Institution: University of Greenwich

Unit of Assessment: 8 - Chemistry

Title of case study: Pheromone Traps: providing new Tools across Europe for the Prevention of Spread of Pine Wood Nematode, an Invasive Disease of Pine Forests

Period when the underpinning research was undertaken: January 2007 – December 2020

Details of staff conducting the underpinning research from the submitting unit:

Name(s):	Role(s) (e.g. job title):	Period(s) employed by submitting HEI:
David R Hall	Professor of Chemical Ecology	01/05/1996 – 30/11/2006; 01/01/2007 – present
Paul Douglas	Research Fellow	19/01/2009 – 30/06/2015
Dudley Farman	Senior Scientist	01/05/1996 – present

Period when the claimed impact occurred: August 2013 – December 2020

Is this case study continued from a case study submitted in 2014? N

1. Summary of the impact

Pinewood nematode (PWN) is a devastating disease of pine trees that was introduced into Portugal in 1999 and subsequently spread through 500,000 ha of forest despite EUR38,000,000 spent on attempted eradication. The nematode is spread by a beetle vector, and research by University of Greenwich academics led to development of a pheromone-based trapping system for the beetle that has significantly **changed policy and practice** for managing the disease and its vector throughout the EU. **Early detection of the disease** and **control of the vector** enabled by this system has contributed to **preventing the spread** of the disease into Spain and the rest of Europe. At least seven SME's internationally have **benefitted economic**ally by commercialising the lures and traps and increasing business by millions of euros.

2. Underpinning research

The Chemical Ecology Group at the University of Greenwich's Natural Resources Institute (NRI), has carried out research on pheromones and other semiochemicals affecting the behaviour of insect pests and development of their use in management of the pests from 1969 to the present (NRI joining the university in May 1996). The chemical structures of attractants for over 60 different species of insect pests or vectors have been identified and synthesised, and research by the Group contributed to the award of the **Queen's Anniversary Prize 2019** to NRI for development and implementation of innovative, "smart" methods of controlling pests and diseases.

The research included identification of pheromones of several cerambycid beetle species, and in 2007 the Group began a collaboration with Prof Juan Pajares, University of Valladolid, Spain, an expert on forest pests, particularly *Monochamus galloprovincialis*, the cerambycid beetle vector of pine wood nematode (PWN). The Spanish group had demonstrated that male beetles produced an aggregation pheromone attracting male and female beetles. In the research at NRI (**3.1**), volatiles were collected from male and female beetles and analysed by gas chromatography coupled to electroantennographic (EAG) recording from receptors on the beetle antennae, a technique pioneered by NRI. A male-specific compound elicited EAG responses from antennae of both female and male beetles This was identified as a novel structure, 2-undecyloxy-1-ethanol, using mass spectrometry, microanalytical techniques and comparison with synthetic standards in the large library of compounds built up previously at NRI. A route for synthesis of the compound was developed suitable for large-scale production, and the synthetic compound was shown to attract *M. galloprovincialis* beetles to traps in field tests in Spain (**3.1**).

This work was taken up by the EU REPHRAME project (2010-2014) involving **Hall** and Pajares and collaborators from five other European countries, China and the US. The attractiveness was shown to be greatly synergised by chemicals from the host plants and also kairomones produced by another beetle pest and host plants - 2-methyl-6-methylene-7-octen-4-ol (ipsenol), 2-methyl-3-buten-2-ol and alpha-pinene (**3.2**). Slow release formulations for the pheromone and the synergists were developed by NRI (**3.1**), and the attractant system was patented in 2011 (**3.3**). Production of lures in Spain was taken up by SEDQ and Econex under licence and the trade name "Galloprotect".

Further research was carried out to minimise the number of non-target insects attracted to the traps. This has included modification of the traps but also investigation of alternative synergists for the pheromone including volatile components of wood smoke such as 2-methoxyphenol, 4-methyl-2-methoxyphenol and eugenol, which were identified and tested by NRI (**3.4**).

The compound identified as the male-produced aggregation pheromone of *M. galloprovincialis* was subsequently shown by NRI to be the pheromone of *M. sutor,* a potential new vector of PWN in Europe and Asia, **(3.5)**, and lures and traps were optimised for this species **(3.6)**. The compound was also identified as a pheromone component in three other species of *Monochamus* and shown to attract eight other *Monochamus* species world-wide, as described in over 30 publications by other researchers. These include the pheromone of *M. alternatus*, the main vector of PWN in Asia. The compound was given the trivial name "monochamol" by Canadian authors in 2012 and is widely used to bait traps to detect the presence of invasive species of *Monochamus* world-wide.

3. References to the research

1. Pajares, J.A., Álvarez, G., Ibeas, F., Gallego, D., **Hall, D.R**. and **Farman, D.I.** (2010). Identification and field activity of a male-produced pheromone in the pine sawyer beetle, *Monochamus galloprovincialis. Journal of Chemical Ecology 36: 570-583 (*DOI: 10.1007/s10886-010-9791-5)
2. Álvarez, G., Gallego, D., **Hall, D.R**., Jactel, H. Pajares, J.A. (2016). Combining pheromone and kairomones for effective trapping of the Pine Sawyer Beetle *Monochamus galloprovincialis*. *Journal of Applied Entomology,* 140:58-71 (DOI: 10.1111/jen.12297)
3. Pajares, A.J.A. and **Hall, D.R**. 2011. Attractant bait for capturing the Coleoptera insect *Monochamus galloprovincialis*, the pine sawyer. PCT WO 2011/048246 A2. https://patentimages.storage.googleapis.com/78/f1/ca/c05d916782b506/EP2517561A2.pdf
4. Álvarez, G., Ammagarahalli, B, **Hall, D.R**., Pajares, J.A., Gemeno, C. (2015). Smoke, pheromone, and kairomone olfactory receptor neurons in males and females of the pine sawyer *Monochamus galloprovincialis* (Olivier) (Coleoptera: Cerambicydae). *Journal of Insect Physiology,* 42:46-55. (DOI: 10.1016/j.jinsphys.2015.08.004)
5. Pajares, J.A., Gonzalo Álvarez, A., **Hall, D.R., Douglas, P.,** Centeno, F., Ibarra, N., Martin Schroeder, M., Stephen A. Teale, S.A., Zhiying Wang, Z., Shanchun Yan, S., Millar, J.G. and Hanks, L.M. (2013). 2-(Undecyloxy)-ethanol is a major component of the male-produced aggregation pheromone of ***Monochamus sutor. Entomologia experimentalis et applicata,* 149:118-127.** (DOI: 10.1111/eea.12113**)**
6. Pajares, J, Alvarez G, **Hall D**, Ibarra N, Hoch G, Halbig P, Cocos D, Johansson H, Schroeder M. (2017) Attractants for management of the pine sawyer beetle *Monochamus sutor*, a potential vector of *Bursaphelenchus xylophilus. Journal of Applied Entomology* 141: 97–111 (DOI: 10.1111/jen.12320)

Research grants:
G1: Prof Hugh Evans, Forest Research; **David Hall; Co-I**; Development of improved methods for detection, control and eradication of pine wood nematode in support of EU Plant Health Policy (REPHRAME); European Union Seventh Framework Programme FP7; March 2011 – November 2014; £2,700,000

G2: Rick Mumford, FERA; **David Hall; Co-I**; Tree Health and Plant Biosecurity Initiative: New approaches for the early detection of tree health pests and pathogens; BBSRC; April 2014-Mar 2017; £423,000

4. Details of the impact

Pine wood nematode (PWN) is the cause of a devastating disease of pine trees. It was introduced into Portugal in 1999 and subsequently spread throughout the country to 500,000 ha of forest. The disease is spread naturally by vector beetles of the *Monochamus* genus, and the only vector identified conclusively in Europe is *M. galloprovincialis*. In the absence of control measures, it was estimated that losses in Europe due to damage by the nematode would be of the order of EUR20,000,000,000 during the period 2008-2030 (**5.1. p1**).

Development of pheromone-baited traps for the vector *M. galloprovincialis*, resulting from this research by NRI and its partners, has changed policy and practice in the European Union (EU) to prevent spread of the disease and the subsequent economic losses, and also benefitted SME's which have commercialised the patented trapping technology.

Impacts of the Research on Policy to Prevent the Spread of PWN in the EU

EU Regulations published in 2012 for dealing with new infestations of PWN required removal of susceptible trees within a radius of 500 m round infested trees and intensive manual surveys for PWN in susceptible trees in a buffer zone of 20 km beyond this. In the light of results of the REPHRAME research project (**5.12. Testimonial 1**) an **EU Task Force** reporting in 2016 (**5.2**) recommended the use of the pheromone baited traps to catch *M. galloprovincialis* beetles, followed by molecular analysis for presence of PWN (**5.2. pp 9, 13, 25, 28, 31**). Based on the availability and effectiveness of the pheromone traps and new, more accurate data on beetle flight derived from research made possible by these pheromone-baited traps, they proposed reducing the clear-cut zone to 100 m and the buffer zone to 6 km (**5.2. p26**). This greatly reduced the cost of the operation and loss of trees, and the trap network had the added benefit of reducing the populations of vector beetles, thereby reducing risks of spread (**5.2. p31**). In 2017, these recommendations were taken up by the **EU via an EC Implementing Decision** (**5.3**). **"***The semiochemical/trap system....is now used across Europe in line with EU policy and this ground-breaking research has made a major contribution to preventing the spread of PWN from Portugal into the rest of Europe"* (**5.12 Testimonial 1,2**).

French policy on measures to prevent spread of PWN was summarised by **ANSES -French Agency for Food, Environmental and Occupational Health and Safety, (2015)** (**5.4**). "*Trapping the insect vector for the purpose of early detection of the presence of the nematode remains an essential part of the control strategy."*(**5.4. p34**) "*This method of trapping insect vectors….is possible because the baits designed to capture them are available and operational"* (**5.4. p35**).

A 2016 policy document from the Euphresco network of 70 organisations in more than 50 countries worldwide **coordinating national phytosanitary research** (**5.4**) laid out the optimal strategy for trans-national monitoring programme of *Monochamus* using pheromone-baited traps based on research in five European countries (**5.5. Exec Summary p4**).

In 2018, the **European and Mediterranean Plant Protection Organization (EPPO),** an intergovernmental organization responsible for cooperation in plant health within the Euro-Mediterranean region with 52 members, revised their "Standard" for control and eradication of PWN (**5.6**). This revision "*takes into account the availability of an effective pheromone/kairomone attractant for trapping it*". *"Early detection of new outbreaks is a very important factor in determining the likelihood of eradication*" (**5.6. p504**). "*Sampling should include...the use of traps containing a mixed pheromone/kairomone attractant, followed by testing of any trapped Monochamus species for the presence of B. xylophilus* (**5.6. p505**)."

In 2017, the **UK Forestry Commission Contingency Plan for PWN** (**5.7**) introduced use of pheromone-baited traps for monitoring introduction of *Monochamus* beetles and PWN into the UK with over 15,000 sq km of conifer forest. The trapping approach is particularly important as trees are likely to be asymptomatic in the UK and hence infection is difficult to detect (**5.7. p 25**).
The European Food Safety Authority (EFSA) Plant Health Panel of Experts (**5.8**) advised "*the most efficient detection method is trapping*" for detection of invasive species of *Monochamus* worldwide (**5.8. p13**).

Impacts of the Research on Practice to Prevent the Spread of PWN in the EU

An EU Report on the Situation in Spain (2016) (**5.9**) reported a network of pheromone-baited traps placed throughout the demarcated area was established for the surveys of trees by the Plant Protection Service Spain and Regional Authorities in controlling an outbreak area in As Neves, Galicia (**5.9. p14; 5.12. Testimonial 2**).

An EU Report on the situation in Portugal (2018) (**5.10**) detailed that since 2016, the survey of the vector had relied exclusively on multi-funnel traps baited with the specific pheromone, which are installed and managed by the Portuguese Institute of Nature and Forests Conservation (ICNF). Over 2,000 traps were installed in 2018 (**5.10. p.13**).

Economic Impacts of the Research

Impact on spread of disease. Use of pheromone traps to monitor and reduce dispersal of the vector and PWN is only part of an integrated programme that has prevented spread of the disease from Portugal throughout Europe. Thus, it is not possible to attribute savings in the forestry sector to this innovation alone. However, the importance of early detection meant that "*by the time pinewood nematode was detected in Portugal, over 500 000 ha were affected, and EUR38,000,000 was granted for an eradication campaign, which ultimately failed. In contrast, three isolated outbreaks of pinewood nematode in Spain were detected early and* eradicated with expenditures of less than EUR5,000,000 (**5.11. p10**). Future impact can be expected wherever PWN is a threat, e.g. Australia (**5.11**).

Sale of lures and traps. Lures for *Monochamus* containing the pheromone identified by NRI are now sold by at least seven different companies, including one in the UK and two in Spain. SEDQ Spain stated that the development of *Monochamus galloprovincialis* dispensers with NRI and University of Valladolid has allowed them to expand their business portfolio in forestry with *product sales at a European level worth EUR1,500,000 from 2013 to the present* (**5.12. Testimonials 3,4**).

5. Sources to corroborate the impact

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2. European Commission (2016) Report of the Task Force on the control of pine wood nematode in Portugal operating between November 2014 and October2015. Ref. Ares(2016)2889630 - 22/06/2016.
3. European Commission (2017) Commission Implementing Decision (EU) 2017/427 of 8 March 2017 amending Implementing Decision 2012/535/EU as regards measures to prevent the spread within the Union of *Bursaphelenchus xylophilus* (Steiner et Buhrer) Nickle et al. (the pine wood nematode) (notified under document C(2017) 1482).
4. ANSES -French Agency for Food, Environmental and Occupational Health and Safety (2015) Evaluation of emergency measures to prevent the spread of the pine wood nematode within the European Union. ANSES Opinion Collective Expert Appraisal Report, Sept 2015.
5. EUPHRESCO (2018) Focusing on *Monochamus* spp., insect vectors of *Bursaphelenchus xylophilus* (MONOCHAMUS). http://doi.org/10.5281/zenodo.2223533

6. European and Mediterranean Plant Protection Organization (EPPO) (2018) PM 9/1 (6) *Bursaphelenchus xylophilus* and its vectors: procedures for official control. https://doi.org/10.1111/epp.12505
7. Forestry Commission (2017). Contingency Plan for the Pine wood nematode (*Bursaphelenchus xylophilus*) and its longhorn beetle (*Monochamus* spp.) vectors. 31pp
8. EFSA Plant Health Panel (EFSA PLH Panel), Bragard C et al. (2018). Scientific Opinion on the pest categorisation of non-EU Monochamus spp. EFSA Journal 2018;16(11):5435, 35 pp. https://doi.org/10.2903/j.efsa.2018.5435
9. European Commission (2016) Final Report of an Audit carried out in Spain from 17 October 2016 to 28 October 2016 in order to evaluate the situation and controls for *Bursaphelenchus xylophilus* DG(Sante) 2016-8803-MR; Ref. Ares(2017)1847284 - 06/04/2017.
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11. A. J. Carnegie, T. Venn, S. Lawson, M. Nagel, T. Wardlaw, N. Cameron & I. Last (2018): An analysis of pest risk and potential economic impact of pine wilt disease to Pinus plantations in Australia, Australian Forestry, https://doi.org/10.1080/00049158.2018.1440467
12. Testimonials **Professor Hugh Evans**, Fellow of UK Forest Research and Coordinator of EU REPHRAME project; **Dr Angel Sanchez,** Head of Environmental Protection Services, Castilla y León, Spain; **Dr Lidia Roura**, Product Control and Regulatory Manager, SEDQ, Spain; **Dr Diego Gallejo**, Technical and R&D Manager, Econex, Spain.