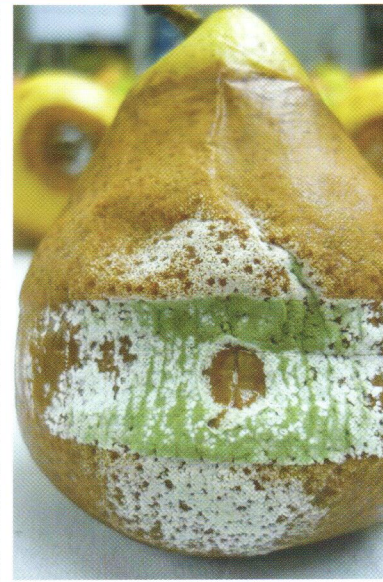


An International Journal of Applied Plant Pathology

plant disease



THE AMERICAN PHYTOPATHOLOGICAL SOCIETY

First Report of Impatiens Downy Mildew Outbreaks Caused by *Plasmopara obducens* Throughout the Hawai'ian Islands. J. A. Crouch, Systematic Mycology and Microbiology Laboratory, USDA-ARS, 10300 Baltimore Avenue, Beltsville, MD 20705; M. P. Ko, Hawaii Department of Agriculture, 1428 S. King Street, Honolulu, HI 96814; and J. M. McKemy, USDA-APHIS-PPQ-National Identification Services, Beltsville, MD 20705. Plant Dis. 98:696, 2014; published online as <http://dx.doi.org/10.1094/PDIS-10-13-1017-PDN>. Accepted for publication 17 October 2013.

Downy mildew of impatiens (*Impatiens walleriana* Hook.f.) was first reported from the continental United States in 2004. In 2011 to 2012, severe and widespread outbreaks were documented across the United States mainland, resulting in considerable economic losses. On May 5, 2013, downy mildew disease symptoms were observed from *I. walleriana* 'Super Elfin' at a retail nursery in Mililani, on the Hawai'ian island of Oahu. Throughout May and June 2013, additional sightings of the disease were documented from the islands of Oahu, Kauai, Maui, and Hawai'i from nurseries, home gardens, and botanical park and landscape plantings. Symptoms of infected plants initially showed downward leaf curl, followed by a stippled chlorotic appearance on the adaxial leaf surfaces. Abaxial leaf surfaces were covered with a layer of white mycelia. Affected plants exhibited defoliation, flower drop, and stem rot as the disease progressed. Based on morphological and molecular data, the organism was identified as *Plasmopara obducens* (J. Schröt.) J. Schröt. Microscopic observation disclosed coenocytic mycelium and hyaline, thin-walled, tree-like (monopodial branches), straight, 94.0 to 300.0×3.2 to $10.8 \mu\text{m}$ sporangiophores. Ovoid, hyaline sporangia measuring 11.0 to 14.6×12.2 to 16.2 (average 13.2×14.7) μm were borne on sterigma tips of rigid branchlets (8.0 to $15.0 \mu\text{m}$) at right angle to the main axis of the sporangiophores (1,3). Molecular identification of the pathogen was conducted by removing hyphae from the surface of three heavily infected leaves using sterile tweezers, then extracting DNA using the QIAGEN Plant DNA kit (QIAGEN, Gaithersburg, MD). The nuclear rDNA internal transcribed spacer was sequenced from each of the three samples bidirectionally from Illustra EXOStar (GE Healthcare, Piscataway, NJ) purified amplicon generated from primers ITS1-O and LR-OR (4). Resultant sequences (GenBank KF366378 to 80) shared 99 to 100% nucleotide identity with *P. obducens* accession DQ665666 (4). A voucher specimen (BPI892676) was deposited in the U.S. National Fungus Collections, Beltsville, MD. Pathogenicity tests were performed by spraying 6-week-old impatiens plants (*I. walleriana* var. Super Elfin) grown singly in 4-inch pots with a suspension of 1×10^4 *P. obducens* sporangia/ml until runoff using a handheld atomizer. Control plants were sprayed with distilled water. The plants were kept in high humidity by covering with black plastic bags for 48 h at 20°C , and then maintained in the greenhouse (night/day temperature of $20/24^\circ\text{C}$). The first symptoms (downward curling and chlorotic stippling of leaves) and sporulation of the pathogen on under-leaf surfaces of the inoculated plants appeared at 10 days and 21 days after inoculation, respectively. Control plants remained healthy. Morphological features and measurements matched those of the original inoculum, thus fulfilling Koch's postulates. To our knowledge, this is the first report of downy mildew on *I. walleriana* in Hawai'i (2). The disease appears to be widespread throughout the islands and is likely to cause considerable losses in Hawai'ian landscapes and production settings.

References: (1) O. Constantinescu. Mycologia 83:473, 1991. (2) D. F. Farr and A. Y. Rossman. Systematic Mycology and Microbiology Laboratory, ARS, USDA. Retrieved from <http://nt.ars-grin.gov/fungalatabases/> July 16, 2013. (3) P. A. Saccardo. Syllogeus Fungorum 7:242, 1888. (4) M. Thines. Fungal Genet Biol 44:199, 2007.

e-Xtra

First Record of Thousand Cankers Disease *Geosmithia morbida* and Walnut Twig Beetle *Pityophthorus juglandis* on *Juglans nigra* in Europe. L. Montecchio, Department of Land, Environment, Agriculture, and Forestry (TeSAF), University of Padova, Italy; and M. Faccoli, Department of Agronomy, Food, Natural Resources, Animals, and Environment (DAFNAE), University of Padova, Italy. Plant Dis. 98:696, 2014; published online as <http://dx.doi.org/10.1094/PDIS-10-13-1027-PDN>. Accepted for publication 25 November 2013.

Thousand cankers disease (TCD) of walnut (*Juglans nigra* L.) in the United States since the mid-1990s (2). The disease is caused by the fungus *Geosmithia morbida* Kolařík (Ascomycota, Hypocreales), vectored by the walnut twig

beetle *Pityophthorus juglandis* Blackman 1928 (Coleoptera, Scolytinae). In September 2013, TCD was observed in northeastern Italy (Bressanvido, Vicenza, $45^\circ39' \text{N}$, $11^\circ38' \text{E}$) in black walnuts of different ages: ~80-year-old plants growing in a garden and 17-year-old trees belonging to a nearby walnut plantation for timber production. Main symptoms were yellowing, wilting, twig and branch dieback, and a high number of small bark cankers (3). Longitudinal and radial sections collected through the cankers revealed gray to brown discoloration of both phloem and outer bark, and the presence of bark beetle galleries radiating from the mating chamber and developing horizontally (across the wood grain), and vertical (along the grain) larval galleries. Occasionally, discoloration involved the outward xylematic tissues. Fungal fruiting bodies were not found on or near the cankers. Whitish mycelium, sometimes producing verticillate conidiophores, was frequently detected inside galleries. A number of 1- to 3-cm diameter twigs showing cankers up to 2 cm long surrounding bark beetle penetration holes were randomly collected. From samples, emerging beetles were identified as *P. juglandis* both morphologically (4) and genetically. DNA extraction was carried following a standard salting out protocol. The barcode region of the mitochondrial gene cytochrome oxidase I was then amplified using universal primers (1) and sequenced, obtaining 627 bp. BLAST analysis showed 100% identity to *P. juglandis*. Sequences were finally deposited in the BoldSystem database (GenBank Accession No. KF725084). From the necrotic margin of six cankers previously surface-sterilized with 3% sodium hypochlorite, two 3-mm-wide chips per canker were placed on potato dextrose agar and incubated at $23 \pm 1^\circ\text{C}$ in the dark. Among a variety of microorganisms, slow growing lobate, plane, yellowish-ochre colonies with hyaline mycelium appeared in 6 days. After subculturing to the same medium, growing features, mycelium, conidiophores, and conidia with morphological characteristics matching Kolařík's description of *G. morbida* (2) were observed. Same result was obtained culturing the mycelium growing inside the galleries. The ITS region of rDNA was amplified using ITS1F and ITS4 primers and sequenced, obtaining 597 bp. BLAST analysis showed 100% identity to *G. morbida* strain U173 (HF546283.1) for 558 bp. To our knowledge, this is the first record of TCD and *P. juglandis* to Europe, where walnut species (mainly *J. regia*, *J. nigra*, and their hybrids) are intensively cultivated for timber production. This finding is therefore of particular importance. An intensive survey of the disease is suggested, both to assess fungus/beetle presence and to verify possible pathways of introduction, likely associated to importation of infested/infected timber from native Nearctic regions. Voucher specimens are stored in the TeSAF herbarium (fungus) and in the DAFNAE insect collection.

References: (1) O. Folmer et al. Mol. Marine Biol. Biotechnol. 3:294, 1994. (2) M. Kolařík et al. Mycologia 103:325, 2011. (3) C. Nischwitz and M. Murray. Utah Pests Fact Sheet, PRP-015pr, 2011. (4) S. L. Wood. Great Basin Naturalist Memoirs 6:1123, 1982.

First Report of Anthracnose of *Alocasia macrorrhiza* Caused by *Colletotrichum karstii* in Guangdong, China. Y. He, C. Shu, J. Chen, and E. Zhou, Department of Plant Pathology, and the Guangdong Province Key Laboratory of Microbial Signals and Disease Control, South China Agricultural University, Guangzhou, Guangdong 510642, China. Plant Dis. 98:696, 2014; published online as <http://dx.doi.org/10.1094/PDIS-10-13-1046-PDN>. Accepted for publication 6 November 2013.

Alocasia macrorrhiza (L.) Schott. (Araceae), native to South America, is a common, herbaceous perennial ornamental plant in tropical and subtropical areas (1). A severe leaf spot disease was observed on this plant in several places on the campus of authors' university in Guangzhou, Guangdong Province, China, in April 2013. Initial symptoms were water-soaked, dark green leaf spots. These small spots gradually expanded to 6- to 11-mm circular lesions. They were grayish-white in color with a yellow halo and many small, black, concentric dots were observed on them. Microscopic examination revealed that these small dots were acervuli, which were 100 to 300 μm in diameter, developing beneath the epidermis and becoming erumpent with age. By using routine tissue-isolation method and single-spore purification technique, four single-conidial isolates were obtained from each of four diseased leaves. These isolates formed a grayish-white colony with numerous pink spore masses on PDA at 28°C . Their mycelial radial growth rate was about 4.5 mm per day. Conidia were single-celled, hyaline, and cylindrical with an obtuse apex and protruding base; they were 12.7 to 14.2×4.8 to $5.9 \mu\text{m}$ in size. Conidial appressoria were irregular in shape, sepia to dark brown, solitary, and 6.9 to 8.5×4.6 to $5.9 \mu\text{m}$. These morphological characteristics were consistent with the