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Mycoviruses an

Although it is less than 20 years since viruses that infect fungi were first described (8), mycoviruses, as they have come to be called, are now known to be widespread in all the major taxonomic groups of fungi. Mycoviruses show several different aspects of particular biological interest. The literature about them is voluminous and has been reviewed so often in recent years (2,4,7,9,11,15,16) that any general account can hardly avoid repetition. Accordingly, I will only briefly mention these well-publicized aspects of mycoviruses and instead concentrate attention on comparing mycoviruses with viruses from other host taxa and on some of the apparent paradoxes that have arisen from recent findings.

So far, mycoviruses have been found in well over 100 fungal species. Independent estimates made in two laboratories, however, from examination of random samples of different fungi indicated that some 10–20% of samples were infected, suggesting that at least 5,000 species of fungi may contain mycoviruses. Viruses are thus as prevalent in fungi as they are in vertebrates and flowering plants (Table 1), although the cynic may well comment that this merely reflects the relative research effort devoted to each group. It is of interest that in some fungal genera, such as *Penicillium* and *Aspergillus*, most of the species examined have been virus-infected, whereas in other fungal genera viruses have not been found so far.

Table 1. Prevalence of viruses in different host phyla

Phylum	Number of species known to be infected
Prokaryotes	
Bacteria	Over 100
Blue-green algae	1–10
Eukaryotes	
Fungi	Over 100
Algae	1–10
Pteridophytes	1–10
Gymnosperms	1–10
Angiosperms	100–1,000
Protozoa	1–10
Nematodes	?1–10
Arthropods	Over 100
Molluscs	1–10
Vertebrates	100–1,000

The Kinds of Viruses in Fungi

What kinds of viruses infect fungi? And how do they compare with viruses of other host taxa? Table 2 gives some idea of the different genome types of viruses in various host phyla. We must remember that very few mycoviruses have so far been adequately characterized, and most are known only as viruslike particles (VLP) in electron micrographs of partially purified extracts from the fungus or, sometimes, from thin-section studies.

Several different morphological types of particle have been found, some corresponding fairly closely with well-known viruses of other host taxa. Thus, rod-shaped particles of tobamovirus type have been noted in *Peziza ostracoderma*, filamentous particles of potexvirus type in *Boletus edulis*, isometric particles of herpesvirus type in *Thraustochytrium* sp. and *Phytophthora infestans*, and bacilliform particles of alfalfa mosaic type in *Agaricus bisporus*. Rod-shaped particles of uncertain affinity have been reported in *Lentinus edodes*, *Mycogone perniciosa*, and *Armillaria mellea*. Some isometric particles that are 105–110 nm in diameter superficially resemble iridoviruses, and some that are 50 nm in diameter resemble caulimoviruses. Tailed DNA-phage particles and paired 20-nm isometric particles of possible geminivirus type have also been reported. The great majority of particles so far recorded in fungi, however, have been isometric, with a genome of several species of dsRNA; a few have undivided genomes.

In other properties these mycoviruses are a very heterogeneous group, with diameters ranging from 25 to 45 nm and with particle weights ranging from 6 to 13 $\times 10^6$ daltons. They have 1–8 segments of dsRNA, with total molecular weights of 2–8.5 $\times 10^6$. Nearly all those examined have had only a single capsid protein, but molecular weights have ranged from 25 to 130×10^3 in the different viruses. Reports of a few mycoviruses with more than one capsid polypeptide merit caution, for proteolytic degradation might be responsible; this occurs surprisingly rapidly in purified preparations of many potyviruses and other viruses of higher plants, as well as in AfV-S of *Aspergillus foetidus* (4).

Mycoviruses pose a frustrating taxonomic problem. No serological relationships have been detected between any mycovirus and morphologically similar viruses in higher plants, and no mycovirus has been shown to infect a higher plant. The isometric dsRNA mycoviruses have no obvious taxonomic affinity with dsRNA viruses of other host taxa. There is no evidence to suggest any taxonomic unity among viruses with a dsRNA genome, any more than the possession of the highly successful ssRNA genome indicates any affinities among the diverse groups of isometric viruses of higher plants or vertebrates. It could equally well be that dsRNA mycoviruses have evolved on more than one occasion and that the dsRNA genome reflects adaptation to conditions within the fungal cell.

For these reasons, we members of the Fungus Virus Sub-committee of the International Committee for Virus Taxonomy resisted the temptation to set up a large taxonomic group to accommodate all dsRNA mycoviruses. Experience with viruses of other hosts has shown the advantages of approaching the problem from the bottom, by creating small groups of viruses that clearly share most properties in vivo and in vitro. Two such groups have now been designated: the *Penicillium chrysogenum* virus group and the *P. stoloniferum* virus S (PsV-S) group (10). Member viruses within each of these groups are serologically related. Far more characterization data are essential, however, before meaningful taxonomic groupings can be considered. In addition to "minimum essential information" (10), the replication strategies would be especially valuable.

Replication Strategies

The major obstacle to knowledge of the replication strategies of the dsRNA mycoviruses is the lack of a technique to obtain experimental infection of a healthy fungus cell by a cellfree virus preparation; this problem has been reviewed by Lecoq and colleagues (14). Early reports of the infection of fungal protoplasts with a range of purified mycoviruses have not been confirmed and need reevaluation. Protoplasts regenerated from virus-infected fungal mycelia have given rise to cultures that were still infected, however, suggesting that the problem may lie in the initial establishment stages of the infection rath-

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er than in subsequent virus replication.

It is widely believed that dsRNA seems unable to function as a messenger RNA (mRNA) in the host cell and may indeed sometimes inhibit protein synthesis. Host-cell enzymes able to transcribe ssRNA and dsRNA in vitro—and probably dsRNA in vivo, although not efficiently—have been reported from various plants and animals (4). But for effective in vivo transcription and replication of dsRNA, highly specific, virus-coded RNA polymerases are necessary, sometimes with additional host polypeptides. Such polymerases have been demonstrated in a number of the dsRNA mycoviruses. The polymerase may possibly remain confined within the virus particle during the replicative cycle of the mycovirus (4), as it does in the dsRNA reoviruses of vertebrates and bacteriophage $\phi 6$. Some purification procedures used for mycoviruses have resulted in the loss of the polymerase activity—one obvious possible reason for the failure to infect fungus cells or protoplasts with purified virus preparations.

Because of these limitations, little is known of the various in vivo stages of the replicative cycle, although surprising progress has been made as a result of some elegant in vitro translation studies. Already it is apparent that different strategies may occur among the various mycoviruses.

With the undivided genome of virus L of *Saccharomyces cerevisiae*, two types of RNA polymerase activity have been detected: ds→ssRNA (transcriptase) and ss→dsRNA. These activities would permit L dsRNA to replicate asynchronously, as occurs in reovirus, although there are several differences between the two systems.

In AfV-S, transcriptase activity has been found and replication is thought to involve a semiconservative strand displacement (3,4), thus differing from the fully conservative reovirus system. In contrast again, PsV-S shows in vitro replicase activity, giving rise to dsRNA progeny molecules that remain encapsidated. Such semiconservative replication and strand displacement, similar to that found with adenovirus DNA, would suggest a synchronous system. However, "diploid" virions have not been detected in vivo and might be merely an artifact of the in vitro conditions. In vivo studies suggest that replication of PsV-S dsRNA may in fact be asynchronous. Comprehensive reviews of the present position have been given by Buck (3,4).

Biological Effects

Uncertainties surrounding the taxonomy and replication of mycoviruses are paralleled by doubts concerning their precise biological significance. Ecologically, mycoviruses are exceptional, possibly unique among viruses, for their existence appears to be entirely intracellular—a life-style for which they are well fitted. Transmission is by hyphal anastomosis, often (although not always) with accompanying heterokaryosis, and dispersal occurs through certain kinds of spores. This contrasts sharply with the situation in other host taxa (Table 3), in which the viruses enjoy several different methods of spread, most involving extracellular routes.

As might be expected, mycoviruses and VLP are particularly prevalent in fungi that are propagated vegetatively or through asexual spores. They are abundant in industrial fungi, and possible effects in those used in antibiotic production were an obvious line of investigation. Partly because of understandable reticence among the pharmaceutical organizations involved but also because specific genetic lines of the fungi concerned could not be infected with cellfree preparations of the different viruses, critical data on controlled comparisons have been few. Circumstantial evidence is consistent, however. All tested races of *P. chrysogenum* produced penicillin, whether or not they were virus-infected. Virus was present in *P. notatum*, believed to derive from the original Fleming isolate, but apparently absent from other cultures that also produced penicillin.

Similarly, the production of mycotoxins, such as aflatoxin, shows no consistent correlation with the presence or absence of viruses (5). Several other aspects of the biological effects of mycoviruses have received more extensive study.

Mycoviruses and Interferon

During the 1960s, there was energetic study of the antiviral and interferon-inducing activities of culture filtrates and cellfree extracts of certain isolates of *P.*

Table 2. Number of viruses, according to genome type, in different host taxa

	Virus genome			
Phylum	**ssDNA**	**dsDNA**	**ssRNA**	**dsRNA**
Vertebrates	23	202	440	86
Invertebrates	6	143	30	12
Flowering plants	9	7	371	9
Bacteria	47	168	27	1
Fungi	?	?2	1	25+[a]

[a] Probably a gross underestimate.

Table 3. Transmission of viruses in different host taxa

	Virus transmitted through:					
Host taxon	**Contact**	**Ingestion**	**Aerosol**	**Arthropod**	**Tissue union**	**Congenital means[a]**
Vertebrates	+	+	+	+	+	+
Invertebrates	+	+	+	(+)	?	+
Higher plants	+	?+[b]	−	+	+	+
Bacteria	+	+	−	−	+	+
Fungi	−	−	−	−	+	+

[a] Includes infection of offspring through seeds, spores, gametes, or cellular fission.
[b] By uptake through roots, eg, tobacco mosaic virus, cymbidium ringspot virus.

stoloniferum and *P. funiculosum*. The detection of virus particles in these isolates and the demonstration of the viral dsRNA as the inducer of interferon when injected into animals led to massive funding of mycovirus research, particularly by the large pharmaceutical companies. VLP were reported from many fungal species, and a number were shown to contain dsRNA and to induce interferon production. The prospects for clinical and veterinary use looked bright, and a number of preparative processes for industrial production of the mycovirus extracts were patented.

Although some successes in suppressing virus infections, such as the common cold, in human volunteers were reported, it was soon evident that there were side effects too serious to be clinically acceptable. The synthetic ribonucleotides such as poly (I):poly (C), which also induced interferon production, had equally unpleasant consequences. It is still unclear whether these effects derive from toxicity of breakdown products of the injected materials or are an integral part of the interferon response. What was clear was that this line of research had little future application, and funding ceased almost as rapidly as it had begun. Perhaps the principal legacy from the brief flowering period of this aspect of mycovirus research (well reviewed by Kleinschmidt [12]), and certainly the one of most interest to plant pathologists, has been the large number of viruses and VLP reported from all manner of fungi.

The Killer Phenomenon

Among the most intensively studied aspects of mycoviruses in recent years has been the connection between the production of "killer toxin" and mycovirus dsRNA segments. Certain strains of brewer's yeast (*S. cerevisiae*) and of corn smut fungus (*Ustilago maydis*) secrete extracellular toxins that kill or suppress growth of sensitive races of the same or related fungus species, but each killer strain is immune to its own toxin. Most sensitive races of *S. cerevisiae* contain virus particles about 40 nm in diameter with a single dsRNA genome species, designated L, of molecular weight variously given as about 2.4, 2.7–3.0, or, most recently, 3.4×10^6. These discrepancies may in part derive from the nonlinear migration of larger molecular weight dsRNA species in polyacrylamide gel electrophoresis (PAGE). Killer strains also contain a dsRNA component of molecular weight about $1.1–1.4 \times 10^6$, designated M. L and M dsRNAs are separately encapsidated in morphologically identical virions, whose coat proteins are apparently indistinguishable in PAGE or in serological tests.

M dsRNA encodes the rather labile glycoprotein killer toxin (confirmed by in vitro translation) and is believed also to determine the immunity factor. M has been found only in cells containing L dsRNA and may be a satellite of L. L is thought to encode both the RNA polymerase and the coat protein for L and M RNAs.

Certain yeast strains contain a third dsRNA segment, designated S for killer suppressor, of molecular weight 0.5×10^6; strains containing L, M, and S lack killer toxin, and S has been regarded as somewhat similar to small interfering particles seen with some animal viruses. Neutral strains of yeast produce no toxin but are immune to it. Superkillers and sensitive toxin producers, which are potentially self-destructive, are also known. Recently, yeast killer toxins of different specificities and produced by different isolates of the fungus have been recorded. A number of nuclear genes are required to maintain M dsRNA and, therefore ultimately, L dsRNA, but cytoplasmically inherited factors are also required. The possibility has also been raised that M dsRNA may exist as a provirus, encoded by the host nuclear gene.

Since the killer phenomenon was first recognized almost 20 years ago, the complexity of the interaction between the two viral dsRNAs (sometimes regarded by geneticists as plasmids), host nuclear genes and cytoplasmic factors, has become increasingly apparent, as indicated in the thorough review by Vodkin and Alianell (20).

The situation in *U. maydis* is equally complex (13). Three killer specificities are known, each killer type resisting its own killer toxin but being sensitive to the other two. Specific dsRNA species are associated with the three killer phenotypes, and the proteinaceous toxins differ from one another serologically and in PAGE. *Ustilago* isolates with different killer specificities may contain 5–7 dsRNA segments, and attempts to combine two different specificities in a single strain of *U. maydis* have led to mutual exclusion to give nonkiller progeny or to unilateral exclusion, with all progeny of one specificity or the other. The similarity of these reactions to the premunity phenomenon with viruses of bacteria and higher plants is food for thought.

These studies are of fundamental biological importance for several reasons. They indicate the suitability of such mycovirus-fungus systems for studying the interactions of host and virus genetic information. They may represent examples of a system that is more widespread than presently realized in fungi and whose ecological significance has only been surmised. It has even been suggested that the dsRNA (or a DNA copy) for the *U. maydis* toxin production might be transferred by genetic manipulation to the corn plant itself. There are, of course, serious doubts as to the feasibility or the practical value of such an approach. These studies have also indicated certain similarities between fungal killer toxins and bacteriocins, which are encoded by plasmids. But perhaps most of all, they sound a warning that any assessment of the effects of a mycovirus on its fungus host must consider the activities of the specific dsRNA segments that are present. The apparent lack of any consistent correlation between the production of mycotoxins and the presence or absence of a particular mycovirus may be worth reexamination on the basis of the individual dsRNA segments that are involved.

Occurrence of Mycoviruses in Plant-Pathogenic Fungi

Isolates of diminished virulence have been reported with many plant-pathogenic fungi, and the possibility that such hypovirulence, or even the death of the fungus, might be induced by mycoviruses was an attractive idea. First reports were encouraging. Two weakly pathogenic isolates of the take-all fungus of cereals (*Gaeumannomyces graminis*) contained isometric virus particles about 29 nm in diameter, whereas five strongly pathogenic isolates were apparently virus-free. Virus-infected cultures grew poorly and were unstable in culture, with poor sporulation. Further tests in another laboratory with 300 isolates of *G. graminis*, however, indicated a more complex situation. At least two viruses (27 and 35 nm in diameter) were present, but no direct evidence was found that virus infection impaired pathogenicity, enzyme production, or saprophytic survival of the fungus or that it had any effect on the tolerance to benomyl. Nevertheless, fungus isolates containing only one kind of virus particle were mostly more pathogenic than were virus-free isolates, whereas isolates with both kinds of virus particles tended to be less pathogenic than either of the other two classes.

More recent reports have shown that a number of different serological groups of mycoviruses occur in various combinations in different isolates of *G. graminis* and that within any serotype there may be considerable differences in the number and molecular weights of the dsRNA segments present. Recent findings by Férault and colleagues in France are also of interest. A highly pathogenic isolate of *G. graminis* from wheat roots gradually lost virulence over a period of 17 months in culture. At first, no virus particles could be detected but as the culture was periodically retested during the subsequent months, 35-nm virions and later also 26-nm particles were observed in increasing quantities, paralleling the decrease in pathogenicity of the fungus during this time.

A possibly similar phenomenon occurred in our laboratory with a 42-nm diameter virus in *M. perniciosa*, the cause of wet-bubble disease of the cultivated mushroom. When virus-infected mycelium was repeatedly subcultured on synthetic media, the virus titer progressively decreased and the mycelial growth rate increased. When the fungus was inoculated to healthy mushrooms or grown on mushroom extract agar, the virus concentration increased while the mycelial growth rate diminished. This apparently reversible process suggests a connection between increasing virus multiplication and loss of vigor and pathogenicity in the fungus. In *Pyricularia oryzae*, the rice blast fungus, experimental virus infection was obtained by fusing infected with healthy protoplasts, but the

resulting mycelial cultures showed no morphological differences, although sporulation was reduced.

The factor causing decreased pathogenicity of *Helminthosporium victoriae*, the cause of victoria blight in oats, could be transferred from affected to normal cultures of the fungus by hyphal anastomosis. Mycelial cultures showed abnormal, stunted growth on agar. Recent work has shown that two serologically unrelated mycoviruses, designated 190 *S* and 145 *S* from their sedimentation values, occur in hypovirulent cultures. Normal, virulent cultures either contain the 190 *S* virus only or are apparently virus-free. By fusing protoplasts treated with partially purified preparations of both viruses with normal protoplasts, hypovirulence was transferred to a normal culture, although the rate of infection was very low (7).

Mycoviruses have now been reported from over 40 plant-pathogenic fungi, but there has been no consistent correlation with hypovirulence. Indeed, several virus-infected isolates of *H. maydis* from six countries were much more pathogenic than virus-free isolates from three other countries. Three different virus types were suspected.

In all these comparisons, however, only available isolates of the fungi could be examined. Controlled inoculations of specific fungus races with purified preparations of individual defined viruses were not possible.

Examples of transmissible hypovirulence have been reported in which no conventional virions have been detected. A degenerative decline in certain cultures of *Rhizoctonia solani* has been associated with an assortment of dsRNA segments of several different sizes; in one isolate, these were of molecular weights 2.2, 1.5, and 1.1×10^6. The decline could be transferred by hyphal anastomosis, but so far no virus particles have been observed. Some apparently normal cultures contained the three dsRNA segments in very low concentration, and once again there is the possibility of a quantitative connection between virus multiplication and the degree of metabolic disturbance in the host. Another suggestion has been that a closed circular DNA plasmid may be involved.

There is strong circumstantial evidence that the cytoplasmically transmissible hypovirulence in the chestnut blight fungus, *Endothia parasitica*, is correlated with the presence of dsRNA segments and, in at least one strain, with unusual club-shaped particles up to 300 nm long (6). Experimental inoculations of diseased chestnut trees with mycelial cultures of hypovirulent strains of appropriate compatibility have resulted in the regression of cankers and even the apparent displacement of the virulent form of the fungus from the treated trees. It is of interest that the hypovirulent form seems to have spread in Italy during recent years, so that chestnut blight is now much less serious a problem there than formerly. It may be that some natural vector is involved.

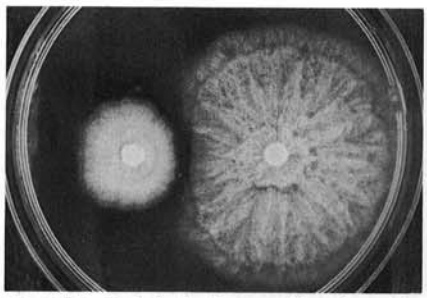

Fig. 1. The Dutch elm fungus (*Ceratocystis ulmi*) is an example of nontransmissible hypovirulence. Note the antagonism between mycelial cultures of the fast-growing aggressive W2 race (right) and the slow-growing hypovirulent W7 race.

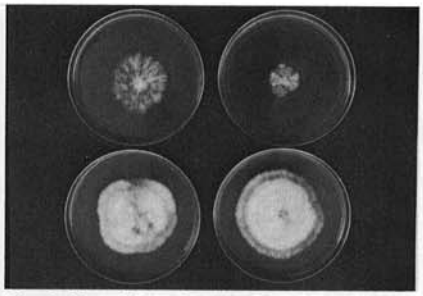

Fig. 2. Mycelial cultures on malt agar from mushroom after 3 weeks at 25 C. The three cultures from virus-infected sporophores show the range of growth rates inversely correlated with the virus concentrations in the relevant sporophores. The least virulent isolate is barely different from the virus-free control culture.

This might seem to offer an ideal system for biological control of chestnut blight disease, but there are practical problems. Vegetative incompatibility between a number of virulent and hypovirulent races of the fungus has been recorded, so that extensive typing might be required before treatment could begin. Because of the restricted invasiveness of the hypovirulent isolates, topical inoculation to individual cankers may be needed—a costly procedure on a commercial forestry scale. There would also seem to be no mechanism for long-term protection against future infection with a virulent race; moreover, the ascospores from hypovirulent isolates do not contain the dsRNA and so give rise to aggressively pathogenic mycelia.

Some of these difficulties apply to all attempts to use possible virus-induced hypovirulence for biological control, but the development of a simple and reliable technique for inoculating virulent fungi with cellfree preparations of the appropriate dsRNA mycoviruses could radically change the situation.

There remain yet other instances of hypovirulence that are apparently not transmissible and in which neither VLP nor specific dsRNA segments have so far been identified. The Dutch elm fungus (*Ceratocystis ulmi*) is an example (Fig. 1). In Europe, the introduced virulent races, which show more vigorous mycelial growth on agar and much greater pathogenicity, have displaced the milder local races, with disastrous consequences for the elm trees. Hypovirulence in these instances appears to be determined solely by the fungus genotype and seems unlikely to be of any value for biological control.

Viruses in Cultivated Mushroom

Virus diseases of the cultivated mushroom (*A. bisporus*) have been recorded from nearly all countries where this crop is widely grown. Detailed accounts of the symptoms, means of spread, and control measures have been extensively reported (7–9,11,15,18,19). The great variations in symptoms, resulting particularly from differences in cultural conditions and in how early the infection enters the crop, gave rise to much early confusion, especially in nomenclature.

At least six viruses and viruslike agents have been reported and extensively studied, but they remain among the most difficult viruses to handle. They nearly always occur in mixtures of two or more and are extremely hard to separate. It has not been possible to consistently obtain high yields of virus, and there is no simple, reliable inoculation technique for transferring cellfree virus preparations. We still do not know what effects the individual viruses cause. With many industrial fungi, high virus yields are readily obtained with shaken-flask cultures of infected mycelium, but this approach has failed with cultivated mushroom; mycelial growth has been accompanied by a steadily decreasing virus titer despite a variety of culture media and conditions.

The evidence that mycoviruses caused serious disease in mushrooms was consistent and results were confirmed in many laboratories. The reported correlation (11) among the concentration of virus particles in the sporophores, the reduction in crop yield, and the decrease in mycelial growth on malt agar of cultures taken from the sporophores (Fig. 2) has been confirmed in some laboratories but disputed by others. There are certainly some anomalous results, and in recent years there have been many reports of normal yields and mycelial growth from virus-infected mushroom crops, with the suggestion sometimes made that perhaps mushroom viruses are not pathogenic after all. There have also been reports that viruses have been present in every sample of mushrooms or spawn culture examined, although these findings conflict with the findings in

a number of laboratories, including our own.

There is, of course, nothing new in latent virus infections in mushrooms. We spent much time examining ways by which severely diseased mycelial cultures could be converted into hypovirulent or apparently symptomless cultures, through partial thermotherapy, selective growth media, and other methods (9,11). In retrospect, we were probably misled by the often dramatic effects of environmental changes on disease severity in the crop into concentrating on the physiological responses of the host to these alterations. The geneticists' approach in studying the killer phenomenon in yeast and corn smut has shown the vital role of individual dsRNA segments within the virus genome and the complex interactions with the host genetic systems.

There are many possible reasons for apparently conflicting reports. First, virus mixtures are common in fungi, with a number of examples of two morphologically indistinguishable viruses occurring together, sometimes in the same cell. All virions of 25-nm diameter may thus not be MV-1 nor those of 35-nm diameter, MV-4. Serologically specific electron microscopy offers greatly increased sensitivity and specificity, especially with the "decoration" method, for detecting such mixtures. Using another approach, however, in limited tests we have so far not detected capsid polypeptides other than those of MV-1 (24,400 mol wt) or MV-4 (63,800 mol wt) by PAGE of purified extracts from infected mushroom samples. Because almost all the dsRNA mycoviruses so far characterized have contained a single coat protein, this approach would seem to offer a fairly simple and sensitive means of screening samples for virus mixtures within a single particle size class.

Second, within one specific virus, variants may exist that contain additional—or fewer—dsRNA segments that are associated with pathogenicity. Parallel situations have been reported in viruses of flowering plants, eg, the RNA 5 segment of cucumber mosaic and the "nettlehead RNA" of arabis mosaic virus in hops. There are some indications of considerable variation in the number and size of dsRNA segments in different mushroom virus isolates, and the wholly intracellular way of life of mycoviruses probably encourages the persistence of such variants. There could be complementation between different dsRNA species from different isolates, whereby their combined presence resulted in disease. These avenues of investigation merit much more study.

Third, the genotypes of cultivated mushroom grown today appear to differ considerably from those of 20 years ago, as indicated by incompatibility responses. Observation suggests that present-day genotypes are much more tolerant of infection with MV-1, MV-3, and MV-4 than were former races. Such tolerance might operate through the suppression of virus replication; several examples of suppression have been seen in killer fungi (13,20) and through the production of patulin in several species of *Penicillium* and *Aspergillus* (5).

Fourth, there is the possibility that one or more mushroom viruses may exist as DNA proviruses that, under certain conditions, can initiate rapid virus replication and associated disease.

A complicating factor of particular interest has been the occurrence of club-shaped particles (Fig. 3), very similar to those in *E. parasitica*, associated with a severe disease of cultivated mushrooms in Europe (1,17) and South Africa.

Conclusions

It is often stated that fungus viruses are typically latent. Evolutionary pressures would greatly favor this trend, unless counterbalanced by major biological advantages associated with disease. This

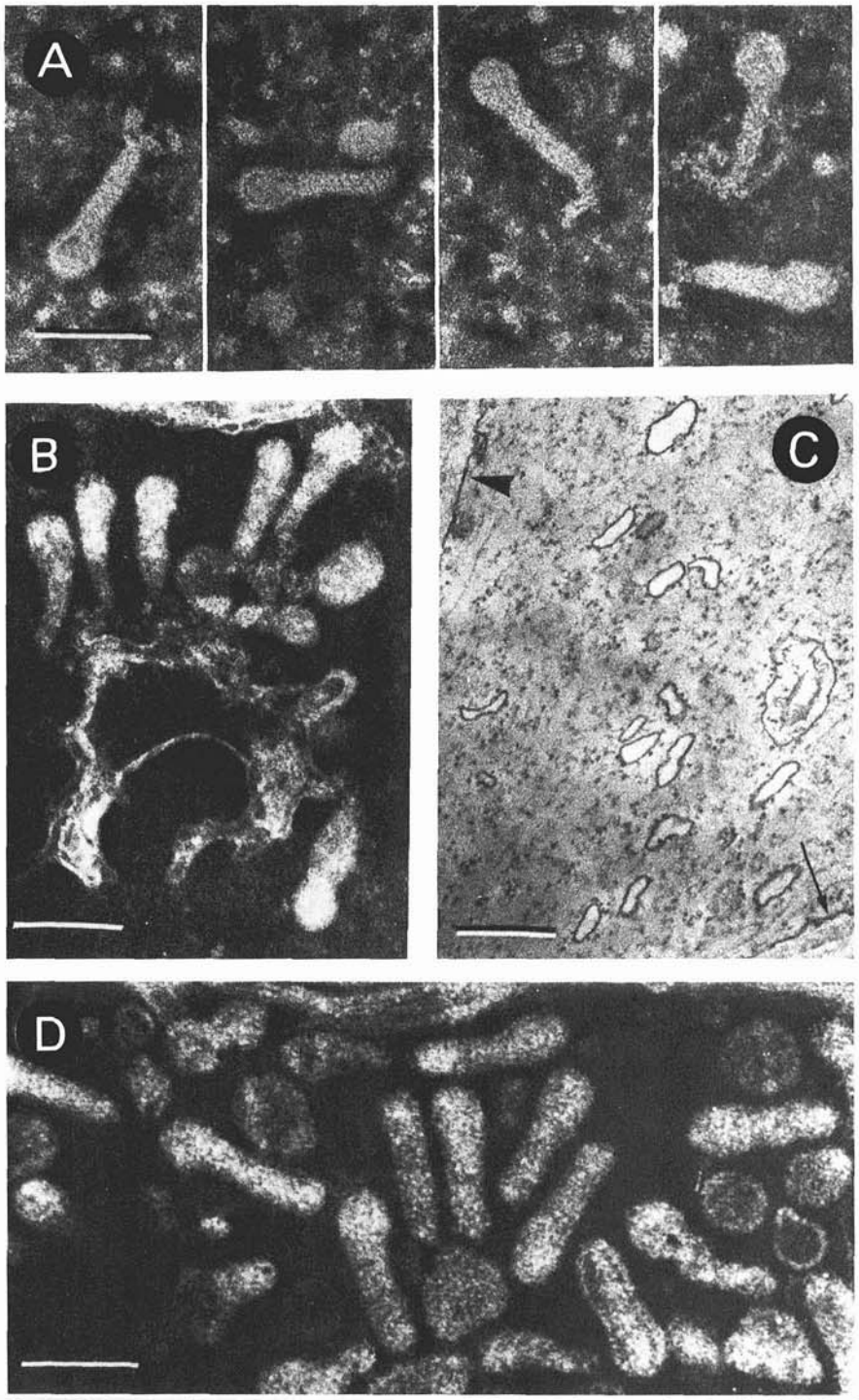

Fig. 3. Club-shaped particles from mushroom: (A) Particles from crude extract of affected sporophore. (B) Particles associated with membrane fragment. (C) Thin section of infected cell, showing several vesicles; arrows indicate cell wall. (D) Partially purified preparation. Scale bars represent 100 nm in A, B, and D and 500 nm in C.

would be hard to envisage. Even the report that spores from virus-infected mushrooms germinate better and more rapidly than those from virus-free sporophores could hardly compensate for loss of sporophore production in diseased mushroom crops. It has also been suggested that killer systems restrict outcrossing and give the killer strains selective advantages over neutral or sensitive races. But in yeast, killer toxin is active only at a small range of pH values around 4.8, whereas in *U. maydis* it is hard to see how any advantage can occur in view of the mating requirements of this fungus.

On the one hand, latency has been inferred from the absence of obvious disease or abnormality in comparison with available races or genotypes of the same species. On the other hand, there are examples of conditional lysis or plaque formation associated with mycovirus infection, and thin-section studies in a wide variety of fungal species have revealed cytopathic effects (1).

We should keep an open mind until the critical infectivity tests can be achieved. There are many parallels in viruses of other hosts. Many chronic virus infections in higher plants cause insidious loss of vigor and crop without evident foliage symptoms. In vertebrates, long periods of latency may be interspersed with phases of acute disease, as in the "cold sores" of herpes simplex virus. Chicken pox virus can remain latent in the dorsal root ganglia of a patient, to resurge many years later as shingles (herpes zoster). A capacity for latency under some conditions or for much of the time would greatly reduce the selection presure against a virus.

Amid the sometimes conflicting evidence, there seems to be emerging what may be a general pattern in mycoviruses, whereby pathogenicity depends on the rate of virus replication, which may itself be mediated by specific dsRNA segments.

There is a paramount need for a simple and reliable inoculation technique for mycoviruses. The bald assertion that they are noninfective is premature. Mycoviruses appear to be highly host-specific, yet reports 10–15 years ago that viruses as diverse as tobacco mosaic, poliomyelitis, polyoma, and endomyocarditis have been induced to infect and multiply in yeast cells have not been confirmed or refuted. It would seem well worthwhile to explore yeast cells and protoplasts as a possible equivalent for mycoviruses to the embryonated hens' eggs and BHK (baby hamster kidney) cells for vertebrate viruses or to *Chenopodium quinoa* and *Nicotiana clevelandii* for plant viruses. Cytoplasmic fractions within infected fungus cells may be required to initiate or maintain mycovirus replication.

All these aspects are of some concern to the plant pathologist. A greater understanding of the underlying mechanisms that regulate mycovirus replication may well explain today's apparent contradictions and paradoxes and may even lead to new virological concepts applicable also to pathogenic viruses of higher plants and vertebrates.

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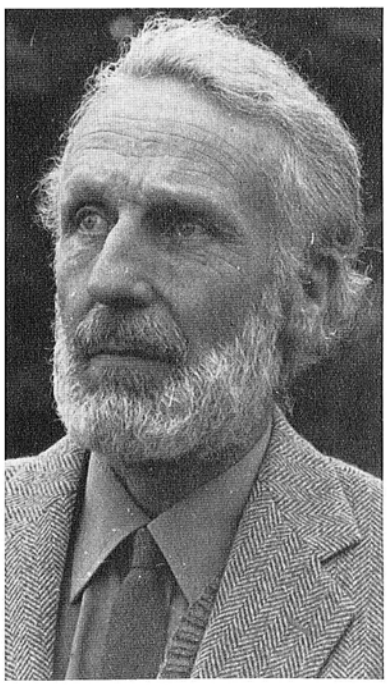

Michael Hollings

Dr. Hollings retired at the end of 1980 from the Glasshouse Crops Research Institute, Littlehampton, England, where he had been head of the Virology Department since May 1959. A graduate of the University of Nottingham, from which he also obtained his doctorate, he received his training in plant virology at Rothamsted Experimental Station. After working for several years on the epidemiology of viruses in seed-potato growing areas in England and Wales, he turned to viruses in glasshouse vegetable and flower crops. This work led to the production of virus-free foundation clones of carnations, chrysanthemums, pelargoniums, lilies, and narcissus and the establishment of nuclear stocks associations for these crops. After publishing the first description of viruses in fungi in 1962, he continued work on viruses in mushroom and other fungi. He intends to concentrate now on ecological research, especially the effects of habitat changes on local bird populations.