DISEASE NOTES

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First Report of Charcoal Rot on Zebra Plant (*Aphelandra squarrosa*) Caused by *Macrophomina phaseolina*

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The Zebra plant (*Aphelandra squarrosa*) is native to tropical forests of Brazil and is a popular decorative pot plant in Serbia. Charcoal rot-like symptoms were first observed on a zebra plant in August 2010 in Novi Sad (Vojvodina Province, Serbia). The diseased plant showed symptoms of stem and root rot, loss of stem turgor, and premature death. In the cross section of the stem, mycelia with black microsclerotia were observed from the base of the plant up to 50% of the plant stem. To isolate the causal agent, 10 cuttings of the infected stem tissue were surface disinfected with 2% sodium hypochlorite solution for 5 min, rinsed three times in sterile distilled water, air dried on sterilized filter paper, and plated on potato dextrose agar (PDA) and water agar (WA) amended with streptomycin sulfate. After 7 days of incubation at 30°C in the dark, colonies with *Macrophomina phaseolina* morphology (gray aerial mycelia with black pigmentation in agar caused by homogenous black microsclerotia formation) were observed from all cuttings. The colonies had average growth rates of 33 mm/day on PDA and 8 mm/day on WA and produced microsclerotia with average diameters of 119.6 µm (PDA) and 49.0 µm (WA), which is in accordance with Watanabe (2010) and

earlier findings in Serbia (Aćimović 1998). A representative isolate was purified by a hyphal tip transfer technique for further analyses (Leslie and Summerell 2006). The pathogenicity was determined according to Koch's postulate. Plants were longitudinally cut at the stem base, artificially inoculated with a 5 mm^2 plug of PDA with developed microsclerotia or without fungus for the control, sealed with Parafilm, and placed in a growth chamber at 30°C with a 12 h/12 h light/dark regime. Plants were regularly watered every third day with 200 ml of water per pot, and the humidity level was artificially raised by humidity trays around the plants. The first charcoal rot symptoms occurred 6 days after inoculation as brown spots on the leaves and stems around the incision site, along with loss of turgor. On day 9, symptoms advanced with gray mycelia present, and browning stems and complete loss of turgor were observed. The fungus was reisolated as previously described. No symptoms were observed on the control plants. To confirm the identification, DNA from the 7-day-old *M. phaseolina* isolate used for inoculation was extracted using the cetyltrimethylammonium bromide protocol (Permingeat et al. 1998). Polymerase chain reaction amplification was performed in 25-µl reaction volume according to the protocol of Nagl et al. (2011). DNA of the *M. phaseolina* isolate originating from the zebra plant was compared with 15 *M. phaseolina* isolates originating from sunflower, soybean, common bean, and flax from different sites in Serbia by random amplification of polymorphic DNA (RAPD) analyses based on 154 polymorphic bands obtained with 14 (10-mer) OPA primers (Operon Technologies, Alameda, CA): OPA-02 to OPA-05, OPA-07 to OPA-13, and OPA-18 to OPA-20. So far, OPA primers and the RAPD technique have proved useful for distinguishing *M. phaseolina* isolates from species common in mixed infections with *M. phaseolina* (Jana et al. 2003). Genetic similarity between the isolate from the zebra plant with other *M. phaseolina* isolates was confirmed through cluster analyses using the unweighted pair group method with arithmetic mean and the Jaccard similarity coefficient. The tested isolate showed 80% genetic similarity with some sunflower-originating isolates. To the best of our knowledge, this is the first report of *M. phaseolina* causing charcoal rot on zebra plant in the world.

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