Fungal Diseases of Grapevine and Management practices

Akif Eskalen[1], Marcelo Bustamante[1], Karina Elfar[1], Robert Blundell[1], Molly Arreguin[1], Rhonda Smith[2], Christopher Chen[2], Lynn Wunderlich[3], Mark Battany[4], Carmen Gispert[5], Monica Cooper[6], George Zhuang[7], Larry Bettiga[8]

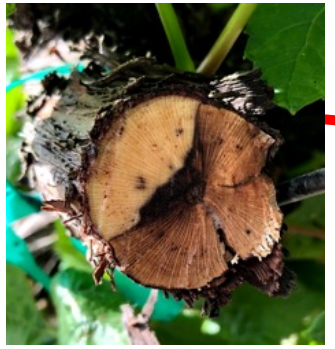
aeskalen@ucdavis.edu

[1]Department of Plant Pathology, [2]UCCE Sonoma, [3]El Dorado, [4]San Luis Obispo, [5]Riverside, [6]Napa, [7]Fresno, [8]Monterey

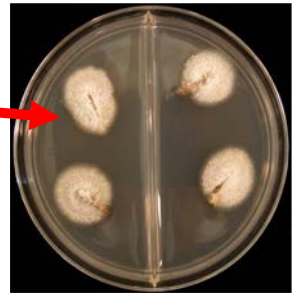
Outline

1. Introduction
2. Fungal diseases of grapevine
3. Grapevine trunk diseases
4. Pruning wound protection trials
5. Use of endophytic beneficials
6. Conclusion

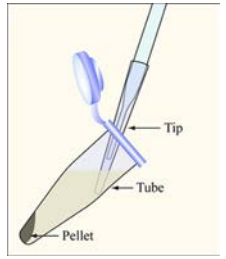
Identification of plant Pathogens

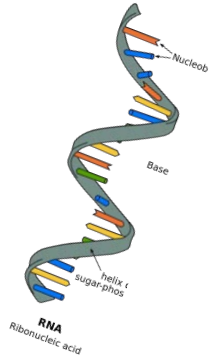


Symptomatic plant tissue

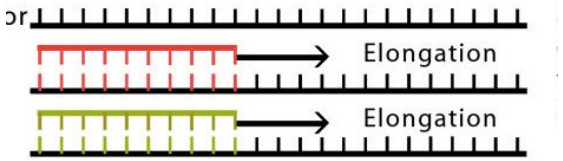


Culture Media



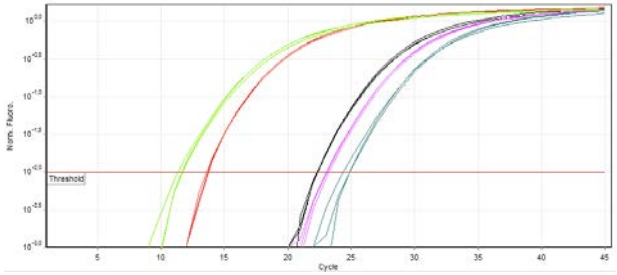


DNA Extraction

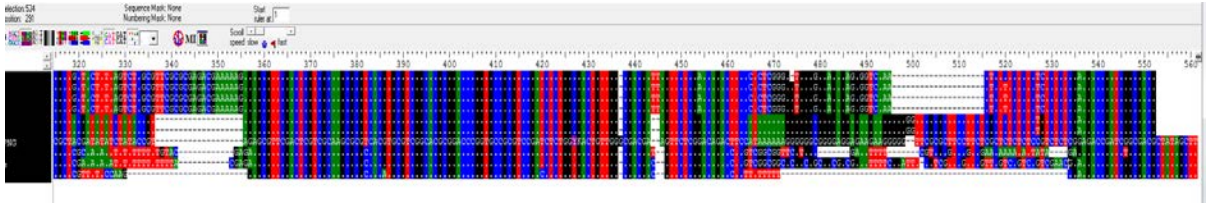


Species Specific Primers

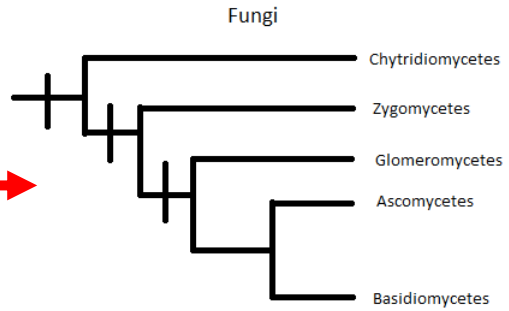
Quantitative Real Time PCR



Identification based on melting curve



Sequencing the DNA Region



Phylogenetic Tree

Field Trial to Evaluate Fungicides to control Powdery

Grape Bunch Rot- Sour Rot Fungicide Efficacy Field Trial

https://ucanr.edu/sites/eskalenlab/Fruit_Crop_Fungicide_Trials/

Eskalen lab website

Flag 18-YKC-2022- Powdery Mildew Trial

Jul 25 – 26, 2022 · Shared

G. Results

Trial I

Table 1. Disease incidence and severity of synthetic fungicides and combinations of soft chemistry and synthetic products. Product names are followed by rate (per acre). Treatment means followed by the same letter are not significantly different according to Fisher's LSD at α=0.05;

Treatment			Application date	Powdery mildew on the cluster [y]	
Pictures	**- Flag**	**Rate/A [z]**	**(Julian day)**	**Incidence, %**	**Severity, %**
18	YKC	Abound 15.5 fl oz + Syl-Coat 4 fl oz	105	0.0 a	0.00 a
		Prolivo 5 fl oz + Syl-Coat 4 fl oz	119		
		Kenja 22 fl oz + Rally 4 oz + Syl-Coat 4 fl oz	132		
		Quintec 4oz + Syl-Coat 4 fl oz	147		
		Torino 3.4 oz + Syl-Coat 4 fl oz	161		
		Merivon 4oz + Syl-Coat 4 fl oz	178		
		Vivando 15.4 oz + Syl-Coat 4 fl oz	193		
37	BC	PureSpray Green 1 gal	103, 110, 117	0.0 a	0.00 a
		Luna Experience 8.6 fl oz	124, 182		
		Pristine 23 oz	138		
		Elevate 16oz	152		
		Parade 3.1 fl oz	166		
41	Pu	Parade 3.1 fl oz + Dyne-Amic 0.25% v/v	108, 122, 136, 150, 165, 179, 194	0.0 a	0.00 a
62	Y+O	Aprovia Top 13.3 fl oz +Syl-Coat 0.125% v/v	122, 179	0.0 a	0.00 a
		Quintec 6.6 fl oz + Syl-Coat 0.125% v/v	136, 194		
		Miravis Prime 13.4 fl oz +Syl-Coat 0.125% v/v	165		
		Inspire Super 20.0 fl oz +Syl-Coat 0.125% v/v	150		
63	Y+W	Aprovia Top 13.3 fl oz + A9180B 0.5 oz +Syl-Coat 0.125% v/v	122, 179	0.0 a	0.00 a
		Quintec 6.6 fl oz + A9180B 0.5 oz + Syl-Coat 0.125% v/v	136, 194		

Grapevine Trunk Diseases

- Young Vine Decline
- Esca

Vascular diseases

- Eutypa Dieback
- Bot Canker
- Phomopsis Dieback

Canker diseases



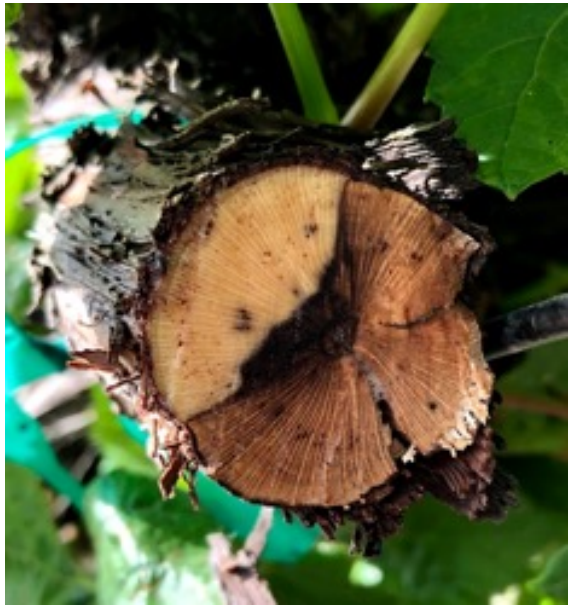

Grapevine Trunk Diseases

- Young Vine Decline
- Esca

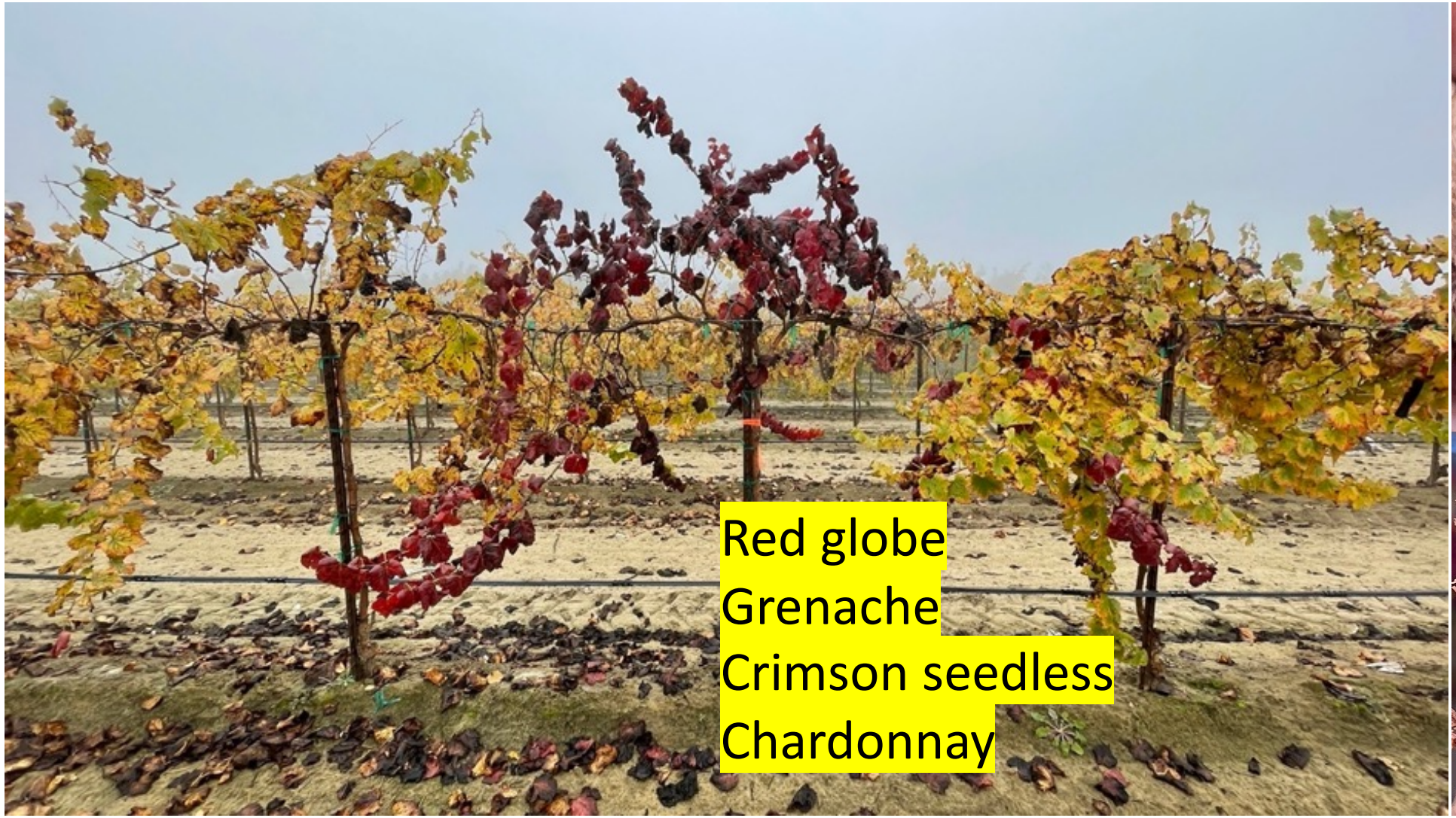
Vascular diseases

- Eutypa Dieback
- Bot Canker
- Phomopsis Dieback

Canker diseases



Unusual fall symptoms on virus free grapevines



Grenache cv./ Freedom

Aspergillus tubingensis

External Symptoms

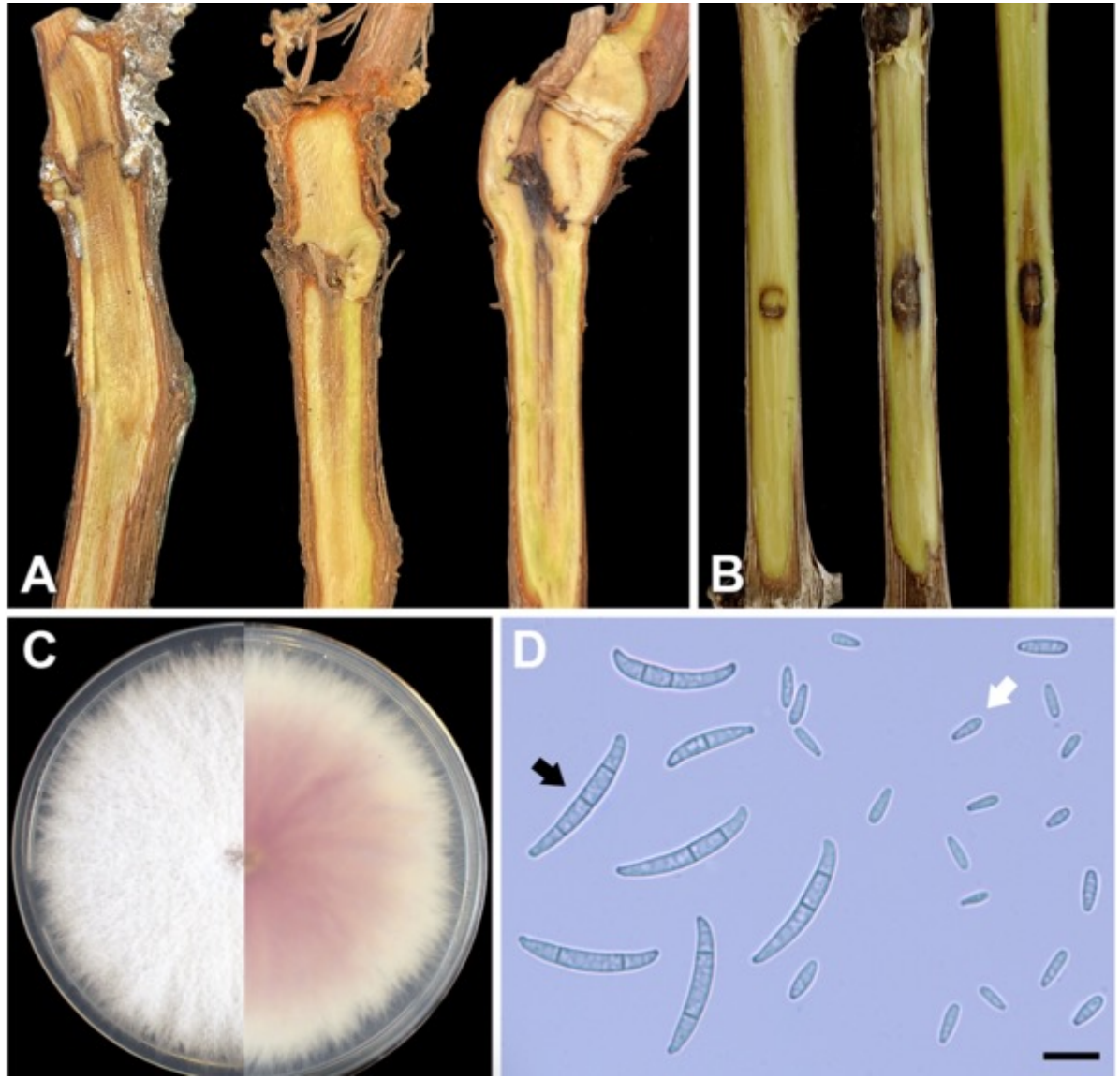
Sour rot complex



Aspergillus tubingensis
Aspegillus niger
Aspegillus carbonarius

Macrophomina Charcoal Rot *(Macrophomina phaseolina)*

Fusarium annulatum

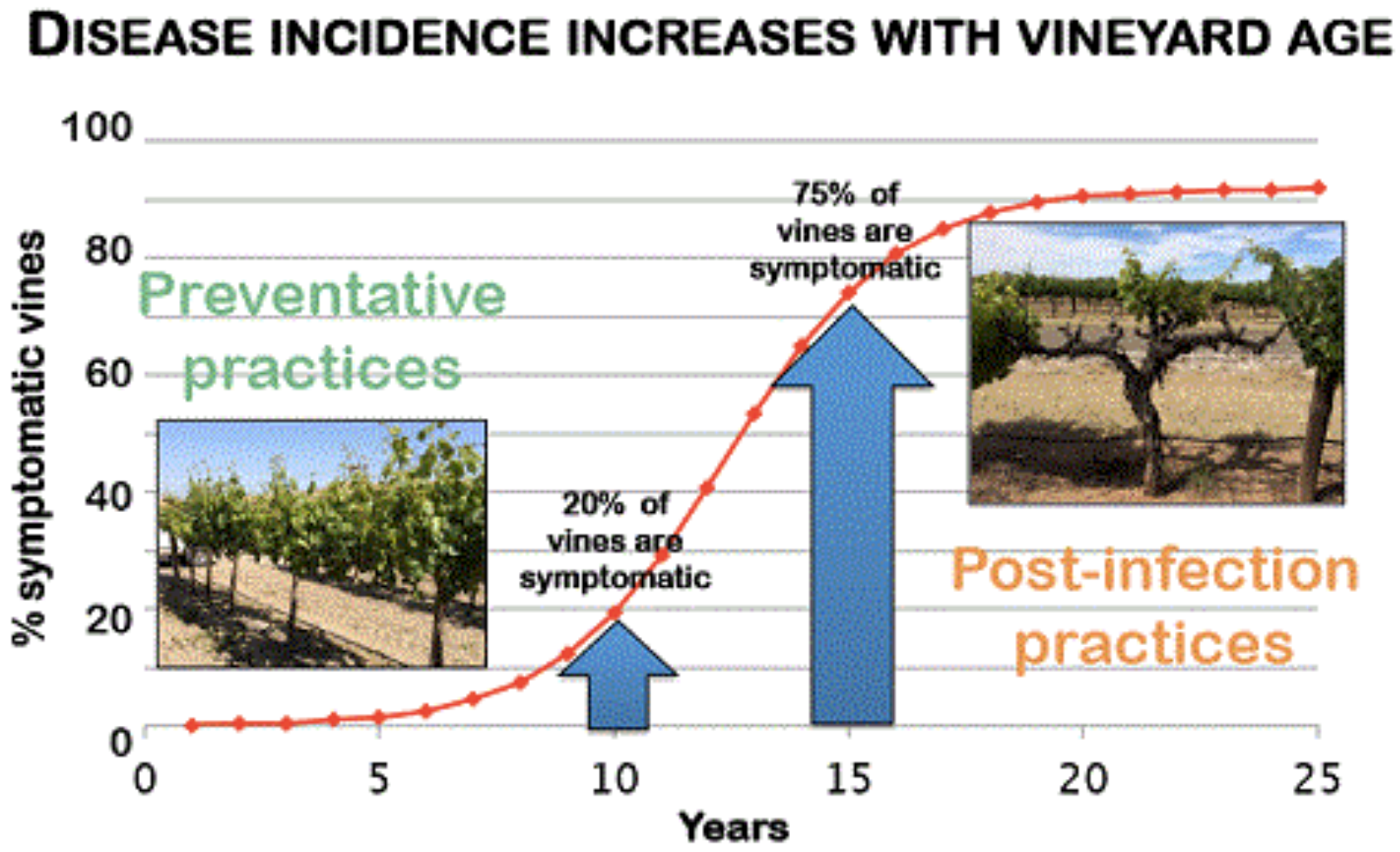


Graft Union

Bustamante et al. 2022 First Report of *Fusarium annulatum* Associated with Young Vine Decline in California. Plant Disease.

Sudden vine collapse: GLRaV-3 + GVA+ Mealybug + Freedom + GTD

Economical Impact



From Duthie et al. 1991 (Colombard vineyards ranging from 5 to 34 years)

Asexual Fruiting body-Pycnidia

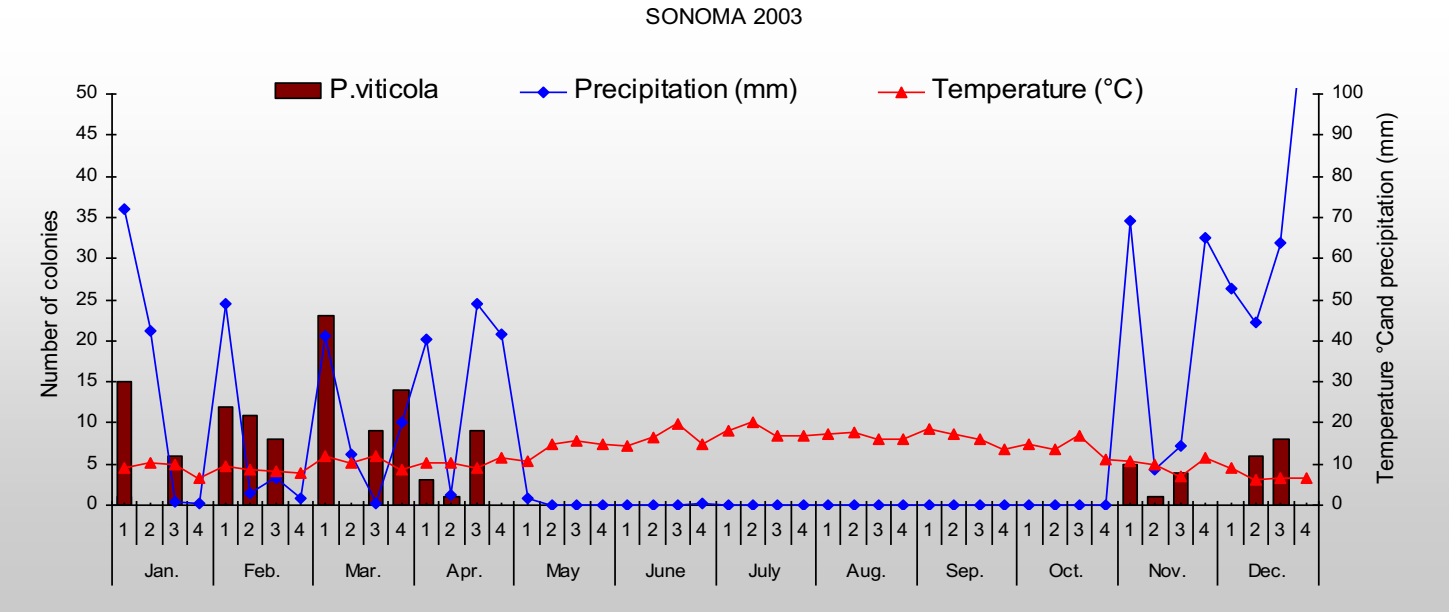
Source of inoculum

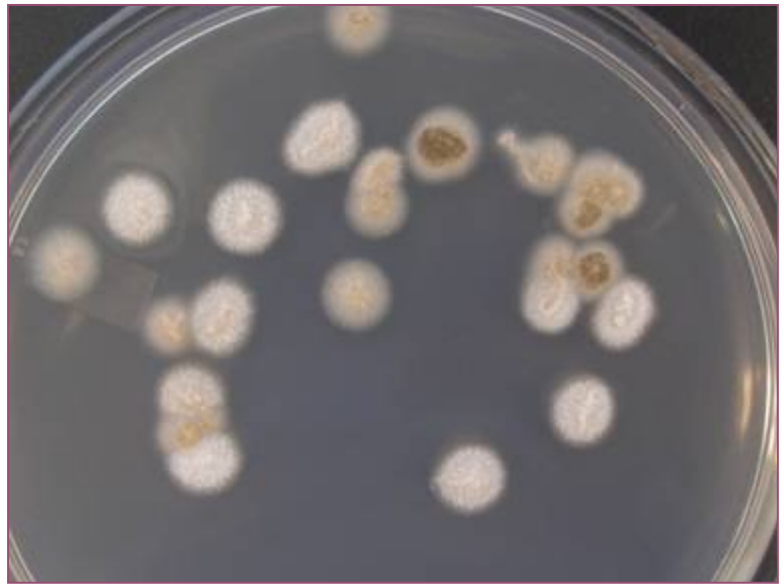
Sexual Fruiting body-Perithecia

Spore Release from Perithecia of Botryosphaeria

APS

Spore dispersal pattern of GTD pathogens

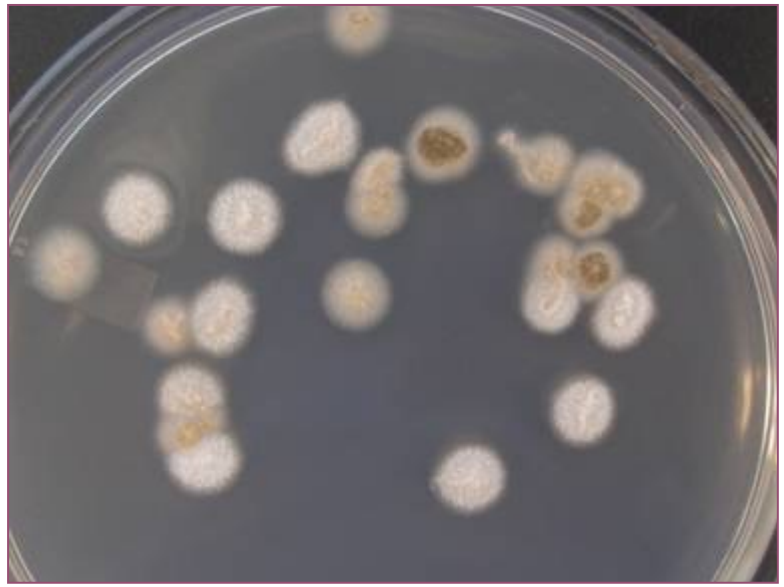




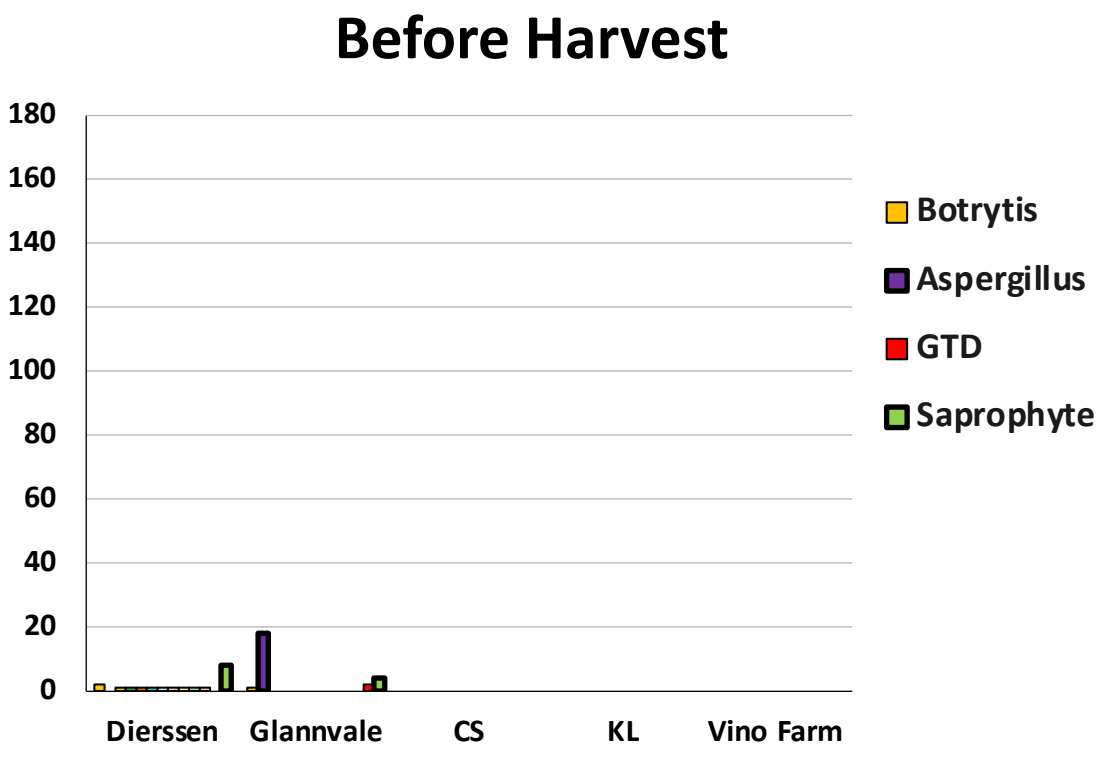
Would machine harvest contribute to the spore dissemination of GRD pathogens?

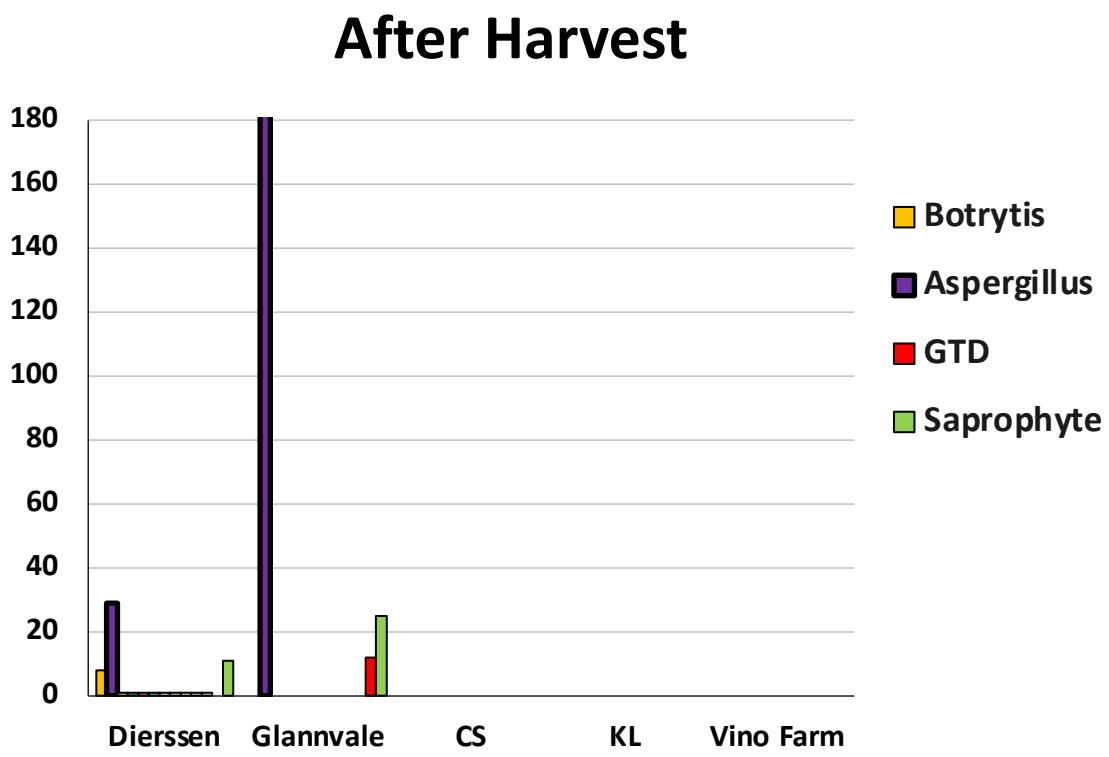
Would machine harvest contribute to the spore dissemination of GRD pathogens?

Spore dispersal of GTD during harvest

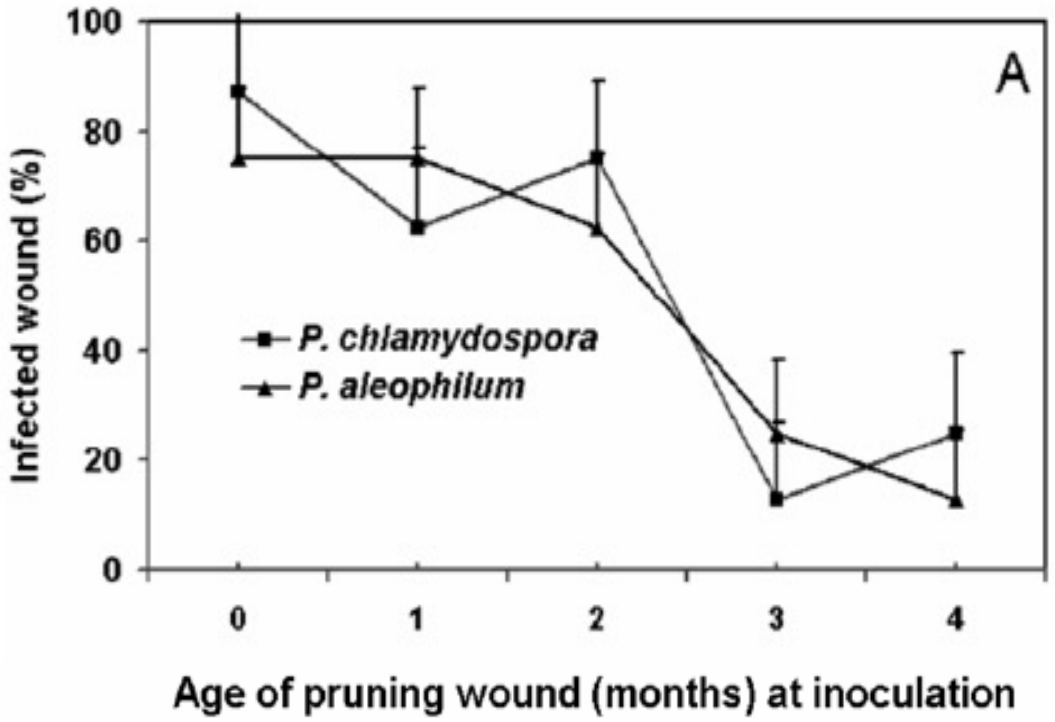


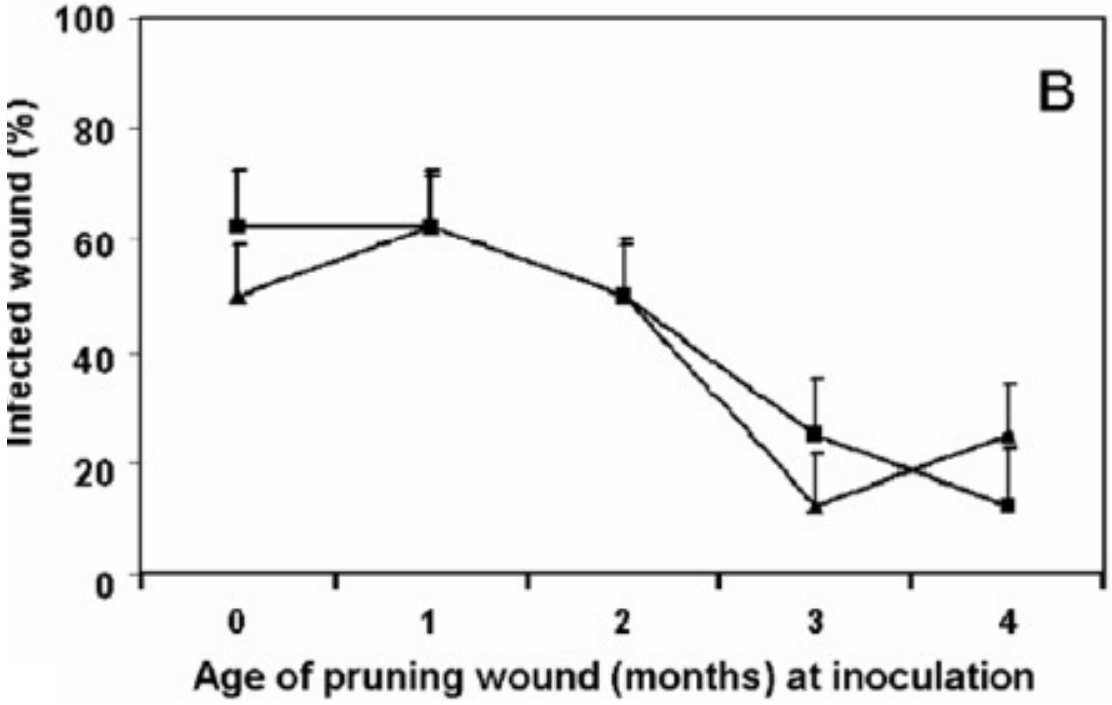
Would machine harvest contribute to the spore dissemination of GRD pathogens?





Pruning wound susceptibility for Esca Pathogens





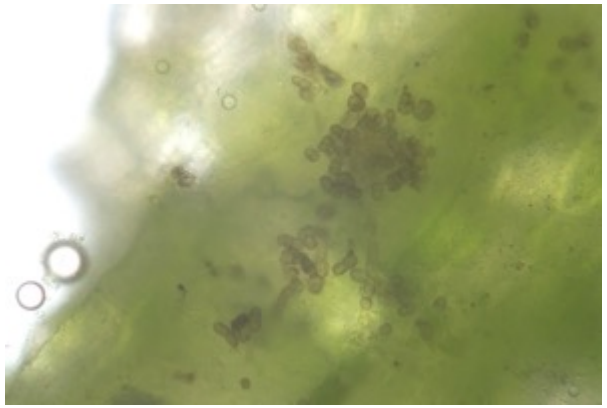
Eskalen et al. Plant Disease, 2007, 91:9, 1100-1104.

How do they infect grapevine?

- Pruning wounds

- Latent Infection

- Endophyte



Infection of GTD on different part of the vine

Spurs

Cordon

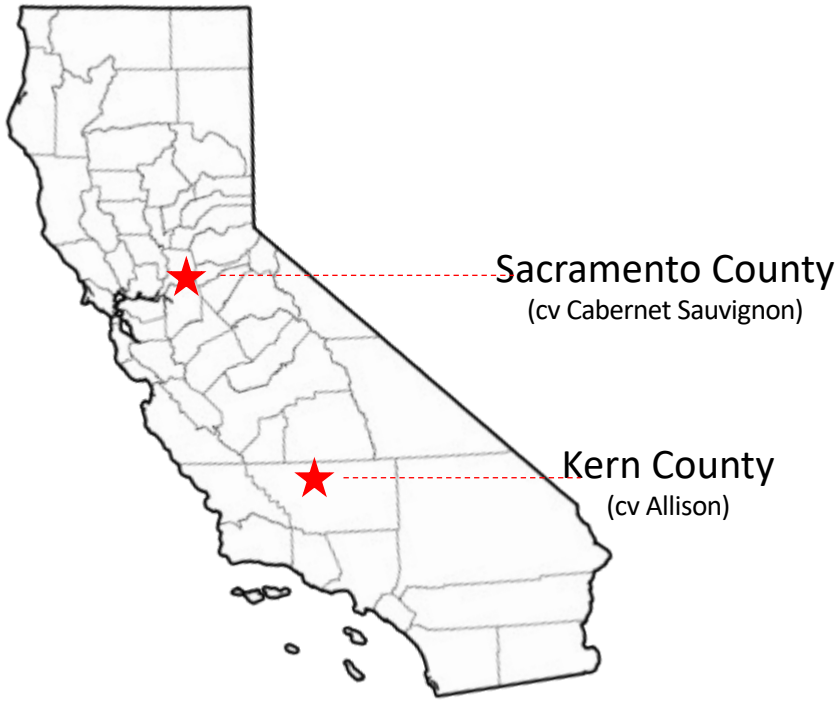
Trunk

Rootstock

Pruning wound protection trials

Product name	Active Ingredient	Manufacturer
Untreated (non-inoculated)	-	-
Esendo, 2.8 lbs	pre-mix of Howler and azoxystrobin	AgBiome Innovations
Parade, 4.7 fl oz	pyraziflumid	Nichino America
Luna Sensation, 7.6 oz	fluopyram (17.54%), tebuconazole (17.54%)	Bayer CropScience
1 L Vitiseal ready-to-use (V-RTU). This is NOT to be diluted.	Acrylic Co-Polymer	VitiSeal International LLC
UCD 8189 + 8344, $1x10^5$ cfu/ml	Aureobasidium pullulans-8189+8344	N/A
Topsin M 1.25 lbs	Triophanate-methyl	United Phosphorus Inc.
Guarda, 2.56 fl oz/ga	thyme oil	BioSafe Systems, LLC
Biotam, 2 lbs	Trichoderma asperellum (ICC 012) + Trichoderma gamsii (ICC 080)	Isagro USA
Vintec, 2.8 oz	Trichoderma atroviride strain SC1	Bi-PA
Botector, 8 oz	Aureobasidium pullulans strain DSM14940/14941 1	Westbridge Agricultural Products
Crab Life Powder, 0.5 lbs	Chitin	Conchazul de Mexico
PerCarb, 4 lbs	sodium carbonate peroxyhydrate (85%)	BioSafe Systems, LLC
2 X 0.5 L experimental new Vitiseal formulation, ready-to-use (X-RTU). This is NOT to be diluted.	Acrylic Co-Polymer	VitiSeal International LLC
Rhyme, 5 fl oz (applied as pruning wound spray)	Flutriafol (22.7 %)	FMC
TrichosSymBio, 25.6 fl oz	Trichoderma harzianum T78 (of 5 x 10^{11} cfu)	Symborg
UCD-10631, 10% fermented product	Bacillus velezensis UCD-10631	N/A
Parade, 3.1 fl oz	Pyraziflumid	Nichino America
UCD 8717, ($1x10^5$ cfu/ml)	Trichoderma hamatum -8717	N/A
Positive Control (Inoculated with N. parvum)	-	-
Baby detergent 2%	Dreft Stage 1 Liquid Detergent	Dreft
microSURE (Agriwash), 4.36 gal	Proprietary	Strategia Project Management Inc
CS2005, 32 fl oz	Copper Sulfate Pentahydrate	Magna-Bon
GCM (Gelatinise and Chitinase Microorganism)	Bacillus velezensis CE100	N/A
Theia, 3 lbs	Bacillus subtilis strain AFS032321	AgBiome Innovations
UCD-10719, 10% fermented product	Serratia plymuthica UCD-10719	N/A
Vitiseal ready-to-use (V-RTU) applied using FELCO 19 - Special application - FELCO 8 with spraying device	Acrylic Co-Polymer	VitiSeal International LLC

Field trial in 2019-2022 to prevent and control GTD pathogens with synthetic, organic and biological fungicides



Field trial in 2019-2022 to prevent and control GTD pathogens with synthetic, organic and biological fungicides

Pruning
(February)

Application of protectant

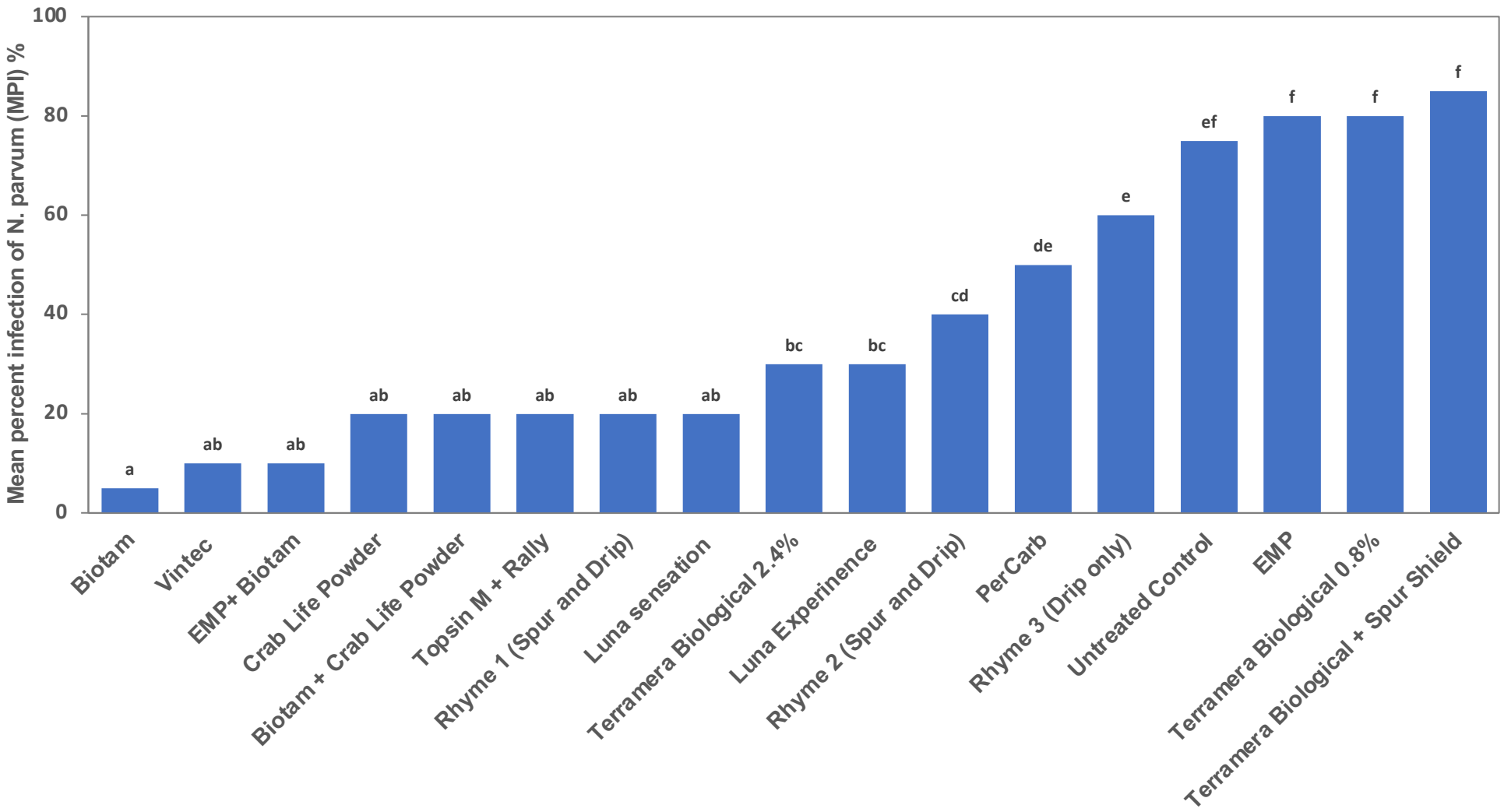
Inoculation of GTDs ($5x10^5$) spores

Evaluation of field trial for pruning wound protection

3 isolations made from pith
+
3 isolations made from areas exhibiting discoloration

PDA-t

Results of pruning wound protection trial for *Neofusicoccum parvum* in 2020



Evaluation of pruning wound treatments mean percent infection (MPI) rates with *N. parvum* located at UC Davis Plant Pathology Field Station, 2020. Bars = standard errors.

Treatments of pruning wound protection trial in 2022

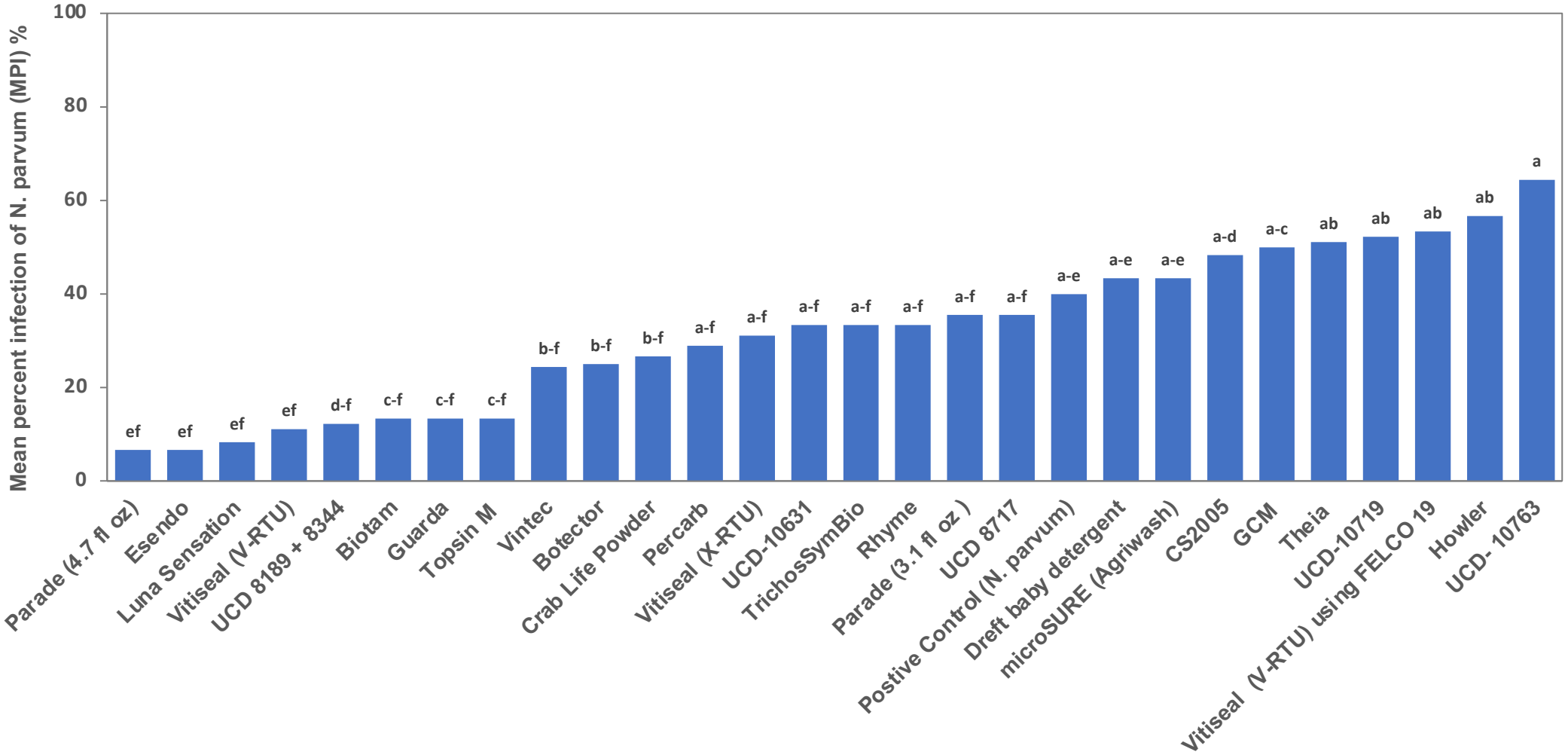
Product name	Active Ingredient	Manufacturer
Untreated (non-inoculated)	-	-
Esendo, 2.8 lbs	pre-mix of Howler and azoxystrobin	AgBiome Innovations
Parade, 4.7 fl oz	pyraziflumid	Nichino America
Luna Sensation, 7.6 oz	fluopyram (17.54%), tebuconazole (17.54%)	Bayer CropScience
1 L Vitiseal ready-to-use (V-RTU). This is NOT to be diluted.	Acrylic Co-Polymer	VitiSeal International LLC
UCD 8189 + 8344, $1x10^5$ cfu/ml	*Aureobasidium pullulans-8189+8344*	N/A
Topsin M 1.25 lbs	Triophanate-methyl	United Phosphorus Inc.
Guarda, 2.56 fl oz/ga	thyme oil	BioSafe Systems, LLC
Biotam, 2 lbs	*Trichoderma asperellum* (ICC 012) + *Trichoderma gamsii* (ICC 080)	Isagro USA
Vintec, 2.8 oz	*Trichoderma atroviride* strain SC1	Bi-PA
Botector, 8 oz	*Aureobasidium pullulans* strain DSM14940/14941 1	Westbridge Agricultural Products
Crab Life Powder, 0.5 lbs	Chitin	Conchazul de Mexico
PerCarb, 4 lbs	sodium carbonate peroxyhydrate (85%)	BioSafe Systems, LLC
2 X 0.5 L experimental new Vitiseal formulation, ready-to-use (X-RTU). This is NOT to be diluted.	Acrylic Co-Polymer	VitiSeal International LLC
Rhyme, 5 fl oz (applied as pruning wound spray)	Flutriafol (22.7 %)	FMC
TrichosSymBio, 25.6 fl oz	*Trichoderma harzianum* T78 (of 5 x 10^{11} cfu)	Symborg

Product name	Active Ingredient	Manufacturer
UCD-10631, 10% fermented product	*Bacillus velezensis* UCD-10631	N/A
Parade, 3.1 fl oz	Pyraziflumid	Nichino America
UCD 8717, ($1x10^5$ cfu/ml)	*Trichoderma hamatum* - 8717	N/A
Positive Control (Inoculated with N. parvum)	-	-
Baby detergent 2%	Dreft Stage 1 Liquid Detergent	Dreft
microSURE (Agriwash), 4.36 gal	Proprietary	Strategia Project Management Inc
CS2005, 32 fl oz	Copper Sulfate Pentahydrate	Magna-Bon
GCM (Gelatinise and Chitinase Microorganism)	*Bacillus velezensis* CE100	N/A
Theia, 3 lbs	*Bacillus subtilis* strain AFS032321	AgBiome Innovations
UCD-10719, 10% fermented product	*Serratia plymuthica* UCD-10719	N/A
Vitiseal ready-to-use (V-RTU) applied using FELCO 19 - Special application - FELCO 8 with spraying device	Acrylic Co-Polymer	VitiSeal International LLC
Howler, 5 lbs	*Pseudomonas chlororaphis* strain AFS009	AgBiome Innovations
UCD-10763, 10% fermented product	*Pseudomonas chlororaphis* UCD-10763	N/A

owed by the same letter within a column are not significantly different according to Fis
=0.05).

https://ucanr.edu/sites/eskalenlab/files/378928.pdf

Results of pruning wound protection trial for *Neofusicoccum parvum* in 2022

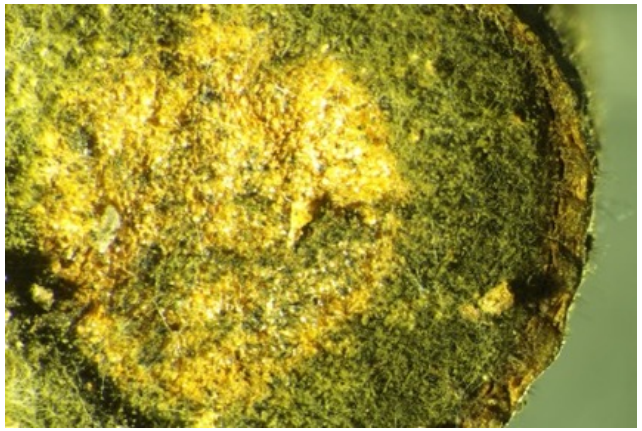


Evaluation of pruning wound treatments mean percent infection (MPI) rates with *N. parvum* located at UC Davis Plant Pathology Field Station, 2022. Bars = standard errors.

https://ucanr.edu/sites/eskalenlab/files/378928.pdf

Recovery of biological treatments from inoculated canes in October 2020

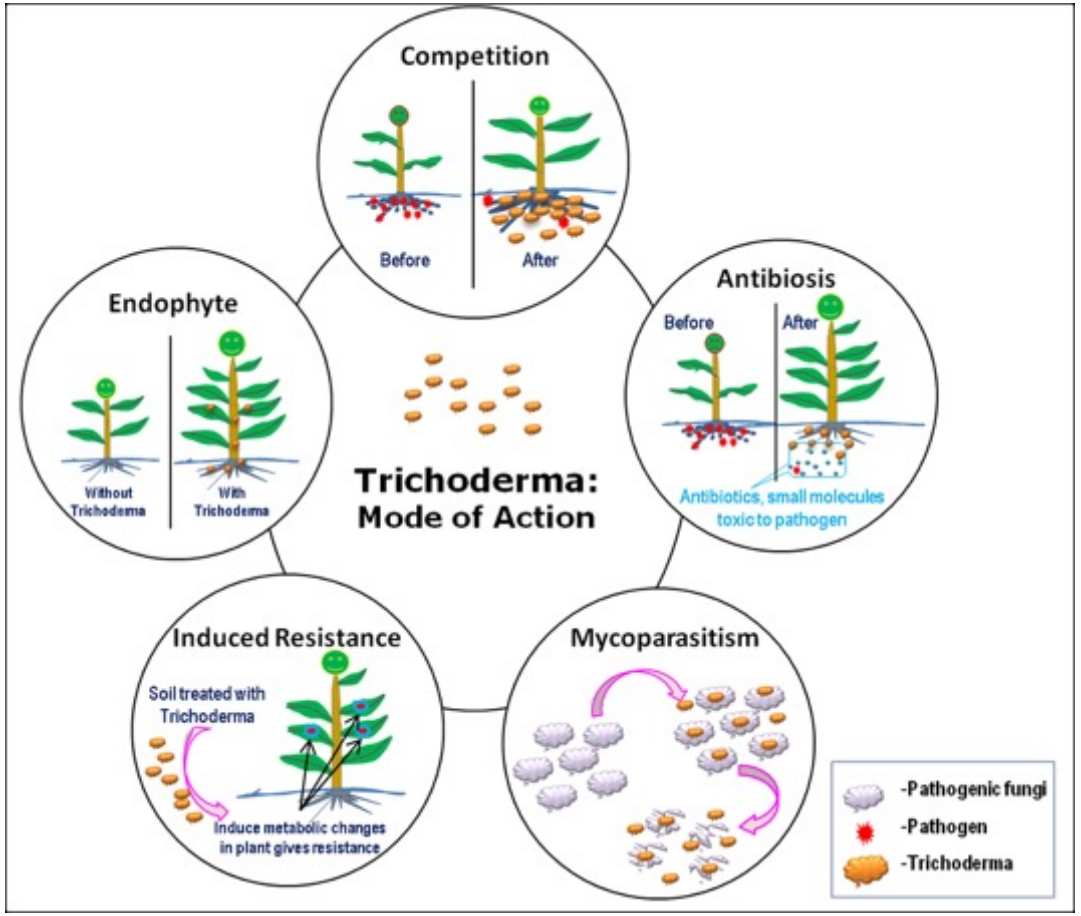
	Recovery %			
	Sacramento County		Kern County	
Treatment	***E. lata***	***N. parvum***	***E. lata***	***N. parvum***
Bacillus velezensis	0	25	25	5
Bacillus subtilis strain QST 713	0	5	0	0
Bacillus sp.	0	5	10	0
Trichoderma hamatum	0	20	20	15
Trichoderma asperellum and *Trichoderma gamsii* + a blend of crab and lonbster shell powder	35	10	30	30
Trichoderma asperellum and *Trichoderma gamsii*	60	45	20	30
Aureobasidum pullulans strain DSM14940/14941	65	100	25	30
Trichoderma atroviride	70	100	45	80
Aureobasidum pullulans	100	100	25	60



https://ucanr.edu/sites/eskalenlab/

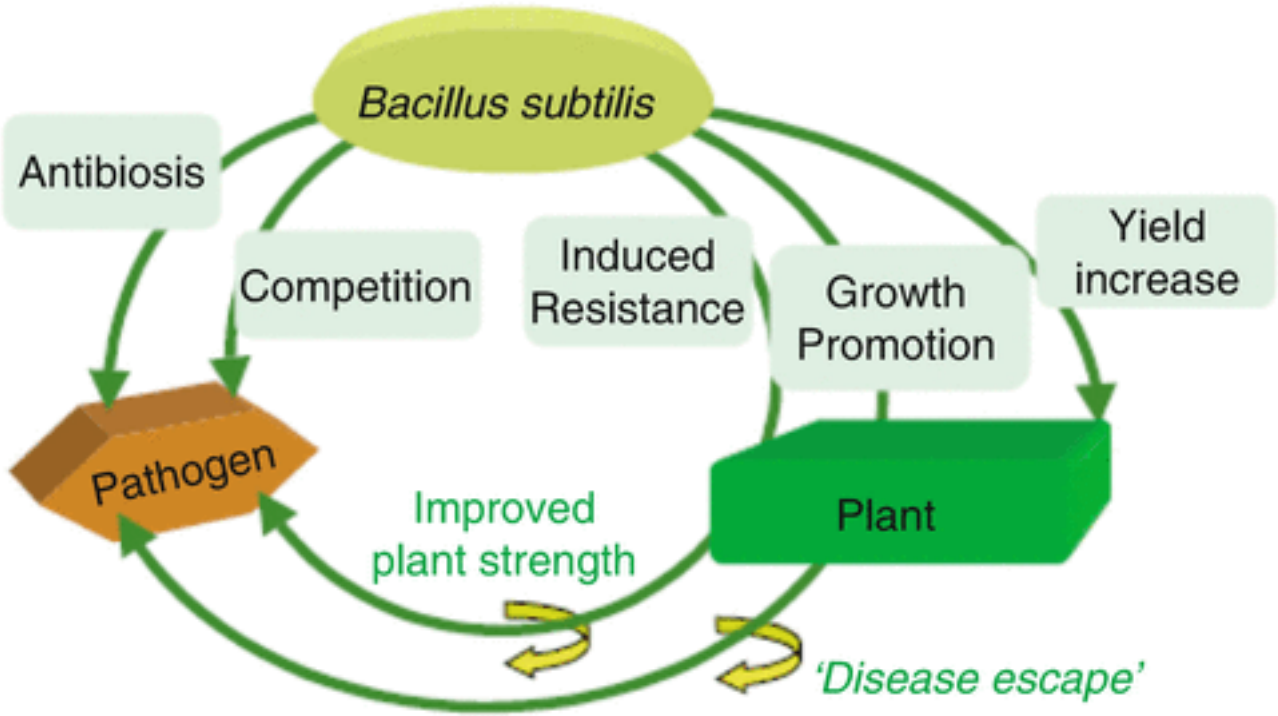
Mode of Action of Biologicals

Trichoderma spp.



Rahul Mahadev Shelake, 2016

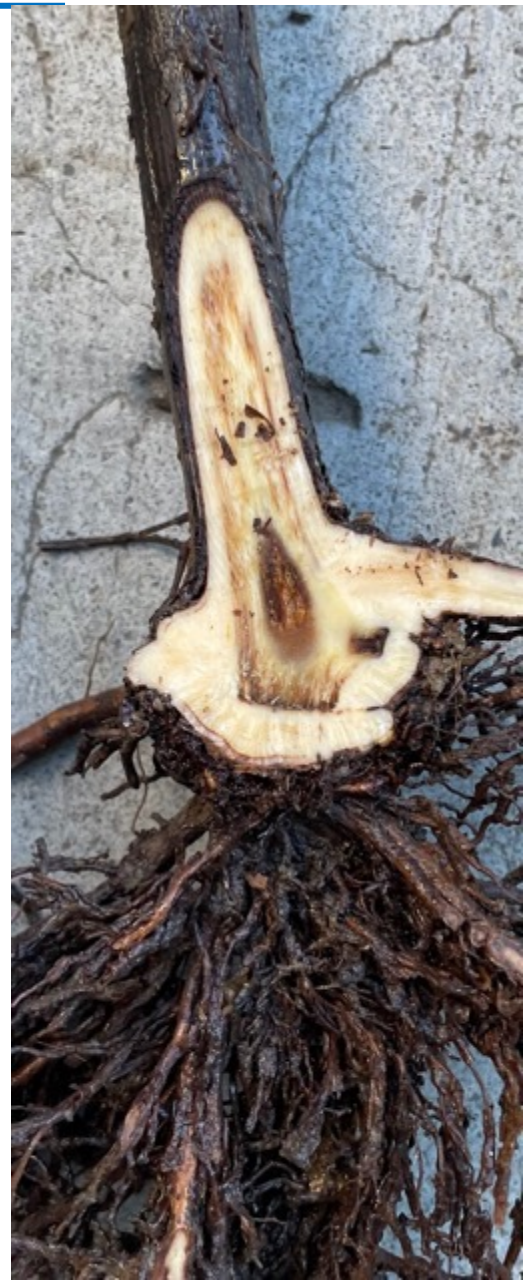
Bacillus subtilis



Bohne and Helmut Junge, 2017

Preventative Management in Vineyards

- Protect pruning wounds
- Use disease free, clean plant materials when establish new vineyards
- Apply good cultural practices to minimize stress on young and mature vines
- Delay dormant pruning to avoid potential pathogen dissemination during winter precipitation and to reduce the susceptibility
- If applicable, consider doing double pruning to reduce fungal spore infection during winter moths



Double Pruning

- Pre pruning about 1-foot-long dormant season (December-February)
- Second prune is late pruning before budbreak

Protecting pruning wound is essential

	Commercial name	Active ingredient	Manufacturer
Biocontrol	Biotam	(*Trichoderma asperellum* + *T. gamsii*	SepRo
	Vintec	*Trichoderma atroviride* SC1	BI-PA
	Botector	*Aerobasidium pullulans*	Westbridge
	GCM	*Bacillus velezensis CE100*	BSR
Plant extract	Guarda	Thyme oil	Biosafe System
Synthetic fungicides	Topsin-M	Triophanate-methyl	United Phosphorous
	Luna sensation	Fluopyram/Trifloxystrobin	Bayer CropScience
	Esendo	Azoxystrobin + Pseudomonas chlororapsis	Agbiome
	Rhyme	Flutriafol	FMC
	Parade	Pyraziflumid	Nichino America
Sealant	Vitiseal	Acrylic C0-Polymer	Vitiseal International
Disinfectant	PerCarb	Sodium carbonate peroxyhydrate (85%)	Biosafe Systems

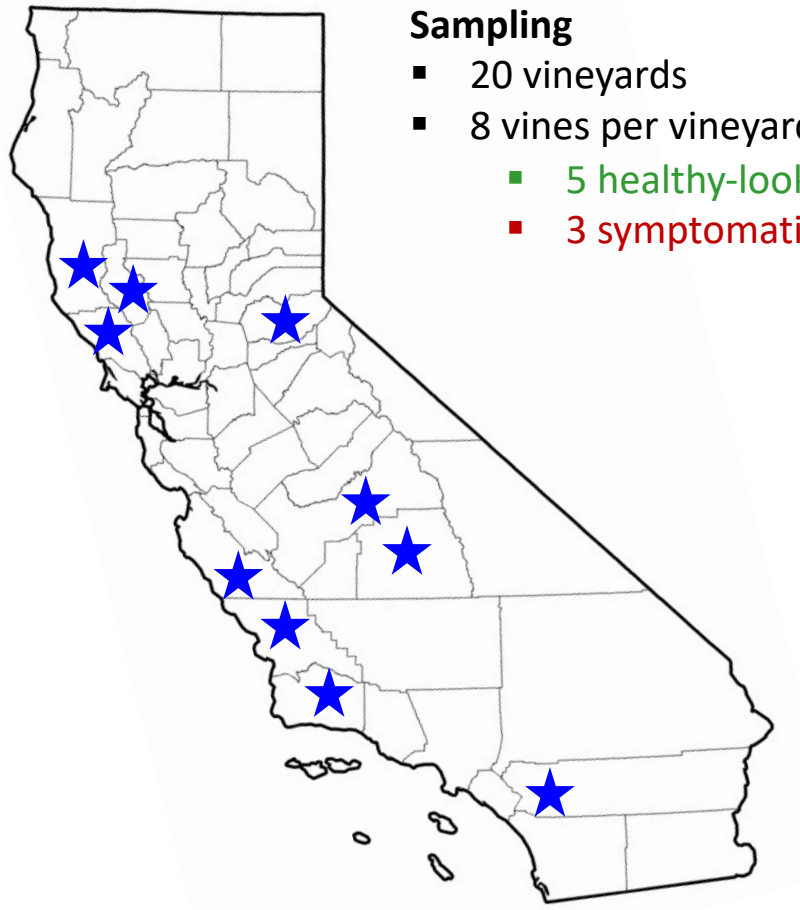
https://ucanr.edu/sites/eskalenlab/

Using Beneficial Endophytes for Controlling Grapevine Trunk Diseases

Objective:

- Screening for grapevine endophytic bacteria as potential biocontrol agents of fungal pathogens of grapevine trunk diseases

Screening for potential BCAs against GTDs



Sampling

- 20 vineyards
- 8 vines per vineyard
  - 5 healthy-looking
  - 3 symptomatic

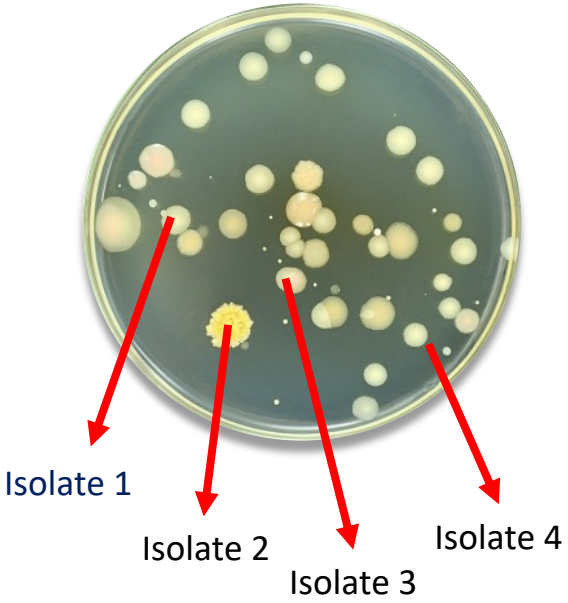
	County	City	Vineyard name	Cultivar
1	Fresno	Fresno	Michael Mehling	Thompson Seedless
2	Fresno	Fresno	J&L	Pinot Gris
3	Mendocino	Ukiah	Nelson Family	Cabernet Sauvignon
4	Mendocino	Ukiah	Nelson Family	Merlot
5	El Dorado	Placerville	Sumu Kaw	Zinfandel
6	El Dorado	Fair Play	Cedarville	Syrah
7	Santa Barbara	Santa Maria	Bien Nacido	Pinot Noir
8	Santa Barbara	Los Alamos	Cat Canyon	Chardonnay
9	San Luis Obispo	Paso Robles	Four Sisters Ranch	Cabernet Sauvignon
10	San Luis Obispo	Paso Robles	Sunnybrook Ranch	Cabernet Sauvignon
11	Tulare	Dinuba	WMJ Farms	Thompson Seedless
12	Tulare	Hanford	TBD	Pinot Gris
13	Monterey	Soledad	Lone Oak vineyard	Chardonnay
14	Monterey	Santa Lucia Highlands	Kimberly vineyard	Chardonnay
15	Napa	Napa	Big Ranch	Chardonnay
16	Napa	Napa	Stags Leap	Cabernet Sauvignon
17	Sonoma	Geysersville	River Oaks (ROV)	Tempranillo
18	Sonoma	Geysersville	Del Rio, Simi	Sauvignon Blanc
19	Riverside	Mecca	Avenue 66	Scarlet Royal
20	Riverside	Mecca	92250 Avenue 66	Scarlet Royal

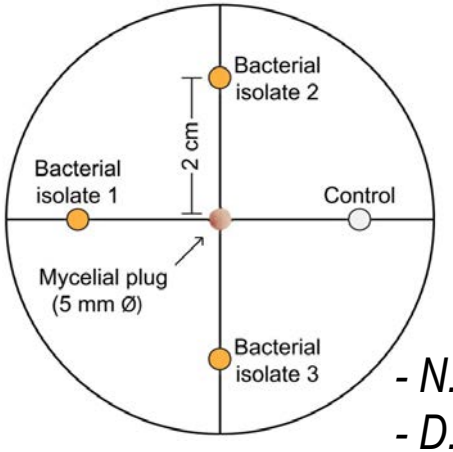
In vitro screening pipeline

1. Sampling of wood and root material → 2. Isolation of bacteria → 3. Antagonism assays

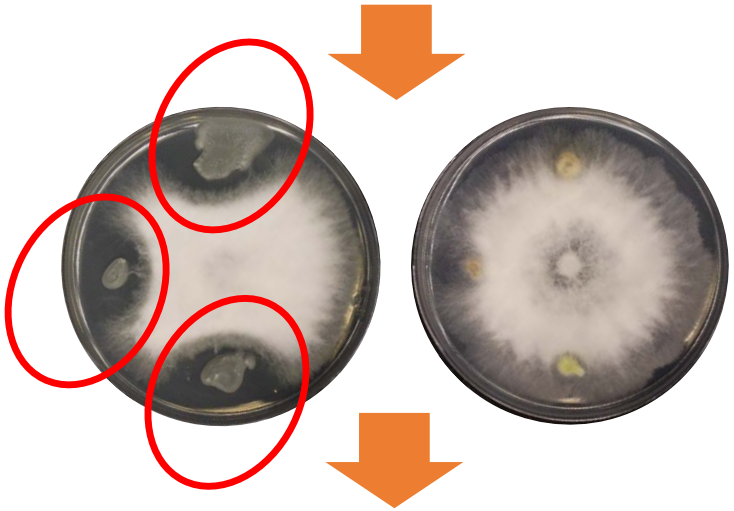
Trunk + cordon

Feeder roots





- *N. parvum*
- *D. seriata*



First selection

Dual antagonism assays (1)

Bacillus *Pseudomonas* *Serratia*

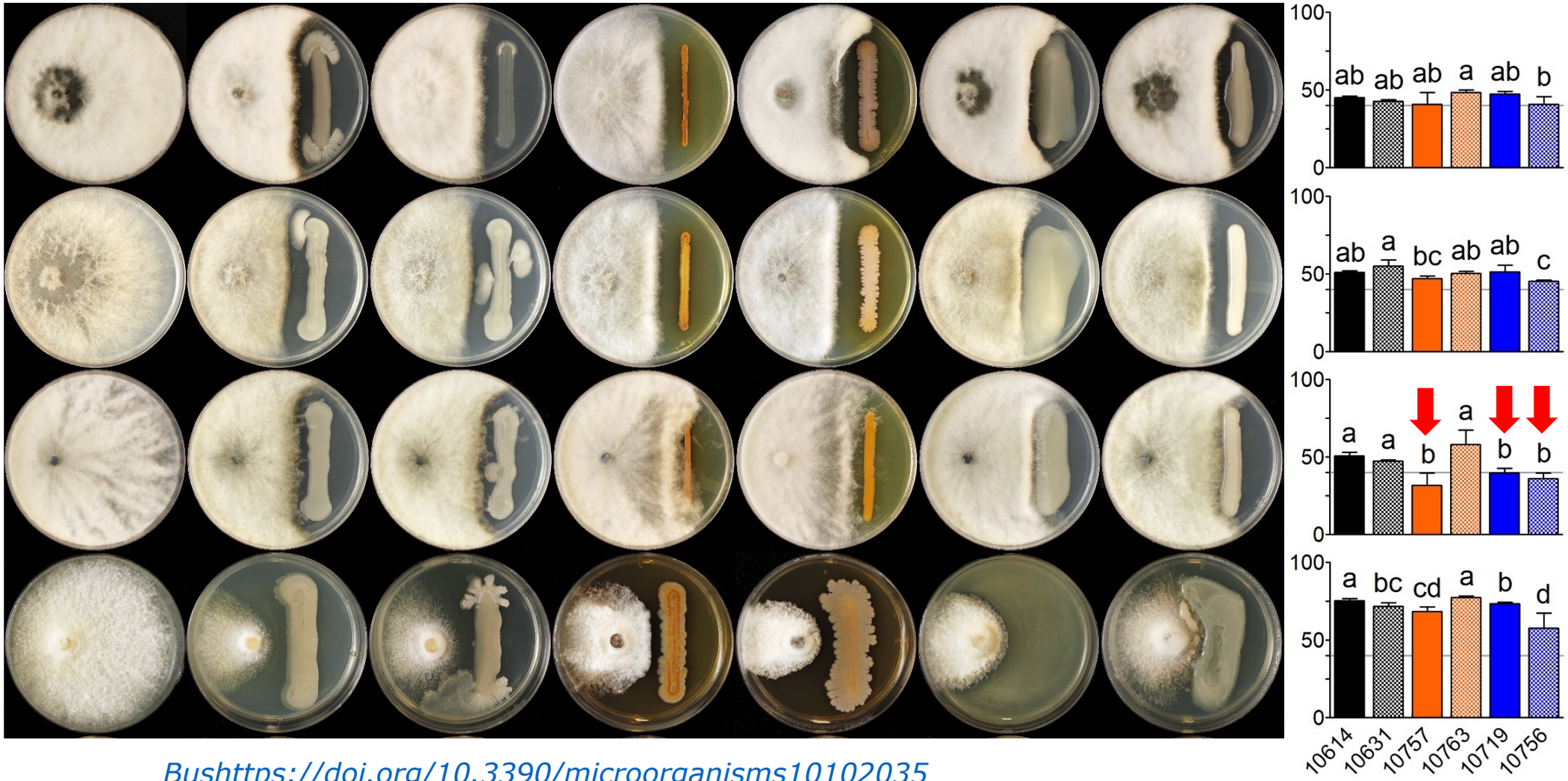
Control | UCD10614 | UCD10631 | UCD10757 | UCD10763 | UCD10719 | UCD10756

Neofusicoccum parvum

Diplodia seriata

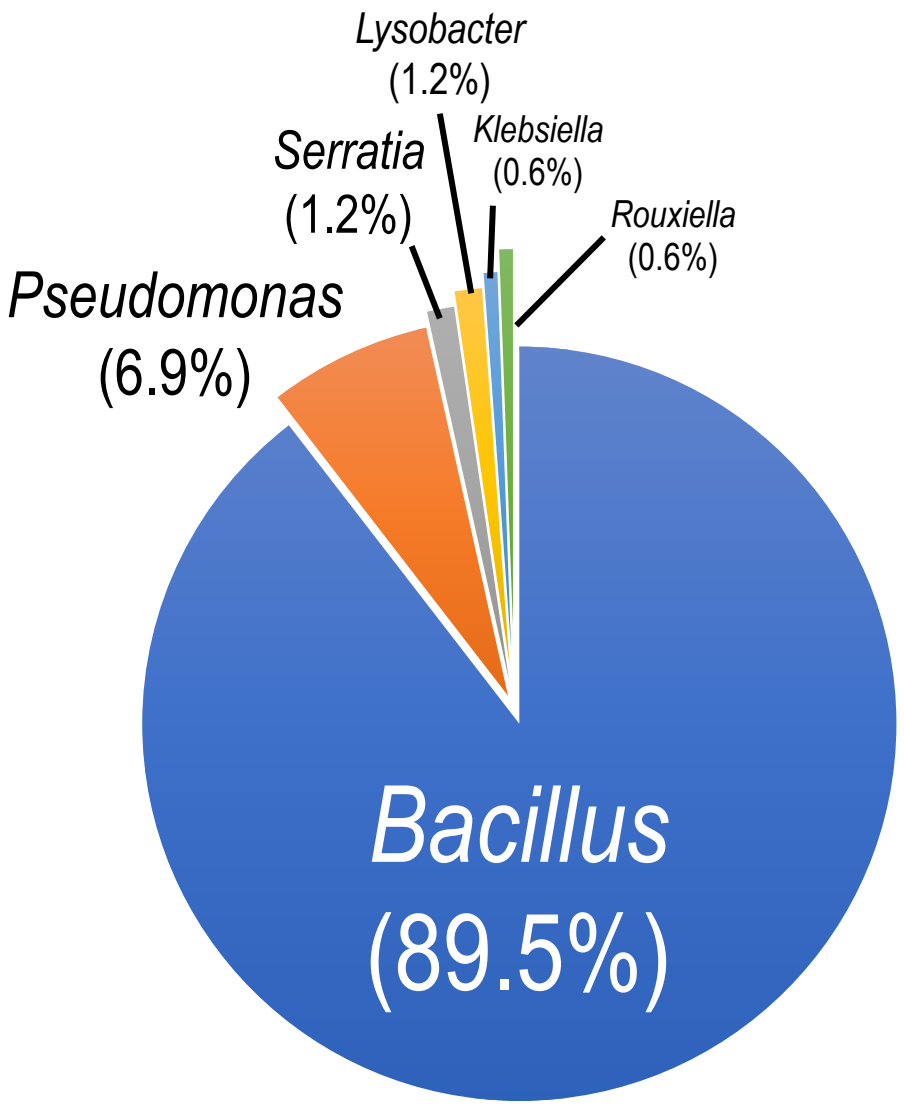
Lasiodiplodia theobromae

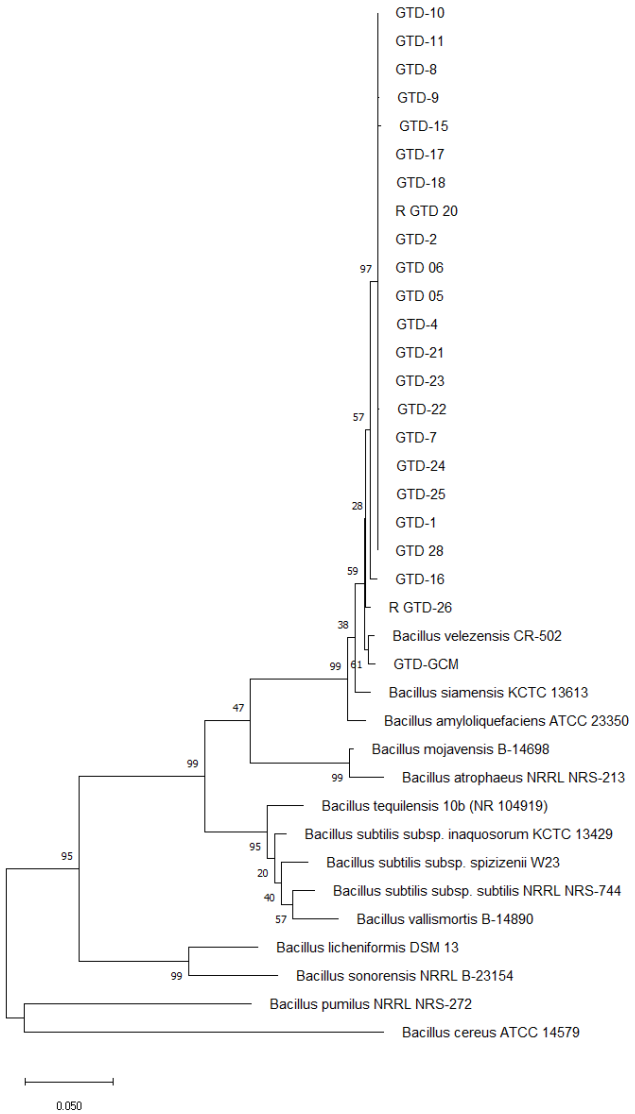
Eutypa lata

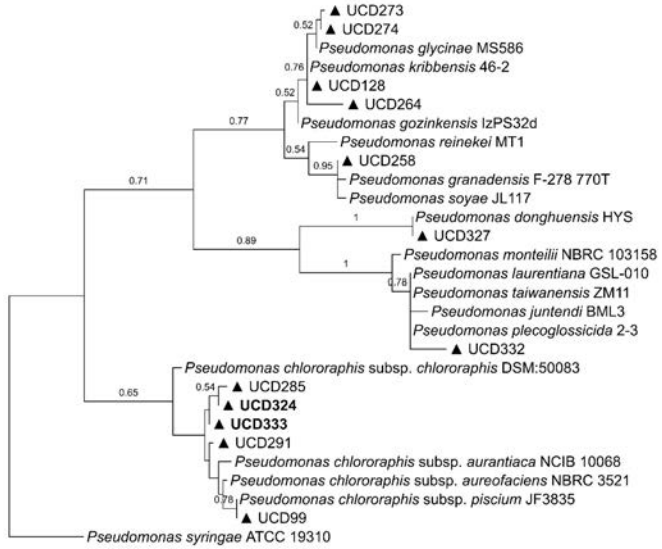


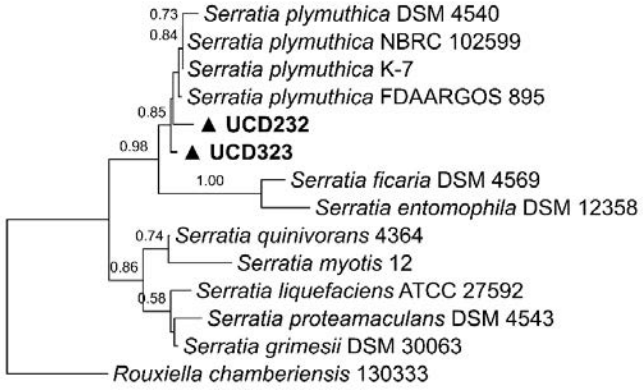
Bushttps://doi.org/10.3390/microorganisms10102035

16S rRNA sequences revealed predominance of *Bacillus* isolates









Field Experiments 2022-23 (Goal 2)

1) Vacuum infiltration of BCA to dormant cuttings in nurseries

2) Trunk and cordon injections in mature vines

3) Soil drench

Acknowledgements

Eskalen Lab Team – Department of Plant Pathology UC Davis

Cooperators and UCCE Farm Advisors
Mark Battany – UCCE San Luis Obispo, Santa Barbara
Larry Bettiga – UCCE Monterey
Monica Cooper – UCCE Napa
Carmen Gispert – UCCE Riverside
Glenn McGourty – UCCE Mendocino
Rhonda Smith – UCCE Sonoma
Gabriel Torres – UCCE Tulare
Jose Ramon Úrbez-Torres – Agriculture and Agri-Food Canada
Lynn Wunderlich – UCCE Central Sierra
George Zhuang – UCCE Fresno

Funding:

aeskalen@ucdavis.edu

https://ucanr.edu/sites/eskalenlab/