A Survey of Pinot Noir Vineyard-Associated *Saccharomyces cerevisiae* strains and Yeast species in the Okanagan Valley 2016

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BACKGROUND

Research Goals

1. Survey *S. cerevisiae* populations in Pinot Noir vineyards of three regions of the Okanagan Valley

2. Use a molecular technique to generate a genetic profile that will differentiate commercial strains from non-commercial

3. Elucidate differences in *S. cerevisiae* populations between regions using genetic profiles

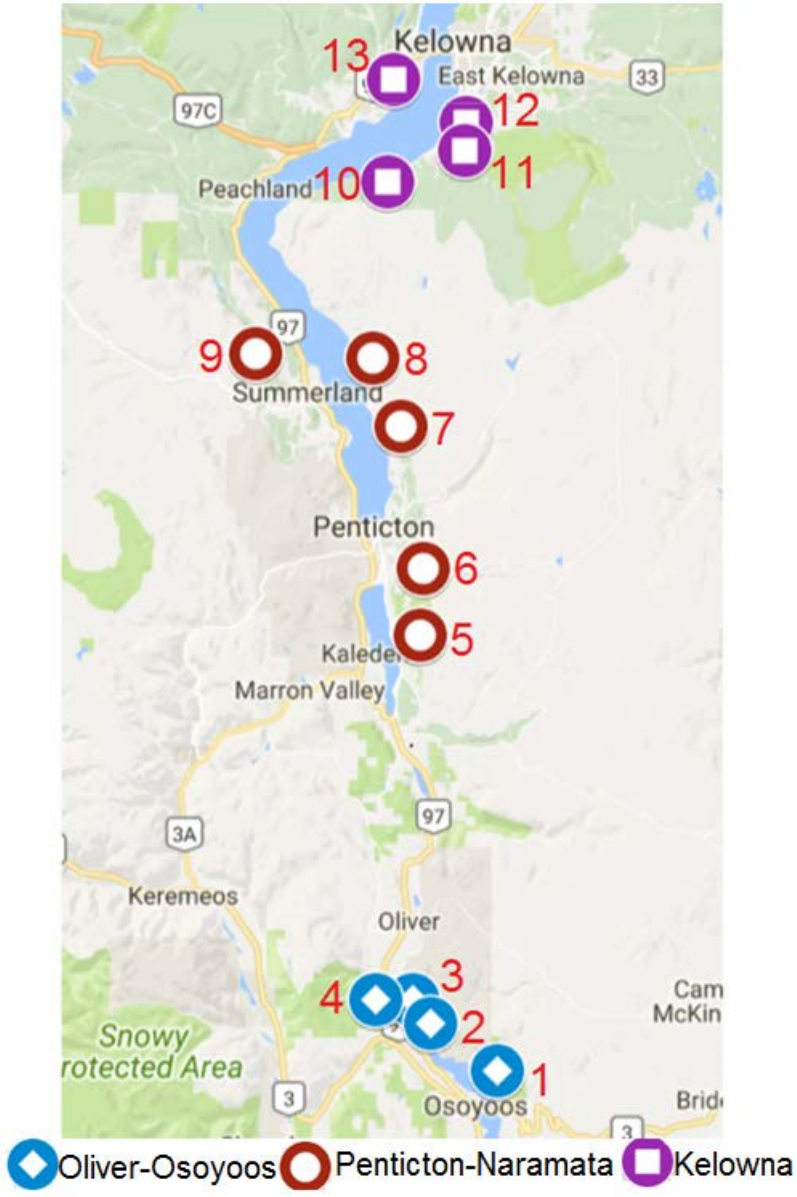
METHODS

13 Pinot Noir vineyards – 2016 vintage

Kelowna

Naramata-Penticton

Oliver-Osoyoos



METHODS

2016 Pinot Noir Vineyard Sampling

- Random Sampling in a 0.25 ha section of each vineyard
- Divided into 32x18m sections (3 post panels x 2 rows)
- 6 randomly chosen sections

Garrett McCarthy

Example of a vineyard plot – red blocks sampled

METHODS

Pinot Noir Crush

- 15 minute crush by hand in bags
- 500ml of juice transferred to pre-weighed fermentation apparatus
- Samples taken for downstream analysis
- Juice plated to obtain initial yeast colony counts
- Initial Brix also taken

Jay Martiniuk

METHODS

Spontaneous Fermentation

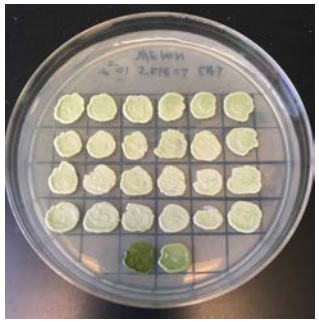
- Incubated flasks at 25°C
- Weighed at least once a day
- 1g of CO_2 lost = 2.118g of sugar
- Used conversion to approximate sugar depletion
- Fermented until dry or up to 40 days

METHODS

Yeast Isolation

- Sampled at 2/3 sugar depletion
- Plated a serial dilution of juice on YPD in duplicate
- Incubated for 48 hours then counted
- 48 yeast randomly selected from each fermentation and grown on YPD
- Isolates plated on Wallerstein/lysine (WL) agar to confirm *Saccharomyces* identity

WL agar

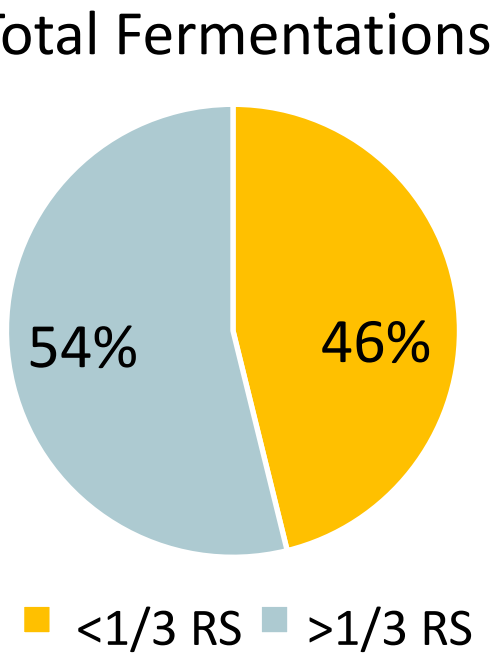


Non-*Saccharomyces* turn green

RESULTS

Fermentation results

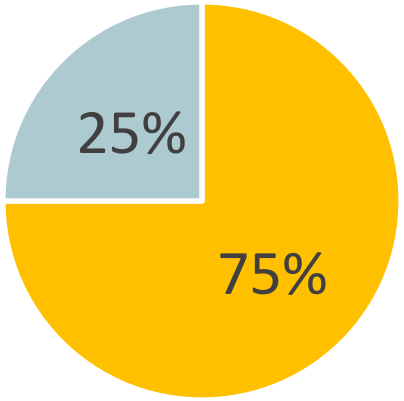
- 78 spontaneous fermentations
- 36 reached the 1/3 residual sugar (RS) mark
- *S. cerevisiae* were isolated from 34/36 fermentations
- 28/36 fermented to dryness



RESULTS

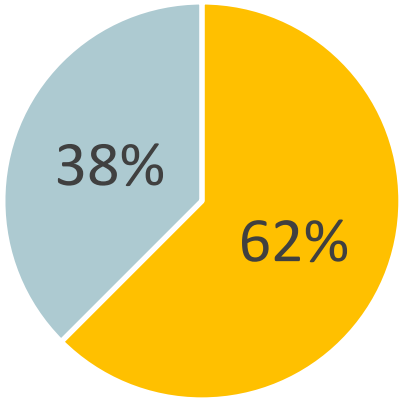
Fermentation Results by region

Kelowna



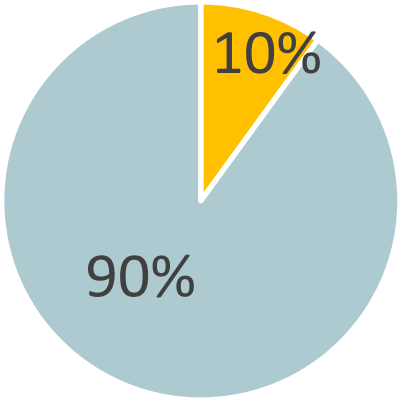
18/24 fermentations

Oliver Osoyoos

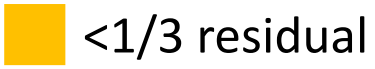


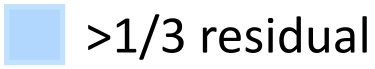
15/24 fermentations

Naramata/Penticton



3/30 fermentations

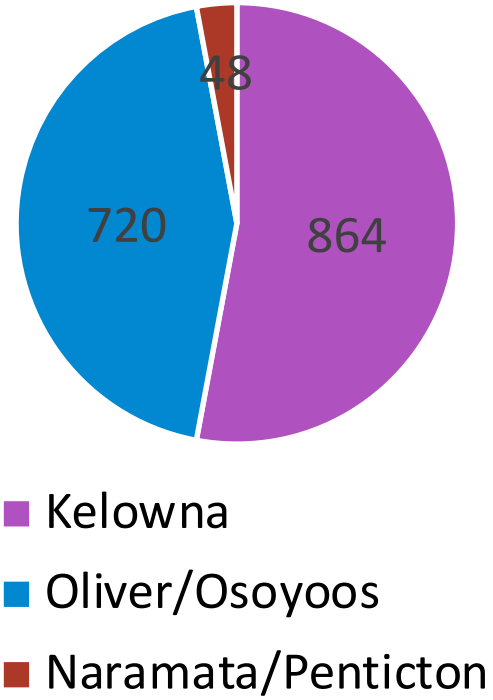




RESULTS

S. cerevisiae isolates by region

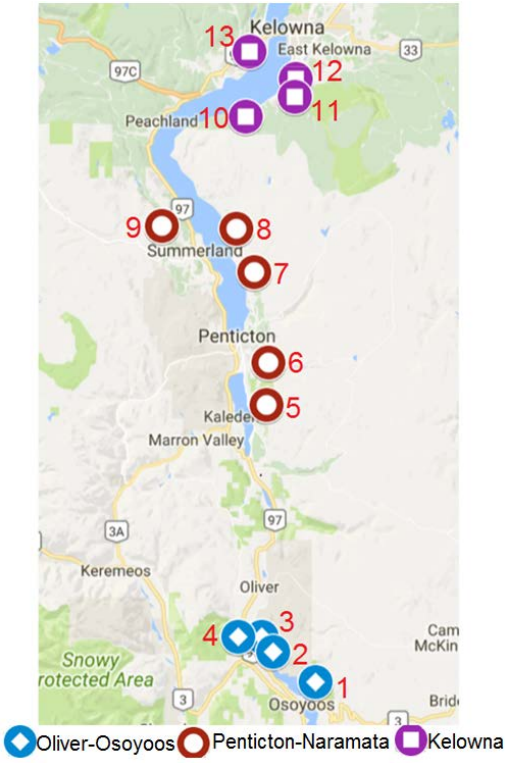
- 1632 total isolates obtained
- Majority of *S. cerevisiae* obtained from Kelowna and Oliver/Osoyoos
- Naramata/Penticton very underrepresented



RESULTS

Summary of yeast isolated from spontaneous fermentation by region and vineyard

Sub-region	#	Fermentations with 2/3 RS	*#S. cerevisiae* Isolates
Oliver-Osoyoos	1	3/6	144
	2	3/6	144
	3	4/6	192
	4	5/6	240
Naramata-Penticton	5	0/6	0
	6	1/6	48
	7	0/6	0
	8	1/6	48* non-Saccharomyces
	9	1/6	48* non-Saccharomyces
Kelowna	10	6/6	288
	11	1/6	48
	12	6/6	288
	13	5/6	240



Non-*Saccharomyces* isolated from Naramata-Penticton at 2/3 residual sugar

Torulaspora delbrueckii

- Used in sequential fermentations
- One of the first commercial non-*Saccharomyces* strains to be released
- Produces lower levels of volatile acidity than *S. cerevisiae*

Kluyveromyces marxianus

- Isolated from food and beverage environments
- Isolated from grapes in Linosa (Mediterannean Island)
- High producer of β-glucosidase which can release flavour and aroma compounds

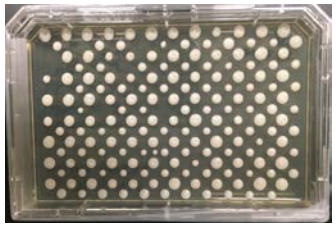
METHODS

Work flow to create *S. cerevisiae* genetic fingerprint

1.

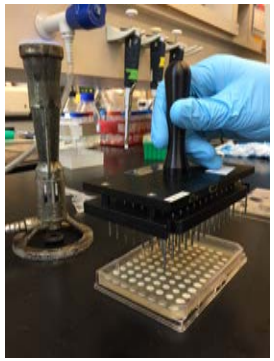
Yeast colonies from a fermentation sample

2.



Array 96 colonies onto plate

3.

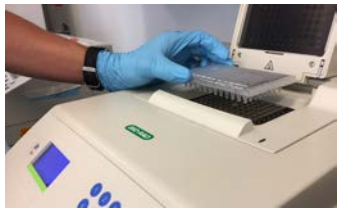


Pin colonies into 96 well plate

4.

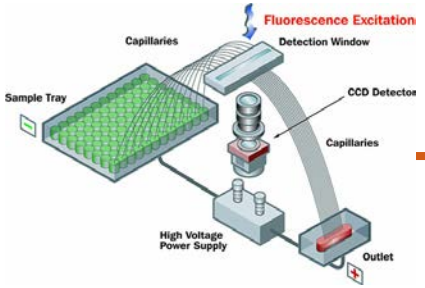
Genomic DNA extraction

5.



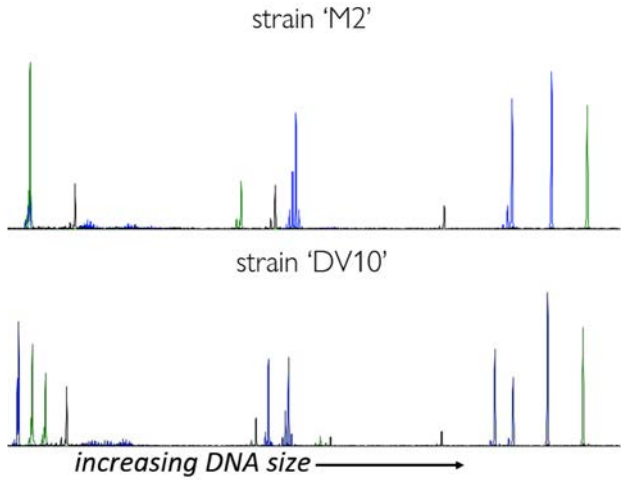
Amplify 11 microsatellite loci with fluorescent primers

6.



Capillary electrophoresis

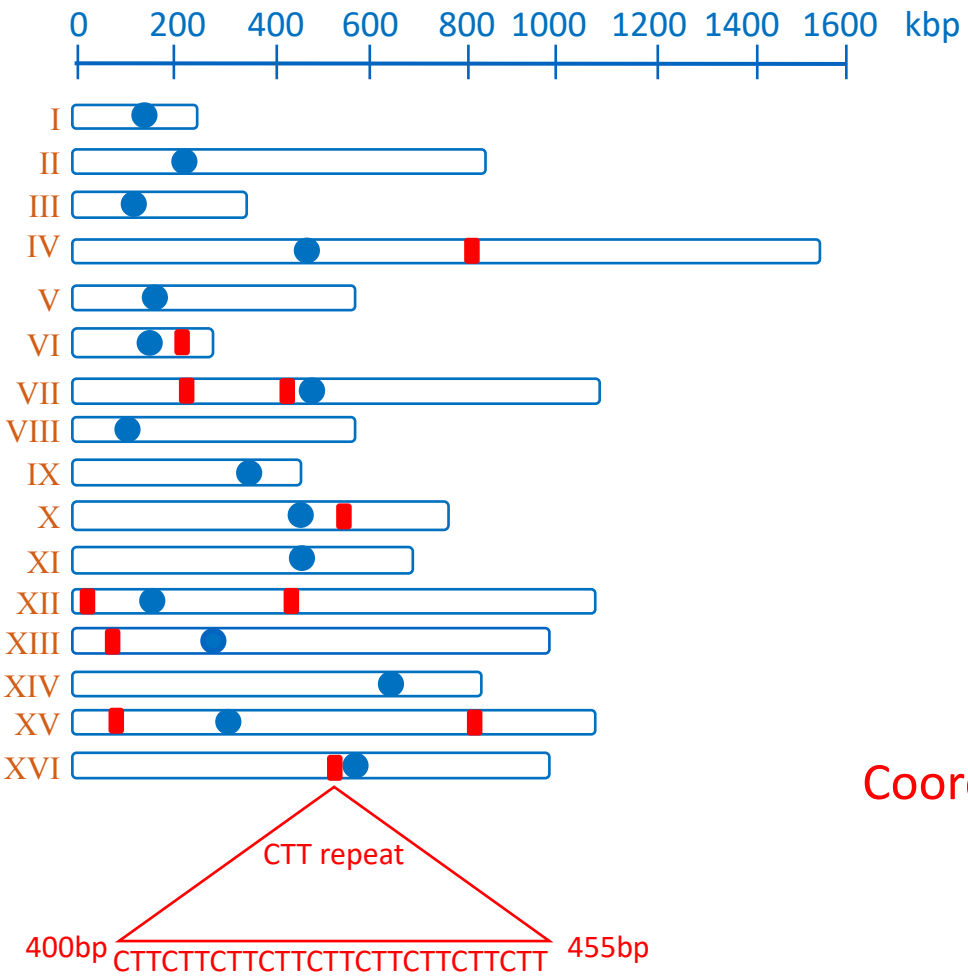
7.

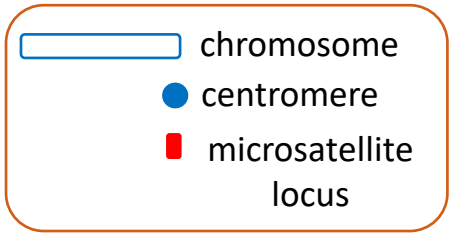


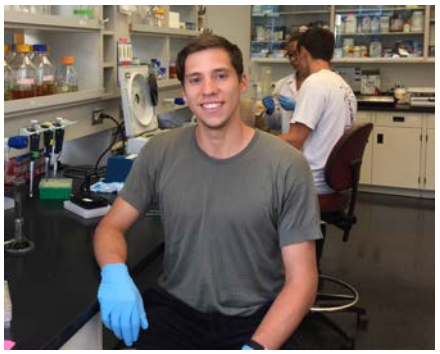
Analyze size of each genetic loci

METHODS

S. cerevisiae genomic loci used for microsatellite genetic fingerprint





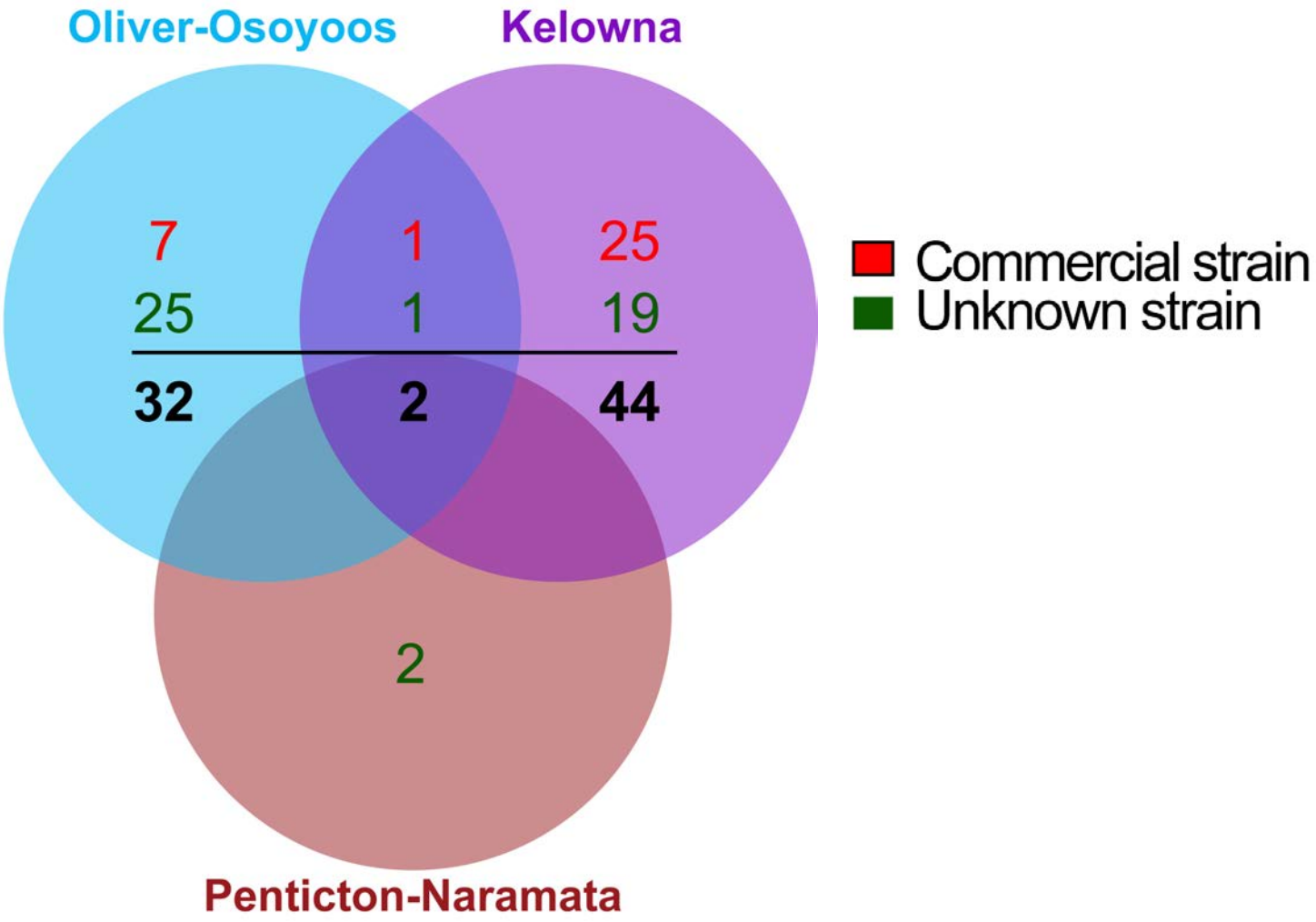


Jonah Hamilton

Coordination of microsatellite loci with
Dan Durall's lab

RESULTS

S. cerevisiae strains are unique to sub-region



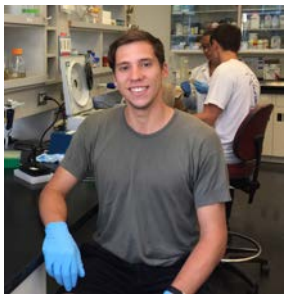
Pinot Noir Vineyard
S. cerevisiae Survey 2017

- Deeper exploration of Okanagan vineyard *S. cerevisiae* diversity
  - Identify vintage effects on strain population structure
  - Isolate other unique *S. cerevisiae* strains
- Profile Pinot Noir grape composition
  - Dr. Simone Castellarin, WRC

Courtesy Google Maps

Acknowledgements

Measday Lab



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Durall Lab

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Breanne Newman

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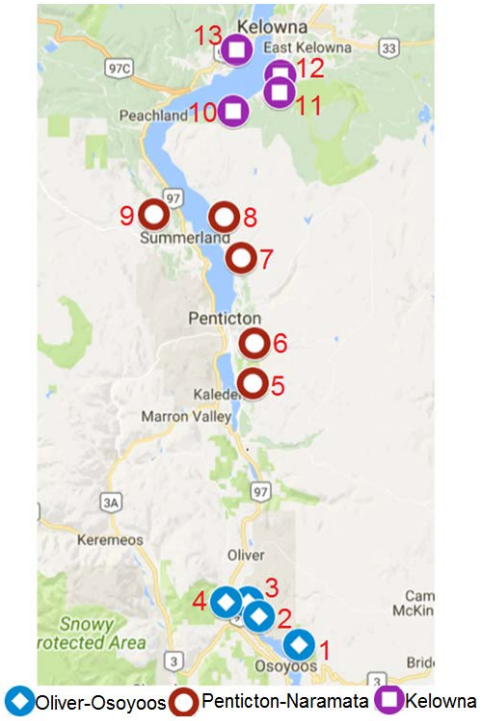
Participating vineyards



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