Report of IMC12 – Maastricht, the Netherlands

Conference

The 12th International Congress of Mycology was held from 11 to 15 August 2024 in Maastricht, the Netherlands. The congress was attended by 1400 participants from 80 countries. This conference is held every four years, though the previous one was held in 2018. There were 62 sessions and 16 workshops.

Programme

Each morning and afternoon there were six concurrent sessions from which one had to choose. Fungal research over a wide variety of topics was presented.

Symposia topics of relevance to plant pathology were the following:

- Global health and challenges to forests: interactions of fungi, climate change and biological invasions
- Biocontrol mechanisms against fungal plant pathogens
- Powdery mildews and the hosts: co-evolutions, systematics and global biodiversity
- Fungal interactions in the phyllosphere: from molecules to communities
- Effects of changing climate on fungal diseases
- Community of ecology of fungi and of fungi and bacteria
- Mycoviruses unmasked: bridging evolution, interactions and ecology
- The silent menace: exploring mycotoxins as a global one health concern

General topics that stood out included the use of fungal genomes and the use microbiome data in research. The importance of DNA was also stressed with the debate of using DNA as type for lodging new descriptions. The influence of climate change on diseases was highlighted in several sessions.

Participation

I had one oral and two poster presentations:

Mostert L, Halleen F, Havenga M. Fungal diversity and relevance of canker and wood rot pathogens of fruit trees in the Western Cape of South Africa. Oral presentation.

Jacobs V, Halleen F, Stempien E, Mostert L. Presence and viability of *Diplodia seriata*, an important dieback and canker pathogen, on chipped apple wood used for mulching. Poster presentation.

Jacobs S, Wood A, Havenga M, Mostert L. Characterisation of bark and wood canker pathogens of dryland trees indigenous to South Africa, *Vachellia karroo, V. erioloba* and *Senegalia mellifera*. Poster presentation.

A session was held entitled "**Fungal diversity and relevance of canker and dieback pathogens of fruit trees and grapevines**''.

Session description: Fungal canker and dieback diseases cause serious yield losses in fruit and nut trees as well as grapevines. These canker diseases are caused by a complex of fungal genera and species. Knowledge regarding the fungal diversity, host associations, and pathological relevance of the species involved aids in understanding their unique ecological niches. This information is required to develop effective management strategies to control canker diseases on these crops.

It was an honour to get an opportunity to hold a session at the IMC12. Myself, and Dr. David Gramaje (Spain) were the chairpersons for the session. Researchers from California, Czech Republic, China and Italy gave presentations on canker diseases associated with various woody crops including: pome fruit, stone fruit, nut crops, grapevines, Perimmons and Kiwifruit vines. Dr. Lennox presented research entitled: "*Cytospora punica* – from stem canker to postharvest stem end rot of pomegranate". The special session provided an opportunity for research on canker and dieback of agricultural important trees to be presented and discussed.

Three of the speakers included research on stone fruit or apple cankers.

Florent Trouillas (USA): Fungal diversity associated with canker diseases in the main fruit and nut crops in California

Aleš Eichmeier (Czech republic): Diversity of Botryosphaeriaceae species associated with canker and dieback of grapevines, stone fruit and nut trees in the Czech Republic

Vladimiro Guarnaccia (Italy): Fungal diversity associated with trunk diseases of apple and hazelnut in Italy

Attached to this report are extracts of these presentations on the crops of relevance to Hortgro. Symptoms and causal pathogens are shown in these presentations.

Speakers of the special session on dieback diseases of fruit trees and vines at the IMC12 in Maastricht. In front Dr. David Gramaje (Spain) and Prof. Lizel Mostert (South Africa). In the back from the left: Prof. Aleš Eichmeier (Czech Republic), Prof. Akif Eskalen (California), Dr. Wei Zhang (China), Dr. Cheryl Lennox (South Africa), Prof. Irene Barnes (South Africa), Dr. Vladimiro Guarnaccia (Italy) and Prof. Florent Trouillas (California).

Presentations and posters of relevance to SA fruit industry (abstracts shortened)

Identification and pathogenicity of *Botrytis cinerea* and *B. prunorum* associated with calyx-end rot in pears during postharvest in Chile. Galdos et al. (poster presentation)

A new species of *Botrytis* was identified from pears in Chile. *Botrytis prunorum* is less virulent than *B. cinerea*. Isolates of *B. cinerea* from grapevine and cherries could form equal size lesions on pears than those that originated from pears. This research is important, since *B. prunorum* has not been found in South Africa. PDF of poster attached.

Influence of rain shelters on fungi on apple tree surfaces. Claudia et al. (poster)

The use of rain shelters/nets has been proposed as an alternative approach to prevent harmful effects of the rainfall and to reduce the application of plant protection products in apple orchards. Higher abundance under the rain shelters compared to no rain shelter condition were found for genera like *Alternaria, Cladosporium*, *Pseudopithomyces*, *Venturia*, and *Vishniacozyma.* Comparing two cultivars (Fuji and Golden) *Hygrophorus*, *Tilleptiopsis*, *Botrytis*

and *Venturia* resulted more abundant in Golden, while *Diplodia*, *Fusarium* and *Podosphaera* were significantly more abundant in Fuji. PDF of poster attached.

Brown seaweed as source of natural compounds against *Stemphylium seicarium*, the causal agent of brown spot disease in pear trees. Vicente et al. (oral presentation)

Stemphylium vesicarium is a fungal phytopathogen responsible to cause brown spot disease in different plant hosts, as pear trees. Rocha pear, a west Portuguese variety worldwide commercialized, has been persistently affected by *S. vesicarium*, reaching economic losses of 30 million euros between 2014 and 2016. Their control by synthetic products is not fully effective and it is known to be prejudicial for non-target organisms, being mandatory to find more sustainable and efficient solutions against this disease. Aiming the improvement of plant defences against brown spot disease, the potential of the brown seaweeds *Fucus vesiculosus* and *Sargassum muticum* extracts as priming agents was investigated. For this, *in vivo* assays with pear trees were performed. The aqueous extracts of both seaweeds were applied before inoculation with *S. vesicarium*, with additional application after inoculation in specific conditions. The most promising result was obtained with a continuous application of the extract of *S. muticum* (before and after inoculation with *S. vesicarium*), where no signs of disease were found, contrary to the results obtained with *F. vesiculosus*. The chemical profile of the extracts, including the phytohormones such as the presence of salicylic acid in the extracts, may help to explain the bioactivities found. This work highlights the agricultural potential of these seaweeds to fight agricultural fungal diseases under sustainable and circular framework, despite more studies are needed to understand the underlying mechanisms to fully potentiate the use these seaweeds in the orchard.

Green-synthesized nanoparticles against post-harvest diseases caused by *Penicillium* spp. Kavroulakis et al. (poster presentation)

Green-synthesized nanoparticles (GNPs) are eco-friendly alternatives to be used against post-harvest pathogens. In this study, GNPs were synthesized from ionic metals using bacterial and plant metabolites as reducing and capping agents, following an eco-friendly method. In this study copper GNPs exhibited good potential to control *Penicillium* spp. isolates and constitute a promising approach for reducing the environmental footprint of chemicals used against postharvest diseases. PDF attached.

Threats of latent, stress related tree pathogens in a changing climate with a special focus on Botryosphaeriaceae. Slippers et al. (oral presentation)

Botryosphaeriaceae is a diverse group of fungi that predominantly infect woody plants. infections. Importantly, many Botryosphaeriaceae species are also known as plant pathogens, and they are amongst the most common fungi associated with diseases such as leaf necrosis, as well as dieback, cankers and blue stain of branches and stems. These disease symptoms typically appear when the plants are under stress. With climate change increasingly placing stress on plant communities worldwide, the threat posed by Botryosphaeriaceae is expected to increase. There is an apparent increase in the number of reports and distribution of some species that appear to support this idea. In this study, we have interrogated the relevant

literature and public global databases to consider how widely distributed species in *Botryosphaeria*, *Diplodia*, *Neofusicoccum* and *Lasiodiplodia* are likely to behave as climates change to favour their spread and emergence as pathogens around the world.

Novel large scale tree mortality in California is caused by latent pathogens triggered by climate change. Garbelotto and Guarnaccia (oral presentation).

Until recently, disease caused by latent pathogens, i.e. pathogens that alternate between endophytic, pathogenic and saprobic phases, has been reported only in localized situations of acute plant stress. Surprisingly, since 2015, latent pathogens have been repeatedly identified as causes of the widespread mortality of trees and shrubs throughout Northern California. Isolations and pathogenicity tests revealed that disease of each of eight tree/shrub species was associated with latent pathogens in the genera *Botryosphaeria*, *Diplodia*, *Neofusicoccum*, *Dothiorella*, *Diaporthe* and *Pseudosysdowia*. California is experiencing a megadrought: we hypothesized that drought may be a driver of the emergence of novel latent pathogens. By maintaining soil moisture just above the wilting point in a set of potted plants, we were able to show that disease severity for almost all pathogens increased significantly in plants that were drought-stressed and inoculated with latent pathogens. Finally, by using a model "host x pathogen" combination, we addressed whether disease severity in potted plants may be positively associated with drought, warmer temperatures, fungal strain and wounding, alone and together. Results showed that disease increased at warmer temperatures, while drought increased disease only at the warmer temperature. Presence of latent pathogens was significant in all treatments, while wounding on its own was significant only in the hot and dry treatment. Results further suggest that warmer temperatures do not increase disease because of their effect on fungal strains, but because of their effect on plant physiology. Our data support a major role of latent pathogens in causing large scale tree mortality in association with climate change.

Fungal diversity associated with canker diseases in the main fruit and nut crops in California. F Trouillas (oral presentation)

Fungal canker diseases that affect these crops have been of increasing concern in recent years. Most California orchards are likely to become affected by at least one or more canker diseases, impacting their yield, longevity and profitability. Phylogenetic inferences revealed 15 *Cytospora* species, including 10 new species, associated with branch and twig cankers and dieback of almond, apricot, cherry, olive, peach, pistachio, plum, pomegranate, and walnut trees in California. In almond, 12 Botryosphaeriaceae species as well as five *Cytospora* species, *Collophorina hispanica*, *Eutypa lata*, and *Pallidophorina paarla* were found to cause different canker diseases. Six-locus phylogenetic analyses and morphological characterization of the North American clade of *Ceratocystis* also identified *Ceratocystis destructans* as the causal agent of Ceratocystis canker of almond, a disease that is unique to California almond. *Calosphaeria pulchella*, *Cytospora sorbicola*, and *Eutypa lata* prevail as the main canker pathogens of sweet cherry and other stone fruits.

Diversity of Botryosphaeriaceae species associated with canker and dieback of grapevines, stone fruit and nut trees in the Czech Republic. A. Eichmeier (oral presentation)

A survey of fungal pathogens associated with the decline of stone fruit trees, analyzing wood samples from symptomatic plum (Prunus domestica L.) and apricot (Prunus armeniaca L.) trees was done. Pathogens identified included *Dothiorella sarmentorum*, *Diplodia seriata* and *Diplodia sapinea*. The survey underscores the critical need for ongoing monitoring and management strategies for fungal trunk pathogens in economically important woody crops in the region.

Relevance to industry

The IMC is a conference conveying the most recent developments related to fungal nomenclature and taxonomy. This information is essential for the use of the correct names for pathogens of concern listed in our phytosanitary information packages. From time to time, I am required to write a letter in support of the occurrence of certain pathogens on fruit trees in South Africa. Having the most up to date information in regards to fungal names is important to provide this technical support.

The influence of climate change on disease severity and occurrence is important. The presentations on this topic at the conference were very insightful. Especially the talk by Garbelotto and Guarnaccia, where they set up trials to investigate the effect of temperature and drought on latent pathogens of trees. Their results showed that disease increased at warmer temperatures, while drought only increased disease at the warmer temperature. Warmer temperatures did not increase disease because of their effect on the fungal strains, but because of their effect on plant physiology.

The latest technologies were employed to answer various diagnostic and disease related questions. Whole genomes were frequently used together with microbiome analyses. In terms of control, does seaweed extracts hold potential for the control of *S. vesicarium*. Green-synthesized nanoparticles hold good potential to control *Penicillium* spp. isolates associated with postharvest diseases.

New contacts were made especially in countries difficult to source information from (India and Japan). The contacts listed below are valuable for plant disease related research. I would recommend Prof. Florent Trouillas from University of California (Davis) as an invited speaker at one of the Hortgro Symposia. He specializes in stone fruit fungal diseases and has more recently also started to work on bacterial diseases of stone fruit. In the unit that he comes from Dr. Themis Michailides is well known for many practical solutions that he has brought to producers for the control of diseases.

Industry contacts

Prof. Florent Trouillas, Plant Pathology, University of California, Davis, USA (stone fruit diseases). Email: lotrouillas@ucanr.edu

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Email: vladimiro.guarnaccia@unito.it

Prof. Aleš Eichmeier, Medeleum – Institute of Genetics, Mendel University in Brno, Lednice, Czech republic (stone fruit diseases)

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Prof. Levente Kiss, Center for Crop Health, Institute for Life Sciences and the Environment, University of Southern Queensland, Toowoomba, Australia (powdery mildew specialist on various crops)

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Prof. Izume Okane, Institute of Life and Environmental Sciences, University of Tsukuba, Japan (using biocontrol for tree diseases)

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Identification and pathogenicity of *Botrytis cinerea* and *B. prunorum* associated with calyx-end rot in pears during postharvest in Chile

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INTRODUCTION

In Chile, the European pear (*Pyrus communis*) has a surface productive area of over 6,000 hectares. Calyx-end rot (CER) is the major postharvest disease of pears, with incidence from 2 to 60% of fruit losses. The major pathogen is *Botrytis cinerea*. Therefore, the aim of this study was to determine the identification and pathogenicity of *Botrytis-like* species associated with CER in pear fruits in Chile.

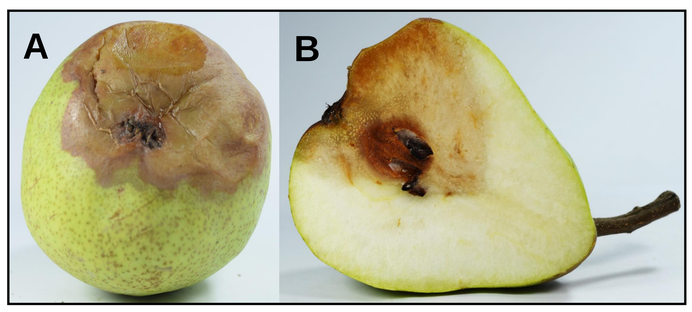

Fig 1. A. Pears fruits cv. Packham´s Triumph show symptoms of CER. **B.** Longitudinal section of affected fruits with CER.

METHODOLOGY

Isolation and identification. The isolations were performed on acidified potato dextrose agar (APDA, 2%) and incubated for 7 days at 20-22°C in darkness. Twelve representative isolates were selected and identified using morphology and multilocus phylogenetic analysis (*RPB2*, *HSP60*, *G3PDH* loci) (Ferrada et al. 2016; Garfinkel et al., 2019).

Pathogenicity and temperature tests. Ten isolates of *Botrytis*-like were inoculated on wounded pears and eight on cherries and grapes. All fruit were incubated for 7 days at 20°C. Growth curves were established from 0 to 40 °C.

RESULTS

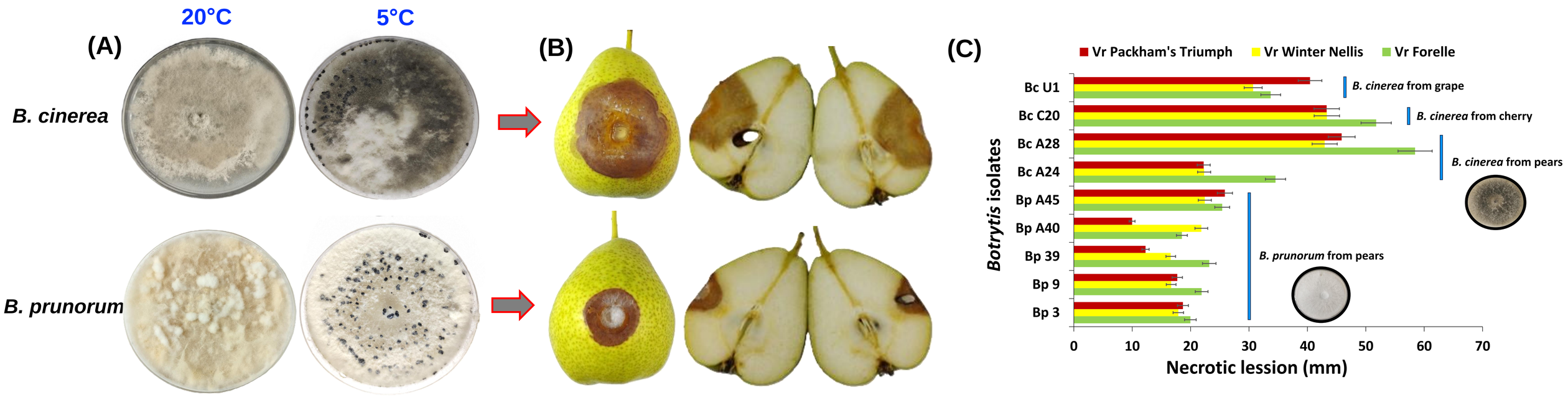

Fig 2. Colonies of *B. cinerea* and *B. prunorum* on APDA (**A**); Pathogenicity on pear Forelle inoculated con mycelial plug de isolates of *B. cinerea* (above) and *B. prunorum* (below) (**B**); Diameter of necrotic lesion on pear inoculated at 20°C for 7 days (**C**).

Based on morphological and phylogenetic studies, **we identified six isolates as *Botrytis cinerea*** (medium to high sporulation level) and **seven isolates as *B. prunorum*** (isolates of low to cero sporulation level) (**Figs. 2 A, 3**) showing an optimal temperature of 20 and 25°C for mycelial growth on APDA, respectively (**Fig. 4**).

All *Botrytis* isolates developed lesions on pear cvs. Forelle (1.8 to 5.9 cm), Packams (1.7 to 3.4 cm) and Winter Nellis (1.6 to 4.5 cm), showing that cv. Forelle was significantly more susceptible ($P< 0.001$) than cvs. Winter Nellis and Packams (**Figs. 2 B, C**). Moreover, rot lesions were also observed in the other host fruit species ($P< 0.001$) (**Fig. 5**). Koch's postulates were fulfilled after the reisolations of the causal agent from the inoculated fruits.

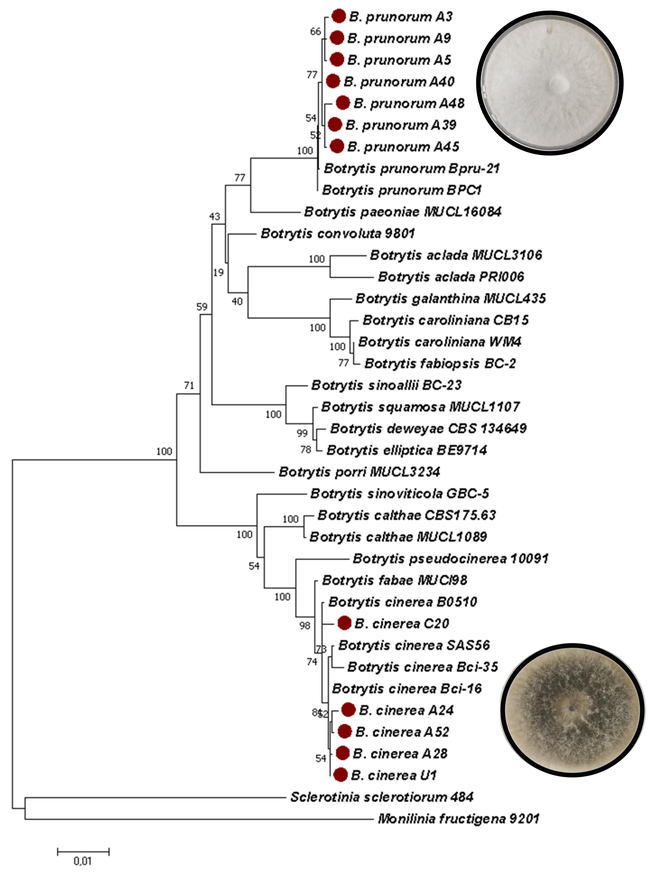

Fig 3. Maximum parsimony tree from concatenated *RPB2*, *HSP60* and *G3PDH* gene sequences of Chilean isolates.

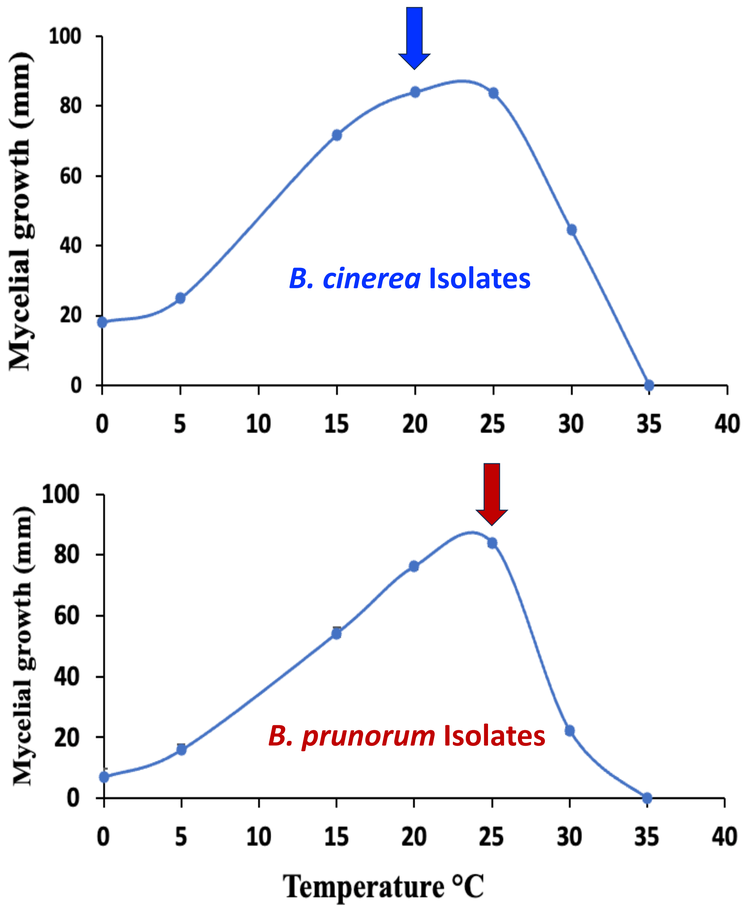

Fig 4. Mycelial growth (mm) of *B. cinerea* (above) and *B. prunorum* (below) after 7 days under darkness incubated from 0 to 40°C.

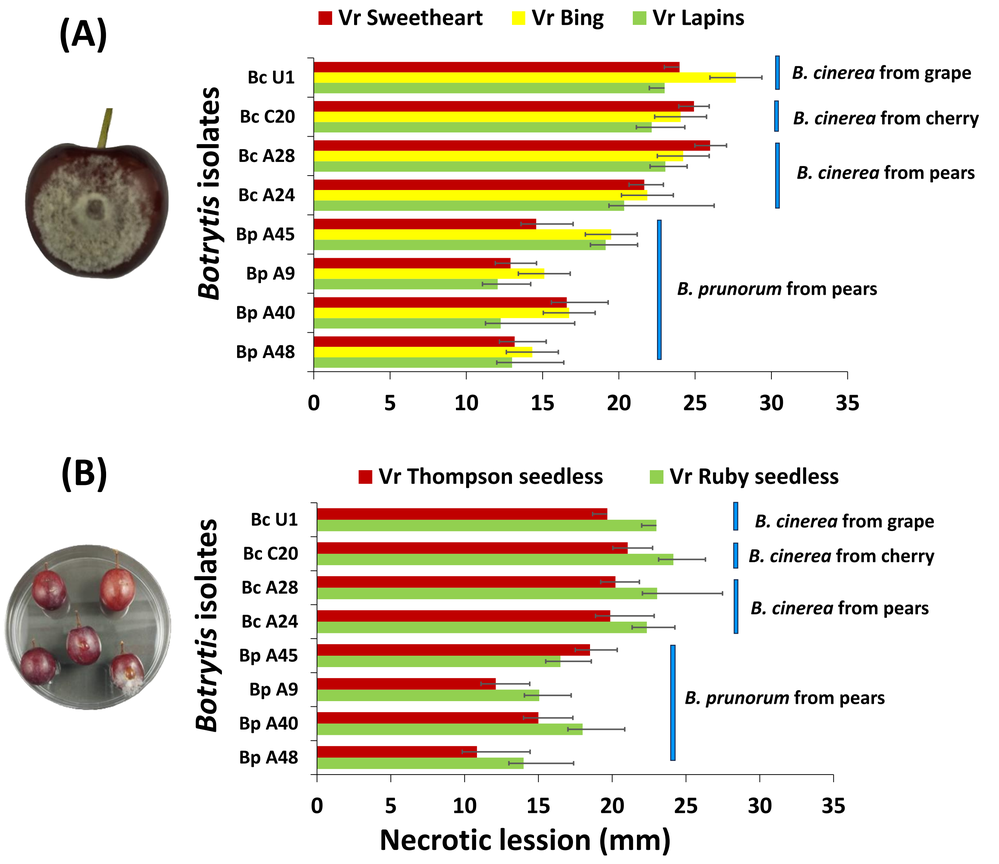

Fig 5. Cross pathogenicity of *Botrytis* isolates on cherry (A) and grape (B) 20°C after 7 days.

CONCLUSION

Botrytis cinerea and *B. prunorum* are the causal agents of CER in pear fruits in Chile. The results obtained show that *B. cinerea* isolates are the most virulent than *B. prunorum* isolates, and their potential cross-infection on other fruit species can occur. More studies are necessary for understand the CER and developed measured of mitigation of this rot disease.

REFERENCES

Ferrada, E. E., Latorre, B. A., Zoffoli, J. P. & Castillo, A. (2016). Identification and characterization of Botrytis blossom blight of Japanese plums caused by *Botrytis cinerea* and *B. prunorum* sp. nov. in Chile. Plant Dis.106:155-165.
Garfinkel, A.R., Coats, K.P., Sherry, D.L. et al. (2019). Genetic analysis reveals unprecedented diversity of a globally-important plant pathogenic genus. Sci Rep 9, 6671.

INFLUENCE OF RAIN SHELTER ON THE FUNGAL BIODIVERSITY OF APPLE ORCHARD

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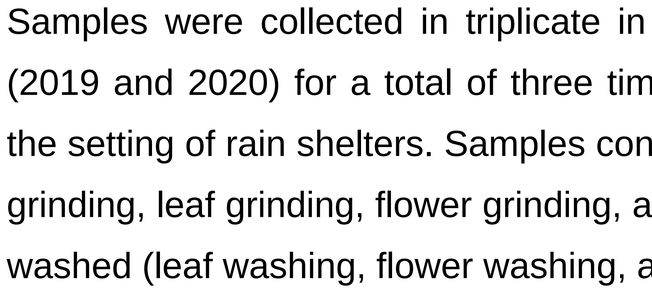
Introduction

The use of rain shelters has been proposed as an alternative approach to prevent harmful effects of the rainfall and to reduce the application of plant protection products in apple orchards. The use of net systems in fruit cultivation presents some advantages such as the protection of fruits from biotic (insect, bird, bats) and abiotic (wind, rain, frost, temperature fluctuations, solar radiation) stresses. The environmental changes induced by rain shelter system, as lower leaf wetness and higher average temperature, can affect the plant-microbe interactions.

Objective

The aim of this work was to understand the impact of rain shelters on fungal communities associated with tissues of two different apple cultivars.

Material and Methods



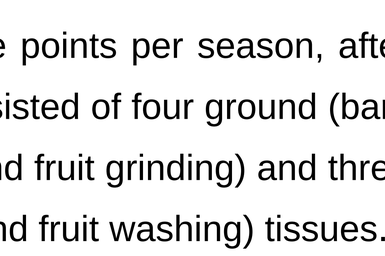


Fig 1. View of the experimental area and detail of the rain shelter cover in the apple orchard.

The research was carried out in an experimental orchard located in northern Italy. The experimental orchard was planted in 2017 with two-year-old clonally propagated apple trees of two cultivars (Golden and Fuji) grafted on the M9 rootstock. In 2019, half of the orchard was covered with a rain shelter (Microtex, patent n. 0001422628) to protect plants against rain (IN) and the remaining part of the orchard was an open-filed not covered (OUT) with the rain shelter.

Samples were collected in triplicate in two consecutive seasons (2019 and 2020) for a total of three time points per season, after the setting of rain shelters. Samples consisted of four ground (bark grinding, leaf grinding, flower grinding, and fruit grinding) and three washed (leaf washing, flower washing, and fruit washing) tissues.

The genomic DNA of each sample was extracted and metabarcoding sequencing of the fungal internal transcribed spacer (ITS2) was conducted to analyse the difference between the fungal communities on apple plants tissues (leaf, bark, flower and fruit) from orchard grown under rain shelter (IN) and open field condition (OUT), across two seasons.

DNA indexing, quantification, and library preparation for the Illumina MiSeq sequencing (PE300) and sequences of the 360 samples were obtained.

Results

The majority of fungal sequences were assigned to Ascomycota and Basidiomycota. The most abundant genera were *Aureobasidium* (14.58%), *Cladosporium* (12.11%), *Alternaria* (3.98%), *Podosphaera* (2.89%), and *Sporidiobolus* (2.43%).

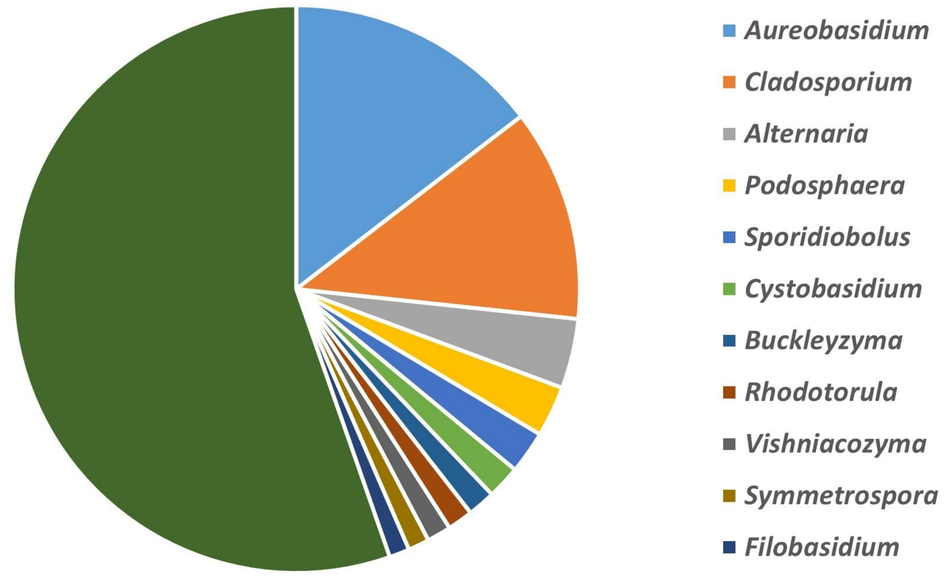


Figure 2: Relative abundance of the most represented fungal genera in apple samples (abundance >1%).

A Significant difference in the beta diversity composition of apple fungal epiphytes and endophytes inside and outside the rain shelter was detected (PERMANOVA, p = 0.02);

ASVs with higher abundance in the IN compared to OUT condition were found, such as *Alternaria, Cladosporium*, *Pseudopithomyces*, *Venturia*, and *Vishniacozyma.*

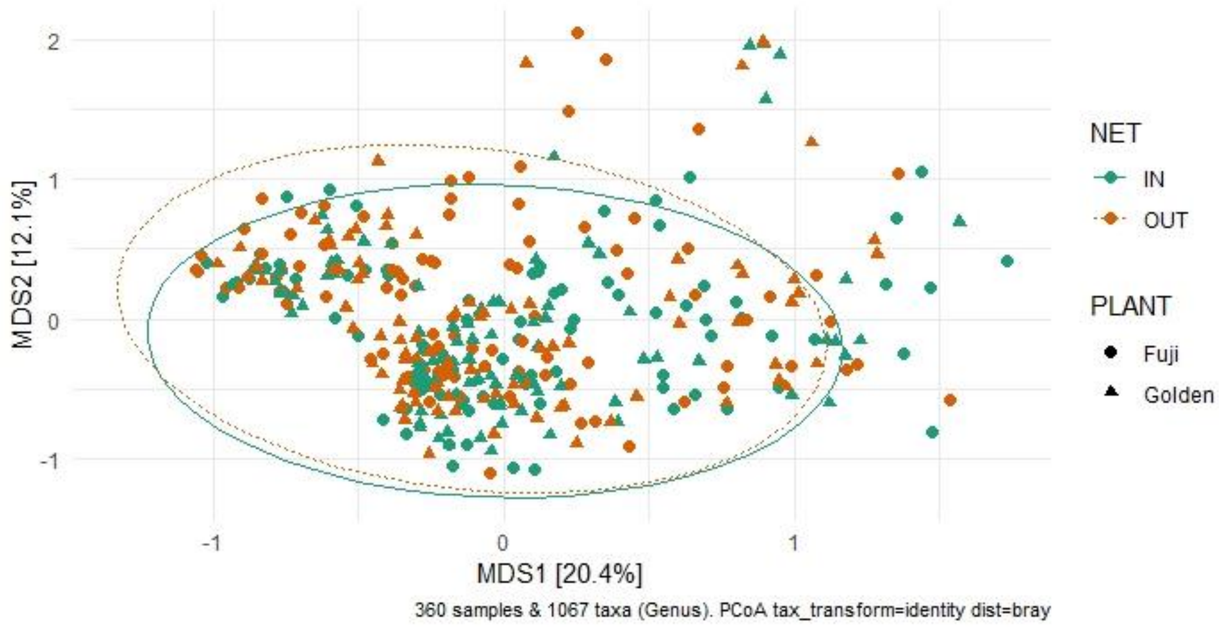


Figure 3: Non-Metric Multidimensional Scaling (NMDS) based on the Bray-Curtis dissimilarity distance matrix of Illumina sequencing data of fungal communities in apple tree tissues inside (IN) and outside (OUT) the rain shelter.

Likewise, a significant difference between the composition of the fungal communities in Fuji and Golden plants was found (PERMANOVA, p = 0.001);

Differential fungal ASVs in Fuji and Golden plant tissues, where *Hygrophorus*, *Tilleptiopsis*, *Botrytis* and *Venturia* resulted more abundant in Golden, while *Diplodia*, *Fusarium* and *Podosphaera* were significantly more abundant in Fuji.

In conclusion, the use of rain shelters can influence the structure and composition of the fungal communities on apple trees. Understanding how the diverse microorganisms within the plant microbiome respond to this management practice, may guide the adoption of a successful strategy, favoring microorganisms with beneficial functions to improve plant health and production.

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Green-synthesized nanoparticles against post-harvest diseases caused by *Penicillium* spp.

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Abstract: To date, synthetic fungicides is the most effective method for controlling these diseases and preventing mycotoxin contamination of fruit. However, chemical control is being challenged by resistance development to most of the fungicides utilized and strict EU legislation due to environmental concerns, causing the withdrawal of a large number of available active ingredients used for post-harvest treatment of fruit. To tackle these challenges, green-synthesized nanoparticles (GNPs) have been proposed as eco-friendly alternatives to be used against post-harvest pathogens. In this study, GNPs were synthesized from ionic metals using bacterial and plant metabolites as reducing and capping agents, following an eco-friendly method. The effectiveness of GNPs was tested against sensitive and fungicide-resistant Penicillium isolates both in vitro and on citrus fruit. GNPs could reduce mycelial growth and disease symptoms more effectively than the reference fungicide containing $Cu(OH)_2$ and their ionic counterparts. A significant synergy was observed when GNPs were combined with conventional fungicides typically used against these pathogens.

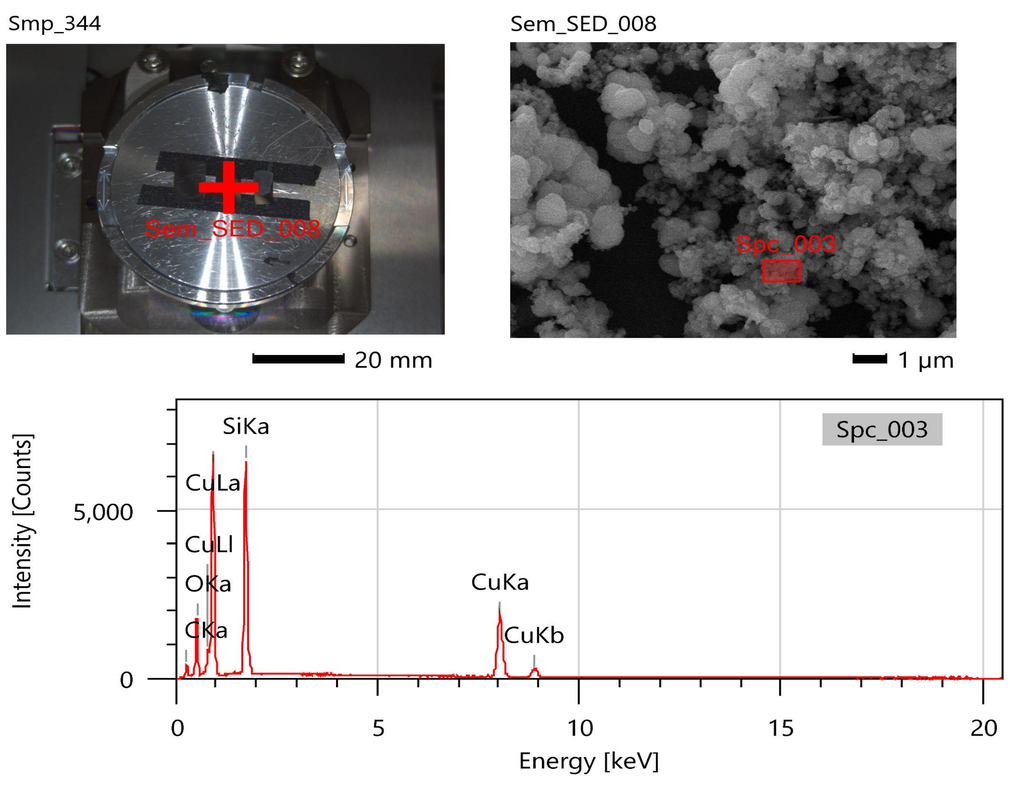

Figure 1. SEM image of Gr10.1 copper-containing nanoparticles**.**

Methods

Green synthesis of Cu-NPs: $CuSO_4 \cdot 5H_2O$ was the precursor for the synthesis. The extract of *Bacillus amyloliquefaciens* strain P.10.1 obtained by centrifugation after 1 day of incubation at 25°C was used a natural reducing and capping agent. A volume of 50 mL of the above copper sulphate solution was mixed with 50mL bacterial extraction and the mixture was steered at 70°C for 1 h until the colour changed from light blue to dark green, indicating the formation of CuO-NPs. Particles were collected by centrifugation on was centrifuged at 10,000 rpm for 10 mins and the supernatant was discarded. Particles were washed trice and transferred to an oven to dry at 80°C overnight. NP morphology, zeta potential and size were analyzed by SEM and a ZetaSizer.

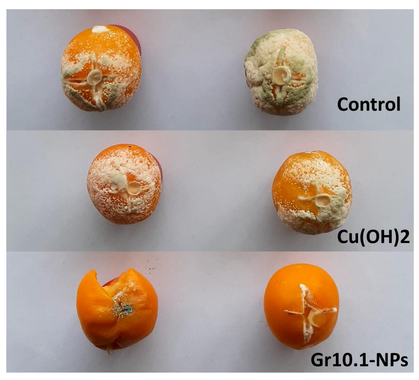

Figure 2. Effect of Gr10.1-NPs, against *Penicillium* mold

In vitro mycelium growth inhibition tests

*The sensitivity of sensitive and fungicide-resistant Penicillium i*solates to NPs were tested by measuring radial growth of strain on PDA. Fungitoxicity was expressed based on EC_{50} values (effective concentration causing 50% inhibition of mycelial growth) for each antifungal agent tested.

Effectiveness of nanoparticles in vivo

The effectiveness of NPs in suppressing blue-mold symptoms caused by *Penicillium spp. in vivo* was tested *in vivo using* Kumquat fruits. The fungicide Copperblau-N containing copper hydroxide was used as a reference. Four fruits per treatment with antifungal agents were used while
the control treatment consisted of fruits sprayed with distilled sterilized water.

Results

Characterization of Gr10.1: *Green synthesized copper* NPs (Gr10.1) were spherical and their size ranged between 250-350 nm as indicated by SEM analysis (Fig.1). The average zeta potential and hydrodynamic diameter of Green35 NPs were -22 and 295 nm, respectively.

Effectiveness of Gr10.1-NPs: Green synthesized copper NPs were more effective against sensitive and fungicide-resistant Penicillium isolates than the reference fungicide containing $Cu(OH)_2$ both *in vitro* Fig.2) and *in vivo* (Fig.3).

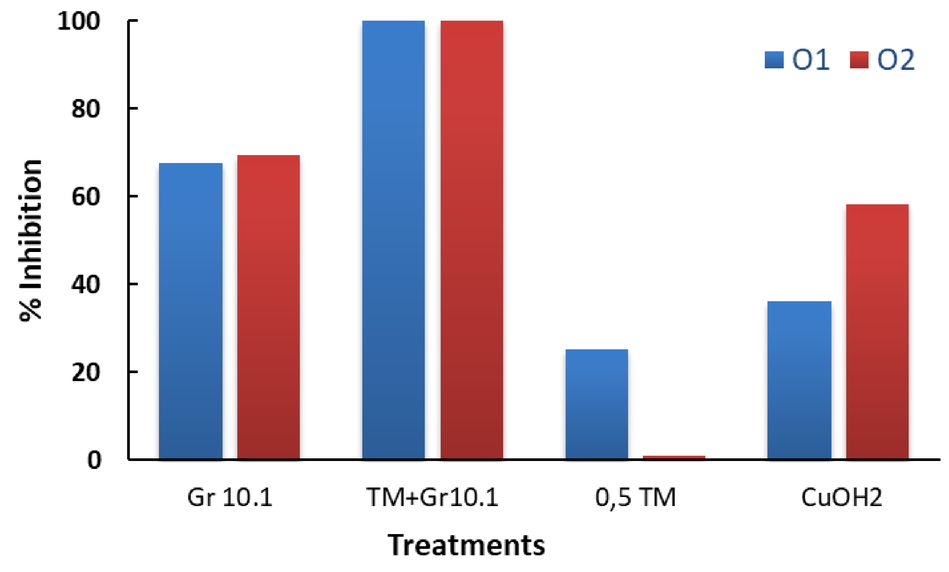

Figure 2. Effectiveness of Gr10.1-NPs and their combination with thiophanate methyl (TM) against *Penicillium spp. isolates* sensitive *(O1)* and resistant to TM (O2).

Conclusion

Copper GNPs have exhibited a great potential to control Penicillium spp. isolates and constitute a promising approach for reducing the environmental footprint of chemicals used against post harvest diseases.

References

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