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The contribution of imperfections in nursery stock to the decline of young vines in California

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Summary. 'Petri disease', as defined at the 2nd International Workshop on Grapevine Trunk Diseases (Esca and Grapevine Declines, September 14–15 2001, Lisbon, Portugal), is a condition associated worldwide with the decline of young vines contaminated by *Phaeoacremonium* and/or *Phaeomoniella* pathogens. Vines exhibit stunted development with vascular tissues characteristically exuding darkened gums when sectioned transversally. 'Young Vine Decline' (YVD), historically including the condition now known as Petri disease, is a term still used widely in California to describe unexpectedly poor performance of young vines exhibiting symptoms that include those associated with Petri disease. Examination of more than eight hundred thousand dormant nursery vines as well as new and established declining vineyards demonstrated that nursery stock defects and mechanical and biotic vineyard stresses were frequently associated with YVD in California. Rootstock shaft lesions and weak roots were most commonly associated with YVD in very young vineyards, while root system contamination by nematodes and fungal pathogens was frequently associated with YVD in older vineyards.

Key words: grapevine nursery stock, defects, vine decline.

Introduction

Much work has been published on the nature and causal agents of Petri disease, a decline of young vines associated with the presence of *Phaeoacremonium* and *Phaeomoniella* species and also referred to, historically, as young vine decline, Phaeoacremonium grapevine decline, black goo, and slow-dieback (Scheck *et al.*, 1998; Surico *et al.*, 1998; Adalat *et al.*, 2000; Rego *et al.*, 2000; Sidoti *et al.*, 2000). In addition to their involvement in Petri disease, *Phaeoacremonium* and *Phaeomoniella* species play a primary role in the development of esca (black measles) in mature grapevine plants (Mugnai *et al.*, 1996; Larignon and Dubos, 1997; Graniti *et al.*, 2000; Pascoe and Cottral, 2000; Sparapano *et al.*, 2000).

Esca is one of the most important causes of vineyard decline in Europe, causing apoplexy and death in vines more than ten years old. Leaves of affected vines develop interveinal chlorosis and become necrotic, turning from yellow to red and brown. Esca is believed to be a complex of at least two diseases (Graniti *et al.*, 2000; Sparapano *et al.*, 2000). Healthy trunk tissues may become infected with *Phaeoacremonium* or related genera, resulting in characteristic brown/black streaking in the xylem. This infection can be accompanied by wood rotting fungi such as *Fomitiporia (Phellinus) punctata*

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which promote the development of white spongy heartwood decay in older vines. Alternatively, *Fomitiporia* may act as a primary pathogen (Mugnai *et al.*, 1999; Graniti *et al.*, 2000).

Vines with Petri disease are usually stunted with shortened internodes and small chlorotic leaves. Irregular cane maturation, severe leaf necrosis, and fruit raisining may also occur. When sectioned transversely, rootstock vascular tissues exude characteristic black and amber coloured gums which, in longitudinal section, appear as dark brown/red/black vascular streaking.

In California, 'Young Vine Decline' (YVD) is a catchall phrase used by vineyard growers and viticulturists to describe declining vineyards of almost any age. Affected vines possess stunted shoots with shortened internodes and chlorotic leaves. Fruit set and yield are often compromised and vine trunk and cordon calliper are significantly reduced compared to non-affected vines. Examination of declining vineyards has revealed that although the Petri disease pathogens *Phaeoacremonium* spp. and *Phaeomoniella chlamydospora* are occasionally involved, frequently other biological and physical stresses play an apparently more important role in YVD. This is especially so in recently established vineyards where observations revealed that severe nursery stock defects and poor vineyard management practices are frequently associated with YVD.

The purpose of this study was to correlate grapevine nursery stock defects with the decline of young and recently established California vineyards.

Materials and methods

Grapevine nursery stock examination

California Department of Food and Agriculture (CDFA) 'number 1 grade' dormant bench-grafted vines and rootstock rootings [CDFA Regulations for Nursery Stock Grades and Standards for 'No.1 grade' Grapevines (CDFA NIPM Item No.5, Article 10, Section 3062d, revised 2/7/96)] were inspected at the time of delivery from California grapevine nurseries to vineyards and wineries. From May 2000–July 2001, Stamp Associates examined more than 800,000 dormant nursery vines (Table 1). Usually, all vines in shipments of fewer than 500 plants were individually examined. Each vine was flexed to help detect hidden rootstock shaft lesions and necroses. In larger lots, representative samples comprising 5–10% of the shipment were individually examined to determine the overall status of the vines. Vines examined from larger lots were selected randomly from different positions within shipping containers. Only those vines without significant physical defects were subjected to visual internal examination. Internal examination was undertaken by cutting vines transversally at 5-cm intervals through shoot, trunk, and root tissues.

Overall quality as judged by CDFA standards for 'No.1 grade' grapevines and the presence of physical and phytopathological defects was recorded (Table 1). Defects detrimental to field performance included small size, incomplete root systems, significant rootstock lesions or cankers, incompletely healed graft unions, and symptoms associated with Petri disease pathogens (Table 1).

Vines were evaluated for the following 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;
- condition of graft union;
- 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;
- condition of graft union;
- visual presence of Petri disease symptoms

Vineyard plant examination

Representative vines from vineyards exhibiting YVD were examined for external and, after they were removed, for internal quality criteria described above.

Laboratory diagnostic procedures

Plant and soil samples were submitted to private, University of California, and CDFA laboratories for analysis of various conditions. Both PCR and culture-based testing procedures were used for

Table 1. Performance-affecting defects in dormant nursery bench-grafted vines and rootstock rootings

Plant material	Rootstock	No. of vines inspected	Defective vines		Nature of defects%				
			No.	%	Weak/ under-sized	Inadequate roots[c]	Rootstock lesion[d]	Partially healed graft union	Petri disease /stress[e]
Dormant bench-grafts	all[a]	171,251	68,496	39	3	9	8	13	6
Dormant rootstock rootings	all[b]	631,806	225,057	35	8	4	8	n.a.	14
	3309C	178,063	51,430	29	10	3	12	n.a.	4
	101-14 MG	191,949	106,379	53	6	3	7	n.a.	37
	420A	105,552	14,790	13	4	6	2	n.a.	1
	110R	70,693	11,797	17	3	3	10	n.a.	0
	44-53 M	44,500	12,343	28	19	3	1	n.a.	6
	Riparia Gloire	35,800	9,882	19	8	3	8	n.a.	0

[a] Includes vines grafted to 140R, 3309C, 44-53M, 101-14 MG, 1103P, Riparia Gloire, 110R.
[b] Includes 3309C, 44-53M, 420A, 101-14 MG, 1103P, Riparia Gloire, 110R, 5C.
[c] Incomplete or weak root system.
[d] Significant rootstock lesions and cankers and/or rootstock failure.
[e] Significant internal discoloration and/or production of gummy exudates.
n.a., not applicable.

identification of *Phaeoacremonium* and *Phaeomoniella* species while PCR and ELISA were employed for grapevine virus identification. Root pathogens and nematodes were identified by cultural and microscopic techniques.

Results and discussion

Finished nursery stock

Table 1 summarises important defects detected in more than 171,000 dormant bench-grafted vines and 631,000 dormant rootstock rootings. Thirty nine percent of benchgrafted vines and 35% of rootstock rootings (Table 1), respectively, failed to meet CDFA standards for 'No.1 grade' grapevines or possessed defects sufficiently significant to negatively affect future vine performance (as determined by field observations).

Three percent of grafted vines and 8% of rootstock rootings were weak or undersized (Table 1). Undersized vines were defined as those with a calliper of 6.35 mm or less immediately below the graft union or rootstock spur. Observations have shown that such vines usually possess weak root systems and minimal carbohydrate reserves.

Grapevine plants should have a full root system, with root development from all sectors of the rootstock base (CDFA Regulations for Nursery Stock Grades and Standards for 'No.1 grade' Grapevines). Not only does a complete root system promote strong development of newly planted vines, providing important carbohydrate resources to the pushing shoot, but it is also an important indicator of the general health status of the vine. In this study, 9% and 4% of bench-grafted vines and rootstock rootings, respectively, possessed incomplete root systems where more than 40% of the rootstock base had failed to initiate roots (Table 1). The absence of roots from a significant sector of the rootstock base often signalled the presence of rootstock lesions.

The extent of callusing of propagation wounds, although influenced by rootstock, is an indicator of the physiological status of parental cuttings. Carefully grown and properly matured cuttings callus more readily than weaker material. Rootstock lesions generally develop from incompletely healed graft unions or overly large basal rootstock disbudding sites. Lesions extended down the rootstock shaft from the graft union (Fig. 1) and up from the rootstock base. Examination of recently grafted potted vines revealed that lesions were initiated from

non-callused propagation wounds (Fig. 2) that continued to extend along the length of the rootstock shaft in the nursery and vineyard, ultimately producing rootstock length cankers. Significant lesions were found in 8% of both dormant bench-grafted vines and rootstock rootings (Table 1).

The graft union should be fully healed when a vine leaves the callusing chamber, 2–4 weeks after grafting. Incompletely callused rootstock and scion graft union tissues never completely heal in the vineyard, leaving the vine exposed to biotic and environmental stresses. According to CDFA standards, graft unions should be completely healed, although in this study, poorly healed graft unions were defined as those with greater than 25% non-callused tissues. Following this definition, thirteen percent of dormant bench-grafted vines possessed inadequately healed graft unions (Table 1). There appears to be a correlation between physiological maturity of the cuttings used for production of grapevine nursery products (as determined by caliper, cross-section, internode length, carbohydrate reserves, and condition of donor mother vines) and successful propagation.

Work in collaboration with the Plant Pest Diagnostics Branch of the CDFA Plant Health and Pest Prevention Services, Sacramento, California has failed to isolate any important primary grapevine pathogen from rootstock lesions on young potted vine and dormant products. Rather, soil borne saprophytes such as *Trichoderma* were found associated with cankers of young and old vines alike, suggesting that the non-healed propagation wounds provide a route of entry for micro-organisms that ordinarily would not present a threat to healthy, intact vines. Rootstock lesions and cankers negatively impact young vine growth and development by severely reducing the effective volume of the plant's vascular system and presenting an opportunity for infection by primary grapevine pathogens. The nature of basal rootstock (pruning wound initiated) cankers is such that affected vines often have inferior root systems, an additional disadvantage.

Healthy 1 year-old dormant nursery stock ordinarily has no significant vascular tissue discoloration. Minimal discoloration adjacent to propagation wounds is the result of the oxidation of naturally occurring tannin and phenolic compounds. Extensive discoloration and the production of black, brown, and amber coloured gummy exudates from transversely cut xylem elements has frequently been associated with contamination by *Phaeomoniella*, *Phaeoacremonium*, and *Cylindrocarpon* species. Such excessive discoloration is also associated with vine stress, resulting from conditions such as extended cold storage, improper planting, or inadequate irrigation. Of the vines examined here, 6% of dormant benchgrafts and 14% of rootstock rootings possessed internal symptoms associated with Petri disease and/or stress (Table 1). These symptoms comprised the production of black, brown, and amber coloured exudates from transversally cut vine shaft tissues distant from the rootstock base and graft union and the presence of overly dry trunk wood. In related studies of nursery stock growing under field finishing conditions in the year prior to delivery, more than 50% of vines expressing identical vascular symptoms tested positive for *Phaeomoniella*, *Phaeoacremonium*, or *Cylindrocarpon* species when subjected to culture-based identification analyses.

Although not the subject of this study, it has been observed that green potted vines possess many of the same defects as dormant products. In one recent study of more than 8000 such vines, 54% possessed an incomplete graft union in association with a significant rootstock lesion. Generally, however, internal symptoms associated with Petri disease have not been observed in recently grafted potted product.

One-two year old vineyards

Evaluation of young (less than two year old) declining vineyards has revealed that many factors are involved in the poor performance of vines. As judged by the absence of vascular discoloration and gummosis, however, it appears that in most cases it is the exception for Petri disease pathogens to play a leading role in the observed decline. Evaluation of declining vineyards has revealed that the most common cause of poor performance is physical defects present in the vines at the time of planting. Most commonly, vines possess significant rootstock shaft lesions, often in association with incomplete root systems (Fig. 3). Frequently more than 50% of the vascular system of such symptomatic vines is missing. In nearly all cases, rootstock lesions have developed directly from non-healed rootstock and graft union propagation wounds.

In recent studies of two-year old Napa County declining vineyards planted with potted vines, extensive imperfections in planting material were observed. In one study of more than 10,000 vines, 45% possessed significant physical defects. The graft union of 32% of the vines was less than 50% callused while 17% possessed substantial rootstock lesions. A second study of a two-year old fifteen-acre vineyard revealed that the graft union of 40% of the vines was less than 50% healed (Fig. 4). Twenty seven percent of the vines possessed a significant lesion extending basipetally from the graft union, and 37% of the vines possessed significant rootstock lesions that had developed upward from the basal rootstock disbudding site. Seventeen percent of this latter group of vines possessed rootstock lesions that extended from rootstock base to graft union. Typically, vines in this condition also exhibit internal vascular gumming symptoms associated with Petri disease pathogens (Fig. 5). These symptoms, however, also develop in vines subjected to stress, and it is hypothesised that the inherent stress resulting from severely damaged vascular and trunk tissues is sufficient to induce these symptoms regardless of the presence of Petri disease pathogens.

Two or more year old vineyards

Extensive rootstock shaft lesions are commonly found in YVD-symptomatic vines greater than 2 years of age. In the most severe cases, more than 50% of the trunk is destroyed and original graft union tissues are exposed (Fig. 6 and 7). Vascular symptoms typically associated with Petri disease may or may not be present in such vines.

Studies of YVD in older vineyards have demonstrated that factors contributing to poor vine performance fall into three basic categories: nursery induced stress, vineyard establishment and management stresses, and biological stresses (Table 2). Nursery induced stresses include structural vine defects, extended cold storage, limited vine carbohydrates, and pot-bound root systems. Vineyard establishment and management stresses contributing to YVD include inadequate ground preparation, incorrect planting, inappropriate irrigation,

Table 2. Stress factors associated with YVD in new and maturing vineyards.

Nursery induced stress
- Rootstock shaft lesion development from:
  - basal disbudding site
  - improperly healed graft union
- Inadequate root systems and other physical defects
- Limited carbohydrate reserves
- Stress from extended cold storage, excessive time in containers
- Petri disease pathogens (*Phaeomoniella, Phaeoacremonium)*

Vineyard establishment and management stress
- Poor preparation of ground – inadequately ripped soils
- Poor planting of vines - 'J' rooting
- Incorrect irrigation practice
- Nutritional deficiency or toxicity
- Frost damage

Biological stress
- Nematodes
- Root rot in association with poor irrigation, emitter placement, and presence of fungal pathogens: *Phytophthora*, *Rhizoctonia*, *Pythium*, *Fusarium*
- Viruses:
- Incompatibility - e.g. Red Globe virus (rootstock stem lesion associated virus)
- *Armillaria* (oak root fungus)
- Petri disease pathogens (*Phaeomoniella, Phaeoacremonium)*
- Black foot pathogens (*Cylindrocarpon* spp.)
- Insect damage

Figure 1. Rootstock and scion trunk lesion initiated from the graft union in a green potted vine.

Figure 3. Extensive rootstock shaft lesion initiated from the basal rootstock pruning wound of a 1-year old bench-grafted vine. Note the incomplete root system.

Figure 5. Transverse section of young vine with YVD growth habit and foliar symptoms showing vascular discoloration and exudates.

Figure 2. Initiation of rootstock shaft lesion from improperly callused graft union in a green potted vine.

Figure 4. Longitudinal section through the graft union of a 21-month old vine with YVD growth habit and foliar symptoms.

Figure 6. Root system and lower trunk of a 5-year old grafted vine with a severe rootstock shaft lesion.

Figure 7. Graft union and upper trunk of a 5-year old grafted vine. Note the extensive rootstock shaft lesion and 'teeth' of the original saw-cut graft union.

and nutritional deficiencies or excesses. Nematode and root colonising fungal pathogens (such as *Phytophthora*) are the two biotic stress factors most frequently associated with YVD in older vineyards (Table 2). These mechanical and biotic factors cause poor vine development that is often identified as YVD.

Conclusions

YVD in California vineyards is the result of a variety of mechanical and biological stresses affecting both nursery stock and vineyard plants. Common to all cases of YVD is some type of stress, be it that typically associated with Petri disease pathogens or that induced by improper nursery or vineyard management practice. Indeed Gubler (personal communication) believes *Phaeoacremonium* and *Phaeomoniella* may be endophytes - organisms that live asymptomatically in grapevine provided the vine is not stressed. It is hypothesised that mechanical or biotic stresses such as water deficit or nematode populations, for example, may predispose Petri disease infected vines to develop disease symptoms.

The significance of many vine product imperfections is clear: vines with inadequate root systems or incompletely healed graft unions cannot compete as effectively for limited nutrients or cope as readily with environmental or biotic stress as properly finished product. Simply put, defective vines are more susceptible to stress. Examination of new and established vineyards indicate that there are very real, but less immediately obvious, consequences to planting inferior vines and that there is a direct relationship between defective nursery stock and the various conditions in California known as YVD. Exercising caution at planting time is doubly important as observations suggest that pathogens associated with grapevine decline could be widely dispersed through certified rootstock mother blocks.

Of equal importance in protecting against YVD in new and established vineyards is the implementation of effective and consistent farming practices that maintain biological and mechanical stress at minimum levels. Procedures such as pre-plant fumigation and careful monitoring of potential biological and mechanical stress factors can be very effective in reducing the incidence of YVD.

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