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SCIENTIFIC OPINION

Scientific Opinion on the phytosanitary risk associated with some coniferous species and genera for the spread of pine wood nematode[1]

EFSA Panel on Plant Health (PLH)[2, 3]

European Food Safety Authority (EFSA), Parma, Italy

ABSTRACT

The European Commission requested the Panel on Plant Health to deliver a scientific opinion on the phytosanitary risk of plants (other than fruits and seeds) of *Pinus pinea* and of the genera *Chamaecyparis*, *Cryptomeria* and *Juniperus* for the spread of pine wood nematode (PWN) via movement of infested plants or untreated plant products or by supporting natural spread of PWN in conjunction with European species of the vector. The Panel analysed the data submitted by Portugal regarding surveys on the Tróia Peninsula where *P. pinaster* and *P. pinea* co-occur, and the related laboratory results of Naves et al. (2006) on feeding and oviposition preferences of *Monochamus galloprovincialis*. The Panel also undertook a comprehensive review of the literature. The zero infestation of PWN recorded on *P. pinea* on the Tróia Peninsula was not significantly different from the result for *P. pinaster*, because of the small *P. pinea* sample. Hence, the conclusion that *P. pinea* is not a host plant for PWN is not supported by the data submitted, principally because of low statistical confidence arising from the few *P. pinea* trees present. Moreover, the limited presence of *P. pinea* in the study areas means that the results are representative neither of the Tróia Peninsula nor of other parts of Portugal. Naves et al. (2006) recorded some oviposition by *M. galloprovincialis* on *P. pinea*, but less than on other hosts. No differences in feeding of *M. galloprovincialis* on *P. pinaster* and *P. pinea* were detected, thus potentially allowing PWN transmission to trees by this route. The available information regarding the genera *Chamaecyparis*, *Cryptomeria* and *Juniperus* as potential hosts of *Monochamus* spp. and PWN suggests overall a low susceptibility to PWN or its vectors; the uncertainty concerning PWN is high and would require supplementary research.

KEY WORDS

Pine wilt disease, *Bursaphelenchus xylophilus*, *Monochamus*, *Pinus pinea*, *Juniperus*, *Cryptomeria*, *Chamaecyparis*

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[1] On request from the European Commission, Question No EFSA-Q-2011-00883, adopted on 22 December 2011.

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SUMMARY

Following a request from the European Commission, the Panel on Plant Health was asked to deliver a scientific opinion concerning the phytosanitary risk of plants (other than fruits and seeds) of *Pinus pinea* and of plants of species belonging to the genera *Chamaecyparis*, *Cryptomeria* and *Juniperus* for the spread of the pine wood nematode (PWN) *Bursaphelenchus xylophilus* via movement of infested plants or untreated plant products or by supporting natural spread of PWN in conjunction with European species of the vector.

The Panel has analysed the data in the document submitted by Portugal (Sousa et al., 2011) and the related evidence in Naves et al. (2006). In connection with this, the Panel has also undertaken a comprehensive review of the literature.

Surveys on the Tróia Peninsula (Sousa et al., 2011) showed a lower yearly mortality rate of *P. pinea* (below 0.03 %) compared with *P. pinaster* (1-2 %). The PWN infestation rate in dead trees (with symptoms) decreased from 80 % to 10 % during the period 2000/01–2007/08, so in later years PWN was not the main cause of tree mortality. Although a zero infestation of PWN was recorded on *P. pinea*, this was not statistically significantly different to the result for *P. pinaster* because of the small number of *P. pinea* trees sampled. Hence, based purely on the data presented by Sousa et al. (2011), the conclusion that *P. pinea* is not a host plant for PWN has not been supported. In addition, no information on PWN infestation of symptomless trees was given. Statistically, therefore, it cannot be stated that there is a difference in PWN infestation between *P. pinea* and *P. pinaster*, but it is clear that at most there would be a very low infestation in the former tree species. A much higher level of sampling would be needed to provide confidence in concluding on whether PWN can survive and breed in living *P. pinea* trees in the field.

P. pinea occurs in many locations in Portugal. From the data presented, it appears that the results from the studies of the pine forest on the Tróia Peninsula cannot be extrapolated to the other parts of the peninsula, nor to other areas of Portugal. In the case of the Tróia Peninsula, the experimental plots had higher densities of pine trees and a higher proportion of *P. pinaster* trees than the average in corresponding forest classes over the remainder of the Tróia Peninsula. Owing to the low relative frequency of *P. pinea* in the studied forest plots, an extrapolation to plantations of this species is questionable, and an extrapolation to other parts of Portugal is not possible because of different conditions of climate and soil. Thus, the very low prevalence of *P. pinea* in the study areas indicates that the results are representative neither of the Tróia Peninsula nor of other parts of Portugal.

With regard to the vector insect *Monochamus galloprovincialis* (the only species of vector considered in the study), the Portuguese document concludes that differences occur in oviposition rate in *P. pinea* and *P. pinaster* under the specific experimental settings. Although the rate of oviposition in *P. pinea* is lower than on other host plants, oviposition on *P. pinea* still remains possible. An extrapolation to forests with different tree compositions and different settings is not possible from the limited data presented. The Portuguese document does not acknowledge the fact that experiments by Naves et al. (2006) did not detect differences in feeding of *M. galloprovincialis* on *P. pinaster* and *P. pinea*. The transmission of PWN from the vector to the two species of pine was not investigated and remains unclear.

M. galloprovincialis is distributed over a vast geographical area and it cannot be excluded that subspecies (*M. galloprovincialis galloprovincialis*, *M. galloprovincialis pistor*) and local populations could have host preferences different from that of the known Portuguese populations. Attacks on *P. pinea* by *M. galloprovincialis* are in fact known from Italy. Observations and, particularly, the studies of Halik and Bergdahl (1994) support the conclusion that some coniferous trees may become infested with PWN, but remain free of pine wilt disease (PWD) symptoms for many years while containing live nematodes. Such trees can act as reservoirs for the nematode over prolonged periods. However, if these trees are weakened sufficiently to become attractive to *Monochamus* for oviposition and larval development, there is a possibility that the nematode could associate with the vector and be

transmitted to other trees. Unfortunately, the relationships between European *Monochamu*s species other than *M. galloprovinciallis*, *P. pinea* and PWN have not yet been studied in sufficient detail to draw firm conclusions on the survival and transmission of PWN.

An absence of apparent wilt symptoms arising from PWN infestation in *P. pinea* would not necessarily indicate that nematodes are unable to invade and survive in such trees. It is possible that the relationship between *P. pinea* and PWN in Portugal may be similar to the situation in North America, where PWN is widely distributed but not frequently reported from indigenous pine species and is associated with saprophytic development in dead trees arising from causes other than wilt caused by the nematode. Furthermore, it cannot be excluded that PWN could be present, but not necessarily causing tree mortality, in *P. pinea* in situations when this species is a dominant tree; however, this would require that *Monochamus* spp. were able to successfully breed in weakened trees. The fact that PWN may reproduce in dead *P. pinea* would allow the nematode to be present in traded lumber and wood products. Plants for planting could also contain living nematodes but for further spread from such trees the vector is needed.

Owing to missing scientific information on the interaction of *M. galloprovincialis*, *B. xylophilus* and *P. pinea*, the risk of PWN spread with plants and wood of *P. pinea* is difficult to assess. However, as long as trade volumes are small, the probability of spread is considered low. Owing to insufficient documentation of the trade volumes and the nematode–beetle interaction on *P. pinea*, the uncertainty is high.

The available information regarding the status of the genera *Chamaecyparis*, *Cryptomeria* and *Juniperus* as regards *Monochamus* spp. and PWN suggests overall a low susceptibility of these taxa to PWN or its vectors, with a low uncertainty concerning the vectors and a high uncertainty concerning PWN. No experimental inoculation of PWN on *Chamaecyparis*, *Cryptomeria* or *Juniperus* spp. has been attempted so far, except one test involving *Chamaecyparis nootkatensis* carried out in laboratory conditions in Canada. No *C. nootkatensis* plant died after inoculation and no nematodes could be detected in the asymptomatic plants. The scant information regarding the plant genera *Chamaecyparis*, *Cryptomeria* and *Juniperus* suggests that they would not suffer from wilt disease and would not function as efficient hosts for PWN, but there is still a possibility that they could be either asymptomatic hosts for PWN in living trees or hosts during the saprophytic phase of the nematode cycle. The information on the interaction between *Monochamus* spp., PWN and species in the genera *Chamaecyparis*, *Cryptomeria* and *Juniperus* is largely missing, without specific surveys or experimental inoculations. Thus it is difficult to make firm statements on the risks of PWN spread in trade from this material. Therefore, the uncertainty of this is high.

As indicated in the evaluations above, there are limited data on the potential for *P. pinea*, *Chamaecyparis* spp., *Cryptomeria* spp. and *Juniperus* spp. to successfully support either or both of *Monochamus* spp. or PWN. The fact that *Monochamus* spp. are known to maturation feed and breed, albeit at low levels, in *Chamaecyparis* spp., *Cryptomeria* spp. and *Juniperus* spp. and possibly in *P. pinea* suggests that PWN could be carried to these host trees and potentially be dispersed further if *Monochamus* breeding is successful. Unfortunately, data to confirm and quantify these potential associations is poor and, therefore, further research is needed to increase the insights into PWN ecology, by studying the development and survival of PWN in artificially inoculated field-grown trees.

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BACKGROUND AS PROVIDED BY THE EUROPEAN COMMISSION

Commission Decision 2006/133/EC requires Member States to take measures against the dissemination of *Bursaphelenchus xylophilus*, the pine wood nematode (PWN), as regards certain demarcated areas in Portugal. The measures concern certain coniferous plant genera considered to be susceptible to PWN and that might support the establishment and spread of PWN, through movement of infested plants or plants products or by supporting natural spread.

The European and Mediterranean Plant Protection Organisation (EPPO) in 1996 published a Pest Risk Assessment for PWN[4]. An updated version of that PRA, published in 2009[5], considers that PWN prefers *Pinus* species, but is also able to attack other Coniferae, in particular *Abies*, *Picea*, *Larix*, *Cedrus* and *Pseudotsuga*, and considers these genera as PWN host plants. It furthermore recalls that "PWN vectors in the genus *Monochamus* can also attack trees of above mentioned species and some other Coniferae: *Juniperus*, *Chamaecyparis*, *Cryptomeria* and sometimes *Tsuga*, but it is uncertain whether these genera are hosts for PWN. They may become infested. Neither *Thuja* nor *Taxus* are regarded as hosts of PWN and its vectors".

Commission Decision 2006/133/EC lists as susceptible to PWN: plants (other than fruit and seeds) of *Abies*, *Cedrus*, *Larix*, *Picea*, *Pinus*, *Pseudotsuga* and *Tsuga*, and as susceptible wood and bark: wood and isolated bark of conifers (Coniferales), except that of *Thuja*. EFSA is requested to clarify whether plants belonging to the genera *Chamaecyparis*, *Cryptomeria* and *Juniperus* should be considered a phytosanitary risk for the spread of PWN via movement of infested plants or plant products or by supporting natural spread.

Furthermore, Portugal and Spain have requested to exempt *Pinus pinea* from the list of plants susceptible to PWN because no PWN-diseased *P. pinea* plants were apparently found in Portugal since the introduction of PWN in 1999. EFSA is requested to clarify the phytosanitary risk of such an exemption.

TERMS OF REFERENCE AS PROVIDED BY THE EUROPEAN COMMISSION

EFSA is requested, pursuant to Article 29(1) and Article 22(5) of Regulation (EC) No 178/2002, to provide a scientific opinion concerning the phytosanitary risk of plants (other than fruits and seeds) of *Pinus pinea* and of plants of species belonging to the genera *Chamaecyparis*, *Cryptomeria* and *Juniperus* for the spread of PWN via movement of infested plants or untreated plant products or by supporting natural spread of PWN in conjunction with European species of the vector.

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[4] Evans HF, McNamara DG, Braasch H, Chadoeuf J, Magnusson C (1996). Pest risk analysis (PRA) for the territories of the European Union (as PRA area) on *Bursaphelenchus xylophilus* and its vectors in the genus *Monochamus*. *Bulletin OEPP/EPPO Bulletin* **26**(2), 199-249.

[5] http://www.eppo.org/QUARANTINE/Pest_Risk_Analysis/PRA_documents.htm

ASSESSMENT

1. Introduction

1.1. Purpose of the opinion

This document presents an assessment prepared by the Panel on Plant Health concerning the potential roles of *Pinus pinea* L. (other than its fruit and seeds) and of plant species belonging to the genera *Chamaecyparis*, *Cryptomeria* and *Juniperus* in relation to the phytosanitary risk posed by the pine wood nematode (*Bursaphelenchus xylophilus* (Steiner et Buhrer) Nickle; hereafter PWN) via movement of infested plants or untreated plant products or by supporting the natural spread of PWN in conjunction with the European species of its insect vector, in response to a request from the European Commission.

1.2. Scope of the opinion

The assessment covers the probability of spread of PWN, after introduction, to the whole EU territory. The plant taxa considered in the assessment are *P. pinea* and all species of the genera *Chamaecyparis*, *Cryptomeria* and *Juniperus*. For the nematode species the assessment considers only the PWN (see section 3.1.1), while for the vector all species of the genus *Monochamus* are included (see section 3.1.2).

1.2.1. The document submitted by the Portuguese authorities in support of their request to exempt *Pinus pinea* from the European Union emergency measures against PWN

With a note (Ref. ARES/2011/743492) dated 8 July 2011, the European Commission – Health and Consumers Directorate General – Plant Health Unit has transmitted to EFSA, as supplementary information to the request reported in the above sections on “Background and terms of reference provided by the European Commission”, a dossier submitted by the Portuguese authorities in support of their request to exempt *Pinus pinea* from the European Union emergency measures against PWN.

This dossier, dated June 2011 and authored by Edmundo Sousa, Pedro Naves and Luis Bonifacio from the National Institute for Biological Resources (Portugal) and by José Manuel Rodrigues from the National Forestry Authority (Portugal), is titled “Risk assessment of *P. pinea* in relation to PWN” (hereinafter referred to as “Sousa et al., 2011”). In this text the authors describe four experiments or survey results which form the basis of the claim that PWN is not able to infest *P. pinea* and this species should be classified as ‘resistant’. The authors provided, on request, further clarifications, on October 2011, which were included in the consideration of the Panel (Edmundo Sousa, Portugal, personal communication, October 2011, hereinafter referred to as ‘Sousa, 2011, clarifications').

2. Data and methodology

2.1. Data used in the assessment

2.1.1. Data from the Portuguese document on “Risk assessment of *Pinus pinea* L. in relation to PWN”

In **survey 1** (survey conducted by the INRB, IP Institute, in Tróia Peninsula: sampling on diseased trees, Sousa et al. (2011), table III) the authors reported the number of dead trees in the pine forests on Tróia Peninsula (South bank of the Sado River, near the city of Setúbal) in the years from 2000/01 to 2007/08. Tróia Peninsula is a tourist settlement within a demarcated area south of Lisbon. The pine forest is mainly composed of maritime (*P. pinaster*) and stone (*P. pinea*) pines of 30-40 years of age, on an area of about 400 ha. The trees that died from pine wilt disease (thereafter PWD) or other reason were felled each year. Between 5 % and 10 % of the dead maritime pines and all dead stone pines were randomly sampled for PWN. In total 20 % of the samples were also taken from symptomless pine trees (Sousa, 2011, clarifications). Sampling was done in winter when the wilting symptoms are most conspicuous. Yearly figures were provided neither for PWN infestation nor for the total number of trees. A figure (figure 1) provided with the clarifications shows a decreasing curve over time of total PWN infestation of the maritime pine samples (80 % in 2001/02 to < 20 % in 2007/08). The authors clarified that, although the PWN had been present in the peninsula since at least 1999, the first sanitary felling of dead pine trees only began in 2000/01. Thus, this initial felling included a number of dead and wilted trees that had died during the previous years but had not been removed. The number of dead pines felled in 2000/01 is therefore inflated.

The authors clarified further that the presence of Aleppo pines in the peninsula is vestigial, being found in two small areas with about 30-40 trees. Over the years none of these trees have wilted or died, and therefore none have been sampled for the PWN. The only other conifers present are two species of Juniper (*Juniperus navicularis* and *Juniperus turbinata*), which are not forestry species and have not suffered of mortality over the years, and therefore have not been sampled for the PWN. (Sousa, 2011, clarifications).

In **survey 2** (survey conducted by the INRB, IP Institute in Tróia Peninsula: Experimental plots, Sousa et al. (2011), table IV) the authors reported the results from three experimental plots of 1 ha each in the Tróia Peninsula pine forest sampled over a four-years period (2001/02 to 2004/05).

In **surveys 1** and **2**, dead trees were checked for the presence of the larval stage of the beetle vector, the pine sawyer, *Monochamus galloprovincialis* Olivier (Coleoptera: Cerambycidae) as well as for infestation with PWN. Using a battery-driven drill at low speed, shavings of wood were taken from the trees at breast height and collected in an unused bag. Four or more drillings per tree taken from a maximum of five trees were combined to one composite sample. In addition, wooden discs were taken at different heights and cut into 1 cm wood pieces to form a sample. When trees were felled, samples from the canopy were collected and kept separate from trunk samples (Sousa, 2011, clarifications).

A minimum amount of 100–200 g of wood was collected per sample. Before analysis, the samples were left for 1–3 weeks at 25 °C under laboratory conditions (Sousa, 2011, clarifications). The samples were placed in a tray with water for 48 h at ambient temperature, then sieved with a 400 mesh (38 µm). The extracted nematodes were identified morphologically and/or by using molecular techniques.

For **survey 1** Sousa et al. (2011) reported (see table 1):

- The yearly count of dead trees for *P. pinaster* and *P. pinea* (Sousa et al., 2011, table III). The number of samples collected for *P. pinaster* and *P. pinea* were reported in the clarification.

- More than 99 % of the dead trees were of *P. pinaster*.

- The infestation of PWN varied between locations and years: 10-75 % (details presented on figure 1 from the clarifications) of all dead *P. pinaster* trees were infested with PWN, and no *P. pinea* trees were infested with PWN.

- 83 % of the individuals of the vector *M. galloprovincialis* were carrying larval instars of the PWN.

The Panel calculated additionally the 95 % confidence intervals for the infestations rates of *P. pinaster* and *P. pinea* (Table 2). Owing to its wide confidence limits, the estimated probability of finding a tree dying from PWD or other reason and being infested with PWN is not different between *P. pinaster* and *P. pinea*. A detailed analysis of the survey results can be found in Appendix A.

Table 1: Number of dead pine trees in Tróia Peninsula between 2000/01 and 2007/08 (Sousa et al., 2011, table III; Sousa, 2011, clarifications)

Year (winter)	***No of dead trees in Tróia Peninsula*** *Pinus pinaster* total				*Pinus pinea* total				Total[2] total	
	total	sampled	infested[1] abs.	rel. (%)	total	sampled	infested abs.	rel. (%)	total	sampled
2000/01	4226	120	unknown		5	5	0	0	4231	125
2001/02	1365	100	77	77	0	0			1365	100
2002/03	636	80	62	77	0	0			636	80
2003/04	1135	80	59	73	0	0			1135	80
2004/05	953	90	52	58	3	3	0	0	956	93
2005/06	1568	80	28	35	0	0			1568	80
2006/07	1337	90	11	12	3	3	0	0	1340	93
2007/08	633	70	9	13	11	11	0	0	644	81
2000-08	11853				22				11875	
2001-05	4089				3				4092	

[1] Estimated from figure 1

[2] Calculated by the Panel

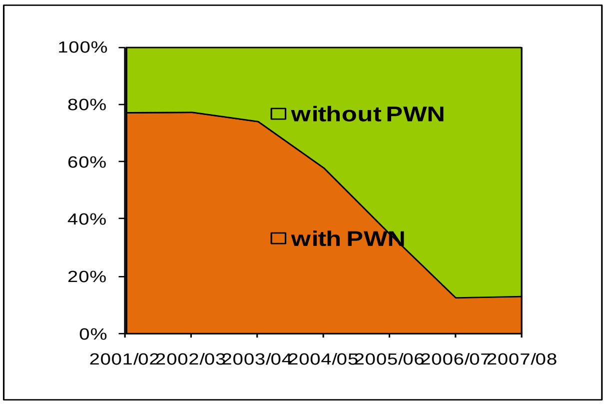

Figure 1: Annual percentage of PWN infested samples of maritime pine (*P. pinaster*) (Sousa, 2011, clarifications).

Table 2: Infestation rates of pine trees in Tróia Peninsula (Sousa et al., 2011, table III; Sousa, 2011, clarifications).

Year (winter)	***No of dead trees in Tróia Peninsula*** *Pinus pinaster* sampled	infested[1] with PWN abs.	rel. (%)	Confidence interval (95 %) (%)		*Pinus pinea* sampled	infested with PWN abs	rel. (%)	Confidence interval (95 %) (%)	
2000/01	120	Unknown				5	0	0	0	45
2001/02	100	77	77	68	85	0				
2002/03	80	62	77	67	86	0				
2003/04	80	59	73	63	83	0				
2004/05	90	52	58	47	68	3	0	0	0	63
2005/06	80	28	35	25	46	0				
2006/07	90	11	12	6	21	3	0	0	0	63
2007/08	70	9	13	6	23	11	0	0	0	24

[1] Estimated from figure 1

For **survey 2** Sousa et al. (2011) reported (see table 3):

- the total number of trees per plot
- the yearly count of dead and live *P. pinaster* and *P. pinea* trees (Sousa et al., 2011, table IV).

Table 3: Number of live or dead pine trees in three experimental plots in Tróia Peninsula between 2001/02 and 2004/05 (Sousa et al., 2011, table IV).

Plot	Year (winter)	***No of dead trees***			***No of live trees***			***Total no of trees[1]***		Gross total[1]
		Pinus pinaster	*Pinus pinea*	Total[1]	*Pinus pinaster*	*Pinus pinea*	Total[1]	*Pinus pinaster*	*Pinus pinea*	
1	2001/02	5	0	5	491	4	495	496	4	500
1	2002/03	24	0	24	467	4	471	491	4	495
1	2003/04	13	0	13	454	4	458	467	4	471
1	2004/05	33	0	33	421	4	425	454	4	458
1	**2001-05**	**75**	**0**	**75**	**421**	**4**	**425**	**496**	**4**	**500**
2	2001/02	15	0	15	330	3	333	345	3	348
2	2002/03	22	0	22	308	3	311	330	3	333
2	2003/04	24	0	24	284	3	287	308	3	311
2	2004/05	2	0	2	282	3	285	284	3	287
2	**2001-05**	**63**	**0**	**63**	**282**	**3**	**285**	**345**	**3**	**348**
3	2001/02	2	0	2	135	43	178	137	43	180
3	2002/03	8	0	8	127	43	170	135	43	178
3	2003/04	4	0	4	123	43	166	127	43	170
3	2004/05	2	0	2	121	43	164	123	43	166
3	**2001-05**	**16**	**0**	**16**	**121**	**43**	**164**	**137**	**43**	**180**
All[1]	2001/02	22	0	22	956	50	1006	978	50	1028
All[1]	2002/03	54	0	54	902	50	952	956	50	1006
All[1]	2003/04	41	0	41	861	50	911	902	50	952
All[1]	2004/05	37	0	37	824	50	874	861	50	911
All[1]	**2001-05**	**154**	**0**	**154**	**824**	**50**	**874**	**978**	**50**	**1028**

[1] Calculated by the Panel

The Panel also compared the density and composition of pine trees in the experimental plots with the average situation on Troía Peninsula (table 4).

Table 4: Comparison between average densities of pine trees on Tróia Peninsula with the selected experimental plots (Sousa et al., 2011, table IV; Sousa, 2011, clarifications).

		Average number of trees/ha in 2011 (%)				Number of trees/ha in 2001/02		
Forest type		*P. pinaster*	*P. pinea*	Total	Experimental plot	*P. pinaster*	*P. pinea*	Total
Dominant *P. pinaster*		177	21	198	Plot 1	496	4	500
	%	89.4	10.6	100		99.2	0.8	100
					Plot 2	345	3	348
	%					99.1	0.9	100
Mixed *P. pinaster* and *P. pinea*		111	48	159	Plot 3	137	43	180
	%	69.8	30.2	100		76.1	23.9	100
Dominant *P. pinea*		27	89	116	none			
	%	23.3	76.7	100				

All plots have higher total pine tree density and fewer *P. pinea* than the average on Tróia Peninsula: plots 1 and 2 were dominated by *P. pinaster* (500 trees/ha and 348 trees/ha, respectively, and about 1 % *P. pinea*), compared with an average of 198 *P. pinaster* trees/ha and 11 % of *P. pinea* on the peninsula. (Sousa, 2011, clarifications). Plot 3 is an example of mixed forest, but *P. pinaster* is the dominant tree species on most of the peninsula. The exact area of the different forest types was not provided by the authors. Thus, the plots clearly have special characteristics and appear not to be representative of the Tróia Peninsula or of the entire North Alentejo coast and the Ribatejo Province. A detailed analysis of the results of survey 2 can be found in Appendix A.

In **experiment 1** (experiment 2 in Naves et al. (2006): same as table VIII in Sousa et al. (2011)), the authors reported on the oviposition of the vector, the pine sawyer, in different types of wood: Aleppo pine (*P. halepensis*), Monterey pine (*P. radiata*). maritime pine (*P. pinaster*), stone pine (*P. pinea*), Scots pine (*P. sylvestris*), Mexican white cedar (*Cupressus lusitanica*) and Rocky Mountain Douglas-fir (*Pseudotsuga menziesii* var. *glauca* (Beissn) Franco).

The trees of **experiment 1** were felled in the last week of June 2003; the bolts used in the experiment were sawn 20 days after felling with the following characteristics: 60 cm long × 6-12 cm diameter; the ends coated with paraffin.

Wood samples that were used in the experiments originated from three different places. The maritime and stone pine from the Tróia Peninsula, Aleppo pine and Mexican white cedar from the Monsanto Park, Lisbon, and Scots and Monterey pines as well as Douglas fir from VN de Cerveira, Minho Province.

All wood was stored in a room maintained at ambient temperature (24 °C).

Insects emerged in June 2003 from *P. pinaster* logs kept in wooden boxes in Tróia. Three pairs of 25 days old unmated adults were randomly chosen and placed in a 0.2 m³ screened wooden box along with a single PWN-free bolt. The oviposition experiment lasted 5 days. Dead insects were immediately replaced by one of the same sex and age. Five replicates of three pairs/bolt of seven tree species were conducted, giving a total of 35 experiments.

After 5 days, the number of oviposition slits was counted, and bolts were incubated at:

- 25 °C, 16 h–8 h light–dark cycle for 80 days
- 8 °C, 0 h–24 h light–dark cycle, for 40 days
- 25 °C, 16 h – 8 h light–dark cycle, for 120 days

The number of emerged adults, their size (length of right elytron) and sex were recorded. After incubation, all wood samples were debarked, and dissected, and any immature life stages were also counted.

The emergence rate was calculated per bolt as ratio of number of emerged beetles divided by number of eggs (equal to the number of oviposition slits, on the assumption that each slit contained one egg).

Sousa et al. (2011) reported the following data from experiment 1: mean values and standard error of the mean (SEM) for characteristics of the bolts (diameter and bark thickness) for each of the seven kinds of bolts. For oviposition, mean and standard error of the mean (SEM) of number of eggs, emergence rate per bolt and elytral length, as well as proportion of females were reported (table 5).

Eggs were laid in all five bolts of *P. sylvestris, P. halepensis, P. pinaster,* in three (of five) bolts of *P. radiata,* two of *P. pinea,* one of *P. menziesii*, none on *C. lusitanica*.

Naves et al. (2006) carried out an analysis of variance test (Kruskal–Wallis test) for differences of the emergence rate. Fisher's least significant difference (LSD) was used to identify differences in the number of eggs, the days to emergence, the male/female elytral lengths:

Oviposition**:** Highest number was laid on *P. sylvestris,* followed by *P. halepensis* and *P. pinaster*, then *P. radiata.* The lowest number of eggs was laid on *P. pinea* and *P. menziesii*, none were laid on *C. lusitanica.*

Emergence rate**:** No difference was found between *P. halepensis*, *P. pinaster, P. radiata* and *P. sylvestris.* No adults emerged from *P. pinea* or *P. menziesii*.

Minor differences were found in development time (no of days until emergence) and in size (elytral length, with the largest individuals emerging from *P. pinaster*).

No correspondence was found between bolt diameter or bark thickness and the numbers of eggs.

Naves et al. (2006) conclude that *P. pinea*, *P. menziesii* and *C. lusitanica* are not adequate hosts for *M. galloprovincialis* and that the breeding success in *P. pinaster* indicates that it is the most suitable host.

In the last part, the authors reported on a preference experiment (**experiment 2**) of Naves et al. (2006) (experiment 3: table IX in Sousa et al., 2011).

In this experiment**,** beetles were first kept for maturation feeding in acrylic boxes (80 cm × 40 cm) along with one branch of *P. pinaster* and one of either *P. halepensis, P. pinea, P. radiata,* or *P. sylvestris*.

After 25 days, four randomly selected pairs of unmated adults were placed in a 0.7 m^3 screened wooden box along with a bolt of *P. pinaster* in one corner and one bolt of another type (same as for maturation feeding) in the opposite corner. Dead insects were immediately replaced by another adult of the same sex and age. Five replicates of four insect pairs per four pairs of bolts were conducted (in total, 20 experiments per combination). After 72 h, bolts were debarked, and the numbers of oviposition slits with eggs were counted.

Mean values and standard errors of the mean (SEM) for each kind of bolts and the bark characteristics (diameter and thickness) were calculated. The same was done for the number of eggs laid per bolt (table 6).

Wilcoxon signed rank test was used to compare the two types of wood in each combination. No differences were found between: *P. pinaster* and *P. halepensis* or between *P. pinaster* and *P. sylvestris*. Beetles preferred *P. pinaster* over *P. pinea* or *P. radiata*. About 10 % of eggs laid (2 out of 21) were, however, laid on *P. pinea.*

Further details on the data from the Portuguese document on 'Risk assessment of *Pinus pinea* L. in relation to PWN' are presented in Appendix A.

Table 5: Bolt characteristics, mean number of eggs laid and emergence on seven conifer species (Naves et al., 2006, table 2).

Pine species	**Bolt diameter** (mm)		**Bark thickness** (mm)		**No of eggs laid**			**Emergence**			**Days until emergence**		**Females %**		**Elytral length** (mm)			
								total	rate per bolt (%)				No[1]		males		females	
	mean	SEM	mean	SEM	total[1]	per bolt			mean	SEM	mean	SEM		(%)	mean	SEM	mean	SEM
						mean	SEM											
P. halepensis	136.6	4.9	1.5	0.1	67	13.4	2.4	28	56.2	9.0	80.7	4.1	14	50	13.8	0.3	14.3	0.4
P. pinaster	86.8	6.4	2.8	0.7	52	10.4	0.8	14	54.6	5.6	76.7	3.2	5	36	14.5	0.2	15.6	0.4
P. pinea	108.4	9.3	2.1	0.2	3[2]	0.6	0.4	0	0		n/a		n/a	n/a	n/a		n/a	
P. radiata	93.3	9.4	1.7	0.3	28	5.6	3.1	18	43.6	20.0	72.1	3.5	10	56	13.8	0.3	14.1	0.4
P. sylvestris	105.6	6.5	1.4	0.0	109	21.8	2.1	44	52.8	9.9	72.1	2.3	17	39	13.0	0.3	14.6	0.4
P. menziesii	88.9	6.8	2.0	0.3	2[3]	0.4	0.4	0	0		n/a		n/a	n/a	n/a		n/a	
C. lusitanica	96.6	5.1	2.6	0.1	0	0		n/a	n/a		n/a		n/a	n/a	n/a		n/a	
Total[1]					**261**			**104**					**46**					

[1] Calculated by the Panel

Individual results: [2]=(0/0/0/1/2); [3]=(0/0/0/0/2)

Table 6: Bolt characteristics and mean number of eggs laid on pines in four paired experiments (Naves et al., 2006, table 3)

Pine species (pairs)	**Bolt diameter** (mm)		**Bark thickness** (mm)		**No of eggs laid**		
	mean	SEM	mean	SEM	total	mean	SEM
P. pinaster	74.0	7.8	1.9	0.1	25	5.0	1.6
P. halepensis	80.1	10.9	1.2	0.1	17	3.4	2.2
Total					**42**	**8.4**	
P. pinaster	85.9	10.5	2.2	0.4	19	3.8	1.2
P. pinea	88.8	9.9	1.7	0.3	2	0.4	0.2
Total					**21**	**4.2**	
P. pinaster	93.2	8.3	2.3	0.1	15	3.0	0.6
P. radiata	95.7	8.2	2.2	0.2	2	0.4	0.4
Total					**17**	**3.4**	
P. pinaster	66.3	8.3	1.7	0.4	44	8.8	2.7
P. sylvestris	68.8	7.5	1.0	0.1	67	13.4	1.1
Total					**111**	**22.2**	

2.1.2. Extensive literature searches on PWN, *Monochamus* spp., and on the four plant taxa

During the literature search, the principles of the extensive literature search (EFSA, 2011), corresponding to the first steps of a systematic review process (EFSA 2010), were followed.

The following information sources were consulted:

- ISI Web of knowledge (Biological Abstracts®, BIOSIS Previews®, Current Contents Connect℠, CABI: CAB Abstracts and Global Health®, Derwent Innovations Index℠, Food Science & Technology Abstracts™, Inspec®, MEDLINE®, Zoological Records®)
- open sources
- Asian data sources
- additional studies identified using the screening of references of the most relevant studies
- expert knowledge
- web based search utilities (e.g. Google…)

The literature search strategy applied on the ISI web of knowledge database was articulated in three parts:

- the host plants, *Juniperus* spp., *Cryptomeria* spp., *Chamaecyparis* spp. and *Pinus pinea*
- the harmful organism, PWN, *Bursaphelenchus xylophilus*
- the vector of the PWN, *Monochamus* spp.

The three individual search algorithms were combined:

- each one of the host plants AND the PWN
- each one of the host plants AND *Monochamus* spp.
- the pinewood nematode AND *Monochamus* spp.

The lists of references resulting from these combinations were screened for relevance by their titles and abstracts by Panel experts. The screening process was unmasked. The full texts of the selected references were considered to produce a set of relevant evidence.

The resulting combined list of 256 publications comprised peer-reviewed articles, PhD theses, technical reports from various organisations at international, regional, and national levels.

Further details on the literature search and the consulted database are presented in Appendix B.

2.1.3. Data maps on the European and world distribution of *P. pinea*, *Chamaecyparis* spp., *Cryptomeria* spp. and *Juniperus* spp.

Any information about the world presence of *P. pinea*, *Chamaecyparis* spp., *Cryptomeria* spp. and *Juniperus* spp. with overlapping ranges with the presence of PWN and/or of its vectors of the genus *Monochamus* could provide interesting evidence on the role of *P. pinea*, *Chamaecyparis* spp., *Cryptomeria* spp. and *Juniperus* spp. as hosts for PWN. However, in addition to the two possibilities, development of PWD or not, the situation in such areas might also be that PWN infests these plant species without expression of symptoms. Nevertheless, if there exist areas of the world where these species have co-occurred, over a longer time period than the areas of the EU territory infested with PWN, more robust evidence could potentially be collected from such areas.

Maps on European and world distribution of *P. pinea*, *Chamaecyparis* spp., *Cryptomeria* spp. and *Juniperus* spp. were generated by the Joint Research Centre of the European Commission, Institute for Environmental Sustainability, Forest Action Group, Ispra (IT) (thereafter JRC-IES).

The European maps have been generated using recently available forest data from European National Forest Inventories and harmonized within the European Forest Data Centre (EFDAC, 2005). Additional datasets which have been considered are the one from the BioSoil Project (Hiederer and Durrant, 2010; Lacarce et al., 2011) and the dataset of the Forest Focus Monitoring Database System (Hiederer et al., 2006; 2007). The maps also allow the comparison with the observations reported by the Global Biodiversity Information Facility (GBIF) datasets (Edwards, 2004; Yesson et al., 2007; Gilman et al., 2009).

The Iberian peninsula has been object of further analysis and the information available from the Spanish Forest Map (“Mapa Forestal de España”, MFE) and from Portuguese regional forest plans (“Planos Regionais de Ordenamento Florestal”, PROF) have been reviewed and the MFE data have been mapped with respect to information derived by aggregating several categories of the Corine Land Cover 2006.

The mapped global distribution is limited to data available from the Global Biodiversity Information Facility (GBIF, online). The CABI Forestry Compendium[6] was also consulted both for European and global distribution and for the main use of these species.

Further details on the data sources and the methodologies used for production of these maps are presented in Appendix C.

2.2. Methodology used in the assessment

The assessment has been conducted in line with the principles described in the documents “Guidance on a harmonised framework for pest risk assessment and the identification and evaluation of pest risk management options” (EFSA Panel on Plant Health (PLH), 2010) and “Guidance of the Panel on Plant Health on the evaluation of pest risk assessments and risk management options prepared by third parties to justify requests for phytosanitary measures under Council Directive 2000/29/EC (EFSA, 2009). When expert judgement and/or personal communication were used, justification and evidence are provided to support the statements.

In order to follow the principle of transparency as described under Paragraph 3.1 of the Guidance document on the harmonised framework for risk assessment (EFSA Panel on Plant Health (PLH), 2010) – ‘…*Transparency requires that the scoring system to be used is described in advance. This includes the number of ratings, the description of each rating…. the Panel recognises the need for further development…*’– the descriptors of qualitative ratings are provided when used.

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[6] http://www.cabi.org/fc/

2.2.1. Evaluation of the document submitted by Portugal on ‘Risk assessment of *Pinus pinea* L. in relation to PWN’

To perform the evaluation of the risk assessment of *P. pinea* submitted by Portugal, the Panel followed the EFSA guidance of the Panel on Plant Health on the evaluation of pest risk assessments and risk management options prepared by third parties to justify requests for phytosanitary measures under Council Directive 2000/29/EC (EFSA, 2009).

The evaluation was done using checklists, which are presented in Appendix A. Quantitative information was extracted in tables and completed if necessary and possible; to express the uncertainty of quantitative estimates, 95 % confidence levels were calculated, especially for infestation rates. The uncertainties were listed (see section 3.3.2). The complete assessment is shown in Appendix A.

2.2.2. Assessment of the probability of spread

The assessment of the probability of spread of PWN via the genera *Chamaecyparis*, *Cryptomeria* and *Juniperus* and *P. pinea* was made following the EFSA ‘Guidance on harmonised framework for pest risk assessment’ (EFSA Panel on Plant Health (PLH), 2010).

The geographic distribution and main uses of the genera *Chamaecyparis*, *Cryptomeria* and *Juniperus* and *P. pinea* as well as their host status with regard to PWN and its vectors were established using information from the available literature (retrieved as in 2.1.2) and expert advice. In a further step, the probability of spread of the PWN via the genera *Chamaecyparis*, *Cryptomeria* and *Juniperus* and *P. pinea* (by infested plants or untreated plant products) was assessed. Finally, the possibility of further spread by natural means, using these tree species as stepping stones, was also analysed.

2.2.3. Level of uncertainties

Uncertainty was estimated following the EFSA guidance on harmonised framework for pest risk assessment, in particular regarding (i) the field data and experimental results provided on the susceptibility of *P. pinea* as a host tree for *Monochamus* spp.; (ii) the nature, extent and precision of the available data relative to the geographical spread of the tree species *Chamaecyparis* spp., *Cryptomeria* spp. and *Juniperus* spp. and *P. pinea* in the EU; (iii) the available information regarding the movements of commercial products made from these species; and (iv) the published records of PWN and/or *Monochamus* spp. infesting these tree species.

The descriptors used for qualitative ratings given for the level of uncertainty are shown in Table 7 below.

Table 7: Descriptors used for qualitative ratings for uncertainty

Rating	**Descriptors**
Low	No or few information or data are missing, incomplete, inconsistent or conflicting. No subjective judgement is introduced. No unpublished data are used.
Medium	Some information or data are missing, incomplete, inconsistent or conflicting. Subjective judgement is introduced with supporting evidence. Unpublished data are sometimes used.
High	Most parts of information or data are missing, incomplete, inconsistent or conflicting. Subjective judgement may be introduced without supporting evidence. Unpublished data are frequently used.

3. Assessment results

3.1. The pine wood nematode and its insect vectors

3.1.1. The pine wood nematode *Bursaphelenchus xylophilus*

PWN is a serious threat to susceptible conifer trees worldwide. PWN is the causal agent of PWD, a severe hypersensitive response resulting in sudden wilting and death of pine trees. This occurs in susceptible tree species in warm climates and is caused by a nematode invasion of healthy trees through the feeding scars of nematode-carrying cerambycid beetles belonging to the genus *Monochamus*.

Threats from PWN must be placed in the context of the natural biology and occurrence of the nematode. In North America, where PWN is native, trees have co-evolved with the nematode and, in general, do not express wilt symptoms and survive introduction of PWN through maturation feeding. In this area, the nematode lives mainly in a non-pathogenic interaction with host trees. In this type of interaction the nematodes are transmitted to the wood during oviposition by the cerambycid vector. In living wood, the nematodes feed on live host cells, but they are also saprophyte, consuming fungi and other sources of food on dead conifers. On some hosts, nematodes introduced by beetle maturation feeding can develop only local pockets of infestation which have little or no impact on the health of the tree. This is also true of infestation of susceptible host tree species in cooler regions of the world.

However, when susceptible conifer species are grown under stressful environmental conditions (e.g. high temperatures and low soil moisture), the nematodes introduced by maturation feeding can survive and move through the tree, ultimately leading to xylem cavitation and PWD. This is the situation in several countries of the world where PWN has been introduced. Massive mortality of native pine trees has been recorded in Japan (Mamiya, 1988) and to a lesser, but still serious, extent in China and Taiwan (Zhao, 2008), Korea (Shin, 2008), and also Portugal (EPPO, 2009). In all such cases, the existing, native species of *Monochamus* in each country has taken the role of the vector.

Although the origin of the PWN is clearly North America, infestations in different parts of the world reveal variation in strains of the nematodes, that is detectable both in pathogenicity (Bolla et al., 1986; Kiyohara and Bolla, 1990; Mota et al., 2006) and in the genetic structure of the nematodes (Bolla et al., 1988). This suggests that either a subset of the total population variation in the native range has arrived in each country or that the nematode is developing new strain characteristics after arrival.

3.1.2. The insect vectors of PWN

The genus *Monochamus* Megerle (Coleoptera: Cerambycidae, subfamily Lamiinae), commonly known as sawyers or pine sawyer beetles, comprises more than 160 species, with a worldwide distribution and different trophic specialisations (Hellrigl, 1971; Goidanich, 1972; Cesari et al., 2005). All species indigenous to temperate regions attack species of Pinaceae, breeding, on trees that are stressed or recently killed. They are mainly *Pinus* feeders, but some may also utilise the genera *Picea, Abies, Larix, Pseudotsuga and Tsuga* (see table 2).

Although pine sawyer beetles are economically insignificant by themselves, some species transport phoretic nematodes belonging to the genus *Bursaphelenchus*, including PWN. PWN is transmitted as J4 dispersing juveniles (dauerlarvae) exclusively by adult beetles of the genus *Monochamus*. In addition to *Monochamus*, other genera of the Cerambycidae (e.g. *Acalolepta*, *Acanthocinus*, *Amniscus*, *Arhopalus*, *Asemum*, *Corymbia*, *Neacanthocinus*, *Rhagium*, *Spondylis*, *Uraecha*, *Xylotrechus*) and other Coleoptera (e.g. *Chrysobothris*, *Hylobius*, *Pissodes*) can carry PWN, but none has been shown to successfully transmit it between host trees (Linit, 1988; EPPO/CABI, 1997; Akbulut and Stamps, 2011). The adult beetles can spread the nematode either to the shoots of trees during maturation feeding (primary transmission), or to suitable oviposition sites in the branches or trunks of stressed, dying or recently dead trees by the females (secondary transmission) (Linit, 1988; EPPO/CABI, 1997).

Many of the *Monochamus* spp. from conifers have been recorded as having non-pathogenic *Bursaphelenchus* spp. as associates. It is, therefore presumed that most, if not all, species would also be capable of transmission of PWN to a greater or lesser extent (EPPO/CABI, 1997).

Transmission of PWN has so far been recorded in *M. alternatus*, *M. carolinensis*, *M. mutator*, *M. scutellatus*, *M. titillator*, *M. saltuarius*, *M. obtusus*, *M. nitens*, *M. marmorator* and *M. galloprovincialis* (Evans et al., 1996; Sousa et al., 2001). The species of *Monochamus* which are of concern as known or possible vectors of the PWN are listed in table 2 and are found only in the Northern Hemisphere, where they are very widely distributed. The genus *Monochamus* is represented elsewhere in the world, but the species concerned do not attack conifers (EPPO/CABI, 1997). Several African species of the genus are pests of broad-leaved trees and some (e.g. *M. scabiosus*) have a tendency to attack healthy trees (Browne, 1968).

Table 8: List of *Monochamus* species from coniferous trees, known to be vectors of PWN or considered to be potential vectors (*) (Sources: Bowers et al., 1992; Akbulut and Stamps, 2011; Leland Humble, Canada, personal communication October 2011; USDA APHIS 2011).

Species	Country (region)	Hosts
America		
M. carolinensis	United States (central and eastern seaboard; 26 states), Mexico, Canada (New Brunswick, Ontario, Quebec)	*Pinus*
M. clamator	Canada (British Columbia)	*Pinus contorta, Pseudotsuga menziesii*
M. scutellatus	United States (35 states), Mexico, Canada (widespread)	*Abies, Larix, Picea, Pinus, Pseudotsuga menziesii,Tsuga*
M. titillator	United States (31 states), Canada (Ontario)	*Abies, Picea, Pinus*
M. mutator	United States (Minnesota), Canada (six provinces)	*Pinus*
M. obtusus	United States, Canada (four states in western British Columbia)	*Abies, Pinus, Pseudotsuga menziesii*
M. notatus	United States, Canada (10 provinces)	*Pinus strobes, Picea glauca, Pinus monticola, Pseudotsuga menziesii*
M. marmorator	United States (19 states), Canada (five provinces)	*Abies, Picea*
Asia		
M. alternatus	China (20 provinces), Japan (widespread), Republic of Korea (Pusan area), Laos, Taiwan, Vietnam	*Abies, Cedrus, Larix, Picea, Pinus*
M. nitens	Japan	*Pinus*
Europe/Asia		
M. saltuarius	China (four provinces), Japan, Europe	*Abies,Larix, Picea, Pinus, Sciadopitys, Tsuga,*
M. rosenmuelleri (=*M. urussovi*) (*)	China (three provinces), Korea, Japan, Europe	*Abies, Betula, Larix, Picea, Pinus*
M. sutor (*)	China (five provinces), Siberia, Mongolia, Korea, Japan, Europe	*Larix, Picea, Pinus,*
Europe/North Africa		
M. galloprovincialis	Europe, Africa (Algeria, Morocco, Tunisia)	*Pinus, Picea*
M. sartor (*)	Europe	*Abies, Picea, Pinus,*

3.1.2.1. Vectors in the native range of PWN

M. carolinensis and *M. scutellatus* are the major vectors of PWN in North America, but many other apparently less efficient vectors have been also recorded there.

The Carolina pine sawyer beetle *M. carolinensis* is native to North America and is one of the most effective vectors of the PWN in the USA. This species colonises and reproduces only in pine species (Akbulut and Stamps, 2011).

The white-spotted sawyer *M. scutellatus* is the second most important vector of the PWN in the USA (Holdeman, 1980; Wingfield and Blanchette, 1983; Akbulut and Stamps, 2011). *M. titillator* is also a known vector in the USA (Luzzi et al., 1984), while *M. marmorator, M. mutator* and *M. obtusus* have also been listed for USA and Canada (EPPO/CABI, 1997).

3.1.2.2. Vectors in Asia

Japanese pine sawyer *M. alternatus*, an Asian species, is the most important vector of the PWN in Asia, where the nematode was introduced first to Japan and then into China and neighbouring countries (see section 3.1.1). The beetle has been recorded on more than 17 species of *Pinus*, including Japanese red pine (*Pinus densiflora*), Japanese black pine (*P. thunbergii*), luchu pine (*P. luchuensis*) (Japan) and Masson pine (*P. massoniana*) (China), three species of spruce (*Picea* spp.) and one species each of fir (*Abies* sp.), true cedar (*Cedrus* sp.) and larch (*Larix* sp.) (Kobayashi et al., 1984; Juan et al., 2008). This beetle is indigenous to China, Taiwan, Korea, Laos and Japan. It is widely distributed in Japan, except in Hokkaido and northernmost Honshu. Its occurrence in both the Ryuku and Ogasawara Islands of Japan is believed to be the result of recent introductions with pine logs (Kobayashi et al., 1984). *M. alternatus* is known to oviposit readily on *P. massoniana*, *Cedrus deodara* and *P. elliottii*, less so on *Podocarpus macrophyllus*, *Juniperus (Sabina) chinensis* and *Cryptomeria fortunei,* and rarely on *Tamarix chinensis* or *Metasequoia glyptostroboides* (Yang et al., 2010; Jianghua Sun, China, personal communication, September 2011).

The Sakhalin pine sawyer, *M. saltuarius*, is a widespread Asian–European species distributed from Austria to Japan, but much more numerous in Siberia (Danilevskaya et al., 2009. It has been reported as vector of the PWN in Japan and Korea (Kobayashi et al., 1984; Sato et al., 1987; Togashi et al., 1994; Kim et al., 2006; Kang et al., 2009) as well as a vector of *B. mucronatus*, a non-virulent species closely related to the PWN (Jikumaru and Togashi, 1995, 2001; Togashi and Jikumaru, 1996; Akbulut and Stamps, 2011). *M. saltuarius* is established in China and Russia (Asian part) – on the western Siberian plain and in the southern Siberian mountains and the Amur and Primorye regions (Danilevsky, 2007; USDA APHIS, 2011) – and is also widely distributed in hills and submontane forests (Brelih et al., 2006) in Europe, including Austria, Belarus, Bosnia-Herzegovina, Croatia, Czech Republic, Germany, Hungary, Italy, Latvia, Poland, Romania, Russia (south and middle of European Russia), Slovakia, Slovenia, and the Ukraine (Danilevsky, 2007; Fauna Europaea, 2011; Telnov, 2004). *M. saltuarius* infests species of the genera *Abies*, *Larix*, *Picea* and *Pinus* that are either dying or were recently felled (USDA APHIS, 2011). *P. densiflora* and *P. thunbergii* are main hosts in Japan (USDA APHIS, 2011), whereas *P. abies* is the main host in Europe (Sama, 2002).

The following hosts have been reported for *M. saltuarius* (USDA APHIS, 2011):[7],

- fir species: silver fir (*Abies alba*), Manchurian fir (*Abies holophylla)*, Khingan fir (*Abies nephrolepis*), Siberian fir (*Abies sibirica*);
- larch species, Dahurian larch (*Larix gmelinii*), Japanese larch (*Larix kaempferi*), Siberian larch (*Larix sibirica*);

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[7] USDA APHIS (2011) reports also Japanese cedar (*C. japonica*) among the hosts of *M. saltuarius*, citing as reference 'Anonymous. n.d. 2001. Illustrations of tree diseases, tree insect pests in Hokkaido: Monochamus saltuarius. Hokkaido Research Center, Forestry and Forest Products Research Institute, Incorporated Administrative Agency, Entomology Laboratory. http://www.ffpri-hkd.affrc.go.jp/group/konchu/Zukan/HTML/Coleo_Kamikiri-e.htm Accessed on March 1, 2010'. However, in the English version of this website, last accessed on December 2011, by selecting "Illustrations of tree diseases, tree insect pests in Hokkaido", then "Cerambycidae – under construction" and then *Monochamus, C. japonica* is presently listed as host of *M. alternatus* but not of *M. saltuarius.* Therefore this citation is considered as doubtful.

- spruce species: Norway spruce (*Picea abies*), dragon spruce (*Picea asperata*), Yeddo spruce (*Picea jezoensis*), Korean spruce (*Picea koraiensis*), Siberian spruce (*Picea obovata*);

- pine species: jack pine (*P. banksiana*), Japanese umbrella pine (*Sciadopitys verticillata*), Corsican pine (*P. nigra* subsp. *laricio*), Japanese white pine (*P. parviflora*), Siberian pine (*P. sibirica*), Scots pine (*P. sylvestris)*, Japanese black pine (*Pinus thunbergii*), Japanese hemlock (*Tsuga sieboldii*).

3.1.2.3. Known vectors in Europe

The pine sawyer *M. galloprovincialis*, a Central Asian-European species, is one of the five European *Monochamus* species breeding in declining, recently dead or recently felled coniferous trees and is usually considered to be a secondary pest in forests. The species is widely distributed throughout Europe (except in Cyprus, Ireland and in the United Kingdom), Russia and Siberia, North Africa, Asia Minor, Mongolia, Korea, and China (Hellrigl, 1971; Francardi and Pennacchio, 1996; Naves et al., 2006). It is a partly colline, rarely submontane and montane silvicole species. The larvae develop in pine, especially *P. sylvestris* and *P. nigra*, occasionally also in spruce (Brelih et al., 2006). Females lay eggs in *P. sylvestris*, *P. halepensis*, *P. pinaster*, *P. radiata*, *P. pinea* and *Pseudotsuga menziesii* (Mirbel) Franco, not all of which are European conifer species. However, in experimental testing larvae successfully completed development only on the first four pines (Naves et al., 2006). *M. galloprovincialis* was identified as the vector of the PWN in Portugal in 1999 (Sousa et al., 2001).

3.1.2.4. Other potential PWN vectors in Europe

The small white-marmorated longicorn *M. sutor*, an Asian-European species, is distributed in Europe from Scandinavia, where it reaches the Polar Circle, the Alps and the Pyrenees to the Urals, Bulgaria and Albania. It is also distributed in Asia from the Urals beyond Siberia to northern Mongolia, northern China, Korea and Japan. It is a partly submontane and montane, rarely colline and high montane silvicole species. In Central Europe, the species mainly attacks spruce and occasionally fir or larch. In Scandinavia, other pine species (*Pinus sylvestris*, *P. mugo, P. nigra*) are also attacked (Cesari et al., 2005; Brelih et al., 2006).

The black fir sawyer *M. rosenmuelleri* (= *M. urussovi* Fischer) is an Asian-European species, living on fir, larch, pines, spruce and occasionally birch (Kolk and Starzyk, 1996). The species has been documented in Europe in the Scandinavian and Baltic regions, particularly in Norway, Sweden and Finland (Bense, 1995) but also in Belarus, Czech Republic, Estonia, Latvia, Lithuania, Poland, central and north Russia and Ukraine (Danilevsky, 2007; Fauna Europaea, 2011), Siberia, Mongolia, north-eastern China, Korea and Japan (Kolk and Starzyk, 1996; Akbulut and Stamps, 2011). The black fir sawyer is a major vector of *B. mucronatus* and potentially could act as a vector for PWN in Eastern Europe (Togashi et al., 2008; Akbulut and Stamps, 2011).

The European longhorn beetle *M. sartor* is widespread across Central and Eastern Europe reaching some countries in Western Europe. It is recorded in Albania, Austria, Bosnia and Herzegovina, Bulgaria, Croatia, Czech Republic, France, Germany, Hungary, Italy, Lithuania, Poland, Romania, Serbia and Montenegro, Slovakia, Slovenia, Switzerland and the Ukraine (FERA, 2009). There are uncorroborated reports of the presence of the species in Belarus, Norway and Sweden. A report from Latvia is not confirmed: the species does not appear in the checklist of Latvian species (Telnov, 2004 cited in FERA, 2009). Its host plants are coniferous trees, especially Norway spruce, very rarely fir and various species of pine (Cesari et al., 2005; Brelih et al., 2006).

M. sartor is a potential vector for the PWN, although at present the distributions of beetle and nematode are widely separated and the two organisms are unlikely to come into contact without significant further spread of the PWN (FERA, 2009).

M. saltuarius is known to be a vector of the PWN in Asia. However, it has not yet come into contact with PWN in Europe and it has never been reported to transmit PWN in Europe.

Table 9: Distribution of *Monochamus* species in Europe

Country/region	**Species**							
	M. galloprovincialis **(Olivier 1795)**	***M. galloprovincialis galloprovincialis*** **(Olivier 1795)**	***M. galloprovincialis*** **pistor (Germar 1818)**	***M. impluviatus*** **(Motschulsky 1859)**	***M. rosenmuelleri*** **(Cederjelm 1798)**	***M. saltuarius*** **(Gebler 1830)**	***M. sartor*** **(Fabricius 1787)**	***M. sutor*** **(Linnaeus 1758)**
Albania	+++	-	+++	-	-	-	++*	+++
Andorra	-	-	-	-	-	-	-	+++
Austria	+++	-	+++	-	-	+++	+++	+++
Belarus	+++	-	+++	-	+++	+++	+++	+++
Belgium	+*	-	-	-	-	-	+*	+++
Bosnia and Herzegovina	+++	-	+++	-	-	+++	+++	+++
Bulgaria	+++	-	+++	-	-	-	+++	+++
Croatia	+++	-	+++	-	-	++*	+++	-
Cyprus	-	-	-	-	-	-	-	-
Czech Republic	+++	-	+++	-	+++	+++	+++	+++
Denmark incl. Borholm Island	-	-	-	-	-	-	-	+++
Denmark: Faroe Islands	-	-	-	-	-	-	-	-
Estonia	+++	-	+++	-	+++	-	-	+++
European Turkey incl. Imroz I. - Gokceada, but not those in the Sea of Marmara	-	-	-	-	-	-	-	-
Finland	+++	-	+++	-	+++	-	-	+++
French mainland	+++	+++	-	-	-	-	+++	+++
Corsica	+++	+++	++*	-	-	-	-	-
Germany	+++	-	+++	-	-	+++	+++	+++
Gibraltar	-	-	-	-	-	-	-	-
Greek mainland incl. Andikithira I., Evvia I., Ionian Is., Samothraki I., Northern Sporades Is., Thasos I.	+++	-	+++	-	-	-	-	-
Greece: Crete, incl. small adjacent islands like Gavdhos. Note that Andikithira I. although being closer to Kriti than to mainland, belongs to a mainland province	+++	+++	-	-	-	-	-	-
Greece: Cyclades Islands, incl. Amorgos, Anafi, Anidros, Andros, Andiparos, Denousa, Folegandros, Ios, Iraklia, Karos, Kimolos, Kea, Kythnos, Milos, Mykonos, Naxos, Paros, Poliaigos, Serifos, Sifnos, Sikinos, Syros, Thira, Tinos, Yiaros and other smaller islands	-	-	-	-	-	-	-	-
Greece: Dodecanese Islands, incl. Alimnia, Arkoi, Astipalaia, Avgonisi, Ankathonisi, Farmakonisi, Ioinianisia, Kalimnos, Kalolimnos, Kandeliousa, Karpathos, Kasos, Khalki, Khamili, Kinaros, Kos, Leros, Levitha, Lipsoi, Meyisti, Nisiros, Ofidousa, Patmos, Rodhos, Saria, Simi, Sirina, Tilos, Tria Nisia, Yiali and other smaller islands	-	-	-	-	-	-	-	-
Greece: North Aegean Islands, incl. Andipsara, Ayios Evstratios, Fournoi, Ikaria, Khios, Lesvos, Limnos, Oinousa, Psara, Samos, Skopelos Kaloyeroi and other smaller islands	-	-	-	-	-	-	-	-
Hungary	+++	-	+++	-	-	+++	+++	+++

Iceland	-	-	-	-	-	-	-	-
Ireland not incl. Northern Ireland (GB-NI)	-	-	-	-	-	-	-	-
Italian mainland	+++	+++	+++	-	-	++*	+++	+++
Italy: Sardinia	-	-	-	-	-	-	-	-
Italy: Sicily incl. adjacent Italian islands (Lipari Is., Ustica I., Egadi Is., Pantelleria I., Pelagie Is.)	++*	++*	-	-	-	-	-	-
Latvia	+++	-	+++	-	+++	+++*	-	+++
Liechtenstein	-	-	-	-	-	-	+++	+++
Lithuania	+++	-	+++	-	+++	-	+?	+++
Luxembourg	-	-	-	-	-	-	-	-
Macedonia	+++	-	+++	-	-	-	-	-
Malta	-	-	-	-	-	-	-	-
Moldova, Republic of	-	-	-	-	-	-	-	-
Monaco	-	-	-	-	-	-	-	-
Northern Ireland	-	-	-	-	-	-	-	-
Norwegian mainland	+++	-	+++	-	+++	-	-	+++
Norway: Svalbard and Jan Mayen incl. Bear I.	-	-	-	-	-	-	-	-
Poland	+++	-	+++	-	+++	++*	+++	+++
Portugal: mainland	+++	+++	-	-	-	-	-	-
Portugal: Azores islands	-	-	-	-	-	-	-	-
Portugal: Madeira	+++	-	-	-	-	-	-	-
Portugal: Selvagens Islands	-	-	-	-	-	-	-	-
Romania	+++	-	+++	-	-	+++	+++	+++
Russia central	+++	-	+++	+++	+++	+++	-	+++
Russia east	+++	-	+++	-	-	+++	-	+++
Russia north	+++	-	+++	+++	+++	-	-	+++
Russia northwest	+++	-	+++	-	-	-	-	+++
Russia south	+++	-	+++	-	-	-	-	+++
Russsia: Kaliningrad Region	-	-	-	-	-	-	-	-
Russia: Novaya Zemlya	-	-	-	-	-	-	-	-
Russia: Franz Josef Land not incl. Ushakova I. and Vize I.	-	-	-	-	-	-	-	-
San Marino	-	-	-	-	-	-	-	-
Slovakia	+++	-	+++	-	-	+++	+++	+++
Slovenia	+++	-	+++	-	-	+++	+++	+++
Spain: mainland incl. Alboran Island.	+++	+++	-	-	-	-	-	+++
Spain: Balearic Islands, incl. Mallorca, Menorca and Pityuses Islands (Ibiza and Formentera)	+++	+++	-	-	-	-	-	-
Spain: Canary Islands.	-	-	-	-	-	-	-	-
Sweden incl. Gotland I.	+++	-	+++	-	+++	-	-	+++
Switzerland	+++	-	+++	-	-	-	+++	+++
The Netherlands	+++	-	+++	-	-	-	-	+++
United Kingdom incl. Shetlands, Orkneys, Hebrides and Man Is.	-	-	-	-	-	-	-	-
UK: Channel Islands.incl. Jersey, Guernsey, Alderney	-	-	-	-	-	-	-	-
Ukraine	+++	-	+++	-	+++	+++	+++	+++
Vatican City	-	-	-	-	-	-	-	-
Former Yugoslavia,incl. Serbia, Kosovo, Voivodina, Montenegro	+++	-	+++	-	-	-	++*	+++

Key: +++ present (listed in Fauna Europaea (2011), +++* present (listed for Latvia in Danilevsky (2007) and Telnov (2004) but not in Fauna Europaea (2011)), ++* present (listed only in Danilevsky (2007)), +* present (Institut Royal des Sciences Naturelles de Belgique (IRScNB) collection, Belgium, as per Alain. Drumont, Belgium, personal communication, October 2011), + present (Hugh Evans, United Kingdom, personal communication, October 2011), - absent, +? doubtful.

3.1.2.5. Conclusions on PWN vectors

Monochamus species living on coniferous trees are the only known vectors of PWN. Currently, transmission of this nematode has been observed in *M. alternatus*, *M. carolinensis*, *M. mutator*, *M. scutellatus*, *M. titillator*, *M. saltuarius*, *M. obtusus*, *M. nitens*, *M. marmorator* and *M. galloprovincialis*.

The most important and therefore the most studied species among vectors of the PWN in Asia is *M. alternatus*, in North America, *M. carolinensis,* while *M. galloprovincialis* is the only known vector in the current range of PWN in Europe.

The other four European *Monochamus* species (*M. rosenmuelleri=urussovi*, *M. saltuarius*, *M. sartor*, *M. sutor*) that are more widely distributed in Europe than the current range of PWN, could be vectors of the PWN or the closely related *B. mucronatus*. *M. saltuarius* has already been documented as a PWN vector in Asia, while *M. sutor* transmits *B. mucronatus* (Schroeder and Magnusson, 1992). Currently, the distributions of these additional *Monochamus* spp. and PWN in Europe are widely separated, and for possible contact the PWN will have to spread considerably beyond its current distribution.

3.2. The plant genera *Chamaecyparis, Cryptomeria* and *Juniperus* and *P. pinea*: geographical distributions and main uses

Information on the European and world distribution of the genera *Chamaecyparis*, *Cryptomeria* and *Juniperus* and the species *Pinus pinea*, and on their main uses, is relevant to the scientific opinion because, provided they can act as host plants by PWN, the distribution of these species would influence the potential for spread of PWN in the EU. Moreover, areas of the world where the distribution of PWN overlaps with that of the plant genera *Chamaecyparis, Cryptomeria* and *Juniperus* and the species *P. pinea*, as well as of suitable vectors, are of special interest, because information from such areas can provide answers to the host–nematode relationship questions addressed by this scientific opinion.

It is particularly important to note that PWN is widely distributed in its native area, and, that the presence of the nematode in host plants in its native area generally does not result in any symptoms being expressed. Asymptomatic host plants of PWN are thus quite common. However, the absence of PWD in PWN infested plant species does not imply that the plant species is safe when assessed as a potential pathway for spread of PWN.

3.2.1. *Pinus pinea*

3.2.1.1. Distribution

According to CABI (2011a), *P. pinea* is a typical Mediterranean species; distributed from Portugal to Syria and along some coastal areas of the Black Sea. Owing to intensive cultivation and diffusion since well before the Roman age, it is now practically impossible to distinguish the indigenous areas from those where it was planted (CABI, 2011a). The European distribution map (figure 1) shows that the main distribution of *P. pinea* in the EU is in Spain and Portugal, but the species also occurs in Italy and the southern and central parts of France. Notably, the GBIF data indicate the presence of *P. pinea* also in the southern United Kingdom and Ireland, Germany and Greece. The differences in *P. pinea* distribution reflected in the forest inventory (NFI) data compared with the GBIF data could be explained by the fact that the NFI data are constrained to forested areas, whereas the GBIF data have no such constraints in types of areas covered. This means that presence of *P. pinea* in arboreta, parks or gardens, where *P. pinea* has sometimes been planted for its ornamental qualities, will be reported in the GBIF data, but not in the NFI data.

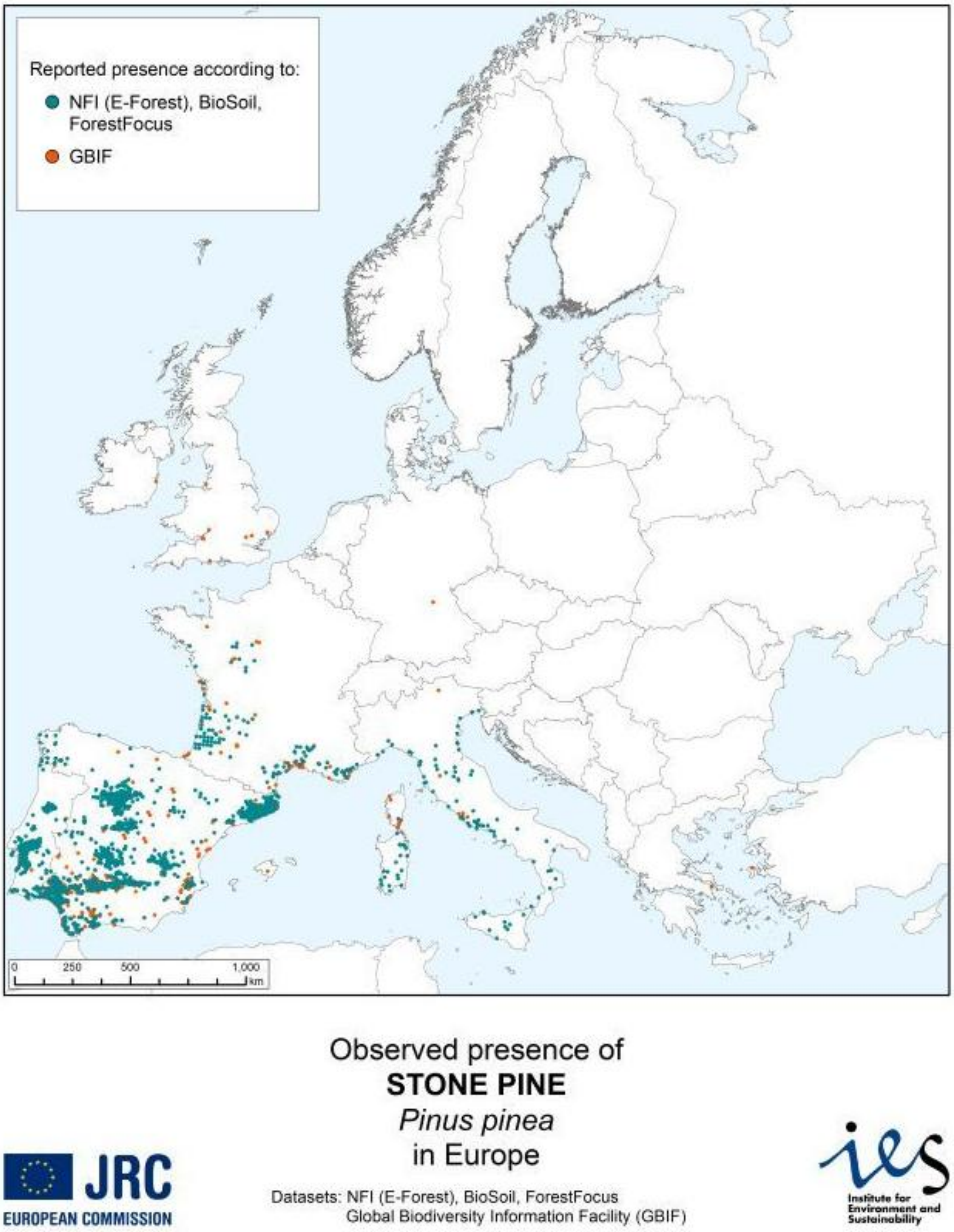

Figure 2: European distribution of *P. pinea* (combined map NFI+GBIF, see Appendix C).

In the Appendix C (*P. pinea* Spain map MFE), a map shows the occurrence of *P. pinea* in various landscape types of Spain. This map further illustrates the differences between the NFI-data and the GBIF-data, and the point that *P. pinea* plants frequently are present outside forest-type areas.

In other parts of the world, the GBIF data report occurrences of *P. pinea* in the United States (California), Australia, Turkey and Israel. According to CABI (2011a), the species has been planted in several countries in Africa (Algeria, Libya, Morocco, South Africa and Tunisia), Asia (Israel, Lebanon, Syria and Turkey) and South America (Argentina).

3.2.1.2. Main uses

Historically, *P. pinea* has been cultivated extensively for the edible seeds, pine nuts, since the Palaeolithic period (Gil, 1999; Badal, 2001) and recognised as one of the most important pine-nut producing species, along with Siberian pine (*Pinus sibirica*), Korean pine (*Pinus koraiensis*), Chilgoza pine (*Pinus gerardiana*), singleleaf pinyon (*Pinus monophylla*), Colorado pinyon (*Pinus edulis*) and other pinyon pine species (Sharashkin and Gold, 2004).

According to Mutke and Gordo (2005), *P. pinea* has never been domesticated (‘despite its interest as a nut tree’: around 70 % of the world production is from Spain and Portugal) and remains a genuine forest tree. The pine nut yield from cones is gathered from forest stands. Fady et al. (2004) indicate that Spain, Portugal, Italy, Tunisia and Turkey are the main countries where pine nuts are traditionally marketed. The Food and Agriculture Organization of the United Nations (FAO, 1995) acknowledges the importance of *P. pinea* in international trade. A moisturising oil is also produced from the edible seeds for cosmetic and skin cares uses (Athar and Nasir, 2005).

P. pinea is also cultivated widely as an ornamental and amenity plant (CABI 2011a; Kew Royal Botanic Garden, online).

P. pinea timber is considered of mediocre quality, very resinous and heavy, and of short durability. It is used as structural timber, sawn timber for light constructions, containers, wood wool, fibreboards, particleboards, mechanical pulp for cellulose and paper. Examples of its uses include pallets, carpentry/joinery, piles, crates and boats (CABI, 2011a).

P. pinea bark can be used as a component of plant growth media (Guerrero et al., 2002; Marfà et al., 2002; Zapata et al., 2005) and scales or pieces of *P. pinea* cones are commercialised for mulches for nurseries[8].

Directive 1999/105/CE of the European Union Council[9] includes *P. pinea* in the list of forest tree species for the commerce of forest reproductive material.Wooded dunes with *P. pineaster* and *P. pinea* have been considered a priority habitat for conservation in Europe (‘Habitat’ Directive n° 92/43/CEE of 21 May 1992[10]) and from an environmental protection perspective, *P. pinea* is relevant for consolidating coastal dunes and also for soil conservation and protection of coastal agricultural crops (Fady et al., 2004).

3.2.2. *Chamaecyparis* spp.

3.2.2.1. Distribution

The presence of *Chamaecyparis* spp. in Europe is reported by NFI and GBIF from 13 European countries (Austria, Belgium, France, Germany, Ireland, Italy, Liechtenstein, Poland, the Netherlands, Norway, Spain, Sweden and the United Kingdom). *Chamaecyparis* spp. can therefore be considered to have a relatively wide presence in the EU and it is reported as very frequent in the UK and with clustered occurrences in Ireland, the Netherlands, Poland and the Basque country of Spain. Maps of the European and world distribution for *Chamaecyparis* spp. are provided in Appendix C.

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[8] E.g. an example from Italy can be found at http://www.clamerinforma.it/servizi/info_aziende/Vigorplant/Vigorplant_Garden_2010/Vigorplant_Linea%20Garden%202010_Terricci_Decorazione%20e%20Pacciamatura.pdf

[9] Council Directive 1999/105/EC of 22 December 1999 on the marketing of forest reproductive material. OJ L 11, 15.1.2000, p. 17-40.

[10] Council Directive 92/43/EEC of 21 May 1992 on the conservation of natural habitats and of wild fauna and flora. OJ L 206, 22.7.1992, p. 1-66.

3.2.2.2. Main uses

Atlantic white cedar (*Chamaecyparis thyoides*) is used for the production of lumber. It is also commonly used for fencing, either as small dimension round timber or as lumber. The wood is used for exterior cladding and interior paneling, shingles, production of lawn or patio furniture, boat building, hand carving of ornamental objects, telephone and power poles and spars (Ward, 1989). A chemical substance (Yoshixol) with antibiotic properties is extracted from wood oil of *Chamaecyparis obtusa* (Koyama et al., 1997). Oil from *C. lawsonia* has properties of interest for aromatherapy[11].The wood of Port Orford cedar (*Chamaecyparis lawsoniana*) is among the most valuable commercially harvested conifer timbers (Hansen et al., 2000) and is also used as a substitute for Japanese hinoki (*Chamaecyparis obtusa*) in traditional construction and reconstruction of temples and shrines in Japan. Port Orford cedar oil is used for products for horse, dog and laboratory animal bedding, as well as for fragrance products and topical applications for human use (Craig et al., 2004). Ornamental *Chamaecyparis* trade is significant (Brasier et al., 2010): *C. lawsoniana*, *C. obtusa* and *C. pisifera* and *C. thyoides* (Hansen et al., 2000; Torchik, 2010; Webber et al., 2011) are the main species of *Chamaecyparis* used for ornamental purposes. In the natural context *C. thyoides* is highly palatable to white-tailed deer and provides cover for a variety of birds and mammals.

3.2.3. *Cryptomeria* spp.

3.2.3.1. Distribution

The *Cryptomeria* genus belongs to the cypress family *Cupressaceae* (formerly belonging to the family *Taxodiaceae*). It is a monotypic genus including only one species, *Cryptomeria japonica*, an evergreen tree (up to 70 m tall), endemic to Japan, where it is known as Sugi. In China, however, *M. alternatus* is noted in laboratory experiment to feed and rest on *Cryptomeria fortunei* (Yang et al., 2010), also described as *C. japonica* var. *sinensis* (USDA ARS GRIN, online). A map of the world distribution of *C. japonica* from the GBIF database is shown in Appendix C. For Europe, the NFI data reports *C. japonica* to be present at five locations in the United Kingdom and at three locations in Denmark. The GBIF data reports the presence of *C. japonica* from 10 European countries (number of locations given in brackets): United Kingdom (130), Spain (14), Ireland (10), France (5), Poland (6), Luxembourgh (4), Austria (2), Sweden (2), Croatia (1) and the Czech Republic (1). These data indicate that *Cryptomeria* spp. are not widely distributed in the EU and will consequently not be an important pathway for spread of PWN in Europe, even if they were a suitable host for PWN.

3.2.3.2. Main uses

Cryptomeria spp. are extensively used in forestry plantations in Japan, China and the Azores islands (Dickens and Neves, 2005), but apparently not in most of the EU. *C. japonica* is used as an evergreen screen or specimen plant for landscapes (Contreras et al., 2010) and as a building material, furniture and as an ornamental tree (Moiteiro et al., 2008) in temperate areas, including the United Kingdom, mainland Europe and North America. There are numerous dwarf cultivars that are widely used in rock gardens and for bonsai, including 'tansu', 'koshyi', 'little diamond', 'yokohama' and 'kilmacurragh’.

C. japonica essential oil has medical and cosmetic uses. It is recommended for skin health because it inhibits the growth of drug-resistant skin pathogens (Yoon et al, 2009). Essential oil extracted from leaves is especially active for other antimicrobial activity in inhibiting the fungus *Botrytis cinerea* and for human pathogenic bacteria (Moiteiro et al., 2008).

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[11] E.g. http://chestofbooks.com/health/aromatherapy/The-Volatile-Oils-Vol2/106-Oil-Of-Chamaecyparis-Lawsoniana.html

3.2.4. *Juniperus* spp.

3.2.4.1. Distribution

The *Juniperus*[12] genus belongs to the *Cupressaceae*. More than 50 species of juniper are classified (with a certain variance depending on taxonomic viewpoint). *Juniperus* spp. are evergreen plants whose size range from shrubs with long trailing branches up to trees. As a consequence, some countries may include information on *Juniperus* tree species distribution in their national forest inventories, whereas in other countries the whole *Juniperus* genus can be excluded by forest inventory surveys (see Appendix C). The genus shows a wide distribution in the northern hemisphere (figure 3). The *Juniperus* spp. considered in this section include the seven species for which datasheets are available in the CABI Forestry Compendium: *J. chinensis*, *J. excelsa*, *J. foetidissima*, *J. oxycedrus*, *J. procera*, *J. scopulorum* and *J. viriginiana*.

Juniperus chinensis occurs naturally in western and northern China, including Shaanxi, Gansu, Nei Menggu, Hebei Province, and also locally in alpine areas at altitudes of 1 000-2 000 m. It has been widely planted as an ornamental in southern Nei menggu, Hebei, Shanxi, Shandong, Henan and Shaanxi Province, and around the world in temperate climates (CABI, 2011b).

Juniperus excelsa grows in the mountains of the east Mediterranean region of Europe and South-West Asia, extending to Central Asia. In Europe, mostly in Macedonia, nothern Greece and southern Bulgaria, *J. excelsa* forms forests at elevations of 300-1 000 m. It is also a component of oak forests or steppe-forest communities (CABI, 2011c).

Juniperus foetidissima grows mostly in the mountains of the eastern Mediterranean region and in South-West Asia. *J. foetidissima* grows as sparse forests or groves on barren lands, traditionally used as pastures for sheep and goats (CABI, 2011d).

Juniperus oxycedrus is widespread on rocky or degraded soils in both lowlands and high mountain zones in the south of the Mediterranean region including the Atlas Mountains of Morocco (where it is found at altitudes over 2 500 m), associated with *Cedrus atlantica*. At higher altitudes in the Mediterranean area, it is replaced by common juniper (*J. communis*) (CABI, 2011e).

Juniperus procera is one of only a few indigenous African conifers. It is the largest juniper species in the world (growing up to 50 m in height) and produces a very valuable timber, being strong, easy to work, extremely durable and resistant to fungi or insect attack as a result of the presence of oleo-resins. It is known commercially as the African pencil cedar because its wood closely resembles that of *Juniperus virginiana*, the Eastern red cedar grown in North America for the pencil-making industry. From the late 1800s onwards, considerable quantities have been exported from East Africa (especially from Kenya) to Europe (CABI, 2011f).

Juniperus scopulorum is a western North America species distributed more in the Cordilleran region than the Pacific region. Within its range the distribution is highly scattered. However, the concentrations, from central British Columbia and southern Alberta, through northwestern Montana and southeastern Idaho into Colorado, and northern New Mexico, generally follow the Rocky Mountains. Owing to its scattered distribution over a broad range, *J. scopulorum* grows in mixture with many other tree species (CABI, 2011g).

Juniperus virginiana is possibly the most widely distributed tree in the world. This circumboreal species occurs across North America, Europe, Northern Asia and Japan. It is the most widely distributed timber-producing conifer in the eastern USA, and is found in every state east of the 100th

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[12] *Sabina* is considered a synonym of *Juniperus* (CABI 2011b; Farjon A. (2011). Conifer Database (version February 2011), *in*: Species 2000 & ITIS Catalogue of Life, 26 July 2011 (Bisby F.A., Roskov Y.R., Orrell T.M., Nicolson D., Paglinawan L.E., Bailly N., Kirk P.M., Bourgoin T., Baillargeon G., Ouvrard D., eds). Digital resource at http://www.catalogueoflife.org/col. Species 2000: Reading, UK.

meridian. The species extends northward into southern Ontario and the southern tip of Quebec in Canada. The range of eastern red cedar has been considerably extended, especially in the Great Plains region, by natural regeneration from planted trees. *J. virginiana* has not been widely planted outside its native range, except for arboreta, ornamental cultivars, or in trial plantations, particularly in eastern and central Europe. It has been much planted in central and northern China (CABI, 2011h).

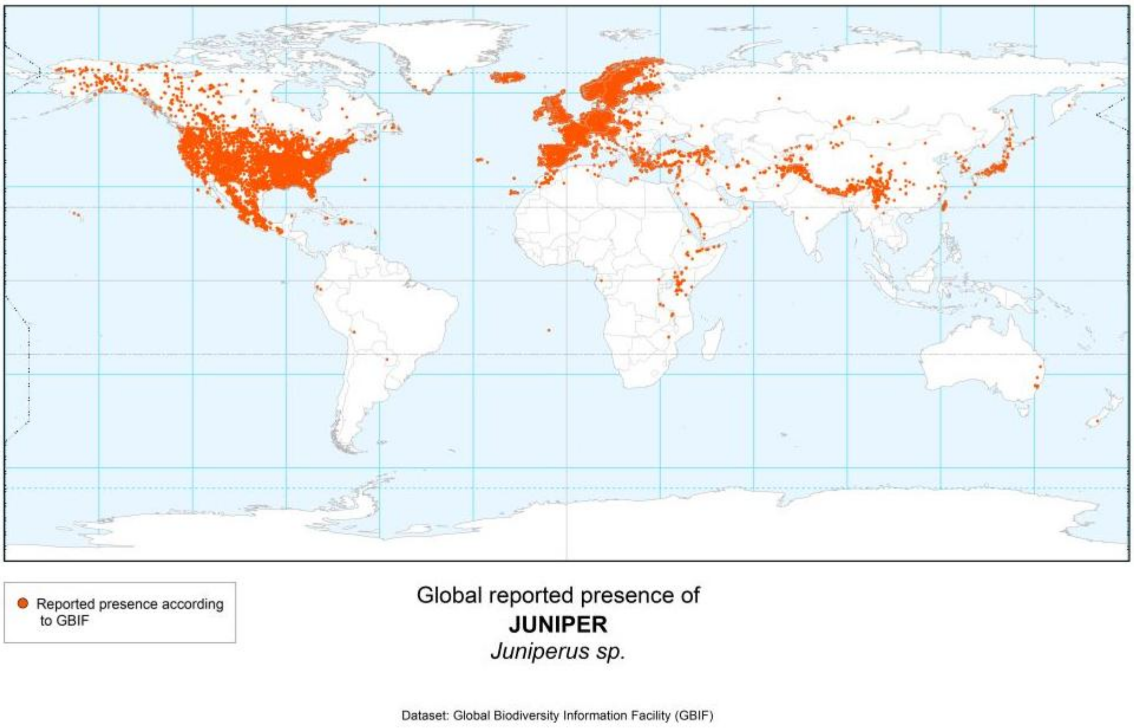

Figure 3: World distribution of *Juniperus* spp.

3.2.4.2. Main uses

Wood from *Juniperus communis* has no important commercial value, although it was used in the past for handicrafts because of its very strong and fragrant properties. Examples of trade of *Juniperus* wood products still exist[13]. It is highly valued as an ornamental. The foliage of *J. communis* may be poisonous to domestic goats, although livestock in parts of Europe have reportedly been fed sprays of common juniper with no ill effects.

Juniperus berries are used to produce oil for cosmetics such as skin care products (Athar and Nasir, 2005). It has historically been used by Native Americans to purify the air because of its anti-septic properties. *Juniperus* berry oil is also used as an astringent for the skin and hair and in massage blends. Western juniper (*Juniperus occidentalis*) is used for products for horse, dog and laboratory animal bedding, as well as for fragrance products and topical applications for humans (Craig et al., 2004). Juniper berries are used in northern European and particularly Scandinavian cuisine to ‘impart a sharp, clear flavour’ to meat dishes, especially wild birds and game meats. A similar purpose is the use of branches of *Juniperus communis* as a common substrate for smoking of fish. *J. communis* is the predominant flavour of gin. Several herbal properties are listed for *J. communis*: e.g. diuretic, stimulant, stomachic, carminative. For this reason *J. communis* is used in aromatherapy.

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[13] For example,. commercial information on juniper wood from Norway and Estonia at http://italian.alibaba.com/product-free/juniper-wood-timber--103873875.html

3.2.5. Overlap in mapped presence of PWN, *Monochamus* spp. and the plant genera *Chamaecyparis*, *Cryptomeria* and *Juniperus* and the plant species *P. pinea*

Within the limits of the current mandate it is not possible to conduct a thorough assessment of every species within the genera *Chamaecyparis*, *Cryptomeria* and *Juniperus*. This applies to the distribution of individual species, their suitability as hosts for PWN and the eventual relationships with vectors that can transmit PWN to them. The literature describes relationships with the vector (*Monochamus* spp.) and *Chamaecyparis obtusa* – Japan; *Chamaecyparis nootkatensis* – Canada; *Cryptomeria japonica* – Japan; *Cryptomeria fortunei* – China; *Juniperus virginiana* – China; *Sabina* (*Juniperus*?) *chinensis* – China, but their suitability as hosts for PWN it is not known. The level of uncertainty regarding the suitability of these plant genera as hosts for PWN is, therefore, generally high. This is because few studies have addressed this topic.

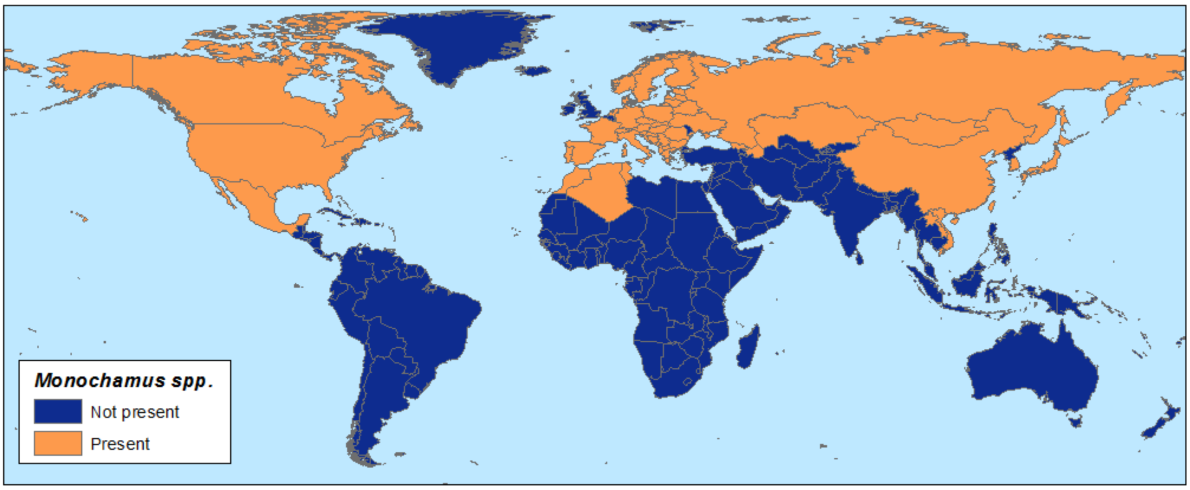

Figure 4: World distribution of *Monochamus* spp. based on countries reported in tables 6 and 7.

Areas of the world where there is a potential overlap in the presence of PWN, its vectors and the plant genera *Chamaecyparis*, *Cryptomeria* and *Juniperus* and the species *P. pinea*, can be considered as representative of a range of situations from an invasion ecology perspective: (1) native presence of potential host plants in a native area of PWN; (2) exotic presence of potential host plants in the native area of PWN; (3) native presence of potential host plants in the exotic area of PWN and (4) exotic presence of potential host plants in the exotic area of PWN.

In all these situations the plant genera genera *Chamaecyparis*, *Cryptomeria* and *Juniperus* and the species *P. pinea*, could either be a non-host, an asymptomatic host or a symptomatic host of PWN.

Outside Europe, the GBIF database indicates that *P. pinea* is present in California, North America, which is the continent of origin of *B. xylophilus*. *P. pinea* is also present in Israel, Turkey and Australia.

Regarding the spatial coexistence of *P. pinea* and PWN on the global scale, no specific information about the susceptibility to PWN of *P. pinea* trees in California is currently known. As North America is the native area of the PWN, its coexistence with introduced *P. pinea* over a much longer time span than in Portugal offers the potential to provide valuable information on the question of whether *P. pinea* is a suitable host. However, no PWD has been reported on *P. pinea* from California, nor it is known whether PWN has infested the *P. pinea* trees that are present there and which may have been asymptomatic or only hosts during the saprophytic phase of the nematode cycle.

Table 10: Countries with known presence of PWN (official status according to EPPO PQR – version 5.0 (2011))[14]

Country	Continent	Situation
Canada	America	Present, widespread
Mexico	America	Present, no details
United States of America	America	Present, widespread
China	Asia	Present, restricted distribution
Japan	Asia	Present,widespread
Korea, Republic	Asia	Present, restricted distribution
Taiwan	Asia	Present, widespread
Portugal (continental Portugal and Madeira island)	Europe	Present, restricted distribution
Spain	Europe	Transient, under eradication

The EUROSTAT and FAOSTAT databases do not provide disaggregated data on trade volumes and flows at species level. The data are available only for the conifers, all genera and species included. In table 11 a compilation from various sources summarises the uses of the plant genera *Chamaecyparis*, *Cryptomeria* and *Juniperus*, and of the species *P. pinea* for ornamental, wood, food and feed, natural and recreational, as well as medical and cosmetic purposes.

Table 11: Summary of the main uses of the plant genera *Chamaecyparis*, *Cryptomeria* and *Juniperus* and the species *P. pinea*

	Species			
Main uses	***Chamaecyparis* spp.**	***Cryptomeria* spp.**	***Juniperus* spp.**	***P. pinea***
Ornamental	+	+	+	+
Wood	+	+	NF.	+
Food and feed	NF.	+	+	+
Natural/recreational	+	NF.	+	+
Medical/cosmetic	+	+	+	+
Horticultural bark	NF.	NF.	NF	+

Key: + reported use; NF. information not found.

3.3. Host status of *Pinus pinea* with regard to PWN and its vectors

3.3.1. Host status of *Pinus pinea* with regard to *Monochamus* spp.

There are few reports in the literature concerning *Monochamus* spp. association with *P. pinea*. In Italy, Campadelli and Dindo (1994) reported that *M. galloprovincialis* attacks *P. pinea* branches and tops of fallen trunks. Similarly Francardi and Pennacchio (1996) state that "*Monochamus galloprovincialis galloprovincialis* is very frequent in the pine forests of *P. pinaster* and not uncommon in forests of *P. halepensis* and, secondarily, of *P. pinea*". In surveys of nematodes and insects in declining pine trees in Italy (68 composite samples from a total of 169 coniferous trees from 38 locations), Caroppo et al. (1998) did not report any association between *M. galloprovincialis* and *P. pinea*. Naves et al. (2006) could not detect a statistically significant difference in feeding activity of *M. galloprovincialis* on *P. pinaster* compared with *P. pinea*, but oviposition was much lower on *P. pinea* than on *P. pinaster*. It seems obvious that *P. pinea* is an inferior host for *M. galloprovincialis* and its capacity to support a full life cycle of the beetle remains poorly characterised or tested.

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[14] European and Mediterranean Plant Protection Organisation (EPPO) Plant Quarantine Data Retrieval System (PQR) version 5.0 (2011). Downloadable at http://newpqr.eppo.org/download.php

3.3.2. Host status of *Pinus pinea* with regard to PWN

In nematode surveys in areas where *P. pinea* occurs naturally (Philis and Braasch, 1996; Caroppo et al., 1998; Escuer et al., 2004; Akbulut et al., 2006; Mota and Vieira, 2008), *Bursaphelenchus sexdentati* has been reported from *P. pinea* in Italy (Caroppo et al., 1998) and Spain (Escuer et al., 2004), and *B. leoni* has been reported on *P. pinea* in Cyprus (Philis and Braasch, 1996). So far, there is scant information on the occurrence of PWN in *P. pinea* from areas where the nematode is prevalent. However, with reference to unpublished data, PWN has been reported to infest, multiply in and kill small plants of *P. pinea* (Mota and Vieira, 2008; Daub, 2009; Manuel M. Mota, University of Évora, ; Évora, Portugal, personal communication, October 2011) and increase its numbers in dead *P. pinea* plants (Daub, 2009). With regard to PWN infestation, *P. pinea* is considered more tolerant than *P. pinaster* or *P. sylvestris* (Franco et al., 2011). However, different isolates of PWN may differ in their host preferences and pathogenicity (Bolla et al., 1986; Kiyohara and Bolla, 1990). According to Valadas et al. (2011), the PWN population of Portugal is of multiple origin, both East Asian and North American. Mota et al. (2006) reported that one Portuguese population of PWN was more pathogenic than several Japanese populations in a laboratory experiment on *Pinus thunbergii*. Consequently, the host quality of *P. pinea* for PWN could vary between isolates.

3.3.3. Evaluation of the Portuguese risk assessment

3.3.3.1. Results of the evaluation of the Portuguese risk assessment

The Portuguese pest risk assessment reports on the number of dead trees in the pine forests on Tróia Peninsula in the period 2000/01–2007/08. In this study 99 % of the dead trees were *P. pinaster*. The average mortality rate of *P. pinea* on Tróia Peninsula in the years from 2000 to 2008 is below 0.06 %, smaller than that of *P. pinaster* (Appendix A, table 2). The analyses of symptomatic and felled *P. pinea* proved negative for *M. galloprovincialis* and PWN. However, this does not necessarily demonstrate that *P. pinea* is not a host of PWN. The low relative frequency of *P. pinea* on the Tróia Peninsula and the high frequency of *P. pinaster* (Appendix A, table 6) may have restricted the transmission of PWN to *P. pinea*. First, if vector beetles select feeding sites at random, they would less frequently encounter *P. pinea* (*P. pinea* density of 3–43 trees/ha versus a *P. pinaster* density of 137–496 trees/ha). Second, the ability to transmit PWN by vector beetles seems to be related to nematode load: higher nematode load gives higher rate of transmission (Linit, 1988) and a higher rate of infestation (Linit, 1990). The expected more frequent transmission of nematodes to the dominant *P. pinaster* may have resulted in rapidly decreasing nematode loads in the beetle population, and consequently low transmission rates to *P. pinea*. In susceptible species of pine, ten to a few hundred nematodes may be sufficient to cause PWD. However, in species previously classified as showing intermediate susceptibility, such as *P. pinea* (Evans *et al*., 1996), a low rate of nematode transmission might have resulted in latent infestations with a weak or a total lack of symptom expression.

From a statistical point of view, the sample size for *P. pinea* is too small to allow for detection of statistically significant differences in rate of PWN infestation between the two pine species. Owing to the wide confidence limits, the estimated probability of finding a PWN infested tree dying from PWD or other reason is not different between *P. pinaster* and *P. pinea* (Appendix A, table 4).

The risk assessment also reports on dead trees in three experimental plots on the Tróia Peninsula in the period 2001/02–2004/05. In the plots 1, 2 and 3 the frequency of *P. pinea* was 0,8 %, 0,9 % and 24 % respectively (Appendix A, table 6). For the whole period of study the number of dead *P. pinea* plants was zero in all plots, whereas the frequency of dead *P. pinaster* in plots 1, 2 and 3 was 3.8 %, 4.6 % and 2.9 % respectively (Appendix A, table 8). No information on the infestation rate of PWN is given, nor on the presence of *M. galloprovincialis*. There is also no information on infestation rates of PWN in symptom-free trees. No connection of PWN to the dead trees is provided for these plots.

All three selected plots showed a distinct higher density of trees than the average on Tróia Peninsula and a lower percentage of *P. pinea* trees (Appendix A, table 7). Plot 1 and 2 were with dominant *P. pinaster* with respectively 500 and 348 trees/ha and about 1 % *P. pinea*, compared with an average of

198 trees/ha and 11 % *P. pinea* (Sousa, 2011, clarifications). Plot 3 is an example of mixed forest, but *P. pinaster* is the dominant species in most of the area on Tróia peninsula. The exact acreage of the different forest types was not provided. Thus, the plots clearly have special characteristics and cannot be seen as representative of the Tróia Peninsula as a whole.

Nevertheless the authors state (Sousa, 2011, clarifications) that the local forest, soil and climate characteristics are representative of the entire North Alentejo coast and the Ribatejo Province, which is where the most important stone pine plantations in Portugal can be found, with high production of edible seeds. However, the selection of the plots represents only pine density and composition on the Tróia Peninsula with dominant *P. pinaster* forests and mixed forest with ca. 70 % of *P. pinaster*.

As regards extrapolation of the results to other areas, pine forests in central and northern Portugal have different characteristics owing to distinct edapho-climatic conditions, and stone pine is usually absent or residual in such areas (Sousa, 2011, clarifications).

The risk assessment reports on results from an oviposition/emergence trial (Naves et al., 2006) with *M. galloprovincialis* on several tree bolts including the five pine species *P. sylvestris*, *P. halepensis*, *P. pinaster*, *P. radiata* and *P. pinea*. The highest number of eggs was laid on *P. sylvestris* followed by *P. halepensis* and *P. pinaster*, then *P. radiata*, while the lowest number was recorded for *P. pinea*. For the emergence of adult beetles, no difference was found between *P. halepensis*, *P. pinaster*, *P. radiata* and *P. sylvestris*, while no adults emerged from *P. pinea*.

Feeding was not considered; hence the experiment does not represent real-life situations, in which PWN infestation of trees occurs through vector feeding. The oviposition of *M. galloprovincialis* is reduced but is shown to be possible in *P. pinea*. The number of eggs laid on *P. pinea* is too small to estimate a rate of emergence. Although the results suggest that further development through to adult stage does not take place under the conditions tested, definitive conclusions on the emergence in *P. pinea* in the field cannot be drawn from this experiment.

The risk assessment also reports on an oviposition preference experiment with *M. galloprovincialis* on bolts of *P. halepensis*, *P. radiata*, *P. sylvestris* and *P. pinea* tested in pairs with *P. pinaster*. No difference was found between *P. pinaster* and *P. halepensis* or between *P. pinaster* and *P. sylvestris*. Beetles preferred *P. pinaster* over *P. pinea* or *P. radiata*. About 10 % of eggs laid were laid on *P. pinea*.

Also in this case feeding by the beetles was not considered, and so the experiment is not valid for real life situations, in which PWN infestation of trees occurs through vector feeding. The experiment does not reflect the situation when the composition of the forest is not an equal 50:50 mix of two species of pines and thus cannot explain host plant choice under monoculture or *P. pinea* dominance.

With regards to sampling, the distribution in wood of PWN transmitted at feeding and, especially, oviposition by *M. galloprovincialis* can be concentrated close to the point of entry to the tree, and this condition needs to be considered in sampling trees and wood (Schröder et al., 2009). Consequently, samples should be taken from several positions along the trunk and in the crown of standing or felled trees.The authors of the Portuguese document have recognised the need for extended sampling of trees in that felled trees were sampled at several positions including the canopy. The sampling also included trees free of symptoms but it is unclear to which extent sampling of symptom free trees included the canopy (Sousa, 2011, clarifications) and whether *P. pinea* was included among the symptoms-free trees. The samples were incubated at adequate temperature for 1–3 weeks before extraction This is of particular importance in *P. pinea*, which can be expected to have received fewer nematodes than the dominant *P. pinaster*.

Regarding the chosen line of reasoning, concerns are mainly focused on the oviposition and development of the vector *M. galloprovincialis* in stone pine wood. The document fails to mention that the vector feeds equally well on *P. pinea* and *P. pinaster* (Naves et al., 2006), and hence is capable of transmitting the infective PWN juveniles to both pine species. Studies on small plants (Daub, 2009) have demonstrated that PWN can increase its numbers in dead *P. pinea* and consequently occurs in cut wood and in traded wood products of *P. pinea*.

The Panel did some further calculations on the reported data of the two surveys. Details can be found in Appendix A.

3.3.4. Uncertainties on the Portuguese risk assessment

Uncertainties of survey 1:

- Specific conditions on Tróia Peninsula might restrict the possibility to apply the results to other areas in Portugal, especially where the composition of the forest or tree density is different. The authors state that the local forest, soil and climate characteristics are representative of the entire North Alentejo coast and Ribatejo Province, which is where the most important stone pine plantations in Portugal can be found, with high production of edible seeds. Nevertheless, forests which have high *P. pinea* presence cover 20 % of the Tróia Peninsula (Sousa, 2011, clarifications; Appendix A, tables 6 and 7)

- With regards to the extrapolation of results to other areas: pine forests in central and nothern Portugal have different characteristics due to distinct edapho-climatic conditions, and stone pine is usually absent or residual in these areas (Sousa, 2011, clarifications).

- The authors concluded that only 0.2 % of the dead pine trees (with symptoms) in the winters 2000/01–2007/08 were *P. pinea*. However, the years are not homogenous (Appendix A, section1.2). The first sampling was in 2000/01, at the start of the programme of felling dead trees, and included an accumulated number of trees that had died in previous years. After 2006/07, an outbreak of bark beetles (*Scolytidae*) contributed an important addition to the general symptoms of tree decline. The proportion of PWN-infested *P. pinaster* trees decreased from about 80 % to 10 % (Appendix A, section 1.2). The connection between dead trees and PWN might, therefore, be weak when there are other compounding factors such as heavy bark beetle attacks. The majority of dead trees were infested with PWN only in the first years (until 2004/05).

- The total number of *P. pinaster* and *P. pinea* trees growing on Tróia Peninsula is unknown. Only rough information was given on the average densities of *P. pinaster* and *P. pinea* for three types of forests: *P. pinaster* dominant, *P. pinea* dominant and mixed woodlands. We calculated two scenarios to estimate the total number of pine trees on the Tróia Peninsula: (1) using the densities of the experimental plots as representative for the whole peninsula (Appendix A, table 2); and (2) assuming that 80 % of the forest is *P. pinaster* and the share of *P. pinea* is 20 % (Appendix A, table 3). The yearly mortality of pine trees was 1–2 % for *P. pinaster* and 0.01–0.02 % (upper 95 % confidence level) for *P. pinea*. Although the concrete values are uncertain, the general difference is consistent for both scenarios.

- The number of PWN-positive dead trees was given only on a graph. Using estimates from this figure, we calculated infestation rates with confidence intervals for both tree species (Appendix A, table 4). The sample sizes for *P. pinea* are too small to provide statistical support for differences in infestation rates between the two species. No information on the presence of *M. galloprovincialis* or of the bark beetles (*Scolytidae*) on individual trees was reported.

Uncertainties of survey 2:

- Specific conditions on the Tróia Peninsula might restrict the possibility to apply the results to other areas in Portugal, especially where the composition of the forest or tree density is different. The authors state that the local forest, soil and climate characteristics are representative of the entire North Alentejo coast and Ribatejo Province, which is where the most important stone pine plantations in Portugal can be found, with high production of edible seeds. Nevertheless, forests where *P. pinea* is dominant cover 20 % of the Tróia Peninsula (Sousa, 2011, clarifications; Appendix A, table 6).

- All plots have higher total pine tree density and fewer *P. pinea* than the average on Tróia Peninsula: plots 1 and 2 were dominated by *P. pinaster* (500 trees/ha and348 trees/ha, respectively, and about 1 % *P. pinea*), compared with an average of 198 *P. pinaster* trees/ha and 11 % of *P. pinea* on the peninsula (Sousa, 2011, clarifications; Appendix A, table 7). Plot 3 is an example of mixed forest, but *P. pinaster* is the dominant tree species in most of the peninsula. The exact area of the different forest types was not provided by the authors. The plots therefore clearly have special characteristics and appear not to be representative of the Tróia Peninsula nor of the entire North Alentejo coast and the Ribatejo Province.

- No information was given on the infestation with PWN, the presence of *M. galloprovincialis* or bark beetles, nor on the infestation of symptomless trees. The connection to PWN is weak. PWN might be present in symptomless trees, and observed mortality could occur for other reasons (e.g. bark beetle infestation).

- No discussion presented on the differences between the years.

- The number of *P. pinea* trees in plots 1 and 2 are extremely small and do not allow statistically valid conclusions to be drawn. Combining all plots and summarising the data over the years allows the estimation of the mortality rate of *P. pinea* which appears to be below 1.45 % (Appendix A, table 8: upper level of 95 % confidence interval). The yearly mortality rate for *P. pinaster* is about 4 % (95 %-CI 3.38–4.55 %) (for details see appendix A, table 8).

In spite of the authors' claim that the local forest, soil and climate characteristics are representative of the entire North Alentejo coast and the Ribatejo Province, the plots are dominated by *P. pinaster* forests or are mixed *P. pinaster* (about 70 %) and *P. pinea* (about 30 %) forest. No information on the *P. pinea*-dominated forests, which are 20 % of the pine forest on Trója Peninsula, is provided and it appears that no sampling was carried out in these stone pine-dominated forests.

Uncertainties of experiment 1:

- The experiment does not take into account the age of the wood, seasonal differences in wood quality for the development of *M. galloprovincialis* (Akubulut et al., 2007), temperature,bark thickness, the diameter of bolts and the pine species from which the adult beetles originally emerged. All these factors are fixed in the experiments.

- The experiment considers only oviposition and emergence, but not feeding (experiment 1 in Naves et al., 2006). The results are not valid for real life situations related to transmission to potential host trees, where PWN infestation is connected to *Monochamus* feeding, but not necessarily to breeding.

- The number of eggs laid on *P. pinea* and *P. menziesii* are too small to estimate a rate of emergence (Appendix A, table 9).

- No information is presented on oviposition when alternative pine species are available. Is the oviposition delayed only when no optimal material is present?

- In summary, oviposition of *M. galloprovincialis* is reduced but remains possible in *P. pinea*. Although the results suggest that further development through to the adult stage does not take place under the conditions tested, definitive conclusions on the emergence in *P. pinea* in the field cannot be drawn from this experiment.

Uncertainties of experiment 2:

- The experiment does not take into account the age of the wood, seasonal differences in wood quality for the development of *M. galloprovincialis* (Akubulut et. al. 2007), temperature,bark thickness, the diameter of bolts and the pine species from which the adult beetles emerged originally. All these factors are fixed. The experiment considers only oviposition, but not feeding (experiment 1 in Naves et al.). The results are not valid for real-life situations related to transmission to potential host trees, where PWN infestation is connected to *Monochamus* feeding, but not necessarily to breeding.

- The design does not allow for the testing of pure preference for oviposition, because the total number of eggs differs between the four paired experiments. The results were not standardised to equalise the total number of eggs per paired experiment.

- Most egg laying takes place on *P. sylvestris*, with much lower numbers on *P. pinea* and *P. radiata* (Appendix A, table 10). The reason for this difference is not presented.

- The experiment found no differences between *P. pinaster* and *P. halepensis*, but it is not known whether this is also reflected in the mortality rates.

The experiment does not reflect the situation when the composition of the forest (Appendix A, table 6) is not an equal 50:50 mix of two species of pines and thus cannot explain host plant choice under monoculture or *P. pinea* dominance.

3.3.5. Conclusions and uncertainties

- Some statements of the Portuguese document rely on circumstantial information. This pertains in particular to information that *P. pinea* is repellent to *M. galloprovincialis* and which relates to other insect species.

- Necessary information is lacking in the document, including:

    - the sampling strategy for *P. pinea* in particular criteria for selecting sample sites in the canopy;

    - the actual infestation pressure of PWN in the Troia Peninsula forest sites;

    - the feeding preferences of *M. galloprovincialis* as reported by Naves et al. (2006).

- Extrapolation beyond datasets.

    - Specific conditions in the experimental plots on the Tróia Peninsula, i.e. the extremely low frequency of *P. pinea* compared with *P. pinaster* and the high density of pine trees, might restrict the possibility of applying the results to other areas of *P. pinea* in Portugal, especially with different forest compositions or densities.

- The absence of PWN in symptomatic *P. pinea* might be related to the low abundance of this pine species on the Tróia Peninsula, making the site a special rather than a typical case.

3.4. Host status of the genera *Chamaecyparis*, *Cryptomeria* and *Juniperus* with regard to PWN and its vectors

The overlap in the mapped presence of PWN, *Monochamus* spp. and these plant genera has been considered in section 3.2.5. It appears difficult to separate tree genera that are suitable for breeding/survival by PWN from those that are suitable for their vectors. Some tree species or genera might be outside the geographical range of some *Monochamus* species, which therefore have never been subjected to beetle pressure. The same situation may occur between potential host trees and *B. xylophilus*. It may thus be dangerous to conclude an absence of risk from absence of observed cases in the field, especially in the absence of statistically valid field surveys. The safest approach would be to consider all tree genera colonised in their range by any *Monochamus* species as susceptible hosts for the PWN. Evans et al. (1996) provide an extensive list of the main host trees for all the known species of *Monochamus*, some of which are not known to be vectors of the nematode. This list includes the genera *Pinus, Abies, Picea, Pseudotsuga, Larix*, and *Cedrus*, and the species *Gingko biloba*. In North-America, 6 out of 10 *Monochamus* species in the native fauna act as vector of PWN: *M. carolinensis, M. marmorator, M. mutator, M. obtusus, M. scutellatus* ssp. *scutellatus* and *M. titillator*. They attack the genera *Pinus*, *Abies, Larix* and *Picea*. In the Palaearctic region overlapping with the occurrence of the PWN, 3 out of 10 *Monochamus* species, *M. alternatus, M. nitens* and *M. saltuarius*, act as vector of *B. xylophilus* and they attack *Pinus* and *Picea* species. Later on, when *B. xylophilus* was found in Portugal, it was observed that *M. galloprovincialis* also vectors the nematode (Schröder et al., 2009). Its main hosts are *Pinus* and rarely *Picea* species; and in Russia occasionally *Cedrus, Abies* and *Larix* (Schröder et al., 2009). An updated list of known host species of *Monochamus* spp. worldwide (table 8) indicates 10 genera of host trees. *Cryptomeria, Chamaecyparis* and *Juniperus* do not appear to have been reported as host trees for PWN vectors in the field, but being studied only in laboratory experiments (see sections 3.4.1.1, 3.4.2.1 and 3.4.3.1 below).

3.4.1. Host status of *Chamaecyparis* spp. with regard to PWN and its vectors

3.4.1.1. Host status of *Chamaecyparis* spp. with regard to *Monochamus* spp.

Yamane (1981) reports experimental results (Yamane and Akimoto, 1974) showing that starved *M. alternatus* can feed on *Chamaecyparis obtusa*. Nakamura and Okochi (2002) investigated the survival and ovarian development of adult *M. alternatus* experimentally fed on non-pine tree species in Japan. Those fed on *C. obtusa* lived as long as 25.6 days in 1996 and 51.3 days in 1997. Two of the 10 female adults fed on Hinoki in the experiment in 1997 had mature ovaries when they died.

3.4.1.2. Host status of *Chamaecyparis* spp. with regard to PWN

The information from the available literature is scarce and often indirect. Fujii (1999) studied pine forest decline in Gifu, Shiga and Wakayama prefectures in Japan in 1998. Extremely high pine mortality and very heavy leaf loss was observed, although some adjacent species, Japanese red cedar (*Cryptomeria japonica*) and hinoki cypress (*Chamaecyparis obtusa*), remained healthy. Morishita and Ando (2002), observed changes in cover types of urban forests damaged by PWD in the northern part of Kyoto City by comparing aerial photographs taken in 1982, 1990 and 1998. In 1982, *Pinus densiflora* forests occupied 30 % of the area in Kamigamo. In 1998, they occupied only 4.6 % or less of the area, and in some of these stands *Chamaecyparis obtusa* quickly appeared in the forest canopy.

Sutherland et al. (1991) inoculated seedlings of 22 conifer species growing in Canada (*Abies amabilis, A. balsamea, A. grandis, Chamaecyparis nootkatensis, Larix laricina, L. occidentalis, Picea glauca (British Columbia and New Brunswick provenances), P. engelmannii, P. mariana, P. rubens, P. sitchensis, Pinus banksiana, P. contorta, P. monticola, P. ponderosa, P. resinosa, P. strobus, Pseudotsuga menziesii* (inland and coastal British Columbia provenances) *Thuja occidentalis, T. plicata, Tsuga heterophylla* and *T. mertensiana*) with m and r form isolates of *Bursaphelenchus xylophilus*. *Chamaecyparis nootkatensis* suffered no mortality and nematodes were not detected from the asymptomatic seedlings of this species.

3.4.1.3. Conclusions and uncertainties

From the scarce literature available, no direct, unambiguous information can be drawn. No *Monochamus* spp. attacks or PWN infestations on *Chamaecyparis* spp. in the field have been described. The only field data available are indirect and suggest that *Chamaecyparis obtusa* survived PWN outbreaks in several locations in Japan. Two laboratory experiments testing the vectors' performances on *Chamaecyparis obtusa* suggests that the insect would have some (limited) chances to establish on this tree genus, or feed on it to some extent but, by contrast, one PWN inoculation experiment (but on seedlings) in Canada involving a local species, *Chamaecyparis nootkatensis* led to no tree mortality at all on this tree species.

In view of the scant but converging evidence available, the susceptibility of *Chamaecyparis* spp. to PWN or its vectors appears thus low, with a medium uncertainty. It remains possible, however, that *M. alternatus* could infest the trees with PWN, even with limited feeding.

3.4.2. Host status of *Cryptomeria* spp. with regard to PWN and its vectors

3.4.2.1. Host status of *Cryptomeria* spp. with regard to *Monochamus* spp.

Yamane (1981) reports information from the literature (Kojima and Hayashi, 1969) according to which the Japanese cedar *Cryptomeria japonica* has been listed as a host of *M. alternatus*. He also reports experimental results (Yamane and Akimoto, 1974) showing that starved *M. alternatus* can feed on *C. japonica*. However the host status of *C. japonica* for *M. alternatus* in Japan is questioned by Makihara (2000), who only found mentions of this possible host tree in general manuals, with no concrete examples.

Zhou and Togashi (2006) tested in the laboratory in Japan whether *Monochamus alternatus* can use *C. japonica* as a host tree. Adult females chose *Pinus densiflora* bolts as oviposition substrate when supplied with cedar and pine bolts simultaneously. Some females from one locality oviposited on cedar bolts in a no-choice experiment. Twenty-nine eggs out of 40 (73 %) hatched in cedar bolts. When first instar larvae were inoculated on cedar bolts, the development was stunted greatly and all died during the larval stage. Two of 20 larvae that were inoculated on cedar bolts at the third instar entered diapause and one larva developed into an adult female, which produced viable eggs but was much smaller than those obtained from pine bolts. The results did not exclude the possibility that *M. alternatus* can use recently killed *C. japonica* trees as a host.

In laboratory tests in China, adult *Monochamus alternatus* beetles preferred to rest, feed, and lay eggs on *Pinus massoniana*, *Cedrus deodara* and *Pinus elliottii*, less on *Podocarpus macrophyllus*, *Juniperus* (*Sabina) chinensis* and *Cryptomeria fortunei* (Yang et al., 2010). Although these authors do not give quantitative tabular information, they provide graphs that show that: an average of ca. 15 adult beetles rested on *Cryptomeria fortunei* (significantly fewer than ca. 80 on *Pinus massoniana*; 60 on *P. eliottii* and 45 on *Cedrus deodara*); an average of ca. 10 cm² of bark was eaten on *Cryptomeria fortunei* (significantly less than ca. 78 cm² on *Pinus massoniana*, 42 cm² on *P. eliottii* and 38 cm² on *Cedrus deodara*); an average of ca. three oviposition scars and one egg were observed on *Cryptomeria fortunei* (significantly fewer than ca. 29 and 22, respectively, on *Pinus massoniana*; 25 and 22, respectively, on *P. eliottii*; and 18 and 16, respectively, on *Cedrus deodara).* These laboratory results thus suggest that *Cryptomeria japonica* is a very poor host for *M. alternatus* which feeds and oviposits very little on this tree species. However, even limited feeding could result in infesting the trees with PWN.

3.4.2.2. Host status of *Cryptomeria* spp. with regard to PWN

Fujii (1999) studied pine forest decline in Gifu, Shiga and Wakayama prefectures in 1998. Extremely high pine mortality and very heavy leaf loss was observed, although some adjacent species, Japanese red cedar (*Cryptomeria japonica*) and hinoki cypress (*Chamaecyparis obtusa*), remained healthy.

3.4.2.3. Conclusions and uncertainties

No *Monochamus* spp. attack or PWN infestation on *Cryptomeria* spp. in the field have been described and the only field data available are indirect and suggest that *Cryptomeria japonica* survived PWN outbreaks in several locations in Japan. The two laboratory experiments described in the literature suggest that *Monochamus alternatus* has a limited propensity for maturation feeding on *Cryptomeria japonica* and a weak capacity to oviposit on this tree species with a low larval survival and development. However, this does not preclude the possibility that *M. alternatus* could infest the trees with PWN, even with limited feeding.

These converging but scant reports suggest a low susceptibility of *Cryptomeria* spp. to PWN or its vectors, with a low uncertainty regarding the vectors but a high uncertainty regarding the nematodes (no experimental testing of the susceptibility to PWN).

3.4.3. Host status of *Juniperus* spp. with regard to PWN and its vectors

3.4.3.1. Host status of *Juniperus* spp. with regard to *Monochamus* spp.

Maturation feeding preferences of *Monochamus alternatus* in forest stands in Nanjing, Jiangsu Province, China, were the following: *P. massoniana* > *P. densiflora* > *Cedrus deodara* > *P. taeda* > *P. elliottii* > *P. thunbergii* > *Sabina virginiana* [*Juniperus virginiana*] > *Cunninghamia lanceolata* (Xu et al., 1994).

Yamane (1981) reports information from the literature (Gressitt, 1951) according to which *Juniperus* sp. has been listed as a host of *Monochamus alternatus* in China.

In laboratory tests in China, *Monochamus alternatus* adults seldom chose to rest or feed on *Juniperus* (*Sabina*) *chinensis* as compared with *Pinus massoniana*, *Cedrus deodara*, *Pinus elliottii*, *Podocarpus macrophyllus* and *Cryptomeria fortunei* (Yang et al., 2010). An average of ca 10 adult beetles rested on *Juniperus* (*Sabina*) *chinensis* (significantly fewer than ca. 80 on *Pinus massoniana*, 60 on *P. eliottii* and 45 on *Cedrus deodara*). An average of ca. 7 cm^2 of bark was eaten on *Juniperus* (*Sabina*) *chinensis* (significantly less than ca. 78 cm^2 on *Pinus massoniana*, 42 cm^2 on *P. eliottii* and 38 cm^2 on *Cedrus deodara*). In another laboratory test in China, Li et al. (2003) tested frequency of visitation and maturation feeding of adult *Monochamus alternatus* between *Pinus massiniana*, *P. densiflore*, *P. taeda*, *P. elliotii*, *P. thunbergii*, *Cedrus deodara*, *Juniperus virginiana* and *Cunninghamia sinensis*. *J. virginiana* was one of the two least preferred species, with *C. sinensis* significantly differing on all criteria from the preferred species, *P. massoniana*. For example, the average feeding areas in three experiments were respectively 139 mm^2, 2097 mm^2 and 3451 mm^2 on *P. massoniana* and 23 mm^3, 544 mm^2 and 660 mm^2 on *J. virginiana*.

3.4.3.2. Host status of *Juniperus* spp. with regard to PWN

No reference was found.

3.4.3.3. Conclusions and uncertainties

No *Monochamus alternatus* attack or PWN infestation has been described on *Juniperus virginiana* and *J. chinensis* in the field, but some degree of maturation feeding was observed in two laboratory and one field studies. The results of all these studies suggest that *Monochamus alternatus* has only a weak capacity to rest and feed on *Juniperus virginiana* and *Juniperus chinensis.* But even a limited capacity to feed on these trees might allow PWN transmission. Nothing is known about the relationships of PWN to this genus.

The susceptibility of *Juniperus* spp. to PWN or its vectors appears low, with a low uncertainty regarding the vectors but a high uncertainty regarding the nematodes (no experimental testing of the susceptibility to PWN).

3.5. Probability of spread of PWN via the genera *Chamaecyparis, Cryptomeria* and *Juniperus* and the plant species *Pinus pinea*

From the information above, the probability of PWN spread via the genera *Chamaecyparis*, *Cryptomeria* and *Juniperus* and *P. pinea* appears limited because of the apparently weak connection between these tree taxa and both the nematodes and their vectors. We shall however examine the possibilities of spread through (i) infested plants and natural spread using these plants as stepping stones between more favourable hosts and (ii) untreated plant products.

3.5.1. Spread via infested plants and natural spread (incl. uncertainties)

The possibility of spread via infested plants of the genera *Chamaecyparis*, *Cryptomeria* and *Juniperus* and the species *Pinus pinea* can be analysed from three standpoints: (i) the information gathered above regarding the geographic distribution of these trees as well as their host status with regard to PWN and its vectors; (ii) the geographic distribution of PWN vectors (*M. galloprovincialis*) or potential vectors (*M. sutor, M. sartor, M. rosenmuelleri* and *M. saltuarius*); and (iii) the commercial movements of infested plants or derived products into or within the EU.

Pinus pinea occurs mainly in Spain and Portugal but is also present in Italy and France and, more locally, in the southern United Kingdom and Ireland, Germany and Greece. It appears to elicit as much maturation feeding as *P. pinaster* but oviposition is low (Naves *et al*., 2006) and, apparently, larval development through to adult was not supported on this species in the experiment in Portugal. The results are inconclusive because of considerable mortality in the control treatment (wood of preferred hosts). In Italy, however, *M. galloprovincialis* was reported to breed in fallen branches and tops of trunks of *P. pinea* lying on the ground (Campadelli and Dindo, 1994), It is also clear that PWN is capable of establishing in young trees of *P. pinea* after artificial inoculation in the laboratory, and so could potentially also occur in plants for planting, and increase in population in such trees when they are dead (Daub, 2009).

Six species of the genus *Chamaecyparis* are found scattered thoughout the EU (see Appendix C). From the information discussed above it seems that the vectors might reproduce on *C. obtusa* in laboratory conditions in Japan but, in Canada, *C. nootkatensis* seedlings inoculated with PWN suffered no mortality.

Cryptomeria japonica has a very limited geographical distribution (see Appendix C). In Japan, *C. japonica* elicited only reduced maturation feeding and weak oviposition of *M. alternatus*.

Juniperus spp. have a wider geographical distribution than the former two genera (see Appendix C). Some limited maturation feeding of *M. alternatus* on *J. virginiana* and *J. chinensis* was observed in the field and laboratory in China.

The flight capacity of some *Monochamus* species has been analysed with tethered beetles in the laboratory and deduced from field observations. A good recent summary is given by Akbulut and Stamps (2011): ‘*Monochamus* beetles are generally poor fliers, although in flight mill experiments, female *M. carolinensis* beetles have flown a maximum distance of 10 km with duration of 115 min’ (Akbulut and Linit, 1999). In a mark and recapture study of the closely related beetle, *M. alternatus*, most beetles were recaptured within 100 m of the release site in one study (Ogawa and Hagiwara, 1980). In another study, the majority of beetles remained within 800 m of their origin, although some flew up to 3.3 km (Kobayashi et al., 1984). Based on field observations, Togashi (1990) calculated that the average distance a *M. alternatus* beetle moves during the first few weeks post-emergence is 10–20 m per week. Assuming an average field lifespan of 7 weeks, dispersal would range from 50 to 260 m. The analysis of Togashi (1990) also suggested increased beetle dispersal with increased ambient air temperature and decreased stand density.

In addition, Zhang et al. (2007) reports that *M. saltuarius* tested in flight mills (where they are flying attached to a rotating arm and have their equivalent flight distances and flight duration recorded) covered a maximum distance of 1 300 m in laboratory tests in China. They also report field observations on *M. sutor* in north-east China, which showed that the beetles usually cover 5–15 m in one flight with maximum distances exceeding 900 m.

The restricted range of these tree genera and species (except *Juniperus* spp.), combined with the weak responses of the vectors to these trees described in the available literature and the limited dispersal capacities of the beetles, suggests with a low uncertainty that the spread of PWN is very unlikely to be favoured by the individuals of the genera *Chamaecyparis*, *Cryptomeria* and *Juniperus* that are presently growing in the EU.

With regard to *P. pinea*, maturation feeding by *M. galloprovincialis* has been reported in laboratory experiments not to differ significantly from feeding on *P. pinaster* (Naves et al., 2006), thus allowing for PWN infestation on *P. pinea* through feeding scars. In addition, the reported breeding of *M. galloprovincialis* in branches and tree tops of *P. pinea* lying on the ground (Campadelli and Dindo 1994) indicates that it would be possible for European vector insects to get infested by PWN from breeding in PWN-infested wood of *P. pinea*. As long as trade volumes are small the probability of spread is low. However, owing to insufficient documentation of the nematode–beetle interaction on *P. pinea*, the uncertainty is high.

3.5.2. Spread via untreated plant products (incl. uncertainties)

Living plants and several untreated plant products could contribute to spreading PWN and its vectors. Table 8 (section 3.2.6) shows that *Chamaecyparis*, *Cryptomeria* and *Juniperus* spp., and *P. pinea*, are all valued as ornamentals. The wood of *Chamaecyparis* is considered as valuable and should therefore be considered as potential commercial item. The wood of *P. pinea* is used for pallets in Italy and has many other uses in Portugal. There is also a recent trend to import *P. pinea* bark into several countries in the EU, either as mulch or, in larger sizes, as decorative elements on their own. Commercial movements of these commodities could thus favour the spread of PWN and its vectors. However, as stated before, the weak link between these tree genera and species with the vectors and with PWN makes this unlikely with a low uncertainty.

CONCLUSIONS AND RECOMMENDATIONS

CONCLUSIONS

The Panel has analysed the data in the document submitted by Portugal (Sousa et al., 2011) and the related evidence in Naves et al. (2006). In connection with this, the Panel has also undertaken a comprehensive review of the literature.

Surveys on the Tróia Peninsula (Sousa et al., 2011) showed a lower yearly mortality rate of *P. pinea* (below 0.03 %) than of *P. pinaster* (1–2 %). The PWN infestation rate in dead trees (with symptoms) decreased from 80 % to 10 % during the period 2000/01–2007/08, so in later years PWN was not the main cause of tree mortality. Although a zero infestation of PWN was recorded on *P. pinea*, this was not statistically significantly different to the result for *P. pinaster* because of the small number of *P. pinea* trees sampled. Hence, based purely on the data presented by Sousa et al. (2011), the conclusion that *P. pinea* is not a host plant for PWN has not been supported. In addition, no information on PWN infestation on symptomless trees was given. Statistically, therefore, it cannot be stated that there is a difference in PWN infestation between *P. pinea* and *P. pinaster*, but it is clear that at most there would be a very low infestation in the former tree species. A much higher level of sampling would be needed to provide confidence in reaching a conclusion on whether PWN can survive and breed in living *P. pinea* trees in the field.

P. pinea occurs in many locations in Portugal. From the data presented, it appears that the results from the studies of the pine forest on the Tróia Peninsula cannot be extrapolated to the other parts of the peninsula, nor to other areas of Portugal. In the case of the Tróia Peninsula, the experimental plots had higher densities of pine trees and a higher proportion of *P. pinaster* trees than the average in corresponding forest classes over the remainder of the Tróia Peninsula. Owing to the low relative frequency of *P. pinea* in the studied forest plots, an extrapolation to plantations of this species is questionable, and an extrapolation to other parts of Portugal is not possible owing to different conditions of climate and soil. So, the very low prevalence of *P. pinea* in the studied areas indicates that the results are representative neither of the Tróia Peninsula nor of other parts of Portugal.

With regard to the vector insect *Monochamus galloprovincialis* (the only species of vector considered in the study), the Portuguese document concludes that differences occur in oviposition rate in *P. pinea* and *P. pinaster* under the specific experimental settings. Although the rate of oviposition in *P. pinea* is lower than on other host plants, oviposition on *P. pinea* still remains possible. An extrapolation to forests with different tree compositions and different settings is not possible from the limited data presented. The Portuguese document does not acknowledge the fact that experiments by Naves et al. (2006) did not detect differences in feeding of *M. galloprovincialis* on *P. pinaster* and *P. pinea*. The transmission of PWN from the vector to the two species of pine was not investigated and remains unclear.

M. galloprovincialis is distributed over a vast geographical area and it cannot be excluded that subspecies (*M. galloprovincialis galloprovincialis*, *M. galloprovincialis pistor*) and local populations could have host preferences different from that of the known Portuguese populations. Attacks on *P. pinea* by *M. galloprovincialis* are in fact known from Italy. Observations and, particularly, the studies of Halik and Bergdahl (1994) support the conclusion that some coniferous trees may become infested with PWN, but remain free of PWD symptoms for many years while containing live nematodes. Such trees can act as reservoirs for the nematode over prolonged periods. However, if these trees are weakened sufficiently to become attractive to *Monochamus* for oviposition and larval development, there is a possibility that the nematode could associate with the vector and be transmitted to other trees. Unfortunately, the relationships between European *Monochamu*s species other than *M. galloprovinciallis*, *P. pinea* and PWN have not yet been studied in sufficient detail to draw firm conclusions on the survival and transmission of PWN.

An absence of apparent wilt symptoms arising from PWN infestation in *P. pinea* would not necessarily indicate that nematodes are unable to invade and survive in such trees. It is possible that the relationship between *P. pinea* and PWN in Portugal may be similar to the situation in North America, where PWN is widely distributed but not frequently reported from indigenous pine species and is associated with saprophytic development in dead trees arising from causes other than wilt caused by the nematode. Furthermore, it cannot be excluded that PWN could be present, but not necessarily causing tree mortality, in *P. pinea* in situations in which this species is a dominant tree; however, this would require that *Monochamus* spp. were able to successfully breed in weakened trees. The fact that PWN may reproduce in dead *P. pinea* would allow the nematode to be present in traded lumber and wood products. Plants for planting could also contain living nematodes but for further spread from such trees the vector is needed.

Owing to missing scientific information on the interaction of *M. galloprovincialis*, *B. xylophilus* and *P. pinea*, the risk of PWN spread with plants and wood of *P. pinea* is difficult to assess. However, as long as trade volumes are small, the probability of spread is considered low. Owing to insufficient documentation of the trade volumes and the nematode–beetle interaction on *P. pinea*, the uncertainty is high.

The available information regarding the status of the genera *Chamaecyparis*, *Cryptomeria* and *Juniperus* as regards *Monochamus* spp. and PWN suggests overall a low susceptibility of these taxa to PWN or its vectors, with a low uncertainty concerning the vectors and a high uncertainty concerning PWN. No experimental inoculation of PWN on *Chamaecyparis*, *Cryptomeria* or *Juniperus* has been attempted so far, except one test involving *Chamaecyparis nootkatensis* carried out in laboratory conditions in Canada. No *C. nootkatensis* plant died after inoculation and no nematodes could be detected in the asymptomatic plants, The scant information regarding the plant genera *Chamaecyparis*, *Cryptomeria* and *Juniperus* suggests that they would not suffer from wilt disease and would not function as efficient hosts for PWN, but there is still a possibility that they could be either asymptomatic hosts for PWN in living trees or hosts during the saprophytic phase of the nematode cycle. The information on the interaction between *Monochamus* spp., PWN and species in the genera

Chamaecyparis, *Cryptomeria* and *Juniperus* is largely missing, without specific surveys or experimental inoculations. Thus it is difficult to make firm statements on the risks of PWN spread in trade from this material. Therefore, the uncertainty of this is high.

RECOMMENDATIONS

As indicated in the evaluations above, there are limited data on the potential for *P. pinea*, *Chamaecyparis* spp., *Cryptomeria* spp. and *Juniperus* spp. to successfully support either or both of *Monochamus* spp. or PWN. The fact that *Monochamus* spp. are known to maturation feed and breed, albeit at low levels, in *Chamaecyparis* spp., *Cryptomeria* spp. and *Juniperus* and in *P. pinea* suggests that PWN could be carried to these host trees and potentially be dispersed further if *Monochamus* breeding is successful. Unfortunately, data to confirm and quantify these potential associations is poor and, therefore, further research is needed to increase the insights into PWN ecology, by studying the development and survival of PWN in artificially inoculated field-grown trees.

DOCUMENTATION PROVIDED TO EFSA

1. Sousa E, Naves P, Bonifacio L and Rodrigues JM, 2011. Risk assessment of *Pinus pinea* L. in relation to pine wood nematode. June 2011. Submitted by DG SANCO.

2. Sousa E, 2011. Personal communication received via email on 21 October 2011. Clarifications.

3. Maps of forestry species distribution from the Joint Research Center of the European Commission, Institute for Environmental Sustainibility (materials and methods are described in Appendix C).

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APPENDICES

A. APPENDIX ON THE STATISTICAL ISSUES OF THE EVALUATION OF THE DOCUMENT ON “RISK ASSESSMENT OF *PINUS PINEA* L. IN RELATION TO PWN

OBJECTIVES AND SCOPE OF THIS APPENDIX

The objective of this appendix is to:

- Evaluate the statistical issues of the RA on Pinus *pinea L.*

It is important to note that the scope of this appendix is limited here to the statistical issues.

1. Survey conducted by the INRB, IP Institute in Tróia peninsula Survey 1: Sampling on diseased trees (Table III)

1.1. Screening of the documentation / description of datasets

Sources:

Sousa E, Naves P, Bonifacio L and Rodrigues JM, 2011. Risk assessment of *Pinus pinea* L. in relation to pine wood nematode. Portuguese National Institute of Biological Resources (INRB) and Portuguese National Forestry Authority, June 2011 (supporting document).

Sousa E, 2011. Personal communication. Clarifications of the authors received on 21 October 2011.

Item	*Description based on the submitted document(s)*	*Comments*
<u>Description of the proposed risk reduction option</u>		
Target pest	**Pine wood nematode (PWN)** *Bursaphelenchus xylophilus* (Steiner et Buhrer, 1934) Nickle 1970	
Vector	**Pine sawyer** *Monochamus galloprovincialis*	
Target plant material/product	**Pine trees,** *Pinus sp.* Susceptible: European black pine, *P. nigra* **Maritime pine, *P. pinaster*** Scots pine, *P. sylvestris* Intermediate: Aleppo pine, *P. halepensis* **Stone pine, *P. pinea***	Proposal of Evans et al. (1996)
Disease	**Pine Wilt Disease (PWD)**	
Origin of plant material/product	**Portugal,** only *P pinaster*, *P. pinea* and *P. halepensis* (scattered) *P. pinea* distributed in whole Mediterranean basin from Portugal to Turkey	

Type of risk reduction option	**Proven resistance:** Pinus *pinea* should be classified as “resistant” instead of “intermediate”.
Place of implementation	**Portugal and Spain**
Other relevant information	The only other conifers present are two species of juniper (*Juniperus navicularis* and *J. turbinata*), which are not forestry species and have not suffered of mortality over the years, and therefore have not been sampled for the PWN.

Experimental assessment of the option efficacy to reduce pest infestation in plant material/product under operational conditions

Plant material information Type of plant material/product used in the experiment	Trees in the Tróia Peninsula pine forest (south bank of Sado river, near city of Setúbal/2 km from Setúbal port as probable pathway/centre of critical zone for PWD / PWN present over a decade causing high mortality)
Plant identity (e.g. botanical name, variety)	Pine forest mainly composed of maritime (*P. pinaster*) and stone (*P. pinea*) The presence of Aleppo pines (*P. halepensis*) is vestigial on the Tróia Peninsula, being found in two small areas with about 30–40 trees. Over the years none of these trees have wilted or died, and therefore were never sampled for the PWN Pines aged 30-40 years, about 400 ha. Dominant *P. pinea* forest about 80 ha (20 % of total forest)
Conditions under which plant materials/products are managed	Tourist area, sandy soil The local forest, soil and climate characteristics are representative of the entire North Alentejo coast and the Ribatejo Province
Conditions of the plant commodity (e.g. degree of ripeness, presence of bark, etc.)	Dead trees with symptoms, felled
Pest information Identity (species- strains biotypes if applicable-)	Checked for vector (*Monochamus galloprovincialis)* and PWN (*Bursaphelenchus xylophilus*) Larval stage of vector Infestation with PWN
Conditions under which the pests are cultured, reared or grown	Natural
Method of infestation	Natural
Level of infestation	Presence, high incidence

	Part of demarcated area of South of Lisbon/centre of critical zone for PWD/PWN present over a decade causing high mortality
Stage of the pest that is most resistant to the treatment	Unknown
Was the most resistant stage used in the experiment?	Sampling were done in autumn and winter, when the wilting symptoms are most conspicuous
Potential development of resistance to the option	Sampling only on trees with symptoms
Experiment(s) description and analysis Variables used to measure efficacy	Counts of dead or wilted trees (with symptoms) for eight years: 2000/01–2007/08 In clarification: 2000/01 first sanitary felling with large number of dead and wilted trees from previous years Bark beetle (Scolytidae) outbreak in 2006 onwards
Factors influencing efficacy which were taken into account in the experiment	None
Factors influencing efficacy which were **not** taken into account in the experiment	Development of infestation (both vector and PWN) Composition of the forest: Density of *P. pinaster* and *P. pinea* in three strata: dominant *P. pinaster*, dominant *P. pinea*, mixed. Only for dominant *P. pinea* is the total acreage given (80 ha)
Description of facilities and equipment	In general, small pieces of wood material collected at the trees diameter breast height with an autonomous slow-rotation drilling device, in to an unused bag, making composite samples, up to a maximum of five trees per composite sample, and of four or more drillings per tree. Also samples from wooden discs, cut at different heights and reduced into small (1 cm) wood pieces are subject to nematode analysis. When trees were felled, samples from the canopy are collected but they are never mixed with the samples collected at the diameter in breast height. A minimum amount of 100 g and up to 200 g material was collected per sample. Before being analysed the wood material was left for up to three weeks at 25 °C under laboratory conditions (Sousa, 2011, clarifications). The samples were placed in a tray of water for 48 h at ambient temperature, then sieved with a 400 mesh (38 µm). The retained nematodes were identified morphologically and/or by using molecular techniques. The method is discussed in Penas et al (2002).
Description of treatment	Two *Pinus* species: *P. pinaster*, *P. pinea*
Monitoring of critical parameters	None
Description of experimental design	Yearly field samples of dead trees Between 5 % and 10 % of the dead maritime pines (P.*pinaster*) and all of the dead stone pines (*P. pinea*) were randomly sampled for PWN. In total 20 % of the samples

	were also taken from symptomless pine trees
Presentation of the data	• Yearly count of dead trees (with symptoms) for *P. pinaster* and *P. pinea* (Table III) • Yearly number of sampled trees for *P. pinaster* and *P. pinea* (Sousa, 2011, clarifications) • Overall ratio of dead trees of different *Pinus* species of (over 99 % of all dead trees were *P. pinaster*) • Infestation of PWN (only range between locations and years: 10–75 % of all dead *P. pinaster* were infested with PWN, 0 % of *P. pinea*) • A figure shows a decreasing curve over time of PWN infestation in *P. pinaster* of the samples (going from about 77 % in 2001/02 down to below 13 % in 2007/08) (Sousa, 2011, clarifications). • Infestation with larval instars of vector (only overall: 83 %)
Description of the statistical analysis	None
Conclusions of the experiment	Additional evidence confirms that the stone pine (*P. pinea*) is not attacked by PWN
Other relevant information	

1.1.1. Extracted data

Table 1: Number of dead pine trees in Tróia Peninsula between 2000/01 and 2007/08 (Sousa et al., 2011, table III; Sousa, 2011, clarifications).

Year (winter)	***No of dead trees in Tróia Peninsula***									
	Pinus pinaster				*Pinus pinea*				Total	
	total	sampled	infested[1] absolute	relative (%)	total	sampled	infested absolute	relative (%)	total	sampled
2000/01	4226	120		unknown	5	5	0	0	4231	125
2001/02	1365	100	77	77	0	0			1365	100
2002/03	636	80	62	77	0	0			636	80
2003/04	1135	80	59	73	0	0			1135	80
2004/05	953	90	52	58	3	3	0	0	956	93
2005/06	1568	80	28	35	0	0			1568	80
2006/07	1337	90	11	12	3	3	0	0	1340	93
2007/08	633	70	9	13	11	11	0	0	644	81
2000-08	11853				22				11875	
2001-05	4089				3				4092	

Grey cells are calculated

[1] Estimated from figure 1

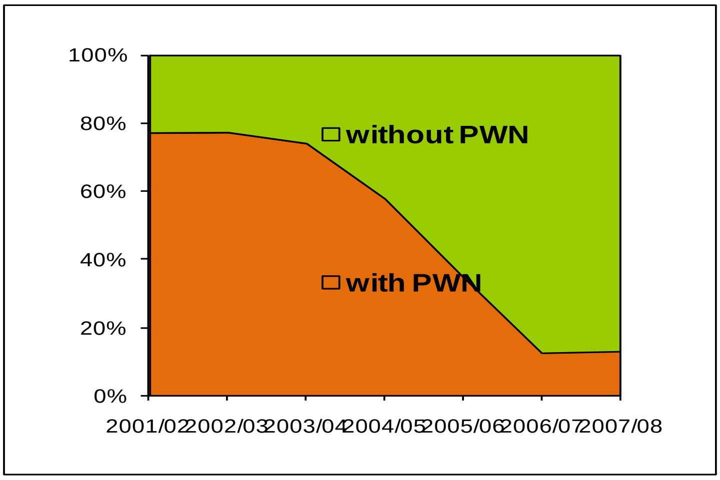

Figure 1: Annual percentage of PWN infested samples of maritime pine (P. pinaster) (Sousa, 2011, clarifications)

1.2. Data analysis/methods

Table 2: Estimated probability to find a specific *Pinus* species, when died (with symptoms) and estimated yearly mortality rates of *P. pinaster* and *P. pinea* using the experimental plots as reference.

	Pine tree mortality					***Estimated no of Pinus trees***		***No of years***	***Estimated mortality rate per year***			
Year	*P. pinaster*		*P. pinea*		All	*P. pinaster*	*P. pinea*		*P. pinaster* estimated (%)	*P. pinea* estimated (%)	confidence interval (95%) (%)	
	absolute	relative (%)	absolute	relative (%)								
2000/01	4226	99.88	5	0.12	4231	130400	6667	1	3.24	0.08	0.02	0.17
2001/02	1365	100.0	0	0.00	1365	126174	6662	1	1.08	0.00	0.00	0.04
2002/03	636	100.0	0	0.00	636	124809	6662	1	0.51	0.00	0.00	0.04
2003/04	1135	100.0	0	0.00	1135	124173	6662	1	0.91	0.00	0.00	0.04
2004/05	953	99.69	3	0.31	956	123038	6662	1	0.77	0.05	0.01	0.13
2005/06	1568	100.0	0	0.00	1568	122085	6659	1	1.28	0.00	0.00	0.04
2006/07	1337	99.78	3	0.22	1340	120517	6659	1	1.11	0.05	0.01	0.13
2007/08	633	98.29	11	1.71	644	119180	6656	1	0.53	0.17	0.08	0.30
2000-08	**11853**	**99.81**	**22**	**0.19**	**11875**	**130400**	**6667**	**8**	**1.14**	**0.04**	**0.03**	**0.06**
2001-05	**4089**	**99.93**	**3**	**0.07**	**4092**	**126174**	**6662**	**4**	**0.81**	**0.01**	**0.00**	**0.03**

Grey cells are calculated

Table 3: Estimated probability to find a specific pine species, when died (pine wilt disease) and estimated yearly mortality rates of *P. pinaster* and *P. pinea* using the average densities on Tróia Peninsula.

Year	***Pine tree mortality***					***Estimated no. of pinus trees***		***no of years.***	***Estimated mortality rate per year***			
	P. pinaster		*P. pinea*		All	*Pinus pinaster*	*Pinus pinea*		*Pinus pinaster*	*Pinus pinea*	confidence interval (95 %)	
	No.	rel (%)	No.	rel (%)					(%)	(%)	(%)	
2000/01	4226	99.88	5	0.12	4231	58800	13840	1	7.19	0.04	0.01	0.08
2001/02	1365	100.0	0	0.00	1365	54574	13835	1	2.50	0.00	0.00	0.02
2002/03	636	100.0	0	0.00	636	53209	13835	1	1.20	0.00	0.00	0.02
2003/04	1135	100.0	0	0.00	1135	52573	13835	1	2.16	0.00	0.00	0.02
2004/05	953	99.69	3	0.31	956	51438	13835	1	1.85	0.02	0.00	0.06
2005/06	1568	100.0	0	0.00	1568	50485	13832	1	3.11	0.00	0.00	0.02
2006/07	1337	99.78	3	0.22	1340	48917	13832	1	2.73	0.02	0.00	0.06
2007/08	633	98.29	11	1.71	644	47580	13829	1	1.33	0.08	0.04	0.14
2000-08	**11853**	**99.81**	**22**	**0.19**	**11875**	**58800**	**13840**	**8**	**2.52**	**0.02**	**0.01**	**0.03**
2001-05	**4089**	**99.93**	**3**	**0.07**	**4092**	**54574**	**13835**	**4**	**1.87**	**0.01**	**0.00**	**0.02**

Grey cells are calculated

The 95 % confidence intervals for the mortality rates were calculated using *F*-quantiles to approximate the binomial distribution (Pearson–Clopper values). In case of no observed mortality the one-sided version was used.

The counts of dead trees were tested for independence of *Pinus* species and year using a χ^2-test. The result shows a large influence of the year 2007/08 ($p < 0.001$), which looks irregular compared with the other years.

The mortality rate was calculated using the estimated number of *Pinus* trees from section 2.2 of this Appendix. Two scenarios were used. It was assumed first that the selected plots are representative of the total Tróia Peninsula and second that the reported densities of dominant *P. pinaster* and dominant *P. pinea* areas could be used to estimate the total number.

The yearly mortality calculated from a longer period is approximated by dividing by the number of years.

Restricting the calculation to the years between 2001 and 2005 the average mortality rate of *P. pinaster* is about 0.81 % per year and for *P. pinea* about 0.01 % per year (95 % CI 0.00 %–0.03 %).

Table 4: Infestation rates of pine trees in Tróia Peninsula (Sousa et al., 2011, table III; Sousa, 2011, clarifications)

Year (winter)	***No of dead trees in Tróia Peninsula***									
	Pinus pinaster					*Pinus pinea*				
	sampled	infested[1] with PWN				sampled	Infested with PWN			
		absolute	relative (%)	Confidence interval (95%) (%)			absolute	relative (%)	Confidence interval (95%) (%)	
2000/01	120	unknown				5	0	0	0	45
2001/02	100	77	77	68	85	0				
2002/03	80	62	77	67	86	0				
2003/04	80	59	73	63	83	0				
2004/05	90	52	58	47	68	3	0	0	0	63
2005/06	80	28	35	25	46	0				
2006/07	90	11	12	6	21	3	0	0	0	63
2007/08	70	9	13	6	23	11	0	0	0	24

[1] Estimated from figure 1

The numbers of infested samples were estimated from figure 1.

The 95% confidence intervals for the infestation rates were calculated using *F*-quantiles to approximate the binomial distribution (Pearson-Clopper values). In case of no observed infestation the one-sided version was used.

The infestation rate of dead trees decreased from 2000/01 (77 %) to 2007/08 (13 %). The sample size for *P. pinea* is too small to detect differences between the species.

1.3. Results / uncertainties

- Specific conditions on Tróia Peninsula might restrict the ability to apply the results to other areas in Portugal, especially one with a different composition of forest or density of trees. The authors state that the local forest, soil and climate characteristics are representative of the entire North Alentejo coast and the Ribatejo Province, which is where the most important stone pine plantations in Portugal can be found, with high production of edible seeds. Nevertheless dominant *P. pinea* forests are only rarely (20 %) found on Tróia Peninsula. (Sousa, 2011, clarifications).

- Concerning extrapolation of results to other areas: pine forests in central and nothern Portugal have different characteristics as a result of distinct edapho-climatic conditions, and stone pine is usually absent or residual in such areas (Sousa, 2011, clarifications).

- The authors concluded that only 0.2 % of the dead pine trees (with symptoms) identified in the winters of 2000/01 to 2007/08 were of *P. pinea*. However, the years are not homogenous. The first sampling in 2000/01 was the start of the felling action and included also dead trees from the previous years. After the year 2006/07, an outbreak of bark beetle (Scolytidae) caused an important part of the symptoms. The proportion of PWN-infested trees in the samples of dead P.pinaster trees fell from about 80 % to 10 %. However, the connection between tree death and PWN might be weak. Only in the first years (until 2004/05) were the main parts of dead pine trees infested with PWN.

- The total number of *P. pinaster* and *P. pinea* trees growing on Tróia Peninsula is unknown. Only approximate information on the average densities of *P. pinaster* and *P. pinea* trees in three types of forests, dominant *P. pinaster*, dominant *P. pinea* and mixed, was given. We calculated two scenarios to estimate the total number of pine trees on Tróia Peninsula: (1) taking the densities of the experimental plots given in section 2.2 as representative of the whole peninsula; and (2) assuming that 80 % of the forest is dominant *P. pinaster*/20 % *P. pine*a. The yearly mortality of pine trees is about 1–2 % for *P. pinaster* and below 0.01% to 0.02 % (upper 95 % confidence limit) for *P. pinea*. Although the concrete values are uncertain, the general difference is consistent for both scenarios.

- Regarding the cause of the mortality, the number of dead trees that tested positive for PWN was only given in a graphic. Using estimates from this figure we calculated infestation rates with confidence intervals for *P. pinaster* and *P. pinea*. The sample sizes for *P. pinea* are too small to prove existing differences. No information on the presence of *M. galloprovincialis* or of the bark beetle (Scolytidae) was reported.

2. Survey conducted by the INRB, IP Institute in Tróia Peninsula Survey 2: Experimental plots (Table IV)

2.1. Screening of the documentation / description of datasets

Sources:

Sousa E, Naves P, Bonifacio L and Rodrigues JM, 2011. Risk assessment of *Pinus pinea* L. in relation to pine wood nematode. Portuguese National Institute of Biological Resources (INRB) and Portuguese National Forestry Authority, June 2011 (supporting document).

Sousa E, 2011. Personal communication. Clarifications of the authors received on 21 October 2011.

Item	***Description based on the submitted document(s)***	***Comments***
	<u>Description of the proposed risk reduction option</u>	
Target pest	**Pine wood nematode (PWN)** *Bursaphelenchus xylophilus* (Steiner et Buhrer, 1934) Nickle 1970	
Vector	**Pine sawyer** *Monochamus galloprovincialis*	
Target plant material/product	**Pine trees,** *Pinus* sp. Susceptible: European black pine, *P. nigra/* **Maritime pine, *P. pinaster/*** Scots pine, *P. sylvestris* Intermediate: Aleppo pine, *P. halepensi/* **Stone pine, *P. pinea***	Proposal of Evans et al. (1996)
Disease	**Pine wilt disease (PWD)**	Not only caused by PWN
Origin of plant material/product	**Portugal,** only *P. pinaster*, *P. pinea* and *P. halepensis* (scattered) *P. pinea* distributed in whole Mediterranean basin from Portugal to Turkey	
Type of risk reduction option	**Proven resistance:** *Pinus pinea* should be classified as ‘resistant’ instead of ‘intermediate’	

Place of implementation	**Portugal and Spain**
Other relevant information	

Experimental assessment of the option efficacy to reduce pest infestation in plant material/product under operational conditions

Plant material information Type of plant material/product used in the experiment	Trees in three experimental plots of 1ha each in the Tróia Peninsula pine forest (south bank of Sado river, near City of Setúbal) Representing dominant *P. pinaster* and mixed *pinaster–pinea* forest
Plant identity (e.g. botanical name, variety)	Pine forest mainly composed of maritime (*P. pinaster*) and stone (*P. pinea*) pines aged 30–40 years, about 400 ha
Conditions under which plant materials/products are managed	Tourist area, sandy soil
Conditions of the plant commodity (e.g. degree of ripeness, presence of bark, etc.)	Dead trees with pine wilt disease (PWD), felled
Pest information Identity (species, strains, biotypes if applicable)	Not sampled
Conditions under which the pests are cultured, reared or grown	
Method of infestation	
Level of infestation	
Stage of the pest that is most resistant to the treatment	
Was the most resistant stage used in the experiment?	Dead and surviving trees were counted in autumn and winter, when the wilting symptoms are most conspicuous
Potential development of resistance to the option	Sampling only on trees with symptoms
Experiment(s) description and analysis	
Variables used to measure efficacy	Counts of dead (diseased) and survived trees for four years: 2000/01–2004/05
Factors influencing efficacy which were taken into account in the experiment	None
Factors influencing efficacy which were **not** taken into account in the experiment	Development of infestation (both vector and PWN) Composition of the plots Tree density
Description of facilities and equipment	
Description of treatment	Two *Pinus* species: *P. pinaster* and *P. pinea*
Monitoring of critical parameters	None
Description of experimental design	Yearly count of dead and survived trees

Presentation of the data	• Number of trees per plot • Yearly count of dead trees for *P. pinaster* and *P. pinea* (Table IV) • No information on PWN or vectors
Description of the statistical analysis	none
Conclusions of the experiment	The dominant *P. pinaster* suffered high mortality over the years: 15 % in a period of 5 years. No *P. pinea* died, despite the high incidence of PWN
Other relevant information	

2.1.1. Extracted data

Table 5: Number of pine trees alive or dead in some experimental plots in Tróia Peninsula between 2001/02 and 2004/05 (Sousa et al., 2011, table IV)

Plot	**Year** (winter)	***No of dead trees***			***No of live trees***			***Total no of trees***		
		P. pinaster	*P. pinea*	Total	*P. pinaster*	*P. pinea*	Total	*P. pinaster*	*Pinus pinea*	Gross total
1	2001/02	5	0	5	491	4	495	496	4	500
1	2002/03	24	0	24	467	4	471	491	4	495
1	2003/04	13	0	13	454	4	458	467	4	471
1	2004/05	33	0	33	421	4	425	454	4	458
1	**2001-05**	**75**	**0**	**75**	**421**	**4**	**425**	**496**	**4**	**500**
2	2001/02	15	0	15	330	3	333	345	3	348
2	2002/03	22	0	22	308	3	311	330	3	333
2	2003/04	24	0	24	284	3	287	308	3	311
2	2004/05	2	0	2	282	3	285	284	3	287
2	**2001-05**	**63**	**0**	**63**	**282**	**3**	**285**	**345**	**3**	**348**
3	2001/02	2	0	2	135	43	178	137	43	180
3	2002/03	8	0	8	127	43	170	135	43	178
3	2003/04	4	0	4	123	43	166	127	43	170
3	2004/05	2	0	2	121	43	164	123	43	166
3	**2001-05**	**16**	**0**	**16**	**121**	**43**	**164**	**137**	**43**	**180**
All	2001/02	22	0	22	956	50	1006	978	50	1028
All	2002/03	54	0	54	902	50	952	956	50	1006
All	2003/04	41	0	41	861	50	911	902	50	952
All	2004/05	37	0	37	824	50	874	861	50	911
All	**2001-05**	**154**	**0**	**154**	**824**	**50**	**874**	**978**	**50**	**1028**

Grey cells are calculated

2.2. Data analysis / methods

Assuming that the selected plots are representative for the whole Tróia Peninsula, the total number of *P. pinaster* is about 130000 and of *P. pinea* 6700. The total number of *P. halepensis* trees is negligible, as below 40 trees.

In a second scenario we assume that 80 % of the forest area is dominant *P. pinaster* and the remaining area is dominant *P. pinea,* we get 60000 *P. pinaster* and 14000 *P. pinea* trees on the Tróia Peninsula. Both scenarios were used to estimate the overall mortality.

Assuming additionally that the distribution is homogenous in the plots the mean distance between two pinus trees is 4m to 8m, between to *P. pinea* trees is 15m to 60m.The 95 %-confidence intervals for the mortality rates were calculated using F-quantiles to approximate the Binomial distribution (Pearson-Clopper values). In case of no observed mortality the one-sided version was used.

Differences between the mortality rate of *P. pinaster* and *P. pinea* are only visible for the cumulated years of plot 3 and all plots. The overall mortality rate of *P. pinea* is less than 1.45 % (upper 95 %-CI limit), while for *P. pinaster* it is 3.94 % (95 %-CI: 3.38-4.55 %).

Table 6: Rough estimation of the mean distance between *Pinus* trees and the total number of *Pinus* trees on Tróia Peninsula

	No of Pinus *trees*					*Size* (ha)	*Mean distance between trees (m)*	
	P. pinaster		*P. pinea*		All		All[1]	*P. pinea*[1]
	absolute	relative (%)	absolute	relative (%)	absolute			
Plot 1	496	99.2	4	0.8	500	1	4.5	50.0
Plot 2	345	99.1	3	0.9	348	1	5.4	57.7
Plot 3	137	76.1	43	23.9	180	1	7.5	15.2
Total[2]	978	95.1	50	4.9	1028	3[2]		
Tróia[2]	130 400	95.1	6667	4.9	137067	400		
Dominant *P. pinaster*	177	89.4	21	10.6	198	1	7.1	21.8
Mixed	111	69.8	48	30.2	159	1	7.9	14.4
Dominant *P. pinea*	27	23.3	89	76.7	116	1	9.3	10.6
Dominant *P. pinaster*	56 640	89.4	6720	10.6	63360	320[3]		
Dominant *P. pinea*	2160	23.3	7120	76.7	9280	80[3]		
Troia[3]	58 800	80.9	13 840	19.1	72640	400		

Grey cells are calculated

[1] Assumed to be a homogeneous distribution: distance = $\sqrt{1/\text{density}}$

[3] Assumed to be a similar composition of the total forest as in the three plots.

[2] Assumed to be a composition of 80 % dominant *P. pinaster* and 20 % dominant *P. pinea* forest. Only the last figure was provided by the authors.

Table 7: Comparison between average densities of pine trees on Tróia Peninsula with the selected experimental plots

		Average number of trees/ha in 2011				Number of trees/ha in 2001/02		
Forest type		*P. pinaster*	*P. pinea*	total	Experimental plot	*P. pinaster*	*P. pinea*	total
Dominant *P. pinaster*	 %	177 89.4	21 10.6	198	Plot 1	496 99.2	4 0.8	500
	 %				Plot 2	345 99.1	3 0.9	348
Mixed *P. pinaster* and *P. pinea*	 %	111 69.8	48 30.2	159	Plot 3	137 76.1	43 23.9	180
Dominant *P. pinea*	 %	27 23.3	89 76.7	116	none			

Table 8: Estimation of the mortality rates of *P. pinaster* and *P. pinea* on specific plots on Tróia Peninsula

Plot	Year (winter)	***No of dead trees***			***No of trees***			***No of years***	***Mortality rate per year***								
		Pinus pilaster	*Pinus pinea*	Total	*Pinus pinaster*	*Pinus pinea*	total		*Pinus pinaster*			*Pinus pinea*			total		
									(%)	confidence interval (95%) (%)		estimated (%)	confidence interval (95%) (%)		estimated (%)	confidence interval (95%) (%)	
1	2001/02	5	0	5	496	4	500	1	1.01	0.33	2.34	0.00	0.00	52.71	1.00	0.33	2.32
1	2002/03	24	0	24	491	4	495	1	4.89	3.16	7.19	0.00	0.00	52.71	4.85	3.13	7.13
1	2003/04	13	0	13	467	4	471	1	2.78	1.49	4.71	0.00	0.00	52.71	2.76	1.48	4.67
1	2004/05	33	0	33	454	4	458	1	7.27	5.06	10.06	0.00	0.00	52.71	7.21	5.01	9.97
1	**2001-05**	**75**	**0**	**75**	**496**	**4**	**500**	**4**	**3.78**	**3.02**	**4.65**	**0.00**	**0.00**	**13.18**	**3.75**	**3.00**	**4.61**
2	2001/02	15	0	15	345	3	348	1	4.35	2.45	7.07	0.00	0.00	63.16	4.31	2.43	7.01
2	2002/03	22	0	22	330	3	333	1	6.67	4.22	9.92	0.00	0.00	63.16	6.61	4.19	9.83
2	2003/04	24	0	24	308	3	311	1	7.79	5.06	11.37	0.00	0.00	63.16	7.72	5.01	11.26
2	2004/05	2	0	2	284	3	287	1	0.70	0.09	2.52	0.00	0.00	63.16	0.70	0.08	2.49
2	**2001-05**	**63**	**0**	**63**	**345**	**3**	**348**	**4**	**4.57**	**3.58**	**5.69**	**0.00**	**0.00**	**15.79**	**4.53**	**3.55**	**5.64**
3	2001/02	2	0	2	137	43	180	1	1.46	0.18	5.17	0.00	0.00	6.73	1.11	0.13	3.96
3	2002/03	8	0	8	135	43	178	1	5.93	2.59	11.34	0.00	0.00	6.73	4.49	1.96	8.66
3	2003/04	4	0	4	127	43	170	1	3.15	0.86	7.87	0.00	0.00	6.73	2.35	0.64	5.91
3	2004/05	2	0	2	123	43	166	1	1.63	0.20	5.75	0.00	0.00	6.73	1.20	0.15	4.28
3	**2001-05**	**16**	**0**	**16**	**137**	**43**	**180**	**4**	**2.92**	**1.71**	**4.57**	**0.00**	**0.00**	**1.68**	**2.22**	**1.29**	**3.51**
All	**2001/02**	**22**	**0**	**22**	**978**	**50**	**1028**	**1**	**2.25**	**1.41**	**3.39**	**0.00**	**0.00**	**5.82**	**2.14**	**1.35**	**3.22**
All	**2002/03**	**54**	**0**	**54**	**956**	**50**	**1006**	**1**	**5.65**	**4.27**	**7.31**	**0.00**	**0.00**	**5.82**	**5.37**	**4.06**	**6.95**
All	**2003/04**	**41**	**0**	**41**	**902**	**50**	**952**	**1**	**4.55**	**3.28**	**6.12**	**0.00**	**0.00**	**5.82**	**4.31**	**3.11**	**5.80**
All	**2004/05**	**37**	**0**	**37**	**861**	**50**	**911**	**1**	**4.30**	**3.04**	**5.87**	**0.00**	**0.00**	**5.82**	**4.06**	**2.88**	**5.55**
All	**2001-05**	**154**	**0**	**154**	**978**	**50**	**1028**	**4**	**3.94**	**3.38**	**4.55**	**0.00**	**0.00**	**1.45**	**3.75**	**3.21**	**4.33**

Grey cells are calculated

2.3. Results / uncertainties

- Specific conditions on Tróia Peninsula might restrict the ability to apply the results to other areas in Portugal, especially those with a different composition of forest or density of trees. The authors state that the local forest, soil and climate characteristics are representative of the entire North Alentejo coast and the Ribatejo Province, which is where the most important stone pine plantations in Portugal can be found, with high production of edible seeds. Nevertheless, dominant *P. pinea* forests are only rarely (20 %) found on Tróia Peninsula (Sousa, 2011, clarifications).

- In all plots the total density of pine trees is higher and the percentage of *P. pinea* lower than the average forest composition on Tróia Peninsula (compared with the corresponding forest type). In plots 1 and 2, *P. pinaster* is dominant (500 and 348 trees/ha, respectively) and *P. pinea* accounts for only about 1% of trees, compared with an average of 198 *P. pinaster* trees/ha and 11 % *P. pinea* (Sousa, 2011, clarifications). Plot 3 is an example of mixed forest, but *P. pinaster* is the dominant species in most of the area on Tróia Peninsula. The exact acreage of the different forest types was not provided by the authors. Thus, the plots clearly display special characteristics and cannot be seen as representative of Tróia Peninsula and thus of the entire North Alentejo coast and the Ribatejo Province.

- No information on the infestation with PWN, presence of *M. galloprovincialis* or the bark beetle is given. No information on the infestation of symptomless trees is given. The connection to PWN is weak. PWN might be present in symptomless trees, but there may also be other reasons (bark beetle) for the observed mortality..

- No discussion on the differences between the years is provided.

- The numbers of *P. pinea* trees in plots 1 and 2 are too small to draw conclusions. Combining all plots and years gives an estimate of the cumulative mortality rate of *P. pinea* of below 1.45 % (upper limit of 95 % confidence interval). The yearly mortality rate for *P. pinaster* is about 4 % (95 % CI 3.38–4.55 %).

- Nevertheless the authors state that the local forest, soil and climate characteristics are representative of the entire North Alentejo coast and the Ribatejo Province, which is where the most important stone pine plantations in Portugal can be found, with high production of edible seeds. However, the selected plots represented only pine density and composition on the Tróia Peninsula with dominant *P. pinaster* forests and mixed *P. pinaster* (about 70 %) *P. pinea* (about 30 %) forest. No information on dominant *P. pinea* forests, which account for 20 % of the pine forest on Trója Peninsula, was provided in this experiment.

3. Experiments reported in Naves et al. (2006) Experiment 2: Oviposition of M. galloprovincialis (Table VIII)

3.1. Screening of the documentation / description of datasets

Sources:

Naves PM, Sousa E and Quartau JA, 2006. Feeding and oviposition preferences of *Monochamus galloprovincialis* for certain conifers under laboratory conditions. Entomologia Experimentalis et Applicata 120: 99-104 (experiment 2).

Sousa E, Naves P, Bonifacio L and Rodrigues JM, 2011. Risk assessment of *Pinus pinea* L. in Relation to Pine Wood Nematode. Portuguese National Institute of Biological Resources (INRB) and Portuguese National Forestry Authority, June 2011. (supporting document).

Sousa E (2011). Personal communication. Clarifications of the authors received on 21 October 2011.

Item	***Description based on the submitted document(s)***
<u>Description of the proposed risk reduction option</u>	
Target pest	**Pine sawyer** *Monochamus galloprovincialis* (Olivier)
Target plant material/product	**Pine trees,** *Pinus* sp., in Portugal Aleppo pine, *P. halepensis/* Monterey pine, *P. radiata/* Maritime pine, *P. pinaster/* Stone pine, *P. pinea/* Scots pine, *P. sylvestris* White cedar, *Cupressus lusitanica/* Mexican cypressm, *Pseudotsuga menziesii*
Disease	**Oviposition** Is vector of PWN for pine wilt disease
Origin of plant material/product	
Type of risk reduction option	Oviposition preferences due to defence abilities of adult pines
Place of implementation	**Portugal**
Other relevant information	
Experimental assessment of the option efficacy to reduce pest infestation in plant material/product <u>under laboratory/controlled conditions</u>	
Plant material information Type of plant material/product used in the experiment	Trees cut in last week of June2003, bolts used 20 days after cutting. 60 cm long × 6-12 cm diameter, ends coated with paraffin Characteristics: → **Diameter** (in mm)/ → **Bark thickness** (in mm)
Plant identity (e.g. botanical name, variety)	Origin of different trees from Tróia Peninsula: Maritime pine, *P. pinaster/* Stone pine, *P. pinea* from Monsanto Park, Lisbon: Aleppo pine, *P. halepensis/*

	White cedar, *Cupressus lusitanica* from VN de Cerveira, Minho province: Scots pine, *P. sylvestris/* Monterey pine, *P. radiata/* Mexican cypressm, *Pseudotsuga menziesii*
Conditions under which plant materials/products are managed	Stored in a room at ambient temperature (24 °C).
Conditions of the plant commodity (e.g. degree of ripeness, presence of bark, etc.)	
Pest information Identity (species, strains, biotypes if applicable)	
Conditions under which the pests are cultured, reared or grown	Insects emerged in June 2003 from *P pinaster* logs kept in wooden boxes in Tróia. Logs were PWN free. Start of experiment in July: bolts 20 days after cutting Incubation: After 5 days bolts were kept: 80 days, 25 °C, 16 h light/8 h dark 40 days, 8 °C, 0 h light/24 h dark 120 days, 25 °C, 16 h light/8 h dark then debarked, dissected
Method of infestation	Three adult insect couples (*ca.* 25 days old) that had not previously reproduced. Randomly chosen and placed in a 0.2 m³ screened wooden box along with a single bolt. Dead insects were immediately replaced by another one of same sex and age
Level of infestation	
Stage of the pest that is most resistant to the treatment	
Was the most resistant stage used in the experiment?	
Potential development of resistance to the option	
Experiment(s) description and analysis	Five replications of three insect couples per bolt of seven pines = 35 experiments
Variables used to measure efficacy	After 5 days Number of oviposition slits were counted → **Number of eggs** There are no external visual differences between empty and egg-containing pits, so it was necessary to debark and analyse the pine logs (at the end of the experiment) to find that on average 75 % of the egg pits contained eggs, on all the tested confers. Only the oviposition pits with eggs were counted. After breeding /return to 25 °C: Number of days until emergence → **Days till emergence**/ Number of emerged beetles → **number emerged beetles**/ Size (length of right elytron) → **Elytral length per sex**/ sex of brood adults recorded

	→ **Percentage females** **Emergence rate** calcutated as ratio of number of emerged beetles divided by number of eggs After debarking: Number of immature organisms counted
Factors influencing efficacy which were taken into account in the experiment	Only one experimental setting
Factors influencing efficacy which were not taken into account in the experiment	Age of the wood, temperature, type of wood from which the beetles emerged, differences due to PWN infestation, thickness of bark, diameter of bolts
Description of facilities and equipment	
Description of treatment	Bolts from seven kind of trees
Monitoring of critical parameters	
Description of experimental design	
Presentation of the data	Mean values and standard error of the mean (SEM) for each of the seven kinds of bolts For oviposition: Eggs were laid in all five bolts of *P. sylvestris*, *P. halepensis*, *P. pinaster*, and in three (of five) bolts of *P. radiata*, two of *P. pinea*, one of *P. menziesii*, and none of *C. lusitanica*
Description of the statistical analysis	Analysis of variance test: Kruskal–Wallis test for differences of: Emergence rate Fisher least significant difference (LSD) for: Number of eggs/ days to emergence/ elytral length of males/ elytral length of females
Conclusions of the experiment	**Oviposition/eggs:** Highest number for *P. sylvestris* Second highest for *P. halepensis* and *P. pinaster* Lower for *P. radiata* Lowest for *P. pinea* and *P. menziesii* None in *C. lusitanica* **Emergence rate:** No difference for *P. halepensis*, *P. pinaster*, *P. radiata* and *P. sylvestris* None for *P. pinea* and *P. menziesii* Minor differences in days until emergence or elytral length (largest *P. pinaster*) No correspondence to bolt diameter or bark thickness Conclusion: *P. pinea*, *P. menziesii* and *C. lusitanica* are not adequate hosts for *M. galloprovincialis* Largest size in *P. pinaster* indicates best adaptation
Other relevant information	*P. halepensis* is similar to *P. pinea* and is not affected by PWN in Portugal, but shows similar characteristics as *P. pinaster* in this experiment.

3.1.1. Extracted data

Table 9: Bolt characteristics, mean number of eggs laid and emergence on seven kind of conifers (Naves et al., 2006, table 2)

Pine species	**Bolt diameter** (mm)		**Bark thickness** (mm)		**No of eggs laid** (mean per bolt/ total = five replicates)			**Emergence** (mean per bolt/total = five replicates)			**Days until emergence**		**Females**		**Elytral length** (mm)			
								total	rate	SEM			absolute	relative	males		females	
	mean	SEM	mean	SEM	total	mean	SEM		(%)		mean	SEM		(%)	mean	SEM	mean	SEM
P. halepensis	136.6	4.9	1.5	0.1	67	13.4	2.4	28	56.2	9.0	80.7	4.1	14	50	13.8	0.3	14.3	0.4
P. pinaster	86.8	6.4	2.8	0.7	52	10.4	0.8	14	54.6	5.6	76.7	3.2	5	36	14.5	0.2	15.6	0.4
P. pinea	108.4	9.3	2.1	0.2	3[1]	0.6	0.4	0	0		n/a		n/a	n/a	n/a		n/a	
P. radiata	93.3	9.4	1.7	0.3	28	5.6	3.1	18	43.6	20.0	72.1	3.5	10	56	13.8	0.3	14.1	0.4
P. sylvestris	105.6	6.5	1.4	0.0	109	21.8	2.1	44	52.8	9.9	72.1	2.3	17	39	13.0	0.3	14.6	0.4
P. menziesii	88.9	6.8	2.0	0.3	2	0.4	0.4	0	0		n/a		n/a	n/a	n/a		n/a	
C. lusitanica	96.6	5.1	2.6	0.1	0	0		n/a	n/a		n/a		n/a	n/a	n/a		n/a	
Total					**261**			**104**					**46**					

Grey cells are calculated
Individual results: [1]=(0/0/0/1/2); [2]=(0/0/0/0/2)

3.2. Data analysis/methods

No additional calculations were performed.

3.3. Results/uncertainties

- The experiment does not take into account the age of the wood, seasonal differences in wood quality for development of *M. galloprovincialis* (Akubulut et. al. 2007), temperature, the thickness of the bark, the diameter of bolts and the pine species from which the adult beetles originally emerged. All these factors are fixed in the experiment.

- The experiment considers only oviposition and emergence, but not feeding (experiment 1 in Naves et al., 2006). The results are not valid for real-life situations, in which PWN infestation is connected to *Monochamus* feeding.

- The number of eggs laid in *P. pinea* and *P. menzielii* are too small to estimate a rate of emergence.

- No information is presented on oviposition when alternative pine species are available. Is the oviposition delayed only when no optimal material is present?

- In summary, the oviposition of *M. galloprovincialis* is reduced but remains possible in *P. pinea*. Although the results suggest that further development through to adult stage does not take place under the conditions tested, definitive conclusions on the emergence in *P. pinea* in the field cannot be drawn from this experiment.

4. Experiments reported in Naves et al. (2006) Experiment 3: Preferences of M. galloprovincialis (Table IX)

4.1. Screening of the documentation/description of datasets

Source:

Naves PM, Sousa EM de and Quartau JA, 2006. Feeding and oviposition preferences of *Monochamus galloprovincialis* for certain conifers under laboratory conditions. Entomologia Experimentalis et Applicata, 120: 99-104 (experiment 3).

Sousa E, Naves P, Bonifacio L and Rodrigues JM, 2011. Risk assessment of *Pinus pinea* L. in Relation to Pine Wood Nematode. Portuguese National Institute of Biological Resources (INRB) and Portuguese National Forestry Authority, June 2011. (supporting document)

Sousa E, 2011. Personal communication. Clarifications of the authors received on 21 October 2011.

Item	***Description based on the submitted document(s)***	***Comments***
	Description of the proposed risk reduction option	
Target pest	**Pine sawyer** *Monochamus galloprovincialis* (Olivier)	
Target plant material/product	**Pine trees,** *Pinus sp.*, in Portugal Aleppo pine, *P. halepensis/* Monterey pine, *P. radiata/* Maritime pine, *P. pinaster/* Stone pine, *P. pinea/*	

	Scots pine, *P. sylvestris*
Disease	**Oviposition** Is vector of PWN for pine wilt disease
Origin of plant material/product	
Type of risk reduction option	Oviposition preferences due to defence abilities of adult pines
Place of implementation	**Portugal**
Other relevant information	

Experimental assessment of the option efficacy to reduce pest infestation in plant material/product under laboratory/controlled conditions

Plant material information Type of plant material/product used in the experiment	Trees cut in last week of June 2003, branches used 3–4 days after cutting, bolts used 20 days after cutting. bolts: 60 cm long × 6–12 cm diameter, ends coated with paraffin Characteristics: → **Diameter** (in mm)/ → **Bark thickness** (in mm) Selected to get similar characteristics of the pairs
Plant identity (e.g. botanical name, variety)	Origin of different trees from Tróia Peninsula: Maritime pine, *P. pinaster/* Stone pine, *P. pinea* from Monsanto Park, Lisbon: Aleppo pine, *P. halepensis* from VN de Cerveira, Minho province: Scots pine, *P. sylvestris/* Monterey pine, *P. radiata*
Conditions under which plant materials/products are managed	Stored in a room at ambient temperature (24 °C)
Conditions of the plant commodity (e.g. degree of ripeness, presence of bark, etc.)	
Pest information Identity (species, strains, biotypes if applicable)	
Conditions under which the pests are cultured, reared or grown	Insects emerged in June 2003 from *P. pinaster* logs kept in wooden boxes in Tróia. Logs were PWN free Beetles were kept for maturation feeding in acrylic boxes (80 × 40 cm) along with one branch of *P. pinaster*

	and one of other type
Method of infestation	After 25 days, four adult insect couples that had not previously reproduced Randomly chosen and placed in a 0.7-m³ screened wooden box along with one bolt of *P. pinaster* in one corner and one bolt of another type (same as for maturation feeding) in the opposite corner Dead insects were immediately replaced by another one of same sex and age
Level of infestation	
Stage of the pest that is most resistant to the treatment	
Was the most resistant stage used in the experiment?	
Potential development of resistance to the option	
Experiment(s) description and analysis	Five replications of four insect couples per four pairs of bolts = 20 experiments
Variables used to measure efficacy	After 72h bolts were debarked Number of oviposition slits with eggs were counted → **Number of eggs**
Factors influencing efficacy which were taken into account in the experiment	Only one experimental setting
Factors influencing efficacy which were not taken into account in the experiment	Age of the wood, temperature, type of wood from which the beetles emerged, differences due to PWN infestation, thickness of bark, diameter of bolts No preference of *P. pinaster* might related to thinner bark (as generally preferred)
Description of facilities and equipment	
Description of treatment	Bolts from *P. pinaster* paired with four other types: *P. halepensis/* *P. pinea/* *P. radiata/* *P. sylvestris*
Monitoring of critical parameters	
Description of experimental design	

Presentation of the data	Mean values and standard error of the mean (SEM) for each kind of bolt
Description of the statistical analysis	Wilcoxon signed-rank test to compare the two types of wood
Conclusions of the experiment	**Oviposition/eggs:** No differences between: *P. pinaster* vs. *P. halepensis* *P. pinaster* vs. *P. sylvestris* Preference of *P. pinaster:* *P. pinaster* vs. *P. pinea* *P. pinaster* vs. *P. radiata*
Other relevant information	*P. halepensis*, like *P. pinea*, is not affected by PWN in PT, but shows similar characteristics to *P. pinaster* in this experiment

4.1.1. Extracted data

Table 10: Bolt characteristics and mean number of eggs laid on pines in four paired experiments (Naves et al., 2006, table 3)

Pine species pairs)	Bolt diameter (mm)		Bark thickness (mm)		**No of eggs laid** (mean per bolt /total = five replicates)		
	mean	SEM	mean	SEM	total	mean	SEM
P. pinaster	74.0	7.8	1.9	0.1	25	5.0	1.6
P. halepensis	80.1	10.9	1.2	0.1	17	3.4	2.2
Total					**42**	**8.4**	
P. pinaster	85.9	10.5	2.2	0.4	19	3.8	1.2
P. pinea	88.8	9.9	1.7	0.3	2	0.4	0.2
Total					**21**	**4.2**	
P. pinaster	93.2	8.3	2.3	0.1	15	3.0	0.6
P. radiata	95.7	8.2	2.2	0.2	2	0.4	0.4
Total					**17**	**3.4**	
P. pinaster	66.3	8.3	1.7	0.4	44	8.8	2.7
P. sylvestris	68.8	7.5	1.0	0.1	67	13.4	1.1
Total					**111**	**22.2**	

4.2. Data analysis/methods

No additional calculations were performed.

4.3. Results / uncertainties

- The experiment does not take into account the age of the wood, seasonal differences in wood quality for development of *M. galloprovincialis* (Akubulut et al., 2007), temperature, the thickness of the bark, the diameter of bolts and the pine species from which the adult beetles originally emerged. All these factors are fixed in the experiment.

- The experiment considers only on oviposition, but not feeding (experiment 1 in Naves et al., 2006). The results are not valid for real-life situations, in which PWN infestation is connected to *Monochamus* feeding.

- The design does not obtain pure preference for oviposition, because the total number of eggs differs between the four paired experiments. The results were not standardised to equal total number of eggs per paired experiment.

- *P. sylvestris* induces egg laying, whereas *P. pinea* and *P. radiata* inhibit it. The mechanism causing this is not presented.

- The experiment found no differences between *P. pinaster* and *P. halepensis,* but it is not known whether this is also reflected in the mortality rates.

- The experiment does not reflect the situation when the composition of the forest is not an equal 50:50 mix of two species of pines and thus cannot explain host plant choice under monoculture or *P. pinea* dominance.

REFERENCES

Akbulut S, Keten A, Baysal I and Yüksel B, 2007. The effect of log seasonality on the reproductive potential of Monochamus galloprovincialis Olivier (Coleoptera: Cerambycidae) reared in black pine logs under laboratory londitions. Turkish Journal of Agriculture Forestry, 31, 413-422.

Naves PM, Sousa EM de and Quartau JA, 2006. Feeding and oviposition preferences of *Monochamus galloprovincialis* for certain conifers under laboratory conditions. Entomologia Experimentalis et Applicata, 120, 99-104.

Sousa E, Naves P, Bonifacio L and Rodrigues JM, 2011a. Risk assessment of *Pinus Pinea L.* in Relation to Pine Wood Nematode. Portuguese National Institute of Biological Resources (INRB) and Portuguese National Forestry Authority, June 2011. (supporting document, accessible at www.efsa.europa.eu).

Sousa E, 2011. Personal communication. Clarifications of the authors received on 21 October 2011.

B. LITERATURE SEARCH

1. Search performed on 15/09/2011 on ISI Web of Knowledge

	Key words	**Synonyms and/or common names**	**Search no**	**No of hits**	**After screening (20 September 2011)**
Organism	*Bursaphelenchus xylophilus*	*B. lignicolus* *B. xylophilus* PWN *Aphelencoides xylophilus* Pine wood nematode Pinewood nematode Pine wilt disease	12	2205	
Host plants	*Juniperus* spp.	Juniper	1	Approx. 13 712	
	Cryptomeria spp.	Sugi Japanese Cedar Dhuppi	2	Approx. 9 478	
	Pinus pinea	Stone pine Umbrella pine Parasol pine *Pinus sativa* (invalid name but used)	3	Approx. 5 678	
	Chamaecyparis spp.	False cypress (incorrect but used) *Retinispora* Siebold & Zucc. *Retinospora* Carr.	4	Approx. 5 841	
Vector	*Monochamus*	Sawyer beetles Sawyers	5	Approx 2 421	
COMBINATIONS					
Organism AND Host plants					
Organism	AND	*Juniperus*	13= 12 AND 1	7	4
	AND	*Cryptomeria*	14= 12 AND 2	10	5
	AND	*Pinus pinea*	15= 12 AND 3	11	7
	AND	*Chamaecyparis*	16=12 AND 4	11	5
		ALL 4	13OR14OR15OR16	**33**	**18**
Organism AND Vector					
Organism	AND	vector	17= 11 AND 5	**499**	**172**
Vector AND Host plants					
Vector	AND	*Juniperus*	6= 5 AND 1	7	4
	AND	*Cryptomeria*	7= 5 AND	10	3

			2		
	AND	*Pinus pinea*	8= 5 AND 3	15	11
	AND	*Chamaecyparis*	9= 5 AND 4	4	1
	AND	ALL 4	9OR8OR7 OR6	**32**	**17**
TOTAL	**for Screening**		17OR13OR14OR15OR16OR9OR8OR 7OR6	**543**	**194**

2. Search strategy

# 18	543	#17 OR #16 OR #15 OR #14 OR #13 OR #9 OR #8 OR #7 OR #6 *Timespan=All Years* *Search language=English Lemmatization=On*
# 17	499	#12 AND #5 *Timespan=All Years* *Search language=English Lemmatization=On*
# 16	11	#12 AND #4 *Timespan=All Years* *Search language=English Lemmatization=On*
# 15	11	#12 AND #3 *Timespan=All Years* *Search language=English Lemmatization=On*
# 14	10	#12 AND #2 *Timespan=All Years* *Search language=English Lemmatization=On*
# 13	7	#12 AND #1 *Timespan=All Years* *Search language=English Lemmatization=On*
# 12	2,205	#11 OR #10 *Timespan=All Years* *Search language=English Lemmatization=On*
# 11	2,033	Topic=(lignicolus OR xylophilus) OR Topic=(PWN) OR Topic=(Aphelenchoides AND xylophilus) *Timespan=All Years* *Search language=English Lemmatization=On*
# 10	1,897	Topic=((Bursaphelenc* xylophilus) OR (("Pine wood" OR Pinewood) AND nematode) OR ("Pine wilt disease")) *Timespan=All Years* *Search language=English Lemmatization=On*
# 9	4	#5 AND #4 *Timespan=All Years* *Search language=English Lemmatization=On*
# 8	15	#5 AND #3 *Timespan=All Years* *Search language=English Lemmatization=On*
# 7	10	#5 AND #2 *Timespan=All Years* *Search language=English Lemmatization=On*
# 6	7	#5 AND #1 *Timespan=All Years* *Search language=English Lemmatization=On*
# 5	2,421	Topic=(Monochamus) OR Topic=(sawyer) *Timespan=All Years* *Search language=English Lemmatization=On*
# 4	Approximately 5,841	Topic=(Chamaecyparis) OR Topic=("false cypress") OR Topic=(retinispora) *Timespan=All Years* *Search language=English Lemmatization=On*
# 3	Approximately 5,678	Topic=(Pinus AND (pinea OR sativa)) OR Topic=((Stone OR Umbrella OR Parasol) AND pine) *Timespan=All Years* *Search language=English Lemmatization=On*
# 2	Approximately 9,478	Topic=(Cryptomeria OR Sugi OR "Japanese Cedar" OR Dhuppi) *Timespan=All Years* *Search language=English Lemmatization=On*
# 1	Approximately 13,712	Topic=(Juniperus OR Juniper) *Timespan=All Years*

3. Screening

First screening was made by the working group on the basis of irrelevance:

- not relevant host species
- not relevant vector
- not relevant organism
- not relevant to the topic (addressing control measures, general documents etc.).

4. Other datasources consulted

1) http://www.cipm.info
2) http://www.greynet.org/greysourceindex.html
3) http://www.opengrey.eu
4) http://www.evaluationcanada.cc
5) http://www.science.gov
6) http://www.scienceaccelerator.gov
7) http://worldwidescience.org
8) http://www.euforgen.org/databases.html
9) http://cordis.europa.eu/library
10) http://www.nyam.org/library/online-resources/grey-literature-report
11) http://www.osti.gov
12) http://highwire.stanford.edu

Thesis and Dissertations:

13) http://www.collectionscanada.gc.ca/thesescanada/index-e.html
14) http://adt.caul.edu.au/homesearch/advancedsearch
15) http://indcat.inflibnet.ac.in/indcat/thesis.jsp
16) http://www.openthesis.org
17) http://www.dissertation.com/browse.php
18) http://www.dissonline.de
19) http://www-apps.crl.edu/catalog/dissertationSearch.asp
20) http://www.ndltd.org
21) http://www.phddata.org
22) http://www.theses.com

Asian literature databases (and full-texts):

23) http://www.journalarchive.jst.go.jp/english/top_en.php
24) http://www.jstage.jst.go.jp/browse
25) http://www.jsnfs.or.jp/english/english_jnsv.html
26) http://synapse.koreamed.org/
27) http://ci.nii.ac.jp

C. HOST DISTRIBUTION MAPS FROM JRC

This section describes the datasets and the data-processing methodology which has been used by the Joint Research Center of the European Commission, Institute for Environmental Sustainability, to generate the maps provided in this opinion. The maps have been generated using recently available forest data from European National Forest Inventories and harmonized within the European Forest Data Centre (EFDAC, 2005).

Additional datasets which have been considered are the one from the BioSoil Project (Hiederer and Durrant, 2010; Lacarce et al., 2011) and the dataset of the Forest Focus Monitoring Database System (Hiederer et al., 2006; 2007).

The maps also allow the comparison with the observations reported by the Global Biodiversity Information Facility (GBIF) datasets (Edwards, 2004; Yesson et al., 2007; Gilman et al., 2009). The Iberian peninsula has been object of further analysis and the information available from the Spanish Forest Map (“Mapa Forestal de España”, MFE) and from Portuguese regional forest plans (“Planos Regionais de Ordenamento Florestal”, PROF) have been reviewed and the MFE data have been mapped with respect to information derived by aggregating several categories of the Corine Land Cover 2006.

European National Forestry Inventories

The maps derived for European areas have been generated using recently available forest data from the E-forest consortium (EFDAC, 2008) which is part of a framework contract to broaden and develop the knowledge base of the European Forest Data Centre (EFDAC, 2005).

The data which have been used refer to the presence/absence of a given forest tree species with a spatial resolution of 1km/pixel brought up into line with an INSPIRE (European Parliament and Council, 2007[15]; INSPIRE, 2007) compliant 1×1 km Grid. The presence/absence records provided by the E-Forest platform have been harmonized from the original one of the National Forest Inventories. They concern the following Countries: Austria, Czech Republic, Denmark, Finland, France, Germany, Hungary, Italy, Latvia, Lithuania, Netherlands, Norway, Portugal, Romania, Spain, Slovak Republic, Sweden, Switzerland, United Kingdom.

The inventory date associated with the records range from 1993 to 2009 with the following distribution[16]:

YEAR	PERC.	YEAR	PERC.
1993	0.5%	2002	9.7%
1994	1.2%	2003	4.3%
1995	4.0%	2004	8.4%
1996	0.6%	2005	10.3%
1997	0.9%	2006	13.5%
1998	4.1%	2007	9.6%
1999	4.7%	2008	9.7%
2000	4.8%	2009	1.7%
2001	11.8%		

Therefore, more than 96 % of the data have been recorded between 1995 and 2008, more than 91 % between 1998 and 2008 and more than 51 % between 2004 and 2008.

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[15] Directive 2007/2/EC of the European Parliament and of the Council of 14 March 2007 establishing an Infrastructure for Spatial Information in the European Community (INSPIRE). Official Journal of the European Communities (25.4.2007), 2007/2/EC.

[16] Statistics from a representative subset of data, to be updated to the complete dataset in the final version.

BioSoil Project

The BioSoil project has been one of the demonstration studies initiated in response to the the “Forest Focus” Regulation (EC) No. 2152/2003 concerning monitoring of forests and environmental interactions in the Community (European Parliament and Council, 2003[17]) to develop the EU forest monitoring scheme “by means of studies, experiments, demonstration projects, testing on a pilot basis and establishment of new monitoring activities” (European Parliament and Council, 2003: art. 6).

The aim of the BioSoil project is to demonstrate how a large-scale European study can provide harmonised soil and forest biodiversity data and contribute to research and forest related policies.

The project comprised two main modules:

a) Soil Module;

b) Biodiversity Module.

Both modules used a common scheme for sampling data, which was also the location in many countries of the existing network of sites for monitoring the forest environment under Forest Focus - International Cooperative Programme on assessment and monitoring air pollution effects on Forests.

Forest Focus Monitoring Database System

The Forest Focus is a Community scheme for harmonized, broad-based, comprehensive and long-term monitoring of European forest ecosystems, normed by EC Regulation No 2152/2003 (European Parliament and Council, 2003). Under this scheme the monitoring of air pollution effects on forests is carried out by participating countries on the basis of the systematic network of observation points (Level I) and of the network of observation plots for intensive and continuous monitoring (Level II).

According to art. 15(1) of the Forest Focus Regulation, Member States shall annually, through the designated authorities and agencies, forward to the Commission geo-referenced data gathered under the scheme, together with a report on them by means of computer telecommunications and/or electronic technology. For managing the data JRC has implemented a Forest Focus Monitoring Database System.

Global Biodiversity Information Facility

In 2001 the Global Biodiversity Information Facility (GBIF) was established by governments. GBIF is intended to encourage free and open access online availability of biodiversity data and consists of a global network of 57 countries and 47 organizations (Edwards, 2004; Yesson et al., 2007; Gilman et al., 2009).

The plotted data extracted from GBIF have been filtered to ensure very elementary quality constraint to be respected:

D. only data with proper coordinates have been selected;

E. only years from 1930 to 2011 have been considered;

F. presumed presences located in impossible locations (e.g. seas) have been removed;

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[17] Regulation (EC) No 2152/2003 of the European Parliament and of the Council of 17 November 2003 concerning monitoring of forests and environmental interactions in the Community (Forest Focus). Official Journal of the European Communities L 324 (11.12.2003), pp. 1-8.

G. two basis of records have been excluded: "fossil" and "germplasm";

H. data categorized within the class "issues detected" have been removed.

The filtered GBIF data have been denominated as "reported presence". As required, we also plotted the subset of GBIF data unable to pass the aforementioned filters. Those data have been denoted as "uncertain presence".

Portugal, Planos Regionais de Ordenamento Florestal

For Portugal, regional forest plans (“Planos Regionais de Ordenamento Florestal”, PROF) are available for 21 sub-regions covering the whole country (Direcção Geral dos Recursos Florestais, 2006, 2007).

Those plans allow to map with variable spatial accuracy (the variability is based on the area of the corresponding minimal administrative units for each plan) the presence of *Pinus pinea*, irrespective of whether the reported presences correspond to forested areas or not.

Unfortunately, no information is available for *Cryptomeria*, *Chamaecyparis* and *Juniperus* genera. While a good assessment of the *Pinus pinea* distribution in Portugal is already available from the previously discussed data sources, PROFs have not been considered in this study. Nevertheless, it is worth noticing that, among the thematic categories made available by PROFs, there is also the one referring to wildlife or green corridors (Corredores Ecológicos), which could be of monitoring interest as possible ways of pest-spread (Williams, 1998; Kurttila, 2001; Kingsland, 2002).

Spain, Mapa Forestal de España

For Spain, Spanish Forest Map (“Mapa Forestal de España", MFE) is available country-wide (Ministério de Agricultura, 2001). It’s provided a dataset with the recorded presence of species - even not directly of forestry interest - on spatial polygons either within forested areas or in other categories of land cover.

The available Spanish data allow to map with variable spatial accuracy (the producer refers to a printed spatial scale of 1:200.000) the presence of *Pinus pinea*. Information appears to also be available for *Cryptomeria*, *Chamaecyparis* and *Juniperus* genera.

The Spanish forest map has been used as additional dataset to complement the information conveyed in the other maps. An additional analysis has been performed by intersecting the reported presence in MFE with the Corine Land Cover 2006 (European Environment Agency, 2011) categories to better classify the presence areas in five main land uses:

- Forested areas,
- Natural non-forested areas,
- Agricultural areas,
- Artificial surfaces (e.g. urbanized areas)
- Mixed landscape

The first category has been further divided in three sub-categories: broad-leaved forest; coniferous forest; mixed forest.

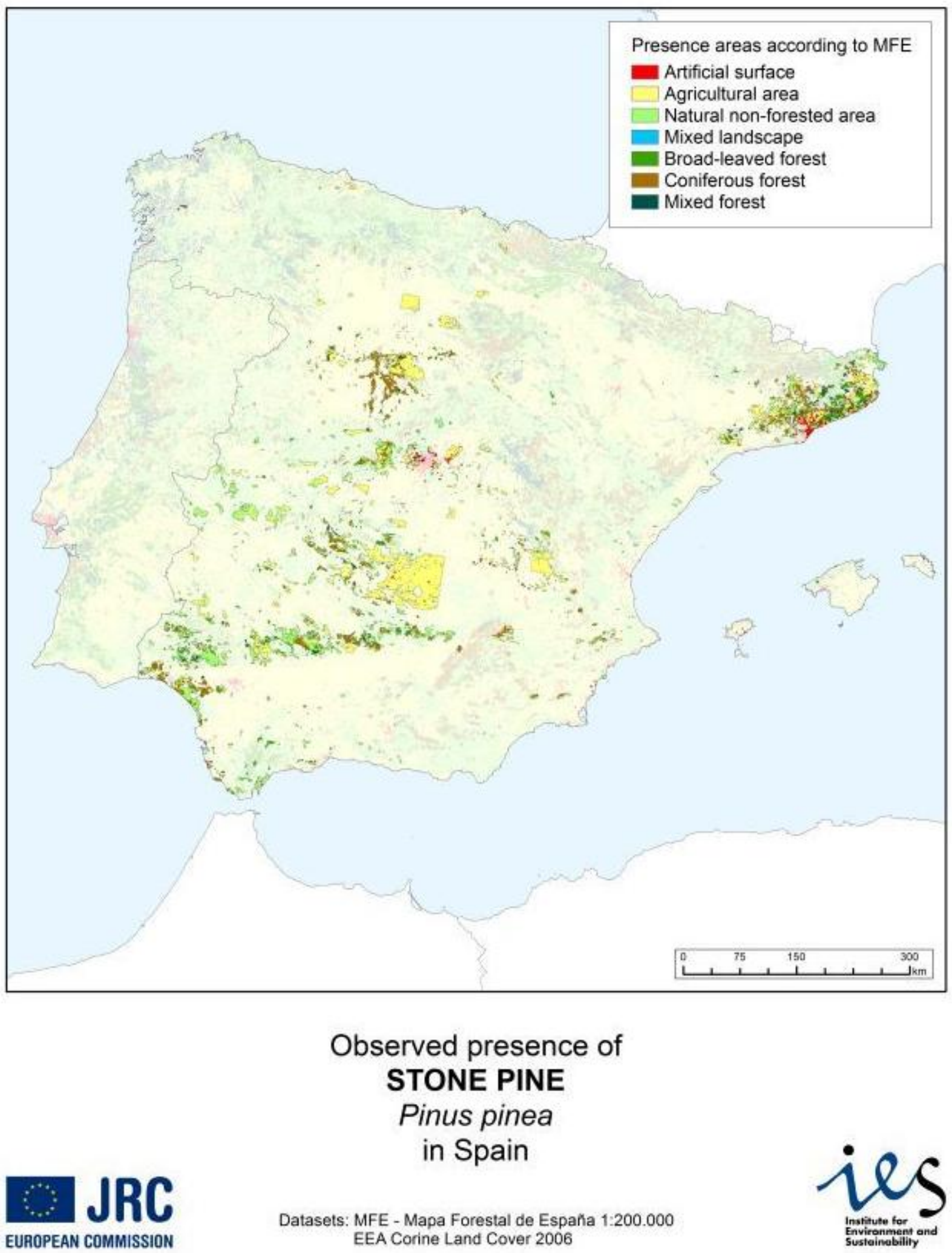
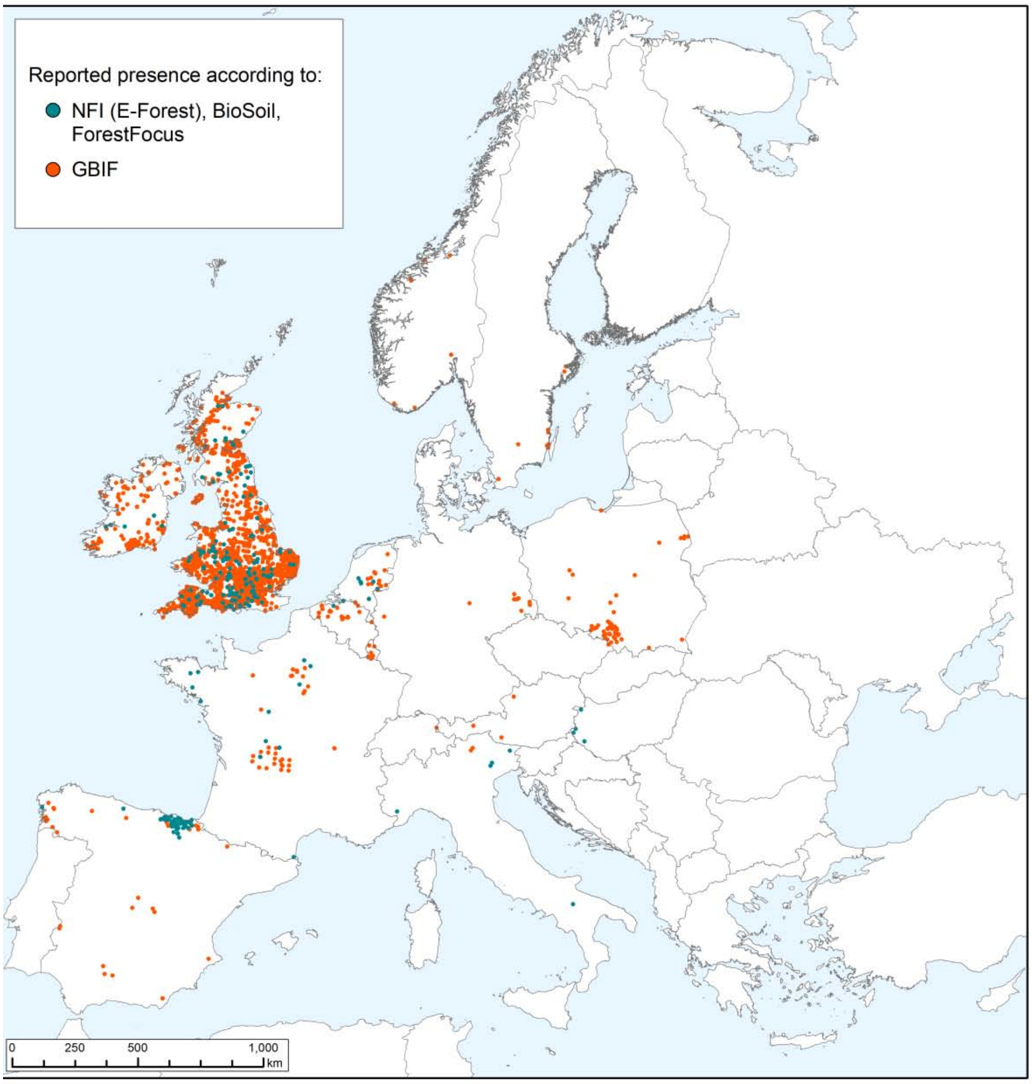


Figure 1: Spanish distribution of *Pinus pinea*



Observed presence of
CHAMAECYPARIS CYPRESS
Chamaecyparis sp.
in Europe

Datasets: NFI (E-Forest), BioSoil, ForestFocus
Global Biodiversity Information Facility (GBIF)

Figure 2: European distribution of *Chamaecyparis* spp

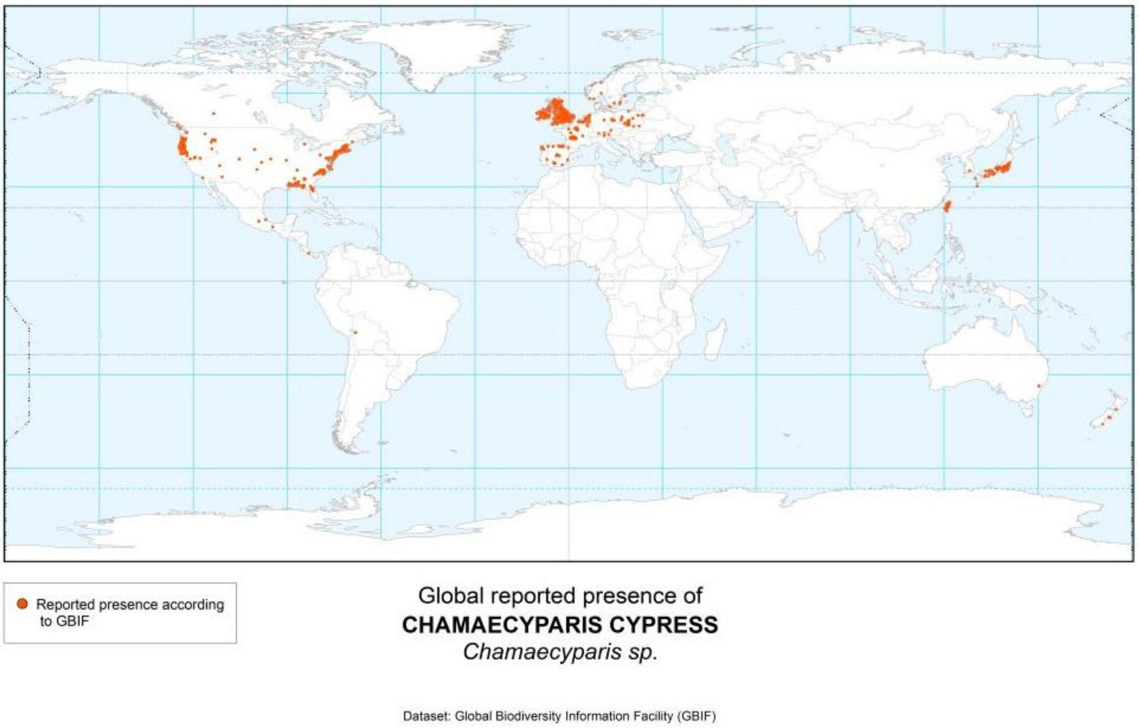


Figure 3: World distribution of *Chamaecyparis* spp.

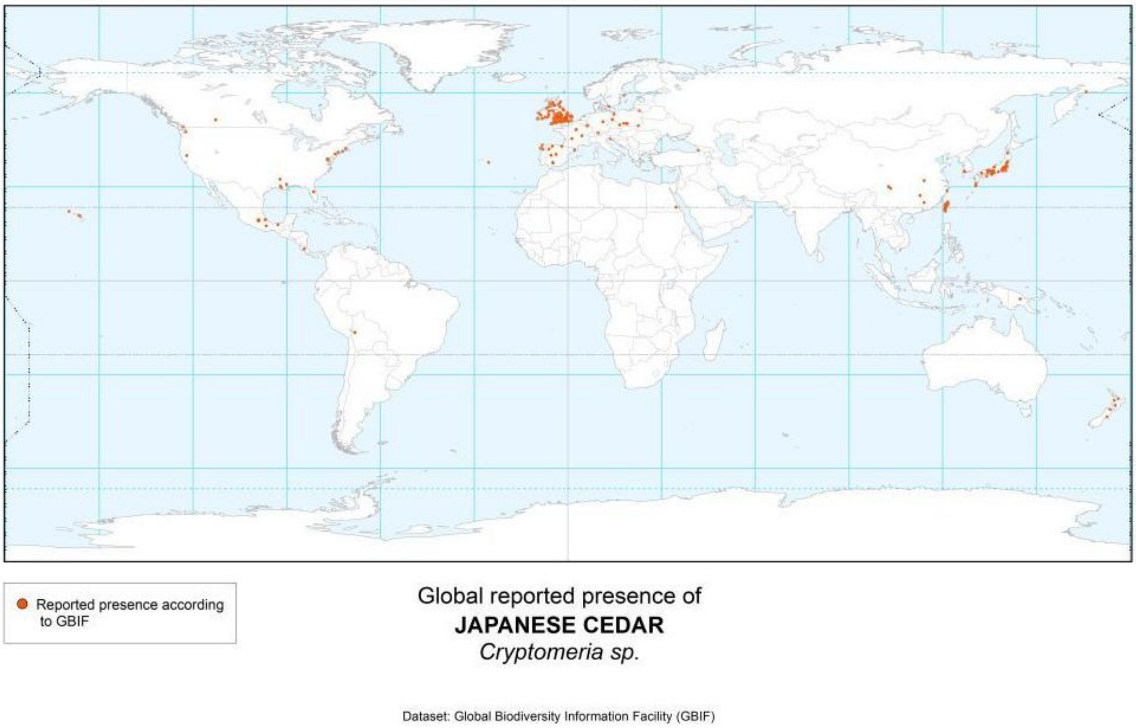


Figure 4: World distribution of *Cryptomeria* spp.

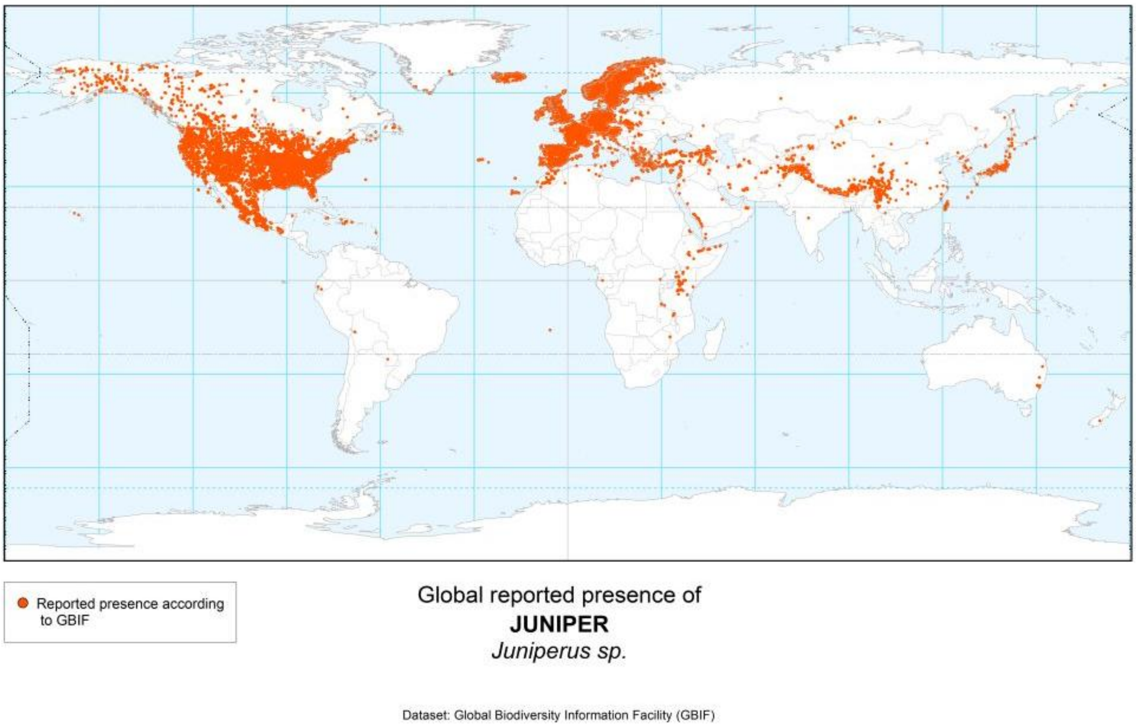


Figure 5: World distribution of *Juniperus* spp.

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BeBIF Provider, Belgian IFBL Flora Checklists (1939-1971)
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Berkeley Natural History Museums, UCBG TAPIR Provider
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Biologiezentrum Linz Oberoesterreich, Biologiezentrum Linz
Bioversity International, EURISCO The European Genetic Resources Search Catalogue
Borror Laboratory of Bioacoustics, Borror Laboratory of Bioacoustics
Botanical Research Institute of Texas, Botanical Research Intitute of Texas
Botanic Garden and Botanical Museum Berlin-Dahlem, Herbarium Berolinense
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GBIF Swiss Node, Global Mountain Biodiversity Assessment GMBA
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GEO-Tag der Artenvielfalt, AKG-Gelände (Bensheim)
GEO-Tag der Artenvielfalt, Aktion der Klasse H2 in Simmelsberg
GEO-Tag der Artenvielfalt, Artenvielfalt am 'Grünen Band' bei Coburg: 20 Jahre Wiedervereinigung
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GEO-Tag der Artenvielfalt, Artenvielfalt auf der Weide - GEO-Hauptveranstaltung in Crawinkel
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GEO-Tag der Artenvielfalt, Streuobstwiese Kugelberg (Ulm)
GEO-Tag der Artenvielfalt, Tage der Artenvielfalt rund um die Naturschutzstation Molsberg
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GEO-Tag der Artenvielfalt, Weide am Ostufer des Zotzensees Müritz-Nationalpark
GEO-Tag der Artenvielfalt, Zitadelle Berlin-Spandau (7b)
Harvard University Herbaria, Vascular plants of south-central China
Herbario SANT Universidade de Santiago de Compostela, SANT herbarium vascular plants collection
Herbarium Hamburgense, Impetus - Herbarium Hamburgense
Herbarium of the University of Aarhus, The AAU Herbarium Database
Icelandic Institute of Natural History, Herbarium (AMNH)
Icelandic Institute of Natural History, Herbarium (ICEL)
Icelandic Institute of Natural History, Observational database of Icelandic plants

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Institute of Dendrology Polish Academy of Sciences, Institute of Dendrology PAS Flora of Sudety Mountains
Institute of Nature Conservation Polish Academy of Sciences, National System of Protected Areas in Poland - Plants
Israel Nature and Parks Authority, Israel Nature and Parks Authority
Jyvaskyla University Museum - The Section of Natural Sciences, Invertebrate collection of Jyvaskyla University Museum
Jyvaskyla University Museum - The Section of Natural Sciences, Vascular plant collection of Jyvaskyla University Museum
Karl Franzens University of Graz Insitute for Botany - Herbarium GZU, Herbarium GZU
KBIF Data Repository, Insect (MNHM-IN)
KBIF Data Repository, Plant (KIWE-PL)
KBIF Data Repository, Plant (MNHM-PL)
KBIF Data Repository, Plant (NSMK-PL)
Louisiana State University Herbarium, Herbarium
Marine Science Institute UCSB, Paleobiology Database
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National Museum of Nature and Science Japan, Plant specimens depodited in Osaka Museum of Natural History Japan.
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Natural History Museum University of Oslo, Vascular plant herbarium Agder naturmuseum og botaniske hage
Natural History Museum University of Oslo, Vascular Plant Herbarium Oslo (O)
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Natural History Museum University of Oslo, Vascular Plants The Norwegian University of Life Sciences (NLH)
Natural History Museum Vienna - Herbarium W, Natural History Museum Vienna - Herbarium W
New Mexico Biodiversity Collections Consortium, New Mexico Biodiversity Collections Consortium database
Nicolaus Copernicus University of Torun, The Distribution Atlas of Butterflies in Poland
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NSW Department of Environment Climate Change and Water representing the State of New South Wales, OEH Atlas of NSW Wildlife
Oregon State University, Vascular Plant Collection
Research Institute for Nature and Forest, Florabank1
Royal Botanic Garden Edinburgh, RBGE Herbarium (E)
Royal Botanic Garden Edinburgh, RBGE Living Collections
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Scientific Research Centre of the Slovenian Academy of Sciences and Arts Institute of Biology, FloVegSI - Floristical and fitocenological database of ZRC SAZU
Service du Patrimoine naturel Muséum national d'Histoire naturelle Paris, Inventaire national du Patrimoine naturel (INPN)
Staatliche Naturwissenschaftliche Sammlungen Bayerns, Epiphytic Lichens of G. Lettau at the Botanical Museum Berlin-Dahlem
Staatliche Naturwissenschaftliche Sammlungen Bayerns, IBF Monitoring of Lichens
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SysTax, SysTax
Taiwan Biodiversity Information Facility (TaiBIF), National vegetation diversity inventory and mapping plan
Taiwan Forestry Research Institute, Herbarium of Taiwan Forestry Research Institute
TELDAP, ENDEMIC SPECIES RESEARCH INSTITUTE
TELDAP, Herbarium Biodiversity Research Center Academia Sinica Taipei
TELDAP, Plantae TAIF (Taiwan e-Learning and Digital Archives Program TELDAP)
Texas A&M University Insect Collection, Texas A&M University Insect Collection
The Danish Biodiversity Information Facility, Botany registration database by Danish botanists
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UK National Biodiversity Network, National Trust - Hatfield Forest species data held by The National Trust.
UK National Biodiversity Network, National Trust - Ickworth species data held by The National Trust.
UK National Biodiversity Network, North & East Yorkshire Ecological Data Centre - North and East Yorkshire Ecological Data Centre - Non-sensitive Records from all taxonomic groups.
UK National Biodiversity Network, Northern Ireland Environment Agency - EHS Species Datasets
UK National Biodiversity Network, Nottinghamshire Biological and Geological Records Centre - UK abstract from Nottingham City Museums & Galleries (NCMG) Insect Collection Baseline database
UK National Biodiversity Network, Phase 2 Lowland Grassland Survey of Wales
UK National Biodiversity Network, Plantlife International - Back from the Brink vascular plant species abundance and distribution for Great Britain for the period 2002-2009
UK National Biodiversity Network, Rotherham Biological Records Centre - Rotherham Biological Records Centre - Non-sensitive Records from all taxonomic groups
UK National Biodiversity Network, Royal Horticultural Society - RHS monitoring of native and naturalised plants and animals at its gardens and surrounding areas
UK National Biodiversity Network, Scottish Borders Biological Records Centre - SWT Scottish Borders Local Wildlife Site Survey data 1996-2000 - species information
UK National Biodiversity Network, Scottish Natural Heritage - Standing Waters Database
UK National Biodiversity Network, Scottish Wildlife Trust - Commissioned surveys and staff surveys and reports for SWT reserves.
UK National Biodiversity Network, Sheffield Biological Records Centre - Sheffield Biological Records Centre- Non-sensitive Records from all taxonomic groups.
UK National Biodiversity Network, South East Wales Biodiversity Records Centre - CCW Regional Data : South East Wales Non-sensitive Species Records
UK National Biodiversity Network, Staffordshire Ecological Record - SER Site-based Surveys
UK National Biodiversity Network, Staffordshire Ecological Record - SER Species-based Surveys
UK National Biodiversity Network, Suffolk Biological Records Centre - Suffolk Biological Records Centre (SBRC) dataset
UK National Biodiversity Network, Thames Valley Environmental Records Centre - Local Wildlife Site Surveys Berkshire
UK National Biodiversity Network, Thames Valley Environmental Records Centre - Local Wildlife Site Surveys Oxfordshire
UK National Biodiversity Network, Thames Valley Environmental Records Centre - Nature Conservancy Council Berkshire Meadows Survey 1984-87 (as held by Thames Valley Environmental Records Centre)
UK National Biodiversity Network, Thames Valley Environmental Records Centre - Nature Conservancy Council Survey of Ancient Woodlands in Berkshire.
UK National Biodiversity Network, Tullie House Museum Natural History Collections.
UK National Biodiversity Network, Tullie House Museum - Tullie House Museum. Cumbria Wildlife Trust survey records from 1970 - 2007 of Cumbria Wildlife Sites. Various.
UK National Biodiversity Network, Vegetation surveys of coastal shingle in Great Britain
UNIBIO IBUNAM, MEXU/Flora de Oaxaca
UNIBIO IBUNAM, MEXU/Tipos de plantas vasculares
University Museums of Norway (MUSIT), University Museums of Norway (MUSIT)
University of Alabama Biodiversity and Systematics, Herbarium (UNA)

University of Alberta, University of Alberta Museums Entomology Collection
University of Alberta, University of Alberta Museums Vascular Plant Herbarium
University of Arizona Herbarium, UA Herbarium
University of Białystok, Institute of Biology, Herbarium of University of Białystok - Vascular Plants
University of Connecticut, CONN GBIF data
University of Helsinki Department of Applied Biology, Lepidoptera collection of Hannu Saarenmaa
University of Kansas Biodiversity Research Center, Botany Vascular Plant Collection
University of Malaga, Universidad de Málaga: MGC-Cormof
University of Silesia Laboratory of Botanical Documentation - Herbarium KTU, KTU Pinophyta
University of Vienna Institute for Botany - Herbarium WU, Herbarium WU
University of Vienna Institute for Botany Research Group for Plant Biogeography, Floristische Kartierung Österreichs - Mapping the Flora of Austria
University of Washington Burke Museum, Vascular Plant Collection - University of Washington Herbarium (WTU)
USDA PLANTS, USDA PLANTS Database
US National Plant Germplasm System, United States National Plant Germplasm System Collection
Utah State University, USU-UTC Specimen Database
Utah Valley State College (UVSC), Utah Valley State College Herbarium
Wrocław University Museum of Natural History, Flora of the Stołowe Mts.
Yale University Peabody Museum, Peabody Botany DiGIR Service
Yale University Peabody Museum, Peabody Paleobotany DiGIR Service
Zoologisches Forschungsinstitut und Museum Alexander Koenig, ZFMK Coleoptera collection
Zoologisches Forschungsinstitut und Museum Alexander Koenig, ZFMK Hymenoptera collection

ABBREVIATIONS

EPPO	European and Mediterranean Plant Protection Organisation
GBIF	Global Biodiversity Information Facility
IES	Institute for Environmental Sustainability, JRC, Ispra (IT)
JRC	Joint Research Center of the EU Commission
PWN	Pine wood nematode
PWD	Pine wilt disease