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Mitigating the pine wood nematode and its insect vectors in transported coniferous wood

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Summary – The pine wood nematode (*Bursaphelenchus xylophilus*) (PWN) has been intercepted in pine chips, unseasoned lumber and packing-case wood. Likewise, PWN insect vectors, *Monochamus* spp., have been found in pallets, crates, and dunnage. As a result of these interceptions and the known consequences of the introduction of the PWN, many countries regulate the import of coniferous wood. Science-based procedures are needed to ensure that globally transported wood is free of the PWN and its vectors. Mitigation measures that have been investigated include prevention, host selection, and treatment by fumigation, irradiation, chemical dips, pressure treatment with preservatives, and elevated temperature. A mill certification programme for lumber (no bark, no grub holes) is rational, but has not got much support. Air-drying wood to its equilibrium moisture content will eliminate the PWN. Switching to hardwood lumber for wood packing material is a short-term solution. Irradiation and dipping wood in borates have shown little promise. The efficacy of pressure-treating green pine lumber with chromated copper arsenate has been shown. Pine chips can be fumigated with phosphine. Logs, lumber, and wood packing material can be fumigated with methyl bromide. Heating coniferous wood to a core temperature of 60°C will eradicate the PWN and its vectors.

In North America, the native pine wood nematode (PWN), *Bursaphelenchus xylophilus* (Steiner & Buhrer) Nickle, is purported to cause the mortality of stressed exotic pines. When inadvertently introduced into Japan and other Asian countries, the PWN became a destructive pest of pines. Over the past 18 years, the PWN has been intercepted in pine chips, unseasoned lumber, pallets, and crating. Likewise, the nematode's

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insect vectors, *Monochamus* spp. (sawyers), have been found in pallets, crates and dunnage. As a result of these interceptions and the known consequences of the introduction of the PWN (*i.e.*, pine wilt disease), many countries regulate the importation of coniferous wood (Dwinell, 1997). The application of sound mitigation procedures can insure that globally transported coniferous wood is free of the PWN and its vectors, and that future pine wilt epidemics, such as those in Japan and China, can be prevented.

WOOD MANAGEMENT SYSTEM

In order to develop control strategies, it is essential to understand the life histories of the PWN and its insect vectors, and how they enter the wood management system. Most of the described species of *Bursaphelenchus* are carried by insects, especially bark beetles and woodborers. The PWN and other closely related species (*i.e.*, *B. mucronatus* Mamiya & Enda) are vectored by cerambycid longhorn beetles in the genus *Monochamus*. The basic biology of Asian, North American, and Euro-Siberian *Monochamus* species are basically the same. Sawyers are opportunistic saprophytic woodborers and the adults are attracted to freshly cut, felled, dying or recently dead conifers, especially pines (*Pinus* spp.). The cause of the tree's mortality is not significant. Sawyers oviposit only in dying trees and logs with bark with phloem and cambium. The sawyer larva feeds for several weeks on the cambial fibre layer under the bark, then bores deep into the sapwood and then turns around and bores back towards the surface, thereby forming a characteristic U-shaped tunnel. The pupal cell is located in the sapwood just beneath the outer bark. Upon emergence the adult beetles move to a suitable host to feed on the bark of young branches. The sawyer's life cycle is normally 1 year in southern areas and 1-2 years in northern areas (Dwinell & Nickle, 1989).

The transmission stage (dauer juvenile) of the PWN invades the callow sawyer adult through the thoracic spiracles and is held in a quiescent state in the tracheae. Nematode dauer juveniles emigrate from the spiracles and enter the tree through wounds caused by the beetle feeding on young branches. Once inside a susceptible host, the nematode feeds on the parenchyma cells of the ray canals and reproduces. Such PWN-infected trees exhibit wilt symptoms and die rapidly. If these events occur, the resultant disease is called 'pine wilt'.

This mode of transmission is called 'primary' because primary infection of the host occurs. The most common means of transgenerational tranfer of species of *Bursaphelenchus*, however, is through secondary transmission. Secondary transmission occurs when the PWN enters a freshly cut, felled, dying or recently dead tree during oviposition of the female sawyer. In this case, the PWN is a secondary associate and feeds largely on the fungi in the wood (Dwinell & Nickle, 1989; Dwinell, 1997).

Secondary transmission has broad implications. For example, if oviposition pits are noted on the trunk of a dead or dying conifer, it cannot be automatically assumed that the tree succumbed to pine wilt. Secondary transmission makes accurate diagnosis of pine wilt extremely difficult. Furthermore, since the PWN normally exists in nature independent of pine wilt, the distribution of the PWN cannot be equated with the distribution pine wilt disease (Dwinell & Nickle, 1989). Anyhow, it is through secondary transmission that species of *Bursaphelenchus* vectored by insects may be found in the wood management system (*i.e.*, logs, lumber, and chips).

Logs

Damage to sawn logs by sawyers and colonisation by the PWN can be prevented by cutting trees outside the flight period of the insect (*i.e.*, generally taken as May to October for *M. tiltillator* (Fab.) and *M. carolinensis* (Olivier) in the southern USA) and removing them from the woods before mid-June. Prompt salvage and utilisation of dead and dying trees, debarking freshly felled logs, and water storage of logs will prevent attacks by sawyers. Since female sawyers require bark for oviposition, debarking is particularly effective for managing both sawyers and PWN in conifer logs.

There is little information on mitigation procedures for eradicating the PWN and its vectors in conifer logs. Sodium borate dips have proved to be ineffective in controlling the PWN or sawyers in debarked pine logs. Heat-treating Virginia pine (*P. virginiana* Mill.) logs infested with sawyers and pine wood nematodes in a 77°C kiln effectively controlled sawyers, nematodes, and fungi. A core temperature of 53°C plus 30 min killed the sawyers, and 60°C plus 30 min eliminated the PWN and fungi. (Since the kiln's temperature schedule exceeded the target wood temperature, the temperature of the wood continues to increase during

the 30 min. The final core temperature was not reported.) (Dwinell, 1997, 2001a).

In order to avoid re-infestation of fresh logs, the mitigation treatment has to kill the phloem and cambium. For example, heat-treating freshly sawn eastern white pine (*P. strobus* L.) and Virginia pine logs in a 77°C kiln until the wood temperature reached 60°C at a depth of 2.5 cm did not reduce their susceptibility to attacks by sawyers or colonisation by the PWN, because the sawyers were still attracted by stimuli released by the logs (Dwinell, 1997, 2001a). In a more recent study, freshly sawn loblolly pine (*P. taeda* L.) logs fumigated for 24 h with methyl bromide at the rate of 49 g/m^3 were attacked by sawyers and colonised by the PWN when placed in the field. However, methyl bromide eradicated sawyers and the PWN from infested logs and, since the bark had separated from the sapwood, the logs were not re-infested (Dwinell, unpubl.). Wang *et al.* (1995) in the People's Republic of China have also demonstrated the efficacy of methyl bromide against the pine wood nematode in naturally infested pine logs.

CHIPS

Phosphine has been used extensively for shipboard fumigation to control insects in US export grains. In 1987, this technology was transferred to the in-transit fumigation of pine chips to eradicate the PWN. The 'Florani' experiment showed that phosphine could be successfully used as an in-transit fumigant for eliminating the PWN from pine chips (Leesch *et al.*, 1989; Dwinell, 2001b).

The PWN can be killed in pine chips by irradiation. Scientists in the southern United States treated PWN-infested pine samples in a caesium-137 irradiator; 9 kGy was the lethal dosage (Dwinell, 1997). Subsequently, Canadian scientists reported that 7 kGy (cobalt 60 gammacell 220 irradiator) will eliminate the PWN in aqueous solution (Smith, 1991). These data support the contention that a higher dosage is necessary to eliminate the PWN *in vivo* than *in vitro*.

PWN can be exterminated in southern pine chips by immersion in 0.15% solution of sodium N-methyldithiocarbamate. However, metam-sodium, which is also efficacious as a fumigant, is probably impractical for large volumes of pine chips (Dwinell, 1997).

Temperature markedly affects the population dynamics of the PWN in piled pine chips. In piled southern pine chips, the PWN primarily

inhabits fresh chips and chips on the outer shell of the pile. The nematode does not inhabit chips in the interior of the pile where oxidative processes cause spontaneous heating to around 60°C (Dwinell & Nickle, 1989).

There have been a number of laboratory studies on using heat to decontaminate PWN-infested pine chips. It has been found, for example, that wet heat (hot water or steam) is more effective than dry heat. Also, these experiments have demonstrated that the PWN population in pine chips rapidly declines at temperatures above 45°C. Mortality of the PWN in chips is primarily a function of type of heat, wood moisture, heat conductivity of wood, time and temperature (Dwinell, 1997; Smith, 1991; Dwinell, 2001).

There have been two studies using elevated temperatures to eradicate the PWN in large volumes of pine chips. Peeples Industries Inc. (Savannah, GA, USA) designed an apparatus that could be used to treat 200 lb lots of wood chips with steam or hot water. Basically, it was a long tube, covered with insulation, housing a screw-type conveyor. The screw could be adjusted to control the attack time (amount of time the chips were in the tube). The chips were placed in a hopper, moved through the tube by the screw for the required time, and discharged at the other end. The minimum temperature-time schedules (hot water, 80°C for 42 s; steam, 100°C for 37 s) resulted in the decontamination of the chips (Dwinell, 1996). The other apparatus evaluated was a Swedish steam-dryer that had been shipped to Savannah, GA. The vessel (2.1 m long, 0.9 m diam.) has a chain-plate conveyor, which acted both as a treatment table and as a throughput carrier. Steam was injected through a three-port manifold. The lowest time/temperature/pressure combination was to increase the vessel temperature from 85-105°C in 5 min at low pressure. No nematodes were recovered from any of the treated samples (Dwinell *et al.*, 1994).

In a study on the use of radio waves to decontaminate pine chips, it was determined that mortality of the PWN in pine chips exposed to radio frequency waves (27.1 MHz) was a function of temperature. In a subsequent study, radio waves and steam, alone or in combination, were evaluated as decontaminators of PWN-infested chips. Radio waves and live steam effectively eradicated the PWN in pine chips when the wood temperature exceeded 57°C. High temperatures (81-85°C) can be reached in less time by combining steam and radio waves (Dwinell & Carr, 1995).

Since the PWN in wood chips is outside the evolutionary biology of the PWN and its insect vectors, it is problematic that the PWN in chips is a measurable pest risk. The PWN would have to infest a new pine host through soil transmission. A PWN 'experts' meeting in Brussels in 1993 concluded that soil transmission was 'an unlikely event in nature' (Dwinell, 1997). Unfortunately, the 'Florani' in 1986 (Leesch *et al.*, 1989) was the last pine chip shipment to Europe from North America. The United States no longer has a state-of-the-art wood chip export terminal.

LUMBER

Since sawyers pupate just under the bark, they are normally removed with the slabs when the log is sawn into a cant. The slabs are usually chipped, but occasionally may be used for pallets. Thus, the occurrence of sawyers in lumber is greatly reduced during manufacture. However, the unseasoned lumber may still harbour the PWN. This led, in part, to a mill certification programme for lumber that states that lumber should be free of bark and grub holes (borer holes created by sawyers). If no grub holes are visible, it is concluded that there are no sawyers in the sawn wood that might emerge and transmit the PWN. Furthermore, the foreign *Monochamus*, which requires bark with phloem and cambium for oviposition, would be unable to breed in the wood, and, therefore, not be infested by the PWN if it were to emerge.

Methyl bromide has been identified as an ozone-depleting substance, and the developed countries signatory to the Montreal Protocol must phase out its use by 2005. The Montreal Protocol presently exempts the use of methyl bromide for pre-shipment and quarantine purposes. Recent studies have shown that methyl bromide is useful in eradicating the PWN and its vectors from pine lumber. Dwinell (2000) reported that a June fumigation with methyl bromide at 150 g/m^3 and 300 g/m^3 eradicated the PWN in shortleaf pine disks, boards, and cants. More recently, Dwinell (unpubl.) fumigated PWN- and sawyer-infested shortleaf pine (*P. echinata* Mill.) lumber with methyl bromide at the rates of 80 g/m^3 (winter) and 46 g/m^3 (summer). The rates are equivalent to US Department of Agriculture Animal and Plant Inspection Service (APHIS) T404 schedule for logs and lumber. In both experiments, no nematodes were extracted from the fumigated lumber. Although the lumber had numerous grub holes caused by sawyer larvae, no sawyers emerged from any

of the boards the summer following fumigation. The sawyer larvae and pupae had probably been removed when the logs were sawn into cants.

The PWN will not survive in air-dried shortleaf pine lumber that has been commercially pressure-treated with chromated copper arsenate (CCA) (Dwinell, 2001). In February 2002, the United States Environmental Protection Agency announced a voluntary decision by industry to move consumer use of treated lumber products away from pressure-treated wood that contains arsenic by 31 December, 2003, in favour of new alternative wood preservative. It should be noted that 90% of southern pine is kiln-dried to a wood moisture content (WMC) of 20% or less prior to pressure treatment with CCA.

Lumber can be seasoned by either air-drying or kiln-drying. When the lumber is air-dried, the PWN population declines when the wood moisture content falls below the fibre saturation point (FSP) (generally taken as 28% on dry weight basis). Furthermore, at the FSP the PWN population is no longer reproducing. When the WMC reaches the equilibrium moisture content (EMC) (10-12% for southern pines), the PWN normally can no longer be extracted from the wood (Dwinell, 1997).

To speed up seasoning, green pine lumber is typically kiln-dried to a WMC of less than 20% (In the United States, over 86% of the pine lumber is kiln-dried). Since kiln temperatures are usually 71-113°C, kiln-drying not only removes water from the wood, but also sterilises it in the process (Dwinell, 1990, 1996, 1997, 2001; Smith, 1991; Tomminen & Nuorteva, 1992).

Mesophilic organisms can be eradicated by kiln-heating the wood without significantly reducing the moisture content. Dwinell (1990) reported that heat-treating dimension southern pine lumber naturally infested with the PWN in a 71°C kiln to a core temperature of 58-60°C was sufficient to kill all nematodes. In a Canadian study, heat-treating artificially infested ponderosa pine (*P. ponderosa* Dougl. Ex Laws.) logs in dehumidification (70°C), high temperature (113°C), and conventional (88°C) kilns to a target temperature of 59°C plus 30 min resulted in total mortality for the PWN (Smith 1991). (The final wood temperature after 30 min was not reported, but would be expected to be significantly higher than 59°C. For example, heating unseasoned pine in a 71°C kiln to 56°C plus 30 min is roughly equivalent to heating wood to 60°C.) In 1991, the European Union PWN Team collaborated with Dwinell on an experiment to kiln-heat loblolly and shortleaf pine lumber naturally infested with PWN and sawyers (EOLAS 1991) in a 71°C kiln. In the

two runs, boards were removed from the kiln once 10% of them had reached 45°C (Run 1) or 60°C (Run 2). In Run 1, the wood temperature ranged from 37.5 to 52.5°C and 67% of the planks were positive for the PWN. In Run 2, the wood temperature ranged from 52 to 66°C and the boards were free of nematodes. In final analysis, this unreplicated experiment indicates that kiln-heating green southern pine lumber to 54-57°C may be sufficient to eradicate the PWN. Finnish scientists have studied the affects of kiln-heating pine lumber from Canada naturally infested with PWN and *Monochamus* imported into southern Finland. In 60 cm boards (50 × 150 mm) all nematodes were killed in two hours in a 70°C sawmill kiln, but the wood temperature was not recorded (Tomminen & Nuorteva, 1992).

Radio frequency heating may have potential use for eliminating the PWN and sawyers in green lumber. Canadian scientists heated ponderosa pine artificially infested with the PWN to 40 and 60°C using a Raytherm radio frequency generator (Smith, 1991). Radio frequency heating to 40°C had little impact on the PWN and a target temperature of 60°C killed PWN in only 44% of the boards. It was concluded that variations in wood moisture content made the method unreliable. This problem, however, may be overcome by using a radio-frequency/vacuum (RF/V) dryer. Dwinell *et al.* (1994) evaluated RF/V kiln equipped with parallel electrodes operating at a frequency of 13.56 MHz. No nematodes were recovered from the slash pine lumber naturally infested with PWN when the core wood temperature reached 75-100°C within 1 h. A live *Monochamus* larva was found in the run in which the wood temperature did not exceed 45°C. The researchers concluded that the RF/V kiln could be used to eradicate pests in green pine; but green lumber should be heat-treated to the thermal death point of the PWN at the beginning of the drying cycle rather than the end.

WOOD PACKING MATERIAL

The risk of the PWN and its *Monochamus* vectors in wood packing material (WPM) has not been fully delineated. The key to determining if WPM is a significant source of the PWN and its insect vectors is by ascertaining whether or not the nematode can be transmitted. Certainly, slabs sawn from fresh logs being cut into cants should not be used in pallets for international trade. Although unseasoned wood without grub holes and bark with phloem and cambium is a dead end for the nematode,

its use for WPM may be complicated by regulatory interpretations. In service WPM that has dried to its equilibrium moisture content is probably of minimal risk. Coniferous wood with grub holes and bark that has been kiln-dried is of no risk. Lumber used in WPM can be fumigated with methyl bromide, kiln-heated, or kiln-dried. Although the use of kiln-heated or kiln-dried lumber may be the preferred, it is necessary to recognise that many of the 100 trading partners of North American countries do not have the necessary timber resource or kiln-capacity. These countries may have to turn to methyl bromide fumigation to meet regulatory demands for pest-free WPM. If methyl bromide fumigation is used, recapture systems should be online to avoid releasing the methyl bromide into the atmosphere. A short-term solution would be to manufacture WPM from hardwoods instead of softwoods for WPM; however, the proposed International Plant Protection Convention Standard for WPM would regulate all species of wood, including hardwoods.

Conclusions

Science-based procedures are needed to ensure that globally transported wood is free of the PWN and its vectors. Mitigation measures that have been investigated include prevention, host selection, alternative materials, and treatment by fumigation, irradiation, chemical dips, pressure treatment with preservatives, and elevated temperature. A mill certification programme for lumber (no bark, no grub holes) is rational, but has not got much support. Air-drying wood to its equilibrium moisture content will eliminate the PWN. Irradiation and dipping wood in borates have shown little promise. The efficacy of pressure-treating green pine lumber with chromated copper arsenate has been demonstrated, but industry is phasing out CCA. Pine chips can be fumigated with phosphine. Infested logs, lumber, and wood packing material can be fumigated with methyl bromide. Heating green coniferous wood to a core temperature of 60°C will eradicate the PWN and its vectors.

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