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Fungal infections of grapevine roots in phylloxera-infested vineyards

by

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S u m m a r y : Wounds caused by feeding of grape phylloxera on grape roots can become infected with a variety of fungi. *Fusarium roseum*, *F. oxysporum* and *Pythium ultimum* are important in *Vitis vinifera* Chardonnay wounds whereas *F. oxysporum* and *Cephalosporium* sp. are important for the moderately tolerant rootstock AXR#1. Proportion of root lengths infected in the phloem parenchyma were measured in two vineyards through the 1996 growing season and into the winter. Infection rates were highest in spring (as measured in May) but decreased to a low level by the end of summer. There was a second infection peak in fall. We suggest that the decline in fungal infections was due to death of highly infected roots and their removal from the sampled pool of roots. Loss of roots is a logical cause of vine decline and explains why there have been poor correlations between phylloxera populations and vine damage symptoms.

K e y w o r d s : grape phylloxera, Homoptera, Phylloxeridae, Daktulosphaira vitifoliae, Fusarium, Pythium, Cephalosporium, Vitis.

Introduction

Grape (*Vitis vinifera* L.) roots damaged by grape phylloxera, *Dactulosphaira vitifoliae* (FITCH) are gnarled with swellings (galls) that are subject to fungal rot (MILLARDET 1892; DAVIDSON and NOUGARET 1921). Galls can be found on mature roots (tuberosities) or on rootlets (nodosities). DAVIDSON and NOUGARET (1921) suggest that high moisture in soil hastens rotting of galls and vine decline. They suggest that rot organisms can infect galls during the wet months of the year and kill roots in a short time. Decay is less during dry months and in years that approach drought conditions. The relationship of high phylloxera populations to dry conditions was noted by HELM *et al.* (1991) though they did not relate populations to fungal conditions.

A question not answered in earlier vineyard research is whether the rot organisms associated with phylloxera galls are pathogenic, saprophytic or both. It may be that the gall itself results in dysfunction of the root and that the rot organisms are saprophytic, not appreciably harming healthy tissues. OMER *et al.* (1995) answered this question for young greenhouse-grown vines: exclusion of fungi from phylloxera-infested roots resulted in substantially less plant damage than when fungi were allowed to grow in conjunction with the phylloxera feeding sites. They showed that key fungi involved were *Fusarium* species and *Pythium ultimum* TROW. and that these taxonomic groups were also present in California vineyards within vine root tissues that had phylloxera galling. Although these fungi are common in vineyard soils, they are not listed as disease causing organisms by PEARSON and GOHEEN (1988) or FLAHERTY *et al.* (1992); apparently they are rarely invasive unless roots are damaged or unless phylloxera galls are present.

The observations of MILLARDET (1892) and DAVIDSON and NOUGARET (1921) on fungal infections in vineyards and the results of OMER *et al.* (1995) with greenhouse plants suggest the hypothesis that fungal infections associated with phylloxera galls are pathogenic and cause the decline observed in vineyards. To test this hypothesis we must first determine the species of fungi involved under different conditions and we must also better understand the temporal relationship between fungi, phylloxera and vine health. This research deals with the fungal species identified from phylloxera feeding sites in various locations in California and the temporal changes in percentage of roots damaged with fungal rots through the growing season in two locations. The temporal studies were at the same locations as the population measurements reported by OMER *et al.* (1998) thus enabling us to relate fungal activity to phylloxera activity.

Materials and methods

F r e q u e n c y o f r o t f u n g i : Roots infested with phylloxera were collected from one commercial vineyard in San Joaquin County, three in Mendocino County and one in Monterey County, California, during summer, 1996. Roots were obtained by digging at the base of vine trunks to a depth of not more than 0.3 m and cutting and withdrawing roots thus exposed. The roots were placed in plastic bags, kept cool and brought to the laboratory for processing. The presence of fungi at phylloxera feeding sites on roots was determined by removing the phylloxera, surface sterilizing the roots and placing root sections on acidified potato dextrose agar (APDA) medium in sterile plastic Petri dishes (TSAO 1970, OMER *et al.* 1995). Plates were incubated at 24 °C for 6 d and fungi identified under a compound microscope based on colony and spore characteristics. For each collection, we evaluated 25 to 100 feeding sites from 5 to 20 sampled vines and determined presence and frequencies of each fungal species.

D y n a m i c s a n d s e v e r i t y : We estimated seasonal changes in root infections in two vineyards at

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3-week intervals between May and November, 1996 with an additional winter sample in February, 1997. The first vineyard (own-rooted Chardonnay) was near Stockton in San Joaquin County, California. Fungal species were identified for this vineyard (Table) and we had a 2-year phylloxera population record for it (OMER *et al.* 1998). The second vineyard was near Ukiah in Mendocino County, California. This vineyard was planted on AXR#1 rootstock. Fungal species were identified from this vineyard (Mendocino vineyard 1, Table) and had a 1-year phylloxera population record for it (OMER *et al.* 1998). On each sampling date vines were chosen randomly in an area ascertained to be infested by phylloxera from preliminary excavations of roots and we collected root samples as described above from 20 vine positions not previously sampled. Root dry weights from each vine position varied between 100 and 300 g. Root weight was determined after air drying of samples for 2 weeks. Freshly excavated 2-15 mm diameter root pieces were cross-sectioned at 4 cm intervals along their entire lengths. Percentage of the circumference of each section that had fungal rot was estimated. Root age ranged from 2 to 5 years; it roughly corresponded with root diameter. We identified rot as a dark-brown to tan discoloration and granulation of the root tissues using a dissecting microscope. The discoloration was primarily seen in the region of the phloem parenchyma. We confirmed that this discoloration was symptomatic of fungal infections by the culturing methods described above. We averaged the percentages of rotted tissue for all sections for each root piece and each vine. Disease symptoms in the xylem region of the root were not quantified because they were uncommon and observed only on the most severely rotted root sections. We also evaluated the severity of rot infections across sampling dates by determining proportions of samples with 0 to 10 %, 11 to 50 % or 51 to 100 % rot classes.

We used a two-way analysis of variance (PROC GLM, SAS Institute 1989) to compare mean percentages of root rot across sampling dates. Percentages were transformed byto $\sqrt{(x+0.5)}$correct for heterogeneity of variance before conducting the analyses. Means were separated by DUNCAN'S (1955) multiple range tests at $\alpha = 0.05$.

Results and Discussion

Frequency of rot fungi: For purposes of this study rot fungi were identified to the genus level except for *Fusarium* and *Pythium* spp. which were identified for comparison with previous isolates from phylloxerated vineyards (OMER *et al.* 1995). Nine taxa of fungi were identified from wounds caused by phylloxera feeding on grapevine roots (Table). *Fusarium roseum* (SCHLECHT.), *F. oxysporum* (MART.) and *Pythium ultimum* TROW. were frequently present in the *V. vinifera*-rooted vineyards. On the other hand, *Cephalosporium* sp. was most common only in the phylloxera-infested AXR#1 root systems. *Fusarium oxysporum* was relatively common in all root systems. *Phytophthora* sp. and *Rhizoctonia* sp. were uncommon in all root systems. *Trichoderma* sp. and *Macrophomina* sp. were only found in the Mendocino County collections. Other species showed no cultivar or geographical patterns of occurrence. If these fungi are pathogenic as suggested by MILLARDET (1892), DAVIDSON AND NOUGARET (1921) and OMER *et al.* (1995), it is likely that each will have a different set of factors influencing growth and damage to vines. Moisture and temperature influence the growth of these fungi. Genetic and physiological characters of the vine host will also affect the infection rate, severity of damage, timing of activity and spread within and between vines. In addition, soil ecology will affect pathogenicity. Until we have information on the influence of these factors we cannot evaluate damage thresholds or propose cultural practices to moderate damage.

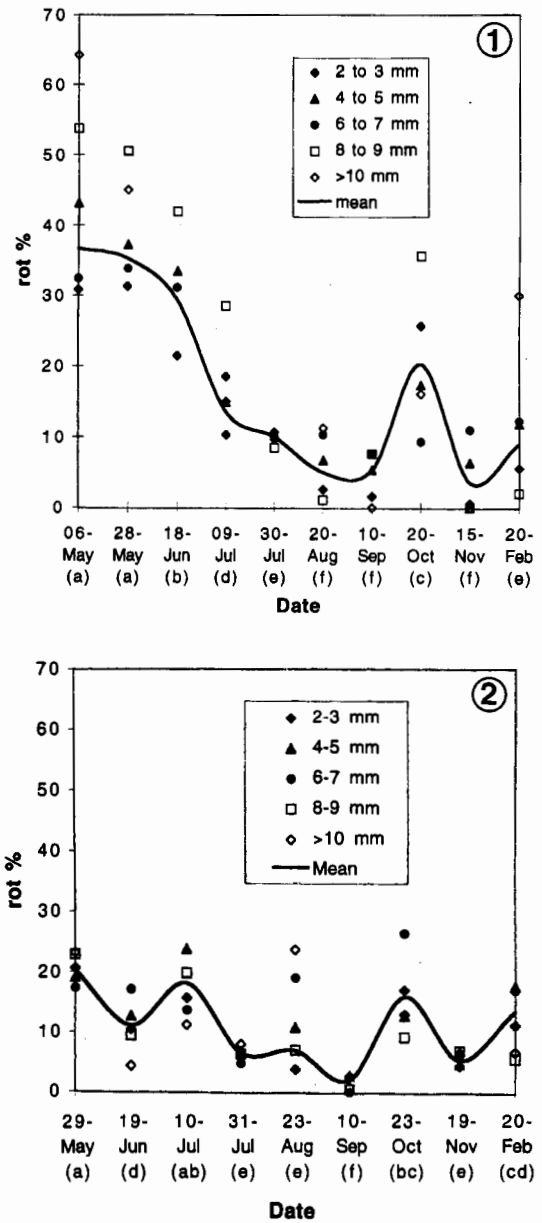
Dynamics and severity: The analysis of variance for the Stockton data showed significant effects

Table

Frequency of fungal infection in root wounds of grape phylloxera feeding in five California vineyards. Values represent frequencies of fungi in 25 to 100 wounds caused by grape phylloxera

	AXR#1		Chardonnay		
	Mendocino County (Vineyard 1)	Monterey County	San Joaquin County	Mendocino County (Vineyard 2)	Mendocino County (Vineyard 3)
Fusarium roseum	.05	.03	.26	.12	.20
Fusarium oxysporum	.24	.12	.19	.16	.18
Fusarium solani	.01	0	.03	.08	.08
Pythium	.04	.03	.22	.20	.05
Cephalosporium	.43	.79	0	.04	.05
Trichoderma	.05	0	0	.08	.08
Macrophomina	.02	0	0	0	.08
Phytophthora	0	0	.01	0	0
Rhizoctonia	0	.01	0	0	0
None	.25	.07	.20	0	.13

for sampling date ($F = 124.35$, $p < 0.0001$) and for root diameter ($F = 14.09$, $p < 0.0001$) on rot infections. Significant effects for sampling date ($F = 35.89$, $p < 0.0001$) and root diameter ($F = 2.80$, $p < 0.0246$) on rot infection were also found in the Ukiah data. Symptoms of rot infection were common in May and June and decreased to low levels by August and September at both locations (Figs. 1 and 2). The Stockton



Figs. 1 and 2: Average percentages of root circumference with fungal rot in the phloem parenchyma in a phylloxera-infested vineyard near Stockton, San Joaquin County (above) and Ukiah, Mendocino County, California (below), by sampling date and root diameter. Values for sample dates associated with similar letters are not significantly different by DUNCAN's (1955) multiple range test, $p < 0.05$.

vineyard was more severely infected than the Ukiah vineyard with the total infection in the former reaching 37 % and the latter reaching only 20 %. By September, however, rot infection decreased to around 5 % in both vineyards. This pattern of fungal infection appeared to have an inverse relationship to the phylloxera population density in the vineyards. Phylloxera populations at both the Ukiah and Stockton locations were very low in May and June and reached their summer peaks by the end of July (OMER *et al.* 1998). In addition, the Ukiah population density at its 1996 peak was twice that observed in Stockton (OMER *et al.* 1998) whereas rot infection at its peak (May observations) was higher in Stockton than in Ukiah.

Both vineyards experienced a second peak in rot infection in October (Figs. 1 and 2). These rises were temporary and occurred just after the root flush which was accompanied by an increase in phylloxera population (OMER *et al.* 1998).

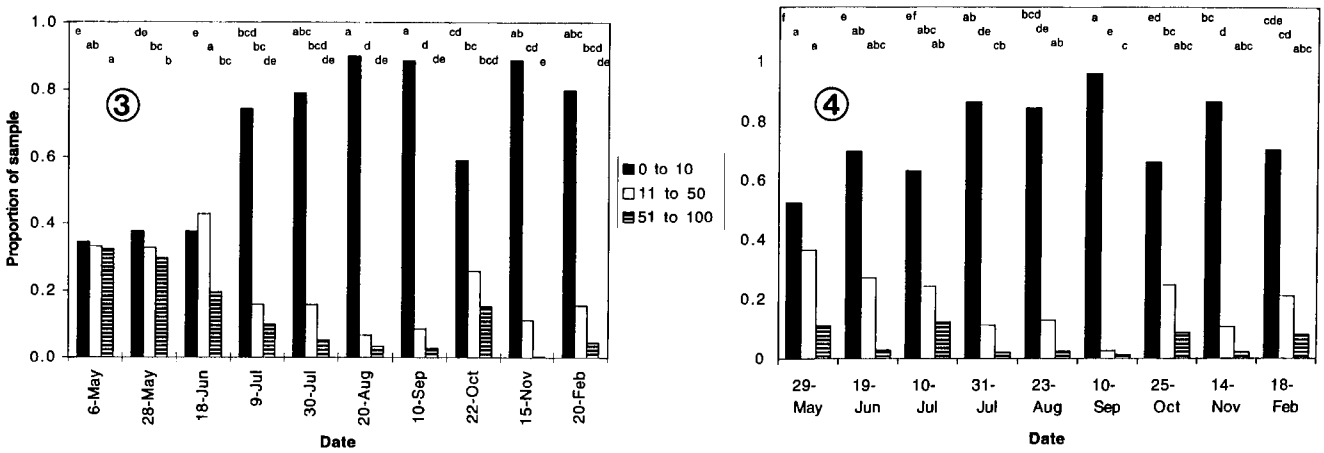
Trends in the rates of infection of roots of different sizes did not account for changes in the seasonal infection rates in either vineyard (Figs. 1 and 2).

From these data and the report of OMER *et al.* (1995), we hypothesize that fungal rot develops rapidly in vineyards and is a measure of root mortality. The rot fungi invade wounds caused by phylloxera and the damage, whether caused by phylloxera directly or indirectly by the fungi, results in mortality of root sections. This mortality is seen in our data from both vineyards as a decrease in the 11 to 50 % and 51 to 100 % rot classes between spring and summer (Figs. 3 and 4). As a result of this loss of highly infected roots, the relative proportion of roots with 0 to 10 % infection tends to increase during this time period. In addition, the increase in proportion of root samples with low levels of infection may occur because of *de novo* growth of small diameter roots, although data to support this idea were not collected. The increase in rot infection observed in fall (Figs. 1 and 2) may be related to the root growth that occurs at this time and parallels the increase in phylloxera populations seen at this time (OMER *et al.* 1998). Increase in root rot during winter is slow.

Our data suggest that loss of living roots is substantial in infected vineyards. In the Stockton vineyard, 32 % of the roots sampled in May were in the 51-100 % rot class. Yet this rot infection class decreased to less than 5 % of the roots sampled by September. The difference in percentages between these two sampling dates indicates that about 27 % of the root system sampled had died. The Ukiah vineyard experienced less loss for this rot class, about 8 % during the similar time period.

It is interesting to note that the infection does not increase from season to season. Thus, the roots appear to die within a relatively short time of becoming highly infected. Similarly, phylloxera populations do not accumulate from year to year (OMER *et al.* 1998). Because the purported causal agents of vine damage do not accumulate, economic thresholds based on populations of either fungi or insects cannot be valid. Our data suggest, however, that root loss can be associated with damage and suggest that quantification of changes in infection level, as we noted between spring and late summer, may estimate root losses and may presage above ground symptoms.

An important issue for vineyard managers is spread rate of phylloxera damage in vineyards. Spread rates of vineyard damage have been estimated at 2- to 4-fold per year (WILDMAN *et al.* 1983; 1988). Spread rates of damage may change from year to year (DAVIDSON and NOUGARET 1921).



Figs. 3 and 4: Proportion of root samples with 0 to 10 %, 11 to 50 % and 51 to 100 % fungal root rot in the phloem parenchyma in a phylloxera-infested vineyard near Stockton, San Joaquin County (left) and Ukiah, Mendocino County, California (right), by sampling date. Proportions are not significantly different by DUNCAN's (1955) multiple range test, $p < 0.05$ if letters within rows are similar.

Our research implies that damage may be modulated by factors which either influence phylloxera population growth, fungal infection or root decay. Factors likely to influence these rates are environmental conditions (e.g., temperature, moisture and soil organic matter), plant physiology (OMER *et al.* 1998) and soil ecology. Research is needed to determine how to manipulate these factors to decrease phylloxera populations, fungal infections and vine damage.

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