

DYER*, ALAN T. and CAROL E. WINDELS, Univ. Minnesota, NW Res. & Outreach Center, Crookston 56716. **Methods for assessing viability of *Aphanomyces cochlioides* oospores.**

Our objective was to identify a method for determining viability of *A. cochlioides* oospores. Oospores were exposed to lethal stresses (35% ethanol for 24 h, boiling water for 20 min, or 0.5% NaOCl 24 h) or were untreated and then assessed for viability by: plasmolysis in 4N NaCl (PLA), tetrazolium bromide stain (TET, 0.1% MTT, pH=6.5, 35°C, 48 h), or microscopic inspection for densely organized, uniform granules (DOUG, typical appearance of oospores). Oospores that were exposed to ethanol, boiling water or untreated and then assessed by PLA resulted in 3, 9 and 85% viability, respectively (viable: cytoplasm detaches from oospore wall). Oospores that were exposed to ethanol, boiling water or untreated and then assessed by TET resulted in 15, 8 and 16% viability, respectively (viable: oospores stain rose or lavender). Walls of oospores treated with NaOCl and then exposed to PLA or TET were distorted and not assessed. Oospores that were exposed to ethanol, boiling water, NaOCl or untreated and then examined for DOUG resulted in 0, 0, 11 and 84% viability, respectively. Oospores without DOUG lacked granules or contained loosely organized, non-uniform granules (LONG) and were assumed to be nonviable. The relationship between number of viable oospores and disease was determined by inoculating a non-pasteurized greenhouse soil mix with a total of ~30 oospores/g in each of seven mixtures of DOUG (viable) + LONG (nonviable) oospores. Mixtures included ~2, 4, 5, 8, 11, 14, 17 DOUG oospores /g soil, with the remainder composed of LONG oospores; the control was uninoculated. Soils were sown with beet seed and kept moist at 25°C for 3 wk. Regression analysis revealed a significant relationship ($r^2=0.61$, $P<0.001$) between number of DOUG oospores and disease severity. Thus, inspection of oospores for densely organized, uniform granules was a fast and easy test compared to PLA or TET and was verified as a reliable indicator of pathogenicity.