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STATEMENT

Panel statement on young pine plants used for afforestation or reforestation as pathway for pinewood nematode (PWN) *Bursaphelenchus xylophilus*

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The declarations of interest of all scientific experts active in EFSA's work are available at https://open.efsa.europa.eu/experts.

Abstract

Pinewood nematode (PWN) (*Bursaphelenchus xylophilus*) is a destructive pest of *Pinus* trees worldwide which depends for transmission on the cerambycid insects of the genus *Monochamus*. EFSA was asked to assess the potential role of young host plants for afforestation or reforestation planting as a pathway for PWN. According to legislation (EU COM/2023/415 final), these seedlings are not older than 2 years. Based on communication with SNPF (Syndicat National des Pépiniéristes Forestiers), such pine seedlings are assumed to have a collar diameter no more than 12 mm. It was evaluated that seedlings, by their physical dimensions, cannot sustain the complete developmental cycle of the vector insect *Monochamus galloprovincialis*. In particular, the reported empirical data on the minimum width of the head capsule of the phloem-based larval stage (L2) of 0.89–1.03 mm would not fit the 0.44-mm thick phloem of a 2-year-old pine tree, and the minimum 15-mm long pupal chamber with minimum observed inclination of 30° from the vertical of the heartwood, where L3 + L4 end feeding, would ultimately stretch beyond the 6-mm radius of the seedling and hence stick outside of the seedling. Observations of over 2000 beetle exit holes revealed that the branch diameter associated with successful insect development was larger than 20 mm, with a median of 90 mm. It was further evaluated that a vector-independent transmission between seedlings would necessitate direct contact of injured roots, which contrasts with prescribed procedures and good practice. The Panel considers the sequence of events leading to the transmission of nematodes through a seedling is highly implausible. Transmission would necessitate (i) nematode infestation of seedlings in a nursery, (ii) trade and planting of a symptom-free but nematode-infested seedling, (iii) its asymptomatic growth in the field to a tree size that allows vector development after years, (iv) the decline of this tree to attract vector egg laying while keeping nematodes in a developmental stage enabling insect vector transmission. Therefore, the Panel does not consider pine seedlings for afforestation or reforestation that are not older than 2 years and that have a root collar diameter of no more than 12 mm a pathway for PWN.

KEYWORDS

Bursaphelenchus xylophilus, pest risk assessment, pine wilt disease, pinewood nematode, plants for planting

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1 | INTRODUCTION

1.1 | Background and Terms of Reference as provided by the requestor

EFSA was requested an extension of the mandate M-2025-00073 on the risks to plant health in the EU territory posed by *Bursaphelenchus xylophilus* (PWN).

Specifically, EFSA is requested to undertake an analysis of the available scientific evidence about the phytosanitary risks linked to young host plants with small diameter (below 3 cm and below 2 cm).

1.2 | Interpretation of the Terms of Reference

The mandate is addressing young host plants as a pathway for the pest. Because PWN was detected in Europe only in *Pinus* spp., no other host plants are considered for this assessment. The mandate was split into two sub-assessments. The first sub-assessment (this document) addresses plants for planting foreseen for afforestation and reforestation. Consequent to EU regulation of the maximum age of seedlings, together with the communication from Syndicat National des Pépiniéristes Forestiers (SNFP), these plants are assumed to have diameters at root collar no more than 12 mm. The second sub-assessment (see EFSA-Q-2026-00324) will refer in the second document to estimation of a critical parameter, namely the minimum stem diameter/age above which young plants could facilitate the developmental cycle of the pest and the vector and therefore lead to the risk of being a pathway for the pest.

2 | DATA

The data chapter collates evidence and expert knowledge found by an ad hoc literature search and informal communications with experts. The subsections cover the characteristics of pine forests, reforestation and management, the details of the pest development and the impact of the nematode on the host, the biological details of the insect vector and its developmental cycle, and the bilateral interaction of the nematode and the vector populations.

2.1 | Definition of Forest reproductive material (FRM) and size measurements of plants for planting

The 'Proposal for a REGULATION OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL on the production and marketing of forest reproductive material'[1] specifies forest reproductive material (FRM) as referring to seeds, plants and parts of tree species that are used to create new forests ('afforestation'), the replanting of areas with trees ('reforestation') and other types of tree planting for different purposes. The proposed regulation also states the principles of registration of 'parent trees' ('basic material') and certification before that FRM can be placed on the market. Certified FRM should be free from pests and their symptoms and adapted to the climatic and ecological conditions of the area where it is planted.

The main characteristics of young plants used as FRM and criteria of quality in nursery stock are height and the root collar diameter (RCD). RCD is defined as the thickness of the main stem just above the roots (Figure 1).

[1]Proposal for a REGULATION OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL on the production and marketing of forest reproductive material, amending Regulations (EU) 2016/2031 and 2017/625 of the European Parliament and of the Council and repealing Council Directive 1999/105/EC (Regulation on forest reproductive material). https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=celex:52023PC0415.

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FIGURE 1 Position of the measurements taken for root collar diameter on a plant for planting (Thomas D. 'Tom' Landis, USDA Forest Service, Bugwood.org).

The physical details of seedlings as used in this document are detailed in Section 2.3.1 and a recently planted *Pinus pinaster* plant in the field during reforestation is shown in Figure 2.

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FIGURE 2 Size comparison of a freshly planted seedling of *Pinus pinaster* on a reforestation area in Portugal (*Source: Hans-Hermann Thulke*).

Annex I of the 'Proposed regulation on production and marketing of forest reproductive material' specifies tree species and artificial hybrids used as FRM out of which the confirmed main hosts of PWN are *Pinus* spp. (*P. brutia, P. canariensis, P. cembra, P. contorta, P. halepensis, P. leucodermis, P. nigra, P. pinaster, P. pinea, P. radiata, P. sylvestris*). Other tree species included in the proposed regulation are identified as PWN hosts but not considered for this assessment, these are: *Abies* spp. (*A. alba, A. cephalonica, A. grandis, A. pinsapo*), *Picea* spp. (*P. abies, P. sitchensis*), *Larix* spp. (*L. decidua, Larix × eurolepis, L. kaempferi, L. sibirica*), *Cedrus* spp. (*C. atlantica, C. libani*) and *Pseudotsuga menziesii* (EPPO, 2023, 2026).

Annex VII part E of Directive 1999/105/CE specifies special requirements for a list of species, categories and types of FRM concerning planting stock to be marketed to users in regions having a Mediterranean climate. It stipulates that the plants must be in good condition, free from defects and pests.

Particularly relevant for this document, the FRM must comply with the size and age requirements listed in Table 1.

TABLE 1 Size of the plants for planting (Directive 1999/105/CE Annex VII part E).

Species	Maximum age (years)	Minimum height (mm)	Maximum height (mm)	Minimum root collar diameter (mm) (**Figure 1**)
Pinus halepensis	1	80	250	2
	2	120	400	3
Pinus leucodermis	1	80	250	2
	2	100	350	3
Pinus nigra	1	80	150	2
	2	100	200	3
Pinus pinaster	**1**	**70**	**300**	**2**
	2	**150**	**450**	**3**
Pinus pinea	1	100	300	3
	2	150	400	4

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2.2 | Life cycle of the pinewood nematode, *Bursaphelenchus xylophilus*, and its insect vector in Europe, *Monochamus galloprovincialis*

2.2.1 | Life cycle of the nematode

The life cycle of *B. xylophilus* is complex, comprising two distinct developmental pathways: the saprophytic and the phytoparasitic phases. Both phases are obligately dependent on insect vectors, primarily *Monochamus* spp. (e.g. *M. galloprovincialis*), for dispersal and transmission between hosts (Figure 3).

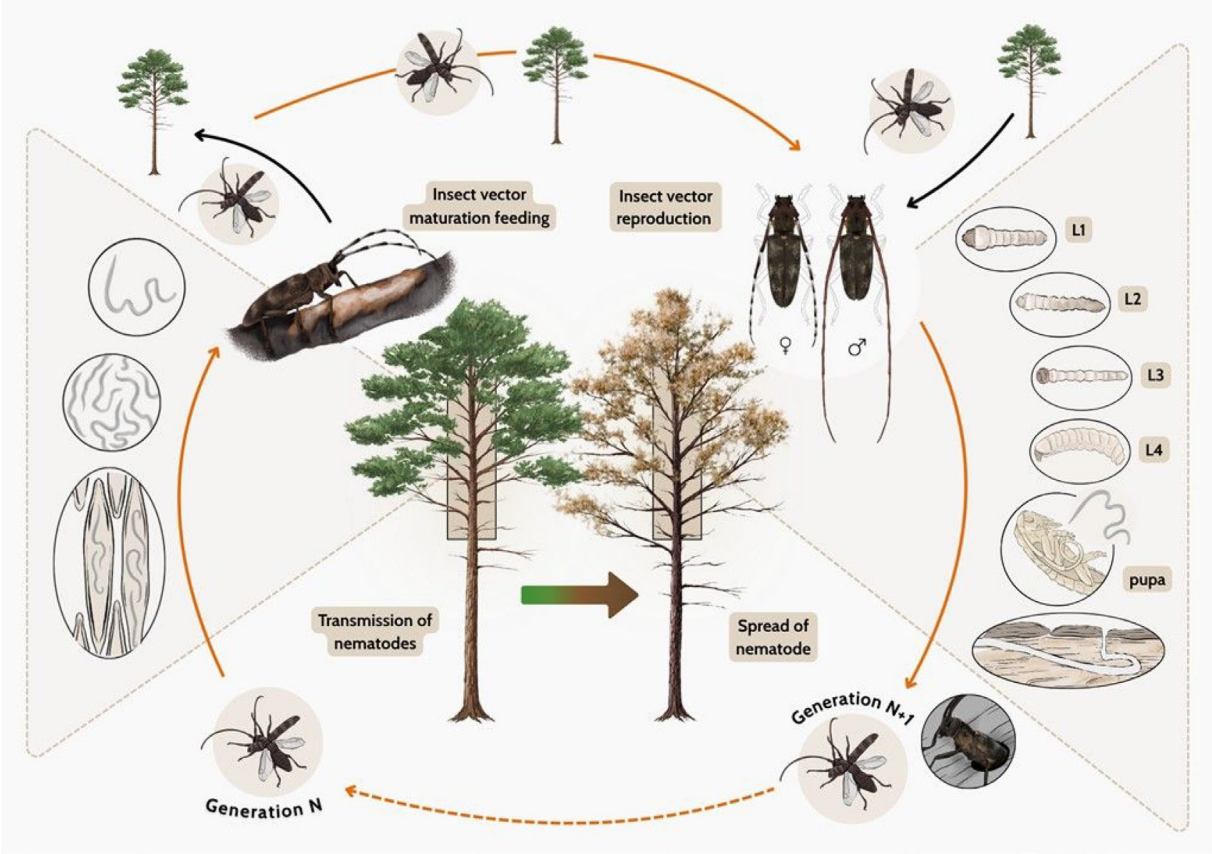


FIGURE 3 Details of the epidemiological cycle of the pinewood nematode (*Bursaphelenchus xylophilus*) including healthy trees for vector feeding and infestation, and weakened trees, maybe due to infestation, for vector reproduction, as well as the vector developmental stages egg, larva L1–L4, pupa and immature young insect and sexually mature adult insect (picture Jactel & Beaufort, 2026, https://doi.org/10.17180/89cg-0271).

During their maturation feeding period, insect vectors *Monochamus* spp. (e.g. *M. galloprovincialis*), which just emerged from nematode-infested trees, feed on the tender bark of multiple healthy conifer branches, thereby transmitting pathogenic JIV dauer larva of the pinewood nematode. Within each infested tree, the phytoparasitic phase of the nematode's life cycle begins. Nematode larvae enter the host tissue through wounds caused by feeding vector beetles and migrate into the resin canals where they start to reproduce. The generation cycle of PWN consists of four larval stages (J1–J4), which moult into male and female adult stages. After several of these generations (each lasting about a week), the nematodes become very numerous and begin to feed on the xylem cells. Around 3 weeks post-infestation, the infested tree weakens and initial symptoms of physiological decline emerge, characterised by reduced oleoresin exudation or a significant decrease in resin secretion. During this developmental phase, the needles on the tree turn intensely yellow, the wilting disease (EPPO, 2023, 2026; Naves et al., 2016). Thirty to 40 days after the initial infestation, infested trees die, with millions of nematodes present in the trunk, branches and roots. The decaying infested tree may even attract adult vector beetles which preferably gather on weakening trees for mating and subsequent egg laying.

After the nematode population in the decaying tree reached its peak, the reproduction intensity of the nematodes diminishes and the resource limitations lead to a slowly declining number of nematodes. This marks a phase of dormancy within the infested trees where limited food availability forces *B. xylophilus* to enter a specialised third-stage dauer larva (JIII), which can survive in dead wood even under extremely unfavourable conditions such as drought, low temperatures and lack of food.

When the nematode's vector beetle *Monochamus* spp. (e.g. *M. galloprovincialis*) is present in the tree, the JIII dauer larva of the nematode moults into the fourth-stage dispersal larva (dauer larva, JIV, specifically adapted for phoretic transport). The larva JIV now feeds on the mycelium of fungi (usually species of the genus *Ceratocystis*), which is introduced

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into the tree tissue by the beetles during egg laying (saprophytic phase). When the least larval stage of the insect vector (L4) pupates, the JIV larvae of the nematode gather around the pupal chamber, probably influenced by secretions from developing beetles. Concurrently, fungal mycelia form elongated perithecia that extend into the pupal chamber, and the nematode larvae aggregate at the tips of the perithecia. Finally, the nematode larvae board the emerging insect vector by aggregating beneath the elytra or within the trachea of the beetle. The emerged beetles depart the tree for maturation feeding on healthy conifers and thereby transport the nematodes to other healthy trees, concluding the cycle (Evans et al., 1996; Vicente et al., 2011).

2.2.2 | Life cycle of *Monochamus galloprovincialis*

Following emergence (May–October), young immature adults of the insect feed on fresh shoots of healthy trees. The maturation feeding period lasts 2–3 weeks during which they fly from tree to tree. If the feeding insect did emerge from a nematode infested tree, the incision damage by the feeding bites fosters transmission of the nematodes onto the tree where the feeding took place (Figure 3).

Mature females lay eggs on weakened trees and incise an entry hole into the phloem (Figure 4A). The tree must still be alive or contain fresh phloem to support the development of the first two larval stages of the insect (L1 and L2, together about 35–55 days). Subsequent larval stages enter the sapwood (Figure 4B) and the stages L3 and L4 tunnel a gallery into the xylem (and sometimes even in the heartwood) of the infested tree. The pupal chamber is built from the gallery towards the outer edge of the stem or branch using the shortest distance, and with a declination angle from the main direction of the stem. The nematode's dispersal stage migrates to the pupal chamber and inserts itself on the body of a young adult just before emergence.

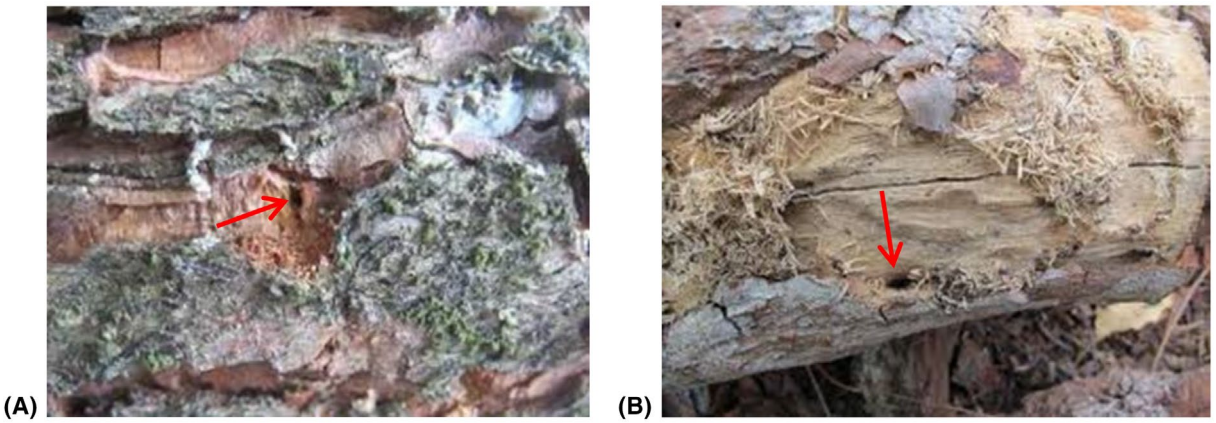


FIGURE 4 Entry holes on *Pinus* spp. (A) Hole in the bark created by the egg-laying female vector insect. (B) Entry hole into the sapwood by the finishing larva L2 when leaving the phloem after 35–55 days. (*Source:* Herve Jactel, INRAE).

2.3 | Data, evidence and documented knowledge

The chapter collates the available data, evidence and knowledge addressing the detection, infestation and transmission of the PWN in the context of afforestation and reforestation of pine forest areas which then will be used as dossier for the assessment. The section is structured into several subsections concerning the dimensions of plants for planting (Section 2.3.1), the pest spread without the vector (Section 2.3.2), the physical dimensions of the vector insect (Section 2.3.3) and the interaction between pest and vector (Section 2.3.4).

2.3.1 | FRM plants for planting

As specified in Section 2.1, FRM species that could host PWN and are used for afforestation/reforestation are no more than 2 years old.

2.3.1.1 | *Root collar diameter*

The literature provides typical root collar diameter of container grown plants for planting for different ages of *Pinus* spp. ranging from 3 to 4 mm up to 12 mm large planting stock (Castro-Garibay et al., 2023; Öner & Eren 2008; Jokanović et al., 2024) (Tables 2 and 3).

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TABLE 2 Average diameter of *Pinus* spp. rootstocks and scions (Castro-Garibay et al., 2023).

Usage	Species	Diameter (mm)
Rootstocks	*Pinus greggii* var. *australis*	3.1
	Pinus teocote	2.4
	Pinus patula	3.1
	Pinus leiophylla	2.9
Scions	*Pinus greggii* var. *australis* (nursery-produced)	2.6
	Pinus greggii var. *australis* (field-produced)	2.1

TABLE 3 Min, max and mean root collar diameter (RCD) of *Pinus nigra* and *Pinus sylvestris for different seedling types and ages* (Öner & Eren, 2008). The term 'X + Y' indicates that the seedling was grown for X year(s) in the seedbed and then for Y years in the transplant bed. The second part of the code is a zero (e.g. 2 + 0) if the stock has not been transplanted.

Species	Seedling type	Age (year)	N	RCD min (mm)	RCD max (mm)	Mean RCD (mm)
Pinus nigra	Bare root	1 + 0	30	3.20	4.10	3.86
Pinus sylvestris	Bare root	1 + 0	30	2.76	3.22	2.97
Pinus nigra	Bare root	2 + 0	30	4.10	5.85	4.90
Pinus sylvestris	Bare root	2 + 0	30	2.95	4.90	4.04
Pinus nigra	Containerised	1 + 0	30	3.20	4.90	4.21
Pinus sylvestris	Containerised	1 + 0	30	3.00	4.20	3.78
Pinus nigra	Containerised	2 + 0	30	5.00	6.80	5.85
Pinus sylvestris	Containerised	2 + 0	30	4.20	5.90	5.19

According to the plants for planting specifications communicated by the French Association of Forestry (SNPF – Syndicat National des Pépiniéristes Forestiers), the maximum RCD for container grown *Pinus* spp. up to 2 years old is 12 mm (Table 4 and in detail Appendix A).

TABLE 4 The maximum root collar diameter for selected conifer seedlings according to French Association of Forestry (SNPF-Syndicat National des Pepinieristes Forestiers) (Appendix 1).

Species	Maximum root collar diameter (mm)
Container-grown plants (potted/motte) – Age 1 + 0	
Cedrus spp.	6
Larix spp.	8
Picea spp.	6
Pinus pinaster/Pinus taeda	6.5
Pinus nigra/Pinus sylvestris	6
Pinus taeda	6
Pseudotsuga menziesii	7
Container-grown plants (potted/motte) – Age 2 + 0	
Cedrus spp.	9
Larix spp.	11
Picea spp.	11
Pinus pinaster/Pinus taeda	11
Pinus nigra/Pinus sylvestris	12
Pseudotsuga menziesii	14
Bare root plants	
Larix spp.	15
Picea spp.	18
Pinus nigra/Pinus sylvestris	15
Pseudotsuga menziesii	18

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For the purpose of this statement, the panel considers FRM as container-grown plants for planting (IPPC Secretariat, 2026), coming from basic material, used for creation of new forests ('afforestation') and for the replanting of areas with *Pinus* spp. trees ('reforestation') not older than 2 years and with a collar diameter of no more than 12 mm. This reflects that PWN was detected in Europe only in *Pinus* spp., the FRM requirements for such plants, and the communication with SNPF. *Cedrus* spp., *Larix* spp., *Picea* spp. and *Pseudotsuga* spp. (Table 4) are not considered.

2.3.1.2 | *Phloem thickness*

Based on the research by Castro-Garibay et al. (2023), the vascular cambium plus phloem (the tissues on which *Monochamus* larvae feed during the L1 and L2 stages) ranged from 45.6 ± 13.9 μm to 51.9 ± 20.9 μm depending on the *Pinus* spp. species (Tables 5 and 6).

TABLE 5 Width of pith, xylem, VC-P, cortex and E-P in rootstock seedlings (15-month-old) (Castro-Garibay et al., 2023).

Species	Pith (μm)	Xylem (μm)	VC-P (μm)	Cortex (μm)	E-P (μm)
P. greggii var. australis	708.7 ± 128.7	685.7 ± 80.8	45.6 ± 13.9	87.3 ± 25.7	30.2 ± 8.0
P. teocote	504.2 ± 101.3	521.7 ± 31.8	42.0 ± 13.3	119.6 ± 48.9	38.9 ± 10.4
P. patula	685.8 ± 185.7	686.5 ± 71.1	49.4 ± 13.3	97.5 ± 34.8	36.4 ± 8.5
P. leiophylla	520.8 ± 98.9	754.4 ± 46.6	51.9 ± 20.9	109.3 ± 23.0	38.4 ± 10.1

Abbreviations: E-P, epidermis + periderm; VC-P, vascular cambium + phloem.

TABLE 6 Width of pith, xylem, VC-P, cortex and E-P in *Pinus greggii* var. australis seedlings (Castro-Garibay et al., 2023).

Scion	Pith (μm)	Xylem (μm)	VC-P (μm)	Cortex (μm)	E-P (μm)
S-NG (nursery-grown)	418.4 ± 107.7	664.4 ± 133.9	47.1 ± 8.9	162.5 ± 53.6	34.1 ± 7.9
S-FG (field-grown)	390.8 ± 164.8	334.6 ± 55.8	54.7 ± 10.5	259.2 ± 50.6	21.8 ± 5.8

Abbreviations: E-P, epidermis + periderm; VC-P, vascular cambium + phloem.

The phloem thickness of maritime pine seedlings with 3–5 mm RCD was measured using X-ray microtomography on average 0.149 mm (± 0.029 standard deviation).

TABLE 7 *Pinus pinaster*'s seedling diameter and phloem thickness measured by X-ray (method and data by Sylvain Delzon, INRAE).

Seedling diameter (mm)	Phloem thickness (mm)
3	0.122
4	0.128
4	0.139
4	0.192
5	0.162

The data presented in Table 7 are used to fit a linear model (without intercept) relating phloem thickness to seedling diameter, expressed as follows:

$$\text{Phloem_thickness} = \beta \times \text{Seedling_diameter} + \varepsilon.$$

The estimated value of β is equal to 0.0367 mm, with a 95% confidence interval of [0.0283; 0.0452]. The fitted model is then used to predict the phloem thickness of maritime pine seedlings with a diameter of 12 mm, and the predicted thickness value is equal to 0.44 mm, with a 95% prediction interval of [0.31; 0.57]. This estimation was performed with the lm() R function.

The data used in this analysis were obtained from X-ray measurements collected on five maritime pine seedlings shown in Figure 5 and in Appendix B.

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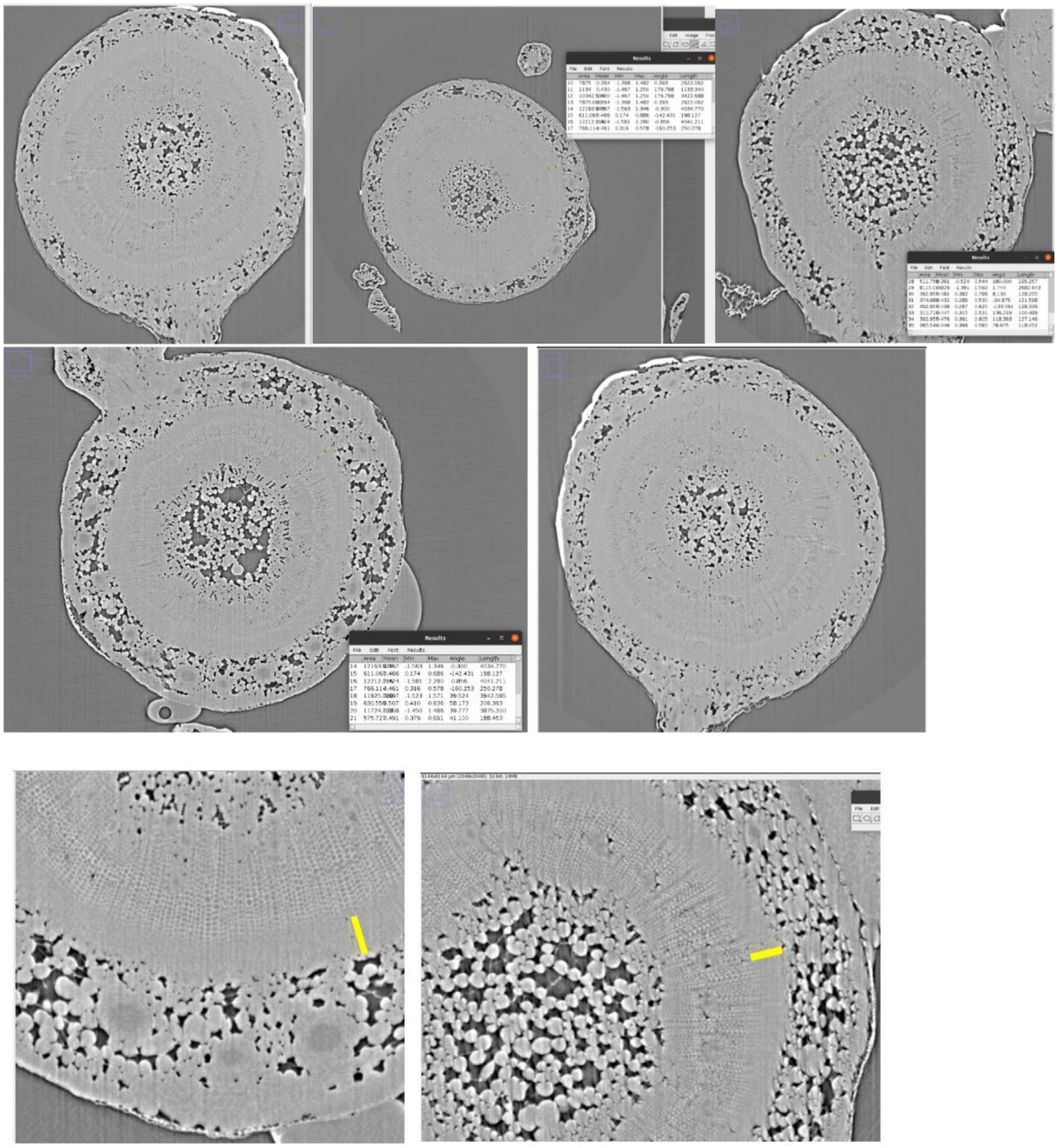


FIGURE 5 X-ray measurements collected on five maritime pine seedlings (Appendix B, source H. Jactel, INRAE). Detail of the phloem thickness measurement (yellow line).

2.3.2 | Nematode

2.3.2.1 | *Non-vectored transmission*

This subsection collates paragraphs regarding evidence and knowledge bits of pest transmission and spread without direct vector insect involvement. Indeed, the literature contains several experimentally confirmed reports of non-vector transmission of *B. xylophilus*, when experimentally damaged or otherwise wounded seedlings are exposed to PWN in the growing substrate, from wood chips, shredded bark, saw dust or directly contaminated soil.

Wood chips

According to Panesar et al. (1994), PWN can survive wood chipping if wood moisture content remains suitable.

Hopf-Biziks et al. (2017) demonstrated that *B. xylophilus* can survive in wood chips for 23.5 weeks when openly stored at 25°C, and above 1 year in sealed chambers at 15°C and 25°C. The same authors demonstrated the possibility of non-vector spread of PWN from wood chips to young trees under laboratory conditions. The temperature, tree health and wood chip distance to seedlings are influencing factors for the infestation. At 25°C, seven out of 12 seedlings developed higher wilt classes and were PWN positive compared to the seedlings treated at 15°C.

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Halik and Bergdahl (1992) showed that numbers of nematodes and percentage of third-stage dispersal larvae were greater in chips without soil and at 12°C. The percentage of juveniles of the propagative cycle was greater in chips with soil and at 20°C. Although *B. xylophilus* survived in chips with soil for 12 weeks, nematode numbers and life stage percentages changed little over the whole period. In the same research, 12 replicates per *Pinus* species were mulched with infested wood chips and examined after 18–25 weeks or when wilting symptoms appeared. Nematodes were recorded in 92% of the treated *P. sylvestris* seedlings and 75% of the *P. strobus*.

Saw dust

Sousa et al. (2011) studied survival and development of *B. xylophilus* for up to 40 weeks in *P. pinaster* sawn wood and branches. 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 (JIII), and in the final sample, nematode abundance was very low. Nematode decline in the wood was correlated with a decrease in the moisture content to below fibre saturation.

According to Arbuzova et al. (2023), 83% of pine seedlings with damaged stems were infested after direct exposure to infested sawdust which was more often than for seedlings without damage ($p < 0.012$). Furthermore, the same authors showed that 50% of seedlings with damaged roots became infested which again was more often than for undamaged seedlings ($p < 0.008$). However, symptoms of disease were observed in only two seedlings in week 7, and these eventually died at week 20. In this study, it was also observed that nematodes could not infest seedlings if the contaminated sawdust occurred on the soil surface at 2.5 cm distance from the stem of the seedling; however, Hopf-Biziks et al. (2017) reported that nematodes still reached the plants from 3.5 cm distance.

Soil

In soil, Mamiya and Shoji (1989) noted that PWN survival does not exceed 72 h. No mortality was recorded when nematodes were applied to the soil just 1 cm from the injured roots, suggesting that PWN have low persistence in soil or do not move actively over 1 cm within soil. Indeed, to date, soil-borne transmission has not been demonstrated in the absence of infested woody debris (i.e. no infested wood chips, sawdust, is present), and therefore, transmission of PWN directly through soil is considered negligible (Dwinell and Nickle, 1989).

Other

Mamiya and Shoji (1989) showed 100% mortality in pine seedlings when roots were injured and treated directly with a PWN aqueous suspension.

2.3.2.2 | *Disease symptoms in young plants – time to wilting*

Infestation of pine trees with the nematodes causes non-specific but strong patterns of desiccation which could be detected by visual inspection. Consequently, the time to symptoms development provides important evidence which is collated in this paragraph.

Arbuzova et al. (2023) observed the wilting symptoms (needle discoloration/yellowing needles), characteristic of pine wilt in the second week after artificial inoculation of 4 years old wounded *Pinus sylvestris* with 100% case lethality 9 weeks after inoculation.

Fang et al. (2022) inoculated aseptic pinewood nematodes onto 4-month-old aseptic seedlings using the top-truncation method. Twenty-five days after inoculation, the wilting rates of *Larix principis rupprechtii*, *Pinus tabuliformis* and *P. massoniana* were 53.3%, 78.3% and 89.2%, respectively, while control seedlings showed no symptoms.

In controlled inoculation assays, on 2-year-old *P. pinaster* visible symptoms (class 1, < 25% needle yellowing) appeared after 14 weeks, with some individuals progressing to class 4 (> 75% needles affected; Ribeiro, 2012).

Similarly, other authors found needle discoloration becoming visible at 14 days after inoculation (DAI), and symptoms progressed rapidly: at 28 DAI more than half of the plants scored symptom classes 3–4, while at 35 and 42 DAI, 77% and 92% of the plants, respectively, showed more than 75% needle desiccation (Modesto et al., 2022; Rodrigues et al., 2021).

In PWN inoculation assays conducted on older *P. pinaster* plants (4-year-old), the first symptoms became visible at 14 DAI, and the first plant mortality occurred at 35 DAI (Modesto et al., 2022).

Menéndez-Gutiérrez et al. (2021) assessed that, in *P. radiate*, wilting symptoms appeared 33 DAI and ended 55 DAI, and with the average duration of the disease of 22 days with the plant's root collar diameter from 18.44 mm ± 0.23 up to 19.32 mm ± 0.23.

Hakamata et al. (2013) measured symptom expression in resistant varieties of *P. thunbergii* in 1-year-old seedlings. Across 3-year study, the percentage of symptomless *P. thunbergii* seedlings inoculated with *B. xylophilus* changed between varieties from 36.4% to 73.3%.

Pimentel et al. (2020) inoculated seedlings of different *Pinus* species under experimental conditions of winter climates and sustained water provision. Average temperatures recorded during experiments differed from 10.1 ± 6.6°C to 13.7 ± 2.5°C

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in Pineville, Louisiana (USA) and Lisbon (Portugal), respectively. During this study, 39.2% of seedlings of *P. sylvestris* (in Portugal) and 9.7% of *P. radiata* (in Louisiana) had completely wilted before the end of the experiment. Visual changes in the canopy of inoculated seedlings, except *P. palustris*, were more significant than in controls. The proportion of seedlings survived the 3 months of the experiment, and several showed a declining nematode population with the highest nematode numbers occurring in *P. sylvestris* and *P. radiata*, followed by *P. pinaster*, and the lowest population levels in *P. pinea* and *P. palustris*.

2.3.2.3 | *Regulatory background to prevent the spread within the Union of* Bursaphelenchus xylophilus

The subsection addresses the regulatory impact of materials that possibly can be contaminated with the pest and in contact with pine seedlings.

Rules for establishment of demarcated areas, eradication and containment, and movement restrictions are set in regulation 2012/535/EU on emergency measures preventing the spread within the Union of *Bursaphelenchus xylophilus*.[2]

According to this regulation, materials such as wood chips or sawdust coming from the demarcated areas should undergo the heat treatment in 56°C for at least 30 min.

EPPO Standard PM 8/2 (1) on commodity-specific phytosanitary measures for *Coniferae* (EPPO, 2009) requires that plants for planting are free from soil according to EPPO Standard PM 3/54 and are harvested and imported only between 1 October and 31 March in conjunction with the vector inactivity period.

Producers are obliged to test wooden packaging materials, subject them to thermal treatment or drying and mark them with the IPPC/ISPM15 symbol according to ISPM No. 15 'Regulation of Wood Packaging Material in International Trade' (FAO, Rome, adopted, 2018).

2.3.3 | Vector

The section collects available evidence on physical needs or biological limitations of the vector that could be compared against the size of seedlings of max 2 years of age and therefore considered as not more than 12 mm diameter at the collar. It should be noticed (see Figures 1 and 2) that all other parts of the seedling will usually be of thinner dimension than the collar diameter.

2.3.3.1 | *Exit holes and penetration to the phloem*

The distribution of branch diameters naturally infected and bearing exit holes of *M. galloprovincialis* (Figure 6 and Appendix C) provides evidence of the wood diameter range preferentially used by the insect to complete its developmental cycle. Exit holes were only marginally observed in branches with diameters slightly greater than 20 mm, whereas already the first percentile exceeded 30 mm in diameter. The distribution showed a median value of about 90 mm, indicating a strong preference for relatively large diameter woody material for successful development and adult emergence.

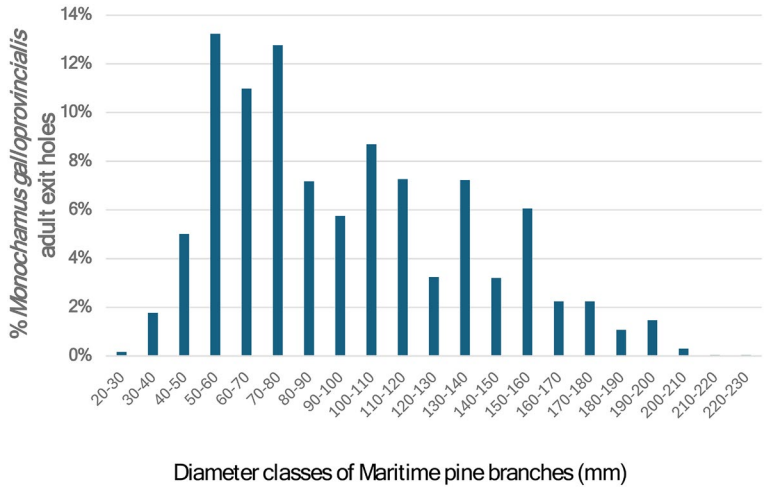


FIGURE 6 The diameter of maritime pine branches at the position of exit holes of the vector insect *Monochamus galloprovincialis*. Data of 236 branches, with 2312 exit holes. The minimum observed were branches of 20–30 mm (*Source:* H. Jactel, INRAE, original data Appendix C).

[2]COMMISSION IMPLEMENTING DECISION of 26 September 2012 on emergency measures to prevent the spread within the Union of *Bursaphelenchus xylophilus* (Steiner et Buhrer) Nickle et al. (the pine wood nematode). https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=CELEX%3A02012D0535-20180423.

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The apparent optimum of branch diameters around 90 mm may be conditioned by the increasing thickness of the bark which, above certain dimensions, may prevent the larva L1 to reach the phloem where L1 + L2 develop. This also may be the reason why higher parts of the tree are preferred, as the higher the wider the choice of diameters. When the diameter exceeds 23 cm, the bark is likely too thick to allow young L1-stage larvae to reach the phloem. Larva L1 must be able to enter through the bark and reach the phloem, which limits the growing dimension of the stem or branch that can be attacked.

This limitation is not relevant for seedling and their limited bark thickness.

2.3.3.2 | *Size of the insect vector*

The size distribution data of emerging *M. galloprovincialis* adults, including both body length and body width measurements (Figure 7), provide evidence of the minimum dimensions required for successful adult emergence. Specifically, body width is indicative of the minimum exit hole diameter necessary to allow adult emergence, whereas body length reflects the minimum pupal chamber length required to accommodate the developing adult prior to emergence.

The minimum observed length of the adult *M. galloprovincialis* was 12 mm, and the width was 3 mm. Median is at lengths of 19 mm and 6 mm width.

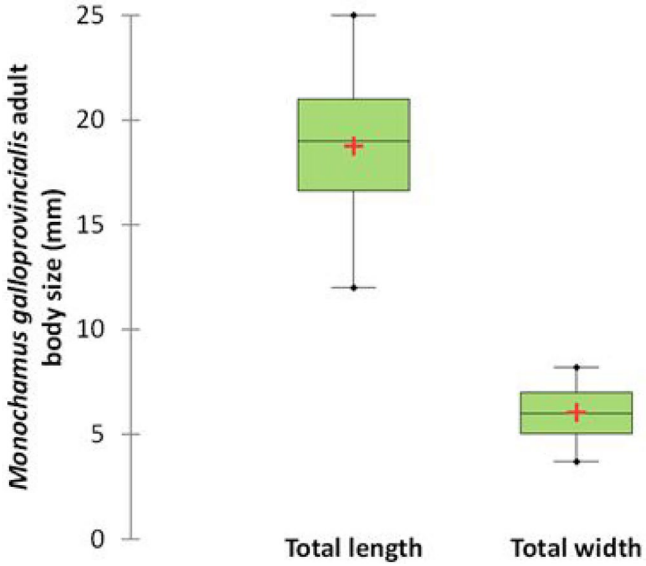


FIGURE 7 Observational data on the total body lengths and widths of adult *Monochamus galloprovincialis* in mm, $n = 122$, 68 females, 54 males (*Source:* H. Jactel, INRAE, original data).

2.3.3.3 | *Size of head and body of larval stages of* Monochamus galloprovincialis

Maximum larval head capsule size and larval gallery lengths across all developmental stages provide evidence of the spatial requirements associated with larval development Koutroumpa et al., 2008. Specifically, maximum head capsule width allows estimation of the minimum phloem thickness required to sustain early larval stages (L1–L2), whereas larval gallery length data enable the estimation of the volume of sapwood required for gallery construction during the later developmental stages (L3–L4). The minimum head capsule width recorded for L2 larvae was 0.89 mm (Table 8; from Koutroumpa et al., 2008).

TABLE 8 Size characteristic of *Monochamus galloprovincialis* (after Koutroumpa et al., 2008).

Larvae			
Stage	**Body length (mm)**	**Limits of the measured head capsule length (mm) at the end of the larval stage**	**Limits of the measured head capsule width (mm) at the end of the larval stage**
L1	2.1 to 9.5–10.4	1.7–1.75	0.5 to 0.89–1.03
L2	3.7–4.3 to 19–21.4	2.76–2.87	1.55–1.62
L3	7.0 to 29.3–32.0	4.5–4.6	2.25–2.33
L4	12.5–13.6 to 42.3	6.38	3.51

2.3.3.4 | *Living location chosen by the different larval stages of* Monochamus galloprovincialis

L1 and L2 larval instars of *M. galloprovincialis* may persist for approximately 35–55 days and develop entirely within the phloem tissue ('bark') (Koutroumpa et al., 2008). L1 and L2 need the sugar of the phloem because their mandibles are not

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strong enough to enter beyond the phloem into the log. Even the L3 stage remains predominantly associated with the phloem during its development (Table 9). These observations provide evidence of the prolonged dependence of the early larval stages on sufficient phloem thickness and quality for successful establishment and development.

TABLE 9 Localisation of developmental stage of *Monochamus galloprovincialis* in the log (observational data $n = 797$; adapted from Koutroumpa et al., 2008).

	Percentage (%) of developmental stages in different positions of the log			
Stage	**Phloem**	**Sapwood**	**Heartwood**	**Pupal chamber (sapwood)**
Egg	100			
L1	100			
L2	100			
L3	78.7	4.8	16.4	
L4	28.1	5.9	62.6	3.4
Pupae				100
Adult				100

2.3.3.5 | *Lengths of the pupal chamber of* Monochamus galloprovincialis

Koutroumpa et al. (2008) reported experimental minimum–maximum data on total larval gallery lengths in various wood types, along with pupal chamber dimensions and exit hole diameter (Table 10). These data provide quantitative evidence of the spatial requirements for the successful development and emergence of *M. galloprovincialis*.

TABLE 10 Measurements of the galleries lengths and the size of the pupal chamber of *Monochamus galloprovincialis* given in mm (observational data; adapted from Koutroumpa et al., 2008).

Species	Type	Sapwood gallery (1)	Heartwood gallery (2)	Total (1) + (2)	Pupal chamber length	Pupal chamber width
Monochamus galloprovincialis	Min	10	10	30	15	5
	Max	45	137	161	64	16

The last larval stage (L4) is feeding through the sapwood of the branch/stem of the seedling with the main gallery being comprised in the heartwood central to the branch or stem; Koutroumpa et al. (2008) observed that *M. galloprovincialis*

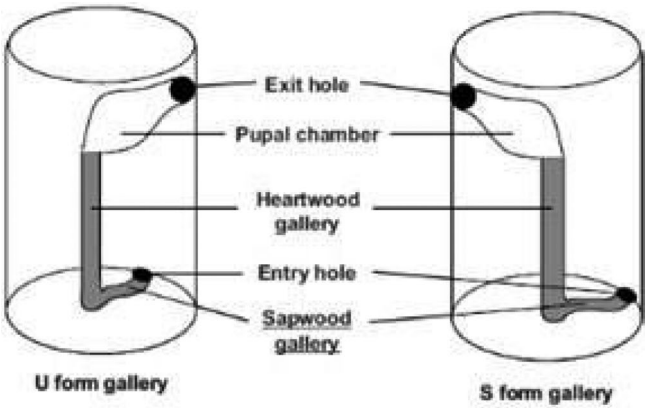


FIGURE 8 Schematic representation of the possible configuration of the *Monochamus galloprovincialis* galleries i.e. into, through and out of the seedling stem via the pupal chamber (observational data $n = 87$; Koutroumpa et al., 2008).

galleries could be both U shaped or S shaped (Figure 8).

2.3.3.6 | *Diameter of exit holes of* Monochamus galloprovincialis

Detailed distributional data on the exit hole dimensions of *M. galloprovincialis* (Figure 9) provide additional evidence regarding the body width of newly emerged adults ('total width' in Figure 7) and thereby the minimum diameter of the pupal

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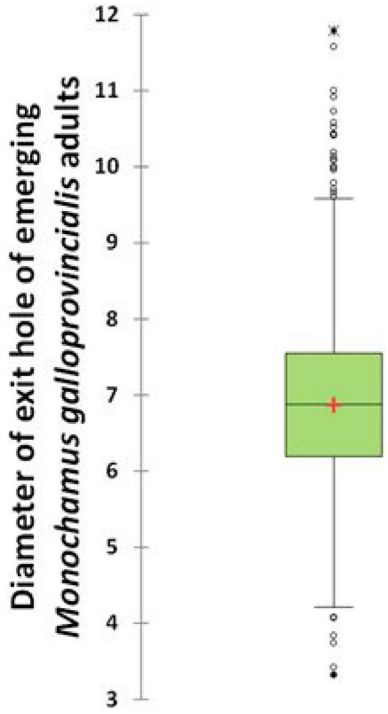


FIGURE 9 Observational data on *Monochamus galloprovincialis* exit hole diameters, $n = 2312$ (Appendix C Source: H. Jactel, INRAE, original data).

chamber required for successful emergence. Notably, all recorded exit holes exceeded 3 mm in diameter, indicating a lower threshold for the structural space necessary to permit adult emergence from the host material.

2.3.3.7 | *Size and positioning of the pupal chamber*

The data set shown in 10 is based on an observational sample comprising 198 pupal chambers and their associated exit galleries of *M. galloprovincialis*. For each of the 198 observations, several morphological and spatial measurements are reported, including pupal chamber length, chamber angle relative to the horizontal axis and log diameter at the location of the exit hole.

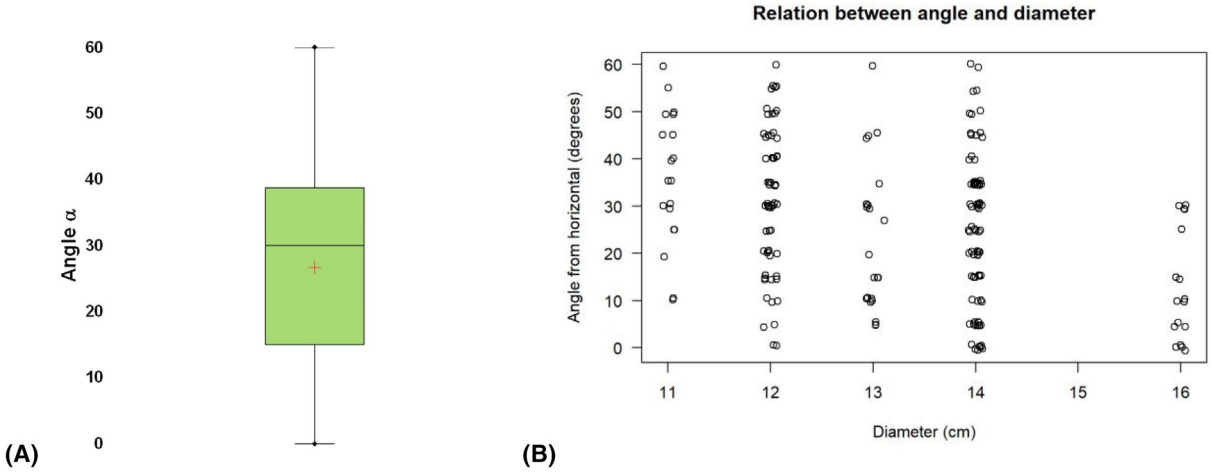


FIGURE 10 (A) Observational data on angle in degree the *Monochamus galloprovincialis* pupal chamber forms against the horizontal of the wood, $n = 198$ (Appendix D, Source: H. Jactel, INRAE original data); (B) scatterplot of the same data with stem diameter on the *x*-axis and the related angle formed by the pupal chamber against the horizontal on the *y*-axis.

Observed angle the pupal chamber forms against the horizontal line:

$$\text{Mean} = 26.67^{\circ} \pm 1.15; \text{max} = 60.00^{\circ}$$

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2.3.4 | Vector–pest interactions

Hypothetically, developmental stages of the vector can survive in wooden products and thereby enter a seedlings nursery accidentally together with the pest. This section collates related experimental evidence on these interactions. Additionally, expert knowledge is provided regarding the timing of pest and vector activity as well as observational characteristics of the vector.

Sousa et al. (2011) assessed the survival of the insect vector *M. galloprovincialis* in sawn boards. Most of the insect larvae were killed when sawing the wood, although some adults successfully emerged from the boards (10% survival) and blocks (37%). These results indicate a hypothetical way to disperse PWN if sawn wood or wood packaging material originates from PWN-affected forests and was not adequately heat treated.

Latitude and altitude influence the length of the life cycle, which is related to changes in mean annual temperatures (Naves et al., 2016). In Central Europe (e.g. Poland), *M. galloprovincialis* has an univoltine life cycle. The beetles fly from early/mid-June to late October, depending on ambient temperature, rainfall and sunlight (Filipiak & Tomalak, 2025). In Portugal, *M. galloprovincialis* flies from May through October and is probably bivoltine. Phoresy by *B. xylophilus* only occurs within a short period in early summer, when infection of new pine hosts most likely occurs. Peaks of *M. galloprovincialis* emergence and *B. xylophilus* phoresy immediately precede onset of the Mediterranean dry season (Firmino et al., 2017).

The following paragraphs report in large part personal communication with entomologists and practitioners in PWN control.

In Portugal, Spain and France, pine tree afforestation and reforestation are primarily performed during the late autumn (November to December) or early spring (February to early April). Planting during these cooler, wetter months ensures the seedlings can establish their root systems without suffering from summer drought stress.

In natural environment, insect vectors choose the crown of the trees for maturation feeding. According to Tomiczek and Hoyer-Tomiczek (2008) during 2 weeks of maturation feeding if young *Pinus* seedlings and branches of older *Pinus* were present together, *M. galloprovinialis* preferred branches over the young plants; however, if only young seedlings were present, these also would be attacked. Young plants were used in this assessment, but no height or age limit was mentioned in the publication. It is plausible that *Monochamus* may use nursery seedlings for maturation feeding only if a nursery forming a complete landscape of seedlings of same height.

No documented evidence was found in the EU that the vectors bred on trees of maximum age of 2 years i.e. max 12 mm diameter; likely the bark is too thin for the oviposition notch. Furthermore, the insect vector has never been observed laying eggs on healthy trees suggesting that the insect has capacity to recognise the unhealthy smell of weakened trees even if the tree is still asymptomatic.

European entomologists recognised that the vector's larval development requires physiologically alive wood until the end of L2 which is about 2 months (L1 + L2 stages lasting 35–55 days). L3 + L4 can develop in decaying trees or dead logs.

3 | ASSESSMENT

3.1 | Compatibility of the physical dimension of the vector stages and the size of the seedling

The assessment of physical compatibility between seedlings intended for afforestation/reforestation and insect vector life stages is based on three lines of evidence. These show that development of vectors in the branches or stems of seedlings with a RCD of less than 12 mm has never been observed and is physically impossible:

- The distribution of the diameters of 236 pine branches with exit holes of *M. galloprovincialis* shows that no exit hole was recorded in branches with diameter lower than 20 mm.
- The 0.9–1.6 mm width of the head capsule of the phloem-based larva L2 is larger than the phloem thickness predicted for a seedling of 12 mm diameter (0.441 mm, with a 95% confidence interval of [0.339; 0.543]), based on a linear extrapolation of X-ray measurements collected from thinner seedlings.
- The observed variation of 0–60° of the angle between horizontal and the pupal chamber does not allow the 5–16 mm long pupal chamber to fit in a log of a diameter of less than 12 mm, i.e. only 6 mm radius.

These lines of evidence are detailed in the three following section.

3.1.1 | Evidence provided by exit holes observed on pine branches

Because of the size of the larvae, pupae and emerging adults, only branches of a certain diameter can host the vector. The branch diameter must be large enough to host not only the larval gallery but also a pupal chamber for a nearly mature adult.

The distribution of the diameters measured on 236 branches of maritime pine tree reveals that the smallest branches with recorded exit holes of *M. galloprovincialis* were of diameter 20–30 mm. No exit hole was found in branches with diameters lower than 20 mm. Less than 1% of the exit holes (among $n = 2312$) were found in the smallest diameter classes

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(20–30 mm). The highest frequencies of exit holes were recorded in branches of intermediate diameters, from 50 to 80 mm (Figure 6).

In addition, all observed exit holes were wider than 3 mm, with most diameters reaching 6–8 mm (Figure 9). The emerging adult thus produces a tunnel and an exit hole that would be large relatively to a total branch diameter of 12 mm, which must severely damage or even kill the plant.

CONCLUSION

Successful vector development in branches smaller than 12 mm in diameter was never observed and would represent a major deviation from observed emergence patterns.

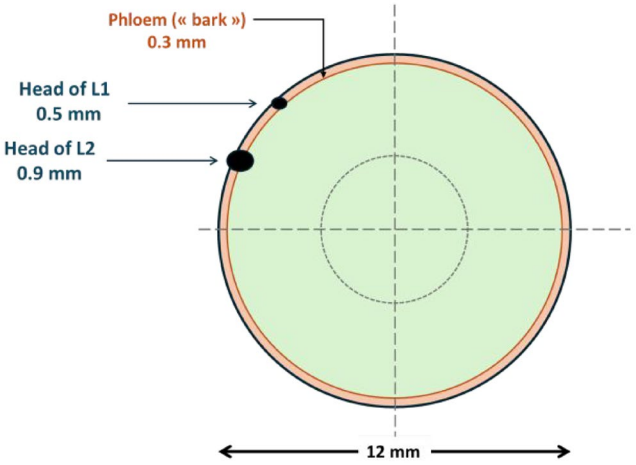


FIGURE 11 Geometric scale drawing of minimum size dimensions of the larval vector developmental stages associated with the phloem (L1 and L2) and the seedlings' maximum diameter at root collar (12 mm) with the surrounding phloem.

3.1.2 | Mechanistical explanation of head size of the larval stages L2 and the thickness of the phloem

Both stages, L1 and L2, of *M. galloprovincialis* are exclusively feeding reside phloem because their weak mandibles do not allow entering beyond the phloem (Section 2.3.3.4). Whether these young instars can physically fit inside the phloem of a small seedling until reaching L3 is not known (Figure 11).

The L1 stage is smaller than the L2 stage, and so it suffices to consider the L2 stage. The cephalic (head) capsule width of L2 larvae ranges from 0.89–1.03 mm (at the start of L2) to 1.55–1.62 mm (at the end of L2) (Section 2.3.3.3). The lower final size measurement of the L2 stage is 1.55 mm in head capsule width, which provides a minimum requirement for the phloem thickness needed for *M. galloprovincialis* to complete its development until the end of its L2 stage.

Additionally, already the head capsule width of L1 larvae ranges from 0.50 mm (at start) to 0.89–1.03 mm before next larval stage (Section 2.3.3.3). Hence, even at beginning of its development, L1 would not fit into the estimated phloem thickness of the seedling at root collar (Section 2.3.1.1), representing a size constraint for development in the phloem too.

CONCLUSION

L1 and L2 larvae of the PWN *M. galloprovincialis* cannot complete their development within the phloem of seedlings with not more than 12 mm diameter at root collar.

3.1.3 | Mechanistical explanation of the fitting the pupal chamber into the physical dimensions of a seedling

Last larval stage will tunnel the pupal chamber towards the closest exit of the log it is in. (H. Jactel, pers. comm.). The longest pupal chamber would result from a larvae feeding in the heartwood i.e. the centre of the seedling. Then, the length of the pupal chamber must run from the centre axis of the seedling to the exit hole (Figures 8 and 10, and Appendix D). Given the angle between the pupal chamber and the central axis, α, and the radius of the seedling, R, the theoretical maximum length of the pupal chamber, L, can be calculated by trigonometry ($L = R/\cos(\alpha)$; Figure 12). Then, by comparing this theoretical maximum length to the observed pupal chamber lengths (Section 2.3.3.7), it is possible to assess whether the pupal chamber could fit within a seedling of radius $R = 6$ mm (i.e. diameter 12 mm).

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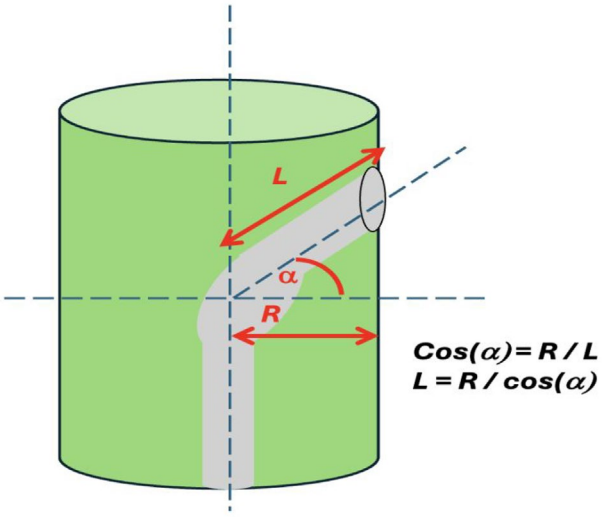


FIGURE 12 Relationship between the length of the *Monochamus galloprovincialis* pupal chamber and the angle of exit gallery. The parameters *R*, *L*, and α are the radius of the plant, the length of the pupal chamber, and the angle of the exit gallery, respectively.

Based on observations from infested *Pinus* trees, the mean and maximum exit angles from 198 pupal chambers are α = 26.67° and α = 60° (Section 2.3.3.7), respectively. For radius *R* = 6 mm, the corresponding theoretical maximum length is 6.71 mm [6/cos(26.67)] and 12 mm, respectively.

According to Koutroumpa et al. (2008), pupal chamber lengths range from 15 to 64 mm (Section 2.3.3.5). Even the minimum of these (15 mm) exceeds the theoretical maximum length allowed by a plant of 12 mm diameter by about 20% or 50% for the observed angles of 60° to 26.67°, respectively (Section 2.3.3.7).

CONCLUSION

The observed size and patterns of orientation of the pupal chamber and exit gallery indicate that the vector cannot fully develop in seedlings no more than 12 mm in diameter at root collar.

3.2 | Compatibility of nursery practice with non-vectored transmission

The assessment of the non-vectored transmission of PWN considers the possibility of infestation to and between seedlings by sawdust or wood chips through soil and survival of the seedling once infested.

Non-vectored transmission in a nursery may hypothetically occur in two ways: (a) entry into the nursery of contaminated material with subsequent transmission to healthy seedlings or (b) transmission of the nematode from an infested to a healthy seedling.

a. The changing end use of wood chips, the increasing amounts in international wood chip trade and the transmission of the nematode to seedlings from infested growing materials (sawdust and wood chips) from up to 3.5 cm distance (Section 2.3.2.1) request consideration. Because nematodes are shown to survive at most 72 h in soil (Mamiya & Shoji, 1989), the entry would be more likely with contaminated plant material used as growing substrate. The entry of the pest into the nursery by contaminated wooden material, however, can be excluded if the required heat-treatment at 56°C for 30 min (Section 2.3.2.3) is performed according to 2012/535/EU: Commission Implementing Decision of 26 September 2012 on emergency measures to prevent the spread within the Union of *Bursaphelenchus xylophilus* (Steiner et Buhrer) Nickle et al. (the pinewood nematode).
b. The onward transmission of nematodes from an (hypothetical) infested seedling in the nursery to a healthy one can be excluded based on nursing practice and the evidence complicating plant-to-plant infestation (Section 2.3.2.1). In nursery settings, pine seedlings (up to 2 years old) are grown in individual containers or pots (pers. comm. SNPF) thereby preventing inter-root contact required for nematode transfer. The plant-to-plant transmission of the nematode between seedlings depends on injuries or wounds which are expected to be limited due to sorting following the requirements for FRM stock. Moreover, an infested seedling (of non-resistant cultivars) will be detected within 2 weeks because of disease symptoms (Section 2.3.2.2) and the seedling discarded by the nursery managers.

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CONCLUSION

Good practice standards for nurseries exclude physical contact between individual (injured) seedlings and thereby prevent transmission of the pest between seedlings. Phytosanitary measures regulated for wood chips and sawdust will prevent introduction into nurseries and the infestation of seedlings from extern.

3.3 | Hypothetical interactions of vector and pest

The outcome of the assessments in the previous sections supports the conclusion that seedlings not older than 2 years and thereby a root collar diameter of not more than 12 mm are unsuitable to allow the vector concluding the part of its developmental cycle inside the wood and the nematode cannot effectively being spread between seedlings without the vector.

This final section looks at the scenario when both the vector and the pest collocate at seedlings of 12 mm root collar diameter.

To finalise the transmission cycle of the pinewood nematode, a host tree needs to be infested and allow the pinewood nematode to be transmitted via the insect vector. To involve a seedling in this process, and accounting for the fact of its immediate size limitation to vector development, the following sequence of undescribed or implausible events must happen.

First, the natural transmission of the nematode requires insect vectors feeding on the succulent seedling as they mature (Section 2.2.2). It has not yet been observed or described, that beetles deviate from attacking larger trees to reach sexual maturity, consuming tender bark, phloem and pine shoots (Sousa et al., 2015 and pers. comm., Luis Bonifacio). This is reasonable, as host selection occurs during flight at canopy level and is guided by volatile cues (terpenes), while landing is influenced by visual stimuli such as tree height and crown structure which results in preferential selection of taller trees (Sousa et al., 2015 and pers. comm., Luis Bonifacio), particularly along forest edges, roads and clearings. Indeed, surveys conducted annually in Portugal over the last 25 years have rarely detected PWN-infected trees with a diameter below 15 cm at breast height (DBH) (pers comm., Luis Bonifacio).

Second, the seedling must be assumed to be infested by another hypothetical source, not by the vector and not via production practice in accordance with regulatory standards (Section 3.2).

Third, the infested seedling must stay apparently asymptomatic to be traded and planted into the pest free region while remaining infested. Laboratory evidence on disease progression in seedlings of different sizes considering relevant climate conditions or vegetation seasons led to infected seedlings wilting and dying within few weeks up to few months (Section 2.3.4) after inoculation (Arbuzova et al., 2023; Hakamata et al., 2013; Melakeberhan & Webster, 1990; Modesto et al., 2022). When using rooted cuttings derived from resistant genotypes, no nematodes were found in asymptomatic or slightly damaged plants even as early as 13 months after inoculation (Díaz et al., 2026).

Fourth, in a seedling that may have sustained disease, the nematode population must persist for months or years, while concurrently asymptomatic seedling grows to a size that can attract the vector for egg laying and accommodate all life stages of the developing vector insect. Although the nematode has a (dauer) developmental stage that allows persistence under unfavourable environmental conditions and allows developmental arrest until cues associated with vector availability lead to a transition to the dispersal stage (Section 2.2.1), there was no evidence found concerning how long that waiting period can last or whether the within-tree nematode population would be eventually reduced to zero if no vector emerges.

Fifth, the growing tree must eventually become suitable for attraction, oviposition or colonisation by a vector which the evidence links with weakening tree condition (not necessarily caused by the nematode).

At that stage, the nematode must still be present to turn into a transmissible larval state when the colonising vector is close to emergence (Section 2.2.1) and allowing the vector acquiring the nematode to close the cycle.

The sequence to involve a seedling in vector-based transmission depends on biologically unsupported or highly implausible events. Furthermore, the seedlings will be planted more than 3.5 cm from each other and without root contact excluding non-vectored transmission (Section 2.3.2.1). Hence, it is implausible that seedlings become involved in closing the transmission cycle of the nematode in a pest-free region.

The time window of vectors flying period and nematode phoresy vs. the climatically driven time of planting of the seedlings (Section 2.3.4) do not overlap which excludes the accidental spread of the pest by the seedlings for forestation planting.

CONCLUSION

To be a pathway, seedlings need to be infested by the nematode via the insect vector feeding in the nursery, planted while asymptomatic into the field and remain asymptomatic for years until they reach a size that allows successful development of the vector. The tree must decline to attract vectors for egg laying. This sequence of events needs to occur while the nematode is still in the suitable developmental phase to conclude the transmission cycle. Based on the collated evidence, this sequence of events is considered highly implausible.

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3.4 | Uncertainty

The noted uncertainty stems from the available evidence on vector-related measurements, which are largely observational. This means that the vectors are not experimentally forced out of their natural behavioural patterns (e.g. exit hole data, data on pupal chamber inclination; Section 2.3.3). Forced choice experiments would be necessary to fully prove the excluding consequence of the evidence.

The maximum age of the seedlings of 2 years is regulated (Section 2.1). Uncertainty relates to the assumed threshold dimension of 12 mm root collar diameter for 2-year-old plants for planting in the context of afforestation and reforestation, which is based on one public document from a national forest nursery association only (Section 2.3.1). The growth of seedling's diameter at the root collar and the age possibly have a functional dependency, but this was not found in the literature search.

Another source of uncertainty is related to the small number of data used to estimate the phloem thickness of a seedling of 12 mm (Section 2.3.3.4). Nevertheless, the conclusion would remain unchanged based on the upper bound of the prediction interval of the estimated value.

The assumption of larvae starting to build the pupal chamber from the heartwood i.e. central to the branch or stem is based on expert knowledge and if it not correct, would relax the strength of the arguments in Section 3.1.3. If hypothetically, the L4 larva tunnels the sapwood right under the phloem and the pupal chamber perfectly straight through the centre towards the opposite side, then for the 15 mm diagonal of the chamber to fit into the 11.1 mm between the two phloem of 0.44 mm each, the angle must be about 42.3 degrees which is inside the observational data range. However, the pupal chamber crossing the heartwood was never observed.

More generally, one may assume values beyond the ranges of the data reported for the vector measurements (e.g. larger angles of exit gallery, longer pupal chamber) to force physical fitting of the vector life stages into the phloem or the stem, but there is no indication why such deviations from the observational data would be useful from the biological point of view.

The identified knowledge gap of long-term longevity of nematodes inside an apparently healthy seedling, which grows under natural conditions to an asymptomatic tree which is sufficiently large to allow insect development, leaves uncertainty and therefore the argument cannot be explored in the assessment.

4 | CONCLUSIONS

There is some residual uncertainty in the assessment, and in certain cases, it would be possible to identify scenarios within which transmission would, perhaps, be possible. However, this would require assumptions or interpretations of the biology and epidemiology of the pest–vector–host system extending well beyond the evidence that is currently available.

Based on the available evidence, the Panel concludes that seedlings for planting in afforestation and reforestation with a root collar diameter of 12 mm or less are not a pathway.

This opinion is based on the following statements:

C1: Successful vector development in branches smaller than 12 mm in diameter was never observed and would represent a major deviation from observed emergence patterns.

C2: L1 and L2 larvae of the vector cannot entirely complete their development within the phloem of seedlings smaller than 12 mm diameter at root collar.

C3: The size and patterns of orientation of the pupal chamber and exit gallery indicate that the vector cannot fully develop in seedlings smaller than 12 mm in diameter.

C4: Good practice standards for nurseries limit physical contact between individual (injured) seedlings and would prevent pest transmission between seedlings. Phytosanitary measures regulated for wood chips and sawdust should prevent introduction into nurseries and the infestation of seedlings from extern.

C5: To be a pathway, seedlings need to be infested by the nematode via the insect vector feeding in the nursery, planted while asymptomatic into the field and remain without symptoms for years until they reach a size that allows successful development of the vector. The tree must decline to attract vectors for egg laying. Moreover, all of this must occur while the nematode is still in the suitable developmental phase to conclude transmission cycle. Based on the collated evidence, this sequence of events is considered highly implausible.

ABBREVIATIONS

EPPO European and Mediterranean Plant Protection Organization
FAO Food and Agriculture Organization
ISPM International Standards for Phytosanitary Measures
PLH Plant Health
PRA Pest Risk Assessment
PWD Pine Wilt Disease
PWN Pinewood Nematode
SNFP Syndicat National des Pépiniéristes Forestiers

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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APPENDICES

Appendices A–D are available under the Supporting Information section on the online version of the scientific output.

Appendix A. Communication with SNPF.
Appendix B. Measurement of phloem thickness in maritime pine seedlings using X-ray microtomography.
Appendix C. Data on diameter of exit holes of *Monochamus galloprovincialis* in maritime pine branches.
Appendix D. Pupal chambers exit galleries *Monochamus galloprovincialis*.

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