GRAPE PHYLLOXERA MEETS DROUGHT: INCREASED RISK FOR VINES UNDER CLIMATE CHANGE?

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Abstract:

Context and purpose of the study - Climate change is increasing the frequency and severity of drought periods leading to significant impacts on agro-economic activities, with particular regard to viticulture. Moreover, in the last decades the wine-making industry is further threatened by new outbreaks of grape phylloxera (*Daktulosphaira vitifoliae* Fitch) which have been reported worldwide. Phylloxera is a galling aphid native to eastern North America that targets grapevines as its single host and source of nutrition. Knowledge on how the aphid affects the whole-plant physiological functions is limited, in particular when the phylloxera attack is accompanied by drought stress. In the light of prolonged drought periods forecasted for the near future in many viticultural regions, it is fundamental to understand and predict eventual negative cumulative effects of a combined biotic-abiotic stress.
Material and methods - In the present study we monitored water and carbon metabolism, gas exchange and photosystem functionality of grapevines subjected to drought stress (D) and/or phylloxera infestation (P). The experiment was carried out in pots using Riesling grafted on Teleki 5C (RR) and own-rooted Teleki 5C (5C, rootstock). P vines were root inoculated with phylloxera eggs collected from a field population. A subset of plants was subjected to an 8 week-long moderate drought stress (PD), while the others were maintained in well-watered conditions (PI). Non-inoculated control plants were also included in the trial for both irrigated (CI) and drought stress (CD) conditions. Non-structural carbohydrates (NSC) were measured in young leaves developed under the treatments. Differences in root infestation (presence of nodosities) were also investigated among experimental treatments.
Results - Drought stress had a significant impact on the plants gas exchange leading to the reduction of NSC in the leaves. On the other hand, infestation with phylloxera did not induce notable shifts in physiological traits with the exception of a marked increase of leaf surface temperature recorded in RR (+1°C recorded in P plants compared to C). The insect induced starch depletion and enhanced glucose synthesis in the leaves. The inoculation efficiency was higher in D plants compared to I ones, suggesting that events of water shortage favor the insect spread.

Keywords: Drought stress, Gas exchange, Carbon metabolism, Biotic stress, Riesling.

1. Introduction

Global change phenomena are posing serious threats to natural and agricultural ecosystems, with particular regard to viticulture (Webb et al. 2007; Nardini et al. 2013). Long and intense drought events are becoming year after year more common with consequences on water and carbon metabolism of vines, as well as on their growth rates and survival (Webb et al. 2007; IPCC 2014). Beside drought stress, the spread of pests is of particular concern, since it further impacts grapevine development and production (Bernardo et al. 2018). Phylloxera (*Daktulosphaira vitifoliae* Fitch) is a worldwide diffused galling insect obligate biotroph of *Vitis* species which is causing significant economic losses to the viticultural industry (Forneck et al. 2002; Powell et al. 2013). Current knowledge suggests that future warming and drying conditions may change the pests' distribution and alter the fitness of populations (Castex et al. 2018). However, studies addressing the interaction between abiotic (drought) and biotic (phylloxera) stress in vines are particularly lacking in the scientific literature.
To fill this knowledge gap, the present work was aimed at investigating the physiology and growth of an economically important rootstock (with or without scion Riesling) under future "natural" growing conditions, characterized by prolonged drought periods and phylloxera spread. Firstly, we wanted to

shed light into the effects of watering on the aggressiveness of grape phylloxera. Secondly, we wanted to monitor vines water and carbon metabolism under root phylloxeration, drought stress, as well as under a coupled biotic-abiotic stress. We hypothesized that the insect attack negatively affects plant metabolism, in particular when it is accompanied by the drought stress.

2. Material and methods

Plant material and growing conditions

The experiments were carried out in the greenhouse of the Institute of Viticulture and Pomology (BOKU University) located in Tulln (Austria). Ownrooted Teleky 5C plants (1 year old) and Riesling scion grafted on Teleky 5C (2 years old, RR) were selected as study genotypes. In spring, a total of 93 plants was potted in 7 pots containing a blend of natural soil and perlite (70% and 30%, respectively) and maintained well watered. After about 3 months of growth, the crown of the plants was uniformed to 15 leaves. The vines were split in 2 groups per spices, i.e. irrigated (I) and drought-stressed (D). The I group was maintained well-watered throughout the study period, while the irrigation volumes were reduced in D vines with the aim to limit the transpiration to about 30% of that recorded in I vines. Within each irrigation treatment, the plants were subdivided into two additional categories: non-phylloxerated (C) and phylloxerated (P). The roots of P plants were inoculated with 100 phylloxera eggs collected from a nearby site, while C plants were considered as control. All pots were enclosed in a bag made of fine mesh (125 µm) to prevent the spread of the insect. The climatic parameters of the greenhouse (air temperature and relative humidity) were recorded on an hourly basis throughout the study period.

Experimental measurements

With the aim to study differences among the experimental categories, six and eight weeks after the beginning of treatments leaf functional traits reflecting the aboveground physiological status were measured. In particular, the temperature of the leaf lamina (T_{leaf}), transpiration rates (E_L) and net photosynthesis (A) were measured using a portable gas-exchange system (LCpro-SD, ADC BioScientific Ltd., Hertfordshire, UK). The efficiency of the PSII was estimated as Fv/Fm recorded with a portable fluorimeter on a dark adapted leaf (Handy Pea, Hansatech, Norfolk, UK; for details see Savi et al. 2016). All measurements were performed on the first fully-expanded young leaf, developed under the treatments application. During measurements performed in the central hours of the day, the greenhouse temperature and relative humidity averaged 32°C and 40%, respectively, while the photosynthetic photon flux density was about 800 µmol m^{-2} s^{-1}.

On the 8th week of treatments application, the leaves used for gas-exchange measurements were detached, immersed in liquid nitrogen and stored at -80 °C until non-structural-carbohydrate analyses (NSC). Plants were gently uprooted and the root systems were observed under a binocular microscope in order to evaluate the actual presence of phylloxera population (root nodosities).

Glucose, fructose, sucrose and starch were measured in the sampled leaves to verify how the Irrigation and/or root phylloxeration influence carbon fixation and sugars storage at aboveground level. The samples were pulverized in liquid nitrogen using a mortar. After a freeze-drying cycle (48 h), about 50 mg of material was washed three times in 80% ethanol (at 90°C) to dissolve soluble sugars (Landhäusser et al. 2018). The supernatant was filtered at 0.45 µm and, after appropriate dilution, analyzed with anion exchange chromatography (Dionex™ ICS-5000, Thermo Fischer Scientific, Waltham, MA). On the other hand, the pellet was treated with α-amilase (70 units per sample) followed by amyloglucosidase (6 units per 0.1 ml of sub-sample; for details see Landhäusser et al. 2018) in order to break down the starch to glucose. After digestion, the samples were treated with chloroform (1:1 v/v), the aqueous layer was filtered, and the glucose hydrolysate measured as described above.

Statistical analyses

Data were analyzed in SigmaPlot (v 13, Systat Software Inc., San Jose, CA). Statistically significant differences were highlighted by Two-way-analysis of variance followed by pairwise multiple comparison procedures (Holm-Sidak method). The effect of different treatments was tested separately for each parameter and for each genotype independently. Each trait was considered as a response variable, while Infection (levels C and P) and Irrigation (levels I and D) were treated as explanatory variables (factors). Results were considered statistically significant at $P \leq 0.05$ and marginally significant at $0.05<P<0.1$. Mean ± standard errors of the mean are reported.

3. Results and discussion

In the present study we monitored water and carbon metabolism, gas exchange and photosystem functionality of grapevines subjected to an eight-week-long moderate drought stress (D) and/or phylloxera infestation (P). At the end of the experiment, the two studied genotypes showed similar degree of root phylloxera infestation; hence data was pooled and reported in Figure 1. A total of 46 out of 57 inoculated plants developed the insect population, i.e. abundantyoung (white to light-yellow color) and old (dark brown) nodosities (Porten and Hubmer 2003). Despite high daily maximum temperatures recorded in the greenhouse (>40°C), the insect found overall favorable environmental conditions for the reproduction, as demonstrated by the high infection frequency (81%). Interestingly, only 40% of all plants showing root galls were grown under irrigated conditions, while the other 60% in drought-stressed pots. These results suggest that prolonged water shortage may favor the insect spread.

Leaf physiological parameters measured 6 and 8 weeks after treatments imposition in 5C (a) and Riesling grafted on 5C (b) plants are reported in Table 1. Irrigation treatment played a fundamental role in modulating gas-exchange of newly-developed young leaves in both study genotypes. Prompt stomatal closure led to a marked reduction of net photosynthesis and transpiration rates, with consequent increase of the leaf temperature. On the other hand, infestation with phylloxera did not induce notable shifts in physiological traits, with the exception of T_{leaf} measured in RR vines on the first day of measurements, when 1°C higher values were recorded in P than in C plants. Only one significant interaction between factors (Irrigation x Infection) was highlighted throughout measured physiological parameters 8 weeks after treatment imposition (Table 1b). In particular, the photosynthetic efficiency of phylloxerated 5C plants under irrigated conditions was higher when compared to control, suggesting that a higher amount of incoming energy was directed toward photosynthetic pathway in the former group compared to the latter. Under drought conditions, vines were water-stressed and the effect of the pest on Fv/Fm could not be detected. However, a trend toward higher E_L, A, and Fv/Fm values could be noticed in RR genotype, especially when subjected to drought. These marginally significant interactions between factors ($0.05<P<0.1$) suggest that the insect may interferes with water uptake and influence the whole-plant physiology by stimulating gas-exchange rates. Similarly, a recent work found an increase of gas-exchange rates in phylloxerated plants (Eitle et al. 2017), while other authors failed in recording such differences (Bates et al. 2001).

Non-structural carbohydrates measured in the leaves of experimental plants are reported in Table 2. The drought stress caused a reduction of about 16% all sugars, but the drop was statistically significant only for fructose recorded in 5C leaves (20.4 *vs* 13.2 mg g^{-1} recorded in C and D plants, respectively). Phylloxeration induced a significant increase of glucose in both study genotypes, supporting the previously formulated hypothesis of enhanced photosynthetic rates under pest attack. The higher glucose concentration suggests that root infestation influences the aboveground physiology by stimulating sugars production. On the other hand, phylloxera led to a 25% reduction of starch content in the leaves (storage of energy). The starch depletion was statistically significant in 5C plants subjected to drought (2.9 and 6.8 mg g^{-1} as measured in P and C group, respectively). Interestingly, previous studies demonstrated that phylloxeration enhances the carbon sink activity of roots, since higher sugars concentrations were measured in the roots of infested plants compared to control ones (Forneck et al. 2002; Kellow et al. 2004). Our data somehow integrate this hypothesis by clearly showing intense sugars mobilization at leaf level (starch degradation), as well as a likely enhanced glucose production in both genotypes (+23%). Hence, we had evidence that the insect is able to trigger changes in leaf physiology with consequent sugars translocation toward belowground organs to support of the galling habit.

4. Conclusions

Our results indicate that events of water scarcity may favor the fitness and spread of phylloxera. Teleki 5C rootstock showed marked tolerance to Phylloxera, since it efficiently buffered intense negative effects of pest infestation on the functionality of the aboveground organs. Glucose accumulation and starch depletion in leaves indicate increased sugars mobilization and translocation toward belowground organs. Hence, we showed that phylloxera is able to re-program whole-plant metabolism in order to provide more food resources for the proliferation of the insect population. In conclusion, root phylloxera infestation imposes a considerable stress to the vines which might exacerbate the negative effects of drought.

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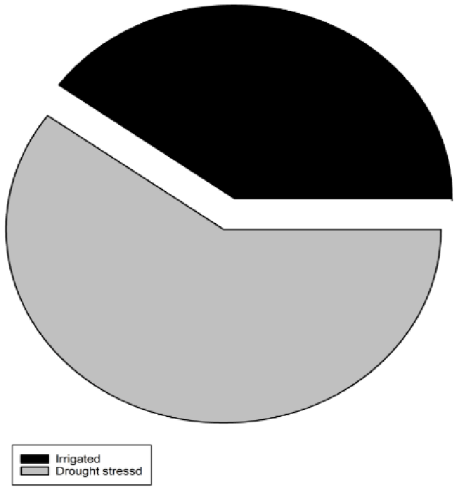

Figure 1: Inoculated potted vines that developed the phylloxera population. The infestation was evaluated 8 weeks after inoculation and divided in the two irrigation treatments, i.e. irrigated (I) and drought stressed (D). Data of the two studied genotypes 5C and Riesling x 5C are pooled together.

(a)	5CIC	5CIP	5CDC	5CDP	RRIC	RRIP	RRDC	RRDP
T_{leaf}, °C	38.0 ± 0.5^{a}	38.5 ± 0.4^{a}	39.9 ± 0.6^{b}	40.3 ± 0.3^{b}	**37.6 ± 0.4^{a}**	**38.4 ± 0.4^{a}**	**40.2 ± 0.4^{b}**	**41.0 ± 0.2^{b}**
E_L, mmol m^{-2} s^{-1}	4.5 ± 0.9^{a}	4.1 ± 0.6^{a}	0.2 ± 0.1^{b}	0.5 ± 0.2^{b}	4.4 ± 0.8^{a}	3.6 ± 0.5^{a}	0.1 ± 0.01^{b}	0.5 ± 0.2^{b}
A, µmol m^{-2} s^{-1}	6.0 ± 1.1^{a}	5.5 ± 0.8^{a}	0.5 ± 0.2^{b}	0.9 ± 0.5^{b}	7.3 ± 2.0^{a}	5.3 ± 1.2^{a}	0.1 ± 0.02^{b}	0.4 ± 0.3^{b}
Fv/Fm	0.82 ± 0.01	0.84 ± 0.001	0.83 ±0.01	0.82 ± 0.003	0.81 ± 0.01^{a}	0.81 ± 0.01^{a}	0.77 ± 0.02^{b}	0.79 ± 0.01^{b}

(b)	5CIC	5CIP	5CDC	5CDP	RRIC	RRIP	RRDC	RRDP
T_{leaf}, °C	32.1 ± 0.9	33.5 ± 0.5	33.8 ± 0.9	32.7 ± 1.3	31.2 ± 1.0	32.0 ± 0.6	34.1 ± 1.0	31.8 ± 1.5
E_L, mmol m^{-2} s^{-1}	2.7 ± 0.3^{a}	2.9 ± 0.3^{a}	1.3 ± 0.2^{b}	1.0 ± 0.1^{b}	2.7 ± 0.2^{a}	3.1 ± 0.3^{a}	0.9 ± 0.2^{b}	1.3 ± 0.1^{b}
A, µmol m^{-2} s^{-1}	5.0 ± 0.3	5.0 ± 0.7	5.0 ± 0.5	3.3 ± 0.8	6.9 ± 0.8^{a}	8.0 ± 1.0^{a}	3.5 ± 0.6^{b}	5.1 ± 0.4^{b}
Fv/Fm	*0.80 ± 0.01	0.81 ± 0.01	0.82 ±0.01	0.81 ± 0.01	0.79 ± 0.02	0.74 ± 0.03	0.73 ± 0.04	0.79 ± 0.02

Table 1: Leaf temperature (T_{leaf}), transpiration rates (E_L), photosynthesis (A), and photosynthetic efficiency (Fv/Fm) measured in 5C and Riesling x 5C (RR) plants 6 weeks (a) and 8 weeks (b) after the beginning of treatments (n = 5-8). I = irrigated plants; D = drought-stressed; C = control, non-phylloxerated; P = root phylloxerated. Lettering and bold numbers indicate significant differences within the factor Irrigation and Infection, respectively. A significant interaction (denoted with the asterisk) between factors (Irr x Inf) was observed 6 weeks after inoculation in Fv/Fm measured in 5C, i.e. within I level: P > C; within P level: I > D.

	5CIC	5CIP	5CDC	5CDP	RRIC	RRIP	RRDC	RRDP
Glucose, mg g^{-1}	***17.0 ± 3.2**	**28.4 ± 1.8**	**19.4 ± 1.5**	**19.3 ± 1.9**	*16.9 ± 1.3	26.5 ± 5.1	20.4 ± 2.1	16.3 ± 2.1
Fructose, mg g^{-1}	17.2 ± 4.0^{a}	23.6 ± 2.1^{a}	12.7 ± 1.2^{b}	13.7 ± 1.1^{b}	12.7 ± 1.8	18.3 ± 3.9	16.6 ± 1.0	13.1 ± 1.9
Sucrose, mg g^{-1}	81.0 ± 4.4	95.7 ± 0.9	102.0 ± 11.8	90.2 ± 7.6	107.1 ± 10.2	120.5 ± 11.6	116.2 ± 19.3	131.2 ± 7.8
Starch, mg g^{-1}	*5.2 ± 1.5	5.9 ± 1.0	6.8 ± 1.4	2.9 ± 0.4	11.0 ± 2.8	8.9 ± 1.6	7.9 ± 1.5	6.0 ± 1.3

Table 2: Glucose, fructose, sucrose, and starch measured in the young leaves of 5C and Riesling x 5C (RR) at the end of 8-weeks-long treatments (n = 5-8). I = irrigated plants; D = drought-stressed; C = control, non-phylloxerated; P = root phylloxerated. Lettering and bold numbers indicate significant differences within the factor Irrigation and Infection, respectively. Significant interactions (denoted with the asterisks) between factors (Irr x Inf) were observed in glucose (within I: P > C; within P: D < I) for both genotypes, and starch measured in 5C (within D: P < C).