MONITORING PINEWOOD NEMATODE (*Bursaphelenchus xylophilus*) IN NORWAY 2017-2018.

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Abstract

The results of the monitoring in Norway of the pinewood nematode *Bursaphelenchus xylophilus* in 2017-2018 is based on 845 wood samples collected from the counties of Telemerk (municipalities Drangedal, Seljord, Hjartdal and Notodden), Agder (municipalities Mykland and Vennesla), Vestfold (municipality of Sandefjord), Østfold (municipalities Råde, Fredrikstad, Sarpsborg and Rakkestad), Akershus (municipalities Nes, Sørum and Ski), Buskerud (municipality Sigdal) and Hedmark (municipality Åmot). All samples were from wood of pine (*Pinus sylvestris*) showing signs of *Monochamus* activity. The dominating material sampled were logging waste and cut tops of trees. In this survey, the pinewood nematode (PWN) *Bursaphelenchus xylophilus* was not detected in wood samples. No nematodes of the *Bursaphelenchus xylophilus*–group were present in the 173 wood samples taken in 2017. In 2018 the species *B. mucronatus kolymensis* was detected on six occasions four of which in logging waste and two from cut tops of trees. In 2017, 49 beetles of *Monochamus sutor* was trapped in the counties of Hedmark, Møre and Romsdal and Østfold. None of the beetles carried nematodes. In 2018 71 specimens of *Monochamus* spp were trapped in Trøndelag, Hedmark and Østfold. Fifty-three of the beetles were *M. sutor* and 18 specimens belonged to *M. galloprovincialis*. The latter species was detected at Halden in Østfold. Six specimens of *M. galloprovincialis* carried dispersal juveniles of *B. mucronatus kolymensis* at densities varying from one to 736. Nematodes were not detected in *M. sutor*.

Background

The detection in May 1999 of the pine wood nematode (PWN), *Bursaphelenchus xylophilus*, in Portugal (Mota *et al*. 1999) and the repeated outbreaks in Spain has 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.). The present report gives information on the Norwegian survey results for 2017-2018. The presented data only reports on finds of nematodes belonging to the *Bursaphelenchus xylophilus*-group.

Materials and methods

The work was carried out in accordance with the Nordic Pine Wood Nematode Survey – Draft 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.

In 2017 the selection of logging sites was made through contacts with forest management offices in selected municipalities, while in 2018 the logging sites 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 2016 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 potential logging sites.

In 2017-2018 a total of 845 samples were collected from wood of *Pinus sylvestris* L. attacked by the cerambycid beetles *Monochamus s*pp. In 2017 a total number of 173 samples were collected in Agder, Telemark and Vestfold. In 2018 672 samples were taken from Telemark, Vestfold, Buskerud, Akershus, Østfold and Hedmark (Fig.1).

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^{o}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).

In 2017 two traps for *Monochamus* spp. were set up in the counties of Hedmark at Åsen, Finnburgruva and in Møre og Romsdal (B. Dynes Aarø). In 2018 traps were set up in Hedmark at Damstuen, Dambrenna and Romedal Almenning, in Østfold at Halden and in Trøndelag at Stjørdal, Elvran and Idar Kvaal. Two kind 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 large collection cups 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. In 2017 Identification was made by morphology according to Wallin *et al*. (2013),and in 2018 this was complemented by molecular PCR methods where the legs of *Monochamus* were used for extraction of DNA. The extractions were made by using the Qiagen DNeasy Blood & Tissue Kit according to the protocols provided by the producer. The PCR was performed at NIBIO and the PCR-product was sent to Eurofins for sequencing. The sequences were trimmed a CLC Main Workbench 8 and searched for in the databases BOLD and BLAST.

The extracts from the beetles were examined in a Leica M10 stereo microscope for the presence of nematodes. In one case the nematode identification was by molecular methods at INIAV in Lisbon, while the other identifications were made on nematode specimens reared on fungal mats of *Botrytina cinerea*.

Results

Source of samples

The sampling volumes and localities for 2017-2018 are shown in Table 1 and Fig. 1. Most samples (278) were from Telemark, followed by Hedmark (200), Østfold (150), Buskerud (90), Akershus (62), Agder (62) and Vestfold (3). During the survey 32 logging sites in 16 municipalities were visited (Tab. 1).

In Telemark 167 samples came from the municipality of Notodden (Tab. 1, Fig 2) with 70 samples from Sejord, 62 samples from Skogen, 19 samples from Hera and 16 samples from Bulkesjø. In Drangedal 84 samples were collected with 56 samples from Gare, 25 samples from Sannes and 3 samples from Etterstad. In the municipality of Seljord 24 samples were taken in Vefall, and in the municipality of Hjartdal 3 samples came from Brekka. In Agder (Tab. 1) the municipality of Mykland contributed with 56 samples and the municipality of Vennesla with 6 samples. In Vestfold 3 samples were collected at Stranda. In Østfold and the municipality of Rakkestad (Tab. 1, Fig.3) 70 samples were collected at Langtjernet, 13 samples at Djupetjernet, 10 samples at Moen and 4 samples at Laksen. In Sarpsborg (Tab. 1, Fig. 4) 37 samples came from Leiråsen and 2 samples from Stensrudmosen. In Råde (Tab. 1, Fig. 5) eight samples were taken in Gillingsrød and 3 samples at Spetalen. In Fredrikstad (Tab. 1, Fig. 6) Havviken, Søndre Torp and Kileløkka each had one sample. In Akershus and the municipality of Nes (Tab. 1, Fig. 7) 30 samples were collected at Finnholt, In Sørum (Tab. 1, Fig. 8) 25 samples were taken at Piggåsen, and in Ski (Tab. 1, Fig. 9) seven samples came from Gjetsjø. In Buskerud and the municipality of Sigdal (Tab. 1, Fig. 10) 90 samples originated in in the area of Myrbråtamyra and Rånåbekk. In Hedmark and the municipality of Åmot (Tab. 1. Fig. 11) 115 samples were taken at Runddalssjøen and 85 samples at Skjerstad.

Material sampled

The kind of wood materials sampled are shown in Table 2 and Figure 12. Samples were collected from wind thrown trees, cut tree tops, other logging waste like branches and lying trunks of trees and cut bolts. Most samples 632 (75 %) of the total 845 samples came from cut tops of trees and logging waste like branches present in the logging sites. The number of samples taken from the different wood objects varied between logging sites (Table 2).The lables of the 62 samples (7 %) taken at Mykland and Vennesla in Agder were wet, damaged and not readable so the sources of the samples remains unknown.

Occurrence of nematodes in wood samples

The pinewood nematode *Bursaphelenchus xylophilus* was not detected in wood samples in this survey (Tab. 1 & 2), but the species *B. mucronatus kolymensis* (Tab. 3) was detected in the county of Østfold in one detached tree top at Djupetjernet and in two pieces of other logging waste at Langtjernet. This nematode was also detected in the county of Hedmark in the municipality of Åmot in one detached tree top at Runddalssjøen and in two pieces of other logging waste at Skjerstad. Apart from this the following nematode groups and genera were present in the wood samples: members of the Rhabditida, *Tylenchus*, *Ditylenchus*, *Aphelenchoides*, Neotylenchidae and *Hexatylus* sp. Forty-five samples (5 %) were without nematodes.

Nematodes in the insect vectors *Monochamus* spp.

In 2017 (Tab. 4) traps were placed in Hedmark and Møre, Romsdal and in Oslo and Akershus. In Hedmark eight beetles were caught at Åsen, 37 beetles at Finnburgruva, and 2 beetles from Romedal Almenning. In Møre and Romsdal one beetle was trapped at Dynes Aarø. In Oslo and Akershus one specimen of *Acanthocinus* was captured close to a ware storage facility in Langhus. In Østfold one beetle was caught by hand and sent for analysis. All 49 *Monochamus* beetles belonged to *M. sutor*. No nematodes were extracted from the beetles in 2017.

In 2018 71 beetles belonging to the genus *Monochamus* were trapped and analysed (Tab. 5). None of these beetles carried *Bursaphelenchus xylophilus*. Fifty-three (75 %) of the beetles were *M. sutor*

and 18 *M. galloprovincialis*. All specimens of *M. galloprovincialis* were only recovered from traps in Østfold, while *M. sutor* was caught at all trapping locations. The pinewood nematode *Bursaphelenchus xylophilus* was not detected in the beetles trapped in 2018. The nematode species *Bursaphelenchus mucronatus kolymensis* was extracted from six beetles of *M. galloprovincialis*. The nematode loads were 1, 7, 9, 11, 75 and 736 ind. per beetle. In *M. sutor* no nematodes were detected in 2018.

Monitoring activities in Norway 2000-2018

In total 7 723 wood samples were analysed in this period. The annual number of samples varies between years (Fig. 13). Due to sampling problems in 2017 only 173 samples were analysed, which is the lowest number in the total monitoring period. More than 500 samples were collected in 2001, 2002, 2003, 2004, 2010, 2012, 2014, 2015 and 2018. Regarding sample volumes in counties (Fig 14) most samples have been taken in Østfold (2088), Hedmark (1960), Agder (1108), Telemark (922) and Buskerud (819).

Discussion

Compared to earlier surveys this period stands out in that Telemark contributed with the highest number of samples. Like in previous surveys in Norway logging waste like cut branches, pieces of stems together with cut tops of pine trees were the main objects sampled. During 2018 672 samples were collected, which is the highest number since the start of the monitoring in 2000. The six detections of *Bursaphelenchus mucronatus kolymensis* this period is higher compared to 2016 when three detections were made (Magnusson et al. 2017). This may be explained by the higher number of samples and the focus on pine wood objects, as well as the high number of samples from areas known to have the nematode. The frequency of detection of *B. mucronatus kolymensis* in wood samples was 0,1 %.

With respect to the *Monochamus* beetles trapped there were differences in identification techniques in 2017 compared to 2018. In 2017 identification relied entirely on morphology, while in 2018 both morphology and PCR was used. It has been considered that *M. galloprovincialis* in Norway has a more south-eastern distribution compared to *M. sutor*. This was clearly confirmed in this study by using molecular methods. Interestingly, at Halden 18 of 29 beetles caught belonged to *M. galloprovincialis*. The remaining 11 beetles were *M. sutor*. Only *M. galloprovincialis* carried dispersal juveniles of *B. mucronatus kolymensis*. No specimens of *M. sutor* carried nematodes at the trapping locations used in 2018. This may suggest *M. galloprovincialis* to be a more efficient nematode vector compared to *M. sutor*. However, in Sweden fourth dispersal juveniles of *B. mucronatus* were detected in both beetle species (Magnusson & Schroeder 1989). Also in Norway the previous detections of *B. mucronatus kolymensis* in wood attacked by *Monochamus* beetles indicate *M. sutor* to be a vector of this nematode. In 2018 the frequency of detection on *B. mucronatus kolymensis* in beetles was 8,5 %.

The surveillance program for PWN in Norway has been carried out in accordance with the Nordic 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 2016 (Magnusson *et al*. 2001, 2002, 2004 ab, 2007, 2009, 2013, 2016 and this report) *Bursaphelenchus mucronatus kolymensis* + *B. macromucronatus,* which both resemble PWN ecologically, have been found in 69 of 7 723 wood samples, which gives a frequency of 0,0010. 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 in Norwegian forests. If we then assume a hypothetical frequency of *B. xylophilus* to be 100 times lower, *i.e*. 0,00010, the number of samples necessary for one positive find of *B. xylophilus* can according to our equation be calculated to 29 956. It seems we have now reached 26 % of the required volume of samples.

Like in the monitoring made in 2013-2014 (Magnusson *et al*. 2014), the frequency of detection of *B. mucronatus kolymensis* was higher in beetles than in wood samples. This demonstrates the importance of beetle trapping in monitoring for PWN.

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Table 1. Monitoring of pinewood nematode (PWN) *Bursaphelenchus xylophilus* at logging sites in Norway 2017-2018. BMK = *Bursaphelenchus mucronatus kolymensis*; BXY = *Bursaphelenchus xylophilus*.

COUNTY	MUNICIPALLITY	LOCATION	NOS SAMPLES	LOGGING SITE	BMX	BXY
Telemark (278)	Drangedal (84)	Etterstad	3		0	0
		Gare	56		0	0
		Sannes	25		0	0
	Seljord (24)	Vefall	24		0	0
	Hjartdal (3)	Bekka	3		0	0
	Notodden (167)	Skogen	45	807-20	0	0
		Skogen	17	807-21	0	0
		Bulkesjø	16	807-9	0	0
		Sejord	70	807-17	0	0
		Hea	19	807-11	0	0
Agder (62)	Mykland		56		0	0
	Vennesla	Skjervedalsmyra	6		0	0
Vestfold (3)	Sandefjord	Stranda	3		0	0
Østfold (150)	Råde (11)	Gillingsrød	4	135-1	0	0
		Gillingsrød	4	135-2	0	0
		Spetalen	3	135-3	0	0
	Fredrikstad (3)	Havviken	1	106-4	0	0
		Søndre Torp	1	106-2	0	0
		Kileløkka	1	106-1	0	0
	Sarpsborg (39)	Leiråsen	37	105-13	0	0
		Stensrudmosen	2	105-12	0	0
	Rakkestad (97)	Moen	10	128-7	0	0
		Laksen	4	128-1	0	0
		Langtjernet	70	128-2	2	0
		Djupetjernet	13	128-3	1	0
Akershus (62)	Nes (30)	Finnholt	30	236-5	0	0
	Sørum (25)	Piggåsen	25	227-8	0	0
	Ski (7)	Gjetsjø	6	213-4	0	0
		Gjetsjø	1	213-6	0	0
Buskerud (90)	Sigdal (90)	Myrbråtamyra & Rånåbekk	90	621-6	0	0
Hedmark (200)	Åmot (200)	Runddalssjøen	115	429-3	1	0
		Skjerstad	85	429-6	2	0
Totalt = 845					6	0

Table 2. Monitoring of pinewood nematode (PWN) *Bursaphelenchus xylophilus* in Norway 2017-2018. Sampled material from pine wood (*Pinus sylvestris*). BMK = *Bursaphelenchus mucronatus kolymensis*; BXY = *Bursaphelenchus xylophilus*

COUNTY (n)	MUNICIPALITY	OBJECTS AND NUMBER OF SAMPLES					SUM	BMK	BXY
		Wind fall	Tops	Logging waste	Bolts	Un-known			
Telemark (278)	Drangedal	0	26	41	17	0	84	0	0
	Seljord	0	18	0	6	0	24	0	0
	Hjartdal	0	0	3	0	0	3	0	0
	Notodden	2	86	42	37	0	167	0	0
Agder (62)	Mykland	-	-	-	-	56	56	0	0
	Vennesla	-	-	-	-	6	6	0	0
Vestfold (3)	Sandefjord	3	0	0	0	0	3	0	0
Østfold (150)	Råde	3	1	5	2	0	11	0	0
	Fredrikstad	1	0	2	0	0	3	0	0
	Sarpsborg	2	12	14	11	0	39	0	0
	Rakkestad	0	35	56	6	0	97	3	0
Akershus (62)	Ski	0	4	2	1	0	7	0	0
	Nes	0	11	9	10	0	30	0	0
	Sørum	1	12	8	4	0	25	0	0
Buskerud (90)	Sigdal	1	36	32	21	0	90	1	0
Hedmark (200)	Åmot	0	73	104	23	0	200	2	0
TOTAL 845		13	314	318	138	62	845	6	0

Table 3. Monitoring of pinewood nematode (PWN) *Bursaphelenchus xylophilus* in Norway 2017-2018. Detection of *Bursaphelenchus mucronatus kolymensis* in wood material of *Pinus sylvestris*.

COUNTY	MUNICIPALITY	LOCALITY	MATERIAL	NUMBER OF DETECTIONS	LOGGING SITE
Østfold	Rakkestad	Langtjernet	Logging waste	2	128-2
		Djupetjernet	Topp	1	128-3
Hedmark	Åmot	Runddalssjøen	Topp	1	429-3
		Skjerstad	Logging waste	2	429-6
TOTAL DETECTIONS				6	

Table 4. Monitoring pinewood nematode (PWN) *Bursaphelenchus xylophilus* in *Monochamus* beetles in Norway 2017 using pheromone traps.

COUNTY	LOCALITY	NOS. Monochamus sutor	NOS. NEMATODES
Hedmark	**Åsen**	**8**	**0**
	Finnburgruva	**37**	**0**
	Romedal Almenning	**2**	**0**
Møre og Romsdal	**Dynes Aarø**	**1**	**0**
Østfold	**Trøgstad**	**1**	**0**
TOTAL		**49**	

Table 5. Monitoring pinewood nematode (PWN) *Bursaphelenchus xylophilus* in *Monochamus* beetles in Norway 2018 using pheromone traps. BMK = *Bursaphelenchus mucronatus kolymensis*; BXY = *Bursaphelenchus xylophilus*.

COUNTY	TRAP POSITION	*Monochamus sutor*		*Monochamus galloprovincialis* BMK		BXY
		Nos. beetles	Nos. BMK	Nos. beetles	Nos. BMK	Nos. nematodes
TRØNDELAG (11)	Stjørdal / Elvran	4	0	0		0
	Levanger / Åsen	0	0	0		0
	Idar Kvaal ?	7	0	0		0
HEDMARK (31)	Stange / Dambrenna	11	0	0		0
	Stange / Damstuen	13	0	0		0
	Romedal almenning	7	0	0		0
ØSTFOLD (11)	Sarpsborg	0	0	0		0
	Halden / Iversen	11	0	18	1, 7, 9, 11, 75, 736	0
TOTAL		53		18		0

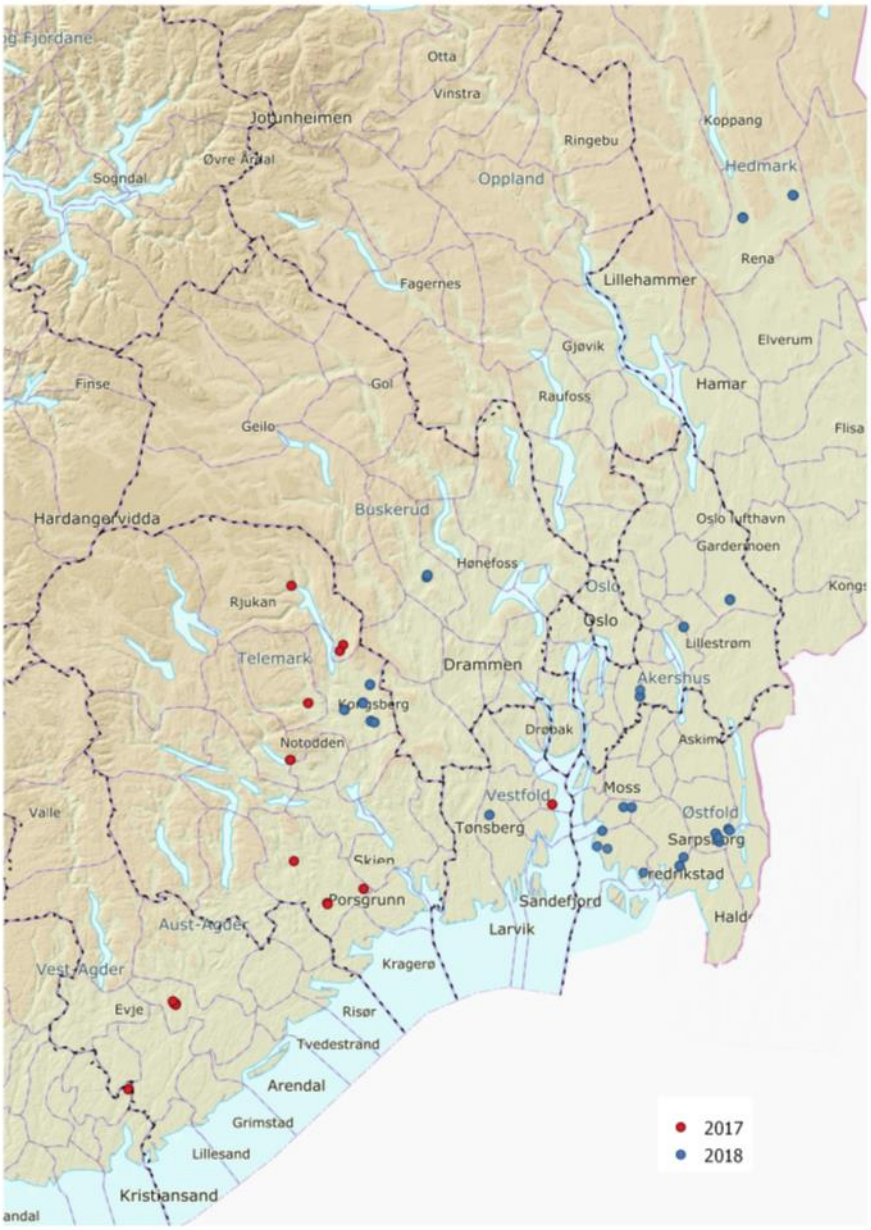

Figure 1. Monitoring pinewood nematode (PWN) *Bursaphelenchus xylophilus* in Norway. The positions of the 845 samples in 2017-2018. In 2017 173 samples were taken and in 2018 the number of samples was 672.

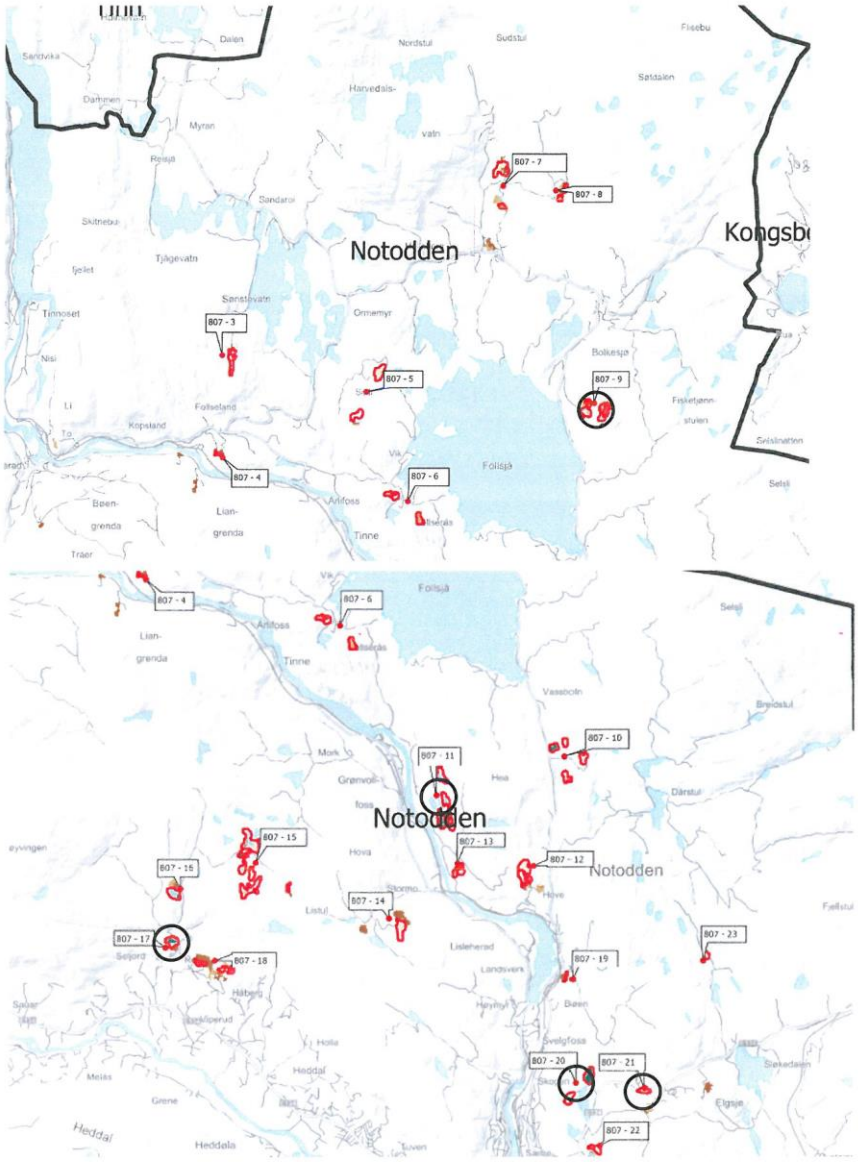

Figure 2. Monitoring pinewood nematode (PWN) *Bursaphelenchus xylophilus* in Norway 2017-2018. Black circles mark the positions of samples taken in Notodden.

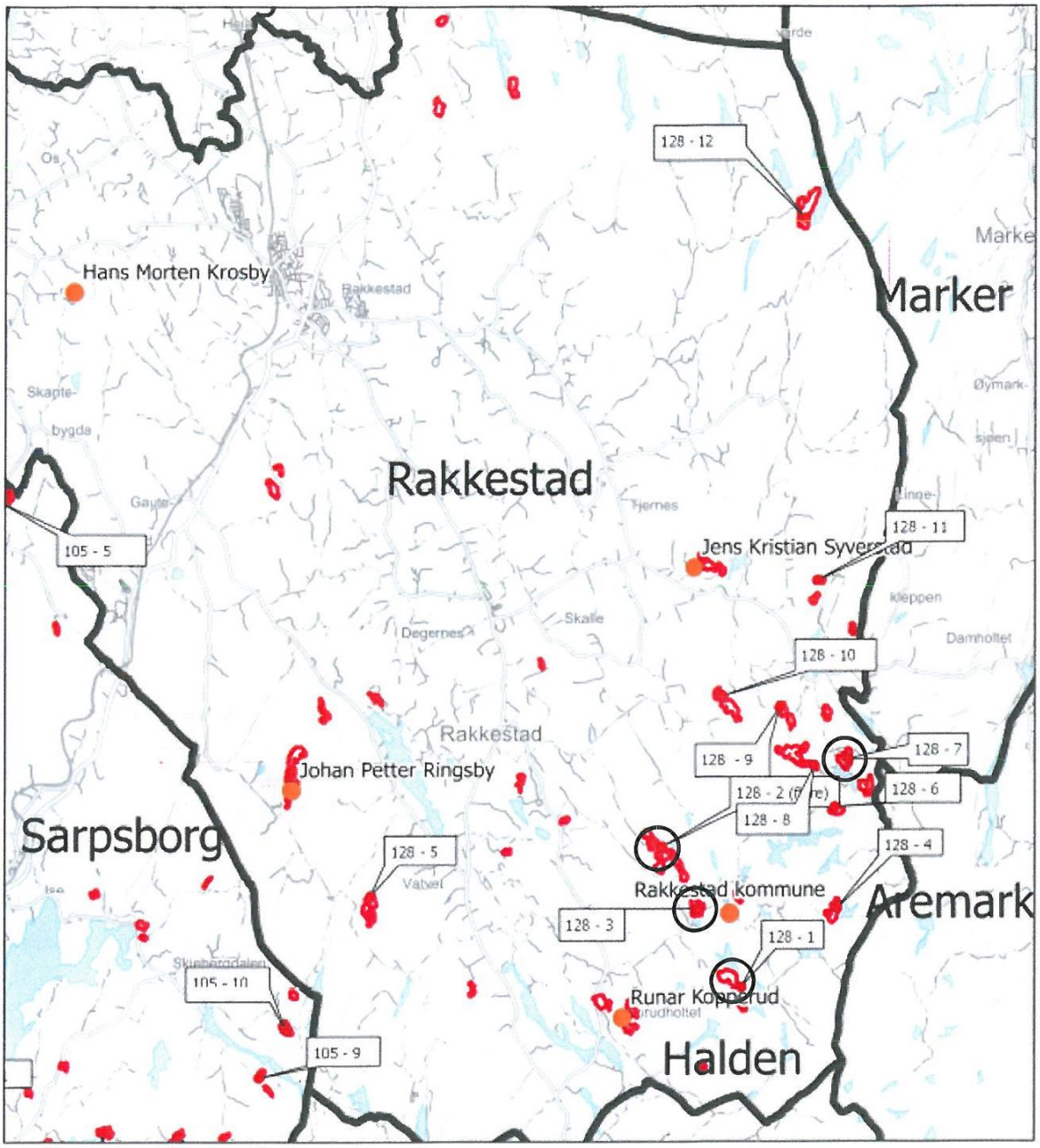

Figure 3. Monitoring pinewood nematode (PWN) *Bursaphelenchus xylophilus* in Norway 2017-2018. Black circles mark the positions of samples taken in Rakkestad.

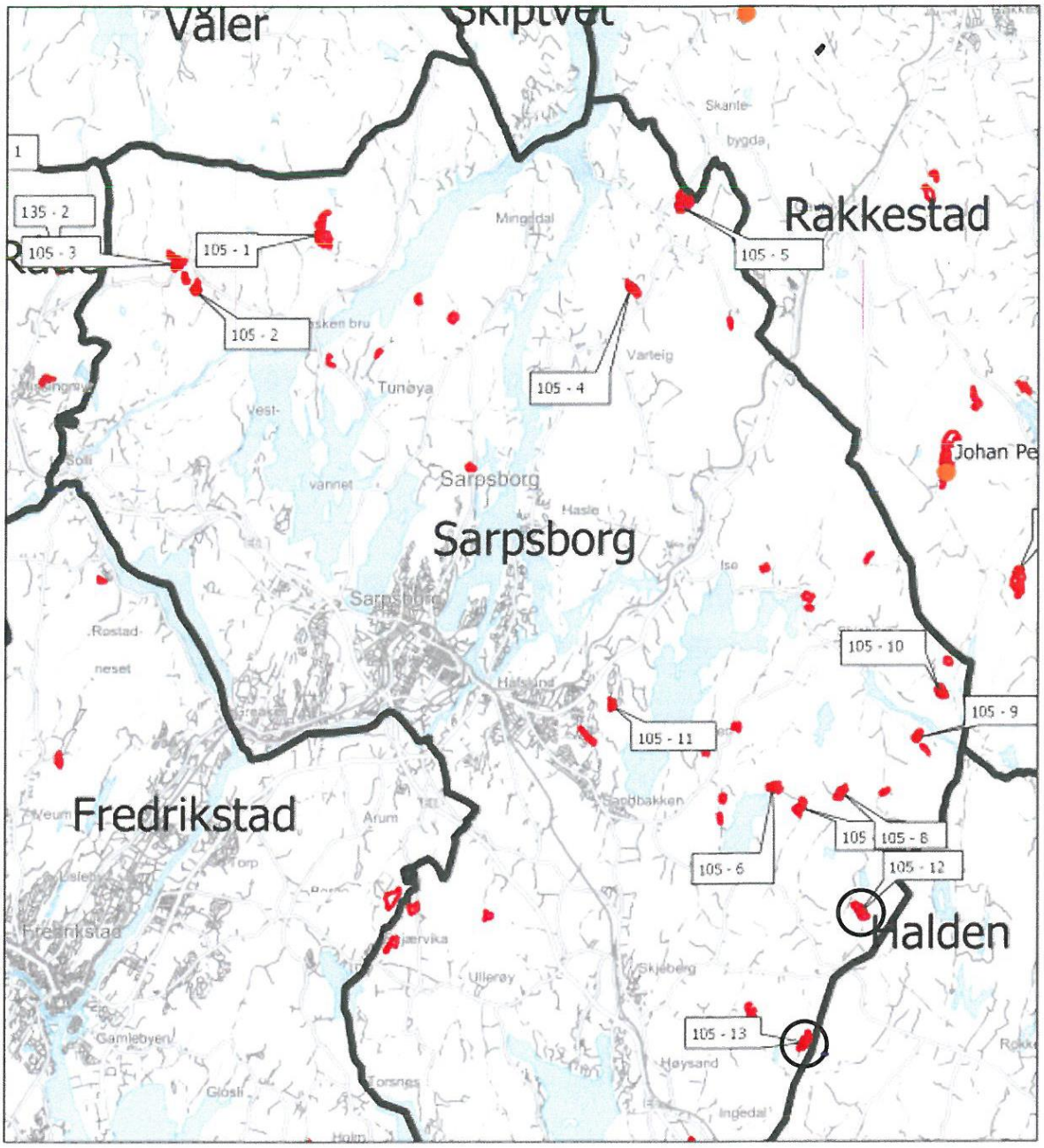

Figure 4. Monitoring pinewood nematode (PWN) *Bursaphelenchus xylophilus* in Norway 2017-2018. Black circles mark the positions of samples taken in Sarpsborg.

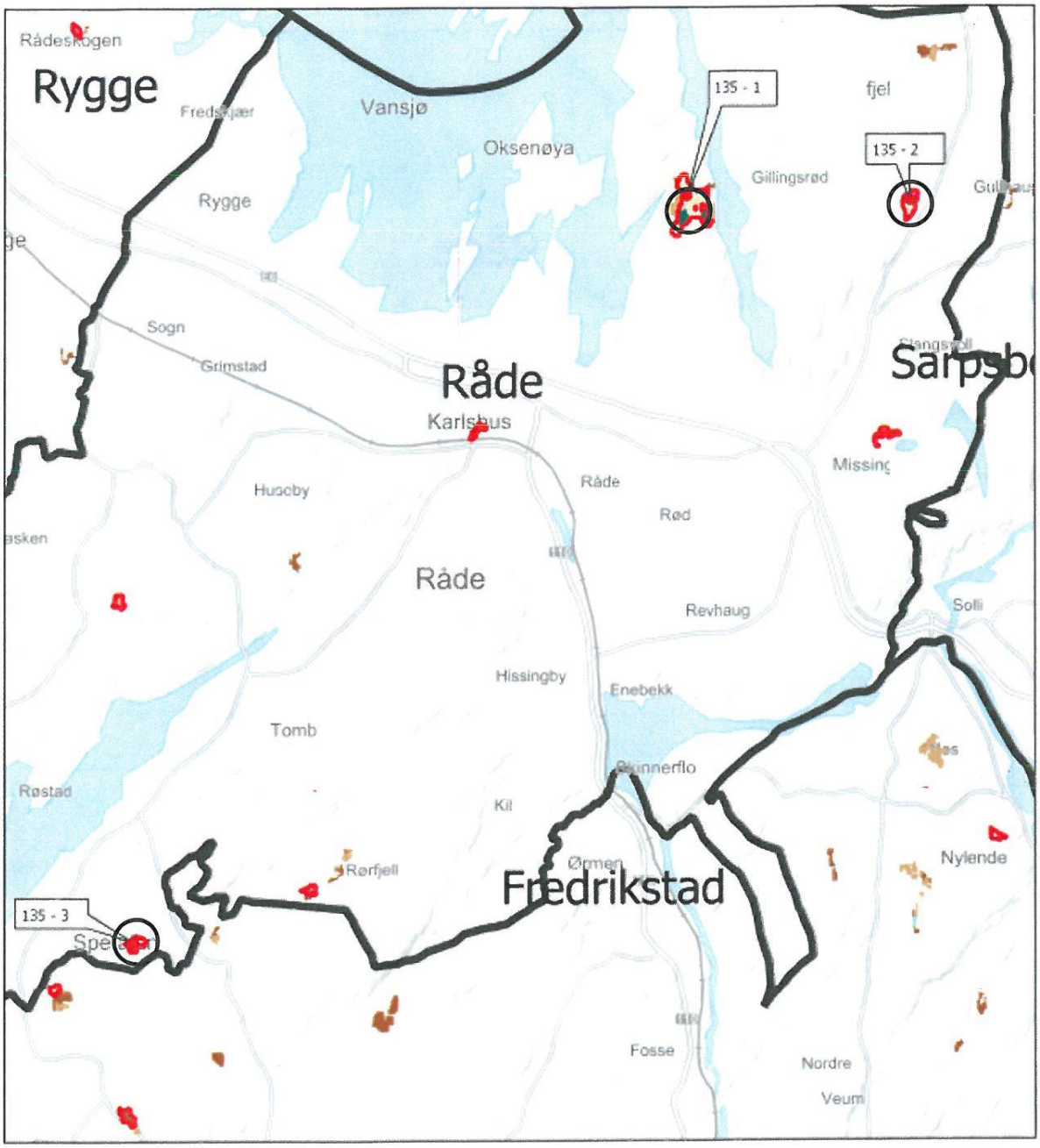

Figure 5. Monitoring pinewood nematode (PWN) *Bursaphelenchus xylophilus* in Norway 2017-2018. Black circles mark the positions of samples taken in Råde.

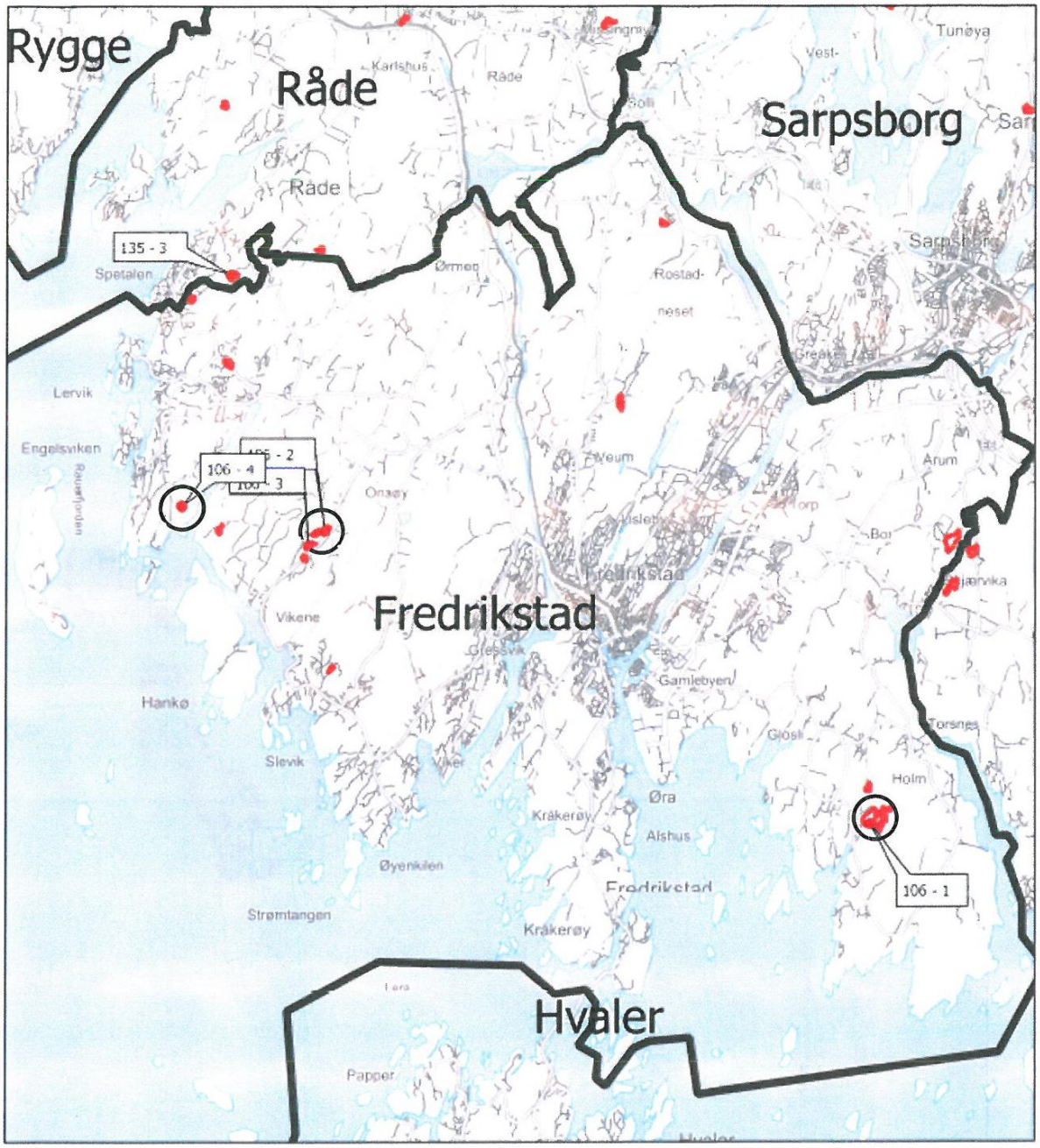

Figure 6. Monitoring pinewood nematode (PWN) *Bursaphelenchus xylophilus* in Norway 2017-2018. Black circles mark the positions of samples taken in Fredrikstad.

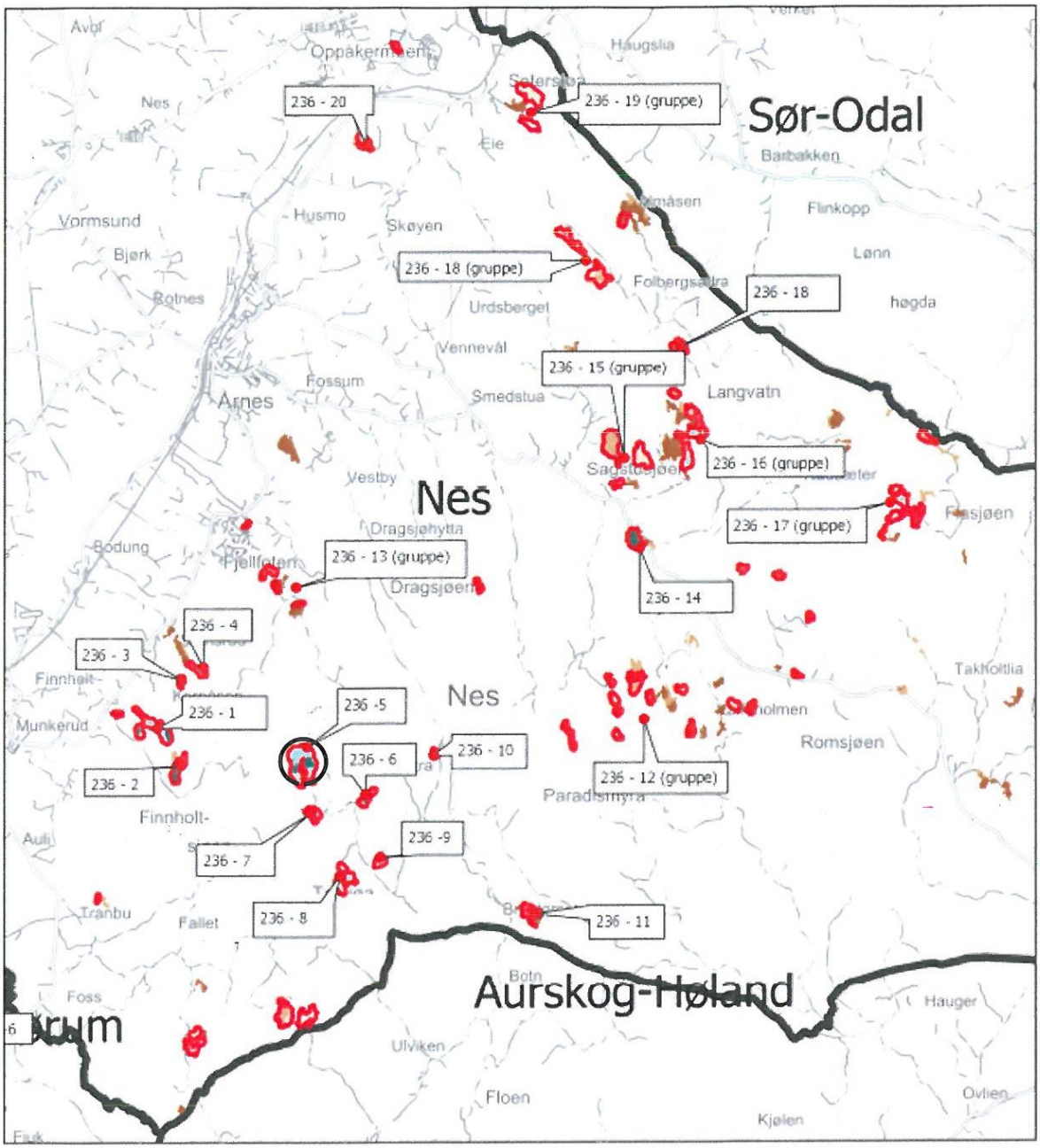

Figure 7. Monitoring pinewood nematode (PWN) *Bursaphelenchus xylophilus* in Norway 2017-2018. Black circle marks the positions of samples taken in Nes.

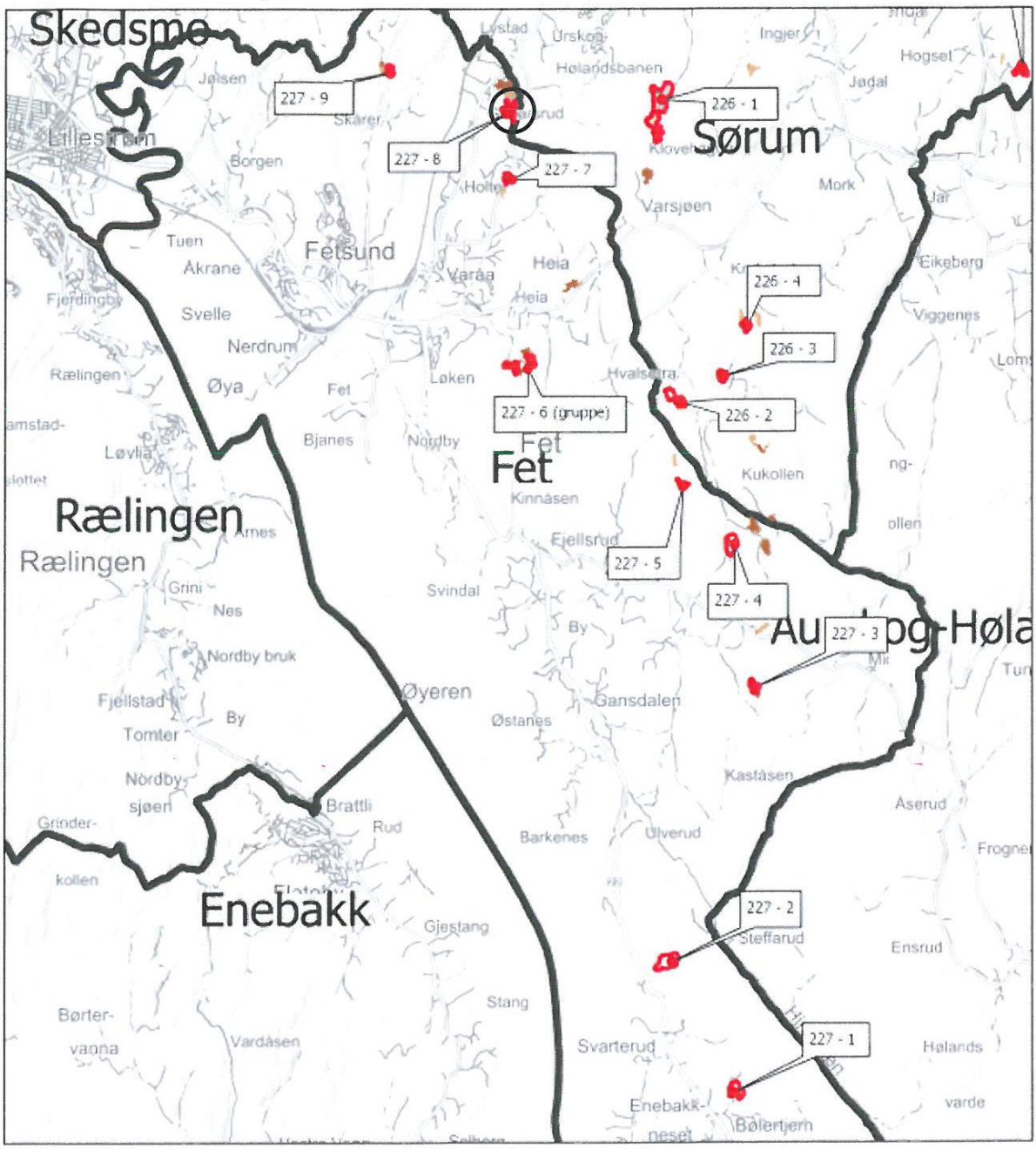

Figure 8. Monitoring pinewood nematode (PWN) *Bursaphelenchus xylophilus* in Norway 2017-2018. Black circle marks the positions of samples taken in Sørum.

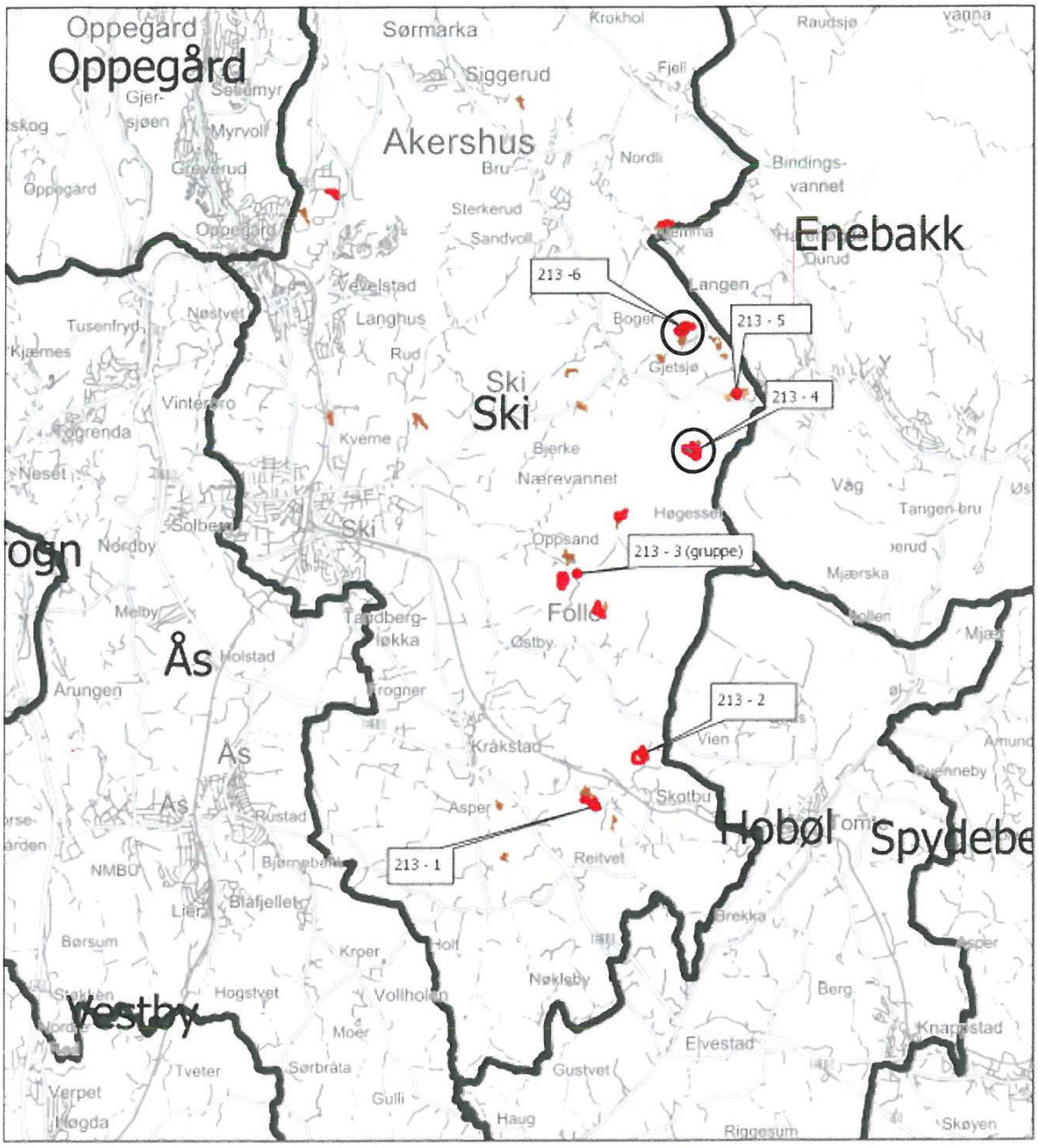

Figure 9. Monitoring pinewood nematode (PWN) *Bursaphelenchus xylophilus* in Norway 2017-2018. Black circles mark the positions of samples taken in Ski.

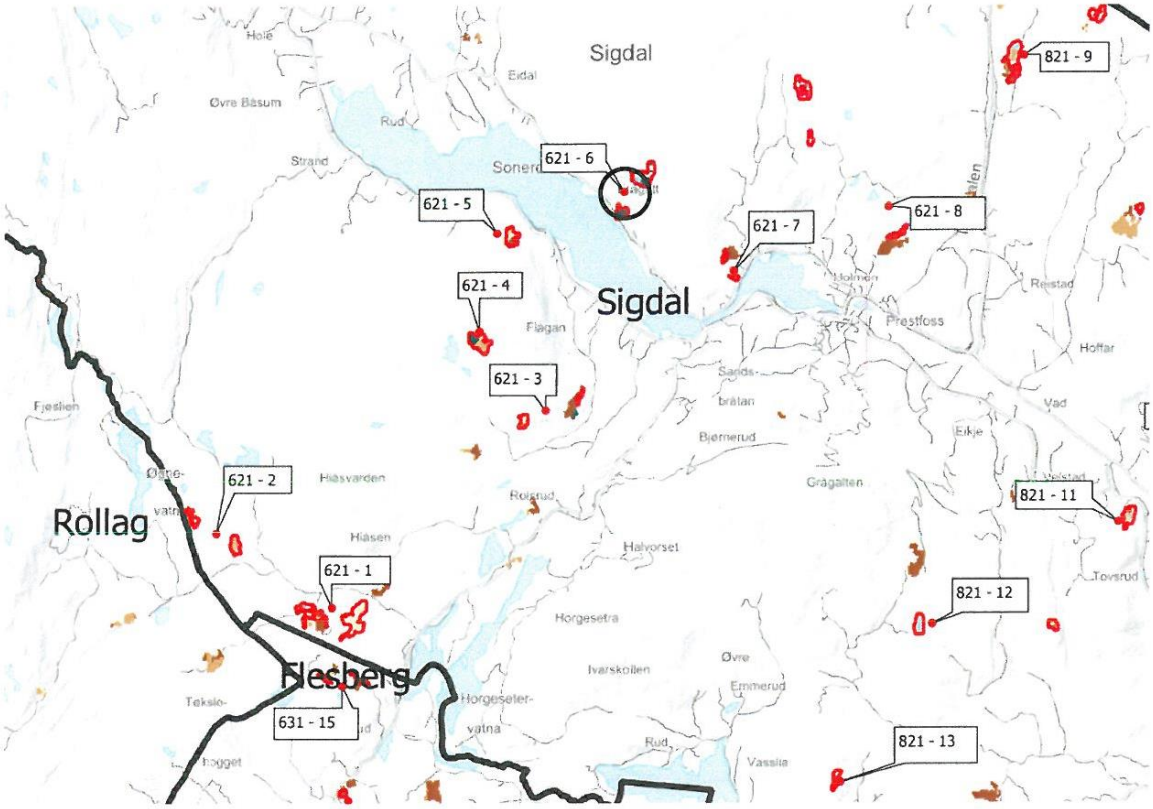

Figure 10. Monitoring pinewood nematode (PWN) *Bursaphelenchus xylophilus* in Norway 2017-2018. Black circle marks the positions of samples taken in Sigdal.

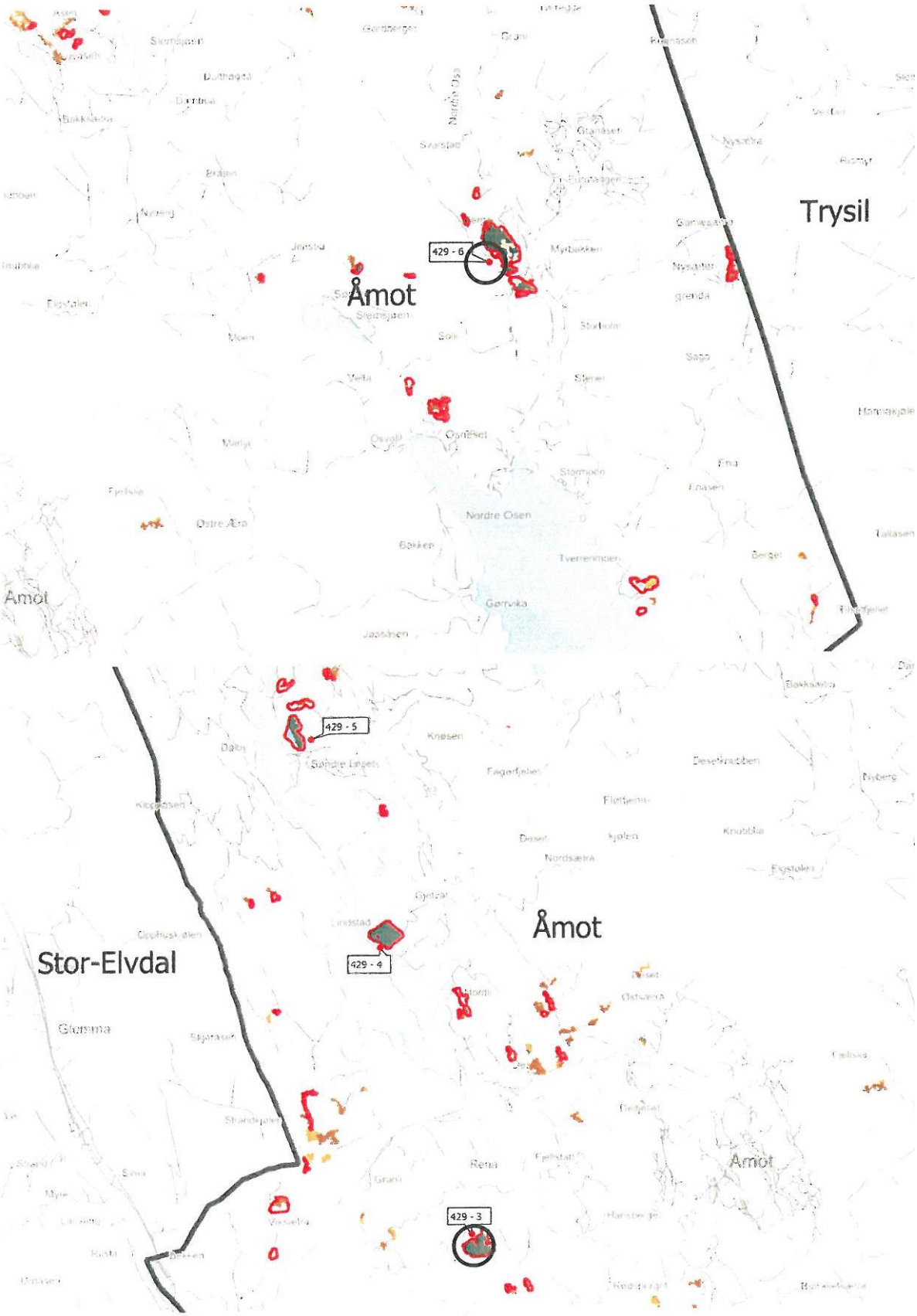

Figure 11. Monitoring pinewood nematode (PWN) *Bursaphelenchus xylophilus* in Norway 2017-2018. Black circles mark the positions of samples taken in Åmot.

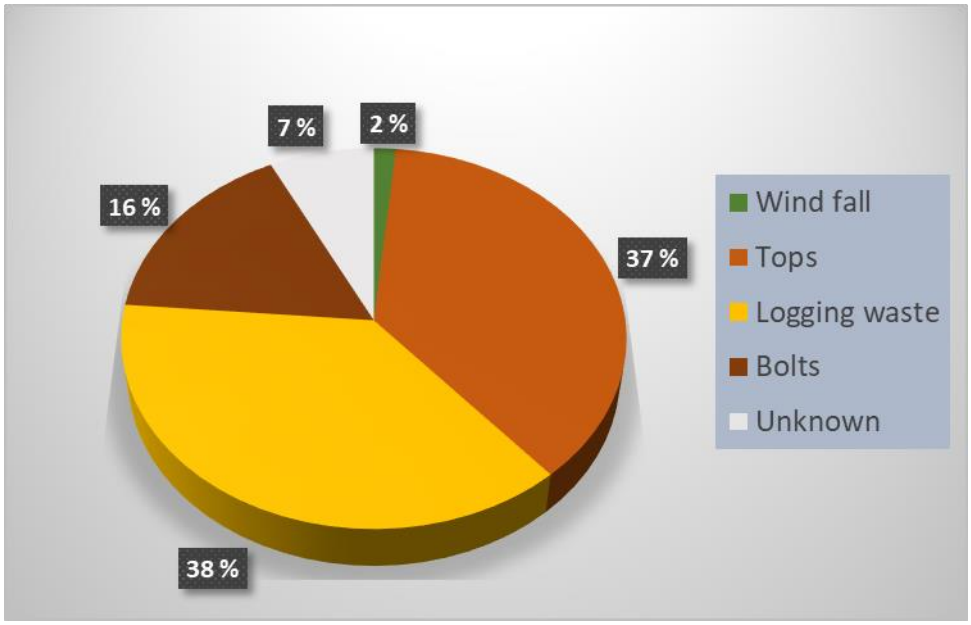

Figure 12. Monitoring pinewood nematode (PWN) *Bursaphelenchus xylophilus* in Norway 2017-2018. Objects of wood material sampled. Total number of samples 845.

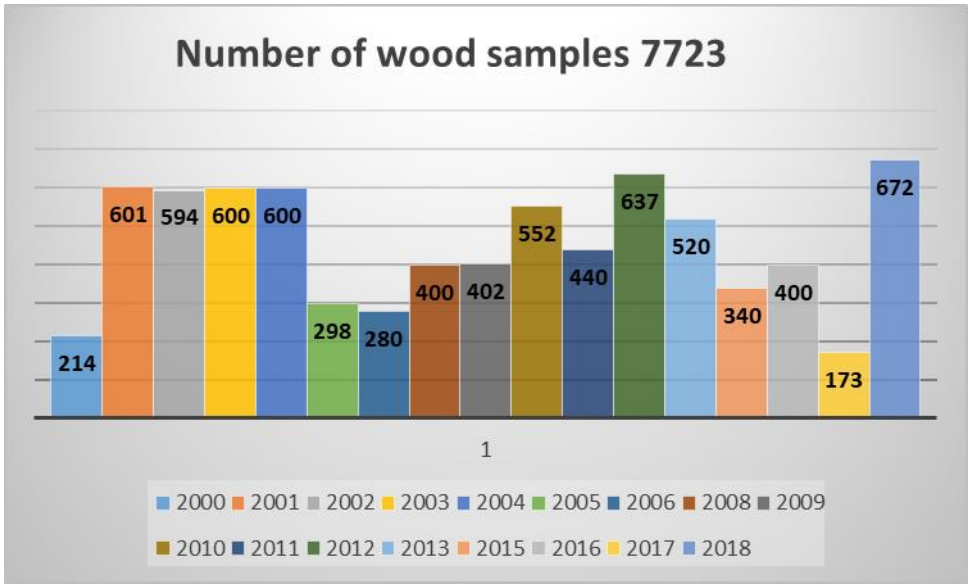

Figure 13. Monitoring pinewood nematode (PWN) *Bursaphelenchus xylophilus* in Norway. Number of wood samples collected in 2000 – 2018.

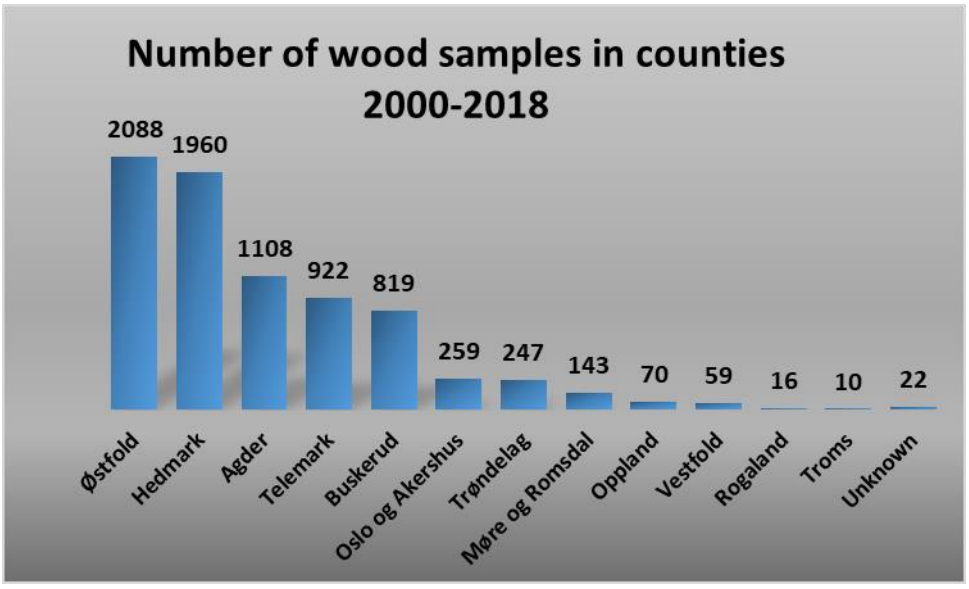

Figure 14. Monitoring pinewood nematode (PWN) *Bursaphelenchus xylophilus* in Norway. Number of wood samples from counties in 2000 – 2018.

Appendix

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 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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