



# Plant health risk and monitoring evaluation (PHRAME)

## Progress report for Year 2

### Results and milestones

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

Using standard protocols from year 1, vector surveys were carried out in Austria, Germany, Portugal and Spain. Overall, *M. galloprovincialis* was again dominant, although *M. sartor* and *M. sutor* were also captured in Austria but only in trap logs, not in cross-vane traps. Other families of Coleoptera, including Scolytidae, Cerambycidae (other than *Monochamus* spp.), Curculionidae, Buprestidae, Cleridae and Opionidae were also found with differing frequencies (especially in Spain). Adult emergence and flight periods of *M. galloprovincialis* were similar to 2003 (peak in July in both Germany and Portugal). Work on trap design and lure components in Portugal confirmed that the transparent cross-vane trap was best and that placing ethanol and turpentine in separate vials was more effective than mixing them directly.

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

Further surveys for *Bursaphelenchus* spp. were carried out in Portugal, Spain and France. *B. xylophilus* was found only in the known infested area of Portugal. However, a number of other *Bursaphelenchus* spp. was recorded in each country, e.g. *B. piniphilus*, *B. eggersi*, *B. fungivorus* and *B. sexdentati* for the first time in Spain. *B. hellenicus*, *B. hylobianum*, *B. leoni*, *B. pinophilus*, *B. sexdentati*, *B. tusciae*, *B. teratospicularis*, *B. xylophilus* and an unidentified *Bursaphelenchus* species were found in Portugal.

Fifteen strains of *Bursaphelenchus* were maintained by Partner 2 and mass produced as necessary on non-sporulating *Botrytis cinerea* cultures. *B. mucronatus* was the most common species in France and some new isolates of *B. mucronatus* and *B. glochis* were added to the collection. Molecular analysis of Portuguese isolates indicated that the previously identified *B. hofmanni* was probably *B. pinasteri* s.cf. Screening of non-*Monochamus* for presence of nematodes indicated that none can be regarded as effective vectors (only one specimen out of 141 had a low number of *B. xylophilus* present). Studies with *M. galloprovincialis* indicated that nematodes continue to be transmitted in maturation feeding throughout their lives with the peak being between 2 and 6 weeks after adult emergence from logs.

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

Portuguese data on temperatures (a range of parameters), soil characteristics, fire outbreaks, tree felling, incidence of *B. xylophilus*, evapotranspiration as well as satellite imagery have been gathered by Partner 3. These data are being collated and included in models by Partner 1 under Work Package 9. Maritime pine (*P. pinaster*) in the two study plots in Portugal (Tróia and Companhia das Lezírias) were monitored; 1 and 4 dead pines were recorded respectively. A study of possible *Monochamus* attack in still attached lower branches of trees indicated that they were caused primarily by carpenter bees (Hymenoptera: Anthophoridae).

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

Further experiments on electrical conductivity were carried out by Partner 5 with Partner 8; these were done in Portugal at Tróia and Companhia das Lezírias. The main differences were found between healthy smaller trees and all other trees. In the cases of trees that eventually died from pine wilt, there were no significant differences until visual symptoms were already strong. Other experiments on resin flow indicated that this ceased well before visual crown symptoms were noticed.

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

Strains of *B. xylophilus* from USA (2), Portugal (2) and Korea (3) were maintained and included in the permanent collection of Partner 2. Mass production of nematodes for use in Work Package 6 was carried out. A literature review was carried out ready for work on cryopreservation of nematodes. In Spain, dauer larvae carried by *Monochamus* were cultured and some of these have not been identified to species. Cultures of Portuguese *B. hylobianum*, *B. sexdentati*, *B. pinastri* s. cf and *B. xylophilus* are being maintained on non-sporulating *B. cinerea* and *Monilinia fructicola*. Partner 7 carried out comparisons of techniques for cryopreservation. Glycerol was used as the cryoprotectant agent and a two-step freezing procedure was tested. More juveniles than adult stages survived after freezing. Preliminary storage experiments demonstrated encouraging rates of viability and reproduction of two *B. xylophilus* and two *B. mucronatus* isolates after 15 and 30 days of storage in liquid nitrogen.

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

Partner 2 carried out three inoculation trials in climate chambers (1210 seedlings) to test relative pathogenicities of the Portuguese strain of *B. xylophilus*. Parameters included (1) inoculation load, (2) distribution and migration of nematodes in the plant and (3) the effects of temperature on host susceptibility of *Pinus sylvestris*, *Larix decidua* and *Picea abies*. Results are still being evaluated. Partner 8 carried out two Portuguese *B. xylophilus* inoculation trials with 4 year old seedlings of *P. pinaster* and *P. sylvestris* in outdoor conditions. Symptoms were classified according to severity at weekly intervals and, when dead, were split into sections for enumeration of nematodes. Preliminary results indicated that *P. pinaster* showed symptoms and died faster than *P. sylvestris*. Nematodes had migrated to most parts of the dead plants but remained at higher densities near the inoculation points. This work continues to provide quantitative information on the rapid early distribution of nematodes when introduced into plants by vector insects and this information is now being incorporated into the process-models being developed by Partner 1 under Work Package 9.

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

Apart from the 26 *B. xylophilus* isolates described in the last reporting period, no further strains have been obtained. Culturing of existing strains continues, although one strain from Taiwan could not be retained because of contamination. Partner 6 carried out genomic DNA isolation of new strains of nematodes from Korea and a series of PCR experiments using different primers was conducted with a total of 30 *B. xylophilus* populations in order to identify DNA markers for future use. All *B. xylophilus* populations tested grouped closely together, whereas other *Bursaphelenchus* species were in outgroups with high genetic distances. Partner 7 continued work on satellite DNA-based procedures for specific identification of single *B. xylophilus* specimens. Both squash-blot and PCR amplification techniques are now fully operational. Positive identification of a range of *B. xylophilus* isolates has been successfully performed. RFLP analysis of genomic DNA from *B. xylophilus* isolates from Japan, Canada and the USA using restriction endonucleases that do not cut within the monomeric unit of the satellite DNA revealed a significant intraspecific polymorphism in the range of 2 to 10 kilobases. Such variability in the distribution of the satellite DNA repeats in the genome of the nematode isolates could be used to analyse how they are related, but this strategy needs too much biological material to be practical for wider use. In order to overcome this limitation, direct sequencing of satellite DNA monomers from a subset of *Bursaphelenchus* species and isolates has been performed. Preliminary experiments showed an unexpectedly high level of polymorphism between monomers, at both the interspecific and the intraspecific levels, suggesting that sequence analysis could be a powerful tool to aid assessment of the origins of the different isolates of nematodes and thus to provide accurate elements for pathway analysis of *B. xylophilus* in Portugal.



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

Further additions of papers and other information on pinewood nematode have been made to the bibliographic database Endnote®. Many of these are now available electronically using PDF file formats. These are being shared between partners on the secure Consortium website.

**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**

Considerable effort has gone into development of a process-based approach to understanding the mechanisms that lead to wilt expression in trees where nematodes have been introduced during maturation feeding. Work has concentrated on the pathways for nematode movement within the tree and the differentiation of physical destruction and blockage of cells (especially the xylem) from possible defensive reactions within the tree induced by the presence of *B. xylophilus*. The principles of a model were presented at an IUFRO conference in New Zealand and are being developed into a scientific paper for publication. The core components of the model are quantification of the discrepancy between Potential Evapotranspiration, PEt, in a plant (essentially maximum theoretical water uptake and usage) and Actual Evapotranspiration, AEt, (actual water utilisation linked to site conditions, tree health, presence of nematode, etc.). All plants show some shortfall in AEt compared with PEt but the ability to control and recover from this is compromised by nematode activity which caused embolism in the xylem tracheids. Using the models, work is now commencing on predicting how particular tree species react under different climatic and soil conditions. Discussions on specific experiments to test hypotheses in the field in Portugal took place between Partners 1, 2, 5 and 8 and plans are being prepared for field experiments to be carried out in 2006.

**Benefits and beneficiaries**

- Rapidly increasing knowledge of the biology, ecology and dynamics of *Bursaphelenchus xylophilus* and its vectors in the genus *Monochamus*. Much confirmatory and new information has been gathered during this period and is being used to design experiments and field observations for 2005/6.
- Aspects of pathogenicity and movement of nematodes within infested plants are providing quantitative data for inclusion in the main risk models.
- Identification of strains of nematodes through a range of molecular techniques is offering the prospect of detailed pathway analysis to aid interpretation of the situation in Portugal and to add to the main risk models.
- Literature and knowledge gathering continues and is being disseminated in a number of written and oral presentations in journals and conferences.
- Process Based 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.



## Further information

### List of partners

1	H. F. Evans (Coordinator)	Forest Research, UK
2	T. Schröder	Federal Biological Research Centre for Agriculture and Forestry, BBA/AG, Germany
3	M. M. Mota	Departamento de Biologia, Universidade de Évora, Portugal
4	M. Arias	Departamento de Agroecologia, CSIC, Spain
5	C. Tomiczek	Institute of Forest Protection, Austria
6	W. Burgermeister	Federal Biological Research Centre for Agriculture and Forestry, BBA/PS, Germany
7	P. Castagnone-Sereno	Interactions Plantes-Micro-organismes et Santé Végétale, INRA, France
8	E. Sousa	Instituto Nacional de Investigação Agrária, Portugal

### Website

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