

	EUROPEAN COMMISSION RESEARCH AND INNOVATION DG	Periodic Report
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Project No: 265483

Project Acronym: REPHRAME

Project Full Name: Development of improved methods for detection, control and eradication of pine wood nematode in support of EU Plant Health policy

Periodic Report

Period covered: from 01/03/2011 to 31/08/2012

Start date of project: 01/03/2011

Project coordinator name:

Dr. Hugh Evans

Version: 1

Date of preparation: 19/12/2012

Date of submission (SESAM): 19/12/2012

Project coordinator organisation name:

FORESTRY COMMISSION RESEARCH AGENCY

Periodic Report

PROJECT PERIODIC REPORT

Grant Agreement number:	265483
Project acronym:	REPHRAME
Project title:	Development of improved methods for detection, control and eradication of pine wood nematode in support of EU Plant Health policy
Funding Scheme:	FP7-CP-FP
Date of latest version of Annex I against which the assessment will be made:	27/07/2011
Period number:	1st
Period covered - start date:	01/03/2011
Period covered - end date:	31/08/2012
Name of the scientific representative of the project's coordinator and organisation:	Dr. Hugh Evans FORESTRY COMMISSION RESEARCH AGENCY
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Declaration by the scientific representative of the project coordinator (1)

I, Dr. Hugh Evans FORESTRY COMMISSION RESEARCH AGENCY , as scientific representative of the coordinator of the project REPHRAME and in line with the obligations as stated in Article II.2.3 of the Grant Agreement declare that:

The project has achieved most of its objectives and technical goals for the period with relatively minor deviations.

The attached periodic report represents an accurate description of the work carried out in this project for this reporting period.

The public website is up to date.

To my best knowledge, the financial statements which are being submitted as part of this report are in line with the actual work carried out and are consistent with the report on the resources used for the project (section 6) and if applicable with the certificate on financial statement.

All beneficiaries, in particular non-profit public bodies, secondary and higher education establishments, research organisations and SMEs, have declared to have verified their legal status. Any changes have been reported under section 5 (Project Management) in accordance with Article II.3.f of the Grant Agreement.

Name	Dr. Hugh Evans FORESTRY COMMISSION RESEARCH AGENCY
Date	19/12/2012

This declaration was visaed electronically byHugh EVANS(ECAS user name nevanshu) on 19/12/2012

1. Publishable summary

Summary description of project context and objectives

Europe's pine forests are a valuable economic, social and environmental resource under threat from the introduction of the pine wood nematode (PWN), *Bursaphelenchus xylophilus*. Significantly, PWN is not a pest in its native North America, which is linked to both tolerance in native conifers and to unsuitable environmental conditions for wilt over much of its range there. Since the arrival of PWN in Portugal, the native maritime pine, *Pinus pinaster*, has proved to be extremely susceptible, with PWN being spread by the local longhorn beetle *Monochamus galloprovincialis*. Previous studies have shown that PWN could spread throughout the Iberian Peninsula and beyond, making it a major threat to European forests.

Effective containment and local eradication of PWN demands a detailed understanding of the behaviour and dynamics of the nematode and its vector insects in infested trees, especially because delayed onset of symptoms (latency) reduces survey accuracy and can compromise containment strategies. Research in REPHRAME concentrates on:

- vector dispersal capacity;
- improved ways to monitor and reduce populations using synthetic chemical lures;
- the potential for PWN transfer between trees in the absence of the *Monochamus* spp. vectors,
- the potential for the introduction of conifers resistant to PWN.
- extending the capability of existing models to identify the risk posed by PWN to the rest of Europe under current and future climates.
- synthesising the results of the project will be into an on-line toolkit for end users.
- extensive dissemination activities to ensure the uptake and application of results across the EU and world-wide.

Description of work performed and main results

WP2 -Behaviour and dynamics of PWN in infested trees

- Different bacterial communities are associated with *Bursaphelenchus xylophilus*, mainly belonging to Enterobacteriaceae, Burkholderiaceae and Pseudomonadaceae family, and appear to augment the deleterious effects of *B. xylophilus*;
- By producing ACC deaminase, wild-type *Pseudomonas putida* UW4 bacterium inoculated into pine seedlings and later inoculated with PWN, acts indirectly as a biological control agent, decreasing pine wilt disease development.
- In China, the community compositions of the PWN-associated fungi indicate that the more severe the region is infested, the higher the frequency of the unknown fungus *S. sp1*. This fungus produces a chemical compound which promotes growth and reproduction of both nematodes and vector beetles.
- Species-specific primers for *B. xylophilus* (PCR-ITS) have been developed that enable accurate identification of *Bursaphelenchus* spp, especially *B. xylophilus* and *B. mucronatus*, and is suitable for use in ground woody tissues and in macerated insect tissue.

WP3 – Assessing phenology and dispersal capacities of PWN vectors

- In nature, *M. galloprovincialis* has only one annual generation, with adults emerging from May onwards throughout the summer.
- Immature *Monochamus* adults do not respond to pheromone-kairomone lures
- Although most mature beetles fly only short distances, some are able to disperse further (for at least 990m and 1.5 km), especially if they are in an area deprived of any living pine trees.
- *Monochamus* spp adults have been collected in Portugal, Spain, France and other locations to analyse population variation and gene flow. Preliminary studies did not find a pattern of between genetic structure and geography, although further studies are required.

WP4: Development of new methods for monitoring and control of *Monochamus* spp and PWN based on vector trapping

- Results in Spain, Portugal and France have confirmed the effectiveness of the developed pheromone-kairomone lure (*M. galloprovincialis* pheromone + ipsenol + methyl-buthenol), commercialized as GALLOPROTECT 2D (SEDQ), in trapping both sexes of *M. galloprovincialis*.
- However, these lures attract only adults older than two weeks and further work is needed to

determine whether younger adults can be attracted.

- The greater capture efficiency of Teflon®-coated multifunnel and crossvane traps, indicates they are suitable for monitoring and for mass trapping of *M. galloprovincialis*. Trapping of live adults is enhanced by the use of the extended collector cup in the multifunnel trap.
- *M. sutor* males produce an aggregation pheromone identified as 2-undecyloxy-1-ethanol, the same alcohol produced by *M. galloprovincialis* and *M. alternatus*.
- Results from Austria show that *M. sartor* responded to 2-undecyloxy-1-ethanol, the *M. galloprovincialis* and *M. sutor* pheromone, combined with bark beetle and host kairomones (Galloprotect 2D + GPlus).

WP5 Determine risk of non-vector spread of PWN through various pathways to healthy forests

- Due to problems with grant allocation and delivery of experimental equipment the planned experiments have been postponed to 2012 or 2013. This causes 1-2 years delay in scheduled deliveries. However, several sub-objectives have so far been successfully addressed, so experiments will start in 2012-2013.
- For field experiments to assess spread of PWN from tree to tree by root grafting, field sites in Portugal have been selected but the experiments were delayed due to problems in equipment delivery.
- Progress in setting up laboratory experiments to study movement of PWN through root grafts has been hampered by unusually hot weather affecting greenhouse plants. The work will now commence in 2013.
- A set of 18 polymorphic loci has been characterized, that are usable on single *B. xylophilus* nematodes in three complementary multiplex reactions. In addition, among the 94 original loci, 8 produced an amplification pattern differentiating *B. xylophilus* from *B. mucronatus*. Further testing of *B. leoni* and *B. tusciae* confirmed the specificity of the amplification pattern obtained with *B. xylophilus*.
- A total of 349 MspI satellite DNA repeats from thirty-five isolates of *B. xylophilus* have been generated and are now available for in-depth studies of the variability of this satellite DNA family which will aid in assessing pathways of entry of PWN to Portugal.

WP6 - Host tree resistance to PWN and its vectors for future planting

- An ITS-PCR based method applied directly to wood from adult maritime pine trees was tested and compared with a standard morphological identification method. PCR provided more rapid and accurate results and will be used in tree breeding for resistance/tolerance to PWD.
- Hybridization between *P. pinaster* x *P. halepensis* was carried out in Portugal. The trees were young and medium age, parasite free, vigorous and with abundant flowers.
- Techniques for inoculation of test trees with PWN have been developed to assess relative susceptibility to the nematode.
- 34 cDNA libraries are being sequenced using ultra high throughput pyrosequencing to characterize transcripts and putative candidate genes involved in tree responses to biotic stress related to PWN infestation.

WP7: Prediction of pine wilt expression across eco-climatic zones, taking account of latency

- The process-based models (Evapo-transpiration – ETP and Forest Growth – FG) have been assessed in detail and a sensitivity analysis of the importance of different parameters in relation to predicted wilt expression is being carried out.
- Currently, simulations have been run using daily data over 3 years. Realistically, this detail of input would be impossible for a general user to obtain and work is progressing to determine what are the minimal inputs needed to provide a realistic simulation to develop simplified models, including latency parameters, for wider use.
- Until March 2012: bioclimatic modelling was based on rapid field assessment and data from national authorities (positive and negative points for PWD) with clear correlations between climate variables and PWN infestations.
- After April 2012: field sampling has been carried out to refine quantitative data regarding the spread of PWD in Portugal (more than 100 field samples so far). These are now being analysed.
- Remote sensing of PWD is being carried out based on Google earth imagery, supported by field verification. This shows promise in relation to determining centres of high nematode activity.
- Models of the spread of PWN are being developed based on local spread (adult flight activity from flight mills and field data) and human-assisted long distance spread (geographic data and genetic

analysis of PWN populations)

WP8: EU and international cooperation and collaboration

- Within the REPHRAME consortium, knowledge gathering, collaborative research and exchange of expertise has been developed among beneficiaries and by expanding collaborative networks internationally.
- Contacts of interest have been maintained with several European and international quarantine and research laboratories, on both scientific and technical issues related with the pinewood nematode and pine wilt disease. Use has been made of the EUMAINE MSc programme.

WP9: Synthesis and development of PWN Tool Kit for monitoring and management of PWN

- Based on progress in the WP2, 4 and 7 in particular, the outline structure of the inter-relationships that will form the basis of the PTK interface has been developed.

WP10: Stakeholder Engagement & Dissemination

- The Consortium website is currently at www.forestry.gov.uk/fr/rephrame but a stand-alone website at www.rephrame.eu was launched in November 2012.
- A project leaflet has been published in printed and electronic form in October 2012.
- The Stakeholder Observer Group met in October 2012 in Portugal.

Expected final results and potential impacts

Work Packages 2-7 are making good progress, despite the delays that have affected WP5 in particular, to address the critical issues identified in the call text. There is a constantly improving understanding of the interactions between PWN and its host trees, taking account of vector and non-vector transmission routes and integrating with environmental and tree/PWN genetic drivers that could lead to wilt expression. This knowledge is being enhanced by international collaboration to extend data gathering and ensure maximum synergy through sharing of existing and new data (WP8).

WP9 is now accelerating to synthesise and use existing and new information to develop the PWN Tool Kit (PTK), which will integrate the strong science being delivered in the other work packages.

WP10 has had a relatively slow start, but is also accelerating activity through its web and other portals, including the Stakeholder Observer Group. As indicated in the main report, publications in peer-reviewed journals are being generated, and the work of REPHRAME publicised in conferences and workshops. Taken together, the final results will address critical issues and be delivered through a range of outputs, but particularly through the PTK and final conference of the project.

REPHRAME will have extensive positive impacts in socio-economic terms by providing substantial improvements to risk assessment and management of the PWN threat to European and global conifer forests and woodlands. As already demonstrated, this will be based on robust science-based methodologies underpinning improved management strategies. This will decrease uncertainty about specific factors that are critical to survey and management of PWN and its vectors.

Project public website address:

www.rephrame.eu

2. Core of the report

Project objectives, Work progress and achievements, and project management during the period

The Project Summary Pdf document contains the core of the report.

3. Deliverables and milestones tables

Deliverables (excluding the periodic and final reports)										
Del. no.	Deliverable name	Version	WP no.	Lead beneficiary	Nature	Dissemination level	Delivery date from Annex I (proj month)	Actual / Forecast delivery date	Status	Comments
1	Minutes of meetings of Management Committee and consortium	1.0	1	FORESTRY COMMISSION RESEARCH AGENCY	Report	PU	36	26/06/2012	Submitted	
2	Consortium Agreement signed	1.0	1	FORESTRY COMMISSION RESEARCH AGENCY	Report	PP	2	22/06/2012	Submitted	
3	Task and milestone reports	0.0	1	FORESTRY COMMISSION RESEARCH AGENCY	Report	PU	36	28/02/2014	Not submitted	
1	Factors governing association of PWN with vector beetles	0.0	2	UNIVERSIDADE DE EVORA	Report	PU	24	28/02/2013	Not submitted	
2	Factors affecting departure of PWN from vector beetles	0.0	2	UNIVERSIDADE DE EVORA	Report	PU	24	28/02/2013	Not submitted	
3	Pathogenicity of PWN in host tree species	0.0	2	UNIVERSIDADE DE EVORA	Report	PU	36	28/02/2014	Not submitted	
4	Methods to detect PWN in trees	0.0	2	UNIVERSIDADE DE EVORA	Report	PU	36	28/02/2014	Not submitted	
1	Vector flight capacity related to physiology	0.0	3	INSTITUTO NACIONAL DE RECURSOS BIOLOGICOS I.P. INRB	Report	PU	24	28/02/2013	Not submitted	
2	Vector dispersal related to forest condition	0.0	3	INSTITUTO NACIONAL DE RECURSOS BIOLOGICOS I.P. INRB	Report	PU	24	28/02/2013	Not submitted	

3	Vector dispersal related to population genetics	0.0	3	INSTITUTO NACIONAL DE RECURSOS BIOLOGICOS I.P. INRB	Report	PU	36	28/02/2014	Not submitted	
4	Climate influences on vector dispersal	0.0	3	INSTITUTO NACIONAL DE RECURSOS BIOLOGICOS I.P. INRB	Report	PU	24	28/02/2013	Not submitted	
1	Lure for <i>M. galloprovincialis</i>	0.0	4	UNIVERSIDAD DE VALLADOLID	Report	PU	24	28/02/2013	Not submitted	
2	Development of traps for monitoring & control	1.0	4	UNIVERSIDAD DE VALLADOLID	Report	PU	12	26/06/2012	Submitted	
3	Effectiveness of mass trapping for vector control	0.0	4	UNIVERSIDAD DE VALLADOLID	Report	PU	36	28/02/2014	Not submitted	
4	Development of lures for other <i>Monoctonus</i> spp.	0.0	4	UNIVERSIDAD DE VALLADOLID	Report	PU	36	28/02/2014	Not submitted	
1	Distribution of PWN in wood and wood chips	0.0	5	JULIUS KUHN INSTITUT BUNDESFORSCHUNGSINSTITUT FUR KULTURPFLANZEN	Report	PU	24	28/02/2013	Not submitted	
2	Transmission of PWN to trees with wood chips/bark	0.0	5	JULIUS KUHN INSTITUT BUNDESFORSCHUNGSINSTITUT FUR KULTURPFLANZEN	Report	PU	36	28/02/2014	Not submitted	
3	Direct tree to tree transmission of PWN	0.0	5	JULIUS KUHN INSTITUT BUNDESFORSCHUNGSINSTITUT FUR KULTURPFLANZEN	Report	PU	24	28/02/2013	Not submitted	
4	Wood to wood transmission of PWN in wood packaging	0.0	5	JULIUS KUHN INSTITUT BUNDESFORSCHUNGSINSTITUT FUR KULTURPFLANZEN	Report	PU	36	28/02/2014	Not submitted	

			KULTURPFLANZEN							
5	Microsatellite markers for PWN identification	1.0	5	JULIUS KUHN INSTITUT BUNDESFORSCHUNGSINSTITUT FÜR KULTURPFLANZEN	Report	PU	12	26/06/2012	Submitted	
6	PWN genetic diversity as indicators of invasion history	0.0	5	JULIUS KUHN INSTITUT BUNDESFORSCHUNGSINSTITUT FÜR KULTURPFLANZEN	Report	PU	36	28/02/2014	Not submitted	
1	Susceptibility of Pinus sylvestris provenances to PWN	0.0	6	INSTITUTO NACIONAL DE RECURSOS BIOLOGICOS I.P. INRB	Report	PU	24	28/02/2013	Not submitted	
2	Construction of cDNA libraries from sensitive and resistant genotypes of Pinus	1.0	6	INSTITUTO NACIONAL DE RECURSOS BIOLOGICOS I.P. INRB	Report	PU	12	08/11/2012	Submitted	
3	Identification of PWN resistance genes in pines	0.0	6	INSTITUTO NACIONAL DE RECURSOS BIOLOGICOS I.P. INRB	Report	PU	36	28/02/2014	Not submitted	
4	Resistance of pines to feeding by Monochamus	0.0	6	INSTITUTO NACIONAL DE RECURSOS BIOLOGICOS I.P. INRB	Report	PU	24	28/02/2013	Not submitted	
5	Host preferences for Monochamus oviposition	0.0	6	INSTITUTO NACIONAL DE RECURSOS BIOLOGICOS I.P. INRB	Report	PU	36	28/02/2014	Not submitted	
6	Hybrid progenies with different tolerance/resistance to the PWN	1.0	6	INSTITUTO NACIONAL DE RECURSOS BIOLOGICOS I.P. INRB	Report	PU	18	08/11/2012	Submitted	

7	Tree species mosaics to reduce PWN impact	0.0	6	INSTITUTO NACIONAL DE RECURSOS BIOLÓGICOS I.P. INRB	Report	PU	36	28/02/2014	Not submitted	
1	Refinement of core model	0.0	7	INSTITUT NATIONAL DE LA RECHERCHE AGRONOMIQUE	Report	PU	35	31/01/2014	Not submitted	
2	Field verification of process model	0.0	7	INSTITUT NATIONAL DE LA RECHERCHE AGRONOMIQUE	Report	PU	36	28/02/2014	Not submitted	
3	Latency sub-model	0.0	7	INSTITUT NATIONAL DE LA RECHERCHE AGRONOMIQUE	Report	PU	35	31/01/2014	Not submitted	
4	Analysis of PWN history in Portugal	0.0	7	INSTITUT NATIONAL DE LA RECHERCHE AGRONOMIQUE	Report	PU	33	30/11/2013	Not submitted	
5	PWN spread model	0.0	7	INSTITUT NATIONAL DE LA RECHERCHE AGRONOMIQUE	Report	PU	35	31/01/2014	Not submitted	
1	Knowledge from previous EU projects	1.0	8	UNIVERSIDADE DE EVORA	Report	PU	12	26/06/2012	Submitted	
2	Interaction with EU/International projects	0.0	8	UNIVERSIDADE DE EVORA	Report	PU	36	28/02/2014	Not submitted	
1	PTK interface	1.0	9	FORESTRY COMMISSION RESEARCH AGENCY	Report	PU	12	08/11/2012	Submitted	
2	Beta testing of PTK modules	0.0	9	FORESTRY COMMISSION RESEARCH	Report	PU	33	30/11/2013	Not submitted	

				AGENCY						
3	Launch of PTK	0.0	9	FORESTRY COMMISSION RESEARCH AGENCY	Report	PU	35	31/01/2014	Not submitted	
1	REPHRAME website launch & maintenance	1.0	10	FORESTRY COMMISSION RESEARCH AGENCY	Other	PU	2	26/06/2012	Submitted	
2	Project leaflet	1.0	10	FORESTRY COMMISSION RESEARCH AGENCY	Other	PU	4	18/10/2012	Submitted	
3	SOG minutes	1.0	10	FORESTRY COMMISSION RESEARCH AGENCY	Report	PU	12	18/10/2012	Submitted	
4	Themed workshop 1	0.0	10	FORESTRY COMMISSION RESEARCH AGENCY	Other	PU	22	31/12/2012	Not submitted	
5	Themed workshop 2	0.0	10	FORESTRY COMMISSION RESEARCH AGENCY	Other	PU	34	31/12/2013	Not submitted	
6	International Conference on PWN	0.0	10	FORESTRY COMMISSION RESEARCH AGENCY	Report	PU	36	28/02/2014	Not submitted	
7	Plan for use & dissemination of foreground	0.0	10	FORESTRY COMMISSION RESEARCH AGENCY	Report	PU	36	28/02/2014	Not submitted	
8	Awareness & wider societal implications	0.0	10	FORESTRY COMMISSION RESEARCH AGENCY	Report	PU	36	28/02/2014	Not submitted	

Milestones

Milestone no.	Milestone name	Work package no	Lead beneficiary	Delivery date from Annex I	Achieved Yes/No	Actual / Forecast achievement date	Comments
1	Consortium Agreement completed	1	1	01/05/2011	Yes	01/05/2011	Subsequently modified with updated version
2	Mid term report delivered	1	1	31/08/2012	Yes	19/12/2012	Final editing after mid-term meeting in October

4. Explanation of the use of the resources

The **explanation on the use of resources** was removed from the scientific periodic reports in SESAM. These details now have to be entered in the cost statement forms in FORCE instead.

Attachments	
Grant Agreement number:	265483
Project acronym:	REPHRAME
Project title:	Development of improved methods for detection, control and eradication of pine wood nematode in support of EU Plant Health policy
Funding Scheme:	FP7-CP-FP
Project starting date:	01/03/2011
Project end date:	28/02/2014
Name of the scientific representative of the project's coordinator and organisation:	Dr. Hugh Evans FORESTRY COMMISSION RESEARCH AGENCY
Period covered - start date:	01/03/2011
Period covered - end date:	31/08/2012
Name	
Date	19/12/2012

This declaration was visaed electronically by Hugh EVANS (ECAS user name nevanshu) on 19/12/2012