Three *Botrytis* species found causing gray mold on industrial hemp (*Cannabis sativa*) in Oregon

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In September and October of 2019, flowers of hemp plants in Polk and Linn counties in Oregon showed symptoms of die-back with necrosis of the tissues, resulting in significant yield reductions. The tops of the inflorescences were often the most severely affected with the infection sometimes moving down into the petiole or stem. Up to 90% of the plants in these fields had at least one flower infection present, however, foliar symptoms (lesions) were not observed. Gray to white mycelium and *Botrytis*-like conidiophores could often be seen arising from host tissue. The fungus was recovered from colonized plant tissues either by placing conidia directly onto a Petri dish containing potato dextrose agar (PDA) or placing a small piece of surface sterilized plant tissue onto PDA. A total of 23 pure cultures were recovered from three fields. All cultures displayed white to gray, fast-growing mycelium within which conidiophores sometimes developed bearing Botryose clusters of conidia, followed by the formation of black sclerotia in all isolates. Since morphology does not allow for distinguishing among cryptic species in the genus, glyceraldehyde 3-phosphate dehydrogenase (G3PDH) gene sequences (Staats et al. 2005) were generated to identify species (GenBank accession nos. MN909293-MN909315). Twenty isolates shared >99 to 100% sequence identity to *B. cinerea* type specimen isolate B05.10 (accession no. JQ036050), two isolates shared >99% sequence identity to *B. pseudocinerea* type specimen isolate 10091 (accession no. JN692414), and one isolate shared >99% sequence identity to isolates representing a published, but unnamed species of *Botrytis* (accession no. KY200370 as an example) (Garfinkel et al. 2019). One isolate from each representative species was selected for pathogenicity trials. Isolates LV05 (*B. cinerea*), LV20 (*B. pseudocinerea*), and LV07 (*Botrytis* sp.) were grown on PDA. After 48 hours, plugs 4mm in diameter were cut from the margin of the actively growing colony and placed mycelium-side down on the inflorescence of potted CBD-type industrial hemp plants. All inoculated flowers were female and in the receptive stage, displaying white stigmas. Uninoculated flowers were used as controls. Plants were placed in grow tents and kept at 20°C in the dark for three days under high humidity conditions. Two plugs per isolate were placed onto four plants each for a total of 8 replicates per *Botrytis* species. After 7 days, a dry necrosis or a wet decay of the flowers and surrounding leaves was visible. Symptoms were often accompanied with profuse epiphytic mycelium surrounding and within the flower clusters. The symptoms seen in pathogenicity trials were consistent with those seen in the field and those described in the literature (McPartland 1996) with no obvious differences in the symptoms or their severity among species. The fungus was re-isolated from plant tissue for every replicate of all of the three *Botrytis* species as per the surface sterilization method; the fungus was not isolated from controls. Although *B*. *cinerea* has been reported to cause disease on *C. sativa* in the state of Oregon, this work is the first to describe the completion of Koch's postulates with *B. cinerea* isolated from Oregon hemp. Additionally, to our knowledge, only B. cinerea has been previously reported to cause disease on *C. sativa*; this is the first time *B. pseudocinerea* or the novel *Botrytis* species have been reported on this crop anywhere in the world. As additional samples are taken from hemp throughout the United States, these and other *Botrytis* species will likely be found in Oregon and other growing regions.

References

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Figure 1. (a) Dieback of a female inflorescence, (b) a stem lesion, and (c) fuzzy gray growth (indicated by arrow) associated with *Botrytis* infection on industrial hemp (*Cannabis sativa*).

254x100mm (150 x 150 DPI)