Evaluating Resistance to Grape Phylloxera in *Vitis* Species with an *in vitro* Dual Culture Assay

WLADYSLAWA GRZEGORCZYK[1] and M. ANDREW WALKER[2]*

Forty-one accessions of 12 *Vitis* L. and *Muscadinia* Small species were evaluated for resistance to grape phylloxera (*Daktulosphaira vitifoliae* Fitch) using an *in vitro* dual culture system. The performance of the species tested in this study was consistent with previously published studies with whole plants and helps confirm the utility of *in vitro* dual culture for the study of grape/phylloxera interactions. This *in vitro* system provides rapid results (8 wk) and the ability to observe the phylloxera/grape interaction without interference from other factors. This system also provides an evaluation that overemphasizes susceptibility, thus providing more confidence in the resistance responses of a given species or accession. Among the unusual responses were the susceptibility of *V. riparia* Michx. DVIT 1411; susceptibility within *V. berlandieri* Planch.; relatively wide ranging responses in *V. rupestris* Scheele; and the lack of feeding on the roots of *V. californica* Benth., in contrast to the severe foliar feeding damage that occurred on this species. *Vitis californica* #11 and *V. girdiana* Munson DVIT 1379 were unusual because phylloxera on them had the shortest generation times. Such accessions might be used to examine how grape hosts influence phylloxera behavior. Very strong resistance was found within *V. aestivalis* Michx. DVIT 7109 and 7110; *V. berlandieri* c9031; *V. cinerea* Engelm; *V. riparia* (excluding DVIT 1411); *V. rupestris* DVIT 1418 and 1419; and *M. rotundifolia* Small. These species and accessions seem to possess enough resistance to enable their use in breeding with minimal concern about phylloxera susceptibility.

KEY WORDS: phylloxera resistance, resistance evaluation, sterile culture, tissue culture, host/pest interactions

One hundred years ago, rootstocks were bred in Europe to combat the inadvertent introduction of grape phylloxera (*Daktulosphaira vitifoliae* Fitch) from North America. Three North American *Vitis* L. species (*V. riparia* Michx, *V. rupestris* Scheele, and *V. berlandieri* Planch.) were used to produce the vast majority of phylloxera-resistant rootstocks. These species have evolved resistance to phylloxera; the first two are easily propagated, and although the third propagates poorly it is well-known for its lime tolerance. The rootstocks produced from crosses with these species were selected for adaptation to European soils and climates, but have proven durably phylloxera resistant in vineyards around the world.

The need for diligence in the control of phylloxera was reemphasized when vineyards planted with the insufficiently resistant rootstock AXR#1 (*V. vinifera* L. Aramon X *V. rupestris* Ganzin) began failing in California [11]. This failure lead to the need for reevaluation of existing rootstocks and the opportunity for developing new rootstocks capable of combating serious soilborne problems that the European rootstocks do not address [21]. Resistance to these soil-borne problems will come from a wide range of *Vitis* species, some having poorly defined phylloxera resistance. Given that phylloxera resistance should be in the background of all new rootstocks and that field evaluations of phylloxera resistance can take many years, a means of rapidly evaluating resistance would accelerate rootstock breeding and improve our understanding of resistance.

Phylloxera resistance has been evaluated under field conditions [3,6,18,20], in the greenhouse [4,7], and under laboratory conditions [3,4,8,11,12]. Tissue culture-based systems have also been developed to study phylloxera/grape interactions [1,10,17,19], although few have remained free of contamination which compromised their results. Tissue culture conditions may not produce a typical host/pest interaction when compared to tests conducted under field conditions. However, tissue culture evaluations allow close examination of the host/pest interaction, produce reliable results in relatively short periods of time with limited space and limited inoculum, and allow pest spread to be contained.

Previous work in our laboratory reported on the inoculation techniques and culture conditions capable of providing a sterile environment and optimal conditions for an *in vitro* grape/phylloxera dual culture system [10,14]. The research presented here examines the ability of this system to accurately evaluate the phylloxera resistance of *Vitis* species by comparing *in vitro*-based responses to those described in past experiments with field grown or potted grapes. Forty one accessions of 12 *Vitis* and *Muscadinia* Small species were inoculated with phylloxera under sterile conditions and the

[1]Graduate student and [2]Associate Professor, Department of Viticulture and Enology, University of California, Davis, CA 95616-8749.

*Corresponding author [FAX: 530-752-0382; E-mail mawalker@ucdavis.edu].

Acknowledgments: The authors gratefully acknowledge the financial support of the California Grape Rootstock Improvement Commission, the American Vineyard Foundation, the California Table Grape Commission, specific cooperative agreements with the USDA National Germplasm Repository-Davis and the USDA-ARS Fresno, and the Andre Tchelistcheff Scholarship. We also thank J. Granett and his laboratory staff for phylloxera eggs and technical consultation.

Manuscript submitted for publication 11 July 1997.

results were compared to previous reports on the resistance of these species.

Materials and Methods

Plant materials and propagation: The *Vitis* and *Muscadinia* germplasm examined in this experiment were collected from the National Clonal Germplasm Repository — Davis (DVIT accession numbers) and vineyards of the Department of Viticulture and Enology, University of California, Davis (Table 1).

Establishment of plants in tissue culture: Plants were established under *in vitro* conditions and examined for phylloxera development following the techniques of Forneck *et al.* [10]. Nodal sections of stems from mother vines in 4-L pots and grown in the greenhouse were surface sterilized for 20 minutes in 30% commercial bleach with two drops of surfactant. Following three sterile water rinses, the cuttings were placed into 25 mm x 100 mm tubes filled with 15 mL of MS media (Sigma N5524), containing 1/2X MS salts (Sigma M-5524), 1/2X MS vitamins (Sigma M 7150), 10 g/L of sucrose, 6 g/L agar (Sigma A-1296), and 1 mL/L indole-3-acetic acid (Sigma I 2886), with a pH adjusted to 5.7. Thirty six tubes were maintained for each genotype and provided sterile source material for the phylloxera evaluation. These were subcultured and maintained until cuttings were taken from them to establish the test plants described below. Growth chambers conditions were set for 25°C with a 16-hour day length (30 - 50 µE/m/s).

Six weeks prior to inoculation with surface-sterile phylloxera eggs, two-node apical cuttings were taken from the tissue-cultured source vines described above and placed into GA-7 Magenta vessels (Sigma T 8654). These vessels were filled with 45 mL of the media described above, and 10 replicates of each accession were established of which five were later used for inoculation. After filling with liquid media, the vessels were placed so that the media solidified at a slant. This provided an area for condensed water, which was found damaging to phylloxera [10], to accumulate.

Inoculation process: The phylloxera eggs used for this experiment were obtained from J. Granett, Department of Entomology, University of California, Davis. One- to seven-day-old eggs were taken from a variety of field collected root-galling colonies, including biotypes A and B, and strains 1, 2, and 3 [9]. Because so many eggs were needed for the test plant inoculations, it was not possible to use eggs from a single colony. Eggs from these various sources were bulked and then used for inoculation as described below.

Eggs were obtained from the Granett lab on a weekly basis. They were surface sterilized following the process outlined in Grzegorczyk and Walker [14] and placed on tissue cultured plants of *V. vinifera* Cabernet Sauvignon growing in 100 x 25 mm petri plates filled with 25 mL of the above media. The inoculated plates were wrapped with Parafilm® M and placed in growth chambers set for the above conditions. Eggs from these Cabernet Sauvignon plants remained surface sterile and were transferred to more Cabernet Sauvignon plants cultured in the same manner to build up the large populations of eggs necessary for inoculation of the test plants. This process produced the eggs that were used to inoculate the test plants.

The test plants growing in the Magenta vessels were ready for inoculation after six weeks of growth. Leaves were removed before infesting with phylloxera to minimize condensation inside the culture boxes as the phylloxera established feeding sites. About 50 eggs from the Cabernet Sauvignon plants described above were placed on the roots and stems of each test plant with a soft brush, after which they were returned to the growth chambers described above. Five replicates of each test plant accession were inoculated in this manner.

A total of 200 plants, five replicates from each accession, were tested. It was not possible to test the 200 plants in one group. Thus, the plants were tested in groups of five at weekly intervals. These groups included four plants from randomly chosen accessions and a plant of Cabernet Sauvignon, making a total of 50 groups. It was possible to evaluate the plants in this fashion because the culturing conditions were standardized and because all of the tested plants were initiated by subculturing from tissue cultured source plants as described above. This subculturing produced test plants that were uniform in terms of health, size, and vigor. The plant of Cabernet Sauvignon was included to provide a susceptible control and to monitor changes or problems in the testing process over time. A total of 50 Cabernet Sauvignon plants were evaluated.

Evaluation process: After eight weeks of co-culture, the test plants were evaluated. The total number of live phylloxera on each plant was recorded including adults, feeding immatures, crawlers, and eggs. Root and leaf feeding sites were examined and recorded. Necrotic areas on the roots were not distinctive, but galling and swelling associated with active feeding sites was. Galling at the root tips was classified as a primary feeding site and appeared to be nodosities. The number of these feeding sites was recorded. Swellings between the root tip and the stem were classified as secondary feeding sites and were considered to be equivalent to tuberosities. The number of these feeding sites was also recorded. Phylloxera feeding on the leaves caused necrotic lesions which ranged from 3 to 5 mm in diameter. The average number of these lesions for each accession was recorded. Five adult phylloxera on each test plant, a total of 25 for each accession, were observed to determine the number of days between egg hatch and egg laying, and the number of eggs laid per day during the first week of egg laying. Analysis of variance was applied to the data and mean differences among the species were distinguished using Fisher's protected LSD.

Results and Discussion

Phylloxera resistance in grape species has been evaluated and categorized since they were recognized

Table 1. Responses of *Vitis* and *Muscadinia* species to grape phylloxera after eight weeks under *in vitro* conditions. Reported values are averages of five phylloxera/plant combinations. Primary feeding site was recorded as the number of root tip feeding sites, secondary feeding site as the number of mid-section or root base feeding sites, leaf damage as the number of 3 - 5 mm necrotic regions surrounding leaf feeding sites, generation time as the number of days from egg hatch to egg laying, and eggs/day as the number of eggs laid per day during the first week of egg laying.

Species	Total phylloxera	Primary feeding sites	Secondary feeding sites	Leaf damage	Generation time	Eggs/day
V. aestivalis DVIT 7026[y]	26 a[z]	4.2 bcd	3.6 cde	0.4 ab	21.8 i	1.8 b
V. aestivalis DVIT 7109	0 a	0.0 a	0.0 a	0.0 a	0.0 a	0.0 a
V. aestivalis DVIT 7110	0 a	0.0 a	0.0 a	0.0 a	0.0 a	0.0 a
V. rufotomentosa DVIT 1416	0 a	0.0 a	0.0 a	0.0 a	0.0 a	0.0 a
V. amurensis 01	875 g	4.0 bcd	7.8 hi	4.2 fgh	19.7 fg	7.7 j
V. berlandieri c9017	71 ab	6.6 def	2.2 bcd	5.8 h	26.0 j	3.5 de
V. berlandieri c9019	167 bc	5.8 cde	3.6 cde	3.4 defg	21.8 i	4.7 f
V. berlandieri c9031	0 a	0.0 a	0.0 a	0.0 a	0.0 a	0.0 a
V. berlandieri c9043	878 g	6.0 cde	6.8 gh	8.6 i	20.5 gh	7.2 ij
V. californica 5	515 d	8.8 ef	0.0 a	10.4 ij	16.0 d	6.9 hi
V. californica 11	653 e	19.0 g	0.0 a	5.2 gh	12.7 b	6.9 hi
V. californica 19	819 fg	6.8 def	0.0 a	16.4 k	14.4 c	6.5 gh
V. californica c9545	94 ab	1.2 ab	0.0 a	1.6 abcd	21.4 hi	1.8 b
V. champinii c9016	116 ab	2.0 ab	0.8 ab	2.0 bcde	25.6 j	4.9 f
V. champinii c9021	296 c	3.0 abc	4.0 def	4.4 fgh	21.7 i	6.9 hi
V. champinii c9035	288 c	0.0 a	4.0 def	0.2 ab	17.3 e	4.8 f
V. champinii c9037	121 ab	1.4 ab	3.2 cde	1.4 abc	19.8 fg	4.3 ef
V. cinerea c9007	0 a	0.0 a	0.0 a	0.0 a	0.0 a	0.0 a
V. cinerea c9008	0 a	0.0 a	0.0 a	0.0 a	0.0 a	0.0 a
V. cinerea c9025	0 a	0.0 a	0.0 a	0.0 a	0.0 a	0.0 a
V. cinerea c9041	0 a	0.0 a	0.0 a	0.0 a	0.0 a	0.0 a
V. girdiana DVIT 1379	872 g	6.2 de	5.6 fg	0.0 a	12.8 b	8.6 k
V. girdiana DVIT 1380	686 ef	9.4 f	7.4 gh	0.0 a	15.0 cd	7.5 ij
V. girdiana DVIT 1387	1071 h	7.6 ef	18.2 j	9.6 ij	21.2 hi	7.0 hi
V. girdiana DVIT 1389	450 d	2.0 ab	3.6 cde	3.6 efg	18.8 f	2.7 cd
V. labrusca DVIT 1391	26 a	0.0 a	1.2 ab	1.4 abc	22.4 i	3.6 e
V. labrusca DVIT 1392	7 a	0.0 a	0.0 a	0.8 ab	22.2 i	4.2 ef
V. labrusca DVIT 1393	5 a	0.0 a	0.0 a	0.0 a	22.3 i	2.1 bc
V. riparia DVIT 1411	1073 h	23.4 g	5.8 fg	14.6 k	19.1 f	7.0 hi
V. riparia DVIT 1423	0 a	0.0 a	0.0 a	0.0 a	0.0 a	0.0 a
V. riparia DVIT 1437	0 a	0.0 a	0.0 a	0.0 a	0.0 a	0.0 a
V. riparia DVIT 1438	0 a	0.0 a	0.0 a	0.0 a	0.0 a	0.0 a
V. rupestris DVIT 1406	789 efg	8.0 ef	4.6 ef	3.0 cdef	21.4 hi	6.0 g
V. rupestris DVIT 1418	0 a	0.0 a	0.0 a	0.0 a	0.0 a	0.0 a
V. rupestris DVIT 1419	0 a	0.0 a	0.0 a	0.0 a	0.0 a	0.0 a
V. rupestris DVIT 1421	727 ef	8.8 def	4.2 ef	3.6 efg	21.3 hi	4.7 f
V. vinifera Cabernet Sauvignon[x]	1081 h	7.0 def	9.6 i	10.6 j	17.6 e	8.3 k
M. rotundifolia DVIT 1706	0 a	0.0 a	0.0 a	0.0 a	0.0 a	0.0 a
M. rotundifolia DVIT 1750	0 a	0.0 a	0.0 a	0.0 a	0.0 a	0.0 a
M. rotundifolia DVIT 1756	0 a	0.0 a	0.0 a	0.0 a	0.0 a	0.0 a
M. rotundifolia DVIT 1768	0 a	0.0 a	0.0 a	0.0 a	0.0 a	0.0 a
F-value	60.68	18.59	27.18	40.388	636.38	317.97
P-value	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001

[z] Selections followed by the same letter are not significantly different according to Fisher's protected LSD ($\alpha = 0.05$)

[y] DVIT numbers are from the National Clonal Germplasm Repository-Davis, all other accessions are from the collections of the Department of Viticulture and Enology, University of California, Davis.

[x] Values for Cabernet Sauvignon are averages of 50 plants.

as serious grape pests. Early studies [6,15,16,20] examined grape species under field conditions and found a wide range of responses from extreme resistance in *M. rotundifolia* to susceptibility in *V. vinifera*. These studies also found that resistance varied from very high to moderate in some species such as *V. riparia*, *V. rupestris,* and *V. berlandieri*. *Vitis californica* Benth. and *V. girdiana* Munson, which might be considered for use in rootstock breeding because of their unique adaptation to Californian conditions, did not exhibit sufficient phylloxera resistance. Boubals [3,4] confirmed these earlier results with tests conducted in the greenhouse and laboratory. He found that the level of phylloxera resistance can vary within a species and is genetically controlled. The results from our survey of grape species and their phylloxera resistance under *in vitro* conditions are in correspondence with these earlier studies.

The dual culture screening system described herein worked well. Results were obtained in eight weeks and many aspects of the grape/phylloxera interaction could be readily evaluated and the degree of damage rated. Testing the plants in groups allowed this study to be accomplished, and the controlled testing conditions and relatively uniform source material enabled comparisons among the genotypes tested. The greatest limitation to the test was producing sufficient quantities of eggs for inoculation. This problem was overcome by maintaining a large number of inoculated Cabernet Sauvignon in tissue culture for the sole purpose of producing eggs.

At the end of the eight-week evaluation period, it was clear which test plants supported phylloxera reproduction and responded negatively to their feeding. Furthermore, only the grape/phylloxera interaction was evaluated and interactions with environmental factors such as soil type or temperature, or from other soil organisms such as fungi or bacteria were eliminated. The reactions of the various species and their accessions will be discussed in alphabetical order and are presented in Table 1.

***Vitis aestivalis* Michx.**: This species from the south-central and southeastern United States is highly variable and is considered to encompass *V. rufotomentosa* Small [5]. Phylloxera feeding occurred on two accessions — DVIT 7026 and *V. rufotomentosa* DVIT 1416. However, few eggs (1.8) were laid per day on DVIT 7026, and all of the adults feeding on *V. rufotomentosa* died before reproducing. Primary and secondary root galling were evident on DVIT 7026, though both galls formed in low numbers and brown spots formed on the roots where feeding occurred. DVIT 7109 and 7110 did not support phylloxera feeding. Viala and Ravaz [20] mentioned that this species had good resistance and rated it 16 on a 20 point scale.

***Vitis amurensis* Rupr.:** This species from northeastern China has not evolved with phylloxera, and as expected, it was susceptible. The number of eggs per adult per day produced on its roots was high, 7.7, and secondary swellings and damage was very evident on older roots. Feeding and subsequent necrosis also occurred on the stem and leaves to the same degree as on the roots. *Vitis amurensis* appeared very susceptible and had an unusually high number of secondary feeding sites. These results confirmed those of Viala and Ravaz [20] who scored it as 2 on a 20-point scale and by Boubals [4] who put it in his most susceptible class.

***Vitis berlandieri*:** This species is found throughout central and southern Texas on limestone soils. The tested accessions were collected in Comanche County, Texas, with the exception of c9031 which was collected in Bell county. Past studies have found variability in the phylloxera resistance of *V. berlandieri* [3], although Viala and Ravaz [20] stated that its resistance is relatively strong. Accessions c9017, c9019 and c9043 supported feeding and reproduction at different levels. The greatest number of phylloxera were produced on c9043 (878), followed by c9019 (167) and c9017 (71). c9043 was the most damaged by feeding and had the greatest number of primary feeding sites. Feeding occurred on roots, leaves, and stems, but damage in terms of necrosis and swellings was more severe on the roots.

***Vitis californica*:** Testing results of this California species were unusual, because feeding only occurred on the stems and leaves; the roots were not fed upon. All four accessions responded in this way and formed leaf galls that became necrotic, eventually killing all plants of #11 and #19. High numbers of phylloxera built up on #5, #11, and #19. However, c9545 had greatly reduced numbers of phylloxera [94], longer generation time, and fewer eggs per day. Studies done by Granett *et al.* [13] found that accessions #5 and #11 were very susceptible and #19 was less susceptible. In our test #19 was the most susceptible, in terms of the number of phylloxera sustained by it. Viala and Ravaz [20] stated that *V. californica* was very susceptible and it received a rating of 5 on their 20-point scale.

***Vitis champinii* Planch.:** Low to moderate levels of feeding and reproduction occurred on all genotypes of this putative natural hybrid between *V. candicans* Engelm. and *V. rupestris* [20]. Accessions c9021 and c9035 had significantly greater numbers of phylloxera. c9016 had fewer secondary feeding sites when compared to the other three, but it supported similar numbers of phylloxera when compared to c9037. c9021 was fed upon more aggressively than the other accessions. It produced the greatest number of eggs per day and the greatest number of primary feeding sites. Feeding on the leaves and stems occurred on all four accessions, but did not seem to cause damage. Primary and secondary root feeding affected all the accessions with the latter being very evident. Roots turned brown at the point of phylloxera feeding and decayed, but none of the accessions died. Viala and Ravaz [20] found that *V. champinii* was variable in terms of resistance and morphology. They rated some forms as 14 and others as 12 on their 20-point scale. However, Boubals [3] rated this species as resistant. *Vitis champinii* is valuable to grape breeders because of its nematode resistance [21], but our results suggest that parents should be selected carefully with regard to phylloxera resistance.

***Vitis cinerea* Engelm.:** Our results confirmed the strong resistance of this species to phylloxera [3,20]. The first instar phylloxera crawlers survived for five to six days and then died without feeding. Evidence of feeding was observed only on c9025, where crawlers began feeding on stems and leaves, but died after one week without damaging the plant.

***Vitis girdiana* Munson:** All of the accessions of this southern Californian species allowed feeding and reproduction on their roots, stems and foliage. However, the responses and total phylloxera produced varied relatively widely. DVIT 1387 sustained almost as many phylloxera as were produced on Cabernet Sauvignon, while DVIT 1389 sustained many fewer phylloxera and fewer feeding sites were recorded. Phylloxera behavior on this species also varied in the number of eggs laid per day, generation times, and the number of primary and secondary feeding sites. DVIT 1379 was unusual because it induced a rapid generation time in phylloxera, equivalent to *V. californica* #11, which was the lowest among all plants tested. This accession could be used in crosses with species that induce longer generation times in order to study phylloxera biology and the host effect on altering development and reproductive rates. Phylloxera preferred the young root tips of DVIT 1379 and DVIT 1380, but fed more at the base and middle root areas on DVIT 1387 and 1389. This difference in feeding behavior may warrant further study to determine whether nodosities or tuberosities are more damaging under field conditions. Leaf galls were formed on all accessions but did not lead to necrosis or decline.

***Vitis labrusca* L.:** Only three accessions of this species from the northeastern United States were available for testing. Feeding and reproduction occurred at low levels and damage was not evident. Few phylloxera were found at the end of eight weeks, few eggs were laid per day and the generation time was long. There was little effect from phylloxera feeding; DVIT 1391 was the only accession with feeding sites and they were relatively few. Limited feeding occurred on the foliage, but no damage was observed. Our results differed from those reported by Viala and Ravaz [20], who found that *V. labrusca* was quite susceptible (5 on their 20-point scale). Boubals [3] classified *V. labrusca* as resistant which our results seemed to confirm.

The resistance of Concord grape has been reported to be relatively weak, particularly on gravely limestone-based soils [20]. However, this variety is regarded as a *V. labrusca* × *V. vinifera* hybrid [2], which likely reduces its phylloxera resistance when compared to *V. labrusca.* Concord was not tested in our study.

***Vitis riparia*:** Only one *V. riparia* accession, DVIT 1411, supported phylloxera feeding and did so at high levels. This accession produced 1073 phylloxera and allowed a relatively high reproduction rate of seven eggs per day. DVIT 1411 appears to be pure *V. riparia* (M. A. Walker, personal observation) and was collected from the most northerly area (Mitchell, South Dakota) of the tested accessions. South Dakota is at the edge of phylloxera's range. The possibility that *V. riparia* from this area may have reduced resistance to phylloxera due the lack of an interaction with the pest should be studied and would add to our understanding of phylloxera/grape interactions. DVIT 1423 and 1438 came from Kansas an area with strong phylloxera pressure. These two accessions might be expected to have evolved a higher level of phylloxera resistance. The source of DVIT 1437 Riparia Gloire is poorly documented, but it also possess strong resistance to phylloxera. The *V. riparia* accessions that Boubals [3] tested ranged from moderate to very resistant, with none rated as susceptible. Viala and Ravaz [20] state that *V. riparia* is very resistant to phylloxera, but the results of our study indicate that there is at least one instance of susceptibility within *V. riparia*, accession DVIT 1411.

***Vitis rupestris*:** The phylloxera resistance of *V. rupestris* has been reported to be moderate [3,20]. Of the four accessions tested, DVIT 1406 (St. George) and DVIT 1421 (Metallique) allowed feeding and reproduction, while DVIT 1418 (Constantia) and DVIT 1419 (Ganzin) did not. The total number of phylloxera, number of feeding sites, and reproductive rates were similar for DVIT 1406 and DVIT 1421. Roots on both of these accessions had browning and necrosis associated with root feeding sites, and more feeding at the root tips. Leaf damage as judged by the number of necrotic regions was relatively low. Viala and Ravaz [20] reported that resistance in *V. rupestris* was variable, but that even when fed upon the plants did not die in the field. Some of this variability may be accounted for by confusion surrounding the origin of these accessions. DVIT 1421 (Metallique) has been reported to be a natural hybrid of *V. rupestris* × *V. candicans* [20], which may reduce its resistance to phylloxera compared to pure *V. rupestris* accessions.

***Vitis vinifera* Cabernet Sauvignon:** As expected, this species was very susceptible, as both the total number of phylloxera and the eggs laid per day were high, and their generation time was relatively rapid. A total of 50 Cabernet Sauvignon plants were evaluated over the testing period. The average values for the evaluation parameters and their standard deviations follow: total number of eggs, 1081 (117); number of primary feeding sites, 7.0 (1.2); number of secondary feeding sites, 9.6 (2.7); number of necrotic areas on leaves, 10.6 (1.1); generation time in days, 17.6 (3.3); and eggs per day, 8.3 (1.7). Feeding occurred on the entire plant and the foliage was damaged as severely as the roots. Feeding girdled the base of the stem which resulted in plant death at the end of the eight-week testing period. The necrotic areas on the leaves expanded and contributed to the overall decline. *Vitis vinifera* has been reported to be relatively resistant to foliar feeding under field conditions [3]; however, under our *in vitro* system the entire plant was susceptible.

***Muscadinia rotundifolia*:** As reported by others [3,4,20], phylloxera were unable to feed on *M. rotundifolia*. Eggs hatched and crawlers searched for feeding sites for five to six days before death. Feeding attempts

were only detected on leaves and stems of DVIT 1706 resulting in very small brown necrotic spots. This feeding lasted five to six days, then the phylloxera died. Resistance to phylloxera seems to be very strong in *M. rotundifolia*.

Conclusions

The performance of the species tested in this study was reasonably consistent with other published studies [3,4,20] and helps confirm the utility of *in vitro* dual culture for the study of grape/phylloxera interactions. Among the unusual responses were the susceptibility of *V. riparia* DVIT 1411, variability or susceptibility within the *V. berlandieri* and *V. rupestris* species tested, and the lack of feeding on the roots of *V. californica*, even though in the latter case the foliage was severely damaged. *Vitis californica* #11 and *V. girdiana* DVIT 1379 were also unusual because phylloxera developed so rapidly on them. These latter accessions might be useful in examinations of how grape hosts influence the developmental rate and reproductive behavior of phylloxera.

No phylloxera survived on the following accessions: *V. aestivalis* DVIT 7109 and 7110, *V. berlandieri* c9031, *V. cinerea*, *V. riparia* (excluding DVIT 1411), *V. rupestris* DVIT 1418 and 1419, and *M. rotundifolia*. The lack of phylloxera on these accessions seems to indicate that they would make ideal parents for rootstock programs.

The dual culture system presented here provides a method for evaluating phylloxera resistance, and studying phylloxera biology and grape/phylloxera interactions. It produces results in eight weeks, takes less space than greenhouse or field-based methods, and provides a means of quarantining aggressive strains of this pest. Testing of material can be done over time because of the relative uniformity of the *in vitro* environment in terms of media and growing conditions. A limitation and benefit of this testing system is that it provides an ideal environment for phylloxera feeding and may overemphasize the apparent susceptibility of some hosts. Thus, when no feeding occurs in this environment it is likely that the host will have very strong resistance in the field. However, the establishment of phylloxera in dual culture with grape is labor intensive, as are the observations needed to detail the process.

The results of this study show that the phylloxera resistance of North American *Vitis* species is variable and that accessions of these species should be evaluated before use in a rootstock breeding program. These results need to be confirmed for North American *Vitis* species which may be tolerant of phylloxera feeding, allowing development of the insect without negative impact on the host, and for those that react differently under field conditions.

Literature Cited

1. Askani, A., and R. Beiderbeck. *In vitro* propagation of *Dactylosphaera vitifolii* Shimer (Homoptera: Phylloxeridae) on shoot and root culture of a *Vitis* hybrid. Vitis 30:223-232 (1991).

2. Bailey, L. H. The species of grape peculiar to North America. Genetes Herb. 3:149-244 (1934).

3. Boubals, D. Etude de la distribution et des causes de la resistance au phylloxera radicicole chez les Vitacees. Ann. Amelior. Plantes 16:145-184 (1966).

4. Boubals, D. Heredite de la resistance au phylloxera radicicole chez la vigne. Ann. Amelior. Plantes 16:327-347 (1966).

5. Comeaux, B. L., W. B. Nesbitt, and P. R. Fantz. Taxonomy of the native grapes of North Carolina. Castanea 52:197-215 (1987).

6. Davidson, W. M., and R. L. Nougaret. The grape phylloxera in California. USDA Bull. 903:1-128 (1921).

7. Davidis, U. X., and H. P. Olmo. The *Vitis vinifera* x *V. rotundifolia* hybrids as phylloxera resistant rootstocks. Vitis 4:129-143 (1964).

8. De Benedictis, J. A., and J. Granett. Laboratory evaluation of grape roots as hosts of California grape phylloxera biotypes. Am. J. Enol. Vitic. 44:285-291 (1993).

9. De Benedictis, J. A., J. Granett, and S. P. Taormino. Differences in host utilization by California strains of grape phylloxera. Am. J. Enol. Vitic. 47:373-379 (1996).

10. Forneck, A., M. A. Walker, and N. Merkt. Aseptic dual culture of grape (*Vitis* spp.) and grape phylloxera (*Dactulosphaira vitifoliae* Fitch.). Vitis 35:95-97 (1996)

11. Granett, J., P. Timper, and L. A. Lider. Grape phylloxera (*Dactulosphaira vitifoliae*) (Homoptera : Phylloxeridae) biotypes in California. J. Econ. Entomol. 78:1463-1467 (1985).

12. Granett, J., A. C. Goheen, L. A. Lider, and J. J. White. Evaluation of grape rootstocks for resistance to type A and type B grape phylloxera. Am. J. Enol. Vitic. 38:298-300 (1987).

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

14. Grzegorczyk, W., and M. A. Walker. Surface sterilization of grape phylloxera eggs in preparation for *in vitro* culture with *Vitis* species. Am. J. Enol. Vitic. 48:157-159 (1997).

15. Hayne, A. P. Resistant vines; their selection, adaptation and grafting. Calif. Exp. Sta. Appendix Viticultural Report 1896. 37 pp. University of California (1897).

16. Hilgard, E. W. Phylloxera-resistant vines. Calif. Exp. Sta. Appendix VI to the Report of the Viticultural Work During the Seasons of 1885 and 1886. pp 139-154. University of California (1886).

17. King, P. D., and G. Rilling. Variations in the galling reaction of grapevines: evidence of different phylloxera biotypes and clonal reaction to phylloxera. Vitis 24:32-42 (1985).

18. Lider, L. A. Phylloxera-resistant grape rootstocks for the coastal valleys of California. Hilgardia 27:287-318 (1958).

19. Rilling, G. Zur frage der direkten oder indirekten schadigung von rebenwurzeln bei befall durch die reblaus (*Dactulosphaera vitifolii* Shimer). Vitis 14:40-42 (1975).

20. Viala, P., and L. Ravaz. American Vines, translation of the Second Edition by R. Dubois and E.H. Twight., 299 pp, Freygang-Leary Co., San Francisco (1903).

21. Walker, A., J. Wolpert, E. Weber, R. Smith, L. Bettiga, and P. Verdegaal. Breeding rootstocks for California's current and impending viticultural problems. Grape Grower 6:11-18 (1994).