

SERIOUS DAMAGE BY *DIPLODIA AFRICANA* ON *PINUS PINEA* IN THE VESUVIUS NATIONAL PARK (CAMPANIA REGION, SOUTHERN ITALY)

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In some municipalities, located within the boundaries of the Vesuvius National Park (Campania Region, southern Italy), several area with forest cover of *Pinus pinea* showed severe withering of the crowns and damage to pine cones. In the present study, we have isolated in the period May 2013 – May 2014 from Ercolano, San Sebastiano, Terzigno, Torre del Greco and Trecase an anamorphic form of *Botryosphaeriaceae*. The latter cause dieback and serious canker on several woody plants, including species of *Pinus*. Morphological and cultural characteristics as well as DNA sequence data (5.8S rDNA, ITS-1 and ITS-4) were made on 30 isolates obtained from 5 municipalities. All strains belonged to only two species: *Botryosphaeria dothidea* and its anamorph, *Diplodia africana*. These two fungi were present on all pine cones collected and analyzed. Finally we carried out growth assays at different temperatures: 8 °C, 18 °C and 28 °C. All fungi found the optimum of growth at 28 °C while at 8 °C we noted the lowest growth. This seems to be the first report of *D. africana* on *Pinus* species in Campania Region.

Keywords: plant pathology, pine, fungi, DNA extraction, *Botryosphaeriaceae*.

Parole chiave: patologia forestale, pino, funghi, estrazione DNA, *Botryosphaeriaceae*.

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1. Introduction

In the last 10 years in some municipalities within the National Park of Vesuvius (Campania Region, southern Italy, (Fig.1) the cone crop for pine nuts was nearly wiped out by serious alterations suffered by *Pinus pinea* forests.

The pine trees showed brown needles on current shoots and great masses of fungal fruiting on pine cones (Fig. 2a and 2b).

These damage shave almost cancelled the production of pine nuts in this area. Commercial pine nuts production has declined up to 20%, leading many dry fruit companies to bankruptcy, with associated job losses.

The main aims of the present study was to detect the pathogens that cause damages to pine cones and branches in *P.pinea* Vesuvius forests.

2. Materials and Methods

We carried out some isolation from pine cone (Fig. 2b) on potato dextrose agar (PDA) (5 g L⁻¹ potato; 20 g L⁻¹ dextrose; 15 g L⁻¹ agar) collected in the period May 2013 – May 2014 from Ercolano, San Sebastiano, Terzigno, Torre del Greco and Trecase municipalities. Then we isolated and identified (using morphological analysis and molecular identification by DNA extraction with NucleoSpin® Plant II kit) fungal strains.

We performed a molecular diagnosis using PCR amplification of the internal transcribed spacer (ITS) regions of the ribosomal DNA with two specific primers, ITS1 (5'-TCCGTAGGTGAACCTGCGG-3') and ITS4 (5'-GCTGCGTTCTTCATCGATGC-3'). Amplicon sequencing was done with the specified universal primers, via a custom sequencing service (Eurofins MWG Ope-ron, Ebersberg, Germany).

The new sequences were then aligned and compared with the GenBank National Center for Biotechnology Information (NCBI) database.

Finally we carried out growth assays for these fungal strains at different temperatures: 8 °C, 18 °C and 28°C.

3. Results and Discussion

All strains belonged to *Botryosphaeria dothidea* and its anamorph, *Diplodia africana* (Fig. 3).

D. africana was present on all pine cones collected and analyzed.

It is well known that many species in the family *Botryosphaeriaceae* cause violent withering and cancers of many plant, wood and fruit species (Mutke *et al.*, 2012; Mutke *et al.*, 2013).

Austrian pine (*Pinus nigra*) and Stone pine (*P. pinea*) are two of the most severely damaged conifer species in Europe (Mutke *et al.*, 2012; Mutke *et al.*, 2013).

The damage caused by *D. africana* in the study area is most severe on old trees and/or trees growing on shallow volcanic soils. Mechanical injury or damage by herbivorous insects may eventually kill the trees (Mutke *et*

al., 2012; Mutke *et al.*, 2013). All strains found the optimum of growth at 28 °C while at 8 °C we observed the lowest growth.

During the summer, in always more zones of the Mediterranean area, the temperatures are often very high and in future an expansion of the pathogen may occur also in other areas.

4. Conclusions

This seems to be the first report of *D. africana* on *Pinus* species in the Campania Region. Further analyses are in progress to verify the actual damage that this pathogen may carry out on plant tissues of *P. pinea*.



Figure 1. Vesuvius National Park (gray area).



Figure 2. Brown needles on current shoot (a) and great masses of fungal fruiting on pine cone of *P. pinea* (b).

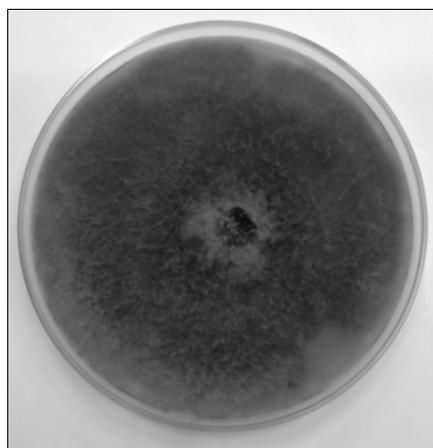


Figure 3. Seven day-old *D. africana* culture on PDA.

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