

Genome editing of filamentous fungi using CRISPR-Cas9 technology has increased in recent years. There are few reports about CRISPR-engineered filamentous fungi related to biocontrol and crop disease. Our goal was to use this technique, as proof of concept of its feasibility, to edit the genome of a *Trichoderma afro-harzianum* and a *T. gamsii* isolate, well known as biocontrol and biostimulating fungi, as well as in a mycotoxigenic *Fusarium graminearum* isolate, the causal agent of Fusarium Head Blight (FHB). A gene encoding a polyketide-synthase, disruption of which can be easily detected phenotypically, was chosen as the target gene in all the three isolates, and used to design the RNA-guide to be included in the RGR-cassette. The cassette was then assembled in a Cas9 expressing plasmid. The resulting vector will be used for fungal transformation by protoplasts. Resulting mutants from all the three fungi will be phenotypically and molecularly analyzed, to verify the knockout of the selected gene. The presence of a shortened AMA1 sequence will allow rapid removal of the plasmid from the edited strains, simply by reducing the selection pressure. Edited strains will be checked for the presence of foreign DNA, to contribute to the debate about the inclusion of this type of genetically manipulated microorganisms within GMOs. The ability to manipulate, beneficial and plant pathogenic isolates at a genetic level with these techniques represents a tool to increase knowledge of how these fungi interact with their hosts.

**Spore trapping and quantitative PCR for monitoring airborne inoculum of *Mycosphaerella nawae* in persimmon.** M. BERBEGAL<sup>1</sup>, J.L. MIRA<sup>2</sup>, J. ARMENGOL<sup>1</sup>, A. VICENT<sup>2</sup>. <sup>1</sup>Instituto Agroforestal Mediterráneo, Universitat Politècnica de València. 46022, Valencia, Spain. <sup>2</sup>Centro de Protección Vegetal y Biotecnología, Instituto Valenciano de Investigaciones Agrarias (IVIA). Moncada 46113, Valencia, Spain. E-mail: mobermar@etsia.upv.es

Circular leaf spot of persimmon, caused by *Mycosphaerella nawae*, includes symptoms of necrotic leaf lesions, defoliation and fruit drop. The disease is widespread in humid regions in Japan and South Korea, and, more recently, also in Mediterranean areas in Spain. The pathogen reproduces in leaf litter through ascospores formed in pseudothecia. Fungicide sprays are scheduled based on ascospore moni-

toring to define the periods of inoculum availability. Airborne ascospores of *M. nawae* are routinely quantified by counting using microscopy. This technique is time-consuming, especially for field sampling for rapid decision making. Monitoring airborne inoculum using spore traps combined with real-time PCR assays for quantification can be rapid, specific, reproducible and reliable. A real-time PCR assay for *M. nawae* quantification (qPCR) was designed and evaluated under laboratory conditions. To validate the technique under field conditions, two Burkard volumetric spore traps were deployed in a 100 m<sup>2</sup> plot. Soil was covered with persimmon leaf litter severely affected by *M. nawae*, and overhead sprinkle irrigation was used to enhance ascospore release. The spore traps were operated during May to July in 2016. Tapes from both spore traps were changed weekly, one was used for microscope counting and the other for qPCR analyses. Ascospore counts were correlated against DNA concentration of *M. nawae* based on Ct qPCR values. Results indicate that monitoring of *M. nawae* ascospores by qPCR may be a more efficient alternative to conventional inoculum counting, based on microscope examination.

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**Root colonization of host (*Cucumis sativus*) and non-host (*Solanum lycopersicum*) species by a DsRed-fluorescent strain of the specific pathogen *Fusarium oxysporum* f. sp. *radicis-cucumerinum*.** M. DE CARA-GARCÍA<sup>1</sup>, C. LECOMTE<sup>2</sup>, M. FERNÁNDEZ-PLAZA<sup>1</sup>, L. MUELA-JORDÁN<sup>1</sup>, A. BOIX-RUIZ<sup>3</sup>, C. STEINBERG<sup>2</sup>. <sup>1</sup>IFAPA Centro La Mojonera, Camino de San Nicolás, 1, 04745, La Mojonera, Spain. <sup>2</sup>I.N.R.A. UMR Agroécologie, Rue Sully, 17, 21065, Dijon, France. <sup>3</sup>University of Almería. Dept. Agronomía, Ctra. Sacramento s/n., 04120, Almería, Spain. E-mail: franciscom.cara@juntadeandalucia.es

A monoconidial *Fusarium oxysporum* isolate (codified as 14/1Fo3), originally collected from sporodochia of a diseased cucumber plant showing root and stem rot, was identified as *F. oxysporum* f. sp. *radicis-cucumerinum*. The isolate was transformed by *Agrobacterium tumefaciens*, by means of 'EHA 105-DsRed2' strain containing the binary vector pAN-DsRed2, carrying red fluorescent insert *DsRed2*, and the *hgh* gene.