Department of Agriculture, Food and the Marine
An Roinn Talmhaíochta, Bia agus Mara

CoFoRD

Final Report

'Multi-agent control options for pine weevil (MCOP)'

DAFM Project Reference No: 10/RD/MCOP/NUIM/720

Start date: 01/02/2012

End Date: 31/05/2016

Principal Coordinator and Institution: Professor Christine Griffin, Maynooth University
Email:Christine.griffin@nuim.ie

Collaborating Research Institutions and Researchers: Prof Kevin Kavanagh and Dr Conor Meade, both of MU

Please place *one* "x" below in the appropriate area on the research continuum where you feel this project fits

Basic/Fundamental → Applied/Pre Commercial

	x				x	

Please specify priority area(s) of research this project relates to from the National Prioritisation Research Exercise* (NRPE) report;

Priority Area (s)	**Sustainable food production and Processing (I)**

Key words: *(max 4). Integrated pest management (IPM); forest protection; biological pest control; Hylobius abietis*

1. Rationale for Undertaking the Research

This section should outline the rationale for carrying out the research and identify the need / problem to be addressed

The large pine weevil is the most devastating insect pest of Irish forestry. There is a need for alternative control strategies for this pest, to reduce reliance on chemical insecticides. Biological control using entomopathogenic nematodes (EPN) applied to stumps is under development in Ireland for suppression of weevil populations, but can best be considered as one weapon in the arsenal against the pest. This leaves Irish forestry undesirably dependant on finding a chemical replacement for cypermethrin (the use of which continues derogation for a limited period), though the use of any replacement chemical will still need to be minimised to comply with forest certification requirements that "management systems shall promote the development and adoption of environmentally friendly non-chemical methods of pest management and strive to avoid the use of chemical pesticides".

Integrated pest management can involve a variety of control options, including biological agents, environmentally acceptable chemicals, and cultural practices. Since two agents with different modes of action often act synergistically (give an outcome greater than that predicted based on the performance of either agent alone) the project involved testing agents alone and in combination. Pine weevil can be targeted at two stages: weevils developing in recently felled conifer stumps can be targeted with EPN and entomopathogenic fungus (EPF) applied to stumps either alone or together. Killing adult weevils, which feed on and can kill newly planted seedlings and against which chemicals are currently targeted, would be an attractive alternative. Entomopathogenic fungi are successfully used against other adult beetles, and synergy with chemical insecticides has been reported. The main aims of the project were to test EPF and EPN against developing weevils, test EPF and chemical insecticides against adult weevils, investigate diversity of EPF in the target ecosystem and develop methods to screen agents for potential synergistic effects.

On a broader perspective: the development of an EPF team integrated with the existing EPN team at NUI Maynooth, and the establishment of a strain bank of Irish EPF will provide a national asset with potential to deliver EPF solutions to other insect pest problems in Irish forestry, horticulture and agriculture.

2. Research Approach

Specify the research methodologies employed, emphasising novel techniques and also outline any modifications from the original approved project proposal

- Isolation and identification of entomopathogenic fungi (EPF) from the target environment (clearfell coniferous forests), providing indigenous isolates and baseline frequency biodiversity data
- Measurement of intraspecific genetic diversity of EPF, providing this biodiversity baseline and also providing a basis for selection of diverse strains for biocontrol screening
- Evaluation, against pine weevil larvae and adults, of indigenous EPF strains together with strains (*Metarhizium anisopliae* and *Beauveria bassiana*) already commercialised for other purposes
- Evaluation of potential of EPF to colonise conifer seedlings endophytically

- Assessment of effects of EPF on pine weevil immune defences, providing understanding upon which management may be based, the basis of a better screening for virulence, and insight into pathogen functional diversity
- Assessment of interactions (synergy, additivity, antagonism) between EPF and nematodes when used against pine weevil larvae
- Assessment of the persistence of applied permitted EPF in the target environment
- Assessment of interactions between EPF and potential new chemicals against adult weevils
- Identification and assessment of the biological activity of a metabolite produced by native EPF *Beauveria caledonica*. This was an addition approach not foreseen in the original proposal.

3. Research Achievements/Results

Outline main results achieved

In a survey of native EPF in the clear-fell forest ecosystem, EPF were recovered from the bark of tree stumps and from soil around stumps, and most promisingly, pine weevil larvae within stumps were found infected with indigenous EPF. Several native species of EPF including *Beauveria bassiana*, *B. caledonica* and *M. anisopliae* were identified by molecular means (sequencing the ITS and EF1 regions) and representative samples are maintained at MU.

In laboratory bioassay, the mortality of pine weevil larvae due to indigenous *B. caledonica* (isolated from naturally infected weevil larvae) was comparable to that due to existing commercial EPF species. EPF were tested as stand-alone agents and in combination with nematodes for action against weevil larvae in stumps. In three years of field trials, application of a combined treatment of nematodes and EPF to stumps reduced the number of adult weevils emerging over the subsequent season by up to 90% relative to untreated stumps. There was no evidence of synergistic effect of the EPN-EPF combination in these trials.

EPF applied to seeds or seedlings colonised both Sitka spruce and lodgepole pine and persisted endophytically (within the seedling) for at least 9 months without any adverse effects on plant growth. This method has potential for longer term plant protection. Laboratory studies showed a synergistic effect between three species of EPF and each of three chemical pesticides, cypermethrin, thiacloprid and acetamiprid against adult weevils. One EPF-chemical insecticide combination was tested in the field. One or both agents were applied to seedlings and weevil damage was compared to that on untreated controls. The approach showed promise but would require further optimisation of fungal strain and application methods.

In order to understand the interaction of EPF with other control agents and to develop a method of screening for strains that may be most effectively combined with other agents, we investigated the effects of EPF on insect defences, both their cellular defences and protein components of the blood system. Alteration was seen in the levels of certain proteins in the insect blood following treatment with EPF, including several with known involvement in the insect immune response.

A novel biochemical produced by the native fungus *B. caledonica* was identified. It did not function as an antifeedant but was found to be a potent immunosuppressant on injection.

Important differences between species of EPF in mode of action, growth characteristics and ecology in the forest ecosystem were detected.

4. **Impact of the Research**

A summary of the impact of the research should be provided through the project outputs and outcomes however please provide a synopsis of the benefits / improvements the research has made to the area under investigation. Outline the benefits of the research to end users, e.g. industry, consumers, regulatory authorities, and scientific community etc

Forest industry: Some very interesting and potentially useful findings are emerging, both in terms of efficacy of EPF species choice and use, alone, or in combination with nematodes or chemicals through laboratory and field trials. The project is of considerable potential and practical relevance for more effective pest control at reforestation with the environmental benefit of increased use of biological agents and reduced insecticide use.

Horticulture/agriculture sector: strain bank of indigenous EPF is available for testing against other pests

Regulatory authorities: information on indigenous species/genotypes of EPF will inform licencing of products for use

Scientific community: The availability of pine weevil transcriptome will facilitate further investigations of this pest's biology.
Comprehensive description of effects of EPF culture filtrate on protein expression in pine weevil and in a model insect (wax moth, Galleria mellonella) will further the understanding of the mode of action of entomopathogenic fungi and ways in which they may facilitate other agents when used in combination.

4(a) Summary of Research Outcomes

(i) Collaborative and Industry links developed during this research

Producers/distributors of plant protection products such as BeckerUnderwood and e-nema were kept up-to-date on results of field trials. Discussions were held with Paul Sopp of Fargro in relation to field trials with Met52.

(ii) Outcomes where new products, technologies and processes were developed and/or adopted

Methods for endophytic colonisation of conifer seeds/seedlings with EPF were developed

(iii) Outcomes with economic potential

Use of EPF alone or in combination has potential to mitigate damage caused by pine weevil in reforestation when derogation for cypermethrin ends

(iv) Outcomes with national/ policy/social/environmental potential

During the reporting period project outcomes were communicated to Coillte for inclusion in their request for a derogation to continue use of cypermethrin. Project outcomes were also communicated directly in writing to FSC (Forest Stewardship Council) in this regard. The derogation was approved.

4 (b) Summary of Research Outputs

(i) Peer-reviewed publications, International Journal/Book chapters.

McNamara, L., Carolan, J., Griffin, C.T. Fitzpatrick, D., and K. Kavanagh. The effect of entomopathogenic fungal culture filtrate on the immune response of the greater wax moth, *Galleria mellonella*. *Journal of Insect Physiology*, accepted

Kapranas, A., Malone, B., Quinn, S, McNamara, L. Williams, C.D., O'Tuama, P., Peters, A. Griffin, C.T. (2017) Efficacy of entomopathogenic nematodes for control of large pine weevil, *Hylobius abietis*: effects of soil type, pest density and spatial distribution. *Journal of Pest Science* 90, 495-505. DOI 10.1007/s10340-016-0823-y

Williams, C.D., Dillon, A.B., Harvey, C.D. Hennessy, R., McNamara, L., Griffin, C.T. (2013). Control of a major pest of forestry, *Hylobius abietis*, with entomopathogenic nematodes and fungi using eradicant and prophylactic strategies. *Forest Ecology and Management* 305, 212-222. DOI: 10.1016/j.foreco.2013.05.055**.**

In preparation

Mc Namara, L., Kapranas, A., O'Tuama, P., Kavanagh, K. and C.T. Griffin
Control of a major pest of forestry, *Hylobius abietis*, with entomopathogenic fungi, alone and in combination with entomopathogenic nematodes.

Mc Namara, L., K. Kavanagh Connell, S., Fitzpatrick, D., Griffin, C.T. Carolan, J., Effect of entomopathogenic fungi on the immune system of large pine weevil, *Hylobius abietis.*

Mc Namara, L., Dolan, S., Walsh, J.M.D., Stephens, J., Glare, T., Kavanagh, K. and C.T. Griffin. Identification of immunosuppressive compounds in *Beauveria caledonica*.

Van Vlaenderen, J., Griffin, C.T., O'Tuama, P. and Meade, C. Identification of entomopathogenic fungi from clearfell coniferous forests.

Van Vlaenderen, J., Meade, C. and Griffin, C.T. Endophytic colonisation of conifer seedlings (lodgepole pine and Sitka spruce) by entomopathogenic fungi.

Van Vlaenderen, J., O'Tuama, P. and Griffin, C.T. Combining chemical insecticides with entomopathogenic fungi: promising control strategy for large pine weevil, *Hylobius abietis.*

(ii) Popular non-scientific publications and abstracts including those presented at conferences

McNamara, L., Griffin, C. and Kavanagh, K. 2015. *Galleria mellonella* as a model for studying insect immune responses to entomopathogenic fungi Royal Entomological Society Annual meeting, Dublin, 2-4 September 2015

McNamara, L., Kavanagh, K. and Griffin, C. 2015. Biocontrol of *Hylobius abietis* (pine weevil) larvae using entomopathogenic fungi and nematodes. Irish Plant Society Annual Meeting, Maynooth, 11-12 May 2015

van Vlaenderen et al 2015 Inoculation and persistence of three entomopathogenic fungi as endophytes in Sitka spruce and lodgepole pine. Irish Plant Society Annual Meeting, Maynooth, 11-12 May 2015

McNamara, L. Griffin, C. & Kavanagh, K. 2014 The immune response of *Hylobius abietis* and *Galleria mellonella* larvae to entomopathogenic fungi. Xth European Congress of Entomology, York, England 3-8th August 2014

VanVlandereren, J. Meade, C. & Griffin, C. 2014. Inoculation and persistence of three entomopathogenic fungi as endophytes in Sitka spruce and lodgepole pine. Xth European Congress of Entomology, York, England, 3-8th August 2014

VanVlandereren, J. Meade C. & Griffin, C. 2014. Inoculation of entomopathogenic fungi as endophytes by treatments of the seeds and rootlets of Sitka spruce and lodgepole pine seedlings. COST FA1103 Application techniques of endophytes, Bielefeld, Germany. 14-15 July, 2014

McNamara, L. Griffin, C. & Kavanagh, K (2013) Screening Indigenous Entomopathogenic Fungi for Control of Pine Weevil larvae. IMPACT Conference "Protecting current and future forests", Dublin, 7 May 2013.

Van Vlanderen, J., Meade, C. and Griffin C. (2013) Survey of the entomopathogenic fungal community in coniferous forests in Ireland : Endemic control agents in the fight against *Hylobius abietis*. IMPACT Conference "Protecting current and future forests", Dublin, 7 May 2013

McNamara, L. Griffin, C. & Kavanagh, K. 2013. Interactions of entomopathogenic fungi and other control agents: mechanism and field potential against pine weevil. 46th Annual Meeting of the Society for Invertebrate Pathology Conference on Invertebrate Pathology and Microbial Control. Pittsburgh, USA, August11-15, 2013

Van Vlanderen, J. Meade, C and Griffin C. (2013) Endophytes for Plant Protection : the state of the art. COST Action FA1103; Berlin, Germany, 27-29 May 2013.

Van Valendern, J., Hennessy, R., Harvey, C., Williams, C., Dillon, A. and Griffin, C. Control of the large pine weevil *Hylobius abietis* with entomopathogens. Royal Entomological Society Annual Meeting, Cambridge UK.18-20 July 2012,

(iii) National Report

(iv) Workshops/seminars at which results were presented

In addition to those with abstracts listed under section (ii) :

Griffin, C.T. (2013). Environmental safety and factors influencing field success of entomopathogens. Talk at Hylobius Stakeholder meeting, National Botanic Gardens, Wales, 23rd January, 2013

(v) Intellectual Property applications/licences/patents

(vi) Other

5. Scientists trained by Project

Total Number of PhD theses: ___2_

Please include authors, institutions and titles of theses and submission dates. If not submitted please give the anticipated submission date

Louise McNamara. Interactions of entomopathogenic fungi and other control agents: mechanism and field potential against *Hylobius abietis* larvae. Maynooth University, 2016

Johan van Vlaenderen. Potential for use of native and non-native entomopathogenic fungi against the large pine weevil, *Hylobius abietis.* Maynooth University. Anticipated submission date: Oct 2017.

Total Number of Masters theses: __0__

Please include authors, institutions and titles of theses and submission dates. If not submitted please give the anticipated submission date

6. Permanent Researchers

Institution Name	Number of Permanent staff contributing to project	Total Time contribution (person years)
NUIM	6	1.0
Total	**6**	**1.0**

7. Researchers Funded by DAFM

Type of Researcher	Number	Total Time contribution (person years)
Post Doctorates/Contract Researchers	0	0
PhD students	2	8.25
Masters students	0	0
Temporary researchers	26	0.820
Other	0	0
Total		

8. Involvement in Agri Food Graduate Development Programme

Name of Postgraduate / contract researcher	Names and Dates of modules attended

9. Project Expenditure

Total expenditure of the project:	€317,211.07
Total Award by DAFM:	€317,031.00
Other sources of funding including benefit in kind and/or cash contribution(specify):	€0

Breakdown of Total Expenditure

Category	Maynooth University Institution 1	Name Institution 2	Name Institution 3	Name Institution 4	Total
Contract staff	0.00				
Temporary staff	17,695.86				
Post doctorates	0.00				
Post graduates	176,288.68				
Consumables	33,535.93				
Travel and subsistence	12,658.66				
Sub total	240,179.13				
Durable equipment	4,978.00				
Other	0.00				
Overheads	72,053.74				
Total	**317,211.07**				

10. Leveraging

Summarise any additional resources'/funding leveraged by this award from other sources i.e. Additional Staff, National/EU funding secured, EI Commercialisation Fund

(1) EU FP7 funding for project "New biological control products for sustainable farming and forestry (BIOCOMES)." 2013-2017. €529,728 to MU. Coillte Teoranta is also involved as partner. Project involves 27 partners (biocontrol industry and research organisations) from 14 countries.
(2) Industry funding (Coillte: €6933 plus in-kind contribution of seedlings, insecticide and personnel) for field evaluation of combined agents
(3) Maynooth University funding (€3000) to cover costs of sequencing pine weevil (*Hylobius abietis*) transcriptome, to enable identification of proteins affected by EPF

11. Future Strategies

Outline development plans for the results of the research.

Maynooth University and Coillte propose to develop some of the more promising approaches further, subject to availability of funding

12. Consent to Publish Final Report on the DAFM Website and/or Through Other Dissemination channels

I consent to this report being made available to the public, through the Department's website and other dissemination channels. *

Yes ☐x No ☐

13. Declaration

I declare that the information contained in this final report is complete and true to the best of my knowledge and belief.

Signed: _____ Project Coordinator

Date: 7th April 2017___

*IPR sensitive information that the coordinator does not wish to make public should be highlighted in red font. All text in red font in this report will not be made publicly available by DAFM.

Guidelines for the Completion of Final Report

The attached Final Report Template should be completed for DAFM projects funded under the **2006, 2007, 2008, 2010 and 2011** Calls.

The aim of the final report is to provide a summary of all aspects of the research project. A final report is required for all projects and a percentage of the grant award will be withheld until it is submitted and deemed satisfactory.

Please note that the Department of Agriculture, Fisheries and the Marine may publicise information included in the Final Report. ***All sections of the report must be completed.*** Incomplete reports will not be accepted by DAFM and will be returned to the project coordinator for completion.

Two copies of the final report are required, 1) a signed hard copy and 2) an electronic copy.

A guideline of up to 400 words per relevant section is recommended.

Project Details

The project title, project reference number and actual start and actual finish date should be noted. Indicate on the research continuum from basic/fundamental research to applied/pre-commercial research where you feel the research project fits by placing **'x'** in the most appropriate box. Indicate in the section provided **the priority area of research this project relates to from the National Prioritisation Research Exercise* (NRPE) report**, There are 14 priority areas of Research as follows;

A Future Networks & Communications	**H Food for Health**
B Data Analytics Management, Security & Privacy	**I Sustainable Food Production and Processing**
C Digital Platforms, Content & Applications	**J Marine Renewable Energy**
D Connected Health & Independent Living	**K Smart Grids & Smart Cities**
E Medical Devices	**L Manufacturing Competitiveness**
F Diagnostics	**M Processing Technologies and Novel Materials**

In addition, key words relating to the project should be included in this section.
***http://www.agriculture.gov.ie/research/14researchprioritisationactionplans**

1. Rationale for Undertaking the Research

This section of the final report should provide background information on why the research was needed. It should clearly outline the reason for carrying out the research and identify the problem / knowledge gap that needed to be addressed. It should address the question '**why was this research needed?**

2. Research Approach

Information provided on research approach should address the questions **'how the research was carried out?'** Details should include work carried out and research methodologies used to address the issues identified in the 'rationale for undertaking the research'. Emphasis should be placed on novel techniques, materials, technology and equipment used. Scientific or technical difficulties encountered in the research and any significant modifications from the original proposal must be noted. Please note that this section does not require fine scientific detail, but is designed to give the reader an overall view of the research methods employed.

3. Research Achievements/Results

This section is simply designed to address the question **'what are the results of the research'**. Emphasis should be placed on novelty and innovation. Tabulated scientific results are not required but a succinct summary of results obtained from each task should be illustrated.

4. Impact of the Research

A summary of the impact of the research should be provided through the project outputs and outcomes. The benefits / improvements the research has made to the area under investigation should be elucidated. Specifically, describe how the outcomes of the research have benefited the end users such as industry, consumers, regulatory authorities, policy makers and the scientific community.

4(a). Summary of Research Outcomes

The outcomes reported must detail the wider effect of the project from a sectoral or national perspective; these may be in the medium or long term. The summary of research outcomes is a critical component of the final report. It is imperative that this section is completed fully and precisely, as DAFM is required to report on the outcomes of all research projects. In addition, this data is essential to DAFM in justifying value for money of its research programmes and in securing future funding. Therefore, please ensure that information in this section is accurately reported.

4(b). Summary of Research Outputs

Research Outputs are what are produced by the project in terms of activities, events, services that reach people. The summary of research outputs is a critical component of the final report as it provides quantitative data on the research. It is imperative that this section is completed fully and precisely, as DAFM is required to report on the outputs of all research projects. In addition, this data is essential to DAFM in justifying value for money of its research programmes and in securing future funding. Therefore, please ensure that information in this section is accurately reported.

5. Scientists trained by the project

The total number of PhD and MSc theses produced as a direct result of work carried out on this DAFM project should be noted. In addition, the authors, institutions and titles of the theses and submission dates should be specified. If theses have not been submitted before completion of the final report, please give details including the anticipated submission date.

6. Permanent Researchers

The number of permanent research staff who contributed to the project (on a cost neutral basis) per institution and associated time contribution must be captured.

7. Researchers Funded by DAFM

Details of numbers, total time contribution (in months) and of all, post doctorates (PD) &contract researchers; PhD students; Masters students; temporary researchers and other staff funded by DAFM should be included.

8. Involvement in Agri Food Graduate Development Programme

The names of students / researchers that participated in the Agri Food Graduate Development Programme should be included in addition to the names and dates of modules undertaken.

9. Project Expenditure

The aim of this section is to provide a summary of expenditure during the lifetime of the project. Please note that it is imperative that all figures included in this section correspond to figures included in the last progress report submitted and evaluated by DAFM. The names of the institutions involved must be included in the tables provided.

10. Leveraging

The aim of this section is to summarise any additional resources'/funding leveraged from this award from other sources e.g. Additional Staff (type of staff, value of staff secured), National/EU funding secured, EI Commercialisation Fund

11. Future Strategies

Future strategies to further develop the outputs of the research should be indicated. If the outputs of the research have not been taken up by end users, explain why this is the case. What further advances / work is required in your area of research in order for the outputs to taken up by industry / consumers / end users? What follow-on research is required in this area to realise an end product? If further funding is required for research in this area, where do you intend to apply for funding e.g. Enterprise Ireland?