

**First Report of Brown Rot Caused by *Monilia polystroma* on Apple in Serbia.** M. Vasić, N. Duduk, and M. S. Ivanović, University of Belgrade, Faculty of Agriculture, Institute of Phytomedicine, Plant Pathology Department, Nemanjina 6, 11080 Belgrade, Serbia. This research was supported by the project III46008 financed by the Ministry of Education and Science, Republic of Serbia. Plant Dis. 97:145, 2013; published online as <http://dx.doi.org/10.1094/PDIS-07-12-0670-PDN>. Accepted for publication 9 August 2012.

*Monilia polystroma* van Leeuwen is a new Japanese species, similar to *M. fructigena* but distinguishable based on morphological and molecular characteristics (3). After its first discovery on apple in Japan, occurrence of *M. polystroma* in Europe has been reported in Hungary, the Czech Republic, and Switzerland (2,3,4). In October 2011, during a survey for apple fungal pathogens in the Bela Crkva district, 15 apple fruit (*Malus domestica* Borkh.) cv. Golden Delicious were collected. Two isolates of *Monilinia polystroma* were obtained from apple fruit showing brown rot, covered with small yellowish sporodochia. The pathogen was identified as *M. polystroma* based on morphological and molecular features (1,3). Upon isolation, colonies cultivated on PDA were white to grayish and the mycelium grew 8.85 mm per day at  $22 \pm 1^\circ\text{C}$  in 12-h light/12-h dark regime. After 6 to 8 days of incubation, black stromatal plates were observed on the reverse sides of the inoculated petri dishes. Conidia were one-celled, limoniform, hyaline,  $14.7$  to  $21.88 \mu\text{m}$  ( $16.2$  mean)  $\times$   $7.85$  to  $12.92 \mu\text{m}$  ( $10.8$  mean), and were produced in branched moniloid chains on inoculated apple fruit. Morphological identification was confirmed by PCR (1) using genomic DNA extracted from the mycelium of pure cultures, and amplified products of 425 bp in length, specific for *M. polystroma* were amplified as expected with primers MO368-5 and MO368-8R. For one isolate, the ribosomal ITS1-5.8S-ITS2 region was obtained, using primers ITS1 and ITS4, and deposited in GenBank (Accession No JX315717). The sequence was 498 bp in length and showed 100% identity with sequences deposited for *M. polystroma* in NCBI GenBank (JN128835, AM937114, GU067539). Pathogenicity was confirmed by wound-inoculating five surface-sterilized, mature apple fruit with mycelium plugs (5 mm in diameter) of both isolates grown on PDA. Control fruit were inoculated with sterile PDA plugs. After 3 days of incubation in plastic containers, under high humidity (RH 90 to 95%) at  $22 \pm 1^\circ\text{C}$ , typical symptoms of brown rot developed on inoculated fruit, while control fruit remained symptomless. Isolates recovered from symptomatic fruit showed the same morphological and molecular characteristics as original isolates. To the best of our knowledge, this is the first report of *M. polystroma* in Serbia. Further studies are necessary to estimate the economic importance and geographic distribution of this organism in Serbia.

**References:** (1) M.-J. Côté et al. Plant Dis. 88:1219, 2004. (2) M. Hilber-Bodmer et al. Plant Dis. 96: 146, 2012. (3) G. C. M. van Leeuwen et al. Mycol. Res. 106: 444, 2002. (4) OEPP/EPPO Reporting Service. Retrieved from <http://archives.eppo.int/EPPOreporting/2011/Rse-1106.pdf>

**First Report of Brown Rot Caused by *Monilinia fructicola* in Sweet Cherry in Maryland.** F. Chen and X. Liu, College of Agriculture and Biotechnology, China Agricultural University, Beijing 100193; and G. Schnabel, School of Agricultural, Forest, and Environmental Sciences, Clemson University, Clemson, SC 29634. Plant Dis. 97:145, 2013; published online as <http://dx.doi.org/10.1094/PDIS-07-12-0675-PDN>. Accepted for publication 4 September 2012.

*Monilinia fructicola* (G. Wint.) Honey is the most important causal agent of brown rot of stone fruits in North America. In July 2010, 20 sweet cherry fruit (*Prunus avium*) of unknown variety with symptoms resembling brown rot were collected from one commercial orchard in Maryland. Each cherry fruit came from a different tree. Symptoms included necrotic areas up to 10 mm in diameter with brown conidia and conidiophores developing from the infection center. Spores from nine symptomatic fruit collected each from different trees of a single orchard were suspended in sterile water, spread onto the surface of 1% agar plates, and incubated at  $22^\circ\text{C}$ . After 12 h, single, germinated spores were transferred onto 9-cm petri dishes with potato dextrose agar (PDA). Nine fungal colonies, each from a different fruit, were investigated in three replicates for cultural characteristics on separate petri dishes containing PDA. They were very similar in morphology and grew 12.4 mm per day on average at  $22^\circ\text{C}$ , forming branched, moniloid chains of grayish colonies with concentric

rings and little sporulation. Rich sporulation was observed on tomato sauce medium (250 ml tomato sauce and 20 g agar in 750 ml water). The lemon-shaped spores had an average size of  $15 \times 10 \mu\text{m}$ , which is consistent with *M. fructicola*. Two colonies were randomly selected to identify the pathogen to the species level using a PCR technique based on cytochrome b sequence amplifications (2). Resulting gel electrophoresis patterns were consistent with *M. fructicola*. Koch's postulates were fulfilled by inoculating 15 mature sweet cherry fruits of cv. Bing with a conidial suspension ( $10^5$  spores/ml) of one of the single-spore isolates from cherry. Fruit were stab-inoculated at a point to a depth of 2 mm using a sterile needle. A 10- $\mu\text{l}$  droplet was placed on each wound; control fruit received sterile water without conidia. After 3 days of incubation at room temperature in airtight plastic bags, the inoculated fruit developed typical brown rot symptoms with lesions that were 20.6 mm in diameter. The developing spores on inoculated fruit were confirmed to be *M. fructicola*. All control fruit remained healthy. The entire detached fruit experiment was repeated 1 week later. *M. fructicola* is assumed to be the main causal agent of brown rot of sweet cherry in the northeastern United States, but recent studies show that *M. laxa* is also causing the disease on sweet cherry in many northeastern states (1). For this reason, it is important to delineate species for accurate disease assessments. This study confirms assumptions that *M. fructicola* is a causal agent of sweet cherry in Maryland.

**References:** (1) K. D. Cox et al. Plant Dis. 12:1584, 2011. (2) J.-M. Hily et al. Pest Manag. Sci. 67:385, 2011.

**First Report of Verticillium Wilt caused by *Verticillium dahliae* Kleb. on New Zealand Spinach (*Tetragonia tetragonioides*) in Italy.** A. Garibaldi and S. Rapetti, Center of Competence AGROINNOVA, University of Torino, Via Leonardo da Vinci 44, 10095 Grugliasco, Italy; P. Martini and L. Repetto, Istituto Regionale per la Floricoltura, Sanremo, Italy; and D. Bertetti, A. Poli, and M. L. Gullino, Center of Competence AGROINNOVA, University of Torino, Via Leonardo da Vinci 44, 10095 Grugliasco, Italy. Plant Dis. 97:145, 2013; published online as <http://dx.doi.org/10.1094/PDIS-07-12-0678-PDN>. Accepted for publication 5 October 2012.

*Tetragonia tetragonioides* (New Zealand spinach, Aizoaceae) is an Australasian annual species that occurs naturally in Italy, where it is cultivated for the edible young shoots and succulent leaves. In September 2011, a previously unknown wilt was observed in 10 private gardens, each 0.1 to 0.5 ha, near Castellaro, Northern Italy, on 7-month-old New Zealand spinach plants. Leaves wilted, starting from the collar and moving up the plant, and vascular tissues showed brown streaks in the roots, crowns, and stems. Diseased plants were stunted with small, chlorotic leaves. Infected stems and leaves then wilted, and plants often died. Of about 500 plants, 30% were affected. Stems of 10 diseased plants were disinfected with 1% NaOCl for 1 min. Sections of symptomatic vascular tissue were plated on potato dextrose agar. After 3 days at  $23 \pm 1^\circ\text{C}$ , colonies developed that were white and turned a grey to dark green color. Irregular, black microsclerotia ( $32.0$  to  $63.1 \pm 16.8 \mu\text{m}$  ( $106.1$ )  $\times$  ( $18.7$ )  $39.1 \pm 12.3 \mu\text{m}$  ( $65.8$ ) developed in hyaline hyphae after 8 days. Hyaline, elliptical, single-celled conidia ( $2.7$ )  $3.8 \pm 0.6 \mu\text{m}$  ( $4.8$ )  $\times$  ( $1.9$ )  $2.6 \pm 0.5 \mu\text{m}$  ( $3.5$ ) developed on verticillate conidiophores with three phialides at each node. Based on these morphological characteristics, the fungus was identified as *Verticillium dahliae* (1). The internal transcribed spacer (ITS) region of rDNA was amplified for one isolate using the primers ITS1/ITS4 (3) and sequenced (GenBank Accession No. JX308315). BLASTn analysis of the 479-bp segment showed 100% homology with the ITS sequence of a *V. dahliae* isolate (AB551206). Pathogenicity tests were performed twice using 60-day-old plants of *T. tetragonioides*. Unwounded roots of eight plants were dipped for 1 min in a conidial suspension ( $5 \times 10^7$  conidia/ml) of one isolate of *V. dahliae* obtained from the original infected New Zealand spinach plants, and grown in potato dextrose broth. The inoculated plants were transplanted into 2-liter pots (1 plant/pot) containing steamed potting mix (sphagnum peat-perlite-pine bark-clay; 50:20:20:10) and maintained in a growth chamber at  $20$  to  $24^\circ\text{C}$  and 50 to 80% RH. Eight

(Disease Notes continued on next page)

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