Plant Pathology (2018) **67**, 1680–1686 Doi: 10.1111/ppa.12889

Distinguishing sporulating and nonsporulating lesions as a method for evaluating the resistance of sugarcane genotypes to orange rust

A. S. Moreira[a]*, A. F. Nogueira-Júnior[b], C. R. N. B. Gonçalves[c], N. A. Souza[c] and A. Bergamin Filho[b]

[a]*Embrapa Cassava & Fruits, Cruz das Almas, BA, 44380-000;* [b]*Plant Pathology and Nematology Department, University of Sao Paulo, CP 9, Piracicaba, SP, 13418-900; and* [c]*Technology Sugarcane Center, CP 162, Piracicaba, SP, 13400-970, Brazil*

Sugarcane breeding programmes rank the resistance of genotypes to *Puccinia kuehnii*, causal agent of orange rust, according to levels of disease severity. However, during the screening stages, this method of assessment can lead to precipitous elimination of genotypes with promising agronomic traits but showing mild symptoms of rust such as flecks or lesions that do not produce spores. This study aimed to propose a new method to classify the resistance of sugarcane genotypes to orange rust by counting sporulating lesions. Five sugarcane varieties with different levels of resistance to *P. kuehnii* were inoculated with two pathogen populations under controlled conditions. The disease severity (SEV), total number of lesions (TNL), and total number of sporulating lesions (TNSL) were evaluated in a 20 cm leaf fragment from the most diseased leaf. The TNL and TNSL evaluations were performed at 11, 16 and 21 days after inoculation (DAI) and SEV at 21 DAI. The thresholds of 80% and 8% of sporulating lesions (SL) separated susceptible from the intermediate varieties and intermediate from the resistant ones, respectively. It is proposed that the method of counting sporulating lesions be used in screening genotypes for resistance to *P. kuehnii* in sugarcane breeding programmes.

Keywords: Puccinia kuenhnii, *Saccharum* spp., sugarcane breeding, sporulating lesions

Introduction

Puccinia kuehnii, the causal agent of sugarcane orange rust (SOR), is distributed in Asia, Africa, the Americas and Oceania. The pathogen is easily spread by wind and wind-blown rain. In 2000, the disease became endemic in Queensland, Australia, due to a wide sugarcane area being planted with a susceptible cultivar. Orange rust quickly spread causing yield losses of up to 40% during the following years (Magarey *et al.*, 2003; CABI, 2016). In Brazil, the sugarcane-producing areas faced the first orange rust report at the end of 2009. However, the losses from orange rust in Brazil were lower than those in Australia due to the wide use of Brazilian varieties resistant to *P. kuehnii*. The disease is widespread in the main Brazilian sugarcane-producing areas, but the major disease incidence occurs in the south-central part of Brazil, such as the states of Sao Paulo, Mato Grosso do Sul, Parana, Espirito Santo, Goias and Minas Gerais. In susceptible varieties, yield losses greater than 50% were reported (Araújo *et al.*, 2013). After detection in Brazil (Barbasso *et al.*, 2010), orange rust was also reported in other South American countries including Colombia (Cadavid *et al.*, 2012), Ecuador (Garcés *et al.*, 2014) and Argentina (Funes *et al.*, 2016).

Generally, the sugarcane host response to rust infections leads to three types of lesion: (i) hypersensitive reaction expressed as flecks or necrotic lesions usually without sporulation; (ii) typical lesions with abundant sporulation; and (iii) depending on the resistance level, an intermediate response of pustule formation, necrotic lesions and nonsporulating lesions in the same leaf. The presence of an intermediate response has been observed in several pathosystems, such as *Phakopsora phachyrhizi*–soybean (Soares *et al.*, 2009), *Puccinia psidii*–Myrtaceae (Junghans *et al.*, 2003; Glen *et al.*, 2007), *Puccinia sorghi*–maize (Reuveni *et al.*, 1996), *Puccinia triticina*–wheat (Agarwal *et al.*, 2003), and *Pyricularia oryzae*–rice (Roumen *et al.*, 1992).

The quantitative traits commonly measured in plant–pathogen systems are infection frequency (efficiency), latent period, spore production rate, lesion size and infectious period (Parlevliet, 1979; Lannou, 2012), which describe well the epidemic phase of polycyclic diseases, such as rusts. These quantitative traits should initially be measured in controlled environments. In field conditions, the expression of these traits in plant–pathogen interactions are strongly influenced by environmental effects and environment-by-genotype interactions (Lannou,

*E-mail: alecio.moreira@embrapa.br

Published online 25 June 2018

Plant Pathology

2012). For example, spore production of *P. triticina* lasts more than 40 days under controlled conditions on wheat plants and around 15 days under field conditions (Robert *et al.*, 2004; Lannou, 2012).

A decreased infectious period, longer latent period, and reduced spore production result in a delay in onset of an epidemic and the slowing down of epidemic development. A shorter infectious period is responsible for delay in epidemic onset, and the combination of shorter infectious period, longer latent period and reduced spore production causes the slowing down of epidemics. A reduction in spore production has a direct effect on the rate of disease progress. These effects lead to fewer lesions, which start to produce spores later at a lower rate. Nonsporulating lesions have epidemiological relevance by causing a decrease in disease progress in areas growing genotypes with quantitative resistance (Parlevliet, 1979). Soybean genotypes with low incidences of *P. pachyrhizi* have shown red-brown reactions followed by the expression of partial resistance, resulting in longer disease latent periods, low rates of increase in pustule number over time, and small lesions (Twizeyimana *et al.*, 2008). This type of quantitative resistance, or partial resistance, contributes to long-term use of additional disease control measures (Ribeiro do Vale *et al.*, 2001). For sugarcane, breeders do not need to look for partial resistance in primitive genotypes from centres of diversity or wild sugarcane species; this resistance is present in almost all varieties, which makes crop breeding much easier (Ribeiro do Vale *et al.*, 2001).

In Brazilian sugarcane breeding programmes, screening for orange rust-resistant varieties is based on a diagrammatic severity scale adapted from the brown rust (caused by *Puccinia melanocephala*) developed by Amorim *et al.* (1987). Klosowski *et al.* (2013) published a similar diagrammatic scale; however, this scale was developed for evaluations of orange rust severity on sugarcane leaves. Both diagrammatic scales are based on disease severity and do not take into account the presence or absence of urediniospore production by the lesions.

The use of other variables in addition to disease severity to evaluate a plant's resistance to pathogens has already been developed. Parlevliet *et al.* (1984) adopted the number of uredia of *Puccinia hordei* per barley plant to separate the varieties according to their degree of horizontal resistance to the pathogen. In studies of common rust on maize, evaluation by counting pustules proved to be more objective, accurate and reproducible than evaluations based on diagrammatic scales only (Bade & Carmona, 2011). Even studies of dispersal of *Puccinia striiformis* f. sp. *tritici* have adopted a lesion count rather than measures of disease severity (Farber *et al.*, 2017).

The use of additional methods for assessment of orange rust besides assessing disease severity may provide a more precise selection of promising sugarcane genotypes, especially those that exhibit nonsporulating flecks. Thus, this work aims to develop and evaluate the use of a sporulating lesion counting method in order to offer an additional tool for selection of orange rust-resistant varieties in sugarcane breeding programmes.

Materials and methods

Puccinia kuehnii isolate collection

The inoculations were performed using two *P. kuehnii* collections: one collected in a sugarcane experimental area planted with the variety SP81-3250 in the municipality of Araras (SP), and the other collected in a commercial sugarcane-growing area planted with the variety CT96-3415, located in the municipality of Paranacity (PR). In each collection, 100 sugarcane leaves with typical orange rust symptoms were collected in order to obtain *P. kuehnii* urediniospores. Furthermore, some of the urediniospores were extracted from the collected leaves and analysed under an optical microscope in order to observe the typical cell wall apical thickening of *P. kuehnii* urediniospores, which is not present in *P. melancephala* cell walls.

Sugarcane varieties

Five sugarcane varieties were selected based on their importance in Brazilian sugarcane-growing areas and on their different resistance levels to *P. kuehnii* under field conditions (Table 1). Seven plants were used for each sugarcane variety (repetitions). The plants were obtained from cane seed pieces with buds and cultivated in pots of 700 mL capacity containing substrate. After planting, all plants received a single fertilization of ammonium sulphate (5 g per plant) and were kept in a greenhouse for 30 days. Plants were then transferred to individual growth chambers for inoculation.

Inoculation with *P. kuehnii*

The inoculations using *P. kuehnii* populations were carried out according to the procedure described by Martins *et al.* (2010). Before inoculation, urediniospore viability was verified in a germination test on water agar (Braithwaite *et al.*, 2009; Fig. 1a). The inoculum consisted of urediniospore suspensions, obtained by shaking infected leaves in water and gently scraping with a brush (Fig. 1b). To assess viability, four replicate samples (50 μL each) of each suspension were spread onto water agar plates, incubated at 22 °C for 8 h, and examined microscopically for germination. The germination rate was calculated based on the observation of 100 urediniospores per replicate sample. Urediniospore concentrations were adjusted to 5×10^4 viable urediniospores mL^{-1} of distilled water according to the germination percentage (Table 2). Inoculations were made by

Table 1 Susceptibility level of variety (SLV) for five sugarcane varieties inoculated with *Puccinia kuehnii* isolates under field conditions.

Sugarcane variety	SLV	Reaction to *P. kuehnii*	Reference
SP89-1115	3	Susceptible	Barbasso *et al.* (2010)
RB85-5156	2	Intermediate	Minchio *et al.* (2011)
RB86-7515	1	Resistant	Klosowski (2012)
CTC 3	3	Susceptible	Nunes Junior (2010)
CTC 6	1	Resistant	Dalri (2012)

spraying 10 mL of each *P. kuehnii* population onto the sugarcane leaves (abaxial and adaxial surfaces). Urediniospores produced at the end of the first infection experiment were used for the repeat infection experiment, employing the same inoculation procedure. The amount of urediniospores collected at the end of the first infection experiment was not sufficient to achieve the standardized concentration of 5×10^4 viable urediniospores mL^{-1} in 70 mL of distilled water (the volume necessary for inoculation of seven plants of each sugarcane variety). Therefore, a correction factor (CF) was applied for equivalence between the two infection experiments. The CF was calculated by dividing the standard concentration of viable urediniospores mL^{-1} (5×10^4) by the concentration of viable urediniospores obtained with the urediniospores collected from the first infection experiment (Table 2). The CF was applied to the variables 'severity' (SEV), 'total number of lesions' (TNL) and 'total number of sporulating lesions' (TNSL), assuming a linear association between these variables and the amount of urediniospores.

Inoculated plants were kept in incubation chambers (individual chambers for each *P. kuehnii* population) under continuous darkness for 24 h to provide sufficient leaf wetness for the infection step. Subsequently, inoculated plants were maintained in separate Conviron growth chambers for each *P. kuehnii* population (Fig. 1c) at a temperature of 25 ± 2 °C until the end of the experiment.

Analysed variables

The following variables were evaluated: disease severity (SEV), total number of lesions (TNL) and total number of sporulating lesions (TNSL). In addition, TNSL/TNL was also calculated, resulting in the percentage of sporulating lesions (%SL). The sporulating and nonsporulating lesions (Fig. 1d) were counted 11, 16 and 21 days after inoculation (DAI) in a 20 cm leaf fragment from the most diseased leaf of each plant. The evaluations were performed with the aid of a stereoscopic magnifying glass with ×40 magnification. The disease severity was quantified by estimating the actual percentage of the leaf area with symptoms (severity) at 21 DAI based on the diagrammatic scale developed by Amorim *et al.* (1987).

Data analysis

The SEV, TNL, TNSL and %SL data obtained in the two successive inoculations of the two *P. kuehnii* populations were submitted to variance analysis followed by the Scott–Knott comparative test of means at 5% significance. The SEV, TNL and TNSL data were transformed by square root of $(x + 1)$, in which x corresponds to the analysed variable, before applying the Scott–Knott test. The susceptibility level of the variety (SLV) was based on the level of the variety's susceptibility to *P. kuehnii* in the field, by assigning the values 3, 2 and 1 for susceptible, intermediate, and resistant varieties, respectively (Table 1).

Results

Disease severity

The estimated disease severity in the susceptible varieties was higher than 5%. For intermediate and resistant varieties the disease severity was lower than 6% in the

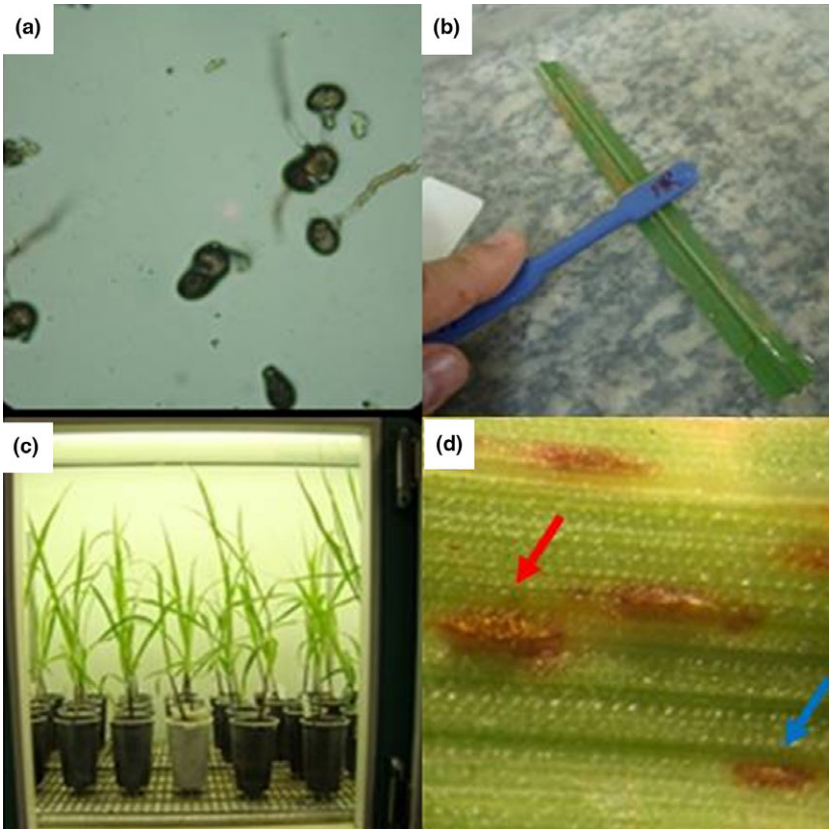

Figure 1 Germinated urediniospores of *Puccinia kuehnii* (a), collection of urediniospores for inoculation of sugarcane plants (b), maintenance of inoculated sugarcane plants (c), sporulating (left arrow) and nonsporulating (right arrow) orange rust lesions (d). [Colour figure can be viewed at wileyonlinelibrary.com]

Table 2 Percentage germination of *Puccinia kuehnii* urediniospores collected from sugarcane areas in Araras and Paranacity, concentrations of total and viable spores used in inoculations, and correction factor used for variables assessed after second inoculation.

	First inoculation			Second inoculation			
Location	Ger. %[a]	Total spores[b] × 10^5	Viable spores[c] × 10^5	Ger. %[d]	Total spores[e] × 10^5	Viable spores[f] × 10^5	CF[g]
Araras	35	1.42	0.5	11	0.69	0.076	6.58
Paranacity	20	2.50	0.5	24	1.21	0.290	1.72

[a]Percentage germination of spores used in first inoculation (%).
[b]Total spore concentration used to obtain 5 × 10^4 viable urediniospores mL^{-1} for inoculation.
[c]Concentration of viable urediniospores used for inoculation (total spore concentration × percentage germination).
[d]Percentage germination of spores used in second inoculation (%).
[e]Total spore concentration obtained for the second inoculation.
[f]Concentration of viable urediniospores used for second inoculation (total spore concentration × percentage germination).
[g]Correction factor = concentration of viable spores in first inoculation (5 × 10^4)/(concentration of viable spores in second inoculation.

inoculations with the population collected in Araras (SP). Susceptible and intermediate varieties showed severities higher than 7%, and the resistant ones lower than 6%, when inoculated with the Paranacity population. Differences in the severity estimates between the two populations were not significant for all varieties except on variety SP89-1115 (Table 3). The correlation coefficient between the laboratory (SEV) and field (SLV) severities was 0.38 (Table 4).

Total number of lesions (TNL)

In all inoculations, the average total number of lesions (TNL) exceeded 289, regardless of the resistance level of the varieties to *P. kuehnii*. No significant difference was found in TNL between varieties inoculated with the Paranacity population; the TNL ranged between 293 (RB86-7515) and 336 (CTC 3). In plants inoculated with the Araras population, TNL ranged from 289 (RB85-5156) to 530 (RB86-7515; Table 3). The correlation coefficient between TNL and SLV was negative (−0.06) but not significant (Table 4).

Table 3 Total number of lesions (sporulating and nonsporulating; TNL) and sugarcane orange rust severity of five sugarcane varieties inoculated with *Puccinia kuehnii* isolates collected in Araras and Paranacity

	Collection location			
Sugarcane variety	Araras TNL[a]	Paranacity	Araras Severity (%)[b]	Paranacity
SP89-1115	394 bA	320 aA	6 aA	12 aB
RB85-5156	289 bA	331 aA	5 bA	8 aA
RB86-7515	530 aA	293 aB	3 bA	2 bA
CTC 3	528 aA	336 aA	9 aA	8 aA
CTC 6	505 aA	297 aB	4 bA	6 bA

For each variable, values in the same row followed by identical uppercase letters and values in the same column followed by identical lowercase letters do not differ according to the Scott–Knott test at 5% significance. Data were transformed by square root of (x + 1) for statistical analysis.
[a]TNL, total number of lesions, both sporulating and nonsporulating.
[b]Severity (%), disease severity quantified by estimating the actual percentage of leaf area with symptoms, based on the diagrammatic scale developed by Amorim *et al.* (1987).

Total number of sporulating lesions (TNSL)

Contrary to TNL, TNSL analysis showed significant differences between the sugarcane varieties. In the susceptible varieties SP89-1115 and CTC 3 inoculated with the Araras population, the TNSL was 374 and 503, respectively. When these two varieties were inoculated with the Paranacity population, the TNSL of both varieties was also >300: 311 (SP89-1115) and 321 (CTC 3). In the varieties RB86-7515 and CTC 6, considered resistant to orange rust, the TNSL was 7 when inoculated with the Araras population and sporulation was absent when inoculated with the Paranacity population. The intermediate variety RB85-5156 showed TNSL of 196 and 239 when inoculated with the Araras and Paranacity populations, respectively (Table 5). The correlation between the TNSL and the SLV was 0.86, in contrast to the low correlations found between SEV and SLV, and TNL and SLV (Table 4).

Table 4 Correlation analysis between susceptibility level of the variety (SLV), disease severity (SEV), total number of lesions (TNL), total number of sporulating lesions (TNSL) and percentage of sporulating lesions (%SL) assessed at 21 days after inoculation of five sugarcane varieties with isolates of *Puccinia kuehnii* collected in Araras and Paranacity

	SLV	SEV	TNL	TNSL	%SL
LSV	–	–	–	–	–
SEV	0.38*	–	–	–	–
TNL	−0.06	0.26	–	–	–
TNSL	0.86*	0.52*	0.22	–	–
%SL	0.97*	0.37*	−0.09	0.86*	–

*Significant correlation according to the Scott–Knott test at 5% significance. Data transformed by square root of (x + 1) for correlation analysis.

Table 5 Total number of sporulating lesions (TNSL) and percentage of sporulating lesions (%SL) at 21 days after inoculation of five sugarcane varieties with *Puccinia kuehnii* populations collected in Araras and Paranacity.

	Collection location			
Sugarcane variety	Araras TNSL	Paranacity	Araras %SL	Paranacity
SP89-1115	374 bA	311 aA	95.3 aA	97.0 aA
RB85-5156	196 cA	239 aA	67.1 bA	67.7 bA
RB86-7515	6 dA	0 bA	0.9 cA	0.0 cA
CTC 3	503 aA	321 aB	95.5 aA	95.4 aA
CTC 6	7 dA	0 bA	1.3 cA	0.0 cA

For each variable, values in the same row followed by identical uppercase letters and values in the same column followed by identical lowercase letters do not differ according to the Scott–Knott test at 5% significance. Data were transformed by square root of ($x + 1$) for statistical analysis.

Table 6 Percentage of sporulating lesions (%SL) evaluated at 11, 16 and 21 days after inoculation (DAI) of five sugarcane varieties with *Puccinia kuehnii* isolates collected in Araras and Paranacity.

	Percentage of sporulating lesions (%SL)		
Sugarcane variety	11 DAI A	16 DAI B	21 DAI B
SP89-1115	55.6 a	94.3 a	95.8 a
RB85-5156	18.5 c	59.3 b	67.3 b
RB86-7515	0.0 d	0.2 c	0.7 c
CTC 3	43.9 b	92.7 a	95.5 a
CTC 6	0.0 d	0.2 c	0.9 c

Values in the same row followed by identical uppercase letters and values in the same column followed by identical lowercase letters do not differ according to the Scott–Knott test at 5% significance.

Percentage of sporulating lesions (%SL)

By 21 DAI, the differences in %SL between susceptible, intermediate and resistant sugarcane varieties were significant in the inoculations with both the Araras and Paranacity populations (Table 5). The %SL in the susceptible varieties SP89-1115 and CTC 3 was 95.3% and 95.5%, respectively, when inoculated with collected urediniospores from Araras. Similarly, for inoculations with urediniospores from Paranacity, the %SL was 97% (SP89-1115) and 95.4% (CTC 3), respectively. The intermediate variety RB85-5156 showed 67.1% and 67.7% sporulating lesions when inoculated with Araras and Paranacity populations, respectively. The %SL in the resistant varieties RB86-7515 and CTC 6 were respectively 0.9% and 1.3% when inoculated with the Araras population. After inoculation with the Paranacity population, there were no sporulating lesions (%SL of 0) in the two rust-resistant varieties (Table 5). The correlation between %SL and SLV was high at 0.97 (Table 4).

The evaluations of %SL at 16 and 21 DAI did not differ among themselves but differed from the first evaluation carried out at 11 DAI. The difference in the %SL between susceptible, intermediate and resistant sugarcane varieties was significant in all three evaluations (11, 16 and 21 DAI). However, the greatest difference between susceptible and resistant varieties was observed at 16 and 21 DAI: more than 90% of the total number of lesions in susceptible varieties had produced and released urediniospores by 16 DAI, whereas resistant varieties showed lower than 0.2%SL at 16 DAI (Table 6).

Discussion

This study has shown that counting the total number of lesions (TNL) on leaves infected with *P. kuehnii* does not distinguish with precision the resistant, intermediate and susceptible sugarcane varieties. In each inoculation, more than 289 sporulating and nonsporulating lesions were observed, regardless of the sugarcane variety and *P. kuehnii* population. The same indistinguishability was observed in analysis of disease severity. Both TNL and laboratory severity (SEV) proved weakly correlated with field severity (LSV): −0.06 and 0.38, respectively. However, sporulating lesions proved discriminant: the percentage of sporulating lesions (%SL) and total number of sporulating lesions (TNSL) precisely separated the three resistance levels, resistant, intermediate and susceptible, of the sugarcane varieties to orange rust. The correlations of these two variables with SLV were the largest: 0.97 and 0.86, respectively. The susceptible varieties (SP89-1115 and CTC 3) showed more than 95%SL at 16 DAI. The TNSL in these sugarcane varieties was higher than 310. The resistant varieties RB86-7515 and CTC 6 showed less than 2%SL in the evaluation at 16 DAI and a TNSL of <7 per 20 cm of leaf fragment. The intermediate variety RB85-5156 showed approximately 67% sporulating lesions and the TNSL in this variety ranged from 190 to 240 per 20 cm of leaf fragment.

The knowledge and adoption of criteria that best separate the sugarcane varieties according to their resistance level to *P. kuehnii* are important. Selecting rust-resistant genotypes in sugarcane breeding programmes is usually based on disease severity evaluation due to the feasibility of this technique (Santos, 2003). However, visual methods that estimate the severity of foliar diseases are subjective and cause imprecise estimates (Bade & Carmona, 2011).

The results of the present study show the efficacy of the %SL variable as a method to separate the sugarcane varieties into different groups for resistance to *P. kuehnii*. The combined use of the variables severity and %SL should be considered for disease evaluation of sugarcane genotypes in breeding programmes. Counting sporulating lesions is simple and inexpensive. If performed under controlled conditions, this methodology provides fast results and favours the selection of promising genotypes. Furthermore, distinguishing resistant varieties from the others is possible as early as 11 DAI and enables separation of the susceptible from the intermediate varieties after only 16 days.

The adoption of %SL of orange rust in sugarcane breeding programmes is a worthwhile alternative to using disease severity for ranking sugarcane varieties, avoiding the elimination of genotypes that only show nonsporulating lesions or a very low number of sporulating lesions. Although the efficiency of quantitative resistance may decrease over time (erosion process) due to the selection of isolates with a high level of aggressiveness on resistant plants (Caffier *et al.*, 2016), this method favours the selection of horizontal resistance-forms usually more durable and effective. Genotypes with resistance components that reduce spore production slow disease progress in the field and contribute to inoculum reduction over time (Parlevliet, 1979). In soybean, the use of rust resistance based on low levels of sporulation, longer latent periods and the presence of red-brown lesions with either no uredinia or only sparsely sporulating uredinia, gave low rates of increase in pustule number over time, and smaller lesions compared with susceptible genotypes. These parameters are characteristic of partial resistance, although in soybean their genetic determinism is based on single-gene resistance (Twizeyimana *et al.*, 2008).

In conclusion, the findings of this study propose a promising, simple and fast method for evaluation of sugarcane resistance genotypes to orange rust in breeding programmes: the assessment of the percentage of sporulating lesions in 20 cm leaf sections at 15–20 days after *P. kuehnii* inoculations. The threshold separating susceptible from intermediate varieties is 80%SL and the threshold separating susceptible from intermediate varieties is 8%SL. A minimum count of 100 sporulating lesions on susceptible varieties and a maximum count of 15 sporulating lesions on resistant varieties is necessary.

Acknowledgements

The authors would like to thank the Sugarcane Technology Centre (CTC) for the multiplication of varieties, collection of *P. kuehnii* populations, and availability of the staff and structure to assist in carrying out this study.

References

Agarwal S, Saini RG, Sharma AK, 2003. Temperature-sensitive adult plant leaf rust resistance in bread wheat (*Triticum aestivum* L.). *Phytopathologia Mediterranea* **42**, 89–92.

Amorim L, Bergamin Filho A, Sanguino A, Cardoso COM, Moraes VA, Fernandes CR, 1987. Metodologia de avaliação de ferrugem da cana-de-açúcar (*Puccinia melanocephala*). *Boletim Técnico Copersucar* **39**, 13–6.

Araújo KL, Canteri MG, Gilio TAS *et al.*, 2013. Resistência genotípica e monitoramento da favorabilidade para ocorrência da ferrugem alaranjada da cana-de-açúcar. *Summa Phytopathologica* **39**, 271–5.

Bade CIA, Carmona MA, 2011. Comparison of methods to assess severity of common rust caused by *Puccinia sorghi* in maize. *Tropical Plant Pathology* **36**, 264–6.

Barbasso D, Jordão H, Maccheroni W, Boldini J, Bressiani J, Sanguino A, 2010. First report of *Puccinia kuehnii*, causal agent of orange rust of sugarcane in Brazil. *Plant Disease* **94**, 1170.

Braithwaite KS, Croft BJ, Magarey C, Scharaschkin T, 2009. Phylogenetic placement of the sugarcane orange rust pathogen *Puccinia kuehnii* in a historical and regional context. *Australasian Plant Pathology* **39**, 380–8.

CABI, 2016. Invasive species compendium. [http://www.cabi.org/isc/datasheet/45818]. Accessed May 2017.

Cadavid M, Ángel JC, Victoria JI, 2012. First report of orange rust of sugarcane caused by *Puccinia kuehnii* in Colombia. *Plant Disease* **96**, 143.

Caffier V, Le Cam B, Al Rifai M *et al.*, 2016. Slow erosion of a quantitative apple resistance to *Venturia inaequalis* based on an isolate-specific quantitative trait locus. *Infection, Genetics and Evolution* **44**, 541–8.

Dalri IB, 2012. Manejo varietal na usina Iracema. Grupo São Martinho. Iracemápolis. [http://www.stab.org.br/ii_usuario_variedades/08_stab_ivan_2012_.pdf]. Accessed 5 February 2014.

Farber DH, Medlock J, Mundt CC, 2017. Local dispersal of *Puccinia striiformis* f. sp. *tritici* from isolated source lesions. *Plant Pathology* **66**, 28–37.

Funes C, Pérez Gómez SG, Henriquez DD *et al.*, 2016. First report of orange rust of sugarcane caused by *Puccinia kuehnii* in Argentina. *Plant Disease* **100**, 861.

Garcés FF, Fiallos FF, Martinez F *et al.*, 2014. First report of orange rust of sugarcane caused by *Puccinia kuehnii* in Ecuador. *Plant Disease* **98**, 842.

Glen M, Alfenas AC, Zauza EAV, Wingfield MJ, Mohammed C, 2007. *Puccinia psidii*: a threat to the Australian environment and economy. *Australasian Plant Pathology* **36**, 1–16.

Junghans DT, Alfenas AC, Brommonschenkel SH, Oda S, Mello EJ, Grattapaghlia D, 2003. Resistance to rust (*Puccinia psidii* Winter) in *Eucalyptus*: mode of inheritance and mapping of a major effect locus with RAPD markers. *Theoretical and Applied Genetics* **108**, 175–80.

Klosowski AC, 2012. *Escala Diagramática, Segregação da Resistência em Progênies de Cana-de-açúcar e Reação de Cultivares à Ferrugem Alaranjada (Puccinia kuehnii)*. Curitiba, Brasil: Universidade Federal do Paraná, MSc dissertation.

Klosowski AC, Ruaro L, Bespalhok Filho JC, May de Mio LL, 2013. Proposta e validação de escala para a ferrugem alaranjada da cana-de-açúcar. *Tropical Plant Pathology* **38**, 166–71.

Lannou C, 2012. Variation and selection of quantitative traits in plant pathogens. *Annual Review of Phytopathology* **50**, 319–38.

Magarey RC, Bull JI, Neilsen WA, 2003. Yield losses caused by several endemic diseases in Queensland. In: Hogarth DM, ed. *Proceedings of the Conference of the Australian Society of Sugar Cane Technologists*. Brisbane, Australia: PK Editorial Services Pty Ltd, 30.

Martins TD, Raid RN, Burnquist WL, Urashima AS, Bergamin Filho A, Comstock J, 2010. Influence of temperature and leaf wetness duration on orange rust of sugarcane. *Phytopathology* **100**, 78.

Minchio CA, Canteri MG, Rocha JA, 2011. Germinação de uredósporos de *Puccinia kuehnii* submetidos a diferentes temperaturas e tempos de incubação. *Summa Phytopathologica* **37**, 211–4.

Nunes Junior D, 2010 A ferrugem alaranjada chegou, e agora? Disponível em [http://dibnunes.wordpress.com/category/opiniao/page/3/]. Accessed 18 December 2013.

Parlevliet JE, 1979. Components of resistance that reduce the rate of epidemic development. *Annual Review of Phytopathology* **17**, 203–22.

Parlevliet JE, Leijin M, Van Ommeren A, 1984. Accumulating polygenes for partial resistance in barley to barley leaf rust. *Puccinia kuehnii*. II: Field evaluation. *Euphytica* **34**, 15–20.

Reuveni R, Reuveni M, Agapov V, 1996. Foliar sprays of NPK fertilizers induce systemic protection against *Puccinia sorghi* and *Exserohilum turcicum* and growth response in maize. *European Journal of Plant Pathology* **102**, 339–48.

Ribeiro do Vale FX, Parlevliet JE, Zambolim L, 2001. Concepts in plant disease resistance. *Fitopatologia Brasileira* **26**, 577–89.

Robert C, Bancal MO, Lannou C, 2004. Wheat leaf rust uredospore production on adult plants: influence of leaf nitrogen content and *Septoria tritici* blotch. *Phytopathology* **94**, 712–21.

Roumen EC, Bonman JM, Parlevliet JE, 1992. Leaf age related partial resistance to *Pyricularia oryzae* in tropical lowland rice cultivars as measured by the number of sporulating lesions. *Phytopathology* **82**, 1414–7.

Santos AS, 2003. Doenças causadas por fungos e bactérias em cana-de-açúcar. In: *Proceedings of IX Reunião Itinerante de Fitossanidade do Instituto Biológico – Fitossanidade Cana-de-açúcar*, 2003. Catanduva, Brazil, 11–7. [http://www.biologico.sp.gov.br/rifib/IX_RIFIB]. Accessed 23 March 2014.

Soares RM, Akamatsu H, Yamanaka N *et al.*, 2009. Variabilidade patogênica do fungo *Phakopsora pachyrhizi*. In: *Reunião do Consórcio Antiferrugem Safra 2008/2009, Londrina. Embrapa Soja. Documentos 315*. Londrina, Brazil: Embrapa Soja, 93–101.

Twizeyimana M, Ojiambo PS, Ikotun T, Ladipo JL, Hartman GL, Bandyopadhyay R, 2008. Evaluation of soybean germplasm for resistance to soybean rust (*Phakopsora pachyrhizi*) in Nigeria. *Plant Disease* **92**, 947–52.