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MUSHROOM VIRUS X (MVX): A NOVEL DISEASE OF MUSHROOMS IN POLAND?

*Krzysztof Pudełko**

Poznań University of Life Sciences, Department of Biochemistry and Biotechnology
Wołyńska 35, 60-437 Poznań, Poland

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Abstract: The emergence of an unusual *Agaricus bisporus* mushroom disease first reported in the UK, and later termed as "mushroom virus X" (MVX), exhibits a wide range of symptoms. Findings from laboratories in the UK, the Netherlands and Ireland indicate that symptomatic MVX mushrooms contain a variable compendium of novel 26 (dsRNA) elements, ranging in size between 20.2 kb and 0.64 kb. Four low molecular weight dsRNA bands (sizes 2.0–0.64 kb) are consistently synchronous to mushroom off-color/browning symptoms. This devastating disease first appeared in 1996 on UK farms. MVX is now more widespread and prevalent in a number of European countries (e.g. Netherlands, Ireland). Symptoms vary and range from the occasional outbreak to severe outbreaks leading to crop losses. Recently a few mushroom farms in Poland reported symptoms of brown-colored mushrooms among the white ones. These observations were very similar to the observations of MVX incidences observed before on Dutch farms. This work is probably the first information about the possible presence of MVX on Polish mushroom farms.

Key words: *Agaricus bisporus*, mushroom virus X, MVX, dsRNA, mushroom browning

INTRODUCTION

Mycoviruses are widespread in fungi, including plant pathogenic fungi. In most cases, they were reported to be cryptic or show few symptoms leading to latent infection in host cells (Pearson *et al.* 2008). However, several mycoviruses associated with fungal diseases were recently reported in *Nectria radicicola* (Ahn and Lee 2001), *Sclerotinia sclerotiorum* (Boland 1992), *Fusarium graminearum* (Chu *et al.* 2002), and so on. The incidence and variability of mycoviruses have most commonly been determined based on the presence of dsRNAs. Mycoviruses are distinct from those viruses that use fungi as vectors because mycoviruses are able to replicate within the fungal host (Rochon *et al.* 2004). Mycoviruses present in mushrooms were found in *Agaricus bisporus* (white-button mushroom) and *Pleurotus ostreatus* (Yu *et al.* 2003). The viral disease of *A. bisporus*, the first viruses found in fungi, was first described in 1950 (Sinden and Hauser 1950) and subsequently shown to be related to the presence of virus particles in 1962 (Hollings 1962). The main symptom was the malformation of fruiting bodies, followed by loss of yield. The disease was named La France disease or Die-back disease.

In 2000 a putative new virus disease of mushrooms was described and identified as mushroom virus X (MVX – unassigned) (Gaze *et al.* 2000; Fauquet *et al.* 2005). The MVX disease of *A. bisporus* encompasses a range of disease symptoms. These include bare cropping areas (primordia disruption), crop delay, premature veil opening, off-colored or brown-colored mushrooms and sporophore malformations. The various symptoms can occur either singularly or in combination but mostly are associated with loss of crop yield or product quality. Because of some similarity in symptoms, the disease was at first linked erroneously to La France Virus disease (LIV) (Green *et al.* 2008). But unlike this known mushroom virus (LIV) that normally carries a specific set of dsRNAs, in the case of MVX the number of dsRNAs, their range, size and distribution over the samples is different. The dsRNAs in their electrophoretic patterns did not resemble those previously described in *A. bisporus* and were substantially different from those characteristic of La France disease.

MVX first appeared on UK farms in the late 1990s but rapidly became prevalent (Adie *et al.* 2004). The first indications of the emergence of a new mushroom virus were the reported cropping problems (restricted pinning) on a single farm in Britain in the autumn of 1996. Gaze *et al.* (2000) outlined the emergence of this phenomenon with the appearance of similar problems on other farms during 1997, 1998 and 1999 when at least 15 farms were reporting similar problems. The reported symptoms where diverse, but the most common visible symptom was the appearance of patches (of various sizes and shapes) on the casing surface devoid of mushrooms. These affected areas consisted of totally underpinned casing, arrested pins or fully formed but late developing mushrooms. Such patches never produce healthy mushrooms. Distorted and discolored mushrooms where common. In contrast

*Corresponding address:
bioline@home.pl

bare patches where absent and mushrooms exhibited premature cap opening often associated with an absence of a veil and other minor distortions of mushrooms. The appearance of 'brown' mushrooms within a white strain is stated to be associated with this virus disease. In some instances all the symptoms described appeared together on one site. In all cases reduced yields occurred (Doyle 2003). In the subsequent years this virus affected the crops of 80% of commercial mushroom growers in Great Britain; losses amounted to £50 million. Such losses resulted in mushroom farm closures and the loss of nearly 800 jobs (NAO 2003).

Farms in The Netherlands, South Africa, New Zealand, and Italy reported symptoms characteristic of MVX and the molecular tests confirmed the presence of MVX in samples from these locations (Kaur 2002).

A complex of up to 23 dsRNAs was associated with the presence of virus-like symptoms such as severe crop loss and crop delay due to the disruption of the pinning process, and poor quality mushrooms. In some cases, brown or off-coloured mushrooms occurred in an otherwise white crop (Gaze *et al.* 2000; Grogan *et al.* 2003). The brown mushroom symptoms were also reported from The Netherlands and Ireland, but in these countries it occurred in the absence of the other symptoms. The brown symptoms were subsequently shown to be correlated to the presence of four or five low molecular weight dsRNAs between 0.64 and 2.0 kilo base pairs (kbp) in size, whereas the pinning disruption and other symptoms were associated with larger molecular weight dsRNAs between 3.6 and 14.4 kbp (Grogan *et al.* 2003; Sonnenberg *et al.* 2004).

Recently a few mushroom farms in Poland reported unusual brown-colored mushrooms appearing among the white ones. The symptoms were very similar to the reports of MVX incidences observed before on Dutch farms. This work presents the case study of symptom dynamics associated with MVX on two mushroom farms. The work is probably the first information about the possible presence of MVX also on Polish mushroom farms.

MATERIALS AND METHODS

MVX symptoms and mushroom samples

Mushroom farms experiencing MVX were visited in order to obtain pictures and details of disease symptoms. Eleven mushroom sample sets (both 'healthy' and diseased) were collected at farms and analyzed for the presence of MVX. For each test a min. of 150 g of mushroom samples were collected. Samples shipped for molecular MVX tests were stored and delivered at 4°C.

MVX detection

The mushroom samples were routinely sent to Plant Research International of Wageningen UR, The Netherlands who offered commercial testing services. The testing services were to determine the presence or absence of MVX via the banding patterns and PCR analyses by their own modified methods, described previously for La France virus (Sonnenberg *et al.* 1995).

Growing tests

In order to check the points of compost infection during the production cycle a growing test was performed. Compost from the same batch was collected at different stages of production and the mushrooms were grown on a separate mushroom farm (samples CP2O, CP3O, CP3T in table 1) or on the suspected farms (CP3I).

The farm which was separated from the others was located ca. 30 km from the suspected ones and routinely used the compost from the different supplier. No MVX symptoms were recorded earlier.

Heavy gauge polythene bags were filled with 15 kg of compost at different stages of production. When Phase II compost was used (sample CP2O) it was hand spawned with 105 g of spawn (0.7%). Four replicate bags of compost were prepared for each of these treatments, and incubated in the cropping room at 25°C for 17 days. At the end of this incubation period, the colonized compost was covered with the casing layer. A 45–50 mm layer of casing soil was applied on top of the compost in all bags and the mushrooms were grown according to the standard procedures. Phase III compost (CP3O,CP3T, CP3I) was spawned with a spawn rate of 0,7% and incubated bulk in the tunnels. Casing layer was applied directly after placing compost in the growing rooms.

On-farm observations

Regular observations were performed on the farms from the onset of the MVX disease to enable a detailed case history study. In every growing room a detailed inspection of the symptoms were made and a simple point system was implemented to follow and compare the infection intensity changes. The growing rooms were given points where 0 pt. mean no symptomatic mushrooms present; 1 pt. – single symptomatic mushrooms in the growing room (1 per 100 m^2); 2 pts. – visible infection (1 symptomatic sporophore per 10 m^2.); 3 pts. – serious infection (> 1 symptomatic sporophores per 10 m^2).

RESULTS AND DISCUSSION

Two mushroom farms in the Wielkopolska region in Poland reported a strange occurrence of brown mushrooms among the white ones. These farms were located a short distance from each other and used compost from the same supplier. Additionally, they closely cooperated in compost and mushroom logistics. The symptoms appeared at the same time on both farms.

Symptoms were very similar (if not identical) to these previously described as mushroom browning caused by Mushroom Virus X (MVX) (Grogan *et al.* 2003; Sonnenberg *et al.* 2004). No other symptoms were reported as correlating to MVX, like pinning disruption and crop delay or sporophore malformations and loss of yield. Only off- or brown-colored mushrooms among the normal crop of white mushrooms were localized on the beds (Fig. 1). Rare incidents of premature veil opening were also noted (Fig. 1B, C) and some symptomatic mushrooms differed from the others on the bed by a sticky, scaly cap texture (Fig. 1D, F). The discolorations of mushrooms represented the whole spectrum from slightly brown tinted to strong

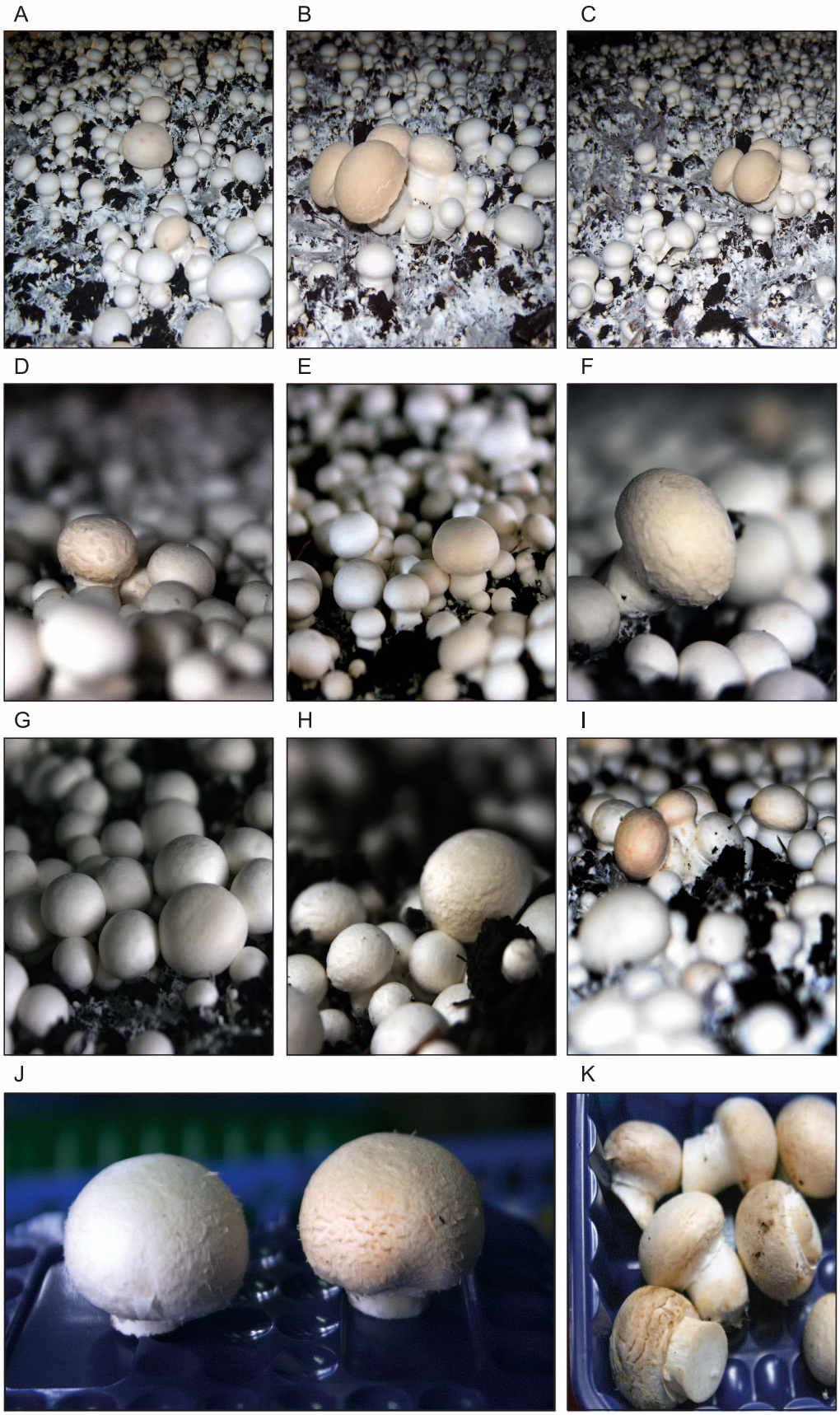


Fig. 1. Various browning symptoms on mushrooms with MVX

brown sporophores (Fig. 1G, H, I respectively). It was also not rare to observe that mushrooms picked as white started to lose their color and turned brown during 2–3 days of cool storage (Fig. 1J, K). The color change from white to brown was accelerated to 1 day when stored at room temperature.

Five consecutive molecular tests were performed to determine the presence or absence of the MVX dsRNAs in the mushroom sporophores. Mushrooms were picked and tested in the 24th 25th, 28th, 32nd and 35th weeks. Week 24 was the first week in which many recognized symptoms showed up. Normal white, well-formed mushrooms as well as well-formed mushrooms showing brown discolorations were sent for MVX determination. Only the first test of the mushrooms collected in the first week of observed infection was positive for both – brown discolored mushrooms and white asymptomatic ones. In all subsequent tests only brown discolored mushrooms were positive for the MVX. This could suggest that the initial infection happened early in the compost production at the moment of spawning. Later cases of MVX symptoms were most likely from late reinfection at filling the growing rooms and casing. Such a conclusion could be based on earlier observations by Grogan who showed that brown mushrooms appeared most consistently when a small quantity of infective material (0.01%) was incorporated into healthy spawn-run compost during bulk handling or into the casing of a healthy crop. But if infection with a small quantity of virus inoculum (0.01%) occurred earlier, at spawning, occurrence of brown mushrooms was much more erratic and in most cases they did not appear on the bed. White mushrooms on such a compost contained the dsRNA virus (Grogan *et al.* 2004).

While the symptoms of MVX persisted on the infected farms, a second set of experiments was performed with the mushrooms grown on compost obtained at different stages of production.

These results showed no MVX symptoms as well as no dsRNA in the samples obtained from mushrooms grown on the compost at earlier stages of production (CP2O, CP3O, CP3T in table 1). MVX tests were positive only with symptomatic mushrooms grown on the infected farms (CP3I), while white mushrooms, even if grown on the infected farms were free of MVX. That could again suggest that the persistence of the infection was caused by the reinfection of compost at the farm level. Despite the implementation of strong hygiene regimes, there were several opportunities for infecting a crop from spawning right through to casing and cropping at farms with a virus problem. That would be particularly true with MVX infection, where disease symptoms could be recognized

Table 1. Compost source description used for growing tests

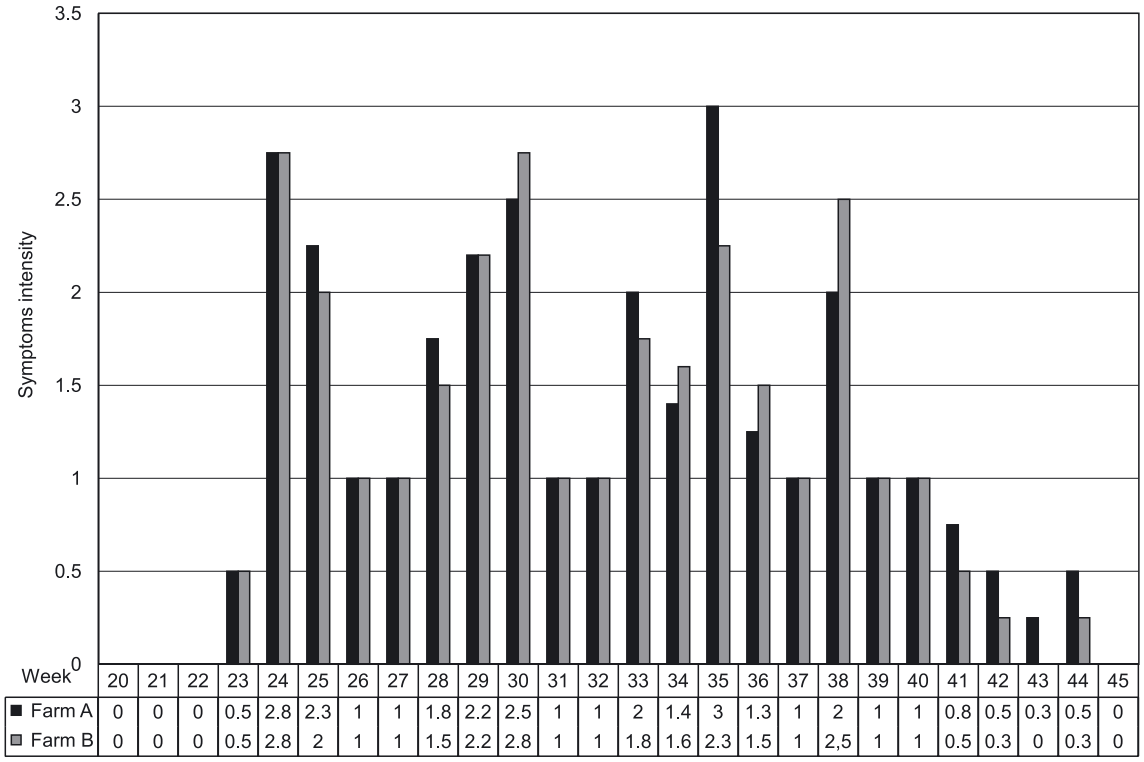
Compost designation	Description
CP2O	phase II spawned compost bagged; mushrooms grown in the test farm
CP3O	phase III compost collected directly from the spawn-runned tunnel, bagged; mushrooms grown in the test farm
CP3T	phase III compost collected from the truck delivering spawn-runned compost in bulk; compost bagged for the experiment; mushrooms grown in the test farm
CP3I	mushrooms grown in the suspected farm on shelves filled in bulk

Table 2. MVX identification in mushrooms from infected growing rooms

Test No.	Week	Virus symptoms	Virus identification
Test I	24	no virus symptoms	confirmed
		mushrooms with virus symptoms: solid, well formed, brown tinted	confirmed
Test II	25	no virus symptoms	not confirmed
		mushrooms with virus symptoms: solid, well formed, brown tinted	confirmed
Test III	28	no virus symptoms	not confirmed
		mushrooms with virus symptoms: solid, well formed, brown tinted	confirmed
Test IV	32	no virus symptoms	not confirmed
		mushrooms with virus symptoms: solid, well formed, brown tinted	confirmed
Test V	35	no virus symptoms	not confirmed
		mushrooms with virus symptoms: solid, well formed, brown tinted	confirmed

Table 3. MVX search in mushrooms grown on compost from different stages of production

Compost designation	Mushroom sample designations	Virus symptoms	Virus identification
CP2O	CP2OW	no symptoms, white mushrooms	not confirmed
CP3O	CP3OW	no symptoms, white mushrooms	not confirmed
CP3T	CP3TW	no symptoms, white mushrooms	not confirmed
CP3I	CP3IW	no symptoms, white mushrooms	not confirmed
	CP3IB	mushrooms with virus symptoms: solid, well formed, brown tinted	confirmed



Week	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45
Farm A	0	0	0	0.5	2.8	2.3	1	1	1.8	2.2	2.5	1	1	2	1.4	3	1.3	1	2	1	1	0.8	0.5	0.3	0.5	0
Farm B	0	0	0	0.5	2.8	2	1	1	1.5	2.2	2.8	1	1	1.8	1.6	2.3	1.5	1	2,5	1	1	0.5	0.3	0	0.3	0

Fig. 2. Weekly intensity of MVX symptoms by farm

even at a very low infection levels. Even 1 ppm contaminated compost mixed with non-contaminated compost was stated to delay cropping (Doyle 2003).

Further on-farm observations showed an interesting periodicity of symptom intensity (Fig. 2). A summary of the weekly symptom levels suggested a time correlation associated with the infection level on both farms. That would be in contradiction with the previous hypothesis of self-reinfection on the observed farms which suggested the same compost supplier as a source of MVX. Two weeks period of symptom intensity reduction (weeks 26th, 27th and 31st, 32nd and 36th, 37th, respectively) could be related to the period of 14–16 days of spawn incubation after spawning the bulk compost in the tunnels. It could also be possible, that simultaneous changes of the MVX symptoms observed in both mushroom farms were related to the growing conditions influenced by the weather changes. It was suggested by other authors, that browning of mushrooms despite being correlated with MVX presence, could be caused by unknown stress rather than a virus. Recent observations suggest that plant pathogenic *Pseudomonas* and their peptides could lead to significant environmentally governed 'stress effects'. These effects culminate in an off- or brown-color response in the host mushroom cap tissues – rather than the four smaller MVX dsRNAs implicated in the browning symptoms within the MVX disease complex (Rao *et al.* 2007). On the other hand, results from a recent study (Duffy *et al.* 2006) showed that varied environmental conditions could play an important role in viral emergence, critical for host shifts in RNA viruses and thereby for disease emergence in novel or 'unselected' hosts.

Also the observed termination of disease symptoms could not be explained in an unambiguous way. Along with an increase in general hygiene standards on both previously infected farms, the weather conditions changed to be more favorable for mushrooms growing. It was recently suggested, that environmental conditions could affect mushroom gene expression during MVX infection and potentially influence the browning symptom occurrence (Green *et al.* 2008). While we do not know the direct causes of the disease emergence and its completion, we were able, probably for the first time, to observe on Polish mushroom farms the presence of the previously described MVX in the mushrooms manifesting symptoms typical for this disease. Although considerable data has already accumulated on the symptoms of infection, etiological sources, epidemiology and molecular characterization of the MVX, dsRNA elements associated with the disease are limited and should be investigated in the future.

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POLISH SUMMARY

MUSHROOM VIRUS X (MVX): NOWA CHOROBA PIECZAREK W POLSCE

W 1996 r. w Wielkiej Brytanii, po raz pierwszy, odnotowano pojawienie się nietypowej choroby w uprawach pieczarek (*Agaricus bisporus*), nazwanej "Mushroom Virus X" (MVX). Choroba ta wykazuje szereg zróżnicowanych objawów. Wyniki prac przeprowadzonych w Wielkiej Brytanii, Holandii i Irlandii wskazują na to, że grzyby z objawami MVX zawierają zróżnicowany zestaw do 26 elementów dsRNA o wielkościach w przedziale 20,2 do 0,64 kpz. Występowanie czterech najmniejszych frakcji dsRNA (2,0–0,64 kpz) jest wyraźnie skorelowane z obecnością charakterystycznych symptomów chorobowych – brązowych, lub przebarwionych kapeluszy pieczarki. Ta groźna choroba, staje się coraz bardziej rozpowszechniona w szeregu innych krajów europejskich (np. w Holandii, Irlandii), wykazując zróżnicowane objawy oraz różne nasilenie – od pojedynczych przypadków aż do istotnej redukcji plonu. W ostatnim czasie kilka gospodarstw w Polsce donosiło o pojawieniu się brązowych grzybów występujących wśród białych owocników. Objawy te były bardzo podobne do wcześniej obserwowanych w holenderskich pieczarkarniach. Prezentowana praca stanowi prawdopodobnie pierwsze doniesienie o możliwej obecność wirusa X w uprawach pieczarek w Polsce.