

Small-spored *Alternaria* species associated with potato leaf spot across the US for nearly two decades

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Abstract

Recent literature reported that brown leaf spot of potato in the Pacific Northwest US is caused by several small spored *Alternaria* spp (SSA). Our laboratory has collected putative *A. alternata sensu lato* isolates from several potato producing states in the US for almost 20 years. An NCBI BLAST nucleotide match from *OPAI-3* gene sequences from a sub-set of two hundred isolates identified three species of SSA as *A. alternata sensu stricto*, *A. tenuissima* and *A. arborescens*. These three species are similar morphologically, displaying overlapping and variable characteristics, and have indistinguishable disease symptomatology. A phylogenetic characterization of sixty-nine SSA isolates was accomplished based on four gene sequences, *OPAI-3*, *Alt a1*, *ITS* and *TEF*. The phylogenetic analysis based on *ITS*, and *TEF* revealed no diversity among the SSA isolates. The *Alt a1* analysis classified the SSA isolates into two major groups, with no distinction between *A. alternata sensu stricto* and *A. tenuissima*. The phylogenetic analysis based on *OPAI-3* gene sequences classified the SSA isolates in three distinct groups as *A. alternata sensu stricto*, *A. tenuissima* and *A. arborescens*. A multiplex real-time PCR was developed based on SNPs identified in the *OPAI-3* gene to distinguish isolates of *A. alternata* (E=100%, R² value=0.97), *A. tenuissima* (E=104%, R² value=0.98) and *A. arborescens* (E=102%, R² value=0.98). Multiplex real-time PCR differentiation was 99%

successful when compared to sequences from the *OPA* gene and closely-related phytopathogenic fungi were not amplified. The multiplex real-time PCR performed on three-hundred and four SSA isolates indicate that all three species were found in association with brown leaf spot of potato in the US and Canada. In both populations, *A. alternata* represented greater than 60% of all SSA isolates recovered. Isolates of all three species were found as early as 2000 in the US; however, the frequency of *A. alternata* isolates has decreased recently. Among US isolates collected in 2000, 2003, 2011, 2013 and 2014, the frequency of recovering *A. alternata* ranged from 75% to 93%. In 2017, 2018 and 2019 that decreased to 47% to 61%. It is unclear if the shift in SSA species associated with brown leaf spot will affect brown spot severity or management. The use of the multiplex PCR assays will facilitate accurate characterization of the potato brown leaf spot pathogen complex in the future.