

SCIENTIFIC OPINION

Scientific Opinion on comments provided by Portugal on the phytosanitary risk associated with *Pinus pinea* for the spread of pine wood nematode¹

EFSA Panel on Plant Health (PLH)^{2, 3}

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ABSTRACT

Following a request from European Commission, the PLH Panel examined the comments of the Portuguese plant health authorities on a previous EFSA opinion with regard to pine wood nematode (PWN) and *Pinus pinea* and a report on PWN surveys in Portugal. Regarding the comment, based on behavioural responses of PWN to CO₂ and to β-myrcene, that *Monochamus galloprovincialis* can feed on *P. pinea* without implying PWN infestation, the Panel noted the uncertainties on how chemical attraction influences the exit of PWN from the vector. The Panel agreed that *P. pinea* is not a preferred breeding host of *M. galloprovincialis*, but it noted the evidence from Italy of breeding of *M. galloprovincialis* in fallen woods of *P. pinea*, suggesting *P. pinea* to be an occasional host. The Panel agreed that, although pathogenicity of PWN has been recorded on *P. pinea* in inoculation experiments on small plants, this does not necessarily relate to pathogenicity on larger trees in the field. The additional information on Portuguese PWN surveys is not sufficient to conclude that *P. pinea* is resistant to PWN. The Panel also noted that asymptomatic infestations by PWN are well known in other areas, and pine species considered tolerant to PWN could still maintain it at low levels in restricted parts of a tree. The Panel concluded that there is high uncertainty concerning the classification of the risk of spread of PWN with plants and wood of *P. pinea*, owing to the scarcity of information on the interaction of *M. galloprovincialis*, PWN and *P. pinea*, as well as on the field resistance of *P. pinea* to PWN. Owing to high uncertainty related to the host potential of *P. pinea*, more studies on the transmission of PWN at feeding wounds and on its survival in trees and wood of *P. pinea* are needed.

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KEY WORDS

Black pine sawyer, *Bursaphelenchus xylophilus*, *Monochamus galloprovincialis*, pine wood nematode, PWN, *Pinus pinea*, stone pine.

¹ On request from the European Commission, Question No EFSA-Q-2012-00561, adopted on 20 March 2013.

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³ Acknowledgement: The Panel wishes to thank the members of the Working Group on name of the WG on pine wood nematode host plants: Hugh Evans, Jean-Claude Grégoire, Gabor Lovei, Christer Magnusson, Trond Rafoss and Gregor Urek for the preparatory work on this scientific opinion, and EFSA staff: Olaf Mosbach-Schultz, Giuseppe Stancanelli, Sara Tramontini and Sybren Vos for the support provided to this scientific opinion.

Suggested citation: EFSA PLH Panel (EFSA Panel on Plant Health), 2013. Scientific Opinion on comments provided by Portugal on the phytosanitary risk associated with *Pinus pinea* for the spread of pine wood nematode. EFSA Journal 2013;11(4):3163, 37 pp. doi:10.2903/j.efsa.2013.3163

Available online: www.efsa.europa.eu/efsajournal

SUMMARY

Following a request from the European Commission, the Panel on Plant Health (the Panel) was asked to deliver a scientific opinion on comments provided by Portugal on the phytosanitary risk associated with *Pinus pinea* for the spread of pine wood nematode (PWN). In particular, the Panel was asked to reply to the comments of the Portuguese plant health authorities with regard to a previous scientific opinion on the phytosanitary risk associated with some coniferous species and genera for the spread of PWN (EFSA PLH Panel, 2012a).

The Panel closely examined the Portuguese technical file (Sousa et al., 2012) and the clarifications received from the Portuguese authorities on the methodology and results of the PWN annual pest surveys conducted by the Portuguese national forestry authorities (Rodrigues et al., 2012). The Panel conducted its work in line with the principles described in the guidance on the evaluation of pest risk assessments and risk management options prepared by third parties (EFSA Panel on Plant Health (PLH), 2009) and in the guidance on evaluation of risk reduction options (EFSA PLH Panel, 2012b).

After consideration of the evidence provided, and after undertaking an additional literature review, the Panel reached the following conclusions on the comments provided by the Portuguese authorities to the previous PLH Panel scientific opinion:

With regard to the assertion that *Monochamus galloprovincialis* can feed on *P. pinea* but this does not imply infestation with the PWN, the Portuguese claim was based on behavioural responses of the nematodes to CO₂ and to β-myrcene. However, the Panel found that the role of CO₂ as an attractant for PWN has not been investigated for different pine species (with different susceptibility to PWN infestation). Also, further conclusive scientific evidence is missing to determine the role of β-myrcene in the exit of PWN from the vector's body. It is uncertain how chemical attraction affects the likelihood and rate of exit of PWN from the vector to the tree, and it may be necessary to examine a complex of chemicals, rather than single compounds. Therefore, the Panel concluded that this claim could not be substantiated.

The Panel agreed with the Portuguese assertion that *P. pinea* is not a preferred host for breeding of *M. galloprovincialis*. It also noted that there is evidence from Italy of breeding of *M. galloprovincialis* in fallen woods of *P. pinea*, suggesting *P. pinea* to be an occasional host for *M. galloprovincialis*.

The Panel agreed with the assertion that, although pathogenicity of PWN has been recorded on *P. pinea* in inoculation experiments on small plants under controlled conditions, this does not necessarily relate to pathogenicity on larger trees in the field.

With regard to the assertion that adult *P. pinea* trees are not adequate hosts for the PWN and do not suffer from wilt disease, the Panel concluded that the additional information provided in Rodrigues et al. (2012) on the national surveys is not sufficient to conclude that *P. pinea* is resistant to PWN. In addition, the Panel noted that asymptomatic infestations by PWN are well known in areas where the nematode occurs. Pine species that are tolerant to the PWN could still allow the nematode to exist at low levels in restricted parts of a tree and thus act as a reservoir.

The overall conclusion of the Panel is that there is high uncertainty concerning the classification of the risk of spread of PWN with plants and wood of *P. pinea*, owing to the scarcity of information on the interaction of *M. galloprovincialis*, *Bursaphelenchus xylophilus* and *P. pinea*, as well as the lack of clear evidence for field resistance of *P. pinea* to PWN.

The Panel also recommended that studies on the transmission of PWN at feeding wounds and the survival of PWN in trees and wood of *P. pinea* are needed to reduce the uncertainties regarding the host potential of *P. pinea* for PWN.

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BACKGROUND AS PROVIDED BY THE EUROPEAN COMMISSION

In a recently published scientific opinion of EFSA in the plant health area, which was prepared upon a request from the Commission (ARES(2011)694631), the phytosanitary risk associated with some coniferous species and genera for the spread of pine wood nematode was evaluated (EFSA Journal 2012;10(1):2553).

One of the coniferous plant species evaluated by EFSA in this opinion was *Pinus pinea*. The Portuguese Plant Health Authorities consider that this species should be exempted from the Union emergency measures against pine wood nematode. A technical file prepared by Portugal in support of this view was made available to EFSA in the context of the above-mentioned scientific opinion (ARES(2011)743492).

Written comments have been prepared by the Portuguese plant health authorities in reply to the above-mentioned EFSA's scientific opinion on pine wood nematode and have been submitted to EFSA with letter ARES(2012)509871 dated 25/04/2012.

In addition, the Commission has provided EFSA, with letter ARES(2012)1487396 dated 13/12/2012, with a document entitled "Risk assessment of *Pinus pinea* L. in relation to pinewood nematode – Additional information – (December 2012)", which was prepared by the Portuguese phytosanitary authorities.

TERMS OF REFERENCE AS PROVIDED BY THE EUROPEAN COMMISSION

With letter ARES(2012)509871 dated 25/04/2012, the Commission asked EFSA to react to the written comments prepared by Portuguese Plant Health Authorities.

With letter ARES(2012)1487396 dated 13/12/2012, the Commission asked EFSA to also take into consideration, in the preparation of the reply to the comments, the document entitled "Risk assessment of *Pinus pinea* L. in relation to pinewood nematode – Additional information – (December 2012)", prepared by the Portuguese phytosanitary authorities.

ASSESSMENT

1. Introduction

The risk associated with *Pinus pinea* for the spread of the pine wood nematode (hereinafter, PWN), *Bursaphelenchus xylophilus*, was discussed within the scientific opinion of the EFSA Panel on Plant Health (hereinafter, the Panel) on the phytosanitary risk associated with some coniferous species and genera for the spread of PWN (EFSA PLH Panel, 2012a). The Panel concluded within that scientific opinion that, owing to the lack of relevant scientific information on the interaction of *Monochamus 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 (EFSA PLH Panel, 2012a).

Following a further request from the European Commission in 2012, the Panel has been asked to react to the comments of the Portuguese plant health authorities, which considered that *P. pinea* should be exempted from the European Union emergency measures against PWN. In these comments, the Portuguese plant health authorities agreed that additional scientific evidence would always be desirable but concluded that, currently, there is sufficient scientific data to argue that *P. pinea* is not a suitable host for the larval development of *M. galloprovincialis* and that grown *P. pinea* do not suffer from pine wilt disease in the field. The Portuguese authorities have made the case that *P. pinea* should be exempted from the Union emergency measures against PWN. A technical file prepared by Portugal in support of its view was made available to EFSA (hereinafter cited, with the authors' names, as Sousa et al., 2012). The Portuguese plant health authorities asserted in this document that:

- *M. galloprovincialis* can feed on *P. pinea* but this does not imply infestation with the PWN.
- *P. pinea* is not a preferred host for *M. galloprovincialis*.
- Artificial inoculation of small plants and seedlings does not necessarily relate to wilt expression in grown trees in the field.
- Grown *P. pinea* trees are not adequate hosts for the PWN and do not suffer from wilt disease.

EFSA has requested from the European Commission additional information on the methodology and results of the PWN annual pest surveys conducted by the Portuguese authorities. In particular, in accordance with the EFSA PLH Panel checklist for evaluating a proposed risk reduction option (EFSA PLH Panel, 2012b), more details have been requested, for example on the following aspects: survey hypothesis; explanation of the applied mathematical background and its justification; sampling methods; confidence level; methodology and instruments for performing an individual observation, including sampling height and laboratory testing; results of the survey, i.e. list and details of observations.

The Panel has closely examined the Portuguese technical file (Sousa et al., 2012) and the clarifications received from the Portuguese authorities on the methodology and results of the PWN annual pest surveys (hereinafter cited, with the authors' names, as Rodrigues et al., 2012). The replies to the Portuguese comments and further analysis on the interactions between *M. galloprovincialis*, *P. pinea* and PWN, *B. xylophilus*, are provided in this opinion.

Due to the additional literature review conducted and to the analysis of the PWN annual survey data and information provided by the Portuguese authorities, this assessment has been conducted in the form of a scientific opinion.

2. Methodology

2.1. The guidance documents

The risk assessment has been conducted in line with the principles described in the guidance on the evaluation of pest risk assessments and risk management options prepared by third parties (EFSA Panel on Plant Health (PLH), 2009) and in the guidance on evaluation of risk reduction options (EFSA PLH Panel, 2012b).

The documentation provided by Rodrigues et al. (2012) on PWN surveys was screened following the EFSA PLH Panel checklist for evaluating a proposed risk reduction option (EFSA PLH Panel, 2012b) and the data provided were analysed by the Panel. The detailed review and analysis of the survey data are presented in Appendix A of this opinion.

2.2. Literature search

An update of the extensive literature search on *M. galloprovincialis*, *P. pinea* and PWN, *B. xylophilus*, was conducted following the first three steps (preparation of protocols and questions, search, selection of studies) of the EFSA guidance on systematic review methodologies (EFSA, 2010), repeating the same questions and search strategy of the search conducted in the previous Panel opinion on PWN host plants (EFSA PLH Panel, 2012a). Further references and information were obtained from experts and from citations within references found.

The methodology and results of the extensive literature search are presented in Appendix B of this opinion.

3. Results

3.1. Host status of *P. pinea* with regard to *M. galloprovincialis*

3.1.1. From the previous EFSA opinion (EFSA PLH, 2012a)

There is evidence of oviposition by *M. galloprovincialis* in *P. pinea* but this is lower than on other host trees. An extrapolation to forests with different tree compositions and different settings is not possible from the limited data presented (EFSA PLH Panel, 2012a).

M. galloprovincialis is distributed over a vast geographical area and it cannot be excluded that subspecies (*M. galloprovincialis* subsp. *galloprovincialis*, *M. galloprovincialis* subsp. *pistor*) and local populations could have host preferences different from those of the known Portuguese populations⁴. Attacks on *P. pinea* by *M. galloprovincialis* are in fact known from Italy. Field studies on *P. koraiensis* in Japan (Futai, 2003) and on *P. sylvestris* in the United States (Bergdahl and Halik, 2003) 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 still containing live nematodes. Such trees can act as reservoirs for the nematode over prolonged periods. However, if these trees are weakened

⁴ Local variations in host preferences, or host shifts, are often observed in insects (Bernays and Graham, 1988). In forest insects, host shifts have been documented, e.g. in *Dendroctonus micans* (Coleoptera: Curculionidae), from spruce to pine (Voolma, 1978) and in *Operophtera brumata* (Lepidoptera: Geometridae), from broadleaved trees to Sitka spruce (Stoakley, 1985).

sufficiently to become attractive to *Monochamus* spp. 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 *Monochamus* species other than *M. galloprovincialis*, *P. pinea* and PWN have not yet been studied in sufficient detail to draw firm conclusions on the survival and transmission of PWN (EFSA PLH Panel, 2012a).

3.1.2. *M. galloprovincialis* feeding on *P. pinea*

3.1.2.1. Portuguese comment on the previous EFSA opinion (EFSA PLH Panel, 2012a) with regard to *M. galloprovincialis* feeding on *P. pinea*

Sousa et al. (2012) consider that there are sufficient scientific data to state that *M. galloprovincialis* can feed on *P. pinea* but that this does not imply infestation with the PWN:

“The suitability of *P. pinea* as feeding host is based on the only available information in the literature, which is the report by Naves et al. (2006) who did not detect statistically significant difference in feeding activity on *Pinus pinaster* and *P. pinea* branches in studies conducted under laboratory conditions. Nevertheless, this does not necessarily imply that nematode-infested beetles would infect this pine when feeding, because the departure of the nematodes from the insect’s body is controlled by an interaction of various factors such as volatile chemicals and CO₂ emitted by the host (Edwards and Linit, 1992; Kishi, 1995). The volatile compound β -myrcene has been found to play a decisive role in promoting the exit of PWN from vector’s body to enter the host (Tominaga et al., 1984; Ishikawa et al., 1986; Stamps and Linit, 1998, 2001), and this volatile is much more abundant in *P. pinaster* needles and branches than in *P. pinea* (Tiberi et al., 1999; Macchioni et al., 2003).”

3.1.2.2. The standpoint of the Panel on *M. galloprovincialis* feeding on *P. pinea*

In an absolute majority of the more than 40 *Pinus* spp., which are susceptible or intermediate susceptible to the PWN (Evans et al., 1996), detailed information on the interaction among PWN, *Monochamus* spp. and the tree hosts during the transmission process is missing. The discussion on the role of chemical cues in *P. pinaster* and *P. pinea* would benefit from evaluation of the chemical cues associated with a wider range of pine species. The factors influencing the exit of dauer larvae (J_{IV}) from beetles are still not well understood. Volatile terpenes such as β -myrcene from pine have been suggested to stimulate exit from beetle vectors, but the detection of these compounds by J_{IV} cannot fully explain the pattern of nematode exit. There is also evidence that large numbers of J_{IV} larvae exit beetles in the apparent absence of pine volatiles (Stamps and Linit, 1998).

The Panel concludes that the assertion, submitted by the Portuguese authorities, that “*M. galloprovincialis* can feed on *P. pinea* but this does not imply infestation with the PWN” was not supported by sufficient evidence for the following reasons:

- It is well known that nematodes are attracted by CO₂ (Johnson and Viglierchio, 1961). However, this has not been investigated for different pine species in relation to PWN.
- Further conclusive scientific evidence is needed to determine the role of β -myrcene in the exit of PWN from the vector’s body.
- It is uncertain how chemical attraction works on the exit of the nematode from the vector to trees, and it may be necessary to examine a complex of chemicals, rather than single compounds.

To reduce the uncertainty, it would be recommended to carry out an experiment to determine whether the nematodes exit the vectors and invade feeding wounds on *P. pinea*.

3.1.3. *M. galloprovincialis* breeding on *P. pinea*

3.1.3.1. Portuguese comment on the previous EFSA opinion (EFSA PLH Panel, 2012a) with regard to *M. galloprovincialis* breeding on *P. pinea*

Sousa et al. (2012) consider that *P. pinea* is not a preferred host for *M. galloprovincialis* breeding:

“There are just two isolated reports in the literature concerning *Monochamus* spp. breeding in *P. pinea* wood, both from Italy. Campadelli and Dindo (1994) and Francardi and Pennacchio (1996) report attack of *M. galloprovincialis* on fallen trees, and state that insects are only secondarily found in *P. pinea* forests. No additional reports on the association with *P. pinea* (either standing or felled) exists from other countries where both species are abundant, such as Spain, Portugal, France or Turkey. In EFSA document it is stated that *P. pinea* is an inferior host for *M. galloprovincialis* and its ‘capacity to support a full life cycle of the beetle remains poorly characterized or tested’ (page 30), although laboratory studies by Naves et al. (2006) found oviposition on *P. pinea* to be infrequent, and even when occurred the larvae were unable to complete their development and successfully emerge, which lead these authors to consider *P. pinea* as unsuitable host.”

3.1.3.2. The standpoint of the Panel on *M. galloprovincialis* breeding on *P. pinea*

The Panel agrees with the Portuguese assertion that *P. pinea* is not a preferred host for the breeding of *M. galloprovincialis*. It also notes that there is information from Italy on the breeding of *M. galloprovincialis* in fallen woods of *P. pinea*, suggesting *P. pinea* to be an occasional host for *M. galloprovincialis*. The position of the Panel is based on the following information:

- The PLH Panel again closely reviewed two Italian papers referring to the breeding of *M. galloprovincialis* in wood of *P. pinea* (Campadelli and Dindo, 1994; Francardi and Pennacchio, 1996) to evaluate the reliability of the data. The second paper does not provide any detailed information and only mentions this association as more rare than with *P. pinaster*. The first paper contains more substantial information, which is also in accordance with the observed patterns of the behaviour of the insect in Portugal, where *M. galloprovincialis* attacks the tops and branches of coniferous trees. These are expected to be reliable observations as it is possible to clearly distinguish between the two species of pine. The PLH Panel therefore concludes that there is evidence of breeding of *M. galloprovincialis* in fallen woods of *P. pinea*.
- According to Naves et al. (2006), oviposition of *M. galloprovincialis* is reduced but remains possible in *P. pinea*. The results of this study suggested that further development through to the adult stage does not take place under the conditions tested; however, the few eggs laid on *P. pinea*, combined with the considerable larval mortality observed also in wood of the preferred host *P. pinaster*, preclude safe conclusions to be drawn on the emergence of *M. galloprovincialis* from *P. pinea* in the field.

The obvious lack of reports on maturation feeding of *M. galloprovincialis* on *P. pinea* in the field may be a consequence of the low priority accorded to such studies, rather than a reflection of a true fact.

3.2. Host status of *P. pinea* with regard to PWN

3.2.1. From the previous EFSA opinion (EFSA PLH, 2012a)

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 would be needed (EFSA PLH Panel, 2012a).

3.2.2. Portuguese comment on the previous EFSA opinion (EFSA PLH Panel, 2012a)

Sousa et al. (2012) consider that artificial inoculation of small plants and seedlings does not necessarily relate to wilt expression on adult trees in the field:

“Although studies with inoculation of young plants are an approach to preliminary evaluate the susceptibility of different species to PWN, there are some differences in the physiology and anatomy of young plants and seedling in opposition to adult plants, and therefore the behaviour and pathogenicity of *B. xylophilus* can differ from young to adult trees. This has already been found with *B. xylophilus*, which killed seedlings of *P. resinosa* while the adult trees are resistant to pine wilt disease (Wingfield et al., 1986; Bedker et al., 1987).

Even considering the inoculation studies, the EFSA document refers to trials where the PWN killed small plants of *P. pinea* (Daub, 2007; Mota and Vieira, 2008), although the authors forgot to quote similar studies from de Guiran and Boulbria (1985) and Franco et al. (2011) where similar inoculations did not kill *P. pinea* trees. In fact, Guiran and Boulbria (1985) inoculated young trees of both *P. pinea* and *P. pinaster* in France, and found that two out of 10 *P. pinea* trees died, although the number of recovered nematodes were very low and the authors concluded that the nematodes did not cause the tree's mortality. All *P. pinaster* trees were dead within 8 weeks. Similarly, Franco et al. (2011) inoculated 20 seedlings of various pine species with Portuguese strains of virulent PWN, and found that *P. pinea* were not killed. All *P. pinaster* seedlings died 20 days after the inoculation. Both papers conclude that *B. xylophilus* is not pathogenic to *P. pinea*.”

3.2.3. The standpoint of the Panel

In connection with the Portuguese comments on the susceptibility of *P. pinea*, reference is made to a number of inoculation experiments. The conditions of these experiments are detailed in Table 1.

- The Panel notes that pathogenicity tests with PWN on four-month- to five-year-old plants of *P. pinea* and related work have reported on mortality rates from 0 % to 60 % (Table 1), and that mortality may be related to the type of nematode isolate. Daub (2007) reported that isolates of PWN from the United States, China and Portugal when inoculated on four-year-old *P. pinea* caused mortality rates of 10, 25 and 60 %. de Guiran and Boulbria (1985) reported 20 % mortality in two-year-old *P. pinea*. Despite these observations the Panel agrees with the Portuguese assertion that pathogenicity recorded in inoculation experiments with PWN on

small plants does not necessarily relate to pathogenicity on larger trees in the field. This fact has been recognised for a long time (McNamara, 2003).

- After the submission of the comments from the Portuguese authorities (Rodrigues et al., 2012; Sousa et al., 2012), a paper was recently published (Santos et al., 2012), demonstrating a differential response of two-year-old potted plants of *P. pinaster* and *P. pinea* to PWN infestation. This study provides insight into the defence related genes of these pine species expressed upon infestation by PWN, but it still does not clarify the degree of tolerance or resistance of *P. pinea* to PWN.
- The Panel would like to emphasise that host status of a pine species for PWN is not necessarily connected with pathogenicity. As cited in Tamura and Dropkin (1984), there was no difference in the population growth of PWN in excised branches or logs of susceptible *P. densiflora* and *P. thunbergii* compared with resistant *P. taeda*. Tamura and Dropkin (1984) also recorded that three-year-old *P. jeffreyi* was resistant to PWN, but still the nematode could reproduce in cut pieces of this species. The Panel would like to draw attention to the fact that detached wood of a tree species may have different characteristics from the living tree with regard to its capacity to support populations of PWN.
- Asymptomatic infestations by PWN are well known in Japan and asymptomatic carrier trees in fact may ensure the continuity of PWN (Futai, 2003), and it is difficult to determine the latent infestation with PWN by external observation or a resin exudation test (Takeuchi and Futai, 2007). Pine species that are considered tolerant to the PWN could still allow the nematode to exist in restricted parts of a tree or at low levels and could serve as “latent” or “asymptomatic” carriers (Futai, 2003). Fonseca et al. (2012) reported that 25 % of PWN-inoculated five-year-old plants of *P. pinea* contained nematodes in the trunk and branches 50 days post inoculation. This suggests that nematodes can survive for long periods within living *P. pinea*. Similarly Bergdahl and Halik (2003) found living nematodes 11 years post inoculation in symptomless *Pinus sylvestris* in the United States.

Studies on the survival of PWN in standing trees of *P. pinea* are needed to provide a higher level of certainty regarding the host potential of *P. pinea*.

Table 1: Summary of inoculation trials with the pine wood nematode (PWN), *Bursaphelenchus xylophilus*, on *Pinus pinea* and other *Pinus* spp.

Reference	Trial duration Temperature	Tree species	Tree age	Treatment	Replicates	Inoculum per tree ^a	Mortality (%) Other results
de Guiran and Boulbria, 1985	10 weeks 20–28 °C	<i>P. pinaster</i>	4 years	PWN	12	10 000 PWN	100 % 7 trees (in quarantine lab): average 20 735 nematodes/tree 5 trees (in greenhouse): average 2 933 nematodes/tree
			4 years	Control	23	Water from <i>Botrytis cinerea</i> culture	0 %
		<i>P. pinea</i>	2 years	PWN	10	10 000 PWN	20 % Respectively 45 and 12 nematodes/tree
			2 years	Control	10	Water from <i>B. cinerea</i> culture	0 %
		<i>P. halepensis</i>	2 years	PWN	10	10 000 PWN	0 %—weak symptoms No nematodes found
			2 years	Control	10	Water from <i>B. cinerea</i> culture	0 %
Mota and Vieira, 2008; PHRAME, 2007	37 days 24.2–31.7 °C	<i>P. pinaster</i>	4 years	PWN	50	5 000 PWN	<i>P. pinaster</i> : “results show a very fast infestation and mortality rate of <i>P. pinaster</i> ”
			4 years	Control	50	Wash water of Petri dishes with non-sporulated <i>B. cinerea</i>	No information
		<i>P. pinea</i>	4 years	PWN	50	5 000 PWN	<i>P. pinea</i> : “Only after many weeks did <i>P. pinea</i> display some symptoms of possible susceptibility”
			4 years	Control	50	Wash water of Petri dishes with non-sporulated <i>B. cinerea</i>	No information
Daub, 2007	12 weeks 25 °C	<i>P. pinaster</i>	2 years	PWN	20	4 000 PWN Three different <i>B. xylophilus</i> origins (PT, US, CN)	75 % (PT); 90 % (US); 95 % (CN) Median value of 944.19 nematodes (PT origin) per gram (fresh weight) after 4 weeks ($n = 4 \times 5$ trees)
			2 years	Control	20	Sterile tap water	0 %
		<i>P. pinea</i>	3–4 years	PWN	20	4 000 PWN Three different <i>B. xylophilus</i> origins (PT, US, CN)	10 % (US); 25 % (CN); 60 % (PT) Median value of 26.98 nematodes (PT origin)
			3–4 years	Control	20	Sterile tap water	10 %
		<i>P. sylvestris</i>	2–3 years	PWN	20	4 000 PWN Three different <i>B. xylophilus</i> origins (PT, US, CN)	100 % (US, CN, PT) Median value of 647.83 nematodes (PT origin)
			2–3 years	Control	20	Sterile tap water	5 %
		<i>P. nigra</i>	3–4 years	PWN	20	4 000 PWN Three different <i>B. xylophilus</i> origins (PT, US, CN)	100 % (US, CN, PT) Median value of 426.27 nematodes (PT origin)
			3–4 years	Control	20	Sterile tap water	0 %

Reference	Trial duration Temperature	Tree species	Tree age	Treatment	Replicates	Inoculum per tree ^a	Mortality (%) Other results
		<i>P. cembra</i>	3–4 years	Control	20	Sterile tap water	0 %
			3–4 years	PWN	20	4 000 PWN Three different <i>B. xylophilus</i> origins (PT, US, CN)	100 % (US, CN, PT) Median value of 5 104.86 nematodes (PT origin)
			3–4 years	Control	20	Sterile tap water	0 %
		<i>P. strobus</i>	3–4 years	PWN	20	4 000 PWN Three different <i>B. xylophilus</i> origins (PT, US, CN)	90 % (US); 95 % (PT); 100 % (CN) Median value of 1,135.94 nematodes (PT origin)
			3–4 years	Control	20	Sterile tap water	0 %
		<i>P. radiata</i>	2–3 years	PWN	20	4 000 PWN Three different <i>B. xylophilus</i> origins (PT, US, CN)	75 % (CN); 95 % (PT, US) Median value of 244.32 nematodes (PT origin)
			2–3 years	Control	20	Sterile tap water	5 %
		<i>P. halepensis</i>	2–3 years	PWN	20	4 000 PWN Three different <i>B. xylophilus</i> origins (PT, US, CN)	0 % (CN); 5 % (US); 15 % (PT) Median value of 9.53 nematodes (PT origin)
			2–3 years	Control	20	Sterile tap water	0 %
		<i>P. mugo</i>	3–4 years	PWN	20	4 000 PWN Three different <i>B. xylophilus</i> origins (PT, US, CN)	55 % (PT); 80 % (CN); 85 % (US) Median value of 134.08 nematodes (PT origin)
			3–4 years	Control	20	Sterile tap water	0 %
Franco et al., 2011	20 days 25 °C	<i>P. pinaster</i>	4 months	Virulent PWN	20	500 PWN	Total discoloration, necrosis and seedling death
			4 months	Avirulent PWN	20	500 PWN	Partial needle discolouration, necrosis and reduction in the resin production
			4 months	Control	20	Sterile water	Partial needle discolouration
		<i>P. pinea</i>	4 months	Virulent PWN	20	500 PWN	Partial needle discolouration
			4 months	Avirulent PWN	20	500 PWN	Partial needle discolouration
			4 months	Control	20	Sterile water	Healthy plant
		<i>P. nigra</i>	4 months	Virulent PWN	20	500 PWN	Partial needle discolouration
			4 months	Avirulent PWN	20	500 PWN	Partial needle discolouration
			4 months	Control	20	Sterile water	Healthy plant
		<i>P. sylvestris</i>	4 months	Virulent PWN	20	500 PWN	Partial discoloration and resin deduction
			4 months	Avirulent PWN	20	500 PWN	Partial needle discolouration
			4 months	Control	20	Sterile water	Partial needle discolouration
		<i>P. pinaster</i>	5 years	25 °C—high water	5	6 000 PWN	Nematodes in “higher numbers”
			5 years	25 °C—low water	5	6 000 PWN	in all trees, stems, branches and roots

Reference	Trial duration Temperature	Tree species	Tree age	Treatment	Replicates	Inoculum per tree ^a	Mortality (%) Other results
Fonseca et al., 2012 ^b	50 days 25 and 30 °C Two watering conditions (high and low)		5 years	30 °C—high water	5	6 000 PWN	
			5 years	30 °C—low water	5	6 000 PWN	
			5 years	25 °C—high water	5	Sterile water	
			5 years	25 °C—low water	5	Sterile water	
			5 years	30 °C—high water	5	Sterile water	
			5 years	30 °C—low water	5	Sterile water	
		<i>P. pinea</i>	5 years	25 °C—high water	5	6 000 PWN	Nematodes in 4 trees (out of 20?), in stems and branches
			5 years	25 °C—low water	5	6 000 PWN	
			5 years	30 °C—high water	5	6 000 PWN	
			5 years	30 °C—low water	5	6 000 PWN	
			5 years	25 °C—high water	5	Sterile water	
			5 years	25 °C—low water	5	Sterile water	
			5 years	30 °C—high water	5	Sterile water	
			5 years	30 °C—low water	5	Sterile water	
		<i>P. radiata</i>	5 years	25 °C—high water	5	6 000 PWN	Nematodes in all trees, stems, branches and roots
			5 years	25 °C—low water	5	6 000 PWN	
			5 years	30 °C—high water	5	6 000 PWN	
			5 years	30 °C—low water	5	6 000 PWN	
			5 years	25 °C—high water	5	Sterile water	
			5 years	25 °C—low water	5	Sterile water	
			5 years	30 °C—high water	5	Sterile water	
			5 years	30 °C—low water	5	Sterile water	
Mendes, 2012 ^b	50 days 25 and 30 °C Two watering conditions (high and low)	<i>P. pinaster</i>	3-4 years	25 °C—high water	5	6 000 PWN	20 % trees with 100 % needles reddish-brown
			3-4 years	25 °C—low water	5	6 000 PWN	60 % trees with 100 % needles reddish-brown
			3-4 years	30 °C—high water	5	6 000 PWN	100 % trees with 100 % needles reddish-brown
			3-4 years	30 °C—low water	5	6 000 PWN	20 % trees with 100 % needles reddish-brown 80 % trees dead
			3-4 years	25 °C—high water	5	Not specified	20 % trees with 100 % needles reddish-brown
			3-4 years	25 °C—low water	5	Not specified	60 % trees with 100 % needles reddish-brown
			3-4 years	30 °C—high water	5	Not specified	20 % trees with 100 % needles reddish-brown
			3-4 years	30 °C—low water	5	Not specified	40 % trees with 100 % needles reddish-brown
		<i>P. pinea</i>	3-4 years	25 °C—high water	5	6 000 PWN	No symptoms
			3-4 years	25 °C—low water	5	6 000 PWN	No symptoms
			3-4 years	30 °C—high water	5	6 000 PWN	No symptoms
			3-4 years	30 °C—low water	5	6 000 PWN	No symptoms
			3-4 years	25 °C—high water	5	Not specified	No symptoms

Reference	Trial duration Temperature	Tree species	Tree age	Treatment	Replicates	Inoculum per tree ^a	Mortality (%) Other results
			3-4 years	25 °C—low water	5	Not specified	No symptoms
			3-4 years	30 °C—high water	5	Not specified	No symptoms
			3-4 years	30 °C—low water	5	Not specified	No symptoms
		<i>P. radiata</i>	3-4 years	25 °C—high water	5	6 000 PWN	20 % trees with 100 % needles reddish-brown
			3-4 years	25 °C—low water	5	6 000 PWN	20 % trees with 100 % needles reddish-brown 80 % trees dead
			3-4 years	30 °C—high water	5	6 000 PWN	20 % trees with 100 % needles reddish-brown 20 % trees dead
			3-4 years	30 °C—low water	5	6 000 PWN	100 % trees dead
			3-4 years	25 °C—high water	5	Not specified	No symptoms
			3-4 years	25 °C—low water	5	Not specified	No symptoms
			3-4 years	30 °C—high water	5	Not specified	No symptoms
			3-4 years	30 °C—low water	5	Not specified	20 % trees dead

^a Inoculations on stems.

^b Similarity in experimental conditions compared with Fonseca et al. (2012) suggests that these studies are related.

3.3. The overall interaction among *P. pinea*, *M. galloprovincialis* and *B. xylophilus*

3.3.1. From the EFSA opinion (EFSA PLH Panel, 2012a)

Owing to the lack of 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 (EFSA PLH Panel, 2012a).

3.3.2. Portuguese comment on the previous EFSA opinion (EFSA PLH Panel, 2012a)

Sousa et al. (2012) consider that adult *P. pinea* trees are not adequate hosts for the PWN and do not suffer from wilt disease:

“This has been proved by the annual surveys conducted by the Portuguese National Forest Authority (NFA), which since 2001 has analyzed more than 1 673 wood samples of *P. pinea* from PWN-infested locations for the presence of *B. xylophilus*, and the PWN was never detected on any occasion. The continuous sampling over a decade is the clearest and most evident result on the non-susceptibility of adult *P. pinea* trees in the field, and although these results were presented in the first report they were surprisingly discarded in the EFSA report, and never commented or discussed. The additional results on the surveys and plots from Tróia peninsula also support the absence on infestation in *P. pinea*, although these are limited by the low number of dead *P. pinea* trees, and were incorporated in the report only as complementary and additional information to the NFA surveys, and not as the supporting argument.”

3.3.3. The standpoint of the Panel

To enable EFSA to reply to the Portuguese comments, additional information on methodology and results of the EFSA annual pest surveys conducted by the Portuguese National Forest Authority have been requested. The Panel reviewed the additional information received from Portugal (Rodrigues et al., 2012), with reference to the “Guidance on methodology for evaluation of the effectiveness of options for reducing the risk of introduction and spread of organisms harmful to plant health in the EU territory” (EFSA PLH Panel, 2012b) and to the EFSA technical assistance report on sampling statistics on emergency measures to prevent the spread of PWN within the European Union (EFSA, 2012). The detailed review is presented in the Appendix of this opinion and the main results are presented below.

The Panel noticed that results are given only for the first phase of the national survey 1999–2007. Therefore, the results are restricted only to the area around Setubal peninsula.

The exact location of the Setubal demarcated area and the characteristics of the forest stands included are not described. Important factors, such as composition and density of the forest stand and climatic and soil conditions, are not included in the analysis. The result could be influenced by these factors, and the Setubal demarcated area does not, perhaps, cover all conditions within Portugal. Climatic and soil conditions and the composition and density of the forest stands in other parts of Portugal may be more favourable for the infestation of *P. pinea* trees than in the Setubal demarcated area.

The data presented are not the most current data from 2008–2012 and do not cover the whole of Portugal. Consequently, the results may not be representative for the situation in the whole of Portugal.

The data presented do not distinguish between the number of trees in the infested zone or in the buffer zone⁵. To show potential resistance or tolerance to infestation, an appropriate infestation pressure for the forest with *M. galloprovincialis* and PWN must be given, which is not expected to occur in the buffer zone for PWN.

The infestation level of PWN and *M. galloprovincialis* in the Setubal demarcated⁵ area is not described fully. Samples could be taken from non-infested areas or areas with negligible infestations of other *Pinus* species (below the threshold of 0.02 %).

The health status of *P. pinaster* is generally weaker than that of *P. pinea* in the Setubal demarcated area (as shown in Section 3.1.1 of Appendix A, the rate of decline, without PWN, in the Setubal demarcated area is about 30 times higher for *P. pinaster* than for *P. pinea*). This difference can cause a preference of infestation of *M. galloprovincialis* for *P. pinaster* trees.

The Panel notes that Rodrigues et al. (2012) do not specify how many *P. pinea* trees were felled and how many samples were taken from the canopy of the tree or the stem. Missing samples from parts of the trees with a higher risk of infestation, such as parts showing signs of decline, signs of activity of *Monochamus* spp. or blue-stain fungi will decrease the sensitivity of the detection method. In addition the minimal amount of material taken from each *P. pinea* tree is not specified. Small or inadequate sampling will decrease the sensitivity of the detection method. Furthermore, samples were taken only from *P. pinea* trees showing symptoms of decline. Latent infestations cannot be detected with this sampling scheme.

It is not reasonable to assume that the sensitivity of the detection via composite samples from up to five trees with a minimal amount of 100 g and unspecified sampling locations on the tree is equal or higher than 99 %. Instead the sensitivity of the detection method is most likely below 99 %, which implies that the number of samples was too low to confirm the absence of infestation with a threshold of 0.02 % and reliability of 99 % as mentioned in Commission Implementing Decision 2012/535/EU (see Appendix A for further details).

Based on the evaluation of the statistical issues related to the additional information provided in Rodrigues et al. (2012) and Sousa et al. (2012) and using the evaluation scheme in the guidance on evaluation of risk reduction options (EFSA PLH Panel, 2012b), the Panel concludes that the additional information presented in Rodrigues et al. (2012) is not sufficient to conclude that *P. pinea* is resistant to PWN. In order to demonstrate resistance by a pest survey, a study should guarantee that the pest is present in sufficient numbers, and consider different key factors influencing the growth of the host, such as climatic and soil conditions and forest characteristics. Furthermore, the sample size must be sufficient to detect small probabilities of infestation ($P=0.02$) and compensate for any loss of sensitivity in the employed detection methods. The Panel considers that these conditions were not fulfilled in the data submitted by Rodrigues et al. (2012) (see Appendix A for further details).

⁵ According to Commission Decision 2006/133/EC of 13 February 2006 requiring Member States temporarily to take additional measures against the dissemination of *Bursaphelenchus xylophilus* (Steiner et Buhrer) Nickle et al. (the pine wood nematode) as regards areas in Portugal, other than those in which it is known not to occur (OJ L 52,23.2.2006, p. 34), "Portugal shall establish areas in which PWN is known not to occur, and demarcate areas (hereinafter called demarcated areas) comprised of a part in which PWN is known to occur and a part designated as buffer zone of not less than 20 km width surrounding that part, taking into account the results of the surveys referred to in Article 4."

CONCLUSIONS AND RECOMMENDATIONS

CONCLUSIONS

Following a request from the European Commission, the Panel on Plant Health was asked to deliver a scientific opinion on comments provided by Portugal on the phytosanitary risk associated with *P. pinea* for the spread of PWN. In particular, the Panel was asked to reply to the comments of the Portuguese plant health authorities with regard to the scientific opinion on the phytosanitary risk associated with some coniferous species and genera for the spread of PWN (EFSA PLH Panel, 2012a).

After consideration of the evidence provided and after undertaking an additional literature review, the Panel reached the following conclusions on the comments provided by the Portuguese authorities on the previous Panel scientific opinion:

The Portuguese authorities asserted that *M. galloprovincialis* can feed on *P. pinea* but that this does not imply infestation with the PWN. This claim was based on behavioural responses of the nematodes to CO₂ and to β -myrcene. However, the Panel found that the role of CO₂ as an attractant for PWN has not been investigated for different pine species (with different susceptibility to PWN infestation). Also, further conclusive scientific evidence is missing to determine the role of β -myrcene in the exit of PWN from the vector's body. It is uncertain how chemical attraction affects the likelihood and rate of exit of PWN from the vector to the tree, and it may be necessary to examine a complex of chemicals, rather than single compounds. Therefore, the Panel concludes that this claim could not be substantiated.

The Panel agrees with the Portuguese assertion that *P. pinea* is not a preferred host for *M. galloprovincialis* breeding. It also notes that there is evidence from Italy of breeding of *M. galloprovincialis* in fallen woods of *P. pinea*, suggesting that *P. pinea* is an occasional host for *M. galloprovincialis*.

The Panel agrees with the assertion that, although the pathogenicity of PWN has been recorded on *P. pinea* in inoculation experiments on small plants under controlled conditions, this does not necessarily relate to pathogenicity on larger trees in the field.

With regard to the assertion that adult *P. pinea* trees are not adequate hosts for the PWN and do not suffer from wilt disease, the Panel is of the opinion that the additional information provided in Rodrigues et al. (2012) on the national surveys is not sufficient to conclude that *P. pinea* is resistant to PWN. In addition, the Panel notes that asymptomatic infestations by PWN are well known in areas where the nematode occurs. Pine species that are tolerant to the PWN could still allow the nematode to exist at low levels in restricted parts of a tree and thus act as a reservoir.

The overall conclusion of the Panel is that there is high uncertainty concerning the classification of risk of spread of PWN with plants and wood of *P. pinea*, owing to the scarcity of information on the interaction of *M. galloprovincialis*, *B. xylophilus* and *P. pinea*, as well as the lack of clear evidence for field resistance of *P. pinea* to PWN.

RECOMMENDATIONS

The Panel suggests that studies on the transmission of PWN at feeding wounds and the survival of PWN in trees and wood of *P. pinea* are needed to reduce the uncertainties regarding the host potential of *P. pinea*.

DOCUMENTATION PROVIDED TO EFSA

1. Sousa E, Naves P, Bonifacio L and Rodrigues JM, 2012. Comment on the document “Scientific Opinion on the phytosanitary risk associated with some coniferous species and genera for the spread of the pine wood nematode (PWN) *Bursaphelenchus xylophilus*” (February 2012).
2. Rodrigues JM, Fialho C, Ferreira T, Sousa E, Naves P and Bonifacio L, 2012. Risk assessment of *Pinus pinea* L. in relation to Pine Wood Nematode — additional information. Portuguese National Forestry Authority/National Institute for Nature Conservation and Forests (ICNF, I.P.) and Portuguese National Institute of Agricultural Research and Veterinary (INIAV, I.P.) (December 2012).

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APPENDICES

Appendix A. Evaluation of risk assessment of *Pinus pinea* L. in relation to pine wood nematode – update of the statistical issues

1. Introduction

1.1. Comments on the EFSA opinion on PWN host plants (EFSA PLH Panel, 2012a)

In their comments on the EFSA opinion on PWN host plants (EFSA PLH Panel, 2012a), Sousa et al. (2012) claim that the surveys of the Portuguese National Forest Authority (NFA) show that adult *P. pinea* trees are not adequate hosts for the PWN and do not suffer from wilt disease.

The authors state that since 2001 more than 1 673 wood samples of *P. pinea* from PWN-infested locations have been tested for the presence of PWN, and “the PWN was never detected on any occasion. The continuous sampling over a decade is the clearest and most evident result on the non-susceptibility of adult *P. pinea* trees in the field, and although these results were presented in the first report (rem. Sousa et al., 2011) they were surprisingly discarded in the EFSA report, and never commented or discussed.” (Sousa et al., 2012, p.3).

The surveys conducted by the Portuguese National Forest Authority (NFA) are described in chapter III.1 of Sousa et al. (2011). Table II refers to the evolution of the area and felled pine trees during the eradication campaign in the affected and buffer zones⁶ in Portugal from 2000 to 2007. In 2007 the demarcated area was increased to 1 010 000 ha with a total of 80 000 ha of pine stands and 28 667 ha of pine stands with symptoms. (DGRF, cited in Sousa et al., 2011) “In the restricted area south of the Tagus river, the PWN occurs in an area with a forest cover dominated by maritime pine (47 000 ha) and stone pine (64 000 ha) [...]” (Sousa et al., 2011, p. 5).

Table 1: Areas in the demarcated areas in Portugal

Name	Area (ha)	No of felled pines
Demarcated area in Portugal in 2007	1 010 000	214 300 ^a
... thereof pine stands (in affected zone)	80 000 = 7.9 %	196 530 ^b
... thereof with symptoms	28 667 = 36 %	
Restricted, affected area south of Tagus River		
... thereof dominated by <i>P. pinaster</i>	47 000	
... dominated by <i>P. pinea</i>	64 000	

a Sum of trees in affected and buffer zone.

b Number of trees in affected zones.

From these data it is not clear how many hectares with forest cover dominated by *P. pinea* are falling within the affected zones.

⁶ According to Commission Decision 2006/133/EC of 13 February 2006 requiring Member States temporarily to take additional measures against the dissemination of *Bursaphelenchus xylophilus* (Steiner et Buhrer) Nickle et al. (the pine wood nematode) as regards areas in Portugal, other than those in which it is known not to occur (OJ L 52,23.2.2006, p. 34) “Portugal shall establish areas in which PWN is known not to occur, and demarcate areas (hereinafter called demarcated areas) comprised of a part in which PWN is known to occur and a part designated as buffer zone of not less than 20 km width surrounding that part, taking into account the results of the surveys referred to in Article 4.”

Nevertheless since 2001 a total of 1 673 wood samples of *P. pinea* “(consisting in mixtures of wood of up to five dead trees)” (Sousa et al., 2011) were taken and analysed for the presence of PWN. Figure 1 shows the annual number of wood samples from *P. pinea* in the demarcated area South of Lisbon. The numbers vary from more than 1 100 samples in 2001 to more than 200 samples in each year from 2002 to 2007, giving at least 2 300 samples in total. There appears to be a discrepancy between the accumulated total from Figure 1 and the reported total of 1 673.

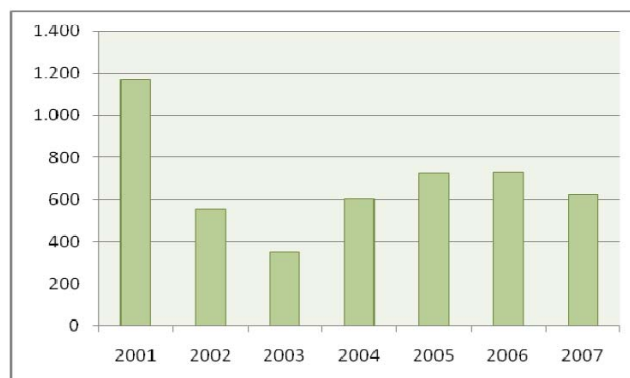


Figure 1: Annual number of wood samples from *P. pinea* of the demarcated area south of Lisbon (from Sousa et al., 2011)

1.2. Request for additional information

Because the information on the surveys conducted by the Portuguese National Forest Authority was incomplete in the paper by Sousa et al. (2011), EFSA requested from the European Commission, in a letter dated 2 July 2012, additional information on the methodology and results of the PWN annual pest surveys. In particular, in accordance with the EFSA PLH Panel checklist for evaluating a proposed risk reduction option (EFSA PLH Panel, 2012b), more details were requested, for example, on the following aspects: survey hypothesis; explanation of the applied mathematical background and its justification; sampling methods; confidence level; methodology and instruments for performing an individual observation, including sampling height and laboratory testing; results of the survey, i.e. list and details of observations.

In the following sections the additional information on Portuguese National Forest Authority PWN surveys, received in December 2012 from the EU Commission (Rodrigues et al., 2012), will be described and analysed in detail.

2. Surveys for hosts associated with the PWN in Portugal

The latest Portuguese National Forest Inventory (FloreStat, 2010) estimated there are about 311.8 million trees of *P. pinaster* and 14.8 million of *P. pinea* in Portugal. Out of these 89.3 % of *P. pinaster* and 62.1 % of *P. pinea*, trees are segregated in pure or dominant forest stands of these species.

90.1 % of the area of dominant forest stands with *P. pinaster* is located in northern (NUTS2: PT11) and central (PT16) continental Portugal, while 97.5 % of the area of the dominant forest stands with *P. pinea* is located around Lisbon (PT 17), Alentejo (PT18) and Algarve (PT15).

While in north continental Portugal (PT11) 44.9 % of all forest stands are dominated by *P. pinaster* and 0.0 % by *P. pinea*, the situation is turned around in the south (Algarve, PT15) with 4.9 % *P. pinaster* and 24.7 % *P. pinea*.

Another difference between forest stands dominated by *P. pinaster* or *P. pinea* is the mean density, which is in general for grown trees about three times higher for *P. pinaster* than for *P. pinea*.

In summary, growing conditions of *P. pinea* and *P. pinaster* trees are different in Portugal. To compare their suitability for infestation by PWN or *M. galloprovincialis*, several confounding factors have to be taken into account, e.g. climatic differences and differences in soil between northern and southern continental Portugal, differences in the composition of the forest stands and differences in the tree density.

The following tables show relevant information from the Portuguese National Forest Inventory (FloreStat, 2010) for the NUTS2 level and selected LAU1 municipalities (“Concelhos – Municípios”).

Table 2: Total number of trees of *P. pinaster* and *P. pinea* and their mean densities in forest stands in Portugal (Source: FloreStat, 2010, table 201)

Dominance	<i>P. pinaster</i>			<i>P. pinea</i>		
	No of trees (10 ⁶)	%	Mean density (trees/ha)	No of trees (10 ⁶)	%	Mean density (trees/ha)
Pure	245.8	78.8	361	7.3	49.3	137
Dominant	32.6	10.5	226	1.9	12.8	63
Dominated	15.3	4.9	129	1.8	12.2	42
Dispersed	13.3	4.3	–	1.7	11.5	–
Pure/dominant young	4.8	1.5	82	2.1	14.2	46
Total	311.8	100	311 ^a	14.8	100	87 ^a

a Estimated mean density from pure/dominant/dominated.

The technical glossary of the Portuguese National Forest Authority (ICNF) gives following definition of the composition of forest stands:

“Composition of a forest stand—In a narrow sense it refers to the individual variety and their specific or cultural nature. It’s possible to distinguish between pure stands, as consisting of a single dominant species, and mixed stands, with individuals from more than one species – dominant and dominated species, presence of shrub of other species. The pure stands are those in which the percentage of other species don’t exceed 25 %.”⁷ (<http://www.icnf.pt/portal/florestas/gf/glossario-tecnico>, translation by EFSA).

Centre region (of Portugal)	Continental Portuga/NUTS region PT1 “Continente”
Setúbal district	“Distrito de Setúbal”/NUTS regions PT172 “Península de Setúbal” and PT181 “Alentejo litoral” without PT181.0211 “Odemira”
Setubal Demarcated Area	(around) Setubal peninsula/NUTS region PT172 “Península de Setúbal”, from 5 640 km ² (2000/2001) to 10 100 km ² (2006/2007).

⁷ Composição de um povoamento — Em sentido restrito, refere-se à variedade e natureza específica ou cultural dos indivíduos de um povoamento. Distinguir-se-á assim entre povoamentos puros, constituídos por uma só espécie florestal dominante, e povoamentos mistos, nos quais coexistem indivíduos pertencentes a mais do que uma espécie florestal — espécies dominantes e espécies dominadas, presença de bosquetes de outras espécies. Considerar-se-ão povoamentos puros aqueles em que a percentagem de outras espécies não ultrapasse 25 %.

Table 3: Areas in Portugal (Sources: Instituto Geográfico Português, 2011; FloreStat, 2010, tables 101, 104, 106, 401, 402)

NUTS	Name	Total area (ha)	Area with forest, total (ha)	Area with forest stands, total (ha)	Area of forest stands with dominant trees						Condition of stands with dominant <i>P. pinaster</i>			
					<i>P. pinea</i> (ha)	% <i>P. pinea</i>	% Forest stands	<i>P. pinaster</i> (ha)	% <i>P. pinaster</i>	% Forest stands	No damage (%)	Light damage (%)	Severe damage (%)	Rate of dead trees (%)
PT	Portugal	9 209 010	3 541 284					892 071	100.0		43	46	11	7.4
PT1	Continental Portugal	8 896 710	3 458 557	3 175 348	130 386	100.0	4.1	885 019	99.2	27.9				
PT11	North continental/“Norte”	2 128 640	680 659	577 212	276	0.2	0.0	259 275	29.1	44.9	45	46	9	6.4
PT15	Algarve	499 600	132 209	121 679	30 044	23.0	24.7	5 973	0.7	4.9				
PT150.0801	Albufeira	14 060	1 564	1 450	174		12.0	21		1.4				
PT150.0802	Alcoutim	57 540	17 817	16 853	9 266		55.0	515		3.1				
PT150.0803	Aljezur	32 350	10 511	9 398	1 898		20.2	1 328		14.1				
PT150.0804	Castro Marim	30 080	6 991	6 482	3 703		57.1	175		2.7				
PT150.0805	Faro	20 160	1 526	1 360	224		16.5	42		3.1				
PT150.0806	Lagoa	8 830	577	480	113		23.5	49		10.2				
PT150.0807	Lagos	21 280	4 485	4 044	1 766		43.7	184		4.5				
PT150.0808	Loulé	76 420	24 862	22 984	2 305		10.0	312		1.4				
PT150.0809	Monchique	39 530	19 952	18 597	958		5.2	935		5.0				
PT150.0810	Olhão	13 090	871	784	75		9.6	15		1.9				
PT150.0811	Portimão	18 210	3 609	3 250	949		29.2	91		2.8				
PT150.0812	São Brás de Alportel	15 340	5 785	5 154	465		9.0	148		2.9				
PT150.0813	Silves	68 000	16 460	14 976	3 993		26.7	888		5.9				
PT150.0814	Tavira	60 700	13 777	12 717	3 358		26.4	423		3.3				
PT150.0815	Vila do Bispo	17 900	2 229	2 008	363		18.1	576		28.7				
PT150.0816	Vila Real de Santo António	6 120	1 192	1 142	431		37.7	270		23.6				
PT16	Central/“Centro”	2 819 850	1 159 494	1 058 533	3 029	2.3	0.3	544 585	61.0	51.4	43	45	12	8.2
PT17	Lisbon/“Lisboa”	293 480	72 211	68 741	9 730	7.5	14.2	15 924	1.8	23.2				

NUTS	Name	Total area (ha)	Area with forest, total (ha)	Area with forest stands, total (ha)	Area of forest stands with dominant trees						Condition of stands with dominant <i>P. pinaster</i>			
PT171	Great Lisbon/"Grande Lisboa"	137 590	16 517	14 689	1 728	11.8	3 204	21.8						
PT172	Setúbal peninsula/"Península de Setúbal"	155 890	55 695	54 052	8 002	6.1	14.8	12 720	1.4	23.5				
PT172.1502	Alcochete	12 840	3 748	3 659	454	12.4	159	4.3						
PT172.1503	Almada	7 020	1 470	1 436	271	18.9	556	38.7						
PT172.1504	Barreiro	3 180	685	678	76	11.2	263	38.8						
PT172.1506	Moita	5 530	331	321	50	15.6	108	33.6						
PT172.1507	Montijo	34 810	17 570	17 195	2 458	14.3	647	3.8						
PT172.1508	Palmela	46 290	14 972	14 535	2 808	19.3	894	6.2						
PT172.1510	Seixal	9 550	2 580	2 520	200	7.9	1 867	74.1						
PT172.1511	Sesimbra	19 500	9 928	9 756	714	7.3	7 211	73.9						
PT172.1512	Setúbal	17 190	4 412	3 952	971	24.6	1 014	25.7						
PT18	Alentejo	3 155 140	1 413 983	1 349 184	87 308	67.0	6.5	59 262	6.6	4.4	40	54	6	3.4
PT181	Alentejo Litoral	525 580	301 206	284 463	36 171	27.7	12.7	33 777	3.8	11.9				
PT181.0211	Odemira	172 060	80 207	74 937	2 487	3.3	4 752	6.3						
PT181.1501	Alcácer do Sal	146 510	95 659	90 793	21 778	24.0	13 353	14.7						
PT181.1505	Grândola	80 770	58 455	55 099	9 859	17.9	7 902	14.3						
PT181.1509	Santiago do Cacém	105 980	58 759	55 884	1 591	2.8	4 355	7.8						
PT181.1513	Sines	20 260	8 126	7 749	456	5.9	3 415	44.1						
Setúbal district		509 410	276 694	263 578	41 686	32.0	15.8	41 745	4.7	15.8				
PT182	Alto Alentejo	624 900	267 048	255 073	6 415	2.5	11 792	4.6						
PT183	Alentejo Central	722 880	366 874	344 570	11 370	3.3	1 930	0.6						
PT184	Baixo Alentejo	854 280	274 592	268 368	17 789	6.6	1 607	0.6						
PT185	Lezíria do Tejo	427 500	204 262	196 710	15 563	7.9	10 156	5.2						
PT2	Açores Islands	232 200	48 503					874	0.1					
PT3	Madeira Islands	80 100	34 224					6 178	0.7					

3. Surveys for PWN and *P. pinea* conducted by the National Forest Authority

The national surveys on PWN were done in two phases.

From 1999 to 2007 the campaigns were focused on the area around the Setubal peninsula and additional locations of higher risk, e.g. points of wood import, handling and storage.

Since 2008 the field plots with coniferous forest cover of the National Forest Inventory (IFN) have been screened for the presence of PWN. These comprised a screening of 10 850 plots in the five years since 2008. Besides that the buffer zones around outbreaks and other locations of higher risk were surveyed.

In the paper by Rodrigues et al. (2012) only results of the first phase are mentioned. Therefore, the results are restricted only to the area around Setubal peninsula.

3.1. Screening of the documentation/description of the datasets

The documentation provided by Rodrigues et al. (2012) on PWN surveys was screened according to the EFSA PLH Panel checklist for evaluating a proposed risk reduction option (EFSA PLH Panel, 2012b). The results of this screening are presented in the tables below.

Table 4: Checklist for evaluating a proposed risk reduction option (EFSA PLH Panel, 2012b)

Item	Description based on the submitted document(s)	Comments
Description of the proposed risk reduction option		
Name	<i>P. pinea</i> resistant host for PWN	
Target pest	Pine wood nematode (PWN) <i>Bursaphelenchus xylophilus</i> (Steiner et Buhner 1934) Nickle 1970	
Vector	Pine sawyer <i>Monochamus galloprovincialis</i>	
Target plant material/product	Stone pines, <i>P. pinea</i>	
Disease	Pine wilt disease (PWD)	
Origin of plant material/product	Portugal <i>P. pinea</i> distributed in the whole Mediterranean Basin from Portugal to Turkey	
Type of risk reduction option	Proven resistance <i>P. pinea</i> should be classified as “resistant” instead of “intermediate” Exempt <i>P. pinea</i> from emergency measures	Evans et al., 1996
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	Survey and sampling during autumn and winter, when wilting symptoms are most conspicuous	
Plant identity (e.g. botanical name, variety)	<i>P. pinea</i>	

Conditions under which plant materials/products are managed	Natural conditions in the forest plots	
Conditions of the plant commodity (e.g. degree of ripeness, presence of bark, etc.)	<i>P. pinea</i> , diameter at breast height ≥ 10 cm, dead or showing symptoms of poor health	
Sampling of material	Small pieces collected at diameter breast height with autonomous slow rotation drilling device, four or more drillings per tree. Also wooden discs cut at different heights and reduced into small pieces (1 cm). Canopy samples from felled trees. Composite sample of (up to) five trees per sample, totalling a minimum of 100 g	
Pest information		
Identity (species—strains biotypes if applicable)	<i>Bursaphelenchus xylophilus</i> (Steiner et Buhner 1934) Nickle 1970	
Conditions under which the pests are cultured, reared or grown	Incubation for three weeks at 25 °C under laboratory conditions	
Method of infestation	Natural infestation through vector <i>M. galloprovincialis</i>	
Level of infestation	1999: first detection in PT 1999–2007: restricted to Setubal peninsula (“ Setubal demarcated area ”) From 2008: whole continental Portugal (“ Centre Region ”) and Madeira Island Indication of level from <i>P. pinaster</i> infestations	
Extraction	Modification of Bearman funnel: tray with water for 48 hours, sieved with a 400 mesh (38 μ m)	
Identification	Experienced nematologist and/or molecular techniques	
Stage of the pest that is most resistant to the treatment	Detection only of alive nematodes	
Experiment(s) description and analysis		
Origin	1999–2007 1. Area around the Setubal peninsula 2. Risk plots in remaining continental Portugal, mainly points of wood import, handling or storage Only results from Setubal peninsula were presented in tables I and II (Rodrigues et al., 2012) 2008–2012 (five years) 1. 2 170 IFN field plots (2 \times 2 km grid: 2 170 plots \times 0.05 ha/plot = 108.5 ha) classified as “coniferous forest cover” were taken in 2008 (year “zero”) and then dislocated by 500 m for the following years (5 years \times 108.5 ha/year = 542.5 ha) 2. Monitoring in buffer zones 3. Other risk areas, e.g. intervention zones, isolated outbreaks, areas showing symptoms of poor health or forest fires, points of wood import, handling or storage Results from 2008–2012 were not provided	

Selection of trees	In buffer zones: Sampling of all <i>P. pinea</i> Other demarcated area, IFN plots, risk areas: All <i>P. pinea</i> showing symptoms of poor health and/or forest fires	
Variables used to measure efficacy	Detection of alive nematodes alive in the material (yes/no)	
Factors influencing efficacy which were taken into account in the experiment	Year: 2000/2001 to 2006/2007	
Factors influencing efficacy which were not taken into account in the experiment	Infestation level of the forest plot (PWN and <i>Monochamus</i>), forest conditions (composition, density), climatic and soil conditions, general health status //Information on the composite sample: number of trees, number of individual samples per tree, location of sample in the tree, amount of material per individual sample //Sensitivity of the detection method	
Monitoring of critical parameters	Unknown	
Description of experimental design	In “Setubal Demarcated Area” all <i>P. pinea</i> in poor health were effectively sampled	
Presentation of the data	Area (estimated number of <i>P. pinea</i> trees), number of trees with decline symptoms/number of composite samples/“no positive findings”/all variables per year	
Description of the statistical analysis	None	
Conclusions of the experiment	<i>P. pinea</i> is resistant to PWN	
Other relevant information		

Source:

Rodrigues JM, Fialho C, Ferreira T, Sousa E, Naves P and Bonifacio L, 2012. Risk assessment of *Pinus pinea* L. in relation to pine wood nematode — additional information. Portuguese National Forestry Authority/National Institute for Nature Conservation and Forests (ICNF, I.P.) and Portuguese National Institute of Agricultural Research and Veterinary (INIAV, I.P.), December 2012

3.1.1. Extracted data

The area of “Setubal demarcated area” is given from 564 000 ha (2000/2001) to 1 010 000 ha (2006/2007), which is larger than the total area of the Setubal peninsula (PT172: 155 890 ha) or the larger Setubal district (PT172 + PT181 – PT181.0211 = 509 410 ha). No clear description on the location of the survey areas over the years is given in the paper.

Using the Setubal district as approximation the National Forest Inventory (FloreStat, 2010) shows that 54 % of the total area is covered by forest and 52 % by forest stands. About 16 % of the area of forest stands is dominated by either *P. pinea* or *P. pinaster*.

Using the whole of Alentejo as an approximation the National Forest Inventory (FloreStat, 2010) shows that 6 % of the forest stands dominated by *P. pinaster* show severe damage and that 3.4 % of the *P. pinaster* trees were dead in the survey period 2005/2006.

Table 5: Development of areas in Setubal demarcated area from 2000 to 2007

	2000 /2001	2001 /2002	2002 /2003	2003 /2004	2004 /2005	2005 /2006	2006 /2007	Setubal peninsula (see Table 3)	Setubal district (see Table 3)

Total area (ha)	564 000	564 000	564 000	617 000	617 000	641 000	1 010 000	155 890	509 410
Total area of pine stands (ha)	60 000	60 000	60 000	69 000	69 000	69 500	80 000		
Area of forest stands dominated by <i>P. pinaster</i> (ha)								12 720	41 745
Area of forest stands dominated by <i>P. pinea</i> (ha)								8 002	41 686
<i>P. pinea</i> area (ha)	48 000	48 000	48 000	50 000	50 000	50 000	64 000		

Grey cells are calculated by EFSA.

Table 6: Numbers of monitored *P. pinea* trees in Setubal demarcated area from 2000 to 2007

	2000 /2001	2001 /2002	2002 /2003	2003 /2004	2004 /2005	2005 /2006	2006 /2007	Total
Estimated ^a number of monitored trees	2 880 000	2 880 000	2 880 000	3 000 000	3 000 000	3 000 000	3 840 000	21 480 000
Number of trees with decline symptoms	1 170	554	352	603	727	732	623	4 761
Number of declining trees with findings of PWN	0	0	0	0	0	0	0	0
Number of declining trees without findings of PWN	1170	554	352	603	727	732	623	4 761
Rate of decline without PWN findings (%)	0.04	0.02	0.01	0.02	0.02	0.02	0.02	0.02

a Estimated by *P. pinea* area multiplied by mean density of *P. pinea* 60 trees/ha (Rodrigues et al., 2012).

Grey cells are calculated by EFSA.

Table 7: Numbers of monitored *P. pinaster* trees in Setubal demarcated area from 2000 to 2007

	2000 /2001	2001 /2002	2002 /2003	2003 /2004	2004 /2005	2005 /2006	2006 /2007	Total
Estimated ^a number of monitored trees	14 928 000	14 928 000	14 928 000	15 550 000	15 550 000	15 550 000	19 904 000	111 338 000
Number of trees with decline symptoms	57 864	49 988	61 106	84 483	102 090	288 253	213 677	857 461
Number of sampled trees	25 637	9 750	10 687	11 465	10 276	11 142	17 155	96 112
Rate of positive PWN samples	19	7.26	18.04	8.71	10.32	39.33	4.77	
Estimated ^b number of declining trees with findings of PWN	10 994	3 629	11 024	7 358	10 536	113 370	10 192	167 103
Estimated ^c number of declining trees without findings of PWN	46 870	46 359	50 082	77 125	91 554	174 883	203 485	690 358
Rate of decline without findings of PWN (%)	0.31	0.31	0.34	0.50	0.59	1.12	1.02	0.62

Grey cells are calculated by EFSA.

- a Assuming that the *P. pinaster* area is as large as the *P. pinea* area in Setubal demarcated area, and estimated by *P. pinaster* area multiplied by mean density of 311 *P. pinaster* trees/ha.
- b Estimated by the number of declining trees times the rate of positive PWN samples.
- c Estimated by the number of declining trees times the rate of negative PWN samples.

Regarding the trees that were showing symptoms of decline in the Setubal demarcated area, but that were not connected to findings of PWN, the rate of decline is about 30 times higher for *P. pinaster* than for *P. pinea*.

3.2. Data analysis and methods

The main conclusion of Rodrigues et al. (2012) is, that “for the total of 4 761 Stone Pine trees identified with decline symptoms, an amount of 1 673 wood samples (consisting in mixtures of wood of up to five trees) were collected and analyzed for the presence of PWN. The pine wood nematode was not detected on those samples.” (Rodrigues et al., 2012, p. 4)

Applying the procedure to determine the minimal number of samples to be taken to confirm the absence of a pest with a detection limit of 0.02 % and a reliability of 99 % described in the technical assistance on sampling statistics to be applied pursuant to Commission Implementing Decision 2012/535/EU on emergency measures to prevent the spread of PWN within the European Union (EFSA, 2012) shows that:

- Without further information on the locations of the *P. pinea* trees in the demarcated area (infested (affected) part vs the buffer zone) a judgement on the resistance of *P. pinea* trees is not possible.
- The examination of 4 761 *P. pinea* trees would be sufficient to state with 99 % reliability that the maximum infestation rate is below 0.1 % in the Setubal demarcated area, but only if the sensitivity of the detection method is close to 100 %. For the composite samples with an undefined amount of material and locations per tree, a lower sensitivity has to be assumed. Owing to the use of a sample of insufficient size and to the detection method implemented by Rodrigues et al. (2012), it is not possible to confirm an infestation level of 0.02 % from the Portuguese surveys.

The following reasoning is applied:

Continental Portugal comprises 130 386 ha of forest stands with *P. pinea* dominating. With a mean density of more than 80 trees/ha and a yearly decline of about 0.02–1 %, the total population of *P. pinea* trees showing symptoms of decline is large enough to calculate the minimal sample size to confirm freedom from the pest using the binomial approximation. To confirm that the infestation rate is below a threshold of 0.02 % with a reliability of 99 % the necessary sample size is larger than 23 000 trees (EFSA, 2012). These samples have to be taken randomly out of the total Portuguese area with an appropriate sampling procedure for each tree.

The most recent surveys conducted by the Portuguese National Forest Inventory may provide these data (from the year 2008), but these were not given in the paper by Rodrigues et al. (2012).

However, the task of the paper by Rodrigues et al. (2012) was not to confirm pest freedom of *P. pinea* trees but to show their resistance. For that a second condition must be fulfilled, namely the proven presence of the pest. Therefore the restriction on the Setubal demarcated area for the survey period 2001–2007 is reasonable. Nevertheless, a demarcated area consists of the infested (affected) area and a buffer zone around it. In the buffer zone it is assumed that the pest is not present. The authors do not distinguish the data into numbers of trees in the infested area and trees in the buffer zone.

Another difference for showing resistance is that the population is not fixed to the number of trees in a specific area. Instead the study is only a sample out of all *P. pinea* trees that are currently are growing in Portugal.

To have a $1 - \alpha = 99\%$ reliability that at least one tree ($k = 1$) within a population of $n = 4\,761$ trees is infested, the minimal probability π of infestation must be:

$$\pi \geq 1 - \sqrt[n]{\alpha} = 0.1\%$$

Otherwise, there is a non-negligible chance that the infestation is not expressed in the population of declined trees.

To have a $1 - \alpha = 99\%$ reliability that at least one detection of $k = 1$ infested samples, the minimal sensitivity σ of the detection method must be

$$\sigma \geq 1 - \sqrt[k]{\alpha} = 99\%$$

Based on the checklist for evaluating a proposed risk reduction option (Table 4), the Portuguese sampling regime would not give a sensitivity of detection equal to or higher than 99 %, also because of the composite samples up to five trees with a minimal amount of 100 g and unspecified sampling locations on the tree.

3.3. Uncertainties

- The data presented are not the most current data from 2008–2012 and do not cover the whole of Portugal but only the Setubal demarcated area. The results may not be representative for the situation in whole of Portugal owing to different climatic, soil and forest stand conditions (the composition and density of the forest stands).
- The data presented do not distinguish between the number of trees in the infested zone and in the buffer zones. To show resistance an appropriate infestation of the forest with *Monochamus* and PWN must be given, which is not expected to occur in the buffer zone for PWN.
- The level of infestation of PWN and *Monochamus* in the Setubal demarcated area is not described fully. It is not known whether some of the samples were also taken from non-infested areas or areas with negligible (below the threshold of 0.02 %) infestations in other *Pinus* species.
- The health status of *P. pinaster* is general weaker than that of *P. pinea* in the Setubal demarcated area, which might cause an infestation preference in *M. galloprovincialis* for *P. pinaster* trees.
- It is not specified how many *P. pinea* trees were felled and how many samples were taken from the canopy of the tree or from the trunk. Missing samples from parts of the trees with a higher risk of infestation, such as parts showing signs of decline, the activity of *Monochamus* spp. or blue-stain fungi will decrease the sensitivity of the detection method.
- It is not known what the minimal amount of material was that was taken from each *P. pinea* tree. Small or inadequate sampling will decrease the sensitivity of the detection method (EFSA, 2012).
- Samples were taken only from *P. pinea* trees showing decline symptoms. Latent infections cannot be observed with this sampling scheme.
- The sensitivity of the detection method is most likely below 99 %, which implies that the number of samples was too low to confirm the absence of infestation with a threshold of 0.02 % and reliability of 99 % as mentioned in Commission Implementing Decision 2012/535/EU.

4. Surveys conducted by the national reference laboratory for plant health in Tróia peninsula

This information, already presented by Sousa et al. (2011) and by Sousa (2011), was discussed in the former EFSA opinion (EFSA PLH Panel, 2012a).

The main conclusions of the analysis of these data in the previous opinion were the following:

Table 8: Summary of uncertainties of the surveys on Tróia peninsula by evaluation criteria (EFSA PLH Panel, 2012a)

Evaluation criteria	Survey 1: Tróia peninsula	Survey 2: Tróia peninsula experimental plots
Representativeness	Specific conditions on Tróia peninsula might restrict the possibility of applying the results to other areas in Portugal, especially where the composition of the forest or tree density is different	

Evaluation criteria	Survey 1: Tróia peninsula	Survey 2: Tróia peninsula experimental plots
Forest composition	<p>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 that have high <i>P. pinea</i> presence cover 20 % of the Tróia peninsula. (Sousa, 2011; Sousa et al., 2011)</p> <p>Concerning an extrapolation of results to other areas: pine forests in Central and Northern Portugal have different characteristics owing to distinct edaphoclimatic conditions, and stone pine is usually absent or residual in these areas. (Sousa, 2011; Sousa et al., 2011)</p>	<p>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 <i>P. pinaster</i> forests or are mixed <i>P. pinaster</i> (about 70 %) and <i>P. pinea</i> (about 30 %) forest. No information on the <i>P. pinea</i>-dominated forests, which are 20 % of the pine forest on Tróia peninsula, is provided and it appears that no sampling was carried out in these stone pine-dominated forests</p>
Density of trees		<p>All plots have higher total pine tree density and fewer <i>P. pinea</i> than the average on Tróia peninsula: Plots 1 and 2 were dominated by <i>P. pinaster</i> (500 trees/ha and 348 trees/ha, respectively, and about 1 % <i>P. pinea</i>), compared with an average of 198 <i>P. pinaster</i> trees/ha and 11 % of <i>P. pinea</i> on the peninsula (Sousa, 2011; Sousa et al., 2011). Plot 3 is an example of mixed forest, but <i>P. pinaster</i> 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 for the Tróia peninsula nor for the entire North Alentejo coast and the Ribatejo Province</p>
Level of infestation	<p>The authors concluded that only 0.2 % of the dead pine trees (with symptoms) in the winters 2000/01 to 2007/08 were <i>P. pinea</i>. However, the years are not homogeneous. 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 <i>P. pinaster</i> trees decreased from about 80 % to 10 %. 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)</p>	<p>No information was given on the infestation with PWN, the presence of <i>M. galloprovincialis</i> or bark beetles, or 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)</p> <p>No discussion was presented on the differences between the years</p>
Accuracy of detection/data analysis	<p>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. The sample sizes for <i>P. pinea</i> are too small to provide statistical support for differences in infestation rates between the two species. No information on the presence of <i>M. galloprovincialis</i> or of the bark beetles (Scolytidae) on individual trees was reported</p>	<p>The number of <i>P. pinea</i> 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 <i>P. pinea</i> which appears to be below 1.45 % (upper level of 95 % confidence interval). The yearly mortality rate for <i>P. pinaster</i> is about 4 % (95 % confidence interval 3.38–4.55 %)</p>

Appendix B. Update of literature search

In the EFSA PLH Panel opinion (2012a), an extensive literature search was performed on the PWN and its vector and on four specific host plants including *P. pinea*. For this scientific opinion the literature search was updated (from 2011 to 14/02/2013), focusing on the PWN and its vector and *P. pinea*. The principles of the extensive literature search (EFSA, 2011), corresponding to the first steps of a systematic review process (EFSA, 2010), were followed.

The information sources consulted were:

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 and Zoological Records).

Web-based search utilities (Google Scholar).

1. Search on ISI Web of Knowledge

The literature search was performed on 14/02/2013 to retrieve the published articles since 2011. The search strategy applied on the ISI Web of Knowledge database was articulated in two searches:

Pinus pinea AND the PWN:

[Topic=(lignicolus OR xylophilus) OR Topic=(PWN) OR Topic=(Aphelenchoides AND xylophilus)

OR

Topic=((Bursaphelench* xylophilus) OR ((“Pine wood” OR Pinewood) AND nematode) OR (“Pine wilt disease”))]

AND

Topic=(Pinus AND (pinea OR sativa)) OR Topic=((Stone OR Umbrella OR Parasol) AND pine)

Pinus pinea AND *Monochamus* spp:

Topic=(Monochamus) OR Topic=(sawyer)

AND

Topic=(Pinus AND (pinea OR sativa)) OR Topic=((Stone OR Umbrella OR Parasol) AND pine)

2. Results

All the references resulting from the search strategy detailed above were screened for relevance by their titles and abstracts. The screening process was unmasked and performed on the basis of irrelevance to the subject of this work. Documents not dealing with *P. pinea* in relation with the PWN were considered irrelevant. The full texts of the selected references were considered to produce a set of relevant evidence.

From these searches in ISI Web of Knowledge the following articles were retained:

- Dayi M and Akbulut S, 2012. Pathogenicity testing of four *Bursaphelenchus* species on conifer seedlings under greenhouse conditions. *Forest Pathology* 42, 213–219.
- Franco AR, Santos C, Roriz M, Rodrigues R, Lima MRM and Vasconcelos MW, 2011. Study of symptoms and gene expression in four *Pinus* species after pinewood nematode infection. *Plant Genetic Resources—Characterization and Utilization* 9, 272–275.
- Sancho dos Santos CS and de Vasconcelos MW, 2012. Identification of genes differentially expressed in *Pinus pinaster* and *Pinus pinea* after infection with the pine wood nematode. *European Journal of Plant Pathology* 132, 407–418.
- Santos CS and Vasconcelos M, 2011. Physiological response of *Pinus* spp. in the first hours after infection with *Bursaphelenchus xylophilus* (Nematoda: Aphelenchoididae). *Silva Lusitana* 19, 99–110.
- Santos CS, Pinheiro M, Silva AI, Egas C and Vasconcelos MW, 2012. Searching for resistance genes to *Bursaphelenchus xylophilus* using high throughput screening. *BMC Genomics* 13, 599–599.

From the searches performed in web-based search utilities (Google Scholar) the following theses were retained:

- Santos CS, 2010. Identification of genes differentially expressed in *P. pinea* and *P. pinaster* after infection with the pine wood nematode (PWD) using the SSH technique. Master of Science thesis, presented to Escola Superior de Biotecnologia of the Universidade Católica Portuguesa.
- Mendes AR, 2012. Avaliação das condições ambientais que contribuem para o estabelecimento do nemátode da madeira do pinheiro (*Bursaphelenchus xylophilus*) Master of Science thesis in Ecology and Environment, presented to the University of Lisbon, Faculty of Science, Department of Animal Biology, Portugal.

ABBREVIATIONS

EU	European Union
IFN	National Forest Inventory
J _{IV}	fourth-stage dispersal juvenile (“dauer larva”)
NFA	Portuguese National Forest Authority
PWN	pine wood nematode (<i>Bursaphelenchus xylophilus</i>)
PWD	pine wilt disease