SCREENING OF A SELECTED SET OF STRAWBERRY GENOTYPES FOR GRAY MOLD RESISTANCE

By

Zehranur Gulbahar

A THESIS

Submitted to
Michigan State University
in partial fulfillment of the requirements
for the degree of

Plant Breeding, Genetics, and Biotechnology – Crop and Soil Sciences – Master of Science

2024

ABSTRACT

Botrytis cinerea is a necrotrophic fungus that causes gray mold disease in strawberries, leading to significant pre- and post-harvest losses. Considering there are no strawberry cultivars that show complete resistance to gray mold, this research is aimed at screening a selected set of germplasm consisting of both strawberry Cultivars and MSU Lines as promising candidates for breeding parents to develop resistance to *B. cinerea,* thereby reducing the use of chemical pesticides on strawberries. Our other goal is to see where advanced MSU Lines stand compared to Cultivars for tolerance to *B. cinerea*. The experiments were conducted in 2021 and 2022 with the fruits harvested from plants maintained in the Pathology Farm at Michigan State University (MSU). In total, 26 different genotypes were examined for their disease severity, Titratable Acidity (TA), and Soluble Solids Content (SSC). The Area Under the Disease Progress Curve (AUDPC) was calculated to quantify the level of tolerance, and the correlation between AUDPC to SSC and TA was analyzed to determine if there is any correlation. Allstar and Camarosa showed moderate tolerance, agreeing with previous studies on determining tolerance in literature. Three MSU Lines, MSU69, MSU78, and 14_30_4 were more tolerant than most of the genotypes evaluated. Although Cultivars performed better in general, many MSU Lines appeared to be significantly better than some Cultivars, such as Earliglow and Jewel. We also observed a positive correlation between SSC and AUDPC for Cultivars; however, TA did not show a correlation with disease severity. Overall, MSU Lines could be stated as moderately susceptible to gray mold; however, MSU 69, 78, and 14_30_4 were found to be promising lines to be used as parents along with cultivars tolerant to *B. cinerea* to increase the level of tolerance in the MSU germplasm.

Dedicated to my family.

ACKNOWLEDGMENTS

I want to express my profound gratitude to the Republic of Türkiye for supporting my education and research. I am particularly thankful for the financial support the Ministry of National Education of Türkiye provided through the YLSY scholarship program. As the Republic of Türkiye commemorates its centennial in 2023, I celebrate this momentous milestone and look forward to the country's continued prosperity and contributions in the centuries to come.

TABLE OF CONTENTS

INTRODUCTION

Strawberry as a Globally Important Crop

Strawberry is the common name of the *Fragaria* genus belonging to the Rosaceae family and the fruits of the species in this genus (Yang & Davis, 2017). Strawberry, an important member of the berry group, is a perennial and evergreen plant species (Cai et al., 2020). It is known to be native to North and South America (Edger et al., 2019) and is widely grown in the Northern and Southern hemispheres, as shown in Figure 1.

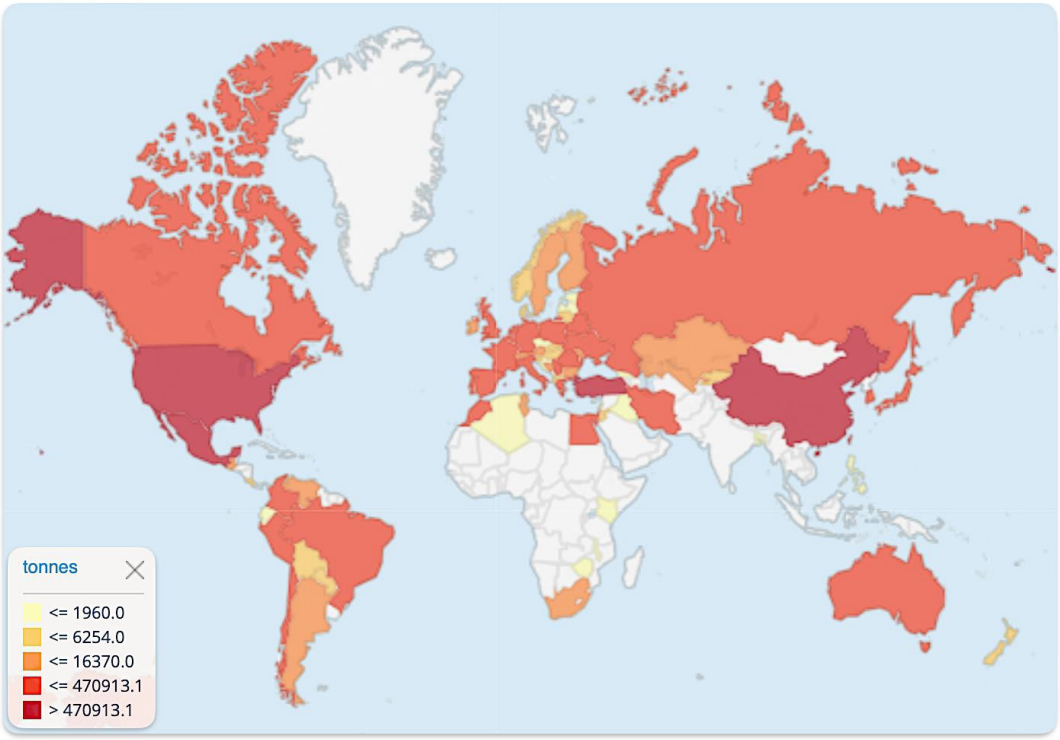

Figure 1. 2021 Global Strawberry Production in tons
Source: FAOSTAT (January 31, 2023)

The cultivated strawberry is an octoploid species resulting from an accidental hybridization that took place in a French botanical garden in the 1800s (Hummer, 2008). Since then, attempts have been made by several strawberry breeders and researchers to

broaden the narrow genetic base of strawberries by first evaluating the progenitor species for agronomically important traits and recreating the hybridization with desirable traits (Davis et al., 2007). Some of the recreated hybrids have been and continue to be used to improve agronomic traits in the cultivated strawberry.

Strawberries have been a preferred fruit for centuries, not only for their fresh and delicious taste but also for their vitamins, minerals, antioxidants, and health benefits. For example, recent studies show how eating strawberries could reduce neurodegenerative diseases in people (Giampieri et al., 2015). Agarwal et al. (2022) suggested that consuming strawberries can decrease brain inflammation associated with Alzheimer's disease. However, the health benefits of strawberries are not new. For example, it is mentioned that the famous Swedish botanist Carolus Linnaeus, known for giving the *Fragaria* name to the genus, cured himself of gout by eating strawberries in the late 1700s (Edger et al., 2019; Hjalmarsson, 2021). Ikram et al. (2019) stated that strawberries were previously only used as a medicinal plant until Carolus Linnaeus presented it as an edible fruit for the first time. However, strawberries are now considered a "functional food" that provides additional health benefits beyond basic nutrition. (Hasler & Brown, 2009). Giampieri et al. (2015) stated that phenolic compounds found in strawberries are able to neutralize free radicals and prevent their formation. They can also influence the expression of genes involved in cellular metabolism, survival, proliferation, and antioxidant protection. In addition, these phenolic compounds are capable of safeguarding and repairing DNA from damage. Strawberries are the fourth-most antioxidant-active common fruit after cranberries, red grapes, and apples (Sun et al., 2002). All these reasons given above enabled the widespread production of strawberries.

Status of Global Strawberry Production

In 2020, the worth of strawberry output globally was estimated at 14 billion USD (Hernández- Martínez et al., 2023). The top three producers of strawberries were China, the United States, and Spain over the 1980 - 2020 period, respectively; however, Türkiye took third place in 2021 (FAOSTAT, 2023).

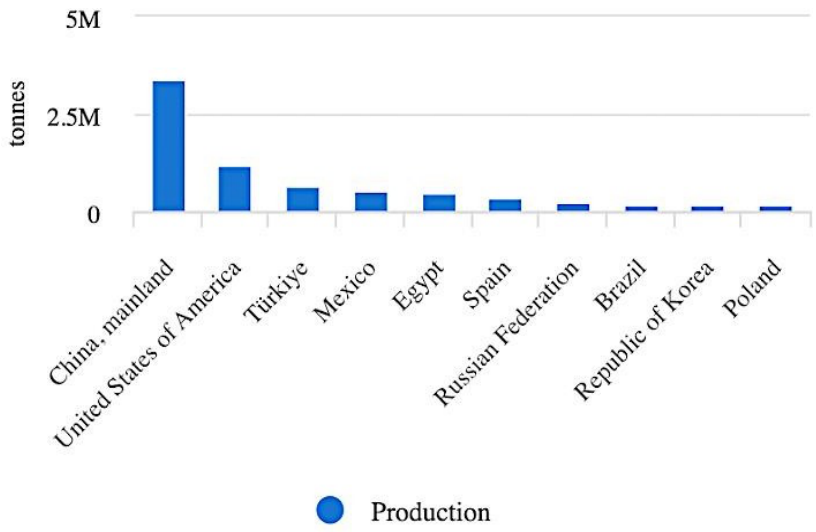

Figure 2. Strawberry production in the ten highest global producers in 2021
Source: FAOSTAT (January 31, 2023)

The United States was the world's largest producer of strawberries for many decades before being surpassed by China in 1994. Over the past decade, Mexico, Türkiye, and Egypt have demonstrated the most significant growth among all countries for strawberry production (Simpson, 2018). Figure 2 shows the top 10 strawberry producers in the world in 2021.

According to FAOSTAT (2023), global strawberry production increased significantly going from the 2000-2010 decade to 2010-2020. Hernández-Martínez et al. (2023) suggest that introducing new technologies such as plastic mulch, drip irrigation, and protected culture

systems has allowed farmers to increase yields and extend the growing season, and the demand for fresh strawberries has risen, leading to more acreage devoted to strawberry cultivation worldwide as shown in Figure 3 (FAOSTAT, 2023).

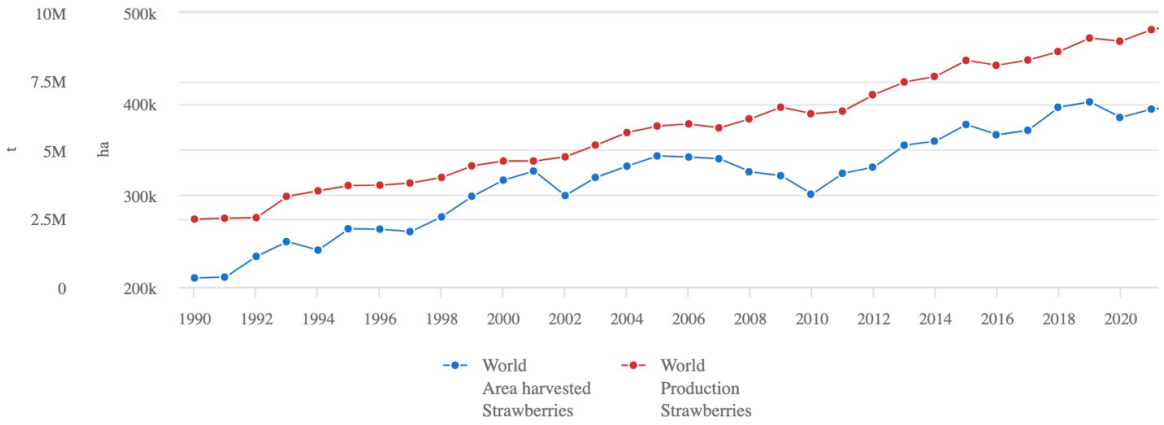

Figure 3. Production and yield quantities of strawberries in the world from 1990 to 2020
Source: FAOSTAT (February 15, 2024)

Status of the US Strawberry Production

In 2021, the US produced 1.3 million tons of strawberries (Statista, 2023). The total acreage of strawberry production in the US was 49,400 acres (USDA, 2023). The average yield of strawberries in the US was around 64,000 pounds per acre (71,450 kg/ha) in 2021. While the average price per hundredweight/centum weight (cwt) of strawberries in US grocery stores was $128, the total value of production reached $3.4 billion (USDA, 2023). According to the Supply Chain Management Education (SCM EDU, 2022), a cwt is referred to as 100 lbs., or approximately 45.36 kg in the US.

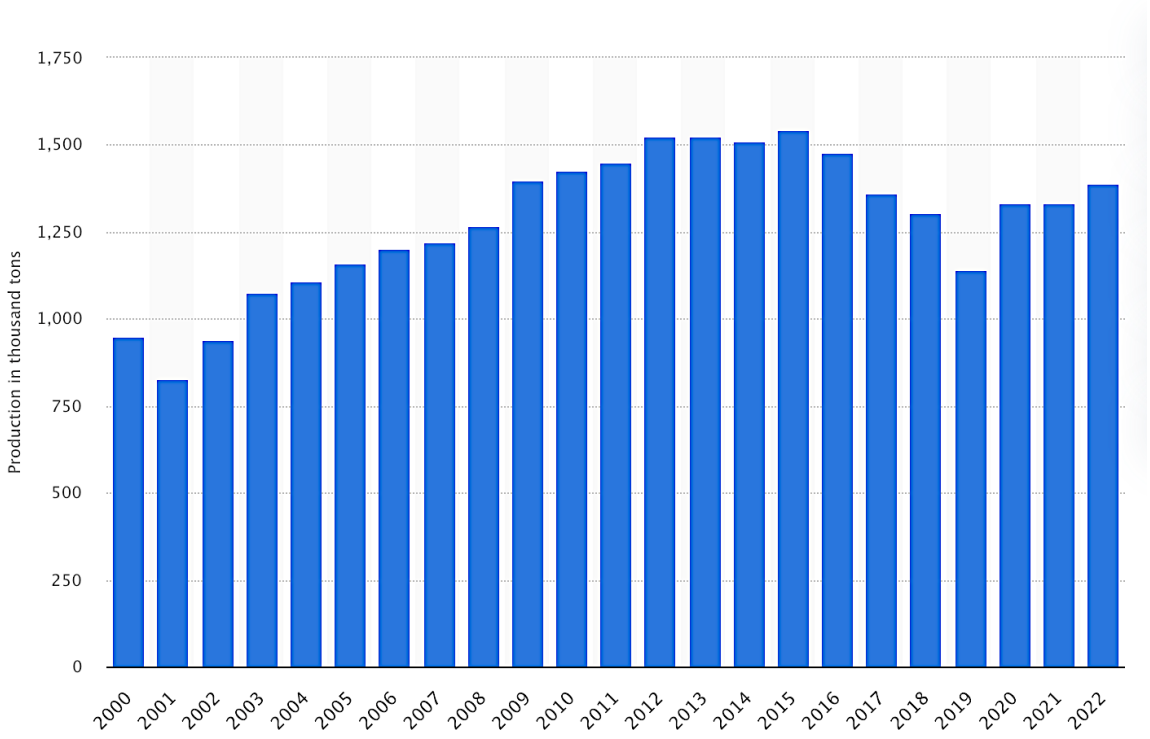

Figure 4. Total strawberry production in the US from years 2000 to 2022 (in 1,000 tons)
Source: (Shahbandeh, 2023)

As can be seen from Figure 4, there has been a steady increase in strawberry production since 2001, and a small drop in production started to be seen since 2015. Among the reasons, one could be because of the banning of Methyl Bromide (MB) as a soil fumigant due to its harmful effects of damaging the skin, eyes, and lungs in addition to causing problems with the respiratory and central neurological systems in humans and ozone-depleting effects (EPA, 2023; Carter et al., 2005). It was prohibited from use with The Montreal Protocol in developed nations starting in 2005 (Nellist, 2018), and EPA began phasing out MB until it was banned entirely in 2016 (Farnsworth, 2017). However, from 2020, an upward trend was observed in strawberry production, which could be due to the usage of new fungicides replacing MB and/or adapting new and better- performing varieties developed and selected in non-fumigated soils (Holmes et al., 2020).

According to the USDA (2023), the United States produced an estimated 6.26 billion pounds / 2.8 megatons of strawberries valued at $3.15 billion in 2021. California was the top-producing state of strawberries in the United States, producing an estimated 2.96 billion pounds / 1.3 megatons, followed by Florida with 1.41 billion pounds / 0.6 megatons and North Carolina with 590 million pounds / 0.2 megatons. Other top ten states producing strawberries were Oregon, Washington, Arkansas, Michigan, Indiana, Ohio, and New York, respectively.

Status of Michigan Strawberry Production

According to the USDA National Agricultural Statistics Service (2023), Michigan ranked as the 7th highest-producing state for strawberries in the United States in 2021, producing an estimated 75 million pounds. Michigan is one of the strawberry states in the Upper Midwest region, including Iowa, Wisconsin, Minnesota, North Dakota, and South Dakota, with cold winters and hot summers (Hancock, 2020). In the Upper Midwest, almost all commercial strawberry producers use the perennial matted row (PMR) technique, contrary to Florida and California, which utilizes the annual production system (APS) (Domoto et al., 2008; Samtani et al., 2019). The PMR system has been using short-day cultivars with a short harvest window from early June through early to mid- July, and strawberries are generally sold to markets and restaurants or sold through Pick Your Own operations (Samtani et al., 2019). Day-neutral varieties have been added to the growers' lists to obtain fruits throughout summer (Demchak & Harper, 2005). Having a longer time for the harvest is demanding; however, when strawberries are grown in a perennial system, tremendous work is expended to control weeds, diseases, and pests, which significantly decrease fruit output and quality.

Challenges of Strawberry Production

Diseases and pests significantly reduce agricultural output globally, resulting in high

expenditures due to yield losses and the cost of using pesticides to avoid or treat conditions. Strawberries face both abiotic (caused by non-living factors) and biotic (caused by living organisms) stresses.

Abiotic Stresses

Abiotic stresses can be caused by floods, drought, heat, cold, soil salinity, etc. Strawberries are sensitive to water stress. Inadequate or excessive watering can cause reduced growth, yield, and fruit quality (Zhang et al., 2020). High temperatures can cause heat stress, which affects plant growth and development by leading to reduced fruit yield and fruit size (Menzel, 2021; Morton, 2017; Palencia et al., 2013). Freezing temperatures of around 0°C (32°F) and frost cause injury to open flowers killing the pistils leading to misshapen berries and reduced yield potential (Shortt et al., 2022). High soil salinity levels can lead to water stress on the plant, impacting plant growth and development and leading to reduced yield and fruit quality (Shamshiri et al., 2018). In contrast, nutrient deficiency, such as nitrogen, phosphorus, calcium, or potassium, can lead to stunted growth, yellowing of leaves, reduced fruit yield, etc. (Lineberry & Burkhart, 1943). Another abiotic stress occurs when the fruit is exposed to too much direct sunlight, resulting in discoloration, sunken areas, and reduced fruit quality due to sunscald (Barber & Sharpe, 1971).

Biotic Stresses

Biotic stresses are caused by viruses and pests along with bacterial and fungal pathogens. One of the most common bacterial pathogens *Xanthomonas fragariae*, causes angular leaf spot, leading to lesions on leaves, which can become necrotic and reduce yield (Kennedy, & King, 1962; Kim et al., 2016). Several viruses, such as Strawberry Mottle Virus and Strawberry Crinkle Virus, can cause damage to the leaves, fruit, and plant development

(Prentice & Harris, 1946; Tzanetakis & Martin, 2013; Zeller, 1933). Various insect pests, such as mites, aphids, and thrips, can damage the leaves and fruits of the strawberry plant, leading to reduced yields and quality (Reddy, 2016).

Fungal pathogens can cause major diseases such as powdery mildew, anthracnose, verticillium wilt, and gray mold, causing significant damage to the plant and fruit if not appropriately managed (Patel, Lee & Peres, 2022). Anthracnose/black spot is caused by *Colletotrichum acutatum* or *gloeosporioides* (Brooks, 1931; Ma, 2022; Denoyes-Rothan et al. 2005; Lerceteau-Köhler et al. 2005), a common disease observed in North America and Europe. Thus far, resistant varieties have only been identified for *Colletotrichum acutatum* (Denoyes-Rothan et al., 2005; Lerceteau-Köhler et al., 2005; Louws & Cline, 2019). Fusarium wilt is caused by *Fusarium oxysporum* f. sp. *fragariae*. Although it is observed in North America, Europe, Asia, and Australia, resistant varieties are now available for this disease (Bancroft, 1876; Dávalos-González et al., 2022; Pincot et al. 2018). Verticillium wilt caused by *Verticillium dahliae* has been an issue for growing strawberries in North America and Europe, and resistant varieties to this disease have also been developed (Antanaviciute et al. 2015; Dávalos-González et al., 2022; Thomas, 1932). The presence of charcoal rot, caused by *Macrophomina phaseolina*, Phytophthora crown rot, caused by *Phytophthora cactorum*, and black root rot, caused by both *Fusarium* spp. and *Rhizoctonia* spp., tend to spread just as gray mold; however, resistant varieties are now available against these diseases as well (Dávalos-González et al., 2022; Golzar et al., 2007; Louws & Cline, 2019; Nelson et al., 2021).

To date, there are no resistant varieties available for several strawberry diseases including the Mucor fruit rot caused by *Mucor* spp., Leaf spot caused by *Mycosphaerella*

fragariae, Powdery mildew caused by *Podosphaera aphanis*, Rhizopus fruit rot caused by *Rhizopus* spp. and gray mold caused by *Botrytis cinerea* (Agyare, Magan, & Xu, 2020; Haller, 1771; Hammel, 2016; Nellist, 2018; Persoon, 1794). Of all these diseases, the presence of gray mold is more common and can be found worldwide. Despite many efforts, no fully resistant varieties have been identified for gray mold yet, although some tolerant varieties exist (Ma, 2022; Nellist, 2018; Petrasch et al., 2019).

To our knowledge, the level of disease tolerance available in current strawberry cultivars is limited, and this is especially true for gray mold. As such, strawberries are treated with fungicides since natural genetic resistance has not been able to prevent the development of postharvest gray mold disease (Petrasch et al., 2022). Even though there are several chemicals to control *Botrytis* incidence, strawberries are not fully protected against *Botrytis cinerea* because the pathogen's airborne inoculum is available at any time, and it could develop resistance rapidly (Petrasch et al., 2022; Hu et al., 2016; Leroch et al., 2013).

Considering that various diseases and pests affect strawberry production, many pesticides are used by strawberry growers to prevent and control diseases and pests. These substances have the function of either repelling or killing bugs, protecting the plants from damage, and ensuring a healthy crop. However, using pesticides excessively can result in environmental contamination and health risks from pesticide residues (Andreotti et al., 2009; Lee et al., 2007).

Pesticide Use for Disease Control

Utilizing the test data from the US Department of Agriculture, the Environmental Working Group (EWG) has been developing and publishing an annual report called "The Dirty Dozen List" since 2004, to classify several agricultural products based on their level of

pesticide residues from higher fungicide residues to lower (LaMotte, 2022). Considering that the strawberry industry tends to rely on chemicals to manage diseases and pests, strawberries have been at the top of the list for over 5 years (Shao, 2021). But stricter regulations on the use of chemicals to control pests and pathogens pose a challenge (Guthman & Jiménez-Soto, 2021).

The production of strawberries has been facing difficulties since the banning of active chemicals, including fungicides and soil fumigants like MB, which have increased the incidence and severity of certain diseases (Nellist, 2018; Samtani et al., 2019). Although there have been transition programs for MB with other alternatives (Holmes et al., 2020), additional limitations on chemical usage on strawberries may be needed to reduce the residues that remain on the harvested fruit and get strawberries off the dirty dozen list. Thus, there is an urgent need to develop and experiment with non-chemical alternatives to fumigation, such as cultivating plants that are resistant to diseases.

Screening MSU Germplasm for Resistance to Gray Mold

There are several commercially grown strawberry cultivars that are resistant or tolerant to the diseases, and breeders are interested in pyramiding these resistance genes. However, very limited cultivars such as San Andreas (Niewczas, 2021) show tolerance to the fungal disease gray mold, caused by *Botrytis cinerea* with the usage of additional products such as essential oils.

The strawberry germplasm pool at Michigan State University has incorporated wild germplasm into the breeding program for the past 30-plus years, and some of these wild progenitor species have shown resistance to various diseases and pests. However, the MSU strawberry germplasm is yet to be evaluated for tolerance to gray mold. As such, the objective

of the current study is to screen a selected set of MSU's advanced strawberry breeding lines for gray mold resistance in the hopes of finding genetic sources of resistance.

Botrytis cinerea

The following briefly describes the biology of *B. cinerea* and methods used to control the pathogen, including screening for resistant strawberry varieties. In summary, it captures the impact of *B. cinerea* on the strawberry industry.

Biology of *Botrytis cinerea*

B. cinerea belongs to the *Botryotinia* genus, Sclerotiniaceae family, in the Ascomycota phylum under the fungi kingdom (Oliveira et al., 2009). Historically, several species of *Botrytis* have been identified, with most of them being specific to certain hosts but some being generalists like *B. cinerea and B. pseudocinerea* (Valero-Jiménez et al., 2019). According to Dewey & Garfinkel (2021), *B. cinerea* has been observed on over 1,400 plant species across almost 600 genera up to date; however, it is believed that the actual number of hosts is likely to be even greater.

B. cinerea is an airborne pathogen that can be transmitted by soil, water, wind, etc. (Bhujel et al., 2022), but it can also survive in the soil for a long period of time (Akça & Tozlu, 2019). It has been known as a necrotrophic fungus since it can kill the plant host; however, recent mentions have considered it to be an endophyte because it can be present in the host undetected, which makes it harder to control (Dewey & Garfinkel, 2021).

Botrytis cinerea in Agriculture

B. cinerea produces gray mold disease in strawberries. In addition, it causes great losses to a wide variety of crops, such as fruits, vegetables, flowers, and ornamental plants, with 1400 identified hosts in 586 plant genera (Rosa et al., 2022). *B. cinerea*, labeled as the most

significant post-harvest pathogen (Hua et al., 2018), also appeared in the second rank in the Top 10 Plant Pathogen list based on its scientific and economic significance (Dean et al., 2012).

Gray Mold in Strawberry

One of the major diseases affecting strawberries is gray mold, which can occur before or after harvesting, leading to a significant reduction in yield or even a total yield loss. *B. cinerea* can infect each part of the plant (Petrasch, 2019; Garrido, 2011), enabling pre-harvest losses. Due to their high respiration rate and physical characteristics, strawberries are also highly susceptible to post-harvest storage issues (Kahramanoğlu et al., 2022). Thus, gray mold is a significant obstacle to the strawberry industry.

Infection Mechanism

B. cinerea has two mechanisms to infect strawberries: conidia and sclerotia (Rhouma et al., 2022). Conidia are asexual spores produced under favorable environmental conditions and facilitate rapid growth and reproduction. Conversely, sclerotia are sexual spores that enable the fungus to endure unfavorable environmental conditions (Willetts, 1971; Brandhoff et al., 2017; Hua et al., 2018).

Gray masses of asexual spores called conidia are produced by the fungus and can be seen on leaves and soft fruits. When these spores land on a host surface, they produce germ tubes within a few hours, allowing the fungus to penetrate the plant directly by degrading the cell walls or indirectly through wounds, hydathodes, and stomata (Bai et al., 2022; Petrasch et al., 2019; Roca-Couso et al., 2021; van Kan, 2003). *B. cinerea* generates toxins, reactive oxygen species (ROS), and enzymes that break down cell walls (Zhao et al., 2022). For example, polygalacturonase (PG) breaks down homogalacturonic acid, a cell wall pectin, which leads

to tissue degradation, making it easier for pathogenic fungi to invade the plant (Hong et al., 2024).

The fungus continues to grow into the epidermal cells and produces chemicals that damage the host cells, causing the plant tissue to die. After the plant tissue dies, *B. cinerea* feeds on the dead tissue and persists as a necrotroph, producing conidiophores that release conidia or conidiospores that can land on other plants or soil and continue the life cycle of the fungus (Rosa et al., 2022).

Environmental Factors Promoting Disease Incidence and Severity

B. cinerea can endure moderate winters in both mycelium and sclerotia forms (Jarvis, 1962). The fungus can survive the winter season outdoors as mycelium on plant debris or irregular sclerotia form in the soil (IPM, 2000). *Botrytis* can also be active at low temperatures and affect vegetables stored at 32 to 50°F (0 to 10°C) for several weeks or months. Higher temperatures above 77°F (25°C) are less favorable for infection. However, once *Botrytis* infects a plant, it can grow between 32 to 96°F (0 to 35°C).

Sclerotia are the primary method that *Botrytis* survives in the field and can remain viable between 39 to 131°F (4 to 54°C). Usually, sclerotia develop conidia, but they can also release directly penetrating infection hyphae (IPM, 2000). Hence, the overwintering stages of the organism are highly significant sources of initial inoculum (Jarvis, 1962; Romanazzi & Feliziani, 2014). The sclerotia can also produce apothecia, a sexual fruiting body that produces infective ascospores (IPM, 2000).

Impact on Production and Fruit Loss

B. cinerea has a detrimental impact on strawberry production, along with its capacity to affect a broad range of hosts. About 80–90% of blooms and fruit might be lost during rainy

seasons on unsprayed plants (Petrasch, 2019; Ries, 1995). Yan et al. (2021) stated that losses ranging from 25% to 55% had been reported during harvest because of *B. cinerea* infection, while post-harvest losses can reach as high as 89%. It is estimated that annual losses because of *Botrytis* spp. range from $10 to $100 billion worldwide (Boddy, 2015; Hua et al., 2018). The estimated average cost of controlling *Botrytis* is $18 per acre (Dewey & Garfinkel, 2021). However, Steiger (2007) noted that the management cost could vary depending on the significance of *Botrytis* diseases for a particular crop and its value, potentially reaching up to €130 per hectare or approximately $57 per acre. Overall, it affects up to 60% of fruits and causes them to rot and become inedible (Lu et al., 2020).

Pesticide Usage to Manage *B. cinerea* and its Negative Impacts

Switch 62.5WG, Merivon, Pristine, Fontelis, Kenja, Luna Sensation, Luna Privilege, Captan 50WP, Thiram Granuflo, Elevate 50WP, Rovral 4F, Topsin-M are some pesticides that have been used to control *B. cinerea* given in the order of effectiveness from highest efficacy and lowest potential for inducing resistance as evaluated by Koike et al. (2018) using the IPM (Integrated Pest Management) value. However, most chemicals are now ineffective against *B. cinerea*, especially Pristine, Elevate, and Rovral 4F, and as such, the order of effectiveness would have changed (Koike et al., 2018; Vischetti et al., 2023). Captan, the primary broad-spectrum fungicide applied to manage gray mold in Florida (Gama et al., 2023), once considered as having a low risk for developing resistance (Petrasch et al., 2019) is not as effective in controlling *B. cinerea* (Amiri et al., 2018; Weber & Petridis, 2023).

The expenses for *Botrytis* control represent about 10% of the fungicide market worldwide (Bestfleisch et al., 2015; Dean et al., 2012; Fillinger & Elad, 2016). Although many fungicides and pesticides have been used against gray mold disease, they have yet to provide

100% protection to date.

Unfortunately, many fungicides applied in fields are known to end up in unintended areas. They can contaminate water bodies or seep into the soil, resulting in environmental pollution, disrupting the ecological balance of entire ecosystems, and adversely affecting non-target organisms. For example, Cyprodinil is very hazardous to freshwater and marine organisms and mildly poisonous to fish (EPA, 1998).

Furthermore, the presence of these chemicals can elevate the residue levels of agrochemicals in the final product (de Moura et al., 2021). Due to the long-term health concerns posed to several life forms, the environmental advantages of synthetic chemical pesticides in agriculture may cause several disadvantages (Ramakrishnan et al., 2021).

Romanazzi & Feliziani (2014) state that the requirement for an appropriate implementation of Fungicide Resistance Action Committee (FRAC) guidelines advising a restricted number of sprays per year is highlighted by the fact that fungicide-resistant *B. cinerea* strains are becoming increasingly common.

Genetic Resistance of Strawberry Cultivars for Gray Mold

Because of the significant effect caused by gray mold on strawberries, scientists have been focusing on how to protect fruits for the strawberry industry. There have been many studies testing the efficiency of fungicides, but as indicated before, there has not been 100% protection. Moreover, *B. cinerea's* resistance to fungicides can change significantly even in a single growing season (Cosseboom et al., 2019; Petrasch et al., 2019). There is disagreement among experts as to whether one cultivar is typically more resistant than another, with evidence that there is diversity in the susceptibility of strawberry genotypes to *B. cinerea* (Reyes, 1990).

LITERATURE REVIEW

Numerous screening attempts have been made to search for *B. cinerea* resistance in strawberries. Preharvest and postharvest screening are the two main methods to screen the strawberry germplasm for *B. cinerea* resistance. Blossom screening, field trials, and yield data are preharvest methods being used to determine resistance, while storage, inoculation, incubation, juice extracts, and fruit firmness are postharvest methods (Maas, 1978; Reyes, 1990).

Firmness of the strawberry fruit was found to be a significant characteristic linked to *B. cinerea* resistance (Hancock et al., 2008; Petrasch et al., 2019; Terry et al., 2004). According to Daubeny and Pepin (1977), firmness and the frequency of fruit rot were correlated and fruits with a firm texture demonstrated postharvest resistance to gray mold. However, firmness does not guarantee that disease will not occur. Postharvest-resistant cultivars had somewhat soft fruit but a low frequency of infection, according to Barritt's research (1980) in comparison to firm fruits against *B. cinerea*.

Seijo et al. (2008) used Camarosa and Albion cultivars in their experiment for screening their germplasm to see the resistance against *Botrytis*, and Camarosa was one of the cultivars that displayed a good level of resistance among other varieties. Camarosa was also found to be more tolerant to gray mold compared to Sweet Charlie and Earlibrite by Chandler et al. (2004).

Although it is one of the oldest experiments, Mass (1978) used 33 different cultivars, and Earliglow was the superior cultivar among them for the postharvest incubation analysis that showed resistance to *B. cinerea*. Additionally, Honeoye (Sanford et al., 1982) had the strongest resistance to fruit rots, including gray mold, that considered 10 cultivars along with

Allstar and Earliglow in experiments. While Allstar was considered less susceptible along with Earliglow and Jewel by Madeiras and Schloemann (2017), more recently, Earliglow and Jewel were found to be less susceptible whereas Allstar was found to be severely impacted by gray mold in experiments conducted by Carroll & Pritts, (2022).

Petrasch et al. (2022) state that although gray mold resistance is heritable, it is quantitative and genetically complicated. Multiple genes are involved in controlling strawberry traits with resistance (Amil-Ruiz et al., 2011; Folta & Davis, 2006). Given that, agronomic traits may play an important role in searching for the resistance in strawberries to *B. cinerea*.

Many strawberry cultivars including Albion, Camarosa, and San Andreas were put to the test under the high tunnel production by Guan & Sutterer (2017). They reported that unmarketable fruits were mostly caused by *Botrytis*. They analyzed agronomic traits such as average fruit weight, soluble solids content (SSC), pH, titratable acidity (TA) and firmness of berries. Generally, San Andreas showed better results among these three cultivars. For disease severity, Albion had a better outcome; however, Camarosa was more competitive for other traits.

Customers notably enjoy strawberry fruits for their sweet flavor, which is connected to their SSC and TA values (Hasing et al., 2013; Jouquand et al., 2008; Kafkas et al., 2007; Shaw, 1990; Sweeney et al., 1970). According to Kubota Lab (n.d.), the SSC to TA ratio appears to be an effective indicator of overall sweetness as the strawberry fruit tends to be viewed as sweet when the Brix to TA ratio is greater than one. Ganhão et al. (2019) state the analysis of the relationship between TA and SSC in several strawberry cultivars revealed a positive correlation between the two quality parameters. Since the findings showed a strong

connection between SSC and *B. cinerea by* Mancini et al. (2023), it would be interesting to see whether screening for BRIX and TA can be used as an indirect method to screen for gray mold resistance.

Also, there has been a search for methods other than fungicides to control the gray mold disease on strawberries, such as using bioagents (Faedo et al., 2022; Yong et al., 2022; Zhang et al., 2022; Moura et al., 2021), thermotherapy (Gahatraj et al., 2023), UV lights (Forges et al., 2018; Jin et al., 2017; Nigro et al., 2000), essential oils (Alves et al., 2022; Hassan et al., 2021), etc. However, these methods have only reduced the incidence and are not accessible to all growers.

The protocols for screening strawberries for *B. cinerea* resistance are not standardized or utilize specific chemicals or varieties. Thus, it is harder to compare the resistance levels among different varieties used in the various studies dedicated to screening for resistance.

Importance of Breeding Resistance

Most of the chemical control, such as the use of Pristine, Elevate 50WD, and Rovral 4F, are now ineffective against *Botrytis cinerea* due to the pathogen acquiring resistance (Koike et. al., 2018; Petrasch et al. 2019). Continued use of pesticides can lead to the development of pesticide-resistant pathogen populations, making it more difficult to control (Deising, et al., 2008). Thus, breeding efforts need to be enhanced to develop resistance against gray mold.

Concluding Remarks

B. cinerea has a destructive impact on strawberry production. Due to the development of resistant pathogen strains and risks to human health and the ecosystem, fungicides are not an appropriate management method long term. Finding strawberry genetic sources with *B. cinerea* resistance within our germplasm has gained our attention due to the lack of fully

resistant cultivars (Carroll & Pritts, 2022). The objective of this study was to A) screen the strawberry germplasm at MSU with *B. cinerea* inoculation to detect any resistance and B) determine if there is a correlation between disease progression of *Botrytis cinerea* and SSC and TA that could be used as a potential method for indirectly selecting for resistance.

MATERIALS & METHODS

Materials

The fruit used for the experiment was obtained from plants established in the Pathology Farm at Michigan State University (MSU) in East Lansing, Michigan. The germplasm used in the study included commercial cultivars and MSU advanced breeding lines that have been obtained over the years.

Ten strawberry cultivars, namely Albion, Allstar, Cabot, Camarosa, Cavendish, Earliglow, Honeoye, Jewel, Redstart, and Wasatch along with 14 MSU advanced breeding lines; MSU44, MSU49, MSU69, MSU71, MSU73, MSU75, MSU76, MSU78, MSU80, MSU81, MSU86, MSU90, 14_30_4, and 16_1_1 were used to comparing tolerance to gray mold in 2021. In 2022, two additional cultivars, Annapolis and San Andreas, were added to the experiment, while two MSU advanced breeding lines, MSU 86 and MSU 90, were not included due to not having sufficient fruit in time for the experiment.

We decided to use popular publicly available commercial strawberry cultivars in our study so that agronomic traits of MSU advanced breeding lines could be compared to existing cultivars to determine which cultivars can be used as breeding parents to further improve existing germplasm at MSU. Following is a brief introduction to each of the cultivars used in the study.

Albion (U.S. Plant Pat. No. 16.228) is a day-neutral cultivar comparable to Aromas (U.S. Plant Pat. No. 10,451) but with larger, higher quality, firmer, and better-flavored fruit. It is also similar to Diamante (U.S. Plant Pat. No. 10,435) but with a lower cull rate, darker fruit, and significantly better resistance to *Phytophthora cactorum*, causing *Phytophthora* crown rot (Shaw & Larson, 2005) but susceptible to gray mold (Moura et al., 2021). Allstar is a

June-bearing cultivar with a sweet and juicy firm texture. It has been reported to be susceptible to many pathogens and diseases like viruses, mites, and nematodes, as well as verticillium wilt, foliage, fruit, and root rot diseases, to name a few (Missouri Botanical Garden, 2023; Gardenia, 2023) although previously recorded to have multiple resistance to red stele and other root and leaf diseases, as well as some tolerance to *Botrytis* fruit rot under lower inoculum conditions (Galleta et al., 1981). Annapolis is a June- bearing variety, being sweet, firm, and cold-hardy, and also known for its good resistance to red stele (Gurney's Seed and Nursery, n.d.). Cabot, known as a short-day strawberry, has a large fruit structure with a big calyx and shows resistance to the red stele root (Jamieson, 2006). The inventors of the Camarosa cultivar, Voth et al. (1994), claim that despite being a short-day cultivar, it has greater early and overall productivity as well as larger and more firm fruits. Cavendish is known for its suitability for the Northeast climatic zone, its resistance to red stele, and its high-yielding midseason (Jamieson et al., 1999). Although Earliglow is not known for its high yield, it is a highly preferred cultivar in the US due to its aroma, early fruiting season, and red stele resistance (Hokanson & Finn, 2000). Honeoye, being one of the early-season cultivars, was introduced in 1979, and it has been produced successfully in MI since then due to its winter hardiness and vigor (Hokanson & Finn, 2000). Although it is susceptible to black root rot disease, it is believed to have some resistance towards powdery mildew (Cornell Fruit Resources: Berries, 2022) and gray mold (Sanford et al., 1982) as well as resistant to red stele (Hokanson & Finn, 2000). Sanford et al. (1987), who are inventors of the cultivar Jewel, claim that it shows better performance than Honeoye in terms of combined scores of fruit rot diseases such as *Botrytis*, soft rot, and white mold. They also stated that Jewel is susceptible to verticillium wilt and red stele; however, it is resistant to mildew and leaf spot. Redstart,

with publication number 20180255673, is one of the day-neutral varieties that James Hancock released from Michigan State University (Justia Patents Search, n.d.). He declared that it proved to be more vigorous, had greater yields, and had better fruit color compared to Albion, although Redstart produced smaller and softer fruits (MSU Technologies, 2015). Shaw & Larson (2009) express that San Andreas is an everbearing cultivar that is similar to Albion; however, it is known to be somewhat tolerant to powdery mildew, common leaf spot, verticillium wilt, *Phytophthora*, and Anthracnose crown rot. San Andreas is also known to be tolerant to *Botrytis* fruit rot (Miles et al., 2017), which we used as a control cultivar for our experiment. Wasatch, publication number 20180255672, offers larger yields, better fruit color, stronger plant vigor, and a similar good flavor to Albion while being less firm and having smaller fruits (Justia Patents Search, 2017).

Our goal is to determine the tolerance level of the MSU strawberry advanced breeding lines against *B. cinerea* compared to existing commercial cultivars mentioned above. As such, we inoculated the fruits with *B. cinerea* spores and provided these spores with ideal conditions to grow and infect the fruit.

Fungal Inoculum

The *B. cinerea* isolate was obtained from Dr. Tim Miles' lab's long-term culture preservation collection at MSU in East Lansing, Michigan. The culture was set up on V8 agar (200 mL V8 juice, 10 g CaCO3, 3 g agar, 800 mL distilled water per liter) (Krasnow et al., 2017; Jeffers, (Ed.) 2015) and preserved for 7 days at room temperature under fluorescent lighting.

The growth media was prepared approximately one week before the experiment for both years to allow time for subculturing. V8 Juice Agar was used as a growth medium to grow *B.*

cinerea in this experiment because the 8-vegetable juice, when used as a substance, mostly tomato, stimulates fungal growth while suppressing bacterial growth (Maung et al., 2021).

Subculturing of the *Botrytis* isolate was done with plate-to-plate method (Jain et al., 2020). Plates were kept for one week for both years to let fungi grow enough hyphae.

To prepare the spore suspension for inoculation, twenty drops of Tween20 were added to 500 mL of autoclaved dH_2O, of which 4000 µL was added to each plate containing the fungus. To remove the spores from the fungal mycelia, a flame-sterilized glass hockey stick was used to carefully rub the plates. Once the spores had been rubbed off, without damaging the agar, the mixture was placed into 50 mL Eppendorf tubes using a pipette with the tip cut off. The Eppendorf tubes were then placed on ice and refrigerated until needed for inoculation.

After removing the spore suspension from the refrigerator, the suspended spores were poured over 2 layers of cheesecloth to filter out sclerotia and hyphae from the spore suspension. Thus, clear liquid was collected from all the tubes with *Botrytis* spores to average the concentration.

Figure 5a. Hemocytometer

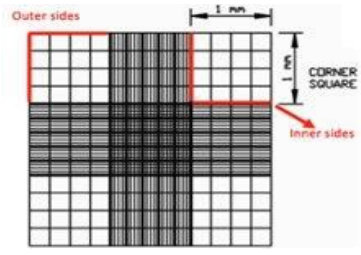

Figure 5b. The grid of a hemocytometer
Source: Chan, 2020

Using the grided slide of the hemocytometer (see Figures 5a and 5b), the concentration of spores was determined by placing 20 µL of the filtered spore suspension and counting the

number of spores as described by Thermo Fisher Scientific - IE. (n.d.). We counted the 4 corners and the middle box on the grid and only counted spores that touched 2 out of the 4 sides of the boxes. This ensures that there is no overestimation. With the help of the dilution formula ($C_1V_1=C_2V_2$), the spore concentrations for sprays were calculated for both years and kept the same amount for accurate comparison.

Experimental setup

The experimental setup was designed to create a micro-environment for the *Botrytis* spores in the inoculum to germinate and infect the fruit. This way, the number of days that the fruit stays uninfected can be considered as a measurement of tolerance of the strawberry genotype being tested.

Figure 6a. The setup in the laboratory

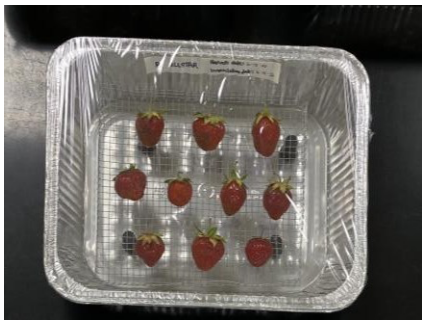

Figure 6b. Replication setup for screening

To create the ideal environment for *Botrytis cinerea*, two nested aluminum pans were placed together and covered with plastic wrap to maintain humidity and prevent the pathogens from spreading. To keep the berries in place, a metal screen was placed on four stoppers in each tray as shown in Figure 6b. A thin layer of water was placed at the bottom of the trays to retain humidity.

Harvesting all the berries for the experiments was done at once for both years instead of

harvesting and organizing different batches depending on when the fruit was available. In 2021, fruits were harvested on June 21; we had to gather fruit from every genotype on the same day in order to reduce the chance of losing fruits to severe rain. In 2022, fruits were harvested as soon as they ripened on June 15 to maintain the experiment setup consistent. Once the fruit was harvested, soil particles adhering to the fruit were gently cleaned using a Kleenex tissue prior to using the fruit in the experiment. In 2021, however, due to heavy rain prior to harvesting, the fruit had more soil particles attached and had to be gently rinsed with de-ionized water using a colander and dried prior to placing the fruits in the trays and inoculation. Because we observed high levels of bruising and some bleeding of the fruit from rinsing, we did not rinse the fruit with water in 2022. Instead, they were placed directly in the trays after gently cleaning the fruit with Kleenex tissue since we did not have a problem due to the weather conditions. Artificial inoculation was done at the same angle for each inoculated tray by spraying the spore mixture 10 times for both years.

Screening the Disease Progression

Over two strawberry seasons, fruits were monitored for fourteen days to observe their decay process after inoculation with *B. cinerea*. Considering that field-grown strawberries already carry multiple pathogens at harvest, and ripe fruits cannot be surface sterilized without damaging the skin, which would impact fruit evaluation, it was expected that other fruit rot pathogens would appear in the experiment, including but not limited to *Rhizopus and Colletotrichum*.

Figure 7a. *Botrytis* signs with gray messes on the berry (Louws, 2014)

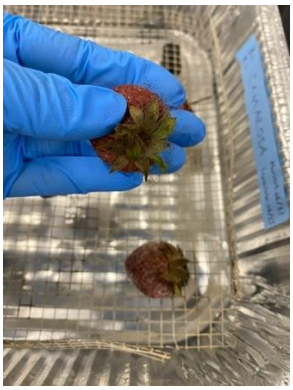

Figure 7b. *Rhizopus* signs with white hyphae ending with black and/or white tips (Koike et al., 2018)

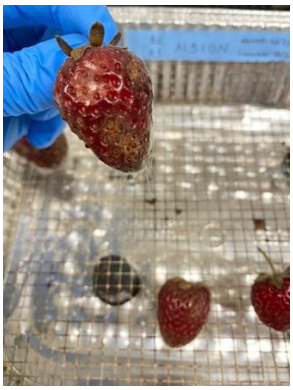

Figure 7c. *Colletotrichum* signs on the fruit with sunken areas covered with orange spores (Demchak et al., 2023)

Since we were particularly evaluating MSU's strawberry germplasm for tolerance to *B. cinerea*, we made sure to identify the mycelia characteristics of both *Botrytis* and *Rhizopus* clearly, as their mycelia tend to look similar to the naked eye. However, *Rhizopus* has black or white spheres at the end of white mycelium (Koike et al., 2018), whereas *Botrytis* has a white to gray cottony mass on the berries (Louws, 2014), as shown in Figures 7a and 7b.

Although most of the disease experiments considered the percentage of disease covering the fruit, observing multiple diseases in the trays, especially *Rhizopus* fruit rot, led us to take steps to minimize cross-contamination of fruit with other pathogens while evaluating disease progress for *Botrytis*. For example, if *Rhizopus* infected the fruits, those fruits had to be removed immediately to prevent the overnight infection of other berries on the tray because sporangia are packed with thousands of spores and tend to spread quickly (Koike et al., 2018).

The berries in all replicates of different genotypes tested were evaluated daily for *Botrytis* symptoms for 2 weeks, and any berries showing enough signs to identify gray mold disease were discarded from the experiment. However, berries that were showing symptoms of anthracnose disease were not removed immediately, considering they may still show signs

of a *Botrytis* infection. The data was collected for each fruit by mentioning which day they were discarded and which diseases they showed symptoms of. However, data analysis was only focused on *Botrytis*- related symptoms.

Our objective for 2021 was to obtain 8 replications for each genotype, with 10 fruits on each tray. However, due to the difficulty of maintaining this for every genotype, we reduced the number of replications to 5 in 2022. Unfortunately, some genotypes experienced fewer fruit numbers than intended because of the weather circumstances. In addition, we had to discard some berries from the experiment due to factors such as *Rhizopus* fruit rot and bruising. If the remaining fruit count fell below 5 on a tray, we decided to eliminate those reps, resulting in some genotypes having less than 3 replications, and those were not included in the analysis to obtain accurate and unbiased results.

Agronomic Traits

Considering the challenges of evaluating strawberries for *Botrytis* tolerance, we considered if there may be agronomically important traits that breeders select for that may correlate with tolerance to *Botrytis*. If such a correlation exists, it may help to indirectly select genotypes for tolerance to gray mold disease using the agronomic trait instead. As such, we decided to measure the Soluble solids content (SSC) using the BRIX value, which determines the sweetness of the fruit, and titratable acidity (TA), which speaks to the sourness or acidity of the fruit, two important indicators of determining the flavor of strawberries (Jouquand et al., 2008; Kubota Lab, n.d.).

Soluble Solids Content

According to Barrett's (n.d.) protocol, Brix values were measured using a Brix Refractometer with ATC (Tiaoyeer, 2018) and reported as °Brix for the germplasm's SSC. To

determine SSC, fresh fruits were used immediately after harvesting, and readings were taken in the laboratory under the optimal temperature frame.

Titratable Acidity

TA measurement was done by using the Mettler-Toledo Titrator (Rondolino, 2017). Between 7 to 15 grams of samples were added to 25-30 mL of deionized water and titrated with 0.1 NaOH (4g/L) to an endpoint of pH 7. The titratable acidity is expressed as a percentage per gram of fruit.

Data Analysis

The tolerance level to *B. cinerea* was determined by the number of days before the fruits started showing gray mold signs. Area under the disease progress curve (AUDPC) is an effective standardized quantitative evaluation method for determining disease severity over time (APS, n.d.). According to Jackson (2017), lower AUDPCs indicate stronger disease resistance and a slower rate of disease progression, while greater susceptibility to the disease and quicker disease progression is indicated by higher AUDPC values. The formula for AUDPC involves adding together the two infection percentages and dividing by two to obtain the mid-value between the two readings, and multiplying the average by the time interval, the number of days between the first and second readings until the last reading. The sum of these trapezoid areas represents the AUDPC value. Time intervals can be for 2, 4, or even more days (Cockerton et al., 2019; Rahman & Louws, 2017); however, we obtained disease incidence daily for 2 weeks to determine the susceptibility of berries and to minimize contamination of fruits by *Rhizopus* in an attempt to obtain accurate data for *Botrytis* disease progression. AUDPC values were individually calculated using the trapezoid area method described above in Excel per each genotype's replicates. We used 8 replicates in 2021 and 5

replicates in 2022.

Statistical analysis was performed using SAS software (version 9.4; SAS Institute Inc., Cary, NC). PROC GLM statement was used for analysis of variance (ANOVA) since our data was unbalanced (SAS Help Center, 2019), and PROC REG was used for linear regression analysis. PROC GLM was also used for Tukey's Studentized Range Test (HSD) and PROC MEANS statement was conducted to compare MSU Lines with Cultivars. A significant difference ($p < 0.05$) between the genotypes for AUDPC 2021 and 2022 values is shown by different letters according to their mean separation test results done by SAS and Phyton (version 3.12; Python Software Foundation).

RESULTS

The results of the AUDPC, BRIX, and TA comparisons, as well as the correlation of AUDPC with BRIX and TA measurements for a selected set of germplasm that includes Cultivars and MSU Lines are given below.

Area Under the Disease Progress Curve (AUDPC)

The AUDPC was used to calculate how the gray mold disease progressed from inoculation to showing signs as a quantitative measurement of disease incidence by percentages over time. We expect a lower AUDPC value for the most tolerant genotypes, while the most susceptible genotypes would show a higher AUDPC value. Each fruit per genotype of every replicate was rated daily for 14 days, and the number of fruits removed due to gray mold symptoms was recorded and plotted against time to obtain AUDPC values for the cultivars and MSU lines in both 2021 and 2022. With the AUDPC values, our goal is to determine the level of tolerance to *Botrytis* in the MSU germplasm compared to commercially available strawberry cultivars that are tolerant or susceptible to gray mold. Considering this is the first attempt to evaluate the tolerance of the current MSU advanced breeding lines to gray mold, we assessed the AUDPC values for both years to see if A) the cultivars that are documented to be tolerant or susceptible to gray mold are consistent in our experiment, and B) whether MSU germplasm shows any tolerance to the disease. Because MSU's advanced breeding lines have wild germplasm incorporated and were bred for over 30 years in non-fumigated soils, we expect to see some disease tolerance. As such, this comparison would inform the breeder whether the tolerance level is significant compared to existing commercial cultivars or if the program needs to assemble tolerant germplasm from other sources to increase tolerance to gray mold in the MSU germplasm pool.

Our initial comparison was to see whether commercial cultivars are more tolerant to gray mold than the MSU germplasm. Although the AUDPC values for Cultivars and MSU Lines in 2021 were not significantly different from each other (F (1, 115) = 0.98, p = 0.32), given that lower AUDPC values mean more tolerance against diseases, MSU Lines showed more tolerance than Cultivars in 2021.

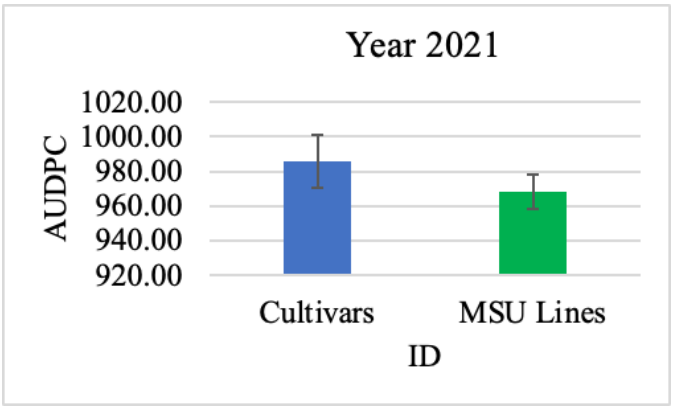

Figure 8. Area Under the Disease Progress Curve (AUDPC) comparison between Cultivars and MSU Lines in 2021

Then, we compared the AUDPC values for individual genotypes to determine which MSU lines were more tolerant or susceptible to gray mold than the commercial cultivars. The results from the 2021 AUDPS values are shown in Figure 9.

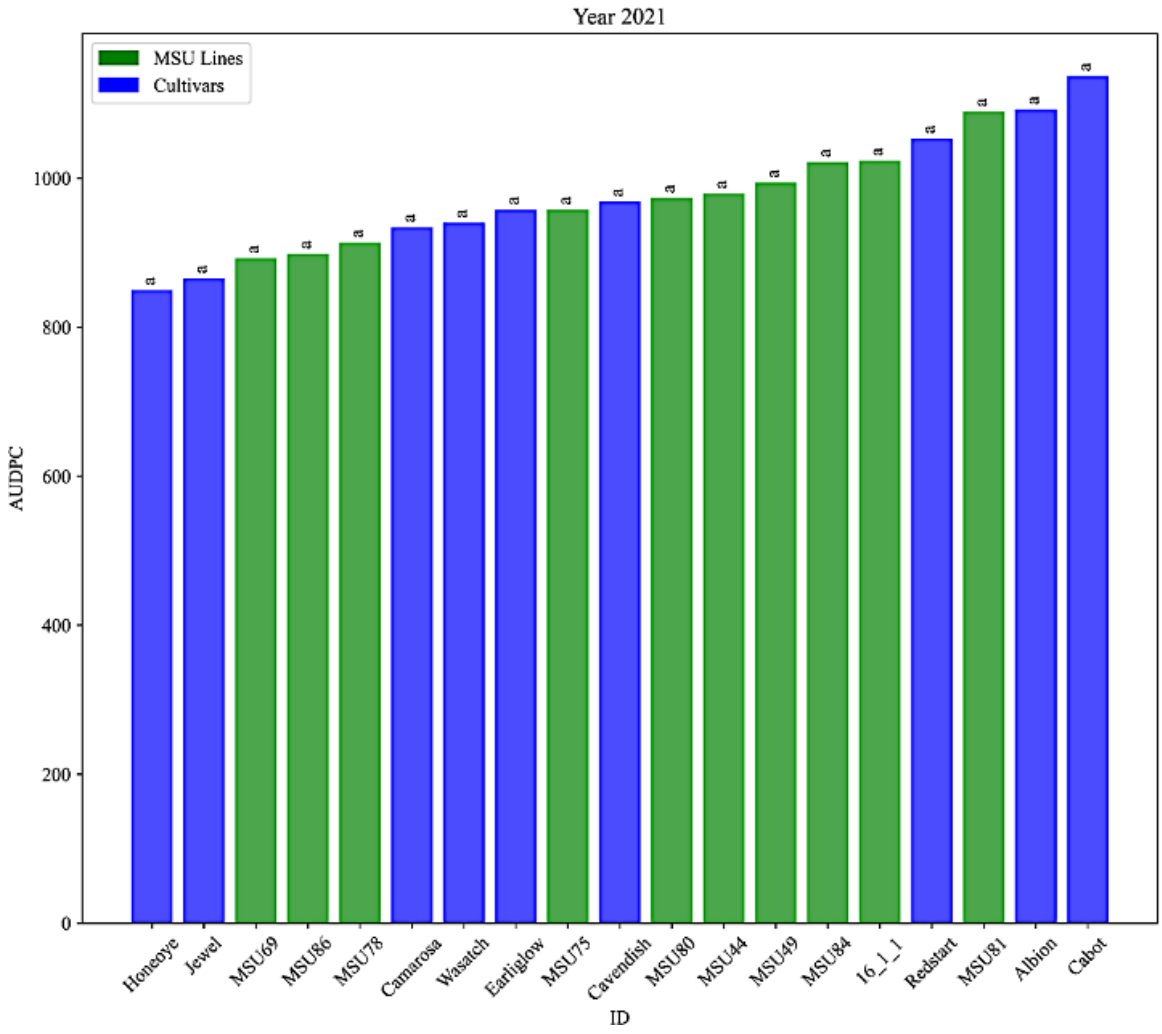

Figure 9. Area Under the Disease Progress Curve (AUDPC) for all genotypes inoculated with *B. cinerea* in 2021

Comparison of Cultivars used in 2021 were ranked based on their AUDPC values. Of the cultivars tested, Honeoye and Jewel were found to be somewhat more tolerant, while Camarosa, Wasatch, Earliglow, and Cavendish were moderately susceptible. The other three cultivars, Redstart, Albion, and Cabot, had higher AUDPC values, indicating greater susceptibility.

MSU69 was the most tolerant MSU Line compared to the rest of the MSU germplasm tested. MSU86, 78, and 75 were comparatively more tolerant than the other MSU Lines.

MSU80, 44, and 49 were moderately susceptible, whereas MSU84, 16_1_1, and MSU81 were highly susceptible to gray mold.

In summary, in 2021, Honeoye, Jewel, and MSU 69 showed more tolerance, and Redstart, MSU81, Albion, and Cabot showed greater susceptibility in comparison to other genotypes.

Cultivars and MSU Lines in 2022 showed a different pattern than in 2021. In 2022, Cultivars showed more tolerance to *Botrytis* than the MSU Lines overall; however, they were not significantly different (F (1, 88) = 2.69, p = 0.10).

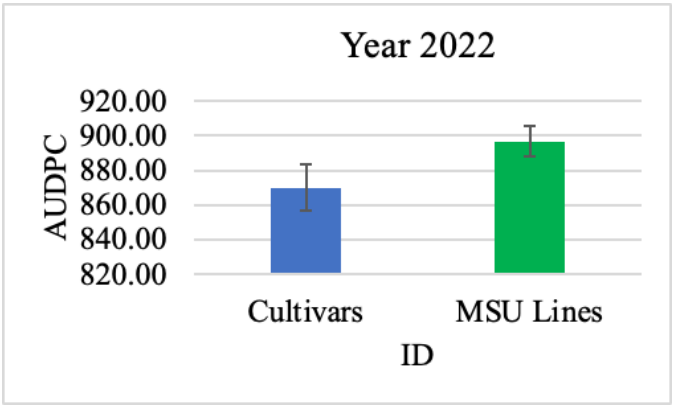

Figure 10. Area Under the Disease Progress Curve (AUDPC) comparison between Cultivars and MSU Lines in 2022

In contrast to 2021, we were able to maintain Allstar in the 2022 experiment, where it showed the highest resistance to gray mold out of all cultivars and MSU lines tested. This was expected because Allstar is considered to be more tolerant to *B. cinerea* unless the inoculum level is too high (Galleta et al., 1981). The rainy weather conditions in 2021 were favorable for the pathogen spread across the field, increasing the inoculum coming from the field prior to the lab inoculation, and the fruits had more bruising and bleeding (an indication of wounding) prior to the experimental set up, which, considering the pathogen enters the fruit through wounds, would have allowed easy entry and infection. In 2022, dryer weather patterns

allowed less bruising of the fruit and perhaps providing a better evaluation of the fruit for tolerance to *Botrytis* with less fruit damage to start with. Among the cultivars' ratings, Camarosa, Albion, Annapolis, and Cavendish were more tolerant than Wasatch, Cabot, and Earliglow, while Jewel was the most susceptible.

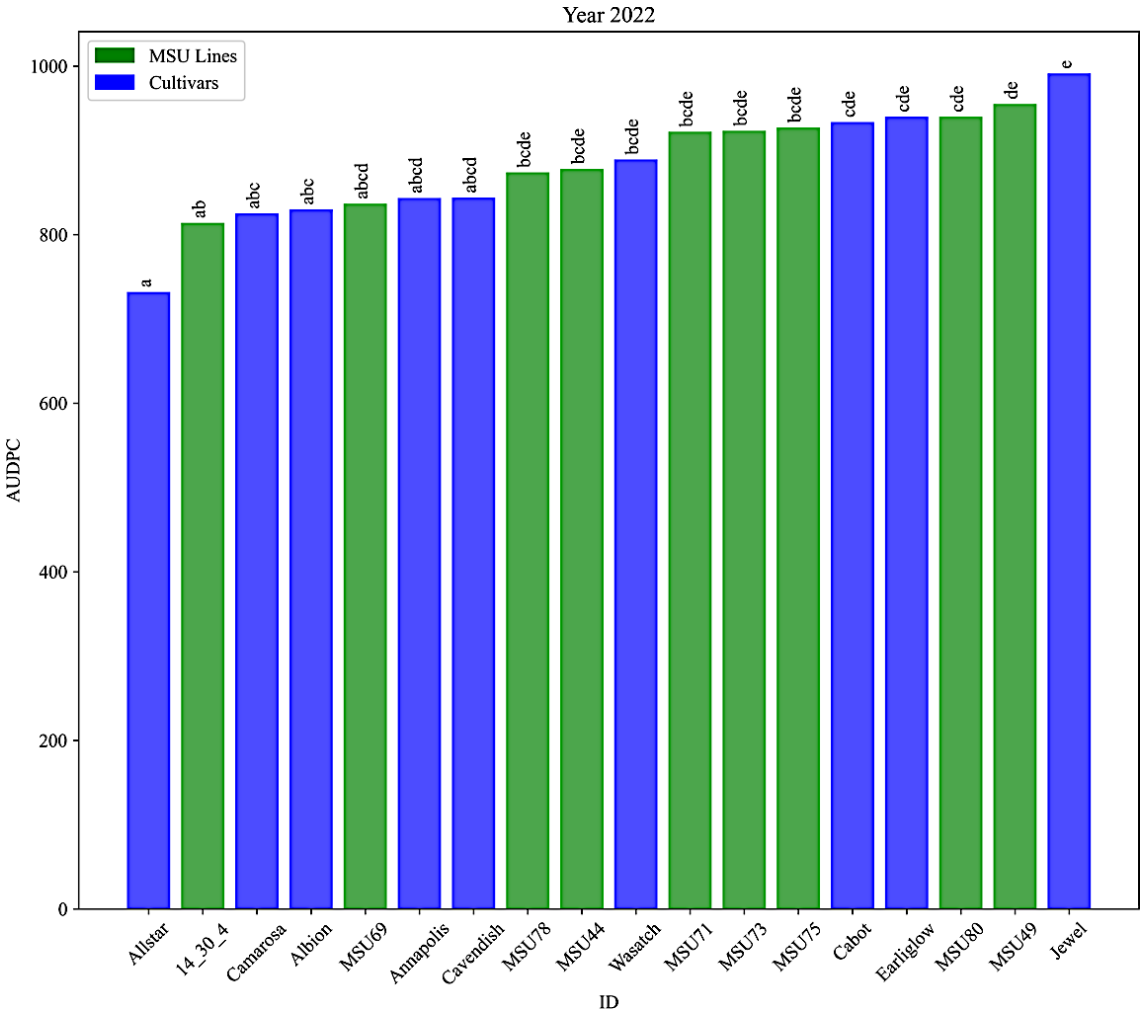

Figure 11. Area Under the Disease Progress Curve (AUDPC) for all genotypes inoculated with *B. cinerea* in 2022

The results of the screening showed that among the different MSU lines that were tested, 14_30_4 had the highest tolerance level, while MSU69 showed moderate tolerance when

compared to the other MSU lines. On the other hand, MSU 78, 44, 71, 73, and 75 all displayed the same level of susceptibility to *B. cinerea*, as moderately susceptible. MSU80 was highly susceptible with MSU49 being the most susceptible.

To summarize 2022, Allstar and 14_30_4 were significantly more tolerant to gray mold while MSU49 and Jewel were the most susceptible. Earliglow also showed higher susceptibility, which is opposed to the literature stating that Jewel and Earliglow are known to be tolerant against *B. cinerea* (Madeiras & Schloemann, 2015).

Soluble Solids Content

We were curious to see whether tolerance to gray mold correlates with any of the common fruit quality measurements that breeders focus on. We first focused on the Soluble Solids Content (SSC) measured as a BRIX value that indicates the sugar content/sweetness of the fruit. Considering fungi feed on sugar, we expected genotypes with higher BRIX values to show less tolerance (Britannica, n.d.).

BRIX analyses were conducted on the available data for genotypes: only Cultivars in 2021, but both MSU Lines and Cultivars in 2022. Comparing cultivars was done for both years, whereas comparing MSU Lines to Cultivars was only obtained for 2022.

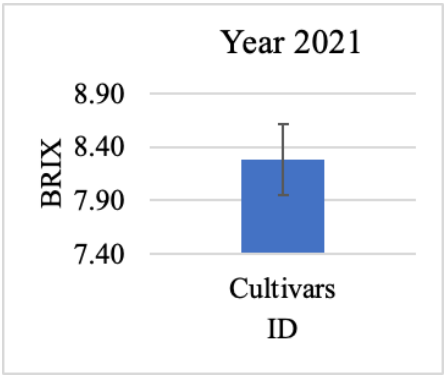

Figure 12. Average Soluble Solids Content (SSC) level for Cultivars in BRIX in 2021

The means of SSC values were 8.28 for Cultivars, whereas the standard error was 0.33. The typical industry standard for BRIX value for advanced selections and cultivars is 8.5 or higher (Personal communication with Driscolls Inc., 2021). However, other factors can affect the BRIX values, including how ripe the fruit is at harvest, age of the plants, and post-harvest conditions (Kleinhenz, 2020). This could be why our analysis of SSC for cultivars does not show BRIX values over the expected BRIX values of above 8.5 for cultivars.

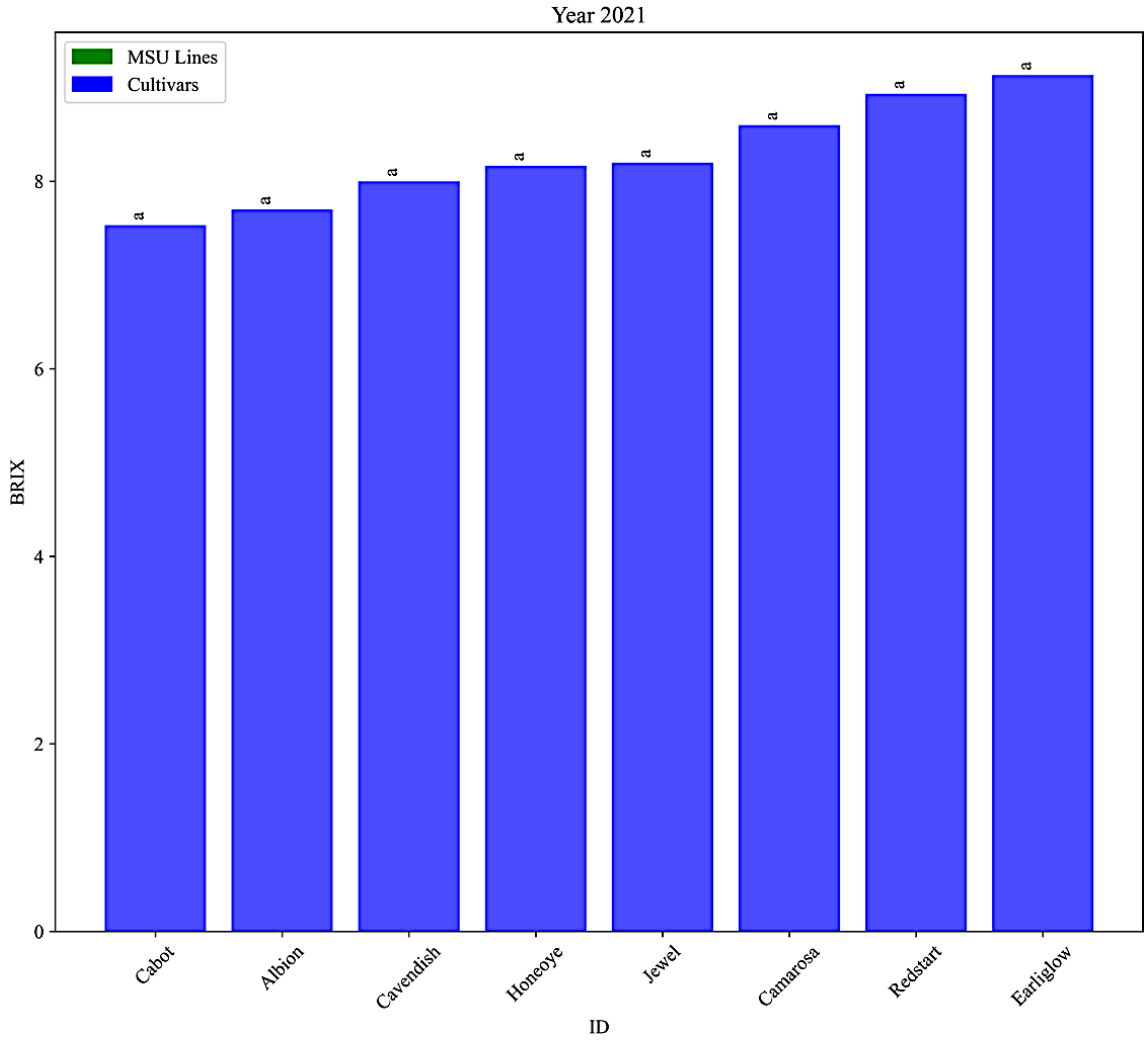

Figure 13. Average Soluble Solids Content (SSC) per Cultivars in BRIX in 2021

In the genotype comparison analysis, Cabot had the lowest sugar content, while Albion, Cavendish, Honeoye, and Jewel were slightly below the overall BRIX average. Camarosa had more sugar content, slightly above the overall Brix mean, while Redstart and Earliglow had the highest sugar content.

Linear regression analysis for Cultivars of 2021 regarding AUDPC vs. BRIX was given below to see whether there is any correlation. Unfortunately, there was no relationship between Brix values and AUDPC (R^2 = 0.003, F (1, 22) = 0.07, p = 0.80).

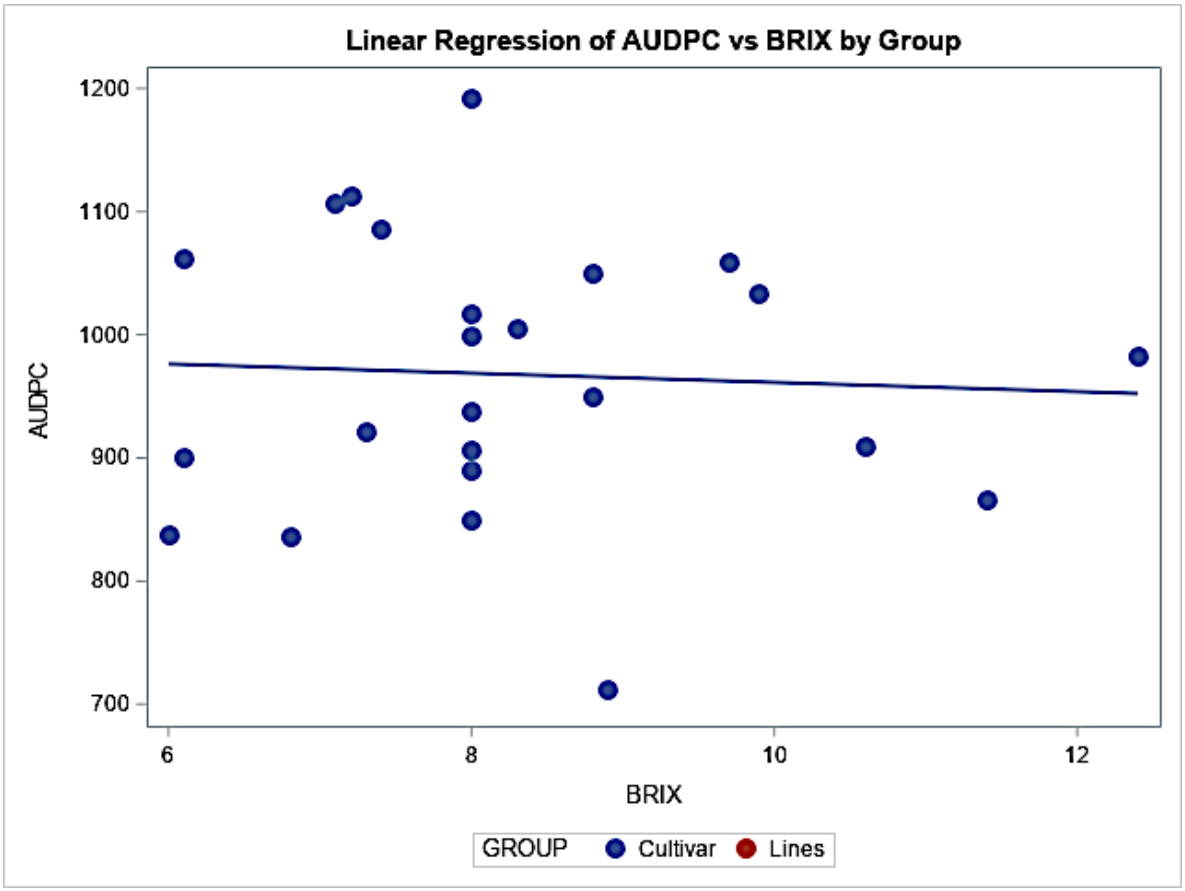

Figure 14. Linear regression of Average Soluble Solids Content in BRIX vs Area Under the Disease Progress Curve (AUDPC) in 2021

In 2022, we obtained BRIX values for both groups. Our comparison of Cultivars and MSU lines as a whole showed there is more sugar content in cultivars compared to MSU lines, and they were significantly different from each other (F (1, 88) = 19.22, p < .001). This agrees with our expectation because MSU germplasm has been bred for a sweet/sour balance for over 30 years, while released varieties tend to be sweeter. In 2022, we also observed expected BRIX values for cultivars (above 8.5 BRIX value).

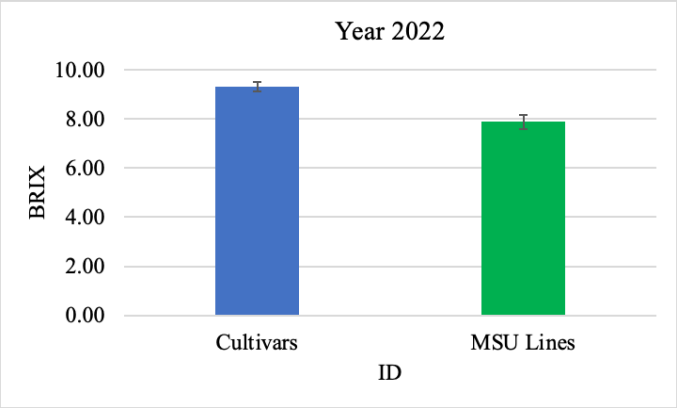

Figure 15. Average Soluble Solids Content (SSC) comparison between Cultivars and MSU Lines in BRIX in 2022

When considering the BRIX values for MSU lines, MSU 78, 49, and 69, in order, had significantly lower sugar content than the rest of the MSU Lines. MSU 73 and 71 stated next, and MSU44 had moderately low sugar content. One of the selections, 14_30_4 had moderately higher sugar content, followed by the highest sugar content found in MSU80 and 75 Lines.

As for the cultivars, Allstar had the lowest sugar content in 2022, followed by Albion, Camarosa, and Annapolis. Wasatch and Jewel had significantly more sugar content, followed by Cavendish and Earliglow, having the most SSC.

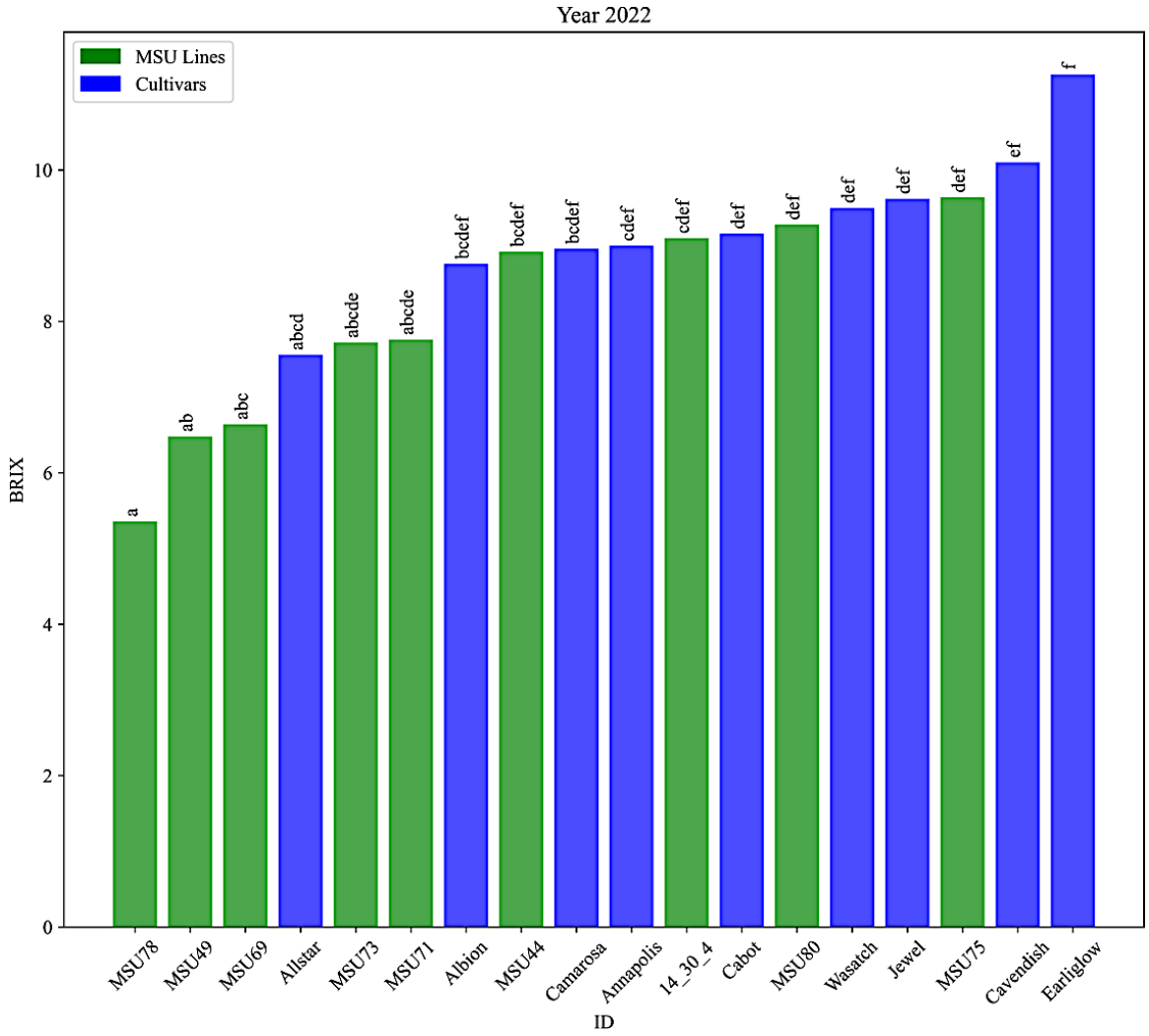

Figure 16. Average Soluble Solids Content (SSC) per all genotypes in 2022

Overall, MSU 78, 49, 69, and Allstar showed the lowest SSC while Cavendish and Earliglow showed significantly higher sugar content. Thus, seeing Earliglow as the most susceptible cultivar agrees with our assumption that susceptibility to gray mold may correlate with high sugar content.

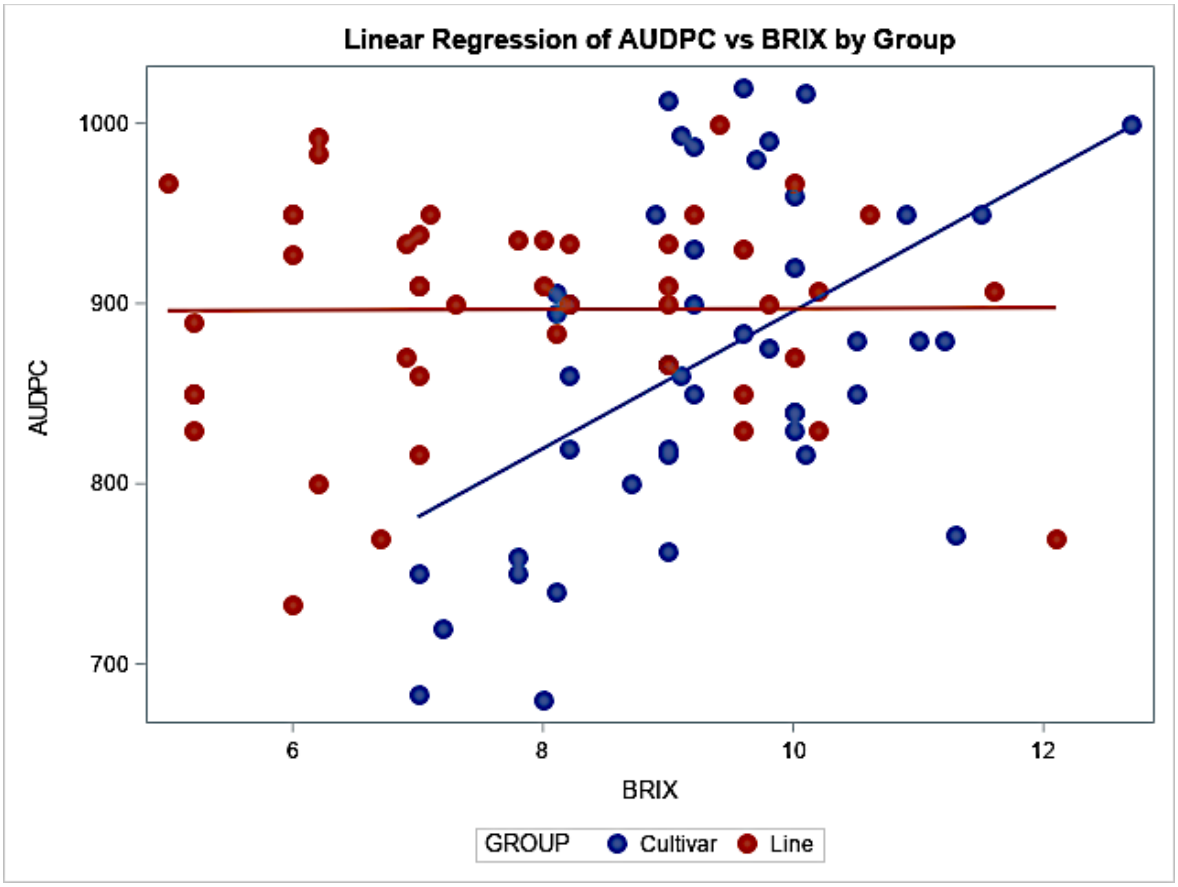

Figure 17. Linear regression of Average Soluble Solids Content in BRIX vs Area Under the Disease Progress Curve (AUDPC) in 2022

A linear regression analysis indicated that while BRIX values had a significant impact on AUDPC values for the Cultivars considered in the study (R^2 = 0.265, F (1,43) = 15.47, p = 0.0003), the BRIX had no significant effect (R^2 = 0.000, F (1,43) = 0.06, p = 0.97) for MSU Lines.

Considering that compared to Cultivars, MSU lines were tarter; we were curious to see whether titratable acidity would correlate with tolerance to gray mold.

Titratable Acidity

We were curious to see whether tolerance to gray mold correlates with any common fruit quality measurements that breeders focus on. After checking on the Soluble Solids Content

(SSC), we compared the TA values that indicate the acidic content/tartness of the fruit. We expected that lines with higher acidic values may show more tolerance in general. Comparisons for both groups and genotypes are as follows.

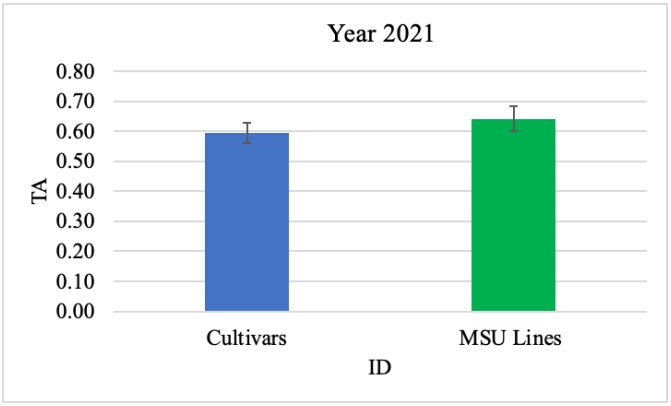

Figure 18. Average Titratable Acidity (TA) comparison between Cultivars and Line in 2021

Although MSU Lines had shown more acidic content than Cultivars, the difference between them was insignificant in 2021 (F (1, 59) = 0.76, p = 0.39).

In 2021, cultivars Earliglow and Camarosa had the least acidic content, followed by Albion and Honeoye. Cavendish and Cabot had shown more acidic content, while Redstart had the most significant acidic level. This is expected since Redstart is a cultivar released from MSU, where the former strawberry breeder focused on sweet/tart balance for selecting varieties.

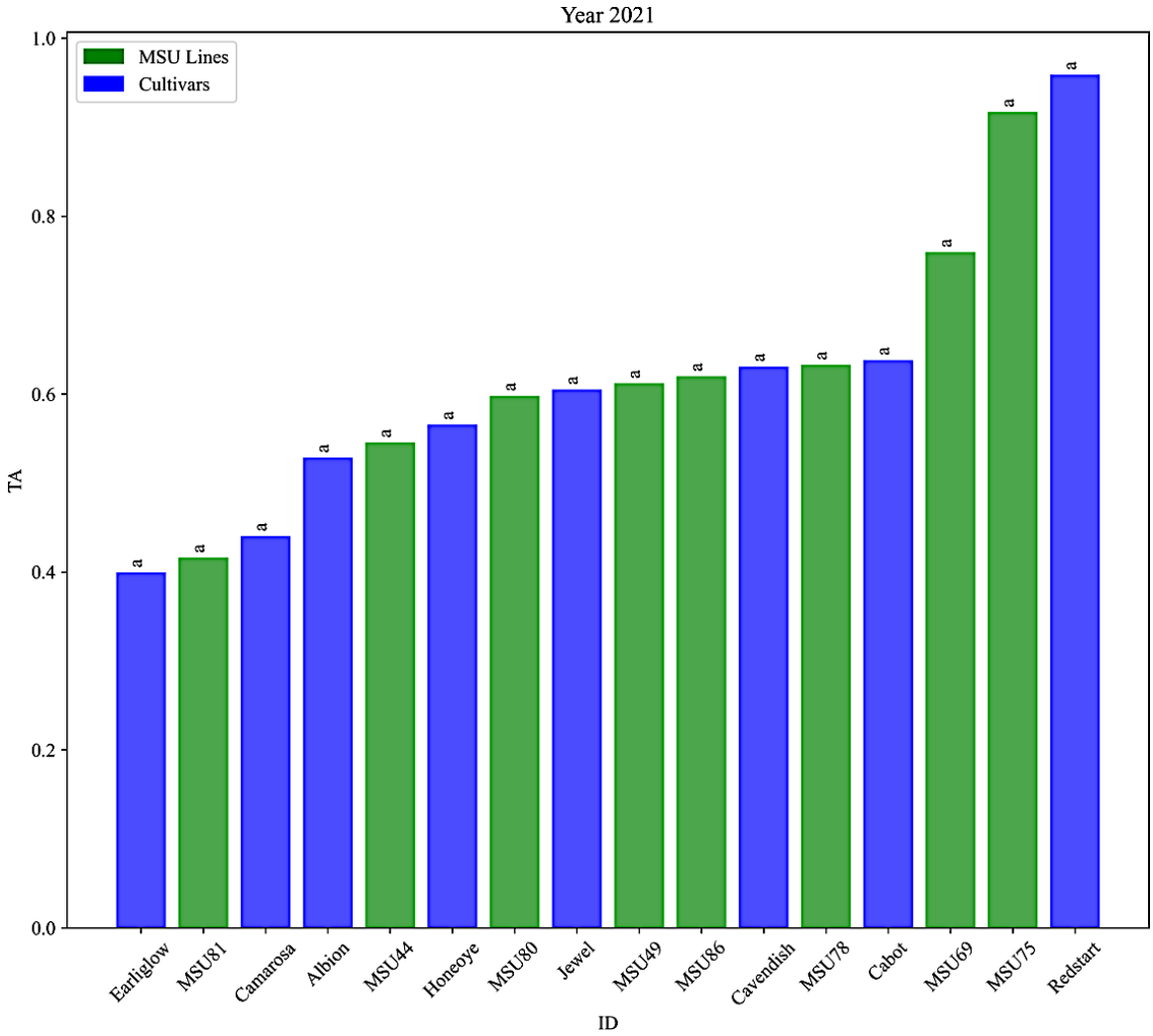

Figure 19. Average Titratable Acidity (TA) values per genotype in 2021

Among the MSU Lines, MSU81 and 44 had the least acidic content. They were followed by MSU80, 49, 86, and 78, which had relatively average acidity levels. On the other hand, MSU 69 showed relatively higher acidic content, and MSU 75 had the most acidic among all MSU Lines.

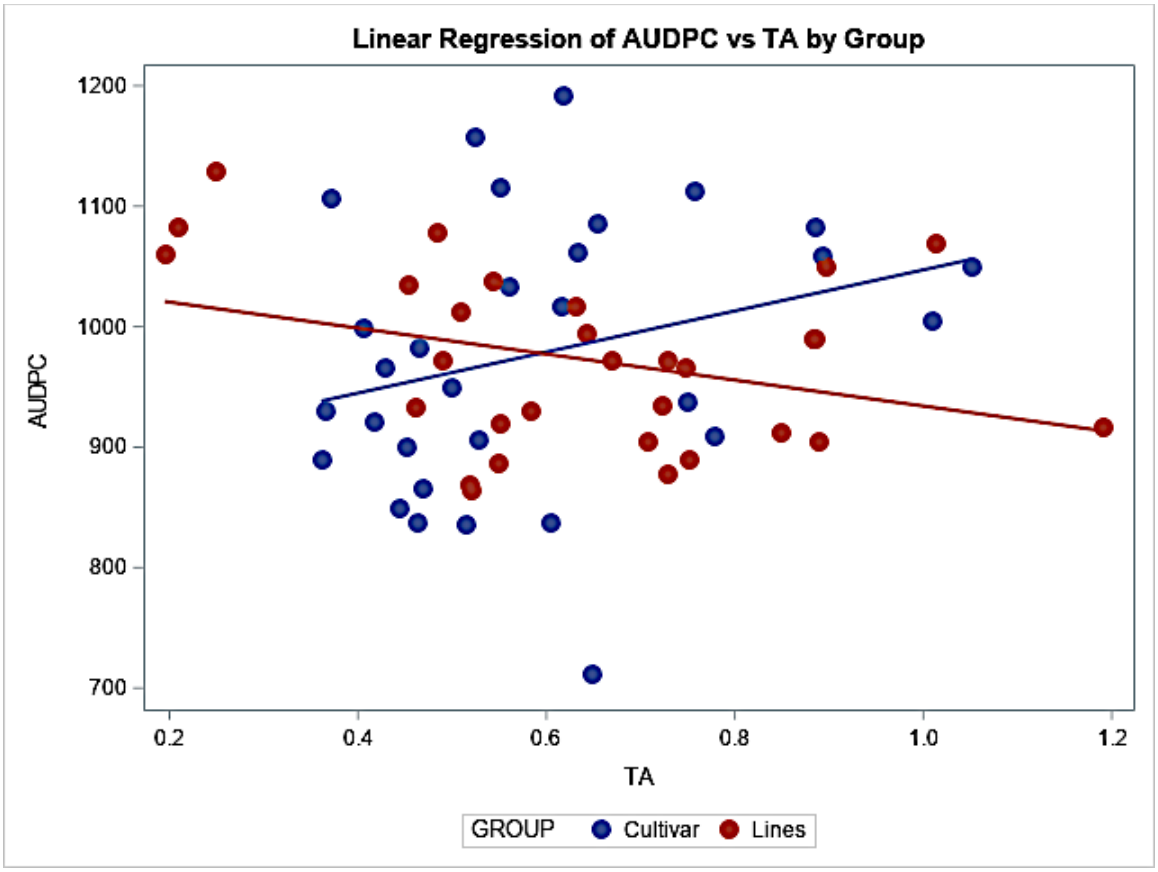

Figure 20. Linear regression of Average Titratable Acidity (TA) vs Area Under the Disease Progress Curve (AUDPC) in 2021

Linear regression analysis was conducted for TA and AUDPC to understand the correlation; however, TA was not a valuable variable for linking acidic level to the tolerance to gray mold in our study. TA of Cultivars ($R^2 = 0.080$, $F\ (1, 28) = 2.43$, $p = 0.13$) and MSU Lines did not show a correlation ($R^2 = 0.115$, $F\ (1, 28) = 3.63$, $p = 0.07$) in 2021.

The MSU Lines had more acid content than Cultivars when taken as a whole in 2022, and unlike in 2021, the difference was statistically significant ($F\ (1, 86) = 10.16$, $p = 0.002$). The MSU Lines consisting of the series of 70s, has more sour-tasting fruits, and as such, this result was expected.

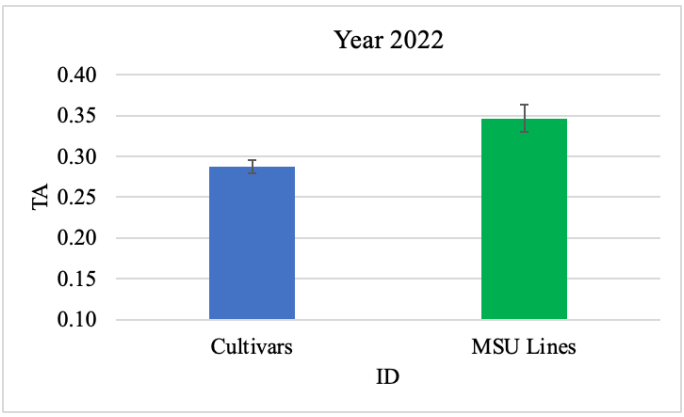

Figure 21. Average Titratable Acidity (TA) comparison between Cultivars and Line in 2022

In 2022, the strawberry varieties with the lowest acidic levels were Cavendish, Jewel, Camarosa, Albion, and Wasatch, respectively, followed by Earliglow, MSU44, Cabot, Allstar, and Annapolis. Once again, as expected, the cultivar released from MSU, Redstart, had the highest acidic content compared to the rest of the Cultivars included in our experiment.

The MSU Lines were grouped based on their acidic content compared to Cultivars. Among them, 14_30_4 and MSU 73 had the least acidic content, while MSU 49 and 44 had relatively low acidic levels. On the other hand, MSU 69, 76, and 78 had relatively higher acidic levels. The genotypes MSU 71, 80, and 81 had the most acidic content, with MSU 75 being the most acidic of them all.

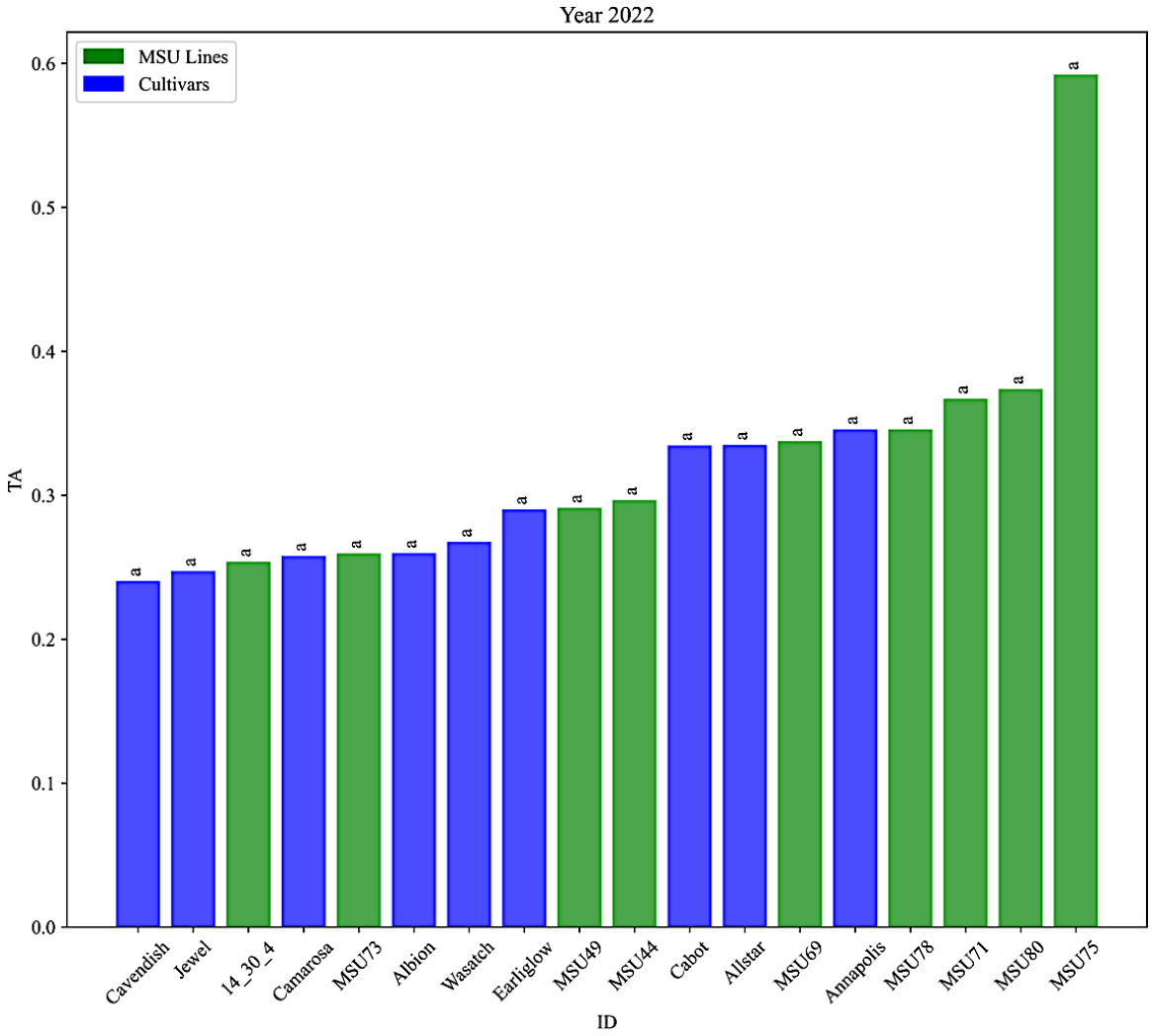

Figure 22. Average Titratable Acidity (TA) values per genotype in 2022

The set of cultivars, including Cavendish, Jewel, Camarosa, Albion, and Wasatch, alongside the MSU lines, 14_30_4 and 73, displayed a lower acidic content. Furthermore, it was observed that the MSU lines generally displayed higher TA values, with the highest TA value being recorded for MSU75.

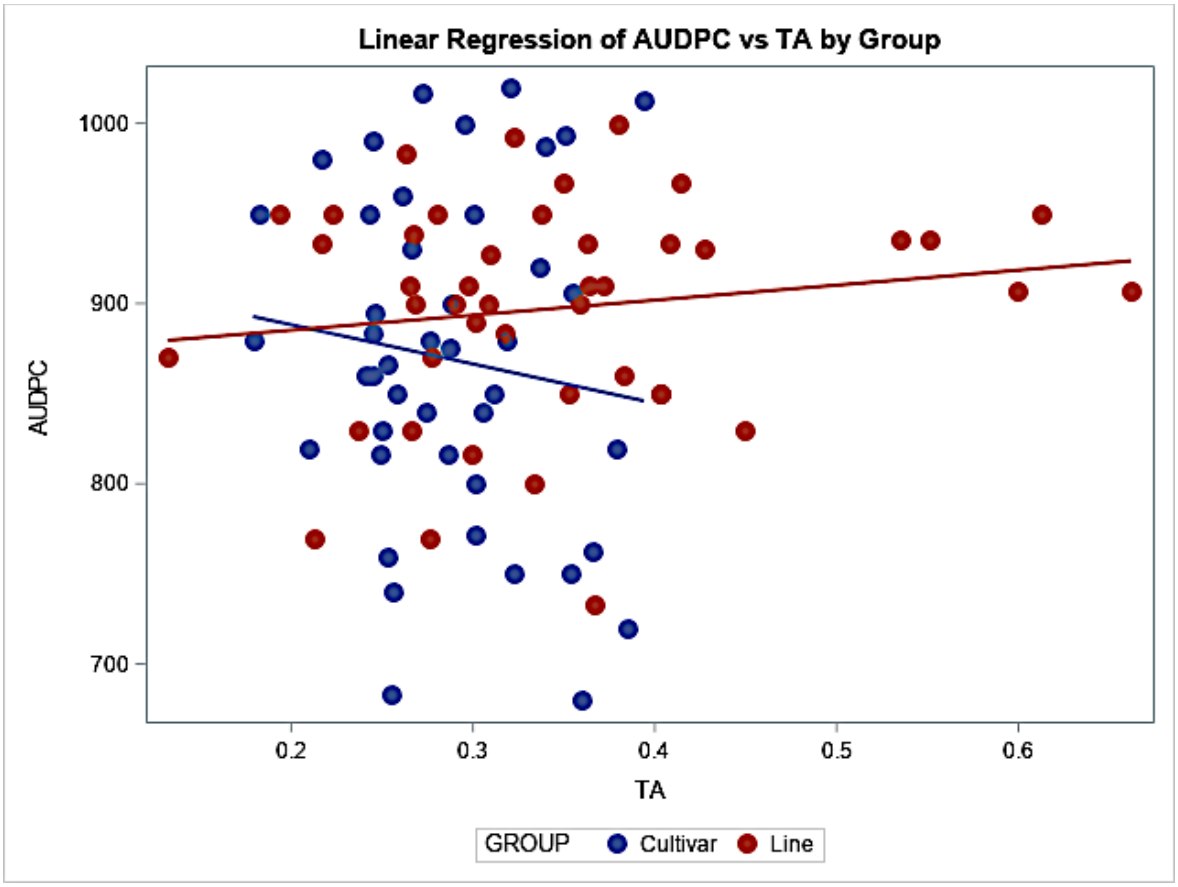

Figure 23. Linear regression of Average Titratable Acidity (TA) vs Area Under the Disease Progress Curve (AUDPC) in 2022

It was determined through linear regression analysis that TA was not a valuable variable for understanding gray mold tolerance in our study. Both the Cultivars ($R^2 = 0.015$, $F\,(1, 42) = 0.65$, $p = 0.42$) and MSU Lines had no significant correlation ($R^2 = 0.023$, $F\,(1, 42) = 1.00$, $p = 0.32$) in 2022.

DISCUSSION

We began our experiment with eight replicates, each consisting of ten fruits per genotype to be artificially inoculated in 2021. However, due to unfavorable weather conditions producing low quality or diseased fruit, there were several genotypes that did not have sufficient harvested fruits required to attain the desired 10 fruits per replication in the experiment. These genotypes had to be removed from the analysis.

In 2021, although we were to harvest the fruit weekly and conduct the experiment in batches, due to heavy rains and weather forecasts, we had to harvest fruit from all the genotypes on the same day to de-risk losing the fruit to heavy rain. In addition, due to the rain, a lot of soil adhered to the fruit, and we were unable to gently wipe the fruit to remove the soil particles. Therefore, we had to gently wash off the soil from the fruits contained in a colander, resulting in bruising of fruits that were not inherently firm. In comparison, 2022 was a drier year and there was less soil adhering to the fruits. Considering the bruising in 2021, we decided not to wash the fruits, but instead, gently wipe the dirt off of the fruits with a Kleenex tissue. As such, the fruit quality going into the experiment was much better than in 2021. Furthermore, rain would spread the *Botrytis* and other pathogens across the field more readily in 2021 than in 2022. Figures 24a and 24b show the daily precipitation received at the Pathology farm located in Okemos, Michigan. Therefore, our initial inoculum could have been higher in 2021 than in 2022. For these reasons, it is difficult for us to compare the tolerance levels of strawberry genotypes between 2021 and 2022. Considering the amount of bruising on fruit, year 2022 results may be more reliable than that of 2021.

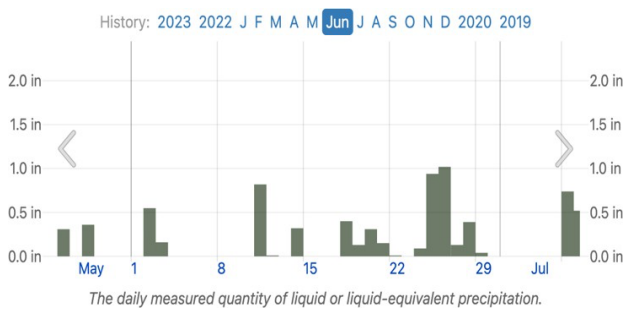

Figure 24a. Daily Precipitation in June 2021 in Okemos
Source: Weather Spark (n.d.)

Figure 24b. Liquid-Equivalent Precipitation on Monday, June 21, 2021, in Okemos
Source: Weather Spark (n.d.)

We also faced difficulties in controlling other pathogens causing fruit rot in strawberries such as *Rhizopus* (causing *Rhizopus* rot) (Bautista-Baños et al., 2014) and *Colletotrichum* (causing anthracnose disease) (Ellis & Erincik, 2008). The plants were maintained in a plot in the pathology farm where pathogen pressure was kept intentionally high to select for resistance to diseases by not spraying any fungicides for control. However, this very fact created challenges in our evaluations with field inoculation being higher – especially when fruit was harvested after heavy rains in 2021. For example, compared to anthracnose disease, *Rhizopus* rot spread faster and affected our methods and results substantially. Fruits had to be removed from the trays at the first sight of *Rhizopus* rot to minimize spread, often before observing the incidence of *Botrytis* on these fruits that were removed, which would affect the overall disease incidence.

During the experiment, numerous berries had to be discarded due to factors such as *Rhizopus* fruit rot and bruising. If the number of remaining fruits on a tray was less than 5, we considered those replications invalid and eliminated them. This process resulted in some genotypes having less than 3 replications, which were not included in the analysis to obtain accurate and unbiased results.

Although we were careful to differentiate and discard fruits with *Rhizopus* immediately, we had to eliminate several genotypes, especially Allstar from 2021, Honeoye, Redstart, and San Andreas from 2022 data analysis, since the loss of fruits due to *Rhizopus* was non-negligible. Honeoye was the most tolerant cultivar in our 2021 experiment, and this data is consistent with published information on Honeoye's resistance to *Botrytis* (Sanford et al., 1982). It is unfortunate that we had to remove the cultivar from our 2022 data analysis due to *Rhizopus* fruit rot incidence. But, considering its susceptibility to *Rhizopus*, the fruit would have succumbed to fruit rot at some point.

To keep the research method somewhat consistent between the years, we harvested all the genotypes at once in 2022 as they ripened. We did not choose any surface sterilization method because there was no optimal standardized protocol, and surface sterilization could have bruised the fruit, making them more susceptible to pathogens.

However, comparing the AUDPC data from 2021 and 2022 shows how the rainy weather negatively impacted the experimental results. For example, Jewel from the Cultivars w as more tolerant to gray mold in 2021; however, the same cultivar was the most susceptible in 2022. Whereas Albion was one of the most susceptible genotypes in 2021, it was one of the most tolerant cultivars in 2022. For this reason, we decided to analyze the datasets separately.

We can state from the common genotypes used for both years that Camarosa and MSU69 showed more tolerance to gray mold. Cavendish, Wasatch, and MSU78 were moderately tolerant, while Earliglow, MSU44, 75, and 80 were moderately susceptible. Cabot and MSU49 were highly susceptible in both 2021 and 2022.

Allstar was one of the more tolerant cultivars to *B. cinerea* in literature (Madeiras & Schloemann, 2015), until recently, it was declared to be grouped with the more susceptible

genotypes (Carroll & Pritts, 2022). In our experiment, Allstar was one of the most affected genotypes for fruit quality prior to the experimental setup in 2021. Fruits were mostly bruised and strongly affected by anthracnose disease and these symptoms allowed fruits to become heavily infected by *Rhizopus* and *Botrytis*. For this reason, it was discarded from the 2021 data analysis. However, the tolerance of Allstar to *Botrytis* was greater than other genotypes in 2022 when conditions were better. Contrary to the literature, Allstar showed more tolerance to gray mold than Earliglow and Camarosa in our study (Carroll & Pritts, 2022; Madeiras & Schloemann, 2015).

Previous studies also indicate that Earliglow and Jewel are tolerant to *Botrytis* (Madeiras & Schloemann, 2015; Carroll & Pritts, 2022); however, we found them highly susceptible in our 2022 study. It is possible that *B. cinerea* has developed resistance to these cultivars.

Based on this study, for resistance breeding purposes for *Botrytis* at MSU, it would be essential to assemble germplasm tolerant to *Botrytis* (e.g., San Andreas or other varieties or advanced breeding lines) to incorporate resistance genes into the MSU germplasm through breeding crosses.

Considering the difficulties in evaluating tolerance to *Botrytis* by observing symptoms of gray mold amid symptoms of other fruit-rotting fungi, we were interested in determining whether any other agronomic traits measured by a breeder would correlate with tolerance to *Botrytis* that could be used for indirect selection. We were particularly interested in seeing whether correlations exist between the soluble solids content (SSC), which speaks to the sweetness of strawberry fruit, and titratable acidity (TA). These are two measurements that strawberry breeders use to get a sense of the flavor of strawberries (Jouquand et al., 2008; Kubota Lab, n.d.). A possible relationship between the TA and tolerance to gray mold disease

was mentioned by Neri et al. (2015). In addition, Petrasch et al. (2022) stated that increasing resistance requires choosing higher levels of TA and firmness while decreasing SSC; nevertheless, these characteristics substantially influence flavor. Thus, we pursued checking SSC/BRIX and TA on our dataset to see the correlations between sugar and acid content with resistance.

Unfortunately, we could not obtain BRIX values for the MSU Lines in 2021. The analysis was done with the available replicates for both years. In 2021, 3 replicates for SSC and 4 replicates for TA could be used in the experiment. In 2022, 5 replicates for each trait were obtained.

Guan & Sutterer's (2017) research had the most comparable cultivar choices to our germplasm with Albion, Camarosa, and San Andreas being evaluated for their SSC and TA. In their experiment, Camarosa had the highest BRIX level, followed by San Andreas and Albion. In our experiment, Camarosa had more sugar content than Albion as well; however, we had to eliminate San Andreas due to the lack of adequate fruit numbers for the replicates. Camarosa and Albion shared the same TA level in their study, likewise, Albion and Camarosa were not significantly different in our analysis; however, Camarosa was slightly less acidic than Albion. Since fungi feed on sugar, it was expected to see less disease severity in Camarosa compared to Albion in our germplasm, supporting Guan & Sutterer's (2017) disease severity analysis, which our experiment supported for both years.

Considering sugar serves as a nutrient for *B. cinerea,* and SSC represents sugar content in strawberries, we expected a positive correlation between AUDPC and the BRIX level (Mancini et al., 2023). In 2021, BRIX level and AUDPC values were in correlation for Redstart's susceptibility and higher sugar content only. It is possible that the absence of an

overall correlation for disease severity could be due to the limited variance in sugar content observed in the genotypes used in 2021. In 2022, we were able to see enough variation in genotypes and Cultivars had a correlation with the AUDPC values; whereas MSU Lines did not support a similar correlation potentially due to their lower sugar content compared to Cultivars. Considering that one of the current objectives of the MSU strawberry breeding program is increasing the sweetness/SSC, it would be interesting to see how this change would affect the correlation with tolerance to *Botrytis* in our most recent advanced selections that have higher SSC levels.

We also noted that lower AUDPC values correlated with higher TA levels, as indicated by the negative slope within the Cultivars in 2021. On the other hand, the MSU Lines exhibited a positive slope, suggesting a correlation between increased acidity and higher AUDPC results. As Ganhão et al. (2019) stated, SSC and TA were positively correlated, so TA and AUDPC were also expected to be positively correlated. While our MSU Lines served this correlation, the Cultivars in the experiment did not, and *p*-values for both were more than 0.05, so their effect was not significant on disease tolerance. However, fungi can be affected by high concentrations of acidity, and it is preferable to have low TA values for both the sweetness of berries and resistance. The relationship between TA and disease tolerance is possibly more complex and needs further study.

Although one of our breeding objectives is to produce more sweet strawberries with a high BRIX and a low TA ratio with better *B. cinerea* resistance, chefs prefer to work with berries with a sweet/sour balance (personal communication with Jim Hancock, 2021). It is interesting that both MSU69 and 78 with more sour-tasting berries, as well as 14_30_4 as a genotype producing sweeter berries, performing comparable to commonly used cultivars such

as Allstar, Camarosa, Earliglow, and Jewel with similar or better tolerance to gray mold. As such, from the MSU lines tested, MSU69, 78, and 14_30_4 could be considered as breeding parents to improve tolerance to *B. cinerea* to develop tolerance to gray mold.

CONCLUSION

Our study found that MSU69 is one of the most tolerant MSU Lines against gray mold disease. Also, we have observed that genotypes MSU78 and 14_30_4 can be promising as a source for tolerance to use as breeding parents along with cultivars that have been reported to be more tolerant to the disease to improve resistance breeding for gray mold while maintaining the good flavor of MSU germplasm.

Seeing MSU Lines falling into the range in Cultivars for different traits gives encouragement to improve them with better parental combinations that could provide compatibility for agronomically important traits. Incorporating germplasm with higher levels of tolerance into MSU germplasm could be a reasonable solution for increasing resistance to *B. cinerea* (Bestfleisch et al., 2015; Edger et al., 2019; González et al., 2009).

In the meantime, we remain hopeful that through the diligent efforts of scientists and researchers, effective measures can be implemented to mitigate *B. cinerea*'s effects as breeders continue to enhance resistance to gray mold.

In conclusion, it would be essential to assemble germplasm tolerant to *Botrytis* in order to introduce resistance genes into the MSU germplasm via breeding crosses for the aim of resistance breeding at MSU.

BIBLIOGRAPHY

Agarwal, P., Holland, T. M., James, B. D., Cherian, L. J., Aggarwal, N. T., Leurgans, S. E., ... & Schneider, J. A. (2022). Pelargonidin and Berry intake association with Alzheimer's disease neuropathology: A community-based study. *Journal of Alzheimer's Disease*, (Preprint), 1-9.

Agyare, S., Magan, N., & Xu, X. (2020). Conditions for infection of strawberry fruit by M. piriformis and Rhizopus spp. *European Journal of Plant Pathology*, 157, 65-75.

Akça, A., & Tozlu, E. (2019). The investigation of biological control opportunities against gray mold causing Botrytis cinerea Pers: Fr in eggplant. *ICOFAAS 2019*, 92.

Alves, J. A. V., Steffens, C. A., da Silva, J. C., Pansera-Espíndola, B., do Amarante, C. V. T., & Moreira, M. A. (2022). Quality of 'San Andreas' strawberries and control of gray mold with essential melaleuca oil. *Journal of Food Processing and Preservation*, 46(1), e16130.

Amil-Ruiz, F., Blanco-Portales, R., Munoz-Blanco, J., & Caballero, J. L. (2011). The strawberry plant defense mechanism: A molecular review. *Plant and Cell Physiology*, 52(11), 1873- 1903.

Amiri, A., Zuniga, A. I., & Peres, N. A. (2018). Potential impact of populations drift on Botrytis occurrence and resistance to multi-and single-site fungicides in Florida southern highbush blueberry fields. *Plant disease*, *102*(11), 2142-2148.

Andreotti, G., Freeman, L. E. B., Hou, L., Coble, J., Rusiecki, J., Hoppin, J. A., ... & Alavanja, M. C. (2009). Agricultural pesticide use and pancreatic cancer risk in the Agricultural Health Study Cohort. *International Journal of Cancer*, 124(10), 2495-2500.

Antanaviciute, L., Šurbanovski, N., Harrison, N., McLeary, K. J., Simpson, D. W., Wilson, F., & Harrison, R. J. (2015). Mapping QTL associated with Verticillium dahliae resistance in the cultivated strawberry (Fragaria× ananassa). *Horticulture research*, *2*.

Antonio Dávalos-González, P., Aguilar-García, R., Rodríguez-Guillén, A., & Estela Jofre-y- Garfias, A. (2023). The genetic diversity of strawberry species, the underutilized gene pool and the need for cultivars with new quality and agronomic attributes. *IntechOpen*. doi: 10.5772/intechopen.102962

APS. (n.d.). Calculating the Area Under the Disease Progress Curve to Quantify Disease Progress. Retrieved November 16, 2023, from https://www.apsnet.org/edcenter/disimpactmngmnt/topc/EcologyAndEpidemiologyInR/D iseaseProgress/Pages/AUDPC.aspx#:~:text=The%20area%20under%20the%20disea

se%
20progress%20curve%20(%20AUDPC%20)%20is%20a,%2C%20locations%2C%20or% 20management%20tactics.

Bai, Y., Liu, H., Lyu, H., Su, L., Xiong, J., & Cheng, Z. (2022). Development of a single-cell atlas for woodland strawberry (Fragaria vesca) leaves during early Botrytis cinerea infection using single-cell RNA-seq. *Horticulture Research*, *9*. https://doi.org/10.1093/hr/uhab055

Bancroft, J. (1876). Report of the board appointed to enquire into the cause of disease affecting livestock and plants. *Votes Proc*, *3*, 1011-1038.

Barber, H. N., & Sharpe, P. J. H. (1971). Genetics and physiology of sunscald of fruits. *Agricultural Meteorology*, *8*, 175-191.

Barrett. (n.d.). How to use a refractometer. *UC Davis Postharvest Technology*. Retrieved October 16, 2023, from https://fruitandvegetable.ucdavis.edu/files/237293.pdf

Barritt, B.H. (1980). Resistance of strawberry clones to Botiytis fruit rot. *Journal of the American Society for Horticultural Science*, 105, 160-164.

Bautista-Baños, S., Bosquez-Molina, E., & Barrera-Necha, L. L. (2014). Rhizopus stolonifer (soft rot). In *Postharvest decay* (pp. 1-44). Academic Press.

Bestfleisch, M., Luderer-Pflimpfl, M., Höfer, M., Schulte, E., Wünsche, J. N., Hanke, M. V., & Flachowsky, H. (2015). Evaluation of strawberry (Fragaria L.) genetic resources for resistance to Botrytis cinerea. *Plant Pathology*, 64(2), 396-405.

Bhujel, A., Khan, F., Basak, J. K., Jaihuni, M., Sihalath, T., Moon, B. E., ... & Kim, H. T. (2022). Detection of gray mold disease and its severity on strawberry using deep learning networks. *Journal of Plant Diseases and Protection*, 129(3), 579-592.

Boddy, L. (2016). Pathogens of autotrophs. In *The Fungi* (pp. 245–292). Academic Press.

Brandhoff, B., Simon, A., Dornieden, A., Schumacher, J. (2017). Regulation of conidiation in Botrytis cinerea involves the light-responsive transcriptional regulators bcltf3 and bcreg1.
Current Genetics, 63, 931–949.

Brooks, A. N. (1931). Anthracnose of Strawberry caused by Colleto trichum fragariae, n. sp. Phytopathology, 21(7).

Basu, A., Nguyen, A., Betts, N. M., & Lyons, T. J. (2014). Strawberry as a functional food: An evidence-based review. *Critical Reviews in Food Science and Nutrition*, 54(6), 790–806. https://doi.org/10.1080/10408398.2011.608174

Britannica. (n.d.). Fungus - Nutrition, Saprotrophs, Mycorrhizae. Retrieved November 29, 2023 from https://www.britannica.com/science/fungus/Nutrition

Cai, C., He, S., An, Y., & Wang, L. (2020). Exogenous 5-aminolevulinic acid improves strawberry tolerance to osmotic stress and its possible mechanisms. *Physiologia Plantarum*, 168(4), 948-962.

Carisse, O. (2016). Epidemiology and Aerobiology of Botrytis spp. *Botrytis–the Fungus, the pathogen and its management in agricultural systems*, 127-148.

Carroll, J., & Pritts, M. (2022). Production and IPM Guide for Organic Strawberries. New York State Integrated Pest Management Program. Ithaca, NY. 69 pages.

Carter, C. A., Chalfant, J. A., Goodhue, R. E., Han, F. M., & DeSantis, M. (2005). The methyl bromide ban: Economic impacts on the California strawberry industry. *Applied Economic Perspectives and Policy*, 27(2), 181-197.

Chan, L. (2020, June 17). How to count cells using a hemocytometer. *Nexcelom Bioscience*. https://www.nexcelom.com/blogs/how-to-count-cells-using-a-hemacytometer/

Chandler, C. K., Mertely, J. C., & Peres, N. (2004, September). Resistance of selected strawberry cultivars to anthracnose fruit rot and Botrytis fruit rot. In *V International Strawberry Symposium* 708 (pp. 123-126).

Chinburg, L. (2022). Perilous Bounty and the future of farming in America. *Journal of Agriculture, Food Systems, and Community Development*, 12(1), 1-3.

Cockerton, H. M., Li, B., Vickerstaff, R. J., Eyre, C. A., Sargent, D. J., Armitage, A. D., ... & Harrison, R. J. (2019). Identifying Verticillium dahliae resistance in strawberry through disease screening of multiple populations and image based phenotyping. *Frontiers in Plant Science*, *10*, 924.

Cornell Fruit Resources: Berries . (2022, January 18). Patent Status of Select Strawberry Varieties. Retrieved November 17, 2023, from https://blogs.cornell.edu/berries/2022/01/18/patent-status-of-select-strawberry- varieties/#:~:text=Earliglow%20–%20patent%20expired%201995.,red%20stele%20and%20verticillium%20wilt

Cosseboom, S. D., Ivors, K. L., Schnabel, G., Bryson, P. K., & Holmes, G. J. (2019). Within- season shift in fungicide resistance profiles of Botrytis cinerea in California strawberry fields. *Plant disease*, *103*(1), 59-64.

Daubeny, H. A., & Pepin, H. S. (1977). Evaluation of strawberry clones for fruit rot resistance. *Journal of the American Society for Horticultural Science*, 102, 431-435.

Davis, T. M., Denoyes-Rothan, B., & Lerceteau-Köhler, E. (2007). Strawberry. In *Fruits and nuts* (pp. 189-205). Berlin, Heidelberg: Springer Berlin Heidelberg.

Dávalos-González, P. A., Aguilar-García, R., Rodríguez-Guillén, A., & Jofre-y-Garfias, A. E. (2022). The Genetic Diversity of Strawberry Species, the Underutilized Gene Pool and the Need for Cultivars with New Quality and Agronomic Attributes.

de Moura, G. G. D., de Barros, A. V., Machado, F., Martins, A. D., da Silva, C. M., Durango, L. G. C., ... & Doria, J. (2021). Endophytic bacteria from strawberry plants control gray mold in fruits via production of antifungal compounds against Botrytis cinerea L. *Microbiological Research*, *251*, 126793.

Dean, R., Van Kan, J. A., Pretorius, Z. A., Hammond-Kosack, K. E., Di Pietro, A., Spanu, P. D., ... & Foster, G. D. (2012). The Top 10 fungal pathogens in molecular plant pathology. *Molecular Plant Pathology*, 13(4), 414-430.

Demchak, Elkner, & Hu. (2023, April 6). Anthracnose on strawberry fruit. Retrieved October 24, 2023, from https://extension.psu.edu/anthracnose-on-strawberry-fruit

Demchak, K. & Harper, J.K. (2005, June 20) Strawberry Production. PenState Extension. Retrieved March 5,2023, from https://extension.psu.edu/strawberry-production

Denoyes-Rothan, B., Guérin, G., Lerceteau-Köhler, E., & Risser, G. (2005). Inheritance of resistance to Colletotrichum acutatum in Fragaria× ananassa. *Phytopathology*, *95*(4), 405- 412.

Deising, H. B., Reimann, S., & Pascholati, S. F. (2008). Mechanisms and significance of fungicide resistance. *Brazilian Journal of Microbiology*, 39, 286-295.

Dewey, F. M.; Garfinkel, A. R. (2021). The history of Botrytis taxonomy, the rise of phylogenetics, and implications for species recognition. *Phytopathology®, 111(3)*, 437- 454.

Domoto, P., Gleason, M., & Lewis, D. (2008). Production guide for commercial strawberries. *Iowa State Univ. Ext.* PM672d.

Denoyes, B., & Baudry, A. (1995). Species identification and pathogenicity study of French Colletotrichum strains isolated from strawberry using morphological and cultural characteristics. *Phytopathology*, *85*(1), 53-57.

Drenth, A., & Sendall, B. (2001). Practical guide to detection and identification of Phytophthora. *Tropical Plant Protection*, 1, 32-33.

Edger, P. P., Poorten, T. J., VanBuren, R., Hardigan, M. A., Colle, M., McKain, M. R., ... & Knapp, S. J. (2019). Origin and evolution of the octoploid strawberry genome. *Nature Genetics*, 51(3), 541-547.

Elad, Y., Williamson, B., Tudzynski, P., & Delen, N. (2007). Botrytis spp. and diseases they cause in agricultural systems–an introduction. *Botrytis: Biology, pathology and control*, *412*.

Ellis, M. A., & Erincik, O. (2008). Anthracnose of strawberry. *The Ohio State University Extension*. Retrieved November 16, 2023, from https://ohioline.osu.edu/factsheet/plpath- fru-16

Extech Instruments. (2003, September 1). Refractometer. *Portable Salinity Refractometer With ATC*. Retrieved October 18, 2023, from https://www.instrumart.com/assets/RF20- manual.pdf

EPA. (1998, April 6). Pesticide Fact Sheet. *United States Environmental Protection Agency*. Retrieved October 15, 2023, from https://www3.epa.gov/pesticides/chem_search/reg_actions/registration/fs _PC- 288202_06-Apr-98.pdf

Faedo, L. F., Verdi, R., Pinto, F. A., Kretszchmar, A. A., & Boff, P. (2022). Use of Trichoderma spp. and high-dynamized dilutions in the control of Botrytis cinerea and strawberry growth. *Revista Brasileira de Agroecologia*, 17(1), 6-19.

Farnsworth. (2017, September 14). Department of Pesticide Regulation. In *Pesticide Programs Division #17-01*. Department of the California Environmental Protection Agency.
Retrieved November 25, 2023, from https://www.cdpr.ca.gov/docs/county/cacltrs/exec/2017/ppd/ppd1701.pdf

Fillinger, S., & Elad, Y. (Eds.). (2016). *Botrytis-The Fungus, The Pathogen and Its Management in Agricultural Systems* (pp. 189-216). Cham, Switzerland: Springer International Publishing.

Folta, K. M., & Davis, T. M. (2006). Strawberry genes and genomics. *Critical Reviews in Plant Sciences*, 25(5), 399-415.

Forges, M., Vàsquez, H., Charles, F., Sari, D. C., Urban, L., Lizzi, Y., ... & Aarrouf, J. (2018). Impact of UV-C radiation on the sensitivity of three strawberry plant cultivars (Fragaria x ananassa) against Botrytis cinerea. *Scientia Horticulturae*, 240, 603-613.

Food and Agriculture Organization of the United Nations. (2023). FAOSTAT statistical database.

Gahatraj, B., Nielsen, K. A., Le, V. H., Sønsteby, A., & Stensvand, A. (2023). Steam thermotherapy strongly reduces Botrytis in strawberry transplants with no or minor negative effects on plant growth and yield. *European Journal of Plant Pathology*, 166(1), 109-121.

Gama, A. B., Cordova, L. G., Baggio, J. S., Mertely, J. C., & Peres, N. A. (2023). Old but Gold: Captan Is a Valuable Tool for Managing Anthracnose and Botrytis Fruit Rots and Improving Strawberry Yields Based on a Meta-Analysis. *Plant disease*, *107*(10), 3071– 3078. https://doi.org/10.1094/PDIS-12-22-2781-RE

Galletta, G. J., Draper, A. D., & Swartz, H. J. (1981). 'Allstar' Strawberry. *HortScience*, 16(6), 792-794.

Ganhão, R., Pinheiro, J., Tino, C., Faria, H., & Gil, M. M. (2019). Characterization of nutritional, physicochemical, and phytochemical composition and antioxidant capacity of three strawberry "Fragaria× ananassa Duch." cultivars ("Primoris", "Endurance", and "Portola") from Western Region of Portugal. *Foods*, 8(12), 682.

Gardenia. (n.d.) Fragaria Allstar (Junebearing Strawberry) Retrieved September 17, 2023, from https://www.gardenia.net/plant/fragaria-ananassa-allstar

Garrido, C., Carbú, M., Fernández-Acero, F. J., González-Rodríguez, V. E., & Cantoral, J. M. (2011). New insights in the study of strawberry fungal pathogens. . *Genes Genomes Genomics*, 5(1), 24-39.

Geisseler, D., & Horwath, W. R. (2014). Strawberry production in California. *Fertilizer Research and Education Program (FREP)*, 4.

Giampieri, F., Forbes-Hernandez, T. Y., Gasparrini, M., Alvarez-Suarez, J. M., Afrin, S., Bompadre, S., ... & Battino, M. (2015). Strawberry as a health promoter: An evidence- based review. *Food & Function*, 6(5), 1386-1398.

Golzar, H., Phillips, D., & Mack, S. (2007). Occurrence of strawberry root and crown rot in Western Australia. *Australasian Plant Disease Notes*, 2(1), 145-147.

Grant-Downton, R. (2016). Botrytis-biology, detection and quantification. In *Botrytis – The Fungus, the Pathogen and its Management in Agricultural Systems* (pp. 17-34).

Guan, W & Sutterer, L. (2017). Strawberry Variety Evaluation for High Tunnel Production in Southwest Indiana. *Midwest Vegetable Trial Reports.* Paper 187. https://docs.lib.purdue.edu/mwvtr/187

Gurney's Seed and Nursery Co. (n.d.). Annapolis Strawberry | Plants for Sale From Gurney's. Retrieved September 17, 2023, from https://www.gurneys.com/product/annapolis-strawberry

Guthman, J., & Jiménez-Soto, E. (2021). Socioeconomic challenges of California strawberry production and disease-resistant cultivars. *Frontiers in Sustainable Food Systems*, 446.

Hammel, J. (2016, October 14). *Common Leaf Spot of Strawberry*. Wisconsin Horticulture. Retrieved January 23, 2024, from

https://hort.extension.wisc.edu/articles/common-leaf- spot-of-strawberry/

Hancock, J.F., Sjulin, T.M. and Lobos, G.A. (2008) Strawberries. In: *Temperate Fruit Crop Breeding: Germplasm to Genomics*. Netherlands: Springer, pp. 393–437.

Hancock, J. F. (2020). Worldwide strawberry industry. In *Strawberries* (pp. 86-108). Wallingford UK: CABI.

Hancock, J. F. (2019). Temperatures and crop plants. In *Encyclopedia of Agriculture and Food Systems* (Second Edition) (pp. 84-90). Elsevier. https://doi.org/10.1016/B978-0-08- 100596-5.21733-4

Handley, D. T. (2023). Strawberry IPM Newsletter No. 1 - May 7, 2020 - Cooperative Extension at Highmoor Farm - University of Maine Cooperative Extension. Retrieved from https://extension.umaine.edu/highmoor/2020/05/07/strawberry-ipm-news-no1-2020/

Hassan, E. A., Mostafa, Y. S., Alamri, S., Hashem, M., & Nafady, N. A. (2021). Biosafe management of Botrytis grey mold of strawberry fruit by novel bioagents. *Plants*, *10*(12), 2737.

Hasing, T. N., Osorio, L. F., & Whitaker, V. M. (2013). Within-season stability of strawberry soluble solids content. *Journal of the American Society for Horticultural Science* J. Amer. Soc. Hort. Sci., 138(3), 190-197. Retrieved Oct 24, 2023, from https://doi.org/10.21273/JASHS.138.3.190

Hasler, C. M., & Brown, A. C. (2009). Position of the American Dietetic Association: functional foods. *Journal of the American Dietetic Association*, 109(4), 735-746.

Hernández-Martínez, N. R., Blanchard, C., Wells, D., & Salazar-Gutiérrez, M. R. (2023). Current state and future perspectives of commercial strawberry production: A review. *Scientia Horticulturae*, *312*, 111893. https://doi.org/10.1016/j.scienta.2023.111893

Hjalmarsson, I. (2021). Strawberry cultivars preserved in the Swedish National Gene Bank. In *Proceedings of the Latvian Academy of Sciences. Section B. Natural, Exact, and Applied Sciences* (Vol. 76, No. 4, pp. 402-408).

Hong, P., Zhang, Z., Zhou, Y., Lu, X., Sadeghnezhad, E., Pang, Q., Tao, Z., Cheng, Y., Wang, B., & Jia, H. (2024). Polygalacturonase inhibiting protein enhances cell wall strength of strawberry fruit for resistance to Botrytis cinerea infection. *Scientia Horticulturae*, *327*, 112850. https://doi.org/10.1016/j.scienta.2024.112850

Hu, M. J., Cox, K. D., & Schnabel, G. (2016). Resistance to increasing chemical classes of fungicides by virtue of "selection by association" in Botrytis cinerea. *Phytopathology*, 106(12), 1513-1520.

Hua, L., Yong, C., Zhanquan, Z., Boqiang, L., Guozheng, Q., & Shiping, T. (2018). Pathogenic mechanisms and control strategies of Botrytis cinerea causing post-harvest decay in fruits and vegetables. *Food Quality and Safety*, 2(3), 111-119.

Hummer, K. E. (2008). Global conservation strategy for Fragaria (Strawberry). *Global conservation strategy for Fragaria (Strawberry)*.

Hokanson, S. C., & Finn, C. E. (2000). Strawberry Cultivar Use in North America. *HortTechnology horttech*, 10(1), 94-106. Retrieved Nov 18, 2023, from https://doi.org/10.21273/HORTTECH.10.1.94

Holmes, G. J., Mansouripour, S. M., & Hewavitharana, S. S. (2020). Strawberries at the crossroads: Management of soilborne diseases in California without methyl bromide. *Phytopathology*, 110(5), 956-968.

Ikram, S., Abassi, U. A., & Khalid, N. (2019). Strawberry (Fragaria ananassa Duch): phytochemicals, nutraceuticals, and health benefits. A brief review. *World Journal of Biology and Biotechnology*, 4(3), 25-34.

IPM. (2000, May). Reports on Plant Diseases: Gray-Mold Rot or Botrytis Blight of Vegetables.Retrieved November 16, 2023, from http://ipm.illinois.edu/diseases/series900/rpd942/

Jain, A., Jain, R., Jain, S., Jain, A., Jain, R., & Jain, S. (2020). Sub-culturing of bacteria, fungi, and actinomycetes. In *Basic Techniques in Biochemistry, Microbiology and Molecular Biology: Principles and Techniques* (pp. 101-103).

Jarvis, W. R. (1962). The infection of strawberry and raspberry fruits by Botrytis cinerea Fr. *Annals of Applied Biology*, 50(3), 569-575.

Jeffers, S. (Ed.). (2015). Protocol 07-11.1: V8 agar (V8A) or broth. In *Laboratory Protocols for Phytophthora Species* (pp. 1-2). The American Phytopathological Society.

Ji, Y., Geng, C., Song, L., Zhang, L., Yang, J., Gao, Q., ... & Duan, K. (2023). Different responses to elevated temperature in the representative strains of strawberry pathogenic Colletotrichum spp. from eastern China. *Mycological Progress*, 22(1), 1-15.

Jin, P., Wang, H., Zhang, Y., Huang, Y., Wang, L., & Zheng, Y. (2017). UV-C enhances resistance against gray mold decay caused by Botrytis cinerea in strawberry fruit. *Scientia Horticulturae*, 225, 106-111.

Jamieson A. R. (2006). USOOPP16971 P3 (U.S. Patent No. US PP16,971 P3) U.S. Patent and Trademark Office. Retrieved September 17, 2023 from https://patentimages.storage.googleapis.com/87/bd/be/9c1624bb3b7bb9/USPP16971.pdf

Jamieson, A. R., Nickerson, N. L., Sanford, K. A. (1999) USOOPP1111OP (U.S. Patent No. Plant 11,110) U.S. Patent and Trademark Office. Retrieved September 17, 2023 from https://patentimages.storage.googleapis.com/11/7b/56/62f00a89041100/USPP11110.pdf

Jouquand, C., Chandler, C., Plotto, A., & Goodner, K. (2008). A sensory and chemical analysis of fresh strawberries over harvest dates and seasons reveals factors that affect eating quality. *Journal of the American Society for Horticultural Science*, 133(6), 859-867.

Justia Patents Search. (n.d.). Everbearing Patents and Patent Applications (Class PLT/209). Retrieved September 17, 2023 from https://patents.justia.com/patents-by-us-classification/PLT/209?page=3

Justia Patents Search. (2017, March 3). US Patent Application for Strawberry plant named "WASATCH" Patent Application (Application #20180255672 issued September 6, 2018)
Retrieved September 17, 2023 from https://patents.justia.com/patent/20180255672

Kafkas, E., Koşar, M., Paydaş, S., Kafkas, S., & Başer, K. H. C. (2007). Quality characteristics of strawberry genotypes at different maturation stages. *Food Chemistry*, 100(3), 1229- 1236.

Kahramanoğlu, İ., Panfilova, O., Kesimci, T. G., Bozhüyük, A. U., Gürbüz, R., & Alptekin, H. (2022). Control of postharvest gray mold at strawberry fruits caused by Botrytis cinerea and improving fruit storability through Origanum onites L. and Ziziphora clinopodioides
L. volatile essential oils. *Agronomy*, *12*(2), 389.

Kennedy, B. W., & King, T. H. (1962). Studies on epidemiology of bacterial angular leafspot on strawberry. *Plant Disease Reporter*, *46*, 360-3.

Kim, D. R., Gang, G. H., Jeon, C. W., Kang, N. J., Lee, S. W., & Kwak, Y. S. (2016). Epidemiology and control of strawberry bacterial angular leaf spot disease caused by Xanthomonas fragariae. *The plant pathology journal*, *32*(4), 290.

Kleinhenz, M. (2020, June 27). *Factors Influencing Measures of °Brix (Soluble Solids), an Indicator of Potential Crop Quality*. Fruit, Vegetable, & Specialty Crop News. Retrieved December 15, 2023, from https://u.osu.edu/vegnetnews/2020/06/27/factors-influencing- measures-of-brix-soluble-solids-an-indicator-of-potential-crop-quality/

Krasnow, C. S., Hammerschmidt, R., & Hausbeck, M. K. (2017). Characteristics of resistance to Phytophthora root and crown rot in Cucurbita pepo. *Plant Disease*, 101(5), 659-665.

Koike, S. T., Browne, G. T., Gordon, T. R., Bolda, M. P., Gubler, W. D., & Wintermantel, W. M. (2018). Botrytis fruit rot / strawberry / agriculture: Pest management guidelines / *UC statewide IPM program (UC IPM)*. Retrieved April 27, 2023, from https://ipm.ucanr.edu/agriculture/strawberry/botrytis-fruit-rot/

Koike, S. T., Browne, G. T., Gordon, T. R., Bold, M. P., Gubler, W. D., & Wintermantel, W. M. (2018, July). Rhizopus fruit rot (LEAK) / strawberry / agriculture: Pest management guidelines. *UC Statewide IPM Program*. Retrieved October 16, 2023, from https://ipm.ucanr.edu/agriculture/strawberry/rhizopus-fruit-rot

Kubota Lab. (n.d.). Fruit quality | Controlled environment berry production information. Retrieved October 24, 2023, from https://u.osu.edu/indoorberry/fruit-quality/

LaMotte, S. (2022, April 7). Dirty Dozen 2022: Produce with the most and least pesticides. CNN. https://www.cnn.com/2022/04/07/health/dirty-dozen-produce-2022-wellness/index.html

Lawrence, D., Brittain, G., Aglave, B., & Sances, F. (2022). First report of Neopestalotiopsis rosae causing crown and root rot of strawberry in California. *Plant Disease*.

Lee, W. J., Sandler, D. P., Blair, A., Samanic, C., Cross, A. J., & Alavanja, M. C. (2007). Pesticide use and colorectal cancer risk in the Agricultural Health Study. *International Journal of Cancer*, 121(2), 339-346.

Lerceteau-Köhler, E., Guerin, G., & Denoyes-Rothan, B. (2005). Identification of SCAR markers linked to Rca2 anthracnose resistance gene and their assessment in strawberry germplasm. *Theoretical and applied genetics*, *111*(5), 862-870.

Leroch, M., Plesken, C., Weber, R. W., Kauff, F., Scalliet, G., & Hahn, M. (2013). Gray mold populations in German strawberry fields are resistant to multiple fungicides and dominated by a novel clade closely related to Botrytis cinerea. *Applied and Environmental Microbiology*, 79(1), 159-167.

Lineberry, R. A., & Burkhart, L. (1943). Nutrient Deficiencies in the Strawberry Leaf and Fruit. *Plant physiology*, 18(3), 324–333. https://doi.org/10.1104/pp.18.3.324

Louws. (2014, April 8). Botrytis fruit rot / Gray mold on strawberry. *NC State Extension Publications*. Retrieved October 16, 2023, from https://content.ces.ncsu.edu/botrytis- cinerea-botrytis-fruit-rot-and-blight-on-strawberry

Louws, F., Ridge, G., Harrison, J., & Cline, B. (2019, July 10). Anthracnose Fruit Rot of Strawberry. *NC State Extension Publications*. Retrieved December 15, 2023, from https://content.ces.ncsu.edu/anthracnose-fruit-rot-of-strawberry

Louws, F. & Cline, B. (2019). Black root rot of strawberry. NC State Extension Publications. Retrieved April 2, 2023, from https://content.ces.ncsu.edu/black-root-rot-of-strawberry-1

Lu, B., Wang, Y., Zhang, G., Feng, Y., Yan, Z., Wu, J., & Chen, X. (2020). Genome-wide identification and expression analysis of the strawberry *FvbZIP* gene family and the role of key gene FabZIP46 in fruit resistance to gray mold. *Plants*, 9(9), 1199.

Ma, L. (2022). Genome-wide analysis of G-type lectin genes in Fragaria vesca and functional characterization of FaMBL1 gene in defense response of F. x ananassa to fungal pathogens.

Maas, J. L. (1978). Screening for resistance to fruit rot in strawberries and red raspberries: A review1. *HortScience*, 13(4), 423-426. Retrieved Oct 14, 2023, from https://doi.org/10.21273/HORTSCI.13.4.423

Mancini, M., Mazzoni, L., Qaderi, R., Leoni, E., Tonanni, V., Gagliardi, F., Capocasa, F., Toscano, G., & Mezzetti, B. (2023). Prediction of soluble solids content by means of NIR spectroscopy and relation with Botrytis cinerea tolerance in strawberry cultivars.
Horticulturae, 9(1), 91. https://doi.org/10.3390/horticulturae9010091

Madeiras, A., & Schloemann, S. (2015, July). *Strawberry IPM- Gray Mold*. Center for Agriculture, Food, and the Environment. UMass Extension Fruit Program. Retrieved November 17, 2023, from https://ag.umass.edu/fruit/fact-sheets/strawberry-ipm-gray- mold

Maung, C. E. H., Baek, W. S., Choi, T. G., & Kim, K. Y. (2021). Control of grey mould disease on strawberry using the effective agent, Bacillus amyloliquefaciens Y1. *Biocontrol Science and Technology*, 31(5), 468-482.

Menzel, C. (2021). Higher temperatures decrease fruit size in strawberry growing in the subtropics. *Horticulturae*, 7(2), 34. https://doi.org/10.3390/horticulturae7020034

Miles, T.D., Koike, S., Olmstead, M. (2017). Evaluation of commonly grown commercial strawberry varieties for susceptibility to Gray mold and Rhizopus fruit rot, 2015 and 2016. *Plant Disease Management Reports* (Report 11:SMF033).

Missouri Botanical Garden. (n.d.). Fragaria “Allstar” - Plant Finder. Retrieved September 17, 2023, from https://www.missouribotanicalgarden.org/PlantFinder/PlantFinderDetails.aspx?kemperco de=b879

MSU Technologies. (2015). 'REDSTART' Day-Neutral Strawberry - 'MSU 67'. Retrieved October 11, 2023 from https://msut.technologypublisher.com/technology/22786

Morton, L. W., Peres, N., Fraisse, C., & Gleason, M. (2017). Climate, weather, and strawberries. *Sociology Technical Report*, 1047, 16.

Moura, G. G. D. D., Barros, A. V. D., Machado, F., Martins, A. D., Silva, C. M. D., Durango, L. G. C., Forim, M., Alves, E., Pasqual, M., & Doria, J. (2021). Endophytic bacteria from strawberry plants control gray mold in fruits via production of antifungal compounds against Botrytis cinerea L. *Microbiological Research*, 251, 126793. https://doi.org/10.1016/j.micres.2021.126793

Nellist, C. F. (2018). Disease resistance in polyploid strawberry. In *The Genomes of Rosaceous Berries and Their Wild Relatives* (pp. 79-94).

Nelson, J. R., Verma, S., Bassil, N. V., Finn, C. E., Hancock, J. F., Cole, G. S., Knapp, S. J., & Whitaker, V. M. (2021). Discovery of three loci increasing resistance to charcoal rot caused by *Macrophomina phaseolina* in octoploid strawberry. *G3: Genes, Genomes, Genetics*, 11(3). https://doi.org/10.1093/g3journal/jkab037

Neri, F., Cappellin, L., Spadoni, A., Cameldi, I., Algarra Alarcon, A., Aprea, E., Romano, A., Gasperi, F., & Biasioli, F. (2015). Role of strawberry volatile organic compounds in the development of Botrytis cinerea infection. *Plant Pathology*, 64, 709-717. https://doi.org/10.1111/ppa.12287

Niewczas. (2021). Niewczas Szkółka. Retrieved October 24, 2023, from https://niewczas.co/en/offer/san-andreas

Nigro, F., Ippolito, A., Lattanzio, V., Di Venere, D., & Salerno, M. (2000). Effect of ultraviolet- C light on postharvest decay of strawberry. *Journal of Plant Pathology*, 29-37.

Noncitrus Fruits and Nuts 2021 Summary. (2022, May). United States Department of Agriculture National Agricultural Statistics Service. Retrieved February 26, 2023, from https://downloads.usda.library.cornell.edu/usdaesmis/files/zs25x846c/4q77gv96p/t722jd7 6c/ncit0522.pdf

Oliveira, M., Guerner-Moreira, J., Mesquita, M. M., & Abreu, I. (2009). Important phytopathogenic airborne fungal spores in a rural area: Incidence of Botrytis cinerea and Oidium spp. *Annals of Agricultural and Environmental Medicine*, 16(2), 197-204.

Palencia, P., Martínez, F., Medina, J. J., & López-Medina, J. (2013). Strawberry yield efficiency and its correlation with temperature and solar radiation. *Horticultura Brasileira*, 31, 93-99.

Ramakrishnan, B., Maddela, N. R., Venkateswarlu, K., & Megharaj, M. (2021). Linkages between plant rhizosphere and animal gut environments: Interaction effects of

pesticides with their microbiomes. *Environmental Advances*, 5, 100091.

Patel, A. M., Lee, W. S., & Peres, N. A. (2022). Imaging and deep learning based approach to leaf wetness detection in strawberry. *Sensors (Basel, Switzerland)*, 22(21), 8558-8558.

Patent Status of Select Strawberry Varieties | Cornell Fruit Resources: Berries. (2022, January 18). Retrieved September 17, 2023 from https://blogs.cornell.edu/berries/2022/01/18/patent-status-of-select-strawberry-varieties/#:~:text=Honeoye%20%E2%80%93%20patent%20expired%20in%201999, susc eptible%20to%20black%20root%20rot.

Peres, N. A., Baggio, J. S., & Mertely, J. C. (2018). Charcoal rot of strawberries caused by Macrophomina phaseolina. Plant Pathology Department, UF/IFAS Extension, Gulf Coast Research and Education Center, Wimauma, Florida 33598, U.S.A.

Persoon C.H. Dispositio methodica fungorum. [(accessed on 7 May 2020)];Neues Mag. Bot. 1794 1:81–128. Available online: http://www.mycobank.org/BioloMICS.aspx?TableKey=14682616000000061&Rec=161 7 9&Fields=All

Petrasch, S., Knapp, S. J., Van Kan, J. A., & Blanco-Ulate, B. (2019). Grey mould of strawberry, a devastating disease caused by the ubiquitous necrotrophic fungal pathogen Botrytis cinerea. *Molecular Plant Pathology*, 20(6), 877-892.

Petrasch, S., Mesquida-Pesci, S. D., Pincot, D. D. A., Fieldmann, M. J., López, C. M., Famula, R., Hardigan, M. A., Cole, G. S., Knapp, S. J., & Blanco-Ulate, B. (2022). Genomic prediction of strawberry resistance to postharvest fruit decay caused by the fungal pathogen Botrytis cinerea. *G3: Genes|Genomes|Genetics*, 12(1). https://doi.org/10.1093/g3journal/jkab378

Pincot, D. D., Poorten, T. J., Hardigan, M. A., Harshman, J. M., Acharya, C. B., Cole, G. S., ... & Knapp, S. J. (2018). Genome-wide association mapping uncovers Fw1, a dominant gene conferring resistance to Fusarium wilt in strawberry. *G3: Genes, Genomes, Genetics*, *8*(5), 1817-1828.

Prasad, R., Lisiecka, J., & Raj, K. (2022). Strawberry–More than a popular summer fruit: A mini-review. DOI: 10.37722/ANAFS.2022201

Prentice, I. W., & Harris, R. V. (1946). Resolution of strawberry virus complexes by means of the aphis vector Capitophorus fragariae Theob. *Annals of Applied Biology*, *33*(1), 50-53.

Rahman, M., & Louws, F. J. (2017). Epidemiological significance of crown rot in the fruiting field in relation to Colletotrichum gloeosporioides infection of strawberry nursery plants. *Plant disease*, *101*(6), 907-915.

Reddy, P. P. (2016). Strawberry. *Sustainable Crop Protection under Protected Cultivation*, 245- 260.

Redmond, R. S., Kalantari, F., Ting, K. C., Thorp, K. R., Hameed, I. A., Weltzien, C., Ahmad, D., & Shad, Z. M. (2018). Advances in greenhouse automation and controlled environment agriculture: A transition to plant factories and urban agriculture. NTNU. Retrieved May 10, 2023, from https://ntnuopen.ntnu.no/ntnu-xmlui/handle/11250/2587902

Reyes, L. (1990). Survey of strawberry genotypes for infection of flower tissues by Botrytis cinerea: Oregon State University.

Rhouma, A., Hajji-Hedfi, L., Ben Othmen, S., Kumari Shah, K., Matrood, A. A., Okon, O. G., & Pant, D. (2022). Strawberry grey mould, a devastating disease caused by the airborne fungal pathogen Botrytis cinerea. *Egyptian Journal of Phytopathology*, 50(2), 44-50.

Ries, S. M. (1995). RPD No. 704 - Gray Mold of Strawberry. Available at http://ipm.illinois.edu/diseases/series700/rpd704/ accessed on Jan 4, 2019.

Roca-Couso, R., Flores-Félix, J. D., & Rivas, R. (2021). Mechanisms of Action of Microbial Biocontrol Agents against *Botrytis cinerea*. *Journal of fungi (Basel, Switzerland)*, *7*(12), 1045. https://doi.org/10.3390/jof7121045

Romanazzi, G., & Feliziani, E. (2014). Botrytis cinerea (Gray Mold). *Postharvest Decay*, 131-146. https://doi.org/10.1016/B978-0-12-411552-1.00004-1

Rondolino. (2017, September 1). *Mettler-Toledo International Inc.* https://www.mt.com/us/en/home/products/Laboratory_Analytics_Browse/Product_Famil y_Browse_titrators_main/automated-titration-systems/Rondolino.html

Rosa, S., Pesaresi, P., Mizzotti, C., Bulone, V., Mezzetti, B., Baraldi, E., & Masiero, S. (2022). Game-changing alternatives to conventional fungicides: Small RNAs and short peptides. *Trends in Biotechnology*, 40(3), 320-337.

Samtani, J. B., Rom, C. R., Friedrich, H., Fennimore, S. A., Finn, C. E., Petran, A., ... & Bergefurd, B. (2019). The status and future of the strawberry industry in the United States. *HortTechnology*, 29(1), 11-24.

Sanford, J. C., Ourecky, D. K., Reich, J. E. II. (1987) (U.S. Patent No. Plant 5,897) U.S. Patent and Trademark Office. Retrieved September 17, 2023 from https://patentimages.storage.googleapis.com/13/be/e9/5057b95f931100/USPP5897.pdf

Sanford, J. C., Ourecky, D. K., Reich, J. E., & Aldwinckle, H. S. (1982). 'Honeoye'and 'Canoga'Strawberries1. *HortScience*, *17*(6), 982-984.

Sangiorgio, D. (2022). Deciphering the role of plant microbiome on strawberry and raspberry growth, resistance, fruit quality, and aroma.

SAS Help Center. (2019, December 13). SAS® Help Center. The GLM Procedure. Retrieved November 17, 2023, from https://documentation.sas.com/doc/en/pgmsascdc/9.4_3.4/statug/statug_glm_overview.ht m

Schilder. (2016, June 24). Warm weather favors anthracnose fruit rot in strawberries. MSU Extension. Retrieved April 1, 2023, from https://www.canr.msu.edu/news/warm_weather_favors_anthracnose_fruit_rot_in_strawb erries

SCM EDU. (2022, April 2). SCM EDU - Supply Chain Management Education. Retrieved October 2, 2023, from https://scmedu.org/hundredweight_cwt_/

Seijo, T. E., Chandler, C. K., Mertely, J. C., Moyer, C., & Peres, N. A. (2008, December). Resistance of strawberry cultivars and advanced selections to anthracnose and Botrytis fruit rots. In *Proceedings of the Florida State Horticultural Society* (Vol. 121, pp. 246- 248).

Shahbandeh, M. (2023, May 16). *U.S. total strawberry production 2000-2022*. Statista. Retrieved February 23, 2024, from https://www.statista.com/statistics/193288/us-total- strawberry-production-since-2000/?gad_source=1&gclid=EAIaIQobChMI4Kv3g5PBhAMVSlFBAh3OigaiEAAYAi AAEgJWnPD_BwE

Shao, W. C., Zang, Y. Y., Ma, H. Y., Ling, Y. E., & Kai, Z. P. (2021). Concentrations and related health risk assessment of pesticides, phthalates, and heavy metals in strawberries from Shanghai, China. *Journal of Food Protection*, 84(12), 2116-2122.

Shaw, D.V., & Larson, K. D. (2009). USOOPP 19975P2 (U.S. Patent No. US PP19,975 P2) U.S. Patent and Trademark Office. Retrieved September 17, 2023 from https://patentimages.storage.googleapis.com/05/25/0e/e1ae08f5cbf0cf/USPP19975.pdf

Shaw, D.V., & Larson, K. D. (2005). US 2005O172374A1 (U.S. Patent No. US 2005/0172374 P1) U.S. Patent and Trademark Office. Retrieved September 17, 2023 from https://patentimages.storage.googleapis.com/64/96/78/c747565d57cf97/US20050172374P1.pdf

Shaw, D. V. (1990). Response to selection and associated changes in genetic variance for soluble solids and titratable acids contents in strawberries. *Journal of the American Society for Horticultural Science*, 115(5), 839-843.

Shortt, Eng, & Pate. (2022, October). Irrigation for frost protection of strawberries. Ontario Ministry of Agriculture, Food and Rural Affairs. Retrieved October 21, 2023, from https://files.ontario.ca/omafra-irrigation-frost-protection-strawberries-22-043-en-2022- 12-16.pdf

Simpson, D. (2018). The economic importance of strawberry crops. In *The Genomes of Rosaceous Berries and Their Wild Relatives* (pp. 1-7).

Sobekova, K., Thomsen, M. R., & Ahrendsen, B. L. (2013). Market trends and consumer demand for fresh berries. *APSTRACT: Applied Studies in Agribusiness and Commerce*, 7(1033-2016-84241), 11-14.

Statistica. (n.d.). U.S. total strawberry production from 2000 to 2021 (in 1,000 tons). Retrieved March 3, 2023, from https://www.statista.com/statistics/193288/us-total-strawberry- production-since-2000/

Statistica. (2022). Top strawberry producing U.S. states. Retrieved from https://www.statista.com/statistics/194235/top-10-strawbery-producing-us-states/

Steiger, D. (2007). Global economic importance of Botrytis protection. In *Book of Abstracts, 14th International Botrytis Symposium, Cape Town, South Africa* (pp. 21-26). AFRICAN SUN MeDIA Pty (Ltd.).

Sun, J., Chu, Y. F., Wu, X., & Liu, R. H. (2002). Antioxidant and antiproliferative activities of common fruits. *Journal of Agricultural and Food Chemistry*, 50(25), 7449–7454. https://doi.org/10.1021/jf0207530

Sweeny, J. P., V.J. Chapman, and P.A. Hepner. (1970). Sugar, acid, and flavor in fresh fruit. *Journal of the American Dietetic Association*, 57:432-435.

Terry, L. A., Joyce, D. C., Adikaram, N. K., & Khambay, B. P. (2004). Preformed antifungal compounds in strawberry fruit and flower tissues. *Postharvest biology and technology*, 31(2), 201-212.

Thermo Fisher Scientific - IE. (n.d.). Counting cells in a hemocytometer. Retrieved October 16, 2023, from https://www.thermofisher.com/us/en/home/references/gibco-cell-culture-basics/cell-culture-protocols/counting-cells-in-a-hemacytometer.html

Thomas, H. E. (1932). Verticillium wilt of strawberries. *California Agricultural Experiment Station 530* (pp 16)

Tiaoyeer. (2018). Brix refractometer with ATC - Tiaoyeer digital handheld refractometer for beerwine brewing, dual scale-specific gravity 1.000-1.130 and brix 0-32%. *Amazon.com*. Retrieved October 25, 2023, from

https://www.amazon.com/Tiaoyeer-Refractometer- Handheld-Scale-Specific-1-000-1-130/dp/B07DL2NJYS?ref_=ast_sto_dp

Tzanetakis, I. E., & Martin, R. R. (2013). Expanding field of strawberry viruses which are important in North America. *International Journal of Fruit Science*, 13(1-2), 184-195.

USDA. (2023, May). Noncitrus Fruits and Nuts 2022 Summary (May 2023). National Agricultural Statistics Service. Retrieved March 3, 2023 from https://downloads.usda.library.cornell.edu/usda-esmis/files/zs25x846c/zk51wx21m/k356bk214/ncit0523.pdf

USDA ERS - Fruit and Tree Nuts Yearbook Tables. (2022). Retrieved March 25, 2023, from https://www.ers.usda.gov/data-products/fruit-and-tree-nuts-data/fruit-and-tree-nuts- yearbook-tables/

van Kan, J. A. (2003, August). Infection strategies of Botrytis cinerea. In *VIII International Symposium on Postharvest Physiology of Ornamental Plants 669* (pp. 77-90).

Valero-Jiménez, C. A., Veloso, J., Staats, M., & van Kan, J. A. (2019). Comparative genomics of plant pathogenic Botrytis species with distinct host specificity. *BMC Genomics*, 20(1), 1-12.

Vinje, E. (2019, November 12). Gray Mold (Botrytis): Symptoms, Treatment and Control. *Planet Natural*. https://www.planetnatural.com/pest-problem-solver/plant-disease/gray- mold/

Vischetti, C., Feliziani, E., Landi, L., De Bernardi, A., Marini, E., & Romanazzi, G. (2023). Effectiveness of Four Synthetic Fungicides in the Control of Post-Harvest Gray Mold of Strawberry and Analyses of Residues on Fruit. *Agronomy, 14*(1), 65.

von Haller, A. (1771). *Bibliotheca antomica qua scripta ad anatomen et physiologiam facientia a rerum initiis recensentur: tempora ante tournefortium* (Vol. 1). apud Orell, Gessner, Fuessli, et socc..

Voth, V., Ana, S., Attorney, D. V., Bringhurst, R. S. (1994). USOOPPO8708P (U.S. Patent No.Plant 8,708) U.S. Patent and Trademark Office. Retrieved September 17, 2023 from https://patentimages.storage.googleapis.com/63/f8/f3/2cb5af668594a9/USPP8708.pdf

Weather Spark . (n.d.). Okemos June 21, 2021 Historical Weather (Michigan, United States) – Weather Spark. Retrieved November 17, 2023, from https://weatherspark.com/h/d/16083/2021/6/21/Historical-Weather-on-Monday-June-21- 2021-in-Okemos-Michigan-United-States#Figures-PrecipitationProbability

Weather Spark . (n.d.). Okemos June 21, 2021 Historical Weather (Michigan, United

States) – Weather Spark. Retrieved November 17, 2023, from https://weatherspark.com/h/d/16083/2021/6/21/Historical-Weather-on-Monday-June-21- 2021-in-Okemos-Michigan-United-States#Figures-PrecipitationProbability

Weber, R. W., & Petridis, A. (2023). Fungicide Resistance in Botrytis spp. and Regional Strategies for Its Management in Northern European Strawberry Production. *BioTech*, *12*(4), 64.

Willetts, H. J. (1971). The survival of fungal sclerotia under adverse environmental conditions. *Biological Reviews*, 46: 387–407.

Yan, J., Wu, H., Chen, K., Feng, J., & Zhang, Y. (2021). Antifungal activities and mode of action of Cymbopogon citratus, Thymus vulgraris, and Origanum heracleoticum essential oil vapors against Botrytis cinerea and their potential application to control postharvest strawberry gray mold. *Foods*, 10(10), 2451.

Yang, Y., & Davis, T. M. (2017). A new perspective on polyploid Fragaria (strawberry) genome composition based on large-scale, multi-locus phylogenetic analysis. *Genome biology and evolution*, *9*(12), 3433-3448.,

Yong, D., Li, Y., Gong, K., Yu, Y., Zhao, S., Duan, Q., Ren, C., Li, A., Fu, J., Ni, J., Zhang, Y., & Li, R. (2022). Biocontrol of strawberry gray mold caused by Botrytis cinerea with the termite associated Streptomyces sp. Sdu1201 and actinomycin D. *Frontiers in Microbiology*, 13, 1051730. https://doi.org/10.3389/fmicb.2022.1051730

Zeller, S. M. (1933). Crinkle disease of strawberry.

Zhao, Y., Vlasselaer, L., Ribeiro, B., Terzoudis, K., Hertog, M., Nicolaï, B., & De Coninck, B. (2022). Constitutive Defense Mechanisms Have a Major Role in the Resistance of Woodland Strawberry Leaves Against Botrytis cinerea. *Frontiers in Plant Science*, 13, 912667. https://doi.org/10.3389/fpls.2022.912667

Zhang, Z., Lu, S., Yu, W., Ehsan, S., Zhang, Y., Jia, H., & Fang, J. (2022). Jasmonate increases terpene synthase expression, leading to strawberry resistance to Botrytis cinerea infection. *Plant Cell Reports*, 41(5), 1243-1260.

APPENDIX

Table A.1: Dataset of 2021 AUDPC Values

GROUP	ID	REP	#FRUIT	AUDPC
Cultivar	Albion	1	7	1107.14
Cultivar	Albion	2	2	1050.00
Cultivar	Albion	3	6	1033.33
Cultivar	Albion	4	8	1062.50
Cultivar	Albion	5	6	1116.67
Cultivar	Albion	6	7	1135.71
Cultivar	Albion	7	5	1180.00
Cultivar	Albion	8	5	1010.00
Cultivar	Allstar	1	1	450.00
Cultivar	Allstar	2	2	600.00
Cultivar	Allstar	3	4	675.00
Cultivar	Allstar	4	3	950.00
Cultivar	Allstar	5	1	1250.00
Cultivar	Allstar	6	6	966.67
Cultivar	Allstar	7	3	883.33
Cultivar	Allstar	8	9	850.00
Cultivar	Cabot	1	7	1085.71
Cultivar	Cabot	2	6	1191.67
Cultivar	Cabot	3	8	1112.50
Cultivar	Cabot	4	6	1158.33
Cultivar	Cabot	5	2	1000.00
Cultivar	Cabot	6	4	1125.00
Cultivar	Cabot	7	3	1116.67
Cultivar	Cabot	8	4	1075.00
Cultivar	Camarosa	1	6	983.33
Cultivar	Camarosa	2	7	921.43
Cultivar	Camarosa	3	6	900.00
Cultivar	Camarosa	4	6	966.67
Cultivar	Camarosa	5	4	900.00
Cultivar	Camarosa	6	6	900.00
Cultivar	Camarosa	7	6	933.33
Cultivar	Camarosa	8	3	1083.33

Table A.1 (cont'd)

Cultivar	Cavendish	1	3	916.67
Cultivar	Cavendish	2	4	987.50
Cultivar	Cavendish	3	7	907.14
Cultivar	Cavendish	4	8	937.50
Cultivar	Cavendish	5	6	1016.67
Cultivar	Cavendish	6	8	1012.50
Cultivar	Cavendish	7	3	883.33
Cultivar	Cavendish	8	3	1016.67
Cultivar	Earliglow	1	5	890.00
Cultivar	Earliglow	2	6	1000.00
Cultivar	Earliglow	3	6	866.67
Cultivar	Earliglow	4	3	983.33
Cultivar	Earliglow	5	3	1016.67
Cultivar	Earliglow	6	5	930.00
Cultivar	Earliglow	7	5	1010.00
Cultivar	Earliglow	8	5	1050.00
Cultivar	Honeoye	1	7	835.71
Cultivar	Honeoye	2	6	950.00
Cultivar	Honeoye	3	8	712.50
Cultivar	Honeoye	4	4	900.00
Cultivar	Honeoye	5	4	850.00
Cultivar	Honeoye	6	8	837.50
Cultivar	Honeoye	7	8	912.50
Cultivar	Honeoye	8	4	825.00
Cultivar	Jewel	1	8	837.50
Cultivar	Jewel	2	4	1050.00
Cultivar	Jewel	3	7	850.00
Cultivar	Jewel	4	3	950.00
Cultivar	Jewel	5	5	910.00
Cultivar	Jewel	6	3	1050.00
Cultivar	Jewel	7	1	950.00
Cultivar	Jewel	8	4	975.00
Cultivar	Redstart	1	9	1005.56
Cultivar	Redstart	2	9	1050.00
Cultivar	Redstart	3	10	1060.00

Table A.1 (cont'd)

Cultivar	Redstart	4	9	1083.33
Cultivar	Redstart	5	8	1075.00
Cultivar	Redstart	6	10	1140.00
Cultivar	Redstart	7	8	975.00
Cultivar	Redstart	8	8	1037.50
Cultivar	Wasatch	1	5	750.00
Cultivar	Wasatch	2	7	935.71
Cultivar	Wasatch	3	2	1100.00
Cultivar	Wasatch	4	5	1090.00
Cultivar	Wasatch	5	10	960.00
Cultivar	Wasatch	6	10	860.00
Cultivar	Wasatch	7	7	992.86
Cultivar	Wasatch	8	7	992.86
Line	MSU44	1	6	933.33
Line	MSU44	2	7	1035.71
Line	MSU44	3	7	935.71
Line	MSU44	4	6	983.33
Line	MSU44	5	6	983.33
Line	MSU44	6	7	1064.29
Line	MSU44	7	7	950.00
Line	MSU44	8	5	950.00
Line	MSU49	1	7	864.29
Line	MSU49	2	9	1038.89
Line	MSU49	3	9	972.22
Line	MSU49	4	9	1050.00
Line	MSU49	5	10	880.00
Line	MSU49	6	9	1050.00
Line	MSU49	7	8	1075.00
Line	MSU49	8	10	1020.00
Line	MSU69	1	7	878.57
Line	MSU69	2	9	905.56
Line	MSU69	3	10	890.00
Line	MSU69	4	9	972.22
Line	MSU69	5	9	850.00
Line	MSU69	6	9	850.00

Table A.1 (cont'd)

Line	MSU69	7	8	925.00
Line	MSU69	8	6	866.67
Line	MSU71	1	4	1050.00
Line	MSU71	2	4	1025.00
Line	MSU71	3	3	983.33
Line	MSU71	4	4	975.00
Line	MSU71	5	4	1125.00
Line	MSU71	6	3	983.33
Line	MSU71	7	3	1016.67
Line	MSU71	8	3	983.33
Line	MSU73	1	3	1116.67
Line	MSU73	2	1	850.00
Line	MSU73	3	1	1050.00
Line	MSU73	4	0	NA
Line	MSU73	5	0	NA
Line	MSU73	6	0	NA
Line	MSU73	7	2	1050.00
Line	MSU73	8	5	1070.00
Line	MSU75	1	6	916.67
Line	MSU75	2	5	990.00
Line	MSU75	3	8	912.50
Line	MSU75	4	6	966.67
Line	MSU75	5	10	970.00
Line	MSU75	6	8	987.50
Line	MSU75	7	3	1050.00
Line	MSU75	8	9	961.11
Line	MSU76	1	0	NA
Line	MSU76	2	1	850.00
Line	MSU76	3	2	1050.00
Line	MSU76	4	2	1000.00
Line	MSU76	5	2	850.00
Line	MSU76	6	4	1075.00
Line	MSU76	7	0	NA
Line	MSU76	8	1	1050.00
Line	MSU78	1	6	1016.67

Table A.1 (cont'd)

Line	MSU78	2	10	920.00
Line	MSU78	3	9	905.56
Line	MSU78	4	9	994.44
Line	MSU78	5	10	920.00
Line	MSU78	6	10	930.00
Line	MSU78	7	9	805.56
Line	MSU78	8	6	816.67
Line	MSU80	1	3	816.67
Line	MSU80	2	8	1012.50
Line	MSU80	3	7	1078.57
Line	MSU80	4	4	1000.00
Line	MSU80	5	5	870.00
Line	MSU80	6	5	990.00
Line	MSU80	7	4	900.00
Line	MSU80	8	6	916.67
Line	MSU81	1	9	1061.11
Line	MSU81	2	10	1130.00
Line	MSU81	3	9	1083.33
Line	MSU81	4	10	1070.00
Line	MSU81	5	10	1130.00
Line	MSU81	6	10	1090.00
Line	MSU81	7	9	1072.22
Line	MSU81	8	10	1080.00
Line	MSU84	1	4	1125.00
Line	MSU84	2	5	990.00
Line	MSU84	3	2	900.00
Line	MSU84	4	4	1000.00
Line	MSU84	5	3	1116.67
Line	MSU84	6	5	1010.00
Line	MSU84	7	7	1078.57
Line	MSU84	8	7	1007.14
Line	MSU86	1	9	972.22
Line	MSU86	2	8	887.50
Line	MSU86	3	10	930.00
Line	MSU86	4	5	810.00

Table A.1 (cont'd)

Line	MSU86	5	9	850.00
Line	MSU86	6	8	887.50
Line	MSU86	7	10	950.00
Line	MSU86	8	10	900.00
Line	MSU90	1	3	783.33
Line	MSU90	2	3	583.33
Line	MSU90	3	2	700.00
Line	MSU90	4	1	850.00
Line	MSU90	5	3	1150.00
Line	MSU90	6	0	NA
Line	MSU90	7	0	NA
Line	MSU90	8	1	1150.00
Line	14_30_4	1	3	1016.67
Line	14_30_4	2	3	816.67
Line	14_30_4	3	3	883.33
Line	14_30_4	4	3	983.33
Line	14_30_4	5	3	883.33
Line	14_30_4	6	3	716.67
Line	14_30_4	7	2	850.00
Line	14_30_4	8	2	800.00
Line	16_1_1	1	4	900.00
Line	16_1_1	2	5	1010.00
Line	16_1_1	3	2	850.00
Line	16_1_1	4	4	1000.00
Line	16_1_1	5	3	816.67
Line	16_1_1	6	5	1030.00
Line	16_1_1	7	2	950.00
Line	16_1_1	8	5	1030.00

Table A.2: Dataset of 2022 AUDPC Values

GROUP	**ID**	**REP**	**#FRUIT**	**AUDPC**
Cultivar	Albion	1	6	816.67
Cultivar	Albion	2	6	866.67
Cultivar	Albion	3	6	883.33
Cultivar	Albion	4	6	683.33

Table A.2 (cont'd)

Cultivar	Albion	5	6	900.00
Cultivar	Allstar	1	10	750.00
Cultivar	Allstar	2	10	720.00
Cultivar	Allstar	3	10	680.00
Cultivar	Allstar	4	10	750.00
Cultivar	Allstar	5	10	760.00
Cultivar	Annapolis	1	10	820.00
Cultivar	Annapolis	2	8	762.50
Cultivar	Annapolis	3	10	850.00
Cultivar	Annapolis	4	10	880.00
Cultivar	Annapolis	5	9	905.56
Cultivar	Cabot	1	8	1012.50
Cultivar	Cabot	2	8	800.00
Cultivar	Cabot	3	8	987.50
Cultivar	Cabot	4	8	875.00
Cultivar	Cabot	5	8	993.75
Cultivar	Camarosa	1	10	860.00
Cultivar	Camarosa	2	10	740.00
Cultivar	Camarosa	3	9	772.22
Cultivar	Camarosa	4	9	894.44
Cultivar	Camarosa	5	10	860.00
Cultivar	Cavendish	1	10	820.00
Cultivar	Cavendish	2	10	830.00
Cultivar	Cavendish	3	10	850.00
Cultivar	Cavendish	4	10	840.00
Cultivar	Cavendish	5	10	880.00
Cultivar	Earliglow	1	10	880.00
Cultivar	Earliglow	2	10	950.00
Cultivar	Earliglow	3	10	920.00
Cultivar	Earliglow	4	10	950.00
Cultivar	Earliglow	5	10	1000.00
Cultivar	Honeoye	1	6	900.00
Cultivar	Honeoye	2	0	NA
Cultivar	Honeoye	3	0	NA
Cultivar	Honeoye	4	5	830.00

Table A.2 (cont'd)

Cultivar	Honeoye	5	4	825.00
Cultivar	Jewel	1	9	1016.67
Cultivar	Jewel	2	10	980.00
Cultivar	Jewel	3	9	950.00
Cultivar	Jewel	4	10	1020.00
Cultivar	Jewel	5	10	990.00
Cultivar	San Andreas	1	1	750.00
Cultivar	San Andreas	2	1	750.00
Cultivar	San Andreas	3	3	1083.33
Cultivar	San Andreas	4	2	850.00
Cultivar	San Andreas	5	2	1000.00
Cultivar	Redstart	1	3	1150.00
Cultivar	Redstart	2	5	910.00
Cultivar	Redstart	3	3	916.67
Cultivar	Redstart	4	3	850.00
Cultivar	Redstart	5	3	816.67
Cultivar	Wasatch	1	9	816.67
Cultivar	Wasatch	2	10	900.00
Cultivar	Wasatch	3	10	930.00
Cultivar	Wasatch	4	10	840.00
Cultivar	Wasatch	5	10	960.00
Line	MSU44	1	7	850.00
Line	MSU44	2	10	860.00
Line	MSU44	3	10	910.00
Line	MSU44	4	10	870.00
Line	MSU44	5	10	900.00
Line	MSU49	1	9	938.89
Line	MSU49	2	6	983.33
Line	MSU49	3	9	950.00
Line	MSU49	4	7	992.86
Line	MSU49	5	5	910.00
Line	MSU69	1	6	733.33
Line	MSU69	2	6	800.00
Line	MSU69	3	6	966.67
Line	MSU69	4	6	816.67

Table A.2 (cont'd)

Line	MSU69	5	6	866.67
Line	MSU71	1	10	910.00
Line	MSU71	2	9	927.78
Line	MSU71	3	10	910.00
Line	MSU71	4	10	930.00
Line	MSU71	5	6	933.33
Line	MSU73	1	8	900.00
Line	MSU73	2	6	933.33
Line	MSU73	3	6	883.33
Line	MSU73	4	5	950.00
Line	MSU73	5	9	950.00
Line	MSU75	1	7	907.14
Line	MSU75	2	7	907.14
Line	MSU75	3	7	935.71
Line	MSU75	4	7	950.00
Line	MSU75	5	7	935.71
Line	MSU76	1	2	800.00
Line	MSU76	2	3	950.00
Line	MSU76	3	3	950.00
Line	MSU76	4	3	750.00
Line	MSU76	5	3	816.67
Line	MSU78	1	5	950.00
Line	MSU78	2	5	890.00
Line	MSU78	3	5	850.00
Line	MSU78	4	5	830.00
Line	MSU78	5	5	850.00
Line	MSU80	1	6	966.67
Line	MSU80	2	6	1000.00
Line	MSU80	3	4	875.00
Line	MSU80	4	6	933.33
Line	MSU80	5	6	900.00
Line	MSU81	1	4	950.00
Line	MSU81	2	5	810.00
Line	MSU81	3	4	900.00
Line	MSU81	4	5	870.00

Table A.2 (cont'd)

Line	MSU81	5	4	900.00
Line	14_30_4	1	5	830.00
Line	14_30_4	2	5	830.00
Line	14_30_4	3	5	770.00
Line	14_30_4	4	5	870.00
Line	14_30_4	5	5	770.00

Table A.3: Dataset of 2021 Soluble Solids Content

GROUP	ID	REP	BRIX
Cultivar	Albion	1	7.1
Cultivar	Albion	2	9.9
Cultivar	Albion	3	6.1
Cultivar	Cabot	1	7.4
Cultivar	Cabot	2	8
Cultivar	Cabot	3	7.2
Cultivar	Camarosa	1	12.4
Cultivar	Camarosa	2	7.3
Cultivar	Camarosa	3	6.1
Cultivar	Cavendish	1	8
Cultivar	Cavendish	2	8
Cultivar	Cavendish	3	8
Cultivar	Earliglow	1	8
Cultivar	Earliglow	2	8
Cultivar	Earliglow	3	11.4
Cultivar	Honeoye	1	6.8
Cultivar	Honeoye	2	8.8
Cultivar	Honeoye	3	8.9
Cultivar	Jewel	1	6
Cultivar	Jewel	2	8
Cultivar	Jewel	3	10.6
Cultivar	Redstart	1	8.3
Cultivar	Redstart	2	8.8
Cultivar	Redstart	3	9.7

Table A.4: Dataset of 2022 Soluble Solids Content

GROUP	ID	REP	BRIX
Cultivar	Albion	1	9
Cultivar	Albion	2	9
Cultivar	Albion	3	9.6
Cultivar	Albion	4	7
Cultivar	Albion	5	9.2
Cultivar	Allstar	1	7
Cultivar	Allstar	2	7.2
Cultivar	Allstar	3	8
Cultivar	Allstar	4	7.8
Cultivar	Allstar	5	7.8
Cultivar	Annapolis	1	8.2
Cultivar	Annapolis	2	9
Cultivar	Annapolis	3	9.2
Cultivar	Annapolis	4	10.5
Cultivar	Annapolis	5	8.1
Cultivar	Cabot	1	9
Cultivar	Cabot	2	8.7
Cultivar	Cabot	3	9.2
Cultivar	Cabot	4	9.8
Cultivar	Cabot	5	9.1
Cultivar	Camarosa	1	8.2
Cultivar	Camarosa	2	8.1
Cultivar	Camarosa	3	11.3
Cultivar	Camarosa	4	8.1
Cultivar	Camarosa	5	9.1
Cultivar	Cavendish	1	9
Cultivar	Cavendish	2	10
Cultivar	Cavendish	3	10.5
Cultivar	Cavendish	4	10
Cultivar	Cavendish	5	11
Cultivar	Earliglow	1	11.2
Cultivar	Earliglow	2	11.5
Cultivar	Earliglow	3	10
Cultivar	Earliglow	4	10.9

Table A.4 (cont'd)

Cultivar	Earliglow	5	12.7
Cultivar	Jewel	1	10.1
Cultivar	Jewel	2	9.7
Cultivar	Jewel	3	8.9
Cultivar	Jewel	4	9.6
Cultivar	Jewel	5	9.8
Cultivar	Wasatch	1	10.1
Cultivar	Wasatch	2	8.2
Cultivar	Wasatch	3	9.2
Cultivar	Wasatch	4	10
Cultivar	Wasatch	5	10
Line	MSU44	1	9.6
Line	MSU44	2	7
Line	MSU44	3	9
Line	MSU44	4	10
Line	MSU44	5	9
Line	MSU49	1	7
Line	MSU49	2	6.2
Line	MSU49	3	6
Line	MSU49	4	6.2
Line	MSU49	5	7
Line	MSU69	1	6
Line	MSU69	2	6.2
Line	MSU69	3	5
Line	MSU69	4	7
Line	MSU69	5	9
Line	MSU71	1	8
Line	MSU71	2	6
Line	MSU71	3	7
Line	MSU71	4	9.6
Line	MSU71	5	8.2
Line	MSU73	1	7.3
Line	MSU73	2	6.9
Line	MSU73	3	8.1
Line	MSU73	4	9.2

Table A.4 (cont'd)

Line	MSU73	5	7.1
Line	MSU75	1	10.2
Line	MSU75	2	11.6
Line	MSU75	3	7.8
Line	MSU75	4	10.6
Line	MSU75	5	8
Line	MSU78	1	6
Line	MSU78	2	5.2
Line	MSU78	3	5.2
Line	MSU78	4	5.2
Line	MSU78	5	5.2
Line	MSU80	1	10
Line	MSU80	2	9.4
Line	MSU80	3	9
Line	MSU80	4	9.8
Line	MSU80	5	8.2
Line	14_30_4	1	10.2
Line	14_30_4	2	9.6
Line	14_30_4	3	6.7
Line	14_30_4	4	6.9
Line	14_30_4	5	12.1

Table A.5: Dataset of 2021 Titratable Acidity

GROUP	ID	REP	TA
Cultivar	Albion	1	0.371
Cultivar	Albion	2	0.561
Cultivar	Albion	3	0.633
Cultivar	Albion	4	0.55
Cultivar	Cabot	1	0.654
Cultivar	Cabot	2	0.618
Cultivar	Cabot	3	0.757
Cultivar	Cabot	4	0.524
Cultivar	Camarosa	1	0.464
Cultivar	Camarosa	2	0.417
Cultivar	Camarosa	3	0.452
Cultivar	Camarosa	4	0.429

Table A.5 (cont'd)

Cultivar	Cavendish	1	0.528
Cultivar	Cavendish	2	0.749
Cultivar	Cavendish	3	0.616
Cultivar	Earliglow	1	0.361
Cultivar	Earliglow	2	0.405
Cultivar	Earliglow	3	0.468
Cultivar	Earliglow	4	0.365
Cultivar	Honeoye	1	0.514
Cultivar	Honeoye	2	0.498
Cultivar	Honeoye	3	0.647
Cultivar	Honeoye	4	0.604
Cultivar	Jewel	1	0.462
Cultivar	Jewel	2	0.443
Cultivar	Jewel	3	0.777
Cultivar	Jewel	4	0.739
Cultivar	Redstart	1	1.009
Cultivar	Redstart	2	1.051
Cultivar	Redstart	3	0.892
Cultivar	Redstart	4	0.884
Line	MSU44	1	0.461
Line	MSU44	2	0.453
Line	MSU44	3	0.723
Line	MSU49	1	0.52
Line	MSU49	2	0.543
Line	MSU49	3	0.489
Line	MSU49	4	0.897
Line	MSU69	1	0.729
Line	MSU69	2	0.888
Line	MSU69	3	0.752
Line	MSU69	4	0.669
Line	MSU75	1	1.19
Line	MSU75	2	0.884
Line	MSU75	3	0.848
Line	MSU75	4	0.747
Line	MSU78	1	0.631

Table A.5 (cont'd)

Line	MSU78	2	0.551
Line	MSU78	3	0.708
Line	MSU78	4	0.642
Line	MSU80	1	0.508
Line	MSU80	2	0.483
Line	MSU80	3	0.518
Line	MSU80	4	0.883
Line	MSU81	1	0.195
Line	MSU81	2	0.249
Line	MSU81	3	0.208
Line	MSU81	4	1.013
Line	MSU86	1	0.728
Line	MSU86	2	0.549
Line	MSU86	3	0.583

Table A.6: Dataset of 2022 Titratable Acidity

GROUP	**ID**	**REP**	**TA**
Cultivar	Albion	1	0.286
Cultivar	Albion	2	0.253
Cultivar	Albion	3	0.245
Cultivar	Albion	4	0.255
Cultivar	Albion	5	NA
Cultivar	Allstar	1	0.354
Cultivar	Allstar	2	0.385
Cultivar	Allstar	3	0.36
Cultivar	Allstar	4	0.323
Cultivar	Allstar	5	0.253
Cultivar	Annapolis	1	0.379
Cultivar	Annapolis	2	0.366
Cultivar	Annapolis	3	0.311
Cultivar	Annapolis	4	0.318
Cultivar	Annapolis	5	0.355
Cultivar	Cabot	1	0.394
Cultivar	Cabot	2	0.301
Cultivar	Cabot	3	0.34

Table A.6 (cont'd)

Cultivar	Cabot	4	0.287
Cultivar	Cabot	5	0.351
Cultivar	Camarosa	1	0.241
Cultivar	Camarosa	2	0.256
Cultivar	Camarosa	3	0.301
Cultivar	Camarosa	4	0.246
Cultivar	Camarosa	5	0.245
Cultivar	Cavendish	1	0.21
Cultivar	Cavendish	2	0.25
Cultivar	Cavendish	3	0.258
Cultivar	Cavendish	4	0.305
Cultivar	Cavendish	5	0.179
Cultivar	Earliglow	1	0.276
Cultivar	Earliglow	2	0.243
Cultivar	Earliglow	3	0.337
Cultivar	Earliglow	4	0.3
Cultivar	Earliglow	5	0.295
Cultivar	Jewel	1	0.272
Cultivar	Jewel	2	0.217
Cultivar	Jewel	3	0.182
Cultivar	Jewel	4	0.32
Cultivar	Jewel	5	0.245
Cultivar	Wasatch	1	0.249
Cultivar	Wasatch	2	0.288
Cultivar	Wasatch	3	0.266
Cultivar	Wasatch	4	0.274
Cultivar	Wasatch	5	0.261
Line	MSU44	1	0.403
Line	MSU44	2	0.383
Line	MSU44	3	0.297
Line	MSU44	4	0.132
Line	MSU44	5	0.268
Line	MSU49	1	0.267
Line	MSU49	2	0.263
Line	MSU49	3	0.338

Table A.6 (cont'd)

Line	MSU49	4	0.323
Line	MSU49	5	0.265
Line	MSU69	1	0.367
Line	MSU69	2	0.334
Line	MSU69	3	0.35
Line	MSU69	4	0.299
Line	MSU69	5	NA
Line	MSU71	1	0.364
Line	MSU71	2	0.309
Line	MSU71	3	0.372
Line	MSU71	4	0.427
Line	MSU71	5	0.363
Line	MSU73	1	0.29
Line	MSU73	2	0.217
Line	MSU73	3	0.317
Line	MSU73	4	0.194
Line	MSU73	5	0.28
Line	MSU75	1	0.6
Line	MSU75	2	0.662
Line	MSU75	3	0.551
Line	MSU75	4	0.613
Line	MSU75	5	0.535
Line	MSU78	1	0.223
Line	MSU78	2	0.301
Line	MSU78	3	0.353
Line	MSU78	4	0.449
Line	MSU78	5	0.403
Line	MSU80	1	0.414
Line	MSU80	2	0.38
Line	MSU80	3	0.408
Line	MSU80	4	0.308
Line	MSU80	5	0.359
Line	14_30_4	1	0.237
Line	14_30_4	2	0.266
Line	14_30_4	3	0.213

Table A.6 (cont'd)

Line	14_30_4	4	0.277
Line	14_30_4	5	0.276