A strategy for delimitation survey in case of an introduction of the pine wood nematode in Sweden

En strategi för avgränsningsinventering om tallvedsnematoden påträffas i svensk skog

- A high quality survey to determine the outer boundaries of an area infested by the pine wood nematode (PWN) is important as it will form the basis for decisions with large consequences.
- A strategy for a delimitation survey is developed taking into account Swedish climate conditions, current forestry operation practices and the biology of beetles vectoring PWN.
- The proposed survey offers the possibility of determining which level of PWN-infested objects that shall be detected and with which statistical reliability. Sampling of *Monochamus*-colonized logging residues on clear-cuts and in pre-commercial thinnings is conducted in zones around the location of the original detection of PWN.

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Cover photo: Martin Schroeder
Female of *Monochamus galloprovincialis*
Hona av kronbock (*Monochamus galloprovincialis*)

Foreword

The Swedish Board of Agriculture is as the Swedish Plant Health Authority responsible for handling detection of plant pests and pathogens that are new for the country and may cause severe damages if established. The capability of handling this kind of situations is currently being developed. One example of such a pest is pine wood nematode, *Bursaphelenchus xylophilus*, that may cause damages to Swedish pine forests if established as well as impacts on trade with wood products.

The specific requirements for control of pine wood nematode in the EU are defined in Commission Decision 2012/535/EU. That Decision includes requirements for all EU Member States to carry out surveys in order to determine whether there is any evidence of the presence of the nematode. Detection of the occurrence of pine wood nematode as early as possible after an introduction would increase the probability of a successful control strategy. In case of a detection, surveying to obtain the delimitation of the area with possible occurrence of pine wood nematode is then one of the crucial steps.

In order to further develop strategies for such a delimitation survey in Sweden, where we primarily expect no expression of wilt symptoms after an introduction of pine wood nematode, several projects financed by the Swedish Civil Contingencies Agency have been set up. This report focuses specifically on sampling efforts during a delimitation survey following a finding of pine wood nematode in a forest area.

In agreement with the Department of Ecology at the Swedish University of Agricultural Sciences that was contracted for the project, it was decided that the report should be written in English and with a summary in Swedish. In this way we offer also a possibility to reach out to an audience outside Sweden as this report addresses a topic that is governed by EU legislation and is thus of interest for the whole EU.

Martin Schroeder professor at the Department of Ecology at the Swedish University of Agricultural Sciences, Uppsala, has written this report and is also responsible for the content.

Karin Nordin

Swedish foreword

Förord

Jordbruksverket är i sin egenskap av Sveriges växtskyddsmyndighet ansvarigt för hantering av upptäckt av växtskadegörare som är nya för landet och som kan orsaka allvarliga skador om de skulle etableras. Förmågan att hantera denna typ av situationer utvecklas för närvarande. Ett exempel på en sådan skadegörare är tallvedsnematoden, *Bursaphelenchus xylophilus*, som kan orsaka skador på svensk skog, i första hand tallskog, och påverka handeln med virkesprodukter.

I EU-kommissionens beslut 2012/535/EU finns särskilda krav för hantering av ett utbrott av tallvedsnematoder. Beslutet innehåller också krav på att alla EU-länder ska utföra inventeringar i syfte att fastställa om tallvedsnematoder förekommer. En så tidig upptäckt som möjligt, efter en introduktion, ökar förutsättningarna för en framgångsrik kontrollstrategi. I händelse av en påvisad förekomst, är inventering i syfte att åstadkomma en avgränsning av ett område med möjlig förekomst av tallvedsnematoder, ett av de avgörande stegen.

I syfte att vidareutveckla strategier för en sådan avgränsningsinventering i Sverige, där vi i första hand inte väntar oss vissnesymptom efter en introduktion av tallvedsnematoder, har flera projekt finansierats genom medel från Myndigheten för Samhällsskydd och Beredskap. Denna rapport fokuserar speciellt på genomförande av provtagning under en avgränsningsinventering efter det att tallvedsnematoder påvisats i ett område med skog.

Institutionen för Ekologi, Sveriges lantbruksuniversitet har kontrakterats för uppdraget. I överenskommelsen beslutades att rapporten skulle skrivas på engelska men med en svensk sammanfattning. På så sätt erbjuder vi också en möjlighet att nå ut till en publik utanför Sverige eftersom den här rapporten tar upp en fråga som styrs av EU-lagstiftningen och därför är av intresse för hela EU.

Professor Martin Schroeder, Institutionen för Ekologi, Sveriges lantbruksuniversitet, Uppsala, har skrivit rapporten och ansvarar också för innehållet.

Karin Nordin

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1 Objectives of the report

The pinewood nematode (PWN), *Bursaphelenchus xylophilus*, is an important forest pest in Asia and now also in Portugal. A pest risk analysis conducted by EPPO concludes that the risk for entry of PWN is high (EPPO 2009a, b). An establishment in Sweden could result in economic losses for forestry and also negatively affect biodiversity and recreational values (Rinman 2008). According to the Commission Decision 2012/535/EU (European Commission 2012) all EU member states are required to adopt contingency plans to be prepared for findings of the presence of PWN. In Sweden such a plan has been developed by the Swedish Board of Agriculture. In a previous report strategies for detection and delimitation surveys of PWN were developed (Schroeder 2012). The objective of the present report was to further develop a strategy for a delimitation survey for PWN. In the report it is assumed that if PWN is established in Sweden it will be asymptomatic (i.e. that an establishment of PWN in Sweden will not result in pine wilt disease) and that it will be detected in the forest.

2 Introduction

2.1 Actions if PWN establish in Sweden

According to the Commission Decision 2012/535/EU (European Commission 2012; hereafter the Decision) member states shall without delay demarcate an area if PWN is detected in a susceptible plant (i.e. tree or part of a tree). The demarcated area shall consist of a zone in which PWN was found to be present (hereafter the infested zone) and a zone surrounding the infested zone (hereafter the buffer zone). The definition of the infested zone shall be based on a delimitation survey. The buffer zone surrounding the infested zone shall be of a width of at least 20 km (could be reduced to 6 km in case of eradication provided that the reduction does not jeopardize eradication). The member state shall take measures to eradicate PWN in the demarcated area. If the annual surveys show the presence of PWN in the demarcated area during a period of at least four consecutive years, and the experience shows that eradication is impossible, the member state may instead decide to contain PWN within the demarcated area. This decision may be taken already before the end of the four year period in case the diameter of the infested zone exceeds 20 km, there is evidence of presence of PWN throughout the infested zone and the experience shows that it is impossible to eradicate PWN in the area.

2.2 PWN and *Monochamus* beetles

PWN is the causal agent of the pine wilt disease (PWD), a serious threat to native pine forests in eastern Asia and Europe (Mamiya 1988, Dwinell 1997, Togashi & Shigesada 2006). The nematode is indigenous to North America where it is never a primary pathogen of native pines. PWN is vectored between host trees by cerambycid beetles of the genus *Monochamus* (Linit 1988, Mamiya 1988, Schröder et al. 2009). The beetles and nematodes develop in conifer trees. Dispersal juveniles of PWN move into the respiratory system of the beetles before they emerge. Directly after emergence the new generation adult beetles feed on bark on living conifers (i.e. maturation feeding). During their life span the beetles will repeatedly feed on living trees. The nematodes leave the beetles and may enter the living trees via the feeding wounds made by the beetles. If it is a susceptible pine species, the climatic conditions are suitable, and the number of transmitted nematodes exceeds a threshold, PWD may develop which generally results in tree death. After feeding the beetles are attracted to dying or recently cut trees, including cutting residues, where they mate. The female beetle excavates egg pits in the bark, in which the eggs are laid, and through which the nematodes may infest the wood. In North America, where PWN does not kill trees, PWN reproduces in cut trees or trees dying for other reasons than PWD. In such a situation (saprophytic life cycle) living trees generally represent a dead end for PWN transmission because *Monochamus* beetles are not able to colonize them (as long as the trees are healthy) and thus there will be no vector to transport the nematode to new host substrates. In contrast, in areas where the nematode is able to kill large number of trees (e.g. Japan, China and Portugal) the beetles, and PWN, primarily reproduce in trees killed by PWN.

2.3 History of PWN in Europe and Sweden

In 1984 PWN was detected by the Finnish Plant Quarantine Service in wood chips imported from North America (Rautapää 1986). As a result, import of untreated conifer wood to Europe from areas infested by PWN was banned. In 1999 PWN was discovered in Portugal where it now has spread to large parts of the country and has killed large numbers of pines. As a result of the establishment in Portugal, yearly PWN surveys in the forest, and of imported wood packaging material, have been conducted in Sweden since 2000. So far more than 2 700 samples from clearing sites in the Swedish forest as well as 1 800 samples originating from Swedish sawmills and paper mills have been processed and all have been negative (Capieau 2014, personal communication). In contrast, PWN has been intercepted at several occasions in wood packaging material imported to Sweden. Since 2008, PWN has been detected at four occasions in Spain, all in dead trees close to the Portuguese border (two times in Extremadura, once in Galicia, and once in Castile and León). After intensive eradication efforts performed by Spain the EU Commission has declared one of the outbreak areas in Extremadura as free from PWN. In 2008 live PWN was also found in wood packaging material used in transport of goods from Portugal to Sweden after which rules for treatment of wood from Portugal were enforced. In 2009 PWN was also recorded from Madeira, Portugal (European Commission 2010).

3 Monochamus species in Sweden

3.1 Species

Three species of *Monochamus*: *M. sutor* L., *M. galloprovincialis* Olivier and *M. urussovii* (Fischer von Waldheim) occur in Sweden. The biology of the species is described in Ehnström & Axelsson (2002) and Ehnström & Holmer (2007). *Monochamus sutor* is distributed all over Sweden and common in many regions. *Monochamus galloprovincialis* is not as common and seems to be mainly distributed in the eastern coastal areas of Sweden. The species is recorded from most parts of Sweden but many of the inland records may instead have been *M. sutor* because it is hard to discern the two species based on morphological characters. In two recent studies identification keys based on male genitalia have been developed which will contribute to better knowledge of the two species distribution in the future (Koutroumpa et al. 2013; Wallin et al. 2013). Both species may occur in the same locality (Schroeder unpublished). *Monochamus urussovii* is a rare species which in recent time only have been recorded from a few localities in the provinces of Ångermanland and Norrbotten in Northern Sweden except for one individual caught in a trap in Uppland in 2012 (Schroeder unpublished). Historical records exist from several provinces. *Monochamus urussovii* and *M. galloprovincialis* are included in the Swedish red list (Gärdenfors 2010). According to Wallin et al. (2013) *M. urussovii* should be considered a subspecies of *M. sartor*, a species distributed in other parts of Europe.

3.2 Breeding substrate

All three species reproduce in newly dead conifer wood: *M. sutor* in both Norway spruce (*Picea abies* L. (Karst.)) and Scots pine (*Pinus sylvestris* L.), *M. galloprovincialis* in Scots pine and *M. urussovii* in Norway spruce. *Monochamus sutor* prefers sun-exposed breeding material (Trägårdh 1929, Schroeder & Lindelöw 2003). Thus, in Sweden the major source of breeding material is constituted by logging residues on clear-cuts (e.g. tops of Norway spruce and Scots pine, and branches of Scots pine) and cut stems of spruce and pine in pre-commercial thinnings. It is generally not found on dead standing trees with the exception of burned trees, which generally are heavily colonized, or high stumps on clear-cuts (Trägårdh 1929, Schroeder et al. 1999). *Monochamus sutor* seems to be a species that is adapted to disturbances like forest fires and storm-fellings. Emergence holes, most probably originating from *M. sutor*, have been recorded from the top of bark beetle killed spruces in one stand in central Sweden (Åke Lindelöw, personal communication). It is not known how commonly this type of substrate is used. Emergence holes have been recorded for substrates with as small diameter as 2.5 cm (Åke Lindelöw, personal communication).

Monochamus urussovii has mainly been recorded within old-growth spruce stands (Ehnström & Axelsson 2002). *Monochamus galloprovincialis* generally attacks branches on dead or dying Scots pine trees in sun-exposed pine forest (e.g. on sandy soils or on rocky grounds) (Ehnström & Axelsson 2002) but has also been found in logging residues in Sweden (Schroeder unpublished) and Finland (Tomminen 1993a). Generally none of the species breed in thinning- or clear-cutting stumps. But, *Monochamus* emergence holes have been recorded from the roots of a sun-exposed fire damaged Scots pine in a stand with very thin soil layer in the province of Gotland in Sweden (Åke Lindelöw, personal communication). The diameter of the colonised parts of the roots was 4 – 5 cm and the roots were partly above the soil.

Several species of *Monochamus* are attracted to host tree volatiles, bark beetle pheromones and a genus-specific *Monochamus* pheromone (Pajares et al. 2013 and references therein). Host volatiles and bark beetle pheromones signal to the beetles the presence of a damaged tree that may be suitable for reproduction. The *Monochamus* pheromone is released by the males to attract females. But also males are attracted. Baits, releasing all these volatiles, are commercially available and can be used in surveys for PWN. All three *Monochamus* species present in Sweden are strongly attracted to these baits (Pajares et al. 2013 and references therein, Gernot Hoch, unpublished).

3.3 Feeding

Directly after emergence from their breeding material adults of all three *Monochamus* species conduct a maturation feeding of bark on branches of living conifers. Maturation feeding by adult *Monochamus sutor* has been observed on both Scots pine and Norway spruce branches and spruce needles in the field (Forsslund 1934) and on branches of both tree species in the laboratory (Schroeder & Magnusson 1992). *Monochamus galloprovincialis* and *M. urussovii* adults presumably conduct most of their maturation feeding on the same tree species as they reproduce in (i.e. pine and spruce respectively) but it cannot be ruled out that they also may feed on spruce and pine respectively. Throughout their life time the adult beetles will repeatedly feed (Schroeder & Magnusson 1992).

3.4 Developmental time

The developmental time of *M. sutor* and *M.galloprovincialis* is generally one to two years, and two years for *M. urussovii*, in Sweden (Ehnström & Holmer, 2007; Schroeder, unpublished). In southern Sweden the one-year developmental time is most common while in northern Sweden the two-year developmental time dominates. A proportion of the adults may emerge later. The adults may be found from May to September with a peak in June and July (Schroeder unpublished). All three species hibernate as larvae.

3.5 Capacity to vector PWN

Of the species present in Sweden *M. galloprovincialis* act as vector for PWN in Portugal (*M. sutor* and *M. urussovii* do not occur in Portugal) while for the other two species no records of association with PWN have been published so far. But it is likely that also *M. sutor* and *M. urussovii* are able to act as vectors for PWN. In Sweden *M. sutor* is associated with the nematode *Bursaphelenchus mucronatus* (Magnusson & Schroeder 1989), a very close relative to the PWN, sharing the same biology but without being pathogenic. In an experiment, *B. mucronatus* was vectored by *M. sutor* to branches of Scots pine and Norway spruce by maturation feeding beetles and to stem sections of both tree species by egg-laying females (Schroeder & Magnusson 1992). In Japan, *M. urussovi* has been demonstrated to be associated with *B. mucronatus* (Togashi et al. 2008). There are examples of other *Monochamus* species which is vectoring both PWN and *B. mucronatus*, e.g., M. *galloprovincialis* (Magnusson & Schroeder 1989) and *M. alternatus*. Thus, it is likely that also *M. sutor* and *M. urussovii* may act as vector for PWN. If this is true a vector which is distributed all over the country (*M. sutor*) is available if PWN is introduced to Sweden. Thus, in the following text the main emphasis is on *M. sutor* as a potential vector of PWN.

3.6 Dispersal biology

An understanding of the dispersal biology of the vector beetles is necessary for estimates of rate of spread of PWN after an introduction. There are three reasons for dispersal in *Monochamus* beetles: finding of living trees for feeding, finding of mates and finding of dying or newly dead trees suitable for egg-laying. Finding of living conifers for feeding will generally not require any long distance dispersal. In contrast, finding of suitable breeding material may require long distance dispersal because newly dead trees are not to be found everywhere, and especially not if they are required to be sun-exposed (preferred breeding material for *M. sutor*). Unfortunately, there is no information about the dispersal capacity of *M. sutor.* In laboratory studies (flight mill) the North American *M. carolinensis* was able to fly up to 10 km during a single flight event (Akbulut & Linit 1999) and *M. galloprovincialis* demonstrated an average distance of 16 km flown over the lifetime of the beetles (David et al. 2014). It is not known how flight performance on a flight mill relates to flight capacity in the field. But the facts that the beetles feed as adults, may survive for several weeks and thus have the opportunity to make several dispersal flights, indicate that they during their lifetime can disperse many km´s. Marc-release-recapture experiments with *M. galloprovincialis* conducted in Spain demonstrated that most beetles could fly at least 3 km and some beetles were recaptured at more than 20 km from their release point (Mas et al. 2013). There is no reason why *M. sutor* should not have about the same dispersal capacity as *M. galloprovincialis*.

3.7 PWN introduction into new areas

The most likely way of introduction is that PWN-infested *Monochamus* beetles emerge from imported wood and fly out in the forest (EPPO 2009a, b). There are several reports of interceptions of living *Monochamus* in imported material (Anonymous 2000), from Austria (living larvae of *Monochamus* spp. and adult *M. alternatus,* (Tomiczek et al. 2003)), France (*M. alternatus* adults, (Christian

Cocquempot personal communication)), Germany (adult *M. alternatus* recorded from wood packaging material originating in China (Cocquempot 2006)). This despite the fact that EU-legislation requires that wood packaging from outside EU (or from Portugal where PWN is established as well as demarcated areas in Spain) must be heat treated to kill accompanying insects (ISPM 15). Another possibility is that PWN could already be established in other European countries without being detected and subsequently spread to more countries via trade of wood within EU (for which treatments to kill insects are not required except for Portugal and the demarcated areas in Spain). Such a scenario is not totally unlikely because in countries where PWD is not expected it may take many years before an establishment of PWN is detected (Økland et al. 2010).

Monochamus beetles emerging from imported wood may introduce PWN into the forest in two different ways: (1) via feeding on the bark of branches on living trees and (2) via attraction to potential breeding material (i.e. newly dead or dying trees or parts of trees). Directly after the emergence from the breeding material the new generation beetles need to do a maturation feeding on the bark of branches of living pines or spruces and thus may transmit PWN to these trees via the feeding wounds. If the transmission results in the development of PWD the nematode population will increase strongly and spread through the dying tree which also will be suitable for colonization by native *Monochamus* beetles. The offspring of these colonizers may then be boarded by PWN before emerging and in their turn kill new trees via transmission of PWN to living trees during their feeding.

If transmission of PWN during feeding on living trees does not result in the development of PWD, as would be most likely the case in Sweden, there is still a possibility that it may result in the establishment of PWN in the living trees. As long as these trees remain healthy they cannot be colonized by *Monochamus* beetles and thus represents a dead end for PWN transmission. But if PWN-infested trees are weakened or die it is possible that the nematodes will multiply and that the trees will be colonized by *Monochamus* beetles which then may be boarded by PWN. Studies in North America have demonstrated that living Scots pine trees which have experimentally been inoculated with PWN can harbour populations of PWN for up to 11 years after inoculation without inciting PWD (Bergdahl & Halik 2003). But, from this experiment it is hard to conclude how likely such a scenario would be in Sweden because the inoculated dose of PWN was very high, they were inoculated directly into the stem of the trees via a 3 cm deep bored hole and several of the inoculated, as well as control trees, died showing that they were susceptible to PWD and stressed. Tomminen (1993b) inoculated *B. mucronatus* in the branches of 10 – 15 year old Scots pine trees in Finland and were unable to extract any nematodes from the branches after one year. To conclude, the possibility of establishment of PWN in living trees in Sweden where we do not expect PWD to develop remains uncertain.

A much more likely way of introduction to Sweden (and other countries where development of PWD is unlikely) is via the sharing of breeding material between introduced beetles (carrying PWN) and native *Monochamus* beetles. The offspring of the native beetles may then be infested and in their turn continue to spread PWN in the native population. Such a scenario does not require successful reproduction of the exotic *Monochamus* beetle. As a result of many different species of *Monochamus* (including all three Swedish species) being attracted to the same attractants (i.e. *Monochamus* pheromone, bark beetle pheromones and host volatiles) there is a risk that exotic beetles will end up on the same breeding material as native beetles. If the exotic beetle is a female PWN may be transmitted to the wood through the oviposition scars. In laboratory experiments even unmated *Monochamus* females readily oviposit despite the fact that no offspring will be produced (Zhang & Linit 1998). Even though unmated females laid fewer eggs than mated they started to oviposit at the same age as mated females and lived longer. Also males may transmit nematodes through bark beetle entrance holes or female *Monochamus* oviposition scars.

To conclude, one single exotic *Monochamus* beetle may be enough to transmit PWN to a dead wood substrate. If the same wood substrate also is colonized by native *Monochamus* beetles their offspring may be boarded by PWN. In the following beetle generations the proportion of native beetles carrying PWN may increase as a result of infested and uninfested parent beetles sharing breeding material.

4 Delimitation survey

The aim of a delimitation survey is to define the outer borders of an area found to be infested by PWN. That the delimitation is correct (i.e. including the total infested area) is of crucial importance because it will be the base for decisions about if to eradicate or to contain PWN. Furthermore, both eradication and containment measures are costly, money that will be wasted if PWN also occur outside the demarcated areas (Eriksson 2013). Such a scenario may involve neighboring countries.

In the Decision there are no specific requirements about how a delimitation survey should be conducted (in contrast to requirements for surveys in demarcated areas subject to eradication or containment) or what kind of substrates that should be sampled. The optimal strategy may also differ between countries and areas.

In the following sections a sampling strategy has been developed for Swedish conditions regarding what to sample and how to sample. Finally, the suggested procedure is applied in a case study.

Directly after detection of PWN an evaluation should be made of the most likely pathway of introduction. Ideally such an evaluation could answer questions about: (1) exactly where the introduction took place, (2) when it was introduced (how many years ago) and (3) from which kind of material it was introduced. Information about the time since introduction is of interest when making assumptions about how far away from the place of introduction PWN may have spread. Information about source of introduction (kind of material and country of origin) could be used for guidance about other possible places of introductions in Sweden and other countries.

When an infested zone has been defined by the delimitation survey it is very important to find out about all transports of potentially PWN-infested wood that have taken place from the infested zone in previous years and evaluate if these may have spread PWN outside the delimited area. These transports may include timber, pulpwood, logging residues for energy purposes, fire wood, wood packaging material, bark and susceptible plants.

4.1 What to sample

In a strict sense according to the Decision an infestation of PWN is defined as infested susceptible plants, i.e. the presence of PWN-infested trees, parts of trees, cut trees or logging residues. Finding of a PWN-infested *Monochamus* beetle in the forest will not, according to the Decision, result in the location being defined as infested, unless a further investigation in the proximity of where the vector was caught proves the presence of PWN in susceptible plants. Thus, the focus of the delimitation survey should be on susceptible plants. Susceptible plants listed in the Decision are (other than fruit and seeds) *Abies* Mill., *Cedrus* Trew, *Larix* Mill., *Picea* A. Dietr., *Pinus* L., *Pseudotsuga* Carr.

and *Tsuga* Carr. In the present study the focus is on Scots pine and Norway spruce which are native and by far the most common of the listed tree species in Sweden. Three types of susceptible plants could be sampled: (1) healthy-looking trees, (2) dead or dying trees and (3) logging residues colonized by *Monochamus*.

Under Swedish conditions, and assuming that *M. sutor* is the most likely vector of PWN, the sampling should be focused on logging residues colonized by *Monochamus*. The advantages and disadvantages of each type are discussed below. In Table 1 - 4 the densities of these substrates in Swedish forest, separated for four different parts of Sweden, are given. These figures can be used for planning the delimitation sampling. Also the benefits of including *Monochamus* beetles in the survey are briefly discussed.

4.1.1 Healthy-looking trees

Sampling of healthy-looking trees cannot be recommended under Swedish conditions. The rationale behind including healthy-looking trees in the sampling is that PWN may be transmitted to such trees during the feeding of adult beetles on the branches. Sampling of living trees is also mentioned in the Decision for sampling in demarcated areas subject to eradication or containment. But, as already mentioned above, good studies demonstrating that PWN really is able to establish in trees without resulting in PWD is still lacking. Other problems are the very large number of living trees available for sampling (Table 1) and that we do not know on which trees the PWN-infested beetles have fed. In addition, nothing is known about if PWN is able to spread within living trees not developing PWD. Thus, if we assume that PWN is able to establish in such trees, they may still only be present in the exact position of the beetle feeding which makes sampling even harder.

Table 1. The average densities of living Scots pines (*Pinus sylvestris*) and Norway spruces (*Picea abies*) per ha of forest land in different parts of Sweden and the national average. Data from the Swedish National Forest Inventory 2008 – 2012.

Region	Pines (per ha)	Spruces (per ha)	Sum (per ha)
Norra Norrland	619	650	1269
Södra Norrland	488	1061	1548
Svealand	616	913	1529
Götaland	310	862	1172
Sweden	519	860	1378

4.1.2 Dead or dying trees

Sampling of dead or dying trees cannot be recommended to be the main focus in a delimitation survey in Sweden. In areas where PWD develops, dead or dying trees is the main substrate sampled for PWN. Although it is not possible just from the appearance of a dying tree to determine if the cause of death is PWN or some other factor (e.g. drought, bark beetles) there is a good chance to detect PWN in this way. But in areas where we do not expect PWD, like in Sweden, sampling of dead or dying trees is less probable to result in findings of PWN. Such trees, if situated in sun-exposed conditions like on clear-cuts or at stand edges, may be used as breeding substrate by *Monochamus* beetles and thus could harbor PWN. But the density of such trees (Table 2), and especially of those colonized by *Monochamus*, is much lower than the density of colonized logging residues.

Table 2. The average densities of newly dead (previous year) Scots pines *(Pinus sylvestris)* and Norway spruces *(Picea abies)* per ha of forest land in different parts of Sweden and the national average. Data from the Swedish National Forest Inventory 2008 – 2012.

Region	Pines (per ha)	Spruces (per ha)	Sum (per ha)
Norra Norrland	0.6	0.3	0.9
Södra Norrland	0.7	0.7	1.3
Svealand	0.7	0.6	1.3
Götaland	0.6	1.2	1.7
Sweden	0.6	0.6	1.3

But, in case of PWN establishment in Sweden dead or dying Scots pine trees should definitively be sampled in the infested zone for evidence of development of PWD. It is not known exactly how many years after tree death that PWN is still present in the wood. Thus, such a survey should focus on dying or newly dead trees.

4.1.3 Logging residues colonized by Monochamus

A delimitation survey conducted in Sweden should focus on logging residues colonized by *Monochamus*. Logging residues on clear-cuts (tops of harvested spruces and pines, and pine branches) and in pre-commercial thinnings (thinned stems of spruce and pine) constitute the major breeding substrate for *M. sutor* in Sweden. Logging residues of pine is also used by *M. galloprovincialis* (even though it is uncertain if this kind of substrate constitutes the major breeding resource for this species). There is a high probability of PWN being transmitted to dead wood by egg-laying *Monochamus* beetles (and probably also by the males). This is the way PWN is vectored in its native range (North America) where it does not cause PWD. As mentioned above it is not known how many years after tree death that PWN is still present in the wood.

The main developmental time for *M. sutor* is one to two years and thus the sampling should be restricted to one to three years (i.e. summers) old logging residues showing signs of *Monochamus* activity.

The advantages with this method are:

- *Monochamus*-colonized wood objects are easy to identify as a result of characteristic larval galleries, larval frass and adult emergence holes.
- Long time period available for sampling (all year around except for when deep snow).
- Many available clear-cuts and pre-commercial thinnings spread out all over Sweden.
- GIS data are available on exactly where clear-cuts are located and with information of their age.
- The annual detection survey in Sweden for PWN is directed to this kind of substrate which means that there is already experience of sampling this kind of substrate.

4.1.4 Monochamus beetles

Trapping of *Monochamus* beetles is an interesting complement to a delimitation survey of logging residues but needs to be followed up by a further investigation verifying the presence of PWN in specified plants (findings of PWN in beetles are not considered as an infestation according to the Decision, see above). With traps baited with the commercially available attractants large numbers of beetles can be caught in a single trap. As a result of the high dispersal capacity beetles caught in a single trap can be assumed to originate from many different localities in the surrounding landscape. The ongoing rapid development of new molecular methods for detection of PWN in beetles will make it possible to make one sample of several beetles which will reduce costs for analyses. Thus, traps may be used outside a delimited area as an extra tool to analyze whether beetles are infested with PWN. If PWN is detected in beetles caught outside a delimited area this is a strong indication of that also PWN-infested host plants may be present there.

Experiments conducted in Sweden, Spain and China have demonstrated that the commercially available bait is as attractive for *M. sutor* as for *M. galloprovincialis* (Pajares et al. 2013). Another study conducted in Sweden demonstrated that the catches are considerably higher on clear-cuts than within forest stands (Schroeder unpublished). Preferably, the trapping should be conducted in the early part of the *Monochamus* flight period before most PWN have left the beetles.

4.2 Sampling strategy

The suggested sampling strategy is based on the survey procedures prescribed for eradication and containment in the Decision. This means that: (1) a statistical reliability of the survey is chosen and (2) the infestation level (i.e. proportion of objects with PWN) at which PWN shall be detected is chosen. In the case of surveys in a PWN infested zone subject to eradication or containment measures the Decision states a reliability of 99% and detection levels of 0.1% and 0.02% respectively. Tables of the sample sizes needed to achieve the chosen levels of reliability and detection levels, as well as an explanation of the statistics behind these tables, can be found in a publication from the European Food Safety Authority (EFSA 2012). The great advantage of this statistical-based approach compared with a non-statistical approach is that the decision-makers have a much more solid base for interpreting the result when PWN is not recorded from a zone, i.e. how sure can we be that the result represents a true absence.

In the case of a delimitation survey the sampling will be conducted in a zone around the location/locations where PWN was first detected. If PWN is detected in the zone, a new zone needs to be established surrounding the first zone. This would follow the logic presented in EPPO standard PM 9/1 on procedures for official control of PWN and its vectors (EPPO 2012). The number of samples needed to achieve the chosen reliability and detection level depends on the total number of objects in the zone. In this case logging residues colonized by *Monochamus* constitutes the population of objects which shall be sampled. The number of such objects in a given location will depend on:

- The area of final cuttings and pre-commercial thinnings in the last three years.
- The area of clear-cuts from which logging residues have been removed to be used for energy purposes.
- The density of tops or stems of spruce and pine, and of branches of pine, with a diameter > 5 cm (although *Monochamus* may colonize thinner objects most of the population could be assumed to breed in objects with a diameter > 5 cm) in the remaining areas.
- The proportion of these substrates colonized by *Monochamus*.

On clear-cuts branches and tops may be removed for energy purposes. This may reduce the population of objects available for sampling (if objects removed before sampling). In Table 3 estimates on the proportion of clear-cuts from which tops and branches have been removed is given for different parts of Sweden. The situation may vary a lot locally and thus in case of a delimitation survey the actual values need to be retrieved.

Table 3. The average percentages of clear-cuts and pre-commercial thinnings from which logging residues have been removed for energy purposes in different parts of Sweden and the national average. Data from the Swedish National Forest Inventory 2009 – 2012.

Region	Clear-cuts (%)	Pre-commercial thinnings (%)
Norra Norrland	17	0
Södra Norrland	20	0
Svealand	27	1
Götaland	44	0
Sweden	27	0

In Table 4 data are presented on the densities of tops on clear-cuts (assuming one top per cut tree) and of stems cut in pre-commercial thinnings in different parts of Sweden. There is no statistics available of densities of pine branches (remaining from cut trees) on clear-cuts. Thus, in the following the density of pine branches is assumed to be twice as high as for pine tops (based on a sampling conducted on six clear-cuts in the province of Uppland in Sweden; including branches with a diameter exceeding 5 cm; Schroeder unpublished).

Table 4. The average densities of tops on clear-cuts and of thinned stems in pre-commercial thinnings of Scots pines *(Pinus sylvestris)* and Norway spruces *(Picea abies)* per ha in different parts of Sweden and the national average. The data for tops is based on number of harvested trees (i.e. one top per harvested tree). Data from the Swedish National Forest Inventory 2008 – 2012.

Region	Clear-cuts		Pre-commercial thinnings	
	Pine tops (per ha)	Spruce tops (per ha)	Pine stems (per ha)	Spruce Stems (per ha)
N Norrland	252	334	128	33
S Norrland	219	469	105	94
Svealand	131	465	167	164
Götaland	93	383	78	167
Sweden	176	414	119	124

There are no published data on the proportion of logging residues colonized by *Monochamus*. Thus, a pilot study was conducted in the province of Uppland in Sweden (Schroeder unpublished). Six clear-cuts and 11 pre-commercial thinnings were included in the study. On each clear-cut 100 tops of pine or spruce, and 100 branches of pine (all with a diameter exceeding 5 cm) were inspected for the presence of *Monochamus* galleries. A total of 401 pine tops, 203 spruce tops and 585 pine branches were inspected. On average 14% (SE[1] = 10%) of the pine tops, 16% (SE = 6%) of the spruce tops and 6% (SE = 3%) of the pine branches were colonized by *Monochamus* (based on the six replicates/clear-cuts). Because the proportions of colonized pine and spruce tops were not significantly different the average (i.e. 15%) is used in the following practical example.

1 Standard Error

On each of the 11 pre-commercial thinnings up to 100 stems of spruce or pine were inspected. In pre-commercial thinnings generally the whole trees are left (because of small diameter) and thus available for inspections. A total of 606 pine stems and 449 spruce stems were checked. On average 8% (SE = 2%) of the stems were colonized by *Monochamus*.

Because these surveys were conducted in only one region it should be noted that the proportions could be different in other regions in Sweden. Thus, in case of a delimitation survey the actual values should be recorded during the sampling (see below).

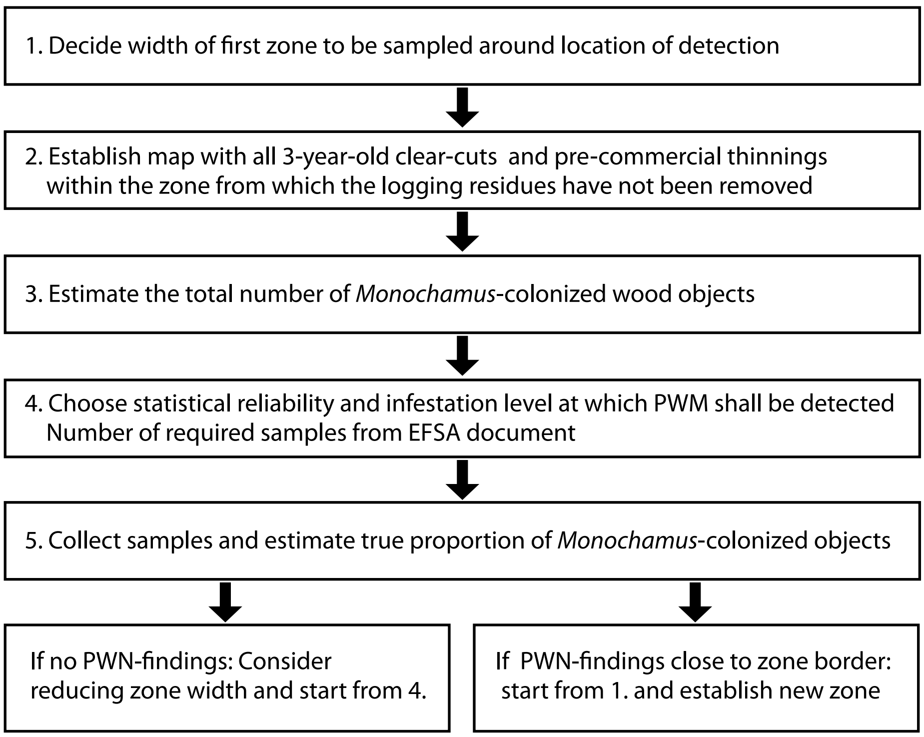

Figure 1. Schematic description of the most important steps when conducting a delimitation survey after an initial PWN detection.

4.2.1 Determining the width of sampling zone

There are no rules or recommendations in the Decision about the radius of the zone to be sampled around a PWN detection during the delimitation survey. Factors in favor of a radius larger than 10 km are:

- The high dispersal capacity of *Monochamus* beetles (see above).
- That individual beetles may transmit PWN in several different locations in a single season.
- That the probability of early detection under Scandinavian conditions is extremely low (Økland et al. 2010) which means that PWN has most probably been established for several years when detected.

- That the Decision states that containment to prevent the spread of PWN, instead of eradication, may be an alternative in case the diameter of the infested zone exceeds 20 km, there is evidence of presence of PWN throughout the infested zone and the experience shows that it is impossible to eradicate PWN in the area. This is important, because in the case of containment no clear-cuttings are required around infested susceptible plants (after eradication strategy has been seriously considered or eradication measures have been attempted under a certain period of time and proven to be ineffective).

With increasing radius there will be a dilution effect of the sampling (i.e. a decreasing proportion of all possible sampling objects will be included in the survey). Thus, a 10 km radius could be a good choice for the first zone to be sampled.

4.2.2 Estimate of size of population of objects to be sampled

The first step will be to establish a map over all clear-cuts and pre-commercial thinnings from the last three years in the landscape surrounding the location or locations of detection of PWN. The Swedish Forestry Agency has information such as location, age and size about all clear-cuts and should be able to get information about most pre-commercial thinnings. They should also be able to retrieve information about clear-cuts from which logging residues have been removed and maybe also about over how large proportion of the logging residues that have been removed from these clear-cuts.

In the second step regional averages for densities (per ha) of spruce and pine tops on clear-cuts, and number of cut pines and spruces in pre-commercial thinnings will be multiplied with the areas without removal of logging residues. Such averages are available from the Swedish National Forest Inventory at the Swedish University of Agricultural Sciences. In Table 1 figures for four different parts of Sweden are presented. When it comes to the number of pine branches on clear-cuts there are unfortunately no statistics available. Thus, based on a pilot study conducted within this project in the province of Uppland in Sweden this figure is assumed to be 2 branches per pine top on clear-cuts before removal (Schroeder unpublished).

In the third step estimates of the proportion of objects colonized by *Monochamus* is required. Such information is at present not available except for a pilot study conducted within this project in the province of Uppland in Sweden (see above). Thus, until more information about possible differences for this variable between regions in Sweden is obtained, the figures for Uppland have been used in this report. However, by keeping track of how many wood objects that are checked for *Monochamus* colonization during the field sampling (initiated in the delimitation survey) the correct figure for the actual sampling zone will be obtained.

4.2.3 How to sample

Because it could be that the first detection of PWN happens to be at the edge of the infested zone the clear-cuts and pre-commercial thinnings chosen to be sampled should be distributed all over the sampling zone.

When analyzing the collected samples for PWN this should be done by starting with the samples collected in the outer part of the zone. If PWN is detected in such a sample it is not necessary to analyze the remaining samples but instead invest efforts in looking for further PWN infestations in areas beyond the new detection. This process is continued until no more PWN findings are recorded. This will reduce the costs for the laboratory analyzes. However, not analyzed samples can be stored if later on there is an interest of studying the frequency of PWN infested wood objects within the infested zone.

If several wood objects are sampled in the same location these samples can be pooled before analyzes (as long as the probability of detection of PWN if present is not reduced) because the delimitation survey only aims at delimiting the infested zone (i.e. not to estimate the proportion of infested objects).

If PWN is detected in Sweden it is also important to find out which *Monochamus* species that act as vector. Of the three native species only *M. galloprovincialis* has been demonstrated to vector PWN so far even though it is likely that also the other two species have this capacity. Thus, if it could be demonstrated that also *M. sutor* and/or *M. urussovii* act as vector this would be important information. In addition, the biology of the three species differs somewhat which could influence control measures. In addition to the native species there is also a possibility that a non-native *Monochamus* species has established and act as vector which in that case would result in eradication measures for this species. The identity of the vector can be demonstrated by rearing of *Monochamus* adults from the infested zone, by molecular analyzes of larvae in wood and by trapping of beetles.

During the field sampling of wood it could also for each object be noted if emergence holes of *Monochamus* are present. If PWN is detected from wood objects with emergence holes this implies that infested beetles may have dispersed to other parts of the surrounding landscape. This also applies to the yearly detection surveys.

4.3 Practical example

In the following example PWN is assumed to have been detected in a forest in the province of Uppland on a clear-cut with a size of 5 ha during the yearly national detection survey. The whole clear-cut is immediately declared as an infested zone before the delimitation survey is initiated. This means that a sampling zone, for the delimitation survey, will be established around the perimeter of the clear-cut. It is decided that the width of this sampling zone should be 20 km. This corresponds to an area of 127 183 ha (circular area width a radius of 20 126 m, 126 m being the radius of the clear-cut, minus the area of the clear-cut where PWN was detected). It is assumed that 70% of the sampling zone is covered by forest, that 1.94% of the forest area has been clear-cut, that 1.93% has been pre-commercially thinned during the last three years (based on national averages) and that the logging residues have been removed from 23% of the clear-cuts (Table 3). In a real case these figures would instead be retrieved for the actual area. Thus, we end up with 1 263 ha of clear-cuts and 1 717 ha of pre-commercial thinnings. By applying the national averages for densities of tops on clear-cuts (Table 4), and the assumption of twice as many pine branches as pine tops, and stems in pre-commercial thinnings (Table 4) this sum up to 745 211 tops, 444 601 pine branches and 417 134 thinned stems. According to the pilot studies (see above) 15% of the tops, 6% of the pine branches and 8% of the thinned stems can be expected to be colonized by *Monochamus* (i.e. 111 782 tops, 26 676 branches and 33 371 thinned stems) summing up to 171 828 colonized wood objects in the sampling zone. After the sampling has been conducted the figures for percentage of logging residues colonized will be adjusted to reflect the situation in the actual area. By applying table 5 in EFSA (2012) the sample size required is 4 570 for a detection level of 0.1% and 22 200 for a detection level of 0.02% with a reliability of 99%. A strong reduction of the estimated number of colonized wood objects (e.g. as a result of a much smaller sampling zone, much lower percentage of colonized wood objects or higher proportion of removed objects for energy purposes) only results in a modest change in number of required samples. For example, if the number of colonized wood objects is 10 000, the sample size required is 4 060 for a detection level of 0.1% and 13 500 for a detection level of 0.02% (with a reliability of 99%). The same is also true if the population of wood objects to sample from is much larger, e.g. > 100 000 the sample size required is 4 603 for a detection level of 0.1% and 23 024 for a detection level of 0.02%.

5 Gaps of knowledge

How much does the proportion of *Monochamus*-colonized logging residues vary between regions in Sweden? Rough estimates would be useful in the initial stage of the delimitation survey for determining the number of samples required. The expectation is that the proportions are higher in the north than in the south of Sweden. This information can be retrieved by repeating the field study already conducted in the province of Uppland in other parts of Sweden.

What are the absolute densities of *Monochamus* beetles and how does it vary between regions? This information could be used in the same way as for wood substrates for determining the number of caught beetles required to achieve a certain level of reliability at a certain detection level.

Is it possible to predict proportions of objects colonized, and beetle densities, based on landscape characteristics (e.g. tree species, forest cover, area covered by clear-cuts)? The expectation is that the population levels of *M. sutor* are higher in landscapes with high conifer forest cover and high proportions of forest land constituted by recent clear-cuts and thinnings from which beetles have been produced in logging residues. This can be tested by sampling either logging residue or beetles (with pheromone-baited traps) in different kinds of landscapes. Predictive model would be useful in the planning of delimitation survey.

Is the presence of PWN in *Monochamus*-colonized logging residues influenced by type of substrate, e.g. tree species, top or branch, diameter and presence of blue-stain fungi? If there are differences they should be accounted for when prioritizing which kind of logging residues that should be sampled. The populations of PWN can be expected to be affected by e.g. the moisture content in the wood because they feed on fungi. This could be tested under Nordic conditions by checking for *B. mucronatus* in different kinds of substrates because also *B. mucronatus* feed on fungi.

For how many years after *Monochamus* colonization can PWN be detected in logging residues? This is very important information because if present many years the number of year-classes of clear-cuts to sample could be extended.

What is the distribution of *M. galloprovincialis* in Sweden? If there is a difference in the ability between *M. sutor* and *M. galloprovincialis* to vector PWN it is important to know their distribution areas. Distribution areas can be checked by pheromone-baited traps.

How rapidly would PWN spread if introduced in an area without development of PWD?

How large proportion of the vector beetles would carry PWN in such a situation?

Can PWN survive in healthy living trees under Swedish conditions? If so, for how many years will it survive in living trees and will it be able to spread within the tree from the point of entry (i.e. the feeding place of the vector beetle)?

6 Conclusions

In case of a detection of PWN in Sweden the first step will be a delimitation survey defining the infested zone as one of the measures to prevent further spread of PWN. It is important that the quality of the survey is high because it will form the basis for decisions with large economic consequences (such as eradication or containment). In the present report a strategy for such delimitation survey in a forest area, is developed for Swedish conditions where we do not expect the development of PWD and taking in account current forestry operation practices. The proposed survey offers the possibility of determining which level of PWN infestation that shall be detected and at which statistical reliability. With such an approach the decision-makers have a much more solid base for interpreting the result when PWN is not recorded from a zone, i.e. how sure can we be that the result represents a true absence. This approach requires an estimate of the size of the population of objects to be sampled in the sampling zone. The sampling should focus on logging residues from clear-cuts and pre-commercial thinnings colonized by *Monochamus*. A procedure for how to estimate the number of such objects in a forest area is described.

7 Swedish summary Svensk sammanfattning

Den första åtgärden att utföra om tallvedsnematoden upptäcks i Sverige, blir en inventering för att definiera gränserna för det angripna området (en så kallad ”avgränsningsinventering”). ”Det angripna området” är det område där nematoden har etablerat sig. Det är viktigt att kvalitén på denna inventering är hög, eftersom den utgör grunden för beslut som har stora ekonomiska konsekvenser (bl.a. beslut om åtgärder för utrotning eller inneslutning).

Rapporten innehåller ett förslag till en strategi för hur en sådan inventering ska utföras under svenska förhållanden, där vi inte förväntar oss att tallvedsnematoden ska döda träd. Förslaget i rapporten erbjuder en möjlighet att definiera hur omfattande angreppet av tallvedsnematod ska vara för att kunna upptäckas och med vilken statistisk tillförlitlighet som det går att fastställa det angripna områdets storlek. I det fall tallvedsnematod inte påträffas i det inventerade området, ger den föreslagna strategin beslutsfattarna ett bättre underlag för att tolka hur säkert det är att resultatet representerar en sann frånvaro av tallvedsnematod.

Under inventeringen är det lämpligast att ta prover på avverkningsrester från slutavverkningar och röjningar som är koloniserade av tallbockar. Det är då nödvändigt att uppskatta hur många objekt som det ska tas prover från.

Rapporten beskriver en procedur för inventeringen.

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