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Çam Odun Nematodu: *Bursaphelenchus xylophilus* (Nematoda: Parasitaphelenchidae)

***Mehmet KARAKAŞ**

Ankara University, Faculty of Science and Engineering, Department of Biology,
06100 Tandoğan-Ankara, TURKEY

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Özet: Çam odun nematodu, *Bursaphelenchus xylophilus* çam solgunluk hastalığına sebep olan bir etkendir. Bu nematodun vektörleri, özellikle *Monochamus* cinsine ait olan uzun-boynuzlu kınkanatlılardır. Bu nematodun Japonya'da tanımlanması, ülkedeki doğal çamlar üzerindeki yıkıcı etkileri sonucu olmuştur. Kuzey Amerika için endemik olan birçok kozalaklı ağaç türü solgunluk hastalığına karşı dirençlidir, fakat birçok egzotik tür ise oldukça hassastır.

Anahtar kelimeler: *Bursaphelenchus xylophilus*, Çam odun nematodu, çam solgunluk hastalığı, *Monochamus* spp., uzun-boynuzlu kınkanatlılar

The Pinewood Nematode: *Bursaphelenchus xylophilus* (Nematoda: Parasitaphelenchidae)

Abstract: The pinewood nematode, *Bursaphelenchus xylophilus* is the causative agent of pine wilt disease. Vectors of this nematode are long-horned beetles especially those belonging to the genus *Monochamus*. The introduction of this nematode into Japan had devastating effects on the native pines in that country. Most species of conifers endemic to North America are resistant to the wilt disease, but many exotic species are highly susceptible.

Keywords: *Bursaphelenchus xylophilus*, the pinewood nematode, pine wilt disease, *Monochamus* spp., long-horned bettles

***E-mail:** mkarakas@science.ankara.edu.tr

Introduction

Pine wilt is a disease of pine (*Pinus* spp.) caused by the pinewood nematode, *Bursaphelenchus xylophilus* (Steiner & Buhrer 1934) Nickle, 1970. The pinewood nematode is native to North America and is not considered a primary pathogen of native pines, but is the cause of pine wilt in some non-native pines (Kondo et al. 1982, Mamiya 1983, OEPP/EPPO 1986, Wingfield et al. 1984, Wingfield 1987, Dwinell and Nickle 1989, Dwinell 1997).

The pinewood nematode (Figure 1) is transmitted to conifers by pine sawyer beetles, *Monochamus* spp. (Figure 2) either when the sawyer beetles feed on the bark and phloem of twigs of susceptible live trees (primary transmission) or when the female beetles lay eggs in freshly cut timber or dying trees (secondary transmission) (Figure 3). Nematodes introduced during primary transmission can reproduce rapidly in the sapwood and a susceptible host can wilt and die within weeks of being infested if conditions are favorable to disease development (Wingfield 1983, 1987, OEPP/EPPO 1986, Schroeder and Magnusson 1992, Evans et al. 1993, Dwinell 1997).

Pinewood nematodes introduced to fresh logs or dying trees during egg laying of sawyer beetles feed on fungi introduced by the sawyer and other bark beetles (Kobayashi et al. 1984, Dwinell 1988, 1997, Bowers et al. 1992, Kishi 1995, Evans et al. 1996, Mota and Vieina 2004).

The common presence of the pinewood nematode from such secondary transmission can confound the diagnosis of pine wilt disease. If a dead or dying pine tree with pinewood nematode also has oviposition pits from sawyer beetles at the time of sampling, then pine wilt disease may not be the cause of mortality. The common presence of the pinewood nematode in forest products due to secondary transmission has resulted in restrictions on wood exports (Mamiya 1983, Wingfield 1987, Dwinell 1997, Takuya 2006).

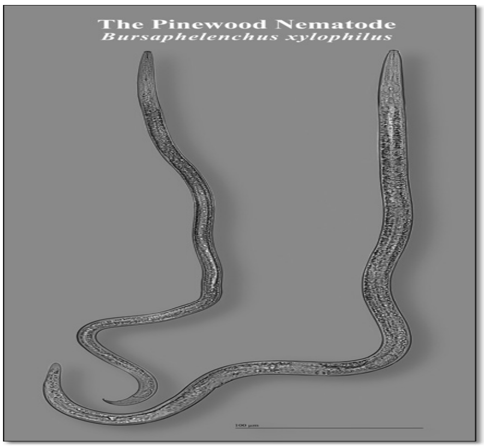

Figure 1. The Pinewood Nematode – *Bursaphelenchus xylophilus*

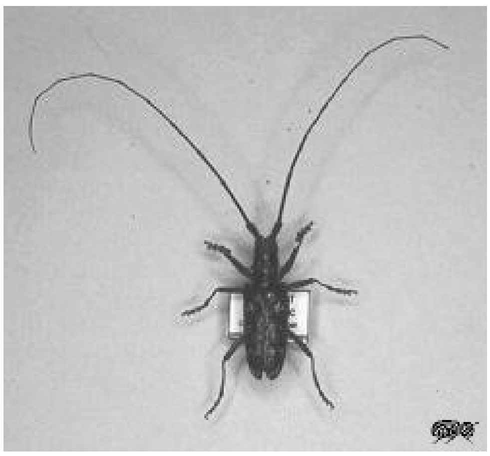

Figure 2. Long-Horned Beetle – *Monochamus* spp.

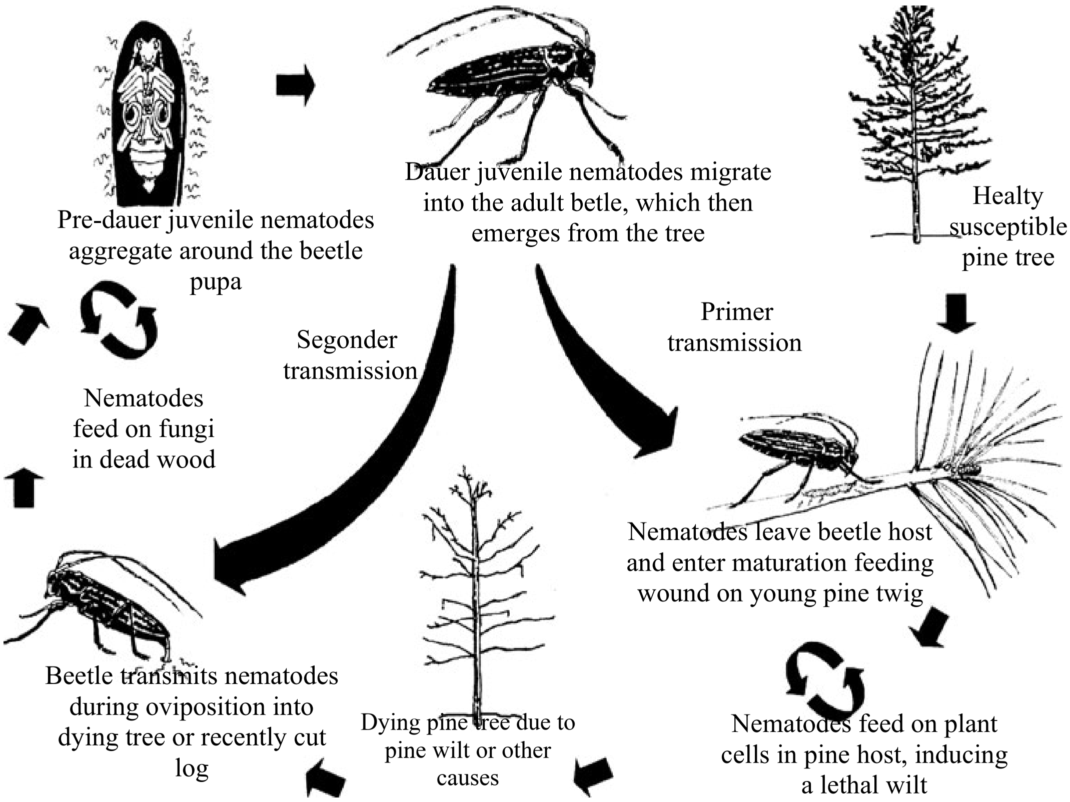

Figure 3. Diagram representing the dispersal mode (primary transmission) and the propagative mode (secondary transmission) of the pinewood nematode, *Bursaphelenchus xylophilus* by its *Monochamus* vector on a susceptible conifer host. (From Giblin-Davis et al., 2003)

History

Pine wilt disease was first described in 1905 in Japan, but the pinewood nematode was not identified as the causal agent of this disease until 1971. Since the pinewood nematode was introduced into Japan, it has extensively damaged Japanese red pines (*Pinus densiflora*) and black pines (*Pinus thunbergii*). Analysis of DNA from several studies indicates that the pinewood nematode was introduced to Japan from the United States (Kondo et al. 1982, Mamiya 1983, Linit 1987, Wingfield 1987, Dwinell 1997).

In the United States, the pinewood nematode was first reported in 1934 associated with fungi in timber, but it was not until 1979 that the nematodes was reported to induce pine wilt disease of non-native pines. Subsequent surveys and studies have established that the pinewood nematode is native to North America and not a threat to native forests. The pinewood nematode has been reported from the United States, Canada and Mexico (Knowles et al. 1983, Wingfield 1984, Kinn 1986, Linit 1987, Bergdahl 1988, Dwinell 1993).

The pinewood nematode is a common secondary colonizer of freshly cut timber and dying conifers. In North America, pine wilt disease occurs predominately in non-native pines that include Austrian (*Pinus nigra*), Scotch (*Pinus sylvestris*) and Japanese red (*P. densiflora*) and black (*P.

thunbergii) pines (Dwinell 1993, Sutherland and Webster 1993).

Asian countries other than Japan began to report presence of the pinewood nematode in the mid-to-late 1980s. Taiwan reported the pinewood nematode in Japanese black pine and luchu pine (*Pinus luchuensis*) in 1985. China and Korea had also reported pinewood nematode in Japanese red and black pines (Li et al. 1983, Tzean and Tang 1985, Yi et al. 1989).

In 1999, Portugal reported the pinewood nematode present in declining maritime pine (*Pinus pinaster*) in the Iberian Peninsula (Mota et al. 1999).

In Turkey

The pinewood nematode, *Bursaphelenchus anamurius* in *Pinus brutia* was reported from Anamur-Mersin (Turkey) (Akbulut et al. 2007).

After this report, *Bursaphelenchus vallesianus* isolated from a dead Scots pine, *P. sylvestris* in the village of Bahçecik Northeastern Turkey in an area managed by the Trabzon Regional Forestry Directorate is described and illustrated (Akbulut et al. 2008).

Nematode Characteristics

B. xylophilus shows the general characters of nematodes. Lips high and offset, weakly developed stylet with reduced basal knobs, median bulb well developed, dorsal oesophageal gland opening inside median bulb. In the female, the post uterine sac is long. In the male, the tail is curved ventrally, conoid and has a pointed terminus. A small bursa is situated terminally. The spicules are well developed, with a prominent rostrum (Mamiya and Enda 1979, Mamiya 1984, OEPP/EPPO 1986).

B. xylophilus can be distinguished by the simultaneous presence of the following three characters. In the male the spicules are flattened into a disc-like structure at their distal extremity. In the female the anterior vulval lip is a distinct overlapping flap and the posterior end of the body is rounded in nearly all individuals. This last character separates *B. xylophilus* from *Bursaphelenchus mucronatus*, a non-pathogenic species in which the female has a mucronate posterior end. However morphological differentiation between *B. mucronatus* and populations of *B. xylophilus* with mucronate tails is very difficult (Mamiya 1984, OEPP/EPPO 1986).

Taxonomy

Pinewood nematodes found associated with fungi in logs in the United States were first reported in 1934 as *Aphelenchoides xylophilus*, later renamed *Bursaphelenchus xylophilus*. In 1972, the pinewood nematode nematode was recovered from Austrian (*P. nigra*) and Scotch (*P. sylvestris*) pine and was referred to as *Bursaphelenchus lignicolus* (Mamiya and Kiyohara 1972). By 1981, *B. lignicolus* was synonymized with *B. xylophilus* and was determined to be native to North America (Nickle et al. 1981).

Over 50 known species of *Bursaphelenchus* inhabit trees. *B. xylophilus* and *B. mucronatus* share several morphological characteristics and are sometimes referred to as members of the pinewood nematode species complex (Mamiya and Enda 1979, Nickle et al. 1981).

B. xylophilus has a pathogenic form “ r ” and a non-pathogenic form “ m ”. The “ r ” form has a round tail and the “ m ” form (mucro form) has a pointed tail. The “ r ” form usually occurs in pine, and the “ m ” form occurs most often on fir and spruce but ossacionally may be found on pine and other conifers. Under natural conditions, most pine wilt disease has been attributed to *B. xylophilus* with the “ r ” form. Webster et al (1990) grouped these morphological forms and species into the pinewood nematode species complex (PWNSC), and several different molecular techniques have been used to learn more about speciation within the PWNSC (Webster et al. 1990, Abad et al. 1991, Tares et al. 1991, Bolla and Bochert 1993, Harmey and Harmey 1993, Castagnone and Castagnone-Sereno 2005).

Hosts

B. xylophilus is found mainly on *Pinus* spp. Apparently, the dead wood of all species of *Pinus* can act as a substrate for the development of *B. xylophilus*. However, only a limited number of species is susceptible to attack as living trees (OOPP/EPPO 1986).

The far eastern species:

(In their native habitats)

Pinus bungeana (Lacebark Pine)

Pinus densiflora (Japanese Red Pine)

Pinus lunhuensis (Luchu Pine)

Pinus massoniana (Masson Pine - China Red Pine - Horsetail Pine)

Pinus thunbergii (Japanese Black Pine)

They are the only species known to be killed by pine wilt disease as mature trees in the field. Many other species have been found to be damaged or killed by the nematode but only under experimental conditions mainly as seedlings in glasshouse (Akbulut et al. 2007).

Other conifers can also act as hosts, primarily *Abies, Larix* and *Picea,* but reports of damage are rare. Isolated cases of death of *Picea* and *Pseudotsuga* due to this nematode have been reported in the USA (Malek and Appleby 1984).

European species:

(Planted in North America)

Pinus nigra (Austrian Pine)

Pinus sylvestris (Scots Pine)

(Planted in China)

Pinus pinaster (Maritime Pine)

Geographical Distribution

It is presumed that *B. xylophilus* originated in North America and was transported from there to the southern Japanese in infested timber (Nickle et al. 1981, Mamiya 1983, Malek and Appleby 1984). The fact that native American conifers are mostly resistant while Japanese species are susceptible. *B. xylophilus* has spread to other Asian countries from Japan (Li et al. 1983).

Asia: China, Hong Kong, Japan, South Korea, Taiwan (Li et al. 1983, Tzean and Tang 1985, Yi et al. 1989, Gu et al. 2006), Turkey-Asia region (Akbulut et al. 2007, 2008).

Europe: Austria, Czech Republic, Finland, France, Germany, Italy, Netherlands, Norway, Poland, Portugal, Russia, Sweden, United Kingdom (Smith 1985, McNamara and Stooen 1988, Tomminen et al. 1989, Kulinich and Kolossova 1995, Mota et al. 1999, Běhalová 2006).

North America: Canada, Mexico, USA (Knowles et al. 1983, Wingfield 1984, Kinn 1986, Linit 1987, Bergdahl 1988, Dwinell 1993).

Life Cycle and Biology

The life cycle of the pinewood nematode involves a propagative cycle and a dispersal cycle. The propagative cycle occurs in the sapwood and involves six life stages:

The egg

Four larval stages

The adult

The sequence of egg to adult takes only 4 to 5 days under favorable conditions of adequate wood moisture, temperature and nutrient availability. The first stage occurs within the egg followed by hatching to the second stage, which soon molts into the third stage. There are two forms of the third stage.

a. Larvae that change into fourth stage larvae, which eventually change into adults that remain in infested trees.

b. A non-feeding dispersal stage. The development of the nematode switches to this dispersal mode in the late stages of tree infection after tree death and occurs only in the presence of *Monochamus* pupae within the wood. These third stage larvae aggregate on the wall of the pupal chamber of the sawyer beetle (*Monochamus* spp.) in the xylem, and then molt to a dauerlarvae. The dauerlarvae is a non-feeding larval stage that is specialized for survival during the transport phase of the life cycle. These fourth stage larvae enter the respiratory system of the young adult beetle and are vectored by the beetle to new hosts. The dauerlarvae can molt into adults within 48 hours after transmission to a conifer host (Mamiya and Enda 1979, Mamiya 1983, Dwinell 1997).

The plant feeding phase occurs when pinewood nematodes are introduced into the branches of a susceptible pine by adult sawyer beetles feeding on the young bark. Once the nematodes are introduced, they feed on the epithelial cells and resin ducts in susceptible host trees and can become distributed throughout the sapwood of the branches, trunk and roots. Vascular dysfunction can occur rapidly under dry conditions or with mean summer temperatures above 20 °C resulting in pine wilt and death of a susceptible pine host (Wingfield 1987).

The fungi feeding phase of the pinewood nematode life cycle occurs in freshly cut softwood and in dead and dying conifers. This is the most common phase and is usually the result of secondary transmission during egg laying by sawyer beetles infested with the pinewood nematode. The pinewood nematodes feed on blue-stain fungi (*Ceratocystis* spp.) and other fungi that typically invade cut timber and dead and dying softwood (Bowers et al. 1992).

Sawyer beetles: Pine sawyer beetles (*Monochamus* spp.), also referred to as long horned beetles, serve as vectors of the pinewood nematode. Primary transmission occurs when sawyer beetles feed on the bark of young branches. Pine sawyers infested with the pinewood nematode can transmit the nematode by the way of feeding wounds to a susceptible host and pine wilt can develop under favorable conditions for the disease (Kobayashi et al. 1984, Bowers et al. 1992, Evans et al. 1993, Kishi 1995).

Pine sawyer beetles are attracted to weakened trees or recently cut logs, where they mate and lay eggs. The beetle, will deposit eggs only on trees or logs with the bark attached. The beetle larvae hatch within a week and feed on the phloem. The larvae tunnel into the xylem to form an oval entrance hole and U-shaped galleries. The sawyer beetles overwinter as larvae and then pupate within an enlarged portion of the gallery. The pinewood nematode larvae, introduced by infested beetles along with their eggs, invade the thoracic spiracles and tracheae of the beetle pupa in numbers as high as 289.000. The adult beetle emerges from the tree, leaving a round ¼ - inch-diameter exit hole. Because beetle development is temperature dependent, the number of generations is lower in cold climates and higher in hot climates. In mild climates, there is generally more than one generation per year (Yi et al. 1989, Smith 1991, Dwinell 1995).

Symptoms

Bursaphelenchus xylophilus: Most pines infected in the spring are often dead by late summer to early fall. Large trees may take two years to die. Vigor of plant does not seem to have a bearing on which plants become infected. Infected plants quickly become stunted. Foliage begins to fade to an off green or slightly yellowish color before turning brown. Dead needles hang on to the branches for a long time. On large trees that take two years to die, the older needles turn yellow first and fall off before the younger needles turn brown. These symptoms can occur any time midsummer to late fall or late winter to spring. Trees infected in the fall do not break bud the following spring (Mamiya and Enda 1979, Mamiya 1983, 1984, Dwinell 1986, Dwinell 1997, Kliejunas et al. 2003, 2006).

Prior to foliar symptoms, the resin content of the wood decreases significantly, but this symptom may go unnoticed unless the tree is pruned. Pines declining from environmental stress or transplant shock will develop symptoms similar to pine wilt. In addition saprophytic nematodes and predator nematodes can be found on the dead pines. Proper identification of the nematodes is important. A laboratory test is necessary to confirm this disease (Mamiya 1983, Zhao et al. 2007, Paulo and Mota 2008).

***Monochamus* spp.:** These insects only oviposit on recently felled trees or trees already under stress. The feeding of the larvae produces feeding tracks on the sapwood under the bark and bore holes into the wood which may make the wood unsaleable (Kobayashi et al. 1984, Gibbons, 1988, Kishi 1995, Togashi 2004).

Management Management procedures investigated over the last 15 years include; prevention, host selection, and treatment by fumigation, irradiation, chemical dips, and elevated temperature. Management of pine wilt disease is primarily limited to prevention. There are no cures for pine wilt disease once a susceptible tree becomes infested with the pinewood nematode. The most effective prevention strategy is to avoid planting non-native pines, such as Scotch (*P. sylvestris*) and Austrian (*P. nigra*) pine, where the mean summer temperature is greater than 20 °C. Where these non-native pines already exist, landowners can reduce susceptibility of high-value landscape trees by watering to avoid drought stress. If they discover infestations landowners can consider removing and chipping infested trees to limit the spread to nearby susceptible trees (Kinn 1986, Dwinell 1994, 1995, 1996, Takeuchi and Futal 2007).

The pinewood nematode can be prevented from infesting softwoods by removing the bark at the time of felling and by avoiding harvesting when the *Monochamus* beetles lay their eggs (July-September). Although most regulations require treatments for all softwoods, some conifer species are rarely colonized by the pinewood nematode. These species include;

Pseudotsuga menziesii – Douglas fir

Sequoia sempervirens – Redwood

Abies concolor – White fir

Thuja plicata – Western red cedar

Tsuga canadensis – Eastern hemlock and

Tsuga heterophylla – Western hemlock.

Many methods have been investigated for treating wood products to eliminate the pinewood nematode. Heat treatments and fumigation currently have some practical use. The pinewood nematode has been eliminated in wood when kiln-dried, or heated to a core temperature of 56 °C or greater for 30 minutes (Kinn 1986, Dwinell 1990, 1994, 1995, Dwinell et al. 1995, Dwinell 1996). Fumigation with aluminum phosphine has been effective in eliminating the nematode from wood chips (Kinn and Springer 1985, Choi et al. 2007). Over time, other treatments (radio-frequency/vacuum dryer, radio waves and steam, radiation etc...) may be discovered or become more practical (Dwinell and Carr 1991, Eichholz et al. 1991, Dwinell and Carr 1995, Dwinell et al. 1995).

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