HETEROSIS AND HETEROBELTIOSIS AMONG SHORT-DAY AND DAY-NEUTRAL MICHIGAN STATE UNIVERSITY STRAWBERRY GERMPLASM THROUGH CHARACTERIZATION OF FRUIT QUALITY AND DISEASE RESISTANCE

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ABSTRACT

Strawberries have rapidly become an important horticultural crop and consumer favorite fruit since its accidental hybridization. Constant efforts are made to improve disease resistance and fruit quality traits with special emphasis on improving flavor. The limited availability of disease-resistant cultivars requires other methods for pest and disease control. Chemical sprays are commonly used to control a wide array of high-priority pathogens. However, an increase in pathogens developing resistance to fungicides, the presence of pesticide residues on harvested fruit, and the harmful environmental effects of routine chemical applications have made resistance breeding a main objective in strawberry breeding programs. Screening for resistance to the fungal pathogen *Mycosphaerella fragariae* that causes Common Leaf Spot (CLS) and characterizing fruit quality traits among short-day (SD) and day-neutral (DN) genotypes in the Michigan State University (MSU) strawberry germplasm were the two main objectives in this study. In addition to high yield and large fruit size, there has been a growing interest in breeding for good flavor in strawberries, which is characterized by a balance between total soluble solids (TSS) content and titratable acidity (TA) with a pleasant aroma. Evaluating progeny from breeding crosses with shared parents, Earliglow proved to be the best parent for improving flavor, while Cabot was the best for improving fruit size and tolerance to CLS. Among the germplasm, short-day individuals outperformed day-neutral individuals for CLS tolerance, TSS/TA, fruit size, and TSS. More crosses with shared parents and at least 20-25 progeny per cross would help determine the inheritance of CLS resistance and quantify the general combining ability for determining the best parents to cross to improve MSU's strawberry germplasm pool further.

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INTRODUCTION

Strawberries are an important and high-value horticultural crop and a good source of nutrition. In 2021, worldwide production of strawberries reached 9.2 million metric tons (Shahbandeh, 2023). In 2022, the United States produced over 1.3 million tons, with California as the top-producing state, yielding over 24.8 million cwt (Shahbandeh, 2023). In 2023, the United States ranked second in strawberry production after China, producing over one million tons (WPR, 2023). Through breeding efforts, the strawberry has quickly transformed from a small, soft, short-season fruit crop to the large, firm, long-season fruit of choice (Simpson, 2018). Consumption of strawberries in the United States averaged eight pounds per person and is projected to increase dramatically over the next several decades (Samtani et al., 2019).

Strawberries rapidly became a cornerstone nutritious snack worldwide. The health benefits and cancer-fighting potential are beginning to become apparent. Strawberries are a low-calorie snack with a generous amount of Vitamin C, higher than some citrus fruits (Giampieri et al., 2013). Studies have shown that consistent consumption of berries in a diet can help lower the risk of cancer, cardiovascular disease, and neurodegenerative diseases (Hannum, 2004; Giampieri et al., 2015). In addition, strawberries have elevated antioxidants and anti-inflammatory properties (Newerli-Guz et al., 2023; Giampieri et al., 2013). The high levels of antioxidants in strawberries can help reduce oxidative stress when berries are consumed fresh (Newerli-Guz et al., 2023). Furthermore, supplementing low doses of freeze-dried strawberry powder into the diets of overweight and obese adults has been shown to help improve cholesterol levels (Richter et al., 2023). The numerous emerging health benefits of strawberry consumption continue to drive demand and influence breeding efforts.

Strawberries are among the youngest crops, with the cultivated strawberry only been bred deliberately for the past 275 years after the initial hybridization event in a French botanical garden (Wilhelm, 1974; Nellist, 2018). One of the parents of the initial hybridization event, *Fragaria chiloensis*, is indigenous to 10,000 miles of the pacific coast spreading from Alaska to Chile and was brought to France by a scientist returning from a voyage to South America (Finn et al., 2013; Wilhelm, 1974). The other parent, *Fragaria virginiana*, originated in North America and was brought back to Europe in the 16th century (Wilhelm, 1974). In 1740, The Botanical Garden of Brest acquired *F. chiloensis* and found the flowers lacked pollen. To remedy the barrenness of the strawberry, they planted *F. virginiana*, next to *F. chiloensis,* which resulted in the initial hybridization event leading to the modern-day *F.* × *ananassa*, the octoploid cultivated strawberry (Wilhelm, 1974; Hancock et al., 1992).

The first formal strawberry breeding program was pioneered by Thomas A. Knight in 1817 (Wilhelm, 1974; Hancock et al., 2005; Hummer & Hancock, 2009). However, he had a very narrow germplasm base to work with and started his work mostly from accidental hybrids since *F. virginiana* and *F. chiloensis* have complementary beneficial traits. In the late 1900s, North American strawberry cultivars were evaluated and it was found that most of the nuclear genes came from only seven genotypes, while most of the cytoplasmic genes came from just ten genotypes (Sjulin & Dale, 1987; Dale & Sjulin 1990; Luby et al., 1992). The narrow germplasm base led to a large collaborative effort to improve the genetic variability available to modern-day strawberry breeders.

Breeders spent decades cataloging the genetic variability that was present among wild relatives that were collected from across the globe. It was soon apparent that the strawberry can vary widely in ploidy from diploid ($2n = 2x = 14$) to decaploid ($2n = 10x = 70$) (Edger et al., 2019). The modern-day cultivated strawberry is an allo-octoploid (2n = 8x = 56) (Edger et al., 2019). Wild relatives can be found in diverse environments across the globe. *Fragaria vesca* is the most widely distributed diploid wild relative that is found in North and South America, Europe, Asia, and Hawaii (Hancock & Luby 1993). *Fragaria chiloensis* wild strawberry is an octoploid and can be found along the California coast, Chile, and the Hawaiian Islands (Hancock & Luby, 1993). Another wild species *F. virginiana,* also an octoploid, is native to areas of Canada and the United States (Hancock & Luby, 1993). Today, strawberries are grown in a variety of environments, including temperate, grassland, Mediterranean, taiga, and subtropical climates (Hancock & Luby, 1993).

The large geographic range of wild relatives aids breeders because of the wealth of genetic potential they exhibit. Evaluations of 5,000-6,000 wild accessions have revealed sources of resistance to numerous abiotic and biotic stresses. For example, some wild relatives showed abiotic resilience through salt, cold, drought, shade and heat tolerance, and low nutrient needs (Hancock, 1990; Hancock et al., 1992; Hancock & Luby, 1993; Luby et al., 1992). Furthermore, wild relatives exhibit resistance to numerous biotic stresses like resistance to aphids, two-spotted spider mites, root rots, leaf spots, and powdery mildew, to name a few (Hancock, 1990; Hancock et al., 1992; Hancock & Luby, 1993).

Numerous efforts have been made to incorporate the wealth of beneficial genetics of wild germplasm into elite and cultivated selections. However, to maintain the wide variety of genetic potential for future breeding efforts, the native octoploids were evaluated for a variety of abiotic traits such as heat, cold, salt, and drought tolerance as well as other important fruit quality traits such as sugar content, fruit firmness, disease, and pest resistance (Hancock et al., 2001). From those evaluations, 38 genotypes were selected to form the “supercore” which encompasses a wide array of genetic diversity and horticulturally important traits from North and South America (Hancock et al., 2001; Luby et al., 1992). The 38 genotypes were propagated and trialed in six different locations, including California, Maryland, Michigan, Minnesota, Oregon, and Pennsylvania, with the help of several breeders (Hancock et al., 2001). The "supercore" is meant to be a toolbox for breeders to use for future climate change and pest challenges that are likely to arise.

Most of the successful incorporations of wild relative's beneficial traits into cultivars are through a backcrossing approach (Hancock et al., 1992). One of the most notable success stories with incorporating wild germplasm was from R.S. Bringhurst and V. Voth. (Hancock et al., 1992; Hancock & Luby 1993: Bringhurst & Voth 1980). They used a wild relative *F. virginiana* glauca, to incorporate the day-neutrality (repeat flowering) trait into three varieties, 'Aptos', 'Brighton', and 'Hecker' for release (Bringhurst & Voth, 1980). The introduction of a wild trait into a releasable variety revolutionized the strawberry industry by showing the power of using wild strawberry traits for direct incorporation to release new varieties.

Other efforts to improve the strawberry genetic diversity and genetic potential include the reconstruction of *F.* × *ananassa*. Several studies have carefully selected elite genotypes of *F. virginiana* and *F. chiloensis* to reconstruct superior *F.* × *ananassa*. populations (Hancock et al., 2002; Hancock et al., 2010; Luby et al., 2008; Stegmeir et al., 2010). Luby et al. (2008) showed that combining *F. virginiana* and *F. chiloensis* parents was relatively easy due to significant levels of general combining ability (GCA). GCA is the average performance of a parent when combined with other parents in a hybrid combination. Using *F. virginiana* and *F. chiloensis* to reconstruct *F.* × *ananassa*. resulted is significantly more variability than using a backcrossing method with *F.* × *ananassa*. populations due to the narrow genetic base (Hancock et al., 2002;

Luby et al., 2008). More recent work used breeding material from *F. virginiana ssp. glauca* to improve fruit nutritional quality (Diamanti et al., 2012). The results from the study showed that strawberry nutritional quality could be improved to marketable standards using inter-species back-crosses and intra-species crosses further supporting the potential for incorporating wild germplasm (Diamanti et al., 2012). The work done by various breeders shows the power and genetic potential available in wild germplasm for the advancement and release of superior strawberry cultivars.

Dr. Jim Hancock also used a similar backcrossing approach to develop the MSU advanced selections while introducing superior wild traits (Luby et al., 2008). He continued crossing wild germplasm with elite selections and popular commercial cultivars to develop the MSU advanced selections. These advanced selections were used as parents for this study because the reconstructed germplasm has exceptional traits especially, flavor (Stegmeir et al., 2010). However, the MSU advanced selections were slightly more acidic and fruit size was smaller which led Dr. Cholani Weebadde to use the reconstructed germplasm as parents for another round of crosses. The crosses were made with cultivars that had complementary desirable traits such as large fruit size, and sweetness, and superior disease resistance to continue enhancing the MSU strawberry germplasm (C. Weebadde, personal communication, December 18, 2023). In addition, Dr. Weebadde made crosses between popular cultivars. As such, the progeny from the cultivar × cultivar, advanced selection × cultivar, and advanced selection × advanced selection were used in this study (Table 1). The goal was to evaluate the progeny for the desired large, sweet, nutritious fruits.

Table 1: The strawberry crosses used to create the breeding populations used for the study, the number of progeny in each family and the reason the cross was performed.

Cross	**# of Progeny**	**Justification**
MSU44 x Allstar	18	Improve flavor
MSU44 x Camarosa	23	Increase yield and improve flavor
MSU44 x Earliglow	16	Improve flavor and earlier flowering
MSU44 x Honeoye	18	Improve flavor
MSU44 x Jewel	45	Improve flavor and apperance
MSU49 x Allstar	1	Improve flavor
MSU49 x Cabot	19	Increase fruit size
MSU49 x Earliglow	14	Improve flavor and earlier flowering
MSU67 x Camarosa	15	Increase yield and improve flavor
71 x Camarosa	11	Increase yield and improve flavor
72 x Camarosa	13	Increase yield and improve flavor
73 x Camarosa	14	Increase yield and improve flavor
76 x Camarosa	37	Increase yield and improve flavor
76 x Earliglow	33	Improve flavor and earlier flowering
77 x Camarosa	10	Increase yield and improve flavor
77 x Earliglow	23	Improve flavor and earlier flowering
79 x 69	5	Improve flavor
79 x Camarosa	14	Increase yield and improve flavor
79 x Earliglow	18	Improve flavor and earlier flowering
79 x Jewel	15	Improve flavor and apperance
81 x 69	10	Improve flavor and day-neutral
81 x Cabot	16	Increase fruit size
81 x Camarosa	15	Increase yield and improve flavor
87 x Allstar	9	Improve flavor
87 x Cabot	30	Increase fruit size
Allstar x 67	12	Improve flavor
Allstar x 68	1	Improve flavor
Allstar x 79	8	Improve flavor
Allstar x 86	4	Improve flavor
Allstar x Cabot	7	Increase fruit size
Annapolis x 73	1	Improve flavor
Annapolis x Camarosa	12	Increase yield and improve flavor
Cabot x 44	3	Increase fruit size
Cabot x 67	28	Increase fruit size
Cabot x 68	60	Increase fruit size
Cabot x 72	41	Increase fruit size
Cabot x 75	5	Increase fruit size
Cabot x 79	26	Increase fruit size
Cabot x 80	46	Increase fruit size
Cabot x Earliglow	16	Improve flavor, earlier flowering, increase fruit size
Cabot x Honeoye	18	Increase fruit size and improve flavor

Table 1 (cont'd)

Earliglow x Camarosa	11	Improve flavor and earlier flowering
Allstar x 73	4	Improve flavor
Cabot x 73	1	Increase fruit size
Allstar x honeoye	12	Improve flavor
84 x Honeoye	1	Improve flavor
Earliglow x 73	2	Improve flavor and earlier flowering
81 x 75	1	Improve flavor and day-neutral
16-1-1 x Camarosa	1	Increase yield and improve flavor
83 x Allstar	1	Improve flavor
Allstar x 69	2	Improve flavor

The commercial strawberry industry depends on extensive inputs of chemicals ranging from fertilizers to pesticides to produce large, nutritious fruits. Heavy reliance on chemical applications such as pesticides and fungicides has consistently placed strawberries at the top of the US Dirty Dozen list (EWG, 2023). The Dirty Dozen is a list released each year by the Environmental Working Group dictating crops that use the most chemicals in commercial production and have significant amounts of chemical residues on fruits at the market. Strawberries remain on the Dirty Dozen due to the number of pesticide residues found (Shao et al., 2021; Chu et al., 2020). Of the strawberry samples from Shanghai, China 49.25% had pesticide levels above the European Maximum residue limit (Shao et al., 2021). Furthermore, another study found that 75% of strawberry samples had two or more detectable pesticides (Chu et al., 2020). However, even though many pesticides are found on a number of strawberry samples, the amount detected poses a low risk to adults and children (Chu et al., 2020; Li et al., 2022). Despite the low risk the numerous pesticide residuses may pose, consumers often fear any amount and have a higher "willingness to pay" if farmers keep pesticide use to a minimum (Milford et al., 2021).

Regardless, strawberries require several pesticide and fungcide applications during production to keep pathogens and pests at bay to achieve large marketable fruits. More fungicide applications are necessary after harvest, in addition to extensive post-harvest management practices to ensure that good quality fruit reaches stores. Furthermore, as more information about climate change emerges, many pesticides, fungicides, and other chemical applications that production systems primarily relied on have been banned or weened out of use due to harmful environmental side effects. For example, methyl bromide, a soil fumigant that was a staple in production, came out as ozone-depleting and was reduced incrementally until it was phased out in 2005 except for critical use exemptions that the California Strawberry industry qualified for (US EPA, 2015; Nellist, 2018). However, all critical use exemptions ended at the end of 2016 (Farnsworth, 2017). The discontinued use of methyl bromide and the lack of a comparable replacement left many strawberry producers vulnerable to pathogen epidemics, which poses a challenge for strawberry growers. A study of Michigan cultivar performance on fumigated compared to non-fumigated soils found all genotypes performed better on fumigated soils (Hancock et al., 2001). Producers are now looking into other control methods for many common pathogens, including using resistant cultivars to overcome the decline in strawberry performance on non-fumigated soils.

This study focuses on one fungal pathogen endemic to strawberry fields: *Mycosphaerella fragariae*, which causes Common Leaf Spot (CLS). One challenge is that no commercial cultivars currently used exhibit resistance to CLS. However, whether any CLS resistance lines exist in the MSU germplasm remains unknown. To further complicate efforts, producers will only use cultivars that meet or exceed current varietal standards for fruit quality. Therefore, the study's main objectives are to a) identify sources of resistance to CLS by developing a simple screening method and b) select parents for crosses to improve fruit size and flavor to enhance the MSU germplasm.

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CHAPTER 1: A NEW PROTOCOL FOR ACCURATE QUANTIFICATION OF COMMON LEAF SPOT FOLIAR DAMAGE ON STRAWBERRY

INTRODUCTION

Common Leaf Spot (CLS) is endemic in North American strawberry fields and can affect both fruit and foliage. Historically, CLS has been a minor concern despite being present in most commercial strawberry production (Carisse & McNealis, 2019). The ascomycete causal agent is *Mycosphaerella fragariae* (Tul.) Lindau (= *Ramularia tulasnei* Sacc.), a late-season pathogen primarily affecting the foliage (Fig. 1) (Delhomez et al., 1995; Carisse & Peyrachon, 1999; Carissa & McNealis, 2019). CLS impacts the yield of both June-bearing repeat flowering (ever-bearing and day-neutral) strawberries. Though disease may become more prevalent in the late-season, symptoms are still observed during the June months when short-day varieties are most productive. In Michigan, longer-season strawberry acceptance remains low due to late-season yield reduction and smaller fruit size. However, breeding efforts to improve day-neutral strawberries may increase the threat posed by CLS. Though this pathogen has been of little priority to some, CLS can reduce photosynthesis and the overall yield of the plant (Carissa & McNealis, 2019). One study found a significant reduction in photosynthesis when a leaf is 16% covered by lesions, resulting in a 13% reduction in fruit size (Carisse & McNealis, 2019).

However, more changes occur in the plant prior to the development of necrotic lesions that, in turn, decrease plant productivity. During *M. fragariae* infection, changes in reactive oxygen species (ROS), superoxide dismutase activity, and the ascorbate–glutathione cycle may support plant defense and reduce damage to strawberry leaves (Ding et al., 2011; Ehsani-Moghaddam et al., 2006; Wang et al., 2015; Wang et al., 2019). ROS are known to play a role in plant-pathogen interactions, and some key ROS in *M. fragariae* infection include superoxide (O_2^-) and hydrogen peroxide (H_2O_2). After pathogen recognition by the plant, superoxide, and hydrogen peroxide are overproduced in an oxidative burst which is one of the most rapid defense responses (Apostol et al., 1989; Wang et al., 2019). Ding et al. (2011) found that ROS involved in the initial oxidative burst, such as H_2O_2, are produced at higher levels in resistant genotypes compared to susceptible genotypes though more studies should be conducted to confirm the observed differences. Regardless, the oxidative burst is toxic to invading pathogens and may lead to cell death (Chamnongpol et al., 1998). However, the excessive ROS is also toxic to the plant itself.

Plants have complex antioxidant systems to defend themselves against collateral damage from the initial oxidative burst (Apostol et al., 1989; Hammond-Kosack & Jones, 1996; Lou et al., 2011). For example, superoxide dismutase (SOD) is an enzyme that plays a role in defending plants against excessive ROS production during periods of biological stress by catalyzing the dismutation of superoxide (O_2^-) to H_2O_2 and O_2 (Wang et al., 2015). Two days after initial infection, superoxide dismutase production peaks in infected strawberry leaves and is produced at significantly higher levels in resistant and moderately resistant cultivars than in susceptible cultivars (Ehsani-Moghaddam et al., 2006; Wang et al., 2015). Another system that defends plants against excessive ROS is the ascorbate-glutathione cycle, in which H_2O_2 is catalyzed into H_2O and O_2 (Wang et al., 2015). The activity of the ascorbate peroxidase (APX) enzyme, a part of the ascorbate–glutathione pathway, plays a critical role in eliminating toxic H_2O_2 (Ding et al., 2011). In resistant cultivars, there was a significant increase in the APX enzyme, which may contribute to less strawberry leaf damage, which would benefit the pathogen (Ding et al., 2011). More research is needed to provide additional evidence of what mechanisms support strawberry resistance to CLS infection.

As the disease continues to develop, the necrotic "bullseyes," indicative of CLS, spread across the leaves (Fig. 1). The "bullseyes" lesions can range from red to purple with white centers and usually appear 12 to 18 days after inoculation or initial infection (Fig.1) (Carisse & Peyrachon 1999; Carisse et al., 2000; Xu et al., 2019). The necrotic lesion decreases photosynthesis because of the increase in dead cells as disease progresses.

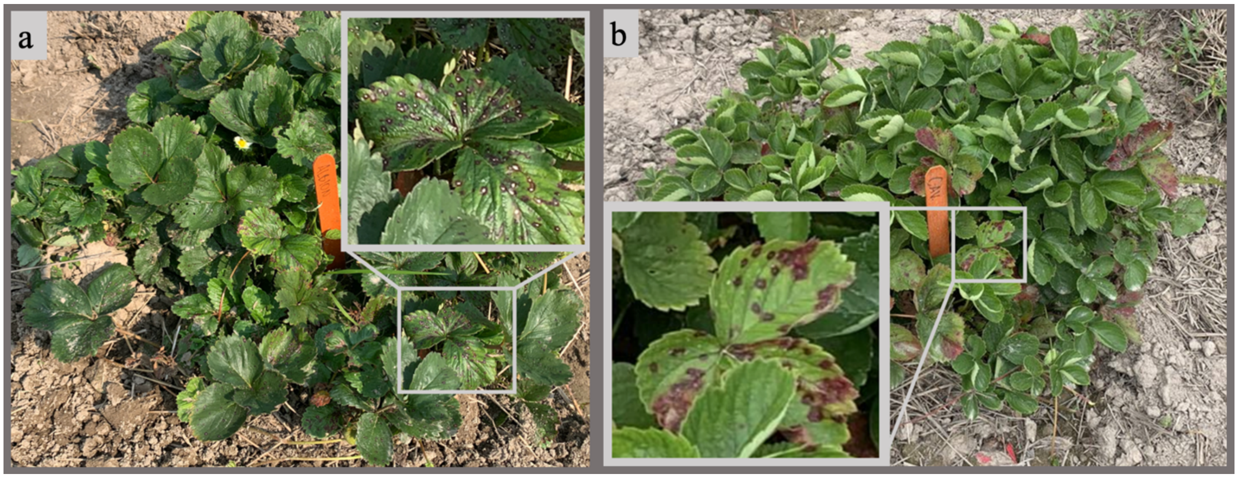


Figure 1: Common Leaf Spot damage on (a) a popular Michigan day-neutral strawberry cultivar 'Albion' and (b) a popular Michigan short-day strawberry cultivar 'Cavendish. Photos taken by Drew Paluda in 2021.

The pathogen's primary inoculum comes from conidia overwintering on leaves, conidia in sclerotia, and ascospores that overwinter on diseased leaves. The conidia are dispersed by rain, which is why heavy rainfalls can result in severe epidemics of CLS (Carisse & McNealis, 2018). Earlier research showed that the ideal temperature for *M. fragariae* conidia germination is between 13°C and 21°C after heavy rains (Paulus, 1990). More recent studies cite 25°C as the optimal temperature for conidia germination with a 12-hour wet period to optimize infestation (Schmid et al., 2005; Carisse et al., 2000). However, under controlled conditions, conidia can germinate between 5°C and 30°C (Carisse & McNealis, 2018). In addition, leaf wetness is a stronger indicator of CLS severity and occurrence. Studies have shown that rainfall <103mm within one month does not result in severe infections, whereas leaf wetness over 24 hours can lead to severe epidemics of CLS (Stegmeir et al., 2010).

To control CLS epidemics and other pathogens, commercial producers rely heavily on pre-plant fumigation, fungicides, pesticides, and plastic mulches to produce marketable strawberries (Samtani et al., 2019). Because strawberries are susceptible to infection by *M. fragariae*, most control efforts of CLS rely heavily on fungicide sprays (Delhomez et al., 1995; Schmid et al., 2005). Plants are frequently over-sprayed for controlling CLS because it is typically sprayed on a calendar basis due to the lack of knowledge of when an application might be most effective. Over-spraying of fungicides often leads to the development of resistance among *M. fragariae* isolates, which could negatively affect disease control in the future (Carisse et al., 2000). Though spraying provides some control, using resistant cultivars is still the most economical approach for growers to control CLS (Carisse et al., 2000). As such, developing resistant varieties for CLS would be an economical and more durable mode of disease control for strawberry growers.

In addition, Michigan strawberry production primarily relies on U-pick farms where consumers pick their own fruit (Michigan Ag Council, 2009). As such, U-pick production systems heavily rely on disease and symptom-free plants to attract repeat consumers. Pathogens that have negative impacts on plant foliage, such as CLS, are more likely to deter consumers from harvesting from such plants (Fig. 1). Thus, CLS-resistant plants are important for Michigan farmers because symptoms are apparent during normal U-pick seasons and then reach a peak at the end of the season causing continued impacts the following year. There are also some sources of resistance already present in released germplasm, which include Dukat, Marmolada, Tenira, Selva, and Elkat (Labanowska et al., 2003). However, they are not among the more popular chosen cultivars for Michigan’s colder climate and lack other necessary agronomic traits like high yield, large fruit size, and good flavor. Some popular Michigan varieties include short-day varieties such as Allstar, Honeoye, Earliglow, Jewel, and Old North Star, in addition to day-neutral varieties including Tribute, Ozark beauty, Tristar, Eversweet, Albion, and White Pineberry (Gardener, 2020; Buck, 2022). However, neither the short day or the day neutral popular and recommended varieties grown in Michigan have resistance to CLS, and little to no breeding efforts have been made to improve CLS resistance. Therefore, there is a need to breed for more acceptable resistant cultivars with attractive foliage that is desirable for Michigan’s U-pick market.

As most breeders would agree, accurate phenotypic selection is the first step to identifying superior genotypes for traits of interest: increased accuracy and reduced time to screen both aid in increasing the response to selection. This is especially true when screening for disease resistance, where an ordinal rating scale with numerical classes of 0-5 or 0-9 has often been used to determine disease severity (Chiang & Bock, 2022). The ratings obtained using an ordinal rating scale, however, are subjective, leading to biases by the person rating the disease. Additionally, traditional scales can be time-consuming, and it is difficult to maintain consistency with a large number of samples (Chiang & Bock, 2022). Research into severity scales or standard area diagrams has been an area of interest for decades (Bock et al., 2022). Chiang & Bock, 2022 concluded a quantitively ordinal scale with sensitivity to low disease severity was more accurate than a traditional rating scale ranging by 10%. As such, the first two objectives of this study are to a) develop a Common Leaf Spot visual assessment protocol similar to standard area diagrams published for early blight of potatoes, rust on soybean leaves, and bacterial spot of yellow passion fruit (Duarte et al., 2013; Bock et al., 2022; Monzani et al., 2018) and b) use this tool to effectively and efficiently screen MSU's strawberry germplasm and breeding populations for the occurrence of CLS, as a means of improving the accuracy and consistency of phenotypic evaluation of disease severity for CLS.

CLS is a simple pathogen to assess compared to other, more economically important fungal pathogens, such as *Botrytis cinerea.* Complex pathogens, like *B. cinerea,* are challenging to screen for because fruit rot assays take more time, materials, and space than a pathogen like *M. fragariae*, which can be screened in the field through leaf assays. Additionally, finding sources of resistance to *B. cinerea* poses further difficulty because it has many different resistance mechanisms involved at various stages of development (Elad & Evensen, 1995). Furthermore, *B. cinerea* is a generalist meaning it has no host specificity and can infect more than 1000 plant species (Fournier et al., 2005; Petrasch et al., 2019). If a correlation between a simple disease like CLS and a complex disease like *B. cinerea*, it could aid breeding efforts. One study found a significant negative correlation between Powdery Mildew and Leaf Scorch of strawberry, which are both fungal pathogens (Hancock et al., 2002). If similar correlations could be found for CLS and *B. cinerea*, screening and selecting for superior resistant genotypes would be significantly easier.

In addition to being a more complex pathogen, a study found significant correlations between disease incidence and some fruit quality traits. Petrasch et al. (2022) found *Botrytis cinerea* mycelium spreads quicker on fruits with a high soluble solid content, and *Botrytis cinerea* lesion diameter increased as titratable acidity decreased. On the other hand, another study found no significant correlations between CLS susceptibility and fruit quality traits (Ukalska et al., 2006). However, little research has been done on the topic. If similar correlations could be found for CLS disease, it may provide breeders with some additional breeding targets to improve resistance to complex fungal pathogens. As such, in addition to the first two objectives mentioned above, as a third objective, this study will determine if there are significant correlations between the CLS lesion area and disease severity of another economically important pathogen, *Botrytis cinerea,* or other important agronomic traits such as soluble solid content (SSC), titratable acidity (TA) and/or the ratio between SSC and TA to determine whether an indirect method to measure resistance is a reliable option.

METHODS

Germplasm

Breeding populations were grown in one field location at the Plant Pathology Research farm at Michigan State University (Latitude: 42.685819°; Longitude: -84.471536°) for four years (2019-2022) (Table 1). Common Leaf Spot incidence and severity were assessed in years three and four, at the end of summer in 2021 and 2022. The field was maintained with minimal inputs since it was planted in June 2019 to maximize the expression of true genotypic tolerance to CLS. Other than testing the field and adjusting the pH prior to planting no fungicides or chemical fertilizers were used since the field was planted. Weed control was done manually and in conjunction with the use of an herbicide once a year. When necessary, hand-weeding, cross-cultivation, and overhead irrigation were used to maintain the field. The herbicide Post was used once in 2019 to control weeds in addition to hand-weeding and cross-cultivation.

Based on the data obtained for fruit quality in the summers of 2020 and 2021, twenty-three families segregating for valuable agronomic traits such as good flavor, larger fruit size, and higher soluble solids content, were selected to be evaluated for CLS lesion coverage so that these populations could be used to study potential correlations of CLS with agronomic traits. The study relied on natural inoculum present in the field since disease was observated after planting. Isolations were not preformed on diseased leaves.

Evaluation Protocol

A visual key to evaluate resistance to CLS in strawberries was developed based off of a standard area diagram used by Monzani et al. (2018) to assess bacterial spot on yellow passion fruit leaves and a diagram for CLS was created by Delhomez et al. (1995). The standard area diagram developed by Monzani et al. (2018) had leaves with a similar shape to strawberry trifoliate leaves, but the area of lesions displayed in the figure were vastly different to how CLS is presented on strawberry foliage. Additionally, the only published diagram or schematic for rating CLS was from 1995 and only provided six rankings and does not accurately represent 5% lesion area.

The new key developed to accurately assess CLS was created showing the percent lesion coverage for ranges of percentages in increments of five (Fig. 2). Using Microsoft Excel, a general strawberry leaf shape was created using 500 square cells. For each 5% increase in lesion area, 25 square cells were colored in grey to represent the area of a lesion on a leaf. This scale continued at 5% increments up to 100% for a total of 20 leaf diagrams. Additionally, each percent range corresponds to a numeric rating for easier data collection. For example, a numeric rating of 1 corresponds to a percent lesion coverage of 1-5% (Fig. 2a). A numeric rating of 5 corresponds to a percent lesion coverage of 21-25% (Fig. 2e). The key was used to assess each leaf. The same individual completed the evaluations to prevent viewer bias throughout the sampling.

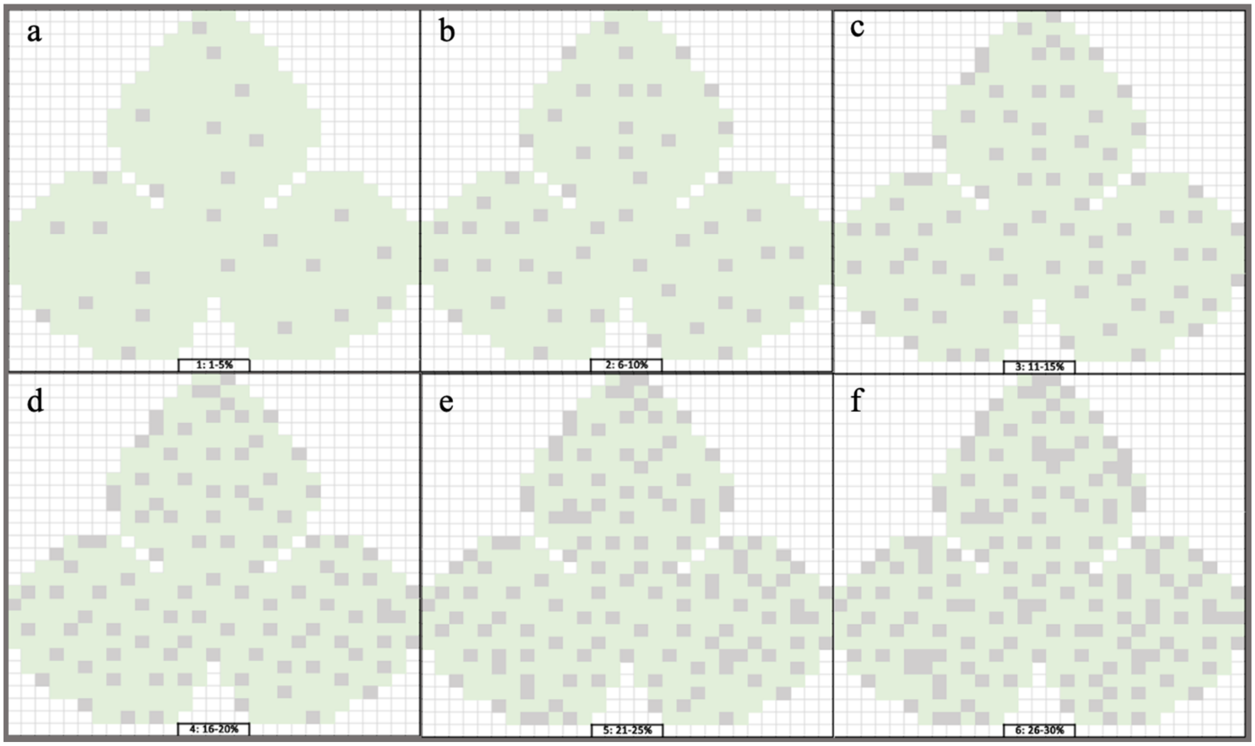


Figure 2: First six diagrams from the new CLS screening method developed by Ashley Wright using Microsoft Excel. The individual diagrams show lesion area with (a) a numeric rating of 1 and a percent range from 1-5%, (b) a numeric rating of 2 and a percent range from 6-10%, (c) a numeric rating of 3 and a percent range from 11-15%, (d) a numeric rating of 4 and a percent range from 16-20%, (e) a numeric rating of 5 and a percent range from 21-25%, and (f) a numeric rating of 6 and a percent range from 26-30%.

Fifteen leaves were randomly collected from each genotype within the families that were maintained in the field. The leaves were placed in a gallon-sized Ziploc bag and transported on ice to the lab for analysis and documentation. The leaf samples were placed and pressed flat on benches for evaluation. The above-mentioned visual key was used for visual identification of the percent lesion coverage of the infected leaves to obtain a numeric rating based on a range of percentages. Each leaf was assessed using the key for percent lesion coverage (PC) and number of diseased leaves (NDL) out of the fifteen assessed, and based on the percent coverage, a numeric rating (NR) was given for the specific range in which the percent assigned placed. After assessing the leaves, images captured each set of 15 leaves on a white background with the family and genotype label and a ruler (Fig. 3).

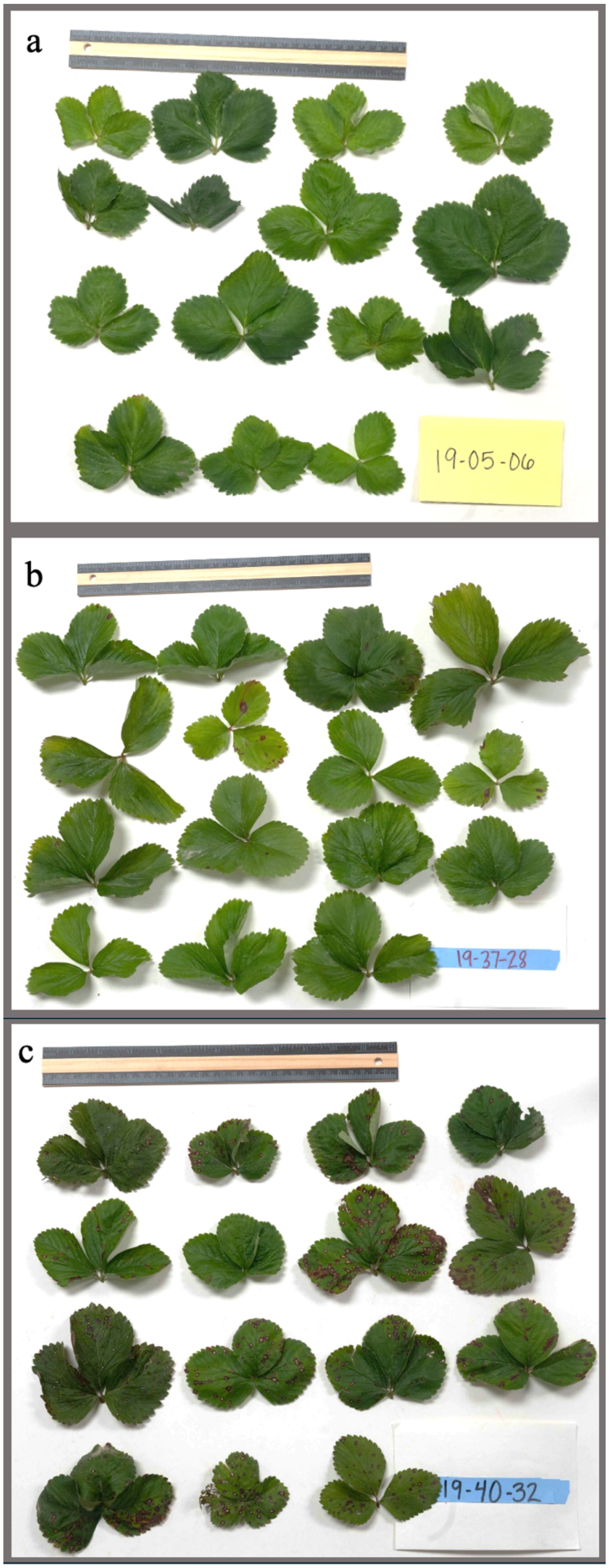


Figure 3: A set of 15 leaves with (a) resistance to CLS – no visible lesions, (b) moderate susceptibility to CLS – a few visible lesions, and (c) high susceptibility to CLS – many small and large visible lesions.

Statistical Analysis

Normality tests were not performed due to the large sample size. However, the trait was viewed graphically to ensure a visual normal distribution. Analysis of variance (ANOVA) was performed in R on the percent lesion area with family and genotype averaged over a year. Twenty-three families were selected to compare means because they had more than 20 progeny per family. Families with fewer than 20 progenies were excluded due to large standard error. Then, the Tukey HSD test was performed to determine which families drive the significant difference and compare population means. Regression analysis of lesion area with TSS, TA, TSS/TA, Weight, and *B. cinerea* was performed based on family and genotype to determine if there was a correlation between the two fungal pathogens and a correlation between *B. cinerea* and fruit quality traits. A correlation between easier-to-screen traits could help target other breeding objectives to improve *B. cinerea* resistance without complex, time-consuming, fruit rot assays.

RESULTS

Many leaves sampled had no or few lesions present resulting from the low disease pressure. Lesion areas significantly varied based on family and genotype. For all regressions, R^2 is low (<0.09), and the trends are highly significant ($p<0.01$). The regression analysis of titratable acidity shows a highly significant ($p<0.01$) slightly positive trend when averaged over 2021 and 2022 for genotypes and families (Fig. 4). The R^2 values of <0.01 and 0.02 for genotype and family, respectively (Fig. 4).

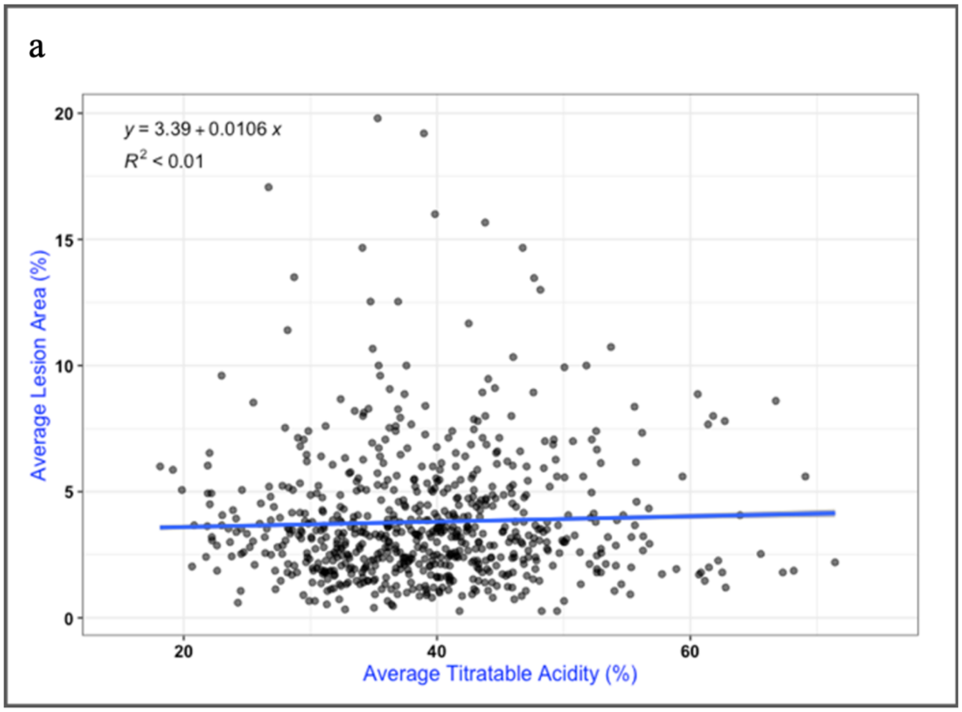
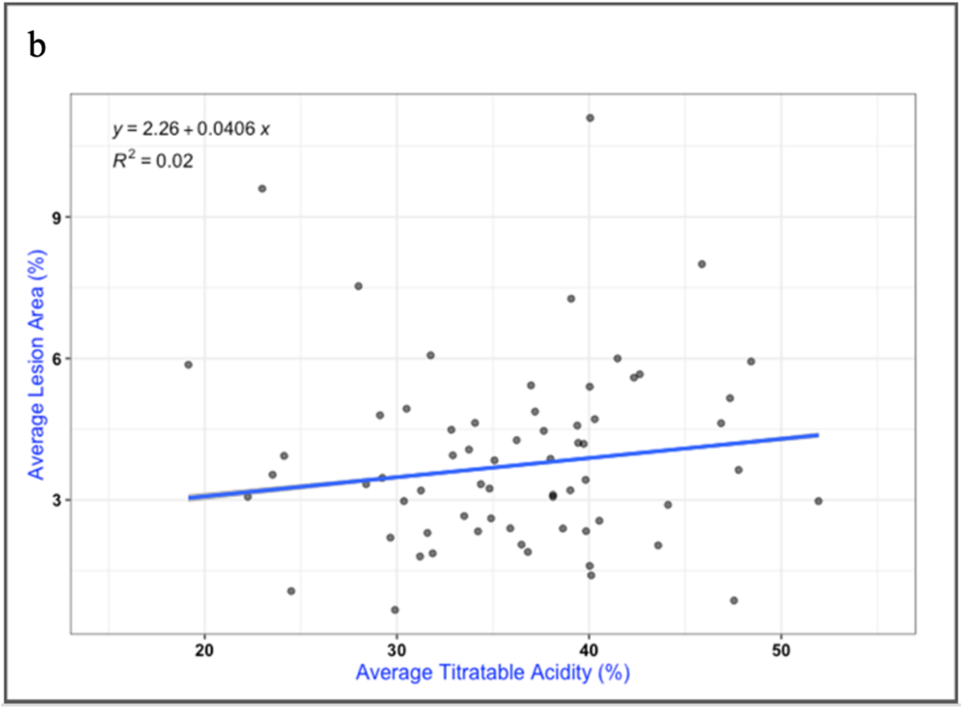


Figure 4: Lesion area (%) model averaged over 2021 and 2022 related to (a) average titratable acidity (%) per genotype (b) average titratable acidity (%) per family.

Figure 4 (cont'd)



The regression analysis of total soluble solids shows a highly significant ($p<0.01$) slightly negative trend when averaged over 2021 and 2022 for genotypes and families (Fig. 5). However, the R^2 values of <0.01 for genotype and family (Fig. 5).

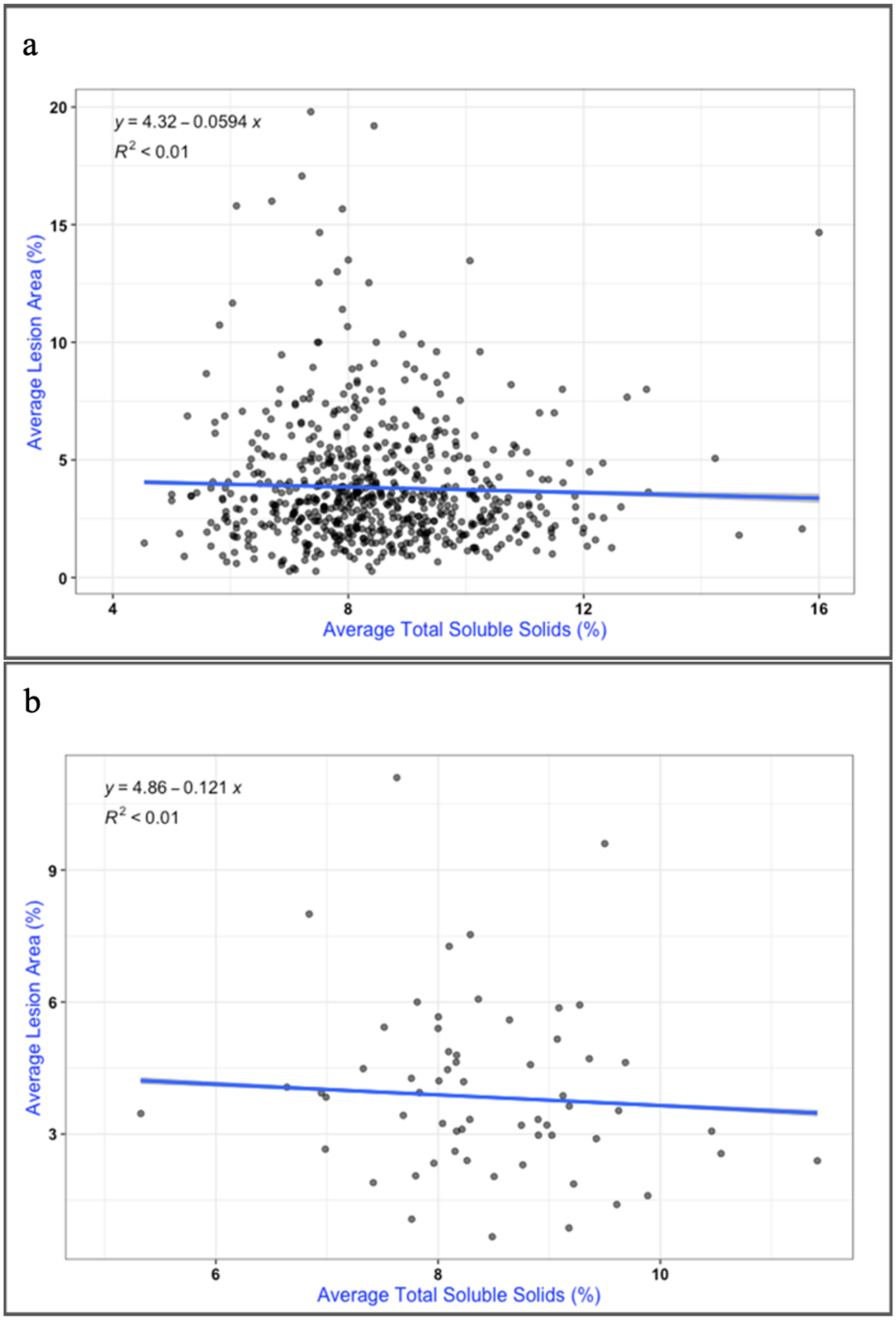


Figure 5: Lesion area (%) model averaged over 2021 and 2022 related to (a) average total soluble solids (%) per genotype and (b) average total soluble solids (%) per family.

The regression analysis of the total soluble solids/titratable acidity ratio shows a highly significant ($p<0.01$) slightly negative trend when averaged over 2021 and 2022 for genotypes and families (Fig. 6). However, the R^2 values of <0.01 and 0.03 for genotype and family respectively (Fig. 6).

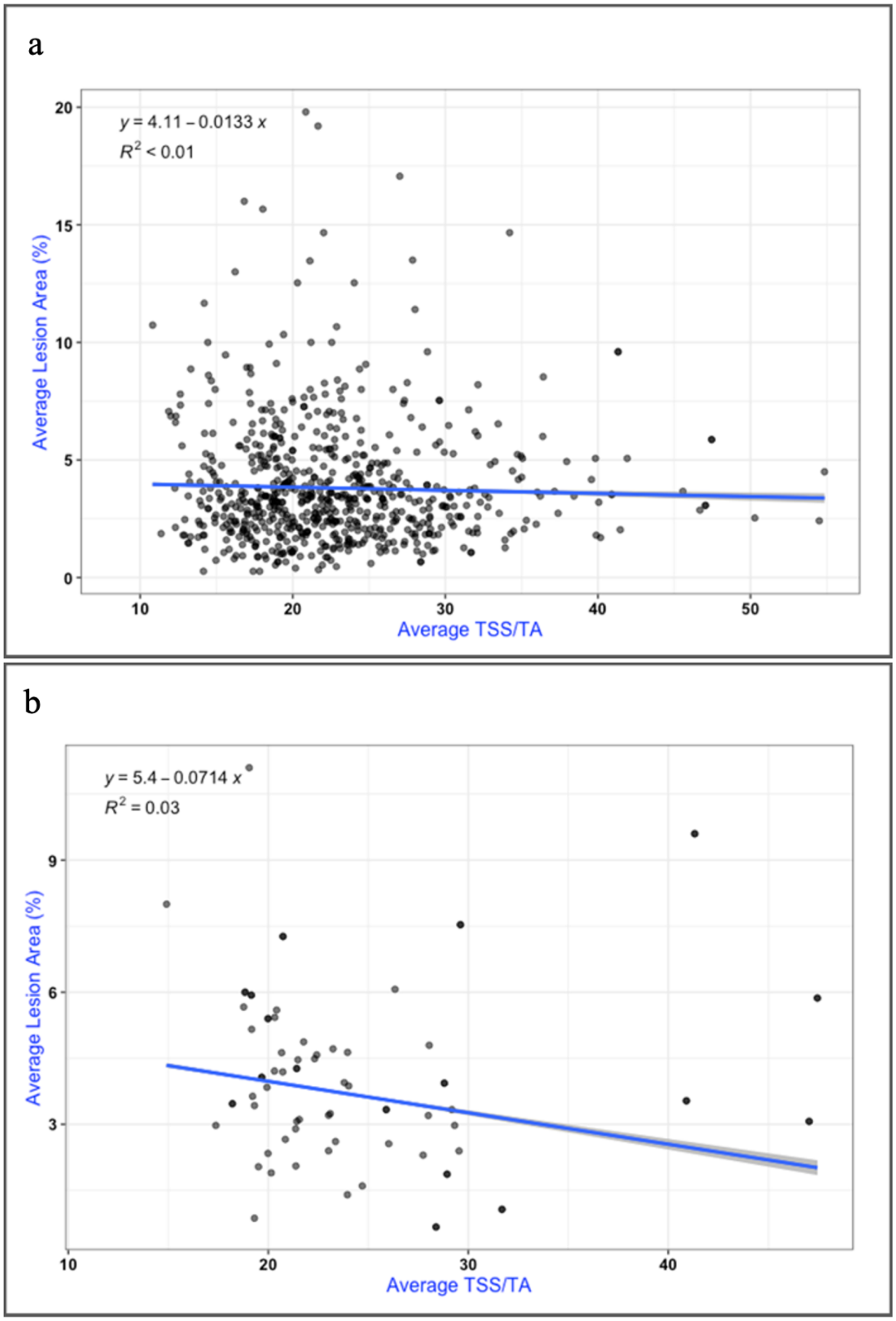


Figure 6: Lesion area (%) model averaged over 2021 and 2022 related to (a) average total soluble solids/titratable acidity ratio per genotype and (b) average total soluble solids/titratable acidity ratio per family.

The regression analysis of fruit weight shows a highly significant ($p<0.01$) slightly negative trend when averaged over 2021 and 2022 for genotypes and families (Fig. 7). However, the R^2 values of <0.01 and 0.08 for genotype and family respectively (Fig. 7).

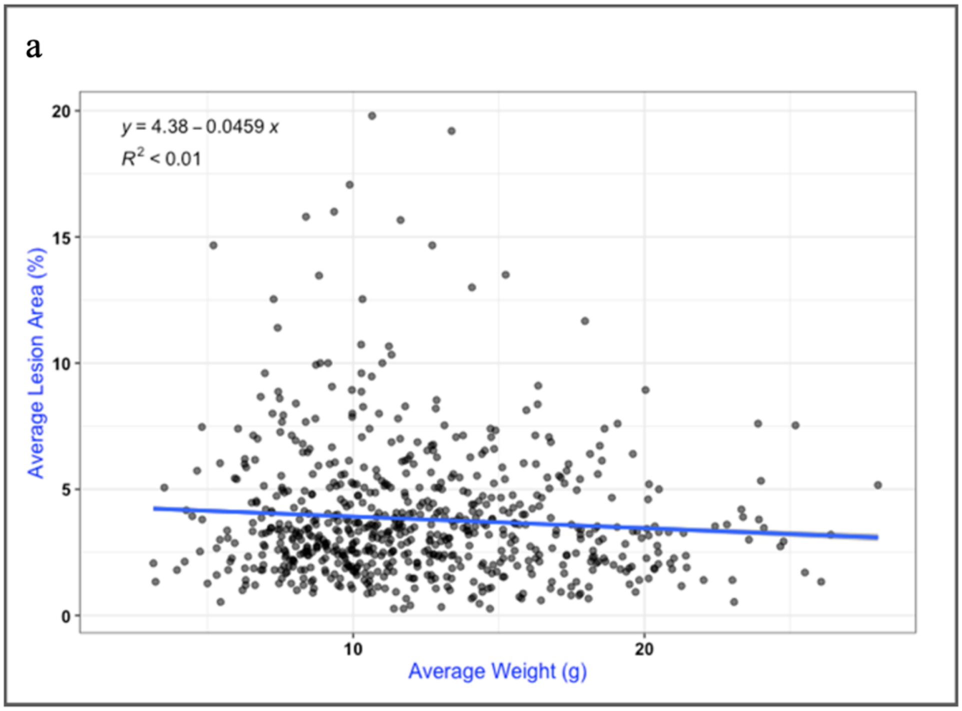
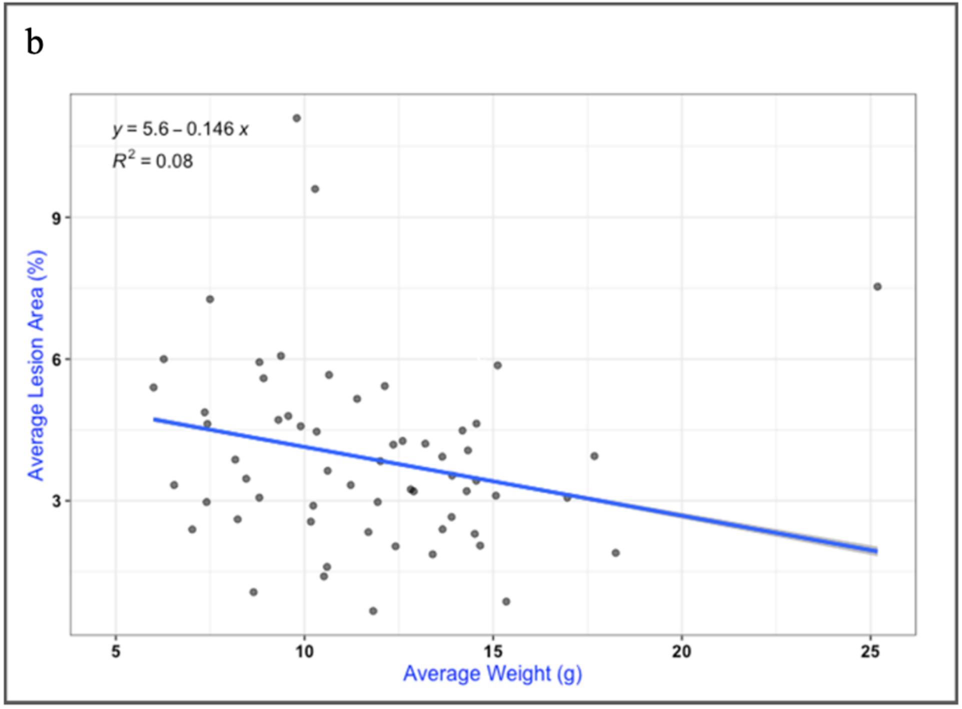


Figure 7: Lesion area (%) model averaged over 2021 and 2022 related to (a) average fruit weight (g) per genotype and (b) average fruit weight (g) per family.

Figure 7 (cont'd)



The regression of *Botrytis cinerea* disease pressure related to the lesion area of CLS showed a significant, slightly positive trend ($p<0.01$) but an extremely low R^2 (Fig. 8).

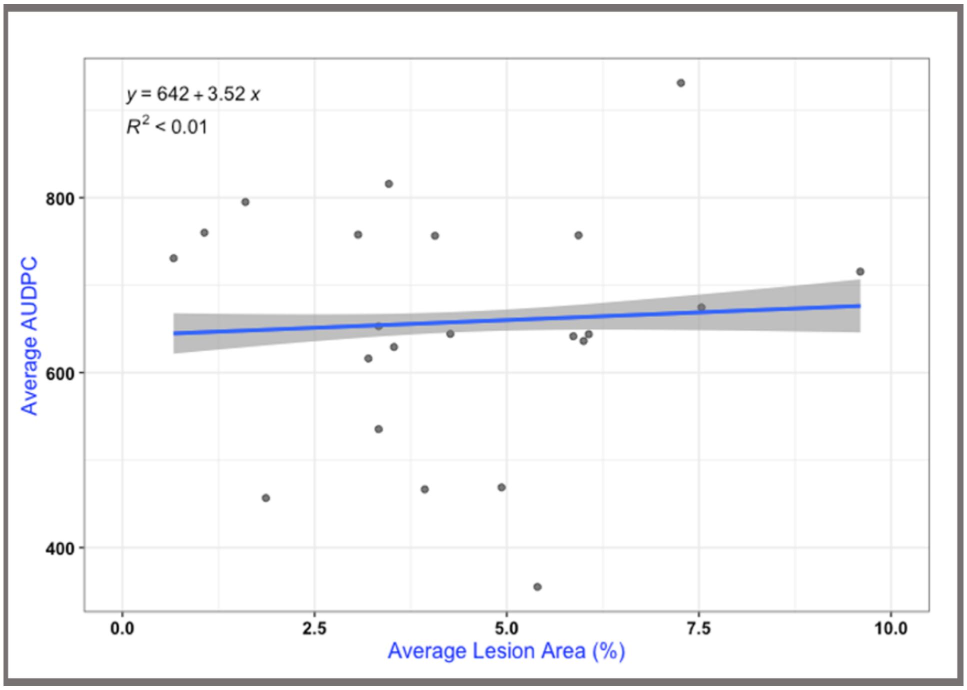


Figure 8: Area under the disease pressure curve (AUDPC) caused by *Botrytis cinerea* related to lesion Area (%) caused by *Mycosphaerella fragariae* averaged over 2021 and 2022.

The lesion area values consist of average lesions over two years of data. The means were then compared using the Tukey HSD test. The results show that there is significant variation between families ranging from an average of 5.66 to 2.04% (Fig. 9). The most susceptible families were crosses between MSU 81 × Camarosa, MSU76 × Camarosa, and Redstart × Camarosa with an average lesion area of 5.66, 5.59, and 5.43% respectively (Fig. 9). The most tolerant families were crosses between MSU 87 × Cabot, Cabot × MSU79, and Annapolis × Camarosa with an average lesion area of 2.04, 2.05, and 2.34% respectively (Fig. 9).

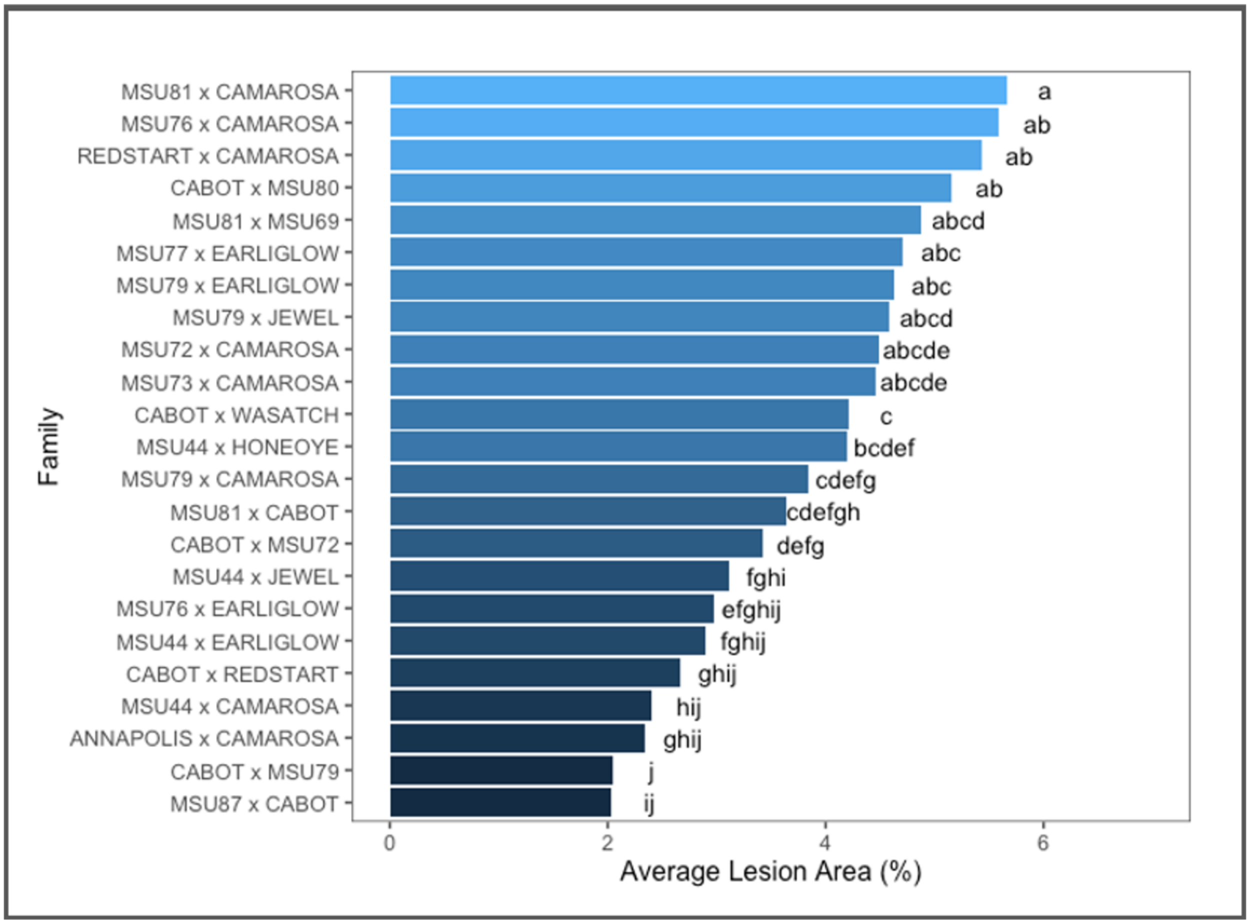


Figure 9: Mean comparisons of average lesion area (%) caused by *Mycosphaerella fragariae* of different families using Tukey HSD test. Different letters represent significant differences between means (α<0.05).

Interestingly, comparison of day-neutral (DN) and short-day (SD) plants showed there is a significant difference (Fig. 10). The lesion area for SD plants was significantly lower than the lesion area for DN plants (Fig. 10). DN plants have an average lesion area of 4.61 ± 0.0832. In contrast, SD plants averaged 3.41 ± 0.0447 (Fig. 10).

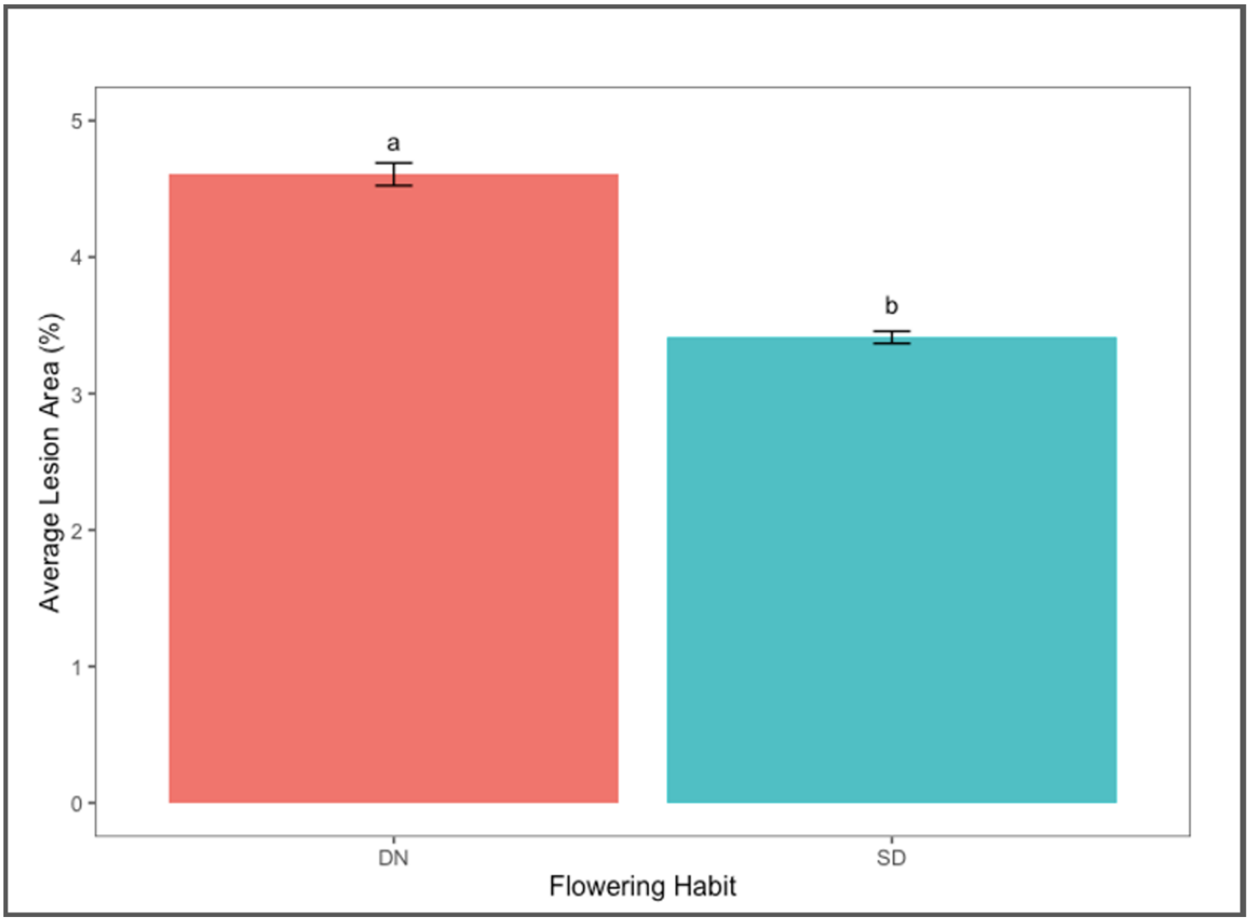


Figure 10: Comparisons of average lesion area (%) caused by *Mycosphaerella fragariae* between day neutral (DN) and short-day (SD) strawberry plants using Tukey HSD test. Different letters represent significant differences between means (α<0.05).

DISCUSSION

The regression of all fruit quality traits and CLS lesion area showed there were significant correlations with low predictive power due to the low regression coefficients. Titratable acidity, total soluble solids, total soluble solids/titratable acidity ratio, and fruit weight still provide valuable information about lesion area but, the data is highly variable and influenced by other outside factors (Fig. 4, Fig. 5, Fig. 6, Fig. 7). The lack of significant correlations is not surprising due to the low disease pressure. In addition, our results line up with the results from Ukalska et al. (2006) where no significant correlations were found. Furthermore, even though one study found some correlation between TSS and TA for *Botrytis cinerea,* similar correlations were not observed in this study for CLS and fruit quality traits (Petrasch et al., 2022). Additionally, there was no correlation between the CLS lesion area and *Botrytis cinerea* disease severity (Fig. 8). As such, CLS lesion area alone is not a good predictor of disease progression of *Botrytis cinerea.* These results do not display the similar significant correlations observed with Powdery Mildew and Leaf Scorch (Hancock et al., 2002).

We found the SD varieties had less disease than the DN varieties (Fig. 10). Though the difference was significant, the average LA for SD plants was 3.41% compared to the LA of DN plants which averaged 4.61%. This is just over 1% difference which is small. The visual difference is not significant between 3.41% and 4.61%. However, one study found that more productive genotypes with larger fruit also showed less incidence of leaf scorch and powdery mildew (Hancock et al., 2002). Therefore, a potential reason the observed difference is present in the MSU germplasm could be that the SD varieties are simply larger-fruited, more productive genotypes than the DN counterparts.

The two families with the lowest lesion area were both crosses with Cabot (Fig. 9). However, Cabot was also involved in some higher lesion area crosses. Cabot does have some known resistant to red stele root rot and other leaf diseases (Jamieson, 2006). Resistance genes (R-genes) are typically clustered in octoploid and diploid strawberry genomes (Barbey et al., 2019). Because R-genes tend to be clustered, it is likely that with more evaluation and screening, significant correlations between easy to screen diseases like CLS could be used as predictors for diseases that are harder to screen for like *Botrytis cinerea.*

CONCLUSION

In conclusion, all regressions failed to find significant predictability due to the low R^2 values observed. Other variables may be more predictive of common leaf spot lesion areas. The two families with the lowest lesion area were both crosses with Cabot. However, Cabot was also involved in some higher lesion area crosses. Selecting progeny from the most tolerant families and crossing them may yield better results for improving resistance to CLS in the Michigan State University breeding program. There are a few lines showing tolerance, but no complete resistance was found against the CLS pathogen. More screening should be done to find strong sources of genetic resistance to introgress into the Michigan State University breeding program. The SD individuals showed more tolerance overall than the DN individuals. More research should be done to determine the root of this difference. Finally, it will be useful to compare the protocol used to screen for lesion area on strawberry in this study with other evaluation methods like providing a 0-9 rating performed in Chiang & Bock (2022) for accuracy and ease of use.

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CHAPTER 2: HETEROSIS AND HETEROBELTIOSIS OF FRUIT QUALITY IN ELITE STRAWBERRY GERMPLASM FAMILIES

INTRODUCTION

Strawberries are an economically important horticulture crop worldwide. The United States produced over one million tons of strawberries in 2023 ranking second in strawberry production after China (WPR, 2023). United States strawberry consumption averaged 8 lbs per person and is projected to continue increasing (Samtani et al., 2019). Strawberries are a high-value crop where consumers and producers expect excellent fruit quality. Consumers and producers regard the content of sugars and acids in strawberries as significant components of determining the flavor of good quality fruit (Kallio et al., 2000). Consumers tend to desire fruits balanced between sweetness and acidity (Rey-Serra et al., 2021). A balance between high titratable acidity, high soluble solids, and an intense aroma characterizes strawberry flavor (Verma et al., 2017; Lerceteua-Köhler et al., 2012; Shaw, 1990).

Breeding programs often focus on improving yield, fruit size, disease/pest resistance, and fruit quality (Barth et al., 2020). Fruit quality traits include appearance, texture, taste, aroma, color, firmness, and more, with sweetness, acidity, and flavor as the most important parameters (Barth et al., 2020; Samykanno et al., 2013). Though many breeders start by focusing on yield, maintaining and improving fruit quality is essential, especially for a high-value crop. For consumers, the size and color of the fruits were less critical than flavor, sweetness, juiciness, freshness, and firmness (Choi et al., 2017). On the other hand, producers preferred firm berries with intense flavor and the classic red color in addition to a long shelf life (Choi et al., 2017). Large fruit size is also a trait of interest to producers because it can help reduce labor costs since fewer berries are required to fill a single clamshell (C. Weebadde, personal communication, January 10, 2024). One thing is consistent – good flavor is always desirable.

The MSU breeding program is a continuation of the work done by its former strawberry and blueberry breeder, Dr. Jim Hancock. His early work focused on reconstructing the cultivated strawberry, which was a result of an accidental hybridization, by crossing superior clones of *Fragaria virginiana* and *F. chiloensis* to reconstruct *F.* × *ananassa*. (Hancock et al., 2002; Hancock et al., 2010; Diamanti et al., 2010). Strawberry improvements have been made from a narrow germplasm base, but horticulturally important traits are thought to be present among wild germplasm (Hancock et al., 2002; Hancock et al., 2010; Stegmeir et al., 2010; Diamanti et al., 2012). Specifically, traits to resist a variety of abiotic and biotic stresses will be present in the wild germplasm because of the wide geographic range (Hancock et al., 2010; Stegmeir et al., 2010). Thus, Dr. Jim Hancock used his knowledge of breeding to introgress wild germplasm into superior genotypes using a four-stage reconstruction process (Luby et al., 2008). The superior genotypes were then evaluated and found to be better sources of genetic variability as well as have fair fruit quality and disease resistance (Stegmeir et al., 2010). Elite germplasm developed was then crossed with commercially available cultivars to create the MSU advanced selections used as parents in the crosses for this study.

Though the reconstructed germplasm had exceptional traits such as flavor, the fruit size and sweetness were not on par with some popular commercial cultivars. This led Dr. Cholani Weebadde to use the MSU advanced breeding lines as parents to make another round of crosses with cultivars that had desirable fruit size, sweetness, and disease resistance traits to further improve MSU's strawberry germplasm. In addition to crosses between the MSU advanced breeding lines and commercial cultivars, she also made additional crosses between popular cultivars, the progenies of which were used in this study. As such, the goal of the crosses evaluated here was to determine the best parental combinations to increase fruit size and fruit quality traits in the existing MSU germplasm.

In the MSU breeding program, the leading fruit quality and agronomic attributes of focus included sugar content, acid content, fruit size, and flowering habit. Due to the significant variation of environmental and genetic factors limiting genetic gains in strawberries, standardized phenotyping protocols are essential for consistent data collection, replication, and comparison of performance among various locations (Davis & Van de Weg, 2013). A study found a significant correlation between brix and sugar content, so brix is used as a good estimator of sugar content and is now considered the standard method for phenotyping, quantifying, and predicting sugars or sweetness in strawberries (Kallio et al., 2000; Davis & Van de Weg, 2013). Currently, phenotyping for sugar content or sweetness is measured using a refractometer. Measuring the g/L citric acid in fruit puree gives a titratable acidity estimate when using an automatic titrator with a pH endpoint of 8.1 (Davis & Van de Weg, 2013). One study estimated average fruit weight (g) from five primary fruits harvested when 30-50% of the fruit on each plant was ripe (Davis & Van de Weg, 2013). To get more accurate data for this study, ten fruits were weighed when the plants reached 50% ripeness to estimate average fruit weight.

Finally, day-neutrality (or repeat flowering) is classified as quantitative weeks of flowering after week 11 from the first bloom (Davis & Van de Weg, 2013). Another way to classify DN strawberries is by photoperiod insensitivity, meaning that the plants will flower both under short-day and long-day conditions (Castro et al., 2015). In Michigan, short days are typically before May (< 14 hours), and long days for DN flowering habit are typically after July (>15 hours) (Serçe & Hancock, 2005). However, day length is not the sole contributor to controlling flowering habit. Temperature also plays a role in the inhibition and initiation of flowering. For example, high temperatures under long-day conditions can inhibit flowering (Heide, 1977; Bradford et al., 2010). However, because of the strong temperature and photoperiod interaction, there is significant variation in the degree of repeat flowering, which is referred to as remontancy (Bradford et al., 2010). Therefore, the DN predictions for Michigan may not exhibit the same flowering habit in warmer climate regions. DN and SD strawberries can also exhibit different fruit quality traits. DN strawberries that flower and fruit, regardless of day length, show significantly lower total soluble solids than SD strawberries that are sensitive to day length (Murti et al., 2012). Studies show that SD cultivars tend to have a higher soluble solids content and color with a lower firmness than DN cultivars (Murti et al., 2012).

Many factors can influence fruit quality traits, such as stage of ripeness, age of plants, soil quality, genotype of variety, and environmental conditions such as temperature and precipitation (Kallio et al., 2000; Wang & Camp, 2000; MacKenzie et al., 2011; Morton et al., 2017; Osatuke & Pritts, 2021; Paul et al., 2017). Furthermore, sweetness, measured by total soluble solids or soluble solid content, varies throughout the season and is controlled by additive and dominance effects of genes (Hasing et al., 2013; MacKenzie et al., 2011). Sugars are also significantly influenced by environmental factors such as light, temperature, and relative humidity (Cervantes et al., 2020). Low temperatures can cause alterations in size, shape, and color, whereas elevated temperatures can cause a decrease in photosynthesis and, in turn, decrease yield and sugar content (Cervantes et al., 2020).

In addition to environmental factors, genetic factors also play a crucial role in sugar content, acid content, and fruit size. The cultivated strawberry is an allo-octoploid showing significant disomic inheritance (Verma et al., 2017). Disomic inheritance means some homologous chromosomes are inherited similarly to a diploid without significant recombination.

Photoperiod sensitivity can impact the accumulation of flavor components. Day-neutral strawberries that flower and fruit, regardless of day length, show significantly lower total soluble solids than short-day strawberries that are sensitive to day length (Murti et al., 2012). Studies show that short-day cultivars tend to have a higher soluble solids content and color with a lower firmness than day-neutral cultivars (Murti et al., 2012). However, breeding can still be challenging due to polyploidy, significant environmental effects, and instability of quantitative trait loci for fruit quality traits (Verma et al., 2017; Rey-Serra et al., 2021).

To make genetic advancements in total soluble solids, acid content, and fruit weight, a good knowledge of how these traits is inherited in the crop is useful. Strawberries exhibit heterosis, or hybrid vigor. Heterosis is the superiority of an F1 hybrid over its parents (Kaczmarska et al., 2016). In other words, the progeny performing better than the average value of the two parents for traits is known as mid-parent heterosis (Kaczmarska et al., 2016). When progeny perform better than the best-performing parent in the cross, this is known as heterobeltiosis (Sahoo et al., 2017). Heterosis and heterobeltiosis determine which parents produce progeny that will outperform the parents in fruit quality and other agronomic traits. Breeders can determine the usefulness of established lines based on the estimated level of heterosis and heterobeltiosis (Kaczmarska et al., 2019). For a hybrid crop like strawberry, understanding heterosis values provides breeders another metric to make informed parental selections.

This study aims to characterize and rank families based on the average sugar content, acid content, flowering habit, and fruit weight for several F1 hybrid families over two years. The degree of heterosis and heterbeltiosis will help inform which parents are used to make genetic gains in strawberry flavor and fruit size. In addition, this study will also seek to identify superior genotypes in the breeding populations to advance to the next stage of the breeding process.

Table 2: Cultivars used in the field to compare agronomic traits along with their desirable traits and whether they were used for creating families (Strawberry Plants LLC, 2023; Shaw & Larson, 2006; Galletta et al., 1981; Jamieson, 2006; Voth et al., 1994; Jamieson et al., 1999; Shaw & Larson, 2009; Hancock, 2015).

Cultivar	**Parents used to create Breeding Populations**	**Desirable Traits**
ALBION	No	Day-neutral, large fruits, firm berries, resistant to verticillium wilt, phytophthora crown rot, partially resistant to anthracnose crown rot
ALLSTAR	Yes	Short day, popular in Michigan, highly resistant to red stele, partially resistant to verticillium wilt, tolerant to *Botrytis cinerea* late to mid-season productivity, large fruits, good flavor, glossy skin
ANNAPOLIS	Yes	Short day, winter-hardy, resistant to red stele, large fruit
CABOT	Yes	Short day, huge berries, excellent flavor, winter-hardy, resistant to red stele
CAMAROSA	Yes	Short day, high yield, early productivity, large fruits
CAVENDISH	No	Short day, mid-season productivity, high yielding, resistant to red stele, partially resistant to verticillium wilt
EARLIGLOW	Yes	Short day, resistant to red stele, partially resistant to verticillium wilt, excellent flavor
HONEOYE	Yes	Short day, vigorous plant, high yield, large berries, firm fruit
JEWEL	Yes	Short day, moderate winter hardiness, large berries, sweet flavor
REDSTART	Yes	Day-neutral, vigorous, high yield, good flavor, smaller fruits, softer berries, heat tolerant
SAN ANDREAS	No	Day-neutral, high yield, larger fruit than Albion, excellent flavor, moderately resistant to powdery mildew, anthracnose crown rot, verticillium wilt, phytophthora crown rot and common leaf spot
WASATCH	Yes	Day-neutral, excellent flavor, high yield, smaller berries, good fruit appearance

METHODS

Germplasm

Breeding populations were grown in one field location (Latitude: 42.685819°; Longitude: -84.471536°) at Michigan State University's Pathology farm for four years (Table 1). The field was maintained with minimal inputs since it was planted in June 2019 to maximize the expression of true genotypic fruit quality. Other than testing the field and adjusting the pH prior to planting no fungicides or chemical fertilizers were used since it was planted. At most only weed control was done manually and the use of an herbicide once a year. When necessary, hand-weeding, cross-cultivation, and overhead irrigation maintained the field. The herbicide Post was used once in 2019 to control weeds in addition to hand-weeding and cross cultivation.

Field Design

In years three and four (summer 2021 and 2022), fruit quality traits were evaluated for the cultivars and breeding populations that were maintained in the Pathology farm at Michigan State University (Latitude: 42.685819°; Longitude: -84.471536°). Fruit quality trait evaluation used a modified approach similar to that used by Davis & Van de Weg (2013). The following fruit quality traits were assessed in 51 families, 17 MSU advanced selections, and 12 cultivars, totaling 1077 unique genotypes in 2021 and 2022.

Fruit Weight

Once the plant reached 50% ripeness, 10-15 berries were randomly harvested from each genotype. The average weight was recorded by weighing ten berries in grams. The total weight was divided by 10 to get an average berry weight per genotype in grams (W).

Total Soluble Solids

The average total soluble solids were collected by cutting 3-5 berries in half and squeezing two drops of strawberry juice on a refractometer. The brix % was recorded to the nearest decimal. Measurements were repeated for 3-5 berries depending on availability and year. All recorded brix percentages were then averaged to estimate the average sugar content per genotype in brix % (TSS).

Acid Content

The average acid content of berries was collected using a Mettler-Toledo Automatic Titrator and Rondolino. First, 5-20g of fruit tissue was weighed. If fruits were frozen, they were thawed at room temperature for approximately 2 hours. The tissue was wrapped in cheesecloth and juiced into a 15 mL centrifuge tube. Distilled water was used to rinse the remaining juice into the centrifuge tube. The juice tubes were frozen until they were processed in the auto titrator. Once the samples were ready for processing, the tubes were thawed in a warm water bath for 30 minutes. The thawed juice was emptied into the Mettler-Toledo Rondolino sample cups. Distilled water was added to get the total volume to approximately 35 mL of liquid. The samples were placed in the Rondolino, and the acid content was measured. Depending on available fruit tissue, the output was recorded for 3-5 replications per genotype. The following equation was used to determine the final proportion of acid:

$$Titratable \quad Acidity = \frac{NaOH_{mL} * 0.1 \frac{meq}{mL} * 0.067045}{Tissue(g)}$$

Flowering Habit

Flowering data was collected on all MSU advanced selections, cultivars, and families in 2020, 2021, and 2022. Starting in early May, each genotype was assessed for flower buds, old flowers, green fruits, white fruits, and red fruits. The genotypes were evaluated weekly through the end of August. If genotypes had a second round or more red fruits present after the end of June, they were considered day-neutral.

Statistical Analysis

Normality tests were not performed due to the large sample size. However, each trait was visualized graphically to ensure normal distribution. Population means, mid-parent values, the proportion of progeny performing better than the mid-parent value, and the proportion of progeny performing better than the best parent were calculated for each family for TSS, TA, and fruit weight. Additionally, the proportion of day-neutral progeny was calculated for each family. Analysis of variance (ANOVA) was performed in R on TSS, TA, and Weight with family averaged over a year as a factor and genotype average over a year as a factor. Then, the Tukey HSD test was performed to determine what families are driving the significant difference. The Tukey HSD test was only performed on families with progeny greater than 20 because the standard error was too high for families with less than 20 progenies. Heterosis and heterobeltiosis were calculated for each family using the equations below.

Mid-Parent Equation

$$MP = \frac{P1 - P2}{2}$$

P1: Parent 1 phenotypic value
P2: Parent 2 phenotypic value
MP: Mid-parent value

Heterosis & Heterobeltiosis Equation (Kaczmarska et al., 2016).

$$Het = \frac{F1 - MP}{MP} x\, 100$$

$$HetB = \frac{F1 - BP}{BP} x\, 100$$

Het: Heterosis % of hybrid vigor in relation to the mid-parent value
F1: Average value of the F1 population
MP: mid-parent value of the two parents of the F1 population
HetB: Heterobeltiosis % of hybrid vigor in relation to the best parent value
BP: best parent value of the two parents of the F1 population

RESULTS

All agronomic traits, TA, TSS, and weight, varied significantly by genotype and family. The mean comparisons show significant variability in TA across families ranging from 0.52 to 0.33%. The families with the highest TA were crosses between MSU76 × Earlilgow, MSU81 × Cabot, and Cabot × MSU80 with an average TA of 0.52, 0.48, and 0.47%, respectively (Fig. 11a). The families with the lowest TA were crosses between MSU72 × Camarosa, Cabot × Redstart, and MSU79 × Camarosa with an average TA of 0.33, 0.34, and 0.35% respectively (Fig. 11a).

Total soluble solids also showed significant variation among families with more than 20 progenies ranging from 9.69 to 6.99% (Fig. 11b). The families with the highest TSS were crosses between MSU79 × Earliglow, MSU44 × Earliglow, and MSU77 × Earliglow with an average TSS of 9.69, 9.42, and 9.36% respectively (Fig. 11b). The families with the lowest TSS were crosses between Cabot × Redstart, MSU79 × Camarosa, and MSU72 × Camarosa with an average TSS of 6.99, 6.99, and 7.33% respectively (Fig. 11b).

There was significant variation in the average weight of 10 fruits across families ranging from 15.07 to 7.35g (Fig. 11c). The families with the largest fruit weight were crosses between MSU44 × Jewel, Cabot × MSU79, and Cabot × MSU72 with an average fruit weight of 15.07, 14.65, and 14.55g respectively (Fig. 11c). The lowest fruit weight families were crosses between MSU81 × MSU69, MSU76 × Earliglow, and MSU79 × Earliglow with an average fruit weight of 7.35, 7.41, and 7.42g respectively (Fig. 11c).

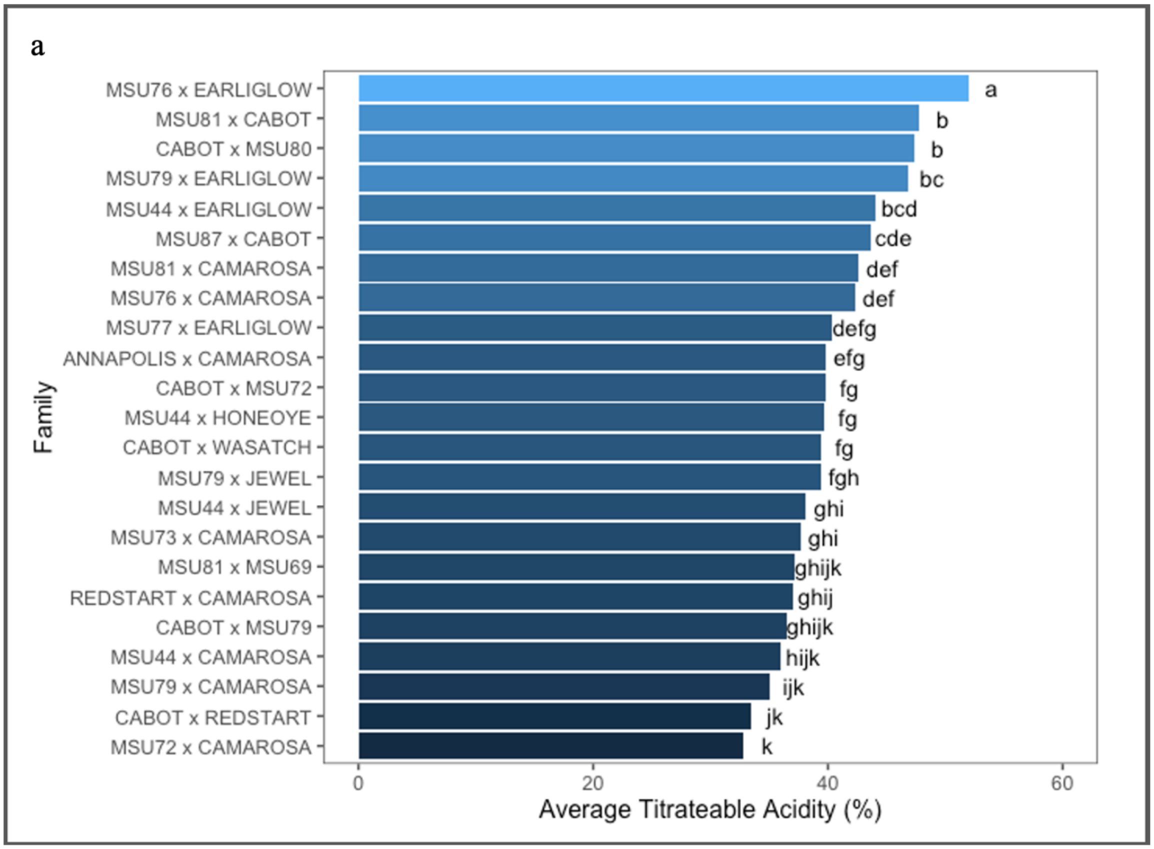
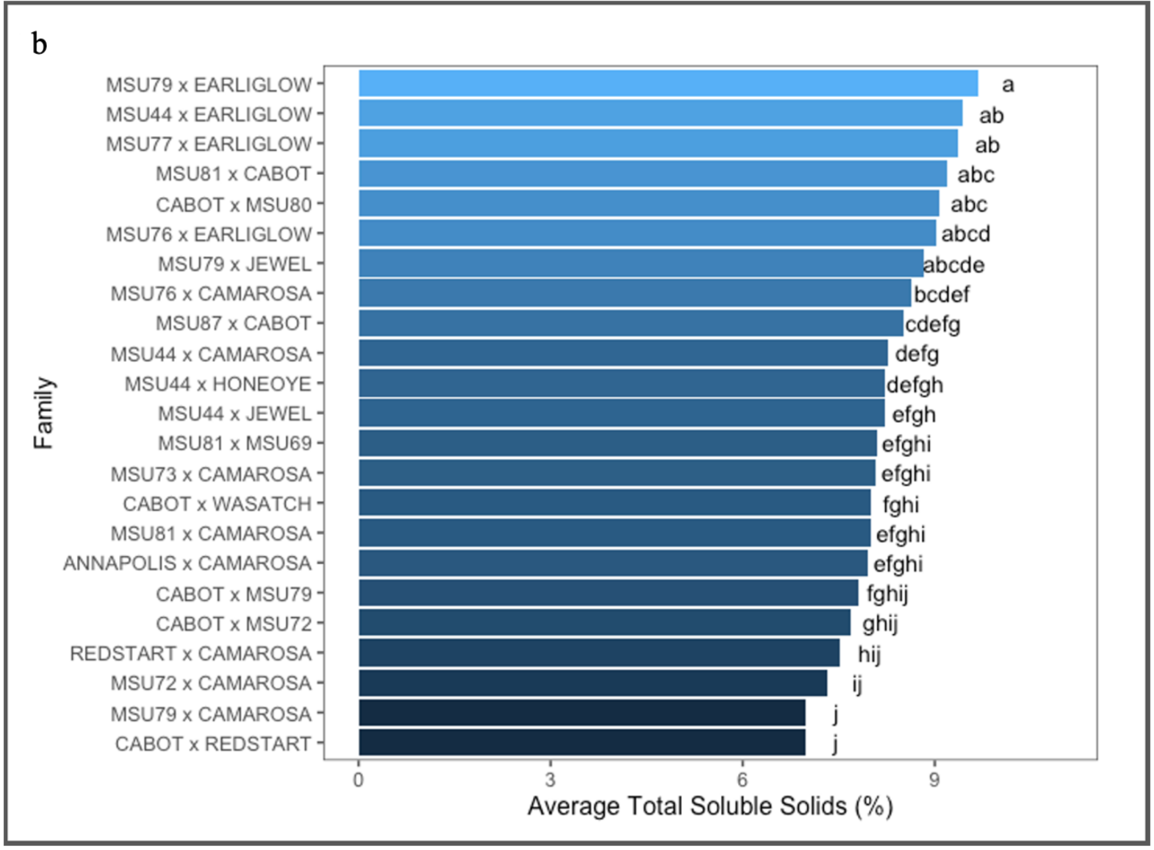


Figure 11: Mean comparisons of (a) titratable acidity (%), (b) total soluble solids (°Brix %), (c) fruit weight (g) of different families using Tukey HSD test. Different letters represent significant differences between means at α=0.05.

Figure 11 (cont'd)



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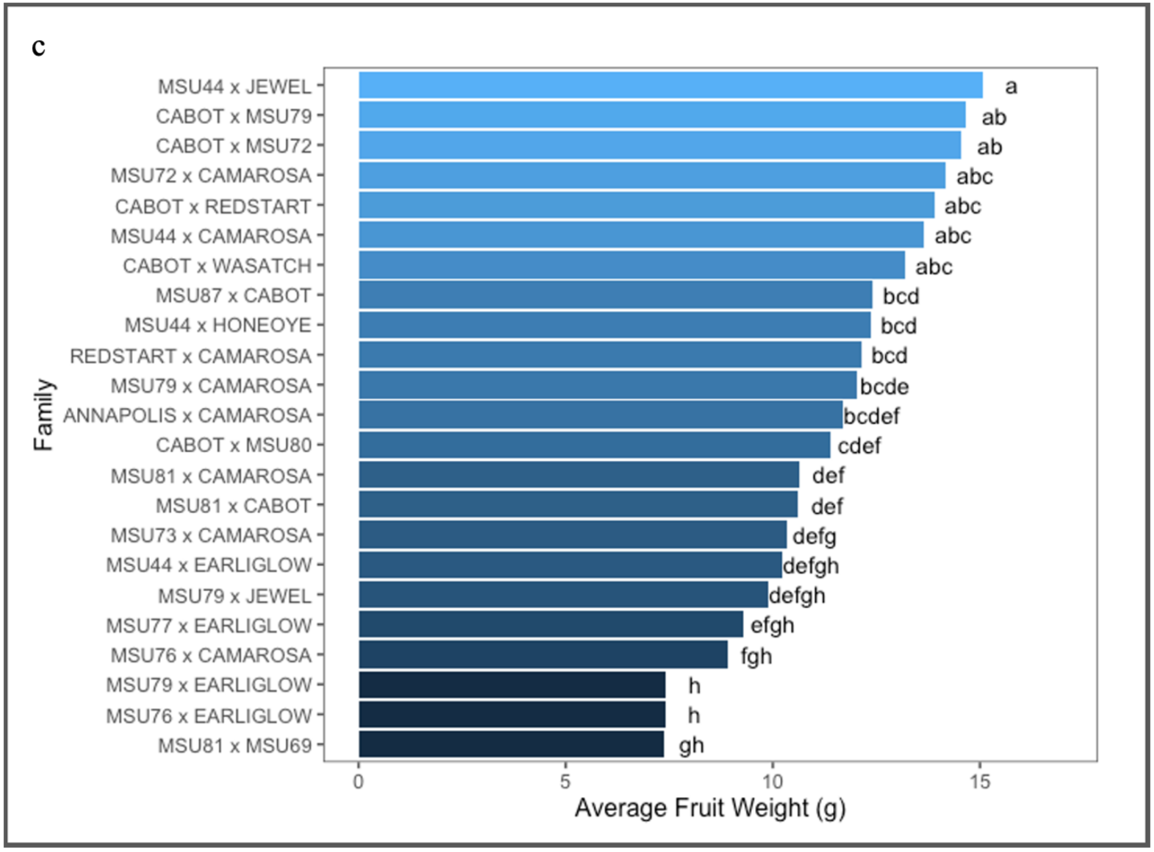

Several crosses resulted in all progenies being DN. Families with 100% DN progeny include MSU77 × Camarosa, MSU81 × MSU69, MSU81 × Cabot, MSU81 × Camarosa, Annapolis × MSU73, and MSU84 × Honeoye (Table 3).

Table 3: Proportion of day-neutral (DN) progeny and number of total progeny per family. Any family not included had 0% DN progeny.

Family	Parental Cross	# of Progeny	Proportion of DN
1912	MSU73 × CAMAROSA	35	51%
1913	MSU76 × CAMAROSA	41	56%
1914	MSU76 × EARLIGLOW	33	79%
1915	MSU77 × CAMAROSA	11	100%
1916	MSU77 × EARLIGLOW	24	92%
1917	MSU79 × MSU69	5	40%
1918	MSU79 × CAMAROSA	43	77%
1919	MSU79 × EARLIGLOW	26	46%
1920	MSU79 × JEWEL	27	48%
1921	MSU81 × MSU69	21	100%
1923	MSU81 × CABOT	38	100%
1924	MSU81 × CAMAROSA	42	100%
1925	MSU87 × ALLSTAR	9	67%
1926	MSU87 × CABOT	30	53%
1929	ALLSTAR × MSU79	14	57%
1930	ALLSTAR × MSU86	4	75%
1932	ANNAPOLIS × MSU73	1	100%
1936	CABOT ×WASATCH	60	60%
1938	CABOT × MSU75	5	40%
1939	CABOT × MSU79	27	37%
1940	CABOT × MSU80	47	64%
1942	CABOT × EARLIGLOW	16	25%
1953	MSU84 × HONEOYE	2	100%

Furthermore, the fruit quality traits of SD and DN plants in the breeding population were compared. When comparing DN and SD plants in the breeding population, titratable acidity was significantly higher in the DN cultivars (Fig. 12a). The DN averaged a TA of 41.6 ± 0.247%. In contrast, the SD plants had a TA average of 37.8 ± 0.165% (Fig. 12a). There was no significant difference between SD and DN plants when comparing total soluble solids (Fig. 12b). This contrasts the significant difference found in Murti et al. (2012) where DN strawberries showed significantly lower TSS than SD. However, there was a significant difference when comparing the TSS/TA ratio. DN plants averaged a TSS/TA of 20.8 ± 0.75, while the SD plants had a higher average of 23.4 ± 0.064 (Fig. 12c). Lastly, the SD plants had a higher average fruit weight than DN plants (Fig. 12d). The SD plants averaged a fruit weight of 12.5 ± 0.146 while DN plants had a lower average of 9.99 ± 0.172 (Fig. 12d). Studies show that short-day cultivars tend to have a higher soluble solids content and color with a lower firmness than day-neutral cultivars (Murti et al., 2012).

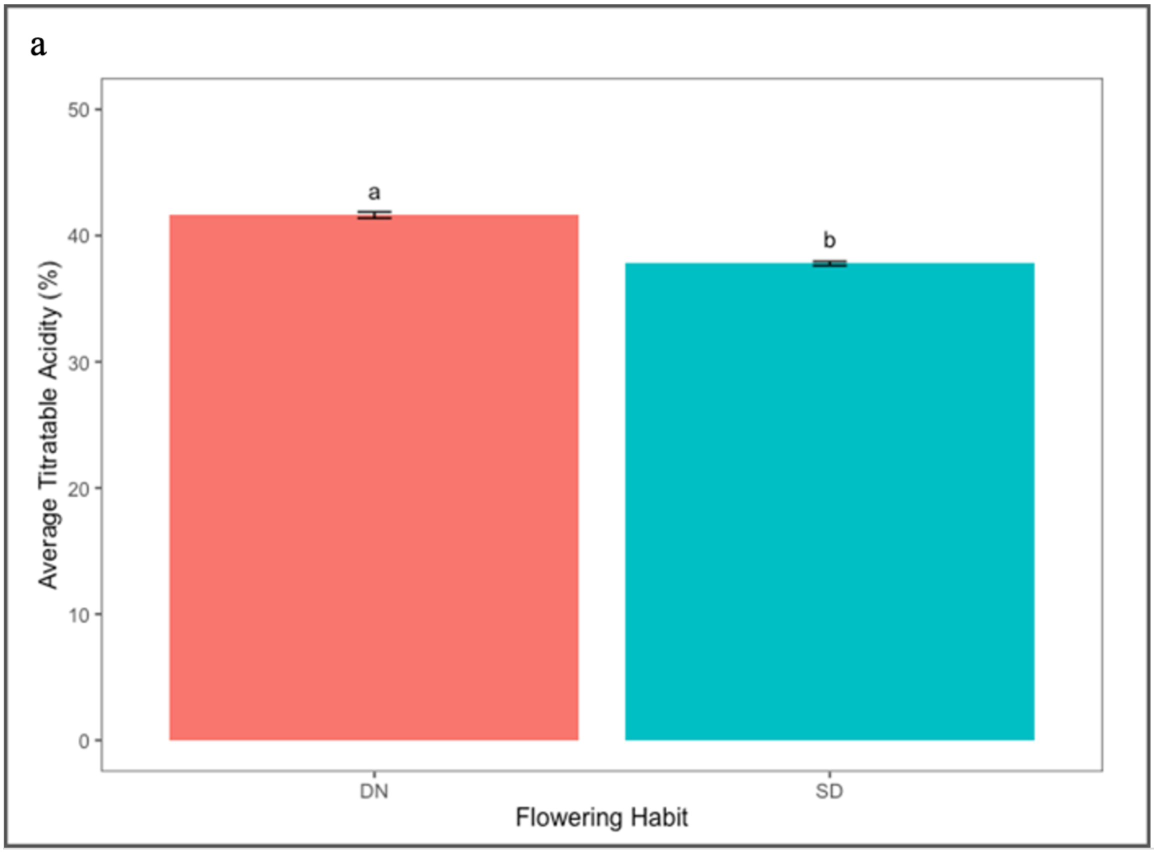

Figure 12: Comparisons of (a) average titratable acidity (%) (b) average total soluble solids (%) (c) average titratable acidity to total soluble solids ratio and (d) average fruit weight (g) between day-neutral (DN) and short-day (SD) strawberry plants using Tukey HSD test. Different letters represent significant differences between means (α<0.05).

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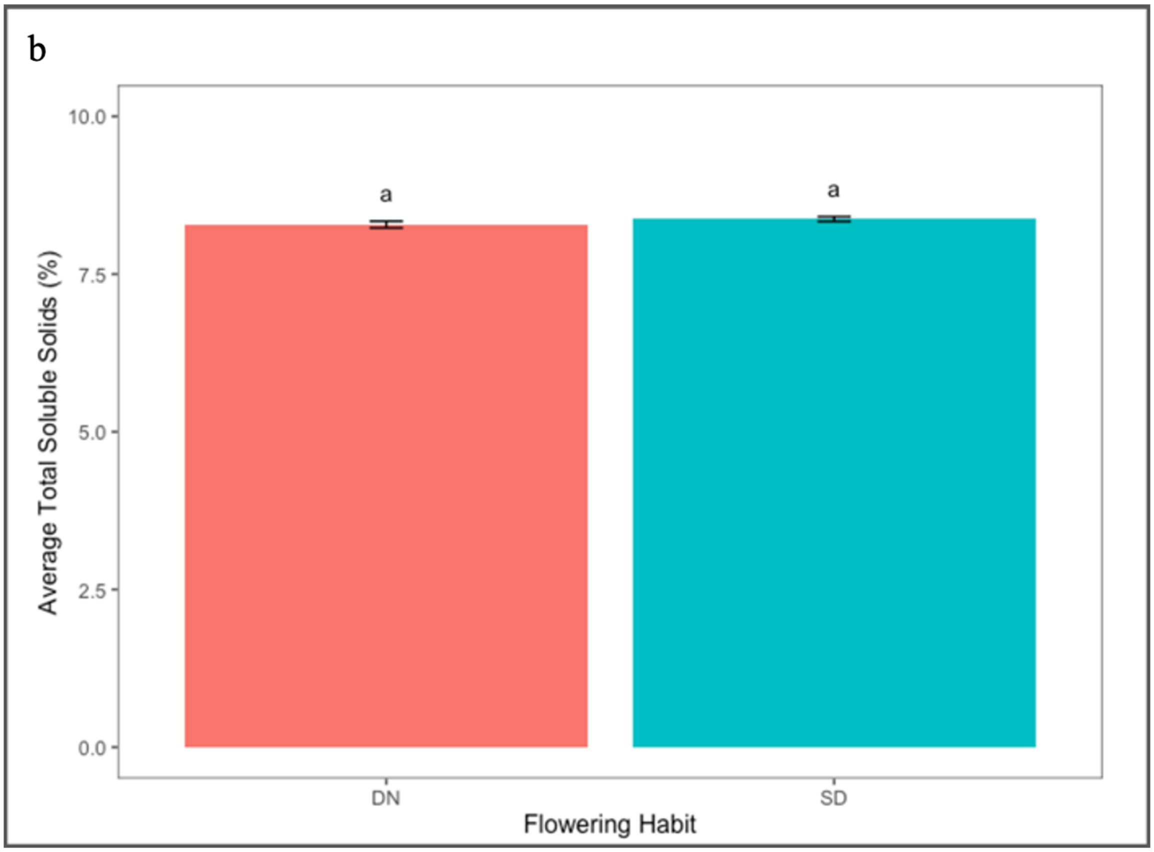

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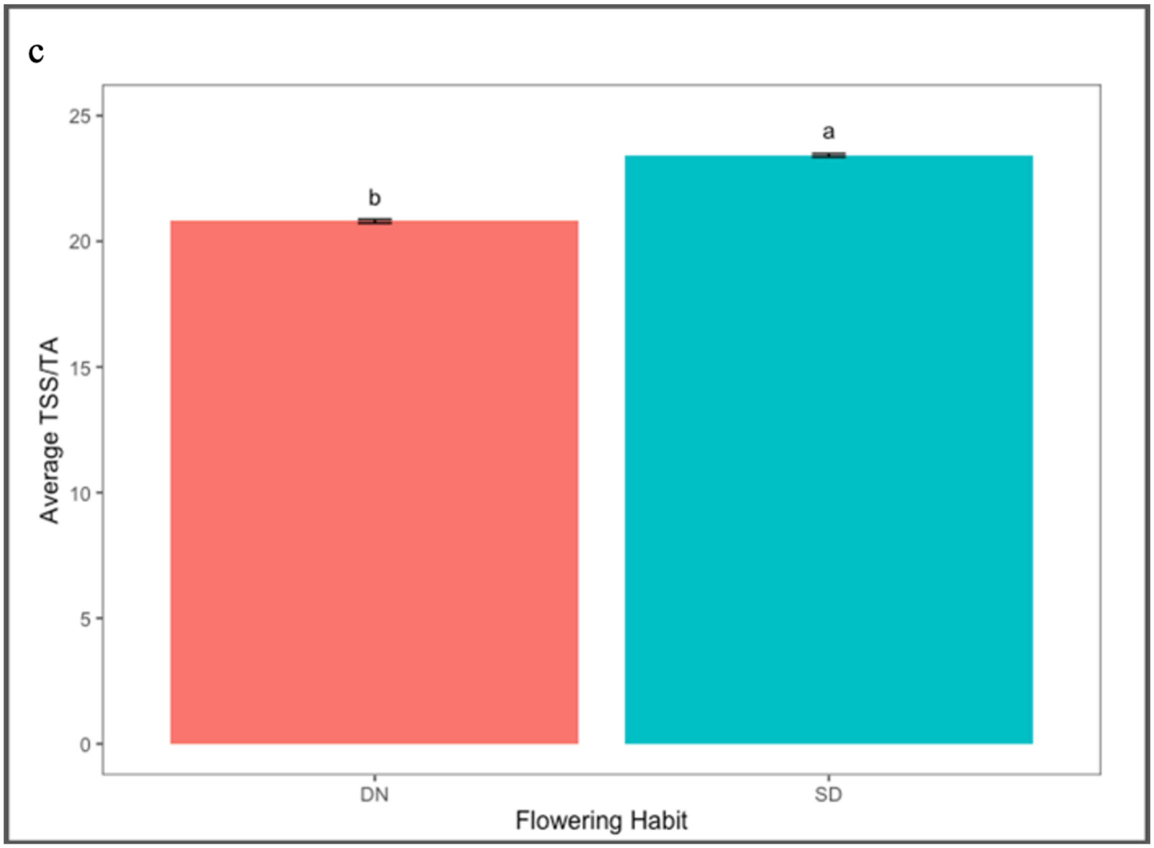

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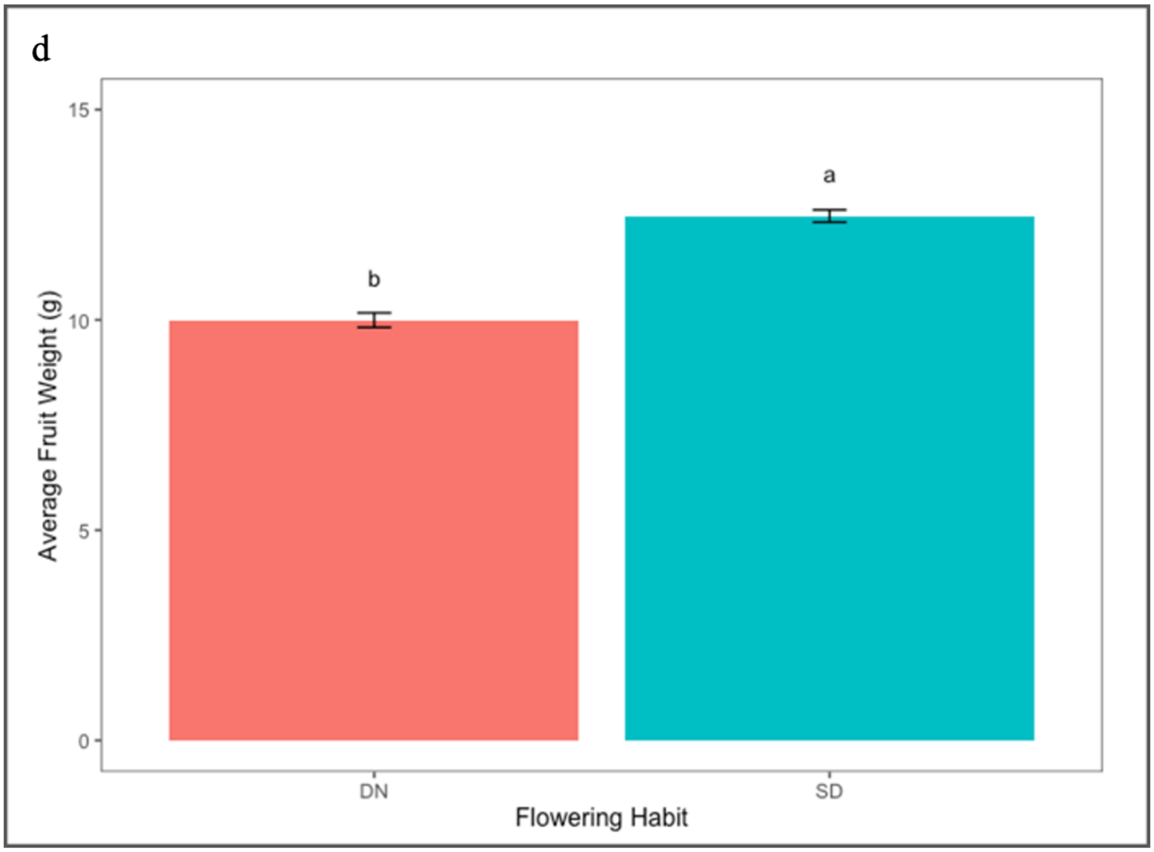


The degree of heterosis and heterobeltiosis varied widely for weight, TSS, TA, and lesion area. The families that showed both heterosis and heterobeltiosis for weight include crosses between MSU49 × Allstar, MSU44 × Camarosa, and Allstar × Redstart (Fig. 13a). The families that only showed heterosis include Redstart × Camarosa, MSU81 × Camarosa, MSU73 × Camarosa, MSU49 × Cabot, MSU44 × Jewel, Annapolis × MSU73, and Allstar × MSU73 (Fig. 13a). No clear pattern emerged from heterosis or heterobeltiosis of fruit weight. However, the cross with the highest degree of heterosis and heterobeltiosis was Allstar × Redstart (Fig. 13a).

The families that showed both heterosis and heterobeltiosis for TSS include crosses between MSU81 × Cabot, MSU49 × Allstar, MSU44 × Allstar, Earliglow × Camarosa, Cabot × MSU80, Cabot × MSU44, Cabot × Earliglow, Allstar × Redstart, and Allstar × MSU69 (Fig.

13b). MSU81 × MSU 69, MSU77 × Earliglow, MSU76 × Earliglow, MSU76 × Camarosa, MSU49 × Earliglow, Earliglow × MSU73, Cabot × Honeoye, Allstar × Wasatch, and Allstar × MSU73 all showed heterosis, however, no heterobeltiosis (Fig. 13b). All but one cross, MSU44 × Earliglow, made with Earliglow, showed some degree of heterosis (Fig. 13b). All but two crosses, Allstar × Honeoye and Allstar × Cabot, showed at least heterosis if not both heterosis and heterobeltiosis. In addition, the MSU49 × Allstar cross showed the highest degree of heterosis and heterobeltiosis for TSS (Fig. 13b).

Titratable acidity showed the most heterosis and heterobeltiosis overall (Fig. 13c). Based on the literature, a higher TA in conjunction with a higher TSS results in good strawberry flavor (Verma et al., 2017; Lerceteua- Köhler et al., 2012; Shaw, 1990). The two crosses that did not result in both heterosis and heterobeltiosis include MSU 71 × Camarosa and Cabot × Redstart (Fig. 13c). The highest degree of heterosis and heterobeltiosis resulted from the cross Earliglow × MSU73.

A negative heterosis and heterobeltiosis are favorable for LA since fewer lesions are desirable (Fig. 13d). The crosses that resulted in negative heterosis and heterobeltiosis include MSU 81 × Cabot, Earliglow × Camarosa, Cabot × Wasatch, Cabot × Redstart, Cabot × MSU79, Cabot × Earliglow and Allstar × Cabot (Fig. 13d). The families that only showed negative heterosis and not heterobeltiosis include MSU84 × Honeoye, MSU81 × MSU69, MSU77 × Earliglow, MSU73 × Camarosa, MSU49 × Cabot, MSU49 × Allstar, MSU44 × Jewel, Cabot × MSU80, Cabot × MSU72, Cabot × MSU44, Cabot × Honeoye, Annapolis × Camarosa, Allstar × Redstart, and Allstar × MSU79 (Fig. 13d). All crosses made with Cabot showed some degree of negative heterosis or heterobeltiosis for CLS lesion area.

The TSS/TA ratio showed the least heterosis and heterobeltiosis overall (Fig. 13e). The families that showed heterosis and heterobeltiosis include MSU81 × MSU69, MSU71 × Camarosa, and MSU44 × Allstar (Fig. 13e). One family, Allster × Redstart, showed only heterosis for TSS/TA (Fig. 13e). The highest degree of heterosis resulted from the MSU71 × Camarosa cross and the highest degree of heterobeltiosis resulted from MSU81 × MSU69 (Fig. 13e).

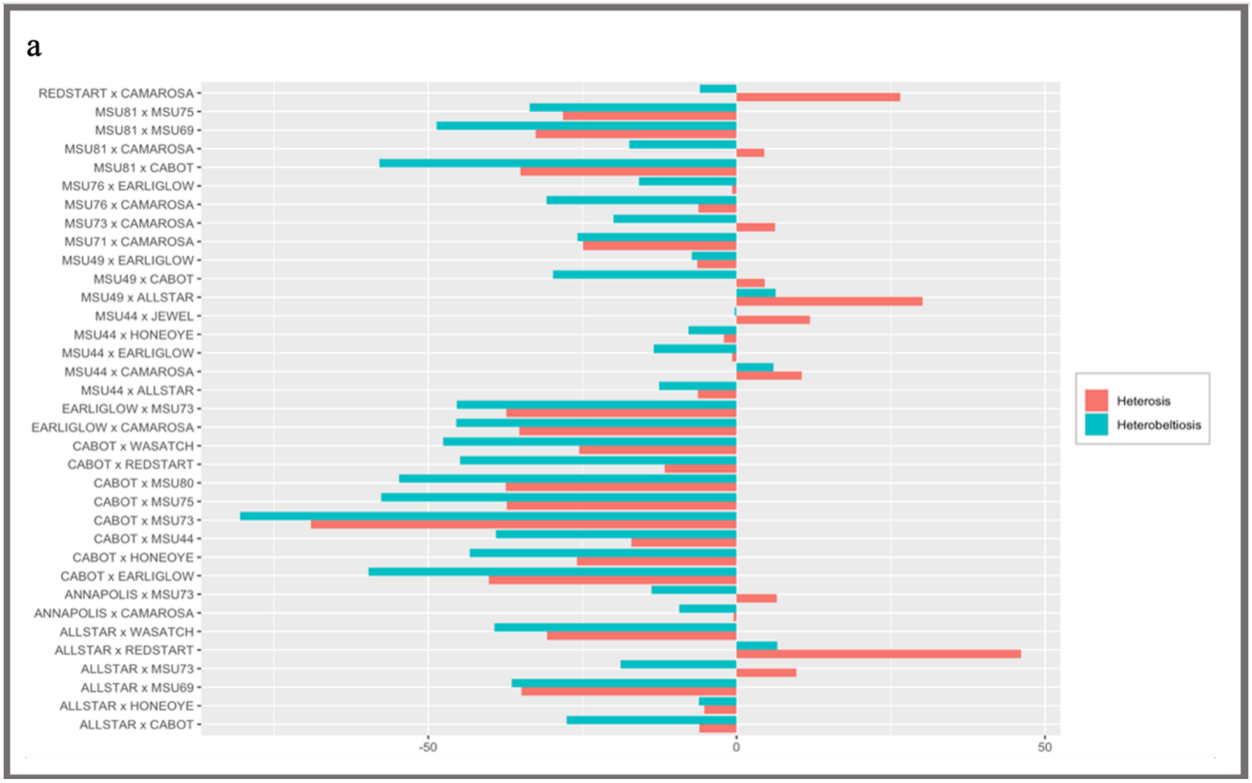
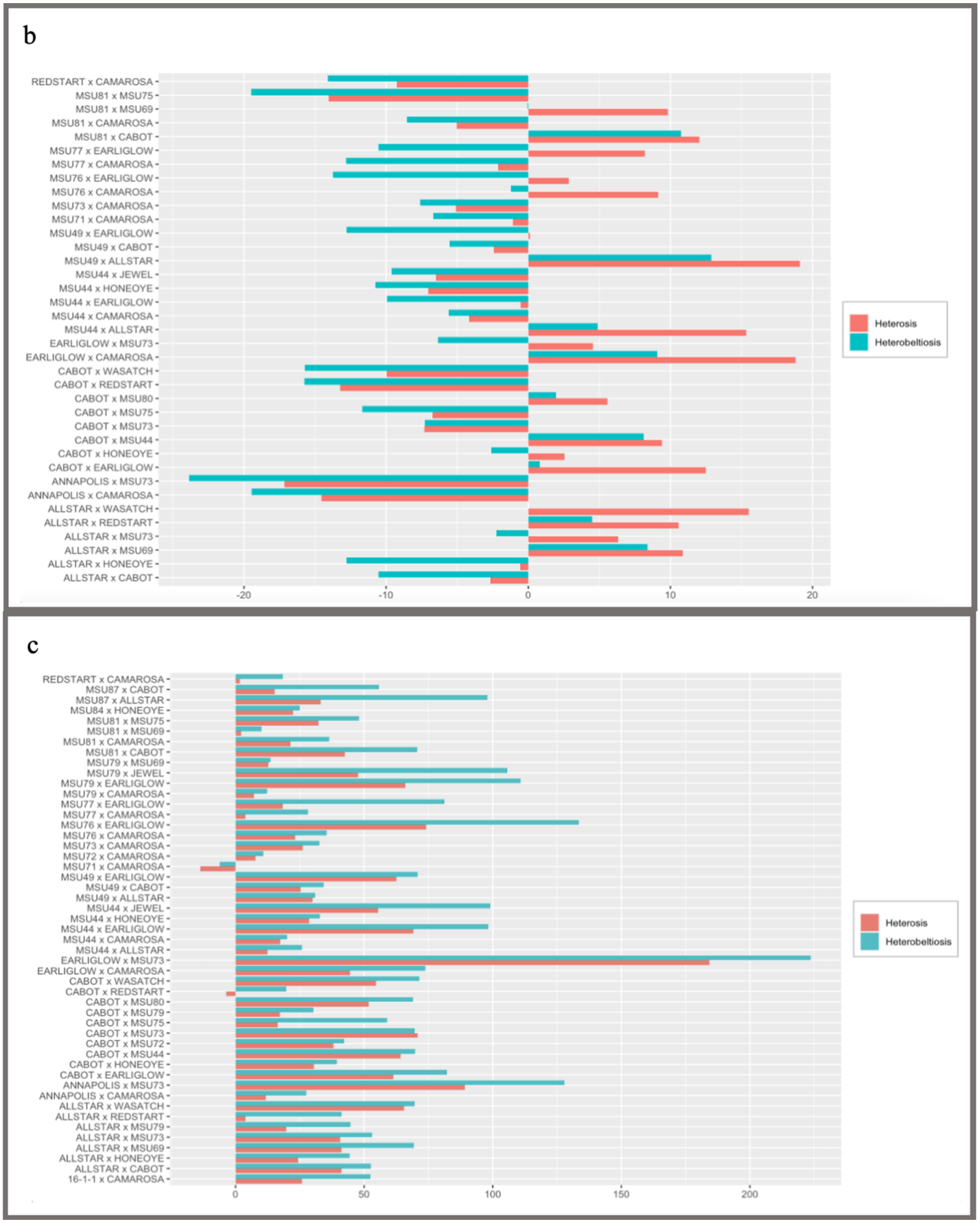


Figure 13: Mid parent heterosis (%) and heterobeltiosis (%) for (a) fruit weight (g), (b) total soluble solids (°Brix), (c) titratable acidity (g/L), (d) Common Leaf Spot lesion area, (e) total soluble solids to titratable acidity ratio. Heterosis is vigor in relation to the mid parent average and heterobeltiosis is vigor in relation to the best performing parent.

Figure 13 (cont'd)



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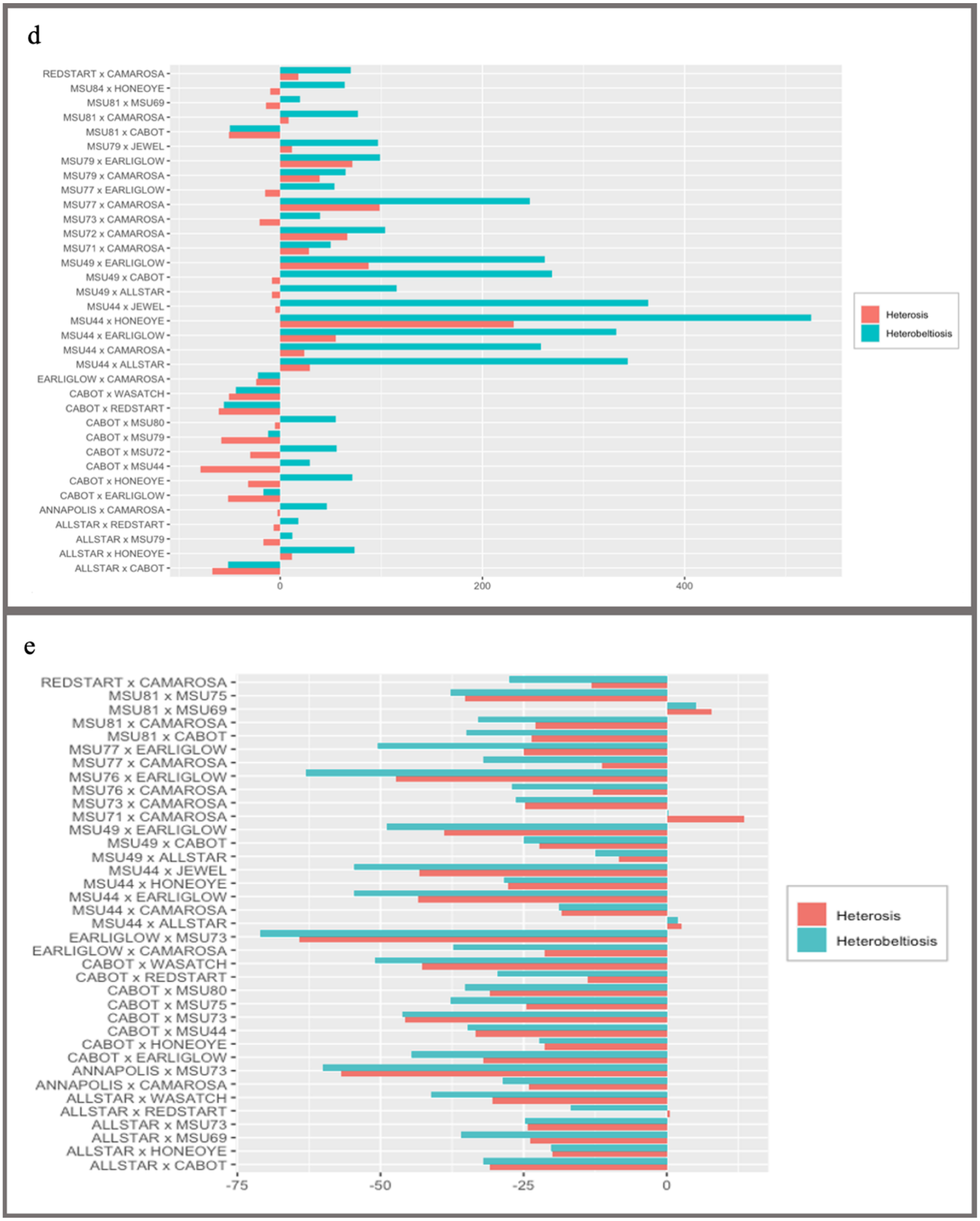

DISCUSSION

The TA found in the MSU germplasm families ranged from 0.52 to 0.33% which is slightly lower than the range reported in other studies (Fig. 11a) (Patel et al., 2023; De Resende et al., 2008; Montero et al., 1996). Interestingly, all crosses made with Earliglow were significantly higher than the lowest average TA families. On the other hand, all crosses with Camarosa had a significantly lower average TA than the average TA of the highest families. However, the crosses made with both Camarosa and Earliglow varied significantly. A balance between acidity and sweetness is desired for consumers therefore both Camarosa and Earliglow could have uses in a breeding program depending on how flavor should be adjusted (Verma et al., 2017; Lerceteua-Köhler et al., 2012; Shaw, 1990).

Total soluble solids ranged from 9.69 to 6.99% across family averages with more than 20 progenies (Fig. 11b). The observed range is narrower than other studies but still in line with other reported TSS ranges (Patel et al., 2023; De Resende et al., 2008; Kallio et al., 2000; Montero et al., 1996). Interestingly, all crosses with Earliglow were not significantly different from each other and were all significantly higher than the families with the lowest TSS. Earliglow may be a beneficial parent for increasing TSS content in strawberries. Historically, Earliglow has been used as a parent in the MSU breeding program to aid in improving flavor (C.Weebadde, personal communication, January 10, 2024). Therefore, Earliglow exhibiting results that suggest it would be a good parent aligns with previously performed crossing and selections with in the MSU germplasm. The current goal of the MSU breeding program is to increase sweetness of berries so Earliglow exhibiting low TA and high TSS in all resulting crosses further emphasizes its potential as a parent for the breeding program.

The average fruit weight among the families ranged from 15.07 to 7.35g with individual progeny ranging from 1.84 to 32.76g which is within the range of other studies (Fig. 11c) (Hummer et al., 2023; Particka & Hancock, 2005). Interestingly, all crosses made with Cabot had a significantly higher fruit weight than the lowest fruit weight families. All crosses made with Earliglow were significantly lower than the highest fruit-weight families and were not significantly different from each other. Earliglow may have a high TSS but may be detrimental if the breeder aims to increase fruit size. Cabot, however, is known for its large fruits and the results show that Cabot is a useful parent for increasing fruit size in a breeding program (Jamieson, 2006).

The proportions of DN progeny did not always follow a typical 3:1 ratio that would be exhibited if it followed the single, dominant gene model which aligns with the results in other studies that DN flowering habit is not regulated by a single locus (Serçe & Hancock, 2005; Castro et al, 2015). In the evaluation of DN proportions, any cross made with MSU81 resulted in 100% of the progeny being DN (Table 3). This suggests MSU81 is a strong DN. MSU81 also has good flavor but small fruits. To maintain the DN flowering habit with good flavor but to increase fruit size, continued crossing and selection with Cabot may yield a large fruited, DN strawberry with good flavor.

All crosses made with Cabot showed some degree of negative heterosis or heterobeltiosis with lesion area (Fig. 13d). Cabot may be a beneficial parent to use when trying to progress CLS resistance since all progeny outperformed the mid-parent value at a minimum. Furthermore, Cabot was a parent to the most resistant families in the MSU breeding program (Chapter 1, unpublished data). These results further support that Cabot may be a beneficial parent to use when breeding form fruit size and CLS resistance.

CONCLUSION

In conclusion, there was much variation in crosses for all agronomic traits observed. However, Camarosa may be a beneficial parent to decrease acid content since all crosses were significantly lower average TA than the average TA of the highest families. Furthermore, Earliglow may be a beneficial parent to increase the fruits' acid content and soluble solids since all crosses were significantly higher than the lowest average TA families. Earliglow is known for its excellent flavor, so, unsurprisingly, Earliglow crosses outperformed others in flavor attributes. However, Earliglow crosses did result in significantly smaller fruits, so it may be a good parent for focusing on flavor, but the size will need to be bigger for current marketable standards.

Interestingly, crosses with Cabot did show significantly higher fruit size than the lowest families for all crosses. Cabot is a beneficial parent to use when focusing on fruit size. More studies should be done on how the general combining ability is reflected in crosses with Earliglow, Camarosa, and Cabot.

Day neutrality has been an interest for strawberry breeders for a while now. Interestingly, all crosses with MSU81 resulted in 100% of the progeny showing DN. MSU81 may have homozygous dominant alleles for DN flowering habit and will be an excellent parent to use for creating all DN progeny. DN progeny showed a higher acid content than SD but a smaller fruit size and TSS/TA ratio. Unfortunately, larger fruits and a higher TSS/TA ratio are desired. So, the DN progeny at Michigan State University are not outperforming the SD progeny. More breeding is needed to improve fruit size and flavor in DN breeding lines.

Earliglow and Allstar showed, in all but one or two crosses, respectively, heterosis for TSS, further supporting the claim that Earliglow is a beneficial parent to use when breeding for fruit flavor. Allstar may also be an excellent parent to use, but due to the small number of progenies from the Allstar crosses, they were not compared using the Tukey HSD test. Furthermore, a cross between Earliglow × MSU73 resulted in the highest heterosis for TA, supporting further that Earliglow can improve fruit flavor. Lastly, all crosses with Cabot showed a degree of negative heterosis or heterobeltiosis for the lesion area for CLS. As such, Cabot may be one of the only cultivars that help breeding efforts to improve CLS resistance in the Michigan State University breeding program.

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FUTURE DIRECTIONS

In the future, more research should be done to screen and identify sources of CLS resistance to introgress into the Michigan State University breeding program. To obtain more consistent and accurate disease ratings for future breeding decisions, inoculated trials should be used. Disease pressure was low and having inoculated trials woul allow for more accurate scores and decisions. However, Cabot could be used as a parent to improve tolerance among current MSU germplasm. Additional crosses should be made between selected individuals of the most tolerant families to see improvement in the existing germplasm. Furthermore, the segregation of CLS resistance should be studied to determine the inheritance pattern of CLS from parent to progeny.

Additionally, more research should be done to quantify the general combining ability of all lines in the Michigan State University breeding program to determine which cultivars and advanced breeding lines could improve future breeding efforts. Lastly, DN cultivars should be improved to match and exceed current SD progeny within the breeding program; MSU81 could be used as a parent to ensure DN progeny while introducing excellent fruit quality traits from other cultivars and advanced breeding selections. Overall, some promising traits exist in the breeding program, and more work is needed to continue improving the germplasm for market standards.