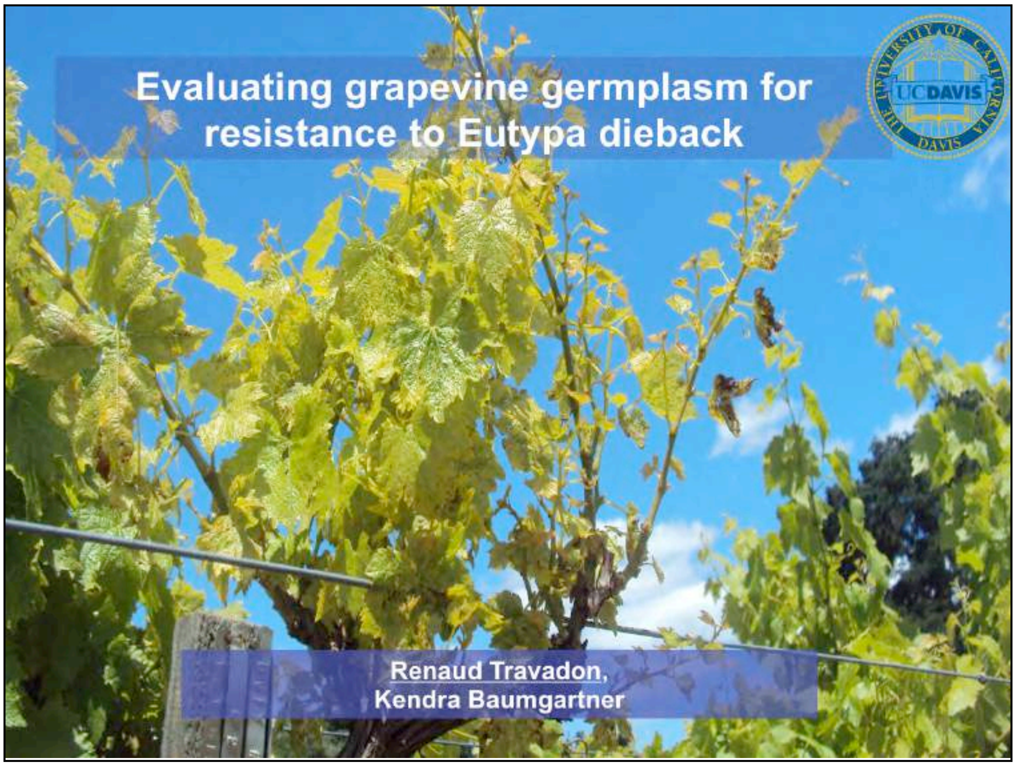

Evaluating grapevine germplasm for resistance to Eutypa dieback
Renaud Travadon,
Kendra Baumgartner

Background

Differential susceptibility of cultivars has been observed in vineyards based on the expression of foliar symptoms.

Dubos (1987)

Class	Cultivars
Very susceptible	Cabernet Sauvignon, Chasselas, Grenache, Mauzac, Negrette, Sauvignon, Ugni Blanc
Susceptible	Alicante Bouschet, Chardonnay, Chenin, Cinsault, Gewurtzraminer, Muscat Ottonel, Pinot Meunier, Pinot Noir, Tannat, Jurancon, Muscadelle, Syrah
Moderately susceptible	Cabernet Franc, Colombard, Malbec, Riesling, Portugais bleu, Gamay, Duras
Resistant	Aligoté, Grolleau, Merlot, Semillon, Sylvaner

Complications with field observations: for a same cultivar, variation in foliar symptom expression:

- Year to year - Plant age - Training system
- Water stress, salinity - Pathogen variability - Presence of other pathogens within the plant

To attenuate such heterogeneity, the use of screening methods in controlled conditions is required.

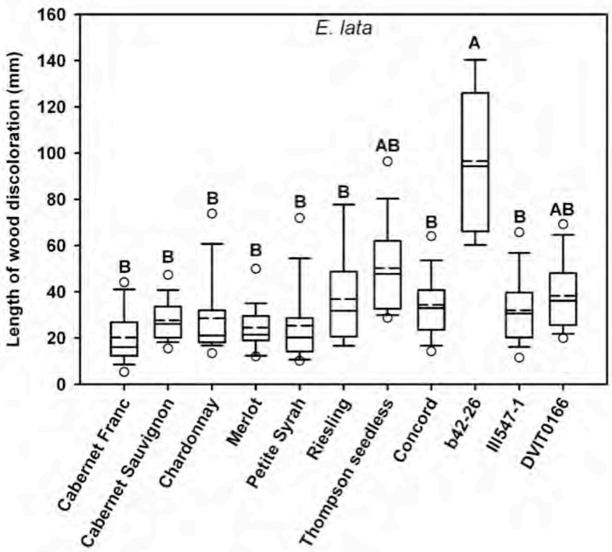
Variation in susceptibility of wine-grape cultivars to the foliar symptoms of Eutypa dieback has been observed in the field. In France, for example, Cabernet Sauvignon and Grenache are ranked as very susceptible, while Merlot and Semillon are considered resistant.

In the field, however, Eutypa symptoms can vary from year to year, depending on plant age, on abiotic factors, and on the pathogen variability.

Evaluating differences in susceptibility could thus benefit from experiments under controlled conditions.

Background

In previous experiments aiming at identifying resistant cultivars to major trunk pathogens (Travadon et al., 2013), we screened seven *V. vinifera* cultivars, the *Vitis* hybrid Concord, and three grapevines accessions used in breeding programs (Pierce's disease, downy and powdery mildew) for resistance to wood symptoms caused by *E. lata* and an undescribed *Eutypa* species that is predominant in eastern North American vineyards (Rolshausen et al., 2013).



- *b42-26 = V. arizonica (*introgressed with *V. girdiana)*
- *III547-1 = V. rupestris* x *V. cinerea*
- DVIT0166 = *V. aestivalis* x *V. vinifera* Tamiami

V. arizonica b42-26 was by far the most susceptible accession. It is resistant to Pierce's disease because of its inability to plug xylem vessels with tyloses and gels, which could explain its higher susceptibility to Eutypa.

V. rupestris x *V. cinerea* 'III5471' and *V. aestivalis* hybrid Tamiami 'DVIT0166' did not show improved resistance to wood symptoms caused by *E. lata*.

In a previous controlled experiment aimed at identifying resistant cultivars (Travadon et al., 2013), we screened:

1. Six wine-grape cultivars,
2. Table-grape cultivar 'Thompson seedless',
3. Juice-grape cultivar 'Concord',
4. three germplasm accessions used in breeding programs

b42-26 was by far the most susceptible. Interestingly, it is resistant to Pierce's disease and does not show foliar symptoms in spite of hosting high *Xylella fastidiosa* populations, possibly because of its inability to plug xylem vessels with tyloses and gels. However, this feature could explain its higher susceptibility to *Eutypa*.

Thompson seedless was slightly more susceptible than the wine-grape cultivars and Concord.

Identify sources of *Eutypa* resistance in grape

- Objective 1: Screen for Eutypa resistance among genetically diverse grapevine cultivars

accession	clone	species	cultivar	Region of origin	Genetic group (Aradhya et al., 2003)	Notes
DVIT 1064	03	*Vitis vinifera*	Carignane	Spain	Central European grape cluster, group 13	test
DVIT 1342	03	*Vitis vinifera*	Primitivo	Croatia	Central European grape cluster, group 13	test
DVIT 1059	03	*Vitis vinifera*	Muscat Hamburg	Germany	Central European grape cluster, group 15	test
DVIT 535	02A	*Vitis vinifera*	Thompson Seedless	Turkey	Mediterranean table-grape cluster, group 1	Susceptible control
DVIT 0419	02	*Vitis vinifera*	Husseine	Afghanista n	Mediterranean table-grape cluster, group 1	test
DVIT 0354	02	*Vitis vinifera*	Black Corinth	Greece	Mediterranean table-grape cluster, group 2	test
DVIT 0882	01A	*Vitis vinifera*	Palomino	Spain	Western European wine-grape cluster, group 12	test
DVIT 826	15	*Vitis vinifera*	Merlot	France	Western European wine-grape cluster, group 8	Resistant control
DVIT 0710	01	*Vitis vinifera*	Peloursin	France	Western European wine-grape cluster, group 10	test

Our next experiment in the greenhouse was focused on screening a more broad set of germplasm, genetically speaking, for resistance to *Eutypa*.

Methods

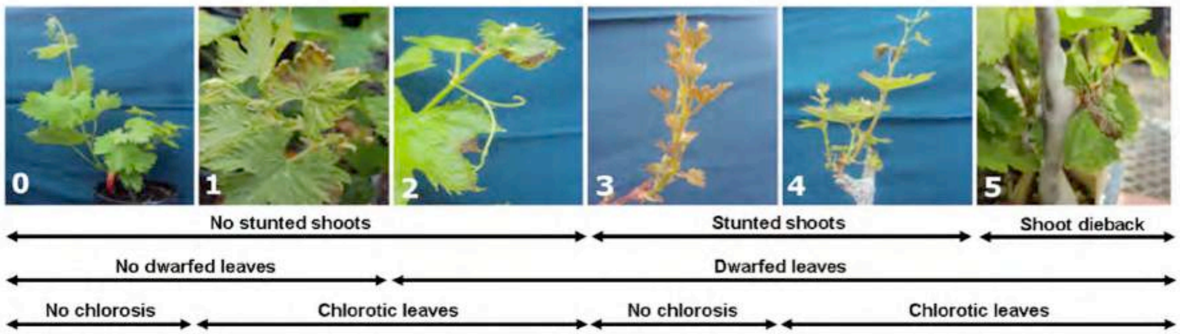
Standardizing methodologies among laboratories

Use of reference isolates:

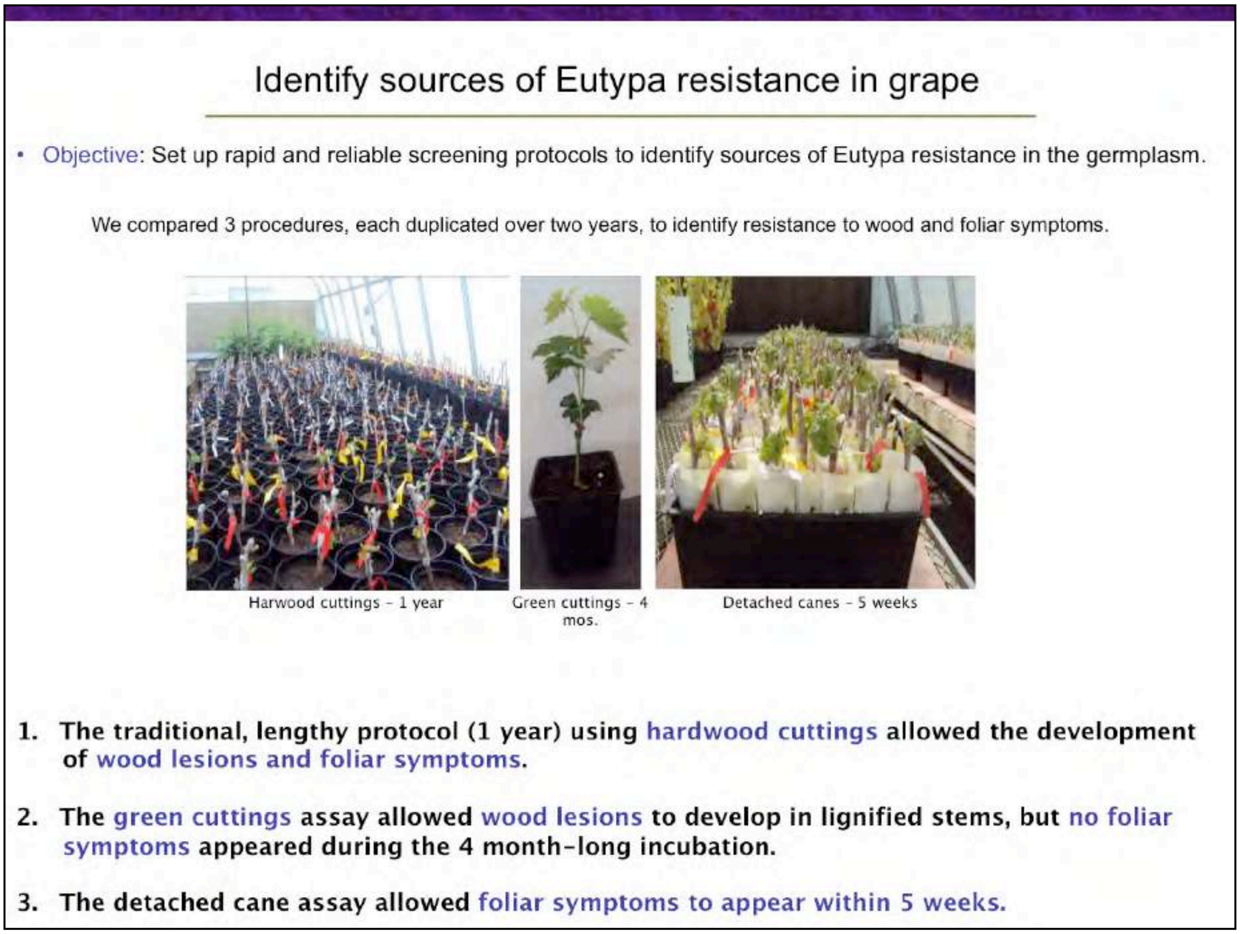
Isolate	species	Region of origin	Cultivar of origin	Notes
M14	*Eutypa lata*	California, Napa Valley	Merlot	reference isolate Baumgartner lab.
BX1-10	*Eutypa lata*	Bordeaux, France	Cabernet Sauvignon	reference isolate INRA France

Uniform notation scale:

(adapted from Péros & Berger, 1994)



In order to develop a standard procedure that could be reproduced among laboratories, we used two *Eutypa* reference isolates used in previous international studies and we developed a uniform notation scale for measuring the severity of foliar symptoms.



We used three distinct protocols to identify rapid and reliable screening assays, one using dormant hardwood cuttings, one using mist-propagated, green cuttings, and then using unrooted and detached dormant canes.

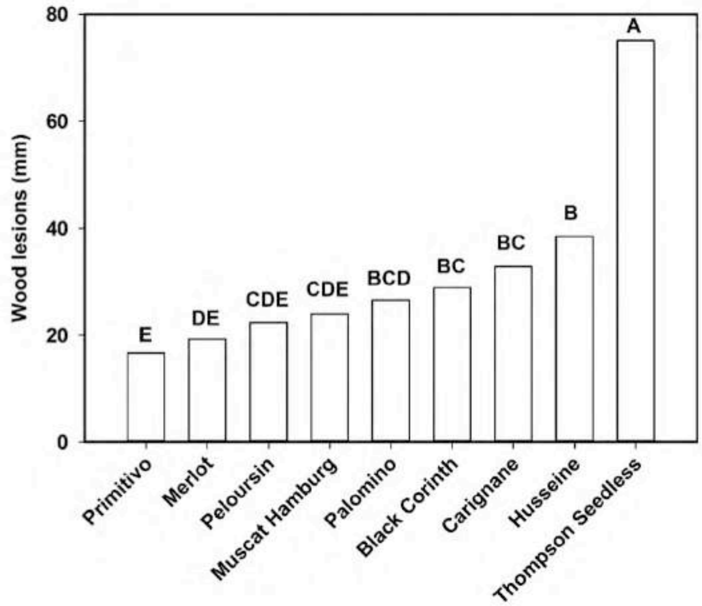
1.The traditional, lengthy protocol (1 year) using hardwood cuttings allowed the development of wood lesions and foliar symptoms.

2.The green cuttings assay allowed wood lesions to develop in lignified stems, but no foliar symptoms appeared during the 4 month-long incubation.

3.The detached cane assay allowed foliar symptoms to appear within 5 weeks.

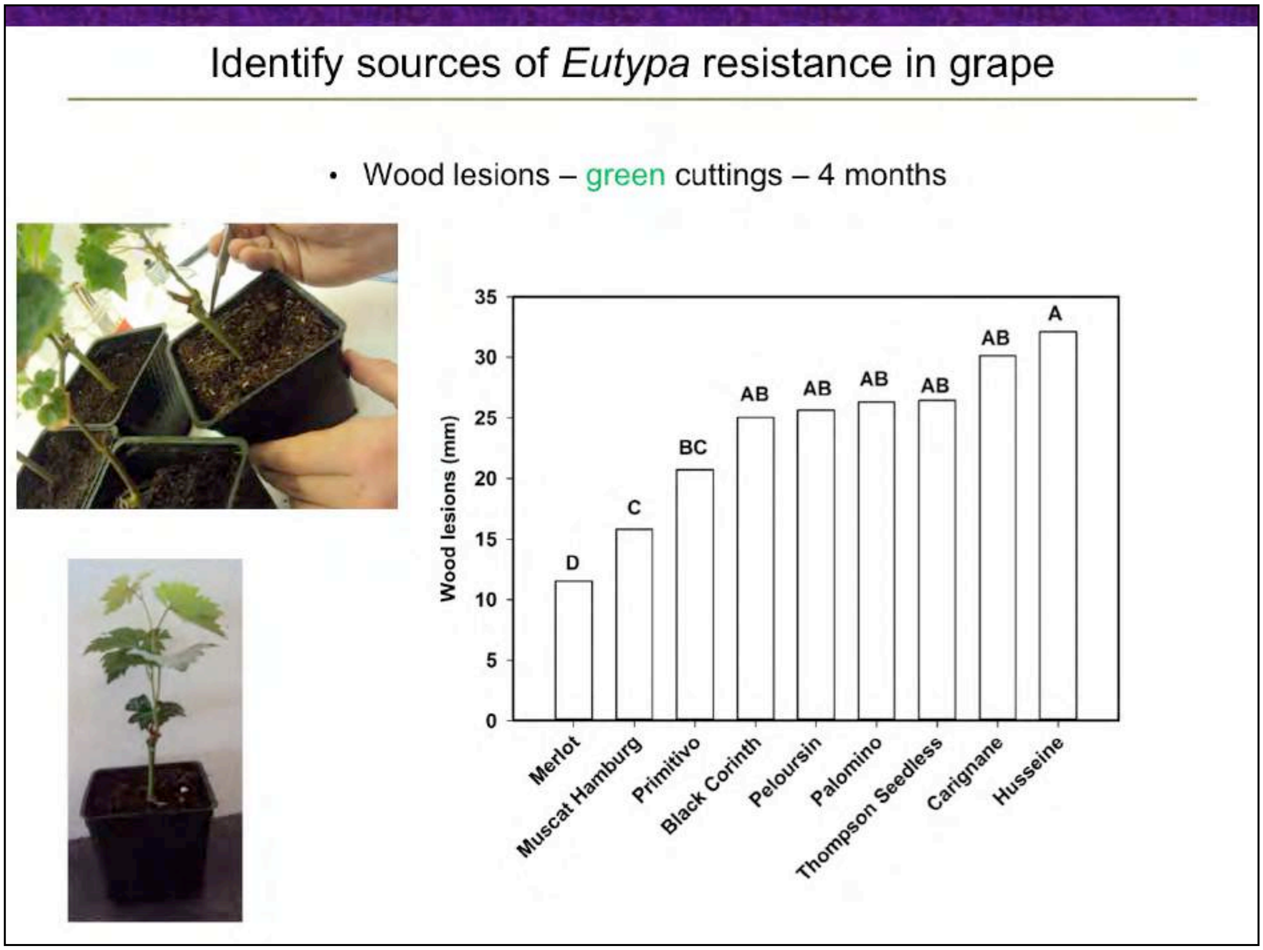
Identify sources of *Eutypa* resistance in grape

- Wood lesions – hardwood cuttings – 12 months



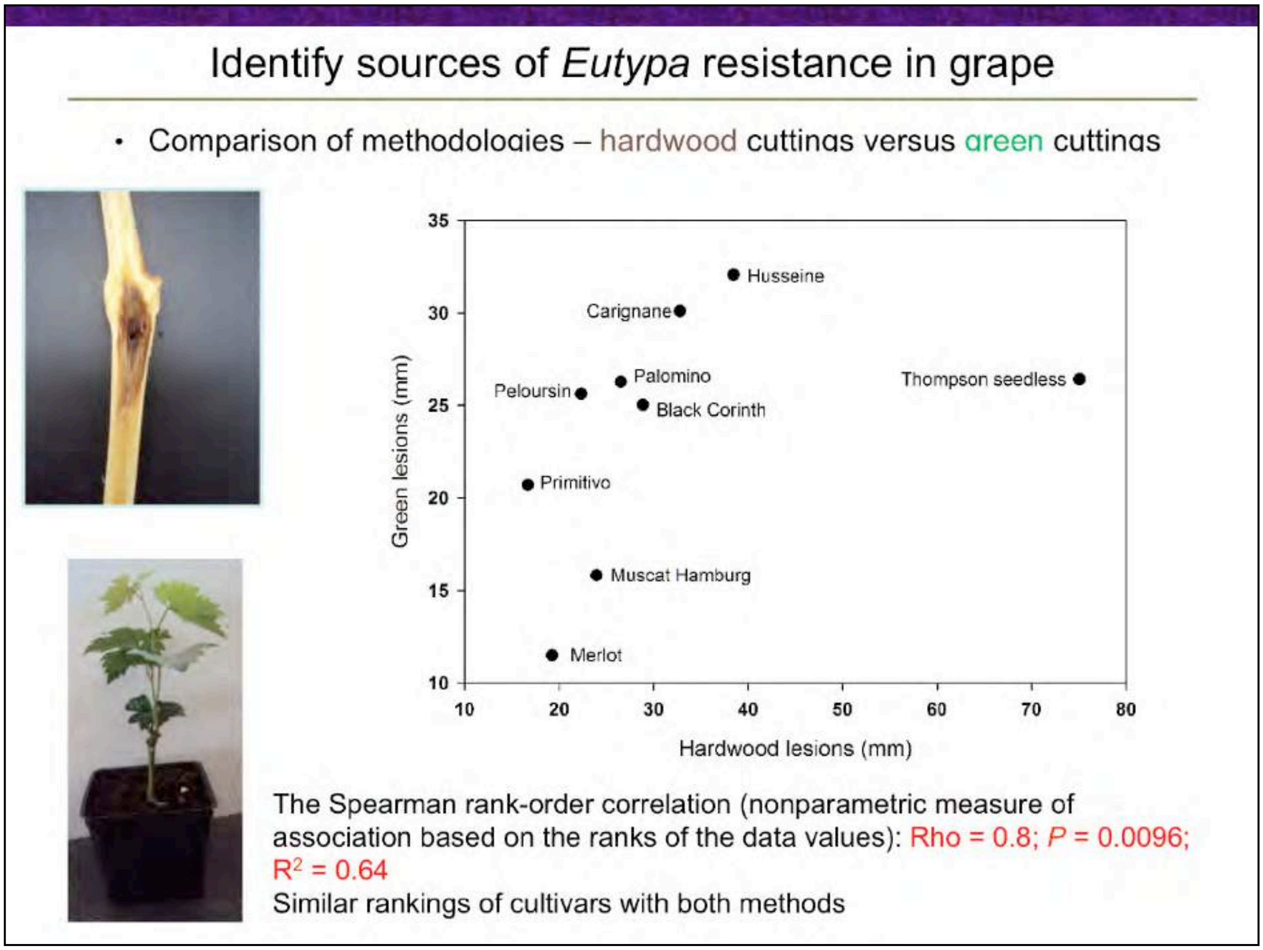
Using hardwood dormant cuttings, we identified differences in susceptibility of cultivars to wood lesions caused by *Eutypa*. After 12 months incubation in the greenhouse, Primitivo was characterized as the most resistant cultivar, while Thompson seedless as the most susceptible.

Large variation in wood symptoms reflect differences in resistance to *Eutypa* among the selected cultivars.



Using green cuttings, we also identified differences in susceptibility of cultivars to lesions developing in lignifying stems. After 4 months incubation in the greenhouse, Merlot was characterized as the most resistant cultivar, while Husseine as the most susceptible.

With green cuttings, no foliar symptoms developed within four months, only lesions developed in stems.



When comparing the results of the “hardwood” assay with those of the “green cuttings” assay, we can observe a similar ranking of cultivars based on the length of the lesions developing in their stems. Positive and significant correlations indicated that both methodologies provided consistent ranking of cultivars (Merlot, Primitivo as resistant; Thompson seedless and Husseine as susceptible).

Foliar symptoms observed after 11 months

Muscat Hamburg

Primitivo (=Zinfandel)

Black Corinth

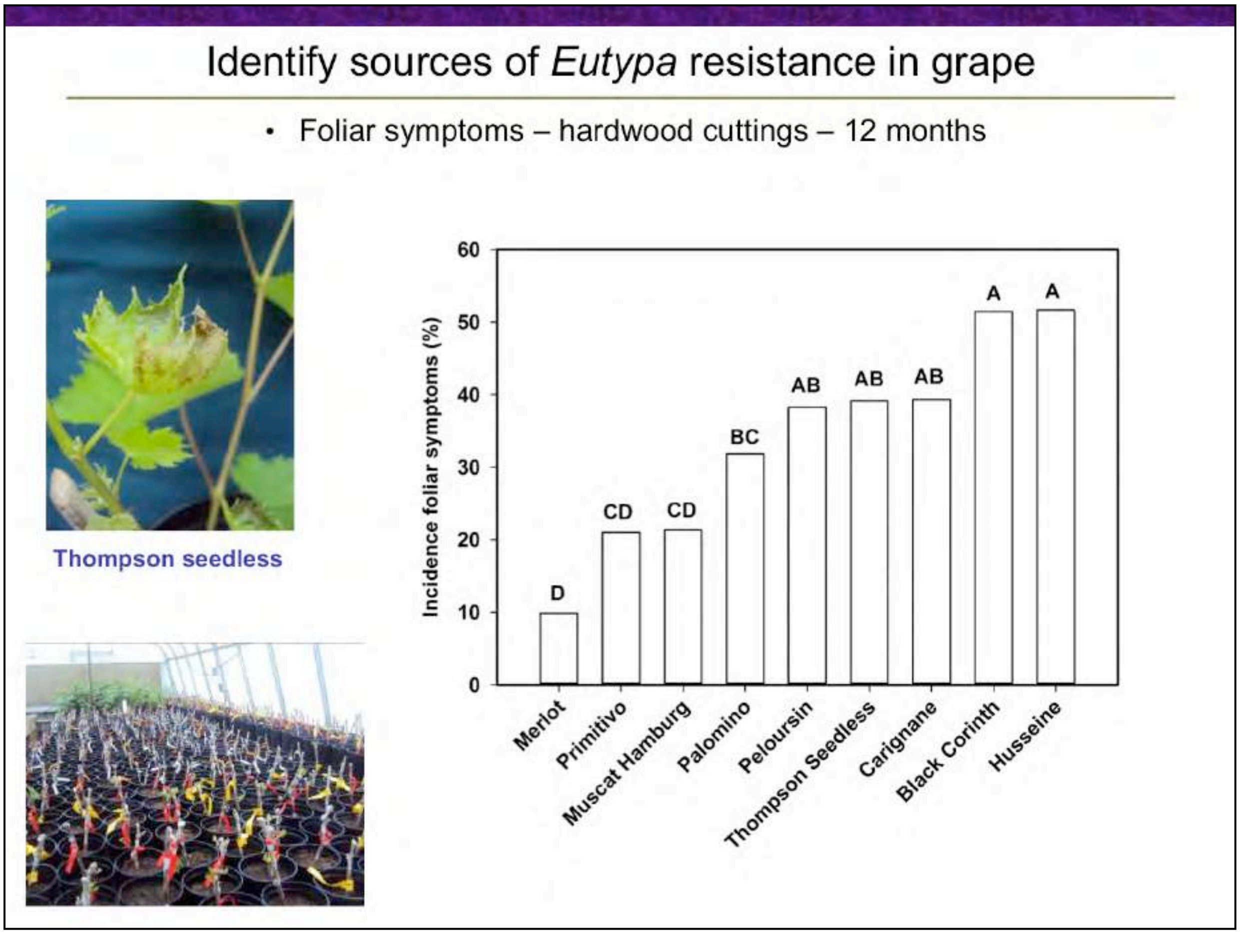
Using hardwood cuttings, *Eutypa* foliar symptoms were visible on each cultivar in the second growth cycle, as illustrated on these photographs.

Foliar symptoms observed after 11 months

Thompson seedless

Peloursin

Husseine

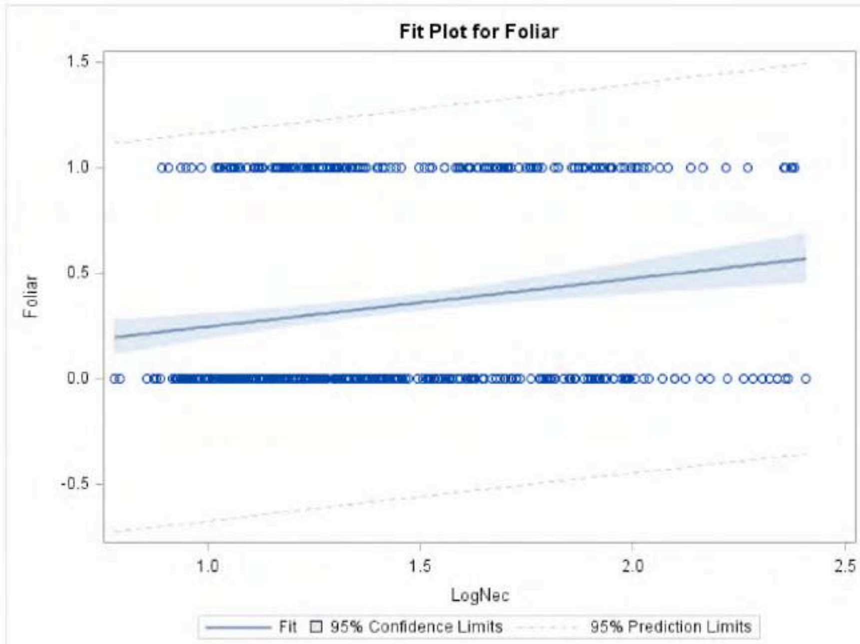


When looking at the incidence of foliar symptoms in hardwood dormant cuttings, we identified differences in susceptibility of cultivars.

After 11 months incubation in the greenhouse, Merlot and Primitivo were characterized as the most resistant cultivars, while Black Corinth and Husseine as the most susceptible.

Identify sources of *Eutypa* resistance in grape

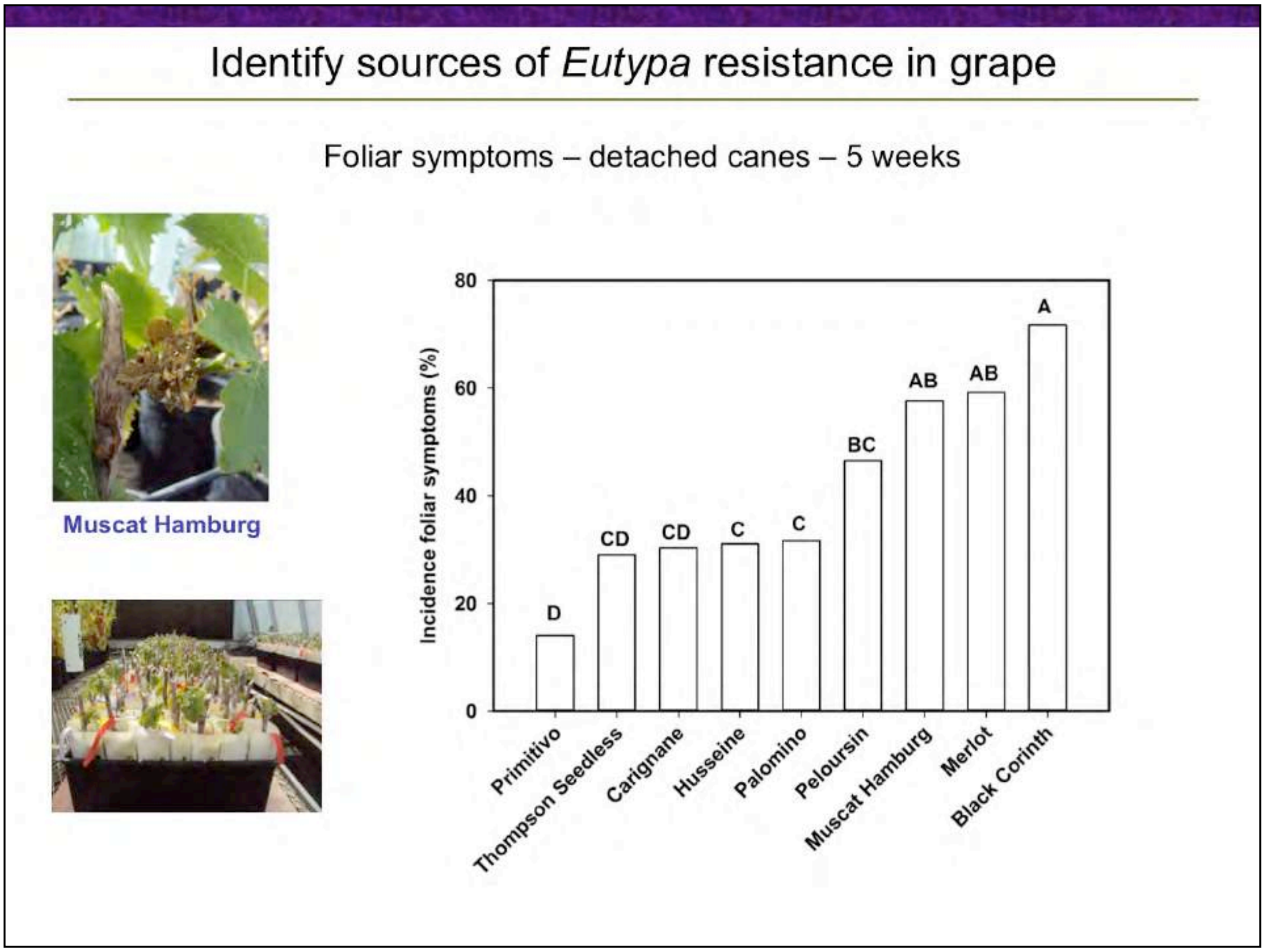
- hardwood cuttings – Relation between presence of foliar symptoms and length of lesions in stems?



No clear relation between wood and foliar symptoms.

In the one-year long traditional assay using hardwood cuttings, we did not observed a significant relation between the length of the lesions and the presence of foliar symptoms.

Plants having large lesions were not more likely to develop foliar symptoms.

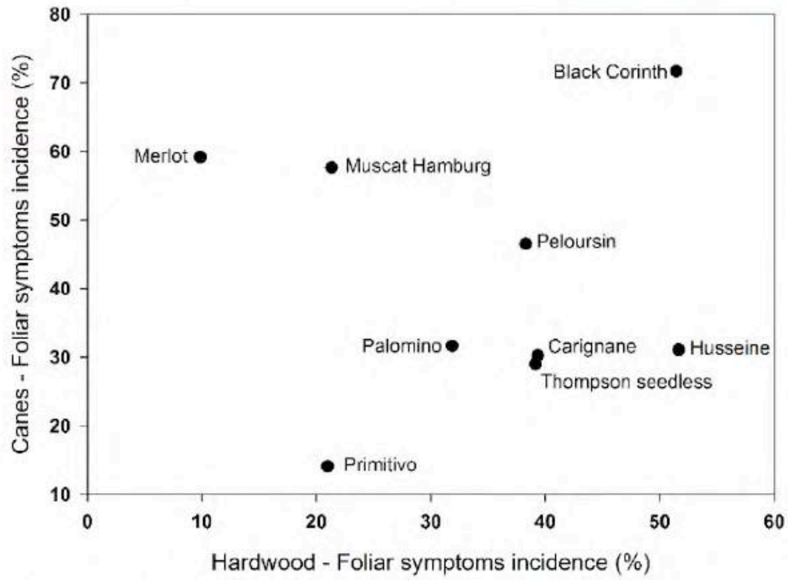


Using detached canes, we obtained foliar symptoms after 5 weeks, and we observed important differences among cultivars.

Primitivo was ranked as most resistant, while Black Corinth was the most susceptible. Merlot was ranked as second most susceptible in this assay.

Identify sources of *Eutypa* resistance in grape

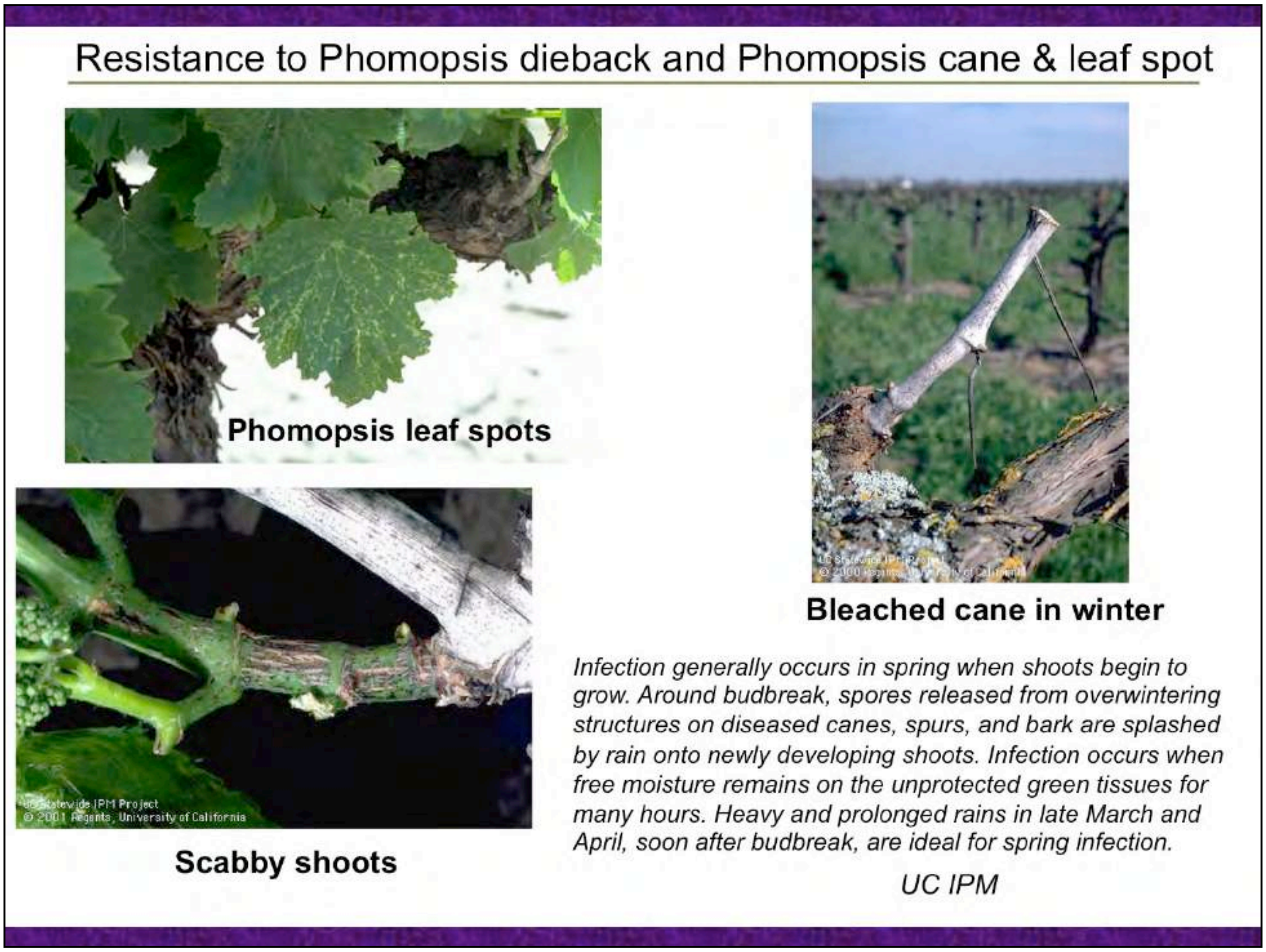
- Comparison of methodologies – hardwood cuttings versus detached canes



The Spearman rank-order correlation (nonparametric measure of association based on the ranks of the data values): Rho = -0.05; *P* = 0.8984

Different rankings of cultivars with the two methods

The assays using hardwood cuttings and detached canes provided different results on the ranking of cultivars based on their level of susceptibility to *Eutypa* foliar symptoms.



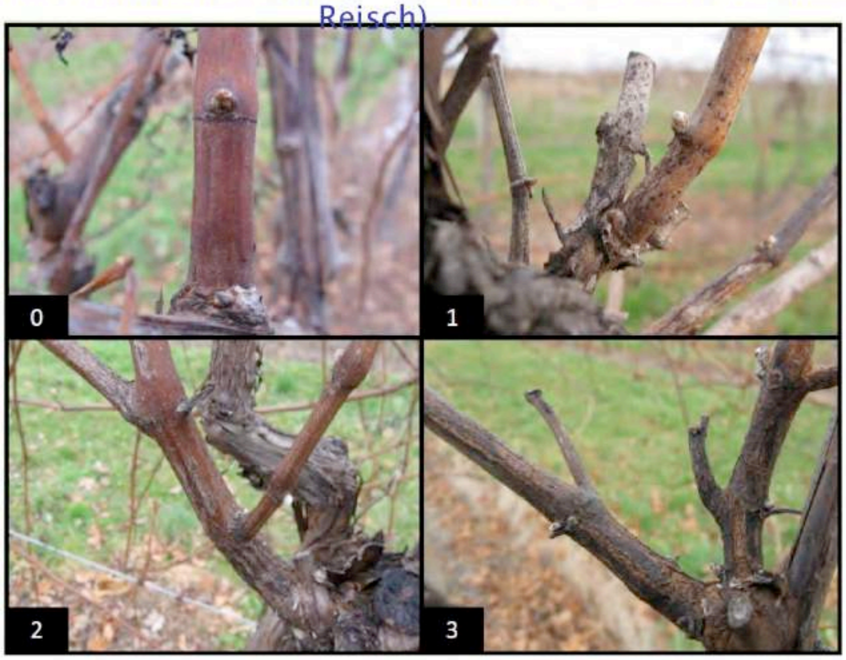
A second project in our lab focuses on resistance to another destructive trunk disease, Phomopsis dieback.

Phomopsis cane and leaf spot disease is a common foliar disease in temperate climates, where rain events throughout the growing season spread spores and initiate infection. It is caused by *Phomopsis viticola* (now named *Diaporthe ampelina*). Typical symptoms include leaf spot, black stem lesions, and bleached canes in winter.

The same fungus causes the trunk disease Phomopsis dieback, which is more common in Mediterranean climates, but we found it also occurs in temperate climates.

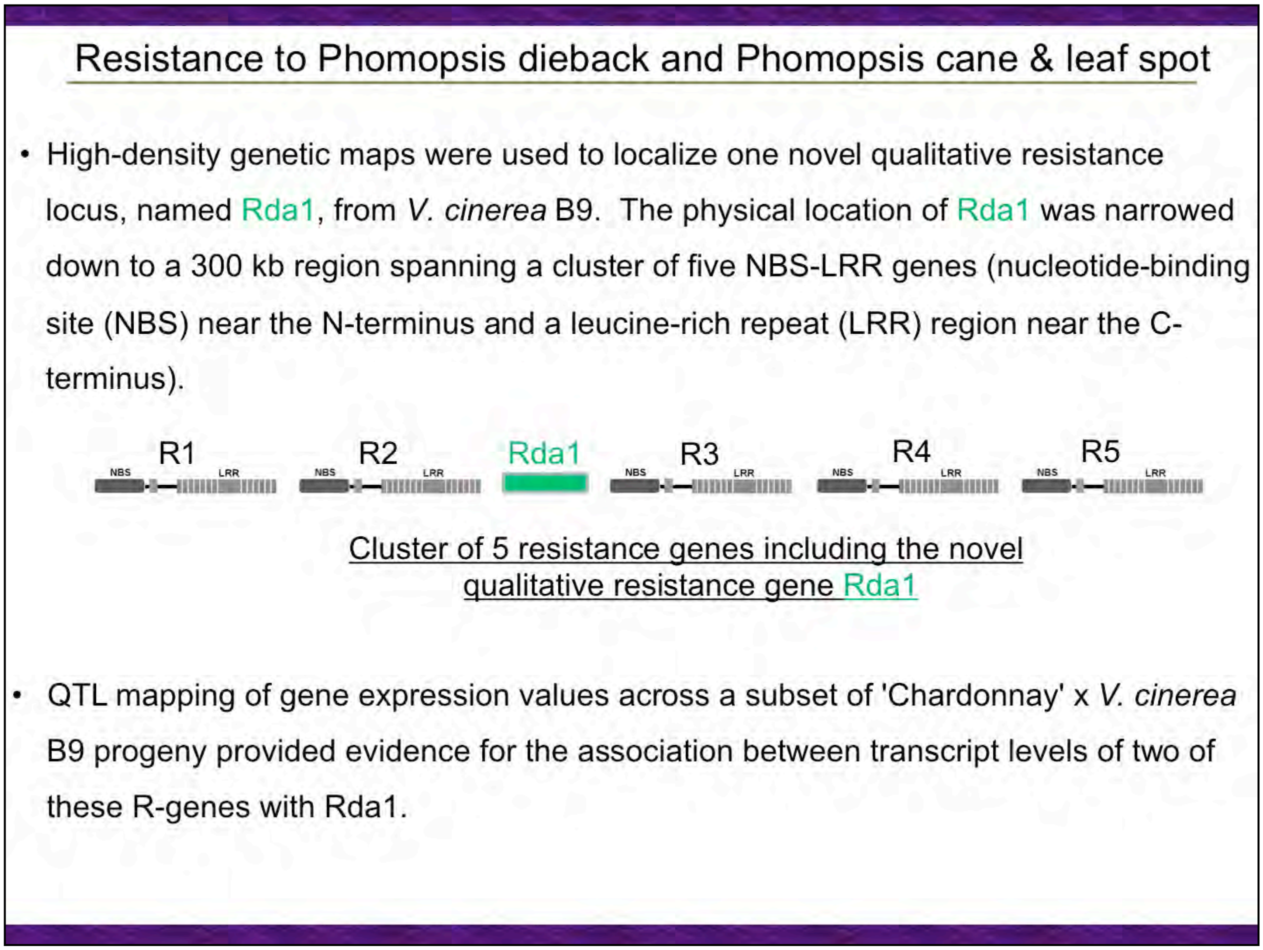
Resistance to Phomopsis dieback and Phomopsis cane & leaf spot

in Geneva, NY, segregation of symptoms was observed and scored on dormant canes on F1 families from Chardonnay * *V. cinerea* B9 crosses (Collaboration B. Reisch).



Disease ratings (0 to 3) of cane symptoms was conducted for 149 siblings from Chardonnay * *V.*

In Geneva, NY, segregation of symptoms was observed and scored on progeny from a cross of Chardonnay x *V. cinerea* B9 (Collaboration with Bruce Reisch, Cornell University, Geneva, NY).

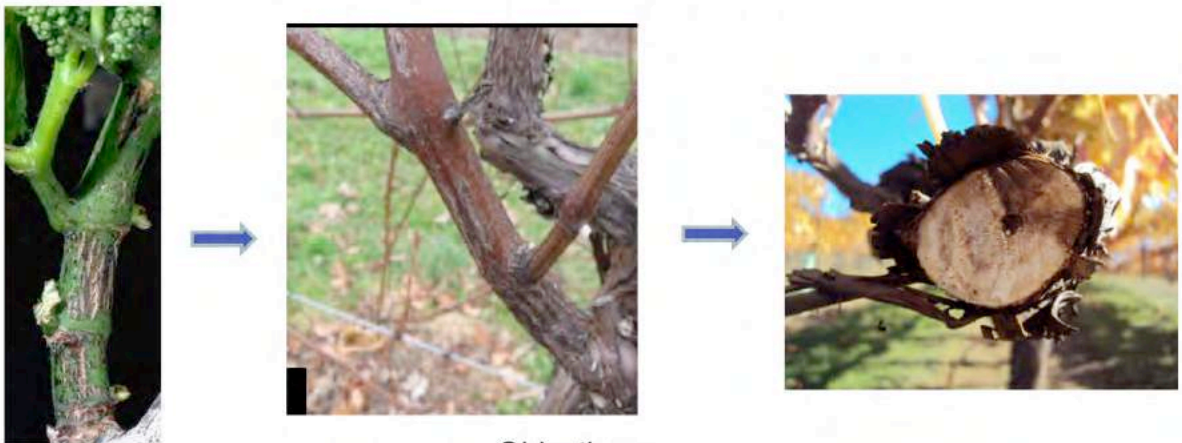


High-density genetic maps were used to localize one novel qualitative resistance locus, named Rda1, from *V. cinerea* B9. The physical location of Rda1 was narrowed down to a 300 kb region spanning a cluster of five NBS-LRR genes (nucleotide-binding site (NBS) near the N-terminus and a leucine-rich repeat (LRR) region near the C-terminus).

QTL mapping of gene expression values across a subset of 'Chardonnay' x *V. cinerea* B9 progeny provided evidence for the association between transcript levels of two of these R-genes with Rda1.

Resistance to Phomopsis dieback and Phomopsis cane & leaf spot

- With dominant effects associated with minimal symptoms on canes, Rda1 is suitable for marker-assisted selection of Phomopsis resistant progeny.



Objective:

Determine if the genetic resistance to Phomopsis cane & leaf spot, expressed in green tissues, provides dual resistance to Phomopsis dieback in woody tissues.

Our objective is to determine if the genetic resistance to Phomopsis cane and leaf spots observed in the field correlates with levels of resistance to the Phomopsis dieback symptoms in woody tissues.

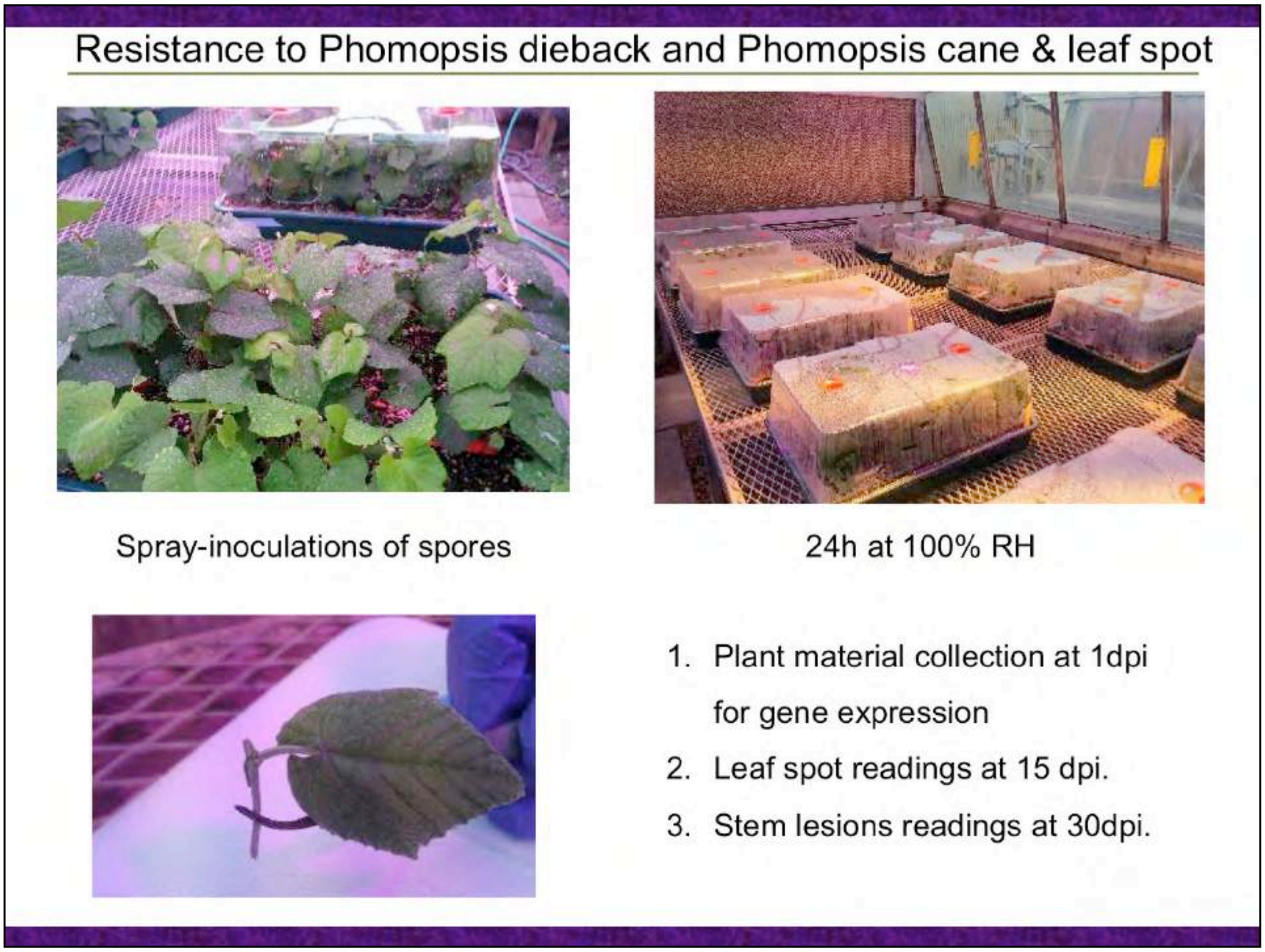
Resistance to Phomopsis dieback and Phomopsis cane & leaf spot

List of siblings and parents that segregate for Phomopsis cane symptoms and will be screened for resistance to wood symptoms.

Vine ID	Phomopsis rating
454064	0
455035	0
454053	1
454058	1
454066	2
454071	2
455072	3
455082	3
454045	3+
454077	3+
V. cinerea B9	1
Chardonnay 95	1

We propagated green cuttings of these 12 accessions for confirming that the phenotypes based on cane lesions in the field are consistent with phenotypes based on green shoot lesions in controlled conditions.

Using the two parents and ten progenies, we first propagated green cuttings to confirm the segregation of resistance to Phomopsis stem lesions observed in the field could be reproduced under controlled conditions.



We inoculated these twelve accessions by spraying Phomopsis spore suspensions on leaves and green stems. One day after inoculations, plant material was collected and flash frozen to measure gene defense expression levels. After 15 days incubation, leaf spots were counted on each accession. After 30 days, stem lesions were quantified.

Resistance to Phomopsis dieback and Phomopsis cane & leaf spot

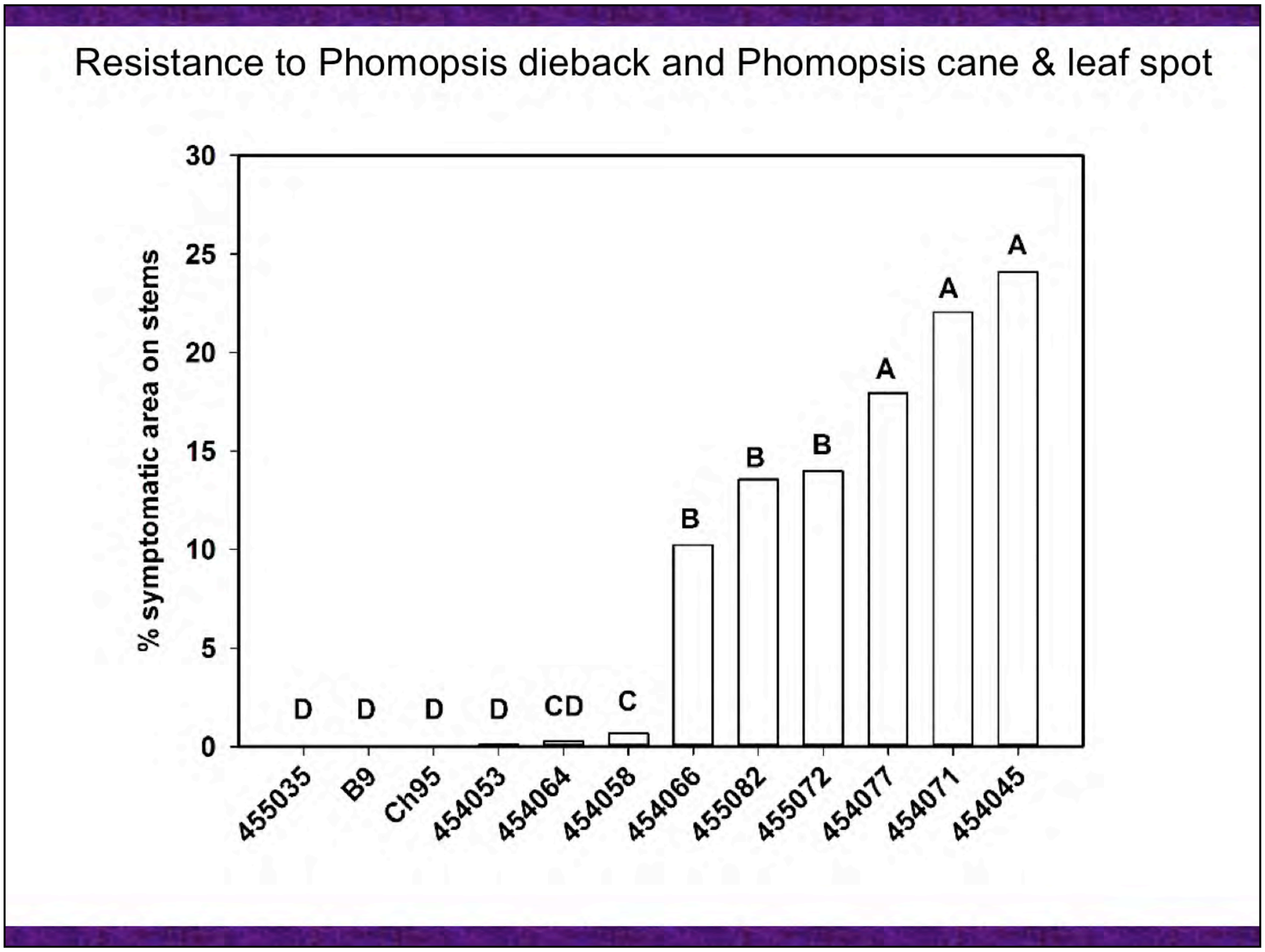
Low levels of leaf spot infections, consistent with field observations in Geneva.

We observed leaf spots on susceptible accessions, but overall levels of leaf spotting were low under our experimental conditions. Observations of low levels of leaf spotting are consistent with field observations in Geneva, NY.

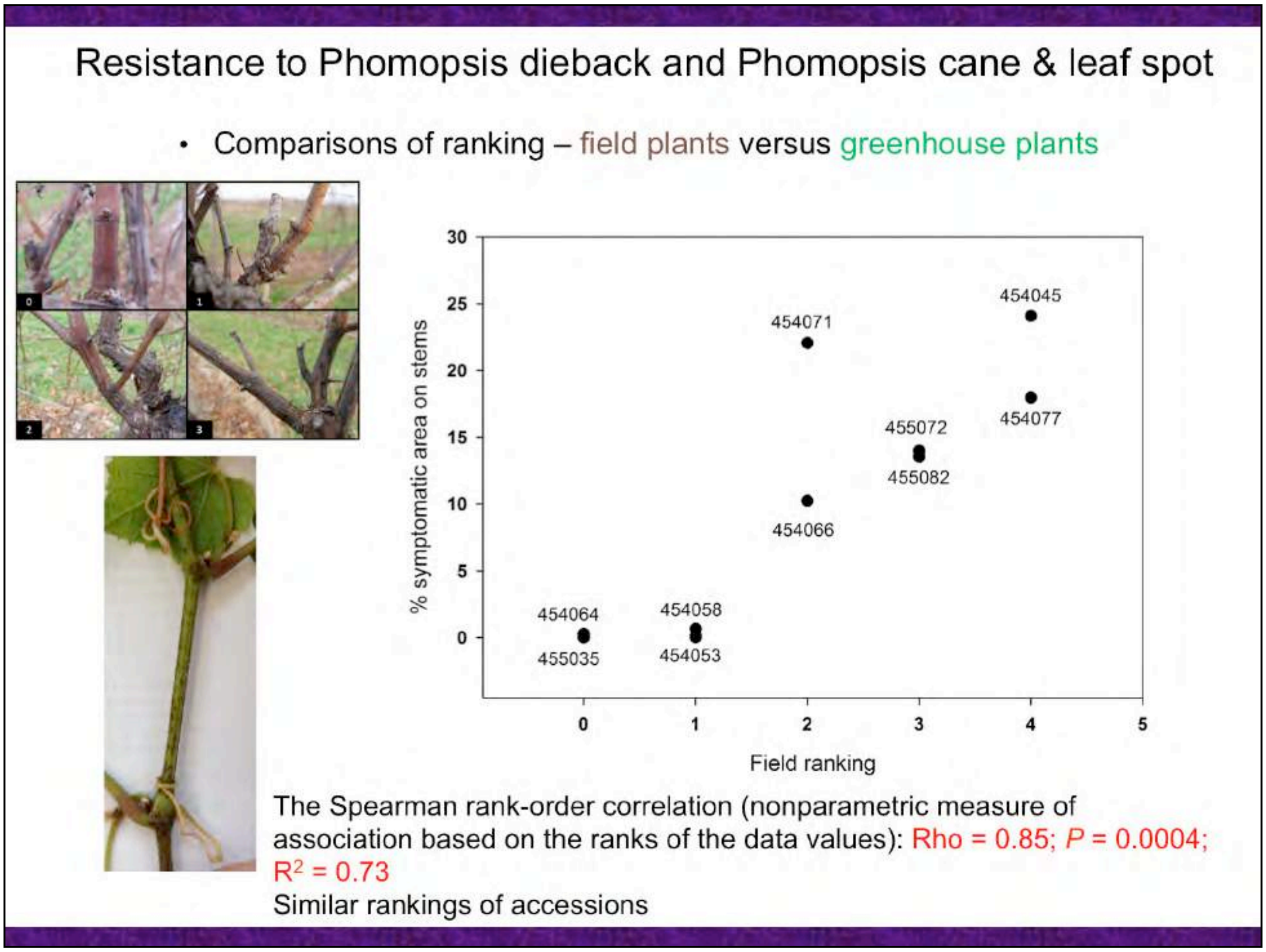
Resistance to Phomopsis dieback and Phomopsis cane & leaf spot

Higher levels of green stem infections, with apparent segregation of symptoms among accessions.

After 30 days incubation in the greenhouse, we observed high levels of green stem infections, with apparent segregation of symptoms among accessions.



When stem lesions were quantified as the proportion of symptomatic area on stems, we observed significant differences among accessions, as illustrated in the above graph, with four resistance classes (distinct letters above columns).



When comparing the resistance ranking observed in the field with the one observed under our greenhouse conditions, we observed a similar ranking of cultivars between the two sets of observations. We were thus able to reproduce the phenotypes observed in the field and confirmed results obtained in Geneva, NY.

Resistance to Phomopsis dieback and Phomopsis cane & leaf spot

- Next steps:
  - Estimate gene expression values of Rda1 across accessions to evaluate association between transcript levels of Rda1 with resistance phenotypes.
  - Inoculate woody stems of these accessions to evaluate their relative susceptibility to Phomopsis dieback (wood cankers).
  - Estimate gene expression values of Rda1 in wody tissues to evaluate if Rda1 provides dual resistance to Phomopsis dieback in woody tissues.

Following these experiments, we will estimate gene expression values of Rda1 across accessions to evaluate association between transcript levels of Rda1 with resistance phenotypes.

Inoculate woody stems of these accessions to evaluate their relative susceptibility to Phomopsis dieback (wood cankers).

Estimate gene expression values of Rda1 in woody tissues to evaluate if Rda1 provides dual resistance to Phomopsis dieback in woody tissues.

Our research is funded by grants from the Specialty Crop Research Initiative, AVF, and the CATGC. Find out more at our website, treeandvinetrunkdiseases.org

Collaborators

Eutypa resistance

- John Preece, USDA-ARS, Davis, CA
- Andrew Walker, UC Davis

Phomopsis resistance

- Bruce Reisch, Cornell University, NY
- Lance Cadle-Davidson, USDA-ARS, Geneva, NY
- Andrew Walker, UC Davis
- Daniel Lawrence, UC Davis

Botryosphaeria resistance

- Philippe Rolshausen, Dept. of Botany, UC Riverside
- Jerome Pouzoulet, Dept. of Botany, UC Riverside
- Daniel Lawrence, UC Davis

Our research team includes a range of plant scientists, as well as social scientists, and extension agents.