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Investigate, evaluate, protect

Evaluation of emergency measures to prevent the spread of the pine wood nematode within the European Union

ANSES opinion
Collective Expert Appraisal Report

September 2015 Scientific publication

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The Director General

Maisons-Alfort, 24 September 2015

OPINION
of the French Agency for Food, Environmental and Occupational Health & Safety

on "the control strategy imposed by Implementing Decision 2012/535/EU of 26 September 2012 on emergency measures to prevent the spread within the European Union of *Bursaphelenchus xylophilus*"

On 24 April 2014, ANSES received a formal request from the Directorate General for Food of the Ministry of Agriculture to conduct the following expert appraisal: request for an opinion on the control strategy imposed by Implementing Decision 2012/535/EU of 26 September 2012 on emergency measures to prevent the spread within the European Union of *Bursaphelenchus xylophilus.*

1. BACKGROUND AND PURPOSE OF THE REQUEST

Implementing Decision 2012/535/EU of 26 September 2012 requires Member States to implement emergency measures to prevent the spread, within the European Union, of *Bursaphelenchus xylophilus*, the pine wood nematode. The introduction of this regulated pest in France could cause major damage to many softwood species. These measures aim primarily to eradicate any detected outbreak of the pest, with containment of such outbreaks only being implemented in areas where eradication would not be a feasible objective.
In this context, the Member States must apply eradication measures for at least four years following detection of an outbreak. This mainly involves implementing clear-cutting (within a radius of 500 m that may be reduced to 100 m subject to conditions) around the infested plants and establishing intensive surveillance within a radius varying from 6 km to 20 km around the infested zone (radii assumed to be sufficient in view of the flight distance of the insect vector) (fig.1).

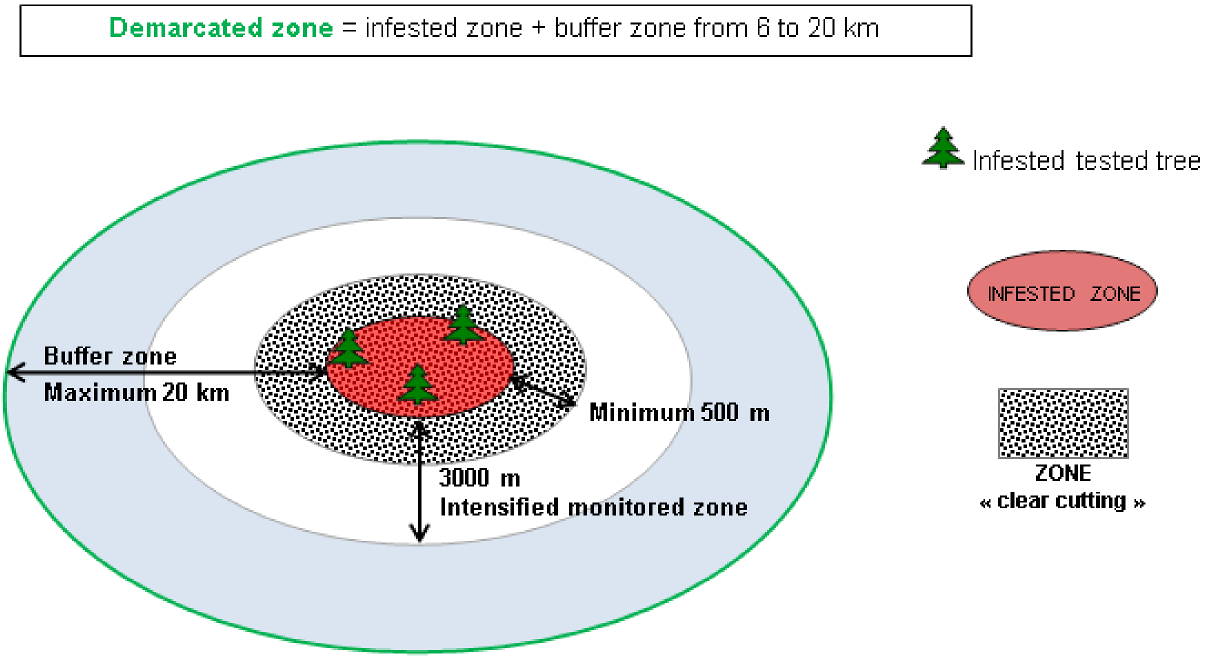

Figure 1: Diagram describing the measures implemented in the context of Implementing Decision 2012/535/EU of 26 September 2012

Such measures can have significant environmental consequences and require considerable human resources, especially if multiple outbreaks are detected. It should be noted that these measures were developed using the scientific and technical information available at the time on the pine wood nematode and its vector in Asia (insects of the genus *Monochamus*).

New studies have recently been published. Some were carried out in Europe and relate in particular to:

- the flight distance of the insect vector in Europe (*Monochamus galloprovincialis*), which appears to be much greater than that of the insect vector in Asia, *Monochamus alternatus* (3 km), which served as a point of reference when drafting the European decision,

- the effectiveness of the method aiming to perform micro-injections of emamectin benzoate for the implementation of preventive treatments (this compound may have both a nematicide effect on the nematode and an insecticide effect on the vectors).

In this context, and in light of these recent studies, a request was made to:

i) review the control strategy as described by Implementing Decision 2012/535/EU of 26 September 2012, in order to determine whether these measures are still suited to the eradication of potential outbreaks,

ii) propose, if necessary, improvements to this control strategy to increase the associated effectiveness/impact ratio.

The answers to these questions could enable discussions to be initiated at European level with the aim of amending, if necessary, Implementing Decision 2012/535/EU of 26 September 2012.

2. ORGANISATION OF THE EXPERT APPRAISAL

The expert appraisal was carried out in accordance with French Standard NF X 50-110 "Quality in Expert Appraisals – General Requirements of Competence for Expert Appraisals (May 2003)".

The expert appraisal falls within the sphere of competence of the Expert Committee (CES) on Biological risks for plant health. ANSES entrusted the expert appraisal to the Working Group on the "Pine wood nematode". The methodological and scientific aspects of the work were presented to the CES between 17 June 2014 and 8 September 2015. They were adopted by the CES on Biological risks for plant health at its meeting of 8 September 2015.

ANSES analyses the links of interest declared by the experts prior to their appointment and throughout the work, in order to avoid potential conflicts of interest with regard to the matters dealt with as part of the expert appraisal.
The experts' declarations of interests are made public *via* the ANSES website (www.anses.fr).

3. ANALYSIS AND CONCLUSIONS OF THE CES

The formal request 2014-SA-0103 concerned the emergency measures designed to prevent the spread, in the European Union, of the pine wood nematode, *Bursaphelenchus xylophilus.* More specifically, the Working Group (WG) set out to: i) review the control strategy based on clear-cutting described by Implementing Decision 2012/535/EU of 26 September 2012, in order to determine whether such measures are still suited to the eradication of potential outbreaks; ii) propose, if necessary, improvements to this control strategy that would increase the associated effectiveness/impact ratio. The work of the WG was based on both an in-depth literature analysis (in particular taking into account recent data concerning the flight distance of the insect vector in Europe and the effectiveness of the method for performing micro-injections of emamectin benzoate for preventive treatments), and on the production of original data (modelling to simulate the dispersal of the insect vector, the transmission of the pine wood nematode and lastly the felling of trees in an increased radius area).

Following detection of an outbreak, Implementing Decision 2012/535/EU advocates eradication measures for at least four years, mainly consisting of the establishment of: i) clear-cuts with a radius of 500 m around the infested plants and ii) intensive surveillance within a radius varying from 6 to 20 km around the infested zone. To estimate the effectiveness of these measures, a simulation model was developed and calibrated using experimental data specifying the dispersal capabilities of the insect vector *Monochamus galloprovincialis.* These data were obtained in the laboratory (on a flight mill) and *in natura* (mark-and-recapture experiments) in the context of the Landes de Gascogne forest, one of the forest areas most exposed to the risk of introduction of the pine wood nematode in France. The simulations that were then carried out under different scenarios (preventive or curative) show that the clear-cuts currently advocated by the European and French regulations would not be effective in a landscape configuration of continuous plantations of maritime pine. Indeed, with the radius of 500 m recommended by the European directive, at best 11% of transmissions would be prevented. Moreover, to obtain a pine wood nematode transmission rate lower than 0.1%, it would be necessary to implement clear-cuts within a radius of between 15 and 38 km. These simulations, carried out in the context of a continuous forest, should however be supplemented by a scenario involving highly fragmented pine forests. In this regard, it would be interesting to include biological data from Spanish forest managers, as they are directly involved in combating the nematode in this type of landscape.

Subsequently, in order to propose possible improvements to the current control measures against B. *xylophilus*, the WG considered the following options:

• Mass trapping of the insect vector:

Mass trapping measures are sometimes considered to eradicate insect populations or reduce their level below an acceptable epidemiological threshold. In practice this entails having to capture more than 90%, or even 99%, of the local population. Three recent studies, based on mark-release-and-recapture of insects, assessed the effectiveness of mass trapping of *M. galloprovincialis* in Europe (Spain and France). These three studies agree on the very limited capture effectiveness of the most commonly used pheromone traps, of the order of 0.5 to 1% of circulating insects. Moreover, it should be remembered that, as with all "curative" methods, it needs to be applied every year, throughout the insect's flight season. Lastly, and even if it were technically feasible, it would be ethically unacceptable to seek to eradicate a native species such as *M. galloprovincialis* which, apart from being the vector of the nematode, is part of the biodiversity of European forests.

• Chemical control:

The technique of micro-injection of a biocidal compound, emamectin benzoate (EB), associated with systemic dissemination of the product in the injected tree trunks, has recently seen renewed interest for combating pine wilt disease due to *B. xylophilus*, following encouraging results on its experimental efficiency obtained both *in vitro* and *in natura*. In fact, this compound is currently being assessed in the context of applying for marketing authorisation in France for this purpose. At the practical level, the injection of EB in tree trunks presents some advantages: absence of phytotoxicity at the recommended doses, safety for the applicator and the environment, and a preventive treatment that is effective for two or three years. On the other hand, its implementation requires considerable manual operations that have to be repeated regularly, which is probably incompatible with the treatment of extended tracts of forest. Nevertheless, it could be an appropriate solution for treating trees with great heritage value, for urban tree planting, or for trees located close to risk areas.

• Individual-centred control:

Given the lack of effectiveness of the methods presented above on the scale of a forest massif, it appears necessary to consider another, more targeted approach. This so-called individual-centred control strategy is based on a three-step approach: i) the early detection of the arrival of the nematode in a new forest region, triggering a series of measures designed to reduce its impact, with ii) the precise identification of individual symptomatic trees, followed by iii) elimination of the nematode. Without going into detail about the actions that could be undertaken to this effect, options on how to implement this individual-centred strategy are listed below.

- Early detection of the presence of the nematode remains the priority of this control strategy. To achieve this, it will be necessary to combine: i) the trapping of insect vectors (mesh size to be defined depending on the context) and the detection (by ground and/or air surveillance) of symptomatic trees with ii) systematic screening for the nematode in insect and tree samples.

- Each time insect vectors of *B. xylophilus* are reported, the position of the traps where they were captured will be triangulated to delineate the likely zone of the presence of the pine wood nematode.

- Each time a tree containing the pine wood nematode has been identified, it will be disposed of individually on site according to the current recommendations, specifically, this will take place between the end of the autumn and the beginning of the spring, when the insect vectors are still in the wood, and in compliance with the regulations concerning transport and management of timber cut in a buffer zone around the destroyed wood. Considering the results of the simulation on the dispersal capabilities of the insect vector, this buffer zone should be expanded to a radius of 40 km.

In conclusion, it is considered that the only currently operational method for limiting the extension of wilt associated with infestation by the pine wood nematode in a contaminated forest stand, combining efficacy and reasonable cost, remains a combination of strengthened resources (both technical and financial) for early detection of the nematode (on insect vectors or in trees) followed by sanitation felling as an outbreak develops. It aims for the targeted elimination of infested trees according to the above recommendations. The objective of control is then no longer to eradicate the disease, but rather to stem its progress on the scale of the forest stand. It is important to reiterate that the measures detailed here, which are the subject of the formal request, primarily target the natural spread of the pathogen by its vector, around a detected outbreak. Containing an outbreak on a wide geographical scale will only be effective if these measures are combined with rigorous application of the regulations concerning the treatment and transport of timber, to avoid introductions over long distances.

4. AGENCY CONCLUSIONS AND RECOMMENDATIONS

The French Agency for Food, Environmental and Occupational Health & Safety reiterates that the impact of the pine wood nematode has been very detrimental in pine forests in Portugal, and that this could also be the case for the whole of the European Union. Effective measures must be implemented very rigorously throughout the territory of the European Union to prevent the pine wood nematode being introduced to new areas.

Applying the clear-cutting measures described by Implementing Decision 2012/535/EU of 26 September 2012 does not enable the eradication of B. *xylophilus* in a landscape of continuous pine forests.

The only currently operational method for limiting the extension of wilt associated with infestation by the pine wood nematode in a contaminated forest stand, combining efficacy and reasonable cost, remains a combination of strengthened resources (both technical and financial) for early detection of the nematode (on insect vectors or in trees) followed by sanitation felling as an outbreak develops. Containing an outbreak on a wide geographical scale will only be effective if these measures are combined with rigorous application of the European regulations in force concerning the treatment and transport of timber, to avoid introductions over long distances.

Marc Mortureux

KEYWORDS

Pine wood nematode, *Bursaphelenchus xylophilus*, *Monochamus galloprovincialis*, pine trees, clear-cut, modelling, alternative management.

Request for an opinion on "the control strategy imposed by Implementing Decision 2012/535/EU of 26 September 2012 on emergency measures to prevent the spread within the European Union of *Bursaphelenchus xylophilus*"

Request No. 2014-SA-0103 Pine wood nematode

Collective Expert Appraisal
REPORT

Expert Committee on Biological risks for plant health

Working Group on the Pine wood nematode

September 2015

Key words

Pine wood nematode, *Bursaphelenchus xylophilus*, *Monochamus galloprovincialis*, pine trees, clear-cut, modelling, alternative management.

Presentation of participants

PREAMBLE: Outside experts, whether Expert Committee and WG members or designated rapporteurs, are all appointed in their personal capacity, *intuitu personae*, and do not represent their parent organisation.

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HEARINGS WITH EXTERNAL FIGURES

Ministry of Agriculture - Department of Forest Health

Mr Dominique PIOU - Forestry Entomologist and Pathologist, National Pine Wood Nematode Expert for the French Ministry of Agriculture

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Acronyms and abbreviations

ANSES: French Agency for Food, Environmental and Occupational Health & Safety
B. xylophilus: *Bursaphelenchus xylophilus*
CES: Expert Committee
cm: centimetre
DSF: Department of Forest Health
EB: Emamectin Benzoate
eff: effectiveness of the trap
FAO: Food and Agriculture Organization of the United Nations
fig: figure
ha: hectare
km: kilometre
L: larval stage
NRL: National Reference Laboratory
m: metre
M. galloprovinciallis: *Monochamus galloprovinciallis*
ISPM 15: International Standard for Phytosanitary Measures no.15
FVO: Food and Veterinary Office
EPPO: European and Mediterranean Plant Protection Organization
REPHRAME: Research Extending Plant Health Risk And Monitoring Evaluation
tab: table

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1 Background, purpose and procedure for handling the request

1.1 Background

Implementing Decision 2012/535/EU of 26 September 2012 requires Member States to implement emergency measures to prevent the spread, within the European Union, of *Bursaphelenchus xylophilus*, the pine wood nematode. The introduction of this regulated pest in France could cause major damage to many softwood species. These measures aim primarily to eradicate any detected outbreak of the pest, with containment of such outbreaks (application of plant protection measures in or around the infested zone in order to prevent the spread of a pest) only being implemented in areas where eradication would not be a feasible objective.

In this context, the Member States must apply eradication measures for at least four years following detection of an outbreak. This mainly involves implementing clear-cutting (within a radius of 500 m that may be reduced to 100 m subject to conditions) around the infested plants and establishing intensive surveillance within a radius varying from 6 km to 20 km around the infested zone (radii assumed to be sufficient in view of the flight distance of the insect vector) (fig. 1).

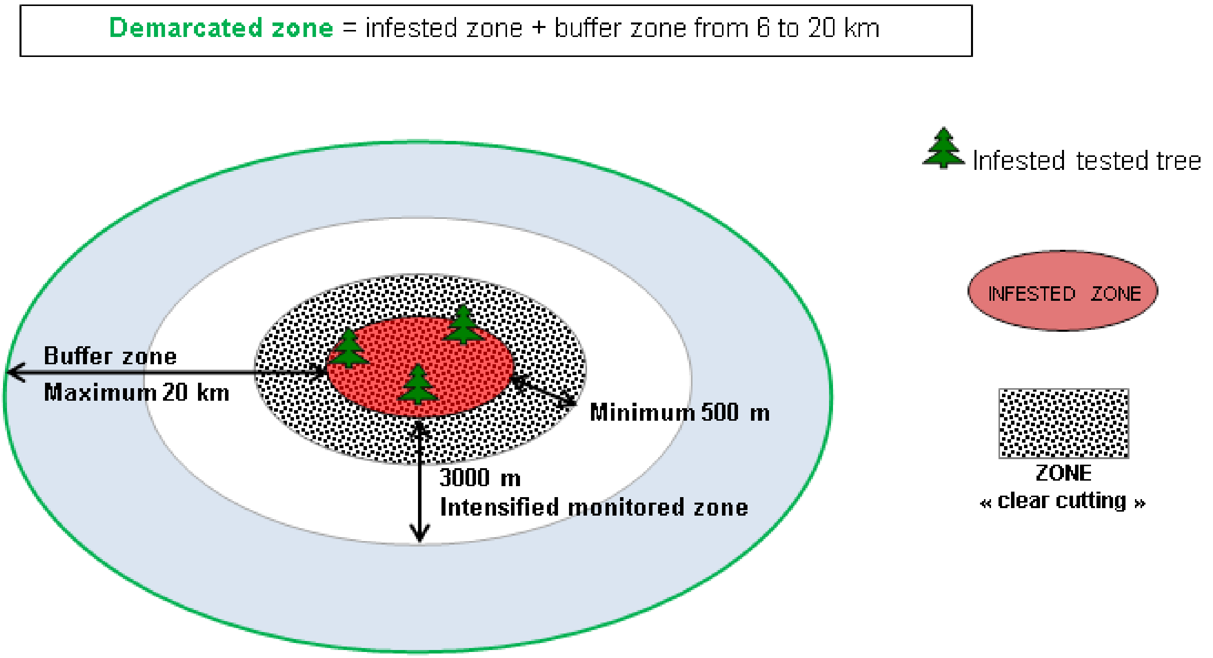

Figure 1: Diagram describing the measures implemented in the context of Implementing Decision 2012/535/EU of 26 September 2012

Such measures can have significant environmental consequences and require considerable human resources, especially if multiple outbreaks are detected. It should be noted that these measures were developed using the scientific and technical information available at the time on the pine wood nematode and its vector (insects of the genus *Monochamus*).

1.2 Subject of the request

New studies have recently been published. Some were carried out in Europe and relate in particular to:

- the flight distance of the insect vector in Europe (*Monochamus galloprovincialis*), which appears to be much greater than that of the insect vector in Asia, *Monochamus alternatus* (3 km), which served as a point of reference when drafting the European decision,

- the effectiveness of the method for performing micro-injections of emamectin benzoate for preventive treatments (this compound may have both a nematicide effect on the nematode and an insecticide effect on the vectors).

In this context, and in light of these recent studies, the Agency was asked to:

i) review the control strategy as described by Implementing Decision 2012/535/EU of 26 September 2012, in order to determine whether these measures are still suited to the eradication of potential outbreaks,

ii) propose, if necessary, improvements to this control strategy that would increase the associated effectiveness/impact ratio.

The answers to these questions could enable discussions to be initiated at European level with the aim of amending, if necessary, Implementing Decision 2012/535/EU of 26 September 2012.

1.3 Procedure for handling the request: resources applied (ANSES, CES, WG, rapporteur(s)) and organisation

ANSES entrusted examination of this request to the Working Group on the pine wood nematode, reporting to the Expert Committee (CES) on Biological risks for plant health.

The methodological and scientific aspects of this group's work were regularly submitted to the CES. The report produced by the Working Group takes account of the observations and additional information provided by the CES members.

This work was therefore conducted by a group of experts with complementary skills.

The expert appraisal was carried out in accordance with French Standard NF X 50-110 "Quality in Expert Appraisals – General Requirements of Competence for Expert Appraisals (May 2003)".

1.4 Prevention of potential conflicts of interest

ANSES analyses the links of interest declared by the experts prior to their appointment and throughout the work, in order to avoid potential conflicts of interest with regard to the matters dealt with as part of the expert appraisal.

The experts declarations of interests are made public via the ANSES website (www.anses.fr).

2 Foreword

Bursaphelenchus xylophilus is the plant-parasitic nematode responsible for pine wilt disease: infected trees turn brownish-red, lose their needles and die within a few weeks (fig. 2).

Photos Setúbal Portugal, G. Roux, INRA Orléans

Figure 2: Symptoms caused by the *B. xylophilus* nematode on pine trees

2.1 Geographical distribution of *B. xylophilus*

B. xylophilus is distributed widely in Canada and the USA from where it originated (Wingfield *et al.*, 1982; Robbins, 1982) and was also reported once in Mexico, in 1992 (Dwinell, 1993). It has been present in Japan since the beginning of the 20th century, and was very likely introduced by contaminated wood imported from the United States (Nickle *et al.*, 1981). It was then detected in Korea, China and Taiwan in the 1980s (EPPO, 1986) and since 1999 in Europe, in Portugal (Mota *et al*., 1999), a country in which this nematode is today considered to be established. It was found on the island of Madeira in 2009 and has been detected several times in Spain since 2008 (four outbreaks currently being managed) (Abelleira *et al.*, 2011). All of these reports are shown on the map in figure 3.

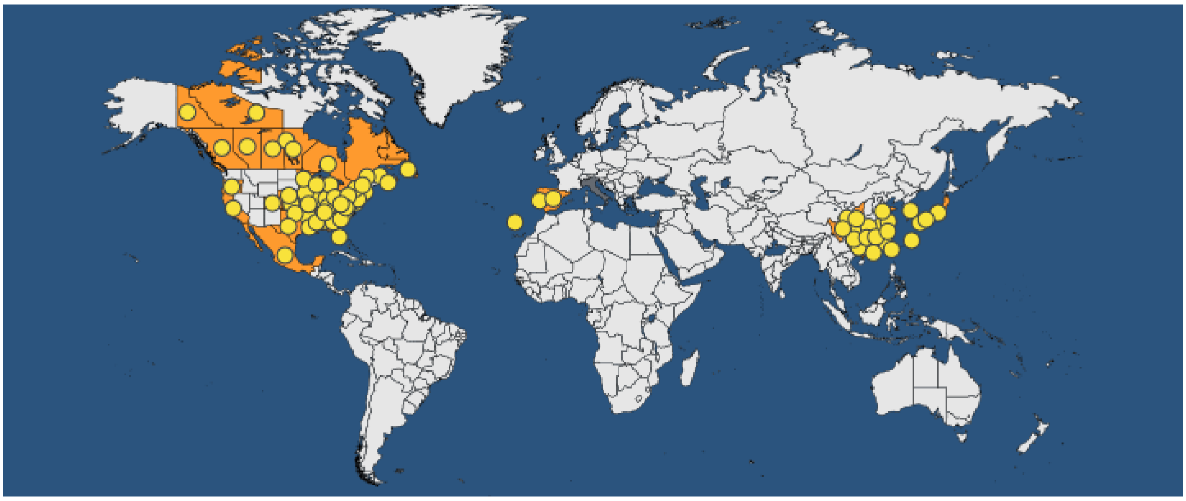

Figure 3: Global distribution of *B. xylophilus* (according to the EPPO global database: https://gd.eppo.int/taxon/BURSXY/distribution)

2.2 Host plants of *B. xylophilus*

B. xylophilus is mainly found on pine trees (*Pinus* spp.). North-American pine species are naturally resistant or tolerant to the nematode (Wingfield *et al.*, 1982; Rutherford *et al.*, 1987). This natural resistance results from a long co-evolution between the nematode and the native species of pine (Lieutier *et al.*, 2009).

It causes major damage in Asia on *Pinus densiflora, P. thunbergii*, P. *luchuensis* and *P. massoniana* (Kwon *et al*., 2011; Togashi and Jikumaru, 2007; Wang *et al*., 2014).

European pine trees are all susceptible to *B. xylophilus*, in particular *P. sylvestris, P. nigra, P. pinaster, P. halepensis* and *P. pinea* (Evans *et al.*, 1996; EPPO, 2009a). European pine trees introduced into the USA also suffer major damage due to the nematode, especially after periods of drought (Randall *et al.*, 2006). In Portugal, the only susceptible species observed is *P. pinaster* (Rodrigues, 2008) but *B. xylophilus* has also been observed on dead black pine (Inacio *et al.*, 2014). Although the nematode can develop on stone pine, this species seems particularly resistant (Silva *et al.,* 2015) and does not seem to be infected *in natura* in Portugal (Santos and Vasconcelos, 2012).

B. xylophilus can also be found on dead trees (without being the cause of their death) of species in the genera *Abies, Chamaecyparis*, *Cedrus, Larix, Picea* and *Pseudotsuga* and other conifers with the exception of *Thuja* spp. (EPPO, 2009b).

2.3 Life cycle of the nematode in relation with its vector

In all the countries where it has been introduced, the pine wood nematode is transmitted from one tree to another by sawyer beetles of the genus *Monochamus*. The only vector currently known in Europe is *Monochamus galloprovincialis* (Sousa *et al.*, 2001, 2002) but *M. sutor* and *M. sartor* are potential vectors, especially in forests of Scots pine (Tomiczek and Hoyer-Tomiczek, 2008). The biological cycle shows the complex interactions between the nematode and its insect vector (fig. 4).

The transmission of nematodes by an insect carrier to a susceptible tree takes place in one of two ways:

- either on healthy trees through wounds caused by insects during maturation feeding on green shoots,
- or on dying or dead trees (or on dead branches in the crown of living trees) by female insects during egg-laying.

With transmission during oviposition (left part of the cycle, fig. 4), nematodes leave the insect and penetrate the tree via the notches bored by the female to lay her eggs. In the wood, the nematodes then feed exclusively on fungal mycelium (most often *Ceratocystis* spp. or *Ophiostoma* spp.).

When the nematodes are transmitted by the young adult insects during maturation feeding, they leave the insect (they are mainly located in the trachea) and penetrate through the feeding wounds (Aikawa 2008). They then multiply in the xylem sieve tubes (sap channels) where they feed on epithelial cells (Takemoto 2008).

Although most of them remain around the inoculation point (around 46% according to Takemoto, 2008), the nematodes also move very rapidly throughout the tree via the vertical xylem channels, and the resulting desiccation and obstruction of the channels causes die-back through embolism, and later the death of the tree within 4 to 5 weeks in favourable climatic conditions. The dying tree then becomes attractive to the *Monochamus* and especially for egg-laying by females.

A close relationship is therefore established between the nematode and its vector: the insect transports the nematode, ensuring its dispersal (phoresis), and the nematode provides the insect with sites favourable for oviposition by causing the death of the trees.

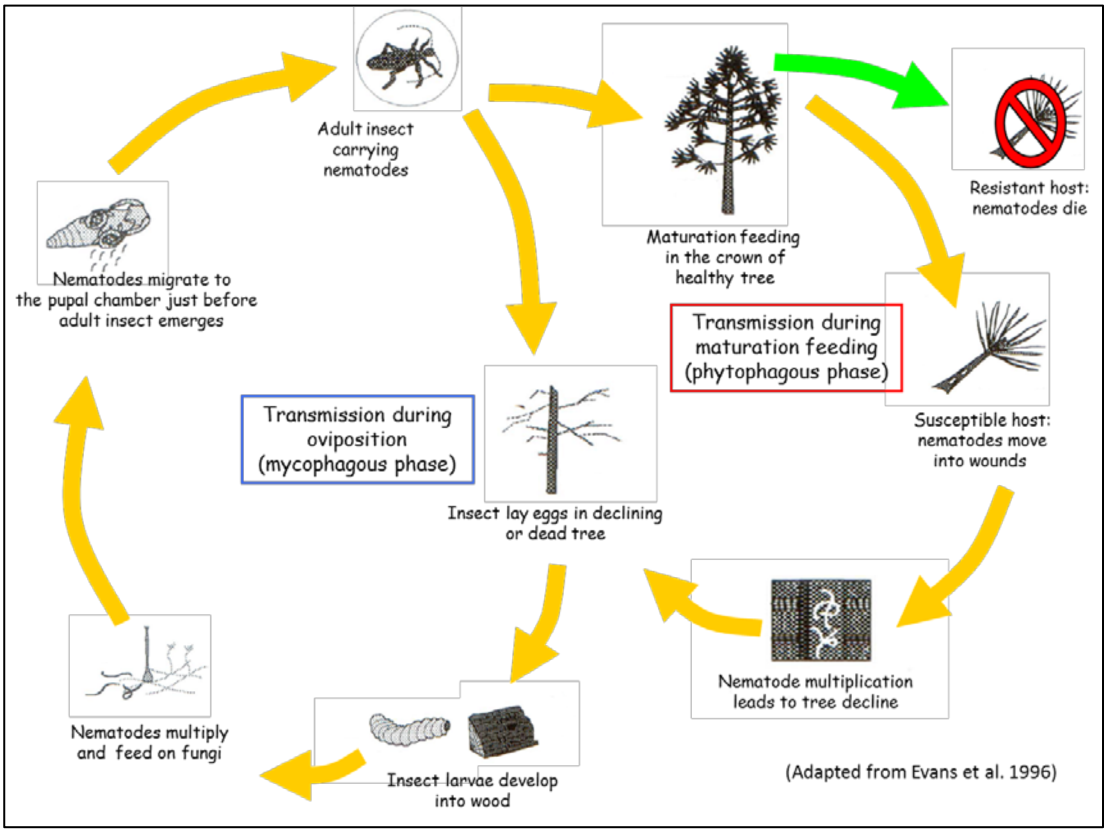

Figure 4: Biological cycle of *B. xylophilus*, in relation with that of its vector *M. galloprovincialis*

2.4 Development cycle of *B. xylophilus*

The development cycle of *B. xylophilus* comprises a propagative cycle and a dispersal cycle (Aikawa, 2008) (fig. 5).

The propagative cycle enables the nematode to reproduce rapidly when environmental conditions are favourable. The dispersal cycle facilitates transmission of the nematode by the vector and enables long periods of quiescence in adverse conditions.

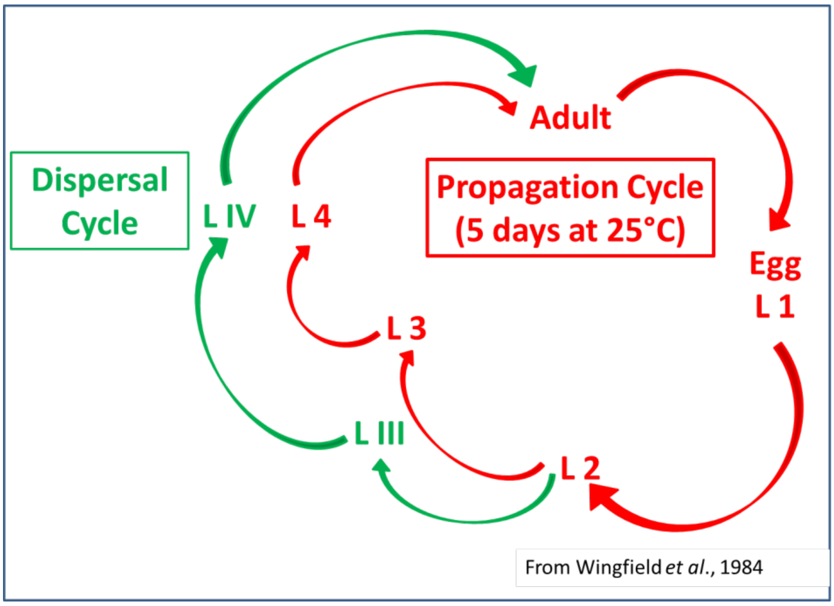

Figure 5: Development cycle of *B. xylophilus*

<u>The propagative cycle</u> of *B. xylophilus* comprises six stages of development: the egg, four larval stages and the adult stage. *B. xylophilus* can complete one cycle in 4 to 5 days at a temperature of 25°C, from the egg to the adult via the four larval stages L1 to L4. The duration of the nematode propagative cycle varies with the temperature: the most favourable temperatures lie between 20 and 30°C. For example, in conditions of artificial culture on the fungus *Botrytis cinerea*, a full cycle is completed in about 3 days at 30°C, 6 days at 20°C, 12 days at 15°C. The temperature thresholds for development of *B. xylophilus* are 6.5°C for the lower limit and 40°C for the upper limit. Exposure to 50°C for a few minutes is lethal for the nematode (Takemoto, 2008).

<u>The dispersal cycle</u> begins when the tree is dying or dead, and only when *Monochamus* pupae are present in the wood: an LIII dispersal stage is formed instead of the L3 propagation stage. This is a survival stage, the nematode does not feed and is able to withstand adverse conditions such as drought, lack of food and low temperatures (Futai, 2013).

These L III larvae aggregate in the wood around the *Monochamus* pupal chamber, probably due to an attraction to substances emitted by the pupa. Just before the emergence of the adult *Monochamus* insect*,* the LIII larvae moult into the fourth stage of dispersal, LIV or "dauer" larvae specialised in survival during transport of the nematode by the insect (Aikawa, 2008). These "dauer" larvae enter the pupal chamber, and when the young adult insect emerges, it is carrying nematodes that have settled beneath its elytra and penetrated its trachea. The infested immature insect then flies toward the crowns of pine trees to feed on young pine shoots (maturation feeding).

Immediately after penetrating a susceptible pine tree, the "dauer" larvae moult into adults and reproduce extremely quickly.

2.5 Development cycle of *Monochamus galloprovincialis*

The development cycle of *Monochamus galloprovincialis* consists of two distinct phases, an internal phase corresponding to development of the larva in dying or recently dead wood, and an external phase of maturation of young adults, followed by the reproduction of sexually mature adults. Egg-laying by adult females does not take place in the branches used by adults for the maturation feeding, but requires a search for favourable host trees, thus contributing to the dispersal of the species (Futai, 2013).

2.5.1 Phase of larval development

During the summer, eggs are deposited individually under the bark of declining trees, in notches made by the female using her mouthparts. These notches are coated with a gelatinous substance secreted by the female, which helps to protect the egg from fungi and prevent egg-laying by other females or cannibalism, which is frequent in this species (Naves *et al.*, 2007a). When eggs hatch, larvae bore tunnels in the phloem, between the bark and the sapwood, before burying themselves into the xylem, from the third larval stage onward, in order to establish a pupal chamber where they will overwinter. The first larval stage develops during the summer and the third larval stage is reached before the winter (fig. 6). The sawdust and faeces regularly produced by the larva as it progresses through the wood clog the tunnels and insulate them from variations in external temperature. The size, shape and distribution of this debris are good indicators of the presence of the species. Pupation occurs the following spring. Larval development, comprising four stages, usually takes place within a year, but can vary depending on temperature conditions, the date of oviposition and the quality of the log, and may extend for up to two years (Tomminen, 1993; Koutroumpa *et al.*, 2008).

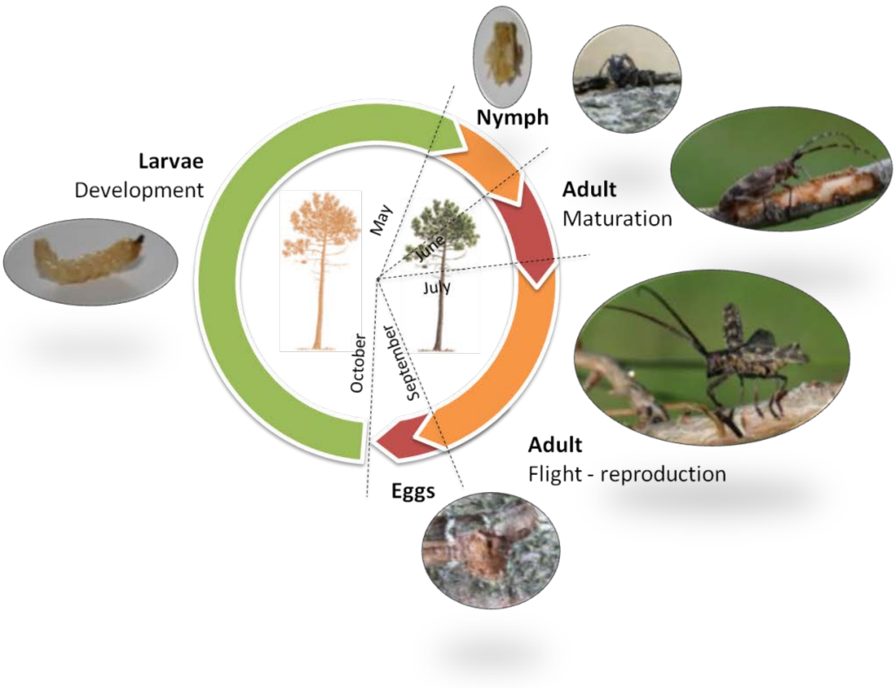

Figure 6: Example of life cycle of *Monochamus galloprovincialis* on maritime pine in Aquitaine

2.5.2 Phase of adult reproduction

At the end of spring or the beginning of summer, depending on climate conditions, young (immature) adults bore a characteristic round exit hole through the bark. The emergences take place throughout the summer, sometimes until September, with a peak between June and July at our latitudes (Naves *et al*., 2008, Koutroumpa *et al*., 2008). In general the males emerge before the females. The young adults then perform their first maturation feeding on the elaborated sap found in the phloem of young pine shoots. The insects are attracted by a combination of visual (the pine tree silhouette) and olfactory signals (smell of resin) (Giffard *et al*., *in prep*.). This feeding period, necessary for the development of the reproductive organs, continues for two weeks (David *et al.,* 2015*;* Naves *et al*., 2006) (fig. 6). The sexually mature adults search for ovipositing sites on dying trees, guided from a distance by volatile substances emitted by weakened pines (example, alpha-pinene) and by the bark beetles that attack these trees (aggregation pheromone containing ipsenol and ipsdienol) (Ibeas *et al.*, 2008; Alvarez *et al*., 2014). In addition to these substances, the females recognise the males from a short distance away by means of a specific male-produced pheromone, a hydrocarbon produced by the males, 2-methyl-3-butanol (Ibeas *et al.*, 2008, Pajares et al. 2010). This species has a high fertility rate, since a female may lay up to 140 eggs throughout her adult life, even though more than 60% of the eggs are deposited during the first half of the oviposition period (Koutroumpa *et al*., 2008). The adults can live for around 4 months (Giffard *et al.*, 2015). Throughout their adult life the insects are able to disperse by flying. Their flight capabilities increase with their age and size, as well as with the lipid reserves accumulated during the shoot feeding phase (David *et al*., 2014). On a flight mill, the insects can cover 2 km per day on average. Mark-and-recapture experiments confirm these considerable dispersal capabilities, with insects frequently recaptured in traps located several tens of kilometres from the point of release (Gallego *et al*., 2012; Mas *et al*., 2013).

2.6 Development of the disease

Once they have penetrated a healthy tree via the feeding wounds, nematodes travel through the resin canals, feeding on living cells by breaking down the cell walls with the cellulases they secrete. They migrate very quickly through the branches and invade the trunk. They can move up to 1.50 m per day (Kuroda *et al.*, 1992) in the horizontal and vertical channels and thus invade an 8 m tree in ten to fourteen days (Takemoto, 2008).

The reduction and then cessation of resin production is the first detectable symptom. The tree defence response to the attack of the nematode is expressed as tracheid dehydration. The cavitation induced in these tracheids causes embolism, as the rise of sap is blocked (Kuroda, 2008). Approximately three weeks after infestation, the tree shows the first signs of dehydration: sudden discolouration of older needles and then discolouration and wilting of young needles. The tree can die thirty to forty days after its infestation, when it contains millions of nematodes that are mainly distributed in the aerial part. The onset of symptoms depends on various environmental factors such as soil water capacity, drought and temperature. For example, symptoms of wilt only appear when the mean summer temperature exceeds 20°C (Rutherford *et al*., 1987, 1990; EPPO, 2009a). It is considered that trees do not exhibit symptoms below the 20°C isotherm in summer.

<u>Cases of infested and asymptomatic trees</u>

Some trees can however survive infestation, for a year, or even longer. This may be due, among other causes, to a late or insufficient (low nematode load) infection, cool climate conditions unfavourable to development of the nematode, mechanisms of resistance (not yet understood) or the interaction of these factors. These infested asymptomatic trees can play an important role in dissemination of the disease because they act as reservoirs of nematodes (Bergdhal, 1999) and also because they emit volatile compounds likely to attract female *Monochamus* (Takeuchi *et al.*, 2007; Futai *et al.*, 2008).

2.7 Economic impact

In Japan, the disease caused losses of up to one million cubic metres of wood per year at the beginning of the 20th century, and two million per year once the trees were no longer being eliminated (since 1970) (Mamiya, 2004). In 1986, the annual budget for control of the disease was fifty million dollars (US), of which more than half was for chemical treatments.

In Korea, some four hundred million dollars (US) have been spent over the past three decades on nematode eradication campaigns (FAO, 2015).

In China, the damage was estimated at three hundred and sixty million dollars (US) (direct cost) between 1982 and 2002 (Shi *et al.*, 2007).

In Portugal, about twenty-four million euros were spent between 2001 and 2009 on control of the nematode (five million trees felled; Rodrigues, 2008). In Spain, the cost of management and eradication of the first outbreak in Extremadura was estimated at nine million euros (EPPO, 2009a). During the period 2001-2012, the European Union spent thirty million euros on support measures for surveillance and control of the pine wood nematode (European Commission data). A recent study estimated that the losses in Europe due to damage by the nematode may be of the order of twenty billion euros during the period 2008-2030 in the absence of effective control measures (Soliman *et al*., 2012).

In the United States, despite there being no symptoms caused by the pine wood nematode on native species, the mere possibility of its presence in the wood has had a significant economic impact on the timber export sector. Indeed, from 1984 onwards, some countries (Finland, Sweden and Norway, and then Korea) decided to impose an embargo on timber from the USA (Bergdahl, 1988). In addition, from 1985 onwards, the EPPO recommended that its member countries impose restrictions on imports of timber from contaminated countries. Despite all these measures, direct damage is regularly observed on Scots pine, an exotic species in the United States which nevertheless has economic value (Christmas trees).

2.8 Environmental impact

In Japan, China and Korea, the rapid spread of the disease has destroyed large areas of pine forests, resulting in significant changes to the local ecosystem. On the archipelago of Ogasawara in Japan, almost all the *Pinus luchuensis* have been destroyed (Mamiya, 1983).

The disappearance of the trees has had a non-negligible impact on soil erosion, stabilisation of the sand and protection against the wind, not to mention on the aesthetic and spiritual value of the site. In most of the forests affected by the disease, the pine trees have been replaced by other forest tree species (for example *Quercus mongollica* in Japan), resulting in a major change in biodiversity.

2.9 Risk of introduction into France

The nematodes can move from one tree to another by means of their insect vector. The natural expansion of the nematode is therefore rather slow, depending on the vector dispersal capabilities and behaviour, as well as the dynamics of its populations. From 2000 to 2007, the area affected by the nematode in Portugal increased from 309,000 ha to 510,000 ha (Rodrigues, 2008). The Pyrenees mountain range could constitute a partial barrier to the migration of insects from areas contaminated by the nematode (Haran *et al*., 2015).

The long-distance dispersal of the nematode takes place through human activity, e.g., the trade in timber from infested areas. There is a danger of introduction if the wood transporting the nematode contains or attracts its insect vectors. These vectors can transmit the nematodes to pine trees after their emergence (Robinet *et al.*, 2011). The nematode and its vector are capable of surviving for forty weeks after felling and processing of wood (Sousa *et al.*, 2011). Thus, wood that has not been treated by heat (56°C internal temperature) or by methyl bromide as required in the regulations (international standard ISPM 15; FAO, 2009), and that is used for packaging (crating, pallets, etc.) or cushioning material, may contain an abundant population of *B. xylophilus* able to survive long enough for the vector to have time to complete its development, if it is present in the wood and has survived the sawing process.

The potential channels for introduction of the nematode in France are, by order of importance (Evans *et al.*, 1996; EPPO, 2009a):

- packaging or crating wood: the presence of the nematode and its vector is highly likely in wood harvested in contaminated areas, with the exception of treated wood (according to standard ISPM 15). The infected wood may contain large quantities of nematodes and *Monochamus*.
- timber from host species, sawn or not, with or without bark: the presence of *B. xylophilus* and *Monochamus* in transported trunks with bark still intact represents a very high potential risk of introduction, limited by bans on intra-EU trade from contaminated areas.
- seedlings for planting: risks exist only in the rare cases where the nematode and its vector are present. The risk is limited to trees with a diameter of more than three centimetres, which are generally at least five years old, and therefore only to ornamental plantings of developed trees.
- wood waste: low probability if the control measures are complied with (dimension of chips less than three centimetres ensuring the destruction of insect larvae), and only in the case where insects and nematodes are present.
- isolated bark: risk virtually non-existent because, although bark may carry the nematode, the vector cannot be present.

The possibility of nematode transmission by contact between planks of healthy and contaminated wood was recently demonstrated if the moisture content of the wood is greater than 20% (EU-funded REPHRAME Project, Final Report 2015).

2.10 Probability of establishment in France

The pine wood nematode could potentially become established on the French territory because:

- The vector is present in all the regions of France, particularly in the Landes forest.
- The French forest area occupied by pine species susceptible to the nematode is around 2.5 million ha (French National Forest Inventory, 2013).

- The climatic conditions in the southern area of France, where summer temperatures are above 20°C, are favourable to development of the nematode and its host, and to the expression of tree decline (fig. 7).

- The presence of other indigenous species of *Bursaphelenchus* which are non-pathogenic for pine trees, such as *B. mucronatus*, found throughout France and occupying the same ecological niche as *B. xylophilus,* does not seem to be an obstacle to the establishment of *B. xylophilus*. Work conducted in the laboratory has even shown that *B. mucronatus* could be displaced from its ecological niche by *B. xylophilus* in the event of introduction of the latter (Vincent *et al.*, 2008).

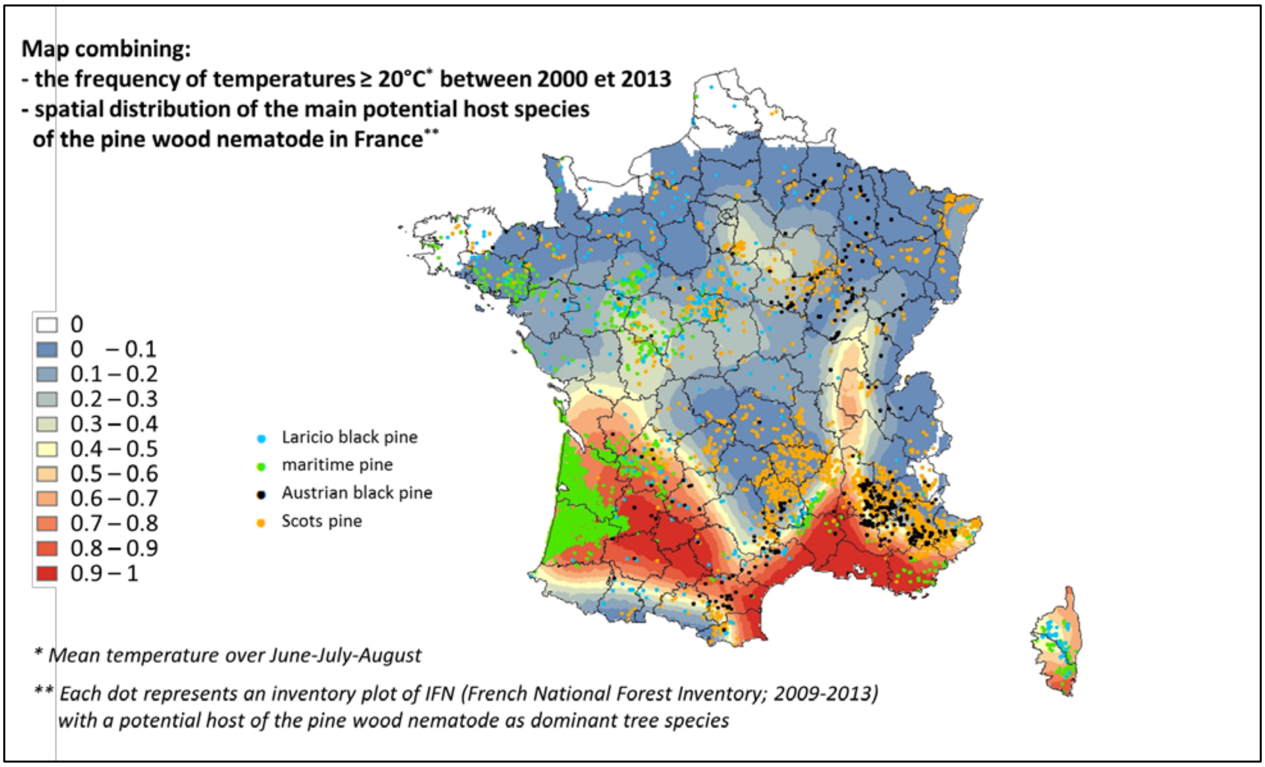

Figure 7: Distribution of the main species of pine trees susceptible to the pine wood nematode and frequency of years with a mean temperature greater than 20°C in June-July-August, between 2000 and 2013.

According to the study by Hannah Gruffudd and Hugh Evans (final report of the European REPHRAME project; http://www.rephrame.eu/pwn.php), places where mean temperatures in June-July-August are ≥ 20°C are at very high risk of developing the disease if the nematode were to be found there. This result is derived from a model of tree evapotranspiration taking into account the effect of the pine wood nematode (ETpN model).

3 Modelling the dispersal of *Monochamus galloprovincialis,* the vector of the pine wood nematode, and assessing the effectiveness of clear-cutting measures

3.1 Background

In order to assess the effectiveness of clear-cutting as a measure for eradicating the pine wood nematode, the WG developed a model to simulate the dispersal of the insect vector, the transmission of the pine wood nematode and lastly the felling of trees in clearcut areas of increasing radius.

To model the flight capabilities of the European insect vector, *Monochamus galloprovincialis*, the WG began by using the data obtained on a flight mill (thesis by Guillaume David, 2014; David *et al.*, 2013). Two mark-release experiments were conducted, one on mature insects (older than 30 days) and another on immature ones (aged less than 20 days). This distinction is important because inoculation of the pine wood nematode in the tree is mainly accomplished by immature insects, during their maturation feeding on green shoots (see Introduction).

Flight capabilities of insect vectors

In the first experiment, the insects were obtained from infested wood in the Landes de Gascogne forest, and raised in the laboratory for 30 days after emergence. They were tested on a flight mill once a week. The results showed that they can fly for an average of 2 km per test day (1.93 km for females and 2.14 km for males; tab. 1).

	Total cumulated flights for flyers		Cumulated flights per recording session		Individual flights	
	Females n = 20	Males n = 27	Females n = 169	Males n = 197	Females n = 325	Males n = 316
Total distance flown (km)						
Mean (± SE)	16.26 ± 2.70	15.59 ± 2.90	1.93 ± 0.12	2.14 ± 0.14	1.00 ± 0.05	1.33 ± 0.08
Median	12.37	10.46	1.53	1.72	0.72	0.81
Maximum	44.85	62.72	6.80	8.54	4.33	8.54
Flight speed (m/s)						
Mean (± SE)	1.43 ± 0.06	1.35 ± 0.05	1.36 ± 0.03	1.36 ± 0.03	1.33 ± 0.02	1.37 ± 0.02

Table 1: Results of the flight mill experiment for mature *Monochamus* (David *et al*., 2013).

A second flight mill experiment was conducted on immature insects. The individuals were collected after emergence in the laboratory and then tested at 0, 5, 10, 20 and 30 days (only once each, for 10 minutes). This experiment showed that: (i) around 45% of insects are capable of flying as soon as they emerge, ii) the flight capabilities are the same for males and females, and (iii) immature insects gradually acquire their flight capabilities until they reach those of mature insects. This experiment also confirmed that the females reach sexual maturity after around three weeks (Naves *et al.*, 2006, David *et al*., 2015).

This type of measurement in the laboratory has several limitations, however. First of all, the flight mills are used to measure potential flight capability, or compare performance between individuals of different sex or physiological states, but do not give any indications on flight behaviour *in natura.* Furthermore, in the conditions of the experiment described above, there was only one test per week: it was therefore impossible for us to define the frequency of the insects' flights, in particular whether they are capable of flying this distance every day (in this case, they could actually fly 2 km each day) or over one week (in this case, they could only fly on average about 2/7 = 0.3 km each day).

In order to reduce these uncertainties and supplement our knowledge of the dispersal of insect vectors, a mark-release-recapture experiment was conducted in the Landes forest with immature (obtained by breeding in the laboratory) and mature insects (obtained by trapping in the forest) (see description below).

These two methods of estimating dispersal yielded complementary results. The data from the flight mill probably overestimated dispersal because the distance is not travelled in a straight line in the field and the insect does not bear its own weight on the flight mill. As for the mark-release-recapture data, they probably underestimated dispersal because the traps can capture individuals that could potentially have flown further, with the maximum measurable dispersal distance being equal to the distance between the point of release and the trap furthest away.

The data provided in the flight mill experiments were used to initialise a numerical model for simulating *M. galloprovincialis* dispersal events in the forest. The data obtained by mark-release-recapture were used to refine the parameters of this model (see description below "3.4.1 Calibration of the dispersal model") in order to make it as realistic as possible.

We therefore first developed this model calibration approach, and subsequently applied the dispersal model to simulate the effect of clear-cutting on PWN dissemination.

3.2 Purposes of clear-cutting

According to the regulations in force, as soon as a tree infested by the nematode is detected, clear-cutting must be implemented around it. The most recent European guidelines recommend a clear-cut radius of 500m. However, the actual objective of this measure was found to be unclear. Two scenarios were therefore considered, in particular on the basis of discussions with experts from the Department of Forest Health at the Ministry of Agriculture and Forestry.

3.2.1 Scenario 1: "preventive action"

In this scenario, it was assumed that the infested tree was detected at an early stage and was eliminated even before the insect vectors could emerge and disperse (fig. 8). However, because of doubts about whether all of the wood bearing *Monochamus* larvae had really been eliminated from this tree (including residuals from cutting), clear-cutting (felling of healthy trees) was implemented around the tree to prevent any remaining carrier insects from dispersing and transmitting the nematode. The clear-cutting radius was therefore assumed to be wide enough to prevent any emerging immature insects from leaving the cleared area alive.

In this first scenario, the tree was infested by the nematode in year N, then symptoms were detected and the tree destroyed in this same year N. Clear-cutting took place during the winter N - N+1 and the remaining insects were supposed to disperse in year N+1 through the clear-cut zone centred on the tree that had been detected as infested. Two strategies can then be considered:

- *Scenario 1-1: Strategy of non-avoidance* of the clear-cut zone

  The clear-cutting has no effect on the dispersal behaviour of the insect: it flies with the same behaviour in the forest environment or in an open environment (e.g. in the clear-cut zone).
- *Scenario 1-2: Strategy of avoidance* of the clear-cut zone

The insect tries to leave the clear-cut zone (if indeed it has the ability to leave) and if it is already outside, it avoids entering it again. This scenario is based on the results of a behavioural test carried out at the Forest Entomology Laboratory of INRA Bordeaux under controlled conditions. In this experiment in a climate chamber, immature and mature insects were released in the presence of maritime pine trees in pots and plastic pine trees of the same colour and size. The insects of both sexes and both stages of physiological development showed a significant tendency to move toward the "real" pines, reflecting an attraction to the scent of the green branches (Giffard *et al*., submitted).

3.2.2 Scenario 2: "curative action"

In this scenario, it was assumed that insects infected by the nematode were dispersed in year N (for example, from infested wood that may have been transported to a given location), the nematode was transmitted to several trees during year N, and an infested tree was detected this same year (fig. 8). Because of doubts about whether any other infected trees had not been detected at the same time, clear-cutting was implemented during the winter N - N+1 in order to eliminate trees that were contaminated but were undetected or asymptomatic. In this scenario, clear-cutting was performed around the tree detected as infested but not necessarily from the moment when the insects emerged (which in reality is still unknown). In principle, this tree could therefore be located anywhere in the dispersal zone of insects emerging from contaminated wood (including on its periphery). In addition, as the dispersal of insects took place before the clear-cutting, there is no avoidance strategy to be considered.

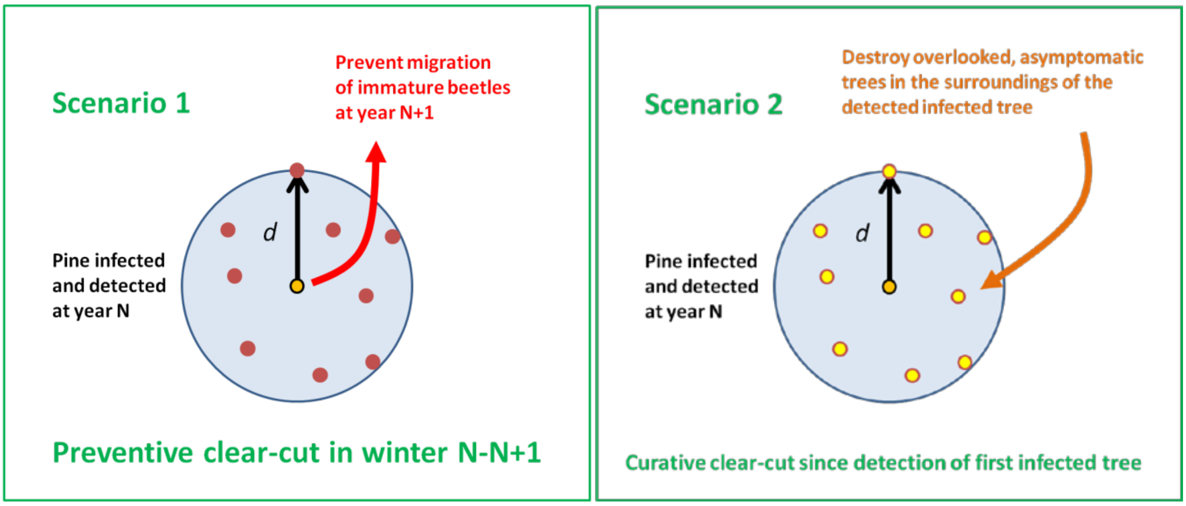

Figure 8: Illustration of Scenario 1 "preventive action" and Scenario 2 "curative action"

In theory therefore, the clear-cutting radius required to eliminate all trees potentially infected in Scenario 2 is around twice that required to prevent infection of new trees by insects "remaining" in Scenario 1 (fig. 9).

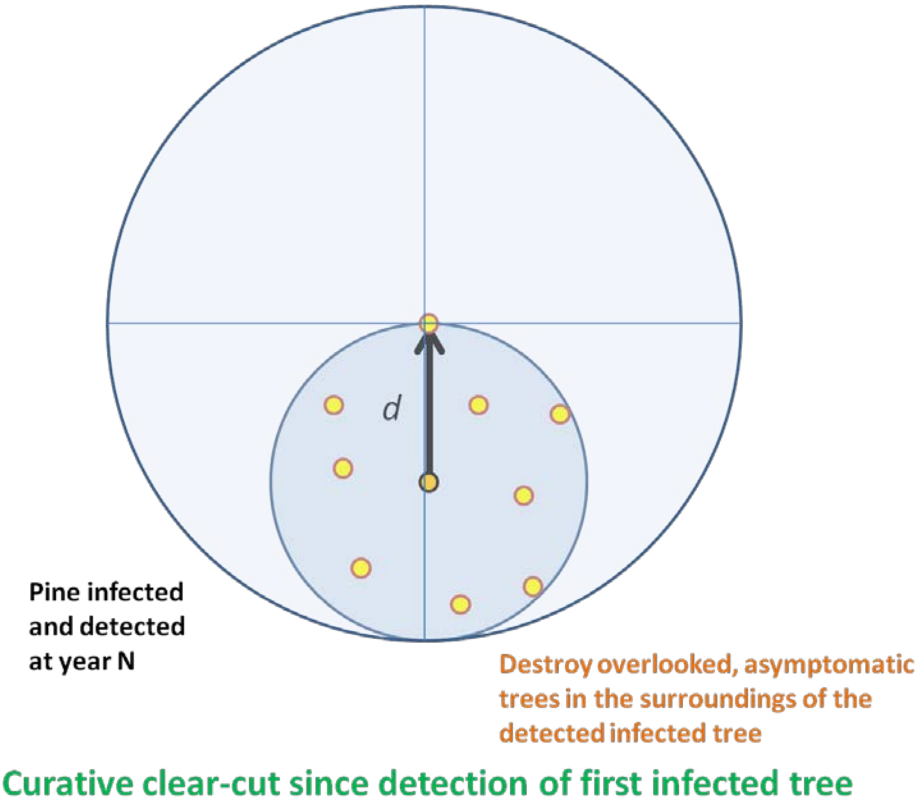

Figure 9: Illustration of the size of clear-cut zone required (large blue circle) to eliminate all trees potentially infected by the nematode if this tree is located on the periphery of the contaminated zone (scenario 2 "curative action").

The dispersal model was therefore applied under these different scenarios in order to assess the effectiveness of clear-cutting.

Important note:

Faced with the complexity of integrating the effect of the landscape in the model, and the lack of calibration/validation data, the assumption was made here that **pine trees are present everywhere** (same probability of presence in every direction). This assumption is consistent with the method of parameterising the dispersal model, based on experiments carried out in the Landes de Gascogne forest with *M. galloprovincialis* from this forest. This forest has landscapes with continuous cover of maritime pine (90% of land cover). The results of this study are therefore valid for the case of the Landes. Their extrapolation to other regions or landscapes, where the pine forest may be less prevalent and/or more fragmented, should therefore be carried out with great caution.

3.3 *Monochamus* mark-and-recapture experiment

In an earlier experiment carried out in 2012, within the framework of the European FP7 REPHRAME project, the interception radius of *M. galloprovincialis* pheromone traps was estimated. This was used to define the circle the insects must enter in order to be able to perceive and respond to the attraction of the aggregation pheromone. The traps tested are those now used routinely by the DSF for surveillance of the insect: a Cross Vane model (Econex ®) baited with the pheromone GalloProtect pack ® (Alvarez *et al*., 2014). In principle, if two identical adjacent traps are separated by a distance of less than twice their interception radius, they are in competition to capture insects (their circles of interception intersect). The mean captures per trap are therefore lower than the mean captures obtained by spatially "independent" traps. Ultimately, if the traps are brought even closer, to the extreme (positioned in the same place), their capture rates are equal to half of the mean captures of spatially independent traps (since any insects intercepted in their attraction radius are divided alternately between the two traps). It was found that the capture curve for each trap as a function of the distance between two adjacent traps has a sigmoid shape, varying asymptotically from *M/2 to M* (where *M* is the mean capture per trap having no interaction with its neighbours*)*. This curve reaches the value of the asymptote for a distance *d* between traps equal to 2*R*, where *R* is the interception radius of the traps (fig.10). A trapping experiment was conducted in the Landes de Gascogne forest in which the mean capture levels for pairs of traps 25, 50, 100, 200 and 300m apart were compared. Indeed a sigmoidal relationship was observed between the mean capture per trap and the distance between the traps, and the interception radius of the traps was therefore estimated at a distance of around 100m (Jactel *et al*., 2013).

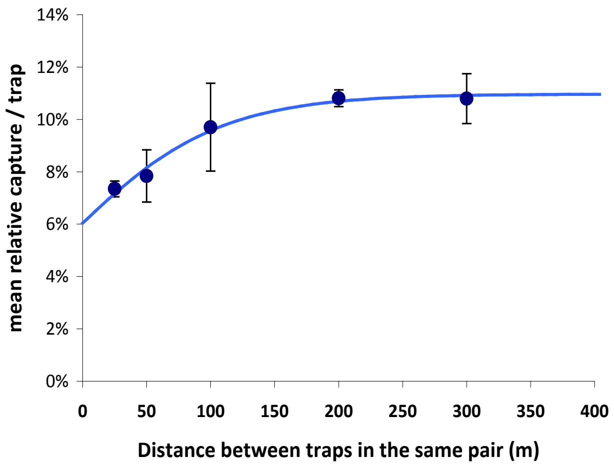

Figure 10: Relationship between mean captures of *M. galloprovincialis* per pheromone trap and distance between neighbouring traps (in the same pair). Note that the mean capture tends toward an asymptote for values of distances greater than 200m, suggesting an interception radius of the traps of around 100m.

Starting from these results, a *M. galloprovincialis* mark-release-recapture experiment was set up. Immature insects were obtained by breeding in the laboratory on pieces of dead wood infested by insect larvae (identified by the presence of characteristic sawdust). Mature insects were obtained by pheromone trapping in the Landes de Gascogne forest. The preliminary observations, as well as those of Spanish and Portuguese colleagues in the REPHRAME project, showed that the immature insects did not respond to the attraction of pheromones on emergence and the first recaptures occurred after around two weeks. This indicates that the vast majority of insects captured in pheromone traps are already mature.

In the field, nine clusters of four traps were installed, so that traps in the same cluster were 200m apart (twice the interception radius). The clusters were arranged at the centre and at the periphery of two concentric circles with radii of 300m and 900m (fig. 11). These traps were the same as those in the previous experiment: Cross Vane ® type and GalloProtect Pack ® dispenser.

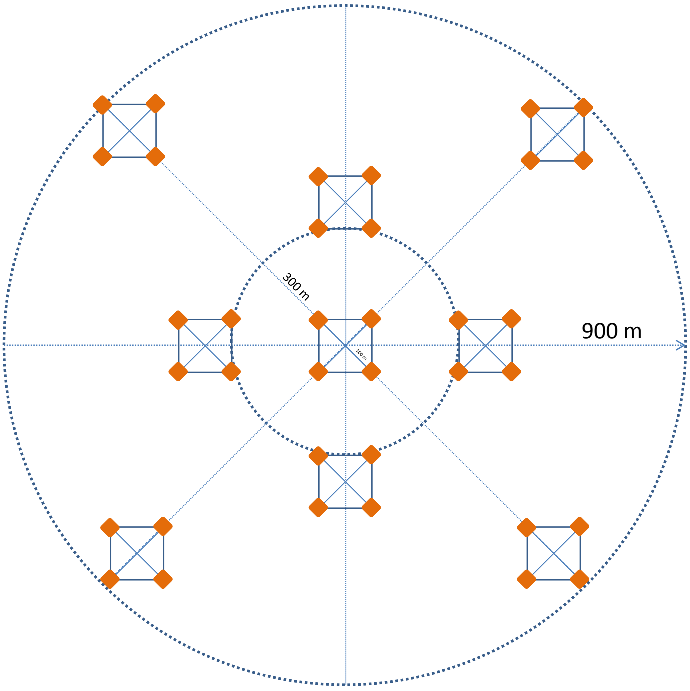

Figure 11: Arrangement of nine clusters of four traps for the mark-release-recapture experiment

The insects were individually marked with numbered tags (originally designed for marking bees) and dots of paint on the elytra in a way that enabled tracking of their physiological state (immature vs. mature), place (in the centre of which cluster) and date of release, and place (in which trap) and date of recapture.

A total of 499 immature and 3085 mature *Monochamus* were marked and released in July - August 2014. Over the duration of trapping (150 days), 17,716 *Monochamus* were captured in the 36 traps, including 37 marked immature and 193 marked mature insects. The average rate of recapture was 6.3% for immature and 5.1% for mature marked insects. The maximum dispersal distance recorded ("as the crow flies") was 1,754m for immature (average of 841m) and 1,886m (average of 483m) for mature insects.

3.4 *Monochamus* dispersal model

It was assumed that one hundred insects carrying the nematode were released in the centre of each of the nine clusters. It was considered that the longevity of adult insects was 120 days (longevity observed in the flight mill experiment of David *et al.*, 2013).

For the mature insects used in the mark-and-recapture experiment (captured initially by a trap), it was assumed that they were more than twenty days old although their exact age was not known. Therefore, we randomly selected the age of each of the hundred insects (in number of days since their emergence) according to a uniform distribution:

U[min = 20, max = 120].

According to the flight mill experiment, not all of the insects flew during a test day. It was assumed, therefore, that there was a daily probability of flying, *pf* = 0.61 (David, 2013). We also tested an initial rest time ("***response time***") before responding to the attraction of the traps (which may be necessary to recover from the stress caused when handling the insects) as well as a rest time after flight to be able to feed ("***rest between two flights***").

For each day ranging from 1 (day of release) to 70 (maximum duration observed between release and recapture):

- We selected flying individuals (i.e. those that had not reached the maximum age of 120 days, those that had not already been captured, those that were actually flying on that day chosen at random according to a binomial distribution, *pf*, those that were not in the initial rest time and those that were not resting to feed).

- For these flying individuals, we knew their position (P1) and we chose the direction in which they would fly randomly according to a uniform distribution U[min = 0, max = 2π] and the distance randomly according to a ***negative exponential distribution with mean α***:

$$k_M(x) = \frac{1}{\alpha}\exp(-\left|\frac{x}{\alpha}\right|)$$

We calculated the new position (P2) of each individual and we looked at whether its trajectory (between P1 and P2) passed close to a trap. The interception radius of the traps was assumed to be 100 m (see experiment described above), i.e. if an insect passed within 100 m of a trap, it was assumed that it could respond to its attraction. For the insects passing within this interception radius, it was assumed that they had only a certain probability of being captured, depending on the ***effectiveness of the trap, eff***. We therefore chose the captured insects at random according to a binomial distribution of probability *eff* from among those that passed close by.

- For the insects that were not caught, it was assumed that they arrived at their position P2.

For the immature insects, we applied a model very similar to the previous one, with the exception of the following points:

- The initial age of the individuals (in number of days since their emergence) was chosen at random according to a uniform distribution:
  U[min = 1, max = 7] because there was one release per week. We therefore released the individuals that emerged in the course of the preceding week.
- The response time here corresponds rather to the time to reach maturity in order to respond to the bait. Disturbance is reduced because the individuals were not previously captured and brought to the laboratory; but they emerged directly in the laboratory.
- The daily probability of flying was *pf* = 0.45.
- The released insects were immature at the start, but became mature during their dispersal. Depending on their age *d* (in days), we calculated the average dispersal distance ***δ(d) α*** considering that beyond 20 days they are mature and their dispersal capabilities have reached the level of mature insects (***α***) (fig. 12). We thus chose the distance according to a negative exponential distribution with mean ***δ(d) α***:

$$k_I(x,d) = \frac{1}{\delta(d)\alpha}\exp(-\left|\frac{x}{\delta(d)\alpha}\right|)$$

$\delta(d) = 0.67 + 0.016 \times d$ (This equation is derived from David *et al.*, 2015)

where $\delta(d = 20) = \alpha$

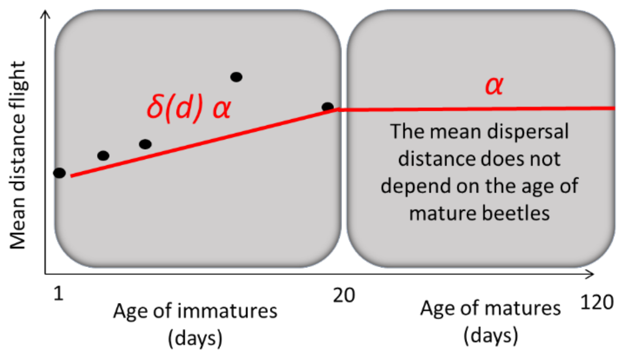

Figure 12: Diagram showing the evolution of the dispersal capabilities of insects based on their age

3.4.1 Calibration of the dispersal model

For each cluster, we can use the dispersal model to calculate the mean capture rate, as well as the mean capture dates, in order to compare them to the observations. The aim is to find the best estimate of the model parameters, i.e. the number of rest days between two flights and the mean daily dispersal distance, *α*. To do this, we must also estimate the response time (inherent to the experiment) as well as the effectiveness of the trap (parameters that are not used in the second part of the study to assess the effectiveness of clear-cutting).

In total we tested five values of α x three values of *eff* x three values for the response time x four values for the rest between two flights = 180 different combinations in order to choose the best one. We thus tested:

- ***α*** = 500, 1000, 1500, 2000, 2500 m
- ***eff*** = 0.5, 1, 2%
- ***response time*** = 4, 8, 12 days
- ***rest between two flights*** 0, 1, 2, 3 days

As this model has a stochastic component, we conducted one hundred repetitions for each combination (180 x 100 = 18,000 simulations).

The observed recapture data were used to calculate:

- the recapture percentage within the same cluster (number of insects released and recaptured in cluster i / total number of insects released in cluster i), both for immature and mature insects: i.e. ***%-immat-intra*** (nine values) and ***%-mat-intra*** (nine values)

- the mean time to recapture these same insects (difference between the release and recapture dates): ***duration-immat-intra*** (nine values) and ***duration-mat-intra*** (nine values)

- the recapture percentage in the other clusters (number of insects released and recaptured in the clusters j≠i / total number of insects released in cluster i): i.e.***%-immat-inter*** (72 values) and %-***mat-inter*** (72 values)

- the mean time to recapture these same insects (difference between the release and recapture dates): ***duration-immat-inter*** (72 values) and ***duration-mat-inter*** (72 values)

To estimate the fit of the data predicted by the model to the observed data, we calculated:

- the relative bias = |mean predicted - mean observed| / mean observed

- the root mean square error: $\sqrt{[\text{mean (predicted value - observed value)}^2]}$

In total, we therefore had 8 variables x 2 estimators = 16 criteria to choose the best of the 180 combinations of parameters. To carry out this optimisation we used a multi-criteria analysis method based on the PROMETHEE algorithm and developed on the Visual-PROMETHEE 1.4 ® platform. We assumed that the objective of the ranking of the 180 combinations was to minimise the criteria (search for the lowest bias and the smallest error), and therefore used linear preference functions and assigned the same weight to all the criteria.

The best fit for the immature insect dispersal model was obtained with the combination of the following parameters (fig. 13):

- ***α*** = 2000m
- ***eff*** = 1%
- ***response time*** = 12 days
- ***rest between two flights*** = 1 day

The best fit for the immature insect dispersal model was obtained with the combination of the following parameters (fig. 13):

- ***α*** = 2000m
- ***eff*** = 1%
- ***response time*** = 8 days
- ***rest between two flights*** = 1 day

The two parameter settings are therefore consistent with each other. They are also consistent with the flight distance data estimated on the flight mill (David *et al.*, 2013) which are 1.93 km for females and 2.14 km for males (Tab1).

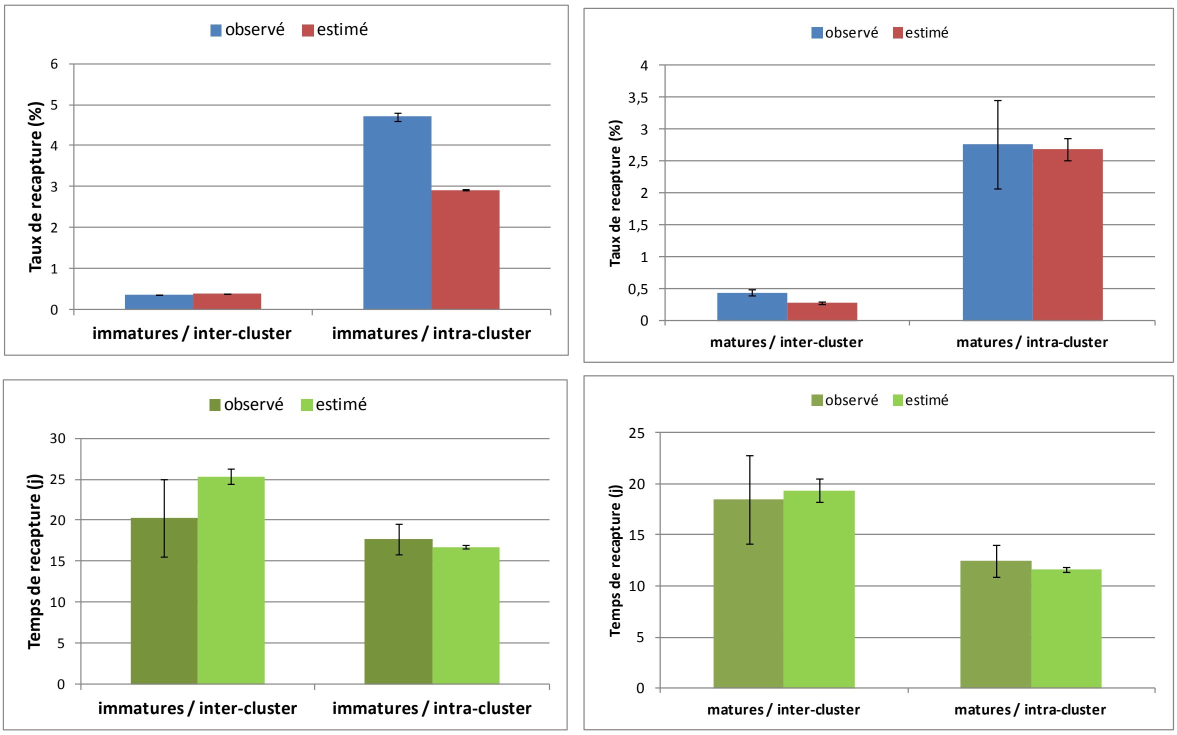

Figure 13: Comparison of mean (± SE) rate and time of recapture for immature and mature beetles, within- and between-clusters, observed in the mark-release-recapture experiment and predicted by the dispersal model parameterised as shown in the text

3.4.2 Simulation of dispersal with clear-cutting

3.4.2.1 Scenario 1 "preventative"

In the dispersal model simulating the effect of clear-cutting, we considered the dispersal of a ***variable number of insects*** (***n***) and we took into account the best combination previously obtained (α = 2000 m, *rest time between each flight* = 1 day). At the initial time, the individuals emerged from the tree, and the age was therefore set at 1 day for all of them. It was assumed that half of the individuals were male and the other half were female.

For each day ranging from 1 (day of release) to 120 (average lifespan of *Monochamus* in Aquitaine, Giffard *et al.*, submitted):

- It was assumed that the insects were immature until the age of 20 days and then became mature.
- We selected flying individuals (i.e. those that had not reached the maximum age of 120 days, those that were actually flying on that day chosen at random according to a binomial distribution of probability *pf*, and those that were not resting to feed).
- For these "flying" individuals, we choose the distance randomly according to a ***negative exponential distribution with mean δ(d) α***:

$$k_I(x,d) = \frac{1}{\delta(d)\alpha}\exp(-\left|\frac{x}{\delta(d)\alpha}\right|)$$

- ***Without avoidance strategy:***
- We chose the direction in which they would fly randomly according to a uniform distribution U[min = 0, max = 2π]

With avoidance strategy:

- We determined the authorised flight directions so that the insect left the clear-cut zone as quickly as possible or did not enter it if the dispersal distance allowed it to cross the edge of the clear-cut zone. We then chose the direction randomly according to a uniform distribution U[min = 0, max = 2π] removing prohibited angles (fig. 14).

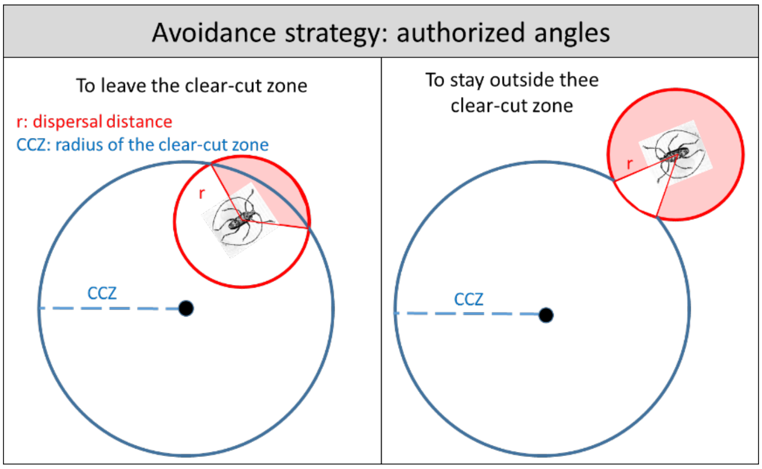

Figure 14: Illustration of the clear-cut zone (CCZ) avoidance strategy and the authorised angles of flight (in red)

- If the insect's dispersal distance did not allow it to leave the clear-cut zone, we counted the number of days that it spent in the clear-cut zone. It was assumed that after 12 days it starved and died. This value was observed in the laboratory (Pers. Comm. Guillaume David, Sanchez *et al*., 2013).
- Thanks to the direction and distance of flight, we were able to calculate the new position of the insect. If it was outside the clear-cut area, it was assumed that it could then transmit the pine wood nematode, either by feeding or, for females, by laying eggs. For transmission of the nematode during maturation feeding, we used the rate of transmission observed in Portugal (Naves *et al*., 2007a; fig. 15). In the model, we considered only the number of transmissions, because we did not have enough information on the number of nematodes carried and transmitted. According to these data (fig. 15), there may therefore be a maximum of 70 transmissions per insect during feeding.

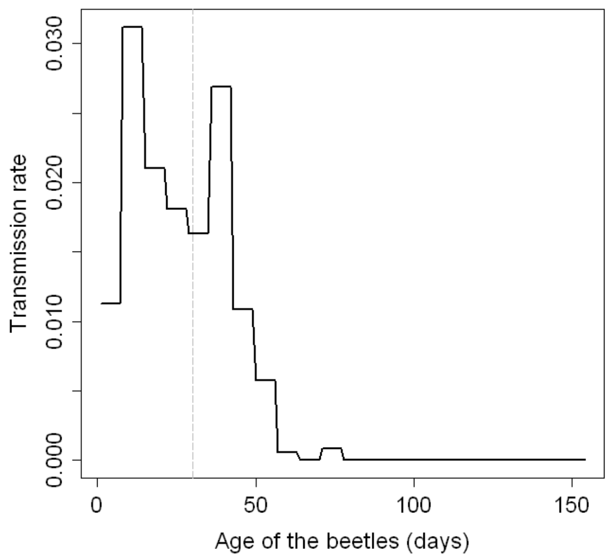

Figure 15: Rate of transmission of the nematode as a function of the age of the insect (modified from Naves *et al.*, 2007a)

It was also assumed that the females could transmit the nematode during egg-laying, although this rate is very low (Naves *et al.*, 2007b). It was assumed that females laid two eggs a day over the laying period, supposed to extend from day 20 to day 53, and that they had a 0.37 probability of transmitting the nematode during this laying.

- We then calculated the total number of transmissions that took place (fig. 16).

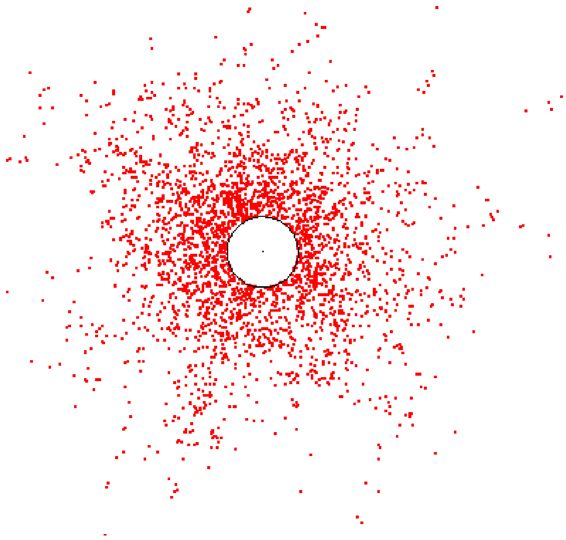

Figure 16: Simulation of the dispersal of insects and the transmission of the nematode (red dots) beyond the radius of the clear-cut zone (black circle)

- As this is a stochastic model, we performed 100 repetitions.
- We tested clear-cut zones with a radius of 0, 100, 500, 1000, 1500,... 29,500, 30,000 m, with a number of insects *n* = 1, 10, 100, and 1000.

3.4.2.2 Scenario 2 "curative"

In this scenario, we used the same model with the exception of a few points.

- There was no avoidance strategy because clear-cutting was performed after dispersal of the insect. There was therefore no famine phenomenon.
- After having dispersed the insects and simulated transmissions of the nematode, we obtained a set of points where the nematode was transmitted. These points represent infected trees. We then chose one of these trees at random and it was assumed that only this symptomatic tree was detected. Clear-cutting was then centred on this point and not on the initial starting point of the insects.
- We then calculated the total number of transmissions that took place outside of this clear-cut zone.
- As the choice of the symptomatic tree around which the clear-cutting was to be implemented added a stochastic component, we performed 10 repetitions for the choice of the tree for the same dispersal simulation.
- As this was a stochastic model, we performed 100 repetitions of these dispersal simulations.
- We tested clear-cut zones with a radius of 0, 100, 500, 1000, 1500,... 39,500, 40,000 m, with a number of insects *n* = 1, 10, 100, and 1000.

For each of the scenarios, we calculated the confidence interval at 99% of the relative number of transmissions of the nematode. This relative number was calculated as the number of transmissions simulated with a clear-cut zone of a given radius, divided by the number of transmissions without clear-cutting (radius = 0).We then identified the confidence interval corresponding to the clear-cut zone radii for a value of 0.001 relative transmissions (0.1%), in order to ensure that the clear-cutting could eliminate 99.9% of infected trees (fig. 17).

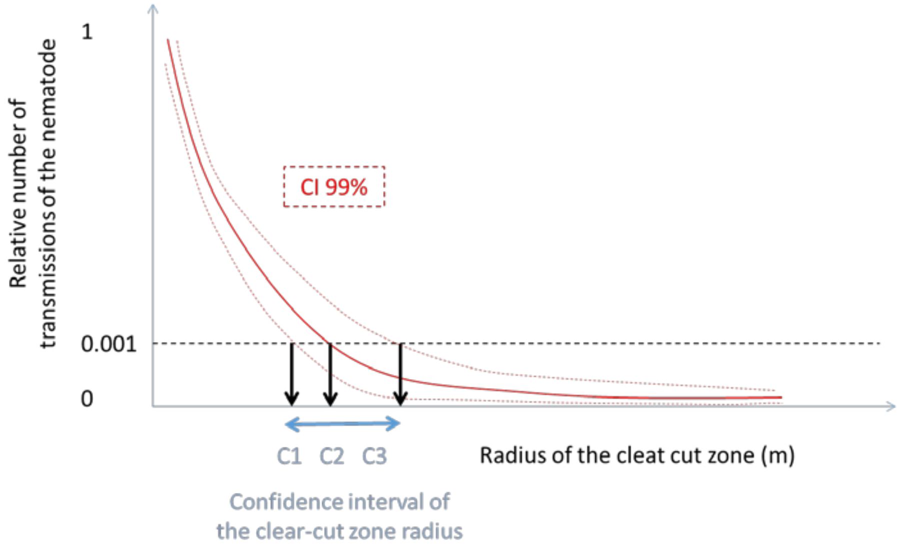

Figure17: Relative number of transmissions of the nematode as a function of the clear-cut zone radius, and calculation of the confidence interval for the recommended radii

3.4.3 Results of the simulation of dispersal and effect of clear-cutting

The results are relatively different depending on the scenarios. Scenario 1 without avoidance is the most optimistic and scenario 2 is the most pessimistic (fig. 18 and 19, tab. 2).

- In scenario 1 without the strategy of avoidance of clear-cut zones by the insect, a clear-cut zone radius of around 15 km is needed to obtain 99.9% effectiveness
- In scenario 1 with the strategy of avoidance of clear-cut zones by the insect, a clear-cut zone radius of around 18 km is needed
- In scenario 2 without the strategy of avoidance of clear-cut zones by the insect, a clear-cut zone radius of around 38 km is needed, or around double scenario 1, as predicted in theory.

If we consider a clear-cutting radius of 500 m, it would avoid at best only 11% of transmissions (scenario 1 without avoidance of clear-cut zones by the insect vectors) and in the worst of cases less than 1% (scenario 2).

The results on effectiveness (relative percentage of contaminated trees eliminated) are basically independent of the number of insects flying (between one and a thousand insects), no doubt due to the model's iterations (simulating ten repetitions of the model for ten insects gives the same result as just one repetition for one hundred insects).

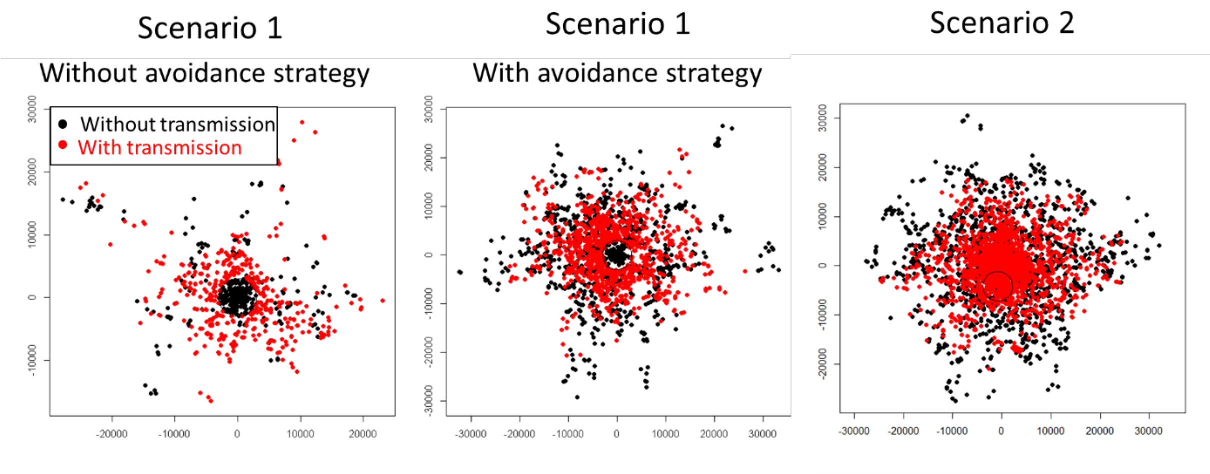

Figure 18: Illustration of the simulations according to each scenario for a clear-cut zone radius of 3000 m and n = 100 insects. The black dots correspond to the position of the insects accumulated over time as a function of their dispersal capacity and behaviour, and the red dots to trees where the insect has inoculated the nematode.

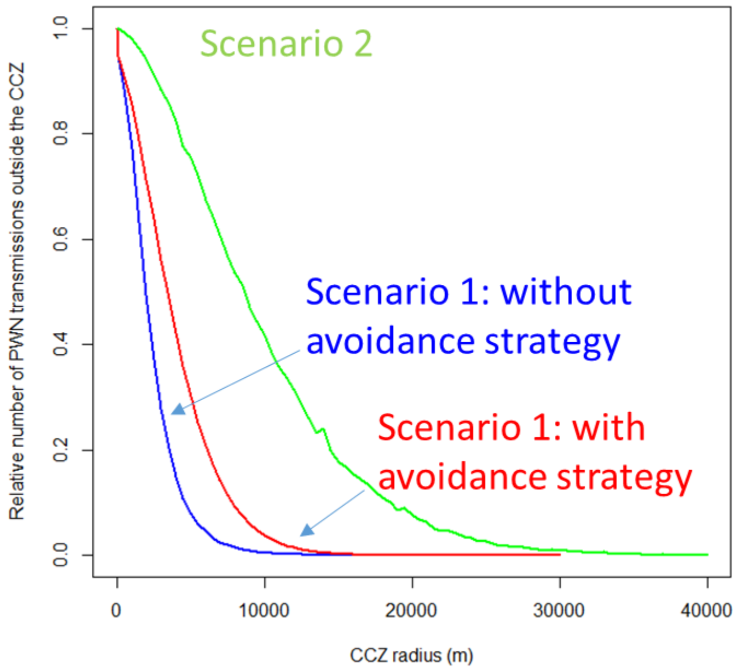

Figure 19: Comparison of the three scenarios in terms of relative number of transmissions outside the clear-cut zone (for 1000 insects) as a function of the clear-cut zone radius

Table 2: Clear-cut zone radius recommended according to the scenarios and the number of insects carrying the nematode in flight

(R = clear-cut zone radius, m = mean, CI = Confidence Interval at 99%)

Number of insects flying	**Scenario 1: no avoidance** R = m (CI)	**Scenario 1: avoidance** R = m (CI)	**Scenario 2** R = m (CI)
1	R = 10,500 m [7,000; 10,500]	R = 12,500 m [9,000; 12,500]	R = 27,500 m [22,500; 27,500]
10	R = 14,500 m [10,000; 16,000]	R =16,500 m [13,500; 17,500]	R = 36,500m [35,500; 36,500]
100	R = 14,000 m [14,000; 15,500]	R = 17,500 m [16,500; 19,000]	R = 38,000 m [37,000; 40,000]
1000	R = 14,500 m [14,000; 14,500]	R = 17,500 m [17,500; 18,500]	R = 38,500 m [36,500; 39,500]

It should be mentioned here that Portugal attempted to apply the preventive clear-cutting method to halt the extension of the outbreak discovered in 1999. The control strategy consisted of four types of actions: i) eliminating the dying trees at the end of the intensive detection programme (autumn - winter); ii) capturing the insect vectors with traps (spring - summer); iii) monitoring the transport of conifer wood on roads and in ports; iv) establishing, in 2007, a clear-cut strip 3 km wide around the contaminated area (Setubal Peninsula) with the help of European funds (Rodrigues *et al*., 2008). This work has not, however, prevented the emergence of new outbreaks of nematodes more than 200 km to the north, in the region of Coimbra. Such long distance dispersal probably results from human-mediated dispersal (see Robinet et al., 2009 for China).

4 Other direct control methods

4.1 Mass trapping of the insect vector

Even if it were technically feasible, it would be ethically unacceptable to seek to eradicate a native species such as *M. galloprovincialis* which, apart from being the vector of the invasive nematode *B. xylophilus*, is part of the European natural heritage.

The few examples of successful eradication of insect pests or disease vectors relate to species that are themselves exotic and in cases where their arrival was detected early enough to intervene quickly and in populations with a low density (Brockerhoff *et al*., 2010; Pluess *et al*., 2012).

In a context where eradication is not the objective but where there is a desire to bring the level of insect populations below an acceptable epidemiological (or economic) threshold, mass trapping measures are sometimes considered. Very few examples of successful mass trapping of forest insects have been documented and even fewer as regards sawyer beetles (Brockerhoff *et al.*, 2010). Thus, the mass trapping of bark beetles has been abandoned virtually everywhere in the world. The failure of these methods is due mainly to the lack of effectiveness of the trapping techniques. Indeed, to achieve a sustainable reduction in the levels of insect populations, it is necessary to reduce local densities enough to go below the threshold of viable reproduction, in order to benefit from the Allee effect. In practice this entails having to capture more than 90%, or even 99%, of the local population. However, bait traps generally have limited capture capabilities, either for technical reasons (attractiveness of the pheromones, saturation of the traps in the event of large catches) or for biological reasons (competition with natural attraction from congeners, obligatory flight or maturation required to allow the insect to respond to trap attraction).

With regard to mass trapping of *M. galloprovincialis* in Europe, we have three studies with which to assess its effectiveness. All three used the insect "mark-release-recapture" method because it makes it possible to determine the quantity of insects present in the environment and therefore to estimate a relative trapping rate.

Sanchez-Husillos *et al*., (2015) conducted their study in Castile (Spain) in 2010, in a stand consisting mainly of black pine. A total of 353 immature individuals obtained by breeding on maritime pine logs were marked and released in the centre of a grid of 52 pheromone traps (multi-funnel traps and GalloProtect Plus bait) positioned every 200 m (0.25 trap/ha). A total of 102 insects were recaptured, i.e. a trapping rate of 29%, with an average 0.56% per trap. A second experiment was conducted in Castile in 2013 in a stand of maritime pine, in which the density of traps was varied (0.02, 0.11, 0.25 and 0.44 trap/ha). To assess the effectiveness of trapping, a model was developed to estimate the total number of insects and the proportion of individuals captured from the number of native insects captured (5,113) in this device. Trapping was then simulated over five consecutive years, making various assumptions. The number of catches was higher for the highest density of traps (2,065 insects captured in the device with 0.44 trap/ha, with an estimate of 21,319 insects present, i.e. an estimate of about 10% of insects captured). To obtain the density of trapping needed to capture 95% of the insects, the authors extrapolated the results over a range of untested values and concluded that repeated trapping for five consecutive years with a density of 0.82 trap/ha might reduce the number of insects by 95%. In our opinion, this estimate of the effectiveness of trapping has several weaknesses: i) it is based on a series of assumptions and estimates whose validity and degree of confidence are not given (in particular the model for estimating the total number of insects is based on the Jolly-Seber method, which needs the same insects to be recaptured twice in a row; here only three insects were recaptured twice out of 2,836 released initially (0.1%); ii) it was not measured in the field and ultimately relies on a very uncertain extrapolation; and iii) if this value were to be confirmed, the density of traps (around every 100m) would be difficult to implement on a large scale. For comparing the studies, we therefore only considered the first experiment, the only one for which we have a direct measure that we can truly compare with the other studies.

Torres-Vila *et al*., (2015) carried out their release-recapture experiments in 2012 and 2013, in a 4 ha plantation of maritime pine in Extremadura (Spain) with Crosstrap Econex ® traps and GalloProtect Plus bait. The traps were laid out on a grid of 25 traps 50 m apart (i.e. a density of 4 traps/ha). The insects were first captured in the traps (and were therefore probably mature individuals), marked and released on the tree nearest the trap where they had been captured (1 to 3 m away from the trap). The recapture rate on the scale of the stand was 14%, or 0.56% per trap. In this study, the effectiveness of mass trapping (36% at best) is regarded as too low to be put in place, and therefore contradicts the effectiveness found in the study by Sanchez-Husillos, despite a much higher trap density (4 traps/ha versus 0.82 trap/ha).

Jactel *et al*. (2015, this report) conducted their mark-release-recapture experiment in a forest of maritime pine in Aquitaine, using the same traps (Crosstrap and GalloProtect Plus) according to a protocol described above (see 3.2.1). In total, 499 marked immature individuals were released in nine clusters of four traps 200m apart (0.25 trap/ha) for a recapture rate of 4.7% at the scale of the cluster (or 1.18% at the scale of the trap). Furthermore, the simulation model developed to explain the dispersal processes of the insect (see 3.3) estimated a trapping efficacy of 1%, i.e. a value close to the 1.18% observed (the difference may be due to the fact that insects may have "passed through" the trap's zone of attraction without being attracted, flown to its periphery and then re-entered this zone and this time been caught). A total of 3,805 mature insects were released in the same conditions. The intra-cluster recapture rate was 2.8%, or 0.7% per trap. This number is lower than that for immature insects, probably because of the mortalities suffered by older insects between the time of release and the time of recapture.

These three studies therefore concur and indicate the very limited effectiveness of capture in the most commonly used pheromone traps (interception traps with the standard GalloProtect ® pheromone), which is of the order of 0.5 to 1% of circulating insects. Unless the density of traps per ha were increased considerably, this method does not therefore appear credible for achieving a significant decrease in local populations of *Monochamus*. In addition, the two Spanish studies assessing the effectiveness of mass trapping arrived at two contradictory results. Moreover, it should be remembered that, as with all "curative" methods, it needs to be applied every year, throughout the insect's flight season (about four months, so four renewals of the bait dispensers).

4.2 Individual-centred control

Given the lack of effectiveness of the mass reduction methods for potentially contaminated trees (see "preventive clear-cutting" method, 3.3.2.1) or insect vectors (see "mass trapping" 4.1), in order to avoid the natural spread of the nematode, it appears necessary to consider another, more targeted approach. This so-called individual-centred control strategy is based on a three-step approach: i) the early detection of the arrival of the pine wood nematode in a new forest region (i.e. "early warning") triggering a series of measures designed to reduce its impact, with ii) the precise identification of individual symptomatic trees, followed by iii) their elimination.

Without going into detail about the actions that could be undertaken, we present options on how to implement this individual-centred strategy below.

4.2.1 Surveillance and detection

4.2.1.1 Trapping of insect vectors

While the effectiveness of mass trapping of the insect vector is uncertain for limiting the spread of the pine wood nematode (see previous section), trapping the insect vector for the purpose of early detection of the presence of the nematode remains an essential part of the control strategy. This surveillance method is equally applicable:

- in zones where the disease cannot develop due to climatic constraints (fig. 7): because the trees infested with the nematode are asymptomatic, it is all the more important to ensure that the vectors are not carrying the nematode because visual surveillance of trees is not possible;

- in zones where the disease can develop, as a complement to the surveillance of trees presenting symptoms of wilting in the crown.

This method of trapping insect vectors for the purposes of surveillance and early detection of the pine wood nematode is possible because:

i) the bait (GalloProtect ®; Ibeas *et al*., 2007, Pajares *et al*., 2010) that attracts mature *M. galloprovincialis* and the trap (Crosstrap, Alvarez *et al*., 2014) designed to capture them are available and operational;

ii) the method of detecting the nematode in the trapped insects (ANSES, 2015) has been developed, validated and is used routinely by the National Reference Laboratory (NRL) - Nematology Unit of the ANSES Plant Health Laboratory, Le Rheu;

iii) the surveillance of conifer stands already takes place in part with the help of a *M. galloprovincialis* trapping network (Guidance Notes DGAL/SDQPV/N2013-8132 and N2013-8102).

The fact that the insect is able to travel great distances before being captured in a pheromone trap seems paradoxically to be an asset for this approach. Indeed the traps "drain" insects from a wide surrounding area, making it possible to limit the number of traps to be installed to cover a large forest area.

According to the current instructions, the observers from the Department of Forest Health (DSF) prioritise sensitive zones at high risk of introduction of the nematode (storage sites for wood or wooden pallets, nurseries, garden centres, sawmills, ports, railway stations, parking areas, DGAL/SDQPV/N2013-8132) as trapping sites. The traps are installed for a minimum of 40 days between 15 May and 15 October and can then be moved. The captured individuals are sent to the NRL (ANSES) for detection of the nematode. The Guidance Note imposes a minimum of 89 trapping operations throughout France.

The current procedure regarding the choice of trapping sites and the time window for trapping for each of the sites makes this method of surveillance random, however. According to the simulations of *M. galloprovincialis* dispersal, the insect can potentially disperse very far (several dozen kilometres from its point of emergence; see 3.3.3). Therefore, even though the sensitive sites provide conditions conducive to the presence of *M. galloprovincialis* and the pine wood nematode, the infested vector can disperse much further. A regular grid of traps would probably help better cover the territory and ensure more systematic surveillance.

Leaving the traps in place for the entire flight season of *M. galloprovincialis* would also reduce the share of uncertainty due to the time window for trapping. Similarly, a finer grid size would be preferable to detect an insect that may be infested with the nematode with greater certainty and also to reduce, by triangulation, the enhanced surveillance zone in the event of detection of the nematode on a captured insect. However, a compromise must be found between the efficiency of surveillance and feasibility. A finer grid size and a longer trapping time imply a greater number of traps to be installed and checked as well as a larger number of insects to be analysed by the laboratory. The limits due to these capacities for analysis and field work must be taken into account when drawing up the protocol. For example, it might be possible to consider grouping some of the catches for a preliminary analysis, reverting to the detail of the individual trap in the event of detection.

The implementation of a regular grid does not call into question trapping at sensitive sites, ports in particular. In addition to these trapping operations targeting insects circulating on French territory and potentially infested when emerging from infested wood, insects migrating from Spain could also bring the nematode and introduce it into France. The recent results on the flows of migrant *M. galloprovincialis* individuals over the entire Pyrenean range show the presence of dispersal corridors to the west and the east of the Pyrenees (Haran *et al*., 2015). Strengthening trapping along main roads, especially the motorways both sides of the Pyrenees, would make it possible to monitor the arrival of the nematode via dispersal of the vector from the Iberian Peninsula.

4.2.1.2 Surveillance and detection of symptomatic trees

4.2.1.2.1 Methods of surveillance on the ground

In areas where the disease can develop (fig. 7), the nematode induces wilting of the crown mainly at the end of summer and in autumn. These wilting symptoms may also appear at the end of winter in Portugal. They reflect colonization of the conductive material by the nematodes, and a lack of water supply to the crown. Unfortunately they are not specific and other parasites (bark beetles, root rot, etc.) can induce similar symptoms. Only an analysis of the wood carried out by laboratories accredited for the detection and identification of nematodes according to the official methods (ANSES, 2011a and b), can confirm or rule out the presence of *Bursaphelenchus xylophilus* in symptomatic trees. Guidance Note DGAL/SDQPV/N2013-8132 defines the sampling methods in forest stands both within and outside sensitive zones. Sampling must be performed i) as a priority in stands with signs of die-back or trees dying within the past year, regardless of the cause of the die-back or mortality, or showing symptoms of attack by *Monochamus*, ii) as a second priority especially in stands that have suffered fire damage, or otherwise on damaged or weakened trees. However, this note does not advocate an increase in sampling in the event of significant die-back following droughts or windfall, for example, even though these scenarios can "hide" mortalities related to the simultaneous arrival of the nematode, making its detection much more random.

The majority of reports of dying trees come from observers from the DSF, as part of their professional visits. In fact, these reports are predominantly made close to roads, which is consistent with the fact that areas of intense human activities coincide with areas where the risk of introduction is highest (Robinet *et al.,* 2009). Regardless of this, in the case of the Landes massif, only 16% of forested areas are visible from trails and roads (Samalens, 2009). A large part of the forest area, is not, therefore, covered by careful surveillance, and yet a *Monochamus* vector could easily contaminate a tree at the centre of a plot in a single flight from an emergence by the side of a road for example. There is therefore a need to develop methods for detecting mortalities using other, more effective and less expensive means than ground detection.

4.2.1.2.2 Aerial methods

Aerial surveillance methods ("sketch mapping") are proving to be more accurate and less expensive when it comes to detecting and mapping forest damage over large surfaces (Ghent *et al*., 1998).

Regarding the detection of trees infested by the pine wood nematode, two major difficulties appear: i) in principle, infested trees are spread out, at least in the early stages of colonisation of a new region, and ii) the symptoms of die-back (reddening of the needles and then mortality of the tree) are not specific to a nematode attack and may therefore be confounded with those of an attack of root rot (e.g. *Fomes, Armillaria*) or bark beetles (e.g. *Ips sexdentatus*). To achieve reliable detection, tools for identifying isolated, recently dead trees (or those suffering from severe die-back) would therefore need to be combined with image analysis methods able to provide information on the cause of the die-back. Regarding image capture tools, it is necessary to compare the respective advantages of conventional methods using Ultra light aircrafts or conventional aircraft with recent developments made possible by progress in very high-resolution satellite imagery and cameras on board drones (Park *et al.,* 2014). For the analysis of these images, a promising option is the taking into account of the spatio-temporal dynamics of damage caused by the pine wood nematode. It is in fact likely that the type of spatial distribution of dead trees differs between those caused by bark beetles (aggregate distribution resulting from mass attacks) and by the nematode (diffused distribution resulting from random attacks). Similarly, temporal dynamics in the development of symptoms, whether at the scale of the season (depending on the phenology of the insect vector for example) or the scale of the year (centrifugal extension of spots of mortality related to root rot for example) should help distinguish between different causes of mortality.

4.2.2 Management of contaminated trees

4.2.2.1 Exploitation of the tree

As in all areas of recent introduction of *B. xylophilus*, each time a tree containing the pine wood nematode is identified (after confirmation by a molecular analysis method), it must be destroyed in situ according to the current recommendations; in particular, this must take place between the end of the autumn and the beginning of the spring, when the insect vectors are still in the wood (Sousa *et al*., 2011).

With regard to the fate of the wood materials exploited, three categories should be distinguished:

- the parts of the tree that may contain larvae of the insect vector: these are the branches and the top of the trunk between 3 cm and 20 cm in diameter on maritime pine (Sousa *et al*., 2011; Schroeder *et al.*, 2009 and Piou, pers. comm.). These pieces must be destroyed in situ.

- the portion of the upper trunk 20 cm in diameter that can contain the nematode but not its insect vector: it can therefore enter the production chain provided that it is heat-treated (ISPM 15). However, it should be verified that this limit of 20 cm, which probably corresponds to a bark too thick to allow egg-laying by *M. galloprovincialis*, is still valid for other species of *Monochamus* (in particular *sartor* and *sutor*) and for tree species other than maritime pine (in particular black pine and Scots pine).

- the stump and the roots are never attacked by the insect vector (*M. galloprovincialis*); they can remain in place and do not require any special treatment as transmission of the disease to neighbouring trees via soil has not been demonstrated.

It would be useful to consider sampling the branches and trunk in the crown of the contaminated tree in order to verify the presence of larval galleries of *Monochamus*. Indeed, in the absence of traces of vector development, the contaminated tree could be regarded as a "dead-end" for the nematode, as it has not been re-used for oviposition by other adult beetles, thus preventing the transport of nematodes by emerging offspring beetles (at the next generation of Monochamus). The risk of spread may therefore be qualified as lower in this case. This is perhaps what happened in the two cases detected in Spain, and might explain the efficacy (unproven) of the clear-cutting performed at the time.

4.2.2.2 Emamectin benzoate

The technique of micro-injection of biocidal compounds in the trunks, associated with systemic dissemination of injected products, has recently seen renewed interest for protecting certain shrub species against various insect pests. Among the products tested, emamectin benzoate (EB) was targeted to combat pine wilt disease due to *B. xylophilus.*

EB is a semi-synthetic active substance belonging to the class of avermectins, which are natural substances produced by soil bacteria. This insecticide has an original mode of action mimicking the action of the GABA neurotransmitter on GABA receptors and glutamate H receptors. This binding leads to the permanent opening of chloride ion channels, causing irreversible muscle relaxation. EB has an ovo-larvicide activity at hatching and on older larvae by ingestion, causing a rapid and irreversible halt to mobility (stops tunnel boring) and nutrition (significant loss of weight) in the insects. The insecticidal efficacy of EB against bark beetles and longhorn beetles was demonstrated in the United States after injections carried out *in situ* in plantations of *Pinus taeda* on trees around 20 years old (Grosman and Upton, 2006). In laboratory conditions, EB affects the nutrition activity and longevity of *M. galloprovincialis* (Sousa *et al*., 2013).

Interestingly, activity against *B. xylophilus* was also demonstrated *in vitro* for EB (Takai *et al*., 2000). In addition, inhibition of the spread of the nematode was observed in branches of Japanese black pine (*P. thunbergii*) previously treated with EB and artificially inoculated with suspensions of nematodes (Takai *et al*., 2003b).

The preventive effects of injecting EB in tree trunks against pine wilt disease were evaluated *in situ.* In Japan, tests on *P. thunbergii* and *P. densiflora* showed an absence of symptoms for three years in 91% of the trees treated with EB (measured at 10g/m^3) and subjected to annual inoculations of the nematode (Takai *et al*., 2003a). In Portugal, no mortality was observed 26 months after the treatment of healthy trees (*P. pinaster*) located in a forest heavily infested with the nematode, whereas one third of the control trees died during this period (Sousa *et al.*, 2013).

These encouraging results in the fight against pine wilt disease due to *B. xylophilus*, and the significant risk of the spread of the disease in Europe, mean that EB is currently being assessed in the context of applying for marketing authorisation in France for this purpose (Bourdrez *et al.*, 2014). At the practical level, injecting EB in tree trunks presents some advantages: absence of phytotoxicity at the recommended doses, safety for the applicator and the environment, preventive treatment that is effective for two or three years. On the other hand, its implementation requires major manual operations (injections tree by tree) that have to be repeated regularly, which is probably incompatible with the treatment of extended areas of forest. In spite of this, it could be an appropriate solution for treating trees with great heritage value, for urban tree planting, or for trees located close to risk areas (ports, sawmills).

4.2.3 Transport and management of wood cut in a buffer zone around the destroyed tree

With regard to the routine exploitation of pine stands located in the vicinity of a tree detected as a carrier of the pine wood nematode, the current recommendations should be followed (Implementing Decision 2012/535/EU of 26 September 2012) but the area concerned (buffer zone) should be extended to a radius of 40 km given the results of the simulation on the dispersal capabilities of the insect vector.

4.2.4 Sanitation felling

To limit the extension of die-back due to infections by the pine wood nematode in a contaminated stand, the only currently operational method, combining efficacy and reasonable cost (which still needs to be assessed under French conditions), remains the sanitation felling (or "salvage harvesting") approach (Waring and O'Hara, 2005).

It is now commonly practised in Portugal (Sousa *et al*., 2011). In particular, it was tested successfully on the Troia Peninsula site by combining three complementary actions after identification of symptomatic trees: (i) felling of individual trees, ii) elimination of contaminated wood material, iii) activation of pheromone traps against the insect vector (Sousa *et al.*, 2011). These control actions were regularly inspected by the Food and Veterinary Office (FVO) of the European Union and its recommendations were followed in Portugal. More recently a "Task Force" on the pine wood nematode involving experts from eight European countries worked together with the Portuguese authorities to limit the extension of the outbreak of the nematode. Among its recommendations was the need to raise awareness among local communities and forest managers to implement these forestry control actions.

Tests conducted in South Korea have also shown the effectiveness of small-scale sanitation felling around the contaminated tree to stem the spread of the disease at the scale of the stand (Kwon *et al*., 2011).

5 Conclusions of the Working Group

The formal request 2014-SA-0103 concerned the emergency measures to prevent the spread, in the European Union, of the pine wood nematode, *Bursaphelenchus xylophilus.* More specifically, the Working Group (WG) set out to: i) review the control strategy based on clear-cutting as described by Implementing Decision 2012/535/EU of 26 September 2012, in order to determine whether such measures are still suited to the eradication of potential outbreaks; ii) propose, if necessary, improvements to this control strategy seeking to increase the associated effectiveness/impact ratio. The work of the WG was based on both an **in-depth literature analysis** (in particular taking into account recent data concerning the flight distance of the insect vector in Europe and the effectiveness of the method for performing micro-injections of emamectin benzoate for preventive treatments) and on the **production of original data** (modelling to simulate the dispersal of the insect vector, the transmission of the pine wood nematode and lastly the felling of trees in zones with an increasing radius).

Following detection of an outbreak, Implementing Decision 2012/535/EU advocates eradication measures for at least four years, mainly consisting of the establishment of: i) clear-cut zones with a radius of 500m around the infested plants and ii) intensive surveillance within a radius varying from 6 to 20 km around the infested zone. To estimate the effectiveness of these measures, a **simulation model** was developed and calibrated using experimental data specifying the dispersal capabilities of the insect vector *Monochamus galloprovincialis*. These data were obtained in the laboratory (on a flight mill) and *in natura* (mark-release-recapture experiments) in the context of the Landes de Gascogne forest, one of the forest areas most exposed to the risk of introduction of the pine wood nematode in France. The simulations that were then carried out under different scenarios (preventive or curative) show that the **clear-cuts currently advocated** by the European and French regulations would **not be effective** in a landscape configuration of continuous plantations of maritime pine. Indeed, with the radius of 500 m recommended by the European directive, at best 11% of transmissions would be prevented. Moreover, to obtain a pine wood nematode transmission rate lower than 0.1%, it would be necessary to implement clear-cuts in a radius of between 15 and 38 km. These simulations, carried out in the context of a continuous forest, should however be supplemented by a case involving highly fragmented pine forests. In this regard, it would be interesting to include biological data from Spanish forest managers, as they are directly involved in combating the nematode in this type of landscape.

Subsequently, in order to propose possible improvements in the current control measures against *B. xylophilus*, the WG considered the following options:

• Mass trapping of the insect vector:

Mass trapping measures are sometimes considered a way of eradicating insect populations or reducing their level below an acceptable epidemiological threshold. In practice this entails having to capture more than 90%, or even 99%, of the local population. Three recent studies, based on mark-release-recapture of insects, assessed the effectiveness of mass trapping of *M. galloprovincialis* in Europe (Spain and France). These three studies agree on the **very limited effectiveness of capture** with the most commonly used pheromone traps, of the order of 0.5 to 1% of circulating insects per trap. Moreover, it should be remembered that, as with all "curative" methods, it would need to be applied every year, throughout the insect's flight season. Lastly, and even if it were technically feasible, it would be ethically unacceptable to seek to eradicate a native species such as *M. galloprovincialis* which, apart from being the vector of the nematode, is part of the biodiversity of European forests.

• Chemical control:

The technique of micro-injection of a biocidal compound, emamectin benzoate (EB), associated with systemic dissemination of the product in the injected tree trunks, has recently seen renewed interest for combating pine wilt disease due to *B. xylophilus*, following encouraging results from experiments obtained both *in vitro* and *in natura*. In fact, this compound is currently being assessed with the aim of applying for marketing authorisation in France for this purpose. At the practical level, the injection of EB in tree trunks presents some advantages: absence of phytotoxicity at the recommended doses, safety for the applicator and the environment, preventive treatment that is effective for two or three years. On the other hand, its implementation requires major manual operations that have to be repeated regularly, which is probably **incompatible with the treatment of extended forest areas.** In spite of this, it could be an appropriate solution for treating trees with great heritage value, for urban tree planting, or for trees located close to risk areas.

• Individual-centred control:

Given the lack of effectiveness of the methods presented above on the scale of a forested area, it appears necessary to consider another, better targeted approach. This so-called individual-centred control strategy is based on a three-step approach: i) the early detection of the arrival of the nematode in a new forest region, triggering a series of measures designed to reduce its impact, with ii) the precise identification of individual symptomatic trees, followed by iii) their elimination. Without detailing the actions that could be undertaken to this effect, options on how to establish this individual centred strategy are listed below.

- **Early detection of the presence of the nematode** remains the priority of this control strategy. To achieve this, it will be necessary to combine: i) the trapping of insect vectors (grid size to be defined depending on the context) and the detection (by ground and/or air surveillance) of symptomatic trees with ii) systematic screening for the nematode in insect and tree samples.

- Whenever insects are reported as vectors of *B. xylophilus*, the **position of the traps where they were captured will be triangulated** to delineate the likely zone of the presence of the pine wood nematode.

- Whenever a tree containing the pine wood nematode is identified, it will be **disposed of individually on site** according to the current recommendations; in particular, this will take place between the end of the autumn and the beginning of the spring, when the insect vectors are still in the wood, and in compliance with the regulations concerning transport and management of timber cut in a buffer zone around the destroyed tree. Given the results of the simulation on the dispersal capabilities of the insect vector, this **buffer zone** should be **expanded to a radius of 40 km.**

In conclusion, the WG considers that the only currently operational method for limiting the extension of wilt associated with infestation by the pine wood nematode in a contaminated forest stand, combining efficacy and reasonable cost, remains a combination of **strengthened resources (both technical and financial) for early detection of the nematode** (on insect vectors and/or in the trees) followed by **sanitation felling as an outbreak develops**. The aim is the targeted elimination of infested trees according to the above recommendations. The objective of control is then no longer to eradicate the disease, but rather to limit its spread on the scale of the forest stand. It is important to reiterate that the measures detailed here, which are the subject of the formal request, primarily target the natural dispersal of the pathogen by its vector, around a detected outbreak. Containing an outbreak on a wide geographical scale will only be effective if these measures are combined with rigorous application of the regulations concerning the treatment and transport of timber or wood packaging, in order to avoid introductions from a great distance.

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6.2 Standards

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6.3 Legislation and Regulations

Commission Implementing Decision 2012/535/EU of 26 September 2012 on emergency measures to prevent the spread within the European Union of *Bursaphelenchus xylophilus* (Steiner and Buhrer) Nickle *et al*. (the pine wood nematode) (notified under document C(2012) 6543) (2012/535/EU). *Official Journal of the European Union* L 266 of 02.10.2012, pages 42-52.

Guidance note DGAL/SDQPV/N2013-8102 of 25 June 2013 - Method of trapping of beetles of the *Monochamus* genus to be implemented in the framework of surveillance of the pine wood nematode (*Bursaphelenchus xylophilus*).

Guidance note DGAL/SDQPV/N 2013-8132 of 31 July 2013 - Annual surveillance plan relative to the pine wood nematode (*Bursaphelenchus xylophilus*) in France.

ANNEXES

Annex 1: Formal request letter

2014 -SA- 0 1 0 3

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Service de la prévention des risques sanitaires de la production primaire

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Agence nationale de sécurité sanitaire
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Copie : Mme. Nathalie FRANQUET

Réf. interne : BSSV / 2014 -

Paris, le 24 AVR. 2014

Objet : Demande d'avis sur la stratégie de lutte imposée par la décision d'exécution 2012/535/UE du 26 septembre 2012 relative à la mise en place de mesures d'urgence destinées à prévenir la propagation, dans l'Union, de *Bursaphelenchus xylophilus*.

La décision d'exécution 2012/535/UE du 26 septembre 2012 (annexe 1) impose aux États Membres, la mise en place de mesures d'urgence destinées à prévenir la propagation dans l'Union, de *Bursaphelenchus xylophilus,* le nématode du pin, un organisme nuisible réglementé dont l'introduction en France pourrait occasionner de gros dégâts sur de nombreux résineux.

Ces mesures visent en priorité à éradiquer tout foyer du nuisible détecté, l'enrayement de tels foyers ne devant être mis en œuvre que dans les zones où l'éradication ne serait pas un objectif réalisable. Dans ce contexte, les États membres doivent appliquer des mesures d'éradication pendant au moins quatre ans suite à la détection d'un foyer. Elle consiste notamment en la mise en place de coupes rases (d'un rayon de 500m réductible sous conditions à 100m) autours des végétaux infestés et à la mise en place d'une surveillance intensive dans un rayon variant de 6Km à 20km autour de la zone infestée (rayon supposés suffisant compte tenu de la distance de vol de l'insecte vecteur). De telles mesures peuvent avoir des conséquences environnementales importantes et nécessiter des moyens humains conséquents en particulier en cas de détections multiples. Il est à noter que ces mesures ont été élaborées à l'aide des éléments scientifiques et techniques disponibles à l'époque sur le nématode et son vecteur (insectes du genre *Monochamus*).

Récemment, de nouvelles études ont été publiées. Certaines ont été réalisées en Europe et portent notamment sur :

- la distance de vol de l'insecte vecteur en Europe (*Monochamus galloprovincialis*) qui serait très supérieure à celle de l'insecte vecteur en Asie *Monochamus alternatus* (3Km) qui a servi de référence pour l'élaboration de la décision européenne

- l'efficacité de la méthode visant à réaliser des micro-injections de benzoate d'émamectine pour la réalisation de traitements préventifs (cette molécule aurait à la fois un effet nématicide envers le nématode et insecticide envers les vecteurs)

Dans ce cadre, au regard de ces récentes études, je vous prie de bien vouloir :

i) réexaminer la stratégie de lutte telle que décrite par la décision d'exécution 2012/535/UE du 26 septembre 2012, afin de déterminer si ces mesures sont toujours adaptées à l'éradication d'éventuels foyers.

ii) proposer le cas échéant des améliorations de cette stratégie de lutte visant à augmenter le rapport efficacité/impact qui lui est associé.

Les réponses à ces questions pourraient permettre d'engager des discussions à l'échelle européenne visant à faire évoluer, si nécessaire, la décision d'exécution 2012/535/UE du 26 septembre 2012. Dans ce cadre, je vous prie de bien vouloir réaliser ce travail pour le 15 décembre 2014, afin que nous puissions disposer d'éléments sur le sujet avant le déroulement du prochain panel de la quarantaine forestière de l'organisation européenne et méditerranéenne pour la protection des végétaux (OEPP).

En cas de difficulté rencontrée dans l'accomplissement de cette mission, je vous prie de m'en informer dans les meilleurs délais.

Mes services se tiennent à votre disposition pour vous apporter toute information complémentaire. Je vous remercie de bien vouloir m'accuser réception de la présente demande.

Le Directeur Général Adjoint
Chef du Service de la Coordination
des Actions Sanitaires - C. V. O.

Jean-Luc ANGOT

Annex 2: Tracking of report updates

Date	Version	Page	Description of the change
08/09/2015	01		First version

Notes

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