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Transmission of the Pinewood Nematode, *Bursaphelenchus xylophilus*, to Slash Pine Trees and Log Bolts by a Cerambycid Beetle, *Monochamus titillator*, in Florida[1]

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Abstract: Field-collected adults of the southern pine sawyer, *Monochamus titillator* (F.) (Coleoptera: Cerambycidae), naturally infested with fourth-stage juveniles (dauerlarvae) of the pinewood nematode, *Bursaphelenchus xylophilus* (Steiner and Buhrer, 1934) Nickle, 1970, were maturation fed on excised shoots of typical slash pine, *Pinus elliottii* Engelm. var *elliottii*, for 21 days. During August 1981, a male and female adult beetle were held in a sleeve cage placed on the terminal of a side branch of each of seven replicate, healthy 10-year-old slash pine trees. All seven branch terminals showed evidence of beetle feeding on the bark after 1 week, and pinewood nematodes were present in wood samples taken near these feeding sites. Four of the seven trees showed wilt symptoms in 4–6 weeks and died about 9 weeks after beetle feeding. Pinewood nematodes were recovered from the roots and trunks of the dead trees. Each of seven replicate slash pine log bolts was enclosed in a jar with a pair of the same beetles used in the sleeve cages. After 1 week, wood underlying beetle oviposition sites in the bark of all replicate log bolts was infested with the pinewood nematode.

Key words: Bursaphelenchus xylophilus, Pinus elliottii, Monochamus titillator, maturation-feeding, oviposition.

Pine wilt disease has killed pine trees in Japan for at least 60 years (8), causing epidemic losses of native Japanese pines during the last 30 years. In 1972 the pinewood nematode, *Bursaphelenchus xylophilus* (Steiner and Buhrer, 1934) Nickle, 1970 (syn. *B. lignicolus* Mamiya and Kiyohara, 1972), was established as the causal agent of pine wilt and found to be vectored by a longhorned beetle called the Japanese pine sawyer, *Monochamus alternatus* Hope (Coleoptera: Cerambycidae) (7,9).

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Development of the nematode and beetle vector are closely synchronized in Japan (4). Following its introduction into pine sapwood, *B. xylophilus* develops through repeated propagative cycles. Each cycle involves eggs, four similar juvenile stages, and adults of both sexes. About the time that foliage wilts on an infected pine tree, the nematode enters a dispersal cycle. This cycle involves development of third- (J3) and fourth- (J4) stage juveniles with thickened cuticles and lipid reserves. Third-stage nematodes move into the pupal chambers of the Japanese pine sawyer, which are located within tunnels bored by sawyer larvae in host tree sapwood. After molting, the non-stylet-bearing J4 nematodes (dauerlarvae) invade the tracheal system of newly formed adult beetles. Upon emerging from the now-dead pine, the nematode-bearing beetles fly to healthy pines where they feed on the bark of young shoots. During this beetle maturation-feeding period, J4 nematodes leave the beetle, enter pine shoot xylem through the beetle's feeding wounds, and molt to adults, thus starting a new series of propagative cycles within the infected host tree. Fourth-stage juveniles also can be transmitted into newly dead or dying pine trees or fresh logs via oviposition by sexually mature female beetles (11).

The susceptibility of pines in the USA to pine wilt has been of concern since this disease was first reported here in 1979 (2). Infections of *B. xylophilus* have been found in stressed pine trees in Minnesota, Iowa, and Wisconsin, where the nematode was found in pine logs and girdled trees in which cerambycid beetles had oviposited (13). The Carolina pine sawyer, *Monochamus carolinensis* (Oliv.) (Coleoptera: Cerambycidae), was found to be a principal carrier of *B. xylophilus* in Missouri (6) where nematode-carrying adult sawyers transmitted *B. xylophilus* to seven small Scotch pine, *Pinus sylvestris* L., seedlings. These subsequently wilted and died, and *B. xylophilus* was recovered from the dead seedlings (5).

Surveys in Florida showed that sand pine, *Pinus clausa* (Chapm. ex Engelm.) Vasey ex. Sarg.; typical slash pine, *P. elliottii* Engelm. var. *elliottii*; longleaf pine, *P. palustris* Mill.; and loblolly pine, *P. taeda* L., were naturally infected with *B. xylophilus*. Adult beetles of the Carolina pine sawyer (newly emerged from trunk sections taken from nematode-infected sand pines) and the southern pine sawyer, *Monochamus titillator* (F.) (similarly emerged from infected slash pines), were found to be carriers of the pinewood nematode in Florida (3). Because typical slash pine is the most important forest crop tree in Florida, our study was confined to the southern pine sawyer as a possible vector of *B. xylophilus,* with slash pine as the host tree.

Although little has been published on the biology and behavior of the southern pine sawyer, what is known parallels that of the Japanese pine sawyer (4). The southern pine sawyer is reported to have one complete generation and a partial second per year in southern Mississippi (12). Adult sawyers are attracted to the trunk and larger branches of weakened, dying, or recently dead pines and to fresh logs. Mating occurs at these sites and the females gnaw crater-like oviposition pits in the outer bark. Up to nine eggs are laid in each oviposition pit by the female extending her abdomen into the bottom of the pit and laying eggs in a radial pattern within the inner bark. The eggs hatch and young larvae feed on inner bark. Older larvae bore into the sapwood, then make a reverse turn and tunnel almost to the surface of the wood. Pupation occurs in an enlarged cavity constructed by the larva near the bottom of the turn. Young adult sawyers develop in this cavity, finish boring out of the tunnel, and disperse to other host trees. We assume that such young beetles next fly to healthy slash pines to feed on shoot bark. This assumption is based on the fact that young sawyer adults feed extensively on the bark of fresh slash pine shoots offered to them in rearing jars.

Objectives of our study were to determine (a) the frequency with which *B. xylophilus* was carried in a field population of newly emerged southern pine sawyer adults; (b) if such beetles transmit the nematode to healthy slash pine trees during maturation feeding on the shoots; and (c) if female sawyers transmit the nematode to fresh slash pine log bolts during oviposition.

Materials and Methods

Wood borings were removed from the trunks of 20 fire-damaged, 17-year-old slash pines in Levy County, Florida, during late

June 1981 and assayed for *B. xylophilus* by means of a modified Baermann funnel extraction technique. Log sections bearing sawyer oviposition pits were removed from a number of the trees and held in insect emergence cages to collect newly emerged sawyer adults. Such beetles were identified as the southern pine sawyer, by the presence of an apical spine adjacent to the rounded tip of each elytron, and sexed according to antenna length and coloration (1).

About 30% (15/53) of the beetles initially were dissected to estimate the incidence of *B. xylophilus* in the beetle population. Beetle elytra and wings were placed in separate Petri dishes containing sterile distilled water and observed for evacuating nematodes. The tracheal system and internal organs were also dissected to determine if nematodes were present. Identification of *B. xylophilus* was based on shapes of the spicule and adult tails and presence of vulval flap in adults obtained by rearing dauerlarvae on *Botrytis* fungus cultures (10).

Beetles to be used in branch-feeding transmission tests were first placed in 3.8-liter glass jars for maturation feeding on fresh slash pine shoots for 21 days. During August 1981, a sleeve cage was placed over a lateral branch terminal on each of seven healthy, 10-year-old slash pine trees. Treatment trees were scattered along the edges of experimental blocks of slash pines planted at 1.5 × 1.5 m. A pair (♀, ♂) of shoot-fed beetles was then placed in each cage and allowed to feed on the branch terminal for 1 week. After the insects were removed, wood samples taken from near the sites of beetle feeding were assayed for *B. xylophilus*. The 7 treated and 21 immediately adjacent control trees in the plantings were monitored for the appearance of wilt symptoms and mortality for 9 months after treatment.

The seven pairs of adult beetles removed from sleeve cages were next placed in individual 3.8-liter jars, each containing a fresh 8 × 25-cm slash pine log bolt to serve as substrate for beetle oviposition. Each bolt was presampled for *B. xylophilus* to ensure nematode-free wood. Fresh pine shoots were also placed in each jar to divert the sawyers from possibly feeding on the bolts. After 1 week, wood underlying beetle oviposition sites was sampled for pinewood nematode.

Results

All 20 fire-damaged slash pines sampled in Levy County were positive for *B. xylophilus*. The 15 southern pine sawyer adults—newly emerged from logs, held in cages, and initially dissected—all carried pinewood nematodes. Masses of this nematode were present under the beetles' elytra and at the bases of their wings and were associated with the tracheal system, especially near the spiracles. Of the remaining 38 beetles collected, 24 died when placed in jars with pine shoots but were infested with *B. xylophilus*. The other 14 beetles that survived also were found to carry the nematode after they had been used in branch-feeding and oviposition tests.

Wood associated with sawyer feeding on branch terminals was infected with *B. xylophilus* in all seven replicates. After 1 week of exposure to the pairs of beetles, 22, 41, 43, 45, 77, and 107 nematodes were extracted from six of the branch terminals. Extraction from the seventh branch terminal after 2 weeks yielded 412 nematodes. The foliage on four of the seven treated trees wilted about 4 weeks later, followed by browning (death) of all needles about 9 weeks after beetle feeding. Random samples of wood from the trunk and root of these four dead trees contained *B. xylophilus*. No wilt symptoms occurred in the three surviving treated trees or in any of the control trees.

Extraction of wood underlying sawyer oviposition pits in the bark of fresh log bolts were positive for *B. xylophilus* in all seven replicates, with 100, 270, 300, 700, 1,000, 2,000, and 6,000 nematodes per bolt extracted after 1 week.

Discussion

This study confirms previous findings (3) that southern pine sawyer adults carry *B. xylophilus* under natural conditions in Florida. All 53 beetles that emerged from nematode-infected pine trees bore populations of the nematode, including a subsample of 15 newly emerged beetles. This indicates that young emerging sawyers already are colonized by *B. xylophilus* present in the host material, as reported elsewhere (3,6,9). It has been suggested that the pinewood nematode may be capable of establishing itself only in stressed parts of a tree (14). The 10-year-old slash pines used in the

present test were healthy; the branches on which the nematode-infested beetles were placed showed no symptoms of pine wilt. Our results indicated that the nematode can be transmitted via feeding wounds made during August by infested beetles, that the nematodes can colonize the wood of nonstressed pine limbs, and that the nematodes so transmitted can cause tree mortality within 9 weeks. The nematode also can be transmitted to fresh log bolts during beetle oviposition. These findings are similar to those reported in Japan (7,9,11).

It is still unknown if the pinewood nematode, vectored by the southern pine sawyer, infects and kills healthy slash pines under wholly natural conditions in Florida. Field studies are needed to determine the timing, frequency, and duration of branch-feeding on slash pine by the southern pine sawyer, how often the nematode is transmitted via beetle feeding wounds to healthy trees, and the incidence of resultant tree mortality under forest conditions. *Monochamus* spp. sawyers in Florida have at times been observed as the only insect species infesting and apparently killing *Pinus* spp. stressed by damage from fire, hail, or sawfly defoliation. The role of *B. xylophilus* infecting such weakened trees should also be investigated.

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