Pine Wilting Disease Caused by the Pine Wood Nematode, *Bursaphelenchus Lignicolus*, in Japan

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The wilting disease of pine trees caused by the pine wood nematode, *Bursaphelenchus lignicolus*, is widespread in stands of Japanese red pine and black pine (*Pinus densiflora* and *P. thunbergii*) throughout central to southwestern Japan. First occurrence of the disease was recorded at a locality in Kyushu nearly 60 years ago. Since then this dreadful disease has been progressively widespread throughout Kyushu, Shikoku, and the coastal areas of the Pacific Ocean restricted to west of Kanto district in Honshu. Recently, however, damages caused by the nematode were found in only scattered points in northern part of Honshu, east of Kanto.

In 1948, it caused the heaviest loss of 1,280,000 m^3 of pine trees in volume. Despite extensive efforts to reduce the losses, more than 400,000 m^3 loss by extreme damage had been annually estimated. In 1975, it caused the loss of approximately 1,000,000 m^3.

Concerning the actual cause of this disease, there was much uncertainty and controversy. Numerous investigations had been conducted mainly on insects associated with deteriorated pine trees as suspected causal agents. Since Kiyohara and Tokushige[2] demonstrated the drastic effect of *B. lignicolus* on pine trees as the result of inoculations, experimental evidence has been presented on the strong correlation betwen nematode inoculation and the death of pine trees by several workers. Not only older pine trees more than 50 years old, but also trees younger than 10-year-old including seedlings were successfully inoculated with *B. lignicolus*. The very same symptoms occurring in naturally infested trees were produced by introducing nematode suspensions to holes or wounds artificially made on stems or branches of healthy trees. Death of trees followed in 40 to 60 days after inoculation when experiments were conducted in summer. Thus th enematode became recognized as the causal agent of pine wilting disease.

Disease development and symptoms

Affected trees are conspicuous because of quick death after the symptoms appear. Pine trees oberved healthy in early summer die in later summer after showing yellowish foliage. The only external outstanding symptom followed by sudden death of pine trees is the yellowing succeeded by browing and eventual death of leaves as if they were subjected to rapid desiccation. It can be concluded that the disease is apparently the wilting one resulted from the abrupt ineffectivity of the tree water economy.

Disease development of the nematode infected pine tree proceeds as follows: (1) Marked reduction of oleoresin yield occurs first in disease tree as a detectable internal symptom during mid July to early August. The rating of oleoresin yield exuding through sapwood in a hole of 10–15 mm in diameter which was made in such a manner as removing only the bark and cambium on the basal portion of trunk was proposed and developed by Oda[1] as an useful method to differentiate diseased trees at the time that no external symptoms of the disease were observed. Oleoresin

exudation from the wound artificially made on the trunk of a inoculated pine tree comes to a complete stop within 2 weeks after inoculation. (2) Transpiration of leaves decreases and then stops. In the case of inoculated trees, transpiration reduction occurs 20 to 30 days after inoculation. During these periods no external symptoms are observed. (3) Foliage wilting and yellowing appear on the diseased trees subsequent to transpiration reduction. The decrease of sap wood moisture content proceeds rapidly, and desiccation of wood is characteristic in this stage of disease development. (4) The diseased tree dies eventually in late August to October, 30 to 50 days after the first occurrence of the internal symptom, marked reduction of oleoresin yield.

As the disease becomes more advanced, large numbers of adult and larval *Bursaphelenchus* are present throughout stems, branches, and roots. They can be recovered easily from pieces of wood by using a Baermann funnel.

Transmission of *Bursaphelenchus lignicolus*

Beetles of the various genera in Cerambycidae, Curculionidae, and Scolytidae, all of which were assoicated with deteriorated pine trees, were examined for the presence of *B. lignicolus*[6,10]. Dauerlarvae of *B. lignicolus* were recovered from adults of eight species

Table 1. Cerambycid beetle species in association with *Bursaphelenchus lignicolus*

Monochamus alternatus Hops.
Monochamus nitens Bates
Acalolepta fraudatrix Bates
Arhopalus rusticus Linne
Corymbia succedanea Lewis
Acanthocinus griseus Fabricius
Uraecha bimaculata Thomson
Spondylis buprestoides Linne

of Cerambycidae. They occurred most frequently and in greatest numbers in *Monochamus alternatus*, the Japanese pine sawyer. More than 75% of examined adults of *M. alternatus* which were collected at a severe damaged pine forest were contaminated with *B. lignicolus*. An average of 15,000 dauerlarvae per insect were recovered. A maximum was 230,000. Adults of *M. alternatus* are contaminated internally prior to emergence from the dead pine tree. Dauerlarvae are found in tracheae throughout the body of an adult beetle, and the largest numbers are in the tracheae directly connected with the methathoracic spiracles.

When nematode-contaminated adult *M. alternatus* were caged on a branch of healthy pine tree, disease transmission occurred in 100% of tested trees. No pine trees on which nematode-free adult *M. alternatus* was caged were affected[1,10].

M. alternatus lays eggs under the bark during July to August. The hatched larvae bore under the bark and then bore their way through wood. In the tunnels, the larvae overwinter and pupate during the following spring. The beetle emerges as an adult during late May to early July. The newly emerged adults attain maturity mainly by feeding on the soft tissue of living branches. After maturation feeding for about 30 days, adults come to full development ready for egg laying. Adults lay eggs successfully only on weakened, suppressed, or dead trees. Adults emerging from nematode-infested pine trees carry many dauerlarvae of *B. lignicolus* to fresh living pine tissue during maturation feeding. It is quite likely that living branches are inoculated with many *B. lignicolus*. An investigation was conducted to follow the chronological development of the disease under natural conditions in a regrowth stand of 25-year-old *P. densiflora* located in Chiba Pref. in Honshu (Fig. 1)[5]. Observations on oleoresin exudation from a punched hole were made on each pine tree at monthly intervals. Disease development was diagnosed from such observations of oleoresin yield as one of noticeable internal symptoms in the early stage. In late June, the first occurrence of diseased trees was observed. During mid July to mid August diseased trees appeared progressively. Eighty three percent of total dead trees throughout this season diagnosed as diseased trees from their internal

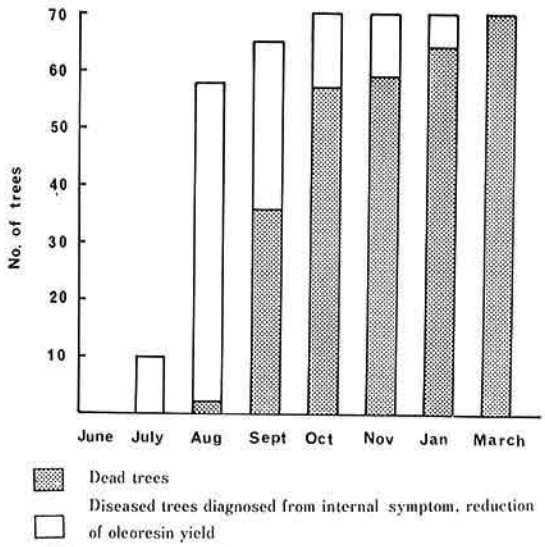

Fig. 1. Natural infection of pine tress with *Bursaphelenchus lignicolus* and disease development.
Total 322 of 25-year-old pine trees (*Pinus densiflora*) were examined at monthly intervals in the middle of each month.

symptoms during this period, and most of them, more than 90%, died until late August to mid October. In late July to mid August, ovipositions of *M. alternatus* were usually observed on diseased trees which had not showed external symptoms yet. There were a few trees which became diseased only after September, no ovipositions of *M. alternatus* were observed on such trees, because it was beyond ovipositing period of the beetle. The mortality rate of total examined 330 trees was 21% in the experimental stand throughout that growing season.

The foregoing remarks indicate that the chronological development of the disease under natural conditions coincides fully with the ecological aspect on the life mode of the vetor, *M. alternatus*.

Population dynamics of *Bursaphelenchus lignicolus* in pine wood

Under natural conditions nematode infection occurs via wounds made by *M. alternatus* on living branches through maturation feeding. Dauerlarvae enter the wood tissues immediately after transmission to branches. Without too much delay they become adults after molting, and reproduction takes place in wood. The rapid movement of nematodes throughout stems, branches, and roots was noticed during the first 24 hr after infection from the results of inoculation experiments on both grown trees and seedlings. Population of nematodes in wood increases more as the disease becomes more advanced. Although *B. lignicolus* is detected rarely in wood of diseased trees at the time of just appearing internal symptoms, large numbers of nematodes are present throughout tissues of a tree as external symptoms, wilting and yellowing of the foliage, become noticeable. Advanced diseased trees, dying or dead trees, contain tens of millions of living nematodes in wood. After reaching a maximum level, population decreases gradually as deterioration of tree becomes advanced. In this stage of population dynamics, "dispersal third stage larvae" which differ from the usual third stage larvae in morphology and biology appear[5] and the proportional numbers of these larvae to the whole population of *B. lignicolus* in wood increases gradually as

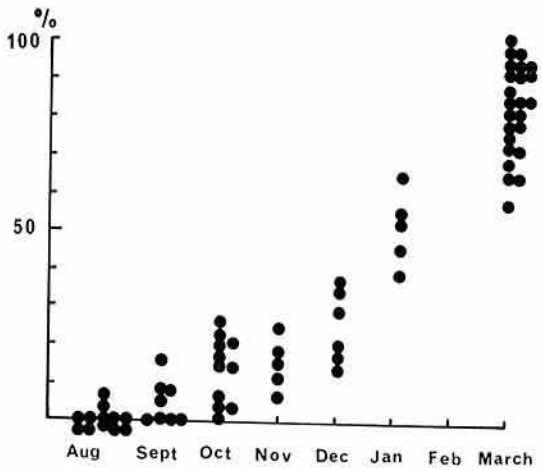

Fig. 2. Proportion of the dispersal third stage larvae to total numbers of *Bursaphelenchus lignicolus*. Mean proportional numbers were obtained from nematode numbers of each examined pine tree, 25-year-old *Pinus densiflora*, on which samples were taken at 2 m intervals.

the time passes (Fig. 2)[8]. Almost no larvae except dispersal third stage larvae are observed in winter and the following spring. Larvae in this stage are resistant to unfavourable environmental conditions, and has its biological significance as the stage preceding dauerlarvae.

Population increase of *B. lignicolus* in wood of inoculated pine trees was investigated in relation with time after inoculation. (Figs 3 and 4)[3,4].

B. lignicolus occurs mostly in both axial and radial resin canals of nematode infected trees. Nematodes multiply in the resin canals and evidence points to the resin canals as the feeding site[7]. Epithelial cells, the resin canal parenchyma cells, are most likely used as food for nematodes, since *B. lignicolus* is successfully cultured on alfalfa callus tissues[12], and also on pine callus tissues[13]. In most earliest stage of disease development, it is characteristic that discolaration in some of ray parenchyma cells and epithelial cells is observed long before localization of nematodes. Epithelial cells of nematode infected resin canals are conspicuously damaged, and in advanced stage of the disease they are completely destroyed.

Migration of *B. lignicolus* occurs in wood during winter to spring, and nematodes consequently gather around pupal chambers of

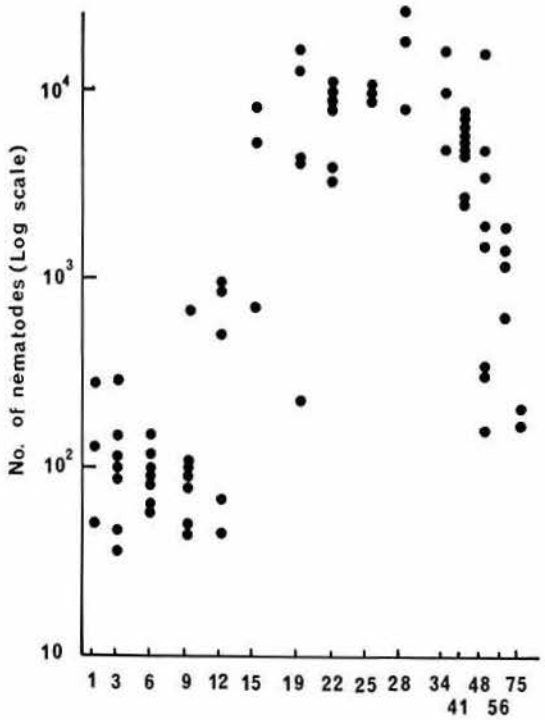

Fig. 3. Population of *Bursaphelenchus lignicolus* in wood of inoculated pine seedlings.
Seedlings were inoculated with 5,000 nematodes per seedling (average 240 nematodes per g of dry wood of seedling). Nematodes were extracted from a whole body of each seedling and then total numbers were converted into numbers per g of dry wood. Average dry weight of a whole body of a seedling (without leaves) was 18.2 g.

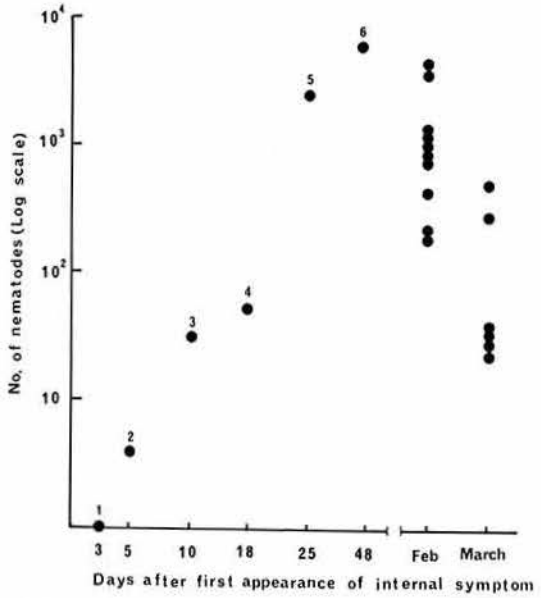

Fig. 4. Population of *Bursaphelenchus lignicolus* in wood of inoculated pine trees (per g of dry wood).
Pine trees (7-year-old *Pinus thunbergii*) were inoculated on July 5, 1971. Nematodes were extracted from pieces of wood sampled at each internode of a tree by boring method. Mean numbers for each tree are shown.
1: Healthy appearance. 2: Healthy appearance. 3: Healthy appearance, transpiration reduction. 4: Healthy appearance, transpiration reduction. 5: Wilting of foliage. 6: Wilting and yellowing of foliage.

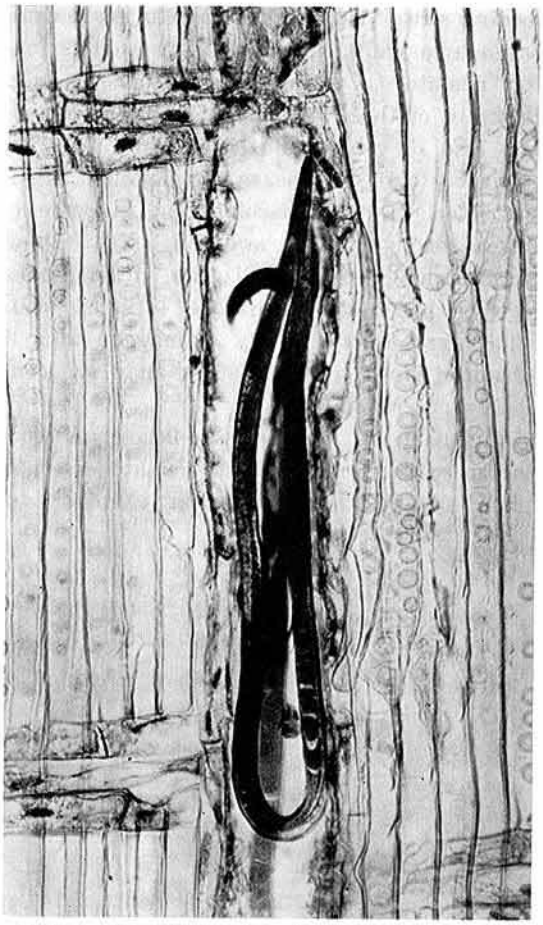

Plate 1. *Bursaphelenchus lignicolus* in an axial resin canal. Epithelial cells are destoryed. (Radial section).

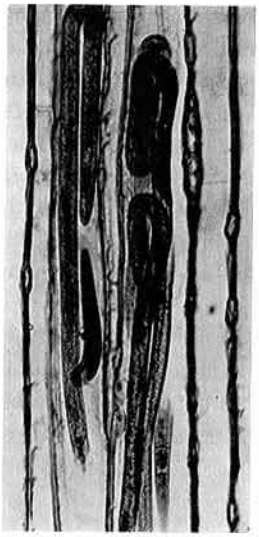

Plate 2. Dispersal third stage larvae of *Bursaphelenchus lignicolus* in tracheids around a pupal chamber of *Monochamus alternatus*. (Tangential section)

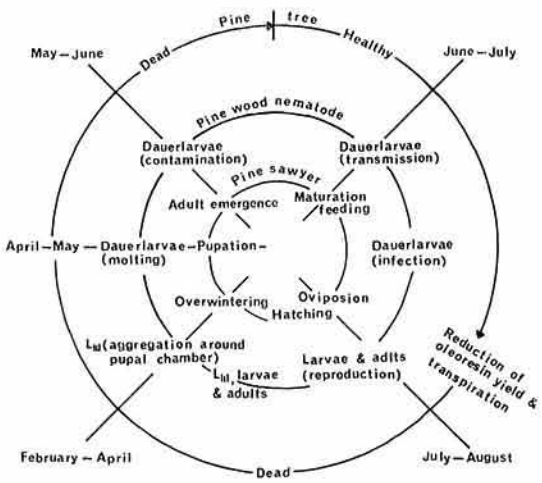

Fig. 5. Biological relationships of *Bursaphelenchus lignicolus*, *Monochamus alternatus*, and pine.

M. alternatus in early spring. All of aggregated nematodes are the dispersal third stage larvae and located within 1–2 mm of wood tissue surrounding the pupal chamber. In late spring they become dauerlarvae after molting there. Contamination with dauerlarvae occurs only on adults just after emerging from pupae.

B. lignicolus is easily grown on cultures of *Botrytis cinerea*, *Pestalotia* spp., and several other fungi. The life cycle of the nematode is completed in 4 days at 25°C on *B. cinerea* cultures[5].

Control of the disease

Effective control of the disease can be achieved by preventing maturation feeding of *M. alternatus* on living fresh branches. Just prior to maturation feeding, the crowns of healthy pine trees are sprayed with organic phosphate insecticides, such as 0.5% emulsion

of fenitrothion in a volume of 2–3 liter per tree, once or twice during late May to mid June. Aerial applications of chemicals for control are also now practical. Emulsion of fenitrothion at 3% contents is commonly used for aerial control, and this chemical is applied at the rate of 60 l/ha twice during late May to mid June.

Trunk injection and soil application of chemicals are investigated to obtain practical methods for direct control of nematodes. In these method certain systemic chemicals such as fensulfothion, disulfoton, and thionazin are effective when they are applied on pine trees 2–4 weeks before nematode infection occurs[9]. There is little value in trying to cure diseased trees, because irreparable damage was already done to tissue by the time external symptoms become visible.

Chemicals are also applied on logs of felled trees to kill *M. alternatus* larvae growing under the bark. Although this method have been used since the time when the actual cause of the disease was not known, it is still useful method to eliminate the disease vectors.

Extensive studies for breeding resistant trees are also in progress as the most promising method for controlling the disease.

According to the results of inoculation tests, pine species vary in their resistance to nematode infection. Native pine species, *P. densiflora*, *P. thunbergii*, and *P. luchuensis*, of which both former two species are very important conifers in our forests, are highly susceptible to the disease. Though *P. pinaster* is susceptible, most of other exotic species are resistant. *P. rigida*, *P. elliottii*, and *P. caribae* are resistant. *P. taeda*, *P. banksiana*, *P. strobus*, *P. pungens*, *P. echinata*, and *P. palustris* are highly resistant.

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