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Aubian wheat mosaic virus, a new soil-borne wheat virus emerging in France

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Abstract

The properties of Aubian wheat mosaic virus (AWMV), a new soil-borne wheat virus in France, were investigated. Symptoms include foliar mosaic and severe stunting of winter wheat. The vector of the disease is unknown but the plants infected carry *Polymyxa graminis* in the roots. AWMV was transmitted mechanically to wheat and to two dicotyledoneous species: *Lactuca sativa* and *Vicia faba*. This virus was transmitted by seed to three winter wheat cultivars tested. Purified preparations contained rod-shaped particles with a variable length of 150–700 nm. Certain particles are very long and appear flexible. Antiserum raised against AWMV reacted specifically with AWMV in both indirect and direct enzyme-linked immunosorbent assays (ELISA). The incidence of AWMV in 26 winter wheat cultivars was investigated in the field during the growing season of 1999–2000. AWMV was detected in roots and shoots of all cultivars regardless of the symptoms. Twelve virus species belonging to the genera *Benyvirus, Bymovirus*, *Furovirus*, *Pecluvirus* and *Pomovirus* did not react with the AWMV antisera. A new tubular virus described in winter wheat in Bedfordshire in England reacted strongly with AWMV in ELISA. It is concluded that AWMV and probably the Bedford-virus constitute a previously undescribed tubular virus biologically and serologically distinct from other soil-borne viruses of wheat.

Introduction

A new virus of winter wheat tentatively called Aubian wheat mosaic virus (AWMV) was described from an infected field located in the eastern part of the French Paris basin (Hariri et al., 1999). Leaf mosaic symptoms and stunting of susceptible cultivars were observed in patches suggesting a soil-borne virus. Purified particles from infected plants were rod shaped and appeared similar to the common wheat *Furovirus* usually found in France referred to as *soil-borne wheat mosaic virus* (SBWMV). Several *Furoviruses* of wheat have been distinguished according to their genome sequences. SBWMV, the type species, has been extensively studied in North America (Shirako and Wilson, 1993). *Chinese wheat mosaic virus* (CWMV) is serologically related to SBWMV and has the same genetic organisation but has been proposed to be a new species because of its distinct genomic sequence (Diao et al., 1999a). In Europe, different wheat *Furovirus* isolates previously described as SBWMV (Lapierre et al., 1985; Huth and Lesemann, 1990; Clover et al., 1999a) have been detected and resistant cultivars have been described (Lapierre et al., 1985; Vallega and Rubies-Autonell, 1985). More recently, from sequence data, it has been proposed to rename all these European isolates *soil-borne rye mosaic virus* (SBRMV) (Koenig et al., 1999), *soil-borne cereal mosaic virus* (Koenig and Huth, 2000) or European wheat mosaic virus (Diao et al., 1999b). Prior to the decision of the ICTV concerning the name(s) of European wheat *Furoviruses*, the French SBWMV-like isolates used in this study

will be called SBWMV-F herein. All European and Asiatic wheat *Furoviruses* as well as *oat golden stripe virus* (Plumb et al., 1977) and *sorghum chlorotic spot virus* (Kendall et al., 1988) are serologically related to SBWMV. By contrast, AWMV and another soil-borne-tubular virus of wheat (Bedford-virus) described in England (Clover et al., 1999b) do not react with *Furovirus* antisera.

The severe symptoms observed in plants of some cultivars infected by AWMV and the susceptibility of the wheat cultivars to this virus compared to SBWMV-F prompted us to determine its biological properties. Its relationship with the English Bedford-virus was studied using a serological diagnosis.

Materials and methods

Virus source

The field infected with AWMV is located at Thil (Aube department, 10, France). The wheat cultivar, Sponsor, which showed mosaic symptoms was used for mechanical inoculation and virus purification. Before each mechanical inoculation and virus extraction, the leaves were checked by enzyme-linked immunosorbent assays (ELISA) to ensure that they were not infected by SBWMV-F or the French *Bymovirus* of wheat: wheat yellow mosaic virus (WYMV-F) (Hariri et al., 1996). These two viruses were frequently found close to the AWMV-infected field. The field infected with SBWMV-F used for comparing cultivar behaviours is located at Chambon sur Cisse (Loir et Cher department, 41, France).

Transmission tests

From infected soil. Soil from the infected field was collected in autumn, dried and mixed with 50% sterile sand. The wheat cultivars, Charger and Sponsor, which are susceptible to AWMV, were sown in pots and maintained at 15 °C. The presence of the virus was checked in the leaves and the roots by ELISA 2 months after inoculation.

From seeds. The ears of plants of two cultivars in 1999 (Sponsor, Charger) and three cultivars in 2000 (Riband, Soissons, Sponsor) showing severe mosaic symptoms were harvested in four distinct areas (25 ears per area and per cultivar) in the AWMV-infected field. The seeds were sown in an AWMV-free field in September (1999 and 2000) and also in pots maintained in a growth cabinet at 15 °C. The leaves and roots of the plants were sampled from December to June and tested in ELISA.

By mechanical inoculation. The leaves of the wheat cultivar Sponsor infected with AWMV were ground in phosphate buffer 0.04 M pH 7.2. The plants of the different species were inoculated mechanically and maintained at 15 °C with 16 h photoperiod and 16,000 lux intensity. The plants were observed periodically for symptom expression during 2 months and tested in ELISA for virus detection. The leaves of cultivar Trémie showing mosaic symptoms were used for successive mechanical inoculations of wheat cultivars. The following plant species representing nine families were assayed: *Tetragonia tetragonioides* (Pall) (Aizoaceae); *Lactuca sativa* L. (Asteraceae); *Brasicca napus*, *B. chinesis* (Brassicaceae); *Cucumis sativus* (Cucurbitaceae); *Beta vulgaris*, *Chenopodium quinoa, C. amaranticolor, Spinacia oleracea* (Chenopodiaceae); *Phaseolus vulgaris*, *Pisum sativum*, *Vicia faba* (Fabaceae); *Datura stramonium*, *Lycopersicon esculentum* var. *Nicotiana benthamiana*, *N. glutinosa, N. tabacum*, *Petunia hybrida* (Solanaceae); *Arachis hypogaea* (Papilionaceae); *Avena sativa*, *Hordeum vulgare*, *Triticum aestivum*, *T. durum, Zea mays* (Poaceae).

Virus purification

Freshly harvested infected wheat leaves were homogenised in a Waring Blender in 0.1 M citrate buffer pH 7.2 containing 0.5 M urea and 1% Sodium sulphite. Generally, 300 ml of the buffer was used per 100 g of tissue. The homogenised extract was filtered through two layers of cheesecloth and then stirred for 2 h at 4 °C in the presence of v/v dichloromethane. The mixture was centrifuged for 30 min at $5000 \times g$. The supernatant was adjusted to 2% Triton X-100 emulsified by stirring the solution at room temperature for 2 min, then centrifuged at $8000 \times g$ for 5 min. The supernatant was ultracentrifuged at $180{,}000 \times g$ for 2 h through a cushion of 30% sucrose in citrate buffer. The pellet was resuspended in 0.1 M citrate buffer pH 7.2 containing 0.5 M urea and centrifuged at $4000 \times g$ for 3 min. The virus was purified by rate-zonal centrifugation in 7.5–30% sucrose density gradient in the same buffer at $140{,}000 \times g$ for 100 min. The fractions containing the viral particles were collected and

centrifuged at 200,000 × *g* for 3.5 h at 4 °C. The purified virus particles were resuspended in 1 ml of distilled water.

Electron microscopy

Virus particles from either leaf extracts or purified preparations were stained with 2% potassium phosphotungstate adjusted to pH 7 and examined by electron microscopy.

Antiserum production

A female New Zealand white rabbit was initially injected intramuscularly with 50 µg of purified virus mixed with Freund's complete adjuvant (v/v). Three intramuscular injections (100 µg purified virus mixed with Freund's incomplete adjuvant (v/v)) were administered 20, 30 and 40 days later, respectively. Antiserum was obtained from bleeds collected as of one week after the last injection.

ELISAs

Immunoglobulins were precipitated with ammonium sulphate, purified by DE 52 cellulose chromatography, and conjugated to alkaline phosphatase as described by Clark and Adams (1977).

The double-antibody sandwich (DAS-ELISA) was carried out essentially as described by Clark and Adams (1977) except that samples from roots and leaves of AWMV and other viruses (Table 1) were prepared by grinding in 0.1 M citrate buffer pH 7.2 containing 0.5 M urea. The wells of microtitre plates were coated with rabbit polyclonal antibodies (1 µg/ml) before overnight incubation at 5 °C with the tissue extracts. After washing the plates with PBS-T, antigen bound by polyclonal IgG was detected using homologous polyclonal IgG alkaline phosphatase conjugate. Following the addition of substrate, the OD value was recorded after 30–180 min at ambient temperature and again after subsequent overnight incubation at 5 °C.

The technique used for the antigen-coated plate (ACP) was similar to that described by Voller et al. (1979). The microtitre plates were coated with the purified particles of AWMV or leaf extract diluted in coating buffer and incubated overnight at 4 °C. After rinsing, the wells were saturated with 1% bovin serum albumin and incubated at 37 °C for 1 h. The plates were rinsed and coated with AWMV and other antibodies and incubated at 37 °C for 1 h. After rinsing, the plates were coated with goat anti-rabbit IgG coupled to alkaline phosphatase, incubated at 37 °C for 3 h, rinsed and finally p-nitrophenyl phosphate substrate was added as in DAS-ELISA.

Field experiments

Winter wheat cultivars were sown during October 1999 in the AWMV infected field at Thil. Two randomised replicates were planted. Samples were collected from the two replicates in April and May. On the different sampling dates, either individual plants or aggregate samples were collected from each cultivar at regular distances.

Table 1. Different viruses or antisera tested

Genera		Viruses	Sources	Antisera	Location	Suppliers
Furovirus	*Oat golden stripe virus*	OGSV-F	Oats (cv. Fringante)	OGSV-F	France	Authors
				SBRMV	Germany	W. Huth
	Soil-borne wheat mosaic virus	SBWMV-F	Wheat (cv. Soissons)	SBWMV-F	France	Authors
		SBWMV-I	Wheat (cv. Valnova)		Italy	V. Valega
Benyvirus	Rice stripe necrosis virus			RSNV	Africa	J.P. Thouvnel
	Beet necrotic yellow vein virus	BNYVV-F3	*Chenopodium quinoa*	BNYVV-F	France	O. Lemaire
Pomovirus	*Potato mop-top virus*	PMTV-S	*Nicotiana benthamiana*	PMTV-S	Scotland	D. Bourdin
		PMTV	Potato		Peru	M. Mayo
Pecluvirus	*Peanut clump virus*			PCV	Africa	J. Dubern
Unassigned		Bedford-virus	Wheat (cv. Riband)		England	G. Clover
	Aubian Wheat mosaic virus	AWMV	Wheat (cv. Sponsor)	AWMV	France	
Bymovirus	*Wheat yellow mosaic virus*	WYMV-F	Wheat (cv. Pernel)	WSSMV-F	France	Authors
	Barley yellow mosaic virus	BaYMV-F	Barley (cv. Plaisant)	BaYMV-F	France	Authors
	Barley mild mosaic virus	BaMMV-F	Barley (cv. Magie)	BaMMV-F	France	Authors

Polymyxa graminis *analysis*

Roots from the plants cultivar Charger and Sponsor infected in the field or in the growth cabinet using the method described by Adams et al. (1986) were mounted in sterile water on a slide and examined with a microscope for the presence of *P. graminis*.

Results

Virus purification

Initial attempts to use the SBWMV purification protocol (Shirako and Brakke, 1984) and other protocols designed for tubular viruses were unsuccessful in purifying the AWMV. Different virus extraction methods were compared using 0.1 M phosphate or citrate buffer containing 1% sodium sulphite, 0.5% 2-mercapthoethanol and 0.5 M urea. Citrate buffer with urea considerably reduced particle aggregation. In these conditions, with a leaf-buffer ratio of 1 : 3 (w/v), it was possible to use differential centrifugation to clarify the virus without unacceptable loss of virus particles. The best virus yield was obtained with fresh leaves at a young stage of development in the beginning of spring. Later in the season, despite severe mosaic symptoms, the yield was very low. From frozen leaves, the virus yield was very low.

Among the different clarifiers tested (chloroform, butanol, carbon tetrachlorure, dichloromethane), only the latter (v/v) gave good virus yields. However, after high speed centrifugation, pellets remained greenish. When 2% Triton X-100 was added after clarification of extracts by dichloromethane treatment, ultracentrifugation pellets were less contaminated.

The purification of the virus particles in CsCl and Cs_2SO_4 gradients was unsuccessful. In the 7.5–30% sucrose gradient, one wide UV-absorbing zone corresponding to the virus particles was usually observed.

Electron microscopy

Rod-shaped particles (150–700 nm in length) were consistently observed in extracts of leaves from infected plants showing symptoms. Certain particles were very long and slightly flexible. No cluster of particle length appeared clearly. The particles showed a central canal unstained by potassium phosphotungstate (Figure 1).

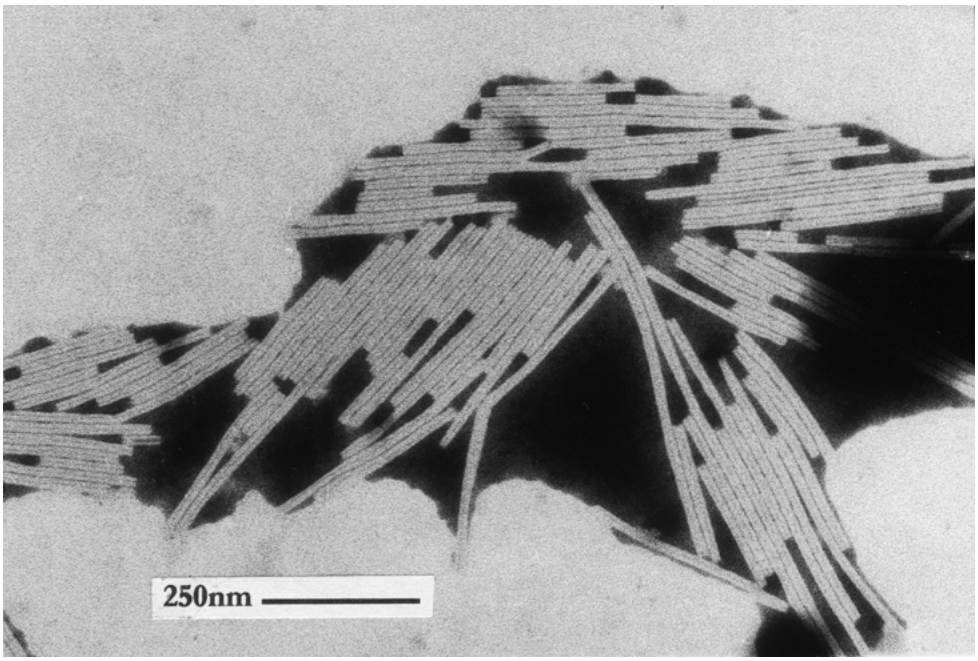

Figure 1. Electron micrograph of purified AWMV stained with 2% potassium phosphotungstate.

Reactivity of polyclonal antibodies raised against AWMV

The prepared antiserum reacted specifically with purified AWMV in ACP and DAS-ELISA. Positive reactions were obtained with infected leaves diluted up to 10^{-3} and 10^{-5} in DAS and ACP-ELISA, respectively.

Serological relationships between AWMV and other viruses

In DAS and ACP-ELISA, AWMV antibodies did not react with French isolates of SBWMV, OGSV, BNYVV, TMV, WYMV, BaYMV1, BaMMV, an Italian isolate of SBWMV and two Scottish and Peruvian isolates of PMTV. In the two ELISA systems, the Bedford-virus showed a strong reaction similar to those obtained with AWMV (Table 2a). The SBRMV antibodies did not detect AWMV, whereas it reacted with SBWMV-F and SBWMV-I in DAS and ACP-ELISA. None of the antibodies prepared against OGSV, BNYVV, WYMV, BaYMV, BaMMV, PMTV, RSNV and PCV detected AWMV in DAS- and ACP-ELISA (Table 2b).

Biological properties of AWMV

Soil transmission
The plants grown in the soil sampled from the infected field at Thil did not express mosaic symptoms after 3 months at 15 °C. However, AWMV was detected in roots and leaves of these plants in DAS-ELISA.

Root analysis
In plant roots infected with AWMV in the field or in the growth cabinet, the presence of numerous cystospores of *P. graminis* were observed.

Symptom expression
Disease symptoms were observed each year in January in the most susceptible wheat cultivars. The mosaic symptoms were indistinguishable from those of the WYMV-F and were characterised by a yellow appearance of the plots and chlorotic to necrotic streaks in the leaves (Figure 2a). Certain cultivars such as Sponsor and Charger showed severe stunting. The cultivar Riband showed mosaic symptoms similar to those caused by the Bedford-virus in England (Clover et al., 1999b).

Mechanical inoculation of winter wheat
AWMV detected by its antibodies was mechanically transmitted to wheat from field-infected plants with low efficiency (Table 3). Purified particles were not tested as inoculum. The percentage of infected plants was increased after three successive mechanical inoculations (Table 4). The cultivar Trémie, which showed low field infection, showed much higher infection after mechanical inoculation than two field-susceptible cultivars Sponsor and Charger.

Host range
Host range was investigated only by mechanical inoculation using field inoculum. The low mechanical transmission efficiency of wheat cultivars may explain the absence of infection of the other *Poaceae* species tested.

Surprisingly, in *V. faba* and *L. sativa*, systemic infection was observed and *V. faba* expressed mild necrotic spots on several leaves (Figure 2b). However, after a susceptible phase of about 30 days, the virus was not detected 50 days later (Table 3).

Seed transmission
From more than 3000 plants observed in the field, no expression of viral symptoms was seen. In the field, positive ELISA tests were obtained only in the roots in the early stages of the developing plants. The cultivar Riband showed the highest rate of transmission with 19% (Table 4). The infection rates of AWMV in the roots of cultivars Soissons and Sponsor were 2% and 10%, respectively. No detection was obtained in the growth cabinet on 2 month-old plants.

Response of wheat cultivars to AWMV
In 1999, of the 26 cultivars tested, 17 showed mosaic symptoms and of these 14 were stunted (Table 5). During the same season, some of the AWMV-susceptible cultivars, e.g. the cultivars Sponsor and Charger, remained resistant to SBWMV-F in a field infected by this virus (Table 5).

AWMV was detected in the leaves of all 26 cultivars tested regardless of the symptoms. However, the number of infected plants and the OD values in ELISA make it possible to distinguish two extreme responses from these cultivars. The cultivars Aztec, Brando, Capvern, Mercury, Noé, Ornicar and Trémie showed low ELISA values and less than 50% of plants were infected. The cultivars Charger, Fronty, Riband, Sponsor and Virtuose showed high ELISA values and more than

Table 2. Serological relationships of AWMV with different tubular and flexuous viruses

ELISA	*Furovirus*			*Benyvirus*	*Pomovirus*		?	?	*Pecluvirus*	*Bymovirus*			Healthy control[a]
	SBWMV-F	OFSV-F	SBWMV-I	BNYVV	PMTV-P	PMTV-S	AWMV	Bedford-virus	PCV	WYMV-F	BaYMV-F	BaMMV-F	
(a) *Reaction of different viruses with AWMV antiserum*													
ACP	0.09[b]	0.08	0.11	0.12	—	—	2.7	2.9	0.15	0.11	0.14	0.11	0.15
DAS	0.05	0.09	0.07	0.04	0.09	0.07	1.9	2.1	0.06	0.09	0	0	0.09
	SBWMV-F	SBRMV-G	OGSV-F	BNYVV-F	PMTV-F								
(b) *Reaction of AWMV with different antisera*													
ACP	0.09[b]	0.08	0.11	0.12	—		2.7			0.09	0.12	0.01	0.2
DAS	0.03	—	0.09	—	0.11		2.2			0.05	0.05	0.05	0.11

[a]Healthy plants of each species were used as control; highest OD values were presented.
[b]OD values present mean of four replicate wells per treatment measured 1 h after addition of substrate; OD values in bold indicate the positive reactions.
Plant extracts were used for all tests and positive controls using the homologous antisera were obtained for all viruses.
—: not tested.

(a)

(b)

Figure 2. (a) Symptoms of AWMV on wheat in the field (cultivar Sponsor). (b) Mechanically inoculated broad bean with AWMV showing systemic, necrotic spots.

Table 3. Reaction of plant species to mechanical inoculation with AWMV

Plant species	Symptoms		DAS-ELISA			
	30[a]	50[a]	30[a]	50[a]	30[a]	30[a]
Lactuca sativa (cv. Appia)	—[b]	—[b]	1/6[b,c]	0/6[b,c]	nt[d]	nt[e]
Triticum aestivum (cv. Trémie)	M	M	3/30	7/30	10/15	25/30
T. aestivum (cv. Soissons)	—	M	0/20	1/20	7/15	nt
T. aestivum (cv. Sponsor)	—	M	0/20	0/20	5/15	nt
T. aestivum (cv. Charger)	—	—	0/20	0/20	5/15	nt
Vicia faba	Sns	Sns	6/6	0/6	nt	nt

M: mosaic symptoms; Sns: systemically necrotic spots; —: no symptoms; nt: not tested.
[a]Days after inoculation.
[b]Inoculum prepared from field infected wheat plants.
[c]Number of infected plants/number of plants tested.
[d,e]Inoculum prepared from first and second mechanically inoculated wheat plants of cultivar Trémie.
OD values present mean of four replicate wells per treatment measured 1 h after addition of substrate.

50% of plants were infected. However, ELISA values were not correlated with leaf symptoms. For example, the two cultivars Fronty and Soissons with high ELISA values did not express any mosaic or stunting symptoms (Table 5). The other cultivars showed intermediate behaviours. AWMV was also detected in the roots of all the cultivars tested. Four of them Aztec, Capvern, Courtot and Trémie showed low ELISA OD values in contrast to the cultivars Charger and Sponsor. The other cultivars showed different levels of OD values and different percentages of root infection irrespective of the leaf reaction (Figure 3).

Discussion

AWMV, a new wheat virus first observed in 1999 in France, is serologically distinct from all soil-borne viruses belonging to the genera *Benyvirus*, *Bymovirus*, *Furovirus*, *Pecluvirus* and *Pomovirus* tested. From an initial survey, AWMV seems restricted to a few fields in France (unpublished results). Bedford-virus, which is serologically related to AWMV, is also restricted to one field in England (Clover et al., 1999b). This type of distribution resembles that of *maize white line mosaic virus* present in Europe and in America (Lapierre et al., 1976; Conti, 1983; de Zoeten et al., 1980).

AWMV was observed in soils containing *P. graminis* but also several fungi and nematodes. The possible role of these wheat parasites as vectors is still to be determined. A study currently being undertaken will examine the geographical distribution of this virus, particularly in fields where known resistant cultivars to SBWMV-F and WYMV-F show mosaic symptoms. In contrast to the Bedford-virus, the infection of wheat by mechanical means with AWMV has been achieved. The mosaic symptoms were obtained and the virus was detected. Although one of the components of Koch's postulates had not been verified (disease reproduction from purified particles), these results indicate that the detected virus was very probably involved in the symptoms obtained. Mechanical inoculation with field material is a very difficult process. However, the successive inoculations from wheat to wheat in the growth cabinet gave good infection rates. This situation is similar to that observed with BaMMV (Dessens and Meyer, 1995; Jacobi et al., 1995; Timpe and Kühne, 1994). In the case of BaMMV, successive passages led to the selection of mutants carrying large viral RNA deletions which were more efficiently transmitted mechanically. The low efficiency of mechanical infection from field material to characterise the experimental host range of this virus has perhaps led to a low estimation of the susceptible species. In contrast to SBWMV, AWMV does not infect the dicotyledoneous species belonging to *Chenopodium* or *Nicotiana* genera. *Vicia faba* mechanically inoculated by this virus shows systemic necrotic spots. This species is known to be the main host plant of *broad bean necrosis virus* (Inouye and Asatari, 1968), a virus tentatively included in the *Pomovirus* genus (Lu et al., 1998). Unfortunately, *V. faba* has not been used as a test plant for other soil-borne tubular viruses except *Nicotiana velutina mosaic virus* (Randles, 1978). The possibility of infection of *V. faba* and *Lactuca sativa* in soil contaminated by AWMV has to be evaluated.

The response of wheat to AWMV is remarkable. Amongst 26 cultivars, of which a number are resistant to SBWMV-F, no root or shoot resistance to AWMV has been characterised. However, different types of wheat cultivar reactions against this virus suggest the existence of polygenic resistance in the *T. aestivum* species.

From seeds of infected plants grown in an AWMV-free field, the virus was detected in roots during the first stages of plant development. Of the possible hypotheses concerning the absence of the virus in the leaves, three have been retained pending further investigation.

Table 4. Field behaviour of different wheat cultivars in soil infected by either AWMV or SBWMV-F

Cultivar	AWMV					SBWMV-F
	Leaf symptoms[a] (Date)		Leaf detection of the virus in ELISA (Date)			
			OD values[b]	Infected plants[c]		
	14.03.00	04.04.00	04.04.00	27.04.00	15.05.00	27.04.00
ALSACE 22	—	—	0.8–1.2	2/5	5/10	nt[b]
APACHE	mM	mM	0.8–0.9	5/5	9/10	3/3
AZTEC	mM	mM-mS	0.5–0.5	3/5	13/50	3/3
BALTIMOR	—	sM-sD	0.9–1.1	5/5	5/10	2/3
BRANDO	—	sM-sS	0.2–0.2	5/5	4/10	0/3
CAPPELLE	—	sM-sS	0.9–1.2	nt	nt	nt
CAPVERN	—	—	0.2–0.2	3/5	4/10	0/3
CHARGER	mM	sM-sS	2.3–2.5	10/10	43/50	0/3
CHATELET	mM	mM	1.2–2.4	5/5	5/10	3/3
COURTEL	—	mM-sS	0.6–0.9	nt	nt	nt
COURTOT	—	—	1.2–1.7	5/5	4/10	nt
ELEPHANT	mM	mM-sS	1.2–1.6	1/5	4/10	3/3
FRONTY	—	—	1.7–2.8	5/5	9/10	1/3
MERCURY	—	—	0.2–0.4	4/5	4/10	0/3
NOE	—	—	0.2–0.5	13/15	9/30	nt
ORNICAR	—	mM-mS	0.4–0.5	3/5	4/10	3/3
RIBAND	—	mM-mS	1.2–2.4	5/5	17/20	3/3
SOISSONS	—	—	0.6–1.1	5/5	5/10	3/3
SPONSOR	mM	sM-sS	1.9–2.4	5/5	10/10	0/3
TALDOR	—	—	0.2–0.3	3/5	6/10	0/3
TEXEL	—	mM-mS	0.9–1.1	5/5	7/10	3/3
THESEE	—	mM	0.2–0.5	5/5	6/10	3/3
TRÉMIE	—	—	0.2–0.2	1/5?	2/10	0/3
TRUST	mM	mM-mS	1.7–2.9	4/5	6/10	3/3
VILMORIN 27	—	mM-mS	1.2–1.7	nt	nt	nt
VIRTUOSE	—	mM-mS	0.6–1.4	5/5	10/10	nt

[a]mM and sM indicated two visual levels of mosaic symptoms, mD and sD indicated two visual levels of stuning (mild and severe).
[b]OD values present mean of four replicate wells per treatment measured 1 h after addition of substrate; OD values of healthy controls are equal or lower than 0.15; two bulks of five plants were tested.
[c]Number of infected plants/number of plants tested.
—: no symptoms.

Table 5. Seed transmission of AWMV

	Field				Growth cabinet[a]
Date	17.05.2000[a]	18.12.2000[b]	07.02.2000[b]	21.02.2000[b]	
Symptoms	0[c]	0[d]	0[d]	0[d]	0[c]
ELISA roots	0	19%	12%	0	0
ELISA leaves	0	nt	nt	0	0
Number of plants tested	1000	120	200	200	200

[a]Seeds from growing season 1998–1999. [b]Seeds from growing season 1999–2000. [c]Charger and Sponsor cultivars. [d]Riband cultivar.

It might be attributed to (i) the absence of the migration factor in the viral genome present in the seeds, (ii) the need to involve a vector and (iii) the climatic conditions. The complete seed transmission of the Bedford-virus may be explained either by the particular properties of this isolate or by the climatic conditions of the experimental study. We conclude that AWMV, like the Bedford-virus, is seed-transmitted,

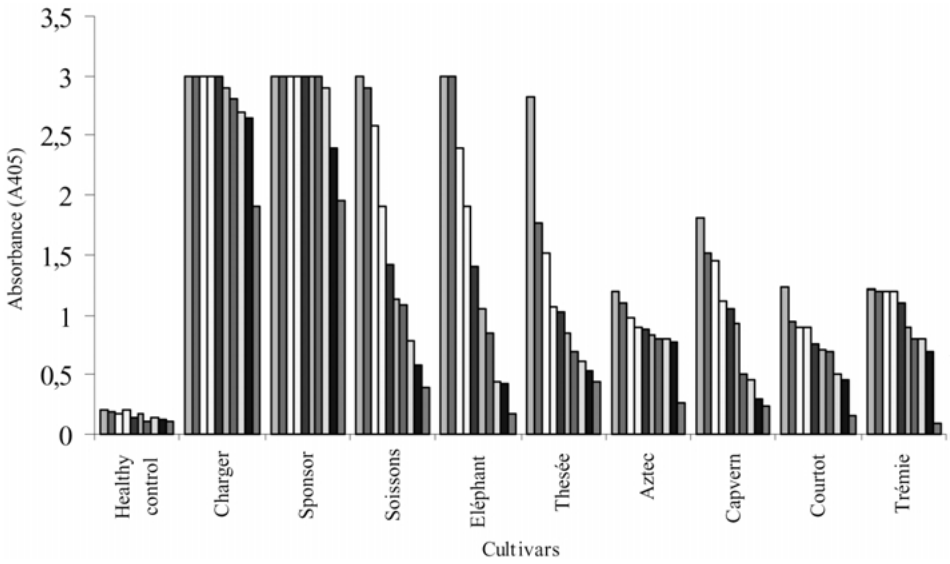

Figure 3. Detection of AWMV in roots of different wheat cultivars infected in the field. OD values present mean of four replicate wells per 10 individual roots of plants measured 1 h after addition of substrate.

but the interactions between these two viruses and the plant are not clear and require more investigation. The susceptibility of the cultivar Riband to both the Bedford-virus in England and AWMV in France reinforces the idea of great similarity between these two viruses. Due to the absence of resistance to this virus in wheat and the risk of transmission through seeds, the study of the geographical distribution of this virus and its biological properties should be undertaken.

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