Molecular Identification of Polish Isolates of *Sclerotinia trifoliorum* and *Sclerotinia sclerotiorum* in the Context of Kura Clover Damage

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Kura clover (*Trifolium ambiguum* M. Bieb.), grown mainly in North America and New Zealand, has several favorable agronomic qualities and is regarded as resistant to infection by *S. trifoliorum* Eriks., one of the most destructive pathogens of clovers in Northern Europe. Attempts to grow Kura clover in Poland were not successful because plants on experimental plots were almost completely destroyed by pathogens resembling *Sclerotinia* sp. Analysis of growth rate of isolates on PDA and their comparison with *S. sclerotiorum* isolates suggested that the damage of *T. ambiguum* was caused by *S. trifoliorum*. Microscopic observation of ascospores confirmed this finding.

Molecular analysis of Internal Transcribed Spacer (ITS) regions did not facilitate species differentiation; although these regions have been used to distinguish species within the *Sclerotinia* genus (Njambere et al., 2008). Species-specific primers developed based on *Aspr* (SSaspr F / SSaspr R) and *Cad* (STCad F/ STCad R) genes were used for further confirmation of isolates of *S. sclerotiorum* and *S. trifoliorum* (Abd-Elmagid et al., 2013) In addition to isolates obtained from *T. ambiguum, S. trifoliorum* isolates from *T. repens, T. pratense* and *T. resupinatum*; and isolates of *S. sclerotiorum* collected from *Brassica napus*, *Daucus carota*, *Helichrysum arenarium* and the other plant species were examined. Primers developed by Abd-Elmagid et al. (2013) proved to be non-specific in the case of Polish isolates. Products with both primer sets were amplified in the case of all samples. All primers used in these analyses were developed in the U.S. Molecular analyses presented here indicate that the isolates from the USA and Poland are gentically different. This is probably the reason for the lack of specificity of primers based on the *Aspr* and *Cad* genes when used to identify isolates from Europe.

Preliminary studies showed that the Intergenic Spacer (IGS) region can be a reliable genetic region to distinguish between *Sclerotinia* species collected in Poland. Single Nucleotide Polymorphisms (SNP) was determined within the fragment amplified with PTR-1a/PTR-1b primers (Andrew and Kohn, 2009). In the case of isolates of *S. trifoliorum* the difference was adenine (A) and in the case of *S. sclerotiorum*, guanine (G).

Njambere et al., 2008. Plant Dis. 92: 917–922; Abd-Elmagid et al., 2013. J Microbiol. Meth. 92: 293–300; Andrew and Kohn, 2009. Appl. Environ. Microbiol. 17: 5600–5606.

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