Identifying biochemical and anatomical markers of resistance in grape, almond and pistachio wood

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Rationale

Cultivars vary in tolerance/susceptibility towards trunk diseases. Based on field observations and bioassays.

(Feliciano et al., 2004; Travadon et al., 2013; Bruez et al., 2013; Murolo and Romanazzi, 2014)

What are the defense mechanisms responsible for these differences?

-Cell walls chemical composition

-Differences in wood anatomy/response of germplasms

Crop	Cultivar	Pathogen
Grape	**Merlot** **Cabernet Sauvignon** **Chardonnay** **Thompson Seedless**	*P. chlamydospora* *T. minima* *E. lata* *N. parvum*
Almond	**Butte, Monterey** **Padre, Non-Pareil**	*N. nonquaesitum* *N. parvum*
Pistachio	**Golden Hills, Randy** **Kerman, Kalaghuchi**	*N. mediteraneum* *B. dothidea*

Organization of defenses in xylem

CODIT model (Compartmentalization Of Decay In Tree) (Shigo et al., 1977)

Wall 1

Wall 1 = Vessel occlusion

n+1

n

n---1

Organization of defenses in xylem

CODIT model (Compartmentalization Of Decay In Tree) (Shigo et al., 1977)

Wall 1

n+1

Wall 2

n

Wall 2

n---1

Wall 1 = Vessel occlusion

Wall 2 = Growth ring

Organization of defenses in xylem

CODIT model (Compartmentalization Of Decay In Tree) (Shigo et al., 1977)

Wall 1

n+1

Wall 3

n

Wall 2

Wall 2

Wall 3

n---1

Wall 1 = Vessel occlusion

Wall 2 = Growth ring

Wall 3 = Ray parenchyma

Organization of defenses in xylem

CODIT model (Compartmentalization Of Decay In Tree) (Shigo et al., 1977)

Wall 1

Wall 4

n+1

Wall 3

Wall 3

n

Wall 2

Wall 2

Wall 4

n---1

Wall 1 = Vessel occlusion

Wall 2 = Growth ring

Wall 3 = Ray parenchyma

Wall 4 = Barrier zone

Phaeomoniella chlamydospora (*Pch*)

One of the causal agent of esca disease, Petri disease

(Mugnai et al., 1999; Feliciano et al., 2004; Úrbez-Torres et al., 2014)

Production of phytotoxins

-Pentaketides (Sparapano et al., 2001; Bruno and Sparapano, 2006)

-Pullulans (Bruno and Sparapano, 2006)

-Secreted polypeptides (Luini et al., 2012)

Colonization strategy

In vitro assays:

-Low enzymatic activity related to cell-wall breakdown (Santos et al., 2006; Valtaud et al, 2009)

-Can use pectin as source of carbon (Surico et al., 2001)

Histological studies:

-Located in vessels and cell lumens (Valtaud et al., 2009; Mutawila et al., 2011; Pouzoulet et al., 2013)

-Colonized occluded vessels (Mutawila et al., 2010; Fleurat-Lessard et al., 2010)

Experimental approach

Selection of cultivars that greatly vary in tolerance (continuum)

(Feliciano et al., 2004; Bruez et al., 2013; Travadon et al., 2013)

Merlot
(Tolerant)

Cab. Sauv./Chardonnay
(Susceptible)

Thompson Seedless
(Highly susceptible)

Increasing susceptibility

a-Experimental validation of this model

b-Screening wood morphological traits > correlation

c-Understanding how these traits could affect the host defense
> host susceptibility to esca disease

Results

Experimental validation of cultivar's susceptibility.

Phaeomoniella chlamydospora infected cuttings 10 weeks post-inoculation

1 - Measure of vascular discoloration.

>Confirm Merlot is the most tolerant cultivars
>BUT, do not allowed a separation of Cabernet Sauvignon, Chardonnay, and Thompson in different groups

2 - Measure of fungal DNA (qPCR).

>Allowed cultivars Cabernet Sauvignon, Chardonnay and Thompson to be classified in different susceptibility group according to the field observations.
>Suggest differences of restriction at the vessel level

5 mm

Results

Characterization of xylem's morphology

6 internodes per cultivars per years (3 stems from 2 mother-vines)

-8 to 10mm in diameter; 100 to 120 internode length

-repeated over two years (2013 and 2014)

	Area	Perim.
1	4748.513	260.989
2	140.617	43.553
3	1022.174	116.940
4	37.858	25.747
5	2563.548	196.905
6	351.541	70.662
7	14510.546	452.114
8	22401.298	574.036
9	18977.826	527.895
10	1471.065	144.847
11	37.858	27.673
12	146.025	43.553
13	535.425	81.891
14	200.108	64.085
…		

1 years old stem cross section, Toluidine O

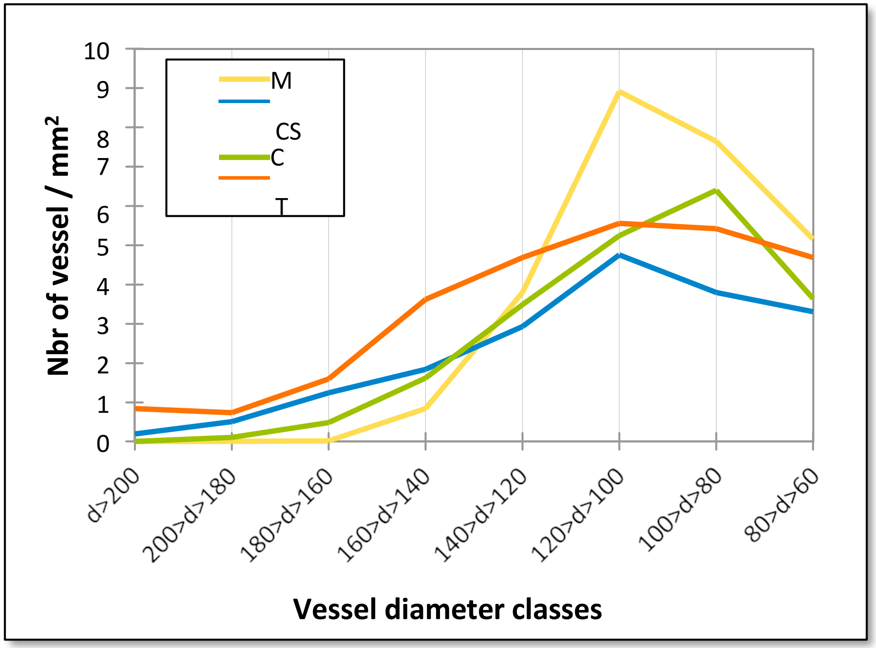
Automated analysis using ImageJ

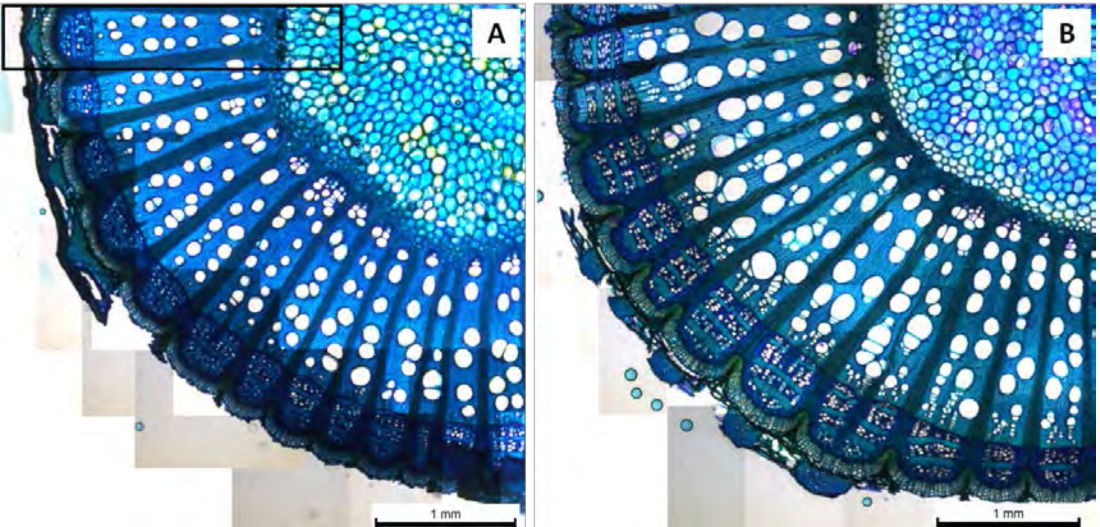
(Scholz et al., 2014)

Data (approx. 100-200 vessels/sample)

Results

Characterization of xylem's morphology





- ➢➢ Vessel diameter distributions vary significantly amongst cultivars

- ➢➢ Tylosis formation and complete vessel plugging requires more time and resources in large vessel cultivars. It provides an opportunity for the fungus to colonize the vascular system faster.

- ➢➢ In plugged vessels, the chemical nature of the tylosis walls provide a suitable food substrate for Pc.

Discussion & perspectives

Can we use this concept for the control of esca disease in field ?

- Selection/use of "narrow" vesseled cultivars > tolerant to esca disease

Asymptomatic
Hidden esca

Abiotic factors
Rainfall
Fertilization

Symptomatic
(Tiger striped leaves)

- **Abiotic** factors during plant growth affect the distribution of vessel diameter (Lovisolo and Schubert, 1998; Solla and Gil, 2002; Fichot et al., 2009)
- Change in vessel diameter mediated water treatment affects expression of DED (Solla and Gil, 2002)
- Does this relationship valid for esca disease pathogens ?
- Can appropriate water and fertilization regimes be used to mitigate the impact of esca disease ?

frontiers in
PLANT SCIENCE

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Can vessel dimension explain tolerance toward fungal vascular wilt diseases in woody plants? Lessons from Dutch elm disease and esca disease in grapevine

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This review illuminates key findings in our understanding of grapevine xylem resistance to fungal vascular wilt diseases. Grapevine (*Vitis* spp.) vascular diseases such as esca, botryosphaeria dieback, and eutypa dieback, are caused by a set of taxonomically unrelated ascomycete fungi. Fungal colonization of the vascular system leads to a decline of the plant host because of a loss of the xylem function and subsequent decrease in hydraulic conductivity. Fungal vascular pathogens use different colonization strategies to invade and kill their host. *Vitis vinifera* cultivars display different levels of tolerance toward vascular diseases caused by fungi, but the plant defense mechanisms underlying those observations have not been completely elucidated. In this review, we establish a parallel between two vascular diseases, grapevine esca disease and Dutch elm disease, and argue that the former should be viewed as a vascular wilt disease. Plant genotypes exhibit differences in xylem morphology and resistance to fungal pathogens causing vascular wilt diseases. We provide evidence that the susceptibility of three commercial *V. vinifera* cultivars to esca disease is correlated to large vessel diameter. Additionally, we explore how xylem morphological traits related to water transport are influenced by abiotic factors, and how these might impact host tolerance of vascular wilt fungi. Finally, we explore the utility of this concept for predicting which *V. vinifera* cultivars are most vulnerable of fungal vascular wilt diseases and propose new strategies for disease management.