Effect of temperature on host plant-specific leaf- and root-feeding performances: a comparison of grape phylloxera biotypes C and G

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Abstract

Grape phylloxera, Daktulosphaira vitifoliae (Fitch) (Hemiptera: Phylloxeridae), is a destructive pest for global viticulture. With the grafting of the susceptible cultivated grapevine (Vitis vinifera L., Vitaceae) on top of tolerant Vitis spp. rootstock, root infestation no longer causes vine death. These tolerant rootstock vines are hybrids of American species that are often highly susceptible to leaf infestation. Though leaf infestation is normally rarely seen on V. vinifera, some commercial vineyards have been showing high intensities of leaf galls for many years. In this study, two possible factors are investigated to explain these anomalies: (1) intra-specific differences in phylloxera host plant specialization, and (2) improved environmental settings for infestation due to temperature increase. To study the former, a phylloxera biotyping assay was conducted after whole-plant (both root and leaf) infestations, and for the latter, a temperature increase simulation was performed with potted plants in climate chambers. Both assays also contained a phylloxera control biotype C, obtained from an American rootstock hybrid (Vitis berlandieri Planch. × Vitis riparia Michx.). The biotyping assay showed that field-sampled populations from V. vinifera leaf galls had innate advantages to infest the leaves of this host plant species compared to those of the American rootstock hybrid. This is therefore the first study to categorize a phylloxera population as biotype G, using controlled experimental conditions with biological pest control. At moderate temperatures (22 °C), infestation was similar as in the biotyping assay, but at higher temperatures (27 °C), biotype G seems to lose its comparative advantage to infest V. vinifera leaves. Specifically, at higher temperatures, insect performance in terms of leaf gall intensity, development, and egg-laying of both biotype G and C is improved on American rootstock hybrids and worsened on V. vinifera compared to infestations at moderate temperatures. We discuss possible explanations for these findings and how these experimental results may be extrapolated to field settings.

Introduction

Grape (Vitis spp., Vitaceae) growers in most regions are obligated to plant their vineyards with grafted vines, evoked by the introduction of the plant sap sucking insect grape phylloxera, Daktulosphaira vitifoliae (Fitch) (Hemiptera: Phylloxeridae), into the wine-producing regions around the world. Even before its anthropogenic dispersal, grape phylloxera covered a wide range of coastal and terrestrial ecosystems from Mexico to Canada (Downie et al., 2001). At lower temperatures, however, insect development is delayed and survivability is reduced (Granett & Timper, 1987). Moreover, low temperatures and bad weather conditions are thought to prevent grape phylloxera to undergo all parts of their life cycle (Forneck & Huber, 2009). Especially in temperate regions where optimum insect temperatures have not been reached yet, global warming is expected to increase pest damage of phytophagous insects (Lehmann et al., 2020).
Grape phylloxera is a major pest throughout the grape-growing regions of the world. This aphid-like insect is an obligate biotroph of *Vitis* spp. and can undergo both belowground (root-feeding) and aboveground (leaf-feeding) life stages. Unlike many North-American grapevine species that co-evolved with this pest, *Vitis vinifera* L. – the grapevine species used for winemaking – greatly suffers when its roots are infested (Wapshere & Helm, 1987). As a solution, *V. vinifera* scions are grafted on top of American rootstock hybrids that tolerate phylloxera root infestation. Though phylloxera populations are still able to feed on the root tips of these rootstock hybrids, grapevines no longer display growth and yield decline. Paradoxically, though the foliage of many tolerant rootstock hybrids is highly vulnerable for phylloxera leaf infestation, the unaccustomed *V. vinifera* is successfully able to suppress leaf gall formation.

Throughout the growing season, phylloxera larvae disperse from existing feeding sites on galled root tips (called nodosities) to other parts of the plant. Besides migrating belowground, these first instars are also able to crawl up the trunk of the vine to initiate feeding on meristematic leaves and thereby create leaf galls (Powell, 2008). In unattended vineyards, where grafted American rootstock hybrids are able to grow shoots and leaves, foliar infestation outbreaks occur that can persist throughout the growing season. The infestation pressure that is created by leaf-feeding phylloxera on American rootstock hybrids also enables the formation of leaf galls on *V. vinifera* leaves (Jubb, 1976; Molnár et al., 2009). Over the last years, the global amount of foliar phylloxera outbreaks in commercial *V. vinifera* vineyards has been increasing for unknown reasons (Forneck et al., 2019). It is often unclear whether such foliar infestations are indirectly initiated through nearby foliage of American rootstock hybrids, and are only able to sustain on *V. vinifera* with the help of a continuous infestation pressure from outside the vineyard, or whether these are directly initiated from root-feeding phylloxera within the vineyard. In this study, we seek to identify the causes of foliar outbreaks, with the focus on specific foliar phylloxera populations that are observed to break with the common concept of indirect leaf infestation. There are individual commercial vineyards throughout the grape-growing region Baden (Germany) that house vast populations of foliar phylloxera on *V. vinifera* scions. These populations persist throughout the growing season and are isolated from any potential foliar infestation source like the mentioned leaf gall populations on American rootstock hybrids (Forneck et al., 2015).

Grape phylloxera populations show inherent differences in host plant-specific infestation performance, which led to the categorization of phylloxera populations into biotypes (Granett et al., 1985, 1996). Unfortunately, to date, there are no genetic markers to define phylloxera biotypes, so they need to be defined by comparing new lines to a standard line using biotyping assays (Forneck et al., 2016). Many biotype experiments have been conducted for phylloxera root infestation traits only, as these are the cause of plant damage and thereby yield reduction (Granett et al., 1985; Eitle & Forneck, 2017). Leaf infestation, on the other hand, has not been studied in such depth and lacks standardized assays for the classification of biotypes. Nonetheless, based on field observations, possible biotypes are mentioned that could develop well on *V. vinifera* leaves (Forneck et al., 2016). This is in contrast with the infestation traits of the most often found biotype in Europe, biotype C, which is adapted to the roots and leaves of American rootstock hybrids (Forneck et al., 2016).

Besides inherent feeding traits, temperature also plays an important role in plant organ-specific feeding and phylloxera development. Briefly, and for the more common non-sexual life cycle, due to rising spring temperatures, phylloxera larvae that hibernated on the vine’s roots resume their feeding. From this point onwards, phylloxera can either stay underground, creating new root-feeding generations throughout the growing season, or, if possible, reproduce in leaf galls (Forneck & Huber, 2009). Together with daylength, temperature is a prime cause for larval transition between above- and belowground life stages (Rilling, 1964). However, the way temperature affects phylloxera development and behavior is complex, because individual peak temperature events alter the temperature-dependent developmental speed by acclimation (Turley et al., 1996). Moreover, each larval stage has a different temperature optimum, deviating actual developmental speed from linear degree-day model predictions (Belcari & Antonelli, 1989; Fisher & Albrecht, 2003). Overall, phylloxera thrives between 21 and 28 °C (Granett & Timper, 1987; Belcari & Antonelli, 1989). Comparing temperature optima for various life table parameters showed that development and fecundity increase with temperature until 30 °C, but that per capita growth rate has a cooler optimum, between 21 and 24 °C (Fisher & Albrecht, 2003). When temperatures rise above 32 °C, high mortality occurs (Granett & Timper, 1987). Interestingly, a study on phylloxera eradication with hot dry air showed that such temperature-dependent mortality rates seem to vary between phylloxera populations (Korosi et al., 2012). This fortifies the assumption that the origin of specific phylloxera populations and biotypes may have an influence on their temperature optimum (Granett & Timper, 1987).

We here hypothesize the above-described phylloxera populations in the grape-growing region Baden, which seem well able to thrive (i.e., better sustain infestation or
develop) on leaves of *V. vinifera* cultivars throughout the vegetative season, to: (1) belong to a biotype that has inherent traits for superior development on *V. vinifera* leaves, and (2) excel at elevated temperatures compared to a phylloxera control biotype C, reared for many generations at 22 °C.

### Materials and methods

#### Phylloxera and plant material

Two phylloxera single founder lineages (SFLs) were compared in this study. The first one, B6, was sampled from the foliage of *V. vinifera* cv. ‘muscat a petits grains blancs’ syn. ‘gelber muskateller’, in Germany’s grape-growing region Baden. In this vineyard, it annually creates vineyard-covering foliar infestations that perpetuate throughout the vegetation period. The second SFL, TT1, is a standard line from the Institute of Viticulture and Pomology in Vienna, Austria. This line belongs to biotype C, the biotype most often found in Europe, and performs well on both roots and leaves of American rootstock hybrids (Forneck et al., 2016). It was originally sampled from the foliage of an American rootstock hybrid (*Vitis berlandieri* Planch. × *Vitis riparia* Michx.) in the grape-growing region Traisental, Austria, and maintained under controlled conditions at (22 °C) on whole vines feeding on roots and leaves of *V. berlandieri × V. riparia* cv. ‘Tel-eki 5C’ for several years. Three grapevine cultivars were employed as host plants: the wine-producing *V. vinifera* cv. ‘Riesling’ (RR), the wine-producing hybrid (*V. vinifera × 101 MG O.P.) cv. ‘Maréchal Foch’ (MF), and the American rootstock hybrid (*V. berlandieri × V. riparia*) cv. ‘Teleki 5C’ (5C). These plants were propagated from dormant single-eyed cuttings under greenhouse conditions as described previously (Forneck et al., 2001c).

#### Biotyping assay

Biotyping assays were carried out in a greenhouse in small isolation chambers with single-plant quarantine conditions, often used for phylloxera root bioassays (for a general description, see Forneck et al., 2001a). This system operates with whole plants in soil medium, which is necessary to obtain non-biased field simulations (Eitle & Forneck, 2017). The analysis of host-specific infestation performance on the three grapevine cultivars cultivated in quarantine systems allows for a biotype classification according to the internationally set standard for grape phylloxera studies (see Forneck et al., 2016). The biotype assays were conducted with a total of 30 plants, 10 of each host grapevine cultivar. After a twofold root-leaf inoculation of 50 eggs, the infestation was run for 5 weeks (until grape phylloxera was in its second asexual cycle). At the end of the biotyping assay, infestation parameters were gathered. For root infestation, the number of nodosities per plant was used.

Because standardized leaf infestation studies are scarce, we conducted a pilot study to assay reliable insect- and plant-based parameters that allow for screening using the biotyping assay set-up, prior to the experimental set-up of this study. We initially included the insect parameters net reproductive rate, fecundity, development, and survivorship, standardized with the plant growth parameters dry weight, shoot length, and leaf area. From these parameters, gall intensity, phylloxera development, and eggs per adult were best compatible with the isolation chambers of Forneck et al. (2001c) and most distinctively visualized biotypes (data not shown). ‘Gall intensity’, the number of leaf galls standardized with plant growth per leaf (Kimberling & Price, 1996) (as was done in this study), per shoot (Granett et al., 2005), or per leaf area (King & Rilling, 1985), is an often used parameter in field studies on foliar phylloxera infestation. The other parameters, ‘phylloxera development’ and ‘eggs per adult’, are often used in phylloxera root bioassays (Granett & Timper, 1987; Fisher & Albrecht, 2003). In root-feeding studies, these are obtained with in vitro experiments (Forneck et al., 1996) or excised root assays (Granett et al., 1983), where continuous observations are possible. In order to cope with the obscuring leaf gall, which makes continuous non-destructive measurements impossible, the relative amount of individual phylloxera stages has sometimes been calculated as a single final measurement in leaf-feeding studies (e.g., Raspi et al., 1987). In line with such studies, we here perform a single destructive counting to calculate the average larval stage as described in the phylloxera population age structure index, used for root-feeding phylloxera (Omer et al., 1999) (only for leaf galls that contained larvae). For similar reasons, phylloxera fecundity is also measured with a single destructive counting of eggs per adult phylloxera (only for leaf galls that contained adults). The three leaf infestation parameters were gathered per leaf, for the six most infested leaves per plant.

#### Temperature experiment

To test the second hypothesis, a temperature experiment was carried out to understand whether SFL B6 is better able to cope with elevated temperatures than TT1. The temperature trials were conducted with 72 potted plants in quarantine systems, growing in climate chambers, set at L16:D8 photoperiod to simulate optimal environmental conditions for the development of leaf-feeding phylloxera (Rilling, 1964) and 85 ± 8% r.h. to prevent drought-induced infestation decline (McLeod, 1990). The quarantine systems were made of 71 × 47 × 136-cm
(length \times width \times height) Plexiglas containers that held six potted plants each and were open at the top and bottom. The bottom was placed between two tubs in a water barrier and the top was covered with a 0.125-mm-meshed phylloxera-proof grid. High-pressure mercury lamps on the ceiling illuminated the systems with a photosynthetic photon flux density of 1350 \mu\text{mol m}^{-2}\text{s}^{-1} at the top of the container. Half of the plants were grown in a chamber at 22 \pm 1 ^\circ\text{C} and the other half in a chamber at 27 \pm 1 ^\circ\text{C}, resembling the lower and higher end for optimal infestation conditions (Powell et al., 2013). Half of the plants in each climate chamber consisted of cv. 5C and the other half of cv. RR, planted in 3-l pots filled with a 9:1 (vol:vol) autoclaved mixture of compost soil and perlite (Eitle et al., 2017).

Within each environmental chamber, 12 plants were inoculated with phylloxera TT1, 12 with B6, and 12 served as control (without phylloxera). The temperature trials simulated a high-infestation scenario, wherein V. vinifera vines are under enhanced infestation pressure from galliform (leaf-galling) phylloxera on rootstock hybrids, as described in the introduction. All plants were therefore arranged so that three 5C and three RR plants shared a quarantine system. The inoculation was carried out by placing 50 phylloxera eggs on a meristematic leaf of each infested plant, moistened with a piece of paper and held in place with aluminum foil (modified from Forneck et al., 2001c). The plants were infested until grape phylloxera underwent two generations (after 7 weeks). This prolonged infestation period was chosen to lower the effects of initial differences in phylloxera fitness (Forneck et al., 2001b). At the end of the experiment, leaves were individually frozen at −20 ^\circ\text{C}, to preserve the phylloxera for stereomicroscopic counting. The leaf infestation parameters gall intensity, phylloxera development, and eggs per adult were also used in the temperature trials (identical to the biotyping experiment, allowing comparison). These were taken from 30 infested leaves per plant. For each leaf, fresh weight was measured directly after picking, and the total number of infested and non-infested leaves were counted per plant. For root infestation, the number of nodosities were counted per plant. Roots were rinsed and, together with shoot and leaves (after stereomicroscopic counting), dried for 72 h at 60 ^\circ\text{C} in an oven, to calculate dry weight.

**Statistical analysis**

Data analyses were carried out with IBM SPSS v.26 (IBM, Armonk, NY, USA). Comparisons between two groups were tested with Welch’s unequal variances t-test, because of its superior robustness compared with student’s t-test (Ruxton, 2006). The biotype tests between three grape varieties, for each phylloxera line separately, were performed with one-way ANOVA, followed with Tukey’s post hoc test with \( \alpha = 0.05 \). MANOVAs were conducted to test the effect of grapevine variety, phylloxera biotype/infestation, and, if relevant, temperature on leaf (not root) infestation and plant weight parameters. Using a Bonferroni correction, it was verified that the MANOVA’s individual two-way and three-way ANOVA results that reject the null hypotheses are valid. Interaction effects between factors are visually highlighted for better interpretation.

**Results**

**Biotyping assay**

In the biotyping assay, 80% of all B6 and 60% of all TT1 inoculations were successful. Phylloxera line TT1 created the most leaf galls on 5C (its original field host plant cultivar) and the least on RR (\( F_{2,72} = 4.53, P = 0.014 \)), whereas B6 created the most galls on MF and the least on 5C (\( F_{2,126} = 7.91, P = 0.001 \)). The overall gall intensity of B6 was higher than that of TT1 on RR, and lower on 5C (Figure 1A). B6 larvae were in a later developmental stage than TT1 larvae on all grapevine cultivars. Development of B6 larvae on the three cultivars was similar (\( F_{2,86} = 2.18, P = 0.12 \)), whereas TT1 larvae were in a later developmental stage on MF than on RR (\( F_{2,65} = 4.91, P = 0.010 \)) (Figure 1B). B6 and TT1 adults laid a comparable total number of eggs (TT1: 8, B6: 9). B6 adults produced a similar number of eggs on the three cultivars (\( F_{2,82} = 1.71, P = 0.19 \)), whereas TT1 adults laid the most eggs on 5C and the least on RR (\( F_{2,31} = 6.41, P = 0.005 \)) (Figure 1C). B6 (\( F_{2,10} = 1.76, P = 0.22 \)) created a similar amount of root galls (nodosities) on all grapevine cultivars. TT1 did not create any nodosities on RR plants; the number of nodosities on the other two grapevine cultivars was similar (\( t = −1.30, \text{d.f.} = 1, P = 0.38 \)) (Figure 1D).

The variation in infestation data of the three leaf infestation parameters (no. galls per leaf, larval stage, and no. eggs per adult) was divided among the independent factors ‘grape variety’ and ‘phylloxera biotype’, verifying the legitimacy of using these parameters for biotyping (Table 1). Two-way ANOVA showed that the variation within the parameter gall intensity was best described by grape variety and, more importantly, also by phylloxera biotype. Larval stage was best explained by phylloxera biotype, and eggs per adult by grape variety and the interaction effect of grape variety and phylloxera biotype. The overall MANOVA showed that all sources of variation were described best when looking at the combined interaction effect of all infestation parameters (Table 1).
Temperature experiment

In the temperature trials, all inoculated plants were successfully infested. From 67% of the infested vines, the intended 30 infested leaves per plant could be harvested, resulting in a total of 1373 analyzed leaves. At the end of the experiment, the infestation frequency (i.e., percentage of infested leaves per plant) was similar for both grapevine species: 64% of all 5C and 54% of all RR leaves were infested by TT1 (t = −1.497, d.f. = 8.4, P = 0.17), and 61% of both 5C and RR leaves were infested by B6 (t = −0.146, d.f. = 19.3, P = 0.89). The infestation frequency was also similar for the trials at both temperatures (t = −1.623, d.f. = 40.8, P = 0.11).

A multivariate analysis showed that all factors – that is, host plant variety, grape phylloxera biotype, and temperature – affected the infestation parameters (Table 2). Three-way ANOVA showed that the number of galls per leaf was differentiated by the independent factors.
temperature, phylloxera biotype, and their interaction effect (and was therefore a valid infestation parameter for the hypotheses of this study). Larval stage was overall the strongest infestation parameter, being differentiated by all main and interaction effects of the three factors, and the strongest parameter to differentiate phylloxera biotypes. Eggs per adult differentiated for the factors temperature and grape variety, but not for phylloxera biotype. MANOVA showed that the overall effect of these three infestation parameters was differentiated for all main factors and interaction effects, except the triple interaction effect (host plant variety*phylloxera biotype*temperature) (Table 2).

In more detail, at 22 °C, B6 produced more galls on RR than on 5C (t = 3.039, d.f. = 252.5, P = 0.003) and TT1 produced more galls on 5C than on RR (t = −3.061, d.f. = 183.6, P = 0.003) (i.e., both biotypes performed best on their original field host plant varieties). Interestingly, for both biotypes, at 27 °C, infestation increased on 5C and decreased on RR. Thus, at 27 °C there were even more TT1 galls on 5C than on RR (t = −5.452, d.f. = 134.3, P<0.001), whereas the amount of B6 galls on both cultivars was similar (t = −0.909, d.f. = 329.2, P = 0.36) (Figure 2A). For TT1, at 22 °C, larvae were further developed on 5C than on RR (t = −15.119, d.f. = 456.4, P<0.001). Temperature increase had no effect on development on 5C (t = 0.614, d.f. = 179, P = 0.54), but slowed down development on RR (t = 9.3, d.f. = 211.9, P<0.001). For B6, at 22 °C, larvae developed equally well on both varieties (t = 0.331, d.f. = 304.8, P = 0.74). Just like for TT1, temperature increase had no effect on development on 5C (t = −0.725, d.f. = 281.5, P = 0.47), but slowed down development on RR (t = 2.021, d.f. = 261.4, P = 0.044). Therefore, larvae reached a later developmental stage on 5C than on RR at 27 °C (t = −2.421, d.f. = 243.5, P = 0.016) (Figure 2B). Overall, the number of eggs per adult was higher on 5C than on RR for both TT1 (t = −5.479, d.f. = 252.1, P<0.001) and B6 (t = −4.896, d.f. = 349.8, P<0.001). At 27 °C, B6 adults laid fewer eggs than at 22 °C on RR (t = 8.082, d.f. = 165.8, P<0.001) and on 5C (t = 4.95, d.f. = 151.7, P<0.001), and TT1 adults laid fewer eggs at 27 °C than at 22 °C on RR (t = 4.025, d.f. = 17.6, P = 0.001), but a similar number of eggs on 5C (t = 0.694, d.f. = 169.6, P = 0.49) (Figure 2C).

Though only leaves were inoculated, root infestation due to migrating larvae was observed on all inoculated plants. Overall, the total amount of nodosities per plant was higher for B6 than for TT1 (t = −2.404, d.f. = 41.8, P = 0.021), and higher on 5C than on RR for both TT1 (t = −2.92, d.f. = 6.1, P = 0.026) and B6 (t = −4.714, d.f. = 12.6, P<0.001). For both temperatures, the number of nodosities per plant was similar for TT1 (t = −0.304, d.f. = 15.6, P = 0.77) and B6 (t = −1.316, d.f. = 15.4, P = 0.21) (Figure 2D). The root infestation parameter was based on infestations per plant, whereas the leaf infestation parameters were based on infestations per leaf (for a total of 30 leaves per plant), which lowered the parameter’s sample size and hence increased the standard error.

Measuring general plant growth parameters revealed that the total amount of leaves was higher for RR (mean ± SE = 111 ± 5 leaves) than for 5C (74 ± 4) and was not affected by phylloxera infestation (t = −0.299, d.f. = 52.2, P = 0.77). The total plant dry weight (leaves, shoot, and roots) was similar for RR (19.9 ± 1.1 g) and 5C (19.8 ± 1.3 g). The leaf fresh weight of 5C (0.659 ± 0.027 g) was higher than RR (0.444 ± 0.016 g). When the infestation parameters were standardized by the fresh weight of the measured leaf, TT1 performed equally well on both plant varieties, whereas B6 had an advantage on the leaves of RR (V. vinifera) over those of 5C (Figure 3A–C). This diverged from the general view on these biotypes in grape phylloxera literature. Root infestation levels between grape varieties also became more similar when standardized by root dry weight, but 5C still

### Table 2 Results of ANOVA and MANOVA (last columns) for three leaf gall characteristics of grape phylloxera (Daktulosphaira vitifoliae) on grape for the temperature experiment

| Source                  | d.f. | No. galls per leaf | Larval stage | No. eggs per adult | MANOVA       |
|-------------------------|------|--------------------|--------------|-------------------|--------------|
|                         |      | F    | P    | F    | P    | F    | P    | λ    | F    | P    |
| Grape variety (GV)      | 1,687| 0.09 | 0.76 | 20.52| <0.001| 46.36| <0.001| 0.92 | 19.54| <0.001|
| Temperature (T)         | 1,687| 37.58| <0.001| 19.30| <0.001| 27.91| <0.001| 0.91 | 22.95| <0.001|
| Phylloxera biotype (PB) | 1,687| 26.41| <0.001| 29.37| <0.001| 0.89 | 0.35  | 0.94 | 15.52| <0.001|
| GV*T                   | 1,687| 1.39 | 0.24 | 13.16| <0.001| 0.54 | 0.46  | 0.98 | 4.46 | 0.004 |
| GV*PB                  | 1,687| 2.53 | 0.11 | 13.33| <0.001| 1.70 | 0.19  | 0.97 | 6.44 | <0.001|
| T*PB                   | 1,687| 8.48 | 0.004| 10.25| 0.001| 3.09 | 0.079 | 0.97 | 7.04 | <0.001|
| GV*T*PB                | 1,687| 0.14 | 0.71 | 5.42 | 0.020| 2.63 | 0.11  | 0.99 | 2.33 | 0.073 |
contained higher amounts of nodosities than RR at both 22 °C \((t = -4.124, \text{ d.f.} = 21.8, P < 0.001)\) and 27 °C \((t = -4.432, \text{ d.f.} = 8.6, P = 0.002)\) (Figure 3D).

When leaf and root dry weight were separately analyzed in a multivariate analysis, temperature showed the strongest effect on plant growth (Table 3). For root dry weight, an interaction effect was detected between temperature and phylloxera infestation (i.e., root dry weight was only higher at 22 °C when plants were not infested with phylloxera). For leaf dry weight, a similar trend was visible. Moreover, when combining leaf and root dry weight, the multivariate analysis showed an effect of phylloxera infestation [i.e., phylloxera infested vines had increased leaf dry weight (from 8.2 ± 0.6 g to 9.2 ± 0.5 g) and decreased root dry weight (from 2.0 ± 0.3 g to 1.6 ± 0.1 g)].

**Discussion**

The effect of climate change on the interaction between pest insects and their cultivated host plants varies greatly between agricultural regions (Lehmann et al., 2020). Understanding individual drivers within this complex helps to explain these observed differences. We here isolated the driver ‘temperature increase’ for the interaction between the phytophagous insect grape phylloxera and two varieties of its grapevine host plant. Based on our standardized biotyping assay and a temperature increase simulation, where individual infestation parameters were taken from 1373 infested leaves, we confirm that temperature increase can have both an increasing and a decreasing effect on the severity of grape phylloxera outbreaks. Both inherent phylloxera infestation traits and host plant variety are fundamental in explaining the effects of temperature on grape phylloxera pest intensity.

The biotyping assay, which was conducted to elucidate inherent differences in host plant-specific infestation traits, showed similar patterns as the temperature experiment at 22 °C. Specifically, the *V. vinifera*-adapted grape phylloxera SFL B6 created a higher number of galls per leaf on RR than on 5C, whereas the American rootstock-adapted SFL TT1 created more galls on 5C than on RR (i.e., both biotypes performed best on
their original field host plant varieties). Larval development was also similar in both experiments; B6 larvae reached a later developmental stage than TT1 larvae on RR. Also, the number of eggs per TT1 adult was higher on 5C than on RR in both experiments. In the temperature experiment, both phylloxera lines perform superiorly on 5C roots compared with RR roots; a similar trend was visible in the biotyping assay. When these results are inserted into the biotype classification table of Forneck et al. (2016), the control biotype TT1 can correctly be classified as biotype C, and B6 can be classified as the V. vinifera foliage-adapted biotype G. This is the first study that classified a phylloxera line into biotype G using a controlled environment, controlled infestation, standardized host plants, and an internal standard biological control.

Table 3 Results of ANOVA and MANOVA (last columns) for two characteristics — leaf and root dry weight — of grape plants infested or not with grape phylloxera (Daktulosphaira vitifoliae) for the temperature experiment

| Source                        | Leaf dry weight | Root dry weight | MANOVA |
|-------------------------------|-----------------|-----------------|--------|
|                               | d.f.            | F               | P      | F     | P    | λ    | F | P |
| Grape variety (GV)            | 1,70            | 1.80            | 0.18   | 0.35  | 0.56 | 0.97 | 0.91 | 0.41 |
| Temperature (T)               | 1,70            | 4.11            | 0.047  | 5.58  | 0.018 | 0.91 | 3.22 | 0.047 |
| Phylloxera infestation (PI)   | 1,70            | 2.16            | 0.15   | 2.68  | 0.11  | 0.86 | 5.41 | 0.007 |
| GV*T                          | 1,70            | 0.30            | 0.59   | 1.15  | 0.29  | 0.96 | 1.51 | 0.23 |
| GV*PI                         | 1,70            | 0.60            | 0.44   | 0.00  | 0.99  | 0.99 | 0.44 | 0.65 |
| T*PI                          | 1,70            | 1.72            | 0.19   | 4.37  | 0.040 | 0.94 | 2.17 | 0.12 |
| GV*T*PI                       | 1,70            | 0.01            | 0.91   | 0.02  | 0.89  | 1    | 0.04 | 0.96 |

Figure 3 Effect of temperature increase, standardized for plant growth parameters — leaf fresh weight (FW) or root dry weight (DW) — on characteristics of two grape phylloxera lines, TT1 and B6, on the grape varieties Teleki 5C and Riesling (RR): mean (± SE) (A) number of galls per leaf (n = 117-180), (B) larval stage in leaf galls (n = 96-164), (C) number of eggs per adult in leaf galls (n = 11-126), and (D) number of nodosities (root galls) on plant roots (n = 6). TT1 is accustomed to 5C, B6 is supposedly accustomed to RR.
Using multivariate analyses, the use of the chosen infestation parameters was statistically validated. In both the biotyping and temperature experiments, larval stage was the strongest dependent variable to differentiate between phylloxera biotypes. Number of galls per leaf also differentiated between biotypes in both experiments, and also distinguished temperature treatments. Eggs per adult was the weakest variable and did not distinguish biotypes. We here showed that our experimental set-up, using a combination of leaf gall intensity, phylloxera development, and egg count per adult measurements, in the isolation chambers of Forneck et al. (2001c), proves to be a robust set-up for biotype assays based on leaf infestation. However, the results also showed that individual infestation parameters do not always yield the exact same results for infestation success and that a relative comparison with an internal control line is crucial for the correct classification of biotypes. Especially for the biotyping assay, which had a lower sample size than the temperature experiment, it was important to combine several dependent variables to distinguish biotypes.

Especially ‘egg count per adult’ deviates from the other two measured infestation parameters. This can be expected from the data acquisition limitations described before. Briefly, the experimental set-up does not allow for a continuous observation of egg laying and hatching over time and relies on a single destructive observation. The parameter ‘egg count per adult’ is therefore less precise for leaf infestation measurements. The overall decrease of counted eggs at 27 °C was not in line with the other two parameters. This decrease could be explained by faster hatching (a shorter developmental time) of eggs, which was also visible in a study with root-feeding phylloxera reared on excised roots at various temperatures (Fisher & Albrecht, 2003).

Comparing the 22 and 27 °C temperature trials, gall intensity and larval development show the same tendencies for both phylloxera lines: the parameters increase with temperature on 5C and decrease on RR. Therefore, unlike the amplifying effect of temperature for TT1, increased temperature diminishes B6’s biotype-specific host plant infestation traits. This is in line with a similar study on soybean aphids (Aphis glycines Matsumura), where increased temperatures had a positive effect on their abundance on susceptible soybean [Glycine max (L.) Merr.] varieties and a negative effect on their abundance on resistant ones (Whalen & Harmon, 2015). These findings were partly attributed to the accelerating effect of a higher temperature on infestation parameters (i.e., soybean aphids would die either way on resistant soybeans, but this process is accelerated for high temperatures). In our experiments and commercial vineyard observations, however, we do not see such a decline in fitness when phylloxera biotype G lives for subsequent generations on V. vinifera leaves. In another study on host-specific feeding traits, the survivability differences of the butterfly Polygonia c-album L. across three host plant species increased with higher temperatures (Braschler & Hill, 2007). For the same butterfly species, an increased difference was observed in larval growth rate at higher temperatures, increasing more on preferred than on less preferred hosts. This was attributed to the quality of host plant material (i.e., C:N-ratio, water content, and tannin concentration) (Audusseau et al., 2013). Through the creation of nutritional plant cells, grape phylloxera is less prone to direct changes in feeding material quality (Powell et al., 2013). A temperature-dependent production of secondary plant metabolites could, however, indirectly influence nutritional quality. An explanation can thus be sought at temperature-dependent alterations at the side of the insect and of its host plant.

For grape phylloxera, energy requirements for insect metabolism may play a role. Metabolism increases exponentially with temperature increase (Gillooly et al., 2001). When ambient temperatures reach the insect’s maximum, reduced performance can be traced back to energy demands that exceed intake (Lemoine et al., 2014). An ambient temperature of 27 °C is known to be the upper limit for optimal grape phylloxera infestation (Powell et al., 2013). It could therefore be that grape phylloxera is not able to acquire adequate energy at higher temperatures when the host plant is less suitable. This may explain why B6 only performs well on RR (V. vinifera) when temperatures are moderate; the acquired ability to infest the leaves of V. vinifera more successfully than other biotypes may have led to an increased metabolic energy demand. In a recent study on the grape phylloxera transcriptome, 44% more differentially expressed genes were upregulated during root-feeding on RR by a V. vinifera-adapted biotype, compared to root-feeding on 5C by a rootstock-adapted biotype (Savoi et al., 2020). These differences were especially pronounced among effector candidates, of which twice as many differentially expressed genes were upregulated when infesting RR, compared to 5C. In a comparative study among pea aphid (Acyrthosiphon pisum Harris) biotypes, those specialized to infest alfalfa, Medicago sativa L. (specific host plant) had many more upregulated differentially expressed candidate salivary effector genes than those specialized to infest pea, Pisum sativum L. (general host plant) (Boulain et al., 2019). These differences were much more specific to pea aphid biotype and genetic line than to the host plant species that was infested. An inherently altered metabolic energy usage of biotype G compared to biotype C may have led to the observed lower
foliar infestation performance on the vulnerable host 5C at higher temperatures.

Besides looking at the insect alone, a reason for our key findings may reside at the side of the host plant. In a study on phylloxera root infestation, local genetic expression for jasmonic acid production increased during insect probing and then decreased at the start of gall formation, to lower levels than the expression in non-infested root tips (Eitile et al., 2019). From this point on, local jasmonic acid and jasmonic acid isoleucine concentrations per amount of plant fresh weight stayed at low levels, whereas high levels of jasmonic acid precursors and a buildup of salicylic acid were observed. In contrast, after foliar infestation, there was an increased expression in mature leaf galls of genes related to jasmonic acid production and the breakdown of salicylic acid. Furthermore, an increased expression of the shikimate and phenylpropanoid pathways was revealed, as well as a reduction in the non-mevalonate pathway in leaf gall tissue (Nabity et al., 2013). Unfortunately, studies on the actual buildup of secondary metabolites in leaf gall tissue are still lacking. Generally, elevated temperature increases plant defense by enhancing plant salicylic acid and jasmonic acid, as well as ethylene production (DeLucia et al., 2012). It is, therefore, still unclear what effects a higher temperature has on grapevine’s defense mechanisms against phylloxera leaf infestation.

In other insect–plant studies, herbivore-specific genes are known that induce resistance, for example, soybean resistance to aphids depends on Rag-genes. In a soybean aphid study, infestation was only impeded (in terms of reduced aphid survival and slower development) on resistant soybean varieties when temperatures were elevated (Hough et al., 2017). These results were attributed to temperature-dependent Rag1 resistance against aphids. In contrast, in another infestation study, several wheat cultivars lost their resistance to the Hessian fly, Mayetiola destructor (Say), at higher temperatures (Chen et al., 2014). Though a temperature-dependent activation of resistance genes was suspected, the exact process was still unclear. Similarly, for our case, it is possible that besides the role of the jasmonic and salicylic acid interplay, putative temperature-dependent genes for grape phylloxera resistance play a role in resistance to leaf galls.

When the infestation parameters used in this study are normalized by the fresh weight of the measured leaf, an apparent shift takes place. The classical view in phylloxera literature is that biotype C performs better and biotype G performs equally well on the leaves of American rootstock hybrids, compared with the leaves of V. vinifera (Forneck et al., 2016). Though having a similar total plant weight, the relatively bigger 5C leaves lower the infestation values relative to individual leaf fresh weight, compared with the smaller leaves of RR. When the difference in leaf size of these plant species is considered, we observed that the infestation performance of biotype C is similar on both grape varieties, whereas performance of biotype G is better on RR than on 5C (at moderate temperatures). The infested leaf’s vigor may therefore be a factor that explains differences in grape phylloxera between and within plant species. A field study on the correlation between grape phylloxera infestation and grapevine shoot vigor confirms this hypothesis on an intra-specific scale (Kimberling et al., 1990). Whether this also plays a role for infestation success in an inter-specific grapevine comparison still requires further research. Taken together, however, we have shown the importance of incorporating individual leaf weight to reduce the risk of experimental errors, when comparing phylloxera leaf infestation between host plant genotypes.

Examining leaf and root growth together, our experiments revealed an infestation-dependent change. Phylloxera infestation caused total leaf dry mass to increase relative to its root dry mass. This disequilibrium in water uptake and evaporation tissue may lead to problems during drought periods. Field observations and experiments attest to this hypothesis, revealing that grapevine phylloxera tolerance decreases in dry years (McLeod, 1990) and on compact shallow soils that restrict rooting (Nougaret & Lapham, 1928). A recent study that investigated the combined effects of drought and phylloxera root infestation showed that root dry weight of RR grafted onto 5C was only reduced during drought, when the roots were infested with phylloxera (Savi et al., 2019).

For the temperature experiments, only leaves were inoculated. Still, the roots of almost all inoculated grapevines of both host plant cultivars were infested at the end of the experiment. This contrasts with laboratory results with Petri dishes, where at L16:D8 photoperiod, gallicole phylloxera that hatched from gallicole eggs did not lay radicole (root-galling) eggs at both 23 and 28 °C (Rilling, 1964). These findings show that besides environmental factors, grape phylloxera leaf-to-root migration is likely to also be influenced by population dynamics and host plant factors. In Australia, such migrations were also observed to take place in vineyard settings throughout the growing season (Herbert et al., 2006). More research is needed to further identify these factors and enable migration forecasts for pest control management.

Finally, the temperature experiments showed that host plant-specific performance of phylloxera biotypes not always alter in the same way due to temperature increase. To enable a comparison between studies and reduce intrabiotype variability, future biotyping assays should be performed at a standard temperature. We see here that
temperatures of 22 °C generate similar results as the field observations in our study area. Moreover, further infestation experiments at more temperatures are needed to identify the temperature optimum for leaf infestation of various biotype and host plant combinations.

Summarizing and extrapolating our simulations to field settings, the biotyping assay and temperature experiment at 22 °C generated similar results as the field observations in Baden/Germany. At 27 °C, both the V. vinifera-adapted biotype G and the American rootstock-adapted biotype C showed the same tendency: higher temperatures increased infestation parameters on 5C and lowered them on RR. The comparative advantage for RR infestation that B6 had at moderate temperatures is apparently not as effective at high temperatures. Furthermore, this experiment was conducted at high relative humidity. Laboratory experiments that simulated larval migration to the grapevine’s shoot tip for leaf gall initiation suggested that, especially at higher temperatures, grape phylloxera is more vulnerable to a dryer environment (McLeod, 1990). However, in the more secluded belowground environment, drought can produce contrary results for grape phylloxera (Savi et al., 2019). Even so, based on our simulations, it is not likely that a temperature shift from 22 to 27 °C will increase foliar infestation in commercial vineyards with V. vinifera scions. On the other hand, grape phylloxera population increase on the foliage of American rootstocks may be more severe due to climate warming. Additional factors, like the length of the growing season and abrupt temperature fluctuations, should also be included to determine the regional effects of a warming climate for grape phylloxera infestation severity. Moreover, to assess whether gall formation in V. vinifera vineyards will be more abundant in the future, due to the dispersal of biotype G, a genetic study on grape phylloxera population dynamics should be conducted.

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Data Availability Statement

The data that support the findings of this study are available in the supplementary material of this article.

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Supporting Information
Additional Supporting Information may be found in the online version of this article:
Tables S1-S4 Complete dataset, file: Dataset EEA-2021-0016.xlsx