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DISEASE NOTES

First Report of *Phakopsora pachyrhizi* Causing Rust on Soybean in Ethiopia

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Soybean rust (SBR), caused by *Phakopsora pachyrhizi*, has been reported in 10 African countries (Murithi et al. 2015) since the first report in Uganda in 1996. Soybean rust was first observed in Ethiopia in October 2011 and again in 2015 and 2016. In mid-October 2016, a severe epidemic caused "clouds," presumably of *P. pachyrhizi* urediniospores, to be observed when walking through fields. In the first week of November, 11 samples (each representing at least five leaflets) were collected from JARC (7°40′N 36°50′E; elevation 1,780 m) and sent by APHIS permit to the USDA-ARS Soybean Disease and Pest Laboratory for further verification. All leaflets had uredinia (145 ± 36 µm in diameter; n = 34) and elliptical, echinulate, hyaline, pale yellowish-brown urediniospores (30.2 ± 3.5 µm length to 22.2 ± 3.2 µm width; n = 50). Uredinial density ranged from 5 to 88 (mean 42) per cm diameter based on counts from the 11 samples. In addition to uredinia, telia were observed in three of the samples. To confirm the pathogen was *P. pachyrhizi*, symptomatic soybean leaf tissue of less than 1 cm^2 was excised from each of the 11 samples. Samples were disrupted in Lysing Matrix A and CLS-Y solution as provided by the FastDNA Spin Kit (MP Biomedicals, Solon, OH). We used two rounds of homogenization in a FastPrep FP120 lemniscate beater (MP Biomedicals) for 40 s at a speed of 6 m/s, with a 10 min incubation on ice before, between, and after. DNA was extracted as instructed by the manufacturer. The resulting eluates were diluted 10-fold with 5 mM tris, pH 8, containing 1 mM NaCl. Duplicate 5 µl subsamples were subjected to multiplex qPCR quantification of *P. pachyrhizi* and *P. meibomiae* (separately) using the primers (Frederick et al. 2002), cycling parameters, reagents, methods, and exogenous internal controls previously reported (Haudenshield and Hartman 2011) in each 25 µl reaction. Serial dilutions of previously

purified SBR DNAs established linear standard curves (R^2 > 0.999), with 94 to 95% amplification efficiency and enabled absolute quantification of the two species. The assay for *P. pachyrhizi* had a lower limit of quantification (LOQ) of 0.05 spore equivalent (SEq), and thus as little as 12.5 SEq DNA in an explant could be detected. Substantial quantities of *P. pachyrhizi* DNA were found in all 11 explants, ranging from 1,200 to 55,000 (mean 12,000) SEq. The assay for *P. mebomiae* DNA had a LOQ of 0.5 pg per assay, and thus as little as 125 pg in an explant could have been detected, but none was found. Control reactions gave the expected results, confirming the validity of the assays. Urediniospores that were dislodged from an infected leaf and inoculated in a detached-leaf assay as previously described (Twizeyimana et al. 2012) on susceptible soybean cultivar Williams 82 resulted in tan lesions after 2 weeks of incubation. This is the first confirmed report of *P. pachyrhizi* causing rust on soybean in Ethiopia, putting at risk 30,000 ha currently under soybean production. The reports of soybean rust in Ethiopia and adjoining countries may alter soybean production practices and research interests. Efforts to understand the virulence and genetic diversity of the pathogen in the region will be useful to develop and deploy resistant soybean cultivars.

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