Investigation of apple fruit rot control and of the diversity of wild yeast populations on cider and dessert apple varieties and outcomes on fermentation

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Final report

PROBLEM STATEMENT. To maintain a growing cider industry in Michigan, cideries rely on a steady supply of both dessert and cider apples. A major issue affecting a reliable apple supply is fruit rots that are often initiated in the orchard and can continue to spread or be exacerbated by storage. There is almost no information on the susceptibility of hard cider apple cultivars to fruit rot infection, and little information on fungicide control and the potential for effective control with reduced fungicide inputs on cider apple cultivars.

Wild yeasts are also of interest to craft style cider makers because of their potential to introduce complexity to flavor profiles to cider during the fermentation process. Wild yeasts may also have value in the biological control of fruit rots in the orchard. Understanding seasonal yeast population dynamics on cider apple cultivars and correlations with rot incidence, and their influence on fermentation will impact the emerging craft cider industry in Michigan.

This project addresses Priorities 1 and 2 of the Michigan Craft Beverage Council.

SPECIFIC OBJECTIVES AND HYPOTHESES. The Objectives of this project are to:

1. Determine the susceptibility of fruit from 29 cider apple cultivars to bitter rot and black rot, and evaluate fungicide programs, especially reduced application programs, for fruit rot control. These evaluations will be compared with dessert fruits.

2. Sample and identify wild yeast strains on cider and dessert fruit and determine the influence of wild yeasts on the polyphenol content of the fermented apple product.

We hypothesize that we will observe a wide range of levels of susceptibility to fruit rot among cider apple cultivars, and that we can identify more disease-tolerant cultivars that would be candidates for reduced-input fungicide control programs.

We also hypothesize that the number of unique yeasts isolated will increase as the growing season progresses, and that fermentations with wild yeast will result in cider with higher polyphenol content compared to cider made with commercial yeast.

RESULTS AND CONCLUSIONS OF THE PROJECT.

This projected initiated during FY22 began a novel area of research in the Sundin lab. We began a promising disease rating and fungicide trial program for cider apple cultivars to fruit rots in Michigan. We have also collected samples of fungi and yeast populations on fruits, the results of which will have implications for the role of carbohydrates in microbial community assembly dynamics. These data will be useful to stakeholders not only in the craft beverage industry, but the plant-microbe interaction research field as a whole. This project provided important preliminary data for both applied and basic research projects in the Sundin lab.

TIME PERIOD OF RESEARCH. April 1, 2022 to August 15, 2023

WORK ACCOMPLISHED.

Materials & Methods

Ingham County isolates of *Colletotrichum acutatum* were used for field experiments. Work was conducted at MSU Plant Pathology Research Farm in East Lansing, MI. Four fruit per tree of cultivar 'McIntosh' were wounded while fruit was attached to the tree, wounds were filled with mycelial macerate. Trees were treated on a 10-to-12-day schedule starting one week after inoculations. Spray schedules and product rates are listed in Table 1. Efficacy of treatments were evaluated by counting symptomatic fruit per 120 fruit per tree at harvest.

Table 1. Experimental treatments for bitter rot control.

Application. Number	Product	Rate per acre
1	Double Nickel 1 QT + Cueva 2 QT	79.2 ml + 158.4 ml
2	Captan 80WDG 2.5 lb	94.5 g
3	Double Nickel 1 QT + Cueva 2 QT	79.2 ml + 158.4 ml
4	Captan 80WDG 2.5 lb	94.5 g
5	Oso 6.5 oz + R-11 0.125%	15.4 oz + 118.3 ml
1	LifeGard 4.5 oz / 100 gal + R-11 0.125%	31.9 g + 118.3 ml
2	Captan 80WDG 2.5 lb	94.5 g
3	LifeGard 4.5 oz / 100 gal + R-11 0.125%	31.9 g + 118.3 ml
4	Captan 80WDG2.5 lb	94.5 g
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3	LifeGard 4.5 oz / 100 gal + R-11 0.125%	31.9 g + 118.3 ml
4	Captan 80WDG 2.5 lb	94.5 g
5	LifeGard 4.5 oz / 100 gal + R-11 0.125%	31.9 g + 118.3 ml
1	Flint Extra 2.5 oz + Captan 80WDG 2.5 lb	5.9 oz + 94.5 g
2	Flint Extra 2.5 oz + Captan 80WDG 2.5 lb	5.9 oz + 94.5 g
3	Flint Extra 2.5 oz + Captan 80WDG 2.5 lb	5.9 oz + 94.5 g
4	Flint Extra 2.5 oz + Captan 80WDG 2.5 lb	5.9 oz + 94.5 g
5	Flint Extra 2.5 oz + Captan 80WDG 2.5 lb	5.9 oz + 94.5 g
1	Captan 80WDG 2.5 lb	94.5 g
2	Captan 80WDG 2.5 lb	94.5 g
3	Captan 80WDG 2.5 lb	94.5 g
4	Captan 80WDG 2.5 lb	94.5 g
5	Captan 80WDG 2.5 lb	94.5 g

Apples from 25 cider and 4 dessert cultivars were collected from six-year-old trees at MSU Plant Pathology Research Farm in East Lansing, MI and placed in cold storage for one week. Replicate sets of apples (8 apples per set) were inoculated with fresh cultures of *Colletotrichum acutatum* across three consecutive days. Fruits were wounded, and wound was filled with mycelial macerate. Wounds were wrapped with parafilm until the first measurements were collected. Fruits were stored in lidded bins to maintain humidity. Lesion diameter was measured at 5-, 10-, and 15-days-post-inoculation. Fruit firmness, lesion characteristics, and spore presence were also recorded. Cultivars were grouped into low, moderate, or high susceptibility based on range of symptoms (Figure 1).

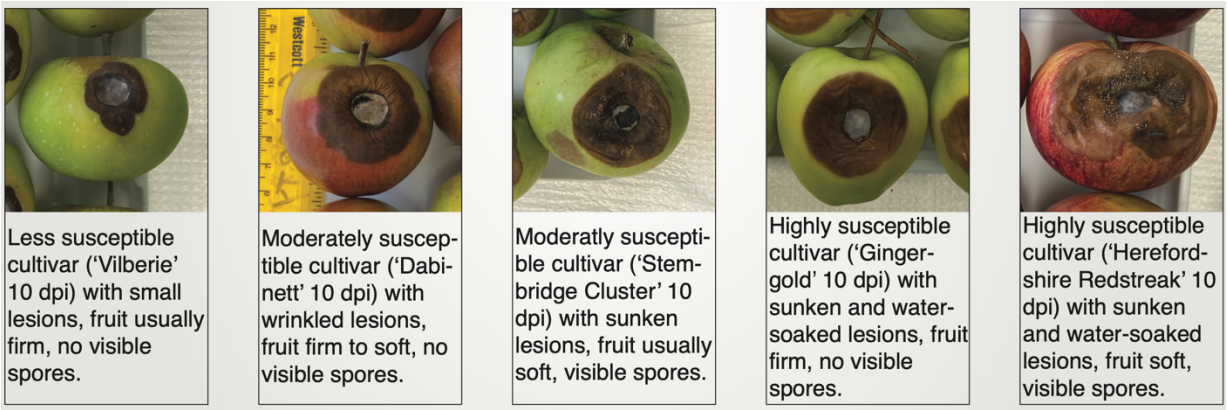

Figure 1. Representative samples of susceptibility assignments of cultivars.

Plant samples were collected from MSU Plant Pathology Research Farm (East Lansing, MI), MSU Clarksville Research Center (Clarksville, MI), and Tandem Ciders (Suttons Bay, MI) for yeast population analysis. Five replicate trees of each cultivar were selected at each stie. Fifteen flower samples from replicate trees of several day-old King Blooms were collected during bloom at each location, May – June 2022. Immature fruit samples, representing cell expansion phases of fruit development, were collected June – July 2022. A second fruit sample, representing starch decline/ripening phases of fruit development, was collected late July 2022. For fruit samples, five fruits were collected from each of the replicate trees. In the laboratory, a peel sample (including skin and ~5 mm of pulp) was taken from each fruit and pooled together. Peels were lyophilized and DNA was extracted. DNA of fungal communities were amplified with ITS primers, and will be sequenced at MSU RTSF Genomics Core.

Yeasts were also isolated from fruit samples from East Lansing and Suttons Bay. Additional peel samples were suspended in sterile half-strength PBS and sonicated to detach cells from plant tissues. The resulting supernatant was used to generate a dilution series from which morphologically diverse colonies were selected. Representatives of these morphotypes were identified with Sanger sequencing at MSU RTSF Genomics Core.

Apples from East Lansing and Suttons Bay were used in fermentation experiments. Apples were harvested at maturity, and placed in cold storage for approximately 1 month. Apples of each cultivar were pressed at Michigan Northwest Horticulture Research Center. Juice was collected and divided into duplicate containers for fermentation. A representative sample of pumice was collected from each cultivar for microbiome sequencing. Due to the limited quantities of some cultivars, fermentation vessel size ranged from pint to gallon. Commercial yeast was added to one container for each cultivar and location combination, and the second container was allowed to ferment spontaneously. Fermentation airlocks were attached to each vessel and allowed to ferment to completion, approximately 6 weeks. The resulting cider was collected for analysis. Juice and cider were measured for the following characteristics: pH, degrees Brix, gallic acid equivalents using Folin-Ciocalteu reagent, malic acid titratable acidity, and nitrogen content. Characteristics of juice and cider will be used as covariates for fungi population analysis.

Results

All tested control methods against bitter rot in orchard were effective (Figure 2A). All experimental treatments reduced bitter rot prevalence relative to untreated trees ($p < 0.001$) as determined by ANOVA and Tukey's HSD. Growers with known bitter rot disease pressure should avoid highly susceptible cultivars. Cider cultivars exhibited a range of susceptibility and symptoms. Symptom progression at 5 dpi was consistent with final susceptibility classifications. Cider cultivars were both more and less susceptible than dessert cultivars with known susceptibility to bitter rot (Figure 2B).

Although the display orchard at Tandem Ciders in Suttons Bay is under a minimal management regime, the population levels of yeasts (total yeast cells per gram of fruit) from these samples were lower than the conventionally managed cider block at the MSU Pathology Farm. Additionally, the East Lansing samples had greater yeast richness (number of unique yeast species) than fruit from Suttons Bay (Figure 3). Characteristics of cider were consistent within cultivar when a given cultivar was grown in multiple locations. Differences in characteristics align with definitions of the British Categorization system (i.e., sharp, bittersweet, etc.) (Table 2).

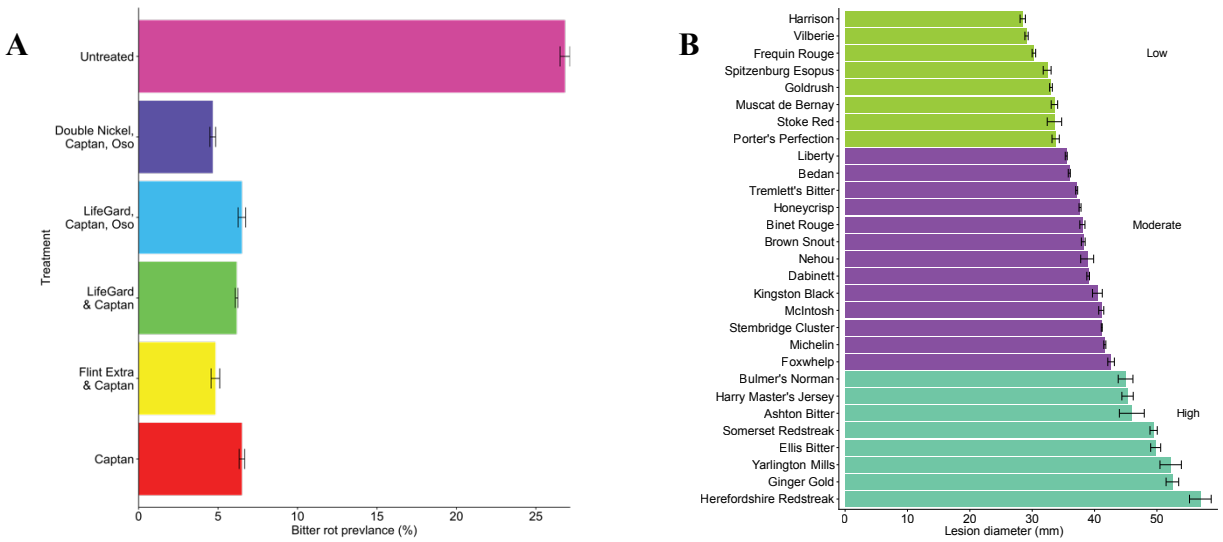

Figure. 2. (A) Control of bitter rot was determined by percent of apples showing symptoms at harvest. Five tested methods effectively reduced bitter rot prevalence (ANOVA and Tukey's HSD, $p < 0.001$) when compared to untreated (pink bar) trees. All of the five fungicide regimes were equally effective at controlling bitter rot. **(B)** Cultivar susceptibility was determined by lesion diameter at 10 days post inoculation. Cultivars were grouped into low (green bars), moderate (mauve bars), or high susceptibility (teal bars).

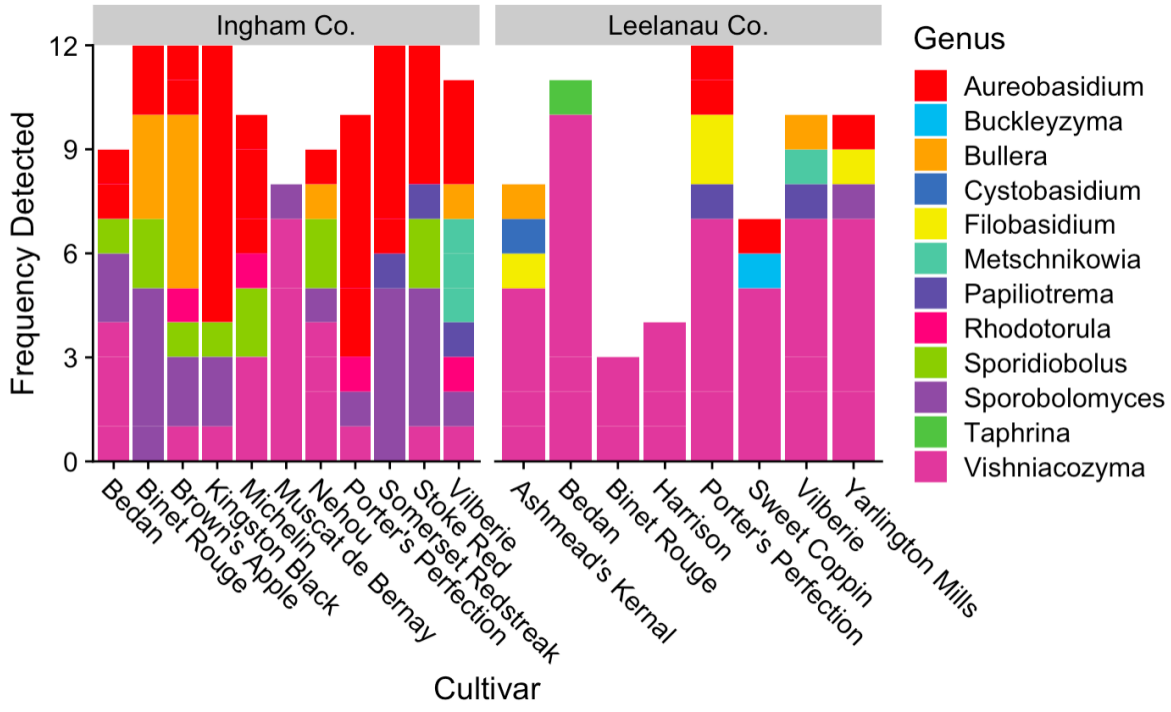

Figure 3. Frequency of yeast morphotypes from East Lansing (Ingham Co.) and Suttons Bay (Leelanau Co.).

Table 2. Juice and cider characteristics measured; numbers represent mean ± standard error.

Location	Fermentation timepoint	Fermentation Condition	Cultivar	Folin-Ciocalteu gallic acid equivalents (mg/L)	Brix (°Bx)	pH	Titratable Acidity (mol/L)	Primary amino nitrogen (mg/L)
East Lansing	before	-	Vilberie	150.9 ± 9.3	13.6 ± 0.7	3.83 ± 0	0.0213 ± 0.0018	4.6965 ± 0.4777
East Lansing	before	-	Stoke Red	140.7 ± 13.2	12.8 ± 0.6	3.48 ± 0.01	0.052 ± 0.005	1.985022 ± 0.3438
East Lansing	before	-	Binet Rouge	42.6 ± 7.8	14.0 ± 0.05	4.17 ± 0.01	0.0213 ± 0.0012	0.6615 ± 0.2737
East Lansing	before	-	Somerset Redstreak	151.1 ± 10.3	13.4 ± 0.4	4.66 ± 0.01	0.0137 ± 0.0007	1.16766 ± 0.966
East Lansing	before	-	Bedan	34.5 ± 2.2	12.3 ± 1.0	4.27 ± 0.01	0.0173 ± 0.0015	1.783925 ± 1.1806
East Lansing	before	-	Michelin	74.7 ± 21.6	12.3 ± 0.05	3.81 ± 0.01	0.022 ± 0.0015	2.315859 ± 0.999
East Lansing	before	-	Nehou	85.5 ± 10.3	16.5 ± 0.1	4.17 ± 0.01	0.1373 ± 0.1014	6.3215 ± 0.1179
East Lansing	before	-	Kingston Black	35.9 ± 0.4	12.5 ± 0.7	3.55 ± 0.01	0.067 ± 0.0012	2.76675 ± 0.2978
East Lansing	before	-	Muscat de Bernay	87.8 ± 3.0	13.8 ± 0.03	3.97 ± 0	0.02 ± 0.0006	0.8822 ± 0.5384
Suttons Bay	before	-	Bedan	39.7 ± 5.3	11.2 ± 0.08	4.33 ± 0	0.03 ± 0.002	4.6965 ± 0.4777
Suttons Bay	before	-	Mettias	19.4 ± 0.5	12.2 ± 0.12	3.41 ± 0.01	0.102 ± 0.0122	0.253 ± 0.1038
Suttons Bay	before	-	Harrison	49.4 ± 6.4	14.9 ± 0.03	3.39 ± 0.01	0.1243 ± 0.0116	1.122251 ± 0.5319
Suttons Bay	before	-	Somerset Redstreak	69.63 ± 8.3	12.3 ± 0.3	3.37 ± 0.01	0.0197 ± 0.0003	0.8628 ± 0.2854
Suttons Bay	before	-	Nehou	63.8 ± 3.1	12.6 ± 0.03	4.19 ± 0	0.0383 ± 0.0044	1.388218 ± 1.239
Suttons Bay	before	-	Porter's Perfection	97.6 ± 9.6	10.9 ± 0.1	3.47 ± 0.01	0.077 ± 0.0059	13.3957 ± 4.0868
Suttons Bay	before	-	Binet Rouge	37.8 ± 5.6	12.1 ± 0.03	4.6 ± 0	0.013 ± 0.0012	1.41425 ± 0.3935
East Lansing	before	commercial	Vilberie	NA	3.8 ± 0.1	3.79 ± 0.01	0.0223 ± 0.0102	-
East Lansing	after	spontaneous	Vilberie	175.2 ± 47.5	7.5 ± 0.06	3.71 ± 0.01	0.017 ± 0.0029	-
East Lansing	after	commercial	Stoke Red	NA	3.6 ± 0.1	3.61 ± 0.01	0.041 ± 0.002	-
East Lansing	after	spontaneous	Stoke Red	51.4 ± 7.5	6.8 ± 0.06	3.5 ± 0.01	0.0603 ± 0.0072	-
East Lansing	after	commercial	Binet Rouge	NA	4.2 ± 0.03	3.94 ± 0.02	0.033 ± 0.0036	-
East Lansing	after	spontaneous	Binet Rouge	27.8 ± 3.2	6.5 ± 0.03	4.32 ± 0	0.015 ± 0.0012	-
East Lansing	after	commercial	Somerset Redstreak	NA	4.4 ± 0.03	4.15 ± 0.01	0.0287 ± 0.002	-
East Lansing	after	spontaneous	Somerset Redstreak	111.6 ± 4.4	9 ± 0.1	5.18 ± 0.01	0.013 ± 0.0015	-
East Lansing	after	commercial	Bedan	NA	4.1 ± 0.05	4.15 ± 0.01	0.0297 ± 0.0019	-
East Lansing	after	spontaneous	Bedan	22.8 ± 1.2	5.2 ± 0.05	4.22 ± 0.01	0.0207 ± 0.0013	-
East Lansing	after	commercial	Michelin	NA	3.6 ± 0.06	3.74 ± 0.01	0.0317 ± 0.0037	-
East Lansing	after	spontaneous	Michelin	34.2 ± 8.4	9 ± 0.05	3.71 ± 0.01	0.025 ± 0.0012	-
East Lansing	after	commercial	Nehou	NA	6.2 ± 0.01	4.18 ± 0.01	0.043 ± 0.0026	-
East Lansing	after	spontaneous	Nehou	81.7 ± 1.2	10.8 ± 0.08	3.94 ± 0.01	0.0257 ± 0.0012	-
East Lansing	after	commercial	Kingston Black	NA	3.3 ± 0.2	3.64 ± 0.02	0.06 ± 0.015	-
East Lansing	after	spontaneous	Kingston Black	43.1 ± 5.7	9.8 ± 0.06	3.47 ± 0.01	0.0417 ± 0.0207	-
East Lansing	after	commercial	Muscat de Bernay	NA	3.3 ± 0.06	3.84 ± 0.01	0.0223 ± 0.00145	-
East Lansing	after	spontaneous	Muscat de Bernay	77.9 ± 14.4	4.9 ± 0.08	3.97 ± 0	0.0193 ± 0.0017	-

Suttons Bay	after	commercial	Bedan	NA	2.8 ± 0.05	4.06 ± 0.01	0.0213 ± 0.0009	-
Suttons Bay	after	spontaneous	Bedan	19.4 ± 1.4	6.7 ± 0.08	4.41 ± 0.01	0.011 ± 0.0006	-
Suttons Bay	after	commercial	Mettias	NA	4.5 ± 0.08	3.39 ± 0.01	0.086 ± 0.0026	-
Suttons Bay	after	spontaneous	Mettias	47.4 ± 0.7	5.7 ± 0.06	3.34 ± 0.02	0.0493 ± 0.0035	-
Suttons Bay	after	commercial	Harrison	NA	5.2 ± 0.2	3.45 ± 0.01	0.0967 ± 0.0098	-
Suttons Bay	after	spontaneous	Harrison	45.0 ± 4.3	9.8 ± 0.1	3.26 ± 0.02	0.095 ± 0.0049	-
Suttons Bay	after	commercial	Somerset Redstreak	NA	2.9 ± 0.08	4.15 ± 0	0.0243 ± 0.0024	-
Suttons Bay	after	spontaneous	Somerset Redstreak	110.9 ± 10.5	12.5 ± 0.3	3.97 ± 0.01	0.0223 ± 0.0013	-
Suttons Bay	after	commercial	Nehou	NA	3.0 ± 0.08	4.05 ± 0.01	0.036 ± 0.007	-

COMMUNICATION ACTIVITIES, ACCOMPLISHMENTS, AND IMPACTS.

Oral presentations documenting project results were given at the following meetings:

1. Great Lakes Fruit Workers meeting, East Lansing, MI, Nov. 2022. Attended by Extension personnel from Michigan, New York, Indiana, and Ontario.

2. Great Lakes EXPO, Grand Rapids, MI, Dec. 2022. Attended by apple growers and extension personnel.

3. American Phytopathological Society North Central meeting, West Lafayette, IN, Jun 2023. Attended by plant pathologists from 10 states in the north central U.S.

BUDGET NARRATIVE. The project was conducted consistent with the budget proposed by the PIs and approved by the State of Michigan.