# INCIDENCE OF THE PINEWOOD NEMATODE[1] IN A SOUTHERN PINE BEETLE[2] INFESTATION IN CENTRAL LOUISIANA

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## ABSTRACT

A large infestation of southern pine beetle, (*Dendroctonus frontalis* Zimm.), located in central Louisiana, was surveyed for the pinewood nematode, *Bursaphelenchus xylophilus* (Steiner and Buhrer) Nickle. Pinewood nematodes were present in 4.2 percent of 94 loblolly pine (*Pinus taeda L.*) trees attacked by *D. frontalis* and were absent from 101 unattacked trees that surrounded the infestation. Monthly sampling of this infestation revealed an increase in the incidence of nematodes as the season progressed. Samples taken from different heights on beetle infested-boles did not differ significantly in the presence of nematodes.

Key Words: Pine wilt disease, *Bursaphelenchus xylophilus, Dendroctonus frontalis,* Cerambycidae.

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## INTRODUCTION

The pinewood nematode, *Bursaphelenchus xylophilus* (Steiner and Buhrer 1934) Nickle 1970, is considered the causative agent of pine wilt disease. This disease was first reported from Japan in 1913 and is now a major threat to Japan's forest industry. In 1980 the Japanese government allocated $35 million to control the disease (Kondo et al. 1982). Results to date have been limited.

Pine wilt disease is thought to be caused by either a phytotoxin produced by the pinewood nematode or a metabolite of an associated bacterium (Bolla et al. 1982; Oku et al. 1980). The disease appears to be endemic and nonepidemic in the U. S. (Dropkin et al. 1981). *Bursaphelenchus xylophilus,* originally described from specimens collected in Louisiana, is native to this continent and was introduced into Japan in the late 1800's. Many North American *Pinus* spp. exhibit some degree of resistance to the disease (Mamiya 1983). However, it is a potential threat to stand survival, especially in areas planted with exotic pines or native pines planted on off-site conditions (Wingfield et al. 1982a). Of 30 pine species tested by Futai and Furuno (1979), *Pinus elliottii* Engelm. var. *elliottii* (slash pine) and *P. taeda* L. (loblolly pine) were found to be highly resistant to pine wilt disease. However, resistance varies inversely with the number of nematodes in the inoculum.

In Japan the nematode is vectored by a cerambycid, *Monochamus alternatus* Hope. The major vectors in the United States are: *Monochamus carolinensis*

[1] *Bursaphelenchus xylophilus* (Nematoda: Aphelenchoididae)
[2] *Dendroctonus frontalis* (Coleoptera: Scolytidae)

(Oliv.), *M. scutellatus* (Say), and *M. titillator* (F.) (Linit et al. 1983; Williams 1980; Wingfield 1983). The dauerlarvae leave the beetle and enter wounds made by these beetles while they feed on succulent bark (Kondo et al. 1982). Inoculations made in this manner are considered primary. Nematodes can also be introduced into dead or dying trees through ovipositional pits produced by female longhorn beetles. In the latter case the trees may have been killed or stressed by fungal disease agents, insects, or physical factors. Inoculations made during oviposition are considered secondary (Wingfield 1983).

This study was undertaken to determine the incidence of *B. xylophilus* in an active southern pine beetle infestation and to note any changes in the occurrence of the nematode infections through time.

## MATERIALS AND METHODS

A large southern pine beetle (SPB) infestation near Williana, LA, (Grant Parish), in the Kisatchie National Forest (Saddle Bayou area) was used for this study. An initial survey for the pinewood nematode was made in July 1983 by sampling 94 loblolly pine trees that had been or were currently under attack by SPB, as well as 101 uninfested trees located just outside the advancing edge of the beetle infestation. One sample was taken from each tree at a height of 1 m using a brace and a 1.9-cm auger bit to drill a 3.8-cm deep hole into the xylem. Before sampling, the outer bark and phloem-cambium interface were removed with a chisel. Borings were collected in Ziploc®[3] bags and held in an ice-chest until processed the next day. In the laboratory the wood borings were wrapped in Kimwipes®[3] and soaked in distilled water for 18 to 24 hours. Any nematodes found in the water were identified as to species [using temporary slide mounts]. To facilitate identification when only immature aphelencoidid worms were present, pieces of xylem and individual worms were transferred to Petri dishes containing fungal cultures in an attempt to rear the worms to adults. *Monilinia fructicola* (Wint.) Honey (brown rot of stone fruits) was used to culture the worms.

Between July 27 and October 24, 1983, an additional 34 SPB-infested loblolly pines were chosen from the same area as the 94-tree sample for additional studies on incidence and within tree distribution of *B. xylophilus*. Five sets of 5 to 10 trees were sampled on two occasions at an interval of about 20 days (Table 1).

Table 1. Number of southern pine beetle infested trees with pinewood nematode present in Louisiana at various dates in 1983.

| Trees sampled | Sampling dates | | | | |
|---|---|---|---|---|---|
| (no.) | 7/27 | 8/16 | 9/6 | 9/27 | 10/21 |
| 5 | 1 | –* | –* | –* | –* |
| 5 | 0 | 1 | –* | –* | –* |
| 10 | –* | 1 | 2 | –* | –* |
| 8 | –* | –* | 0 | 3 | 7 |
| 6 | –* | –* | –* | 0 | 1 |

* No trees from this set were sampled at this date.

[3] Use of trade, firm, or corporation names is for the reader's information and convenience. Such use does not constitute official endorsement or approval by the U. S. Department of Agriculture of any product or service to the exclusion of others that may be suitable.

One set of 8 trees was sampled on three occasions because of delayed beetle development. Trees were sampled by ascending the standing trees. The area of the bole infested by SPB was divided into five equal-length sections to sample at five different heights, and xylem samples were taken from each section using a brace and bit. Maximum sampling heights varied among trees but ranged between 15.6 and 16.5 m. Trees were chosen for the first sampling when bark beetle oviposition was complete. The second sampling, 20 days later, was set to coincide with the presence of SPB pupae or brood adults. Using a chi-square test, a comparison was made between the observed and calculated, theoretical values for each of the five tree sections, in order to determine if the nematode is preferentially localized at a particular height on the bole.

## RESULTS AND DISCUSSION

Only 4 of the 94 infested trees (4.3%) in the initial survey revealed the presence of pinewood nematodes, and the density of nematodes per sample was low. None of the 101 samples from uninfested-trees contained *B. xylophilus*. Of the 34-tree study, pinewood nematodes were found in 5.9% (2 trees) on the first sampling date, and 32.4% (11 trees) eventually became infested with *B. xylophilus*. Nematodes were seldom present in all samples from an individual tree on any given sampling date. *Bursaphelenchus xylophilus* was absent from two sections on one tree and one section on another that had tested positive for *B. xylophilus* on the first sampling date. Of the set of trees sampled on three successive dates none were infected on the first sampling date, three tested positive for pinewood nematodes on the second visit 20 days later, and seven contained *B. xylophilus* on the third sampling date 51 days after the first sampling date. The increase may be the result of the time it takes for *B. xylophilus* to migrate through the tree and reproduce, or may be the result of the introduction of more nematodes through additional beetle oviposition. Although it has been reported that *B. xylophilus* feeds on blue stain fungi (Wingfield et al. 1984), blue stain (*Ceratocystis* spp.) was associated with pinewood nematodes in only 18.4% of the positive samples.

No significant differences were noted in the incidence of worms among the five sample heights ($X^2$, $P > 0.05$). The incidence of pinewood nematodes may have been higher than indicated in this study, since some fungal cultures did not produce worms and others developed high populations of the nematode, *Rhabdontolaimus frontali* Massey 1974. When present in our samples, *R. frontali* appeared to reproduce more rapidly than *B. xylophilus* and tended to suppress the numerical increase of *B. xylophilus*.

The results indicate that the trees were not primarily infected, and thus not stressed by pine wilt disease prior to being attacked by bark beetles. Evidence for this conclusion comes from the fact that the pinewood nematode was absent from healthy trees surrounding the SPB infestation, and their density in trees killed by beetles prior to July was very low. The lower incidence of the nematode on the first sampling date compared to the second is probably the result of increased cerambycid activity on these trees coupled with numerical increases in the nematode populations. The rapid increase in the size of the SPB infestation precludes the possibility that most of the trees were asymptomatically stressed by pine wilt disease prior to attack by SPB. Wingfield et al. (1982b) found pinewood nematodes only in trees or the portion of trees killed by pathogenic fungi or

insects. Marshall and Favinger (1980) suggest that *Ips* spp. may have been responsible for killing the trees infested with *B. xylophilus*. Nematodes were most likely introduced into these dying and dead trees by ovipositing cerambycids. Transmission of this worm to dead and dying trees is an efficient means of perpetuating the population until vectored to healthy host trees (Wingfield 1983). Secondary infestations such as those found in the present study at Saddle Bayou may also provide a reservoir in which cerambycids and pinewood nematodes reproduce and from which infected sawyers may migrate, vectoring pine wilt disease to healthy stands, nursery plantations, or logs at sawmills. Importation of infested coniferous products, especially woodchips, is of great international concern. Finland and Sweden have curtailed imports of these products from North America.

The spotty distribution of *B. xylophilus* along the bole and the failure to find worms at sample heights previously found to be infected indicate the need for better sampling procedures. There is definitely a need for additional studies on the epidemiology of the disease in conifers native to North America.

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