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## Pine wilt disease – a threat to pine forest in Europe

**Abstract:** Pine wilt disease caused by pine wood nematodes is endemic to North America. Pine wood nematodes have already spread to East Asia, including China, Taiwan and Korea in addition to Japan. The pine wood nematode, *Bursaphelenchus xylophilus*, was discovered in Portuguese pine forests in 1999. If it were to become established in the pine forests in Europe, it could become one of the most serious threat to coniferous forests worldwide.

**Key words:** *Bursaphelenchus xylophilus*, pine stands, pine wood nematodes (PWN), wilting mechanisms, disease control

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### Introduction

The 21st century is the age of environment. “Green” will be a key word all over the world. The people's feelings toward the forest and trees is increasing more than ever before. Several years ago, in the world famous scientific journal *Nature*, the economic value of forests was estimated to be worth 4.7 trillion US\$ per year that is 14% of total global flow value.

In Japan, the most beautiful sceneries are built up along the seaside by pine trees. In addition, these pine forests actually cover 10% of the forest land and play an important role in both society and environment in addition to timber products.

In recent years, the decline of forests worldwide has been raised as a problem. In the Northern Hemisphere, the major forests are composed of pines. Pines have spread all over the Northern Hemisphere since their incipience in the Mesozoic era. The original birth place of pines was in the Bering Sea where the land was connected with Alaska and Siberia.

Plant diseases first became a concern to the public in the mid-19th century, where potato blight had

spread mainly in Ireland. This famine forced a large number of people to immigrate to the US. Then 1.7 million Europeans moved to the US. As to the forest diseases in the 20th century, three major epidemics occurred; Dutch elm disease, Chestnut blight, and White pine blister rust spread around the world. First, the Dutch elm disease which broke out in Holland at the beginning of the 20th century. The money spent against it has now reached 15 billion US\$. Second, the Chestnut blight caused the almost complete loss of the American chestnut by the introduction of the pathogens from East Asia, probably Japan. In those days American chestnut was a major timber species in the American East. Third, White pine blister rust also spread from Far East Asia. And, then, the most serious epidemic disease broke out in the late 20th century. This is the Pine wilt disease (Fig. 1).

### Background

Since the end of World War II in 1945, pine wilt disease has caused the loss of 26 million m<sup>3</sup> of timber in Japan. This volume is equivalent to the timber required for the construction of 1.7 million wooden



Fig. 1. Actual damage of the Japanese red pine forest by pine wilt disease



Fig. 2. Pine wood nematode, *Bursaphelenchus xylophilus*



Fig. 3. Distributuion of Pine wilt disease

houses in Japan. Initially, the disease was recognized as an epidemic in 1905 in the major port city of Nagasaki, located in southern Japan. Pine wilt disease spread from many port cities in western Japan both before and after World War II. Subsequently, the damage increased to 1 million m<sup>3</sup> per year.

In 1950, in order to control this disease, recommendations were made to develop a pine bark beetle control program. The overall recommendations were the direct control of pine bark beetle by felling, de-barking, and burning. As a result of the recom-

mendations, pine timber losses fell to 200–300 thousand m<sup>3</sup> annually. However, pine timber losses again increased from the late 1970's, reaching a maximum of 2.4 million m<sup>3</sup> in 1979.

Up until now, more than two thousand papers have been published on pine wilt disease. The pine wood nematode (PWN), *Bursaphelenchus xylophilus*, was recovered from dead Japanese black pines in 1969, and was determined as the causal agent of pine wilt disease by the application of Koch's postulates in 1971 (Fig. 2). The pine species susceptible to pine wilt disease are Japanese black pine (*Pinus thunbergii*), Japanese red pine (*P. densiflora*) and *P. luchuensis*, which are also the predominant pine species in Japan. In 1979 the PWN was discovered in Missouri, USA. This was the first report of PWN in the US published in the journal *Plant Disease*. Most pine species seem to be moderately resistant to the PWN. Following an investigation of pine wilt disease distribution, the PWN was determined as being endemic to the US.

In recent years, pine wilt disease has spread to Nanjing, China in 1982, on Japanese black pine and southern red pine (*P. massoniana*) and to Taiwan in 1985, on *P. luchuensis* and Japanese red pine, and to Pusan, Korea in 1988, on Japanese black pine and Japanese red pine (Fig. 3). Recently the PWN was found in dead maritime pine (*P. pinaster*) in Portugal in 1999. This is the first report of the occurrence of PWN in Europe. The outbreak is at the port of Setubal, near Lisbon in maritime pine forests.

Up to now, the PWN has been found in many pine species in Asia and North America. In addition to these pine species, the PWN is also naturally found in blue spruce and white spruce (*Picea* spp.), Eastern larch and European larch (*Larix* spp.), Douglas fir (*Pseudotsuga* spp.), balsam fir (*Abies* spp.), and Atlas cedar and deodara cedar (*Cedrus* spp.) in North America.

A closely related wood nematode, *Bursaphelenchus mucronatus* is frequently recovered from declining pine trees. Its morphology closely resembles PWN. Tails of female PWN round, however, those of *B. mucronatus* have distinctive characteristics, showing a mucronate tail. The hosts of *B. mucronatus* are *Pinus* spp. in Japan, China, Korea, Russia, France, Austria, Italy, Finland, Sweden, Norway, and Canada. In addition to *Pinus* spp., *Abies* sp., *Cedrus* sp. *Larix* sp. and *Pseudotsuga* sp. are its hosts in the US. *B. mucronatus* is distributed over a geographically wider area than *B. xylophilus*.

The PWN is vectored by cerambycid beetles (*Monochamus* spp.) which are inhabitants of coniferous forests worldwide. However, neither the PWN nor *Monochamus* species are known to occur in the Southern Hemisphere.

In 1989 Dr. G. de Guiran of France proposed that, based on hybridization and phylogeny of the pine wood nematode, the Japanese and American strains

of pine wood nematode are derived from common stock originating in western Europe. PWN was introduced to the US and differentiated into *B. mucronatus* and *B. xylophilus*. *B. xylophilus* is the most virulent species to pine trees.

## Pine wilt disease control program

Before the discovery of pine wood nematode as the causal agent of pine wilt disease, nobody knew the actual pathogen of pine wilt disease. However, felling, de-barking and burning of damaged pines immediately proved effective against the disease. In practice, these control methods required a huge input of manpower. With the high level of economic growth in the 1960's, felling and burning were replaced by the spraying of BHC insecticide for the direct control of the Japanese pine sawyer. Since 1971, when BHC was banned, other organophosphate insecticides having a shorter residual activity, such as fenitrothion (sumithion) have been used. Following the discovery of the pine wood nematode as the pathogen responsible for pine wilt disease, the Japanese pine sawyer, *Monochamus alternatus*, was determined to be a vector of the pine wood nematode. Accordingly, control measures were redirected to the destruction of the Japanese pine sawyer by the spraying of insecticide.

A large scale, five year control project for pine wilt disease was initiated in 1977 by law. Aerial spraying was implemented to prevent Japanese pine sawyer maturation feeding. In spite of many efforts severe damage was not completely controlled and the disease spread widely from the southern to the northern part of Japan, with the exception of Hokkaido, the northern island of Japan. The reasons for failure of complete control are thought to be: 1) the limitations of aerial spraying, 2) too much reliance on the Law, and 3) not recognizing the severity of the threat to pine forests. In 1997, the Law came to an end after three revisions and enforcement for 20 years. Currently, annual timber losses still amount to slightly below 1 million m<sup>3</sup> annually. The pine wilt disease is still a great threat to community and landscape in Japan.

## Wilting mechanism and environmental conditions

PWNs introduced into the shoots of young pine trees during maturation feeding of Japanese pine sawyer migrate rapidly into the whole trunk. After the entry of PWN into living pines, a slight reduction in the flow of oleoresin exudate is observed as a unique symptom at early stages of the disease. This is due to the movement of PWN through resin canals at early stages of the disease development and epithelial cells around resin canals are destroyed. Nematodes then

eventually move from rays to tracheids through pits. At the same time, enhanced ethylene production is observed 2–3 days after invasion by PWN. While the exact mechanism remains unclear, this increase is incited by the excretion of a considerable amount of cellulase by the pine wood nematodes. The nematode density is very low at the early stage of disease development, often as low as a few nematodes per 100 gram fresh weight of wood, even following highly concentrated inoculations.

As a general rule, symptom development may be divided into two stages following invasion of the wood by nematodes, namely an early stage and an advanced stage. In the early stage, cytological changes occur in the xylem parenchymatous cells, and these are soon followed by cavitation and embolism formation within a number of tracheids. Dysfunction of conduction in vascular system is observed by acid fuchsin absorbed from the roots. Such internal symptomatology is induced not only in compatible but also in incompatible combinations of pine tree and nematode isolates. However, growth of the nematode population in living pines under conditions unfavorable to the nematode is not assured, even if a high concentration of nematodes is inoculated. Therefore, this stage is considered to be latent, that is, denaturation of parenchymatous cells by nematode invasion results in cavitation and embolism of some tracheids.

At the onset of the advanced stage of disease development, visible symptoms are expressed as a severe reduction of the oleoresin exudation rate and chlorosis of 2–3 year-old needles, accompanied by a decrease in transpiration. This phenomenon is unique characteristics of pine wilt disease, accompanied by a further increase in ethylene production. Furthermore, cambial death and cavitation occur within a large part of the outer xylem, and result in a water deficiency which induces decreases in both transpiration and photosynthesis in leaves. At this time, other pathophysiological phenomena are observed, for example, electrolyte leakage from pine tissues occurs, and a number of abnormal metabolites such as benzoic acid are produced. From the onset of water stress, the nematode population begins to increase rapidly with time.

In terms of the disease development, the water status of pines plays a very important role in the pine-nematode relationship. Experimental results suggest that pine seedlings do not wilt solely by virtue of the number of nematodes 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 summers with little rainfall. Therefore, these two factors, the physiological water status and a particular nematode

population density, are considered to be key factors in the disease development.

### Present pine wilt control programs and the threat of epidemics

Control measures against pine wilt disease are aimed at breaking the pine tree – pine wood nematodes – pine sawyer disease triangle. Present control measures consist of aerial spraying of insecticides effective against the pine sawyer as a preventative of disease to cut the pine tree-pine sawyer relationship, spraying of insecticides on timber damaged by infestation to kill the pine sawyer, and trunk injection of chemicals active against the pine wood nematode to cut the pine tree-PWN relationship. In spite of various efforts, the total amount of pine timber lost to the disease is not decreasing dramatically.

The incident of the pine wilt disease is related to environmental conditions as mentioned before. Environmental changes, such as increasingly warm and unusual weather conditions which are expected in the near future will affect the susceptibility of pine trees. Changes in forest ecosystems resulting from the deposition of acidifying substances may be significantly influence to pine wilt disease. Furthermore, pine wilt disease has the potential to become a major threat if it were to be exported to European countries, because Scotch pine (*P. sylvestris*) and maritime pine (*P. pinaster*) are very susceptible pine species to this disease.

In conclusion, pine wilt disease, which is endemic to North America, has already spread to East Asia, including Japan, China, Taiwan and Korea epidemically, and now in Portugal in Europe. If it were to become established in the pine forests of Europe, it could become one of the most serious threats to pine forests worldwide in the 21st century.

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