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# *Phytophthora* diversity in a protected natural area and in a botanical garden

**Mario Riolo[1,2,3†], Federico La Spada[1†], Francesco Aloi[1,4†], Giampietro Giusso del Galdo[5], Elena Santilli[2], Antonella Pane[1*], Santa Olga Cacciola[1*]**

[1] Department of Agriculture, Food and Environment (Di3A), University of Catania, Catania, Italy;

[2] Department of Agriculture, University Mediterranea of Reggio Calabria, Reggio Calabria, Italy;

[3] Council for Agricultural Research and Agricultural Economy Analysis, Research Centre for Olive, Citrus and Tree Fruit-Rende CS (CREA-OFA), Rende, Italy;

[4] Department of Agricultural, Food and Forest Sciences, University of Palermo, 90128 Palermo, Italy;

[5] Department of Biological, Geological and Environmental Sciences, Catania University, 95125 Catania, Italy.

* Correspondence: apane@unict.it; olga.cacciola@unict.it

† These authors contributed equally to the study.

**Abstract:**
Most soilborne *Phytophthora* species are invasive plant pathogens and nursery plants for transplanting are considered a primary pathway for the introduction of exotic *Phytophthora* species into plant diversity conservation sites. As a preliminary contribute to the study of *Phytophthora* populations in plant conservation sites, we compared the diversity of *Phytophthora* in the protected natural area Complesso Speleologico Villasmundo S. Alfio Nature Reserve (NR) (Siracusa) and the botanical garden (BG) of the University of Catania, in eastern Sicily (Italy). Sampling was carried out in spring 2019. Overall 29 rhizosphere soil samples were collected, 17 from different types of vegetation in NR and 12 from different plant species in BG. *Phytophthora* species were recovered from soil samples by leaf baiting and isolation on a selective medium. Isolates were identified by combining morphological features with phylogenetic inferences from ITS-rDNA sequence analysis. Overall 82 *Phytophthora* isolates, 30 from NR and 52 from BG, were characterized. Five *Phytophthora* species, *P. pseudocryptogea*, *P. cryptogea*, *P. bilorbang*, *P. plurivora* and *P. gonapodyides*, were recovered from NR, while only three species, *P. nicotianae*, *P. multivora* and *P. parvispora*, were found in BG. Factors contributing to shape *Phytophthora* populations of rhizosphere soil in these two vegatational contexts are discussed.

**Keywords:** Nature Reserve | Botanical garden | leaf baiting | molecular analysis | ITS-rDNA | morphological characters | monitoring

# Results

**Table 2**. *Phytophthora* spp. recovered from plant rhizosphere and physico-chemical soil properties in samples collected in two different plant diversity conservation sites.

| | | | | Soil properties | | | | |
|---|---|---|---|---|---|---|---|---|
| **Sampling site** | **Rhizosphere soil sample ID.** | **Host** | **Baited *Phytophthora* spp.[a]** | **pH** | **Electrical conductivity at 25°C (µS/cm)** | **Soil texture** | **Nitrates (mg/Kg)** | **Organic matter (%)** |
| Complesso Speleologico Villasmundo S. Alfio Regional Nature Reserve | NR_1903_S1 | *Salix pedicellata* | PSC | 7.6±0.1 | 1497.0 ± 49 | Sandy clay loam | 11.0 ± 1 | 6.5 ± 0.3 |
| | NR_1903_S2 | *S. pedicellata* | CRY | 7.7 ± 0.1 | 938.0 ± 43 | Sandy clay loam | 1.6 ± 0.2 | 2.8 ± 0.1 |
| | NR_1903_S3 | *Platanus orientalis* | - | 7.0 ± 0.1 | 913.0 ± 43 | Sandy clay | 7.1 ± 0.7 | 4.9 ± 0.2 |
| | NR_1903_S4 | *P. orientalis* | BIL | 7.1 ± 0.1 | 1023.0 ± 44 | Sandy clay loam | 6.9 ± 0.7 | 5.4 ± 0.3 |
| | NR_1903_S5 | *Euphorbia dendroides* | - | 7.3 ± 0.1 | 976.0 ± 44 | Sandy clay | 5.9 ± 0.6 | 7.1 ± 0.4 |
| | NR_1903_S6 | *Cynara cardunculus* | - | 7.5 ± 0.1 | 822.0 ± 41 | Sandy clay | 7.3 ± 0.7 | 5.5 ± 0.3 |
| | NR_1903_S7 | *Asphodelus* sp. | - | 7.5 ± 0.1 | 1122.0 ± 45 | Sandy clay | 7.2 ± 0.7 | 5.4 ± 0.3 |
| | NR_1903_S8 | *Quercus ilex* | GON | 7.3 ± 0.1 | 1463.0 ± 48 | Clay loam | 13.0 ± 1 | 13.1 ± 0.7 |
| | NR_1903_S9 | *Q. ilex* | PLU | 7.4 ± 0.1 | 1617.0 ± 53 | Loamy sand | 17.0 ± 2 | 21.0 ± 1 |
| | NR_1903_S10 | *Q. ilex* | - | 7.6 ± 0.1 | 1397.0 ± 46 | Sandy loam | 11.0 ± 1 | 16.3 ± 0.8 |
| | NR_1903_S11 | *Q. pubescens* sensu latu | GON | 7.2 ± 0.1 | 1174.0 ± 45 | Clay loam | 11.0 ± 1 | 11.4 ± 0.6 |
| | NR_1903_S12 | *Sarcopoterium spinosum* | - | 7.2 ± 0.1 | 922.0 ± 42 | Sandy clay | 6.1 ± 0.5 | 5.1 ± 0.2 |
| | NR_1903_S13 | *S. spinosum* | - | 7.3 ± 0.1 | 1102.0 ± 49 | Sandy clay | 7.1 ± 0.7 | 4.2 ± 0.1 |
| | NR_1903_S14 | *Pistacia lentiscus* | - | 7.4 ± 0.1 | 831.0 ± 41 | Sandy clay | 6.7 ± 0.7 | 8.2 ± 0.4 |
| | NR_1903_S15 | *P. lentiscus* + *Pyrus* sp., mixed sample | - | 7.2 ± 0.1 | 856.0 ± 43 | sandy clay loam | 5.3 ± 0.7 | 7.2 ± 0.2 |
| | NR_1903_S16 | *P. lentiscus* | - | 7.3 ± 0.1 | 796.0 ± 41 | Sandy clay | 1.7 ± 0.2 | 7.7 ± 0.4 |
| | NR_1903_S17 | *P. lentiscus* | - | 7.3 ± 0.1 | 1056.0 ± 44 | Sandy clay | 3.6 ± 0.4 | 8.7 ± 0.4 |

[a] BIL = *P. bilorbang*; CRY = *P. cryptogea*; GON = *P. gonapodyides*; MUL = *P. multivora*; NIC = *P. nicotianae*; PLU = *P. plurivora*; PSC = *P. pseudocryptogea*; PAR= *P. parvispora*

# Results

*Con. Table 2.*

| Sampling site | Rhizosphere soil sample ID. | Host | Baited *Phytophthora* spp.[a] | Soil properties: pH | Soil properties: Electrical conductivity at 25°C (µS/cm) | Soil properties: Soil texture | Soil properties: Nitrates (mg/Kg) | Soil properties: Organic matter (%) |
|---|---|---|---|---|---|---|---|---|
| Botanical garden of Catania | BG_1903_S1 | *Eucalyptus citridora* | MUL, NIC | 7.99 ± 0.1 | 877.5 ± 48 | Loamy sand | 145.3 ± 0.4 | 1.07 ± 0.2 |
| | BG_1903_S2 | *Araucaria cooki* | MUL, NIC | 8.19 ± 0.1 | 3437.5 ± 46 | Sandy loam | 1210.9 ± 0.6 | 1.29 ± 0.1 |
| | BG_1903_S3 | *Gravillea robusta* | - | 8.14 ± 0.1 | 852.5 ± 40 | Loamy sand | 145.3 ± 0.6 | 0.86 ± 0.1 |
| | BG_1903_S4 | *Phytolacca dioica* | - | 8.26 ± 0.1 | 997.5 ± 43 | Sandy clay loam | 188.2 ± 0.7 | 1.01 ± 0.1 |
| | BG_1903_S5 | *Pistacia atlantica* | MUL | 7.43 ± 0.1 | 3945.0 ± 45 | Loamy sand | 1076.6 ± 0.1 | 1.49 ± 0.1 |
| | BG_1903_S6 | *Quercus suber* | - | 8.14 ± 0.1 | 765.0 ± 45 | Loamy sand | 45.3 ± 0.2 | 1.48 ± 0.2 |
| | BG_1903_S7 | *Zelkova sicula* | MUL | 8.55 ± 0.1 | 970.0 ± 44 | Sandy clay loam | 103.9 ± 0.8 | 0.73 ± 0.1 |
| | BG_1903_S8 | *Sterculia diversifolia* | MUL, NIC | 8.10 ± 0.1 | 1675.0 ± 48 | Clay loam | 1366.6 ± 1.0 | 0.11 ± 0.05 |
| | BG_1903_S9 | *Mangifera indica* | MUL, NIC | 8.60 ± 0.1 | 765.0 ± 41 | Clay loam | 176.7 ± 0.4 | 1.0 ± 0.1 |
| | BG_1903_S10 | *Olea europaea* | - | 7.10 ± 0.1 | 1540.0 ± 43 | Sandy clay loam | 55.3 ± 0.4 | 0.66 ± 0.1 |
| | BG_1903_S11 | *Pistacia lentiscus* | PAR | 8.64 ± 0.1 | 867.5 ± 46 | Sandy loam | 148.2 ± 0.5 | 0.99 ± 0.1 |
| | BG_1903_S12 | *Coffea arabica* | - | 8.40 ± 0.1 | 677.5 ± 50 | Loamy sand | 41.0 ± 0.3 | 0.82 ± 0.1 |

[a] BIL = *P. bilorbang*; CRY = *P. cryptogea*; GON = *P. gonapodyides*; MUL = *P. multivora*; NIC = *P. nicotianae*; PLU = *P. plurivora*; PSC = *P. pseudocryptogea*; PAR= *P. parvispora*

# Discussion

**Differences in the variability of *Phytophthora* species from both sites:**

- the presence in the restricted area of the botanical garden of the University of Catania of different potential woody host-plants in close proximity to each other could favor the spread and prevalence of invasive as well polyphagous *Phytophthora* species, such as *P. multivora* and *P. nicotianae*.

- in the nature reserve of Villasmundo the presence of different vegetational types and peculiar ecological niches may have favored the diversity of *Phytophthora* community even in a relatively restricted area.

# Discussion

**Effects of different soil properties in the occurrence of *Phytophthora* spp.:**

- The widespread occurrence of *Phytophthora* species in soils with different physico-chemical characteristics in both surveyed sites confirms the ability of these oomycetes to adapt to different environments and thrive in a wide range of ecological conditions

# Conclusions

- Most of the recovered species are aggressive plant pathogens and two of them, in particular, *P. multivora* and *P. nicotianae* are invasive and polyphagous.

- The isolation methods based on leaf baiting, which have the advantage of recovering living and culturable isolates, might have contributed to isolate some species selectively. This limit can be excluded using in parallel detection methods based on next generation sequencing (NGS) technology which are more sensitive to detect *Phytophthora* species in environmental samples and are less influenced by environmental conditions.

- NGS-based methods can help the fine tuning of studies aimed at exploring the complexity of *Phytophthora* communities in different ecosystems and the effects of ecological factors driving the diversity and the structure of these communities.

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