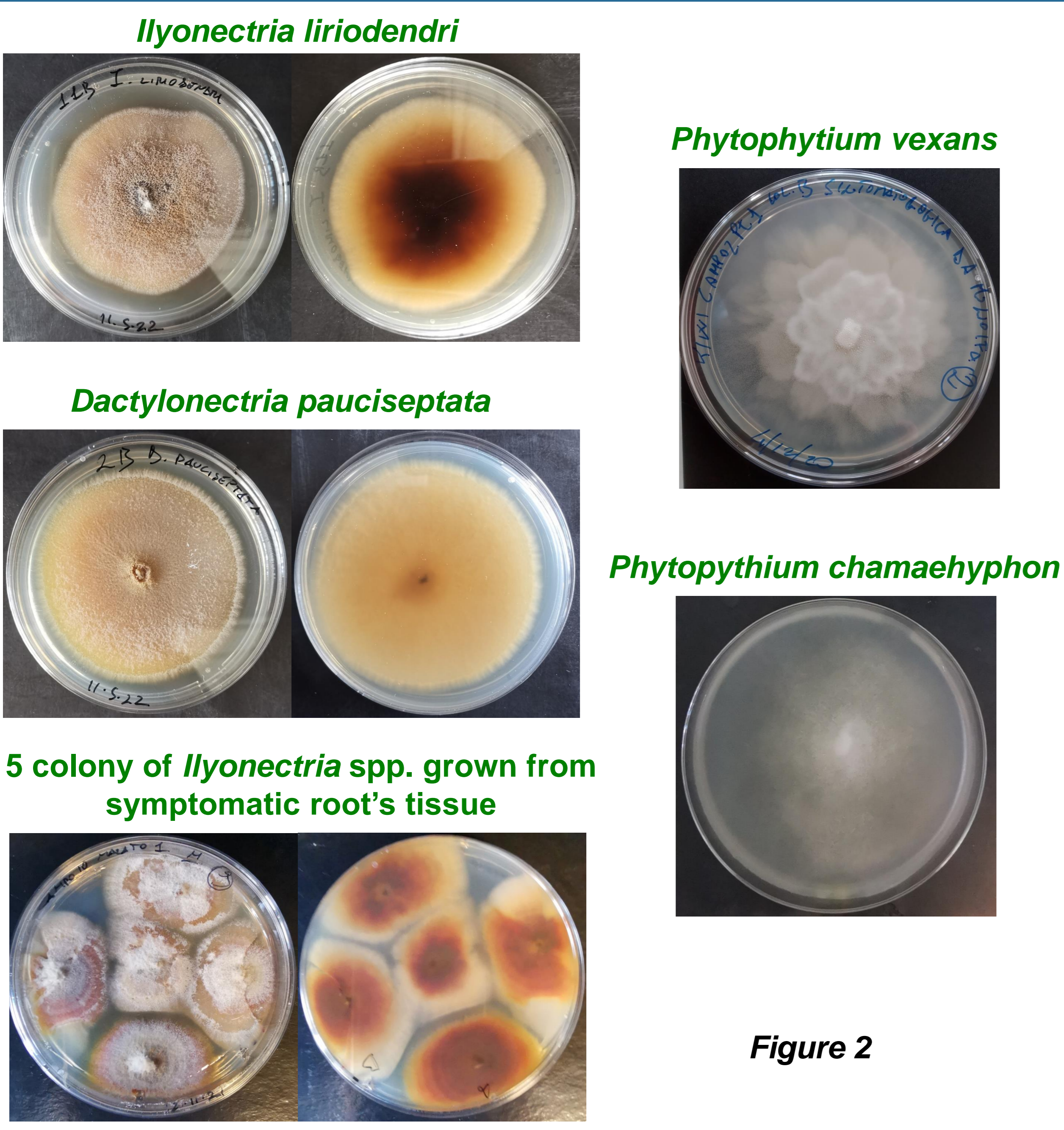
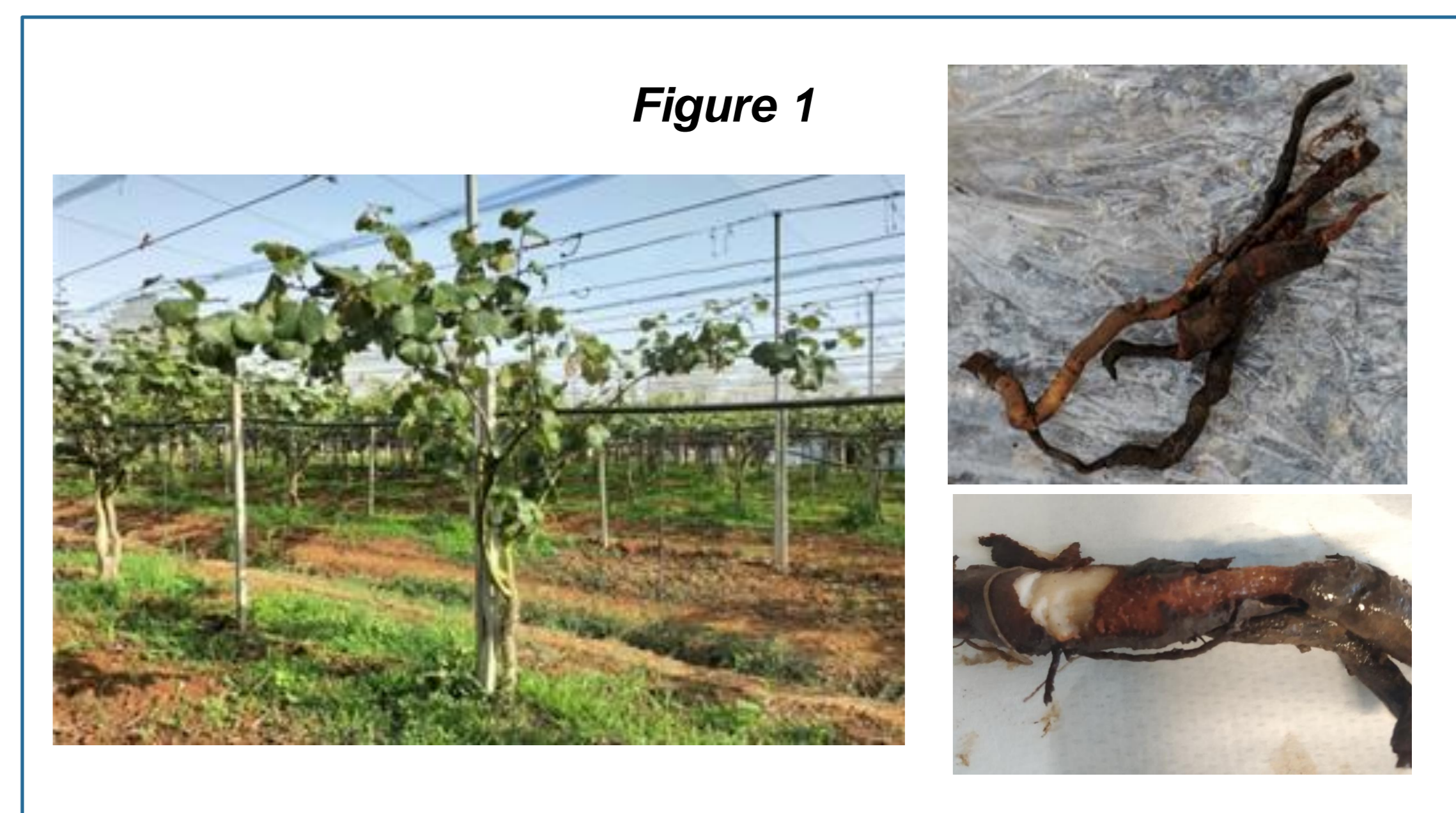


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INTRODUCTION

Kiwifruit Vine Decline Syndrome (KVDS) is a disease that affects *Actinidia chinensis* (kiwifruit) causing plant decline. Symptoms appear as root rot and branch wilting (figure 1). The disease spread out in Lazio (central Italy) in 2017, while in northern Italy appeared as early as 2012 [1, 2]. The disease probably is caused by abiotic [3] and biotic factors [4, 5, 6]. In this study, we aim to investigate the aetiological or associated agents causing the symptoms, using traditional, molecular and Next Generation Sequencing (NGS) approaches.

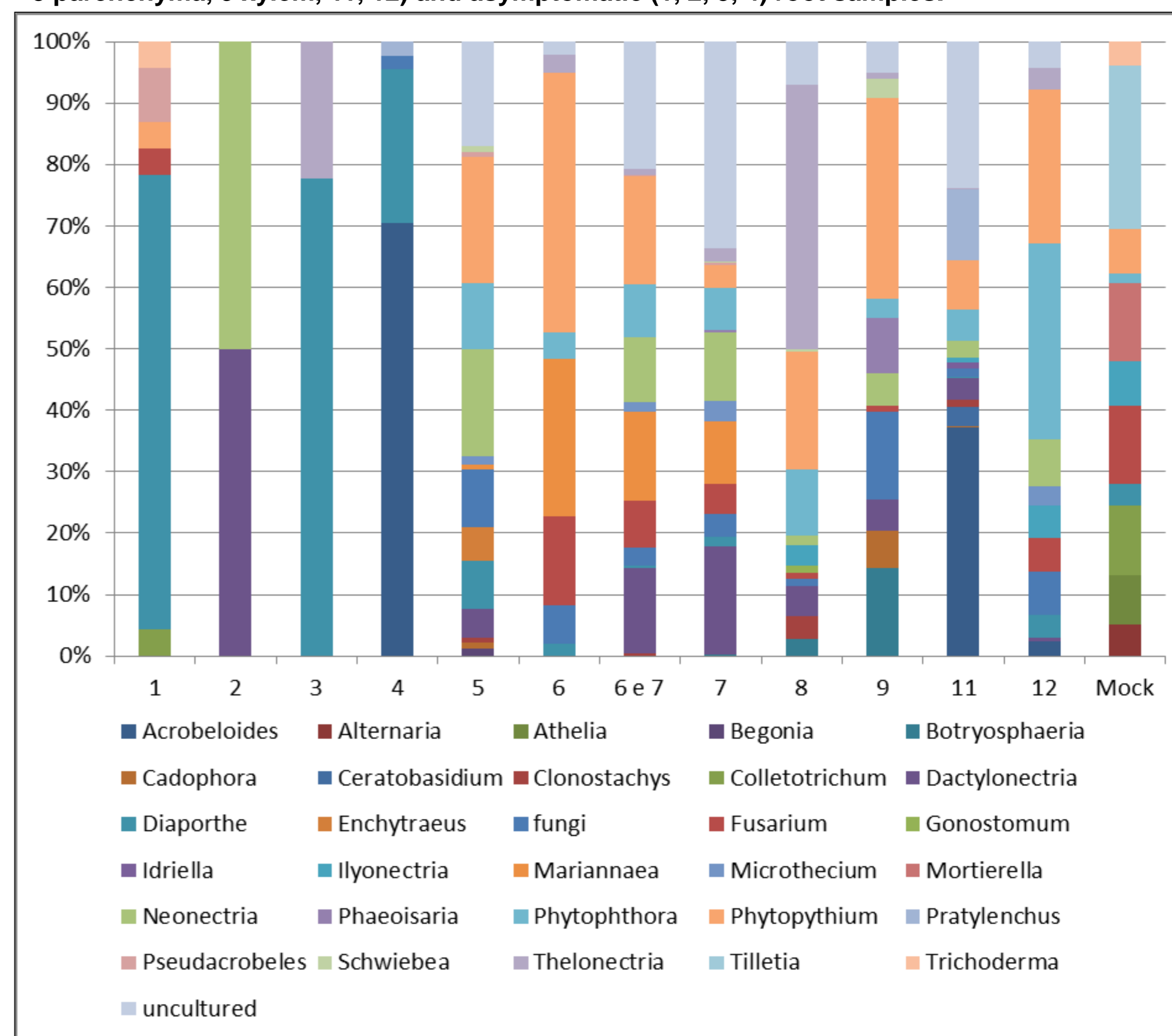


MATERIALS AND METHODS

Root and rhizosphere samples were collected from 20 kiwifruit orchards localized in Lazio region. The samples were analyzed at the CREA-DC laboratories in Rome. The roots were first washed under running water to remove the soil.

Then, samples were obtained from healthy and infected root tissue and grown on two culture media: P5ARP [H] selective for the growth of Oomycetes and PDA for the development of most fungi in plant tissue (figure 2). A method of DNA extraction from roots and soil was developed and a metabarcoding analysis was performed using ITS2 for fungi and oomycetes in order to understand the differences in the microbiome between kiwi plants affected or not by KVDS.

Figure 3: Fungal percentage (at genera level) of the symptomatic (5, 6 xylem, 7 parenchyma, 8 parenchyma, 9 xylem, 11, 12) and asymptomatic (1, 2, 3, 4) root samples.



PRELIMINARY RESULTS AND CONCLUSIONS

Isolation from symptomatic roots revealed the presence of *Cylindrocarpon* spp. and oomycetes such as *Phytophthium* spp. and *Phytophthora* spp. and these were confirmed by Sanger sequencing and by the Illumina sequencing.

Preliminary results show that the DNA extraction method from root and soil samples works properly despite the high polysaccharides content in roots.

Furthermore, NGS sequencing confirms the differences in the microbial community of asymptomatic and symptomatic samples (figure 3). The next step will be to confirm these differences analysing further soil and root samples in order to associate a pool of potential pathogens to the disease and/or a pool of microorganisms to the healthy root and rhizosphere.

References:

- Tosi, L., Tacconi, G., Giacomini, A. (2015). The die-off of kiwi, status and prospects. *Informatore Agrario*, 71(44), 67-70.
- Tacconi, G et al. (2015) Vine decline in kiwifruit: Climate change and effect on waterlogging and *Phytophthora* in North Italy. *Acta Hort.* 2015, 1096, 93-97.
- Bardi, L. (2020). Early Kiwifruit Decline: A Soil-Borne Disease Syndrome or a Climate Change Effect on Plant-Soil Relations. *Front. Agron.* 2: 3. doi: 10.3389/fagro.
- Donati, I. et al. (2020). Pathogens associated to kiwifruit vine decline in Italy. *Agriculture*, 10(4), 119.
- Savian, F. et al. (2020). Studies on the aetiology of kiwifruit decline: interaction between soil-borne pathogens and waterlogging. *Plant and Soil*, 456(1), 113-128.
- Prencipe, et al. (2020). First report of *Phytophthium vexans* causing decline syndrome of *Actinidia deliciosa* 'Hayward' in Italy. *Plant Disease*, 104(7), 2032.

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