

Biolog Microbial Identification System, Version 4.2 (Biolog Inc., Hayward, CA), the isolates were identified as *Pseudomonas viridiflava* with a Biolog similarity index range of 0.52 to 0.72 after 24 h. Results of LOPAT tests (2) of isolates were identical to that of atypical *P. viridiflava* reported by Gonzalez et al. (1). Levan production and pectolytic activity of the isolates were variable. All isolates were positive for tobacco hypersensitivity and negative for oxidase reaction and arginine dihydrolase production. The 16S rDNA region (1,442 bp) of the isolates (GenBank Accession Nos. HM190218-HM190224; *P. viridiflava* CFBP2107<sup>T</sup> = HM190229), amplified by using universal PCR primers, shared 100% sequence identity with atypical *P. viridiflava* (GenBank Accession No. AM182934) (1). The *gyrB* sequence (638 bp) from the isolates (GenBank Accession Nos. HM190232-HM190238; *P. viridiflava* CFBP2107<sup>T</sup> = HM190239), amplified by using previously reported PCR primers (3), had a distance index value range of 0.029 to 0.031 with that of the *P. viridiflava* CFBP2107<sup>T</sup> (=BC2597) as determined by Jukes-Cantor model using MEGA Version 4.1 (4). On the basis of phenotypic characteristics and the sequences, the seven isolates were identified as atypical *P. viridiflava*. The disease is named "bacterial leaf spot". To our knowledge, this is the first report of bacterial leaf spot of rape caused by atypical *P. viridiflava*.

**References:** (1) A. J. Gonzalez et al. *Appl. Environ. Microbiol.* 69:2936, 2003. (2) R. A. Lelliott et al. *J. Appl. Bacteriol.* 29:470, 1966. (3) H. Sawada et al. *J. Mol. Evol.* 49:627, 1999. (4) K. Tamura et al. *Mol. Biol. Evol.* 24:1596, 2007.

\* The e-Xtra logo stands for "electronic extra" and indicates this Disease Note online contains supplemental material not included in the print edition.

**First Report of Brown Ring Patch Caused by *Waitea circinata* var. *circinata* on *Poa annua* in Wisconsin and Minnesota.** J. P. Kerns and P. L. Koch, Department of Plant Pathology, University of Wisconsin-Madison; B. P. Horgan, Department of Horticultural Science, University of Minnesota. St. Paul; and C. M. Chen and F. P. Wong, Department of Plant Pathology, University of California, Riverside. *Plant Dis.* 94:1165, 2010; published online as doi:10.1094/PDIS-94-9-1165A. Accepted for publication 22 June 2010.

In summer of 2008, two turfgrass samples were submitted to the Turfgrass Diagnostic Lab at the University of Wisconsin-Madison. The samples were from golf courses in Beaver Dam, WI on 12 June and Minneapolis, MN on 14 July. Both samples were collected from 40-year-old native soil putting greens mowed at 3.2 mm that had received annual sand topdressing since 1992. The putting greens were a mixture of approximately 75% annual bluegrass (*Poa annua* L.) and 25% creeping bentgrass (*Agrostis stolonifera* L.). Stand symptoms observed in the field were bright yellow, sunken rings that were approximately 5 cm thick and 15 to 35 cm in diameter. Some rings were incomplete, giving a scalloped appearance. Affected plants were severely chlorotic and lacked any discrete lesions or spots. Symptoms were more prominent on annual bluegrass than creeping bentgrass. Upon incubation of samples at room temperature in a moist chamber for 24 h, fungal mycelia with septations and right-angle branching were observed in the foliage and thatch layer. Two isolates were obtained from affected annual bluegrass in each sample. Isolations were performed by washing affected leaves in 0.5% NaOCl solution for 2 min, blotting the tissue dry, and plating the tissue on potato dextrose agar (PDA) amended with chloramphenicol (0.05 g/liter), streptomycin (0.05 g/liter), and tetracycline (0.05 g/liter). After incubation for 2 days at 23°C, isolates were transferred and maintained on PDA. All four isolates had multinucleate hyphae and displayed sclerotial characteristics similar to those reported for *Waitea circinata* var. *circinata* (2). Sequencing the ITS1F/ITS4-amplified rDNA internal transcribed spacer (ITS) region confirmed the isolates as *W. circinata* var. *circinata*, with ≥99% sequence similarity to published *W. circinata* var. *circinata* ITS sequences (GenBank Accession No. FJ755849) (1,2,4). To confirm pathogenicity, isolates were inoculated onto 6-week-old annual bluegrass (True Putt/DW184) grown in 10-cm-diameter pots containing calcined clay (Turface; Profile Products LLC., Buffalo Grove, IL). Two 4-mm-diameter agar plugs for each isolate were removed from the margins of 3-day-old colonies grown on PDA and placed near the soil surface to ensure contact with the lower leaf blades. Each isolate was placed in four separate pots to have four replicated tests per isolate, and four noninfested pots were utilized as negative controls. All pots were placed in moist chambers at 28°C with a 12-h light/dark cycle. Within 4 to 6 days, inoculated plants exhibited severe chlorosis and a minor amount of aerial mycelium was

observed. Inoculated plants became necrotic after 15 to 20 days, while the noninoculated plants remained healthy. *W. circinata* var. *circinata* was reisolated from inoculated plants and its identity was confirmed by morphological and molecular characteristics. This pathogen was previously reported as a causal agent of brown ring patch of creeping bentgrass in Japan and annual bluegrass in the western United States (2,4). To our knowledge, this is the first report of brown ring patch in Minnesota and Wisconsin. Intensive fungicide practices are needed to control brown ring patch; therefore, this disease could have significant economic impact throughout the Upper Midwest (3).

**References:** (1) C. M. Chen et al. *Plant Dis.* 93:906, 2009. (2) K. de la Cerda et al. *Plant Dis.* 91:791, 2007. (3) J. Kaminski and F. Wong. *Golf Course Manage.* 75(9):98, 2007. (4) T. Toda et al. *Plant Dis.* 89:536, 2005.

**Differential Response by *Melaleuca quinquenervia* Trees to Attack by the Rust Fungus *Puccinia psidii* in Florida.** M. B. Rayamajhi, P. D. Pratt, T. D. Center, and G. S. Wheeler, USDA-ARS, Invasive Plant Research Laboratory, Fort Lauderdale, FL. *Plant Dis.* 94:1165, 2010; published online as doi:10.1094/PDIS-94-9-1165B. Accepted for publication 3 June 2010.

*Melaleuca quinquenervia* (melaleuca) is an exotic invasive tree in Florida, Hawaii, and some Caribbean islands (1,2). *Puccinia psidii* (rust fungus) attacks melaleuca as well as other plants in a few genera of the Myrtaceae and Heteropyxidaceae, both members of the Myrtales (1,2). Disease occurs on succulent stems and foliage of melaleuca, causing twig dieback and defoliation (3). Melaleuca trees growing under similar field conditions exhibit susceptible or resistant reactions toward this fungus. To document this differential susceptibility of melaleuca to *P. psidii*, we visually evaluated 331 field-grown melaleuca trees from southeast Florida for occurrence of disease attributes: pustules (susceptible), nonpersistent halos (resistant), or asymptomatic (no macroscopic symptoms) conditions on leaves and succulent twigs during February and March when symptoms were at their peak. Percentages of trees manifesting susceptible, resistant, and asymptomatic responses to this fungus were 85.8, 13.0, and 1.2%, respectively. A screenhouse study was conducted to corroborate these observations by raising plants from composite seed sources and maintaining them in seven 3.8-liter plastic pots that were filled with commercial potting media. Nine to eleven plants per pot (with new foliage) were individually tagged, grown to 30 to 45 cm high, and spray inoculated (during February and March) with uredospores ( $\sim 2 \times 10^6$ /ml) obtained from melaleuca trees and suspended in water. Inoculated plants were placed on a screenhouse bench under infected trees and subjected to additional inoculum, thereby simulating field conditions. Evaluations made weekly during a 4-week period revealed that susceptible, resistant, and asymptomatic seedlings constituted 63.3, 33.6, and 3.2%, respectively, of the tagged plants. To assess the stability of these fungal and host attributes over time and space, we multiplied two *P. psidii* susceptible and two resistant plants from cuttings. We spray inoculated 6 to 13 rooted cuttings from each plant types with uredospores (0.8 to  $2 \times 10^6$ /ml) obtained from diseased melaleuca trees and suspended in water. These plants were incubated in a dew chamber for 72 to 96 h under 100% relative humidity at 19 to 23°C maintained with a 12-h fluorescent light cycle. After incubation, plants were placed randomly on a bench in a screenhouse (21 to 23°C) and evaluated weekly for symptom development during a 4-week experimental period. Noninoculated controls were maintained as well. The experiment was repeated twice. Foliage of the resistant plants developed a few incipient halos whereas 100% of the susceptible plants developed erupted uredinia and were defoliated in both replications. No detectable change in *P. psidii* virulence and melaleuca susceptibility patterns was observed. Despite wide host range within Myrtales, resistance to *P. psidii* exists within *M. quinquenervia*. Other *P. psidii* susceptible host systems of economic and environmental importance may have host/pathogen relationships similar to that of melaleuca and the selection of resistant individuals from their affected populations may be possible. Additional studies will be needed to ascertain the attributes of virulence or resistance in this rust fungus-melaleuca association.

**References:** (1) M. Glen et al. *Australas. Plant Pathol.* 36:1, 2007. (2) P. D. Pratt et al. *J. Aquat. Plant Manag.* 45:8, 2007. (3) M. B. Rayachhetry et al. *Biol. Control* 22:38, 2001.

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