Diseases Caused by Fungi and Fungus-Like Organisms

First Report of *Colletotrichum fructicola*, *C. perseae*, and *C. siamense* Causing Anthracnose Disease of Avocado (*Persea americana*) in New Zealand

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In January and March 2019, inspection of 11 commercial ‘Hass’ avocado orchards in mid-North and Tauranga (New Zealand [NZ]) was conducted by NZ Avocado Growers Association Inc., and samples were sent to Plant Diagnostics Limited to investigate a newly observed fruit staining symptom, “tannin stain”. Symptoms consisted of areas of minute spots that coalesced into areas of tear staining associated with water movement over the fruit’s surface. Up to seven trees per orchard were sampled, targeting symptomatic fruit. Fruit was surface disinfected for 4 min in 1% NaOCl, and sections from lesions were plated on prune extract agar to isolate plant pathogens. The predominant fungi isolated represented species in the *Colletotrichum acutatum*, *C. gloeosporioides*, and *C. boninense* species complexes. Because morphological characters within these complexes overlap (see supplementary material), the isolates were differentiated by amplification and sequencing of the glyceraldehyde-3-phosphate dehydrogenase (GPDH) gene and, where necessary, the calmodulin (CAL) gene and/or the Apn2-Mat1-2 intergenic spacer region (ApMat) (Rojas et al. 2010; Weir et al. 2012). Sequence analysis revealed eight *Colletotrichum* species: *C. alienum*, *C. aotearoa*, *C. cigarro*, *C. fiorinae*, *C. fructicola* (Cf), *C. karstii*, *C. perseae* (Cp), and *C. siamense* (Cs). Three species were not previously recorded in NZ: Cf, Cp, and Cs. Colonies for all three fungi were white to gray with salmon-colored and black acervuli. Conidia were aseptate, hyaline, straight, cylindrical, with broadly rounded ends, forming on cylindrical conidiogenous cells. The GPDH, CAL, and/or ApMat sequences of the Cf, Cp, and Cs isolates were identical to reference sequences of representative isolates in GenBank (e.g., ApMat: Cf,

KX620181; Cp, KX620177; Cs, KP703788). An isolate for each species is stored in the International Collection of Microorganisms from Plants (Cf, ICMP22409; Cp, ICMP22431; Cs, ICMP22411), and sequences are in GenBank (MT522858 to MT522865). Pathogenicity of each newly recorded species was confirmed on freshly picked ‘Hass’ avocado fruit. After surface disinfection with 1% NaOCl for 5 min, fruit was triple washed with sterile water (SW) and air dried. Five fruits per species were pinpricked and inoculated with 10 µl of conidial suspension (7×10^6 to 1×10^7 conidia/ml) prepared with SW containing Tween 20 (1 µl/ml H₂O) from 6-day-old cultures grown on PDA. Control fruit was pinpricked and mock inoculated with SW containing Tween 20 (1 µl/ml H₂O). All fruit was incubated in moist chambers at 25°C for 7 days. The three *Colletotrichum* species produced anthracnose symptoms on inoculated fruit; no symptoms were observed on control fruit. Each species was successfully reisolated from symptomatic tissue and identified using the methods described above, fulfilling Koch’s postulates. Although Cf and Cs have been reported from several hosts and countries (Weir et al. 2012), Cp has only been found from avocado in Israel (Sharma et al. 2017) and grape in Japan (Yokosawa et al. 2020). Although several species from the *C. gloeosporioides* species complex (i.e., *C. alienum*, *C. aotearoa*, *C. cigarro*, and *C. gloeosporioides*) have been previously associated with avocado diseases in NZ, the detections of Cf, Cp, and Cs represent first records. In this study, eight *Colletotrichum* species were associated with tannin stain fruit symptoms in NZ avocado orchards. The individual contribution of Cf, Cp, and Cs to the observed symptoms was not determined, but their detection highlights the importance of sequence-based identification of *Colletotrichum* species, as morphological characters are insufficient to separate cryptic species. Accurate identification of pathogens provides knowledge of species biodiversity that may be useful in biosecurity decision making. Because fungicide treatment efficiencies differ for some closely related *Colletotrichum* species on grape (Yokosawa et al. 2020), accurate identification might also contribute to establishing effective management strategies.

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