



# First detection of *Fusarium oxysporum* f. sp. *radicis-lycopersici* across major tomato-producing regions in Brazil

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**Abstract** *Fusarium oxysporum* f. sp. *lycopersici* (FOL) races 1 and 2 were, up to recent years, the only pathogenic Fusaria associated with tomatoes (*Solanum lycopersicum* L.) in Brazil. However, recent outbreaks of *F. oxysporum* isolates with the ability to breakdown the *I-2*-mediated resistance prompted us to conduct nationwide surveys of the pathogens associated with vascular wilt of tomatoes. During these surveys, eight atypical *F. oxysporum* isolates (collected across five tomato-producing Brazilian states) displayed a peculiar ability of infecting accessions carrying the *I-3* and *I-7* genes (controlling resistance to FOL race 3 isolates). These observations suggested the involvement of either a new FOL race or of *F. oxysporum* f. sp. *radicis-lycopersici* (FORL) isolates. Koch's postulates were fulfilled for these eight atypical isolates via root dipping inoculation ( $10^6$  conidia mL<sup>-1</sup>) of seedlings from two tomato cultivars 'Ponderosa' and 'Dominador'. All eight isolates induced crown-rot symptoms only in

'Ponderosa', but not in 'Dominador' (which is resistant to FORL). To confirm the FORL identity, PCR assays were done using a set of race-specific and *forma specialis*-specific primers. A single amplicon of  $\approx 947$  bp was observed with the primer pair Spr1 only with DNA template obtained from standard FORL isolates and from the eight atypical isolates, confirming the first detection of this *forma specialis* in Brazil. The geographical distribution of FORL isolates across major tomato-producing areas may require the incorporation of resistant factors into adapted cultivars.

**Keywords** *Solanum lycopersicum* · Soil-borne pathogen · New disease

## Introduction

*Fusarium* species are among the most cosmopolitan and genetically diverse groups of plant pathogens, displaying a broad profile of pathogenicity and virulence. These fungi have a huge economic importance as causal agents of root-rot and vascular wilt diseases in a wide range of host plants (Leslie and Sumerell 2006). *Fusarium oxysporum* f. sp. *lycopersici* (Sacc.) Snyder & Hansen (FOL) is the causal agent of vascular wilt in tomatoes (*Solanum lycopersicum* L.), which is one of the major diseases affecting this vegetable crop at a global level. The interaction between FOL isolates and *Solanum* (section *Lycopersicon*) accessions is race-cultivar specific, displaying a typical gene-for-gene relationship (Catanzariti et al. 2017). So far, there are

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three described FOL races, which are defined according to their ability of infecting (or not) a distinct set of *S. lycopersicum* and *S. pennellii* Correll, accessions carrying race-specific resistance factors. Four dominant race-specific resistance genes (*I-1*, *I-2*, *I-3*, and *I-7*) have been characterized in accessions of *Solanum* (section *Lycopersicon*) and introgressed into commercial varieties (Gonzalez–Cendales et al. 2016). The *I-1* and *I-2* genes are derived from *S. pimpinellifolium* L. and they control resistance to FOL races 1 and 2, respectively. The *I-2* was the first FOL resistance gene cloned (Simons et al. 1998). More recently, the *I-1* gene was also cloned (Catanzariti et al. 2017). The *I-3* gene confers resistance to FOL race 3 gene and was introgressed from *S. pennellii* (Catanzariti et al. 2015). The *I-7* gene displays a wide spectrum resistance being effective against all FOL races (Gonzalez–Cendales et al. 2016).

Tomato is also infected by *F. oxysporum* (Schlechtendahl: Fries) f. sp. *radicis-lycopersici* (FORL), which is the causal agent of the root-rot and crown-rot disease (Jarvis and Shoemaker 1978). FORL is more prevalent in tomato-producing regions with mild climates or in high elevations (Davis and Paulus 2014; McGovern 2015; Farr and Rossman 2018). Under open-field conditions, yield losses due to the crown-rot disease may range from 20–40% (Davis and Paulus 2014). However, this disease is more problematic in tomato plants cultivated under protected conditions, especially if they are grown on sterilized soil or substrate with reported losses ranging from 20–60% (Katan and Katan 1999; Davis and Paulus 2014; McGovern 2015). Nowadays, this pathogen is present in most of the tomato-producing regions around the world (Katan and Katan 1999; Davis and Paulus 2014). There are no reports of FORL races and the dominant root rot resistance gene/locus *Frl* is thus far effective against all variants of this pathogen (Fazio et al. 1999; Devran et al. 2018). Thus far, FORL has not been reported in Brazil, being classified as a quarantine pest (MAPA 2018).

The features of FOL and FORL in culture media are quite similar (Leslie and Summerell 2006), precluding a reliable identification based solely upon morphometrical traits. The initial discrimination of FOL and FORL isolates under field conditions is done mainly by the type of symptom they induce in tomato (vascular wilt versus crown-rot). Another striking difference between FORL and FOL is the ideal

temperature range required to each pathogen to cause disease. FOL is favored by warm temperatures (25–27 °C), whereas FORL is a more serious problem in mild climates (15–20 °C). More recently, PCR assays using a set of race-specific and *forma specialis*-specific primers have been employed as a reliable method to discriminate these *Fusaria* associated with tomatoes (Hirano and Arie 2006).

In Brazil, FOL race 1 and race 2 isolates were, up to recent years, the only pathogenic *Fusaria* associated with tomatoes. However, a recent high number of simultaneous outbreaks of *F. oxysporum* isolates with the ability to break down the *I-2* mediated resistance prompted us to carry out nationwide surveys of the pathogens associated with vascular wilt of tomatoes (Gonçalves et al. 2018). During these surveys, eight atypical *F. oxysporum* isolates (collected across distinct tomato-producing regions) displayed a peculiar ability of also infecting accessions carrying the *I-3* and *I-7* genes (both controlling resistance FOL race 3 isolates). In the present work, we report the biological and molecular characterization of these atypical isolates and the first confirmation of the occurrence of FORL isolates associated with tomatoes throughout five major production states of Brazil.

## Materials and methods

**Field collection of atypical *Fusarium oxysporum* isolates** During nationwide field surveys, eight (Table 1) *F. oxysporum* isolates (viz., ‘Fus 022’, ‘Fus 189’, ‘Fus 190’, ‘Fus 289’, ‘Fus 293’, ‘Fus 444’, ‘Fus 446’, and ‘Fus 447’) were obtained from tomato plants displaying atypical crown-rot and vascular discoloration symptoms (Fig. 1) with incidence ranging from 10 to 50% in the three Southeast Brazilian states (São Paulo-SP, Espírito Santo-ES, and Minas Gerais-MG) and in two Northeast states (Ceará-CE and Pernambuco-PE). In addition, these atypical *Fusarium* isolates displayed in subsequent bioassays a peculiar ability of infecting accessions carrying the *I-3* and *I-7* genes (data not shown).

**Morphological/morphometrical analysis of the atypical *Fusarium oxysporum* isolates** For morphological characterization analysis, one atypical *F. oxysporum* isolate from each Brazilian state was used. Length and width of 30 macroconidia and microconidia were measured. The

**Table 1** Description of the *Fusarium oxysporum* f. sp. *lycopersici* (Sacc.) Snyder & Hansen (FOL) and *F. oxysporum* (Schlechtendahl: Fries) f. sp. *radicis-lycopersici* (FORL) isolates obtained in the Brazilian states of São Paulo (SP), Espírito Santo

(ES), Bahia (BA), Ceará (CE), Distrito Federal (DF), Pernambuco (PE) and Minas Gerais (MG). These isolates were employed in pathogenicity or/and in PCR assays

Isolate code	Collection site	Collection year	Pathogen variant
'Fus 63k'*	Canada	---	FORL
'Fus 66k'*	Crete	---	FORL
'Fus 022'	Botucatu-SP, Brazil	1998	FORL
'Fus 119'	Venda Nova Imigrante-ES, Brazil	2005	FOL race 3
'Fus 189'	Venda Nova Imigrante-ES, Brazil	2009	FORL
'Fus 190'	Venda Nova Imigrante-ES, Brazil	2009	FORL
'Fus 204'	Venda Nova Imigrante-ES, Brazil	2010	FOL race 1
'Fus 216'	Gama-DF, Brazil	2010	FOL race 3
'Fus 229'	Jaguaquara-BA, Brazil	2011	FOL race 3
'Fus 289'	Várzea Alegre-CE, Brazil	2012	FORL
'Fus 293'	Camocim São Félix-PE, Brazil	2000	FORL
'Fus 444'	Araguari-MG, Brazil	2017	FORL
'Fus 446'	Araguari-MG, Brazil	2017	FORL
'Fus 447'	Araguari-MG, Brazil	2017	FORL

\*Standard FORL isolates maintained in the collection of quarantine pathogens of Embrapa

presence and type of chlamydo spores as well as the type of phialide and conidiogenesis were also observed for each isolate (Leslie and Summerell 2006).

**Pathogenicity bioassays using root-dipping method** Pathogenicity tests were carried out with these atypical *F. oxysporum* isolates with two contrasting tomato cultivars 'Ponderosa' (susceptible) and 'Dominador' (which is resistant to FORL due to the presence of the *Frl* locus and resistant to FOL races 1 and 2 due to the presence of the genes *I-1* and *I-2*, respectively). In this assay, the pathogenicity of all atypical *F. oxysporum* isolates ('Fus 022', 'Fus 189', 'Fus 190', 'Fus 289', 'Fus 293', 'Fus 444', 'Fus 446', and 'Fus 447') was evaluated. The reaction of eggplant (*Solanum melongena* L.) 'Ciça', scarlet eggplant (*S. aethiopicum* var. *gilo* L.) 'Morro Redondo', and sweet-pepper (*Capsicum annuum* L.) 'Cascadura Ikeda' was also evaluated, but only with a subset of four isolates ('Fus 022', 'Fus 189', 'Fus 190', 'Fus 289'). Inoculation protocol employed in these bioassays was the root-dipping method. Conidia were produced in Potato Dextrose broth under standard conditions (12 h of light and  $22 \pm 2$  °C) for seven days. The suspension was filtered and adjusted to  $10^6$  conidia mL<sup>-1</sup>. The accessions were sown in trays with 128 cells, filled with

sterile substrate (Plantmax®). Plants with the first two pairs of true leaves fully expanded were removed from the tray cells with gentle sprays of water in order to preserve root integrity. The tip of the root system (about 2 cm) was removed with scissors and then the roots were dipped into a spore suspension for 3 min. Afterward, the plantlets were transplanted to 2 L plastic pots filled with sterile soil and maintained in a growth chamber under 18 °C and 12 h of light. The experimental plots were composed by four replicates (= four 2 L-pots filled with sterile substrate) with two plants each. Two mL of the conidia suspension were added in the crown of each plantlet after transplanting. Disease incidence was assessed 14 days after inoculation by counting the number of plants with root or crown rot and dead plants. The isolates were classified as pathogenic only when they were able to induce characteristic crown-rot symptoms. At the end of the evaluation, the fungus was reisolated from symptomatic plants and PCR assays (see section below) were carried out again with some isolates to confirm the identification.

**Pathogenicity bioassays using chlamydo spores** A pathogenicity assay was also carried out employing chlamydo spores as inoculum. For this assay, only the pathogenicity of a subset of four isolates ('Fus 022', 'Fus



**Fig. 1** Symptoms of crown-rot in a field tomato plant, caused by *Fusarium oxysporum* f. sp. *radicis-lycopersici*

189', 'Fus 190', and 'Fus 289') was carried out employing only two tomato cultivars ('Ponderosa' and 'Dominador'). The production of chlamydospores was made in a mixture of 150 g of dry sand, 17 g of corn meal and 34 mL of distilled water (Nene and Haware 1980). This admixture was placed into a glass Erlenmeyer flask and sterilized in autoclave twice (121°C for 60 min). Ten mycelial discs (of each isolate) obtained from seven-day old fungal colonies growing on Potato Dextrose Agar (PDA) were placed on this sterilized substrate. The Erlenmeyer flasks were then kept in a growth chamber (25 °C and 12 h photoperiod) for 15 days. The flasks were shaken every two days in order to obtain a homogenous colonization of the mixture. The soil infestation was carried out five days before planting by mixing sterilized soil with the medium (substrate) containing the *F. oxysporum* chlamydospores in the proportion of 100 g of substrate / 20 kg of soil,

corresponding to approximately  $5 \times 10^4$  cfu mL<sup>-1</sup>. In the control plots, the substrate was mixed to the soil without infestation by the pathogen. The colonized and non-colonized soil were transferred to 2 L plastic pots. Then, tomato plants with the first two pairs of true leaves fully open were removed from the cells with a gentle jet of water to preserve root integrity. The apical sector of the root system (about 2 cm) was removed and the plantlets were transplanted to the plastic pots, containing inoculated and non-inoculated soil. The experimental plots were composed by four replicates (= four 2 L-pots filled with sterile substrate) with two plants each. Pots were maintained in a growth chamber under 20 °C and 12 h of light. Disease was assessed 21 days after inoculation by counting the number of plants with root or crown rot and dead plants. The isolates were classified as pathogenic only when were able to induce characteristic symptoms of the disease. At the end of the evaluation the fungus was reisolated from symptomatic plants and PCR was carried out (see section below) again with a subset of isolates in order to confirm their identification.

*Molecular characterization of the atypical F. oxysporum isolates* All eight atypical *F. oxysporum* isolates (viz. 'Fus 022', 'Fus 189', 'Fus 190', 'Fus 289', 'Fus 293', 'Fus 444', 'Fus 446', and 'Fus 447') were employed in this study. In addition, two standard and bona fide FORL isolates ('Fus 63k' and 'Fus 66k') were employed as controls in the molecular assays (Table 1). One FOL race 1 isolate ('Fus 204') and one FOL race 3 ('Fus 229') were also employed as controls in the molecular assays (Table 1). Total DNA of all isolates was extracted by means of a microtube-adapted 2X CTAB (pH = 8.0) method with organic solvents (Boiteux et al. 1999). PCR assays were carried out for molecular identification and differentiation of FORL and FOL races using the following set of primer pairs: Uni-F (5'-ATC ATC TTG TGC CAA CTT CAG-3') / Uni-R (5'-GTT TGT GAT CTT TGA GTT GCC A-3'), Sp13-F (5'-GTC AGT CCA TTG GCT CTC TC-3') / Sp13-R (5'-TCC TTG ACA CCA TCA CAG AG-3'), Sp23-F (5'-CCT CTT GTC TTT GTC TCA CGA-3') / Sp23-R (5'-GCA ACA GGT CGT GGG GAA AA-3'), Spr1-F (5'-GAT GGT GGA ACG GTA TGA CC-3') and Spr1-R (5'-CCA TCA CAC AAG AAC ACA GGA-3') (Hirano and Arie 2006; van der Does et al. 2008; Lievens et al. 2009). PCR reactions were composed by 5 µL genomic DNA



(20 ng/ $\mu$ L), 1.25  $\mu$ L 10X buffer (100 mM Tris-HCl, 500 mM KCl, pH 8.3), 0.6  $\mu$ L MgCl<sub>2</sub> (50 mM), 0.5  $\mu$ L of dNTPs (2.5 mM each), 0.2  $\mu$ L *Taq* DNA polymerase (5U/ $\mu$ L, Invitrogen), 1  $\mu$ L of each primer (2.5 nM each), 5.95  $\mu$ L Milli-Q water with a final volume adjusted to 15.5  $\mu$ L. The program for DNA amplification using the primer pairs Uni, Sp13, Sp23, and Sprl (Hirano and Arie 2006) consisted of an initial cycle of 94 °C for 1 min, followed by: 50 cycles of 94 °C per 1 min for denaturation, 62 °C for 1 min for annealing, and 72 °C for 2 min for elongation, and final cycle at 68 °C for 7 min. The genomic DNA of the following samples were included as controls in the PCR assays: two standard FORL isolates (named as ‘Fus 63k’ from Canada and ‘Fus 66k’ from Crete), maintained at the fungi collection of Embrapa Cenargen (Brasília–DF, Brazil), and previously characterized isolates of *F. oxysporum* f. sp. *lycopersici*, ‘Fus 204’ classified as race 1 (Reis and Boiteux 2007) and ‘Fus 229’ classified as race 3 (Barboza et al. 2013).

## Results

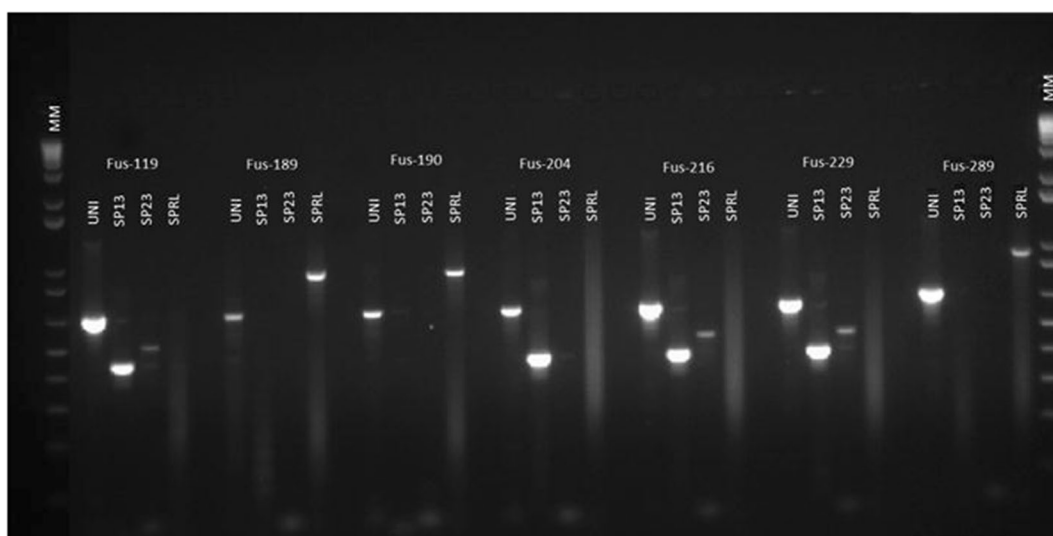
All eight atypical isolates (‘Fus 022’, ‘Fus 189’, ‘Fus 190’, ‘Fus 289’, ‘Fus 293’, ‘Fus 444’, ‘Fus 446’, and ‘Fus 447’) were initially identified as *Fusarium oxysporum*. These isolates were classified as atypical because they induce severe disease symptoms (data not shown) in seedlings of the tomato differential lines ‘Viradoro’ (with the *I-1* gene), ‘Floradade’ (with the *I-2* gene) as well as *S. pennellii* LA 716 (with the *I-3* gene) and the hybrid ‘BRS Imigrante’ (carrying the *I-7* gene) after root dipping inoculation assays ( $10^6$  conidia mL<sup>-1</sup>). In the morphological characterization, the isolates presented short monophialides and production of three types of spores: microconidia, macroconidia, and chlamydo spores (Leslie and Summerell 2006). The microconidia were produced on false heads, oval to ellipsoid, measuring 2.8–3.7  $\times$  5.4–9.3  $\mu$ m. Macroconidia were curved, fusoid, pointed in both ends, two to five septa, measuring 3.6–4.9  $\times$  26.2–63.0  $\mu$ m. The chlamydo spores were formed in old mycelia, at the terminal or intercalary positions, usually single or in pairs. Total DNA of these isolates as well as the standard FORL isolates (‘Fus 63k’ and ‘Fus 66k’) was obtained and used as template in PCR assays employing set of primers Uni, Sp13, Sp23, and Sprl (Hirano and Arie

2006). Amplification patterns indicated the presence of single bands only when employing the prime pair Uni ( $\approx$  850 bp) and the primer pair Sprl ( $\approx$  947 bp) in all eight isolates and in the standards FORL isolates (Fig. 2). This peculiar amplification pattern is in complete agreement with the one reported to FORL isolates by Hirano and Arie (2006).

Koch’s postulates were fulfilled for all eight atypical *F. oxysporum* isolates employing the root dipping inoculation method (Table 2). Symptoms (21 days after inoculation) were similar to those observed under field conditions. In this inoculation method, the tomato cultivar ‘Ponderosa’ was found to be susceptible to all isolates, but the aggressiveness varied among the isolates (Table 2). The tomato hybrid ‘Dominador’ did not display any conspicuous symptoms for these eight atypical isolates, except for the isolate ‘Fus 289’ for which two plants died (Table 2). Disease symptoms were not observed in the mock-inoculated (control) plants. The fungus was re-isolated of symptomatic plants and the PCR assays were carried out again, confirming that the obtained fungi were FORL isolates. Eggplant, scarlet eggplant, and sweet-pepper were not susceptible to the FORL isolates. Tentative of re-isolation of the pathogen of these non-symptomatic solanaceous species were unsuccessful, indicating no latent infection. In the bioassay using chlamydo spores as inoculum, the four evaluated isolates were pathogenic to the tomato cultivar ‘Ponderosa’ (100% of plants displaying symptoms), but not to the cultivar ‘Dominador’. Control plants were also free of symptoms. The FORL was also re-isolated of symptomatic plants in this assay. A second round of pathogenicity assay using root-dipping inoculation was carried out only with the cultivar ‘Ponderosa and identical symptoms were observed (data not shown). The results obtained in the two pathogenicity experiments confirm the results of morphological and molecular tests, verifying that the eight atypical isolates can be unequivocally classified as *F. oxysporum* f. sp. *radicis-lycopersici*.

## Discussion

Eight *F. oxysporum* isolates found in association with tomatoes in five Brazilian states were initially classified as atypical due to the fact that they were able to induce crown-rot symptoms in all *Solanum* (section *Lycopersicon*) accessions carrying either *I-1*, *I-2*, *I-3*



**Fig. 2** Agarose-gel electrophoresis of the amplicons obtained with DNA extracted from Brazilian isolates of *Fusarium oxysporum* f. sp. *lycopersici* (FOL) and *Fusarium oxysporum* f. sp. *radicis-lycopersici* (FORL) after PCR assays with the primer pairs Uni, Sp13, Sp23, and Sprl (first, second, third, and fourth lane for each isolate, respectively). The PCR protocol was identical to that described by Hirano and Arie (2006). The figure illustrates the amplicon profile obtained for the isolates ‘Fus 204’

(= FOL race 1 isolate), ‘Fus 119’, ‘Fus 216’ and ‘Fus 229’ (= FOL race 3 isolates); and ‘Fus 189’, ‘Fus 190’ and ‘Fus 289’ (= FORL isolates). A single amplicon of  $\approx 947$  bp was observed with the primer pair Sprl only with DNA template obtained from standard FORL isolates and from eight atypical isolates. MM = 1 Kb Plus marker (with the respective major bands of 1500 bp, 1000 bp, 850 bp, 650 bp, 500 bp, 400 bp and 300 bp)

or *I-7* FOL resistance genes (data not shown), suggesting the involvement of either a new FOL race or of FORL isolates. Koch’s postulates were fulfilled for all eight atypical isolates via root-dipping method (Table 2), employing the contrasting cultivars ‘Ponderosa’ (susceptible) and ‘Dominador’ (resistant).

In order to confirm the identity of these isolates, PCR assays using a set of race-specific and *forma specialis*-specific primers were employed (Hirano and Arie 2006). The two standard FORL isolates (‘Fus 63k’ and ‘Fus 66K’) employed as controls and all eight atypical isolates displayed the presence of single amplicons only

**Table 2** Incidence of plants with crown rot and wilt symptoms after root-dipping inoculation assay with  $10^6$  conidia mL<sup>-1</sup> of *Fusarium oxysporum* f. sp. *radicis-lycopersici* (FORL) isolates from different regions of Brazil

FORL isolate	Plants with symptoms (%)				
	Tomato (‘Ponderosa’)	Tomato (‘Dominador’)	Eggplant (‘Çiça’)	Scarlet Eggplant (‘Morro Redondo’)	Sweet Pepper (‘Cascadura Ikeda’)
‘Fus 022’	37.5	0.0	0.0	0.0	0.0
‘Fus 189’	62.5	0.0	0.0	0.0	0.0
‘Fus 190’	75.0	0.0	0.0	0.0	0.0
‘Fus 289’	87.5	25.0	0.0	0.0	0.0
‘Fus 293’	50.0	0.0	NE*	NE	NE
‘Fus 444’	50.0	0.0	NE	NE	NE
‘Fus 446’	87.5	0.0	NE	NE	NE
‘Fus 447’	75.0	0.0	NE	NE	NE

\*NE = Not evaluated. Isolates were not available when the assays were carried out

when employing the prime pair Uni ( $\approx 850$  bp) and with the primer pair Spri ( $\approx 947$  bp), confirming the occurrence of this *forma specialis* in Brazil. To our knowledge, this is the first formal report of FORL in the Brazilian territory. One of these FORL isolates (named as ‘Fus 289’) was able to induce severe symptoms in two out of eight ‘Dominador’ plants in the root-dipping inoculation assay (Table 2). However, no symptomatic ‘Dominador’ plant was observed for this isolate in the bioassay using chlamydospores as inoculum. The presence of some symptomatic plants carrying the *Frl* locus can be explained by either the harsh root-dipping inoculation conditions (carried out with high inoculum pressure and at very early seedling stage) or by some incomplete penetrance of the resistance gene. Another possibility is the presence of genetic variability of this FORL isolate in terms of its ability to infect plants carrying the *Frl* locus. However, no information is thus far available about the variation in the virulence profile of FORL isolates.

Currently, FORL has a cosmopolitan geographic distribution with reports across major tomato-producing regions around the globe (Katan and Katan 1999; Davis and Paulus 2014). However, up to the present work, FORL was not reported under Brazilian conditions, being till recently considered as a quarantine pest (MAPA 2018). FORL is seed-transmissible, therefore, fungal isolates can be dispersed over long distances via contaminated seeds (Davis and Paulus 2014), which may explain the multiple and geographically distinct sites of fungal occurrence reported here. It is interesting to point out that we have in our collection FORL isolates that were obtained in the early 1990s (Table 1), indicating multiple and consecutive events of introduction of this fungus into the country (more likely via contaminated seeds). The delay in formally detecting FORL isolates in Brazil may be explained by the fact that FOL and FORL are morphometrically quite similar. Even though the symptoms they cause in tomato are very peculiar (vascular wilt for FOL and crown-rot for FORL) both pathogens are able to induce plant death as the disease progresses. FOL is favored by warm temperatures (25–27 °C) whereas FORL is more problematic at mild temperatures (15–20 °C). However, somewhat surprising, we found FORL isolates also infecting tomatoes in the warm Northeast region of Brazil, including the Ceará and Pernambuco states (Table 1).

Our data indicated that the FORL isolates have been associated with the tomato crop in Brazil since the early 1990s. However, FORL has not been able to cause perceptible outbreaks in the country, indicating that this pathogen may not be well-adapted to the prevalent conditions. Brazil is predominantly a tropical/subtropical country and this pathogen is reported to cause serious problems predominantly under mild temperature conditions. Although the pathogen still does not represent a great risk for Brazilian tomato growers, in the future this scenario may change. Thus far, FORL has not yet been detected in the mild climate South Region of Brazil, where this pathogen can find more favorable environmental conditions and have a greater potential of inducing severe epidemics and economic damages to the tomato growers.

Pathogenicity of FORL to other solanaceous species (the potato is an exception) as well as to spinach, beet, cabbage, carrot, onion, wheat, beans, soybeans and some Cucurbitaceae species has been reported (Szczechura et al. 2013). There are also reports of weed hosts of FORL, including *Amaranthus* spp., red mastic (*Schinus terebinthifolium*) and *Stellaria media*. However, the cultivars of eggplant, sweet pepper, and scarlet eggplant (all Solanaceae species) that we evaluated here were not susceptible to a subset of four Brazilian isolates of FORL. This may be due to the isolate(s) of the pathogen introduced in Brazil not being able to infect these plants or the evaluated cultivars are, coincidentally, all resistant to this pathogen.

In the case of tomatoes, the use of resistant cultivars is the most effective and economically feasible method for control of the crown-rot disease (Szczechura et al. 2013; Manzo et al. 2016). The geographical distribution of FORL isolates across five Brazilian states suggest that employment of resistant tomato cultivars may be required. In this context, it is important to recommend to tomato breeding programs the incorporation of resistance factors to this pathogen in the new hybrids for the Brazilian market. In conclusion, the present work represents the first report of this pathogen in Brazil (Mendes and Urben 2019) and it must be therefore removed from the list of quarantine pests A1 of MAPA and kept in the A2 list of quarantine pests (pests present in some geographic region of the country but not in others). In fact, our data indicated that the pathogen appears to be already widely distributed in Brazilian territory and measures of eradication (in the strict sense of the word) or containment would no longer be feasible.

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### Compliance with ethical standards

**Conflict of interest** The authors do not have any conflict of interest.

**Research involving Human Participants and/or Animals** Not applicable.

**Informed consent** All authors have reviewed the manuscript and approved its submission to **Phytoparasitica**.

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