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Mushroom Virus X (MVX) - Investigating gene expression in infected crops

Key external stakeholders:

Mushroom industry, mushroom compost producers, researchers, Disease diagnosis service laboratories

Practical implications for stakeholders:

Mushroom virus X (MVX) disease causes significant economic losses to the mushroom industry due to the production of poor quality and brown off-coloured mushrooms. This project used a range of molecular technologies to gain an understanding of the genetic make-up of the virus and how it affects the normal functioning of mushrooms. The main outcomes are:

- MVX infection causes significant disruption to the normal processes within the mushroom, which is likely to reduce mushroom quality. Thus it is important to ensure that crops remain virus-free.
- Strong evidence suggests that the mushroom-cap-browning symptom is caused by a distinct virus-like entity that is tentatively named Brown Cap Mushroom Virus (BCMV).
- Two test methods were developed that can detect BCMV. A colorimetric test can detect subtle changes in the whiteness of a mushroom due to the presence of the BCMV before the development of any brown discolouration. This test is suitable for use on mushroom farms. A second method uses a highly sensitive molecular-based PCR test.

Main results:

- **Normal mushroom cell processes are disrupted**. MVX infection causes 32 mushroom genes to be significantly suppressed resulting in the disruption of many normal functions in the mushroom. This is likely to reduce mushroom quality.
- **Confirmation that MVX disease is caused by viruses.** Strong evidence suggests that the mushroom-cap-browning symptom is caused by a distinct virus-like entity that is tentatively named Brown Cap Mushroom Virus (BCMV)
- **A new on-farm colorimetric test** has been developed which can discriminate between infected white mushrooms and non-infected mushrooms.
- **MVX molecular diagnostic test available.** Unique MVX dsRNA sequences have been validated in a new highly-sensitive QPCR diagnostic test which can detect the presence of the BCMV associated with MVX disease in mushrooms.

Opportunity / Benefit:

This work has led to the development of a new QPCR diagnostic test for BCMV. Growers and composters can have mushrooms tested to indicate if BCMV is present or not, and obtain advice on how the virus is being transmitted. In addition an on-farm colorimetric test is available to identify potential disease symptoms with greater accuracy.

Collaborating Institutions:

University of Warwick, UK; Horticulture Research International, Warwick, UK, mushroom growers.

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1. Project background:

The white commercial mushroom *A. bisporus* is prone to a viral disease known as MVX, which causes white mushrooms to develop a brown or off-white cap colour as they grow. Cap discoloration can also take place post-harvest, when mushrooms are being stored or transported. The MVX browning symptom is a serious economic problem as it severely reduces mushroom quality, resulting in product rejection or downgrading of up to 80% of a harvest. There is a poor understanding of how the virus affects the mushroom, as the symptoms of the disease can be irregular and transient. This project used a range of molecular technologies to gain an understanding of how the virus affects the mushroom.

2. Questions addressed by the project:

This project concentrated on identifying what was happening at a genetic level in virus-infected mushrooms, compared with non-infected mushrooms. The key questions were: (1) What is the genetic response of the mushroom when it is infected with MVX? and (2) Can we identify more specifically the virus elements that are responsible for the brown-cap-colour symptoms associated with MVX?

3. The experimental studies:

Brown symptom development during MVX infection was investigated using gene expression profiles from mushrooms in different pathological states. Genes associated with virus-induced browning were successfully identified by using molecular techniques including Suppression Subtractive Hybridization (SSH), micro-array and quantitative reverse transcriptase PCR (Q-PCR) analyses. MVX infected mushrooms were collected from industry. A time-course progression of brown-symptom development was conducted in conjunction with MVX-infected crops grown at the Teagasc Mushroom Unit at Kinsealy.

4. Main results:

Microarray analysis identified 25 genes up-regulated and 32 genes down-regulated in MVX infected mushrooms. Thus MVX-infection drives widespread suppression of host genetic processes and their associated functions, thereby putting the organism under stress. Sixteen up-regulated elements (transcripts) were not part of the *A. bisporus* genome and were thus inferred to be viral. They were found to accumulate to very high levels in MVX-infected brown mushrooms and QPCR indicated they were up-regulated by 24,000 to 1 million-fold compared with non-infected controls. Thus the viral genome is preferentially replicated while there is a general inactivation and disruption of host protein synthesis.

Northern analysis demonstrated that all 16 viral transcripts hybridized to a subset of known MVX dsRNA elements of size 2.0 Kbp, or smaller. Thus, it appears that the mushroom cap browning symptom is caused by a distinct and apparently unencapsidated partitivirus-like entity of at least two dsRNA segments ≤ 2.0 Kbp, tentatively named Brown Cap Mushroom Virus (BCMV).

A time-course study of an infected crop displaying brown-cap mushroom symptoms indicated that although viral transcripts accumulated in all mushrooms, only some went on to develop a “visible” brown-cap symptom. The reason for this is as yet unknown but it may be associated with the time a particular mushroom initial or mycelium becomes infected. This has resulted in a new on-farm colorimetric test being developed which can detect subtle changes in whiteness levels, due to the presence of the BCMV in mushrooms prior to the development of the brown discolouration. This test can discriminate between non-infected white mushrooms and white BCMV infected mushrooms and is suitable for use on mushroom farms.

5. Opportunity/Benefit:

The on-farm colorimetric test can be used to identify BCMV-infected crops before symptoms can be seen by the human eye. Unique MVX dsRNA sequences identified in this studentship are the basis of a new highly-sensitive QPCR diagnostic test which can detect the presence of the BCMV associated with MVX disease in mushrooms. Growers and composters can have mushrooms tested (Teagasc Horticultural Dept.) to indicate if BCMV is present or not, and if there is a need determine how the virus is being transmitted on their facilities.

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6. Dissemination:

Results from this project have been disseminated to the mushroom industry through the All Ireland Mushroom Conference, Monaghan, May 21st 2009. The outcomes were also disseminated to mushroom composters and key staff through a series of MVX Seminars on individual stakeholder premises during 2011. Mushroom growers and their staff were reached via Disease Control seminars in 2011 and 2012, which were organised by CMP, in conjunction with Teagasc, at venues in Cavan, Monaghan, Westmeath and Tipperary. Teagasc advisory staff deal with grower queries on this topic and, when necessary, farm visits are made to provide one to one advice tailored for specific farms.

A follow-up project involving Drs Grogan and Burton has validated the use of the highly sensitive PCR test for compost samples so the presence of disease infection can now be detected earlier in the cropping cycle, before mushrooms are produced.

Teagasc offer MVX diagnostics services to industry and technical support on MVX control in mushroom crops.

Formal links with Industry: Work on MVX epidemiology and control continues with national and European industry and scientific partners via EU FP7 Project MushTV (286836) 2012- 2014 (www.MushTV.eu)

Main publications:

Burton, K., Green, J., Baker, A., Eastwood, D. and Grogan, H. (2011). Mushroom Virus X – The Identification Of Brown Cap Mushroom Virus And A New Highly Sensitive Diagnostic Test For Phase Iii Compost. Proceedings of the 7th International Conference on Mushroom Biology and Mushroom Products (ICMBMP7) 2011, 466-473.

Deakin GL, Edward Dobbs E, Bennett JM, Green J, Jones IM, Grogan HM and Kerry S. Burton KS (2012) Genomic Studies to characterise the viruses of Mushroom Virus X and responses of the host Agaricus bisporus to infection. Proceedings of the 18th Congress of the International Society for Mushroom Science (Beijing August 2012), 329-335.

Green, J. M., Grogan, H., Eastwood, D. C. and Burton, K. S. (2008). Investigating genetic and environmental control of brown colour development in the cultivated mushroom *Agaricus bisporus* infected with Mushroom virus X, pp. 536-553. In: Science and cultivation of edible and medicinal fungi (ed. M. van Gruening). Proceedings of the 17th Congress of the International Society for Mushroom Science, Cape Town, South Africa, 20-24 May 2008.

Green, J. M., Burton, K. S. Eastwood, D. C. and Grogan, H. (2009). Brown colour development in the edible mushroom *Agaricus bisporus* infected with mushroom virus X. Abstract and presentation for the Agricultural Research Forum, Co. Offaly, Ireland, 12-13 March 2009.
http://www.agresearchforum.com/publicationsarf/2009/proceedings2009.pdf

Popular publications:

Green, J. M., Burton, K. S. Eastwood, D. C. and Grogan, H. (2009). An elusive virus. TResearch, Vol4, No. 4: 16-17. http://www.teagasc.ie/publications/2009/3/3_tresearch200910.pdf

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