

Pathogenic Status of High Quality Grapevine Nursery Stock

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Editor's Note: Petri Disease, also called Young Vine Decline, is a significant problem for grapegrowers (see "Black Goo, From Mystery Disease to Discovery of Pathogens," *WBM*, December 2001). Unfortunately the fungi associated with the disease are widespread and are present in some of the materials sold to nurseries. The following article does indicate that while the pathogens are pervasive, "high quality" grapevine nursery stock is less likely to be contaminated. More surprising, however, is that laboratory analyses indicate that some economically important grapevine viruses are also present in some rootstock varieties produced under the guidelines of the California Department of Food and Agriculture nursery certification program. Growers should be concerned about these results. More testing would likely benefit all concerned.

Young Vine Decline

'Young vine decline' is used to describe the condition where vines of up to 10-years-old produce stunted shoots with shortened internodes and small chlorotic leaves. Irregular cane maturation, severe leaf necrosis, and fruit raising typify this condition. When sectioned transversally, rootstock and trunk vascular tissues exude characteristic black and amber colored gums that, in longitudinal section, appear as dark brown-black vascular streaking.

Investigation of the 'young vine decline' condition has shown that it is frequently associated with the presence of fungal pathogens, in particular *Phaeoconiella chlamydospora*, *Phaeoacremonium aleophilum*, and *Phaeoacremonium inflatipes*. Other fungal pathogens commonly found in young declining vine-

yards include *Cylindrocarpon* and *Verticillium* species. At the 2nd **International Workshop on Grapevine Trunk Diseases: Esca and Grapevine Declines**, held in Lisbon, Portugal, in September 2001, it was decided that the grapevine-decline condition specifically involving *Phaeoconiella chlamydospora* and *Phaeoacremonium* species would henceforth be called Petri Disease, in honor of Lionel Petri who first described the decline of young grafted vines in 1912 (Petri 1912).

Petri Disease pathogens cause decline in young and older vines by interfering with the movement of water, photosynthates, and mineral nutrients through the trunk of the vine. The fungal pathogens reside in the plant's vascular system and cause the production of tyloses and gums that subsequently block the vascular elements responsible for moving solutions around the plant. It has been demonstrated that toxins produced by these pathogens can induce the development of leaf symptoms associated with Petri Disease in experimental systems.

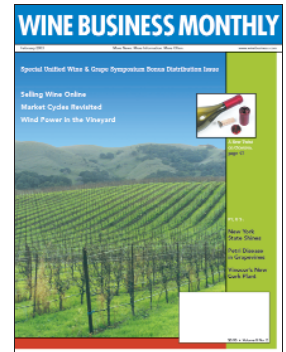
Contamination of grapevines by Petri Disease pathogens is understood to be the first step in the development of the disease known as Esca in Europe and Black Measles in California. Esca is a significant disease problem in Europe, responsible for reduced yields and vineyard replanting throughout France, Italy, Portugal, and Spain. It is now known that *Phaeoconiella chlamydospora* and *Phaeoacremonium* species are also directly involved in the development of Esca and that they are primary pathogens responsible for invasion of healthy grapevine wood. This initial weakening of the vine provides the opportunity for contamination by secondary colonizing fungi, such as *Fomitiporia punctata*,

that induce the characteristic wood rotting associated with Esca. Esca tends to affect vines more than 10-years-old inducing shoot stunting and wilting in the most severe cases. Leaves undergo a characteristic interveinal chlorosis before reddening, and berries develop minute black spots, hence the name Black Measles.

As reported at the First International Workshop on Grapevine Trunk Diseases in 1999 (documented in volume 39 of *Phytopathologia Mediterranea*), the disease previously known by the name 'young vine decline', but now referred to as Petri Disease, has been reported to be present in vineyards from all major world viticultural regions including California, Australia, New Zealand, and all important European grape producing countries.

Stress Induces Petri Disease

Although the causative agents associated with Petri Disease have been identified, 'young vine decline' is still the catchall phrase most commonly used in California to describe the condition in new and established vineyards suffering decline due to unknown or non-obvious causes. Examination of numerous California vineyards purported to be suffering from 'young vine decline' has shown that a wide array of biological and non-biological factors contribute to the observed decline each, either singly or in combination, exerting its effect by applying stress to the grapevine plant. It is now understood that Petri Disease pathogens are opportunistic species, frequently inducing the development of disease symptoms only when vines are under stress.



The Petri Disease pathogens, *Phaeoconiella chlamydospora* and *Phaeoacremonium* species, are termed endophytic because they can survive harmlessly within the grapevine until the plant is stressed, usually by some external trigger, which then induces the development of symptoms. It is clear, therefore, that Petri Disease pathogens may be present in non-symptomatic vines and that only under conditions of stress will disease symptoms develop.

Studies of declining vineyards have shown that stress factors associated with the development of 'young vine decline' and Petri Disease symptoms can be divided into three major groups: nursery-induced stress, vineyard installation and management stress, and biological stresses (Table 1).

Table 1. **Stress factors associated with 'young vine decline' and Petri Disease in new and maturing vineyards.**

Nursery induced stress

- Rootstock shaft lesion development from basal disbudding sites and improperly healed graft unions
- Inadequate root systems and other physical root defects
- Root-bound potted vines (excessive time in containers, e.g. potted dormant vines)
- Inadequate carbohydrate reserves
- Extended cold storage
- Petri Disease pathogens (*Phaeoconiella chlamydospora*, *Phaeoacremonium* species)

Vineyard establishment and management stress

- Poor preparation of ground: inadequately ripped soils
- Poor planting of vines: 'J' rooting
- Late planting of potted and dormant vines
- Incorrect irrigation practice
- Nutritional imbalances: zinc deficiency, boron toxicity, magnesium or potassium deficiency
- Frost damage

Biological stress

- Nematodes
- Root rot in association with poor irrigation, emitter placement, and presence of fungal pathogens: *Phytophthora*, *Rhizoctonia*, *Pythium*, *Fusarium*, *Verticillium*
- Viruses: especially leafroll, grapevine virus B (corky bark), and Fanleaf.
- Fungi: *Armillaria* (Oak root fungus), *Phaeoconiella chlamydospora*, *Phaeoacremonium* species, *Cylindrocarpon* species (Black foot disease)
- Insect damage: mites, thrips, ants
- Animal feeding: voles

Source of Petri Disease Contamination

Laboratory analysis during the 1999-2002 growing seasons of new and maturing California vines with 'young vine decline' or exhibiting symptoms of Petri Disease showed that approximately half of those exhibiting vascular discoloration tested positive for *Phaeoconiella chlamydospora* and/or *Phaeoacremonium aleophilum*.

Although it is widely acknowledged that the pathogens associated with Petri Disease are found in most viticultural regions, the source of contamination of vines is the subject of considerable discussion. While it has been proven that *Phaeoconiella chlamydospora* and *Phaeoacremonium aleophilum* spores are spread by rain and wind in vineyards (Rooney et al 2001), there is substantial evidence that contaminated nursery stock is a key source of inoculum for the establishment of Petri Disease in new and maturing vineyards (Pascoc and Cottral 2000, Rego et al 2000, Zanzotto et al 2001).

It has been demonstrated that nursery derived imperfections in grapevine stock are a critical source of stress for new and established vineyards (Stamp 2001) and it is clear that planting vines without significant defects will result in healthier vineyards that are less likely to succumb to stress-induced Petri Disease. The types of defects most commonly found in nursery stock include improperly healed graft unions, lengthy rootstock shaft lesions derived from poorly healed graft unions or nodal disbudding sites, and incomplete root systems. It is frequently observed that vines with such defects also show vascular symptoms associated with the presence of Petri Disease pathogens. Not only can such defects significantly compromise the vine's vascular system but they also represent an opportunity for pathogen contamination in the vineyard.

Pathogenic Status of High Quality Nursery Stock

As part of an ongoing vineyard and winery client service directed toward identification of grapevine nursery stock of the highest physical quality,

Stamp Associates has screened over one million grapevine plants including dormant bench-grafts, dormant rootstock rootings, and current season and dormant (year-old) potted grapevine plants. Inspection of vines for physical quality criteria including condition of root system, rootstock shaft, and graft union, and vascular symptoms of Petri Disease resulted in the detection of significant defects in 35 percent of dormant rootstock rootings and 39 percent of dormant bench-grafted vines in a two season study of nursery stock (Stamp 2001).

It is clear that planting high quality vines with few or no physical imperfections is advantageous in that they will more readily withstand biotic and mechanical stresses. There are no studies, however, on the pathogenic status of vines that have been pre-screened for physical quality. In the study reported here, several lots of vines from California and Oregon nurseries were examined for physical quality. Vine lots that did not meet strict quality criteria were rejected. Only those lots that met physical quality criteria were subjected to laboratory testing for grapevine pathogens.

Plant Material

Vine orders for more than 35,000 dormant rootstock rootings were placed with four leading California grapevine nurseries and one Oregon nursery for delivery in spring 2002. The California stock was produced through the **California Department of Food and Agriculture** (CDFA) nursery certification program and as such was certified free of important grapevine viral pathogens including those tested here (CDFA Regulations for Nursery Stock Grades and Standards for #1 grade Grapevines: CDFA NIPM Item #5, Article 10, Section 3062d, revised 2/7/96). Vines were inspected before planting by examining randomly selected bundles from each storage container. Each selected bundle was opened and every vine was examined for conformity to the quality criteria identified in Table 2. Only vines with acceptable external physical features were examined internally.

Vine Evaluation

The proportion of vines meeting particular quality criteria or possessing specific defects was calculated for each vine lot. In addition, the overall batch quality was determined by calculating the proportion of vines that fell into four CDFA-based grading standards: poor, acceptable, good, and very good. Using these two scores

Table 2. **Vine Quality Criteria****External quality criteria**

- Root development and condition
- Condition of rootstock base
- Condition of rootstock disbudding sites
- Presence of rootstock shaft wounds and lesions
- Matching of rootstock and scion calliper
- Shoot development, condition, and basal calliper
- Overall vine strength
- General condition of rootstock and scion

Internal quality criteria

- Condition of roots
- Condition of rootstock shaft and shoot tissues
- Rootstock wood:pith ratio
- Vascular discoloration
- Visual presence of Petri Disease symptoms

an overall 'vine quality' grade was calculated ranging from 1-100 points, with 1 point representing the worst quality and 100 points the best possible quality (Table 4). Scores 85 and over represent vines of very good quality; 70-84: good quality; 55-69 acceptable quality, and vines scoring less than 55 points were of poor quality and were not recommended for planting. In addition, a proportion of vines that proved to be physically acceptable were examined internally by transverse dissection from shoot spur to rootstock base and observed for visual symptoms of stress and Petri Disease. From this examination a score was calculated for the internal condition of the vines ranging from 0 to 100 where a score of zero represented zero vascular discoloration and higher scores represent proportionately greater discoloration (Table 4).

Vascular discoloration of nursery stock is a result of the plant's response to the propagation process, stress, or contamination by pathogens associated with Petri Disease and other rootstock trunk diseases. Experience has shown that a certain degree of vascular discoloration is usually observed at the graft union and adjacent to the rootstock base and appears to be associated with the propagation process. It is very unusual to find vines with no vascular discoloration. Excessive discoloration is defined as that where the vascular tissues are stained along the length of the rootstock, and this condition is usually associated with vines of poor physical quality or those that have been excessively stressed during the growing period or subsequently, during storage. Such vines frequently

test positive for Petri Disease pathogens and were rejected in this study and not subjected laboratory analysis.

Laboratory Analyses

Whole vine samples were collected from each lot and submitted to an independent commercial laboratory for virus and fungal pathogen analysis. Depending on lot size, from 2 to 9 whole plant samples were analyzed from each batch of vines. Laboratory samples were not examined internally. Each vine was tested for the pathogens indicated in Table 3 using the identified laboratory techniques.

Results and observations

A summary of the results of the examination of more than 35,000 dormant rootstock rootings is presented in Table 4. Table 4 lists the rootstock under evaluation, the source nursery (by code), vine quality and vascular discoloration scores, and the pathogens for which sampled vines tested positive. With the exception of vines from the Oregon nursery (E), samples were only submitted for laboratory analysis from vine batches that were of acceptable internal and external quality. As a general rule, vine lots with greater than 40 percent defective plants were rejected outright because of the dif-

good caliper with respect to CDFA specifications.

Of the 12 batches of vines examined, seven were positive for one or both of the Petri Disease pathogens (Table 4). *Phaeomoniella chlamydospora* was found in seven vine lots while *Phaeoacremonium aleophilum* was found in three lots and only in association with *Phaeomoniella chlamydospora*.

Although exceptions occur, there appear to be some general trends associating vine quality, vascular discoloration, and the presence or absence of Petri Disease pathogens. For example, the three vine lots with the highest vine quality scores (all greater than 80 points) exhibited no vascular discoloration (Table 4). However, three of the five vine samples tested from lot #2 (possessing the second highest quality score) tested positive for one or both Petri Disease pathogens while the other two highest quality lots tested negative for these pathogens.

Two vine lots (#4, #8) scored 79 quality points, one with vascular discoloration and one without (Table 4). Vine lot #4, with vascular discoloration, tested positive for both Petri Disease pathogens while lot #8, without discoloration, tested negative. All seven vine lots scoring less than 79 quality points possessed some degree of vascular discoloration, with five testing positive for one or both Petri Disease

Table 3. **Pathogen tests and laboratory techniques**

Pathogen	Associated Disease	Laboratory Technique
<i>Phaeomoniella chlamydospora</i>	Petri Disease	PCR
<i>Phaeoacremonium aleophilum</i>	Petri Disease	PCR
Grapevine Leafroll associated virus 1	Leafroll virus	ELISA
Grapevine Leafroll associated virus 2	Leafroll virus	PCR
Grapevine Leafroll associated virus 3	Leafroll virus	ELISA
Grapevine virus B	Corky bark	PCR
Grapevine Fanleaf virus	Fanleaf	ELISA

PCR: polymerase chain reaction, ELISA; enzyme linked immunosorbent assay

ficulty and time involved in identifying substandard plants in large batches of nursery stock at the time of delivery to the vineyard.

With the exception of dormant rootstock rootings from the Oregon nursery, most vines were of generally good physical quality usually scoring more than 70 points on the vine quality evaluation scale (Table 4). Such vines usually had good root and shoot systems and well-healed rootstock disbudding sites, and were of

pathogens and two testing negative for all Petri Disease pathogens.

No conclusions can be drawn regarding the propensity of different rootstocks to show vascular discoloration or to be contaminated with Petri Disease pathogens as only single vine lots were examined for some rootstocks while multiple lots were examined for others (Table 4).

Two vine lots tested positive for grapevine virus pathogens (Table 4). Three of seven lot #1,

Table 4. Contamination of high quality grapevine stock by fungal and virus pathogens

Rootstock	Vine Lot	Nursery*	Physical Vine Quality (1-100)**	Vascular discoloration (0-100)***	Detected pathogens (by ELISA or PCR)§				Total pathogen positive vines§§
					<i>Phaeomoniella chlamydospora</i> (PCR)	<i>Phaeoacremonium aleophilum</i> (PCR)	GVLrAV 3 (ELISA)	GVB (PCR)	
101-14 MG	1	A	85	0	negative	negative	POSITIVE 3/7*	negative	3/7
101-14 MG	2	B	89	0	POSITIVE 1/5	POSITIVE 2/5	negative	negative	3/5
101-14 MG	3	C	72	20	POSITIVE 3/5	POSITIVE 1/5	negative	negative	3/5
101-14 MG	4	D	79	41	POSITIVE 2/10	POSITIVE 1/10	negative	negative	2/10
3309C	5	A	67	31	negative	negative	negative	negative	0/3
3309C	6	E	9	38	POSITIVE 3/9	negative	negative	negative	3/9
1103P	7	A	72	36	negative	negative	negative	negative	0/3
110R	8	A	79	0	negative	negative	negative	negative	0/2
St. George	9	B	92	0	negative	negative	negative	negative	0/10
Riparia Gloire	10	C	55	23	POSITIVE 4/5	negative	negative	negative	4/5
Riparia Gloire	11	D	49	22	POSITIVE 5/5	negative	negative	POSITIVE 1/5	5/5
Riparia Gloire	12	E	6	31	POSITIVE 3/9	negative	negative	negative	3/9

Legend *A-D: California nurseries, E: Oregon nursery **1: very poor, 100: excellent ***0: no vascular discoloration, 100 extreme vascular discoloration

§All whole vine samples were tested for: Grapevine leafroll virus (GVLrAV) types 1, 3 (ELISA), 2 (PCR); Grapevine virus B (GVB) (PCR); Grapevine fanleaf virus (ELISA); Grapevine rootstock stem lesion associated virus (PCR) §§Total pathogen positive vines out of total samples submitted

101-14 MG vine samples tested positive by ELISA for grapevine leafroll virus type 3. These three positive vine samples were re-tested by polymerase chain reaction diagnostic technology (PCR) and two of them tested positive for the virus. This vine lot (#1) tested negative for Petri Disease pathogens.

One of five vines of Riparia Gloire vine lot (#11) tested positive by PCR for grapevine virus B, the virus agent associated with the development of corky bark disease in grapevines. All five tested vines were positive for *Phaeomoniella chlamydospora* (Table 4).

Discussion of Results

The role of Petri Disease pathogens in vineyard decline is a relatively new field of research in California and consequently CDFA nursery grades and standards do not govern contamination of nursery stock by these organisms. At the 2nd International Workshop on Grapevine Trunk Diseases, however, several international experts on grapevine trunk diseases recommended that European nursery certification and quarantine authorities consider adding Petri Disease pathogens to those already identified as non-permissible in grapevine nursery stock.

The results support earlier studies (Stamp 2001) that the majority of dormant rootstock

and bench-grafted vines exhibit some degree of internal discoloration associated with propagation and grafting. In fact, during the course of examination of more than one million vines, fewer than five percent of all batches did not show some level of vascular discoloration and it is highly unusual, therefore, for four of the 12 batches examined in this study to be without symptoms of vascular discoloration (Table 4). This may reflect a tougher market for grapevine nursery stock and the omission of lower quality materials from propagation programs.

High quality vines tended to be less frequently associated with vascular discoloration. This may be correlated with the presence of Petri Disease pathogens because it was noted that 100 percent of vine lots (4/4) with a quality score of less than 60 points tested positive for Petri Disease pathogens (ranging from 33 percent to 100 percent incidence of sampled vines) while only 38 percent of vine lots (3/8) scoring more than 60 points tested positive for Petri Disease pathogens (ranging from 20 percent-60 percent incidence of sampled vines) (Table 4).

This limited survey indicates that high quality vines tend to be associated with a lower incidence of pathogen contamination while lower quality vines tend to be associated with a higher incidence of pathogen contamination. There is also evidence to support a direct correlation between the vascular discoloration typi-

cally associated with the propagation process and the presence of Petri Disease pathogens. 75 percent of vine lots (3/4) without vascular discoloration tested negative for Petri Disease pathogens while 75 percent of vine lots (6/8) with vascular discoloration tested positive for at least one Petri Disease pathogen (Table 4).

It may be that there is a cause and effect relationship between the presence of Petri Disease pathogens and vine quality. As Petri Disease pathogens cause vine decline and associated slow-down in vine growth it is to be expected that stronger cuttings (produced from stronger vines) are less likely to be contaminated with growth limiting pathogens. In support of this hypothesis it has been observed that cuttings contaminated with Petri Disease pathogens undergo weaker graft union callus formation.

It is not possible to draw conclusions about the relative contamination of different rootstocks by Petri Disease pathogens from this study because multiple lots of vines were only available for three of the six varieties tested. However, where more than two lots were available the majority of lots tested positive for one or both pathogens (Table 4) and when only two lots were available (3309C), one was positive for *Phaeomoniella chlamydospora*. Because of the greater popularity of certain rootstocks, field studies of their relative susceptibility to Petri

Disease pathogens have proven impractical. Although in experimental systems, cuttings of 3309C, 420A, and 110R, among others, proved least susceptible to *Phaeoconiella chlamydospora* inoculation, no correlation with field contamination of vines could be drawn (Eskalen et al 2001).

With respect to the influence of source nursery on rootstock contamination, only the several rootstock varieties of one nursery tested negative for Petri Disease pathogens (California nursery A). This finding may be even more significant because this nursery contributed four of the 12 batches, twice as many as any other nursery. Interestingly, however, the 101-14 MG batch from this nursery (A) tested positive for grapevine leafroll virus type 3 (Table 4).

It is very surprising to find that vines from two of 12 lots tested positive for economically important grapevine viruses. Two of three of the ELISA leafroll positives from vine lot #1 (101-14 MG) were confirmed by PCR. The grapevine virus B PCR positive from lot #11 was not retested. It is possible that these results represent false positives but this is a much less likely event than the occurrence of false negatives. Taken at face value the results show that CDFA-certified rootstock rootings may be contaminated with important grapevine viruses. Additional studies have provided supporting evidence that CDFA-certified grapevine nursery stock may be contaminated with economically important grapevine pathogens (Stamp, unpublished results). In one such study of rootstock 1616C propagation cuttings, 3 of 4 samples submitted for PCR analysis tested positive for grapevine leafroll associated virus type 4.

Both grapevine leafroll virus and grapevine virus B are among the most debilitating grapevine viruses, and these pathogens could present a serious problem for growers, especially those choosing to graft non-certified field scion selections to rootstock that may be contaminated with the same or different viruses. Contamination of plant stock with more than

one virus can result in the development of symptoms far more significant than those associated with the individual virus types. Similarly, it is to be expected that vines contaminated with an array of pathogens including potentially damaging endophytic fungi such as the Petri Disease pathogens and viruses are more likely to succumb to mechanical and biotic stresses.

Conclusions

As noted here, it is very unusual to examine grapevine nursery stock that does not exhibit some degree of vascular discoloration. In fact, based on past experience, it would be impossible to fill orders for grapevine nursery stock with material free of vascular discoloration because it simply does not exist in sufficient quantity. Less than five percent of all batches of examined grapevine stock have proven to be free of vascular discoloration. This study has shown that although limited amounts of vascular discoloration may appear to be associated with the propagation process it may also be an indicator of possible contamination by Petri Disease pathogens. And although 75 percent of vines without vascular discoloration tested negative for Petri Disease pathogens, *Phaeoconiella chlamydospora* and *Phaeoacremonium*

aleophilum may still be present in symptom-free material.

The results support the conclusion that careful selection of grapevine nursery stock will result in the planting of significantly higher quality material that has a greater chance of being free of Petri Disease pathogens. This study also suggests that nursery stock may be contaminated to a lesser or greater degree with Petri Disease pathogens and that it is possible to find material that is pathogen-free, although possibly not for all rootstock varieties.

Given the wide range of stresses that new vines are submitted to before and after planting, ranging from nursery derived defects and extended cold storage to improper planting, insufficient irrigation, and biotic stress, it is obvious that any measures that result in the planting of vines with superior physical quality and reduced pathogen contamination represent a worthwhile investment. **wbm**

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Literature Cited

- Eskalen, A., W.D. Gubler and A. Khan. (2001). Rootstock susceptibility to *Phaeoconiella chlamydospora* and *Phaeoacremonium* spp. *Phytopathologia Mediterranea*, 40: S433-S438.
- Pascoe, I. and E. Cottral. 2000. Developments in grapevine trunk diseases research in Australia. *Phytopathologia Mediterranea*, 39: 68-75.
- Petri, L. 1912. Osservazioni sopra le alterazioni del legno della vite in seguito a ferite. *Le Stazioni Sperimentali Agrarie Italiane*, 45: 501-547.
- Rego, C., H. Oliveira, A. Carvalho and A. Phillips. 2000. Involvement of *Phaeoacremonium* spp. and *Cylindrocarpum destructans* with grapevine decline in Portugal. *Phytopathologia Mediterranea*, 39: 76-79.
- Rooney, S.N., A. Eskalen and W.D. Gubler. 2001. Recovery of *Phaeoconiella chlamydospora* and *Phaeoacremonium inflatipes* from soil and grapevine tissues. *Phytopathologia Mediterranea*, 40: S351-S356
- Stamp, J.A. 2001. The contribution of imperfections in nursery stock to the decline of young vines in California. *Phytopathologia Mediterranea*, 40: S369-S375
- Zanzotto, A., S. Serra, W. Viel and M. Borgo. 2001. Investigation into the occurrence of Esca-associated fungi in cuttings and bench-grafted vines. *Phytopathologia Mediterranea*, 40: S311-S316