

MOLECULAR IDENTIFICATION AND FUNGICIDE TOLERANCE OF RHIZOCTONIA FROM TURFGRASSES

Bimal S. Amaradasa¹, Dilip K. Lakshman^{2*} and Brandon Horvath³. ¹Dept. Plant Pathology, Physiology and Weed Science, Virginia Tech, Blacksburg, VA, ²Floral & Nursery Plants Research Unit, Beltsville Agricultural Research Center, Beltsville, Maryland, ³Dept. of Plant Sciences, Univ. of Tennessee, Knoxville, TN.

Patch diseases, caused by a plethora of genera, species and subgenomic groups of *Rhizoctonia*, are considered one of the most severe problems of turf management. The patch syndromes are variously named as brown patch (BP), large patch (LP), yellow patch (YP), brown ring patch (BRP), and leaf and sheath spots (LSP). BP is caused by multinucleate *R. solani* (Tel: *Thanatephorus cucumeris*) isolates. Anastomosis groups (AG) 1, 2, 3, 4, 5 and 6 have been isolated from blighted grasses. LP is caused by *R. solani* AG2-2LP. LSP is caused by multinucleate *R. oryzae* and *R. zaeae* (Tel: *Waitea circinata* var *oryzae/zaeae*), whereas BRP is caused by a newly reported species *W. circinata* var *circinata* (Wcc). YP is caused by the binucleate *R. cerealis* (Tel: *Ceratobasidium cereale*). Various *Rhizoctonia*-like fungi (RLF) of patch diseases are also reported. *Rhizoctonia* isolates are known to differ in sensitivity to fungicides and virulence among various grass cultivars, resulting in inconsistent management of patch diseases. For better identification, we have compared the conventional procedures (i.e., anastomosis grouping, morphology) with more reliable molecular techniques like Internally Transcribed Spacer-PCR (ITS-PCR) as well as the Universally Primed PCR (UP-PCR) analysis and UP-PCR cross hybridization assays on randomly selected field isolates of *Rhizoctonia* from several brown patch affected golf courses and lawns in Virginia and Maryland. The respective AGs were determined, ITS-PCR product sequencing and homology were conducted, and the genomic fingerprinting and cross-hybridization patterns were evaluated. Of the 54 isolates analyzed for rDNA-ITS sequence polymorphism, 33 (61%) isolates belong to multinucleate *R. solani*, 9 isolates (17%) belong to binucleate RLF and 12 isolates (22%) belong to multinucleate *R. zaeae*/ Wcc. Among the 33 identified *R. solani* isolates, 20, 12 and 1 belonged to AG2-2IIIB, AG 1-1B and AG-5, respectively. The cladistic analysis of UP-PCR genome fingerprints supported seven clades including 3 clades of *R. solani* (AG1-1B, AG2-2IIIB and AG5), 1 clade of binucleate RLF and 3 clades of *Waitea circinata* (varieties *zaeae*, *oryzae* and *circinata*). The UP-PCR and ITS DNA analyses corresponded well with traditional AGs by grouping isolates of similar AGs together. In the UP-PCR cross hybridization analysis, genetically related isolates belonging to the same AG subgroups cross hybridized strongly while isolates of different AGs gave weak or no signal. These results confirmed that the UP-PCR and cross hybridization can assist in determining accurate species distinction of *T. cucumeris*, *W. circinata* and *C. cereale*. We are testing the sensitivity of selected molecularly identified *Rhizoctonia* isolates to three routinely

used fungicides to choose appropriate fungicides in turf disease management practices.