Pine Wilt and the Pine Wood Nematode

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Introduction

Pine wilt is caused by the pine wood nematode *Bursaphelenchus xylophilus* (**Figure 1**). This pine wilt disease, which is endemic to North America, has already spread epidemically to East Asia, and recently to Portugal in Europe (**Figure 2**). *Monochamus* spp. beetles (pine sawyer beetles) are the only insects that disperse the pine wood nematode. The incidence of the disease is closely related to environmental conditions such as high temperature with little rainfall. Pine wilt disease is becoming a serious threat to pine forests in the northern hemisphere.

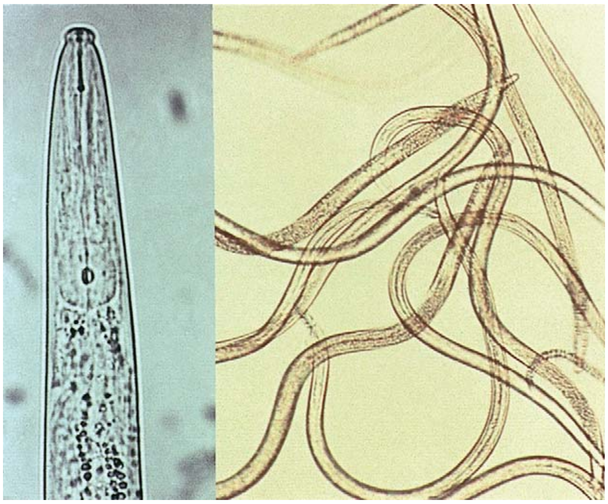

Figure 1 The pine wood nematode, *Bursaphelenchus xylophilus*. The left image shows the head of a nematode. The right image shows the larvae in a cultural media.

History

Pine wilt was initially recognized as an unusual wilt of pines in 1905 in the port city of Nagasaki in southern Japan, which was the only port open to foreign culture in the nineteenth century. Pine wilt spread thereafter from many port cities in southern Japan. This suggests that the disease is artificially distributed by the shipment of infested logs. The loss of timber by pine wilt showed an abnormal increase in the late 1940s soon after World War II and then pine wilt became a matter of public concern. Thereafter, the damage was called 'matsukuimushi,' which is a general term covering more than 70 species of pine bark beetles and pine wood borers thought to be concerned with pine wilt in those days.

As a causal agent of pine wilt, the pine wood nematode was recovered from a dead Japanese black pine, *Pinus thunbergii*, in 1969. The pine wood nematode is associated with cerambycid beetles, the Japanese pine sawyer, which is a matsukuimushi (**Figure 3**). The pine species susceptible to the disease are Japanese red pine, *P. densiflora*, and the Ryukyu pine, *P. luchuensis*, in addition to Japanese black pine.

In 1979, the pine wood nematode was discovered in Missouri, USA. Most pine species in the USA are moderately resistant to the disease. The pine wood nematode is naturally found in diseased *Abies* spp., *Cedrus* spp., *Larix* spp., *Picea* spp., and *Pseudotuga* spp. in the USA and Canada. It is hypothesized that

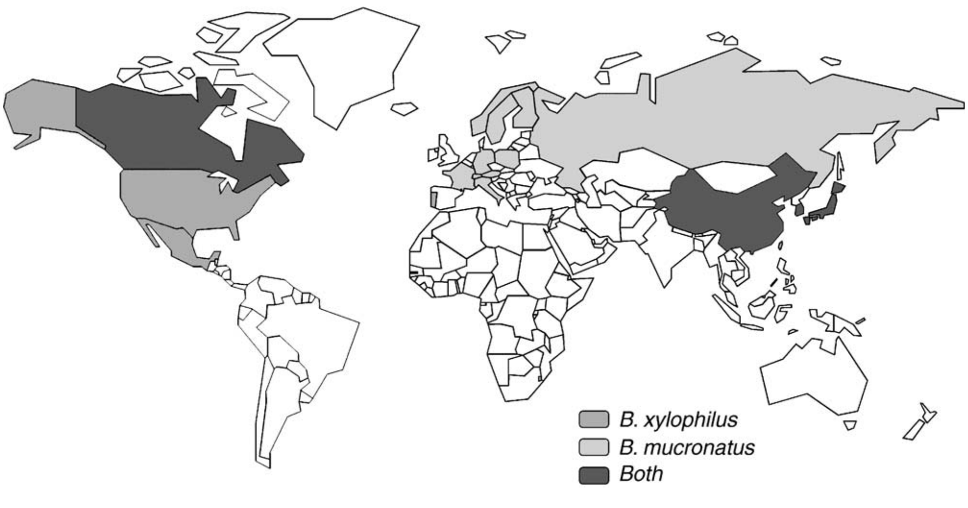

Figure 2 The distribution of *Bursaphelenchus xylophilus* and *B. mucronatus* in the northern hemisphere.

Figure 3 The Japanese pine sawyer, *Monachamus alternatus*.

the pine wood nematode was introduced in pine logs to Japan possibly from the USA early in the twentieth century.

Pine wilt disease had spread to Nanjing, China, by 1982 on Japanese black pine and southern red pine, *P. massoniana*, to Taipei, Taiwan, by 1985 on Japanese red pine and *P. luchuensis*, and to Pusan, Korea, by 1988 on Japanese black pine and Japanese red pine. Recently, pine wilt disease was found at Setubal, Portugal, in 1999 on maritime pine, *P. pinaster.*

The Pathogen, the Pine Wood Nematode

The pine wood nematode was first named as *B. lignicolus* in Japan in 1972, but was morphologically similar to the timber nematode, *B. xylophilus,* in the USA. The genus *Bursaphelenchus* belongs to the family Aphelenchoididae, and *B. xylophilus* was initially described as *Aphelenchoides xylophilus* from the wood of long-leaf pine, *P. palustris,* in 1934. Thereafter, *A. xylophilus* was transferred to the genus *Bursaphelenchus* in 1970. Based on genetic crossing between *B. lignicolus* and *B. xylophilus*, it was elucidated that they are the same species. Therefore, the name of *B. xylophilus* is valid based on the priority of nomenclature for a causal agent of pine wilt disease.

All species of the genus *Bursaphelenchus* have a relation with bark beetles and wood borers. As the pine wood nematode is mycophagous, it can propagate easily on cultures of *Botrytis cinerea* and other fungi. The pine wood nematode is also cultured on callus tissues of Japanese red and Japanese black pines grown on artificial media. This indicates that the pine wood nematode is able to feed on parenchymatous cells of living pine wood.

The pine wood nematode has four larval stages (L_1, L_2, L_3, L_4) and two different forms in its life cycle, that is, a propagative form (L_3, L_4) and a dispersal form (L_{III}, L_{IV}). The former develops under favorable conditions and the latter under unfavorable conditions. As to the propagative stage, females of the pine wood nematode lay an average of 80 eggs during a 30-day oviposition period. The pine wood nematode completes its life cycle in 3 days at 30°C, 4 days at 25°C, 6 days at 20°C, and 12 days at 15°C. No reproduction occurs at a temperature higher than 33°C and the limit of low temperature is 9.5°C for growth on *B. cinerea*. The dispersal stage is adapted to surviving unfavorable conditions, such as low temperature and lack of food. The larva of this stage, which is called the dispersal third-stage larva (L_{III}), is different in its morphological and biological features from the propagative third-stage larva (L_3). Larvae molt to become the dispersal fourth-stage larvae (L_{IV}) in wood under natural conditions. They have specific features such as a dome-shaped head, lack of stylet, degenerate esophagus, and subcylindrical tail. The fourth-stage larvae (L_{IV}, dauerlarvae) are adapted to being carried by the insect vector and are easily transmitted to healthy pines. When dispersal third-stage larvae (L_{III}) are placed under favorable conditions, such as on fungal culture at 25°C, they molt soon after to the propagative fourth-stage larvae (L_4) and multiply rapidly.

Meanwhile, a closely related pine wood nematode, *Bursaphelenchus mucronatus,* is found in declining pines, and is distributed over a geographically wider area than *B. xylophilus* (**Figure 2**). The hosts of *B. mucronatus* are *Pinus* spp. in Austria, Canada, China, Finland, France, Italy, Japan, Korea, Norway, Russia, and Sweden. In addition to *Pinus* spp., *Abies* spp., *Cedrus* spp., *Larix* spp., and *Pseudotsuga* spp., are its host species. *Bursaphelenchus mucronatus* has a similar life cycle to *B. xylophilus*; however, its pathogenicity is very weak. Based on hybridization and phylogeny of the pine wood nematode, the Japanese and the American strains of the pine wood nematode, *B. xylophilus*, are supposed to be derived from common stock of *B. mucronatus* originating in western Europe.

The Vector, the Pine Sawyer

Most species of *Bursaphelenchus* are associated with cerambycid beetles. *Monochamus* spp. called pine sawyer beetles are the only means for dispersal of the pine wood nematode in Asia and USA. *Monachamus alternatus*, the Japanese pine sawyer, is the major vector for *B. xylophilus* throughout Japan, and is widely distributed in Southeast Asia, including China, Korea, Laos, Taiwan, and Vietnam.

Japanese pine sawyer grows and develops each stage linearly in relation to effective temperature. The threshold temperature to commence development of overwintering larvae is 12.5°C. Japanese pine sawyer beetles often complete a single generation in 2 years in a cool climate in northern Japan. In this case, the larvae overwinter as immature larvae and become fourth-instar larvae in pupal chambers after feeding again in the following spring.

Before the emergence of the Japanese pine sawyer in the wood, the dispersal fourth-stage larvae (L_{IV}) of the pine wood nematode enter a body of the adult beetle through the abdominal spiracles and are held in the tracheae. The number of nematodes varies greatly in the body of the beetle. The maximum number of nematodes was recorded as 289 000 per beetle. The adult beetle emerges from May to July. Larvae (L_{IV}) have been observed on the body surface of beetles, mostly on the abdomen, with the largest numbers at the tail tip. The number of nematodes in a beetle's body decreases gradually with time after emergence. The Japanese pine sawyer has been shown to fly a maximum distance of 2.4 km by mark-recapture tests; however, most adult beetles are found around 100–200 m from the release point. Emerging adults of the beetle feed on the bark of pine twigs for maturation and the larvae (L_{IV}) of the pine wood nematode invade the living pine tree from the scars of maturation feeding.

Symptoms and Development of Disease

Pine wood nematodes are introduced into the shoots of pine trees during maturation feeding of Japanese pine sawyer beetles. They move initially through cortical resin canals and then migrate rapidly into the whole trunk through xylem resin canals at a maximum speed of 40–50 cm day^{-1} (**Figure 4**). A slight reduction in the flow of oleoresin exudate is observed as a unique symptom at an early stage of the disease. This symptom is due to the destruction of epithelial cells around resin canals by the invasion of the nematode. The nematodes then eventually move from resin canals to adjacent rays, from rays to tracheids through cross-fields, and from tracheids to tracheids through pits. At the same time, enhanced ethylene production is observed 2–3 days after an invasion of pine wood nematodes. The ethylene increase is partially caused by an excretion of a considerable amount of cellulase by the pine wood nematode. The nematode density is very low at an early stage of disease development, often as low as a few nematodes per 100 g fresh weight of wood, even following highly concentrated inoculations.

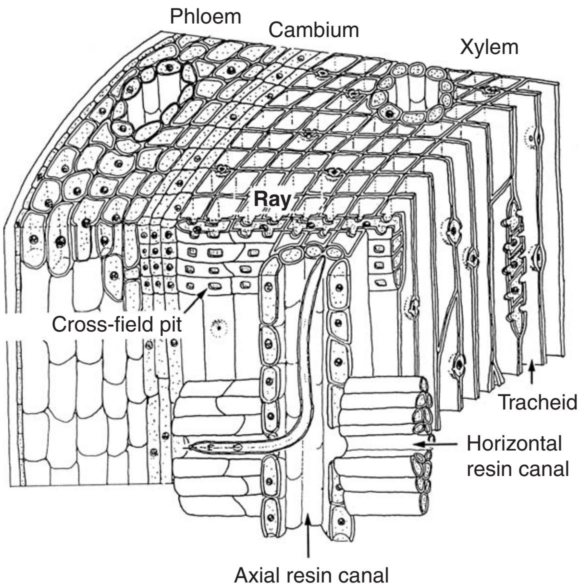

Figure 4 Main anatomical features involved in the movement of the pine wood nematode, *Bursaphelenchus xylophilus*, in Japanese black pine, *Pinus thunbergii*.

As a general rule, disease developments following an invasion by the pine wood nematode are divided into two stages: an early stage and an advanced stage (**Figure 5**). In the early stage, cytological changes in the xylem parenchymatous cells occur, and these are soon followed by cavitation and embolism formation in tracheids, which cause dysfunction of conduction in the vascular system of the pine tree. Such internal symptomatology is induced not only in compatible but also in incompatible combinations of pine trees and nematode isolates. However, growth of the nematode population is not assured in pine trees under conditions unfavorable to the nematode, even if a high concentration of nematodes is inoculated. Therefore, this stage is considered to be latent, that is, denaturation of parenchymatous cells by a nematode invasion results in cavitation and embolism of some tracheids.

At the onset of the advanced stage, visible symptoms appear as a severe reduction of the oleoresin exudation rate and a chlorosis of 2–3-year-old needles, accompanied by a decrease in transpiration. This phenomenon is a unique characteristic of pine wilt disease, accompanied by a further increase in ethylene production. Furthermore, death of cambial cells and cavitation of tracheids occur within a large part of the outer xylem, and result in water deficiency, which induces a decrease in both transpiration and photosynthesis. At the same time, other pathophysiological phenomena are observed, for example, electrolyte leakage from pine tissues and production of abnormal metabolites such as benzoic acid. From the onset of

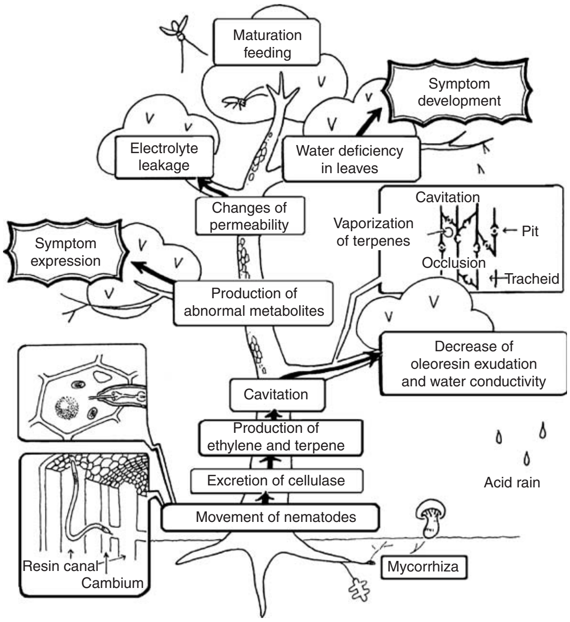

Figure 5 Schematic representation of the wilt mechanisms in the development of pine wilt disease.

water stress, caused by the cavitation of tracheids, the nematode population begins to increase rapidly.

In terms of disease development, the water status of pine trees plays a very important role in the pine tree–pine wood nematode relationship. Experimental results suggest that a lot of pine seedlings do not wilt solely by virtue of numbers of nematodes existing under conditions unfavorable to them, such as a well-watered environment. Empirically, pine wilt disease seems to occur more frequently and to be more destructive in high temperatures with little rainfall. Therefore, these two factors, physiological water status and nematode population density, are considered to be the decisive factors in the development of pine wilt disease.

Disease Control

Before the discovery of pine wood nematode as a causal agent of pine wilt disease, nobody knew an actual pathogen of the disease. However, felling, debarking, and burning of damaged pines immediately proved effective against the disease. Following the discovery of the pine wood nematode as a pathogen of pine wilt disease, the Japanese pine sawyer beetle, *M. alternatus*, was determined as a vector of pine wood nematode. Accordingly, control measures were directed to the Japanese pine sawyer by insecticide spraying.

A large-scale 5-year control project for pine wilt disease was initiated in 1977 by a special law in force in Japan. The project involved aerial spraying to prevent maturation feeding of the Japanese pine sawyer. In spite of these efforts, severe damage was not completely controlled and the disease spread widely from the south to the north of Japan. The reasons for incomplete control are thought to be the limitations of aerial spraying, relying too heavily on the special law in force, and failure to recognize the severity of the disease. In 1997, the special law in force came to an end after three revisions and having been enforced for 20 years since 1977, because considerable damage was continuing and it is still a great threat to the community and the landscape in Japan. Thus, the measures included in the special law in force were incorporated in the common law on forest pest control in 1997.

Control measures against the disease are aimed at breaking the pine tree–pine sawyer beetle–pine wood nematode disease triangle. Present control measures consist for the most part in aerial spraying of insecticides that are effective against the pine sawyer (to break the pine tree–pine sawyer relations), trunk injection of chemicals active against the pine wood nematode (to break the pine tree–pine wood nematode relations), and spraying of insecticides on timber damaged by infestation (to kill the Japanese pine sawyer).

Meanwhile, present control measures are classified under three categories: (1) direct control measures of felling and extermination; (2) preventive control measures of aerial/ground spraying and trunk injection; and (3) biological control measures of pathological microorganisms (*Beauveria bassiana*), predaceous insects (*Dastarcus longulus*), and predatory birds such as woodpeckers (*Dendrocopos major*).

Future Prospects for Pine Wilt Disease

It is generally believed that the pine wood nematode was introduced into Japan from North America a century ago. Subsequently it was introduced into eastern Asian countries and Portugal. European countries show great interest in avoiding the introduction of the pine wood nematode into Europe by timber imports. At present they impose an embargo on all raw softwood materials from North America.

The incidence of pine wilt disease is closely related to environmental conditions such as high temperature with little rainfall as mentioned before. Environmental changes such as increasingly warm and unusual weather conditions which are expected in the near future will most certainly affect the susceptilbility of pine trees. Changes in forest ecosystems resulting from the deposition of acidifying substances may significantly influence pine wilt disease. Pine wilt disease could become the most serious threat in pine forests in European countries, because Scots pine (*P. sylvestris*) and maritime pine (*P. pinaster*) are very susceptible to it.

See also: **Pathology**: Insect Associated Tree Diseases; Vascular Wilt Diseases.

Further Reading

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Leaf and Needle Diseases

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Overview of Foliage Diseases

Foliage diseases of forest trees can be caused by a variety of biotic agents: fungi, bacteria, algae, phytoplasmas, and viruses, as well as abiotic agents such as air pollutants, chemical deposition (e.g., salt injury, acid precipitation), thermal injury, and nutritional deficiencies, both individually and in combinations. Recognition of the primary cause of diseased plants is often a difficult puzzle for the diagnostician, who must consider interactions between host plant, pathogens, environmental conditions, and management history to determine accurately the underlying cause of a foliar disorder. Often, unhealthy-looking foliage may actually indicate a root or stem disease problem.

Foliage diseases affect trees in nurseries and greenhouses, forest plantations, and urban and natural forests worldwide. Foliage pathogens, like their hosts, can occur either as natural components of a forest ecosystem or as exotics introduced through human activity. Exotic pathogens on endemic hosts, and endemic pathogens on exotic hosts, are often more destructive because host plants have no coevolved resistance to the pathogens. Diseases affecting trees in managed situations, such as nurseries and plantations, tend to be more uniformly distributed than in natural forests. Uniform host populations (with respect to species, clone, and age), i.e., monocultures, are more prone to damaging epidemics than plantations and forests managed for genetic diversity.

The inventory of pathogens that pose a threat to forest health is constantly expanding. The lists of forest foliage pests given below are a necessarily selective attempt to enumerate the most common and important foliage pathogens and their current known distributions, recognizing that forest pathogens and pests are constantly changing. New pest organisms are continually being discovered and described. Probably fewer than 20% of the pathogens that pose a threat to forest health have been recognized. Very little information is available on foliage diseases of several important forest trees such as dipterocarps, *Metrosideros*, and *Nothofagus*. Biological evolution continues to shape the interactions between plants and pathogens, as evidenced by development of pesticide resistance and the emergence of aggressive strains and races in pathogen species. Interspecific hybridization and resultant changes in host range have been documented for *Melampsora* and *Phytophthora* pathogens affecting forest trees. Despite international phytosanitary regulation, international trade involving plant and forest products continues to provide an avenue for introduction of insects and pathogens to new hosts and environments. The changing state of scientific knowledge also affects our understanding of pathogen distribution and importance. As increasingly powerful methods of population genetic analysis are applied to the study