

## EFFECT OF SUBLETHAL FUNGICIDE EXPOSURE ON GENOMIC VARIATION IN *Sclerotinia sclerotiorum*

S.E. EVERHART<sup>1</sup>; N.K. GAMBHIR<sup>1</sup>; Z.N. KAMVAR<sup>1</sup>. <sup>1</sup>University of Nebraska, Lincoln, NE, USA. E-mail: everhart@unl.edu

Emergence of novel genetic variants is dependent on mutation rates. Exogenous stress is known to increase mutational frequencies; however, little is known about the role of sublethal exposure to fungicides as a mechanism for mutation and subsequent adaptation. Our work sought to examine the effect of sub-lethal fungicide exposure on the genome of *Sclerotinia sclerotiorum*. Eight wild-type isolates were exposed to five fungicides with different modes of action: boscalid, iprodione, thiophanate methyl, azoxystrobin and pyraclostrobin. Mycelium was grown on a logarithmic fungicide gradient and sub-cultured from the 50-100% inhibition zone for 12 consecutive generations. A selection of fungicide-exposed isolates (n=17) showing mutations at SSR loci were selected for further analysis, in addition to pre-exposure (n=8) and non-exposed control isolates after 12 generations on PDA (n=8). Amplified fragment length polymorphism (AFLP) analysis was performed using three primers and resulted in a set of 602 polymorphic alleles. Cluster analysis with PCoA and DAPC showed fungicide-treated isolates formed a distinct group from pre- and non-exposed control isolates (PhiPT=0.151,  $P=0.001$ ). Whole-genome sequence data were obtained for a selection of 5 isolates and their corresponding treated isolates. Preliminary analysis of genomes from isolates exposed to Thiophanate Methyl showed 317 indels and 157 SNPs. Most variants (405) were in intergenic regions and 69 were in genic regions, all coding hypothetical proteins. No mutations were identified in the  $\beta$ -tubulin gene (target of MBC fungicides). This is the first study to characterize genomic alterations of a plant pathogen after exposure to a sublethal fungicide. Future analysis will determine if sublethal fungicide stress leads to random or directional mutations.

**Keywords:** Whole-genome sequencing; Mutagenesis; Pathogen evolution.