

Indications for rootstock related ecological preferences of grape phylloxera (*Daktulosphaira vitifoliae* Fitch)

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Summary

By parasitizing the roots of *Vitis* species, grape phylloxera (*Daktulosphaira vitifoliae* Fitch, Phylloxeridae) is one of the most devastating pests in viticulture. Grafting susceptible scions (*Vitis vinifera*) to tolerant *Vitis* rootstocks is a common practice to control grape phylloxera in wine growing regions worldwide. However, grape phylloxera populations still develop on the roots of most grafting combinations. Questions remain as to whether or not the impact of environmental factors on grape phylloxera population dynamics is related to *Vitis* rootstock cultivars. In the presented field study, we investigated the influence of two closely related mature *Vitis berlandieri* x *Vitis riparia* rootstock varieties on the relations between the dynamics of root feeding grape phylloxera populations, root morphology and abiotic factors. The investigation took place on a commercial vineyard in Geisenheim/Germany on Teleki 5C/*V. vinifera* 'Weisser Riesling' and Kober 125AA/*V. vinifera* 'Weisser Riesling'. Samples of roots, attached grape phylloxera populations and soil were taken in the field 19 times between August 2007 and August 2009. Grape phylloxera population structure was recorded by the occurrence of root-feeding wingless females, fundatrices and nymphs and the pigmentation and position of root galls were assessed. Root morphological parameters were assessed using WinRhizo Pro. Soil abiotic parameters were assessed in the laboratory. Results of a principal component analysis showed rootstock related differences considering the impact of abiotic factors on grape phylloxera population structure. Especially soil temperature and soil organic matter were indicated to have a lower impact on grape phylloxera population structure on roots of 5C than on roots of 125AA. Our data indicate that ecological factors have a lower impact on the development of grape phylloxera on more supportive rootstocks.

Key words: *Vitis berlandieri* x *Vitis riparia*; insect population dynamics; Phylloxeridae, rootstocks.

Introduction

It is widely accepted that coevolutionary processes such as host switches can lead to host-associated ecologi-

cal traits in herbivorous insects (SINGER 1982, SINGER and McBRIDE 2012, SEVERNS and BREED 2014). Particularly resource mediated processes are known to influence the ecological dynamics of plant feeding insects (MORET and SCHMID-HEMPEL 2001, COTTER *et al.* 2011, SINGER *et al.* 2014). These adaptive processes are suggested to lead to different ecological responses of a parasite, depending on their host and nutrition (COTTER *et al.* 2011). Grape phylloxera (*Daktulosphaira vitifoliae* Fitch) is a root and leaf sucking phylloxerid with its native host range on native North and Central American *Vitis* species (WAPSHERE and HELM 1987). Most of the American *Vitis* species are considered to be tolerant or resistant to grape phylloxera, due to their coevolution with the parasite (DOWNIE *et al.* 2001). However, the overwhelming part of berry and wine production throughout the world relies on European *Vitis vinifera* cultivars. With introduction of grape phylloxera to Europe in the 19th century, it became the most devastating pest in viticulture within less than a decade (BIADA-MIRO *et al.* 2010). Nowadays, in most wine growing regions grape phylloxera populations are controlled by grafting tolerant American rootstocks cultivars to *V. vinifera* scions (POWELL *et al.* 2013). On such grafted combinations, grape phylloxera only develops a belowground lifecycle and does not parasitize leaves of the scion (FORNECK and HUBER 2009). But up to today, root feeding grape phylloxera morphotypes are predominant in grafted vineyards world-wide, representing a permanent soilborne stress factor for grapes (POWELL *et al.* 2013).

Although factors such as temperature (TURLEY *et al.* 1997), host variety (GRANETT *et al.* 2007), root gall development and crowding (HOFFMANN *et al.* 2015) or grape phylloxera genotype (HERBERT *et al.* 2010) are known to impact performance and dynamics of grape phylloxera populations, little is known about the impact of the host plant on ecological responses of grape phylloxera. The objective of this study was to investigate the influence of closely related mature rootstocks on the relations between the dynamics of root feeding grape phylloxera populations, root morphology and abiotic factors. For several reasons, we decided to address this objective in the field. Root systems of perennial plants change their physiology and biochemistry over time (HODGE 2009). Due to the size of grape root systems, trials in pots or growth chambers would target younger plants and would restrict the growth of a mature grape root system, which can grow more than 6 m deep (RICHARDS 1983). Grape phylloxera was found

in several meters of depth (POWELL *et al.* 2013). To paint a picture close to the agricultural conditions in a vineyard, we investigated a local grape phylloxera population on a low sloped, commercial vineyard in Germany. Neither productivity nor growth of the vines were affected by the local grape phylloxera populations (HUBER *et al.* 2007). The vineyard was planted with Teleki 5C/*V. vinifera* 'Weisser Riesling' and Kober 125AA/*V. vinifera* 'Weisser Riesling'. 5C and 125AA are widely used rootstocks in viticulture. Both rootstocks are based on *Vitis riparia* x *Vitis berlandieri* crossings and are considered as tolerant to grape phylloxera (GRANETT *et al.* 1996).

Material and Methods

Study site: The study site is located in the wine-growing district of the Upper Rheingau in Germany and is part of the location "Burgweg/Moenchspfad" in Geisenheim/Germany (N 49 59.748', E 007 57,105') on 157 m height (± 9.3 m) above sea level. The soil is a Rigosol (terrestrial Kulturosoles). The parent rock material is formed by alluvial gravel in the subsoil and loess, partly with alluvial gravel in the Rigosolhorizon. In the subsoil, the type of soil is sand, gravel and sandy clay (concentration of lime 0-15 %). In the Rigosol horizon, the type of soil is a sandy clay without a measurable concentration of lime (FRIEDRICH and SABEL 2004).

The study site is a commercially used and conventionally managed vineyard, planted in 1985 with *Vitis vinifera* 'Weisser Riesling' grafted on Teleki 5C (*V. berlandieri* x *V. riparia*) and Kober 125AA (*V. berlandieri* x *V. riparia*) rootstocks. Teleki 5C/*Vitis vinifera* 'Weisser Riesling' is planted in rows one to 32, Kober 125AA/*Vitis vinifera* 'Weisser Riesling' is planted in rows 33 to 56. The total area of the vineyard is 2500 m², the row distance is 200 cm, distance between single vines is 135 cm. The vineyard is located on a flat location (slopes between 0-4 degrees). During this study, the vineyard was fertilized with manure (cow dung, 40 t·ha⁻¹) in August 2008. Grape phylloxera was first detected on the study site 1990.

Sampling: Grape phylloxera, root and soil samples were collected 19 times between August 2007 and August 2009 (n = 10 per rootstock, Table). The sampling based on a random design within each rootstock (5C, 125AA). All samples from the upper soil layer up to 20 cm depth were collected next to a vine and each plant was sampled only once during the whole study. Roots were extracted with a

quadrangular metal box (2 L volume) by using a hammer to push the metal box into the soil. Roots and attached grape phylloxera were stored in water at 7 °C for transportation. To assess abiotic and biotic soil conditions, separate soil samples were taken of the top 20 cm soil layer and stored in plastic bags at 7 °C for transportation. All samples were stored in the laboratory at 4 °C until processing.

Parameter assessment: Root samples were investigated under a microscope for grape phylloxera population and gall formation within the following days after the field sampling. Grape phylloxera instars were classified into root feeding wingless females, fundatrices and nymphs. The number of root galls was characterized according to their pigmentation. In subsamples, the pigmentation of root galls was estimated by visual survey. Afterwards, the same subsamples were analyzed with the color analysis function of Win Rhizo Pro V2005b (Regent Instruments, Nepean, Canada). Color classes were adjusted until the amount of detected root galls in one color class fitted to the previously assessed estimate (HOFFMANN *et al.* 2011). Three pigmentation classes were established: ld (light pigmentation), md (brown pigmentation) and hd (dark pigmentation). The assessed color ranges in each class were printed on PVC and used for the visual survey of the rest of the root samples. Additionally, the position of a root gall in the root system (terminal, not terminal) was recorded. After the visual survey, root samples were gently washed under tap water. Root surface area [cm²] and root diameter [mm] were assessed with Win Rhizo Pro V 2005b (Regent Instruments, Nepean, Canada).

To assess abiotic soil parameters, all soil samples were sieved through a 5 mm mesh. Soil water content [%] was assessed by weight before and after drying (105 °C / 24 h). Soil pH was determined with a pH Meter, 30 min after suspending 10 g of soil sample in 25 ml of 0.01 M CaCl₂ solution. Soil organic matter (SOM) [%] was assessed by detection of the loss of ignition (LOI) after the incineration of dry (105 °C) soil samples (400 °C / 24 h).

Following abiotic environmental parameters for the location Geisenheim were provided by Deutscher Wetter Dienst (DWD): hourly soil temperature in -10 cm [°C], precipitation per day [mm]. We calculated daily mean temperatures and monthly precipitation. Coordinates of the measuring unit were 49° 58' 59" N, 7° 57' 00" E.

Statistical analysis: To illustrate the mean monthly development of the root surface area, mean values of root surface were calculated. Moreover, monthly densities of the grape phylloxera population were calculated per cm² root surface. Crowding effects were calculated by counting the neighbors of an individual phylloxerid per root gall (see also HOFFMANN *et al.* 2015). To indicate differences in grape phylloxera population densities between 5C and 125AA, a Kurskal Wallis ANOVA was conducted ($\alpha = 0.05$).

To indicate the impact of rootstock variety on the dynamics between grape phylloxera population, root gall pigmentation and position, root morphology, soil and environmental parameters, principal component analyses (PCA) were conducted and eigenvalues were extracted. Prior to all PCAs, the data were log-transformed. Graphical illus-

Table

Number of samples per month, evenly distributed among the rootstocks '5C' and '125AA'

Year	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov
2007	-	-	-	-	-	10	-	10	-
2008	20	20	20	40*	20	40*	20	20	20
2009	20	20	20	20	20	20	-	-	-

* two sample dates per months.

trations and descriptive statistics were calculated in Excel 2010 (Microsoft, Redmond, WA). All analytical calculations were conducted in R 3.0.2 (www.r-project.com).

Results and Discussion

We investigated the influence of two mature rootstocks on relations between the dynamics of grape phylloxera population, root system and abiotic factors. For this purpose, we examined mature *Vitis berlandieri* x *V. riparia* rootstocks in the field (Teleki 5C/'Weisser Riesling' and Kober 125AA/'Weisser Riesling'). In our study we analyzed a two year dataset (with 19 different sampling times) in terms of possible correlations between abiotic soil and environmental factors, root surface area, root gall and grape phylloxera abundance. Studies suggest that 5C is the more supportive rootstock (GRANETT *et al.* 1996, RITTER *et al.* 2007). To answer the questions whether or not grape phylloxera population densities differ between rootstocks,

we analyzed seasonal dynamics of root surface and population densities in both rootstocks (Fig. 1 A-B). Root surface did not differ between rootstocks (Fig. 1 A), and also the seasonal dynamics of fundatrix and nymph densities did not differ between 5C and 125AA (data not shown). But 5C provided significant higher population densities of wingless females than 125AA (Fig. 1 B; Kruskal-Wallis $\chi^2 = 4.36$; $p = 0.037$). The main population increase was observed between May and July, with a second peak of wingless females in October (see also HOFFMANN *et al.* 2015). By observing only one rootstock, OMER *et al.* (1997) and PORTEN and HUBER (2003) observed similar dynamics in grape phylloxera population development in earlier field studies. Both studies were positive about the impact of temperature on grape phylloxera population dynamics. To investigate crowding of wingless females, we counted the number of neighbors per root gall on 5C and 125AA rootstocks. Wingless females tend to crowd more on 5C than on 125AA, which leads to higher overwintering rates in 5C than on 125AA (HOFFMANN *et al.*, 2015). Especially in

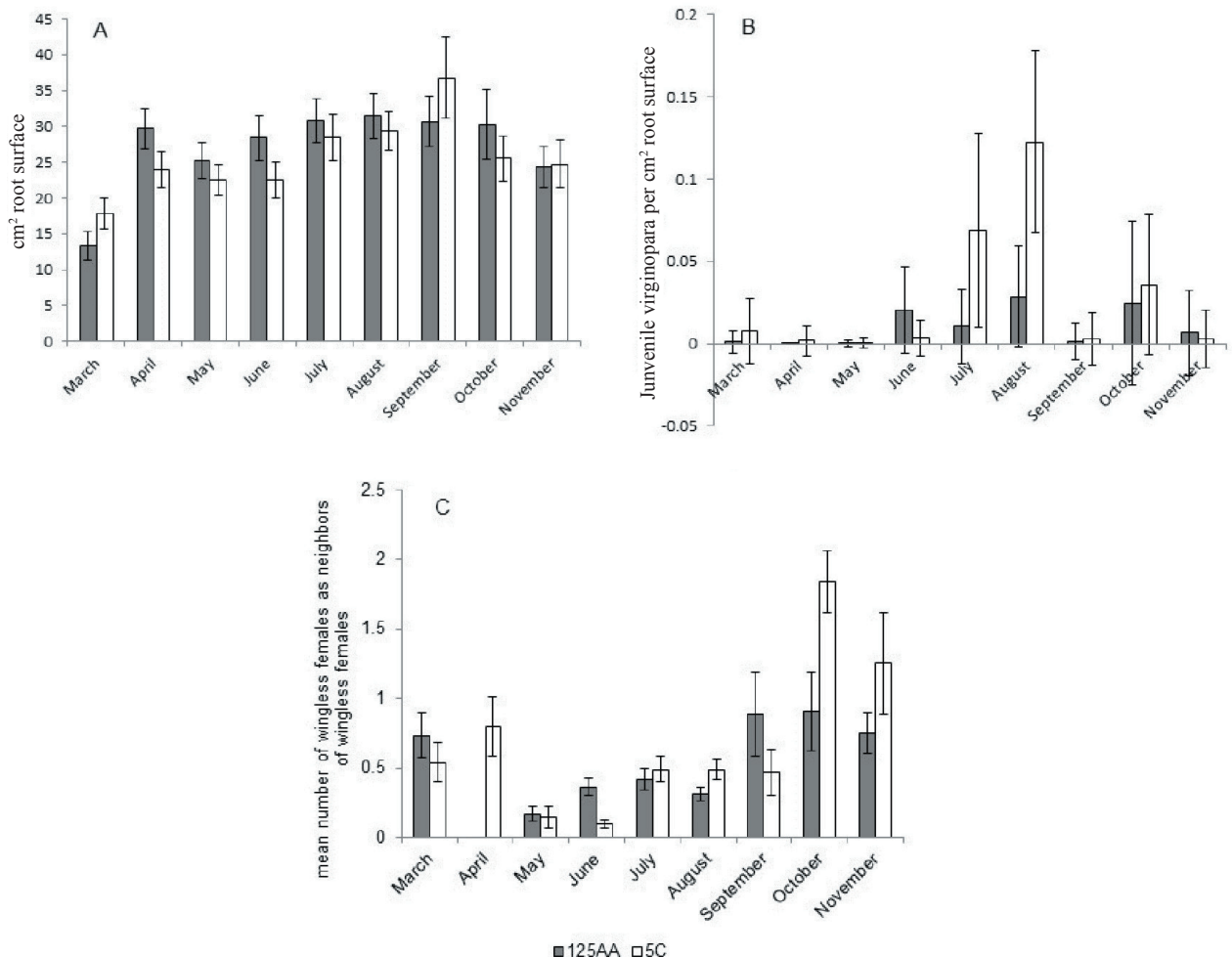


Fig. 1: **A:** Monthly development of root surface between 0 and 0.5 mm diameter (mean \pm SE). Similar amounts of root surface were measured in 125AA (grey) and 5C (white) in every month. **B:** Monthly measurements of wingless females per cm² root surface area (mean \pm SE). 5C (white) provided significant higher rates of wingless females than 125AA (grey) (Kruskal-Wallis $\chi^2 = 4.36$; $p = 0.037$). Although no significant effects could be calculated in a TukeyHSD post hoc test ($p = 0.05$), 5C showed a higher tendency of wingless females per cm² root surface in July and August than 125AA. **C:** Crowding of wingless females per root gall (mean \pm SE); numbers of neighbors per wingless female are shown. Significant effects of month ($p < 0.001$, $F = 10.78$, $df = 10$) and month combined with rootstock ($p = 0.048$, $F = 1.00$, $df = 8$) were calculated in a two way ANOVA. No direct significant differences between month/rootstock could be calculated (TukeyHSD, $p = 0.05$). But wingless females tend to crowd more on 5C (white) in April, October and November.

the months October and November higher crowding rates of wingless females were observed on 5C than on 125AA (Fig. 1 C).

However, to indicate differences in the effects of rootstocks on the relations between root gall development, grape phylloxera population structure and abiotic soil and environmental parameters, we performed separated principal component analyses (PCA) for both rootstocks ('5C', '125AA'; Fig. 2 A-D). First, we analyzed the assessed root

gall parameters (pigmentation and position) with abiotic and environmental parameters and the surface area in different root diameter classes for both rootstocks (Fig. 2 A, B). The results of both analyses were very similar. 47.13 % of total variance was explained by the first two extracted dimensions in 125AA (Fig. 2 A) and 46.29 % of the total variance was explained in 5C (Fig. 2 B). In both rootstocks, soil water content (SWC) and precipitation did not group with either root surface, root gall or other abiotic pa-

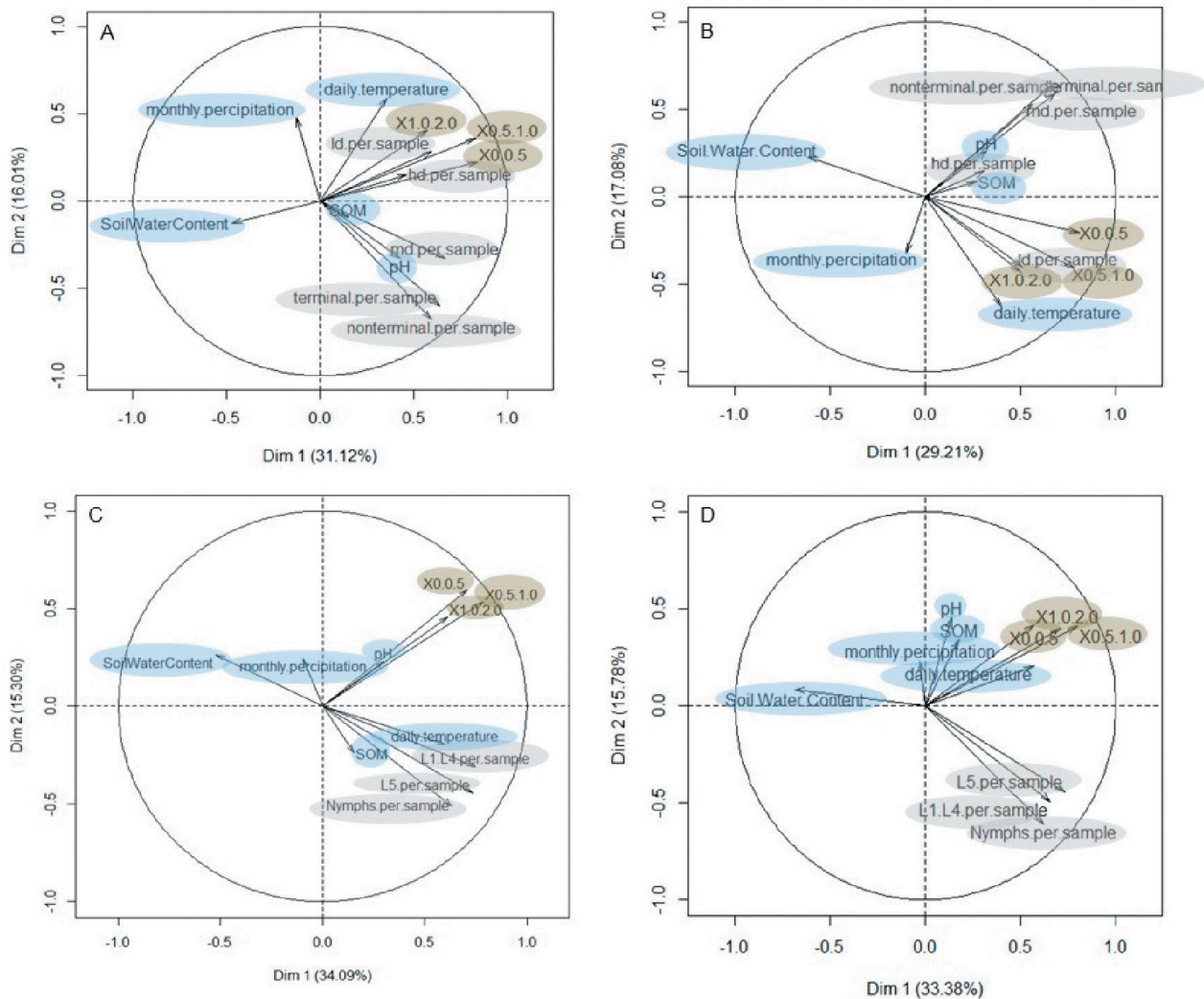


Fig. 2: Factorial maps of the conducted Principal Component Analyses (PCA). Abiotic factors are marked in blue (Soil Water Content, monthly precipitation, daily temperature (soil), SOM (= soil organic matter), pH (= soil acidity)). Root morphological parameters are marked in brown (X0.0.5 = surface of roots < 0.5 mm diameter; X0.5.1.0 = roots between 0.5 and 1 mm diameter; X1.0.2.0 = roots between 1 and 2 mm diameter). Grape phylloxera population related parameters are marked in grey (terminal per sample = amount of terminal root gall in a 2 L soil sample, nonterminal.per.sample = amount of nonterminal root galls in a 2 L soil sample, ld per.sample = amount of pale root galls in a 2 L soil sample; md per sample = amount of brown root galls per sample; hd per sample = amount of dark root galls per sample; L1 L4 per sample = amount of wingless females per 2 L soil sample; L5 per sample = amount of fundatrices per 2 L soil sample; nymphs per sample = amount of nymphs per 2 L soil sample. **A:** 2-dimensional factor map of abiotic, root and root gall parameters for 125AA. Dim1: 4.04 Eigenvalue, 31.12 % of total variance; Dim2: 2.08 Eigenvalue, 16.01 % of variance; 47.13 % of total variance explained. Temperature groups with ld and hd root galls and root surface. **B:** 2-dimension factor map of abiotic, root and root gall parameters for 5C. Dim1: 3.79 Eigenvalue, 29.21 % of total variance; Dim2: 2.22 Eigenvalue, 17.08 % of variance; 46.29 % of total variance explained. Temperature groups with root surface and ld root galls. Temperature seems to have a higher impact on root pigmentation on 125AA than on 5C. **C:** 2-dimensional factor map of abiotic, root and grape phylloxera parameters for 125AA. Dim1: 3.74 Eigenvalue, 34.08 % of total variance; Dim2: 1.68 Eigenvalue, 29.89 % of variance. Population densities of grape phylloxera group with temperature and SOM. **D:** 2-dimensional factor map of abiotic, root and grape phylloxera parameters of 5C. Dim1: 3.67 Eigenvalue, 33.38 % of total variance; Dim2: 1.74 Eigenvalue, 15.77 % of variance. Population densities group separately. Population densities on 5C are less influenced by temperature and SOM than on 125AA.

rameters. Soil temperature grouped in both rootstocks with root surface parameters and pale (light pigmented (ld)) root galls. This implies that in both rootstocks the development of roots and the development of fresh induced root galls depends on environmental temperature parameters. Temperature-dependent induction of root galls was seen before in laboratory assays by (TURLEY *et al.* 1997). In both rootstocks, brown root galls (md), terminal and nonterminal root galls grouped with soil acidity (pH) and soil organic matter (SOM). Dark pigmented (hd) root galls, however, grouped with soil temperature, pale root galls and root surface area in 125AA (Fig. 2A), whereas in 5C dark root galls grouped with pH and SOM (Fig. 2B). Higher pigmentation of root galls can be related to an advanced stage of degradation (HOFFMANN *et al.* 2011). Soil acidity and SOM are often related to microbial soil community parameters (YU *et al.* 2015). Our data analysis implied that root gall degradation processes seemed to be more affected by acidity and organic compounds in the soil on 5C rootstocks, whereas on 125AA rootstocks temperature seems to have a higher impact on root gall degradation (Fig. 2 A, B). However, generally the position of root galls seemed to be more related to SOM and pH than to temperature. This indicates that rather microbial factors than temperature affecting root gall development under field conditions. This in accordance with earlier suggestions made by HUBER *et al.* (2009). However, little is known about how and which environmental factors regulate the growth of a root after gall induction (LAWO *et al.* 2011). Particularly the ecological factors which influence gall induction and development are unknown for both grapes and grape phylloxera. Our analysis suggests that these interactions are mainly related to soil acidity and the amount of organic compounds on both 5C and 125AA rootstocks.

To investigate the relations between abiotic, root system parameters and grape phylloxera population development, we analyzed the population densities of wingless females, fundatrices and nymphs, abiotic and environmental parameters and the surface area in different root diameter classes for both rootstocks in two separate PCAs (Fig. 2 C, D). 49.12 % of total variance was explained by the first two extracted dimensions in 125AA (Fig. 2 C), 49.16 % of the total variance was explained in 5C (Fig. 2 D). In both rootstocks, root surface area did not group with the densities of grape phylloxera population. This implies that densities of grape phylloxera are not directly related to the available amount of root surface on both rootstocks. SWC, precipitation and pH also grouped separately in both rootstocks and were neither related to root surface parameters nor to grape phylloxera population densities. SOM and soil temperature grouped with population parameters on 125AA (Fig. 2 C). However, grape phylloxera population development densities grouped separately from all assessed abiotic parameters on 5C (Fig. 2 D). Our data strongly imply that grape phylloxera population development on 5C is not highly related to temperature and organic compounds in the soil, but on 125AA. By observing higher population densities in winter, already OMER *et al.* (1997) suggested a lower impact of temperature on grape phylloxera population dynamics under field conditions than proposed for example

by TURLEY *et al.* (1997). Our results suggest different ecological preferences of grape phylloxera, depending on the variety of the host plant. On 5C, grape phylloxera is able to crowd in higher rates (Fig. 1 C) and wingless females are able to overwinter in higher densities on roots of 5C (HOFFMANN *et al.* 2015).

This study highlights different ecological preferences of grape phylloxera, even on the same vineyard. In our study, these preferences could be related to the rootstock. However, the knowledge of the ecological preferences of grape phylloxera depends on the knowledge of the complex ecosystem of the specific vineyard. Those ecosystems might be very different between regions and/or management systems. Our study shows that the knowledge of the local agroecosystems is crucial to achieve a precise regional grape phylloxera management in grafted vineyards.

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