

	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 01/03/2014 to 30/11/2014

**Start date of project:** 01/03/2011

**Project coordinator name:**

Dr. Hugh Evans

**Version:** 1

**Date of preparation:** 28/01/2015

**Date of submission (SESAM):** 31/01/2015

**Project coordinator organisation name:**

FORESTRY COMMISSION RESEARCH AGENCY

# Periodic Report

## PROJECT PERIODIC REPORT

<b>Grant Agreement number:</b>	265483
<b>Project acronym:</b>	REPHRAME
<b>Project title:</b>	Development of improved methods for detection, control and eradication of pine wood nematode in support of EU Plant Health policy
<b>Funding Scheme:</b>	FP7-CP-FP
<b>Date of latest version of Annex I against which the assessment will be made:</b>	25/02/2014
<b>Period number:</b>	3rd
<b>Period covered - start date:</b>	01/03/2014
<b>Period covered - end date:</b>	30/11/2014
<b>Name of the scientific representative of the project's coordinator and organisation:</b>	Dr. Hugh Evans FORESTRY COMMISSION RESEARCH AGENCY
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<b>Project website address:</b>	www.rephrame.eu

## **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 fully achieved its objectives and technical goals for the period.

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.

<b>Name</b>	Dr. Hugh Evans FORESTRY COMMISSION RESEARCH AGENCY
<b>Date</b>	31/01/2015

This declaration was visaed electronically byHugh EVANS(ECAS user name nevanhu) on 31/01/2015

# 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 of PWN

- Vector development and role of CO<sub>2</sub> in nematode entry elucidated.
- Dynamics of PWN in *P. sylvestris* and effects on plant tissues elucidated. Further analysis of histopathology of PWN in *P. pinaster*, *P. pinea* and *P. halepensis*
- Further assessment of bacteria associated in nematode pathogenicity; some aid and others hinder pine wilt.
- RNAseq dataset has been used to identify candidate effectors in nematodes

### WP3 Phenology and dispersal of PWN vectors

- Further analysis of *M. galloprovincialis* adult fat content confirms decline after emergence
- Tethered adult *M. sartor* flew equivalent to 1.46 km in single flight and 8.5 km over lifetime
- *M. galloprovincialis* flight mill data were, on average, 15.6 km for males and 16.3 km for females with max of 62.7 km for a male, with no detectable effect of nematode load
- No effect of exposure to sun on *M. galloprovincialis* development in logs; day degrees were identical from shaded and sun-exposed logs

### WP4 Methods for monitoring and control of *Monochamus* spp and PWN

- Smoke volatiles gave small, but not significant, increase in attraction to Galloprotect 2D G 2D but reduced catch of non-targets
- Volatile Organic Compounds investigated as potential to improve lure performance; some differences recorded for further investigation using electro antennograms
- Further studies of mass trapping indicated 60% reduction of *M. galloprovincialis* adults at trap density of 0.44 traps/ha for a beetle density of 82 beetles/ha
- Role of cuticular hydrocarbons in mate recognition elucidated for *M. galloprovincialis* and *M. sutor*
- Standard pheromone plus bark beetles kairomone blend can be used for *M. sartor* monitoring and control

### WP5 Risk of non-vector spread of PWN through various pathways to healthy forests

- PWN infested *P. sylvestris* wood chips can infest *P. sylvestris* saplings when buried around roots, especially if recipient is damaged
- PWN infested *P. pinaster* wood boards attached to live trees resulted in nematode transmission when cambium of xylem exposed, but not when bark intact

- Non-vector spread of PWN from infested to uninfested *P. pinaster* trees through the soil or by root contact could not be demonstrated in laboratory or field

- New microsatellite DNA analysis using fresh isolates of PWN from China, Japan, USA and Portugal indicate that Portuguese isolate is from USA

WP6 Host tree resistance to PWN and its vectors

- Botrytis fungus used to grow PWN does not directly influence pathogenicity

- Ninety-six half-sib progenies obtained from 120 plus trees selected for tolerance/resistance and being assessed for wilt after inoculation with PWN

- Some tolerance being shown but further analysis needed

- Preferences of *M. galloprovincialis* females for oviposition indicates wood between 7 and 31 days after death were preferred, with decreasing oviposition at 74 and 115 days

- *P. halepensis* x *P. pinaster* hybrids being grown on to assess susceptibility to PWN

WP7 Prediction of pine wilt expression, taking account of latency

- ETpN model for wilt further improved and validated using Japanese and Portuguese data on wilt occurrence

- The model has been run for current and future climates and maps produced of zones of wilt expression

- Latency (delayed expression by 1 or 2 years) can be predicted by the model and is driven by Mean Summer Temperature and date of nematode introduction to tree

- Simplified models developed for general use and available on [www.rephrase.eu](http://www.rephrase.eu)

- Further analysis of the bio-climatic model for Portugal indicates continentality, summer temperature and precipitation are important. Areas of expected wilt are highest inland and in southern and central Portugal.

- Spread model of PWN refined and uses vector, wilt likelihood, trade routes, human population density and wood processing capacity as drivers.

- PWN could spread from Portugal even with current control measures but Pyrenean barrier should contain in Iberian Peninsula

- Human spread could accelerate invasion of rest of Europe and containment essential

WP8 EU and international cooperation and collaboration

- Strong interaction and collaboration between Beneficiaries and with wider scientific community

- +ve interactions continue with EU, EPPO, IFQRG and IPPC, including International Standard of Phytosanitary Measures (ISPM)

- Many peer-reviewed and other papers produced from project collaboration

WP9 Synthesis and development of PWN Tool Kit

- Online PTK further developed especially through inputs from SOG

- PTK has been placed on REPHRAME website and draft version demonstrated at EU Task Force meeting in Spain, REPHRAME Final Workshop in Madrid and Seminar in Brussels. Also described in REPHRAME Webinar in November

- PTK also has on-line user-friendly interactive version of wilt expression model applicable to whole of Europe

WP10 Stakeholder Engagement & Dissemination

- > 60 publications; others under preparation

- SOG includes 22 scientists, plant health regulators, practitioners and timber trade representatives from 12 countries

- REPHRAME Final Workshops in Spain and Portugal from 30 Sept to 2 Oct 2014. Well attended and included formal presentations in Madrid and two full days in the field in Spain and Portugal

- REPHRAME featured in joint Webinar with FP7 ISEFOR project on 4 Nov 2014r. Well attended and presentations still available <http://view6.workcast.net/?pak=3315733587423686>.

- REPHRAME Seminar to invited audience in Brussels 13 Nov 2014. This provided a full synthesis of the key findings from the project to key stakeholders in the EU

## Expected final results and potential impacts

### VECTOR MONITORING

REPHRAME confirmed that *Monochamus galloprovincialis* is the only vector of PWN in Europe; managing vectors remains core to reducing impacts of PWN and minimising spread. Galloprotect 2D (SEDQ, Barcelona Spain), composed of the *M. galloprovincialis* pheromone plus two kairomones was identified by Beneficiaries 2 and 9. It attracts both sexes of *M. galloprovincialis* as well as *M. sutor* and *M. sartor* and is recommended for all European *Monochamus* spp.

Traps for *Monochamus* must be Teflon coating to prevent escape of captured beetles. The trap and lure combination now provides a standardised method for capturing vector beetles and for mass trapping. In Spain a reduction of around 60% was achieved with 0.44 traps per ha.

#### IMPLICATIONS OF FLIGHT DISTANCES OF MONOCHAMUS VECTORS

*Monochamus* flights of >2 km are common and up to 40 km or even longer during the lifetime of an adult. A tree-free zone increases likelihood of longer flights since there are no stimuli to retain the vector. This influences local design of felling regimes, with possible retention rather than felling of host trees.

Dispersal models have been produced, including both vector-driven and human-assisted spread of the nematode; they provide risk profiles of likely spread of PWN across Europe. PWN is not likely to progress by vector dispersal alone beyond the Iberian Peninsula due to the natural barrier of the Pyrenees. Human spread could threaten European countries outside the Iberian Peninsula by 2020.

#### OPTIMISING SAMPLING TO DETECT PWN

It is recommended that sampling for PWN should be carried out in the upper part of a tree when *M. galloprovincialis* is the vector.

#### SOURCE OF THE PORTUGUESE PWN

Prior to the REPHRAME project, the assumption was that the origin of the Portuguese PWN infestation was Asia. New microsatellite analysis indicates that USA is the origin.

#### TRANSMISSION OF PWN IN THE ABSENCE OF THE VECTOR BEETLE

Transmission from PWN-infested wood chips through the roots of trees has been demonstrated, especially through wounds. Direct transfer from infested sawn wood to living trees is possible by direct contact with under-bark exposed tissues of the recipient.

It is recommended that end use of PWN-infested wood chips moved to non-PWN areas should be regulated to ensure that the chips are either heat treated or burned. Untreated PWN-infested chips should not be deployed in direct contact with the ground near susceptible tree species.

#### IMPROVED MODELS FOR PINE WILT DISEASE

New, improved, models of the likelihood of pine wilt occurring under current and future climates have provided risk maps of Europe. A simple on-line tool on the REPHRAME website provides outputs. Latent expression of wilt has also been modelled and has important implications for symptom-based surveys.

#### SOCIO-ECONOMIC AND SOCIETAL IMPLICATIONS

Direct costs of dealing with the outbreaks in Portugal and Spain are already substantial. In addition, there are societal implications through loss of visual amenity and potential effects on water flow, soil stability, etc. Results from REPHRAME could provide tailored management with selective felling in infested areas and less reliance on clear felling as the main tool.

#### DISSEMINATION OF THE RESULTS FROM REPHRAME

The main results from the project, as well as links to world literature on PWN and its vectors, have been brought together in the PWN Tool Kit (PTK), an on-line resource on the main topics that affect the PWN-vector-tree-environment relationship.

#### KNOWLEDGE SHARING AND COLLABORATION

REPHRAME extended existing well-established collaborations with researchers and other stakeholders globally, sharing information from previous and ongoing projects on PWD.

A good example of educational benefit was the EU Erasmus Mundus programme “EUMAINE”, exposing international post-graduate students to the PWD issue.

IUFRO/REPHRAME “International Conference on Pine Wilt Disease 2013” in Braunschweig included 87 participants from 23 countries with 41 talks and 22 posters. There were two workshops (Spain and Portugal), a webinar and a seminar in Brussels during 2014.

Stakeholder Observer Group membership increased gradually through the project involving 22 representatives from 12 countries.

**Project public website address:**

[www.rephrame.eu](http://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	45	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	1.0	1	FORESTRY COMMISSION RESEARCH AGENCY	Report	PU	45	30/11/2014	Submitted	
1	Factors governing association of PWN with vector beetles	1.0	2	UNIVERSIDADE DE EVORA	Report	PU	33	26/10/2014	Submitted	
2	Factors affecting departure of PWN from vector beetles	1.0	2	UNIVERSIDADE DE EVORA	Report	PU	33	22/05/2014	Submitted	
3	Pathogenicity of PWN in host tree species	1.0	2	UNIVERSIDADE DE EVORA	Report	PU	45	30/11/2014	Submitted	
4	Methods to detect PWN in trees	1.0	2	UNIVERSIDADE DE EVORA	Report	PU	45	30/11/2014	Submitted	
1	Vector flight capacity related to physiology	1.0	3	INSTITUTO NACIONAL DE RECURSOS BIOLOGICOS I.P . INRB	Report	PU	33	22/05/2014	Submitted	
2	Vector dispersal related to forest condition	1.0	3	INSTITUTO NACIONAL DE RECURSOS BIOLOGICOS I.P . INRB	Report	PU	33	22/05/2014	Submitted	



3	Vector dispersal related to population genetics	1.0	3	INSTITUTO NACIONAL DE RECURSOS BIOLOGICOS I.P . INRB	Report	PU	45	30/11/2014	Submitted	
4	Climate influences on vector dispersal	1.0	3	INSTITUTO NACIONAL DE RECURSOS BIOLOGICOS I.P . INRB	Report	PU	33	22/05/2014	Submitted	
1	Lure for <i>M. galloprovincialis</i>	1.0	4	UNIVERSIDAD DE VALLADOLID	Report	PU	33	22/05/2014	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	1.0	4	UNIVERSIDAD DE VALLADOLID	Report	PU	45	30/11/2014	Submitted	
4	Development of lures for other <i>Monochamus</i> spp.	1.0	4	UNIVERSIDAD DE VALLADOLID	Report	PU	45	30/11/2014	Submitted	
1	Distribution of PWN in wood and wood chips	1.0	5	JULIUS KUHN-INSTITUT BUNDESFORSCHUNGSINSTITUT FUR KULTURPFLANZEN	Report	PU	33	22/05/2014	Submitted	
2	Transmission of PWN to trees with wood chips/bark	1.0	5	JULIUS KUHN-INSTITUT BUNDESFORSCHUNGSINSTITUT FUR KULTURPFLANZEN	Report	PU	45	30/11/2014	Submitted	
3	Direct tree to tree transmission of PWN	1.0	5	JULIUS KUHN-INSTITUT BUNDESFORSCHUNGSINSTITUT FUR KULTURPFLANZEN	Report	PU	36	22/05/2014	Submitted	

				NZEN						
4	Wood to wood transmission of PWN in wood pack aging	1.0	5	JULIUS KUHN-INSTITUT BUNDESFORSCHUNGSINSTITUT FÜR KULTURPFLANZEN	Report	PU	45	30/11/2014	Submitted	
5	Microsatellite markers for PWN identification	2.0	5	JULIUS KUHN-INSTITUT BUNDESFORSCHUNGSINSTITUT FÜR KULTURPFLANZEN	Report	PU	12	25/06/2013	Submitted	
6	PWN genetic diversity as indicators of invasion history	1.0	5	JULIUS KUHN-INSTITUT BUNDESFORSCHUNGSINSTITUT FÜR KULTURPFLANZEN	Report	PU	45	30/11/2014	Submitted	
1	Susceptibility of <i>Pinus sylvestris</i> provenances to PWN	1.0	6	INSTITUTO NACIONAL DE RECURSOS BIOLÓGICOS I.P . INRB	Report	PU	45	30/11/2014	Submitted	
2	Construction of cDNA libraries from sensitive and resistant genotypes of <i>Pinus</i>	1.0	6	INSTITUTO NACIONAL DE RECURSOS BIOLÓGICOS I.P . INRB	Report	PU	12	08/11/2012	Submitted	
3	Identification of PWN resistance genes in pines	1.0	6	INSTITUTO NACIONAL DE RECURSOS BIOLÓGICOS I.P . INRB	Report	PU	45	30/11/2014	Submitted	
4	Resistance of pines to feeding by <i>Monochamus</i>	1.0	6	INSTITUTO NACIONAL DE RECURSOS BIOLÓGICOS I.P . INRB	Report	PU	33	22/05/2014	Submitted	

5	Host preferences for <i>Monochamus oviposition</i>	1.0	6	INSTITUTO NACIONAL DE RECURSOS BIOLOGICOS I.P . INRB	Report	PU	45	30/11/2014	Submitted	
6	Hybrid progenies with different tolerance/ resistance to the PWN	2.0	6	INSTITUTO NACIONAL DE RECURSOS BIOLOGICOS I.P . INRB	Report	PU	18	26/10/2014	Submitted	
7	Tree species mosaics to reduce PWN impact	1.0	6	INSTITUTO NACIONAL DE RECURSOS BIOLOGICOS I.P . INRB	Report	PU	45	30/11/2014	Submitted	
1	Refinement of core model	1.0	7	INSTITUTO NACIONAL DE LA RECHERCHE AGRONOMIQUE	Report	PU	44	31/10/2014	Submitted	
2	Field verification of process model	1.0	7	INSTITUTO NACIONAL DE LA RECHERCHE AGRONOMIQUE	Report	PU	45	30/11/2014	Submitted	
3	Latency sub-model	1.0	7	INSTITUTO NACIONAL DE LA RECHERCHE AGRONOMIQUE	Report	PU	44	31/10/2014	Submitted	
4	Analysis of PWN history in Portugal	1.0	7	INSTITUTO NACIONAL DE LA RECHERCHE AGRONOMIQUE	Report	PU	42	31/08/2014	Submitted	
5	PWN spread model	1.0	7	INSTITUTO NACIONAL DE LA RECHERCHE AGRONOMIQUE	Report	PU	44	31/10/2014	Submitted	
1	Knowledge from previous EU projects	2.0	8	UNIVERSIDADE DE EVORA	Report	PU	12	25/06/2013	Submitted	

2	Interaction with EU/International projects	1.0	8	UNIVERSIDADE DE EVORA	Report	PU	45	30/11/2014	Submitted	
1	PTK interface	2.0	9	FORESTRY COMMISSION RESEARCH AGENCY	Report	PU	12	22/05/2014	Submitted	
2	Beta testing of PTK modules	1.0	9	FORESTRY COMMISSION RESEARCH AGENCY	Report	PU	39	31/05/2014	Submitted	
3	Launch of PTK	1.0	9	FORESTRY COMMISSION RESEARCH AGENCY	Report	PU	44	31/10/2014	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	2.0	10	FORESTRY COMMISSION RESEARCH AGENCY	Report	PU	12	22/05/2014	Submitted	
4	Workshop 1 Spain	1.0	10	FORESTRY COMMISSION RESEARCH AGENCY	Other	PU	38	30/04/2014	Submitted	
5	Workshop 2 Portugal	1.0	10	FORESTRY COMMISSION RESEARCH AGENCY	Other	PU	45	30/11/2014	Submitted	
6	International Conference on PWN	1.0	10	FORESTRY COMMISSION RESEARCH AGENCY	Report	PU	32	22/05/2014	Submitted	
7	Plan for use & dissemination of foreground	1.0	10	FORESTRY COMMISSION RESEARCH	Report	PU	45	30/11/2014	Submitted	

				AGENCY					
8	Awareness & wider societal implications	1.0	10	FORESTRY COMMISSION RESEARCH AGENCY	Report	PU	45	30/11/2014	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	05/05/2011	Yes	31/01/2015	
2	Mid term report delivered	1	1	01/05/2013	Yes	31/01/2015	
3	Final report delivered	1	1	31/01/2015	Yes	31/01/2015	After agreed 9 month extension
4	Report on PWN interaction with host trees	2	7	31/01/2015	Yes	31/01/2015	In Final Report
5	Rapid diagnostic PWN diagnostic method produced and demonstrated	2	7	31/01/2015	Yes	31/01/2015	Mostly completed - in Final Report
6	Lifetime audit of flight capacities of Monochamus spp	3	6	31/01/2015	Yes	31/01/2015	
7	Validated lures and traps for vector monitoring.	4	9	31/01/2015	Yes	31/01/2015	
8	Mass trapping protocols for vector management	4	9	31/01/2015	Yes	31/01/2015	
9	Risk profiles of non-vector dispersal pathways quantified	5	5	31/01/2015	Yes	31/01/2015	See Final Report
10	Advice on potential of tree resistance or tolerance to PWN	6	6	31/01/2015	Yes	31/01/2015	Partially completed, see Final Report
11	Full process model a	7	4	31/01/2015	Yes	31/01/2015	

	ccounting for latency and climate change						
12	Light version of model for stakeholder use	7	4	31/01/2015	Yes	31/01/2015	On-line version available
13	Sharing knowledge with international research community	8	7	31/01/2015	Yes	31/01/2015	
14	Sharing staff resources by exchange internationally	8	7	31/01/2015	Yes	31/01/2015	
15	Delivery of PWN Tool Kit for structured output from REPHRAME	9	1	31/01/2015	Yes	31/01/2015	On-line PTK available

#### **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.

<b>Attachments</b>	REPHRAME 3rd periodic report final.pdf
<b>Grant Agreement number:</b>	265483
<b>Project acronym:</b>	REPHRAME
<b>Project title:</b>	Development of improved methods for detection, control and eradication of pine wood nematode in support of EU Plant Health policy
<b>Funding Scheme:</b>	FP7-CP-FP
<b>Project starting date:</b>	01/03/2011
<b>Project end date:</b>	30/11/2014
<b>Name of the scientific representative of the project's coordinator and organisation:</b>	Dr. Hugh Evans FORESTRY COMMISSION RESEARCH AGENCY
<b>Period covered - start date:</b>	01/03/2014
<b>Period covered - end date:</b>	30/11/2014
<b>Name</b>	
<b>Date</b>	31/01/2015

This declaration was visaed electronically by Hugh EVANS (ECAS user name nevanshu) on 31/01/2015