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PROJECT FINAL REPORT

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Final Report

Please note that the contents of the Final Report can be found in the attachment.

4.1 Final publishable summary report

Executive Summary

REPHRAME has successfully addressed many of the key issues that underpin the pine wood nematode problem in Europe and the results will aid refinement of future management of this important pest. The key findings are summarised under the following headings:

VECTOR MONITORING AND MASS TRAPPING

Monochamus galloprovincialis is the only demonstrated vector of PWN in the affected area in Portugal and Spain. M. galloprovincialis pheromone was identified and is now available as Galloprotect 2D (SEDQ, Barcelona Spain). It is effective for monitoring other European Monochamus species, notably M. sutor and M. sartor and is recommended for all European Monochamus spp. Recommended traps for vector monitoring and mass trapping include Teflon-coated multi-funnel and cross-vane designs.

Mass trapping of vectors has been investigated in France, Portugal and Spain. In general, it appears that a network of traps baited with Galloprotect 2D can result in population reduction. Mass-trapping is likely to be effective but requires further field verification.

FLIGHT DISTANCES OF MONOCHAMUS VECTORS

Short flights of Monochamus spp are common in dense woodland but, in more open areas, long distance flight is likely; >2 km is common and up to 40 km or even longer is possible during the lifetime of a beetle. It now appears that a tree-free zone actually increases the likelihood of longer flights and a strategy based on vector behaviour would be to ensure that there are both feeding and oviposition resources left in an area to attract and retain vectors.

New models account for both vector-driven and human-assisted spread of the nematode, providing risk profiles of likely spread of the nematode across Europe. The nematode could spread from Portugal even under current control measures but not by vector dispersal alone, due to the natural barrier of the Pyrenees. Human-assisted spread could threaten European countries outside the Iberian Peninsula by 2020.

OPTIMISING SAMPLING WITHIN TREES TO DETECT PWN

Laboratory experiments on the pathology of PWN in susceptible trees succumbing to pine wilt confirmed the rapid movement of nematodes throughout an infested tree, detectable by trunk sampling. However, nematodes introduced by M. galloprovincialis egg-laying will be concentrated near to vector breeding sites and sampling should target this part of the tree.

NEW INSIGHTS ON SOURCE OF THE PORTUGUESE PWN

Improved microsatellite analysis of newly acquired fresh nematode isolates have been used to assess the origin of the European PWN. The results have provided new insights into the movement of PWN globally; Japan - origin USA, China origin USA and Japan, Portugal origin USA. Further analysis of pathways will help determine how the nematode arrived.

TRANSMISSION OF PWN IN THE ABSENCE OF THE VECTOR BEETLE

Rapid transfer of PWN from infested to non-infested sawn wood takes place if recipient wood has moisture content greater than 20%. Transmission from nematode-infested wood chips through the roots of trees has been demonstrated, especially if there is any wounding. Direct transfer from

infested sawn wood to living trees is possible if the wood makes direct contact with under-bark exposed tissues of the recipient tree. This needs to be accounted for in defining the end uses of potentially infested wood and wood products.

IMPROVED MODELS OF PINE WILT DISEASE

Sophisticated process and correlation models of the likelihood of pine wilt occurring under current and future climates have enabled risk maps of Europe to be produced. Simplified models enable end users to estimate the potential for wilt in a given area or region using easily available parameters such as Mean Summer Temperature or knowledge of the precise location and are supplemented by a simple on-line tool on the REPHRAME website. Latent expression of wilt (delayed by one or two years) has also been modelled and has important implications for carrying out surveys based on symptoms of affected trees.

KNOWLEDGE SHARING AND 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 on-line PWN Tool Kit (PTK). An international conference and several workshops provided direct end-user knowledge transfer. REPHRAME built on the existing well-established collaborations with researchers and other stakeholders globally.

Summary description of project context and objectives

Pine wood nematode (PWN), Bursaphelenchus xylophilus, is rightly regarded as a major threat to European forests, particularly following its establishment in Portugal and the fact that, even with application of stringent measures, the pest has continued to spread and kill pine trees in that country. The basis for classifying PWN as a highly significant threat is its well documented track record of killing pine trees in Japan (late 19th century), China (from 1982), Korea (from 1988), Taiwan (from 1985) and, most recently, Portugal (continental PT 1999; Madeira 2009) with 4 incursions in Spain (2008 onwards). In all countries where wilt expression has been recorded (leading to the description of the syndrome pine wilt disease) there has been extensive tree mortality. For example, in Japan annual losses peaked at 2.5 million m3 in the early 1980s but appear to be fluctuating around an average of about 1.0 million m3 to the present time. The recent outbreak in Portugal illustrates the high costs of attempting eradication of a newly discovered organism (appr. €100million from 1999 – 2013; source EU DG SANCO), which must also be added to direct losses from tree mortality. The campaign to eradicate the findings of PWN in Spain, as well as intensive ongoing surveys, has also incurred high management costs of at least €16 m (EU DG SANCO). Faced with this evidence of direct and indirect financial effects, combined with environmental and social impacts, there is an urgent need to develop the most effective pest management strategies to deal both with established outbreaks and, particularly, for early detection and eradication of new infestations.

Considerable progress has been made in understanding the relationships between PWN, its vector insects and the host tree/environmental factors that result in pine wilt in all countries affected but, especially, in Portugal. Apart from research and management initiated within Portugal directly, the EU project PHRAME addressed a number of important issues on B. xylophilus, its vectors in the genus Monochamus and the factors leading to wilt expression and tree mortality. This has been extended considerably in the follow-up REPHRAME project which is described in this report. REPHRAME has addressed the key interactions between the four main elements that determine risks from PWN namely: the nematode, its vectors, the host tree, and the immediate eco-climatic environment.

In North America, where B. xylophilus is native, there is very little wilt expression or tree mortality and the nematode persists primarily through transfer during oviposition by female Monochamus spp. Under these circumstances, B. xylophilus breeds on any residual intact cells in the dying or dead tree and, particularly, on fungi that exploit the dead tree. This cycle is, therefore, saprophytic and is exhibited at all locations where PWN exists, even when trees are not killed by the nematode as is the case in North America, including its northernmost range in Canada. A critical factor in this cycle is the fact that Monochamus spp. females are unable to lay eggs in living host trees and, consequently, the transfer of PWN during egg-laying is the primary mechanism that both ensures survival of the nematode and retains it in close proximity to the breeding site of the vector for subsequent transfer to new hosts. In this latter context, it is also significant that virtually all conifer hosts (with the exception of the genera Thuja and Taxus) are suitable for this saprophytic phase of the nematode cycle, which extends the availability of suitable host trees in Europe significantly.

Expression of pine wilt, leading to tree mortality, is a phenomenon that is only noted under particular combinations of susceptible tree species and eco-climatic conditions, principally high temperatures and low soil moisture contents. The precise conditions leading to pathological impacts from PWN are not fully understood, although many of the factors have been now been elucidated within REPHRAME.

In relation to the specific requirements of KBBE.2010.1.4-09, the key questions posed by the call text are interpreted in relation to how they relate to PWN/vector/tree interactions. By relating to the saprophytic and pathogenic components of the nematode life cycle and, critically, to the interaction (or not) with the vector Monochamus spp., the gaps in current knowledge are highlighted and have formed the basis of the REPHRAME research programme through a series of questions and Work Packages.

Question 1, Work Package 2: Where do the nematodes live in the tree and how do symptoms relate to infestation over time?

This question relates particularly to the entry and subsequent fate of PWN after they first enter the tree during maturation feeding by Monochamus spp. in the tree crown. It is known from analysis of effects on trees expressing pine wilt that nematodes can be found throughout the tree in a very short time after entering branch wounds, possibly within hours. A detailed audit of the fate of nematodes within host trees and, particularly, linkage to whether wilt expression takes place is, has been carried out and linked to environmental parameters.

Question 2, Work Package 3: Monochamus flight; how far over the adult lifetime?

There is overwhelming evidence that transfer of PWN from host tree to host tree (whether living or dead) is through the close interaction between the nematode and adult vectors of the genus Monochamus. Indeed, within the host tree B. xylophilus migrates to the Monochamus spp. pupal chamber where it develops into a specific larval stage called the dauer larva (IVth stage juvenile). This is adapted to move onto the newly formed adult beetle where it enters the tracheae (breathing tubes) and is transported with the flying adult to new locations. Maturation feeding by both beetle sexes delivers some nematodes to the living tree through wounds made on branch tissues in the crowns of trees. When environmental conditions are suitable (high temperatures and low soil moisture), wilt expression can follow. Evidence from Portugal and from studies in China and Japan indicates that local flights in pine wilt areas lead to patches of wilting trees in close proximity to each other, suggesting that Monochamus spp. adults do not fly far under these circumstances. However, the fact that isolated trees and forest blocks physically separated from known infested areas can become infested by PWN, also suggests that some flights by the adult beetles must extend for considerable distances, perhaps several kilometres from the point of origin. In these cases, it becomes increasingly difficult to separate beetle-mediated dispersal from human-assisted dispersal through transport of infested wood.

In relation to strategies for management of the PWN threat, both by attempting to eradicate local infestations and, particularly, by prevention of further dispersal from a geographically limited infestation, it is essential to know how far a dispersing population of beetles can fly over the lifetime of the beetles (usually around 12 weeks). The question needs to account for the influences of local environment (e.g. does flight propensity change in relation to whether a beetle is in contiguous forest or in a landscape with fragmented availability of host trees?) and for the age of the beetle (does flight tendency change with age, frequency of maturation feeding, frequency of mating, etc?).

Question 3, Work Package 4: Monochamus adults: can new traps/lures improve monitoring and mass capture?

Progress in development of improved traps, enhanced by the addition of chemical lures to mimic host trees, have been made through the PHRAME project and in routine use in the Portuguese campaign to manage PWN. There has also been a more recent specific evaluation of the precise chemical composition of tree host and beetle aggregation and sex attractants in relation to Monochamus galloprovincialis (work in Spain and UK). The combination of refined biochemical analysis tools, linked to electro-antennogram studies to determine beetle responses provides a basis for rapid evaluation of candidate attractant compounds. These can then be assessed in field situations along with a range of potential beetle trap designs.

There is sufficient promise in existing developments to suggest that significant advances in trap-lure combinations can be made relatively quickly and the knowledge used to assess their roles in survey and in potential for mass capture as a population reduction measure. The answers to Question 3 relate closely to those from Question 2, particularly in provision of accurate monitoring tools for studies of adult beetle dispersal.

Question 4, Work Package 5: Can nematodes transfer or be transferred between trees without Monochamus vectors?

In carrying out Pest Risk Analysis (PRA) for B. xylophilus and its vectors in the genus Monochamus, all possible pathways for transfer of PWN between trees have been evaluated (Evans et al. 1996 EPPO Bulletin 26: 199-249). The best defined pathway, with clear evidence of transfer within and between countries, is through the actions of vector beetles in the genus Monochamus. However, the PRA carried out by Evans et al (1996) and the more recent EPPO PRA carried out by EPPO Forest Quarantine Panel, also considered the possibility that wood or host plant material without Monochamus spp. but containing B. xylophilus could present a risk of transfer if the infested material came into contact with potential host trees, living or dead. There have been some laboratory-based studies that suggest transfer of PWN from wood chips buried in soil to seedling trees through their roots. REPHRAME has carried out definitive studies on whether direct transfer of PWN to living trees, either through the roots or by wood to stem contact can take place or what this represents in overall risk.

Studies of non-vector transmission included easily investigated and manipulated laboratory scale studies where the parameters of potential transfer can be closely monitored and quantified. Data from such studies defined baseline risks and provided the basis for field experimentation using larger host trees but ensuring that vector transmission cannot take place on the test trees.

Question 5, Work Package 6: Are some tree species resistant, or is pine wilt environmentally driven?

Although it is clear that the expression of pine wilt disease is heavily influenced by environmental conditions, particularly temperature, there is evidence from the literature that some tree species appear to be intrinsically resistant to the nematode, regardless of environmental drivers. Also, within a tree species, some trees survive PWN pressure and, therefore, present possible opportunities for selective breeding, e.g. researchers in China have taken those trees or their seed to start a selection process to propagate the most tolerant individuals of a tree species population. Those trees have been termed tolerant because the nematode survives in the tree but does not harm them, which is comparable to the situation in North America. Although very long-term in practicality, some of the short-term analyses and tests to assess possible tolerance or resistance have been done in REPHRAME.

Question 6, Work Package 7: Which parts of Europe are vulnerable to pine wilt expression?

As indicated earlier, the presence of PWN in a living tree does not always result in wilt expression and tree mortality. There have been a number of attempts to quantify the environmental conditions that can lead to wilt in intrinsically susceptible Pinus species and, at a simplistic level, it appears that summer temperatures around 20oC are important precursors for wilt. These conclusions have been based principally on correlation analysis in relation to the known distribution of PWN and of wilt expression globally. While valuable, they did not provide explanatory variables that could allow more accurate linkage to both average temperatures and site conditions, such as soils and aspect, etc. These parameters were assessed specifically in the PHRAME project where both statistical, correlative models and tree growth process models were developed to provide a stronger explanatory framework to predict wilt expression in susceptible trees. The process model extended existing tree growth models and provided remarkably accurate predictions of wilting and tree mortality for Portugal. However, further validation and refinement of the models is needed to account fully for the eco-climatic variation across Europe and internationally.

Development of models to improve knowledge on the vulnerability of host trees to pine wilt in Europe have focussed on eco-climatic drivers as well as tree species and soil types at a range of scales. Validation of the model drivers, especially linkage to symptom expression over time (including latency) have refined the model predictions and also support research to answer Questions 2 to 4. A particular focus for model refinement was provision of modules to account for future climate change scenarios across Europe and internationally. Delivery of nematodes to areas likely to express wilt is an important part of assessing risk and this has been addressed through new models for both vector- and human-assisted dispersal.

Description of main S & T results/foregrounds

THE PWN/VECTOR/TREE/ENVIRONMENT RELATIONSHIP

In order to address the critical questions raised in the current project, the important first step is to provide a clear picture of the nature of the Bursaphelenchus xylophilus/vector/tree/environment relationship. Whilst there is understandable concern about the likelihood of trees being killed by pine wilt caused by PWN, the dynamics of the PWN/tree interaction are driven by the life cycle and biological characteristics of its vector beetles in the genus Monochamus. Underlying this relationship is the fact that, especially in its native range in North America where native conifers are rarely killed, the nematode has a co-evolved relationship with the vector, dominated by the breeding biology of Monochamus spp. In this situation, transmission of PWN from tree to tree is primarily through egg-laying by the female beetle with nematodes migrating into the exposed tree tissues as she cuts a hole in the bark to lay an egg. Survival and breeding of the nematode takes place on fungal contaminants associated with the larval feeding chambers of the vector. At the beetle pupal stage, the nematode moults to a specific dauer fourth larval stage which congregates around the pupal chamber and, late in the cycle, enters the tracheae (breathing tubes) of the newly formed adult and exits the tree with it. This part of the cycle takes place throughout the range of the vector, including areas in its native range that have very low temperatures, such as Northern USA and Canada. The saprophytic cycle can, therefore, be regarded as the underpinning component of the PWN/vector relationship and ensures a high probability of nematode survival in close association with the vector to virtually guarantee that PWN survives and moves from tree to tree.

Although the saprophytic phase is, therefore, prominent and widespread, it is the relatively unusual phase of nematode entry to living trees and subsequent impact on those trees through blockage of water conductance – the syndrome called Pine Wilt Disease – that is of particular concern in those countries where the nematode has established as an invasive organism. Driving this part of the cycle is the universal characteristic of adult Monochamus spp. to carry out feeding in the canopies of living trees. This is called maturation feeding and, in the early stages after emergence, is essential to help the beetles reach sexual maturity and to provide food to strengthen flight muscles for further dispersal. However, this feeding takes place throughout adult life and would more accurately described as shoot feeding. Shoot feeding is universal, regardless of beetle geographic location, but it is only under certain combinations of tree species and environmental conditions, especially high temperatures, that nematodes can enter the tree, breed, and potentially cause pine wilt. Extensive mortality in China, Korea, Japan, Taiwan and, most recently, Portugal has confirmed that highly susceptible tree species, combined with relatively high temperatures have provided ideal conditions for pine wilt to build up. The large numbers of trees killed rapidly by pine wilt also have the unfortunate consequence of providing abundant breeding material for Monochamus vectors and this, in turn, leads to more vectors and more transmission of nematodes; a positive feedback loop leading to increasing tree mortality.

In the original proposal we posed a number of questions to address the call text and to build on the knowledge already available on PWN and its vectors both from the previous PHRAME project and from international literature. Progress in answering these questions has been good and the outputs have been brought together in detailed periodic reports and through the PWN Took Kit (PTK) to improve understanding and to provide recommendations for monitoring and managing the PWN threat.

QUESTION 1: WHERE DO THE NEMATODES LIVE IN THE TREE AND HOW DO SYMPTOMS RELATE TO INFESTATION OVER TIME?

The emphasis in answering this question has been on wilt expression arising from introduction of nematodes into living trees, as is the case during maturation feeding by vector insects. In work carried out in Germany under quarantine conditions, particular focus has been on inoculation experiments on relatively large trees (7-8 year old Scots pine, Pinus sylvestris; 2.5 m tall) to study the distribution of nematodes and development of wilt symptoms over a 10 week period.

To assess the movement of PWN in the trees, on each weekly sampling occasion, one tree was divided into 46 wood segments, including root collar and root, and nematode density per gram dry matter was determined. Wilt symptoms appeared within 3 weeks and increased over time. The last tree was sampled shortly before tree death after 67 days. The first nematode infested segments were at the inoculation site and adjacent segments, after which nematodes spread to all tree parts, including the roots, after 16 days. The moisture content (MC) of wood and needles decreased while nematode density increased.

Pathogenicity of PWN was investigated in both healthy and drought-stressed trees under the same test conditions. Water regimes for the test trees were controlled and assessed by weighing the plants with soil. Drought stress was induced by stopping watering after the test start and assessed using a Scholander bomb. At tree death, or after three months, stem parts were sampled for nematodes. The normally watered control trees showed constant water potential. Water potential of the PWN inoculated pines dropped suddenly approximately three weeks after inoculation of PWN, which coincided with the first appearance of wilt symptoms. By contrast water potential of the drought-stressed trees decreased slowly but gradually. PWN-inoculated trees suffered 100% mortality and PWN could be extracted from them all.

Under optimal conditions PWN migrated to all segments of the almost mature P. sylvestris trees within a few weeks and resulted in tree death within three months. These results are very similar to results obtained for young pine saplings in the previous PHRAME project. We conclude that PWN population dynamics and pathogenicity results from experiments using small P. sylvestris saplings are transferable to large P. sylvestris trees, even within forests, under similar climatic circumstances. In addition, these results confirm the finding that this pine species is highly susceptible to pine wilt and should be classified in the PWD-endangered species.

The rapid movement of PWN through the trees from the point of inoculation confirms previous findings both within REPHRAME and in the literature that indicates nematodes can disperse throughout a host tree within days or a low number of weeks when tree species and environmental conditions are suitable. It is not entirely clear how the nematodes move so quickly through the tree, especially since tree defences such as resin production could act as a barrier to movement. Histopathology studies, using thin sections of wood tissues, differential staining and use of microscopes for examination, were carried out in order to observe possible differences between Pinus pinaster (susceptible to PWN wilt) and Pinus halepensis (tolerant to PWN with no wilt).

Anatomical differences were noted, which could help improve our understanding of susceptibility/tolerance mechanisms in different pine species:

- P. pinaster resin canals are greater in number than in P. halepensis;
- Distances between cortex resin canals of P. pinaster are smaller than in P. halepensis;
- P. pinaster xylem resin canal density by surface area is greater than in P. halepensis.

The two pine species, after inoculation with PWN, also showed different characteristics including:

- 48h after inoculation there was noticeable tissue damage caused by nematodes in P. pinaster;
- Damage in cortex and xylem ray parenchyma cells were also noticeable, along with degradation of cortex and xylem resin canals, thickening of xylem cell walls and damage of pit cells.
- By contrast in P. halepensis there was no visible extensive damage at the cellular level, indicated by intact structures, intact pit cells and some of the resin canals plugged by tylosoid formation.

Further evidence in tolerant species, such as P. halepensis, inoculated with PWN was that among the parenchyma cells there were clear tannin idioblasts, showing that plant defence mechanisms, including production of secondary metabolites, were activated. Furthermore, the lumens of the resin canals in the cortex were delimited by two layers of epithelial cells. These cells are rich in cytoplasm with secretory function where biosynthesis of terpenes (defensive compounds) takes place. Biochemical response together with anatomical differences could be a partial explanation for different responses, including resistance, by different tree species to nematodes. The results for P. sylvestris, a highly susceptible species, were similar to those obtained for P. pinaster.

As part of the improved understanding of the PWN-tree interaction, studies were carried out on the potential roles of associated bacteria on pathogenesis, based on previous Chinese information to indicate that increased impact of PWN could be linked to presence of such bacteria.

Initial investigations were carried out on nematode-bacteria interactions under severe oxidative stress (OS) conditions (pro-oxidant hydrogen peroxide) focussing on the adhesion ability of these bacteria to fix to the nematodes' cuticle. Results showed clearly a beneficial effect of Serratia spp. (isolates LCN-4, LCN-16 and PWN-146) towards B. xylophilus under OS conditions. In this scenario, Serratia spp. were extremely OS-resistant, and contributed to considerably lower mortality in the B. xylophilus population and down-regulation of B. xylophilus catalase genes (Bx-ctl-1 and Bx-ctl-2). In addition, we assessed two isolates of B. xylophilus, virulent (Ka4) and avirulent (C14-5), and observed that Ka4 showed better fitness under OS conditions than C14-5. The bacterial effect was similar for both B. xylophilus isolates. However, we did not observe strong and specific adhesion of these bacteria to the B. xylophilus surface. New insights into nematode-bacteria interactions were provided in this study. We report, for the first time, that B. xylophilus associated bacteria may assist the nematode opportunistically in disease progression, and that virulent B. xylophilus were better able to tolerate OS conditions than avirulent.

In further experiments, different bacterial isolates were phenotypically characterized in terms of: fitness (tolerance to oxidative stress, antibiotic resistance, antifungal activity), metabolic (protease production; phosphate solubilisation, siderophores production, IAA production and biofilm formation) and pathogenicity features (nematicidal activity and gnotic root elongation). From these studies, it is apparent that the bacterial communities associated with the system (nematode-pine-insect) are highly diverse and seem to have different roles in the ecology of pine wilt. Significantly, we have been able to demonstrate the role of some bacterial species in providing protection to PWN from the oxidative stress created by the plant. On the other hand, some bacterial mutants (Pseudomonas putida UW-4) may be helpful in protecting the plant against the invasion of the pinewood nematode.

Variable pathogenicity has been demonstrated for B. xylophilus and, therefore, one of the important questions on pathogenicity is where did the Portuguese isolate of PWN come from and is it a single or multiple introduction? This has been addressed through molecular genetics studies using a wide range of techniques. In particular, through work by beneficiary 4 in France, a set of 15 polymorphic loci has been characterised and validated; these are usable on single B. xylophilus nematodes in three complementary multiplex reactions which can be used to assess the variability and origins of PWN isolates.

Thanks to intense sampling effort from several international collaborators in infested pine forests in the native (USA) and invaded (Japan, China, Portugal) areas, fresh isolates of PWN (i.e. not grown over many generations in the laboratory) were obtained from these sources. The 15 microsatellites were then used to genotype more than 1,000 individual nematodes. Samples from the USA displayed significant genetic differences, highlighting the existence of a strong spatial genetic make-up of the nematode in its native area. Spatial differentiation was detected over a very short scale, with PWN populations from neighbouring trees differentiated significantly. This suggests that PWN dispersal, whether active or passive, can be spatially limited even over a short distance and that genetic drift may play an important role at a local scale. In some cases, different genetic clusters were identified within a single tree, suggesting that different beetles carrying genetically differentiated nematode populations infested a single tree. Furthermore, some nematodes sampled in different US States were assigned to a unique genetic cluster despite the large geographical distance between them (more than

500 km). This result is in agreement with the potentially important role of the human-induced long-distance dispersal of PWN.

Conversely, we found very low levels of polymorphism in the invaded areas (Japan, China and Portugal, including Madeira Island), suggesting single introduction events together with intense demographic and genetic bottlenecks. In the European context, these findings firmly suggest that the second outbreak detected in the centre of mainland Portugal in 2008 resulted from expansion of the first outbreak detected close to Lisbon in 1999. Our results also suggest that the PWN populations on Madeira originated from mainland Portugal, given their virtual near identity.

Classical population genetics methods were then applied to identify the origin of the Portuguese populations. However, these analyses inferred inconclusive results, since they alternatively suggested either an American or a Japanese origin for all the Portuguese samples tested. Given the limitations of these methods, we further used on the same data set a recently developed model-based method, the approximate Bayesian computation and this produced a more definitive result on the origin of the Portuguese isolate, strongly indicating that it came directly from the native range in the USA.

The results of genetic analysis of PWN variability and origins of the nematodes in invaded areas can be summarised as follows:
1. At least three independent introduction events occurred from the USA into the invaded areas at a worldwide scale.
2. The origin of the Japanese PWN invasive populations is the USA. However, a second introduction event probably occurred, whose origin remains to be elucidated.
3. Two independent introductions occurred in China, one from the USA and one from Japan.
4. The most probable origin of the invasive PWN population in Portugal is the native area of the nematode (USA).
5. The origin of the Madeira outbreak is continental Portugal.

QUESTION 2: MONOCHAMUS FLIGHT; HOW FAR OVER THE ADULT LIFETIME? AND QUESTION 3: MONOCHAMUS ADULTS: CAN NEW TRAPS/LURES IMPROVE MONITORING AND MASS CAPTURE?
These questions have been addressed together in inter-related studies on trap design, flight capacity, population genetics and potential for management of beetle populations using mass trapping.
Detailed assessment of literature and field surveys where PWN has been found in Portugal and Spain, confirm that the only demonstrated vector is Monochamus galloprovincialis. This species is widespread and common in the Iberian Peninsula with population size being determined by a range of factors that reduce the health of trees sufficiently to make them suitable for breeding. Factors such as fire, drought, and biotic damage (especially from bark beetles and pine processionary moth) can weaken trees sufficiently to enable breeding by the vector beetles. For M. galloprovincialis, death of branches in healthy trees also provides breeding resources. Since PWN in now relatively widespread in Portugal, the dynamics of M. galloprovincialis as the key driver for dispersal are important. Several characteristics have been studied during the project and these are important components in understanding and potentially managing the vector beetle populations:

LURES AND TRAPS TO MONITOR MONOCHAMUS BEETLE POPULATIONS IN EUROPEAN FORESTS

LURES

Project Partners in Spain and UK previously identified the pheromone of M. galloprovincialis as 2-undecyloxy-1-ethanol and showed blends of this compound plus two kairomones (bark beetle pheromones ipsenol and methyl-butenol) were highly attractive to both male and female M. galloprovincialis. The commercial lure derived from this work - Galloprotect 2D (SEDQ, Barcelona Spain) - has been shown to be highly efficient in attracting both sexes of M. galloprovincialis. It is now recommended as the standard lure for M. galloprovincialis monitoring. More detailed studies have been carried out to further improve the efficiency of the lure. For example, alpha-pinene has been shown to be the best synergist of the G2D standard lure, with up to 20% increase in attraction (but not on every occasion). However, it is not target specific and attracts many xylophagous (wood dwelling) beetles as well as bark beetle predators. It is included in the commercial lure Galloprotect PACK (= G2D plus alpha-pinene; SEDQ, Barcelona Spain). In order to overcome the impact of capturing non-target species, it was found that some smoke compounds (methoxyphenols) were equally good synergists of the standard lure compared with terpenes, but without attracting non target insects. It is expected that they will soon replace alpha-pinene in the commercial lures.

An interesting finding that is still under investigation is that immature beetles, those that have fed for less than 10 days, were not attracted by any of the volatiles tested (pheromone, bark beetle kairomones, terpenes), either within forests or in areas deprived of hosts. Thus, a lure for these is still lacking and field monitoring has to allow for a lag in captures for each set of adults that emerges.

During the project both M. sutor and M. sartor were shown to produce the same pheromone as M. galloprovincialis, and the blends of pheromone and kairomones attracted these species. Similar results were obtained in the Pyrenean black pine forests in Spain and in spruce forests in the eastern Alps in Austria from experiments in 2012, 2013 and 2014, using the pheromone for both M. sutor and M. sartor. Addition of the pheromone significantly increased the attractiveness of bark beetle kairomones alone. The host tree volatile alpha-pinene increased trap catches but not always significantly. The bark beetle kairomone Chalcogran did not increase attractiveness. Teflon-coated multifunnel traps hung from 2-m poles in open areas in spruce forests yielded good catches of both species. Overall, the traps and lures developed for M. galloprovincialis proved to be excellent tools for trapping M. sutor and M. sartor.

TRAPS

In extensive tests in France, Portugal and Spain, two commercial Teflon-coated traps performed most effectively in trapping M .galloprovincialis; significantly higher numbers of insects were caught and the non-stick coating prevented escapes and enabled the trapping of live beetles in the Teflon-coated, wire screened collecting containers. This was confirmed by work in Germany using both Teflon-coated and non-coated traps. In 2012 only 29 M. galloprovincialis were captured in non-coated traps compared with 442 M. galloprovincialis in 2013 (using Teflon coated traps and cups). The majority of this species was captured in Lower Saxony in dying pine stands. Captures of non-target predators (especially Spondylis buprestoides and Thanasimus formicarius) were high. Investigations concerning the effect of Teflon-coated traps and collection cups confirmed that 100% of beetles can escape from non-coated traps and collecting cups, regardless of beetle age and sex.

Taken together, our studies provide the following recommendations for monitoring and for control of M. galloprovincialis and other European Monochamus species: CROSSTRAP® (with Crosstrap Collection 2 litre Cup) and ECONEX MULTIFUNNEL-12® (with Econex Multifunnel Extended Collection Cup) (Econex SL, Murcia, Spain). In relation to efficiency, the durability of the Teflon coating has been demonstrated for two seasons. However, it is not known whether this remains durable for longer periods and, therefore, an easy way to re-coat the traps with Teflon by users is being developed by the manufacturer.

In UK and Spain, further studies were carried out to understand the roles of contact sex pheromones for lure improvement. Experiments have shown that males of M. galloprovincialis and M. sutor rely for sex discrimination on a pheromone present on the female cuticle. Cuticular analysis showed that two male-specific peaks occurred in both species. Separation from the rest of the extract of the two male peaks was achieved by fractionation of cuticular extracts. Fractionated extracts applied to freeze killed females or female decoys (glass rods) were bioassayed and results showed that these male-specific peaks are key in M. galloprovincialis and M. sutor discrimination of males during mating.

Further work to assess which compounds are most significant in potential attraction of M. galloprovincialis to Scots pine (Pinus sylvestris) was carried out in Germany. Volatile organic compounds (VOCs) of nearly mature P. sylvestris were compared in relation to host selection by M. galloprovincialis. P. sylvestris trees (2.50 m high) were tested at 25C. Controls, drought stressed trees (inoculated with water) and PWN-inoculated trees were sampled from either inoculated shoots or from unwounded branches over time. Chemical compounds emitted from the branches were analysed by gas chromatography. Potential differentiation by M. galloprovincialis of different host odours was studied by electroantennography using antennae removed from M. galloprovincialis captured in traps as well as beetles emerged from infested pine logs. Preliminary results showed some VOC differences between the tested pine groups and collection dates. Chemical compounds stimulating antennal reactions by M. galloprovincialis were identified and could give indicators for pine group differentiation by the adult beetles and lead to further improvement of the lures.

FLIGHT PERIODS OF EUROPEAN MONOCHAMUS SPP.

Detailed studies have been carried out in Austria, Germany, Portugal and Spain to determine the main emergence and flight periods of the local species of Monochamus.

M. galloprovincialis: Mid-May to late October, peak in June/July, earlier emergence in Madeira and mainland Portugal compared with Austria and Germany.
M. sutor: Early June to late October, peak in July
M. sartor: Early June to late October, peak in July
M. saltuarius: Early June to late October, peak in July

Although the data indicate considerable similarity in activity periods for the four Monochamus species trapped, there were regional differences related to local weather patterns, with low flight during cool and rainy periods, especially in Austria where trap catches were positively correlated with mean air temperatures. Early warning of adult emergence is valuable in relation to timing of trap/lure placement in the field. Building on preliminary studies in the PHRAME project on the required temperatures for larval development, it was confirmed that the development threshold was around 12.2C with an average of 822 degree-days (DD) required for full development to adult emergence. New experiments investigated whether DD changed with exposure of vector infested wood to sunlight (which would have had higher radiant heat exposure). DD accumulations were calculated from 1 March onwards and the emergence dates predicted by a sine wave model (University of California IPM online degree-day model). Model outputs were compared with observed cumulative emergences for five dates.

The emergence pattern of adults was not significantly different for the shaded and sun-exposed wood, with the first beetles appearing in mid-May and the last ones in mid-August in both cases, well within the normal pattern of M. galloprovincialis emergence in Portugal. The peak of emergence took place throughout the months of July and August. Overall, the sine wave method forecasted the emergence pattern of M. galloprovincialis with relatively high accuracy, resulting in similar deviations for the shaded and sun-exposed bolts. Apparently, the accumulation of heat by the sun-exposed wood does not result in a significantly earlier emergence period, and therefore the model can be employed to forecast the emergence pattern of M. galloprovincialis from field populations of sun-exposed trees in the natural environment.

DISPERSAL OF PWN THROUGH FLIGHT BY ADULT MONOCHAMUS SPP

With improved knowledge on emergence patterns and lifespans of European Monochamus spp., the project also assessed the behaviour of adults as the key factors in potential dispersal of PWN. Hence, flight capacities, including individual flights and accumulated distance flown over the lifetime of an adult, have been elucidated.

Flight dispersal studies, using flight mills (laboratory) or baited traps plus a combination of mark-release-recapture studies (field), have provided new insights into maximum natural dispersal which have major implications for assessment and development of future strategies to manage PWN infestations.

Dispersal by the newly emerged adults is the most important component driving natural spread of PWN in pine and other conifer forests. New information on flight characteristics of Monochamus galloprovincialis and other European Monochamus species indicates that previous understanding of this means of dispersal has under-estimated potential flight distances over the lifetime of an adult vector.

FLIGHT MILLS TO ASSESS FLIGHT POTENTIAL

A valuable technique to assess flight potential is the use of ‘flight mills’ in which an adult beetle is attached to a rotating arm and can be induced to fly so that each rotation of the arm can be measured and converted to distance flown. It also provides an estimate of the duration of each flight. Studies in Austria (M. sutor, M. sartor) and France (M. galloprovincialis) have provided information on flight capacities of these species of adult vectors.

M. sutor males and females covered distances of 1272 ± 348 m and 2008 ± 510 m, respectively, in individual flight events. Distances of individual flights of M. sartor were shorter; males travelled 810 ± 97 m and females 689 ± 82 m. Moreover, flight speed of the large M. sartor was slower. Nevertheless, this species is also able to travel more than 500 m in an individual flight.

For M. galloprovincialis, likelihood of flight, flight speed and total distance flown was investigated throughout the adult lifespan, and included effects of age, sex and body weight. A total of 77% of M. galloprovincialis adults flew on the flight mill with no significant difference between sexes. Total distance flown over the adult lifespan (99.5 ± 4 days since emergence) was 15.6 km, on average, for males, and 16.3 km for females (no significant difference between sexes), with a maximum flight distance of 62.7 km recorded for a male. Half of the tested population covered total flight distances exceeding 11.4 km. The average speed was similar in males and females, at about 1.4 m/s or 5 km/h. For both males and females, total distance flown increased significantly with body weight at emergence, but was not affected by relative weight gain over a 30-day period; this was also correlated to flight speed.

Beetle age had a significant negative effect on the number of individual flights per recording session. However the average distance covered per individual flight per session also increased significantly with beetle age. As a result, the insects flew on average the same distance per recording session (of 2h) irrespective of their age; they performed fewer but longer individual flights. In summary, individual flights were an average of 1.2 km (distance) and 5 km/h (speed) for both males and females. Presence of nematodes in the beetles was also investigated. From the 98 insects tested there was no significant difference in distance flown between the beetles with and without nematodes. However, only 15 beetles were carrying nematodes and with very small loads. Thus it was not possible to test the effect of high nematode loads on the flight capacities of M. galloprovincialis.

Using basic biochemical analysis the link between energy allocation and dispersal was explored. Distance flown on flight mills increased significantly with increasing proportion of energy reserves, but not with proportion of muscle mass in the thorax. This might be explained by an accumulation of energy reserves during insect maturation, which is essential to sustain flight activity. Studies in Spain showed that adults emerged with an average lipid content of 12.3% of their Dry Weight; this remained fairly constant during the initial 18 days of feeding. Wing muscle content in the thorax of adults at emergence was 26%/20% DW (males/females), and it increased to 36/39% DW after 18 days feeding. Further studies in Portugal indicated that fat content of newly emerged adults declined after maturation feeding commenced, whereas flight muscle increased after feeding.

USE OF TRAPS TO STUDY DISPERSAL IN THE FIELD

A study of dispersal behaviour of sexually immature and mature adult M. galloprovincialis in a low density P. pinaster forest was carried out in Spain employing mark, release and recapture. A sampling network grid of 200mx200m containing 64 multi-funnel traps baited with Galloprotect 2D + alpha-pinene (SEDQ), over an area of 256 ha was set up. 111 immature and 112 mature beetles were released from the centre of the grid from June 22nd to July 6th. 137 native insects caught were also marked and released. The experiment was sampled weekly over 15 weeks. Live recaptured insects were released again. Distances of recapture ranged from 141m to 990m. 26% of immature beetles and 32.1% of mature beetles were re-captured, with an average of 29.15%. Similar results were obtained in a similar experiment carried out in Portugal These data represent local flight distances which would accumulate to greater distances of the lifetime of an individual adult.

Studies in Spain were aimed to determine the distances that immature beetles may fly if they emerge in areas deprived of pines for feeding (i.e. ports on entry, warehouses, recently cut forests, etc.). Immature M. galloprovincialis adults were released downwind at each of 100m, 250m, 500m, 750m, 1000m, 1500m, 2000m, 2500m and 3000m distance from an isolated small pine stand (4 ha). A network of multi-funnel traps baited with Galloprotect 2D + alpha-pinene (SEDQ, Spain) was set up in the pine stand to trap any beetles reaching it. It was shown that adults released up to 2000m away reached the stand, confirming that recently emerged immature adults can perform flights of at least such distances in field areas without pines.

A further study in Portugal during 2014 used mark-recapture of M. galloprovincialis in an area without pine trees (agricultural land with rice, sunflowers and pastures); the closest pines were over 10km away. Traps baited with Galloprotect 2D alpha-pinene and containers loaded with fresh cut P. pinaster logs covered with a net with Temocid (TM) glue were place at 1 km, 2 km and 3 km from the release point and visited weekly to assess captures.

Most of the M. galloprovincialis beetles flew within 30 minutes after release. No beetles were recaptured and no other cerambycids were caught in the traps. After the removal of the traps from the site, the wood placed in the containers was debarked and no bark or wood boring beetle were found, confirming the absence of these insects in the test area and the inefficiency in recapturing the marked M. galloprovincialis. Placement of the traps on the floor (containers) and at 2 metres height (multifunnel) seems to be incompatible with the observed flight pattern of the M. galloprovincialis adults, since the released insects flew vertically on release and, therefore, at a higher altitude and probably spread out of the range of the traps' odour emissions. It is also possible that in the absence of a tree silhouette the insects had no visual stimuli to arrest the initial flight.

A mark-release-recapture study on a mountain slope in Austria showed that M. sartor and M. sutor dispersed in all directions, uphill as well as downhill and crossed mature spruce stands as well as open areas; one M. sutor male and one female were recaptured in the most distant trap at 380 m whereas the maximum recapture distance for M. sartor was 220 m. Median time between release and recapture was 7 days in both years of the study (maximum 25 days).

From these studies, especially the flight mills, it is clear that local flights can average 1.2 km (but can be up to 8.5 km) and can accumulate to around 16 km (but up to 63 km) over the lifetime of an adult. This has very strong implications for management of PWN infestations where a survey and clearfell regime is being employed. The situation is made more complex by the stimuli that either induce flight or minimise the need for dispersal by the adults. In dense forests where there is an abundance of trees for either maturation feeding (particularly) or egg laying, the adult vectors tend not to fly long distances; evidence from clustering of wilting trees in Japan and Portugal based on field surveys supports this finding. However, when there are very few trees, or where there has been a substantial felling regime to manage the PWN infestation, adult beetles tend to fly long distances (as demonstrated in the mark-recapture work in Portugal and Spain). Flight in such situations is initially vertical and is then followed by a horizontal dispersal flight. Field experiments using traps with lures supported by flight mill experiments in the laboratory indicate that flights of several km are likely in open areas without the stimulus of host trees.

DEVELOPMENT OF MOLECULAR TECHNIQUES TO ASSESS VECTOR DISPERSAL AND DISPERSAL ROUTES

A population genetic study, by the way of the development of 12 highly polymorphic molecular markers, offered an alternative for obtaining information about individual movements and effective dispersal over geographic and temporal scales. It provides valuable diagnostic tools for tracing movements of individuals in order to characterize long distance human-mediated transportation events.

Phylogeography revealed that four main lineages occurred in the Eastern and Western parts of the Iberian Peninsula and in Central and Eastern Europe (islands not included). Populations of the vector are highly structured at the European scale, reflecting the existence of natural barriers to gene flows. Even though the Pyrenean chain appears to constitute a barrier to dispersal of the vector, the genetic structure analysis revealed a signature of migration along the Atlantic coast and, to a lesser extent, along the Mediterranean coast; these could act as corridors for natural dispersal. At fine-scale analysis, we are able to detect patchy genetic structuring that could reveal zones of population admixture and/or long distance movements of the vector.

This study provides valuable information of potential pathways for the PWN in order to define suitable management strategies.

NEMATODE TRANSFER BY ADULT MONOCHAMUS

Our knowledge of flight characteristics and distances flown has increased significantly and, in order to assess the implications of such flights in relation to transfer of nematodes in the field, studies were also carried out on this aspect.

During the management of PWN in Portugal, populations of M. galloprovincialis adults have been assessed for presence of the nematode. Up to 75% of adults in areas with the highest incidence of PWN have been shown to carry the nematode. More recent, detailed, work in Portugal indicated that virtually all the nematodes carried were within the adults in the tracheae (breathing tubes). Around 60% of adults carrying nematodes had less than 1000 per adult, but a small proportion (approximately 5%) had an average of 45000 nematodes. These data fit with observations in other countries where a very wide range of nematode densities were found in the local Monochamus species. For example, studies in the USA by Marc Linit and colleagues have provided useful information on the carrying and transmission capacity of M. carolinensis in relation to PWN transfer to exotic Scots pine. An average of 6915 nematodes was recorded per adult beetle and these results were compared with previous studies indicating over 19000 PWN per adult. Transmission of nematodes takes place over most of the adult vector flight period such that, for M. galloprovincialis, nematodes were transferred during maturation feeding for up to 8 weeks, with a decline from the sixth week (work by Naves and colleagues). It was also found that nematodes were transmitted during oviposition by the female M. galloprovincialis confirming that both feeding and egg-laying are part of the tree to tree transfer of PWN by the vector insects in Europe. This mirrors the findings in all other countries where PWN has been recorded and where the local species of Monochamus has taken on the role of vector.

It follows, also, from the above findings that relatively low numbers of nematodes during each transmission event are sufficient to ensure survival and growth of PWN. Breeding by the nematode in trees succumbing to pine wilt or in proximity to the larval development sites of the vector maintains the presence of the nematode in close proximity to the developing and emerging adult beetles. The change in the development stage of the nematode to the dauer fourth stage larva and aggregation near the pupal chamber in readiness to migrate to the newly formed adult appears to be triggered by specific host volatiles and an increase in the concentration of CO_2 as the beetle becomes a callow (newly formed) adult. This has been confirmed by recent work in Portugal within REPHRAME which found a significant increase in the CO_2 release rate of over 1.6 times from pupae and larvae to the callow adult stage.

POTENTIAL OF MASS TRAPPING TO REDUCE MONOCHAMUS POPULATIONS

Experiments have been carried out in Spain using a network of the recommended traps and lures at different densities in forest locations and analysis of captures relative to estimate local population size of M. galloprovincialis. These data have been used to fit a mathematical function relating the population expected to be removed by mass trapping to the intensity of trapping (trap density) for moderate beetle population density. This relationship indicated that a mass trapping campaign might be able to remove a high proportion of the local beetle population; i.e. for the sites in Spain, a set of regularly spaced traps at 0.44 traps/ha (i.e. traps spaced every 150m) was able to remove 60% of M. galloprovincialis adults from a moderate population (82 beetles/ha).

Similar relationships should extend the range to other beetle densities, including low densities, for which a response curve is being developed. In combination, this approach should provide a tool to help managers to determine a suitable trapping intensity in mass trapping programmes dependent on their objectives and resources.

QUESTION 4: CAN NEMATODES TRANSFER OR BE TRANSFERRED BETWEEN TREES WITHOUT MONOCHAMUS VECTORS?

Although it is clear that the transfer of B. xylophilus from tree to tree in nature is through the activity of vector insects in the genus Monochamus, the movement of PWN-infested wood by human activity introduces another potential mechanism for transfer in the absence of insect vectors. This has been investigated both within REPHRAME and through a separate study carried out by beneficiary 6 (with input from beneficiary 1) funded by CHEP Europe (a pallet logistics company). These combined studies looked at direct transfer of nematodes from infested wood (either sawn wood or wood chips) to living trees or to other pieces of wood with no vector present.

WOOD TO WOOD TRANSFER OF PWN

Although originally planned in REPHRAME, it was felt that the work carried out in Portugal on wood to wood transfer funded by CHEP and published in the EPPO Bulletin had covered this topic well and no further work was done in the project. In summary, the results indicated there was successful nematode transfer from donor (wood derived from pine wilt killed trees in forests in Portugal) to recipient (non-infested) wood materials kept at 25°C. Donor wood had initial moisture content (MC) of 70% or more whereas recipient wood (range of sizes typical of pallets) had a range of MCs from high (>70%) to fibre saturation (around 30%) to dry (<20%), including heat treated wood. Nematodes transferred rapidly and reproduced in recipient wood provided that the MC of the recipient was >20% (partial transfer) or above 30% (full transfer). Overall, there was no nematode transfer to kiln dried or in-service boards (i.e. wood taken from pallets that were naturally at <20% MC), or in the board-to-board treatments at 10°C. When it occurred, nematode transfer took place within the first week in all experiments, being more successful and abundant in freshly cut recipient wood with high MC. The nematode load of the donor wood appeared to play a role in nematode transfer, as higher donor densities resulted in more frequent B. xylophilus transfers and significantly higher nematode densities in adjacent wood pieces. Initially, nematode populations increased (breeding in residual wood cells) but after peaking, nematode numbers declined gradually until the final sample, being virtually zero by week 40 in both donors and recipients. Overall, a weak but significant correlation was detected between the development and survival of the nematode population and the MC in the wood ($r = 0.1312$, $P = 0.0001$). Nematode decline coincided with a generalised decrease in MC to about or below the fibre saturation point, when rehydration of the wood was no longer possible.

This result, therefore, indicates that when a PWN-infested piece of wood comes into contact with wood with MC above fibre saturation (e.g. as would be the case in a living tree), there appears to be no major barrier to nematode movement into the recipient wood. This was investigated further within REPHRAME in spring 2014 by experimentation to assess possible transfer of PWN from infested wood boards to living P. pinaster trees (known to be nematode-free). Fresh pine boards were inoculated with PWN under controlled conditions, yielding infested boards averaging 100 nematodes per gram (fw) wood. The boards were tied to healthy pines (27-41 cm DBH) in three different arrangements: (1) board tied to tree on intact bark, (2) board tied to tree with exposed cambium and (3) board tied to tree with exposed inner wood (xylem). Fresh non-infested pine boards were used as controls in the same configurations. The trial took place before the flight period of M. galloprovincialis in order to ensure that no insect-vectored nematode infestation of the pines occurred. 45 days after the establishment of the experiment in the field, the trees were felled and both the boards and the trunk adjacent to the board were sampled for PWN infestation.

When boards were attached to exposed cambium or to exposed inner wood, nematode transmission took place in a single pine for both configurations, with nematode densities in the trunk of 100-300 per 100 g stem wood (fw). All boards initially inoculated with nematodes were still infested at the end of the trial. The results demonstrate the capacity of B. xylophilus to infest living trees through wood contact, provided there is no bark to act as a barrier to invasion.

This is a unique study and revealed that infestations can take place in situations when nematode-infested lumber comes into contact with exposed cambium or xylem of a living tree. One out of five trees (20 %) was infested in this experiment. For technical reasons the trial had to be restricted to 45 days only. If the experiment had been continued for a longer period, there is a possibility that the frequency of infestation could have been higher. Although studies in Japan have shown that B. xylophilus is capable of moving over bark surfaces, infestation of trees through intact bark has not been demonstrated. In the REPHRAME experiments, the presence of intact bark on the recipient trees appears to have protected them from nematode infestation. While it is not clear what the bark barrier contributes to preventing transfer, the information on moisture content in the wood to wood transfer studies described earlier suggests that provision of a moisture ‘pathway’ is needed for nematode transfer. Infestation of trees as a result of contact transmission has previously been observed in dense natural stands of P. sylvestris where branches come in close physical contact (Malek & Appleby 1982 in the USA). Although the likelihood of PWN-infested wood being placed in direct contact with exposed tissues of living trees is low, these new results indicate that consideration needs to be given to managing the end use of wood coming from nematode infested areas.

SURVIVAL OF PWN IN WOOD CHIPS AND POTENTIAL TRANSFER FROM CHIPS TO LIVING TREES

SURVIVAL IN WOOD CHIPS OVER TIME

The potential effects of different temperatures occurring in wood chip piles over time in relation to survival of PWN were determined in Germany using PWN in pure culture multiplied on the fungus medium Botrytis cinerea on agar Petri dishes. After PWN reproduction at 25C for 14 days, the plates were incubated at different test temperatures (from 25C to 60C in 5C steps) and a range of time periods (24/ 48/ 72/ 96/ 120 h) followed by nematode extraction to assess survival or reproduction. At the beginning of the experiment and after PWN reproduction, 5% males, 11% females and 84% juveniles were recorded. PWN did not survive temperatures of 50C, 55C and 60C for 24 h. By contrast at 25C, 30C, 35C and 40C very high numbers (1000s) of living PWN could still be found after 5 days. At 45C after 72 h no PWN could be extracted.

Natural composting processes in wood chip piles can result in a wide range of elevated temperatures but not throughout a given chip pile. The results from this study on survival of PWN at different temperatures over time indicate that composting of wood chips is likely to include both lethal and non-lethal temperatures for PWN. These data therefore confirm the increasing view that natural composting is not a reliable procedure for killing PWN in wood chip piles unless rigorous and reliable ways can be found to turn the pile to ensure high temperatures throughout.

Experiments carried out in Germany studied survival of PWN directly in wood chips that were exposed to different temperatures and either allowed to air dry or retained in a plastic bag to keep higher moisture content. Nematode densities declined over time of wood chip storage; nematode density decreased from 374 nematodes/gdry matter initially to 0 nematodes after 369 days for the wood chips allowed to air-dry at 15C and 25C. Nematodes could be extracted from the 25C air-dried wood chips up to 123 days (over 17.5 weeks) after the test start. Sealing of the bags to retain higher MC had a positive influence on nematode survival. At the end of the test, after more than one year, nematodes were still present in sealed variants at both temperatures. For most test dates, the 15C variant had higher nematode densities, but at the beginning and end of the experiment, the 25C variant showed larger values. Therefore at the end of the experiment, the highest nematode density was found for wood chips in plastic bags at 25C with 57 nematodes/gdry matter (median).

These results confirm that if moisture content is retained above ambient air-dried values (which would reach equilibrium MC of around 16%) then nematode survival for at least one year is possible, although there is a decline over this period. Such conditions could be found within the core of a wood chip pile which would also provide conditions suitable for fungal growth to aid nematode survival and reproduction. However, this is balanced against natural composting processes that could elevate temperatures to lethal levels for the nematode. In conclusion, it appears that nematode survival is possible in certain parts of a chip pile (especially a large pile) which would have sub-lethal temperatures but retain sufficient moisture to support nematodes.

POTENTIAL TRANSFER FROM PWN-INFESTED WOOD CHIPS TO LIVING TREES

There is some evidence from the literature that PWN can move from infested wood chips and enter living seedling trees if the roots of those trees are damaged. This is a potential route of non-vector transmission and, therefore, work was carried out in Germany and in Portugal to evaluate this as part of the non-vector study.

In Germany, non-vector transfer of PWN from infested wood chips to non-infested P. sylvestris saplings was investigated under quarantine containment. Wood chips were produced from artificially PWN-inoculated pine logs. At 15C and 25C stem-injured, root-injured or uninjured and severed saplings were tested without wood chips (controls) and wood chips either with direct contact to the stem, mixed in the soil or on the soil surface. The trees were evaluated for pine wilt symptoms over three months. PWN were extracted shortly before tree death, otherwise at the end of the experiment. PWN transmission from chips to trees was influenced significantly by temperature and the occurrence of wounds either on the stem or to the roots. While at 15C only 1.5% of the variants showed successful transmission, this rose to 18% at 25C. PWN-infested wood chips placed directly on stem wounds led to PWN infestation in more than 80% of the tested trees. At both temperatures almost no PWN transmission could be observed in non-damaged trees and in the variants containing severed trees.

Similar studies in Portugal assessed potential transfer of PWN from infested wood chips to 5-year-old P. pinaster with intact and artificially wounded roots. Chips were produced from PWN-inoculated logs. Trees were planted in sand mixed with 2l of chips containing 300 nematodes per gram (fw) wood. In controls, sand was mixed with fresh chips. The plants were checked for wilt symptoms for 14 months and were all sampled for PWN infestation regardless of the symptoms. Periodically, in the first 90 days of the experiment, the nematode community in the soil was analysed. By the end of the experiment, 33% of the pines that received PWN-infested chips were dead, with nematode densities ranging from 0 to 16200 per 100 g stem wood (fw). A single tree in the PWN set and one in the control set with intact roots was dead, although the presence of PWN was not detected. PWN was not detected in all the symptomless plants in the four experiment treatments that were analysed. In the soil, saprophagous nematodes in the order Rhabditida dominated the material, followed by the miscellaneous feeders (omnivores) in the family Dorylaimidae. Fungal feeders, i.e. genera Aphelenchus, Aphelenchoides, Boleodorus and species in Tylenchus sensu lato were noted less frequently. Strict root-parasites (i.e. genera Tylenchorhynchus, Pratylenchus, Rotylenchus and Helicotylenchus) occurred in low frequencies. Strict predators in the family Monochidae were rare. Bursaphelenchus xylophilus was absent in the soil material sampled.

In the literature dealing with B. xylophilus, studies on nematode transmission from infested wood to living trees are uncommon. In most experiments, wounded roots have been a prerequisite for successful transmission from nematode infested soil (Mamiya & Shoji 1989) or chips (Halik & Bergdahl 1992) to roots. Our experiment also demonstrated that PWN can infest small trees of P. pinaster with artificially damaged roots with 4 out of 12 trees with damaged roots becoming infested by the nematode.

Nematode densities in infested trees were significant, ranging from 420-16,200 individuals per 100 gram fresh stem wood. Nematode infestations of plants with damaged roots planted in soil-chips mixtures have previously been reported in 3-5 year-old plants of Pinus sylvestris, P. strobus, and P. resinosa (Halik & Bergdahl 1992). Early experiments in Japan demonstrated that PWN infestation of the trees could be established from nematode-infested wood discs buried in soil around roots on 14-year-old P. thunbergii; however in this case the roots were reported as undamaged. Kiyohara & Tokushige (1971) reported an infestation frequency of 50%, while Halik and Bergdahl (1992) recorded frequencies of 50% in P. sylvestris and 8% in P. strobus and P. resinosa. The 30% infestation reported in the present study is the first record of soil transmission of PWN to P. pinaster.

In previous studies the occurrence of B. xylophilus in soil during the infestation process has not been studied in detail, but it has been noted that the nematode can survive in soil for 48 hours to 1 month (Mamiya & Shoji 1989). In the present study we were unable to detect PWN in soil samples taken regularly up to 90 days post-infestation. The nematode fauna recorded in this experiment showed a normal composition and occurrence of nematode groups and genera.

The results from Germany on P. sylvestris and from Portugal on P. pinaster demonstrate that non-vector spread of PWN from wood chips to trees is possible under certain circumstances. Tree wounds, direct wood chip contact and an optimal temperature increase the likelihood of this occurring. PWN-infested wood chips used as a surface layer on paths and as soil mulch with direct contact to trees could serve as a potential infestation pathway. This is a more likely use of wood in a woodland situation than attaching wood boards to trees and, therefore, should be considered carefully in future management of pathways to reduce the likelihood of human-assisted PWN spread.

POTENTIAL TRANSFER FROM PWN-INFESTED TREES TO ADJACENT LIVING TREES

The clustering of PWN-infested trees in a forest situation can be attributed mainly to local dispersal by Monochamus spp during maturation feeding. However, another possibility is that nematodes can move directly from an infested tree to a neighbouring tree by movement through the soil or through root grafting by adjacent trees. This has been investigated in Germany and Portugal within the current project.

In Germany PWN spread from infested trees to neighbouring trees through the soil or by root contact was examined using pairs of P. sylvestris saplings grown at 25C in a quarantine greenhouse. A nematode suspension was inoculated in one of the pair of trees and evaluated over more than one year for pine wilt symptoms. In the variant where both trees were planted in one pot to investigate migration in the soil or by natural root grafts, all PWN inoculated pines died and PWN could be re-isolated, while none of the neighbouring trees died or contained PWN. Artificial root grafting was not successful, however. Therefore, transmission of PWN from tree to tree by root grafting or via soil migration did not take place under the given test conditions.

Similar experiments were carried out in Portugal using 5 year old P. pinaster as the host tree species. The trees were potted in pairs in 80l boxes. In the nematode treatment one tree in each pair was inoculated with 6,000 PWN at 6 points along the stem. For controls, one tree in each pair was inoculated with water. The trees were kept under observation for wilting symptoms and were sampled for presence of PWN. The experiment has been running for 16 months. In this period 69% of the inoculated pines died with nematode densities ranging from 500 to over 5000 per 100 g stem wood (fw). None of the neighbours (recipients) of the inoculated trees showed symptoms. Mortality occurred in both donor trees and in some recipient control trees, but no PWN was detected in these. The regular observation of the pines for wilt symptoms and sampling is still in progress. Air and soil temperature conditions favourable for the development of PWN were present throughout the experiment.

No mortality was observed in the recipient trees even though 11 out of 16 donor trees died from the nematode infestation with high nematode densities in the stem wood. One possible route for infestation between infested trees and healthy neighbour trees would be through root grafts which are known to occur in at least 26 Pinus spp. However, the frequency of root grafting may vary between species of pine. Roof grafts have not been detected in maritime pine P. pinaster in Portugal (PHRAME project 2007.

Experiments on root transmission in the field were carried out in the Lisbon area and were located at Companhia das Lezírias and Mata da Machada. Each experimental plot contained healthy trees with ca. 40 cm DBH with smaller trees nearby. The absence of B. xylophilus on those trees was confirmed by laboratory analysis and they were covered with nets to prevent access by vector insects. The healthy trees were ring barked to cause weakening stress and were inoculated at the base with 50,000 PWN (10 x 5 ml of a suspension of 1000 nematodes/ ml) using Arbojet Quick-Jet kit. The trees inoculated with B. xylophilus were sampled one month after inoculation to confirm the effectiveness of this process. The covered pines are being observed regularly for wilt symptom detection and for PWN sampling. Four months after inoculation the inoculated pines exhibited wilting symptoms. In Mata da Machada, one small covered tree died but no B. xylophilus was detected. Observation of the covered trees is still in progress.

Although still continuing, these laboratory and field observations indicate that direct tree to tree transmission of PWN in either P. pinaster or P. sylvestris does not take place. This suggests that such a transmission route is of very low significance relative to the natural transmission by vector insects and, in any event, further transfer would require re-connection with the vector to ensure wider dispersal.

QUESTION 5: ARE SOME TREE SPECIES RESISTANT, OR IS PINE WILT ENVIRONMENTALLY DRIVEN?

In its natural range in North America, pine wilt is a relatively unusual occurrence on native pine trees and only appears to express to a significant extent in non-native pines such as P. sylvestris. This indicates that some pine species are highly tolerant or fully resistant to the nematode. For example, sand pine (P. clausa), slash pine (P. elliottii), pitch pine (P. rigida) and Virginia pine (P. virginiana), native North American pines, are all regarded as resistant to pine wilt. An important question for Europe is whether any of the native pine species are resistant and, even among the susceptible pines, is there scope for selecting and propagating some species with higher tolerance or resistance? Since the pine wilt disease syndrome is a combination of transmission of PWN arising from host tree selection by the vector as well as intrinsic susceptibility to PWN itself, investigation of possible resistance includes susceptibility both to the vector and to the nematode.

IDENTIFICATION OF PUTATIVE RESISTANCE GENES IN PINES

Ninety-six half-sib progenies were obtained from 120 plus trees selected for tolerance/resistance, at Comporta, and sown at Obidos nursery (Quinta do Furadouro) in November 2012. In April 2014, all the plants were transferred to Oeiras nursery. A randomized complete block design was applied for the 96 half-sib families, with 4 blocks and 15 plants per family. All the 5760 plants were measured for total height and diameter at the base and inoculation tests were done in August/September 2014 using 500µl/tree, of a suspension of 1000 PWN/ml. Symptom evaluation began 27 days after the inoculation. Three evaluations were done until the end of November 2014 (27, 38 and 57 days after inoculation). Four symptom levels were considered: 1-healthy or less than 25% of dead needles; 2- 25%-50% of dead needles; 3- 51-75% of dead needles; 4- More than 75% of dead needles or dead. Every week the air temperature in the greenhouse was recorded.

The results are being analysed, but the final results will be obtained only in spring 2015, when the air temperature will be higher. Over winter 2014, the air temperature is low which may influence PWN behaviour inside the plant. However, some families have already shown more tolerance than others. Statistical analyses, logistic and ordinal regression, will be applied at the end of the trial.

Other studies have been carried out to assess whether crosses between susceptible and resistant pines can provide resistance in the progeny. In this study artificial pollination between two pine species was carried out to obtain hybrid seedlings for testing potential resistance to B. xylophilus. Artificial hybridization between P. pinaster x P. halepensis and P. halepensis X P. pinaster was performed. In both species, seed maturation takes approximately 20 months. 1332 seeds from 19 fully mature seed cones from P. pinaster x P. halepensis crosses were obtained. These were manually extracted, examined, and a buoyancy test used to discard empty seeds, which totalled 497. Of the remaining seeds, 835 were sown in containers from January 30-February 4, 2014. Germination of each batch ranged from 0-100% and the seedlings are being grown on for growth characteristics, genetic analysis and susceptibility to PWN. This is ongoing since the seedlings are still too small for inoculation.

Pathogenicity of PWN towards different German P. sylvestris provenances was evaluated. Saplings of the German provenances 851 02, 03, 08, 13, 14, 15, 20 and 22 which covered origins from north, south, east and west of Germany were tested at 25C over three months. For each provenance, PWN-inoculated and water-inoculated plants were studied for wilt symptoms and nematode density. All PWN-inoculated pines died, with some mortality of non-inoculated control trees. In addition, there was no significant difference in the development of wilt symptoms between the PWN inoculated provenances. PWN was extracted from the inoculated saplings of all tested provenances but not from the controls. The MC of the plants inoculated with PWN was reduced compared to the control trees. To exclude the influence of the non-sporulating Botrytis cinerea strain used to multiply PWN, inoculations using either a filtrate or direct stem contact of fungal mycelium to the wounded stem were carried out. No mortality or effects on growth and physiological conditions of the trees was recorded.

As will be apparent, the long-term nature of research into potential resistance to PWN has limited the data that could be gathered within the time frame of the project. However, further work will continue in Portugal using the established crosses.

POTENTIAL RESISTANCE OF PINES TO MONOCHAMUS FEEDING OR OVIPOSITION

Field observations suggest that umbrella pine (P. pinea) is avoided by M. galloprovincialis for both maturation feeding and oviposition in areas of Portugal with and without PWN present. However, laboratory experiments in Spain to evaluate the suitability of P. pinea as a host of M. galloprovincialis showed that P. pinea was accepted as a host for both feeding and oviposition and that M. galloprovincialis progeny could complete its development to adult on this pine species. A detailed assessment by EFSA (European Food Safety Authority) concluded that further field evidence is needed to confirm this finding. However, in practical terms, it appears that P. pinea is highly tolerant and receives very little pressure from resident M. galloprovincialis populations in Portugal.

For P. pinaster there is no evidence for resistance to attack by M. galloprovincialis but studies in Portugal indicate that the females have distinct preferences for oviposition depending on the freshness of stressed or newly dead pines. In this experiment M. galloprovincialis females were allowed to lay eggs on P. pinaster bolts from healthy trees at 4 different times after being cut (7, 31, 74 and 115 days) to simulate different ages of breeding resources in the field. Overall, a total of 100 eggs were laid in 28 out of 60 available wood logs (47%), with significant differences depending on age of the wood. The logs that were 7 and 31 days after cutting were preferred for oviposition (both total egg numbers and number of separate oviposition events).

Although this finding is not related particularly to pine species differences, the information has implications for the silvicultural management of vector populations, since there is a lower risk for wood that has been dead for more than two months (either by felling or from other biotic or abiotic factors) to be used for egg laying. Therefore, there is a prolonged period from mid-autumn to early spring when healthy trees can be felled and remain in the field for the subsequent months, because when the beetles emerge in the following spring, this wood will have a low attractiveness for the breeding Monochamus females.

QUESTION 6: WHICH PARTS OF EUROPE ARE VULNERABLE TO PINE WILT EXPRESSION?

One of the major concerns in relation to the future impacts of PWN in Europe is the likelihood of wilt expression in infested trees and where wilt will be apparent under current and future climates. In addition, any projection of wilt expression needs to combine with likelihood of B. xylophilus reaching different regions of Europe to give an overall risk rating for the future. These aspects have been studied in detail within REPHRAME and new models produced to quantify likely wilt expression and spread of PWN in Europe.

A PROCESS MODEL TO PREDICT PINE WILT UNDER CURRENT AND FUTURE CLIMATES

A process-based ETpN (Evapo-Transpiration + Nematode) model has been developed to model the interaction between B. xylophilus and living host trees. Nematode population density (with a temperature dependent growth rate) affects the amount of photosynthesis that can take place (where it is assumed that number of losses of water conductance (cavitations) inside the xylem of the tree depends on the number of nematodes present). As part of the model, we have introduced an available energy element, positively increased by photosynthesis but reduced by tree defence against PWN. If photosynthesis and available energy become zero this is interpreted as tree death from pine wilt disease (PWD). The ETpN model has been calibrated using data from Portugal (where PWD has affected many regions) and, particularly, data from Japan where PWD is severe and has a long history. The model output is in close agreement with the known distribution and history of PWD in Japan which provides confidence that it provides an effective simulation of pine wilt.

Although an accurate and sophisticated predictor of pine wilt, the ETpN model requires extensive climatic data and site parameters to run, which make it less useful for wide application by non-specialists. To overcome this, we have developed a user-friendly simplified model that will allow a general user to determine the likelihood of PWD at their location/or for particular climates. Following sensitivity and statistical analyses, the key climatic variables and parameters that drive the model have been determined. Although several parameters have some effect on the model output, climate data are the key driving variables. A User-friendly sub-model has, therefore, been developed, using two different approaches.

The first, and most accurate, method is to use Mean Summer Temperature (MST), the average of mean daily temperature over June, July and August, to predict the likelihood of wilt at a particular location, where:

Locations with MST 20°C are at a high risk of PWD
Locations with MST < 19.31°C are at a low risk of PWD, and
Locations with 19.31°C   MST < 20°C are at risk of some PWD.

When a user does not have any information about average climate parameters, a simple location model can be used (for -9   Longitude   55 degrees), where we predict (x and y represent longitude and latitude respectively):

a high risk of PWD if $y \leq f(x)$,
a medium risk of PWD if $f(x) < y \leq f(x)+1.2$,
a medium risk of PWD if $y \leq f(x)$ and altitude is 800m, and
a low risk of PWD if $y > f(x)+1.2$,

where

$$f(x) = a13x^{13} + a12x^{12} + a11x^{11} + a10x^{10} + a9x^9 + a8x^8 + a7x^7 + a6x^6 + a5x^5 + a4x^4 + a3x^3 + a2x^2 + a1x + a0$$

and

a13 = -4.2716235E-17   a6 = -2.0946235E-5
a12 = 1.2184542E-14   a5 = 0.000069825976
a11 = -1.4265794E-12   a4 = 0.0024502893
a10 = 8.6199246E-11   a3 = -0.014679433
a9 = -2.6591771E-9   a2 = -0.1129682
a8 = 2.5733313E-8   a1 = 0.68441653
a7 = 7.3633335E-7   a0 = 46.391586

Although this looks to be a complex formula, it can easily be included in a spreadsheet to produce the location-based outputs.

LATENCY IN EXPRESSION OF PINE WILT

The ETpN model provides accurate output and also an indication of how quickly the trees are likely to die in the period following introduction to the tree (inoculation) by maturation feeding by Monochamus spp. When conditions are suitable, trees exhibit pine wilt very quickly and both symptom development and tree death occur within a few weeks in the year of inoculation. However, the model also indicates when symptom development is delayed and tree death occurs in the year following inoculation, or even later. Although symptoms are delayed, the model indicates that physiological processes within the tree are already compromised and this could affect its susceptibility to oviposition by Monochamus spp or to attacks by other biotic agents such as bark beetles.

Taking account of this ‘lag’, we have developed a latency sub-model to determine which areas of Europe are likely to experience delays (latency) in wilt symptoms and tree death. Model parameters, location parameters and climate have a combined effect on latency. The key model parameters driving symptom development are inoculation day, initial nematode number and tree tolerance. Latency is more likely for later inoculation dates (i.e. late in the flight period of Monochamus spp), lower initial nematode numbers and higher tree tolerance. Model simulations, where initial nematode numbers are used in the interval 10-10000, predict PWD symptoms in the year of infestation for an increasing number of locations as initial nematode numbers are increased. With regards to the inoculation day, the model predicts PWD in the year of infestation at 62% of locations when inoculation day is Julian day 160 (9th June), while for Julian day 200 (19th July), the model predicts

PWD in the year after infestation for over 75% of locations and when inoculation day is Julian day 240 (28th August), the model predicts PWD in the year after infestation for 100% of locations. There is some evidence that latitude, longitude and altitude have an effect on latency but these co-vary with climate variables which have a significantly greater effect on latency. The principal climate drivers for latency are mean summer temperature (MST) and mean annual temperature (MAT). MST, MAT (both in degrees C) and inoculation day are used to predict the year in which we expect to see wilt, while MST is used to predict the number of days/months from inoculation to death. An interactive model (which is available on the REPHRAME website) uses these results to predict the risk of PWD and likelihood of latency.

With regard to possible wilt expression under future climates, the ETpN model has been run using two climate models for Europe; A1B - medium-high emissions scenario with relatively high temperature increases and E1 – a mitigation scenario with moderate temperature increases.

Under current climate (2009-2011 data), PWD symptoms are predicted for Lisbon, Portugal but not for Bourges, France or Junsele, Sweden. In Lisbon we see death in the year following inoculation. For E1 2070-2100 climate projections, the model predicts PWD symptoms and death in Lisbon 3 months after inoculation and PWD symptoms for Bourges two years after inoculation. No PWD symptoms are expected in Junsele. For the higher temperatures expected under the A1B 2070-2100 climate projections the model predicts PWD symptoms in Lisbon a month earlier than for the E1 climate projection. In Bourges, PWD symptoms are expected over two months earlier than for the E1 scenario (in third year), although there are signs of PWD in the second year. No PWD symptoms are expected in Junsele.

The REPHRAME website also includes risk maps for simplified climate assumptions for current temperature and +1C, +2C and +3C. As might be expected, wilt expression and the zone of expected latency moves northward and eastward with increasing Mean Summer Temperature.

CORRELATION MODELS EMPLOYING FIELD DATA ON FOREST LOCATIONS, CLIMATE AND KNOWN PWN DISTRIBUTION IN PORTUGAL AND SPAIN

From 2008 to 2011, 11425 samples from symptomatic trees in Portugal were assessed for PWN, with 10597 negative results and 828 positive results. This information has enabled quantification of PWD severity throughout Portugal on a geographic grid basis, which has been combined with climate drivers for each grid. There is a good correlation between climate and observed PWD incidence, especially for central Portugal, around Sines, and Nazaré. This model approach predicts light PWD incidence and tree mortality in north Portugal, which fits with observations and with outputs from the ETpN model.

The model predicts a low incidence of PWD in Spain, except in the regions around Cadiz, near Vigo and in the Basque country (around 20-25% of PWD). It is interesting that it predicts low PWD (around 1%) near the coast in almost all the Iberian Peninsula. The Vigo location and Extremadura are consistent with the observed first entry points of PWN in Spain.

A more complex pattern is apparent in Central Portugal, where relatively high percentage mortality is predicted, with summer precipitation between 71 and 89 mm. Continentality (the difference between inland and coastal temperatures), distance from the coast and summer precipitation proved to be the main variables for PWD quantitative distribution. Interestingly, even with high summer temperatures (>30C) if there is low continentality (<25C) and low distance from the coast the model predicts low PWD. The highest predicted PWD of 41% is for moderate summer precipitation (21-25mm), highest summer temperature <28C and continentality <25C. Essentially, the variables explaining PWD mortality distribution interact in different ways in order to produce the observed distribution of PWD in Portugal. Nevertheless, these complex interactions determine that continentality, distance to the coast and summer precipitation are the main drivers in explaining PWD in Portugal.

Along with similar outputs from ETpN, this simplistic modelling approach offers the possibility of having low impact zones of PWD in Portugal. Such an approach can be augmented by use of remote sensing to track past and new areas with symptoms across the Iberian Peninsula and should be included in future management options.

MODELLING POTENTIAL SPREAD OF PWN ACROSS EUROPE

The spread model comprises several sub-models describing various mechanisms involved in the spread of the pine wood nematode and calibrated to the spread mechanism observed in Europe. These sub-models have been integrated and simulations of spread at small scale (Iberian Peninsula) to validate the model were carried out, followed by some future projections at the European scale.

VECTOR DISPERSAL:

A dispersal kernel was fitted to flight mill data using three scenarios:
D (“daily”): the mean daily dispersal distance is 2268 m
M (“moderate”): the mean daily dispersal distance is 1296 m
W (“weekly”): the mean daily dispersal distance is 324 m

The results were converted to population projections for a grid of 1 km x 1 km over the Iberian Peninsula (for validation) and a grid of 10 km x 10 km over Europe (for projections).

VECTOR EGG-LAYING:

Each female lays eggs related to tree species:
P. pinaster - 2 eggs every day from day 20 to 53
P. sylvestris – 4 eggs every day from day 17 to 48
Other pines - 3 eggs every day from day 15 to 33

PWN TRANSMISSION:

This includes transmission by maturation feeding or through oviposition related to age of the beetle, according to published studies. Data are extrapolated to population level according to the proportion of healthy trees relative to declining trees.

PWD EXPRESSION:

Simplified from ETpN using Mean Summer Temperature of 20C.

PWN CONTROL:

Given the detection effort on symptomatic and non-symptomatic trees, given the radius of the clear cut belt (tree removal from 0 to 3000 m tested), we calculate the transmission rate of the pine wood nematode. According to the dispersal scenario (D, M or W), the efficiency of the control varies considerably.

ACCIDENTAL (HUMAN-ASSISTED) TRANSPORTATION:

Three factors assumed to play a role in nematode dispersal over long distances: human population density, road density and wood factory density.

VECTOR EMERGENCE:

Degree-day model to calculate development time and thus the proportion of the beetle population that emerges as an adult within one year, versus one that requires two years to complete development.

The model was run for Europe using a range of parameters and the principal outputs were:

- Pine wilt disease expression could be expected in northern Portugal and possibly in Central Spain by 2020 (even under current control measures);
- Pine wilt disease is unlikely to be expressed in most of Europe in the immediate future, unless there are secondary introductions elsewhere (especially in Hungary or Slovenia)

- Consequently, it is very important to avoid additional introductions;
- A map showing the risk of introducing pine wood nematode in Europe was constructed based on human population density, road density and wood factory density. Intensifying controls in these high risk areas is necessary to avoid other introductions and rapid spread throughout Europe.

Another model was developed specifically for the Pyrenean chain in combination with genetic analysis of the vector. It shows that:
- This mountainous area separating the Iberian Peninsula from the rest of Europe could play a role as a partial barrier to the spread of PWN due to its high elevation and low temperatures
- However, infested vectors could eventually cross this zone on the lower elevation western or eastern edges of the Pyrenean Mountains
- Intensifying controls on western and eastern sides of the Pyrenean chain is necessary to avoid the “natural” spread of the vector carrying the nematode to the rest of Europe.

Under warmer future climates, the potential spread of PWN and associated expression of pine wilt disease would be more rapid:

- for instance, an introduction of the nematode to Belgium may not result in expression of PWD under current climate conditions, but with a 2°C temperature increase, this could lead to tree mortality and an accelerated range expansion throughout Europe
- Moderate warming will reduce the extent of the Pyrenean barrier and will facilitate the “natural” spread across this mountainous chain and thus the spread to the rest of Europe.

Potential impact and main dissemination activities and exploitation results

As indicated in the main report, there are a number of keynote findings that, when brought together and interpreted, have provided valuable new insights into the causes of, and likely extent of, pine wilt disease in a European context. This information provides a knowledge base for improved management of the Bursaphelenchus xylophilus problem in Europe. The key findings as a basis for management improvement and long-term impact are summarised below.

VECTOR MONITORING

REPHRAME has confirmed that Monochamus galloprovincialis is the only demonstrated vector of PWN in the affected area of Europe, a finding that fits with global experience that it is only this genus of beetle that carries PWN from tree to tree. Therefore, attention to managing the vectors remains core to reducing the impacts of PWN and minimising the likelihood of spread. Effective monitoring of Monochamus vectors is now well developed with highly efficient attractant lures and traps. Galloprotect 2D (SEDQ, Barcelona Spain), composed of the M. galloprovincialis pheromone (2-undecyloxy-1-ethanol) plus two kairomones (bark beetle pheromones ipsenol and methyl-butenol), identified by REPHRAME beneficiaries 2 and 9, attracts both sexes of this species of beetle. It is now recommended as the standard lure for M. galloprovincialis monitoring. Research in REPHRAME has indicated that the lure and trap combination is also effective for monitoring other European Monochamus species, notably M. sutor and M. sartor and the lure is, therefore, recommended for all European Monochamus spp. With regard to further improving trap captures and avoiding accidental captures of non-target beetles and beneficial predators, it was found that some smoke compounds (methoxyphenols) were good synergists of the standard lure compared with terpenes, but without attracting non target insects. Smoke volatiles are likely to be included in future composition of the lures.

Effective and, therefore, recommended traps for vector monitoring and mass trapping include multi-funnel and cross-vane designs (both commercially available; e.g. CROSSTRAP®, with Crosstrap Collection 2 litre Cup or ECONEX MULTIFUNNEL-12® with Econex Multifunnel Extended Collection Cup). Regardless of source of trap, it was shown that it is essential to have Teflon coating to prevent escape of captured beetles. The trap and lure combination now provides a standardised method for capturing vector beetles and, with careful placement, can provide a quantitative assessment of the size of beetle populations in a given area.

Improved and reliable trapping of the vectors has also offered the opportunity for mass trapping and vector population reduction. This has been investigated in France, Portugal and Spain. In general, it appears that a network of traps baited with Galloprotect 2D can result in population reduction, at least within the populations in the test areas. For example, in Spain a reduction of around 60% was achieved with 0.44 traps per ha (i.e. every 150m) in a moderate population of 82 beetles per ha. Whilst not yet a fully proven technology, the lure/trap tool combined with selective felling (see below) offers improved potential to reduce vector populations and minimise the risk of vector spread of PWN.

FLIGHT DISTANCES OF MONOCHAMUS VECTORS AND IMPLICATIONS FOR SPREAD OF PWN AND MANAGEMENT OF SANITATION FELLING

New information on flight capacities of Monochamus spp indicates that short flights are common in dense woodland but, in more open areas, long distance flight is likely. Distances of >2 km are common and up to 40 km or even longer (up to 63 km in a flight mill) is possible during the lifetime of a beetle. This information has major implications for management of felling operations in areas where PWN has been identified. To place this in context, most of the current statutory or recommended regimes for reducing PWN populations include the establishment of demarcated areas and clear-fell zones around infested trees. The scale of felling varies but, under current EU Directives, is at least 500m in radius around the infested area (subject to local interpretation, with reduced areas being possible provided that equivalent protection is achieved). Although principally to reduce PWN sources and vector breeding, this strategy is also to reduce spread by setting up a tree-free belt over which vectors are not expected to fly. From the results of this project, it now appears that a tree-free zone actually increases the likelihood of longer flights since there are no stimuli to retain the vector in the immediate area.

Therefore, a more effective strategy based on vector behaviour would be to ensure that there are both feeding and oviposition resources left in an area to attract any vectors that might have been missed during felling regimes. This is counter-intuitive to the current strategy and would require a more rigorous survey regime in the immediate area to ensure that any breeding populations of the vector are located and destroyed before emergence of nematode-carrying adults. It would, however, retain vectors in a more manageable area and enable resources to be concentrated in that area. There would still need to be general surveys in the immediate vicinity (the buffer zone) as in the current approach, but the need for an extensive buffer zone would be reduced, although areas naturally free of host trees would extend the buffer width.

Based on improved information on vector flight distances and also on various elements of human-aided dispersal of infested wood, new models of spread have been produced. These models account for both vector-driven (local) dispersal and human-assisted (long distance) spread of the nematode. Linked to the wilt expression models (see below), this provides a risk profile of likely spread of the nematode across Europe and identifies where the impacts are likely to be greatest under current and future climates. These models, along with more detailed studies of the role of the Pyrenean Mountains as a natural barrier to vector spread, lead to several inter-related conclusions on future dispersal of PWN:

The nematode could spread from Portugal even under current control measures due to natural spread of the vector, although the tailored strategy suggested above could reduce the rate of dispersal. PWN is not likely to progress by vector dispersal alone beyond the Iberian Peninsula due to the natural barrier of the Pyrenees.

However, accidental introduction by humans could threaten European countries outside the Iberian Peninsula by 2020, with higher risks for those countries with suitable climate and on the ends of trade routes likely to deliver higher volumes of potentially infested wood from Portugal.

This indicates, strongly, that it is essential to apply rigorous containment measures to avoid human-assisted range expansion of the pine wood nematode. Although this would naturally focus on Portugal as a source, it is also essential to prevent any additional introduction into Europe.

OPTIMISING SAMPLING WITHIN TREES TO DETECT PWN

Laboratory experiments on the pathology of PWN in susceptible trees succumbing to pine wilt confirmed the rapid movement of nematodes throughout an infested tree. In such situations, where the tree has actually been killed by PWN, sampling in all parts of the tree should provide positive confirmation that the nematode is present. However, when a tree has been weakened or killed by other factors (or in the case of M. galloprovincialis even when branches have died naturally) nematodes will not be distributed throughout the tree and will be concentrated near to the vector breeding sites. In such situations, sampling at breast height will not provide confirmation of nematode presence and it would be necessary to sample in the upper parts of the tree near to signs of vector activity. It is, therefore, recommended that sampling should be carried out in the upper part of a tree when M. galloprovincialis is the principal local vector species. Additional sampling in the trunk would provide further confirmation that a tree has been killed by PWN but a negative result does not always indicate absence of the nematode.

SOURCE OF THE PORTUGUESE PWN; NEW INSIGHTS ON ORIGIN AND POSSIBLE PATHWAYS

Prior to the REPHRAME project, the assumption, which was partially supported by the molecular techniques available at the time, was that the origin of the Portuguese PWN infestation was either Japan or China. Improved molecular diagnostic techniques, notably microsatellite analysis of newly acquired fresh nematode isolates from China, Japan, Portugal and USA, have been used to assess the origin of the European PWN population(s). The results have provided new insights into the movement of PWN globally:

Japan - the origin of the original PWN isolate is the USA, although a second introduction event probably occurred, whose origin remains to be elucidated;
China - two independent introductions, one from the USA and one from Japan;
Portugal - the probable origin of the PWN population in Portugal is the USA; the origin of the Madeira PWN population is continental Portugal.

Although it is not possible to be certain about the pathway for arrival, or even the date of the original arrival, further assessment of trade patterns with the USA may indicate when and how the nematode was moved to Portugal. In keeping with interception data on Monochamus spp. internationally, wood packaging is the most likely pathway. Since ISPM15 (Regulation of Wood Packaging Material in International Trade) was not implemented by the EU until 2005, the import of wood used as packaging would have been through bilateral agreement with the USA. In general, this would have required absence of grub holes but no direct treatment of the wood (e.g. heat treatment) and so was likely to be less effective than ISPM15 compliant wood packaging.

TRANSMISSION OF PWN IN THE ABSENCE OF THE VECTOR BEETLE

Rapid transfer of PWN from infested sawn wood to non-infested sawn wood takes place provided that the recipient wood has moisture content greater than 20%. This represents a possible means of dispersal of nematodes if wood to wood (whether sawn of living) direct contact takes place. The fact that PWN remains in wood and can actually multiply if food sources are available, means that it also survives well over considerable time periods; at least 40 weeks in solid wood and longer (minimum 1 year) in wood chips. These remain possible sources of non-vector transmission and this has been investigated in detail.

Transmission from vector-free, nematode-infested wood chips through the roots of trees has been demonstrated, especially if there is any wounding of the roots. This was shown in both laboratory and field situations. Similarly, direct transfer from infested sawn wood to living trees is possible if the wood makes direct contact with under-bark exposed tissues of the recipient tree (e.g. if it is nailed to a tree).

Whilst these remain potential pathways, the new information needs to be accounted for in defining the end uses of potentially infested wood and wood products. The highest risk would be from wood chips where end use as mulch or ground cover is likely to bring chips into contact with tree roots. In areas where pine wilt can be expected (see pine wilt modelling) this could represent a significant means of entry to a susceptible tree species and the tree could die from pine wilt. In such situations, the tree would need to be visited by a Monochamus adult for further transmission but, since they are attracted to newly killed trees, this represents a relatively high risk. It is, therefore, recommended that the end use of wood chips created from PWN-infested wood and then moved into 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 in areas where pine wilt is predicted and where the nematode has not been found previously. Conversely, the risk of new PWN establishment and further transfer by local vectors is lower in areas where pine wilt is not predicted (i.e. the trees would not be likely to die from pine wilt and contact with the vector would be unlikely). However, this would be difficult to manage in relation to guaranteeing the final destination of the chips. With regard to the fate of PWN-infested wood chips in forest locations already infested (as is the case in Portugal currently), the results from REPHRAME suggest that indiscriminate scattering of wood chips may lead to some trees becoming infested if chips remain in contact with roots and lower stems of healthy trees. It is uncertain whether this would give a net increase in trees infested, relative to vector-delivered PWN, but the rate of reduction in infestation could be slowed.

IMPROVED MODELS PROVIDE ACCURATE PREDICTION OF AREAS OF EUROPE LIKELY TO SUFFER FROM PINE WILT DISEASE

New, improved, models of the likelihood of pine wilt occurring under current and future climates have enabled risk maps of Europe to be produced. This has been done using sophisticated process models of tree physiology, augmented by detailed correlation analysis of the PWN infestation in Portugal. In addition, simplified models have been produced that will enable end users to estimate the potential for wilt in a given area or region. These employ easily available parameters such as Mean Summer Temperature or knowledge of the precise location (longitude and latitude) and are supplemented by a simple on-line tool on the REPHRAME website. Latent expression of wilt (delayed by one or two years) has also been modelled and has important implications for carrying out surveys based on symptoms of affected trees.

The likelihood of symptom expression (or its absence) is an important part of survey methodology and is the central basis for both EU and EPPO recommended schemes. We can now provide high confidence in predicting where either rapid or delayed wilt expression and, therefore, PWN-related symptoms can be expected in susceptible pine species. When wilt is rapid, as is predicted for many areas in Portugal and on Madeira, surveys can be based on presence of foliar symptoms and there is high confidence that PWN can be detected easily from trunk sampling. However, when latent expression of wilt is predicted, the tree will not show visual symptoms in the year when nematodes are introduced (inoculated) by vectors and so symptom-based surveys in the winter following will not detect infested trees. Symptoms will generally express in the following year and will usually be detected in the next round of winter surveys. This will not matter if the trees are only colonised by vectors in the year of wilt expression. However, if the trees are colonised by vectors in the same year as nematode inoculation they may be detected too late to remove them before the next generation of vectors emerges. Therefore, in areas where latent expression is predicted, a more stringent survey regime augmented by other measures to detect reduced tree health, such as reduced resin flow or signs of Monochamus breeding activity, should be implemented. In such cases it would also be prudent to implement immediate felling rather than delaying until after the vector flight period.

SOCIO-ECONOMIC AND SOCIETAL IMPLICATIONS

Direct costs of dealing with the outbreaks in Portugal and Spain are already substantial (at least 120 million Euros and continuing). Much of these costs have been incurred on surveys and through premature felling of trees from infested forests (direct felling costs and potential loss to timber value through lower yields and changes in end use of the wood to a lower or nil return market).

In addition to direct costs, this has societal implications through loss of visual amenity (large clearfells and potential effects on water flow, soil stability, etc.). Public awareness of the effects of PWN is, therefore, affected both by the nematode-induced losses of trees (wilt expression and poor appearance of standing trees) and the large landscape changes arising from premature felling of trees. Results from REPHRAME provide new information to make changes to the current survey and felling regimes (as outlined above). This knowledge could provide a more tailored approach with selective felling in infested areas and less reliance on clear felling as the main tool in management.

This would also have the effect of reducing biodiversity losses through habitat alteration and enable a more structured approach to retaining tree cover in the affected areas. Although survey costs would remain high, there should be reduced costs from premature felling and a better balance between supply and demand for timber.

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) which is an on-line resource to guide end-users on the main topics that affect the PWN-vector-tree-environment relationship. This is available at www.rephrame.eu. PTK provides summaries of the main findings through a series of modules which can then be explored further to give greater detail and links to other information sources. The main modules in the PTK are: The PWN Problem; Detection; Survey Methodology; Management; Modelling; Nematode Transmission; Visualisation. Although these are shown as separate modules, the results are highly inter-related and there are internal links between the modules to share information between them.

KNOWLEDGE SHARING AND COLLABORATION

An important part of work within REPHRAME was to build on the existing well-established collaborations with researchers and other stakeholders globally. All partners benefitted from a thorough sharing of information generated from previous and ongoing projects (EU and worldwide) related to pine wilt disease (PWD). Ample opportunities were provided for exchange visits to benefit from each partner´s know-how and to provide a range of field and laboratory conditions for experimentation or observation. Partners took the opportunity to develop joint research, which is clearly demonstrated in the most significant outputs, namely publication in internationally refereed journals as well as presentations at national and international meetings. The report demonstrates the interaction with numerous international phytosanitary and research institutions (EU and otherwise) working on PWD, with useful exchange of information and biological material (such as nematode and insect specimens and populations). For example, there was highly effective collaboration with researchers to provide new, field collected, isolates from China (4 isolates), Japan (7 isolates), USA (28 isolates) and Portugal (9 isolates). This was achieved through both existing and new collaboration and illustrates how well connected the scientists in REPHRAME are in relation to this important problem.

The project was also mindful of the educational opportunities arising from the collaborations and Beneficiary linkages internationally. A good example is the provision of The European Union Erasmus Mundus programme “EUMAINE” was a good platform for exposing international post-graduate students to the PWD issue; in fact, one of the courses taught was precisely “Pine wilt disease: bioecological and global issues”. The programme also allowed for the production of an MSc thesis (Silva, A., 2014, UGent) in support of a PhD thesis (Espada, M., Univ. Évora, started 2012) which has been identifying candidate genes for pathogenicity (“effectors”).

The major dissemination event from the project was the joint IUFRO/REPHRAME International Conference entitled “International Conference on Pine Wilt Disease 2013” in October 2013 organised by JKI (Beneficiary 5) and the German Scientific Society for Plant Protection and Plant Health (DPG) in Braunschweig, Germany. There were 87 participants representing 23 countries, including Europe as well as Russia, Asia, North America and Australia. In total, 41 talks were given and 22 Posters were presented. Information on the conference and the extended abstracts are available at http://pub.jki.bund.de/index.php/BerichteJKI/issue/view/858.

Further dissemination was achieved through two back-to-back workshops (Spain and Portugal), a webinar and a seminar in Brussels during 2014. These events are described in the third periodic report and on the REPHRAME website.

A Stakeholder Observer Group was established through the international collaboration within REPHRAME. Membership increased gradually through the project and was aided by the following events:

June 2012: IUFRO Alien Invasive Species & International Trade meeting, Tokyo, Japan. Discussions and field visits to see PWN affected sites and resistance breedin programme.
October 2013: Joint REPHRAME/IUFRO PWN Group International Conference Braunschweig, Germany in October 2013 (see above). A short formal session to encourage additional membership of the SOG and to extend collaboration was held in addition to the normal scientific interchange at the meeting.
October 2013: 2nd International Congress on Biological Invasions in Qingdao, China. Several participants in REPHRAME presented papers at the Congress and Prof Hugh Evans gave an overview of the European situation on PWN at a special session on the nematode. During this presentation, he developed discussion with existing members of the SOG and encouraged further membership, which has proved fruitful in developing the PTK.
November 2013: International Forest Quarantine Research Group in Qingdao, China. A specific presentation and discussion session on PWN was included in the agenda and further interest shown in REPHRAME and the SOG, including additional membership.

Current membership of the SOG includes 22 scientists, plant health regulators, practitioners and timber trade representatives from Canada, China, France, Japan, South Korea, Netherlands, Portugal, South Africa, Spain, UK, USA, Vietnam.
Communication has been mainly through exchange of emails and face to face meetings at international conferences and we are pleased to acknowledge their contribution of ideas for the PTK.

Members of REPHRAME are also active contributors to international panels and other fora on phytosanitary issues, most of which have dealt with PWN. For example, IUFRO - 7.02.10 Pine Wilt Disease: Chaired by B5, 7.03.05 Ecology and Management of Bark and Wood Boring Insects, 7.03.12 Alien Invasive Species and International Trade: Deputy Chair B1; International Forestry Quarantine Research Group (IFQRG) - several members of REPHRAME have contributed to this important group over many years, providing regular reports on the work of REPHRAME to the annual meetings of IFQRG; European Food Safety Authority (EFSA) - contributions to European phytosanitary development has been done as experts working within EFSA groups, with recent contributions by REPHRAME members on PWN and its vectors; EPPO Forest Quarantine Panel – contributions by several REPHRAME members especially on PWN. In addition, several of the REPHRAME team (B1, B5, and B6) are contributing to the newly formed EU Task Force on PWN, which provides a direct opportunity to share our outputs and to help develop new strategies for dealing with PWN.

Address of project public website and relevant contact details

www.rephrame.eu

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Beneficiary 11: Norwegian Institute for Agricultural and Environmental Research – Bioforsk
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4.2 Use and dissemination of foreground

Section A (public)

Publications

LIST OF SCIENTIFIC PUBLICATIONS, STARTING WITH THE MOST IMPORTANT ONES										
No.	Title / DOI	Main author	Title of the periodical or the series	Number, date or frequency	Publisher	Place of publication	Date of publication	Relevant pages	Is open access provided to this publication ?	Type
1	2-(Undecyloxy)-ethanol is a major c omponent of the male-produced aggregatio n pheromone of Monochamus sutor	Juan A. Pa jares, Gonzalo Ylvarez, D avid R. Hall, Paul Douglas, Felix Centen o, Nieves Ibarra, Martin Schroede r, Stephen A. Teale, Zhiyin g Wang, Sh anchun Yan, Jocelyn G. Mi llar and L awrence M. Hanks	Entomologia Experimentali s et Applicata	-	Blackwell Publishing	United Kingdom	01/08/2013	-		Peer revie wed
2	Worldwide invasion routes of the pinewoo d nematode: What can we infer from popul ation genetics analyses? 10.1007/s1053 0-014-0788-9	Sophie Mallez , Chantal C astagno ne , Margarida Espada , Paul o Vieira , Jona than D. Ei senbac k , Mark Harrell ,	Biological Invasions	Vol.16	Springer Netherlands	Netherlands	28/09/2014	2247-2261	No	Peer revie wed

		Manuel Mota , Takuya Aika wa , Mitsuter u Akiba , Ha jime Kosaka , Philippe Ca stagno ne-S ereno , Th omas Guill ema ud								
3	Dispersal capacity of Monochamus gallopr ovincialis, the European vector of the pine wood nematode, on flight mills	G. David, B. Giffard, D. Piou and H. Jactel	Journal of Applied Entomology	Vol. 138/I ssu e 8	Blackwell Publishing		19/12/2013	566-576	Yes	Peer revie wed
4	Development of 12 microsatellites loci for the longhorn beetle Monochamus gallop rovinc ialis (Coleoptera Cerambycidae), v ector of the Pine Wood Nematode in Europ e 10.1007/s1268 6-014-0262-0	Julien Haran, Geraldin e R oux-Mor abito	Conservation Genetics Resources	Vol. 6/Iss ue 4	Springer Verlag		17/07/2014	975-977	No	Peer revie wed
5	A native fungal symbiont facilitates the prevalence and development of an invasive pathogen?native vector symbiosis 10.1890/12-22 29.1	Lilin Zhao , Min Lu , Hon gtao Niu , Guofei Fang , Shuai Zhang , Jianghua Sun	Ecology	Vol. 94/Is sue 12	Ecological Society of America	United States	01/12/2013	2817-2826	No	Peer revie wed
6	Chemical Signals Synchronize the Life Cy cles of a Plant-Parasi tic Nematode and Its Vector Beetle 10.1016/j.cub .2013.08.041	Lilin Zhao , Shuai Zhang , Wei Wei , Hai jun Hao , Bin Zhang , Reb ecca A. Bu tcher , Jianghu a Sun	Current Biology	Vol. 23/Is sue 20	Cell Press	United States	01/10/2013	2038-2043	No	Peer revie wed
7	Catalases induction in high virulence pi newood nematode Bursaphelench us xyl ophilus under hydrogen peroxide-induced stress	V. Vicente, Y. Ikuyo, R. Shinya, M. Mota and K. Hasegawa	PLoS One	-	Public Library of Sc ience		01/09/2014	-		Peer revie wed
8	First detection of Bursaphelenc hus xylop hilus associated with Pinus nigra in Por tugal and in Europe	M.L. Inacio, F. Nabrega, P. Vieira, L. B onifaci o, P.	Forest Pathology	Online ver sion publi she d before inc	Blackwell Publishing	United Kingdom	01/12/2014	n/a-n/a	Yes	Peer revie wed

		Naves, E. So usa and M. Mota		lusion in an issue						
9	Efficacy of sulfuryl fluoride against the pin ewood nematode, Bursaphelench us xyl ophilus (Nematoda: Aphelenchida e), in Pi nus pinaster boards	Luis F Bon ifacio, Ed mundo Sousa, Pedro Naves, Maria L I nacio, Joana Henrique s, M anuel Mota, Pedro Barbosa , Mike J D rinkall and Sta nislas Buckley	Pest Management Science	Vol. 70/Is sue 1	John Wiley and Sons Ltd	United Kingdom	01/01/2014	6-13	Yes	Peer revie wed
10	The mitochondria l genome of the pin ewood nematode (Bursaphelen chus xyl ophilus) lineage introduced in Europe	Claudia Mo reira, Barbara van Asch, Luis Fonseca, Isabel Pere ira-C astro, Raquel Silva, Luisa Aze vedo, Manuel Mota, Isabel Abrantes, Antonio A morim, and Filipe Pereira	Mitochondrial DNA	Vol.25/Is sue 6	Informa Healthcare	United Kingdom	01/12/2014	420-421	No	Peer revie wed
11	New Insights into the Phylogeny and Worl dwide Dispersion of Two Closely Related Nematode Species, Bursaphelenchus x ylophilus and Bursaphelenc hus mucro natus 10.1371/journ al.pone.0056288	Filipe Pereira , Clyia Mo reira , Luis Fo nseca , Ba rbara van Asch , Manuel Mota , Isabel Abrantes , A nty Amorim	PLoS One	Vol. 8/Iss ue 2	Public Library of Sc ience	United States	08/02/2013	e56288	Yes	Peer revie wed
12	First Insights into the Genetic Diversity of the Pinewood Nematode in Its Native Area Using New Polymorphic Microsatellite Lo ci 10.1371/journ al.pone.0059165	Sophie Mallez , Chantal C astagno ne , Margarida Espada , Paul o Vieira , Jona than D. Ei	PLoS One	Vol. 8/Iss ue 3	Public Library of Sc ience	United States	15/03/2013	e59165	Yes	Peer revie wed

		senbac k , Manuel Mota , Thomas Guil lemau d , P hilippe Ca stagno ne-S ereno								
13	Characterizat ion of Bacteria Associated with Pinewood Nematode Bursaphelenchus xylophilus 10.1371/journ al.pone.0046661	Claudia S. L. Vicente , F rancisc o N ascimen to , Margarida Espada , Pedr o Barbosa , Manuel Mota , Bernard R. Glick , Solan ge Oliveira	PLoS One	Vol. 7/Iss ue 10	Public Library of Sc ience	United States	16/10/2012	e46661	No	Peer revie wed
14	Pine wilt disease: detection of the pine wood nematode (Bursaphelen chus xylo philus) as a tool for a pine breeding pr ogramme	B. Ribeiro, M. Espada, T. Vu, F. No brega, M. Mota, I. C arrasqu inho	Forest Pathology	Vol. 42/Is sue 6	Blackwell Publishing	United Kingdom	01/12/2012	521-525	Yes	Peer revie wed
15	The pinewood nematode, Bursaphelenchus xylophilus, in Madeira Island 10.2478/s1168 7-012-0020-3	L. Fonseca , J. M. S. Cardoso , A. Lopes , M. Pestana , F. Abreu , N. Nunes , M. Mota , I. Abra ntes	Helminthologi a	Vol. 49/Is sue 2	Versita	United Kingdom	01/06/2012	96-103	No	Peer revie wed
16	Bacterial role in pine wilt disease deve lopment - review and future perspectives 10.1111/1758- 2229.12202	Francisco X. Nascimen to , Koichi Ha segawa , M anuel Mota , Cláudia S. L. Vicente	Environmental Microbiology Reports	NA	Wiley-Blackwe ll	United States	01/10/2014	n/a-n/a		Peer revie wed
17	Pinewood nematode-ass ociated bacteria co ntribute to oxidative stress resistance of Bu rsaphelenchus xylophilus 10.1186/1471- 2180-13-299	Claudia S L Vicente , Yor iko Ikuyo , Manuel Mota , Koichi Ha	BMC Microbiology	Vol. 13/Is sue 1	BioMed Central	United Kingdom	01/01/2013	299	Yes	Peer revie wed

		segawa								
18	Characterizat ion of bacterial communities associated with the pine sawyer beetle 10.1111/1574- 6968.12232	Claudia S.L. Vicente , Fr ancisc o X. Nasciment o , Margarid a E spada , Pedro Barbosa , K oichi Hase gawa , Man uel Mota , Solange O liveira	FEMS Microbiology Letters	347	Blackwell Publishing	United Kingdom	01/08/2013	n/a-n/a	Yes	Peer revie wed
19	Pine Wilt Disease: a threat to European forestry 10.1007/s1065 8-011-9924-x	Claudia Vi cente , Ma rgarid a Es pada , Paulo Vieira , Man uel Mota	European Journal of Plant Pathology	Vol. 133/I ssu e 1	Springer Netherlands	Netherlands	01/05/2012	89-99	No	Peer revie wed
20	Bursaphelench us pinophilus Brzeski & Baujard, 1997 (Nematoda: Parasitaphele nchinae) associated with nematangia on P ityogenes bidentatus (Herbst, 1783) (Col eoptera: Scolytinae), from the Czech Rep ublic 10.1163/15685 4111X614502	Vaclav Cer mak, Katerina Siroka, Maria Cudejkov a, P aulo Vieira, Manuel Mota, Jiri Foit, Vla dimir Gaar	Nematology	Vol. 14/Is sue 3	Brill Academic Publi shers	Netherlands	01/02/2012	385-387	No	Peer revie wed
21	The pine wood nematode, Bursaphelenc hus xylophilus, in Portugal: possible introd uction s and spread routes of a serious b iological invasion revealed by molecular methods 10.1163/15685 4112X632673	Vera Valadas , Solange Ol iveira , M argarid a E spada , Marta Laranjo , M anuel Mota , Pedro Barbosa	Nematology	Vol. 14/Is sue 8	Brill Academic Publi shers	Netherlands	02/05/2012	899-911	No	Peer revie wed
22	Molecular characteriza tion of Portuguese populations of the pinewood nematode Bu rsaphelenchus xylophilus using cytochrome b and cellulase genes 10.1017/S0022 149X12000673	V. Valadas , M. Laranjo , M. Mota , S. Oliveira	Journal of Helminthology	Vol. 87/Is sue 04	Cambridge University Press	United Kingdom	01/12/2013	457-466	No	Peer revie wed
23	Bioassays Against Pinewood Nematode: Assessment of a Suitable Dilution Agent and Screening for Bioactive Essential Oils	Pedro Barbosa , Jorge M. S. Faria , Marta	Molecules	Vol. 17/Is sue 12	Molecular Diversity Preservation Interna tional	Switzerland	01/12/2012	12312-123 29	Yes	Peer revie wed

	10.3390/molec ules171012312	D. Mendes , L uySilva Dias , Maria Teresa Tinoco , Josy. Barroso , Luis G. Pedro , A na Cristina Figueiredo , Manuel Mota								
24	Evidence for the involvement of ACC deaminase from Pseudomonas putida UW4 in the biocontrol of pine wilt disease ca used by Bursaphelenchus xylophilus 10.1007/s1052 6-012-9500-0	Francisco X. Nascimen to , Clyia S. L. Vi cente , Pedro Barbosa , M argarid a E spada , Be rnard R. Glick , Manuel M ota , Solange Oliveira	BioControl	Vol. 58/Is sue 3	Springer Netherlands	Netherlands	01/06/2013	427-433	No	Peer revie wed
25	On the genus Bursaphelenchus Fuchs, 1937 (Nematoda: Parasitaphele nchinae) associ ated with wood and insects from declining forest trees in the Czech Republic	V. ?Ermak, P. Vieira, V. Gaar, M. U dejkova , J. Foit, M. Zouh ar, O. Douda, M. Mota	Forest Pathology	Vol. 43/Is sue 4	Blackwell Publishing	United Kingdom	01/08/2013	306-316	Yes	Peer revie wed
26	Survey of the genus Bursaphelenc hus Fuchs, 1937 (Nematoda: Aphelenchoid idae) in Romania	M. Calin, P. Vieira, C. C ostache , H. Braasch, J. G u, J. Wang and M. Mota	EPPO Bulletin	Vol. 43/Is sue 1	Blackwell Publishing	United Kingdom	01/04/2013	144-151	Yes	Peer revie wed
27	On the track of Bursaphelench us pinophil us Brzeski and Baujard, 1997 (Nematoda: Aphelenchoid idae) in Portugal	P. Vieira and M. Mota	Forest Pathology	Vol. 43/Is sue 5	Blackwell Publishing	United Kingdom	01/05/2013	433-436	Yes	Peer revie wed
28	Bioactivity against Bursaphelenc hus xylo philus: Nematotoxics from essential oils, essential oils fractions and decoction wate rs 10.1016/j.phy tochem.2013.06.005	Jorge M.S. Faria , Pedro Barbosa , Richard N. Bennett , Manuel Mota , A. Cristina Figueiredo	Phytochemistr y	Vol. 94	Elsevier Limited	United Kingdom	01/10/2013	220-228	No	Peer revie wed

29	Sequence variability of the MspI satellite DNA family of the pinewood nematode B ursaphelenc hus xylophilus at different g eographic scales 10.1016/j.ymp ev.2013.09.017	Paulo Vieira , Chantal Ca stagno ne , Sophie Mallez , Margarida Espada , Alfo nso Navas , Manuel Mota , Philippe Castagno ne -Seren o	Molecular Phylogenetic s and Evolution	Vol. 70	Academic Press Inc.	United States	01/01/2014	120-129	No	Peer revie wed
30	Bursaphelench us hofmanni Braasch, 1998 associated with peat growth substrate in hops nurseries in the Czech Republic 10.1163/15685 411-00002801	Vyav ?ermy, Vladimir Gaar , KateYina Mikuakovy Mya ?ude jkovy Manuel Mota , KateY ina Tomyovy Paulo Vieira , Jonathan D. E isenbac k	Nematology	Vol. 16/Is sue 6	Brill Academic Publi shers	Netherlands	18/07/2014	739-742	No	Peer revie wed
31	Interspecific communicatio n between pine wood nematode, its insect vector, and as sociated microbes 10.1016/j.pt. 2014.04.007	Lilin Zhao , Manuel Mota , Paulo Vieira , Rebecca A. Butcher , Ji anghua Sun	Trends in Parasitology	Vol. 30/Is sue 6	Elsevier Limited	United Kingdom	01/06/2014	299-308	No	Peer revie wed
32	New reports of Bursaphelenc hus species a ssociated with conifer trees in Romania	M. Calin, C. Costache , H. Braasch, M. Zaulet, L. B uburuza n, V. Petrovan , M. Dumitru, M. Mota and P. Vieira	Forest Pathology	Online ver sion publi she d before inc lusion in an issue	Blackwell Publishing	United Kingdom	16/12/2014	n/a-n/a	Yes	Peer revie wed
33	In vitro co-cultures of Pinus pinaster with Bursaphelenc hus xylophilus: a biotechnolo g ical approach to study Pine Wilt Disease	J.M.S. Faria, I . Sena, I. Viei ra da Silva, B. Ribeiro, P. Ba rbosa, L. Ascens#o , R. Bennett, M. Mota, A.C.S.	Planta	-	Springer Verlag		12/01/2015	-		Peer revie wed

		Figueire do								
34	An update on the occurrence of nematodes belonging to the genus Bursaphelenc hus in Mediterranean area	G. d'Errico , B. Carletti, T. Schröder , M. Mota, P. Viei ra and P.F. Roversi	Forestry	-	Oxford University Pr ess		01/11/2014	-		Peer revie wed
35	Development of 12 microsatellites loci for the longhorn beetle Monochamus gallop rovinc ialis (Coleoptera Cerambycidae), v ector of the Pine Wood Nematode in Europ e 10.1007/s1268 6-014-0262-0	Julien Haran , Geraldin e Roux-Mor ab ito	Conservation Genetics Resources	Vol. 6/Iss ue 4	Springer Verlag	Germany	01/12/2014	975-977	No	Peer revie wed
36	Ghost mtDNA haplotypes generated by fort uitous NUMTs can deeply disturb infraspe cific genetic diversity and phylogeographic pattern	Haran J., Koutroum pa F., Magnoux E., Roques A., Roux- Morabi to G.	Journal of Zoological Systematics and Ev olutionary Research	-	Blackwell Publishing		01/01/2015	-		Peer revie wed
37	Rearing the Pine Sawyer Monochamus galloprovinc ialis (Olivier, 1795) (Coleo ptera: Cerambycidae) on Artificial Diets	Petersen- S ilva R., Naves P., Sousa E. & Pujade-V illar J	Journal of the Entomologica l Research So ciety	Vol. 16/Is sue 1	Gazi Entomologica l Research Society		01/03/2014	61-70	Yes	Peer revie wed
	Flight performances of Monochamus g alloprovinc ialis, insect vector of the Pine Wood Nematode	David G, J actel H, Piou D, Naves P & Sousa E	Schroder, T. (ed.) IUFRO / REPHRAME International Conference on Pine Wilt D isease 2013, Abstracts, Berichte aus dem Julius K#hn-Institut 169		Julius Kuhn-Institut e	Braunschweig	15/10/2013	20		Conference
	The current situation on pine wood nemat ode, Bursaphelenc hus xylophilus in Europ e; from research to management	H. F. Evans	Abstracts of the 2nd International Congress of Biological Invasions, Qingdao, China		-	Qingdao, China	23/10/2013			Conference
	How Could Climate Change Affect the Potential Spread of Pine Wilt Disease in Europe?	Gruffudd, H., Evans, H., Haran, J., Roux-Mora bito, G., Roques, A., Robinet, C	ClimTree 2013. Internationa l Conference on Climate Change and Tree Responses in Central European Forests		Swiss Federal Instit ute for Forest, Snow and Landscape Resea rch WSL, Birmensdor f (Suisse)		01/09/2013			Conference
	Using an evapo-transp iration model to pr edict the current and future range and s everity of pine wilt disease caused by pine	Gruffudd, H., Evans, H.F. and Jenkins, T	Abstracts of the 2nd International Congress of Biological Invasions, Qingdao, China		-		23/10/2013			Conference

	wood nematode, Bursaphelenc hus xylop hilus in Europe	.								
	Using an evapo-transp iration model to pr edict the current and future range and s everity of pine wilt disease caused by pine wood nematode, Bursaphelenc hus xylop hilus in Europe	Gruffudd, H., Evans, H.F. and Jenkins, T .	Schroder, T. (ed.) IUFRO / REPHRAME International Conference on Pine Wilt D isease 2013, Abstracts, Berichte aus dem Julius K#hn-Institut 169		Julius Kuhn-Institut e		15/10/2013			Conference
	Flight activity of longhorn beetles Mono chamus sartor and M. sutor: Attractiveness of insect and tree produced volatiles	Halbig, P., Hoch, G, Menschhor n, P., Hall, D.R., Krehan, H.	Meeting of the Deutsche Gesellschaft f#r allgemeine und angewandte Entomologie		-		18/03/2013			Conference
	Assessing dispersal routes and ongoing g ene flow of the vector of the Pine Wood Nematode, Monochamus galloprovinci alis, at different spatial scales	Haran J., Roques A., Robinet C. & Roux- Morab ito G.	XXIV IUFRO World Congress, 2014 Salt Lake City, United States		-		05/10/2014			Conference
	Assessing potential expansion of the Pine Wood nematode (Bursaphelenc hus xy lophilus) from the spatial genetic structure of the vector (Monochamus galloprovinci a lis)	Haran J., Roques A., Roux- Mor abito G.	Schroder, T. (ed.) IUFRO / REPHRAME International Conference on Pine Wilt D isease 2013, Abstracts, Berichte aus dem Julius K#hn-Institut 169		-		15/10/2013			Conference
	Evolutionary history and ongoing gene fl ow of Monochamus galloprovinci alis (Coleoptera, Cerambycidae), vector of the Pine Wood Nematode	Haran J., Roques A., Roux- Mor abito G	IUFRO 7.03.14, 7.03.06, 7.03.01 Joint Me eting, Antalya, Turkey.		-		09/04/2014			Conference
	Assessing potential expansion of the Pine Wood Nematode (Bursaphelenc hus xy lophilus) from the spatial genetic structure of the vector (Monochamus galloprovinci alis): First results of a STSM stay in Portug al	Haran J., Roux- Mora bito G., R oques A	PERMIT meeting, Treviso		-		01/10/2013			Conference
	Flight activity of sawyer beetles Monoch amus sartor and Monochamus sutor: Attrac tivene ss of insect and host tree volatiles.	Hoch, G., Halbig, P., Menschhorn, P., Hall, D.R., Krehan, H.	24th USDA Interagency Research Forum on Invasive Species		-		08/01/2013			Conference
	Testing attractants for trapping Monocha mus sartor and Monochamus sutor.	Hoch, G., Halbig, P.,	Schroder, T. (ed.) IUFRO / REPHRAME International Conference on Pine Wilt D		-		15/10/2013			Conference

		Menschhorn, P., Hall, D.R., Krehan, H.	isease 2013, Abstracts, Berichte aus dem Julius K#hn-Institut 169							
	Non vector spread of Bursaphelench us xyl ophilus via wood chips	Hopf, A., Schroder , T	Schroder, T. (ed.) IUFRO / REPHRAME International Conference on Pine Wilt D isease 2013, Abstracts, Berichte aus dem Julius K#hn-Institut 169		-		15/10/2013	46-47		Conference
	Development of Bursaphelenc hus xylo philus-sp ecific microsatellit e markers to assess the genetic diversity of populations from European forests	Mallez S., Castagnon e C., Espada M. , Mota M., Guillemau d T. and Castag no ne-Sereno P.	Proceedings of the 31st Internationa l Sy mposium of the European Society of Nematologist s, Adana, Turkey: 88		-		23/09/2012			Conference
	Genetic diversity of the pinewood nemato de around the world – First insights in its i nvasion routes	Mallez S., Castagnon e C., Espada M. , Vieira P., Ei senbac k J., Mota M., Aikawa T., Akiba M., Kosaka H., Ca stagno ne-S ereno P. and Guillema ud T .	Abstracts of the 2nd International Congress of Biological Invasions, 23-27 October, Q ingdao, China : 96		-		23/10/2013			Conference
	Simulating the dispersal of Monochamus g alloprovinc ialis based on its flight mill per formance and testing several pest manage ment scenarios	Robinet C, David G, Piou D, Roques A, Jactel H .	Schroder, T. (ed.) IUFRO / REPHRAME International Conference on Pine Wilt D isease 2013, Abstracts, Berichte aus dem Julius K#hn-Institut 169		-		15/10/2013			Conference
	Efficacy of kiln drying as phytosanitary treatment against wood borne nematodes	Schroder, T., Welling J., Aukamp-Ti mmreck C.	Schroder, T. (ed.) IUFRO / REPHRAME International Conference on Pine Wilt D isease 2013, Abstracts, Berichte aus dem Julius K#hn-Institut 169		-		15/10/2013	51-52		Conference
	Landscape genetics of Monochamus ga lloprovincialis, vector of the pine wood nematode in Europe	Haran J.	International Conference on Insect Invas ions. Le Studium, Orl#ans, France		-		17/12/2014			Conference
	Assessing the invasion probability of the pine wood nematode with imported wood	Robinet C.	International Conference on Insect Invas ions. Le Studium, Orl#ans, France		-		17/12/2014			Conference

	Pine wilt disease, and the pinewood nema tode: a worldwide issue, a nematological challenge	Mota, M.	6th Internationa l Congress of Nematology, Capetown, S. Africa		-		05/05/2014			Conference
	Ecological role of bacteria asociated with the pine wilt disease system	Nasciment o, FX, Cl#udia S .L. Vicente, Pedro Barbos a, Margarid a Espada, Paulo Vieira, Koichi Hasegawa and Manuel Mota	6th Internationa l Congress of Nematology, Capetown, S. Africa		-		05/05/2014			Conference
	Virulence and oxidative stress response ofthe pine wood nematode Bursaphelenc hus xylophilus	Vicente, CV, Yoriko Ikuyo, Ryoji Shinya, Manuel Mota, Koichi Hase gawa	6th Internationa l Congress of Nematology, Capetown, S. Africa		-		05/05/2014			Conference
	Ectopic expression of cell cycle inhibitor genes effectively interferes with root-knot nematode feeding site development	Vieira, P., Nat alia Rodiuc, Lieven De Ve ylder, Gilbert Engler and Pierre Abad, J anice de A lmeida Engler	6th Internationa l Congress of Nematology, Capetown, S. Africa		-		05/05/2014			Conference
	The pinewood nematode, Bursaphelenchus xylophilus, and pine wilt disease: a serious forest threat to Turkey and Europe	Mota, M., Francisc o X. Nascimen to, Cl#udia S.L. Vicente, Joa na Henrique s, Pedro Barbo sa, Margarid a Espada, Paulo Vieira, and Koichi Has egawa	2nd Symp. Turkey Forest Entomology and Pathology. and IUFRO Joint Meeting A ntalya, Turkey		-		07/04/2014			Conference
	Preliminary genetic analysis of the bacteria associated with Monochamus galloprovinci alis from Turkey	Dayi, M., Henrique s, J., Alves, M., Henriques , I., Mota, M., Akbulut, S., C	2nd Symp. Turkey Forest Entomology and Pathology. and IUFRO Joint Meeting A ntalya, Turkey		-		07/04/2014			Conference

		orreia, A.								
	Histological changes in stems of Pinus s ylvestris seedlings infected with a virulent isolate of the pinewood nematode Bursaph elenchus xylophilus	Vieira da Silva, I., Barb osa, P., Mota, M. and Asc ens#o, L.	International Conference on Microscopy a nd Microanalysis XLVIII Congress of the Portuguese Microscopy Society, Oporto, P ortuga		-		06/11/2014			Conference
	Nematotoxic activity from essential oils and own hydrocarbons and oxygen-contai n ing molecules fractions against the pine wood nematode Bursaphelenc hus xylop hilus	Barbosa, P., Ana M. Rodri gue s, Jorge MS Faria, Lu# s S. Dias, Lu#s G. P edro, Jos# G. Barroso, Ana C. Figueire do and Manuel Mota	62nd Int. Cong. and Ann. Meeting, Soc. M ed. Plant and Natural Prod. Res, Guimar# es, Portugal		-		31/08/2014			Conference
	Volatile response of Pinus halepensis and Pinus sylvestris after inoculation with the pinewood nematode Bursaphelenc hus xylophilus	Rodrigues , AM., Pedro M. Barbosa, Ana S. Lima, Lu#sa Mota, J os# G. Bar roso, Luis G. Pedro, Lia M. Ascens#o , Manuel Mota, A. Cristina Fi gueire do	XVIII Congresso da Sociedade Portuguesa de Bioqu#mica, Coimbra, Portugal		-		17/12/2014			Conference
	Plant volatile compounds to control the pinewood nematode in Portugal	Barbosa, Ana M. Rodrigues , Jorge M.S. Faria, Marta D. Mendes, Ana S. Lima, Luís S. DiaS, P. Vieira, MT Tinoco, Rich ard N. Ben nett, Luis G. P edro, José G. Barroso, A. Cristina Figue ire do and Manuel Mota	1º Simpósio SCAP “Novos desafios na prot ecção das plantas” e 7º Congresso SPF, O eiras, Portugal		-		20/11/2014			Conference

	An integrative approach on Pine wilt dis ease	Espada, M, C. Vicente, F. Nasciment o, M. Mota.	1º Simpósio SCAP "Novos desafios na prot ecção das plantas" e 7º Congresso SPF, O eiras, Portugal		-		20/11/2014			Conference
	Histopatholog y of Bursaphelenchus x ylophilus-i nfected seedlings of two pine species grown in Portugal	Vieira da Silva, L. Mota, P. B arbosa, M. Mota, and L. Ascensão	1º Simpósio SCAP "Novos desafios na prot ecção das plantas" e 7º Congresso SPF, O eiras, Portugal		-		20/11/2014			Conference
	The pinewood nematode problem: current s tatus of research in Europe	Castagnon e -Sereno P.	Abstracts of the XLIII ONTA Annual Meeting, 4-8 September 2011, Coimbra, Po rtugal: 84		-		04/09/2011			Conference
	Deciphering the invasion routes of Bursa phelenc hus xylophilus: a population gene tics approach	Mallez S., Castagnon e C., Guillemau d T. and C astagno ne-Sereno P.	Proceedings of the 6th International Con gress of Nematology, 4-9 May 2014, Cape Town, South Africa: 77		-		04/05/2014			Conference
	Bursaphelench us xylophilus (Steiner & Bu hrer, 1934) Nickle 1970 ? hyatko borovic ova: hrozba pre eurapske lesy / Bursaphe lench us xylophilus (Steiner & Buhrer, 19 34) Nickle 1970 ? pinewood nematode: a t hreat to European forests	A. ?Erevkov a, M. Mota, and P. Vieira	Lesnicky Casopis For. J.	Vol. 60	National Forest Cent re - Forest Research Institute in Zvolen		01/03/2015		Yes	Monogram
	Monochamus-Ar ten als potenztielle V ektoren des Kiefernholzne matoden in Oste rreich: Lockstofffallen zum Monitoring des Flugverhaltens Fluges [Monitoring flight of Monochamus spp., potential vectors of the pine wood nematode in Austria using phero mone-ka iromone baited traps]	Hoch, G., Mitterma yr, D., Krehan, H .	Forstschutz Aktuell	IN PRESS	BFW	Wien	02/02/2015		Yes	Monogram
	Der Kiefernholzn ematode ? ein Notfallpla n fur Osterreich [The pine wood nematode ? a contingency plan for Austria]	Tomiczek, C.	Forstschutz Aktuell	59	BFW	Wien	07/07/2014		Yes	Monogram
	Flugaktivität der Bockkafer Monochamus s artor und Monochamus sutor: Attraktivitat insekten- und baumbürtiger volatiler S ubstanzen [Flight activity of sawyer beetles Monochamus sartor and Monochamus su tor: Attractivity of insect and host tree vol	Halbig, P., Menschhorn, P., Krehan, H., Hall, D.R., Hoch, G.	Silva Fera	3	Wildnisgebiet s Durre nstein		07/07/2014		Yes	Monogram

	atiles]									

LIST OF DISSEMINATION ACTIVITIES								
No.	Type of activities	Main Leader	Title	Date	Place	Type of audience	Size of audience	Countries addressed
1	Flyers	FORESTRY C OMMISSION RESEARCH AGENCY	REPHRAME Projec t Leaflet	24/09/2012	Aberystwyth, UK	Scientific comm unity (higher educat ion, Research) - Ind ustry - Civil society - Policy makers - Medias		All
2	Organisation of Conference	JULIUS KUH N-INSTITUT BUNDESFOR SCHUNGSINS TITUT FUR KULTURPFLA NZEN	Pine Wilt Disease Conference 2013	15/10/2013	Braunschweig Ge rmany	Scientific comm unity (higher educat ion, Research)	90	DE, CA, SE, JP, FR, BE, CN, PL, NL, US, UK, NO, CH, PT, AU, ES, R U, TR, SI, HR, IT, AT
3	Flyers	JULIUS KUH N-INSTITUT BUNDESFOR SCHUNGSINS TITUT FUR KULTURPFLA NZEN	Kiefernholznema tode, Informationsbl att des JKI	06/01/2014	Braunschweig Ge rmany	Civil society		DE
4	Organisation of Workshops	AGENCIA ES TATAL CONSEJO SUPERIOR DE INVESTIGACIONE S CIENTIFICAS	REPHRAME Final Workshop	30/09/2014	Madrid	Scientific comm unity (higher educat ion, Research) - Ind ustry - Policy makers	25	PT, AU, ES, FR, ET, MA, UK, D, BE, NL
5	Organisation of Workshops	INSTITUTO NACIONAL DE RECURSOS BIOL OGICOS I.P. INRB	REPHRAME Final Workshop 2	01/10/2014	Portugal field sites	Scientific comm unity (higher educat ion, Research) - Ind ustry - Civil society - Policy makers - Medias	37	PT, AU, ES, FR, ET, MA, UK, D, BE, NL
6	Web sites/Appli cations	FORESTRY C OMMISSION RESEARCH AGENCY	The Future of E uropean Forests	04/11/2014	Webinar (on-line presentation)	Scientific comm unity (higher educat ion, Research) - Ind ustry - Civil society - Policy makers - Medias	118	Global by online registration
7	Organisation of	FORESTRY C	REPHRAME S	13/11/2014	Brussels, Belgium	Policy makers	6	BE, IRE

	Workshops	OMMISSION RESEARCH AGENCY	eminar to the EU					

Section B (Confidential or public: confidential information marked clearly)

LIST OF APPLICATIONS FOR PATENTS, TRADEMARKS, REGISTERED DESIGNS,UTILITY MODELS, ETC.					
Type of IP Rights	Confidential	Foreseen embargo date dd/mm/yyyy	Application reference(s) (e.g. EP123456)	Subject or title of application	Applicant(s) (as on the application)

OVERVIEW TABLE WITH EXPLOITABLE FOREGROUND								
Type of Exploitable Foreground	Description of Exploitable Foreground	Confidential	Foreseen embargo date dd/mm/yyyy	Exploitable product(s) or measure(s)	Sector(s) of application	Timetable for commercial use or any other use	Patents or other IPR exploitation (licences)	Owner and Other Beneficiary(s) involved

ADDITIONAL TEMPLATE B2: OVERVIEW TABLE WITH EXPLOITABLE FOREGROUND	
Description of Exploitable Foreground	Explain of the Exploitable Foreground

4.3 Report on societal implications

B. Ethics

1. Did your project undergo an Ethics Review (and/or Screening)?	No
If Yes: have you described the progress of compliance with the relevant Ethics Review/Screening Requirements in the frame of the periodic/final reports?	

2. Please indicate whether your project involved any of the following issues :

RESEARCH ON HUMANS	
Did the project involve children?	No
Did the project involve patients?	No
Did the project involve persons not able to consent?	No
Did the project involve adult healthy volunteers?	No
Did the project involve Human genetic material?	No
Did the project involve Human biological samples?	No
Did the project involve Human data collection?	No
RESEARCH ON HUMAN EMBRYO/FOETUS	
Did the project involve Human Embryos?	No
Did the project involve Human Foetal Tissue / Cells?	No
Did the project involve Human Embryonic Stem Cells (hESCs)?	No
Did the project on human Embryonic Stem Cells involve cells in culture?	No
Did the project on human Embryonic Stem Cells involve the derivation of cells from Embryos?	No
PRIVACY	
Did the project involve processing of genetic information or personal data (eg. health, sexual lifestyle, ethnicity, political opinion, religious or philosophical conviction)?	No
Did the project involve tracking the location or observation of people?	No
RESEARCH ON ANIMALS	

Did the project involve research on animals?	No
Were those animals transgenic small laboratory animals?	No
Were those animals transgenic farm animals?	No
Were those animals cloned farm animals?	No
Were those animals non-human primates?	No

RESEARCH INVOLVING DEVELOPING COUNTRIES

Did the project involve the use of local resources (genetic, animal, plant etc)?	No
Was the project of benefit to local community (capacity building, access to healthcare, education etc)?	No

DUAL USE

Research having direct military use	No
Research having potential for terrorist abuse	No

C. Workforce Statistics

3. Workforce statistics for the project: Please indicate in the table below the number of people who worked on the project (on a headcount basis).

Type of Position	Number of Women	Number of Men
Scientific Coordinator	0	1
Work package leaders	0	6
Experienced researchers (i.e. PhD holders)	9	25
PhD student	5	7
Other	18	15

4. How many additional researchers (in companies and universities) were recruited specifically for this project?	8
Of which, indicate the number of men:	4

D. Gender Aspects

5. Did you carry out specific Gender Equality Actions under the project ?	Yes

6. Which of the following actions did you carry out and how effective were they?	
Design and implement an equal opportunity policy	Very effective
Set targets to achieve a gender balance in the workforce	Effective
Organise conferences and workshops on gender	Not Applicable
Actions to improve work-life balance	Effective
Other:	

7. Was there a gender dimension associated with the research content - i.e. wherever people were the focus of the research as, for example, consumers, users, patients or in trials, was the issue of gender considered and addressed?	No
If yes, please specify:	

E. Synergies with Science Education

8. Did your project involve working with students and/or school pupils (e.g. open days, participation in science festivals and events, prizes/competitions or joint projects)?	Yes
If yes, please specify:	EU student exchanges, especially

9. Did the project generate any science education material (e.g. kits, websites, explanatory booklets, DVDs)?	Yes

F. Interdisciplinarity

10. Which disciplines (see list below) are involved in your project?	
Main discipline:	1.5 Biological sciences (biology, botany, bacteriology, microbiology, zoology, entomology, genetics, biochemistry, biophysics, other allied sciences, excluding clinical and veterinary sciences)
Associated discipline:	4.1 Agriculture, forestry, fisheries and allied sciences (agronomy, animal husbandry, fisheries, forestry, horticulture, other allied subjects)
Associated discipline:	1.1 Mathematics and computer sciences [mathematics and other allied fields: computer sciences and other allied subjects (software

	development only; hardware development should be classified in the engineering fields)]

G. Engaging with Civil society and policy makers

11a. Did your project engage with societal actors beyond the research community? (if 'No', go to Question 14)	Yes
11b. If yes, did you engage with citizens (citizens' panels / juries) or organised civil society (NGOs, patients' groups etc.)?	No
11c. In doing so, did your project involve actors whose role is mainly to organise the dialogue with citizens and organised civil society (e.g. professional mediator; communication company, science museums)?	

12. Did you engage with government / public bodies or policy makers (including international organisations)	Yes, in communicating /disseminating / using the results of the project

13a. Will the project generate outputs (expertise or scientific advice) which could be used by policy makers?	Yes - as a primary objective (please indicate areas below multiple answers possible)
13b. If Yes, in which fields?	
Agriculture	Yes
Audiovisual and Media	No
Budget	No
Competition	No
Consumers	No
Culture	No
Customs	No
Development Economic and Monetary Affairs	No
Education, Training, Youth	Yes
Employment and Social Affairs	No
Energy	No
Enlargement	No
Enterprise	No
Environment	No
External Relations	No
External Trade	No
Fisheries and Maritime Affairs	No
Food Safety	No

Foreign and Security Policy	No
Fraud	No
Humanitarian aid	No
Human rightsd	No
Information Society	No
Institutional affairs	No
Internal Market	No
Justice, freedom and security	No
Public Health	No
Regional Policy	No
Research and Innovation	Yes
Space	No
Taxation	No
Transport	No
13c. If Yes, at which level?	European level

H. Use and dissemination

14. How many Articles were published/accepted for publication in peer-reviewed journals?	74
To how many of these is open access provided?	18
How many of these are published in open access journals?	18
How many of these are published in open repositories?	0
To how many of these is open access not provided?	17
Please check all applicable reasons for not providing open access:	
publisher's licensing agreement would not permit publishing in a repository	Yes
no suitable repository available	No
no suitable open access journal available	No
no funds available to publish in an open access journal	Yes
lack of time and resources	No
lack of information on open access	Yes
If other - please specify	
15. How many new patent applications ('priority filings') have been made?	0

("Technologically unique": multiple applications for the same invention in different jurisdictions should be counted as just one application of grant).	

16. Indicate how many of the following Intellectual Property Rights were applied for (give number in each box).	
Trademark	0
Registered design	0
Other	0

17. How many spin-off companies were created / are planned as a direct result of the project?	0
Indicate the approximate number of additional jobs in these companies:	0

18. Please indicate whether your project has a potential impact on employment, in comparison with the situation before your project:	Increase in employment, None of the above / not relevant to the project

19. For your project partnership please estimate the employment effect resulting directly from your participation in Full Time Equivalent (FTE = one person working fulltime for a year) jobs:	4Difficult to estimate / not possible to quantify

I. Media and Communication to the general public

20. As part of the project, were any of the beneficiaries professionals in communication or media relations?	No

21. As part of the project, have any beneficiaries received professional media / communication training / advice to improve communication with the general public?	Yes

22. Which of the following have been used to communicate information about your project to the general public, or have resulted from your project?	
Press Release	Yes
Media briefing	Yes
TV coverage / report	Yes
Radio coverage / report	Yes
Brochures /posters / flyers	Yes
DVD /Film /Multimedia	Yes
Coverage in specialist press	Yes
Coverage in general (non-specialist) press	Yes

Coverage in national press	Yes
Coverage in international press	Yes
Website for the general public / internet	Yes
Event targeting general public (festival, conference, exhibition, science café)	Yes

23. In which languages are the information products for the general public produced?	
Language of the coordinator	Yes
Other language(s)	Yes
English	Yes

Attachments	REPHRAME supporting information.pdf
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:	30/11/2014
Name of the scientific representative of the project's coordinator and organisation:	Dr. Hugh Evans FORESTRY COMMISSION RESEARCH AGENCY
Name	
Date	31/01/2015

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