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Effects of fungal root infections on the vigor of grapevines infested by root-feeding grape phylloxera

by

A. D. Omer[1]), J. Granett[1]), J. A. De Benedictis[1]) and M. A. Walker[2])

[1]) Department of Entomology, University of California, Davis, USA
[2]) Department of Viticulture and Enology, University of California, Davis, USA

Summary: The role of fungal pathogens in damage of grapevines (*Vitis vinifera* L.; cv. Chardonnay) associated with grape phylloxera (*Daktulosphaira vitifoliae* Fitch) was investigated. Seven different genera of secondary fungi were isolated from surface-disinfected feeding sites of phylloxera but none from surface-disinfected root tissues undamaged by phylloxera. Damage in vines infested with phylloxera and infected with *Fusarium solani* (Mart.) or with *F. solani* and *Pythium ultimum* Trow. was significantly greater than damage in vines infested with phylloxera only. In a greenhouse experiment, total biomass was reduced by 16 % in vines infested with phylloxera and 24 to 29 % in vines infested with phylloxera and infected with fungus in comparison with control vines. Chlorophyll content, average internode length, shoot biomass, and root biomass in the uninfested, uninfected vines were significantly greater than vines infested with phylloxera or vines infested with phylloxera and infected with *F. solani* or *P. ultimum*, or both. Preventative treatment with metalaxyl, benomyl or copper quinolinolate fungicides significantly decreased damage in phylloxera-infested vines in comparison with untreated vines. The implications of this research with respect to management of grape phylloxera are discussed.

Key words: grape phylloxera, Daktulosphaira vitifoliae, damage, secondary fungi, infection, Vitis vinifera, grapevine, fungicides.

Introduction

Grape phylloxera, *Daktulosphaira vitifoliae* (Fitch), is a major insect pest of grapes worldwide. More than 2 million ha of European vineyards were destroyed by phylloxera between 1869 and 1900 (Ordish 1987). Phylloxera has also caused considerable losses in the United States, Australia, New Zealand, South Africa, and Argentina (Coombe 1963; Commonwealth Institute of Entomology 1982). Recently, phylloxera outbreaks have been devastating vineyards in the California counties of Napa and Sonoma (Weber 1992), leading to losses currently in the hundreds of millions of dollars (Anonymous 1994). Despite the long history of losses due to phylloxera, the causes and progression of root damage and vine decline are poorly understood.

In previous studies (Boubals 1966; King and Rilling 1985), phylloxera damage to grape rootstocks was assessed by examining root swellings induced by phylloxera feeding. Omer *et al.* (1995) demonstrated that swellings on mature roots (tuberosities) formed by phylloxera feeding enhanced the vulnerability of grape roots to subsequent attack from phylloxera. Feeding of phylloxera on grape roots may provide avenues for infections by secondary rot fungi which lead to increased damage. Millardet (1892) suggested that secondary pathogens which require avenues of entry caused by phylloxera feeding may be responsible for damage attributed to phylloxera. Rilling (1975) demonstrated that phylloxera alone was capable of damaging grape roots. Vannacci *et al.* (1984) presented limited evidence of association of microflora with grape roots infested with phylloxera, but the nature and causes of damage associated with grape phylloxera were not determined.

The microflora associated with grape roots infested with phylloxera could be involved with phylloxera in damaging grape roots, so we investigated whether fungicides could lessen or prevent vine decline. This led to greenhouse and field research to isolate and identify fungal pathogens from feeding sites of phylloxera on roots and determine the effects of fungal infection with and without the presence of phylloxera on grapevine vigor. Here, we describe our research and discuss the implications of our findings.

Materials and methods

We investigated the effects of fungal infections on the vigor of grapevines infested by root-feeding grape phylloxera in three experiments.

Experiment 1. The effects of fungicidal treatments on the vigor of phylloxera-infested, potted plants were evaluated. We first determined whether 3 fungicides, metalaxyl 2 E (Ridomil , Ciba-Geigy, Greensboro, NC), benomyl 50 % WP (Benlate , Du Pont, Wilmington, DE), and copper quinolinolate 80 % WP (Cidal Systems, San Jose, CA), altered the establishment of phylloxera on fungicide-treated root pieces using a 25-d bioassay (De Benedictis and Granett 1992). We then treated *Vitis*

Correspondence to: Dr. A. D. Omer, University of California, Department of Entomology, Davis, CA 95616, USA.

vinifera L. cv. Chardonnay grapevines from the Foundation Plant Material Service (University of California, Davis) with fungicide by dipping roots in a solution of 2,285 ppm metalaxyl, 600 ppm benomyl, or 958 ppm copper quinolinolate for 10 min. We planted vines in 3.8 l plastic pots in steam-sterilized clay soil from vineyards in Napa County, California. These vines were compared with untreated controls planted in unsterilized soil from the same source.

Potted vines were infested by burying paper packets containing surface-sterilized phylloxera eggs and root pieces infested with phylloxera adults and nymphs at the periphery of the roots. Phylloxera eggs or root pieces infested with phylloxera were disinfected with 1.6 % sodium hypochlorite solution for 5 min, then rinsed with sterile distilled water. Twenty replicates, one potted vine to a replicate, were established for each of the 3 treatments and the control. The total of 80 potted vines were arranged in a completely randomized design in a greenhouse maintained between 21-32 °C. Treated and control vines were equally watered as needed. Phylloxera establishment was verified one month after infestation by examining the roots of 2 randomly selected potted vines from each of the 3 treatments and the control.

Chlorophyll content, shoot biomass, shoot length, and root biomass are important measures of vine growth and productivity (WINKLER *et al.* 1974). We assessed vine vigor using these measures 2 months after grapevines were infested with phylloxera. Chlorophyll content was measured with SPAD 502 Chlorophyll Meter (Minolta Camera Co., Ltd., Osaka, Japan). Because leaves from the middle of the vine canopy tended to give high and consistent chlorophyll values, we randomly selected 3 middle leaves and recorded their average chlorophyll content for each vine. At the termination of the experiment, approximately 3.5 months after phylloxera infestation, all vines were cut at soil level and shoot length and biomass were determined. Root biomass, counts of feeding populations of phylloxera, numbers of tuberosities and nodosities (swellings on growing rootlets) were also determined for each vine.

Association of fungi with phylloxera feeding sites was examined in the laboratory by placing surface-disinfected sections of tuberosities, nodosities, and undamaged roots from each treatment onto acidified potato dextrose agar (APDA) media in sterile plastic Petri plates using the methods of TSAO (1970). Inoculated plates were incubated at 24 °C for 6 d. Fungi isolated from these samples were examined under a compound microscope and identified based on colony and spore characteristics. Pure cultures of the most frequently isolated fungi in this experiment were prepared and retained in the laboratory for further assays.

E x p e r i m e n t 2. The effects of the two most frequently detected fungal pathogens from our first experiment, *Fusarium solani* (Mart.) and *Pythium ultimum* Trow., on phylloxera-infested and -uninfested grapevines were investigated. We began by testing the inoculation procedures of TUITE (1969) and DE VAY *et al.* (1982) on Chardonnay roots. We punctured the roots with a sterile syringe to simulate a phylloxera feeding site, exposed the root system to fungus by dipping into a conidial suspension of each fungus for 10 s, then planted the vines in 3.8 l pots of sterilized soil moistened with the fungal suspension. Fungal inocula were prepared by blending 1-week-old pure cultures in sterile distilled water in a food-preparation blender for 1 min. A control was established in a similar manner but dipped in a suspension of plain agar. There were 8 vines in each treatment or control. 3 weeks later, vines exposed to the fungus were considered to be infected by the isolation of the pathogen and development of dark discoloration around the puncturing point.

Next, we set up 5 different treatments and a control to assess vine damage due to phylloxera alone and damage due to phylloxera and infections by *F. solani*, *P. ultimum*, or both. 18 Chardonnay grapevines were randomly assigned to each of the following treatments: I, a control in which vines were not infested with phylloxera and not exposed to the fungus; II, vines infested with phylloxera only; III, vines infested with phylloxera and exposed to *F. solani*; IV, vines infested with phylloxera and exposed to *P. ultimum*; V, vines infested with phylloxera and exposed to both *F. solani* and *P. ultimum*; and VI, vines exposed to both *F. solani* and *P. ultimum* without phylloxera. Vines were planted in 3.8 l pots with sterilized clay soils as in the first experiment. Roots were dipped in 10 % copper quinolinolate solution which inhibited growth of *F. solani* and *P. ultimum* in the laboratory, and washed in sterile distilled water before planting.

Vines exposed to fungus were infested with phylloxera as described in Expt. 1. All vines were maintained in the greenhouse. To control for potential temperature gradients in the greenhouse, the total of 108 potted vines were arranged in 6 replicated blocks. Vines were watered and fertilized with liquid plant food (10-15-10 [N/P/K]; Schultz-Instant, St. Louis, MO) as needed. Chlorophyll content, shoot length, number of shoot nodes, and shoot biomass were determined 7 weeks after planting. To prevent damage from leaf pests and pathogens, all vines were pruned at the 4th or 5th stem node from soil level at this time.

Pruned vines were held in the greenhouse for an additional 6 weeks during which vines were treated weekly with 1.3 g/l sulfur for powdery mildew control. Wetting of soil with sulfur solution was kept minimal. When the experiment was terminated at week 13, we measured chlorophyll content, shoot length, number of nodes, and shoot biomass for each vine as done in the initial evaluation. We combined data from shoot length and number of shoot nodes in a single estimate, the average internode length, by dividing shoot length by number of shoot nodes for each vine. We also determined root biomass and numbers of tuberosities and nodosities. We estimated number of feeding phylloxera by examining all roots of all vines. Two vines from the uninfested control group were found to be contaminated with only one phylloxera each and were excluded from the analysis. Infectivity of *F. solani* and *P. ultimum*, was confirmed by placing root samples from each of the 18 vines of each treatment or control onto APDA media as previously described.

Data collected were subjected to analysis of variance (ANOVA) with means separation by Duncan's multiple

range tests at $\alpha = 0.05$ to identify differences among the treatments. Data were analyzed using the SAS statistical package (Sas Institute 1989). We used the ANOVA to test for treatment effects, block effects, and treatment by block interactions in Expt. 2, on chlorophyll content measurements, average internode length, shoot biomass, root biomass, phylloxera populations, and numbers of tuberosities and nodosities formed. Data from phylloxera populations and numbers of tuberosities and nodosities were transformed (square root [x + 0.5]) to stabilize the variance prior to ANOVA. For tests of significance, the 'H=' option was used to specify the appropriate error term (see STEEL and TORRIE 1980). Means separation was made by Duncan's multiple range tests at $\alpha = 0.05$.

Experiment 3. Twenty Ganzin 1 (*V. vinifera* x *V. rupestris* SCH.) root samples infested with phylloxera from different sites in a Napa County vineyard. We examined association of fungi with phylloxera in these samples by placing surface-disinfected sections of damaged tissues with tuberosities or nodosities and undamaged tissues without tuberosities or nodosities onto APDA media in sterile plastic Petri plates. The inoculated plates were held at 20 °C for 6 d before results were assessed. Fungi isolated from these field samples were identified by colony and spore characteristics, and their incidence in damaged and undamaged roots was recorded.

Results and discussion

Experiment 1. Phylloxera established at similar rates (mean % ± SD) on roots treated with metalaxyl (71.0 ± 16.5), benomyl (64.0 ± 13.0), copper quinolinolate (66.0 ± 11.5), or on untreated roots (72.5 ± 11.5) indicating that fungicide treatment has no detrimental effects on phylloxera establishment. There were no significant differences in numbers of tuberosities ($P = 0.59$) or nodosities ($P = 0.25$) among treatment and control vines. Nodosities were more abundant than tuberosities. Chlorophyll content, shoot length, shoot biomass, and root biomass were significantly higher ($P < 0.05$) in fungicide-treated than untreated vines (Tab. 1). The reduced vigor of untreated vines suggests that fungicides decrease damage of phylloxera-infested grapevines.

Secondary fungi are weak pathogens incapable of penetrating the epidermal layer of their host plants (AGRIOS 1988). Our results revealed that secondary fungi are associated with phylloxera feeding sites. We isolated seven different genera of secondary fungi from surface-disinfected tuberosities and nodosities but none from surface-disinfected undamaged root tissues (Tab. 2). Isolation of secondary fungi from nodosities and tuberosities but not from undamaged root tissues implicates fungal infections in the etiology of vine damage and suggests that the observed decline in vigor of the untreated vines is not solely due to phylloxera. In other studies, it has been shown that root feeding by nematodes predispose beans (HUTTON *et al.* 1973) and citrus rootstocks (GRAHAM and TIMMER 1992) to root rot infections.

In this study, feeding of phylloxera on grape roots may have created entry ports for infections with these fungi and resulted in depressed vine growth. Secondary fungi were isolated at higher frequencies from roots of untreated than treated vines. Lower incidence of fungi in the treated

Table 1

Effect of fungicides on vine damage associated with grape phylloxera

Treatment	Growth measures evaluated						
	Chlorophyll content (SPAD units)	Shoot biomass (g)	Shoot length (cm)	Root biomass (g)	Phylloxera population (n)	Tuberosities formed (n)	Nodosities formed (n)
Control	35a*	28c	25a	17a	30a	2.5a	17a
Cu quinolinolate	43b	32a	35b	21b	21b	2.2a	15a
Metalaxyl	40b	31ab	35b	20b	22b	2.3a	13a
Benomyl	41b	30b	33b	20b	24ab	1.9a	17a

* means in columns followed by the same letters are not significantly different at $\alpha = 0.05$ using Duncan's multiple range test.

Table 2

Incidence of secondary fungi in phylloxera-damaged and undamaged grape roots

Treatment	Frequency of secondary rot fungi/20 samples							
	Undamaged root tissues	Damaged root tissues						
		Fusarium	*Trichoderma*	*Rhizoctonia*	*Alternaria*	*Pythium*	*Mucor*	*Penicillium*
Control	0	20	9	5	8	9	5	3
Cu quinolinolate	0	5	0	2	2	0	3	2
Metalaxyl	0	7	5	3	2	1	3	2
Benomyl	0	10	3	3	3	0	2	2

Table 3

Effect of grape phylloxera infestation and fungal infection on grapevine vigor before pruning at week 7

Treatment[a]	Growth measures evaluated			
	Chlorophyll content (SPAD units)	Shoot length (cm)	Shoot nodes (n)	Shoot biomass (g)
I	35.1a*	45.7a	13.4a	25.0a
II	35.3a	45.0a	13.1a	24.1a
III	35.1a	42.7a	12.9a	24.1a
IV	34.9a	43.4a	13.2a	23.5a
V	35.2a	41.8a	12.9a	23.9a
VI	36.1a	43.9a	13.2a	25.3a

[a] I, uninfested, unexposed control; II, infested with phylloxera only; III, infested with phylloxera and exposed to *F. solani*; IV, infested with phylloxera and exposed to *P. ultimum*; V, infested with phylloxera and exposed to both fungi; VI, uninfested with phylloxera but exposed to both fungi.
* means in columns followed by the same letters are not significantly different at $\alpha = 0.05$ using Duncan's multiple range test.

vines suggests that fungicidal treatment suppresses secondary fungi and protects grapevines against root infections.

Experiment 2. Infestation with phylloxera alone or combined with exposure to *F. solani* or *P. ultimum* did not show detrimental effects on vine growth at the time of the initial evaluation at week 7 (Tab. 3). No significant effects of block (P ranged from 0.08 to 0.76) or block by treatment interaction (P ranged from 0.13 to 0.87) on chlorophyll content, shoot length, number of shoot nodes or shoot biomass were detected. We attribute the lack of significant differences in plant growth among treatments during the first 7 weeks of the experiment to rapid growth after potting. Also, phylloxera may have required longer time to establish feeding sites and provide avenues for fungal infections.

Evaluation of plant growth at week 13 showed differences among the treatments (Tab. 4). Chlorophyll content, average internode length, shoot biomass, and root biomass in the uninfested, unexposed control vines (treatment I) were significantly greater ($P < 0.05$) than vines infested with phylloxera (treatment II) or vines infested with phylloxera and exposed to *F. solani* (treatment III) or *P. ultimum* (treatment IV) or both (treatment V). Vines not infested with phylloxera but exposed to *F. solani* and *P. ultimum* (treatment VI) did not differ significantly from the uninfested, unexposed control vines in these growth measures. No significant block effects (P ranged from 0.07 to 0.77) or block by treatment interaction (P ranged from 0.25 to 0.99) on chlorophyll content, average internode length, shoot biomass or root biomass were detected. Average internode length, shoot biomass, and root biomass were significantly lower in vines infested with phylloxera and exposed to *F. solani* or to *F. solani* and *P. ultimum* than vines infested with phylloxera only. Chlororophyll content was significantly higher in vines infested with phylloxera only than in vines infested with phylloxera and exposed to both fungi. Chlorophyll content, average internode length, shoot biomass, and root biomass were low in vines infested with phylloxera and exposed to *P. ultimum* but not significantly different from vines infested with phylloxera only. Exposure to fungus in the absence of phylloxera did not significantly impair vine growth. Total biomass was reduced by 16 % in vines infested with phylloxera and 24 to 29 % in vines infested with phylloxera and exposed to fungus in comparison with control vines.

The significantly greater growth of vines exposed to both fungi but not infested with phylloxera than of vines infested with phylloxera and exposed to either or both fungi demonstrates that phylloxera feeding provides entry ports for infections by secondary fungi, and suggests that the observed vine damage cannot be attributed to the activity of phylloxera alone. Fungal infections appeared to worsen damage to phylloxera-infested grapevines. Damage in vines infested with phylloxera only was observed confirming the results of RILLING (1975). However, this damage was significantly less than damage in vines infested with phylloxera and exposed to *F. solani* or to *F. solani* and *P. ultimum*. Damage to vines that were infested with phylloxera and exposed to *F. solani* and *P. ultimum* was greater than damage to vines infested with phylloxera and exposed

Table 4

Effect of grape phylloxera infestation and fungal infection on grapevine growth after pruning at week 13

Treatment[a]	Growth measures evaluated				
	Chlorophyll content (SPAD units)	Average internode length (cm)	Shoot biomass (g)	Root biomass (g)	Total biomass[b] (% of control)
I	32.8a*	3.4a	17.4a	20.1a	-
II	28.1b	3.0b	15.1b	16.3b	84
III	24.8bc	2.4c	12.8c	13.9c	71
IV	25.5bc	2.7bc	13.7bc	14.6bc	76
V	24.1c	2.2c	12.9c	13.7c	71
VI	31.4a	3.5a	17.1a	19.0a	96

[a] I, II, III, IV, V, VI: see Tab. 3.
* means in columns followed by the same letters are not significantly different at $\alpha = 0.05$ using Duncan's multible range test.
[b] shoot biomass and root biomass combined.

to *F. solani* but not to *P. ultimum*. Decreased effects on growth due to *P. ultimum* infection may be attributed to varietal susceptibility, fungal strain, or both. The lowest average internode length in vines infested with phylloxera and exposed to *F. solani* and *P. ultimum* suggests that fungal infections associated with phylloxera infestations disrupts vine physiology and stunts growth.

There were no significant differences in numbers of nodosities, tuberosities or feeding phylloxera among the infested treatments (Tab. 5). No significant block effects (P ranged from 0.08 to 0.89) or block by treatment interaction (P ranged from 0.76 to 0.99) on numbers of nodosities, tuberosities or phylloxera populations were detected. Phylloxera produced more nodosities than tuberosities in the infested vines as they did in Expt. 1.

Table 5

Effect of grape phylloxera infestation and fungal infection on grapevine root system

Treatment[a]	Growth measures evaluated		
	Nodosities formed (n)	Tuberosities formed (n)	Phylloxera population (n)
I	0.0a*	0.0a	0.0a
II	15.0b	1.3b	28.7b
III	12.4b	1.3b	23.2b
IV	13.7b	1.1b	26.8b
V	12.9b	1.4b	23.3b
VI	0.0a	0.0a	0.0a

[a] I, II, III, IV, V, VI: see Tab. 3.
* means in columns followed by the same letters are not significantly different at a = 0.05 using Duncan's multiple range test.

Fusarium solani or *P. ultimum*, or both fungi were re-isolated from vines exposed to fungus but not from the unexposed control vines (Tab. 6). *Fusarium solani* and *P. ultimum* were re-isolated from 11 and 13 vines of the 18 phylloxera-infested vines initially exposed to each fungus, respectively. We were unable to re-isolate fungi from only 2 of the 18 vines that were infested with phylloxera and exposed to both *F. solani* and *P. ultimum*. Fungi not re-isolated from the exposed vines may have lost their infectivity before phylloxera established feeding sites or before the entry sites became available for the fungus. Infection in vines that were not infested with phylloxera but exposed to fungi may have occurred at sites with other types of root injury.

Table 6

Fungi re-isolated from roots of potted grapevines in APDA media

Vine	Treatments[a]					
	I	II	III	IV	V	VI
1	-	-	-	P[b]	F	-
2	-	-	F	P	F	-
3	-	-	F	P	P	-
4	-	M	F	P	-	-
5	-	-	F	P	P+F	P
6	-	-	-	-	P+F	P+F
7	*[c]	-	F	P	P	-
8	*	-	-	P	F	-
9	M	M	-	-	P+F	-
10	-	-	F	P	-	P
11	-	-	-	-	F	F
12	-	-	F	P	F	-
13	-	-	F	P	P+F	-
14	-	-	-	P	P+F	Pn
15	-	-	F	-	P	-
16	-	-	F	P	P+F	-
17	-	-	F	P	P+F	-
18	-	-	-	-	P+F	-

[a] I, II, III, IV, V, VI: see Tab. 3.
[b] P, *P. ultimum*; M, *Mucor*; F, *F. solani*; Pn, *Penicillium*.
[c] excluded from assays because of phylloxera infestation.

Experiment 3. Assay for secondary fungi in grape root samples collected from the field indicated that more fungi were isolated from surface-disinfected roots damaged with tuberosities or nodosities than from surface-disinfected undamaged root tissues (Tab. 7), suggesting that phylloxera infestation promotes fungal invasion and infection. In Italy, VANNACCI *et al.* (1984) surveyed microorganisms in vineyards infested with phylloxera and isolated more fungi from roots infested with phylloxera than from uninfested roots. Results of fungal isolation from the field samples suggest that *P. ultimum* and *F. solani* are in

Table 7

Incidence of secondary fungi in grape roots from a phylloxera-infested vineyard, Napa County, California

Root tissue assayed	Frequency of secondary rot fungi/20 samples								
	Cylindrocarpon	*Macrophominia*	*Fusarium*	*Trichoderma*	*Rhizoctonia*	*Alternaria*	*Pythium*	*Mucor*	*Penicillium*
damaged	3	2	9	3	4	2	14	1	2
undamaged	1	1	0	5	0	0	2	0	1

the sampled Napa County vineyard. Although we cannot predict damage in phylloxera-infested vineyards due to these fungi with certainty, data presented here suggest that combined infection by *F. solani* and *P. ultimum* would be more damaging to phylloxera-infested grapevines. Different grape cultivars and rootstocks may respond differently to grape phylloxera and the presence of fungal pathogens in different soil types. Damage in the field must be quantified to understand cultivar-phylloxera-fungal interactions.

Our research has several implications for management of grape phylloxera. We demonstrated that infections by secondary fungi contribute substantially to damage in phylloxera-infested vines, and showed that prophylactic fungicidal treatment decreased infections and damage. These greenhouse experiments suggest that use of fungicides in vineyards could moderate or reverse phylloxera-related damage symptoms, but this possibility is probably not realistic. Treating the entire volume of soil encompassing grape root systems is prohibitively difficult as is the case with insecticides which usually do not control the insect because of their uneven coverage and inability to penetrate to the depths occupied by phylloxera. In addition, soil treatments might not enable the fungicide to reach internal root infections unless the fungicide had systemic activity. Systemic fungicides would obviate treatment of soil mass, but none has been tested on grape phylloxera-infested vines. Even if fungal pathogens were eliminated, our greenhouse results suggest that unless phylloxera is controlled substantial damage would still occur. An alternative chemical-based strategy might be to emphasize the prophylactic rather than curative use of fungicides and/or insecticides. A preventative strategy might keep the infections from occurring in the first place. We are currently testing such a strategy.

Damage at vineyard sites depends on susceptibility of roots to phylloxera, ability of roots to tolerate their wounds, prevalence of pathogens at wound sites, and susceptibility of the cultivar to infections. If we are to develop economic injury levels for phylloxera, we must consider all of these factors. Because each of these factors is affected by the environment and the physiology of the vine, developing a comprehensive economic injury level may be impossible.

Lastly, this research suggests that grape rootstock breeding for grape phylloxera resistance can be improved by selecting for resistance to secondary pathogens. Such a resistance factor has not been considered crucial in the past.

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References

Agrios, G. N.; 1988: Plant Pathology. 3rd ed. Academic Press Inc., San Diego, CA, USA.

Anonymous; 1994: California wine industry winning battle against phylloxera. Amer. Vineyard. **3** (8), 6-7.

Boubals, D.; 1966: Étude de la distribution et des causes de la résistance au phylloxéra radicole ches les vitacées. Ann. Amélior. Plantes. **16**, 145-185.

Commonwealth Institute of Entomology; 1982: Distribution Maps of Pests, Series A, CAB International, Wallingford, UK, Map No. 339.

Coombe, B. G.; 1963: Phylloxera and its relation to South Australian viticulture. Tech. Bull. No. 31. Dept. of Agricult., South Australia.

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

DeVay, J. E.; Garber, R. H.; Matheron, D.; 1982: Role of *Pythium* species in the seedling disease complex of cotton in California. Plant Dis. **66**, 151-154.

Graham, J. H.; Timmer, L. W.; 1992: *Phytophthora* diseases of citrus. In: Kumar, J.; Chaube, H. S.; Singh, U. S.; Mukhopadhyay, A. N. (Eds.). Plant Diseases of International Importance. Vol. III. Diseases of Fruit Crops, 250-269. Prentice-Hall, Englewood Cliffs, NJ, USA.

Hutton, D. G., Wilkinson, R. E.; Mai, W. F.; 1973: Effect of two plant-parasitic nematodes on *Fusarium* dry rot of beans. Phytopathology **63**, 749-751.

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

Millardet, A.; 1892: New researches upon the resistance of, and exemption from, phylloxera. In: Annual Report of the Board of State Viticultural Commissioners for 1891-92, 265-279. State Printing, Sacramento, CA, USA.

Omer, A. D.; De Benedictis, J. A.; Granett, J.; 1995: Grape phylloxera, *Daktulosphaira vitifoliae* (Fitch), population response to preformed tuberosities. J. Appl. Entomol. (in press).

Ordish, G.; 1987: The Great Wine Blight. J. M. Dent & Sons, London.

Rilling, G.; 1975: Zur Frage der direkten oder indirekten Schädigung von Rebenwurzeln bei Befall durch die Reblaus (*Dactylosphaera vitifolii* Shimer). Vitis **14**, 40-42.

Sas Institute Inc.; 1989: SAS/STAT Users Guide, version 6, 4th ed. SAS Institute Inc., Cary, NC, USA.

Steel, R. G. D.; Torrie, J. H.; 1980: Principles and Procedures of Statistics, 2nd. ed., New York, McGraw-Hill, NY, USA.

Tsao, P. H.; 1970: Selective media for isolation of pathogenic fungi. Ann. Rev. Phytopathol. **8**, 157-186.

Tuite, J.; 1969: Plant Pathological Methods. Burgess Pub. Co., Minneapolis, MN, USA.

Vannacci, G.; Triolo, E.; Lorenzini, G.; 1984: First report on the microflora associated to grapevine roots infested by phylloxera. Riv. Ortofloro-frutticolt. **68**, 21-23

Weber, E.; 1992: Phylloxera survey results. Vineyard Views. 29 January 1993. Newsletter.

Winkler, A. J.; Cook, J. A.; Kliewer, W. M.; Lider, L. A.; 1974: General Viticulture. 2nd ed. University of California Press, Berkeley, CA, USA.

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