European Journal of Plant Pathology (2006) 114:127–137
DOI 10.1007/s10658-005-8913-3

Attenuation of virulence in *Sclerotinia homoeocarpa* during storage is associated with latent infection by *Ophiostoma mitovirus 3a*

F. Deng and G.J. Boland
Department of Environmental Biology, University of Guelph, N1G 2W1, Guelph, ON, Canada
E-mail: gboland@uoguelph.ca

Accepted 13 June 2005

Key words: dollar spot, double-stranded RNA, dsRNA, hypovirulence, latent infection, mitochondrial virus, mitovirus

Abstract

The hypovirulence-associated mitovirus, *Ophiostoma mitovirus 3a* (OMV3a), has been shown to be widespread in eastern Canadian populations of *Sclerotinia homoeocarpa* in the form of latent infection. Latent infection by OMV3a was not associated with an apparent phenotype and did not significantly reduce the growth and virulence of the pathogen. In the present study, we found that isolates of *S. homoeocarpa* latently infected by OMV3a can change to hypovirulent isolates after storage at 4 °C, and that this attenuation of virulence was associated with increased concentration of the OMV3a virus. Recurrent observations revealed that up to 29.8% of latently infected isolates changed to hypovirulent isolates after 21 months of storage. Transmission of OMV3a dsRNA from latently infected isolates to virus-free isolates resulted in latent infection of the recipient isolates, indicating that latent infection by OMV3a was not associated with genetic differences in the fungal host. The RNA genomes of the OMV3a virus in an isogenic pair of latently infected and hypovirulent isolates were sequenced and compared. Each of the two RNAs contained an open reading frame of 726 amino acids with conserved motifs typical of RNA-dependent RNA polymerase (RdRp). The OMV3a RNA sequences in these two isolates share 95.1% nucleotide and 94.6% amino acid sequence identities. The development of hypovirulence from latent infection by OMV3a virus may provide new strategies to improve the biological control efficacy of hypovirulence in dollar spot management.

Introduction

The term 'hypovirulence' was introduced in the 1960s to describe isolates of a fungal plant pathogen that exhibited attenuated virulence to their host plants (Grente, 1965). Hypovirulence in plant pathogenic fungi can be caused by several infectious cytoplasmic factors, including viruses, virus-like agents, and plamids (Elliston, 1982), but most commonly, hypovirulence has been associated with double-stranded RNA (dsRNA) genetic elements. The hypovirulence-associated mycoviruses in several plant pathogenic fungi have been characterized and classified into five virus families, the *Chrysoviridae, Hypoviridae, Narnaviridae, Reoviridae, and Totiviridae,* based on genome structure, particle morphology, or absence of an encoded coat protein (Buck, 1998; Hillman et al., 2000; Wickner et al., 2000; Ghabrial, 2002; Nuss, 2005).

Hypovirulence-associated dsRNAs are receiving increased attention because of their potential use as probes to elucidate the molecular basis of fungal virulence and as biological control agents (Nuss, 1996; Dawe and Nuss, 2001;. Buck and Brasier, 2002). The successful control of chestnut blight in Europe and some regions of north America is a well-documented example of using such a hypovirulence-mediated biocontrol strategy

(MacDonald and Fulbright, 1991; Dawe and Nuss, 2001; Milgroom, 2004). Partial and effective field control of several other plant pathogenic fungi using hypovirulent isolates has also been reported (Bandy and Tavantzis, 1990; Bissegger et al., 1997; Zhou and Boland, 1998).

Sclerotinia homoeocarpa causes dollar spot of turfgrass, and the effective suppression of dollar spot by hypovirulent isolates of *S. homoeocarpa* offers a promising strategy for dollar spot management (Zhou and Boland, 1998). A hypovirulence-associated dsRNA in hypovirulent isolate Sh12B was found to be a mitovirus that was conspecific with *Ophiostoma mitovirus 3a* (OMV3a)-Ld from a diseased isolate of *O. novo-ulmi*, the causal agent of Dutch elm disease. This was the first report of a fungal virus found in another host, and because this virus was previously described, it was assigned *Ophiostoma mitovirus 3a*-Sh12B (OMV3a-Sh12B) (Deng et al., 2003). In addition to this single-stranded RNA virus, a satellite RNA was also found in some hypovirulent isolates of *S. homoeocarpa* although this RNA was not related to the hypovirulent phenotype (Deng and Boland, 2005).

The prevalence of the OMV3a virus was investigated in 116 isolates of *S. homoeocarpa* from eastern Canada. Initially, these isolates were screened using agarose gel electrophoresis and only four isolates contained detectable concentrations of the OMV3a dsRNA and were hypovirulent. Subsequently, all isolates were screened using reverse transcription-polymerase chain reaction (RT-PCR) and the OMV3a dsRNA was detected in 49% of the isolates. Isolates containing OMV3a virus that were only detectable using RT-PCR displayed typical colony growth and virulence, and were considered to be asymptomatic, virulent isolates that were latently infected by OMV3a (Melzer et al., 2005).

In many plant pathogenic fungi, isolates tend to lose their ability to cause plant disease after short periods of storage and this attenuation of virulence is often accompanied by slow or abnormal growth, or both (Russell, 1939; Chambers, 1970; Férault et al., 1979; Asher, 1981; Cook and Naiki, 1982). There are several explanations for this phenomenon, including expression of latent mycoviruses (Férault et al., 1979). This phenomenon was also recently observed in *S. homoeocarpa*. The prevalence of latent infection by OMV3a provided a model system for studying the mechanisms of virulence attenuation in *S. homoeocarpa*. Therefore, the objectives of this study were to determine if isolates latently infected by OMV3a could change to hypovirulent isolates, and to clarify if latent infection by OMV3a was associated with genetic differences in the virus or the fungal host.

Materials and methods

Isolates

One hundred and sixteen samples of dollar spot were collected from nine golf courses in southern Ontario and one golf course in Nova Scotia. Isolates of *S. homoeocarpa* were recovered into pure culture in the laboratory. The presence of OMV3a viral dsRNA in these isolates has been reported by Melzer et al. (2004).

Recurrent assessment of colony morphology, growth rate, and presence of OMV3a viral dsRNA

Two complete sets of the 116 isolates of *S. homoeocarpa*, all growing on potato dextrose agar (PDA) medium in Petri dishes (90 mm diam), were stored at 4 °C for up to 21 months. The Petri dishes were sealed with parafilm and enclosed in plastic bags during storage. The colony morphology and colony diameter of each isolate were examined every 4–6 months by subsampling a 6 mm diam agar plug from each culture and transferring this to fresh PDA. The original culture was immediately returned to storage. All isolates were assessed for colony morphology and colony diameter after culturing on PDA for 5 days at 20 °C, and all isolates with abnormal colony morphology were further tested for virulence and the presence of OMV3a dsRNA, using methods described by Zhou and Boland (1997).

Transmission of latent infection by OMV3a virus to virus-free isolates

A transmission experiment was conducted to determine if latent infection by OMV3a was associated with genetic differences among isolates of *S. homoeocarpa*. Transmission of OMV3a dsRNA from latently infected isolates to vegetatively compatible, virus-free isolates of

S. homoeocarpa was performed by pairing these two types of isolates, as described by Melzer et al. (1997). Hypovirulent isolates were also included as positive controls in this transmission experiment. After incubation for 60–72 h agar plugs containing mycelium were sampled from the colony of the dsRNA-free isolate. The growth rate, virulence, and presence of OMV3a dsRNA in the donor and recipient isolates were tested. The growth of these isolates was assessed by measuring the colony diameters of four cultures of each isolate 72 h after incubation on PDA at room temperature (20–22 °C). The virulence of these isolates was assessed using detached leaves of creeping bentgrass (cv. Penncross), as described by Zhou and Boland (1997). Nine lesions were measured for each isolate and the test was repeated once. Statistical analysis was performed using the *Proc glm* procedure of SAS ver. 6.12 (SAS institute, Cary NC), and the type I error rate (α) was set at 0.05.

The presence of OMV3a viral dsRNA was detected by agarose gel electrophoresis and RT-PCR. DsRNA was extracted from donor and recipient isolates using DNAzol (Molecular Research Center, Inc., Cincinnati, OH), according to the manufacturer's instructions. For detection using agarose gel electrophoresis, nucleic acids were separated by 1% agarose gel electrophoresis, and visualized under ultraviolet light after ethidium bromide staining.

For detection of OMV3a dsRNA using RT-PCR, the primer pair 1137F (5-GCC TTCCAATGGATGGAAC-3) and 1675R (5-TGTCACCAATCTCTTAGCAAG-3) was used to directly amplify a 539 bp cDNA fragment from samples containing OMV3a dsRNA. Samples were incubated at 65 °C for 30 min for the reverse transcription reaction. The PCR reaction was started with an initial denaturation step for 2 min at 94 °C, followed by 40 cycles of 30 s at 94 °C, 30 s at 60 °C, and 45 s at 72 °C, and a final extension cycle of 7 min at 72 °C. Positive and negative control reactions were included in each reaction.

Sequence comparison of OMV3a genomes in latently infected and hypovirulent isolates

To determine if latent infection in *S. homoeocarpa* was associated with genetic differences in the OMV3a virus, the entire genome of the OMV3a virus in a hypovirulent isolate (Sh13H) and a latently infected isolate (Sh13L) were sequenced and compared. Sh13H was derived from Sh13L after 11 months of storage at 4 °C. Therefore, these two isolates are considered isogenic. The dsRNAs were extracted from these two isolates using DNAzol as described previously, and cDNA clones were generated using RT-PCR primers specific to the OMV3a dsRNA genome. RT-PCR reactions were conducted using the *C. therm.* Polymerase One-Step RT-PCR System (Roche Diagnostics, Laval, Quebec) according to the manufacturer's instructions. The PCR products were directly sequenced by the Sanger chain termination method (Sanger et al., 1977) using an ABI PRISM sequencer and the sequences were completed by overlapping the cDNA clones. Sequence analysis was performed using the Needle programme of the European Molecular Biology Open Software Suite (EMBOSS), and multiple alignments were done with Clustal W (Thompson et al., 1997). Additional sequences for characterized strains of OMV3a were obtained from GeneBank database (Hong et al., 1998; Deng et al., 2003).

Results

The association of latent infection by OMV3a with attenuation of virulence in S. homoeocarpa

One set of the 116 isolates was recurrently assessed for colony morphology and growth on PDA after storage at 4 °C. Immediately after the cultures of *S. homoeocarpa* were recovered into pure culture, no hypovirulent isolates were observed based on the cultural phenotype associated with hypovirulent isolates. At the start of the experiment, which was after 4 months of storage at 4 °C, four hypovirulent isolates were observed based on cultural morphology and virulence, and confirmed by detection of OMV3a dsRNA using agarose gel electrophoresis (Figure 1; Tables 1, 2). Using the same methods, 11 and 14 hypovirulent isolates were detected after 10 and 16 months of storage, respectively. In total, 17 hypovirulent isolates were recovered after 21 months of storage at 4 °C, comprising 14.7% of the total isolates (Table 2). Analysis of experimental results revealed that all these isolates were obtained from isolates latently infected by OMV3a and no isolates were derived

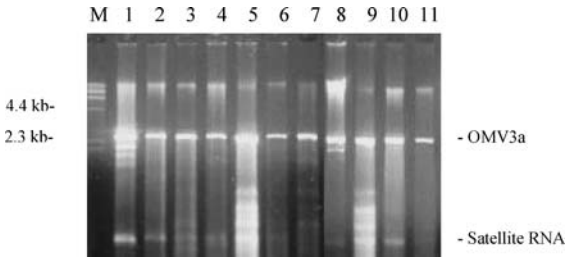

Figure 1. Agarose gel banding patterns of dsRNAs in selected hypovirulent isolates of *Sclerotinia homoeocarpa* that developed from latently infected isolates. Lane M, (-*HindIII* molecular marker, sizes were indicated on the left. Lanes1–11. dsRNA extracts from isolates Sh4, Sh5, Sh13, Sh35, Sh41, Sh48, Sh70, Sh111, Sh116, Sh126, and Sh128, respectively. All the hypovirulent isolates contained OMV3a dsRNA and some isolates contained the satellite RNA and defective forms of OMV3a dsRNA.

Table 1. Virulence and growth rate of hypovirulent isolates of *Sclerotinia homoeocarpa* that developed from latently infected isolates

Isolate	dsRNA	Colony diameter (mm)	Lesion length (mm)
Sh4	+	19.2	1.1
Sh5	+	17.3	1.0
Sh13	+	15.2	0.7
Sh35	+	15.0	2.3
Sh41	+	30.9	3.1
Sh48	+	31.8	3.9
Sh70	+	26.0	2.9
Sh111	+	19.5	5.7
Sh116	+	19.0	1.8
Sh126	+	21.3	0.0
Sh128	+	22.5	3.9
Sh94	–	69.8 *	17.0*
		SE = 2.24	SE = 0.44

*Indicates significant difference between dsRNA-containing isolates and dsRNA-free isolate Sh94. All hypovirulent isolates contained gel detectable concentrations of OMV3a dsRNA.

from the virus-free, virulent isolates. These results indicated that all hypovirulent isolates were derived from isolates that were latently infected by OMV3a, and the percent of isolates that changed from latently infected to hypovirulent was as high as 29.8 % (Table 2). All these new hypovirulent isolates contained the OMV3a dsRNA, but some also contained the satellite RNA and other defective RNAs of the OMV3a virus. No new dsRNA elements were found in these hypovirulent isolates (Figure 1).

As two sets of the 116 isolates were stored at 4 °C, several isolates were found in two forms, hypovirulent in one set but latently infected in another (Figure 2). These pairs of isolates were considered isogenic except for the differential infection by the OMV3a virus. Detection of OMV3a dsRNA and virulence evaluation of these isolates showed that the increased concentrations of OMV3a dsRNA from RT-PCR detectable to gel detectable levels were closely associated with the attenuation of virulence (e.g. hypovirulence) (Figure 3).

Transmission of latent infection by OMV3a to virus-free isolates in S. homoeocarpa

The transmission of OMV3a dsRNA from two latently infected isolates (Sh55 and Sh60) to three virus-free isolates (Sh10, SH84, and Sh125) was investigated, and two hypovirulent isolates (Sh70 and Sh126) were used as positive controls. All these isolates were in the same VCG group (Deng et al., 2002). The OMV3a dsRNA in hypovirulent isolates Sh70 and Sh126 was transmitted to the three virus-free isolates, and the OMV3a dsRNA was detected by agarose gel electrophoresis. Transmission of the dsRNA resulted in conversion of virus-free, virulent isolates to hypovirulent isolates, as the converted isolates grew slowly and their virulence was reduced (Table 3). Similarly, the OMV3a dsRNA was also transmitted from latently infected isolates to virus-free isolates, but the transmission did not result in the hypovirulent phenotype. Instead, the viruses remained latent and could only be detected by RT-PCR (Table 3; Figure 4). More isolates were tested in this experiment and similar results were obtained.

Sequence comparison of OMV3a virus in hypovirulent and latently infected isolates

The nucleotide sequences of OMV3a-Sh13H and OMV-Sh13L were 2636 and 2635 bp, respectively, and their putative RdRp-like proteins were both 726 amino acids. The overall nucleotide and amino acid sequence identities between the OMV3a in these two isogenic isolates were 95.8 and 94.9%, respectively. Nucleotides were more conserved in the coding (96.1% identity) and 3′-non-coding regions (98.3% identity), and less conserved in the

Table 2. Number and percent of hypovirulent isolates derived from latently infected isolates of *Sclerotinia homoeocarpa* after storage at 4 °C

Type of isolates	No. of isolates	Number of hypovirulent isolates				Percent (%) after 21 months)
		4 months	10 months	16 months	21 months	
Latently infected	57	4	11	14	17	29.8
dsRNA free	59	0	0	0	0	0
Total	116	4	11	14	17	14.7

Latently infected isolates of *S. homoeocarpa* contained OMV3a dsRNA that was only detected by RT-PCR. Hypovirulent isolates are characterized by reduced virulence and growth, and containing gel-detectable OMV3a dsRNA.

Figure 2. Comparison of cultural morphology between isogenic pairs of hypovirulent and latently infected isolates of *Sclerotinia homoeocarpa*. L, latently infected by OMV3a. H, hypovirulent. Hypovirulent isolates were all debilitated and grew poorly on PDA medium.

5′-non-coding region (91.1% identity) (Figure 5a). This region was also less conserved among the four characterized OMv3a virus strains (Figure 5b).

Discussion

Fungal viruses or mycoviruses, mostly with dsRNA genomes, are widespread and have been reported in all classes of fungi. However, the majority of these mycoviruses appear to have no detrimental effect on their hosts. Such infections have been termed latent, even though the virus may replicate to high titers (Buck, 1986). Several mycoviruses can induce hypovirulence in plant pathogenic fungi and have been extensively studied as they have the potential to identify unique physiological pathways that affect virulence in fungal plant pathogens, and as biological control agents in plant disease management (Dawe and Nuss, 2001; Buck and Brasier, 2002; Ghabrial et al., 2002; Tavantzis et al., 2002; Nuss 2005).

In *S. homoeocarpa*, studies on the prevalence of hypovirulence-associated OMV3a virus revealed that 49% of the isolates were latently infected by OMV3a (Melzer et al., 2005). In the present study, we found that isolates latently infected by the hypovirulence-associated OMV3a virus could change to hypovirulent isolates after storage at 4 °C, and the percent of isolates that changed could reach as high as 29.8% after 21 months of storage. In *C. parasitica* and *O. ulmi*, generation of hypovirulence by mitochondrial mutation has been reported and this type of mitochondrial hypovirulence was also found in natural populations of *C. parasitica* (Brasier et al., 1993; Monteiro-Vitorello et al., 1995; Baidyaroy et al., 2000). However, detailed analysis has shown that these hypovirulent isolates were dsRNA or virus-free, and that development of hypovirulence was not associated with the occurrence of dsRNA or mycoviruses (Baidyaroy et al., 2000). In contrast, all hypovirulent isolates that developed in the present study contained increased concentrations (e.g. detectable by agarose gel electrophoresis) of

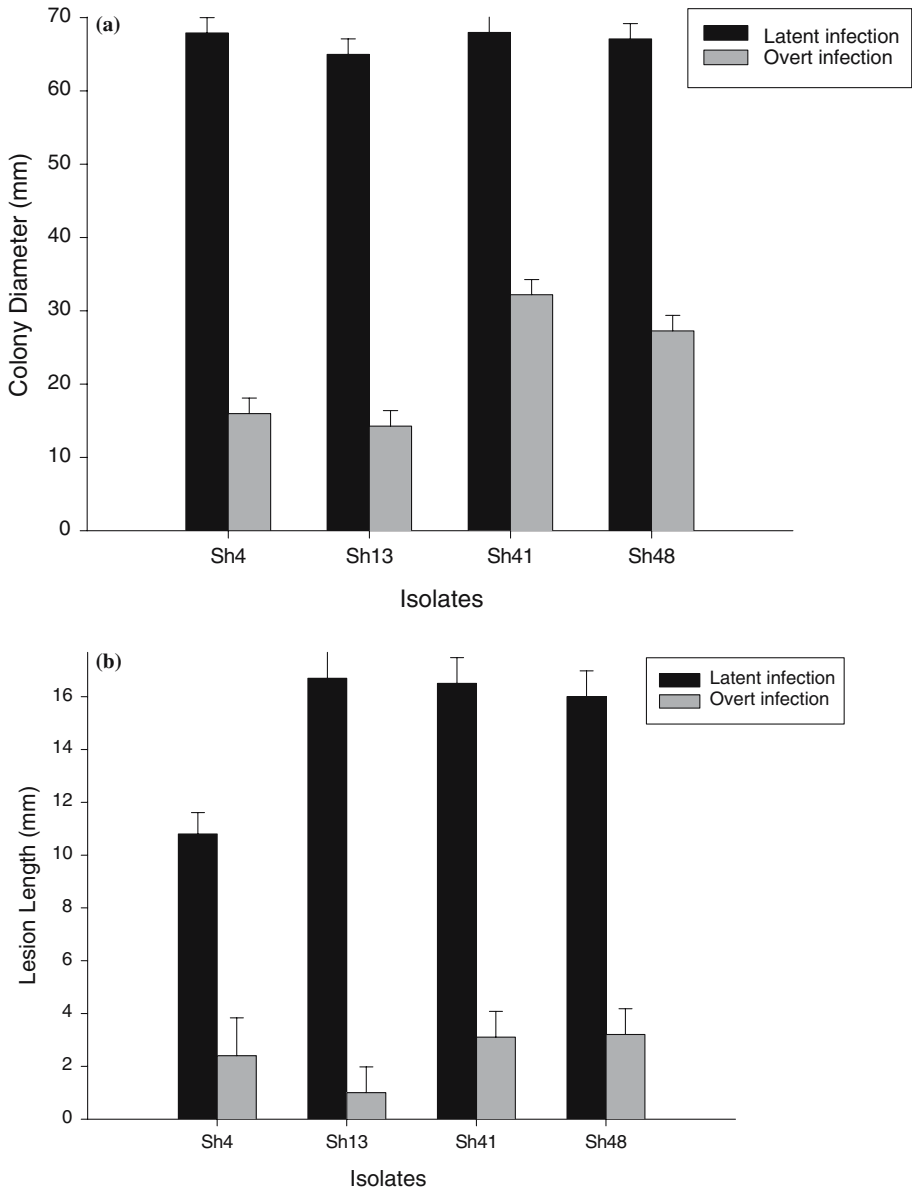

Figure 3. Effect of latent and overt (hypovirulent) infections of OMV3a on growth (colony diameter, (a)) and virulence (length of lesion, (b)) of *Sclerotinia homoeocarpa*. Colony diameters were measured on PDA plates 3 days after incubation at 22 °C, lesion lengths were measured on detached leaves of creeping bentgrass (cv. Penncross) 3 days after inoculation at 22 °C.

OMV3a dsRNA. Analysis of nucleic acid extracts from all these hypovirulent isolates did not detect fungal plasmids. In addition, all the hypovirulent isolates were recovered from the 57 latently infected isolates and none developed from the 59 virus-free isolates. Therefore, it appeared that attenuation of virulence or development of hypovirulence was associated with latent infection by the hypovirulence-associated OMV3a virus in *S. homoeocarpa*.

There are several types of evidence that hypovirulence can result from expression of latently infected viruses or virus-like dsRNAs. In *O. ulmi*, the hypovirulent (diseased) phenotype was also

Table 3. Transmission of latent infection of OMV3a and its effect on virulence and growth of the virus-free recipient isolates of *Sclerotinia homoeocarpa*

Pairings	Presence of dsRNA[a]	Colony diameter[b] (mm)	Lesion length[c] (mm)
Sh70	+	37.3	1.8
Sh70 × Sh10	+	37.3	1.7
Sh70 × Sh84	+	36.8	1.6
Sh70 × Sh125	+	36.3	1.7
Sh126	+	31.1	3.1
Sh126 × Sh10	+	36.6	1.6
Sh126 × Sh84	+	38.8	1.7
Sh126 × Sh125	+	32.1	1.3
Sh55	L	72.6	16.6
Sh55 × Sh10	L	71.5	16.9
Sh55 × Sh84	L	70.9	16.4
Sh55 × Sh125	L	72.5	15.9
Sh60	L	71.7	16.3
Sh60 × Sh10	L	71.1	16.8
Sh60 × Sh84	L	70.3	16.9
Sh60 × Sh125	L	71	16.6
Sh10	−	73.2	16.0
Sh84	−	73.3	16.4
Sh125	−	72.6	16.3
		LSD = 6.1	LSD = 1.8

[a] +, L, and − indicate gel-detectable OMV3a, RT-PCR detectable OMV3a and OMV3a-free, respectively.
[b] Colony diameters were measured after 72 h of growth on PDA at room temperature (20–22 °C).
[c] Lesion length was measured 72 h after inoculation.

associated with infection by mitoviruses (Hong et al., 1998, 1999). Latent infections by mitoviruses have been observed in single conidial progeny derived from mitovirus-infected isolates. These latently infected isolates had a phenotype similar to that of healthy isolates; however, after a period of growth in culture, a second cycle of conidiogenesis or sometimes storage at 4 °C, these isolates invariably changed to the hypovirulent phenotype. Of 50 viable single conidial isolates derived from the mitovirus-infected isolate L8d[2], 20 were latently infected, i.e. initially appeared to be healthy but later changed to the hypovirulent phenotype (Rogers et al., 1986).

The spontaneous appearance of genetically distinct dsRNA elements in *Rhizoctonia solani* was another example of latent infection (Lakshman and Tavantzis, 1994). The emergence of three novel dsRNAs in subculture Rh1A1 coincided with change of the parental virulent isolate Rhs1AP to hypovirulent Rhs1A1. PCR and southern blot analysis showed that two dsRNA-related sequences were present in the genomic DNA of both Rhs 1AP and Rh1A1. The third dsRNA was not present in DNA form in either of the two isolates. However, this dsRNA was found in total dsRNA preparations from parental isolate Rhs1AP in very low concentrations, and could only be detected by RT-PCR. This is similar to the latent infection by OMV3a in *S. homoeocarpa*. It was also noteworthy that the parental isolate Rhs1AP of *R. solani* gave rise to three subcultures containing novel dsRNAs during a 12-year storage, which involved numerous subculturings (Lakshman and Tavantzis, 1994).

Narnaviruses are phylogenetically closely related to mitoviruses in the family *Narnaviridae* (Wickner et al., 2000). Synthesis of both single- and double-stranded forms of the *Saccharomyces cerevisiae* 20S and 23S narnaviruses (ScNY-20S and ScNY-23S) was induced by exposing yeast cells to stressful conditions, such as heat shock or nutritional starvation (Kadowaki and Halvorson, 1971; Wesolowski and Wickner, 1984; Matsumoto et al., 1990). The copy number of the dsRNA forms (W and T dsRNA) of ScNY-20S and ScNY-23S was amplified 10-fold by growth of yeast cells at 37 °C compared to that in stationary phase cell growth at 30 °C (Wesolowski and Wickner, 1984). The single-stranded form of ScNY-20S (20S RNA) was amplified 10,000-fold on acetate medium and the same phenomenon also was observed for ScNY-23S (23S RNA) (Matsumoto et al., 1990; López et al., 2002). Therefore, latent infection by viruses or virus-like dsRNAs appears to be an explanation for the spontaneous emergence of novel dsRNAs and the development of hypovirulence in these fungi.

In the present study, low concentrations of OMv3a virus were transmitted from latently infected isolates to virus-free isolates. OMV3a remained latent in these recipient isolates, as the virus did not cause the hypovirulent phenotype. In contrast, transmission of OMV3a virus from hypovirulent to virus-free isolates resulted in attenuated virulence of the recipient isolates and these recipient isolates contained gel-detectable concentrations of OMV3a viral dsRNA. These results indicate that latent infection by OMV3a in *S. homoeocarpa* is not associated with genetic differences in the fungal hosts.

Nucleotide and amino acid sequence comparisons between OMV3a-Sh13L (latent) and OMV3a-Sh13H (hypovirulent) in *S. homoeocarpa* revealed high sequence identities at the nucleotide

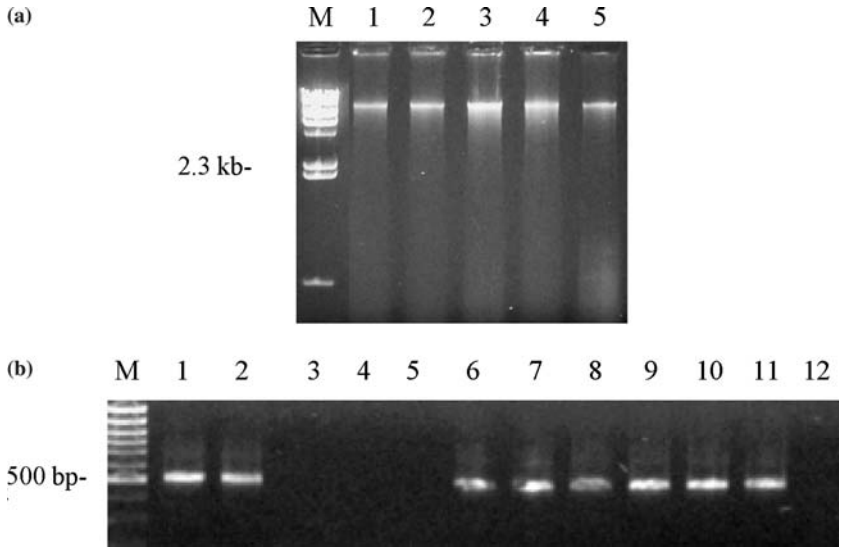

Figure 4. Transmission of latent infection by OMV3a dsRNA to virus-free isolates of *S. homoeocarpa*. (a) agarose gel electrophoresis of nucleic acid extracts of isolates, Lane M, molecular weight marker (size indicated on the left). Lanes 1-5: isolates Sh55, Sh60, Sh10, Sh84, and Sh125. No dsRNAs were detected. (b) RT-PCR detection of the OMV3a virus using primers 1137F and 1675R (see Materials and methods), Lane M: molecular weight marker (size indicated on the left). Lanes 1-5: isolates Sh55, Sh60, Sh10, Sh84, and Sh125. Isolates Sh55 and Sh60 are latently infected whereas isolates Sh10, Sh84, and Sh125 are virus-free. Lanes 6-8: isolates Sh10, Sh84, Sh125 after pairing with isolate Sh55. Lanes 9–11: isolates Sh10, Sh84, and Sh125 after pairing with isolate 60. Latently infected OMV3a viruses were transmitted from isolates Sh55 and Sh60 to virus-free isolates Sh10, Sh84, and Sh125. Lane 12, virus-free isolate Sh94 as negative.

(95.8%) and amino acid (94.9%) levels. Relatively low nucleotide sequence identity (91.1%) was found on the 5′-non-coding region between the two viral dsRNAs, and there were fewer conserved nucleotide sequences before the translation start codon. However, sequence comparison of the four characterized OMV3a strains also revealed high nucleotide polymorphism in this region, including three strains in hypovirulent isolates. RNA-dependent RNA polymerases generally lack proofreading activities (Domingo and Holland, 1994) and viral RNA typically consists of populations of closely related molecules or 'quasi-species' (Eign and Biebriecher, 1988). The RNA genome and the mitochondrial localization of the mitovirus make it difficult to characterize important point mutations. Therefore, further molecular characterization is needed to confirm if latent infection is associated with genetic differences in the virus.

The expression of latent infections by mycoviruses may involve interactions among the mycovirus, fungal host, and environment. It has been reported that host genes were required for replication of viruses or virus-like dsRNAs. In *Saccharomyces cerevisiae*, a system of six superkiller (SKI) genes (SK12, Sk13, SK14, SK16, Sk17 and SK18) negatively controls the copy number of the ScV-L-A totivirus, and its satellite and defective dsRNAs. The only essential function of these genes is to block virus multiplication. Mutations in any of these SKI genes can lead to an increased copy number of M dsRNA (Wickner, 1996).

The replication of viral dsRNAs in fungi may be under strict host control and, therefore, the amount of virus or dsRNA produced in actively dividing cells may be insufficient to interfere significantly with host metabolism. Therefore, viruses or virus-like dsRNAs would not cause any disease symptoms. However, continued virus replication after host cell division has ceased (e.g. in storage) would lead to higher virus levels in older resting cells, and this may attenuate the virulence of their fungal hosts. Still et al. (1975) reported that, in liquid cultures of *Penicillium stoloniferum* infected with PsV-S and PsV-F, maximum rates of dsRNA synthesis were not achieved until host growth had slowed. Moreover, as fungal growth moved

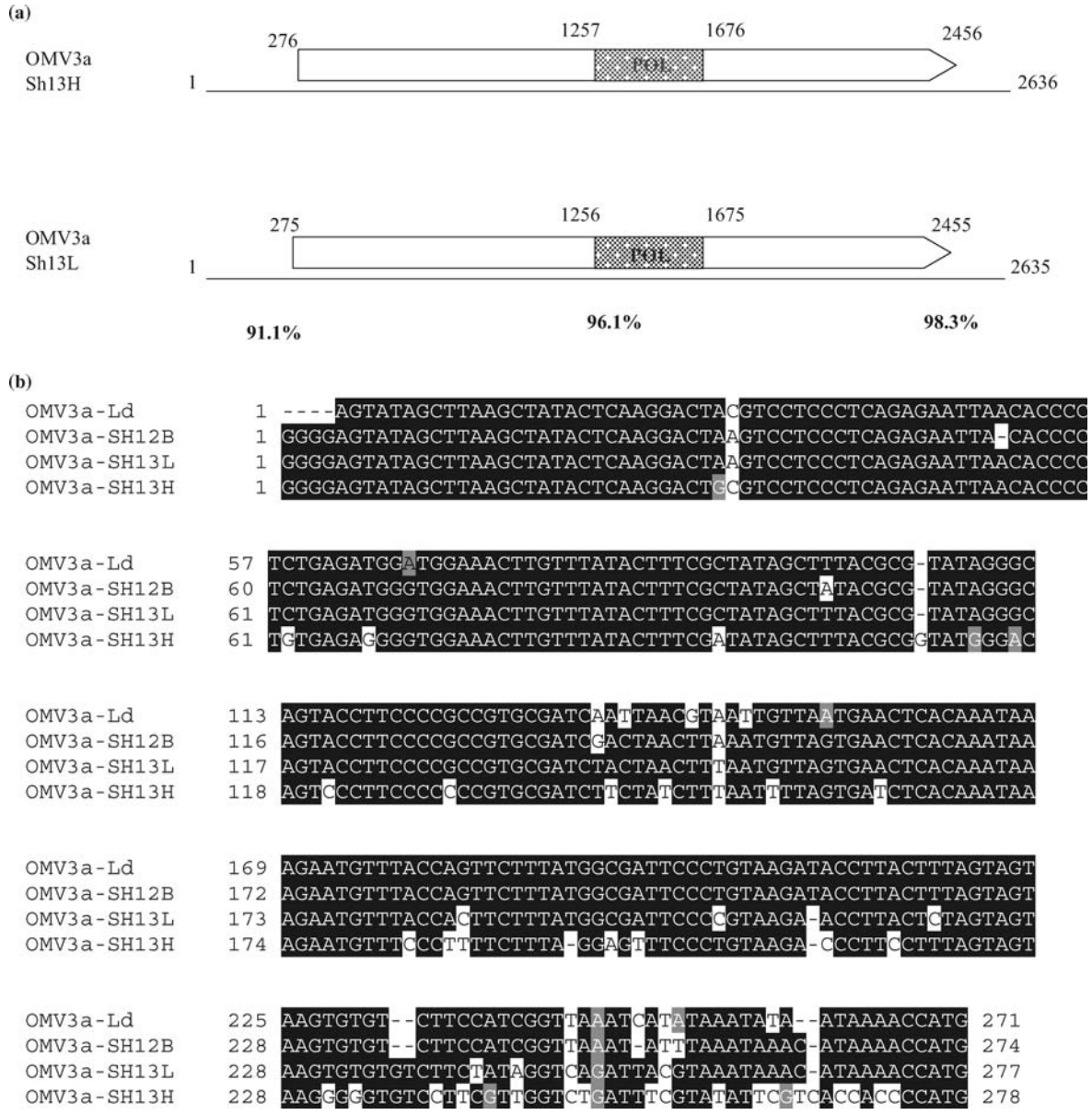

Figure 5. (a) Diagram of open reading frames for OMv3a-Sh13H and OMV3a-Sh13L. The shaded regions, designated Pol, contain the RdRp motifs I–VI. Numbers at bottom are nucleotide sequence identities for the 5′-non-coding sequences, coding sequences, and 3′-non-coding sequences between the OMv3a viruses in the two isogenic isolates Sh13H and Sh13L. (b) Nucleotide sequence alignment of four characterized OMV3a viruses, OMV3a-Ld (*Ophiostoma mitovirus 3a*-Ld, Hong, et al., 1998), OMV3a-Sh12B (*Ophiostoma mitovirus 3a*-SH12B, Deng et al., 2003), OMV3a-Sh13H and OMV3a-Sh13L (present study). Identical nucleotides are black-shaded.

towards the stationary phase, PsV-F levels increased threefold while biomass increased by only 40%. Only about 20% of the virus particles were synthesized during exponential phases of fungal growth, the remainder being produced during the deceleration or stationary phases of growth (Buck, 1977). It is possible that the growth and division of host cells and replication of the OMV3a virus were both reduced by low temperature, but the OMV3a replication was less affected and the virus could gradually increase its infection level. In addition, low temperature may affect the expression of host

genes which control replication of the virus. Therefore, the virus could accumulate to a higher concentration, which results in attenuation of virulence.

Infection by hypovirulence-associated viruses or virus-like dsRNAs can severely affect the virulence, growth, and survival of their fungal hosts. This, in turn, would lead to the elimination of the viruses or virus-like dsRNAs as they lack extracellular infectivity. Therefore, from a biological perspective, latent infection could help to ensure the survival and spread of the viruses in a fungal population. In this regard, latent infection benefits both the viruses and their fungal hosts.

In *S. homoeocarpa*, a change from latent infection by OMV3a to the hypovirulent phenotype may also occur in nature, as low temperatures during winter can provide suitable conditions for this change. Therefore, this phenomenon may play an important role in the natural biocontrol of dollar spot and provide new strategies to improve the biocontrol efficacy of hypovirulence in dollar spot management. However, more work is needed to understand the relationship between latent and overt infection by the OMv3a virus, and the mechanisms regulating this change before this phenomenon can be explored to maximum advantage.

Acknowledgements

This research was financially supported by the Natural Science and Engineering Research Council of Canada (NSERC) and the Ontario Ministry of Agriculture and Food (OMAF).

References

Asher MJC (1981). Pathogenic variation. In: Asher MJC and Shipton PJ (ed) Biology and Control of Take-all (pp. 999–218) Academic Press, London.

Baidyaroy D, Huber DH, Fulbright DW and Bertrand H (2000) Transmissible mitochondrial hypovirulence in a natural population of *Cryphonectria parasitica*. Molecular Plant-Microbe Interactions 13: 88–95.

Bandy BP and Tavantzis SM (1990) Effect of hypovirulent *Rhizoctonia solani* on Rhizoctonia disease, growth, and development. American Potato Journal 67: 189–199.

Bissegger M, Rigling D and Heiniger U (1997) Population structure and disease development of *Cryphonectria parasitica* in European chestnut forests in the presence of natural hypovirulence. Phytopathology, 87: 51–59.

Brasier CM, Bates MR and Chater NW (1993). DNA polymorphism, perithecial size, and molecular aspects of D-factors in *Ophiostoma ulmi* and *O. novo-ulmi*. In: Sticklen MB and Sherald JL (ed) Dutch Elm Disease Research (pp. 308–321) CAB Int, New York.

Buck KW (1977). Biochemical and biological implications of double-stranded RNA mycoviruses. In: Hems DA (ed) Biologically Active Substances: Exploration and Exploitation (pp. 121–148) John Wiley, Chichester.

Buck KW (1986). Fungal virology- an overview. In: Buck KW (ed) Fungal Virology (pp. 1–84) CRC Press, Boca Raton.

Buck KW (1998). Molecular variability of viruses of fungi. In: Bridge P, Couteaudier Y and Clarkson J (ed) Molecular Viability of Fungal Pathogens (pp. 53–72) CAB International, Wallingford, Oxfordshire, England.

Buck KW and Brasier CM (2002). Viruses of the Dutch elm disease fungi. In: Tavantzis SM (ed) dsRNA Genetic Elements: Concepts and Applications in Agriculture, Forestry, and Medicine (pp. 165–190) CRC Press, Boca Raton.

Chambers SC (1970) Pathogenic variation in *Ophiobolus graminis*. Australian Journal of Biological Science, 23: 1099–1103.

Cook RJ and Naiki T (1982) Virulence of *Gaeumannomyces graminis* var. *tritici* from fields under short-term and long-term wheat cultivation in the Pacific Northwest, USA. Plant Pathology, 31: 201–207.

Dawe AL and Nuss DL (2001) Hypoviruses and chestnut blight: exploiting viruses to understand and modulate fungal pathogenesis. Annual Review of Genetics, 35: 1–29.

Deng F and Boland GJ (2004) A satellite RNA in hypovirulent isolates of *Sclerotinia homoeocarpa*. Phytopathology, 94: 917–923.

Deng F, Melzer MS and Boland GB (2002) Vegetative compatibility and transmission of hypovirulence-associated dsRNA in *Sclerotinia homoeocarpa*. Canadian Journal of Plant Pathology, 24: 481–488.

Deng F, Xu R and Boland GJ (2003) Hypovirulence-associated dsRNA in *Sclerotinia homoeocarpa* is conspecific with *Ophiostoma* mitovirus 3a-OnuLd. Phytopathology, 93: 1407–1414.

Domingo E and Holland JJ (1994). Mutation rates and rapid evolution of RNA viruses. In: Morse SS (ed) The Evolutionary Biology of Viruses (pp. 161–184) Raven Press, New York.

Eign M and Biebriecher C (1988). Sequence space and quasispecies distribution. In: Domingo E, Holand JJ and Ahlquist P (ed) RNA Genetics (pp. 211–245)3 CRC Press, Boca Raton, FL.

Elliston JE (1982). Hypovirulence. In: Ingram DS and Willians PH (ed) Advances in Plant Pathology (pp. 1–33)1 Academic Press, Toronto, Canada.

Férault AC, Tivoli B, LeMaire JM and Spire D (1979) Etude dé l'volution comparee du niveau d'agreesivité et du contenu en particules de type virul d'une souche de *Gaeumannomyces graminis* (Sacc.) Arx et Olivier (*Ophiobolus graminis* Sacc.). Annales de Phytopathologie, 11: 185–191.

Ghabrial SA, Soldevila AI and Havens WM (2002). Molecular genetics of the viruses infecting the plant pathogenic fungus *Helminthosporium victoriae*. In: Tavantzis SM (ed) dsRNA Genetic Elements: Concepts and Applications in

Agriculture, Forestry, and Medicine (pp. 213–236) CRC Press, Boca Raton.

Grente J (1965) Les formes hypovirulentes *d'Endothia parasitica* et les espoirs de lutte contre le chancre du chataignier. Comptes Rendus de Acadamie d'Agriculture de France, 51: 1033–1037.

Hillman BI, Fulbright DW, Nuss DL and vanAlfen NK (2000). Family *Hypoviridae*. In: van Regenmortel MHV, Fauquet CM, Bishop DHL, Carstens EB, Estes MK, Lemon SM, Maniloff J, Mayo MA, McGeoch DJ, Pringle CR and Wichner RB (ed) Virus Taxonomy: Seventh Report of the International Committee on Taxonomy of Viruses (pp. 515–520) Academic Press, London.

Hong Y, Cole TE, Brasier CM and Buck KW (1998) Evolutionary relationships among putative RNA-dependent RNA polymerases encoded by a mitochondrial virus-like RNA in the Dutch elm disease fungus, *Ophiostoma novo-ulmi*, by other viruses and virus-like RNAs and by the *Arabidopsis* mitochondrial genome. Virology, 246: 158–169.

Hong Y, Dover SL, Cole TE, Brasier CM and Buck KW (1999) Multiple mitochondrial viruses in an isolate of the Dutch elm disease fungus *Ophiostoma novo-ulmi*. Virology, 258: 118–127.

Kadowaki K and Halvorson HC (1971) Appearance of a new species of ribonucleic acid during sporulation in *Saccharomyces cerevisiae*. Journal of Bacteriology, 105: 826–830.

Lakshman DK and Tavantzis SM (1994) Spontaneous appearance of genetically distinct double-stranded RNA elements in *Rhizoctonia solani*. Phytopathology, 84: 633–639.

López V, Gil R, Carbonell JV and Navarro A (2002) Occurrence of 20S RNA and 23S RNA replicons in industrial yeast strains and their variation under nutritional stress conditions. Yeast, 19: 545–552.

MacDonald WL and Fulbright DW (1991) Biological control of chestnut blight: Use and limitations of transmissible hypovirulence. Plant Disease, 75: 656–661.

Matsumoto Y, Fishel R and Wickner RB (1990) Circular single-stranded RNA replicon in *Saccharomyces cerevisiae*. Proceedings of the National Academy of Sciences of the United States of America, 87: 7628–7632.

Melzer MS, Deng F and Boland GJ (2004) Asymptomatic infection and distribution of *Ophiostoma mitovirus 3a* (OMV3a) in populations of *Sclerotinia homoeocarpa*.Canadian Journal of Plant Pathology (submitted).

Melzer MS, Dunn M, Zhou T and Boland GJ (1997) Assessment of hypovirulent isolates of *Cryphonectria parasitica* for potential in biological control of chestnut blight. Canadian Journal of Plant Pathology, 19: 69–77.

Milgroom MG (2004) Biological control of chestnut blight with hypovirulence: a critical analysis. Annual Review of Phytopathology, 42: 311–338.

Monteiro-Vitorello CB, Bell JA, Fulbright DW and Bertrand H (1995) A cytoplasmically-transmissible hypovirulence phenotype associated with mitochondrial DNA mutations in the chestnut blight fungus *Cryphonectria parasitica*. Proceedings of National Academy Science of the United Sates of America, 92: 5935–5939.

Nuss DL (1996) Using hypoviruses to probe and perturb signal transduction processes underlying fungal pathogenesis. The Plant Cell, 8: 1845–1853.

Rogers HJ, Buck KW and Brasier CM (1986). The d^2-factor in *Ophistoma ulmi*: expression and latency. In: Bailey J (ed) Biology and Molecular Biology of Plant-Pathogen Interactions (pp. 393–400) Springer-Verlag, Berlin.

Russell RC (1939) Pathogenicity tests with cultures of *Ophiobolus graminis* Sacc. Scientific Agriculture, 19: 662–669.

Sanger F, Nicklen S and Coulson AR (1987) DNA sequencing with chain-termination inhibitors. Proceedings of National Academy Science of the United Sates of America, 74: 6463–5467.

Still PE, Detroy RW and Hesseltine CW (1975) *Penicillium stoloniferum* virus: altered replication in ultraviolet derived mutants. Journal of General Virology, 27: 275–281.

Tavantzis SM, Lakshman DK and Liu C (2002). Double-stranded RNA elements modulating virulence in *Rhizoctonia solani*. In: Tavantzis SM (ed) dsRNA Genetic Elements: Concepts and Applications in Agriculture, Forestry, and Medicine (pp. 191–211) CRC Press, Boca Raton.

Thompson JD, Gibson TJJ, Plewniak F, Jeanmougin F and Higgins DG (1997) The ClustalX windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. Nucleic Acids Research., 24: 4876–4882.

Wesolowski M and Wickner RB (1984) Two new double-stranded RNA molecules showing non-Mendelian inheritance and heat inducibility in *S cerevisiae*. Molecular and Cellular Biology, 4: 181–187.

Wickner RB (1996) Double-stranded RNA viruses of *Saccharomyces cerevisiae*. Microbiological Review, 60: 250–256.

Wickner RB, Esteban R and Hillman BI (2000). Family *Narnaviridae*. In: Van Regenmortel MHV, Fauquet CM, Bishop DHL, Carstens EB, Estes MK, Lemon SM, Maniloff J, Mayo MA, McGeoch DJ, Pringle CR and Wichner RB (ed) Virus Taxonomy: Seventh Report of the International Committee on Taxonomy of Viruses (pp. 651–656) Academic Press, London.

Zhou T and Boland GJ (1997) Hypovirulence and double-stranded RNA in *Sclerotinia homoeocarpa*. Phytopathology, 87: 147–153.

Zhou T and Boland GJ (1998) Suppression of dollar spot by hypovirulent isolates of *Sclerotinia homoeocarpa*. Phytopathology, 88: 788–794.