Delimiting of biological invasions

– an overview of strategies and tools with pine wood nematode as a case study

- This report presents general Pros and Cons of different strategies and survey tools for delimiting surveys of invasive species.
- If pine wood nematode was to establish in Sweden, it is most likely that it would have time to spread over a considerable area before it would be detected.
- The strategy for delimiting survey of pine wood nematode in Sweden should be to survey sequential zones around the detection point until there are no more records of the species.

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The Swedish Board of Agriculture is as the Swedish Plant Health Authority continuously developing its capability for handling detection of plant pests and pathogens that are new for the country and may cause severe damages if established. One example of such a pest is pine wood nematode Bursaphelenchus xylophilus that if established may have significant impact on Swedish pine forests and trade of wood products.

The binding requirements for control of an outbreak of pine wood nematode in the EU are specified in Commission Implementing Decision 2012/535/EU. This Decision includes requirements for all EU Member States to carry out surveys in order to detect any occurrence of pine wood nematode as early as possible after an introduction and in case of an outbreak to delimit the geographical area, which must be subject to official measures.

One crucial aspect in a contingency plan for pine wood nematode and other quarantine pests, is how to delimit the infested area. A specific project was set up, in order to further develop strategies for such delimiting surveys using pine wood nematodes as a case study. The task to carry out the study was given to professor Martin Schroeder at the Department of Ecology at the Swedish University of Agricultural Sciences, Uppsala. Professor Schroeder is responsible for the contents of this report. The conclusions are not the official position of the Swedish Board of Agriculture. The project was financed by the Swedish Civil Contingencies Agency. This report includes the results of the project.

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Sammanfattning

När en för landet ny växtskadegörare upptäcks så är första åtgärden att definiera gränserna för artens förekomst. Detta görs genom en så kallad ”avgränsningsinventering”. Det är viktigt att kvalitén på denna inventering är hög, eftersom den utgör grunden för beslut som kan medföra stora ekonomiska och ekologiska konsekvenser. Det kan vara t.ex. beslut om åtgärder för utrotning eller inneslutning och det är avgörande att dessa åtgärder dimensioneras rätt. I rapporten ges först en överblick över de olika strategier och metoder som kan användas vid en avgränsningsinventering. Rapporten avslutas sen med ett förslag på hur en avgränsningsinventering skulle kunna utföras.

Med strategier avses hur man rumsligt och tidsmässigt lägger upp en inventering medan metoder avser på vilket sätt man identifierar arten när man bestämt var man skall leta efter den. Större delen av rapporten ägnas åt tallvedsnematoden *Bursaphelenchus xylophilus* som en fallstudie. Den innefattar en detaljerad beskrivning av tallvedsnematodens biologi, på vilka sätt den skulle kunna bli introducerad, etablera sig och sprida sig i Sverige, och vad konsekvenserna skulle kunna bli av en etablering.

Tallvedsnematoden är inhemsk i Nordamerika där den huvudsakligen lever saprofytiskt i nyligen döda barrträd och därför inte är någon viktig skadegörare. Den är för sin spridning mellan träd helt beroende av tallbockar (skalbaggar av släktet *Monochamus*) som också utvecklas i nyligen döda barrträd. Tallvedsnematoden har etablerat sig i Japan, Kina, Sydkorea och Portugal där den dödar stora mängder av inhemska tallar. Det troligaste sättet som den skulle kunna sprida sig till Sverige är genom träemballage med levande tallbockar och tallvedsnematoder. Om tallvedsnematoden skulle lyckas etablera sig i Sverige är det troligaste att den i vårt klimat inte skulle döda tallar i någon större utsträckning. Den troligaste vektorn i Sverige är vanlig tallbock *Monochamus sutor* som förekommer i hela landet.

I rapporten föreslås strategin för avgränsningsinventering av tallvedsnematoden i Sverige vara att inventera ett antal zoner kring den första fyndplatsen tills man inte längre påträffar arten. Det vill säga avgränsning via dokumenterad frånvaro i en zon kring det ”smittade” området. Denna metod erbjuder också en möjlighet att för varje zon anpassa antalet prov till en vald statistisk signifikansnivå och detektionsnivå. Eftersom tallvedsnematodens vektorer (tallbockar) sprider sig över stora områden, och det mest sannolika är att tallvedsnematoden redan är spridd över ett stort geografiskt område när den upptäcks, föreslås radien på den första zonen vara i storleksordningen 25 km. Eftersom tallvedsnematoden inte förväntas döda träd i Sverige bör provtagningen istället huvudsakligen vara inriktad på avverkningsrester på hyggen (toppar och grenar) som visar spår av att vara koloniserade av tallbockar. Vedprover från sådana objekt skickas till laboratorium för analys av förekomst av tallvedsnematod. Som ett komplement bör även fällor betade med doftämnen som lockar tallbockar användas. De fångade tallbockarna analyseras för förekomst av tallvednematod.

Summary

In case of a detection of an invasive pest species in Sweden the first step will be a delimiting survey defining the infested area. It is important that the quality of the delimiting survey is high because it will form the basis for decisions that can have large economic and ecological consequences. It can concern e.g. decisions about eradication or containment and it is crucial that they are dimensioned correctly. The report gives an overview of strategies and survey tools used in delimiting surveys.

Strategy denotes the principle after which the spatial and temporal allocation of sampling resources is decided (i.e. where and when to search for the species) while survey tools denotes the methods used for documenting presence of the species in a locality chosen for survey (e.g. traps or visual inspections). Special focus is on the pine wood nematode *Bursaphelenchus xylophilus* (PWN hereafter) as a case study. A detailed description of the biology of PWN and its vectors, in which ways PWN may be introduced, establish and spread in Sweden, and how a delimiting survey could be conducted is given in the report.

PWN is native in North America where it mainly develops in newly dead conifers (saprophytic life cycle) and thus not is an important pest. It is totally dependent on pine sawyers (beetles of the genus *Monochamus*), which also breed in newly dead conifers, for its spread between trees. PWN has established in Japan, China, South Korea and Portugal where it also kills large numbers of native pine trees. The most probable way of introduction into Sweden is by wood package material infested by PWN and its vector. In Sweden the most likely scenario is that PWN would not be an important tree killer, because of the rather cool summers. The most probable vector in Sweden would be *M. sutor*, which is common in large parts of the country.

The suggested strategy for delimiting a PWN invasion in Sweden is by survey of sequential zones around the detection point until the species is not recorded anymore (i.e. delimiting by documented absence in a zone surrounding the infested area). This strategy also offers the possibility to adapt the number of samples to a chosen level of statistical reliability and detection level. Because the vector beetles disperse large distances and PWN is not expected to kill large number of trees, it is most likely that PWN is already established over a considerable area before being detected. Thus, a radius of 25 km is suggested for the first zone around the point of detection. Because PWN is not expected to generally kill trees in Sweden the sampling should instead be directed towards logging residues (tops and branches) on clear-cuts with signs of vector colonization. Wood samples from such objects are analyzed in laboratories for the presence of PWN. Trapping of vector beetles in traps baited with attractants is used as a complement. Also the beetles are analyzed at laboratory for PWN.

Innehåll

1 Background and aim of the study

Surveillance of invasive species is a crucial component of prevention and management of biological invasions. Surveillance programs have different objectives at different stages of invasion. They may be conducted to

(i) demonstrate that an area is free from a particular species before an invasion has been detected,

(ii) detect introductions and/or establishments as early as possible,

(iii) delimit the infested area after an establishment has been detected (as a base for decisions about eradication or containment measures) and

(iv) prove absence after eradication programs or absence in a buffer zone around an infested area in case of containment.

This study focus on the delimiting of the infested area after an invasive species has been detected. The aim of a delimiting survey is to establish the boundaries of the area in which a specific non-native species has established. Generally, a delimiting survey is conducted after an invasive pest has been recorded in a detection survey or by chance. It is a crucial part in any eradication or control program by ensuring that mitigation efforts are directed to the entire pest population (or at least most of it considering that Allee effects may lead to extinction at low densities) while not wasted in locations far outside the infested area (Leung et al. 2010). The results of the delimiting survey may also be used for assessments of eradication feasibility or for planning of alternative strategies like containment, slowing down the expansion of infested area and mitigation efforts to reduce damages within infested area (Tobin et al. 2013, Kean et al. 2015). It is important that the delimiting of biological invasions is as accurate as possible and thus considerable efforts should be spent on their design. The problem can be conceptualized as a trade-off between the probability of unsuccessful eradication (or control) if the delimited area underestimates the true infested area (i.e. the invader is also present outside the delimited area) and wasted mitigation resources if the delimited area is too large (i.e. the true infested area is much smaller). In addition, it is also important that the delimiting is rapid because the invasive species may continue to spread during the delimiting survey.

When planning the response to an invasion the first step may be to decide the size of the budget that can be spent and the second to decide how resources should be divided between the delimiting survey and the subsequent eradication or control measures. One way of deciding the budget is by conducting a cost – benefit analysis in which the costs for delimiting surveys and control measures are balanced against the predicted economic gains (i.e. reduction in damages caused by the pest) if action is taken. Alternatively, the budget may already be fixed as a result of a budget constrain decided on beforehand. When a total budget is decided the second step is to decide how to allocate the money between the delimiting survey and the subsequent eradication or control measures. Different methods may be used to optimally allocate the resources between these activities (e.g. Bogich et al. 2008, Yemshanov et al. 2017a, b).

Neither the first nor the second step will be covered in the following text. Mainly because it in many cases is hard to predict several of the parameters required for such analyses like e.g. the damages caused if established, and costs and not the least the efficiency of control and eradication measures. Instead, the focus will be solely on strategies and tools that can be used in delimiting surveys. Delimiting of infested areas is generally based on surveys generating data on presence/absence, or densities, of the invasive species across space. Different strategies and survey tools can be used in such surveys. In the following, strategy denotes the principle after which the spatial and temporal allocation of sampling resources is decided (i.e. where and when to search for the species) while survey tools denote how presence, and density, of a species in a locality chosen for survey is determined (e.g. by traps or by visual inspections of host trees).

The aim of the study is to review the literature on different strategies and survey tools that can be used for the delimiting of the infested area after detection of a non-native species. Special focus will be on the pine wood nematode *Bursaphelenchus xylophilus* (PWN hereafter). Because the choice of strategy and survey tools to a large extent depends on the biology, population build up after establishment and spread of the organism after establishment these subjects are discussed in detail for PWN and its beetle vectors. The study is a further development of two earlier reports on this subject (Schroeder 2012, 2014).

2 Delimiting survey strategies

Generally it takes several years after establishment before non-native species are detected. But, in extreme cases such species may be detected early after establishment. These two cases require different delimiting strategies.

2.1 Early detection

In the most favorable case the establishment is detected early after introduction. If so, it may be possible to trace back exactly when and where the species was first introduced. Estimate of the species´ dispersal capacity, in combination with the short time since introduction, and known habitat preferences can be used for deciding at which distance from the detection site a delimiting survey should be conducted and in which habitat types (Gust & Inglis 2006, Hauser et al. 2016). In some cases detailed knowledge of the species´ dispersal behavior, trap efficiency (Mangel et al. 1984) and meteorological data can be used to predict the infested area (Guichard et al. 2012). Also the possibility of human-mediated dispersal from the area of establishment should be considered. It might also be possible to determine the pathway of the introduction (i.e. how the species was introduced), especially if the exact point of entry is known. Pathway information may be used to estimate the risk that other introductions in other locations already have occurred via the same pathway and to prevent subsequent introductions.

2.2 Late detection

Unfortunately, early detection is rarely the case. There is generally a considerable time lag between a species´ establishment and detection which means that the invasion pathway, the first locality of establishment and time (i.e. number of generations) for subsequent population increase and spread is uncertain. In addition, capacity of dispersal and population growth is generally unknown for species in novel environments which further add to uncertainty about how large the infested area may be. Furthermore, long distance dispersal events (human mediated or natural) and variation in habitat suitability may result in populations that are patchily distributed with a fragmented and irregular range limit. Two main strategies that may be applied for determining the boundaries of an invaded area by sampling are:

1. to document absence in a zone around the infested area and
2. to utilize a declining density of the invader towards the range limit to estimate the location of the range boundary.

Several other approaches have also been proposed. Below follows short descriptions of these strategies and approaches.

2.2.1 Delimiting by documented absence in a zone around the infested area

2.2.1.1 Evaluation based on the rate of discovered new occupied localities

This approach was suggested by Panetta and Lawes (2005) for invasive weeds in Australia. They used two indicators for deciding if the total, or close to total, infested area had been documented. The first one is based on to what extent new occupied localities are found over time during a survey. If no new localities are discovered for a certain period (not specified) despite search efforts it indicates that the total extent of the invasion has been determined (assuming efficient survey tools). The second indicator utilize the change in detection rate (i.e. detected infested area per area searched) over time during the survey. A strong decrease to a low detection rate indicates that the major part of the infested area has been delimited.

2.2.1.2 Evaluation based on selected statistical confidence and infestation level

This approach has earlier been suggested for PWN in Sweden (Schroeder 2014). The method allows for a statistical estimate of the number of samples required for a certain statistical reliability of the results at a chosen infestation level to be detected. The strategy is adopted from the survey procedures prescribed for eradication (to prove absence after eradication measures) and containment (to prove absence in a buffer zone around the infested area) of PWN in EU (EFSA 2012, European Commission 2012). To be applicable, the size of the target population to be sampled must be known or estimated.

2.2.2 Utilizing a declining density of the invader towards the range boundary

This approach, suggested by Leung et al. (2010), is based on probability and sampling theory and continuously uses data collected during a survey for inferences about the extent of the invasion. The method (denoted ADD hereafter) is based on three steps: (1) Approach towards the boundary, (2) measure the Decline in density of occurrences when moving towards the edge of the invasion and (3) use the rate of decline to Delimit the invasion.

(1) Approach, moving towards the boundary: Localities along a transect, starting from the point of the first detection, are surveyed. The distance between localities to be inspected may be adapted to assumptions of the size of the infested area. If invaded area is assumed to be large it would be a waste of resources to use small distances between searched localities and instead an exponential model could be used (e.g. 2, 4, 8, 16 distance units). When an unoccupied locality is recorded the search may be moved back until an occupied locality is found. This procedure is repeated in four cardinal directions. When the epicentre is unknown the second transect may be started at the midpoint of the first transect.

(2) Decline, estimating the proportion of invaded localities: To find the last invaded locality is difficult because many gaps in distribution and a low infestation level are expected when approaching the boundary. This problem is addressed by estimating the proportion of localities invaded at a given distance from the epicentre (with a new sampling). Guided by Bayesian statistics the sampling can be moved out to farther distances, with a desired degree of confidence, each time an invaded site is found.

(3) Delimit, fitting the rate of decline: Where the number of invaded localities is very low, which may be expected at the boundary, an unrealistic large sampling effort may be required to find even a single invaded locality. This problem can be approached by fitting a curve to the estimated (in stage 2) densities of occurrences at each distance.

The performance of ADD was tested in a modelling exercise and the method was less accurate when densities are low (i.e. low proportion of localities with occurrence) and for long distance kernels (i.e. when a few occupied localities exist far away from the main distribution area). There are so far no published studies in which ADD has been applied for real biological invasions. In addition, it is unclear to what extent biological invasions really are characterized by "regularly" declining densities when approaching the invasion boundary.

2.2.3 Allocation of survey resources based on other principles

2.2.3.1 Adapt survey to legislative mandatory responses

For some regulated invasive species the specific mandatory actions that must be taken after detection depends on the size of the infested area. For example, for the PWN it is stated that containment may be an alternative to eradication if the diameter of the infested area exceeds 20 km, there is evidence of the presence of PWN throughout the infested zone and the experience gathered shows that, in the situation concerned, it is impossible to eradicate PWN in that area (European Commission 2012). This is important, because in the case of containment no clear-cuttings are required around each record of PWN in the infested area. Thus, in such cases it might be a good idea to initially focus on establishing if the species already have spread beyond such regulated limits to avoid unnecessary costs.

2.2.3.2 Allocation of resources based on prior knowledge of human-mediated spread

If long distance spread by human transportation is an important factor, this should be taken into account when planning a delimiting survey. In this case there could be both a short distance natural spread from the core area and human-mediated establishments of isolated populations far from the core area to consider (Tobin et al. 2013, Yemshanov et al. 2014, Yemshanov et al. 2015).

2.2.3.3 Initial study for increasing efficiency of a subsequent delimiting survey

The probability of detection of an invasive species within its infested area may to a large extent depend on the habitat types included in the survey. The species may have a higher probability of colonizing, occur in higher abundances and/or be more easily detected in certain habitat types. Thus, in cases when there is little knowledge about these relationships it may be a good idea to initially invest resources to increase the knowledge about in which habitats to most efficiently search for a specific invasive species and not only select location that are initially assumed to be the best (Gust & Inglis 2006, Rout et al. 2017). This kind of information should also continuously be collected during a survey to increase the efficiency.

3 Survey tools

Several tools have been developed for detection of non-native insect species (for a review see Poland & Rassati 2019). Two main types are: (1) visual inspection of host material (e.g. trees or parts of trees) for colonization and (2) trapping of dispersing adult individuals outside their host material. Presence in host material may be established by finding the organism itself or characteristic galleries or emergence holes. In case the species is causing considerable tree mortality inspections may be focused on dying trees, if not, on suitable breeding material in the form of e.g. logging residues, wind-felled or otherwise stressed trees. Also sniffer dogs (i.e. dogs trained to recognize odors released from colonized trees) can be used for locating infested trees. For these kinds of methods it generally pays off to initially invest some extra time in developing an efficient search method regarding suitable habitats and substrate types.

When using traps both the type of trap (e.g. funnel trap, barrier trap, trap color) and the trapping location (e.g. stand type and if the trap is placed at ground level or in tree crowns) may influence trapping efficiency (Allison & Redak 2017). Ideally, attractive baits (pheromones and/or host tree volatiles) are available to increase probability of catch. There are advantages and disadvantages with both types of methods which are discussed below.

3.1 Interpretation of positive records

If the surveyed species itself, or its galleries or emergence holes, is found in its host tree it undoubtedly represents a colonization event at that locality. In contrast, if the species is caught in a trap it does not necessarily mean that the individual(s) originate from that specific locality (i.e. have developed in a host tree there) or that the species is able to colonize substrates there. Many insects have high dispersal capacities which mean that a trapped individual may originate from a distant colonized host tree or even from wood package material for imported goods. In addition, if a colonized wood object is found it does not necessarily mean that a population has successfully established there because of Allee effects (see below) operating at small populations or low population densities.

3.2 Detectability

For species utilizing living trees but not necessarily killing them or causing apparent visible damage it might be quite difficult to detect them by inspecting host material because of the large amounts of potential host material available (i.e. all living individuals of the host tree species). Also species that do not have species-specific galleries may be hard to detect by visual inspections.

For such species trapping may be the most efficient way of delimit areas where the species may be established (but see above). Trapping may also be an efficient way of detecting species at low population densities if strong attractants are available.

3.3 Time window for survey

Trapping is restricted to the flight period of the surveyed species which depending on the species may extend over only a few days to several months. Even if the flight period is extended over several months there is generally a much shorter peak period with high flight activity and thus also increased probability of catch. In contrast, inspections of host tree material may be conducted year around except for when deep snow (covering objects to be sampled) or frozen conditions (preventing debarking in search for insects or their galleries).

3.4 Continuous update of infested area during on-going survey

One advantage of inspecting host material for individuals or characteristic galleries is an immediate identification which means that after a positive record the plan for which localities to check may be changed accordingly by skipping localities inside the new boundary of the infested area in favor of localities outside the new boundary. In contrast, trap catches generally take time to process and, depending on the length of the flight period, after a positive record it may be too late to move the traps to new areas.

4 Case study pine wood nematode (PWN)

4.1 Background

4.1.1 PWN in Europe and Sweden

In 1984 the 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 dying trees in Portugal where it now has spread to large parts of the country and has killed large numbers of pines (Fuente et al. 2018). 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 4 000 wood samples (generally with *Monochamus* galleries) from clear-cuts in the Swedish forest as well as more than 200 *Monochamus* beetles caught in traps (including trapping since 2015) have been processed and all have been negative for PWN (Kristof Capieau personal communication). In contrast, PWN has been intercepted at several occasions in wood packaging material imported to Sweden. 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 exported from Portugal were enforced.

Since 2008, PWN has been detected in several cases in the forest in Spain, all in newly dead trees rather close to the Portuguese border (two times in Extremadura, once in Galicia, and twice in Castile and León). After eradication efforts performed by Spain the EU Commission has declared three of the outbreak areas as free from PWN (the two areas in Extremadura and one of the areas in Castile and Leon). In 2009 PWN was also recorded from dead pine trees in Madeira, Portugal (European Commission 2010).

4.1.2 Risk for pine wilt disease caused by PWN

Pine wilt disease (PWD) represents the situation when PWN, transmitted to live trees by vector beetle maturation feeding, results in tree mortality. PWN is native to North America where it is distributed over most of the continent while PWD is only expressed in regions with warm summers and mainly affects non-native pine species like Scots pine (Rutherford & Webster 1987, Hirata et al. 2017, EPPO/CABI 2015). Outside its native area PWN has established in Japan, China, South Korea, Taiwan and Portugal, and in all these regions PWD is expressed and has resulted in large tree mortality (Rutherford & Webster 1987, Mamiya 1988, Dwinell 1997, Togashi & Shigesada 2006, Ikegami & Jenkins 2018).

Several factors may influence the expression of PWD like tree species susceptibility, temperature during summer, drought, occurrence of suitable beetle vector species and nematode load carried by the beetle vectors. Many pine species occurring in the native area of PWN (North America) seems to be resistant against PWD even though also some pine species native to North America are classified as being susceptible (Rutherford & Webster 1987). In contrast the European Scots pine, maritime pine (*P. pinaster*) and black pine (*P. nigra*), and several pine species in Asia are highly susceptible to PWD (Rutherford & Webster 1987, Mamayia 1983, Nakamura & Tabata 2014).

It is uncertain if PWD will develop or not if PWN establish in Scandinavia despite the fact that Scots pine is highly susceptible. The general opinion is that the present climate is too cold. Based on the northern range limit of PWD in Japan (northern part of Honshu) several studies suggest that the average temperature during the summer months must exceed about 20–22˚C for expression of PWD (Rutherford & Webster 1987, Rutherford et al. 1990, Hirata et al. 2017). This is also roughly in accordance with a study based on the summer temperatures in all areas where PWD occur which demonstrated that summer temperature explained most of the variation in occurrence of PWD (Ikegami & Jenkins 2018). The same study concluded that the occurrence of PWD starts at about 15˚ C, with disease expression increasing dramatically at 19˚C. In a rearing experiment PWN population build up (which may be important for overcoming host tree defenses) was highest at temperatures between 25–31˚C which indicates that mortality caused by PWD will be higher in areas with such high summer temperatures (Pimentel & Ayres 2018).

Apart from temperature also drought increases the risk of PWD (Ikegami & Jenkins 2018). The magnitude of damage caused by PWD in Japan is known to increase during drought years (Suzuki & Kiyohara 1978).

In Japan it has also been suggested that the occurrence of the main vector beetle *M. alternatus* is a factor that limits the northerly distribution of PWD. In a study conducted at different altitudes on Mount Fuji *M. alternatus* could not develop above an altitude of about 1000 m while PWN in stem sections survived at also higher altitudes (Ohsawa & Akiba 2014). The occurrence of PWD-killed trees on the mountain also corresponded to the presence of the vector beetle (i.e. almost no PWD-trees above 1000 m altitude).

Several studies have demonstrated that there is a threshold regarding the number of nematodes that must be transmitted during beetle feeding for PWD to be expressed (Kishi 1995). Thus, feeding by either one beetle with a very high nematode load or several beetles with lower nematode loads are required to induce PWD.

To conclude, the summer temperature is generally too low for development of PWD in Sweden (Hirata et al 2017, Ikegama & Jenlins 2018). On the other hand we have a highly susceptible pine species and also suitable beetle species that may act as vectors (see below). With the on-going temperature warming the situation may change dramatically. The study by Ikegami & Jenkins (2018) predicts that large parts of southern and central Sweden may be suitable for development of PWD in 2070 while the study by Hirata et al. (2017) predicts that smaller areas in Southern Sweden will be suitable.

4.1.3 Actions if PWN is detected

According to the Commission Decision 2012/535/EU (European Commission 2012) 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 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 despite eradication measures, 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.

In the Commission Decision 2012/535/EU (European Commission 2012) it is not stated how the delimiting of the infested zone shall be conducted. But in the EPPO standard PM 9/1 there are some suggestions about how a delimiting survey for PWN should be conducted (EPPO 2018) which are presented below (see 4.4.2 Strategy for delimiting infested area).

4.2 Biology of PWN and its vector beetles

4.2.1 Association between PWN and *Monochamus* beetles

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 of adult beetles feed on bark (generally on branches) of 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. PWN has both a phytophagous and mycetophagous phase during its life cycle. This unusual feature distinguishes it from other plant parasitic nematodes and requires profound changes in biology (Espada et al. 2016). During the phytophagous stage, the nematode migrates within pine trees, feeding on parenchymal cells. Subsequently, PWN feeds instead on fungi in the dying or dead tree.

After maturation feeding the *Monochamus* beetles are attracted to dying or recently dead trees, including cut trees and cutting residues, where mating and oviposition takes place. 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 generally kill trees, PWN reproduces in newly dead trees (dying for other reasons than PWD) or cut trees and logging residues. In such a situation (i.e. 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 available 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 reproduces in trees killed by PWN.

4.2.2 *Monochamus* species present in Sweden

Three species of *Monochamus* occur in Sweden: *M. sutor* L., *M. galloprovincialis* Olivier and *M. urussovi* (Fischer von Waldheim). The general biology of the species is described in Ehnström & Axelsson (2002) and Ehnström & Holmer (2007). *Monochamus sutor* is the most common species. It is distributed all over Sweden and common in many regions. *Monochamus galloprovincialis* is not as common and seems to be more common in the eastern coastal areas of Sweden. In a trapping study conducted in eastern Uppland and Gästrikland 13% of the 3725 caught *Monochamus* beetles were *M. galloprovincialis* and the rest *M. sutor* (Schroeder 2019). In 46% of the trapping locations the two species occurred together (*M. sutor* were caught in all locations). *Monochamus galloprovincialis* is reported from most parts of Sweden but due to identification problems (hard to discern from *M. sutor*) the true distribution is uncertain.

In a trapping study in Norrbotten *M. galloprovincialis* was caught which indicates that the species is widely distributed (Schroeder 2019). 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). In addition, the two species may be identified without dissection by visual inspection of the hairs on the sides of pronotum (Wallin et al. 2013). The third species, *Monochamus urussovi*, 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 urussovi* is included in the Swedish red list (Swedish Species Information Center 2015). According to Wallin et al. (2013) *M. urussovi* should be considered a subspecies of *M. sartor*, a species distributed in central Europe.

4.2.3 Breeding substrate of Swedish *Monochamus* species

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.), and also in the introduced lodgepole pine (*P. contorta*) (Schroeder & Cocos 2018). *Monochamus galloprovincialis* breeds in Scots pine and *M. urussovi* 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 (Schroeder 2014). It is generally not found on dead standing trees with the exception of burned trees, which can be 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 because of the rare inspections of tops of standing dead trees. Emergence holes have been recorded for substrates with as small diameter as 2.5 cm (Åke Lindelöw personal communication).

Monochamus urussovi 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, Foit 2010) but has also been found in logging residues in Sweden (Schroeder 2014) and Finland (Tomminen 1993a). Generally none of the three species breed in 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 colonized parts of the roots was 4–5 cm and the roots were partly above the soil.

Many species of *Monochamus* have been demonstrated to be attracted to host tree volatiles, bark beetle pheromones and a genus-specific *Monochamus* pheromone (Pajares et al. 2013, Ryall et al. 2015). 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 occurring in Sweden are strongly attracted to these baits (Pajares et al. 2013, 2017, Ryall et al. 2015).

4.2.4 Feeding behavior of adult *Monochamus* beetles

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 *M. 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. urussovi* 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).

4.2.5 Developmental time of *Monochamus* beetles

The developmental time of *M. sutor* and *M. galloprovincialis* is generally one to two years, and two years for *M. urussovi*, in Sweden (Ehnström & Holmer 2007, Schroeder unpublished). There is no evidence from Scandinavia that the new generation emerge already during the first summer of development. Thus, they will always hibernate at least one winter before emergence. This is probably a result of an obligatory diapause in the last larval stage. All three *Monochamus* species hibernate as larvae. In southern Sweden the one-year developmental time is most common while in northern Sweden the two-year developmental time dominates. A minor proportion of the *M. sutor* (no data for the other species) adults emerge one year later than the main part of the beetles (Schroeder unpublished). This may be because of a late-summer oviposition or poor quality of the breeding material. The *M. sutor* adults may be caught in traps from May to end of August with a peak in June and July while the flight period of *M. galloprovincialis* is about one month later with a peak in July and early August (Schroeder 2019).

4.2.6 Capacity of Swedish *Monochamus* species to act as vectors of PWN

Of the species present in Sweden *M. galloprovincialis* act as vector for PWN in Portugal while for the other two species no records of association with

PWN have been published so far (*M. sutor* and *M. urussovi* does not occur in Portugal). But it is likely that also *M. sutor* and *M. urussovi* 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 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* and *M. alternatus*. Thus, it is likely that also *M. sutor* and *M. urussovi* may act as vector for PWN. If this is true a vector which is distributed all over the country (i.e. *M. sutor*) is available if PWN is introduced to Sweden. Also *M. galloprovincialis* is recorded from many localities even though not as common as *M. sutor*. Thus, in the following text the main emphasis is on *M. sutor* as a potential vector of PWN but also *M. galloprovincialis* is considered.

4.2.7 Dispersal of *Monochamus* beetles

An understanding of the dispersal 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 living trees for feeding, finding mates and finding dying or newly dead trees suitable for egg-laying. Finding 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 not much information about the dispersal capacity of *M. sutor* based on direct studies of flight capacity or flight distance. Indirect data of strong dispersal capacity for *M. sutor* are observations that colonization rates can be high in damaged trees after large disturbances that cannot be explained by local populations (Forsslund 1934, Schroeder & Lindelöw 2003). 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. Mark-release-recapture experiments with *M. galloprovincialis* conducted in Spain demonstrated that most beetles could fly at least 3 km and some beetles were recaptured more than 20 km from their release point (Mas et al. 2013). Because recapture of marked beetles at large distances from release sites are very unlikely even larger dispersal distances for some individuals are most likely. There is no reason why *M. sutor* should not have about the same dispersal capacity as *M. galloprovincialis*.

4.3 PWN invasion into new areas

When planning a delimiting survey after a detection of an invasive species in a new area it is helpful to have some knowledge about the characteristics of the invasion by the specific species in that area. But, generally detailed knowledge about what to expect regarding e.g. speed of spread, spatial distribution and population density is lacking (except for when prior establishments have occurred and been studied). In such a situation it may still be helpful to try to predict the characteristics of the biological invasion based on available knowledge about the biology in combination with the environmental conditions in the new area (e.g. habitat distribution, climate, human-mediated spread). Regarding PWN, no information is available about establishment in new areas where PWD is not expressed (as expected for Sweden, see above). Thus, in the following text predictions are made for PWN if established in Sweden.

Biological invasions are generally divided into three consecutive steps: introduction, establishment and spread. Introduction represents the arrival of a propagule of individuals to the new area. Establishment represents the stage when a local reproducing population, that will not get extinct in the near future, is formed after an introduction. Spread represents the last step when a locally established population increases in size and starts to expand its distribution area. Of these three steps spread is of especially interest when planning delimiting surveys. It should be noted that only a fraction of all introductions result in establishment of local populations and that the rate of spread may vary a lot between species and geographical areas.

4.3.1 Introduction of PWN

The most likely way of introduction is that *Monochamus* beetles carrying PWN emerge from imported wood, including wood packaging, and disperse out in the forest (EPPO 2009a, b). There are several reports of interceptions of living *Monochamus* in material imported from outside EU (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. Another possibility is that PWN could already be established within Europe without being detected and subsequently spread to more countries via trade of wood within EU (for which treatments to kill insects and PWN are not required except for Portugal and the demarcated areas in Spain) or by natural spread with the vector beetles. 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). For Sweden natural spread is possible from Norway and Finland but unlikely from Denmark where vector beetles (*Monochamus*) are lacking.

Also non-vector introduction of PWN to new areas is possible. PWN-infested solid wood, wood chips or wood packaging material may be left out in the forest or close to trees in urban areas.

PWN may be introduced to many different locations within a country as a result of complex within-country human transportation of e.g. imported solid wood (before and after processing), wood for energy purposes and wood packaging (Douma et al. 2017).

4.3.2 Establishment of PWN

Most introductions of non-native organisms do not result in establishment of local populations. One reason for this may be strong Allee effects (Courchamp et al. 1999, Courchamp et al. 2009) acting at low densities and that introduced propagules are generally small (Tobin et al. 2011). An Allee effect is defined as 'a positive relationship between any component of fitness of a species and either numbers or densities of conspecifics' meaning that an individual of a species that is subject to an Allee effect will suffer decrease in some aspect of its fitness when conspecific density is low. Examples of Allee effects are genetic inbreeding leading to decreased fitness, problems of finding suitable breeding material and mates at low densities.

After introduction of PWN to a new area a necessary first step for establishment is colonization of host tree material by PWN for reproduction. The most probable way of colonization is by the beetle vector but also non-vector colonization is possible. The vector may transmit PWN both to living trees during feeding (which in Sweden is not expected to result in PWD at present climatic conditions) and to dying or newly dead trees (or parts of trees) during mating and egg-laying. But for establishment it is not enough with only a first successful colonization of host material but PWN must be able to subsequently spread to and reproduce in new wood substrates for a local population to persist over time (according to the definition of establishment used for biological invasions). In the following the different ways of colonization and establishment are discussed in more detail.

If PWN is introduced by *Monochamus* beetles emerging from imported wood or wood packaging the beetles must directly do a maturation feeding on the bark of branches of living conifers and thus may transmit PWN to these trees via the feeding wounds. If the transmission results in the development of PWD (like e.g. in Portugal) the nematode population will increase strongly and spread through the dying tree. The tree will also become a suitable breeding material for the native *Monochamus* beetles in the area which may lay eggs in it. The resulting new generation *Monochamus* beetles may then be boarded by PWN before emerging and in their turn transmit PWN to other living trees during their feeding which will result in new dying trees suitable as breeding substrate for subsequent *Monochamus* beetle generations. If tree mortality caused by PWN is high this scenario will result in increased populations of the vector beetle of which most will carry PWN (because most beetles origin from PWN-killed trees).

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 (Gruffudd et al. 2016), there is still a possibility that it may result in PWN persisting in low numbers in the trees (Bergdahl & Halik 2003). But, as long as these trees remain healthy they cannot be colonized by *Monochamus* beetles and thus represents a dead end for PWN (because they cannot be vectored to new wood substrates). But if such PWN-infested trees are weakened or die (e.g. as a result of wind-throw) before the stand is harvested the nematodes may multiply and the trees may be colonized by *Monochamus* beetles and their offspring may vector PWN to new trees. An even larger risk in this respect would be thinnings and final cuttings which result in large amounts of branches and tops that are left in the forest and in which PWN if present in low numbers in the living tree could build up high populations after cutting. These kinds of substrates are also highly attractive for vector beetles (see above).

So an important question is how probable long-time survival of PWN in living trees is in regions where PWD is not developing. Studies in North America have demonstrated that living Scots pine trees which have experimentally been inoculated with PWN can harbor 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. In addition, 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. In a study conducted in Canada on young healthy balsam firs (*Abies balsamea*) produced as Christmas trees, PWN was extracted from 9–50% of branches with feeding scars of *Monochamus* (Blatt et al. 2019). But, in that study most feeding scars were probably recent. To conclude, the possibility of establishment of PWN in living trees in Sweden (where we do not expect PWD to develop) remains uncertain and new studies are needed to finally solve this question.

A more likely way of establishment in Sweden, and other countries where development of PWD is unlikely, is via the sharing of breeding material (e.g. wind-felled trees, logging residues) between introduced (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. As a result of many different species of *Monochamus* (including all three Swedish species) being attracted to the same attractants (*Monochamus pheromone*, bark beetle pheromones and host volatiles) there is a risk that introduced *Monochamus* beetles carrying PWN will end up on the same breeding material as native beetles. If the non-native beetle is a female, PWN may be transmitted to the wood through 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. Thus, if the breeding material is also colonized by native *Monochamus* beetles their offspring may carry PWN and in their turn spread it to new living trees and dead wood objects.

None of the scenarios described above require establishment of the introduced non-indigenous *Monochamus* species as long as native *Monochamus* species that can act as vectors of PWN are present. Actually, neither in Japan (PWN introduced from North America, the native *M. alternatus* the main vector) nor in Portugal (PWN probably introduced from Asia, the native *M. galliprovincialis* the vector) establishment of non-indigenous *Monochamus* species have occurred. This may be a result of Allee effects, i.e. that the per capita growth rate is negative below a critical population density threshold (Courchamp et al. 1999) which may often be the case when the introduced propagule size is small (generally only a few *Monochamus* individuals are present in imported wood package material). In such a case the individuals may have problems to find each other for mating because they are too diluted.

Colonization of living trees (or dead wood) by PWN without the vector beetle may be possible if imported wood (infested by PWN) is stored or left close to living trees or dead wood substrates. In laboratory experiments three to four year old Scots pine saplings with root and stem damages became infested when in direct contact or close to PWN-infested wood chips (Hopf-Biziks et al. 2017). If also more mature trees (i.e. potential breeding material for *Monochamus*) could be colonized in this way is still unclear. If so, establishment in this way would require either introduction into a region where PWD develops, or that the living tree for some other reason are weakened, and thus become suitable as breeding material for native *Monochamus* beetles (see above).

The probability of establishment will differ a lot between the different scenarios described above. The highest probability of establishment could be expected in regions where PWN-infested *Monochamus* beetles feeding on living trees results in PWD. The main reasons for this expectation are: (1) Both maturation feeding on living trees and egg-laying on weakened trees or parts of trees (including logging residues) by introduced *Monochamus* beetles may result in breeding material colonized by PWN suitable for native *Monochamus* beetles. (2) The beetle population will increase strongly because large numbers of native

Monochamus beetles carrying PWN can be produced from a single tree dying from PWD which increases the probability of more trees being killed and thus also of establishment of PWN. In regions where PWD is not expected the most likely scenario for establishment is by non-native and native *Monochamus* beetles sharing breeding material in the form of weakened trees and logging residues. But the probability of establishment should be much lower compared with the scenario described above because the introduced beetle must find a suitable breeding material which is much less probable than finding a living tree. In addition, because the only way in which the proportion of native beetles carrying PWN can increase over time is by sharing breeding substrate with uninfested beetles because there is no increase in *Monochamus* population density (breeding material for the beetles will not increase because no trees are killed). Thus, there should initially be a considerable chance that the few individuals that carry PWN will not be able to produce offspring (adults could die before finding breeding material, PWN not transmitted to the breeding substrate during egg-laying, *Monochamus* developmental stages could die in the breeding material).

4.3.3 Spread of PWN after establishment

The potential spread of PWN in Sweden, where we do not expect PWD and thus not an increase in beetle populations, may be predicted based on what we know about the spatial and temporal occurrence of suitable breeding material for the vector, the dispersal behavior of the vector and how fast PWN will spread within the vector population. The breeding material of *M. sutor* consists mainly of logging residues on fresh clear-cuts and pre-commercial thinnings (Schroeder 2014). Because the breeding material generally is not suitable during the second summer after cutting when the new generation beetles emerge (because of drying out and already utilized by a variety of insects) each beetle generation is forced to disperse to new locations with fresh clear-cuts and thinnings. The nearest new suitable locations may thus be located several km away and the distances dispersed may be considerably longer because the beetles cannot be expected to always find the nearest clear-cut or thinning. Thus, the beetles in most cases have to fly several km before finding breeding material (Økland et al. 2010).

As mentioned above the beetles are capable of flying long distances. Results from a trapping study (Schroeder 2019) conducted on fresh and old clear-cuts indicate that when the beetles find a suitable clear-cut for oviposition they will not remain on the same clear-cut during the whole season but disperse to new locations. Thus, to conclude the vector beetles can be expected to colonize breeding material in several places and far away from the location where they developed.

Unfortunately, there are no data available on how PWN will spread within the population of vector beetles after establishment (i.e. how the proportion of beetles carrying PWN, or proportion of beetle-colonized wood objects infested by PWN, will change over time) in areas where PWD is not expressed (as most probable for Sweden). In such a situation the beetle population will not increase (because no increase in breeding material in form of trees dying from PWD). Furthermore, there is no reason why beetles carrying PWN should have a higher reproductive output than beetles without PWN. Thus, the only way in which PWN can spread within the beetle population is if beetles carrying PWN share breeding materials with uninfested beetles. As an example, if a beetle carrying PWN oviposits in a newly dead tree (e.g. a wind-felled tree), and PWN is successfully transmitted, the nematodes will disperse within the whole tree and also multiply to large numbers. If such a tree (offering colonization space for many beetles) is also colonized by uninfested beetles all the offspring, irrespective if their parents carried PWN or not, may be boarded by PWN before they emerge. Studies conducted in Japan, Portugal and North America shows that 50–100% of *Monochamus* beetles emerging from trees colonized by PWN carry PWN (Linit et al. 1983, Wingfield & Blanchette 1983, Bergdahl et al. 1991, Kishi 1995, Sousa et al. 2001). But, in Sweden the major breeding material for *M. sutor* is constituted by branches and tops after clear-cuttings and thinnings (Schroeder 2014). These kind of breeding substrates are rather small, and thus, cannot be expected to offer oviposition sites for several beetles on individual wood objects. This may slow down the spread rate of PWN within the beetle population. This in combination with the large dispersal distances of the beetles (see above) should result in low proportions of PWN-infested beetles, and PWN-colonized wood substrates, spread out over large areas after an establishment. In North America, where PWN is native, the proportion of *Monochamus* caught in traps carrying PWN ranges from 0–71% in different regions (Pimentel et al. 2014, Blatt et al. 2019). In the situation described above on average a lower proportion of beetles carrying PWN can be expected.

In regions where PWD is expressed the spread of PWN in the vector beetle population after an establishment will be much faster compared to areas where PWD is not expressed. In this situation the major breeding material for the vector beetles will be trees dying as a result of PWD. This results in a rapidly increasing beetle population (because of an increasing amount of breeding material). But also in an increasing proportion of beetles carrying PWN because a high proportion of beetles that emerges from PWD trees will carry PWN. Studies conducted in Japan, Portugal and North America generally showed that 50–100% of *Monochamus* beetles emerging from PWD trees carried PWN (Linit et al. 1983, Wingfield & Blanchette 1983, Bergdahl et al. 1991, Kishi 1995, Sousa et al. 2001). Both the increase of beetle population and the high proportion of beetles carrying PWN are factors that should increase the spread rate of PWN. Unfortunately, the data of spread in regions where PWD is expressed is solely based on the distribution of PWD-killed trees. Natural yearly spread of PWD in invaded areas have been estimated to 5.3 km (maximum 8.3 km) in Portugal (Fuente et al. 2018), and a mean of about 6 km (from a number of different studies) in Japan (Togashi & Shigesada 2006, Osada et al. 2018) and 7.5 km in

China (Robinet et al. 2009). But, it is important to note that the distribution of PWD is not necessarily the same as the distribution of PWN. Because the numbers of PWN transmitted to a live tree must exceed a threshold value for PWD to be expressed (Kishi 1995) it could be that PWN is established in logging residues and wind-felled trees (no threshold value of transmitted PWN required for PWN colonization) beyond the range limit of PWD.

In regions where PWN has established also human-mediated spread has been demonstrated resulting in establishment of PWD-killed trees further away than the rate of natural spread (Robinet et al. 2009, Fuente et al. 2018). This may be explained by the PWD-killed trees that may have been logged and transported to saw-mills and other kinds of facilities where new generation beetles carrying PWN may have emerged and spread out in the forest. Also in Sweden this is a possibility that should be considered even though PWD is not expected. Logging residues are extracted from many clear-cuts for energy purposes which mean that in case of a PWN establishment there is a risk that also human mediated spread will contribute to the spread of PWN. But, in most cases the logging residue will be stored on the clear-cut to dry out before being chipped (either at the clear-cut or at the industry) in the autumn or winter after the first summer after cutting which means that the new generation beetles will not have time to emerge (because they hibernate as larvae at least one winter before emergence).

4.4 Delimiting PWN-infested area

In the following a suggestion for how to conduct a delimiting survey in case of detection of PWN in Swedish forest is presented. It is impossible to develop a survey that covers all possible scenarios regarding the circumstances of detection and the wishes of the decision makers. Thus, the survey is developed for the most probable scenario: that PWN establishment in Sweden will not result in PWD (i.e. asymptomatic), that it has been established for some time before being detected and that *M. sutor* (the most common *Monochamus* species in Sweden) is the main vector. In case of a different scenario the detailed description of the biology of PWN and its vectors, and the predicted characteristics of a PWN invasion under Swedish conditions, can be utilized for adjustments of the survey model suggested below. Before developing a delimiting survey it is important to clarify the principles for definition of PWN colonization and PWN-infested area. Thereafter, a survey strategy and the tools to be used can be decided upon.

4.4.1 Definition of PWN infestation and PWN-infested area

4.4.1.1 Interpretation of a positive PWN record

In a strict sense according to Commission Decision 2012/535/EU (European Commission 2012) 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. Thus, finding of a PWN in a *Monochamus* beetle trapped in the forest will not necessarily according to the Commission Decision result in the location being defined as infested by PWN (unless a by the Decision required further investigation proves the presence of PWN in susceptible plants) while a finding in a wood object will. *Monochamus* beetles caught in traps may originate from imported wood objects in which case a PWN record i.e. positive detection of PWN in the caught *Monochamus* beetles represents an introduction and not necessarily that PWN has been transmitted and colonized a host tree. In contrast, if PWN is recorded from native wood objects it means that PWN has been able to colonize at least that wood object and possibly also is established (i.e. has established a population that will sustain at least for some time). Thus, when interpreting PWN records from trapped beetles it is important to consider trapping location in relation to possible occurrence of imported wood from which beetles may have emerged. If trapping locations are situated out in the forest, far away from any possible destinations of imported wood, catches of introduced (i.e. emerging from imported wood objects) *Monochamus* beetles are unlikely and the finding of PWN in a beetle is a strong indication of establishment.

4.4.1.2 Definition of infested area

This question is highly relevant because the area defined as infested is crucial when planning subsequent eradication (or containment) measures. PWN colonization will be patchy as a result of the main breeding material for the vector *M. sutor* being constituted by logging residues on clear-cuts and pre-commercial thinnings which are spread out as patches in the forest landscape in Sweden. There are several reasons why each PWN-colonized clear-cut (or thinning) should not be defined as a separate infested area. (1) Each year new clear-cuts and thinnings (in new locations) will be colonized by the vector beetle and PWN (if carried by the beetles) because the logging residue is only suitable for one summer. (2) Within a given year the vector beetles may colonize logging residues in more than one location. (3) Even though clear-cuts and thinnings constitute the major source of breeding material for the vector also some wind-felled trees and weakened standing trees in the forest landscape between the clear-cuts and thinnings may be colonized. Altogether this means that the vector cannot be assumed to form closed populations in different parts of a forest landscape. Thus, if PWN is recorded from a number of clear-cuts, spread out in a forest landscape, these findings will not be interpreted as separate infestations from a biological point of view but instead as belonging to the same infestation. This would mean that the infested area will be defined from the outermost records of PWN during a delimiting survey even though the distances between records may be several km´s.

4.4.2 Strategy for delimiting infested area

In Commission Decision 2012/535/EU (European Commission 2012) there are no specific requirements about how a delimiting 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 in such a survey. But, in the EPPO standard PM 9/1 on procedures for official control of PWN and its vectors (EPPO 2018, hereafter EPPO standard) suggestions for how to conduct a delimiting survey are included. The optimal strategy may differ between countries and areas. Thus, in the following sections a sampling strategy, and what to sample, is suggested for Swedish conditions. Finally, the suggested procedure is applied in a practical example.

4.4.2.1 If documented recent introduction

Directly after detection of PWN an attempt should be made to pin down the most likely pathway of introduction. In the best case it is a recent introduction and it will be possible to answer questions about: (1) exactly where the introduction took place, (2) how many years ago it was introduced 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). If a recent introduction is the case the answers to the questions above will be the base for decisions about how to conduct a delimiting survey. But, as discussed above, the most likely scenario is that PWN has already been present for a long time in Sweden when detected and thus is spread over a large area. Thus, the following is based on this assumption and that *M. sutor* (the most common *Monochamus* species in Sweden) is the main vector.

4.4.2.2 Choice of strategy if not a recent introduction

Of the two main delimiting strategies for invasive organisms described above (see section 2), the procedure Utilizing a declining density of the invader towards the range boundary” (Leung et al. 2010) is unlikely to work in the case of PWN. This is because of the predicted patchy occurrence of PWN (clear-cuts and pre-commercial thinnings) and predicted low proportions of colonized objects. Thus, the strategy “Delimiting by documented absence in a zone around the infested area” is the suggested procedure to use. It is based on subsequent surveys of zones around the detection point until no more findings are recorded. This strategy follows the logic presented in the EPPO standard.

For this strategy two approaches are available: (1) evaluation based on the rate of discovered new occupied localities (Panetta and Lawes 2005) and (2) evaluation based on selected statistical confidence and infestation level to be detected (Schroeder 2014). The second approach is based on the survey procedures prescribed for eradication and containment of PWN in the Commission Decision 2012/535/EU (European Commission 2012) and in EFSA (2012). This means that: (1) a statistical reliability of the survey result and (2) the infestation level (i.e. proportion of objects with PWN) at which PWN shall be detected is chosen. From the perspective of the responsible authority it may be an advantage to be able to select a statistical confidence level and at what infestation level PWN should be detected. The great advantage of this statistical-based approach compared with a non-statistical approach is that the decision-makers have a more solid base for interpreting the result when PWN is not recorded from a zone. In addition, the statistical approach will in most situations not require a lot of extra work (see below). In the following both approaches are included.

4.4.2.3 Size of the first sampling-zone around detection point

Before deciding on the size of the initial (and eventual subsequent) zone to be surveyed it is helpful to try to predict the characteristics of the infestation. In the case of an PWN detection in Sweden the most probable scenario is that PWN already has been established for a number of years because only a very small fraction of all possible PWN-infested substrates are sampled in detection surveys each year (Økland et al. 2010) and because the invasion most probably will be asymptomatic (i.e. no large-scale tree mortality that can be observed). This in combination with the high dispersal capacity of the vector indicates that the PWN infested area may already be large at the time of detection. In addition, the main habitat colonized by PWN can be assumed to be clear-cuts and pre-commercial thinnings which are spread out in the landscape forcing the vectors to fly considerable distances to find breeding material. Based on the reasoning above the initial delimiting survey should cover a considerable area around the point of first PWN detection. A suitable radius could be about 25 km because: (1) an even larger area would dilute the proportion of sampled objects which is a problem if the proportion of PWN-colonized objects is low and (2) in the Commission Decision 2012/535/EU (European Commission 2012) it is stated that containment may be an alternative to eradication if the diameter of the infested area 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 which means that clear-cutting (which is expensive and resource demanding) of forest around the point of detection could be avoided. The EPPO standard suggests a delimiting survey with a radius of at least 10 km.

4.4.2.4 Number of samples

After the size of the zone to be surveyed has been determined the next step is to decide the number of samples to take. Unfortunately there is no data available about infestation levels in *Monochamus*-colonized wood objects or beetles from invaded regions where PWN does not result in PWD to base a decision about sample size on. As discussed above for Swedish conditions it may be that despite if it is assumed that PWN has been established for a number of years the proportion (or density) of PWN-colonized wood objects and beetles could still be low as a result of most breeding substrate for the vector being rather small (i.e. tops and branches on cut areas) which delays the spread of PWN within the vector population. In North America, where PWN is native, the proportion of beetles (no data for colonized logging residues) caught in traps carrying PWN ranges from 0–71% in different regions (Pimentel et al. 2014, Blatt et al. 2019). Thus, under Swedish conditions (assuming a limited number of beetle generations since introduction and low rate of spread in the beetle population) a much lower infestation level can be expected. On the other hand, if assuming that the infestation is spread over a large area when detected (as assumed here) an extremely low infestation level is unlikely (if a very recent introduction the situation may be different with extremely low infestation levels). Because lower detection levels require larger sample sizes (which will be more costly) it may also be poor use of resources to sample very intensively in the first zone in case PWN is spread over a much larger area (requiring establishment of subsequent zones outside the first zone) and/or occurring at much higher prevalence than the chosen detection level. In the end, available resources to spend on sampling may also determine the detection level. A goal may be to detect PWN if the infestation level is 1%. In case the sampling results in no positive records a smaller zone around the point of detection may be established and a lower detection level chosen, e.g. 0.1%. As a comparison, in the case of surveys in a PWN infested zone subject to eradication or containment measures the Commission Decision 2012/535/EU (European Commission 2012) states detection levels of 0.1% and 0.02% respectively (and a reliability of 99%). If using the statistical approach, tables of the sample sizes needed to achieve the chosen levels of reliability and detection levels can be found in Appendix 1. An explanation of the statistics behind these tables can be found in a publication from the European Food Safety Authority (EFSA 2012).

One special feature of PWN survey is that presently, at a practical scale, there is no methods available that directly in the field can conclude if a sampled wood object represents a positive PWN record. Instead the samples are generally sent to a laboratory for analyzes. Because the main aim of a delimiting survey is to determine the boundary of infestation many “unnecessary” (i.e. samples taken in-between the original location of detection and a location with a positive record) locations may be sampled and analyzed. One way of avoiding the latter is to start analyzing the samples furthest away from the original point of detection. On the other hand it may also be of interest to get data on the density of infested objects/localities within the infested area in which case all collected samples should be analyzed.

4.4.2.5 Choice of sampling tool

Both sampling of wood objects colonized by *Monochamus* and trapping of flying *Monochamus* beetles should preferably be used. This is in accordance with the recommendation in the EPPO standard for areas where PWD is not expected (EPPO 2018). For a detailed description of the two methods and their advantages and disadvantages see below (section 4.4.3 Tools). Within the decided zone the main focus should be on *Monochamus*-colonized wood object on clear-cuts. There are several reasons for this: (1) Sampling can be conducted year around except when snow (wood objects hard to find because covered) and because detection surveys in Sweden generally are conducted during summer trapping of beetles cannot be conducted until next season. (2) Logging residues on clear-cuts (and pre-commercial thinnings) is the major breeding substrate for *M. sutor* and also at least to some extent used by *M. galloprovincialis*. (3) Locations, and ages, of all clear-cuts in Sweden are available from the Swedish Forest Agency.

In addition to sampling wood objects also trapping of *Monochamus* beetles should be conducted during the summer. Within the zone trapping can be used to identify the vector species: which of the native species, could be more than one, or if it is a non-native species. Trapping of *Monochamus* beetles outside the zone can be used as a complement to the sampling of wood objects within the zone. Traps could be placed on clear-cuts at a distance of at least 30 km from the outer boundary of the zone. For shorter distances there is a risk that some of the caught beetles originate from the zone. If PWN is recorded it is a strong indication that PWN is spread even outside the zone and a new zone for sampling of *Monochamus*-colonized wood objects can be established accordingly. Trapping has the advantages that beetles originating from many different localities may be caught in a single trap. If more than one trap is placed on each clear-cut a minimum distance of about 100 m between traps can be used to reduce competition between traps (Jactel et al. 2019).

4.4.2.6 After delimiting

When an infested zone has been defined it is 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, firewood, wood packaging material.

4.4.3 Tools

PWN can be sampled both in its host plant and in its vector beetles. Below are the two methods of sampling for PWN described and their merits and disadvantages discussed.

4.4.3.1 Sampling of wood

Susceptible tree genera for PWN listed in the Commission Decision 2012/535/EU (European Commission 2012) are *Abies, Cedrus, Larix, Picea, Pinus, Pseudotsuga* and *Tsuga*. In Sweden the focus is on Scots pine and Norway spruce which are native and by far the most common of susceptible species present in Sweden. Three types of trees could be sampled: (1) healthy-looking trees, (2) dead or dying trees and (3) wood colonized by *Monochamus*.

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 Commission Decision 2012/535/EU (European Commission 2012) for sampling in demarcated areas subject to eradication or containment. But, as already mentioned above, reliable studies demonstrating that PWN really is able to survive in trees without resulting in PWD is still lacking. Other problems are the very large number of living trees available for sampling and that we do not know on which trees 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 survive in such trees, they may still mainly be present close to the position of the beetle feeding which makes detection even harder.

Dead or dying trees: Sampling of newly dead or dying pines cannot be recommended to be the main focus in a delimiting survey in Sweden where we do not expect PWD. But still, such trees should be sampled if found during the survey to establish if PWD may develop under Swedish conditions. In areas where PWD develops, dead or dying trees are 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 in such areas. But in areas where we do not expect PWD, like in Sweden, a main focus on 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 still harbor PWN. But the density of such trees, and especially of those colonized by *Monochamus*, is much lower than the density of colonized logging residues (Schroeder 2014). But, in case of PWN establishment in Sweden dead or dying Scots pine trees should definitively be sampled in the infested zone, as a complement to the main sampling of wood colonized by *Monochamus*, for evidence of development of PWD. A study conducted in Japan on trees killed by PWD demonstrated that PWN population in the trees peaks in the months after the tree has died and then decline sharply, and is undetectable after one to two years (Kanetani et al. 2011). Even though the process may be slower in the cooler climate of Sweden, sampling should focus on dying or newly dead trees.

Logging residues colonized by *Monochamus*: A delimiting 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 it is the major breeding resource). 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. Regarding the age of the clear-cuts to be sampled (i.e. after how many years can PWN still be detected in substrates colonized by *Monochamus* beetles) a study conducted in Japan on trees killed by PWD demonstrated that PWN population in the trees peaks in the months after the tree has died and then decline sharply, and is undetectable after one to two years (Kanetani et al. 2011). In Scandinavian climate this process may be slower but the study indicates that sampling should mainly focus on one to three year old clear-cuts to be on the safe side. The main developmental time for *M. sutor* in Sweden is one to two years but a proportion of the offspring may emerge one year later.

4.4.3.2 Sampling of Monochamus beetles with traps

Trapping is a powerful tool for collecting *Monochamus* beetles. With traps baited with the commercially available *Monochamus* attractants large numbers of beetles can be caught in a single trap. In Sweden the catches of *M. sutor* was 25–30 times higher in baited compared with in unbaited traps (Schroeder 2019). In addition, many different species of *Monochamus* occurring in Asia, North America and in Europe (including all three native species present in Sweden) are attracted by the same attractants (Boone et al. 2019 and see above).

As a result of the generally patchy occurrence of *Monochamus* breeding material, and the beetles' high dispersal capacity, beetles caught in a single trap can be assumed to originate from many different localities in the surrounding landscape. Depending on the location of the traps also *Monochamus* beetles originating from imported wood material may be caught in which case the beetle, and PWN if carried, will represent an introduction and not an establishment. However, if the traps are placed out in the forest, distant from locations with imported goods, this is unlikely.

When planning a survey with traps there are several factors to consider like choice of trapping locations, type of trap, trap density, time period of trapping and method of analyzing the beetles for PWN. Trapping location may strongly influence catches of insects. Studies conducted in Sweden showed that the catches of *M. sutor* were four to six times higher on clear-cuts than within pine stands (Schroeder 2019). For clear-cuts there was no significant difference in catch between fresh ones (cut during the previous winter) and two-year-old ones. Thus, if *M. sutor* is the target traps should be placed on fresh or older clear-cuts. In the same study there was no significant difference in catch of *M. galloprovincialis* between clear-cuts and pine stands. All these studies were conducted with black funnel traps (Econex, Murcia, Spain) baited with Galloprotect (SEDQ, Barcelona, Spain) and placed at ground level. In a study conducted in the Czech Republic catches of *M. galloprovincialis* in old pine stands were 7 times higher in cross-vane traps (Crosstrap, Econex) placed 18 – 24 m above ground compared with traps placed at ground level (Foit et al. 2019). Thus, if *M. galloprovincialis* is the target traps can also be placed in pine stands and higher catches can be expected for traps in tree crowns than at ground level. In the study by Foit et al. (2019) also two commercial European baits for *Monochamus* were compared and Galloprotect Pack (SEDQ, Barcelona, Spain) caught three times more *M. galloprovincialis* than Gallopro Pinowit (WITASEK Pflanzenschutz GmbH, Feldkirchen, Austria) and the difference was even higher for traps at ground level.

Also if trapping is conducted with wet or dry collecting cups may influence catches. In a study conducted in Sweden on fresh clear-cuts the catches of *M. sutor* were two times higher with wet collecting cups compared with dry as a result of the beetles being able to escape from "dry" traps (Schroeder 2019). Also the density of traps influence the number of *Monochamus* beetles caught per trap. At higher trap densities the catches per trap decrease as a result of competition between traps.

Monochamus sutor may be caught in traps from May to end of August with a peak in June and July (Schroeder 2019). In the same study the flight period of *M. galloprovincialis* was about one month later than for *M. sutor* with peak catches in July and early August. Based on studies on *M. galloprovincialis* in Portugal we know that the number of PWN per beetle decrease during the season (because PWN leave the beetles during beetle feeding and oviposition). Thus, trapping should preferably be conducted during the early part of the flight periods.

Generally caught beetles are sent to a laboratory for analyses of PWN. This means that there is a delay between catches and results. This in combination with the rather short optimal time window for trapping means that it is usually not possible to change trap positions during the season as a result of positive records of PWN. But, portable equipment that can be used in the field for testing *Monochamus* beetles for presence of PWN (based on DNA) have been developed and have been used in detection surveys in some countries (Kikuchi et al. 2009, Andrea Battisti personal communication). The use of such a method would give an immediate result at the emptying of traps and thus enable moving traps to new locations outside the known infested area within a season. Ideally, part of the beetle may be saved and sent to the lab for a backup molecular test (and also morphological identification if necessary) of PWN. But this may require a rapid transport to the lab and processing of the beetle parts to ensure that the nematodes are still in condition for the analyses. Also the *Monochamus* species can be identified in the lab to save time in the field. Even if the lab generally not has the expertise for beetle identification this should be the case during a delimiting survey.

4.4.3.3 Comparison of sampling of wood and beetles

Legislative significance of record: According to the Commission Decision 2012/535/EU (European Commission 2012) a finding of PWN from a trapped vector beetle is not interpreted as a PWN infestation (i.e. requiring delimiting and eradication) while this is the case for PWN records from wood objects. The Commission Decision 2012/535/EU (European Commission 2012) however requires a further investigation.

Resources required for sampling: Generally it is more resource demanding to use traps than to sample *Monochamus*-colonized wood objects on clear-cuts because it is expensive to buy large number of traps and baits, takes time to install and take down traps, short period during when all traps need to be set up, frequent emptying for collection of live beetles, need to contact landowners for permission to use traps on their land and cost for cleaning and storage of traps between seasons.

Time window for survey: Sampling of wood objects colonized by *Monochamus* can be conducted all year around except for when snow. In contrast, sampling with traps is much more restricted in time because of the restricted main flight period of *Monochamus* and the fact that the number of PWN carried by the beetles decrease over time (see above).

Selection of sampling locations: For clear-cuts GIS data are available from the Swedish Forest Agency on exactly where they are located and with information of their age which can be used both for sampling of *Monochamus*-colonized wood objects and for choosing trapping positions.

Identification of sampling objects: *Monochamus*-colonized wood objects are easy to identify as a result of characteristic larval galleries, larval frass and adult emergence holes. Also trapped beetles are fairly easily identified as belonging to the genera *Monochamus*.

Possibility to roughly estimate the total number of colonized wood objects and *Monochamus* beetles: In case of the statistical approach of the strategy "Delimiting by documented absence in a zone around the infested area" an estimate of the total population to be sampled is required for deciding the sample size required for a chosen significance and detection level. For *Monochamus*-colonized wood objects the total number can be estimated based on earlier field studies and data collected during sampling for PWN during the delimiting. It is harder to estimate the total population of beetles (requires data on number of beetles emerging per colonized wood object).

Geographic representability of records: The two sample types differ in two ways regarding their geographic representability:

> (1) A record of PWN from a colonized wood object means that a colonization event has taken place at that specific locality while this is not necessarily true for trapped beetles carrying PWN (even though if trapping is conducted on fresh clear-cuts with logging residues there is a high probability that colonization will take place if many beetles are caught).
>
> (2) Traps may sample beetles originating from many different localities in the adjacent landscape because generally several beetles are caught in each trap. This could be viewed as an advantage because of the possibility to detect presence of PWN also from localities that are not surveyed. But, the same is true if several wood objects are sampled at the same clear-cut because the origin of the *Monochamus* beetle transmitting PWN to them is also not known and can be expected to originate from different localities.

Continuous update of infested area during on-going survey: There is a rapid development of methods for molecular determination of PWN including portable equipment that can be used in the field and thus providing direct results (instead of sending the material to a lab and waiting for analyzes). This is a great advantage because the choice of locations to sample during a delimiting survey can be continuously adapted to the last records of PWN. Such a method has already been developed for detecting PWN in trapped *Monochamus* beetles in the field and it should also be possible to develop for wood samples (Andrea Battisti personal communication).

Detectability: At low beetle density trapping may be more efficient than searching for *Monochamus*-colonized wood objects that are scarce and spread out in the landscape. Another advantage with trapping compared with sampling wood is that traps can be set out everywhere in the landscape (although catches of *M. sutor* will be lower in forest than on clear-cuts) while sampling of wood is most efficient in recent clear-cuts and thinnings. Thus, in landscapes with few clear-cuts or thinnings (where it may be problematic to sample *Monochamus*-colonized wood objects) trapping should be a good complement. Also in a situation when the main vector is not *M. sutor* (instead *M. galloprovincialis* or less likely *M. urussovi* or very unlikely non-indigenous *Monochamus* species) the main reproductive substrate may not be logging residues and thus sampling with traps would be a good complement.

Identification of vector species: Because trapped *Monochamus* beetles can be identified to species when analyzing them for PWN, a finding of PWN in a beetle individual will also reveal which *Monochamus* species that act as the vector. This is generally not the case when PWN is recorded from wood samples because it is not possible to identify the *Monochamus* species from larval galleries or adult emergence holes when collecting the wood sample in the field. Thus, for *Monochamus*-colonized wood samples identification of beetle species requires either that beetle larvae are collected and identified or that the wood objects are individually placed in emergence cages in the lab for extraction of *Monochamus* adults. This will be impracticable in a survey when maybe thousands of wood objects are sampled. In addition, many colonized wood objects may be too old for *Monochamus* to remain. To identify which *Monochamus* species that act as vector is important because it will influence what are the optimal wood substrates to sample and where traps should be deployed.

Proportion of wood objects or beetles with PWN: If individual *Monochamus* beetles are analyzed separately it will give information about the prevalence of PWN within the beetle population. If wood samples are analyzed separately it will reveal the proportion of wood objects harboring PWN which is not necessarily the same as proportion of infested beetles. Not all *Monochamus* egg-layings will result in transmission of PWN to the wood object and not all beetles emerging from wood objects harboring PWN will be infested. In addition, also the method of analyses of wood samples and beetles may differ in detectability of PWN. The prevalence of PWN in beetles and wood objects is interesting because it indicates the size and age of the infestation and may also indicate if the sampled site is located close to the border of the infested area. A high prevalence of PWN indicates that the infested area may be large and that the establishment took place many years ago. A low prevalence could either be a result of a recent small infestation or that only the outer zone of the infested area has been sampled yet.

Population density of vector beetles: If traps are set out in standardized locations like clear-cuts the numbers of caught *Monochamus* beetles will for each area represent a relative estimate of vector density (c.f. Schroeder 2013). Because traps sample beetles from a large area of the surrounding landscape (high dispersal capacity and the beetles will move to several locations during their life span) traps are much more accurate than data on colonized wood samples (which may be affected of differences between clear-cuts in amount of suitable breeding material). Thus, by trapping the density of *Monochamus* can be compared between localities and also over time which may be of interest when managing a PWN infestation. In addition, high *Monochamus* densities will increase the rate of spread of PWN outside the infested area which should be accounted for during an ongoing delimiting survey. Vector density in a specific area may also be used for planning of trap densities in subsequent trapping surveys (i.e. number of traps requested to trap a given number of beetles).

Determination of the age of PWN infestation: By sampling *Monochamus*-colonized wood objects on clear-cuts of different ages it may be possible to trace back to some extent the age of the infestation. One weak point is that we do not know for how many years after colonization PWN remains in a wood object. But a reasonable guess is at least two years in southern areas and three years in northern areas in Sweden (based on the number of years when *Monochamus* beetles may emerge from the objects).

Prior experience of sampling: The annual detection survey in Sweden for PWN is using both sampling of *Monochamus*-colonized wood objects and trapping of *Monochamus* beetles.

5 Practical example

In the following example PWN is assumed to have been detected in a sample from a wood object colonized by *Monochamus* on a clear-cut in Sweden during the yearly national detection survey conducted during summer. The total area of the clear-cut is immediately declared as an infested area before the delimiting survey is initiated. This is because the exact position of the sampled wood object is not known and/or the positive record may be constituted of samples from several wood objects (i.e. a pooled sample) to reduce costs of analyses or to generate sample sizes large enough for lab diagnostics. It is assumed that there are no indications that the infestation may be very recent (e.g. nearby facility importing goods from abroad). It is also assumed that it is too late for starting trapping of *Monochamus* beetles in the same season. Thus, the focus of the following text is on sampling of wood objects. But, the following summer traps should be included in the survey as described above.

5.1 Step 1: Establishment of a sampling zone

A first circular zone to be sampled is established around the central point of the clear-cut. The radius of this zone is set to 25 km. This corresponds to an area of 196 250 ha. It is assumed that 70% of the zone is covered by forest which corresponds to 137 375 ha. In a real case all conifer forests within the zone would be mapped which rapidly can be done by the Swedish Forest Agency based on available GIS-layers.

5.2 Step 2: Deciding what to sample

It is assumed that the detection occurred in summer/autumn (when the national detection inventory generally is conducted) which means that it is too late to start sampling *Monochamus* beetles with traps the same season. Thus, it is decided to focus on sampling wood colonized by *Monochamus* beetles. It is also assumed that no practical method for PWN diagnosis in wood in the field is available. This means that all samples must be sent to a laboratory for analyzes before any results will be available.

It is assumed that PWN will not result in PWD (and tree mortality) based on the most probable scenario under present Swedish climatic conditions. But, because this is an assumption, dying pine trees should still be sampled if found in the zone. Only recently dead or dying trees should be included in the sampling. Samples should preferably be taken in the crown of the trees where *Monochamus* beetles conduct their feeding on branches.

5.3 Step 3: Deciding the number of samples

The statistical approach is chosen which means that a statistical reliability and a minimum infestation level to be detected are chosen. In this case 99% reliability (P = 0.01) and 1% infestation level is chosen. The number of samples needed to achieve the chosen reliability and detection level depends on the size of the population to be sampled, i.e. total number of *Monochamus*-colonized wood objects in the zone (see Appendix 1). One interesting feature of the tables in Appendix 1 is that for a large range of sizes of populations to be sampled (e.g. 10 000 to 1 000 000 objects) the resulting sample size is about the same for detection levels from 5%–0.1% (larger differences for the detection level 0.01 %) within each significance level. This means that in most situations a very coarse estimate of the size of the population to be sampled is sufficient which should be kept in mind when reading the detailed description below about how to estimate population size in this case. Thus, even if the statistical approach is not chosen it is recommended to check the tables in Appendix 1 to get an overview of the chosen sample size correspond to regarding significance and detection levels.

Because we assume that *M. sutor* is the main vector, colonized logging residues on clear-cuts and pre-commercial thinnings constitute the population to be sampled. The number of such objects can be estimated from: (1) the area of final cuttings and pre-commercial thinnings in the last three years (because we do not know if PWN will remain in wood on older clear-cuts), (2) 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), (3) the proportion of these substrates colonized by *Monochamus* and (4) the area of clear-cuts from which logging residues have been removed to be used for energy purposes.

In a real case data on clear-cuts in the zone would be provided by the Swedish Forest Agency on a short notice while data on pre-commercial thinnings will take longer time to get (requires contacts with all forest owners in the zone). Thus, in the initial stage an estimate based on data from the Swedish National Forest Inventory (Riksskogstaxeringen) can be used for pre-commercial thinnings. In this example it is assumed that 1.94% of the forest area has been clear-cut and that 1.93% has been pre-commercially thinned during the last three years based on national averages (Schroeder 2014). Thus, we end up with 2 665 ha of clear-cuts and 2 651 ha of pre-commercial thinnings. By applying the national averages for densities of tops on clear-cuts (Table 4 in Schroeder 2014, based on national averages 2008–2012), and the assumption of twice as many large pine branches as pine tops, and stems in pre-commercial thinnings (Table 4 in Schroeder 2014) this sum up to 1 572 394 tops, 938 106 pine branches and 644 275 thinned stems. According to a pilot study (Schroeder 2014) 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. 235 859 tops, 56 286 branches and 51 542 thinned stems) summing up to 343 688 colonized wood objects in the sampling zone.

Removal of logging residues from clear-cuts (removal from pre-commercial thinnings is unusual) for energy purposes is a common practice in Sweden. Generally the material is stacked along the forest road at the time of cutting and chipped in late summer (or autumn) of the first summer after cutting. Thus, this material is not available for sampling and should be subtracted from the total figure above. In a real case the true figure for removal of logging residues should be retrieved because this figure varies a lot locally. In this example we assume that the logging residues have been removed from 23% of the clear-cuts (Table 3 in Schroeder 2014, based on national average 2009 - 2012). Thus, the estimate of the total number of colonized wood objects will be reduced to 276 494 wood objects.

By applying table 2 in Appendix 1 the sample size required is 458 for a detection level of 1% (i.e. 0.01 of objects infested) with a reliability of 99% (i.e. significance level 0.01). 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 small change in number of required samples. For example, if the total number of colonized wood objects is 10 000, the sample size required is still 453. But, if the detection level is reduced it will require a strong increase in sample size. For example, if the detection level is set to 0.1% instead of 1% the sample size will increase to 4 590 in the example above and for a detection level of 0.01% to 44 744 (Table 2).

5.4 Step 4: Deciding where to sample

Sampling should mainly be conducted on one to three year old clear-cuts (i.e. first to third summer after cutting). The advantage of clear-cuts over pre-commercial thinnings is that the Swedish Forest Agency already has information about location, time of cutting and size for all clear-cuts in Sweden while this is not the case for thinnings. After a map has been created with all the clear-cuts preferably information about from which clear-cuts logging residue has been removed should be added (with the help of the Swedish Forest Agency). On such clear-cuts it will be harder to find suitable wood objects for sampling. It is good to have a spread of sampling locations in all directions and distances from the point of detection. There is a kind of trade-off between the number of clear-cuts to sample and the number of wood objects to sample on each clear-cut. Because it takes time to travel to the clear-cuts a number of samples should be taken on each visited clear-cut. It is not necessary to sample all the suitable clear-cuts within the zone because the *Monochamus* beetles most probably will visit more than one clear-cut during their life-span. When planning the sampling operation it would be a good idea to start sample clear-cuts at the outer part of the zone (although accessibility from roads may be as important when deciding where to start sampling) and rapidly send these samples to the laboratory for analyzes. If PWN is present there a new zone should immediately be established and sampled.

5.5 Step 5: Sampling and analyzes of samples

The samples from individual wood objects colonized by *Monochamus* collected on an individual clear-cut can either be analyzed separately or several samples from the same clear-cut can be pooled (as long as the probability of detection of PWN if present is not reduced). The advantage with separate analyses is that you will get data on the proportion of infested objects while the advantage of pooled samples is that the costs for analyzes will be lower (because of fewer samples).

When analyzing the samples for PWN at the laboratory 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 may not be necessary to analyze all the remaining samples but instead invest efforts in looking for further PWN infestations in clear-cuts beyond the new detection. 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.

5.6 Step 6A: If no positive record

If the survey does not result in any positive PWN records it may be because the infestation level is very low or that only a minor part of the sampling zone is infested. To exclude these possibilities a smaller sampling zone around the point of detection should be established. The radius of this zone could be e.g. 10 km and the level of detection could be changed from 1% to 0.1%. With the same assumptions as above this would require 4 470 sampled objects according to table 2 (a zone with 10 km radius includes 21 980 ha forest land and a total population of objects to be sampled of 44 239).

5.7 Step 6B: If positive records

If the outermost PWN-findings are close to the border of the first zone a new sampling zone needs to be established outside the first zone. The width of this zone depends on how many localities PWN is recorded from within the first zone. If many it indicates that PWN is common and may be spread over a much larger area than the first zone. If this is the case an even wider zone than 20 km may be established. This process is continued until no more PWN findings are recorded.

5.8 Step 7: Trapping of beetles

There are several reasons why it is a good idea to include trapping of *Monochamus* beetles in the survey: (1) as a complement to sampling wood for delimiting the infestation, (2) to determine which *Monochamus* species that act as the vector of PWN and (3) in case there are few clear-cuts in the area or if the vector is a species for which logging residues is not the major source of breeding material.

If the delimiting survey is still going on during early spring (i.e. PWN-infested area not yet delimited by the on-going sampling of *Monochamus*-colonized wood objects on clear-cuts) trapping of *Monochamus* beetles may be conducted on some clear-cuts far outside the current sampling zone. A suitable distance should be at least 30 km to reduce the risk for trapping beetles originating from inside the sampling zone. A positive record from a trapped beetle means that a new much wider sampling zone should be established for the sampling of *Monochamus*-colonized wood 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 will influence survey methods and control measures. 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 also for this species. The most accurate way of demonstrating the identity of the vector is by species identification of adult beetles carrying PWN.

In case there are few clear-cuts and pre-commercial thinnings in the area where PWN is detected trapping may be an important complement to sampling of *Monochamus*-colonized wood objects. In such a situation it is very time consuming to search for wind-felled trees or broken branches that may be colonized by *Monochamus*. Also in a situation when *M. sutor* is not believed to be the main vector trapping should be used as a complement to increase the probability to sample other *Monochamus* species.

6 Gaps of knowledge

The most important gap is that we know very little about invasion of PWN into areas where it would be asymptomatic, i.e. not resulting in PWD and tree mortality. All invasions documented so far have resulted in PWD and large tree mortality. Knowledge about asymptomatic PWN invasions would be a large advantage when planning delimiting survey for regions where PWD is not expected. Examples of questions in an asymptomatic situation are: How probable are establishment of PWN if introduced? How rapidly would it spread and what would the proportions of PWN-infested wood objects (colonized by *Monochamus*) and beetles be? Would the proportions of PWN-infested wood objects and beetles decrease when closer to the range limit? Such studies could be conducted in Portugal, China or Japan beyond the range limit of PWD where PWN may occur without causing PWD.

Other questions are: Can PWN survive in healthy living trees? 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)? This is an important question because if PWN can survive for many years in living trees it may enter the vector beetle populations after the forest stands are thinned or clear-cut as a result of the beetles colonizing branches and tops from the cut trees.

How common is PWN in logging residues colonized by *Monochamus* in regions where PWN is established? Most studies of PWN have focused on killed trees and thus we know little about the prevalence of PWN in logging residues.

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

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.

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 respective areas of distribution. This can be studied by trapping *Monochamus* in different areas in Sweden and/or by checking already collected beetles in collections.

Which is the most cost efficient method of detecting PWN in regions where PWD does not occur when comparing trapping and sampling of *Monochamus* colonized wood objects?

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Appendix 1.

Below are tables presented for the sample sizes (K) required at different total number of objects in the population to be sampled (W) and at four different minimum detection levels (π0) to achieve a statistical significance level of 0.05 (Table 1), 0.01 (Table 2) and 0.001 (Table 3). The tables are based on the last formula on page 14 (Section 3.3.2) in an EFSA report (EFSA 2012). For practical use the sample size (K) could be rounded up to next 10 as suggested in the report. The calculations were conducted by Jesper Ryden at the Swedish University of Agricultural Sciences.

***Table 1.** Sample sizes (K) required at different total number of objects in the population to be sampled (W) and at four different minimum detection levels (π0) at a statistical significance level of 0.05.*

	W	K	K	K	K
		π0 = 0.05	π0 = 0.01	π0 = 0.001	π0 = 0.0001
1	1:999	58	282	1000	1000
2	1000:1999	58	290	1899	2000
3	2000:2999	58	293	2328	3000
4	3000:3999	58	294	2525	4000
5	4000:4999	58	295	2634	5000
6	5000:5999	58	296	2703	6000
7	6000:6999	58	296	2750	7000
8	7000:7999	58	296	2784	8000
9	8000:8999	58	296	2810	9000
10	9000:9999	58	297	2830	10000
11	10000:10999	58	297	2846	10449
12	11000:11999	58	297	2859	11399
13	12000:12999	58	297	2871	12349
14	13000:13999	58	297	2880	13299
15	14000:14999	58	297	2888	14249
16	15000:15999	58	297	2895	15199
17	16000:16999	58	297	2901	16149
18	17000:17999	58	297	2907	17099
19	18000:18999	58	297	2912	18049
20	19000:19999	58	297	2916	18999
21	20000:20999	58	297	2920	16303
22	21000:21999	58	297	2923	17079
23	22000:22999	58	297	2927	17856
24	23000:23999	58	297	2929	18632
25	24000:24999	58	297	2932	19409
26	25000:25999	58	298	2935	20185
27	26000:26999	58	298	2937	20961
28	27000:27999	58	298	2939	21738
29	28000:28999	58	298	2941	22514
30	29000:29999	58	298	2943	23291
31	30000:30999	58	298	2945	19578

32	31000:31999	58	298	2946	20210
33	32000:33999	58	298	2949	21473
34	34000:35999	58	298	2952	22736
35	36000:38999	58	298	2955	24631
36	39000:41999	58	298	2958	25263
37	42000:44999	58	298	2960	23720
38	45000,47999	58	298	2963	25301
39	48000:52999	58	298	2966	26355
40	53000:57999	58	298	2968	26140
41	58000:63999	58	298	2971	27042
42	64000:71999	58	298	2973	27511
43	72000:81999	58	298	2976	27852
44	82000:94999	58	298	2978	28110
45	95000:112999	58	298	2981	28474
46	113000:138999	58	298	2983	28718
47	139000:181999	58	298	2986	29080
48	182000:261999	58	298	2989	29360
49	262000:465999	58	298	2991	29624
50	466000:999999	58	298	2993	29805

Table 2. *Sample sizes (K) required at different total number of objects in the population to be sampled (W) and at four different minimum detection levels ($\pi 0$) at a statistical significance level of 0.01.*

	W	K	K	K	K
		$\pi 0 = 0.05$	$\pi 0 = 0.01$	$\pi 0 = 0.001$	$\pi 0 = 0.0001$
1	1:999	87	399	1000	1000
2	1000:1999	89	428	1979	2000
3	2000:2999	89	438	2699	3000
4	3000:3999	89	443	3137	4000
5	4000:4999	89	446	3417	5000
6	5000:5999	89	448	3610	6000
7	6000:6999	89	450	3749	7000
8	7000:7999	90	451	3854	8000
9	8000:8999	90	452	3937	9000
10	9000:9999	90	452	4003	10000
11	10000:10999	90	453	4057	10889
12	11000:11999	90	453	4103	11879
13	12000:12999	90	454	4141	12869
14	13000:13999	90	454	4174	13859
15	14000:14999	90	454	4203	14849
16	15000:15999	90	454	4228	15839
17	16000:16999	90	455	4250	16829
18	17000:17999	90	455	4269	17819
19	18000:18999	90	455	4287	18809
20	19000:19999	90	455	4303	19799
21	20000:20999	90	455	4317	18899

22	21000:21999	90	456	4330	19799
23	22000:22999	90	456	4342	20699
24	23000:23999	90	456	4353	21599
25	24000:24999	90	456	4363	22499
26	25000:25999	90	456	4372	23399
27	26000:26999	90	456	4381	24299
28	27000:27999	90	456	4388	25199
29	28000:28999	90	456	4396	26099
30	29000:29999	90	456	4403	26999
31	30000:30999	90	456	4409	24320
32	31000:31999	90	456	4415	25104
33	32000:33999	90	456	4426	26673
34	34000:35999	90	457	4436	28242
35	36000:38999	90	457	4449	30596
36	39000:41999	90	457	4460	31381
37	42000:44999	90	457	4470	30768
38	45000,47999	90	457	4478	32819
39	48000:52999	90	457	4490	34187
40	53000:57999	90	457	4499	34908
41	58000:63999	90	457	4509	36112
42	64000:71999	90	457	4520	37507
43	72000:81999	90	457	4530	38562
44	82000:94999	90	458	4540	39387
45	95000:112999	90	458	4550	40593
46	113000:138999	90	458	4560	41461
47	139000:181999	90	458	4570	42712
48	182000:261999	90	458	4580	43739
49	262000:465999	90	458	4590	44744
50	466000:999999	90	458	4597	45449

***Table 3.** Sample sizes (K) required at different total number of objects in the population to be sampled (W) and at four different minimum detection levels (π0) at a statistical significance level of 0.001.*

	W	K	K	K	K
		π0 = 0.05	π0 = 0.01	π0 = 0.001	π0 = 0.0001
1	1:999	128	533	1000	1000
2	1000:1999	131	607	1997	2000
3	2000:2999	133	633	2904	3000
4	3000:3999	133	646	3598	4000
5	4000:4999	133	654	4109	5000
6	5000:5999	134	660	4491	6000
7	6000:6999	134	664	4784	7000
8	7000:7999	134	666	5015	8000
9	8000:8999	134	669	5202	9000
10	9000:9999	134	671	5356	10000
11	10000:10999	134	672	5484	10988

12	11000:11999	134	673	5593	11987
13	12000:12999	134	674	5687	12986
14	13000:13999	134	675	5768	13985
15	14000:14999	134	676	5839	14984
16	15000:15999	134	677	5902	15983
17	16000:16999	134	677	5958	16982
18	17000:17999	134	678	6007	17981
19	18000:18999	134	678	6052	18980
20	19000:19999	134	679	6093	19979
21	20000:20999	134	679	6130	20334
22	21000:21999	134	680	6164	21303
23	22000:22999	134	680	6195	22271
24	23000:23999	134	680	6223	23240
25	24000:24999	134	681	6250	24208
26	25000:25999	134	681	6274	25176
27	26000:26999	134	681	6296	26145
28	27000:27999	134	681	6317	27113
29	28000:28999	134	682	6337	28081
30	29000:29999	134	682	6355	29050
31	30000:30999	134	682	6373	27898
32	31000:31999	134	682	6389	28798
33	32000:33999	135	682	6418	30598
34	34000:35999	135	683	6445	32398
35	36000:38999	135	683	6479	35098
36	39000:41999	135	683	6509	35998
37	42000:44999	135	684	6535	36996
38	45000,47999	135	684	6558	39462
39	48000:52999	135	684	6590	41107
40	53000:57999	135	684	6616	43429
41	58000:63999	135	685	6643	44926
42	64000:71999	135	685	6672	47862
43	72000:81999	135	685	6700	50177
44	82000:94999	135	686	6728	52045
45	95000:112999	135	686	6755	54867
46	113000:138999	135	686	6783	57293
47	139000:181999	135	686	6812	60102
48	182000:261999	135	687	6840	62767
49	262000:465999	135	687	6868	65457
50	466000:999999	135	687	6887	67393

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