

## Properties of Thirteen Viruses and Virus Variants Obtained from Eight Isolates of the Wheat Take-All Fungus, *Gaeumannomyces graminis* var. *tritici*.

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(Accepted 4 November 1980)

### SUMMARY

The properties of polyhedral double-stranded (ds)RNA virus particles obtained from eight isolates of the wheat take-all fungus, *Gaeumannomyces graminis* var. *tritici*, have been investigated. Thirteen viruses and virus variants were distinguished and these were classified into three groups on the basis of serological and physical properties of the virus particles; viruses in a group were related serologically to other members of the same group, but unrelated serologically to members of other groups. Group I viruses had particles of diam. 35 nm sedimenting at 109 to 126S; the virus capsid contained one polypeptide species, mol. wt.  $54 \times 10^3$  to  $60 \times 10^3$  and virus dsRNA consisted of two to four components, mol. wt.  $1.0 \times 10^6$  to  $1.3 \times 10^6$ . Group II viruses had particles of diam. 35 nm sedimenting at 133 to 140S; the virus capsid contained one polypeptide species, mol. wt.  $68 \times 10^3$  to  $73 \times 10^3$  and virus dsRNA consisted of two to four components with mol. wt.  $1.39 \times 10^6$  to  $1.60 \times 10^6$ . Group III viruses had particles of diam. 40 nm sedimenting at 159 to 163S; the virus capsid contained three polypeptide species, mol. wt.  $78 \times 10^3$ ,  $83 \times 10^3$  and  $87 \times 10^3$  and virus dsRNA consisted of two components, mol. wt.  $3.2 \times 10^6$  to  $4.3 \times 10^6$ .

Five of the fungal isolates contained only single viruses. However, one isolate contained viruses from groups I and II, and two isolates each contained viruses from groups I, II and III. Investigation of virus from ten single conidial isolates derived from one of the latter isolates has shown that all three viruses were present in a single cell.

### INTRODUCTION

Isometric virus particles containing double-stranded (ds)RNA are of common occurrence in the wheat take-all fungus, *Gaeumannomyces graminis* var. *tritici* (Lapierre *et al.*, 1970; Rawlinson *et al.*, 1973; Frick & Lister, 1978). Lapierre *et al.* (1970) and Lemaire *et al.* (1970) correlated the presence of virus with low pathogenicity of the fungus, but Rawlinson *et al.* (1973) in a study of 156 isolates of the take-all fungus, of which 114 contained virus particles, found no consistent association of virus infection with weak pathogenicity. In a more recent study of 20 isolates of *G. graminis*, Frick & Lister (1978) found serotype variation in virus particles obtained, not only from isolates of different geographical origins, but also from isolates taken from the same field in the same year. They suggested that the frequency and diversity of such serotype variation may reflect similar biotype variation in these viruses and could offer an explanation for inconsistencies in reports of the association of *G. graminis* viruses with fungal pathogenicity.

Table 1. *Fungal isolates from G. graminis var. tritici studied*

Fungal isolate	Geographical origin	Source	Year of isolation
O1-1-4	Japan, Gunma, Tokyo	S. Yamashita	1950
3b1a	England, Rothamsted	M. Chu Chou	1970
45/9	England, Rothamsted	C. J. Rawlinson	1972
O19/6	England, Rothamsted	C. J. Rawlinson	1972
OgA	England, Selby	J. E. E. Jenkins	1972
38-4	England, Rothamsted	D. B. Slope	1973
T1	Australia, Carnamah	P. T. W. Wong	1974
F6	France, Le Rheu, near Rennes	C. J. Rawlinson	1974

In previous studies the viruses were not extensively characterized and it was not determined whether the isolates contained one or more viruses. To provide a firm foundation for studies of biotype variation in *G. graminis* viruses, we have isolated and characterized virus particles from eight isolates of the take-all fungus. Thirteen viruses and virus variants have been distinguished and classified into three groups on the basis of physical and serological properties.

#### METHODS

**Buffers.** P buffer: 0.03 M-NaH<sub>2</sub>PO<sub>4</sub>, adjusted to pH 7.6 with NaOH; PK buffer: P buffer containing 0.15 M-KCl; TBE-1 buffer: 0.1 M-tris + 0.07 M-boric acid + 4 mM-Na<sub>2</sub>EDTA, pH 8.3; TBE-2 buffer: 0.03 M-tris + 0.02 M-boric acid + 0.4 mM-Na<sub>2</sub>EDTA, pH 8.3; SB buffer: 0.05 M-sodium tetraborate, adjusted to pH 8.5 with HCl; V buffer: 0.05 M-sodium diethylbarbiturate, adjusted to pH 8.5 with HCl.

**Fungal isolates.** The sources of the isolates of *G. graminis* var. *tritici* used for virus preparation are given in Table 1. For brevity these isolates will be described as isolates of *G. graminis* throughout the paper.

**Preparation and purification of virus.** *G. graminis* isolates were grown in shaken flasks for 7 days, or in 60 l fermenters for 3 days at 24 °C in a glucose-corn-steep liquor medium using the methods described by Banks *et al.* (1971). Preparation and purification of virus by polyethylene glycol precipitation, ultracentrifugation and sucrose density-gradient centrifugation were carried out using the methods described by Buck & Girvan (1977), except that PK buffer was used throughout.

**Separation of viruses by DEAE-cellulose chromatography.** This was carried out essentially as described by Buck & Kempson-Jones (1973), except that TBE-2 buffer was used throughout.

**Agarose gel electrophoresis.** Electrophoresis of intact virions was carried out on horizontal slab gels of 0.5% agarose in TBE-1 buffer at 3 V/cm for 18 h. Virus bands were located by staining with ethidium bromide (1 µg/ml) with u.v. detection, or by staining with Coomassie blue (Buck & Kempson-Jones, 1974).

**Isolation and analysis of dsRNA.** Virus RNA was prepared by phenol extraction and shown to be double-stranded as described by Buck & Ratti (1975). Analysis and mol. wt. determinations of dsRNA components were carried out by polyacrylamide gel electrophoresis (PAGE) as described by Buck & Ratti (1977), using internal dsRNA standards covering the mol. wt. range  $0.9 \times 10^6$  to  $6.3 \times 10^6$  from the following viruses: *Penicillium stoloniferum* viruses S and F (Bozarth *et al.*, 1971); *Aspergillus foetidus* viruses S and F (Buck & Ratti, 1977); bacteriophage  $\phi 6$  (Semancik *et al.*, 1973); *Helminthosporium maydis* virus (Bozarth, 1977).

**Analysis of virus polypeptides.** The mol. wt. of capsid polypeptides were determined by SDS-PAGE in 8% gels as described by Buck & Ratti (1975) and in 12% gels by the method

of Laemmli (1970) using the following internal standards (subunit mol. wt. in parentheses are those given by Weber *et al.*, 1972): *Escherichia coli* RNA polymerase  $\beta'$  subunit (160 000),  $\beta$  subunit (150 000), phosphorylase A (100 000), bovine serum albumin (68 000), glutamate dehydrogenase (55 000) and lactate dehydrogenase (36 000). Similar values were obtained by both methods. In the case of virus F6-B, attempts to increase the proportion of monomeric polypeptide subunit in the sample were as follows: performic acid oxidation (Hirs, 1967); reduction and carboxymethylation (Van Etten *et al.*, 1976); treatment with alkali (Sehgal & Hsu, 1977); dialysis against 6 M-guanidine chloride followed by dialysis against 8 M-urea containing 0.1% SDS; inclusion of 0.1 M-sodium thioglycollate in the gel and running buffer; inclusion of 8 M-urea in the gel. SDS (2%) and 2-mercaptoethanol (2%) were added to all samples, which were then boiled for 3 min before SDS-PAGE.

*Sedimentation coefficients.* These were carried out with a Beckman model E analytical ultracentrifuge as described by Buck & Kempson-Jones (1973).

*Preparation of antisera.* Rabbits were immunized with one intravenous injection (1 ml containing approx. 1 mg virus in P buffer), followed by two intramuscular injections (each of approx. 1 mg virus emulsified with Freund's complete adjuvant; Difco Bacto) over a period of 3 weeks. Mice were immunized with three intramuscular injections (each of approx. 10  $\mu$ g virus emulsified with Freund's complete adjuvant) over a period of 5 weeks. For both animals sera were obtained 1 to 2 weeks after the final injection and stored either at  $-20^{\circ}\text{C}$  or at about  $2^{\circ}\text{C}$  with an equal volume of glycerol as preservative.

*Serological tests.* Purified virus preparations (0.5 to 1 mg/ml) were used as antigens. Gel diffusion tests were done on microscope slides using 1% Ionagar no. 2 (Oxoid) in PK buffer. Wells were 5 mm in diam. and 3 mm apart. For determining antiserum titres, twofold serial dilutions of sera in PK buffer were used. For comparing antigens several dilutions of antisera were tested to obtain optimum conditions for interaction of precipitin lines. Gels were examined after 48 h at  $20^{\circ}\text{C}$  using dark-ground illumination or after staining of precipitin lines with Coomassie blue.

*Electron microscopy.* Samples were negatively stained with 1% potassium phosphotungstate pH 7 and examined with a Siemens Elmiskop 1A electron microscope, internally calibrated with a  $69.6\ \mu\text{m}$  aperture.

*Germinable microconidia.* These were produced from flooded cultures by the method of Deacon (1976).

## RESULTS

### *Purification and separation of viruses from G. graminis isolates*

Crude virus preparations were obtained from homogenized mycelium of each of the *G. graminis* isolates listed in Table 1 by polyethylene glycol precipitation and differential centrifugation, and purified by velocity centrifugation in sucrose density-gradients. A typical purification, exemplified by virus from *G. graminis* isolate T1, is shown in Fig. 1. In the first virus preparation made from *G. graminis* isolate 45/9 the virus remained in suspension in P or PK buffers long enough to permit sedimentation analysis to be carried out. However, in six subsequent preparations from this isolate, the virus precipitated out of solution just before the sucrose density-gradient stage of purification. The virus could not be resuspended in P, PK, TBE-1, SB or V buffers, preventing analysis of intact virions by gel electrophoresis or gel immunodiffusion. Virus preparations from the other eight *G. graminis* isolates formed stable suspensions in PK buffer.

Virus preparations from *G. graminis* isolates O19/6, 38-4, O1-1-4, 45/9 and T1 each contained only one virus because (i) they contained polyhedral particles of diam. 35 nm, which sedimented as single boundaries on ultracentrifugation and (ii) analysis of virus protein

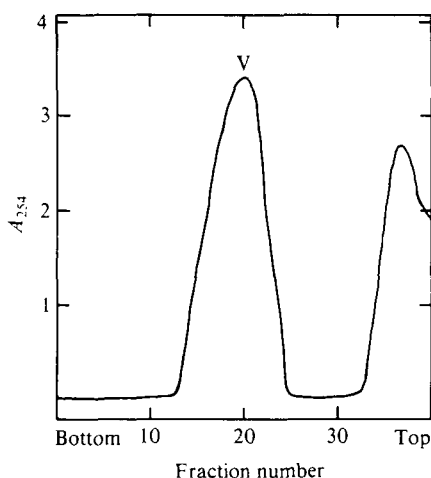


Fig. 1. Purification of virus from *G. graminis* fungal isolate T1-A. The virus preparation (50 ml.  $A_{260}$  of 10) was loaded on to a 20 to 50% (w/w) sucrose density-gradient (500 ml) in an MSE BIV zonal rotor and centrifuged at 40000 rev/min for 2.5 h. After separation the gradient was pumped through an ISCO u.v. monitor. V denotes the virus peak.

by SDS-PAGE gave a single polypeptide band in each case. Additional evidence for homogeneity was obtained for viruses from isolates O19/6, 38-4 and T1, which gave single precipitin lines in gel diffusion tests against homologous antisera and for viruses from isolates O19/6 and 38-4 which migrated as single bands in agarose gel electrophoresis (viruses from isolates O1-1-4 and T1 precipitated during gel electrophoresis, precluding their analysis by this technique).

Virus preparations from *G. graminis* isolate OgA contained polyhedral particles of diam. 35 nm, but migrated as two components in agarose gel electrophoresis and gave two precipitin lines in gel diffusion tests against homologous antiserum, indicating the presence of two viruses. Separation of these viruses was achieved by salt gradient elution from a DEAE-cellulose column. Viruses OgA-B and OgA-A were eluted at 0.15 M-NaCl and 0.5 M-NaCl respectively. Each virus migrated as a single band in agarose gel electrophoresis. In gel immunodiffusion tests against the same antiserum, each virus gave a single precipitin line.

Virus preparations from *G. graminis* isolate 3b1a contained polyhedral particles of two sizes, 35 nm and 40 nm diam., which in agarose gel electrophoresis migrated as three distinct bands and gave three precipitin lines in gel immunodiffusion tests against antiserum, prepared against the virus mixture, indicating the presence of three viruses. The 40 nm particles (virus 3b1a-A) were obtained free from the other two viruses by sucrose density-gradient centrifugation. Separation of the two 35 nm viruses was achieved by salt gradient elution from a DEAE-cellulose, viruses 3b1a-C and 3b1a-B being eluted at 0.2 M-NaCl and 0.3 M-NaCl respectively. Each of the separated viruses migrated as a single band in agarose gel electrophoresis and gave a single precipitin line in gel immunodiffusion tests against the antiserum described above.

Virus preparations from *G. graminis* isolate F6, and from ten single microconidial isolates (SM1 to SM10) derived from F6, contained polyhedral virus particles of three sizes (diam. 27, 35 and 40 nm). After purification, virus preparations contained only the 35 and 40 nm particles, indicating that the 27 nm particles were either unstable to centrifuging in sucrose gradients or were empty particles containing no nucleic acid. Three viruses, one of 40 nm diam. (F6-A) and two of 35 nm diam. (F6-B and F6-C) were isolated by sucrose density-gradient centrifugation and DEAE-cellulose chromatography and shown to be

Table 2. *Physicochemical properties of G. graminis viruses*

Group	Virus	Particle diam. (nm)*	$s_{20}$ (Svedbergs)†	Mol. wt. of dsRNA components ( $\times 10^{-6}$ )‡	Mol. wt. of capsid polypeptides ( $\times 10^{-3}$ )‡	Electrophoretic movement (mm/h) towards anode
I	O19/6-A	35	126	1.27, 1.19	60	1.25
	38.4-A	35	115	1.27, 1.19, 1.09	55	1.18
	O1-1.4-A	35	109	1.22, 1.14	55	P§
	45/9-A	35	117	1.30, 1.22, 1.14, 1.11	55	NT
	OgA-B	35	125	1.30, 1.22	55	0.58
	3b1a-C	35	115	1.27, 1.19, 1.11	55	1.10
	F6-C	35	128	1.27, 1.19	54	2.20
II	T1-A	35	133	1.49, 1.47	73	P
	F6-B	35	133	1.60,¶ 1.56, 1.45	73	1.10
	OgA-A	35	135	1.49, 1.39	68	2.40
	3b1a-B	35	140	1.60, 1.54, 1.45, 1.43	68	1.38
III	3b1a-A	40	163	4.1, 3.5	87, 83, 78	2.10
	F6-A	40	159	4.3, 3.2	87, 83, 78	1.14

\* Modal values. The diam. of between 75 and 250 particles of each virus were measured and histograms were constructed of numbers of particles of each diam. in 1 nm intervals. Clear modes were obtained in every case.

† Measured in PK buffer at very low concentrations ( $A_{260}$  0.5 to 0.7). Values are means of three determinations. In no case did any of the three values differ by more than 2 units from the mean value.

‡ All dsRNAs and polypeptides have been tested for identity or non-identity of mol. wt. by co-electrophoresis. The dsRNA species which differ in mol. wt. by  $0.02 \times 10^6$  and polypeptide species which differ in mol. wt. by  $1 \times 10^3$  have been shown to be separated in gel electrophoresis.

§ P, Virus precipitated during electrophoresis.

|| NT, Not tested because virus was obtained as a precipitate.

¶ This dsRNA component was present in virus isolated from the *G. graminis* isolate F6 and from nine single microconidial cultures (SM1 to SM7, SM9 and SM10) derived from F6; however, it was absent in virus from one derived microconidial culture (SM8).

homogeneous, in an analogous way to those of isolate 3b1a. From DEAE-cellulose, viruses F6-B and F6-C were eluted at 0.2 M-NaCl and 0.5 M-NaCl respectively.

#### *Physicochemical properties of G. graminis viruses*

The physicochemical properties of 13 viruses obtained from the eight *G. graminis* isolates are given in Table 2. On the basis of some of these properties the viruses were placed in three groups. It is noteworthy that when comparing the three groups there is a good correlation between the different properties of the viruses; the viruses with the greater dsRNA and capsid polypeptide mol. wt. also had greater sedimentation coefficients and, in the case of group III, particle diameter.

The capsids of viruses in groups I and II were constructed from a single polypeptide species. A second polypeptide of mol. wt.  $66 \times 10^3$ , found in virus 3b1a-B, varied significantly in amount (from 5 to 30% of the capsid protein) in different virus preparations and probably arose by proteolytic cleavage of the  $68 \times 10^3$  mol. wt. polypeptide during virus purification. The two viruses in group III had three capsid polypeptides; although possible relationships between them have not been investigated, the ratio of the polypeptides was similar in several different virus preparations.

The capsid polypeptides of some of the viruses in group II showed a tendency to form stable aggregates which migrated close to the top of the gel in SDS-PAGE, even after boiling the sample with 2% SDS and 2% mercaptoethanol. The degree of such aggregation varied with different virus preparations; it was always negligible in the case of virus OgA-A, but accounted for 10 to 30% of the capsid protein in virus 3b1a-B, 30 to 60% in virus T1-A and

Table 3. *Antiserum titres in gel diffusion tests with G. graminis viruses*

Group	Virus	Maximum reacting dilution of antiserum to							
		O19/6(M)*	O19/6(R)*	38-4(M)	OgA(M)	3b1a(M)	F6(R)	T1(M)	T1(R)
I	O19/6-A	64†	16 384	64	2	8	0‡	0	0
	38-4-A	<u>32</u>	<u>8192</u>	<u>128</u>	2	8	64	0	0
	O1-1-4-A	0	16 384	<u>64</u>	0	4	0	0	0
	OgA-B	0	16	0	<u>64</u>	0	16	0	0
	3b1a-C	16	4096	64	<u>0</u>	<u>16</u>	128	0	0
	F6-C	0	0	0	0	<u>0</u>	<u>1024</u>	0	0
II	T1-A	0	0	0	0	0	0	128	<u>4096</u>
	F6-B	0	0	0	0	16	<u>4096</u>	<u>0</u>	<u>16</u>
	OgA-A	0	0	0	<u>64</u>	0	<u>16</u>	0	16
	3b1a-B	0	0	0	<u>0</u>	<u>128</u>	128	0	0
III	3b1a-A	0	0	0	0	<u>512</u>	512	0	0
	F6-A	0	0	0	0	<u>128</u>	<u>4096</u>	0	0

\* M, Mouse antiserum; R, rabbit antiserum.

† Homologous titres are underlined.

‡ 0 indicates no detectable precipitin line formation at all antiserum concentrations tested (undiluted and twofold serial dilutions down to 1:1024).

90 to 100% in virus F6-B. In the case of virus F6-B, the proportion of monomeric polypeptide subunits detected by SDS/PAGE was not increased by prior treatment of virus protein with 8 M-urea or 6 M-guanidine chloride, followed by electrophoresis in 8 M-urea-SDS gels, by prior oxidation with performic acid, by prior reduction and carboxymethylation, by treatment with 0.5 M-sodium hydroxide or by several combinations of these treatments. The possibility that a proportion of polypeptide subunits may have become covalently cross-linked during virus purification as a result of the action of polyphenol oxidases (Bancroft *et al.*, 1971) was considered; however, inclusion of inhibitors, such as thioglycollate and diethyldithiocarbamate, in the virus extraction buffer led to a marked reduction of virus yield without increasing the proportion of monomeric polypeptide subunits detected.

In sucrose density-gradient centrifugation each of the 13 viruses sedimented as a single peak. However, when dsRNA components of fractions taken across a virus peak were analysed by gel electrophoresis it was found that their proportions varied. The slower sedimenting side of the peak contained a larger proportion of the dsRNA species of lower mol. wt. and the faster sedimenting side of the peak contained a larger proportion of the dsRNA species of higher mol. wt. In some cases, fractions only containing one dsRNA component were obtained, e.g. fractions of virus T1-A only containing the  $1.49 \times 10^6$  mol. wt. dsRNA and fractions of virus OgA-B only containing the  $1.22 \times 10^6$  mol. wt. dsRNA. These results indicate that the multiple dsRNA components are not enclosed in a single particle, but are separately encapsidated as found with most other isometric dsRNA mycoviruses (Hollings, 1978). The sedimentation coefficients given in Table 2 are therefore averages of the different component particles in each virus. Differences in sedimentation coefficients of individual component particles, however, were never large enough to result in the formation of more than one sedimenting boundary in analytical ultracentrifugation.

#### *Serological relationships among G. graminis viruses*

All serological studies were carried out using gel double-diffusion tests with broad-spectrum antisera, prepared by immunizing mice (M) or rabbits (R) with three injections of virus. None of the antisera reacted with dsRNA. Antiserum titres obtained with both homologous and heterologous viruses from the three virus groups are given in Table 3. In the

Table 4. Serological cross-reactions of *G. graminis* viruses in double-diffusion tests with pairs of viruses from one group\*

Group	Virus	Antiserum to						
		O19/6(M)	O19/6(R)	38-4(M)	OgA(M)	3b1a(M)	F6(R)	T1(R)
I	O19/6-A v 38-4-A	Spur	Spur	Spur	Fusion	Fusion		
	O19/6-A v O1-1-4-A		Fusion	Fusion		Fusion		
	O19/6-A v OgA-B		Spur		Spur			
	O19/6-A v 3b1a-C	Spur	Spur	Spur		Spur		
	38-4-A v O1-1-4-A		Spur	Spur		Fusion		
	38-4-A v OgA-B		Spur		Spur		Spur	
	38-4-A v F6-C						Spur	
	38-4-A v 3b1a-C	Fusion	Fusion	Fusion		Spur	Fusion	
	O1-1-4-A v OgA-B		Spur					Spur
	O1-1-4-A v 3b1a-C		Spur	Fusion		Fusion		Fusion
	OgA-B v F6-C						Spur	
	OgA-B v 3b1a-C		Spur				Spur	
	3b1a-C v F6-C						Spur	
	II	T1-A v OgA-A						
T1-A v F6-B								Spur
OgA-A v F6-B							Spur	Fusion
3b1a-B v F6-B						Spur	Spur	
OgA-A v 3b1a-B						Spur	Spur	
III	F6-A v 3b1a-A					Spur	Spur	

\* In the cases where no entry is made no test was carried out, because one or both of the viruses did not react with the antiserum.

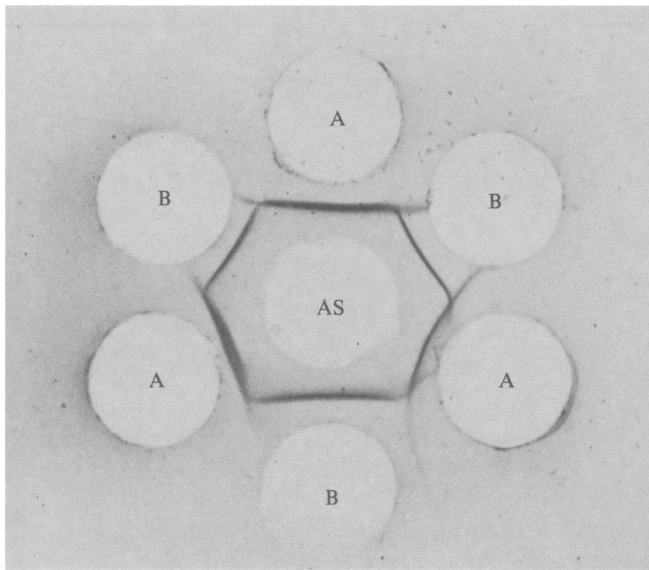


Fig. 2. Gel diffusion serological tests with viruses from *G. graminis* fungal isolates O19/6 and 38-4. A, Virus O19/6-A; B, virus 38-4-A; AS, rabbit antiserum prepared against virus O19/6-A.

case of antisera raised to single viruses, i.e. O19/6(M), O19/6(R), 38-4(M), T1(M) and T1(R), cross-reactions were only detected with heterologous viruses from the same group. Similarly, antiserum raised to a mixture of viruses from two groups, i.e. OgA(M), only showed cross-reactions with heterologous viruses from these two groups.

Table 5. *Serological tests with pairs of G. graminis viruses from different groups using homologous antisera prepared to the virus mixtures*

Antiserum	Pairs of viruses tested	Reaction observed
OgA(M)	OgA-A v OgA-B	C*
3b1a(M)	3b1a-A v 3b1a-B	C
	3b1a-A v 3b1a-C	C
	3b1a-B v 3b1a-C	C
F6(R)	F6-A v F6-B	C
	F6-B v F6-C	C
	F6-A v F6-C	C

\* C. Crossing of precipitin lines.

The lower titres of the mouse antisera compared to those of the rabbit antisera could be due to the viruses having different immunogenicities in the two animals or to the different modes of injection (three intramuscular injections in mice and one intravenous, followed by two intramuscular injections in rabbits). The disparate reactions of antisera O19/6(M) and O19/6(R) with virus O1-1-4-A may be due to different responses of different virus immunogenic determinants in the two animals, an effect which could also be accentuated by the different modes of injection.

Further tests were carried out by placing viruses in adjacent wells and, after diffusion against an antiserum to which both viruses reacted, examining the type of interaction between the precipitin lines. When pairs of viruses from the same group were tested in this way, either spur formation, or, less frequently, fusion of precipitin lines, occurred (Table 4). When a pair of viruses gave rise to a spur, this was formed by the virus which gave the higher antiserum titre, except in the case of O19/6-A v 3b1a-C against 38-4(M) antiserum when a short spur was formed by 3b1a-C even though the two viruses gave the same antiserum titre. A typical spurring reaction, exemplified by viruses O19/6-A and 38-4-A tested against O19/6(R) antiserum, is shown in Fig. 2. When a pair of viruses gave fusing precipitin lines their antiserum titres were either the same or within a factor of four of each other. When pairs of viruses from the same *G. graminis* isolates, but from different virus groups were tested against their homologous antisera, crossing precipitin lines were obtained (Table 5).

#### DISCUSSION

This study shows that the *G. graminis* viruses examined fall into three groups on the basis of physicochemical properties (sedimentation coefficient, dsRNA and polypeptide mol. wt.; Table 2). The grouping is supported by the serological studies; with the eight antisera used, no serological relationship between viruses of different groups could be detected. This conclusion was based on (i) the absence of cross-reactions between viruses from one group and antisera raised to single viruses of other groups (Table 3) and (ii) the formation of crossing precipitin lines when two viruses from different groups reacted with sera containing antibodies to both viruses (Table 5).

In contrast to the absence of serological relationships between viruses of different groups, relationships between viruses within a group were established by (i) reaction of one virus with antiserum to another (Table 3) and (ii) formation of single spurs or fusing precipitin lines when two viruses diffused against antiserum heterologous to both (Table 4). Serological relationships established in these two ways are listed in Table 6. In each group at least one virus had an antigenic determinant in common with each of the other viruses in the group (virus 45/9-A was not included in the gel immunodiffusion tests because it was obtained as a



Table 6. *Serological relationships established between viruses within groups*

Group	Test virus	Viruses having an antigenic determinant in common with the test virus
I	O19/6-A	38-4-A, O1-1-4-A, OgA-B, 3b1a-C
	38-4-A	O19/6-A, O1-1-4-A, OgA-B, 3b1a-C, F6-C
	O1-1-4-A	O19/6-A, 38-4-A, OgA-B, 3b1a-C
	OgA-B	O19/6-A, 38-4-A, O1-1-4-A, 3b1a-C, F6-C
	3b1a-C	O19/6-A, 38-4-A, OgA-B, O1-1-4-A, F6-C
II	F6-C	38-4-A, OgA-B, 3b1a-C
	T1-A	F6-B, OgA-A
	F6-B	T1-A, OgA-A, 3b1a-B
	OgA-A	T1-A, F6-B, 3b1a-B
	3b1a-B	F6-B, OgA-A
III	3b1a-A	F6-A

precipitate). The determinant in common, however, was not the same in every case. For example in group I virus OgA-B had antigenic determinants in common with virus O19/6-A and virus F6-C, but these two viruses themselves had no antigenic determinant in common. In fact, all viruses within a group were serologically distinguishable, either by differing antiserum titres (Table 3) or by formation of spurs, when pairs of viruses diffused against antiserum homologous to one of the viruses (Table 4). Thus, no two viruses had all their antigenic determinants in common. Viruses within a group were also distinguishable by electrophoretic mobility (Table 2), reflecting their differing net surface charges.

The serological relationships established suggest that the viruses within a group may be all variants of a single virus. Furthermore, within a virus group there is a striking conservation of capsid polypeptide mol. wt. In group I five out of seven viruses had a polypeptide mol. wt. of  $55 \times 10^3$ . The four viruses in group II had a polypeptide mol. wt. of either  $73 \times 10^3$  or  $68 \times 10^3$  and the two viruses in group III each had three polypeptides of identical mol. wt. Similarly, certain dsRNA mol. wt. appear to be conserved. For example, in group I four out of seven viruses have dsRNA components of mol. wt.  $1.27 \times 10^6$  and  $1.19 \times 10^6$ . The dsRNA mycoviruses do not lyse their hosts and transmission only occurs intracellularly either within a host during growth and cell division or between hosts via hyphal anastomosis (cell fusion). The viruses can therefore persist indefinitely in their infected hosts, creating an opportunity for the survival and selection of virus mutants. Hence, a virus found in a particular isolate of *G. graminis* may be the variant which replicates the most efficiently against the genetic background of that isolate.

Viruses O19/6-A, 38-4-A and 3b1a-C, all from English isolates of *G. graminis*, were the most closely related. However, it is noteworthy that virus O1-1-4-A (from *G. graminis* isolated in Japan in 1950) is serologically related to virus O19/6-A (from *G. graminis* isolated in England in 1972). These two viruses had identical titres and gave fusing precipitin lines, with no spur, with the rabbit anti-O19/6-A serum, indicating identity of antigenic determinants recognized by antibodies in this serum. However, virus O1-1-4-A did not react with the mouse anti-O19/6-A serum, indicating that virus O19/6-A had an antigenic determinant not possessed by virus O1-1-4-A. Other fairly close serological relationships between viruses from English isolates and a French isolate (F6) of *G. graminis* were also established (Tables 3 and 4). Virus T1-A from an Australian isolate of *G. graminis* was distantly related to viruses from an English isolate (OgA) and a French isolate (F6) of this fungus. These results imply transmission between continents of virus-infected propagules of *G. graminis* or, since virus infection may have arisen early in the phylogeny of the organism, of one of its ancestors.

Out of the eight *G. graminis* isolates studied, five isolates each contained only one virus, one isolate contained two viruses and two isolates each contained three viruses. Since field isolates were used which had not been cloned it was uncertain in the latter three isolates whether all viruses were present together in a single cell or whether these isolates were mixtures of *G. graminis* strains growing in close association, each with one virus. This possibility was tested in the case of the French isolate F6 by examining virus from 10 single microconidial cultures derived from this isolate. All 10 single microconidial isolates contained the three viruses found in the original isolate F6, indicating that all these viruses are present together in a single cell, since microconidia of *G. graminis* have been shown to be uninucleate (Deacon, 1976). Accumulation of two or more viruses by a fungus is a likely consequence of the intracellular mode of transmission of these viruses and the absence of cell lysis: mixed virus infections have been found in several other fungi (Hollings, 1978).

Isometric dsRNA mycoviruses appear to be of (at least) two basic types (Buck, 1980). In the first type the virus has an undivided genome of polycistronic dsRNA with a mol. wt. in the range  $3 \times 10^6$  to  $6.5 \times 10^6$ . Although a single dsRNA segment is sufficient for virus replication, in some fungal strains infected with this type of virus there are additional dsRNA segments, which may be satellite dsRNAs encoding specific proteins, e.g. M dsRNA of yeast which encodes killer protein (Bostian *et al.*, 1980), or may be deletion mutants, e.g. S dsRNAs of yeast (Bruenn & Kane, 1978), which may be similar to defective interfering RNAs of animal viruses (Huang & Baltimore, 1977). The *G. graminis* viruses of group III are probably of this first type, although whether one or both of the two dsRNAs found in these viruses are required for virus replication is unknown. In the second type of isometric dsRNA mycovirus the genome is divided, consisting of a minimum of two segments of monocistronic dsRNA, each with a mol. wt. in the range  $1 \times 10^6$  to  $2 \times 10^6$ , one encoding the single capsid polypeptide and the other the virus RNA-dependent RNA polymerase. The *G. graminis* viruses of groups I and II may be of this type. The capsid polypeptide requires the whole, or most, of the coding capacity of one dsRNA molecule and at least one further dsRNA molecule would be required to encode an RNA polymerase. The possibilities for the function of the additional dsRNAs associated with the viruses in some isolates are the same as discussed above. Because of the existence of satellite and/or defective RNAs in fungal viruses, we do not regard the number of dsRNA components, *per se*, in a virus as a useful property for mycovirus grouping.

The present work has revealed the existence of three different groups of *G. graminis* viruses, with serotype, dsRNA and polypeptide variation within each group. The particles of 27 nm diam. found in isolate F6, and in many other isolates of *G. graminis* (Rawlinson *et al.*, 1973), may require a further group. It is clear that future studies concerning possible biological effects of these viruses on their hosts must take the multiplicity and variation of the viruses into account.

We thank the Science Research Council for studentships (to M.A.R., M.R.A. and J.J.P.M.), Mr R. Woods for electron microscopy, and Dr J. L. Van Etten and Dr R. F. Bozarth for generous gifts of phage  $\phi$  dsRNA and *Helminthosporium maydis* virus dsRNA respectively.

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(Received 9 September 1980)