Diseases Caused by Fungi and Fungus-Like Organisms

First Report of Leaf Rust on Blackberry (*Rubus* spp.) Caused by *Kuehneola uredinis* in Florida

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During the fall of 2020 and summer of 2021, leaf rust was observed on blackberry plants of cultivar 'Kiowa' and breeding line 1734 (BL1734) ('Natchez' × 'Arapaho') in a field trial in Wimauma, FL. Symptoms consisted of chlorotic spots (1 to 3 mm) on the upper side of the leaves, while the underside had yellow-orange pustules. Disease incidence (number of symptomatic leaves) was up to 100% on Kiowa and BL1734, and severity (percentage of diseased area) was up to 20%, with most of the symptoms observed on older leaves. Two isolates collected from Kiowa and one from BL1734 were maintained on healthy Kiowa plants in growth chambers (25°C and 12/12 h photoperiod). Uredinia ($n = 30$) were erumpent and ranged from 90 to 320 µm (Average = 285 µm, SD = 5.3 µm) in diameter. Urediniospores ($n = 50$) were obovoid, yellow, and 24 to 36 µm long (Average = 32 µm, SD = 3.2 µm) and 22 to 30 µm wide (Average = 28 µm, SD = 2.5 µm). Based on morphology and literature, the pathogen was tentatively identified as *Kuehneola uredinis* (Link) Arth (Arthur 1906; Shands et al. 2018). Urediniospores from a single uredinium of each isolate were collected and suspended in 50 µl of molecular biology-grade water ($\sim 5 \times 10^4$ spores/ml), and 2 µl was directly used for the PCR reactions. DNA fragments were amplified using the primers Rust2inv and LR6, and Rust18S-R and NS1 for the 5.8S-ITS2-28S gene region of rDNA (1,755 bp) and partial 18S gene region of rDNA (2,684 bp), respectively. The amplified products of the partial 28S gene region were sequenced with the primers LR3 and LR0R, and the 18S gene region with NS5, NS6, and NS4 (Aime 2006). Sequences were deposited in GenBank (accession nos. OK509845 to OK509848). BLASTn searches revealed that the isolates were 100% identical to *K. uredinis* reported

on blackberry in California (1044/1044 bp and 1540/1540 bp for MF158087 and MF158088, respectively). To test for pathogenicity, blackberry cultivars Kiowa, Natchez, Osage, Ouachita, Ponca, Prime-Ark 45, Prime-Ark Freedom, Prime-Ark Traveler, and Prime-Ark Horizon were inoculated. Five plants of each cultivar were inoculated with a mixture of the three isolates, and two plants per cultivar were used as controls. Spores were washed from sporulating Kiowa leaves with a solution of 1% Tween 20 in deionized water and adjusted to 10^4 spores/ml. Plants were inoculated in the greenhouse with a spray bottle until run-off and kept inside clear plastic boxes for 48 h. Controls were sprayed with 1% Tween 20 in deionized water. Plants were watered by mists of 3 s every 10 min twice a week. Disease incidence and severity were evaluated weekly on five leaves per plant that had been tagged before inoculation. The experiment was repeated once. Symptoms were only observed in Kiowa and Prime-Ark Freedom. One week after inoculation, incidence was 100% in both cultivars, with at least one pustule on all the inoculated leaves, and 6 weeks later severity was up to 50% (Average = 35%, SD = 2.4%). To our knowledge, this is the first report of *K. uredinis* causing leaf rust on blackberry in Florida. This pathogen was reported on *Rubus* spp. in Europe, South Africa, Australia, New Zealand, South America, Canada, and the United States (Farr and Rossman 2021; Laundon and Rainbow 1969; Shands et al. 2018). Blackberry is an emerging crop in Florida, and efforts should be implemented to monitor the occurrence and spread of leaf rust considering that urediniospores disperse long distances by wind, especially if growers choose susceptible cultivars such as Kiowa and Prime-Ark Freedom. The resistance observed in other commercial cultivars may contribute for developing new cultivars with resistance to leaf rust.

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