Management through the Microbiome: How Manipulating Grape Endophytes Can Affect Berry Development

Megan E. Hall, Ph.D.

Assistant Research Professor

University of Missouri – Columbia

Show Me Grape and Wine Conference, March 5, 2020

Where we're going

- Current Research in my Lab
- Background on the Microbiome
- Modifying the Microbiome
- Results
- Future Avenues of Research
- Questions

Current Research in my Lab

- Sour rot
  - Fine tuning spray timings
  - Implicating larvae in SR progression
- Grapevine Trunk Disease
  - Prevalence in MO
  - Variety variation
- Ripe rot
  - Prevalence
  - Management strategies
- Phomopsis
  - Prevalence
  - Management strategies
- Trialing 5 MN varieties
- Good luck with Viognier and Petit Manseng

Overwintering stage of Phomopsis

Bleaching
Black spots

Management:

Dormant spray: Sulforix or
lime sulfur

1” – 3” shoot growth: Mancozeb or
Captan

Background on the Microbiome

Yeast and Bacteria are prevalent in healthy grapes

Research Note

Identification and Frequencies of Endophytic Microbes within Healthy Grape Berries

Megan E. Hall[1]* and Wayne F. Wilcox[1]

Abstract: Intact, healthy grape berries were sampled from vineyards in the states of Washington and New York; in Tasmania, Australia; and from bunches of table grapes exported from Chile that were purchased on two occasions in a United States supermarket. Endophytic microbes were isolated on media conducive to fungi or bacteria and subsequently identified by Illumina sequencing of their DNA. Species of the yeast genera *Metschnikowia*, *Pichia*, and *Hanseniaspora* were recovered from every set of samples, as were species of the bacterial genera *Acinetobacter*, *Burkholderia*, *Bacillus*, *Acetobacter*, and *Gluconobacter*. Multiple other fungal and bacterial species were recovered less often. When quantified for the Washington samples and one set from the supermarket, non-*Saccharomyces* yeast species represented the vast majority of fungal identifications, while the distribution of various bacterial species varied widely between and within the two sources. The endophytic presence of these microbes within grape berries has implications with respect not only to the potential development of sour rot, but also to the broader concept of microbial terroir in wine quality.

Key words: bacterial endophytes, microbial populations, yeast ecology

The composition of epiphytic microbes on the surface of ape berries has been researched extensively, with studies using on grapes sampled in the days leading up to harvest osini et al. 1982, Parish and Carroll 1985, Yanagida et al. 92, Martini et al. 1996, Sabate et al. 2002, Combina et al. 05, Raspor et al. 2006, Setati et al. 2012, Brysch-Herzberg d Seidel 2015, Drożdż et al. 2015, Garofalo et al. 2016, a et al. 2016). In contrast, endophytes of grape berries and ier plant reproductive organs are rarely addressed (Com- at et al. 2011). *Firmicutes*, primarily *Bacillus* spp., were orted with grape berries, in the only reports of endo- ytic microbes within the fruit of this crop (Compant et al. 11, 2012). Nevertheless, individual species and groups of crobes inhabiting the pulp of healthy grape berries could potentially have a significant practical impact under son conditions, e.g., as pathogens or in enological processes aft harvest. While many researchers have explored the microbi communities within grape musts after crushing (Bokulich al. 2014, 2016, Gilbert et al. 2014, Pinto et al. 2015, Setati al. 2015), there has been no effort to determine whether the organisms originated on the surface of the harvested cluste or within the pulp.

In a study examining potential causes of the disease so rot, we wounded intact healthy table grape berries obtain from a supermarket, inoculated them with various cand date microbes, and measured the evolution of ethanol ar acetic acid after five to eight days of incubation. In repeat experiments, we routinely found detectable levels of eth nol (and less often, acetic acid) in wounded but otherwi healthy control fruit, which had been handled aseptical but not inoculated with microbes (Hall et al. 2018). Becau these results suggested the possible endophytic presence yeast (and less often, acetic acid bacteria) within the berrie we undertook the following study to investigate both t ubiquity and diversity of these and other microbes prese within the pulp of healthy grapes from different geograph cal locations.

Materials and Methods

Detection of endophytic microbes. Grape clusters we sampled from three vineyards in Tasmania, Australia; a si gle vineyard in Kennewick, WA; two vineyards in Genev NY; and from a supermarket in Geneva, NY, on two separa occasions (Table 1). All grapes examined were cultivars *Vitis vinifera* except *Vitis* × *labruscana* Concord. All vin yard samples were obtained from vines exhibiting no ove symptoms of disease. Clusters were intact and uninjured, a maturity stage corresponding to approximately one to fi

tion of Plant Pathology and Plant-Microbe Biology, School of Integrative st Science, Cornell University, NYS Agricultural Experiment Station, eva NY 14456.

rresponding author (hallmegan@missouri.edu)

nowledgments: The authors thank the Cornell Genomics Facility for their stance with Illumina sequencing and Dr. Lance Cadle-Davidson and Isabelle ryon for help in analyzing sequencing data. Thanks to Dr. Kathy Evans niversity of Tasmania for assistance in finding commercial collaborators to Dr. James Fredrickson for collecting samples in Kennewick, WA. This k was supported by the NY State Department of Agriculture and Markets, Wine and Grape Foundation, USDA Specialty Crops Block Grant, and Dyson Fund.

nuscript submitted March 2018, revised Aug 2018, Oct 2018, accepted 2018

10.5344/ajev.2018.18033

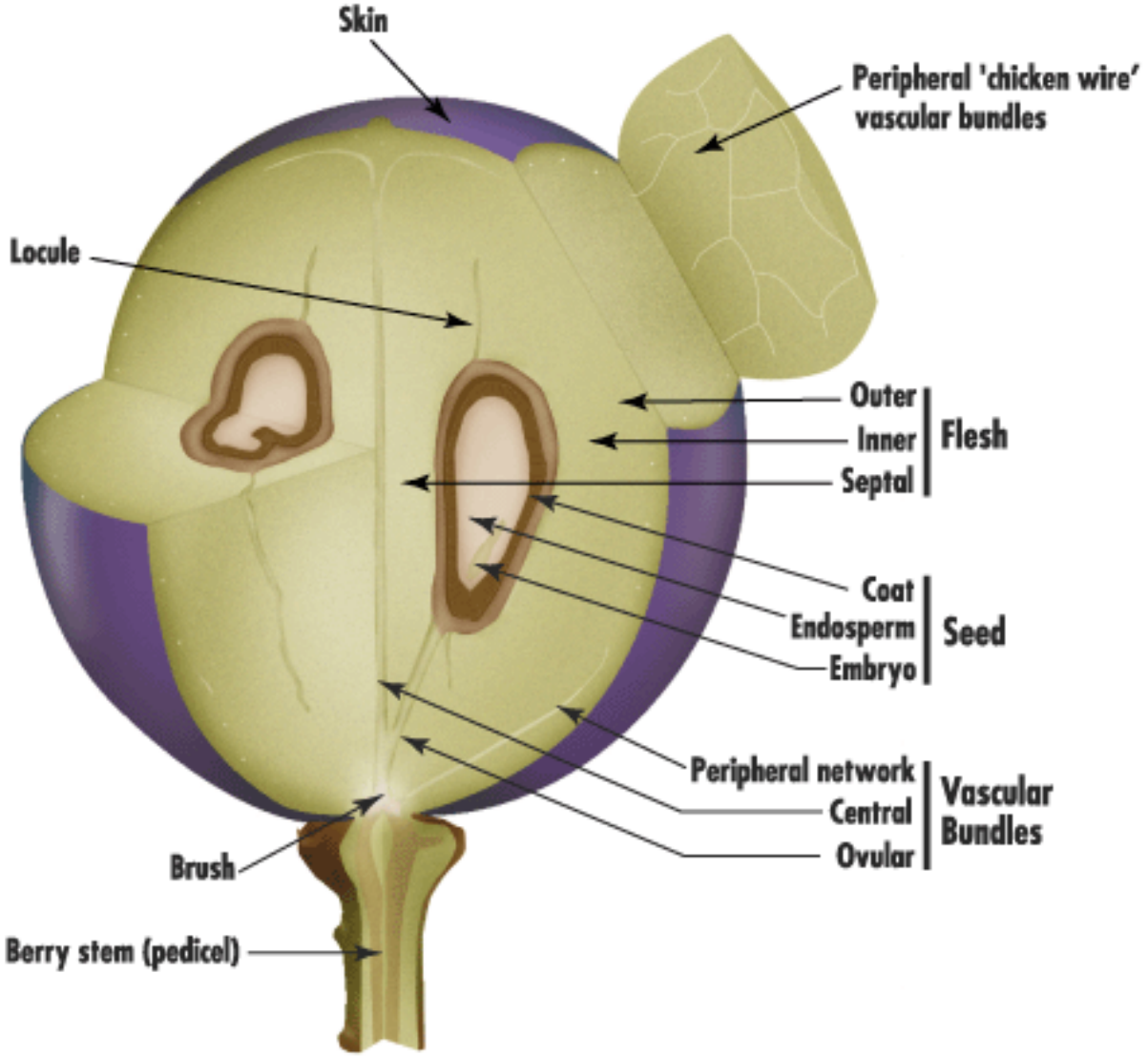


Figure I: Structure of a ripe grape berry partially sectioned on the long and central axis to show internal parts. Illustration by Jordan Koutroumanidis, Winetitles.

- The grape berry is a factory for various biochemical compounds
- Flesh contains the most juice
- Seed:flesh ratio depends on variety and climate
- Number of seeds depends on variety and climate

Flavonoids are produced in seeds and skins

- Important for the color and taste of wine
  - **Tannins** and **anthocyanins** are the major flavonoids

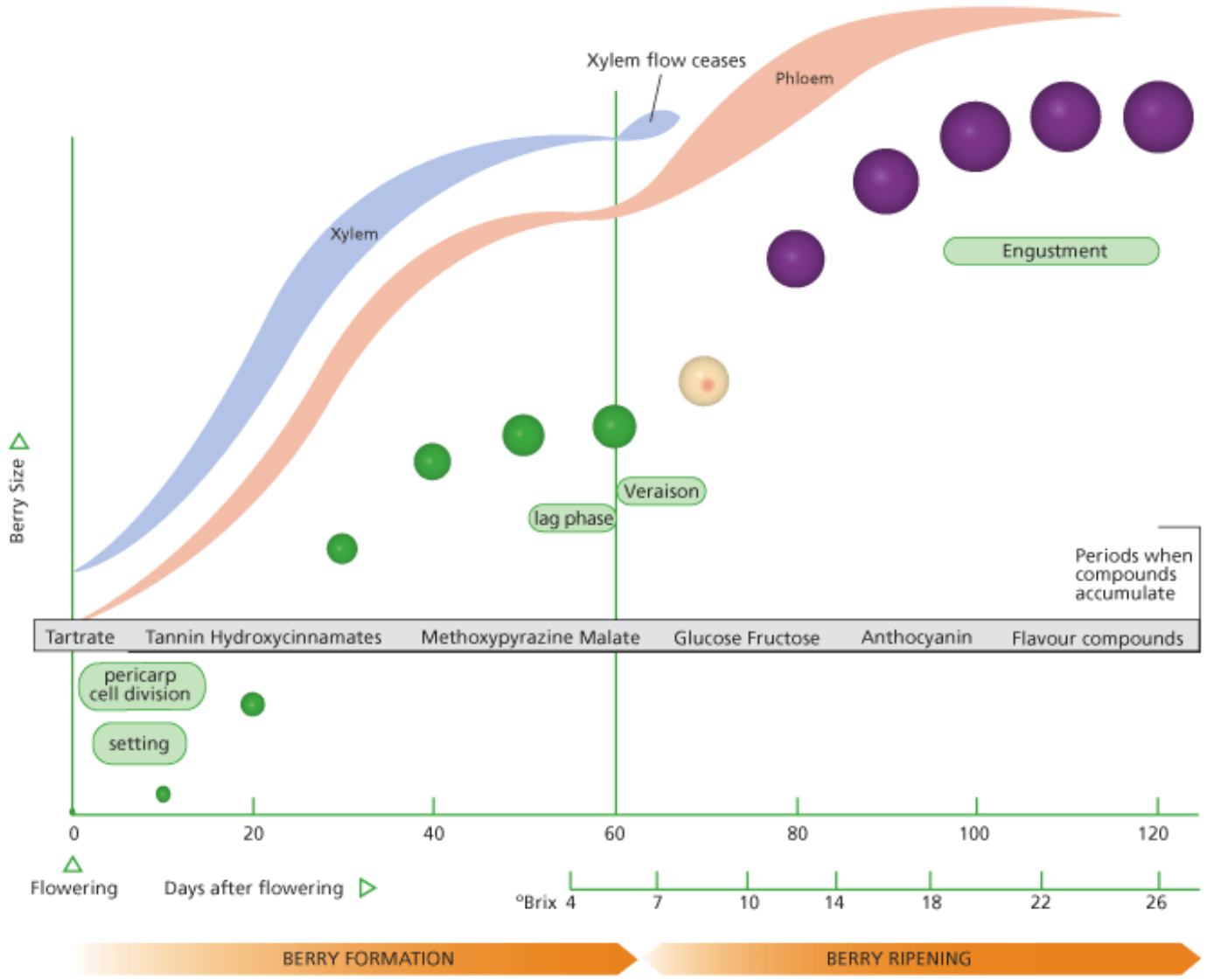


Figure 2: Diagram showing relative size and color of berries at 10-day intervals after flowering, passing through major developmental events (rounded boxes). Also shown are the periods when compounds accumulate, the levels of juice °brix, and an indication of the rate of inflow of xylem and phloem vascular saps into the berry. Illustration by Jordan Koutroumanidis, Winetitles.

- Two stages:
  - Berry formation
  - Berry Ripening
- Xylem supplies berry early in season (water, minerals, nutrients)
- Phloem supplies berries after Veraison (photosynthates/ sugars)

Berry formation
First stage of growth : Bloom + 60 days

- Berry expands
- Tartaric and malic acids accumulate
  - Provide the acidity in wine
- Hydrocinnamic acids accumulate
  - Precursors to volatile phenols
    - Each volatile phenol has a distinct aroma (cloves, sweat, etc…)
- Tannins begin to accumulate
  - In seed and skin, not flesh
  - Contribute to color stability

Berry Ripening
Second stage of growth: softening & color change

- Berry doubles in size from Veraison to harvest
- Malic acid, tannins, certain volatiles (methoxypyrazines) decline (and not just by dilution)
- Huge increase in glucose and fructose
  - Sucrose produced by photosynthesis
  - Transported into the berries
  - Hydrolized into glucose & fructose
- Secondary metabolite production
  - Anthocyanins (skin)
  - Volatiles (flesh and skin)

Even though everyone claims that wine is made in the vineyard…

- Overcropping/undercropping
- Pyrazines are thought to decline with sunlight exposure (leaf pulling)
- Hanging fruit longer (more sugar)
- Earlier harvest (more acid)

…the real toolbox has generally been in the winery

- Yeast impart many characteristics (aroma, mouthfeel)
- Enzymes
- Adding tannin (tannin products derived from grapes and oak are common)
- Adding acid
- Adding sugar
- Maceration (pump-overs, punchdowns, submerged cap, pulsed air)
- Oak chips
- Fining (bentonite, PVPP, gelatin, egg whites, casein)
- Aging

But terroir is important, right?
…so what is it?

Terroir

Three components:

Soil

Climate

Cultivar

- The environment relates to sensory attributes in wine, but how?
- Terroir is hard to study!
- Microbes are one way of studying terroir
  - High-throughput sequencing

What about microbes?

They aren't usually mentioned when discussing the grape berry biochemistry

But they must be playing a role in grape growing

Most research has been on must and grape surface

Challenges isolating DNA from the grape surface

Thick, waxy cuticle and limited amount of DNA

Development of DNA extraction technique

Microbes vary by region

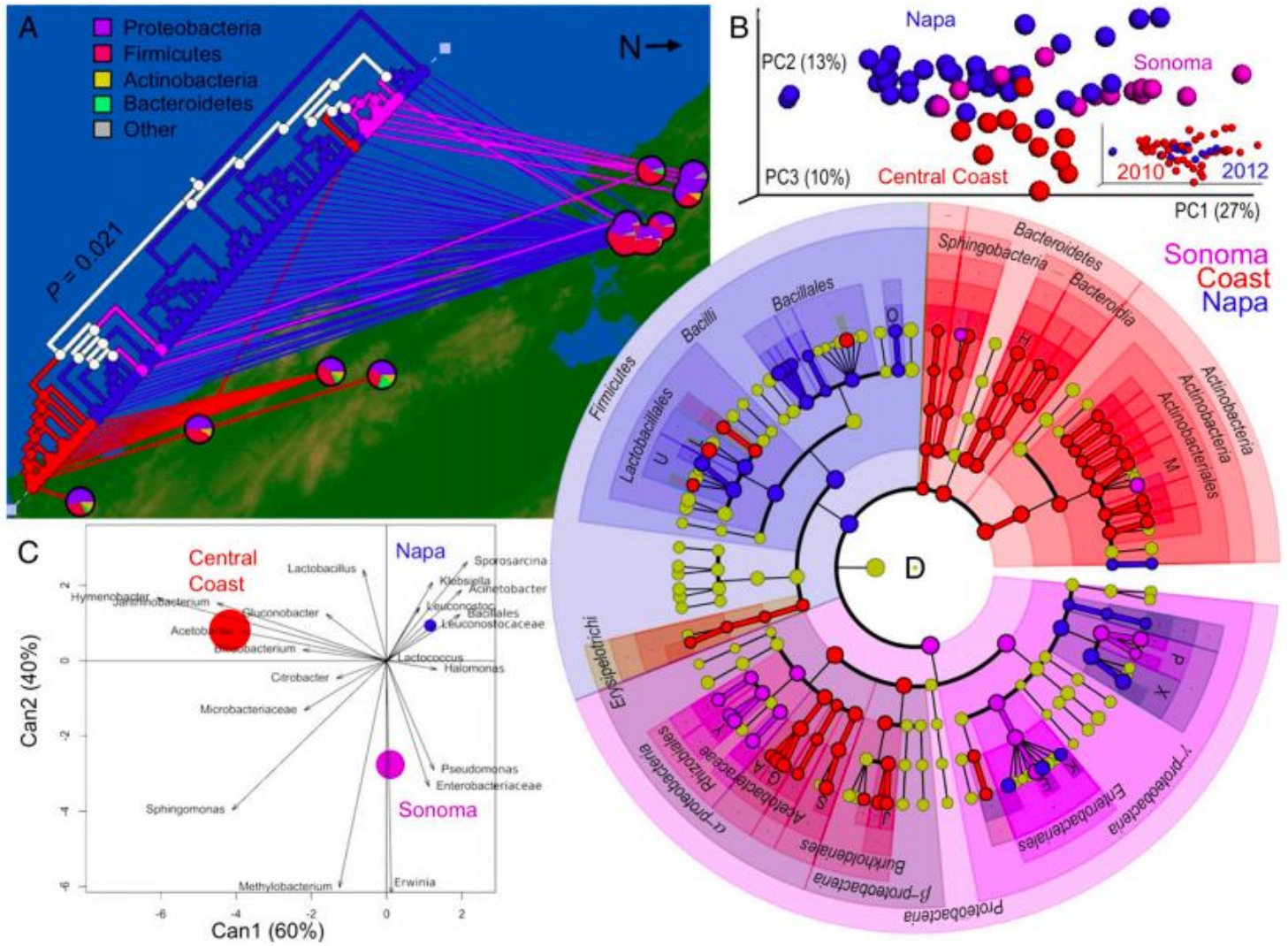


Fig. 1. Grape must bacterial communities demonstrate distinct regional patterns. (*A*) Weighted UniFrac distance dendrogram comparing bacterial communities of Chardonnay musts from across California. Branches are colored by the growing regions they represent, white branches encompass

Bokulich et al. 2014

Microbes vary by cultivar

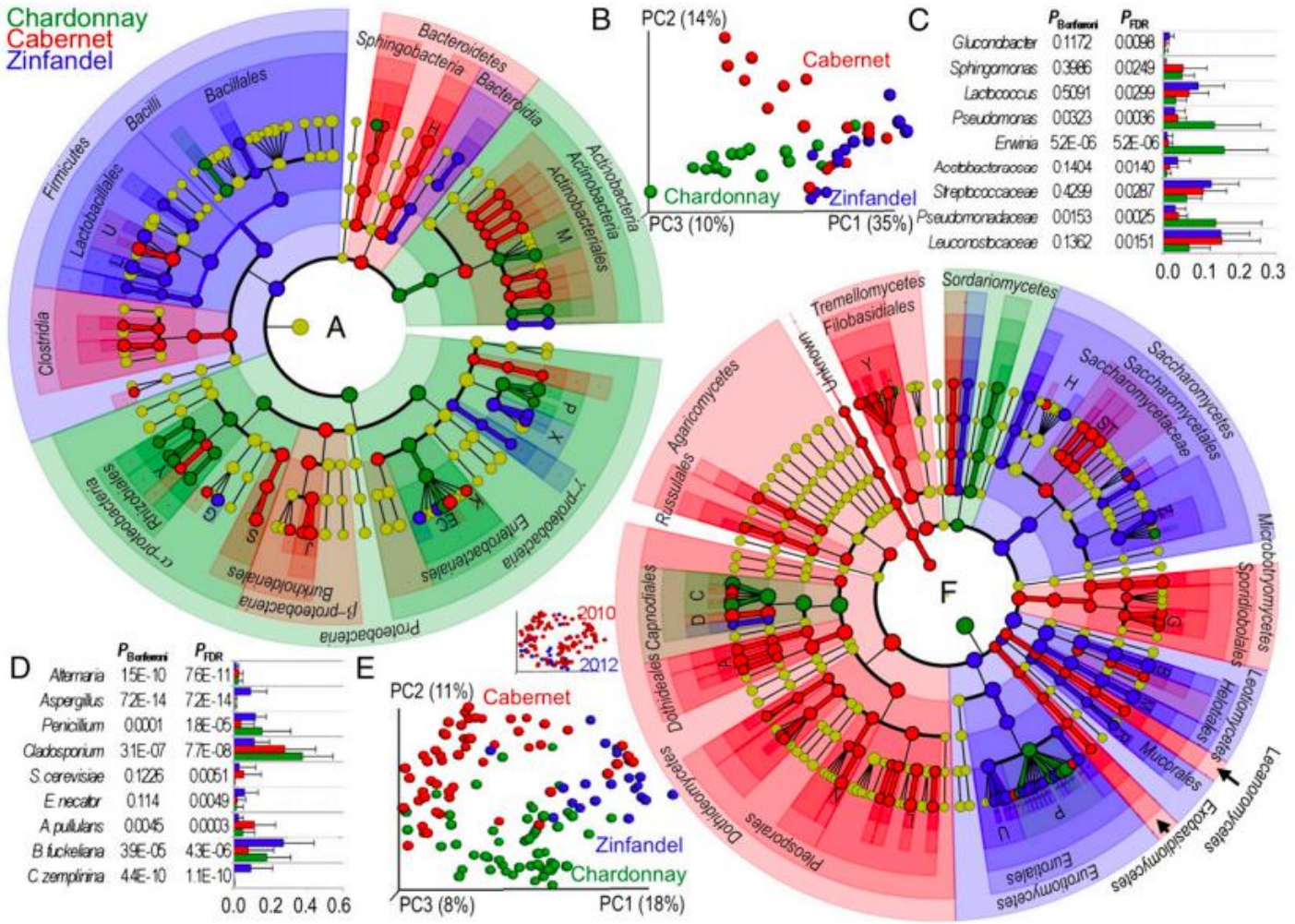


Fig. 2. Varietal variation in bacterial (*Left*) and fungal (*Right*) communities of Zinfandel, Cabernet Sauvignon, and Chardonnay grape musts. (*A*) LDA effect size taxonomic cladogram comparing bacterial communities in all Sonoma Cabernet Sauvignon, Chardonnay, and Zinfandel musts. Significantly discriminant taxon nodes are colored and branch areas are shaded according to the highest-ranked variety for that taxon. For each taxon detected, the corresponding node in the taxonomic cladogram is colored according to the highest-ranked group for that taxon. If the taxon is not significantly differentially represented between sample groups, the corresponding node is colored yellow. Highly abundant and select taxa are indicated: C, *Citrobacter*; E, *Erwinia*; G, *Gluconobacter*; H, *Hymenobacter*; J, *Janthinobacterium*; K, *Klebsiella*; L, *Lactococcus*; M, *Microbacteriaceae*; P, *Pseudomonadaceae*; S, *Sphingomonas*; U, *Leuconostocaceae*; X, *Moraxellaceae*; Y, *Methylobacterium*. (*B*) Weighted UniFrac distance PCoA of bacterial communities in all Sonoma Cabernet Sauvignon, Chardonnay, and Zinfandel musts. (*C* and *D*) One-way ANOVA of select bacterial (*C*) and fungal taxa (*D*) exhibiting significant differences

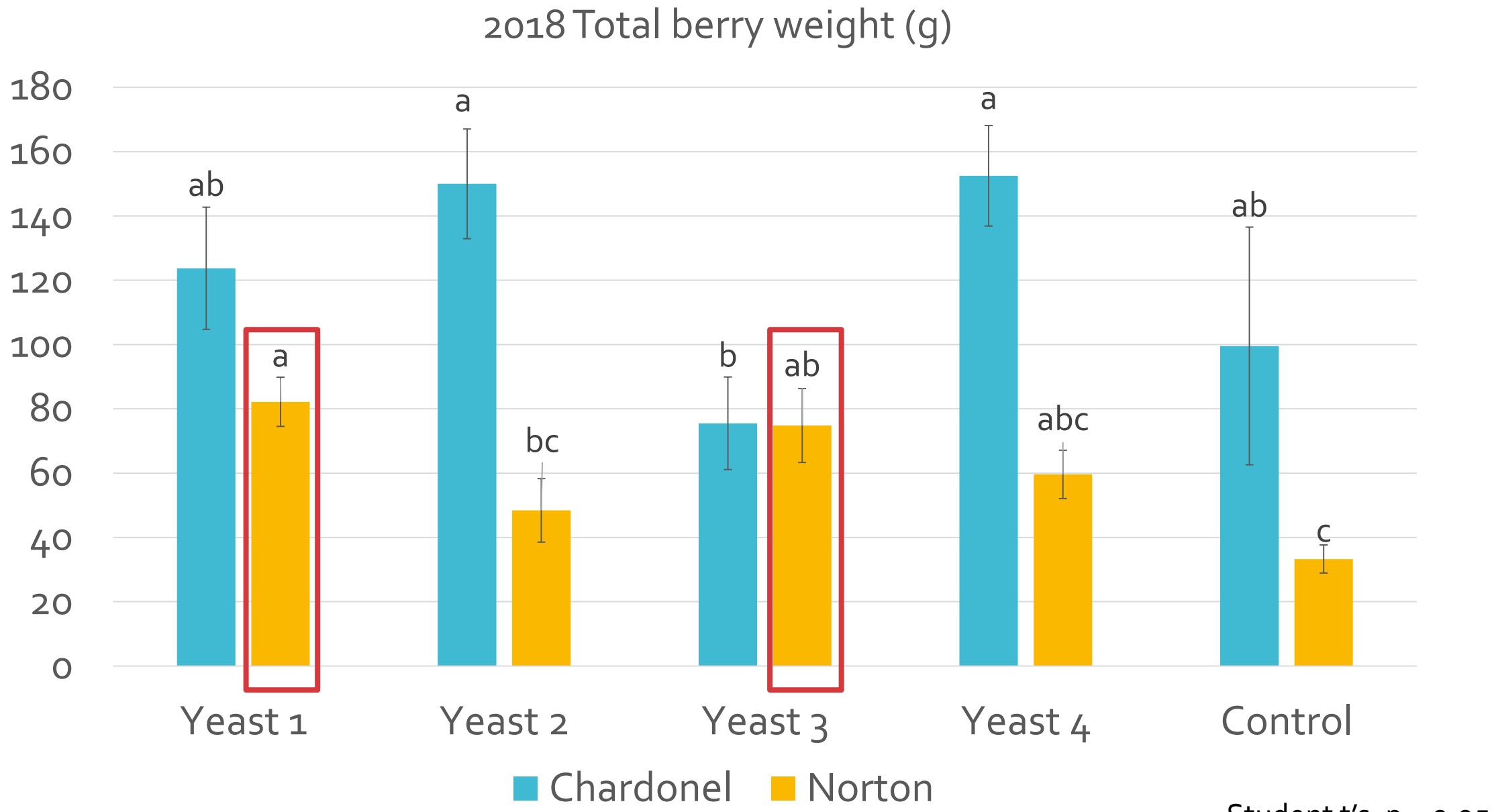
Bokulich et al. 2014

Grape Microbiome Affecting Physiology

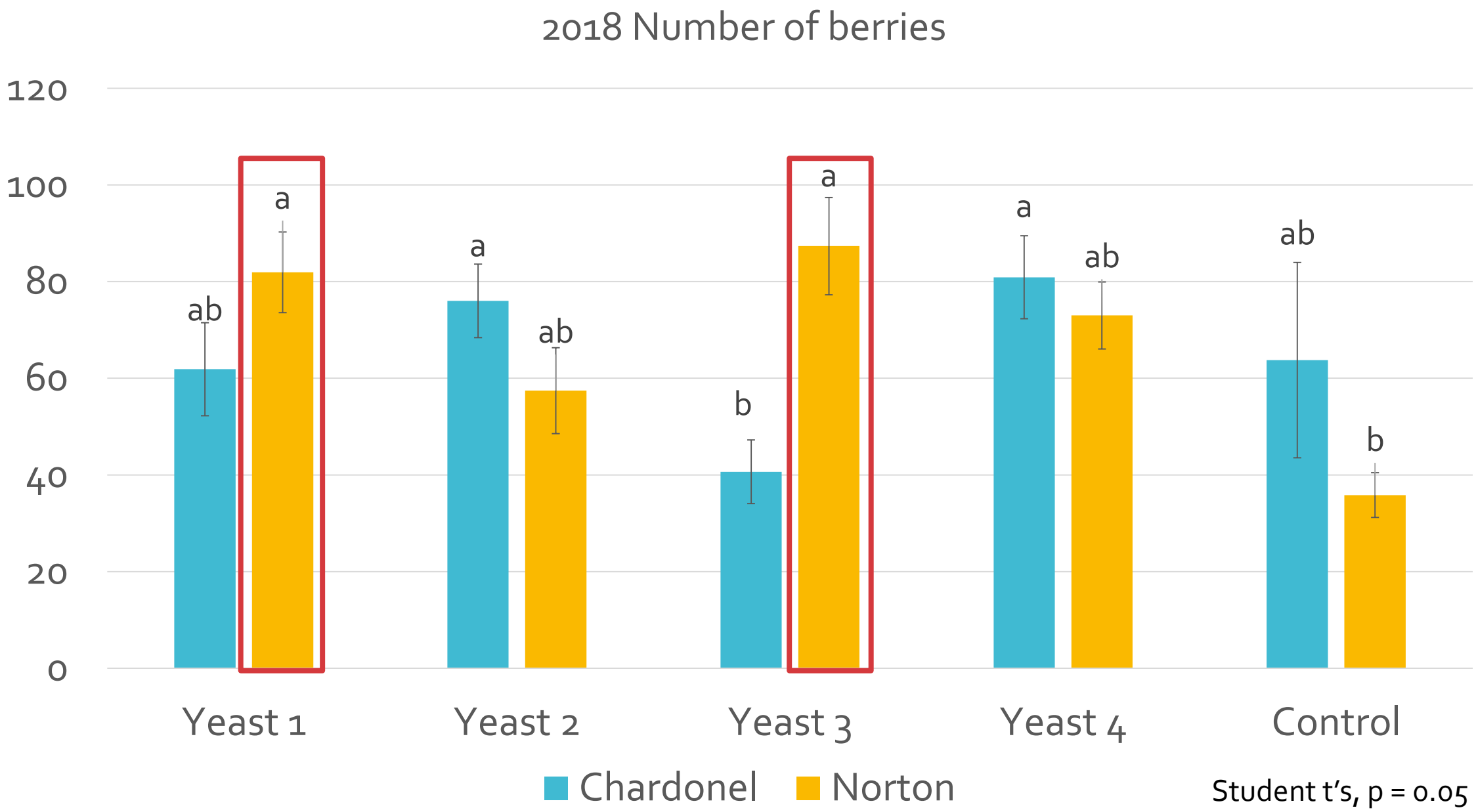
- Healthy grapes have an abundance of microbes in their pulp
- **Can we manipulate the grape microbiome?**
- What's the best timing?
- Will manipulating the microbiome change the berries in any way?

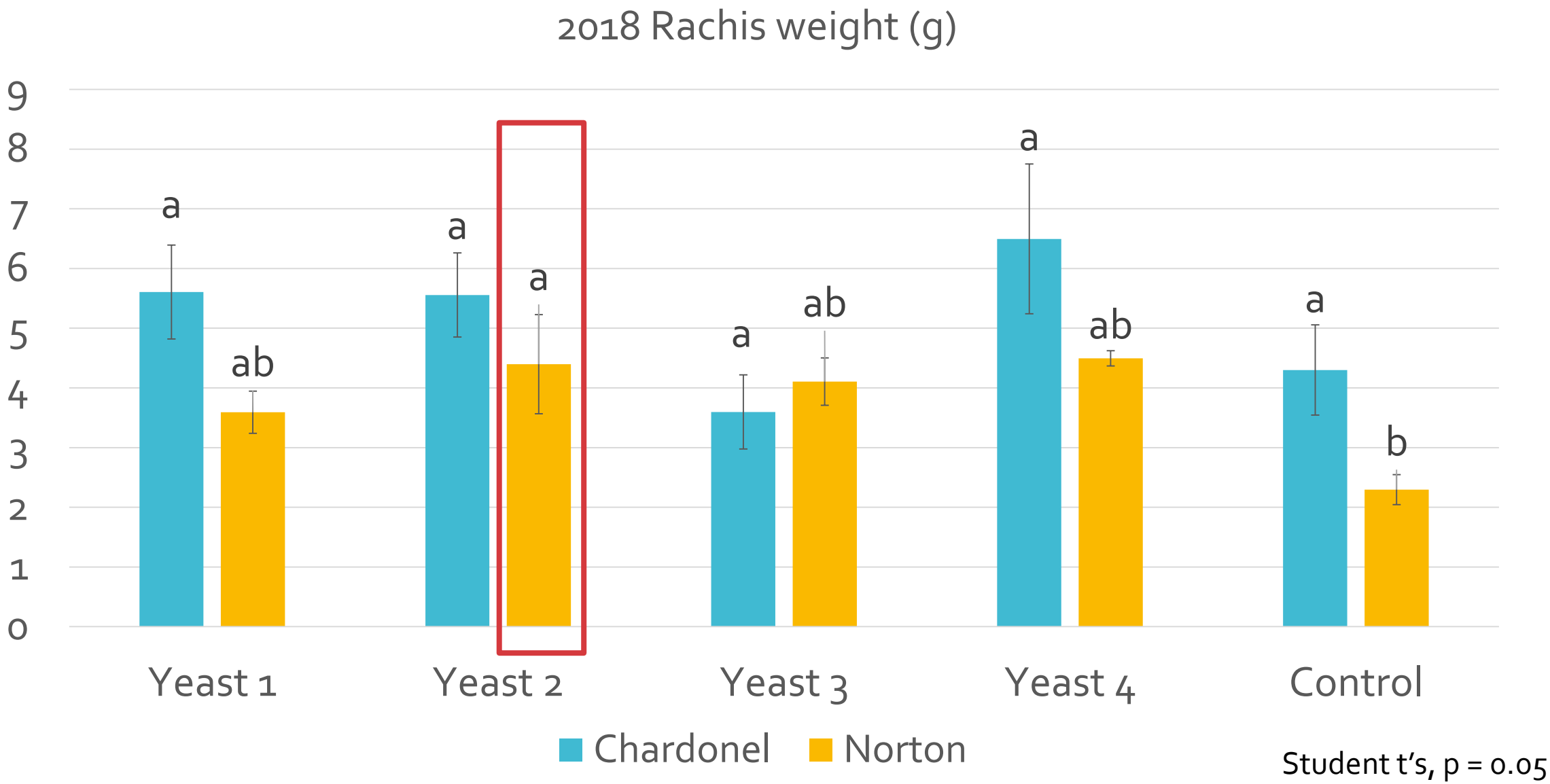
Manipulation study

- **2018:** Research vineyard: 3 reps of vines each of *Vitis* interspecific hybrid cvs. Chardonel and Norton sprayed at Bloom with 4 different single-species active yeast or water (control)
- Berry weight, rachis length, cluster compactness all done at Veraison and Harvest

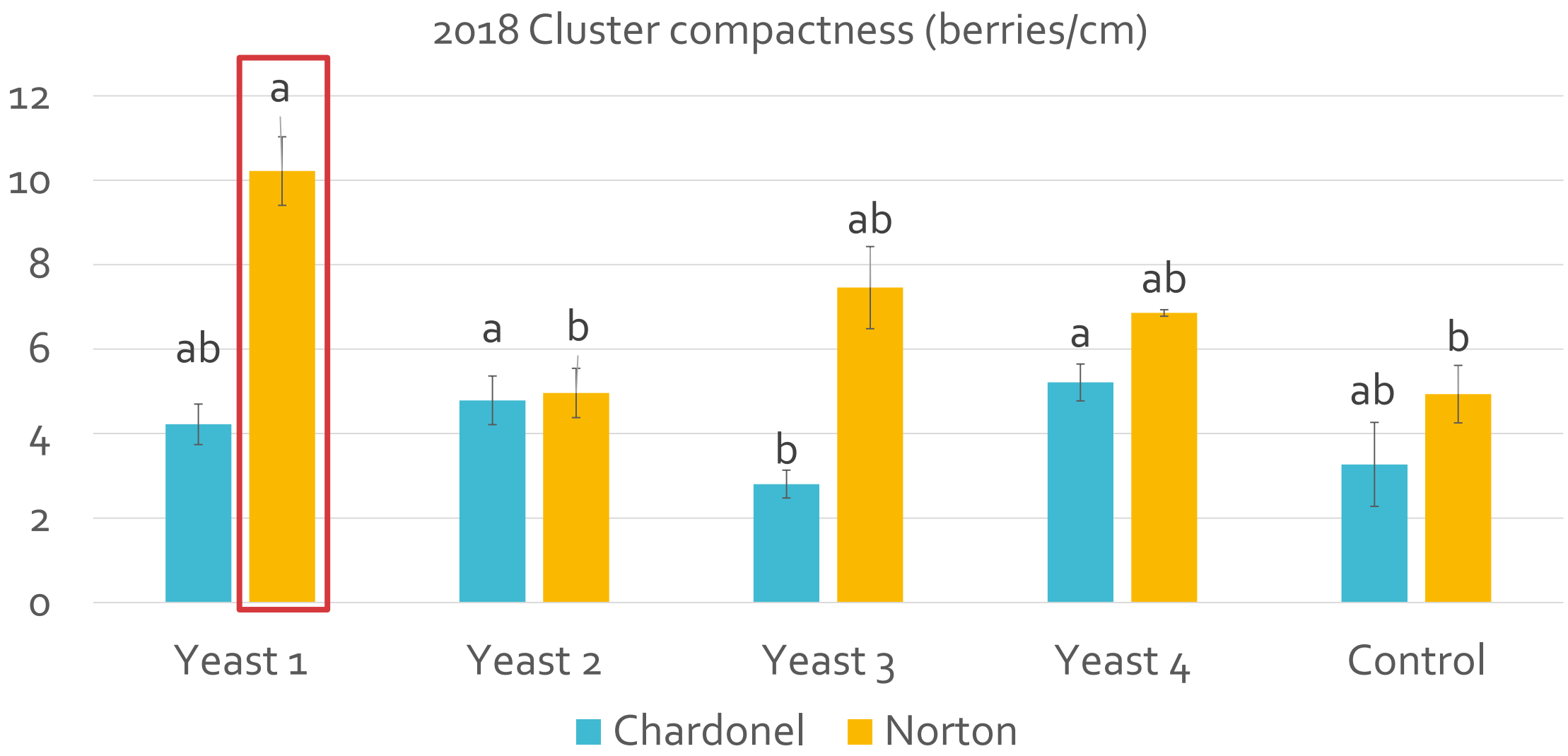


Student t's, p = 0.05


2018 Number of berries
120
100
80
60
40
20
0
ab
a
a
ab
b
a
a
ab
ab
b
Yeast 1
Yeast 2
Yeast 3
Yeast 4
Control
Chardonel
Norton
Student t's, p = 0.05



Student t's, p = 0.05

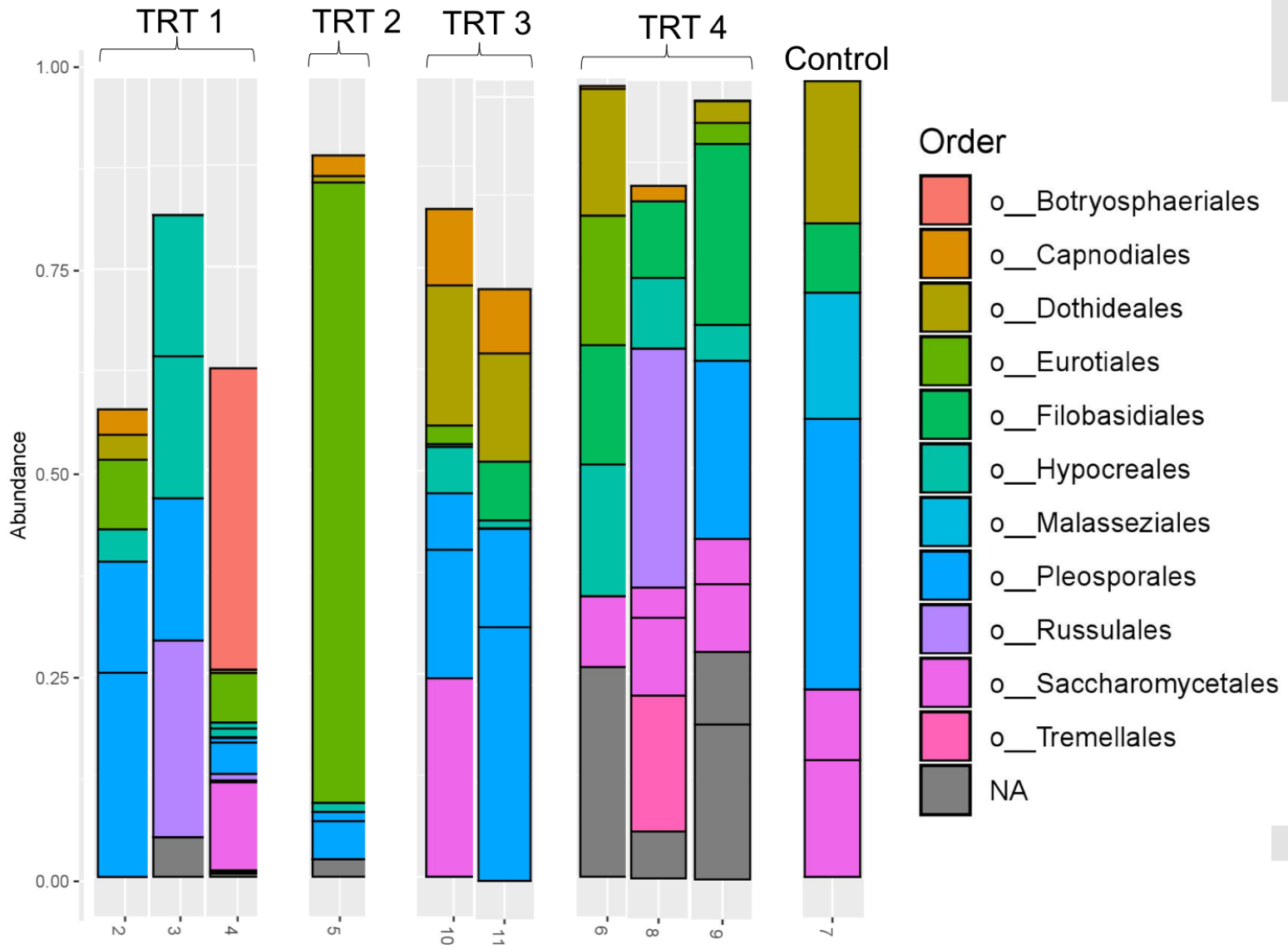


Student t's, p = 0.05

- Yeasts 1 and 3 made an impact on Norton
  - Larger and more berries
- No *significant* impacts on Chardonel, but impacts nonetheless
  - Yeast 2 and 4 showed larger and more berries

Relative Distribution of Orders of the 20 most Abundant Taxa

- Similarities within treatments
- Variation between treatments
- Treated vines looks different than the Control



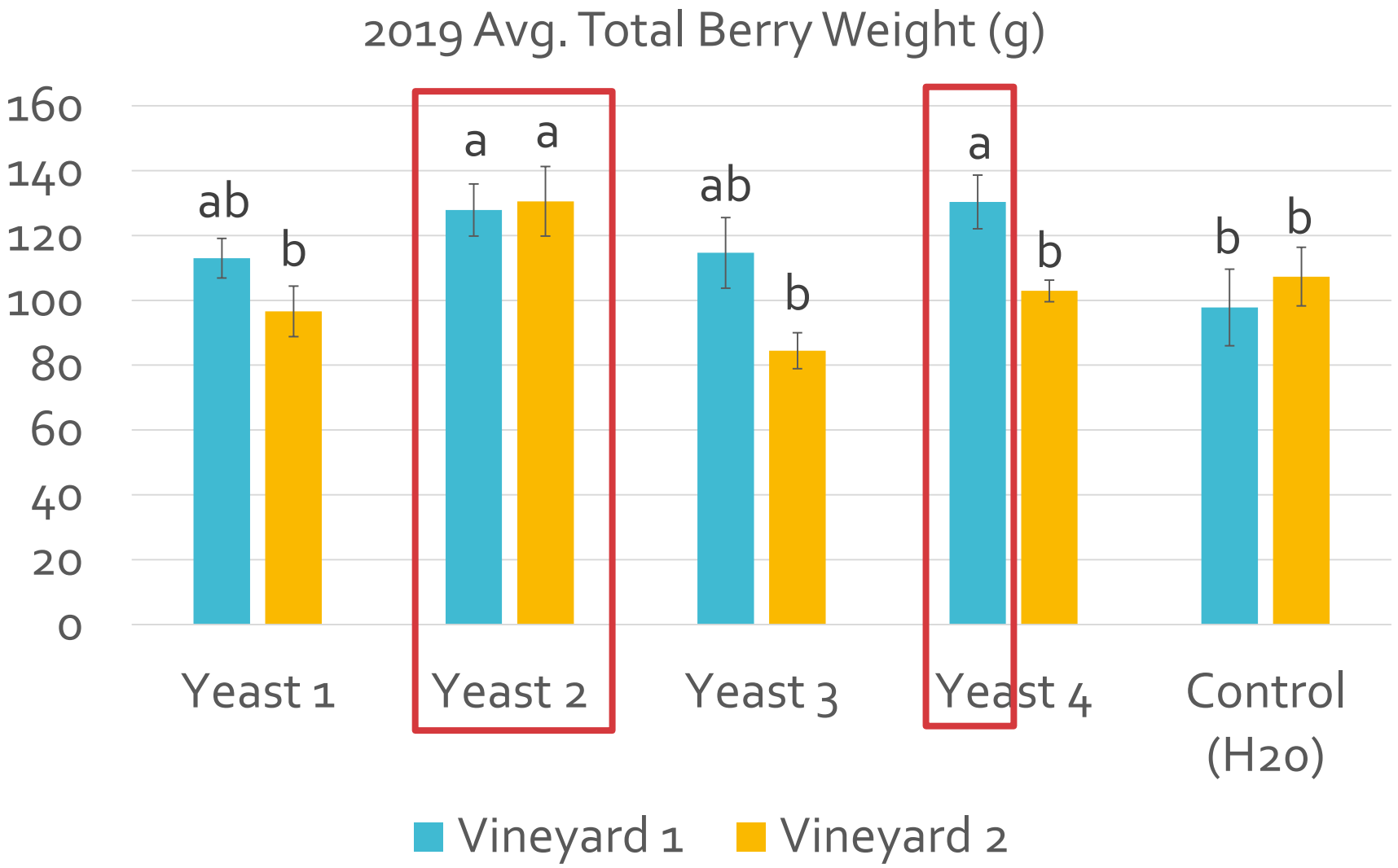
2019: Garnering grower support

- **Missouri Department of Agriculture, Specialty Crop Block Grant:** "Determining the impact of the grape endophytic microbiome on grape physiology." M. E. Hall (PI). Requested years 1 and 2(10/01/19-09/30/21): $38,010. Awarded.

2019: Commercial collaboration

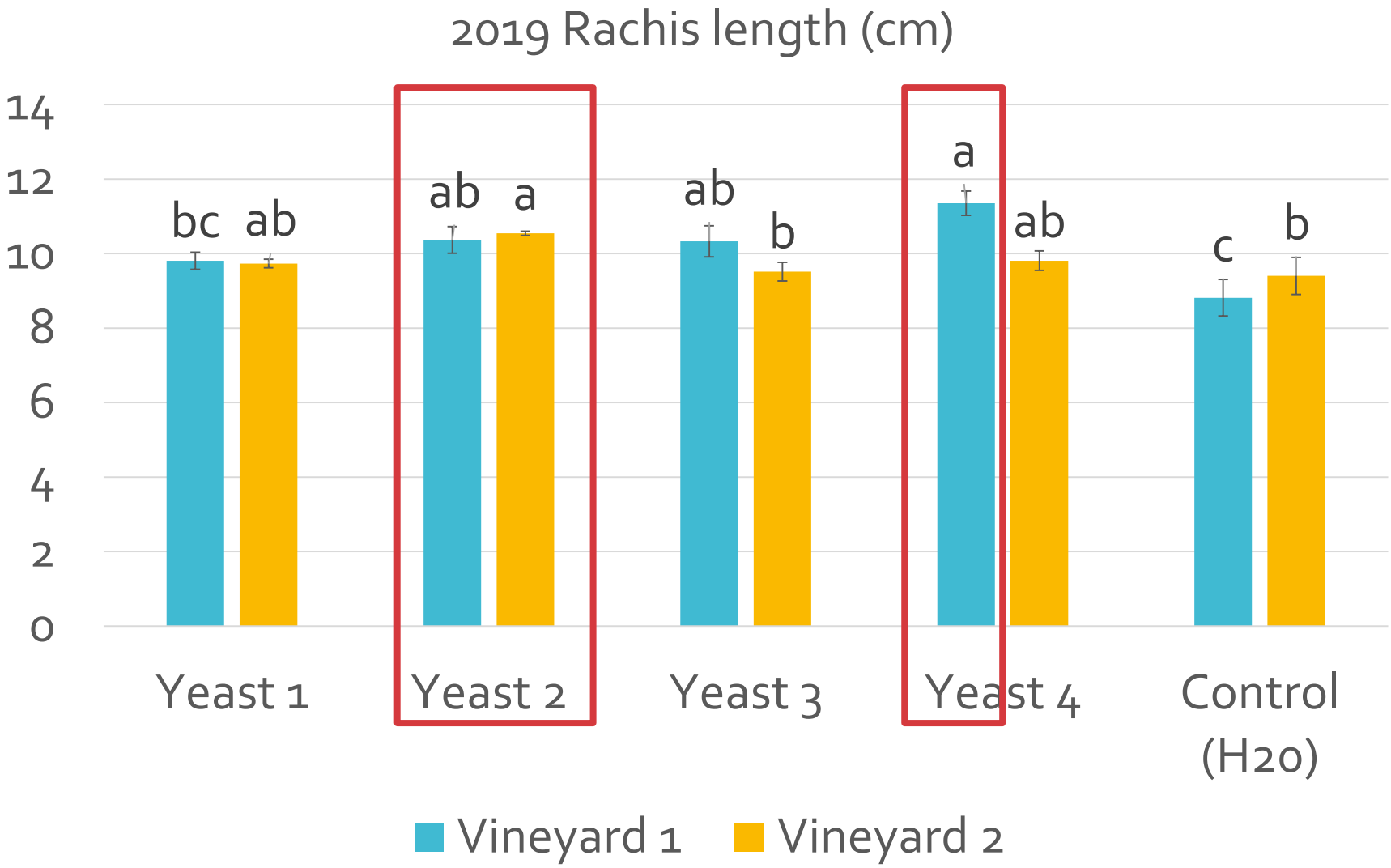
- **2019:** 2 Commercial Vineyards: 5 reps of single vines of Vitis interspecific hybrid cv. Vignoles sprayed at Bloom with 4 different single-species active yeast or water (control)
- 10 clusters harvested from each vine for all 25 vines
- For 15 vines (3 reps), entire vine was harvested
- Cluster weight, berry weight, rachis length, cluster compactness all done at Veraison and Harvest
- Wines made from each treatment using inoculation of commercial yeast

Average Total Berry Weight affected

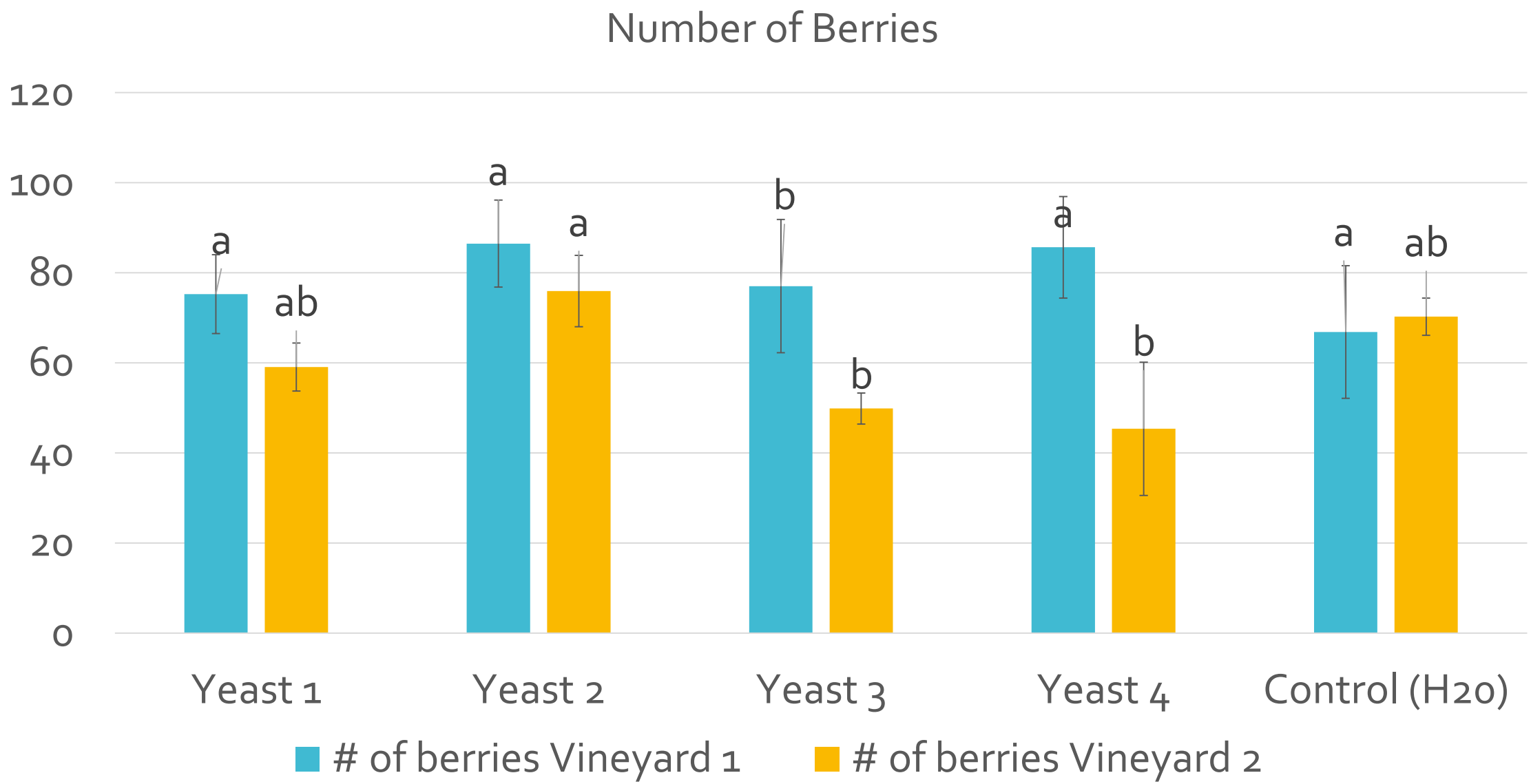


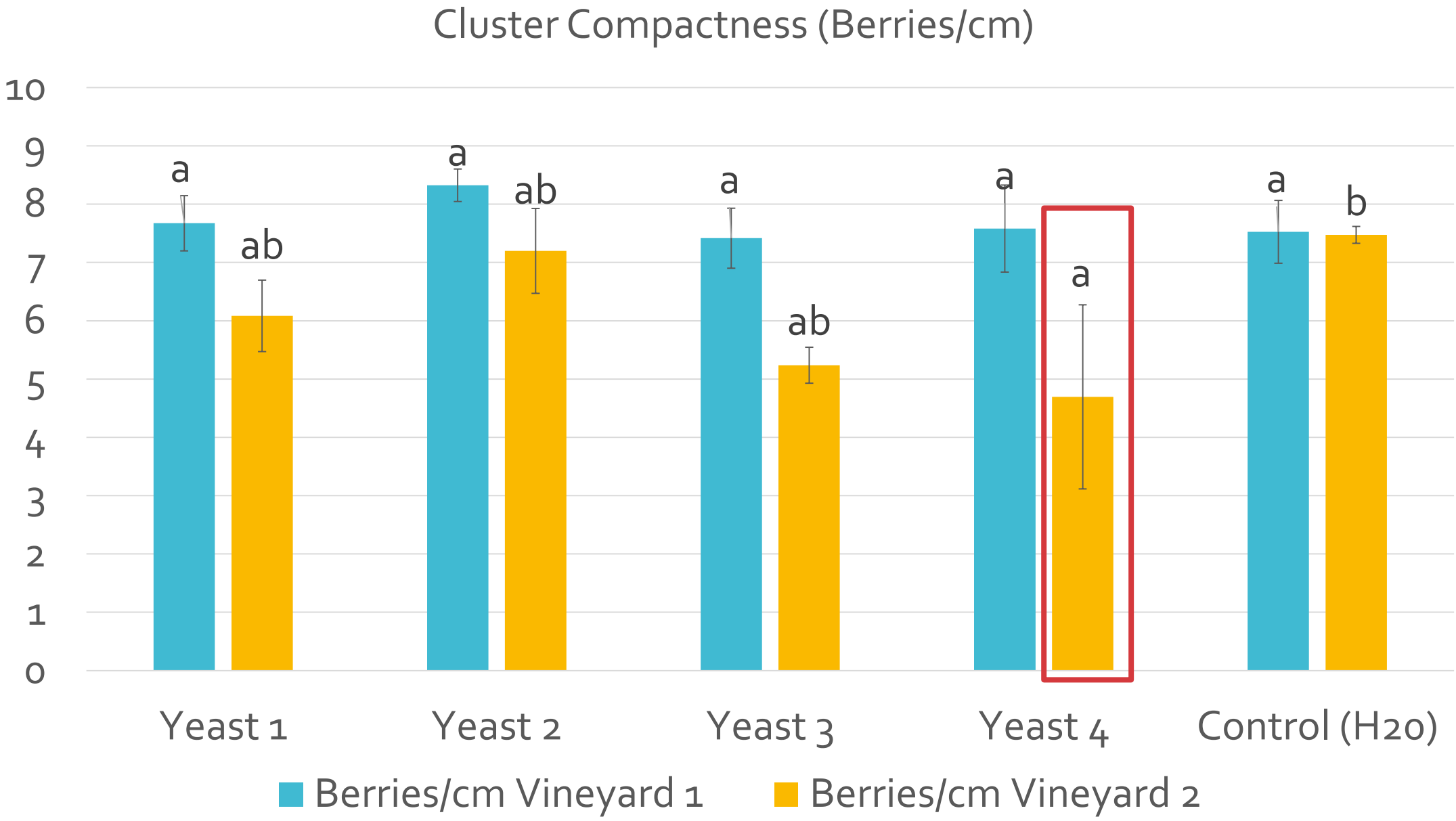
Student t's, p = 0.05

Rachis length affected



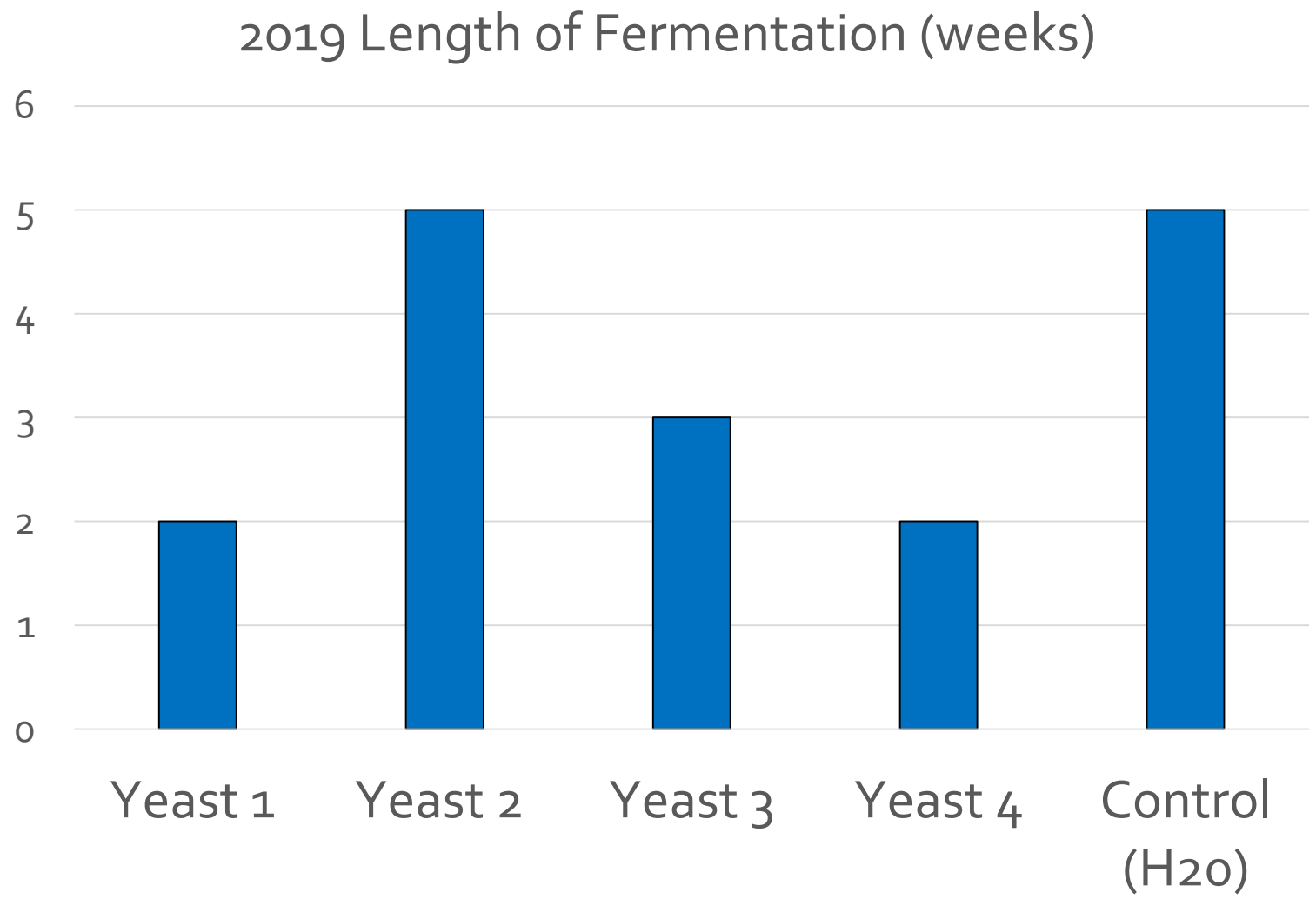
Student t's, p = 0.05


Number of Berries
120
100
80
60
40
20
0
a
ab
a
a
b
b
a
b
a
ab
Yeast 1
Yeast 2
Yeast 3
Yeast 4
Control (H20)
# of berries Vineyard 1
# of berries Vineyard 2


Cluster Compactness (Berries/cm)
10
9
8
7
6
5
4
3
2
1
0
a
ab
a
ab
a
ab
a
a
a
b
Yeast 1
Yeast 2
Yeast 3
Yeast 4
Control (H20)
Berries/cm Vineyard 1
Berries/cm Vineyard 2

- Yeasts 2 and 4 significantly affected the Vignoles
  - Larger berries, longer clusters, and more berries
- Yeast 4 reduced cluster compactness in Vineyard 2
- Consistent with the Chardonel results

Fermentation time affected



No error bars because all samples finished on the same days in each treatment

Ruling out Nitrogen addition

- Yeast suspensions of each species, let them sit for 8 hours
- Sent for YAN analysis at Iowa State's Midwest Grape and Wine Industry Institute
  - Free Amino Nitrogen (for yeast growth)
  - Ammonium (Nitrogen available for plant growth)

FAN was low but present (between 5 – 37 mg/L)

*in wines, a healthy fermentation has >150 mg/L

No Ammonia/Ammonium

We weren't just spraying Nitrogen on the vines

So what?

- Spraying the yeast did something substantial!
  - Growers are engaged and interested in more research
- But what did it do?
  - Move on to the more complicated
    - Hormonal shift?
    - Affecting gene expression?

Future Research Avenues

- Manipulating the microbiome with a specific goal in mind
- Using the microbiome to reduce pesticide applications
- Speeding up the process of microbiome data collection

Manipulating the microbiome with a goal in mind

- Can we encourage a microbial shift through management practices?
  - Cover crops
  - Sunlight exposure
  - Source-sink relationships
  - Livestock or silvopasture
- Or does the microbiome have to affected through directly applications?
- Can we use the microbiome to cut down on pesticide applications?

Using the microbiome to reduce pesticide applications

- No one wants to spray
- Can we get the microbiome to work to our advantage?

Using the microbiome to reduce pesticide applications

- "Bolstering" the microbiome
- Will an abundance of certain microbes prevent infection by certain pathogens?
- Insects deterred by some plant and microbial volatiles
  - Using this to our advantage

Thank you!

Questions?

Missouri Grape Growers

Missouri Wine and Grape Board

My lab:
Dr. Zhiwei Fang
Patrick Kenney
Emily Serra

Dr. Misha Kwasniewski

Dr. Wayne Wilcox
Judy Burr

Isabelle O'Bryon

Dr. Kathy Evans (UTAS)

UM Informatics Core

UM DNA Core

Tasmania Grape Growers

Washington Grape Growers

California Grape Growers

Finger Lakes Grape Growers

MISSOURI WINES™

Mizzou
University of Missouri