Screening for biologicals to control *Rumex lunaria* in Timanfaya National Park

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**INTRODUCTION**

*Rumex lunaria*, a Polygonaceae shrub, has been colonizing the Timanfaya National Park, in Lanzarote (Canary Islands), from the 1980s to the present day, revealing a behavior as an invasive species. For a couple of years, the appearance of leaf spots, the lack of turgidity in the trunks and other symptoms of disease have been observed in the specimens, which led to collecting samples of different parts of plants from several areas of the National Park and Lanzarote Island for being analyzed. Our goal is the optimization of potentially biological control agents to be used in a strategy to control *Rumex lunaria* in Timanfaya National Park, and our objective is the detection and selection of fungal strains with phytopathogenic traits isolated from plants with disease symptoms using different screening techniques.

**METHODS**

To isolate the potential fungi causing disease symptoms, samples were processed using mycological classical techniques. Identification of fungi was based on morphological and molecular analysis using DNA fungal barcode ITS (ITS1 and ITS4 primer pair to amplify the 5.8S rDNA and the two internal transcribed spacers ITS1 and ITS2).

Five screening techniques were designed to select the most pathogenic strain: method 1 — both sides of the midrib of a cut leaf were inoculated with spores solution; method 2 — a branch of 5 or 6 leaves maintained in water was sprayed with spores solution; method 3 — the aerial part of a seedling was sprayed with spores solution; method 4 — the aerial parts of adult plants were partially sprayed with spores solution; method 5 — the aerial parts of adult plants were entirely sprayed with spores solution. Several scales for symptoms were developed according to the identity of the strains and their effects on tissue morphology, symptoms, necrosis. In addition, the different size of leaves spots produced by the strains with phytopathogenic traits were recorded.

We performed a linear mixed effects analysis to determine the effect of the different strains on leaf spot size (represented as a scale). As fixed effects, we entered strain and time (without interaction term) into the model. As random effects, we had intercept for leaves. To determine differences among treatments, we used Tukey post-hoc test. In all analysis, level of significance was set to 0.05. The statistical analyses were performed with R (RCoreTeam, 2020).

**RESULTS & DISCUSSION**

Of 100 fungal isolated strains, twenty-six genera were identified, with *Alternaria* and *Penicillium* being the most frequent. In the last screening with entire leaves on adult plants, the strain identity and the factor time showed an effect on the leaf spots size, *Alternaria tenuissima* being the strain with the highest leaf spot size values along the time (Tukey 4th: 0.05). The three strains caused larger leaf spots after the second monitoring (6 days). Afterwards, the spots size did not change in relation to any of the strains.

**CONCLUSIONS**

This study contributes to the knowledge on the fungal communities of *Rumex lunaria*. It supports the conservation and management of the singular volcanic landscape and the natural ecosystem of the Timanfaya National Park focusing on the search of the fungal phytopathogenic traits that may become biological control agents of this invasive plant species.

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