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Incidence of *Soil-borne wheat mosaic virus* in mixtures of susceptible and resistant wheat cultivars

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Abstract

The multiplication of *Soil-borne wheat mosaic virus* (SBWMV) was studied in mixtures of two winter wheat (*Triticum aestivum*) cultivars, one susceptible (Soissons) and the other resistant (Trémie). Two seed mixtures of susceptible and resistant varieties in ratios of 1 : 1 and 1 : 3 and their component pure stands, i.e. each variety grown separately, were grown in a field infected with SBWMV. The presence of the virus was detected using DAS-ELISA from January to May. The resistant cultivar Trémie showed no foliar symptoms nor could the virus be detected in the leaves or roots. In May, about 88% of plants of susceptible cultivar Soissons grown in pure stands were infected. At this time, the disease reduction relative to pure stands was 32.2% in the 1 : 1 mixture and 39.8% in the 1 : 3 mixture. Optical density (OD) values from ELISA of the infected plants in the two mixtures were consistently lower than that of the infected plants in cultivar Soissons in pure stands. The ELISA index (EI) calculated using three scales of OD values was 65.5% in the susceptible cultivar in pure stands. The value for this index was 19.1% in the 1 : 1 mixture and 7.9% in the 1 : 3 mixture. The plants of the resistant cultivar Trémie infected in the same field and transferred in January to a growth cabinet at 15 °C multiplied the virus and produced viruliferous zoospores. These results show that the resistant cultivar Trémie plays a role in disease reduction in the cultivar mixtures in field conditions. Possible reasons for this are discussed.

Introduction

Soil-borne wheat mosaic virus (SBWMV) is a member of the *Furovirus* genus which is characterised by rigid, rod-shaped viruses and a divided genome (Shirako et al., 1990). The virus causes yield losses in winter wheat in many areas in the world. SBWMV is transmitted by a soil-borne plasmodiophoromycete, *Polymyxa graminis*, more recently classified as a protist (Estes and Brakke, 1966; Rao and Brakke, 1969). In France, SBWMV is essentially found in the central part of the country (Lapierre et al., 1985). The virus has also been detected in Italy (Canova, 1966), in England (Clover et al., 1999) and in Germany where the name *Soil-borne rye mosaic virus* was proposed (Koenig et al., 1999). All of these viruses have been tentatively grouped into one species referred to as the *Soil-borne cereal mosaic virus* (Koenig and Huth, 2000) or the European wheat mosaic virus (Diao et al., 1999). No variety immune to this virus has been found, but field resistance is commonly encountered in soft winter wheat. This resistance could be monogenic (Dubey et al., 1970; Modawi et al., 1982) or polygenic (Nakagawa et al., 1959; Shaalan et al., 1966; Merkle and Smith, 1983) depending on the material studied.

The use of mixed host populations is a possible solution for protecting a single host plant genotype to disease (Browning and Frey, 1969; Garrett and Mundt, 2000). The effect of genetic diversity has been studied for several air-borne pathogens such as wheat yellow rust (*Puccinia striiformis*), wheat brown rust

(*Puccinia recondita*), barley powdery mildew (*Erysiph graminis*) and rice blast (*Magneporthe grisea*) (Calonnec et al., 1996; van Asch et al., 1992; Finckh et al., 1999; Zhu et al., 2000). A study of the impact of mixed cultivations on a soil-borne virus was undertaken, first, to assess the agronomic potential as a control measure and secondly to evaluate the role of secondary infection during the crop cycle of wheat. This paper describes viral multiplication in the roots and leaves of the susceptible cultivar Soissons and the resistant cultivar Trémie in pure stands and in two mixtures in an infected field during 1998–1999.

Materials and methods

Field experiments

Two winter wheat cultivars, Soissons and Trémie, susceptible or resistant to SBWMV were sown in an infested field in France (Neuville, Department 41). The two cultivars were sown in pure stands or in mixtures of Soissons: Trémie (1 : 1) or Soissons: Trémie (1 : 3). There were three randomised replicates for each cultivar and mixture of cultivars, and these were sown at a density of 280 seeds/m^2 on 10 October 1998 in plots measuring 1.5 m by 12 m. At each sampling, 90 plants per replicate, including the roots to a soil depth of about 25 cm, were collected.

Plants were sampled four times between January and May. The plants were washed by ultrasonication in 1% Sodium metaphosphate. After removing excess moisture with paper towels, the roots and leaves were separated with a razor. Virus detection was performed using the enzyme-linked immunosorbent assay (ELISA) as described below. At ear emergence, a large number of plants from each of the three replicates of the mixed treatments were sampled, and the number of each cultivar was counted (ears of cv. Soissons and cv. Trémie are easy to distinguish).

Effect of temperature on multiplication of SBWMV

The plants of cv. Trémie and Soissons sampled in the pure stands in the same field in January were washed with 1% sodium metaphosphate and planted at either 6 °C or 15 °C in pots containing sterile sand. Some of these plants were used as inoculum. Healthy seedlings of cv. Soissons (10/pot) were inoculated in climatic chambers at 15 °C with the roots of these plants.

Enzyme-linked immunosorbent assay

Double antibody sandwich ELISA (DAS-ELISA) was performed (Clark and Adams, 1977) at each sampling time using a polyclonal antiserum prepared at INRA, Versailles. The root and leaf samples were ground in 1 : 10 (w/v) of 0.1 M citrate buffer pH 7.2 containing 0.5 M urea. Root and leaf saps from infected and healthy plants were added to six wells in each test as a control. ELISA index (EI) was calculated for each plot using a scale of 0–3 where 0 is not infected (Optical density (OD) less than three times that of the healthy plant); 1–3 are the different classes of OD (1 includes between $> 3\times$ OD of the healthy plant and 1; 2 from 1 to 2; 3 from 2 to > 3) and calculated using an equation described by Ohto and Naito (1997): $\mathrm{EI} = 100 \times \Sigma$ (OD value (0 to 3)$\times$ number of plants with each OD value)/(3$\times$ total number of plants tested).

Results

Frequency of infected plants in two mixtures and cv. Soissons in pure stands

In cv. Trémie, the virus was not detected in the roots or the leaves in any sample (Figure 1). In the cv. Soissons in pure stands, low positive ELISA values were first recorded in some root samples in December. In January, the virus concentrations in the roots had increased and the virus was detected in leaves without symptoms. At this time, SBWMV was detected in 54% of the roots and 25% of the leaves of cv. Soissons in pure stands. In May, the number of infected plants had reached 88%.

In the 1 : 1 mixture, the frequency of infected plants was 15% in the roots and 6% in the leaves in January. In the 1 : 3 mixture, this frequency was 4% for the roots and 2% for the leaves. At the 3rd sampling, the frequency of infected plants in the 1 : 1 mixture was 31% in the roots and 15% in the leaves. In the 1 : 3 mixture the proportion of infected plants remained low and did not exceed 9% for the roots and 8% for the leaves. In May, the virus was very difficult to detect in the roots, but in the leaves 24% and 12% of the plants were infected in the 1 : 1 and 1 : 3 mixtures (Figure 1).

ELISA index in two mixtures and cv. Soissons in pure stands

The EI was calculated from the OD of infected plants given in Figure 1. In the four tests, the OD values in

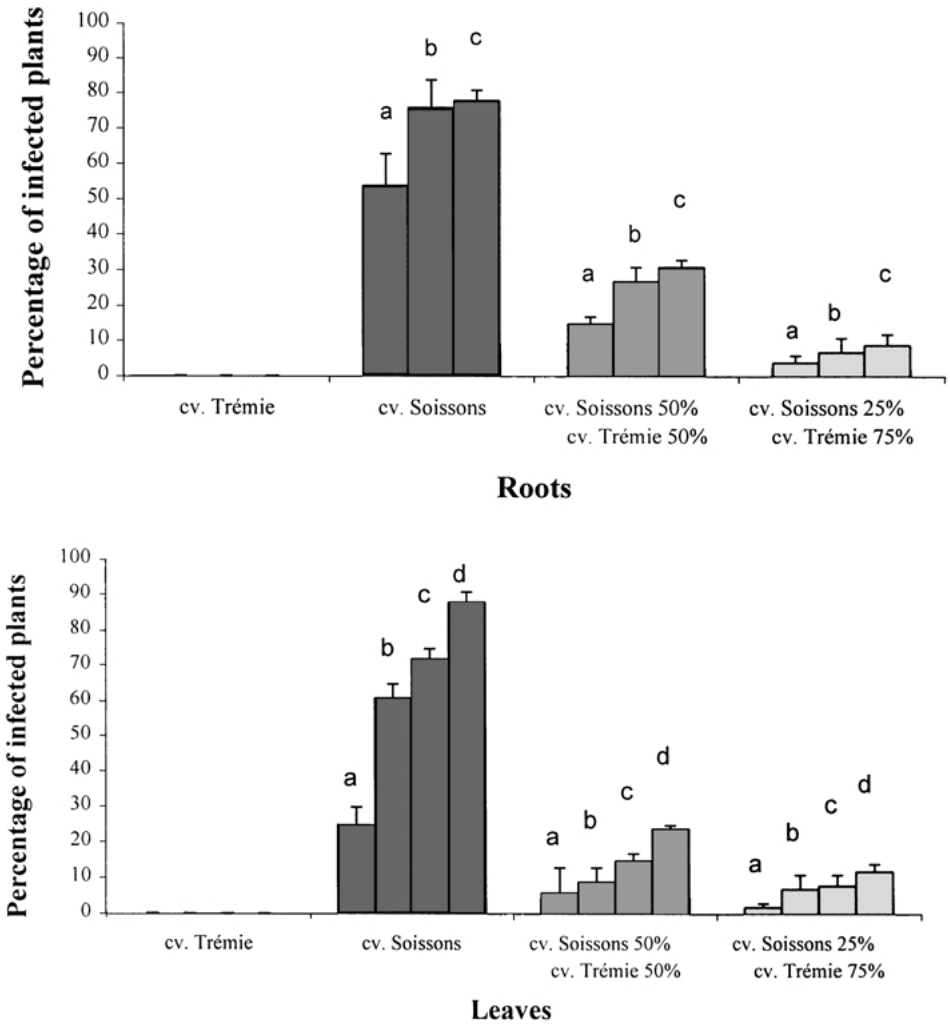

Figure 1. Percentage of infected plants with SBWMV in roots and leaves of two winter wheat cultivars in two mixtures and in pure stands. For each replicate, 90 plants were tested in DAS-ELISA. OD values represent mean values of four replicate wells per individual root measured 90 min after addition of substrate. Samples are considered to be positive when the OD value is equal or more than three times that of the healthy control. Bars represent the mean percentage ± standard error (SE) of infected plants from three replicate pots calculated using the Microsoft Excel software package, a–d are different sampling dates: a, January 18; b, February 18; c, April 4; d, May 22.

ELISA in the roots and leaves of the two mixtures were low compared to the OD values in the cv. Soissons in pure stands. In the roots of cv. Soissons in pure stands, the EI was 33.3% in January and reached 68.2% in April. In leaves, the mean EI was 14.8% in January and reached 65.5% in May. In January, for the 1 : 1 mixture, this index was 9.1% in the roots and 2.4% in the leaves. In April, the EI reached 19.7% and 11.3% in the roots and leaves, respectively. The EI was even lower in the 1 : 3 mixture, with a maximum of 6.6% in the roots and 7.9% in the leaves (Figure 2).

Disease losses in two mixtures compared to cv. Soissons in pure stands

A slight decrease and increase in the proportion of the susceptible cultivar were observed in the 1 : 1 and 1 : 3 mixtures, respectively. The corrected percentage of plants of cv. Soissons in the two mixtures were 48.7% and 26.1% in the 1 : 1 and 1 : 3 mixtures respectively (Table 1). Comparison of the percentage of infected plants in the cv. Soissons in pure stands and that expected for the two mixed treatments

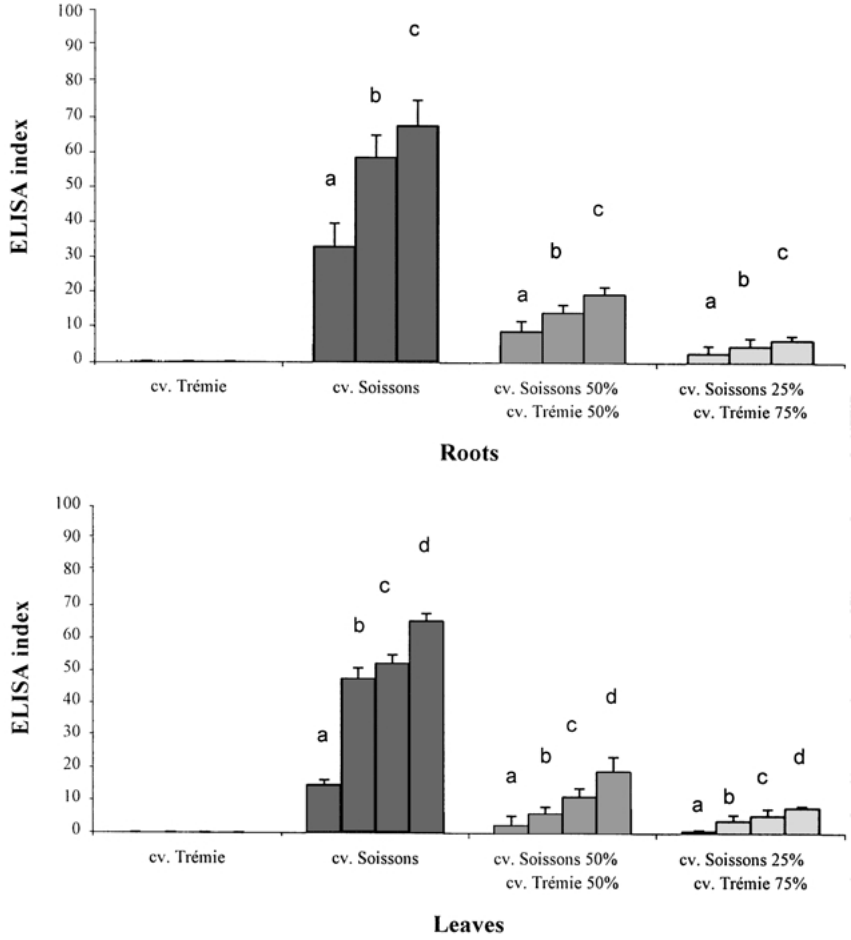

Figure 2. ELISA index (EI) of SBWMV in roots and leaves of two winter wheat cultivars in two mixtures and in pure stands. Bars represent the mean percentage ± standard error (SE) of infected plants from three replicate pots calculated using the Microsoft Excel software package. EI was calculated for each replicate using a scale of 0–3 where 0 is not infected (OD less than three times that of the healthy plant): 1–3 are different classes of OD (1 is between >3× OD of the healthy plant and 1; 2 from 1 to 2; 3 from 2 to >3) and calculated using the following equation: EI = 100 × Σ (OD value (0–3)× number of plants with each OD value)/(3× total number of plants tested). a–d are different sampling dates: a, January 18; b, February 18; c, April 4; d, May 22.

Table 1. Evaluation of the percentage of susceptible and resistant cultivars in the two mixtures

Date	Replicates	Number of plants	Soisons 50%/Trémie 50% (1 : 1)		Number of plants	Soisons 25%/Trémie 75% (1 : 3)	
			Soissons%	Trémie%		Soissons%	Trémie%
June 18	1	546	47.4	52.5	338	25.9	74.1
	2	704	53.2	47.8	443	24.2	75.8
	3	679	46.3	53.7	476	28.4	71.6
	Means		48.7	51.3		26.1	73.8

revealed decreases of 16.1% and 44.1% in the number of infected roots (in April) and 33.2% and 39.8% in the number of infected leaves (in May) in the 1 : 1 and 1 : 3 mixtures, respectively (Table 2). The effect of the two mixed treatments on the EI was also significant with reductions of 27.1% and 33.7% in the leaves and 28.9% and 39.4% in the roots of the 1 : 1 and 1 : 3 mixtures, respectively.

Table 2. Measured percentage of infected plants with SBWMV and the ELISA index (EI) in two mixtures compared with expected values based on pure stands

	Cultivar mixtures	Measured[a]		Expected[b]		Reduction percentage[c]	
		4 April Roots	22 May Leaves	4 April Roots	22 May Leaves	4 April Roots	22 May Leaves
Infected plants	1 : 1	30.1	26.6	37.9	42.8	16.1	33.2
	1 : 3	9.3	12.5	20.8	22.9	44.1	39.8
ELISA index	1 : 1	19.1	18.6	33.2	31.8	28.9	27.1
	1 : 3	6.6	8.2	17.8	17	39.4	33.7

[a]Average of three replicates calculated with corrected number of cv. Soissons in the mixtures.
[b]Half or Quarter of data obtained for cv. Soissons in pure stands.
[c]Difference between measured and expected percentage multiplied to two for 1 : 1 mixture and to four to 1 : 3 mixture.

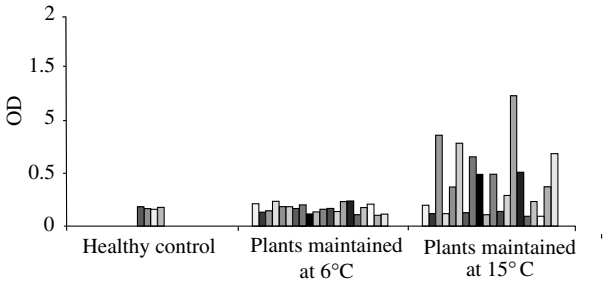

Figure 3. Detection of SBWMV in roots of resistant cultivar Trémie sampled in an infected field and maintained at either 6 °C or 15 °C. OD values represent mean values of four replicate wells per individual root measured 90 min after addition of substrate.

Effect of temperature on the multiplication of SBWMV in the resistant cultivar Trémie

The plants of cv. Trémie, sampled in January from the pure stands in the infected field and maintained in growth cabinets at either 6 °C or 15 °C, were tested in ELISA. No virus multiplication was detected in roots after four weeks at 6 °C. However, at 15 °C, 10 plants out of 20 contained SBWMV in their roots (Figure 3).

Plants of cv. Soissons inoculated with roots of either cv. Trémie or cv. Soissons at 15 °C were tested in ELISA. Four weeks after inoculation, SBWMV was detected in the roots of some plants. The number of infected plants and the ELISA values varied depending on the plant used (Table 3). Two plants of Trémie multiplied SBWMV after having been grown at 15 °C for four weeks and each infected four plants. One plant showed no reaction in ELISA and infected no plants. Of the three plants of cv. Soissons only two plants infected three and five plants, respectively.

Discussion

Analysis of plants from mixtures of the susceptible wheat cultivar Soissons and the resistant wheat cultivar Trémie, showed a reduction in both the frequency of infected plants and the virus concentration compared to the susceptible cultivar in pure stands. At the four sampling dates for both mixtures there was a regular increase in infection in the roots and leaves during the season.

In the case of diseases caused by air-borne pathogens, the beneficial effects of mixed host populations was attributed to barrier effects of resistant plants (Trenbath, 1977), increased distance between susceptible plants (Burdon and Chilvers, 1982), competitive interactions among host plants (Finckh and Mundt, 1992) and induced resistance by the development of nonvirulent races or diverse pathogen populations (Lannou et al., 1995; Zhu et al., 2000). In the case of SBWMV, more complex interactions may be attributed to the necessity of *Polymyxa graminis* transmission. Two hypotheses concerning Polymyxa–plant interactions can be proposed to explain the results obtained in this study. Firstly, it might be the effect of root development and possibly root exudate of the resistant cv. Trémie. The cv. Trémie may produce a large root volume and/or a large quantity of root exudates which specifically attracts a large number of zoospores released from cystosori or zoosporangia. This hypothesis remains to be proven. Preliminary observations from plants in the field show that there was no difference in root development between the two cultivars and no information is available concerning specific root exudates of the two cultivars.

The second hypothesis taking into account the thermosensitivity of wheat resistance to SBWMV (Hariri et al., 1987; Armitage et al., 1990; Himmel et al., 1991;

Table 3. Transmission of SBWMV to cv. Soissons from roots of susceptible and resistant cultivars infected in the field

Cultivars	Plant	OD										
		Plants used as inoculum		Plants of cv. Soissons inoculated at 15 °C								
		After sampling	After 4 weeks at 15 °C									
Trémie	1	0.2	0.78	0.83	0.72	0.78	0.1	0.05	0.22	0.15	0.42	0.1	0.1
	2	0.18	0.91	1.07	0.23	0.96	0.35	0.14	0.4	0.4	0.17	0.24	0.1
	3	0.18	0.19	0.12	0.14	0.11	0.1	0.1	0.1	0.13	0.15	0.07	0.1
Soissons	1	1.75	1.86	0.19	0.77	0.1	0.72	0.3	0.19	0.4	0.39	0.14	0.1
	2	1.34	1.12	0.1	0.1	0.1	0.1	0.03	0.1	0.05	0.1	0.09	0.09
	3	1.23	1.45	0.37	0.11	0.19	0.18	0.12	0.38	0.72	0.25	0.04	0.07
Healthy control		0.19	0.17	0.12	0.12								

OD values present mean values of four replicate wells per individual roots measured 90 min after addition of substrate.
OD values equal or above three times the healthy control absorbance is considered as positive.

Myers et al., 1993), might be attributed to a decrease in the infectivity rate of zoospores from the zoosporangia formed in the cv. Trémie. In our experiment, in field conditions, SBWMV was not detected in the cv. Trémie from October to April, whereas at 15 °C in a growth cabinet this cultivar multiplied the virus and produced viruliferous zoospores. Consequently, in field conditions, the secondary infections probably occurred only through the viruliferous zoospores from the cv. Soissons. A study of the Polymyxa cycle in the resistant wheat cultivars under field conditions and at different temperature should allow us to verify this hypothesis.

In our experiments, in 1998–1999, not all plants of the cv. Soissons in pure stands were infected, either because the primary inoculum in the soil was insufficient or the climatic conditions were unfavourable. Further research is necessary to study the effect of mixed cultivation in susceptible and resistant cultivars in soil with high concentrations of the primary inoculum.

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