Department of Agriculture, Fisheries and Food
An Roinn Talmhaíochta, Iascaigh agus Bia

Research Stimulus Fund

Final Report

'Mushroom Virus X disease: Understanding the factors which trigger "brown mushroom" symptom expression as a means to improved diagnosis and control

DAFF Project Ref No:	RSF 07 547
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End date:	30th Nov 2010

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Please tick below the appropriate area on the research continuum where you feel this project fits

BASIC/FUNDMENTAL →		APPLIED/PRE COMMERCIAL
		X

Key words: *(Mushroom, compost, disease, diagnostics)*

NDP NATIONAL DEVELOPMENT PLAN

1. Rationale for Undertaking the Research

Mushroom Virus X (MVX) is a relatively new virus disease of mushrooms that has affected the Irish mushroom industry since late 1990's. The main symptom is "brown" or "off coloured" mushrooms of reduced quality, which leads to the dumping of produce by growers or the rejection of produce by the retailers, thereby disrupting the supply chain and causing economic losses for the growers. As the bulk of Ireland's mushroom crop is exported (about 80%), this is not desirable for the image of Irish mushrooms abroad or the economic health of the growers. Brown mushroom symptom expression is sporadic, transient and unpredictable, suggesting a complex aetiology involving unknown factors. Mushroom viruses are transmitted within the mushroom tissue (mycelium) that grows through the mushroom compost as well as through the spores produced by the mushrooms. Thus the problem needs to be addressed by both mushroom compost producers and mushroom growers. In order to control a disease it is essential to understand how if affects the host organism – the mushroom. At the start of this project there were many gaps in our knowledge of the epidemiology and aetiology of brown mushroom symptom expression associated with MVX.

This project aimed to clarify the complex aetiology of brown mushroom symptom expression associated with MVX infection and to identify possible critical factors that would result in consistent symptom expression. Sections of the industry believed that stressful environmental conditions (e.g excessive watering, humidity, etc), as well as particular crop management practices (e.g. ruffling) influenced the appearance of symptoms, and these hypotheses were examined. There was no quantitative measure to detect "brownness" for MVX infected mushrooms so objective methods to both quantify and detect brown-mushroom symptoms in mushrooms were also needed. As the mushroom virus can be carried in the mycelium that grows throughout the mushroom compost, the industry also needed a test that could detect MVX in colonised compost. Molecular diagnostic techniques are the most appropriate to detect the genetic material of MVX in mushroom compost. However, mushroom compost is high in humic acids, which are inhibitory to molecular testing methods, so an effective test was always going to be a challenge.

A successful outcome to this research would lead to increased knowledge about how MVX affects the mushroom crop as well as diagnostic tests to detect its presence in mushrooms and compost.

2. Research Approach

i. Standard laboratory procedures were used to (a) culture and store MVX-infected *Agaricus* isolates and (b) produce sterilised media, substrates and inoculum.

ii. Experimental cropping trials were planned and designed to maximise the chances of detecting significant treatment effects. These were conducted at the experimental mushroom growing facilities at both Teagasc, Kinsealy and AFBI, Loughgall. Cropping experiments included treatments such as (a) the introduction of MVX-infected material into either compost or casing at different rates and times; (b) the use of a ruffling technique; (c) the manipulation of environmental factors such as humidity, evaporation, watering management etc. to induce "stressful" conditions; (d) point inoculation.

iii. Mushroom "colour" was measured quantitatively using a colorimeter to quantify the level of brown mushroom symptoms observed in a crop.

iv. Standard and newly-reported molecular biology techniques were used for nucleic acid extraction, purification, electrophoresis, and PCR.

v. Measurement of post harvest quality was done by the DIT team (Dr Jesus Frias) using a wide array of analytical tools, and the most up to date techniques and methodologies such as Colorimetry, RGB image acquisition, hyperspectral imaging, , Fourier Transform Infra Red microscopy (FT-IR). External quality indexes (colour, density, weight loss) were analysed using wet lab and image analysis together with biochemical quality indexes (enzyme activity, browning pigments).

3. Research Achievements

- **Critical infection time identified.** The critical infection time and conditions that lead to consistent appearance of MVX brown mushroom symptoms were identified. A low rate of infected material (0.01%) that is incorporated into compost or casing at the end of the compost incubation period gave the most consistent symptoms. Thus it is imperative that mushroom compost and casing does not come into contact with any infective material (from previously infected crops) at this time. Composters and growers should enhance hygiene measures to minimise any cross-contamination at this critical time.
- **MVX moves through compost very rapidly.** Following a point-infection of MVX into mushroom compost it was found to move at least 4 m (length of compost studied) within a single cropping period. This contrasts with a <1 m spread for fungal diseases following a point-infection.
- **Agronomic factors do not influence MVX symptoms expression.** Crops grown under stressful environmental conditions were no more likely to develop brown mushroom symptoms than crops grown under non-stressful conditions. MVX does not appear to be "triggered" by environmental factors.
- **Effective molecular diagnostic test to detect MVX in mushrooms.** A PCR based diagnostic test for mushrooms was effective and reliable and detected MVX in infected mushrooms that showed no symptoms. An industry wide survey also detected MVX at a level higher than the occurrence of symptoms suggested. This would explain the persistence and transience of the symptoms within the Irish industry since the late 1990's.
- **Molecular diagnostic test to detect MVX in mushroom compost**. Although a test to detect MVX in mushroom compost was developed it was only moderately successful and not entirely reliable due to the humic acid content of compost.
- **FT-IR detection of MVX in post-harvest mushrooms shows promise**. Detection of MVX in post-harvest mushrooms using spectroscopic methods and its discrimination from other causes of browning proved difficult but FT-IR microimaging method shows promising results. This technique may be useful to rapidly detect MVX within a few hours in mushroom samples and might develop into a complementary technique to the molecular diagnostic.

4. Impact of the Research

- The outcomes of this research have had a significant impact on the Irish and European mushroom industries. Growers and composters in Ireland and Europe are more aware of the complex aetiology behind MVX and brown mushroom symptoms as a result of scientific presentations at meetings and seminars. Compost producers and growers are requesting compost testing and advice when outbreaks occur as well as technical seminars for their key staff.

- The outcomes of this research have had a significant impact on the mushroom scientific community. Several peer-reviewed publications are in an advanced stage of preparation. A significant bank of knowledge has been acquired. A collaborative research project with HDC and University of Warwick (UK) on MVX detection in compost was undertaken as a follow on from this project. There has been a major advancement in the reliability of detection of MVX in compost as a result, although the testing methodology is still very laborious.
- This research project has fostered and developed close formal links between Teagasc, Kinsealy; AFBI, Northern Ireland and DIT, Dublin, where complimentary expertise has been applied to a single subject.
- This Stimulus-funded research project underpinned a successful bid by the Co-ordinator for EU funding in Dec 2010, which will continue aspects of this project with regard to MVX epidemiology and control, in collaboration with European industry and research partners. A new 3-year EU funded project is due to start in Jan 2012 (See section 11 below).

5. Exploitation of the Research

- The intellectual property produced from this research is primarily in the form of Knowledge. No patents or licences have been generated
- Compost producers and mushroom growers are engaging with researchers to inform themselves in detail of the research findings and to learn how to tackle MVX control on their facilities.
- CMP (Commercial Mushroom Producers) – the organisation representing mushroom growers in Ireland, held a series of disease seminars for growers in 2011 at which the project co-ordinator presented information arising from this research.
- Composters and mushroom growers contact the Co-ordinator when they have samples for testing. At the moment there is no commercial testing service available other than what is offered by Teagasc. There is a need to identify a service provider to provide this service to the industry. This will be addressed in the forthcoming EU project due to start in Jan 2012.

6. Summary of Research Outputs

(a) Intellectual Property applications/licences/patents

1. None

(b) Innovations adopted by industry

1. In-house seminars for composters on “MVX epidemiology and control” for key technical staff.
2. Regular disease updates and seminars for growers to inform them of latest research developments.
3. Improved understanding by the industry of the need for thorough and efficient hygiene measures to be in place when handling bulk phase 3 compost (equipment, machinery, surfaces, etc)

4. Mushroom and compost samples sent in to Teagasc for testing (this service can only be offered on an occasional basis).

(c) Number of companies in receipt of information

- All major Irish composters
- Sylvan, the main spawn manufacturer in Ireland (and Europe)
- All Irish mushroom growers (~100) through the activities of CMP as well as via industry newsletters and industry publications in both Northern Ireland and the Republic of Ireland.

(d) Outcomes with economic potential

1. The major economic benefit is through achieving MVX-free crops. However, this would require regular testing and further research to identify weak links in the system that allow MVX to continue to proliferate. Further research is planned in these areas in the forthcoming EU project due to start in Jan 2012 (See Section 11 below)

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

1. MVX has the potential to cause significant losses in mushroom businesses as there are strong competitive pressures on all growers and composters. The Irish mushroom industry needs to have the eradication of MVX as an objective if it is to prosper and retain employment in rural areas over the next 10 years.

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

(g) Scientific abstracts or articles including those presented at conferences

1. Fleming-Archibald, C., Ruggiero A., Kilpatrick, M. and Grogan, H.M (2009). Factors which influence the expression of "brown mushroom" symptoms in crops infected with Mushroom Virus. Molecular Biology of Plant Pathogens, Oxford, 21 September 2009.
2. Fleming- Archibald, C., Ruggiero A. and Grogan, H. (2009). Identifying factors which influence the expression of "brown mushroom" symptoms in crops infected with Mushroom Virus X. Proceedings of the Agriculture Research Forum, Tullamore, Co. Offaly, 12-13 march, 2009, P124.
3. Fleming- Archibald, C., Ruggiero A. and Grogan, H. (2009). Identifying factors which influence the expression of "brown mushroom" symptoms in crops infected with Mushroom Virus X. Society of Irish Plant Pathologists Spring Meeting, Backweston, Celbridge, Co Kildare, 25 March, 2009.
4. Grogan, H. (2010) Unraveling the epidemiology of a new virus disease of mushrooms. Mycoviruses. Special Interest Group. 9th International Mycological Congress, Edinburgh, 1-6th Aug 2010. Abstract.
5. Fleming-Archibald, C., Ruggiero A., Kilpatrick, M. and Grogan, H.M (2009). Epidemiological studies of the factors influencing brown mushroom symptom expression associated with Mushroom Virus X. Mycoviruses. Special Interest Group. 9th International Mycological Congress, Edinburgh, 1-6th Aug 2010. Abstract.
6. Grogan, H., Fleming-Archibald, C., Ruggiero A. & Kilpatrick, M. (2010). Mushroom virus X disease: a whiter shade of pale brown? TResearch Vol 5, No 4, pp14-15.

7. E. Gaston, J.M. Frías, P.J. Cullen, C.P. O'Donnell and A.A. Gowen (2010) Vis-NIR hyperspectral imaging for the identification of damaged or infected mushrooms. Poster presentation at the International Association for Spectral Imaging Meeting IASIM-10, Ashtown, Dublin, Ireland, 18-19th of November 2010.
8. L. Alvarez-Jubete, F. Bonnier, H. Byrne, H. Grogan and J. M. Frias (2011) DETECTION OF MUSHROOM VIRUS X (MVX) INFECTION IN ASYMPTOMATIC MUSHROOMS USING FTIR MICROSCOPIC IMAGING. Poster accepted for presentation at the International Conference of Engineering and Food (ICEF11), Athens, Greece, 24th May 2011.

(h) National Report

(i) Popular non-scientific publications

1. Grogan, H. (2008). Brown Mushroom Virus Research. Teagasc Mushroom Newsletter (Kinsealy, Dublin), No 30. P2.

(j) Workshops/seminars/ open days at which results were presented (excluding those in (g))

1. Aguirre, L., Frias, J. & Grogan, H. (2009). Quality assessment in mushroom (*Agaricus bisporus*) infected with Mushroom Virus X. Poster. All Ireland Mushroom Conference and Trade Show, Monaghan, Co Monaghan, 21 May 2009, P72.
2. Fleming- Archibald, C., Ruggiero A. and Grogan, H. (2009). Identifying the optimum conditions for development of brown mushroom symptoms in a crop infected with Mushroom Virus X. Poster. All Ireland Mushroom Conference and Trade Show, Monaghan, Co Monaghan, 21 May 2009, P72.
3. Grogan, H. (2009) Research Update, Teagasc, Kinsealy. All Ireland Mushroom Conference and Trade Show, Monaghan, Co Monaghan, 21 May 2009, pp15-18.
4. Grogan, H. (2009) Disease threats to mushroom production. Seminar to Sylvan International technical management meeting, Chateauform, Paris, France. 23/24 Sept 2009.
5. Grogan, H. (2009) Disease threats to mushroom production - Virus X update and Diagnostics. Seminar to Polish Mushroom Growers Association, Skierneweitze, Poland, 18/19 Nov 2009.
6. Kilpatrick, M *et al* (2009) R&D in support of the Mushroom Industry – Issues with MVX. Northern Ireland Institute of Agricultural Science visit to AFBI, Loughgall, 8th September 2009.
7. Grogan, H. (2010). Stimulus-funded MVX Research update. Oral presentation at Mushroom Stakeholder meeting, Teagasc, Kinsealy, to discuss Research needs for the Industry. 26 Jan 2010.
8. Grogan, H. (2010). In house seminar on Stimulus-funded MVX Research update given to technical staff at major compost facilities, 2 Feb 2010 and 22 July 2010.
9. Grogan, H. (2010). Mushroom Virus X – Epidemiology and Control. Oral presentation at South African Mushroom farmers Congress 2010, Centurion, South Africa. 16/17 Feb 2010.
10. Grogan, H. (2010). Seminar on Stimulus-funded MVX Research – update on industry survey. Oral presentation given to stakeholders (Mushroom Enterprise Group) Teagasc, Kinsealy, 8th October 2010.

11. Grogan, H. (2011). Mushroom Disease Control. Seminar series for mushroom industry organized by CMP at four venues around the country:- 11th, 14th & 21st March and 4th April 2011.
12. Grogan, H. (2011). Research Update – Teagasc, Kinsealy. Proceedings of the 2011 All Ireland Mushroom Conference, Hillgrove Hotel, Monaghan, October 20th & 21st 2011, pp14-20.
13. Kilpatrick, M., Fleming-Archibald, C, Ruggiero, A., Burns, D., McPoland, P., & Grogan, H. (2011). The effect of agronomic factors on the expression of brown mushroom symptoms in Mushroom Virus X (MVX) infected crops in Ireland. Poster presentation at the 2011 All Ireland Mushroom Conference, Hillgrove Hotel, Monaghan, 20th October 2011.
14. L. Alvarez-Jubete, F. Bonnier, H. Byrne, H. Grogan and J. M. Frias (2011) Detection of mushroom Virus X (MVX) infection in asymptomatic mushrooms using FTIR microscopic imaging. Poster presentation at the 2011 All Ireland Mushroom Conference, Hillgrove Hotel, Monaghan, 20th October 2011.

7. Permanent Researchers

Institution Name	Number of Permanent staff contributing to project	Total Time contribution (months)	Average time contribution per permanent staff member
Teagasc	5	15	3
DIT	1	1.44	1.44
AFBI	7	40.2	5.74
Total	**13**	**56.64**	**4.36**

8. Researchers Funded by RSF

Type of Researcher	Number	Total Time contribution (months)	Average time
Post Doctorates	3	49	16.3
Contract Researchers			
PhD postgraduates			
Masters postgraduates			
Temporary researcher	1	20.3	20.3
Other			
Total	**4**	**69.3**	**17.3**

9. Postgraduate Research

Total Number of PhD theses:	0
Total Number of Masters theses:	0

10. Project Expenditure

Total expenditure of the project:	€	434,459.26
Total Award by RSF	€	446,914.79
Other sources of funding (specify) 1. In-kind industry contribution	€	(40,000)

Breakdown of Total Expenditure

Category	Teagasc Institution 1	DIT Institution 2	AFBI Institution 3	Total
Contract staff				
Temporary staff	67,618.00			67,618.00
Post doctorates	111,894.00	91,083.15		202,977.15
Post graduates				
Consumables	25,683.27	2,992.93	16,828.30	45,504.50
Travel and subsistence	8,549.56	2,419.78	481.73	11,451.07
Sub total	213,744.83	96,495.86	17,310.03	327,550.72
Durable equipment				
Other	8,643.66			8,643,66
Overheads	64,123.44	28,948.45	5,192.99	98,264.88
Total	**286,511.93**	**125,444.31**	**22,503.02**	**434,459.26**

11. Future Strategies

- Three peer-reviewed publications are at an advanced stage of preparation for submission to international scientific journals
- The results of this research will be advanced in a recently awarded EU FP7–funded research project entitled "MushTV - Solutions for the mushroom industry to emerging disease threats from *Trichoderma* and Virus". This project is a collaboration of 16 mushroom-industry and mushroom-research partners across five European countries with a €2.5 m research budget over 3 years. Further research will be conducted on (a) identifying weak links in the MVX infection pathway, (b) industry screening to identify actual presence of non-symptomatic MVX infections within the European industry and (c) evaluating and improving diagnostic tests that growers can avail of commercially.

12. Industry Collaboration

Industry collaboration on this project consisted of the following:

- Discussions were undertaken with mushroom composters and growers in the early stages of the project to identify their views on how MVX affected crops and what possible factors they believed might correlate with MVX outbreaks.

- Discussions were undertaken with the technical representatives of Sylvan Spawn, a company who provide technical support to the mushroom industry and who have extensive knowledge of how diseases affect the industry from month to month.
- An industry wide survey of mushrooms and compost was undertaken in the final year of the project to get (a) a perspective on how well the diagnostic tests developed during the project were working and (b) to see if MVX was more widespread than symptoms indicated.
- When MVX outbreaks occurred during the period of the project, growers and composters usually called the research team for help and advice. At these times they were updated with whatever new information was emerging from the research to date.
- A number of international mushroom organisations sought advice on MVX epidemiology and control during the project.