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Impacts of soil bicarbonate ($KHCO_3$) on growth and leaf-level parameters of different grapevine rootstocks

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“Life is not a problem to be solved,

but a reality to be experienced.”

(Søren Aabye Kierkegaard)

Abstract

Iron deficiency chlorosis is a common nutritional disorder in grapevine (*Vitis* ssp.) and occurs particularly in wine growing regions with calcareous soils as iron is less plant available under such conditions. Plants evolved various strategies to cope with this situation and grapevine is assumed to acidify the rhizosphere (strategy I plant). Grapevine rootstocks differ in their ability to prevent scion chlorosis, yet biochemical and physiological mechanisms are not fully understood. The thesis was to analyse the growth adaptations of different rootstocks to different levels of bicarbonate (HCO_3^-) under semi-controlled conditions and to relate this behaviour to physiological leaf parameters. Our hypothesis was that different rootstocks would adapt their growth pattern to stress severity, depending on their genetic background.

Rooting cuttings of four rootstocks (Fercal, K5BB, T5C, 3309C) were cultivated under semi-hydroponic conditions (pots, sand culture). Plants were fertilized daily with a ½-strength Hoagland solution and, after growth establishment, five treatments were applied: 0, 5, 7.5, 10, 12.5 mM $KHCO_3$ (daily application). Plant growth and physiological parameters (SPAD, chlorophyll fluorescence, gas exchange, hyperspectral reflectance) were measured on a weekly basis for 8 weeks.

Our results showed that root growth (Fercal) was stimulated, while growth of shoots and leaves was hardly influenced by stress treatments. Severest leaf symptoms were observed for 3309C, K5BB and T5C as shown by ≈ 30% lower SPAD values and deteriorated spectral indices (NDVI, PRI, MCARI). Symptoms were first observed for rootstock 3309C.

We could show that rootstocks adapt to carbonate stress levels differently and that measured leaf parameters facilitate an early detection of symptoms. Future studies should consider such differences in growth behaviour and symptom development and translate this information into biochemical processes of root exudation and nutrient uptake.

Keywords: grapevine rootstock, Fe deficiency, bicarbonate, chlorophyll, semi-hydroponic

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Abbreviations

i.e.	that is („*id est"*)
e.g.	example given
pH	power of hydrogen
e^-	electron (negatively charged subatomic particle)
ATP	Adenosine triphosphate
PS	phytosiderophore(s)
PS I	Photosystem 1
PS II	Photosystem 2
TCA	Tricarboxylic acid cycle
PEPC	Phosphoenolpyruvate carboxylase
OA	organic acids
CS	citrate synthase
MA`s	mugineic acid family phytosiderophores
CPI	chlorotic power index
HCO_3^-	bicarbonate
$KHCO_3$	Potassium bicarbonate
K5BB or 5BB	rootstock Kober 5BB
T5C or 5C	rootstock Teleki 5C
3309C or 3309	rootstock 3309 Couderc
Control	Control group
T-1 / T-2 / T-3 /T-4	treatment group 1 / 2 / 3 / 4
mM	millimole
ANOVA	Analysis of variances
KW	Kruskal-Wallis Test
n	sample size
$\bar{x}$ / Ø	mean value
SD	standard deviation
FW	fresh weight
DW	dry weight
SPAD	soil plant analysis development index
g_s	stomatal conductance (in mol H_2O m^{-2} s^{-2})
E	transpiration rate (in mmol H_2O m^{-2} s^{-2})

A	assimilation rate (µmol CO_2 m^{-2} s^{-2})
F_v/F_m	maximum quantum yield of PSII chemistry
F_0	emission by excited chlorophyll a molecules
F_m	maximum fluorescence value obtained for continuous light intensity
F_v	relates to the maximum capacity for photochemical quenching; calculated by subtracting F_0 from F_m
Tfm	time until the maximum fluorescence value (F_m) is reached
PI total	Performance index; sensitive and reliable index for physiological stress
NDVI	Normalized difference vegetation index
MCARI	Modified chlorophyll absorption in reflectance index
PRI	Photochemical reflectance index
NS	nutrient solution
DI-H_2O	deionized water
PAR	photosynthetic active radiation

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Introduction

Overview: Soil bicarbonate, Iron (Fe) and chlorosis

This Master Thesis deals with several physiological responses of different grapevine rootstocks in order to take up Fe when exposed to soil bicarbonate (HCO_3^-), mostly present in alkaline or calcareous soils (Dell`Orto et al., 2000). Many studies observed that high levels of HCO_3^- are associated with Fe deficiency of the grapevine, mainly because HCO_3^- acts as a "buffer" and hinders the Fe uptake of the plant (Dell'Orto et al., 2000; Gruber and Kosegarten, 2001). The micronutrient Fe plays a major role in the photosynthesis of green plants, especially in the biosynthesis of the chlorophyll pigments and in the electron transport chains (Ksouri et al., 2004; Spiller and Terry, 1980). Therefore, a deficiency of this micronutrient could lead to lower photosynthetic rates which would result in less growth (yield) or even may cause severe health issues (e.g. chlorosis) of the plants.

Strategies and approaches towards the problem of Fe deficiency

From the economic point of view of a winegrower, a short-term approach towards the problem of Fe deficiencies of grapevines could consist in applying foliar sprays containing Fe chelates[1]. Nevertheless, those applications often showed ineffective results leaving green spots where drops of Fe penetrated the leaf on a chlorotic background. The translocation rate of foliar applied Fe within the plants is relatively poor and make continuous and multiple applications necessary. This explains the rather high costs and only short term reliefs for plants when choosing this strategy (Chen and Barak, 1982).

A long-term approach could entail to initially plant grapevine rootstocks which are more tolerant to Fe deficient soils (Dell'Orto et al., 2000). However, the susceptibility of different grapevine varieties and/or rootstocks strongly depends on their genotypes, with some of them being more resistant towards Fe deficiencies of the soil/substrate than others (Dell'Orto et al., 2000; Ksouri et al., 2004; Erdem et al., 2010; Ksouri et al., 2007). Studies showed that genotypes more tolerant towards the above described soil conditions adapt themselves by showing several chemical responses, such as an increased total root biomass, acidification of the rhizosphere or the release of organic compounds (Covarrubias and Rombolà, 2013; Erdem et al., 2010).

[1] This method dates back to 1843 when Gris first applied ferrous sulphate ($FeSO_4$) in vineyards with chlorotic symptoms (Chen and Barak, 1982).

Approaches of this study

So far, physiological adaptions of grapevine rootstocks exposed to high bicarbonate concentrations (in this study: $KHCO_3$) are not fully understood. It is thus the aim of this thesis to characterize the main physiological responses of four different grapevine rootstocks in their capability to prevent scion chlorosis by applying different levels of bicarbonate in the soil solution. Rootstocks' adaptation strategies in terms of growth parameters will be analysed and described. In addition, parameters related to their photosynthetic activity will be examined and discussed.

Expected results

Since *Vitis vinifera* L. belongs to the „Strategy I"-plants[2] (described in detail in the chapter "Literature overview"), it is very likely that the rootstocks used in this experiment will increase both Fe-reductase activity and the net release of protons as well as the amount of organic compounds in their roots. This would lead to a lower pH, increasing the solubility of Fe^{3+} (Covarrubias and Rombolà, 2013). However, it is likely that - under the conditions of this experiment with sterilized siliceous acting as soil substrate - the rootstocks show severe Fe deficiency symptoms due to the missing (phyto-) siderophores produced by microbes in "normal" soil. Marsalha et al. (2000) observed similar effects in maize and sunflower plants.

According to Covarrubias and Rombolà (2013) grapevine rootstocks grown in Fe-deficient environment increased their root biomass. Building on these findings, plants would have a bigger total rhizosphere and therefore could possibly absorb more Fe. Moreover, since superficial plant biomass (i.e. leaves and shoots) is likely to be decreased (Gruber and Kosegarten, 2001), this would result in lower photosynthetic activity and subsequently in less sugar compounds produced. However, the biomass of the different plant organs might differ widely for the different rootstock genotypes used in our experimental setup.

Keeping the above described information in mind, it should be interesting to see to which extent the rootstocks will be able to compensate for the missing generated compounds of photosynthesis with nutrients taken up by a possibly higher root biomass.

[2] Most non-graminaceous plants are „Strategy 1"-plants, which mainly acidify the rhizosphere to reduce Fe^{3+}.

Hypothesis

To summarize, three main hypotheses can be formulated for the experiment:

H1: Grapevine rootstock genotypes adapt the growth behaviour of their roots and shoots differently when coping with bicarbonate induced stress.

H2: In the presence of bicarbonate, grapevine rootstocks tolerant to bicarbonate induced Fe deficiency adapt their metabolism differently than susceptible ones. These adaptions can be measured in a non-destructive way.

H3: In the presence of bicarbonate, rootstocks adapt the amount of root exudation. In general, more total carbon will be exudated by rootstocks with a higher root biomass.

Literature overview

Photosynthesis

The main process of dry matter accumulation of plants is called photosynthesis. Characterized by the capability of plants to generate sugar-molecules with the input of water, CO_2 and (sun-)light, this process generally allows plants to build new cells and therefore to grow (Figure 1[3]).

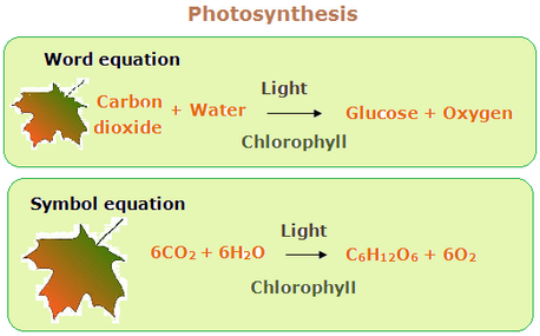


Figure 1: Photosynthesis

Chlorophyll

In order to perform photosynthesis, light energy is absorbed by chlorophyll, carotenoids and other pigment molecules present in the photosynthetic antenna in the thylakoid membranes of green plants. It can be divided in two different reactions: In the first one, the "light-dependent reactions", chlorophyll molecules in the cell membranes of the chloroplasts capture light and produce chemical molecules and coenzymes. In the second chain of reactions ("dark reactions"), the newly produced molecules then synthesize carbohydrates with CO_2 of the surrounding atmosphere (Misra et al., 2012).

The above mentioned chlorophyll is one of the key elements in order to execute photosynthesis. Those molecules can be found in and around pigment protein complexes called photosystems which are located in the thylakoid membranes of the chloroplasts. The functions of chlorophyll consist of absorbing light and transferring the captured light energy by so called "resonance energy transfer" to a specific chlorophyll pair in the reaction centre of a photosystem (Misra et al., 2012; Lumitos, 2020). Currently, we know of two

[3]https://biology-igcse.weebly.com/the-equation-for-photosynthesis.html

photosystems with specific wavelength selectivity of the chlorophyll involved: Photosystem I and Photosystem II (P680 and P700[4]).
Light energy absorbed by the chlorophyll molecule can undergo three different processes:

1 -> drive photosynthesis ("photochemistry")
2 -> dissipate excess energy as heat
3 -> re-emit excess energy as chlorophyll fluorescence

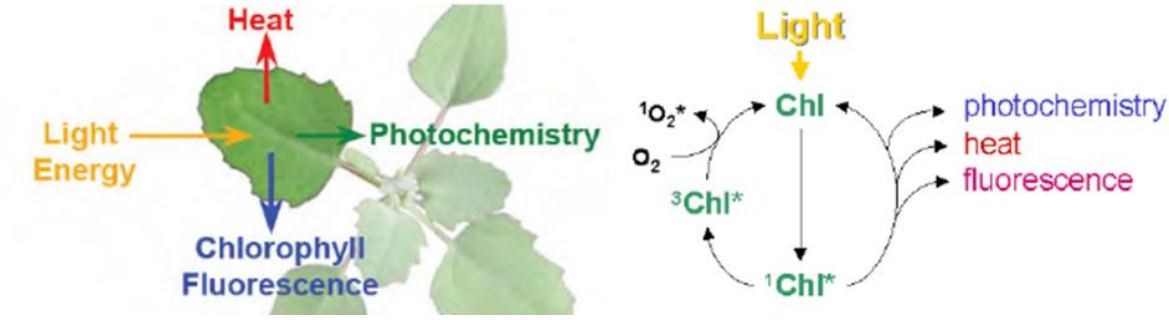


Figure 2: Possible pathways of light energy absorbed by plants (Misra et al., 2012)

These three processes always occur in competition, as shown in Figure 2. Therefore, under optimum conditions for photosynthesis (i.e. pathway for plant growth), only a tiny proportion of absorbed light is lost as heat or as red chlorophyll fluorescence (Pandey and Gopal, 2011). Still, various stress conditions, such as drought stress or nutrient deficiencies, can reduce the rate of photosynthesis and therefore lead to a decreased CO_2-assimilation. In such a case the light-driven photosynthetic e^--transport as well as the photosynthetic pigment apparatus would be blocked or disturbed without affecting the process of light absorption itself. An increased de-excitation of absorbed light energy via the chlorophyll fluorescence and/or heat emission would be the result (Pandey and Gopal, 2011).

The main function of the chlorophyll of the reaction-centre is undoubtedly to drive photosynthesis, which means that the initially absorbed energy is being transferred to other chlorophyll pigments within the photosystem. When driving photosynthesis, the reaction-centre chlorophyll additionally undergoes a charge separation, which can be described as a specific redox-reaction in which the involved chlorophyll donates an electron (e^-) into a series of molecular intermediates, known as "electron transport chain" (Pandey and Gopal,

[4] wavelength of their red-peak absorption maximum in nm

2011). The newly charged chlorophyll of the reaction-centre ($P680^+$) will be reduced back to its ground state simply by accepting an e^-. Meanwhile, in Photosystem II, the e^- which has reduced $P680^+$ ultimately, originates from the oxidation of H_2O and H^+ through several intermediates. The hereby resulting flow of e^- produced by the reaction-centre chlorophyll pigments is indispensable in order to shuttle H^+-ions across the thylakoid membrane, which - in turn - sets up a chemi-osmotic potential mainly used to produce ATP (chemical energy). Amongst other processes, those e^- are used to reduce $NADP^+$ to NADPH which is a universal reductant used to reduce CO_2 into sugars as well as for driving other biosynthetic reductions (Lumitos, 2020).

Bavaresco et al. (2006) found out that chlorotic plants had lower iron content in their leaves, lower leaf area, and lower fresh and dry matter. They conclude that these facts are the reason of a reduced CO_2-fixation. The authors clarify their thesis by showing that chlorotic plants had lower rates of photosynthesis and transpiration and a lower stomatal conductance in comparison to the control plants.
The described functions of chlorophyll and the photosynthesis in general and their impacts on grapevines' metabolism highlight the life-essential importance they have for plants' metabolism. This explains and justifies why those parameters not only are incorporated in the conducted experiment of this Master thesis, but play a major role in the analysed parameters.

Iron (Fe)

Physiological importance of Fe for plants

Among all essential micronutrients for plants, Fe is required most since it is an important component in heme, the Fe-sulphur-cluster or other Fe-binding sites. Additionally, it is indispensable for processes such as photosynthesis, respiration or the chlorophyll biosynthesis (Kobayashi and Nishizawa, 2012).

Thus, Fe deficiency could lead to a (lime-induced) Fe chlorosis since chlorosis is linked to the lack of bivalent Fe, which is – as already mentioned - a main driver in the biosynthesis of chlorophyll pigments. In total, up to 60% of all leaf iron happens to be concentrated in the chloroplasts (Ksouri et al., 2004). Consequently, Fe deficiency can reduce both, the number of chloroplasts per cell, as well as the chlorophyll content per chloroplast (Chen and Barak, 1982). Spiller and Terry (1980) cite evidence that the whole light harvesting apparatus[5] would also be retarded in plants with Fe deficiencies. Furthermore, Fe is involved in several other biochemical processes, such as the electron-transport chains in the mitochondria and the chloroplasts, with both of them being essential processes in the metabolism of plants in general (Bavaresco et al., 2006). Chen and Barak (1982) describe that there is evidence that ferrous iron (Fe^{2+}) is involved in the condensation of succinic acid and glycine to form γ-aminolevulinic acid, which is also necessary in the process of capturing CO_2. Finally, Bar-Akiwa and Lavon (1968) revealed, that the Fe containing heme compounds (e.g.: cytochromes, peroxidase, catalase, ferredoxin) may have reduced activities under Fe deficiency. Since Fe deficient chlorotic (i.e. yellow) leaves might not be able to produce enough energy for the H^+-ATPase, negative effects on photosynthetic rates and therefore less growth (yield) are often a directly correlated consequence of Fe deficiency (Gruber and Kosegarten, 2001). Bearing all the above mentioned involvements of Fe in plants' metabolism in mind, this highlights the importance of this micronutrient for plants.

Fe abundance in the soil

Although Fe is very abundant in the soil[6], it is only slightly soluble under aerobic conditions and even less plant-available in soils with high pH and in calcareous soils respectively (Marschner, 1995). According to Colombo et al. (2013) the chemical species of Fe present in the soil environment can be summarized as follows:

[5] includes chloroplast membranes, chlorophyll-protein-complexes, carotenoids, reaction-centres and electron carriers amongst others.

[6] Iron is the fourth most abundant element in the earth's lithosphere, following oxygen, silicon, and aluminium (Chen and Barak, 1982).

- Fe II (Fe^{2+}) in primary minerals
- Fe III (Fe^{3+}) in secondary minerals, as Fe crystalline minerals and poorly ordered crystalline (hydro)oxides
- soluble and exchangeable Fe
- Fe bound to organic matter in soluble or insoluble forms

Under aerated conditions and pH values above 7, it has been estimated that the total concentration of inorganic (soluble) Fe species in the soil solution is, 10^4–10^5-fold lower than that required for an optimal growth of most of the plants (Römheld and Marschner, 1986). Therefore, Fe deficiency is a frequent problem for many crops, particularly in calcareous soils[7] (Mengel et al., 2001).

The vast majority of iron in the earth`s crust is present in the form of ferromagnesium silicates which precipitate as oxides or hydroxides by weathering processes (Chen and Barak, 1982). According to Lindsay (1979) the most abundant soluble iron species present in solutions in the pH-range of 7-9 are $Fe(OH)_2^+$, $Fe(OH)_3$ and $Fe(OH)_4^-$.

Fe uptake: different strategies

In order to be taken up by plants, all nutrients present in the soil solution must first be transported to the root surface (i.e. rhizosphere) somehow. The two main (passive) mechanisms driving this transport are called mass flow and diffusion (Oliveira et al., 2010). Barber (1974) defines mass flow as "the total potential gradient regulating the water movement in the soil-plant-atmosphere. Thus, (primarily) the soil solution concentration and plant transpiration rate determine the quantity of ions transported through this mechanism." Nevertheless, mass flow strongly depends on the plants species' demand, which is characterized by different transpiration rate, root activity and nutrient selectivity. Additionally, soil components and the analysed nutrients' properties itself influence mass flow (Marschner, 1995). Concerning the interaction of nutrients with plants, two terms have to be distinguished: The efficiency of nutrient acquisition by the roots is defined by the total nutrient uptake per plant, while the nutrient-use efficiency describes the nutrient utilization within the plant and is evaluable by the dry matter produced per unit nutrient in the dry matter (Schroth et al., 2003).

[7] The effect of the "HCO_3^--buffer", which hinders Fe uptake of plants in calcareous soils will be described more detailed in following chapters.

Looking at the Fe uptake of plants in general, there are two different plant strategies, classified as "Strategy I and Strategy II plants". In this context, Naranjo Arcos and Bauer (2016) describe that Strategy I plants, such as grapevine, can take up Fe in the following ways: In order to liberate and solubilise Fe^{3+} ions, a proton pump acidifies the rhizosphere. The secretion of phenolic compounds increases the solubilisation of Fe. Another possibility would consist in the Fe reductase (Fe(III)-reductase), reducing Fe^{3+} to Fe^{2+} which is finally being transported into the epidermis cell by an Fe transporter. Inside of the plant, citric acid or nicotian-amine ($C_{12}H_{21}N_3O_6$) chelate Fe^{2+} for further transport within the plant via xylem or phloem. The Fe uptake in Strategy II[8] plants, exemplified by *Zea mays* and *Oryza sativa* consists of two steps: firstly, phytosiderophores (PS) are exported into the rhizosphere to solubilize Fe^{3+} ions. The Fe^{3+} /PS complex is then being transported by protein carriers. The pathways of these different strategies is displayed in Figure 3.

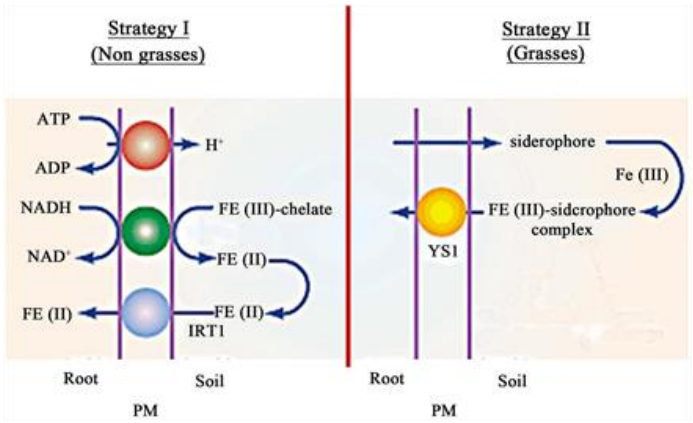


Figure 3: Acquisition of Fe in Strategy I and Strategy II – plants (Seraj and Rahman, 2018)

Physiological response: increased Fe (III)-reductase activity

Specifically looking at the uptake of the nutrient Fe of grapevine, Chaney et al. (1972) have stated that Fe, for example present in the form of ferric chelate Fe^{3+}-EDTA, is first being reduced to Fe^{2+} at the root surface so that it can be absorbed by grapevine roots at all. Consequently, this means that Fe is taken up actively by grapevine and not only via passive mass-flow or diffusion. Bradford et al. (1971) concluded that ½ to ¾ of the absorbed Fe is "pre-processed" and taken up by several other processes prior to mass-flow and Oliver and Barber (1966) even state that only 3-9% of Fe are exceptionally taken up via mass-flow, while the vast majority is taken up by root interception, which permits a microenvironment

[8] Basically only species belonging to the order of *Poales*

favourable to Fe uptake afterwards. The uptake-strategy of the plant reducing ferric chelates (Fe^{3+} -> Fe^{2+}) at the root surface with an absorption of the bioavailable, newly generated ferrous ions across the root plasma membrane is also supported by Kobayashi and Nishizawa (2012). This process is characterized by increased activities of the plasmalemma-linked H^+-ATPase and an increased root Fe (III)-reductase activity (Dell'Orto et al., 2000; Ksouri et al., 2004).

Physiological response: acidifying the rhizosphere

Another pathway of Fe uptake - usually occurring parallel to the one described above - consists in the excretion (i.e. release) of proton and phenolic compounds from the roots to the rhizosphere in order to increase the solubility of ferric ions (Fe^{3+}) by an acidified rhizosphere. Kashirad and Marschner (1974) revealed that roots of the relatively Fe deficient sunflower plant (*Helianthus annuus*) were able to strongly decrease the pH-value of a nutrient solution when being confronted with Fe stress. They were therefore able to mobilize Fe from Fe oxides more easily. Ksouri et al. (2004) revealed that, while grapevine rootstock control plants, growing in neutral commonly used nutrient solutions and thus not suffering Fe deficiency, did not acidify the rhizosphere, the rhizosphere was strongly acidified by Fe deficiency tolerant rootstocks in Fe deficient environments, and environments with high contents of bicarbonate in the soil solution respectively. Ksouri et al. (2007) made the same findings while using different rootstocks in some other experiment. Covarrubias and Rombolà (2013) found out that in an Fe deficient environment, the grapevine rootstock 140 Ruggeri - which is considered as lime (i.e. bicarbonate) tolerant (Ksouri et al., 2004) - showed an increased activity in the Phosphoenolpyruvate carboxylase (PEPC) and increased citric acid concentration in its roots when bicarbonate was added to the nutrient solution. Other plant responses in Fe-deficient environments can also be related to tricarboxylic acid cycle (TCA) related enzymes (Covarrubias and Rombolà, 2014). Different patterns in the accumulation, translocation or exudation of organic acids (OA) from plants' roots were observed under stressful situations, i.e. when bicarbonate was present (Covarrubias and Rombolà, 2014). In the same study the authors revealed that the PEPC activity did not increase in genotypes susceptible to Fe deficiency when bicarbonate was present, thereby clarifying the influence of the soil composition towards physiological plant responses in general. High bicarbonate concentrations in the soil induce Fe chlorosis very easily in sensible grapevine rootstocks, mainly through the "HCO_3^--buffer" effect which neutralises

the root H^+-ATPase activity and decreases the Fe^{3+}-reductase, hence the Fe reduction (Ksouri et al., 2007).

Fe uptake in the presence of soil bicarbonate: rootstock genotypes

The capability to acidify the rhizosphere in general varies strongly from genotype to genotype, with the more tolerant ones being able to decrease (i.e. acidify) the pH-value of the rhizosphere by 1.0 units or more (Dell`Orto et al., 2000). The authors mentioned that rootstocks of *Vitis riparia* were very inefficient in acidifying the rhizosphere, hence rootstocks containing genetic material of *Vitis riparia* would be less successful in Fe deficient environments. Erdem et al. (2010) state, that – amongst others – especially the grapevine rootstock Fercal would display remarkable tolerance to lime-chlorosis when being irrigated with a bicarbonate-containing solution. This, once more, leads to the conclusion that the susceptibility of chlorosis strongly varies among different grapevine rootstock genotypes. Covarrubias and Rombolà (2013) revealed that the Fe chlorosis tolerant grapevine rootstock Ruggeri 140 changed its response mechanisms towards Fe deficiency strongly when bicarbonate, acting as a buffer, was present. They observed an increased root malic acid concentration as well as an increased total root biomass, which could be interpreted as the plant`s intention of increasing the total absorbance area for nutrients under stressful conditions. Moreover, the activity of PEPC and TCA related enzymes (CS, $NADP^+$-IDH) increased, as well as the accumulation and translocation of organic acids in the Fe deprived plants. In another experiment Ksouri et al. (2007) analysed Tunisian autochthonous grapevine varieties which were considered as not tolerant towards Fe deficiency. They showed severe deteriorations in growth parameters, leaf aspect and chlorophyll status of the grapevines grown in environments where bicarbonate was present. These authors stated that Fe deficiency tolerant genotypes where able to acidify the medium by 0,5 pH units and showed markedly stimulated activity of Fe(III)-reductase and PEPC, which underlines the findings of the importance of these physiological plant responses once more.

Interestingly, Ksouri et al. (2007) stated that winegrowers should bear in mind that irrigation water used in vineyards could contain high levels of bicarbonate[9], which could become another undesired source of bicarbonate, adding to soil composition.

[9] Depending on the geographical region of the vineyard and its predominant water-attributes.

Fe translocation in the plant

After Fe has been taken up by the plant, various types of influx and efflux transporters as well as suitable chelators help to translocate the micronutrient within the plant. According to Kobayashi and Nishizawa (2012) the translocation consists of the following processes:

- radial transport across the root tissues (includes symplastic[10] transport through the Casparian strips[11]);
- xylem loading, transport and unloading;
- xylem-to-phloem transfer;
- phloem loading, transport and unloading;
- symplastic movement toward the site of demand; and
- retranslocation from source or senescing tissue.

The authors list citrate, nicotianamine and mugineic acid family phytosiderophores (MA's) as the principal chelators and mention that due to xylem and phloem consisting of dead and living cells respectively, xylem loading is assumed to require efflux transporters, while phloem loading would require influx transporters. Furthermore, they highlight the essential need of Fe being delivered to appropriate plant compartments in order to be utilized there and to prevent it from being accumulated in excess in certain plant tissues (cytotoxicity). While the chloroplast and the mitochondrion are the two main Fe requiring sites in the cell, the vacuole generally functions as a metal-pool to avoid cytotoxicity (Kobayashi and Nishizawa, 2012).

Methods to measure Fe deficiencies

From the viewpoint of a scientist it is of great interest to know how to measure whether plants are suffering from Fe deficiency. The following list gives a short overview of the most commonly used ways to evaluate the Fe status of a plant and it also highlights the weaknesses of each of the approaches:

[10] The movement of water and solubles happens in between the cytoplasm and the vacuoles through the plasma membranes and plasmodesmata and beyond the cortex of plant cells. The pathway is slower when compared to the apoplastic pathway (Byjus, 2020).
[11] water-impermeable deposits of suberin that regulate water and mineral uptake by the roots (Encyclopaedia Britannica, 2020)

1. **Approach:** Total Fe of plant tissue or total chlorophyll content or Fe:chlorophyll ratio of leaf tissue

- **Theory:** Since lime-induced chlorosis can be linked to Fe deficiency (as described previously), chlorosis inducing low levels of Fe content should be examined.
- **Strengths / Weaknesses:** In spite of high absolute concentrations of Fe in roots and leaves of vines grown in calcareous soils, plants suffered from Fe deficiency. This means that iron – even if it is present within the plant – can be inactivated in the leaf (Gruber and Kosegarten, 2001). The variance in the Fe:chlorophyll ratio is in fact very high, and indicates that chlorotic leaves may contain as much or even more iron than healthy leaves. Therefore, total iron may show no relationship to the chlorotic or healthy appearing of the plant (Chen and Barak, 1982). Furthermore, the chlorophyll measurements must be checked against the possibility that low values are due to leaf senescence or nutrient stress other than Fe. This could be executed by the use of a control treatment using other remedies, such as FeEDDHA (Chen and Barak, 1982).

2. **Approach:** Total Fe:total N or cation:anion ratios

- **Theory:** North and Wallace (1952) found that a high absorption of nitrate (NO_3^-) caused chlorosis in avocado and suggested the use of Fe:N as a means to measure Fe deficiency. Chen and Barak (1982) report same findings in tobacco, macadamia and soybeans, which all belong to the Strategy I plants.
- **Strengths / weaknesses:** No_3^- is the principal anion absorbed by roots and therefore governs the cation:anion ratio as well as the bicarbonate excretion into the rhizosphere. An increased accumulation of organic anions with increasing NO_3^- concentrations in the nutrient solution can be explained as a result of reduction of NO_3^- in plant shoots in the course of assimilating NO_3^-. Therefore, simply linking Fe chlorosis with organic anion accumulation does not suggest a cause and effect phenomenon, but rather that both (i.e. chlorosis and organic anion accumulation) are different effects of high NO_3^- adsorption rates (Kirkby and Knight, 1977). Moreover, sorghum in Steinberg`s nutrient solution (contains the cation NH_4^+) reduced the pH 2.5 units, whereas in Hoagland's No. 1 solution (contains NO_3^-) the initial pH increased by 2.5 units (Esty et al., 1980). The amount of Fe was the same in both nutrient solutions, which does not allow to draw conclusions from the total Fe content itself, but necessitates looking at the chemical form of iron present in the soil solution.

3. **Approach:** Activity of peroxidase

- **Theory:** Peroxidase is an Fe hemoprotein in leaf tissue. Measurements of peroxidase activity can be used to distinguish Fe deficiency from manganese deficiency (Bar-Akiwa, 1961).
- **Strengths / weaknesses:** Varietal differences in peroxidase levels may exist independent of Fe nutrition status, as concluded by Chen and Barak (1982).

The methods mentioned above would only serve to measure Fe deficiencies directly and therefore are different approaches than the methods used to evaluate grapevine rootstocks tolerant to lime induced Fe deficiencies. Nevertheless, evaluating chlorosis tolerance levels of rootstocks requires a detailed knowledge of the whole system involving the interdependencies of a plants' nutritional status, the Fe content in the soil and the plant tissues and the signs and reasons of chlorosis.

Rootstocks

Historical overview of rootstocks

Upon the accidental introduction of the American aphid pest grape phylloxera (*Daktulosphaira vitifoliae F.*) to Europe in the late 19th century, nearly all European vineyards were destroyed because the roots of European *Vitis vinifera* varieties lack phylloxera tolerance. However, introducing the technique of grafting European grape varieties onto naturally phylloxera-resistant native American grape varieties[12], helped to save and maintain Europe's viticulture and became an integral part of successful modern growing of grapevine. Thus, phylloxera tolerance was combined with fruit quality (Gautier et al., 2020). The grapevine varieties that we nowadays know under the name "rootstocks", are the ancestors of such native American grape varieties.

Influence on the vine

Today, vineyards exist on nearly all continents and are widely distributed in very different climatic, geological and topographical regions, consequently resulting in different requirements of the entire plants and the rootstocks. Rootstocks play an important and often underestimated role, although they have to react towards these different climatic and soil specific parameters. Since rootstocks form the connection between the soil and the rest of the engrafted plant organs, they directly influence vigour, phenology, resistance to pests, fruit quality, yield, and tolerance to deleterious environmental conditions such as water deficit and nutrient limitations (Warschefsky et al., 2016).
Therefore, if a rootstock is chosen adequately, it contributes not only to a healthy vine but also influences the quality of the grape and eventually the wine. (Bavaresco et al., 2005).

The use of rootstocks for several different crops originates from the desire to combine desirable traits of two different genotypes in the same plant. For vine, the "scion variety" (e.g. Chardonnay, Grüner Veltliner, Pinot gris) which produces the aboveground parts (i.e. trunk, shoots and fruit) is grafted onto the rootstock variety (e.g. Fercal, 5BB, 5C, 3309) which provides the root system and the lower part of the trunk. The position where scion and rootstock are joined by grafting grows together and is called grafting union (Goldhammer, 2018).

[12] e.g.: *Vitis rupestris, Vitis berlandieri, Vitis riparia*

Main breeding material for rootstocks worldwide

According to Gautier et al. (2020), there are about 30 different American *Vitis spp.*, however, the majority of breeding partners for viticulture are a result of breeding programs between just three American *Vitis spp.*: *Vitis riparia, Vitis rupestris* and *Vitis berlandieri*. The Vitis International Variety Catalogue database –VIVC (2020) states that 47% of the currently 83 different rootstocks used in Europe are the result of interspecies crosses between the three species mentioned above and that all rootstocks used in Europe have at least one of these three *Vitis spp.* in their genetic background[13]. Those three American *Vitis spp.* all have different properties and requirements, which are listed below.

Vitis rupestris

- deep root system and vigorous growth
- often selected for use in warm regions with long seasons

Vitis riparia

- roots grow laterally
- prefers cool, naturally fertile soils well supplied with water
- does not do well in calcareous soils

Vitis berlandieri

- fleshy deep root system and vigorously climbing vine
- very drought and frost tolerant

As listed above, all of the three *Vitis* spp. have quite different desirable and undesirable attributes which is why cross-breeding was implemented to combine the desirable traits.

[13] 47 % with *V. berlandieri*, 52 % with *V. riparia* and 30 % with *V. rupestris*

Characteristics of the rootstocks used in this experiment

In the following list the main traits of the rootstocks used in the experiment will be shown.

Fercal

- **1B** (*Vitis berlandieri* x *Vitis vinifera* cv. Ugni blanc) x **31 Richter** (*Vitis berlandieri* cv. Rességuier number 2 x *Vitis longii* cv. Novo-mexicana)
- Fercal features both, a good drought and soil wetness resistance given that roots are properly developed, thus it is susceptible to drought issues particularly in juvenile stages (Pl@ant grape, 2019-20).
- The rootstock does not tolerate compacted soils and has problems absorbing Mg, especially in soils with high contents of K (Pavloušek, 2008; Martínez et al., 1990; Carbonell-Bejerano et al., 2016).

The most important attribute of Fercal is its resistance to limestone: It is resistant to active limestone contents in the soil of up to 45% and an CPI[14] of up to 120 (Morrisson Couderc – Grapevine Nurseries (2017).

3309 Couderc (3309)

- *V. riparia* x *V. rupestris.*
- 3309 prefers deep, fertile and moist soils since it features low drought resistance.
- It offers low vigour to the scion but may accelerate ripening.
- 3309`s resistance towards active limestone concentration in the soil is very low since it only tolerates a content of about 11% or a CPI of 10 (Morrisson Couderc – Grapevine Nurseries (2017).

Kober 5BB (5BB) and Teleki 5C (5C)

- *V. berlandieri* x *V. riparia*.
- They offer high vigour to the scion, with 5BB being one of the most vigorous rootstocks known. 5C may accelerate ripening of the berries.
- Both rootstocks are very susceptible to drought stress.
- 5BB and 5C are moderately resistant towards active limestone content of the soil; 5C tolerates up to 17% and 5BB up to 20% of this soil parameter. Both rootstocks tolerate a CPI of up to 40 (Morrisson Couderc – Grapevine Nurseries (2017).

Figures 4-8 provide an overview of some important parameters of rootstock attributes.

[14] Chlorotic power index (CPI = ((active lime content (in %) / Fe^2) x 10.000)
→ Fe^2 refers to the amount of easily extractable Fe in mg/kg

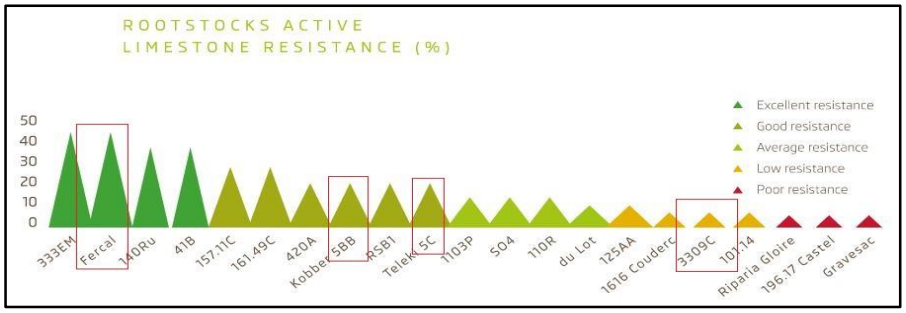


Figure 4: Resistance towards active limestone (Morrisson Couderc – Grapevine Nurseries, 2017)

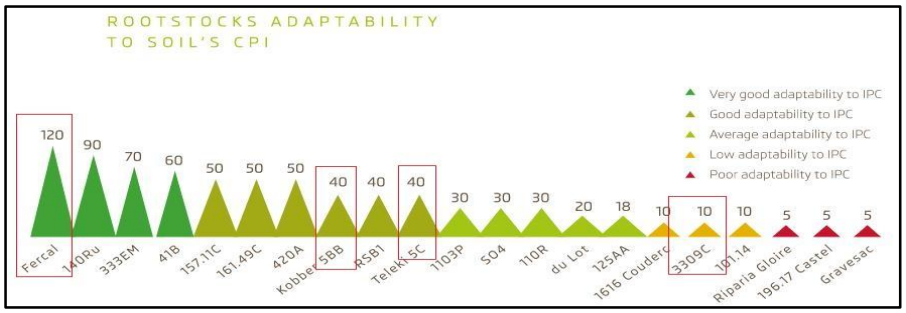


Figure 5: Adaptability to soil`s CPI (Morrisson Couderc – Grapevine Nurseries, 2017)

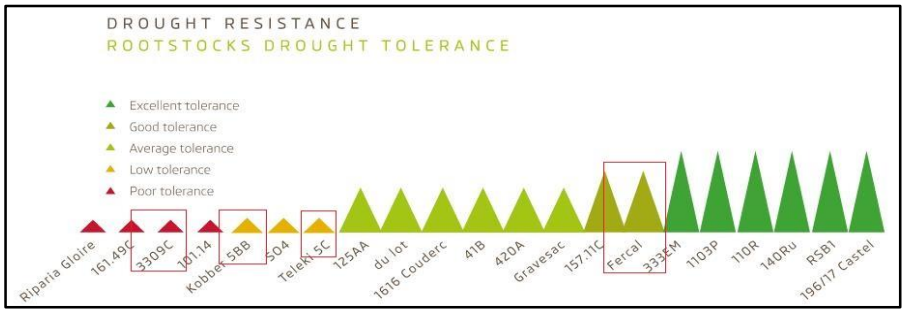


Figure 6: Drought resistance (Morrisson Couderc – Grapevine Nurseries, 2017)

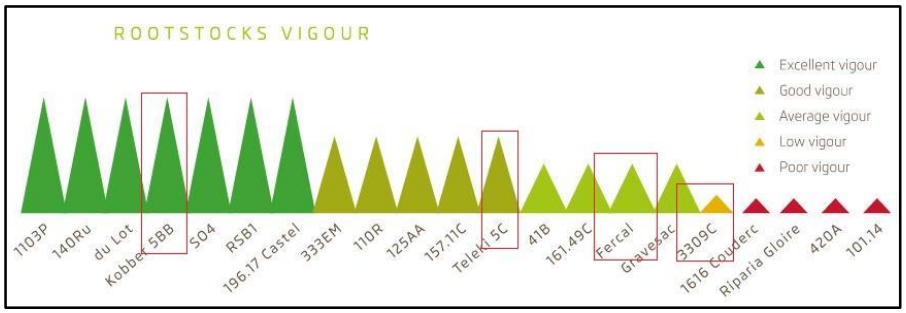


Figure 7: Vigour (Morrisson Couderc – Grapevine Nurseries, 2017)

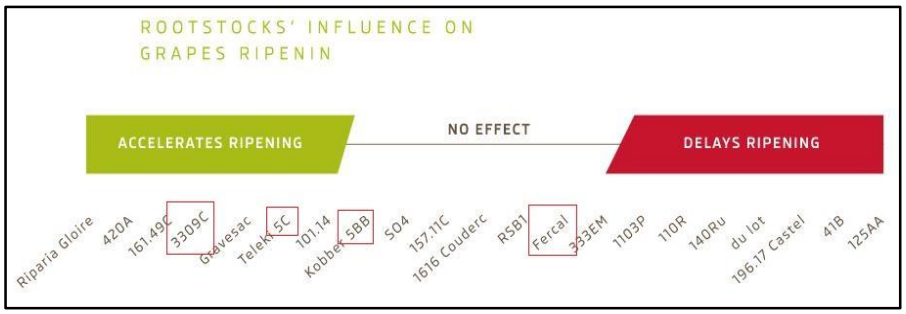


Figure 8: Influence on grapes ripening (Morrisson Couderc – Grapevine Nurseries, 2017)

Soil attributes in general and in regions with abundance of limestone in their soils

As briefly indicated in chapter “Iron (Fe)” it is not only the nutrient content and composition of a soil that influences nutrient uptake of plants. Soil parameters, such as parent material, density (structure texture and particle distribution), water content, pH, aeration and temperature, clay-mineral content and cation-exchange-capacity (CEC), and/or mycorrhiza development influence the bioavailability of nutrients as well (El Ramady et al., 2014). Bearing these influences in mind, it is close to impossible to find the same environmental conditions twice on earth. This stresses the importance for winegrowers to first know about them and secondly, to choose the right plant material (i.e. grapevine rootstock and scion) for the region concerned.

In many famous winegrowing regions such as the Champagne, Cognac or Burgundy in France or some important winegrowing regions in Spain (e.g. Valencia) a relatively high limestone content in the soils can be observed. Therefore, the need to find adequate rootstocks which are able to acquire Fe even though bicarbonate locks it up, is an important and current issue in many countries worldwide, since the precautionary and long-term approach of choosing rootstocks tolerant to Fe deficiencies would be significantly less cost-intense than applying Fe with foliar sprays.

Material and methods

Plant setup

Location, environmental surroundings and climatic conditions

The entire practical part of the experiment was conducted in greenhouses, laboratories and other premises within the facilities of BOKU's site in Tulln (UFT – "Universitäts- und Forschungszentrum")[15] and lasted from the beginning of May until mid-August of 2020.

From 19 June onwards the plants were located in a greenhouse with "semi-controlled conditions". This means that no drastic adjustments concerning temperature, humidity or light intensity were made. During June, July and August 2020 the weather in Tulln showed strong oscillations resulting in temperature maxima and minima in the greenhouse of about 40° C and 20° C respectively.
Light intensity during the extremely hot and sunny days was eased by closing the sunshade underneath the greenhouse roof, however, since during measurements plants had to be exposed to full sunlight, this sunshade was kept open during them.

Rootstocks and experimental design

The following 4 different grapevine-rootstocks with different levels of chlorosis-susceptibility were chosen for this experiment:

Fercal	(1B x R 31[16])	= tolerates active lime content of 45%[17]
Kober 5BB	(*Vitis berlandieri* x *Vitis riparia*)	= tolerates active lime content of 20%[18]
Teleki 5C	(*Vitis berlandieri* x *Vitis riparia*)	= tolerates active lime content of 17%[19]
3309 Couderc	(*Vitis riparia* x *Vitis rupestris*)	= tolerates active lime content of 11%[20]

[15] coordinates of the site: **48° 19′ 12″ N, 16° 3′ 59″ O**
[16] breeding partners described more in detail in chapter "Literature overview"
[17] Martínez et al., 1990
[18] Mercier, 2020
[19] Wineplant, 2020
[20] Martínez et al., 1990

Woody cuttings of those four rootstocks were obtained from the following institutions:

Fercal	"G&H Scheiblhofer Reben GmbH[21]" and own production
Kober 5BB	„Hochschule Geisenheim University[22]" and own production
Teleki 5C	own production
3309 Couderc	„Hochschule Geisenheim University"

The roots of small plantlets of the above described rootstocks were tipped into a growth-stimulating solution on 7 May 2020, put into Perlite and kept in a sufficiently watered plastic box inside a cultivation room. The growth-stimulating solution was prepared as follows:

Table 1: Preparation of the growth-stimulating solution

❖ **Preparation of the two stock solutions**	
stock solution "IBA"	stock solution "NAA"
weigh in 200 mg of Indole-3-butyric acid (IBA) and put it into a 15 ml Falcon™ centrifuge tube	weigh in 200 mg of 1-Naphthaleneacetic acid (NAA) and put it into a 15 ml Falcon™ centrifuge tube
add 8 ml of Ethanol (EtOH) and 2 ml of DI-H_2O and mix it	add 8 ml of Ethanol (EtOH) and 2 ml of DI-H_2O and mix it
wrap the Falcon™ with aluminium foil and store it at 4° C	wrap the Falcon™ with aluminium foil and store it at 4° C
❖ **Finalization of the growth stimulating solution**	
mix 2,575 ml of the "IBA" stock solution with 1,650 ml of the "NAA" stock solution	
add 45,775 ml DI-H_2O and mix everything	
wrap the centrifuge tube with aluminium foil and store it at 4° C	

With regard to some of the results, it is highly important to mention that small plantlets of rootstock Fercal were only rooting very slowly in comparison with the other rootstocks. Hence, Fercal started the experiment in a stressed condition and was less developed (underground organs) than the other plants.

[21] https://www.scheiblhofer-reben.at/
[22] https://www.hs-geisenheim.de/

On 19 June 2020, when the rootstocks had built proper roots and first leaves, they were translocated into a greenhouse on the campus of the BOKU-University in Tulln, Lower Austria. They were planted in 1 litre pots filled with a thin layer of Perlite at the bottom and a mixture of sterilized fine siliceous quartz-sand[23] acting as soil-component. All of the pots were placed onto plastic plates.

Six rooted cuttings of each of the four rootstocks were chosen and separated into 5 different treatment groups, resulting in 120 plants in total and 24 plants (4 different rootstocks x 6 plants) within each of the following groups:

- Control group: ½-strength Hoagland solution, 0 mM $KHCO_3$
- Treatment-1 (T-1): ½-strength Hoagland solution, 5 mM $KHCO_3$
- Treatment-2 (T-2) ½-strength Hoagland solution, 7,5 mM $KHCO_3$
- Treatment-3 (T-3) ½-strength Hoagland solution, 10 mM $KHCO_3$
- Treatment-4 (T-4) ½-strength Hoagland solution, 12,5 mM $KHCO_3$

Each rootstock was marked with a label giving information about the rootstock genotype, the treatment group, and a number from 1-6 in order to distinguish every single plant (Figure 5).

Figure 9: Plant #4 of rootstock 5BB within the red-marked treatment group 1 (T-1)

Plants of the same treatment group were kept together on two moveable metallic trays, however within each individual treatment group, the plants of different rootstocks were positioned randomly, as visible in Figure 10 below. Figure 11 gives an additional overview of the plants' grouping from a bird's eye view.

[23] 1:1 blend of Quartz-sand purchased from „Casafino" and "Quarzwerke Österreich" with diameters ranging from 0,3-1 mm and 0,5-2mm respectively.

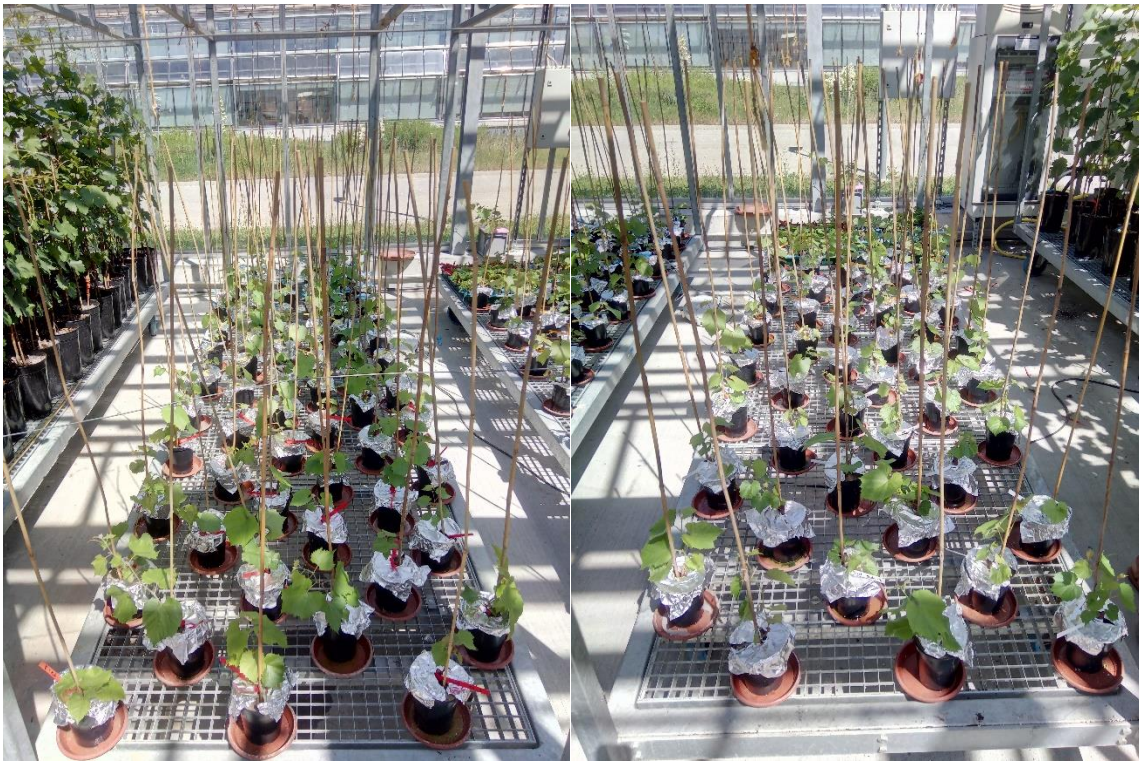


Figure 10: Experimental setup: the two metallic trays with all 120 plants on them

Row #						
24		3309 / 3		3309 / 6		Fercal / 2
23	3309 / 2		5BB / 4		5BB / 3	
22		5BB / 6		5BB / 2		Fercal / 1
21	5C / 4		Fercal / 4		5BB / 5	
20		Fercal / 5		5C / 6		3309 / 5
19	Fercal / 6		5C / 5		5C / 2	
18		3309 / 4		5C / 1		3309 / 1
17	5BB / 1		Fercal / 3		5C / 3	
16		5BB / 2		5C / 3		5C / 1
15	5C / 5		3309 / 6		Fercal / 2	
14		5BB / 3		Fercal / 5		3309 / 2
13	3309 / 1		3309 / 5		5C / 6	
12		5BB / 1		Fercal / 3		Fercal / 1
11	Fercal / 6		3309 / 4		5C / 4	
10		Fercal / 4		5BB / 6		3309 / 3
9	5BB / 4		5BB / 5		5C / 2	
8		Fercal / 5		Fercal / 1		5BB / 6
7	5C / 4		5BB / 4		5BB / 2	
6		3309 / 5		5C / 2		5C / 6
5	3309 / 6		Fercal / 2		3309 / 2	
4		5BB / 1		Fercal / 4		3309 / 3
3	5BB / 3		5BB / 5		3309 / 1	
2		Fercal / 6		Fercal /3		5C / 5
1	5C / 3		5C / 1		3309 / 4	

Legend:	Control group
	Treatment group 1
	Treatment 2
	Treatment 3
	Treatment 4

Row #						
16	Fercal / 3		5BB / 6		5C / 5	
15		5C / 3		Fercal / 2		Fercal / 1
14	5BB / 5		5C / 2		3309 / 3	
13		3309 / 6		5BB / 1		5C / 1
12	5C / 4		Fercal / 5		5BB / 3	
11		3309 / 4		3309 / 1		5BB / 4
10	Fercal / 4		5BB / 2		3309 / 2	
9		Fercal / 6		3309 / 5		5C / 6
8	5BB / 2		5C / 3		5BB / 6	
7		Fercal / 1		Fercal / 2		3309 / 4
6	5C / 4		Fercal / 4		5BB / 3	
5		Fercal / 5		5BB / 1		5C / 2
4	3309 / 5		5BB / 5		3309 / 1	
3		3309 / 6		5C / 1		Fercal / 6
2	5C / 5		Fercal / 3		5BB / 4	
1		3309 / 3		5C / 6		3309 / 2

Central aisle of the glasshouse

Figure 11: Experimental setup: bird`s eye view

Plant replacements

When plants had died before the treatments with $KHCO_3$ started (i.e. 20.7.2020), they were replaced by young cuttings of the same rootstock in order to have six plants per rootstock per treatment group before starting with the bicarbonate treatments. Replacements were necessary on the following six dates:

29 June	Fercal T1-1, T2-1, T2-5 & T4-5;	3309 T3-1
3 July	Fercal Control-5, T1-4, T3-3, T3-5, T3-6;	5C T3-5
6 July	Fercal Control-6, T1-2, T1-5, T2-6;	5C Control-3, T4-5
7 July	Fercal T2-3, T4-4;	5BB T4-2
10 July	Fercal T4-3	
14 July	Fercal T4-6	

Additional details

On **26 June** the sandy surface of each pot was covered with aluminium foil in order to prevent excessive algae to grow which possibly could have distorted nutrient availability for the rootstocks and may have distorted results. Additionally, plants of the Control group were moved onto another moveable metallic tray due to a restricted availability of space.

On **3 July** plants of T-4 were moved onto the same table as the plants of the Control group, resulting in the final arrangement of plants, as shown in Figures 10 and 11.

On **10 July** all plants were fixed with woody sticks and attached onto them in order to maintain an erect position of the shoot.

Nutrient solution and treatments

During the first month (19 June 2020 - 20 July 2020) all plants were equally watered between 5-6 times per week with 50 mL or 100 mL[24] of a nutrient solution, as described in the following paragraph. No bicarbonate treatments were applied during the first month to allow the plants to reach a certain resistance. On the day of their translocation - the 19 June - they were additionally watered with 150 mL of deionized water (DI-water) to ensure a sufficiently wet soil at the beginning. From 20 July until 12 August the first, second, third and fourth treatment group received an extra 5 mM, 7.5 mM, 10 mM and 12.5 mM of $KHCO_3$[25] (potassium bicarbonate) respectively, while the control group received 50 or 100 mL of the

[24] amount depending on water-saturation of the substrate and excess water in the plates; evaluated by visual evaluation.

[25] $KHCO_3$ was prepared in advance every two weeks; it was always stored in a cool-room (+4° C).

nutrient solution, as described above. The amounts of $KHCO_3$ added to the nutrient solution of the treatment groups was established based on a study of Ksouri et al. (2007). They had a similar experimental setup and revealed that rootstocks showed severe differences in the analysed parameters already at amounts of 4, 8 and 10mM of bicarbonate ($NaHCO_3$). A study of Covarrubias and Rombolà (2013) supported these findings, since their analysed plants showed severe differences in physiological responses already at the threshold of 5mM $KHCO_3$ added to their nutrient solution.

Between 19 June 2020 and 28 June 2020 all plants received a slightly modified ½–strength HOAGLAND-solution, prepared as described by Hoagland and Arnon (1938). Figure 12 below details the molecules which were added to the solution in order to obtain the nutrient abundance according to a ½-strength HOAGLAND-solution.

1/2 - strength Hoagland Nutrient solution

Name		Formula	(mM)	M.Wt g/mol	(g/L)	1/100 stock (g/L)	Name of solution	
Calcium nitrate tetrahydrate	Macronutrient	$Ca(NO_3)_2{\cdot}4H_2O$	1,5000	236,1500	0,3542	35,42	A	A1
Magnesium sulfate heptahydrate		$MgSO_4{\cdot}7H_2O$	1,0000	246,4800	0,2465	24,65	B	B3
Potassium sulfate		K_2SO_4	0,7500	174,2600	0,1307	13,07		B2
Ammonium nitrate		NH_4NO_3	0,7000	80,0400	0,0560	5,60		A2
Potassium dihydrogen phosphate		KH_2PO_4	0,7500	136,0900	0,1021	10,21		B1
Name		**Formula**	**(µM)**	**M.Wt g/mol**	**(g/L)**	**1/100 stock (g/L)**	**Name of solution**	
Boric acid	Micronutrient	H_3BO_3	23,20000	61,83000	0,00143	0,1434	B	B5
Copper sulfate monohydrate		$CuSO_4H_2O$	0,31000	159,60000	0,00005	0,0049		B6
Manganese (II) Chloride Tetrahydrate		$MnCl_2(H_2O)_4$	4,60000	197,91000	0,00091	0,0910		B4
Zinc sulfate monohydrate		$Zn\ SO_4\ H_2O$	0,40000	179,46000	0,00007	0,0072		B8
Sodium molybdate dihydrate		$Na_2MoO_4(H_2O)_2$	0,06000	241,95000	0,00001	0,0015		B7
Sodium Iron EDTA		FeNaEDTA	50,00000	421,10000	0,02106	2,1055	A	A3
Name		**Formula**	**(mM)**	**M.Wt g/mol**	**(g/L)**	**1/100 stock (g/L)**	**Name of solution**	
Potassium bicarbonate	Treatment	$KHCO_3$	5	100,12	0,50	50,06	T1	
			7,5	100,12	0,75	75,09	T2	
			10	100,12	1,00	100,12	T3	
			12,5	100,12	1,25	125,15	T4	

Figure 12: Modified ½-strength Hoagland solution

On 29 June the amounts of nutrients in the solution were doubled ("modified full strength Hoagland nutrient solution") because a higher demand for nutrients was suggested for the plants. Nevertheless, after 1,5 weeks, strange leaf-symptoms occurred on some plants' leaves. The reasons for this phenomenon could have ranged from toxicity to wrong pH-values or excessive N-contents and is being discussed in chapter "Discussion" in more detail. Consequently, on 16 July all nutrients remaining in the pots were washed out with 2 x 200

mL of normal water before the plants continued receiving the ½-strength Hoagland nutrient solution again from 17 July onwards.

To get a better understanding of irrigation amounts and dates as well as the timeline of the treatments with $KHCO_3$, Figures 13 and 14 provide an overview.

Irrigation with NS - amounts and dates	
19 June - 28 June	50 ml NS half strength
29 June - 15 July	10-100 ml NS full strength
16 July	2x 200 ml DI-H_2O
17 July - 12 August	50-150 ml NS half strength
13 August	150 ml DI-H_20

Figure 13: Irrigation with NS: amounts and dates

Irrigation with $KHCO_3$ - timeline	
19 June - 19 July	plants were only fed with NS in order to prepare them for the treatments
20 July - 12 August	treatments with $KHCO_3$

Figure 14: Irrigation with $KHCO_3$: timeline

Measurements

Measurements related to biomass and growth

Fresh weight (FW) and Dry weight (DW)

At the beginning of the experiment (19 June) fresh weight (FW) and dry weight (DW) of three woody cuttings of each rootstock (Fercal, 5BB, 5C, 3309) was evaluated and the values for arithmetic mean ($\bar{x}$) and standard deviation (SD) were calculated.

On the last day of the experiment, roots, shoots and leaves were separated manually from the plants. For each plant they were then weighed separately on a common laboratory-scale in order to obtain the FW. Additionally, fresh weight of one single young and one single old leaf of each plant was measured. Figure 15 shows pictures of old (left leaf) and young leaves (right leaf) of the rootstocks Fercal, 5C and 3309.

Figure 15: Old and young leaf of 3309, 5C and Fercal (from left to right); end of the experiment

Subsequently, all the measured samples were put into separate paper bags and stored in an oven (60° C) for 10 days. Measurements for dry weight (DW) of the oven-dried samples were conducted with the same laboratory-scale.

Concerning FW and DW measurements at the end of the experiment, only three rootstocks were analysed: rootstock 5BB was excluded from the analysis because of its similarity to 5C regarding the level of limestone resistance. Therefore, in the analysis of FW and DW, values of 5C can be seen as a representative for 5BB.

In chapter "Results" values will mostly be discussed for DW, since the FW-values are likely to be influenced by different external water contents of plant tissues.

Shoot length and leaf number

Between 19 June and 13 August the shoot length (cm) of each plant[26] was measured manually with a common measuring stick once per week; resulting in 9 measurement dates in total (dates A – I) . Leaf numbers were counted manually on the same dates, however on the penultimate measurement date (5 August; date H) no leaf numbers were counted, resulting in 8 measurement dates in total.

Leaf-level measurements

Chlorophyll content

Measurements of the chlorophyll content were carried out using a Chlorophyll Meter (SPAD 502PLUS; Konica Minolta[27]). One developed and sunlight-exposed leaf of each plant was measured on three different leaf-positions and the arithmetic mean ($\bar{x}$) was evaluated. The measurements with the SPAD 502PLUS were taken on 17 July, 24 July, 31 July, 7 August and 12 August. In the majority of cases values of all 120 plants were evaluated.

[26] Due to practical and organisational issues, on 13 August (later: date "I") not every plants' shoot length could be measured.

[27]https://www5.konicaminolta.eu/fileadmin/content/eu/Measuring_Instruments/2_Products/1_Colour_Measurement/6_Chlorophyll_Meter/PDF/Spad502plus_EN.pdf

In addition to the non-destructive and weekly measurements with SPAD, chlorophyll and carotenoid content were extracted from selected young and old leaves at the end of the experiment from rootstocks 3309, 5C (as a representative for 5BB) and Fercal. Extractions and calculations were carried out as described by Bappa et al. (2015).

Chlorophyll fluorescence

Measurements of the chlorophyll fluorescence were carried out with "Handy Pea +" (Hansatech Instruments[28]), an advanced continuous excitation chlorophyll fluorimeter. Since this device is able to measure several different parameters concerning chlorophyll fluorescence, it was of importance to know which parameters are commonly used to detect these special stress situations of leaves.

Amongst other authors, Murchie and Lawson (2013) described that the parameter F_v/F_m is the most robust indicator of the maximum quantum yield of photosystem II (PSII) chemistry. The official parameters list published on the homepage of Hansatech Instruments[27] affirms this statement. Since F_v is calculated by substracting F_0 (emission by excited chlorophyll a molecules in the antennae structure of PSII) from F_m (maximum fluorescence value obtained for a continuous light intensity), the values F_0 and F_m were not analysed separately. Furthermore, values of PI_{total} – an indicator of sample vitality – were compared. PI_{total} offers an overall expression of resistance of the sample plants towards constraints from outside. Table 2 summarizes the parameters analysed[29].

Table 2: Chlorophyll fluorescence: analysed parameters

F_v/F_m (=$(F_m-F_0)/F_m$)	Indicator of max. quantum yield of PSII considered to be a sensitive indicator of plant photosynthetic performance
PI_{total}	Performance index of light reactions; indicator of sample vitality, which expresses the internal force of the sample to resist constraints from outside

Measurements with Handy Pea + were executed on 16 July, 24 July, 30 July and 12 August. One developed and sunlight-exposed leaf per plant had been chosen and dark-adapted with

[28] https://www.hansatech-instruments.com/product/handy-pea/
[29] Analyses regarding parameter Tfm are provided in the attachment

a clip for about 15-30 minutes before the chlorophyll fluorescence was measured. On 16 July only 40 plants were measured due to the same reason as described above for LCpro-SD. On all other dates three plants per rootstock and treatment were measured resulting in 60 plants measured per date.

Hyperspectral reflectance

Hyperspectral reflectance of the leaves was measured with a PolyPen RP 410 UVIS (Photon System Instruments, Czech Republic[30]) on 17 July, 24 July 31 July, 5 August and 12 August. One developed and sunlight-exposed leaf per plant was measured on three different positions of the leaf, resulting in three measurements per plant. On 17 July 40 plants (i.e. 10 per rootstock) were measured since all 120 plants had been irrigated with the same nutrient solution until then. On all other dates three plants per rootstock and treatment were measured, resulting in 60 plants measured per date. Measurements always were carried out between 10:00 am. and 16:00 pm. The suggested parameters of importance for this experiment are provided in Table 3:

Table 3: Hyperspectral reflectance: analysed parameters

NDVI	Normalized difference vegetation index	Structure, good correlation with green biomass (LAI, chlorophyll)
MCARI	Modified chlorophyll absorption in reflectance index	Chlorophyll, potential for LAI prediction
PRI	Photochemical reflectance index	Chlorophyll, correlation with growth parameters

Gas exchange measurements

The following gas exchange parameters of the plants were measured using the infrared gas exchange analyser model "LCpro-SD" (ADC Bioscientific Ltd.[31]):

- E (transpiration rate in µmol H_2O m^{-2} s^{-1})
- A (assimilation rate in µmol CO_2 m^{-2} s^{-2})
- g_s (stomatal conductance in mol H_2O m^{-2} s^{-1})

[30] https://handheld.psi.cz/products/polypen/#details
[31]https://www.adc.co.uk/

Measurements with the "LCpro-SD" were taken on 10 July, 16 July, 23 July and 30 July on one developed and sunlight-exposed leaf per plant. Light conditions inside the leaf chamber were adjusted so that the emitted photosynthetic active radiation (PAR) constantly was at 1000 µmol m^{-2} s^{-1}. All measurements were carried out from 11:00 a.m. to 15:00 p.m. when leaves were suggested to show highest photosynthetic activity. Each plant (i.e. leaf) was kept inside the LCpro-SD measuring chamber for about 3 minutes in order to avoid irregular values resulting from calibration problems at the start of each measurement.
On 10 July and 16 July 20 (i.e. 5 per rootstock) and 40 (i.e. 10 per rootstock) plants were measured respectively, because on these dates the treatments with $KHCO_3$ had not started yet and all plants had received the same nutrient solutions so far. On 23 July and 30 July three plants of each rootstock and treatment were measured, resulting in 60 plants measured on each of those dates.
It is worth mentioning, that gas exchange measurements were incorporated to the experimental design even though these measurements are more commonly used to analyze water stress in plants and not Fe deficiency (Bojovic et al.; 2017). However, in this experiment such measurements were undertaken because the means were available.

A chronological overview of all measurements taken is provided in Figure 16 below. The dates of measurements and the correlating letters used for each of the dates in the charts of the chapter "Results" are provided as well.

Shoot lengths		Leaf numbers		Polypen		Handypea		LCPRO	
Date	code	Date	code	Date	code	Date	code	Date	code
19 June	A	19 June	A	17 July	A	16 July	A	10 July	A
26 June	B	26 June	B	24 July	B	23 July	B	16 July	B
3 July	C	3 July	C	31 July	C	30 July	C	23 July	C
9 July	D	9 July	D	5 August	D	6 August	D	30 July	D
17 July	E	17 July	E	12 August	E	12 August	E		
24 July	F	24 July	F						
31 July	G	31 July	G						
5 August	H	13 August	I						
13 August	I								
SPAD		FW		DW		Root exudates			
Date	code	Date	code	Date	code	Date	code		
17 July	A	19 June	A	13 & 14 August	A	13 & 14 August	A		
24 July	B	13 & 14 August	B						
31 July	C			Start / End of treatment with $KHCO_3$:				20 July / 12 August	
7 August	D			Measurements before 20 July ->					
12 August	E			Measurements during the treatment ->					

Figure 16: Chronological overview of all measurements taken

Statistical analysis

Data preparation

The measured values of each of the above described relevant parameters were assigned to the correlating factors of "rootstock", "treatment" and "date of the measurement". Additionally, two factors were combined to one, particularly "rootstock & treatment"; "rootstock & date"; "treatment & date", in order to do analyses on a broader base. Figure 17 gives an overview of the data processing with the single factors being displayed in the first three rows, the combined factors in rows four to six, and the values of the parameter (in this case: SPAD Ø) in row 7.

rootstock	treatment	date	rootstock/treatment	rootstock/date	treatment/date	SPAD Ø
Fercal	Control	A	Fercal/Control	Fercal/A	Control/A	29,6
Fercal	Control	A	Fercal/Control	Fercal/A	Control/A	29,9
Fercal	Control	B	Fercal/Control	Fercal/B	Control/B	35,4
Fercal	Control	B	Fercal/Control	Fercal/B	Control/B	28,6
Fercal	Control	B	Fercal/Control	Fercal/B	Control/B	31,9
Fercal	Control	C	Fercal/Control	Fercal/C	Control/C	37,2
Fercal	Control	C	Fercal/Control	Fercal/C	Control/C	26,8
Fercal	Control	C	Fercal/Control	Fercal/C	Control/C	30,2
Fercal	Control	D	Fercal/Control	Fercal/D	Control/D	32,4
Fercal	Control	D	Fercal/Control	Fercal/D	Control/D	31,2
Fercal	Control	D	Fercal/Control	Fercal/D	Control/D	23,9

Figure 17: Method of data processing

Statistical approach

Analysis of data was conducted with the "R-commander" of the statistical program "R" (Rcmdr, version 2.3-0).

First of all, a Levene Test was performed in order to evaluate whether the sample groups were homogenous or not. If the Levene Test showed no significant differences of the sample groups ($p > 0,05$) a one- or multi-factorial ANOVA (Analysis of Variances) with the significance level of $\alpha = 0,05$ was conducted. Furthermore, a Post-Hoc Test (Tukey's honest significant difference test) was executed in order to compare the means of each factor-group with each other and see which groups' mean values differed significantly. If the Levene Test showed significant differences of the sample groups ($p < 0,05$) a non-parametric Kruskal-Wallis-Test (KW) was undertaken followed by the same Post-Hoc Test as described above.

For each of the above described parameters of each measurement device values of the following factors were compared and analysed[32]:

- 1 factorial analysis:
    - rootstock groups, treatment groups, date (of measurement) groups
    - combined factors (as shown in Figure 17)
- 2 factorial analyses:
    - rootstock- and treatment-, rootstock- and date-, date- and treatment groups

The Figures presented in the Chapter "Results" all follow the same patterns: All figures provide mean values ($\overline{x}$) and Standard deviations (SD) for the analysed groups and small letters inside or above the grouped columns highlight if significant differences between the analysed groups were detected. In case of coloured small letters, the comparison of means was made within the groups that display letters of the same colour. Sample sizes (n), as well as the results of the executed statistical tests (ANOVA or KW) are provided below the graphical presentations for each of the figures.

[32] only reasonable and interesting analyses are discussed in the chapter "Results".
Due to the constant chronological increase of shoot length and leaf numbers and the single date measurement of FW and DW, a statistical comparison of date groups was not taken into account in those analyses.

Results

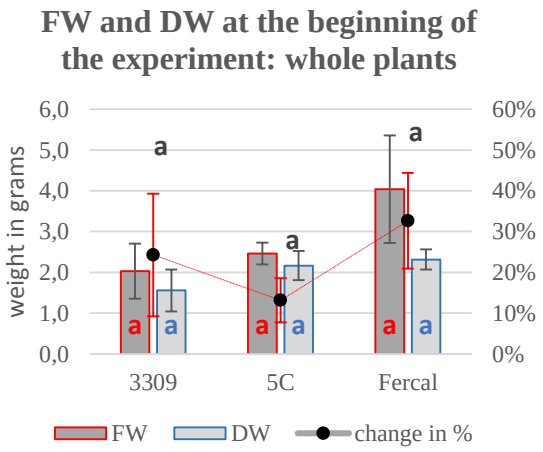
Growth parameters

Beginning of the experiment

FW and DW

Rootstocks showed no significant differences regarding their fresh or dry weight at the beginning of the experiment, nor did they show significant differences in the weight change measured in % of FW (Figure 18).

In absolute numbers, rootstock Fercal weighed most (FW: Ø 4,04g; DW Ø: 2,31g) followed by rootstock 5C (FW: Ø 2,46g; DW: Ø 2,17g) and rootstock 3309 (FW: Ø 2,03g; DW: Ø 1,56g).



comparison of $\bar{x}$ with SD; executed separately according to the different colors of the letters above / inside the bars; same letters ("a") indicate that no significant differences were observed

FW: KW (p= 0,3932); n= 9; red letters

DW: ANOVA (p= 0,4900); n= 9; blue letters

change (in % of FW): ANOVA (p= 0,6050); black letters

Figure 18: FW and DW at the beginning of the experiment

End of the experiment (biomass of roots, leaves and shoots in % of total plants' biomass)

DW

Figure 19a-f provide an overview of the different percentages ($\bar{x}$ with SD) of plant organs (in DW) in relation to the total plants' DW. In the comparison of rootstocks with all treatment groups consolidated in Figure 19a, 5C had the highest values with 18%, followed by Fercal (15%) and finally 3309 whose roots contributed only 12% to the total DW-biomass. Rootstock 5C differed significantly from rootstock 3309. In a more detailed analysis shown in Figure 19b – where all individual treatment groups of all rootstocks were compared - no significant differences were observed. However, roots of the treatment groups tend to contribute more to total DW than the Control group in all the rootstock groups.

Figure 19c compares the contribution of leaves to total plants DW and shows that rootstock 5C has a significantly higher contribution (32%) than the other two rootstocks (both less than 25%). In this analysis all treatment groups were consolidated for each rootstock. A HSD-Tukey Test for the individual treatment groups of all rootstocks only resulted in significant differences ($p < 0,05$) between 3309 T-2, 5C Control and 5C T-4 (Figure 19d)

Shoots of rootstock 3309 contributed 63% to total plants' DW, which differed significantly from rootstocks Fercal and 5C, whose shoots only contributed 60% and 50% to total plants' DW, respectively (Figure 19e; all treatment groups consolidated). A comparison of all of the individual treatment groups shows that the Control group of rootstock 3309 was significantly higher than T-4 of rootstock 5C, whereas all other treatment groups of the rootstocks didn`t display significant differences (Figure 19f).

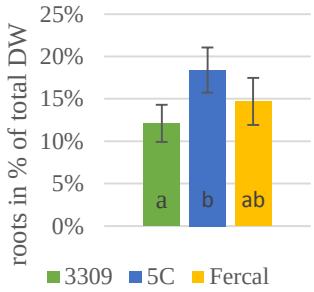


Figure 19a:

ANOVA (p= 0,0063); n= 45

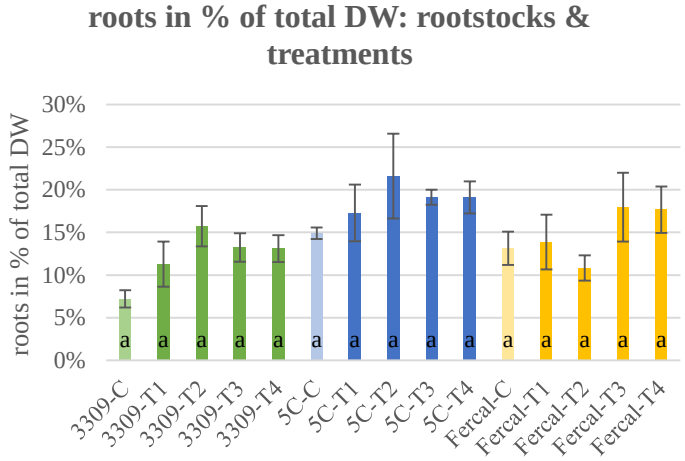


Figure 19b:

ANOVA (p= 0,126); n= 45

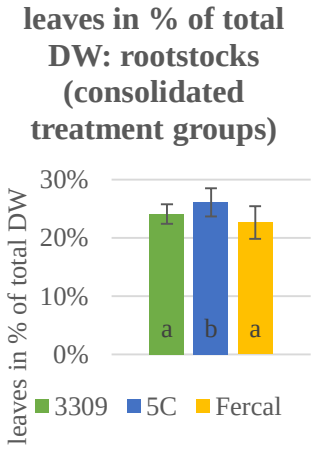


Figure 19c:

ANOVA (p= 0,0002); n= 45

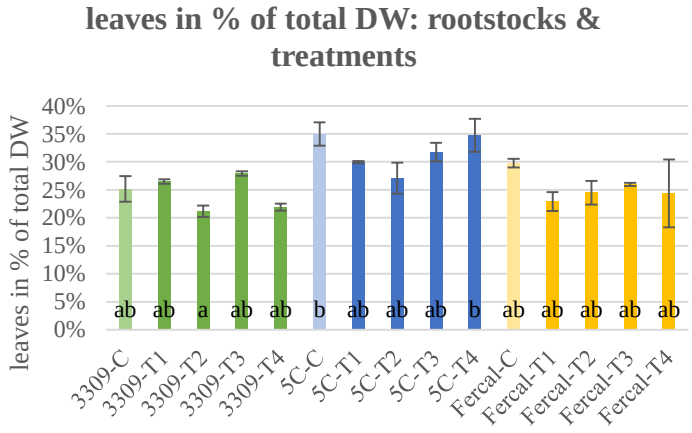


Figure 19d:

KW (p= 0,0079); n= 45

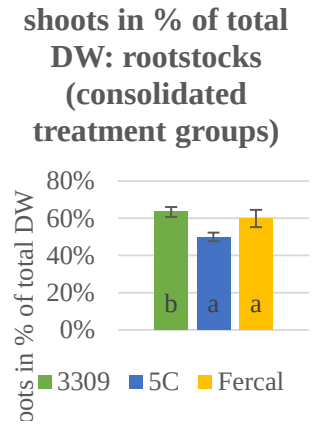


Figure 19e:

ANOVA (p= 5,83 * 10^{-6}); n= 45

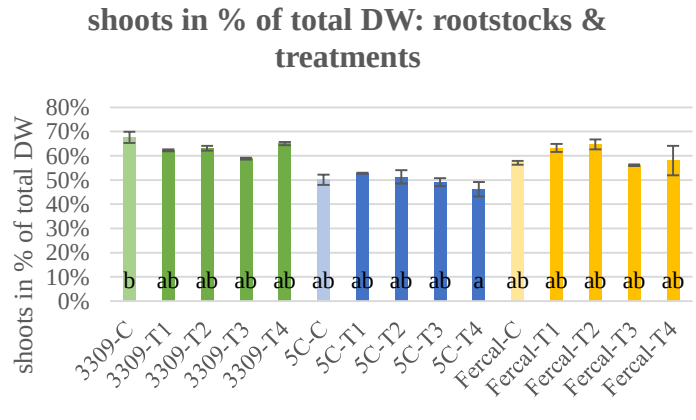


Figure 19f:

KW (p= 0,023); n= 45

Figures 19a-f: comparison of $\bar{x}$ with SD; significant differences are indicated with different letters

Figure 19: Roots, leaves and shoots in % of total DW

Young leaf and old leaf (FW and DW)

Significant differences between the rootstock groups (consolidated treatment groups) were observed in the FW and DW of old and young leaves (Figure 20). While Figure 20a facilitates a direct comparison of FW with DW (old leaf: left; young leaf: right), Figure 20b alleviates a comparison young with old leaves (FW: left; DW: right). Results of a pairwise comparison of rootstock groups are marked with letters in colors red, orange, green and blue. The colors indicate which samples had been compared. The changes between FW and DW (in % of FW; Figure 20a) and between young and old leaf (in % of young leaf; Figure 20b) are marked with a red spot in both of the figures[33]. In all comparisons, leaves of rootstock 5C were significantly heavier than those of Fercal, whose leaves weighed significantly more than those of rootstock 3309 in turn. Regarding the percentage changes, two outliers –both from rootstock 3309 - can be detected: Its young leaf had a smaller decrease from FW to DW than those of the other rootstocks (Figure 20a), and the FW increase from young to old leaf was a lot higher for 3309 than it was for the other rootstocks (Figure 20b).

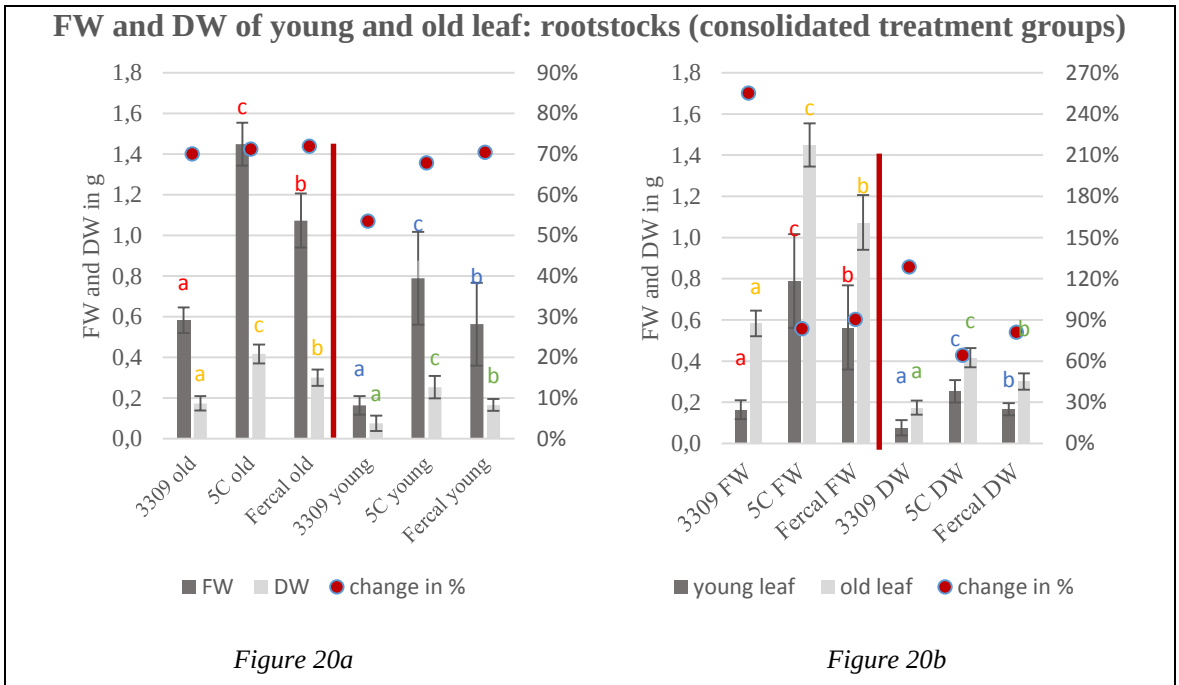


Figure 20a *Figure 20b*

comparison of $\bar{x}$ with SD; executed separately according to the different colors of the letters above the bars in Figures 20a and 20b; significant differences are indicated with different letters

FW old leaf: KW ($p= 8{,}026*10^{-8}$); **FW young leaf**: KW ($p= 1{,}528*10^{-7}$), n= 45;

DW old leaf: ANOVA ($p= 2{,}11*10^{-9}$); **DW young leaf**: ANOVA: ($p= 4{,}6*10^{-6}$), n= 45;

Figure 20: FW and DW of young and old leaf: rootstocks

[33] Regarding the changes in %, no comparison of means ($\bar{x}$) was executed.

Comparisons of the individual treatment groups (i.e. Control group, T-1, T-2, T-3, T4) of each rootstock only showed significant differences (ANOVA) for rootstock 3309 (DW old leaf; p= 0,00987) and Fercal (DW young leaf; p= 0,0108). However, since no explicit trend was observed between the treatment groups (e.g. constant increase/decrease from Control group to T-4), no additional data will be shown with regard to these analyses.

Shoot length and leaf number

Shoot length

Figure 21 provides an overview of the development of the shoot length (in cm) within the rootstock- and treatment-groups. Comparing rootstock groups separately for each date, significant differences were observed between them on all of the single dates A - I (KW or ANOVA: $p < 0,05$), except for date B (Figure 21a). The main trend amongst the rootstocks can be described as: 5BB > 3309 > 5C > Fercal. The trend within the treatment groups with consolidated values of all rootstock groups (Figure 21b) – particularly on the last 3 dates of measurements (G, H and I) when plants were already exposed to the $KHCO_3$ solutions for one, two and three weeks respectively - is: Control > T-1 > T-2 > T-3 > T-4. However, analysing the treatment-groups, no significant differences were observed on any of the single dates, even though the values in Figure 21b seem to differ a lot, particularly at the end of the experiment: Significant differences were neither observed on date G (ANOVA: p= 0,313;), nor on date I (ANOVA: p= 0,06).

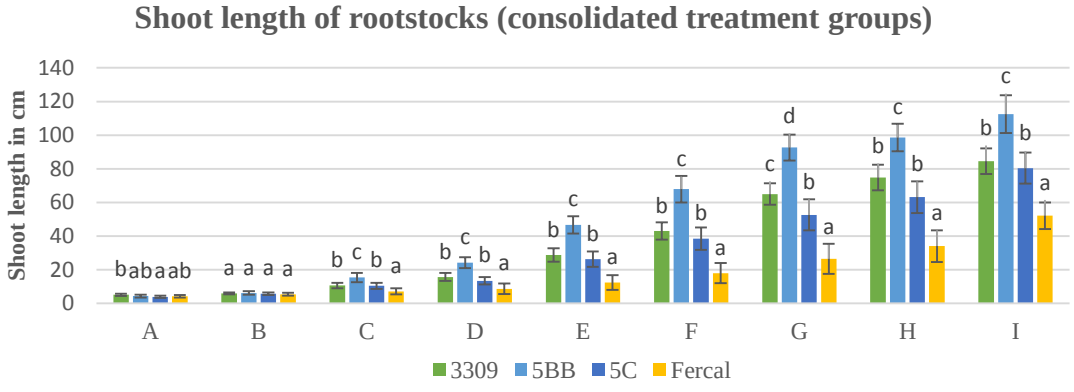


Figure 21a

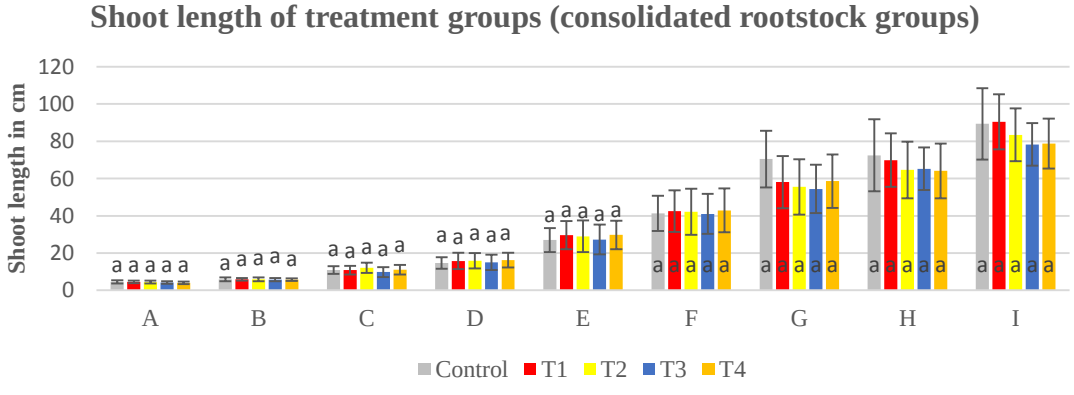


Figure 21b

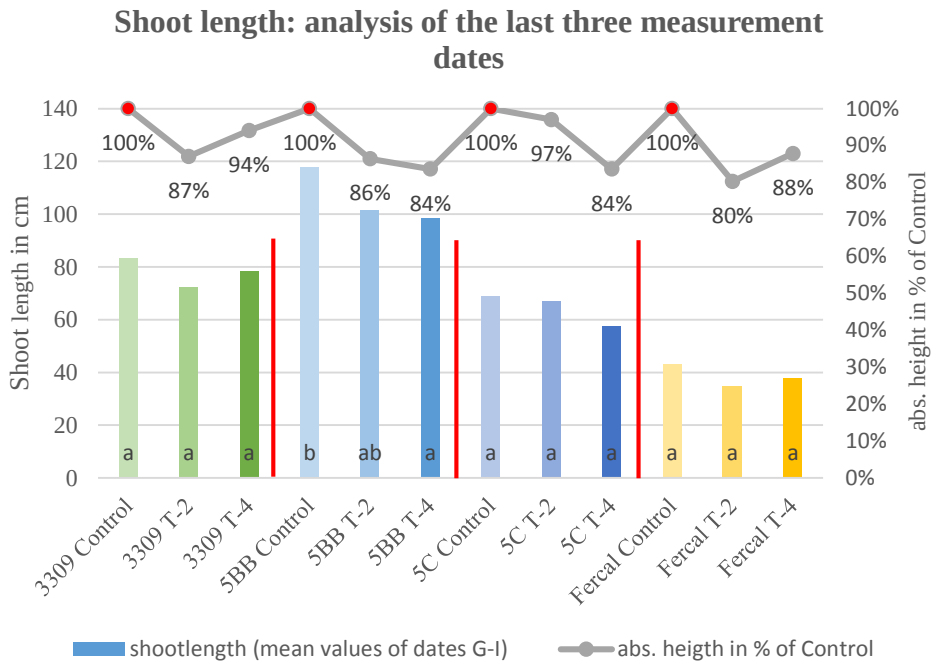
comparison of $\bar{x}$ with SD; executed separately for each group of bars in both figures

significant differences are indicated with different letters; **n**: A-D= 120, F-G= 119, H= 109, I= 103

Figure 21: Shoot length of rootstocks and treatment groups: overview

Figure 22 provides an analysis in order to facilitate a comparison of the shoot length of each rootstock group at the end of the experiment, uncoupled from the vastly different absolute shoot length of each rootstock displayed in Figure 22. Coloured bars in Figure 22 display the mean shoot length of dates G-I, while the grey line displays the shoot length of treatment groups T-2 and T-4[34] in % of the Control group (100%).

A similar decline in all of the rootstocks – for T-2 as well as for T-4 – with values ranging between 80% – 97% of the Control group can be observed. Significant differences between the treatment groups were only observed for rootstock 5BB between T-4 and the Control group (ANOVA: p= 0,0207).



comparison of $\bar{x}$; executed separately for each rootstock
significant differences are indicated with different letters
(**3309**: n= 43; **5BB**: n= 49; **5C**: n= 49; **Fercal**: n= 37)

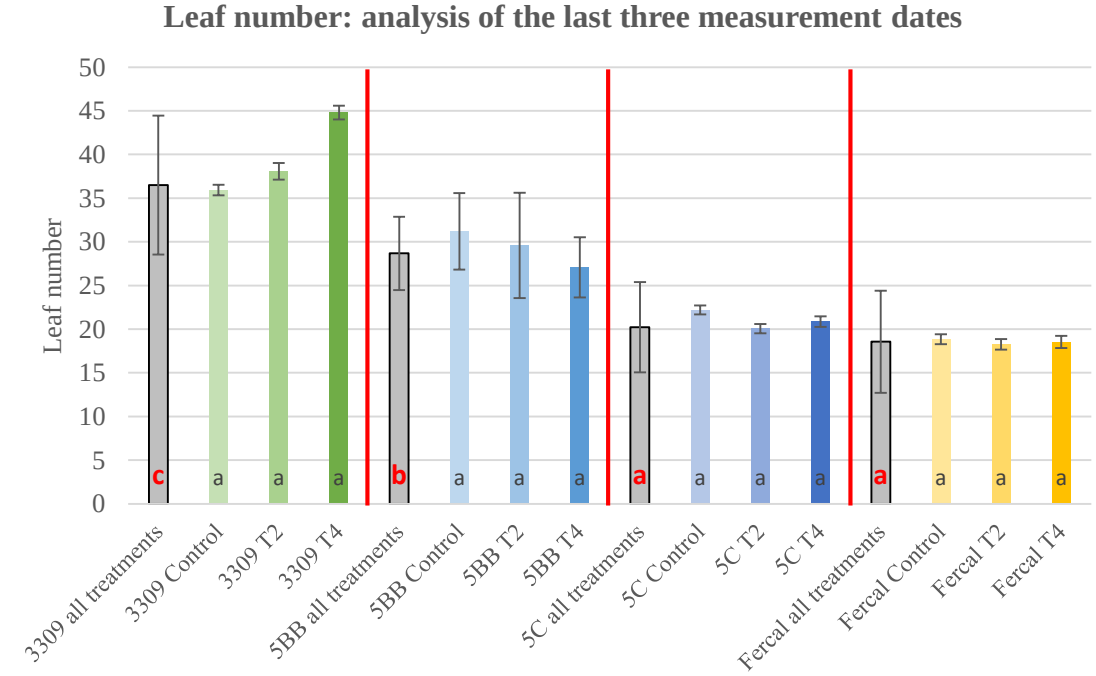
Figure 22: Shoot length: rootstocks on the last three measurement dates

[34] Plants considered as medium stressed (T-2) and maximal stressed (T-4) were chosen for this comparison.

Leaf number

Figure 23 shows the mean value of leaf number of the rootstocks for the last three dates of measurements, in the same way and reasons as previously explained for Figure 22 regarding shoot length. Furthermore, the mean value of all treatment groups are shown for the four rootstocks (grey columns); those were subject to a separate statistical analysis[35]. Comparisons of the treatment groups (Control, T-2, T-4) were also made separately for each rootstock.

Leaf number at the end of the experiment was significantly different between the rootstocks. Rootstock 3309 resulted to have the highest leaf number (Ø: 36,5) followed by rootstocks 5BB (Ø: 28,7); 5C (Ø: 20,2) and Fercal (Ø: 18,6). No significant differences between the treatment groups of the individual rootstocks could be observed.



comparison of $\bar{x}$ with SD; executed for "rootstocks all treatments" (red letters in grey bars) and separately for each of the four rootstocks (treatment groups Control, T-2, T-4; black letters)

significant differences are indicated with different letters

rootstocks all treatments: KW: ($p < 2{,}2 * 10^{-16}$); n= 279

3309: n= 42; **5BB**: n= 45; **5C**: n= 44; **Fercal**: n= 42 (ANOVA: $p > 0{,}05$ for all the individual rootstocks)

Figure 23: Leaf number: rootstocks on the last three measurement dates

[35] Red letters in the grey columns display the levels of significance for the four rootstocks. For representational issues, the separately analysed grey columns were grouped on the left side of the more detailed analyses of treatment groups of each individual rootstock.

Physiological plant parameters

Chlorophyll content (SPAD)

Treatment groups showed significant differences overall (i.e. measured values of all rootstocks and dates consolidated for each treatment group), with the Control group displaying higher values than T-1 (0,1 > p > 0,05) and T-2 to T-4 (p < 0,05). Figure 24a provides a graphical overview for this analysis.

Figure 24b shows the comparison of SPAD-values of the rootstock groups overall (i.e. measured values of all treatment groups and dates consolidated for each rootstocks). Fercal had the highest SPAD-values (i.e. chlorophyll content), followed by 3309. These two rootstocks had significantly higher SPAD-values than 5C (p < 0,05) and higher values than 5BB (0,1 > p > 0,05).

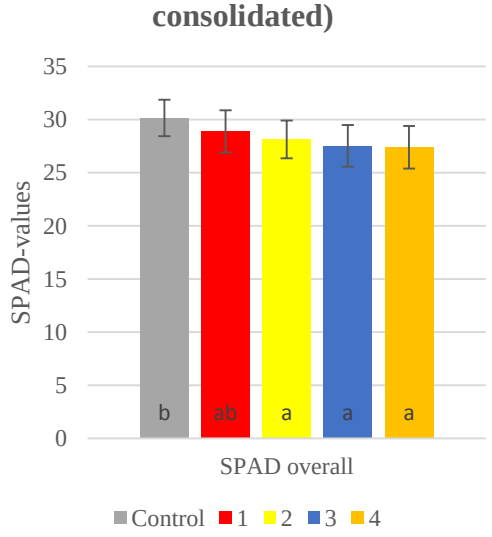


Figure 24a

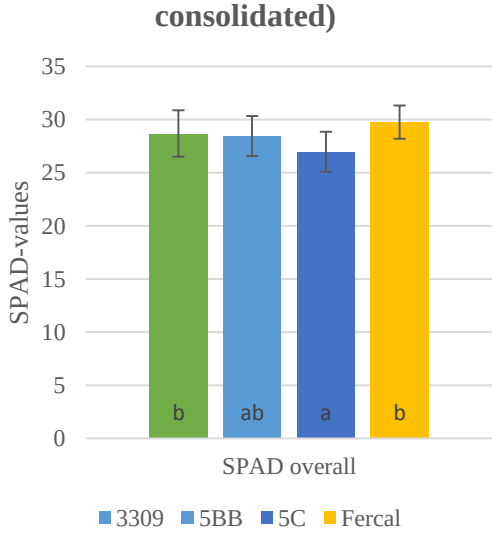


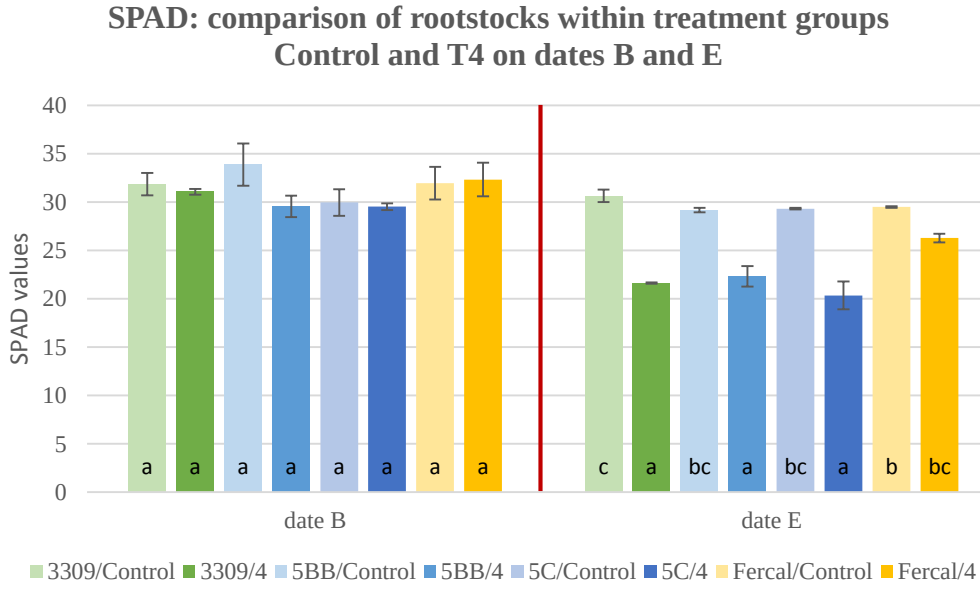
Figure 24b

ANOVA (p= 0,0004) comparison of $\bar{x}$ with SD (n= 287)	KW (p= 0,0009) comparison of $\bar{x}$ with SD (n= 287)

significant differences are indicated with different letters in both figures

Figure 24: SPAD: treatments and rootstocks overall

Furthermore, a comparison of the rootstocks within the treatment groups Control and T-4 was conducted for the dates B[36] and E in order to be able to see the effects of bicarbonate on the chlorophyll content (Figure 25). While on date B none of the rootstock and/or treatment groups displayed significant differences, a significant drop of SPAD-values within treatment group T-4 was observed at the end of the experiment (date E) in all of the rootstock groups. The only exception from this phenomenon was rootstock Fercal, whose relatively high SPAD-value within T-4 differed significantly from the other rootstocks within T-4 on date E ($p < 0,05$).



comparison of $\bar{x}$ with SD; executed separately for dates B and E

significant differences are indicated with different letters

date B: ANOVA (p= 0,513), n= 24

date E: KW (p= 0,0054), n= 24

Figure 25: SPAD: rootstocks within treatment groups Control and T-4 on dates B and E

[36] In this case date A – as the very beginning of the experiment - was not chosen, because of missing measurements within some of the treatment groups on that date. However, since date B had also been prior to the beginning of the treatment with $KHCO_3$, values of date B veritably represent a neutral status of the experiment.

Chlorophyll and Carotenoid content (extractions)

Results of the analyses of the chlorophyll extractions were very similar to the ones obtained from the non-destructive SPAD-method (Figure 26a). However, since extractions were taken from young and old leaves, additional information was gained.

Comparing the total chlorophyll content (i.e. chlorophyll a and b) of the young leaf for rootstock 3309 showed a constant and significant decline in chlorophyll content the more the bicarbonate content was increased within the treatment groups. Rootstock 5C showed a similar (significant) pattern, although the decrease of the total chlorophyll content within the treatment groups was not as much as it was for rootstock 3309. Fercal, in contrast, maintained the chlorophyll content of young leaves on about the same level within all the individual treatment groups – they did not differ significantly from each other and were on a higher level than they were for rootstocks 3309 and 5C.

A comparison of the chlorophyll content of the old leaf did not show such big changes between the treatment groups of any of the rootstocks. The only exception was T-2 of rootstock 3309, which differed significantly from Control group and T-1 ($p< 0,05$), as well as from T-3 and T-4 ($p< 0,1$)[37]. In comparison to the young leaves, the chlorophyll content of the old leaves was higher for all the rootstocks, especially within T-3 and T-4 of rootstocks 3309 and 5C.

Analyses of total carotenoid content revealed similar results as mentioned above for the total chlorophyll content, and more or less showed the same levels of significance between treatment groups of each individual rootstock (Figure 26b): A comparison of the young leaves resulted in significant differences between the Control group and T-4 of 3309 and between T-1 and T-4 of 5C. Treatment groups of Fercal were not significantly different.

The carotenoid content of the old leaves was only significantly different in the treatment groups of 3309, with the Control group having a higher content than all other treatment groups.

[37] However, since only 3 leaves of each treatment group were measured, it is quite probable that the results of T-2 of the old leaf of rootstock 3309 were distorted by one "irregular" leaf.

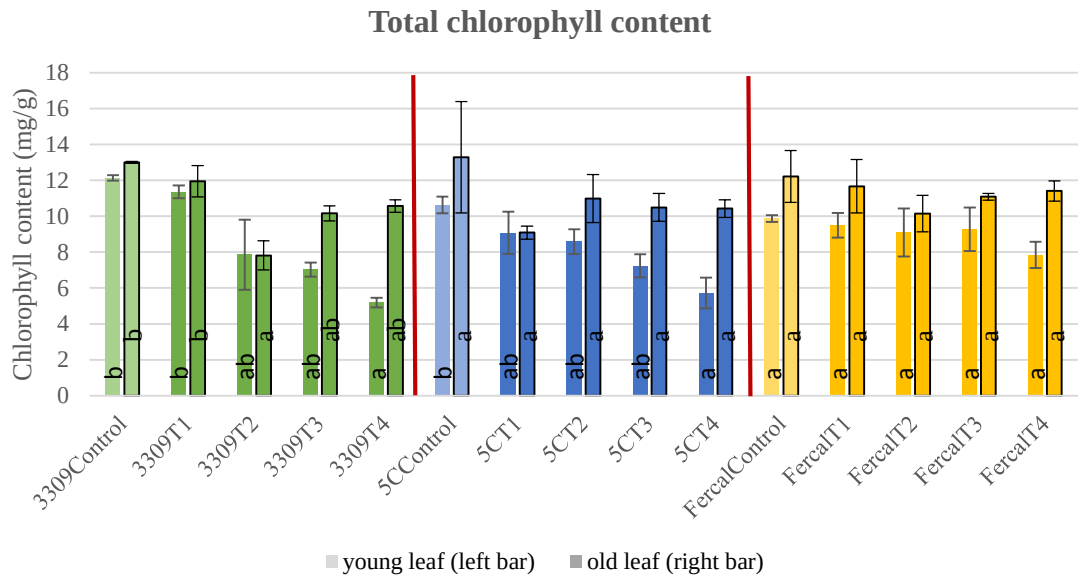


Figure 26a

3309: young leaf (y.l.) ANOVA (p= 0,0111); old leaf (o.l.) ANOVA (p= 0,0093); n= 14

5C: y.l. ANOVA (p= 0,0332); o.l. ANOVA (p= 0,605); n=15

Fercal: y.l. ANOVA (p= 0,726); o.l. ANOVA (p= 0,814); n=15

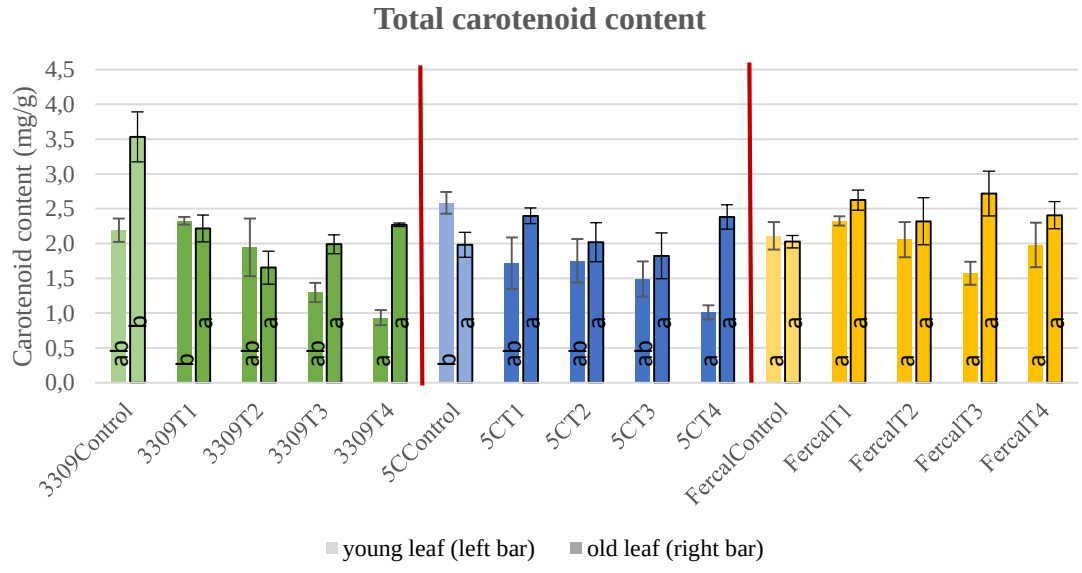


Figure 26b

3309: young leaf (y.l.) ANOVA (p= 0,0185); old leaf (o.l.) ANOVA (p= 0,0067); n= 14

5C: y.l. ANOVA (p= 0,0435); o.l. ANOVA (p= 0,478); n=15

Fercal: y.l. ANOVA (p= 0,371); o.l. ANOVA (p= 0,466); n=15

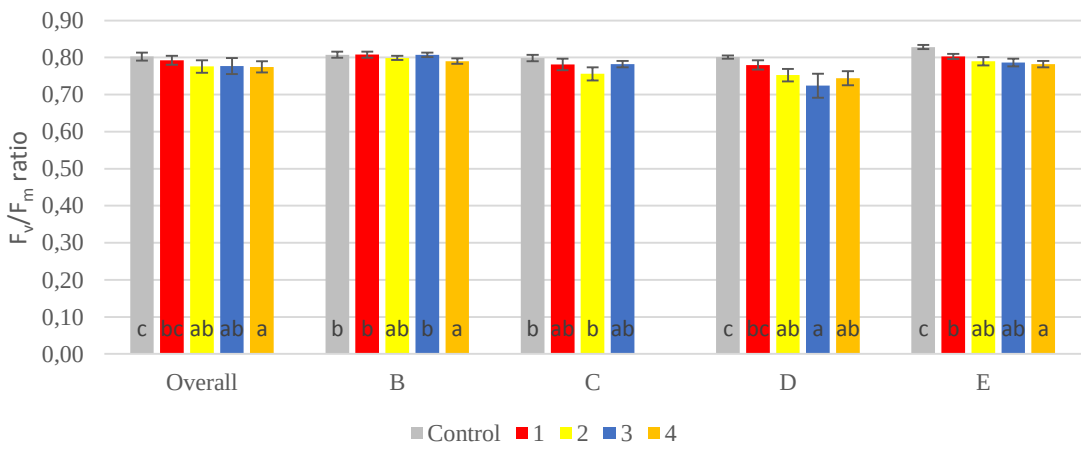
Figure 26a and b: comparison of $\bar{x}$ (letters) with SD; executed separately for young and old leaf within every rootstock;
significant differences are indicated with different letters

Figure 26: Chlorophyll and carotenoid content at the end of the experiment

Chlorophyll fluorescence

Parameter "F_v/F_m"

Analyses of the plant photosynthetic performance indicating parameter F_v/F_m, revealed significant differences between the treatment groups. Figure 27 shows the analysis of the treatment groups for all dates and rootstock groups consolidated (left group of bars; overall). Furthermore, it shows the analyses for all of the individual dates except for date A, on which no significant differences between the treatment groups were observed. Nearly on all of the analysed dates the Control group had the highest values, followed by a constant decline until T-4, which had the lowest values. The level of significance constantly increased from date A until date E, reflecting the different levels of physiological stress responses of the treatment groups. On date C no measurements were taken for treatment group T-4.



comparison of $\bar{x}$ with SD; executed separately for each group of bars

significant differences are indicated with different letters

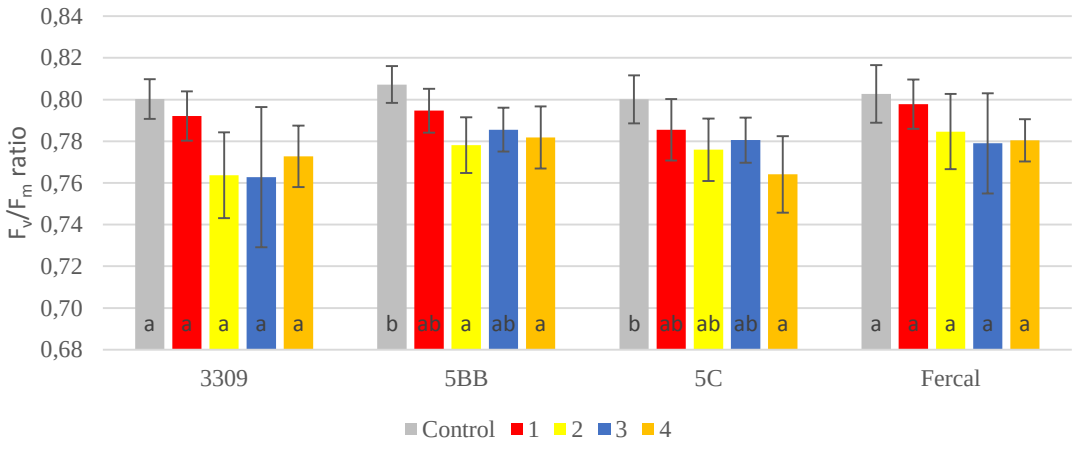
overall: n= 265; consolidated rootstock groups and dates

date B: n= 59; **date C**: n= 46; **dates D and E**: n=60; consolidated rootstock groups

Dates analysed	*Levene Test*	ANOVA (KW for dates C and E)
Overall	*p= 0,0546*	p= $1,47 * 10^{-6}$
A	*p= 0,2587*	p= 0,3880
B	*p= 0,4594*	p= 0,0163
C	*p= 0,0144*	p= 0,0116
D	*p= 0,0002*	p= $1,80 * 10^{-5}$
E	*p= 0,0580*	p= $7,85 * 10^{-8}$

Figure 27: F_v/F_m: separate analysis of dates (consolidated rootstock groups)

Furthermore, Figure 28 provides an overview of the different values of F_v/F_m when comparing the treatment groups within each of the rootstock groups. Even though more or less the same trend can be observed within all of the rootstock groups – the Control group has the highest values followed by T-1 until T-4 in a decreasing order - significant differences existed only within rootstocks 5BB and 5C. Treatment groups within rootstocks 3309 and Fercal did not show any significant differences in a comparison of means.



comparison of $\bar{x}$ with SD; executed separately for each group of bars

significant differences are indicated with different letters

3309: ANOVA (p= 0,05), n= 67; **5BB**: ANOVA (p= 0,0089), n= 65;

5C: ANOVA: (p= 0,029), n= 67; **Fercal**: ANOVA (p= 0,26), n= 66

Rootstocks analysed	*Levene Test*	ANOVA
3309	*p= 0,0194*	p= 0,0520
5BB	*p= 0,4590*	p= 0,0089
5C	*p= 0,8260*	p= 0,0290
Fercal	*p= 0,2681*	p= 0,2600

Figure 28: F_v/F_m: separate analysis of rootstocks (treatments)

Rootstocks additionally grouped by treatment showed significantly different values of F_v/F_m in a comparison of date E, while doing the same analysis for date A did not lead to significant differences (Figure 29a). On date E, all rootstocks had significantly lower values within treatment group T-4 in a comparison with the Control group.

When treatment groups within each of the rootstocks were consolidated and analysed for each date separately (Figure 29b), significant differences could only be observed on date E. On this date the mean value of all treatment groups of rootstock Fercal had the highest F_v/F_m-value (0,8121), followed by 5BB (0,7995), 3309 (0,7927) and 5C (0,7873).

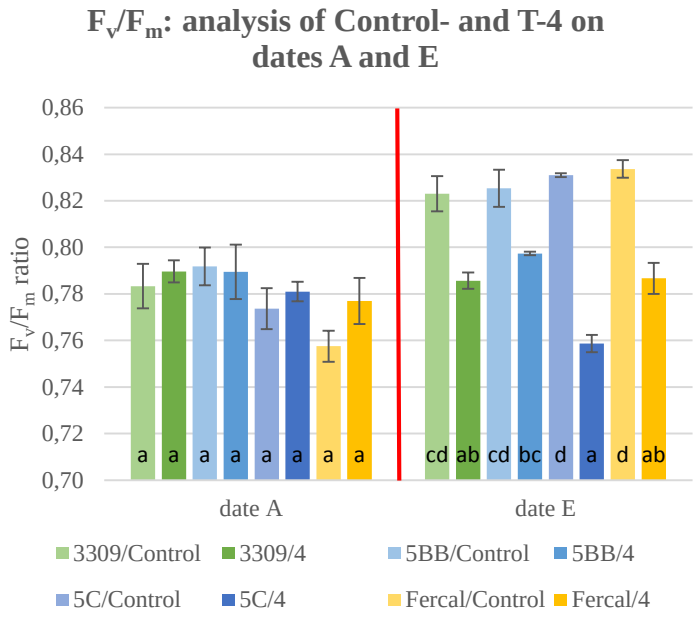


Figure 28a

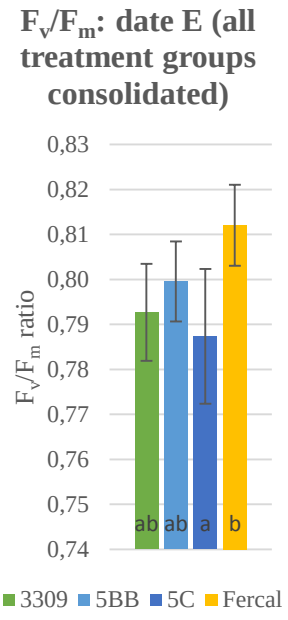


Figure 28b

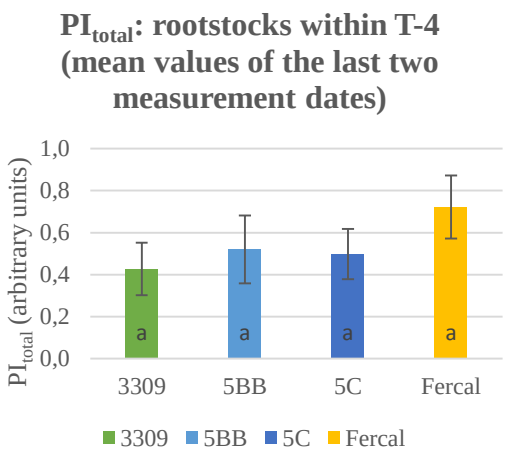
comparison of $\bar{x}$ with SD; executed separately for each group of bars **date A**: ANOVA (p= 0,374); n= 22 **date E**: ANOVA (p=5,24 * 10^{-7}); n= 24	comparison of $\bar{x}$ with SD KW (p= 0,0319); n= 60
significant differences are indicated with different letters	

Figure 29: F_v/F_m: rootstocks (Control vs T-4) and on date E (consolidated treatment groups)

Parameter "PI_{total}"

None of the conducted analyses regarding the sample-vitality indicating parameter "PI_{total}" that would have been relevant for this study, resulted in significant differences. However, Figure 30 provides a comparison of the rootstocks within T-4 of the last two measurement dates (dates D and E). Rootstock Fercal clearly differed from the other ones – although not significantly.

Similar patterns could be observed within other treatment groups and/or dates, which is why this parameter will not be discussed more in detail.



comparison of $\bar{x}$ with SD; same letters ("a") indicate that no significant differences were observed

ANOVA: (p= 0,327); n= 24

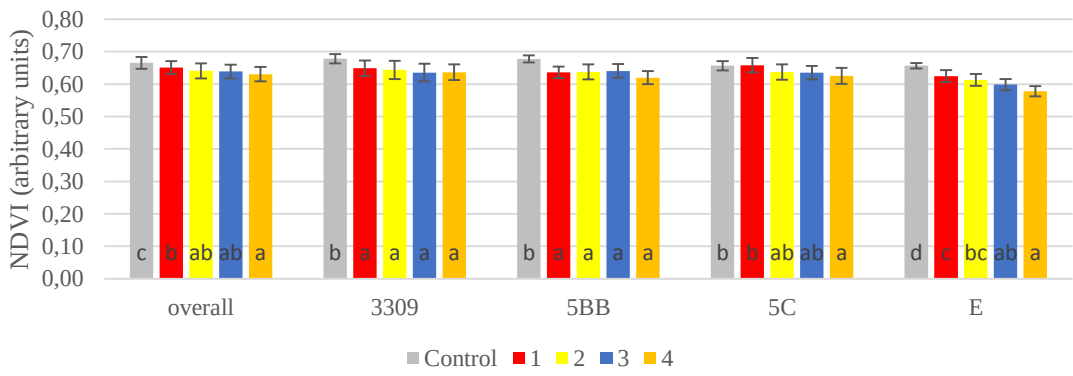
Figure 30: PI total: rootstocks within T-4 on the last two measurement dates

Hyperspectral reflectance

Parameter "NDVI"

Treatment groups with consolidated measurements of all rootstock groups and dates (i.e. overall) showed significant differences in the parameter NDVI, with the Control group having the highest, and T-4 the lowest values respectively (Figure 31).

In comparison to rootstocks 3309, 5BB and 5C, where treatment groups followed the same trend as visible in the overall comparison, treatment groups of Fercal were not significantly different and thus are not displayed in Figure 31. Furthermore, treatment groups were analysed for dates A and E since they mark the beginning and the end of the experiment. In contrast to date E at the end of the experiment, on date A no significant differences between the treatment groups were observed.



comparison of $\bar{x}$ with SD; executed separately for each group of bars

significant differences are indicated with different letters

overall: n= 816; consolidated rootstock groups and dates

3309 and 5BB: n= 207; **5C**: n= 198; consolidated dates

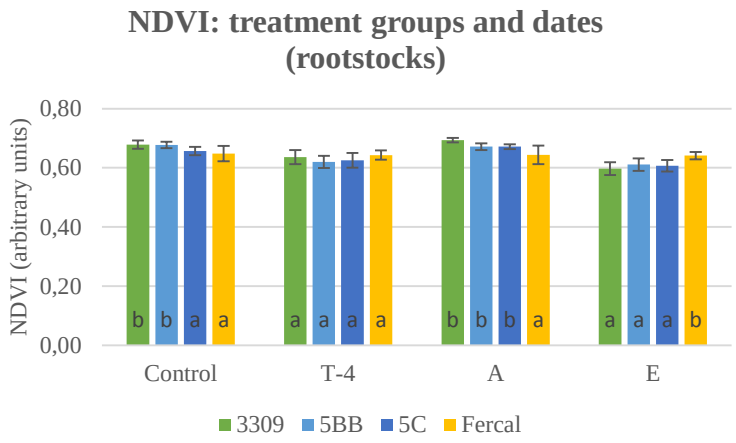
dates A and E: n= 180; consolidated rootstock groups

Rootstock groups and dates analysed	*Levene Test*	KW
Overall	*p= 1,342 * 10^{-5}*	p= 1,248 * 10^{-14}
3309	*p= 6,226 * 10^{-7}*	p= 0,0003
5BB	*p= 0,0015*	p= 1,407 * 10^{-11}
5C	*p= 0,0018*	p= 0,0040
A[38]	*p= 0,0166*	p= 7,818 * 10^{-5}
E	*p= 0,0003*	p= 1,027 * 10^{-15}

Figure 31: NDVI: rootstock groups and dates (treatments)

[38] However, an ANOVA followed by a Post-Hoc Test (Tukey comparison of means) resulted in no significant differences between the treatment groups (p= 0,232).

Although no significant differences were detected between the four rootstock groups when consolidating all treatment groups and dates, significant differences between the rootstock groups were prevalent within single treatment groups and dates (Figure 32). Due to their characteristics of differing most from each other in the experimental design, the comparisons of the rootstock groups within the two treatment groups Control and T-4 on the dates A and E are shown as a representative for the other treatment groups and dates. Within the Control group and on date A, rootstock Fercal displayed the lowest values of all rootstocks, while it did not anymore differ significantly from the other rootstocks within treatment group T-4 and even accounted for the significantly highest NDVI-values on date E.



comparison of $\bar{x}$ with SD; executed separately for each group of bars

significant differences are indicated with different letters

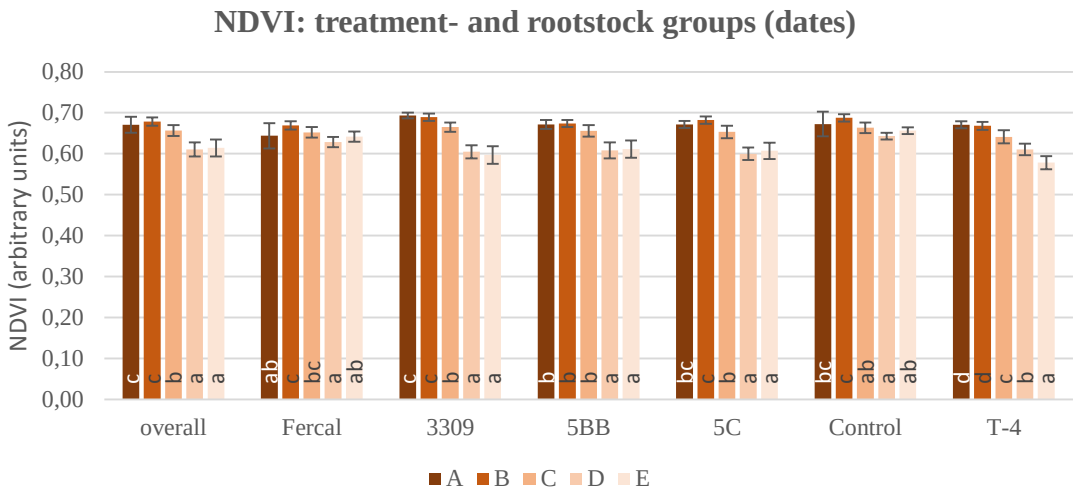
all treatment groups consolidated for each rootstock

Control: KW (p= 0,0920), n= 177; **T-4:** KW (p= 0,0412), n= 180

date A: KW (p= 7,818 *10^{-5}), n= 120; **date E**: KW (p= 1,027 * 10^{-15}), n= 180

Figure 32: NDVI: treatment groups and dates (rootstock groups consolidated)

In addition, a comparison of the chronological development of NDVI-values (dates A to E) shows how the treatment with $KHCO_3$ influenced this parameter over the time (Figure 33). An overall analysis (consolidated treatment- and rootstock groups for each of the dates), as well as separate analyses of the dates within every rootstock- and treatment group, showed a more or less significant decline in the values of NDVI over the time starting with the highest values for date A and the lowest values for the dates D and E. Rootstocks Fercal and the treatment group Control were an exception and showed a slightly different pattern, since those two groups were capable of maintaining relatively high NDVI-values for dates D and E.



comparison of $\bar{x}$ with SD; executed separately for each group of bars

significant differences are indicated with different letters

overall: n= 816; consolidated rootstock and treatment groups

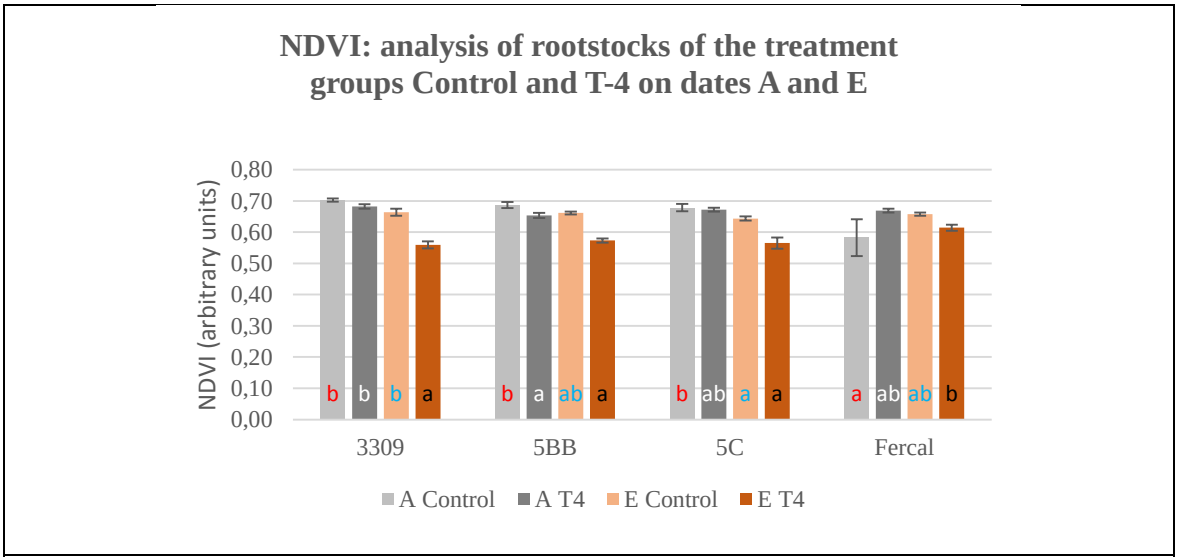
Fercal: n= 204; **3309 and 5BB**: n= 207; **5C**: n= 198; consolidated treatment groups

Control: n= 177; **T-4:** n= 180; consolidated rootstock groups

Treatment- and rootstock groups analysed	*Levene Test*	KW
Overall	$p= 4{,}515 * 10^{-16}$	$p= <2{,}2 * 10^{-16}$
Fercal	$p= 3{,}943 * 10^{-5}$	$p= 1{,}039 * 10^{-10}$
3309	$p= 3{,}501 * 10^{-9}$	$p= <2{,}2 * 10^{-16}$
5BB	$p= 4{,}016 * 10^{-9}$	$p= <2{,}2 * 10^{-16}$
5C	$p= 5{,}684 * 10^{-7}$	$p= <2{,}2 * 10^{-16}$
Control	$p= 0{,}0001$	$p= 2{,}441 * 10^{-5}$
T-4	$p= 0{,}0293$	$p= <2{,}2 * 10^{-16}$

Figure 33: NDVI: treatment- and rootstock groups (dates)

Figure 34 shows more detailed comparisons of the rootstocks, with a special focus on dates A and E and the treatment groups Control and T-4. Firstly, NDVI-values of the four rootstocks within the Control groups as well as within T-4 for date A were compared, then the same comparisons were done for date E. Each of those four analyses resulted in significant differences between the rootstocks, however, the relation amongst them was not the same. Especially rootstock Fercal, which had the significantly lowest values on date A within the Control group, displayed the highest values on date E within T-4 and differed significantly from all the other rootstocks. The different colours of the small letters – indicating the levels of significance between the rootstocks – show which columns were compared, since they were grouped differently (i.e. according to rootstock groups).



comparison of $\bar{x}$ with SD

bars (and letters, indicating the level of significance) of the same colour were compared separately; significant differences are indicated with different letters

The arrangement of bars provides an additional overview of the differences between the dates/treatment groups within each of the four rootstocks.

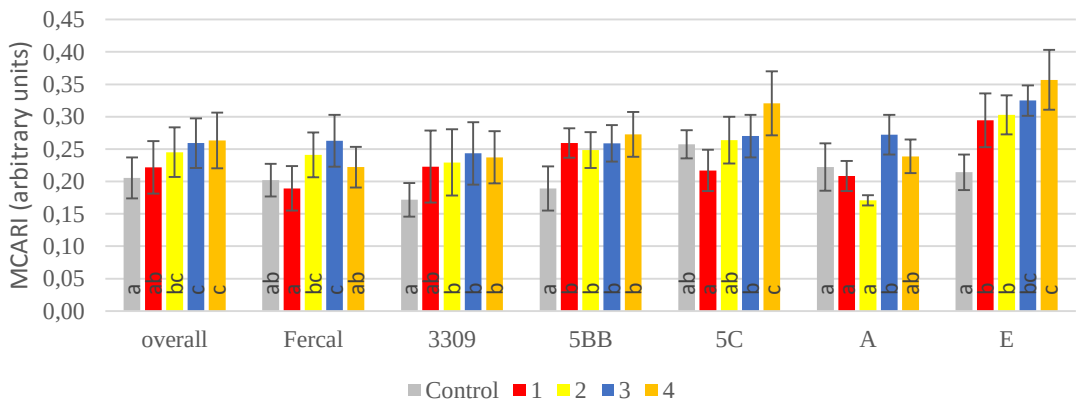
date A & Control: ANOVA (p= 0,0002), n= 39; **date A & T-4**: ANOVA (p= 0,0072), n= 27;

date E & Control: ANOVA (p= 0,0344), n= 36; **date E & T-4**: ANOVA (p= 0,0001), n= 36

Figure 34: NDVI: rootstocks within treatment groups Control and T-4 on dates A and E

Parameter "MCARI"

When the chlorophyll-related parameter MCARI[39] of the five treatment groups was compared, significant differences between them were visible - not only in an analysis where all rootstock groups and dates were consolidated (i.e. overall), but also within the single rootstock and date groups respectively (Figure 35). The main pattern observed is that values of the Control group are the lowest ones, while T-4 tends to display the highest values. Treatment groups within the rootstock Fercal and on date A showed slightly different patterns, since in those groups T-4 was not responsible for the highest values.



comparison of $\bar{x}$ with SD; executed separately for each group of bars

significant differences are indicated with different letters

overall: n= 816; consolidated rootstock groups and dates

Fercal: n= 204; **3309 and 5BB**: n= 207; **5C**: n= 198; consolidated dates

date A: n= 120; **date E**: n= 180; consolidated rootstock groups

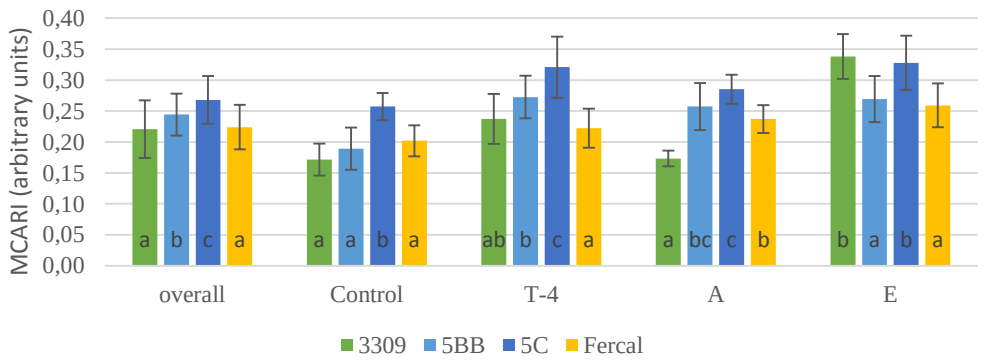
Rootstock groups and dates analysed	*Levene Test*	KW (ANOVA for 5BB and A)
Overall	*p= 0,0036*	p= 1,864 * 10^{-7}
Fercal	*p= 0,0147*	p= 2,84 * 10^{-5}
3309	*p= 5,306 * 10^{-9}*	p= 0,0035
5BB	*p= 0,5308*	p= 6,05 * 10^{-10}
5C	*p= 0,0205*	p= 7,867 * 10^{-6}
A	*p= 0,0522*	p= 0,0002
E	*p= 0,0211*	p= 8,423 * 10^{-12}

Figure 35: MCARI: rootstock groups and dates (treatments)

[39] Higher values of "MCARI" indicate lower Chlorophyll contents in the leaves (Daughtry et al.; 2000).

Figure 36 provides an overview of the comparison between the rootstocks when all treatment groups and dates were consolidated (i.e. overall), as well as for the rootstocks within the treatment groups and on individual dates. Similar to prior analyses, special attention was paid to treatment groups Control and T-4 and dates A and E due to their highest dissimilarities between treatment groups (no $KHCO_3$ vs. 12,5mol of $KHCO_3$) and dates (no time vs. longest period of time exposed to $KHCO_3$) in the experiment.

The values of MCARI of rootstocks 5BB and 5C were significantly higher than those of the other two rootstocks in the analysis "overall", as well as within treatment group T-4 and on date A. Rootstock Fercal had the lowest MCARI-values within treatment group T-4 and on date E, however, it only differed significantly from all other rootstocks within treatment group T-4.



comparison of $\bar{x}$ with SD; executed separately for each group of bars

significant differences are indicated with different letters

overall: n= 816; consolidated treatment groups and dates

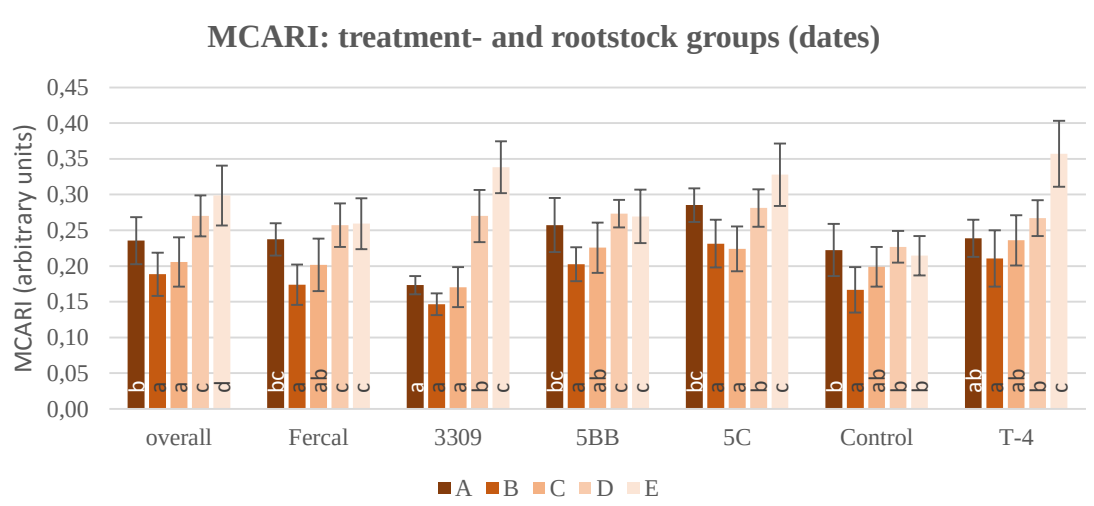
Control: n= 177; **T-4:** n= 180; consolidated dates

date A: n= 120; **date E**: n= 180; consolidated treatment groups

Treatment groups and dates analysed	*Levene Test*	ANOVA (KW for Overall and T-4)
Overall	*p= 1,864 * 10^{-7}*	p= 2,474 * 10^{-11}
Control	*p= 0,1692*	p= 2,37 * 10^{-11}
T-4	*p= 0,2432*	p= 7,66 * 10^{-8}
A	*p= 9,498 * 10^{-6}*	p= 1,143 *10^{-11}
E	*p= 0,8433*	p= 2,39 * 10^{-7}

Figure 36: MCARI: treatment groups and dates (rootstocks)

Figure 37 illustrates the chronological development of the values of MCARI with consolidated values of all treatment- and rootstock groups (i.e. overall), as well as within the single treatment- and rootstock groups. Summarizing the main pattern for all analyses, date A had significantly higher values than dates B and C except within rootstock group 3309 and treatment group T-4. Date E revealed significantly higher values than all other dates in most of the groups analysed, however, within the rootstock groups Fercal and 5BB and the treatment group Control, no significant peaks on date E were observed.



comparison of $\bar{x}$ with SD; executed separately for each group of bars

significant differences are indicated with different letters

overall: n= 816; consolidated rootstock and treatment groups

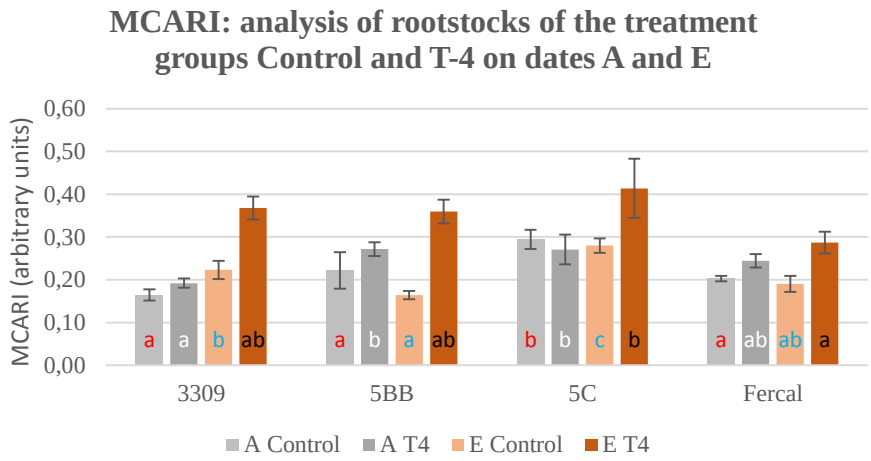
Fercal: n= 204; **3309 and 5BB**: n= 207; **5C**: n= 198; consolidated treatment groups

Control: n= 177; **T-4:** n= 180; consolidated rootstock groups

Treatment- and rootstock groups analysed	*Levene Test*	KW (ANOVA for 5C)
Overall	*p= 0,0001*	p= <2,2 * 10^{-16}
Fercal	*p= 0,0055*	p= 8,477 * 10^{-11}
3309	*p= 2,813 * 10^{-8}*	p= <2,2 * 10^{-16}
5BB	*p= 0,0005*	p= 4,594 * 10^{-8}
5C	*p= 0,4107*	p= 1,15 * 10^{-12}
Control	*p= 0,0322*	p= 0,0006
T-4	*p= 0,0499*	p= 6,593 * 10^{-13}

Figure 37: MCARI: treatment- and rootstock groups (dates)

Another more detailed comparison of rootstock groups was done within the two antipodes regarding treatment groups (Control and T-4) and dates (A and E) in order to highlight the response of the rootstock groups towards the impact of the treatment itself as well as in relation to the duration of the treatment (Figure 38). Although significant differences between the rootstocks were detected in all of the four conducted analyses, the major finding can be seen on date E within T-4: Rootstock Fercal managed to keep "MCARI"-values significantly lower than all other rootstocks. Colours of the letters, as well as the grouping of columns are displayed identically and with the same intentions as explained previously in the comparison of NDVI in Figure 34.



comparison of $\bar{x}$ with SD

bars (and letters, indicating the level of significance) of the same colour were compared separately; significant differences are indicated with different letters

The arrangement of bars provides an additional overview of the differences between the dates/treatment groups within each of the four rootstocks.

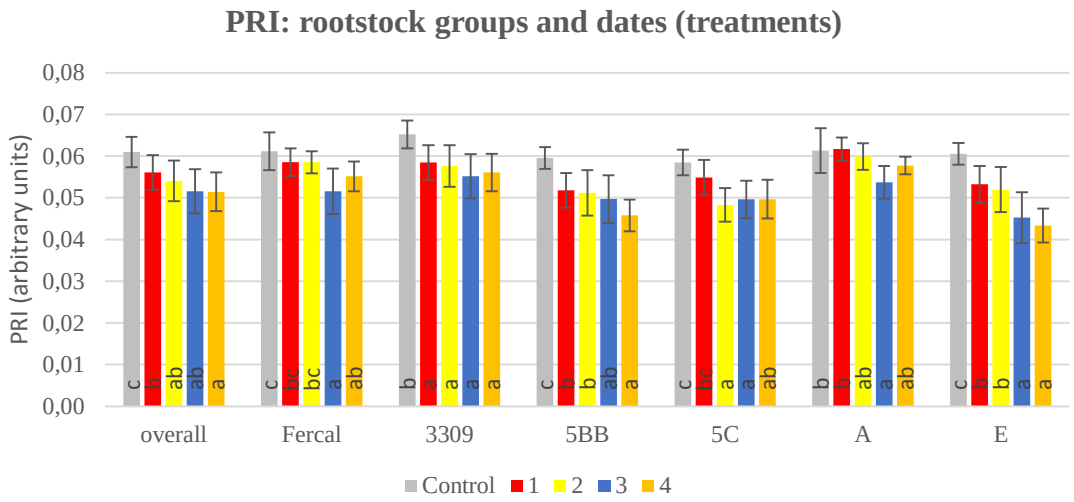
date A & Control: ANOVA (p= 0,0006), n= 39; **date A & T-4**: ANOVA (p= 0,0023), n= 27;

date E & Control: ANOVA (p= 3,01 * 10^{-7}), n= 36; **date E & T-4**: ANOVA (p= 0,0250), n= 36

Figure 38: MCARI: rootstocks within treatment groups Control and T-4 on dates A and E

Parameter "PRI"

The analysis of parameter PRI revealed a statistical significance between treatment groups when values of all rootstocks and dates were consolidated (i.e. overall). Furthermore, PRI was significantly different within the single rootstock groups and dates (Figure 39). The Control group was responsible for the highest PRI-values in all conducted analyses while especially T-3 and T-4 accounted for the lowest values. However, rootstocks 5C and Fercal, as well as date A displayed the narrowest deviations between the treatment group with the highest PRI-value and the one with the lowest value (Δ= 0,0097, Δ= 0,0095 and Δ= 0,0080 respectively).



comparison of $\bar{x}$ with SD; executed separately for each group of bars

significant differences are indicated with different letters

overall: n= 816; consolidated rootstock groups and dates

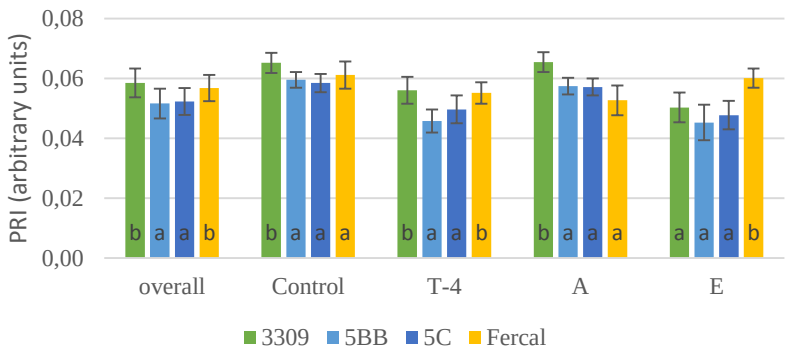
Fercal: n= 204; **3309 and 5BB**: n= 207; **5C**: n= 198; consolidated dates

date A: n= 120; **date E**: n= 180; consolidated rootstock groups

Rootstock groups and dates analysed	*Levene Test*	KW (ANOVA for 5C)
Overall	*p= 2,287 * 10^{-7}*	p= < 2,2 * 10^{-16}
Fercal	*p= 0,0004*	p= 1,534 * 10^{-6}
3309	*p= 0,0133*	p= 1,318 * 10^{-5}
5BB	*p= 0,0003*	p= 9,421 * 10^{-13}
5C	*p= 0,1449*	p= 2,88 * 10^{-8}
A	*p= 0,0213*	p= 5,762 * 10^{-5}
E	*p= 4,49 * 10^{-5}*	p= 1,434 * 10^{-11}

Figure 39: PRI: rootstock groups and dates (treatments)

Comparing the rootstock groups with consolidated values of all treatment groups and dates (i.e. overall), as well as the values within the single treatment groups and dates revealed statistical significances (Figure 40). In all of the analyses, rootstocks 3309 and/or Fercal showed significantly higher PRI-values, while rootstocks 5BB and/or 5C had the lowest values. On date E, when plants had already been exposed to $KHCO_3$ for the longest period of time, rootstock Fercal showed significantly higher values than all other rootstocks, while within the Control group and on date A, where plants had only received neutral nutrient solution, rootstock 3309 had significantly higher PRI-values than the other rootstocks.



comparison of $\bar{x}$ with SD; executed separately for each group of bars

significant differences are indicated with different letters

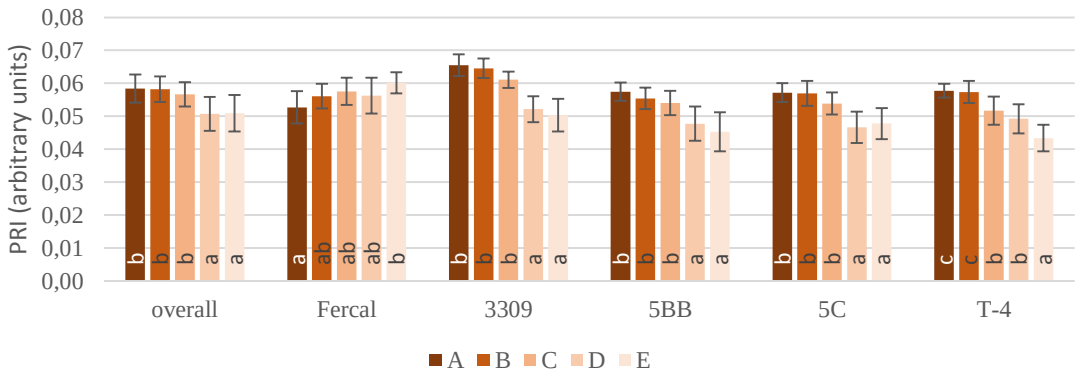
overall: ANOVA ($p= 2 * 10^{-16}$), n= 816; consolidated treatment groups and dates

Control: ANOVA ($p= 5 * 10^{-5}$), n= 177; **T-4:** ANOVA ($p= 7{,}66 * 10^{-8}$), n= 180; consolidated dates

date A: KW ($p= 1{,}416 * 10^{-7}$), n= 120; **date E**: KW ($p= 2{,}{,}39 * 10^{-7}$), n= 180; consolidated treatment groups

Figure 40: PRI: treatment groups and dates (rootstocks)

Figure 41 provides an analysis of the five dates of measurements. In the analysis "overall" (left group of bars), the measured values of all treatment and rootstock groups were consolidated. A comparison between dates A and E in all of the conducted analyses revealed that date A had significantly higher PRI-values than date E, except within rootstock group Fercal where this phenomenon was reversed. A comparison of the dates within the Control group did not result in significant differences (KW: p= 0,7553) and therefore will not be discussed in this paper.



comparison of $\bar{x}$ with SD; executed separately for each group of bars

significant differences are indicated with different letters

overall: n= 816; consolidated rootstock and treatment groups

Fercal: n= 204; **3309 and 5BB**: n= 207; **5C**: n= 198; consolidated treatment groups

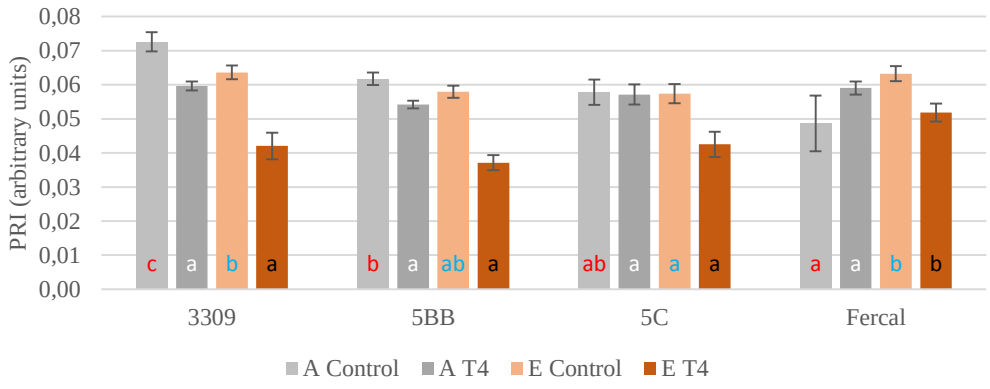
T-4: n= 180; consolidated rootstock groups

Treatment- and rootstock groups analysed	*Levene Test*	KW
Overall	*p= 2,495 * 10^{-10}*	p= < 2,2 * 10^{-16}
Fercal	*p= 0,0281*	p= 0,0088
3309	*p= 1,831 * 10^{-5}*	p= < 2,2 * 10^{-16}
5BB	*p= 1,63 * 10^{-6}*	p= 6,071 * 10^{-8}
5C	*p= 0,0077*	p= 6,472 * 10^{-9}
T-4	*p= 0,0006*	p= 9,794 * 10^{-12}

Figure 41: PRI: treatment- and rootstock groups (dates)

Finally, a more detailed comparison between rootstocks for the antithetical treatment groups Control and T-4, and the dates A and E was conducted (Figure 42). Colours of the letters, as well as the grouping of columns are displayed identically and with the same intentions as explained previously in the comparison of NDVI and MCARI in Figures 34 and 38 respectively.

While rootstock Fercal had the significantly lowest values on date A within the Control group, it differed significantly from the other rootstocks on date E within T-4 by having the highest values. According to parameter PRI, this highlights that rootstock Fercal was better able to sustain high $KHCO_3$ concentrations than the other rootstocks.



comparison of $\bar{x}$ with SD

bars (and letters indicating the level of significance) of the same colour were compared separately; significant differences are indicated with different letters

The arrangement of bars provides an additional overview of the differences between the dates/treatment groups within each of the four rootstocks.

date A & Control: ANOVA ($p= 1{,}77 * 10^{-5}$), n= 39; **date A & T-4**: ANOVA (p= 0,0666), n= 27;

date E & Control: ANOVA (p= 0,0064), n= 36; **date E & T-4**: ANOVA (p= 0,0003), n= 36

Figure 42: PRI: rootstocks within treatment groups Control and T-4 on dates A and E

The three analysed parameters of hyperspectral reflectance (NDVI, MCARI and PRI) are shown in a Scatterplot matrix which is to serve as a summary for this subchapter (Hyperspectral reflectance). Figure 43a presents the rootstocks within treatment group T-4 on date A (beginning of the experiment), while Figure 43b does so on date E (end of the experiment).

It is clearly visible that - in contrast to date A - on date E, rootstock Fercal had the best values in all of the three analysed parameters, namely the highest ones in NDVI and PRI and the lowest ones in MCARI. On date A rootstock 3309 had the best scores in all parameters, while on date E rootstocks 3309, 5BB and 5C were worst regarding these three parameters.

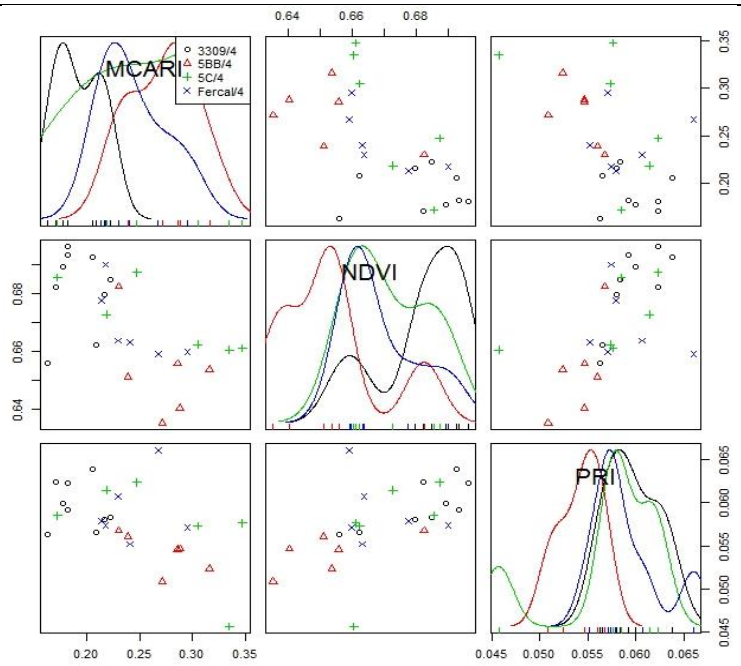


Figure 43a: MCARI, NDVI, PRI (T-4 of each rootstock on date A)

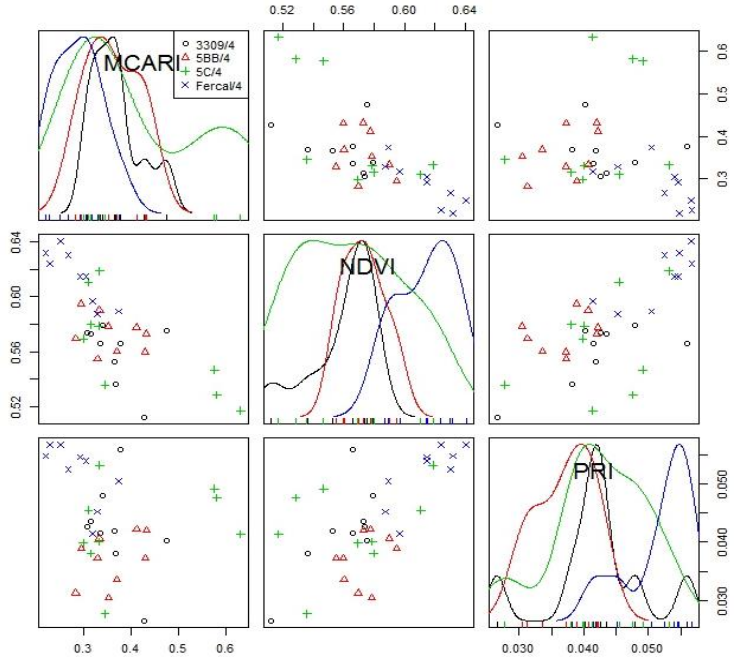


Figure 43b: MCARI, NDVI, PRI (T-4 of each rootstock on date E)

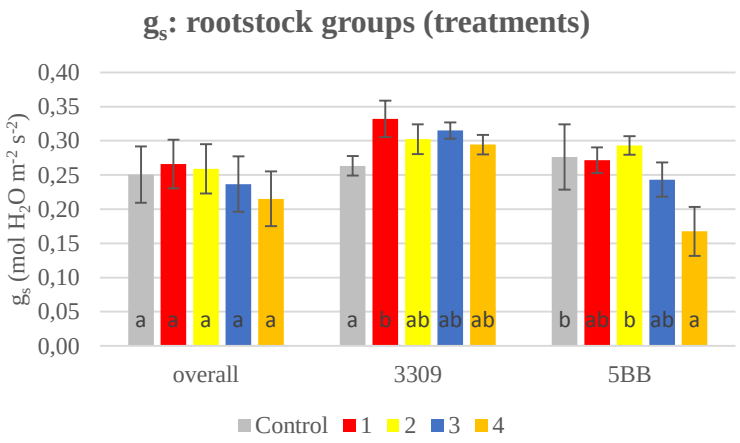
Figure 43a and b: coloured lines show the probability of distribution for the measured values of each parameter, with peaks reflecting most frequently measured values for each rootstock

Figure 43: NDVI, MCARI, PRI: comparison of rootstocks within T-4 for dates A and E

Gas exchange measurements

Parameter "g_s"

Measurements of the parameter "g_s" revealed no significant differences of the treatment groups overall (all rootstocks consolidated; Figure 44). However, a comparison of the treatment groups within the rootstock groups resulted in significant differences for the two rootstocks 3309 and 5BB. Treatment groups within the rootstocks Fercal and 5C did not reveal any statistical significances. Unlike to previously analysed parameters, no explicit trend can be noticed in Figure 44.



comparison of $\bar{x}$ with SD; executed separately for each group of bars

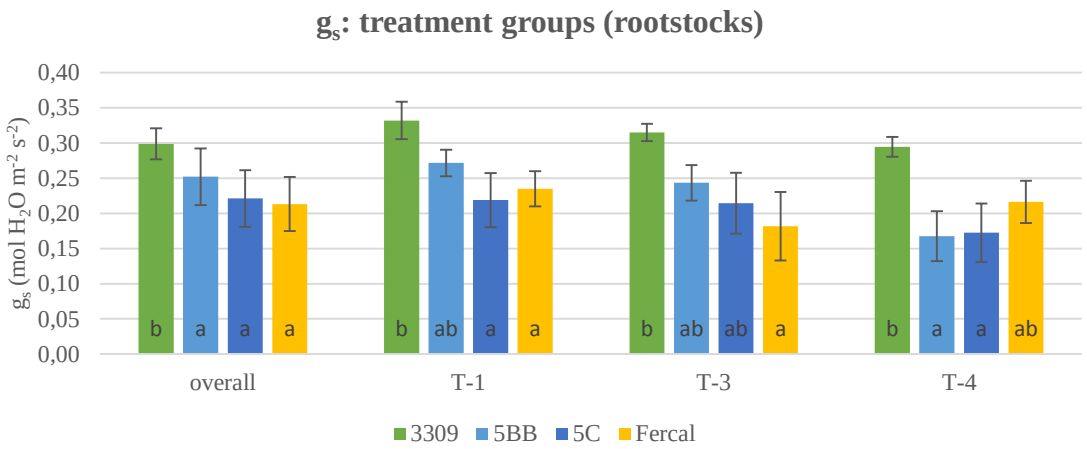
significant differences are indicated with different letters

overall: ANOVA (p= 0,0662), n= 180; consolidated rootstock groups and dates

3309: ANOVA (p= 0,0026), n= 45; **5BB:** KW (p= 0,0124), n= 45; consolidated dates

Figure 44: g_s: rootstock groups (treatments)

Figure 45 provides a comparison of parameter "g_s" of the rootstock groups with values from all treatment groups and dates consolidated (i.e. overall), as well as within each individual treatment group and revealed significant differences between the rootstocks. In all of the conducted analyses, rootstock 3309 showed the highest values while rootstocks 5C and Fercal had the lowest values. However, within T-4 values of Fercal were in between the ones from 3309 and the other rootstocks. It is worth noticing, that Fercal was the only rootstock without lower values in T-4 than in the overall analysis (comparison of absolute values, no statistical verification).



comparison of $\bar{x}$ with SD; executed separately for each group of bars

significant differences are indicated with different letters

overall: n= 180 (for each rootstock: n=45); consolidated treatment groups and dates

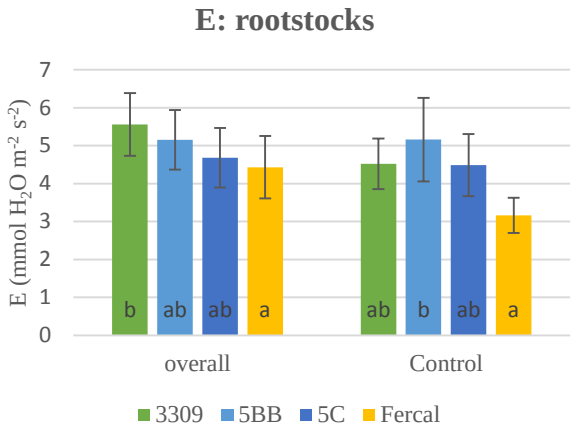
T-1, T-3 and T-4: n= 36 (for each rootstock: n= 9); consolidated dates

Treatment groups analysed	Levene Test	ANOVA (KW for Overall and T-4)
Overall	*p= 0,0080*	p= 1,254 * 10^{-9}
T-1	*p= 0,7503*	p= 0,0008
T-3	*p= 0,1588*	p= 0,0182
T-4	*p= 0,0454*	p= 0,0008

Figure 45: g_s: treatment groups (rootstocks)

Parameter E

Regarding parameter E – the transpiration rate (mmol H_2O m^{-2} s^{-2}) - significant differences were only observed between the rootstocks when all treatment groups and dates were consolidated (i.e. overall, left group of bars) and within the Control group (Figure 46). Rootstock 3309 had the highest values overall, with rootstocks 5BB and 5C following. Fercal displayed significant differences ($p< 0,05$) when being compared with rootstock 3309 and a statistical significance of $p< 0,1$ when being compared with rootstocks 5BB and 5C. Within the Control group, rootstock 5BB had significantly higher values than Fercal, while the other two rootstocks had values in between them. Neither within the other treatment groups (T-1, - T-4), nor in any other statistical setup, significant differences between rootstocks and/or treatment groups were observed.



comparison of $\bar{x}$ with SD; executed separately for each group of bars

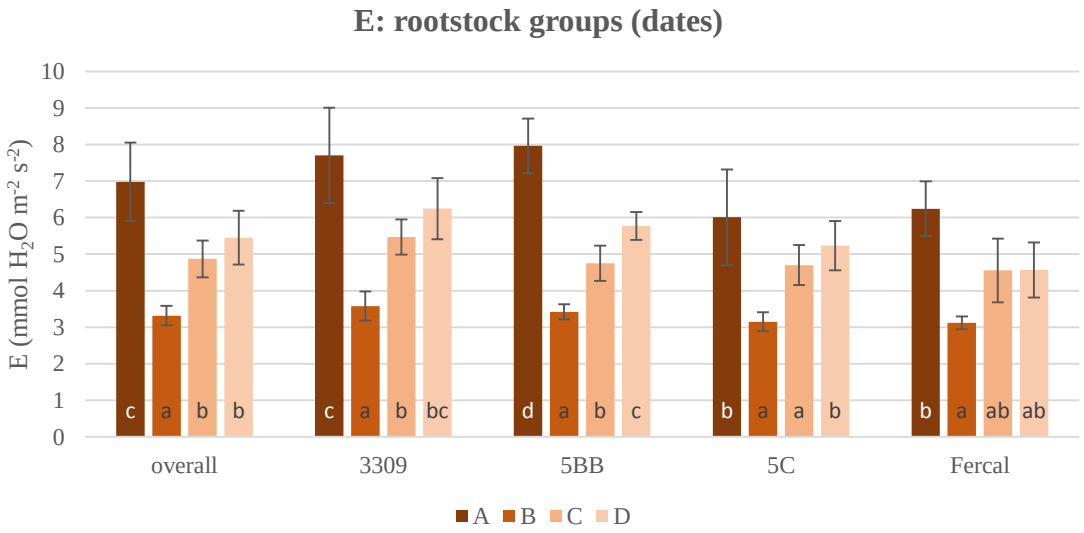
significant differences are indicated with different letters

overall: ANOVA ($p= 0,0055$), $n= 180$; consolidated treatment groups and dates

Control: ANOVA ($p= 0,0434$), $n= 50$; consolidated dates

Figure 46: E: rootstocks

Finally, dates of measurement displayed significantly different values of parameter E, as shown in Figure 47. Looking at the left group of bars, values of all rootstock and treatment groups for each of the dates were consolidated (i.e. overall) and compared with each other. Not only in this analysis, but also in the analysis within each of the rootstock groups, date A was responsible for the highest values followed by a significant drop on date B and a constant recovery on dates C and D respectively. However, similar to Figure 44, no similar pattern to previously discussed parameters could be observed.



comparison of $\bar{x}$ with SD; executed separately for each group of bars

significant differences are indicated with different letters

overall: n= 180 (A: n= 20; B: n= 40; C and D: n= 60); consolidated treatment and rootstock groups

3309, 5BB, 5C, Fercal: n= 45 (A: n= 5; B: n= 10; C and D: n= 15); consolidated treatment groups

Rootstocks analysed	*Levene Test*	ANOVA (KW for overall, 3309 and 5C)
Overall[40]	*p= 6,995 * 10^{-10}*	p= 0,1658
3309	*p= 6,778 * 10^{-5}*	p= 1,492 * 10^{-5}
5BB	*p= 0,0609*	p= 3,8 * 10^{-11}
5C	*p= 0,0002*	p= 0,0011
Fercal	*p= 0,0021*	p= 0,0195

Figure 47: E: rootstock groups (dates)

Parameter "A" – the assimilation rate (µmol CO_2 m^{-2} s^{-2}) – was also subject to different analyses, however, no significant or meaningful findings that were considered worth mentioning were found in any of them.

[40] A KW-Test showed no levels of significance, however an ANOVA with a subsequent Post-Hoc Test (Tukey comparison of means) resulted in a level of significance of p= 2 * 10-^{16} and thus revealed significant differences between the dates of measurement.

Cross connection: chlorophyll content and chlorophyll fluorescence

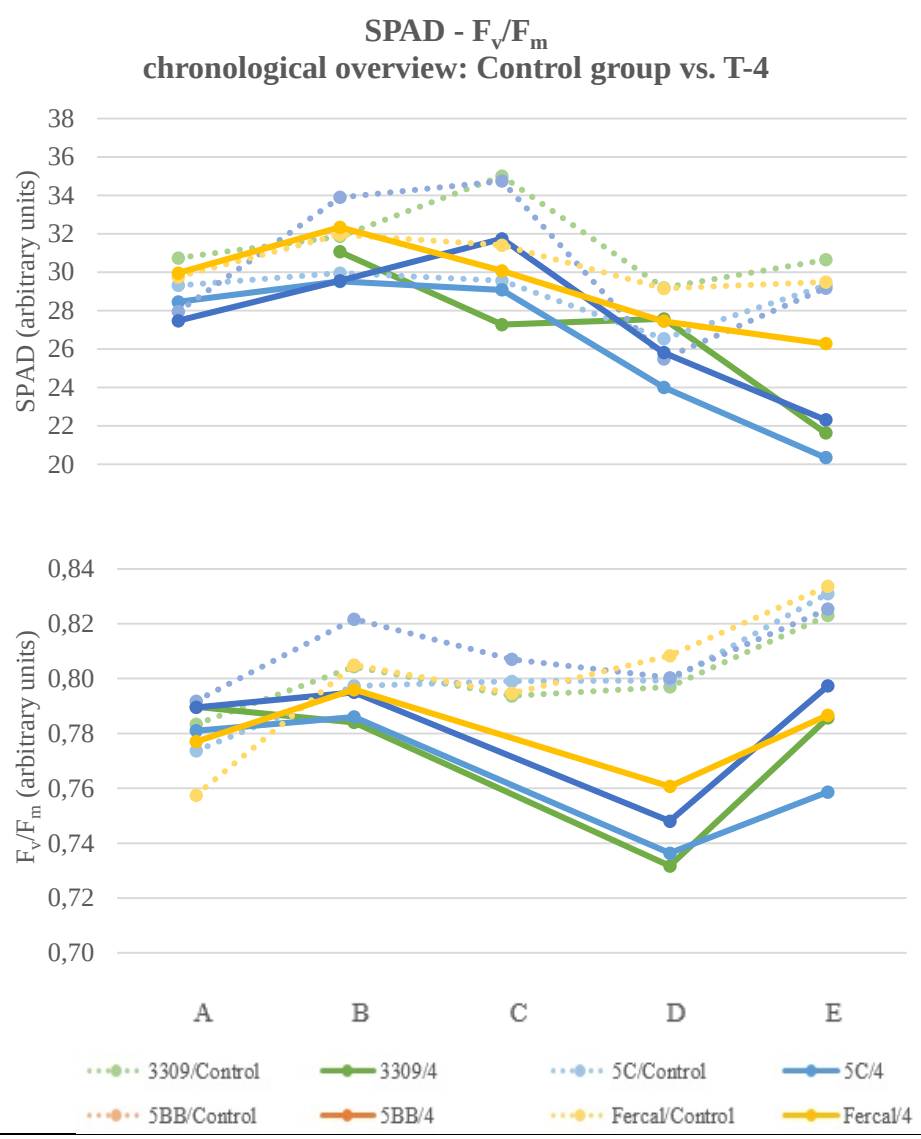
Concluding the chapter "Results", a chronological overview of two parameters (SPAD and F_v/F_m) is provided for the Control group and T-4 of each of the rootstocks (Figure 48). Each of the four rootstocks is represented by lines of the same color (3309 = green, 5BB = light blue, 5C = dark blue, Fercal = yellow). While the Control group is displayed with dotted lines, the connected lines show T-4 of each rootstock.

In both figures the values of the Control groups clearly differ from the ones of T-4, especially on the last measurement dates. Separate comparisons of each date (A-E) confirm this observation, since there were no significant differences between rootstocks of Control group and T-4 each before date E (SPAD) and date D (F_v/F_m).

The straightness of a line indicates how consistent the values of a certain rootstock-/treatment group were maintained during the course of the experiment – thus, oscillations or strong increases or decreases can be observed if values could not be maintained on a similar level.

A statistical analysis of such oscillations for SPAD measurements showed that within T-4 all rootstocks except Fercal underwent significant changes (i.e. decreases) over the time. At the same time, it was only rootstock 5BB showing significant changes within the Control group. F_v/F_m measurements showed a different pattern, since the Control group of all rootstocks except 3309 differed significantly during the course of the experiment and rootstock 3309 was the only one which oscillated in a significant way within T-4.

Since results of SPAD measurements and chlorophyll extractions correlated in this study, the SPAD results can be considered as a representative for the results obtained in the analyses of extracted chlorophyll.



above: chronological development of SPAD and F_v/F_m values ($\bar{x}$) for Control group and T-4 of each rootstock; SD not displayed to provide a clearer view

below: levels of significance between the rootstock-/treatment groups on dates A-E (columns; comparison of all measured values for the individual dates) and within Control group / T-4 of each rootstock (dotted lines; chronological comparison of each individual line);

n.s. (not significant) p > 0,05; * 0,01 < p < 0,05; ** 0,001 < p < 0,01; *** p < 0,0001

Con. = Control; Ferc. = Fercal

	A	B	C	D	E	3309 Con.	3309 T-4	5C Con.	5C T-4	5BB Con.	5BB T-4	Ferc. Con.	Ferc. T-4
SPAD	n.s.	n.s.	n.s.	n.s.	***	n.s.	**	n.s.	*	*	*	n.s.	n.s.
***n*=**	*18*	*24*	*24*	*26*	*24*	*16*	*12*	*15*	*14*	*17*	*13*	*14*	*15*
F_v/F_m	n.s.	n.s.	n.s.	*	***	n.s.	*	*	n.s.	*	n.s.	**	n.s.
***n*=**	*22*	*23*	*12*	*24*	*24*	*15*	*12*	*15*	*11*	*17*	*11*	*13*	*11*

Figure 48: SPAD – Fv/Fm: Control group vs. T-4 (chronological overview)

Root exudates

Due to technical problems and some unforeseen incidents related to Covid-19, an analysis of root exudates could regrettably not be done early enough so as to be presented in this thesis. However, since root exudate measurements were taken at the end of the experiment, this topic will be dealt with in future papers as soon as the personnel and technical situation in the responsible institutes will have returned to normal working conditions.

Discussion

This study addresses problems of vineyards located in regions abundant of calcareous soils with high bicarbonate content and highlights the importance of choosing an adequate rootstock for these regions. A better understanding of weaknesses and physiological responses of rootstocks in calcareous soils could help winegrowers making the right decisions, possibly making expensive foliar Fe applications redundant.

As described in detail in this paper, parameters related to growth and biomass (i.e. FW and DW, shoot length and leaf number), as well as parameters reflecting leaf-level performance (chlorophyll content and fluorescence, hyperspectral reflectance and gas exchange fluxes), of four rootstocks commonly used in worldwide viticulture (Fercal, Kober 5BB, Teleki 5C and 3309 Couderc) were analysed. In total, 120 young plants (30 of each rootstock) were split into five treatment groups, which received different levels of $KHCO_3$ (Control group: 0mM; T-1: 5mM; T-2: 7,5mM; T-3: 10mM; T-4: 12,5mM). Plants were cultivated in a greenhouse in Tulln in semi-hydroponic and semi-controlled conditions for two months.

Results of this study show to which extent the different rootstocks were stressed (i.e. affected) by the presence of bicarbonate, and whether they adapted their metabolism. At the end of the experiment, rootstock 5C was the one which invested most in leaf biomass (32%), a value significantly higher than the other two rootstocks 3309 (25%) and Fercal (25%). Although rootstock 3309 had the highest leaf number (36) on the last measurement dates, its leaves were the lightest and smallest, which explains, why its leaves did not contribute as much to total plant biomass (DW) as the ones of 5C. The highest contribution of shoots to total plant DW was accomplished by rootstock 3309 (63%), which can be explained by the tendency of this rootstock to build lateral shoots (Pl@ant grape, 2019-20).
Keeping the overall (underdeveloped) root status of rootstock Fercal at the beginning of the experiment in mind, it is worth mentioning, that its roots contributed nearly as much to total plant DW as 5C at the end of the experiment[41]. At the same time, Fercal was the rootstock with the lowest and second-lowest contribution of leaves and shoots to total plant DW respectively. The fact that at the beginning of the experiment Fercal weighed most (total plant DW) – presumably thanks to well-developed leaves and shoots, since its roots were

[41] Fercal had the worst developed root system at the beginning (visual testing) of the experiment, it continued to develop very slowly during the first few weeks. Since the duration of the experiment was relatively short, it is very likely, that at a later point bigger differences between the rootstocks would have been observed.

rather tiny – suggests the following: Fercal had a different strategy than rootstocks 3309 and 5C regarding its distribution of assimilates into different plant organs - namely mostly into the roots. Gruber and Kosegarten (2001) described similar observations for rootstocks tolerant to Fe deficiencies. Since the timespan of this experiment was only relatively short, it is suggested that in the long term Fercal would have been able to survive better in Fe deficient environments due to an easier Fe uptake capacity, thanks to the relatively big root surface (rhizosphere). This consideration would confirm the hypothesis, that rootstocks tolerant to Fe deficiency (in this case: Fercal) are able to adapt their growth of roots and shoots in order to survive in environments which hinder the uptake of Fe (i.e. presence of bicarbonate).

In this context, a study by Marastoni et al. (2020) revealed that grapevine rootstocks follow vastly different strategies of Fe uptake by the roots, even if all of them belong to the same plant genus (*Vitis*). The authors claim that these strategies would even contradict the commonly accepted distinction of Strategy I (non gramineous plants; e.g.: Vitis) and Strategy II plants (gramineous plants), since some grapevine rootstocks show mechanisms typically related to Strategy II plants (e.g. the release of MA`s). They conclude that rootstocks tolerant to environments with high content of bicarbonate appear to stimulate Fe acquisition by reducing the microbial competition for Fe. Furthermore, such rootstock genotypes exhibit a greater ability to increase Fe availability at the rhizosphere by an increased H^+ extrusion and the secretion of larger amounts of ferulate conjugates, which can play a role in Fe(III) reduction (Marastoni et al., 2020).

Connecting findings of this Master thesis with findings of the above mentioned studies can lead to the following considerations: Bicarbonate not only affects the growth behaviour of shoots, leaves and roots, but has also an impact on several processes related to photosynthesis. Despite many physiological processes/environmental interactions of a single rootstock, the plant must be considered as one whole connected system. It is hence very likely that single adaptions at one intersection would affect the whole plant and subsequently lead to trade-offs, since resources are always endless. For example, more root biomass could lead to an easier nutrient absorption in the future which could facilitate the future biosynthesis of chlorophyll and increase the activity of photosynthesis. However, at first, the commitment of the plant to invest currently available assimilates into the root system instead of the cell division (i.e. growth) of aboveground organs slows down the short-term biosynthesis of chlorophyll. In contrast, investing in the growth of the current aboveground

biomass would certainly result in more voluminous plants (i.e. longer shoots, bigger leaves) in the short term. The crucial question is whether rootstocks following this strategy are able to absorb enough - and the right - nutrients in order to meet the (higher) needs of the newly built aboveground cells in the mid- and long term and, furthermore, if the absorbed nutrients are sufficient in order to execute photosynthesis with the same efficiency. Especially in environments where certain nutrients, such as Fe[42], are present in very low amounts, different strategies can have significant impacts on the health status of plants.

Although the experimental setup of this study did not allow for constant (destructive) evaluations of root-, shoot- and leaf biomass, such trade-offs and their consequences on measured parameters - especially when comparing the status of the susceptible rootstock 3309 with the tolerant rootstock Fercal – can be drawn into consideration: Rootstock 3309 seemingly invested a lot more into the biomass of leaves (i.e. increase in current biosynthesis) during the first weeks of the experiment and subsequently performed very well in several growth and photosynthetic parameters at the beginning of the experiment. At the end of the experiment this rootstock presumably paid tribute to its initial "investment strategy" of the assimilates and underperformed in many photosynthetic parameters. Contrary to this, rootstock Fercal, invested relatively more in root biomass[43] (probably already during the first days/weeks of the experiment), which could explain the better performance in nearly all photosynthetic performance parameters at the end of the experiment. In the case of this study, the relation of biomass vs. nutrients of Fercal in the photosynthetic active plant tissues was presumably more equilibrated than it was for the other rootstocks, with a (relatively) higher amount of Fe in the relatively lower biomass of leaves and chloroplasts. Future studies should evaluate when such different growth adaptations first become visible/measurable and relate them to nutrient contents of the different plant organs.

[42] As explained previously in this study, less Fe content within the chloroplasts would lead to a lower efficiency of the light harvesting complexes and thus reduce the efficiency of the photosystems.
[43] It is very likely that the bigger root biomass went hand-in-hand with an increased extrusion of phenolics and a more efficient acidification of the rhizosphere as well. Fe would not only become more bioavailable but could at the same time be taken up in higher amounts due to the increased root surface.

However, following the considerations of this study, tolerant rootstocks can be separated from susceptible ones by their behaviour/strategy of providing for a sufficient nutrient supply in the future, while at the same time increasing the current biomass of the different plant organs only as much as the current nutrient availability allows for. Keeping this suggestion in mind, should help to better understand the interpretations of some of the results of this study described in what follows:

Despite having underdeveloped (in size, length and biomass) aboveground plant organs, rootstock Fercal managed to be the only rootstock without significant changes in chlorophyll content of the leaves, when comparing T-4 and Control group at the end of the experiment. A comparison of treatment groups showed a certain impact of $KHCO_3$, since T2, T-3 and T-4 all had a significantly lower chlorophyll content than the Control group. Rootstock 3309 was apparently affected most by bicarbonate, since it revealed the highest decrease (absolute values) between Control group and T-4, the latter was considered to be the treatment group affected most by the presence of bicarbonate. Livigni et al. (2019) and Bavaresco et al. (2006) describe that the total chlorophyll content strongly depends on the uptake of Fe and Magnesium (Mg), since those elements are main components of the chloroplasts, the chlorophyll molecule and the chlorophyll biosynthesis. These observations of the chlorophyll content with SPAD were fortified by the results of chlorophyll extractions, which revealed more or less the same levels of significance between the treatment groups of each individual rootstock. Fanizza et al. (1991) already described a good correlation between SPAD results and the "real" (extracted) chlorophyll content of grapevine leaves.

Again, the different growth strategies of the rootstocks could explain why the chlorophyll content of the young leaves differed significantly within the different treatment groups, while the chlorophyll content of the old leaves did not: Old leaves were reflecting the health status of the plants during the first few weeks of the experiment, when they had been irrigated only with nutrient solution, since Fe is not translocated within the plant and stays within the old leaves even if future Fe availability and supply are decreased. Thus, young leaves are better able to reflect the current health status of the plants, because they have to rely on current availability and supply of Fe during their formation process. Young leaves showed significantly lower contents of chlorophyll for rootstocks 3309 and 5C, which both invested more into aboveground biomass. Rootstock Fercal, which invested relatively more into root biomass, was able to maintain similar chlorophyll contents of the young leaves in all

treatment groups. This indicates that this rootstock was yet able to maintain an equilibrated nutrient (Fe) status at the end of the experiment. Results of the total carotenoid content showed similar relations between the Control group and stressed treatment groups[44].

Parameter F_v/F_m, which reflects the maximum quantum efficiency of photosystem II (Murchie and Lawson; 2013), also displayed the impact of $KHCO_3$ on plants well: A constant decline from the Control group towards T-4 of the values of this parameter could be observed. While the decrease of values of T1 – T-4 for rootstocks 5BB and 5C were significant, this was not the case for 3309 and Fercal. However, 3309 had lower absolute values than Fercal in all treatment groups. Furthermore, rootstock Fercal (with all treatment groups consolidated) had the highest values at the end of the experiment and even differed significantly from 5C. A possible explanation for 5C being the worst rootstock with regard to this parameter could be the fact that 5C built the biggest and relatively heaviest (DW) leaves. It may be concluded from a finding of Covarrubias et al. (2014), that the requirement of Fe is relatively higher to keep a plant (i.e. grapevine rootstock) healthy and vital if it builds up a lot of aboveground biomass in a short time. Suggesting that Fe assimilation of 5C could not keep up with its growth, could be a possible explanation as to why this rootstock not only was the significantly worst in F_v/F_m, but also had the significantly lowest chlorophyll content. Bavaresco et al. (2006) observed correlations between F_v/F_m and chlorophyll content.

Analyses of PI_{total} on the last two measurement dates revealed a similar pattern as already observed for chlorophyll content: Fercal performed best, followed by 5BB, 5C and 3309 respectively. Although differences between rootstocks were not statistically significant, it can be assumed that Fercal managed to conserve the energy absorbed by PS II better than other rootstocks (Samborska et al.; 2019). This consideration can be fortified by the fact that Fercal did not invest as much energy in the growth of leaves (DW biomass) as the other rootstocks, and therefore presumably could translocate a sufficient amount of assimilated nutrients (in this case especially Fe) to the photosynthetic active leaves.

Hyperspectral reflectance parameters confirmed a general impact of $KHCO_3$ on the plants as well, since especially parameter NDVI revealed significantly lower values within T-1 -

[44] Additional information about chlorophyll a:b and total chlorophyll:total carotenoid ratios are provided in the attachment.

T-4 in a comparison with the Control group at the end of the experiment. Rootstock Fercal was not only the only rootstock without statistical differences between treatment groups in general, but also managed to differ significantly from the other rootstocks with the highest NDVI-values within T-4 at the end of the experiment.

Similar conclusions can be drawn with regard to parameters MCARI and PRI[45], in relation of which stressed plants of rootstock Fercal outperformed the others at the end of the experiment. Plants within T-4 of the rootstocks 5BB, 5C and 3309 were all stressed to a similar extent at the end of the experiment, irrespective of which reflectance parameter was observed. Parameter NDVI is commonly used in science to evaluate general canopy features, however, Gamon et al. (1992) allude that it would be a poor indicator of real-time photosynthetic fluxes or other dynamic physiological processes which occur on fine temporal and spectral scales. The authors` suggestion of using parameter PRI instead of / or additionally to NDVI was respected in this experiment. Since the hyperspectral analyses of this study showed similar and robust results in all parameters (NDVI, MCARI, PRI), it is highly probable that together they would reflect the physiological status of the plants in a reliable way.

Referring once more to parameter F_v/F_m, Murchie and Lawson (2013) describe healthy plants having values of around 0,83. Although nutrient availability was equilibrated and guaranteed (Hoagland solution), not many plants reached the value of 0,83 in this experiment, which suggests that plants were water-stressed. A closer look at the experimental set up supports this suggestion: For practical reasons plants were only watered five to six times per week during some very hot summer days and thus didn`t receive a constant water supply, which would presumably have ameliorated their physiological processes. Such water stress could also have been a reason for different development of the aboveground plant organs. Rootstocks 5BB and 3309 are described as "well adapted to sandy soils" and rootstocks 5BB and 5C are known for their good wood production (i.e. shoots) amongst rootstock producers (Pl@ant grape, 2019-20). These three rootstocks (5BB, 5C, 3309) were the ones with the longest shoot length in this experiment.

Fercal, in contrast, has only a moderate wood production and its drought tolerance can only be considered as moderate to good, if its roots are well-developed (Pl@ant grape, 2019-20), which was not the case at the beginning of this experiment.

[45] Indicator of photosynthetic light use efficiency, since it is sensitive to changes in carotenoid pigments in live foliage (Gamon et al.; 1992).

The additional unplanned water-stress could furthermore explain why correlations between reflectance, fluorescence and gas exchange parameters could not be confirmed in this experiment, although such were described by Bavaresco et al., (2006).
In fact, gas exchange parameters revealed completely different findings than discussed until now. Rootstock 3309 had the best stomatal conductance (g_s) and was significantly higher than Fercal in all treatment groups except T-4, in which it still performed better than Fercal in absolute values. The transpiration rate (E), as well as the assimilation rate (A) either showed results without comprehensible patterns, or did not reveal statistical significances between rootstock and/or treatment groups. Another possible explanation for the results of gas exchange measurements could be that measurements were regrettably not always taken AFTER plants had been irrigated, which presumably distorted results. In this context, Bojovic et al. (2017) reported that around noon[46] water-stressed plants have small peaks in all gas exchange parameters, while well-watered plants have a so-called "midday depression". Following these findings, chronological comparisons are not very reliable if the water-status of plants differs between the measurement dates.

Finally, it is of relevance to remember that the primary goal of this study was to analyse the effects of bicarbonate on physiological processes of the rootstocks and not water-stress. In this context - neglecting the seemingly backfired gas exchange results – rootstock Fercal proved to have the healthiest plant development and would presumably have managed best to survive in the presence of bicarbonate. In most of the discussed parameters, Fercal outperformed the other rootstocks at the end of the experiment and has managed to do so by a different adaptation of its physiological processes and investments in other parts of the plants – for example the roots. Rootstock 3309 had a similar strategy in the build-up of plant organs as 5BB and 5C, and performed similarly in respect to parameters related to chlorophyll content, chlorophyll fluorescence and hyperspectral reflectance[47]. Generally spoken, observations of this study confirm the hypothesis, that rootstocks tolerant to high bicarbonate contents in the soil adapt their metabolism and physiological processes in a different way than susceptible rootstocks.

[46] Gas exchange parameters were constantly measured around noon in this experiment.
[47] It was assumed that rootstock 3309 would perform worst in this respect, since this rootstock was described to have the highest susceptibility to Fe deficiency in relation to the other three rootstocks analysed in this experiment.

Future studies analysing to which extent responses/strategies of rootstocks are predetermined by the genetics will be necessary, since it is not yet fully understood which gene loci are responsible for certain strategies or responses that are targeted to prevent Fe deficiency.

Last but not least, this experiment was set up in semi-hydroponic conditions where plants neither had the possibility to absorb nutrient from the soil itself, nor the advantage of microorganisms or fungi to be supporting them. This set up presumably affected the development of the rootstocks too. Thus, more studies which analyse effects of bicarbonate on physiological and growth-related parameters of different rootstock genotypes could help to understand the processes within the plants even better. Moreover, since it is highly likely that plants were stressed by the high bicarbonate content AND by an unintentional irregular water deficit, some results of this study – especially gas exchange fluxes - could be distorted at least at some point.

Summary

Small grapevine rootstocks of *Vitis vinifera* with a different susceptibility to Fe deficiency, namely **Fercal** (tolerant), **3309 Couderc** (susceptible), **5BB Kober** and **5C Teleki** (both: medium tolerant), were cultivated in a greenhouse in Tulln in semi-controlled and semi-hydroponic conditions between 19 June and 13 August 2020. Plants were split into five treatment groups, consisting of 24 plants each (i.e. 6 plants of each rootstock). While the Control group plants were irrigated with a neutral nutrient solution only, treatment groups 1 (T-1), T-2, T-3 and T-4 received 5mM, 7,5mM, 10mM and 12,5mM of bicarbonate ($KHCO_3$) respectively from 20 July onwards in order to induce Fe deficiency through the elevated levels of bicarbonate.

Literature highlights the importance of an adequate Fe supply for grapevine since it influences physiological properties of the plants such as yield, resistance to abiotic and biotic stresses, berry quality, ripening time, etc. In the presence of $KHCO_3$ (abundant in calcareous soils), Fe uptake of plants is buffered, because several Fe uptake strategies of plants are disturbed by bicarbonate. According to literature, different rootstock genotypes display different levels of susceptibility to a bicarbonate induced Fe deficiency, which highlights the importance of choosing an adequate rootstock for such environments. As a consequence of choosing the most suitable rootstock, commonly used expensive foliar applications of Fe could subsequently become redundant and significantly alleviate the vineyard management for winegrowers.

In this study parameters related to growth and biomass (i.e. FW and DW, shootlength and leafnumber) as well as parameters reflecting leaf-level performance (chlorophyll content – SPAD and extractions; chlorophyll fluorescence – HandyPea; Hyperspectral reflectance – PolyPen; and gas exchange measurements – LCpro-SD) were weekly measured so as to evaluate to which extent the four rootstocks were stressed by the presence of bicarbonate and whether they adapted their metabolism.

Results of parameters related to growth revealed that rootstock 5BB built the longest shoots, followed by 3309, 5C and – with a significant gap – Fercal. However, rootstock Fercal managed to adapt its metabolism in such a way that, at the end of the experiment, it had better results than the other rootstocks in all parameters related to leaf-level (photosynthetic) performance, with the exception of gas exchange. A possible explanation for the divergence

of results related to gas exchange measurements could be the fact that Fercal had an underdeveloped root system at the beginning of the experiment. Since this rootstock is known for its drought problems in juvenile stages when not having a proper root system, water stress could not only have effected its growth development, but also its performance in gas exchange.
Rootstocks 3309, 5BB and 5C all performed at similar levels in the leaf-level parameters, not confirming findings in literature which describe rootstock 3309 as being more susceptible to Fe deficiency than 5BB and 5C.

The majority of the results of this study are in accordance to prior studies, which found out that the presence of bicarbonate induced several changes in the plant physiology and growth of rootstocks, mainly due to a limited capacity to drive photosynthesis. The amplitude of these changes is reported to strongly depend on the rootstock genotype, which was confirmed by this study.

Since the semi-hydroponic setup of this experiment did not reflect the natural environment, studies with a different experimental setup are required in order to fully understand the adaptations of physiological processes within the different rootstock genotypes in the presence of bicarbonate.
Moreover, studies analysing the real nutrient content (e.g. Fe and Mg) within the different plant organs in addition to the parameters discussed in this study could lead to new and more precise findings.

Bibliography

McGovern, P.; Jalabadze, M.; Batiuk, S.; Callahan, M. P.; Smith, K. E.; Hall, G. R.; Kvavadze, E.; Maghradze, D.; Rusishvili, N.; Bouby, L.; Failla, O.; Cola, G.; Mariani, L.; Boaretto, E.; Bacilieri, R.; This, P.; Wales, N.; Lordkipanidze, D. (2017): Early Neolithic wine of Georgia (online). PNAS 2017 (48), 114, 10309-E10318.

Gautier, A. T.; Cookson, S. J.; Lagalle, L.; Ollat, N.; Marguerit, E. (2020): Influence of the three main genetic backgrounds of grapevine rootstocks on petiolar nutrient concentrations of the scion, with a focus on phosphorus (online). OENO One 2020 (1), 1-13.

Warschefsky, E. J.; Klein, L. L.; Frank, M. H.; Chitwood, D. H.; Londo, J. P.; von Wettberg, E. J.; Miller, A. J. (2016) Rootstocks: diversity, domestication, and impacts on shoot phenotypes (online). Trends Plant Sci. 21 (5), 418-437.

Spiller, S., and Terry, N, (1980): Limiting factors in photosynthesis. II. Iron stress diminishes photochemical capacity by reducing the number of photosynthetic units (online). Plant Physiol. (65), 12l-125.

Chen, Y. and Barak, P. (1982): Iron Nutrition of Plants in Calcareous Soils (online). in Agronomy (35), 217-240.

Pandey, J. and Gopal, R. (2011): Laser-induced chlorophyll fluorescence and reflectance spectroscopy of cadmium treated *Triticum aestivum* L. plants (online). Spectroscopy (26), 129-139.

Bar-Akiva, A. and Lavon, R. (1968) Peroxidase activity as an indicator of the Fe requirements of citrus plants (online). Isr. J. Agric. Res. Ktavin (18), 145–153.

Marschner, H. (1995): Mineral nutrition of higher plants (online). London: Academic Press (2), 889.

Colombo, C.; Palumbo, G.; He, J-Z.; Pinton, R.; Cesco, S. (2013): Review on iron availability in soil: Interaction of Fe minerals, plants, and microbes (online). Journal of Soils and Sediments (14), 3.

Lindsay, W. L. (1979): Chemical Equilibrium in Soils (online). John Wiley & Sons, N.Y. (448)

Mengel, K.; Kirkby, E.; Kosegarten, H.; Appel, T. (2001): Iron. In: Mengel, K. and Kirkby, E. A. (eds.): Mineral nutrition (Vol. 5). Dordrecht: Kluwer Academic Publishers, 553-571

Römheld, V. and Marschner, H. (1986): Mobilization of iron in rhizosphere of different plant species. In: Tinker, P. B. H.; Laüchli, A. (eds.): Advances in plant nutrition (Vol. 2.). pp 155–204

Misra, A. N.; Misra, M.; Singh, R. (2012): Chlorophyll Fluorescence in Plant Biology. In: Misra, A. N. (ed.): Biophysics (7). Intech,171-192

Barber, S. A. (1974): Influence of the plant root on ion-movement in soil. In: Carson, E. W. (ed.): The plant root and its environment. Charlottesville: University Press of Virginia, 525-564.

Oliveira, E. M. M.; Ruiz, H. A.; Alvarez V., V. H.; Ferreira, P. A.; Costa, F. O.; Almeida, I. C. C. (2010): Nutrient supply by mass flow and diffusion to maize plants in response to soil aggregate size and water potential (online). Revista Brasileira de Ciência do Solo, 34 (2), 317-328.

Naranjo Arcos, M. and Bauer, P. (2016): Iron Nutrition, Oxidative Stress, and Pathogen Defense. In: Erkekoglu, P. and Kocer-Gumusel, B. (eds.): Nutritional Deficiency (4). Intech, 63-98.

Schroth, G.; Lehmann, J.; Barrios, E. (2003): Soil nutrient availability and acidity. In: Schroth, G. and Sinclair, F. L. (eds.): Trees, crops and soil fertility: concepts and research methods (Chapter 5). Wallingford: CABI Publishing, 93-130.

Seraj, F. and Rahman, T. (2018): Heavy Metals, Metalloids, Their Toxic Effect and Living Systems (online). American Journal of Plant Sciences (9), 2626-2643.

Chaney, R.L.; Brown, J.C.; Tiffin, L.O. (1972): Obligatory reduction of ferric chelates in iron uptake by soybeans (online). Plant Physiol. (50), 208–213.

Bradford, G. R.; Bair, F.L.; Hunsaker, V. (1971): Trace and major element contents of soil saturation extracts (online). Soil Sci. (112) 225–230.

Oliver, S. and Barber, S. A. (1966) Mechanisms for the movement of Mn, Fe, B, Cu, Zn, Al, and Sr from one soil to the surface of soybean roots (online). Soil Sci. Soc. Am. Proc. (30), 468–470.

Kashirad, A. and Marschner, H. (1974): Iron nutrients of sunflower and corn plants in mono and mixed culture (online). Plant Soil (41), 91-101.

North, C. P. and Wallace, A. (1952): Lime induced chlorosis in avocado and a possible method of control (online). Yearbook Calif. Avocado Soc. (37), 177–186

Kirkby, E. A. and Knight, A. H. (1977): Influence of the level of nitrate nutrition on ion-uptake and assimilation, organic Acid accumulation, and cation-anion balance in whole tomato plants (online). Plant physiology, 60 (3), 349–353.

Esty, J. C.; Onken, A. B.; Hossner, L. R.; Matheson, R. (1980): Iron use efficiency in grain sorghum hybrids and parental lines (online). Agron. J. (72), 589–592.

Bar-Akiwa, A. (1961): Biochemical Indications as a means of distinguishing between Iron and Manganese Deficiency Symptoms in Citrus Plants (online). Nature (190), 647–648.

El-Ramady, H.; Alshaal, T.; Amer, M.; Domokos-Szabolcsy, E.; Elhawat, N.; Joe, P.; Fári, M. (2014): Soil Quality and Plant Nutrition. In: Lichtfouse, E. (ed.): Sustainable Agriculture Reviews (Vol. 14), 11. Switzerland: Springer International Publishing, 345-447.

Austrian Wine (s.a.): Geology of the winegrowing regions. https://www.austrianwine.com/our-wine/climate-soil/geology/geology-of-the-wine-growing-regions (5.10.2020).

TerroirWines (s.s.): Austria. https://www.terroirwines.us/austria. (5.10.2020).

Der Standard (2010): Einzige Millionenstadt mit Weinbau. https://www.derstandard.at/story/1288659542604/einzige-millionenstadt-mit-weinbau. (6.10.2020).

Morisson Couderc – Grapevine Nurseries (2017): Analysis. http://morissoncouderc.com/wp-content/uploads/2016/02/Analyses-GB-Morisson-Couderc.pdf. (6.10.2020).

Vitis International Variety Catalogue database ("VIVC") (2020): Statistical information based on VIVC records. https://www.vivc.de/index.php?r=passport-statistic%2Fresult&PassportStatisticSearch%5Bstinfo%5D=gattung_id&page=1. (6.10.2020).

Goldhammer, T. (2018): The Grapegrowers handbook – A guide to viticulture for wine Production (online). Virginia: Apex Publishers.

Lumitos (2020): Chlorophyll. https://www.chemeurope.com/en/encyclopedia/Chlorophyll.html. (28.9.2020).

Byjus (2020): Symplast. https://byjus.com/biology/symplast/. (25.9.2020).

Encyclopædia Britannica (2020): Casparian strip – plant structure. https://www.britannica.com/science/Casparian-strip. (25.9.2020).

Dell'Orto, M.; Zocchi, G.; Brancadoro, L. (2000): Use of biochemical parameters to select grapevine genotypes resistant to iron-chlorosis (online). Journal of Plant Nutrition 23 (11-12), 1767-1775.

Gruber, B.; Kosegarten, H. (2001): Depressed growth of non-chlorotic vine grown in calcareous soil is an iron deficiency symptom prior to leaf chlorosis (online). Journal Plant Nutr. Soil Sci. 165, 111-117.

Ksouri R, K.; Mahmoudi, H; Gharsalli, M.; Lachaâl, M. (2004): Physiological responses of native Tunisian grapevines and some rootstocks to direct iron deficiency (online). Vitis 43 (2), 97-98.

Bavaresco, L.; Vezzulli, S.; Ferrari, F. (2005): Grape production, technological parameters, stilbenic compounds as affected by lime-induced chlorosis (online). Vitis 44 (2), 63-65.

Bavaresco, L.; Bertamini, M.; Iacono, F. (2006): Lime-induced chlorosis and physiological responses in grapevine (*Vitis vinifera* L. cv. Pinot blanc) leaves (online). Vitis 45 (1), 45-46.

Ksouri, R.; Debez, A.; Mahmoudi, H.; Ouerghi, Z.; Gharsalli, M.; Lachaâl, M. (2007): Genotypic variability within Tunisian grapevine variabilities (*Vitis vinifera* L.) facing bicarbonate-induced iron deficiency (online). Plant Physiology and Biochemistry 45, 315-322.

Erdem, H.; Sabir, A.; Bilir-Ekbic, H.; Tangolar, S. (2010): Response of four grapevine (*Vitis* spp.) genotypes to direct or bicarbonate-induced iron deficiency (online). Spanish Journal of Agricultural Research 8 (3), 823-829.

Kobayashi, T. and Nishizawa, N. K. (2012): Iron Uptake, Translocation and Regulation in Higher Plants (online). Annual Review of Plant Biology 63, 131-152

Covarrubias, J. I. and Rombolà, A. D. (2014): Evaluation of sustainable management techniques for preventing iron chlorosis in the grapevine (online). Australian Journal of Grape and Wine Research (20), 149-159.

Covarrubias, J. I.; Rombolà, A. D. (2013): Physiological and biochemical responses of the iron chlorosis tolerant grapevine rootstock 140 Ruggeri to iron deficiency and bicarbonate (online). Plant Soil (370) 305-315.

Martínez C. A.; Erene Arrabal, M.; Carreño Espín, J.; Fernández Rubio, J. (1990): Patrones de la vid. Serie: Divulgación Tècnica 9. Consejería de Agricultura, Murcia: Ganadería y Pesca de la Region Murcia.

Marsalha, J.; Kosegarten, H.; Elmaci, Ö.; Mengel, K. (2000): The central role of microbal activity for iron acquisition in maize and sunflower (online). Biol. Fertil. Soils (30), 433-439.

Pavloušek, P. (2008): Preliminary results of tests of grapevine rootstocks resistance to lime-induced chlorosis (online). Acta Universitatis Agriculturae et Silviculturae Mendelianae Brunensis (56), 299-301.

Mercier (2020): Portainjertos certificados disponibles. http://www.mercier-groupe.com/es/portainjertos-certificados-disponibles. (20.9.2020)

Wineplant (2020): Vine, clone and rootstock. https://www.wineplant.bz.it/en/rootstocks/. (20.9.2020)

Hoagland, D. R. & Arnon, D. I. (1938): The water culture method for growing plant without Soil (online). Circular 347. University of California – Agricultural Experiment Station, California: University of California.

Pl@ntGrape (2019-2020): Catalogue of Vines Cultivated in France - IFV – INRAE – l'Institut Agro, Montpellier SupAgro. https://plantgrape.plantnet-project.org/en/ (3.12.2020)

Bojovic, M.; Nikolic, N.; Borisev, M.; Pajevic, S.; Zupunski, M.; Horák, R.; Pilipovic, A.; Orlovic, S.; Stojnic S. (2017): The Diurnal Time Course of Leaf Gas Exchange Parameters of Pedunculate Oak Seedlings Subjected to Experimental Drought Conditions (online). Baltic Forestry 23(3): 584-594.

Livigni, S.; Lucini, L.; Sega1, D.; Navacchi, O.; Pandolfini, T.; Zamboni, A.; Varanini1, Z. (2019): The different tolerance to magnesium deficiency of two grapevine rootstocks relies on the ability to cope with oxidative stress (online). BMC Plant Biol. 2019 19: 1–17.

Murchie, E.H. and Lawson, T. (2013): Chlorophyll fluorescence analysis: a guide to good practice and understanding some new applications (online). Journal of Experimental Botany, 64(13): 3983–3998.

Samborska, I. A.; Kalajia, H. M.; Sieczkoc, L.; Boruckid, W.; Radosław, M.; Kouzmanovaf, M.; Goltsev, V. (2018). Can just one-second measurement of chlorophyll a fluorescence be used to predict sulphur deficiency in radish (*Raphanus sativus L. sativus*) plants? (online). Current Plant Biology 19(2019): 100096.

Daughtry, C.; Walthall, C.; Kim, M. S.; Colstoun, E. B.; McMurtrey, J. E. (2000): Estimating Corn Leaf Chlorophyll Concentration from Leaf and Canopy Reflectance (online). Remote Sensing of Environment. 74: 229-239.

Gamon, J.; Peñuelas, J.; Field, C. (1992): A narrow-waveband spectral index that tracks diurnal changes in photosynthetic efficiency (online). Remote Sensing of Environment, 41: 35-44.

Carbonell-Bejerano, P.; Carvalho, L.; Eiras-Dias, J.; Martínez-Zapater, J.; Amancio, S. (2016): Exploiting Vitis genetic diversity to manage with stress. In: Gerós, H.; Chaves, M. M.; Gil, H. M., Delrot, S. (eds.): Grapevine in a changing Environment: A molecular and Ecophysiological perspective. John Wiley and Sons. First edition (15): 347-381.

Marastoni, L.; Lucini, L.;Miras-Moreno, B.; Trevisan, M.; Sega, D.; Zamboni, A.; Varanini, Z. (2020): Changes in physiological activities and root exudation profile of two grapevine rootstocks reveal common and specific strategies for Fe acquisition (online). Sci Rep 10:18839.

Belkhodja, R.; Morales, F.; Quilléz, R.; Lopéz-Millan, A.F.; Abadia, A.; Abadia, J. (1998): Iron deficiency causes changes in chlorophyll fluorescence due to the reduction in the dark of the Photosystem II acceptor side (online). Photosynthesis Research 56:265-276.

Mänd, P. (2013): Light use capacity and carbon and nitrogen budget of plants: remote assessment and physiological determinants (Diss.). Department of Botany, Institute of Ecology and Earth Sciences, Faculty of Science and Technology, University of Tartu, Estonia

Colom, M.R. and Vazzana, C. (2003): Photosynthesis and PSII functionality of drought resistant and drought-sensitive weeping lovegrass plants (online). Environmental and Experimental Botany 49(2):135-144.

Fanizza, G.; Dellagatta, C.; Bagnulo, C. (1991): A non-destructive determination of leaf chlorophyll in *Vitis vinifera* (online). Ann Appl Biol 119:203–205.

Bappa, D.; Sahoo, R.; Pargal, S.; Krishna, G.; Verma, R.; Tiwari, R.; Chinnusamy, V.; Sehgal, V.; Gupta, V. (2015). Spectral Based Non-Invasive Estimation of Plant Chlorophyll Content (online). Journal of Agricultural Physics 15(1):88-102.

Attachment

Vitis vinifera L. belongs to the order of *Vitales* and the family of *Vitaceae* and is a perennial liana. Although thousands of different varieties of *Vitis vinifera* are known (Goldhammer, 2018), *Vitis vinifera ssp. vinifera* is by far the most important one used in viticulture. Latest archeological findings reveal that varieties of *Vitis vinifera* were cultivated by mankind in the Eurasian region already about 8.000 years ago (McGovern et al., 2017).

Austrian wine regions:

Since this paper has been published in Austria, the following short overview of the vast soil diversities focusses on Austria`s main winegrowing regions as a representative for the diversity worldwide. A special focus will be given to the presence of limestone because of its relevance for the experiment of this thesis.

Austria consists of four main winegrowing regions, namely Lower Austria, Burgenland, Styria and Vienna (AUSTRIAN WINE, s.a.). All of the four regions fall into some sub-regions, but these details would lead too far away.

Lower Austria typically has top-soils of Quaternary deposits such as fine-grained loess in more than 50 % of the regions' surface area and coarse-grained terrace gravels. Especially loess can show high calcareous dolomitic content, with the "Leitha limestone" being a representative for calcareous soils. Nevertheless, unconsolidated rocks in this wine-growing region differ greatly in carbonate content (AUSTRIAN WINE, s.a.).

In **Burgenland** over 60 % of vineyards are dominated by calcareous sandy gravels deposited along the ancient course of the river Danube. However, the soil spectrum ranges from partly silty non-calcareous clays in central Burgenland to the consolidated calcareous "Leitha limestone" (AUSTRIAN WINE, s.a.).

The region of **Styria**, located in the area of the central eastern Alps has most of its vineyards within the "Styrian basin". Special features of this region are the volcanic basalts present in the soil of some vineyards, however, there is some limestone contents prevalent as well (AUSTRIAN WINE, s.a.).

Vienna, as the only major city with viticulture that is worth mentioning within its boundaries[48], has its vineyards situated upon the consolidated rocks of the Penninic Flysch

[48] About 700 ha of vineyards are cultivated in the districts of Vienna (Der Standard, 2010).

as well as on marginal marine sediments of the “Vienna basin”. While Flysch consists of partly calcareous and partly quartz-rich sandstones with clay layers, the deposits of the “Vienna basin” mainly consist of consolidated limestone - once more, the “Leitha limestone” (AUSTRIAN WINE, s.a.).

Figure 49 shows Austria`s main winegrowing regions.

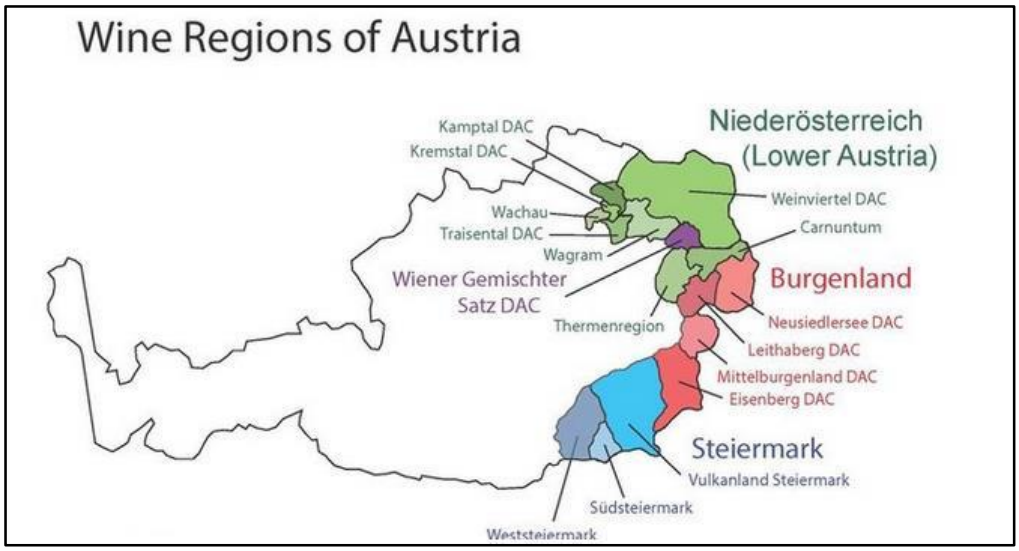
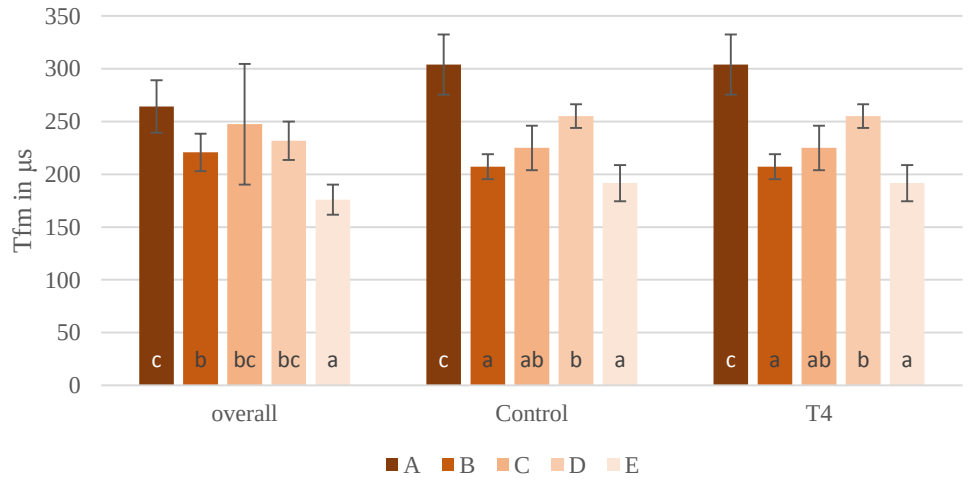


Figure 49: Wine regions of Austria (Terroir-wines, s.a.)

Parameter Tfm

The parameter "Tfm" - with lower values indicating sample stress – turned out to significantly differ between the dates of measurement: Analysing all treatment groups (i.e. overall), date "A" - when plants had not yet been exposed to $KHCO_3$ solutions - displayed the highest values, while "Tfm" was on the lowest level on date "E", when plants had been irrigated with $KHCO_3$ for the longest period of the experiment. Dates "B", "C" and "D" all displayed values in between the ones for "A" and "E" with date "C" and "D" having significantly higher values ($p < 0,05$) than just after the beginning of irrigation with $KHCO_3$ after date "B", as shown in Figure 50. Analysing the chronological development of "Tfm" for each of the treatment groups separately, highlighted significant differences only within the Control group and T-4 ($p < 0,05$), where the values for date "B" were at the same (low) level as the ones of date "E". "Tfm" of the plants within T-1, T-2 and T-3 did not differ significantly over the time. A comparison of the four rootstock groups did not show significant differences between them neither (ANOVA: p= 0,63)



comparison of $\bar{x}$ with SD; executed separately for each group of bars

significant differences are indicated with different letters

overall: n= 265; consolidated rootstock groups and dates

Control: n= 60; **T-4**: n= 45

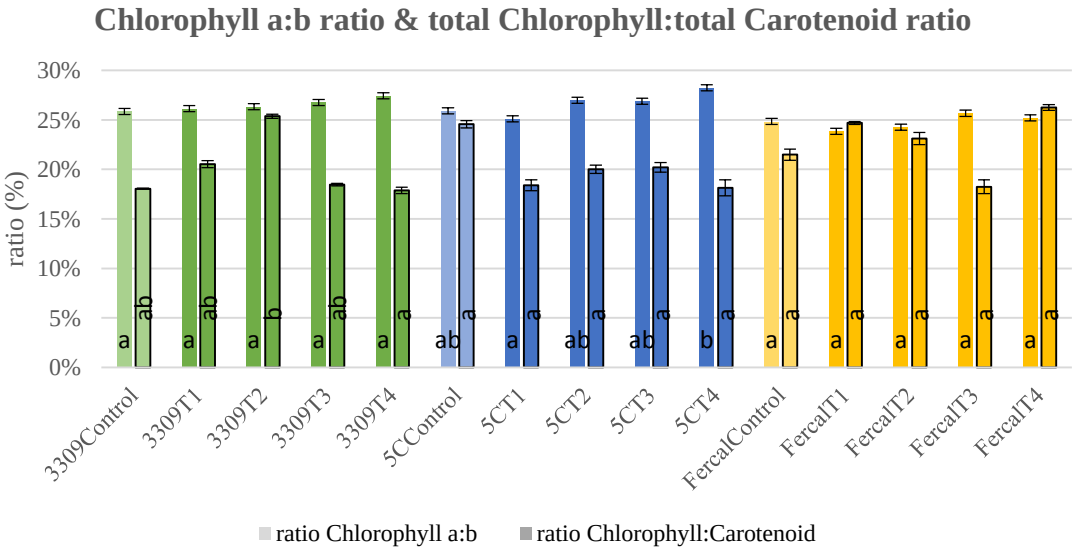
Figure 50: Tfm: separate analysis of treatments

Chlorophyll a:b ratio and total Chlorophyll:total Carotenoid ratio

According to Mänd (2013), light harvesting complexes (LHC) in plant photosystems are rich in chlorophyll b (chl b), while the "core reaction centre complexes" consist only of chlorophyll a (chl a). Subsequently, an increase in antenna size (LHC) would be reflected as a decrease in the chl a:b ratio. Such an increase in antenna size was observed in plants suffering iron deficiency by Belkhodja et al. (1998). Mänd (2013) describes that in addition to light harvesting, the carotenoids are important in order to protect the photosystems of excess light and are responsible to redirect it in order to avoid photo-damage. Hence, the content of photo-protective pigments (in this case: carotenoids) tends to increase in stressed environments, as observed in cases of drought (Colom and Vazzana, 2003).

Results of this study show that treatment groups of rootstocks 3309 (p= 0,0439) and 5C (p= 0,0499) had significant differences regarding the chlorophyll a:b ratio, with higher values in the treatment groups that were exposed to high bicarbonate concentrations (Figure 51). Treatment groups of Fercal were not significantly different.

The ratio of total chlorophyll content:total carotenoid content within the treatment groups of rootstock 3309 was significantly different, however no meaningful pattern can be interpreted. Treatment groups of 5C and Fercal did not differ significantly in this parameter.



comparison of $\bar{x}$ (letters) with SD; executed separately for the two ratios (Chlorophyll a:b and total Chlorophyll:total Carotenoid) and within every rootstock

significant differences are indicated with different letters

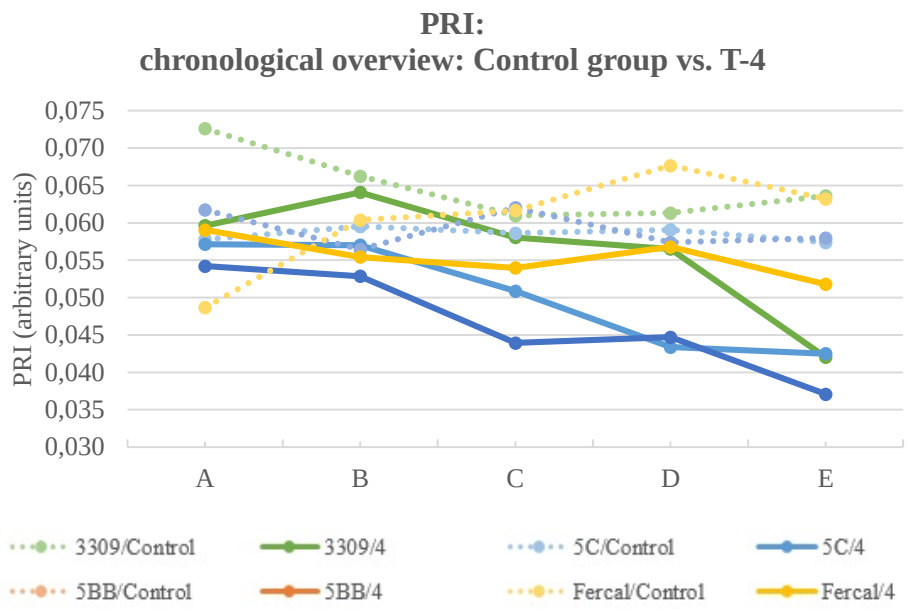
3309: a:b ANOVA (p= 0,0439); Chl.:Carot. ANOVA (p= 0,0350); n= 14

5C: a:b ANOVA (p= 0,0499); Chl.:Carot. ANOVA (p= 0,287); n=15

Fercal: a:b. ANOVA (p= 0,26); Chl.:Carot. ANOVA (p= 0,643); n=15

Figure 51: Chlorophyll a:b ratio & total chlorophyll:total carotenoid ratio

PRI: chronological overview



above: chronological development of PRI values ($\bar{x}$); SD not displayed to have a clearer view

below: levels of significance between the rootstocks and treatment groups on dates A-E (columns; comparison of all measured values for the individual dates) and within the Control group / T-4 of each rootstock (dotted lines; chronological comparison of each individual line)

Con. = Control; Ferc. = Fercal

n.s. (not significant) p > 0,05; * 0,01 < p < 0,05; ** 0,001 < p < 0,01; *** p < 0,0001

	PRI	***n*=**
date A	***	*66*
date B	***	*72*
date C	***	*72*
date D	***	*75*
date E	***	*72*
3309 Control	***	*42*
3309 T-4	***	*45*
5C Control	n.s.	*45*
5C T-4	***	*42*
5BB Control	*	*48*
5BB T-4	***	*51*
Fercal Control	***	*42*
Fercal T-4	n.s.	*42*

Figure 52: PRI: Control group vs. T-4 (chronological overview)