Grower Summary

M 51

Developing an accurate, quantitative and predictive test for Mushroom Virus X.

Final Report 2010

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Before using all pesticides check the approval status and conditions of use.

Read the label before use: use pesticides safely.

Further information

If you would like a copy of the full report, please email the HDC office (hdc@hdc.ahdb.org.uk), quoting your HDC number, alternatively contact the HDC at the address below.

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Headline

- A highly sensitive diagnostic test for Mushroom Virus X has been developed which detects both forms of the disease (brown and pinning disruption symptoms) in spawn-running Phase III compost.

Background and expected deliverables

Mushroom Virus X disease syndrome (known as MVX) causes a number of symptoms, including pinning disruption, crop delay, premature veil opening, various fruitbody abnormalities and brown-coloured mushroom caps. The form of the disease which causes the brown symptom is currently prevalent. However the industry should always be wary of the virus form that causes bare patches (pinning disruption). This form of MVX has been so devastating that some farms have gone out of business as disinfection measures appear to have limited effect.

MVX disease is thought to be spread within the mushroom industry by contaminated compost, with re-infection occurring from contaminated compost, equipment and farm debris. The disease is associated with the presence of double-stranded RNA (ds-RNA) molecules. Identification of the disease is hampered by the current detection method (separation and observation of ds-RNA on gels) which is slow and difficult to interpret. In addition, this current test is so insensitive that a large quantity of material is required (i.e. mushroom fruitbodies) to provide any indication of infection. Using mushrooms for the test is too late in the cropping cycle when the costs of production,, picking and sales commitments have already been incurred.

This project (M51) aimed to develop a detection method which has increased sensitivity to detect the virus. It is based on the technique called Quantitative PCR. PCR is a molecular technique that involves amplification of single-stranded RNA and it is this amplification that allows very low levels of RNA to be detected. Quantitative PCR (also known as ‘real-time’ PCR) is, as the name suggests a quantitative technique and so provides a gauge of the degree of MVX infection.

Summary of the project and main conclusions

Before Quantitative PCR can be adapted into a reliable technique for use for the industry to detect MVX in compost or casing, three key technical questions had to be addressed and solved:

1) Can purified RNA be extracted from compost?

It is relatively easy to extract RNA with high purity from mushroom fruitbodies. It is more difficult to extract RNA from casing material, but this technique has been achieved previously by the scientists undertaking this project. However extracting RNA from compost is a more difficult task, as compost contains large amounts of humic and phenolic compounds which stick to the RNA and interfere with the manipulations as part of the Quantitative PCR methodology.

- A successful method has been designed in this project which extracts purified RNA from compost and without significant RNA degradation.

2) Does mycelium infected with MVX *also* have raised levels of MVX RNA that can be measured by quantitative PCR?

Previous research has shown that MVX-infected mushroom fruitbodies have raised levels of single-stranded RNA (from MVX) compared with non-infected mushrooms.

- This project found that infected **mycelium** in compost and casing also had higher levels of RNA (of MVX) than non-infected compost or casing.

3) Can the new quantitative PCR method detect all forms of MVX?

Recent research suggests that MVX is a syndrome caused by a collection of viruses which may explain the large number of ds-RNA bands and the diverse symptoms associated with MVX including tissue browning and pinning disruption. The key question to answer is: ‘Can quantitative PCR tests be developed that detect all forms of the MVX infections that are commonly found and economically important to the industry’?

- The diagnostic test for MVX developed in this project is based on two MVX sequences. This combined test was able to detect MVX in a range of strains producing the browning symptom and the pinning disruption symptom from spawn-running compost.

This project has therefore developed a detection method for Mushroom Virus X with such an increased sensitivity that it can detect the virus at low levels in **compost prior to cropping**. It can detect the MVX strains that cause browning and pinning disruption. The test will benefit the industry in two ways:

- It will enable detection of early infection when virus levels are low and so give advanced warning to growers.

- It will identify the sources of infection and so enable disease control.

The new diagnostic test is quantitative and indicates the amount of active virus present in a sample. The results indicate that active MVX levels are much higher in the compost than the casing. Therefore compost is a better substrate for testing the presence of the virus. The level of virus detected in compost increases enormously from spawn-run to compost producing mushroom fruitbodies. Virus levels are not affected by the amount of infection introduced by a crop. Even small amounts of infection at spawning leads to approximately the same virus levels during spawn-run and during cropping as high amounts of infection at spawning.

Financial Benefits

Mushroom Virus X is a significant and occasionally devastating disease for the industry. MVX causes financial losses through both yield loss and/or product rejection due to quality issues (brown colouration). The current test for MVX is too insensitive to detect the disease early during mycelial growth in compost and so too late in the cropping cycle to be of much use in real time. The new diagnostic test developed in this project can detect MVX in compost which will give (1) advanced warning to growers of MVX infection and so allow action to be taken to reduce losses due to the disease and (2) the ability to trace the cause of infection which is likely to lead to fewer outbreaks with the knock-on financial benefits. The new test also opens up the possibility of regular compost testing which will give greater confidence that future crops will be MVX-free

Action Points for Growers

- Growers and compost producers can now use a test to detect MVX and ensure crops and compost are free from infection.

- When future MVX outbreaks occur, this test can be used as a diagnostic tool to ascertain the source of infection and to test infected equipment and machinery to minimise the risks of re-infection.

- Growers and composters are invited to consider to what extent they may wish to use this test. This information will be necessary for the business case to make the test commercially available. Kerry Burton now of East Malling Research (kerry.burton@emr.ac.uk) would like to make this test available to the industry and to deliver results in a timely fashion. There are financial planning implications however and he requests that growers and composters contact him directly if they are interested.