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Survey of the pine wood nematode (PWN) *Bursaphelenchus xylophilus* in Norway 2003

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Summary
In this survey of 2003, 600 samples were collected from 96 forest blocks in the counties of Aust-Agder and Vest-Agder in southern Norway. The sampling activity involved 19 municipalities situated mainly within the two zone sites D and E close to Kristiansand and Arendal. Samples from Scots pine (*Pinus sylvestris*) formed 92%, while samples of Norway spruce (*Picea abies*) made up 8% of the total sample volume. Timber and forest debris was the most common objects sampled. Ninety-eight percent of the samples, regardless of tree species, showed signs of *Monochamus* activity. Nematodes were common and occurred in 90% of the samples analysed. Eight samples of pinewood were positive for the genus *Bursaphelenchus*. This genus did not occur in spruce. *Bursaphelenchus mucronatus* was detected in 6 samples of forest debris of pine attacked by *Monochamus* and collected in the county of Aust-Agder. In the municipality of Evje and Hornes *B. mucronatus* was detected at Skjerkelia and Sutestad. In the municipality of Froland the nematode was found in two samples from Budalsfjellet, and in one sample from Mjålandsvatn. In the municipality of Birkenes one sample from Vågsdalen contained *B. mucronatus*. This is the fourth report on the occurrence of *B. mucronatus* in Norway. The pine wood nematode *Bursaphelenchus xylophilus* was not detected.

BACKGROUND

The detection in 1999 of the pine wood nematode (PWN), *Bursaphelenchus xylophilus*, in Portugal (Mota *et al*. 1999) has changed the earlier view on Europe as an area free from this pest (Evans *et al.* 1996). In 2000 the Standing Committee on Plant Health of the European Union (EU) decided on obligating each member state to conduct a survey of their territories for PWN (Anon. 2000).

A survey protocol by Magnusson *et al.* (2000) (Appendix 1) has been agreed upon between the Nordic countries, and with some modifications this protocol was adopted by the EU (Anon. 2000). Based on the biology of PWN and statistical criteria, this survey protocol suggests 3000 wood samples to be the minimal number required for the safe assessment of the presence or absence of PWN in each country. This report gives information on the Norwegian survey results for the year 2003.

MATERIALS AND METHODS

The survey strategy aims at increasing the probability of detection of the PWN by taking advantage of: (a) the natural association of the nematode with its vector insects (*Monochamus* spp.); (b) the time lag in spread of the nematode from the point of a possible introduction; (c) the transmission of PWN at oviposition of the vector insects on weakened trees or detached wood; and (d) the capacity of PWN to increase its population in wood;

Wood samples were collected within or close to two circular areas D and E, each with 50 km radius, centred in points of exposure to wood import materials (i.e. zone sites) in southern Norway (Fig 1). Zone site D was centred in Hunsfoss in the municipality of Vennesla, and zone site D was centred in Rykene in the municipality of Arendal. (Fig 2).

In the survey of 2003, 600 samples of wood were taken from 96 forest blocks. The sampling in forest blocks was focused on Scots pine (*Pinus sylvestris*) with a volume of 552 samples, while Norway spruce (*Picea abies*) was represented by 48 samples. The ambition was to collect samples from wood attacked by the cerambycid beetles *Monochamus* spp. Each sample consisted of 500 – 1000 ml wood

shavings, obtained by a portable Bosch GSR 24 VE-2 and a Milwaukee LokTor S18TX reversible electric drilling machine fitted with a 20 mm diameter bite. The geographical coordinates of each sample was determined by GPS-equipment, and logged into Mallegan MapSend WorldWide Basemap. The wood shavings of each sample was put into a plastic bag and transported to the nematode laboratory of the Norwegian Crop Research Institute, Plant Protection Centre in Ås.

In the laboratory, all wood samples were incubated at +25°C in their plastic bags for two weeks prior to extraction in Baermann funnels. After 24 hours the water was removed and the nematode suspension was allowed to settle. The nematodes were killed at +65°C and mounted in water.

Samples were screened in a Leica M10 stereomicroscope, and nematodes picked for closer investigation were mounted on objective slides and examined in a Leitz DMRB interference microscope fitted with the Leica Q500MC and Leica Qwin image processing and analysis system. Specimens identified as belonging to the *Bursaphelenchus xylophilus*-group were frequently analysed also by molecular techniques involving a multiplex PCR reaction with specific primers for the species *B.xylophilus*, *B. mucronatus* and *B. fraudulentus* (Planteforsk unpubl. data).

RESULTS

Sampled areas and localities

The sampling activity of 2003 started June 23th and finished October 31th and concerned almost exclusively forest blocks situated within the zone sites D and E (Fig. 2). Most samples (Tab. 1) were taken in the county of Aust-Agder (364) with slightly fewer samples originating from Vest-Agder (236). The material from Aust-Agder formed 61% of the total sampling volume (Fig. 3). In the survey, 600 samples were collected from 96 forest blocks distributed on 19 municipalities (Fig. 4). In Aust-Agder samples were taken from 55 forest blocks in 10 municipalities. In Vest-Agder the activity involved 9 municipalities and in total 41 forest blocks (Tab. 1).

Kind of material sampled

Samples from Scots pine (*Pinus sylvestris*) formed the major part, i.e. 552 samples corresponding to 92% of the total sample volume, while the 48 samples from Norway spruce (*Picea abies*) represented 8% (Fig.5 A). With regard to the kind of objects sampled (Fig. 5 B-C) "timber and debris" formed the largest fraction of both pine (95%) and spurce (96%). Lying trees, often wind thrown, formed 4,9% of the pine samples (Fig. 5 B) and 4% of the spruce samples (Fig. 5 C). Only one sample was taken from a pile of pine timber (Fig. 5 B). Ninety-eight percent of the pine (542 samples) and spruce (47 samples) material was taken from wood showing clear *Monochamus* activity (Tab. 2-3).

Occurrence of nematodes

For the total material of pine, nematodes were recorded in 93% of the 552 samples (Tab. 2). The microbivorous nematodes were the most common group and occurred in a frequency of 83%. Plant and insect nematodes were recorded in 61% of the material. Nematodes belonging to the order Aphelenchida and to the subfamily Tylenchinae were registered in frequencies of 51 and 21%. In the former group the genus *Aphelenchoides* (fungal feeders) was the most frequent with a relative frequency of 50 %. Insect associated nematodes, in the family Neotylenchidae and the genus *Cryptaphelenchoides* occurred in 2,2 and 1,1 % of the samples respectively. The family Seinuridae and the genera *Ditylenchus* and *Ektaphelenchoides* were absent. The genus *Bursaphelenchus* was recovered in 8 samples, equal to 1,4% of the total number of pinewood samples. *Bursaphelenchus mucronatus* was recorded for the fourth time in Norway, and was detected in 6 samples of forest debris attacked by *Monochamus*. The finds all were made in the county of Aust-Agder. In the municipality of Evje and Hornes *B. mucronatus* was detected at Skjerkelia and Sutestad. In the municipality of Froland the nematode was found in two samples from Budalsfjellet, and in one sample from Mjålandsvatn. In the municipality of Birkenes one sample from Vågsdalen contained *B. mucronatus*. The pine wood nematode *Bursaphelenchus xylophilus* was not detected.

In the 48 samples of sprucewood analysed (Tab.3), nematodes were present in 63% of the samples. As with pine samples, microbivorous nematodes were dominating at a frequency of 60%. The group of plant and insect nematodes occurred in 21 % the

material, with the subfamily Tylenchinae and the order Aphelenchida (i.e. the genus *Aphelenchoides*) recorded present in 8 and 19 % of the samples. The family Seinuridae and the genera *Cryptaphelenchoides*, *Ektaphelenchoides* and *Bursaphelenchus* was absent in the samples from spruce. This is also true of the family Neotylenchidae and the genus *Ditylenchus*.

The comparison of samples from pinewood showing clear signs of *Monochamus* activity, and samples from wood without clear symptoms (Tab. 2) is difficult because few samples were taken from wood with no such signs. However, the family Neotylenchidae and the genera *Cryptaphelenchoides* and *Bursaphelenchus* were only detected in wood attacked by *Monochamus*. For spruce (Tab. 3), the single sample from wood without signs of *Monochamus* activity was negative for nematodes, so no comparison is possible.

In the case of pine and sample categories (Tab.2), there are no differences in frequencies of nematodes as a group, microbivorous nematodes and the subfamily Tylenchinae between "lying trees" and "timber and debris". Plant and insect nematodes, the group of Aphelenchid nematodes, the genera *Aphelenchoides* are twice as common in samples from "timber and debris" than in samples taken from lying trees. Also the genera *Cryptaphelenchoides* and *Bursaphelenchus* are completely restricted to "timber and debris", while nematodes in the family Neotylenchidae seem more frequent in "lying trees". One sample from a timber pile contained microbivorous nematodes. For spruce (Tab. 3) the material contains only 2 samples from lying trees, which both were negative for nematodes, so statements on patterns of nematode occurrence between kind of objects can not be made.

The genus *Bursaphelenchus* was only detected in "timber and debris"of pine. Nematodes recorded in this genus included members of the "*borealis*" and the "*xylophilus*"- groups". The only representative of the "*xylophilus*-group" was *B. mucronatus*, which was noted in 6 samples of forest debris attacked by *Monochamus*. These finds corresponds to a frequency of 1,1% of the pine samples.

DISCUSSION

For the sampling activity the category "timber and debris of pine" is the main object, and this is in line with the strategy of optimising for the detection of the pine wood nematode, should it be present in the area.

The nematodes were broadly classified into microbivorous (bactetrial feeders) and into a group designated as "plant and insect"-nematodes, i.e. stylet-bearing plant parasites, mycophages, predators and insect parasites, i.e. the subfamily Tylenchinae, the family Neotylenchidae, the genera *Aphelenchoides*, *Seinura*, *Ektaphelenchoides*, *Cryptaphelenchoides* and *Bursaphelenchus*. Within the group of "plant and insect nematodes" the primary ambition was to recognize the genus *Bursaphelenchus* and to identify the species belonging to the *B. xylophilus*-group, which include species like *B. mucronatus* and *B. xylophilus*.

As shown in previous work (Magnusson *et al*. 2001, 2002, 2004), the occurrence of nematodes in deteriorating wood like forest debris is a normal condition. The high frequency of microbivores reflects the importance of this group in decomposition processes. These nematodes are suspected to have an important key-function in the normal decomposition process. Pinewood seems to be a more suitable habitat for many nematodes than spruce wood. All categories of nematodes reached higher relative frequencies in timber and debris of pine compared to spruce, which indicates that pine may offer a better habitat for wood nematodes.

In this survey the genus *Bursaphelenchus* was only detected in samples of pinewood. Eight samples were positive for the genus *Bursaphelenchus.* This corresponds to 1,3% of the total number of samples. This is more than recorded in the surveys of 2000 and 2001(Magnusson *et al*. 2001, 2002), but slightly less than in 2002 (Magnusson *et al*. 2004). In 2003 we were successful in detecting *B. mucronatus* in 6 samples of forest debris attacked by *Monochamus* sp. These finds corresponds to 1,1% of the pinewood samples, which is the highest frequency recorded so far in Norwegian surveys (McNamara & Støen 1988, Magnusson *et al*. 2001, 2002, 2004). This report is the fourth record of *B. mucronatus* in Norway. Earlier this species was detected in Hanestad in the

province of Hedmark (McNamara & Støen 1988), at Ombudstvedt in the municipality of Våler (Magnusson *et al*. 2002), and at Bjørndalen in the municipality of Eidsberg (Magnusson *et al*. 2004) in the county of Østfold.

In our neighbour-countries, Sweden and Finland, *B. mucronatus* is common. In Sweden (Magnusson & Schroeder 1989, Schroeder & Magnusson 1992) *B. mucronatus* occurs from the county of Småland in the south to the county of Lappland in the north. Swedish data demonstrates that the area of Särna and Idre, in the county of Dalarna, has a high population of *B. mucronatus*, with 41 % of the *Monochamus* individuals recorded to carry "dauer juveniles" (Schroeder & Magnusson 1989). Also in Finland (Tomminen *et al*. 1989, Tomminen 1990) *B. mucronatus* occurs in the whole country. The data obtained so far may indicate that *B. mucronatus* could have a more restricted occurrence in Norway.

In the present material 98% of the samples were collected from wood showing attack by *Monochamus*. This proportion is much higher than in the surveys of 2000, 2001 and 2002. In the survey of 2000 most of the insect damage observed was considered as a result of *Monochamus* activity (Magnusson *et al*. 2001). However, with the subsequent increase in the skill of the field personnel that statement seems doubtful. Similarly, in 2001 43% of the pinewood samples were reported attacked by "wood boring insects" (Magnusson *et al*. 2002), a category which probably also includes wood with signs of insects other than *Monochamus*. In 2002 65% of the pine samples were taken from wood showing signs of *Monochamus* activity (Magnusson *et al*. 2004).

So far the total sampling program 2000-2003 has resulted in 2015 samples collected from 310 forest sites in 54 municipalities and 8 counties. As pointed out above the Nordic sampling protocol stipulates that a volume of 3000 samples negative for *B. xylophilus* would allow for a safe statement on its absence from the territory. Due to the fact that the initial sampling (2000-2001) in many instances failed in detecting clear signs of *Monochamus* activity, it can be estimated that about 1000 more samples are needed to reach a safe level of information.

CONCLUSIONS

- In 2003, 600 samples were collected from 96 forest blocks in southern Norway mainly situated within the two zone sites D and E centred close to Kristiansand and Arendal. In the county of Aust-Agder 364 samples were collected, while the county of Vest-Agder was represented by 236 samples.

- Samples from Scots pine (*Pinus sylvestris*) formed 92% of the collected material, while spruce (*Picea abies*) was represented by a smaller 8% fraction. Timber and forest debris were the most common object sampled. Ninety-eight percent of the pine and spruce samples showed signs of *Monochamus* activity.

- Nematodes were common and occurred in 90% of the samples analysed. Eight samples of pinewood were positive for the genus *Bursaphelenchus*. All of these samples were collected from timber and forest debris.

- *Bursaphelenchus mucronatus* was recorded in 6 samples of pinewood, collected in the municipalities of Evje and Hornes (2), Froland (3) and Birkenes (1) in the county of Aust-Agder. This is the fourth report on *B. mucronatus* in Norway.

- The pine wood nematode *Bursaphelenchus xylophilus* was not detected in this survey.

- A safe conclusion on the absence of *B. xylophilus* from the woodlands of Norway would require approximately 1000 additional samples free of this nematode.

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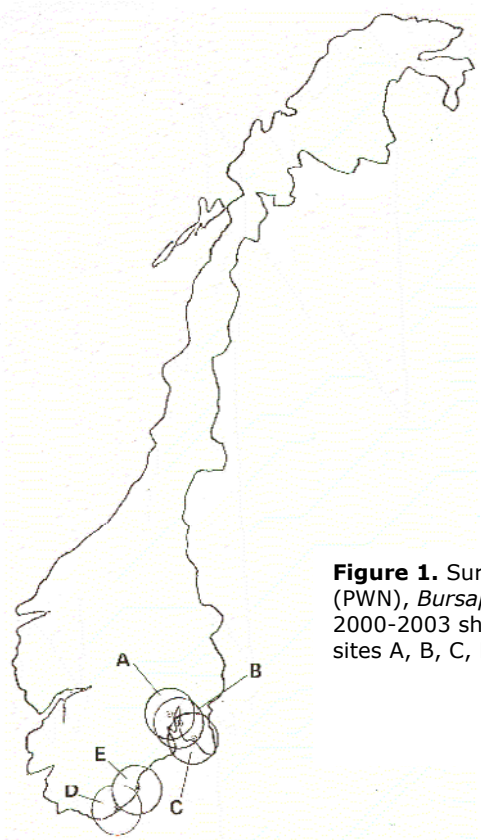

Figure 1. Survey of the pine wood nematode (PWN), *Bursaphelenchus xylophilus*, in Norway 2000-2003 showing the position of the five zone sites A, B, C, D and E.

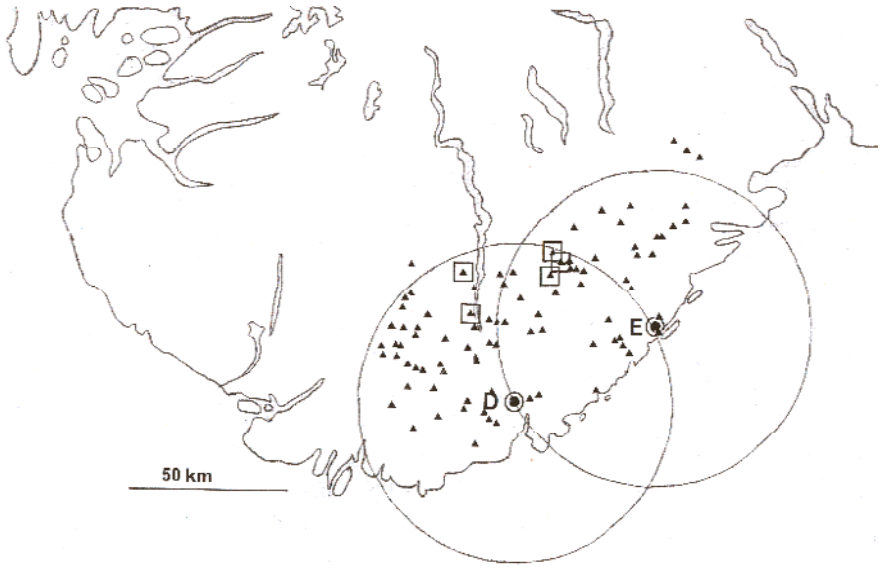

Figure 2. Survey of the pine wood nematode (PWN), *Bursaphelenchus xylophilus*, in Norway 2003. The zone site D is centred in Hunsfoss (municipality of Vennesla) and the zone site E is centred in Rykene (municipality of Arendal). Positions of sampling localities are indicated by triangles. Finds of *B. mucronatus* are shown in squares.

Table 1. Number, locality and kind of wood samples collected in the survey of the pine wood nematode (*Bursaphelenchus xylophilus*) in Norway 2003. *L* = lying tree; *TD* = timber and debris; *TP* = timber pile;

COUNTY	**MUNICIPALLITY**	**LOCATION**	**PINE**			**SPRUCE**		**TOTAL**
			L	***TD***	***TP***	***L***	***TD***	
Aust-Agder (n=364)	**Arendal (15)**	**Grødvika (1)**					**1**	**1**
		Mjåvann (1)		**1**				**1**
		Røynevannet (6)		**6**				**6**
		Styggheia (7)		**7**				**7**
	Birkenes (58)	**Dovland (16)**	**1**	**15**				**16**
		Langetjønna (3)		**3**				**3**
		Lauvraksdalen (5)		**5**				**5**
		Murtetjønn (3)		**3**				**3**
		Orrheim (1)		**1**				**1**
		Store Sundstøl (2)		**2**				**2**
		Tereknipen (8)		**8**				**8**
		Vågsdalen (20)	**2**	**17**			**1**	**20**
	Evje og Hornnes (70)	**Abusland (2)**	**1**	**1**				**2**
		Austerhus (9)		**9**				**9**
		Kjetså (15)		**15**				**15**
		Skjerkelia (17)		**17**				**17**
		Storøygard (2)		**2**				**2**
		Sutestad (9)		**9**				**9**
		Vardeheia (16)		**16**				**16**
	Froland (38)	**Budalsfjellet (14)**		**14**				**14**
		Heidal (1)			**1**			**1**
		Jomås (1)					**1**	**1**
		Lillemykland (2)	**2**					**2**
		Løvjomås 2 km N (1)		**1**				**1**
		Mjåland (2)		**2**				**2**
		Mjålandsvatn (5)		**5**				**5**
		Ytre Lauvrak (2)		**2**				**2**
		Øvre Lauvrak (10)		**10**				**10**
	Gjerstad (13)	**Fossbrekka (4)**		**4**				**4**
		Krossdalen (5)		**3**			**2**	**5**
		"Langs veg" (1)		**1**				**1**
		Materialen (1)		**1**				**1**
		Meånsknuten (2)	**2**					**2**
	Grimstad (37)	**Arnevik (16)**		**16**				**16**
		Bjørnetrø (10)		**9**			**1**	**10**
		Hemningtveitvatnet (5)		**4**			**1**	**5**
		Kilandsvannet (6)		**4**			**2**	**6**
	Risør (2) (1 location) Iveland (36)	**Barmen Felt 4 (2)**		**1**		**1**		**2**
		Dalane (7)	**2**	**5**				**7**
		Grossås (3)	**2**	**1**				**3**
		Hellerheia (17)		**17**				**17**
		Hviltveit (7)		**7**				**7**
		Øynavatn (2)		**2**				**2**
	Vegårshei (45)	**Grindtjønn (15)**		**9**			**6**	**15**
		Moen (10)		**10**				**10**
		Olimstad (11)		**5**			**6**	**11**
		Skarkmyrheia (2)	**1**	**1**				**2**
		Stormyrlia (7)		**2**			**5**	**7**
	Åmli (50)	**Berås (8)**		**8**				**8**
		Gjøvland (5)	**3**	**2**				**5**
		Holmvatn (4)	**1**	**3**				**4**
		Hovde (10)		**7**			**3**	**10**
		Håkedalsåna (10)		**4**		**1**	**5**	**10**
		Nelaug (6)		**5**			**1**	**6**
		Tveit (7)		**6**			**1**	**7**

Vest-Agder (n=236)	**Audnedal (42)**	**Ekra (1)**					**1**	**1**
		Grindheim (15)		**14**			**1**	**15**
		Hisåsen (3)		**3**				**3**
		Høyland (7)		**7**				**7**
		Kollungtveit (4)	**2**	**2**				**4**
		Mosvannet (2)		**2**				**2**
		Solbjør (4)	**1**	**3**				**4**
		Vårdal (5)	**2**	**3**				**5**
		Ås (1)	**1**					**1**
	Hægebostad (28)	**Birkeland (4)**		**4**				**4**
		Godstad (12)		**12**				**12**
		Homestøl (2)	**1**	**1**				**2**
		Tingvatn (10)		**10**				**10**
	Kristiansand (33)	**Krokevatnet (9)**		**9**				**9**
		Langemyr (2)		**2**				**2**
		Rebbåsheia (7)		**6**			**1**	**7**
		Sandnesvannet (15)		**15**				**15**
	Lindesnes (2)	**Barstøl (1)**					**1**	**1**
		Vågestad (1)		**1**				**1**
	Marnardal (27)	**Bjørnevollen (5)**	**3**	**2**				**5**
		Bustatjørna (4)		**4**				**4**
		Kleveland (2)		**2**				**2**
		Langevatnet (5)		**5**				**5**
		Nedre Haraldstad (10)		**10**				**10**
		Ravnåsen (1)		**1**				**1**
	Songdalen (11)	**Fidje (1)**		**1**				**1**
		Godåsen (1)		**1**				**1**
		Grødal (1)		**1**				**1**
		Hagen (2)		**2**				**2**
		Solheia (1)		**1**				**1**
		Åmsland (5)		**5**				**5**
	Søgne (2)	**Hellersdalen (2)**		**2**				**2**
	Vennesla (55)	**Homme (19)**		**19**				**19**
		Homsteane (9)		**9**				**9**
		Karten (14)		**14**				**14**
		Kiledalen (3)		**3**				**3**
		Skarpengland (3)		**3**				**3**
		Åmdal (7)		**7**				**7**
	Åseral (36)	**Madland (14)**		**13**			**1**	**14**
		Tverrhei (12)		**12**				**12**
		Åsen (10)		**5**			**5**	**10**
PINE OBJECT S			**27**	**524**	**1**			
SPRUCE OBJECTS						**2**	**46**	**600**
TOTAL MATERIAL			**552**			**48**		

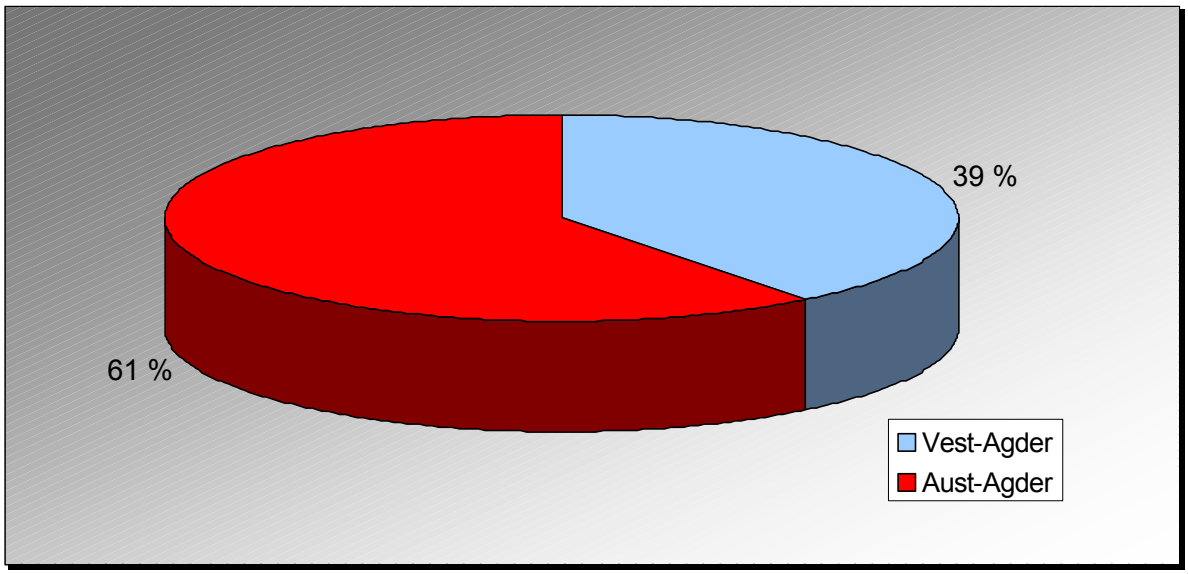

Figure 3. Survey off the pine wood nematode (PWN), *Bursaphelenchus xylophilus*, in Norway 2003. The relative distribution of 600 samples on counties.

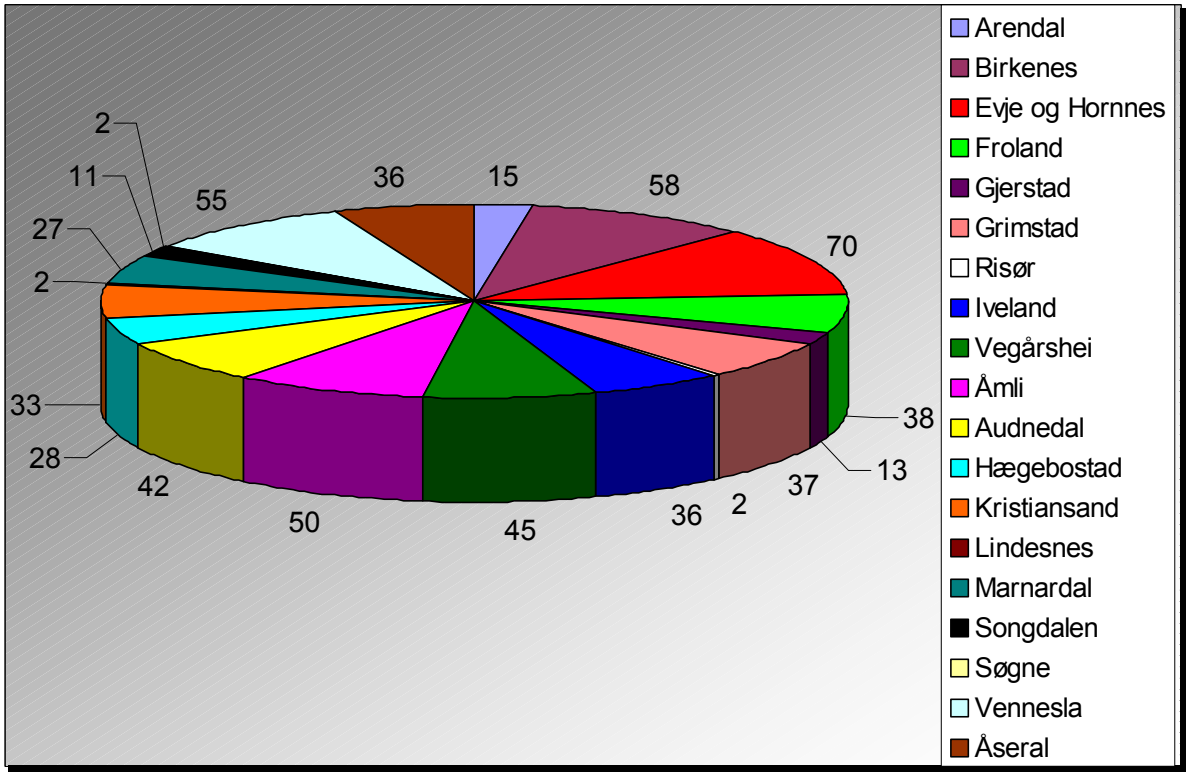

Figure 4. Survey of the pine wood nematode (PWN), *Bursaphelenchus xylophilus*, in Norway 2003. Number of samples distributed on municipallities.

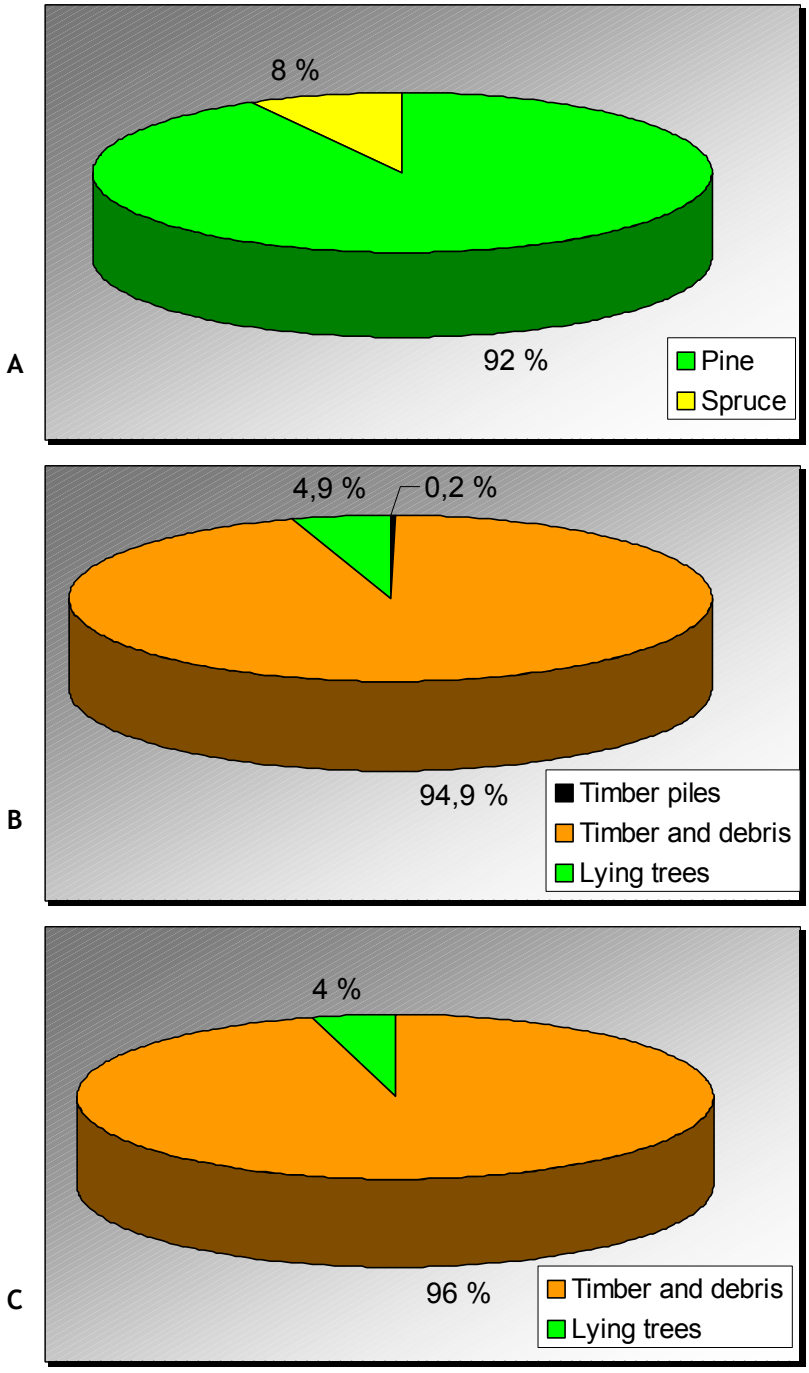

Figure 5. Survey of the pine wood nematode (PWN), *Bursaphelenchus xylophilus*, in Norway 2003. **A**. Distribution of samples on Scots pine (*Pinus sylvestris*) and Norway spruce (*Picea abies*); **B.** Pine samples distributed on objects; **C.** Spruce samples distributed on objects.

Table 2. Nematode frequencies (%) in samples (n=552) of Scots pine (*Pinus sylvestris*) analysed in the Norwegian survey of the pine wood nematode (*Bursaphelenchus xylophilus*) in 2003.

Nematode category	**Pine wood total**	**Wood with *Monochamus* activity** n=542 (98%)	**Wood without clear *Monochamus* activity** n=10	**Lying trees** n=27	**Timber and debris** n=524	**Timber piles** n=1
Nematodes total	93	93	100	96	93	100
Microbivorous	83	83	90	93	83	100
Plant & insect nematodes total	61	61	50	48	62	0
Tylenchinae	21	21	20	22	21	0
Neotylenchidae	2,2	2,8	0	7	2,7	0
Ditylenchus	0	0	0	0	0	0
Aphelenchida	51	51	30	26	52	0
Aphelenchoides	50	50	40	26	51	0
Cryptaphelenchoides	1,1	1,1	0	0	1,1	0
Ektaphelenchoides	0	0	0	0	0	0
Seinuridae	0	0	0	0	0	0
Bursaphelenchus	1,4	1,5	0	0	1,5	0
B. mucronatus	1,1	1,1	0	0	1,1	0
B. xylophilus	0	0	0	0	0	0

Table 3. Nematode frequencies in samples (n=48) of Norway spruce (*Picea abies*) analysed in the Norwegian survey of the pine wood nematode (*Bursaphelenchus xylophilus*) in 2003.

Nematode category	**Spruce wood total**	**Wood with *Monochamus* activity** n=47 (98%)	**Wood without clear *Monochamus* activity** n=1	**Lying trees** n=2	**Timber and debris** n=46
Nematodes total	63	64	0	0	65
Microbivorous	60	62	0	0	63
Plant parasitic total	21	21	0	0	24
Tylenchinae	8	9	0	0	9
Neotylenchidae	0	0	0	0	0
Ditylenchus	0	0	0	0	0
Aphelenchida	19	19	0	0	20
Aphelenchoides	19	19	0	0	20
Cryptaphelenchoides	0	0	0	0	0
Ektaphelenchoides	0	0	0	0	0
Seinuridae	0	0	0	0	0
Bursaphelenchus	0	0	0	0	0
B. mucronatus	0	0	0	0	0
B. xylophilus	0	0	0	0	0

Appendix 1.

Nordic Pine Wood Nematode Survey - Draft Manual

2000-03-20

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Background
The recent detection of the pine wood nematode (PWN) *Bursaphelenchus xylophilus* in Portugal has changed the earlier view on Europe as an area free from this pest (Evans *et al.* 1996), and made the European perspective on forest health considerably wider. The infestation in Portugal is suspected date back 2-3 years (Anonymous 1999) and there is now a growing concern about the possible presence of PWN also in other countries. The Standing Committee on Plant Health of EU has reached a decision on obligating each member state to conduct a survey of their territories for PWN. The Nordic countries, including Norway, strongly support that decision.

In previous surveys in the Nordic countries (McNamara & Støen 1988, Magnusson & Schroeder 1989, Tomminen *et al.* 1989, Tomminen 1990) PWN was not detected. However, in the Nordic area PWN is not expected to cause a large-scale pine mortality. Because of this PWN infections could easily be overlooked. Only sampling strategies designed to give a high probability of detection can form the basis for confident statements on the presence or absence of PWN. This document presents the outlines of an extended and coordinated PWN survey of the Nordic forests.

Objectives
The objectives of the proposed work are to survey:

1. Zone sites:
   - 1.1. Forests adjacent to points of wood import (harbours).
   - 1.2. Forests adjacent to points of handling and storage of imported wood (saw mills and pulp mills).
   - 1.3. Forests adjacent to points of handling and storage of imported wood packaging material.

2. General sites:
   - 2.1. Forests in general.
   - 2.2. Clear cuts burnt for nature conservation
   - 2.3. Areas of forest decline.

Sites of sampling.
Zone sites:
Forests situated within an area with a 50 km radius centred in points of handling and storage of high risk wood import materials. Before the survey activity starts each country shall identify such centres and map the potential sampling objects within each zone. The sites for sampling are forest blocks logged 1-2 year before sampling. The sampling shall be focused on cutting wastes of *Pinus sylvestris* oviposited by *Monochamus* spp , or any other conifer wood showing *Monochamus* activity.

In winter or spring, after the first sampling of year 2000 and 2001, each forest block should be provided with four bait-logs of freshly cut *P.sylvestris*, preferably felled in exposed situations. Bait-logs would serve as traps for *Monochamus* spp. and should be sampled after one year.

This strategy allows for an increased probability of detection by taking advantage of the natural association of PWN with its vector insect, and the lag phase of spread from the point of a possible introduction. It is essential that sample sites are distributed as even as possible over the circular area.

General sites:
Forest sites of normal health, clearings burnt for natural conservation and sites of forest decline. As a consequence of the sampling

strategy, general forest sites will be selected in areas naturally infested by *Monochamus* spp. Instances of forest decline could be identified by Forest Service officers. Samples should be taken from trees showing various symptoms of branch die-back and wilt. If detected, trees, logs or any conifer wood showing *Monochamus* activity is a primary target for sampling.

Local conditions

Conditions may vary between countries, with regard to acerage of pine forests, density of vector insects ect. Therefore, each country need to decide how the sampling activity should be allocated to sampling objects. In a situation where *P.sylvestris* is a minor forest tree species the sampling activity may be directed towards stands of another conifer host plant, like *Picea abies*. Wood of *P. abies* will provide suitable conditions for reproduction of PWN.

Sample size

It is essential to find a way of a correct assessment of the sample size required to allow for confident statements on the hypothetical presence of PWN in each of the Nordic countries.
It is also of outmost importance that the sample size is determined primarily on biological criteria. Economy is of secondary importance, as it relates to political concerns.

Assumptions:

- There is a similar probability of finding PWN regardless of the region sampled.
- In wood attacked by *Monochamus* spp. PWN is assumed to occur in a frequency 0,001, equal to one find out of 1000 samples of wood showing *Monochamus* activity.

The minimal number of samples (n) required is defined from the probability of a positive find (p) and the degree of confidence (ε) according to:

$$n = \ln \varepsilon / \ln (1-p)$$

Table 1. Minimal number of samples determined by the value of ε and p.

p \ ε	0,10	0,05	0,01	0,001	0,0001
0,25	8	11	16	24	32
0,10	22	29	44	66	88
0,05	45	59	90	135	180
0,01	230	298	459	688	917
0,001	2302	2995	4603	6905	9206

According to Tab.1. the selection of $\varepsilon = 0,05$ and $p = 0,001$ gives a sample size of 2995. Hence, the suggestion is to take 3 000 samples for each country, regardless of differences in the forest area between countries

Allocation of sampling activities

Each country should define and map the zones of interest. Within each zone at least 10 locations for sampling collection should be selected. Zone sites have the highest priority for sampling, and equal interest should be paid to the categories 1.1., 1.2. and 1.3. General sites (forests in general, clear cuts burnt for nature conservation and areas of forest decline) are of second priority.

The whole survey includes 3000 samples per country. This sampling activity should be executed preferably during a period of 3 years. The duration of the project depends, however, on funding and the capacity for sampling and analysis.

Sample collection, handling and extraction.

Samples should be collected in May - October 2000, 2001 and 2002.

Logs, branches and cutting wastes : A suitable spiral drill (diameter 25 mm) should be used to obtain wood chips in a minimal volume of 100 ml from each object sampled. Samples should be packed in plastic bags, marked and sent for analysis. Samples should be incubated for a minimum of 2 weeks at +25°C before extraction in water by immersion.

Trees: For each area of forest decline, 5 symptomatic trees are felled. From each tree

trunk and branch wood is sampled. The trunk is sampled with a spiral drill (diameter 25 mm) to obtain wood chips in a minimal volume of 100 ml per drill hole. Ten such drillings evenly distributed along the trunk are combined to form one 1 000 ml trunk sample. Wood from branches showing symptoms of die-back is sampled with the similar technique, and 10 subsamples of 100 ml are combined to form one 1 000 ml branch sample per tree. The samples are packed in plastic bags, marked and sent for analysis. Samples should be incubated for a minimum of 2 weeks at +25°C before extraction in water by immersion. The survey efforts should be focused on *Monochamus*/wilt symptomatic trees regardless of the nature of the site, decline or not decline.

Identification of PWN
PWN can be identified on morphological criteria, or by various techniques based on DNA. For the time being, morphology is considered to support to the molecular techniques.

Preliminary results from the year 2000 survey will be made available before October 15th 2000.

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