Project number: 6271
Funding source: 10/RD/PHYTOFOR/DAFM/721
Date: November, 2016
Project dates: Dec 2011 – Oct 2015

Understanding *Phytophthora ramorum (Sudden Larch Death)* in Irish forests

Key external stakeholders:

Forest owners (public, private), phytosanitary agencies, Department of Agriculture, Food and the Marine (Ire), Department of Agriculture, Environment and Rural Affairs (NI), plant nursery owners

Practical implications for stakeholders:

The plant pathogen *Phytophthora ramorum* has caused serious damage to Japanese larch forests in Ireland and phytosanitary policy to stop the spread of the disease has led to the removal of over 1300 ha of Japanese larch on the island of Ireland. The key outcomes from this research are:

- *Phytophthora ramorum* is spread in rain, in streams, and to a lesser extent, in soil and can be present on boots worn in infected forests. Clear felling infected trees stops aerial spread. However, this research has also demonstrated that two years after eradication treatments in infected sites, the spread of *P. ramorum* in rainwater, plant material and soil is low to negligible.
- Workers and visitors in forests should ensure that they do not inadvertently move material (plants, soil, debris) from one forest to another on boots and equipment as this can spread disease.
- Multi-laboratory ring tests have confirmed the importance of using molecular tests to detect *P. ramorum* in suspect samples.
- An updated Pest Risk Analysis has been prepared for forestry and plant health stakeholders

Main results:

- **Two genetic lineages present on the island of Ireland.** Of the four genetic lineages of *P. ramorum*, only the EU1 lineage is present in Ireland, while EU1 and EU2 lineages are present in Northern Ireland, with most being EU2.
- **Pathogen is detectable all year**. *Phytophthora ramorum* was detected over two years at a site with an infected Japanese larch. However spread of *P. ramorum* in rainwater reduces drastically after removal of infected hosts. Spread in soil is low to negligible in Japanese larch sites cleared of infected hosts.
- **European larch is more resistant.** RNA sequencing experiments showed that one of the reasons why European larch may be more resistant than Japanese larch to infection by *P. ramorum* is because it shows a larger and faster response in its gene up- and down-regulation.
- **Multi-laboratory ring tests.** Multi-laboratory ring tests confirmed the importance of using molecular tests to detect *P. ramorum* in suspect samples.

Opportunity / Benefit:

This project has resulted in new knowledge on *P. ramorum* epidemiology on larch in Ireland which will inform the forestry community and phytosanitary agencies of the risks associated with replanting areas that were clear felled due to *P. ramorum*.

Collaborating Institutions:

DAFM, AFBI-NI, UL, UCD, Coillte

Contact Helen Grogan Email: Helen.grogan@teagasc.ie.

Teagasc project team: Helen Grogan (Teagasc PI)
Richard O' Hanlon

External collaborators: Department of Agriculture, Food and the Marine, Kildare, Ireland (Dr. Josephine Brennan (PI), Dr. James Choiseul (PC)
Agri-Food and Biosciences Institute (AFBI), Belfast, Northern Ireland (Dr. Alistair McCracken, Ms. Lourdes de la Mata Saez)
University College Dublin (UCD), Dublin, Ireland (Prof. Fiona Doohan, Mr. Jianguang Jia)
University of Limerick, Limerick, Ireland (Dr. Tom Harrington)
Coillte
Steering Committee

1. Project background:

Phytophthora ramorum was first detected in Ireland in 2002 on the ornamental host plants *Rhododendron* and *Viburnum*. In 2010 in the UK, and 2011 in Ireland the pathogen was found to have spread to the forest species Japanese larch, on which it caused an epidemic of Sudden Larch Death. *Phytophthora ramorum* is a notifiable quarantine pathogen at EU level, with infected plants and trees subject to destruction notices in order to eradicate the disease. The host jump of *P. ramorum* from *Rhododendron* to the commercially important forest species Japanese larch in 2011 caused concern among the forest industry in Ireland. While research was available on *P. ramorum* in Europe and North America, little was known about the pathogen on Japanese larch or on the island of Ireland. This project conducted research into both the basic biology and genetics of the disease, along with applied epidemiological trials to understand how the disease was behaving and spreading in the forest environment.

2. Questions addressed by the project:

- What is the nature of the *P. ramorum* population on the island of Ireland?
- How does the pathogen spread and disperse in the field?
- What occurs at a molecular level when a susceptible larch host is infected with *P. ramorum*?
- Can we optimize the detection protocols to provide a reliable and robust detection service?

3. The experimental studies:

Desk based reviews were used to analyze the historical records of the pathogen in Ireland. This was followed by a scientific literature review of the pathogen. In parallel, genetic and phenotypic analyses were conducted on a large set of isolates of the pathogen. Over the course of two years, three field plots were monitored in Ireland in order to assess the modes of spread of the pathogen in the wider environment. Host range trials were conducted, to assess if infection could occur in a number of different tree species, and using a number of different infection routes (e.g. leaf, stem and root infections). RNA sequencing was used to concurrently record the defensive responses of the host to infection, and the offensive actions used by the pathogen during infection of larch species with *P. ramorum*. Multi-laboratory ring tests were conducted to evaluate the ability of traditional and molecular tests to detect *P. ramorum* in suspect samples. Finally, the results of all parts of the project were used to update the PRA for *P. ramorum* from an Irish and Northern Irish context, and provide scientific evidence to directly inform policy.

4. Main results:

Population studies. From the review of the historic survey data, and the microsatellite genotyping analyses, we have developed an understanding of the *P. ramorum* population in Ireland, its likely origins and its spread. While there are four genetic lineages of *P. ramorum* worldwide, only the EU1 lineage is present in Ireland, while EU1 and EU2 lineages are present in Northern Ireland, with most being EU2. A single introductory event is the best explanation for the current EU2 forest epidemic in Northern Ireland. This lineage may have entered N. Ireland on infected plants collected by a plant collector, which then spread to Japanese larch forests. In Ireland, the analyses seem to point to several discrete introductions of the EU1 lineage of the pathogen. The horticultural trade in ornamental plants is the most likely route for such introductions. Based on phenotypic characterisation experiments (linear growth rate, pathogenicity to *Rhododendron*), the EU1 and EU2 lineages could not be distinguished from each other. The two lineages are also of the same mating type, preventing mating between them. Based on genetic characterisation, the lineages could be separated using molecular RFLP and microsatellites. There was low fungicide resistance in the Irish isolates, with only 2 of 20 showing resistance to Metalaxyl. Trials on the efficacy of biocides for destroying infective potential of infected leaf litter found that neither Jeyes fluid or dilute bleach were effective

Contact Helen Grogan Email: Helen.grogan@teagasc.ie.

at killing the pathogen in leaf material at recommended rates. Thus Physical removal of all debris followed by cleaning with a suitable biocide is important to stop human mediated spread of the pathogen.

Field trials. The field trials found that rainwater, soil, plant material and watercourses provided sources of infective material in an infected Japanese larch forest. Monitoring over two years found that infective potential existed all year, with most of the sporulation on larch happening in the autumn/winter. Spread of the pathogen in watercourses was confirmed by stream baiting at two of the three sites. However, this research has demonstrated that two years after eradication treatments in infected sites, the spread of *P. ramorum* in rainwater, plant material and soil is low to negligible. Furthermore, the preliminary results of replanting trials using 9 species (*Quercus petraea*, *Fagus sylvatica*, *Picea abies*, *P. sitchensis*, *Pinus sylvestris*, *Pseudotsuga menziesii*, *Larix kaempferi*, *L. decidua* and *Rhododendron caucasicum* × *ponticum*) in 2 previously infected sites in Ireland and 1 site in Northern Ireland indicate that disease is only noticeable in the Larch and Rhododendron hosts. There were occasions where the material collected from boots used in an infected site was found to carry infection. Laboratory trials and results from different hosts sampled in infected forests, found that some hosts (e.g. Sitka spruce, Norway spruce) could not be infected, while others (Japanese larch, beech) were found to be infected on several occasions.

Host response to infection. The RNA sequencing experiments showed that one of the reasons why European larch may be more resistant than Japanese larch to infection by *P. ramorum* is because European larch shows a larger and faster response in its gene up- and down-regulation. Many of the genes that are switched on/off have important functions in plant defence and resistance signalling. This work also highlighted a number of genes that play an important role in *P. ramorum* overcoming and/or avoiding detection and defensive response by the host.

Multi-laboratory ring tests. Multi-laboratory ring tests confirmed the importance of using molecular tests to detect *P. ramorum* in suspect samples as traditional plant pathology methods (e.g. plating onto selective media) often resulted in false negatives.

5. Opportunity/Benefit:

This project has resulted in new knowledge on *P. ramorum* epidemiology on larch on the island of Ireland which will inform the forestry community and plant health stakeholders of the risks associated with replanting areas that were clear felled due to *P. ramorum*. This research has demonstrated that from 2 years after eradication treatments in Larch forests, spread of *P. ramorum* infection in rainwater, plant material and soil is low to negligible. This finding provides hope for the management of the epidemic, as under Irish law all forest land must be replanted with trees within 2 years of harvest.

6. Dissemination:

The knowledge and information gained from this project has been transferred in formal and informal settings during the three years of the project and beyond. There were two formal presentations made to the Forestry Development Department of Teagasc, as they are a key stakeholder and disseminator to farm forestry stakeholders. Open meetings were also held for all stakeholders. Peer reviewed scientific publications are in press and in progress. Details are outlined below.

Main publications:

O'Hanlon, R., Choiseul, J., Grogan, H. and Brennan, J.M. (2016). *In-vitro* characterisation of the four lineages of *Phytophthora ramorum*. *European Journal of Plant Pathology,* (in press), DOI 10.1007/s10658-016-1019-2.

O'Hanlon, R., Choiseul, J., Grogan, H. and Brennan, J.M. (2016). Assessment of the eradication treatments applied to the notifiable plant pathogen *Phytophthora ramorum* in Irish *Larix kaempferi* forests. *Forest Pathology* (under review).

Popular publications:

O'Hanlon, R., Grogan, H., McCracken, A., de la Mata Saez, L., Doohan, F., Jia, J., Harrington, T., Brennan, J. and Choiseul, J. (2014) Understanding *Phytophthora ramorum* in Irish forests. Factsheet printed and published via Teagasc. http://www.agriculture.gov.ie/forestservice/foresthealthandseeds/

O'Hanlon, R., Grogan, H., McCracken, A., de la Mata Saez, L., Doohan, F., Jia, J., Harrington, T., Brennan, J. and Choiseul, J. (2014) PhytoFor- Protecting Irish forests from disease. *TResearch,* 9 (4): 28-29. https://www.teagasc.ie/publications/2014/tresearch-autumn-2014.php

Workshops/seminars:

Contact Helen Grogan Email: Helen.grogan@teagasc.ie.

Forest fungi in Ireland – Opportunities and threats'. 3rd April 2013 Teagasc, Ashtown, Co. Dublin. http://www.rohanlon.org/downloads/Report_on_SIPP_Spring2013_seminar.pdf
PHYTOFOR: Detection, epidemiology and control of *Phytophthora ramorum and P. kernoviae* in Irish forests – Final conference. 29th September 2015, Backweston, Celbridge, Co. Kildare. https://www.eventbrite.ie/e/phytofor-detection-epidemiology-and-control-of-phytophthora-ramorum-and-p-kernoviae-in-ireland-tickets-18344491869

Conference participation:

O'Hanlon, R., Grogan, H., Choiseul, J. and Brennan, J.M. (2014). Basic and applied research on *Phytophthora ramorum* in Irish forests – the PHYTOFOR project. IUFRO congress on *Phytophthoras* in forests and natural ecosystems. Esquel, Argentina. November 2014.
O'Hanlon, R. (2014). Overview of the PHYTOFOR research project. Department of Agriculture Seminar series. Celbridge, Kildare, Ireland. April 2014.
O'Hanlon R. (2013) Sudden oak death (*Phytophthora ramorum*) in Ireland. World Forestry Centre, Portland, Oregon, USA. November 2013.
O'Hanlon, R. (2013) The PHYTOFOR Project- Detection, Epidemiology and control of *Phytophthora ramorum* and *P. kernoviae* in Irish Forests. Seminar entitled 'Forest Fungi in Ireland – Opportunities and Threats' Teagasc, Ashtown, Dublin, Ireland. April 2013.

Future leverage from PHYTOFOR:

The PHYTOFOR project has laid the ground-work for future projects into *Phytophthora* and forest pathology research in Ireland. As a direct result of the PHYTOFOR project, a culture collection of over 400 isolates, including over 24 species of *Phytophthora* has been amassed at DAFM. These isolates can be used in future studies. This research has highlighted that a large diversity of *Phytophthora* species exists across several habitats in Ireland. The threat of these species is yet to be realised. One species found in the sampling of this project, *Phytophthora pseudosyringae*, has since been shown to be a threat to Japanese larch forestry in Ireland. The expertise built in in this project will support future projects into *Phytophthora* diversity and horizon scanning for future threats from non-native pathogens.

7. Compiled by: Dr. Richard O'Hanlon and Dr. Helen Grogan

Contact Helen Grogan Email: Helen.grogan@teagasc.ie.