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Identification of fungal species causing canker disease on Redbud (*Cercis* sp.) and development of an effective detection tool

Redbud (*Cercis* sp.) is a popular ornamental flowering tree that is commonly used in landscaping due to its attractive appearance and relatively low maintenance requirements. Large-scale production of redbud has been challenged by its susceptibility to canker pathogens, one of the most damaging diseases of redbud. The typical symptom of canker is dieback, foliage on the affected branch dries out and turns orangish brown. It also causes dried, discolored, and dead barks, as well as cracked, sunken, or raised appearance on stems, trunks, branches, or even twigs. Canker pathogens can girdle the main stem of a tree and result in weakened trees with deformed architecture. Cankers are most prevalent and obvious on trees that are predisposed to stress and are usually difficult to treat. Currently, the management options for cankers on redbud trees are limited and primarily consist of reducing tree stress and implementing sanitation protocols. It is critical to accurately identify the canker pathogens of redbud and to implement a timely disease management strategy to minimize economic losses. In this current study, redbud samples with canker symptoms received from commercial nurseries in Tennessee were processed for the identification of the fungal microbial diversity associated with redbud plants. Samples were processed as 1-2 cm segments and surface sterilized with 1% sodium hypochlorite for 2 min, rinse with sterilized distilled water two times and blot dry on sterile filter paper. Sterilized plant tissue was cultured on various media, including potato dextrose agar, *Fusarium* selective medium, water agar, V8-PARPH, *Verticillium* selective media, and *Rhizoctonia* semi-selective media, and kept under laboratory conditions at 25°C with a 12-hour fluorescent light and dark cycle for one week. Initial microscopic observations were made based on the pathogen morphological characteristics (pigmentation, growth patterns, spores, hyphae, and conidia formation). To confirm pathogen identity, total DNA was extracted from week-old cultures using DNeasy powerlyzer microbial kit (Qiagen) kit. The ribosomal DNA internal transcribed spacer (ITS) region was amplified using the primer pairs ITS1/ITS4 and the PCR products were sequenced. Various fungal isolates such as *Botryosphaeria dothidea*, *Diaporthe* spp., *Didymella* spp., *Fusarium* spp., were found to be associated with the redbud plants with canker symptoms. This information on fungal diversity was linked with the existing information on redbud canker-causing pathogens and was used to design canker-specific primers that can be used in multiplex qPCR, enabling the detection of two or more fungi in the same reaction. Future experiments will be conducted to determine the pathogenicity of the identified isolates. Early and accurate detection of canker pathogens are important in implementing timely management approaches to minimize the damage caused by these pathogens.