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Organic farming research project report submitted to the Organic Farming Research Foundation:

Project Title:

Soil ecology of grape phylloxera and the potential for biological control:

Differences in root damage caused by grape phylloxera in organic vs. onventionally managed Northern California vineyards

FINAL PROJECT REPORT

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Abstract. To test the hypothesis that the root-feeding insect, grape phylloxera, does less damage in organically managed vs. conventionally managed vineyards, soil and root samples were taken from vineyards in northern California. Greenhouse grown grape plants, inoculated with phylloxera showed less root rot when grown in soils from organically managed vineyards. Greater phylloxera numbers in the organic treatment can be attributed to the better nutritional status than the more deteriorated roots in the conventional treatments. Root samples from organically managed vineyards showed significantly less rot (11.8%) than roots from conventionally managed vineyards (27.1%). Phylloxera numbers in the vineyard root samples were not significantly different between organic and conventional, however there is a trend towards higher numbers in the organically managed vineyards, again most likely due to the better nutritional status of the less deteriorated roots.

The problem of phylloxera in California vineyards and existing, strategies for control

Grape phylloxera *Daktulosphaira vtlifoliae* (Fitch) (Homoptera: Phylloxeridae) is potentially the most serious grape *(Vtlis vinifera)* pest in the world (Granett et al. 1996). A native parasite of North American *Vitis* species, phylloxera is an aphid-like insect that feeds voraciously on the roots of European grapevines causing decline and eventual death. Resistant rootstocks developed by the French in the late 1800's, originating from selections of North American *Vitis* or crosses between North American *Vitis* species and *V. vinifera,* almost completely eliminated vineyard losses caused by phylloxera. One of these rootstocks, AXR#1, once constituted about 70% of the plantings in Napa and Sonoma counties of California (Granett et al. 1996). Populations of phylloxera known as Biotype B, capable of infesting the AXR#1 rootstock, were discovered in California and by the late 1980's vineyards in eight California counties were infested with it. Resistant rootstocks with no *V. vinifera* parentage are the only strategy being focused on for control of phylloxera, a process which entails ripping out phylloxera infested vineyards and replanting the stronger phylloxera resistant stock, often using methyl bromide for nematode and pathogen control.

In California, anecdotal accounts suggest that vineyard soils may be developed which are suppressive of phylloxera damage (Cantisano 1996, Leeds 1996) and in some cases phylloxera infested vineyards recovered to full production by implementation of specific cultural practices such as cover cropping in vineyards, use of organic amendments, and the elimination of pesticides and synthetic fertilizers. (Cantisano 1996). Although these practices are associated with organic farming methods as outlined in Drinkwater et al. (1995) and have been shown to be associated with increased soil microbial diversity, abundance, and activity as well as root pathogen suppression (Drinkwater et al. 1995, van Bruggen 1995), there has been virtually no assessment of the effects of soil microbial activity and organic soil management methods on grape vine root damage by insects or nematodes.

The California viticultural system is optimal for selection of phylloxera genotypes, which are tolerant of currently resistant rootstocks. Hundreds of thousands of acres of resistant stock interspersed by some 10,000 acres of infested vineyard on susceptible stock allows for intense selection pressure for tolerance.

Many vineyards have AXR#1 and new rootstocks interspersed and adjacent to each other, with roots of the respective stocks intermingling or just a few feet apart. The development of ecological methods for controlling phylloxera, which would not be subject to genotypic selection pressure, would allow for alternatives should new biotypes of phylloxera develop in California. Such a scenario as a tolerant phylloxera biotype invading recently (and expensively) replanted vineyards would likely generate intense grower pressure for pesticides. Already the potential exists for such a scenario. The 5C rootstock, one of the commonly planted phylloxera resistant stocks, has been reported in Germany to be breaking down in its resistance. The ancestry *(V. riparia X V. berlandieri)* of 5C may be partially *V. vinifera* which confers susceptibility to phylloxera, as one of the ancestors is believed to have been open pollinated, leaving the possibility of cross pollination with *V. vinifera* having occurred.

Potential for biological control of phvlloxera damage

Very little work has been done on either the soil ecology or biological control of phylloxera. No natural enemies of significance are known for phylloxera in California (Granett et al. 1996), but no concerted work has been done in this area. Researchers in the former Soviet Union, where phylloxera is an exotic, identified 20 species of arthropod natural enemies of the pest (Gorkavenko 1976). However, the work is non-quantitative and therefore hard to evaluate. Soviet and German researchers correlated increased natural enemy density and reduced phylloxera damage in vineyards with proximity to undisturbed natural vegetation, inter-row cover crop use, additions of organic materials, and vineyards untreated with pesticides (Gorkavenko 1976, Moser 1969). These methods were considered to be as or more effective at controlling infestations of phylloxera than fumigation treatments (Gorkavenko 1976). In some cases, recovery of phylloxera infested vineyards was achieved by cover crop use and compost amendments (Riabchun 1971). No work has been done on the community ecology and natural enemies of the insect.

Soil suppressiveness to phylloxera damage may be related to soil microbial activity. Soils which are suppressive to plant pathogens, known as "suppressive soils", have been amply documented (van Bruggen 1995, Voland and Epstein 1994, Cook and Baker 1983, Schneider 1982). Ukrainian researchers showed that three common species of entomopathogenic fungi, *Beauvaria bassiana, Metarhiium ainisopliae and Paecilomyces farinosus* inhibited phylloxera, particularly its nymphal stage. Effects on adults varied. *Paecilomyces farinosus* had the greatest effect on population numbers (Goral 1975).

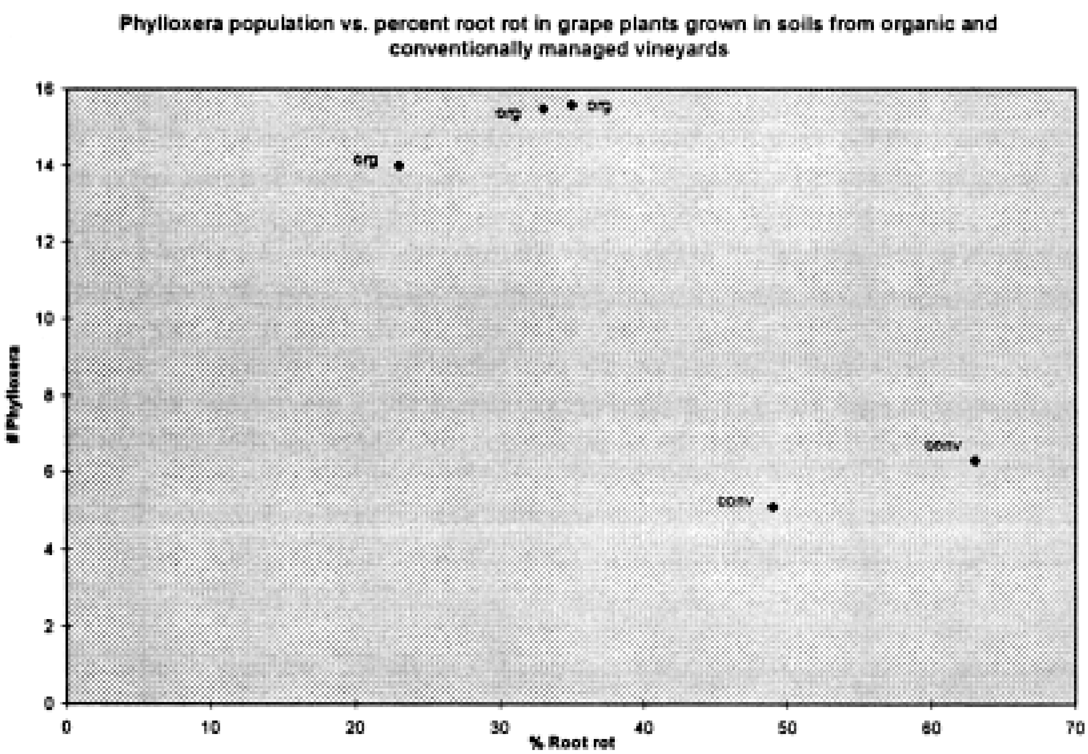
Damage to phylloxera infested grape roots has been shown to be the result of both phylloxera feeding and from secondary fungal infection, primarily *Fusariuni and Pythium* (Omer et al. 1995). Soil mediated suppression of phylloxera damage to grape roots may be in part the result of suppression of secondary pathogen infection of phylloxera feeding wounds. Inhibition of fungal pathogens of plant roots has many sources, the common element being a high level of soil microbial activity associated with decomposing organic matter. In soils suppressive to *Fusarium solani* numbers of total bacteria and total fungi were higher than soils that were less suppressive, while organic matter content of suppressive soils was twice that of nonsuppressive soils (Shim and Lee 1990). *Trichoderma spp.,* a naturally occurring soil inhabiting fungus, has been shown to be an important antagonist to plant root pathogenic fungi, including *Fusarium and Pythium,* lysing host hyphae (Chet 1987). Additional mechanisms of soil suppressiveness have been posited. Induction of resistance to pathogenic *Fusarium* by previous exposure to non-pathogenic *Fusarium* species is believed to be one mechanism. Plants grown in *Fusarium* suppressive soils were shown to have enzymes that, in the absence of non-pathogenic *Fusarium,* inhibited the growth of pathogenic *Fusarium* (Tamietti et al. 1993). Root camouflage has been hypothesized as a mechanism of soil suppressiveness to root pathogens, in which the "rhizosphere effect", where root exudates attract pathogens, is reduced by the increased numbers and types of microorganisms around the root (Gilbert et al. 1994).

Materials and methods

Greenhouse experiment. Soils from five vineyards were used as potting media with phylloxera infested grape plants to determine if differences exist in suppressiveness/conduciveness of soils to phylloxera damage. Two conventionally managed (no cover crops, synthetic inorganic fertilizers, chemical pest and weed control) and three organically managed (composts, cover crops, no synthetic fertilizers, no pesticides, organically managed for at least 5 years) vineyards were selected for soil samples. All organic vineyards were California certified. Soils were collected from around the roots of vines and transported to UC Davis where they were planted to 3 month old grape plants in 3-liter pots in a greenhouse. Plants were infested with phylloxera at the time of transplanting by placing a piece of laboratory cultured Biotype A phylloxera on a root piece at the base of the plant. Each piece of inoculum carried approximately 30 phylloxera individuals. Each of the five vineyard soils was replicated eight times for a total of 48 plants. At 2 months plants were harvested and the nodosities removed from the roots. Nodosities were assessed for phylloxera populations and percent rot, then dried and weighed.

Table 1.

Vineyard	Phyllox	AveRot
Organic	43	35
	46.2	23
	40.8	33
Ave	43.3	30.3
Convent'l	17.1	49
	16.2	60
Ave.	16.6	54.5



Vineyard survey. Using the same criteria as outlined above for conventional and organic vineyards, two cycles of vineyard sampling were carried out, a summer cycle and a fall cycle. In the summer cycle 13 phylloxera infested vineyards were selected, 9 conventionally managed and 4 organically managed. Phylloxera infested organically certified vineyards are few in number, thus the skewed ratio. Of the thirteen vineyards, four were situated in the San Joaquin Valley, six in the Napa/Sonoma area, and one in Mendocino County. In the summer sampling cycle, 4 organic and 5 conventional vineyards were selected. No organic/infested vineyards have been located in the upper San Joaquin Valley. All vines were on AXR41 rootstock except for the San Joaquin Valley vines which were own-rooted. Root samples were taken from vines by digging a hole approximately 60mm long, 30mm wide and 20mm deep at the base of the vine and collecting all roots. Root samples were kept in coolers and taken to the laboratory and examined for phylloxera within 24 h. Phylloxera eggs and individuals were counted using a microscope and classed as egg, 1st, 2nd, 3rd, 4th instars, and adult. Root damage was assessed by examining the roots for necrosis of phloem tissue. All samples showed signs of phylloxera infestation, either tuberosities, nodosities, or phylloxera individuals. Roots were washed and cross-sectioned at 4cm intervals. Percent rot of the phloem tissue was recorded for each cross-section along with root diameter and length. Samples of rotted tuberosities were collected from the roots of each vineyard for culture and identification of microbial pathogens.

Soil samples were sent to the UC DANR laboratories for analysis of percent organic matter, total and nitrate nitrogen, percent sand.

Results and discussion

Greenhouse experiment with vineyard soils. Nodosities from roots grown in soils from organically managed vineyards had significantly less rot (30.3%) than nodosities from roots from conventionally managed soils (54.5%). Phylloxera populations per unit of root tended to be greater in the organic soils and were inversely correlated with root rot. This is probably a result of the weakened condition and poor nutrition of the roots with higher levels of rot, which are unable to support high populations of phylloxera.

Vineyard survey. Two vineyard sampling cycles, summer and fall, in which a total of 212 samples were taken and assessed for phylloxera and percent rot (Table 2) show, as in the greenhouse experiment, decreased rot in the organically managed vineyards (Ave = 11. 8%) relative to conventionally managed vineyards (Ave = 27.1%). Unlike the greenhouse experiment, phylloxera numbers are not significantly different between organic and conventional vineyards. Lower ratios of juveniles and eggs to adults in the conventional vineyards indicates a declining population, generally limited by nutrition, which would be consistent with higher root rot levels.

As in the greenhouse study, there may be a trend toward higher numbers of phylloxera in the organic vineyards, probably due their to better nutritional value and ability to support higher populations.

Summer Sampling

Vineyard	Region	n	RtWdth	TotRtLen	%Rot	Root DW	Egg/100g	Juv/100g	Phy/100g
Organic									
FL	Napa	10	3.4	380	9.2	6.7	8.3	47.3	78
FE	Mendo	15	5.4	352	10.5	20.5	1767.8	533.3	163
RA	Sonoma	10	4.7	730	11.8	12.6	38.6	394.1	479
EI	Napa	10	4.4	644	10.1	12.5	132.1	5814.3	1512
	Tot/Avg.	**45**	**4.4**	**2320**	**10.1**	**12.5**	**78.9**	**1401.0**	**1511.6**
Conventional									
VP	San Joaquin	10	5.9	352	22.0	11.6	11.3	101.3	115
WW	San Joaquin	11	4.8	357	21.9	21.4	87.2	0.8	99
SF	Sonoma	9	4.9	232	14.9	5.2	130.1	354.8	550
HH	Napa	9	5.0	392	20.5	10.6	52.1	40.7	105
VI	San Joaquin	10	5.9	228	28.5	24.4	27.1	27.9	62
HW	Sonoma	9	7.7	240	21.9	20.2	94.8	172.6	294
CH	Sonoma	8	4.4	228	56.3	7.5	45.3	1499.5	1618
EL	Napa	9	4.2	456	7.1	20.5	39.5	28.0	79
LA	San Joaquin	8	9.8	200	30.7	21.8	22.3	689.2	822
	Tot/Avg.	**83**	**6**	**2705**	**23.9**	**16**	**56.0**	**288.0**	**375.4**

Fall Sampling

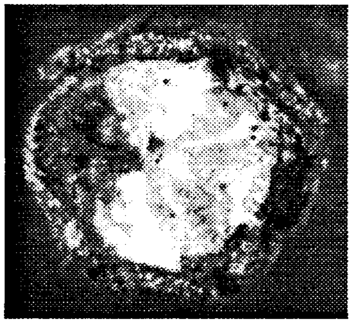
Vineyard	Region	n	RtWdth	TotRtLen	%Rot	Root DW	Egg/100g	Juv/100g	Phy/100g
Organic									
FL	Napa	10	6.2	424	9.0	28.3	13.5	430.4	456.2
FE	Mendo	9	4.8	672	11.2	34.0	1.8	1.8	3.6
RA	Sonoma	10	9.1	344	19.1	21.6	152.9	456.2	636.8
KW	Sonoma	9	4.2	512	13.7	18.0	27.2	57.3	85.2
	Tot/Avg.	**38**	**6.2**	**51.4**	**12.8**	**26.6**	**46.0**	**225.7**	**288.9**
Conventional									
VP	San Joaquin	10	3.8	404	29.8	42.7	111.9	141.8	266.2
SF	Sonoma	10	3.4	432	40.3	19.9	47.8	325.6	400.8
HH	Napa	6	5.3	644	36.3	24.0	338.8	280.2	687.1
CH	Sonoma	10	3.1	536	24.7	18.3	37.0	41.2	78.2
EL	Napa	10	4.9	632	23.7	20.1	60.2	365.6	460.4
	Tot/Avg.	**46**	**4.0**	**2188**	**30.1**	**24.3**	**102.0**	**225.7**	**354.8**

Total % Rot:

Organic	**11.8**
Conv	**27.1**

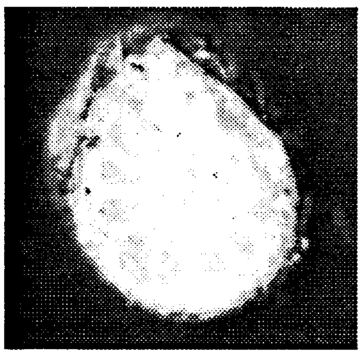
t-Test %Rot: highly significant ($p = 0.00004$)

Table 2. Summer and fall sampling for phylloxera and phylloxera damage to grapevine roots in organic and conventional vineyards in Northern California, 1997.

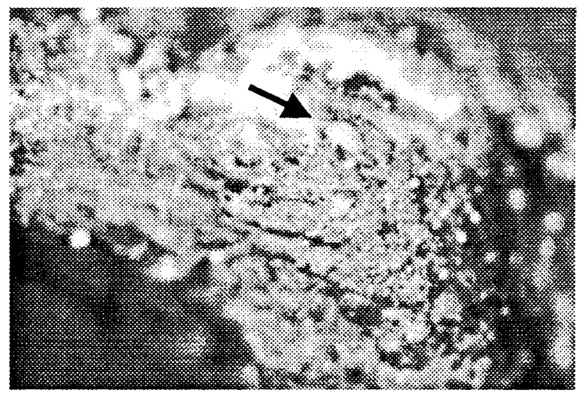


Extensively rotted root, diameter 5mm. Former tuberosity at left.

Moderately rotted root, tuberosity (phylloxera feeding site) at left. Diam. 5mm.



Root with tuberosity at top, very little rot.



Tuberosity with juvenile phylloxera (arrow).

Conclusions

More work needs to be done on the relationship between root rot and plant performance. We would also like to elucidate the species complex in vineyard soils to determine possible associations between microbial species and reduced root rot. We plan to collaborate with Dr. Kate Scow in the Soils Dept. at UCD on looking at soil microbial complexes in vineyard soils during the 1998 growing season.

Work remains to be done on predation on phylloxera. Phylloxerated vineyards which have low levels of root rot combined with low or moderate levels of phylloxera may be candidates for this focus, since vineyards with low levels of root rot have been characterized by higher levels of phylloxera.

Recently discovered susceptibility to phylloxera of current "resistant" rootstocks, the 5C rootstock in particular, in Germany, make research on biological control of phylloxera more important. California is said to be at a stage of phylloxera infestation where Germany was 40 years ago, with "resistant" rootstocks beginning to show nodosities but lacking dai-nage. Geri-nan vines have developed phylloxera dai-nage in the last two decades on rootstocks which are currently being used as phylloxera resistant replanting stock in California (Porten 1997). The current system of replanting vineyards in California is optimal for selection for phylloxera virulence. It is common for growers to replant into infested vineyards such that infested roots intertwine with the newly planted roots, giving an optimal environment for selection.

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