Strain-specific effects of the herbicide RoundUp™ on diverse natural isolates of *Saccharomyces cerevisiae*

by

Savannah Taggard

A THESIS

submitted to

Oregon State University

Honors College

in partial fulfillment of
the requirements for the
degree of

Honors Baccalaureate of Science in Biology
(Honors Scholar)

Presented May 28, 2021
Commencement June 2022
AN ABSTRACT OF THE THESIS OF

Savannah Taggard for the degree of Honors Baccalaureate of Science in Biology presented on May 28, 2021. Title: Strain-specific effects of the herbicide RoundUp™ on diverse natural isolates of Saccharomyces cerevisiae.

Abstract approved:_____________________________________________________

Molly Burke

The safety of the chemical glyphosate, the active ingredient in the herbicide RoundUp™, has been questioned on numerous occasions. Previous studies suggest that glyphosate-containing herbicides have deleterious effects on a variety of organisms; other studies suggest that these effects may be minor, transient, or non-existent altogether. Proper maintenance of microbial soil communities is imperative to agricultural success; yeasts can play an important role in these communities. Studies involving the brewer's yeast Saccharomyces cerevisiae have the ability to model eukaryotes, microbes, genetics, systems-level genomics, as well as to inspect yeasts themselves. Therefore, studying the effects of RoundUp™ on S. cerevisiae may provide direct insight into the potential effects of RoundUp™ on soil yeast health, and indirect insight into the effects of RoundUp™ on non-target organisms including other soil microbes and eukaryotes. Here, we characterize the effects of RoundUp™ on 12 genetically diverse isolates of S. cerevisiae. Growth assays in media with and without RoundUp™ indicate that the herbicide inhibits growth in all strains, but the magnitude of this inhibition is variable among strains. Our results suggest that the genetic basis for RoundUp™ resistance is strain-specific in S. cerevisiae, inviting further study into genetic variants that may be useful in applied contexts.

Key Words: RoundUp™, glyphosate, Saccharomyces cerevisiae

Corresponding e-mail address: taggards@oregonstate.edu
Strain-specific effects of the herbicide RoundUp™ on diverse natural isolates of *Saccharomyces cerevisiae*

by
Savannah Taggard

A THESIS

submitted to

Oregon State University
Honors College

in partial fulfillment of
the requirements for the
degree of

Honors Baccalaureate of Science in Biology
(Honors Scholar)

Presented May 28, 2021
Commencement June 2022
Honors Baccalaureate of Science in Biology project of Savannah Taggard presented on May 28, 2021.

APPROVED:

_____________________________________________________________________
Molly Burke, Mentor, representing Department of Integrative Biology

_____________________________________________________________________
Mark Phillips, Committee Member, representing Department of Integrative Biology

_____________________________________________________________________
Kimberly Halsey, Committee Member, representing Department of Microbiology

_____________________________________________________________________
Toni Doolen, Dean, Oregon State University Honors College

I understand that my project will become part of the permanent collection of Oregon State University, Honors College. My signature below authorizes release of my project to any reader upon request.

_____________________________________________________________________
Savannah Taggard, Author
I. Introduction

Glyphosate, the active ingredient in RoundUp™, is by far the most commonly used herbicide worldwide (Benbrook, 2016). Still, the safety of this chemical has been called into question on numerous occasions, and it has been labelled as a carcinogen by the state of California (OEHHA, 2017; Cohen, 2020). Since the introduction of RoundUp™-resistant crops in the 1990s, glyphosate use has increased dramatically, to nearly 300 million pounds per year in the US (Benbrook, 2016). The manufacturer of RoundUp™, Monsanto, asserts that the herbicide does not pose a health risk to non-target (i.e., non-plant) organisms, including humans (Bayer, 2021). In fact, RoundUp™ is often considered by farmers to be a benign alternative to harsher anti-weed practices of the past (Cohen, 2019). Glyphosate was found to be less acutely toxic to rats than 94% of herbicides found in the USDA-NASS data set (Kniss, 2017). Nevertheless, studies have repeatedly indicated harmful effects of RoundUp™ on a variety of non-target organisms, including honey bees, earthworms, frogs, rats, and numerous microbes (reviewed by Gill et al., 2018).

In order to fully access soil-borne nutrients, plants are dependent on the metabolic activities of the soil microbiome (Jacoby et al., 2017). Therefore, proper maintenance of microbial soil communities is imperative to successful, sustainable agricultural practices. Many studies indicate that glyphosate-containing herbicides have only minor, transient effects on microbial soil communities (Weaver et al., 2007; Dennis et al., 2018; Hagner et al., 2019). However, RoundUp™ has been shown to affect the composition of certain microbial soil communities; in the bacterial communities associated with the roots of RoundUp Ready® soybeans, glyphosate increased the abundance of Gemmimonas bacteria, decreased the abundance of Burkholderia bacteria, and increased overall bacterial diversity with unknown
impacts on plant and soil health (Arango et al., 2014). Within soil microbiomes, fungi are important to the maintenance of agricultural soil health through decomposition, nutrient delivery, and plant protection against pathogenic microorganisms (Frąc et al., 2018); RoundUp™ has been shown to be lethal to the soil filamentous fungus Aspergillus nidulans at 100 times less than the recommended application concentration (Nicolas et al., 2016). In particular, soil yeasts have been associated with decomposition (Mestre et al., 2011), plant and microbial growth, nitrogen and sulfur cycles, and maintenance of soil structure (reviewed by Botha, 2011). The yeast S. cerevisiae is a popular and useful organism for basic research, used to model biological processes in eukaryotes (including human health-related studies), especially those pertaining to genetics and systems biology (Botstein et al., 1997; Siggers & Lesser, 2008; Botstein & Fink, 2011). Therefore, studying the effects of RoundUp™ on S. cerevisiae may provide direct insight into the potential effects of RoundUp™ on soil yeast health, and indirect insight into the effects of RoundUp™ on non-target organisms.

In target organisms (plants), glyphosate acts as an herbicide by disrupting the shikimate pathway via inhibition of the 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS) enzyme. This pathway is involved in the synthesis of aromatic amino acids, including tryptophan, tyrosine, and phenylalanine (Powles and Preston, 2006; Ravishankar et al., 2020). However, the shikimate pathway is also present in many non-target organisms (Herrmann and Weaver, 1999), including S. cerevisiae (Averesch and Krömer, 2018). In S. cerevisiae, resistance to glyphosate-containing herbicides is a polygenic trait that varies notably between strains (Ravishankar et al., 2020). Strains of S. cerevisiae are highly genetically and phenotypically divergent, and phenotypic variance between strains correlates with genome-wide phylogenetic relationships (Liti et al., 2009) and is tied to population history (Warringer et al., 2011).
Previously, six genetically distinct *S. cerevisiae* strains (AWRI1631, RM11, YJM789, S288c, YPS128, UWOPS05_127_3) have been explored regarding resistance to RoundUp™ and glyphosate-containing herbicides (Rong-Mullins et al., 2017; Ravishankar et al., 2020). These studies observed marked variation in responses to RoundUp™, including 10-fold increases in glyphosate concentration required to produce similar levels of growth inhibition in separate strains. Because genotype and phenotype are closely intertwined in *S. cerevisiae*, studying RoundUp™ responses in a number of yeast strains may help to elucidate genetic variants underlying RoundUp™ sensitivity and resistance. Especially helpful in this type of work is a characterization of genotype-by-environment interactions; “Genotype-by-environment interactions consist of differences among individuals, encoded in the genome, in the way the environment influences the phenotype” (Herron & Freeman, 2014). This study seeks to characterize RoundUp™ resistance in and genotype-by-environment interactions among the *S. cerevisiae* strains listed in Table 1; these strains constitute a variety of geographical and contextual origins (Linder et al. 2020), and distinct phylogenetic relationships (Figure 1). While two of these strains (YPS128 and UWOPS05_127_3) have been evaluated for glyphosate resistance previously, the other 10 strains of Table 1 have responses to glyphosate and/or RoundUp™ that are unknown. Unlike previous studies, we seek to report responses RoundUp™ both quantitatively and relative to other strains in the study. By quantifying and ranking RoundUp™ responses in these strains, we can gain insight into the genotypes and phenotypes that influence RoundUp™ resistance. Growth assays of each strain in rich media and RoundUp™-containing media show that RoundUp™ inhibits growth in all 12 strains, and that the magnitude of this growth inhibition varies across the strains. We also seek to characterize RoundUp™ response via flocculation, a general, communal stress response in *S. cerevisiae* that
involves visible clumping. Flocculation assays of each strain in rich media and RoundUp™-containing media demonstrate that RoundUp™ induces flocculation in almost all strains, and that the magnitude of this induction varies across the strains.
II. Methods

i. Yeast cultures

The yeast strains used in this experiment are listed in Table 1. These strains were originally acquired from Dr. Anthony D. Long (UC Irvine) in October 2017 and stored in 25% glycerol for long-term storage at -80°C. In November 2020, frozen archives of each strain were thawed and used to streak for single colonies. Using a wooden applicator, the archives of each strain were streaked onto 2% agar plates containing rich media, or YPD (1% yeast extract, 2% peptone, 2% dextrose) in increasing dilution factors in sections around each plate. These plates were incubated at 30°C for 48 hours, before being sealed with Parafilm and stored at 4°C. These ‘strain plates’ were refreshed every ~4 weeks by sampling one colony from the existing strain plate, inoculating cultures in 10 mL YPD that were incubated for 12-24 hours at 30°C with constant shaking at 200 rpm, and repeating the process of streaking for single colonies on YPD plates.

ii. Assays of population growth

Each of the 12 strains was sampled and assayed for growth in two conditions: rich YPD media, as well as YPD supplemented with 1% of a commercial formulation of RoundUp™. RoundUp™-containing media was produced by adding 1% RoundUp™ Ready-to-Use Weed and Grass Killer III (2.0% glyphosate, isopropylamine salt; 2.0% pelargonic acid and related fatty acids; 96% other ingredients) to YPD by volume, and was sterilized by filtration using 0.2 μm pore polyethersulfone filter units. Standard YPD media was produced using steam sterilization. Each strain was measured in triplicate by sampling three distinct colonies from the respective strain plate to inoculate three overnight cultures (one colony per culture) of 10 mL YPD (Figure 2A). These cultures constitute three biological replicates, capturing possible variation within
strains and increasing statistical power. The cultures were incubated overnight at 30°C with shaking at 200 rpm. The following morning, to estimate the density of yeast cell suspensions, a 1/100 dilution of each suspension was measured for absorbance at 600 nm (OD$_{600}$) using an Eppendorf Biophotometer D30 (Figure 2B). These readings were used to standardize the cultures to a specific OD$_{600}$ in liquid media to a total volume of 3000 μl (Figure 2C). For the YPD (control) treatment assays, a target OD$_{600}$ of 0.05 was used for standardization, and the cultures were diluted in YPD. For the RoundUp™ treatment assays, a target OD$_{600}$ of 0.06 was used, and the cultures were diluted in YPD with 1% RoundUp™ by volume. An increased target OD$_{600}$ for the RoundUp™ assays was chosen to ensure measurable growth under the more stressful condition. For each dilute culture, 10-30 μl of overnight culture was diluted in 2970-2990 μl YPD; due to the small volumes of culture used, any dilution of the RoundUp™ in solution is negligible. These cultures were then used to fill a 96-well plate, with two duplicate wells (technical replicates) per biological replicate (Figure 2D). The arrangement of strains on the plate was carried out in multiple orientations in an attempt to control for possible “edge effects,” in which technical replicates in wells at the edge of the plate may exhibit different growth patterns compared to replicates in central wells. The plate reader assay was carried out in a Tecan Spark Multimode Microplate Reader, set to record the absorbance at 600 nm for each well every 30 minutes for 48 hours at 30°C, without plate agitation/aeration.

iii. Flocculation assays

After each growth assay, a photo was taken of the 96-well plate, capturing the flocculation patterns of each technical replicate. These images were adjusted for color and contrast using the photo editing software ImageJ (imagej.nih.gov/ij/) version 1.53e (Schneider,
Each strain was assigned a ‘flocculation score’, where 1 indicates little to no visible flocculation, and 5 indicates extreme flocculation.

iv. **Statistical analysis**

All statistical tests were performed with R (www.r-project.org) version 4.0.3 (R Core Team, 2020). The raw data from each plate reader run were organized into matrices and processed by row using the R-package “matrixStats”. The R-package “Growthcurver” (Sprouffske and Wagner, 2016) was used to estimate population growth parameters from the raw data. In order to determine the carrying capacity and doubling time of each technical replicate for each plate reader assay, the absorbance measurements taken during the assay were fit to Equation 1:

$$N_t = \frac{N_0 K}{N_0 + (K-N_0)e^{-rt}}$$

Where $N_t$ is the absorbance reading at time $t$, $N_0$ is the initial absorbance, $K$ is the carrying capacity, and $r$ is the growth rate, or doubling time. Here, doubling time is synonymous with generation time, or the time necessary for the size of a population to double under non-restricted conditions, while carrying capacity is the maximum population size under the given conditions (YPD or 1% RoundUp™). Population size is estimated via OD$_{600}$. For subsequent analysis, the values for each biological replicate were determined by averaging across technical replicates, and the values for each strain were determined by averaging across biological replicates. Shapiro-Wilk normality tests were performed by strain for each assay to determine if the data met the requirements for parametric tests (n = 3 biological replicates per strain). For the RoundUp™ assays, significant $p$-values (<0.0001) determined that the data were not normally distributed, and that non-parametric tests should be used to determine differences in growth rate.
between strains. Consequently, Kruskall-Wallis tests were performed comparing the 12 strains (n = 3 biological replicates per strain) for each assay. The Kruskall-Wallis test is a nonparametric test of ranks that compares the medians of two or more groups and determines if there is a significant difference between any of the groups. Following a significant Kruskall-Wallis test, an ad-hoc test of multiple comparisons (in this case, we used a Dunn test implemented with the R-package “FSA”) to make pairwise comparisons between strains. Finally, the R-package “rcompanion” was used to produce compact letter displays for the pairwise comparisons developed by the Dunn test. These displays summarize the comparisons by assigning each strain a set of letters; strains that do not share any letter are significantly different (α = 0.05). These letter designators were then translated to shape designators for ease of interpretation and visualization. Figures displaying carrying capacity, doubling time, and comparisons of these parameters were generally made using the R-package “ggplot2”, which provides the resources for quality, aesthetically appealing data visualization. Linear regression was used to assess the reproducibility of the assay results by comparing calculated doubling time by strain for YPD and 1% RoundUp™ assays (two assays per treatment, one regression per treatment).
III. Results

i. Assays of population growth

The growth curves observed for each strain in rich YPD and 1% RoundUp™ are illustrated in Figures 3 and 4. Growth is plotted as the mean log OD$_{600}$ value averaged across biological replicates (n = 3) for each strain, and is plotted against time. Error bars indicate standard deviation. Due to unavoidable variation among technical replicates (resulting from technical limitations such as edge effects), mean OD$_{600}$ of biological replicates were treated as independent measurements of each strain.

Doubling times in YPD and 1% RoundUp™ were determined for each strain (Figures 5 and 6). Kruskall-Wallis rank sum tests indicate a significant effect of strain on growth rate for both the YPD ($p = 0.001184$) and 1% RoundUp™ assays ($p = 0.001787$). For each treatment, less variation was noted within strains than between strains. For the YPD assay, average within-strain standard deviation was 0.06131 hr, while between-strain standard deviation was 0.1264 hr. For the 1% RoundUp™ assay, average within-strain standard deviation was 0.5984 hr, while between-strain standard deviation was 2.3239 hr.

Post hoc Dunn tests determined the ranking and grouping of strains based on doubling time (n = 3, $\alpha = 0.05$, Tables 2 and 3). Strains that share a shape designator belong to the same group, while strains that do not share a shape are significantly different based on doubling time. For growth in YPD, three distinct groups of strains were determined, as defined by strains that share the circle, square, and diamond shape designators. The circle group includes 9 strains with slower growth, the square group includes 10 strains with intermediate growth, and the diamond group includes 10 strains with faster growth. Strains UWOPS05_217_3 (K), YPS128 (G), and L_1528 (J) were the most quickly growing strains, and DBVPG6044 (B) and YJM981 (L) were
the most slowly growing strains (Table 2). For growth in 1% RoundUp™, three distinct groups were also determined as defined by the circle, square, and diamond shape designators. The circle group includes 9 strains with slower growth, the square group includes 10 strains with intermediate growth, and the diamond group includes 10 strains with faster growth. Strains L_1528 (J), UWOPS05_217_3 (K), L_1374 (E), and DBVP6044 (B) were the most quickly growing strains, and Y12 (H) and SK1 (D) were the most slowly growing strains (Table 3). Doubling time was increased for each strain under 1% RoundUp™ compared to YPD, although to differing extents (Figure 7).

Linear regression analysis comparing duplicate growth assays under YPD and 1% RoundUp™ produced significant regression models (YPD: adjusted \(R^2 = 0.9164, p = 6.463e-07\); 1% RoundUp™: adjusted \(R^2 = 0.6095, p = 0.002777\)). Here, significant \(p\)-values indicate that the doubling time values calculated in one assay are correlated with the doubling time values calculated in the duplicate assay; in other words, the results from the growth assays under each treatment are consistent and reproducible.

We noted that the first time point in each growth assay (cf. hour 0 of Figures 3 and 4) appeared to be unusually high compared to the rest of the growth curves. We determined that this irregularity is likely an artifact of the assay preparation methods, perhaps due to bubbles and unsettled samples. In order to establish that our doubling time calculations and strain rankings were accurate in spite of this disturbance, we ran our full statistical analysis with the first time point removed. The changes in doubling time calculations were insignificant: strain rankings and groupings were identical in both analyses. This analysis indicates that the model-fitting methods of the R-package “Growthcurver” are robust to perturbations that may affect doubling time calculations, and that these calculations are likely highly reliable.
ii. Flocculation assays

Strains displayed distinct flocculation patterns under YPD and RoundUp™ conditions, and were assigned a score based on visual flocculation patterns (Table 4). Images of each strain were captured after the 48 hr population growth assays. A score of 1 indicates little to no visible flocculation, while a score of 5 indicates extreme visible flocculation. The change in flocculation between treatments for each strain is shown in Figure 8. All but two strains (SK1, D and L_1528, J) experienced increases in flocculation under RoundUp™ compared to YPD, with strains YJM975 (F) and Y12 (H) experiencing both the highest flocculation scores (5) and the greatest increases in flocculation score (from 1 to 5). All other strains increased in flocculation under RoundUp™ conditions, but to milder extents.
IV. Discussion

We determine that RoundUp™ increases doubling time (decreases growth rate) for all 12 *S. cerevisiae* strains studied. However, the degree to which doubling time increases varies among strains. We also determine that RoundUp™ induces flocculation in 10 of the 12 studied *S. cerevisiae* strains, to varying extents. Similar to our observations of doubling time, the degree to which RoundUp™ induces flocculation is strain-specific. Because RoundUp™ has an effect on the strains, we infer that there is some genetic basis for RoundUp™ resistance in *S. cerevisiae*. If this genetic basis were the same across all strains (i.e., there is no variance in RoundUp™ resistance), we would expect RoundUp™ to have an equal effect on all 12 strains in this study. In other words, we would expect the doubling time strain rankings to be the same between YPD and 1% RoundUp™ treatments, and we would expect flocculation scores to change uniformly across all strains between treatments. Instead, we observed distinct genotype-by-environment interactions, in which different strains responded differentially to each treatment. Because RoundUp™ affected the strains differently, we conclude that certain strains are more RoundUp™-resistant than others, and that there are unique, strain-specific genotypes that confer differing levels of RoundUp™ resistance.

The doubling time rankings we observed for growth in YPD generally did not correspond to the doubling time rankings we observed for growth in RoundUp™ media. While the two wild strains (UWOPS05_217_3, K and YPS128, G) grew most rapidly in YPD, they exhibited intermediate doubling times when growing in RoundUp™. Strain YJM981 (L) was the slowest growing strain in YPD, and an intermediate strain in RoundUp™. Strain L_1528 (J), a quickly growing strain in YPD, was the most quickly growing strain in RoundUp™. The sole laboratory strain (SK1, D), an intermediate strain in YPD, grew the most slowly under RoundUp™.
Otherwise, the distribution of geographical and other contextual origins among the rankings for each treatment appear to be random. Likewise, the phylogeny of these strains (Figure 1) did not reveal obvious large-scale patterns regarding the ranking of strains in each treatment. However, certain closely-related strains, namely L_1374 (E) and L_1528 (J), clustered together in both treatments. Similarly, changes in flocculation score for each strain between treatments also appears to be unrelated to geographical, contextual, or phylogenetic origin.

Interestingly, there also does not appear to be a relationship in RoundUp™ resistance when comparing doubling time and flocculation. Strain SK1 (D) was the slowest growing strain in RoundUp™, and yet did not experience any induction of flocculation. Meanwhile, L_1528 (J) was the fastest growing strain in RoundUp™, and also did not experience induction of flocculation. All other strains experienced an increase in flocculation in RoundUp™ compared to YPD, despite various doubling time responses. In brewer’s yeast, flocculation varies between strains and can serve as a communal stress response, in which cells on the exterior of a ‘floc’ protect the cells inside from some environmental threat (Soares, 2010). Given that RoundUp™-containing media is a stressful environment for yeast, it is probable that the strains would flocculate under this treatment. Although RoundUp™ does appear to widely induce flocculation, the mechanisms of this response are unknown. Many cellular pathways in S. cerevisiae are involved in flocculation, including cell-wall-associated signalling and regulatory pathways involved in flocculation-associated gene expression (Soares, 2010). Previous research indicates that cell-wall-associated pathways are involved in RoundUp™ resistance in S. cerevisiae (Ravishankar, 2020); it is reasonable to speculate that these pathways may also be involved in flocculation response. However, considering that two of the strains did not flocculate
more intensely in RoundUp™ compared to YPD, and that increases in intensity varied among the other strains, these target pathways must vary between strains.

Exposure to RoundUp™ and glyphosate has been associated with evolution of glyphosate resistance in plants and microbial plant pathogens (Meftaul et al., 2020). Consequently, we explored the possibility that strains with possible previous exposure to RoundUp™ might be more RoundUp™-resistant than other strains due to evolutionary history. Despite increased likelihood that strains of a certain origin (most notably fermentation strains) may have had previous exposure via involvement with agriculture, no such pattern of increased resistance was present in the data. Fermentation strains are scattered among ranks of doubling time under YPD and RoundUp™ and among ranks of differential flocculation scores. Likewise, wild strains may have also come into previous contact with glyphosate and glyphosate-containing herbicides via agricultural runoff and other environmental pollution. Still, while the two wild strains (UWOPS05_217_3, K and YPS128, G) grew quickly in YPD, they grew at an intermediate pace in RoundUp™. Therefore, differences in RoundUp™ resistance between strains is likely not due to some prior evolutionary adaptation to the herbicide, but rather due to other stress-resistant mechanisms present in the strains.

Further research involving the growth response of these strains to a variety of stressful conditions would help elucidate whether the most rapidly growing strains in RoundUp™ are uniquely RoundUp™-resistant, or simply generally stress-resistant. Regardless, knowledge of how RoundUp™ affects these strains may have a variety of applications. Ultimately, it is clear that RoundUp™ deleteriously affected all 12 studied strains, so the herbicide may similarly affect other wild yeasts. RoundUp™ has been shown to persist in certain soils for up to a year or longer and, despite high soil sorption and immobility, is found frequently in runoff and surface
waters surrounding areas exposed to the herbicide (Medalie et al., 2020; Meftaul et al., 2020). Given the deleterious effects that RoundUp™ has on many microbes, including those associated with soil and agriculture, the ubiquity of the herbicide may limit agricultural productivity by reducing soil health. Of course, this study characterizes RoundUp™ response in liquid media only, rather than soil; it is possible that substrate has a significant effect on the impacts of RoundUp™ on microbes, including S. cerevisiae. Within this discussion, we assume that the physiological and genetic mechanisms underlying RoundUp™ resistance under these liquid media assays are at least somewhat reflective of in-soil responses, and therefore relevant to real-world applications. Still, it is worth noting that the effects of substrate on RoundUp™ response in S. cerevisiae have yet to be fully characterized.

Nevertheless, our results suggest that RoundUp™ resistance has a unique genetic basis in diverse strains of S. cerevisiae. The full sequencing and heavy annotation of the yeast genome makes S. cerevisiae an ideal model organism for determining genetic traits associated with certain phenotypes (Botstein & Fink, 2011). It has been proposed that microbiome engineering may serve to enhance agricultural sustainability and yield by optimizing plant-microbe interactions (Trivedi et al., 2017). Perhaps, RoundUp™-resistant soil communities can be engineered to improve agricultural outcomes with this herbicide. Future research may determine which genes are responsible for RoundUp™ resistance in brewer’s yeast, and these genes may be used to engineer microbial soil communities that can engage in beneficial plant-microbe interactions even in the presence of RoundUp™ and other glyphosate-containing herbicides. Thus, agricultural success could be maintained without excessive fertilizer application, as the soil microbiome would be able to function and provide the resources necessary for plant-nutrient absorbance even with regular herbicide application. Of course, the mechanism of RoundUp™
resistance in *S. cerevisiae* is still unknown. It is quite possible that RoundUp™ resistance in *S. cerevisiae* is due to metabolism of the herbicide; in soil, glyphosate degradation is largely microbial (Meftaul *et al*., 2020). In such case, RoundUp™ resistance genes may be used to develop bioremediation systems, producing microbial communities that reduce agricultural pollution. These engineered soil communities (for either purpose) could be developed via genetic modification, or by searching relevant microbes for species or strains that possess homologs for *S. cerevisiae* RoundUp™ resistance-associated genes.

While previous studies have made significant inroads into determining the relationships between genotypes and phenotypes regarding RoundUp™ response in yeast, we expand on the literature here by characterizing the RoundUp™ resistance phenotype of 12 (10 previously uncharacterized) diverse strains. Future work investigating the functional genetics of these strains may help determine which underlying genetic factors contribute to RoundUp™ response, which pathways these factors are involved in, and whether these factors are relevant to other species with orthologous genes in *S. cerevisiae*. 
V. References


Dennis, P.G., Kukulies, T., Forstner, C. et al. (2018). The effects of glyphosate, glufosinate, paraquat and paraquat-diquat on soil microbial activity and bacterial, archaeal and nematode diversity. *Scientific Reports, 8,* 2119. [https://doi.org/10.1038/s41598-018-20589-6](https://doi.org/10.1038/s41598-018-20589-6)


Hagner, M., Mikola, J., Saloniemi, I. et al. (2019). Effects of a glyphosate-based herbicide on soil animal trophic groups and associated ecosystem functioning in a northern agricultural field. *Scientific Reports, 9,* 8540. [https://doi.org/10.1038/s41598-019-44988-5](https://doi.org/10.1038/s41598-019-44988-5)


Kniss, A. (2017). Long-term trends in the intensity and relative toxicity of herbicide use. *Nature Communications, 8,* 14865. [https://doi.org/10.1038/ncomms14865](https://doi.org/10.1038/ncomms14865)


doi:10.1614/WT-04-142R.1

Ravishankar, A., Pupo, A., & Gallagher, J. (2020). Resistance Mechanisms of Saccharomyces cerevisiae to Commercial Formulations of Glyphosate Involve DNA Damage Repair, the Cell Cycle, and the Cell Wall Structure. *G3: Genes, Genomes, Genetics, 10*(6), 2043–2056. [https://doi.org/10.1534/g3.120.401183](https://doi.org/10.1534/g3.120.401183)


Table 1: Characteristics of 12 *S. cerevisiae* strains. The first column indicates the internal identifier for the strain, the second column indicates the standard strain name; the third column indicates the geographical and contextual origin of the strain; the fourth column indicates the strain genotype; the fifth column indicates the yeast mating type. All of these strains are haploid.

<table>
<thead>
<tr>
<th>Internal ID</th>
<th>Strain</th>
<th>Origin</th>
<th>Genotype</th>
<th>Mating Type</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>DBVPG6765</td>
<td>Europe; fermentation</td>
<td><em>ho::HygMX,ura3::KanM</em></td>
<td>a</td>
</tr>
<tr>
<td>B</td>
<td>DBVPG6044</td>
<td>West Africa; wine</td>
<td><em>ho::HygMX,ura3::KanM</em></td>
<td>a</td>
</tr>
<tr>
<td>C</td>
<td>BC187</td>
<td>USA; wine</td>
<td><em>ho::HygMX,ura3::KanM</em></td>
<td>a</td>
</tr>
<tr>
<td>D</td>
<td>SK1</td>
<td>USA; soil</td>
<td><em>ho::HygMX,ura3::KanM</em></td>
<td>a</td>
</tr>
<tr>
<td>E</td>
<td>L_1374</td>
<td>Chile; wine</td>
<td><em>ho::HygMX,ura3::KanM</em></td>
<td>a</td>
</tr>
<tr>
<td>F</td>
<td>YJM975</td>
<td>Italy; vaginal clinical isolate</td>
<td><em>ho::HygMX,ura3::KanM</em></td>
<td>a</td>
</tr>
<tr>
<td>G</td>
<td>YPS128</td>
<td>USA; soil beneath <em>Q. alba</em></td>
<td><em>ho::HygMX,ura3::KanM</em></td>
<td>α</td>
</tr>
<tr>
<td>H</td>
<td>Y12</td>
<td>Japan; sake</td>
<td><em>ho::HygMX,ura3::KanM</em></td>
<td>α</td>
</tr>
<tr>
<td>I</td>
<td>273614N</td>
<td>UK; fecal sample, clinical isolate</td>
<td><em>ho::HygMX,ura3::KanM</em></td>
<td>α</td>
</tr>
<tr>
<td>J</td>
<td>L_1528</td>
<td>Chile; wine</td>
<td><em>ho::HygMX,ura3::KanM</em></td>
<td>α</td>
</tr>
<tr>
<td>K</td>
<td>UWOPS05_217_3</td>
<td>Malaysia; nectar, Bertram palm</td>
<td><em>ho::HygMX,ura3::KanM</em></td>
<td>α</td>
</tr>
<tr>
<td>L</td>
<td>YJM981</td>
<td>Italy; vaginal clinical isolate</td>
<td><em>ho::HygMX,ura3::KanM</em></td>
<td>α</td>
</tr>
</tbody>
</table>
Table 2: Strain doubling time rankings for growth in YPD. The first column indicates the internal identifier for the strain, the second column indicates the standard strain name; the third column indicates the shape designator as produced from Dunn testing (n = 3, α = 0.05); the fourth column indicates the median doubling (DT) time in hours. The strains are ordered down the table in increasing doubling time, or slower growth rate. The doubling time for strains that do not share a shape designator in the third column are significantly different (α = 0.05). The fifth column indicates origin as per Figure 1, with the geographical origin written out and the contextual origin color-coded (red = clinical, orange = fermentation, green = laboratory, blue = wild).

<table>
<thead>
<tr>
<th>Internal ID</th>
<th>Strain Name</th>
<th>Group</th>
<th>Median DT (hr)</th>
<th>Origin</th>
</tr>
</thead>
<tbody>
<tr>
<td>K</td>
<td>UWOPS05_217_3</td>
<td>•</td>
<td>1.237160</td>
<td>Oceania</td>
</tr>
<tr>
<td>G</td>
<td>YPS128</td>
<td>■</td>
<td>1.254002</td>
<td>Americas</td>
</tr>
<tr>
<td>J</td>
<td>L_1528</td>
<td>■</td>
<td>1.272078</td>
<td>Americas</td>
</tr>
<tr>
<td>E</td>
<td>L_1374</td>
<td>●</td>
<td>1.304997</td>
<td>Americas</td>
</tr>
<tr>
<td>D</td>
<td>SK1</td>
<td>●</td>
<td>1.307291</td>
<td>Africa</td>
</tr>
<tr>
<td>A</td>
<td>DBVP6765</td>
<td>●</td>
<td>1.345506</td>
<td>Europe</td>
</tr>
<tr>
<td>I</td>
<td>273614N</td>
<td>●</td>
<td>1.403170</td>
<td>Europe</td>
</tr>
<tr>
<td>H</td>
<td>Y12</td>
<td>●</td>
<td>1.431442</td>
<td>Asia</td>
</tr>
<tr>
<td>F</td>
<td>YJM975</td>
<td>●</td>
<td>1.448955</td>
<td>Europe</td>
</tr>
<tr>
<td>C</td>
<td>BC187</td>
<td>●</td>
<td>1.475464</td>
<td>Americas</td>
</tr>
<tr>
<td>B</td>
<td>DBVP6044</td>
<td>●</td>
<td>1.531365</td>
<td>Africa</td>
</tr>
<tr>
<td>L</td>
<td>YJM981</td>
<td>●</td>
<td>1.669390</td>
<td>Europe</td>
</tr>
</tbody>
</table>
Table 3: Strain doubling time rankings for growth in 1% RoundUp™. The first column indicates the internal identifier for the strain, the second column indicates the standard strain name; the third column indicates the shape designator as produced from Dunn testing (n = 3, α = 0.05); the fourth column indicates the median doubling time (DT) in hours. The strains are ordered down the table in increasing doubling time, or slower growth rate. The doubling time for strains that do not share a shape designator in the third column are significantly different (α = 0.05). The fifth column indicates origin as per Figure 1, with the geographical origin written out and the contextual origin color-coded (red = clinical, orange = fermentation, green = laboratory, blue = wild).

<table>
<thead>
<tr>
<th>Internal ID</th>
<th>Strain Name</th>
<th>Group</th>
<th>Median DT (hr)</th>
<th>Origin</th>
</tr>
</thead>
<tbody>
<tr>
<td>J</td>
<td>L_1528</td>
<td>●</td>
<td>1.805329</td>
<td>Americas</td>
</tr>
<tr>
<td>K</td>
<td>UWOPS05_217_3</td>
<td>● ■ ◆</td>
<td>2.264037</td>
<td>Oceania</td>
</tr>
<tr>
<td>E</td>
<td>L_1374</td>
<td>■ ◆</td>
<td>2.283104</td>
<td>Americas</td>
</tr>
<tr>
<td>B</td>
<td>DBVPG6044</td>
<td>■ ◆</td>
<td>2.491339</td>
<td>Africa</td>
</tr>
<tr>
<td>G</td>
<td>YPS128</td>
<td>● ■ ◆</td>
<td>2.628444</td>
<td>Americas</td>
</tr>
<tr>
<td>L</td>
<td>YJM981</td>
<td>● ■ ◆</td>
<td>2.666134</td>
<td>Europe</td>
</tr>
<tr>
<td>C</td>
<td>BC187</td>
<td>● ■ ◆</td>
<td>2.759916</td>
<td>Americas</td>
</tr>
<tr>
<td>F</td>
<td>YJM975</td>
<td>● ■ ◆</td>
<td>3.122003</td>
<td>Europe</td>
</tr>
<tr>
<td>I</td>
<td>273614N</td>
<td>● ■ ◆</td>
<td>3.342001</td>
<td>Europe</td>
</tr>
<tr>
<td>A</td>
<td>DBVPG6765</td>
<td>● ■ ◆</td>
<td>4.957155</td>
<td>Europe</td>
</tr>
<tr>
<td>H</td>
<td>Y12</td>
<td>● ■</td>
<td>6.935511</td>
<td>Asia</td>
</tr>
<tr>
<td>D</td>
<td>SK1</td>
<td>●</td>
<td>9.939535</td>
<td>Africa</td>
</tr>
</tbody>
</table>
Table 4: Flocculation under YPD and RoundUp™ treatments. The first column indicates the internal strain identifier. The second and fourth columns contain images of each strain after 48 hrs of growth under YPD or 1% RoundUp™ conditions, respectively. These images are grouped by biological replicate (two technical replicates per biological replicate). The third and fifth columns indicate the ‘flocculation score’ assigned to the strain, where 1 indicates little to no visible flocculation, and 5 indicates extreme flocculation. The sixth column indicates the difference in flocculation score between YPD and 1% RoundUp™ treatments (1% RoundUp™ - YPD).

<table>
<thead>
<tr>
<th>Internal ID</th>
<th>YPD Score</th>
<th>1% RoundUp™ Score</th>
<th>Difference</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>1</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>B</td>
<td>1</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>C</td>
<td>1</td>
<td>4</td>
<td>3</td>
</tr>
<tr>
<td>D</td>
<td>3</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>E</td>
<td>2</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td>F</td>
<td>1</td>
<td>5</td>
<td>4</td>
</tr>
<tr>
<td>Internal ID</td>
<td>YPD</td>
<td>Score</td>
<td>1% RoundUp™</td>
</tr>
<tr>
<td>------------</td>
<td>-----</td>
<td>-------</td>
<td>-------------</td>
</tr>
<tr>
<td>G</td>
<td><img src="image" alt="YPD" /></td>
<td>1</td>
<td><img src="image" alt="1% RoundUp" /></td>
</tr>
<tr>
<td>H</td>
<td><img src="image" alt="YPD" /></td>
<td>1</td>
<td><img src="image" alt="1% RoundUp" /></td>
</tr>
<tr>
<td>I</td>
<td><img src="image" alt="YPD" /></td>
<td>2</td>
<td><img src="image" alt="1% RoundUp" /></td>
</tr>
<tr>
<td>J</td>
<td><img src="image" alt="YPD" /></td>
<td>3</td>
<td><img src="image" alt="1% RoundUp" /></td>
</tr>
<tr>
<td>K</td>
<td><img src="image" alt="YPD" /></td>
<td>2</td>
<td><img src="image" alt="1% RoundUp" /></td>
</tr>
<tr>
<td>L</td>
<td><img src="image" alt="YPD" /></td>
<td>3</td>
<td><img src="image" alt="1% RoundUp" /></td>
</tr>
</tbody>
</table>
Figure 1: Phylogenetic tree of 12 strains. Genetic relationships between strains are demonstrated by branching patterns. Contextual origin for each strain is indicated by node color, and geographical origin for each strain is indicated by text highlighting color.
**Figure 2: Growth assay schematic.** This flowchart indicates the process of transitioning from a single strain plate to the 96-well plate used for the growth assays. Firstly, three colonies are samples from a strain plate to inoculate three biological replicate overnight cultures (A). Then, the OD$_{600}$ of each culture is recorded in a 1:100 dilution (B) and used to develop a dilute culture in YPD or 1% RoundUp™ media (C). Each dilute biological replicate is used to fill two wells in the 96-well plate, constituting two technical replicates per biological replicate (D). This workflow was used for all 12 strains for each growth assay. The filled plate is then placed in the microplate reader and the 48 hr growth assay is performed (E).
Figure 3: Growth in rich YPD. Here shown is the mean log OD$_{600}$ for all 12 strains, as averaged across biological replicates (n = 3), plotted against time. Error bars show standard deviation.
Figure 4: Growth in RoundUp™-containing YPD. Here shown is the mean log OD₆₀₀ for all 12 strains, as averaged across biological replicates (n = 3), plotted against time. Error bars show standard deviation.
Figure 5: Doubling time for growth in YPD. Growth rate calculated for each strain as averaged across biological replicates (n = 3). Error bars indicate standard error of the mean.
Figure 6: Doubling time for growth in 1% RoundUp™. Growth rate calculated for each strain as averaged across biological replicates (n = 3). Error bars indicate standard error of the mean.
Figure 7: Doubling time under YPD and RoundUp™ treatments. Doubling times for each strain under YPD and RoundUp™ treatments. The scores for each strain are connected by a coordinated colored line. The y-axis is log_{10} transformed for better visibility.
**Figure 8: Flocculation under YPD and RoundUp™ treatments.** Flocculation scores for each strain under YPD and RoundUp™ treatments. The scores for each strain are connected by a coordinated colored line. The plot is jittered horizontally to distinguish each point.