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GROWER SUMMARY

Headlines

- Virus X can be transmitted to healthy *Agaricus* mycelium at any stage in the growing cycle from spawning to post-casing.
- Extremely low levels of infected mycelium, generated during bulk handling of infected spawn-run compost[1], can contaminate healthy compost[1] in the vicinity.

Background and expected deliverables

Virus X has been a major problem for the British mushroom industry since 1998. An HDC funded research project has provided the industry with a reliable test to diagnose Virus X in mushrooms, as well as providing research-based information on cultural and hygiene measures that can limit the spread of the virus on farms (HDC Project M 39, various HDC News articles). At present spores and mycelial fragments are implicated as important factors in Virus X transmission. This observation is speculative, although it is backed by circumstantial evidence. This project aims to determine:

- Spore Transmission - can Virus X-infected mushroom spores infect healthy compost[1]? How many spores are needed and at what time in the growing cycle?
- Mycelium transmission - can Virus X-infected compost[1] infect healthy compost[1]? How much is needed and at what time in the growing cycle?
- Are airborne mycelial fragments generated during bulk handling of spawn-run compost? Can they transmit Virus X to healthy compost[1]?

Working on virus diseases in mushrooms has always been problematical. While this project endeavours to answer the above questions, this will depend on successful methodologies being established which give reproducible results. Further work is likely to be required in this area.

Summary of results and conclusions

Spore Transmission

Initial experiments were carried out following an existing methodology using spore suspensions to infect compost, but these were unsuccessful. An additional experiment was then carried out whereby fresh Virus X-infected spores were used to infect compost during spawn-running. Virus X symptoms of crop delay, bare areas and reduced yields occurred during the crop, and genetic material of Virus X (dsRNAs) was detected in harvested mushrooms. Further work is necessary to determine how many spores are needed to transmit Virus X dsRNAs, if symptom expression is related to quantity of infected spores applied and if crops are vulnerable to infection by Virus X-infected spores throughout the cropping period from spawning to post-casing.

Mycelial Transmission

The results from all experiments using Virus X-infected compost[1] or mycelium indicated that:

- Virus X-infected compost[1] or mycelium readily transmits Virus X dsRNAs into healthy *Agaricus* mycelium, resulting in Virus X-infected mushrooms, irrespective of whether the contamination occurs at spawning, during bulk handling, or post-casing.

[1] The term "infected compost" means compost that contains *Agaricus* mycelium infected with Virus X dsRNAs. "Healthy compost" is compost that is spawned, or spawn-run with *Agaricus* mycelium that is free of Virus X dsRNAs.

- The expression of Virus X symptoms such as crop delay, bare areas, poor quality and brown mushrooms depends on both the time of infection and quantity of infective material. A summary of Virus X transmission and symptoms is shown in Table 1.
- Exposure of healthy spawn-run compost[1] to extremely low levels of Virus X-infected mycelium fragments during bulk handling, can result in poor quality mushrooms that contain Virus X dsRNAs, but the crop is not delayed and yields are normal. Higher levels of contamination (1%) during bulk handling of compost cause significant crop delay.
- When casing is contaminated with moderate levels of infected compost[1] or mycelium, there may be no noticeable symptoms in the crop, but the mushrooms will contain Virus X dsRNAs.

Mycelial fragment detection

Airborne mycelial fragments of *Agaricus* were detected during the bulk handling of spawn-run compost. Close to 3,000 fragments /1000 litres ($1m^3$) of air were detected during a 30 minute period of bulk handling 90 kg of spawn-run compost. The viability of such fragments has yet to be determined.

Circumstantial evidence suggests that mycelial fragments generated during the bulk handling of Virus X-infected spawn-run compost[1], can transmit Virus X dsRNAs to *Agaricus* mycelium in healthy compost[1], which was bulk handled alongside, but separated from, the infected compost[1].

Table 1. Summary of Virus X transmission routes and symptoms using different types of infected material at different times and levels of contamination. The term "infected compost" refers to compost that has been colonised by an *Agaricus* culture known to contain Virus X dsRNA (Virus X isolate 1283).

Infective material	**Time of contamination (and system tested)**	**Level of Contamination**	**Symptoms**	**Virus X DsRNAs in 1st + 2nd flush mushrooms**
Spores (from Virus X-infected mushrooms)	Throughout spawn-run (in situ)	High	Crop delay Yield reductions Bare areas	**Yes**
Spawn (made from Virus X isolate 1283)	At spawning (bulk and in-situ spawn-run)	High (100%)	Crop delay of 5-7 days Yield reduction Bare areas	**Yes**
Spawn (made from Virus X isolate 1283)	At spawning (bulk and in-situ spawn-run)	Low (5%)	Crop delay of 2-4 days Yield reduction Bare areas	**Yes**
Infected compost (colonised by Virus X isolate 1283)	End of spawn-run (bulk handled spawn-run)	Low (1%)	Crop delay of 1-4 days Yield reduction Bare areas	**Yes**
Infected mycelial fragments from bulk handled compost, spawn-run with Virus X isolate 1283	End of spawn-run (bulk handled spawn-run)	Extremely low. (airborne mycelial fragments generated during bulk - handling)	No crop delay Slight yield reduction Reduced quality Brown mushrooms	**Yes**
Infected compost (colonised by Virus X isolate 1283) used as "cacing"	Post-casing (in-situ spawn-run)	Medium to High	No crop delay No yield reduction Good quality	**Yes**
Controls (for all of the above) Un-infected commercial spawn and un-infected sources of compost	As all of the above	As all of the above	No crop delay No yield reduction Good quality	**No**

[1] The term "infected compost" means compost that contains *Agaricus* mycelium infected with Virus X dsRNAs. "Healthy compost" is compost that is spawned, or spawn-run with *Agaricus* mycelium that is free of Virus X dsRNAs.

Commercial benefits of the project

Virus X is currently the single largest cause of crop loss to the mushroom industry. Three major farm-closures up to 2001 can be attributed primarily, if not solely, to the effects of Virus X disease, resulting in an estimated £21m loss of production. Further losses in production, as well as loss of revenue from inferior quality mushrooms from remaining affected farms, has resulted in an estimated loss of £29m, giving an approximate total loss of about £50m/year.

The current project provides extensive information on the how Virus X can be transmitted by mycelium and spores, and gives an estimate of the likelihood of symptom expression occurring, depending on when Virus X infection takes place. This information has focussed growers' attention to vulnerable areas on a farm, and to closing off possible routes of infection. Many growers are achieving partial control by adopting more stringent virus control hygiene measures, as a result of the information generated by this project. However, the losses being experienced are still not acceptable. The intractability of the problem demonstrates that Virus X is operating at a much lower level than 35 nm virus (La France disease). New virus hygiene guidelines need to be developed to take into consideration the difficulties encountered in controlling Virus X.

Action points for growers

- Protect spawning-halls from any sources of potentially infected spawn-run compost[1], spores or mycelial fragments.
- Ensure spawning-halls for bulk spawn-run tunnels are effectively over-pressured. Do not use machinery for spawning which may be contaminated with spawn-run compost fragments.
- Ensure spawn-running rooms and tunnels are well sealed and that all air entering them is efficiently filtered to exclude mushroom spores and mycelial fragments.
- Contain and filter all air arising from bulk-handled spawn-run compost so as to prevent it contaminating other areas of the farm (ie spawning halls, casing stores, equipment).
- Protect casing material from contamination by mushroom spores, compost fragments and shared machinery that may have handled spawn-run compost.
- Be aware that spawn-run compost used as "cacing" in casing can, if it is infected, transmit Virus X.
- Have mushroom samples tested on a regular basis to pick up any signs of Virus X dsRNAs that may be building up on the site without any obvious symptom expression.
- Have filters, seals, overpressure systems, machinery disinfection programs, etc. regularly overhauled in order to detect any breakdowns in hygiene measures.
- Do not allow mushrooms to open on the bed and release their spores. If open mushrooms are grown commercially then ensure that exhaust-air from growing rooms is filtered.

SCIENCE SECTION

Epidemiology of Virus X complex

1. Introduction

This project began in April 1999 to investigate an expanding problem, which at that time was of unknown cause. There were early indications from Pennsylvania State University that the cause was viral, based on the presence of novel "double stranded ribo nucleic acid" (dsRNA) elements that had not been seen in mushrooms before. HDC report M39 confirmed the correlation between the novel dsRNA elements and Virus X disease. A diagnostic test was subsequently developed and offered to the industry, which was similar to that used to detect 35 nm virus in the 1980's (Wach et al, 1987). The test is based on a total dsRNA extraction from mushrooms. The dsRNA extract is separated out into its component elements on agarose gel by electrophoresis, which are visualised as bands. These can be sized according to their molecular weight by comparison with marker bands of known molecular weight. This correlation between the presence of novel dsRNAs and "Virus X" problems on farms has been consistently confirmed and validated at HRI and the putative virus complex has been called "Virus X" (Gaze et al., 2000; Grogan et al., 2001).

Early work at HRI and elsewhere indicate very strongly that Virus X can be transmitted both by mushroom spores and mushroom mycelium. However, the answer to the basic question of how many Virus X-infected spores or mycelial fragments, at what stage, will cause what effect, is unknown. Schisler et al. (1967) demonstrated that pure cultures and spores from La France infected mushrooms could transmit symptoms to healthy crops with as little as 100 spores, applied to spawn-running compost, capable of causing symptoms to be expressed in the crop. Major investment to exclude spores or mycelial fragments with the current lack of knowledge concerning Virus X epidemiology is therefore risky and hesitant. If, for example very small quantities of spores, or mycelial fragments, at particularly vulnerable stages are all that is required to cause a problem, then expensive control measures put in position elsewhere will have limited, if any, benefit. Alternatively, if large quantities of spores or mycelial fragments introduced at casing were to have a large effect, a period previously considered to be "safe" from virus, then it would be financially worthwhile inserting control measures at this point.

A great deal of remedial action has now been taken by industry. This is based on the (now) confident assumption that the many symptoms that have been observed are indeed characteristic of a viral disease (inhibition and/or retardation of sporophore development, premature opening, brown mushrooms, distortions and quality loss). Existing traditional tests have clearly shown that the cause is not 35-nm virus, but a new virus (or viruses), "Virus X", which has yet to be characterised. Nonetheless, the experience gained in the 60s, 70s and 80s when dealing with 35-nm virus disease, is now of enormous benefit in helping to combat this new problem.

Despite this, the disease is more widespread than it was in April 1999. It can, however, be argued that without such remedial action the situation would be far worse. To support this argument many farms would claim that control, whilst only partial, reduces symptoms to a commercially acceptable, but not desirable, level. There have also been two or three notable success stories where the disease (if not the presence of virus) has been eradicated, at least for the present.

An explanation for not achieving better levels of control, which would probably have been achieved were the problem due to 35nm virus, illustrates how the new virus appears to be operating at a different level. Additionally, other explanations are probably to be found in modern cultural and marketing practices, which make the achievement of virus control "exclusion-hygiene" extremely difficult. These are:

- the widespread use of bulk-compost (phase II and III) and

- the production of a relatively high percentage of open, spore-producing mushrooms.

Phase III has proved in the past to be extremely vulnerable to virus disease due to the production of mycelial fragments capable of forming quick, efficient and continuous virus-loops back to spawning operations. Thus, wherever high spore loads are generated, or wherever bulk operations are carried out, particularly with phase III, the "exclusion-hygiene" approach, which was previously relatively easily achieved, will now be very much more difficult to achieve.

To date, the diagnostic test, based on total dsRNA extraction, has proved effective. There are suspicions that it may lack a desired amount of sensitivity but in its favour there are indications that to some extent it may be both qualitative and quantitative. There remains a need for a more sensitive, precise, less expensive, higher-volume test, based on PCR (polymerase chain reaction) technology. The previous HDC project M39 (Grogan et al., 2001) completed stage 1 of this process by producing PCR primer pairs for three Virus X specific dsRNAs. DEFRA (Department for Environment, Food and Rural Affairs) has funded research at HRI to continue this work and validated PCR primer pairs for specific Virus X dsRNAs have been developed. However, their commercial usefulness will depend on having a greater understanding of the significance and relationships between the many and various dsRNAs that have been associated with Virus X. The most cost-effective course in the short term is to study the transmission of Virus X using the slightly cumbersome electrophoretic test until such a time when a better test becomes available. DEFRA have continued to support the molecular characterisation of Virus X for another year, 2002/2003, which should advance the likelihood of a commercially useful diagnostic PCR test.

The aim of this project is to understand the factors that are important in the transmission of Virus X in order to formulate effective control measures. The experimental programme seeks to demonstrate the routes of transmission of Virus X contaminants (mushroom spores and mycelial fragments) in order to develop effective control strategies. Four main research objectives were set as follows:

1. To demonstrate that *Agaricus* spores, from a crop positive for Virus X, will transmit dsRNA into a new crop. Investigate the effects of (i) time of spore contamination, and (ii) quantity of spore contamination on transmission of Virus X dsRNA.

2. To determine if mycelial fragments, associated with the bulk handling of spawn-run compost, can be detected and quantified

3. To demonstrate that *Agaricus* mycelial fragments, from compost yielding mushrooms which are positive for Virus X, will transmit dsRNA into a new crop. Investigate the effects of (i) time of contamination, and (ii) quantity of mycelial fragments applied on transmission of Virus X dsRNA.

4. To determine if dsRNA bands increase in number and intensity if infected propagules from one crop are continually introduced into the compost of the following crop.

Experimental work was designed to address each of these topics and the methods; results and discussion are presented in the following chapters.

2. General Materials and Methods

2.1 Mushroom crops

All mushroom crops were grown on the HRI Mushroom Unit using a standard wheat straw and chicken manure compost and following standard procedures within the British industry. Phase I compost is produced in non-aerated stacks, which is turned regularly. After about 14 - 19 days on the yard, it is filled into bulk pasteurisation tunnels and subjected to a standard pasteurisation and conditioning regime. The phase II compost is then spawned with a standard commercially available spawn and filled into either wooden trays, holding 50kg, or plastic bags containing up to 20kg. Spawn-running is done in dedicated spawn-running rooms for a period of 17 days, at which time the compost is cased with a commercially bought casing product. Cased trays and bags are then moved into temperature controlled cropping rooms and cropped for up to three flushes.

2.2 Virus X cultures

A large collection of Virus X-infected *Agaricus* cultures have been produced at HRI, which were derived from Virus X-infected mushrooms, compost or casing. Corresponding mushroom samples were analysed for the presence of Virus X associated dsRNAs so that the Virus X profile of each culture at the time of sampling is known. During the course of these experiments one particular Virus X "strain" was used predominantly, culture 1283. In some experiments spores derived from mushrooms grown from strains 1283 and 1282 were also used. Details of the cultures are given in Table 1. The dsRNA banding profiles are given in Table 2.

2.3 Virus X inoculum preparation

Virus X inoculum was prepared in three ways as follows:.

2.3.1 Virus X-infected spawn.

Six plugs of freshly grown Virus X-infected *Agaricus* cultures were used to inoculate jars containing about 150g of sterilised rye grain. Jars were incubated at 25°C for two to four weeks and shaken weekly to ensure good growth of the Virus X-infected *Agaricus* on the rye grain. When the rye was well colonised the jars of Virus X-infected *Agaricus* were stored in the fridge at about 4°C until needed.

2.3.2 Virus X "infected compost" [1].

Bags containing about 150g of pre-chopped Phase II compost were sterilised by autoclaving at 120°C for 1 hour on two consecutive days. The compost was then spawned under clean room conditions with Virus X-infected spawn (see 2.3.1). The spawned compost was then incubated at 25°C until fully spawn-run. When needed the spawn-run compost was gently broken up and weighed out into the required quantities under clean room conditions.

2.3.3. Virus X-infected spores.

Mushroom crops of Virus X strains 1282 and 1283 were grown in Phase II compost using 100% Virus X spawn prepared as described in 2.3.1 above. Maturing closed cup mushrooms were harvested, and taken to the laboratory where spore prints were collected into sterile Petri dishes. Several spore

[1] The term "infected compost" means compost that contains *Agaricus* mycelium infected with Virus X dsRNAs. "Healthy compost" is compost that is spawned, or spawn-run with *Agaricus* mycelium that is free of Virus X dsRNAs.

prints from 1st, 2nd and 3rd flush mushrooms were obtained in this way and were stored in the dark at room temperature until needed.

Table 1. Details of source material for Virus X containing *Agaricus* cultures

Strain	Details of source material
1282	Strain 1282 was subcultured from mushrooms from a farm which was producing large quantities of prematurely opening mushrooms as the dominant symptom.
1283	Strain 1283 was derived from a farm that suffered 40-80% yield reductions, had large bare areas of unproductive bed and crop delay.

Table 2. dsRNA banding pattern of mushrooms from which Virus X strains 1282 and 1283 were taken.

	Strain 1282	Strain 1283
DsRNA bands		
1		
2		
H-1	X	
3		XS
4		
5	X	
6		
H-2	X	X
7		
8	X	X
9	X	X
10		
11		
12	X	
13	X	
14		
15	X	X
16		X
H-3	X	X
17		
18		X
19	X	X
20		
21		
22		
23		

H1, H-2 and H-3 are generally found in healthy mushrooms from sites with no history of Virus X symptoms.

2.3.4. Generation of Virus X-infected mycelial fragments

Microscopic mycelial fragments were generated by disturbing and continuous flipping of fully spawn-run compost, infected with Virus X strain 1283. This was on a large scale on the mushroom unit in a sealed chamber, using bags of spawn-run compost.

2.4 Detection of mycelial fragments

Two pieces of equipment were used to detect microscopic mycelial fragments, a Burkard air sampler and an Andersen viable microbial sampler. The methodologies for these are given in Section 5

2.5 Determination of Virus X dsRNA profiles.

All mushroom samples (150 g fresh weight) for Virus X testing were done via the HRI Mushroom Diagnostic Clinic. Mushroom samples were frozen immediately, and then freeze dried prior to analysis. Total nucleic acids were extracted and purified from 1g samples of freeze-dried mushrooms. The purified extract was subjected to agarose gel electrophoresis and the resultant gels, depicting dsRNA bands, were photographed and scored for the presence of novel Virus X-related dsRNAs.

3. Spore Transmission Experiments

Two spore transmission experiments were carried out, the first in pots containing 3kg compost, and the second in trays containing 50kg compost. The objectives of these experiments were:

- To determine if Virus X dsRNA is transmitted via spores to a crop of mushrooms
- To determine if the quantity of inoculum affects transmission of Virus X dsRNA
- To determine if time of inoculation affects Virus X dsRNA transmission

Two further experiments were carried out as part of six rollover crops designed to see if Virus X titre levels in mushroom built up over time as spores from one crop contaminated the next crop.

3.1 Materials and Methods:

3.1.1. Experiment 1.

Seventy two pots were filled with HRI Phase II compost, which were individually spawned with 0.5% Sylvan A15 spawn. Spore inoculation treatments consisted of either *Agaricus* spores harvested from a non-Virus X source growing A15 or spores harvested from a crop infected with Virus X strain 1283 (see section 2.3.3). A concentrated spore suspension from each source was prepared and diluted to give three concentrations of 10^6, 10^4 and 10^2 spores/ml. Spore suspensions (1ml) were applied to the surface of the compost in the pots either at spawning, mid-way during spawn-run or prior to casing. A fourth treatment consisted of pots receiving spore suspensions at all three times. The treatments are summarised as follows:

- Two sources of spores: Healthy A15; Virus X strain 1283
- Three concentrations of spores: 10^6 spores/ml; 10^4 spores/ml; 10^2 spores/ml.
- Four times of inoculation: at spawning; mid spawn-run; prior to casing; at spawning, mid spawn-run and prior to casing
- Three replicates per treatment

This gives a total of 2 x 3 x 4 x 3 pots = 72 plots.

The pots were positioned on aluminium shelves, according to a factorial design, based on a 6 x 4 array of plots per shelf. Each of three shelves contained one complete replicate of all treatments.

At the end of spawn-run all pots were cased with a commercial casing mix (TunnelTech English) with A15 casing inoculum added. Mushrooms were harvested as closed cups over three flushes, and 1st and 3rd flush mushrooms were analysed for the presence of dsRNA.

3.1.2 Experiment 2.

A second spore inoculation experiment was carried out using 50kg trays of compost. Eight trays were filled with HRI Phase II compost spawned with Sylvan A15. Spore inoculation treatments consisted of *Agaricus* spores harvested from a crop infected with Virus X strain 1283 (see section 2.3.3). The control treatment on this occasion was water only, without spores. A concentrated spore suspension of strain 1283 was prepared and diluted to give three concentrations of 2 x 10^6, 4 x 10^6 and 2 x 10^7 spores/ml. Eight 1ml aliquots of spore suspension were applied to the compost in the trays at

spawning only. The number of treatments in this trial was limited by the number of trays, and size of house available. The treatments are summarised as follows:

- One sources of spores: Virus X strain 1283
- Four concentrations of spores: 2×10^6 spores/ml
  4×10^6 spores/ml
  2×10^7 spores/ml.
  None (Control - water only)
- One inoculation time: at spawning
- Two replicates per treatment

This gives a total of 1 x 4 x 1 x 2 trays = 8 plots.

The trays were positioned randomly in two blocks, in a cropping house, with one replicate of each treatment in each block.

At the end of spawn-run all trays were cased with a commercial casing mix (TunnelTech English) with A15 casing inoculum added. Mushrooms were harvested as closed cups over two flushes, and 1st and 2nd flush mushrooms were analysed for the presence of dsRNA.

3.1.3 Experiments 3 & 4

Two crops, one following the other, were set up along the lines of the experiments described above but using spores from two strains of Virus X, namely Strain 1282 and Strain 1283 (see Table 2). The numbers of spores applied was 2×10^4 spores, in a water suspension, and this was applied at one end of a 50kg tray of compost on each of three occasions: (a) at spawning, (b) mid spawn-run and (c) prior to casing. Healthy spores from un-infected mushrooms were also applied as a Control treatment, and an untreated control of a healthy spawn-run was also included. Two replicate trays were prepared for each treatment. All trays were cased with commercial casing, and case run and cropped according to standard conditions. Mushrooms from the first crop were harvested and tested for Virus X dsRNAs. Spores were collected from first flush mushrooms and were used to make a new suspension to inoculate the second crop. Mushrooms were harvested from the second crop and tested for dsRNAs. The remaining four crops of this experiment were not carried out based on the results obtained from the first two.

3.2 Results

There was no obvious expression of Virus X symptoms following any spore inoculation treatment in either pots or trays. Similarly no symptom expression was recorded in either of the two rollover crops set up.

There was a small effect of spore inoculation on the 1st flush and third flush yields from the pot experiment but no overall significant effect (Fig. 1). There was no effect on the yield from the tray experiment (Fig. 2). There were no significant effects of different concentrations of spores or of different inoculation times on yield.

No dsRNAs were detected in mushrooms from any of the mushrooms examined, from either the straight spore-inoculation experiments or the rollover experiments.

Fig. 1. Yield following inoculation with either Non-Virus X or Virus X infected (strain 1283) *Agaricus* spores

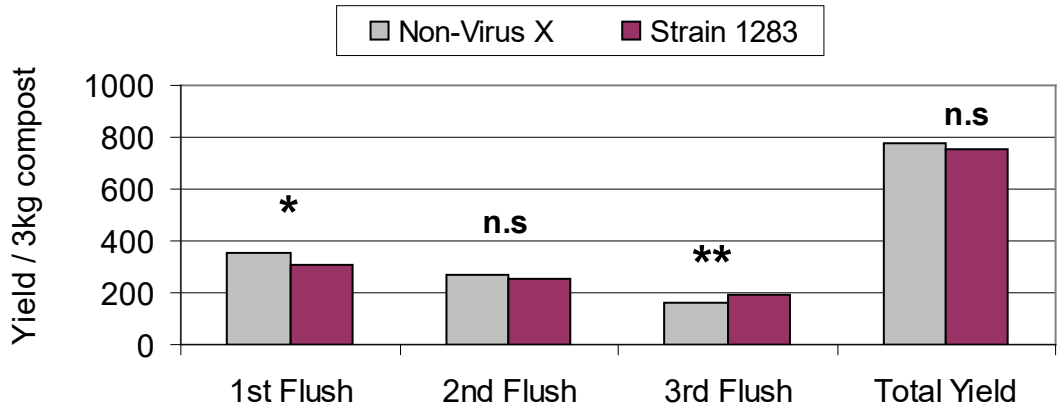
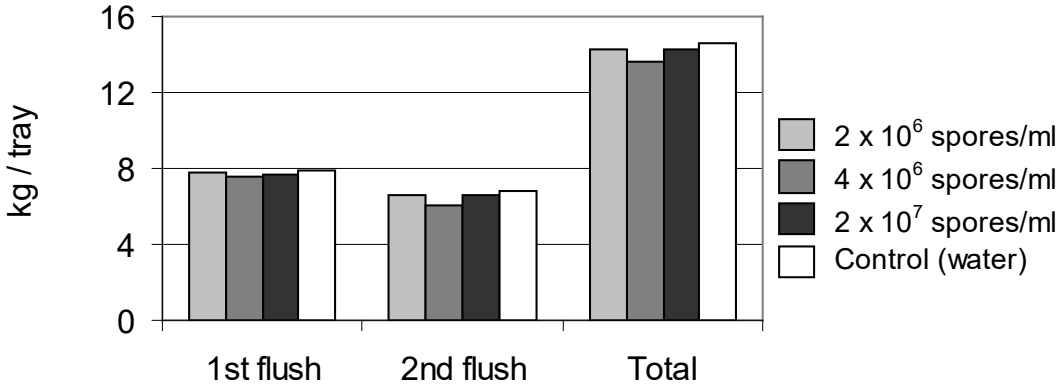


Fig. 2. Yield of mushrooms from 50kg trays inoculated with Virus X infected spores (strain 1283).



3.3 Discussion

There was no evidence of Virus X-infected spores transmitting Virus X dsRNAs in any of the four experiments described above, despite the fact that this method of inoculation was successful in transmitting La France 35 nm virus (Schisler et al., 1967). However, there is significant circumstantial evidence from within Britain to suggest that Virus X-infected spores do transmit dsRNAs to new crops (R. Gaze, pers. comm.). The reason why the experiments described above were unsuccessful may be due to the fact that the inoculation method, although effective for La France transmission, diverged significantly from what would happen normally on a farm. On a farm, spores are likely to be freshly produced on a continuous basis, and would infect compost by falling directly onto compost. The inoculation method in the experiments described above used harvested stored spores, which were suspended in water, then applied to the compost surface. A small unreplicated experiment was carried out at a later stage whereby up to 15 freshly harvested Virus X-infected mushrooms (strain 1283) were allowed to drop their spores directly onto compost throughout the spawn-running period. Mushrooms harvested from this compost did contain Virus X dsRNAs. The crop was also significantly delayed, compared with an uninoculated control, and the yield was reduced by around 20% (R. Gaze, pers.

comm.). Further experimentation using this method of inoculation is required, to try and quantify the number of spores required to transmit dsRNA to a new crop, and to determine if the time at which compost (or casing) is infected, and the quantity of infected spores applied, affects the severity of the disease and the symptoms expressed.

3.4 Conclusions

- Spores from freshly harvested, Virus X-infected mushrooms, dropping onto spawn-running compost, can cause Virus X symptoms (and dsRNAs) to occur in the subsequent crop.

4 Mycelial transmission experiments

Four experiments were carried out using Virus X-infected mycelium, in different forms. Mycelium carryover is known to be very effective at transmitting La France 35 nm virus from crop to crop and the likelihood is that it is also important in transmitting Virus X. The objectives of these experiments were:

- **Experiment 1. Virus X infection at spawning.** To determine the effects of (a) different rates of inclusion of Virus X-infected spawn and (b) disturbance of spawn-run compost (simulated bulk phase III handling) on the transmission of Virus X into mushrooms.

- **Experiment 2. Virus X infection at end of spawn-run (during bulk-handling Phase III).** To determine if infected compost added to both healthy spawn-run compost at the end of spawn-running (ie during bulk handling of phase III), and to casing transmits Virus X.

- **Experiment 3. Virus X infection during bulk handling of spawn-run compost.** To determine if airborne fragments of Virus X-infected mycelium, generated during bulk handling of infected spawn-run compost, can transmit Virus X to healthy compost.

- **Experiment 4. Virus X infection of casing.** To determine if infected compost or mycelial fragments applied to casing only, transmit Virus X to the subsequent crop of mushrooms.

4.1 Experiment 1: Virus X infection at spawning

The objective of this experiment was to determine the effects of (a) different rates of inclusion of Virus X-infected spawn and (b) disturbance of spawn-run compost (simulated bulk phase III handling) on the transmission of Virus X into mushrooms.

4.1.1 Materials and Methods

Thirty-six polythene bags were each filled with 12kg of HRI Phase II compost (batch 01/02). Six spawning treatments were used which consisted of a Control , using 100% commercial spawn (Sylvan A15), followed by different combinations of healthy spawn : Virus X-infected spawn in the range 50:50, 75:25, 90:10, 95:5, 0:100. Six bags of compost were hand spawned for each spawning rate with pre-weighed out spawn mixtures. All bags were spawn-run for 17 days. At casing half the bags for each treatment were cased in-situ with a commercial casing containing no casing inoculum, simulating tray and bag growing systems. The remaining bags were removed, starting with the controls, and subjected to a bulk handling treatment, whereby the spawn-run compost was emptied out of the bag, broken up and then replaced back into the bag, simulating bulk phase III handling. The bags of bulk handled compost were then also cased as described above. The treatments are summarised as follows:

- Six types of inoculum
  - 100% Virus X strain 1283 spawn
  - 50:50 Virus X strain 1283 spawn: A15
  - 25:75: Virus X strain 1283 spawn: A15
  - 10:90 Virus X strain 1283 spawn: A15
  - 5:95 Virus X strain 1283 spawn: A15
  - 100% A15 spawn (Control)
- Two spawn-run handling treatments
  - Spawn-run compost left undisturbed (in-situ)
  - Spawn-run compost bulk handled in isolation
- Three replicates per treatment

This gives a total of 6 x 2 x 3 bags = 36 plots.

The bags were positioned within the house in a Trojan square design consisting of 3 rows x 3 columns, with 2 main plots at each position. Each plot was divided into two subplots to include disturbed and undisturbed treatments. Design allows for spatial variation in two dimensions (rows and columns), and allows equal comparison of all treatments.

All bags were then case-run and cropped for three flushes. Mushrooms were harvested as closed cups or large buttons so as to avoid the production of open mushrooms, which could confound the results by contaminating the controls (A15) via spore transmission. Mushrooms from each treatment were tested for the presence of Virus X dsRNAs.

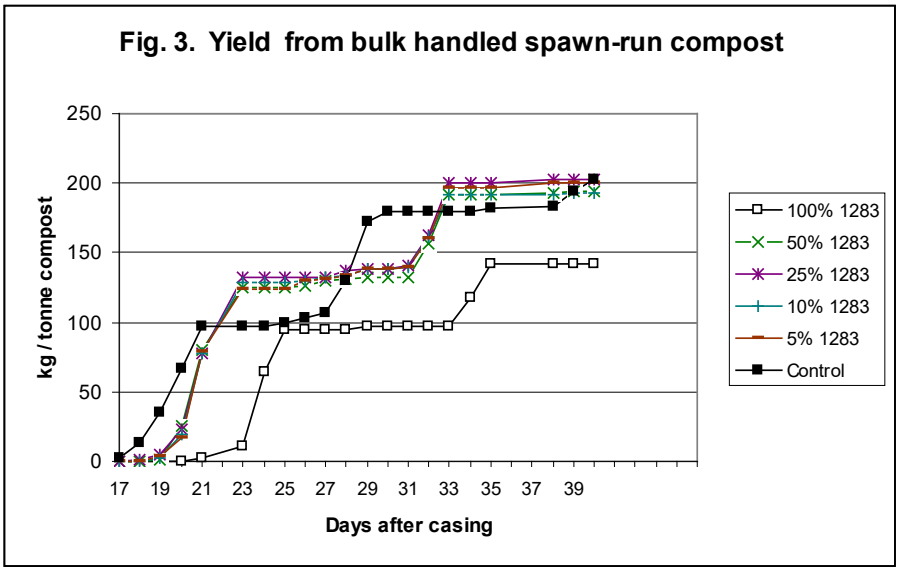
4.1.2 Results

Crop Timing
Only the control treatments cropped according to a normal schedule with the flushes being roughly a week apart. The flushes of all treatments containing Virus X strain 1283 were later than the controls. Treatments containing 5-50% Virus X spawn were one to two days behind, in the first flush and 3-4 days behind in the second, while treatments containing 100% Virus X spawn were a full five days behind in the first flush, and 6-7 days behind in the second. This meant that a third flush could not be taken from Virus X treatments without delaying the crop schedule for the following crop.

This pattern was very similar whether or not the spawn-run compost had been bulk handled (Fig. 3) or left undisturbed (Fig. 4).

The yield data in Figures 3 & 4 suggest that the 1st flush controls yielded less than Virus X treatments. This is due in part to the fact that while control plots were stripped on a Friday, Virus X plots continued to crop and were only picked once over the weekend, resulting in larger mushrooms being picked for those treatments.

Transmission of Virus X dsRNAs.
The control mushrooms remained free of Virus X dsRNAs during the course of the crop. First flush mushrooms from all Virus X treatments had dsRNA profiles consistent with Strain 1283 (Fig. 5, Table 2). Analysis of third flush mushrooms gave a similar result.



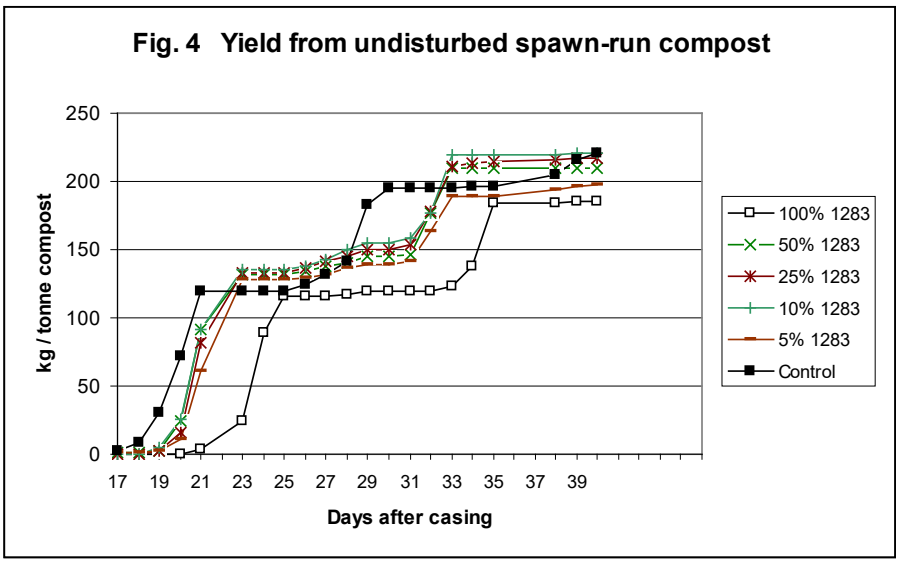
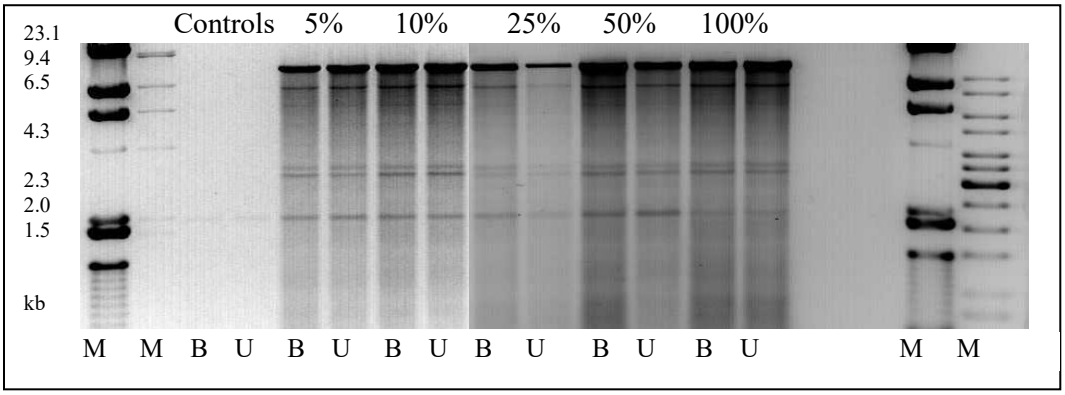


Fig. 5. DsRNA analysis of 1st flush mushrooms from compost inoculated with different rates of Virus X spawn. Compost was either Bulk handled (B) after spawn-run or undisturbed (U). M = molecular weight marker bands of known size in kilo base (kb) pairs



4.2 Experiment 2. Virus X infection at end of spawn-run (bulk handling Phase III)

4.2.1 Materials and methods

Eighteen polythene bags were each filled with 15kg HRI spawn-run compost (batch 16/01 using commercial Sylvan 737 spawn). Virus X-infected compost produced in the laboratory (section 2.3.2) was added at a rate of 1% (150g/15kg bag) to half the bags while uninfected compost was added to the remaining bags. Three casing treatments were then also applied. A standard casing treatment consisted of commercial casing with Casing Inoculum (C.I.) included at the standard rate (0.6% v/v), a cacing treatment consisted of adding chopped Virus X-infected compost (section 2.3.2) to commercial casing at a rate of 2.2% v/v, and a third treatment consisted of half rates of 0.3% v/v C.I. and 1.1% v/v virus X-infected compost. All bags were then case run, aired, and cropped for two flushes. Mushrooms were harvested as closed cups or large buttons so as to avoid the production of open mushrooms, which could confound the results by contaminating the controls via spore transmission. The treatments are summarised as follows:

- Two types of compost treatments
  - -1% Un-infected spawn-run compost (Control) added during bulk handling of Phase III
  - 1% Virus X-infected spawn-run compost added during bulk handling of Phase III
- Three types of casing treatments
  - -Standard C.I. treatment at 0.6%v/v (Control)
  - -Cacing treatment at 2.2% v/v compost/casing (ie Virus X-infected cacing to Virus X-infected crop; un-infected cacing to un-infected crop)
  - -Mixed treatment of Cacing at 1.1% v/v compost (Virus X-infected or un-infected) + C.I. at 0.3% v/v treatment
- Three replicates per treatment

This gives a total of 2 x 3 x 3 bags = 18 plots

The 18 bags were arranged in a Trojan Square design of three columns, each divided into three rows, with 2 main plots at each position. One replicate of each treatment was present in each column, and each row. The design allows for spatial variation in two dimensions (rows and columns), and allows equal comparisons of all treatments.

4.2.2 Results

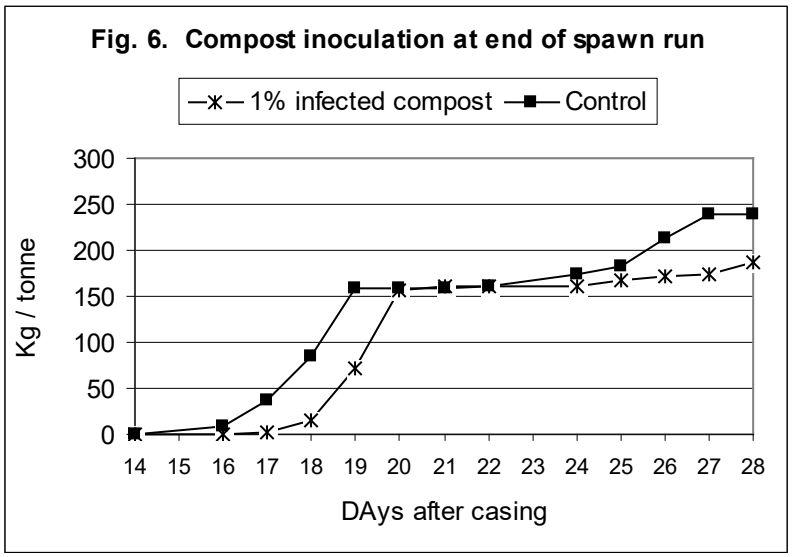
Crop Timing

There was a one day delay in the timing of the first flush when 1% Virus X-infected compost was added to healthy compost at the end of spawn-run (Fig 6). This increased to a three day delay in the second flush. When healthy compost was used in the casing (cacing) instead of commercial casing inoculum (C.I.), the control crop came in a day earlier (Fig 7) however when Virus X-infected compost was used on top of 1% infected compost, their was an increase in the delay of first flush to three days, and the second flush was four to five days delayed. (The "C.I. + cacing" treatments gave similar results to the "cacing only" treatments and are not presented).

By the end of the second flush, yields were significantly lower from Virus X treatments, although given extra time the Virus X-infected crops gave similar yields. However, quality was usually poorer than the controls.

Transmission of Virus X dsRNAs.

The control mushrooms remained free of Virus X dsRNAs while mushrooms from all Virus X treatments had dsRNA profiles similar to strain 1283 used as inoculum (Fig. 8).



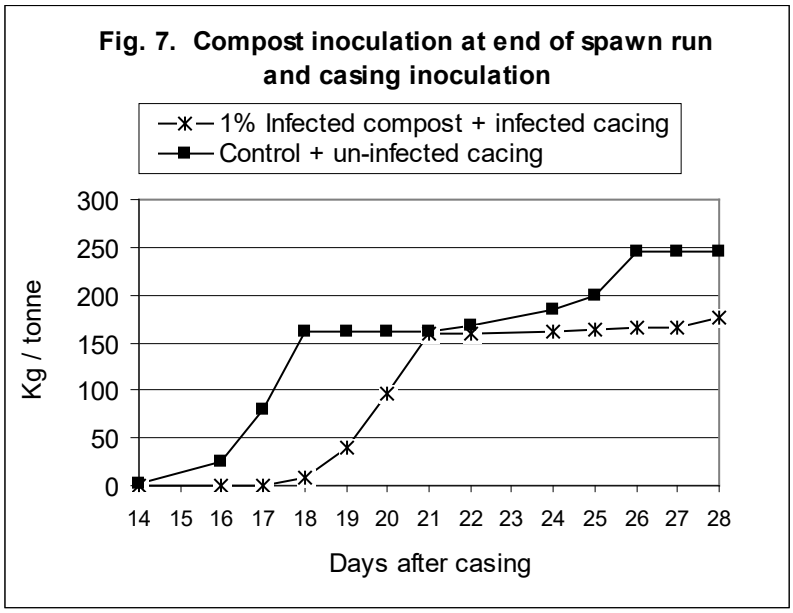
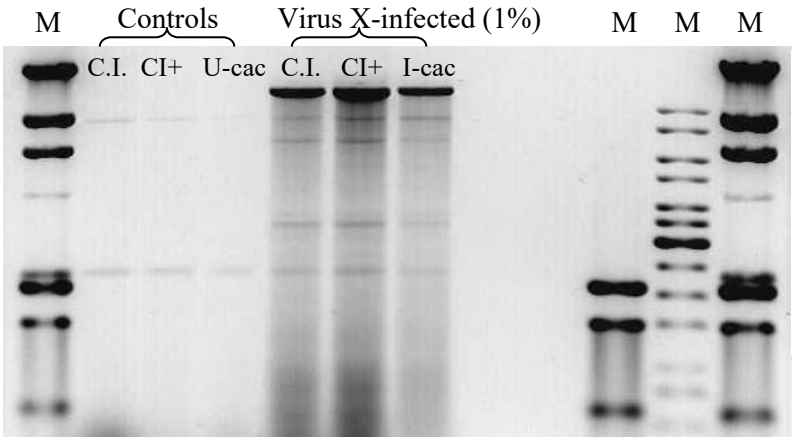


Fig. 8. DsRNA analysis of 2nd flush mushrooms from compost inoculated at end of spawn-run with un-infected compost (Controls) or Virus X-infected compost added at 1% at the end of spawn-run (first flush mushrooms gave similar results). M = molecular marker lanes.



CI = casing inoculum in casing

CI+ = CI + cacing

U-cac. = un-infected compost used as cacing material;

I-cac = Virus X-infected compost used as cacing material

4.3 Experiment 3. Virus X infection during bulk handling of spawn-run compost

4.3.1 Materials and Methods

Eighteen polythene bags were each filled with 15kg HRI phase II compost (batch 14/01). Half were spawned using commercial A15 spawn as Controls, and the remaining half were spawned using 100% Virus X spawn made from strain 1283 (see section 2.3.1). All spawned compost was then spawn-run in the same room under standard conditions. At the end of the spawn-run, 3 replicate bags of each inoculum type (Control and 100% infected) were cased, in-situ, with a commercial casing to which no casing inoculum was added (Fig. 9). Three further Control bags and 100% infected bags were then removed to separate chambers for each spawn type, where the bags of spawn-run compost were emptied out, broken up and then refilled into the original bag again, to simulate the bulk handling associated with the bulk phase III production system. These bulk-handled bags were then cased and put back into the same chamber as the in-situ cased bags. The remaining 3 Control bags and 100% infected bags, were bulk handled (emptied and refilled) in the same chamber and then cased (Fig. 9). (During bulk handling, the air was monitored for the presence of *Agaricus* mycelial propagules. This work is presented in Section 5). All bags were then case-run and cropped for three flushes. Mushrooms were harvested as closed cups or large buttons so as to avoid the production of open mushrooms, which could confound the results by contaminating the controls via spore transmission. The treatments are summarised as follows:

- Two types of inoculum — Control (A15 spawn); 100% Virus X strain 1283 spawn
- Three spawn-run handling treatments — Spawn-run compost left undisturbed; Spawn-run compost bulk handled in isolation; Spawn-run compost bulk handled together
- Three replicates per treatment

This gives a total of 2 x 3 x 3 bags = 18 plots

The 18 bags were arranged in a Trojan Square design of three columns, each divided into three rows, with 2 main plots at each position. One replicate of each treatment was present in each column, and each row. The design allows for spatial variation in two dimensions (rows and columns), and allows equal comparisons of all treatments.

4.3.2 Results

Crop Timing

All control treatments, regardless of whether the spawn-run was undisturbed (in-situ spawn-run) or bulk handled, cropped on time and gave total yields of between 272 and 286 kg/tonne over three flushes. All 100% infected treatments, regardless of whether the spawn-run was undisturbed (in-situ spawn-run) or bulk handled, showed a four day crop delay in the first flush, which increased to between five and seven days in the second and third flushes. The data for bulk handled treatments are presented in Fig. 10.

The control compost that was bulk handled in the presence of 100% Virus X-infected compost (Control +), showed no delay in crop timing but the overall yield was 5% lower (Fig. 10). In addition the quality of the mushrooms was poor and a few mushrooms had the appearance of being "off-coloured" or "brown" (Fig. 11). This was the only time we have seen this symptom during all the cropping experiments carried out to date.

Fig. 9. Different treatments of spawn-run compost prior to casing.

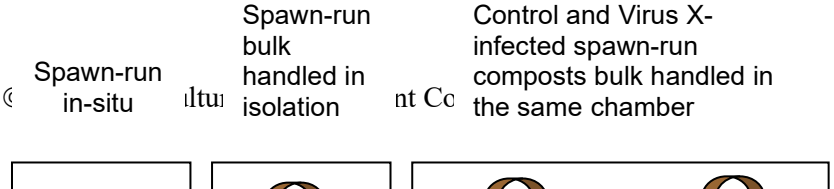
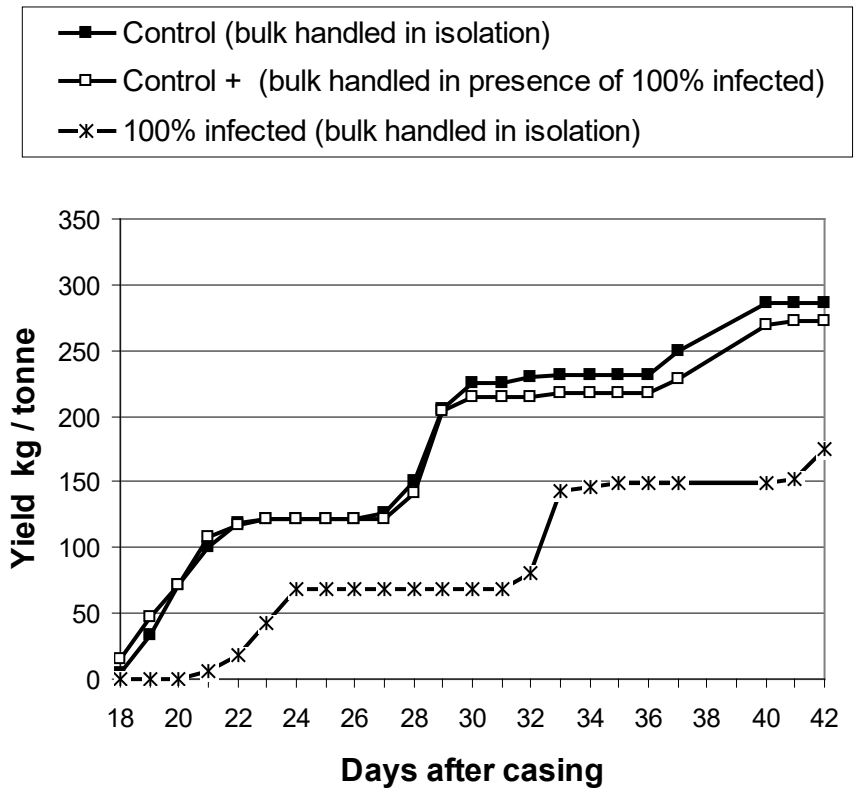


Figure 10. Compost contamination during bulk handling of spawn-run compost



Transmission of Virus X dsRNAs.
The control mushrooms bulk handled in isolation (or in situ) remained free of Virus X dsRNAs while mushrooms from all Virus X treatments had dsRNA profiles similar to strain 1283 used as inoculum. However, the mushrooms harvested from the Control + treatment, where compost was bulk handled in the presence of 100% Virus X-infected compost, all contained Virus X dsRNAs (Fig. 12). This suggests that the contamination of the control compost took place during the bulk handling of the composts in the same room. There was no physical contact between the two sets of compost (Fig. 9) but mycelial fragments were detected in the air during the bulk handling process (see Section 5). If these fragments were responsible for the transmission of Virus X dsRNAs to healthy compost, then the level of inoculum would have been quite low.

Fig. 11. Mushrooms harvested from bulk handled compost. Note off-coloured mushrooms from the Control + treatment.

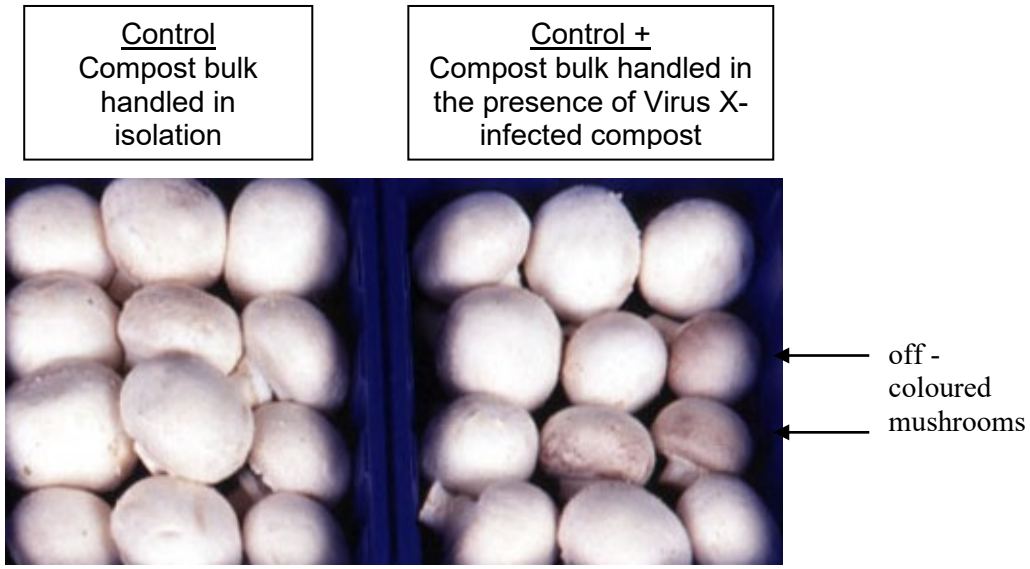
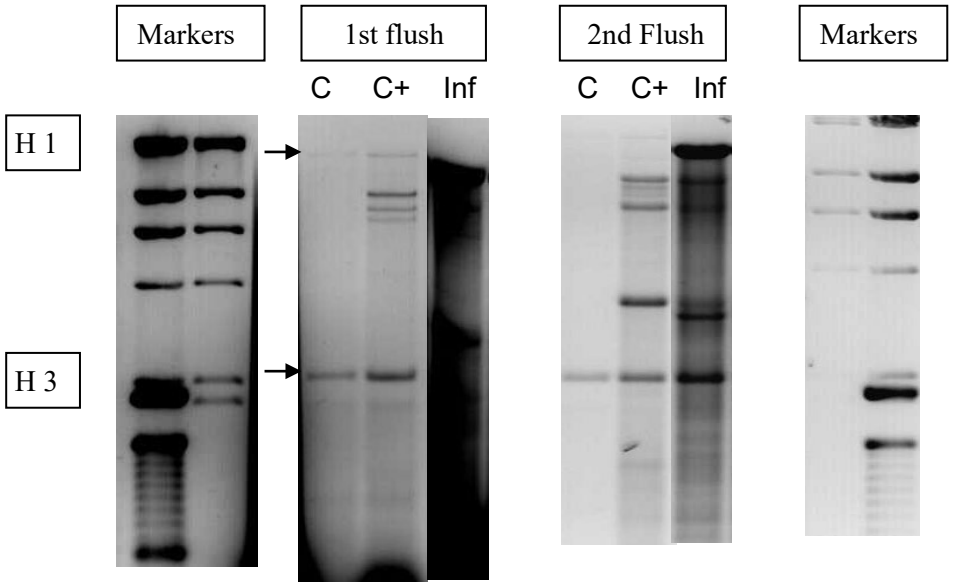


Fig. 12. DsRNA analysis of 1st and 2nd flush mushrooms from bulk handled compost. C = Control compost bulk handled in isolation; C+ = control compost bulk handled in the presence of, and at the same time as 100% Virus X-infected compost; Inf = 100% Virus X-infected compost bulk handled in isolation; H1 and H3 are dsRNAs found routinely in healthy crops; Markers = molecular weight marker bands of standard size.



4.4 Experiment 4. Virus X transmission via casing

The objective of this experiment was to determine if applying Virus X-infected compost or mycelium to casing on top of a healthy, un-infected spawn-run, could transmit the virus to the subsequent crop.

4.4.1 Materials and methods.

Eight wooden trays were each filled with 50 kg HRI phase II compost (batch 14/01), which was spawned using commercial A15 spawn. At the end of a standard spawn-run, all trays were cased with a standard casing mix containing commercial casing inoculum. Virus X-infected compost, prepared as described in section 2.3.2, was roughly chopped into 2 cm lengths in the laboratory, and applied to two replicate trays at a rate of 300g/m^2 (1.2%v/v approx.). A mycelium suspension was prepared from the same quantity of compost, and was prepared as follows: 20 g of compost was steeped in 200ml sterile water for 1 hour. The compost was then gently blended for 1 minute using a Stomacher 400 Circulator, rested for 5 minutes then blended again for 1 minute. The supernatant, containing mycelial fragments, was poured off the compost and applied to the casing at a rate of 2 litres/m^2. Control compost, from an uninfected source, was also prepared in the same way. Four treatments were therefore set up as follows:

- Four treatments
  - Control - healthy spawn-run compost added to casing
  - Control - healthy mycelium suspension added to casing
  - Virus X-infected compost (strain 1283) added to casing
  - Virus X-infected mycelium suspension (strain 1283) added to casing
- Two replicates per treatment

This gives a total of 2 x 4 = 8 plots

The 8 trays were arranged randomly in a simple design of two blocks, each divided into four rows. One replicate of each treatment was present in each block, and each row.

All trays were then case-run and cropped for two flushes. Mushrooms were harvested as closed cups or large buttons so as to avoid the production of open mushrooms, which could confound the results by contaminating the controls via spore transmission.

4.4.2 Results

Crop timing

All trays cropped normally with no delays in timing observed for the Virus X treatments. Yields for all treatments were very similar with no significant differences observed (Fig. 13; only compost result shown). Mushroom quality from all treatments was good.

Transmission of Virus X dsRNAs.

The control mushrooms remained free of Virus X dsRNAs. However, despite the fact that the Virus X treatments had no visible or noticeable effect on either yield, timing or quality, Virus X dsRNAs were clearly detectable in the mushrooms from all Virus X treated plots (Fig. 14).

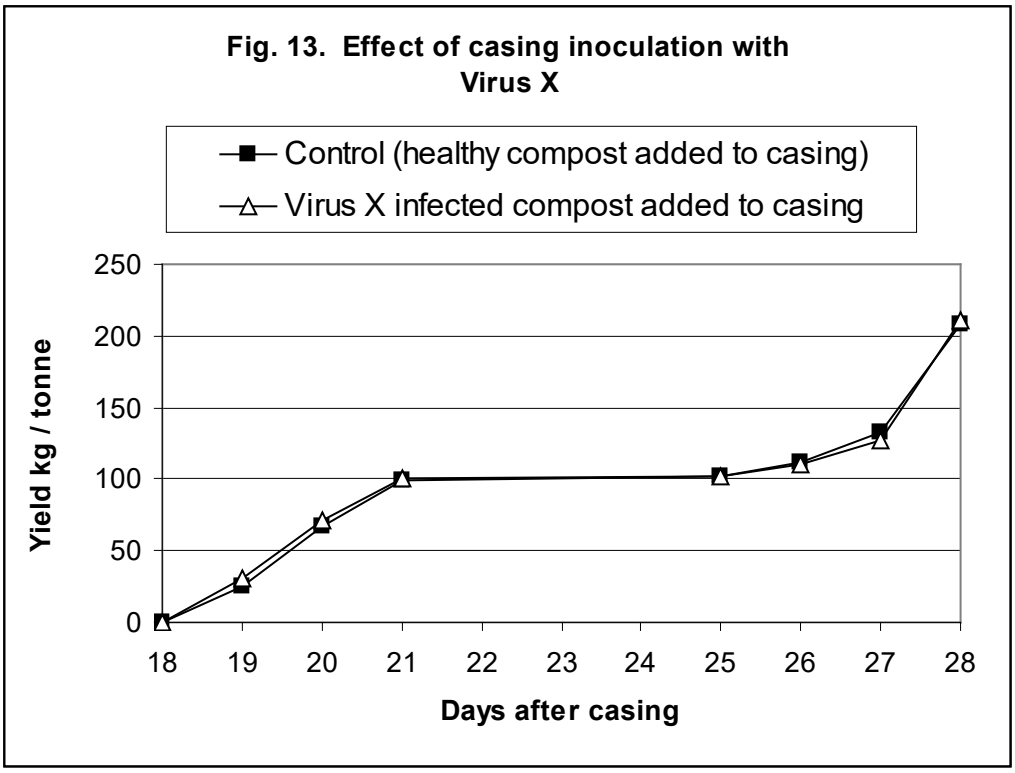
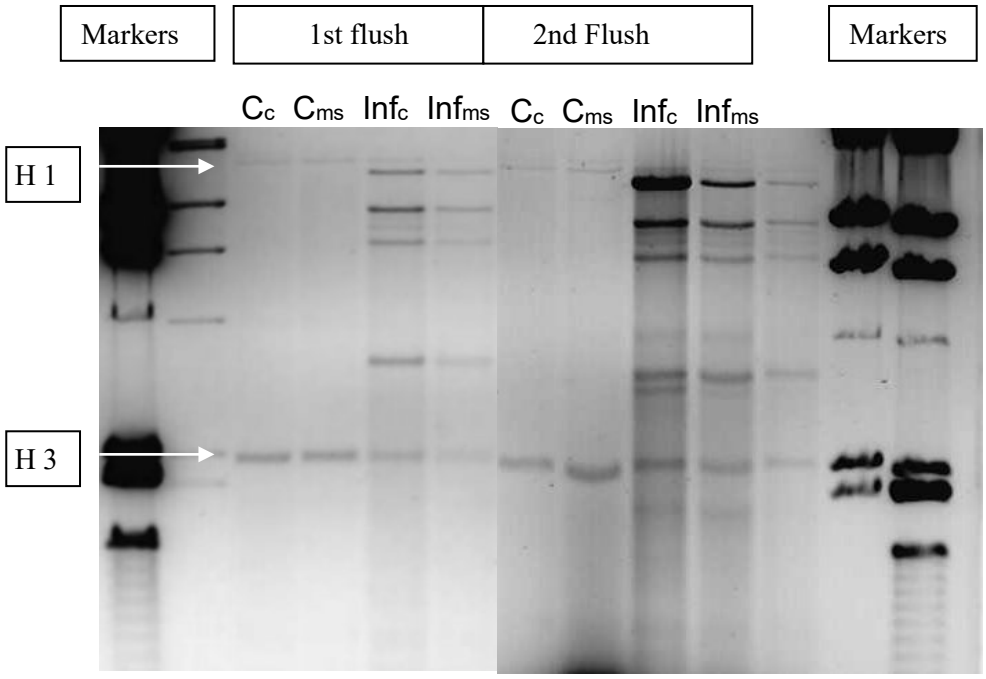


Fig. 14. DsRNA analysis of 1st and 2nd flush mushrooms from healthy compost. The compost was then cased using standard casing with: healthy (control) compost added (C_c); a healthy (control) mycelium suspension added (Control, C_{ms}); Virus X-infected compost added (Inf_c); or a Virus X-infected mycelium suspension (Inf_{ms}). H1 and H3 are dsRNAs found routinely in healthy crops; Markers = molecular weight marker bands of standard size.



4.5 Summary of Results

In all the experiments where Virus X-infected compost was applied to compost or casing, Virus X dsRNAs were detected in first flush mushrooms from those treatments. None of the control mushrooms, which had received a corresponding treatment with healthy un-infected compost, contained any novel dsRNA bands. Although the dsRNAs were always transmitted into crops infected with Virus X-carrying material, the effect on the actual crops differed depending on when the infective material had been applied to the crop.

Fully infected compost, which had no healthy *Agaricus* material present, exhibited the most severe effects, with the first flush struggling to pin and being delayed by 4 days or more. The second flush was delayed by 7 days. If timing is not taken into account, then yields can sometimes eventually recover but, invariably, houses need to be cleared in preparation for the next crop, so that significant a crop delay will usually also convert into a significant yield reduction.

When only small amounts of Virus X-infected material were incorporated into an otherwise healthy crop at spawning, significant effects on crop timing were still observed, with a crop delay of around 2 days being recorded for the first flush. Second flushes still struggled to come in on time with delays of 4 days occurring, while third flushes could not be harvested, as houses needed to be cleared in preparation for the next crop.

Effects on cropping were still evident when Virus X-infected compost was incorporated into healthy compost at the end of the spawn-running period, during bulk handling of spawn-run compost into bags. On this occasion a 1% addition of Virus X-infected compost into otherwise healthy spawn-run compost resulted in a 1 day delay in the timing of the first flush,. Again the second flush struggled to come in on time with a 4 day delay occurring in the harvesting of the second flush. (If the casing on such a crop was also infected with Virus X material then the first flush was delayed by a further day or two).

The lowest level of inoculation used in these experiments consisted of the bulk handling of healthy spawn-run compost in the presence of Virus X-infected material being bulk-handled at the same time, in the same room. Although both composts were kept well clear of each other, mycelial fragments were detected in the air at the time. It is reasonable to assume that both composts were open to contamination by mycelial fragments from the other compost, generated during the bulk handling process. On this occasion the "healthy" compost went on to produce mushrooms which contained Virus X dsRNAs, while healthy compost bulk-handled in isolation, did-not. It is reasonable to suggest therefore that mycelial fragments in the air from Virus X-infected compost contaminated the "healthy" compost on this occasion. This compost did not exhibit any delay in crop timing or yield but the mushrooms were of poorer quality than the uninfected controls, and a few mushrooms exhibited off-colour or "brown" mushroom symptoms. These results suggest that very little infective material is required to transmit Virus X dsRNAs.

The latest time of Virus X inoculation examined in these experiments was post-casing. Virus X-infected compost, or a mycelium suspension derived from Virus X-infected compost, was applied to the casing of a series of healthy spawn-run and cased trays of compost. The infected trays cropped normally with no delay or yield reductions, and they produced good quality mushrooms. However, the mushrooms all tested positive for the presence of Virus X dsRNAs, while control trays, which had received healthy compost or mycelium suspensions, did not.

4.6 Discussion

The results from this series of experiments suggest that Virus X-infected compost or mycelium readily transmits Virus X dsRNAs into mushrooms, irrespective of whether it occurs at spawning, during bulk handling, or post-casing. The expression of Virus X symptoms however such as crop delay, bare areas, poor quality and brown mushrooms, seems to depend on both the time and quantity of infective material, however it was not possible to cover all possible combinations of cropping conditions to provide clear evidence of such relationships.

Contamination of compost at spawning with large or small quantities of highly infected material (strain 1283), caused significant crop delay. This happened whether or not the compost was spawn-run and cased in situ, or subsequently bulk handled. Therefore, the spawning operation is highly vulnerable and needs to be very well protected.

Contamination at the end of spawn-running was only examined for bulk handled compost. Under these conditions moderate levels of contamination, whereby 1% infected compost was added, caused significant crop delay while very very low (infected mycelial fragments in the air when infected compost is bulk handled) did not cause crop delay or yield loss. However, this very very low level of contamination during bulk handling resulted in poor quality and off coloured mushrooms, as well as transmission of Virus X dsRNAs. Such low levels of virus expression might go undetected but the infected mushrooms would produce spores which are positive for Virus X, which could go on to infect other crops and/or compost and casing, thus building up inoculum levels on a farm. Containment of air during bulk handling is therefore recommended to prevent contamination of adjacent un-infected compost and casing. The effect of very very low levels of contamination on in-situ spawn-run compost (blocks or trays) was not examined.

Casing contamination was shown to transmit Virus X dsRNAs into mushrooms but did not produce any symptoms in the crop. This type of contamination is therefore going to be undetected unless mushrooms are routinely tested for Virus X dsRNAs. Again non-symptomatic mushrooms will produce spores which can then contaminate other areas of the crop production cycle, such as spawning and casing. It is important therefore that effective spore-filtration is in place in vulnerable areas to prevent a gradual build up of virus on the farm.

4.7 Conclusions

- Virus X-infected compost or mycelium readily transmits Virus X dsRNAs into mushrooms, irrespective of whether the contamination occurs at spawning, during bulk handling, or post-casing.
- The expression of Virus X symptoms such as crop delay, bare areas, poor quality and brown mushrooms depends on both the time of infection and quantity of infective material. A summary table of Virus X transmission and symptoms is shown in Table 3.
- Exposure of healthy compost to very very low levels of Virus X-infected mycelium fragments during bulk handling, can produce poor quality mushrooms which contain Virus X dsRNAs, but the crop is not delayed and yields are normal. Higher levels of contamination during bulk handling of compost causes significant crop delay.
- Moderate levels of casing contamination with infected compost or mycelium produced no symptoms but successfully transmitted Virus X dsRNAs into mushrooms.
- Spawning operations must be protected from potentially infected compost fragments.
- Bulk handling operations must be protected from sources of potentially infected compost fragments.
- Bulk handling operations must not allow air from bulk handling areas to escape unfiltered.
- Casing and casing operations must be protected from sources of potentially infected compost fragments.

Table 1. Summary of Virus X transmission routes and symptoms using different types of infected material at different times and levels of contamination. The term "infected compost" refers to compost

that has been colonised by an *Agaricus* culture known to contain Virus X dsRNA (Virus X isolate 1283).

Infective material	**Time of contamination (and system tested)**	**Level of Contamination**	**Symptoms**
Spores (from Virus X-infected mushrooms)	Throughout spawn-run (in situ)	High	Crop delay Yield reductions Bare areas
Spawn (made from Virus X isolate 1283)	At spawning (bulk and in-situ spawn-run)	High (100%)	Crop delay of 5-7 Yield reduction Bare areas
Spawn (made from Virus X isolate 1283)	At spawning (bulk and in-situ spawn-run)	Low (5%)	Crop delay of 2-4 Yield reduction Bare areas
Infected compost (colonised by Virus X isolate 1283)	End of spawn-run (bulk handled spawn-run)	Low (1%)	Crop delay of 1-4 Yield reduction Bare areas
Infected mycelial fragments from bulk handled compost, spawn-run with Virus X isolate 1283	End of spawn-run (bulk handled spawn-run)	Extremely low. (airborne mycelial fragments generated during bulk -handling)	No crop delay Slight yield reduct Reduced quality Brown mushroom
Infected compost (colonised by Virus X isolate 1283) used as "cacing"	Post-casing (in-situ spawn-run)	Medium to High	No crop delay No yield reduction Good quality
Controls (for all of the above) Un-infected commercial spawn and un-infected sources of compost	As all of the above	As all of the above	No crop delay No yield reduction Good quality

5. Quantifying and detecting mycelial fragments

During experiment 3, section 4.3, with bulk-handled spawn-run compost, the source of Virus X-infected material was believed to be mycelial fragments in the air emanating from Virus X-infected compost. It was therefore necessary to monitor the air to verify that such propagules were present. This was done using two methods (a) a Burkard spore trap and (b) an Andersen Viable Sampler.

5.1 Materials and Methods

5.1.1 Burkard air samplers

The type of Burkard air sampler used was a 24h Continuous Recording Air Sampler. It draws air over a glass slide at a rate of 10 litres/minute. Propagules in the air then impact and stick onto the slide, which has been coated with a Vaseline smear. When sampling is completed the slide is treated with a permanent mounting fluid and then propagules are counted at a later stage. Propagule numbers are expressed as "Number of propagules/ 1000 litres (m^3) of air".

5.1.2 Andersen Viable Sampler.

The type of Andersen sampler used was a six-stage viable sampler for determining the number and size of viable microbial propagules in the air. Air is drawn in through the top of the apparatus and drawn over six stages stacked on top each other at a rate of 28.3 litres/minute. Each stage is separated by a plate containing a series of holes, which decrease in size with each successive stage, and through which the propagule-carrying air is drawn. Each stage houses a Petri-dish filled with a nutrient medium. The medium used in these studies was a compost based agar medium containing antibiotics, which enhanced *Agaricus* growth and reduced bacterial growth.

The larger the propagule, the sooner it impacts on a Petri-dish in the upper stages. Small and very small propagules and spores are carried further down through the sampler where they impact on the appropriate Petri dish in the lower stages (Table 4). After the air sampling is finished, Petri-dishes are removed and incubated and the colonies that develop are identified and counted. Mushroom mycelial fragments are most likely to impact onto stage 1 and 2 plates.

Table 4. Sizes of propagule retained on different stages in an Andersen 6-stage

Stage Number	Size of propagule retained on stage
1	7.1 microns and above
2	4.7 - 7.1 microns
3	3.3 - 4.7 microns
4	2.1 - 3.3 microns
5	1.1 - 2.1 microns (not included in studies)
6	0.65 - 1.1 microns (not included in studies)

5.2 Results and Discussion

During experiment 3 described in section 4.3 (Virus X infection during bulk handling of spawn-run compost) both the Andersen sampler and Burkard spore trap were used to monitor the propagules present in the air.

No viable *Agaricus* propagules were detected using the Andersen sampler during the bulk handling of composts in experiment 3 (section 4.3) (Table 5). The presence of large numbers of other airborne moulds, such as *Penicillium* spp. and *Cladosporium* sp., may have inhibited the growth of *Agaricus* and the medium may have to be refined further to be more selective for *Agaricus*. Some *Agaricus* growth was detected on Petri-dishes left exposed in the room at the same time as the bulk handling and

air sampling but these colonies were associated with minute but identifiable compost fragments which had landed on the plates during compost disturbance.

A significant number of mycelial fragments were picked up by the Burkard spore trap (Fig. 15). The number ranged from 486 to 2960 /1000 litres of air, with the highest number being recorded when more compost was bulk handled (Table 6). Very few mycelial fragments were detected in the air four hours after the bulk handling operation had stopped.

Other experiments, not reported here, suggest that the viability of such mycelial fragments may be in the region of 1% but this work needs to be repeated and verified. It is important to establish definitively if such fragments are viable, as they are much lighter and more easily airborne than compost fragments, which would settle out very close to the area where they were generated. Very very few compost fragments were picked up on either the Andersen sampler plates or the Burkard spore traps.

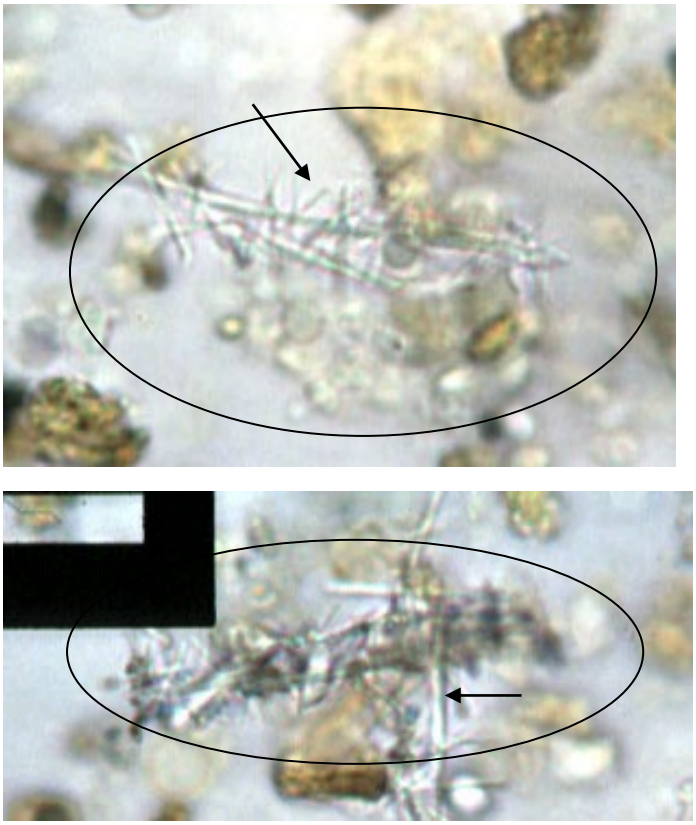
Table 5. Organisms recovered from Andersen sampler plates exposed for 15 minutes during the bulk handling of Control and Virus X-infected compost either in isolation or together.

Stage Number	**Organisms Recovered**		
	Control compost bulk handled in isolation	**Virus X-infected compost bulk handled in isolation**	**Control & Virus X-infected compost bulk handled together**
1	*Cladosporium* sp. *Penicillium* spp.	*Cladosporium* sp. *Penicillium* spp.	*Cladosporium* sp. *Penicillium* spp.
2	*Cladosporium* sp. *Penicillium* spp. *Mucor* sp.	*Cladosporium* sp. *Penicillium* spp.	*Penicillium* spp. *Mucor* sp.
3	*Penicillium* spp.	*Penicillium* spp.	*Penicillium* spp.
4	*Penicillium* spp.	*Penicillium* spp.	*Penicillium* spp.

Table 6. *Agaricus* mycelial propagules detected in the air during and after bulk handling of spawn-run compost using a Burkard spore trap.

Number of *Agaricus* propagules / 1,000 litres (m^3) of air				
Control compost bulk handled in isolation (45 kg of compost)	Virus X-infected compost bulk handled in isolation (45 kg of compost)	Control & Virus X-infected compost bulk handled together (6 x 15 kg = 90 kg)	Empty room immediately after bulk handling	Empty room 4 hours later
486	**1073**	**2960**	**326**	**7**

Fig. 15. Mycelial fragments present on slide from Burkard spore trap (note oxalate crystals covering mycelium, which are characteristic of *Agaricus*).



5.3 Conclusions

- Mycelial fragments are generated in moderate numbers during bulk handling of spawn-run compost. The viability of such fragments has yet to be determined.
- Circumstantial evidence suggests that mycelial fragments generated during the bulk handling of Virus X-infected spawn-run compost, can transmit Virus X dsRNAs to healthy compost. The resultant crop is likely to yield normally, crop on time, but poor quality or "brown" mushrooms may develop.

6. References

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