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Pest risk assessment of the Pine Wood Nematode (PWN) *Bursaphelenchus xylophilus* in Norway - Part 1

Opinion of the Panel on plant health of the Norwegian Scientific Committee for Food Safety 26.09.08

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SUMMARY

Pine Wood Nematode (PWN, *Bursaphelenchus xylophilus*) is the causal organism of Pine Wilt Disease (PWD), the worst forest pest of Japan. In Europe PWN is known to exist in Portugal. The Norwegian Food Safety Authority (Mattilsynet) is concerned about the plant health risks and the consequences to the society if PWN should establish in Norway. Mattilsynet needs a scientific assessment of the proposed measures in a contingency plan for PWN. Mattilsynet also needs the risks connected with recent spread of PWN in Portugal to be evaluated before possible changes can be made in the current phytosanitary policy of Norway.

On this background Mattilsynet requested a pest risk assessment of PWN from the Norwegian Scientific Committee for Food Safety (Vitenskapskomiteen for mattrygghet, VKM). To answer the request, VKM commissioned a draft pest risk assessment report from the Norwegian Institute for Agricultural Sciences and Environmental Research (Bioforsk). A working group appointed by VKM's Panel on Plant Health (Panel 9) has been involved during Bioforsk's work on the report. VKM's Panel 9 has used the report as a basis for VKM's opinion. The current document answers Part 1 of Mattilsynet's request, and was adopted by Panel 9 on a meeting 3rd September 2008.

VKM's Panel 9 gives the following main conclusions of the risk assessment: 1) The PRA area of this assessment is Norway. PWN is not known to occur in Norway. 2) With present trade pattern the probability of entry of PWN into Norway is expected to be high. The most probable pathway for entry of PWN into Norway would be wood packaging material (WPM). 3) The probability that PWN will establish and spread in Norway is considered as high. 4) With regard to the so-called Pest Free Areas (PFAs) of Portugal, the criteria given in ISPM No. 4 (FAO 1995) for establishing and maintaining PFAs have not been met, and the data available is not sufficient to confirm the existence of PFAs. Acceptance of untreated conifer wood from all parts of Portugal will result in a very high probability of entry and a high probability of establishment and spread of PWN and its vector to Norway. 5) Uncertainty factors: To the best of our knowledge PWN is absent from the PRA area. The beetle *M. sutor* is regarded as a potential vector or PWN, but this has so far not been demonstrated in nature. The currently low vector densities may retard establishment of the PWN and PWD, but it will probably not stop establishment in a longer perspective. Lack of information on the dynamics of PWN populations in cool climates complicates estimates of the spread of the nematode and PWD. Custom routines may fail in their detection of PWN. Import of a seemingly harmless material might therefore pose an unknown risk. WPM follows consignments of all kinds and is a good example of a hazardous material, which often escapes plant health inspections. 6) Detailed assessments of economic consequences of a possible establishment and spread of PWN in Norway, the effects of global warming and other climate changes on the probability for PWD outbreaks, and the effect of possible phytosanitary measures, will be given in Part 2.

KEY WORDS

Bursaphelenchus xylophilus; Pest Risk Analysis (PRA); pest risk assessment; Pine Wilt Disease (PWD); Pine Wood Nematode (PWN); Wood Packaging Material (WPM).

ASSESSED BY

Persons working for the Norwegian Scientific Committee for Food Safety (Vitenskapskomiteen for mattrygghet, VKM), either as appointed members of the Committee or as ad hoc-experts, do this by virtue of their scientific expertise, not as representatives for their employers. The Civil Services Act instructions on legal competence apply for all work prepared by VKM.

Panel on plant health (Panel 9):

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ACKNOWLEDGEMENTS

VKM has asked the Norwegian Institute for Agricultural and Environmental Research (Bioforsk), Plant Health and Plant Protection Division, to make a draft pest risk assessment report on Pine Wood Nematode (PWN) in Norway. VKM has used this draft as a basis to answer the request from the Norwegian Food Safety Authority (Mattilsynet). Christer Magnusson and Trond Rafoss are acknowledged for their valuable work with the draft pest risk assessment report.

A working group appointed by VKMs Panel on plant health (Panel 9) has been discussing the draft assessment with Bioforsk during Bioforsk's work on the report. The members of the working group, Leif Sundheim, Christer Magnusson, Trond Rafoss, and Bjørn Økland, all members of Panel 9, are acknowledged for their valuable work with the pest risk assessment.

CONTENTS

1. BACKGROUND

Pine Wood Nematode (PWN, *Bursaphelenchus xylophilus*) is the causal organism of pine wilt disease (PWD), the worst forest pest of Japan (Mamiya 1984). In Europe PWN is known to exist in Portugal, where it was detected in 1999. The pest is not known to exist in Norway. The Norwegian Food Safety Authority (Mattilsynet), in a letter of 21st February 2008, requested a pest risk assessment of PWN from the Norwegian Scientific Committee for Food Safety (Vitenskapskomiteen for mattrygghet, VKM). The current document is VKM's answer to Part 1 in terms of reference, and was adopted by VKM's Panel 9 on a meeting 3rd September 2008. For more background information about the initiation of this pest risk assessment, see section 3.1 Initiation points.

Be aware that the current document is a pest risk assessment, and not a Pest Risk Analysis (PRA). A PRA consists of both a risk assessment and a risk management part. VKM performs purely the risk assessment, whereas Mattilsynet is responsible for the risk management. However, since this pest risk assessment is part of a PRA process, the current document refers to the PRA term in several contexts, like the identification of the PRA area and referrals to former PRAs. This is in accordance with the international standard ISPM No. 11 (FAO 2004).

2. TERMS OF REFERENCE

Mattilsynet requests a pest risk assessment of PWN (*B. xylophilus*), in accordance with the international standard ISPM No. 11 (FAO 2004).

Mattilsynet wishes VKM to assess the following aspects in particular:

Part 1

a. The probability of introduction (entry and establishment) and spread of PWN through import of different types of plants and wood products under the current Norwegian phytosanitary regulations.
b. How will a possible change in the regulations, to allow import of conifer plants and plant parts, and untreated conifer timber and wood products from Pest Free Areas (PFAs) in Portugal, affect the probability for introduction of the pest?

Part 2

a. Which consequences in forest production and economy might a possible future introduction and spread of PWN have if no control measures are imposed? What might be the effects of expected climatic changes during the next 10, 30, 60 and 80 years on the pest, provided that no control measures are imposed?
b. Following a possible introduction of PWN into Norwegian landscapes, what control effects will the measures in the preliminary Contingency Plan, chapter 6.2, have, provided that control is implemented according to the Plan? What is the probability for eradication of the pest by the proposed measures? What will be the economic consequences of the control measures?

Mattilsynet might raise additional questions later, including environmental and social consequences of a possible future establishment and spread of PWN.

3. INITIATION

3.1. Initiation points

3.1.1. PRA initiated by the review or revision of a policy

The current pest risk assessment (and the corresponding PRA) was initiated by Mattilsynet as a basis for a review and possible revision of its policy.

An interception of PWN in Portugal in 1999 lead to surveys in several other Member States of the European Union (EU). PWN was not detected in these surveys. In Norway PWN has so far, not been recorded, neither in surveys made from 2000 to 2006, nor in imported commodities.

Based on experiences from Japan and Portugal among others, and based on discussions in the Nordic collaboration within the plant health area, Mattilsynet is concerned about the plant health risks posed by PWN should the pest establish in Norway. Mattilsynet therefore decided to make a scientific contingency plan for PWN. The work was started in spring 2007, and a draft Contingency Plan is now available (Mattilsynet 2008).

In October 2007 a contingency exercise was carried out, in which representatives from involved ministries, directorates, forest industries, and research institutes, participated. After this exercise all involved parties found it necessary to continue the work on the Contingency Plan, especially with regard to which measures should be put into effect at a possible future outbreak, and how these measures should be carried out in practice if the pest is to be eradicated. The exercise also indicated that the proposed measures might have extensive social, environmental and economic consequences. Mattilsynet therefore needs to document whether such measures are necessary, and that the effect of the measures, with regard to eradication and prevention of further spread, is scientifically assessed and documented.

According to the regulation relating to plants and measures against plant pests (FOR 2000-12-01 nr 1333: Forskrift om planter og tiltak mot planteskadegjørere) it is prohibited to import plants and parts of plants of conifers (except seeds and fruits), wood with bark, chips from wood with bark, isolated bark and wood waste from Non-European countries and Portugal. Import of conifer wood chips from countries where PWN is known to occur, *i.e.* Canada, China, Japan, Korea, Mexico, Portugal, Taiwan and U.S.A is prohibited.

The European Commission (EC) has pointed out that Norway should consider accepting the phytosanitary protection provided by the control programme for PWN in EU/Portugal and the establishment of PFAs in Portugal (EC 2006a), and as a result of this allow import from PFAs in Portugal. However, Mattilsynet needs the risks connected with the recent spread of PWN in Portugal to be analysed and evaluated before changes can be made in the current phytosanitary policy of Norway.

To be able to reach a final scientific contingency plan for PWN, and to be able to evaluate the necessity of suggested measures in the proposed Contingency Plan, Mattilsynet needs a more detailed assessment of the risk that PWN might represent for Norwegian forestry and nature, and of the general risk-reducing effect of current phytosanitary regulations. Moreover, there is a need of a special assessment of risk associated with import of conifer plants and plant parts and untreated wood and timber from conifers originating from PFAs in Portugal. Furthermore, there is a need to assess what effect and consequences the suggested eradication and control measures in the contingency plan will have, and the probability of a successful eradication.

3.2. Identification of PRA area

The PRA area is Norway.

3.3. Information

Information sources utilised for this pest risk assessment are published material available in international scientific journals, books and reports, as well as personal communications with persons involved in the area, geographic data, and unpublished results that have been made available to the risk assessors, like trade statistics and documents from Ministerio da Agricultura of Portugal and EC directives and mission reports. Where these information sources have been used, this is indicated in the text by references enclosed in brackets.

The current pest risk assessment is made according to the international standard ISPM No. 11 (FAO 2004).

3.3.1. Previous PRAs

Evans *et al.* (1996) published a PRA on *B. xylophilus* and its vectors (*Monochamus* spp.) for the territories of EU. The key-conclusion of the PRA was that PWN is a quarantine pest, justifying the use of phytosanitary measures to exclude it from the territories of EU. This was based on the fact that the pest did not occur in the territories of EU, the entire area was suitable for establishment of PWN, the universal occurrence of susceptible hosts, the occurrence of suitable vector insects, and that PWN is of potential economic importance to the territories of EU.

In a PRA on PWN for New Zealand (Sathyapala 2004) it was concluded that PWN could enter the country. Establishment and further spread, however, would require a vector. Vectors (*Monochamus* spp.) are not known to occur in New Zealand. Under the phytosanitary measures in force, the likelihood of establishment of PWN and *Monochamus* spp. from imported forest produce was considered low. In order to control Pitch Canker Disease (*Fusarium circinatum*) the mandatory requirements for heat treatment (HT) in New Zealand is 70^{o}C for 4 hours, which would result in complete kill of PWN and its vectors. It was concluded that the phytosanitary measures to exclude PWN and its vectors from New Zealand, need to continue.

Within the EU-project PHRAME (Plant Health Risks and Monitoring Evaluation) 2003-2006 the importance of forest conditions for the expression of the pathosystem PWN-*Monochamus galloprovincialis-Pinus pinaster* has been studied (PHRAME 2007). A model was developed for estimating the consequences of PWN establishment in Portugal, and elsewhere (Evans *et al.* 2007). This model will be an important tool for future estimates on the regional effects in forestry of a hypothetical establishment of PWN. The application of this model under Swedish conditions (Jordbruksverket 2008 in prep.) is of great importance and will be considered in the present pest risk assessment.

3.4. Conclusion of initiation

The pest of concern in the current pest risk assessment is the Pine Wood Nematode (PWN) *B. xylophilus*. The initiation point for this risk assessment is the review or revision of a policy by Mattilsynet. The PRA area is Norway. Previous PRAs of PWN for the territories of EU and New Zealand do only in part cover conditions applicable to Norway, and since the detection of PWN in Portugal in May 1999 new concerns must be given to the status of erected PFAs in

Portugal and the new information on risks associated with wood packaging material (WPM). These changes motivate a new pest risk assessment with focus on Norwegian conditions and concerns**.**

4. PEST RISK ASSESSMENT

4.1 Pest categorization

4.1.1. Identity of pest

4.1.1.1 Scientific name

Bursaphelenchus xylophilus (Steiner & Buhrer, 1934) Nickle, 1970.

4.1.1.2 Synonym

Aphelenchoides xylophilus Steiner & Buhrer, 1934.

Bursaphelenchus lignicolus Mamiya & Kiyohara, 1972.

4.1.1.3 Common name

English: Pine Wood Nematode (PWN) / = “Timber nema” of Steiner & Buhrer (1934).

Norwegian name: Furuvednematode (FVN).

4.1.1.4 Taxonomic position

Nematoda, Secernentea, Tylenchida, Aphelenchina, Parasitaphelenchoididae, Burspahelenchinae.

4.1.1.5 Biological information

PWN (Fig. 1) is a microscopic plant parasitic roundworm with a phoretic relationship with cerambycid beetles, *i.e.* pine sawyers in the genus *Monochamus* (Fig. 2). Kiyohara and Tokushige (1971) demonstrated the PWN as the causal agent of the pine wilt disease (PWD) or "matsukui-mushi", which after its first occurrence in Japan in the early 1900 has become increasingly severe.

Figure 1. Pine Wood Nematode (PWN) Bursaphelenchus xylophilus. Adult stages (Length 0.8 mm).

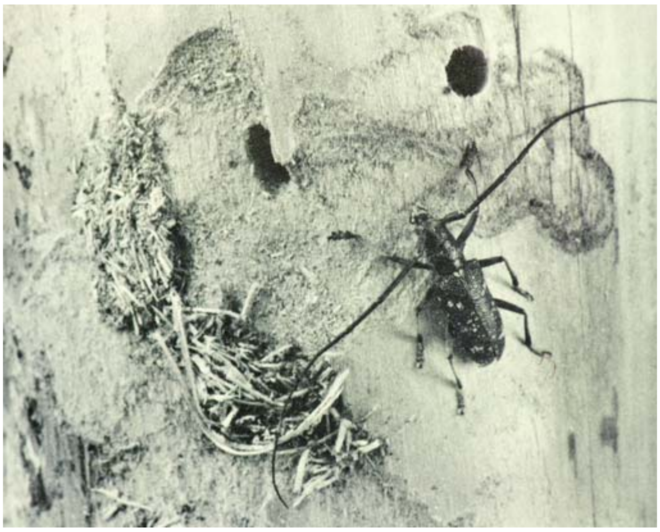


Figure 2. Pine sawyer Monochamus sutor on pine bolt showing typical symptoms of the insect activity. Photo: Rune Axelsson SLU Uppsala,

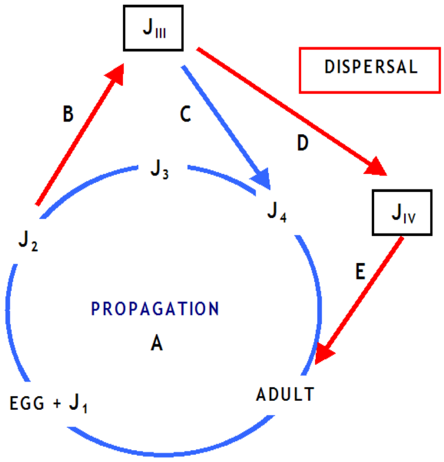


Figure 3. Pine Wood Nematode (PWN) Bursaphelenchus xylophilus life strategy. A. Propagative phase (blue). B, D & E. Dispersal phase (red). B. Induction of spreading and resting stage JIII. C. Reversion to propagative J4. D. Induction of phoretic dispersal stage JIV. E. Molt to adult in feeding wound or oviposition scar made by Monochamus spp.

PWN inhabits the wood of pine trees and reproduce on plant tissue or fungal mycelia. In this propagative phase (Fig. 3 A), the first juvenile stage J_1 molts in the egg and hatches as J_2. Each subsequent stage J_3 to adult is preceded by a molt. Adults mate and lay eggs in the wood tissues.

The threshold temperature for development of PWN is +9.5^{o}C, and the generation time is 12 days at 15^{o}C and only 3 days at +30^{o}C (CABI/EPPO 1997). PWN reproduces well also at higher temperatures, and optimum temperatures of +35-40^{o}C have been reported from pine chip piles in Savannah USA (Dwinell 1986). PWN has a short generation time compared to many other plant parasitic nematodes, and the propagative life cycle results in a very rapid population build-up even at moderate temperatures.

The dispersal phase starts with the molt of a propagative J_2 to the third dispersal stage J_{III} (Fig. 3 B). This stage has the thickest cuticle of all developmental stages of PWN (Kondo & Ishibashi 1978) and is a resting stage in the life cycle (Mamiya 1984). Its development seems to be triggered by starvation, drought or low temperatures (Ishibashi & Kondo 1977). The J_{III} stage is very persistent and can be recovered as long as 3 years after the death of a tree (Malek & Appleby 1984). If the conditions of the microhabitat improve, the J_{III} will molt to J_4 (Fig. 3 C) and continue its propagation.

Development of the J_{III} in wood (Fig. 3 B) is not only triggered by abiotic factors, but most importantly also by the pupation of cohabitant *Monochamus* spp. In early spring J_{III} accumulates in a 1-2 mm thick wood layer surrounding the pupal chamber. Here J_{III} molts to the “dauerlarva” J_{IV} (Fig. 3 D), which invades the pupal chamber and spread out over the inner walls. After the emergence of the adult beetle from the pupa, J_{IV} invades its tracheal system (Mamiya 1984).

Pathogenic life cycle: Nematode infections of trees occur when beetles carrying the nematode J_{IV} dispersal stages do their maturation feeding on the thin bark of new shoots and branches. In this situation J_{IV} leaves the beetle and invades the feeding wounds where they molt to adults, invade resin ducts and enter the propagative life cycle. In temperate climates healthy trees are normally not damaged by this latter type of infection events, and Scots pine (*Pinus sylvestris*) can harbour such infection for at least 14 years without showing symptoms of PWD (Bergdahl & Halik 2003; Bergdahl pers. comm.). The maximal time for latent infections to persist in a living tree is not known. Such infections could possibly persist for the entire life of a tree. In warmer climates, however, like in East Asia and Portugal, nematodes transmitted to the tree crowns often rapidly kill susceptible species of pine by inducing PWD.

Saprophytic life cycle: After the feeding period of the beetles, female beetles locate weakened trees or fresh timber for oviposition. Studies on the dispersal biology of *Monochamus sutor* and *M. galloprovincialis* in the PRA area are lacking. In Europe (Portugal) dispersal of *M. galloprovincialis* has been studied at a local scale (PHRAME 2007). However, several approaches have been used to study dispersal of *Monochamus alternatus* in Japan; showing that many individuals stay in their parental habitat or move only short distances, while some individuals can traverse long distances (see review in Togashi and Shigesada 2006). Eggs are deposited into the cambium through oviposition pits. During oviposition the remaining nematode J_{IV} stages leave the beetle and invade the oviposition pits. Here they molt to adults and enter the propagative phase. In the saprophytic life cycle the nematode propagates on living plant cells and wood living fungi. More than 20 genera of microfungi contain good hosts for PWN (Magnusson 2001). The fungal diet of PWN may also explain its capacity to reproduce in bark (Forge & Sutherland 1996)

Pine Wilt Disease: PWD results from a failure in water conduction arising in susceptible species of pine infested by PWN. One mechanism behind this is irreversible xylem cavitation, which can be a result of evaporation of terpenoids inside the tracheids under the negative trunk pressure resulting from high summer temperatures. This would cause the formation of a hydrophobic coating on tracheid walls and pit membranes preventing refilling with water at falling temperatures (Kuroda *et al.* 1988; Kuroda 1989). In warm climates, like in central and southern parts of USA and Japan, susceptible pine trees often die in the same year as they were infected by PWN, and often very rapidly (within 2 months) with all needles retained (Fig. 4). In more northern locations tree death in a majority of infested trees may occur the year after the infection. In such trees symptoms can be unsynchronized, gradual and very different (Fig. 5) from the classical PWD of the south.

Although there is a positive relationship between the size of the nematode inoculum and the severity of PWD symptom expression (Mamiya 1983), it is difficult to determine the lowest number of nematodes required to induce disease. Field inoculations in Japan with 30 000 – 50 000 nematodes per tree have resulted in high mortality rates (Mamiya 1972; Kiyohara & Susuki 1978), but also as few as 30 nematodes have been reported to kill trees (Mamiya 1983). In Japanese pine trees the reduction in resin flow starts before the nematode population reach detectable levels (Kiyohara & Susuki 1978). This fact and the fact that cell death occurs before nematode population build-up and spread, indicates the involvement of some biochemical factors (Mamiya 1983). Toxic products produced in the nematode infested host by the nematode activity (Oku *et al*. 1979; Shaheen *et al*. 1984) or by accompanying bacteria (Oku *et al*. 1980; Zhao *et al*. 2003), have been suggested to play a role in PWD. This could help to explain the observations from northern Japan, where nematodes are scarcely detectable in wood of trees which die in spring after being infected the previous year (Mamiya pers. comm. cited by Magnusson 1986). Other observations suggesting the nematode activity at the infection site to be important for tree mortality, are from Illinois USA where chlorotic winter flags in the tree canopy preceded spring mortality of *P. sylvestris* (Malek & Appleby 1984). In summary, there seems not always to be no immediate connection between nematode population increase and pine mortality. This might have special relevance to northern locations.

In Japan, Korea, China, Taiwan and Europe (Portugal), where most endemic pine species are highly susceptible, PWN causes a severe PWD epidemic with extensive and increasing damage. PWN was detected in Portugal in 1999 (Mota *et al*. 1999), and the situation recently has become aggravated as a result of the spread of the nematode infection to the central region of the country and the main area maritime pine *Pinus pinaster* (Mota pers. comm.). The total area in East Asia (Mamiya 2004; Yang 2004) and Europe (DGRF 2006; Mota pers. comm.) suffering from PWD is about 1.2 million ha. Annual losses in Japan reach approximately 1 million m^3 of timber (Mamiya 2004).

Figure 4. Pine trees killed by Pine Wood Nematode (PWN) Bursaphelenchus xylophilus transmitted by Monochamus alternatus (blue net bags) in Kyoto central Japan.
Photo: C. Magnusson-November-1989.

Figure 5. Pine trees showing unsynchronized and gradual wilt symptoms. Artificial inoculation of Pine Wood Nematode (PWN) Bursaphelenchus xylophilus for resistant screening in Morioka northern Japan.
Photo: C. Magnusson-November-1989.

4.1.2 Presence or absence in PRA area

Mattilsynet in 2000 commissioned Bioforsk to survey selected areas of Norway for *B. xylophilus*. The Norwegian Forest and Landscape Institute was partner in the survey. Magnusson *et al.* (2007) presented the results from the years 2000-2006. Samples were collected from 10 circular areas (zone sites), with 50 km diameter, around points of import of risk materials (Fig. 6). Most samples were collected in central and southern Norway (Fig. 7). The survey included 3165 wood samples (2880 from *P. sylvestris*, 279 from *P. abies* and 6 from unknown wood) that were collected from 446 logging sites in 84 municipalities, located in 13 of Norway's 19 counties. Of the samples 85% came from cutting wastes, timber or lying trees. Wood showing signs of insect activity (incl. *Monochamus*) formed 73% of the total material. Nematodes were recorded in 85% of the samples, but the genus *Bursaphelenchus* was detected in only 1% of the samples.

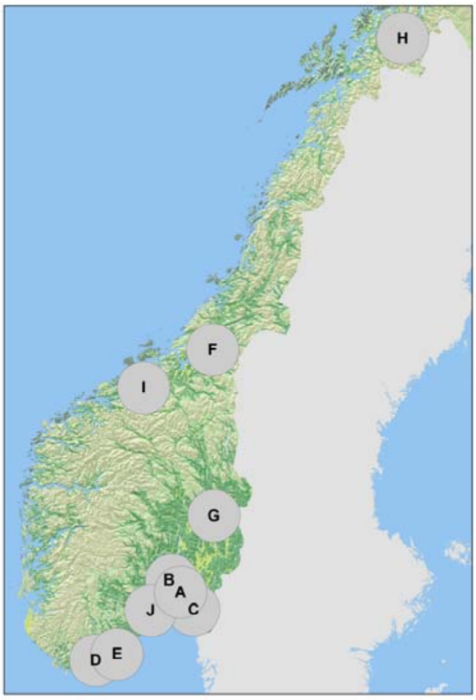


Figure 6. Survey of the pine wood nematode (PWN), Bursaphelenchus xylophilus, in Norway 2000-2006. The 10 zone sites sampled are shown as grey circular areas with a radius of 50 km. A. Tofte; B. Drammen; C. Greåker; D. Hunsfoss; E. Rykene; F. Skogn; G. Elverum; H. Skjold; I. Surnadalsøra; J. Skien. Dark green colour shows the forest area. Magnusson et al. 2007

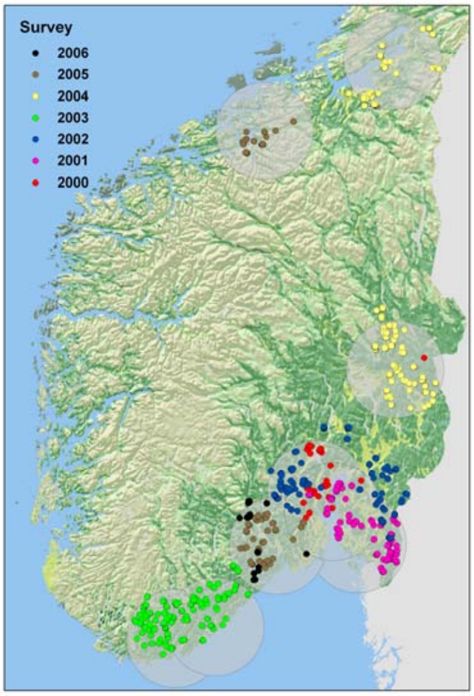


Figure 7. Survey of the pine wood nematode (PWN), Bursaphelenchus xylophilus, in Norway 2000-2006. Map showing the zone sites and sampling positions in the central and southern regions the country. Dark green colour shows the distribution of forests. Magnusson et al. 2007

B. mucronatus was found in 11 samples from central and southern Norway (Fig. 8), and most often in cutting waste of pine. This corresponds to 0.3% of the total sample volume. The low occurrence of *B. mucronatus* indicates that the number of potential niches available for *B. xylophilus* is lower than expected. The pest *B. xylophilus* has so far not been detected in the PRA area (Magnusson *et al*. 2007).

Recorded field frequencies of endemic *B. mucronatus* (Tab. 1) reflect the frequencies of habitats and vectors in the PRA area. Assuming a hypothetical frequency (p) for PWN of 1/10 of that for *B. mucronatus* and a confidence level (ξ) of 0.05, the number of samples (n) can be calculated according to the equation $n = \ln \xi / \ln (1-p)$ (Magnusson 2008). The calculations show that more than 24 0000 samples need to be analysed to fulfil the requirements above.

Figure 8. Survey of the Pine Wood Nematode (PWN), Bursaphelenchus xylophilus, in Norway 2000-2006. Map showing the position of samples positive for B. mucronatus. The find at Hanestad in Østerdalen was reported previous to the present survey by McNamara and Støen (1988).
Magnusson et al. 2007

Table 1. Field frequencies of native Bursaphelenchus mucronatus recorded in Norway 2000-2006 and the estimated number of samples required to detect Bursaphelenchus xylophilus (PWN) should it occur at frequencies of 1/10 of native B. mucronatus.

Region	Recorded field frequency of *B. mucronatus* 2000 - 2006	Hypothetical frequency of *B. xylophilus*	Minimal number of samples for detection of *B. xylophilus*
Aust-Agder	0.0165	0.00165	1800
Østfold	0.0029	0.00029	10320
Hedemark	0.0028	0.00028	10680
Møre & Romsdal	0.0196	0.00196	1510
Total	**0.0035**	**0.00035**	**24310**

PWN is considered absent from the PRA area. However, it has been argued that timber trade and movement of wood from North America to the Nordic area has a history reaching many hundreds of years back in time, and that PWN already must have entered and probably also is established in the PRA area and in neighbouring countries. The argument does not reflect the full perspective of the dynamics in trade. The volume of trade has increased considerably in recent time, and a growing volume of high-risk material traded, like wood chips lumber and various kinds of WPM, is in an historical perspective a very recent event. Many circumstances like availability of vectors, climate, transport etc. need also to be in place for an introduction to be successful.

4.1.3 Regulatory status

The first outbreak of PWD in Japan occurred in 1905 in Nagasaki on the southern island Kyushu. For more than 60 years the disease was thought to be caused by insects. However, in 1971 PWN was demonstrated to be the causal agent of the disease (Kiyohara & Tokushige 1971; Mamiya 1988). Before 1979 European scientists looked upon the occurrence of PWN and the PWD in Japan as an "exotic" situation with little relevance to European countries. In 1979, however, PWD was diagnosed in USA, and soon it was realised that PWN was widespread and endemic to USA (Dropkin *et al*. 1981). Later PWN was also recorded to be widespread in Canada (Anonymous 1989). In fact, a nematode identical to PWN was found already in 1929 in wood of *Pinus palustris* from Texas (Nickle *et al*. 1981), and later described as the "timber nema" *Aphelenchoides xylophilus* (Steiner & Buhrer 1934). *A. xylophilus* was later transferred to the genus *Bursaphelenchus* by Nickle (1970).

Before 1984 PWN was not regarded as a quarantine pest organism in Europe. However, in May 1984 the Plant Inspection Service of Finland intercepted PWN in a consignment of pine wood chips imported from USA. Later the same year a new interception was made in pine wood chips imported from Canada (Rautapää 1986). In September 1984 the government of Finland temporarily prohibited import of conifer wood products from Japan and North America. In 1985 Finland and Norway placed permanent embargoes on import of conifer wood products from areas where PWN was known to occur. The same action was taken by Sweden in 1986. In 1985 EPPO recommended member countries to ban conifer products, except kiln-dried lumber, from areas where PWN was known to occur, and PWN was subsequently in 1986 put on the EPPO A1 list of quarantine pests. In 1989 also the European Community (now EU) imposed import restrictions on PWN (Braasch & Mota 2008). These actions brought the nematode into the arena of international trade relations and sparkled intensive discussions between exporting and importing countries on the regulations of conifer imports mainly from North America.

Current regulatory status

Norway: The pest is listed in Annex 2 "Pests which are prohibited to introduce and spread in Norway if these are present in certain plants or other regulated articles" of the regulation relating to plants and measures against plant pests (FOR 2000-12-01 nr 1333: Forskrift om planter og tiltak mot planteskadegjørere). In the same regulations non-European *Monochamus* spp. are listed in Annex 1 "Pests which are prohibited to introduce and spread in Norway".

EPPO: *B. xylophilus* and its vectors in the genus *Monochamus* are A1 quarantine pests: *M. alternatus, M. carolinensis, M. marmorator, M. mutator, M. nitens, M. obtusus, M. saltuarius, M. scutellatus, M. titillator* (CABI/EPPO 1997).

EU: *B. xylophilus* and non-European *Monochamus* spp. are A1 quarantine pests.

4.1.4 Potential for establishment and spread in PRA area

There is a good supply of host plants, *i.e.* Scots pine (*Pinus sylvestris*), Norway spruce (*Picea abies*) and European larch (*Larix decidua)*, for PWN in the PRA area (see point 4.2.2.1).

The principal vector in Portugal, *Monochamus galloprovincialis*, is found in Østfold in the south-eastern part of Norway. The pine sawyer *M. sutor* is the most widespread species, while there are scattered old records and one new record of *M. urussovii* from the south-eastern part of the PRA area (Bakke & Kvamme 1992; Ehnström & Holmer 2007). The suitability of *M. sutor* as a vector for PWN is indicated by the biological information on *M. sutor* (see 4.2.3.) and its similarities with other *Monochamus* species that are documented vectors for PWN (such as M. *galloprovincialis* that is recorded as vector in Portugal). Furthermore, in areas where PWN occurs most species of *Monochamus* have served as vectors for PWN.

Winter mortality is not expected to limit PWN from living in the PRA area as the nematode already lives in areas in Canada and northern Japan where winter temperatures are similar or lower (Magnusson 1986) than in the commercially important forested areas of Norway. Field infections of Scots pine, *Pinus sylvestris*, have been successful in the State of Vermont, USA (Bergdahl & Halik 2003; Bergdahl pers. comm.), an area with a climate much similar to Norway.

4.1.5 Potential for economic consequences in PRA area

The ForestETp-model developed within the European PHRAME Project (PHRAME 2007) was used by Sweden to estimate the consequences of a hypothetical PWN establishment. The result of the simulation indicated the damage from PWD under current climatic conditions to be small. A small incidence of PWD could probably occur in years when summer temperatures are higher than normal (Jordbruksverket 2008 in prep.).

Magnusson (1992) investigated the effect of an exceptionally hot summer in a growth chamber study (Fig. 9). The study simulated the summer of 1947 as recorded at the meteorological station in Målilla, in eastern Sweden (57°24´N; 15°20´E; elevation 100 m). The pathogenicity of two populations of *B. xylophilus* from USA and a Swedish isolate of *B. mucronatus* was investigated on 5 years-old trees of Scots pine.

The period May-October was simulated in detail by coupled diurnal temperature and humidity programs. In the simulated climate trees were inoculated mid-June with initial population levels (Pi) of 100 or 2 000 nematodes per tree. The maximum temperature during the trial was +38°C, and maximum day temperatures higher than +30°C were reached on eight days of the 137 day-long post-inoculation period. The mean and maximum temperature amplitudes were 13°C and 27°C, with a mean temperature of approximately +14°C.

Typical pine wilt symptoms appeared from day 34 to day 84 post-inoculation. Maximal mortality rates of 26% (Pi=100) and 35% (Pi=2 000) were recorded (amended data) for one of the *B. xylophilus* isolates. Mortality rates recorded for the Swedish isolate of *B. mucronatus*, did not differ markedly from the 9% control mortality. These results suggest that in northern areas the development of typical pine wilt symptoms would require exceptionally high summer temperatures.

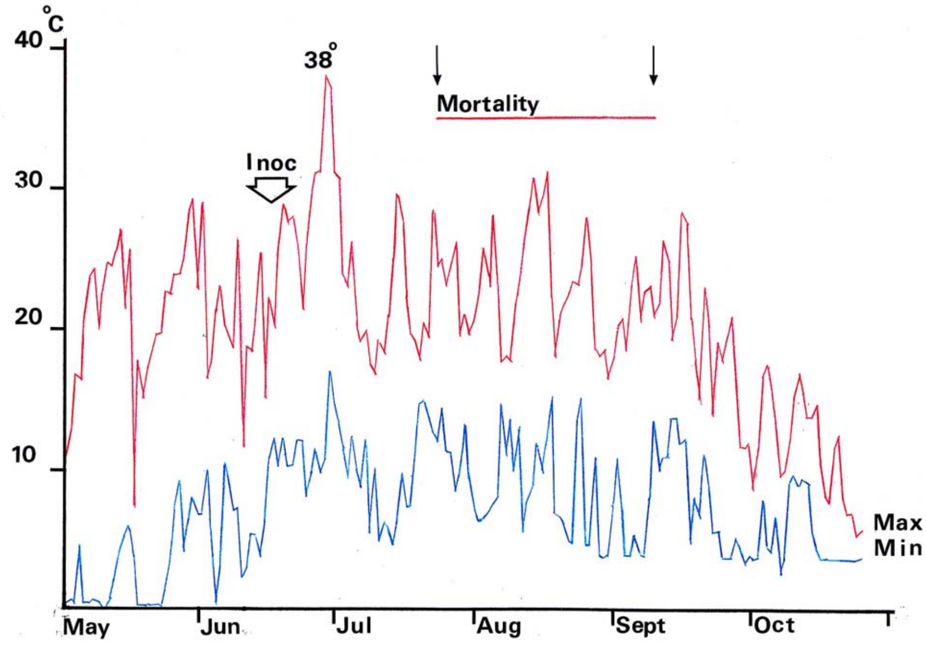


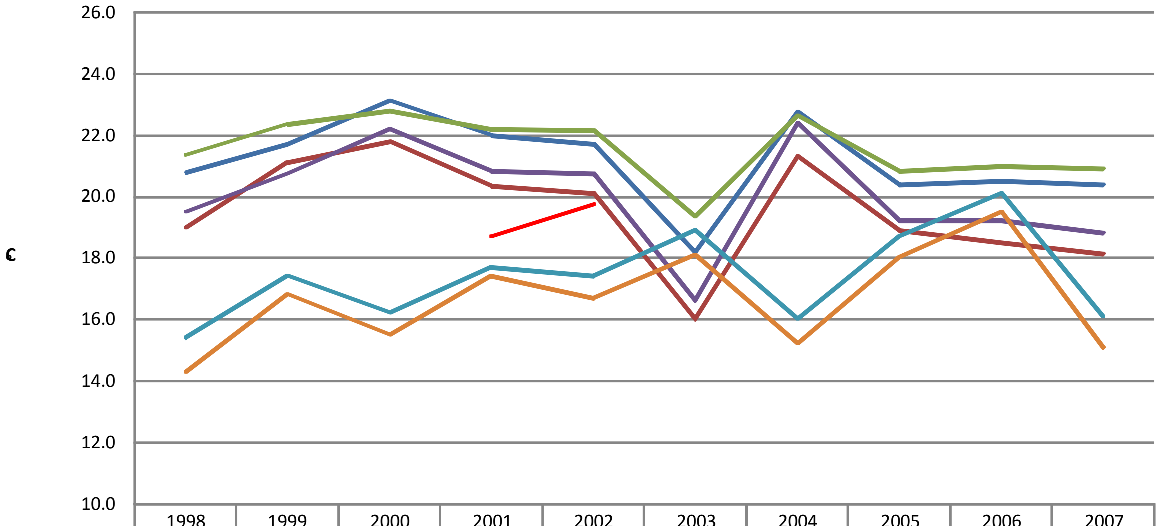
Figure 9. Inoculation experiment with Pine Wood Nematode (PWN), Bursaphelenchus xylophilus, in a growth chamber simulation of the growth season 1947 at Målilla, Småland, eastern Sweden (Magnusson 1992).

The probability of PWD expression in northern Europe is considered to be low (De Guiran & Boulbria 1986; Evans *et al*. 1996; Braasch and Enzian 2004), while pine forests situated in areas in central and southern Europe with a current average summer temperature above 20°C are more likely to fall victim to the disease (Rutherford and Webster 1987).

In Japan the PWD has reached northern Honshu, *i.e* the border of Aomori, the northernmost prefecture of the Tohoku region. To assess the suitability of the environment for PWD in the PRA area, climate data for the last ten years were obtained from four locations in the Aomori prefecture of Japan (NARC 2008), from two locations (Oslo and Hønefoss) in southeast Norway (The Norwegian Meteorological Institute and Bioforsk) and from the experimental field plot in Wolcott USA (Bergdahl & Halik 2003; Bergdahl pers. comm.) where PWD did not develop (Fig. 10 and 11).

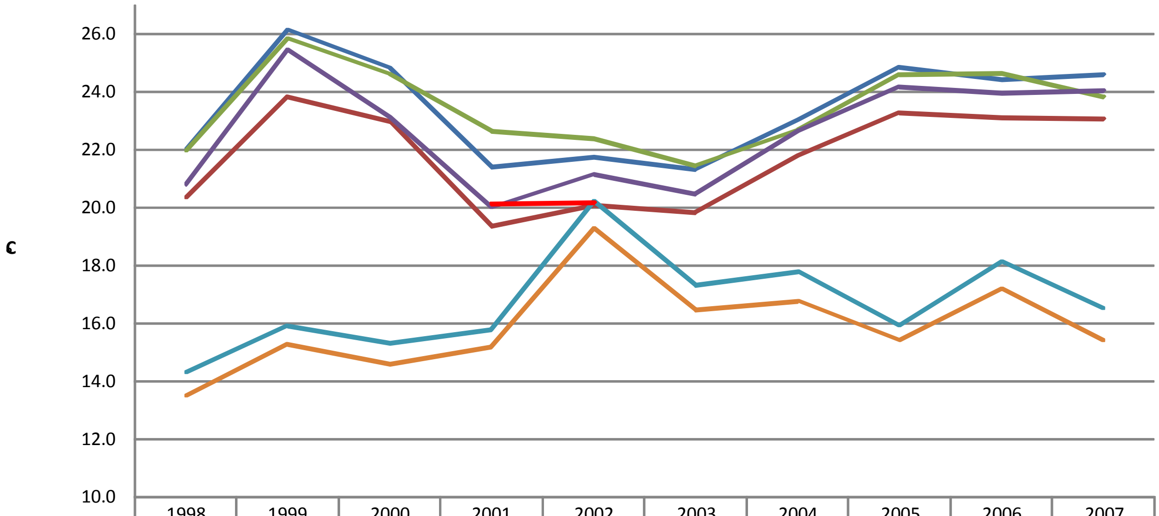
The climate data from Japan shows that the four locations in the Aomori prefecture lie on or above the 20°C summer temperature isotherm, while the Norwegian locations lie just below. From Wolcott there is only data available for the years 2001 and 2002, placing Wolcott between the Japanese and Norwegian locations with respect to air temperature. Moreover, it could be observed that monthly mean air temperature in Norway is higher in July than in August, while the opposite is the case in Japan.

The comparison of the current climatic data suggests the conditions in PRA area to be largely non-conducive for PWD. Recent simulations of forest and climate data from Sweden in the ForestETp-model, developed within the PHRAME-project (Evans *et al.* 2007; PHRAME 2007), suggested an average mortality of 1% of the pine trees infected by PWN (Jordbruksverket 2008 in prep.). For Norway an infection rate of 1% and a mortality rate of 1% would probably result in an annual loss smaller than 50 000 m^3 of wood, which corresponds to less than 0.8% of the annual growth of pine.



	1998	1999	2000	2001	2002	2003	2004	2005	2006	2007
Aomori	20.8	21.7	23.1	22.0	21.7	18.2	22.7	20.4	20.5	20.4
Mutsu	19.0	21.1	21.8	20.3	20.1	16.0	21.3	18.9	18.5	18.1
Fukaura	21.3	22.4	22.8	22.2	22.2	19.3	22.6	20.8	21.0	20.9
Haochinohe	19.5	20.7	22.2	20.8	20.7	16.6	22.4	19.2	19.2	18.8
Oslo	15.4	17.4	16.2	17.7	17.4	18.9	16	18.7	20.1	16.1
Hønefoss	14.3	16.8	15.5	17.4	16.7	18.1	15.2	18	19.5	15.1
Wolcott				18.7	19.8					

Figure 10. July mean air temperatures from four locations in the Aomori prefecture of Japan, from two locations in Norway, and from Wolcott in the state of Vermont, USA.



	1998	1999	2000	2001	2002	2003	2004	2005	2006	2007
Aomori	22.0	26.1	24.8	21.4	21.8	21.3	23.1	24.9	24.5	24.6
Mutsu	20.4	23.8	23.0	19.4	20.1	19.8	21.8	23.3	23.1	23.1
Fukaura	22.0	25.8	24.6	22.6	22.4	21.5	22.7	24.6	24.6	23.8
Haochinohe	20.8	25.5	23.1	20.0	21.2	20.5	22.6	24.2	24.0	24.1
Oslo	14.3	15.9	15.3	15.8	20.2	17.3	17.8	15.9	18.1	16.5
Hønefoss	13.5	15.3	14.6	15.2	19.3	16.5	16.8	15.4	17.2	15.4
Wolcott				20.2	20.2					

Figure 11. August mean air temperatures from four locations in the Aomori prefecture of Japan, from two locations in Norway, and from Wolcott in the state of Vermont, USA.

A hot summer in Sweden, as simulated by the ForestETp-model, is assumed to give less than 10% mortality of infected trees (Jordbruksverket 2008 in prep.), and maybe similar levels of damage can be expected in Norway. However, a detection of PWN in Norway is also anticipated to result in an immediate closure of the Norwegian border with regard to movement of untreated wood. This might cause a certain loss in volume of trade, with a varying degree of impact depending on region. A global warming may allow for PWD expression in the future. In figure 12 the summer temperatures for the last thirty years recorded in Oslo, Norway, is displayed. For this data, the trends in July and August air temperatures were estimated by linear regression. Provided the trends

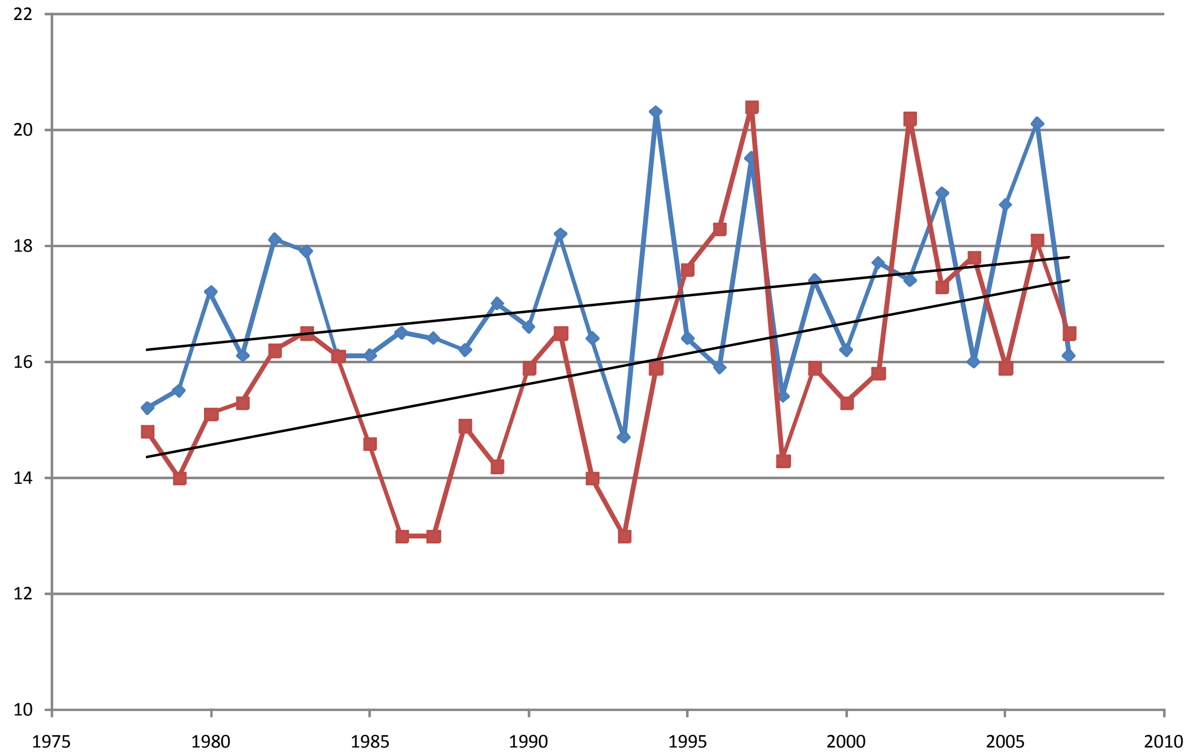


Figure 12. Trends in monthly mean air temperature for July (blue) and August (red) in Oslo during the last 30 years (1978 – 2007). Linear regression indicates annual increase of 0.05^{o}C and 0.1^{o}C for July and August respectively.

continue, the 20^{o}C isotherm for August will be reached in slightly more than 20 years, and for July this isotherm will be reached in 30 years, *i.e.* in less than half a forest cycle. This also means that current forest stands of southern Norway before harvest may experience temperatures similar the current temperatures to the Tohoku region of Japan and regions of Portugal where new outbreaks of PWD recently have occurred. Braasch and Enzian (2004) consider summer temperatures above 20^{o}C for 8 weeks to be required for the expression of PWD. Extrapolating the trends in the future temperature increase in the Oslo area indicates that this situation will be reached in less than half a forest generation.

PWD is strongly aggravated by low soil humidity (Suzuki & Kiyohara 1978). Mamiya (1984) pointed out that the highest losses of timber in Ibaraki Japan occurred in years with unusually high temperatures and low rainfall, bringing losses up to 30 times the normal level.

Another climate factor that could play an important role, if PWN were introduced to Norway, is precipitation. The disease is more severe in stands on dry and exposed sites (Suzuki & Kiyohara 1978). In a survey of PWD in Illinois, Malek and Appleby (1984) noticed that a high proportion of wind-break trees died in PWD. Also high temperatures (Malek & Appleby 1984; Mamiya 1984) favour the expression of PWD. This could relate to higher transpiration rates in trees exposed to wind. In a warmer climate, the hilly topography and shallow soils could make the PRA area vulnerable to PWD, but high rainfall in summer could mitigate disease expression. However, variation in the soil water is thought to be more conducive to PWD than constant low water levels (Jordbruksverket 2008 in prep.).

A more detailed assessment of economic consequences of a possible establishment and spread of PWN in the PRA area, and of the effects of global warming and other climate changes on the probability for PWD outbreaks, will be given in Part 2 of the pest risk assessment.

4.1.6 Conclusion of pest categorization

PWN is not known to occur in the PRA area. As a result of global warming, PWD (caused by the nematode) could become a serious disease in forests of the PRA area within the present forest generation. The detection of PWN in the PRA area might cause significant disturbance of wood trade. PWN is a serious invasive pest species, and the present regulatory status of PWN as an A1 quarantine pest is valid. Thus, the current pest risk assessment continues.

4.2. Assessment of probability of introduction and spread

4.2.1 Probability of entry of the pest

4.2.1.1 Identification of pathways

PWN has repeatedly been intercepted in wood chips (Rautapää 1986; Dwinell 1987; McNamara & Støen 1988; Schroeder & Magnusson 1992), as well as in imports of conifer lumber (Tomminen & Nuorteva 1992) and packing case wood (Tomminen 1991) imported to the Nordic countries. Also vector insects of PWN, cerambycids in the genus *Monochamus* have been reported to occur in wood imports (Lindeløw 2000; Siitonen 2000) and as hitchhikers in containers with import machinery (Kvamme & Magnusson 2006).

The presence of PWN together with its vector insect in a commodity may lead to introduction of PWN. Individuals of *Monochamus* spp. carrying the dispersal J_{IV} stage of PWN may leave the commodity and spread the nematode to nearby trees at maturation feeding or transmit the nematodes to lying trees of timber at oviposition. This makes it necessary to consider the probability of a pathway to harbour also *Monochamus* spp.

It is important to note that the phytosanitary risk from PWN being present in a commodity is not completely dependent on the coincident occurrence of vector insects. PWN may disperse between trees via temporary stem and root grafts (Malek & Appleby 1984) and through soil from infested wood mixed into soil (Kiyohara & Tokushige 1971) or mulched around trees (Halik & Bergdahl 1992). Maturation feeding and oviposition by *Monochamus* spp carrying PWN dispersal J_{IV} stage on trees and timber will most likely result in nematode infection of the wood, with or without successful breeding of the beetles. After introduction of PWN into trees the nematodes may spread throughout the entire tree, and consequently occur also in parts of the tree not colonised by the beetles. Hence, pathways for introduction and establishment of PWN in Norway include wood where the nematode is present with or without its vector insects. Hitchhiking *Monochamus* beetles can be associated with all possible commodities, travelling in hatches and containers of aircraft, ships *etc*.

Commodities/pathways differ with regard to their capacity of carrying PWN, *Monochamus* or both (Tab. 2). The kinds of commodities traded are subject to a considerable variation due to changing availability, demand and market prices. The numbers on volumes given in table 2 refer to the conditions of 2006.

4.2.1.2 Probability of the pest being associated with the pathway at origin

Plants for planting:

This pathway has a medium innate probability of PWN association. *Monochamus* spp. can only breed in large trees, which are expected to have a low volume of trade. It is conceivable that plants, which grow in an area where PWN occurs, may be an object for beetle maturation feeding and consequently potential carriers of the nematode.

Bonsai trees:

This pathway has a very low innate probability of PWN association. Bonsai trees are too small for beetle breeding and do not represent a pathway for *Monochamus* spp. The trees are kept under strict care, and consequently are not likely to be attacked by feeding *Monochamus* spp. Bonsai trees are kept under observation for a time long enough for PWD symptoms to develop in PWN infested trees.

Cut branches:

Branches harvested in areas where PWN is present are pathways with a medium innate probability of PWN association. Although oviposition of the Asian pine sawyer *Monochamus alternatus* can occur on branches down to a diameter of 2 cm (Kobayashi *et al*. 1984), small dimensions and thin bark may not allow *Monochamus* spp. to breed successfully. Cut branches, however, might have been an object for feeding by the beetles before being cut. This means that J_{IV} juveniles may have been introduced into the feeding scars, and that the nematode, by the time of cutting may be well established. It is known that latent infections of PWN may persist at least for 14 years in Scots pine (Bergdahl & Halik 2003; Bergdahl pers. comm.), so feeding scars may not be visible to indicate potential infections when branches are harvested. The role of asymptomatic carrier trees in the spread of PWD has been recognized for years (Futai 2004; Takeuchi & Futai 2007).

Roundwood with bark:

This pathway has a high innate probability of PWN association. Both *Monochamus* spp. and PWN should be expected to occur in roundwood with bark from areas where PWN occurs. The presence of a continuous bark layer provides the best opportunities for successful oviposition and breeding of the beetles in logs. In areas of PWD trees infested by PWN emit volatile compounds, which attract egg laying females of *Monochamus* (Takeushi *et al*. 2006). The egglaying beetles are also attracted to freshly cut logs. At oviposition J_{IV} juveniles are transmitted to trees and logs. The beetle larval development will take 1-2 years depending on location. PWN will develop rapidly through its propagative life cycle and will over time spread throughout the wood. Roundwood with bark from areas where PWN occurs may therefore contain *Monochamus* specimens infested by PWN. When the beetles fly out from the breeding substrate they may transmit PWN to nearby trees. In Japan this pathway is the most important for the spread of PWN into new areas (Mamiya 1988).

Roundwood without bark:

This pathway has a high innate probability of PWN association. Complete removal of bark on logs gives an effective protection from *Monochamus* oviposition (Trägårdh 1929), and hence from nematode infection. Early debarking, within 7 days from entry of *Monochamus* larvae into the wood, will reduce their occurrence by the physical removal of the insects and the cambium upon which they feed (Evans *et al.* 1996). Provided the timber is debarked early enough this is not a pathway for *Monochamus* spp. If the timber is debarked too late there is a possibility that the wood could harbour *Monochamus*, because it penetrates into the wood for pupation. Early debarking will be of little importance for reducing levels of PWN.

Table 2. Expected probability of Bursaphelenchus xylophilus (PWN) and its vector insects Monochamus spp. (MON) to be associated with pathways of wood originating in Canada, China, Japan, Korea, Mexico, Portugal, Taiwan and USA, and effects of phytosanitary measures on level of probability. Probability levels: ++++ very high; +++ high; ++ medium; + low; and – very low; Phytosanitary measures: DB=Debarked wood, GHF=Grub hole free, HT=Heat treatment, KD=Kiln drying, MB=Methyl bromide fumigation, PC= Phytosanitary certificate, PRO=Prohibition.

PATHWAY	PWN	MON	INNATE PROBABILITY OF PWN AT ORIGIN	PHYTOSANITARY MEASURES	IMPORT VOLUME IN 2006	RELATIVE PROBABILITY OF PWN AT ENTRY	
						PRESENT [1]	FUTURE [2]
Plants for planting	X		++	PRO	None	-	-
Bonsai trees	X		-	PC	Small*	-	-
Cut branches	X		++	PRO	None	-	-
Roundwood with bark	X	X	+++	PRO	None	-	-
Roundwood without bark	X	X	+++	PRO	20 m^3 [3] *	-	-
Squared wood [4]	X	X	+++	HT PC	1381 m^3 *	+	+
Non-squared boards [5]	X	X	+++	PRO		-	-
Wood packaging material (WPM) as a commodity	X	X	+++	HT PC	37 tons *	+	+
Wood packaging material (WPM) in service	X	X	+++	DB[1] GHF[1] KD[1] HT[2] MB[2]		+++	++
Dunnage	X	X	++++	DB[1] KD[1] GHF[1] HT[2] MB[2]		+++	++
Wood chips	X		++++	PRO	None	-	-
Sawdust, shavings etc.	X		++	PRO		-	-
Isolated bark	X		++	PRO	None	-	-
Green wood products	X	X	++	PC	62 tons *	+	+
Hitchhiking non-European *Monochamus* [6]	X	X	++	PRO		++	++

[1] Norwegian regulation relating to plants and measures against plant pests (FOR 2000-12-01 nr 1333).
[2] From January 1st 2009: ISPM No. 15 (FAO 2006).
[3] Pulp wood other than pine.
[4] Sawn wood which has not retained its natural round surface.
[5] Sawn wood which has retained its natural round surface.
[6] Not specified in regulation.
* Willumsen Food Safety Authority, pers. comm.

Squared wood:

This commodity contains sawn wood, which has not retained its natural round surface. This pathway has a high innate probability of PWN association. The innate probability of *Monochamus* association is medium due the removal of bark and superficial wood in the squaring process. Sawmill processes will not affect PWN. This is supported by interceptions of these pests in conifer green lumber imports to Finland (Tomminen & Nuorteva 1992) and France (EOLAS 1991).

Non-squared boards:

This commodity contains sawn wood, which has retained it natural round surface. There is a medium probability of *Monochamus* being present in sawn wood, while the presence of PWN has a high probability. Sawing wood will much reduce the occurrence of *Monochamus* spp. by physically killing most of the insects. Sawmill processes will not affect PWN.

Wood packaging material (WPM):

This pathway has a high innate probability of PWN association. WPM include a variety of wood products like packaging cases, crates, drums, pallets, etc., made from sawn wood. WPM has repeatedly been demonstrated to harbour *Monochamus* spp. (EOLAS 1991; Tomminen 1991; Sathyapala 2004; Biosecurity Australia 2006), so this is without doubt a pathway for these beetles. Chinese surveys reported PWN to occur in 1.2% of inspected pallets (Gu *et al.* 2006). This high frequency demonstrates the high innate probability of PWN association with WPM.

Dunnage:

Dunnage may be produced from the lowest grades of wood, so this pathway has a very high innate probability of PWN association. Dunnage is normally contained within WPM. Dunnage is used as spacers and supports for cargo during transportation and may take many different forms and shapes. This type of WPM may be associated with any type of commodity. Dunnage may occur in all possible sizes (even roundwood). Although all life stages of *Monochamus* spp. have been intercepted in dunnage (Sathyapala 2004), the often small size of this kind of material makes the occurrence of the beetles equal or less probable than many other types of WPM.

Wood chips:

This pathway has a very high innate probability of PWN association. Like dunnage, wood chips typically are made from low-grade wood often recovered from salvage operations after storm felling. The small size of chips makes it unlikely that *Monochamus* could survive the chipping process. PWN, on the other hand, may often occur in the raw material (Dwinell 1987), and indeed increase in superficial layers of chip piles (Dwinell 1986) awaiting loading into transport vessels.

Sawdust:

This pathway has a medium innate probability of PWN association. Physical stress from sawing process will cause complete kill of *Monochamus*. PWN might also be reduced in numbers due to the frictional heat generated in the process. However, PWN may reach high numbers by reproducing on various fungi present in sawdust provided the fungal growth is not restricted by lack of water.

Isolated bark:

This pathway has a medium innate probability of PWN association. *Monochamus* requires access to wood and is not able to live in isolated bark. PWN, on the other hand, has been reported to reach up to hundreds of individuals per gram dry weight of bark on artificially inoculated stem segments of *Pinus contorta*, *Pseudotsuga menziesii*, *Abies grandis*, *Tsuga heterophylla* and *Thuja plicata*. In Isolated bark PWN numbers reached thousands of individuals (Forge & Sutherland 1996). In isolated bark PWN can live on rests of the living cambium and develop further on any suitable fungi present, but also in branch segments and wood occurring in bark products (Bioforsk unpubl. data). In a screening of fungi living in soil and litter as hosts for PWN Magnusson *et al*. (1988) reported a PWN multiplication factor of 694 for the common fungus *Auerobasidium pullulans*. The possible occurrence of PWN in bark has not been recognized much.

Green-wood products:

This pathway has a medium innate probability of PWN association. This category includes specialized products like rustic garden furniture, summerhouses sold as a finished commodity. The dimensions of such products often are large enough for harbouring *Monochamus* spp. PWN may also occur in this type of products. It is conceivable that the raw material used in manufacturing these high-value products would hold a higher grade than roundwood and most other categories mentioned above and hence a lower probability of infestation.

Hitchhiking *Monochamus* spp.:

This pathway has a medium innate probability of PWN association. Hitchhiking *Monochamus* spp. can be expected in any form of cargo. Due to the intimate association between these beetles and PWN, the presence of the nematode in beetles from areas with PWN is not unexpected.

4.2.1.3 Probability of survival and multiplying during transport or storage

The probability of PWN surviving and multiplying during transport or storage is high. Published evidence indicates a considerable capacity of PWN to survive, and even increase in transport and storage. The expected survival time in living plants would be at minimum 14 years, as evidenced by field inoculations of *P. sylvestris* in Vermont USA (Bergdahl & Halik 2003; Bergdahl pers. comm.). Nematodes in cut trees or in timber will survive for at least 2 years (Halik & Bergdahl 1994), and this is probably also the case for sawn wood, WPM and dunnage. PWN in the J_{III} resting stage has been demonstrated to survive storage at $-17^{o}C$ for 5 months suffering a minimal degree of mortality (Kondo *et al*. 1982), so winter temperatures seem to have little or no effect on PWN in wood. With regard to wood chips PWN has been reported to increase its populations in the shipload during transport (Dwinell 1987). The survival time of PWN in pine chips is not known.

PWN in *Monochamus* beetles is expected to survive normal air and sea transports. During transports *Monochamus* will continue normal larval development, and all life-stages of the beetles have been intercepted on several occasions by the Chilean plant inspection in packaging wood, and *Monochamus* was the most frequent insect genus encountered in the border inspections 1995-2005 (Ferrada *et al*. 2007). Also New Zealand border controls have intercepted all life stages of *Monochamus* spp. in WPM (Sathyapala 2004.). *Monochamus* spp. are not expected to multiply during transport or storage.

4.2.1.4 Probability of pest surviving existing pest management procedures

Effective and economically feasible treatments are not available to all consignments in international trade (Tab. 2). The only existing effective treatments are heat treatment (HT) and methyl bromide fumigation (MB), which are specified in ISPM No. 15 (FAO 2006). HT should reach a minimum temperature of 56°C throughout the wood including its core for 30 continuous minutes (“56/30”). MB should follow a specific scheme of concentration-time for a 24 hours treatment period and requirements for final concentrations dependent on the ambient air temperature. Removal of bark is stipulated to precede fumigation. Correctly performed HT and MB are highly efficient in killing PWN and its vector in wood. Kiln drying (KD) is a wood treatment performed in order to meet the technical quality requirement of less than 20% humidity on a dry weight basis, and does not necessarily involve temperatures, which would kill PWN. However, any method capable of meeting the temperature-time requirements of HT can, based on experimental evidence, be approved as a method for wood treatment against PWN and its vectors (FAO 2006).

It should be noted that the HT treatment “56/30” according to Chinese research does not result in 100% kill of PWN. Chinese scientists suggest that eradication of PWN would require a temperature of 65°C for more than 30 minutes (Qi *et al*. 2005). This may be one reason behind the interception of PWN in 1.2% of pallets inspected by the Chinese inspection service in Ningbo. Interceptions of PWN were made also in pallets accompanied by HT certificate, and in pallets arriving from countries where PWN is not known to occur (Gu *et al.* 2006). Although not perfect, the present HT schedule of “56/30” definitely will much reduce the survival of PWN and effectively reduce the probability of introducing PWN with in-service WPM.

4.2.1.5 Probability of entry into PRA area

With the present trade pattern the probability of entry of PWN into the PRA area is expected to be high. The relative probability of entry through each pathway is summarised in table 2. The probability of entry into an area depends on the probability of PWN to be associated with various pathways at origin, the efficacy of phytosanitary treatments and the volume of trade in various commodities. The total import of conifer wood to Norway in 2006 was 1 752 702 m^3. The largest volume of this was from Nordic countries, the Baltic States and other countries of Western Europe. An insignificant volume originated in areas where PWN is known to occur (Willumsen pers. comm.).

Import of conifer plants for planting and untreated conifer wood, chips, waste and isolated bark to Norway from areas where PWN occurs is prohibited (Tab. 2). The importation of squared wood is allowed provided the material has been HT according to “56/30” and followed by a phytosanitary certificate (PC). Bonsai pine trees are kept under EU quarantine for 6 months before being exported to Norway under a PC. Green wood products may only be imported if accompanied by a PC.

WPM imported as a commodity also requires a PC stating pest freedom. Today in-service WPM (including dunnage) can be imported to Norway provided the material is free from bark and grub holes from *Monochamus* spp., and has been kiln dried. For WPM as a commodity HT and PC is required for import to Norway. Freedom of signs of *Monochamus* activity is no guarantee for absence of PWN. Kiln-drying procedures, which do not meet the requirements for HT, are not efficient in eradicating PWN. The J_{III}-stage tolerates dehydration well, and can be extracted from what appears to be completely dry pallets (Schröder pers. comm.). The present Norwegian directive for WPM, therefore, does not necessarily reduce the probability of PWN being present in imported in-service WPM to a level lower than the level at the origin of the pathway. According to future Norwegian directives, which will be effective from January 1st 2009, in-service WPM must be treated and marked in accordance with ISPM No.15 (FAO 2006).

It can be concluded that the phytosanitary measures laid down in the present directives are effective in reducing probability of PWN being present in commodities imported to Norway, except for WPM, dunnage and possible hitchhiking *Monochamus* spp. The future adoption of ISPM No. 15 (FAO 2006) will help in further reducing the probability of PWN being present in in-service WPM and dunnage.

WPM of various origin enter Norway at a rate of 5 000-10 000 units per day throughout the year. Gu *et al*. (2006) reported that 1.2% of inspected pallets in Ningbo were infested with PWN. The pallets sampled were mostly of pine wood (Gu pers. comm.). With the assumption of a similar infection rate in pallets arriving in Norway, it can be calculated that maybe 60-120 units arrive each day potentially infested by PWN (predominantly J_{III} resting stages). Pallet wood is a popular resource for people, so the end-use of this commodity is completely out of control. Due to the high probability of innate infestation of PWN, the large volumes imported and the uncontrolled end use of WPM, this material probably is the commodity with the highest probability for PWN transfer to ecosystems of the PRA area. So far, the analyses of about 100 samples from WPM arriving in Norway have been negative. This, however, is a very small fraction of the in-service pallets entering the PRA area in one day.

4.2.1.6. Probability of transfer to a suitable host

Norwegian directives reduce the probability of entry of PWN in most pathways through prohibitions or requirements of phytosanitary certificates. It is evident, however, that the highest probability for entry of PWN to the PRA area comes from WPM and dunnage (Tab. 2), and it has been speculated that infested WPM could have been the source of introduction of PWN in Portugal (FVO 1999). WPM contains elements with dimension large enough to accommodate also live individuals of *Monochamus* from the area of origin.

Beetles emerging from material infested by PWN would have the capacity of transmitting J_{IV} "dauerlarva" stages to nearby trees. In the present climate of Norway this could result in the establishment of latent infections of PWN in trees. Such infections could persist for at least 14 years (Bergdahl & Halik 2003; Bergdahl pers. comm.). Upon storm felling of infested trees the number of PWN would rapidly increase in wood. The same trees would be attractive for oviposition by native *M. sutor*, which could carry PWN to new trees and breeding material. Exotic *Monochamus* spp. emerging from pallets could of course spread PWN also to timber and cutting debris through oviposition. Reproducing on living plant cells and wood fungi, PWN could transfer to native *M. sutor* and hence enter the forest ecosystem.

From pallets without vector insects PWN can only spread to native trees by close contact. Any piece of PWN-infested wood that comes in wood-to-wood contact with native trees or fresh timber represents a possibility for transfer of the nematode. It is known that PWN may disperse between trees via temporary stem and root grafts (Malek & Appleby 1984), and through soil from infested wood mixed into soil (Kiyohara & Tokushige 1971) or mulched around trees (Halik & Bergdahl 1992). PWN infested wood coming in contact with freshly cut stumps was demonstrated to cause infection of the stump (Braasch 1996).

4.2.2 Probability of establishment

4.2.2.1 Availability of suitable hosts, alternate hosts and vectors in the PRA area

Host plants of PWN are listed in table 3 and 4. Pine trees (*Pinus spp*.) are the most important host plants and species differ in their susceptibility to PWD (Tab. 3). Of the 44 *Pinus* species listed as hosts most fall into the category of intermediate susceptibility, while 16 species are considered susceptible. Seven species regarded as resistant. In the case of Chinese red pine (*P. massoniana*),

Table 3. Susceptibility of Pinus spp. for the Pine Wilt Disease (PWD) caused by the Pine Wood Nematode (PWN), Bursaphelenchus xylophilus. (FVO 2001a; Sathyapala 2004).

SUSCEPTIBLE	INTERMEDIATE	RESISTANT
P. armandii (Chinese white pine)	*P. banksiana* (Jack pine)	*P. clausa* (sand pine)
P. ayacahuite (Mexican white pine)	*P. bungean*	*P. eliottii* (slash pine)
P. densiflora (Japanese red pine)	*P. caribaea* (Caribbean pine)*	*P. fenzeliana* (Hainan white pine)
P. kesiya (Khasi pine)	*P. contorta* (lodgepole pine)	*P. morrisonicola* (Taiwan white pine)
P. koraiensis (Korean pine)	*P. cooperi* (Cooper´s pine)	*P. rigida* (pitch pine)
P. leiophylla (chihuahua pine)	*P. echinata* (shortleaf pine)*	*P. taiwanensis* (Taiwan red pine)*
P. luchuensis (Luchu pine)	*P. engelmannii* (Apache pine)	*P. virginiana* (Virginia pine)
P. mugo	*P. halepensis ssp. halepensis* (aleppo pine)	
P. muricata (Bishop pine)	*P. halepensis ssp. Brutia*	
P. nigra (Austrian pine)	*P. jeffreyi* (Jeffrey pine)	
P. pinaster (maritime pine)	*P. lambertiana* (sugar pine)	
P. sylvestris (Scots pine)	*P. massoniana* (Chinese red pine)*	
P. taiwanensis (Taiwan red pine)*	*P. monticola* (western white pine)	
P. massoniana (Chinese red pine)*	*P. montezumae var. hartweigii* (Montezuma pine)	
P. thunbergii (Japanese black pine)	*P. oocarpa* (Mexican yellow pine)	
P. yunnensis (Yunnan pine)	*P. palustris* (longleaf pine)*	
	P. patula (Mexican weeping pine)	
	P. pinea (umbrella pine)	
	P. ponderosa (Ponderosa pine)	
	P. pentaphylla (Japanese white pine)	
	P. pungens (table mountain pine)*	
	P. radiata (Monterey pine)*	
	P. rudis	
	P. resinosa (red pine)	
	P. stobiformis (southwestern white pine)*	
	P. strobus (weeping white pine)	
	P. tabulaeformis (Chinese pine)	
	P. taeda (loblolly pine)*	
	P. wallichiana (blue pine)	

* equivocal status.

this species suffers heavy mortality in pine forests of southern China although experiments have ranked this species as intermediately susceptible to PWD (Zhou & Cheng 1993). Also *P. caribaea*, *P. echinata*, *P. palustris*, *P. pungens*, *P. radiata*, *P. strobiformis, P. taeda* and *P. taiwanensis* have an equivocal status with regard to susceptibility. Host plants also occur in the genera *Abies*, *Cedrus*, *Larix*, *Pseudotsuga*, *Chamaecyparis* and *Picea* (Tab. 4).

Table 4. Conifer host, other than pine, for the Pine Wood Nematode (PWN) Bursaphelenchus xylophilus. (Sathyapala 2004).

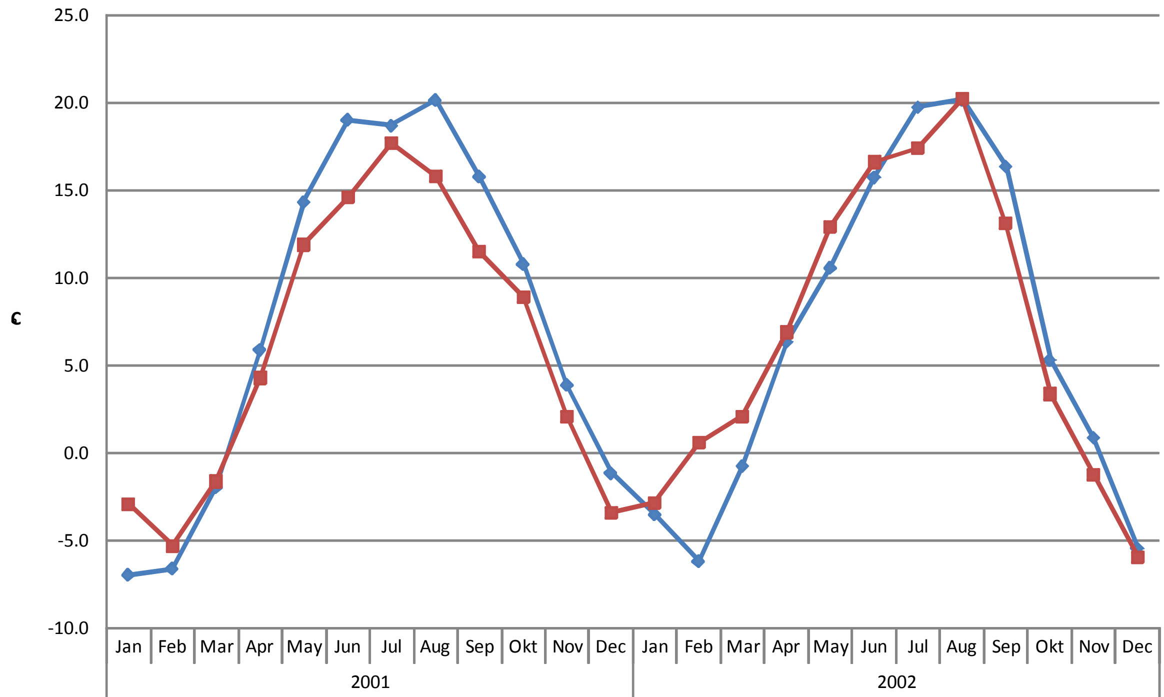
Abies ambilis	*Larix decidua*	*Picea abies*
Abies balsamea	*Larix kaempferi*	*Picea engelmannii*
Abies firma	*Larix laricina*	*Picea Canadensis*
Abies grandis	*Larix occidentalis*	*Picea glauca*
Abies sachalinensis	*Pseudotsuga meziesii*	*Picea jezoensis*
Cedrus atlantica	*Chamaecyparis nootkatensis*	*Picea mariana*
Cedrus deodara		*Picea pungens*
		Picea rubens
		Picea sitchensis

The total forest cover of the PRA area is 9.5 million ha with approximately 2.3 million ha pine (*P. sylvestris*) and 2.8 million ha Norway spruce (*P. abies)* (Larsson & Hylen 2007). In addition, there is restricted occurrence of *Larix decidua*. Some other susceptible host plants have limited occurrence in the PRA area, such as *Pinus mugo* that was planted in the western and northern part of Norway in the period 1860-1960 (6000 ha) and other pine and spruce species that have been planted for amenity purposes.

4.2.2.2 Suitability of environment

PWN occupies today geographic areas with winter temperatures similar to Norway (Magnusson 1986). Field infections of Scots pine, *Pinus sylvestris* have been successful in Wolcott in the State of Vermont, USA (Bergdahl & Halik 2003; Bergdahl pers. comm.).

A detailed comparison (Fig. 13) of air temperatures between Oslo, the capital of Norway, with Wolcott shows a very similar pattern, with only slightly warmer summers and colder winters in Wolcott the two years of 2001 and 2002. This suggests that latent infections can establish in trees in the southeastern part of the PRA area, and probably elsewhere.



	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Okt	Nov	Dec	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Okt	Nov	Dec
	2001												2002											
Wolcott	-7.0	-6.6	-2.0	5.9	14.3	19.0	18.7	20.2	15.8	10.8	3.9	-1.2	-3.5	-6.2	-0.8	6.3	10.6	15.8	19.8	20.2	16.4	5.3	0.9	-5.5
Oslo	-2.9	-5.3	-1.6	4.3	11.9	14.6	17.7	15.8	11.5	8.9	2.1	-3.4	-2.8	0.6	2.1	6.9	12.9	16.6	17.4	20.2	13.1	3.4	-1.2	-5.9

Figure 13. Monthly mean air temperatures in Oslo (Norway) and Wolcott (USA).

4.2.2.3 Cultural practices and control measures

There are today no silvicultural practices that would restrict the establishment of PWN. On the contrary, in the PRA area the annual input of dead wood is 2.9 million m^3, corresponding to an annual increase of 4.5%. The presence of dead wood contributes to an increased biodiversity, which is an important current management priority (Larsson & Hylen 2007). This, however, would in fact support the establishment and spread of PWN by increasing the availability of breeding substrates for the vector *M. galloprovincialis* and for *M. sutor*, the most abundant potential vector of the PRA area.

4.2.2.4 Other characteristics of the pest affecting the probability of establishment

Biological traits making PWN a successful invasive species include high mobility in thin water films, high intrinsic invasion rate, mycophagy, propagation at the point inoculation, high rate of multiplication, high rate of dispersal in host trees, and a high genetic plasticity (Magnusson 2001).

4.2.2.5 Conclusion of the assessment from 4.2.2.1-4.2.2.4

The conditions of the PRA area and the innate traits of an invasive pest found in the PWN give many opportunities for establishment. Hence, the probability that PWN will establish in the PRA area is considered as high.

4.2.3 Probability of spread after establishment

The documentation (Sousa *et al*. 2001) of *B. xylophilus* dauer juveniles (J_{IV}) within tracheae of *M. galloprovincialis* emerging from logs of *P. pinaster* in Portugal, and the consistent association in the field (Sousa *et al*. 2002) between this beetle and the nematode, are the first reports on an European *Monochamus* species serving as vector for PWN. In Europe *M. galloprovincialis* previously was reported to carry dispersal stages of *B. mucronatus* (Magnusson & Schroeder 1989; Tomminen *et al.* 1989; Tomminen 1990; Braasch *et al.* 1999).

At present *M. galloprovincialis* has a restricted occurrence in the PRA area. *M. sutor* is the most widespread species of pine sawyer (Bakke & Kvamme 1992; Ehnström & Holmer 2007), and *M. urussovii* has only old scattered records from the southern part of the PRA area (Ehnström & Holmer 2007). *M. sutor* is recorded from the entire PRA area, but is most abundant in the south-eastern districts. This species is not known to occur in areas of the world infested by PWN so its status as a vector is not known. There is a high probability for *M. sutor* to be a vector of PWN due to its biological similarity to *M. galloprovincialis* and its close association with *B. mucronatus.* This close association has been observed repeatedly in the PRA area (Magnusson *et al*. 2007), and in Sweden, where as much as 41% of *M. sutor* may carry dispersal juveniles of *B. mucronatus* (Schroeder & Magnusson 1989) and transmit the nematode to *P. sylvestris* and *P. abies* at feeding and oviposition (Schroeder & Magnusson 1992). This indicates the existence in the PRA area of a potential biological system for transmission of PWN.

M. sutor has a flight period from June to September (Bakke & Kvamme 1992). During this time adults visit pine and spruce to feed on fresh needles, shoots and the thin bark on twigs. Feeding and oviposition alternates during this time. Oviposition occurs on fresh timber, cutting wastes, wind-thrown trees, high stumps etc. At oviposition the female makes an oviposition pit in the bark and deposits eggs in the cambium layer. The first instar larvae feed from the subcortical layer. The second instar penetrates into the sapwood, where also the third and fourth instar develops (Bakke & Kvamme 1992). The larvae often return to feed in their subcortical galleries. During the autumn the adults die, and the beetles overwinter as larvae in wood. In May next year the fourth instar pupates in a pupal chamber close under the wood surface. In June the adults make their exit holes and fly out to near-by trees to feed (Jordbruksverket 2008 in prep.). The distance beetles move varies from 800 m to 3.3 km in Japan (Kobayashi *et al.* 1984). In southern (Åmli, Aust-Agder County) and south-eastern (Grue, Hedmark County) Norway there is 1 year life cycle, while beetles in the higher inland districts need 2 years for development (Bakke & Kvamme 1992).

Recent laboratory experiments (Vincent *et al.* 2008) on the competitive interaction between PWN and native *B. mucronatus* demonstrate the superior competitiveness of PWN on fungi in wood and in the boarding of beetles of *M. galloprovincialis*. This indicates that the presence of *B. mucronatus* in key-habitats of PWN would not interfere with establishment and spread of the pest. Hence, the probability that PWN will spread after an establishment in the PRA area is considered as high.

4.2.4 Conclusion on the probability of introduction and spread

Under the present regulations the most probable pathway for entry of PWN into the PRA area would be WPM. This will be the case even when ISPM No. 15 (FAO 2006) comes into force. The fact that all life stages of *Monochamus* spp. have been intercepted in WPM, and the high probability of WPM being infested by PWN, are reasons to be concerned. This is because of the large volumes in-service WPM reaching Norway at any point in time, and the complete lack of end-use control of WPM. Host plants of pine, spruce and larch occur abundantly in the PRA area.

Latent infections of host trees in the PRA area would require maturation feeding by *Monochamus* individuals leaving PWN-infested WPM, or transmission of nematodes by wood-to-wood contact or through soil. Seen isolated all these may be rare events, but considering the very large volumes of WPM involved these routes of establishment can not be ignored. Trees harbouring latent PWN infections could deteriorate from any cause, attracting vector beetles and allowing nematode populations to increase. It is highly probable that the domestic species *M. sutor* would be a suitable vector for further spread of PWN.

Thus, the probability that PWN will enter the PRA area, the probability that PWN will establish in the PRA area, and the probability that PWN will spread after an establishment in the PRA area, are all considered as high.

4.2.5 Assessment of the probability of introduction and spread – of PWN from untreated timber and wood of conifers originating in PFAs of Portugal

4.2.5.1 Chronology of the PWN infestation in Portugal and measures taken

The chronology of activities and measures related to the outbreak of PWN in Portugal is summarised in table 5. PWN was detected in May 1999 in recently dead maritime pine *Pinus pinaster* in two sites in the Setúbal Peninsula south-east of Lisbon (FVO 1999; Mota *et al*. 1999, 2004). In order to eradicate PWN Portugal designed and started the eradication program “National Action Plan for Eradicating the Pinewood Nematode (PROLUNP)”.

After being notified on June 25th 1999 of the detection of PWN in Portugal, EC made suggestions and requirements with regard to the Portuguese eradication activities through Commission Decisions. The progress of the eradication was monitored by inspection missions organized by the Food and Veterinary Office (FVO) of the Directorate General for Health and Consumers DG (SANCO). The EC Pinewood Nematode Survey Protocol was issued early in 2000 (FVO 2000a).

The Portuguese action plan PROLUNP (DGRF 2006) contains 4 sub-programs:

1. Survey: To identify, locate and analyse trees with possible PWN infestation in order to define areas where PWN is known to occur and where PWN is known not to occur.
2. Eradication: to eliminate the pine tree that can be sites for the spread of PWN in affected zones.
3. Vector-control: to control vector populations
4. Research: To increase the capacity for making analyses to identify PWN, to identify vector insects, to find methods of controlling the vector, and to promote research related to the biology of the nematode and its vector.

There are two categories of surveys, surveys of the demarked area (DA), including the Affected Zone (AZ) and the Buffer Zone (BZ), and surveys of the Free Zone (FZ).

In AZ a number of plots will be selected of 1 ha each and not having less than 50 trees of *P. pinaster*. Decline symptoms should be surveyed for all trees. For each symptomatic tree two samples, one from stem and one from the canopy, should be taken and analysed.

To eradicate PWN all deteriorating trees in a plot should be eliminated. The erection of a Clear-Cut Belt (CCB) 3 km wide to stop the spread of the nematode and its vector was suggested (DGRF 2006), and required in Commission Decision 2006/923/EC (EC 2006b). In 2007 the strategy of "local eradication" was introduced. This means cutting down of all trees within a circular area of 50 m radius centred in an infested tree (FVO 2008).

The survey activity of the FZ, *i.e.* areas of Portugal outside the DA, is focussed on survey plots within the existing 44 "Risk Areas" (RA), as well as new plots to be defined during the survey work. There are 32 permanent sample plots within each RA. A RA is delimited by a circle with 5 km radius and centred in a point where risk material is handled. Sixteen of the sample plots should be located within a distance of 1.5 km from the centre of RA. Samples should be collected from 50 trees showing symptoms of decline.

Over time it became apparent that the survey activity was insufficient (FVO 2006, 2007a). Many mission reports also mention the financial difficulties of the control operations. In addition there were legal problems, and the legislation of Portugal had to be changed to allow for the felling of symptomatic trees and trees in poor health, which had tested negative for PWN (FVO 2001b, 2002, 2003, 2006, 2007a). In spite of this, symptomatic trees still were standing in the flight period of the vector *M. galloprovincialis* (FVO 2000b, 2001b, 2003, 2006, 2007a, 2008) reducing the desired level of vector and nematode control. Regarding the DA, the inspectors (FVO 2000b, 2007a) also remarked on inappropriate sampling techniques, *i.e.* non-compliant with the EC protocol (FVO 2000a), with to few samples per tree and insufficient sampling of the tree crown. Although several laboratories took part in the sample analyses, the results occasionally were late (FVO 2008).

Table 5. Chronology of activities and measures in relation to the outbreak of Pine Wood Nematode (PWN) Bursaphelenchus xylophilus in Portugal. AZ = Affected Zone; BZ = Buffer Zone; CCB = Clear-Cut Belt; DA = Demarked Area; DGRF = Direccão Geral dos Recursos Florestais; EC = European Commission; FVO = EC/Food and Veterinary Office; FZ = Free Zone; PFA = Pest Free Area; PROLUNP = National Action Plan for Eradicating the Pinewood Nematode; WPM = Wood Packaging Material

YEAR	PORTUGAL	EC DECISIONS	EC-FVO COMMISSION INSPECTORS
1999	PWN was detected in recently dead maritime pine *Pinus pinaster* on the Setúbal peninsula. (FVO 1999; Mota *et al*. 1999, 2004); Portugal notified the EC in June about the detection of PWN on its territory. A central zone with 10 km radius was erected, and a national action plan (PROLUNP) was initiated.		At the first inspection in September it was concluded that the nematode infection may have been present for more than 2 years, and the movements of wood during this time, as well as recent transport activities were unclear. It was recommended to extend surveys to areas outside the currently infested zone, being aware of the likelihood of latent infections (FVO 1999).
2000		<u>Decision 2000/58/EC of 11 January</u>: Portugal should in general surveys establish areas free of PWN. Symptomatic trees should be felled before 1. March. Host plants and wood from infested areas should be inspected and treated, and be followed by a plant passport (EC 2000). The EC Pinewood Nematode Survey Protocol was issued early in 2000 (FVO 2000a).	In May 2000 it was noticed that 2/3 of the symptomatic trees, which according to Decision 2000/58/EC (EC 2000) should have been felled by 1 March still were standing in the infested zone. Also it was remarked that the sampling techniques were inadequate (FVO 2000b).
2001		<u>Decision 2001/218/EC of 12 March</u> considers the phytosanitary situation in Portugal to have improved. EC requires the establishment in Portugal of PFAs, and DA, consisting of the AZ and a BZ 20 km wide surrounding the AF (EC 2001).	In October 2001 cutting operations were still lagging behind in the AZ (FVO 2001b). Symptomatic trees, which had tested negative for PWN, were not felled in the BZ due to the absence of a formal obligation in the national legislation. This was considered a serious shortcoming in the eradication strategy (FVO 2001b).
2002	The government changed the legislation to allow felling of trees on symptoms alone (FVO 2002).		
2003	New infections in BZ made it necessary to extend AZ to cover 258 000 ha. In the legislation the definition of trees in poor health was improved and strict rules were adopted for proper cleaning up after cutting activities.		The significant increase of trees in poor health in the whole demarked area is a worrying condition (FVO 2003).

Table 5 continues.

2004	The felling activity has improved and the total number of infested trees has decreased		The monitor and eradication programme is well organised and executed, but the presence and prevalence of PWN in AZ is of concern and the prospect of complete eradication is far from being achieved. There has been a decrease in the percentage of trees tested positive to PWN (FVO 2004).
2005			
2006	In June an updated version of PROLUNP was presented, with new borders for the AZ and BZ. New outbreaks had resulted in an increase of AZ to 510 000 ha and DA to 1 01 000 ha (DGRF 2006)	Decision 2006/133/EC of 13 February concludes that PWN still is restricted to the DA. The desired level of control is below expectations, and Portugal should present an amended plan for control (EC 2006a). Decision 2006/923/EC of 13 December defines the conditions for the erection of a CCB of 3 km width on the outer border of DA (EC 2006b).	In June the felling of symptomatic trees still were lagging behind schedule and the spreading of the disease to large parts of the old BZ was evident. Survey activities in FZ needed to be increased with due concern paid to the removal of symptomatic trees (FVO 2006).
2007			The survey of DA is lagging behind and will make decisions on the optimal position of CCB difficult (FVO 2007a). For FZ survey results were not available in January 2007. Sampling of symptomatic trees at only 1 point per tree is not in compliance with the EC pinewood nematode survey protocol. Cutting operations in the CCB are not working in an optimal way (FVO 2007b). Later in 2007 sampling techniques are in accordance with the EC survey protocol. The pest free status of the BZ still is unclear. The CCB and the FZ have been demonstrated to be free from PWN (FVO 2007b, 2007c).

Table 5 continues.

2008	"Local eradication" (cutting an area with 50 m radius centred in an infested tree) was introduced as a new technique. On 11 April Portugal informed the EC of new outbreaks of PWN in the FZ. Adoption on 12 May of a ministerial order prohibiting movement of susceptible wood and plants out of continental Portugal unless wood has been heat-treated and plants duly inspected. A new survey plan for the entire territory was presented to EC 26-27 May. On 5 June Portugal again informed the EC of additional outbreaks of PWN in the FZ.	Decision 2008/378/ECof 15 May required Portugal immediately to carry out an additional risk-based survey of its entire territory, and on detection of PWN erect DA consisting of AZ and BZ. The proposed survey plan for Portugal was not approved due to insufficient intensity of monitoring (EC 2008a). Decision 2008/489/EC of 27 June concludes: The data available are not sufficient to confirm the existence of PFAs in Portugal. Community and international measures are not fully implemented. Member States other than Portugal are allowed to control import of susceptible wood, bark and plants originating in all parts of Portugal. Interim protective measures should be taken immediately to safeguard the territory of other Member States and protect Community trade interests in relation to third countries (EC 2008b). Decision 2008/684/EC of 19 August: Interim measures of decision 2008/489/EC are confirmed with regard to testing consignments of susceptible wood coming from Portugal. Newly produced WPM originating in demarked areas should be treated and marked according to ISPM No. 15 (EC 2008c).	Inspections in April demonstrated that the implementation of PROLUNP was late and certain parts had been suspended. This has resulted in the status of BZ as free from PWN is uncertain. Incomplete eradication of symptomatic trees in AZ may create a severe infection pressure on the BZ. Late lab. results on BZ will reduce the effect of "local eradication". Natural regeneration in CCB could soon reduce the effect of the belt (FVO 2008). New inspections in June concluded that the data available are not sufficient to confirm the existence of PFAs in Portugal (EC 2008c).

In figure 14 the temporal change in number of symptomatic trees registered in the surveys of the AZ from 1999 to early 2008 is presented. In the felling season 2005/2006 there was a dramatic increase in the number of symptomatic trees (268 211), and also in 2006/2007 felling the number is very high (196 530). This indicates that the control efforts had been insufficient in the preceding years.

In 2008 EU inspectors reported PROLUNP to be insufficiently implemented, with symptomatic trees still standing in the AZ (FVO 2008). Portugal reported new outbreaks 300 km northeast of the DA, in the largest area of *P. pinaster* (Mota pers. comm.). These outbreaks were detected outside the survey programme of the FZ. The Commission turned down a new version of PROLUNP presented by Portugal due to insufficient intensity of monitoring (EC 2008a). Portugal prohibited movement of untreated wood out of continental Portugal. In Commission Decision 2008/489/EC EU allowed Member States to control wood imports from all parts of Portugal (EC 2008b). There is a requirement that wood and bark of susceptible plants from Portugal must be treated to kill PWN and its vectors (EC 2008b). On 5 June 2008 Portugal again reports on new PWN outbreaks in the FZ. In August 2008 the interim measures of decision 2008/489/EC are confirmed with regard to testing consignments of susceptible wood coming from Portugal and laid down in Commission Decision 2008/684/EC. The Commission Decision also requires treatments according to ISPM No. 15 (FAO 2006) of newly produced WPM originating in demarked areas (EC 2008c).

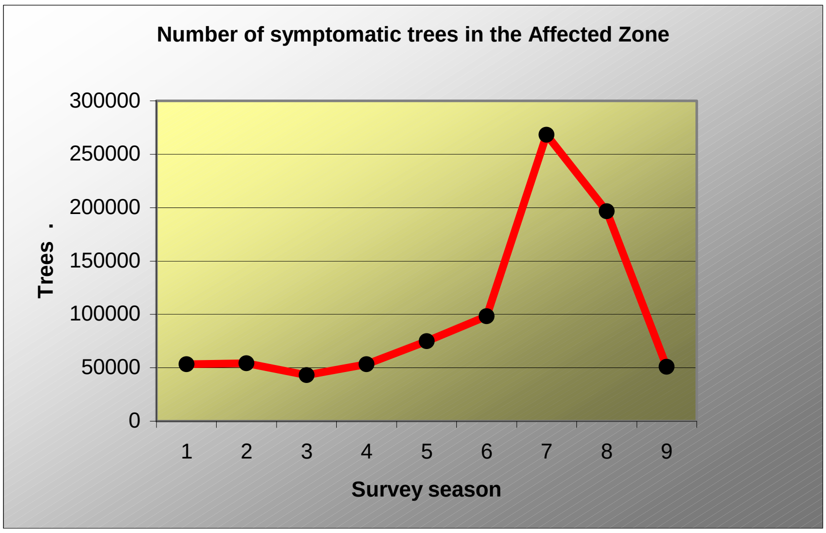


Figure 14. Number of symptomatic trees recorded in the surveys of the Affected Zone (AZ) in Portugal 1999-2008. 1=1999/2000; 2=2000/2001; 3=2001/2002; 4=2002/2003; 5=2003/2004; 6=2004/2005; 7=2005/2006; 8=2006/2007; 9=2007/2008 (preliminary data).

4.2.5.2 Status of PFA in Portugal

According to ISPM No. 4 "Requirements for the establishment of Pest Free Areas" (FAO 1995) there are three main components in the establishment and the maintenance of PFAs: a) systems to establish pest freedom; b) phytosanitary measures to maintain freedom; and c) checks to verify that freedom has been maintained.

Failure in detecting latent infections is the driving mechanism behind the spread of PWN and PWD (Futai 2004; Takeuchi & Futai 2007). Already at the beginning of the survey activities in Portugal the importance of latent infections was stressed by EU inspectors (FVO 1999). In various inspection reports the attention was drawn to the need for incubation of samples and sampling from the tree canopy. In Portugal improved sampling techniques and incubation routines started late in the eradication campaign.

By consequence the maintenance of freedom in specific areas in a country, which has been infested requires the definite containment of existing infestations, including latent infections. The erection of the AZ in Portugal was based on the occurrence of symptomatic trees, and it was in retrospect too small for the effective containment of PWN. Also the CCB was erected quite late in the campaign (2006), and the "local eradication" (2007) implying cutting of all host trees within a circular area of 50 m radius is clearly insufficient with regard to vector flight distances of 3 km (Kobayashi *et al*. 1984). The drastic increase in symptomatic trees in 2006-2007 (Fig. 14) demonstrates that eradication is far from being achieved and that containment most likely will fail. Some problems with controlling wood transports out from the DA is an additional concern.

Systems to verify freedom for PWN in the PFA must take account of the possible occurrence of latent infections. This requires composite samples from trees including material from the canopy, followed by incubation at +25°C for 2 weeks. New molecular techniques to detect very small amounts of PWN in wood (Takeuchi *et al*. 2005; Leal *et al.* 2007) have been developed and used for locating asymptomatic carrier trees (Takeuchi & Futai 2007).

However, timber and waste in 1-2 year old logging sites could carry detectable levels of PWN long before symptom expression of PWD is apparent in an infested area. This is the prime object focused on in the surveillance activities in the Nordic area (Magnusson *et al.* 2000, 2007). Dead wood is mentioned also in the EC Pine Wood Nematode Survey Protocol 2000 (FVO 2000b), but not pointed out as a target for sampling. A strong focus on symptomatic trees (FVO 2000b; EPPO 2003) will even in geographic locations where the climate is highly conducive for disease expression, like in Japan, fail in detecting latent infections (Futai 2004). Since the position of logging operations change constantly, the use of permanent observation plots may be inappropriate.

The suggested sampling of asymptomatic trees in situations where symptomatic trees are absent (FVO 2000b) will give less information on the possible occurrence of latent infections than sampling dead wood oviposited by *Monochamus*. Although Portugal probably did not allocate enough resources to the sampling of the FZ, the protocols used, *i.e*. FVO (2000a) and EPPO (2003), would be of little help in detecting latent infections of PWN.

Obviously the criteria of ISPM No. 4 (FAO 1995) necessary for establishing and maintaining PFA have not been met in Portugal. Hence, as stated in Commission Decision 2008/489/EC, the data available is not sufficient to confirm the existence of PFAs in Portugal.

4.2.5.3 Identification of pathways

The pathways will be the same as identified in section 4.2.1.1 (Tab. 6). Under the assumption of a change in the Norwegian regulations, to allow import of conifer plants and plant parts, and untreated conifer timber and wood products from PFAs in Portugal, phytosanitary measures would not be applicable for such material, and the probability of introduction of PWN will increase drastically.

Table 6. Expected probability of Pine Wood Nematode (PWN), Bursaphelenchus xylophilus, and its vector insects Monochamus spp. (MON) to be associated with pathways of wood originating from Pest Free Area (PFA) of Portugal. Probability levels: ++++ very high; +++ high; ++ medium; + low; and – very low; PC= Phytosanitary certificate.

Pathway	PWN	MON	INNATE PROBABILITY OF PWN AT ORIGIN	PHYTOSANITARY MEASURES	RELATIVE PROBABILITY OF PWN AT ENTRY	
					PRESENT [1]	FUTURE [2]
Plants for planting	X		++	None	++	++
Bonsai trees	X		-	PC		
Cut branches	X		++	None	++	++
Roundwood with bark	X	X	+++	None	+++	+++
Roundwood without bark	X	X	+++	None	+++	+++
Squared wood [3]	X	X	+++	None	+++	+++
Non-squared boards [4]	X	X	+++	None	+++	+++
Wood packaging material (WPM) as a commodity	X	X	+++	None	+++	+++
Wood packaging material (WPM) in service	X	X	+++	None	+++	+++
Dunnage	X	X	++++	None	++++	++++
Wood chips	X		++++	None	++++	++++
Sawdust, shavings etc.	X		++	None	++	++
Isolated bark	X		++	None	++	++
Green wood products	X	X	++	None	++	++
Hitchhiking *Monochamus* [5]	X	X	++	None	++	++

[1] Norwegian regulation relating to plants and measures against plant pests (FOR 2000-12-01 nr 1333).
[2] From January 1st 2009: ISPM No. 15 (FAO 2006).
[3] Sawn wood which has not retained its natural round surface.
[4] Sawn wood which has retained its natural round surface.
[5] Not specified in regulation.

4.2.5.4. Conclusion on probability of introduction and spread of PWN from PFAs in Portugal

- The criteria of ISPM No. 4 (FAO 1995) necessary for establishing and maintaining PFAs have not been met in Portugal. Hence, as stated in Commission Decision 2008/489/EC, the data available is not sufficient to confirm the existence of PFAs in Portugal.
- There is a high probability for initial association of PWN and its vector with pathways originating in “PFAs” of Portugal, *i.e.* similar as for corresponding pathways from any region of the world where PWN is present.
- Probability for survival of PWN and the vector, and reproduction of PWN during transport in consignments from PFAs in Portugal is high, *i.e.* similar as for consignments originating in other areas of the world infested with PWN.
- Probability of entry of PWN into the PRA area with consignments from PFAs in Portugal, when consignments are accepted without phytosanitary treatments, is very high. This also concerns the entry of the *M. galloprovincialis*.
- Untreated wood in the form of roundwood with bark, WPM and dunnage from PFAs in Portugal, have a high probability of transfer of PWN to a suitable host in the PRA area. Roundwood without bark, sawn wood, wood chips and bark has a medium probability for transfer of PWN.
- The probability of establishment and spread of PWN is high due to the abundance of hosts and climatic conditions conducive for establishment. This is also true for the vector *M. galloprovincialis*, which already is known to occur in the PRA area.
- Probability of spread after establishment is high due the likelihood of *M. sutor* to function as a vector.
- Import of untreated wood products from “PFAs” in Portugal would result in a very high probability of entry, and a high probability for establishment and spread of PWN in the PRA area.

4.3. Degree of uncertainty

The freedom from PWN in the PRA area relies on negative information. The sampling techniques followed existing protocols (Magnusson *et al*. 2000; FVO2000a) and seem adequate since nematodes as a group was recorded in most samples. However, as stated in section 4.1.2 more samples are needed for a more safe statement on freedom from PWN.

The vector *M. galloprovincialis* occurs locally in the PRA area and is known as a vector of both PWN and *B. mucronatus*. The species *M. sutor* is more abundant in the PRA area. Since *M. sutor* successfully transmits *B. mucronatus* it is regarded as a potential vector also for PWN. *M. sutor* is not known to occur in areas where PWN is present, and consequently this species has not been demonstrated as a vector of PWN. In North America and Japan, however, six and three local species of *Monochamus* are known as vectors for PWN (CABI/EPPO 1997). Therefore it is highly probable that *M. sutor* would have the same capacity as *M. galloprovincialis*.

The density of vector beetles also may influence the process of establishment. Japanese results indicate a critical lower threshold in density of vector beetles and/or host trees below which PWN and PWD fails to establish (Yoshimura *et al*. 1999; Takasu *et al*. 2000). This “Allee-effect” may retard establishment of the PWN and PWD, but it will probably not stop establishment in a longer perspective. In Japan in 1989 the spread of PWN and the PWD was thought to stop in southern Miyagi and central Akita prefectures because the vector insects *M. alternatus* were regarded to be absent from more northern locations (Forestry and Forest Products Research Institute, Japan pers. comm.). However, the unknown low density of vector beetles did not prevent the nematode from establishing throughout Akita and in reaching the border of the northern prefecture Aomori.

As pointed out in the recent Swedish report (Jordbruksverket 2008 in prep.) the lack of information on the population dynamics of PWN in colder areas makes firm statements on the probability of PWD outbreaks in the Nordic area difficult. Cold weather would trigger the development of the cold resistant J_{III}- stage, which has been reported to survive –17°C for 5 months (Kondo *et al*. 1982). Also the fact that the activity of PWN at the site of infection may induce PWD even in situations where temperatures do not allow for a general nematode population increase in the tree is of concern. Furthermore, the importance of maximum temperatures and diurnal temperature amplitudes for nematode activity and PWD expression largely remains to be evaluated.

Most global warming scenarios predict significant temperature increase even though the magnitude differs between the scenarios. However, in 100 years time the southeastern part of the PRA area is expected to have summer mean temperatures in the range of 15-20°C (as in parts of Portugal where PWD is present today) compared to 10-15°C at present time (Jordbruksverket 2008 in prep). It is uncertain whether drought stress due to shallow soils on bedrock (which is common in the PRA area) could increase the probability of PWD expression compared to the simulation results under Swedish conditions (Jordbruksverket 2008 in prep), and how an eventual increase of precipitation in future would modify the probability of PWD.

Although pathways are well known, custom routines may fail in their detection of PWN. This especially pertains to WPM, which accompanies a variety of commodities. Customs routines seldom include systematic inspection of WPM. Isolated bark of Portuguese *P. pinaster* imported, repacked and sold to a third country as decorative soil cover is a recent example on import of a seemingly harmless material with a medium probability to harbour PWN.

5. CONCLUSION

- The pest of concern is the Pine Wood Nematode (PWN) *B. xylophilus* and the PRA area is Norway. PWN is not known to occur in the PRA area.
- With the present trade pattern the probability of entry of PWN into the PRA area is expected to be high. The most probable pathway for entry of PWN into the PRA area would be wood packaging material (WPM). This is because of the common beetle and nematode infestation of this type of material, the large amount of WPM reaching Norway, and the lack of end-use control.
- The probability that PWN will establish and spread in the PRA area is considered as high. This would be primarily in the saprophytic life cycle, but also as latent infections of trees caused by maturation feeding of the beetles. Host plants (pine, spruce and larch) occur abundantly in the PRA area. The vector insect *Monochamus galloprovincialis* has a restricted occurrence, while the potential vector *M. sutor* is widely distributed in the PRA area.
- With regard to the so-called PFAs of Portugal, the criteria given in ISPM No. 4 for establishing and maintaining PFAs have not been met, and as stated in Commission Decision 2008/489/EC, the data available is not sufficient to confirm the existence of PFAs. Acceptance of untreated conifer wood from all parts of Portugal will result in a very high probability of entry and a high probability of establishment and spread of PWN and its vector to the PRA area. This in particular pertains to all wood packaging material, which has arrived before September 2008. From Commission Decision 2008/684/EC, which requires treatment of WPM originating in demarked areas, it appears that previous treatment activities have not been implemented consistently.
- Detailed assessments of economic consequences of a possible establishment and spread of PWN in Norway, the effects of global warming and other climate changes on the probability for PWD outbreaks, and the effect of possible phytosanitary measures, will be given in Part 2.

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