USE OF MICROORGANISMS IN THE DISINFECTION/PROTECTION FROM WOOD PATHOGENS OF GRAPEVINE ROOTED-CUTTINGS FOR ORGANIC PRODUCTION

G. Carella[1], F. Burroni[2], A. Ciofini[3], L. Ghelardini[1], R. Perria[3], W.A. Petrucci[3], P. Storchi[3] and L. Mugnai[1].
[1] Department of Agriculture, Food, Environment and Forestry (DAGRI), University of Florence, Piazzale delle Cascine 28, I-50144, Florence, Italy,
[2] Studio Associato Agroniminvigna, Via de' Buondelmonti 62, 50125 Firenze, Italy,
[3]Council for Agricultural Research and Economics (CREA), Research centre for Viticulture and Enology, Viale Santa Margherita 80, 52100 Arezzo, Italy.
Email: giuseppe.carella@unifi.it

Introduction

Life Green Grapes is a demonstration project involving both research institutions and farms. The project deals with topics concerning the whole wine-growing chain: on one hand it involves the production of wine and table grapes, on the other one the production of rooted cuttings.

In experimental fields dedicated to the production of grapes for both wine (in Tuscany - Italy) and table grapes (in Apulia - Italy and in Cyprus), the implemented strategies aim at reducing the quantity of fungicides applied to control the main foliar diseases in the vineyard, by combining field monitoring, with the correct use of DSS and the stimulation of the plant's defense responses, all three management options well recognised as pillars of a low impact disease control strategies. The potential and the limits of integrating these approaches was investigated applying 5 different management protocols in the vineyards, while in the grapevine nursery the Project identified some of the crucial phases of grafted cuttings production, in which it is possible to intervene to reduce the presence of pathogens. The final aim was to produce plants with an inner ability of being productive without suffering for an excessive post-transplant stress and able to gain adequate longevity.

Within this frame one of the scopes of the project was to evaluate the positive effect of microorganisms applied to the roots at different stages of the production process: through immersion of plantlets roots in a water solution of the product and through fertigation.

Materials and Methods

Demonstration trials in commercial conditions were set up at the nursery "Vivaio Moroni", a partner of the project. The propagation material was treated by following three different application protocols, which involved the use of natural products with a disinfectant antimicrobial activity, and the use of microorganisms, such as *Trichoderma* sp. and mycorrhizal fungi.

Treatments

1st trial. In the nursery trial three treatments were applied: 1) the standard protocol applied by the company (Organic Management) including propolis as a natural disinfectant; and two commercial products: 2) Product A, containing spores and mycelium of consortium of microorganisms including arbuscular mycorrhizal fungi of the genus *Glomus* (*G. mosseae, G. viscosum, , G. coronatum*), *Rhizophagus* sp., *G. caledonium*) and saprophytic fungi of the genus *Trichoderma* (*Trichoderma*

harzianum and *Trichoderma viride*); 3) Product B containing different species of saprophytic fungi belonging to the genus *Trichoderma* (*T. harzianum*, *T. asperellum*, *T. gamsii*).
2nd trial. For the root treatment two products were applied (with the aid of bentonite to improve attachment) and compared: (1) Product A (as above), and (2) Product C containing different species of arbuscular mycorrhizal fungi of the genus *Glomus* (*G. mossae* and *G. intraradices*) and *Trichoderma atroviride*.

Experimental design
The tests carried out were aimed at 1) evaluating the effect of microorganisms-based products applied during the nursery process on the fungal microflora inhabiting the wood of rooted cuttings, and 2) assessing the most suitable time for application to improve rooting.
The effect of the treatments on the fungal wood-colonizing microflora (1st trial) was examined by comparing three different protocols: Company Organic Management (the standard protocol applied by the company), Product A and Product B.

The products used were applied a) in sawdust at the callusing phase, b) at the hydration phase and c) in fertigation 15 days after transplant in the vineyard (Table 1).
For evaluating the most suitable time point for treating the roots (2nd trial), two products, Product A and Product C, were applied in different phases of the production process: 1) before storage in a cold room at 4 °C; 2) during the packaging phase before delivery; 3) just before planting (Table 2).

Table 1. Application protocol used in the different stages of vine cuttings production.

Work phase	Treatment 1: Organic Management	Treatment 2: Mycorrhizae + *Trichoderma* sp.	Treatment 3: *Trichoderma* sp.
a Callusing in sawdust (about a month)	Propolis extract	Propolis extract + Mycorrhizae + *Trichoderma* sp.	Propolis extract + *Trichoderma* sp.
b Before planting (hydration)	Water for 4-5 days	Water for 4-5days + Mycorrhizae + *Trichoderma* sp.	Water for 4-5 days + *Trichoderma* sp.
c Rooting in nursery soil (after 15 days from planting)	/	Fertigation (Mycorrhizae + *Trichoderma* sp.)	Fertigation *Trichoderma* sp.

Table 2. Application protocol for the evaluation of the most suitable time point for treatment.

Work phase	Treatment 1: Organic Management	Treatment 2: Mycorrhizae + *Trichoderma* sp.					Treatment 3: *Trichoderma* sp.
Before packaging	Propolis extract	Product 1 Mycorrhizae + *Trichoderma* sp.	Propolis extract	Propolis extract	Propolis extract	Propolis extract	Propolis extract + *Trichoderma* sp.
Storage in a cold room (about 1 month)	/	/	/	/	/	/	/
Treatment	VM1	VM2A	VM2B	VM2C	VM2D	VM2E	VM3
Application on the roots	/	/	Product A Mycorrhizae + *Trichoderma* sp.	Product A Mycorrhizae + *Trichoderma* sp.	Product B Mycorrhizae + *Trichoderma* sp.	Product B Mycorrhizae + *Trichoderma* sp.	/
			Before delivery	**Before planting**	**Before delivery**	**Before planting**	

Analysis of the wood-inhabiting fungal microflora and of the root system

After uprooting, the rooted cuttings were taken to the laboratory (DAGRI) - University of Florence), where the analyses were carried out.

After a first wash with water, the sample was disinfected by immersion in 4% sodium hypochlorite for 2 minutes, than it was debarked and immersed for a minute in the same disinfectant solution in order to eliminate pollutants or microorganisms present on the external surface of the sample.

Thirty cuttings per treatment were used. Five slices about 2 mm thick were cut from 5 points (Fig. 1): above the graft point, at the graft point, below the graft point, at rootstock, at collar.

Each section was divided into 5 fragments that were placed in a Petri dish (90 mm diam.) containing MEA. The plates were incubated at 25 °C for 4 weeks, pure cultures were obtained from developing fungi, and morphological identification was carried out.

For the evaluation of the root system, 30 cuttings were analyzed per treatment. The roots were washed with water and air dried. Subsequently, the primary and capillary roots were counted and weighed. The dry weight of the roots was assessed after oven-drying for 72 hours at 50 °C.

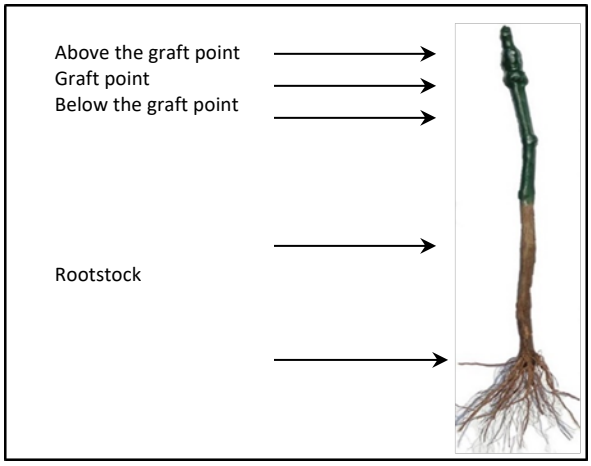

Fig. 1 Sampling points of the analyzed sections

Results

1st trial. The treatments applied in the nursery process on the fungal microflora tended to reduce wood pathogens colonization when *Trichoderma* only (Product B) or *Trichoderma* + Mycorrhizae (Product B) were applied. Althogh the effect was statistically non significant (p=...), the number of pathogens tended to decrease both in samples treated with Product A and in those treated with Product B compared to the control. This trend was observed for "*Cylindrocarpon like*" species , *Phaeomoniella chlamydospora* (Pch), *Phaeoacremonium minimum* (Pmin) and *Ilyonectria* spp. as already reported by other Authors (Carro-Huerga 2020) (Fig. 2).

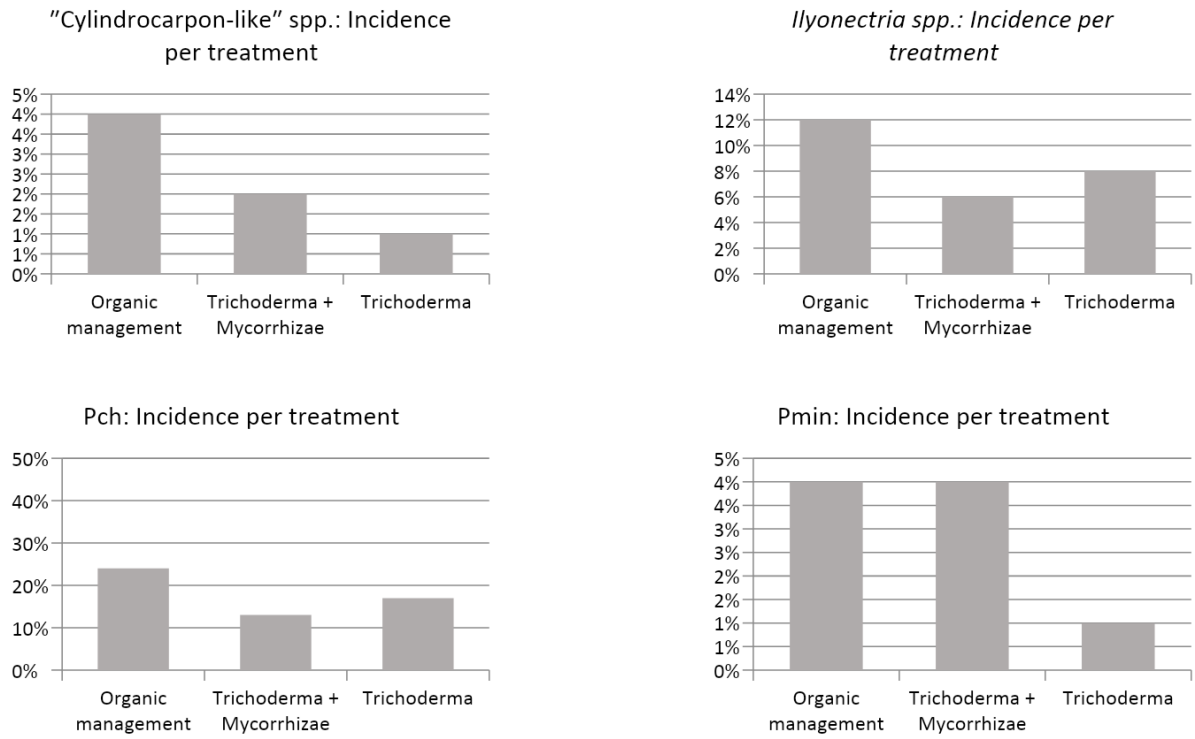

Fig. 2 Fungal wood microflora following treatments with Product A and Product B. The incidence of the fungal pathogens detected (natural infections) tended (although the effect was statistically non-significant) to be lower compared to the Company Organic management treatment (control). For Pmin the only incidence reduction has been in the "Trichoderma" treatment

2nd trial. From the visual comparison between root systems, a greater root mass in the proximal area was evident in all the samples treated with *Trichoderma* and mycorrhizae (Product A and Product C) compared to the untreated control (Fig. 3).In particular, the best results were obtained from the application of Product C when applied both in the phase before the packaging and in the phase before planting.
The analyses carried out on growth parameters showed statistically significant differences between control and treatments. The weight of the root system, both primary and capillary roots, significantly increased in the treatment "*Trichoderma* only". The increase in weight involved both the root system for single plant and for single root. The number of primary roots per single plant did not vary significant between treatments. The number of capillary roots varied significantly between treatments and the highest number of capillary roots was recorded in the Company Organic Management control treatment (Fig. 4).

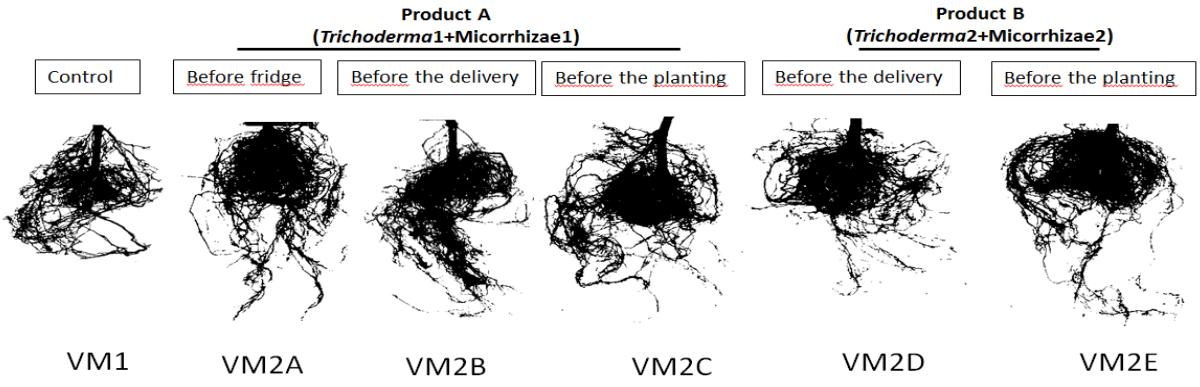

Fig. 3 The root mass in the proximal area was greater in all the treatments compared to the control.

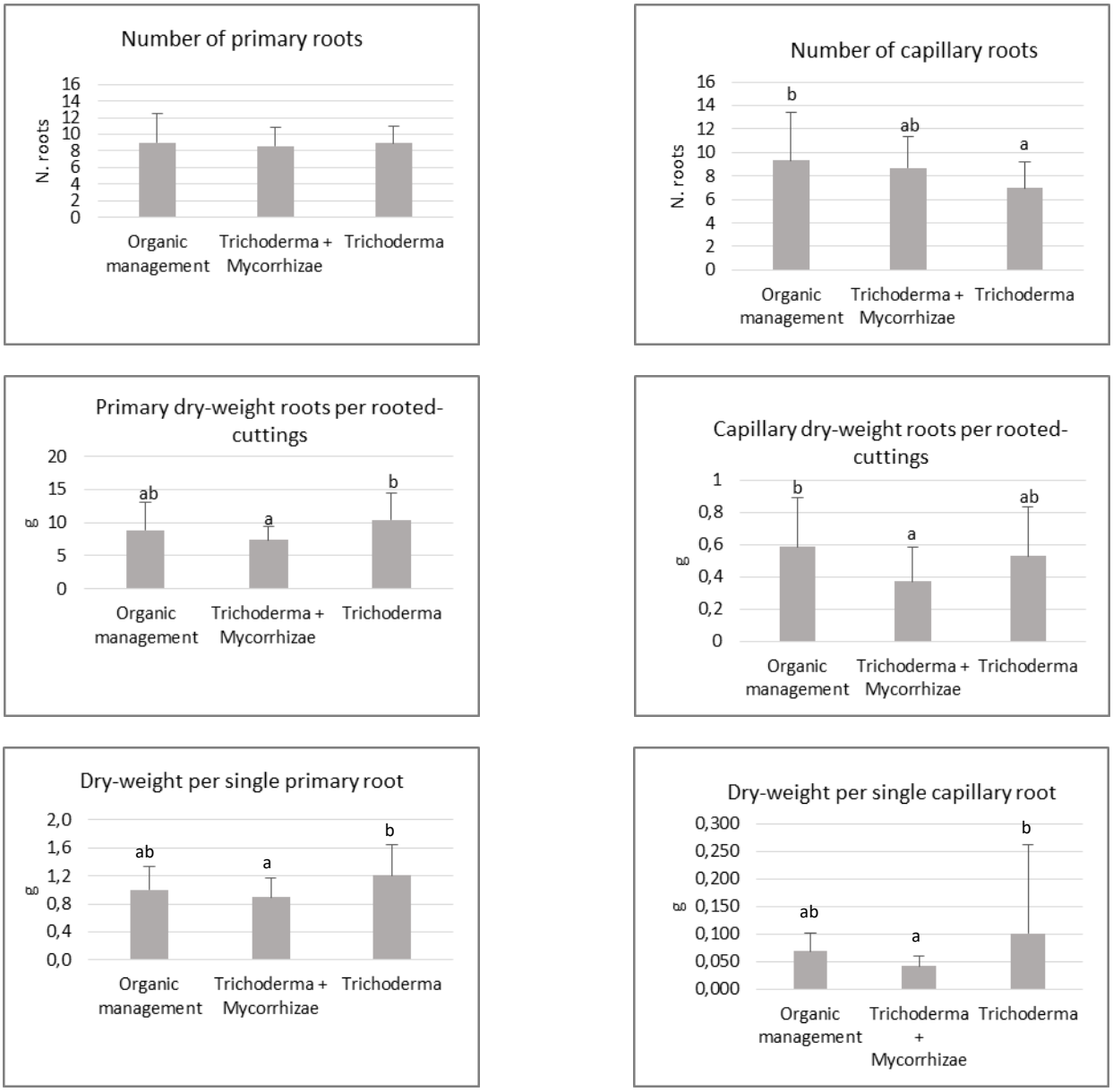

Fig. 4 Effects of treatment application (significant according to one-way ANOVA) on the root system (weight and number of roots). The treatment with Company Organic Management and Product C showed significantly higher dry weight of primary and capillary roots for cutting and for single root than the treatment with Product A. The treatment with Product A significantrly increased the total number of capillary roots.The means followed by the same letter in the bars are not significantly different (Duncan test P <0.05).

In addition,growth parameters were analyzed when cuttings were rooted in pots (Fig. 5). One-way ANOVA showed statistically significant differences in growth traits between the treatments. The treatment with Product C increased growth at both application time points, i.e. at delivery or at plantation, with a slightly higher effect when applied at plantation.

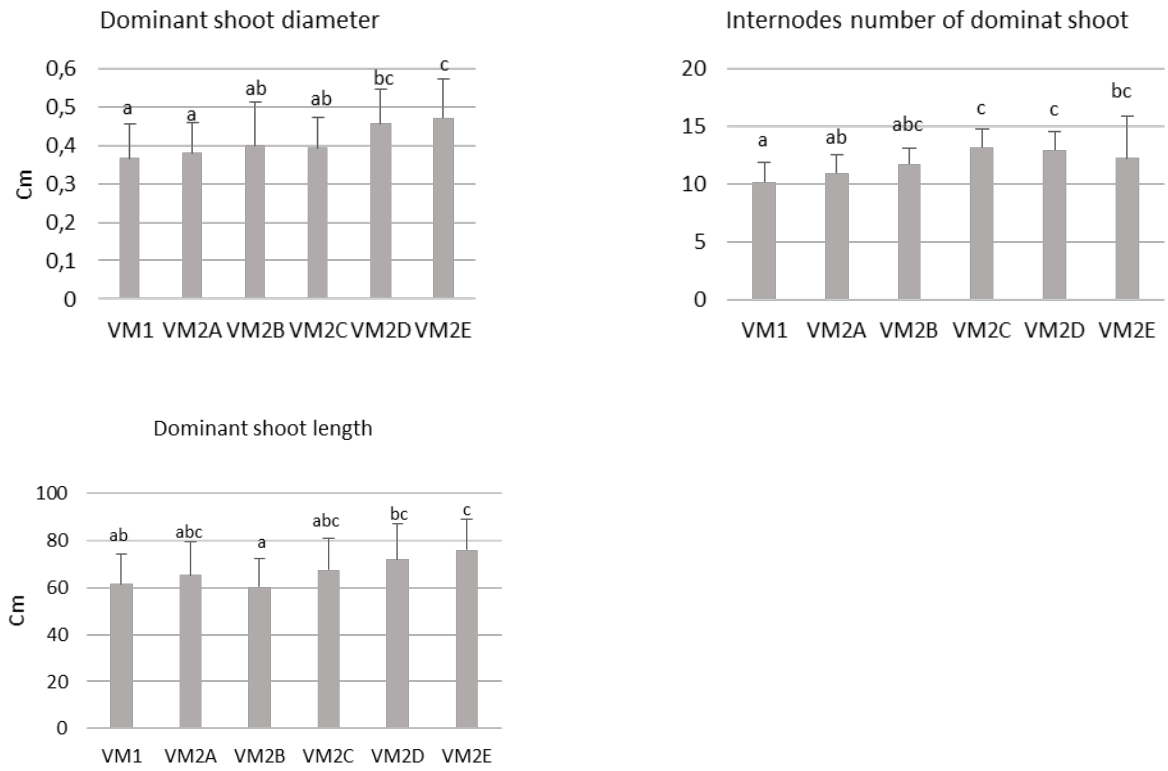

Fig. 5 Growth parameters measured in the cuttings treated and grown in pots. The One way ANOVA statistical analysis highlighted statistically significant differences between the treatments. The means followed by the same letter in the bars are not significantly different (Duncan test P <0.05).

Discussion and conclusion

The results of this demonstration study showed some positive effects of the treatments applied on reducing the fungal wood pathogens colonization, confirming previous findings (Berbegal et al., 2020; María del Pilar Martínez-Diz et al., 2021), and on improving the quality of the root system and on influencing the growth parameters, as recently reported by Tsvetko et al. (2017).

The interaction between the root system and the microorganisms applied is well known to stimulate the activity of the roots, and therefore to improve the water and nutrients absorption, eventually making the cuttings more resistant to biotic and abiotic stresses (Gramaje & Armengol, 2011). With these demonstration trials in the Life Green Grapes project, we aimed at showing that the whole production chain can have access to and be improved by environmentally friendly approaches starting from the very first steps of plant production in the nursery. The trials, carried out in commercial conditions, did show the efficacy of products containing beneficial microorganisms and the relevance of application time in determinating the final efficacy of the treatment.

A trend in the reduction in the incidence of pathogenic wood fungi was recorded confirming the results recently obtained by other researchers (Carro-Huerga et al., 2020; Urbez Torres et al., 2020; Pintos et al., 2018). Furthermore, the application of microorganisms produced positive effects on dry weight of both capillary and primary roots and increased root mass in the proximal zone, as observed by Luciani et al., (2019), and produced in addition a positive effect on growth of aboveground organs (dominant shoot length, dominant shoot diameter, internode number of dominant shoot) of the cuttings.

As for preliminary observations on root system development, the products were applied at different stages before plantation in order to favor rooting, thus showing the positive influence of the treatment. Currently, the application of microorganisms-based products is the most promising

solution available for disinfection of the plant material and should become a routine procedure in the optimized management of nursery production, given the many beneficial effects it produces on the quality and phytosanity status of the final product. These benefits in the long term will eventually affect the entire productive life of the vineyard and most probably its very longevity.

References

1. Berbegal, M., Ramón-Albalat, A., León, M., & Armengol, J. (2020). Evaluation of long-term protection from nursery to vineyard provided by Trichoderma atroviride SC1 against fungal grapevine trunk pathogens. *Pest management science*, *76*(3), 967-977.
2. Carro-Huerga, G., Compant, S., Gorfer, M., Cardoza, R. E., Schmoll, M., Gutiérrez, S., & Casquero, P. A. (2020). Colonization of Vitis vinifera L. By the Endophyte Trichoderma sp. Strain T154: Biocontrol Activity Against Phaeoacremonium minimum. *Frontiers in plant science*, *11*, 1170.
3. Gramaje, D., & Armengol, J. (2011). Fungal trunk pathogens in the grapevine propagation process: potential inoculum sources, detection, identification, and management strategies. Plant Disease, 95(9), 1040-1055.
4. Luciani e., E., Frioni, T., Tombesi, S., Farinelli, D., Gardi, T., Ricci, A., & Palliotti, A. (2019). Effects of a new arbuscular mycorrhizal fungus (Glomus iranicum) on grapevine development. In BIO Web of Conferences (Vol. 13, p. 04018). EDP Sciences
5. Maria del Pilar Martínez-Diz, M., Díaz-Losada, E., Andrés-Sodupe, M., Bujanda, R., Maldonado-González, M. M., Ojeda, S., ... & Gramaje, D. (2021). Field evaluation of biocontrol agents against black-foot and Petri diseases of grapevine. *Pest Management Science*, *77*(2), 697-708.
6. Pintos, C., Redondo, V., Costas, D., Aguin, O., & Mansilla, P. (2018). Fungi associated with grapevine trunk diseases in nursery-produced Vitis vinifera plants. *Phytopathologia Mediterranea*, *57*(3), 407-424.
7. Tsvetkov, I., Georgieva, L., Tsvetkova, D., Michailova, V., & Georgiev, D. (2017). Benefits of the micorrhizal fungi Glomus spp. For grapevine nutrient uptake, biocontrol and microbial ecology. *J Mt Agric Balk*, *20*(1), 227-250.
8. Úrbez-torres, J. R., Tomaselli, E., Pollard-Flamand, J., Boulé, J., Gerin, D., & Pollastro, S. (2020). Characterization of Trichoderma isolates from southern Italy, and their potential biocontrol activity against grapevine trunk disease fungi. *Phytopathologia Mediterranea*, *59*(3), 425-439.

Abstract

One of the major problems in viticulture is the great quantity (excess) of chemical pesticides (especially copper products) used to control foliar diseases of vine.

The Life Green Grapes project enters in the production context with the aim of reducing the use of fungicides throughout the production cycle, starting from mother plants protection in the field up to the production of wine and table grapes.

The process starts in nurseries where the project aims at improving both the phytosanitary state of rooted cuttings, reducing the endophytic presence of potentially pathogenic wood inhabiting fungi, and the quality of plant material, through the application of a consortium of microorganisms that increases microbial biodiversity in the rhizosphere.