Multiplication of soilborne wheat mosaic virus (SBWMV) in wheat roots infected by a soil carrying SBWMV and wheat yellow mosaic virus (WYMV)

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SUMMARY

Soft winter wheat cultivars were infected in the field or in a growth chamber with an inoculum consisting of soilborne wheat mosaic virus (SBWMV) and wheat yellow mosaic virus (WYMV). Only SBWMV was screened by ELISA in the leaves and roots of plants. Among 33 wheat cultivars studied in the field, 9 (Capitole, Cargo, Fortuna, Moulin, Rescler, Albatros, Fidel, Rempart, Tarasque) were resistant to the SBWMV-WYMV complex. However, the first five cultivars mentioned above showed transitory mosaic symptoms. Cultivar Pernel, which is susceptible to WYMV and resistant to SBWMV in the field did not show a breakdown of resistance to SBWMV. This might be accounted for by the spread of SBWMV in the plant shoots. In the cultivars resistant to SBWMV, virus could not be detected in the leaves, but was clearly identified in the roots. The virus could be detected only during a part of the life cycle of the plant. Upon completion of the tillering stage, virus concentrations were equally high in the resistant and in the susceptible cultivars. SBWMV associated with all the resistant cultivars studied could be carried to healthy plants by zoospores of *Polymyxa graminis* attacking the roots at least during the tillering stage. However, the level of inoculum present in the plants of each cultivar was not evaluated. At the end of the life cycle of wheat, resting spores of *P. graminis* do occur in all wheat cultivars resistant to the SBWMV-WYMV complex. In a growth chamber after inoculation by a soil carrying SBWMV and WYMV, root multiplication of SBWMV has been tested in eleven field-resistant cultivars : Albatros, Apexal, Avalon, Cocagne, Capitole, Fleuron, Fortuna, Moulin, Pernel, Sabre, Tarasque. Sabre and Avalon showed the same virus concentration as Hardi the susceptible cultivar. In contrast, Albatros and Cocagne showed a lower virus concentration in their roots (the concentration was 10 to 200 times lower in Cocagne than in Hardi). The other cultivars showed an intermediate behavior. The genetic origin of this resistance is discussed.

Additional key words : ELISA, *Polymyxa graminis*, resistance.

RÉSUMÉ

Multiplication du virus de la mosaïque du blé (VMB = SBWMV) dans les racines de blé infectées par un sol portant le VMB et le virus de la mosaïque jaune du blé (VMJB = WYMV).

Des blés tendres d'hiver ont été soumis au champ ou en enceinte climatique à un inoculum constitué par le complexe virus de la mosaïque du blé (VMB), virus de la mosaïque jaune du blé (VMJB). Seul le VMB a été recherché à l'aide d'un test immunoenzymatique dans les feuilles et les racines des plantes.

Au champ, sur 33 cultivars étudiés, 9 d'entre eux : « Capitole, Cargo, Fortuna, Moulin, Rescler, Albatros, Fidel, Rempart, Tarasque » sont symptomatologiquement résistants au complexe VMB-VMJB. Cependant les 5 premiers blés cités présentent des symptômes fugaces de mosaïque.

Pour le cultivar « Pernel », sensible au VMJB et résistant au VMB au champ, on n'a pas observé de rupture de résistance qui se serait exprimée par une multiplication du VMB dans la partie aérienne des plantes. Si pour les cultivars résistants au champ au VMB on ne peut mettre en évidence une multiplication foliaire, le virus est nettement caractérisé dans les racines. Cette détection n'est possible sur une grande partie des plantes que pendant une période assez brève de la vie de la céréale. Au stade plein tallage, les concentrations virales sont aussi élevées dans les cultivars résistants que dans les cultivars sensibles.

Au moins au stade du tallage, le VMB est présent dans tous les cultivars résistants et peut être transmis à des plantes saines par les zoospores de *Polymyxa graminis* hébergées par leurs racines. Les quantités d'inoculum présentes dans les plantes de chaque cultivar n'ont pas été évaluées.

En fin de cycle végétatif du blé, on peut trouver les formes de conservation de *P. graminis* chez tous les cultivars de blé résistants au complexe VMB-VMJB.

On a recherché le VMB dans les racines des plantes de 11 cultivars (Albatros, Apexal, Avalon, Cocagne, Capitole, Fleuron, Fortuna, Moulin, Pernel, Sabre, Tarasque) inoculés en enceinte climatique par l'intermédiaire d'un sol contenant le VMB et le VMJB. On observe de grandes différences de niveau de la multiplication du VMB dans les racines de ces différents cultivars. Sabre et Avalon multiplient aussi bien le virus que le cultivar sensible Hardi. Au contraire Albatros et surtout Cocagne multiplient très peu de VMB dans leurs racines ; suivant les essais, la concentration en VMB est de 10 à 200 fois plus faible chez Cocagne que chez Hardi. Les autres cultivars ont des comportements intermédiaires. Les bases génétiques de ces résistances sont discutées.

Mots clés additionnels : ELISA, *Polymyxa graminis*, résistance.

I. INTRODUCTION

Soilborne wheat mosaic virus (SBWMV), which is transmitted by *Polymyxa graminis* Led. and belongs to the proposed Furovirus group (SHIRAKO & BRAKKE, 1984), is spreading across Europe (LAPIERRE *et al.*, 1985). Presently, the sites most severely infested with SBWMV in France are restricted to the central part of the country. However, additional sites were detected in 1985, which suggests that the virus is spreading into southern France.

Field resistance to SBWMV is commonly encountered in soft winter wheat, but neither the mechanisms of this resistance nor the genes controlling the resistance have been clearly identified. Depending on workers and plant material used, a single gene (DUBEY *et al.*, 1970, MODAWI *et al.*, 1982) or several genes (NAKAGAWA *et al.*, 1959, SHAALAN *et al.*, 1966, MERKLE & SMITH, 1983) are assumed to be involved in this resistance.

In central France, SBWMV is frequently associated with wheat yellow mosaic virus (WYMV), a virus closely related to wheat spindle streak mosaic virus (WSSMV) (USUGI & SAITO, 1979). Both viruses are transmitted by *P. graminis*, and possible breakdown of resistance to SBWMV in the presence of WSSMV has been reported for the cultivar Newton (LOMMEL & WILLIS, 1984, LOMMEL *et al.*, 1986).

In a previous study of twelve soft winter wheat cultivars showing field resistance to SBWMV, we reported (LAPIERRE *et al.*, 1985) a low virus concentration or absence of virus in shoots and a low virus concentration in roots of naturally infected plants. The purpose of this study was to compare multiplication of SBWMV in the roots of cultivars resistant or susceptible to this virus when infected in the field or in a growth chamber through soil carrying SBWMV and WYMV.

II. MATERIALS AND METHODS

A. Field experiments

Plants and inoculum were taken from an experimental plot at Onzain, Loir-et-Cher. In this plot which was heavily infested with both SBWMV and WYMV, a wheat cultivar susceptible to both viruses was sown in 1983. For the present investigation, thirty three cultivars of wheat and three of barley were sown during October 1984. Two randomized replicates were planted. Phenological stages were characterized using Feeke's scale. In order to characterize the reaction of 33 wheat cultivars to SBWMV and WYMV, different categories were used, based on the presence or absence of mosaic symptoms, on the time of symptom appearance, and on the percentage of infected plants.

B. Inoculation of SBWMV in growth chamber

Soil (inoculum source) collected in March, 1984 was sieved (1 × 1 cm mesh), placed in 5 kg containers and stored at 4 °C. This inoculum remained highly infective for 4-6 months.

Wheat seeds were germinated in Petri dishes for 2 days on moistened filter paper. Germinating seeds were transplanted into flats (30 × 50 × 6 cm) containing a mixture of soil and sterile sand (W/W), and were arranged in separate rows. The susceptible cultivar (Hardi) was included in each experiment. Flats were kept on trays at 15 °C in a growth chamber with 16,000 lx light intensity and a 16 h photoperiod.

Flats were watered frequently during the first 5 days after transplanting. In the tests designed for biological detection of viruliferous *P. graminis*, the seedlings of Hardi to be inoculated were held in separate containers. Under these experimental conditions, mosaic symptoms were observed on only a few plants one to 2 months after exposure to the soil or root inocula.

C. Detection of SBWMV and WYMV and comparison of SBWMV concentration in different plant extracts

Implementation of the ELISA procedure (CLARK & ADAMS, 1977) for detecting SBWMV has been described previously (LAPIERRE *et al.*, 1985). Plants from the experimental plots were harvested with their roots intact down to 25 cm below ground level. Roots were washed, rinsed carefully and plants stored at 4 °C. Roots and leaves were ground for 20 s in a roller press in the presence of 10 volumes of the buffer per unit weight of plant material. Fractions to be tested were diluted with extracts of healthy plants. A range of infected plant dilutions allowed the measure of the relative virus concentrations for the various extracts to be determined. Optical densities (O.D.) were recorded either after 1-h or 8-h hydrolysis of the substrate. For detection of SBWMV and WYMV under the electron microscope, leaf fragments were dilacerated in the presence of 2 % phosphotungstic acid, pH 7. The extracts obtained were placed on carbon-coated grids. Observations were made with a Philips 300 transmission electron microscope at 80 kV.

D. Detection of the cystosori of *Polymyxa* sp.

The presence of cystosori of *Polymyxa* sp. in field-infected wheat or barley roots was tested as follows: for each cultivar, root samples from 5 to 10 plants were obtained. From this sample 40 to 70 fragments of 8 mm average length (including branches) and 200 µm average diameter were taken. These fragments with a total length of 30 to 60 cm were examined under the light microscope after staining with methyl blue.

III. RESULTS

A. Development of mosaic symptoms in wheat cultivars grown in a field infested with SBWMV and WYMV

Initial mosaic symptoms were observed in the field in late January (stage 2) (table 1). Most plants of the highly susceptible cultivars expressed mosaic symptoms on February 20 (stage 3), whereas plants of

TABLE 1

Development of mosaic symptoms on winter wheat cultivars grown in a soil carrying SBWMV and WYMV (percentage estimated from about 1,000 plants).

Evolution des symptômes de mosaïque chez différents cultivars de blé d'hiver cultivés dans un sol contenant le VMB et le VMJB (estimation du pourcentage de plantes malades à partir d'environ 1 000 pieds).

Lines or cultivars of soft wheat	Percentage of plants with mosaic symptoms				Susceptibility of wheat cultivars to the SBWMV-WYMV complex
	Janv. 28	Febr. 20	April 17	June 10	
PERNEL	20	> 50	> 50	> 50	high
BEAUCHAMP	15	> 50	> 50	> 50	
CHOPIN	30	> 50	> 50	> 50	
BERLIOZ	0	> 50	> 50	> 50	
CATON	0	> 50	> 50	> 50	
CAMP-REMY	0	> 50	> 50	> 50	
GALAHAD	0	> 50	> 50	> 50	
HARDI	0	> 50	> 50	> 50	
PLUTON	0	> 50	> 50	> 50	
SENSOR	0	> 50	> 50	> 50	
TENOR	0	> 50	> 50	> 50	
777	0	> 50	> 50	> 50	
6.69	0	> 50	> 50	> 50	
FCS 80	0	> 50	> 50	> 50	
CARAT	0	20	> 50	> 50	moderate
CHAMPLEIN	0	15	> 50	> 50	
EPIROUX	10	40	> 50	> 50	
PRIAM	0	30	> 50	> 50	
RIOL	0	10	> 50	> 50	
IC 8114	20	40	> 50	> 50	
GALAXIE	0	30	> 50	> 50	
FESTIN-FRANDOC	0	5	> 50	> 50	
COURTEL	0	5	30	5	mild
TRACY	0	10	30	20	
CAPITOLE	0	< 5	0	0	very mild
CARGO	0	0	< 5	0	
FORTUNA	0	0	< 10	0	
MOULIN	0	0	< 10	0	
RESCLER	0	0	< 1	0	
ALBATROS	0	0	0	0	0
FIDEL	0	0	0	0	
REMPART	0	0	0	0	
TARASQUE	0	0	0	0	

more resistant cultivars showed similar symptoms one month later. For other cultivars, symptoms, if present, were more or less transitory. Since climatic conditions were favourable for the expression of SBWMV and WYMV symptoms, the 4 symptomless cultivars (Albatros, Fidel, Rempart, Tarasque) may be regarded as resistant to this association of viruses. No symptoms were observed on the 3 barley cultivars (Illia, Panda, Plaisant).

Further tests were conducted on a few cultivars with mosaic symptoms (ELISA for SBWMV and leaf dip in electron microscopy for WYMV). Pernel was the only cultivar resistant to SBWMV leaf multiplication and susceptible to WYMV.

B. Concentration of SBWMV in roots of soft winter wheat during the life cycle of the plant

Nine wheat cultivars field-resistant to SBWMV were compared with susceptible cultivars and the 3 barley cultivars. Six samples were collected between late

January (stage 2) and late June (stage 10.5.3). The percentages of infected plants for the different cultivars are listed in table 2. SBWMV was detected in roots of the susceptible cultivar Hardi up to the 1/1000 dilution of crude extracts (fig. 1) in the first 3 samples.

In the first samples, the percentage of infected plants was very high in cv. Hardi (90 %) and much lower (0-30 %) in the other cultivars. This difference can be expressed as the quantity of O.D. units for 10 plants separately analyzed (table 3). From this first estimation the cultivar classification agrees partially with the levels of leaf susceptibility already known. For cultivars Chopin, Carat and Beauchamp, O.D. measurement by ELISA procedure provided an estimate value inferior to susceptibility rating. For cultivars Moulin and Fidel which are resistant in the field, the O.D. measured was more than three times that of the healthy control ; these cultivars can be rated similar as to the hybrids Courtel and Festin-Frandoc.

Samples II did not reveal any great increase in the percentage of plants exhibiting mosaic symptoms ; but a considerable increase of this percentage was observed

TABLE 2

Percentage of SBWMV infected roots in different cultivars of wheat and barley grown in a field carrying SBWMV and WYMV.
ELISA detection of SBWMV.
(—) No analysis, * barley cultivars, nb : number of plants tested.
Evolution, pour différents cultivars de blé, du pourcentage de plantes pour lesquelles on détecte le VMB dans les racines après une infection au champ dans un sol contenant le VMB et le VMJB.
(—) Non analysés, * cultivar d'orge, nb : nombre de plantes éprouvées.

Sample	I		II		III		IV		V		VI	
Date	20.1		12.2		25.2		19.3		7.6		28.6	
Stage	2		3		3		3		10.5.2		10.5.3	
	%	nb	%	nb	%	nb	%	nb	%	nb	%	nb
<i>Cultivars</i>												
ALBATROS	10	(10)	0	(20)	50	(18)	0	(10)	0	(10)	0	(10)
CARGO	0	(10)	10	(20)	30	(20)	10	(10)	0	(10)	0	(10)
CAPITOLE	0	(10)	10	(20)	70	(14)	20	(10)	0	(10)	0	(10)
FORTUNA	0	(10)	0	(20)	50	(18)	10	(10)	0	(10)	0	(10)
PERNEL	0	(10)	30	(10)	60	(10)	10	(10)	10	(10)	10	(10)
REMPART	0	(10)	20	(20)	70	(10)	40	(10)	20	(10)	0	(10)
MOULIN	20	(10)	20	(20)	100	(18)	20	(10)	0	(10)	0	(10)
TARASQUE	10	(10)	10	(20)	20	(10)	(—)		10	(10)	10	(10)
RESCLER	0	(10)	5	(20)	40	(10)	20	(10)	0	(10)	0	(10)
FIDEL	30	(10)	40	(20)	20	(15)	(—)		0	(10)	0	(10)
HARDI	90	(10)	100	(10)	100	(8)	100	(10)	70	(10)	40	(10)
PANDA *	0	(10)	0	(10)	0	(8)	(—)		(—)		(—)	
PLAISANT *	0	(10)	0	(10)	0	(8)	(—)		(—)		(—)	
ILLIA *	0	(10)	0	(10)	0	(8)	(—)		(—)		(—)

Figure 1
ELISA detection of SBWMV in field-contaminated roots of cv. Hardi.
Infected roots Racines infectées ●—●
Healthy roots Racines saines ○—○
Détection en ELISA du VMB dans des racines de plantes du cv. Hardi contaminé au champ.

TABLE 3

SBWMV in the roots of wheat cultivars at the early tillering stage (sample 1). O.D. in ELISA measured after 60 mn of substrate hydrolysis.
VMB dans les racines de différents cultivars de blé au stade début tallage (prélèvement 1).

CULTIVARS	Sum of O.D. (10 plants)
PERNEL	1.115
RESCLÉR	1.151
REMPART	1.151
CARGO	1.400
CAPITOLE	1.803
FORTUNA	1.661
ALBATROS	2.130
TARASQUE	2.359
MOULIN	3.647
FIDEL	3.807
COURTEL	3.885
FESTIN-FRANDOC	3.784
TRACY	3.997
CHOPIN	6.622
CARAT	6.636
BEAUCHAMP	7.486
GALAXIE	8.695
CAMP-REMY	10.661
BERLIOZ	11.122
777	> 12
PLUTON	> 14
IC 8114	> 15
CATON	> 20
SENSOR	> 20
HARDI	> 20
CHAMPLEIN	> 20
EPIROUX	> 20
RIOL	> 20
TENOR	> 20
GALAHAD	> 20
PRIAM	> 20
6.69	> 20
FCS 201	> 20
HARDI (non-infected)	1.009

TABLE 4

ELISA detection of SBWMV in the roots of few wheat cultivars (sample III). O.D. measured after 60 mn substrate hydrolysis.

Détection en ELISA du VMB dans les racines de quelques cultivars de blé (prélèvement III).

Sample n°	Cultivars tested				Control
	MOULIN	PERNEL	RESCLER	HARDI	HARDI
1	1.623	0.237	0.760	1.270	0.204
2	1.239	1.230	0.708	0.831	0.249
3	1.374	1.215	0.211	1.264	0.240
4	1.489	1.269	0.237	1.266	0.210
5	1.654	1.502	1.055	0.832	—
6	1.058	1.689	0.212	0.837	—
7	1.059	0.236	0.211	1.323	—
8	1.406	0.190	0.259	1.045	—

13 days later. At this time the virus concentration of field-resistant cultivars was close to that of the susceptible ones (table 4).

The percentage of plants in which SBWMV was detected decreased gradually from sample IV (stage 3, March 19). At the same time, the O.D. Values measured in the resistant cultivars dropped to a much lower level than those of the susceptible control (table 5). Sample V (stage 10.5.1) showed that SBWMV could still be detected in a rather large percentage of plants from susceptible cultivars, whereas it was detected in an average of 2 out of 100 among resistant ones (table 2).

The roots of 3 barley cultivars grown under similar conditions, showed no SBWMV-specific reaction in the ELISA test for the first 3 samples.

C. Ability of SBWMV-resistant wheat roots to host viruliferous *P. graminis* in the field

Seedlings of cv. Hardi were infected with SBWMV in a growth chamber using soil containing variable amounts of plant roots from infested plots. Unlike the barley cultivars Illia and Plaisant, all wheat cultivars tested carried viruliferous *P. graminis* (table 6).

TABLE 5

Root detection of SBWMV in wheat. Groups of plant roots in relation to O.D. in ELISA for 3 consecutive field samplings of cultivars resistant or susceptible to SBWMV. (c) : Control.

Détection du VMB dans les racines de différents cultivars de blés pour 3 prélèvements successifs. Groupes de plantes en fonction des D.O. en ELISA. (c) : Témoin.

CULTIVARS	REMPART			MOULIN			PERNEL			RESCLER			CAPITOLE			HARDI		
Sample Echantillons	II	III	IV	II	III	IV	II	III	IV	II	III	IV	II	III	IV	II	III	IV
O.D. groups																		
O.D. < 1.5 × C	13	2	6	13	0	7	3	3	9	16	5	8	18	1	8	0	0	0
1.5 × C ≤ O.D. < 2.5 × C	3	1	0	3	0	1	4	0	0	3	0	0	0	3	0	0	0	0
2 × C ≤ O.D. < 4 × C	4	1	4	4	0	2	3	0	1	1	2	2	2	4	2	2	0	0
O.D. ≥ 4 × C	0	7	0	0	8	0	0	5	0	0	1	0	0	5	0	8	8	10
Total number of plants tested Nombre total de plantes éprouvées	20	11	10	20	8	10	10	8	10	20	8	10	20	13	10	10	8	10

TABLE 6

Transmission of SBWMV to cv. 'Hardi' seedlings by root fragments of wheat and barley plants grown in SBWMV — WYMV contaminated field.

Essai de transmission du VMB à de jeunes plantes du cv. « Hardi » par l'intermédiaire de racines de différents cultivars de blé et d'orge (1) provenant d'une parcelle contenant le VMB et le VMJB () sensible.*

Cultivars tested as inoculum source	Number of seedlings (cv. Hardi)	
	Infected	Total tested
TARASQUE	1	6
FIDEL	1	21
RESCLER	2	4
CARGO	6	13
ALBATROS	5	11
FORTUNA	8	13
PERNEL	5	6
MOULIN	6	6
CAPITOLE	12	14
REMPART	13	15
COURTEL (*)	5	11
HARDI (*)	1	6
PLAISANT (1)	0	8
ILLIA (1)	0	13

Because of poor test replicability (no correlation between amount of root inoculum and number of infected plants), no difference could be distinguished among cultivars.

D. Field presence of cystosori of *P. graminis* in roots of various resistant soft wheat cultivars

Analyses were made in June (stage 10.5.2) and July (stage 11.1) (table 7). The number of cystosori observed varied considerably between the 2 samplings. For example in June, no cystosori were observed in Gala-had plants showing foliar symptoms of SBWMV. All resistant cultivars tested carried *P. graminis* cystosori. However, the method described does not permit a comparison between the different cultivars.

TABLE 7

Estimation of proportion of *Polymyxa graminis* cystosori on roots of several wheat and one barley cultivar grown on a plot contaminated with both SBWMV and WYMV. (*) Slightly susceptible, (**) susceptible, (***) highly susceptible. (l) = Barley.

0 = undetected non détecté
1 = rare elements in a single fragment rares éléments dans un seul fragment
2 = rare elements in several fragments or rather abundant elements in a single fragment rares éléments dans plusieurs fragments ou éléments assez fréquents dans un fragment
3 = abundant elements in 2 or 3 fragments éléments abondants dans 2 ou 3 fragments
4 = abundant elements in 4 to 6 fragments éléments abondants dans 4 à 6 fragments
5 = abundant elements in 7 to 9 fragments éléments abondants dans 7 à 9 fragments
6 = abundant elements in 10 to 15 fragments éléments abondants dans 10 à 15 fragments
7 = abundant elements in more than 15 fragments éléments abondants dans plus de 15 fragments
— = not analysed non analysé.

Abondance des cystosores de *Polymyxa graminis* dans les racines de différents cultivars de blé tendre et d'orge installés sur une parcelle portant le VMB et le VMJB. (*) Peu sensible, (**) sensible, (***) très sensible. (l) = Orge.

CULTIVARS	Date of sample	
	June 10, 1985	July 4, 1985
ALBATROS (*)	3	3
CAPITOLE (*)	4	—
CARGO (*)	3	7
FIDEL (*)	2	6
FORTUNA (*)	4	—
MOULIN (*)	3	1
REMPART (*)	4	4
RESCLER (*)	2	6
TARASQUE (*)	2	4
TRACY (**)	4	2
COURTEL (**)	3	—
GALAHAD (***)	0	4
ILLIA (l)	1	—

E. Multiplication of SBWMV in soft wheat roots grown in a controlled environment chamber

1. Infection rate

Of the 11 field-resistant cultivars used in this analysis, 5 had already been characterized previously (LAPIERRE *et al.*, 1985). Two other wheat cultivars served as controls : Tracy, slightly susceptible and Hardy, highly susceptible.

SBWMV was generally detected 5 days after seedling exposure to the inoculum. After 10 days, all roots of Sabre and Fleuron carried the virus (table 8). Complete infection was only observed after 20 or 30 days in the other 9 cultivars which are field-resistant or susceptible. The primary inoculum might have been insufficient or some cultivars express some resistance to inoculation. All field-resistant cultivars can actually be infected.

TABLE 8

Kinetics of SBWMV infection in the roots of various soft wheat cultivars (rate of infected plants calculated on 12 plant samples). (Growth chamber conditions, soil inoculum, ELISA detection).
* Susceptible cultivar.

Cinétique de l'infection par le VMB de racines de différents cultivars de blé tendre. Le taux d'infection est calculé à partir de l'analyse de 12 plantes (contamination en chambre climatique par un sol contenant le VMB et le VMJB). * : Cultivar sensible.

CULTIVARS	Number of days after infection		
	10	20	30
SABRE	100	100	100
FLEURON	100	100	100
TARASQUE	91	100	100
TRACY (*)	91	100	100
AVALON	83	100	100
APEXAL	75	100	100
PERNEL	75	100	100
CAPITOLE	83	83	100
HARDI (*)	91	91	100
FORTUNA	66	75	100
ALBATROS	58	83	100
MOULIN	58	75	100
COCAGNE	58	68	100

2. Estimated SBWMV concentration in field-resistant cultivars

In the same experiment described above an estimation of SBWMV concentration in root extracts was made from 12 plants of 6 different cultivars (fig. 2).

Figure 2

Estimation of SBWMV relative concentration in the roots of soft wheat cultivars (30 days after root infection in a growth chamber, 12 roots samples, O.D. measures in ELISA).

Estimation de la concentration relative du VMB dans les racines de différents cultivars de blé (30 jours d'infection en chambre climatique, échantillon de 12 racines, mesure des D.O. en ELISA).

Figure 3

Distribution of SBWMV in the roots of 11 field-resistant and 2 susceptible wheat cultivars. Inoculation in a growth chamber, ELISA measured 30 days after exposure of seedlings to soil inoculum.

- 1) O.D. = 0 to < 0.5 3) O.D. = 1.0 to < 1.5 5) O.D. ≥ 2.0
2) O.D. = 0.5 to < 1.0 4) O.D. = 1.5 to < 2.0

Thirty days after exposure to the inoculum the roots of Cocagne contained about 32 times less virus than those of Hardi. None of the Cocagne plant roots showed high virus concentration (fig. 3). Tracy and the other cultivars showed an intermediate behaviour. A similar result was obtained in 2 other independent experiments.

All our results (2 to 4 test depending on cultivars) can be summarized in the form of a temporary classification of resistance of soft wheat cultivars to SBWMV as a function of their ability to multiply the virus in their roots in a growth chamber (table 9).

TABLE 9

Classification of field-resistant wheat cultivars for SBWMV multiplication in the roots of inoculated plants in a growth chamber. (1) Ratio of virus concentration between a tested cultivar and cv. 'Hardi'.

Classement des blés tendres résistants au VMB en fonction de leur capacité à multiplier le virus dans les racines de plantes inocuées en enceinte climatique. (1) Rapport de concentration relative du VMB dans un cv. donné par rapport au cv. « Hardi ».

CULTIVARS	Ratio concentration (1)
SABRE, AVALON	1,2 à 0,5
TARASQUE MOULIN FLEURON TRACY APEXAL PERNEL FORTUNA CAPITOLE	0,5 à 0,1
ALBATROS	0,2 à 0,03
COCAGNE	0,1 à 0,005

Distribution du VMB dans les racines de blé de différents cultivars sensibles ou résistants au champ à ce virus. Contamination en enceinte climatique, mesures en ELISA 30 jours après l'apport de l'inoculum.

IV. CONCLUSIONS

This study of the field response of 33 wheat cultivars to SBWMV and WYMV in a soil carrying both these viruses showed that 9 cultivars were resistant to this virus association.

In the case of cv. Pernel, susceptibility to WYMV caused no breakdown of SBWMV resistance under field conditions. This result seems opposed to the behaviour of cultivar Newton (LOMMEL *et al.*, 1986), but further observations are needed in more numerous cultivars to characterize eventual possible breakdown of resistance to WYMV in the presence of SBWMV or vice versa.

For 5 cultivars, only transitory mosaic symptoms could be observed, possibly corresponding to temporary leaf multiplication of 1 of the 2 viruses.

There was little or no virus multiplication in leaves of field-resistant cultivars, as shown previously (LAPIERRE *et al.*, 1985). Similar observations were made in WSSMV field-resistant wheat cultivars (HAUFLE & FULBRIGHT, 1986).

SBWMV was found in the roots of all resistant cultivars in amounts similar to those detected in susceptible cultivars during a short period of cereal growth. At other stages of the life cycle of the plant, the root virus content of field-resistant cultivars was much lower than in susceptible ones or was undetectable in ELISA.

At least close to stage II, the roots of field-resistant cultivars give rise to viruliferous zoospores. Because of the limited or zero spread of SBWMV in the shoots of these cultivars, the virus particles synthesized in the cell roots are likely to be associated with the viruliferous forms of *P. graminis*.

Cystosori are found in the roots of all resistant cultivars by the end of the life cycle of the plant, but no information is available on the field susceptibility of

seedlings to *P. graminis* nor on the viruliferous capacity of the *P. graminis* resting forms that accumulate in the roots. The reduced percentage of plants with SBWMV-infected roots and the low virus concentration after the critical period might characterize this field resistance of soft winter wheat since such a situation does not occur in susceptible wheat. A possible relationship between this phenomenon and the lack of virus movement to the plant shoots remains to be established.

During present investigations, SBWMV concentration in the roots of resistant cultivars did not develop as described in 1983-1984 in another soil containing only SBWMV (LAPIERRE *et al.*, 1985). Under the latter conditions, root virus concentration remained low in all resistant cultivars. This difference in behaviour remains unexplained.

In the growth chamber, when all 11 field resistant wheat cultivars were tested, SBWMV was detected in their roots. These results and those from field tests show that *P. graminis* can infect all the cultivars studied.

The percentage of infected plants and the root virus concentration varied according to trials. Field-resistant cultivars can, however, tentatively be classified into several categories: Sabre and Avalon are the wheat cultivars that best multiply SBWMV and have a root virus concentration close to that of the susceptible cultivar Hardi.

For 8 field-resistant cultivars, virus root concentration is slightly inferior to that of the susceptible cultivar. In Albatros and especially in Cocagne, SBWMV concentration is at least 10 times less than that of Hardi. A partial resistance to virus inoculation and or virus multiplication might account for the behaviour of Cocagne roots.

In the greenhouse or growth chamber test, other approaches of resistance have been used such as: rating of root symptoms (LARSEN *et al.*, 1985), leaf symptoms (BOCKUS, 1984, HUNGER & SHERWOOD, 1985) and leaf virus concentration (HUNGER & SHER-

WOOD, 1985), all of which deal with the final stages of the virus cycle or of the disease. All field-resistant cultivars tested are susceptible to leaf inoculation (McKINNEY, 1948; LARSEN *et al.*, 1985).

Three stages of the virus cycle occur before SBWMV could multiply and produce symptoms in leaves: 1) virus inoculation by *P. graminis*, 2) virus multiplication in roots, 3) virus movement to plant shoots. For all the resistant soft wheat cultivars tested, it is the third stage of the virus cycle which is affected in the field. This conclusion was one of the hypotheses suggested by LARSEN *et al.*, 1985 as a consequence of root symptoms observed in resistant cultivars after leaf inoculation.

We do not have enough evidence that plant genes could control the 3 stages described above. The gene most commonly encountered in soft wheat is probably responsible for the lack of virus migration in the shoots in field conditions. Cultivar Newton, which was found to have a resistance gene different from that of Homestead (MERKLE & SMITH, 1983), may be a candidate for the control of resistance to one of the first two stages of the virus cycle.

Cocagne is a good example of a wheat cultivar with resistance characterized by root screening for SBWMV. Among the parents of this cultivar either one, 2 or 3 (Heine VII. blé des Domes, 515) possess genes controlling the resistance to this virus while the fourth (Champlein) is susceptible. The field response of Cocagne roots is yet to be studied and the characters of associated *P. graminis* populations also. If a significantly low number of viruliferous cystosori are synthesized, then growing this type of cultivar would permit a rapid decrease of SBWMV soil inoculum.

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