Assessing risks of pine wood nematode *Bursaphelenchus xylophilus* transfer between wood packaging by simulating assembled pallets in service

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The risks of *Bursaphelenchus xylophilus* (pine wood nematode) transfer in relation to wood material were assessed. Combinations of infested and non-infested adjacent boards, long-blocks and blocks of *Pinus pinaster*, simulating assembled pallets, were assessed. For the recipient wood, pieces with natural moisture content (MC), heat-treatment (56°C for 30 min in the core) and kiln-drying to <20% MC were tested, along with in-service boards from pallets. Donor and recipient wood materials were kept in direct contact at 25°C or 10°C, with nine replicates per treatment. *Bursaphelenchus xylophilus* was found to transfer rapidly at 25°C when the wood had an MC above fibre-saturation point (>30%). Nematode reproduction was rapid and sustained, gradually declining to zero at 40 weeks. *Bursaphelenchus xylophilus* did not transfer to kiln-dried or to in-service wood with an MC below fibre-saturation point, or to wood at 10°C. The key factors determining nematode transfer were the ambient temperature, the nematode load of the donor wood and the MC of the recipient wood, with a 'barrier' of 20% MC below which it becomes unsuitable for nematode transfer. This finding indicates that there is a limited risk of spread of *B. xylophilus* in treated and untreated solid wood packaging materials.

Introduction

Pine wood nematode, *Bursaphelenchus xylophilus*, is one of the most important pests of pine trees and can also be found on other conifers worldwide (Kobayashi *et al.*, 1984; Kishi, 1995). The nematode is native to North America, where it does not cause significant mortality to native conifers (Linit, 1988). It has, however, resulted in serious losses of pine trees when it has established in other countries, including China (Yang & Qouli, 1989), Japan (Kishi, 1995), Taiwan (Tzean & Tang, 1985), Korea (La *et al.*, 1999) and Portugal (Mota *et al.*, 1999).

In view of the serious consequences arising from *B. xylophilus* infestation, a pest risk analysis (PRA) was carried out in 1996 (Evans *et al.*, 1996). This concluded that transmission of the nematode from tree to tree was through the activities of *Monochamus* spp. vectors, which has been confirmed in Portugal for *Monochamus galloprovincialis* (Sousa *et al.*, 2001, 2002; Naves *et al.*, 2006, 2007). However, the PRA also considered the possibility that nematodes could be transmitted to living trees directly from infested wood without the presence of *Monochamus* spp. It was noted that evidence for such a hypothesis was scarce and supported only by small-scale laboratory or greenhouse experiments, not through field observation or experimentation. A thorough revision of the PRA was carried out by an EPPO (European and Mediterranean Plant Protection Organization) expert panel in 2009, also concluding that there was a theoretical risk of transmission of nematodes to trees via infested wood in the absence of *Monochamus* spp. No new field-based evidence to support this possible pathway had been accumulated since the first PRA in 1996.

In the context of pathways recognized by the two PRAs, round and sawn wood are regarded as presenting the highest risks of transmission of *B. xylophilus*, overwhelmingly through the ability of *Monochamus* spp. vectors to survive, complete their life cycles and emerge as adults at the end of the pathway. Within these pathways, packaging wood is regarded as very high risk, but that risk is mitigated by application of ISPM15 treatments, which have been established to reduce the risk of introduction and/or spread of quarantine pests associated with wood packaging material, pallets included (ISPM 2002).

Among the treatments recommended, the correct use of heat treatment kills both vector and nematodes as the wood reaches a core temperature of 56°C for a minimum of 30 min (Dwinell, 1990, 1997). The theoretical direct wood-to-wood transmission of pine wood nematode, including transmission into wood that has been decontaminated by ISPM15 treatments, remains an unresolved question and one that has not previously been studied specifically. It is known that *B. xylophilus* can move in water films, both in artificial media such as agar plates and for short distances on the surface of wood, and to and from adult *Monochamus* spp. (Bolla *et al.*, 1989; Stamps & Linit, 1998; Togashi & Arakawa, 2003; Yamane *et al.*, 2003). However, there is no information on whether nematodes can move from one piece of wood to another when these are either in direct contact or separated by short distances.

Data to answer questions on these possible non-vector transmission pathways are essential to quantify the risks from these pathways, especially for the wood packaging industry, particularly for pallet wood. In the latter case, the issues relate both to new pallets entering circulation and also to whether older pallets, which could have been infested by *B. xylophilus* prior to implementation of decontamination procedures, could represent a risk of transfer to new pallets by direct contact.

The objectives of the current study were to evaluate the risk of pine wood nematode transfer in relation to wood material and pallets in service without the presence of vectors in the genus *Monochamus*. Different combinations of adjacent boards, long-blocks and blocks simulating assembled pallets were assessed for transfer and survival of *B. xylophilus* between infested wood sawn to size and non-infested wood.

Materials and methods

Experiments consisted of a donor piece of wood (board or long-block) infested with *B. xylophilus* in direct contact with a nematode-free recipient piece of wood (board, long-block or block).

To obtain the donor wood, dead mature maritime pine (*Pinus pinaster*) trees were sampled for the presence of the pine wood nematode in Herdade da Comporta, Portugal. Trees positive for pine wood nematode were felled in February 2009 and sawn to obtain boards, long-blocks and blocks sized as for pallet construction. The recipient wood was obtained from nematode-free and healthy maritime pines felled in February 2009. After being sawn to size, recipient wood with natural MC, heat-treated to ISPM15 standard (56°C for 30 min) or kiln-dried (to obtain an MC below 20%) was selected for the experiments. Additionally, in-service boards recovered from existing pallets (over 6 months old) were also selected to simulate pallet repairs with new wood, which is common practice for reusable pallets. Used boards were previously sampled for nematode presence, and only nematode-free wood was selected for this trial.

Experiments were conducted at the INRB, IP laboratories at Oeiras, Portugal. Donor and recipient wood pieces were maintained in direct contact (except at sampling occasions), both pieces being held together with plastic strapping, with donors always placed above recipients. Wood was kept in large chambers with controlled temperature and humidity, under favourable (25°C and ≥70% RH) or unfavourable (10°C and ≥70% RH) conditions for nematode movement and reproduction. A range of size combinations were tested, representing boards (1920 × 100 × 25 mm), long-blocks (1920 × 95 × 95 mm) and blocks (160 × 95 × 95 mm) along and across the grain of the wood, with nine replicates per treatment (Table 1).

Samples to assess for nematode survival and transfer were taken periodically in weeks 0 (immediately before the wood pieces were joined), 1, 2, 4, 6 and 8 for the experiments with recipient heat treatment and kiln-dried wood. For the remaining experiments, additional samples were taken in weeks 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 chainsaw. 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 afterwards recovered from the liquid with a 38 μm wire mesh.

Samples were observed under a stereoscopic microscope to detect and identify any nematodes present. Only *B. xylophilus* were counted in the experiments, and they were quantified using a scoring system rather than precise counts. The scoring system used six classes of abundance: class 0: absence of nematodes; class 1: 0.01–1 nematodes per g wood; class 2: 1–5 nematodes per g wood; class 3: 5–10 nematodes per g wood; class 4: 10–20 nematodes per g wood; class 5: 20–50 nematodes per g wood; class 6: >50 nematodes per g wood.

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

For statistical analysis, mean values of the nematode abundance classes were used. For the highest class (class 6, >50 individuals per g wood), the mean value of 391 nematodes g^{-1} was estimated from direct counts made in the selected pine wood. Parametric and non-parametric statistical analyses were used to compare the data.

Table 1 Summary of treatments and combinations tested for *Bursaphelenchus xylophilus* transfer between adjacent wood pieces

			Recipient wood						
			Freshly cut						Used
			Natural MC				Heat-treated	Kiln-dried	Natural MC
			Board	Long-block across grain	Long-block along grain	Block across grain	Board	Board	Board
Donor wood	25°C	Board	X	X			X	X	X
		Long-block			X	X			
	10°C	Board					X	X	

MC, Moisture content.

Results

There was successful nematode transfer from donor to recipient wood materials kept at 25°C, as pine wood nematodes were detected in recipient boards, long-blocks and blocks (with natural MC) and in heat-treated boards. There was no transfer to kiln-dried or in-service boards, or in the board-to-board treatments at 10°C (Table 2).

When it occurred, nematode transfer took place within the first week in all experiments, being more successful and abundant in recipient wood with high MC, such as the freshly cut boards and long-blocks (Fig. 1).

The nematode load of the donor wood appeared to play a role in nematode transfer, as higher donor densities resulted in more frequent *B. xylophilus* transfers and significantly higher nematode densities in adjacent wood pieces ($t = 3.1821$; d.f. = 5; $P = 0.0245$). The *B. xylophilus* populations responded extremely rapidly to the favourable conditions in the recipients with high MC, and a rapid increase in their numbers was observed within a few weeks following the initial transfer. Nematode reproduction was detected from the first samples, with the observation of eggs and juveniles of *B. xylophilus*.

At 25°C, nematode numbers continued to increase to a peak between the eighth week (for the recipients) and the twelfth week (for the donors) (Tables 3 and 4). As mean nematode load of the recipient long-blocks along the grain and blocks across the grain did not differ (Mann–Whitney U test, $P = 0.5470$), results of these two experiments are grouped in Table 3.

Even when kept under unfavourable conditions (10°C), nematode populations in the donor wood remained fairly stable during the 8 weeks of assessment (Table 5).

After populations reached a peak, nematode numbers declined gradually until the final sample, being virtually zero by week 40 in both donors and recipients. Overall, a weak but significant correlation can be detected between the development and survival of the nematode population and the MC in the wood ($r = 0.1312$, $P = 0.0001$), the nematode decline coinciding with a generalized decrease of the MC to about or below the fibre-saturation point, when rehydration of the wood was no longer possible.

Along with the nematode load of the donor wood and the ambient temperature, the MC of the recipient wood was the most decisive factor for nematode transfer in all treatments. Transfer occurred consistently to freshly cut wood recipients with high initial MC (mean of 83.9 ± 17.8% at week 1) and, although heat treatment significantly ($F = 21.1$; d.f. = 3; $P < 0.0001$) lowered the MC of the wood (mean of 44.16 ± 12.5%), such a decrease did not impede successful nematode transfer, which took place until an absolute minimum MC of 21.5% (Table 6).

Table 2 Pine wood nematode transfer in the treatments and combinations tested (number of recipient replicates infested/number of recipient replicates periodically sampled)

			Recipient wood						
			Freshly-cut						Used
			Natural MC				Heat-treated	Kiln-dried	Natural MC
			Board	Long-block across grain	Long-block along grain	Block across grain	Board	Board	Board
Donor wood	25°C	Board	9/9	9/9			8/9	0/9	0/9
		Long-block			9/9	9/9			
	10°C	Board					0/9	0/9	

MC, Moisture content.

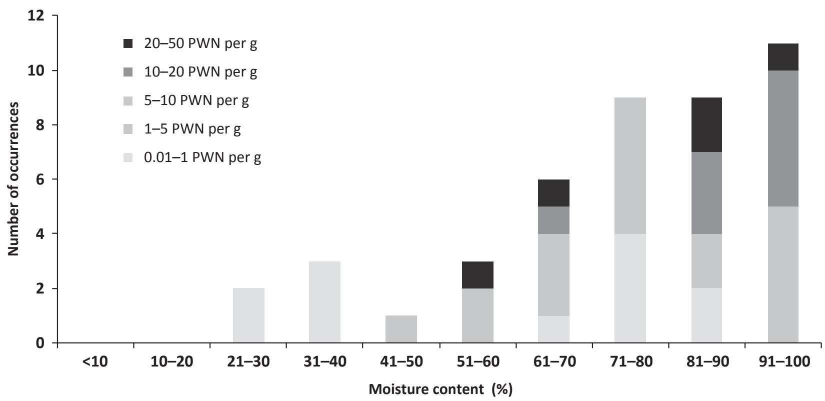

Fig. 1 *Bursaphelenchus xylophilus* density (in classes) in recipient wood at a range of moisture contents during the first week of the experiment. PWN, pine wood nematode.

Table 3 Pine wood nematode (PWN) abundance (per g wood) and moisture content (MC) in donor and recipient wood with natural MC at 25°C (mean values ± SD)

	Donor board		Recipient board		Donor board		Recipient long-block across grain		Donor long-block		Recipient long-block along grain and block across grain	
	PWN	MC	PWN	MC	PWN	MC	PWN	MC	PWN	MC	PWN	MC
Sampling week												
0	25.3 ± 11.8a	43.8 ± 24.2a	0.0 ± 0.0a	nr	29.7 ± 10.6ab	39.8 ± 16.4a	0.0 ± 0.0a	nr	27.0 ± 13.6ab	47.5 ± 18.2a	0.0 ± 0.0a	nr
1	26.1 ± 10.5a	38.5 ± 15.0a	14.7 ± 15.8a	75.9 ± 17.6a	107.4 ± 161.0abc	37.5 ± 12.4a	4.8 ± 5.9a	83.0 ± 19.6a	99.2 ± 160.9bc	42.1 ± 11.9b	9.8 ± 10.9a	88.4 ± 16.4a
2	114.1 ± 157.0ab	36.9 ± 13.6a	222.2 ± 200.2cd	67.6 ± 15.7b	272.3 ± 178.0def	38.4 ± 13.1a	27.0 ± 12.5a	87.7 ± 18.0a	248.8 ± 183.6d	41.9 ± 12.7b	43.4 ± 87.5abc	86.7 ± 16.7a
4	232.8 ± 187.6bcd	21.6 ± 1.2b	105.2 ± 162.3abc	23.0 ± 2.1c	145.3 ± 184.6abcd	23.5 ± 1.7b	13.3 ± 13.3a	49.7 ± 8.7b	105.5 ± 157.4bc	28.6 ± 5.3c	103.0 ± 158.7bc	51.4 ± 16.7b
6	270.1 ± 181.4cde	19.1 ± 0.7bc	184.3 ± 196.2bcd	21.2 ± 0.7cd	228.3 ± 193.0cdef	21.5 ± 0.7bc	109.7 ± 159.7ab	27.9 ± 2.2c	120.4 ± 173.1c	25.8 ± 2.9c	127.7 ± 168.3c	28.9 ± 5.2c
8	351.4 ± 118.7de	14.9 ± 1.8bc	311.9 ± 157.0d	15.1 ± 2.3de	309.7 ± 161.5ef	16.6 ± 2.0cd	272.3 ± 178.0c	17.5 ± 1.4d	311.9 ± 152.3de	19.3 ± 2.6d	349.2 ± 121.6e	19.0 ± 2.3d
12	391.0 ± 0.0e	12.7 ± 1.1c	188.8 ± 192.0bcd	13.7 ± 0.9e	311.9 ± 157.0ef	12.9 ± 1.1d	187.1 ± 193.7bc	13.3 ± 0.8d	391.0 ± 0.0e	14.8 ± 1.3de	249.9 ± 182.1d	14.2 ± 1.4d
16	309.7 ± 161.5cde	16.3 ± 0.9bc	222.1 ± 200.5cd	16.2 ± 0.6de	309.7 ± 161.5f	16.9 ± 2.0bcd	185.7 ± 195.0bc	17.3 ± 0.8d	291.0 ± 166.0d	17.1 ± 1.0de	249.5 ± 182.7d	17.7 ± 0.6d
20	229.7 ± 191.4bcd	15.6 ± 0.8bc	62.2 ± 124.1ab	15.1 ± 0.5de	349.2 ± 125.3def	15.3 ± 1.0cd	103.1 ± 163.7ab	15.5 ± 0.6d	329.9 ± 140.8de	16.2 ± 0.8de	288.8 ± 169.7de	16.0 ± 0.6d
24	226.7 ± 195.1bc	17.1 ± 0.8bc	51.9 ± 127.3a	16.6 ± 0.6cde	264.3 ± 190.0def	17.2 ± 1.2bcd	96.9 ± 167.0ab	18.0 ± 1.2d	266.4 ± 181.5d	17.7 ± 1.1de	105.1 ± 157.6bc	17.4 ± 0.6d
32	12.6 ± 16.9a	15.9 ± 1.5bc	0.8 ± 0.8a	18.9 ± 1.1cde	177.7 ± 203.3bcde	11.3 ± 1.6d	5.6 ± 6.1a	17.9 ± 0.9d	31.5 ± 90.5abc	13.8 ± 2.2e	30.3 ± 90.4ab	18.4 ± 1.1d
40	0.0 ± 0.0a	16.6 ± 0.6bc	0.0 ± 0.0a	15.5 ± 0.5de	0.0 ± 0.0a	17.6 ± 1.0bcd	0.0 ± 0.0a	15.9 ± 0.9d	0.0 ± 0.0a	18.1 ± 1.5de	0.0 ± 0.0a	16.0 ± 0.6d
	$F = 10.312^*$	$F = 12.456^*$	$F = 5.286^*$	$F = 87.071^*$	$F = 4.899^*$,	$F = 18.694^*$	$F = 4.832^*$,	$F = 95.697^*$,	$F = 17.542^*$,	$F = 45.902^*$,	$F = 16.227^*$,	$F = 185.869^*$,
	$P < 0.0001$	$P < 0.0001$	$P < 0.0001$	$P < 0.0001$	$P < 0.0001$	$P < 0.0001$	$P < 0.0001$	$P < 0.0001$	$P < 0.0001$	$P < 0.0001$	$P < 0.0001$	$P < 0.0001$
	t-test = 1.34152; d.f. = 22; $P < 0.1934$				t-test = 2.91995*; d.f. = 22; $P < 0.0079$				t-test = 1.433109; d.f. = 46; $P < 0.4585$			

nr, Value not recorded.
Means within each column followed by the same letter do not differ.
Fisher's LSD test after ANOVA, marked * if significant.
t-test for comparison of nematode abundance between donor and recipients, marked * if significant.

Table 4 Pine wood nematode (PWN) abundance (per g wood) and moisture content (MC) in donor and recipient wood after heat-treatment (HT), kiln-drying (KD) and natural drying at 25°C (mean values ± SD)

	Donor board		Recipient HT board		Donor board		Recipient KD board		Donor board		Recipient used board	
	PWN	MC	PWN	MC	PWN	MC	PWN	MC	PWN	MC	PWN	MC
Sampling week												
0	351.4 ± 118.7a	16.4 ± 0.5a	0.0 ± 0.0	nr	222.6 ± 199.8	18.0 ± 1.8a	0.0 ± 0.0	nr	188.8 ± 192.0a	32.9 ± 11.7a	0.0 ± 0.0	nr
1	228.9 ± 192.5b	19.4 ± 1.1b	2.2 ± 3.1	44.1 ± 12.5a	181.9 ± 198.6	17.6 ± 1.4a	0.0 ± 0.0	18.2 ± 0.8a	309.7 ± 161.5abc	26.1 ± 3.1b	0.0 ± 0.0	21.8 ± 2.3a
2	391.0 ± 0.0a	18.9 ± 1.3b	130.6 ± 195.3	20.6 ± 0.8b	266.6 ± 186.8	17.6 ± 1.9a	0.0 ± 0.0	17.8 ± 0.6ab	193.2 ± 187.6a	27.0 ± 2.7b	0.0 ± 0.0	24.3 ± 3.0b
4	391.0 ± 0.0a	13.9 ± 1.2c	130.6 ± 195.3	14.7 ± 0.5c	264.3 ± 190.0	13.4 ± 0.7c	0.0 ± 0.0	13.3 ± 0.5c	351.4 ± 118.7bc	19.9 ± 1.0c	0.0 ± 0.0	20.4 ± 1.6a
6	391.0 ± 0.0a	16.4 ± 0.6a	130.6 ± 195.3	18.1 ± 0.6bc	226.7 ± 195.1	16.8 ± 1.1ab	0.0 ± 0.0	18.1 ± 1.3ab	230.6 ± 190.4abc	19.9 ± 1.3c	0.0 ± 0.0	20.2 ± 1.9a
8	391.0 ± 0.0a	15.7 ± 0.6a	130.9 ± 195.1	17.3 ± 0.7bc	220.1 ± 202.8	15.9 ± 1.1b	0.0 ± 0.0	17.3 ± 0.9b	272.3 ± 178.0abc	18.4 ± 0.8cd	0.0 ± 0.0	17.6 ± 1.5c
12									391.0 ± 0.0c	12.9 ± 1.0e	0.0 ± 0.0	13.0 ± 1.8d
16									311.9 ± 157.0abc	16.2 ± 0.5de	0.0 ± 0.0	16.6 ± 1.2c
20									351.4 ± 118.7bc	16.5 ± 0.5d	0.0 ± 0.0	16.1 ± 1.2c
24									272.3 ± 178.0abc	17.6 ± 0.5cd	0.0 ± 0.0	17.8 ± 1.5c
32									4.9 ± 11.3d	15.3 ± 0.9de	0.0 ± 0.0	16.3 ± 1.9c
40									0.0 ± 0.0d	15.4 ± 0.6de	0.0 ± 0.0	16.9 ± 1.3c
	$F = 4.447$*, $P < 0.0021$	$F = 43.920$*, $P < 0.0001$	$F = 1.586$, $P < 0.1819$	$F = 41.098$*, $P < 0.0001$	$F = 0.235$, $P < 0.9452$	$F = 13.403$*, $P < 0.0001$		$F = 52.150$*, $P < 0.0001$	$F = 6.953$*, $P < 0.0001$	$F = 23.203$*, $P < 0.0001$		$F = 27.160$*, $P < 0.0001$
	t-test = 7.09246*; d.f. = 10; $P < 0.0001$				*t*-test = 17.85355*; d.f. = 10; $P < 0.0001$				*t*-test = 6.53859*; d.f. = 22; $P < 0.0001$			

nr, Value not recorded.
Means within each column followed by the same letter do not differ.
Fisher's LSD test after ANOVA, marked * if significant.
t-test for comparison of nematode abundance between donor and recipients, marked * if significant.

Table 5 Pine wood nematode (PWN) abundance (per g wood) and moisture content (MC) in donor and recipient wood after heat-treatment (HT) and kiln-drying (KD) at 10°C (mean values ± SD)

	Donor board		Recipient HT board		Donor board		Recipient KD board	
	PWN	MC	PWN	MC	PWN	MC	PWN	MC
Sampling week								
0	265.7 ± 188.0	17.3 ± 0.8a	0.0 ± 0.0	nr	270.1 ± 181.4a	18.8 ± 1.5a	0.0 ± 0.0	nr
1	222.2 ± 200.2	22.0 ± 1.9b	0.0 ± 0.0	62.2 ± 15.0a	106.6 ± 161.6b	19.7 ± 2.0a	0.0 ± 0.0	18.4 ± 1.3a
2	263.5 ± 191.3	21.7 ± 0.6b	0.0 ± 0.0	30.1 ± 6.0b	348.4 ± 127.8a	19.7 ± 1.4a	0.0 ± 0.0	19.7 ± 0.9b
4	264.2 ± 193.3	16.8 ± 0.8a	0.0 ± 0.0	19.7 ± 0.8c	309.7 ± 161.5a	16.2 ± 1.8b	0.0 ± 0.0	16.1 ± 1.0c
6	264.8 ± 189.3	19.5 ± 1.1c	0.0 ± 0.0	21.9 ± 0.7c	351.4 ± 118.7a	18.6 ± 1.6a	0.0 ± 0.0	18.7 ± 1.7ab
8	307.4 ± 165.8	18.9 ± 0.7c	0.0 ± 0.0	21.5 ± 0.6c	311.9 ± 157.0a	19.1 ± 1.4a	0.0 ± 0.0	19.4 ± 1.1ab
	F = 0.185, P < 0.9669	F = 35.140*, P < 0.0001		F = 54.959*, P < 0.0001	F = 3.220*, P < 0.0138	F = 5.683*, P < 0.0003		F = 12.01*, P < 0.0002
	t-test = 23.99697*; d.f. = 10; P < 0.0001				t-test = 7.58341*; d.f. = 10; P < 0.0001			

nr, Value not recorded.
Means within each column followed by the same letter do not differ.
Fisher's LSD test after ANOVA, marked * if significant.
t-test for comparison of nematode abundance between donor and recipients, marked * if significant.

Table 6 Frequency of pine wood nematode successful transfers to recipient wood kept at 25°C in relation to its moisture content (MC). Recipient long-blocks along the grain and blocks across the grain are grouped as 'long-block'

	Natural MC				Heat-treated	Kiln-dried
MC (%)	Board	Long-block	Block	Used block	Board	
<10						
10–20						
21–30					2	
31–40					3	
41–50					1	
51–60	1	1			1	
61–70	3	2	1		1	
71–80	3	5	3			
81–90	1	3	2			
91–100	1	7	3			
Total	9	18	9	0	8	0

In wood with an MC below the critical values of fibre saturation, such as the used boards (mean of 18.3 ± 3.5%) and the kiln-dried boards (mean of 17.7 ± 2.0%), no transfer of *B. xylophilus* was detected. A dense clustering of recipient wood pieces with such low moisture and no nematode transfer can be observed in Fig. 2, reinforcing the decisive role of moisture in permitting or impeding nematode transfer.

Discussion

A key question in the current study is the potential for pine wood nematode to survive and transfer to non-infested wood in the absence of its known vectors. A consistent pattern of nematode transfer, particularly in relation to the MC of recipient pieces of wood, has been demonstrated; *B. xylophilus* transferred very rapidly from donor to adjacent recipient wood under favourable conditions (25°C and >70% RH), and when the MC of the recipient wood was above fibre-saturation point, which is around 30% for pines (Simpson & Barton, 1991; Wiberg & Morén, 1999; Berry & Roderick, 2005).

In general, the mean nematode density in the wood greatly increased during the first 8–12 weeks in both donor and recipient wood. This initial rise in nematode populations from the background level present from the field infestation of the wood coincided with the increase in temperature in the laboratory compared with ambient external winter temperatures, and was presumed to be due to accelerated reproduction in the *B. xylophilus* population as eggs and juvenile stages were systematically detected in the first samples. Under favourable conditions (25°C), *B. xylophilus* can complete its life cycle from egg to adult in just 4–5 days (Ishibashi & Kondo, 1977; Mamiya, 1984).

Subsequently, nematode populations gradually declined to zero, confirming that, despite their significant resilience, there appears to be a finite capacity in any given piece of wood to support nematode breeding and survival, even under favourable conditions. The progressive decrease in the MC of the wood may have affected nematode survival more through reducing the availability of fungal food sources than through direct effects on the nematode itself, as fungi are the principal food source once the living cells of the host tree are dead and the nematode enters a mycophagous phase (Fukushige & Futai, 1987; Wingfield, 1987; Fukushige, 1991; Warren *et al.*, 1995; Maehara & Futai, 1996).

Results from this study suggest that if pine wood containing *B. xylophilus* is moved in trade, whether as a wood product or as packaging, it will have a finite period of <40 weeks during which it poses a risk of transfer if it comes into contact with a suitable recipient piece of wood. During this risk period, if *B. xylophilus*-infested wood comes into contact with wood with an MC around or above 30%, then nematode transfer can take place rapidly. In such cases, there is a further risk period of up to 40 weeks in the newly infested batch of wood, during which it also presents a risk of transfer to other suitable recipient pieces of wood.

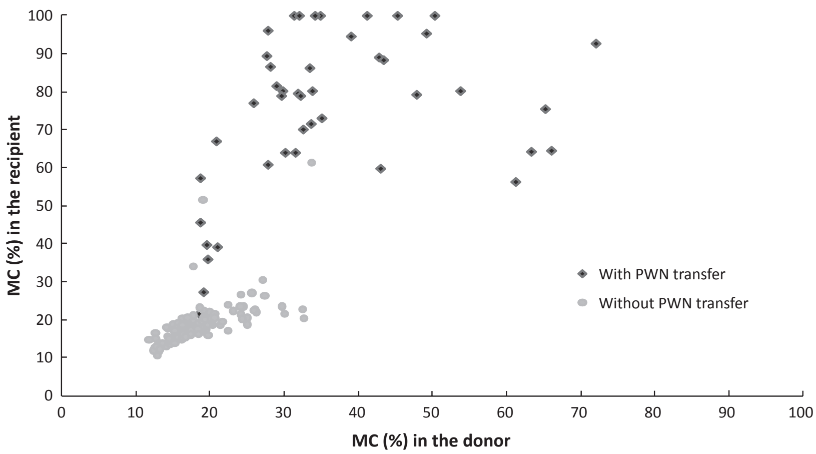

Fig. 2 Moisture content (MC, %) of donor and adjacent recipient wood in the first week of experiments at 25°C, when nematode transfers occurred. PWN, pine wood nematode.

It can be concluded that wood packaging material subjected to ISPM15-compliant heat treatment or fumigation can subsequently become infested with pine wood nematode under favourable ambient conditions, therefore the detection of *B. xylophilus* in ISPM15-marked wood packaging materials cannot always be considered as evidence of incorrect treatment or failed policy. As heat treatment remains an effective process of killing the *Monochamus* vectors in the wood, this process diminishes the threat of introducing the nematode to new locations through wood packaging materials, even if the wood is infested with the *B. xylophilus* after treatment.

Wood unsuitable for nematode transfer was found to have an MC around or below 20%, regardless of having been kiln-dried or dried naturally, as was the case for boards taken from pallets in service. Once wood is sawn, and especially when converted into wood in service (e.g. as constructed pallets), it loses moisture at a rapid rate and, from the current experiments with fresh pine wood from recently felled trees, consistently drops to below fibre-saturation point within approximately 6 weeks. Therefore wood in service (as traded wood or as packaging wood) also has a risk period of about 6 weeks or less as a potential recipient of *B. xylophilus*.

Results from this study represent the first quantification of the risks of non-vector transfer of *B. xylophilus* between infested and non-infested pieces of wood in direct contact with each other. They demonstrate the rapid transfer and successful breeding of nematodes in recipient pieces of wood under experimental conditions. In many respects, these results represent the worst case in relation to nematode transfer; temperatures were highly suitable for nematode reproduction, and donor and recipient wood was kept in constant contact for the entire period of the experiments. When the wood was kept in conditions unfavourable for nematode development and movement (10°C), no transfer of *B. xylophilus* took place, even when the MC of the recipient wood was greater than fibre-saturation point.

Questions remain as to whether the MC 'barrier' demonstrated in these experiments can be overcome if conditions change. For example, further assessment of transfer from donor wood with a very high MC to recipient pieces with an MC of 30% or less should be carried out to assess whether any surface moisture film can overcome the low MC barrier in the core of the recipient wood. The period of contact between donor and recipient wood should also be assessed to mimic more closely the likely much shorter duration of contact for wood in service. Finally, in assessing the consistency of the MC barrier combined with short periods of contact, more detailed studies should be carried out on the fate of nematodes at the interface between donor and recipient wood to determine whether there is any transfer of nematodes and, if so, how deeply they penetrate into the recipient piece.

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Risques de transfert du nématode du pin *Bursaphelenchus xylophilus* entre le bois d'emballage simulant des palettes assemblées en service

Les risques de transfert de *Bursaphelenchus xylophilus* (nématode du pin) en relation avec le bois ont été évalués. Des combinaisons d'objets adjacents infestés et non-infestés (planches, blocs longs et blocs) de *Pinus pinaster*, simulant des

palettes assemblées ont été évaluées. Pour le bois « receveur », des morceaux avec teneur en humidité naturelle, traités par la chaleur (56°C pendant 30 min à cœur) et avec une teneur en eau inférieure à 20%, ont été testés, ainsi que des planches de palettes en service. Les bois « donneur » et « receveur » ont été gardés en contact direct à 25°C ou 10°C, avec neuf répétitions par traitement. Il a été observé que *B. xylophilus* se transfère rapidement à 25°C quand le bois a une teneur en humidité supérieure au point de saturation des fibres (>30%). La reproduction du nématode est rapide et soutenue, puis décline progressivement entre zéro et 40 semaines. *B. xylophilus* ne se transfère pas au bois séché ou en service qui a une humidité inférieure au point de saturation des fibres, ou au bois à 10°C. Les facteurs clés déterminant le transfert du nématode étaient la température ambiante, la charge en nématodes du bois « donneur » et la teneur en humidité du bois « receveur » avec une « frontière » à 20% en dessous de laquelle il devient inadapté au transfert du nématode. Cette découverte indique qu'il existe un risque limité de dissémination de *B. xylophilus* via le bois d'emballage traité et non traité.

Риск передачи сосновой стволовой нематоды *Bursaphelenchus xylophilus* через древесный упаковочный материал, имитирующий готовые поддоны в условиях их эксплуатации

В статье дается оценка риска передачи *Bursaphelenchus xylophilus* (сосновой стволовой нематоды). Оценке подвергались различные сочетания прилегающих друг к другу зараженных и незараженных досок, брусков и блоков из древесины *Pinus pinaster*, имитирующие реальные поддоны. Вместе с находившимися в эксплуатации досками от поддонов в качестве реципиентов тестировались куски древесины с естественным влагосодержанием (MC), после тепловой обработки (56ºC в течение 30 минут в сердцевине) и после сушки в камере с доводкой до менее 20%-ой влажности. Донорская и реципиентная древесина находились в прямом контакте при температуре 25ºC или 10ºC, с девятью повторами каждой обработки. *B. xylophilus* быстро перемещалась при 25°C, когда влажность древесины была выше точки насыщения волокон (>30%). Репродукция нематод была быстрой и устойчивой, и постепенно за 40 недель сводилась к нулю. *B. xylophilus* не перемещалась в высушенную или использованную древесину с влагосодержанием ниже точки насыщения волокон при 10ºC. Ключевыми факторами, определяющими перемещение нематоды, являлись: окружающая температура, насыщенность нематодами донорской зараженной древесины и влажность реципиентной древесины с 'барьером' 20%-ой влажности, ниже которой передача нематоды становится невозможной. Эти результаты указывают, что существует ограниченный риск распространения *B. xylophilus* в обработанном и необработанном древесном упаковочном материале.

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