



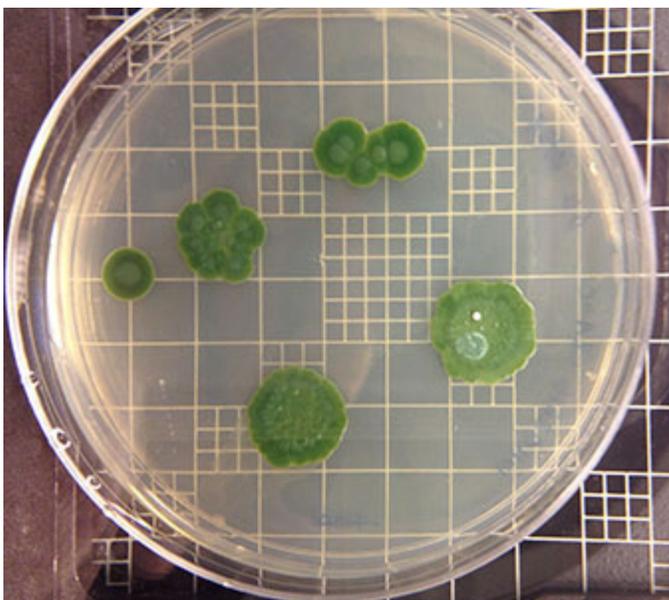
RESEARCH FOCUS

The Wild, Wild Yeast: An Ecological Survey of Yeast Species and Strains in Finger Lakes Riesling

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Introduction. Wine quality and sensory characteristics are influenced by the number and type of yeast species that participate in fermentation. In uninoculated, ‘spontaneous’ fermentations, active yeast strains may originate in the vineyard or on winery equipment. This work is intended as an initial ecological survey of yeast species diversity on Riesling grapes sourced from different Finger Lakes vineyards, and is the first study to investigate the source of *S. cerevisiae* strains in uninoculated Riesling fermentations. Results suggest that both the number of yeast species and the strain diversity within the *S. cerevisiae* species varies by vintage and vineyard location. Ongoing work will continue to chart commonly observed *S. cerevisiae* strains over additional vintages and locations.



Example of a fermentation sample diluted, plated, and allowed to incubate for 10 days. Three unique looking colonies can be seen in this sample.

Photo by Marie Guido-Miner

KEY CONCEPTS

- The yeast species isolated from grape samples differed among vintages and vineyards.
- Yeast species observed on equipment differed between vintages and types of equipment.
- The yeast species observed in the fermentation samples differed between vintages, and between and within the vineyard where the grapes were sourced.
- Non-*Saccharomyces* yeast are more prevalent early in the fermentation but persist throughout the fermentation.
- Over 100 *S. cerevisiae* strains were observed, a majority of which did not match commercially available strains. The strains observed in the fermentation samples differed between vintages, and among the vineyards where the grapes were sourced.
- Yeast strains observed in the fermentation samples did not line up exactly with those observed in the corresponding grape or equipment samples – indicating that the neither the grapes nor the equipment are the only sources of yeast species.
- Different yeast species and *S. cerevisiae* strains were observed in fermentations processed at the same winery with grapes from different vineyards - indicating that the winery was not the sole source of yeast.

The rise of uninoculated fermentations. Spontaneous fermentations, or those performed without the addition of a commercial yeast inoculum, have shown a resurgence in popularity recently, partially in response to consumers' desire for terroir-driven, non-interventionist 'natural wines'. The choice to eschew inoculation is also driven by research showing that uninoculated wines may be perceived as having greater sensory complexity and higher quality. Increased sensory complexity is likely due to greater diversity of yeast species and *S. cerevisiae* strains present during spontaneous fermentation.

Unfortunately, uninoculated fermentations also carry higher risks of problems – such as stuck or sluggish fermentations – compared to fermentations that are inoculated with reliable, tested commercial yeasts. This ecological survey of single-vineyard, uninoculated Riesling fermentations is the first step in identifying yeast species present in the Finger Lakes, with the potential of identifying *S. cerevisiae* strains unique to the region.

Grapes. A total of seven different yeast species were observed on grape samples. The majority of the yeast colonies from the grape samples were identified as *Hanseniaspora uvarum*, commonly present on many types of fruit. *S. cerevisiae*, which is rarely found on undamaged grapes, was not isolated from any samples. The number and type of yeast species present varied from vintage 2015 to 2016. Differences in the yeast species were also observed on grapes from different vineyards, although they were not as pronounced as the differences observed between vintages. Overall, this suggests that a single vineyard may not have a stable set of yeast species that persist from year to year.

Equipment. Sixteen different yeast species were observed on winery equipment swab samples. No single species was dominant in the colonies identified on winery equipment, though *H. uvarum* was widely observed. Several different types of equipment were swabbed each year, and no apparent correlation between type of equipment

and yeast species was observed. Similarly, different yeast species were observed in each vintage and at each winery. *S. cerevisiae* was only observed on two pieces of equipment at two different wineries, both in 2016. In one case, the *S. cerevisiae* strain observed on the equipment was also found in the fermentation. In the other case, the strains observed on the equipment did not match any found in the resulting fermentations.

The variations observed at each winery on different pieces of equipment and across years suggests that no stable set of yeast species persisted year to year at any single winery participating in the study.

Fermentations. Each vineyard-designated Riesling was fermented separately each year, resulting in a total of 10 fermentations. Fourteen different yeast species were observed among these fermentations, most of which were *S. cerevisiae*, which tends to outcompete other yeast. More yeast species, and a higher percentage of non-*Saccharomyces* yeasts, were observed during the 2015 vintage.

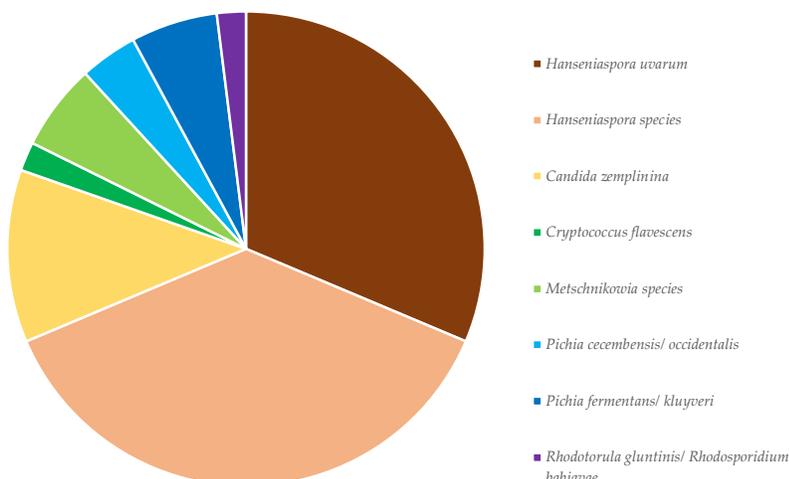
Though a different set of yeast species were identified in each fermentation, a *S. cerevisiae* was present in all, and *H. uvarum* in most. There was some overlap in fermentation yeast species when grapes from the same vineyard were fermented in different years, but it was far from complete. Further study is required to determine which yeasts, if any, persist over time, and if they contribute unique flavor properties of wines produced from a particular vineyard. Samples taken in subregions of one vineyard suggest that yeast species are unevenly distributed throughout a vineyard, and that different lots of grapes may be exposed to different yeast species while traveling through the winery prior to fermentation. In general, this means that each transport load of grapes may carry different yeast species, even if all the grapes come from the same vineyard.

In addition to these vintage and vineyard variations, observed yeast species also varied throughout fermentation. As expected, non-*Saccharomyces* yeast dominate during the first week of fermentation, after which a majority of

Sampling Methods

Grapes, winery equipment, and fermentations associated with uninoculated, single-vineyard Riesling wines were sampled during the 2015 and 2016 vintages at several wineries. A representative sample of individual grape berries was collected from the entire vineyard used for the uninoculated Riesling wine. The grapes were collected about a week before harvest and hand crushed in a bag. Select winery equipment was swabbed during harvest, after the equipment had been cleaned/sanitized. The winery equipment swabs were placed into a minimal growth media. The fermentations were sampled from pressing until the fermentations had less than 5g/L of sugar remaining. All samples were diluted and plated onto three different media types – one that favored yeast growth, one that favored bacterial growth, and one that favored non-*Saccharomyces* yeast growth. The plates were incubated at room temperature for at least 10 days and resulting colonies were separated by how they looked. Each unique looking colony was isolated and subjected to DNA analysis for yeast species and *S. cerevisiae* strain identification. This method is very good for obtaining detailed yeast species and strain information but not as accurate at estimating the total abundance of each yeast species or strain in a sample. All results refer to the species or strain identity of the isolated colonies, not their relative abundance in the sample.

A. Microbes Observed - All Grapes



B. Microbes Observed - All Fermentations

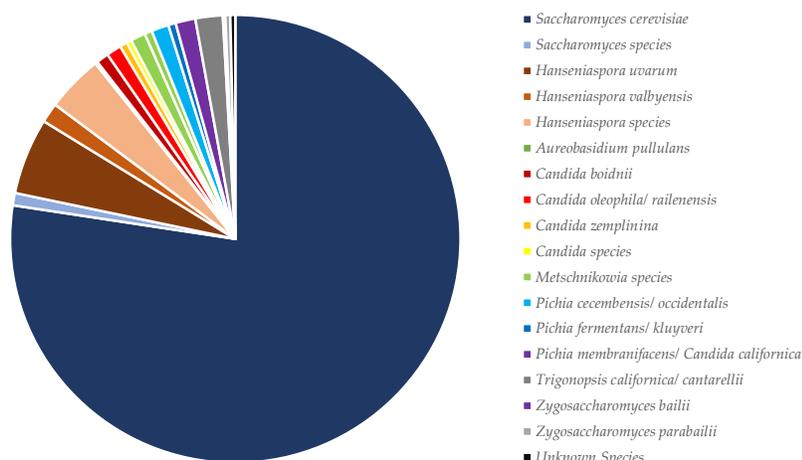


Figure 1. Percentage of colonies identified as yeast species. (A) Yeast species observed in grape samples, 68% of colonies identified were *Hanseniiaspora* spp. (B) Yeast species observed in fermentations, 77% of colonies identified were *S. cerevisiae*. Data is combined from 2015 and 2016.

the colonies were identified as *S. cerevisiae*. However, non-*Saccharomyces* yeast were observed throughout the fermentation, showing up as late as four weeks after fermentation started. In these fermentations, *S. cerevisiae* did not completely outcompete all other yeast species during fermentation.

Comparison of grapes and equipment to fermentations.

There was some overlap in the yeast species observed in the grapes and on the winery equipment and in the corresponding fermentations. However, not all yeast species observed in the fermentations were observed in either the corresponding grapes or winery equipment. There are several possible explanations for this discrepancy, including but not limited to: the lack of sensitivity of the plating method to small abundances of yeasts, yeast being introduced by insect or human vectors, and yeast being outcompeted between sampling time points. Further re-

search is needed to determine the cause of these disparities and the ultimate source of the yeast species observed in the fermentations. Regardless of the reason, it was clear that, in this work, neither the vineyard or the winery was the sole source of yeast in the tracked fermentations.

***S. cerevisiae* strains.** A majority of the colonies identified as *S. cerevisiae* did not match any commercially available strains in the comparison database, and were tentatively presumed to be wild strains. Over 100 unique wild strains were identified, and a majority of these wild strains were observed only a few times, but several were seen multiple times over different vintages, wineries, or individual fermentations.

An additional 20 different strains were identified as genetically similar to existing commercial strains, and these were more likely than the wild strains to be seen multiple times, generally within the same fermentation. The total number of strains observed in 2015 was about the same as 2016, though different strains were observed in each vintage. Each fermentation had a unique set of strains. As with yeast species, there was some overlap in the *S. cerevisiae* strains observed in annual fermentations using grapes from the same vineyard. There were some trends in when and how often *S. cerevisiae* strains were seen over time during the fermentations.

Generally speaking, a majority of the fermentations did not have a single dominant strain, and when a dominant strain



Vineyard overlooking Seneca Lake.

Photo by Tim Martinson

Microbes Observed by Fermentation Stage

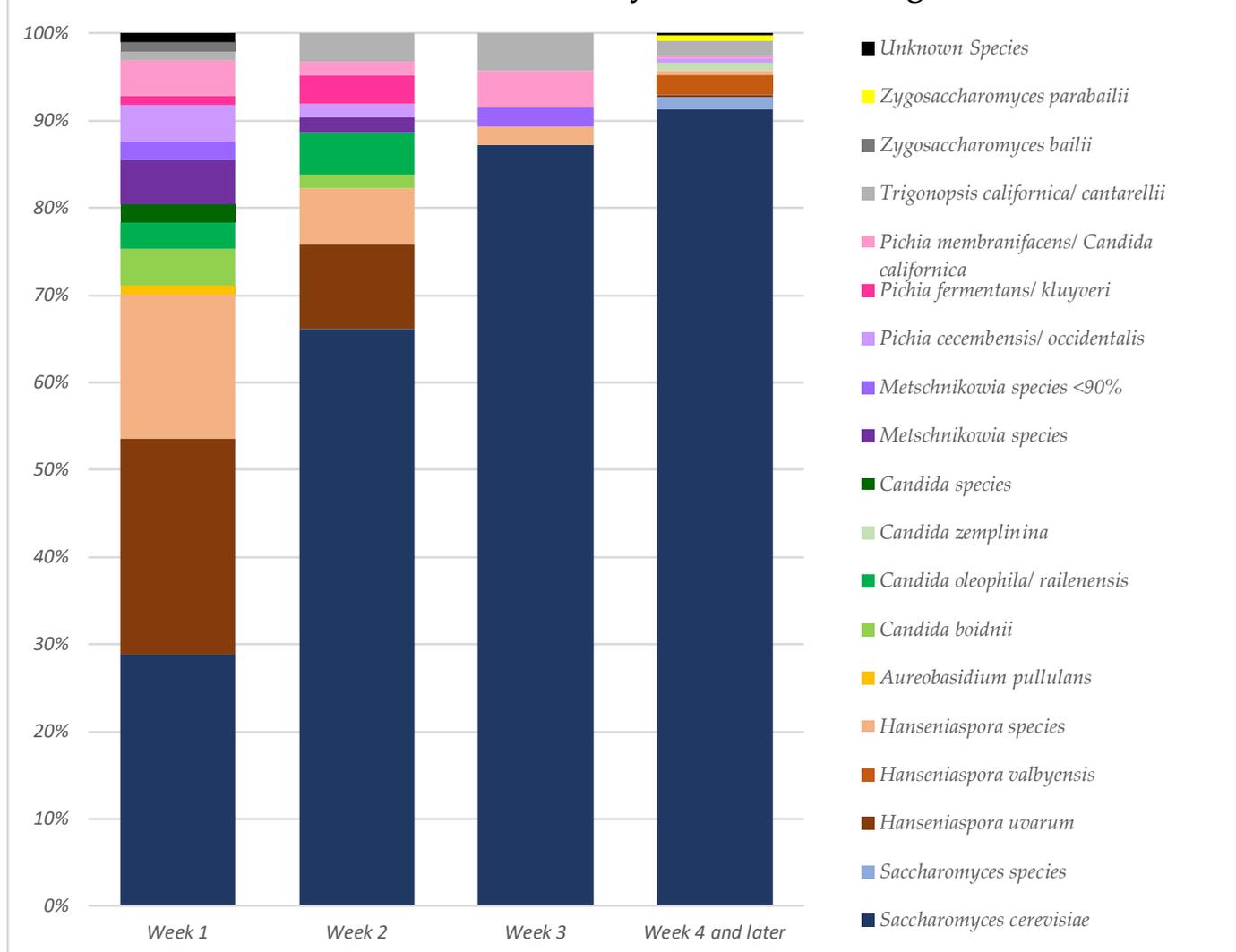


Figure 2. Yeast species observed during the course of fermentation by fermentation stage. Over 90% of colonies observed after week 4 were *S. cerevisiae*. Data is combined from 2015 and 2016.

did exist, it was likely to be similar to a commercial strain and increase in frequency of observation over time. In all fermentations with a dominant yeast strain, however, it was not the only strain observed, suggesting some level of diversity persisted through the end of fermentation.

Practical applications. What we know is that vintage matters: differences in both yeast species and *S. cerevisiae* strains were observed over the two vintages. Geography also matters: differences between grapes from different vineyards, and fermentations were observed. Even small differences in fermentations – such as having fruit from a different part of a single vineyard – can lead to differences in the observed yeast species and *S. cerevisiae* strains.

S. cerevisiae dominates fermentations over time, but does not completely exclude all non-*Saccharomyces* yeast even after four weeks of fermentation. Fermentations are not always dominated by a single *S. cerevisiae* strain. A majority of the *S. cerevisiae* strains observed do not match commercial strains.

Further research. Covering a small area of the Finger Lakes for only two years, this research only provides a starting place for further work. While it is possible to conclude that uninoculated fermentations include yeast species and strains from various sources, additional work is necessary to understand the contribution of vineyard, winery, and other vectors. The link between the observed



Riesling berries.

Photo by Tim Martinson

yeast and the wine flavor characteristics also warrants further exploration. These initial conclusions show that there are differences between vineyards, wineries, and vintages, paving the way for broader investigation. By examining the commonly observed *S. cerevisiae* wild strains, further work may establish these as viable for commercial release.

Acknowledgements. Undergraduate research assistants Patrick Commane, Andrea Torzala, Claire Riedman, Grace Engels, Justin Choi, and Rafia Rifa were essential in completing the plating and colony categorizing of the samples. Dr. Jenny Kao-Kniffen assisted in the yeast species identification procedure and generously allowed the use of her lab space. Dr. Richard DeScenzo of ETS Laboratories was essential to the *S. cerevisiae* strain analysis as he developed the DNA analysis technique and the database of commercial yeast strains. The authors gratefully acknowledge the funding provided by the New York State Agricultural Experiment Station, Canandaigua Wine Company, Dyson and Lacroute Funds.

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