



Grapevine pruning systems and cultivars influence the diversity of wood-colonizing fungi



Renaud Travadon^a, Pascal Lecomte^b, Barka Diarra^b, Daniel P. Lawrence^a,
David Renault^{b,c}, Hernán Ojeda^d, Patrice Rey^{b,c}, Kendra Baumgartner^{e,*}

^a Department of Plant Pathology, University of California, Davis, CA 95616, USA

^b INRA, UMR 1065 SAVE, Université de Bordeaux, ISVV, CS 20032, 33882 Villenave d'Ornon Cedex, France

^c Université de Bordeaux, ISVV, UMR 1065 SAVE, Bordeaux Sciences Agro, CS 20032, 33882 Villenave d'Ornon Cedex, France

^d INRA, UE0999, Unité expérimentale de Pech Rouge, 11430 Gruissan, France

^e United States Department of Agriculture - Agricultural Research Service, Crops Pathology and Genetics Research Unit, Davis, CA 95616, USA

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ABSTRACT

Grapevine wood hosts diverse fungal species, including pathogens that cause grapevine trunk diseases and wood decomposers, with detrimental effects on yields. This study focuses on the effects of two pruning systems, minimal (min-) or spur-pruning, on the community of trunk pathogens and other wood-colonizing fungi in the trunks of two cultivars, Mourvèdre and Syrah. Culture and DNA-based methods were used to describe the fungal communities. In both cultivars, especially Syrah, spur-pruned vines had more wood necrosis than min-pruned vines, and the community of spur-pruned Syrah was distinguished by its single-stranded conformational polymorphism (SSCP) profile. Diversity profiles of all 88 cultivated taxa and canonical correspondence analyses of the 15 most frequently isolated taxa revealed differences in community structure due to pruning system, trunk location, and/or wood type. Greater levels of wood necrosis may be due to the composition of the fungal community rather than to a greater diversity of taxa.

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1. Introduction

Characterization of fungal communities that colonize woody hosts, and how species of wood-colonizing fungi collectively decompose wood, is a topic of study typically focused on forest ecosystems, (e.g., Lindner et al., 2011; Rajala et al., 2011). Wood decomposition, accomplished primarily by fungi, provides the ecosystem service of nutrient cycling. Forest management practices, such as partial thinning and the presence of canopy gaps, have been shown to influence the diversity of wood-colonizing fungi (Junninen et al., 2006; Lindner et al., 2006; Brazee et al., 2014). Because the diversity and composition of such communities impacts the rate of wood decomposition, (e.g., Fukami et al., 2010), it is important to identify management practices that promote forest sustainability.

Wood is usually colonized by communities of wood-decomposing fungi, the diversity of which is thought to influence wood decomposition (van der Wal et al., 2013). White-rot and brown-rot fungi (largely basidiomycete species), and soft-rot fungi (ascomycete species), differ in the types of cell wall-degrading enzymes they secrete, and thus they decompose wood at varying rates and to varying degrees (Worrall et al., 1997). Positive interactions between species can be due to substrate-related niche differentiation (resource partitioning); when fungal species decompose different fractions of substrate, a greater number of species can enhance substrate decomposition (Tiunov and Scheu, 2005; LeBauer, 2010). More diverse fungal communities are more likely to contain strong decomposers, i.e., sampling effect (Loreau and Hector, 2001). Also, synergistic interactions among fungal species (e.g., facilitation of cellulose-degrading species by lignin-degrading species) are more likely in communities harboring more species (LeBauer, 2010). However, interactions among fungal species may be negative, in particular when fungal species and/or individuals within a species compete for woody substrates. Such

* Corresponding author.

E-mail addresses: rtravadon@ucdavis.edu (R. Travadon), Kendra.Baumgartner@ars.usda.gov (K. Baumgartner).

antagonistic interactions may divert their metabolic energy away from decomposition and instead toward defense mechanisms (van der Wal et al., 2013).

In agro-ecosystems, wood-decomposer fungi are present in perennial crops, but wood decomposition is not considered an ecosystem service, as it is in forests. Instead, wood-colonizing fungi can disrupt the vasculature and kill fruiting positions of tree crops, thereby impacting yields and reducing the productive lifespan of the orchard, e.g., panicle blight of pistachio (Michailides and Morgan, 2004). In grapevines, some wood-colonizing fungi cause internal infections that appear as necrotic wood cankers or discoloration of the wood, which are thought to be due to a combination of enzymatic decomposition of the wood by the fungi (Rolshausen et al., 2008; Valtaud et al., 2009), secretion of fungal toxins (Abou-Mansour et al., 2015), and/or production of phenolic compounds by the host (Lambert et al., 2012). These fungi are, for the most part, taxonomically unrelated ascomycetes, which cause what are collectively known as ‘trunk diseases’ (Bertsch et al., 2013), including *Botryosphaeria* dieback (main causal agents are *Diplodia seriata*, *Neofusicoccum parvum*), *Eutypa* dieback (*Eutypa lata*), *Phomopsis* dieback (*Diaporthe ampelina*), and Petri disease and Esca (*Phaeomoniella chlamydospora*, *Phaeoacremonium minimum*).

Grapevine trunk pathogens often cause mixed infections; it is rare to encounter a single grapevine infected with a single species (Péros et al., 1999; Urbez-Torres et al., 2006; Luque et al., 2009; Baumgartner et al., 2013). Virulence varies within and among species of trunk pathogens (Urbez-Torres and Gubler, 2009; Travadon and Baumgartner, 2015), and a common perspective on such mixed infections is that certain combinations are lethal. Multiple trunk pathogens and other wood-colonizing fungi may interact in the process of wood decomposition (Sparapano et al., 2000). Resource partitioning might be relevant in the process of such wood decomposition because different pathogens may decompose distinct woody substrates, leading to facilitative interactions. A case of facilitative interactions among grapevine trunk pathogens has recently been demonstrated, where co-inoculations of *Ilyonectria* and *Botryosphaeria* isolates resulted in more severe grapevine decline than inoculations with *Ilyonectria* alone (Whitelaw-Weckert et al., 2013). Nonetheless, very little is known regarding the relationship between fungal diversity and wood decomposition in agricultural settings in general and in vineyards in particular.

Just as certain logging practices affect communities of wood-colonizing fungi in forest trees (Lindner et al., 2006), so may vineyard practices affect fungal colonization of grapevine wood. The influence of vineyard management practices on communities of endophytic fungi in green stems has been illustrated (Pancher et al., 2012), though no such study has examined the effects of vineyard practices on the wood mycobiota. Nonetheless, modifications to the timing of dormant-season pruning, practices known as ‘delayed pruning’ (Petzoldt et al., 1981; van Niekerk et al., 2011) and ‘double pruning’ (Weber et al., 2007), have been shown to minimize pruning-wound infections by trunk pathogens. Another approach to minimize infection is to adopt training and pruning systems that require fewer pruning wounds, thereby reducing the number of possible infection courts. Vines trained to a head, rather than to bilateral cordons, for example, have been shown to have a lower incidence of *Eutypa* dieback (Gu et al., 2005). We tested the hypothesis that a minimal pruning system, with fewer pruning wounds per vine, is also associated with less wood necrosis and fewer trunk pathogens than a standard, spur-pruning system. Levels of wood necrosis and communities of cultivable fungi were compared in both pruning systems, which were replicated in separate vineyards planted with different wine-grape cultivars,

Mourvèdre and Syrah. Understanding how the diversity and composition of fungal communities in the vine wood is affected by pruning, and in turn how the fungal community affects wood decomposition or vascular dysfunction, might help us identify more effective management practices for trunk diseases.

2. Materials and methods

2.1. Study site

Vines were sampled at Pech Rouge Experimental Station (French National Institute for Agricultural Research (INRA)), Montpellier, located on the Mediterranean Sea in Gruissan, France (43°07′52.94″N; 3°04′55.31″E). The study was replicated in two vineyard sites at the station, planted with two different cultivars and separated by ~300 m. *Vitis vinifera* ‘Mourvèdre’ was planted in 1999 and ‘Syrah’ was planted in 1994. Both cultivars were grafted onto rootstock 140 Ru (*Vitis. berlandieri* × *Vitis. rupestris*) and vines were originally trained to a bilateral-cordon system. Half of the vinerows in each cultivar were either maintained as spur-pruned or converted to minimal pruning, starting in 2003 for the Mourvèdre vineyard and 2002 for the Syrah vineyard. Spur-pruned vines had three to five spurs per cordon, with two to three buds per spur

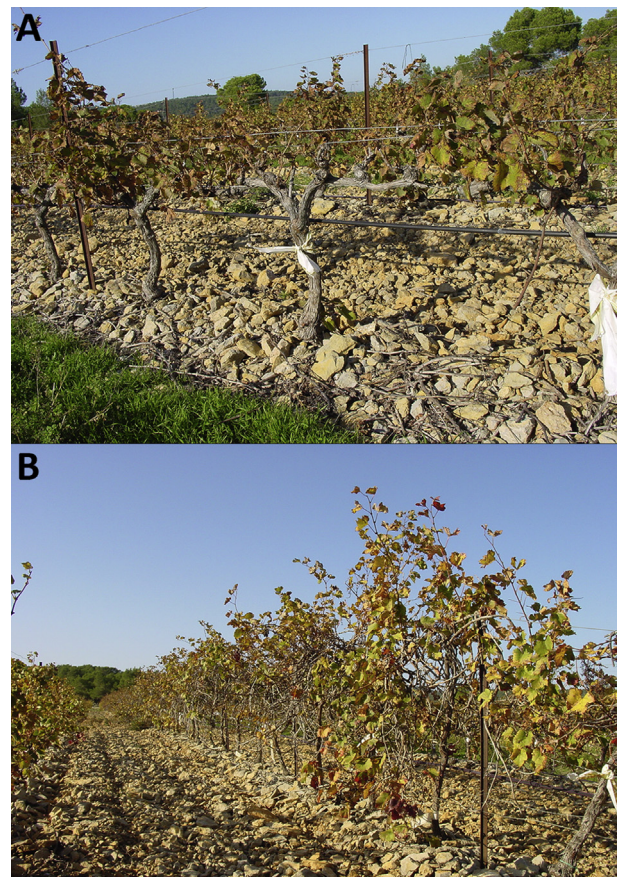


Fig. 1. Grapevines were either spur-pruned (A) or min-pruned (B). Shown here is Mourvèdre, photographed when vines were sampled in November 2012. Vines were selected for sampling the preceding September, when the foliar symptoms of Esca were apparent. We selected vines from sections of the Mourvèdre and Syrah vineyards with no foliar symptoms of Esca and no foliar or canopy symptoms (dead spurs, stunted shoots, shoot dieback) of other trunk diseases (*Botryosphaeria* dieback, *Eutypa* dieback, *Phomopsis* dieback). Spur-pruned vines had two cordons with a trunk height of approximately 50 cm, whereas min-pruned vines had no cordons and a trunk height of approximately 100 cm.

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