



# Plant health risk and monitoring evaluation (PHRAME)

## Progress report for Year 1

### Results and milestones

#### Work Package 1: Vector surveys and biology

Sampling protocols for vectors were developed and surveys carried out in Portugal, Spain and Austria. Several species of *Monochamus* were captured, although *M. galloprovincialis* was dominant, reflecting the incidence of dying trees in Portugal. In Spain, the most frequent beetles captured were Cerambycidae (60.4% of the total of 131 specimens), with the most common being *Spondylis buprestoides* (67 specimens), *Monochamus galloprovincialis* (44), *Arhopalus fesus* (8) and *Rhagium bifasciatum* (6). There were also 67 specimens of Curculionidae and 17 specimens of Buprestidae. Although up to 16.6% of the Cerambycidae carried nematodes, mostly in the Aphelenchoididae, none carried *Bursaphelenchus* spp. Sampling in Austria indicated that on the trap trees only 1 *Monochamus galloprovincialis* *pistor*, 4 *Monochamus sartor* and 7 *Monochamus sutor* were collected.

A total of 123 adult *M. galloprovincialis* were caught in the two main study plots in Portugal during the period to early September, of which 53% were females and the rest males. Studies of vertical distribution in Portugal indicated that >75% of *M. galloprovincialis* were captured in the canopy. Of the beetles captured, up to 76% were shown to be carrying *Bursaphelenchus xylophilus*. Studies were also carried out on host tree preferences, emergence and flight pattern and population dynamics of the vectors. Emergence and flight periods lasted for over four months with peaks in June and July. Traps baited with turpentine (tree species specific) and ethanol were the most effective in capturing beetles. Bark and wood boring beetles of several different species belonging mainly to Cerambycidae, Scolytidae, Buprestidae and Curculionidae, totalling 816 insects, have been screened for the presence of PWN. Some of the scolytids (*Orthotomicus erosus* and *Hylurgus ligniperda*) have been found associated with *Bursaphelenchus* species but, other than *M. galloprovincialis*, no insects have been detected transporting *B. xylophilus*. *Monochamus* has been detected only on *P. pinaster*, which undoubtedly appears to be the main (if not the only) host in Portugal. Using mark-recapture techniques, a population density of 28 *M. galloprovincialis* per hectare was estimated.

#### Work Package 2: Survey of *Bursaphelenchus* spp., wilt expression and quantification of nematode-vector relationships

Surveys for *Bursaphelenchus* spp. were carried out in Austria, Portugal, Spain, France and UK. Apart from the known infested area, no *B. xylophilus* were found, although a number of other *Bursaphelenchus* spp. was recorded in each country, apart from the UK. Seven new strains of *Bursaphelenchus xylophilus* were added to the existing collections and trials on mass production were initiated. Fifteen strains of different *Bursaphelenchus* species were cultured on sporulating *Botrytis cinerea*. Morphological examination using light and scanning electron microscopy was used to confirm the identities of *Bursaphelenchus* spp.; results were linked to DNA techniques in Work Package 7. During the reporting period, 102 samples of at least 5 *Bursaphelenchus* and 2 *Aphelenchoides* species were obtained from Germany (31), Austria (29) and Switzerland (42). Of these 102 samples, 53 have been classified conclusively using DNA techniques.

#### Work Package 3: Collection of eco-climatic data, construction of sub-models and prediction of wilt-risk areas

Eco-climatic data, including maps, National Forestry Inventory, land use cover, forest fire outbreaks and hazards are being gathered. There were some delays in obtaining suitable data, some of which were due to the high costs of climatic data. However, the flow of information in Portugal is now good and will aid research in the next period. GIS databases are being constructed using the eco-climatic data obtained so far. Specific study plots to



assess the dynamics of wilt expression in the affected area of Portugal have been set up. Detailed silvicultural and site factor measurements are being gathered to extend the data and fit to the models being developed in Work Package 9. Preliminary results of *Monochamus galloprovincialis* distribution within the affected zone show a heterogeneous distribution pattern. Data from the pine plots show that PWN dying trees were localised close to previous year's infestations, and therefore contribute to the cluster distribution of pine wilt disease. Also, the number of infested dead trees inside the plots is similar to previous years' tendency of slight decrease (20-38% yearly) at Tróia and stable at Companhia das Lezírias.

#### **Work Package 4: Development of methods for accurate early detection of wilt expression in standing trees**

Extensive literature surveys have been used to assess potential methods for accurate early detection of wilt expression in standing trees. Tests with instruments to measure water/nutrient flow, electrical resistance and sound were evaluated in both the laboratory (acoustic tests only carried out in the laboratory) and the field. Tests on oleoresin flow in Portugal showed that the method has promise, although it is important to use the correct technique. Measurements of the electrical conductivity conducted in September on all the trees of the experimental plots of Tróia and Companhia das Lezírias gave expected significant differences between healthy and dying or dead trees

#### **Work Package 5: Establishment of nematode cultures, mass production and cryopreservation**

Cultures of *B. xylophilus* have been established and include two populations from Portugal, one from China, one from Canada and several other *Bursaphelenchus* spp. from China, Italy, Germany and Turkey. In addition to the existing collection of living *Bursaphelenchus* species of Partner 2, seven new strains of *Bursaphelenchus xylophilus* from the USA (2), Portugal (2) and Korea (3) have been obtained. Initial trials on mass production of *B. xylophilus* on agar plates both with sporulating and non-sporulating *Botrytis cinerea* have been carried out.

#### **Work Package 6: Evaluation of relative pathogenicity of Portuguese *Bursaphelenchus xylophilus* in European pines and model construction**

Protocols for inoculation of seedling trees were developed and tests carried out on 13 conifer species (9 of *Pinus*, 2 of *Larix*, 1 each of *Abies* and *Picea*). They were inoculated, in a randomised block design, with *B. xylophilus* strains from Portugal, China and USA. Plants have been evaluated in relation to development of symptoms up to tree death and presence of nematodes after 4 weeks and at the date of trees death. Reaction of pine species differs between the species. Up to 100% of seedlings of *P. nigra*, *P. cembra*, *P. sylvestris*, *P. strobus*, and *P. pinaster* died within 3 months. For *P. pinea* and *P. halepensis* up to 60% and 15% respectively died. *Abies alba* and *Picea abies* showed almost no symptoms within the 3 months period whereas *Larix decidua* was the most susceptible tested seedling species. Nematode multiplication differed between tested trees to a great extent. No significant difference could be observed between the three nematode strains tested. Different methods for inoculation of nematodes into branches to assess growth of *B. xylophilus* were assessed in Portugal. Results from this study indicate that higher numbers of nematodes are produced with smaller inoculation loads. *P. pinaster* is the best host for nematode reproduction.

#### **Work Package 7: Use of *B. xylophilus* isolates to define pathways for entry to Portugal and construction of pathway sub-model**

Reference isolates of *B. xylophilus* have been obtained from China, Canada, Japan, Korea, Portugal and USA and further work to obtain isolates from Mexico and Taiwan is underway. Identification of nematode samples using ITS-RFLP has resulted in 53 out of 102 samples being classified conclusively. Twenty-three samples were *B. mucronatus* with European and East Asian characteristics in ITS-RFLP profiles. Good progress has been made in identifying DNA markers for phylogeny analysis to aid assessments of the origins of different isolates of nematodes.



### **Work Package 8: New literature review and use of phenology data to construct sub-model**

Revision of papers and other information on pinewood nematode has been completed. Using the bibliographic database Endnote®, 1230 references have been filed and made available to the Consortium. Progress is being made in obtaining electronic copies of papers for ease of transfer using PDF file formats.

### **Work Package 9: Develop GIS and CLIMEX models and incorporate into new PRA process model to be tested and verified using *Bursaphelenchus xylophilus* and *Monochamus* spp. as test organisms**

A secure website to allow data transfer and confidential information flow between partners has been set up. This is accessed by username and password and has proved useful in transfer of large documents. The Consortium website describing the work of the project and providing links to the websites of the individual partners has been set up (<http://www.forestresearch.gov.uk/fr/INFD-63KGEF>). This will be updated at frequent intervals. Work on production of models has concentrated on evapotranspiration models to aid prediction of tree growth parameters by use of a range of entry variables, including solar radiation, precipitation, ambient CO<sub>2</sub>, rain throughfall, soil water uptake, drainage and runoff. The model accounts for photosynthesis and water movement by transpiration and evaporation. The process-based approach is now being adapted to the pinewood nematode-host tree interaction in Portugal and elsewhere.

### **Benefits and beneficiaries**

- Rapidly increasing knowledge of the biology, ecology and dynamics of *Bursaphelenchus xylophilus* and its vectors in the genus *Monochamus*. Progress has been extremely good and there is already much increased information on the biology of *Monochamus galloprovincialis* in Portugal and its capacity to carry *B. xylophilus* between trees.
- Pathogenicity and expression of wilt symptoms in susceptible trees is now being studied in great details, aided by the refined DNA techniques to provide certainty in the identities of species and races of nematodes.
- Techniques to assess early expression of wilt symptoms will aid both survey and experimental work in the affected zone of Portugal and elsewhere.
- Improved literature collation and development of improved means of electronic communication will help to disseminate results of the work of the Consortium both directly and through its website.
- Models to link tree growth processes to environmental parameters and climatic variables are being developed and will be applied to the pinewood nematode system.

Beneficiaries at this stage are mainly the scientific community through contacts by members of the Consortium. An important additional benefit is good interaction with the Portuguese authorities who are fully aware of the progress of the work and are contributing to the information flow in both directions.

### **Future Actions (if applicable)**

Work is progressing as planned. However, the Consortium is also considering a series of specific experiments in the field in Portugal that will add to the work originally conceived. Presentations to conferences, including the International Plant Protection Congress in China, are planned.



## Further information

### List of partners

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### Website

<http://www.forestresearch.gov.uk/fr/INFD-63KGEF>