Viral dsRNAs in *Agaricus bisporus:* Transmission, Symptom Expression and Control

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Abstract: Mushroom Virus X (MVX) is a new viral disease of mushrooms affecting *Agaricus bisporus* production in some European countries. Epidemiological studies have been done using pure isolates of MVX-infected *Agaricus* cultures, derived from infected mushrooms. Infected cultures added to sterilised compost produces MVX-infected compost for use in inoculation experiments. Infection of a healthy crop with very small amounts (0.01%) of isolate MVX 1961, at any time from spawning through to casing, resulted in significant crop delay and pin suppression. On-farm trials were conducted in conjunction with a large bulk phase III composter/grower who was experiencing severe MVX symptoms and crop loss. A common winch and conveyor cassette was used to handle both phase II and phase III compost on this site. Phase II compost samples were taken (a) directly from the phase II tunnel, (b) after passing along the spawning winch, and (c) following transport along the compost conveyor cassette prior to filling the phase III tunnels. All three compost samples were then removed and cropped in isolation. Phase II compost removed directly from the tunnel was shown to be clear but compost that had passed along the winches and conveyors had become infected with MVX. The winches and conveyors were identified as the source of the MVX contamination and the disinfection of the equipment was reviewed. Following a period of methyl bromide fumigation the incidence of MVX declined.

Key words: Viral dsRNAs, mushroom virus X, *Agaricus bisporus*, transmission, symptoms, expression, control

1 Introduction

Novel viral dsRNAs in *Agaricus bisporus* have been associated with a new disease that has affected mushroom production in a number of European countries.[1] Up to 26 dsRNAs have been detected in mushrooms from symptomatic crops but it is not yet known how many are directly involved in the disease.[2, 3] The symptom most commonly seen on farms in Britain is a suppression of pinning, resulting in delayed cropping.[3] This can manifest itself as a bare area or "patch" within an otherwise normal bed of mushrooms and, in extreme cases, can reduce yields by up to 60%. When mushroom pins are eventually formed in the patched areas, they tend to be crowded and develop into poor quality mushrooms (which can open prematurely). In the Netherlands, Belgium and Ireland, the primary symptom has been the appearance of brown discoloured mushrooms in what should be a white crop.[1] In extreme situations they can account for 20-40% of yield. Crop delay and pinning disruption have not normally been associated with the brown mushroom symptom in these countries. The brown mushroom symptom has been associated with a discreet group of small dsRNAs.[1, 2] Until the complex of viral dsRNAs have been more fully characterised, this new dsRNA associated disease has been termed Mushroom Virus X, (MVX).

Studies on farms that have experienced severe MVX symptoms have highlighted that, while breeches in standard virus hygiene measures have occurred on some farms, in other cases standard virus hygiene measures, which were sufficient to keep La France disease caused by a 35nm particulate virus at bay, have not been effective in preventing the spread of MVX on the farm. This suggested that current virus hygiene measures

were ineffective against this new viral complex. Thus it is important to establish the aetiology of MVX disease and to identify vulnerable stages in the crop cycle so that control measures can be more efficiently targeted.

2 Materials and Methods

2.1 Epidemiology experiments

A series of inoculation experiments were undertaken using MVX infected *Agaricus* cultures and spores to determine if such material could transmit MVX dsRNAs to a new crop and if such transmission of MVX dsRNAs was associated with symptom expression. *Agaricus* cultures were obtained from mushrooms originating from various farms experiencing virus symptoms and each culture was identified with a unique number. Pure cultures were obtained and used to infect sterilised compost, thereby providing inoculum associated with a specific MVX-infected *Agaricus* culture (MVX 1283, 2735, 1961). MVX-infected mushrooms and spores were obtained from experimental crops that had been infected with a specific MVX strain. Mushroom compost or casing was infected at different times during the crop cycle with either spores or mycelium (in the form of laboratory made spawn using MVX-infected cultures or MVX-infected sterilised-compost). Infection times included (a) at spawning, (b) at the end of the spawn-run, (c) during bulk handling of spawn-run compost, or (d) just after casing.

2.2 On-Farm studies

Epidemiology studies were also made on a farm that was producing bulk spawn-run (phase III) compost, and which was suffering severely as a result of MVX. Samples of the phase III compost that were cropped off-site indicated that the compost was already heavily infected with MVX prior to filling onto shelves. On this farm, a single winch and conveyor cassette was used to remove both pasteurised phase II compost and fully spawn-run phase III compost in the same emptying and filling hall, with a wash-down and formaldehyde-fogging treatment occurring between the different uses. Once MVX was confirmed to be present in the phase III tunnels, the common winches and conveyors for both phase II and phase III handling could potentially be a source of MVX infected material if it was not thoroughly cleaned and disinfected. To test this hypothesis, pasteurised phase II compost was removed (a) directly from the phase II tunnel and hand spawned, (b) after passing along the spawning winch and (c) just prior to filling the phase III tunnel after further transport along the conveyor cassette. All the compost samples were then removed from the farm, cropped in isolation away from the farm, and the mushrooms tested for the presence of MVX dsRNAs in order to determine where the MVX infection was getting into the system.

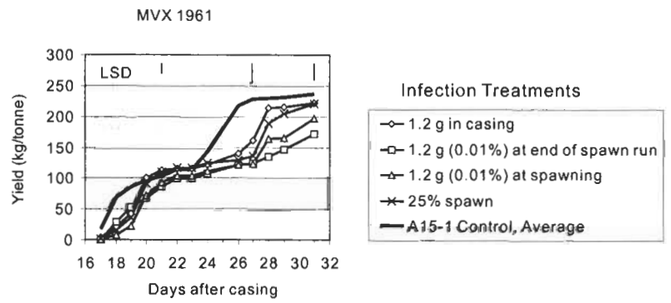

Figure 1. Yield and timing of mushroom production from crops infected with MVX 1961 at different times and dsRNA profiles of mushrooms from compost infected with MVX isolate 1961 at different times during the crop cycle

3 Results and Discussion

3.1 Transmission

Experimental work has demonstrated that MVX dsRNAs are transmitted via both mycelium and spores into healthy crops for a number of different MVX isolates and the results for one isolate MVX 1961 are shown here (Figures 1, 2 and 3). Very low levels of infected material, incorporated at any time from spawning through to casing, appear to be capable of transmitting the dsRNAs into healthy crops and resulting in crop delay. However, the expression of symptoms following casing infections can be variable. Isolates with different dsRNA profiles can give different symptoms.[4] Alternatively sometimes symptoms are expressed and sometimes they are not. However, in all cases, MVX dsRNAs are present in mushrooms. Thus, presence or absence of symptoms is not a reliable diagnostic for the presence of MVX. Once MVX dsRNAs have been detected in mushrooms, growers should investigate where are the likely routes of entry into the system. On sites where compost is made and mushrooms grown, there is a real likelihood of MVX symptoms developing, as there are more opportunities for infected material to enter the crop cycle. Where growers buy in compost from different sources, and produce MVX positive mushrooms, there is a distinct possibility that the compost production site is contaminated.

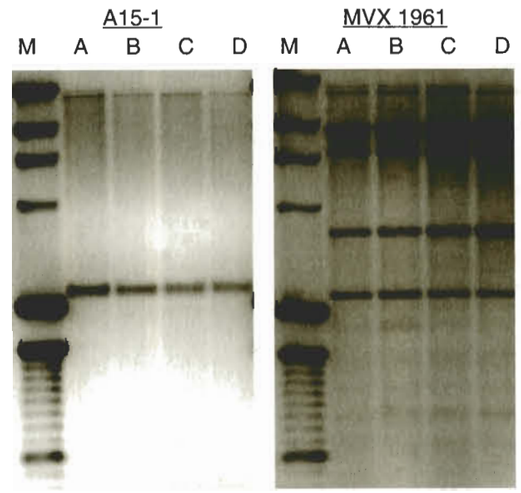

Figure 2. DsRNA profile of mushrooms from crops treated with MVX 1961 compared with an A15-1 Control treatment
M = Marker lane; A, 25% infected spawn added; B, 0.01% infected compost added at spawning; C; 0.01% infected compost added at end of spawn run; D, 1.2 g infected compost added to casing.

That only a very small amount of infective material is needed to transmit MVX dsRNAs was confirmed by the on-farm study. Phase II compost that had not been through the spawning winch produced mushrooms that were clear of MVX dsRNAs (Figure 4), but phase II compost that had travelled a short distance along the winch to have spawn added, or spawned compost that had travelled further along the conveyors to the point where phase III tunnels were being filled, both produced mushrooms with MVX dsRNAs, with the latter containing more dsRNAs than the former. This suggested that the winch and conveyors were the source of MVX contamination. On this farm, the one winch handled both pasteurised phase II compost and bulk spawn-run phase III compost, with a wash-down and formaldehyde-fogging treatment occurring between the different uses. This cleaning procedure however was not effective at eliminating MVX contaminated material from the machinery. Subsequent enhanced cleaning of the winch using methyl bromide fumigation was effective and the level of MVX infection dropped considerable until mushrooms were clear of MVX dsRNAs (Figure 4).

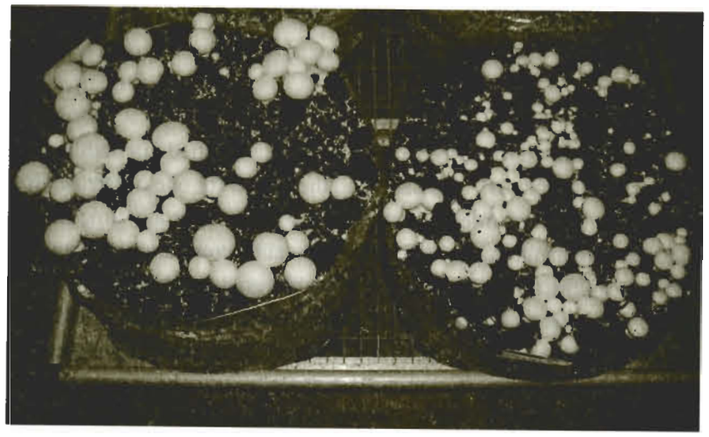

Figure 3. Plots for A15-1 (left) and MVX 1961 (right) during the first flush
Note fewer, better quality mushrooms on the control plot compared with the dense pin-set and crowded small mushrooms on the MVX 1961 plot.

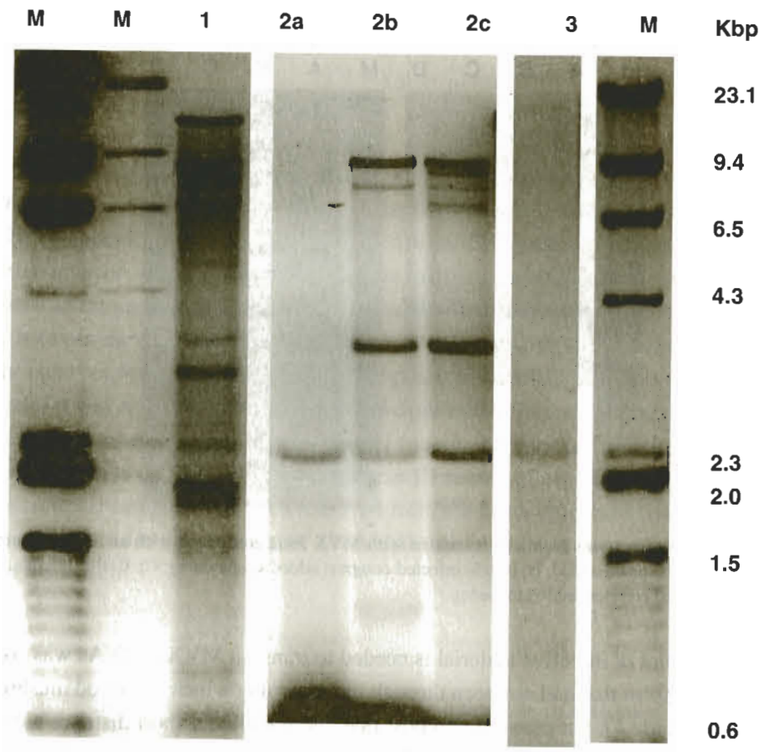

Figure 4. DsRNA profiles of mushrooms from on-farm trail
M = Marker lanes; 1 = Mushroom sample during MVX problem period; 2, Mushrooms from phase II compost removed and cropped off site taken at different locations: (a) pre spawning winch (spawned by hand), (b) post spawning winch, (c) post tunnel-filling cassette; 3, mushrooms following period of methyl bromide fumigation of the spawning and emptying hall and equipment.

These results highlight the necessity for extreme hygiene measures when sharing equipment between phase II and phase III operations. It also highlights the minute amount of material that is required to transmit MVX dsRNAs as the hygiene procedures that were already in place at this site had successfully prevented phase II

compost contamination at spawning with respect to La France 35 nm virus for many years.

References

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