Effects of Grape Phylloxera Leaf Infestation on Grapevine Growth and Yield Parameters in Commercial Vineyards: A Pilot Study

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Abstract

Grape phylloxera (*Daktulosphaira vitifoliae*) can infest both roots and leaves of *Vitis* species. In commercial vineyards planted with *Vitis vinifera* scions grafted on rootstocks, grape phylloxera infestation is generally limited to root feeding. Vineyards are, however, increasingly subjected to vineyard-wide foliar infestations that last throughout the growing season. While some vineyards are affected by the infestation pressure of external leaf-feeding populations, other annually affected *V. vinifera* vineyards do not have these in their vicinity. Much is known about the damage potential of grape phylloxera root feeding; however, data on how phylloxera leaf infestation affects *V. vinifera* grapevines in commercial vineyards are lacking. This study, therefore, aimed to assess whether grapevine growth and yield are affected due to leaf infestation as it occurred in three commercial vineyards in the study area. Treatments were based on phylloxera leaf infestation and additional defoliation. Single-leaf carbon acquisition was measured with gas exchange analyses on healthy and galled leaves. Pruning weight and internode length were measured to assess the effect of leaf infestation and the effect of plant growth and vigour on leaf gall outbreaks. Yield quantity and quality were measured, and grapes were vinified for sensory analyses. Furthermore, using enzymatic analyses, non-structural carbohydrates were analysed in perennial wood. A significant

decrease in sugar content in grapes (10 %) and starch reserves in perennial wood (11 %) was found in the most heavily infested vineyard. Grape must of infested plants in another vineyard furthermore showed a significantly higher level of titratable acid (7.5 %). Significant infestation effects seen in one vineyard were not significant in the other two vineyards. No significant differences were seen for carbon acquisition, harvest quantity, wine sensory analysis, pruning weight or internode length. The overall effect of phylloxera leaf infestation in the studied vineyards was, therefore, marginal. Grapevine vigour did not differ between infested vines, insecticide-sprayed vines, and vines on which no leaf infestation outbreaks took place. By analysing phylloxera leaf infestation under field conditions, these preliminary results form a basis for future long-term field studies about phylloxera leaf feeding on *Vitis vinifera* within the context of other biotic and abiotic plant stresses.

Keywords : Viticulture, non-structural carbohydrates, gas exchange, *Vitis vinifera*, *Daktulosphaira vitifoliae*, galling insect, vigour

Introduction

Grape phylloxera (*Daktulosphaira vitifoliae* FITCH) (from here on phylloxera) is a destructive pest for global viticulture. This plant sap-sucking and gall-forming insect is an obligate biotroph of *Vitis* L. spp, on which it can infest both roots and leaves. Notorious for its root feeding, phylloxera almost collapsed the French viticulture sector in the late 19th century, destroying a third of all vineyards (Gale, 2002). Most plant damage is caused when phylloxera infests mature roots and creates tuberosities. When these are formed, the root vascular system is laid bare, disrupting water and nutrient flows and enabling the entry of soil pathogens (Niklowitz, 1954; Powell *et al*., 2013). Susceptible grapevines that cannot wall-off their vascular system show severe symptoms, losing vitality that can lead to vine death (Boubals, 1966; Granett *et al*., 2001). Though nodosity feeding (root tip galling) does not pose this extent of host plant damage, it can amplify the extent of other biotic (Edwards *et al*., 2007) and abiotic (Savi *et al*., 2019; Savi *et al*., 2021) plant stresses. Furthermore, through root necrosis, nodosity feeding can negatively affect plant vigour (Granett *et al*., 2001). Phylloxera induced leaf galls, on the other hand, were documented to positively impact grapevine vigour (Kimberling *et al*., 1990). Root and leaf galls are moreover known to impact the functioning of the grapevine, modifying primary and secondary host plant metabolism, to alter plant

defences and promote carbon importation into the gall tissue (Eitle *et al*., 2017b; Eitle *et al*., 2019a; Eitle *et al*., 2019b; Griesser *et al*., 2015; Nabity *et al*., 2013).

To preserve wine quality traits while benefiting from phylloxera infestation tolerance, the cultivated grapevine (*Vitis vinifera* L.) is grafted onto American rootstock hybrids that can cope with the pest by suppressing tuberosity formation (Boubals, 1966). The pest's ability to establish tuberosities on specific host plant varieties can, however, not be generalised. In the late 20th century, California's vineyards that were planted with the rootstock hybrid AxR#1 (60 to 70 % of Napa and Sonoma Counties) suffered a total collapse, generating region-wide yield losses and replanting costs of over a billion US-dollar (Gale, 2002). The reason for this sudden collapse resided at differences in hostplant-specific feeding traits among phylloxera populations (i.e., some phylloxera populations can create tuberosities on AxR#1, whereas others are not) (Granett *et al*., 1985). Indeed, over the years, phylloxera populations have been categorised by their innate feeding performance on roots (King and Rilling, 1985) and leaves (Stevenson, 1970b), and a global concept of biotype categories has been introduced (Forneck *et al*., 2016).

Normally, phylloxera rarely creates leaf galls on *V. vinifera* (Powell, 2008). Some phylloxera populations have, however, been documented to infest *V. vinifera* leaves, in Australia, France, Italy, Peru and the USA (Granett *et al*., 2001), Uruguay (Vidart *et al*., 2013), Austria (Könnecke *et al*., 2011) Germany (Kopf, 2000) and Hungary (Molnár *et al*., 2009). A high external infestation pressure from leaf-feeding phylloxera on nearby susceptible *Vitis* species can explain the results of some of these studies. Infestation assays under controlled conditions, however, showed that some phylloxera populations from this study area have a significantly higher rate of infestation and faster development on *V. vinifera* leaves compared to less adapted phylloxera populations (Wilmink *et al*., 2021a). Instead of temporal and marginal foliar outbreaks, some commercial vineyards in Baden, Germany, were subjected to vineyard-wide leaf infestations perpetuated throughout the growing season. In some cases, the origin of leaf-feeding phylloxera could be identified as parthenogenic offspring of the vine's root-feeding phylloxera (Wilmink *et al*., 2021b).

With the onset of vineyard-wide leaf infestation outbreaks, it is crucial to consider their impact on grapevine growth and yield. Unfortunately, the historic rarity of leaf infestations on *V. vinifera* grape varieties has made it a scarce subject of study. Field studies that were conducted with *V. vinifera* hybrids that are more vulnerable to leaf infestation show contradicting results. The eradication of leaf-feeding phylloxera on the hybrid "Seyve-Villard 18-315" increased the amount of yield and the grapes' soluble solids (Schvester, 1959). Conversely, a study with a similar experimental setup with

a natural infestation in commercial “Maréchal Foch”, “Seibel 5279” and “Seibel 7053” vineyards observed no consistent differences between sprayed and unsprayed plots for yield quantity, quality or pruning weight (Stevenson, 1970a). A study with artificial phylloxera infestations on “Seyve-Villard 5276” hybrid vines at different early phenological stages found effects for leaf infestations that were initiated two or three weeks before bloom (Mcleod, 1990). For these artificially established infestations, cluster weight, number of berries per cluster and soluble solids were lowered. In field trails with the hybrid “Frontenac”, cluster weight was significantly higher for sprayed vines compared with vines where more than 50 % of the leaf area of leaves on young shoots were covered with galls (Yin *et al.*, 2021). These authors did not find significant differences between sprayed vines and vines with less than 30 % of the leaf area covered with galls. However, to our knowledge, there is no field study about the impact of phylloxera leaf galls on the vegetative and generative growth of the cultivated grapevine, *V. vinifera*, in commercial vineyards.

Therefore, this study was conducted to investigate the effects of phylloxera leaf infestation outbreaks in commercial vineyards. We aimed to identify whether vineyard-wide leaf gall outbreaks, as they naturally occur in some commercial vineyards in Baden, Germany, can impact different growth and yield parameters. We thereby conducted a fine-scale sampling in three pilot vineyards with different viticulture, environmental and phylloxera infestation scenarios. For phylloxera leaf-infested and control plants, gas exchange, yield quantity and quality, wine sensory analyses, plant vigour, and the storage of non-structural carbohydrates in perennial wood were analysed. We studied this wide array of parameters to provide the first insights for further in-depth research in field settings. Furthermore, additional treatments were created to simulate growing seasons with high plant stress, whereby grapevines were subjected to increased defoliation, reducing photosynthetic source material. We hypothesised that phylloxera leaf infestation only significantly affects grapevine growth and yield parameters in commercial vineyards when they are subjected to severe additional stress (i.e., the defoliation treatment).

Materials and methods

1. Experimental Setup

This study’s experiments were conducted in three commercial vineyards in Baden's wine-growing region in the southwestern part of Germany. Two vineyards were studied in 2018 and one in 2019. These commercial vineyards all showed vineyard-covering phylloxera leaf infestations, albeit with different intensities (Table 1). Besides the alterations made for the treatments, these vineyards were under standard viticulture management by the winegrowers (cane pruning, grass and cover crops in

alternating rows, defoliation of most basal leaves, hedging, (biological) fungicides, no insecticides). The experimental treatments were implemented from the start of July 2018 and from the end of July 2019. At this time, phylloxera larvae were in their second leaf-feeding generation and the grapevines had developed pea-sized berries (BBCH 75).

Table 1. Overview of experiment plots and pre-treatment phylloxera infestation.

		Pfaffenweiler 2018	Britzingen 2018	Bahlingen
Vineyard	Topography	Hilltop	Slope	Terrace
	Management	Conventional	Conventional	Organic
	Soil texture	Loam	Silt loam	Silt
	Planting year	2008	2008	2015
Grapevine	Scion species	*V. vinifera*	*V. vinifera*	*V. vinifer*
	Scion variety	"Muscat a Petits Grains"	"Chasselas"	"Muscari
	Rootstock hybrid	*V. berlandieri* x *V. riparia*	*V. berlandieri* x *V. riparia*	*V. berland* x *V. ripar*
	Rootstock variety	"Kober 5BB"	"Kober 5BB"	"SO4" "Sele Oppenheim
Phylloxera infestation[2]	Infested shoots (%)	1–25	26–50	51–75
	Galls per leaf	1–5	11–20	21–30

[1] Although specified as *V. vinifera*, due to fungus resisting traits, about 10 % is of non-*V. vinifera* ancestry (Maul *et al*., 2021).

[2] Gall count on the vine's most infested leaf in mid-June (2018) or start of July (2019) during the second foliar generation, according to the infestation categories of Mueller and Bogenrieder (2010).

Before the start of the experiment (mid-June 2018 and start of July 2019), the second-generation phylloxera leaf infestation was monitored for all grapevines in each of the vineyards. For every vine, the infestation frequency (i.e., the percentage of infested shoots) and intensity (i.e., number of galls on the most infested leaves) were counted (Table 1), according to Mueller and Bogenrieder (2010). In detail, the number of galls on the vine's most infested leaf was categorised as 0: 0 galls, 1:1–5 galls, 2: 6–10 galls, 3: 11–20 galls, 4: 21–30 galls, 5: 31–50 galls or 6: > 50 galls. The

percentage of infested shoots was categorised as 0: 0 %, 1: 1–25 %, 2: 26–50 %, 3: 51–75 %, 4: 76–100 %. This was done for all 556 vines in the vineyard in Pfaffenweiler, 1670 vines in the vineyard in Britzingen and 196 vines in the vineyard Bahlingen.

This monitoring enabled visualisation of the local infestation intensities within each vineyard. Based on this monitoring data, treatment blocks (total of 96 vines per vineyard) with the same infestation intensity were chosen. In the two conventionally managed vineyards, half of the blocks were sprayed twice with 50 mgl^{-1} Imidacloprid (Confidor® WG 70) to eradicate leaf-feeding phylloxera. The organically managed vineyard could, for legal reasons, not be sprayed with Imidacloprid. The non-infested control blocks in the organically managed vineyard were, therefore, chosen based on natural leaf infestation patterns (Table S1).

To ensure no bias between control treatments, a plant growth comparison was made between control vines based on insecticide spraying (conventionally managed vineyards) and naturally non-infested grapevines (organically managed vineyards). To do this, insecticide-sprayed grapevines in the two conventional vineyards were compared with naturally non-infested grapevines within the same vineyard.

Each of the infested and non-infested treatments was divided into a normal and a defoliation treatment. In the defoliated treatments, 50 % of the leaves were removed by picking every other leaf (on the main and lateral shoots). The leaf removal treatments were implemented to create extra stress on the vines by simulating a lower source-sink ratio. This defoliation intensity was chosen because grapevines normally use up to 50 % of their photosynthetic capacity preveraison (Howell, 2001). The defoliation treatment would therefore help to elucidate any additional phylloxera-induced plant stress. Based on these two parameters (leaf infestation and partial leaf removal), four different treatments were created. Each treatment consisted of four blocks of six vines, resulting in 16 blocks and 96 vines per vineyard.

2. Yield and Growth Parameters

In the most infested vineyard (in Bahlingen), carbon acquisition was measured with gas exchange measurements (Walz® GFS-3000) to identify whether there are any differences in carbon acquisition at this level of infestation. The youngest fully developed leaf of four different vines was measured per treatment, conducted between 10 am and 2 pm. For the vines from treatments infested with foliar phylloxera, measurements were taken from an infested leaf (including gall area) and a non-infested leaf from a non-infested shoot (the vines that were measured are numbered in Table S2 and correspond to the vineyard overview in Table S1). The measurement settings were set at 25 °C

cuvette temperature, 60 % relative air humidity, 400 ppm CO_2 and 1200 µmole $m^{-2}s^{-1}$ PAR in an 8 cm^2 leaf chamber.

Grape clusters of the treatment blocks were harvested in the same week as the grape grower harvested the rest of the vineyard. At this stage, the grape bunches of the treatment blocks were counted, harvested and weighed. The grapes harvested in Britzingen and Pfaffenweiler were transported in small boxes for vinification. For grapes harvested in Bahlingen, only grape quality samples were taken. The vineyards in Britzingen and Pfaffenweiler were harvested in the third week of October in 2018 and the vineyard in Bahlingen in the second week of September 2019.

Vegetative growth was measured using two parameters: internode length and pruning weight (González-Fernández *et al*., 2012). Both parameters were obtained in December (dormancy). Internode length was calculated by measuring the internode length of five internodes, starting at the fourth node of the main shoot. These measurements were performed on three shoots per vine for all 336 vines of this study. Pruning fresh weight was measured directly in the vineyard. Pruned dry weight was calculated by drying a single shoot per vine for 72 hours at 60 °C and calculating the fresh weight to dry weight ratio. Crop load was estimated according to the Ravaz index (i.e., the ratio of fruit yield to dormant pruning weight per vine) (Ravaz, 1911).

3. Sample Processing

An FTIR analysis (Foss, Grapescan™) was conducted to calculate must density, glucose, fructose, titratable acid, volatile acidity, pH, tartaric acid and malic acid of grape must (Bauer *et al*., 2008). This analysis was conducted for a sample that consisted of 100 randomly picked berries per treatment block of six vines (picked during harvest time, to resemble harvest conditions). For the vinification, the grapes of the four repetition blocks of each treatment were put together. After destemming and maceration, the must was pressed in small pneumatic presses and fermented in 25-litre glass carboys. The wine was filtered with a two-step candle filter (1 and 0.65 µm cellulose) and bottled with 90 ppm SO_2. The wines were then tested by a panel of 23 experts with the CATA (check all that apply) method (Valentin *et al*., 2012), using the white wine aroma wheel of Noble *et al*. (1987).

After pruning in December, the pruned two-year-old wood of the lateral cane of twelve plants per treatment and vineyard was enzymatically tested for the concentration of stored sugar and starch reserves. Directly after pruning, the 144 samples were transported in cooled boxes, microwaved for 90 s at 600 W to stop enzymatic activity and subsequently oven-dried for 72 h at 70 °C, according to Landhäusser *et al*. (2018). The samples were stored with silica beads in 50 ml Falcon® tubes

until the start of the enzymatic analysis. This was done according to the protocols and materials of the Megazyme enzyme kits “K-TSHK 01/20” for starch and “K-SUFRG 04/18” for total sugar.

The samples were cryo-homogenised with liquid nitrogen and a ball mill and subsequently sieved through a 0.5 mm meshed sieve. According to Landhäusser *et al*. (2018), the samples were weighed and subsequently heated for 10 min at 90 °C in 80 % ethanol in screw-capped reaction tubes. After 1 min 13k rpm centrifuging, soluble sugars were extracted from the supernatant. Following the Megazyme “K-SUFRG 04/18” protocol, these sugars were converted by adding invertase (converting sucrose to glucose and fructose), hexokinase (phosphorylating glucose and fructose into G6P and F6P) and phosphoglucose isomerase (interconverting G6P and F6P). The total amount of converted sugar was quantified with the 340 nm light absorbance of NADPH before and after adding 6-phosphogluconate dehydrogenase, using the Specord® 50 photometer from Analytik Jena (Jena, Germany).

After the extraction of soluble sugars, the extraction with ethanol was repeated two more times, according to Landhäusser *et al*. (2018), disposing of the supernatant. Following the Megazyme “K-TSHK 01/20” protocol, the remaining starch was extracted from the dried pellet by subsequently incubating with α-amylase (6 min, 90 °C) and amyloglucosidase (30 min, 50 °C). The starch that was converted into glucose (anhydro-glucose) was then quantified in the same way as the total sugars (adding hexokinase and 6-phosphogluconate dehydrogenase and measuring NADPH light absorbance at 340 nm).

4. Statistical Analyses

Statistical analyses were carried out with IBM SPSS 26®, with $\alpha = 0.05$. Throughout this paper, statistical significance is indicated as “*” for $P < 0.05$ and “n.s.” for $P \geq 0.05$. Comparisons between two groups were tested with Welch’s unequal variances t-test, comparisons between three groups with ANOVA, followed with Tukey’s post-hoc test. A non-linear correlation analysis was conducted with Spearman’s rank correlation coefficient with $\alpha = 0.05$. The CATA sensory analysis was carried out with XLSTAT 2018.1. The statistical analysis of CATA output was performed according to Varela and Ares (2012). Briefly, first, a Cochran’s Q-test was carried out to analyse whether individual sensory attributes were significantly distinct between treatments. A contingency table was then created for all significant attributes, followed by a chi-square test of independence between treatments and attributes with $\alpha = 0.05$. When comparing the treatments shown in Tables 2, 3 and 4, all infested treatments were compared with all non-infested treatments, and all non-

defoliated treatments were compared with all defoliated treatments. Additionally, the interaction effect between both treatments was calculated.

Results

The insecticide application in the two conventionally managed vineyards successfully eradicated all leaf-feeding phylloxera for the rest of the growing season. In the organically managed vineyard, the treatment blocks that should have been without phylloxera leaf infestation did not develop any leaf galls throughout the rest of the growing season. Lastly, all treatment blocks that underwent leaf gall infestations housed active phylloxera populations for the rest of the growing season.

1. Carbon Assimilation

The gas exchange analyses in the most-infested vineyard did not reveal a significant reduction in net carbon acquisition of measured galled leaves compared to non-galled leaves (defoliation treatments are nested within infestation data) (Figure 1). However, a non-significant trend of a lower carbon assimilation of infested leaves was visible compared with measured leaves of non-infested plants (Table 2). Furthermore, a Spearman correlation test did not show any significant correlation between the number of galls on an infested leaf and its reduced carbon assimilation ($n = 114$, $r_s = –0.14$, $P = 0.224$). Though some effects were visible on leaf level, it is unclear whether a whole-plant compensation effect took place for the tested grapevines. Raw data from the gas exchange analyses are provided in Table S2.

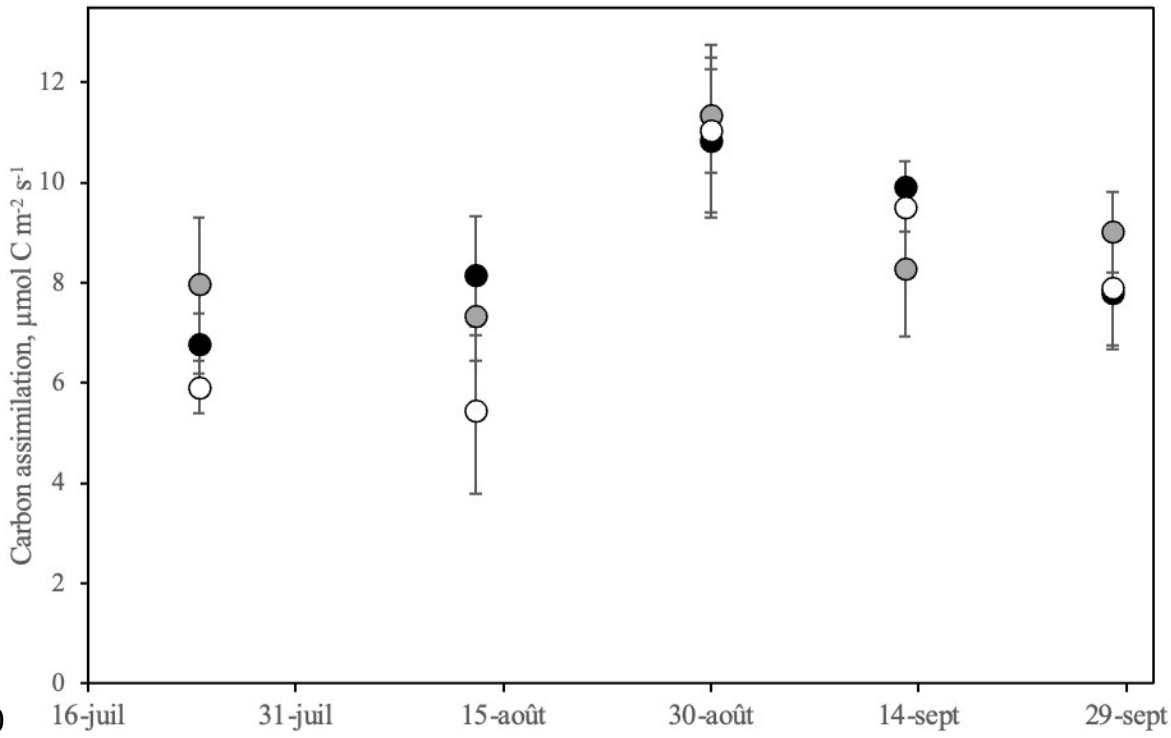

Figure 1. Gas exchange analysis of grapevine plants in vineyards in Bahlingen, based on natural infestation patterns. Black dots represent non-galled leaves on infested plants, grey dots non-infested plants and white dots galled leaves; n = 6 to 8 (defoliation treatments are nested within infestation treatments).

Table 2. Carbon assimilation results from the gas exchange measurements of grapevine plants in vineyards in Bahlingen (n = 19); A = CO_2 assimilation rate.

	Infestation		Defoliation		Interaction
	Phylloxera	Control	50%	Control	Inf. x defol.
A (µmol m^{-2} s^{-1})	7.7 ± 0.5	8.9 ± 0.5	8.6 ± 0.4	8.1 ± 0.6	n.s.

2. Must and Wine

During harvest, grapes were handpicked into small plastic containers and were weighed per treatment block. The total harvest weight of the 96 vines was 212, 560 and 183 kg for the vineyards in Pfaffenweiler, Britzingen and Bahlingen, respectively (Table 4). In the vineyard in Bahlingen, phylloxera infestation caused a significant reduction of must soluble solids, glucose and fructose (Table 3). These same parameters showed significant interaction effects between infestation and defoliation in the vineyard in Britzingen. Against expectation, this interaction effect was that the sugar content for the defoliated treatments was higher for the infestation treatments and lower for the non-defoliated ones. This same interaction effect was also visible for tartaric acid in the grape must of the vineyard in Pfaffenweiler. Tartaric acid levels were furthermore significantly lower for the grape must of the defoliated treatments in the vineyard in Britzingen.

Table 3. FTIR must analysis of treatment blocks (n = 8) in the vineyards in Pfaffenweiler, Britzingen and Bahlingen. All interactions: for 50 %, P > C; for control, C > P. Significantly different numbers are written in boldface.

		Infestation		Defoliation		Interaction
		Phylloxera	Control[1]	50 %	Control	Inf x defol
Pfaffenweiler	Sol. Solids, °Bx	25.2 ± 0.28	25.4 ± 0.26	24.9 ± 0.21	25.7 ± 0.26	n.s.
	Glucose, gl^{-1}	127.7 ± 1.84	128.5 ± 1.57	125.8 ± 1.27	130.3 ± 1.69	n.s.
	Fructose, gl^{-1}	133.5 ± 1.38	134.2 ± 1.48	131.9 ± 1.11	135.8 ± 1.34	n.s.
	pH	3.3 ± 0.01	3.3 ± 0.01	3.3 ± 0.01	3.3 ± 0.01	n.s.
	Tartaric acid, gl^{-1}	4.8 ± 0.09	4.6 ± 0.05	4.7 ± 0.11	4.6 ± 0.04	*

	Malic acid, gl^{-1}	1.6 ± 0.05	1.5 ± 0.07	1.5 ± 0.05	1.6 ± 0.07	n.s.
Britzingen	Sol. Solids, °Bx	16.4 ± 0.33	16.6 ± 0.26	16.5 ± 0.21	16.5 ± 0.37	*
	Glucose, gl^{-1}	79.6 ± 1.70	80.5 ± 1.29	80.2 ± 1.19	79.9 ± 1.78	*
	Fructose, gl^{-1}	81.8 ± 1.97	83.2 ± 1.62	82.4 ± 1.36	82.6 ± 2.19	*
	pH	3.1 ± 0.01	3.2 ± 0.01	3.2 ± 0.01	3.1 ± 0.01	n.s.
	Tartaric acid, gl^{-1}	5.5 ± 0.10	5.6 ± 0.10	**5.3 ± 0.05**	* **5.8 ± 0.09**	n.s.
	Malic acid, gl^{-1}	1.3 ± 0.11	1.2 ± 0.15	1.3 ± 0.15	1.2 ± 0.12	n.s.
Bahlingen	Sol. Solids, °Bx	**22.4 ± 0.33 ***	**24.6 ± 0.54**	23.6 ± 0.56	23.4 ± 0.64	n.s.
	Glucose, gl^{-1}	**103.5 ± 1.74 ***	**115.6 ± 3.09**	110.4 ± 3.10	108.7 ± 3.64	n.s.
	Fructose, gl^{-1}	**112.8 ± 1.87 ***	**125.2 ± 3.07**	119.5 ± 3.20	118.5 ± 3.70	n.s.
	pH	3.2 ± 0.02		3.2 ± 0.01	3.2 ± 0.02	n.s.
	Tartaric acid, gl^{-1}	7.7 ± 0.06		7.6 ± 0.03	7.6 ± 0.12	n.s.
	Malic acid, gl^{-1}	4.7 ± 0.18		4.4 ± 0.15	4.5 ± 0.33	n.s.

[1] Instead of insecticide spraying, the control in the organic vineyard in Bahlingen was based on naturally non-infested grapevine foliage.

For the total sugar content and total titratable acids (expressed as gl^{-1} tartaric acid), we saw similar patterns as before (Figure 2). In all three vineyards, the average total sugar content appeared to be lower in must from infested vines; however, this reduction was only significant for the vineyard in Bahlingen. Here, the sugar content in must was 10 % lower for infested grapevines. The titratable acid showed an opposite and therefore phenologically similar pattern. This increase was statistically significant for the vineyard in Pfaffenweiler, where the grape must of infested vines had 7.5 % more titratable acid.

After harvest, the grapes of the vineyards in Pfaffenweiler and Britzingen were vinified and underwent testing from a sensory panel. The panel found no significant difference between the wines of the different treatments in the vineyard in Pfaffenweiler ($\chi^2 = 16.8$ df = 18, P = 0.537). There was a significant difference between the wines from the vineyard in Britzingen ($\chi^2 = 33.3$ df = 15, P = 0.004). However, the significantly different attributes were the result of vinification (ethyl acetate and hydrogen sulphide) and were not directly related to grape quality. The volatile acidity was 0.25 gl^{-1} must in the vineyard in Bahlingen, 0.11 gl^{-1} must in the vineyard in Britzingen and 0.30 gl^{-1} must in the vineyard in Pfaffenweiler, whereby no significant differences were found between treatments.

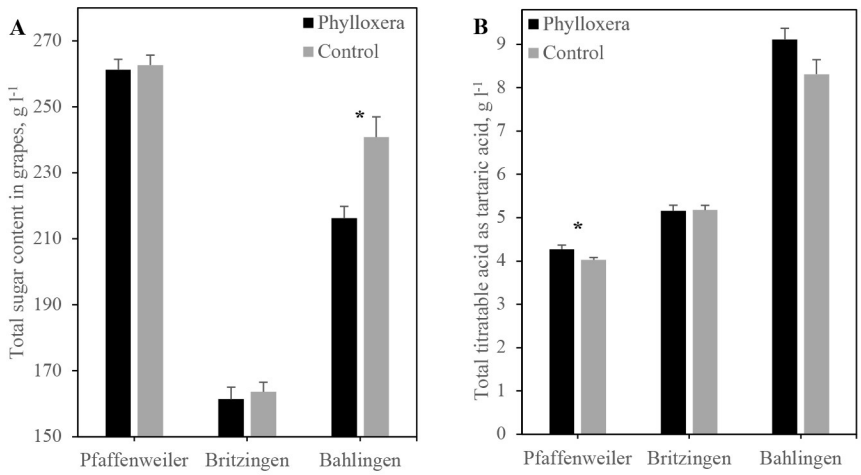

Figure 2. FTIR analyses per treatment block of harvested grapes in the vineyards in Pfaffenweiler, Britzingen and Bahlingen, comparing infested and non-infested grapes (defoliation treatments are nested within infestation treatments). A, total sugar content; B, total titratable acid, depicted as tartaric acid; n = 8.

3. Yield, Vigour and Carbon Reserves

After the growing season, plant vigour analyses were performed per grapevine (Table 4). There were no significant differences for both the phylloxera and defoliation treatments in all three vineyards. There was a significant interaction effect for the parameter yield per vine in the vineyard in Britzingen. This parameter was lower for defoliated infested vines than non-defoliated vines, which may be expected.

An additional treatment was included in the vineyards in Pfaffenweiler and Britzingen, where leaf phylloxera was eradicated with imidacloprid. Vines from this treatment did not develop any foliar phylloxera during the growing season (like the phylloxera control treatment in the vineyard in Bahlingen). A vigour comparison between this additional treatment and the non-defoliated insecticide sprayed treatment revealed non-significant differences in average internode length and pruning weight. Therefore, there was no visible bias in plant growth between the insecticide sprayed control vines and control vines based on naturally absent leaf infestation.

Table 4. Grapevine yield and vigour parameters in the vineyards in Pfaffenweiler, Britzingen and Bahlingen (n = 48). FW = Fresh weight. Interaction: for 50 %, C > P; for control, P > C.

		Infestation		Defoliation		Interaction
		Phylloxera	Control[2]	50 %	Control	Inf x defol
Pfaffenweiler	Pruning FW, kg	0.6 ± 0.03	0.6 ± 0.04	0.5 ± 0.03	0.6 ± 0.04	n.s.

	Intern length, cm	6.8 ± 0.20	7.3 ± 0.22	7.1 ± 0.21	7.0 ± 0.22	n.s.
	Yield, kg/vine[1]	2.5 ± 0.14	2.2 ± 0.12	2.3 ± 0.10	2.3 ± 0.17	n.s.
	Bunch weight, kg[1]	0.2 ± 0.03	0.2 ± 0.04	0.2 ± 0.03	0.2 ± 0.04	n.s.
	Ravaz index[1]	5.1 ± 0.33	5.1 ± 0.54	5.1 ± 0.37	5.1 ± 0.52	n.s.
Britzingen	Pruning FW, kg	0.6 ± 0.02	0.5 ± 0.02	0.6 ± 0.02	0.5 ± 0.02	n.s.
	Intern length, cm	9.0 ± 0.17	8.8 ± 0.15	9.0 ± 0.15	8.7 ± 0.17	n.s.
	Yield, kg/vine[1]	5.8 ± 0.24	6.2 ± 0.39	6.4 ± 0.35	5.5 ± 0.19	*
	Bunch weight, kg[1]	0.3 ± 0.04	0.3 ± 0.04	0.3 ± 0.05	0.3 ± 0.02	n.s.
	Ravaz index[1]	11.0 ± 0.46	12.7 ± 0.79	12.6 ± 0.77	11.2 ± 0.50	n.s.
Bahlingen	Pruning FW, kg	0.2 ± 0.01	0.2 ± 0.01	0.2 ± 0.01	0.2 ± 0.01	n.s.
	Intern length, cm	5.6 ± 0.10	5.9 ± 0.12	5.8 ± 0.12	5.7 ± 0.11	n.s.
	Yield, kg/vine[1]	2.0 ± 0.18	1.9 ± 0.26	2.0 ± 0.24	1.8 ± 0.21	n.s.
	Bunch weight, kg[1]	0.1 ± 0.01	0.1 ± 0.01	0.1 ± 0.01	0.1 ± 0.01	n.s.
	Ravaz index[1]	10.0 ± 0.71	9.0 ± 0.66	9.3 ± 0.64	9.7 ± 0.74	n.s.

[1] During harvest, the grape bunches were pooled per block; therefore, n = 8.

[2] Instead of insecticide spraying, the control in the organic vineyard in Bahlingen was based on naturally non-infested grapevine foliage.

Finally, after leaf senescence at the end of the growing season, the content of non-structural carbohydrates was measured in two-year-old pruning wood. These enzymatic analyses showed no significant differences in total sugars due to infestation. However, the content of starch significantly decreased by 11 % for infested vines in the vineyard in Bahlingen (Figure 3). Calculating the non-structural carbohydrates per vine by multiplying with pruning dry weight, no differences were found between total sugars in all vineyards (Pfaffenweiler: $t = -0.72$, $P = 0.477$ Britzingen: $t = 1.14$, $P = 0.262$, Bahlingen: $t = -0.77$, $P = 0.442$) or starch (Pfaffenweiler: $t = -0.54$, $P = 0.592$ Britzingen: $t = 1.79$, $P = 0.081$, Bahlingen: $t = -1.78$, $P = 0.081$).

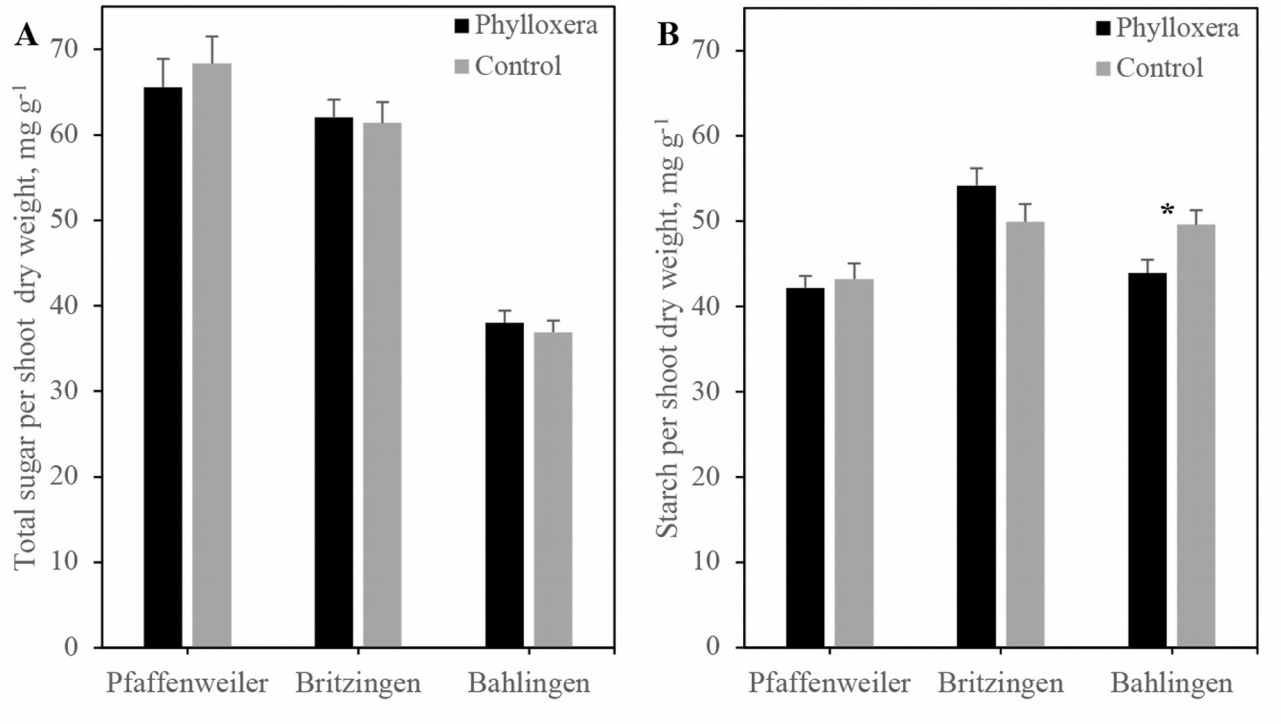

Figure 3. Enzymatic analyses of non-structural carbohydrates in the two-year-old pruning wood of the vine's lateral cane of grapevines with and without phylloxera leaf infestation in the vineyards in Pfaffenweiler, Britzingen and Bahlingen. A, total sugar content per shoot dry weight; B, starch content per shoot dry weight; n = 24 (defoliation treatments are nested within infestation treatments).

Discussion

The presence of perpetual vineyard-wide leaf gall outbreaks in commercial vineyards in Baden, Germany, revealed the knowledge gap on quantifying phylloxera leaf infestation effects on *V. vinifera* growth and yield under field conditions. In a first step to fill this gap, this study investigated the consequences of phylloxera leaf infestation under standard viticulture management, comparing 336 individual grapevines in three pilot vineyards during a single growing season. The three commercial vineyards acted as pilot plots with different local settings each. Although all three vineyards were submitted to vineyard-wide phylloxera infestations, these differed in leaf gall intensity per plant and leaf. The leaf gall intensity was highest in the tested vineyards in Bahlingen, with an average of 20 to 30 galls on the highest infested mature leaf, and the lowest in Pfaffenweiler, with up to 10 galls per leaf, counted at the start of the experiments. Due to the differences in vineyard settings between the pilot plots, direct comparisons should be made with caution. The insecticide spraying in the vineyards in Pfaffenweiler and Britzingen were highly

effective in removing leaf-feeding phylloxera. Imidacloprid is a systemic insecticide with both upward and downward plant mobility that was shown to suppress both leaf- and root-feeding phylloxera (Benheim *et al*., 2012). Although the population size of root-feeding phylloxera was unknown, *V. berlandieri* × *V. riparia* rootstock hybrids are known to successfully tolerate phylloxera root feeding (Eitle *et al*., 2017a). For most of the measured vegetative and generative growth parameters, no difference could be found due to phylloxera leaf infestation. However, our study suggests that yield quality and non-structural carbon reserves could, in single cases, be affected in commercial vineyards.

To measure the impact of leaf infestation on the carbon acquisition of individual leaves, a gas exchange analysis was conducted in the pilot vineyard in Bahlingen. The measurements were made from the start of the leaf infestation outbreaks, measuring galled and non-galled leaves of infested vines and on non-galled control vines. Though a pattern was visible, these analyses did not show a significant reduction of carbon assimilation for galled leaves and no significant overcompensation for the measured non-infested leaves on infested plants. Conversely, in a study with gas exchange measurements on field-grown *Vitis* hybrid “Frontenac” vines, the net carbon assimilation per leaf area was reduced, which the authors partly appointed to the reduced photosynthetically active area (Nabity *et al*., 2013). In a whole-plant hydroponic experiment with *V. rupestris* vines that were infested with 250 to 300 galls per plantlet of eight leaves, a higher retention of ^{14}C-labeled carbon was visible in galled leaves (Steffan and Rilling, 1981). Without a net difference in carbon assimilation per plant, the authors concluded that the plantlet non-galled leaves overcompensated this local effect with a higher photosynthetic rate in healthy leaf tissue. In gas exchange measurements with artificial inoculations on one-year-old greenhouse and three-year-old field-grown vines in July and August on “Seyval” hybrid grapes, the carbon assimilation was reduced on infested leaves, without a visible compensation effect on non-infested leaves (Mcleod, 1990). Interestingly, non-infested “Seyval” leaves that neighboured infested leaves (with up to 200 galls) also showed a temporal lower assimilation rate, which adjusted back to normal in the fourth week of infestation. In this artificial inoculation study, a logarithmic reduction of net photosynthesis correlated with an increased number of galls per leaf. In our study, with a natural leaf infestation of fewer than 20 galls per measured leaf, we did not find such a correlation.

In a study with a galling aphid (*Melaphis rhois* FITCH) and a galling mite (*Eriophyes cerasicrumena* WALSH), no photosynthetic compensation effect was seen for neighbouring non-galled leaves (Larson, 1998). For the mite-infested plants, the author saw a photosynthetic reduction of neighbouring leaves, which she explains by either a competition effect between newly developing

leaves and gall sinks or a biochemical disruption of the photosynthetic machinery. This process may also take place during phylloxera infestation. From phylloxera root galls, it is known that every gall creates high levels of starch that is consumed on demand by the larvae (Griesser *et al*., 2015). A delayed carbon acquisition into the feeding larvae after incorporation into the leaf gall material suggests that these processes also occur during leaf feeding (Nabity *et al*., 2013). Besides the gall tissue itself, the pecan phylloxera (*Phylloxera notabilis* PERGANDE) lowers the photosynthetic capacity on healthy tissue that surrounds the leaf gall (Andersen and Mizell, 1987). Such lowering of adjacent leaf area also appears for grape phylloxera, although these changes were not significant (Nabity *et al*., 2013). These authors, however, did link increased phylloxera sink strength due to locally up-regulated defence mechanisms (jasmonic acid synthesis and vacuolar invertase activity). This verifies that the sink competition is not only based on dietary requirements of phylloxera but is, among others, also defence induced.

There was no significant difference for all tested plant vigour and yield quantity parameters due to infestation or defoliation alone. It is known that grapevine photosynthesis is not source-limited pre-veraison, at which time, merely up to 50 % of the photosynthetic capacity is used (Howell, 2001). Single-leaf photosynthesis measurements furthermore do not correlate with whole-plant photosynthesis. This, unfortunately, means that the gas-exchange measurements on the effect of leaf galls cannot be extrapolated for the whole vine. A greenhouse study with potted plants also found no differences in single-leaf photosynthetic assimilation between phylloxera leaf infested, leaf and root-infested, and control plants (Savi *et al*., 2021). Nevertheless, the leaf infested potted plants in this study showed a significantly reduced total biomass, visualising that single-leaf assimilation measurements and whole-plant carbon assimilation do not always relate to phylloxera-infested plants. Moreover, besides increased stomatal and mesophyll conductance, grapevines compensate a low source-sink ratio by delaying the leaf senescence of main leaves, which would not be visible with gas exchange measurements of young mature leaves (Candolfi-Vasconcelos and Koblet, 1991). The combined non-significant differences of internode length and pruning weight parameters suggest that infested grapevines did not compensate with a higher leaf area.

When defoliation and infestation effects were combined, there was an interaction effect on the yield per vine in the vineyard in Britzingen. Here, the yield was lower on defoliated vines compared with non-defoliated vines. The severity of the effect that phylloxera infestation has on yield may depend on the relation between the plant's source and sink material. However, even though the vineyards in Britzingen and Pfaffenweiler had a much higher crop load (i.e., higher Ravaz index) than the one in Pfaffenweiler, this was not visible from the yield quantity and quality data. For the hybrid vine

"Seyve-Villard 18-315", yield increased by 15 to 20 % after phylloxera eradication (Schvester, 1959). In a pesticide spraying experiment on "Maréchal Foch" and two "Seibel" hybrids that were infested with 15 to 20 galls per leaf on the first five apical leaves, no consistent differences were found for pruning weight, yield and sugar content (Stevenson, 1970a). In an experiment with artificial infestations, economic damage on the hybrid vine "Seyve-Villard 5276" started above 150 galls on the first five apical leaves per shoot at the time of bloom (Mcleod, 1990). The vineyards in our study just started developing leaf galls at bloom, developing in numbers only after this crucial time frame.

Furthermore, the viticulture practice of hedging was observed to diminish phylloxera leaf populations effectively. Due to the pruning of main and lateral shoots, plant growth was temporarily stopped and meristematic leaves were removed. As phylloxera can only create galls on growing meristematic leaves, populations locally collapsed and larval numbers dwindled. The non-pruned shoots that grew horizontally within the canopy especially suffered from the highest intensities of galls. Other studies found that without hedging, phylloxera populations were the biggest on long shoots (Kimberling *et al*., 1990). The effect of phylloxera infestation on shoot elongation is recognised by Kimberling *et al*. (1990) but remains inconclusive in the study of Mcleod (1990). In our study, no significant differences were found in pruning weight or internode length between naturally non-infested vines, insecticide sprayed vines or infested vines. Phylloxera larvae were therefore not more likely to infest leaves of vigorous grapevines, and vines were not more vigorous due to leaf infestation. Although these numbers represent field conditions under standard vineyard management, hedging prevented the measurement of actual differences in shoot length.

The vineyard in Bahlingen suffered the highest intensity of leaf galls and showed the most significant differences in yield quality. The vineyard was organically managed, a trait that suppresses phylloxera root populations (Huber *et al*., 2003). However, the grafted vineyard was planted with scions of the fungus-resisting variety "Muscaris", a scion that was found to be more easily infested by root-feeding phylloxera that migrates from roots to leaves compared with *V. vinifera* scions (Wilmink *et al*., 2021b). Throughout the experiment, the vineyard in Bahlingen received more precipitation and, through cover crops, presumably had a higher local relative humidity than the other two vineyards that were studied in the dry growing season of 2018. The lower damage threshold to phylloxera leaf-feeding that was found in dry years on "Seyval" hybrid grapes (Mcleod, 1990) was, therefore, not visible for our experiments.

In the vineyard in Bahlingen, soluble solids, glucose, fructose and total sugar content were significantly reduced in grape must due to phylloxera infestation. This sink competition with grapes is remarkable because grapes are known to be very strong sinks that can draw photo-assimilates from compensating leaves on other shoots (Mansfield and Howell, 1981). A study with CFDA-marked sucrose confirmed this high sink strength of phylloxera leaf and root galls, being the single destination of the petiole-inserted sucrose (Wieczorek *et al*., 2014). The lack of compensation effects, which are normally seen in defoliation studies, indicates that the effects of phylloxera infestation cannot be simplified as a source-sink issue but should be answered on a biochemical level. Indeed, huge differences in secondary metabolites and host plant reprogramming were seen during phylloxera root- and leaf-feeding (Eitle *et al*., 2017b; Eitle *et al*., 2019a; Nabity *et al*., 2013).

The 10 % soluble solid reduction in grape must in the vineyard in Bahlingen was lower than for hybrid “Seyve-Villard 18-315” vines, where eradication of foliar phylloxera increased soluble solids by up to 20 % (Schvester, 1959). For the hybrid vine “Seyve-Villard 5276”, a soluble solid reduction of about 10 % was found for artificial pre-bloom phylloxera infestation and no differences for post-bloom infestations (Mcleod, 1990). Contrary to these studies, we also found a significant increase of titratable acid in grape must (7.5 %) due to phylloxera infestation in the vineyard in Pfaffenweiler. Through defoliation, we also saw a significant decrease in tartaric acid in grape must in the vineyard in Britzingen, a tendency that was not seen in the other two vineyards. Overall, the combination of a decreased sugar content and increased acidity indicates that phylloxera leaf infestation can delay the ripening of grapes. Though this pattern was visible for all vineyards, the effect was often non-significant or lower than in studies with hybrid grapevines that were subjected to higher rates of leaf infestation. The sensory panel also did not find any differences in wine quality, which was in line with a study with infestation levels of 15 to 20 galls per leaf on the first five apical leaves of hybrid vines (Stevenson, 1970a). After the growing season, the level of stored non-structural carbohydrates in perennial wood was similar to other grapevine literature (Herrera *et al*., 2015; Savi *et al*., 2019). The analysis revealed no significant differences in stored soluble sugars for any vineyard and a significant decrease of starch for phylloxera infested vines in the vineyard in Bahlingen. In a defoliation study with potted *V. vinifera* plants, total sugars and starch were only lowered when 85 % of the leaves were removed, not when 50 % of the leaves were removed (Silva *et al*., 2017). We also did not find any differences based on leaf removal. Similarly, in a dual leaf and root phylloxera infestation, ^{14}C-carbohydrates were transferred to galled leaves at the cost of the roots (Steffan and Rilling, 1981). A study on phylloxera root infestation alone did not reveal differences in non-structural carbohydrates in the plant stem, though a reduction in leaf starch and

root sugar content was evident (Savi *et al*., 2019). Indeed, a gradual carbon concentration build-up from non-infested roots through infested roots to root galls was observed (Eitle *et al*., 2017a). Root infestation, therefore, likely plays a smaller role for starch reserves in perennial wood compared to the effect of phylloxera leaf infestation. Similarly, the perennial wood of plantlet cuttings accumulated the least ^{14}C-carbohydrates during phylloxera leaf infestation compared to root infestation, dual leaf and root infestation and control (Steffan and Rilling, 1981). This shows the importance of phylloxera leaf galls on the storage of starch in perennial wood.

Conclusion

The overall effect of leaf infestation at the intensity seen in the commercial vineyards in Baden, Germany, was low. Many of the effects that are seen in studies with augmented phylloxera infestation on hybrid grapes were not significant in this study, though some significant changes were visible. We conclude that phylloxera leaf infestation at this intensity may marginally affect commercially cultivated grafted *V. vinifera* grapevines, although no effects were seen for yield quantity or wine sensory analyses. Through diminished storage of carbohydrates and altered biochemical processes, there may be an effect on grapevine longevity and resilience against abiotic and biotic stresses (e.g., frost tolerance). Although the number of vines that were tested was high, the results stem from vineyards under different viticulture and environmental settings conducted in a single growing season. These first preliminary results, therefore, form a basis for further in-depth research on phylloxera leaf infestation in commercial vineyards planted with *V. vinifera*.

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