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

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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-α sequences) and comparison of the mating type genes (*MAT1-1* and *MAT1-2*) grouped isolates from the EU (n = 28) and OC (n = 4) 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.

Fig. 1. Light leaf spot symptoms on turnip (*Brassica rapa*) plants inoculated in a growth chamber. European *Pyrenopeziza brassicae* isolates produced white conidiomata on leaves without distinct lesions (left, inset for close-up of conidiomata). North American isolates caused distinct yellow lesions with necrotic centers and numerous acervuli (right).

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 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, β-tubulin gene, and translation elongation factor 1-α gene (TEF1-α); 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 β-tubulin gene sequence (Carter et al. 2013).

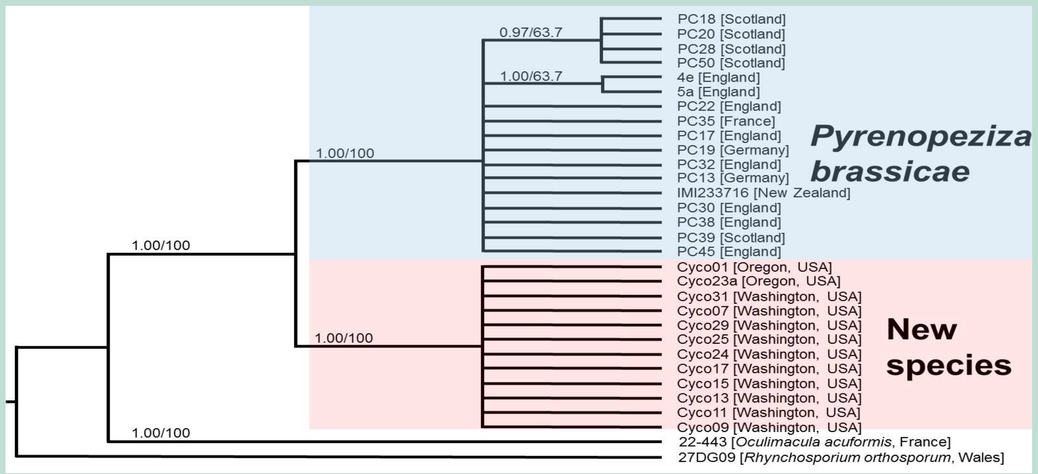

Fig. 2. Phylogenetic tree based on multilocus sequence analysis of the ITS rDNA, β-tubulin gene, and TEF 1-α gene of *Pyrenopeziza brassicae* isolates from Europe and Oceania (blue shading), and isolates associated with LLS in North America (pink shading).

RESULTS

- **Molecular comparison:** North American isolates of both mating types were detected. MLSA and phylogenetic analyses of the ITS rDNA, β-tubulin gene, and TEF 1-α 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.

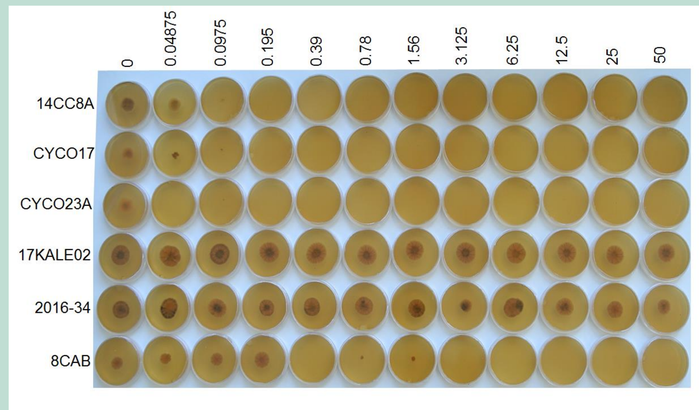

Fig. 3. Sensitivity of isolates of the brassica LLS pathogen to the fungicide carbendazim. North American isolates (top 3 rows) and European isolates (lower 3 rows) were plated on malt extract agar amended with carbendazim at concentrations ranging from 0 to 50 ppm.

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.

SELECT REFERENCES

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