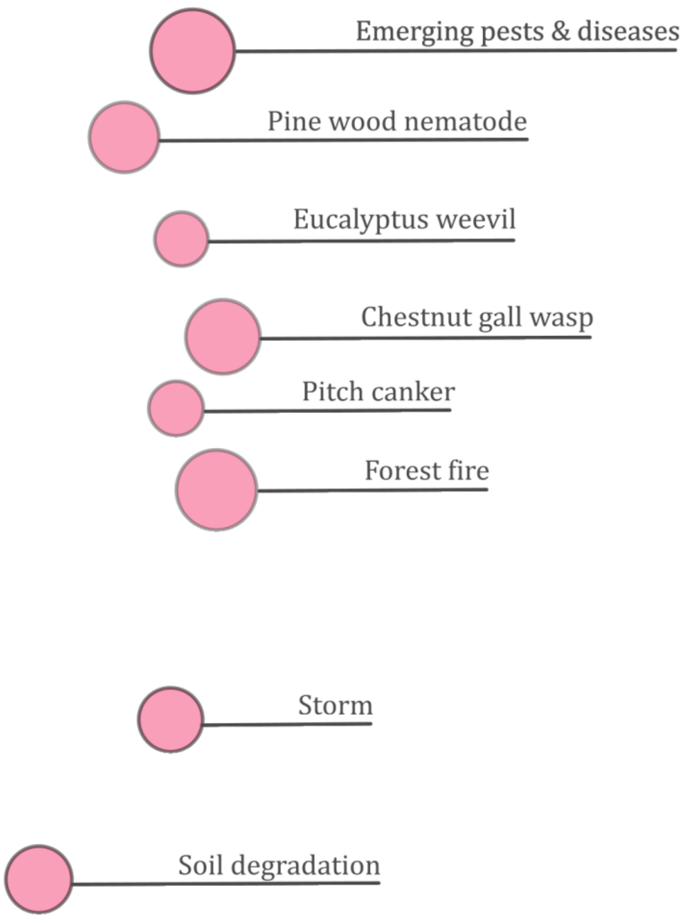
# Minutes of the pine wood nematode (*Bursaphelenchus xylophilus*) workshop

## *Towards early warning detection methods*




**INIAV - Instituto Nacional de Investigação Agrária e Veterinária**
**Oeiras, Portugal**
**26 January 2018**

**Author of the minutes**: Eduard Mauri (EFIATLANTIC)

**Reviewer of the minutes**: Pedro Naves (INIAV)

**Workshop organisers**: Edmundo Sousa (INIAV), Manuela Branco (ISA)

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# Agenda

**PLURIFOR PROJECT**
**PINE WOOD NEMATODE WORKSHOP:**
**TOWARDS EARLY WARNING DETECTION METHODS**

| | |
|---|---|
| **FRIDAY**<br>**26 JANUARY 2018** | **Organisers:** Edmundo Saousa edmundo.sousa@iniav.pt (INIAV) & Manuela Branco mrbranco@isa.ulisboa.pt (ISA)<br>**Language:** Portuguese - English - Spanish<br>**Venue:** CAP Meeting room<br>INIAV - Instituto Nacional de Investigação Agrária e Veterinária<br>Av. da Republica, Nova Oeiras, 2780 Oeiras, Portugal |

| | |
|---|---|
| **9:00** | **Welcome and workshop objectives**<br>Edmundo SOUSA (INIAV), Manuela BRANCO (ISA) |
| **9:15** | **WP2: Review of the tools used for the early detection of trees infested by the pine wood nematode** |
| | List of tools in use or requested by the different partners (15 min presentation)<br>09:15 Portugal<br>09:30 Castilla y Leon<br>09:45 Aquitaine<br>10:00 Discussion on expectations for methodological improvement<br>*11:00 Coffee break* |
| **11:30** | **Case study on remote sensing** |
| | Presentation of PLURIFOR tools: the case study in Nazaré where complementary remote sensing techniques are compared:<br>- Ground survey: INIAV<br>- Drone survey: TRAGSA<br>- Satellite survey: TELESPAZIO |
| **13:00** | **Lunch break** |
| **14:00** | Discussion with attendees about the relevance of new, proposed tools |
| **15:00** | **Case study on pheromone trapping** |
| | 15:00 Pheromone trap monitoring in Portugal<br>15:15 Pheromone trap monitoring in Spain<br>15:30 Pheromone trap monitoring in Aquitaine<br>15:45 Presentation of PLURIFOR tools: the case study on trapping networks in Aquitaine: INRA |
| **16:30** | **Coffee break** |
| **17:00** | Discussion about the potential for different trapping strategies to improve the early detection of PWN transported by *Monochamus galloprovincialis* |
| **17:30** | Definition of the next steps and products resulting from GT2 for the PWN survey |
| **18:00** | **End** |

**PLURIFOR PROJECT**
**PINE WOOD NEMATODE WORKSHOP:**
**TOWARDS EARLY WARNING DETECTION METHODS**

**Requested participants:** partners working on the pinewood nematode risks, PLURIFOR associated partners, and guests invited by the different teams.

Workshop registration before 19 January 2017. Limited places.

# Pine wood nematode WP2 objectives

## Pine wood nematode risk partners and associated partners

| Region | Organisation | Contact person | Associated partners |
|---|---|---|---|
| Portugal | INIAV | Edmundo Sousa | Altri Florestal<br>Instituto da Conservação da Naturesa e das Florestas<br>RAIZ - Instituto de Investigação da Floresta e Papel |
| Castilla y Leáon | TRAGSATEC | Jorge Casado | Junta de Castilla y León<br>Empresa de Transformación Agraria |
| Aquitaine | INRA | Hervé Jactel | Caisse de Prévoyance et de Protection des Forêts du Sud-Ouest<br>Direction régionale de l'alimentation, de l'agriculture et de la forêt<br>Association Régionale de Défense des Forêts Contre l'Incendie |

## Tools and risk management plans to be developed within PLURIFOR project

As decided by the PLURIFOR Technical committee n°2 meeting (25-26 January 2017 at NEIKER, Parque Tecnológico de Bizkaia, Parcela 812, calle Berreaga 1, Derio, Spain), the following tools and risk management plans will be developed by the pine wood nematode risk team in WP2:

- Improve methods of detection of infected trees based on remote sensing;
- Improve simulation model of the *Monochamus* sp. dispersal flight to optimize the design of the trapping network.

# Attendees

## Attendees

### Participants

| First name | Last name | Organisation |
|---|---|---|
| Albertino | Teixeira | APFCAN |
| Alda | Antunes | ICNF-DCNFLVT |
| Alejandro | Cantero | HAZI |
| Ana | Fernandes | ICNF |
| Ana Margarida | Fontes | INIAV |
| Ana Paula | Ramos | Instituto Superior de Agronomia, Universidade de Lisboa |
| Ana Sofia | Domingues | ICNF |
| António | Borges | Instituto da Conservação da Natureza e das Florestas |
| Bruno | Ferreira | Instituto Superior de Agronomia |
| Christophe | Orazio | EFIATLANTIC |
| Edmundo | Sousa | INIAV |
| Eduard | Mauri | EFIATLANTIC |
| Edurne | Lacalle | USSE |
| Emmanuel | Kersaudy | DRAAF |
| Fliomena | Nobrega | INIAV |
| Gaëlle | Burlot | Caisse Phyto Forêt |
| Helena | Marques | ICNF |
| Helena | Vicente | INIAV-LabNematologia |
| Horacio | Peralta | ISA |
| Ilidio | Loução | ICNF |
| João | Silva | icnf |
| João | Rafael | APFCAN |
| José | Campos | ICNF |
| Laura | Luquero Ramos | TRAGSA |
| Leire | Salaberria | USSE |
| Luís | Caparica | ICNF |
| Maria | Inacio | INIAV |
| María | Castaño Diaz | Institute Superior of Agronomy |
| Maria do Rosário | Amaral | ICNF |
| María José | Checa | Grupo Tragsa (Tragsatec) |

| First name | Last name | Organisation |
|---|---|---|
| Marta | Rocha | Instituto Superior de Agronomia |
| Miguel | Pintos | INIAV |
| Olga Verónica | González | ASFOSA |
| Oriencio | Pereira | Floresta Projetos |
| Paula | Soares | Centro Estudos Florestais/ISA |
| Pedro | Marques | Individual |
| Susana | Carneiro | Centro PINUS |
| Thierry | Bélouard | Département santé des forêts |
| Vasco | Oliveira | ICNF/DCNFLVT |

## Speakers

| First name | Last name | Organisation |
|---|---|---|
| Francisco José | Lario Leza | TRAGSA |
| Hervé | Jactel | INRA |
| Jean-Charles | Samalens | Telespazio |
| Jorge | Casado Álvarez | TRAGSATEC |
| Luís Felipe | Bonifácio | INIAV |
| Pedro Miguel | Naves | INIAV |
| Telma | Ferreira | ICNF |

## Organisers

| First name | Last name | Organisation |
|---|---|---|
| Edmundo | Sousa | INIAV |
| Manuela | Branco | ISA |

# Apologies

| First name | Last name | Organisation |
|---|---|---|
| Amaia | Ortiz | NEIKER |
| Ander | Arias | NEIKER |
| Manuela | Branco | ISA |
| Nahia | Gartzia | NEIKER |

# Absent

| First name | Last name | Organisation |
|---|---|---|
| António | Nora | Floresta Atlântica |
| Dina | Ribeiro | ICNF |
| Diogo Neves | Proenca | University of Coimbra |
| Filipa | Maia | LPVVA-ISA |
| Helena | Martins | ICNF |
| Paula | Morais | University of Coimbra |
| Rui | Tujeira | Soluções Tecnicas em Fitossanidade Unipessoal, Lda |
| Rui | Delgado | Syngenta |
| Susana | Brigido | 2bforest |
| Tatiana | Valada | LPVVA, ISA |
| Teresa | Vasconcelos | ESAC |

# Presentation of the tools

## WP2: Review of the tools used for the early detection of trees infested by the pine wood nematode

### Goal

List of tools in use or requested by the different PLURIFOR associated partners. Tools for early warning detection are crucial as it is known that the faster a threat is detected and the sooner the reaction against it happens, the higher are the chances to eradicate it. The tools presented are around two main issues: detection of symptomatic trees and detection of the presence of the nematode, in pine trees and in the vector insect (*Monchamus* sp.).

### Portugal

By Pedro Miguel Naves, INIAV

According to observations on the field, in the Iberian Peninsula the pinewood nematode (*Bursaphelenchus xylophilus*, agent of the pine wilt disease) affects mainly *P. pinaster*, and also *P. radiata* and *P nigra*. *Pinus sylvestris* is likely to be a very susceptible species, but so far has not been affected by the nematode. *P. pinea* and *P. halepensis* appear to be resistant or tolerant species.

*Monochamus* sp. (the vector insect) feeds on healthy trees but lays its eggs on unhealthy or stressed trees. Adult *Monochamus* sp. emerge from larval galleries carrying the nematode and contaminate the healthy pines where they eat. The used method to control the disease is by detecting and destroying the wilted pines when *Monochamus* sp. larvae and nematodes are still inside the pines, during the autumn and winter months. The pine wilt disease has no exclusive symptoms. The trees die rapidly, in some months or 1-2 years. Main symptoms are:

1. Oleoresin flow decreases or stops even before the first visual symptoms;
2. Flag of dead needles; as visual symptom of evaluation. Can be just one branch, where the insect has fed (as it will be the first part infested), or can be the whole canopy;
3. Brown canopy, when pine wood nematode has spread in the pine;
4. Death of the trees.

Symptomatic trees survey takes a lot of time. Trees are marked and a portion of them are sampled and analysed to detect the pine wood nematode, but all symptomatic trees are felled and destroyed.

The whole continental Portugal is considered infested, although not all pine stands are affected. A buffer zone 20 km wide along the border with Spain is the area of highest priority for survey, to avoid spreading the pine wood nematode the country. In the whole country, an assessment of pine wood nematode risk is done to set the level of risk according to:

| Proximity to pine wood nematode affected areas | Presence of conifers | Proximity to forest fires | Risk level of pine wood nematode |
|---|---|---|---|
| Yes | Yes | Yes | Very high |
| Yes | Yes | No | High |
| No | Yes | Yes | Moderate |
| Yes | No | No | Reduced |
| No | No | Yes | Reduced |
| No | No | No | Low |

Efforts are done to detect pine stands free of nematode and keep them free of it.

Field surveys request a lot of effort, and are coordinated by the National Forest Authority. GPS location of symptomatic pines is centralised in an Internet database. Insect traps are also installed and checked to detect the presence of the nematode in the vector insect. Identification of the nematode in a wood sample is very difficult because other *Bursaphelenchus* species exist and can be present. Identification is either done by visual inspection by experienced taxonomists or by DNA analysis.

To reduce survey efforts, Portuguese PLURIFOR partners have selected forest stands with the following criteria where drone remote sensing will be tested to detect symptomatic trees:

- Stand within an affected area,
- Single flight zone with about 15 ha with trees showing different degrees of symptoms,
- Suitable slope of the ground, angle of incidence of sunlight and angle of view of the sensor,
- Monospecific stands with homogeneous characteristics in terms of age, forest structure and spatial distribution,
- A composition of the understory that allows to discriminate the pine canopy,
- The area must have an open surface close to the drone taking off and landing point,
- Easiness to set up and transport the equipment,
- Nearby weather stations (which provide auxiliary information),
- An area free of drone flight constraints, as: at least 2.5 km away from and aerodrome or airport, no inhabited areas or buildings, no more than 12 people below the flight path.

## Castilla y León

By Jorge Casado Álvarez, TRAGSATEC

The national contingency plan for Spain is adapted by regional forest administrations. In Castilla y León, four operational protocols exist for: field sampling, trapping the evictor insect, inspection from roads, and sampling in wood industries.

The objective of the field sampling is to locate and destroy the trees affected by the pine wood nematode. The whole territory is divided in three systematic sampling zones, each one with a given density of sampling plots, located in a grid which density varies according to the distance to Portugal:

1. Close to Portuguese border: the narrowest grid measures 2 x 2 km;
2. Further from Portugal: a larger grid of 4 x 4 km;
3. In the rest of the territory: a grid of 8 x 8 km.

Directed sampling is done in areas with symptomatic trees, in timber markets, along roads used for timber transportation from Portugal and in timber industries.

In the demarcated areas and in areas of special interest, trees are always cut and destroyed and samples are taken to be analysed at the laboratory. Action plans are developed for the demarcated areas. It mainly implies the elimination of all conifers in the infested core area and the elimination of all symptomatic and dead conifers in the buffer are around the core area.

The Valverde del Fresno demarcated area was considered eradicated at the beginning of 2017. The Sancti Spiritus demarcated area has been considered eradicated in January 2018.

## Aquitaine

By Hervé Jactel, INRA

In Aquitaine, the main susceptible area is the Landes de Gascogne, a forest area of more than one million hectares of *P. pinaster* plantations. It would be very difficult to survey everywhere; therefore remote sensing survey would be very useful, as pine wood nematode could appear just in isolated trees and pine wilt disease symptoms are not specific. Questions around symptomatic trees survey are:

- What is the accuracy of the survey methods, as the symptoms are not specific?
- Which is the ideal survey frequency and effort to detect local mortality?
- Is there any spatial or temporal pattern of the symptoms?
- Which is the best location to collect the samples from the tree in order to avoid false negatives? In Portugal, sample collection is done at the top of the tree because is the point of entrance of the nematode, but reaching the highest branches is costly.
- Is there any possibility to perform DNA analyses *in situ* to diagnostic pine wood nematode in real time?
- Which is the optimal organisation of the trapping system of *Monochamus* sp.: should priority be given to pathways (roads), close to facilities where pinewood is processed, or in the core of the forest?
- Can we distinguish the origin of the *Monochamus* sp. so we can figure out the pathway of the vector insect?

## Discussion on expectations for methodological improvement

Pedro Miguel Naves: Aerial photography flights for the forest inventory have to be modified if we want to be able to use them for detection of symptomatic trees. In Portugal, priority areas to perform aerial surveys are defined and published every year by the ICNF.

Infestation rates: in an area subjected to high-intensity management over several years (Troia Peninsula), at the most 10% of sampled trees are true positives during the last years (in the early years that infestation rate was about 70%). For the rest of 90% symptomatic trees, there are several other causes, mainly bark beetles. In early season, there are higher rates of false negatives because the pine wood nematode is not causing a lot of symptoms yet, and the nematode is only in the tips, where it is difficult to collect samples. From May to September, the control of the evolution of the symptoms is done, considering climate variations, and at the end of the period the most probable positive trees are cut.

*In situ* detection of pine wood nematode in Portugal does not work. They do not have a solution for *in situ* detection yet, but they are working on it, as it would be a very useful tool. It takes, at least 48 hours to detect nematode at the lab, plus the time to send the sample from the forest. Usually, the time elapsed from sample collection to have the answer is a week or maximum two weeks.

Hervé Jactel: In Aquitaine, lab analyses use the entire buck of the same insect trap to perform DNA analysis in order to detect the presence of the pine wood nematode in one of the vector insects.

Jorge Casado Álvarez: In Castilla y León, in the 8 x 8 km grid sample plots there are nearly no symptomatic trees, so it supposes a lot of sampling efforts that lead to no results. Directed sampling would be more effective: on the roads, trucks, timber markets and sawmills. However, more systematic and directed samplings are both requested by EU regulation.

Edmundo Sousa: 1) Early detection of pine wood nematode can be possible thanks to the detection of *Monochamus* sp. when there are no symptomatic trees in areas without pine wood nematode like in Spain and France; 2) in Portugal, forest managers and authorities want to know which is the proportion of the *Monochamus* sp. than transports pine wood nematodes to know if there is more or less presence of pine wood nematode carried by the vector insect. Currently, 1/3 of the *Monochamus* sp. captured by buck traps in Portugal carry the pine wood nematode.

Another problem is that the nematodes are clustered distributed in a pine, so how to sample a tree to avoid a false negative is a crucial question.

Luís Bonifácio: 75% of the nematodes would have left the *Monochamus* sp. during the first three weeks after emerging from the pine stem as imago, when they are not mature yet, so not attracted by the pheromone traps. Consequently, this method of survey underestimates the number of nematodes carried by a *Monochamus* sp., but it is correct to use it to survey the presence of pine wood nematode, as DNA sampling can detect the presence of the nematode even if there is only one left; it is very unprovable that all nematode would have left the *Monochamus* sp. after three weeks, we fairly can suppose there would be at least one nematode in the *Monochamus* sp.

Pedro Miguel Naves: It is known that the scorched pines by fires attract *Monochamus* sp. from very far and that they lay eggs on them. So, these trees would be the first priority to be sampled/felled to avoid attracting the vector insect.

Edmundo Sousa: As the *Monochamus* sp. is attracted by the smell of scorched trees, it would have to be test if scotched trees can be used as trap trees: let the *Monochamus* sp. lay their eggs on them and cut and destroy the trees during winter before adults emergence.

After a windthrow, once on the ground, pine bark is still suitable during three to four months for the larvae to complete their stages to imago, and then it dries. Consequently, early blowdowns (in winter) pose no problem because in summer the bark would have dried before the emergence of the adult *Monochamus* sp, which would not be able to complete their cycle. However, blown down trees by late storms (in spring) arrive to summer with fresh bark, offering larvae a suitable medium to reach their imago stage. Consequently, blown down pines must be managed differently depending on the period of the year: high priority should be given to harvest pines blown down by late (spring) storms.

# Case study on remote sensing in Nazaré (Portugal)

## Goal

Show how Portuguese, Spanish and French experts collaborated in the projet to test different pine wood nematode survey techniques.

## Ground survey – conducted by INIAV

By Luís Felipe Bonifácio, INIAV

Following the criteria for site selection presented previously by Pedro Miguel Naves (INIAV), a study area of 5.5 ha of *P. pinaster* plantations in central costal Portugal, in Nazaré, was selected for the test. At the end of May 2017, 15 trees with symptoms were found in the study area: most of them had been infests the previous year, showing advanced symptoms, mainly they were nearly dead. After sampled to detect nematode, 53% of them were infested by the pine wood nematode.

TRAGSA did the drone flight in October 2017, and 25 new symptomatic pines were found, 22 of them (88%) with pine wood nematode inside.

## Drone survey – conducted by TRAGSA

By Francisco José Lario, TRAGSA

The objective of this drone survey was to test and evaluate the results from different remote sensing sensors to identify symptomatic trees caused by the pine wood nematode, in the previous study area. The specific objectives were to:

- Use spectral data taken in August to predict the wilt of pines in December (four months anticipation): prediction model;
- Use remote sensing data to explore the landscape and detect symptomatic trees: extension model.

The work plan was: plan the flights -> calibrate the sensors and on-field geolocation -> take the aerial images -> quality control of the images' radiometry and geometry -> build the orthomosaic with the images -> do the radiometric correction -> calculate the vegetation indexes (for a 1.5 m radius circle around the centre of the pine crowns). NDVI, CARI, ARI, SAVI, MTVI indexes used. Also, field measurements to characterise the wilt of the pine canopies were performed.

**Prediction model**

There were not found any significant linear correlations between the tree physiological parameters and the vegetation indexes, so no prediction model could be built. This is maybe because 2017 had been a warmer and drier year than usual in Portugal, so tree physiological parameters may have been altered.

**Extension model**

Pearson correlation coefficients were calculated between the on-field physiological and dendrometrical measurements and the vegetation indexes obtained by remote sensing, in order to select for the extension model the remote sensing parameters significantly correlated with some of the on-field parameters. The retained explanatory variable was Fv/Fm (photosynthetic efficiency of the II photosystem in the dark, an indicator of the plan stress). Ten trees were used to build a binary logistic regression model.

The model was tested model with the other symptomatic trees (ten other trees not used to build the model) and reached a sensitivity of 80%, a specificity of 60%, and a success of prediction of 70%. A more robust data and more observations would be necessary to build a better model.

**Preliminary conclusions**

- Predicting pine wilt four months in advance using remote sensing techniques has not been proved successful.
- When predicting pine wilt, sampled trees could have not been infested at the moment of the measurements, in August.
- The use of remote sensing methods for and operative identification of wilted pines is possible.
- The sensitivity of the model could be increased by increasing the amount of data used to build it.

## Satellite survey – conducted by Telespazio

By Jean-Charles Samalens, Telespazio

Not all satellite images are usable to distinguish single trees because they usually have a resolution of about 5 m/pixel. Panchromatic bands can achieve a 31 cm/pixel resolution, and multispectral bands up to 1.24 m/pixel resolution. The other constraint of satellite images is a time constraint: the requested images must fit in a one to two months window time, so they cannot be ordered to be taken at a specific date.

The minimum tasking is 100 km², at 55 euros/km². For this project, images were taken on 23 August 2017. September to November would have been better months to take the images.

The prevalence of pine trees with projected crown diameters of 2 m or less dictates the use of very high resolution imagery (5 to 20 cm/pixel) for tree-level forest monitoring in the context of pine wood nematode threat.

After image processing, it is easy to distinguish between dead and healthy trees, but it is more difficult to distinguish between unhealthy trees and the understory. ACP 1, ACP 2, SAVI and SVM indexes have been used, filtering and pinpointing dead trees. In about 1,000 ha, 437 dead trees were detected.

**Conclusions**

To provide a detailed record of individual coniferous tree crowns in the study area, the area should be imaged in colour at 10 cm/pixel or higher spatial resolution.

Nowadays, the potential use of satellite images acquired for forest monitoring in the context of the pine wood nematode hazard is mainly for:

- Monitoring, more than early detection;
- Detecting change, more than a single tree damage classification;

Perfect georegistration is needed to unsure these uses.

UAV and aerial images have the suitable spatial resolution to detect unhealthy pine trees, but they have their own operational limits: extent, flight duration, aviation rules and legal restrictions. UAV are more affected by adverse weather conditions than planes. On-board sketch mapping can be more cost-effective.

## Discussion

Luís Bonifácio: *P. pinea* has set of volatiles that make it not attractive to *Monochamus* sp., and pine wood nematode does not develop inside it. *P. halepensis* is attractive to *Monochamus* sp., so it is not a susceptible pine species maybe because pine wood nematode is not able to develop inside it.

Hervé Jactel: Naturally resistant individual trees exist but are very rare. There is no suitable way to bring this resistance to other pines of the same species. In Aquitaine, there have been tests to find more tolerant pines to the pine wilt disease, like *P. taeda*, but they are more susceptible to the pine processionary moth (*Thaumetopoea pityocampa*) and to bark beetles. Moreover, *P. taeda* individuals can be asymptomatic, so they can be a reservoir of pine wood nematode towards other pines.

Jean-Charles Samalens: Satellites require no authorisation, they are more secure than drones, but have less spatial resolution, cloud coverage can cause problems in image acquisition and it is not possible to ask for an image for a particular date (one to two moths interval). Satellite images can be used to track the evolution of the susceptibility of a forest, but not the pine wood nematode hazard itself. Satellite images are better to detect symptoms when they are shown in grouped trees, not for single tree detection. In this case, then aerial remote sensing is better.

Question from the participants: Is it possible to compare several satellite images in a time series?

Jean-Charles Samalens: This is one of the next steps in satellite imagery use: to detect changes in a time series of images. Copernicus program provides free images and time series nearly in real-time, but spatial resolution is low: 10 m/pixel, so useless for this hazard. However, they could be used to monitor the risk factors. High resolution satellite images start to compete with aerial images taken from planes. However, images from UAV have no competition for resolution, they are the best.

Christophe Orazio: A guidelines should be written to explain which early detection methods should be used for every case situation, including human visual survey and detection from helicopter for important and sensitive areas that are currently only covered from ground survey. This would allow an immediate implementation of aerial detection, without long delays for image acquisition, process and analyses.

Edmundo Sousa: To continue on the idea of the survey from helicopter, it would be possible to have real-time visual inspection of video images taken from the drone. Of course, the limit of a maximum radius of 1 km around the operator poses a problem to the extent of the flight. However, it would be cheaper than a helicopter flight, even if the helicopter has more coverage and fewer flying restrictions. It is important to focus on the most risky areas, where aerial survey of symptomatic trees would be used.

Emmanuel Kersaudy: Aquitaine region is too big to be completely surveyed. It would be more cost-efficient to aim the points of entry of infested material and define risk areas for inspection. In order to define risk areas to proceed to proper survey, it is necessary to know where the pine trees are. For this, satellite remote sensing can be a useful tool, as it also allows locating pine trees in cities (urban forests, usually not surveyed in forest inventories) as well as in mixed stands.

Luís Bonifácio: The size and the shape of the pine crown as seen by the flying *Monochamus* sp. is important for the spread of the vector insect. It orientates itself with a mixture of visual and chemical stimuli. When *Monochamus* sp. emerges, it spends two or three days feeding on the same pine and then disperses to nearby trees. Some *Monochamus* sp. do not move too much (less than 100 m) if there is food nearby.

Edmundo Sousa: *Monochamus* sp. population increased after the pine wood nematode arrival because the weakened pines trees by the nematode, that are an ideal medium to lay the eggs, became more frequent. So, with more *Monochamus* sp. individuals in the ecosystem, they become less selective on pines.

Jorge Casado: In regular ground survey by the forest service, forest technicians have to be ready to suspect from any diseases or pest. For example, in Santi Spiritus demarcated area, forest technicians were searching for *Fusarium*, but found pine tree with the pine wilt disease. Updated training is crucial for ground survey crews.

Edmundo Sousa: Autumn and winter are the best seasons to do the survey if it is decided to do it only once a year. Then, it should be inspected if *Monochamus* sp. larvae are inside the pine stems. If there are emerging holes, it means that the *Monochamus* sp. adults have already emerged and spread around the territory. So, when monitoring for this pest, do not search only for pine wood nematode symptoms, but also for the presence of *Monochamus* sp. This is not done in Spain.

Portugal uses systematically remote sensing only in the 20 km buffer zone along the Spanish border. In the rest of territory, only ground survey is performed.

# Cases studies on pheromone trapping

## Goal

Present the current use and the last knowledge on pheromone trapping concerning the vector insect *Monochamus* sp. in Portugal, Castilla y León and Aquitaine.

## Portugal

By Telma Ferreira, ICNF

Main goals in Portugal concerning pine wood nematode are:

- Maintain a buffer zone free from pine wood nematode and pine wood nematode susceptible plants in decline;
- Contain pine wood nematode in the areas where it is present and reduce the incidence of the pine wilt disease;
- Eradicate pine wood nematode isolated cases;
- Promote monetarization and inspection of susceptible plants and wood, and also related economic operators;
- Ensure the effective implementation of heat treatment to wood, WPM and bark.

The establishment of a network of traps is important to:

- Complement the prospection (and sampling) of symptomatic trees;
- Control the populations of the vector insect in areas where they are actively breeding and are likely to contain pine wood nematode;
- Prevent dispersion flights of infested insect vectors to non-infested areas.

The buffer zone covers 2.3 million ha, of which about 100.000 ha are covered with *P. pinaster*. Due to limited resources, the following locations receive high priority for pheromone trapping:

- Surroundings of burnt areas (100 m buffer);
- Surroundings of areas affected by wind damage (100 m radius);
- Places with high density of pine wood nematode vector insect (*Monochamus* sp.) or bark beetles;
- Timber processing places or yards, and their surroundings;
- Surroundings of positive cases in 5 km distance of the buffer zone and intervention zones;
- Declining trees near the inner border of the buffer zone (after the first km).

As a result:

- This is a non-systematic approach;
- There are not identical sampling efforts (in space and in time);
- ICNF focuses on the traps role as a complement to symptomatic trees sampling;
- The use of different traps and lures are subjected to methodological shifts and they are not intended to perform field test to provide strong scientific data.

Nearly all (97.4%) trapped insects were *Coleoptera*: 90.1% *Scolitydae*; 4.1% *Cerambicydae*, 3.1% other *Coleoptera*, and 0.1% *Curculionidae*. In average, the pine wood nematode was detected in up to 29% of the *Monochamus* sp. individuals caught in the traps located in intervention zones. No pine wood nematode was found on beetles caught in the traps located in the buffer zone.

In Portugal, there are guidelines for the survey of *Monchamus* sp. using pheromone traps. Trapping takes place during the period when the insect flies: from end of May to beginning of October.

Further improvements include: increase the density of traps, improve traps design to be more selective, avoid saturation and avoid trapping other small insects. The main problem is the very large are to sample for *Monchamus* sp.: a full country. It is important to set priorities and that the priority maps are regularly updated.

## Castilla y León

By Jorge Casado Álvarez, TRAGSATEC

**Objectives**

In Spain, trapping specifications for *Monchamus* sp. are included in the National contingency plan for the pine wood nematode. The purposes of trapping in Spain are:

- Track the populations of the vector insect;
- Control the vector insect;
- Detect the presence of pine wood nematode.

For traps which objective is the detection of the pine wood nematode: the use of traps with sliding application is recommended, wet catches must be avoided, and the traps must be visited periodically, at least once a week.

**Placement**

The traps can be hung to tree branches, between two trees or supported by other structures. The distance between the trap and the trunk of the tree or its support must be at least equal to the length of the trap. The bottom of the trap must be located at about 50 cm above the ground. Traps must be easily accessible and placed in open and predominant places. It is recommended to be georeferenced.

**Location**

The location of the traps will be decided according to the presence of sensitive trees, taking into account that it will be avoided to put traps in areas where they can cause involuntary dispersion of the vector insect from potentially infested zones towards non-infested areas.

There are two different locations:

- Demarcated areas: a mesh of traps will be established in the perimeter of intensive surveillance areas, with the following intensity:
    - In closed forests, 1 trap/ha minimum;
    - In open forests, sparse or isolated trees, 1 trap/50 ha minimum.

- Sawmills and timber industries in demarcated areas: three traps in a triangular shape within the perimeter of the facilities.

**Types of traps and attractants**

May be used:

- Multi-trick trap (Lindgren funnel, 12 units)
- Vain type trap (Crosstrap), especially suitable for live captures.

It is recommended the traps to be anti-adherent and have draining containers to evacuate rainwater.

Attractants to use are:

- Aggregation pheromone: 2-undecyloxy 1-ethanol);
- Cairomonal substances: ipsenol and 2-methyl-3-buten-1-ol, α-pinene.

**Trapping calendar and trap visits**

The trap network will be maintained during the flight period of the *Monochamus* sp., at least from the earliest start of the catches (May) until the end of them (October). These tentative dates are adapted to local circumstances. Attractant is added every six weeks (according to manufacturer's specifications).

In order to avoid losing captured insect, traps will be grouped into itineraries and visited every one or two weeks. If the objective of the traps is to detect the presence of pine wood nematode, traps must be visited once a week.

**Particularities of trapping in Castilla y León**

- Traps located in areas of special interest;
- Traps placed in accessible areas: along roads and firewalls;
- Variable trap density;
- Always placed between two masts of 2.5 m, hanging from a rope, to improve the approach by flying insect and the diffusion of the attractants;
- Two types of non-adherent traps with a collector of at least 2 litres with a bottom net:
  - Crosstrap
  - Multi-funnel trap (always 12 funnels)
- Always weekly revisited;
- Analyses of all captured *Monochamus* sp.;
- Use of the pheromone-cairomone compound named Galloprotect 2D;
- Always dry traps without insecticide;
- Release of the natural enemies of bark beetles;
- Traps set from 1st April to 15 November.

## Nouvelle-Aquitaine: pine wood nematode surveillance plan and *Monochamus* sp. trapping strategy

By Hervé Jactel, INRA

**Current situation**

In Nouvelle-Aquitaine, traps used are the model Crosstrap with a draining collector, from ECONEX (42.17 €/trap), lured with the attractant Galloprotect plus, from SEDQ (44.65 €/lure). Each trap collector is treated as a single sample. In 2013 and 2014, a group of 10 vector insects were sampled from each trap. The tracheas were observed to detect the presence of pine wood nematode. Since 2015, all vector insects are analysed by DNA extraction using the real-time PCR method to detect the pine wood nematode into the insect.

In the region, surveillance is done on sites of risk: points of import of timber from infested countries, timber processing industries and roads used for timber transportation. A surveillance buffer 3 km wide is set around these sites. The traps are installed from May to November (during the flight season of the vector insect), visited every 10 days and moved seven times during the season if less than five *Monochamus* sp. are captured. This surveillance mission is done by FREDON, a professional agricultural union for the protection of crops against pathogens. The vector insect has been detected in the south of France, especially in the south-west.

In Nouvelle-Aquitaine region, the objectives for 2018 are to increase the number of traps to 50, increase the number of visits to the traps every 10 days (for a total of 17 visits per year) and maintain the traps near certain identified sites. However, these objectives may be superior to the laboratory capacity to treat the samples, will increase the costs and it is unknown the evolution of the number of captured *Monochamus* sp. In 2017, 850 samples were collected, of which 400 contained the vector insect, with an average of 40 of them. The 2017 trapping campaign had a cost of 31,000 € and the laboratory analyses summed 24,000 €. In Nouvelle-Aquitaine, *Monochamus galloprovincialis* is the only species of vector insect that has been found, however, in other French regions other *Monochamus* species are present.

In conclusion, in such a big region, targeted surveillance is necessary and the network of traps has to be optimised by using multi-insects attractants and through a better knowledge of the *Monochamus* sp. distribution.

**Proposal of a systematic grid of pheromone traps in areas free of pine wood nematode**

Capture-recapture information of flying *Monochamus* sp. would be used to create a model of its dispersal and then simulate the spread of a population of insects through the landscape. The results of this model could be used to optimise the systematic distribution of pheromone traps. Systematic location of traps can fill the gaps between traps located in risk sites. Both methods are complementary, because systematic traps capture the vector insect when it is already established in the territory.

Using a grip of 20 x 20 km to systematically distribute the pheromone traps, only 44 of them would be necessary to cover the whole Landes de Gascogne forest. Knowing that the closer to the focus of dispersion the higher is the probability to capture the vector insect, the focus could be approximately located by triangulation, also taking in consideration the landscape heterogeneity. Field agents could then be dispatched to exactly locate the source of the detected *Monochamus* sp.

For this proposal to be effective we need more than one beetle caught and carrying the pine wood nematode. So, this method would be more suitable in a scenario where pine wood nematode would have been introduced into the landscape in the previous two or three years.

The following parameters have been used to build the individual-based model to simulate the vector insect dispersal:

- Beetles are aged from 1 to 120 days (life expectancy);
- Beetles are immature for the first 20 days;
- The daily probability of flying is 0.45;
- Each day the beetles can change the flight direction and distance of flight;
- The flight distance is taken randomly within a kernel distribution (mean daily flight 2km);
- The direction of flight is taken randomly within a uniform distribution;
- 50% of the insects can fly at least 10 km away from its emergence point;
- After 12 days of starvation they die;
- They fly every second day (or feed).

Simulations were made in a theoretical landscape of non-fragmented maritime pine plantations.

## Discussion

Edmundo Sousa suggests that after the first detection in directed traps along roads, ports and timber processing industries, a dense systematic trap grid should be immediately established to set up control measures. He proposes a grid of 100 x 100 m d around the detection point over a 500 m radius buffer, plus the general grid on all territory previously proposed by Hervé Jactel.

Question from the participants: How far from the risk zone should we put the traps based on the behaviour of the insect?

Edmundo Sousa: Traps should be close to the first detection point, because if they are too far they may attract the insects into surrounding pine stands. Hervé Jactel agrees: the closer the better.

Luís Bonifácio: The problem is that if the first detection is a fresh emerged *Monochamus* sp., they are not mature and are not attracted by the pheromones. The beetles fly away and find the nearby pine stands. So, it is important to know the maturity of the arriving beetles. Because they can be mature and non-mature, the strategy should be double: for mature insects, closer traps are better; for non-mature insects, farther traps are more suitable, to capture them after the first dispersal.

Edmundo Sousa suspects that forest management in Spain maintains high levels of *Monochamus* sp. populations. Spanish authorities have to detect which are these management technics and reduce them to lower *Monochamus* sp. populations,

Jorge Casado: In Spain forest stands are closer to unmanaged forests, with less human interventions because forests are less productive and play a role mainly for biodiversity conservation. Therefore, there is more dead wood and dying trees, caused by overtopped trees.

Hervé Jactel adds that *Monochamus* sp. population can be advantaged because thinned trees and top of the crown are left on the ground after thinning or harvesting. His recommendation is not to leave thinned trees and tree tops in the forest.

Emmanuel Kersaudy: However, by collecting thinned trees and tree tops we would stop an important source of organic matter for the soil. This is a very important issue, as soils in the Landes de Gascogne forest are sandy and with a low content of organic matter.

Edmundo Sousa proposed that branches should be chipped and spread over the ground.

Question from the participants: Can pheromone traps be used to reduce vector insect populations?

Hervé Jactel: Pheromone traps will never work to reduce the population of an insect because the percentage of adults captured is very low compared to the whole population. They should be used only for monitoring and survey.

Pedro Miguel Naves: Traps should be in the same place for several years for long term survey and comparisons between years of population trends.

# Conclusions

## Wrap-up

The design of a network for monitoring the pine wilt disease would include:

1. Definition of **risk areas**: PLURIFOR should work more on the criteria used to define risk areas of *Monachamus* sp. spread in order to adjust the pheromone trap network and to guide directed surveillance and monitoring.
2. Remote sensing: PLURIFOR should investigate the use of **sketch mapping**, or visual inspection from helicopter or real-time drone images to detect symptomatic trees.
3. Remote sensing: we are currently struggling with the **bands** to be used. It is recommended to continue working to find which sensors we should the best ones.
4. Remote sensing should be **combined with other monitoring techniques** depending on the case, and produce **guidelines about how to combine all the techniques**.

Hervé Jactel proposed that in the future it would be useful to identify the origin of trapped *Monochamus* sp. based on genetic markers. This information could lead to know from where and how the vector insect has been introduced in an area, when it has not been detected along the pathway.

Christophe Orazio proposes to monitor in a more concerted way between Spain and France. Both countries should cover the main dissemination pathways in south-west Europe and use the same strategy in the regions covered by these pathways (mainly transport facilities for timber transportation).

# General workshop evaluation questionnaire

## Questions

### Workshop content

| | | Strongly disagree | Partially disagree | Partially agree | Strongly agree | Not applicable | No opinion |
|---|---|---|---|---|---|---|---|
| 1. | I was well informed about the objectives of this workshop and they were clear to me. | | | 2 | 3 | | |
| 2. | This workshop fulfilled my expectations. | | | 2 | 3 | | |
| 3. | The content is relevant to my job tasks concerning forest risks management. | | | 1 | 4 | | |
| 4. | The quality and depth of knowledge of this workshop were appropriate and represented state-of-the-art tools/technologies. | | | 1 | 4 | | |

### Workshop design

| | | Strongly disagree | Partially disagree | Partially agree | Strongly agree | Not applicable | No opinion |
|---|---|---|---|---|---|---|---|
| 5. | The workshop activities/case studies stimulated my learning. | | | 1 | 4 | | |
| 6. | The activities/case studies in this workshop gave me sufficient practice and feedback. | | | 3 | 2 | | |
| 7. | It was easy for me to understand the messages of the professionals/lecturers, they were good communicators. | | | 3 | 2 | | |
| 8. | The pace of this workshop was appropriate. | | | 3 | 2 | | |

### Workshop instructor/facilitator/lecturer

| | | Strongly disagree | Partially disagree | Partially agree | Strongly agree | Not applicable | No opinion |
|---|---|---|---|---|---|---|---|
| 9. | The instructor/facilitator/lecturer was well prepared. | | | | 5 | | |
| 10. | The instructor/facilitator/lecturer was helpful. | | | 1 | 4 | | |

## Workshop results

| | | Strongly disagree | Partially disagree | Partially agree | Strongly agree | Not applicable | No opinion |
|---|---|---|---|---|---|---|---|
| 11. | I accomplished the objectives of this workshop. | | | 2 | 3 | | |
| 12. | I would be able to use the tools that I learned in this workshop on my tasks concerning forest risks management. | | | 1 | 3 | 1 | |
| 13. | The exchanges with other professionals/instructors/lecturers were fruitful and will be useful for accomplishing my tasks concerning forest risks management. | | | 1 | 3 | | 1 |

## Self-paced delivery

| | | Strongly disagree | Partially disagree | Partially agree | Strongly agree | Not applicable | No opinion |
|---|---|---|---|---|---|---|---|
| 14. | The workshop was a good way for me to learn its content. | | | 2 | 3 | | |

# Improvements and values

**How would you improve this workshop?** (Check all that apply)

_2_Provide better information before the workshop.
___Clarify the workshop objectives.
___Reduce the content covered in the workshop.
_1_Increase the content covered in the workshop.
___Update the content covered in the workshop.
___Improve the instructional methods.
_1_Make workshop activities more stimulating.
___Improve workshop organization.
___Make the workshop less difficult.
___Make the workshop more difficult.
___Slow down the pace of the workshop.
___Speed up the pace of the workshop.
_1_Allot more time for the workshop.
___Shorten the time for the workshop.
___Improve the tests used in the workshop.
_2_Add (more) video to the workshop.

**What other improvements would you recommend in this workshop?** *The order of the answers is not relevant.*

Add a field trip.

Make the workshop more interactive.

**What is least valuable about this workshop?** *The order of the answers is not relevant.*

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**What is most valuable about this workshop?** *The order of the answers is not relevant.*

The opportunities to meet new people and to share information. The diversity of speakers.
The subject, the collaboration between countries.