Pine Wilt Disease : Various Biological Relationships and Resulting Events

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Pine Wilt Disease:
Various Biological Relationships and Resulting Events

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Abstract – Pine wilt disease is one of the most serious forest diseases in East Asian countries. The infection chain of this disease, which is filled with various biological relationships, is briefly reviewed. The affinity between host pine trees and pathogenic nematodes, one of the most decisive phases of the disease, is subsequently examined in a series of experiments.

I. Introduction

Pine wilt disease is one of the most serious forest epidemics in the world, especially in East Asian countries. Since the first discovery of the pathogenic nematode in 1970, a great number of papers have been published to explain the biological relationships included in this epidemic. Among them are the relationships between the pathogenic nematode and its' host pine species, those between the pathogenic nematode and its' vector beetle, and those between the beetle and pine species. In all of these relationships, microorganisms play important roles as food sources of the nematode, or as natural enemies of the organisms in these relationships.

II. The Epidemic Manner of Pine Wilt Spread

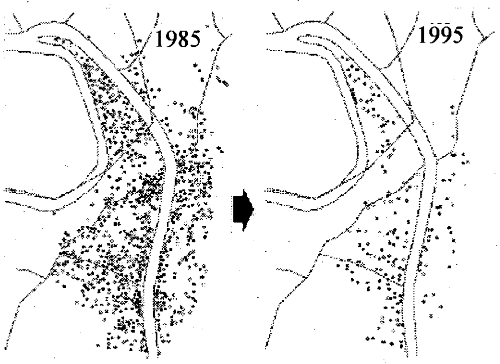
After the first invasion by this disease, most pine stands seem to be devastated within 10 years. To evaluate the precise manner of disease spread, we have to survey the number and the location of diseased trees for many years. So, in 1995, I placed an experimental plot on the ridge of a low mountain at an experimental forest station of our university forest, located 7 km north in the suburb of Kyoto city. The area of the experimental plot is about 1.8 hectares. Then I determined the distribution of all surviving pine trees in that plot.

As shown in Fig. 1, only 178 trees were alive in 1995; then I put the locations of the trees killed in the preceding 10 years on the same map (Fig. 1). This figure shows 810 pine trees were alive in the same area in 1985. Thus, almost 80% of the pine trees alive in 1985 were killed in the following 10 years [2].

The pine wilt disease epidemic has prevailed everywhere in Japan with a few exceptions -- namely the northern-most prefecture of the mainland and the North island, Hokkaido.

II. The Infection Chain of Pine Wilt Disease.

When pine trees are killed and have fallen down, the bark is often peeled off. Beneath the bark, we often found a lot of swarming larvae of beetles and their feeding debris on the log surface. Before pinewood nematode was found as the causal agent of pine wilt disease, massive pine death had been attributed to these beetle larvae. Bark beetles, however, lay their eggs only on diseased or weakened trees to avoid the potential danger of their eggs being killed by the vigorous resin. This implied that some unknown factor(s) must have caused the trees to become weakened or diseased.



810 trees 178 trees

Fig. 1 The decline of a Japanese red pine forest over 10 years.

In the course of surveys to find the true causal agent of pine wilt disease, a tree pathologist, Tokushige, found numerous nematodes in his fungal cultures. He and his colleague, Kiyohara, ventured to inoculate the nematodes into healthy pine trees, though common sense suggested that plant parasitic nematodes could never kill big trees such as pines. Contrary to their presupposition, the pine trees inoculated with the newly-found nematodes were killed in the same manner observed in the field [4].

The pathogenic nematode was described as *Bursaphelenchus lignicolus* [8] and this scientific name was changed into *B. xylophilus* after intensive taxonomical review in 1981 [9].

Taxonomic characteristics of this nematode are:

1. Stylet without basal knob,
2. High lip
3. Vulbal flap of females
4. Clear and large median bulb
5. Characteristic hook-shaped spicule, male sex organ,
6. Wing-like extension of cuticle at the end of male tail,

and so on.

This nematode can feed on both fungal and plant cells by thrusting its' stylet into fungal or plant cells. Thus this nematode is myco- and phytophagous, so it can be cultured on some fungal species such as *Botrytis, Fusalium, Pestalotia*, and so on [5, 6]. This characteristic enabled us to carry out mass-culture, and thereby we could easily do inoculation tests.

The method we use to inoculate nematodes on saplings of Japanese black pine is as follows:

1. First, we peel off a small part of bark, which simulates feeding wounds made by the vector beetle, *M. alternatuss*,
2. Fix a cotton swab on the scar,
3. Pipett an aliquot of nematode suspension into the cotton swab,
4. Then, cover with parafilm so it is not washed away by rain.

When inoculated in July, early symptoms appear in late August to September. Old needles change their color to yellow, then to brown. In September to October, needles droop and change their color to brown, suggesting they are dead.

Thus, pathogenicity of the newly-found nematode was confirmed. But their size of just 1 mm long is too tiny to explain the epidemic spread of this disease, which had been reported to extend 10 km or more a year. So, people started to search for the vector of the nematode.

Among the various species of insects related to dead pine trees, three groups of beetles are dominant; they are longhorn beetles, bark beetles, and weevils.

After intensive surveys for nematode vectors, only one species of longhorn beetle, *Monochamus alternatus*,was identified as the effective vector of the pinewood nematode.

M. alternatus belongs to tribe Lamini of the family Cerambicidae, and is distributed over eastern Asia, including Japan, Korea, China, and Taiwan. Adults of *M. alternatus* feed on the twigs of some genera of Pinacea including *Pinus, Larix, Picea,* and *Cedrus,* and lay their eggs on the trunks of such host trees after cessation of resin exudation.

When the adult *Monochamus* beetles emerge, their reproductive organs are not yet well-developed. So they have to keep on feeding to make their reproductive organs mature. The beetles move from dead pine trees to healthy ones, and feed on young branches for this maturation feeding.

Several days after the maturation feeding of the beetle *M. alternatus* starts, pathogenic nematodes of *B. xylophilus* harbored in the trachea of the beetle are transmitted to the pine trunk and they invade the host tree through the feeding wound.

Three to four weeks after nematode infection, the pine tree ceases its' resin exudation, and it starts to emit volatiles such as ethanol and monoterpenes. These volatiles attract matured *M. alternatus* to the diseased pine trees, where they mate and then lay their eggs after making egg niches.

Female *Monochamus* lay one egg per a nitch, producing about 100 eggs in total during three months. After a week, the eggs hatch into larvae. Ten days later the 1st instar larva molts into a 2nd, then 3rd, and 4th instar larva. Juvenile larvae make shallow tunnels just beneath the bark, but in September to October, matured larvae bore deep into the sapwood.

In late autumn, the mature 4th instar larva makes a large cavity at the end of the tunnel, then it plugs the outlet of the cavity with wood debris, and overwinters there. We call this cavity a "pupal chamber", because the larvae pupate in this cavity the following spring. A few species of fungi proliferate over the wall of this pupal chamber,. Among them are blue stain fungi, *Ophiostoma* spp., which are early colonizers of dead pine trees. The pinewood nematodes feed on the fungi, and propagate around the pupal chamber. But some fungi such as *Trichoderma* spp. and *Verticillium* spp. cohabitate with the *Ohiostoma* fungi, and they suppress the propagation of *Ophiostoma* and/or *B. xylophilus* as antagonists. So, the number of nematodes carried by a vector beetle is partly determined by the fungal flora around the pupal chamber [7].

When nematodes become overpopulated, their food becomes limited, and their environment deteriorates due to their own excrement. Under these conditions, a special stage of nematodes appear in the population. This stage is called the dispersal 3rd juvenile; it contains a lot of fatty granules in its' body. When the 3rd stage juveniles aggregate around the vector's body, they molt again to another characteristic stage, the so-called "dauer juveniles or disparsal 4th juveniles".

In the next year, from May throughout June, *Monochamus* beetles pupate in their pupal chambers. After pupation the beetle's body color changes from white to amber, and eye color changes from red to black. Two to three weeks later, the pupa ecloses in the chamber. It stays in the chamber for several days as a callow adult. During this period, nematodes of the dauer juvenile stage (= dispersal 4th stage) transmit into the *Monochamus* vector's respiratory organ, the tracheal system. When the tracheal system was first dissected from the *Monochamus* beetle's body under water, the whole trachea began to move just like a living organism. At first, the insect's trachea seemed to keep its activity even after death. After a while, however, it was disclosed that this movement was due to the nematodes occupying the tracheal tubes.

The dauer larvae of *B. xylophilus* are a really characteristic stage, which lack digestive organs such as stylets, median bulbs, and mouth openings, suggesting they are not parasitic to the vector beetle but are mere passengers. Their body is covered with a thick cuticle and a sticky surface substance. These must be adaptive characteristics for being vectored.

From June to early July, *Monochamus* beetles emerge from dead pine tree through round holes, carrying pinewood nematodes in their respiratory organ.

Thus, the infection chain of Pine Wilt Disease consists of three major organisms; the pine tree as a host, the pinewood nematode as a fatal pathogen, and the *Monochamus* beetle as the vector of the pathogen. Various species of fungi are also included in this infection chain as food resources, predators, antagonists, and so on.

III. Host resistance and susceptibility.

Once, in an experimental forest station of Kyoto University at Wakayama prefecture, some exotic pine species were planted in the field. A stand of slash pine, *P. elliottii* and another stand of Japanese black pine, *P. thunbergii* were located side by side. The stand of native pine, *P. thunbergii,* had been damaged very seriously, while the neighboring stand of slash pine had scarcely been damaged. This difference in damage between two pine species could be attributed to the vector's host preference. But there was no difference in the number of feeding marks on the young branches between the two pine species. Differences in damage could also be attributed to differences in resistance of the host pine species to the pathogenic nematode.

Actually, when inoculated with pine wood nematodes, *P. thunbergii* showed high susceptibility to nematode infection. Alternatively, *Pinus taeda,* loblolly pine, was very resistant to inoculation with nematodes even when they received three times as many nematodes as *P. thunbergii* trees did.

Which factor(s) determine host resistance to Pine Wilt Disease? To examine the role of host substances in Pine Wilt Disease resistance, I used the folloing method as shown in Fig. 2 and have reported the results elsewhere [1]. Here, I would like to briefly summarize the methods and the results.

(1) Shoots of pine species 8 to 10 mm in diameter were cut into 10 mm long segments. One third of the shoot segments were immersed in absolute ethyl ether, another one third in 95% ethyl alcohol and the remaining one third in distilled water.
(2) Two shoot segments treated with each solvent and two untreated segments were placed on an agar plate 3 cm from the center in a 9 cm diameter Petri dish.
(3) A known number of nemtodes of either *B. xylophilus* or *B. mucronatus* was placed in the midst of the four shoot segments, then incubated for 12 hours at 25° C.
(4) Nematodes were recovered from bark, wood, and the agar disk beneath the segment, separately.

As shown in Figure 3, for *P. thunbergii*, the ethyl ether- and ethyl alcohol-treatments caused a decrease in aggregation of both *Bursaphelenchus* species nematodes. Here I have showed only the results for *P. thunbergii.* As for the results for *P. taeda*, the effects of the solvent treatments is almost the same.

To compare the effect of extraction of host substances on the nematode invasion rate, the differences in the number of nematodes that aggregated to the segments were too large among the three solvent-treatments. So, the results were converted into % base data, and are shown in Fig. 4.

After immersing in distilled water, the invasion rates of both nematode species into the shoot segments of either pine species decreased significantly. This suggests some water soluble substance(s) may induce the nematodes to invade host tissue.

To confirm the role of host substances in PWD resistance, I examined the behavior of nematodes toward several host extracts. Shoots of *P. thunbergii* and *P. taeda* were cut into small pieces, and divided into two parts, bark and wood. One hundred grams of each part were immersed in ethyl ether or in distilled water. Then filter paper disks 8 mm in dia. were immersed in either one of these solvents, then placed in an agar plate as shown in Fig. 5 for nematode preference tests.

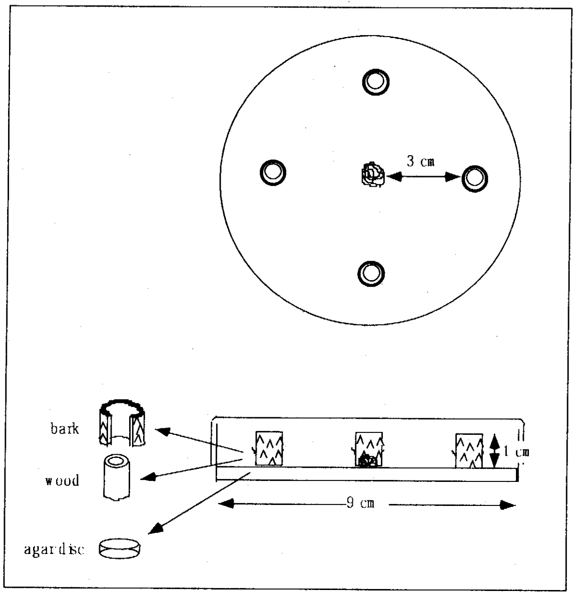


Fig. 2 A simple method to examine nematode behavior to pine segments pretreated with various solvents.

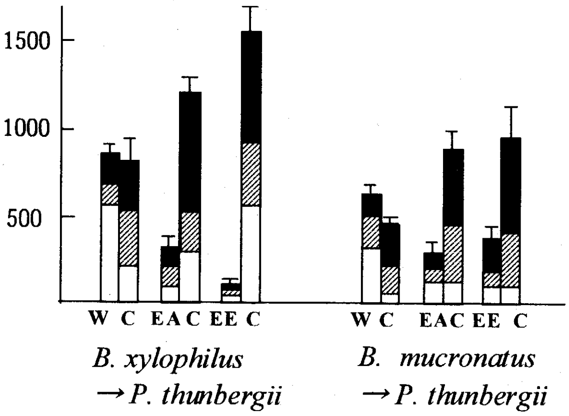


Fig. 3 The response of *B. xylophilus* and *B. mucronatus* to segments of *Pinus thunbergii* pretreated with either of three solvents.

The parts of black, gray or white in columns mean the number of nematodes recovered from bark of the segment, wood of the segment, and the agar disc 10 mm diameter beneath the segment, respectively.

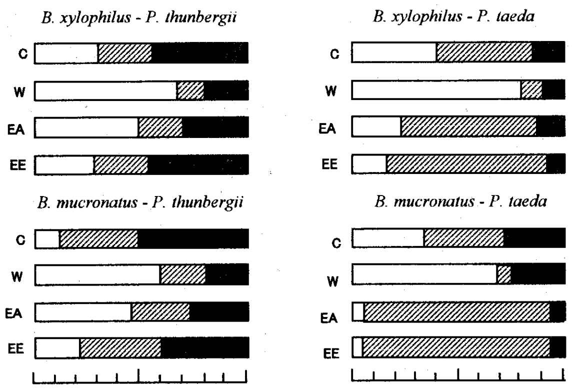


Fig. 4 The invasion rates of *B. xylophilus* and *B. mucronatus* into pine segments previously immersed in distilled water (w), ethyl alcohol (EA) and ethyl ether (EE) and into ones with no treatment (C).

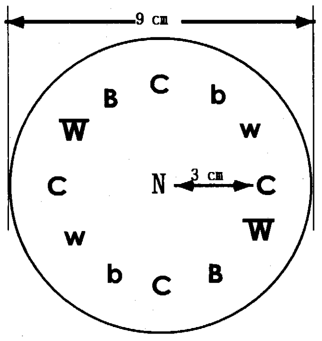


Fig. 5 Arrangement of paper disks holding various pine extracts on agar plate to examine the preference of *B. xylophilus and B. mucronatus* for the pine extracts. N: nematode inoculum, B: water extract of bark, b: ethyl ether extracts of bark, W: water extract of wood, w: ethyl ether extracts of wood, C: control (treated with water).

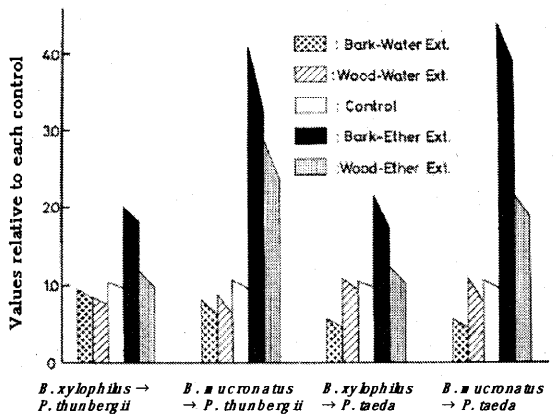


Fig. 6 The aggregation of two *Bursaphelenchus* species to paper disks holding different extracts from *P. thunbergii* and *P. taeda*. The distance from the height of right shoulder of each column to that of left one means the standard error of 10 replicates.

Preferences of both of the *Bursaphelenchus* nematodes is represented in Fig. 6 as values relative to the number of nematodes that aggregated to the disks with the distilled water treatment (control).

As shown in Figure 6, (1) both *B. xylophilus* and *B. mucronatus* preferentially aggregated to the ethyl ether extracts from the wood of both *P. thunbergii* and *P. taeda.* (2) Water extact from the bark of *P. taeda* repelled the nematodes of both *Bursaphelenchus* species.

From the above-mentioned results, I conclude that aggregation and invasion by two species of *Bursaphelenchus* seems to be controlled by separate substances; i.e., both *Bursaphelenchus* species aggregate to the hydro-phobic host substance(s), while their invasion of host tissues is controlled by hydrophilic host substance(s).

The resistance of *P. taeda* could partly be explained by the hydrophilic repellants contained in its' bark.

When I conducted inoculation tests, and compared resistance among pine species, I found that host resistance to Pine Wilt Disease is different among tree species in the genus *Pinus*, and the differences seem to reflect their phylogenetic relationships in the genus. For example, many species of subsection Sylvestres such as *P. densiflora* and *P. thunbergii* are susceptible to this disease. .Furthermore, most specie of subsection Australes such as *P. taeda* and *P. rigida* are highly resistant [3].

If we plot pine species with various resistances on a map of the world, susceptible pine species are distributed both in east Asia and Europe. In North America, most pine species distributed along the East coast are resistant, whereas, those distributed along the West coast are rather or highly susceptible to Pine Wilt Disease.

As I mentioned previously, a forest epidemic, "Pine Wilt Disease" includes various biological relationships. Most of them may seem to be understood. However, several important issues remain unresolved, and wait for challenge by scientists.

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