III. VIRAL DISEASES

a) Button Mushroom

INTRODUCTION

In recent years, viruses have increasingly been found in association with fungi, an association that has taken one of the two forms. In the first, the fungus is the vector of the virus and in the second, fungus is the host of the virus. Here only the second form of association i.e. fungi, especially the mushrooms, as hosts of viruses will be considered in detail which has been reviewed earlier by Raychaudhury (1978), Sharma (1991) and Sharma and Kumar (2000). Although the presence of viruses in fungi has long been suspected (Sinden and Hauser, 1950) experimental evidence was not forthcoming until 1962 when virus particles were demonstrated in diseased mushroom (Gandy and Hollings, 1962; Hollings, 1962). To date viruses or virus-like particles (VLPs) have been reported to occur in over 100 species from 73 genera of fungi, but only a small number of them have been isolated and characterised. Several terms have been used for the viruses of fungi including mycoviruses, fungal viruses, mycophages, double stranded RNA (dsRNA) plasmids and virus-like particles (VLPs). The term mycophage is clearly unsuitable since virus infection has very rarely been associated with lysis in fungi. Although mycoviruses may share some of the characteristics of plasmids, their morphology, nucleoprotein composition and the possession of virion-associated RNA polymerase activity are consistent with a viral nature. The term plasmid has already been abused in current literature as pointed out by Reanney (1976) and to denote the viruses of fungi as plasmids would not find ready acceptance. The term VLPs and mycoviruses have been used by some authors (Bozarth, 1972; Saksena and Lemke, 1978), with the understanding that the first term applies to those particles occurring in fungi and having a virus-like appearance in electron-micrographs but which have not been isolated and characterised, whereas the second term denotes those which have been isolated and shown to have the morphology and nucleoprotein composition generally

attributed to viruses. This distinction offers an operational convenience and has been widely adopted. Since mycoviruses have not conclusively proven to be infectitious as purified particles, some workers prefer to apply the term VLPs in all cases.

HISTORY AND GEOGRAPHICAL DISTRIBUTION

In 1948 a very serious infectitious disease of white button mushroom (*Agaricus bisporus* (Lange) Sing.) was observed in the United States of America on a farm in Pennsylvania run by the La France brothers, and thus became known as La France disease (Sinden and Hauser, 1950). In England, a disease inducing brown staining on the stipe was named as 'brown disease' by Storey (1958). Gandy (1958) observed the most common symptom in the form of large water logged patches on stipes of mushroom from diseased beds and proposed the name 'watery stipe'. A similar, possibly the same disease was observed in the mushroom industry throughout Pennsylvania. The cause of this disease was unknown and no existing description appeared to fit the disorder, hence the name 'X-disease' was coined by Kneebone and co-workers (1961). Since 1959 a similar disease 'mushroom-die-back' has been studied in England wherein degeneration of mycelium rather than the symptoms on fruit bodies were more predominent and has been attributed to a complex of atleast three different viruses (Gandy and Hollings, 1962). Schisler and co-workers (1967), reported that one of these viruses had been isolated from a white isolate of La France. They advocated the name X-disease and die-back be dropped. In the Netherlands, disorders of this type were not reported until 1964 when a heavy outbreak occurred causing significant yield losses (Dieleman Van Zaayen and Temmink, 1968). In Austrailia, mushroom diseases of viral nature probably dated back to the early days of mushroom growing in open ridge and disused railway tunnels in the 1930s but confirmation of existence of die-back was reported in 1968 (Paterson, 1968). Virus disease in button mushroom has been reported from India by Tewari and Singh (1984; 1985) and have also been described from New Zealand, Polland, German Democratic Republic, China, Denmark, Sweden and Canada. More recently La France disease has also been reported from Spain.

Viral particles have been reported, recently in *Pleurotus ostreatus* and *P.sapidus* from China and in *P.pulmonarius*, *P.ostreatus* and *P.columbinus* from France (Table-2). A double stranded RNA polyhedral virus has also been reported in *Volvariella volvacea* from China, Virions of different shapes and sizes have been detected in *Lentinus edodes* from Japan and USA. In India, virus and virus like disease have been on button mushroom (Tewari and Singh, 1984; 1985; Gottapeh and Kapoor, 1990) and oyster mushroom (Krishna Reddy *et al*. 1993). General symptoms, transmission and control

Table -2: Virus and VLPS reported in different mushrooms

S. No.	Host/Disease	Shape	Size	Country
I	*Agaricus bisporus* La France, Watery stipe, X-Disease, Die-back, mushroom disease	Spherical	25nm 29nm 35nm 40-50nm	Australia, England, Holland, America, France GDR, India
		Bacilliform	18x50nm	U.K.
		Club shaped	60-70nm dia or 120-170 long with a spherical body of 40-50 nm & a cylindried tail 20-30nm in dia	France, W Germany S. Africa
		Rods of varing longth	19x9-90nm 19x35nm 20x130nm	Poland GDR China
II	*Pleurotus* spp.			
	P. colombinus	Spherical	26+ 2nm	France
	P. ostreatus			India
	P. pulmonarius	Spherical	24nm	China
	P. sapidus			China
	P. florida	Flexuousrods	40-600nmlong	
III	*Volvariella volvacea*	Spherical	35nm	China
IV	*L.edodes*	Spherical	20nm, 23nm 36nm, 45nm 30nm	China, Japan
		Stiff or	17x200x1200nm 15x700-900nm 18x1500nm 15x16x200-300nm	Japan China

measures of button mushroom viruses are discussed below:

SYMPTOMS

The various early names coined for mushroom virus disease give some indication of the diversity and variation of symptoms caused by viral infection. The symptoms, which have frequently been described may be expressed individually or in various combinations and in a wide range of severity. The symptoms of virus disease vary from reduced yield to distorted mushrooms. During the spawn run period, there is no visible indication of the disease however, once casing is applied, distinctive symptoms may be restorted when symptomless or slightly affected mycelial isolates are grown in compost and induced to fruit. The full range of symptoms that are encountered in non-hybrid mushrooms are also seen in hybrid mushrooms. In extreme cases all sporophore initiation is inhibited and the vigour of the mycelium is severely reduced while in other cases it is difficult to detect these symptoms. This variation depends upon a number of factors which include virus concentration, time of infection, strain of spawn used and cultural conditions. The general symptoms observed are as below:

1. Mycelium does not permeate or hardly permeates the casing layer or disappears after the normal spread. Mushrooms appear only in dense clusters, maturing too early.

2. Mycelium isolated from diseased sporophores on agar shows a slow and degenerated growth as compared with healthy mycelium.

3. The delayed appearance of the pinheads of the first flush can be an important indication of the disease as well as the formation of fruiting primordia below the surface of the casing layer. As soon as these mushrooms appear above the casing soil, their pilei are already opened.

4. Symptoms of sporophores are highly variable. The following abnormalilties can be found separately or in combination:

   a) Slow mycelial growth, development of abnormal mushrooms.

   b) Slow development of pinheads, dwarfing.

c) Delay in appearance of sporophores, reduced yield.

d) Off-white colour of the cap and early maturity.

e) Sporophores with elongated stems and small caps.

f) Elongated slightly bent stipes, sometimes with small early maturing pileus.

g) Premature opening of veil.

h) Mushrooms are loosely attached to the substrate and at the slightest touch are pushed over.

i) Accelerated post-harvested deterioration.

j) Watery stipes, streaking in the stipes

k) Stipes are spongy and quickly turn brown on cutting and show an abnormal structure.

l) Thickened barrel-shaped stipes; the veil is attached to the thickest part of the stipe, thus lower than usually. Pilei are small and fat.

m) Brown, slimy, cap occur owing to a secondary bacterial rot, stipes are sometimes tapering downwards during the first flush, sometimes a few light brown caps can be observed.

n) Veils abnormal or absent, hard gills are common.

5) A specific musty smell can be perceived in a growing room infested with the disease. Whereas in *Pleurotus*, virus infection causes dwarfing or elogation of stipe. However, no distinct symptoms are visible in *Volvariella*.

Severe and total crop losses have been reported due to club-shaped virus reported in *A.bisporus* (Albouy *et al.* 1973). It has been shown to be very difficult to produce spawn from mushroom infected with this club-shaped virus. The symptoms induced by virus disease in *L.edodes* include dwarfing, early maturity, hardened gills and thickened, elongated or barrel shaped stipes (Deahl *et al*. 1986).

Causal organism

Several viruses of different shapes and sizes have been reported on different mushrooms. In India, virions measuring 29nm and 35 nm in diameter have been found associated with a virus disease of

button mushroom. Virus like particles measuring 29nm in diameter have also been reported in button mushroom as revealed by immunosorbent electron-microscopy (Goltapeh and Kapoor, 1990).

CHARACTERIZATION OF VIRUSES AND VLPs

Morphology of Viruses

Experiments by Gandy and Hollings (1962) and Hollings (1962) demonstrated the presence of three types of virus particles associated with the diseased mushrooms having die-back symptoms. Two of the viruses had isometric particles with a diameter of 25nm and 29nm while the third was bacilliform with a diameter of 18nm and a length of 50nm. A fourth virus type was later reported from England (Hollings *et al*., 1968) and Holland (Dieleman-van Zaayen and Temmink, 1968) having 35nm diameter. Another spherical virus having a diameter of 40 to 50 nm was later reported from England by Hollings and co-workers (1968). Thus, five different types of viruses have been reported in England and the accepted nomenclature for these viruses in UK is as below:

MV-1 : Spherical particles, diameter 25nm.

MV-2 : Spherical particles, diameter 29nm.

MV-3 : Bacilliform particles, 19x50nm.

MV-4 : Spherical particles, diameter 35nm.

MV-5 : Spherical particles, diameter 50nm.

In addition to these five viruses, two more types having club-shaped and rod-shaped particles have been reported in *A.bisporus* from different parts of the world.

Recently, spherical viral particles of 24 to 26nm in diameter have been shown to exist in *Pleurotus ostreatus, P.sapidus, P.columbinus* and *P.florida* from China and France (Liang *et al*. 1987, 1990; Liu and Liang, 1986; Molin and Lapierre, 1989) and flexuous rods measuring 40-600 nm long from China (Liang *et al*. 1990). From China, polyhedral virus measuring 34 nm in diameter has been reported in *Volvariella volvacea* (Chen *et al*., 1988). Rods as well as spherical types of viruses have also been reported in *Lentinus edodes*

from China, USA and Japan. Different viruses and VLPs reported from different parts of the world have been summarised in Table-2. In India, viruses and VLPs have been reported infecting *A.bisporus* (Tewari and Singh, 1984; 1985; Goltapeh and Kapoor, 1990) and *P.florida* (Krishna Reddy *et al.*, 1993).

Physico-chemical Properties

As is evident from the reports that several viruses having various shapes and sizes have been found associated with diseased mushrooms. However, the role of individual virus or VLPs in inducing the typical symptoms of the disease has proved inconclusive. Recent biochemical studies have significantly advanced our understanding of the viral nature of the diseases or VLPs. Further, the widespread occurrence of VLPs in healthy basidiocarps and mycelium (Passmore and Frost, 1974, 1979) has raised questions concerning the etiological role of viruses in disease (Frost and Passmore, 1980). Because mycoviruses typically possess double stranded RNA (ds RNA) genomes, the discovery of discrete ds RNA molecules in diseased tissues constitutes the most convincing evidence for the viral etiology of La France disease (Hicks and Haugton, 1986; Lomke, 1976; Marino *et al.*, 1976; Ross *et al.*, 1986; Wach, *et al.*, 1987).

It was also reported that a viral complex (Sonnenberg and Griensven, 1991; Romaine and Schlagnhaufer, 1991) involving a ss RNA virus and unrelated ds RNA virus (es) plays a role in etiology of La France disease. *A.bisporus* fruit bodies affected by La France disease contain the specific set of 9 ds RNA molecules which is genome of 36nm isometric virus (Van der Lende *et al.*, 1994; Revill *et al.*, 1994; Zobalgogeazcoa *et al.*, 1995; Goodin *et al.*, 1992). The nucleotide sequence of dsRNAs M2 (1.3kb) and L3 (2.8 kb) is invariably associated with the disease. The average G+C content of these ds RNAs was 43 percent close to that of *A.bisporus* nuclear DNA. S3 ds-RNA (0.39 kb) is occasionally found in large amounts in diseased mushrooms (Harmsen *et al.*, 1991). Harmsen and Wessels (1991) reported that La France disease was associated with 10 differently sized dsRNAs, which appeared to be encapsidated by virus particles of 25 and 34 nm. One of these dsRNAs was also present in healthy mushrooms. Recently, it has also been shown that dsRNAs L5 and M2 are encapsidated by 34 nm

particle (Ven der Lende *et al*., 1994). Reverse transcription-polymerase chain reaction assay (RT-PCR) showed the diseased mushrooms to be either singly infected by La France isometric virus (LIV) or doubly infected by La France isometric virus and mushroom bacilliform virus (MBV). La France disease is associated with the infection by two autonomously replicating viruses in which LTV is the primary causal agent and MBV, possibly pathogenic, capable of modulating symptoms, is not required for pathogenesis (Romaine and Schlagnhaufer, 1995). MBV was found to have a monopartite ssRNA genome of positive sense. The putative RNA-dependent RNA polymerase and coat protein displayed homology with protein encoded by plant viruses particularly luteoviruses and carmoviruses.

Transmission of LIV during basidiosporogenesis together with spore-borne nature of causal agent playes etiologic role of virus in La France disease (Romaine *et al*., 1993). In two separate trials an average of 75 and 65 per cent of the viable basidiospores discharged from diseased basidiocarp were infected by LIV. Basidiocarp showing the presence of dsRNAs in the stipe tissue produce LIV infected basidiospores. Double stranded RNA having molecular weight of 3.2x10 dalton has been demonstrated with 35nm virus particles in *Volvariella volvacea* (Chen *et al*., 1988) and 0.85x10 daltons with 24nm particles in *P.sapidus* and *P.ostreatus* (Liange *et al*., 1987). In *A.bisporus*, dsRNA has been demonstrated with 25 and 34nm particles (Hicks and Haughton, 1986; Romaine and Schlagnhaufer, 1989) whereas with bacilliform particles measuring 19x50nm, single sRNA has been reported (Molin and Lapierre, 1973; Tavanizis *et al*., 1980). In *L.edodes* dsRNA has been demonstrated with 39nm spherical particles. Most of the spherical VLPs or viruses are isometric, with sizes between the limits of 25 and 45nm diameter. Many have never been transmitted even by hyphal anastomosis to a healthy mycelium and nothing is known about their sedimentation characteristics, number of components or the composition of the viral nucleic acid and polypeptide moieties. They are still only the VLPs. The status of other particles is uncertain for different reasons. These are regarded by some workers as artifacts, fragments from 19x50nm, MV-3 virions, seen transversely (Hollings *et al*., 1971).

These particles, derived only from preparations containing MV-3, had a UV absorption spectrum lacking the 260nm peak of nucleoprotein virions and could not be transmitted. However, some workers regarded these as virions of a distinct type.

From *L.edodes* isometric particles 25, 30 and 39nm (Ushiyama and Nakai, 1975) and 30, 36 and 45nm (Yamashita *et al.*, 1975) have been recorded in Japan, but whether these refer to the same three viruses or to four different viruses, is not known. Too little is known about the rods from *L.edodes* measuring 38x300nm in dip preparation and 15x200nm in thin sections to decide whether or not these could be tobamovirus particles.

Purification Procedures

It has proved much difficult to obtain consistently good preparations of mushroom viruses. In repeated tests mycelium from agar or from liquid cultures has been reported wholly unsatisfactory as the source of virus (Hollings and Stone, 1971) for virus extraction. Most of the virus is lost during the different steps in purification and therefore, sporophores with higher concentrations of the virus should be taken for grinding. Mushrooms contain powerful polyphenols oxidase system and often copious amount of polyphenolic complexes in virus extraction. Several methods of extraction, clarification and purification of viruses or VLPs have been tried in *A.bisporus* with varying degree of success. Some of these procedures are:

1. Hollings and co-workers(1971) attempted extraction with phosphate buffer and precipitation of virus with citric acid which gave best yield of viruses MV-1, MV-3 and MV-4. Precipitation of virus by ammonium sulphate or by sodium chloride plus polyethylene glycol(PEG) gave unsatisfactory results(Hollings and Stone, 1971).

2. Extraction in borate or phosphate buffer and clarification with butanol gave preparation of viruses MV-1, MV-2, MV-3 and MV-5and proved very satisfactory for virus 2but virtually destroyed MV-4(Hollings, 1962).

3. Extraction in phosphate buffer, clarification with ethoxy and butoxy and butoxy-ethanols has yielded MV-1, MV-3 and MV-4 (Kitano *et al.*, 1961)

4. Dieleman-van Zaayen and Temmink (1968) used the methods of Hollings and co-workers (1965) and Kitano and co-workers (1961) with slight modifications followed by differential centrifugation and obtained good yields of MV-1, MV-3 and MV-4.

5. In case of *Pleurotus ostreatus* and *P. sapidus*, Liang and co-workers (1990) used 0.03 M phosphate buffer (pH 7.0) for extraction followed by low speed centrifugation and density gradient centrifugation and a fairly high concentration of both spherical as well as rod shaped virions was obtained. Molin and Lapierre (1973) have also used a similar method for purifying spherical virus from *P. pulmonarius*.

6. Chen and co-workers (1988) used Tris-HCl buffer (pH 7.6) for extracting the spherical virus from *V.volvacea* followed by three centrifugation at 5000g each for clarification. PEG 6000 and 0.1M sodium chloride were used for precipitation and pellets were again suspended in 0.05M Tris-HCl and 1M and NaCl buffer. Concentrated preparations of the virus were obtained by further differential centrifugation.

It can not be disputed that very pure virus preparations are essential for chemical, physical and biochemical studies and that many biological investigations are dependent on the availability of atleast partially purified preparations. It must be stressed here that no two viruses are exactly alike and consequently there are about as many purification procedures as there are viruses which have been purified. To achieve a purified virus preparation one has to take into account several factors like selection of propagating host, conditions affecting virus multiplication, selection of proper host tissue for extraction, extracting media (buffers, pH and molarity) method of extraction, clarification procedures and methods of isolation, concentration and further purification.

EPIDEMIOLOGY

The wide variation in symptoms reflect the variation in the economic impact of viruses on mushrooms. It is possible to have yield losses so slight that they are masked by other factors and the growers remain

unaware of them. Alternatively, the infection may be so severe that virtually no marketable mushrooms are produced. The first appearance of pinheads is delayed by several days and they remain as a small grey-fawn clump without further growth, although some may shed spores. Loss of crop varies from slight to 95 per cent (Barton, 1985; Dieleman-van Zaayen, 1970; Hollings *et al.*, 1963; Rasmussen *et al.*, 1969; Schisler *et al.*, 1967). Various factors like time of infection, cultural conditions and the strains of the spawn used greatly affect the loss in yield. Dieleman-van Zaayen (1972) reported that when artificial inoculation was done from 0 to 12 days after spawning, the extent of loss due to dieback varied from 37.5 to 95.6 per cent over uninoculated control. He also concluded that: a) the time of infection is much more important than the amount of inoculum; b) with early infection, the amount of inoculum is of no consequence, and c) the amount of inoculum has a slight negative influence with later infection, which, by itself, causes a small loss in yield. A survey among more than 1000 Dutch growers showed that in 1967 and in the first half of 1968, one out of three mushroom farms was contaminated and on these farms average yield loss was 15 per cent. Thus in 1967, in the Netehrlands, 4.5 per cent or about 7,90,000 kg of mushroom were lost (Dieleman-van Zaayen, 1972a).

Detection Methods

Diagnosis of virus infection in mushrooms is not easy because of two reasons. The first reasons is that mushroom being an anatomically simple organism, responds to a range of adverse stimuli in only a limited number of ways. For example systems like elongation of the stipe, water logging of stipe, general loss in yield and bare patches in the mushroom beds may be induced by virus infection as well as a variety of other biotic and abiotic factors. The second difficulty with diagnosis is generally the low virus concentration. The different approaches adopted for the diagnosis of virus infection in mushroom are:

1. Symptoms on the bed.
2. Comparative growth rates of mycelium on agar.
3. Direct electron-microscopic examination (EM).

4. Immunosorbent electron-microscopy (IEM or ISEM).

5. Polyacrylamide gel electrophoresis (PAGE).

6. Enzyme-linked immunosorbent assay (ELISA).

7. Reverse transcription-polymerase chain reaction assay (RT-PCR).

Symptoms on the beds

Symptomatology has been discussed in detail earlier and they certainly indicate that something is wrong. However, it is difficult to conclude with authenticity that a particular abnormally in mushroom is only due to virus. Moreover, viruses have been detected by other methods in apparently healthy mushrooms.

Agar growth test

This was the first test to be devised and depends upon the facts that affected mushroom mycelium has a slower growth rate than otherwise identical healthy mycelium. Tissue cultures of healthy mushrooms in 2.5 per cent malt agar at 25°C grow aggressively and achieve a diameter of 80 to 100mm in 21 days. The periphery of the colony will have bare dense aerial hyphae which are white or pale cream in colour and resemble cotton wool. In the centre of the colony, the aerial hyphae largely disappear as they amalgamate to form rhizomorphs which have the appearance of fine white threads on the surface of the agar. In comparison, virus-infected mushrooms cultured in the same manner will achieve a colony diameter of 5mm upto 80to 100mm. If the mushroom is severely infected colony will be flat and slightly waxy in appearance with few aerial hyphae. The colour is usually a deep cream or even light brown and can sometimes be dark in the Centre. Rhizomorphs do not generally form on diseased colonies and are often replaced by very flat aggregates of tissue which give a speckled or pepper and salt appearance to the centre of the colony (Gandy and Hollings, 1962, Nair, 1973). The advantage of this system is that it does not require expensive equipment. The disadvantages are, firstly, the long time needed to obtain a result and, secondly, the relative insensitivity of the test.

Electron microscopy

Gandy and Hollings (1962) first observed virus-like particles in

transmission electron microscope while examining purified and concentrated sap from mushrooms exhibiting dieback symptoms. However, virus purification and concentration was a complex and time consuming process which was not suitable for large number of diagnostic tests and Hollings and co-workers (1965) found that virus particles could be detected more quickly if the juice from diseased mushrooms, disrupted by ultra-high frequency sound waves, was examined under the electron microscope. Hollings and co-workers (1967) modified that detection procedure further wherein the juice from the suspected sporophore was squeezed through a piece of fine cloth. The juice thus expressed was mixed with 2 per cent PTA (pH 7) and mounted on carbon coated grids for examining in electron microscope. Thereafter the use of electron-microscopy allowed several viruses to be found in either purified preparations or ultrathin sections of diseased mushrooms (Barton, 1985; Barton and Hollings, 1979; Chen *et al*., 1988; Dieleman-van Zaayen, 1972b; Dieleman-van-Zaayen and Igesz, 1969; Dieleman-van Zaayen and Temmink, 1968; Hollings *et al*., 1968; Koons *et al*., 1983; Leistner, 1980; Lesemann and Koenig, 1977; Liang *et al*., 1990; Molin and Lapierre, 1989; Mori and Mori, 1974; Mori *et al*., 1978; Passmore and Frost, 1979; Tavanizis *et al*., 1980; Tewari and Singh, 1984; Ushiyama, 1975). Virus particles (MV-1, MV-2 and MV-3) could be detected with reasonable certainly in severely affected mycelium in as little as 1mg (fresh weight) of mycelium disrupted by sonication (Hollings *et al*., 1965). One of the advantages of this technique is the speed in the detection in samples when large number of virus particles are present. The disadvantage is its uncertainty of detecting levels of virus too low to cause disease at the time of examination but which may indicate a potential problem.

Immunosorbent electron microscopy

ISEM, originally developed by Derrick (1973) is a rapid method of detection and cheap to perform. Although it is a serological method, monospecific sera are not necessary its first use with mushrooms was reported by Del Vecchio and co-workers (1978). In this procedure, electron microscope grids are coated with carbon which behaves much like activated charcoal. It strongly absorbs proteins and by floating

these grids on antiserum droplets they become coated with antibody molecules. This coating can then selectively adsorb virus from mushroom extract and the antigen-antibody aggregates can be easily seen in EM. ISEM is almost 5000 times more sensitive than direct EM.

Polyacrylamide gel electrophoresis

This is a highly sensitive and specific detection technique, and is used for detecting double stranded RNA (dsRNA) in diseased mushrooms (Marino *et al*., 1976). In order to use this technique, the viral RNA must be extracted from diseased mushrooms. This can be identified by applying the preparation to an agar gel column which is subjected to an electric field. By staining the column after a predetermined time, the RNA, if present, can be identified. This technique is about 20 times more sensitive than direct EM and can also be used for detecting specific viruses. However, the drawback is that it is dependent upon the stability of the virions during the extraction of the dsRNA. This technique has been widely used in detecting dsRNA in virus infected *A.bisporus*, especially *Lentinus edodes* (Ushiyama *et al*., 1977), *Pleurotus ostreatus, P.sapidus* (Liang *et al*., 1990) and *Volvariella volvacea* (Chen *et al*., 1988).

Enzyme-linked immunosorbent assay

Virus detection by ELISA (Voller *et al*., 1976) had become widespread among plant and animal virologists and was a simple and fairly rapid (1-2 days) detection method. Its drawback was that a monospecific (perfectly pure) antivirus serum was required.

Any antibody to normal mushroom constituents gave very strong, nonspecific, false positive tests. Mushroom was detected in preference to or as well as, virus. The great difficulty in adequately purifying most of the mushroom viruses for antiserum production resulted in limited use of this technique for detecting MV-3 and a spherical virus in *P.pulmonarius* (Barton, 1985; Liu and Liang, 1986).

Tests have shown that direct electron microscopy can detect MV-1 at a concentration of 1mg/ml (micro-gram per ml). A little better is dsRNA at concentration 250mg/ml. Most ELISA tests with plant and animal viruses can detect down to 2ug/ml, an increase in sensitivity

over direct electron microscopy of 5,000 times (Barton, 1985).

Reverse transcription-polymerase chain reaction assay (RT-PCR)

Harmsen (1990) described RT-PCR detection method for the presence of dsRNA in spawn run compost. This is a sensitive and reliable test available for detection of dieback disease virus at any stage of cultivation of *A.bisporus*. This method, in principle, could be applied to mycelium in the compost since dsRNA L3 encodes for one of the coat protein of 34nm particle. However, it is especially important to test atleast two dilutions of each compost extract in a range equivalent to 0.5-5 ug freeze dried compost per RT-PCR reaction. Low dilution of the samples inhibit the RT-PCR by the presence of inhibitory compounds and high dilutions lower the concentration of dsRNAs beyond the detection limit.

Transmission and spread

Through mycelium

It was revealed at an early stage that viable mycelium could transmit the 'dieback' disease (Gandy, 1960). Viable diseased mycelium would remain behind in trays after a crop and after inadequate disinfection, would anastomose with healthy mycelium in the following crop and thus transmit the virus.

This is the most common method of transmission and has been confirmed by several workers (Dielman-van Zaayen, 1986; Hollings, 1962, 1972, 1982; Hollings *et al.*, 1963). However, this is possible only among the compatible strains and not in others. For example, *A.bitorquis* was not infected after exposure to diseased *A.bisporus* mycelium and spores (Van Zaayen, 1976). Although *A.bitorquis* has been regarded as highly resistant or immuse to mushroom viruses, it may escape infection by incompatibility with *A.bisporus*, for heterokaryosis did not occur between the two species (Raper, 1976). *Mycogone perniciosa* heterokaryosis did not occur between the two species (Raper, 1976). *Mycogone perniciosa* and *Verticillium fungicola*, both parasitize *A.bisporus* and their mycelial strands penetrate the intercellular spaces in mushroom sporophores, the known viruses of *M.perniciosa* and *V.fungicola* are serologically unrelated to any of the known viruses of *A.bisporus* and there is no evidence of any virus

transmission occurring between them. Tubular virus particles were found in *Plicaria* sp., a weed mould growing among the mushrooms in trays, and in very low concentration in the mushroom sporophores (Dielman-van Zaayen, 1967) but there is no evidence to suggest that any transfer of the virus took place between the two fungi.

Through spores

This was first demonstrated for mushroom virus (MV-1) by Schisler and co-workers (1963, 1967) and subsequently for mushroom viruses 2,3 and 4 (Hollings *et al*., 1971). Mushroom virus 4 particles have also been visualized in thin sections of mushroom spores and germtubes (Dielman-van Zaayen, 1972b). Last and co-workers (1967) isolated 25nm virus particles from some of Schisler's spore-derived cultures and confirmed the transmission of the disease. Spores can infect the compost at any stage before and/or after spawning and after germination the mycelium anastomose with disease-free mycelium thereby resulting in virus transmission. Infected mushrooms usually mature too early and growers can not pick them all before they open and release the spores. Spores from diseased mushrooms often germinate better and faster than uninfected spores (Dielman-vaan Zaayen, 1970, Schisler *et al*., 1967). Over 40 per cent of spores from infected mushrooms germinate within a week on agar medium compared with none of the healthy spores. The spore load within a mushroom house fluctuates greatly; over 3 million per minute were recorded in the exhaust air from a mine shaft with an accumulation of unpicked mushrooms (Schisler *et al*., 1967) under ordinary cropping conditions, 1000 to 10000 spores per m3 were estimated from cascade impactor and volumetric spore traps (Gandy, 1971). Spores were detected 5cm away from exit ventilators but further away no spores were trapped (Gandy, 1971) but Frost and Passmore (1979) have reported that daily mean concentration of order of 10-10 were present in the compost yard at distances of 10 to 20m from the nearest growing room exhaust. Mushroom spores have been detected in fairly good concentration in air samples taken from the pasteurized filtered air-zone of the farm where peak heating, spawning, spawn run and casing were done (Frost and Passmore (1979). Gandy (1971) also detected basidiospores

upto 16 per m3 in a spawn run room at GCRI where the air was filtered to exclude particles greater than 2 um and detected similar concentrations on a commercial mushroom farm with a similar air-filtration system. Since minimum dose of basidiospores necessary for transmission of the disease lay between 1 to 10 spores per tray (Schisler *et al*., 1967) the efficiency of transmission of virus diseases in mushrooms through spores will be very high. Thirty years or more is the estimate given by Schisler and co-workers (1967) as the life of healthy spores although they did not specify the conditions of storage. Van Zaayen (1979) claimed a life of 14 years of spores stored at 4C. Atkey and Barton (1978) found that virus-infected spores stored under more stringent conditions of normal room-temperature (20C) on a window sill in full sunlight were viable and able to transmit 25 nm and 35 nm viruses after 6.5 years although the efficiency of transmission had declined somewhat compared with that of fresh spores. Nair (1976) observed that infected basidiospores were smaller and had thin walls but this was not verified by other workers (Stalpers and Van Zaayen, 1981). Isometric virus particles measuring 25, 30 and 39 nm in diameter have also been transmitted through basidiospores of *Lentinus edodes* (Ushiyama and Nakai, 1975).

Transmission through vectors

There is no any report about the involvement of any vector for the transmission of mushroom viruses. However, a very low level of transmission of MV-1 by mushroom phorid fly (*Megaselia halterata*: Diptera) was obtained when aseptically reared insects were allowed to feed first on purified virus from a sucrose density-gradient and then on healthy mushroom mycelium. There is no evidence, however, that *M.halterata* can acquire the virus from infected mushroom mycelium. Hollings and Gurney (1973) also failed to transmit MV-1 and MV-4 from sterile virus-infected mycelial cultures to healthy ones, using aseptically reared mites (*Tarsonemus myceliophagus*). However, both phorid flies and mites do carry the mushroom spores from one place to another within a tray or from tray to tray, thereby resulting in introduction of virus inoculum. Such agencies may be important carriers but not vectors.

Mechanical transmission

Many workers have attempted to infect mycelial cultures by applying

cell-free virus preparations but none has succeeded so far even when the cultures were abraded or shaken with carborundum powder or glass blades. Very low transmission has been obtained when purified preparations of mushroom viruses 1, 2 and 3 were hypodermically injected into sporophore initials of healthy *A.bisporus* grown in screened isolation chambers (Hollings, 1962; Holling and Stone, 1971). The viruses were not subsequently detected in the injected sporophores but were confirmed in the mycelium growing beneath them. This has been confirmed with MV-4 with very low levels of transmission (Dieleman-van Zaayen and Temmink, 1968).

Temperature and time of inoculation had great effect on symptom development in inoculations at casing the cream X-disease and the La France isolates produced more severe symptoms when held at a cropping temperature of 20 to 21°C than when held at 15 to 16°C (Hager, 1968). Disease severity was also observed to be correlated with time of inoculation. Inoculations at spawning were more damaging than inoculations at casing (Hager, 1968; Last *et al*., 1967; Schisler *et al*., 1967). Contamination of trays or shelves with fragments of mycelium provides a very important means of spread of all the viruses to the next crop causing maximum damage. For detecting viral infections and predicting percentage of yield losses on the basis of dsRNA bands, Batterley and Olson (1989) have standardized the sampling technique from the mushroom beds/ cropping rooms. It is not unusual to get positive detection by PAGE or ELISA tests for mushroom viruses without observing any virus symptoms in the sporophores. But positive detection results using agar growth test and direct EM are almost always associated with yield reduction or symptoms of the disease.

MANAGEMENT OF MUSHROOM VIRUSES

For adopting suitable management strategies for mushroom viruses, one has to keep in mind that the disease is spread by viable mycelium and spores of diseased mushrooms; early infection is dangerous, especially an infection simultaneous with or shortly after spawning. Upto the time of casing, the compost and mycelium must be protected. Owing to the lack of useful resistance with the species, control of the disease is based largely on the use of hygienic practices

directed at the elimination of diseased mycelium and basidiospores from the production (Schisler *et al*., 1967, Van Zaayen, 1976). Dieleman/van Zaayen (1970, 1986) has suggested various approaches to reduce the spread of mushroom virus diseases which have been summarized below:

When the disease is not present

1. Steam the compost for 12 hours at a temperature of 70°C. At emptying, remove the compost quickly.

2. Spray the wood with 2 per cent sodium pentachlorophenate to which 0.5-1.0 per cent soda (sodium carbonate) has been added, after drying spray with water.

3. Disinfect doors, little holes in the floor, shutters, racks, floors and walls with formaldehyde (not with sodium pentachlorophenate). Also clean the manure yard and adjacent patches of ground with formaldehyde.

4. Before filling, fit spore filters, during growing time these spore filters should be replaced once or twice according to the amount of dust in the air. Use a fan for extracting air.

5. Immediately after spawning, use a pesticide against flies and cover the compost with paper. Keep the paper moist. Wet the paper twice a week with a 2 per cent solution of the 40 per cent commercial formaldehyde. Repeat till a few days before casing. Never use sodium pentachlorophenate here. Moisten the paper before removing it carefully.

6. Quickly remove cuttings and litter and destroy.

7. The entire farm and its surroundings should be maintained very clean and stay so. In the working corridor formaldehyde should be sprayed. Machines, refrigerator and other utilities should be disinfected with a formaldehyde solution.

8. At the first sight of contamination, the disease can be controlled best by immediately steaming out the concerned room.

When the disease is already present

1. Adopt practices 1,3 and 4 mentioned under when the disease is not present.

2. Immerse the wood in a 4 per cent sodium pentachlorophenate solution to which 0.5-1 per cent sodium carbonate has been added.

3. Pick the mushrooms when still closed.

4. Keep each room as a separate entity with separate clothes, shoes, steps, buckets, picking knives, picking racks, fans etc. Kill off diseased patches with salt and cover with plastic, make the limits of the patches rather big. First pick from the healthy parts then from the diseased patches. Wash hands often.

5. Admit as few visitors in the diseased rooms as possible and keep the door towards the working corridors closed. Kill off pests in particular. Have a short picking period only (not more than 4 weeks).

Heat Therapy

When infected cultures were grown at 33C for 2 weeks, and hyphal tips then sub cultured and returned to 25C, many of the latter showed normal growth and did not contain virus (Gandy and Hollings, 1962). However, these findings were not conclusively proved by Dieleman-van Zaayen (1970). Rasmussen and co-workers (1972) also obtained increased sporophore yields when tissue and spore cultures derived from symptomatic sporophores of white and two cream strains were incubated at 32C for 2 weeks. Wuest and Mataka (1989) have observed more extensive spawn run on horse manure compost with the symptomatic spawn incubated at 30C than the spawn incubated at 23 or 27C.

Spawn Strains

Immunity to the virus disease of the cultivated mushroom, *A.bisporus* has been found in several strains of the white mushroom, *A.bitorquis*, collected from nature. Some strains of *A.bisporus* do not show symptoms as markedly as others. These are the brown, cream and off-white strains, or some smooth-white strains known to anastomose less frequently with others, or *A.bitorquis* can help to reduce the general virus inoculum and can enable economically worthwhile crops to be grown. Hybrid strains can anastomose with both white and off-white strains and therefore, their widespread culture may reduce the effectiveness of strain alteration as a means of virus control (Fletcher *et al.*, 1989; Romaine, 1987).

b) OYSTER MUSHROOM

A mycovirus affecting *P.florida* has been detected by immunodiffusion and ELISA tests and found related to *P.ostreatus* virus (Krishna Reddy *et al*. 1993). But varions measuring 26± 2nm and 21nm in diameter have been reported associated with virus disease of *P.ostreatus* and it is not clear as to which virus is affecting *P. florida* in India.

Symptoms induced in *P.florida* include; pileus curling upwards, swollen stalks and greatly distorted basidiocarps. Premature spore shedding and elongation of stalk are typical symptoms of the disease. Management practices are almost same as described in white button mushroom.