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Development of a PCR assay for detection of the geranium rust fungus *Puccinia pelargonii-zonalis*

E.A.Scocco[1], S.N. Jeffers[2], R. Walcott[1], J.W. Buck[1]
[1]University of Georgia, Dept. of Plant Pathology, Griffin, GA 30223. [2]Clemson University, Department of Entomology, Soils, & Plant Sciences, Clemson, SC 29634

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BACKGROUND

Geranium rust caused by the fungus *Puccinia pelargonii-zonalis* affects zonal geraniums (*Pelargonium ×hortorum*). Symptoms start as yellowish-white spots on the upper leaf surface followed by the production of dark brown lesions on the under surfaces of the leaves after 10-14 days (Figs 1,2)

Fig. 1 – Geranium rust lesions on upper surface of a leaf

As the disease progresses, concentric rings of lesions are produced (Fig. 2).

Geranium rust was first reported in the U.S. in 1967. The pathogen is now endemic in California and outbreaks occasionally occur in the southeast U.S. Geranium rust is considered a quarantine pathogen in Ohio. Many popular geranium cultivars are susceptible to the rust and outbreaks can result in large financial losses to growers since rust-infected plants are not marketable and must be destroyed.

Fig. 2 – Concentric rings of rust lesions on the underside of a leaf

Currently, geranium rust can only be detected based on the presence of disease symptoms. Pre-symptomatic stages of the disease and plants simply exposed to rust spores are not easily detectable.

Our objective was to development a real-time PCR assay for *P. pelargonii-zonalis*. The use of a molecular tool allows for a more rapid and precise confirmation of the fungus on propagative materials.

MATERIALS AND METHODS

DNA was extracted from urediniospores (Fig. 3) of three isolates of *P. pelargonii-zonalis* (Georgia, South Carolina, California). The internal transcribed spacer (ITS) region was amplified using the basidiomycete-specific primer pair ITS1-F and ITS6R2. The 427 bp purified product was cloned into pCR®8/GW/TOPO vector using a TOPO TA Cloning Kit and transformed into TOPO 10 chemically competent *E. coli* per manufacturer's instructions. Plasmid DNA from 10 clones per rust isolate was extracted and sequenced. Sequences were aligned using ClustalW and a consensus sequence was used in a BLAST search of the NCBI nucleotide

database. The primer pair GRF (5' – TTA TAC TTG TGT TGA TTC – 3') and GRust-R2 (5' – TGT CTT TTT TAT AAG TGC – 3') was designed for specific detection of *P. pelargonii-zonalis*.

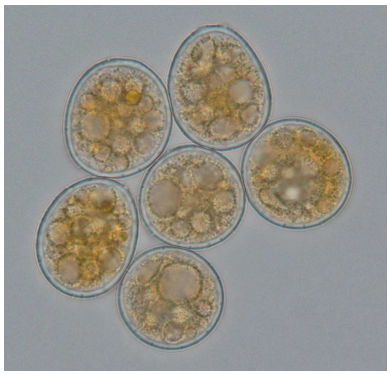

Fig. 3 – Urediniospores of *P. pelargonii-zonalis*

To ensure that the primer pair only amplified the ITS region of *P. pelargonii-zonalis,* we tested them against DNA of 12 other rust fungi and four additional plant pathogenic fungi: *Puccinia sorghi, P. helianthus, P. oxalis*, *P. hemerocallidis*, *P. antirrhini*, *P. iridis*, *P. striiformis*, *P. triticina, Phakopsora pachyrhizi, Pucciniastrum vaccinii, Uromyces phaseoli,* Aster rust, *Ustilago* sp., *Botrytis cinerea*, *Rhizoctonia* sp., *Fusarium* sp.

The sensitivity of real-time PCR was tested by using a ten-fold serial dilution of *P.pelargonii-zonalis* urediniospores (10^5 to 10^1 urediniospores/ml). Each dilution was subjected to bead-beating to liberate spore contents, DNA extraction, and analyzed by real-time PCR using our primer pair.

RESULTS AND CONCLUSIONS

The primer pair GRF and GRust-R2 amplified a 131-bp product from the three isolates of *P. pelargonii-zonalis* (black arrow; Fig 4) and did not amplify a product from the 12 different rust fungi and four additional fungi (white arrow; Fig 4). This suggests that our primers are specific for geranium rust.

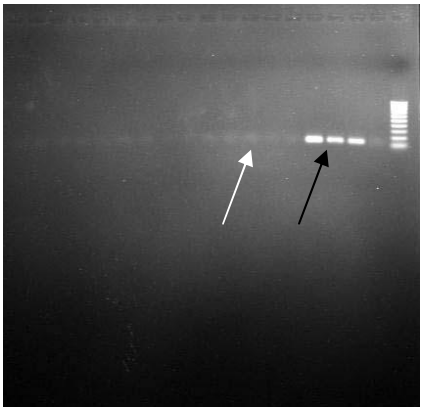

Fig. 4 – Amplification of *P. pelargonii-zonalis* using primer pair GRF and GRust-R2 (black arrow). Right lane: 100 bp ladder.

Primer sensitivity was tested by amplifying 1 ng, 100 pg, 10 pg, 1 pg, and 100 fg concentrations of *P. pelargonii-zonalis* DNA in both conventional and real-time PCR. *P. pelargonii-zonalis* DNA was amplified consistently at 1 ng and 100 pg concentrations in conventional PCR and at 1 pg using real-time PCR. The lowest detection threshold for real-time PCR was 10^2 urediniospores/mL.

The primer pair GRF and GRust-R2 specifically amplify the ITS region of *P. pelargonii-zonalis*.

IMPACT TO THE INDUSTRY

The ability to effectively manage geranium rust in greenhouses is largely dictated by when the problem is discovered. We have observed that a single rust lesion can produce 1,500 urediniospores per day. Thus, even moderately infected plants have the ability to produce tens of thousands of spores. If diseased plants are present in a greenhouse operation, it is almost certain that healthy plants have been infested. Use of molecular tools such as the PCR assay that we have developed for geranium rust will allow for earlier detection and the ability for growers to proactively manage the disease.

For additional information, contact jwbuck@uga.edu.