Grape Phylloxera Populations Adapted to *Vitis berlandieri* × *V. riparia* Rootstocks

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Seven grape phylloxera, *Daktulosphaira vitifoliae* (Fitch), colonies collected from populations on root or leaf galls on the *Vitis berlandieri* × *V. riparia* rootstocks SO4, 5BB, and 5C, one colony from the rootstock *V. rupestris* St. George, and one colony collected from *V. vinifera* Cabernet Sauvignon were compared in bioassays with excised roots. The colonies were collected in Hungary, Germany, and the United States. The colonies had differing survival, developmental and reproductive capacities when tested on Cabernet Sauvignon, SO4 and 5C in laboratory bioassays. The colonies collected from rootstocks utilized SO4 and 5C roots better than the *V. vinifera*-collected colony. The performance of the colonies on 5C and SO4 roots was better on callus and nodosities than on tuberosities. This level of utilization of the rootstock roots is likely a result of selection for individuals possessing such traits from a variable population in viticultural situations. Colonies arising from roots or leaves were similarly adapted in the root bioassays. RAPD DNA analysis suggests that the rootstock-collected phylloxera are genetically remote from the *V. vinifera* collected colony.

KEYWORDS: phylloxera, rootstock, biotype

Intensive grape breeding programs began as a result of the invasion and spread of grape phylloxera, *Daktulosphaira vitifoliae* (Fitch), in Europe during the late 1800s. Grape breeders Baco, Couderc, Seibel, Seyve, Villard, Millardet, Grasset, Richter, Paulsen, Teleki, and Kober became widely known for their successful rootstock cultivars. Most of the currently used *Vitis berlandieri* Planch. × *V. riparia* Michx. rootstocks were selected by Z. Teleki or were selected from Teleki's accessions by other breeders. These include the rootstocks SO4, 5BB, and 5C [1,18]. These rootstocks have resisted phylloxera since their selection in the 1920s. Although their resistance has been stable, their parentage is not completely known, thus opening questions regarding the continued stability of their resistance. When Teleki [17] first characterized the original seedling populations, he noted morphological types consistent with *V. berlandieri* × *V. riparia* hybrids, but also found pure and hybrid forms of *V. berlandieri*, *V. riparia*, *V. rupestris* Scheele, and *V. vinifera* L. In addition, the seedling populations came from parents in a locality that was surrounded by *V. vinifera* vineyards, leaving open the possibility of additional undetected *V. vinifera* parentage and, therefore, the potential of phylloxera susceptibility genes from that source. Hirschmann and Schlamp [10] suggested that these and other rootstocks have considerable susceptibility to grape phylloxera in Germany, although experimental data are not available. De Benedictis *et al.* [3] found low, but significant, levels of phylloxera susceptibility in 5C as indicated by insect feeding and the initiation of tuberosities (feeding sites on mature roots) on rootstock pieces in laboratory assays suggesting that phylloxera are capable of adapting to this rootstock.

In this study, we compared survival, development, and reproduction of phylloxera colonies collected from SO4, 5C, St. George, and Cabernet Sauvignon on tuberosities, nodosities (feeding sites on immature roots), and callus tissues of SO4, 5C, and Cabernet Sauvignon. We determined the relationship between origin of phylloxera colonies and the potential for host utilization as represented by laboratory bioassays. We also used RAPD (Random Amplified Polymorphic DNA) assays to determine the genetic diversity among these insects.

Materials and Methods

Nine colonies of grape phylloxera with different histories were used; these colonies had single-founder origins and were maintained by parthenogenesis. Two of the colonies originated from California, biotype A from own-rooted Cabernet Sauvignon, and a colony from Strain 2 [3] descended from roots of a vineyard planted on St. George rootstock. One colony was collected from the roots of SO4 in Germany (German). The Hungarian colonies were HUN-1R and HUN-1G from radicicoles (root forms of the insect) and gallicoles (leaf-gall forms of the insect), respectively, collected from the rootstock 5C from the Villány region of southern Hungary, HUN-2R and HUN-2G from 5BB from Nemesgulács, Badacsony region on the northern side of Lake Balaton in western Hungary, and HUN-3R and HUN-3G from SO4 from the rootstock collection at the Pannon University of Agricultural Sciences, Keszthely. The collection sites for HUN-1 and HUN-2 are sepa-

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rated from each other by 180 km; collection sites for HUN-1 and HUN-3 are separated from each other by 160 km; and 20 km separates the HUN-2 and HUN-3 sites.

Eggs were obtained from each of the field-collected leaf galls and root sections and used to establish laboratory colonies on *V. vinifera* Merlot root pieces [6]. The colonies were established using the methods of De Benedictis and Granett [2] and Omer *et al.* [12] and maintained on Merlot roots for more than three generations to eliminate maternal effects. Roots of SO4, 5C, and Cabernet Sauvignon were obtained from the vineyard of the Department of Viticulture and Enology, University of California, Davis. Ten root pieces 4 cm long and 3 to 5 mm diameter of each root type were infested each with 10 zero- to three-day-old eggs. Infested roots were maintained in 9-cm diameter ventilated plastic Petri dishes sealed with Parafilm 'M' laboratory film (American National Can, Greenwich, CT), two root pieces per dish. Dishes were placed in airtight boxes and held in darkness at 24°C.

The infested root pieces were examined after 18, 25, and 29 days. We recorded the number of eggs associated with tuberosity-, callus-, or nodosity-feeding sites and the number of immature and mature phylloxera at those feeding sites. For analyses, we combined data from callus and nodosities and will refer to these tissues as C/N. Eggs were removed on each examination date without disrupting the feeding and laying individuals. Development to the adult stage was previously measured as >16 days, and time for egg hatch was measured as six days [5]; however, for some rapidly developing phylloxera colonies, eggs laid during the experiment hatched and resulted in a contribution of a partial second generation to phylloxera counts. Second generation insects were noted in situations where oviposition occurred along with more first instars than second instars. As an index of survival (% surv.), we determined the percentage of the original insects placed on each root that were living on day 18 of the assay. Survival percentages on tuberosities and C/N were calculated separately for each root and these values combined for total survival. A partial second generation would inflate the survival index.

As an index of the developmental rate (D) we determined the average day upon which immatures became adults by counting the number of new adults that were found to have developed on each root on day 18 (A_{18}), day 25 (A_{25}) and day 29 (A_{29}) and entering the data in the following equation:

$$D = \frac{(A_{18})\,(18\text{ days}) + (A_{25})\,(25\text{ days}) + (A_{29})\,(29\text{ days})}{(A_{18} + A_{25} + A_{19})}$$

Developmental rate was calculated separately for phylloxera on tuberosities and C/N on each root and a total developmental rate was calculated based on all adults. For purposes of this experiment, we considered colony-root type combinations with < 5 individuals as insufficient to base a developmental rate.

Average fecundity (F) was estimated as the number of eggs per adult per day as measured during the time between days 18 and 25 and between days 25 and 29 using the equation:

$$F = \frac{E_{25} + E_{29}}{(\bar{X}_{A18..25})\,(7\text{ days}) + \bar{X}_{A25..29})\,+ (4\text{ days})}$$

where E_{25} and E_{29} are the number of eggs counted on day 25 and 29, respectively, and $\bar{X}_{A18..25}$ and $\bar{X}_{A25..29}$ are the average number of adults living between days 18 and 25, and days 25 and 29, respectively. Fecundity was calculated separately for phylloxera from tuberosities and C/N on each root and values combined for total fecundity. For purposes of this experiment, we considered colony-root type combinations with < 5 individuals as an insufficient basis for a fecundity rate.

Total egg production per starting egg (Σ eggs) is the total number of eggs produced for the entire 29-day bioassay divided by the starting population of eggs (=100 eggs). Egg production was calculated separately for phylloxera on tuberosities and C/N on each root and values combined for total egg production at all feeding sites. It should be noted that eggs laid prior to day 18 could not be used for determination of fecundity rate but were used to determine total egg production.

We used one-way analysis of variance (ANOVA) to compare differences in survival, development, fecundity and total egg production of each colony on the three different root types. Separation of means was determined by Duncan's [4] multiple range tests at $\alpha = 0.05$. Survival data were arcsine-square root transformed before the analyses were made.

DNA samples were extracted from about 50 eggs from each of the phylloxera colonies. Eggs were considered genetically uniform because the colonies reproduced parthenogenetically. Eggs were washed once with sterile water and then transferred to a microgrinder (Radnoti Glass, Arcadia, CA). DNA was extracted according to the procedure described by Lin and Walker [11]. DNA concentrations were determined by spectrophotometer and were adjusted to a final concentration of 10 ng/mL for polymerase chain reaction (PCR) with random amplified polymorphic DNA (RAPD) primers.

A 20-μL reaction mixture including 2 μL of 10X buffer (Promega, Madison, Wisc.), 0.1U of *taq* polymerase (Promega, Madison, Wisc.), 20 ng DNA with 2 m*M* $MgCl_2$, 0.2 m*M* of dNTPs (Boehringer Mannheim), 0.5 μ*M* primer (Operon Technologies, Alameda, CA: A2, A3, A4, A7, A9, A10, A11, A13, A14, A15, A16, A18, A19, B7, I3, I6, I7, I8, I9, I10, I11, I12, and I13) at final concentration was prepared according to the protocol described by Williams *et al.* [20]. PCR was performed in a PTC-100 thermal controller (MJ Research Inc., USA) preheated to 94°C using the following cycling program; 4 cycles of 1 minute at 94°C, 1.5 minutes at 37°C, and 2 minutes at 72°C, followed by 30 cycles of 0.5 minutes at 94°C, 0.5 minutes at 37°C, and 1 minute at 72°C. Amplified products were separated by 1.5% agarose gel

Table 1. Survival, development, fecundity, and total egg production of grape phylloxera colonies on tuberosities of Cabernet Sauvignon, SO4, and 5C.

Colony	Cabernet Sauvignon				SO4				5C			
	% surv.	D	F	Σ eggs	% surv.	D	F	Σ eggs	% surv.	D	F	Σ eggs
California A	65 a*	26 b	6.26 a	35.4 a	0 b	—	—	0.0 a	0 b	—	—	0.0 a
Strain 2	53 ab	26 b	3.43 bc	6.1 d	13 a	24 a	—	2.2 a	2 b	—	—	0.2 a
German	47 ab	26 b	2.10 cd	6.5 d	2 b	—	—	0.0 a	4 b	—	—	0.0 a
HUN-1R	59 a	26 b	4.46 b	17.0 b	0 b	28 a	2.03 a	0.6 a	3 b	—	—	0.5 a
HUN-1G	28 b	29 a	1.05 d	0.4 e	6 ab	—	—	0.0 a	1 b	27 a	0.28 a	0.1 a
HUN-2R	58 a	25 b	2.52 cd	4.5 cde	0 b	—	—	0.4 a	6 b	—	—	0.6 a
HUN-2G	47 ab	26 b	2.92 bc	5.5 d	1 b	—	—	0.3 a	15 a	—	—	0.1 a
HUN-3R	65 a	26 b	2.20 cd	3.8 cde	9 ab	25 a	3.82 a	1.9 a	0 a	28 a	1.84 a	0.4 a
HUN-3G	68 a	26 b	3.81 bc	12.3 c	3 ab	—	—	0.4 a	1 b	—	—	0.0 a

*Same letter following variables indicates colonies are not significantly different on the root type at $\alpha = 0.05$.

and viewed under UV light after ethidium bromide staining.

RAPD markers were scored based on the presence or absence of polymorphic bands. These DNA profiles were used to construct a rectangular data matrix which was converted into a similarity matrix using the simple matching coefficient technique [15]. The genetic distances from each site were then clustered using the unweighted pair-group method (UPGMA) [15] and NTSYS-PC (version 1.80) software.

Results

Partial second generation: A partial second generation was seen on all root types for the 18-day observations. However, on Cabernet Sauvignon such rapid development occurred only with the California A colony, but with none of the colonies arising from rootstocks. California A on SO4 and 5C did not have a partial second generation, but other colonies did have a partial second generation on one, the other or both rootstocks, with the exception of HUN-3G which did not produce a second generation.

Survival (% surv.): Survival of phylloxera on Cabernet Sauvignon tuberosities was relatively high for all colonies (between 28% and 68%) (Table 1). In contrast, survival on tuberosities of SO4 and 5C was low (between 0 and 15%). On C/Ns, the colonies originating from rootstocks (Strain 2, German the HUN colonies), exhibited the reverse survival pattern. Survival was higher on the rootstock roots (17% to 49%) than on Cabernet Sauvignon (1% to 15%) (Table 2). The California biotype A colony on Cabernet Sauvignon tended to have a higher survival rate on C/N than the other colonies. On the other hand, the colonies arising from rootstocks tended to have higher survival on rootstock C/Ns than did the California A colony (Table 2). When total survival independent of the nature of the feeding site is considered, the rootstock roots appeared to be reasonably good hosts (17% to 59% survival) for all colonies with the exception of California biotype A which did poorly on the rootstocks (8% survival on SO4 and 1% on 5C) (Table 3).

Developmental rate (D): The total mean developmental time to the adult stage ranged from 18 to 27

Table 2. Survival, development, fecundity, and total egg production of grape phylloxera colonies on nodosities or callus tissue of Cabernet Sauvignon, SO4, and 5C.

Colony	Cabernet Sauvignon				SO4				5C			
	% surv.	D	F	Σ eggs	% surv.	D	F	Σ eggs	% surv.	D	F	Σ eggs
California A	24 a*	22 a	—	0.5 a	8 b	—	—	0.0 c	1 b	—	—	0.0 b
Strain 2	15 ab	23 a	—	0.1 a	18 ab	22 ab	1.91 a	3.3 abc	28 a	20 a	—	4.0 ab
German	10 bc	—	—	0.0 a	33 ab	21 ab	2.07 a	7.7 a	28 a	22 a	1.40 a	7.8 ab
HUN-1R	1 c	—	—	1.1 a	17 ab	25 a	3.65 a	3.6 abc	41 a	22 a	1.75 a	10.6 a
HUN-1G	8 bc	—	—	0.0 a	18 ab	18 b	—	2.4 bc	27 a	20 a	1.35 a	7.1 ab
HUN-2R	2 bc	25 a	2.23 a	0.1 a	40 a	20 ab	1.26 a	7.3 a	49 a	22 a	1.78 a	10.4 a
HUN-2G	13 abc	23 a	1.93 a	1.5 a	29 ab	23 ab	4.67 a	6.0 ab	23 a	22 a	2.48 a	8.2 ab
HUN-3R	5 bc	24 a	—	0.9 a	17 ab	22 ab	2.32 a	2.5 bc	38 a	20 a	1.01 a	8.6 a
HUN-3G	5 bc	23 a	2.31 a	1.8 a	17 ab	21 ab	1.49 a	4.4 abc	46 a	19 a	0.66 a	9.1 a

*Same letter following variables indicates colonies are not significantly different on the root type at $\alpha = 0.05$.

Table 3. Total survival, development, fecundity and total egg production of grape phylloxera colonies on Cabernet Sauvignon, SO4, and 5C independent of nature of the feeding site.

Colony	Cabernet Sauvignon				SO4				5C			
	% surv.	D	F	Σ eggs	% surv.	D	F	Σ eggs	% surv.	D	F	Σ eggs
California A	89 a	25 a	6.17 a	35.9 a	8 b	—	—	0.0 b	1 c	—	—	0.0 b
Strain 2	68 b	26 a	3.34 bc	6.1 c	31 ab	22 ab	1.78 a	5.6 ab	30 b	20 a	1.46 a	4.2 ab
German	57 b	26 a	2.10 cd	6.5 c	35 a	21 ab	2.07 a	7.7 a	32 b	22 a	1.40 a	5.8 ab
HUN-1R	60 b	25 a	5.45 ab	18.1 b	17 ab	26 a	3.11 a	4.2 ab	44 ab	22 a	1.75 a	11.1 a
HUN-1G	36 c	27 a	0.84 d	0.4 d	24 ab	18 b	—	2.4 ab	28 b	23 a	0.99 a	7.1 ab
HUN-2R	60 b	25 a	2.37 cd	4.6 cd	40 a	21 ab	1.67 a	7.7 a	55 a	22 a	1.73 a	11.0 a
HUN-2G	60 b	25 a	2.75 bcd	7.0 c	30 ab	23 a	4.67 a	6.3 a	38 ab	22 a	2.34 a	8.4 a
HUN-3R	70 b	26 a	3.33 bc	4.8 cd	26 ab	22 ab	2.00 a	4.4 ab	38 ab	21 a	0.63 a	9.0 a
HUN-3G	73 b	26 a	3.76 bc	14.0 b	20 ab	21 ab	1.53 a	4.8 ab	59 a	19 a	0.66 a	9.1 a

*Same letter following variables indicates colonies are not significantly different on the root type at $\alpha = 0.05$.

days (Table 3). Times were not different among phylloxera colonies within Cabernet Sauvignon and 5C host types, but there were significant differences for phylloxera on SO4. None of the California biotype A phylloxera developed to the adult stage on SO4 or 5C, while all of the colonies from rootstocks had survival to the adult stage. The colonies behaved differently on tuberosity- and C/N-feeding sites. Tuberosities of Cabernet Sauvignon allowed all colonies to develop ≥ 5 adults (Table 1); however, the German, HUN-1R and HUN-1G colonies developed ≤ 5 adults on Cabernet Sauvignon C/N (Table 2). Phylloxera on tuberosities failed to develop ≥ 5 adults on 13 of the 18 rootstock – colony combinations (Table 1), whereas phylloxera from C/Ns failed to develop ≥ 5 adults only with California biotype A (Table 2). The apparent anomaly where ≥ 5 adults developed to the adult stage on tuberosities of HUN-1R but for which there was 0% survival (Table 1), is due to the movement of insects from N/C feeding sites to tuberosity sites.

Fecundity (F): Total fecundity on Cabernet Sauvignon was highest for California biotype A and HUN-1R (Table 3). Total fecundity on SO4 was not recorded for California biotype A and HUN-1G and on 5C for California biotype A because ≤ 5 adults had produced eggs for the counts on days 25 and 29. For colonies that had ≥ 5 egg-laying adults on those dates, total fecundity on SO4 ranged from 1.53 to 4.67 eggs/female/day and on 5C measurable total fecundity ranged from 0.66 to 2.34 eggs/female/day with no statistical differences between the colonies within root type. On SO4 and 5C tuberosities, only a few of the colonies of rootstock origin had measurable fecundity, whereas on N/Cs, most of these colonies had measurable fecundity (Tables 1 and 2).

Total egg production (Σ eggs): Egg production is a function of survival, developmental rate and fecundity and is limited by any of these factors. For example, California biotype A laid no eggs on SO4 or 5C because no individuals developed to the adult stage. All other colonies produced eggs on all root types. California biotype A had the highest egg production on Cabernet Sauvignon and its egg production was greater on tuberosities than on C/Ns (Table 1, 2). HUN-1G was almost incompetent on Cabernet Sauvignon with about 1% of the egg production seen with California biotype A (Table 3) but this colony was competent on both SO4 and 5C. On one or both rootstocks, total egg production for the colonies strain 2, German, HUN-1G, HUN-2R, HUN-2G and HUN-3R exceeded their egg production on Cabernet Sauvignon (Table 3). Total egg production

HUN-1G	1.0000								
HUN-1R	0.9738	1.0000							
HUN-2G	0.9346	0.9477	1.0000						
HUN-2R	0.9281	0.9411	0.9542	1.0000					
HUN-3G	0.8954	0.8954	0.9084	0.8888	1.0000				
HUN-3R	0.9411	0.9411	0.9281	0.9215	0.9542	1.0000			
German	0.8888	0.9019	0.9150	0.9215	0.8888	0.9084	1.0000		
Strain 2	0.8888	0.9019	0.9150	0.9477	0.8888	0.9084	0.9215	1.0000	
Calif. A	0.7058	0.7058	0.6928	0.6862	0.7320	0.7254	0.6993	0.7124	1.0000
	HUN-1G	**HUN-1R**	**HUN-2G**	**HUN-2R**	**HUN-3G**	**HUN-3R**	**German**	**Strain 2**	**Calif. A**

Fig. 1. Distance matrix calculated using the simple matching algorithm for six phylloxera colonies originating in Hungary (HUN-1G, HUN-1R, HUN-2G, HUN-2R, HUN-3G, HUN-3R), one colony originating in Germany (German) and two colonies originating in California (Strain 2 and Calif. A) generated from RAPD data after PCR with 23 Operon primers (A2, A3, A4, A7, A9, A10, A11, A13, A14, A15, A16, A18, A19, B7, I3, I6, I7, I8, I9, I10, I11, I12, I13) from which 180 markers were scored.

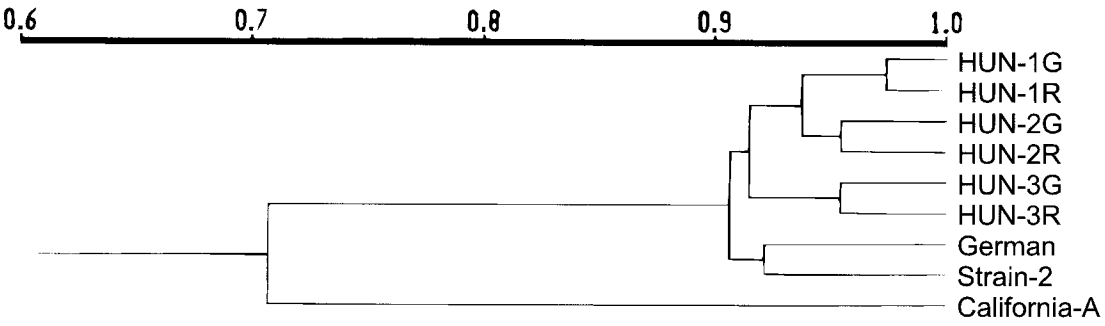

Fig. 2. Dendrogram representing genetic similarity among six phylloxera colonies originating in Hungary (HUN-1G, HUN-1R, HUN-2G, HUN-2R, HUN-3G, HUN-3R), one colony originating in Germany (German), and two colonies originating in California (Strain 2 and Calif. A). Genetic similarity coefficients were calculated using the simple matching algorithm and dendrogram clusters were constructed by the UPGMA method.

for the colonies arising from rootstocks occurred mostly on C/Ns (Table 2).

Colonies arising from radicicoles *vs.* gallicoles: The total egg production for colonies arising from rootstock radicicoles was not generally higher than the egg production for colonies arising from rootstock gallicoles on any root type.

Genetic diversity: A total of 180 RAPD markers were used to construct a simple matching distance matrix representing similarity values for the nine phylloxera colonies (Fig. 1). A dendrogram based on the UPGMA method of genetic distances among the different phylloxera colonies is presented (Fig. 2). The similarity coefficients of different Hungarian colonies ranged from about 0.97 (HUN-G1 vs HUN-R1) to about 0.89. Analyses of colonies originating in Hungary did show genetic differences in the phylloxera genome among locations (Fig. 1, 2). The German colony and Strain 2 were relatively similar (0.9215), and clustered with the Hungarian colonies. The California biotype A colony was very different from all the other colonies.

Discussion

The partial second generation elevated the survivorship values to an unknown extent in the situations where it was seen. However, we saw little evidence that the second generation reached the adult stage, and therefore, it did not influenced the other demographic measures.

We can view total egg production as a measure of the fitness of colonies under conditions of the bioassay. Each of the phylloxera colonies arising from rootstocks were able to survive, develop and reproduce on SO4 and 5C and several of the combinations had measured values statistically better than those of California A. These laboratory data, however, do not necessarily predict demise of SO4 and 5C in the vineyards where these phylloxera types exist. First, populations in the field are governed by many factors [see 13] not represented in a Petri dish, and therefore, high populations in these assays would not necessarily presage high populations on vines under field conditions; second, field damage generally has been associated with tuberosities and the colonies arising from rootstocks tend to be successful on SO4 and 5C tuberosities to a limited extent. Hirschmann and Schlamp [10] suggested that field damage occurred with Teleki rootstocks in Germany, and Walker *et al.* [19] associated this damage with nodosities. We do not know whether viticulturally significant damage can be caused by nodosities in other localities or under other conditions. Third, damage to phylloxera-infested grapevines is strongly influenced by plant pathogens [7]. Damage to vines by pathogens can not be projected from laboratory assays which measure the utilization of rootstocks by phylloxera.

Because of the world-importance of Teleki rootstocks to viticulture, it is clear that further definition of conditions of virulence and damage is needed. We need to understand the frequency of adapted individuals or strains in viticultural regions, how well these strains are adapted (*i.e.,* the intensity of the adaptation) and whether that intensity of adaptation is changing with continued selection under viticultural conditions.

Selection of adapted strains has been used to explain observations of Song and Granett [16], De Benedictis *et al.* [3] and Hawthorne and Via [9] who noted that particular phylloxera colonies are better able to utilize their hosts of origin than are populations from different hosts of origin. In this regard, Strain 2 is an anomaly; De Benedictis *et al.* [3] reported that it had its origin in a *V. rupestris* St. George vineyard, but it is more competent on the *V. berlandieri* × *V. riparia* rootstock 5C than it is on St. George. This might suggest that mechanisms of resistance for St. George and 5C are partially similar and selection on one adapts the colony to the other. But there must be other factors involved because of the low virulence previously observed for Strain 2 on St. George [3].

An alternate hypothesis is that though *frequency* of adapted types might be selected in vineyards, *intensity* of adaptation is not. That is, this virulence may be characteristic of the genetic background of the colony at the time it was introduced into California from wild *Vitis* and that the frequency of this characteristic was amplified or maintained in the phylloxera's genome because of linkage to other genetic characteristics related to the viticultural situation. This issue is important because of its relevance to the question of the

stability of rootstock resistance. If a low intensity of adaptation on wild American *Vitis* is a characteristic that is inherent to the native range of phylloxera, it is logical that rootstocks derived from these *Vitis* as parents could select for that trait and the frequency of the trait would increase. Although it is clear that such selection can increase the frequency of virulent genomes, we have no indication that the intensity of adaptation will be increased by such viticultural selection even in Europe where sexual recombination of phylloxera is known. Song and Granett [16] provided evidence for lack of change in intensity of virulence of phylloxera over a 72-year period to the rootstock 3309C in France.

RAPD DNA analysis of the colonies produced results that bear upon this issue. The Hungarian colonies, the German colony and Strain 2 were clustered in contrast to California biotype A which was substantially different (Figures 1 and 2). The apparent clustering of these SO4- and 5C-competent phylloxera colonies without regard to geographic origin or host of origin suggests the hypothesis that their linkage is based on their adaptation to these rootstocks. Further worldwide sampling is needed to substantiate this hypothesis. Analysis of many such host specific populations from a wide geographical distribution using RAPD or other DNA markers could lead to the development of markers linked to feeding ability.

In contrast to the above discussion, phylloxera feeding on the roots of SO4 and 5C may be a function of the unknown parentage of the Teleki rootstocks [17,18]. The actual cross is unknown and only appears to be *V. berlandieri* × *V. riparia*. Relative to this uncertainty, Ravaz [14] observed susceptibility in *V. riparia* , and Grzegorczyk and Walker [8] observed susceptibility of both *V. berlandieri* and *V. riparia* in tissue culture-based assays. Further tests with accessions of *V. berlandieri* and *V. riparia* are needed to confirm the nature and extent of their interactions with phylloxera.

The RAPD data support the idea that phylloxera move between roots and leaves of grapevines in Hungary. These data do not support the existence of separable adaptations to root or leaf tissue in a region such as Hungary where phylloxera have the opportunity to move between roots and leaves. This does not preclude the existence of root- or leaf-adaptations where opportunity to move is not present (as in California's viticultural regions where root forms are found but not leaf forms or in Death Valley, California, and in Arizona, where leaf forms are found but not root forms) [6].

Literature Cited

1. Bakonyi, K., L. Bakonyi, and L. Kocsis. Hungarian breeding of grape rootstock celebrates its 100th anniversary. Zsigmond Teleki, his life and results. Hortic. Sci. (Kertészeti Tudomány) 28(3-4):87-91 (1996).

2. De Benedictis, J. A., and J. Granett. Variability of responses of grape phylloxera to bioassays that discriminate between California biotypes. J. Econ. Entomol. 85:1527-1534 (1992).

3. De Benedictis, J. A., J. Granett, and S. P. Taormino. Differences in host utilization by California strains of grape phylloxera. Am. J. Enol. Vitic. 47:1-7 (1996).

4. Duncan, D. B. Multiple range and multiple F tests. Biometrics 11:1-42 (1955).

5. Granett, J., B. Bisabri-Ershadi, and J. Carey. Life tables of phylloxera on resistant and susceptible grape rootstocks. Entomol. Exp. Appl. 34:13-19 (1983).

6. Granett, J., J. A. De Benedictis, and J. Marston. Host suitability of *Vitis californica* Bentham to grape phylloxera, *Daktulosphaira vitifoliae* (Fitch). Am. J. Enol. Vitic. 43:249-252 (1992).

7. Granett, J., A. D. Omer, *et al.* Fungal infections of grapevine roots in phylloxera infested vineyards. Vitis 37 (1):39-42 (1998).

8. Grzegorczyk, W., and M. A. Walker. Evaluating resistance to grape phylloxera in *Vitis* species with an *in vitro* dual culture assay. Am. J. Enol. Vitic. 49:17-22 (1998).

9. Hawthorne, D. J., and S. Via. Variation in performance on two grape cultivars within and among populations of grape phylloxera from wild and cultivated habitats. Entomol. Exp. Appl. 70:63-76 (1994).

10. Hirschmann, J., and W. Schlamp. Alter Schädling, Neuer Ärger. Weinbauamt mit Wiebauschule Eltville. Wiesbaden (1994).

11. Lin, H., and M. A. Walker. Extraction of DNA from eggs of grape phylloxera (*Daktulosphaira vitifoliae* Fitch) for use in RAPD testing. Vitis 35:87-89 (1996).

12. Omer, A. D., J. A. De Benedictis, and J. Granett. Grape phylloxera, *Daktulosphaira vitifoliae* (Fitch) (Hom., Phylloxeridae), population response to preformed tuberosities. J. Appl. Entomol. 119:653-657 (1995).

13. Omer, A. D., J. Granett, *et al.* Population dynamics of grape phylloxera in California vineyards. Vitis 36(4): 199-205 (1997).

14. Ravaz, L. Contribution á l'étude de la résistance phylloxérique. Rev. Vitic. 7:137-142 (1897).

15. Sneath, P. H. A., and R. R. Sokal. Numerical Taxonomy. 573 pp. Freedman, San Francisco (1973).

16. Song, G. C., and J. Granett. Grape phylloxera (Homoptera: Phylloxeridae) biotypes in France. J. Econ. Entomol. 83: 489-493 (1990).

17. Teleki, A. A szölök felújitása (Reconstruction of vineyards). pp 35-37. "Pátria" Irodalmi Vállalat és Nyomdai RT, Budapest (1928).

18. Walker, M. A., and J. A. Wolpert. History of Teleki rootstocks and origin of 5C, SO4. Pract. Winery Vineyard (March/April): 47-49 (1992).

19. Walker, M. A., J. Granett, *et al.* Are phylloxera feeding on 5C rootstocks in Europe? PVW 1998 (March/April): 21-26 (1998).

20. Williams, J. G. K., A. R. K. Kubelik, *et al.* DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. Nucleic Acids Res. 18:6531-6535 (1990).