DISEASE NOTES

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# First Report of *Diaporthe eres* Species Complex Causing Seed Decay of Soybean in Serbia

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**Published Online:** 26 Jun 2015 | https://doi.org/10.1094/PDIS-01-15-0056-PDN

One of the most important disease of soybean (*Glycine max* (L.) Merr.) is *Phomopsis* seed decay (PSD) (Sinclair 1993). The disease is caused primarily by *Diaporthe longicolla*, along with *D. sojae*, *D. caulivora*, and *D. aspalathi* (Li 2011). Screening of soybean seeds during 2008 to 2010 showed that other *Diaporthe* species were associated with well-known agents of PDS. Using standard phytopathological procedures, 88 *Diaporthe* single-conidial isolates were obtained, and 23 of them were identified morphologically and molecularly as *D. eres* species complex. Seven-day-old colonies showed white, fluffy, aerial mycelium with dark pigmentation developing in the center (Udayanga et al. 2014). Pycnidia formed within black stroma and sporulation was very abundant. Alpha conidia were 4.8 to 9.7 × 1.5 to 2.9 µm, unicellular, biguttulate, and elongated. Beta conidia were 15.6 to 38.8 × 0.8 to 1.0 µm, unicellular, filiform, and curved at one end. Perithecia were not formed. The identification of *D. eres* species complex was determined by sequence comparison of the internal transcribed spacer (ITS1-5.8S-ITS2) region of the rDNA, partial translation elongation factor 1 alpha (EF-1 alpha), and partial large ribosomal subunit (LSU) using the primers ITS4/ITS5, EF1-728F/EF1-986R, and LR0R/LR3, respectively. BLAST analyses showed 99 to 100% identity with three different ITS groups (GenBank Accession Nos. JF430491 and JF430492 as *Phomopsis occulta* HM439635; JF430487 and JF430488 as *D. eres* KC343074; and JF430493

and JF430494 as *Diaporthe conorum* DQ116551). However, EF-1 alpha (JF461473 to JF461478) and LSU (JF704172 to JF704176) sequences of these three ITS groups were 99 to 100% identical, respectively, suggesting that they belong to the same species *D. eres* as demonstrated by Udayanga et al. (2014). To verify the pathogenicity, thirty soybean plants cv. Balkan were inoculated with six isolates at V2 growth stages by the plug method (Vidić et al. 2013). An equal number of plants treated with sterile agar plugs were used as negative controls. After 30 days in a humid chamber, all inoculated plants showed pycnidial lesions on stems near the inoculation site. Only isolates identified as *D. eres* by ITS sequences caused the wilting of plants by 40%. Soybean seeds were inoculated using conidial suspensions ($10^6$ conidia/ml) (Vidić et al. 2013). The seeds were placed on wet filter paper in 90-mm petri dishes, 25 seeds in each dish, and incubated at 24°C in the dark. The experiment was set up in four replications. An equal number of soybean seeds treated with sterile water were used as negative controls. After 7 days, the number of germinated and decayed seeds were counted. Results showed that isolates of *D. eres* caused 72% seed decay, while isolates identified as *P. occulta* and *D. conorum* by ITS sequences, did not cause seed decay. The control plants and seeds were symptomless. Those results demonstrated that isolates from the *D. eres* complex differ in aggressiveness toward soybean. Koch's postulates were fulfilled by reisolation and molecular identification of *D. eres* isolates from the symptomatic stems and seeds. To our knowledge, this is the first report of seed decay caused by *D. eres* species complex on soybean.