GENETIC ANALYSIS OF PYRENOPEZIZA BRASSICAE, CAUSE OF LIGHT LEAF SPOT OF BRASSICAS, IN THE EUROPEAN UNION, OCEANIA, AND NORTH AMERICA

S. M. Carmody1, K. M. King2, B. J. Claassen3, B. A. Fraaije2, J. S. West2, C. M. Ocamb3, and L. J. du Toit1

1Washington State University, Mount Vernon, WA, USA; 2Rothamsted Research, Harpenden, United Kingdom; 3Oregon State University, Corvallis, OR, USA.
Email: kevin.king@rothamsted.ac.uk / dutoit@wsu.edu

ABSTRACT (APS annual meeting, San Antonio, TX, USA, 5-9 Aug. 2017)
Light leaf spot (LLS), caused by Pyrenopeziza brassicae, is an important disease of Brassica napus (canola and oilseed rape) and B. oleracea (vegetable brassicas) in Europe (EU) as well as New Zealand and Australia (Oceania, OC). LLS was first reported in North America (NA) on B. juncea, B. napus, and B. rapa in six counties in western Oregon in 2014; and on B. juncea cover crops and wild B. rapa in three counties in northwestern Washington in 2016. Multi-locus sequence analysis (ITS ribosomal DNA, beta-tubulin, and elongation factor 1-alpha sequences) and comparison of the mating type genes (MAT1-1 and MAT1-2) grouped isolates from the EU (n = 28) and NA (n = 16) with the P. brassicae type specimen, IMI 204290, whereas isolates from NA (n = 16) represented a novel genotype. Sexual compatibility of NA and EU strains of complementary MAT1-1 and MAT1-2 genotypes is being determined to assess if NA isolates represent a distinct evolutionary lineage or a cryptic sibling species. Fungicide resistance has been documented in some EU populations of P. brassicae, but none of the NA isolates possessed amino acid substitutions E198A and L240F in the beta-tubulin sequences that confer resistance to benzimidazole fungicides; comparison of these sequences for the NA isolates revealed 100% identity to wild type EU P. brassicae isolates and the closely related fungus Rhynchosporium commune; and 98 and 99% identities to Sclerotinia sclerotiorum and Venturia inaequalis, respectively.

RESULTS
- **Molecular comparison**: North American isolates of both mating types were detected. MLSA and phylogenetic analyses of the ITS rDNA, beta-tubulin gene, and TEF 1-alpha gene sequences; as well as MAT1-1 and MAT1-2 sequences, grouped isolates from Europe and Oceania with P. brassicae. Isolates from North America formed a distinct clade (Fig. 2). Clades were not associated with the original Brassica species.
- **Biological analysis**: European isolates of opposite mating type were sexually compatible (formed apothecia and ascospores). North American isolates were not sexually compatible with isolates of opposite mating type from Europe or North America, or with isolates of the same mating type from North America.
- **Pathogenicity test**: European and North American isolates caused different symptoms and signs on inoculated B. rapa plants (Fig. 1).
- **Fungicide-sensitivity test**: North American isolates were sensitive to carbendazim, unlike some European isolates (Fig. 3). None of the North American isolates possessed amino acid substitutions E198A and L240F that confer resistance to carbendazim.

CONCLUSIONS
- Molecular, biological, and pathogenicity comparisons indicated isolates of Pyrenopeziza associated with LLS outbreaks in North America are a different species than P. brassicae, the LLS pathogen in Europe and Oceania.
- The species Pyrenopeziza cascadia is proposed for the North American isolates based on the geographic ecoregion (Cascade Mountains) where the isolates were found.

INTRODUCTION
- Pyrenopeziza brassicae causes light leaf spot (LLS) on many Brassicaceae genera and species (Fig. 1, left) (Rawlinson et al. 1978).
- LLS has been documented in Europe and Oceania for >80 years, particularly on Brassica napus and B. oleracea crops (Karolewski et al. 2010).
- LLS was first found in North America in 2014 on various Brassicaceae crops and weeds in the Willamette Valley of Oregon, where the disease has become widespread (Ocamb et al. 2015). LLS was detected in northwestern Washington in 2015 on B. juncea crops and B. rapa weeds (Carmody et al. 2016).
- **Objective**: Compare fungal isolates associated with LLS in Europe, Oceania, and North America using molecular, sexual compatibility, pathogenicity, and fungicide sensitivity tests.

METHODS
- LLS isolates from Europe (n = 28), North America (16-20), and Oceania (4) were compared (number of isolates varied depending on the test):
  1. **Molecular comparison**: Multilocus sequence analysis (MLSA) of the ITS rDNA, beta-tubulin gene, and translation elongation factor 1-alpha gene (TEF1-alpha); and phylogenetic analyses of mating type genes MAT1-1 and MAT1-2 (Foster et al. 2002).
  2. **Biological analysis**: In vitro sexual compatibility test of isolates of the two mating types from Europe and North America (test for heterothallism and homothallism).
  3. **Pathogenicity test**: On turnip (B. rapa) plants in a growth chamber.
  4. **Fungicide-sensitivity test**: Sensitivity of isolates to the fungicide carbendazim by agar plating and testing for amino acid substitutions E198A and L240F in the beta-tubulin gene sequence (Carter et al. 2013).

**SELECT REFERENCES**
Ocamb et al. 2015. Phytopathology 105:S4.103 (Abstr.)