Pseudocercospora actinidiae causes sooty spot disease on kiwifruit in Santa Catarina, Brazil

Leonardo Araujo¹ · Felipe Augusto Moretti Ferreira Pinto¹ · Camila Cristina Lage de Andrade² · Larissa Bitencourt Gomes² · Tatiana Mituti² · Valmir Duarte²

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Abstract

Sooty spot disease in kiwifruit is caused by *Pseudocercospora actinidiae* and is being reported here for the first time in Brazil. Morphological and molecular observations were consistent with those previously reported for *P. actinidiae* that have been associated with sooty spot disease on kiwifruit. To the best of our knowledge, this is the first report of *P. actinidiae* causing sooty spot disease on kiwifruit outside of the Asian continent.

Keywords *Actinidia chinensis* · *Actinidia deliciosa* · *Leptosphaeria* sp. · Black spot · Diseases kiwifruit

The sooty spot disease of kiwifruit, which is caused by *Pseudocercospora actinidiae* is of great concern to the Asian continent because of its difficulty to control and its potential to cause economic losses. The development of the disease causes symptoms in the leaves and fruit of kiwifruit vines (*Actinidia chinensis* and *Actinidia deliciosa*) (Kikuara and Nakashima 2008; Huang 2016; KVA 2020). Under high disease incidence in orchards and under favorable conditions, sooty spot can produce early defoliation (weakening vines and reducing yield) and fruit abscission (Huang 2016).

In Brazil, about 60 kiwifruit vines (*Actinidia deliciosa*) of the cultivar Hayward showing sooty spot symptoms were observed in a commercial orchard (established during the winter of 2018), during the summer of 2019 (Fig. 1A–C). Yellowish leaf spots and necrosis were observed on the adaxial surface (Fig. 1C). On the abaxial leaf surface, the first symptoms were small, dark spots (Fig. 1A and B). Later, these expanded and coalesced until dark spots with intense conidial masses were present in all leaves (Fig. 1A and B). Symptoms on the fruit were not observed until the harvest, although almost there was not fruit on kiwifruit vines. (Data not shown). Thus, the objective of the present study was to

confirm that *P. actinidiae* is the causal agent of the symptoms observed in kiwifruit vines in Brazil.

Leaves from symptomatic *A. deliciosa* cv. Hayward vines were collected from a commercial orchard in Bom Retiro, Santa Catarina (27°45'51"S, 49°43'44"W), Brazil. To obtain a pure isolate, leaf spots containing fungal structures (Fig. 1A and B) were examined under a stereomicroscope (Leica EZ4E, Germany) fitted with a digital camera (5.0 Megapixel resolution). Conidia were dissected under a microscope and placed on a glass slide in a drop of sterile water, according to Park et al. (2017). After four days, the germinated conidia were transferred to Petri dishes containing potato dextrose agar (PDA). PDA plates were incubated at 25 °C with photoperiod of 12 h for 15 days to develop colonies. The fungal isolates was preserved and deposited in the Micological Collection of the "Santa Verônica Giuliani" in Porto Alegre, RS, under registration number SVG00078-F. Pathogenicity tests on kiwifruit leaves and fruit from *A. deliciosa* cv. Hayward were performed according to Kikuara and Nakashima (2008).

Pseudocercospora actinidiae was confirmed by characterizing the length and width of the conidia (Kikuara and Nakashima 2008; Braun et al. 2015). Morphological observations were made under a BX41 microscope (Olympus, Japan), and images of the conidia were acquired digitally (Q-Color™, 5.0 Megapixel resolution) and further processed with Olympus cellSens Dimension software.

Mycelial disks from isolate were transferred into the malt extract broth (20 g L⁻¹, Difco) by aseptically punching 5 mm

✉ Leonardo Araujo
leonardoaraujo@epagri.sc.gov.br

¹ Empresa de Pesquisa Agropecuária e Extensão Rural de Santa Catarina (EPAGRI), Santa Catarina, Brazil

² Agronômica Laboratório de Diagnóstico Fitossanitário e Consultoria, Porto Alegre, RS, Brazil

Fig. 1 Symptoms of sooty spot disease which is caused by *Pseudocercospora actinidiae* in the leaves of kiwifruit vines of the *Actinidia deliciosa* cv. Hayward. Disease symptoms on the abaxial leaves were small or coalesced, dark spots (A to B). Yellowish leaf spots and

necrosis were observed on the adaxial surface (C). Fifty-days-old colonies of *P. actinidiae* growing on a potato dextrose agar dishes (D). Conidia of *P. actinidiae* (E). Bar: 50 µm (C). Photos: J. O. Vieira (A) and L. Araujo (B to E)

disks of the agar plate culture. A shake flask culture was carried out in 250 mL flasks containing 50 mL of the medium at 110 rpm and incubated at 25 °C for four days. The mycelial growth was filtered, and the mycelial pellet was repeatedly washed with distilled water before being stored at -20 °C. For DNA extraction, the harvested fungal material was ground to a fine powder, in liquid N₂, using a frozen pestle and mortar. Total DNA extraction using 100 mg of homogenized ground material was performed using the Wizard Genomic DNA Purification Kit (Promega, Madison, USA). DNA integrity and concentration were assessed using a NanoDrop™ spectrophotometer (ND-1000; Thermo Scientific, New Hampshire, USA). The DNA was stored at -20 °C until the analysis was performed.

The molecular identity of the fungal isolate was determined using PCR amplification and sequencing of the internal transcribed spacer regions (ITS) according to White et al. (1990). The ITS primers used were ITS-1 and ITS-4. The PCR amplification program consisted of 94 °C for 2 min, followed by 35 cycles of 94 °C for 30 s, 54 °C for 30 s and 72 °C for 1 min, with a final extension of 72 °C for 10 min. The sequences obtained were

edited using the BioEdit 7.0.5.3 software, and consensus sequences were analyzed using the Molecular Evolutionary Genetics Analysis (MEGA X) software (Kumar et al. 2018). The similarity of the nucleotide sequences of the isolate was calculated using the BLAST algorithm (Basic Local Alignment Search Tool). Sequences from reference strains of *Pseudocercospora* spp. (Silva et al. 2016) available in GenBank were added to the analyses. *Cercospora eucalypti* (CBS 11,318) was selected as an outgroup taxon. The Bayesian phylogenetic phylogenetic tree was reconstructed using the ITS data. The best nucleotide substitution models (SYM + G) for ITS were determined using the MrModeltest software (Posada and Buckley 2004). The CIPRES web portal (Miller et al. 2010) was used to run MrBayes v3.2.1 (Ronquist and Huelsenbeck 2003). The Markov Chain Monte Carlo (MCMC) analysis was run with a total of 10 million generations, sampling every 1,000 generations. The first 25% of the sampled vines were discarded as burn-in, and the posterior probability values were calculated using the remaining trees (Rannala and Yang 1996). The phylogenetic tree was visualized using the FigTree software (<http://tree.bio.ed.ac.uk/software/>

Fig. 2 The Bayesian phylogenetic tree based on ITS sequence of *Pseudocercospora* spp. The Bayesian posterior probabilities values are indicated above the nodes, and the scale bar represents the

figtree/). Sequences generated in this study were deposited in GenBank.

After 15 days of incubation, fungal colonies were darker, with smooth, lobate margins and erumpent with moderate aerial mycelia was observed. The surface appearance was pale olivaceous-gray and reverse iron gray (Fig. 1D). Conidiophores were not observed in fungal colonies developed on PDA plates. Koch's postulates were fulfilled with the kiwifruit leaves and fruit. The first symptoms in leaves were small and dark spots on the abaxial surface at 50 days after inoculation (dai). In fruit internal necrosis tissue in wound fruit were observed at 90 dai. Conidia were solitary, pale olivaceous brown, broadly obclavate-subcylindrical, $37.4\text{--}79.8$ ($\bar{x}=57.4$) $\mu\text{m} \times 5.3\text{--}8.3$ ($\bar{x}=7.0$) μm , with a base $1.98\text{--}2.58$ ($\bar{x}=2.2$) μm wide and 6–8 septe ($\bar{x}=6.8$) ($n=30$) (Fig. 1E). The *Pseudocercospora* isolate SVG00078-F was identical to *P. actinidiae* (accession number MK927158.1) (Fig. 2). The sequence was deposited in GenBank under accession number MT679154. The classification of *Pseudocercospora* species is based on a combination of morphological characteristics, host specificity, and molecular analyses (Stewart et al. 1999; Tang et al. 2009). According to the key of *Pseudocercospora* species in the Actinidiaceae the fungus *P. actinidiae* shows conidia broadly obclavate-subcylindrical, $25\text{--}85$ $\mu\text{m} \times 5\text{--}8.5$ μm , with a base that is $2\text{--}2.5$ μm wide (Braun et al. 2015). Similarly Kikura and Nakashima (2008) observed that conidia were pale olivaceous brown, obclavate to cylindrical, with unthickened,

number of expected changes per site. The reference specimen CBS 111,138 of *Cercospora eucalypti* was used as outgroup

and truncate or obconically truncate basal ends, obtuse tips, $41\text{--}78$ $\mu\text{m} \times 4.3\text{--}5.8$ μm , 6–8 septe. Molecular techniques are commonly used to overcome taxonomic problems posed by the limitations of morphological analysis or in cases where morphological characteristics are in conflict, ambiguous, or missing (Tang et al. 2009; Park et al. 2017). Thus, in the present study, morphological and molecular observations indicate that *P. actinidiae* is the causal agent of the symptoms observed in kiwifruit vines in Brazil.

To our knowledge, this is the first report of *P. actinidiae* causing sooty spot disease in kiwifruit outside of the Asian continent. Little is known about the behavior of *P. actinidiae* in the Brazilian climate and additional studies are required to understand the potential economic impacts of this pathogen.

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Declarations

Informed consent Informed consent was obtained from all individual participants included in the study.

Conflict of interest Leonardo Araujo declares that he has no conflict of interest. Felipe Augusto Moretti Ferreira Pinto declares that he has no conflict of interest. Camila Cristina Lage de Andrade declares that she has no conflict of interest. Larissa Bitencourt Gomes declares that she has no conflict of interest. Larissa Bitencourt Gomes declares that she has no conflict of interest. Tatiana Mituti declares that she has no conflict of interest. Valmir Duarte declares that he has no conflict of interest. Ethical approval: This article does not contain any studies with human participants or animals performed by any of the authors.

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