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Review

Polymyxa graminis and the cereal viruses it transmits: a research challenge

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SUMMARY

Polymyxa graminis is a eukaryotic obligate biotrophic parasite of plant roots that belongs to a poorly studied discrete taxonomic unit informally called the 'plasmodiophorids'. *P. graminis* is non-pathogenic, but has the ability to acquire and transmit a range of plant viruses which cause serious diseases in cereal crop species and result in significant yield reductions. The viruses are protected from the environment within *P. graminis* resting spores ('cysts') that may remain dormant but viable for decades (probably until a suitable host plant is encountered). The persistent, soil-borne nature of these diseases makes the use of virus-resistant crop varieties currently the only practical and environmentally friendly means of control.

Useful websites: http://www.rothamsted.bbsrc.ac.uk/ppi/links/pplinks/plasmod/index.html, http://www.dpvweb.net/, http://www.rothamsted.bbsrc.ac.uk/ppi/Iwgpvfv/index.html, http://www.rothamsted.bbsrc.ac.uk/ppi/links/pplinks/bymoviruses/index.html, http://oak.cats.ohiou.edu/~braselto/plasmos/

INTRODUCTION

A serious 'mosaic-like leaf mottling' or 'rosette disease' of winter wheat was first reported from the USA in the early 1920s (McKinney, 1923a). It was noticed that the causal agent of this disease appeared to be carried over from year to year in the soil, and that soil treatments with formaldehyde or steam prevented infection (McKinney, 1923b). In 1925, McKinney demonstrated that this disease could also be transferred from diseased to healthy wheat in infectious sap by needle-pricking inoculation, proving that it is caused by a virus, now named *Soil-borne wheat mosaic virus* (SBWMV). It was not clear, however, how this virus could survive in soil for many years and more importantly how it gained an entrance to the root cells. Various soil organisms and micro-organisms such as nematodes, fungi and bacteria were considered as possible carriers of SBWMV. It took more than 40 years from the first report of disease to establish a correlation with the presence of *Polymyxa graminis* (Canova, 1966; Estes and Brakke, 1966; Rao, 1968). It was only in 1969 that Rao and Brakke demonstrated in controlled laboratory conditions that *P. graminis* grown in roots of plants mechanically inoculated with SBWMV could acquire the virus and transmit it to healthy plants.

At present SBWMV is considered as one of the most important diseases in winter wheat in central and Eastern USA, because it can practically destroy an entire crop of a susceptible variety. SBWMV or similar viruses are also known to occur in Italy, France, Germany, Brazil, Argentina, China and Japan. *Soil-borne cereal mosaic virus* (SBCMV), a virus that shares ≈ 70% genome identity with SBWMV and by some authors is considered as a strain of SBWMV, was recently reported from several Southern locations in England (Clover *et al.*, 1999).

P. graminis is now recognized as a vector for many other plant viruses that belong to at least three separate genera, and are serious pathogens of several cereal crop species (Table 1). For example, the winter barley disease caused by *Barley yellow mosaic virus* (BaYMV) and/or *Barley mild mosaic virus* (BaMMV), is widespread in Europe, Japan and China where it is of great concern to farmers and the agricultural industry. Yield losses of > 50% may occur when susceptible barley varieties are grown on severely infested soils (Plumb *et al.*, 1986).

Chemical control of these soil-borne virus diseases is neither efficient nor acceptable for economic and ecological reasons. Therefore, considerable scientific efforts have been directed in recent years mainly towards breeding for disease resistant varieties of cereal crops. However novel pathotypes of the viruses that can overcome resistance genes available in breeding programmes are continually emerging (Adams, 1991; Hariri *et al.*, 1990, 2003; Huth, 1989; Steyer *et al.*, 1995). Therefore, more detailed knowledge of the biology and molecular interaction between soil-borne viruses, *P. graminis* and cereal crops is urgently required to help

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Table 1 Cereal viruses transmitted by *Polymyxa graminis*

Virus*	Acronym	Genus	Natural hosts	Distribution
Rice stripe necrosis virus	RSNV	*Benyvirus* (?)	Rice	West Africa, South and Central America
Barley mild mosaic virus	BaMMV	*Bymovirus*	Barley	Europe, Japan, China, Korea
Barley yellow mosaic virus	BaYMV	*Bymovirus*	Barley	Europe, Japan, China, Korea
Oat mosaic virus	OMV	*Bymovirus*	Oats	Europe, USA
Rice necrosis mosaic virus	RNMV	*Bymovirus*	Rice	Japan, India
Wheat spindle streak mosaic virus	WSSMV	*Bymovirus*	Wheat, rye, triticale	North America, Europe
Wheat yellow mosaic virus	WYMV	*Bymovirus*	Wheat	Japan, China
Chinese wheat mosaic virus	CWMV	*Furovirus*	Wheat	China
Oat golden stripe virus	OGSV	*Furovirus*	Oats	Europe, USA
Soil-borne cereal mosaic virus†	SBCMV	*Furovirus*	Wheat, rye, tricale	Europe
Soil-borne wheat mosaic virus	SBWMV	*Furovirus*	Wheat, barley, rye, triticale	North America, ?elsewhere
Sorghum chlorotic spot virus	SrCSV	*Furovirus*	Sorghum	USA
Peanut clump virus	PCV	*Pecluvirus*	Peanut, sorghum	India, West Africa
Aubian wheat mosaic virus	AWMV	?	Wheat	France, UK

*Formally accepted virus species appear in italics, and tentative species are in the regular font.
†Includes isolates previously named European wheat mosaic virus or Soil-borne rye mosaic virus.

develop novel options for control or even to prevent these diseases before they become widespread.

POLYMYXA GRAMINIS

Taxonomy

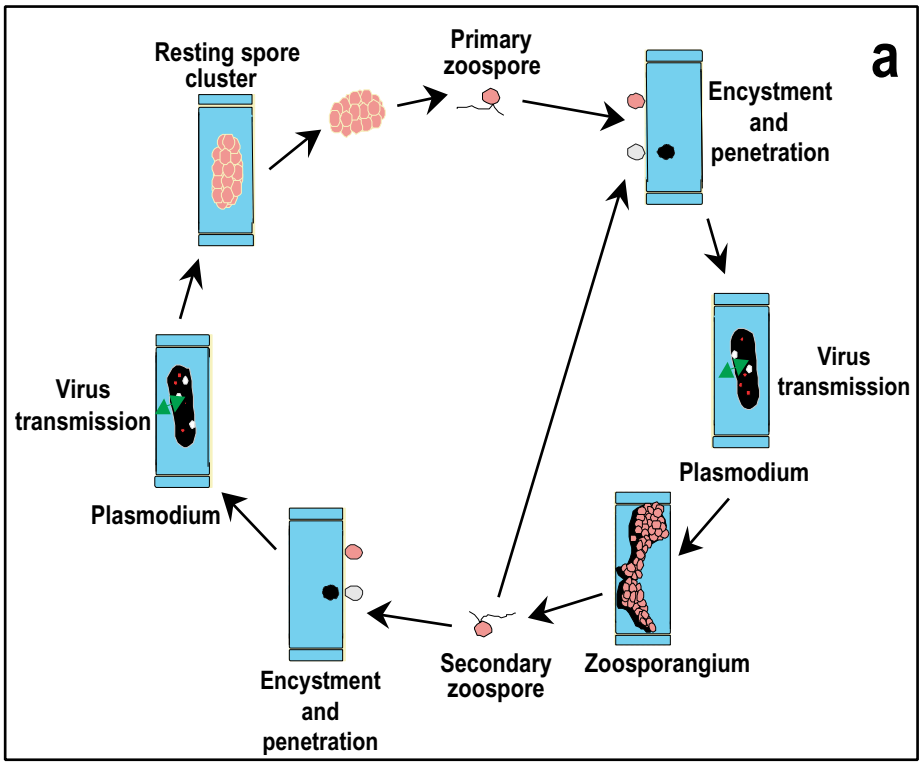
After more than a century of research on plasmodiophorids, their systematic affinities are still a matter for debate. Traditionally considered as fungi, analysis of their small subunit rDNA sequences has shown that they are a monophyletic group that is not closely related to the true Fungi nor to other zoosporic plant parasites (the straminipiles, which include the oomycetes, and the chytridiomycetes). Their most appropriate classification appears to be within the protists, a highly diverse group of eukaryotic organisms. They seem to be most closely related to the *Phagomyxida* (*Phagomyxa* sp.) and *Maullinia ectocarpii*, parasites of diatoms and filamentous algae (Bulman *et al.*, 2001; S. Bulman, E. Ward and I. Maier, unpublished; Maier *et al.*, 2000). There are more distant relationships to the chlorarachneans and sarcomonads, e.g. *Chlorarachnion* sp., *Cercomonas* sp., *Heteromita globosa*, *Paulinella chromatophora* and *Euglypha rotunda* (Bulman *et al.*, 2001; Cavalier-Smith, 2000; van de Peer *et al.*, 2000).

Plasmodiophorid taxonomy and morphology have been most recently reviewed by Braselton (1995) and Dick (2001). They have been classified in the order *Plasmodiophorales* (*Plasmodiophorida*), and family *Plasmodiophoraceae* (*Plasmodiophoridae*) and a total of 10 genera are recognized: *Polymyxa*, *Spongospora*, *Plasmodiophora*, *Ligniera*, *Membranosorus*, *Octomyxa*, *Sorodiscus*, *Sorosphaera*, *Tetramyxa* and *Woronina*. *Polymyxa graminis* and several other species of the first three genera are of significant agronomic importance. For example *Plasmodiophora brassicae* causes the important clubroot disease of brassicas, whilst *Spongospora subterranea* is the agent of powdery scab of potato and is also a virus vector. Phylogenetic analysis of rDNA suggests that *Ligniera* and *Sorosphaera* are very closely related to *Polymyxa*, while *Spongospora* and *Plasmodiophora* are more distantly related (Bulman *et al.*, 2001; Ward and Adams, 1998).

Within the genus *Polymyxa*, two species have been recognized largely on the basis of host range. *P. graminis* primarily multiplies in grass and cereal species while *P. betae* is a parasite of species in the family *Chenopodiaceae* and some related plants, and is also a virus vector, e.g. of rhizomania disease in sugar beet.

All plasmodiophorids share the following distinctive features: (i) an unusual 'cruciform' type of nuclear division whereby the nucleolus is elongated perpendicularly to the plane of the metaphase chromatin (Braselton, 1995); (ii) zoospores with two, anterior whiplash flagella of unequal length (Fig. 1); (iii) multinucleated plasmodia (protoplasts) (Fig. 1); (iv) obligate, intracellular parasitism; and (v) resting spores ('cysts'; Fig. 1) that can survive for many years in various environments (Braselton, 1995).

Fig. 1 *Polymyxa graminis*. (a) Diagram of the life cycle; (b) scanning electron micrograph of a single biflagelate zoospore; (c) scanning electron micrograph of a single sporosorus; (d) multilobed zoosporangium in a barley root (Trypan Blue staining); (e) wheat root cells packed with clusters of resting spores (acid fuchsin staining); (f) sporangial and sporogenic development may occur in the wheat root in adjacent cells (rs: clusters of immature resting spores; sp: sporogenic plasmodium; zs: zoosporangium); (g) diagram of zoospore encystment and penetration of root cells (S: Stachel, R: Rohr, A: adhesorium, N: nucleus, V: vacuole, L: lipid droplet). Re-drawn after Williams (1973).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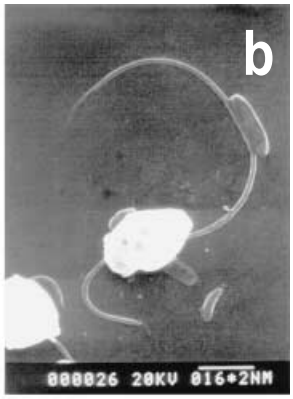
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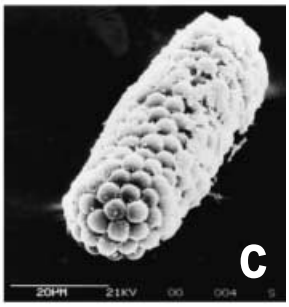
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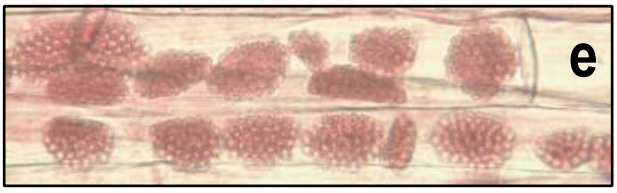
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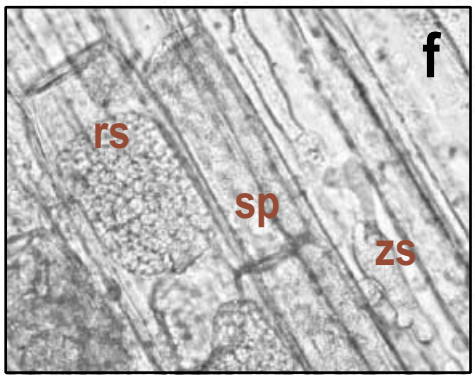
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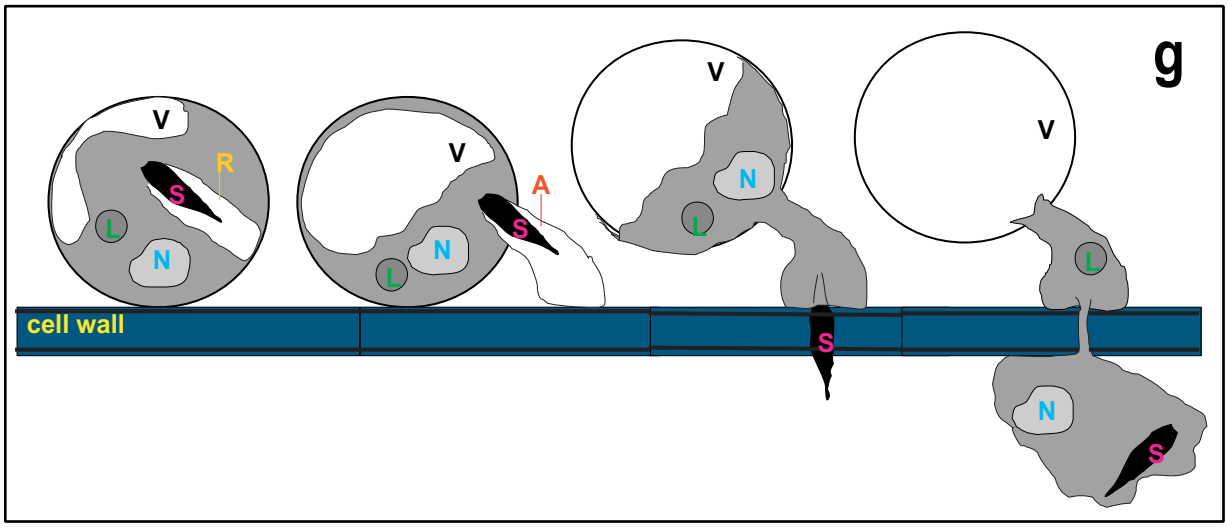
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Life cycle

P. graminis has a life cycle characteristic for most of the plasmodiophorids which consists of two phases (Fig. 1): (i) sporangial (primary), resulting in the production of zoospores, and (ii) sporogenic (secondary), resulting in the production of resting spores.

Each phase is initiated by the attachment of zoospores and penetration of epidermal or root hair cells (Fig. 1). This latter process is unique for plasmodiophorids and involves: (i) encystment of the zoospore at the surface of the host cell wall; (ii) development in the encysted zoospore of a tubular structure (Rohr) that contains a dense dagger-like body (Stachel); (iii) production of an adhesive outgrowth (adhesorium/appresorium) from the encysted zoospore; (iv) almost instantaneous injection of the Stachel and zoospore contents through the adhesorium, host cell wall and plasma membrane into the cytoplasm of plant root cell (Aist and Williams, 1971; Keskin and Fuchs, 1969).

The zoospore contents enlarge within the host cell, undergo several cycles of synchronous mitotic 'cruciform' nuclear divisions and reduction in nuclear size (Braselton, 1995), and eventually develop into a multinucleate sporangial plasmodium separated from the host cytoplasm by a distinct cell wall. Several septa are formed within the zoosporangium, dividing it into lobes that expand in volume followed by several cycles of 'non-cruciform' mitotic nuclear divisions, and many form exit tubes extending to the host cell wall. Then the secondary zoospores are cleaved apart, become rounded when they mature, and the septa between zoosporangial segments disintegrate. Mature secondary zoospores are released either outside of the root, or into the adjacent root cells via specialized exit tubes that dissolve an opening in the host cell wall (Littlefield *et al.*, 1998).

Secondary zoospores either initiate a new sporangial phase resulting in the production of a new generation of secondary zoospores, or develop into sporogenic plasmodia and resting spores. Factors that determine the sporangial vs. sporogenic development phase for *P. graminis* are unknown, and there is an apparent overlap of the presence of the two phases. During the first 3–5 weeks after primary infection, both types of plasmodia can often be seen within the same host root in adjacent cells (Fig. 1). At early developmental stages, sporangial and sporogenic plasmodia are anatomically very similar, but at later stages sporogenic plasmodia can be distinguished as they frequently fill the whole cross-section of host cells. Moreover, sporogenic plasmodia are separated from the host cell cytoplasm by only a thin membrane layer (Littlefield *et al.*, 1998). In addition, the 'non-cruciform' meiotic nuclear divisions characterized by the formation of synaptonemal complexes can only be observed in mature sporogenic plasmodia immediately prior to or during cleavage into immature resting spores (Braselton, 1995). Immature resting spores are tightly packed and angular shaped, but they become more rounded with a multilayered cell wall when they mature. Mature resting spores are usually grouped in clusters (cystosori/sporosori) with characteristic morphology (Fig. 1). The resting spores can survive in soil for several decades, and in suitable environmental conditions they each release one primary zoospore upon germination.

Virus acquisition

How and when *P. graminis* acquires viruses, and how viruses enter the host plant cell cytoplasm is unknown. However, it is likely that these processes are taking place either when zoospores penetrate the host cells and transfer their contents into the host cell cytoplasm, or at the sporogenic plasmodia stage of *P. graminis* development when there is only a thin membrane boundary separating the plasmodiophorid from the host cell cytoplasm. Acquired viruses are thought to be carried inside the *P. graminis* resting spores and zoospores. Viruses cannot be removed from zoospores by washing or inactivated by application of antiserum. Moreover, resting spores remain viruliferous, even after treatments with diluted NaOH and HCl (Rao and Brakke, 1969). BaMMV particles have been observed inside zoospores and zoosporangial plasmodia (Chen *et al.*, 1991), but this has not been demonstrated for resting spores mainly because of the impermeability of their multilayered wall that renders their ultrastructure difficult to study.

It is not known whether the viruses are able to multiply inside *P. graminis*, but there is indirect evidence that suggests that they do not, at least for BaMMV. When viruliferous isolates of *P. graminis* have been grown in the roots of a virus-resistant host, the released zoospores no longer contain or transmit virus (Adams *et al.*, 1987; McGrann and Adams, 2003).

Molecular diversity and phylogenetic relationships

Polymyxa sp. are obligate biotrophs and can only be maintained in the roots of host plants. Therefore it is generally difficult to obtain good quality DNA for molecular studies that is free from the contaminating DNA of the host plant or other root parasites, especially when samples are collected in the field. Moreover, even in experimental glasshouse conditions, *P. graminis* multiplication is slow, taking 3–4 weeks to produce zoospores and approximately 2–3 months to produce resting spores. *Polymyxa* isolates for molecular studies preferably need to be initiated from a single cystosorus, and propagated on host plant roots in semi-sterile sand cultures (Adams *et al.*, 1986). Several reliable protocols have been developed for the preparation of DNA from zoospores and resting spores of *Polymyxa* sp. (Mutasa *et al.*, 1993; Ward *et al.*, 1994). A subtractive hybridization approach has also been used in an attempt to obtain *P. graminis*-specific DNA from plant roots (Subr *et al.*, 2002).

Molecular diversity and phylogeny of isolates of *Polymyxa* species have been studied using RFLPs and sequencing of transcribed

Table 2 Subgroups of *Polymyxa graminis* (after Legrève *et al.*, 2002)

Proposed *forma specialis**	rDNA subgroup*,†	EMBL accession no.	Natural host	Optimum temperature	Distribution
temperata	I	Y12824, AJ311572-4	barley, Poa sp.	15–20 °C	Belgium, Canada, China‡, France, Germany , UK
tepida	II	Y12826	barley‡, wheat, oats, rye§	15–20 °C	Canada, UK, France, Germany§
tropicalis	III	Y12825, AJ311575-6, AJ311580	sorghum, pearl millet, maize	> 23 °C	Tropical regions—India, Senegal
subtropicalis	IV	AJ311577-9	sorghum, pearl millet	> 23 °C	Subtropical regions—India, Pakistan
colombiana	V	AJ010424	rice¶	> 23 °C	Colombia¶

*Legrève *et al.* (2002).
†Ward and Adams (1998).
‡Subgroup inferred by RFLP analysis only, not from sequencing (Ward *et al.*, 1994).
§Subgroup inferred by Pgfwd2/Pxrev7 PCR product size only (Subr *et al.*, 2002).
¶Morales *et al.* (1999).

regions of ribosomal RNA genes (nuclear ribosomal DNA, rDNA). Fragments of nuclear 18S, 5.8S and internal transcribed spacers (ITS1 and ITS2) rDNA regions have been analysed (Legrève *et al.*, 2002; Morales *et al.*, 1999; Ward and Adams, 1998; Ward *et al.*, 1994). In these studies, the two *Polymyxa* species can be clearly distinguished, and there are several subgroups of *P. graminis*. These distinct rDNA sequence types appear (to some extent at least) to be related to the host range, temperature requirements and geographical origin, and this has prompted Legrève *et al.* (2002) to propose classifying them as different *formae speciales* (Table 2). The variation observed is far greater than would generally be expected between isolates of the same species; sequence differences of more than 20% across the two ITS regions can be seen between some isolates.

Ribosomal DNA sequences have also been used to develop *Polymyxa*- and *P. graminis*-specific PCR tests that allow *P. graminis* subgroup determination by amplicon size or RFLP analysis (Morales *et al.*, 1999; Ward and Adams, 1998; Ward *et al.*, 1994; E. Ward, unpublished).

Polymyxa-specific antibodies have also been developed (Delfosse *et al.*, 2000; Mutasa-Gottgens *et al.*, 2000). These antibodies showed little cross-reactivity to other root parasites and may be useful in the quantification of *Polymyxa* in plants and soil, and in aiding localization of *Polymyxa* sp. in their hosts by microscopy.

Epidemiology

Effects of environmental factors on the development of *P. graminis* have been studied in glasshouse experiments using irrigated sand cultures. Temperate isolates were able to grow and infect barley roots over a wide temperature range, but the fastest development occurred at 17–20 °C with a minimum time from inoculation with resting spore to secondary zoospore production of about 2 weeks (Adams and Swaby, 1988). Tropical isolates associated with *Peanut clump virus* had a higher temperature optimum of 27–30 °C (Legrève *et al.*, 1998).

P. graminis has been detected in roots of cultivated wheat, barley, rice, oat and rye, from *Zea and Sorghum* species (Langenberg, 1984; Thouvenel and Fauquet, 1980), Bermuda grass and creeping bent grass (Britton and Rogers, 1963; Dale and Murdock, 1969) as well as various temperate *Agrostis*, *Dactylis*, *Festuca* (turf grass), *Poa and Phleum* species (Canova, 1964). While this is worrying, because all these wild grass species might serve as reservoirs for *P. graminis*, the extent to which different isolates can infect all these hosts is largely unknown. Most isolates studied in detail have originated from barley and these multiplied best on barley and usually less well on wheat; isolates from wheat may have multiplied better in wheat than did the barley isolates and none of the isolates multiplied appreciably in oats (Adams and Jacquier, 1994). Further work is needed to determine the degree of specialization to different hosts and to explore further the susceptibility of weed hosts to the viruses that *P. graminis* transmits.

SOIL-BORNE CEREAL VIRUSES

Taxonomy and genome organization

The best studied cereal viruses known to be transmitted by *P. graminis* have single-stranded RNA genomes divided into two components and are classified in the genera *Bymovirus* and *Furovirus* (Table 1). RSNV is an important emerging disease in South America and probably has more RNA components (Morales *et al.*, 1999), but there is limited information on the virus and it will not be considered further here.

Table 3 Percentage nucleotide identity between the complete genomes of the viruses in the genera *Bymovirus* and *Furovirus*

Bymovirus	BaYMV	OMV	WSSMV	WYMV
BaMMV	48.2	47.3	46.9	48.1
BaYMV		59.2	64.6	65.6
OMV			59.5	59.4
WSSMV				65.5
Furovirus	OGSV	SBCMV	SBWMV	SrCSV
CWMV	63.2	66.2	70.3	55.6
OGSV		62.5	63.3	56.0
SBCMV			68.8	55.0
SBWMV				54.9

The genus *Bymovirus*, family *Potyviridae*, is a well-defined group of viruses that resemble the aphid-transmitted potyviruses and other members of the family in having flexuous filamentous particles (12–13 nm in diameter) (Fig. 2) and in causing 'pinwheel' inclusions in infected leaf cells. The family members have many similarities in genome organization but the bymoviruses are distinctive in having bipartite genomes and *P. graminis* transmission (Berger *et al.*, 2000). Each RNA encodes a single polyprotein that is subsequently processed by viral-encoded proteases (NIa-Pro on RNA1, P1 on RNA2) into (probably) 10 functional proteins (Fig. 3). Within the genus, many of the viruses are serologically related but there are substantial differences in their nucleotide sequences and also in their host range (Tables 1 and 3).

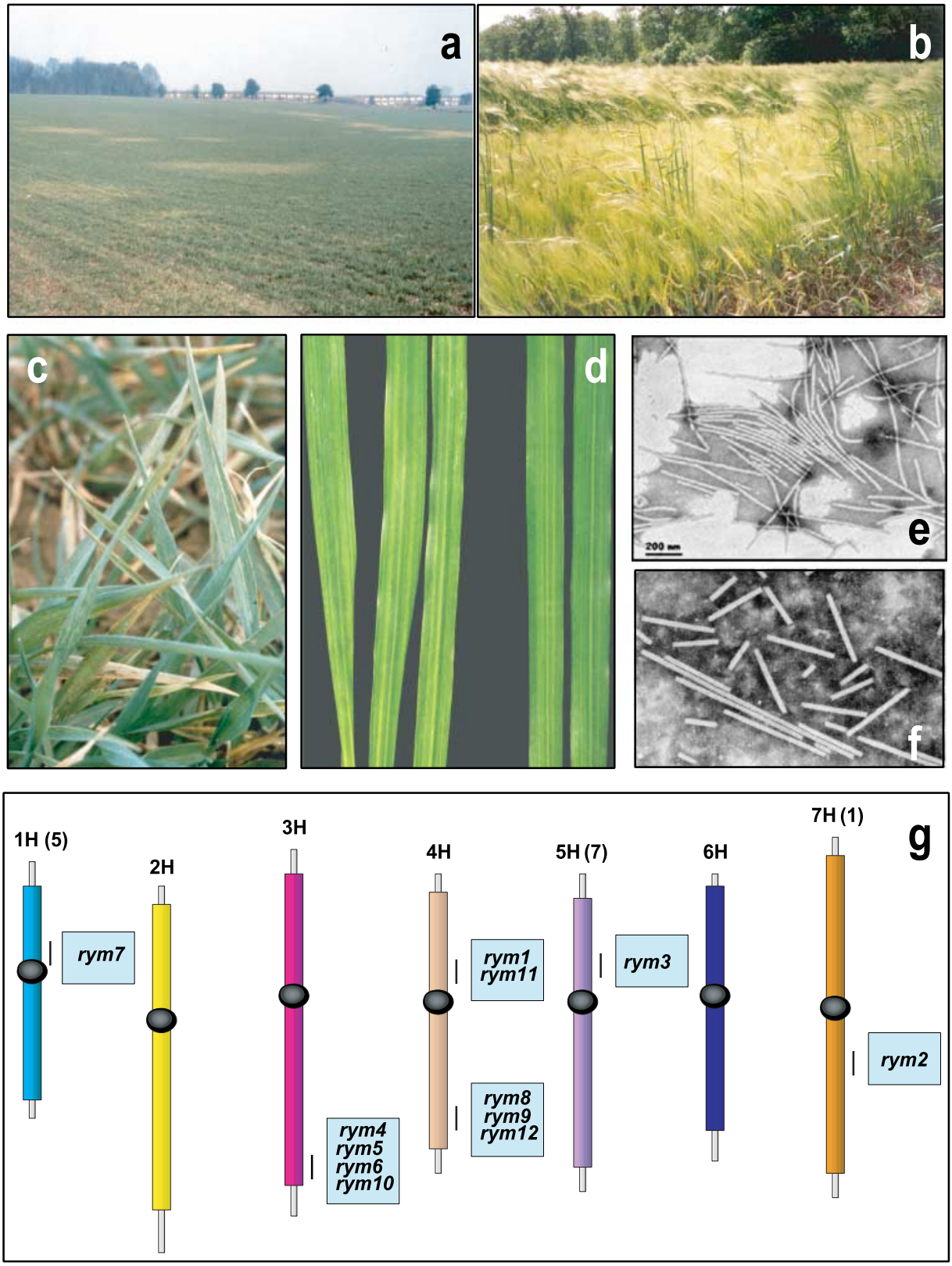
The genus *Furovirus* has not yet been assigned to a family and consists of viruses with bipartite genomes, *P. graminis* transmission, rod-shaped particles (*c.* 20 nm in diameter) and a consistent genome organization (Figs 2 and 3). RNA1 encodes a large replication protein, with helicase and methyl transferase domains, which can be extended through a 'leaky' stop codon to incorporate an RNA-dependent RNA polymerase that is most closely related to those in the genera *Pomovirus*, *Pecluvirus* and *Hordeivirus* (Diao *et al.*, 1999). A gene towards the 3′-terminus of RNA1 is probably translated from a subgenomic mRNA and encodes a movement protein that is a member of the '30K' superfamily (Melcher, 2000) and has some relationship to that of the dianthoviruses. RNA2 encodes the coat protein (with read-through domain) and, probably translated from a subgenomic mRNA, a small cysteine-rich protein that is related to those encoded by members of the genera *Pecluvirus* and *Hordeivirus* (Diao *et al.*, 1999). Within the genus, the viruses are serologically related to one another and there is still some discussion about the criteria for distinguishing species, particularly amongst those isolates from wheat (Shirako *et al.*, 2000). However the large differences in nucleotide identity (Table 3), and the absence of intermediate isolates, convincingly suggests that the viruses from USA, Europe and China should be considered distinct.

Biology and molecular biology

The distinctive biology of the viruses is largely related to their mode of transmission. All the viruses cause symptoms in the late winter and spring on cereal crops sown in the previous autumn (Fig. 2). The primary zoospores of the vector penetrate root hairs or epidermal cells in the autumn, while the soil is moist but before it becomes too cold, and the virus is subsequently introduced into the host cytoplasm. The manner in which the virus moves (as virions or as RNA) has not been determined but its RNA and coat protein can be detected in root cells before symptoms appear in the leaves (Peerenboom *et al.*, 1996). Systemic symptoms typically appear in the newly emerging leaves when plants begin to grow again after a period of cold weather and this may be related to a temporary reversal of the major direction of phloem transport (Schenk *et al.*, 1995). There are also reports suggesting that SBWMV moves upward within the xylem of the host plant (Verchot *et al.*, 2001) but the details of virus movement and of loading/unloading at the vascular system require further investigation. Infected plants have yellowed leaves, typically with distinctive mosaic or stripe symptoms and are usually seen as irregular patches in the field, typical of a soil-borne disease (Fig. 2). As temperatures rise in late spring, symptoms may fade and upper leaves can be symptom-free. However, crops may remain stunted, and yield losses of 30% in infected patches are not uncommon. The viruses mostly have an extremely restricted host range and survive for many years between crops in the resting spores of their vector. Seed transmission has occasionally been claimed for SBWMV (or a similar furovirus) in rye (e.g. Garbaczewska *et al.*, 1997) but does not usually seem to be a major factor in virus epidemiology.

Progress in understanding the molecular biology of the viruses has been limited by the difficulties in obtaining infectious clones that can be reliably used in experiments. Some advances have been made in understanding potyvirus molecular biology (e.g. Revers *et al.*, 1999) and it is likely that bymoviruses will prove to be similar but there are no experimental data on the role of the different functional proteins in, for example, virus replication, cell-to-cell movement or long distance transport. Following repeated

Fig. 2 *P. graminis*-transmitted viruses of cereals. (a) irregular yellow patches in a barley field, typical of BaYMV infection in the winter; (b) a patch of stunted barley plants in the field (front), typical of BaMMV infection in the late spring; (c) barley leaves showing typical BaYMV symptoms; (d) wheat leaves showing typical SBCMV symptoms (left); (e) electron micrograph of flexuous rod-shaped virions of BaMMV (stained with sodium phosphotungstate); (f) electron micrograph of rigid rod-shaped virions of SBCMV (stained with sodium phosphotungstate); (g) schematic diagram of the barley genome showing the map location of the *rym* genes which confer resistance to BaYMV and/or BaMMV.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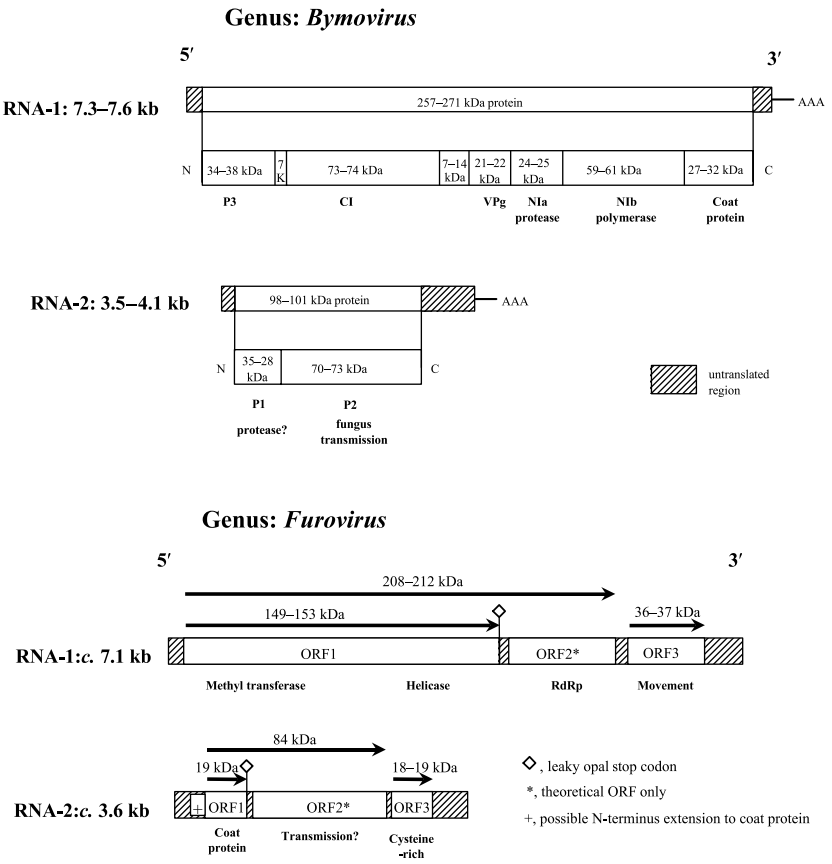

Fig. 3 Genome organization of viruses in the genera *Bymovirus* and *Furovirus*.

mechanical inoculation, it is common to find shorter forms of BaMMV RNA2 as a result of deletions of 400–1200 nucleotides in the P2 cistron (Jacobi *et al.*, 1995; K. Kanyuka, unpublished; Timpe and Kühne, 1995) and similar events appear to occur in OMV (Zheng *et al.*, 2002) and in BaYMV (Kühne *et al.*, 2003). One of the deletions in BaMMV results in a loss of vector transmission (Adams *et al.*, 1988).

There are also relatively few experimental data for the furoviruses, although Yamamiya and Shirako (2000) have reported the construction of infectious clones to SBWMV. They used mutant clones to investigate the significance of a 120 nt N-terminal extension to the coat protein, initiated at a CUG codon, that had been detected by *in vitro* translation and in infected plants (Shirako, 1998). In their experiments, abolition of the N-terminal extension had no effect on virion assembly or systemic movement in wheat. They also investigated the 'leaky' UGA termination codon that results in an 84 kDa rather than a 19 kDa coat protein (Fig. 3) with a frequency of 10–20%, and showed that the read-through domain was also unnecessary for virion assembly or systemic movement. This was unexpected, because the coat protein read-through is known to be necessary for particle assembly in two other viruses transmitted by plasmodiophorids, *Beet necrotic yellow vein virus* (BNYVV) and *Potato mop-top virus* (PMTV), and antibody labelling experiments have demonstrated that it is predominantly located near one end of the virus particles (Cowan *et al.*, 1997; Haeberlé *et al.*, 1994; Schmitt *et al.*, 1992). Spontaneous deletions have been detected in the CP read-through domains of SBWMV (Chen *et al.*, 1994, 1995a,b) and OGSV (Diao *et al.*, 1999) following mechanical transmission or growth of plants at high temperatures. Although there are no experimental data for the cereal viruses, work with BNYVV and PMTV have demonstrated that deletions in this region abolish vector transmission (Reavy *et al.*, 1998; Schmitt *et al.*, 1992; Tamada *et al.*, 1996).

It therefore seems likely that the P2 protein of bymoviruses and the coat protein read-through domain of furoviruses, encoded by RNA2 components of these viruses, are involved in vector transmission. There is very little direct sequence similarity between these proteins but computer analysis predicts some structural similarity between them, and in particular the presence of two transmembrane regions. Examination of the amino acid sequences in these regions suggests that they could be closely paired within a membrane through structural and electrostatic complementarity (Adams *et al.*, 2001; Diao *et al.*, 1999). Non-transmissible deletion mutants lack the second of these regions. It therefore seems possible that these regions are involved in

attachment to the zoosporangial plasmalemma and assist virus particles to move between the cytoplasm of the plant host and that of the vector.

Pathogenicity

Disease control relies almost exclusively on the deployment of resistant crop cultivars (see below). For many of the viruses, there little is known about the interactions between virus strains and host genotypes or the viral determinants of virulence. The only significant exception is for the bymoviruses of barley, BaMMV and BaYMV. Seven different strains of BaYMV (in four groups) have been identified in Japan based on the response of differential cultivars: I-1, I-2, I-3, II-1, II-2, III and IV (Kashiwazaki and Hibino, 1995; Kashiwazaki *et al*., 1989). In Europe, two strains are recognized on the basis of the response of cultivars carrying the *rym4* resistance gene and the resistance-breaking strain, usually named BaYMV-2, is becoming increasingly important (Adams, 2002; Hariri *et al*., 1990; Huth, 1991). Several strains probably occur in China but these are less well defined (Chen *et al*., 1996). For BaMMV, two strains have been identified in Japan based on virulence to cultivars carrying the *rym5* resistance gene (Nomura *et al*., 1996) and a European isolate able to infect *rym5* cultivars has recently been reported (Hariri *et al*., 2003). Experimental evidence indicates that the virulence determinant is carried on the RNA1 of both viruses (Kashiwazaki and Hibino, 1996; Kühne *et al*., 2003) and comparisons of sequences of both European BaYMV strains strongly implicate the VPg cistron as the virulence determinant (Kühne *et al*., 2003).

DISEASE RESISTANCE

Although *P. graminis* is an obligate parasite of cereal roots, it is not considered a pathogen because it does not cause any disease, and does not seem to reduce crop yield. However *P. graminis* transmits several plant viruses that do cause serious diseases (Table 1). Since virus-containing resting spores of *P. graminis* persist in soil and crop debris for several decades, cultural practices for virus control such as crop rotations or delayed planting are of little value, whilst chemical control measures (methyl bromide fumigation of soil) are unacceptable for ecological reasons. Therefore, planting of resistant crop varieties offers the only practical and ecologically friendly method of control. Cereal crop varieties with good resistance to one or more of their respective soil-borne virus pathogens are commercially available, but to the best of our knowledge cereal genotypes with resistance to *P. graminis* have not been identified. Nevertheless, resistance to the vector could provide the opportunity to control more than one virus simultaneously and is therefore an attractive idea. Some *Hordeum bulbosum* lines appear to be resistant to *P. graminis* and might provide a source of resistance for barley breeding (Proeseler *et al*., 1999).

BaYMV and BaMMV

Considerable scientific efforts have been directed in recent years towards identification of resistant barley genotypes and breeding for resistant varieties to two bymoviruses, BaYMV and BaMMV. Exotic barley germplasms proved to be a rich source of resistance to these viruses. Interestingly, the resistance is nearly always inherited as a recessive trait (Ordon *et al*., 1996). So far, 11 recessive *rym* genes from various sources have been identified and genetically mapped. Some of these genes seem to map to the same genetic intervals and may well be allelic (see Fig. 2), but the experimental proof for this is missing. Each *rym* gene confers resistance to one or more pathotypes of the same virus, or to both viruses. For example, in Europe one recessive gene, *rym4*, confers resistance against all common strain isolates of BaYMV and BaMMV, and the majority of resistant commercial varieties carry this gene (Graner and Bauer, 1993). The *rym4* genotypes are known to be completely immune to these viruses but the mechanisms of action of the other *rym* genes are less well understood. McGrann and Adams (2003) have recently reported that *rym1*, *rym2*, *rym5 and rym11*, are similar to *rym4* in conferring immunity against BaMMV. On the other hand *rym7*, *rym8* and *rym10* specify a partial resistance that can be completely broken at higher temperatures (> 20 °C), and these genes are likely to act by restricting virus movement from the root system to the aboveground parts of the plant and/or by reducing virus multiplication. This is supported by the fact that these latter genotypes are completely susceptible to the virus when the leaves are mechanically inoculated (McGrann and Adams, 2003). The *rym9* gene is interesting and unusual, as it seems to be tissue specific, preventing leaf (but not root) infection (McGrann and Adams, 2003). In Europe two pathotypes of BaYMV, and two pathotypes of BaMMV have been described that are able to overcome *rym4*- and *rym5*-specified resistance, respectively (see above). Fortunately barley germplasms with other *rym* genes are available and can be used for improving resistance of European barley crops, for example by pyramiding of resistance genes (Ordon *et al*., 1999; Pellio *et al*., 2000; Werner *et al*., 2000).

None of the *rym* genes have been isolated so far, and therefore the nature of recessive resistance in barley to bymoviruses is unknown. The genus *Bymovirus* belongs to the family *Potyviridae*. It is interesting that amongst other members of the family, particularly the aphid-transmitted potyviruses, host plant resistance is more frequently recessive than dominant (Provvidenti and Hampton, 1992). The recessive inheritance and the lack of HR in resistant varieties when challenged by avirulent virus strains suggests that these represent a novel class of disease resistance genes. It has been suggested that this type of resistance is passive

and may be caused by the lack of a host factor essential for a particular step in infection (Revers *et al.*, 1999). It was recently demonstrated that a natural recessive resistance gene *pvr2* against potato virus Y (PVY, the type member of the genus *Potyvirus*) corresponds to the eukaryotic translation initiation factor eIF4E (Ruffel *et al.*, 2002), whilst a knock-out mutation in another member of *eIF4E* family in *Arabidopsis*, *eIF(iso)4E*, results in complete immunity to *Tobacco etch virus* (TEV) and *Turnip mosaic virus* (TuMV) (Lellis *et al.*, 2002). Therefore, by extrapolation it is conceivable that eIF4E- and/or eIF(iso)4E-mediated resistance also occurs in bymovirus–monocot interactions. This hypothesis is currently being tested in our laboratory.

WSSMV and OMV

WSSMV and OMV are bymoviruses that infect wheat and oat, respectively. Interestingly, although their genomes are similar to those of BaYMV and BaMMV, the mechanisms and inheritance of available resistance genes are different to the barley *rym* genes described above.

Many sources of resistance to WSSMV have been identified in hard red, soft red, and soft white winter wheat commercial varieties (Haufler and Fulbright, 1986; Jackson *et al.*, 1976), and in wild *Triticum* species (Cox *et al.*, 1994). The WSSMV resistance in commercial varieties is a highly heritable trait controlled either by a single dominant gene (Wiese *et al.*, 1974) or in an additive dominant manner by two loci (van Koevering *et al.*, 1987). This has been recently confirmed by Khan *et al.* (2000), who demonstrated that WSSMV resistance in the variety Geneva is predominantly controlled by a major gene on the long arm of chromosome 2D, and associates with molecular markers Xbcd1095 and Xcdo373. However, although resistance to WSSMV is effective in the field, it is not immunity, because the resistant varieties are readily infected via mechanical inoculation to the leaves. Moreover, even in the field, the virus and virus symptoms can be occasionally detected in a small proportion of tillers in some resistant individuals (Carroll *et al.*, 2002). Therefore, the WSSMV resistance probably acts by limiting distribution of the virus in the root system and/or subsequent virus transport from roots into the tillers.

Extensive screening programmes have identified resistance to OMV in various *Avena* species (Catherall and Boulton, 1969; Catherall and Valentine, 1987; Graham *et al.*, 1969; Uhr and Murphy, 1992). In contrast to the other bymoviruses discussed above, OMV resistance is inherited as a quantitative polygenic trait (Uhr and Murphy, 1992). Moreover, this resistance is a tolerance, because high titres of virus can be detected in roots and leaves of resistant plants, but the symptoms are either completely absent or only very mild, and the virus has little or no effect on yield and grain quality (Friedt and Ordon, 1995; Walker *et al.*, 1998).

RSNV

Crinkling disease of rice caused by RSNV was first reported in West Africa in the late 1970s, and a similar disease called 'entorchamiento' appeared in Colombia in 1991 (Morales *et al.*, 1999). This disease has spread rapidly throughout Colombia and is present in most of the important rice growing regions. It was also recently found in Panama and Brazil (Sedano Cruz & Calvert, 2003). High levels of resistance to 'entorchamiento' have not yet been detected in continuous evaluations of the commercial rice varieties. However, high levels of tolerance have been detected in the wild relative of rice *Oryza glaberrima* in several independent trials (Correa *et al.*, 2002). The resistance from *O. glaberrima* is easily transferable to cultivated rice, and several breeding lines have been produced. The genetic control of 'entorchamiento' resistance seems to be very simple and likely to be controlled by one or a few major genes.

SBWMV and related viruses

SBWMV and closely related viruses (SBCMV, CWMV) are serious pathogens of wheat that can cause dramatic yield losses in susceptible crops. In Europe, Latin America, USA, China and Japan, locally adapted disease resistant varieties are available. However all the resistant genotypes reported thus far display tolerance rather than resistance or immunity to the virus. All tolerant varieties are known to contain high virus levels in the root system, and low to moderate levels in the leaf tissues (Driskel *et al.*, 2002; Hunger and Sherwood, 1985). Resistant plants show lower disease severity and lesser reductions in height, grain yield and kernel weight than susceptible varieties (Hunger *et al.*, 1989). The SBWMV resistance is temperature-dependent, at least in some resistant varieties (e.g. Hawk, Newton), and is overcome when the plants are grown at temperatures > 23 °C (Myers *et al.*, 1993). The disease resistance in wheat is likely to operate in the roots and restricts either virus multiplication or virus vascular transport from the roots to the leaves (Driskel *et al.*, 2002; Hariri *et al.*, 1987; Myers *et al.*, 1993).

Inheritance of SBWMV resistance has been studied for several commercial wheat varieties from the USA, Brazil and Japan (Barbosa *et al.*, 2001; Dubey *et al.*, 1970; Merkle and Smith, 1983; Miyake, 1938; Modawi *et al.*, 1982; Nakagawa *et al.*, 1959). The results of these studies are contradictory. Some authors suggest resistance to SBWMV is controlled by a single dominant gene (Miyake, 1938; Modawi *et al.*, 1982). Other authors propose that two (Barbosa *et al.*, 2001; Merkle and Smith, 1983; Shaalan *et al.*, 1966) or even three genes (Nakagawa *et al.*, 1959) are required for the resistance. It is possible that these results reflect genuine differences between the different sources, but it is also possible that resistant and susceptible phenotypes were incorrectly identified because they were judged solely on the presence or absence of visible leaf symptoms and on growth habit. From our own experience, SBWMV symptoms in wheat may vary

considerably, depending on the plant genotype, the concentration and aggressiveness of the virus strain, as well as the environmental conditions (temperature, moisture, etc.). In fact, some genotypes, especially under glasshouse conditions, may show no visible symptoms in spite of the presence of high virus titres in both leaves and roots (K. Kanyuka, unpublished). Moreover, an uneven distribution of fertiliser, a nutrient deficiency or winter injuries may cause symptoms in the resistant genotypes that could easily be mistaken for SBWMV. Therefore it is very important for such genetic studies to combine a visual scoring of phenotypes with virus detection by ELISA or RT-PCR. Using such an approach it was possible to determine that the resistance to SBCMV in one of the wheat varieties commonly used in UK wheat breeding programmes is controlled by a single gene (K. Kanyuka, M. J. Adams and K. E. Hammond-Kosack, unpublished).

FUTURE DIRECTIONS

Progress in understanding and controlling the virus diseases has been hampered by the difficulties of working with an obligate, root-infecting, vector. However, these challenges must be met if many of the intriguing questions about the diseases are to be addressed. The following areas appear to us to be of particular interest and relevance:

(i) Molecular studies are required to clarify various aspects of the taxonomy and phylogeny of the plasmodiophorids.

(ii) The biological differences between the various subgroups of *P. graminis* need to be studied further (e.g. what is their host range, and do they transmit different viruses?).

(iii) Where and when does karyogamy occur in the *P. graminis* life cycle, and, if it does not occur, how do nuclei become capable of undergoing meiosis?

(iv) What controls the germination of resting spores?

(v) How do zoospores recognize host plants?

(vi) How and when does *P. graminis* acquire viruses, and release them into the host cell cytoplasm?

(vii) Does the double infection of barley with BaYMV and BaMMV, or wheat with SBWMV and WSSMV or WYMV require inoculation with zoospores carrying both viruses simultaneously or each virus separately?

(viii) Do the viruses replicate inside their vector?

(ix) What virus genes determine pathogenicity?

(x) What is the nature of recessive resistance genes that control bymoviruses of barley?

(xi) What is the nature of dominant resistance genes that control virus movement from root to shoot in wheat, barley and rice?

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