View metadata, citation and similar papers at core.ac.uk
brought to you by CORE
provided by NIBIO Brage

Plante forsk
Grønn kunnskap@
Vol.8 Nr.104 – 2004

# Survey of the pine wood nematode (PWN) *Bursaphelenchus xylophilus* in Norway 2002

**Magnusson, C.**[(1)], **H. Overgaard**[(2)], **H. Nyeggen**[(2)],**K. Thunes**[(2)], **S. Haukeland Salinas**[(1)] **& B. Hammeraas**[(1)]
(1)The Norwegian Crop Research Institute, Plant Protection Centre (2) Norwegian Forest Research Institute, Division of Ecology
*E-mail: plantevernet@planteforsk.no*

Summary
In this survey of 2002, 600 samples were collected from 83 forest blocks in the counties Akershus, Buskerud, Oppland and Østfold. The sampling activity involved 16 municipallities situated mainly within the three zone sites A, B, and C. Samples from Scots pine (*Pinus sylvestris*) formed 89%, while samples of Norway spruce (*Picea abies*) made up 10% of the total sample volume. Timber and forest debris were the most common objects sampled. Sixty-five percent of the pine samples and 81% of the spruce samples showed signs of *Monochamus* activity. Nematodes were common and occurred in 94% of the samples analysed. Thirteen samples of pinewood were positive for the genus *Bursaphelenchus*. *Bursaphelenchus mucronatus* was recorded for the third time in Norway, and was detected in forest debris attacked by *Monochamus* at Bjørdalen in the municipality of Eidsberg in the county of Østfold. The pine wood nematode *Bursaphelenchus xylophilus* was not detected in this survey.

## 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 2002.

## 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 three circular areas, 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 A was centred in Tofte, the location of a major pulp mill, and zone site B was centred in a major timber yard facility in Drammen. Zone site A and B were established in 2000, while the third zone site (C) was established in 2001 and centred in Greåker - a major timber yard facility situated close to Sarpsborg (Fig 2-4).

In the survey of 2002, 600 samples of wood were taken from 83 forest blocks. The sampling in forest blocks was focused on Scots pine (*Pinus sylvestris*) with a volume of 532 samples, while Norway spruce (*Picea abies*) was represented by 62 samples. Six samples were not identified to tree species. 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 12 VE-2 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 Garmin MapSource WorldMap version 3.00 or 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 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 for three minutes and fixed in TAF. Samples were screened in a Leica M10 stereomicroscope, and aphelenchid nematodes were mounted on objective slides for closer examination in a Leitz DMRB interference microscope fitted with the Leica Advanced Quips image processing and analysis system.

## RESULTS

### Sampling activity

In the period 14.08.2002 – 08.11.2002 the first 391 samples were collected. At this time the sampling activity was stopped by heavy snowfall. The fieldwork was resumed 06.05.2003 and finished 06.06.2003.

### Sampled areas and localities

The survey activity of 2002 mainly concerned forests located within the zone sites A, B and C (Fig.1). In 2002, the sampling (Fig. 2) was extended mainly to the west and to the north of the areas covered by the surveys of 2000 and 2001 (Fig. 3). The majority of municipalities visited this year had not been investigated earlier. In the municipalities of Marker and Eidsberg the sampling involved new forest blocks. In the former, as well as in Rømskog and Aurskog-Høland the sampling was extended beyond the areas of the zone sites (Fig. 2). The distribution of the sampling locations for the period 2000-2003 is shown in figure 4.

In the survey, 600 samples were collected from 83 forest blocks. In the county of Buskerud samples were collected from 46 forest blocks. In Akershus, Østfold and Oppland 16, 15 and 6 forest blocks were sampled (Tab. 1). Most samples were taken in the county of Østfold (289) followed by Buskerud (161), Akershus (80) and Oppland (70) (Tab.1), and corresponds to 48, 27, 13 and 12% of the total sample volume (Fig. 5). The survey activities involved forest areas of 16 municipalities (Tab.1 and Fig.6). Most samples were collected in the eastern municipalities of Marker and Eidsberg, with sample volumes of 134 and 116 respectively.

### Kind of material sampled

Samples from Scots pine (*Pinus sylvestris*) formed the major part, i.e. 532 samples corresponding to (89%) of the total sample volume, while the 62 samples from Norway spruce (*Picea abies*) represented 10%. A small fraction, 6 samples (1%) was collected from wood not determined to species (Tab. 1). With regard to the kind of objects sampled (Fig. 7) "timber and debris" formed the largest fraction of both pine (69%) and spurce (75%). The next object by volume was lying (often wind thrown) trees, which formed 21% of the pine samples (Fig. 7A) and 18% of the spruce samples (Fig. 7B). A small part of the sampled material came from standing dead or dying trees, high and low stumps, and from piles of timber (Fig. 7). In the pine material 348 samples (65%) was taken from wood showing clear *Monochamus* activity (Tab. 2), and for spruce wood 50 samples (81%) originated from trees attacked by *Monochamus* (Tab. 3).

### Occurrence of nematodes

For the total material of 532 samples of pine (Tab. 2), nematodes were recorded in 94%. The microbivorous nematodes were the most common group and occurred in a frequency of 87%. Plant and insect nematodes were recorded in 52% of the material. Nematodes belonging to the order Aphelenchida and the subfamily Tylenchinae were registred in frequencies of 33 and 23%. In the former group the genus *Aphelenchoides* (fungal feeders) was the most frequent with a relative frequency of 28 %. Insect associated nematodes, most probably belonging to the genera *Ektaphelenchoides* and *Cryptaphelenchoides* occurred in small frequencies, as did predatory nematodes in the genus *Seinura*. The genus *Bursaphelenchus* was recovered in 13 samples, equal to 2,4% of the total number of pinewood samples. One sample from forest debris at Bjørdalen in the municipality of Eidsberg contained a population of *Bursaphelenchus mucronatus*. This corresponds to a relative frequency of 0,2%. The pine wood nematode, *B. xylophilus*, was not detected. The family Neotylenchidae and

the genus *Ditylenchus* also belong to the group of plant and insect nematodes, and were recorded in 1,5 and 0,4 % of the pinewood samples (Tab. 2).

In the 62 samples of sprucewood analysed (Tab.3), nematodes were present in 94% of the samples. As with pine samples, microbivorous nematodes were dominating at a frequency of 84%. The group of plant and insect nematodes occurred in 40% of the material with the subfamily Tylenchinae and the order Aphelenchida recorded present in 21 and 24% of the samples. *Aphelenchoides* was the dominating genus and occurred in 21% of the samples. The genus *Ektaphelenchoides* was observed in a low frequency, while the genera *Cryptaphelenchoides* and *Seinura* were absent. Also the genus *Bursaphelenchus* was absent in the samples from spruce. This is also true of the family Neotylenchidae and the genus *Ditylenchus*.

In comparing samples from pinewood showing clear signs of *Monochamus* activity, and samples from wood without clear symptoms (Tab. 2), few differences could be detected in the occurrence of nematode groups, genera and species. The genus *Aphelenchoides* seems, however, to be more common in wood with symptoms of *Monochamus* (29%) than in wood without symptoms (19%). The only clear difference is the presence of *B.mucronatus* only in wood attacked by *Monochamus*.

For spruce (Tab. 3), microbivorous nematodes seemed to be less frequent (78%) in samples taken from wood attacked by *Monochamus* than in samples without symptoms of *Monochamus* activity (92%). The same was true for the subfamily Tylenchinae.. The genus *Aphelenchoides* was only detected in wood showing signs of *Monochamus* activity.

In the case of pine samples (Tab.2), there are no differences in frequencies of most nematode groups and genera between the major categories of "lying trees" and "timber and debris". Microbivorous nematodes and the genus *Aphelenchoides* seem to be less frequent in standing trees, while the opposite is true for nematodes in the family Neotylenchidae, all in comparison with the former major categories of objects. All samples from timber piles contained nematodes, with plant and insect nematodes being less frequent (33%) than microbivorous nematodes (96%). The genus *Bursaphelenchus* was detected in the categories "lying trees", "timber and debris" and "timber piles". Nematodes recorded in this genus included members of the "*sexdentati*-" and "*borealis*- groups". The only representative of the "*xylophilus*-group" was *B. mucronatus*, which was noted in one sample of forest debris attacked by *Monochamus*. This single find corresponds to a frequency of 0,3% of pine samples.

In spruce (Tab. 3) the material is smaller, so statements on patterns of nematode occurrence become more uncertain. The material suggests nematodes as a group to be more common in category "timber and debris" (87%) than in "lying trees" (16%). This also is true of the subgroups microbivorous nematodes and plant and insect nematodes, as well as members of the subfamily Tylenchinae, the order Aphelenchida and the genus *Aphelenchoides*. With regard to timber and debris, all categories of nematodes were more commonly recorded in samples from pine than from spruce.

## DISCUSSION

The sampling program was complementary with regard to the areas investigated in 2000 and 2001 and completes the general survey of the eastern territory "Østlandet", and when the three-year sampling activity is considered there is a good cover of the area.

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.

In comparison with the surveys of 2000 and 2001 the proportion of samples that were attacked by *Monochamus* was higher regardless of tree species. In 2001 43% of the pinewood samples were reported attacked by "wood boring insects" (Magnusson *et al*. 2002), compared to 65% of *Monochamus* attacks in the present material. For spruce the frequency of insect attacks was 72% in 2001 and 81% for *Monochamus* specifically in the present survey.

The nematodes were broadly classified into microbivorous (bactetrial feeders) and into a group designated "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 the primary ambition was to recognize the genus *Bursaphelenchus* and to identify the two species *B. mucronatus* and *B. xylophilus*.

As shown in previous work (Magnusson et al. 2001, 2002) 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. Pine wood seems to be a more suitable habitat for many nematodes than spruce wood. As all categories of nematodes reached higher relative frequencies in timber and debris of pine compared to spruce indicates that pine may offer a better habitat for wood nematodes than spruce.

In this survey the genus *Bursaphelenchus* was only detected in samples of pinewood. Thirteen samples were positive for the genus *Bursaphelenchus.* This corresponds to 2% of the samples, which is slightly more than reported earlier (Magnusson *et al*. 2001, 2002). We were successful in detecting *B. mucronatus* in forest debris attacked by *Monochamus* sp. at Bjørdalen in the municipality of Eidsberg. This is the third record of *B. mucronatus* in Norway. Earlier this species was detected in Hanestad in the province of Hedmark (McNamara & Støen 1988) and at Ombudstvedt in the municipality of Våler, Østfold county (Magnusson *et al*. 2002).

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 indicate that *B. mucronatus* may have a very restricted occurrence in eastern Norway.

The planned sampling volume for 2003 is 600 samples and will focus on areas situated in the southern region of Norway.

## CONCLUSIONS

- In 2002, 600 samples were collected from 83 forest blocks situated mainly within the three zone sites A, B, and C. Most samples (289) were collected in the county of Østfold in south-eastern Norway.
- Samples from Scots pine (*Pinus sylvestris*) formed 89% of the collected material. Timber and forest debris were the most common objects sampled. Sixty-five percent of the pine samples and 81% of the spruce samples showed signs of *Monochamus* activity.
- Nematodes were common and occurred in 94% of the samples analysed. Thirteen samples of pinewood were positive for the genus *Bursaphelenchus*. Eight of these samples were collected from timber and forest debris, three samples from lying pine trees and two samples from timber piles.
- *Bursaphelenchus mucronatus* was recorded for the third time in Norway, and was detected in forest debris attacked by *Monochamus* at Bjørdalen in the municipality of Eidsberg in the county of Østfold.
- The pine wood nematode *Bursaphelenchus xylophilus* was not detected in this survey.

## ACKNOWLEDGEMENTS

The Norwegian Agricultural Inspection Service funded this survey, and this support is greatly

acknowledged. We are thankful to the staff of The Norwegian Forest Research Institute, Aas for assistance in the fieldwork. The laboratory technicians, Irene Rasmussen and Kari Ann Strandenæs, at the Nematode Laboratory of The Norwegian Crop Research Institute are greatly acknowledged for their efficient handling of samples and extractions.

## REFERENCES

**ANONYMOUS (2000)**. E. C. Pinewood Nematode Survey Protocol 2000. European Commission, Directorate-General Health and Consumer Protection, Directorate E- Public, animal and plant health. Unit E1. Legislation relating to crop products and animal nutrition. *SANCO E/1D(00)*: 7 pp.

**EVANS, H.F., McNAMARA, D.G., BRAASCH, H., CHADOEUF, J. & MAGNUSSON, C. (1996)**. Pest Risk Analysis (PRA) for the territories of the European Union (as PRA area) on Bursaphelenchus xylophilus and its vectors in the genus Monochamus. *EPPO Bulletin*26: 199-249.

**MAGNUSSON, C. & SCHROEDER, L.M. (1989)**. First record of a Bursaphelenchus species (Nematoda) in Monochamus beetles in Scandinavia. *Anzeiger für. Schädlingskunde, Pflanzenschutz und Umweltschutz* 62: 53-54.

**MAGNUSSON, C., THUNES, K., HAUKELAND SALINAS, S, & ØKLAND, B. (2001)**. Survey of the pine wood nematode B*ursaphelenchus xylophilus* in Norway 2000. *Planteforsk Rapport* 07/2001. 20pp. + iv.

**MAGNUSSON, C., THUNES, K., HAUKELAND SALINAS, S, & HAMMERAAS, B. (2002)**. Survey of the pine wood nematode *Bursaphelenchus xylophilus* in Norway 2001. *Planteforsk Rapport* 26/2002. 14pp. + iv.

**McNAMARA, D.G. & STOEN, M. (1988)**. A survey for Bursaphelenchus spp. in pine forests in Norway. *EPPO Bulletin* 18: 353-363.

**MOTA, M. M., BRAASCH, H., BRAVO, M. A., PENAS, A. C., BURGERMEISTER, W., METGE, K. & SOUSA, E. (1999)**. First report of Bursaphelenchus xylophilus in Portugal and Europe. *Nematology* 1: 727-734.

**SCHROEDER, M. & MAGNUSSON, C. (1989)**. Tallvednematoden – ett hot mot svensk skog? *Skogsfakta, Biologi och skogs-skötse* 64. SLU Uppsala, Sweden: 4 pp.

**SCHROEDER, L.M. & MAGNUSSON, C. (1992)**. Transmission of Bursaphelenchus mucronatus (Nematoda) to branches and bolts of Pinus sylvestris and Picea abies by the cerambycid beetle Monochamus sutor. *Scand. J. For. Res*. 7: 107-112.

**TOMMINEN, J. (1990)**. Presence of Bursaphelenchus mucronatus (Nematoda: Aphelenchoididae) fourth dispersal stages in selected conifer beetles in Finland. *Silva Fennica* 24: 273-278.

**TOMMINEN, J., NUORTEVA, M., PULKKINEN, M. & VÄKEVÄ, J. (1989)**. Occurrence of the nematode Bursaphelenchus mucronatus Mamiya & Enda 1979 (Nematoda: Aphelenchoididae) in Finland. *Silva Fennica* 23: 271-277.

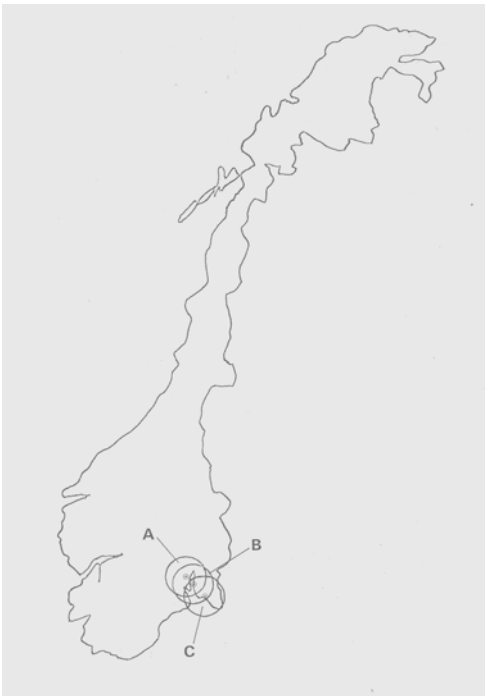


**Figure 1.**
Survey of the pine wood nematode (PWN), *Bursaphelenchus xylophilus*, in Norway 2002. Position of the three zone sites A, B and C.

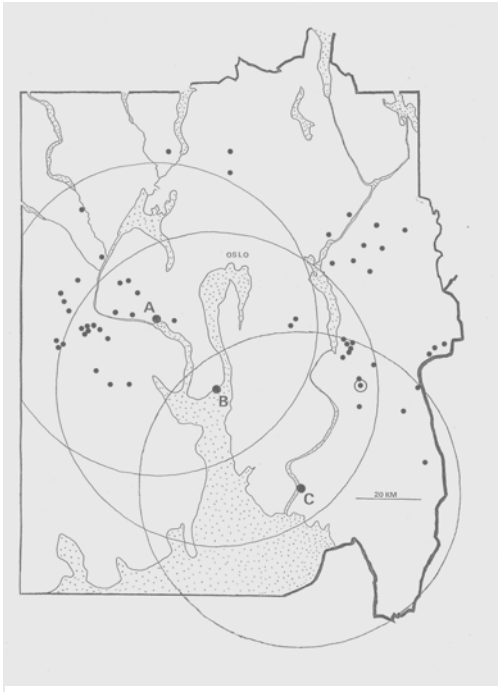


**Figure 2.**
Survey of the pine wood nematode (PWN), *Bursaphelenchus xylophilus*, in Norway 2002. Location of sampling areas. Find of *B. mucronatus* encircled.

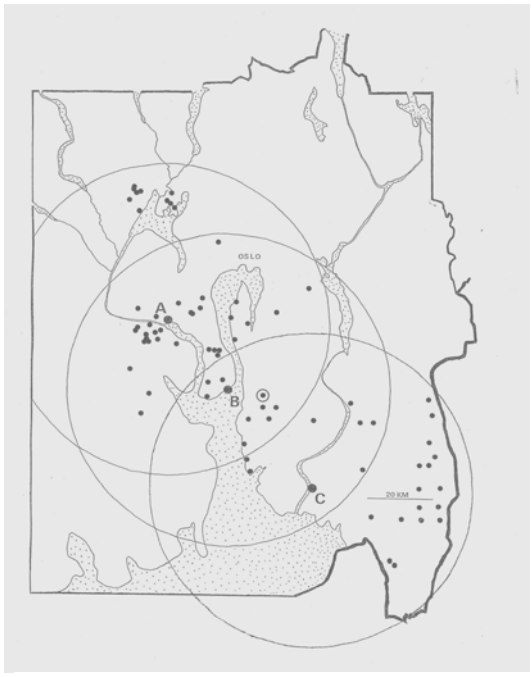


**Figure 3.**
Survey of the pine wood nematode (PWN), *Bursaphelenchus xylophilus,* in Norway 2000-2001. Location of sampling areas. Find of *B. mucronatus* encircled.

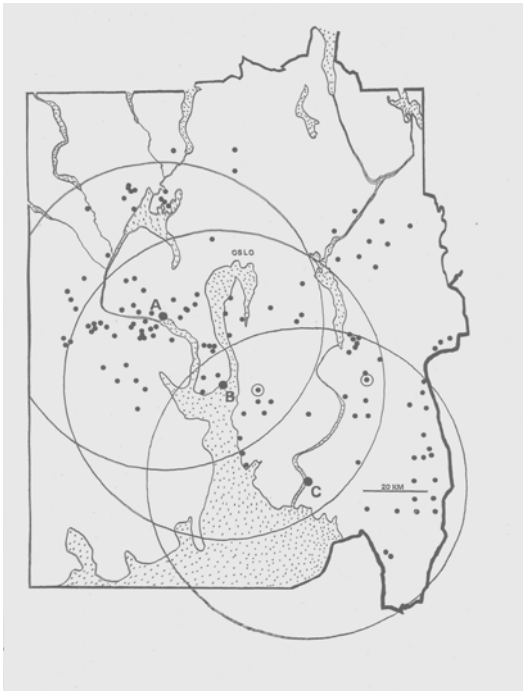


**Figure 4.**
Survey of the pine wood nematode (PWN), *Bursaphelenchus xylophilus,* in Norway 2000-2002. Location of sampling areas. Finds of *B. mucronatus* encircled.

**Table 1.** Number, locality and kind of forest wood samples for the pine wood nematode (PWN), *Bursaphelenchus xylophilus,* in Norway 2002. s = standing trees; l = lying trees; td = timber and debris; tp = timber piles; hs = stumps/high stumps;

| COUNTY | MUNICIPALLITY | LOCATION | PINE Pinus sylvestris | | | | | SPRUCE Picea abies | | | | | UNID. | TOTAL |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | s | l | td | tp | hs | s | l | td | tp | hs | td | |
| Akershus (80) | Aurskog-Høland (40) | Bjørnemyrhøgda | | 8 | 16 | | | | | | | | | 24 |
| | | Bogerud | | 1 | | | | | | | | | | 1 |
| | | Fløyta | | 1 | | 2 | | | | | | | | 3 |
| | | Grøtli | | 2 | | | | | | | | | | 2 |
| | | Gyrileet | | | 1 | | | | | | | | | 1 |
| | | Morttjernmyra | | 3 | | | | | | 2 | | | | 5 |
| | | Skogen | | 1 | | | | | | | | | | 1 |
| | | Strandsmosen | | 1 | | | | | | | | | | 1 |
| | | Tyrihjell | | | 2 | | | | | | | | | 2 |
| | Enebakk (9) | Durud | | | 1 | | | | | | | | | 1 |
| | | Høyde 227 | | | 8 | | | | | | | | | 8 |
| | Fet (10) | Hvaltjern | | 2 | 7 | | | | | | | | | 9 |
| | | Tunnerud | | | 1 | | | | | | | | | 1 |
| | Sørum (21) | Engeråsen | | | 10 | | | | | 1 | | | | 11 |
| | | Jultonåsen | | | 2 | | | | | | | | | 2 |
| | | Skytebane | | 5 | 3 | | | | | | | | | 8 |
| Buskerud (161) | Øvre Eiker (33) | Brennåstjern | | 1 | 2 | | | | | | | | | 3 |
| | | Himsjø | | | 1 | 1 | | | | | | | | 2 |
| | | Lunde | | 1 | | | | | | | | | | 1 |
| | | Løkka | | 4 | 1 | | | | | | | | | 5 |
| | | Løkka felt 2 | | | | | | | | | 1 | | | 1 |
| | | Myrvang | | | | 3 | | | | | | | | 3 |
| | | Nedre Lurdalen | | 1 | | | | | | | | | | 1 |
| | | Rørås | | 1 | | | | | | | | | | 1 |
| | | Rørås felt 2 | | 1 | | | 1 | | | | | | | 2 |
| | | Skomakerplassen | | | 1 | | | | 1 | | | | | 2 |
| | | Tørbekk | | 4 | | | | | | | | | | 4 |
| | | Ulleråsen | | | 2 | | | | | | | | | 2 |
| | | Yttervatnet | | | 6 | | | | | | | | | 6 |
| | Nedre Eiker (12) | Borgetjern | | 1 | | | | | | | | | | 1 |
| | | Kobberdokkfjellet | | | 4 | | | | | | | | | 4 |
| | | Tjurueldsåsen | | 1 | 2 | | | | | | | | | 3 |
| | | Ulevann | | | 4 | | | | | | | | | 4 |
| | Kongsberg (50) | Barmsskarvane | | 4 | 7 | | | | | | | | | 11 |
| | | Brånåbekken | | 1 | 1 | | | | | | | | | 2 |
| | | Godokken | | 1 | | | | | | | | | | 1 |
| | | Jondalen | 4 | 1 | | | | 1 | | | | | 2 | 8 |
| | | Meheia | | 1 | 9 | | 1 | | | | | | | 11 |
| | | Pikerfoss | 2 | | | | | | | | | | | 2 |
| | | RV37 200m N | 3 | 2 | | | | | | | | | 1 | 6 |
| | | Storemyr | | 1 | | | | | | | | | | 1 |
| | | Vettestadmoen | | | 8 | | | | | | | | | 8 |
| | Flesberg (44) | Belgen | | | 4 | | | | | 1 | | | | 5 |
| | | Flatstrand | 3 | 2 | | | | | | | | | 1 | 6 |
| | | Foss | | 2 | | | | | | | | | 2 | 4 |
| | | Fosshølen | | 4 | 1 | | | | | | | | | 5 |
| | | Grønbu | | 3 | | | | | | | | | | 3 |
| | | Lampeland | | | | | 1 | | 1 | | | | | 2 |
| | | Rambergtangen | | 2 | | | | | | | | | | 2 |
| | | Skipsvika | | 1 | | | | | | | | | | 1 |
| | | Skutsvika | | 4 | 2 | | 2 | | | | | | | 8 |
| | | Steinset | 1 | 1 | 2 | 3 | | | | | | | | 7 |
| | | Øygarden | | 1 | | | | | | | | | | 1 |
| | Modum (20) | Bingen | | 1 | | | | | | | | | | 1 |
| | | Branntjern | | 1 | 1 | | | | | | | | | 2 |
| | | Kaggefoss | | 1 | | | | | 1 | | | | | 2 |
| | | Kringlemoen | | 3 | | | | | | | | | | 3 |
| | | Markmoveien | | | | 2 | | | | | | | | 2 |
| | | Mortenstjern | | 2 | 1 | | | | | | | | | 3 |
| | | Thorudveien | | 3 | 1 | | | | 1 | | | | | 5 |
| | | Tuvtjern | | 1 | 1 | | | | | | | | | 2 |
| | Lier (2) | Skapertjern | | | | | | | | 2 | | | | 2 |
| Oppland (70) | Jevnaker (24) | Borstuvatnet | | | 4 | | | | | | | | | 4 |
| | | Svarttjernet | | | 14 | | | | | | | | | 14 |
| | | Svarttjernsmyra | | | 6 | | | | | | | | | 6 |
| | Lunner (46) | Grøa | | | 8 | | | | | | | | | 8 |
| | | Gullerudtjern | | | 29 | | | | | | | | | 29 |
| | | Viubråtan | | | 9 | | | | | | | | | 9 |

| | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Østfold (289) | Eidsberg (116) | Bergsvannet | | 2 | 2 | | | | | | 1 | | 5 |
| | | Bjørdalen | | 2 | 58 | 1 | | | 1 | 13 | | | 75 |
| | | Ertevannet | 3 | 5 | 17 | 3 | 1 | | 2 | | | | 31 |
| | | Stortjern | | | 4 | | | | | 1 | | | 5 |
| | Marker (134) | Lindalsåsen | | 3 | 13 | 5 | | | | | | | 21 |
| | | Mærrakleiva | | | 87 | | | | 2 | 24 | | | 113 |
| | Rømskog (16) | Bjørntjern | 1 | 1 | 3 | | 1 | | | | | | 6 |
| | | Bunes | | 1 | | | | | | | | | 1 |
| | | Gryttjern | | | | 2 | | | | | | | 2 |
| | | Langvik | | | 4 | | | | | 3 | | | 7 |
| | Trøgstad (23) | Håtjern | | 3 | | 1 | 2 | | | | | | 6 |
| | | Jordbrånen | | 1 | | | | | | | | | 1 |
| | | Kløvsrøys | | 2 | | | | | 1 | | | | 3 |
| | | Kverneåsen | | 7 | | | | | | | | | 7 |
| | | Årmomåsan | | 2 | | 1 | 1 | | 1 | | | | 6 |
| TOTAL MATERIAL | | | 17 | 111 | 370 | 24 | 10 | 1 | 11 | 47 | 1 | 2 | 6 | 600 |
| | | | 532 (89%) | | | | | 62 (10%) | | | | | 6 (1%) | |

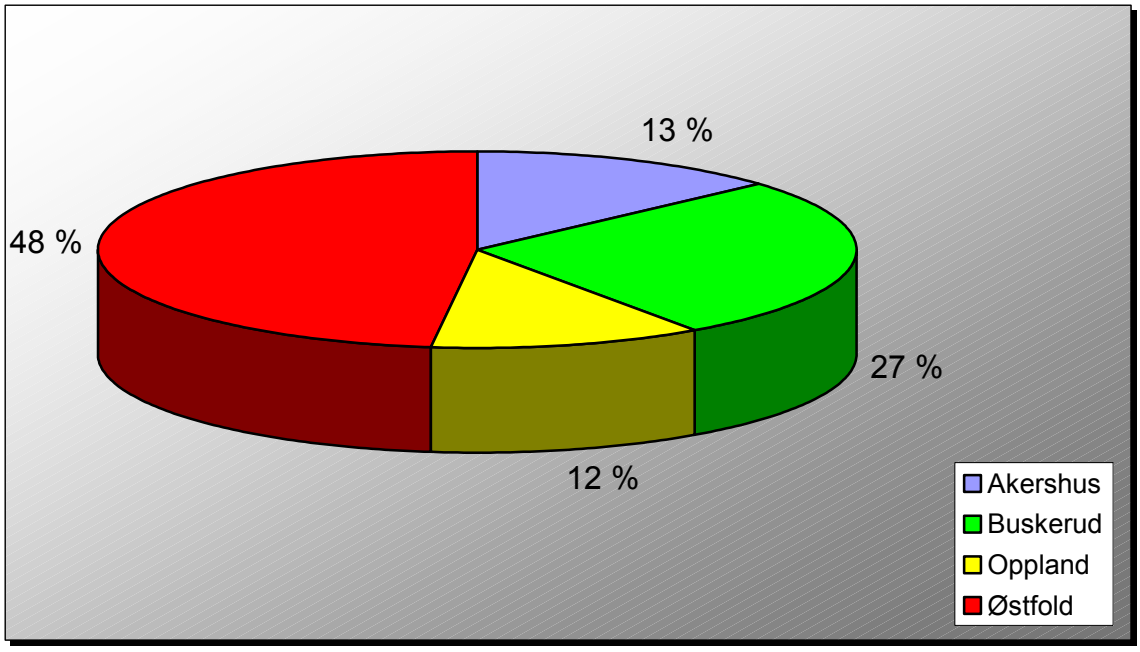


**Figure 5.** Survey of the pine wood nematode (PWN), *Bursaphelenchus xylophilus*, in Norway 2002. The relative distribution of 600 samples on counties.

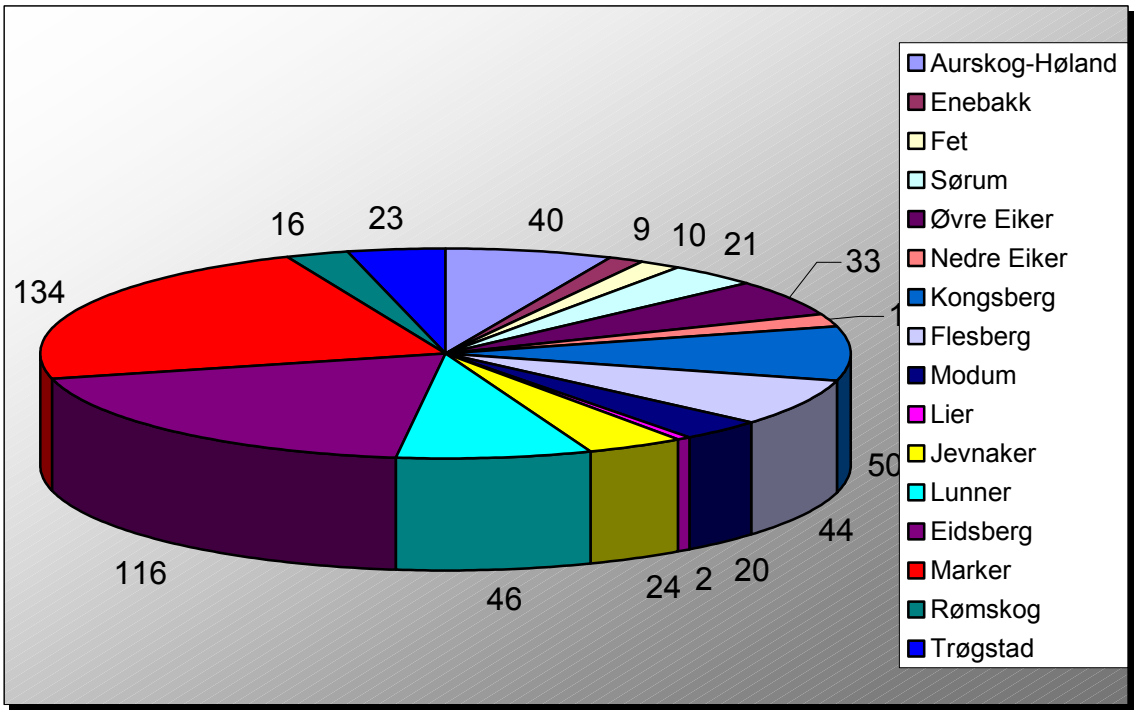


**Figure 6.** Survey of the pine wood nematode (PWN), *Bursaphelenchus xylophilus*, in Norway 2002. Numbers of samples distributed on municipalities.

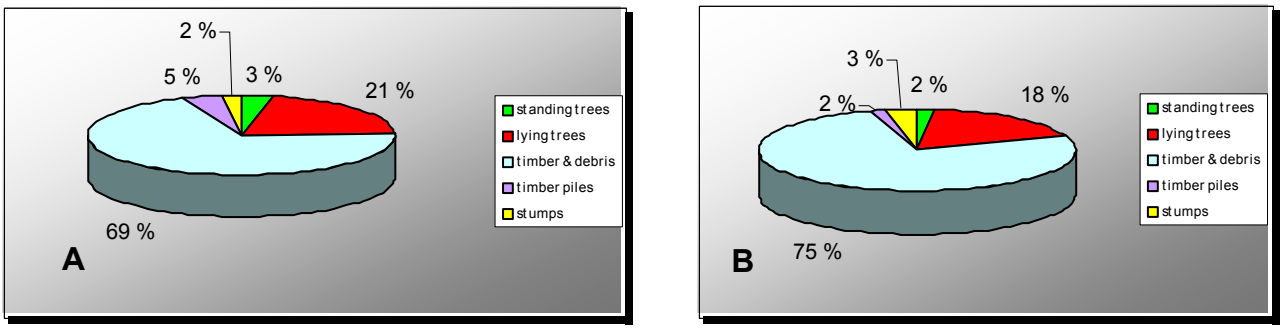


**Figure 7.** Survey of the pine wood nematode (PWN), *Bursaphelenchus xylophilus*, in Norway 2002. Relative distribution of samples on objects. **A**. Scots pine (*Pinus sylvestris*) n=532. **B**. Norway spruce (*Picea abies*) n=62. In this survey 6 samples were not identified to tree species.

**Table 2**. Nematode frequencies (%) in samples (n=532) of Scots pine (*Pinus sylvestris*) analysed in the Norwegian survey of the pine wood nematode (PWN), *Bursaphelenchus xylophilus,* in 2002.

| Nematode category | Pine wood total | Wood with Monochamus activity n=348 (65%) | Wood without clear Monochamus activity n=184 | Standing trees n=17 | Lying trees n=110 | Timber and debris n=367 | Stumps n=11 | Timber piles n=24 |
|---|---|---|---|---|---|---|---|---|
| Nematodes total | 94 | 93 | 91 | 65 | 90 | 96 | 91 | 100 |
| Microbivorous | 87 | 89 | 85 | 47 | 84 | 90 | 82 | 96 |
| Plant & insect nematodes total | 52 | 51 | 52 | 59 | 51 | 52 | 55 | 33 |
| Tylenchinae | 23 | 28 | 35 | 35 | 35 | 30 | 27 | 13 |
| Neotylenchidae | 1,3 | 0,8 | 2 | 12 | 0,9 | 0,8 | 0 | 1 |
| *Ditylenchus* | 0,4 | 0,3 | 0,5 | 0 | 0 | 0,5 | 0 | 0 |
| Aphelenchida | 33 | 32 | 26 | 24 | 26 | 32 | 45 | 13 |
| *Aphelenchoides* | 28 | 29 | 19 | 12 | 24 | 29 | 18 | 8 |
| *Bursaphelenchus* | 2,4 | 2,3 | 3 | 0 | 3 | 2 | 0 | 8 |
| *B. mucronatus* | 0,2 | 0,3 | 0 | 0 | 0 | 0,3 | 0 | 0 |
| *B. xylophilus* | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |

**Table 3**. Nematode frequencies in samples (n=62) of Norway spruce (*Picea abies*) analysed in the Norwegian survey of the pine wood nematode (PWN), *Bursaphelenchus xylophilus,* in 2002.

| Nematode category | Spruce wood total | Wood with Monochamus activity n=50 (81%) | Wood without clear Monochamus activity n=12 | Standing trees n=1 | Lying trees n=11 | Timber and debris n=47 | Stumps n=2 | Timber piles n=1 |
|---|---|---|---|---|---|---|---|---|
| Nematodes total | 94 | 88 | 92 | 100 | 16 | 87 | 100 | 100 |
| Microbivorous | 84 | 78 | 92 | 100 | 16 | 78 | 100 | 100 |
| Plant parasitic total | 40 | 32 | 33 | 0 | 3 | 32 | 50 | 100 |
| Tylenchinae | 21 | 16 | 33 | 0 | 6 | 15 | 0 | 100 |
| Neotylenchidae | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| *Ditylenchus* | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Aphelenchida | 24 | 26 | 8 | 0 | 3 | 21 | 50 | 100 |
| *Aphelenchoides* | 21 | 24 | 0 | 0 | 1 | 19 | 50 | 100 |
| *Bursaphelenchus* | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| *B. mucronatus* | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| *B. xylophilus* | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |

Plante forsk
Grønn kunnskap
Vol.8 Nr.104 – 2004

*Appendix 1*.

# Nordic Pine Wood Nematode Survey - Draft 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-750 07 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:
   - 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 ($\varepsilon$) according to: $n = \ln \varepsilon / \ln (1-p)$

**Table 1.** Minimal number of samples determined by the value of $\varepsilon$ and p.

| p \ $\varepsilon$ | 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.

## REFERENCES

**Anonymous (1999)**. Draft report of a mission carried out in Portugal from 12-16 September 1999 to assess the measures taken by the Portuguese authorities in response to the outbreak of pine wood nematode (*Bursaphelenchus xylophilus* (Steiner et Buhrer) Nickle). European Commission, Directorate-General Health and Consumer Protection, Directorate D-Food and Veterinary Office. Doc. DG (SANCO)/1223/99-MR-Draft 23/09/1999: 18 pp.

**Evans, H.F., McNamara, D.G., Braasch, H., Chadoeuf, J. & Magnusson, C. (1996)**.
Pest Risk Analysis (PRA) for the terri-tories of the European Union (as PRA area) on Bursaphelenchus xylophilus and its vectors in the genus Monochamus. *EPPO Bulletin 26*: 199-249.

**McNamara, D.G. & Støen, M. (1988).**
A survey for Bursaphelenchus spp. in pine forests in Norway. *EPPO Bulletin 18*: 353-363.

**Magnusson, C. & Schroeder, LM. (1989)**.
First record of a Bursaphelenchus-species (Nematoda) in a Monochamus beetles in Scandinavia. *Anzeiger für Schädlingskunde,Pflanzenschutz und Umweltschutz 62*: 53-54.

**Tomminen, J. (1990)**. Presence of Bursaphelenchus mucronatus (Nematoda: Aphelenchoididae) fourth dispersal stages in selected conifer beetles in Finland. *Silva Fennica 24*: 273-278.

**Tomminen, J., Nuorteva, M., Pulkkinen, M. & Väkevä, J. (1989)**. Occurrence of the nematode Bursaphelenchus mucronatus Mamiya & Enda 1979 (Nematoda: Aphelenchoididae) in Finland. *Silva Fennica 23*: 271-277.

Ansvarlig redaktør:
Forskningsdirektør Arne Stensvand
Fagredaktør for denne utgaven:
Forskningssjef Trond Hofsvang


ISBN 80-479-0460-8