Phylogenetic relationships among isolates of *Uromyces transversalis* from *Gladiolus ×hortulanus* based on ITS sequence data

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**Background**

Gladiolus rust caused by *Uromyces transversalis* is considered a pest of quarantine significance in the United States on cultivated gladiolus plants (*Gladiolus ×hortulanus*), which are grown primarily as a cut-flower crop but also in landscapes.

Recent introductions of the pathogen into California and Florida have resulted in the implementation of quarantine and eradication measures at great cost to commercial growers. This pathogen is established in several gladiolus-exporting countries, including Mexico, which are potential avenues for the fungus to move into the U.S.

Symptoms of the disease are small, yellowish- or yellow-orange colored pustules which develop on either side of the foliage or, less commonly, appear on flowers. Pustules tend to form in transverse lines across the foliage, which is one character used for visual identification in the field (Fig. 1).

**Objective**

The objective of this study was to genetically infer the putative origin of *U. transversalis* in the U.S. by generating DNA sequence data for the internal transcribed spacer (ITS1, 5.8S gene and ITS2) region of the ribosomal DNA (rDNA) for isolates from different geographical locations and determining their phylogenetic relationships.

**rDNA-ITS amplification, cloning, and sequencing**

Twenty-seven isolates of *U. transversalis* were collected from commercial fields in Mexico in 2010-11 for comparison to isolates or specimens from the U.S. (five), South Africa (one), New Zealand (one), and Australia (two) (Table 1). To amplify the rDNA-ITS regions, a nested PCR strategy was used (Fig. 2). A first round amplification reaction was performed with the universal primer ITS5 (5'-GGTTAACCAAGGCTGTAACAAAG-3') and a rust-specific primer, Rustl (5'-GCTTACTGCTCTCCTCAATC-3'). Following this first PCR, a second round PCR was conducted to obtain the ITS region (including 5.8S rDNA) by using the universal primers ITS4 (5'-TCTTCCGCTTAATTGATATGC-3') and ITS5 (White et al 1990). Amplified products were purified, cloned, and sequenced.

**Phylogenetic analysis**

Preliminary phylogenetic (neighbor-joining tree) (Figure 3) analysis revealed that all the isolates clustered into 3 different groups regardless of their geographic origin. Mexican isolates were distributed throughout the trees indicating some level of intraspecific variation within this population. rDNA-ITS sequences from the U.S. isolates and those from isolates from the other different continents were similar with sequences of Mexican isolates, and clustered together in the trees.

**Conclusions**

- These genetic similarities evidenced here underline the disseminations of *U. transversalis* through the inter-country and intercontinental trade of contaminated gladiolus plants.
- The extensive ITS sequencing, in addition to resolving phylogenetic relationship among isolates, also showed informative sites where ITS primers specific to *U. transversalis* could be designed that would provide mycologists and microbiologists efficient diagnostic molecular tools to quickly and accurately identify this quarantine-significant pathogen.

**References**