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First report of *Neofusicoccum australe* causing canker disease and branch dieback on *Arctostaphylos glauca* in California

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In February of 2014, severe dieback of the manzanita species *Arctostaphylos glauca* was observed in the Santa Ynez Mountain front range in Santa Barbara County, California. Symptoms of branch dieback and cankers were consistent with infection by *Botryosphaeriaceae* fungi. Samples were retrieved from cankered branches on each of 60 individual shrubs across six sites in the field. Branches were surface-sterilized, and small cross-sections (~1-2mm diameter) were cut and plated onto potato dextrose agar (PDA) amended with streptomycin, wrapped in Parafilm, and stored at room temperature. Fungal cultures became dense with aerial hyphae and gray-black in color within one week, which is consistent with general descriptions of *Botryosphaeriaceae* members by Slippers and Wingfield (2007). Hyphae from all cultures were isolated and re-plated onto half-strength, acidified PDA amended with streptomycin, and stored for approximately 8 weeks. The internal transcribed spacer region (ITS) and alpha-elongation factor-1 (EF1) gene were amplified using PCR primer pairs ITS1F/ITS4, and EF1-728F/986R, respectively, according to Slippers *et al* (2004), and sequenced at UC Berkeley DNA Sequencing Facility, CA. BLAST searches of ITS and EF1 confirmed, with at least 99% similarity in GenBank, 48% of sequences to be *Neofusiccocum australe* (matched accession nos. KF766200.1 and KT440981.1, respectively) and 32% to be *Botryosphaeria dothidea* (KF766151.1 and KT440953.1). Our sequences were deposited in GenBank under accession nos. MH776991-MH777017 (ITS), and MH754915-MH754937 (EF1).

While *Botryosphaeriaceae* fungi, including *B. dothidea*, have been retrieved on manzanita species (Brooks and Ferrin, 1994; Swiecki and Bernhardt, 2003), to the authors' knowledge this is the first report of *N. australe* being recovered from branch cankers in *Arctostaphylos* hosts in California, and on any naturally occurring species in Santa Barbara County. In November 2016, pathogenicity of *N. australe* was tested using approximately 2-year old *A. glauca* plants in the greenhouse. Cultures were made from re-isolations of field samples that were positively identified to be *N. australe* and plated onto half-strength PDA amended with streptomycin. Using a 3 mm cork borer, mycelial plugs were taken from the advancing margin of eight-day old *N. australe* cultures, placed on strips of Parafilm using sterile petroleum jelly for adhesion, and attached to 3 mm superficial wounds made at the stem base by gently wrapping the Parafilm strips 2-3 times around the stem. Those plants not receiving fungal inoculation received a control inoculation with sterilized uncultured half-strength PDA using the same techniques. Over three months, plants were monitored for disease symptom onset including browning/discolored leaves and fungal cirrhi. Symptoms appeared and fungi were successfully re-isolated from just above the POI of inoculated plants, confirming Koch's postulates. No symptoms appeared and no pathogenic fungi were isolated from the controls. Mean lesion length from the POI on diseased plants upon harvesting was 24.5±15.2cm (mean±SD). These results are significant because *A. glauca* is a dominant and widespread species in coastal and inland mountains across much of central and southern California. Its continued dieback could have important consequences for the native species that rely on *A. glauca* for food and structure. Additionally, dieback can increase fire risk in these fire-prone regions.

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