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 technical report

Objective 1: Determine the susceptibility of fruit from 28 cider apple cultivars to black rot and evaluate fungicide programs for bitter rot control

Ingham County isolates of *Colletotrichum acutatum* were used for field experiments. Work was conducted at MSU Plant Pathology Research (PLP) Farm in East Lansing, MI. Immature fruit from cultivar 'McIntosh' were wounded, wounds were filled with mycelial macerate, and hung in trees from onion bags. Trees were treated on an 8- to 14-day schedule starting three weeks after inoculations. Spray schedules and product rates are listed in **Table 1**. Efficacy of treatments were evaluated by counting symptomatic fruit per 100 fruit per tree at harvest. Treatments containing 5 sprays were determined to be effective at disease control, resulting in a 50% decrease in disease incidence (**Table 1**).

Due to biennial bearing, only nine cultivars ('Brown's Apple', 'Dabinett', 'Frequin Rouge', 'Foxwhelp', 'Kingston Black', 'Liberty', 'Nehou', 'Somerset Redstreak', 'Spitzenburg Esopus') had enough fruit across all replicate trees to conduct yeast population experiments. A yeast cocktail made of isolates collected from cider fruit at MSU PLP Farm during FY22 were sprayed onto trees using a backpack sprayer to ensure high population levels of yeast before treatments. Four fruit per tree were collected 24 hours prior to spray applications. Trees were sprayed with Mastercop (1.5 pint per acre) or Captan 80WDG (2.5 pounds per acre). Fruit was collected again at 7-, 14-, and 21-days post spray. Fruit samples were freeze-died, and DNA extractions will be conducted following same protocols from FY22. Diversity and population recovery of fungi on fruit over time post spray will be analyzed, in addition to differences in population recovery between each fungicide type.

Apples from 3 dessert and 19 cider cultivars were collected from seven-year-old trees at MSU PLP Farm. Replicate sets of apples (8 apples per set) were inoculated with fresh cultures of *Botryosphaeria obtusa* 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 sanitized lidded plastic bins to maintain humidity. Lesion diameter was measured at 5-, 10-, and 15- days post inoculation (**Figure 1**). Fruit firmness and lesion characteristics were also recorded. Cultivars were grouped into low, moderate, or high susceptibility based on range of symptoms (**Figure 2**).

***Objective 2: Determine the genetic diversity and susceptibility to fungicide of Michigan isolates of the bitter rot pathogen* Colletotrichum acutatum.**

Colletotrichum isolates were obtained from MSU Clarksville Research Center in Ionia County in November 2022. We were unsuccessful in attempting to collect *Colletotrichum spp*.

isolates from fruit grown in greater Grand Rapids area and Leelanau County during the 2023 or 2024 growing seasons.

Objective 3: Determine the impact of different fungicide treatments on yeast populations, and influence of individual yeast on bitter rot pathogen.

Initial samples of fruit to survey fungal microbial communities were collected on Day 0 before fungicide treatment. The cider block at MSU PLP Farm was divided into two blocks with five replicate trees per block. Each block received one of two fungicide treatments, Captan or Copper. Additional samples were collected weekly for 3 weeks post-treatment. DNA sequencing was used to identify fungi residing on the surfaces of cider fruit. Initial microbial community analysis indicates that non-pathogenic fungi associated with apple fruits are resilient to fungicide treatment, as there is not a large shift in numbers of unique fungi (richness) as calculated by Shannon Index after fungicide application (**Figure 3**). A core group of fungi are consistently found on all cider fruits across cultivars and the timepoints in this study with fluctuations in the proportion of the total community during sampling (**Figure 4**). Response of fungal communities to fungicides is variable within a single orchard. Some samples of communities before sprays (+) were distinct from communities post-spray (circle, triangle, square). However, this trend was not consistent across cultivar or in response to fungicide type (**Figure 5**).

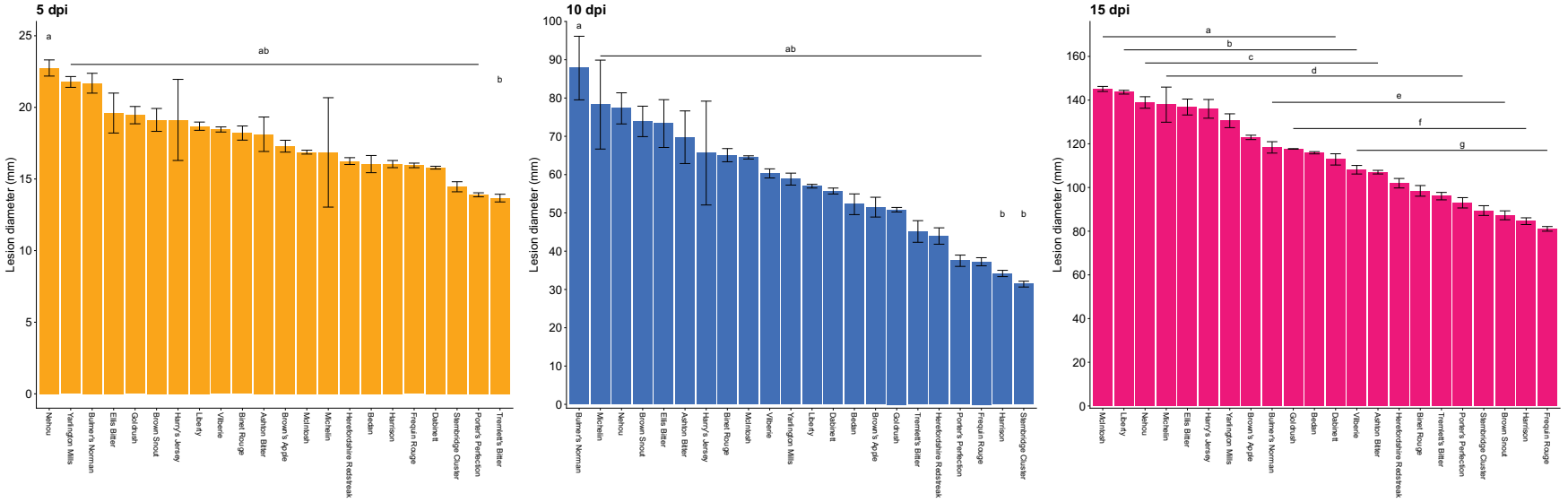

Figure 1. Progression of susceptibility to black rot of each cultivar as measured by lesion diameter. Bars represented by same letter are not significantly different as determine by ANOVA and Tukey's HSD (p = 0.05).

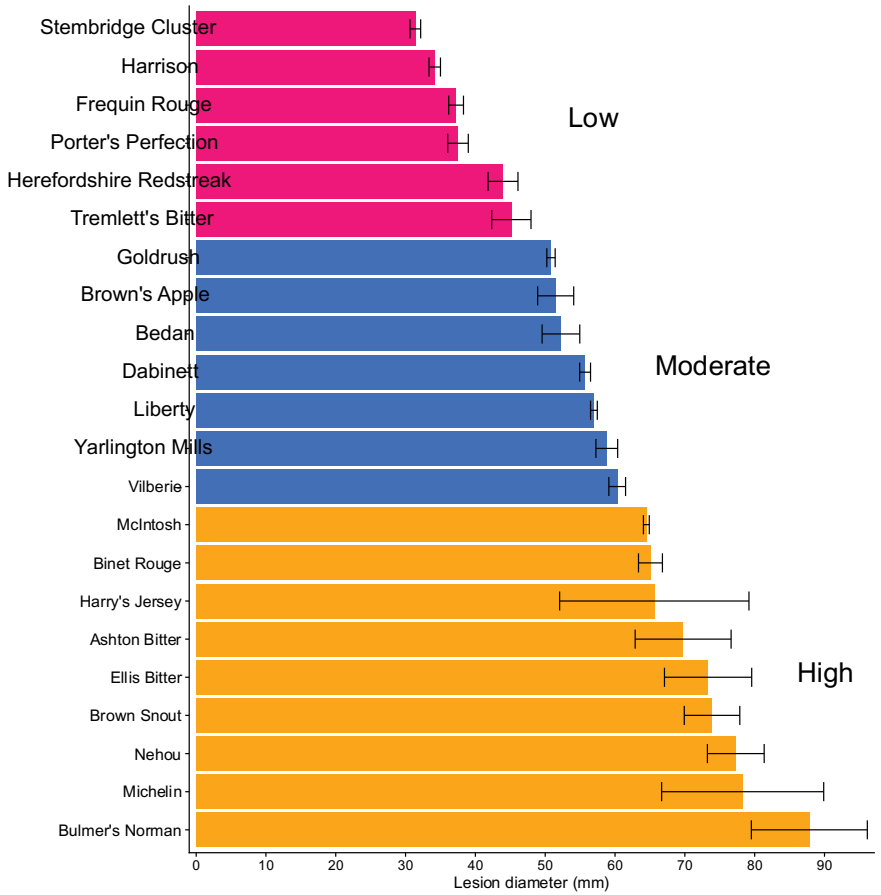

Figure 2. Final categorization of relative cultivar susceptibility. Bars represented by same letter are not significantly different as determine by ANOVA and Tukey's HSD (p = 0.05).

Table 1. Bitter rot control.

Application Number	Product and rate per acre	Mean disease incidence (%)*
1 2 3 4 5	Captan 80 WDG 2.5 lb Captan 80 WDG 2.5 lb Rhyme 13.0 fl oz Rhyme 13.0 fl oz Captan 80 WDG 2.5 lb	3.3 b
1 - 5	Captan 80 WDG	5.1 b
1 2 3 4 5	Merivon 5.5 fl oz Captan 80 WDG 2.5 lb Merivon 5.5 fl oz Captan 80 WDG 2.5 lb Merivon 5.5 fl oz	6.8 ab
1 2 3 4 5	Rhyme 13.0 fl oz Rhyme 13.0 fl oz Captan 80 WDG 2.5 lb Captan 80 WDG 2.5 lb Captan 80 WDG 2.5 lb	7.0 ab
1 2 3 4 5	Double Nickel 1 qt + Cueva 2 qt Captan 80 WDG 2.5 lb Double Nickel 1 qt + Cueva 2 qt Captan 80 WDG 2.5 lb Double Nickel 1 qt + Cueva 2 qt	7.5 ab
1 2 3 4 5	LifeGard 4.5 oz / 100 gal + R-11 0.125% v/v Captan 80 WDG 2.5 lb LifeGard 4.5 oz / 100 gal + R-11 0.125% v/v Captan 80 WDG 2.5 lb LifeGard 4.5 oz / 100 gal + R-11 0.125% v/v	8.2 ab
1 2 3 4 5	3X Captan 80 WDG 2.5 lb 3X Captan 80 WDG 2.5 lb 3X Captan 80 WDG 2.5 lb	12.2 ab
1 - 5	Untreated	21.8 a

* Treatments followed by the same letter are not significantly different as determined by ANOVA and Tukey's HSD ($p < 0.05$).

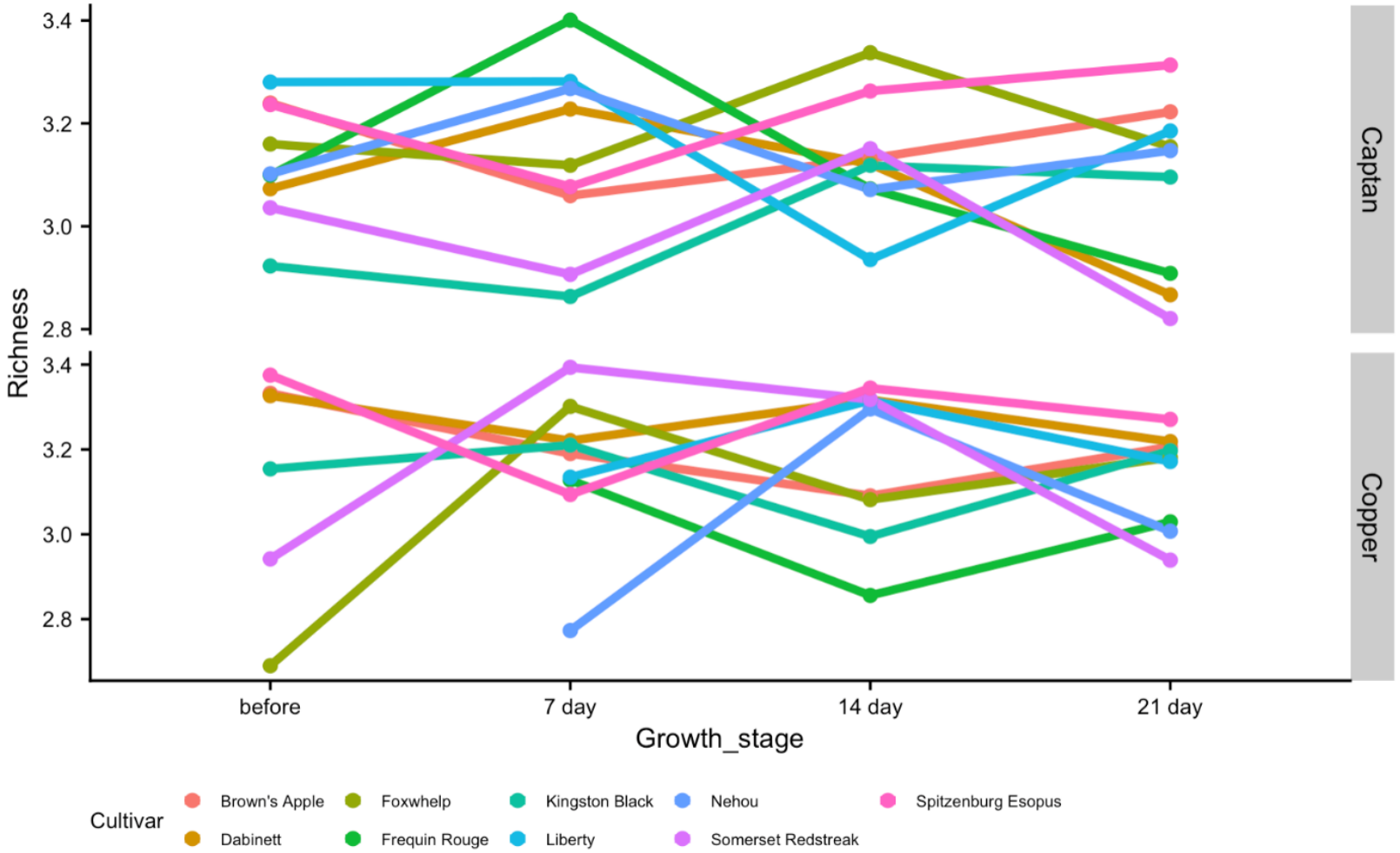

Figure 3. Richness (number of unique species) of fungal sequences from cider apple cultivars before and after applications of two fungicides at MSU Plant Pathology Farm.

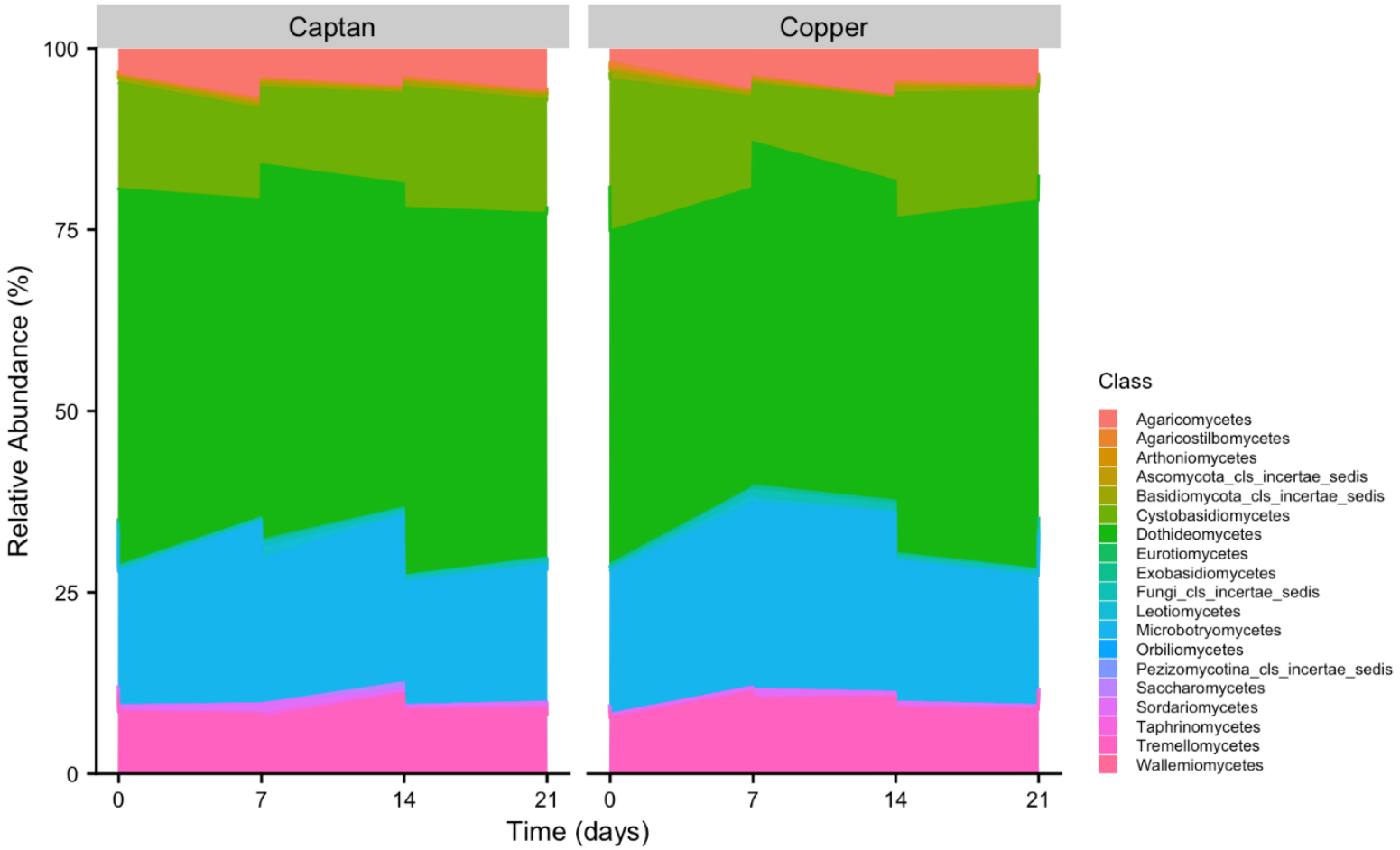

Figure 4. Relative abundance (percent of total sequences) of fungi based on DNA sequencing at Class level. Day 0 = before fungicide applications. Class assignments ending in "incerate sedis" are not able to be assigned at Class level, and are grouped based on their assignments at Phylum or Kingdom levels.

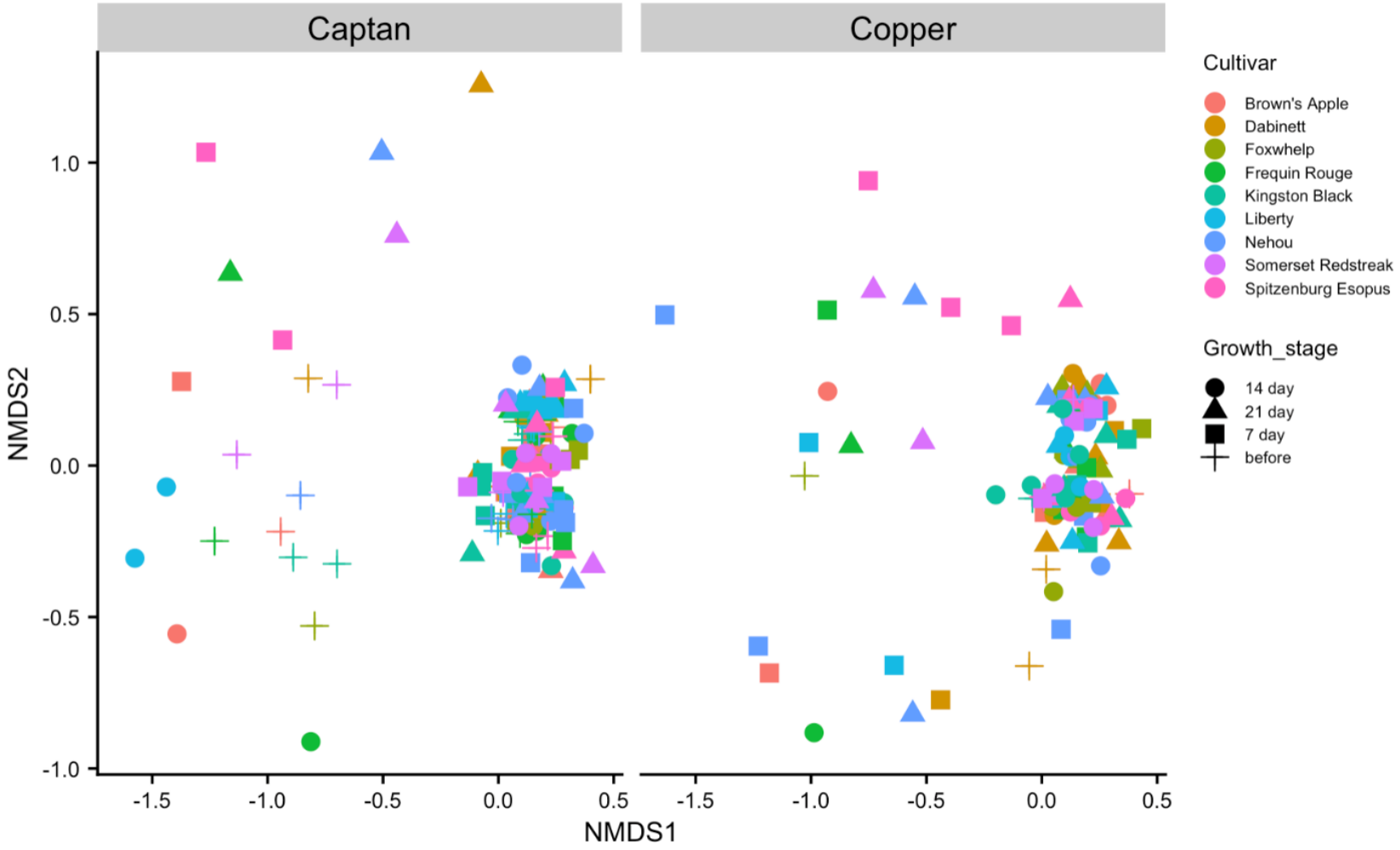

Figure 5. Non-metric multidimensional scaling (NMDS) of fungal communities on cider fruit. Color indicates cultivar, and shape of points indicate time point of sample. Each individual point represents an aggregation of the total microbial community. Points closer together are more similar to each other than distant points.