Survival of *Bursaphelenchus xylophilus* and *Monochamus galloprovincialis* in pine branches and wood packaging material

E. Sousa[1], P. Naves[1], L. Bonifácio[1], L. Inácio[1], J. Henriques[1] and H. Evans[2]

[1]*INRB, IP (National Institute of Biological Resources), Unidade de Silvicultura e Produtos Florestais, 2780-159 Oeiras (Portugal); e-mail: edmundo.sousa@inrb.pt*

[2]*Forest Research, Rhodfa Padarn, Llanbadarn Fawr, Aberystwyth SY23 3UR (UK)*

Survival and development of *Bursaphelenchus xylophilus* (pine wood nematode) was studied for up to 40 weeks in *Pinus pinaster* sawn wood and branches: 30 boards (1200 × 100 × 25 mm), 30 long-blocks (1200 × 95 × 95 mm), 10 pine branches with bark and nine branches without bark (1200 mm long). The nematode was found in all materials and through the entire sampling period, with higher abundance in the sawn wood (boards and long-blocks). In the initial period *B. xylophilus* reproduced abundantly and a population peak was detected at 8–12 weeks. Subsequently, the populations declined and became dominated by third-stage resistant larvae (J_{III}), and in the final sample nematode abundance was very low. Nematode decline in the wood was correlated with a decrease in the moisture content (MC) to below fibre saturation. Survival of the insect vector *Monochamus galloprovincialis* was also assessed in sawn boards (1200 × 100 × 25 mm, $n = 31$) and blocks (160 × 95 × 95 mm, $n = 40$). The majority of the larvae were killed when sawing the wood, although some adults successfully emerged from the boards (10% survival) and blocks (37%). These results represent a contribution to the quantification of the risks of dispersing pine wilt disease through wood packaging materials, confirming that untreated wood can support healthy and abundant *B. xylophilus* populations for sufficient time for vectors surviving the sawing process to complete their development, to emerge and disperse the nematode.

Introduction

Although native to North America (Dropkin & Foudin, 1979; Robbins, 1982; Sutherland & Peterson, 1999), *Bursaphelenchus xylophilus* (pine wood nematode) has been introduced to Eastern Asia and Western Europe by human activities and international trade of untreated and contaminated wood. Outside its native range, the nematode is one of the most important pests of pine trees and other conifers worldwide, being the casual agent of pine wilt disease in certain conditions leading to death of susceptible tree hosts once infested (Kobayashi *et al.*, 1984; Kishi, 1995; Evans *et al.*, 1996).

Short-distance dispersal of the nematode from affected to non-affected hosts is by adults of the genus *Monochamus* (Coleoptera: Cerambycidae), with the role being taken by local species of *Monochamus* once *B. xylophilus* is introduced and established in a new location. In Portugal, where *B. xylophilus* was first detected in 1999, the pine sawyer beetle *Monochamus galloprovincialis* is the sole vector species (Sousa *et al.*, 2001; Naves *et al.*, 2007). However, for long-distance dispersal of the nematode, round or sawn wood is regarded as having very high risk, overwhelmingly through the ability of the insect vectors to survive and emerge at the end of the pathway (Evans *et al.*, 1996; Gu *et al.*, 2006; Haack, 2006). Nematodes of the genus *Bursaphelenchus* are very often detected in surveys and sampling of wood packaging materials worldwide (Tomminen, 1991; Tomiczek *et al.*, 2003; Braasch *et al.*, 2005, 2007; Gu *et al.*, 2005, 2006; Li *et al.*, 2008, 2009), further confirming the risks associated with this specific material and the importance of this pathway for the introduction and spread of alien pests and pathogens.

Despite the worldwide importance of *B. xylophilus* as a quarantine pest and its economic importance for forestry and associated industries, including the wood packaging industry, the biology, ecology, long-term survival and development of *B. xylophilus* in wood packaging materials is still largely unknown. The objectives of the current study were to assess the survival ability over time of *B. xylophilus* in both pine branches and wood as used for wood packaging and pallet construction, and to confirm if *Monochamus* larvae can survive the sawing of wood into boards and blocks and subsequently emerge from these processed wood materials.

Materials and methods

Dead adult maritime pine trees (*Pinus pinaster*) were sampled for the presence of the pine wood nematode in Herdade da Comporta, Portugal. Six mature pine trees infested with *B. xylophilus* were felled in February 2009. The trunks were sawn at a commercial sawmill to produce boards and long-blocks sized to dimensions used for pallet construction and taken to the INRB, I.P. laboratories at Oeiras.

The survival and development of *B. xylophilus* was assessed in 30 boards (1200 × 100 × 25 mm), 30 long-blocks (1200 × 95 × 95 mm), 10 pine branches with bark and nine pine branches without bark (average 8 mm diameter and 1200 mm long), which were kept isolated from each other in a large room with variable ambient temperature (15–26°C, 50–70% humidity). Samples to assess for nematode survival were taken at the beginning of the experiments ('week 0') and periodically in weeks 1, 2, 4, 6, 8, 12, 16, 20, 24, 32 and 40. On each sampling occasion, pre-marked sections of wood were cut and divided into smaller pieces with a vertical chain saw. Each sample consisted of a unit of 100 ± 1 g. Nematodes were extracted from the wood using the modified tray method, in which wood is soaked in water for 48 h and nematodes are recovered from the liquid with a 38 μm wire mesh. Samples were observed under a stereoscopic microscope to detect and identify nematodes. Only *B. xylophilus* was considered, with counts made in a counting chamber using known volumes of nematode suspension. Whenever possible, different life stages where identified and recorded, although not quantified precisely.

To determine the moisture content of the wood after nematode extraction, wood samples were weighed, oven-dried and weighed again, and the MC was calculated by subtracting the initial weight from the dry weight and dividing the result by the dry weight.

To study the survival of *M. galloprovincialis* in sawn wood, dead *P. pinaster* trees containing larvae of the insect vector were sawn into standard-pallet boards (1200 × 100 × 25 mm, $n = 31$) and blocks (160 × 95 × 95 mm, $n = 40$) at the saw mill. At the INRB laboratories at Oeiras the wood was kept separated in meshed wood boxes to allow the development and emergence of the insects. The boards and blocks were analysed and dissected 30-days after the emergence of the last insect, to quantify insect mortality.

Analysis of variance (ANOVA) was used to compare *B. xylophilus* abundance and moisture content of branches and sawn wood.

Results

Pine wood nematode survival in pine branches and wood packaging material

The pine wood nematode was detected in all tested wood materials and throughout the entire sampling period of 40 weeks. Nematode numbers increased during the first 8–12 weeks at an ambient temperature of 20.7 ± 1.7°C and 64 ± 4.2% relative humidity, with the initial samples being dominated by eggs and *B. xylophilus* juveniles. In the subsequent weeks (from 16 to 40, mean temperature of 21.9 ± 2.7°C and 63 ± 5.0% relative humidity), nematode populations gradually declined and were dominated by third-stage resistant larvae (J_{III}). Nematode populations reached very low numbers in the final sample, and disappeared completely in 48% of the replicates (Table 1). Overall, and considering the whole period, the sawn wood (boards and long-blocks) had significantly higher nematode loads than the branches with and without bark ($F = 4.3254$; d.f. = 1; $P = 0.0378$).

The moisture content (MC) of the wood differed significantly between treatments ($F = 19.9350$; d.f. = 3; $P < 0.0001$) and could be divided into three groups: boards (with the lowest MC), long-blocks and branches without bark (intermediate MC) and branches with bark (with the highest MC). Despite variations in the initial moisture content, a common trend for all wood materials was the decrease in the MC during the first 12–16 weeks, when it reached values below the fibre saturation point of the wood at approximately 28% MC (Table 2).

The decline of the nematode population coincided with the decrease of the moisture content to below 15%, resulting in a weak but significant correlation between the MC and the trend of the nematode population in the wood ($r = 0.1312$; $P = 0.0001$). In the final weeks of assessment, the MC stabilized and fluctuated within a narrow range around the equilibrium MC of 10–15%, in accordance with the ambient temperature and humidity.

Table 1 PWN abundance per gram of wood (mean ± SD) in branches (with and without bark) and sawn wood (boards and long-blocks) over a 40 week period. Values within each column followed by the same letter do not differ statistically, Fisher LSD test after ANOVA ($P \leq 0.05$). Number of replicates infested with *Bursaphelenchus xylophilus* in parentheses

	Branches							
Week	With bark		Without bark		Boards		Long-blocks	
0	21.3 ± 18.7ab	(10)	36.2 ± 24.8ab	(9)	61.6 ± 58.9a	(30)	36.0 ± 35.6a	(30)
1	183.2 ± 276.9abc	(10)	197.5 ± 215.2bcd	(9)	467.0 ± 491.7bcd	(30)	125.5 ± 170.9ab	(30)
2	159.5 ± 109.6abcd	(10)	250.5 ± 152.3c	(9)	479.7 ± 507.0bd	(30)	148.1 ± 144.4ab	(30)
4	147.6 ± 151.2abc	(10)	311.9 ± 207.1c	(9)	270.2 ± 291.9e	(30)	239.8 ± 287.0abc	(30)
6	340.7 ± 176.8d	(10)	232.7 ± 271.5cd	(9)	289.8 ± 267.0ce	(30)	702.2 ± 1464.7d	(30)
8	246.6 ± 160.6cd	(10)	378.3 ± 332.9c	(9)	543.9 ± 579.9b	(30)	467.0 ± 779.3cd	(30)
12	293.7 ± 363.1cd	(10)	303.8 ± 262.4c	(9)	643.5 ± 748.4b	(30)	516.5 ± 950.1cd	(30)
16	234.6 ± 227.3cd	(10)	262.3 ± 230.0c	(9)	295.8 ± 244.2cde	(30)	403.1 ± 609.3bcd	(30)
20	214.9 ± 253.3cd	(10)	303.2 ± 294.6c	(9)	153.3 ± 122.7ae	(30)	303.8 ± 466.5abc	(29)
24	196.2 ± 215.0bcd	(10)	47.0 ± 50.0abd	(9)	62.9 ± 66.7a	(30)	119.5 ± 191.2ab	(30)
32	129.2 ± 225.1abc	(10)	41.9 ± 51.7abd	(9)	6.6 ± 6.4a	(30)	43.4 ± 76.2a	(30)
40	4.0 ± 5.7a	(10)	0.5 ± 0.9a	(2)	0.1 ± 0.2a	(7)	0.6 ± 1.5a	(16)

Table 2 Moisture content (mean ± SD) in branches (with and without bark) and sawn wood (boards and long-blocks) over a 40 week period. Values within each column followed by the same letter do not differ statistically, Fisher LSD test after ANOVA ($P \leq 0.05$)

	Branches			
Week	With bark	Without bark	Boards	Long-blocks
1	42.2 ± 13.6a	36.5 ± 7.1a	29.1 ± 11.1a	39.9 ± 13.3a
2	37.1 ± 13.4a	26.2 ± 6.6b	18.2 ± 3.8b	35.2 ± 12.6b
4	26.9 ± 11.2b	29.1 ± 24.2ab	15.5 ± 1.5c	23.5 ± 5.0c
6	24.7 ± 9.7b	21.0 ± 12.0bc	14.4 ± 1.0cd	18.4 ± 2.0d
8	20.3 ± 5.5bc	15.0 ± 1.4cd	13.2 ± 0.8de	17.4 ± 1.2de
12	12.4 ± 2.9d	10.5 ± 1.6d	9.9 ± 2.1f	10.6 ± 1.4g
16	13.8 ± 1.2cd	12.2 ± 0.9d	9.6 ± 2.1f	9.9 ± 2.5g
20	14.6 ± 0.8cd	13.5 ± 0.6cd	11.8 ± 0.5fe	12.8 ± 1.2fg
24	15.8 ± 4.0cd	13.7 ± 0.7cd	12.9 ± 1.0de	14.4 ± 1.1f
32	16.0 ± 3.8cd	13.7 ± 1.1cd	12.6 ± 0.7de	14.6 ± 0.5ef
40	13.9 ± 0.8cd	13.1 ± 0.5cd	12.3 ± 1.0fe	12.9 ± 1.7fg

Monochamus survival in wood packaging material

The majority of the larvae and pupae of *M. galloprovincialis* present in the wood were destroyed in the process of sawing the pine trunks to obtain standard pallet-sized boards and blocks. Nevertheless, a few insects survived and completed their life cycle, resulting in adult beetles emerging from both wood packaging materials. After destructive sampling of the wood it was possible to quantify the overall survival rate of the *M. galloprovincialis* larvae and pupae post-sawing. Survival varied from 10% in the boards (seven adults emerged out of 67 larvae) to 37% in the blocks (18 out of 49).

Discussion

This study has demonstrated that when trees infested by *Bursaphelenchus xylophilus* are felled, the nematodes can survive and continue their development both in branches and sawn wood for prolonged periods of time, which in the current experiments was over 40 weeks from being brought to the laboratory. Halik & Bergdahl (1990) found high populations of *B. xylophilus* in wood chips after 12 weeks and suggested that under favourable conditions nematode abundance could remain high for well beyond that period, while Tomminen *et al.* (1991) detected the *B. xylophilus* in wood chips after 130 days.

The initial rise in nematode populations from the background level present from the field infestation of the pines coincided with a higher temperature in the laboratory relative to the ambient external temperatures. The favourable conditions promoted accelerated reproduction in *B. xylophilus*, resulting in abundant eggs and juveniles in the first samples. The pine wood nematode has a high reproductive potential and under favourable conditions (25°C) is capable of developing from egg to adult in just 4–5 days (Mamiya, 1984; Tomminen, 1993). Rapid population peaks of the pine wood nematode under favourable conditions have also been reported in previous studies (e.g. Halik & Bergdahl, 1990).

In the first 6 weeks there was a rapid decrease in moisture content to well below fibre saturation in all categories of wood. Nematode survival in wood with low moisture is known to occur and, as was observed in the current study, is partially achieved by a shift in the proportion of the population that enters the juvenile third stage larvae (J_{III}). This developmental stage is known for its resistance to temperature extremes, desiccation and food shortage, and therefore is adapted for surviving for considerable periods of time within dead trees (Ishibashi & Kondo, 1977; Mamiya, 1984; Kishi, 1995; Zhao *et al.*, 2007).

Despite the confirmed resilience of the J_{III} larvae, the populations eventually declined to near zero over time, confirming that once removed from the field there appears to be a finite capacity in any given piece of wood to support continued breeding and survival of *B. xylophilus*. As the ambient conditions were favourable for development, the decrease in the nematode populations probably reflected a decline in food availability in wood over time, as fungi are an essential food source for the mycophagous life stages of *B. xylophilus* once the living cells of the host tree are dead (Mamiya, 1983, 1984; Halik & Bergdahl, 1990; Kishi, 1995; Maehara *et al.*, 2005). This was further enhanced by the decrease of the MC in all wood materials, because wood with low moisture has lower capacity to support mycophagous populations of *B. xylophilus* as the blue-stain fungi require MC above fibre saturation to develop (Viitanen, 1997; Schmidt, 2006).

Concerning the survival of the insect vector, there was high mortality of *M. galloprovincialis* larvae when the wood was sawn, although successful emergence occurred in both boards and blocks. The higher survival in blocks reflects their shape and larger size, which favoured larval survival during the sawing operations in contrast with the thinner boards.

Our results suggest that if pine wood infested with both the *B. xylophilus* and its insect vector is moved in trade, whether as a wood product or as pallet/packaging material, there will be a finite period of over 40 weeks (over 10 months) during which it poses a risk of disseminating *B. xylophilus*. This period is sufficient for *M. galloprovincialis* surviving the initial sawing of the wood to emerge and transfer *B. xylophilus* to susceptible hosts in new locations. Nevertheless, this dispersion pathway can be prevented by application of ISPM 15 phytosanitary measures such as heat-treatment, which is effective in killing the *Monochamus* larvae and the pine wood nematode in the wood (Dwinell, 1997; Wang, 2010), although nematodes of the genus *Bursaphelenchus* are sometimes detected in wood despite having phytosanitary certificates indicating that heat-treatment has apparently been carried out (Gu *et al.*, 2006; Zahid *et al.*, 2008).

These results represent a contribution to the quantification of the risks of dispersing *B. xylophilus*, the causal agent of pine wilt disease, through pallets and wood packaging materials. Additional studies are required on the longevity and resilience of the pine wood nematode under a wider range of temperatures and wood moisture contents. The main factors regulating the life his-

tory of *B. xylophilus* and the interactions with the fungal complex in the wood also need to be further studied and understood. The effectiveness of controlling pine wood nematode by manipulation of the wood MC to below the fibre saturation point, and the possibility of non-vector wood-to-wood direct transfer of *B. xylophilus* also need to be investigated. There are strong indications that very low wood MC acts as a barrier to transfer of *B. xylophilus* into the wood (Sousa, *et al.* submited) and this can be exploited to ensure absence of the nematode in pallets in service.

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Survie de *Bursaphelenchus xylophilus* et *Monochamus galloprovincialis* dans les branches de pin et le bois d'emballage

La survie et le développement de *Bursaphelenchus xylophilus* (nématode du pin) ont été étudiés pendant 40 semaines dans du bois scié et des branches de *Pinus pinaster*: 30 planches (1200 × 100 × 25 mm), 30 blocs longs (1200 × 95 × 95 mm), 10 branches avec écorce et 9 branches sans écorce (1200 mm de long). *B. xylophilus* a été trouvé dans tous les matériaux et pendant toute la période d'échantillonnage, avec une abondance plus élevée dans le bois scié (planches et blocs longs). Au début, les nématodes se reproduisent abondamment et un pic de population a été détecté à 8–12 semaines. Ensuite, les populations déclinent et sont dominées par des larves résistantes de troisième stade (J_{III}) et, dans l'échantillon final, l'abondance du nématode est très faible. Le déclin du nématode dans le bois était corrélé à la diminution de la teneur en humidité en dessous du point de saturation des fibres. La survie de l'insecte vecteur *Monochamus galloprovincialis* a également été évaluée dans les planches sciées (1200 × 100 × 25 mm, $n = 31$) et les blocs (160 × 95 × 95 mm, $n = 40$). La majorité des larves ont été tuées lors du sciage du bois, même si certains adultes ont réussi à émerger des planches (10% de survie) et des blocs (37%). Ces résultats représentent une contribution à la quantification des risques de dispersion de la maladie du dépérissement du pin par le bois d'emballages, et confirment que le bois non traité peut permettre le maintien de populations saines et abondantes de *B. xylophilus* pendant un temps suffisant pour que les vecteurs survivant au processus de sciage terminent leur développement, émergent et dispersent le nématode.

Выживание *Bursaphelenchu xylophilus* и *Monochamus galloprovincialis* в сосновых ветках и древесном упаковочном материале

Выживание и развитие *Bursaphelenchus xylophilus* (сосновой стволовой нематоды) исследовались в течение 40 недель в пиленой древесине и в ветках *Pinus pinaster*: 30 досках (1200x100x25 мм), 30 брусах (1200x95x95 мм), 10 сосновых ветках с корой и 9 ветках без коры (длиной 1200 мм). *B. xylophilus* была найдена во всех этих древесных материалах и в течение всего периода осуществления выборки, при более высокой плотности популяции в пиленой древесине (досках и брусах). В начальный период нематоды размножались в изобилии, причем пик популяции приходился на период 8-12 недель. Затем популяции сокращались и в них начинали преобладать резистентные личинки третьего возраста (JIII), а в заключительной выборке обилие нематод было крайне низким. Снижение численности нематод в древесине коррелировало с уменьшением содержания влаги (MC) до уровня ниже насыщенности волокон. Выживание насекомого переносчика *Monochamus galloprovincialis* также оценивалось в пиленых досках (1200x100x25 мм, n=31) и в брусах (160x95x95 мм, n=40). Большинство личинок погибло при распиливании древесины, хотя некоторые взрослые особи успешно появились из досок (10%-ое выживание) и брусов (37%). Эти результаты представляют собой вклад в количественную оценку риска распространения болезни увядания сосны через древесные упаковочные материалы, подтверждая тем самым, что в необработанной древесине могут поддерживаться здоровые и обильные популяции *B. xylophilus* в течение достаточного времени для переносчиков, выживших после процесса распиливания, чтобы завершить свое развитие, появиться и распространить нематоду.

References

Braasch H, Gu JF, Burgermeister W & Zhang JC (2005) *Bursaphelenchus doui* sp. n. (Nematoda: Parasitaphelenchidae) in packaging wood from Taiwan and South Korea – a new species of the xylophilus group. *Russian Journal of Nematology* **13**, 19–27.

Braasch H, Gu J, Burgermeister W, Brandstetter M & Metge K (2007) Description of *Bursaphelenchus africanus* sp. n. (Nematoda: Parasitaphelenchidae) – found in packaging wood exported from South Africa to Ningbo/China. *Journal of Nematode Morphology and Systematics* **9**, 71–81.

Dropkin VH & Foudin AS (1979) Report of the occurrence of *Bursaphelenchus lignicolus* induced pine wilt disease in Missouri. *Plant Disease Reporter* **63**, 904–905.

Dwinell LD (1997) Pinewood nematode: regulation and mitigation. *Annual Review of Phytopathology* **35**, 153.

Evans HF, McNamara DG, Braasch H, Chadoeuf J & Magnusson C (1996) Pest risk analysis (PRA) for the territories of the European Union (as PRA

area) on *Bursaphelenchus xylophilus* and its vectors in the genus *Monochamus*. *Bulletin OEPP/EPPO Bulletin* **26**, 199–249.

Gu J, Braasch H, Burgermeister W & Zhang J (2006) Records of *Bursaphelenchus* spp. intercepted in imported packaging wood at Ningbo, China. *Forest Pathology* **36**, 323–333.

Gu JF, Zhang JC, Braasch H & Burgermeister W (2005) *Bursaphelenchus singaporensis* sp. n. (Nematoda: Parasitaphelenchidae) in packaging wood from Singapore – a new species of the *B. xylophilus* group. *Zootaxa* **988**, 1–12.

Haack RA (2006) Exotic bark- and wood-boring Coleoptera in the United States: recent establishments and interceptions. *Canadian Journal of Forest Research* **36**, 269–288.

Halik S & Bergdahl DR (1990) Development of *Bursaphelenchus xylophilus* populations in wood chips with different moisture contents. *Journal of Nematology* **22**, 113–118.

Ishibashi N & Kondo E (1977) Occurrence and survival of the dispersal forms of pine wood nematode, *Bursaphelenchus lignicolus* Mamiya and Kiyohara. *Applied Entomology and Zoology* **12**, 293–302.

Kishi Y (1995) *The Pine Wood Nematode and the Japanese Pine Sawyer*. Thomas Company Limited, Tokyo (Japan).

Kobayashi F, Yamane A & Ikeda T (1984) The Japanese pine sawyer beetle as the vector of pine wilt disease. *Annual Review of Entomology* **29**, 115–135.

Li HM, Trinh PQ, Waeyenberge L & Moens M (2008) *Bursaphelenchus chengi* sp n. (Nematoda: Parasitaphelenchidae) isolated at Nanjing, China, in packaging wood from Taiwan. *Nematology* **10**, 335–346.

Li HM, Trinh PQ, Waeyenberge L & Moens M (2009) Characterisation of *Bursaphelenchus* spp. isolated from packaging wood imported at Nanjing, China. *Nematology* **11**, 375–408.

Maehara N, Hata K & Futai K (2005) Effect of blue-stain fungi on the number of *Bursaphelenchus xylophilus* (Nematoda: Aphelenchoididae) carried by *Monochamus alternatus* (Coleoptera: Cerambycidae). *Nematology* **7**, 161–167.

Mamiya Y (1983) Pathology of the pine wilt disease caused by *Bursaphelenchus xylophilus*. *Annual Review of Phytopathology* **21**, 201–220.

Mamiya Y (1984) The pine wood nematode. In: *Plant and Insect Nematodes* (Ed. Nickle WR), pp. 589–626. Marcel Dekker, New York (USA).

Naves P, Camacho S, Sousa E & Quartau J (2007) Transmission of the pine wood nematode *Bursaphelenchus xylophilus* through feeding activity of *Monochamus galloprovincialis* (Coleoptera; Cerambycidae). *Journal of Applied Entomology* **131**, 21–25.

Robbins K (1982) Distribution of the pinewood nematode in the United States. In: *Proceedings of the National Pine Wilt Disease Workshop* (Ed. Appleby JE & Malek RB), pp. 3–6. Illinois Natural History Survey , Champaign, IL (USA).

Sousa E, Bravo MA, Pires J, Naves PM, Penas AC, Bonifácio L & Mota M (2001) *Bursaphelenchus xylophilus* (Nematoda; Aphelenchoididae) associated with *Monochamus galloprovincialis* (Coleoptera; Cerambycidae) in Portugal. *Nematology* **3**, 89–91.

Sousa E, Naves P, Bonifácio L, Henriques J, Inácio ML & Evans H. Riks of pine wood nematode *Bursaphelenchus xylophilus* transfer between wood packaging simulating assembled pallets in service (submited).

Sutherland JR & Peterson M (1999) The pinewood nematode in Canada: history, distribution, hosts, potential vectors and research. In: *Sustainability of Pine Forests in Relation to Pine Wilt and Decline* (Ed. Futai K, Togashi K & Ikeda T), pp. 247–253. Shokado Shoten, Kyoto (Japan).

Schmidt O (2006) *Wood and Tree Fungi. Biology, Damage, Protection, and Use*. Springer, Berlin (Germany).

Tomiczek C, Braasch H, Burgermeister W, Metge K, Hoyer U & Brandstetter M (2003) Identification of *Bursaphelenchus* spp. isolated from Chinese packaging wood imported to Austria. *Nematology* **5**, 573–581.

Tomminen J (1991) Pinewood nematode, *Bursaphelenchus xylophilus,* found in packing case wood. *Silva Fennica* **25**, 109–111.

Tomminen J (1993) Reproductive success of *Bursaphelenchus xylophilus* and *B. mucronatus* at constant and fluctuating temperatures. *European Journal of Forest Pathology* **23**, 342–352.

Tomminen J, Halik S & Bergdahl DR (1991) Incubation temperature and time effects on life stages of *Bursaphelenchus xylophilus* in wood chips. *Journal of Nematology* **23**, 477–484.

Viitanen HA (1997) Modelling the time factor in the development of brown rot decay in pine and spruce sapwood – the effect of critical humidity and temperature conditions. *Holzforschung* **51**, 99–106.

Wang X (2010) Heat sterilization of wood. In: *General Technical Report FPL-GTR-190, U.S. Department of Agriculture, Forest Service, Forest Products Laboratory*. pp. 1–13. Madison, WI (USA).

Zahid MI, Grgurinovic CA & Walsh DJ (2008) Quarantine risks associated with solid wood packaging materials receiving ISPM 15 treatments. *Australian Forestry* **71**, 287–293.

Zhao LL, Wei W, Kulhavy DL, Zhang XY & Sun JH (2007) Low temperature induces two growth-arrested stages and change of secondary metabolites in *Bursaphelenchus xylophilus*. *Nematology* **9**, 663–670.