

1 First Report of Orange Rust caused by 2 *Puccinia kuehnii* on sugarcane on the Island 3 of Reunion

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12 *Puccinia kuehnii* (W. Krüger) E. J. Butler, the causal agent of orange rust, is reported as
13 one of the two known widely spread rust species on commercial sugarcane (*Saccharum*
14 spp.). This pathogen affects plant foliage, and the symptoms are characterized by the
15 appearance of small elongated uredinial lesions (pustules), typically light orange to
16 yellowish-brown, oval in shape, producing urediniospores. On highly susceptible cultivars,
17 abundant pustules develop and coalesce on lower leaf surface, causing tissue death. On
18 such cultivars, orange rust has severe yield and economic impact (Magarey et al. 2001). This
19 pathogen has expanded its range of distribution over the last ten years, now affecting
20 America and Africa. In July and August 2018, in different areas of Reunion Island, typical
21 rust symptoms were observed on leaves of R062006 and R062007 sugarcane varieties in
22 selection. The intensity of the disease was low to moderate. Samples were collected on both
23 varieties in fields from ERCANE breeding station in Saint-Louis (Le Gol) in the southwest
24 (-21.265027; 55.379830) and in Saint-Philippe (Le Baril) in the southeast (-21.359220;
25 55.731959) for laboratory analysis. The lesions were examined under a dissecting
26 microscope. Microscopic slides were prepared from material by carefully picking up spores
27 in rust sori with a needle. The presence of the causal fungus of orange rust was confirmed
28 by the observation of typical microscopic features. Urediniospores were mostly obovoid to
29 pyriform, echinulate, variable in size, 35.9 - 57.1 x 16.4 - 29.5 μ . Walls were 1- 2.5 μ
30 thick, most often with apical thickening up to 8.3 μ . Few inconspicuous hyaline
31 paraphyses with thin wall (1-2 μ) were present. No teliospore could be observed
32 (Virtudazo et al. 2001; Dixon et al. 2010). Identity of the species involved was determined by
33 sequencing the internal transcribed spacer (ITS) region of the rDNA followed by comparison
34 with reference sequences available on GenBank. Leaf material containing uredinial lesions
35 was cut into pieces about 2x3 mm and transferred into 2 mL microtubes. Total DNA was
36 then extracted using a commercial plant DNA extraction kit (DNeasy Plant Mini Kit, Qiagen)
37 and the fungal ITS region was amplified by PCR using specific primers PkPm-F/R, located
38 respectively in ITS1 and ITS2 (Glynn et al. 2010). Nucleotide sequence was determined and
39 deposited on GenBank (MK578656-MK578657). Analysis of the sequence by BLAST showed
40 100% and 99% identity with *P. kuehnii*, over a 494 and 493 bp length, respectively, which
41 was consistent with the morphological features observed. This is the first report of *P.*
42 *kuehnii* in Reunion Island. Up to now, the disease has only been found in cultivar trials in
43 Reunion. Orange rust has spread rapidly in the sister mascarene Island of Mauritius since
44 it was initially detected in March 2018. Out of four cultivars present in Mauritius and planted
45 on Reunion, three tend to be moderately or slightly susceptible and one is symptomless
46 (S. Saumtally, personal communication). Following the emergence of brown rust caused
47 by *Puccinia melanocephala* Syd. & P. Syd. in Reunion in 1965 (Boyer de la Giroday et al. 1979),
48 resistant cultivars, such as R570 bearing the *BruI* resistance gene (Costet et al. 2012),
49 have been bred and planted to control this rust species. The same work will have to be

50 conducted by the breeders in the years to come in order to take into account this new
51 threat.

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