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# Monitoring pinewood nematode

## Bursaphelenchus xylophilus IN NORWAY 2019

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Magnusson, C., [1)] Hietala, A.,[1)] Hansen, P. M.,[2)] Heggem, E.,[3)] Rasmussen, I.,[1)]
Birgit Schaller,[1)] Marte Persdatter Tangvik,[1)] Ben Hellal, Z.[1)]

Divisjon for biologi og plantehelse[1)], Divisjon for matproduksjon og samfunn[2)], Divisjon for kart og statistikk[3)],

**TITTEL/TITLE**

# Monitoring pinewood nematode - Bursaphelenchus xylophilus IN NORWAY 2019

**FORFATTER(E)/AUTHOR(S)**

Magnusson, C., Hietala, A., Hansen, P. M., Heggem, E., Rasmussen, I., Schaller, B, Persdatter Tangvik, M & Ben Hellal, Z.

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**SAMMENDRAG/SUMMARY:**

I 2019 års kartlegging av furuvednematoden *Bursaphelenchus xylophilus* i Norge ble 400 prøver tatt fra hogstavfall og vindfall av *Pinus sylvestris* L. med angrep av furubukk *Monochamus* spp. Prøvene ble tatt ut i Akershus, Buskerud, Østfold, Telemark, Aust-Agder og Vest-Agder. Prøvene som besto av flis ble inkubert ved +25°C i to uker før de ble ekstrahert med Baermanntrakt og undersøkt i mikroskop. Furuvednematoden *B. xylophilus* ble ikke påvist i prøvene, men den naturlig forekommende arten *Bursaphelenchus mucronatus kolymensis* ble oppdaget i fire prøver fra Agderfylkene. Feller med feromoner for fangst av furubukk ble satt opp i Hedmark (Elverum, Romedal, Stange og ved Geitholmsjøen), Møre og Romsdal (Kvanne) og Østfold (Fredrikstad og Vestby). I laboratoriet ble billene kuttet i biter og ekstrahert med en modifisert Baermanntrakt. Suspensjonen fra ekstraksjonene ble undersøkt i stereomikroskop for forekomst av infektive stadier av *Bursaphelenchus* spp.. Ingen nematoder kunne påvises i de 106 undersøkte billene. I perioden 2000 – 2019 er totalt 8123 vedprøver analysert. Flest prøver er tatt i Østfold, fulgt av Hedmark, Telemark, Buskerud og Aust-Agder. I kartleggingen 2019 ble *B. mucronatus kolymensis* påvist i fire av de 400 vedprøvene, tilsvarende en frekvens på 0,01 (1 %). For hele perioden 2000 - 2019 ble *Bursaphelenchus mucronatus kolymensis* + *B. macromucronatus,* oppdaget i 73 av 8123 vedprøver som gir en eteksjonsfrekvens på 0,009 (ca. 1 %). I perioden 2014-2019 har *Bursaphelenchus mucronatus kolymensis* blitt påvist fem av totalt 581 biller, som gir den samme frekvensen som for vedprøver. *B. mucronatus kolymensis og B. macromucronatus* likner på *B. xylophilus* i generell biologi og habitatvalg. Hvis vi antar en hypotetisk frekvens i forekomsten til *B. xylophilus* som er 100 ganger lavere enn for disse naturlig forekommende nematodene, dvs. 0,00009, kan det antall prøver som trengs for en påvisning av *B. xylophilus* med 95 % konfidensintervall estimeres til 30 801. Dette

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indikerer at vi i dag hypotetisk sett har nådd bare 26 % av det antall prøver som trengs for å kunne erklære Norge fri for furuvednematoden *B. xylophilus*.

| | |
|---|---|
| **LAND/COUNTRY:** | Norge |
| **FYLKE/COUNTY:** | Viken |
| **KOMMUNE/MUNICIPALITY:** | Ås |
| **STED/LOKALITET:** | Høgskoleveien 7 |

| **GODKJENT /APPROVED** | **PROSJEKTLEDER /PROJECT LEADER** |
|---|---|
| **HANNE SKOMEDAL** | **CHRISTER MAGNUSSON** |

# Preface

As a consequence of the detection in 1999 of the pine wood nematode (PWN) *Bursaphelenchus xylophilus* in Portugal, the view of Europe as an area free from this quarantine pest changed. This made the European perspective on forest health considerably wider. Since the infestation in Portugal was suspected to date back several years, the Standing Committee on Plant Health of the European Union reached a decision on obligating each member state to conduct a survey of their territories for PWN. This activity was started the year 2000 in Norway. This report present the the results of the monitoring of the Norwegian forests in 2019.

Ås, 23.02.21

Christer Magnusson

# Innhold

# Abstract

The results of the monitoring in Norway of the pinewood nematode *Bursaphelenchus xylophilus* in

2019 is based on 400 wood samples collected from the counties of Akershus (municipalities Nes and Sørum), Buskerud (municipalities Flesberg, Ringerike and Øvre Eiker), Østfold (municipality Aurskog-Høland), Telemark (municipalities Nissedal, Drangedal and Kragerø), Aust-Agder (municipalities Åmli and Evje), Vest-Agder (municipality of Vennesla). All samples were from wood of pine (*Pinus sylvestris*) showing signs of *Monochamus* activity. The dominating material sampled were cut tops of trees and other types of logging waste. In this survey the pinewood nematode (PWN) *Bursaphelenchus xylophilus* was not detected in wood samples. The domestic species *Bursaphelenchus mucronatus kolymensis* was detected on four occasions in one sample of logging waste and in one sample of a topp in Åmli Aust-Agder, as well as in two tops sampled in Vennesla Vest-Agder. In 2019 106 beetles of *Monochamus* were trapped in the counties of Hedmark, Møre and Romsdal and Østfold. None of the beetles carried nematodes. During the monitoring in the 20-year period 2000-2029 8 123 wood samples have been collected in Norwegian forests without detecting the pinewood nematode *B. xylophilus*.

# 1 Background

The detection in May 1999 of the pine wood nematode (PWN), *Bursaphelenchus xylophilus*, in

Portugal (Mota *et al*. 1999) changed the earlier view of Europe as an area free from this forest pest. In the year 2000, in response to the outbreak in Portugal, the Standing Committee on Plant Health (SCPH) of the European Union (EU) obliged each member state to conduct a survey of their territories for PWN. After consultations between the Nordic countries, a draft manual for a Nordic PWN survey was developed (Appendix). Norway has 5.14 mill ha productive forests of conifers, which represents a significant monetary value. Surveillance of Norwegian forests for pine wood nematode has been carried-out every year since 2000 and the results are presented in Magnusson *et al*. (2001, 2002, 2004 a b, 2007, 2009 and Magnusson *et al*. 2013, 2014 unpubl., 2016 unpubl., 2017 unpubl., 2019 unpubl.). The present report gives information on the Norwegian survey results for 2019. The presented data only reports on finds of nematodes belonging to the "*Xylophilus* group" of the genus *Bursaphelenchus*.

# 2 Materials and methods

The work was carried out in accordance with the Nordic Pine Wood Nematode Survey Manual (Appendix). 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.

The logging sites for sampling were identified by assistance of the Department of Geomatikk of the Norwegian Institute of Bioeconomy Research (NIBIO) in Ås. In order to find potential logging sites in pine forest areas two data sources where used; The NIBIO SAT-SKOG map showing satellite-based information on tree species, tree age and tree cover and the satellite-based dataset Global Forest Change (GFC). Using a GIS overlay analysis of SAT-SKOG areas having > 60 % pine and GFC forest cover loss events from 2018 a set of potential logging sites were extracted. In order to reduce the number of sites and errors due to dataset noise all areas < 1 ha where removed. The new logging site dataset where presented on maps combined with road network, topography and orthophotos to allow for an easy access to the selected locations.

A total of 400 samples from wood of *Pinus sylvestris* L. attacked by the cerambycid beetles *Monochamus s*pp. were collected in Akershus (85), Buskerud (119), Østfold (10), Telemark (95), Aust-Agder (70) and Vest-Agder (21) (Fig.1). For biological information the diameters of sampled tops and logging waste were recorded.

Each sample consisted of 300-500 ml of wood shavings, obtained by a portable reversible electric drilling machine fitted with a 17 mm diameter bite. The wood shavings of each sample were put into a plastic bag and transported to the nematode laboratory at NIBIO. The samples were incubated at +25°C in plastic bags for two weeks prior to extraction by Baermann funnels. After 24 hours the water was collected and the nematode suspension was allowed to settle. Nematode suspensions were screened in a Leica M10 stereo microscope. Nematodes for closer examination were killed in hot water, mounted in fixative and examined in a Leica 6000 B differential interference contrast microscope equipped with LAS soft-wear modules. The identification was made according to EPPO (2013).

Traps for *Monochamus* spp. were set up in the county of Hedmark in Elverum, Romedal, Stange and at Geitholmsjøen. In Møre og Romsdal traps were installed at Kvanne. In Østfold traps were located in Fredrikstad and Vestby. Two kinds of Econex traps were used, either the Multi-funnel 12 teflon-coated trap with extended collection cup, or the Cross Van trap with teflon coated funnel with a large collection cup fitted with a bottom net. The traps were supplied with the pheromone pack Galloprotect 2D composed of the two dispensers: Galloprotect F: an aggregation pheromone (2-undecyloxy-1ethanol) and Galloprotect A: kairomonal (ipsenol and 2-methyl-3-buten-1-ol). Trapped insects were brought to the laboratory in boxes with fresh branch segments and shoots of *P. sylvestris* as feed, together with pieces of torn egg packages for shelter and protection against cannibalism. In the laboratory each specimen was given a code. The beetles were decapitated, cut into pieces and extracted by a modified Baermann funnel for 24 hours. Identification was made by morphology according to Wallin *et al.* (2013). The extracts from the beetles were examined in a Leica M10 stereo microscope for the presence of nematodes.

# 3 Results

## 3.1 Source of samples

The sampling volumes and localities in 2019 are shown in Table 1 and Figure 1. Most samples (119) were from Buskerud, followed by Telemark (95), Akershus (85), Aust-Agder (70), Vest-Agder (21) and Østfold (10). During the survey 21 logging sites in 12 municipalities were visited (Tab. 1).

In Akershus 62 samples were collected in the municipality of Sørum and 23 in Nes. In Buskerud 58 samples came from the municipality of Ringerike, 50 samples from Flesberg and 11 samples from Øvre Eiker. In Østfold 10 samples were taken from Aurskog-Høland. In Telemark 54 samples were from Drangedal, 40 from Nissedal and one sample from Kragerø. In Aust-Agder 40 samples came from Evje and 30 samples from Åmli. In Vest-Agder 21 samples were collected in Vennesla (Tab. 1).

Objects sampled

The kind of wood objects sampled are shown in Table 2. Two-hundred and twelve samples were taken from tops, 126 samples from other logging waste, 57 from cut bolts and wind thrown trees, cut tree tops, followed by 126 samples from logging waste, 57 samples from cut bolts and 7 samples from wind-thrown trees.

## 3.2 Occurrence of nematodes in wood samples

The pine wood nematode *B. xylophilus* was not detected, while the domestic nematode *B. mucronatus kolymensis* was recorded in 4 samples (Tab. 3). This species was detected in one sample from logging waste (diameter 7 cm) and in one sample from a cut top (diameter 10 cm), both samples from the logging site 929-1 in Åmli Aust-Agder. This nematode was also detected at the location of Ratnedalen in the municipality of Vennesla in Vest-Agder. Both finding were from cut tops of diameter 10 and 12 cm at the logging site 1014-8.

Nematodes in the insect vectors *Monochamus* spp.

In this survey no nematodes were detected in 20 *Monochamus* beetles from Østfold, 35 from Møre og Romsdal, and 51 from Hedmark.

Monitoring activities in Norway 2000-2018

By now 8 123 wood samples have been analysed in the period 2000 - 2019. The annual number of samples varies between years (Fig. 2). More than 500 samples were collected in 2001, 2002, 2003, 2004, 2010, 2012, 2013 and in 2017/2018. In 13 years sampling volumes of 400 samples or more have been collected and analysed. Regarding sample volumes in counties (Fig. 3) most samples have been taken in Østfold (2 098), Hedmark (1 960), Telemark (1 017), Buskerud (938) and Aust-Agder (942).

# 4 Discussion

The survey in 2019 is rather normal with regard to the number of wood samples taken. This year the counties Buskerud and Telemark have been more in focus for the sampling. For the period 2000 – 2019 Hedmark, Østfold, Agder and Telemark are the four most sampled counties in Norway, with total sample numbers of more than 1 000 samples each. Unlike the sampling of 2017-2018 the focus this year has been more on cut tops. In 2019 four detections were made of *B. mucronatus kolymensis* in 400 samples, which is a frequency of 1 %. All detections were from the region of Agder, and this region together with Hedmark and Østfold are the areas with most detections of the domestic species in the "*Xylophilus*-group" in the genus *Bursaphelenchus* (*i.e. B. mucronatus kolymensis* and *B. macromucronatus*).

This year no nematodes were detected in the 106 *Monochamus* specimens analysed. In the period 2014-2019 a total number of 581 beetles have been analysed and nematodes recorded in 5 specimens corresponding to a frequency of 0,009, which is similar to that recorded in wood samples.

The surveillance program for PWN in Norway has been carried out in accordance with the Nordic Pine Wood Nematode Survey Manual (Appendix). In this manual the number of samples (n) is calculated from the assumed probability for a find (p) and the required statistical confidence level (ε) according to the equation: $n = \ln \varepsilon / \ln (1-p)$. In the monitoring of Norwegian forests from 2000 to 2019 (Magnusson *et al.* 2001, 2002, 2004 ab, 2007, 2009, 2013, 2016, 2019 and this report) *Bursaphelenchus mucronatus kolymensis* + *B. macromucronatus,* which both resemble PWN in biology and habitat selection, have been found in 73 of 8 123 wood samples, which gives a detection frequency of 0,009. Due to the relatively high total number of samples we regard this frequency as a good estimate of the frequency of habitats potentially suitable for PWN *B. xylophilus* in Norwegian forests. If we then assume a hypothetical frequency of occurrence of PWN to be 100 times lower, *i.e.* 0,00009 the number of samples necessary for one positive find of *B. xylophilus* can according to our equation be calculated to 30 801. It seems we so far have reached only 26 % of the required volume of samples.

**Table 1. Monitoring of pinewood nematode (PWN) *Bursaphelenchus xylophilus* at logging sites in Norway 2019.**

| COUNTY | MUNICIPALLITY | LOCATION | NOS SAMPLES | LOGGING SITE |
|---|---|---|---|---|
| **Akershus (85)** | Nes (23) | Dragsjøhytta | 23 | 0236-2 |
| | Sørum (62) | | 62 | 0226-1 |
| **Buskerud (119)** | Flesberg (50) | Lauvhaugen | 18 | 0631-1 |
| | | Huslendsetra | 19 | 0631-3 |
| | | | 13 | 0631-6 |
| | Ringerike (58) | Fjellveggtjern | 11 | 0605-2 |
| | | Mastedalen | 36 | 0605-4 |
| | | | 11 | 0605-5 |
| | Øvre Eiker (11) | | 5 | 0624-2 |
| | | Østermoen | 6 | 0624-6 |
| **Østfold (10)** | Aurskog-Høland (10) | | 5 | 0221-4 |
| | | | 5 | 0224-1 |
| **Telemark (95)** | Nissedal (40) | Ånundsbubukta | 40 | 0830-6 |
| | Drangedal (54) | Bekksta | 31 | 0817-2 |
| | | Øvre Garevann | 10 | 0817-3 |
| | | Nordumheia | 13 | 0817-4 |
| | Kragerø (1) | Solli | 1 | 0815-1 |
| **Aust-Agder (70)** | Åmli (30) | | 30 | 0929-1 |
| | Evje (40) | Tolleivsdal | 10 | 0937-2 |
| | | Peekleiv | 30 | 0937-3 |
| **Vest-Agder (21)** | Vennesla (21) | Ratnedalen | 21 | 1014-8 |
| **Total : 400** | | | 400 | |

**Table 2. Monitoring of pinewood nematode (PWN) *Bursaphelenchus xylophilus* in Norway 2019. Sampled material from pine wood (*Pinus sylvestris*). BMK = *Bursaphelenchus mucronatus kolymensis*; BXY = *Bursaphelenchus xylophilus***

| COUNTY | MUNICIPALITY | OBJECTS AND NUMBER OF SAMPLES | | | | SUM | BMK | BXY |
|---|---|---|---|---|---|---|---|---|
| | | Wind fall | Tops | Logging waste | Bolts | | | |
| **Akershus (85)** | Nes (23) | | 13 | 10 | | 23 | 0 | 0 |
| | Sørum (62) | | 39 | 4 | 21 | 62 | 0 | 0 |
| **Buskerud (119)** | Flesberg (50) | | 23 | 23 | 4 | 50 | 0 | 0 |
| | Ringerike (58) | | 37 | 12 | 9 | 58 | 0 | 0 |
| | Øvre Eiker (11) | | 5 | 6 | | 11 | 0 | 0 |
| **Østfold (10)** | Aurskog-Høland (10) | | 9 | 1 | | 10 | 0 | 0 |
| **Telemark (95)** | Nissedal (40) | 2 | 29 | | 9 | 40 | 0 | 0 |
| | Drangedal (54) | 2 | 16 | 33 | 3 | 54 | 0 | 0 |
| | Kragerø (1) | 1 | | | | 1 | 0 | 0 |
| **Aust-Agder (70)** | Åmli (30) | | 8 | 17 | 5 | 30 | 2 | 0 |
| | Evje (40) | | 25 | 10 | 5 | 40 | 0 | 0 |
| **Vest-Agder (21)** | Vennesla (21) | 2 | 8 | 10 | 1 | 21 | 2 | 0 |
| **TOTAL 400** | | 7 | 212 | 126 | 57 | 400 | 4 | 0 |

**Table 3. Monitoring of pinewood nematode (PWN) *Bursaphelenchus xylophilus* in Norway 2019. Detection of *Bursaphelenchus mucronatus kolymensis* in wood material of *Pinus sylvestris*.**

| COUNTY | MUNICIPALITY | LOCALITY | MATERIAL | NUMBER OF DETECTIONS | LOGGING SITE |
|---|---|---|---|---|---|
| **Aust-Agder** | Åmli | Not specified | Logging waste Ø = 7 cm | 1 | 929-1 |
| | | Not specified | Topp Ø = 10 cm | 1 | 929-1 |
| **Vest-Agder** | Vennesla | Ratnedalen | Topp Ø = 12 cm | 1 | 1014-8 |
| | | Ratnedalen | Topp Ø = 10 cm | 1 | 1014-8 |
| **TOTAL DETECTIONS** | | | | 4 | 2 |

**Table 4. Monitoring pinewood nematode (PWN) *Bursaphelenchus xylophilus* in *Monochamus* beetles in Norway 2019 using pheromone traps.**

| COUNTY | LOCALITY | NOS. *MONOCHAMUS* | NOS. NEMATODES |
|---|---|---|---|
| **Østfold (20)** | Fredrikstad | 18 | 0 |
| | Vestby | 2 | 0 |
| **Møre og Romsdal (35)** | Kvanne | 35 | 0 |
| **Hedmark (51)** | Elverum | 1 | 0 |
| | Romedal | 26 | 0 |
| | Stange | 8 | 0 |
| | Geitholmsjøen | 15 | 0 |
| **TOTAL** | 7 | 106 | 0 |

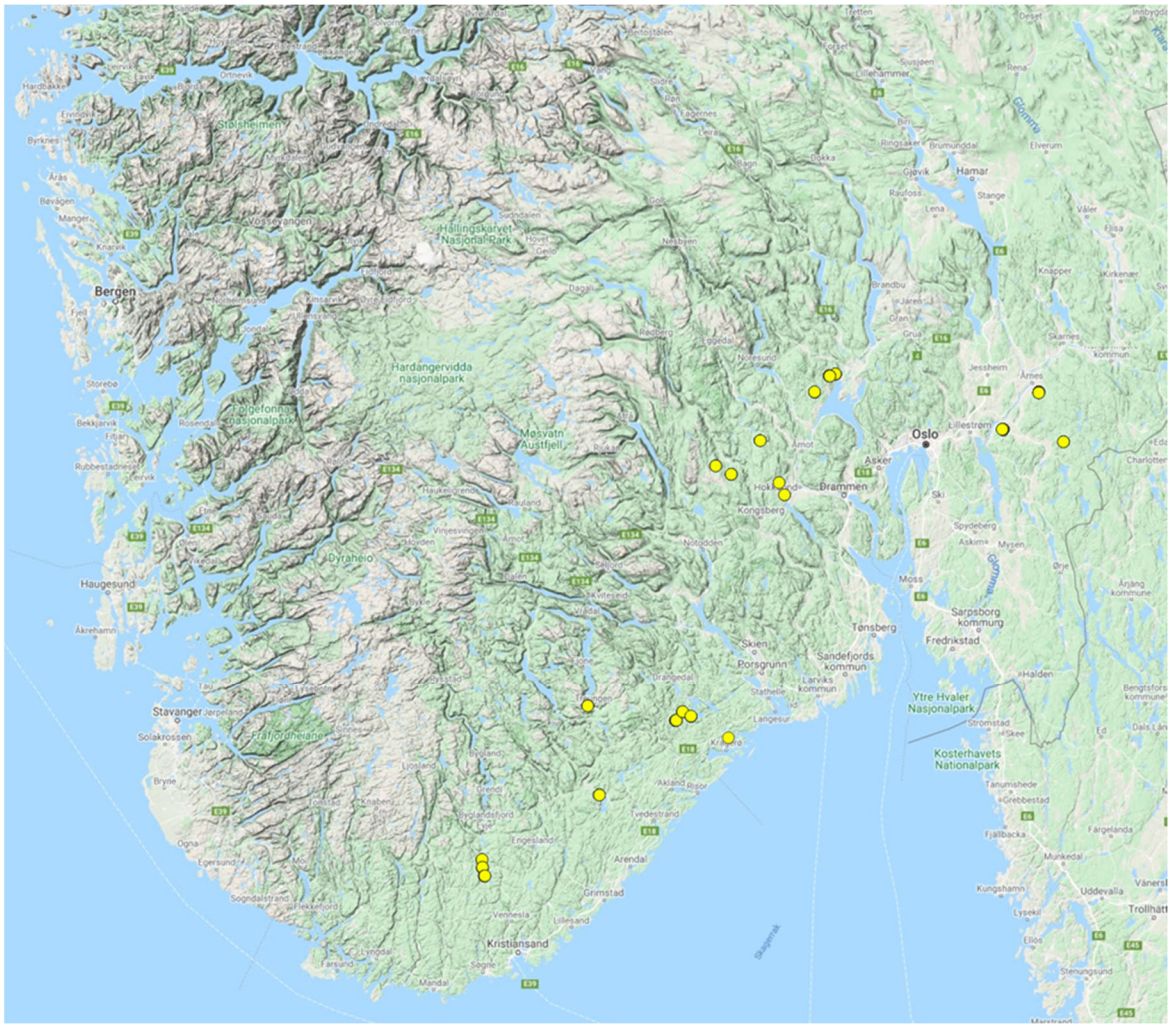


**Figure 1. Monitoring pinewood nematode (PWN) *Bursaphelenchus xylophilus* in Norway 2019. The positions of the 400 samples are marked by yellow points.**

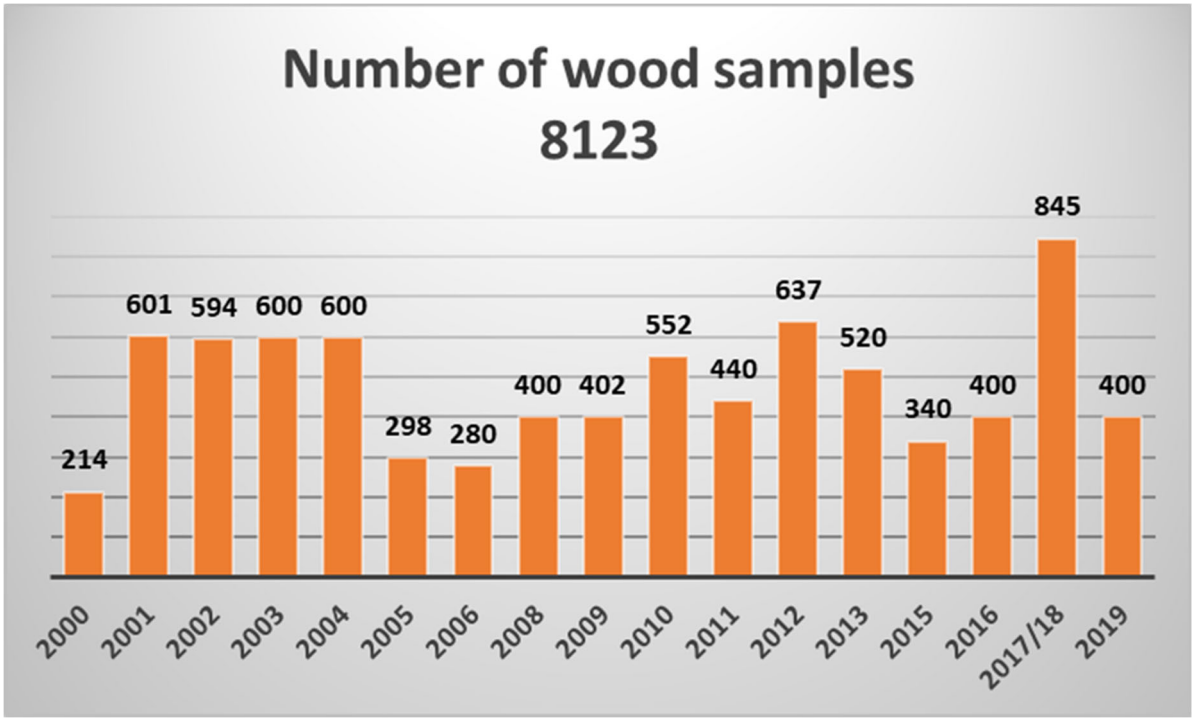


Figure 2. Monitoring pinewood nematode (PWN) *Bursaphelenchus xylophilus* in Norway 2000 - 2019. Annual volumes of samples analysed.

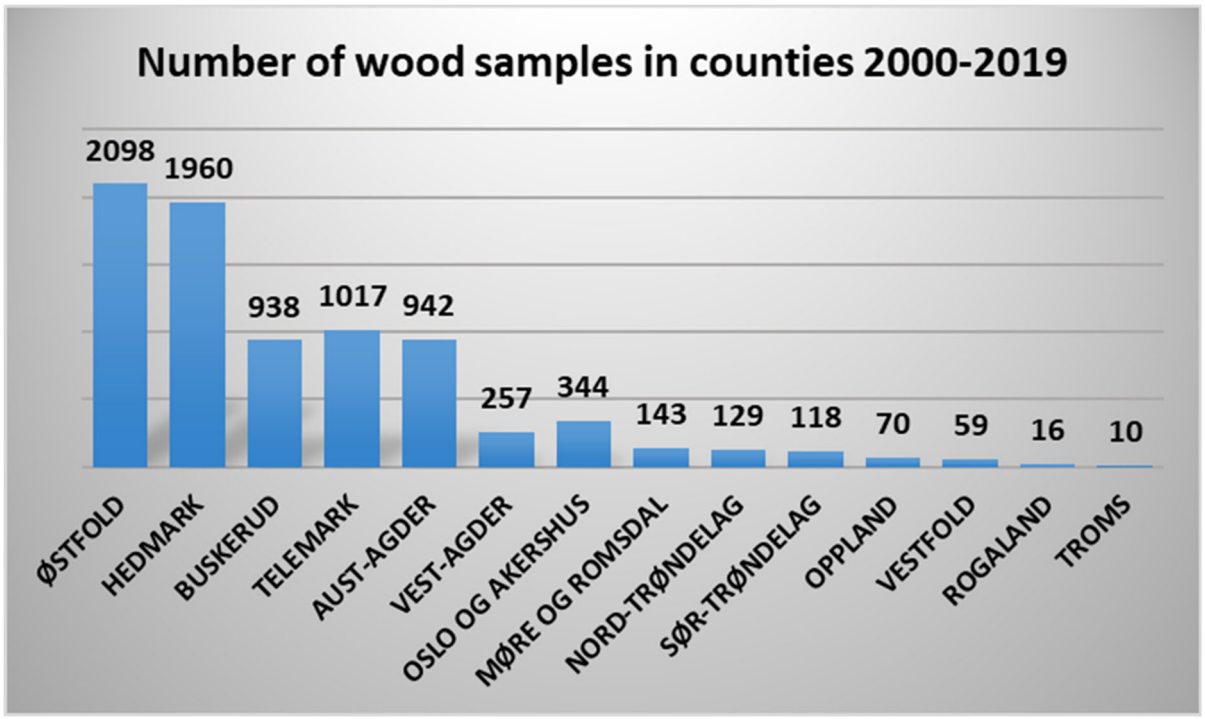


Figure 3. Monitoring pinewood nematode (PWN) *Bursaphelenchus xylophilus* in Norway 2000 - 2019. Total numbers of samples analysed in counties.

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# Appendix

# Nordic Pine Wood Nematode Survey Manual

*2000-03-20*

Christer MAGNUSSON (1), Martin SCHROEDER (2) and Jyrki TOMMINEN (3)
(1)*The Norwegian Crop Research Institute, Høgskoleveien 7, NO-1432 Aas, Norway*
(2) *Swedish University of Agricultural Sciences, Department of Entomology, Box 7044, SE-75007 Uppsala, Sweden*
(3) *KTTK-Plant Protection Service, Box 42, FIN-00501 Helsinki, Finland*

## 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:

   1.1 Forests in general.

   1.2 Clear cuts burnt for nature conservation

   1.3 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 centers 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 ε = 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 +25oC 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.

Progression of survey

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

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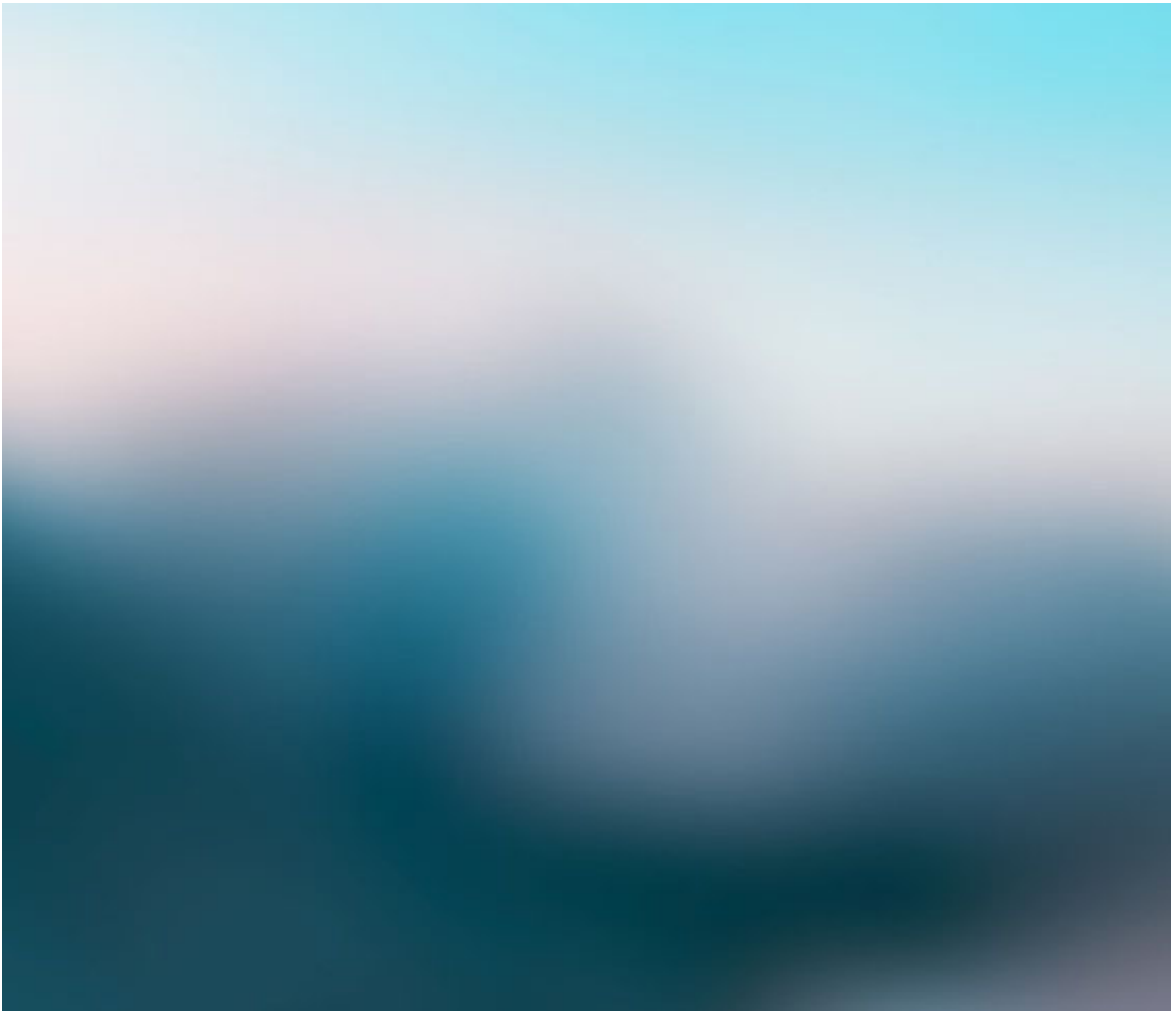
NIBIO
NORSK INSTITUTT FOR BIOØKONOMI

Norsk institutt for bioøkonomi (NIBIO) ble opprettet 1. juli 2015 som en fusjon av Bioforsk, Norsk institutt for landbruksøkonomisk forskning (NILF) og Norsk institutt for skog og landskap.

Bioøkonomi baserer seg på utnyttelse og forvaltning av biologiske ressurser fra jord og hav, fremfor en fossil økonomi som er basert på kull, olje og gass. NIBIO skal være nasjonalt ledende for utvikling av kunnskap om bioøkonomi.

Gjennom forskning og kunnskapsproduksjon skal instituttet bidra til matsikkerhet, bærekraftig ressursforvaltning, innovasjon og verdiskaping innenfor verdikjedene for mat, skog og andre biobaserte næringer. Instituttet skal levere forskning, forvaltningsstøtte og kunnskap til anvendelse i nasjonal beredskap, forvaltning, næringsliv og samfunnet for øvrig.

NIBIO er eid av Landbruks- og matdepartementet som et forvaltningsorgan med særskilte fullmakter og eget styre. Hovedkontoret er på Ås. Instituttet har flere regionale enheter og et avdelingskontor i Oslo.



Forsidefoto: Christer Magnusson (NIBIO) Infective stages of *Bursaphelenchus mucronatus* from *Monochamus sutor*.

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