

# EFFECT OF FUNGI ADDITION, ROOT PREPARATION, AND OTHER FACTORS ON THE SUCCESS OF VINE REPLACEMENT IN AN ESTABLISHED VINEYARD

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## Abstract

Dead or dying vines must be replaced regularly in order to ensure the sustainability of a vineyard. Successful plant replacement is crucial to maintain yield and quality by encouraging balanced root and leaf development in vines. However, young vines planted within an established vineyard encounter several problems, ranging from poor soil conditions to competition with older vines with well-established root systems.

**Aim:** The aim of this project is to study the impact of plant material and plant replacement practices, including the addition of microorganisms.

**Methods and Results:** Three trials were set up in the Médoc region, north of Bordeaux. The first trial had four modalities in which two rootstocks, previously inoculated with arbuscular mycorrhizal fungi (AMF) or not, were planted in April 2019. The second trial was designed to study the influence of root preparation (bare roots versus balled roots) and the period of replacement of the plants, while the third trial, set up in 2018, crossed the modalities of the root preparation and mycorrhization. Measurements of plant recovery rate, nitrogen status of the leaves and development of shoots were carried out.

In the first trial, the mycorrhization of the plants did not reveal differences in terms of recovery. It did not induce a better development of the shoots or differences in nitrogen status in leaves. In contrast, the other mycorrhizal trial found shoots from mycorrhized plants had better growth, although this result was not confirmed in the second year. The two trials addressing root preparation found that plants with balled roots had better shoot growth and nitrogen status compared to bare rooted plants. However, these results are moderated by other parameters of the trials such as planting period, soil maintenance practices or choice of the rootstock.

**Conclusions:** These results are a first step to understand the effect of fungi addition and root preparation on success of vine replacement. To conclude on the interest of balled plants or mycorrhization, these measures on development and production should be continued until the first years of grape production.

Keywords: Mycorrhization, rootstock, planting practices, shoots growth

### Introduction

For more than ten years, French viticulture has had to deal with the problem of vineyard decline leading to a consequent loss of annual yield (estimated at 4.6hL /ha in 2015). The decline of the vineyard is defined by the mortality of the vines or the premature decrease in their productivity, and has multiple causes, such as diseases linked to pathogens, plant feeding, climatic modifications or the impact of winegrower practices. One of the consequences of this progressive decline is the increased need to replace dead vines within a plot, which represents a significant cost for winegrowers. Many parameters can influence the success of the replacement that can be grouped into two main categories: the environment of the plant and the practices of the winegrower.

The quality of the soil, and more particularly the microbiological life of the soils, is often considered as a factor in the proper functioning of the soil, and the microbiological activity of the soils would be a good indicator of the overall health of the vineyard. More precisely, the rhizosphere, which is the portion of soil around the roots (a few millimeters) exhibits an abundance of microbial activity (Compant *et al.*, 2010). All the microorganisms (bacteria, fungi and viruses) present in the rhizosphere form the rhizospheric microbiota. Some of these microorganisms can be pathogenic and cause disease, others are neutral, and many of them are beneficial for plant growth. They can, among other things, promote access to mineral and water resources such as arbuscular mycorrhizal fungi (CMAs), or produce hormone-like compounds that promote plant growth. This is the case for certain bacteria called Plant Growth Promoting Bacteria (PGPB).

In the absence of pathological causes, one hypothesis would be that a deficiency in the functioning of the rhizospheric microbiota could be the cause of the plot's decline. Changes in the balance between positive and negative microorganisms, and in particular the absence or smaller populations of beneficial individuals in some plots could contribute to their decline. Thus, the addition of beneficial microorganisms (CMAs or PGPBs) during planting or replacement could be a promising practice. The objectives of our project, Vitirhizobiome, are (I) to study the influence of the rhyzospheric microbiota on the development of the scion according to the soil and the rootstock, and (II) to explore the interest of the addition of beneficial microorganisms during replacement.

#### **Materials and Methods**

## **Plot Characteristics**

To study the impact of the choice of plant material and plant replacement practices, including the addition of microorganisms, three trials were set up in the Médoc region, north of Bordeaux. The first trial was situated in the Saint-Julien appellation in a plot of Cabernet-Sauvignon on the 3309C rootstock planted in 1995 at the density of 10 000 plants/ha. A total of 200 plants were replaced in spring 2019 under four modalities with two different rootstocks, the initial rootstock of the plot (3309C) and the 420A MGt which is a rootstock conferring slightly stronger vigor than 3309C. Each rootstock has been previously inoculated with arbuscular mycorrhizal fungi (AMF) by the nurseryman or not. The second trial was designed to study the influence of root preparation and the period of replacement of the plants. It was carried out in Saint-Julien appellation, in plot planted in 1985 at the density of 8700 plants/ha with Cabernet-Sauvignon grafted on 101-14MGt. Four technical options were tested for replanting in 2018: two types of plant, bare roots versus balled roots, crossed with two periods of replacement, fall or spring. The third trial, set up in a Cabernet-Sauvignon plot planted in 1954 with a vine density of 8700 plants/ha in the Pauillac appellation, crosses modalities of the root preparation and mycorrhization. Three types of plant were used in 2018 to replace dead vines: bare root plants, balled root plants and plants in pots with mycorrhizal fungi.

#### Measurements

Twice a year, in spring and in autumn, the survival of replaced plants was noted. During summer, the number and the length of new shoots were measured on each plant replaced. At mid-veraison the nitrogen status of leaves was assessed with a Chlorophyll SPAD meter (Konica Minolta) at a rate of 3 measurements per plant in the first season and 5 measurements per plant during the second season.

#### **Statistical Analyses**

The number of healthy young plants and dead young plants were defined as a matched pair of counts. This variable was analysed as a proportion data using a GLM with binomial errors and logit link (Crawley, 2013). Overdispersion was checked by comparing residual deviance and residual degrees of freedom (R software; R Development Core Team 142 2010). Datasets were analysed separately for each of the three trials. We respectively tested the effect of AMF inoculation in interaction with rootstock type in the 1<sup>st</sup> trial, the interaction

between replanting period and plant type for the 2<sup>nd</sup> trial and the simple effect of plant type in the 3<sup>rd</sup> trial (3 modalities). Multiple comparisons were conducted using Tukey's HSD tests when we found a significant interactive or simple effect of treatments on the survival rate, shoot lengths or nitrogen status.

## **Results and Discussion**

# Survival

The mean survival rate (% +/-SE) was 97.9 % (+/-0.85) for the first trial, 91.7% (+/-1.1) for the second and 86.6% (+/-3.5) for the third trial. The first trial showed no difference between survival rate (LR Chisq=3.0, Df=3, p=0.4) because a few young plants were dead at the end of the first year (maximum of 2 dead plants per modality). On the second trial, we did not show any effect of plantation period and type of plants in the survival rate neither after one season nor one year after replanting (plantation period: LR Chisq=0.04, p=0.8; plant type: LR Chisq=0.07, p=0.8). In the third trial, we found a significant effect of the type plant used for replanting on survival rate (LR Chisq = 29.1, Df=2, p<0.0001). Bare-root plants had a lower survival rate than balled plants or plants in pot with mycorrhizae and *Trichoderma* (Figure 1).



**Figure 1:** Mean ( $\pm$ sd) survival rate after the first season for the first trial and after the first season and one year after plantation for the second and the third trials. Different letters above bars indicate significant differences (at P < 0.05). A specific model was used for each trial and for each survival notation.

## Growth

The first trial showed a significant effect of rootstock on growth but not of mycorrhizae inoculation (Rootstock effect: F=15.8, Df=1,  $p=1.0*10^{-4}$ ; Mycorrhizae effect: F=1.9, Df=1, p=0.2). Plants grafted on 3309C showed longer shoots than plants grafted on 420A MGt. The inoculation of mycorrhizae on plants did not induce differences in shoot length after the first season. On the second experimentation, neither plantation period nor plant type influenced plant growth (plantation period: F=0.3, Df=1, p=0.6; plant type: F=2.2, Df=1, p=0.1). However, in fall, balled plants showed slightly longer shoots than bare-root plants (non-significant trend). In the third trial, the same trend was observed. Shoots of balled plants are slightly longer than those with bare rooted plants or plants in pot with mycorrhizae and *Trichoderma* (F=2.4, Df=2, p=0.09) (Figure 2).



**Figure 2:** Mean (±sd) of shoots length at mid- veraison of the first season for the first trial and after one year for the second and the third trial. Different letters above bars indicate significant differences between modalities (at P < 0.05). A specific model was used for each trial.

### Nitrogen Status

The first trial showed an effect of rootstock on nitrogen status of young plants. Plants grafted on 3309C showed higher values of nitrogen content than plants grafted on 420A MGt (Chisq =19.8, Df=1, p=8.7\*10<sup>-6</sup>). Mycorrhizae inoculation did not influence plant nitrogen status (Chisq =0.9, Df=1, p=0.9). The interaction effect between rootstock and mycorrhizae inoculation is significant (F=5.4, Df=1, p=0.02) and highlighted that there were two opposite trends with the effect of mycorrhizae depending on rootstock. On 3309C, plants with mycorrhizae had slightly higher nitrogen status than plants without mycorrhizae. On 420A MGt, plants with mycorrhizae had slightly lower nitrogen status than plants without mycorrhizae. The second trial did not show difference of nitrogen status between plantation period and plant types (plantation period: Chisq=0.4, Df=1, p=0.5; plant type: Chisq=0.02, Df=1, p=0.9). No difference between plant types were shown in the third trial (Chisq= 4.6, Df=2, p=0.1) (Figure 3).



**Figure 3:** Mean (±sd) of nitrogen status at mid-veraison of the first season for the first trial and after one year for the second and the third trial. Different letters above bars indicate significant differences between modalities (at P < 0.05). A specific model was used for each trial.

#### Conclusion

Adding mycorrhizae to the roots of replacement plants does not induce any benefit in terms of either survival or plant development during the first year after planting.

In all three trials, the survival rate was very good. However, in the third trial, the mortality of the bare-rooted plants was higher than the other two types of plants. Practices of planting in fall rather than spring or using a more vigorous rootstock for replacement did not have an effect on mortality rates in our trials. These differences in practices or the addition of mycorrhizae could be more interesting on plots where the survival rate of the replacement plants is lower. Regarding the development of the plants, although no significant difference could be demonstrated, the balled root plants seem to have better growth. This improved growth cannot be linked to

better nitrogen supply to the plants. The genotype of the rootstock has a significant influence on plant growth which appears to be directly related to nitrogen supply. In our trial, the growth of young plants grafted on the same rootstock as the rest of the vineyard was better than those grafted on a slightly more vigorous rootstock.

The impact of the addition of mycorrhizae and the practices on the success of the replacement will have to be confirmed in the following years, going as far as the evaluation of the quantity and the quality of the grape. Only a few factors influencing the successful of replacement were tested in these trials. However, another focus of the project aims to understand the multitude of plot situations including the effects of more practices and the pedoclimatic context.

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