase again after sunset, and reached over 10 times of normal case on the next day (Fig. 3). AE production

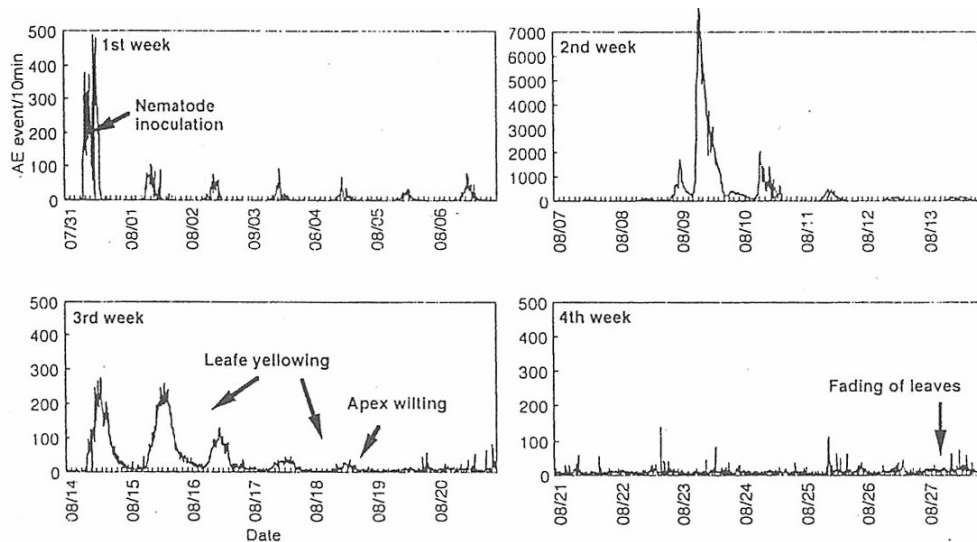


Fig. 2 AE production from the *Bursaphelenchus xylophilus* inoculation to the complete death of *P. thunbergii*.

continued during nights also on that day. I cut down one of the inoculated trees immediate after the highest AE production. Many white spots, the cavitated areas, were found throughout a tree (Fig. 4a). Tracheids of white parts are dehydrated by air, and the ascent of xylem sap was blocked. Those very small patches means that cavitation has just initiated by this time.

This extremely increased period of AE production was around 3 days, and then AE events gradually decreased during third or fourth week, although AE production continued during night (Fig. 2). This pattern of AE increase was completely same with all nematode-inoculated samples. I assumed that tracheid cavitation should have developed drastically in the trunks during the period of this highly frequent AE events.

After the high AE period, when the AE production become lower than normal frequency, disease symptom became visible, first, the yellowing of old leaves, and then apex wilting from 3 to 4 weeks after infection. Also on this period, I cut down a sample tree and observe on cross section. Half or two third of the disks were already white and dysfunctional by cavitation (Fig. 4b). Cambium and phloem were partly necrotic. This tree is already close to the death.

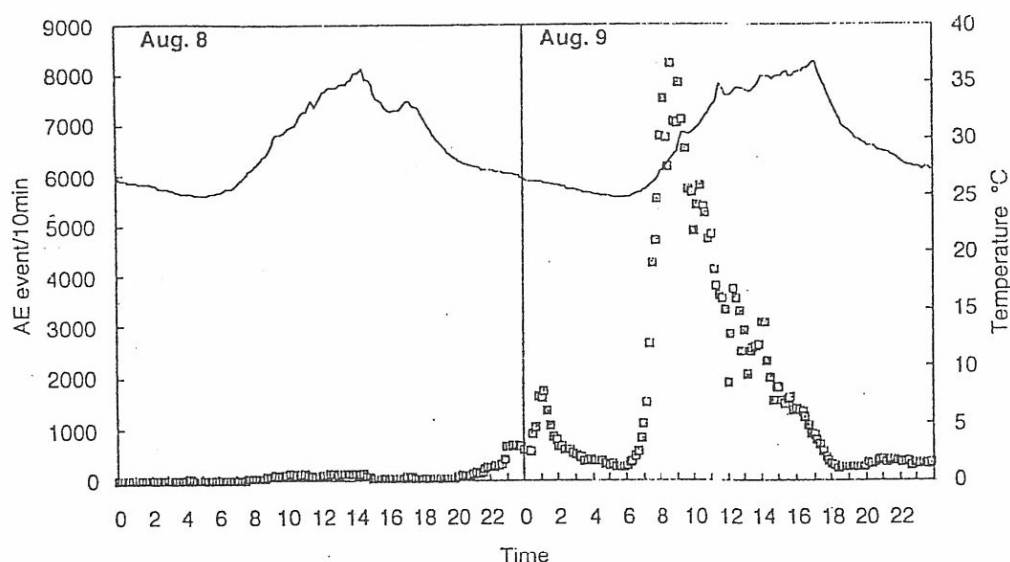


Fig. 3 Abnormal and abrupt increase of AE production during second week of nematode inoculation, August 8 and 9, 1995.

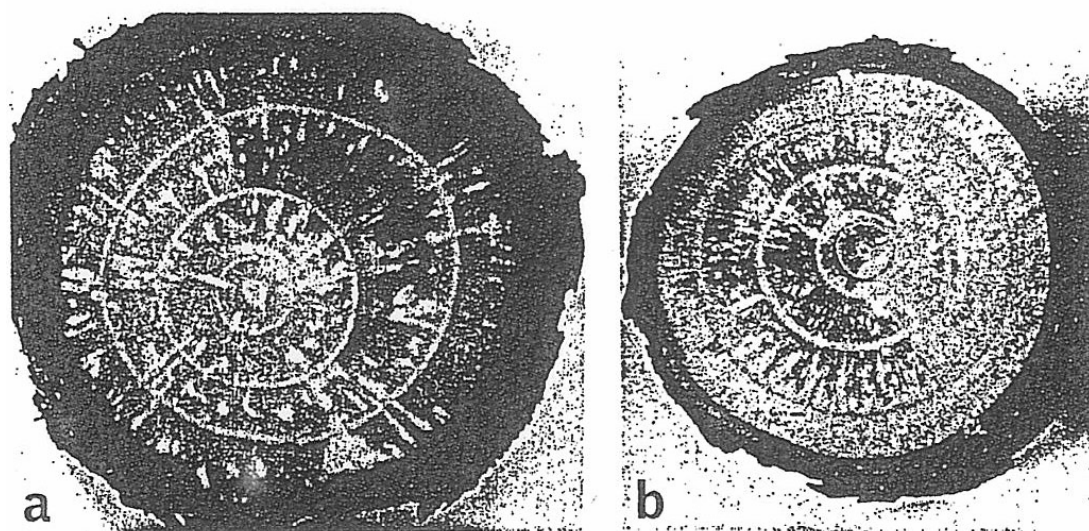


Fig. 4 Cavitation areas on the trunk cross-sections of nematode inoculated *P. thunbergii*, immediate after the highest AE-event production (a) and at the start of leaf yellowing (b).

After the apexes wilting, AE production became very low and random (Fig. 5). These AE events are not related to transpiration. Water contents of xylem was extremely low, about 25% of healthy trees. Water conduction almost completely stopped. Then, leaf color gradually faded. This is complete death of the tree.

As the conclusion, the following facts were clarified: Very high AE production during second week means continuous dehydration from tracheids by cavitation. Therefore, xylem dries incredibly fast. Visible symptom initiates after the

high AE production period. Final decrease of AE events and its random pattern means almost all tracheids had dysfunctional.

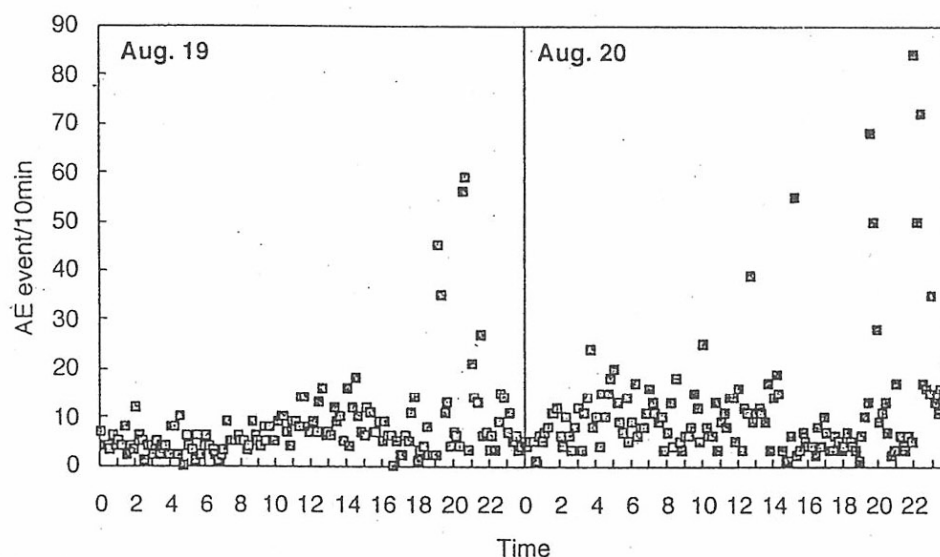


Fig. 5 Random AE production at the start of wilting symptom, August 19, 1995.

In this experiment, AE technique indicated the timing of abnormal cavitation development very clearly without cutting nematode-infected trees. This technique may be also useful for the experiments of cavitation events in the pine clones resistant to pine wilt which were selected by the breeders.

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