Management of wheat stripe mosaic virus by crop rotation

**Lucas Antonio Stempkowski · Fernando Sartori Pereira · Juliana Borba Valente ·
Monica Farias · Douglas Lau ⓘ · Genei Antonio Dalmago · Anderson Santi · Talita
Bernardon Mar · Paulo Kuhnem · Ricardo Trezzi Casa · Amauri Bogo · Fábio
Nascimento da Silva**

Abstract Soil-borne wheat mosaic disease (SBWMD) caused by wheat stripe mosaic virus (WhSMV) is one of the most important viral diseases of wheat in Brazil. Farmers have used crop rotation (CR) successfully to reduce the incidence of many diseases in wheat. CR can contribute along with genetic resistance in the management of SBWMD. The objective of this study was to evaluate the effect of CR in SBWMD incidence and wheat yield as compared with wheat monoculture (W). During 2017 and 2018 growth seasons, in a long-term experiment, W and three CR: W1 – white oats-wheat; W2 – vetch-white oats- wheat; and W3 – black oats-barley-black oats-wheat were evaluated using three wheat cultivars: BRS Parrudo (resistant), BRS Reponte (moderately resistant) and BRS Guamirim (susceptible). The rainiest season (2018) was more favorable to the development of the disease. In this year, CR W2 and W3 significantly decreased SBWMD incidence by 90% compared to wheat monoculture. Also, grain yield (GY) differences between CR were higher in the rainiest season. GY increases up to 58% (BRS Reponte), 81% (BRS Guamirim) and 106% (BRS Parrudo) in W2 and

W3. The use of plant species that are non-hosts of WhSMV may be one of the main factors that contribute to the reduction of SBWMD levels.

Introduction

Diseases and pests cause yield losses of about 20% to the global wheat production every year (Figueroa et al. 2018). Among them, viral diseases provide a major challenge to agriculture worldwide (Burrows et al. 2016; Jones and Naidu 2019). Soil-borne transmitted virus cause soil-borne wheat mosaic disease (SBWMD) in cereal crops and result in significant grain yield reductions worldwide (Kanyuka et al. 2003). Among the etiologic agents of the SBWMD were described *Soil-borne wheat mosaic virus* (*Furovirus* genus, *Virgaviridae* family) and *Wheat spindle streak mosaic virus* (*Bymovirus* genus, *Potyviridae* family). In Brazil, SBWMD was first described in wheat (*Triticum aestivum* L.) in 1978 (Caetano et al. 1978) and was formerly attributed to soil-borne wheat mosaic virus (SBWMV).

However, recent genome sequencing results reveled that, in Brazil, SBWMD is caused by a new species tentatively named Wheat stripe mosaic virus (*Benyviridae* family) (Valente et al. 2019). Wheat stripe mosaic virus (WhSMV) was found widely disseminated

L. A. Stempkowski · F. S. Pereira · J. B. Valente ·
M. Farias · R. T. Casa · A. Bogo · F. N. da Silva
Crop Production Graduate Program, Santa Catarina State
University/UDESC, Lages, SC 88520-000, Brazil

D. Lau (✉) · G. A. Dalmago · A. Santi · T. B. Mar
Embrapa Trigo, Passo Fundo, RS 99050-970, Brazil
e-mail: douglas.lau@embrapa.br

P. Kuhnem
Biotrigo Genética LTDA, Passo Fundo, RS 99050-970, Brazil

through the main wheat-growing regions of southern Brazil (Valente et al. 2019). After being described, WhSMV was also reported in Paraguay (Esquivel-Fariña et al. 2019) and South Africa (Terefe et al. 2019). SBWMD symptoms include yellow mosaic in leaves and stems, also plants may exhibit stunting or rosette formation, characterized by excessive tillering or reduced development of the root system (Barbosa et al. 2001). Severe infections can cause losses of up to 50% of total grain yield (Barbosa et al. 2001). Also, a reduction of 4–26% in the thousand-kernel weight has been reported (Dalbosco et al. 2002b).

Since SBWMD was reported in Brazil, efforts have been made to manage the disease, but the epidemiological complexity of this pathosystem has made disease management a major challenge. WhSMV is vectored by plasmodiophorid root-infecting *Polymyxa graminis* Ledingham (Valente et al. 2019). Because *P. graminis* cannot be manage by chemical control and the viruses retain their infectivity in the resting spores for many years (Kanyuka et al. 2003; Kühne 2009; Tamada and Kondo 2013), the use of resistant cultivars is the main measure of preventing severe losses. However, although the level of variability of the WhSMV has not yet been studied, it is already known that viruses with an RNA genome have high genetic variability due to high rates of mutation and recombination that can result in overcoming resistance (García-Arenal et al. 2001; Holmes 2009). In addition, there are few WhSMV resistance sources in Brazil and the continuous cultivation of susceptible wheat cultivars year after year probably increased the inoculum in the field.

Due to the increasing spread of WhSMV in wheat-growing regions, farmers and agricultural technicians have demanded solutions to overcome its negative impacts. Crop rotation (CR) is a common practice in agriculture and contributes to reduce the incidence of plant diseases. Although, CR has been cited in some parts of the world as a strategy to reduce the incidence of *P. graminis*-transmitted viruses (Chen and Wilson 1995; Rubies-Autonell et al. 1995), studies about this practice for virus disease management are poorly explored. In Brazil, the first evidences of CR effect on the reduction of SBWMD incidence and yield losses were obtained from a long-term experiment implanted in 1980 (Reis et al. 1985, 1990). This long-term experiment has been maintained for almost 40 years, with plots under wheat monoculture and different CR. Despite being ideal for studying the effects of CR on

SBWMD, little progress has been made in quantifying the CR effects by associating them with probable chemical, physical or biological causes.

The effectiveness of a CR systems is determined by the host range and the time that the virus remain infective in natural soil (Curl 1963). Both serological and molecular diagnosis of the viruses associated with SBWMD in Brazil were not possible before the report of WhSMV (Valente et al. 2019). Therefore, information about the host range of the virus can now be better determined by molecular detection. Although *P. graminis* has resting spores, CR with non-host species of the virus can help reduce the viruliferous population, producing virus-free zoospores.

This work is based on the hypothesis that a CR that consists of non-host plants of the virus, can decrease viral inoculum in the field, reducing disease levels and, consequently, increasing yield. This study aimed to measure the effect of different plant species and CR duration on SBWMD incidence and severity, as well as on the grain yield of wheat cultivars with different degrees of resistance. Host range of WhSMV were performed with the species used in the rotation systems in order to test hypotheses that explain how crop rotation reduces the incidence and severity of the disease. This knowledge will assist the development of efficient and sustainable crop production systems.

Materials and methods

Characterization of the experimental site

The effect of CR on development of SBWMD was evaluated during 2017 and 2018 on a long term field experiment conducted at the Brazilian Agricultural Research Corporation, Embrapa Wheat, located in Passo Fundo municipality, Rio Grande do Sul state, Brazil (28°13′58.78″ S, 52°24′13.83″ W, 672 m altitude). Previous studies reported the occurrence of SBWMD in the experimental field (Reis et al. 1985, 1990). The soil at the experimental site is an Oxisol of clayey texture. The local climate is of the Cfa type, with rains well distributed throughout the year (annual average of 1918 mm for the series 1981 to 2010) and average air temperature in the hottest month (January) above 22 °C. In the coldest month (June), the average air temperature is 13.2 °C and the soil temperature at 10 cm is close to 14 °C.

Field layout

The experimental design was a split plot with four replicates. CR treatments were the whole-plot factor and wheat cultivar the split-plot factor. Each whole plot had three randomized subplots of wheat (*Triticum aestivum* L., Poaceae) cultivars: BRS Parrudo (resistant to SBWMD), BRS Reponte (moderately resistant to SBWMD) and BRS Guamirim (susceptible to SBWMD). All wheat cultivars have similar production cycle. This study was carried out in a long-term experiment, composed by wheat monoculture and three CR systems established in the 1980s (Table 1). Cropping history defined the CR: W = wheat monoculture; W1 = wheat following white oat (*Avena sativa* L., cv URS Guapa, Poaceae); W2 = wheat following vetch (*Vicia sativa* L., Fabaceae) and white oat and; W3 = wheat following black oat (*Avena strigosa* Schreb, cv BRS Neblina, Poaceae), barley (*Hordeum vulgare* L., cv BRS Korbel, Poaceae) and black oat (Table 1). In the summer, the plots were normally cultivated with species such as soybean (*Glycine max* L., Fabaceae), sorghum (*Sorghum bicolor* L., Poaceae), and bean (*Phaseolus vulgaris* L., Fabaceae) as succession crops.

Each split-plot was 40 m^2 (2 m wide × 20 m long). Sowing was carried out mechanically on June 22, 2017 and June 21, 2018, with rows at 0.17 m spacing. The sowing rate was adjusted in terms of seed weight and germination percentage of individual cultivars to provide 300 viable seeds/m^2. In both years, the seeds were previously treated with pyraclostrobin (0.05 g a.i. kg^{-1}) + methyl thiophanate (0.45 g kg^{-1}) and fipronil (0.5 g kg^{-1}). Basal fertilization was performed according to the soil analysis results and consisted of 25 kg ha^{-1} of N and 50 kg ha^{-1} of P$_2$O$_5$ and K$_2$O in both years. Nitrogen was applied as the top dressing at 80 kg N ha^{-1} (urea 45% N) at stage 5 of the Feekes scale. The other cultural treatments were performed in

Table 1 Characterization and sequence of plants used in crop rotation systems

Crop rotation	Crop rotation systems			
W - Monoculture	wheat			
W1 - One-year	white oats	wheat		
W2 - Two years	vetch	white oats	wheat	
W3 - Three years	black oats	barley	black oats	wheat

accordance with Brazilian technical indications for wheat crops.

Disease assessments

SBWMD incidence was evaluated at stage 10 of the Feeke's scale in both years. Owing to the low occurrence of the disease in 2017, the whole plot was evaluated by counting the number of symptomatic plants and calculated considering of the density of 300 plants/m^2. In 2018, evaluation of the number of symptomatic plants was carried out in five representative sample points distributed in each subplot (distribution of the sample points as the five-point face of a die). At each sampling point, a linear meter of plants (*n* = 60) was evaluated, and the number of plants with SBWMD-like symptoms was recorded. The disease incidence (I = Σx / N) was calculated as the proportion of symptomatic plants, which consisted of the number of symptomatic plants (x) divided by the total number of evaluated plants (N). The three external lines of each subplot were not considered for evaluation.

Disease severity was scored in each linear meter of plants evaluated in 2018, using a graded scale of classes 1–5 of severity adapted from Barbosa et al. (2001) as follows: 1 = absence of SBWMD symptoms; 2 = plants with mild symptoms; 3 = plants often with obvious symptoms, with no apparent developmental impairment; 4 = plants with typical SBWMD symptoms, with yellowish streaks on the leaves and stems, without stature or spike reduction; and 5 = plants with SBWMD symptoms on leaves and stems, with compromised growth, stature reduction, and ear development. Plants displaying SBWMD-like symptoms were sampled and stored at −80 °C for confirmation of the WhSMV infection by RT-PCR.

Determination of grain yield (GY), thousand-kernel weight (TKW) and test weight (TW)

Plots were harvested using a Classic ST-Stationary Thresher (Wintersteiger, Ried, Austria) at maturity. Grain moisture and TW were estimated and recorded directly using the grain moisture meter Al-101 portable (Agrologic®). GY, adjusted to 13.5% moisture content, was then converted to kilograms per hectare (kg ha^{-1}), and TW to kilograms per hectoliter (kg hl^{-1}). Two grain subsamples were collected in each subplot for TKW determination.

Statistical analysis

A data analysis was carried out using R software version 3.5.1 (Vienna, Austria) (R Core Team 2020). The number of WhSMV symptomatic plants (count data) was tested for differences between treatments using generalized linear models (GLM) with a Poisson distribution. The statistical analysis of GY, TWK, and TW was performed with ANOVA, and the significance of differences was tested using Fisher's method. Prior to the analysis, data were checked for normality of residuals (Shapiro-Wilk test) and homogeneity of variance (Bartlett test), and transformations were not required. Multiple comparisons were assessed by Tukey's test ($p < 0.05$). The association of SMBWD incidence and wheat GY was calculated by a regression analysis. ANOVA and multiple comparison analysis were carried out using the ExpDes.pt. package (Ferreira et al. 2018) implemented in R software.

Characterization of the WhSMV host range

This experiment was conducted to determine the natural host range of WhSMV and to identify hosts that could serve as inoculum source. The same set of species used in CR systems were sown in an area (28°13'S; 52°24'O) with higher inoculum potential. In this area, historically, field trials had been carried out to assess the susceptibility and resistance of wheat cultivars to SBWMD (Rio Grande do Sul State Assay) and the presence of *P. graminis* and WhSMV has already been described (Valente et al. 2019). Rye (*Secale cereale*, cv BRS Serrano, Poaceae) and triticale (*Triticosecale* Wittmack, lineage PFT1802, Poaceae) were included in the test because they are winter grasses commonly cultivated in southern Brazil. Because rice (*Oryza sativa* L., Poaceae) is the host of the rice stripe necrosis virus (RSNV), a member of the *Benyviridae* family that occurs in Brazil and, like WhSMV, is transmitted by *P. graminis* (Maciel et al. 2006; Valente et al. 2019) two rice cultivars (cv Epagri 109 and cv. SCS 121 CL) were also tested as WhSMV hosts. For this, duplicate lines 0.5 m long were sown with approximately 70–80 seeds produced from virus-free source plants of each species. The plants were monitored in the field for 6–8 weeks. Finally, samples composed of 10 symptomatic plants were collected randomly. However, due to possibility of asymptomatic virus infection, asymptomatic plant species were also collected. Additionally,

asymptomatic soybean (*Glycine max* (L.) Merrill, Fabaceae) volunteer plants and ryegrass (*Lolium multiflorum* L., Poaceae) a common weed in the fields were also collected. The roots were washed individually and the plant material (roots and leaves) was stored at −80 °C until RNA extraction. The root and leaf samples were studied separately. Root samples were also used for vector detection.

WhSMV and *Polymyxa graminis* molecular detection

Wheat stripe mosaic virus (WhSMV) was diagnosed by RT-PCR. Total RNA was extracted from 100 mg of frozen root or leaf samples using Tri reagent (Sigma Aldrich), following the manufacturer's recommendations. cDNA synthesis was performed using ImProm II Reverse Transcription System kit and oligo dT primer (Promega), in accordance with the manufacturer's recommendations, from 1 μg of extracted total RNA. The PCR reaction was performed in a 25 μl reaction volume containing 1 μl cDNA (~1 μg), 2.5 μl of a 10 × Taq reaction buffer, 0.75 μl of MgCl2 (25 mM), 0.5 μl of dNTPs (10 mM), 0.5 μl of each primer Beny CP-F/Beny CP-R (Valente et al. 2019), 1 U Platinum Taq DNA polymerase (Invitrogen), and ultra-pure water to make up the final volume of the reaction. Reaction conditions were in accordance with the work of Valente et al. (2019). A wheat plant infected with WhSMV and a virus-free plant were used as positive and negative controls of the PCR reaction, respectively.

For *P. graminis* detection, total DNA was extracted from 100 mg of root samples ground with liquid nitrogen in accordance with the protocol described by Doyle and Doyle (1987), followed by PCR using the primers Pgfwd2/Pxrev7 (Ward and Adams 1998). The PCR mixture consisted of 5 μl of extracted total DNA (~1 μg), 2.5 μl of 10 × Taq reaction buffer, 0.75 μl of MgCl2 (25 mM), 0.5 μl of dNTPs (10 mM), 0.5 μl of each primer, and 1 U Platinum Taq DNA polymerase (Invitrogen). PCR conditions were in accordance with the work of Tyagi et al. (2016). A sample of wheat root infected with *P. graminis* and a sample of *P. graminis*-free root were used as positive and negative controls for PCR, respectively. The RT-PCR and PCR amplified fragments were subjected to 1% agarose gel electrophoresis, stained with GelRed (Biotium), visualized under a UV light transilluminator, and photographed.

Results

Weather conditions

Total and monthly rainfall varied from year to year during the experimental period (Fig. 1). The winter crop season of 2018 (June–October) was rainier (total precipitation: 1000.4 mm) than the same period in 2017 (total precipitation: 763 mm). July was the driest month in 2017 (21.3 mm). Rainfall in 2018 was more equally distributed between the months. Less rainfall was recorded in July (107.5 mm). Regarding the mean soil temperature (10 cm in depth) and air temperature, 2018 was colder than 2017, especially in June and August (Fig. 1).

SBWMD incidence and severity

In 2017, SBWMD incidence was less than 1% and there was no difference between the factors (data not shown). On the other hand, in 2018 SBWMD incidence ranged from 0.3 to 19.2%, so it was possible to detect differences in the disease incidence comparing cultivars and CR according to GLM outputs ($p < 0.05$, Table 2). By the ranking of cultivars, the susceptible BRS Guamirim (10.2%) had the highest average SBWMD incidence across CR systems, followed by moderately resistant BRS Reponte (6.0%) and resistant BRS Parrudo (2.5%).

Disease incidence was strongly affected by CR (Table 2). SBWMD incidence in BRS Guamirim cultivar was about 90% lower in 3-years black oats-barley-black oats rotations (1.35%; W3) and 2-years vetch-white oats rotations (2.25%; W2) compared with wheat monoculture (19.2%; W; Table 2; Fig. 2). The effect of CR systems was similar to other cultivars, although at lower levels of disease incidence (Fig. 2). The 1-year CR with white oats was not sufficient for disease reduction compared to wheat monoculture ($p < 2e-16$; Table 2; Fig. 2).

Disease severity had similar pattern to incidence (Fig. 3). The highest values of the severity scores were recorded with more frequency in BRS Guamirim cultivar, followed by BRS Reponte and BRS Parrudo. Also, highest severity scores were more frequent in wheat monoculture (W) and 1-year of CR with white oats (W1) compared with 3-years of CR with black oats-barley-black oats (W3) and 2-years of CR with vetch-white oats (W2; Fig. 3).

Grain yield (GY), thousand-kernel weight (TKW) and test weight (TW)

In 2017, GY and TW differed by CR and cultivar while TKW differed only across cultivars (Table 3). Wheat monoculture (W) presented lower GY (2566 kg ha^{-1}) while rotations for 2-years with vetch and white oats (W2) increased GY in 19% compared to W (Table 4). Intriguingly, 3-years of CR with wheat following black oat, barley and black oat (W3) did not differ from wheat monoculture in GY (Table 4). Likewise, the TW was lower in W3 and the other rotations did not differ from the wheat monoculture in this variable (Table 4). Overall, grain yield was about 35% higher in BRS Reponte compared to other cultivars. Also, BRS Reponte had higher TW (78.4) than either BRS Guamirim (76.3) or BRS Parrudo (74.6; Table 4). BRS Parrudo had TKW 4 to 6% lower than BRS Guamirim and BRS Reponte, respectively (Table 4).

In 2018, there was a CR-cultivar interaction for the GY and TKW ($P = 0.018$; Table 3). When compared to wheat monoculture, the increase in CR period rise GY in all cultivars, (Fig. 4). For BRS Reponte CR increases of 40% (W1), 49% (W3) and 58% (W2) were noted in GY compared to W. However, not significant differences were noted between them, except in relation to W (Fig. 4). BRS Parrudo, had highest GY in rotations with vetch-white oats (W2) and with black oat-barley-black oat (W3) that generate increases of 106% and 73.7% in GY, respectively, compared with wheat monoculture, but W3 rotation did not differed from 1-year rotation with white oats (W1).

On the other hand, in BRS Guamirim, GY was higher (81%) in 3-years rotations (W3), compared with wheat monoculture (W), but not different from 2-years rotations (W2) (70% of increase; Fig. 4). The main effects of the factors affected TKW and TW (Table 4). The TKW was higher in 2-years rotations, while TW did not differ across rotations (Table 4). BRS Guamirim had TKW around 4% higher than others cultivars, while the cultivar BRS Reponte had higher TW (79.4) than either BRS Guamirim (77.4) or BRS Parrudo (74.8; Table 4).

Association of SBWMD incidence and GY

The relationship between SBWMD incidence and GY described by regression model (Fig. 5), accounted for 42 and 45% of the variance. The estimated slope of the regression line obtained for cultivar BRS Reponte was

Fig. 1 Daily rainfall, daily mean air temperature and daily mean soil temperature (10 cm of depth) during experimental period over 2017 **a** and 2018 **b** crop seasons. Black bars correspond to daily rainfall, dotted line corresponds to the average daily soil temperature at a depth of 10 cm (°C) and; continuous line corresponds to the average daily air temperature (°C)
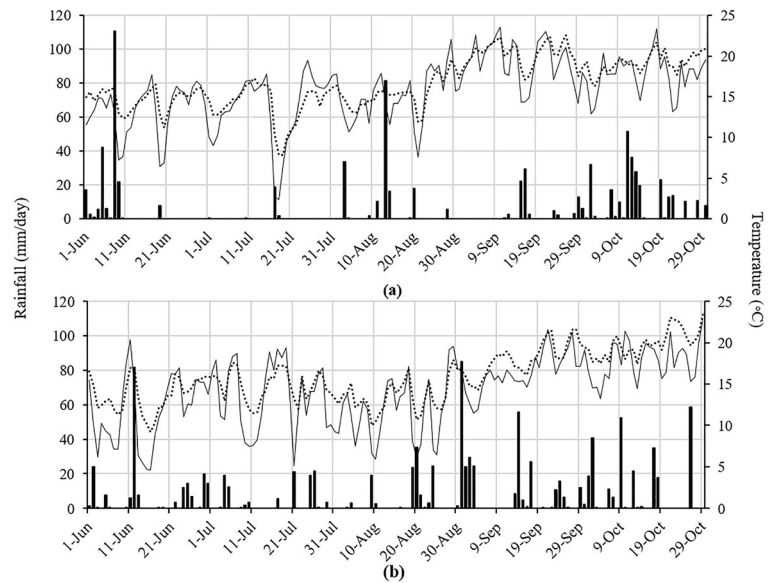
−65.5 ($P < 0.01$), and that for BRS Guamirim was −44 ($P < 0.01$), indicating that for each percentage unit of increase in SBWMD incidence, there were an estimated reductions of 65 and 44 kg ha^{-1} in GY for BRS Reponte and BRS Guamirim, respectively (Fig. 5). By contrast, there was no significant relationship for BRS Parrudo (Fig. 5).

Natural WhSMV host range

The same set of species used in CR systems with wheat were sown in an area with high inoculum potential. Symptoms of WhSMV on collected plants of the wheat cultivars BRS Guamirim, BRS Reponte, and BRS Parrudo were evident, and viral infection was confirmed by RT-PCR (Fig. 6a). Barley (cv. BRS Korbel), black oat (cv. BRS Neblina) and white oat (cv. URS Guapa), did not present any symptoms of viral infection (Fig. 6b). However, WhSMV was detected in tissues of the barley root system by RT-PCR but not in the leaves (Fig. 6d). Among the additional species tested, symptoms of viral infection were detected in rye (BRS Serrano) and triticale (lineage PFT 1802) (Fig. 6b). In rye, the symptoms were weak, with only a few contrasts between shades of dark green and light green on the leaves (Fig. 6b). In triticale, symptoms were more

Table 2 Summary of the results of the GLM model for explaining the variability in the soil-borne wheat mosaic disease incidence in cultivars and crop rotation (CR) systems in 2018

Parameter	Parameter estimated	Standard error	Z value	P value[a]
Intercept	2.535	0.071	35.605	**<2e-16**
Block 1-Block 2	0.011	0.076	0.152	0.879
Block 1-Block 3	−0.146	0.079	−0.1855	0.063
Block 1-Block 4	−0.110	0.078	−1.409	0.158
W-W1[b]	−0.103	0.059	−1.737	0.082
W-W2	−2.318	0.159	−14.508	**<2e-16**
W-W3	−2.712	0.191	−14.146	**<2e-16**
cv. Guamirim - cv. Parrudo	−1.366	0.083	−16.367	**<2e-16**
cv. Guamirim - cv. Reponte	−0.541	0.062	−8.720	**<2e-16**

[a] Significant *P* values (p < 0.05) are indicated in bold

[b] Wheat monoculture (W) and three CR systems: 1-year, white oats-wheat, W1; 2-years, vetch-white oats, W2; and 3-years, black oats-barley-black oats, W3)
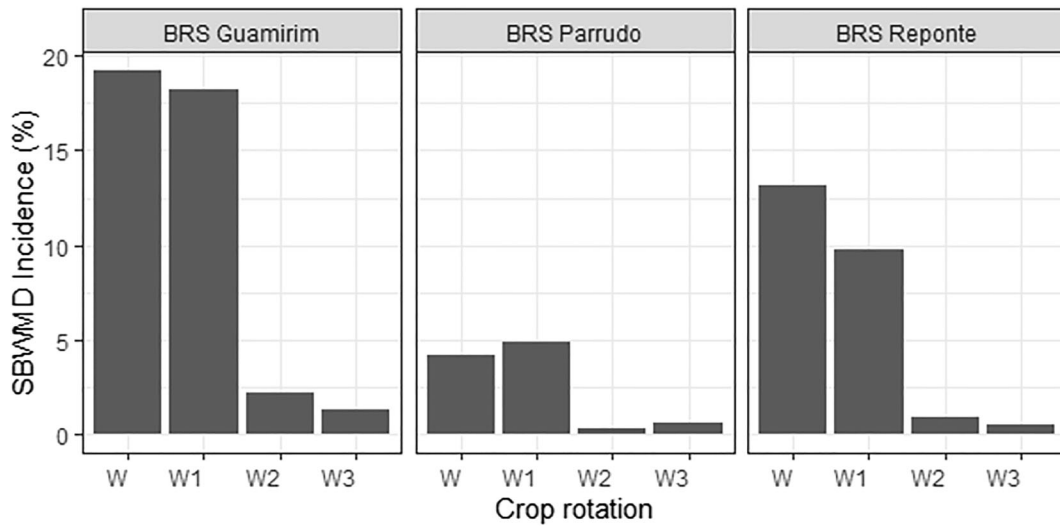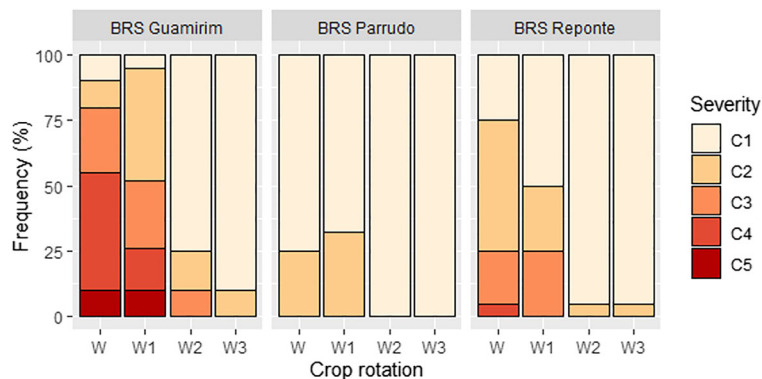
Fig. 2 Mean values of SBWMD incidence of wheat cultivars under wheat monoculture (W) and three crop rotation systems: 1-year, white oats-wheat, W1; 2-years, vetch-white oats, W2; and 3-years, black oats-barley-black oats, W3) in 2018

evident, indicating yellowish longitudinal streaks in the leaves and stems (Fig. 6b). WhSMV infection was confirmed by RT-PCR for both species in roots and leaves (Fig. 6d). Besides rice being a host of another species member of the family *Benyviridae*, no WhSMV symptoms were observed in rice (CS 121 CL and Epagri 109), and no RT-PCR amplification was detected. None of the other species was infected by WhSMV (Fig. 6c and d). Notably, *P. graminis* was detected only in the roots of white oats cv URS Guapa and rye cv BRS Serrano (data not presented). Not detecting the vector

does not necessarily reflect its absence because detection depends on the sampling of the root segment containing the microorganism.

Discussion

The CR systems evaluated in the present study were established in the early 1980s. Since that time, significant variations in wheat yield between rotation systems have been reported. Physical, chemical and biological

Fig. 3 Severity scores frequency of SBWMD of wheat cultivars under monoculture (W) and three crop rotation systems: 1-year, white oats-wheat, W1; 2-years, vetch-white oats, W2; and 3-years, black oats-barley-black oats, W3) in 2018. Severity grade scale: C1 = absence of symptoms; C2 = plants with mild symptoms;

C3 = plants often with obvious symptoms, with no apparent developmental impairment; C4 = plants with typical symptoms, with yellowish streaks on the leaves and stems, without stature or spike reduction; and C5 = plants with symptoms on leaves and stems, with compromised growth, stature reduction, and ear development

Table 3 Mean squares from repeated measures of Anova for wheat grain yield (GY), thousand-kernel weight (TKW) and test weight (TW) over 2017 and 2018

Treatments	GY (kg ha^{-1})	TKW (g)	TW (kg hl^{-1})
2017			
Crop rotation (CR)	616,243 **(0.039)**[a]	1.915 (0.155)	**(0.000)**
Cultivars (C)	5,467,643 (<2e-16)	20.2911 **(0.000)**	69.705 (<2e-16)
CR x C	20,766 (0.947)	2.771 (0.082)	2.490 (0.051)
2018			
Crop rotation (CR)	7,555,696 **(0.000)**	23.5367 **(0.000)**	7.061 (0.724)
Cultivars (C)	4,861,198 (<2e-16)	10.1811 **(0.000)**	109.413 **(0.000)**
CR x C	228,088 **(0.018)**	2.1463 **(0.009)**	12.272 (0.102)

[a] Significant P ($p < 0.05$) values are indicated in bold

factors associated to the soil may cause these differences. Among the possible explanations for yield oscillation are the levels of diseases whose epidemiology is linked to the soil. Like other virus transmitted by *P. graminis,* soil water is critical for the swimming zoospore to reach a host root and transmit WhSMV (Cadle-Davidson and Bergstrom 2004; Kanyuka et al. 2003). Therefore, rainier years are more favorable for the transmission of the virus and the occurrence of the disease. The observed increase in SBWMD and accentuated yield differences between rotation systems observed in 2018 that coincided with heavier rainfall, further supports this hypothesis.

In an epidemiological report from 1983, one of the rainiest years since 1980 until today, a high incidence of SBWMD and wide variation in grain yield between CR compared to wheat monoculture was reported (Reis et al. 1985, 1990). Already in that time, there was enough inoculum in the area to compromise wheat yield by almost 90%. However, plots under CR for 3-years with wheat followed by rape (*Brassica napus* L., Brassicaceae), flax (*Linum usitatissimum* L., Linaceae) or barley, lupine (*Lupinus albus* L, Fabaceae) had 54–70% lower incidence in relation to wheat monoculture. Since then, significant differences in wheat yield and between the rotation systems have been demonstrated, mainly in years with disease-favorable environmental conditions, corroborating the hypothesis that SBWMD is one of the main factors affecting productivity (Reis et al. 1985, 1990; Santos et al. 1996). The present study is in accordance with previous results obtained regarding the effect of CR on the incidence of SBWMD. In

Table 4 Mean wheat grain yield (GY), 1000-kernel weight (TKW) and test weight (TW) for three wheat cultivars cultivated in three crop rotation systems: 1-year, white oats-wheat, W1; 2-years, vetch-white oats, W2; and 3-years, black oats-barley-black oats, W3

Principal effects	Growth season 2017			Growth season 2018		
	GY (kg ha^{-1})	TKW (g)	TW (kg hl^{-1})	GY (kg ha^{-1})	TKW (g)	TW (kg hl^{-1})
Crop rotation						
W	2566b[a]	32.0a	77.3a	2291c	29.0c	76.3a
W1	2789ab	32.8a	76.7a	3379b	31.1b	77.0a
W2	3055a	32.6a	77.9a	4018a	32.3a	77.7a
W3	2594b	32.3a	73.4b	3929ab	31.3ab	78.0a
Wheat cultivar						
BRS Parrudo	2482b	31.4b	74.6c	3320b	30.5b	74.8c
BRS Reponte	3362a	32.7a	78.4a	3926a	30.6b	79.4a
BRS Guamirim	2434b	33.4a	76.3b	2949b	31.8a	77.4b

[a] Means followed by different letters in the columns are significantly different ($p < 0.05$)

Fig. 4 Box plots of grain yield values for the crop rotation x cultivars interaction in 2018. In the boxplots, the central line in the boxes represents the median, and the top and bottom are the 75th and 25th quartiles. The whiskers are the upper and lower limits based on the interquartile ranges (IQRs; Q ± 1.5 × IQR)
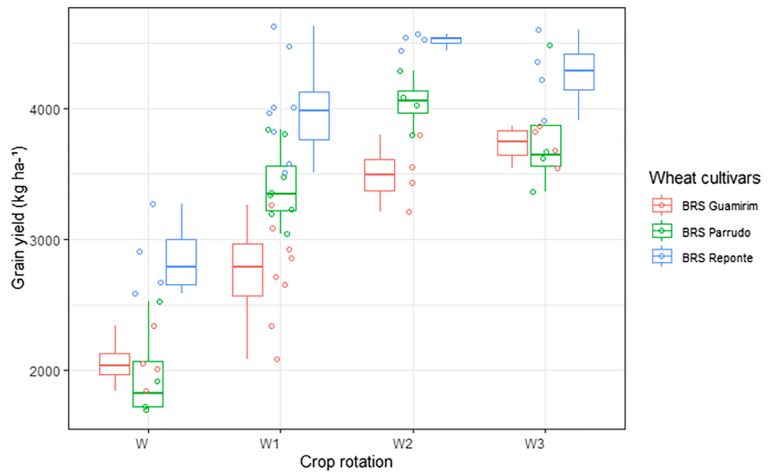

2018 (higher rainfall), conditions were more favorable for disease development and as expected, GY varied more between CR systems than in 2017. In rotations for 3 and 2-years with wheat followed by black oats, barley and black oats and wheat followed by vetch and white oats, respectively, presented SBWMD incidence up to 90% lower compared with wheat monoculture. Also, it was demonstrated a negative correlation between SBWMD levels and yield. Consequently, GY was higher in rotations for 2 and 3-years compared to wheat monoculture.

To begin to explain how CR affects SBWMD levels, this work evaluated the host range of the virus in more detail. Until recently, there were no molecular tools to precisely demonstrate if SBWMD etiological agent was infecting a presumable host. Using RT-PCR to detect WhSMV potential hosts, it was demonstrated that, of the plants grown in winter and therefore used as an alternative to growing wheat, black oats, white oats and vetch were non-host plants. Only barley was naturally infected by WhSMV in the field. Like oats and vetch, barley was asymptomatic. The detection of the virus in barley was confirmed only in the roots, suggesting that the virus does not move to the aerial part of the plant. Notably, the only system in which barley was present is the system with 3-years of CR (W3) presented the lowest levels of incidence of the disease. It is possible that the viral load and vector multiplication in this host were limited, with no significant increase of the inoculum in the system, but additional studies should be performed to test this hypothesis. In addition, the cultivation of two non-hosts (black and white oats) before and after the cultivation of barley might have significantly reduced the inoculum in the system.
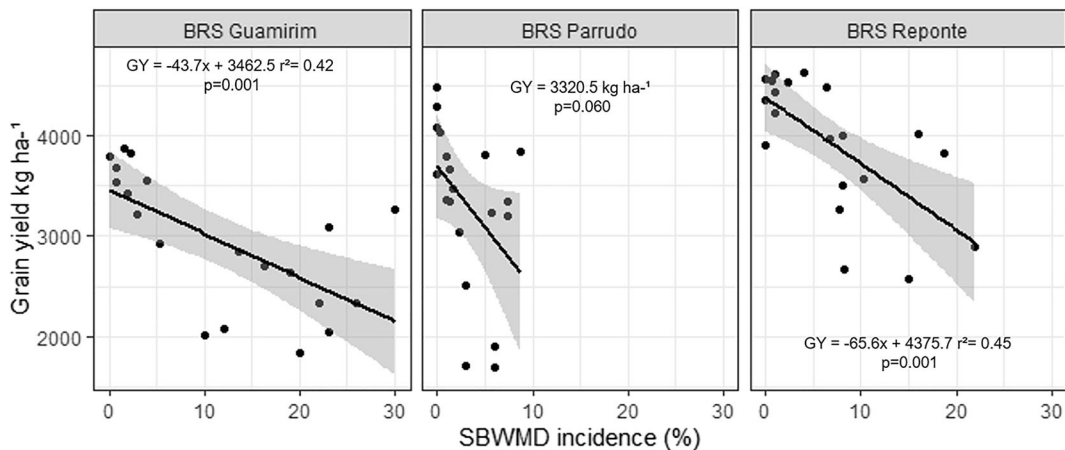
Fig. 5 Correlations between SBWMD incidence and grain yield from wheat cultivars BRS Reponte, BRS Guamirim, and BRS Parrudo in 2018. Gray shadows correspond to the 95% confidence interval
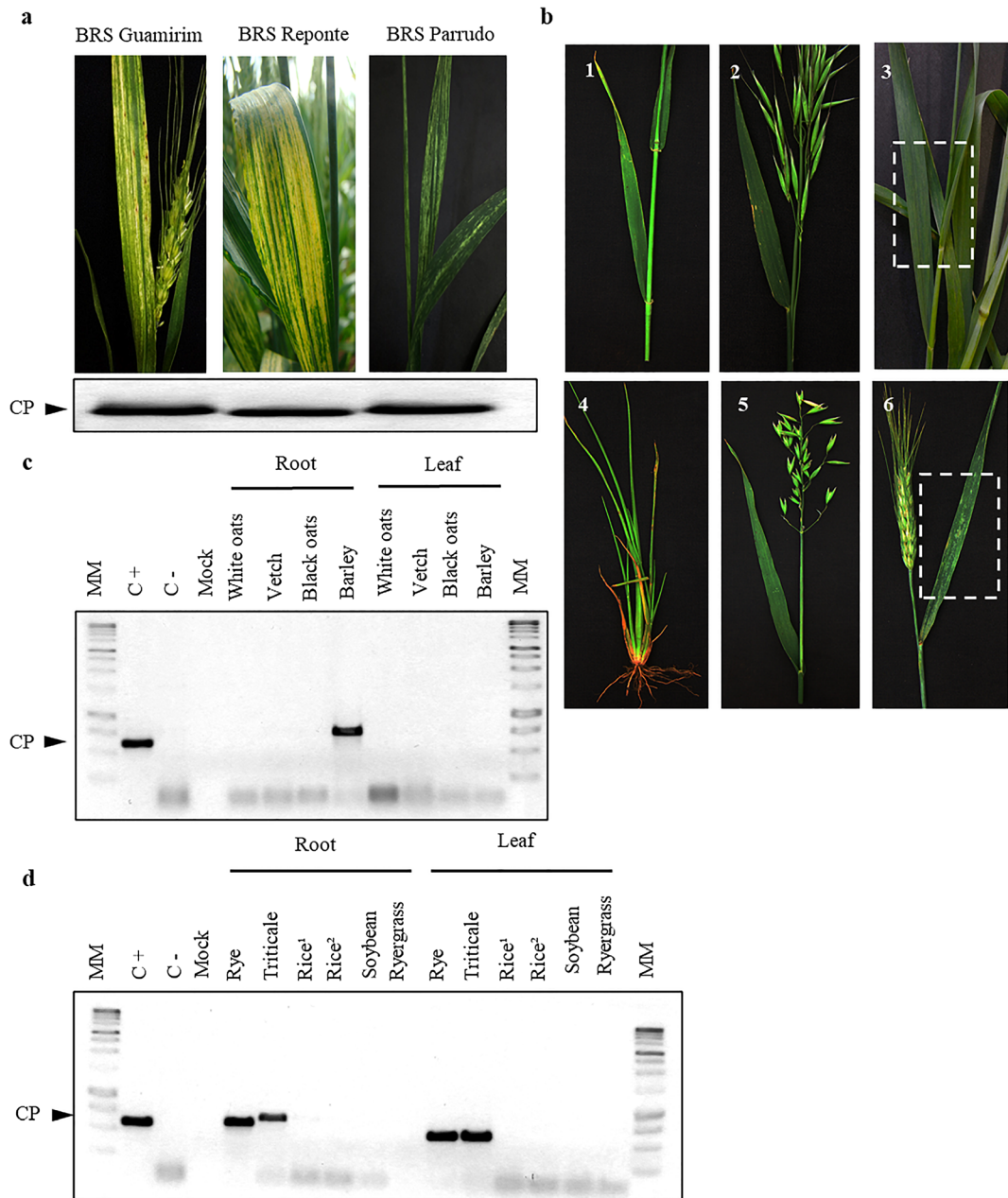
Fig. 6 Symptoms induced by wheat stripe mosaic virus (WhSMV). **a**, Symptoms of WhSMV on wheat cultivars BRS Guamirim, BRS Reponte, and BRS Parrudo. Bands below represent WhSMV coat protein (CP) gene detection by RT-PCR in each cultivar collected in experiment 1. **b**, Partial characterization of the natural host range 1: barley (BRS Korbel), 2: black oat (BRS Neblina), 3: rye (BRS Serrano), 4: rice (Epagri 109), 5: white oat (URS Guapa), and 6: triticale (lineage PFT 1802). Dotted rectangles indicate the symptoms of WhSMV infection. **c** and **d**, Detection of the WhSMV coat protein (CP) gene by RT-PCR in roots and leaves of winter rotation plant species and additional plant species tested. MM is the molecular marker 1 kb DNA ladder, and C+ and C− are positive and negative controls of RT-PCR with one plant WhSMV infected and one plant healthy, respectively. Mock treatment is the additional control with water. [1]rice cultivar CS 121 CL, [2]rice cultivar Epagri 109

Although CR is seldom included in recommendations for controlling diseases caused by viruses (Curl 1963), results of the present study suggest that not only is CR sequence determinant in reducing SBWMD levels, but the rotation period as well. One year rotation may not be enough time to reduce the inoculum.

P. graminis produces resting spores and has a wide range of hosts (Adams and Jacquier 1994; Legréve et al. 2000). *P. graminis*-transmitted virus are associated with the resting spores of the vector (Kanyuka et al. 2003; Kühne 2009). So, the way to decrease virus infection is by producing aviruliferous spores. An efficient way to do this is to use plant species that are good hosts for *P. graminis*, but that do not host and multiply the virus. In this study the vector was detected only in white oat and rye, possibly because of some failure in the sampling process, since the infection by the virus suggests the presence of the vector. In Brazil, *P. graminis* had already been detected in wheat, barley, triticale, and rice roots (Dalbosco et al. 2002a; Maciel et al. 2006). *P. graminis* is also reported to infect several other important species cultivated around the world, such as rye, sorghum (*Sorghum bicolor* (L.) Moench), millet (*Pennisetum glaucum*), corn (*Zea mays* L.) including wheat, triticale, and rice (Adams and Jacquier 1994; Legréve et al. 2000).

Adams et al. (1987) reported that after zoospores carrying the barley yellow mosaic virus (BYMV) colonized roots of immune or virus-resistant barley cultivars, their progeny were unable to transmit the virus again to susceptible hosts. In agreement with these results, other researchers found a lower incidence of BYMV and barley mild mosaic virus (BaMMV) with 3-years of CR and attributed this effect only to the non-host species used (Rubies-Autonell et al. 1995). In addition, continuous cultivation of SBWMV-resistant cultivars significantly reduced the incidence of viral infection in a susceptible cultivar after 8 years (Gitton et al. 1995). By contrast, Adams et al. (1993) observed that the cultivation of BaMMV-resistant barley cultivars for 3 years on infested soil did not have a significant effect on reducing the incidence of a susceptible cultivar and inoculum, contrary to their own results observed years earlier in the laboratory (Adams et al. 1987). This contradiction may be related to the different cultivars used by the authors in the two studies, since some resistant cultivars may have no visible symptoms but a high viral titer in leaves and roots (Kanyuka et al. 2004) and, consequently, serve as an inoculum multiplier.

In the present study, WhSMV was detected by RT-PCR in the three wheat cultivars studied, independent of the level of resistance. These results need to be analyzed with caution, since only symptomatic plants have been analyzed. However, subsequent studies are required to understand the mechanisms of resistance and verify whether the level of resistance of Brazilian wheat cultivars has any effect on inoculum reduction. Also, positive productivity responses should be more noticeable for susceptible cultivars, such as BRS Guamirim, due to the greater variation in the incidence of SBWMD between the systems with higher incidence (W) and lower incidence (W2 and W3). However, unexpectedly, the highest positive yield response was observed with the resistant cultivar BRS Parrudo. Some resistant cultivars may not have visible symptoms of viral infection; however, they allow high replication rates and viral accumulation in tissues (Kanyuka et al. 2004). Although this is possible, further studies are needed to prove whether this is occurring and affecting the cultivar BRS Parrudo.

Among other winter cereal species tested, rye and triticale were found to be hosts of WhSMV. So, these species should not be used in rotation systems with wheat. Ryegrass (*Lolium multiflorum*) was also analyzed because is one of the main weeds of winter crops in Brazil (Lamego et al. 2013), and one of the main weeds found in the field area. This study demonstrated that ryegrass is not a host of WhSMV and also *P. graminis* was not detected. From an epidemiological point of view, this is very important because ryegrass together with black oats occupy a significant area of winter fields in southern Brazil. It is reported that many wild grass species such as *Agropyron repens* (L.) Beauv., *Bromus inermis* Leyss., *Bromus tectorurn* L., and *Hordeum jubatum* L. are also reported as hosts of *P. graminis* (Barr 1979). So, the host range of *P. graminis* and WhSMV in Brazil should be investigated in more detail since wild grasses may serve as a source of inoculum of the vector and virus. In fact, Valente et al. (2019) raised the hypothesis that WhSMV could be a native virus from South America that acquired the ability to infect wheat.

Among summer crops, soybean was not a WhSMV host and unlikely a host of *P. graminis*. Therefore, soybeans should not interfere significantly in the epidemiology of this virus, and the vector spores remaining at rest during the summer period in soybean areas. On the other hand, the use of grasses in the summer can interfere in the growth of inoculum depending on whether they are able to host *P. graminis* and WhSMV. Unfortunately, in this work, the main summer-cultivated grass in this region, corn, was not tested. The effects of corn crop in areas with SBWMD have yet to be established. Rice was tested since it is a host of RSNV in Brazil, which is also transmitted by *P. graminis*. Although

WhSMV is from the same family as RSNV and has the same vector, WhSMV was not able to infect rice. This fact may be important, as it represents a barrier to the recombination of these viruses.

The 2-year test results described in this work represent the current point of a system established almost 40 years ago. Whether CR systems have had a cumulative and progressive effect or the differences between systems have stabilized at some point in time is difficult to ascertain. It is not possible to resolve with certainty the number of years after establishment of a CR system in which there is a significant reduction of the disease. It is also difficult to determine the effect of the SBWMD factor and the effects of other factors from the rotation systems on productivity. Nonetheless, the precise measurements performed in this work agree with the observations that there is a difference in the SBWMD incidence in the different CR systems, and the incidence decreases as the rotation time with non-hosts species of the virus increases. Under favorable conditions, the impact of CR becomes more evident when susceptible wheat cultivars are used. Therefore, these results confirm that the species used in the crop rotation systems studied here can be safely used for modeling a CR system for SBWMD management. From the point of view of future perspectives, it is important to evaluate the regions that are more favorable to the disease and to implement CR together with the use of resistant cultivars for the adequate management of this disease.

Authors' contributions Douglas Lau, Fábio Nascimento da Silva, Lucas Antonio Stempkowski, Ricardo Trezzi Casa and Paulo Kuhnem, planned and designed experimental work. Data collection were performed by Lucas Antonio Stempkowski, Fernando Sartori Pereira, Mônica Farias, Juliana Borba Valente, Douglas Lau and Fábio Nascimento da Silva. Lucas Antonio Stempkowski and Douglas Lau conducted data analysis. Lucas Antonio Stempkowski, Douglas Lau, Fábio Nascimento da Silva and Talita Bernardon Mar proposed and written first draft of the manuscript. All authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

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Compliance with ethical standards

Conflict of interest The authors declare that there are no conflicts of interest.

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