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Elucidation of Avocado Fruit Maturation Mechanisms Under Different Environmental Conditions

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Elucidation of Avocado Fruit Maturation Mechanisms Under Different Environmental Conditions

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ABSTRACT OF THE DISSERTATION

Elucidation of Avocado Fruit Maturation Mechanisms Under Different Environmental Conditions

by

Eric Focht

Doctor of Philosophy, Graduate Program in Plant Biology
University of California, Riverside, December 2024
Dr. Mary Lu Arpaia, Chairperson

The ‘Hass’ avocado, originating from La Habra Heights, California, is a globally significant crop. Over time, somatic mutations have arisen from repeated grafting, leading to the development of two notable cultivars from Chile: ‘Flavia’ and ‘Eugenin’. Both cultivars produce larger fruit than ‘Hass’, with ‘Flavia’ fruit also reported to mature earlier. Avocado maturity is measured by dry weight, which represents the non-water mass of the fruit. Different dry weight standards are necessary for various cultivars and growing regions, as climate significantly influences the maturation rate of avocado fruits.

This study investigates the fatty acid composition of ‘Hass’, ‘Flavia’, and ‘Eugenin’, given that lipids constitute the majority of the avocado fruit’s dry weight and are its most nutritionally important component. Over two years, it was found that palmitoleic acid consistently had higher levels in ‘Eugenin’ and ‘Flavia’ fruit compared to ‘Hass’. The study also examined the environmental effects on fatty acid composition by comparing these cultivars in two different locations. Significant environmental effects were observed, consistent with existing literature, showing lower levels of oleic acid and higher levels of palmitic acid in fruit from the warmer trial site.

Sensory panels conducted over two years assessed whether participants could distinguish between the cultivars. While differences were detectable, consistent differentiation was only achieved with statistical significance in the second year. The findings confirmed that 'Flavia' and 'Eugenin' produce larger fruits than 'Hass'. Cultivar differences in dry weight accumulation were also noted in the sensory panel portion of the trial, where a larger sample size revealed these differences.

In conclusion, detectable differences in the maturity metrics and composition of 'Flavia' and 'Eugenin' compared to 'Hass' fruits were found, but these differences were not of the same nature as those resulting from environmental conditions that hasten fruit maturation. While further research is needed, this study provides a promising start in understanding both the distinct characteristics and environmental responses of 'Flavia' and 'Eugenin' compared to 'Hass'.

Table of Contents

List of Figures

List of Tables

Introduction to the Current Study

The History of the California Avocado Development, 'Hass', and the Modern Commodity Market

When one considers the California germplasm and its manifestation in a typical, emblematic avocado fruit, it is impossible to ignore the 'Hass' avocado, originating in La Habra Heights in the mid to late 1920s. The place of California and its typical varieties in the overall global population structure is one of a subpopulation of a majority Guatemalan minority Mexican makeup(Ashworth and Clegg, 2003; Chen et al., 2009; Solares et al., 2023) and this can be seen in the models of maturation and dry weight accumulation that typify the 'Hass' and its descendants. The history of California avocado domestication, however, is more than just a story of the inevitable rise of the 'Hass' avocado from an introduced pool of sourced plant material, and yet we must start at the beginning in Mesoamerica before arriving at the present situation, nonetheless.

The current understanding of the avocado species complex is based on a tripartite taxonomy from the Nahuatl as recorded in the Florentine Codex(De Sahagun, 1577). This understanding sorts the complex into three distinct subspecies, roughly endemic to the Mexican central highlands (the *Persea americana* var. *drymifolia* ecotype), the Guatemalan highlands (the *Persea americana* var. *guatemalensis* ecotype), and the more lowland tropical regions (the *Persea americanca* var. *americana* ecotype) surrounding these high altitude regions(Bergh and Ellstrand, 1986; Popenoe, 1915; Storey et al., 1986). When considering the adaptability of these populations to the Southern California coastal climate, late 19th Century and early 20th Century plant collectors and horticulturalists focused on the Mexican and Guatemalan highlands to discover varieties that could do well 1000's of miles from their origins. This initial focus was on cold tolerance, but immediately upon its heels was a desire for a larger fruit with a thicker peel to allow better storage and shipping of the fruit. This resulted in an immediate focus on the *drymifolia* subspecies for its very high cold tolerance, but this ecotype has the smallest average fruit mass of the three ecotypes along with the thinnest peel. This is contrasted in the fruit characteristics of the *guatemalensis* ecotype, which typically has a very large green skinned fruit with a thick peel and somewhat more moderate cold tolerance than the *drymifolia*(Popenoe, 1917, 1939). The more tropically adapted *americana* ecotype, common in the Caribbean basin, was never very well suited to the general mediterranean type climate stretching from San Diego to Santa Barbara counties, let alone further north and so early California avocado growers did not consider it and in the few instances where it appears that *americana* seed was brought up to California, it seems to have never performed satisfactorily(Popenoe, 1915, 1936).

Of the collection trips to Mexico and Guatemala in the early 1900s, the most successful single location for sourcing material for California's developing industry was Atlixco, Mexico where several important varieties were collected in 1911(Popenoe, 1915, 1920). Of these, the 'Fuerte' was the most successful and this variety became the leading cultivar from the 1920s to the 1970s and it is still a regionally important cultivar in some countries(Coit, 1968; Shepherd and Bender, 2002). Despite its ascendency, problems with the 'Fuerte' were found with respect to alternate bearing, where fruit set could be irregular from year to year, as well as the large, spreading canopy of the mature tree and nurserymen, hobbyists, and researchers were always on the lookout for an improved variety of avocado.

Although it may not have initially fit the full definition of what the 'Fuerte' era California avocado industry was looking for, this improvement came about in the form of a seed planted in the yard of Rudolph Hass, a postal employee living in La Habra Heights, California(Griswold, 1945).

At the time of its accidental inception, the 'Hass' avocado represented the combined focus on *drymifolia* and *guatemalensis* traits in establishing a California subpopulation: adaptation to climate from *drymifolia*, and the larger fruit size and thicker peel of *guatemalensis*. These two different genetic backgrounds also had effects on the fruit maturation profile of California avocados with fruit such as 'Fuerte' being identified as a winter fruit or as a summer fruit for all of those fruit maturing after 'Fuerte' season. A *drymifolia* type fruit typically matures on the tree within a few months, becoming ready to eat in California by late summer or fall. In contrast, a California-grown *guatemalensis* fruit may need to remain on the tree for over a year, often maturing 400 to 600 days after flowering (DAF), well into the summer or even fall/winter of the following year. The 'Hass' avocado demonstrates an intermediary maturation timeline as it can be ready to harvest within a year of flowering but will also hang on the tree well into the summer under some environmental conditions. It is also important to note that 'Hass' retains a mix of other ancestral traits such as a dark-skinned ripening fruit typical of *drymifolia* with a thicker rougher *guatemalensis* type peel.

In the roughly century since the planting of the original 'Hass' seed, this variety has gone from being an interesting fruit with a potential fatal flaw of ripening dark (as enthusiasts and industry interests of the time preferred a green skinned fruit)(Griswold, 1945) to a global commodity crop that sets a standard for what an "ideal" avocado is for the majority of the global population. From the discovery of the seedling in the 1920s, and patent filing in 1935(Hass, 1935), it took roughly another 35 years for the ‘Hass’ to supersede the ‘Fuerte’ variety as the primary commodity avocado in the California industry, and from there it took over the global market as well. Today this variety accounts for well over 85% of global trade in avocado fruit and more than 95% of the US market(Ayala Silva and Ledesma, 2014; Cavaletto, 2015; Naamani, 2007).

History of Maturity Metrics

Before considering the history of avocado maturity metrics, one must understand an important fact about the physiology of the fruit: although the avocado is a climacteric fruit, meaning that it continues to ripen after it is harvested, an added complication is that while the fruit is still attached to the tree, the ripening process is arrested although the fruit will continue to mature. In practice, this means that fruit, no matter the degree of maturity, will not soften on the tree and so are picked hard regardless of any measurable level of maturity(Lewis, 1978). After harvest, if left under normal environmental conditions, the fruit will gradually soften over a period of time until it is judged of acceptable eating quality. The post-harvest ripening process, however, can be interrupted with refrigeration and controlled atmospheric conditions that, depending on the maturity level of the fruit, can inhibit softening for a period of weeks.

Following the widespread introduction and cultivation of the avocado in California in the first decades of the 20th century, a nascent industry began to form and the question of when to harvest and market the fruit of these first available varieties became an area of active research. The first serious attempt to quantify the traits and composition of avocado fruit in California was undertaken by Church and Chace in a 1922 USDA bulletin where they evaluated fruit from trees throughout Southern California. The investigators noted that “no large planting is old enough yet to tell what the trees will do at an advanced age under orchard conditions” yet they were able to source fruit from eight trees in eight locations and they recorded such general fruit characteristics as specific gravity, weight, and ratios of seed to skin to "edible portion". From the "edible portion" or mesocarp, they measured the following chemical composition characteristics: moisture content, ash, fat, sugar, nitrogen, and crude fiber. This early study did not find enough evidence to recommend a metric as they simply did not have enough data in the form of actual fruit to measure at this early date, but they were able to state that mature avocados "rich in fat usually contain at least 70 per cent on a water free basis", but that there were also avocados that appeared to be low in fat at perceived maturity where this "rule does not hold."(Church and Chace, 1922).

Despite their lack of recommendation for any of the measurements as a maturity metric, the only figure in USDA Bulletin 1073 was that of fat content (as a percentage of fresh weight of the mesocarp)(Church and Chace, 1922) and later industry professionals and researchers quickly realized that fat or oil was a major component of non-water portion of the fruit and so began attempts to quantify it for use as a metric. The first meeting of the California Avocado Society to discuss the standardization of the fruit being sold in market occurred in May 1923(Coit, 1923) and by 1925, avocado fruit was added to the California Fruit and Vegetable Standardization Act with avocado mesocarp requiring a minimum oil content of 8% in marketed fruit (Wolff, 1926). Although this was great step forward, the process of testing required Soxhlet extraction in petroleum ether and took "two to three days" to complete(Wolff, 1926), and a faster method of oil extraction was quickly developed that involved refractometry and was referred to as "the Halowax method"(Hodgkin, 1939, 1928; Lesley and Christie, 1929; Porter, 1947; Shannon, 1949).

The current dry weight method of indexing avocado maturity is based on the ratio of water to non-water mass in the fruit's mesocarp. Aside from dry weight, it is also known as "dry matter" or "dry matter percentage" and is expressed as a percentage(Lee et al., 1983; Lee and Coggins, Jr., 1982). The current minimum maturity standard for fruit from the 'Hass' cultivar is 20.8%(Anonymous, 2011), but this percentage will continue to rise throughout the winter, spring and summer and can exceed 30% by the end of the season. The initial methodology for dry weight sampling involved cutting the fruit longitudinally into halves and then cutting two "opposing eighth" sections from the half pieces. These pieces were then combined into a pooled sample of other fruit and ground together. They were then subsampled and weighed before and after drying in a microwave oven(Anonymous, 1983). Initially, a conventional oven was used as an alternative to a microwave oven(Morris and O'Brien, 1980), but cost, ease of use, and speed have favored the microwave oven in the intervening decades. The current method in California for measuring dry weight is still destructive and results in extracting a core from the fruit's mesocarp with either a cork borer or a coring machine (specially designed for this purpose)(Anonymous, 2002; Arpaia et al., 2001) that allows for the remaining fruit to be carefully ripened and evaluated as a soft fruit, if so desired. Recently, a food desiccator has become available as an alternative to a microwave oven in research settings where an immediate value is not needed.

Dry weight is an extremely useful tool to the fruit handler or grower, as it is a metric that can be measured with commonly available tools (oven and scale), however it is an imprecise method when extended across different growing regions and additionally needs to be adjusted for different varieties.

Despite the imprecision and need for calibration of the dry weight metric to new varieties and growing regions, it is a vast improvement on the previous metric, oil content, in its ease of application. This earlier method required greater chemistry knowledge as well as more strict lab safety controls due to some of the dangerous chemicals needed to efficiently separate the fats from the rest of the mesocarp(Lee, 1981a, 1981b). This oil content analysis also required the creation of separate oil percent standards for different varieties, and so, in that way was not substantially different from dry weight in its need for calibration to new cultivars. Its reliability across different ranges and environments remains unstudied as the expense and effort involved in its implementation limited its application over the decades that it was in use. In contrast, since the introduction of Lee's 1981 dry weight methodology, avocado researchers and industry interests around the world have been collecting and utilizing dry weight as a way of understanding fruit maturation under their local conditions and also of their local or non-standard cultivars(Carvalho et al., 2014; Pak and Dawes, 2002; Ranney, 1991; Rodriguez et al., 2018; Salameh et al., 2022). Dry weight has become a global standard for avocado maturity metrics and is used much more broadly than the previous oil percentage methodology.

Although there is a broader ability to sample more fruits with the dry weight methodology than the old oil percentage technique, the UCR breeding program's post 1999 historic data set for this metric in 'Hass' has a strong bias towards fruit at the end of the season, or at the very least, a focus on fruit that is very close to or exceeding the 'Hass' 20.8% minimum maturity index for dry weight, even at Irvine, California, where a majority of the measurements were made (Fig.1.1). Part of the reason behind this was that in this data set, 'Hass' was being used as a standard to compare to other potential cultivars and the majority of these cultivars did not reach acceptable eating quality until the late fall or winter, when Hass was close to its minimum maturity index. A better understanding of the 'Hass' fruit physiology from well before this threshold would be a good step towards elucidating the difference between fruit that is of acceptable eating quality and those which are not.

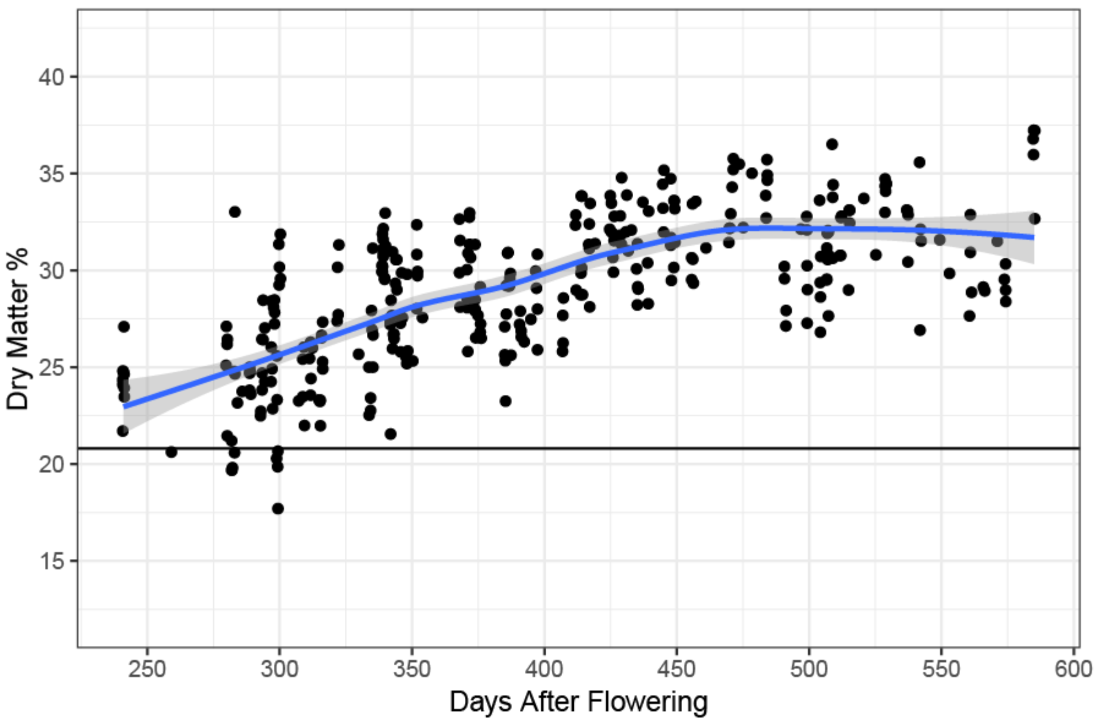

Fig. 1.1 Historic Hass Data from UCR Breeding Program Files
Dry matter data from 1999-2014 at SCREC site in Irvine, California. Days after flowering uses an April 1st date as day zero as that is typically peak flowering at this site, horizontal line at 20.8 represents California minimum maturity value for Hass avocado fruit, N=333.

Environmental Conditions Effect Dry Weights and Other Metrics

Aside from maturity metrics, avocado exhibits considerable plasiticity in its response to environmental factors and the same avocado cultivar can have very different seasonality and length of time to reach maturity under different environments(Donetti and Terry, 2014). In particular, it is well documented that in warmer climates, many different avocado cultivars reach an acceptable eating quality (and a minimum dry weight index) earlier than in cooler climates. A different ratio of fatty acids is also found in the same cultivar when it is grown in different climates(Donetti and Terry, 2014): higher levels of saturated fatty acids and poly unsaturated fatty acids and lower levels of monounsaturated fatty acids are found in warmer climate fruit than in those same fruit grown in cooler climates(Kaiser and Wolstenholme, 1994).

Avocado Somatic Mutations and Their Increasing Prevalence as New Cultivars

As the avocado became increasingly common globally, farmers and researchers began to notice differences between apparently clonally derived populations. The earliest incidences of these were with the 'Fuerte' variety in California, but the technology of the time did not allow for genomic sequencing to assess potential differences between different 'Fuerte' populations(Anonymous, 1939; Hodgson, 1945). Additionally, the lack of a clonal rootstock propagation system made separating environmental effects from scion genetic effects much more difficult and time consuming. In the modern 'Hass' era, however, there is a better ability to assess differences between supposedly identical field trees. This has led to the discovery of several putative somaclonal 'Hass' varieties such as the 'Mendez No. 1', 'Eugenin', and 'Flavia' in addition to others yet to be released(Eugenin, 2014; Mendez Vega, 2000; Schiappacasse Macchiavello, 2014). As these new cultivars are generally discovered by growers in their fields, they are noticed because of specific favorable and obvious traits. In the case of 'Mendez No. 1', this is a different canopy structure and a higher propensity for out of season flowering. For the current study, the 'Eugenin' and 'Flavia' are notable for having a noticeably larger fruit as well as reaching a higher dry weight metric earlier than the standard 'Hass' variety. This presents an excellent opportunity to study differing avocado fruit maturation rates with similar genetics; and with multiple field sites, it also allows one to consider the effect of environmental factors in these reported differences.

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Environmental and Agronomic Field Conditions at the Study Sites

Introduction

The avocado breeding program at the University of California, Riverside is structured as a three tier system(Arpaia, 2012, 2009, 2007, 2000). The initial planting of seeds from promising parents, referred to as “Tier 1”, has occurred at a single location for ease of oversight and evaluation since the program was revamped and restarted in 1999. Promising selections from these thousands of seedlings are moved into “Tier 2” where they are grafted (clonally propagated) onto multiple trees and further evaluated for other agronomic traits before moving into the final tier, “Tier 3”. In “Tier 3” where the elite selections are planted out in different environments to evaluate how these trees will respond to those different environmental conditions, and it is also in these “Tier 3” trials where ‘Hass’ and other commercial varieties are planted as a control to compare adaptability and agronomic traits. It is also in this “Tier 3” phase where evaluation of non-breeding program varieties would be considered and two of these selections were the ‘Eugenin’ and ‘Flavia’ from Chile(Arpaia, 2008; Eugenin, 2014; Schiappacasse Macchiavello, 2014) which are part of this current study.

These “Tier 3” field plantings are carried out on the same rootstock for consistency of results; in the case of the years 2011 through 2015, this rootstock was ‘Merensky 2’, a disease resistant rootstock from South Africa commonly trademarked as “Dusa” in the industry(Kohne, 2004). Originally there were four total “Tier 3” planting locations, but two were lost to either fire or management decision, and the remaining two serve as the basis for the current study of avocado trees under different environmental conditions.

The two locations in this study are both in California but represent two very different environments separated by roughly 320km with the South Coast Research and Extension Center

(SCREC) field site (33°41'40.8"N 117°43'11.8"W) in Irvine, California, representing what is typically considered common historic Southern California growing conditions for avocado. The location (36°21'17.8"N 119°03'27.5"W) in the Central Valley at Exeter, Lindcove Research and Extension Center (LREC), is a much more extreme environment yet represents a region where the early avocado industry did plant acreage and trialed more cold hardy varieties. Central Valley farmers' attention shifted towards other crops after those initial plantings, but they have shown increased interest in growing avocado as a commercial crop more recently. Both sites are field stations in the University of California Agricultural and Natural Resources (UCANR) system, but this does not mean that both sites are standard in all aspects of agronomy, with the staff and resources in both locations relying heavily on historic practices and regional expertise and land use knowledge. Additionally, each of the field stations has an onsite weather station that is part of the California Irrigation Management System (CIMIS), enabling the environmental conditions at the two sites to be assessed with consistent parameters("CIMIS," 1982). Trees were planted at both sites from 2011 through 2015 with an eye to minimizing differences between them in field design and planting.

Despite attempts to minimize variation in trees between the sites, varying success in nursery propagation necessitated planting cohorts with different cultivar compositions at the two sites in some years (Table 2.1). All trees were commercially sourced through Brokaw Nursery LLC and all varieties propagated on 'Merensky 2' rootstock. For the current studies, the focus will be on the previously mentioned cultivars, 'Hass', 'Flavia', and 'Eugenin', but the Tier 3 field plantings at both sites also contain other varieties, both commercial and experimental (Fig. 2.1a, Fig. 2.1b). In addition to some small differences in field variety composition, differing mortalities and field conditions affecting tree health are noticeable at each of these locations and those are noted in this report as relevant.

An issue specific to SCREC is the use of reclaimed water beginning in roughly 2012-2013, initial testing of the water yielded an average of ~100ppm chlorides with peaks close to 300ppm, whereas over the same period these measurements at LREC seldom exceeded 10ppm. This high chloride content caused issues in the SCREC field when combined with a high pH (8.2) calcareous soil(USDA NRCS, 2002) and the Dusa rootstock used proved to be especially sensitive to these conditions, and often trees showed signs of iron chlorosis. There were positional field effects noted, with the Southwest and especially South sides of the site more impacted. A regimen of annual iron chelate spring fertigation was initiated after outside consultation in 2016, and this has proved to be a successful remedy.

Negative field soil conditions at LREC, related to water logging, occur primarily at the bottom portion of the field, downslope along the western edges of the field. During years of heavy rain, the station has occasionally trenched the bottom of this field and used gasoline engine pumps to remove excess water into the station's canal. Prolonged waterlogging has impacted this portion of the field. This area has suffered the highest mortality of trees in either location, particularly in the Northwestern corner with many trees planted in 2012 and 2013, these are the years where 'Eugenin' and 'Flavia' were planted in this field, and lower productivity in these plantings may be attributed to these conditions. Additionally, surviving trees in this section of the field tend to be smaller and have lower productivity than those in other parts of the field.

Table 2.1 Number of Trees Planted and Years Planted at Both Sites

		Number of trees										
		SCREC						LREC				
Measurement		Planting year						Planting year				
Year	Cultivar	2011	2012	2013	2014	2015	Cultivar	2011	2012	2013	2014	2015
2011	Eugenin	15					Eugenin					
	Flavia	15					Flavia					
	Hass	15					Hass	15				
2012	Eugenin	15	15				Eugenin		15			
	Flavia	15					Flavia					
	Hass	15	3				Hass	15	3			
2013	Eugenin	15	15				Eugenin		15			
	Flavia	15		15			Flavia			15		
	Hass	15	3	3			Hass	15	3	3		
2014	Eugenin	15	14				Eugenin		14			
	Flavia	15		15			Flavia			14		
	Hass	15	3	3	15		Hass	15	2	3	15	
2015	Eugenin	15	14				Eugenin		14			
	Flavia	15		15			Flavia			14		
	Hass	15	3	3	15	15	Hass	15	2	3	15	3
2016	Eugenin	15	14				Eugenin		14			
	Flavia	15		15			Flavia			14		
	Hass	15	3	3	14	15	Hass	15	2	3	15	3
2017	Eugenin	15	14				Eugenin		14			
	Flavia	15		15			Flavia			14		
	Hass	15	3	3	14	15	Hass	15	2	3	15	3
2018	Eugenin	15	14				Eugenin		14			
	Flavia	15		15			Flavia			11		
	Hass	15	3	3	14	15	Hass	15	2	3	15	3
2019	Eugenin	15	14				Eugenin		14			
	Flavia	15		15			Flavia			11		
	Hass	15	3	3	14	15	Hass	15	2	3	15	3
2020	Eugenin	15	14				Eugenin		14			
	Flavia	15		15			Flavia			11		
	Hass	15	3	3	14	15	Hass	15	2	3	15	3
2021	Eugenin	15	14				Eugenin		14			
	Flavia	15		15			Flavia			11		
	Hass	15	3	3	14	15	Hass	15	2	3	15	3
2022	Eugenin	15	14				Eugenin		14			
	Flavia	15		15			Flavia			11		
	Hass	15	3	3	13	15	Hass	15	2	3	15	3
2023	Eugenin	15	14				Eugenin		14			
	Flavia	15		15			Flavia			11		
	Hass	15	3	3	13	14	Hass	15	2	3	15	3

The experimental design for testing new varieties was to plant 5 replicates of 3 trees each in randomized complete blocks; however Hass was considered as the control and to save space, in intermediate years, was only planted as a single replicate of 3 trees. Material for Eugenin and Flavia was hard to source in the first 3 years of this trial and only enough trees for one full planting at SCREC were available in 2011. Follow up plantings in 2012 and 2013 introduced Eugenin and Flavia trees to both sites as available.

Fig.2.1a South Coast Research and Extension Center (SCREC) Field Map

2015 planting (columns 19–15) · 2014 planting (columns 14–12) · 2013 planting (columns 11–8) · 2011 planting · 2012 planting

Northeast ←

	19	18	17	16	15	14	13	12	11	10	9	8	7	6	5	4	3	2	1	
1	UCR V	Hass	non UCR V	M Hass	UCR V	UCR V	Hass	Mendez No. 1	Flavia	dead	Flavia	XX3	"3-29-5	Eugenin	UCR V	UCR V	Flavia	Eugenin	Hass	1
2	UCR V	Hass	non UCR V	M Hass	UCR V	UCR V	Hass	Mendez No. 1	Flavia	XX3	Flavia	XX3	"3-29-5	Eugenin	UCR V	UCR V	Flavia	Eugenin	Hass	2
3	UCR V	Hass	non UCR V	M Hass	UCR V	UCR V	Hass	Mendez No. 1	Flavia	XX3	Flavia	XX3	"3-29-5	Eugenin	UCR V	UCR V	Flavia	Eugenin	Hass	3
4	Hass	non UCR V	RV Hass	UCR V	Hass	Hass	Mendez No. 1	non UCR V	UCR V	BL516	dead	Hass	UCR V	"3-29-5	UCR V	Flavia	Eugenin	Hass	UCR V	4
5	Hass	non UCR V	RV Hass	UCR V	Hass	Hass	Mendez No. 1	non UCR V	UCR V	dead	BL516	Hass	UCR V	"3-29-5	UCR V	Flavia	Eugenin	Hass	UCR V	5
6	Hass	non UCR V	RV Hass	UCR V	Hass	Hass	Mendez No. 1	non UCR V	UCR V	BL516	BL516	Hass	UCR V	"3-29-5	UCR V	Flavia	Eugenin	Hass	UCR V	6
7	M Hass	UCR V	UCR V	non UCR V	M Hass	Mendez No. 1	non UCR V	UCR V	BL516	Flavia	UCR V	BL516	deac	UCR V	Flavia	Eugenin	Hass	UCR V	UCR V	7
8	M Hass	UCR V	UCR V	non UCR V	M Hass	Mendez No. 1	non UCR V	UCR V	BL516	Flavia	UCR V	BL516	Eugenin	UCR V	Flavia	Eugenin	Hass	UCR V	UCR V	8
9	M Hass	UCR V	UCR V	non UCR V	M Hass	dead	non UCR V	UCR V	BL516	Flavia	UCR V	BL516	Eugenin	UCR V	Flavia	Eugenin	Hass	UCR V	UCR V	9
10	non UCR V	dead	Hass	RV Hass	UCR V	non UCR V	UCR V	Hass	XX3	UCR V	XX3	UCR V	UCR V	"3-29-5	Eugenin	Hass	UCR V	UCR V	Flavia	10
11	non UCR V	M Hass	Hass	RV Hass	UCR V	non UCR V	UCR V	dead	XX3	UCR V	XX3	UCR V	UCR V	"3-29-5	Eugenin	Hass	UCR V	UCR V	Flavia	11
12	dead	M Hass	Hass	RV Hass	UCR V	non UCR V	UCR V	Hass	XX3	UCR V	XX3	UCR V	UCR V	"3-29-5	Eugenin	Hass	UCR V	UCR V	Flavia	12
13	X	dead	M Hass	UCR V	non UCR V	Hass	dead	Mendez No. 1	non UCR V	Flavia	BL516	Flavia	Eugenin	UCR V	Hass	UCR V	UCR V	Flavia	Eugenin	13
14	X	UCR V	M Hass	UCR V	non UCR V	Hass	UCR V	Mendez No. 1	non UCR V	Flavia	BL516	Flavia	Eugenin	UCR V	Hass	UCR V	UCR V	Flavia	Eugenin	14
15	X	UCR V	dead	UCR V	non UCR V	Hass	UCR V	Mendez No. 1	non UCR V	Flavia	BL516	Flavia	Eugenin	UCR V	Hass	UCR V	UCR V	Flavia	Eugenin	15
16	X	X	dead	Hass	RV Hass	Mendez No. 1	dead	Hass	UCR V	UCR V	XX3	X	"3-29-5	Eugenin	UCR V	Eugenin	Hass	"3-29-5	X	16
17	X	X	dead	Hass	RV Hass	Mendez No. 1	non UCR V	Hass	UCR V	UCR V	dead	X	"3-29-5	Eugenin	UCR V	Eugenin	Hass	"3-29-5	X	17
18	X	X	UCR V	Hass	RV Hass	Mendez No. 1	non UCR V	Hass	UCR V	UCR V	XX3	X	deac	Eugenin	UCR V	Eugenin	Hass	"3-29-5	X	18
		18	17	16	15	14	13	12	11	10	9	8	7	6	5	4	3	2	1	

Spacing : 20' x 15'

Imagery ©2024 Airbus, Maxar Technologies, Map data ©2024 Google 50 ft

Fig.2.1b Lindcove Research and Extension Center (LREC) Field Map

North ←

2011 planting (columns 1–3); 2012 planting (columns 4–5); 2013 planting (columns 6–7); 2014 planting (columns 8–10); 2015 planting (columns 11–12)

	1	2	3	4	5	6	7	8	9	10	11	12
1	Hass	UCR V	UCR V	dead	"3-29-5	dead	BL516	Hass	UCR V	Hass	dead	UCR V
2	Hass	UCR V	UCR V	Eugenin	"3-29-5	Hass	BL516	Hass	UCR V	Hass	UCR V	UCR V
3	Hass	UCR V	UCR V	Eugenin	"3-29-5	Hass	BL516	Hass	UCR V	Hass	dead	UCR V
4	UCR V	Hass	UCR V	"3-29-5	UCR V	Hass	Flavia	non UCR V	Hass	Mendez No. 1	dead	non UCR V
5	UCR V	Hass	UCR V	"3-29-5	dead	ZUTANO	dead	non UCR V	Hass	Mendez No. 1	UCR V	non UCR V
6	UCR V	Hass	UCR V	"3-29-5	UCR V	UCR V	Flavia	non UCR V	Hass	Mendez No. 1	UCR V	non UCR V
7	UCR V	UCR V	Hass	UCR V	Eugenin	UCR V	UCR V	UCR V	Mendez No. 1	non UCR V	non UCR V	UCR V
8	UCR V	UCR V	Hass	UCR V	Eugenin	UCR V	UCR V	UCR V	Mendez No. 1	non UCR V	non UCR V	dead
9	UCR V	UCR V	Hass	UCR V	Eugenin	ZUTANO	UCR V	UCR V	Mendez No. 1	non UCR V	non UCR V	dead
10	UCR V	Hass	"3-29-5	UCR V	XX3	Flavia	XX3	Mendez No. 1	non UCR V	UCR V	Hass	X
11	UCR V	Hass	"3-29-5	UCR V	XX3	Flavia	XX3	Mendez No. 1	non UCR V	dead	Hass	X
12	dead	Hass	"3-29-5	UCR V	dead	Flavia	XX3	Mendez No. 1	non UCR V	dead	Hass	X
13	Hass	UCR V	UCR V	"3-29-5	BL516	ZUTANO	BL516	Hass	Mendez No. 1	UCR V	dead	X
14	Hass	UCR V	UCR V	"3-29-5	BL516	XX3	BL516	Hass	Mendez No. 1	UCR V	UCR V	X
15	Hass	UCR V	UCR V	"3-29-5	BL516	XX3	BL516	Hass	Mendez No. 1	UCR V	UCR V	X
16	UCR V	UCR V	Eugenin	Eugenin	Flavia	XX3	XX3	non UCR V	UCR V	non UCR V	UCR V	X
17	UCR V	UCR V	Eugenin	dead	Flavia	ZUTANO	XX3	dead	UCR V	non UCR V	UCR V	X
18	ZUTANO	UCR V	Eugenin	Eugenin	Flavia	BL516	XX3	non UCR V	UCR V	non UCR V	UCR V	X
19	"3-29-5	Eugenin	Hass	dead	UCR V	BL516	Flavia	Mendez No. 1	Hass	UCR V	non UCR V	X
20	"3-29-5	Eugenin	dead	UCR V	UCR V	BL516	Flavia	Mendez No. 1	Hass	UCR V	non UCR V	X
21	"3-29-5	Eugenin	Hass	dead	dead	ZUTANO	Flavia	Mendez No. 1	Hass	UCR V	non UCR V	X
22	BL516	XX3	UCR V	dead	X	X	UCR V	UCR V	non UCR V	non UCR V	X	X
23	BL516	XX3	dead	dead	X	X	UCR V	UCR V	non UCR V	non UCR V	X	X
24	BL516	XX3	dead	dead	X	X	dead	dead	non UCR V	non UCR V	X	X
	1	2	3	4	5	6	7	8	9	10	11	12

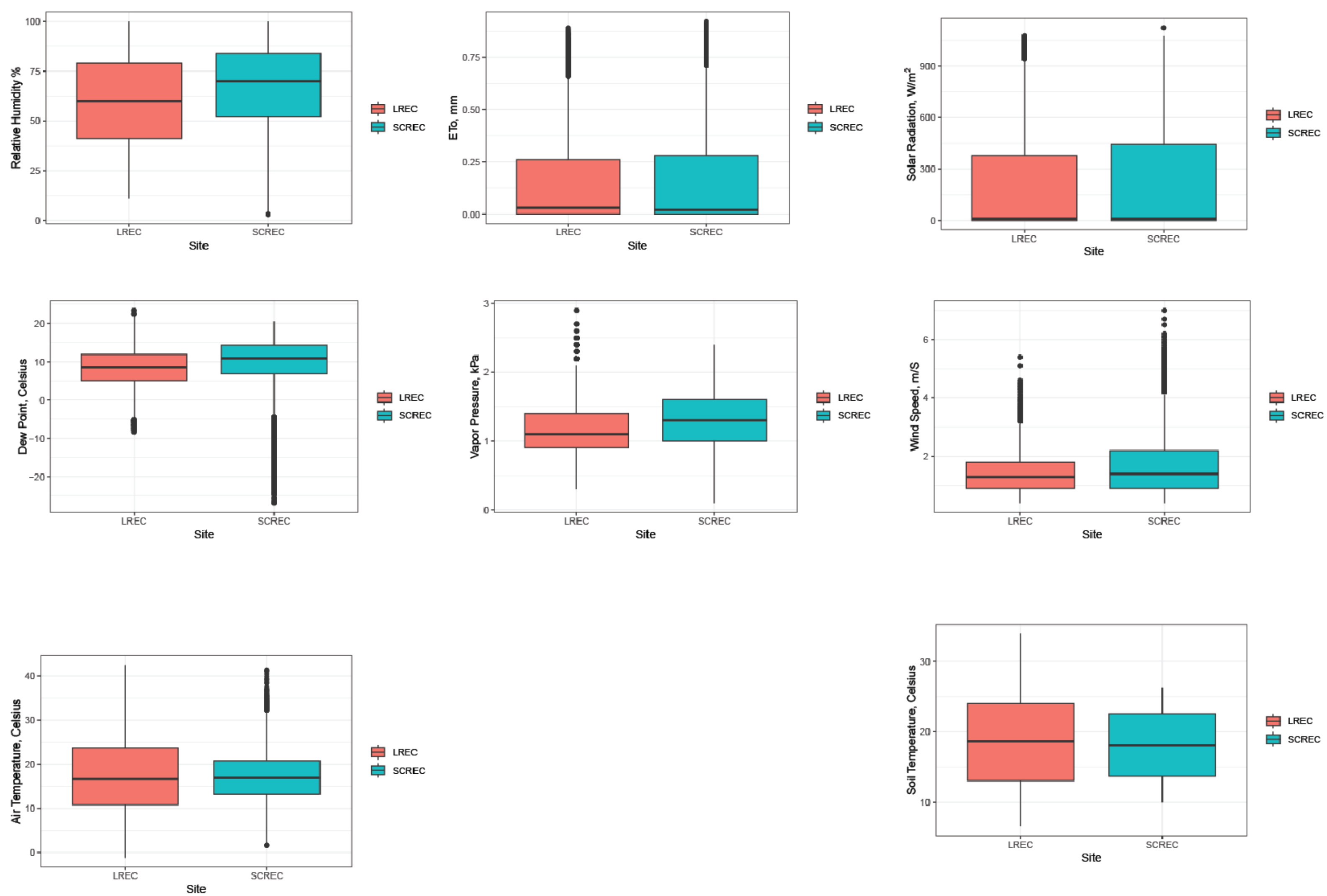
Spacing : 18' x 16'

Imagery ©2024 Airbus, Maxar Technologies, Map data ©2024 50 ft

Climate and Weather: California Irrigation Management Information System (CIMIS) Data

The climate conditions between LREC (USDA Zone 9B) and SCREC (USDA Zone 10A) are quite different(“USDA Plant Hardiness Zone Map,” 2023). The USDA Plant Hardiness Zone Map focuses on average lowest winter temperatures, defining Zone 10A as -1.1°C to -1.7°C and Zone 9B as -1.3°C to -3.9°C. The Köppen–Geiger classification method provides another way to understand the climate of the two sites(Geiger, 1961, 1954). Under this system, LREC is classified as a “Hot-Summer Mediterranean Climate” and SCREC as a “Cold Semi-Arid Climate”. Key differences between the sites include more extreme temperatures at LREC, with hotter summers and colder winters, compared to SCREC (Fig. 2.2). At SCREC, the interplay between coastal and inland climates occasionally leads to extreme weather events like Santa Ana winds (hot, dry winds moving coastally across Southern California) (“Santa Ana Winds - Wildfires,” 2011, “Santa Anas,” 2015) (Fig. 2.2). These sudden weather events in SCREC are best seen in the plots for dewpoint, and windspeed where the outlying data points deviate significantly from the general seasonal trends (Fig.2.3). Another Southern California-specific climate condition is the low ETo outliers in late spring and early summer, colloquially known as “May Gray” and “June Gloom,” representing cool, foggy mornings(“California May Grey / June Gloom,” 2010). These are visible in the low ETo values for May and June in SCREC versus LREC scatterplots (Fig. 2.3). Among these climatic variations at SCREC, the dry Santa Ana winds are particularly stressful to avocado trees. Plantings without wind protection can suffer significant leaf and fruit loss on the northeast-facing top of the field, which bears the brunt of these winds.

Fig. 2.2 CIMIS Station Measurement Comparisons at Both Sites, 2019-2021



An important weather variable is the daily cumulative precipitation totals (Fig. 2.3f). This plot shows that both sites generally have a Mediterranean climate, with most precipitation occurring in late fall or winter. As these are irrigated fields, management uses CIMIS data to calculate weekly irrigation volumes and rates. Specifically, ETo is applied in the following formula to determine the water volume to add to the field:

$$ETc = {}^{ETo \times Kc}/_{DU}$$

Here, ETo is the evapotranspiration measured by CIMIS, Kc is the crop coefficient (0.86 for avocado), and DU represents distribution uniformity, a measure of how evenly the soil and terrain disperse the applied irrigation water. Using these measurements and the irrigation system's flow rates per area, a field manager can apply the precise amount of water to the trees daily, weekly, or biweekly, depending on the management regime (Bender, 2013; “Irrigation Calculator,” 2007). Cumulative precipitation during the irrigation period can be subtracted from the ETc if more than 6.35 mm of rain occurs. This adjustment primarily affects field conditions from an economic perspective for the grower or manager.

Beyond economic or management concerns, cumulative precipitation mainly affects yield, fruit quality, or tree vigor by improving soil in the root zone through leaching accumulated salts from the previous spring and summer's irrigation. This issue is more pronounced at SCREC, where reclaimed water usage results in higher irrigation water salinity compared to LREC.

Fig. 2.3a

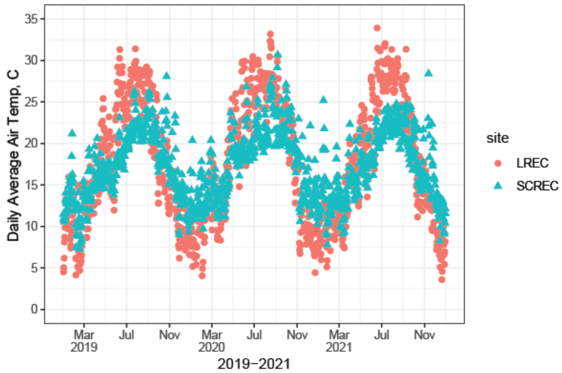


Fig. 2.3b

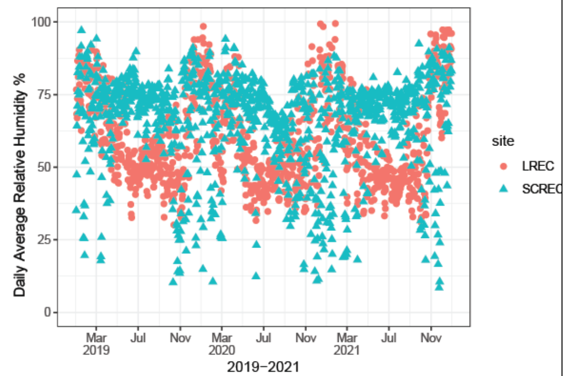


Fig. 2.3c

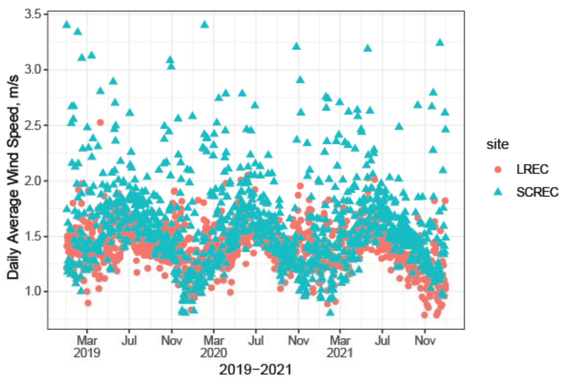


Fig. 2.3d

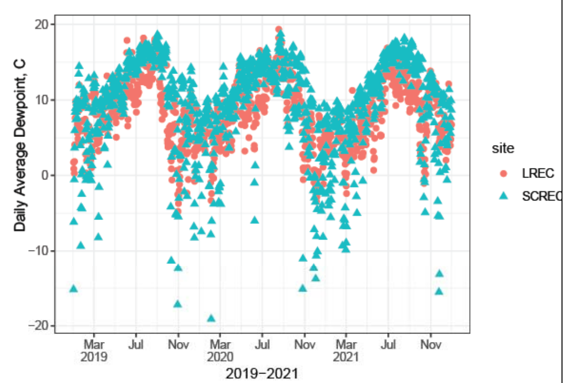


Fig. 2.3e

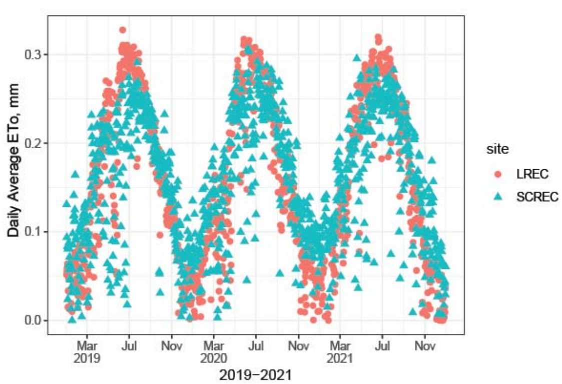


Fig. 2.3f

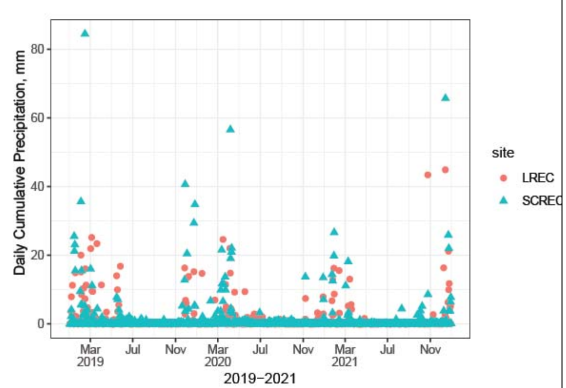


Fig. 2.3a-f Selected CIMIS Seasonal Trends at Both Sites

CIMIS weather station data at two sites, Lindcove Research and Extension Center (LREC) and South Coast Research and Extension Center (SCREC) for period January 1, 2019 through December 31, 2021. Fig. 2.3a through Fig. 2.3e represent weather data averaged by day, Fig. 2.3f represents daily cumulative precipitation.

Considering the sporadic deviations at the SCREC site and the offsetting effect of irrigation on precipitation differences at the two locations, it becomes apparent that the most challenging and stressful conditions for developing avocado fruit occur in the summer and winter months at LREC. In these periods at the Exeter location, temperatures more regularly extend beyond the ranges proposed in literature as the ideal growing temperature range for avocado(Lahav and Trochoulias, 1982; Liu et al., 2002, 1999; Mandemaker, 2008). Although the differences in daily air temperature averages shown in Fig. 2.3a may not be immediately apparent, they become more obvious when examining the daily variance and swings from daytime to nighttime conditions (Fig. 2.4a-c). In these plots, LREC experiences a broader range of temperatures daily, with summer days often exceeding 30°C, a threshold where avocado leaves typically reduce or cease photosynthetic activity. Conversely, in Fig. 2.4c the nighttime temperatures in the summer seem to be closer to the ideal photosynthetic temperature of 27°C at LREC than at SCREC. The specific effects of nighttime temperatures on carbon assimilation in avocados are not well understood. In fact, avocados exhibit unique responses to light as compared to many other agronomic crops(Cran and Possingham, 1973; Scholefield et al., 1980). Observations have noted a rise in turgor pressure in the tree trunk overnight(Carr, 2013; Winer et al., 2007), which have implications for overall tree health and fruit development. However, research has primarily focused on leaf-level carbon assimilation and photosynthetic efficiency, leaving a gap in understanding how these processes affect fruit development under varying nighttime temperatures(Heath et al., 2006, 2005).

Fig. 2.4a

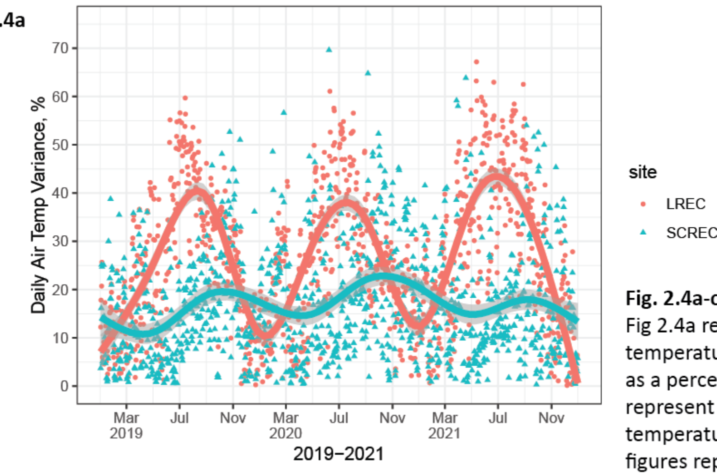


Fig. 2.4a-c Air Temperature Trends at Both Sites
Fig 2.4a represents the daily variance in air temperature at the two sites and is expressed as a percentage, whereas Fig 2.4b and 2.4c represent the averaged daytime and nighttime temperatures in Celsius, the trend lines in all figures represent seasonal and annual trends through a local regression line color coded for site to match data points.

Fig. 2.4b

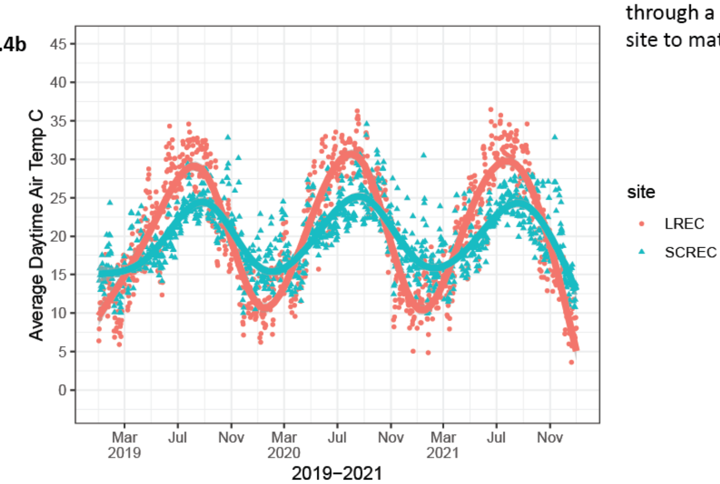
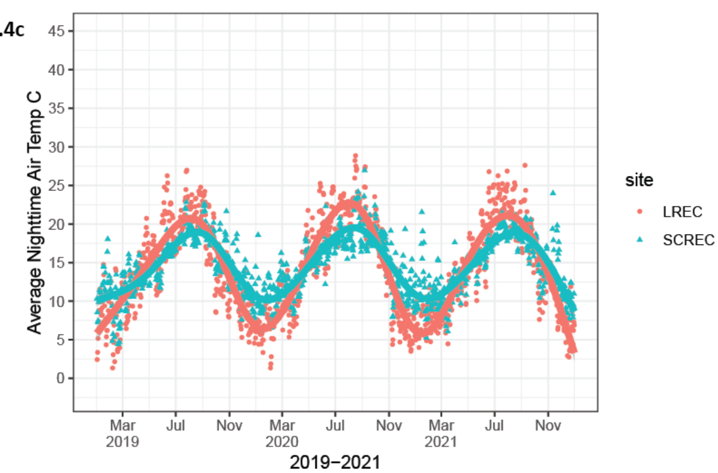


Fig. 2.4c



Canopy Size, Yield Characteristics, Fruit Size

Canopy measurements were undertaken annually in the fall or winter after the previous year's growth was complete. A laser range finder was used to measure length and width of each tree, while a telescoping height pole was used to measure height of the tree. The canopy volume was calculated from these measurements as the area of a cone. The two cultivars, 'Eugenin' and 'Flavia' are not previously reported to have noticeable differences in canopy size or tree architecture between themselves and the standard 'Hass' cultivar(Eugenin, 2014; Schiappacasse Macchiavello, 2014) and our current trials support these findings. At the SCREC and LREC field sites, no statistically significant differences for canopy volume or rate of canopy growth were found between cultivars using the Mann-Whitney U test or Kruskal-Wallis test (Table 2.2). Significant differences between sites were not found with the Mann-Whitney U test except in the case of 2011 planted 'Hass' ($U = 8$, $p = 8.639e-07$), 2012 planted 'Eugenin' ($U = 12$, $p = 8.528e-05$) and 2013 planted 'Flavia' ($U = 5$, $p = 4.918e-06$).

Table 2.2 Average Annual Growth and Canopy Volume, Both Sites

LREC	Cultivar	average annual % increase	9 year average canopy volume (m3)
2011-2019	Eugenin	NA	NA
	Flavia	NA	NA
	Hass	135.3	31.5±9.7
	Cohort	85.8	23.2±8.9
2012-2020	Eugenin	178.9	32.3±5.8
	Flavia	NA	NA
	Hass	206.9	36.5±9.8
	Cohort	142.5	27.7±7.7
2013-2021	Eugenin	NA	NA
	Flavia	63.1	45.5±8.2
	Hass	94.3	44.3±6.3
	Cohort	60.4	28.4±11.6

SCREC	Cultivar	average annual % increase	9 year average canopy volume (m3)
2011-2019	Eugenin	187.1	53.9±18.5
	Flavia	162.4	57.9±27.0
	Hass	157.8	58.6±12.4
	Cohort	131.7	47.2±20.2
2012-2020	Eugenin	379.9	74.3±22.1
	Flavia	NA	NA
	Hass	284.9	55.8±24.3
	Cohort	281.4	59.3±27.9
2013-2020[a]	Eugenin	NA	NA
	Flavia	158.8	87.8±26.7
	Hass	136.1	60.6±32.2
	Cohort	92.7	45.9±31.8

Canopy measurements were conducted beginning the year of planting and following at roughly annual intervals as possible; canopy data for LREC represents measurements of 2011, 2012, 2013, 2014, 2015, 2016, 2018, 2019, 2020, 2021, 2022, canopy data for SCREC represents measurements of 2011, 2012, 2013, 2014, 2015, 2016, and 2020, partial canopy measurements for the field were taken in 2019 representing 2011 and 2012 plantings. [a]2013-2020 data represents an 8 year size average rather than a 9 year average as this was the closest canopy measurement year for that location

The only statistically significant difference between cultivars for a yield value is the Alternate Bearing Index (ABI) between 'Eugenin' and 'Hass' planted in 2012 at SCREC, as indicated by the Mann-Whitney U test ($U = 13$, $p = 0.01856$) (Table 2.3, Fig. 2.6). Otherwise no statistically significant differences were found between cultivars for cumulative yield or ABI at each site with the Mann-Whitney U test (Kruskal-Wallis test for 2011 SCREC cultivars). There were differences in cumulative yield between sites detected by the Mann-Whitney U test for 2011 Hass ($U = 19$, $p = 1.375e-05$), 2014 Hass ($U= 42$, $p = 0.005062$), 2013 Flavia ($U = 25$, $p = 0.0002215$) (Table 2.3, Fig. 2.5). Additionally, statistically significant differences between sites for ABI were detected by Mann-Whitney U test for 2014 Hass ($U = 7$, $p = 1.16e-06$), 2015 Hass ($U = 0$, $p = 0.002451$), 2012 Eugenin ($U = 0$, $p = 0.002451$), and 2013 Flavia ($U = 45$, $p = 0.007903$) (Table 2.3, Fig. 2.6).

Table 2.3 Average Cumulative Yield and Alternate Bearing Index, Both Sites

LREC	Cultivar	Number of trees	Average Cumulative Yield(kg)	Alternate Bearing Index
2011	Hass	15	88.2±65.6	0.70±0.13
2012	Eugenin	14	108.8±44.7	0.69±0.12
	Hass	2	172.2±59.9	0.66±0.01
2013	Flavia	14	100.9±86.6	0.68±0.19
	Hass	3	63.1±32.1	0.62±0.03
2014	Hass	15	96.7±50.4	0.61±0.10
2015	Hass	3	140.7±7.9	0.69±0.07

SCREC		Number of trees	Average Cumulative Yield(kg)	Alternate Bearing Index
2011	Eugenin	15	220.9±53.6	0.72±0.14
	Flavia	15	236.7±42.1	0.80±0.12
	Hass	15	273.9±83.4	0.77±0.10
2012	Eugenin	14	173.6±77.5	0.84±0.07[a]
	Hass	3	215.8±16.6	0.95±0.06[b]
2013	Flavia	15	241.5±89.0	0.86±0.12
	Hass	3	185.1±100.1	0.87±0.09
2014	Hass	14	157.6±47.8	0.91±0.11
2015	Hass	15	147.4±35.0	0.93±0.07

[a]Different letters in same column in a cell represent statisically significant differences. Number of trees, average cumulative yield, and alternate bearing index (ABI) separated by planting year. Statistically significant difference in cumulative yield between Lindcove Research and Extension Center (LREC) and South Coast Research and Extenstion Center (SCREC) sites found for 2011 planted 'Hass' ($U = 19$, $p = 1.375e-05$), 2014 planted 'Hass' ($U = 42$, $p = 0.005062$), and 2013 planted 'Flavia' ($U = 25$, $p = 0.0002215$) with Mann-Whitney U test. Statistically significant difference for ABI between LREC and SCREC sites found for 2012 planted 'Eugenin' ($U = 0$, $p = 0.002451$), 2013 planted 'Flavia' ($U = 45$, $p = 0.007903$), 2014 planted 'Hass' ($U = 7$, $p = 1.16e-06$), and 2015 planted 'Hass' ($U = 0$, $p = 0.002451$) with Mann-Whitney U test.

Fig. 2.5 Cumulative Yield by Cultivar and Planting Year, Both Sites

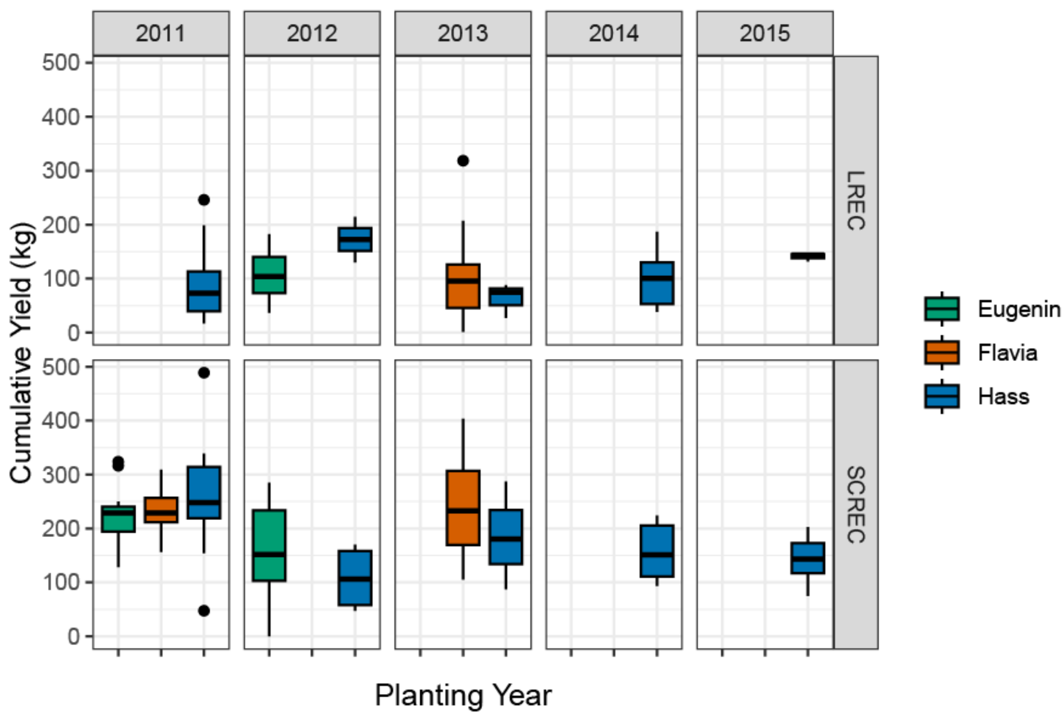
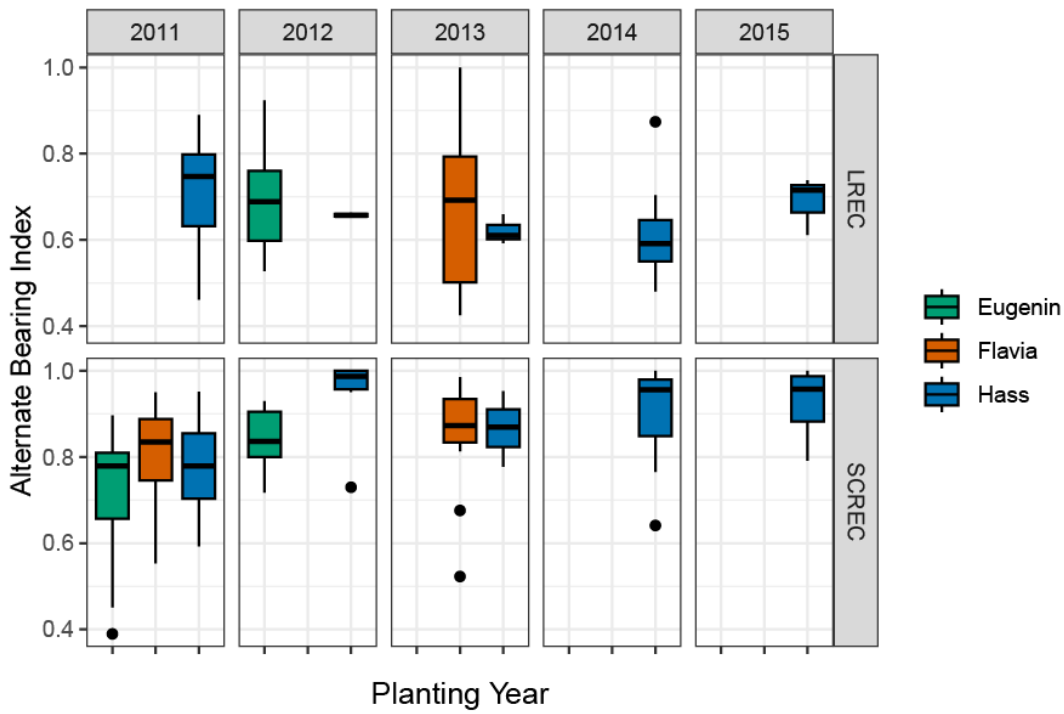


Fig. 2.6 Alternate Bearing Index by Cultivar and Planting Year, Both Sites



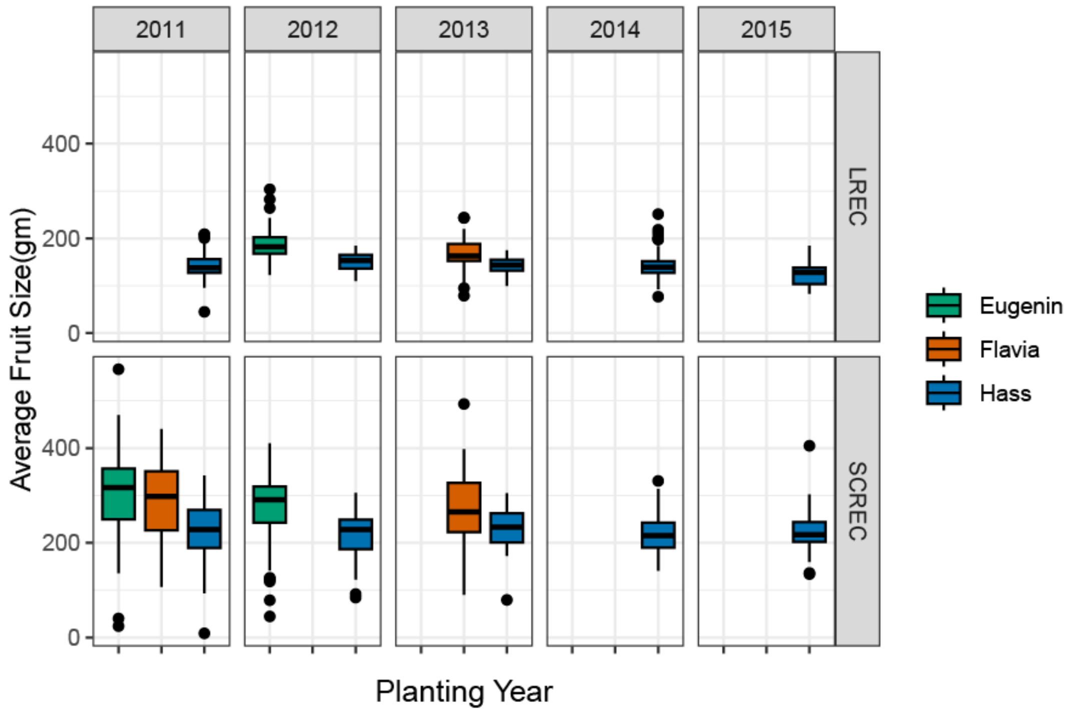
An area where there is a statistically significant difference between ‘Hass’ and the other two cultivars is in the average size of the individual harvested fruit (Table 2.4). As stated in their patents(Eugenin, 2014; Schiappacasse Macchiavello, 2014), both ‘Flavia’ and ‘Eugenin’ bear statistically larger fruit than ‘Hass’ at a given location. The difference between ‘Flavia’ and ‘Eugenin’ themselves under the same environmental conditions is a little more complicated in that there appears to be a significant difference between these varieties in their average fruit mass at LREC, but not at SCREC. However, as these LREC cohorts were planted in different years, with the ‘Flavia’ trees being a year younger than the ‘Eugenin’ trees at that location, a direct comparison is difficult to assess. Additionally, the site effect is still a very strong factor in influencing fruit size and SCREC fruit is consistently larger than LREC fruit for each cultivar.

Table 2.4 Average Fruit Size at Both Sites

Average fruit size (gm)	Cultivar	LREC	SCREC
All trees	Eugenin	187.1±29.3[a]	290.2±76.3[a]
	Flavia	167.0±31.1[b]	280.1±74.9[a]
	Hass	141.0±26.3[c]	223.4±52.7[b]
2011 planting	Eugenin	NA	300.7±79.2[a]
	Flavia	NA	287.5±77.3[a]
	Hass	141.6±26.2	226.1±58.3[b]
2012 planting	Eugenin	187.1±29.3[a]	275.1±69.4[a]
	Flavia	NA	NA
	Hass	150.9±21.5[b]	214.2±64.0[b]
2013 planting	Eugenin	NA	NA
	Flavia	167.0±31.1[a]	270.7±71.0[a]
	Hass	141.6±21.4[b]	225.7±53.1[b]

[a]Different letters in same column in a cell represent statistically significant cultivar differences for Kruskal-Wallis (2011 SCREC planting), or Mann-Whintney U test (all others)

Fig. 2.7 Average Fruit Size by Cultivar and Planting Year, Both Sites



Discussion

Despite the distinct climatic and field conditions at the two sites, rigorous efforts were undertaken to minimize variable influences during the experimental design phase and prior to the field management and assessment of the trees at both locations. Consequently, equivalent trait measurements were conducted on the three cultivars, facilitating the isolation of environmental effects from genetic differences. There was no significant genotype-environment interaction that differentiated ‘Eugenin’, ‘Flavia’, and ‘Hass’ in terms of canopy volume, average cumulative yield, or alternate bearing. Notably, the fruit size of ‘Eugenin’ and ‘Flavia’ was consistently larger than that of ‘Hass’ within the field sites, corroborating the patent descriptions of ‘Flavia’ and ‘Eugenin’ fruit being significantly larger than ‘Hass’ fruit.

Another area that bears mentioning is fruit shape. Although lengths or diameters of individual fruits at both sites were not measured in this study, the general appearance of LREC fruit is of a narrower diameter compared to SCREC fruit. SCREC fruit, regardless of cultivar, appears more round than LREC fruit. Additionally, 'Flavia' fruit tends to have a longer neck above the equator, resembling a pear-shaped avocado more than a typical 'Hass'. This is trait specific to the ‘Flavia’ variety, and 'Eugenin' does not exhibit it: ‘Eugenin’ more typically resembles a large 'Hass' in appearance. This elongated neck phenotype of 'Flavia' is persistent in both locations: although the LREC environment generates smaller, narrower fruit than the SCREC environment for all cultivars in this study, 'Flavia' at LREC is still readily distinguishable from 'Hass' and 'Eugenin' in this manner.

The impact of environmental conditions on fruit development and maturation remains challenging to delineate. However, the significantly warmer summers at the LREC site warrant further investigation as a potential contributing factor. The elevated nighttime temperatures during this period may have enhanced carbon assimilation rates, potentially facilitating fruit development. In contrast, similar temperatures (~27°C) were predominantly observed under daylight conditions at the SCREC site and were less frequent from June through September, a critical period for rapid fruit growth and maturation. It is also plausible that temperature influences overall plant growth and fruit maturation through distinct mechanisms, potentially resulting in smaller fruits that mature more rapidly. Nevertheless, this temperature influence does not entirely override genetic effects, as evidenced by 'Eugenin' and 'Flavia' producing larger fruits than 'Hass' at the warmer LREC site.

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Dry Weight, Oil Content, and Fatty Acid Profiles of the Three Cultivars at Lindcove Research and Extension Center (LREC) and South Coast Research and Extension Center (SCREC) Sites

Introduction

Avocado fruit differs from other fruits in that its caloric value is primarily sequestered as fats rather than sugars. This distinction renders metrics such as Brix, titratable acids, and other measures of fruit maturity, which are useful in stone fruits, citrus, and other pomological crops, poorly suited for assessing the eating quality or acceptability of avocados. Initially, scientific and commercial groups employed an oil percentage system to track fruit maturity and establish a minimal standard for the commercial sale of avocados(Hodgkin, 1939, 1928; Lee, 1981a, 1981b; Lewis, 1978). However, this method was knowledge and technique-intensive, requiring the use of hazardous chemicals and specialized laboratory facilities(Lee, 1981b). Consequently, researchers at the University of California, Riverside, developed a strong correlation between oil percentage and dry weight (non-water mass, also known as dry matter percentage) and established dry weight standards for different varieties. These standards were easily verifiable without the need for specialized laboratory training and equipment(Lee, 1981b, 1981a; Lee et al., 1983; Lee and Coggins, Jr., 1982).

Although the dry weight system is consistently replicable and reliable for determining minimum fruit maturity for sales or consumption, it has limitations. Specifically, it must be established as a separate percentage for each cultivar(Anonymous, 2011, 1983, "Maturity Standards," 2024). Additionally, the same cultivar grown in different environments often requires different dry weight percentage thresholds tailored to the specific combination of genetics and environment.

The recent and ongoing introduction of multiple putative 'Hass' somaclonal mutations as new cultivars for the global avocado industry presents an interesting opportunity to elucidate the nature of avocado fruit maturation. Some of these new cultivars are reported to have an earlier season than the standard ‘Hass’ cultivar(Eugenin, 2014; Mendez Vega, 2000; Schiappacasse Macchiavello, 2014). In particular, the ‘Flavia’ cultivar is noted for reaching a dry weight percentage several weeks earlier than standard ‘Hass’(Schiappacasse Macchiavello, 2014). However, in this 2019-2021 study conducted at two locations in California, the differences in dry weight measurements were small enough to be undetectable with the pooled samples methodology used. Environmental effects continue to be the main driver of fruit qualitative traits. This research group found that in a hot Central Valley location, Lindcove Research and Extension Center (LREC), the rates of fruit maturation in ‘Hass’, ‘Flavia’, and ‘Eugenin’ were flattened, with all cultivars reaching acceptable eating maturity in the same late fall or early winter season. This contrasts with the field site in Southern California, South Coast Research and Extension Center (SCREC). At SCREC ‘Flavia’ and ‘Eugenin’ both reach acceptable eating quality at a similar time to all the study’s cultivars at LREC. In contrast, ‘Hass’ in Southern California typically has a season stretching from winter well into spring. Additionally, ‘Flavia’ fruit does not remain on the tree in Irvine as long into the spring as standard ‘Hass’ fruit and begins to drop as early as March

Materials and Methods

Sampling occurred over 2 seasons; 2019-2020 and 2020-2021, with the initial season serving as a training season for methods with only 2 cultivars, ‘‘Flavia’’ and ‘‘Hass’’, considered instead of the full complement of three cultivars in the second season. Methodology including sampling, sample preparation, Soxhlet processing, and final oil sample desiccation were all modified from previous studies(Hausch et al., 2020), with training undertaken in these methods occurring in the late summer and early spring of 2019. Due to the different lab equipment available to the avocado program at University of California, Riverside's location, some of the techniques and tools were in need of modification and this occurred throughout the sampling and sample preparation process over the roughly 3-year period of collection and processing of samples. These modifications are noted within the body of the text below.

Sample Collection: During the 2019-2020 sampling period, two avocado cultivars, 'Flavia' and 'Hass', were sampled from three trees per cultivar at two distinct locations: LREC in Exeter, California, and SCREC in Irvine, California. Trees were chosen as biological replicates from plantings of comparable age ('Flavia' and 'Hass were both planted in 2011 and 2013). From each biological replicate, five fruits were randomly selected from various positions around the canopy to serve as a pooled source of the sampled tissue. Sampling was conducted monthly from August 2019 through January 2020 for both cultivars.

Table 3.1 Harvest Dates for Both Harvest Seasons and Both Sites

Year 1, 2019-2020

Sampling date		Date
AUGUST 2019		
	LREC	August 22, 2019
	SCREC	August 23, 2019
SEPTEMBER 2019		
	LREC	September 18, 2019
	SCREC	September 19, 2019
OCTOBER 2019		
	LREC	October 21, 2019
	SCREC	October 22, 2019
NOVEMBER 2019		
	LREC	November 18, 2019
	SCREC	November 19, 2019
DECEMBER 2019		
	LREC	December 16, 2019
	SCREC	December 12, 2019
JANUARY 2020		
	LREC	January 21, 2020
	SCREC	January 15, 2020

Year 2, 2020-2021

Sampling date		Date
AUGUST 2020		
	LREC	August 26, 2020
	SCREC	August 20, 2020
SEPTEMBER 2020		
	LREC	September 24, 2020
	SCREC	September 29, 2020
OCTOBER 2020		
	LREC	October 27, 2020
	SCREC	October 20, 2020
NOVEMBER 2020		
	LREC	November 23, 2020
	SCREC	November 16, 2020
DECEMBER 2020		
	LREC	December 29, 2020
	SCREC	December 22, 2020
JANUARY 2021		
	LREC	January 26, 2021
	SCREC	January 19, 2021
FEBRUARY 2021		
	LREC	February 25, 2021
	SCREC	February 17, 2021
MARCH 2021		
	LREC	NA
	SCREC	March 24, 2021
APRIL 2021		
	LREC	NA
	SCREC	April 27, 2021

Samples from the two sites were collected within one week of each other and transported to the University of California, Riverside (UCR) laboratory for processing the following day. Each individual hard fruit was weighed, and two sample coring locations on opposite sides of the fruit's equator were marked with a marker before coring with a cork borer. In the 2020-2021 sampling year, these marked locations were additionally analyzed using a Felix Instruments F750 spectrophotometer. Following the marking (and spectrophotometer analysis), the hard fruit were cored using a cork borer (2019-2020 sampling year) or the borer included with the F750 (2020-2021 sampling year). The mesocarp sections of the samples were meticulously stripped of any adhering seed coat material and peels. The five individual fruits from each tree were combined into a single sample weighing between 30-35 grams of finely minced mesocarp, which was then packed into labeled 55 ml Pyrex® No. 9820 glass tubes and capped with a folded Kimwipe secured with a standard-sized rubber band.

The glass tubes containing the mesocarp samples were then placed in a prepped lyophilizer and lyophilized for 72 hours until the weight stabilized, at which point a dry weight measurement was taken. After recording the dry weight, the tubes were sealed and stored in a -20°C freezer until they were retrieved for Soxhlet extraction.

Soxhlet extraction was performed using a methodology adapted from Hausch et al(Hausch et al., 2020). Two samples were processed simultaneously. The glass tubes containing the samples were removed from the freezer and allowed to reach room temperature in a sealed plastic tub with desiccant to minimize moisture accumulation. The finely diced lyophilized mesocarp samples were ground in a ceramic mortar and pestle until a fine dust or paste was achieved. This material was then placed into a 33 mm x 80 mm thimble for Soxhlet extraction.

The Soxhlet apparatus, set up in a fume hood, was loaded with the thimble and sample. Initially, 180 ml of petroleum ether was added to the bottom of the apparatus (a 250 ml round-bottom flask with a 24/40 taper fitting). A portion of this solvent was used to clean and wash any remaining sample residue from the mortar, pestle, and other grinding tools into the thimble in the Soxhlet upper chamber. During the Soxhlet process, adjustments to the solvent volume were made as needed to ensure continuous cycling, with the initial 180 ml sometimes proving insufficient.

The Soxhlet apparatus operated at 125°C for 18 hours to ensure complete extraction of all lipid components from the mesocarp samples. The thimble and its contents were then left to dry in the fume hood for 24 hours before weighing to calculate the lipid percentage of the mesocarp sample.

In the initial sampling year, the solvent and sample in the bottom of the Soxhlet apparatus were filtered and transferred to a smaller 100 ml round-bottom flask. The mixture was then subjected to rotary evaporation (rotovapping) for approximately 2 hours or until the weight stabilized to within three significant figures. The resulting extract was placed in 8 ml glass vials and stored at -20°C. These vials were kept frozen until they could be transferred to the Selina Wang lab at the University of California, Davis for Fatty Acid Methyl Esters (FAMEs) analysis(Green, 2022; Green and Wang, 2022a, 2022b).

In the second sampling year, several modifications to the above protocol were made. A Kyocera mandolin was utilized at its narrowest slicing setting to expedite the processing and desiccation of samples in the lyophilizer. The mesocarp samples were sliced at the 1/8" setting of the Kyocera mandolin, producing 1/8" discs which were then finely diced into short "matchsticks" to facilitate faster lyophilization. The five individual fruits from each tree were combined into a single sample weighing between 30-35 grams of finely minced mesocarp, which was then packed into labeled 55 ml Pyrex® No. 9820 glass tubes and capped with a folded Kimwipe secured with a standard-sized rubber band.

Monthly fruit samples in the second year were collected from nine trees, with three trees representing each of the three different cultivars at the two field sites, from August 2020 until Winter/Spring 2022. For the LREC samples, the sampling period spanned from August 2020 to February 2021, while for the SCREC samples, the period extended an additional two months due to the slower maturation of fruit at this location compared to LREC. The trees sampled were planted in 2011, 2012, or 2013, with a similar ratio of 'Hass', 'Eugenin', and 'Flavia' trees from each year considered as biological replicates.

Due to the time-consuming nature of the rotary evaporation process under the available vacuum power (generated by a siphon valve), starting in April 2021, samples were rotovapped for 90 minutes until the mass stabilized to approximately 0.1 grams. Subsequently, samples were stored in 8 ml glass tubes, capped, and placed in a -20°C freezer until all 2020-2021 samples had been processed similarly. At this point, all samples were finally dried in a Savant rotary speedvac for 72 hours. The samples were then triple-labeled and stored in a -20°C freezer until they were ready for transport to the University of California, Davis for Fatty Acid Methyl Esters (FAMEs) analysis.

Results

Dry weight (also referred to as dry matter percentage or DM%), crude fat, and oil percentage are presented in Table 3.2 for both the first and second sampling periods. These metrics are calculated as follows:

$$Dry\ Weight = \left(\frac{mass\ of\ fully\ dried\ mesocarp\ sample}{mass\ of\ fresh\ mesocarp\ sample}\right) \times 100$$

$$Crude\ Fat = 1 - \left(\frac{mass\ of\ fully\ dried\ mesocarp\ sample\ post\ soxhlet}{mass\ of\ fully\ dried\ mesocarp\ sample}\right) \times 100$$

$$Oil\ Percentage = 1 - \left(\frac{mass\ of\ fully\ dried\ mesocarp\ sample\ post\ soxhlet}{mass\ of\ fresh\ mesocarp\ sample}\right) \times 100$$

Dry weight represents the non-water mass of the mesocarp sample, calculated post-lyophilization. Crude fat indicates the percentage of dry weight composed of non-polar fat-soluble material, extracted via Soxhlet. Oil percentage represents the same non-polar fat-soluble material but as a percentage of the fresh mesocarp sample weight prior to desiccation. While prior literature typically reports dry weight and oil percentage, crude fat is included as a useful and quick metric for understanding the composition of the dry weight (Table 3.2).

Throughout the sampling period, all cultivars at both locations were observed to follow a similar trend: an early developmental stage in August and September, followed by a transition between September and October to an immature or early season fruit profile. During this period, dry weights began to approach, but did not consistently reach, the 20.8% minimum maturity standard for 'Hass' in California(Anonymous, 2011, 2002, 1983). However, in the October sampling window, a divergence between the two sites, LREC and SCREC, became apparent. In the 2020-2021 sampling period, LREC fruit reached or surpassed the 20.8% threshold, indicating a new phase in fruit development where it meets the industry's minimum maturity standard and can be considered early season, legal-to-harvest fruit. This stage also represents a product likely to be encountered by consumers in the market.

This transition to early season, legal-to-harvest fruit typically occurs in late fall, although it did not occur in the 2019-2020 SCREC season until after December 2019. Dry weight continued to increase throughout the harvest season, reaching approximately 30% by the end of the sampling period at both sites. This range of dry weight indicates the end of the "peak season" for 'Hass' and 'Hass'-type fruit, where the eating quality is considered ideal. No significant cultivar effects on dry weight were found in this study, so the observed trends apply equally to 'Eugenin', 'Flavia', and 'Hass'. The only significant factor was the environment or site, with LREC fruit achieving minimum maturity earlier than SCREC fruit.

Table 3.2 Average Dry Matter (DM%), Crude Fat and Oil Percentage by Cultivar, Both Sites and Both Harvest Seasons[a]

SCREC:	August 2019-January 2020						
	CV	AUGUST	SEPTEMBER	OCTOBER	NOVEMBER	DECEMBER	JANUARY
DM%	Flavia	12.9±0.4	13.3±0.4	17.5±1.7	18.0±0.5	19.5±0.5	24.5±0.7
	Hass	12.9±0.3	13.9±1.1	16.7±1.0	18.3±1.3	19.6±0.7	22.5±0.9
Crude Fat	Flavia	11.1±0.9	16.5±2.8	34.8±8.7	34.4±2.5	40.3±4.9	51.2±1.5
	Hass	16.1±6.2	18.3±6.1	26.7±4.5	40.5±1.8	38.4±3.0	48.0±0.6
Oil %	Flavia	1.4±0.2	2.2±0.4	6.4±2.2	6.2±0.6	7.9±1.2	12.6±0.5
	Hass	2.1±0.8	2.6±1.0	4.5±1.0	7.4±0.6	7.5±0.7	11.0±0.2

LREC:	August 2019-January 2020						
	CV	AUGUST	SEPTEMBER	OCTOBER	NOVEMBER	DECEMBER	JANUARY
DM%	Flavia	14.8±0.3	15.2±0.6	19.2±1.3	21.5±0.7	24.4±0.4	25.5±2.6
	Hass	15.7±0.7	15.8±1.5	16.4±4.5	23.9±1.1	24.2±2.2	26.4±1.5
Crude Fat	Flavia	5.6±1.5	14.4±6.9	32.1±3.5	35.5±6.6	44.2±2.4	47.1±7.4
	Hass	6.2±4.3	17.2±7.1	25.6±2.2	37.6±4.3	42.2±2.1	52.9±1.9
Oil %	Flavia	0.8±0.2	2.2±1.1	6.4±1.1	7.7±1.7	10.8±0.7	12.1±3.1
	Hass	1.0±0.7	2.8±1.4	4.8±0.9	9.0±1.4	10.2±1.5	14.0±1.1

SCREC:	August 2020-April 2021									
	CV	AUGUST	SEPTEMBER	OCTOBER	NOVEMBER	DECEMBER	JANUARY	FEBRUARY	MARCH	APRIL
DM%	Eugenin	13.3±0.5	16.6±1.0	18.1±1.1	20.9±2.1	24.6±1.2	26.7±1.7	28.8±1.2	29.8±2.4	30.5±0.8
	Flavia	13.6±0.2	16.8±0.2	18.1±0.5	21.4±1.3	25.7±1.4	28.1±1.1	30.5±1.2	29.8±2.7	30.3±1.1
	Hass	13.0±0.6	16.0±1.3	16.9±1.0	19.9±0.5	21.8±0.8	23.4±0.6	26.7±1.4	30.5±2.5	30.6±1.7
Crude Fat	Eugenin	14.6±3.5	24.1±4.0	37.8±5.2	45.7±2.9	50.0±4.2	56.1±1.2	54.5±0.7	56.5±2.8	54.4±1.0
	Flavia	13.9±1.6	25.2±1.9	38.3±4.6	46.4±4.3	52.6±1.6	55.0±3.1	55.3±0.5	55.2±3.0	56.2±1.5
	Hass	14.1±1.3	21.6±4.4	31.8±0.9	43.3±0.7	44.3±3.4	49.5±0.7	50.6±3.3	57.3±3.4	55.5±2.7
Oil %	Eugenin	2.0±0.5	4.0±0.9	6.9±1.3	9.6±1.5	12.4±1.7	15.0±1.0	15.7±0.7	16.9±2.1	16.6±0.4
	Flavia	1.9±0.2	4.2±0.4	7.0±1.0	10.0±1.5	13.5±1.1	15.4±1.2	16.9±0.8	16.5±2.4	17.0±1.0
	Hass	1.8±0.2	3.5±0.9	5.4±0.3	8.6±0.2	9.7±0.9	11.6±0.4	13.5±1.5	17.5±2.5	17.0±1.7

LREC:	August 2020-February 2021							
	CV	AUGUST	SEPTEMBER	OCTOBER	NOVEMBER	DECEMBER	JANUARY	FEBRUARY
DM%	Eugenin	14.6±0.1	15.1±0.6	20.4±0.4	23.9±0.3	26.4±0.8	28.6±1.3	30.3±2.1
	Flavia	15.0±0.1	15.0±0.6	21.5±0.7	24.4±1.1	27.8±0.8	29.4±2.2	30.0±2.0
	Hass	15.4±0.8	16.5±1.6	22.7±2.2	25.6±1.5	29.7±3.0	32.6±3.6	35.9±3.2
Crude Fat	Eugenin	6.5±2.2	17.1±4.2	35.5±4.7	40.8±3.2	49.0±2.1	52.0±1.8	57.0±2.6
	Flavia	10.4±5.9	19.7±3.2	37.2±5.3	45.4±4.4	50.5±4.5	53.2±4.5	52.5±3.9
	Hass	11.2±0.8	20.9±6.3	36.0±4.1	44.1±4.3	51.0±5.5	55.5±5.7	61.7±2.5
Oil %	Eugenin	0.9±0.3	2.6±0.7	7.3±1.0	9.7±0.7	12.9±0.7	14.9±1.1	17.3±1.8
	Flavia	1.6±0.9	2.9±0.5	8.0±1.4	11.1±1.5	14.0±1.7	15.7±2.5	15.8±2.2
	Hass	1.7±0.2	3.5±1.4	8.2±1.7	11.3±1.7	15.3±2.9	18.2±3.4	22.2±2.8

[a]n=3 unless otherwise noted; for LREC October 2019 'Flavia' Crude Fat and Oil %, n=2; for LREC October 2019 'Hass' Crude Fat and Oil %, n=2; for SCREC October 2019 'Flavia' Crude Fat and Oil %, n=2; for SCREC January 2020 'Hass' Crude Fat and Oil %, n=2

Very early in both sampling periods, oil content was very low. The 30 grams of wet mesocarp collected and lyophilized provided the bare minimum for two replicates of FAMES GCFID. LREC August samples consistently exhibited lower crude fat content than SCREC samples in both years but reached parity with or exceeded SCREC values by October of both sampling periods. From October through the end of the sampling periods, crude fat content continued to increase, reaching the mid to high 50 percentiles in the 2020-2021 sampling period, and the high 40s to low 50s in the 2019-2020 sampling period. No significant differences were observed between cultivars in terms of crude fat or oil percentage levels or accumulation in either sampling period.

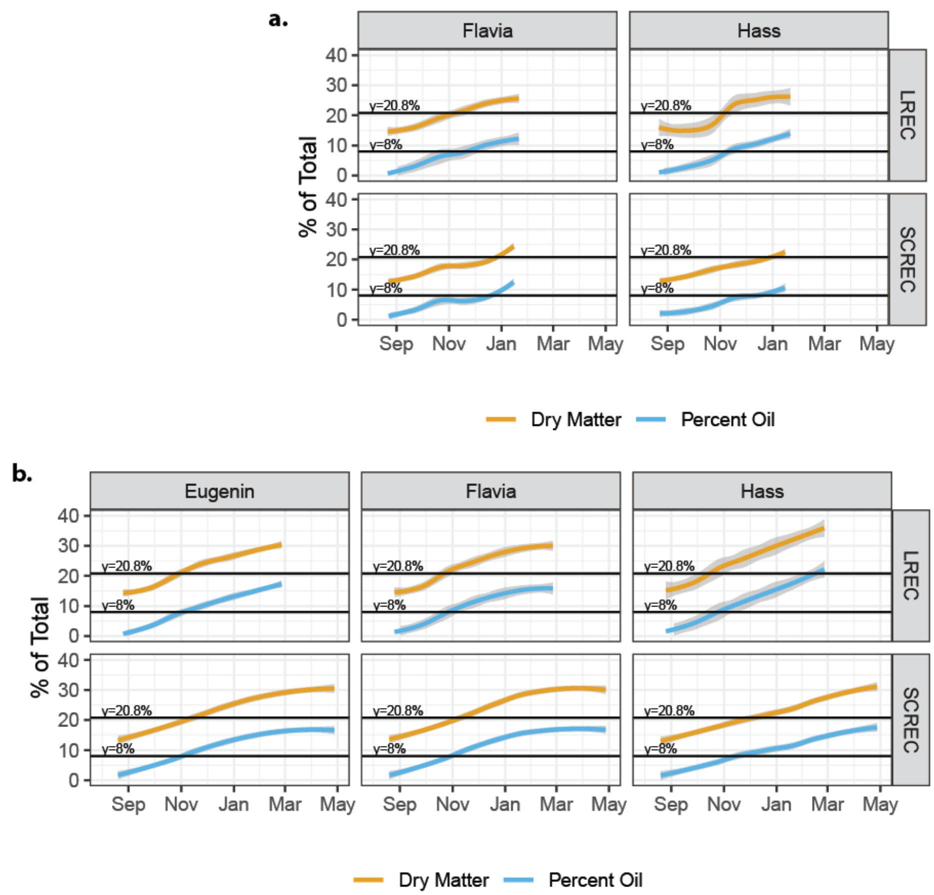


Fig. 3.1a-b Dry Matter and Oil Percentage for Combined Cultivars, Both Years and Sites
Dry matter and oil content for Aug. 2019 - Feb. 2020 (a) and Aug. 2020 - Apr. 2021 (b). Historic and current standards for dry matter of 20.8%(current California Hass minimum standard) and percent oil of 8% (previous minimum standard set for avocados marketed in California) represented with horizontal lines. Relevant y-value noted for each minimum standard marker line. No significant difference between cultivars noted: dry matter and oil content values represent average of all samples for given site and date.

Fatty Acid Profiles: In both years, fatty acid profiles (FAP) changed throughout the season as the fruit matured into and beyond the acceptable minimum dry weight (20.8%) or oil percentage (8%) (Table 3.2, Fig. 3.1)(Anonymous, 2011). Previous studies have shown that in 'Hass', levels of oleic 18:1(n9) fatty acid increased over the maturation period, while levels of palmitic 16:0 and palmitoleic 16:1 decreased over the same period. The results of this study align with this reported literature(Eaks, 1990). Other researchers have consistently found that oleic acid is the primary fatty acid in the avocado mesocarp, although the ratio of oleic to other fatty acids can vary between cultivars.

In this study, a statistically significant difference in oleic fatty acid profiles between cultivars was observed in the second year, but not in the first year. Other significant cultivar differences were found in palmitic and linoleic 18:2(n6) fatty acids in the first year, and in cis-vaccenic 18:1(n7) and linolenic 18:3(n3) fatty acids in the second year. The only fatty acid with a consistent cultivar effect in both years was palmitoleic (Fig. 3.3).

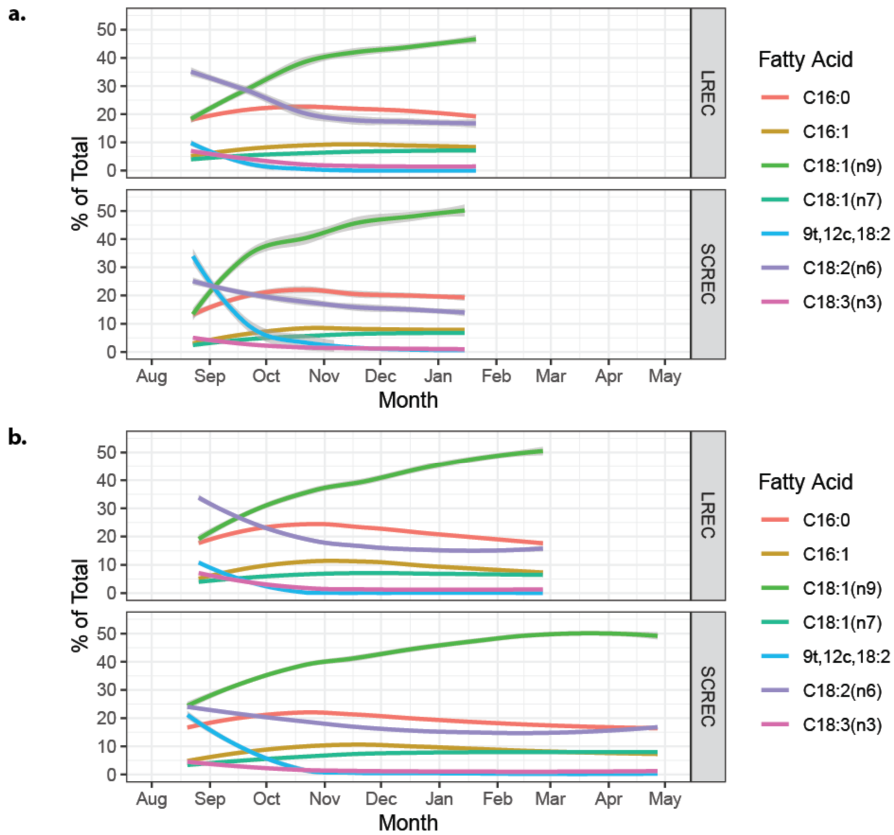


Fig. 3.2a-b Fatty Acid Profiles of Major Constiuents for Combined Cultivars, Both Years and Sites
Fatty acid profiles of major constituents for Aug. 2019 - Feb. 2020 (a) and Aug. 2020 - Apr. 2021 (b). Local regression lines represent average of all cultivars per site and sampling date;

Table 3.3 Fatty Acid Profiles of 'Flavia' and 'Hass' Avocados over the August 2019 - January 2020 Harvest Season, Both Study Sites[a]

SCREC:

Fatty Acid	CV	AUGUST	SEPTEMBER	OCTOBER	NOVEMBER	DECEMBER	JANUARY
14:0	Flavia	0.3±0.1	0.1±0.0	0.1±0.0	0.1±0.0	trace[d]	trace
	Hass	0.4±0.1	0.2±0.1	0.1±0.0	0.1±0.0	0.1±0.0	0.1±0.0
16:0	Flavia	14.6±3.5	20.0±0.8	22.5±1.1	20.4±0.7	20.0±1.2	19.3±1.1
	Hass	11.7±4.1	19.6±2.6	21.5±3.2	20.5±1.5	20.4±0.8	19.2±0.3
16:1	Flavia	3.6±1.4	6.2±0.3	8.9±0.8	8.0±0.2	8.1±0.5 a[b]	8.6±0.3 a
	Hass	2.5±1.4	5.9±1.3	7.9±2.2	8.3±0.6	7.6±0.1 b	7.0±0.7 b
17:0	Flavia	n.d.[c]	n.d.	n.d.	n.d.	n.d.	n.d.
	Hass	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
18:0	Flavia	n.d.	0.6±0.0 a	0.6±0.0	0.5±0.0 a	0.5±0.0	0.5±0.0
	Hass	n.d.	0.5±0.3 b	0.5±0.0	0.5±0.0 b	0.5±0.0	0.5±0.0
18:1(n9)	Flavia	15.1±7.1	36.9±1.2	42.1±1.0	46.8±1.4	47.9±2.9	50.4±0.6
	Hass	9.5±6.4	36.4±3.5	38.6±2.2	45.0±1.9	47.1±0.8	50.5±1.0
18:1(n7)	Flavia	2.6±0.7	4.5±0.2	5.6±0.7	6.1±0.6	6.7±0.2	7.0±0.6
	Hass	2.2±0.6	4.6±0.3	5.6±0.3	6.6±0.3	6.5±0.2	6.5±0.4
9t,12c 18:2	Flavia	33.2±11.3	7.8±0.6	2.1±1.0	1.8±0.6 a	1.1±0.1	0.5±0.1
	Hass	36.6±9.5	7.6±3.5	4.5±4.0	1.0±0.3 b	0.8±0.2	0.5±0.1
18:2(n6)	Flavia	21.9±2.1 a	19.5±0.7 a	16.2±0.8 a	14.9±0.8 a	14.5±1.3 a	12.9±0.4 a
	Hass	28.4±4.0 b	21.9±1.9 b	19.2±1.7 b	16.7±0.3 b	16.0±0.4 b	14.8±0.1 b
18:3(n3)	Flavia	4.4±0.5	2.8±0.1	1.5±0.2	1.3±0.1	1.2±0.1	0.9±0.1
	Hass	5.7±1.2	2.6±0.8	1.6±0.4	1.2±0.1	1.1±0.1	1.0±0.0
20:0	Flavia	4.2±0.9	1.4±0.4	0.3±0.3	0.2±0.2	n.d.	n.d.
	Hass	3.1±2.6	0.8±0.8	0.4±0.4	0.1±0.2	n.d.	n.d.

LREC:

Fatty Acid	CV	AUGUST	SEPTEMBER	OCTOBER	NOVEMBER	DECEMBER	JANUARY
14:0	Flavia	0.6±0.1	0.3±0.0	0.1±0.0	0.1±0.0	0.1±0.0	0.1±0.0
	Hass	0.7±0.1	0.4±0.1	0.1±0.0	0.1±0.0	0.1±0.0	0.1±0.0
16:0	Flavia	19.6±1.0 a	22.3±0.6	23.5±0.5 a	22.2±0.3	21.6±0.7	19.3±0.5
	Hass	16.4±1.9 b	20.6±1.9	21.9±1.1 b	21.6±0.7	21.0±0.2	19.1±1.4
16:1	Flavia	5.9±0.8 a	8.1±0.3	9.9±0.3 a	10.1±0.2 a	8.9±0.1	8.6±0.3
	Hass	4.4±0.8 b	7.0±1.4	7.9±1.0 b	8.5±0.5 b	8.5±0.6	8.2±0.6
17:0	Flavia	0.4±0.2	0.2±0.1	trace	n.d.	n.d.	n.d.
	Hass	0.2±0.2	0.2±0.1	n.d.	n.d.	n.d.	n.d.
18:0	Flavia	0.2±0.3	0.5±0.3	0.6±0.0	0.5±0.0	0.5±0.0	0.4±0.0
	Hass	0.2±0.3	0.6±0.1	0.6±0.0	0.5±0.0	0.5±0.0	0.4±0.0
18:1(n9)	Flavia	19.8±1.6	27.9±3.3	38.0±0.6	41.0±0.8	43.1±1.8	46.6±1.6
	Hass	17.4±5.1	26.3±5.1	39.0±0.1	43.0±0.7	43.7±0.2	46.8±1.4
18:1(n7)	Flavia	4.2±0.2 a	5.5±0.3	6.3±0.0 a	6.9±0.2 a	7.1±0.3 a	7.3±0.5
	Hass	3.7±0.3 b	5.2±0.3	5.9±0.3 b	6.5±0.4 b	6.8±0.1 b	7.0±0.2
9t,12c 18:2	Flavia	9.1±1.5	2.7±1.4	0.1±0.1	n.d.	n.d.	n.d.
	Hass	10.8±5.7	2.2±1.2	1.0±0.8	n.d.	n.d.	n.d.
18:2(n6)	Flavia	31.4±1.2 a	28.0±0.8	19.5±0.4	17.4±0.4	17.4±1.4	16.2±0.8
	Hass	38.1±2.8 b	32.3±5.6	21.2±2.1	18.3±0.6	18.0±0.8	17.1±0.6
18:3(n3)	Flavia	7.0±0.9	4.4±0.8	2.0±0.3	1.7±0.0	1.4±0.2	1.5±0.2
	Hass	6.8±1.2	4.6±1.6	2.3±0.4	1.6±0.1	1.5±0.2	1.4±0.1
20:0	Flavia	1.8±0.3	0.3±0.3	n.d.	n.d.	n.d.	n.d.
	Hass	1.3±1.0	0.6±0.2	n.d.	n.d.	n.d.	n.d.

[a]n=3 unless otherwise noted; LREC October 2019 'Hass', n=2; LREC October 2019 'Flavia', n=2.
[b]Different letters in each row denote significance at the $\alpha=0.05$ using Pairwise Wilcoxon Ranked Sum test. [c]n.d. indicates that the area was below the integration level. [d]Trace indicates that for at least one replication, the area was below the integration level

Table 3.4 Fatty Acid Profiles of 'Eugenin', 'Flavia', and 'Hass' Avocados over the August 2020 - April 2021 Harvest Season, Both Study Sites[a]

SCREC: Fatty Acid	CV	AUGUST	SEPTEMBER	OCTOBER	NOVEMBER	DECEMBER	JANUARY	FEBRUARY	MARCH	APRIL
14:0	Eugenin	0.2±0.0	0.1±0.0	0.1±0.0	0.1±0.0	0.1±0.0	trace[d]	trace	0.1±0.0	0.1±0.0
	Flavia	0.3±0.0	0.1±0.0	0.1±0.0	0.1±0.1	trace	trace	trace	trace	0.1±0.0
	Hass	0.3±0.0	0.2±0.0	0.1±0.0	0.1±0.0	trace	0.1±0.0	trace	trace	0.1±0.0
16:0	Eugenin	17.5±1.6	22.5±0.9	22.6±0.8	21.9±0.5	20.0±0.2	18.5±0.8	18.0±0.5	17.8±1.0	17.0±0.8
	Flavia	17.8±0.6	22.9±0.6	22.9±0.6	21.5±0.3	20.2±0.3	18.6±0.2	17.8±0.4	16.0±0.5	16.3±0.6
	Hass	12.3±1.0	20.1±0.6	21.2±1.0	20.0±0.7	19.4±0.4	18.3±0.4	17.4±0.9	16.9±0.6	15.9±0.7
16:1	Eugenin	5.3±0.8 a	9.0±1.1 a	10.7±1.5 ab	11.4±1.0 a	10.8±0.4 a	9.7±0.8 a	8.9±0.6 a	8.7±0.7 a	8.3±1.0
	Flavia	5.6±0.1 a	9.4±0.6 a	10.8±0.4 a	11.2±0.6 a	10.5±0.8 a	9.5±0.9 a	9.5±0.6 a	6.6±0.6 b	7.0±1.2
	Hass	3.0±0.3 b	6.5±0.8 b	8.3±0.9 b	9.2±0.1 b	8.4±0.9 b	7.6±0.5 b	7.3±0.7 b	7.2±1.1 ab	6.8±1.0
17:0	Eugenin	0.2±0.2	0.1±0.1	0.1±0.0	trace	trace	trace	n.d.[c]	trace	trace
	Flavia	0.4±0.1	0.1±0.1	trace	trace	trace	trace	n.d.	n.d.	trace
	Hass	0.1±0.2	0.1±0.1	0.1±0.1	trace	trace	n.d.	trace	trace	n.d.
17:1	Eugenin	trace	0.1±0.1	0.1±0.0	0.1±0.0	0.1±0.0	0.1±0.0	0.1±0.0	0.1±0.0	0.1±0.0
	Flavia	n.d.	0.1±0.1	0.1±0.1	0.1±0.1	0.1±0.1	0.1±0.0	0.1±0.0	0.1±0.1	0.1±0.0
	Hass	n.d.	0.1±0.1	trace	trace	trace	0.1±0.1	0.1±0.1	0.1±0.1	0.1±0.1
18:0	Eugenin	0.7±0.1	0.7±0.1	0.6±0.1	0.5±0.1	0.5±0.0	0.4±0.0	0.4±0.1	0.4±0.0	0.4±0.0
	Flavia	0.7±0.1	0.6±0.0	0.6±0.0	0.5±0.0	0.5±0.0	0.4±0.0	0.4±0.0	0.4±0.0	0.4±0.0
	Hass	0.3±0.3	0.6±0.0	0.6±0.0	0.5±0.1	0.5±0.1	0.5±0.1	0.4±0.2	0.4±0.0	0.4±0.1
18:1(n9)	Eugenin	28.5±2.2 a	37.5±0.7	38.0±0.6	39.9±1.3 a	43.7±1.5	47.3±1.8 ab	48.2±1.0 a	47.6±2.5 a	46.2±3.6
	Flavia	24.5±4.2 a	36.2±1.1	37.7±0.8	40.1±0.5 a	44.4±0.8	47.1±1.0 a	47.8±1.0 a	53.5±1.0 b	49.9±2.4
	Hass	15.6±4.5 b	37.0±2.7	40.1±1.8	43.7±1.6 b	45.8±1.7	48.9±0.5 b	50.2±0.2 b	51.8±1.7 ab	50.3±2.4
18:1(n7)	Eugenin	3.8±0.1 a	5.3±0.1 a	6.6±0.2 a	7.3±0.2 ab	8.1±0.2 a	8.1±0.3	8.2±0.2 a	8.3±0.2 a	8.3±0.2 a
	Flavia	3.6±0.5 a	5.3±0.1 a	6.4±0.1 a	7.5±0.2 a	7.8±0.3 ab	8.0±0.2	8.2±0.3 a	7.6±0.3 b	8.0±0.2 ab
	Hass	2.7±0.3 b	4.9±0.1 b	5.9±0.1 b	7.0±0.3 b	7.3±0.3 b	7.5±0.4	7.7±0.1 b	7.6±0.2 b	7.8±0.2 b
9t,12c 18:2	Eugenin	16.2±3.3	3.2±0.8	1.0±0.4	0.8±0.1	0.5±0.1	0.4±0.1	0.3±0.1	0.1±0.0	0.4±0.1
	Flavia	20.9±6.5	3.5±0.3	1.4±0.5	0.9±0.4	0.5±0.2	0.3±0.0	0.3±0.0	0.1±0.1	0.4±0.0
	Hass	32.5±8.0	4.3±1.8	1.7±0.2	0.8±0.1	0.6±0.1	0.4±0.0	0.2±0.1	trace	0.3±0.0
18:2(n6)	Eugenin	23.2±1.8	19.7±1.7	18.6±1.3	16.5±1.6	15.0±1.2	14.3±0.4	14.5±0.3	15.6±0.9	17.5±1.4
	Flavia	22.0±2.2	19.9±1.4	18.5±1.0	16.7±0.9	14.9±0.4	14.7±0.2	14.5±0.1	14.5±0.7	16.3±0.7
	Hass	27.6±2.1	23.7±1.4	20.2±0.6	17.3±0.7	16.7±0.3	15.6±0.8	15.5±0.7	14.8±1.4	16.9±1.0
18:3(n3)	Eugenin	4.4±0.7 a	1.9±0.2	1.6±0.2	1.3±0.2	1.1±0.1	1.0±0.0	0.1±0.0	1.1±0.1 a	1.3±0.2
	Flavia	4.2±0.6 ab	1.9±0.1	1.6±0.1	1.3±0.1	1.1±0.0	1.0±0.0	1.1±0.0	1.0±0.1 ab	1.2±0.1
	Hass	5.7±0.3 b	2.5±0.7	1.7±0.1	1.3±0.0	1.1±0.0	1.0±0.1	0.1±0.1	0.9±0.1 b	1.1±0.1
20:0	Eugenin	n.d.	trace	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.1±0.1
	Flavia	n.d.	n.d.	n.d.	n.d.	n.d.	trace	n.d.	n.d.	trace
	Hass	n.d.	trace	trace	n.d.	trace	trace	trace	0.1±0.1	trace
20:1	Eugenin	n.d.	0.1±0.1	0.1±0.0	0.1±0.1	0.2±0.0	0.2±0.0	0.2±0.1	0.2±0.0	0.2±0.0
	Flavia	n.d.	0.1±0.1	0.1±0.1	0.1±0.1	0.1±0.1	0.2±0.0	0.2±0.0	0.2±0.1	0.2±0.0
	Hass	n.d.	trace	0.1±0.1	trace	trace	0.1±0.1	0.1±0.1	0.1±0.1	0.1±0.1
22:0	Eugenin	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	Flavia	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	Hass	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
24:0	Eugenin	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	Flavia	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	Hass	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.

LREC: Fatty Acid	CV	AUGUST	SEPTEMBER	OCTOBER	NOVEMBER	DECEMBER	JANUARY	FEBRUARY
14:0	Eugenin	0.8±0.1	0.2±0.0	0.1±0.0	0.1±0.0	0.1±0.0	0.1±0.0	0.1±0.0
	Flavia	0.6±0.2	0.2±0.0	0.1±0.0	0.1±0.0	0.1±0.0	0.1±0.0	0.1±0.0
	Hass	1.0±0.1	0.2±0.0	0.1±0.0	0.1±0.0	0.1±0.0	0.1±0.0	trace
16:0	Eugenin	17.6±0.4	24.0±0.7	24.7±0.7	23.2±0.9	21.4±0.3	18.7±0.8	17.0±0.2
	Flavia	19.4±2.7	25.0±0.2	24.5±2.1	22.2±1.1	20.9±0.4	19.3±1.2	18.3±1.3
	Hass	14.8±1.2	22.4±2.5	24.3±1.2	23.2±0.8	21.1±0.4	19.5±0.6	17.8±0.4
16:1	Eugenin	5.2±0.4 a	9.3±0.9 a	12.1±0.5	10.8±1.4 ab	9.7±0.1 a	8.3±0.5	7.3±0.5 a
	Flavia	6.3±2.0 ab	9.6±0.4 a	12.6±2.2	11.3±1.2 a	10.2±0.6 a	9.4±1.5	8.1±1.0 a
	Hass	3.9±0.3 b	7.7±0.9 b	11.1±0.9	9.8±0.1 b	8.8±0.6 b	7.4±0.7	6.5±0.5 b
17:0	Eugenin	0.5±0.3	0.3±0.0	0.1±0.0	0.1±0.1	0.1±0.0	0.1±0.0	0.1±0.0
	Flavia	0.7±0.1	0.3±0.0	0.1±0.0	0.1±0.0	0.1±0.1	0.1±0.0	0.1±0.0
	Hass	0.6±0.1	0.2±0.0	0.1±0.0	0.1±0.0	0.1±0.0	trace	trace
17:1	Eugenin	0.1±0.3	0.1±0.0	0.1±0.0	0.1±0.0	0.1±0.0	0.1±0.0	0.1±0.0
	Flavia	trace	0.1±0.0	0.1±0.0	0.1±0.0	0.1±0.0	0.1±0.0	0.1±0.0
	Hass	trace	0.1±0.0	0.1±0.0	0.1±0.0	0.1±0.0	0.1±0.0	0.1±0.0
18:0	Eugenin	0.9±0.1	0.8±0.1	0.7±0.1	0.7±0.1	0.6±0.1	0.6±0.0	0.6±0.0
	Flavia	0.8±0.1	0.7±0.0	0.7±0.1	0.6±0.1	0.6±0.1	0.6±0.0	0.6±0.0
	Hass	0.7±0.2	0.7±0.1	0.6±0.1	0.6±0.1	0.6±0.1	0.6±0.1	0.6±0.0
18:1(n9)	Eugenin	18.8±2.3	30.7±2.3 a	35.1±1.8	39.6±2.0	44.7±0.8	49.3±2.1	51.6±2.6
	Flavia	19.9±2.2	30.0±2.5 ab	35.4±5.4	39.2±2.9	43.9±2.7	46.2±4.2	46.4±5.7
	Hass	15.9±4.0	34.7±2.9 b	35.9±0.5	41.2±0.4	45.9±1.3	50.7±1.3	53.0±1.4
18:1(n7)	Eugenin	3.8±0.1	5.6±0.3	7.0±0.5	7.1±0.5 ab	6.9±0.3	6.7±0.3 ab	6.6±0.5
	Flavia	4.7±1.1	5.7±0.5	7.0±0.7	7.5±0.5 a	7.1±0.6	7.0±0.6 a	6.8±0.8
	Hass	3.5±0.1	5.3±0.3	6.5±0.4	6.6±0.3 b	6.5±0.4	6.1±0.3 b	6.0±0.2
9t,12c 18:2	Eugenin	14.8±2.9	2.1±0.2	0.3±0.1	0.3±0.0	0.1±0.0	0.1±0.0	0.1±0.1
	Flavia	8.7±4.2	1.3±0.2	0.2±0.1	0.1±0.1	0.1±0.0	0.1±0.1	trace
	Hass	11.3±2.4	1.3±0.7	0.2±0.0	0.2±0.1	0.1±0.0	0.1±0.0	trace
18:2(n6)	Eugenin	30.5±0.6	23.9±0.6	17.9±0.8	16.6±0.8	14.9±0.4	14.6±0.7	15.2±1.4
	Flavia	31.6±2.9	23.4±1.5	17.6±1.5	17.0±0.7	15.4±1.2	15.6±1.5	17.6±2.2
	Hass	40.3±2.5	24.3±1.4	19.2±1.5	16.6±0.8	15.4±1.1	14.3±1.1	14.7±1.0
18:3(n3)	Eugenin	7.0±0.2 a	2.9±1.3	1.7±0.1	1.4±0.1 ab	1.2±0.0	1.2±0.2	1.3±0.3 ab
	Flavia	7.2±0.4 ab	3.4±0.4	1.6±0.3	1.6±0.2 a	1.3±0.2	1.4±0.3	1.6±0.5 a
	Hass	7.8±0.7 b	2.8±0.6	1.6±0.2	1.2±0.1 b	1.1±0.2	1.0±0.1	1.0±0.1 b
20:0	Eugenin	n.d.	0.1±0.1	n.d.	n.d.	n.d.	n.d.	n.d.
	Flavia	0.1±0.1	0.1±0.0	n.d.	0.1±0.1	trace	trace	trace
	Hass	trace	0.1±0.1	trace	trace	n.d.	n.d.	n.d.
20:1	Eugenin	n.d.	0.1±0.0	0.2±0.1	0.1±0.1	0.2±0.0	0.2±0.0	0.2±0.0
	Flavia	trace	0.1±0.0	0.1±0.1	0.1±0.1	0.2±0.0	0.2±0.0	0.2±0.0
	Hass	trace	0.1±0.0	0.2±0.0	0.2±0.0	0.2±0.0	0.2±0.0	0.2±0.1
22:0	Eugenin	n.d.	trace	n.d.	n.d.	n.d.	n.d.	n.d.
	Flavia	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	Hass	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
24:0	Eugenin	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	Flavia	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	Hass	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.

[a]n=3 unless otherwise noted; SCREC August 2020, 'Hass', n=2; SCREC September 2020, 'Hass', n=2; SCREC October 2020, 'Hass', n=2.
[b]Different letters in each row denote significance at the α=0.05 using Pairwise Wilcoxon Ranked Sum test. [c]n.d. indicates that the area was below the integration level. [d]Trace indicates that for at least one replication, the area was below the integration level.

Aside from cultivar interaction, the site had a strong influence on fatty acid profiles and accumulation. In all cases except for palmitoleic in the second year, a statistically significant difference was observed between the LREC and SCREC sites. In both years, levels of linoleic acid were much higher at the LREC site than at the SCREC site early in the sampling periods. Countering this trend of higher polyunsaturated fatty acids at the LREC site, the presence of an unexpected transfat, 9t, 12t, 18:2, was detected early in both sampling years, with this transfat being more prevalent in the SCREC fruit (Fig. 3.4). No statistically significant cultivar effects were observed with this transfat.

Previous studies have detected an unknown compound that peaked earlier in the season of avocado fruit(Gaydou et al., 1987), which was posited to result from oxidation of linoleic acid during the Soxhlet process(Kaiser and Wolstenholme, 1994). However, it cannot be stated with certainty whether the current transfat is related, as Gaydou et al. did not have the tools available to better categorize their unknown compound.

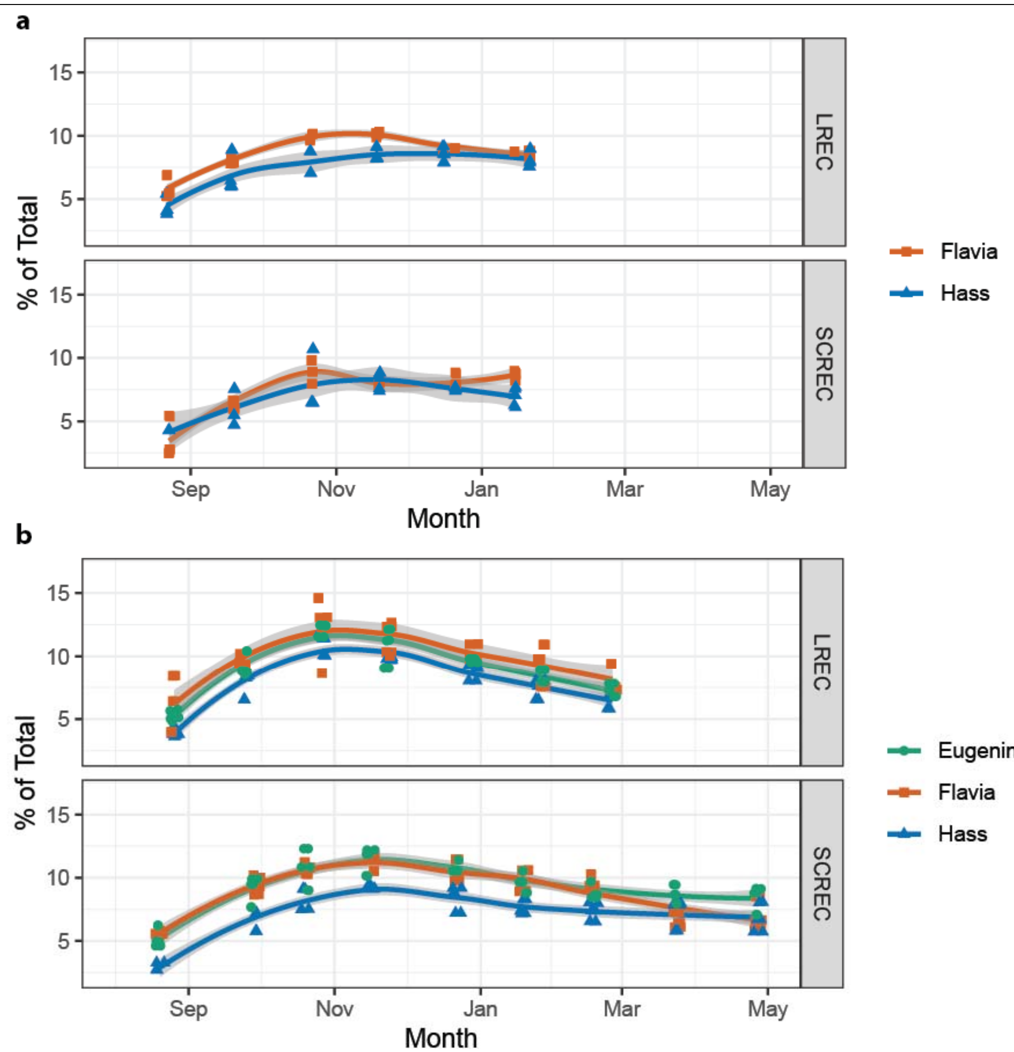
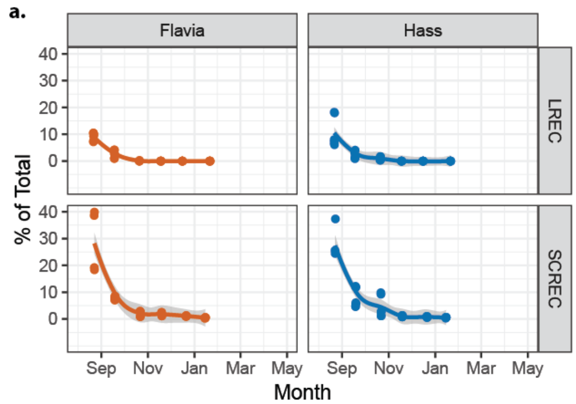


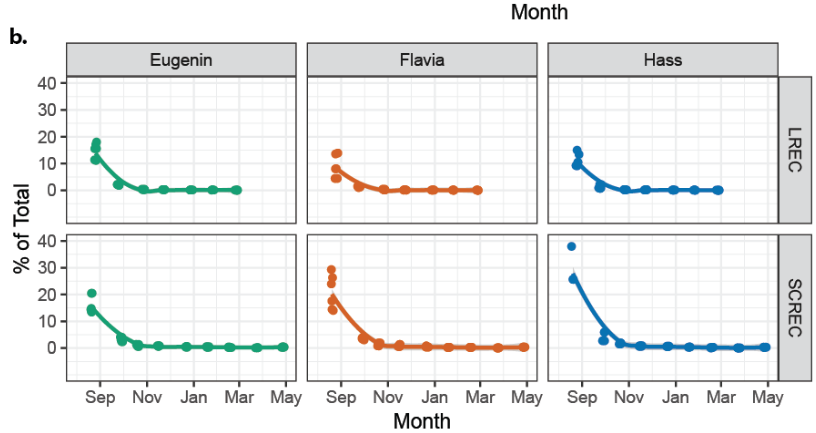
Fig. 3.3a-b Palmitoleic Percentage of Fatty Acid Profile by Cultivar

Palmitoleic fatty acid percentage of total oil content for Aug. 2019 - Feb. 2020 (a) and Aug. 2020 - Apr. 2021 (b). In the first year the Mann-Whitney U test observed a signifcant difference between 'Flavia' and 'Hass' ($U = 3196$, $p = 0.001889$). In the second year the Kruskal Wallis observed a significant difference between 'Eugenin', 'Flavia', and 'Hass' ($H = 33.106$, $p = 6.474e-08$). A significant difference between the two sites was only observed by Mann-Whitney U test in the first year ($U = 3282.5$, $p = 0.0005064$).

Fig. 3.4a-b 9t 12C C18:2 Trans Fatty Acid Percentages

Percentage of 9t 12c C18:2 fatty acid for Aug. 2019 - Feb. 2020 (a) and Aug. 2020 - Apr. 2021 (b). 9t 12c C18:2 is a previously undescribed transfatty acid in avocado. It was transiently detected early in the sampling period for both years. In both years, there was no discernable cultivar difference, but Mann-Whitney U test detected a significant difference between sites, Lindcove Research and Extension Center (LREC) and South Coast Research and Extension Center (SCREC) in the first year ($U = 1179.5$, $p = 9.049e-08$) and again in the second year ($U = 6193.5$, $p = 9.36e-08$)





General profiles of total monounsaturated, polyunsaturated, and saturated fatty acids followed the trends predicted in the literature. Low levels of monounsaturated fatty acids increased from early low levels to represent the majority of the entire profile. Conversely, polyunsaturated fatty acids followed a reverse trend, being the predominant component in August and continually decreasing in proportion from September through the rest of the sampling period. These polyunsaturated fatty acids were more prevalent throughout the sampling period at LREC than at SCREC, especially in the first year of sampling, 2019-2020 (Fig. 3.5).

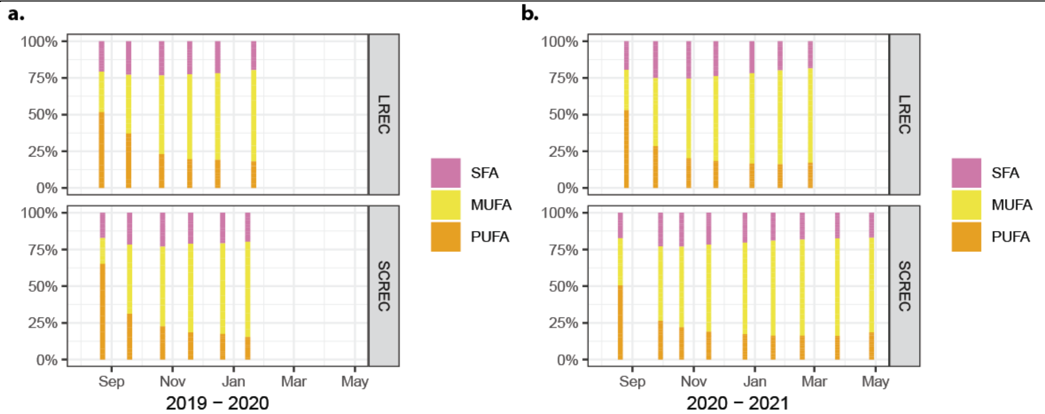


Fig. 3.5 Saturated Fatty Acid (SFA), Monounsaturated Fatty Acid (MUFA) and Polyunsaturated Fatty Acid (PUFA) Percentages of Overall Fatty Acid Profiles
Saturated/Unsaturated fatty acid profiles for Aug. 2019 - Feb. 2020 (a) and Aug. 2020 - Apr. 2021 (b). Saturated fatty acids (SFA) represented in pink, monosaturated fatty acids (MUFA) in yellow, and polyunsaturated fatty acids (PUFA) in orange. Trends show higher PUFA in earlier season than in later season and generally increasing MUFA over the same period.

Discussion

The most profound change in fatty acid profiles occurs between August and September in both years. From the perspective of saturated, monounsaturated, and polyunsaturated fatty acid profiles, as well as the composition of the main individual fatty acids, the August sample dates represent a fruit (in all cultivars) that is quantitatively different from what is considered a typical avocado fruit. This difference in composition is so significant that the initial sampling date could be considered either the end of a period of immature fruit development or the initiation of the period of mature fruit development that follows. It represents a threshold between the beginning of a maturing avocado fruit and a finishing "fruitlet," corresponding to BBCH717-718 in the 2013 methodology developed to describe avocado flower to fruit development(Alcaraz et al., 2013).

An interesting aspect of the cultivar interaction of C16:1 fatty acid profile is its potential relationship to cis-vaccenic (18:1(n7)) fatty acid(Barthet, 2008; Green and Wang, 2022b). The pathway from palmitic to stearic to oleic acid is well researched, and there is evidence of gene expression in avocado mesocarp that explains the prevalence of oleic acid through the relative expression levels of KASII (C18) and Stearoyl-ACP Desaturase (SAD)(Kilaru et al., 2015). This understanding explains the relative paucity of stearic acid in avocado mesocarp, as any C16:0 fatty acid lengthened to 18 chains is quickly desaturated to C18:1(n7) due to the relative abundance of SAD. The difference in expression levels between KASII and SAD was found to be orders of magnitude higher for SAD than for KASII, almost guaranteeing that all 18:0 fatty acids are quickly converted to 18:1-ACP. Expression levels for acyl-ACP thioesterases A and B (FATA and FATB) were roughly equivalent to those for KASII, so the fatty acids generated in this biosynthetic pathway were not made available for Long-chain acyl-CoA synthases (LACS) any faster than they could be desaturated. Very few C18:0 fatty acids are sequestered away into DAGs or TAGs or other molecular structures where SAD cannot reach them(Horn et al., 2013; Kilaru et al., 2015).

When considering the relationship of different fatty acids in the data, some of these putative relationships are stronger in the first year than in the second year of sampling (Fig. 6). In particular, the palmitic/palmitoleic and palmitoleic/cis-vaccenic relationships are much stronger in the first year than in the second year. The reason for this difference is unclear, although it is apparent from other aspects of the dry weight, oil percentage, and other fatty acid data reported above that the two years were different from one another. Previous research has shown that the environment plays a significant role in fatty acid profiles and other traits(Donetti and Terry, 2014; Ferreyra et al., 2016; Kaiser and Wolstenholme, 1994; Rodriguez et al., 2018).

Therefore, environmental and climatic conditions are likely responsible for the different relationships between these two mesocarp components in 2019-2020 versus 2020-2021.

Later research in this area found increased evidence for the efficacy of avocado transcript products, PaDGAT1, PaDGAT2, and PaPCDAT, in initiating the sequestration of oleic and linoleic fatty acids into triacylglycerides (TAGs). Although the overall percentage of palmitoleic acid is low in relation to the mesocarp fresh weight, the avocado acyltransferase system favors oleic and linoleic fatty acids(Behera et al., 2023). It may be that palmitic and palmitoleic fatty acids are relatively more available in the acyl-CoA pool, as free fatty acids, and as non-TAG associated fatty acids. Free fatty acids have been found to be detectable in sensory trials and distinguishable to an extent based on their relative chain lengths(Running et al., 2015; Running and Mattes, 2014). However, untrained human panelists do not seem able to distinguish between similar length free fatty acids(Jaime-Lara et al., 2023). Therefore, although palmitic and palmitoleic acids are potentially more abundant in the intercellular and intracellular lipid pools, their influence on perceptible eating quality would likely be as substrates for pathways into the avocado fruit volatile profile(Behera et al., 2023; Horn et al., 2013; Kilaru et al., 2015).

The relationship and pathway between palmitic and palmitoleic acids are not as well documented as the palmitic to stearic to oleic pathway, and there is a further lack of understanding regarding what occurs between palmitoleic and cis-vaccenic C18:1(n7). This may be compounded by the fact that cis-vaccenic fatty acid is a relatively rare component of the lipidome of most oil-rich plants. In fact, it is currently considered a good biomarker for determining the purity of putative extra-virgin avocado oil due to its relatively high levels in the fruit's mesocarp(Green and Wang, 2022b).

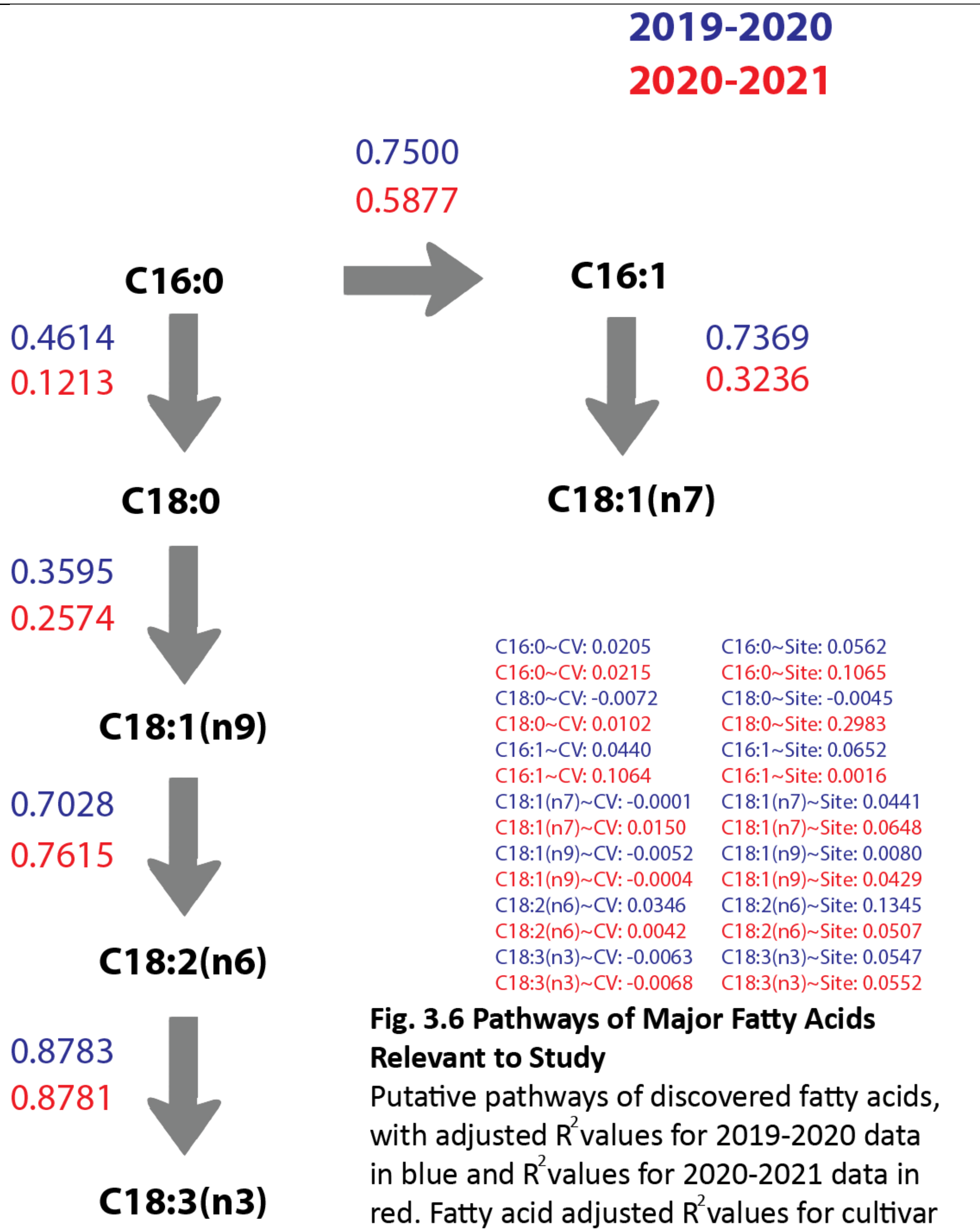


Fig. 3.6 Pathways of Major Fatty Acids Relevant to Study

Putative pathways of discovered fatty acids, with adjusted R^2 values for 2019-2020 data in blue and R^2 values for 2020-2021 data in red. Fatty acid adjusted R^2 values for cultivar and site in inset with identical color coding for year. Adjusted R^2 values indicate percentage of variance in fatty acid explained by precursor fatty acid, site, or cultivar.

Another relatively little-researched area is the later lipid pathways located inside the plastid. Much work has been done on the formation of DAGs and TAGs from the acyl-CoA pool and its products just downstream, but this occurs in the endoplasmic reticulum (ER). It is not clear whether the putative transition from palmitoleic to cis-vaccenic occurs after palmitoleic acid has left the plastid as C16:1-CoA or before. There are also two different pathways from oleic to its polyunsaturated downstream products, linoleic and linolenic acids. Although some exploration of fatty acid desaturase genes is currently underway in 'Hass', 'Flavia', and 'Eugenin' (along with other putative somaclonal 'Hass' cultivars), this work is just beginning, and the genomic aspects of the different desaturases in the ER versus those in the plastid remain an important frontier in avocado fruit physiology.

Unpublished data from collaboration with Dr. Edwin Solares demonstrate that for 'Hass' and its somaclonal variants (including but not limited to 'Flavia' and 'Eugenin'), many transposable elements are associated with putative fatty acid desaturase genic regions. Moreover, multiple potential copies of some of these desaturase orthologs appear to exist in the avocado genomes under investigation by Solares' group. This seems to be an important aspect of avocado fruit physiology, likely to be highly complex with many potential entry points for regulation and modification factors(Sanchez and Solares, 2024).

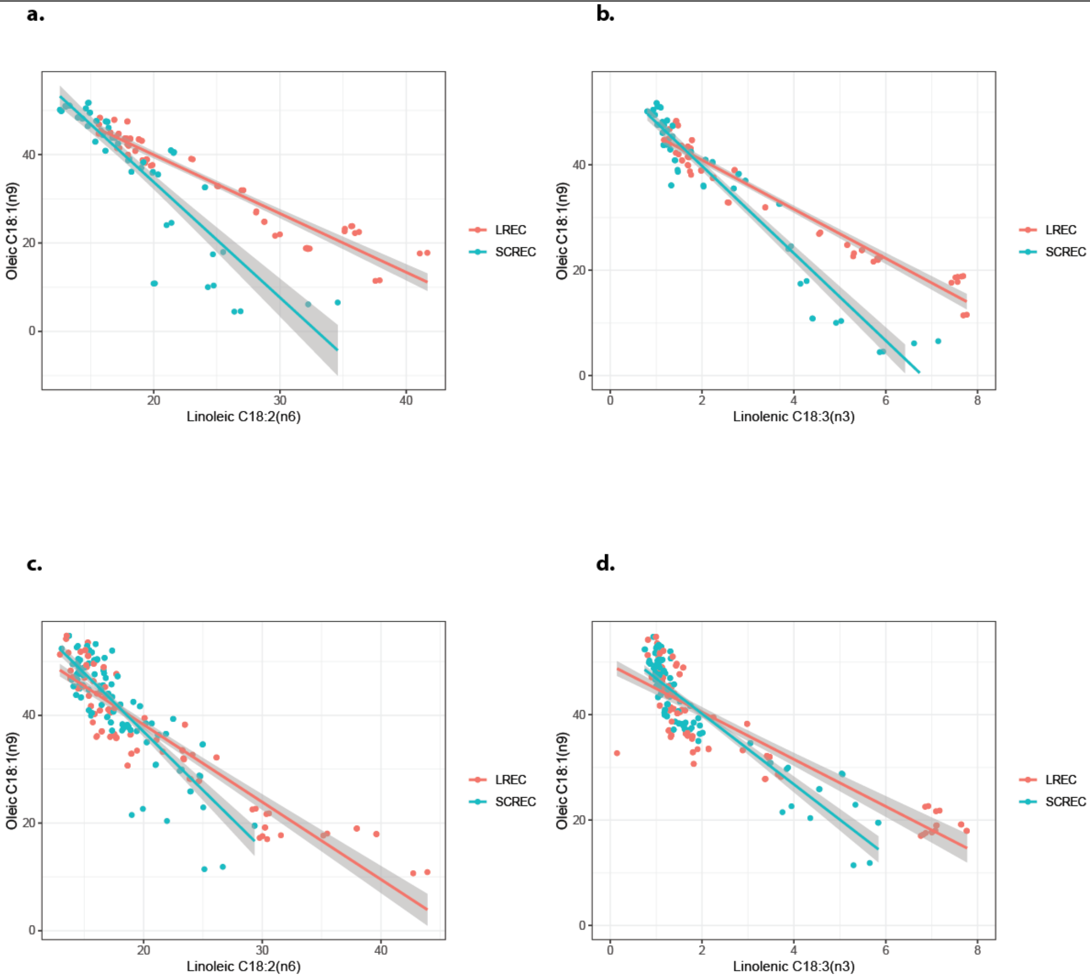


Fig. 3.7a-d Oleic vs Linoleic and Linolenic Profiles by Site
Oleic monounsaturated vs linoleic (a,c) and linolenic (b,d) polyunsaturated fatty acids, linear models by site for 2019-2020 (a, b) and 2020-2021 (c, d). Data represents combined cultivar data, no significant difference detected between cultivar forthese fatty acid profiles. Oleic is upstream in the biosynthetic pathway which generates linoleic and linolenic fatty acids. Relationship between linoleic and oleic 2019-2020 (a): adjusted R^2 for Lindcove Research and Extension Center (LREC) is 0.92 and adjusted R^2 for South Coast Researchand Extension Center (SCREC) is 0.78. Relationship between linolenic and oleic 2019-2020 (b): adjusted R^2 for LREC is 0.94 and adjusted R^2 for SCREC is 0.92. Relationship between linoleic and oleic 2020-2021 (c): adjusted R^2 for LREC is 0.83 and adjusted R^2 for SCREC is 0.71. Relationship between linolenic and oleic 2020-2021 (d): adjusted R^2 for LREC is 0.77 and adjusted R^2 for SCREC is 0.78.

Figure 3.6 illustrates that the oleic to 18C PUFA pathway maintains a stable output of linoleic and linolenic to oleic ratios. Although a notable and predictable site effect is observed, the linear relationship is strong, and R^2 values are correspondingly validating when the site is considered as a factor (Fig. 3.7). However, despite this strong correlation, the FAMES GCFID methodology used does not provide the resolution needed to determine the intracellular localization of the desaturation of oleic fatty acids into PUFAs, or their organization in TAGs, DAGs, or other larger molecules. In addition to lipid droplets or oil bodies scattered throughout the avocado mesocarp, this tissue also contains an intensive system of idioblasts, which are plastids specializing in the storage of large lipid droplets(Platt and Thomson, 1992; Woolf et al., 2009). This further complicates the question of where the various fatty acid pathways occur, as it represents a novel system compared to more well-researched oilseed lipid storage systems(Theodoulou and Eastmond, 2012).

Interestingly, much of the work on the oleic to linoleic pathway has focused on the eukaryotic pathway in the endoplasmic reticulum (ER) and fatty acid desaturase-2 (FAD2), which has been found to have increased expression under cold stress or plant wounding conditions(Dar et al., 2017; Guan et al., 2012; Wang et al., 2004; Yang et al., 2024). Yet, in the current study, linoleic levels are higher at the warm LREC site than at the cool coastal SCREC site. This may indicate that this portion of the mesocarp lipidome is generated through the prokaryotic plastidial pathway with fatty acid desaturase-6 (FAD6). At the very least, it suggests a different environmental effect on the relationship between these fatty acids than has been reported in FAD2 studies.

The work presented herein builds upon existing literature and research on avocado fatty acid biosynthesis and mesocarp fatty acid profiles. It points towards a better understanding of the differences between early season and late season fruit, as well as between different avocado cultivars. Additionally, understanding the effects of environmental factors on avocado oil profiles and content will improve through these results. Researchers, managers, and growers will be better able to predict some aspects of fruit quality when planting in novel environments, such as those presented at the Central Valley site. These novel environments in avocado production are likely to increase due to the expansion into new cultivation areas and the alterations in existing planting sites as climate change becomes the new reality.

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Sensory Characteristics of the Three Cultivars from SCREC Site

Introduction

Although the avocado (Persea americana) is predominantly composed of lipids and its maturity is traditionally assessed by dry matter percentage, the determinants of acceptable eating quality extend beyond these primary components and the water-to-non-water content ratio. A significant focus of current research is the volatile and sensory profiles of this unique fruit. Notably, there are discernible differences in the eating quality among various avocado cultivars, even those originating from similar geographic regions and genetic backgrounds(Hausch et al., 2021).

Recent advancements have introduced numerous tools for studying the sensory attributes of avocado fruit. However, the role of a consistent and dedicated sensory panel remains crucial for accurately determining and describing fruit quality. Sensory trials typically employ two types of panels: trained panels, where panelists are calibrated using specific solutions, compounds, or foodstuffs, and consumer panels, which involve panelists who provide feedback based on their familiarity with the product without undergoing specialized training.

Despite the heavy reliance on sensory evaluations by panelists, ongoing research into the food chemistry underlying the eating experience has yielded valuable insights(Hausch et al., 2020; Obenland et al., 2012). Nevertheless, the volatiles identified in avocados exhibit unique expressions and interactions within this lipid-rich fruit, necessitating a deeper understanding of how these chemical compounds interact with each other and with the fruit mesocarp.

Materials and Methods

‘Eugenin’, ‘Flavia’, and ‘Hass’ fruits at the South Coast Research and Extension Center (SCREC) in Irvine, California were picked monthly from October 2021 to May 2022 and again in October 2022, November 2022, and March 2023 (Table 4.1). These fruits were driven up to UC Kearney Agricultural and Extension Center (UC KARE) the same day for initial evaluation and storage prior to the sensory panels. The e-tongue and volatiles analysis was only performed on the fruit harvested from October 2021 through May 2022 as the research group did not have access to the equipment after this period. The dry weight of each individual fruit was performed according to previous studies(Arpaia et al., 2001; Obenland et al., 2012; Woolf et al., 2003), and the dry matter percentage was obtained. After each harvest fruits were kept in cold storage at 5°C for a week and later were ripened with ethylene (0.3 mL/ min) at 20 °C until they reach 1-1.5 lb firmness. Firmness was measured using a manual penetrometer with an 8 mm tip-. Avocados were kept in cold storage until the sensory evaluation session.

Avocados were maintained at room temperature (21°C). Only the equatorial section of each fruit was peeled and cut into approximately 1 cm^3 cubes. Samples were presented to panelists in 3 ¼ fl oz paper cups, each coded with a unique three-digit number and placed in plastic trays. The presentation order was completely randomized, with codes changing across sessions. To cleanse their palates, panelists were provided with baby carrots and water, and were instructed to rinse their mouths after evaluating each sample.

The test design and data collection were facilitated using Compusense® software. Initially, panelists recorded their gender, age, ethnicity, and frequency of avocado consumption. Two sets of samples were presented. In the first set, panelists rated their liking of each sample on a 9-

point hedonic scale (1 = extremely dislike, 9 = extremely like) and described the samples by selecting all applicable attributes from a pre-defined list (Check-All-That-Apply, CATA) developed by a descriptive panel(Hausch et al., 2021), with modifications including "other" and "none" options. The second set employed a Duo-Trio test (100% balanced), where panelists identified the sample differing from a reference.

Analysis of variance (ANOVA, α = 0.05) and Fisher’s Least Significant Difference (LSD) test were conducted to determine significant differences between means.

Table 4.1. Harvest and Sensory Test Dates of ‘Eugenin’, ‘Flavia’, and ‘Hass’ fruit by Harvest Number, Both Years

Year	Harvest Number	Harvest Date	Sensory Test Date
2021-2022	1	10/26/2021	11/17/2021-11/19/2021
2021-2022	2	11/17/2021	12/12/2021-12/14/2021
2021-2022	3	12/13/2021	1/4/2022-1/6/2022
2021-2022	4	1/10/2022	1/25/2022-1/28/2022
2021-2022	5	2/14/2022	3/1/2022-3/3/2022
2021-2022	6	3/7/2022	3/13/2022-3/15/2022
2021-2022	7	4/11/2022	4/27/2022-4/29/2022
2021-2022	8	5/16/2022	5/27/2022-5/29/2022
2022-2023	1	10/18/2022	11/4/2022-1/6/2022
2022-2023	2	11/29/2022	12/8/2022-12/10/2022
2022-2023	4	3/13/2023	3/30/2023-4/1-2023

When preparing samples for for analysis with the Insent SA-402B electronic tongue, avocados were peeled, and 50 g of sections from multiple fruits were added to a blender. Heated deionized water (40°C, 200 mL) was added, and the mixture was homogenized until smooth. The homogenate was then centrifuged at 10,000 x g for 10 minutes. The middle layer of the resulting three layers was extracted for measurement. Samples were typically frozen to allow for batch processing.

Electronic tongue measurements were conducted using an Insent SA-402B, equipped with probes for umami/richness, bitterness, and astringency. The resulting data were transformed into taste values using Insent software. Only the umami/richness probe provided responses adequate for further analysis.

In preparation for volatile analysis of taste panel fruit, portions from five peeled fruits were combined and homogenized in a blender (20 g tissue + 40 mL water) at high speed for 30 seconds for each replication. A 5 mL aliquot of the homogenate was transferred to a 20 mL volatile vial and allowed to stand for 1.5 minutes. Subsequently, 5 mL of saturated $CaCl_2$ and a linalool internal standard were added, the vial was capped, and the mixture was vortexed for 10 seconds. Samples were then frozen until volatile analysis.

Upon thawing, samples were analyzed using solid-phase microextraction (SPME) and gas chromatography-mass spectrometry (GC-MS). The obtained values were adjusted using the internal standard. Compound identifications were performed using the mass spectrometry (MS) library and retention indices. Four replications were conducted for each cultivar across the first six harvests, except for 'Hass' in November 2021, where only three replications were performed.

Results

Taste panels were conducted across eight harvests for 'Eugenin', 'Flavia', and 'Hass' during the 2021-2022 year, and across three harvests in the 2022-2023 year. For comparative analyses, only equivalent harvests from both years were considered, corresponding to the months of October, November, and March. At the South Coast Research and Extension Center (SCREC) in Irvine, these periods represent an early maturity phase for 'Hass' in October and November, and a typical peak season in March.

Fruit characteristics varied significantly between the two years of the study, as evidenced by both analytical measurements and consumer panel acceptability ratings. Generally, fruit size for each variety was larger in the 2022-2023 sampling year compared to the 2021-2022 year, as indicated by analysis of variance (Table 4.2). Similarly, dry weight values were higher for all varieties in the second year across the October-November-March sampling period (Table 4.2).

When comparing fruit characteristics across varieties, the results were more complex. Over the six sampling periods, 'Hass' fruit was consistently neither the largest nor the heaviest in terms of dry weight. 'Flavia' and 'Eugenin' exhibited significantly higher mass than 'Hass' throughout the study period. Differences in dry matter percentage (DM%) between cultivars were more variable. In the four months with significant DM% differences, 'Flavia' had the highest values, although it was not always statistically distinct from 'Eugenin'.

Table 4.2. Fruit Attributes of Taste Panel Fruit: Mass, Dry Matter (DM%), and Acceptability

		CV	N	October Harvest	N	November Harvest	N	March Harvest
2021-2022	Fruit mass(g)	Eugenin	10	198.7±28.8[a]	12	202.7±15.6[a]	14	266.9±32.0[a]
		Flavia	13	184.2±28.8[a]	10	261.6±22.5[b]	11	233.4±29.1[a]
		Hass	12	124.9±20.2[b]	16	136.4±10.3[c]	10	161.9±12.5[b]
	DM%	Eugenin	10	17.03±0.92	12	18.25±0.87[a]	14	23.79±1.30
		Flavia	13	16.46±0.78	10	19.59±0.76[b]	11	21.55±1.77
		Hass	12	16.86±0.73	16	17.60±1.18[a]	10	21.46±1.17
	Acceptability	Eugenin	40	6.3±1.6	69	6.2±1.6	84	6.8±1.7
		Flavia	60	6.5±1.5	69	6.4±1.4	84	7.0±1.6
		Hass	80	6.4±1.7	69	6.1±1.7	84	6.7±1.6
2022-2023	Fruit mass(g)	Eugenin	20	278.3±41.3[a]	18	277.3±26.3[a]	21	317.7±40.7[a]
		Flavia	21	285.6±43.3[a]	20	283.7±27.8[a]	20	379.7±33.1[b]
		Hass	21	182.7±12.6[b]	21	192.2±27.0[b]	21	244.0±23.1[c]
	DM%	Eugenin	20	19.14±1.32[a]	18	22.13±2.12[a]	21	28.69±1.02[a]
		Flavia	21	20.05±1.24[b]	20	23.63±1.55[b]	20	29.32±2.00[a]
		Hass	21	18.60±0.77[a]	21	22.85±1.02[ab]	21	27.27±1.40[b]
	Acceptability	Eugenin	60	6.3±1.6	60	6.3±1.6	60	6.7±1.5
		Flavia	60	6.6±1.6	60	6.5±1.6	60	6.6±1.6
		Hass	60	5.9±1.8	60	6.0±1.7	60	6.6±1.3

[a]Different letters in each row denote significance as detected by a generalized linear model and followed by using Tukey's test at the α=0.05 level.

The hedonic ratings for acceptability between avocado varieties were influenced by the sampling year. In the first year, panelists consistently rated the varieties as equivalent across all three harvests. However, in the second year, panelists detected a slight difference between 'Flavia' and 'Hass' harvested in October 2021, with 'Flavia' receiving the highest scores. It is important to note that this distinction made by the October 2022 panelists between 'Flavia' and 'Hass' was not statistically significant at $\alpha \leq 0.05$. Although 'Flavia' generally scored higher in most taste panels, this difference was not statistically significant throughout the trial period. The largest difference in eating acceptability was observed at the earliest point in the 2022-2023 season ('Hass' vs. 'Flavia', $\alpha = 0.08$), a period when 'Flavia' is expected to have higher eating quality than 'Hass'. Future panels with a larger number of panelists are necessary to achieve statistically significant results in subsequent trials.

Regarding the interaction between dry matter percentage (DM%), fruit mass, and hedonic acceptability, it was observed that as dry weight increased over both seasons, fruit mass also increased (Fig. 4.1a, Fig. 4.1b). However, the hedonic acceptability responses from the panelists remained relatively high and consistent across all trial periods, showing little interaction between the substantially increasing dry weight and the modest increase (where present) in hedonic acceptability (Fig. 4.1c, Fig. 4.1d).

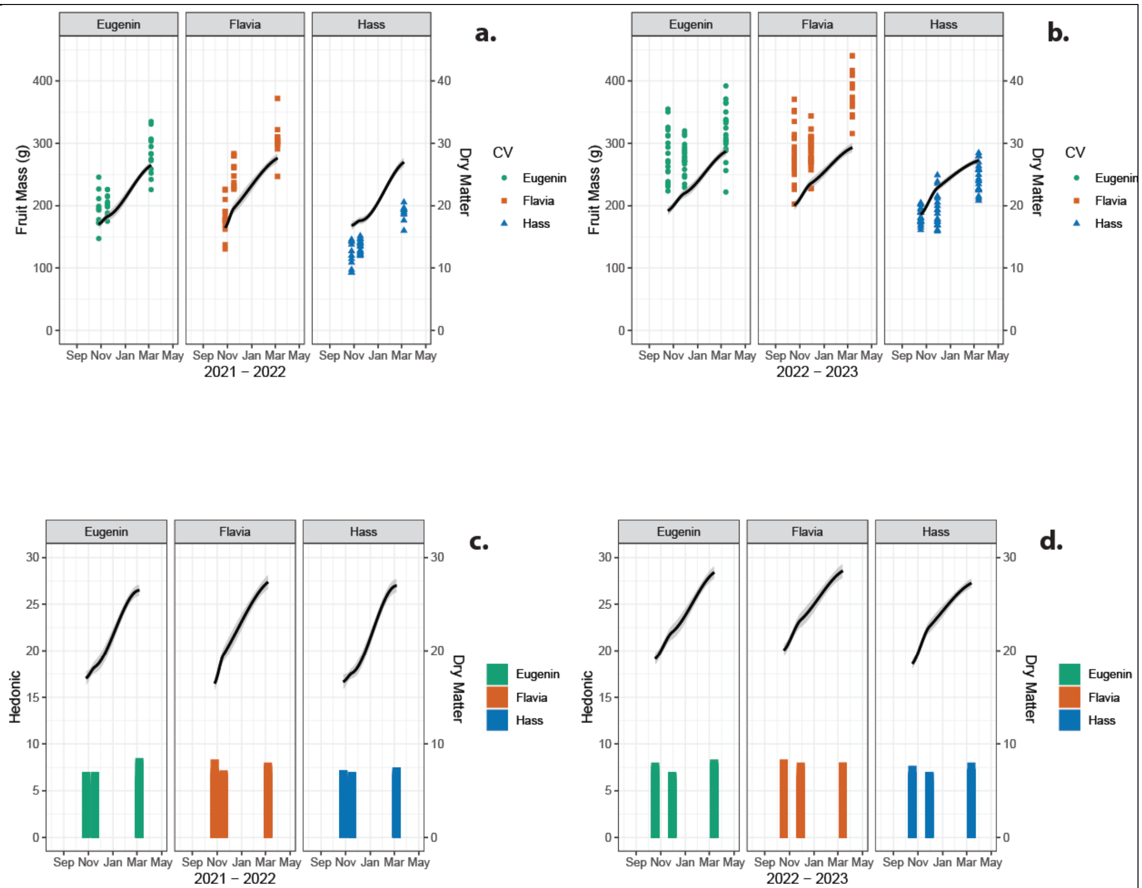


Fig. 4.1a-d Dry Matter in Sample Fruit as Related to Fruit Mass and Hedonic Acceptability Ratings
Dry matter line and fruit mass (gm) scatter graph (a, b) or dry matter line and hedonic bar graph (c, d) for fruit in the study. Fruit from the 2021-2022 season (a, c) and fruit from the 2022-2023 season (b, d) were weighed and a section of mesocarp collected for dry matter analysis before softening and used in taste panel trials. Number of fruit in this study noted in Table 4.2 Fruit Attributes, but each fruit was presented to multiple taste panelists; N for dry matter and mass range from 10-21, while N for hedonic ratings range from 40-80. Spearman's Rank Correlation shows significant interaction between dry matter and hedonic acceptability for 'Eugenin' in November 2021and March 2022. 'Hass' showed significant correlation in March 2023. Significant interaction between fruit mass and dry matter found in all cultivars in October 2021, for 'Hass' and 'Eugenin' in November 2021, for 'Hass' and 'Flavia' in March 2022, for 'Eugenin' in October 2022, and for 'Hass' in both November 2022 and March 2023.

Sensory descriptors for avocados can be categorized into two groups: flavor-related (Table 4.3) and texture-related (Table 4.4). Texture descriptors include "creamy," "dry," "mushy," "smooth," "stringy," and "watery." Flavor descriptors encompass "acid," "astringent," "bitter," "floral," "green/grassy," "herbaceous," "nutty," "oily," "salty," "savory," "spicy," and "sweet." While texture descriptors remained consistent between the two sampling years, flavor descriptors varied, with "herbaceous," "spicy," and "floral" added in the second year. However, "spicy" was excluded from the analysis due to a lack of response, and "floral" and "herbaceous" did not show sufficient correlation with acceptability but were retained in the correlation matrix for comprehensive analysis.

Comparing the two years of sensory trials, panelists in the first year detected no significant differences in the overall texture profiles of 'Eugenin,' 'Flavia,' and 'Hass' across all eight harvests, including the three harvests corresponding to October, November, and March of the second year (Fig. 4.2a). In contrast, in the second year, panelists discerned differences between 'Hass' and 'Flavia'/'Eugenin,' with increased reports of "watery" for October and November 2022. During this period, 'Eugenin' and 'Flavia' were more frequently described as "creamy" and, in October, as "smooth" compared to 'Hass' (Fig. 4.2b).

Table 4.3 Texture Check All That Apply (CATA) Panelist Responses, by Cultivar and Harvest, Both Years

	2021-2022	Percentage response		
		October	November	March
	CV	Harvest	Harvest	Harvest
Creamy	Eugenin	24.8^{a}	31.1^{a}	41.2^{a}
	Flavia	35.0^{a}	30.2^{a}	47.7^{b}
	Hass	28.1^{b}	22.3^{b}	36.7^{a}
Mushy	Eugenin	17.3^{a}	18.0	12.4
	Flavia	14.4^{b}	12.1	10.4
	Hass	15.4^{b}	14.2	12.6
Smooth	Eugenin	32.1	29.8^{a}	37.8^{a}
	Flavia	33.0	33.2^{b}	36.6^{a}
	Hass	26.6	25.8^{c}	28.2^{b}
Stringy	Eugenin	3.0	2.8	0.9
	Flavia	3.4	4.3	1.0
	Hass	4.2	4.4	4.0
Dry	Eugenin	4.0	0.9	3.2
	Flavia	2.3	4.9	2.0
	Hass	1.0	5.3	6.4
Watery	Eugenin	18.7^{a}	14.4^{a}	3.2^{ab}
	Flavia	11.9^{b}	11.8^{a}	1.1^{a}
	Hass	22.6^{ab}	27.2^{b}	8.2^{b}
None	Eugenin	0.0	2.9	1.2
	Flavia	0.0	3.5	1.1
	Hass	2.1	0.9	3.9

	2022-2023	Percentage response		
		October	November	March
	CV	Harvest	Harvest	Harvest
Creamy	Eugenin	30.8^{ab}	34.0^{a}	41.2
	Flavia	33.5^{a}	35.7^{a}	42.1
	Hass	23.4^{b}	25.3^{b}	38.4
Mushy	Eugenin	8.3	13.2	11.8
	Flavia	10.6	20.9	18.3
	Hass	10.2	14.1	11.7
Smooth	Eugenin	35.5^{a}	31.0	35.0
	Flavia	37.3^{a}	31.6	27.3
	Hass	25.5^{b}	25.3	33.1
Stringy	Eugenin	1.1	1.0	1.2
	Flavia	1.0	0.0	0.0
	Hass	4.0	2.0	1.0
Dry	Eugenin	5.5	1.1	6.8
	Flavia	6.8	3.9	4.5
	Hass	6.4	6.1	5.3
Watery	Eugenin	17.7^{a}	16.8^{a}	3.0
	Flavia	9.9^{a}	5.0^{b}	4.4
	Hass	30.5^{b}	24.2^{c}	8.5
None	Eugenin	1.1	3.1	1.0
	Flavia	0.9	3.0	3.4
	Hass	0.0	3.0	2.1

Panelist's Check All That Apply (CATA) scores, as a percentage of all panelists over the three day taste panel period for October, November and March harvested fruit, both years.[a]Different letters within a column demonstrates significance with Chi Squared Z test at the α=0.05 level.

The selection of flavor descriptors by panelists is more complex than that of texture descriptors, partly due to the different descriptors available and chosen by panelists across the two years (Table 4.4). Similar to the first-year texture descriptors, panelists did not find significant differences between 'Eugenin,' 'Flavia,' and 'Hass' during this period (Fig. 4.3a). However, in the second year, panelists identified differences in the October and November panels, associating 'Flavia' more frequently with "Nutty" in the first and last months, and generally finding 'Flavia' to be less "Green grassy" (Fig. 4.3b).

Table 4.4 Flavor Check All That Apply (CATA) Panelist Responses, by Cultivar and Harvest, Both Years

	2021-2022	Percentage response		
		October	November	March
	CV	Harvest	Harvest	Harvest
Astringent	Eugenin	1.7	4.8	4.6
	Flavia	1.7	2.6	2.5
	Hass	1.7	5.8	6.1
Bitter	Eugenin	4.8	6.6	3.4
	Flavia	5.7	6.2	3.4
	Hass	1.7	9.4	7.4
Oily	Eugenin	13.2	13.6 ^{a}	14.7 ^{a}
	Flavia	12.6	14.8 ^{a}	14.7 ^{a}
	Hass	14.3	8.7 ^{b}	8.2 ^{b}
Green grassy	Eugenin	26.2 ^{a}	22.2	9.0 ^{a}
	Flavia	25.3 ^{b}	17.0	12.3 ^{ab}
	Hass	29.7 ^{b}	21.7	18.4 ^{b}
Nutty	Eugenin	17.5 ^{a}	18.7 ^{ab}	24.2
	Flavia	15.2 ^{b}	22.1 ^{a}	23.6
	Hass	17.1 ^{b}	14.6 ^{b}	21.8
Savory	Eugenin	16.2 ^{a}	14.8	17.3
	Flavia	18.0 ^{ab}	13.6	19.8
	Hass	16.8 ^{b}	12.8	13.6
Herbaceus	Eugenin	NA	NA	NA
	Flavia	NA	NA	NA
	Hass	NA	NA	NA
Spicy	Eugenin	NA	NA	NA
	Flavia	NA	NA	NA
	Hass	NA	NA	NA
Floral	Eugenin	NA	NA	NA
	Flavia	NA	NA	NA
	Hass	NA	NA	NA
Sweet	Eugenin	4.5	2.5	6.8 ^{ab}
	Flavia	7.4	6.4	9.1 ^{a}
	Hass	7.9	7.9	4.6 ^{b}
Acid	Eugenin	2.8	3.2	4.4
	Flavia	3.6	2.4	3.4
	Hass	1.0	3.3	4.4
Salty	Eugenin	6.2	7.0	9.9
	Flavia	8.7	7.3	9.5
	Hass	7.2	6.8	9.8
None	Eugenin	6.9	6.8	5.7
	Flavia	1.9	7.7	1.8
	Hass	2.6	9.1	5.7

	2022-2023	Percentage response		
		October	November	March
	CV	Harvest	Harvest	Harvest
Astringent	Eugenin	5.5	6.1	6.2
	Flavia	7.6	4.5	8.2
	Hass	4.9	6.4	8.9
Bitter	Eugenin	6.8	3.6	3.5
	Flavia	4.5	6.8	6.5
	Hass	4.0	6.6	5.4
Oily	Eugenin	12.9	12.5	14.1 ^{a}
	Flavia	16.3	15.3	11.8 ^{a}
	Hass	15.0	10.8	6.4 ^{b}
Green grassy	Eugenin	26.5	18.9 ^{ab}	8.9
	Flavia	19.7	11.9 ^{a}	10.4
	Hass	25.0	24.3 ^{b}	10.9
Nutty	Eugenin	10.6 ^{a}	23.3	18.3 ^{a}
	Flavia	19.1 ^{b}	21.0	25.9 ^{b}
	Hass	14.4 ^{ab}	19.6	19.5 ^{a}
Savory	Eugenin	10.6	12.2	12.6
	Flavia	11.8	13.3	9.4
	Hass	9.8	11.2	11.8
Herbaceus	Eugenin	0.0	0.0	0.9
	Flavia	0.0	0.0	2.6
	Hass	0.0	0.0	3.3
Spicy	Eugenin	0.0	0.0	0.0
	Flavia	0.0	0.0	0.0
	Hass	0.0	0.0	0.0
Floral	Eugenin	0.0	0.0	7.1
	Flavia	0.0	0.0	3.5
	Hass	0.0	0.0	4.3
Sweet	Eugenin	4.7	4.5	8.8
	Flavia	4.1	4.9	4.8
	Hass	5.8	2.6	7.6
Acid	Eugenin	0.0	0.0	0.0
	Flavia	0.0	0.0	0.0
	Hass	5.8	6.2	2.3
Salty	Eugenin	10.1	6.9	8.0
	Flavia	8.7	10.1	8.8
	Hass	7.9	5.6	6.7
None	Eugenin	9.2	8.1	10.5
	Flavia	5.3	5.3	6.5
	Hass	7.4	6.7	13.0

Panelist's Check All That Apply (CATA) scores, as a percentage of all panelists over the three day taste panel period for October, November and March harvested fruit, both years.[a] Different letters within a column demonstrates significance with Chi Squared Z test at the α=0.05 level.

Fig. 4.2a Radar Plots of Texture Check All That Apply (CATA) Ratings, October 2021 Through March 2022

Percentage of panelists responding with listed descriptors for taste panel fruit in the first year of sensory trials. Significant differences detected with Chi Squared Z test at the α=0.05 level for Creamy and Watery in all months, Mushy in October, and Smooth in November and March.

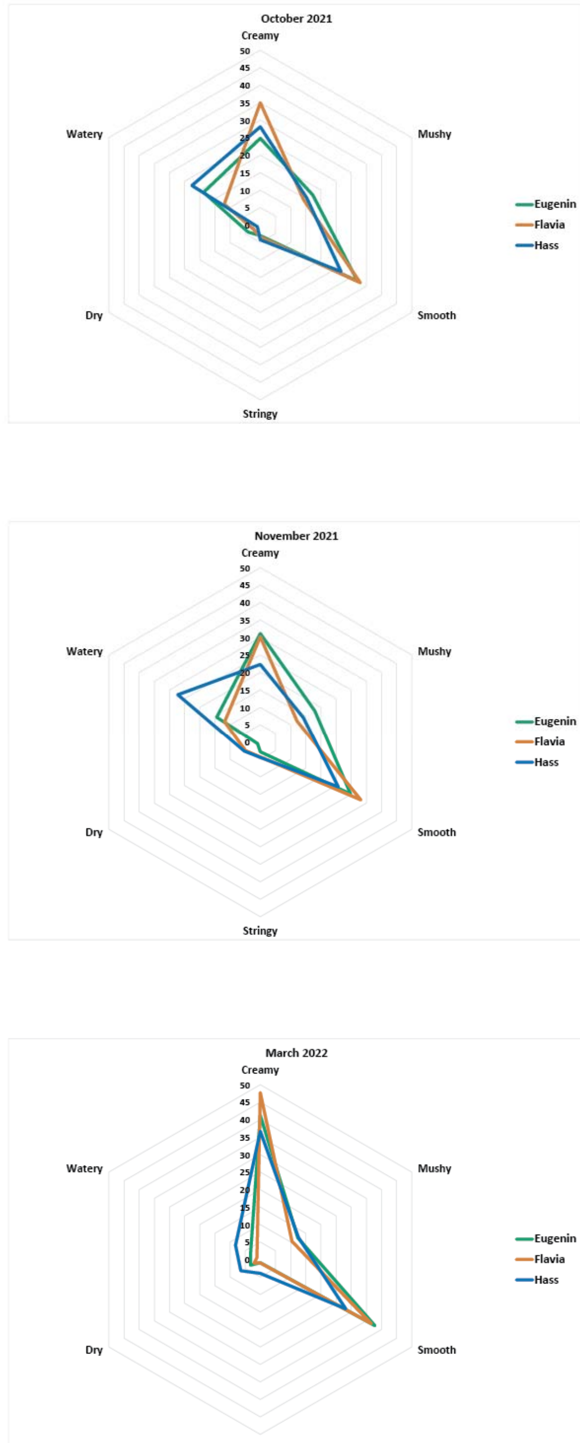
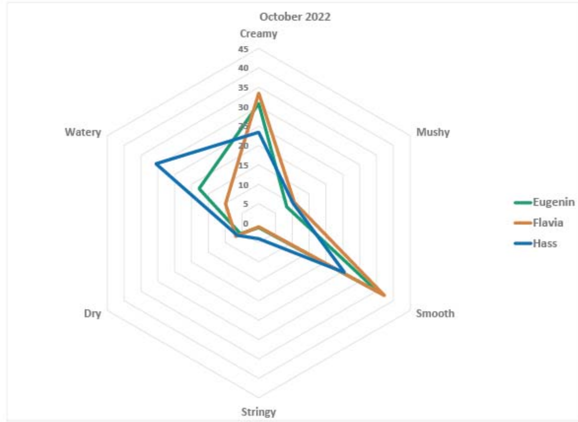
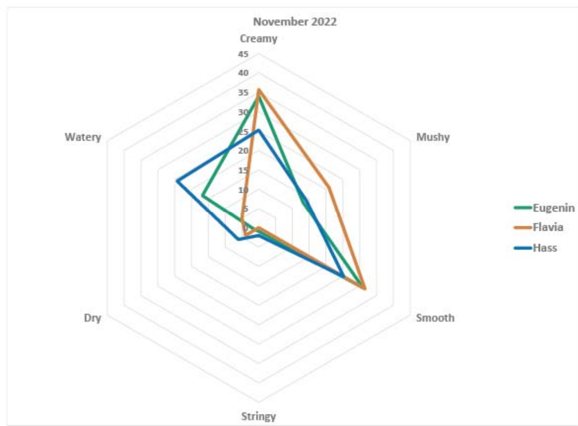
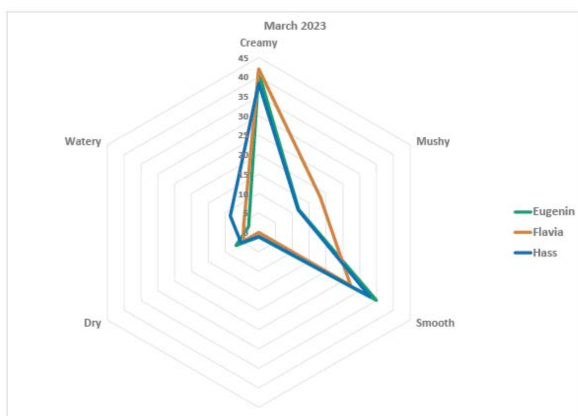


Fig. 4.2b Radar Plots of Texture Check All That Apply (CATA) Ratings, October 2022 Through March 2023

Percentage of panelists responding with listed descriptors for taste panel fruit in the second year of sensory trials. Significant differences detected with Chi Squared Z test at the α=0.05 level for Creamy and Watery in October and November, and Smooth in October.







The early fruit maturation season typically shows the highest mentions of grassiness or wateriness in fruit flavor (Fig. 4.3a, Fig. 4.3b) and texture (Fig. 4.2a, Fig. 4.2b) profiles, which was expected. Notably, in the 2022-2023 period, 'Hass' maintained a relatively high green/grassy perception, ranging from 25.0% to 24.2% from October to November 2022. Panelists generally rated 'Flavia' as more “Nutty”, less “Green grassy”, and occasionally more “Oily” than 'Hass' or 'Eugenin' (Fig. 4.3b). The response to 'Eugenin' was more intermediate, sometimes resembling 'Hass' and other times 'Flavia.'

Certain Check-All-That-Apply (CATA) descriptors are more positively associated with fruit acceptability (Fig. 4.4, Fig. 4.5), while others are negatively associated (Fig. 4.6, Fig. 4.7). For fruit texture (Fig. 4.6, Fig. 4.7), "Creamy" and "Smooth" were positively associated with acceptability, whereas "Watery" and "Stringy" were negatively associated. For flavor descriptors (Fig. 4.4, Fig. 4.5), "Green grassy" and "Bitter" were negatively associated with acceptability, while "Nutty," "Savory," "Salty," and "Sweet" were positively associated.

Fig. 4.3a Radar Plots of Flavor Check All That Apply (CATA) Ratings, October 2021 Through March 2022

Percentage of panelists responding with listed descriptors for taste panel fruit in the first year of sensory trials. Significant differences detected with Chi Squared Z test at the α=0.05 level for Green grassy in October and March, Nutty in October and November, Oily in November and March, and Sweet in March.

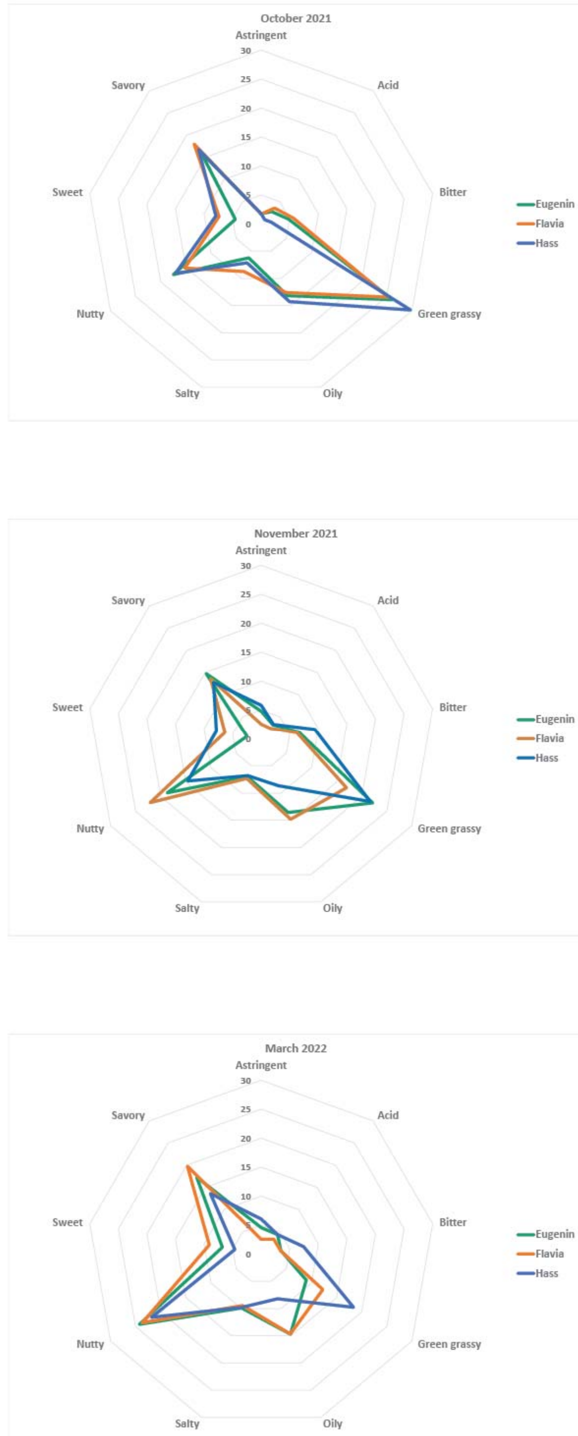
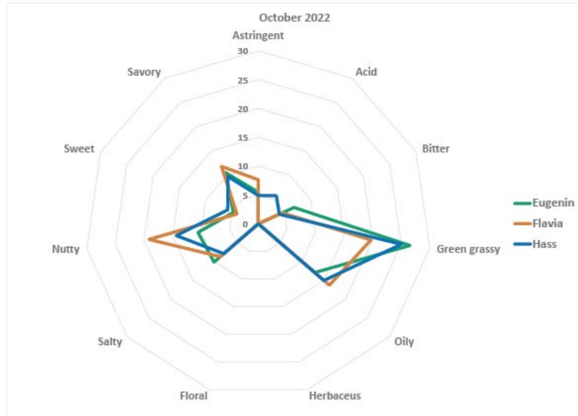
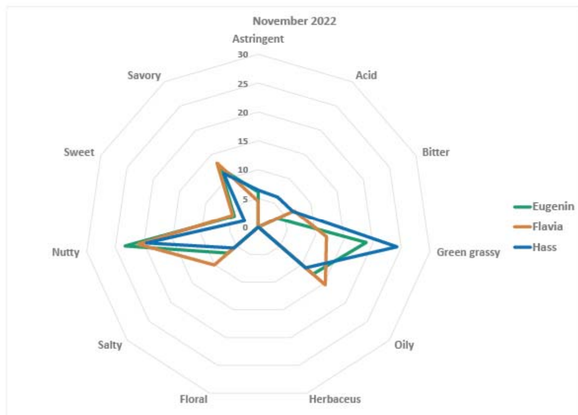
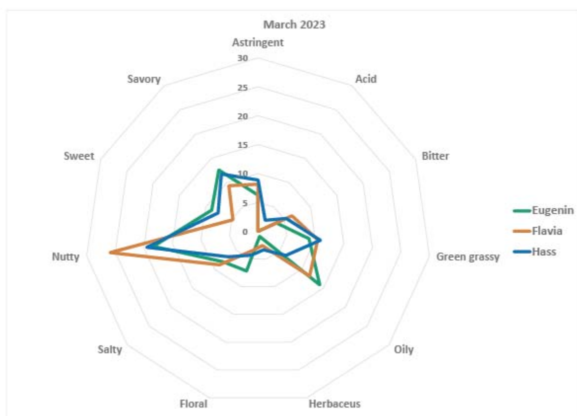


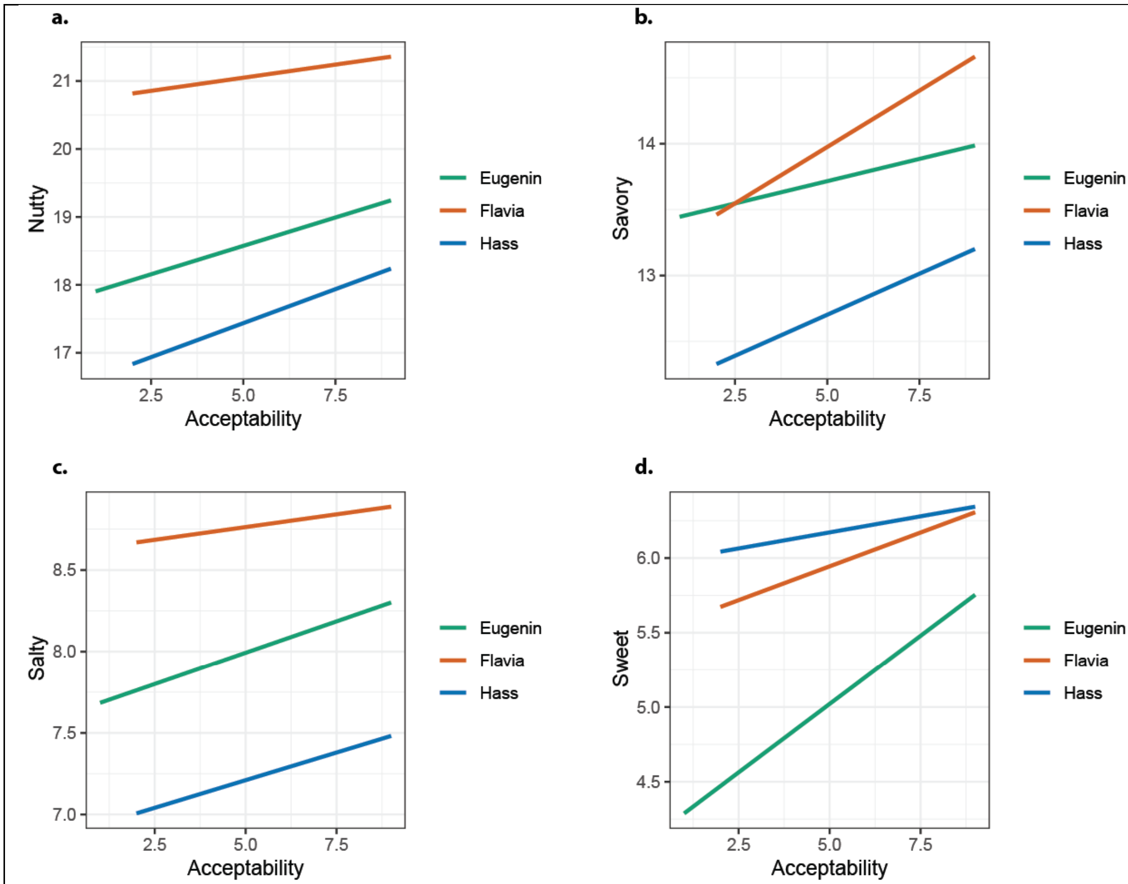
Fig. 4.3b Radar Plots of Flavor Check All That Apply (CATA) Ratings, October 2022 Through March 2023

Percentage of panelists responding with listed descriptors for taste panel fruit in the second year of sensory trials. Significant differences detected with Chi Squared Z test at the α=0.05 level for Green grassy in October, Nutty in October and March, and Oily in March.









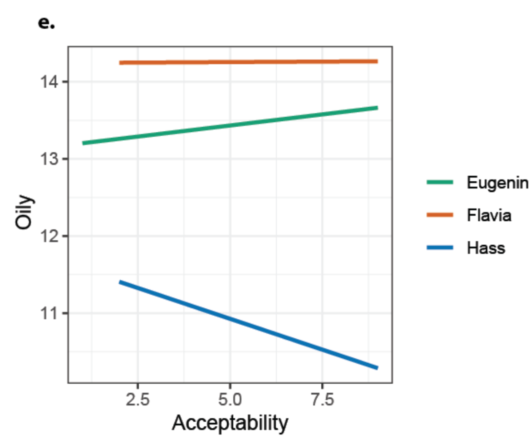


Fig. 4.4a-e Flavor Descriptors Positively Correlated with Increasing Acceptability
Correlation coefficients reported for cultivars combined; no statistically significant cultivar effects detected between hedonic acceptability and descriptors detected by panelists. Salty vs Acceptability: $r = 0.61$ (a), Nutty vs Acceptability ($r = 0.54$)(b), Savory vs Acceptability: $r = 0.49$ (c), Sweet vs Acceptability: $r = 0.48$ (d), Oily vs Acceptability: $r = 0.11$ (e).

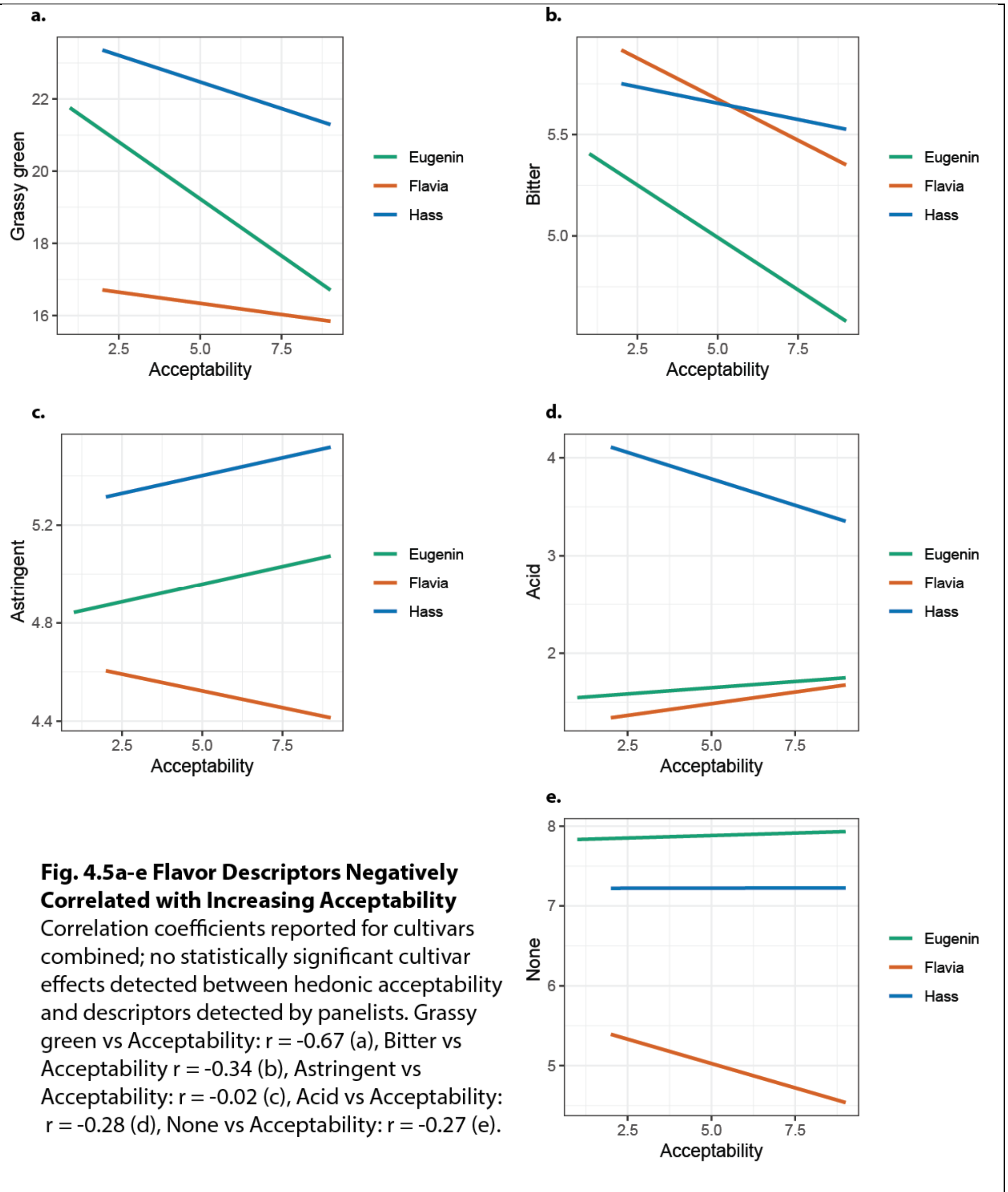


Fig. 4.5a-e Flavor Descriptors Negatively Correlated with Increasing Acceptability
Correlation coefficients reported for cultivars combined; no statistically significant cultivar effects detected between hedonic acceptability and descriptors detected by panelists. Grassy green vs Acceptability: r = -0.67 (a), Bitter vs Acceptability r = -0.34 (b), Astringent vs Acceptability: r = -0.02 (c), Acid vs Acceptability: r = -0.28 (d), None vs Acceptability: r = -0.27 (e).

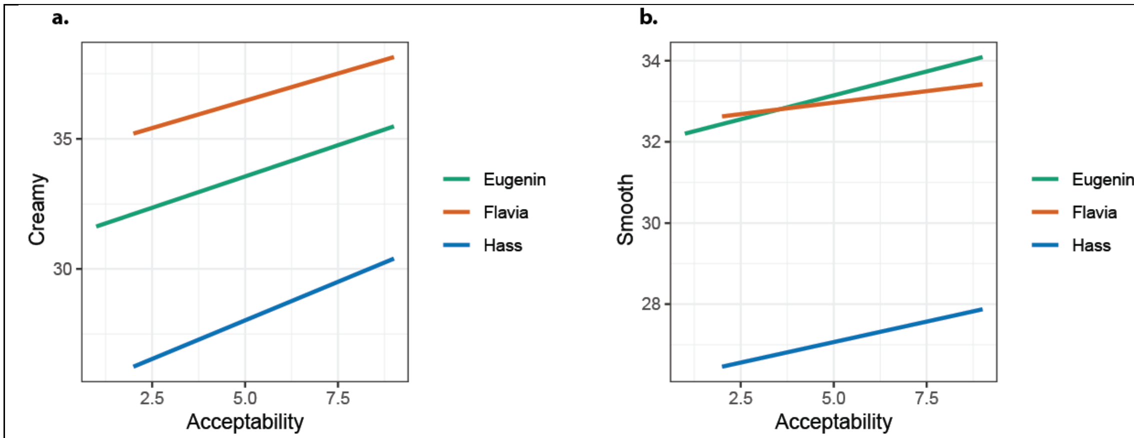
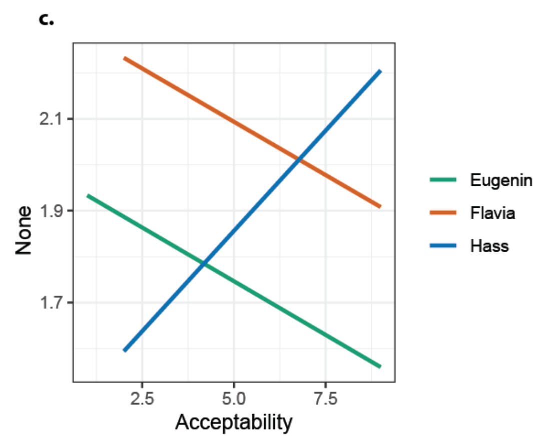


Fig. 4.6a-c Texture Descriptors Positively Correlated with Increasing Acceptability
Correlation coefficients reported for cultivars combined; no statistically significant cultivar effects detected between hedonic acceptability and descriptors detected by panelists. Creamy vs Acceptability: r = 0.91 (a), Smooth vs Acceptability r = 0.70 (b), None vs Acceptability: r = 0.05 (c).



Correlations within the Check-All-That-Apply (CATA) texture descriptors provided substantial insights, revealing a consistent pattern. The most pronounced negative correlations were observed between “Creamy” and “Smooth”, descriptors that were most positively associated with acceptability and “Stringy” and “Watery”, which were most negatively associated with acceptability (Fig. 4.8). Notably, the texture descriptor "Mushy" exhibited either negative correlations or negligible correlations with all other CATA texture descriptors. This suggests that "Mushy" may be a redundant descriptor and could be excluded from future CATA lists, alternately, it may be that “Mushy” represents a textural descriptor that contains a negative association while overlapping with other descriptors in the perceptual space. This descriptor, "Mushy", requires further consideration in how it is used in future panels.

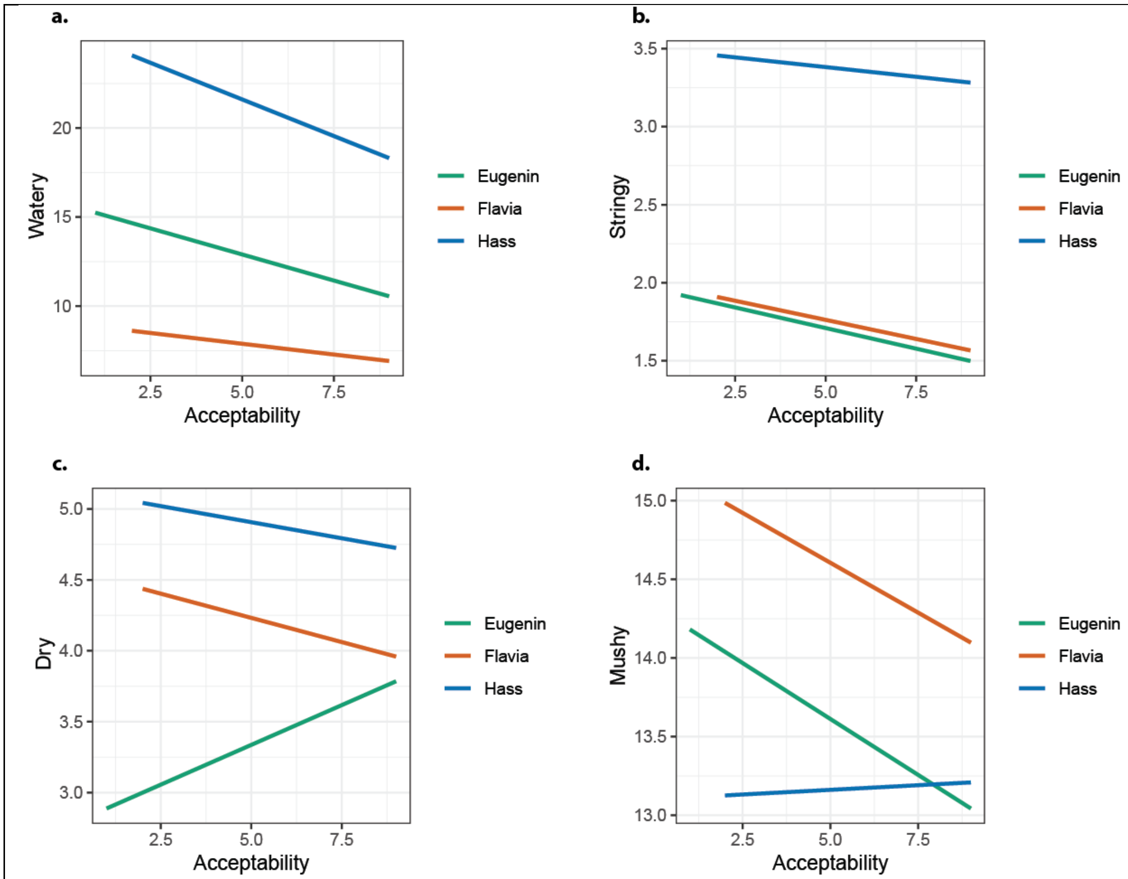
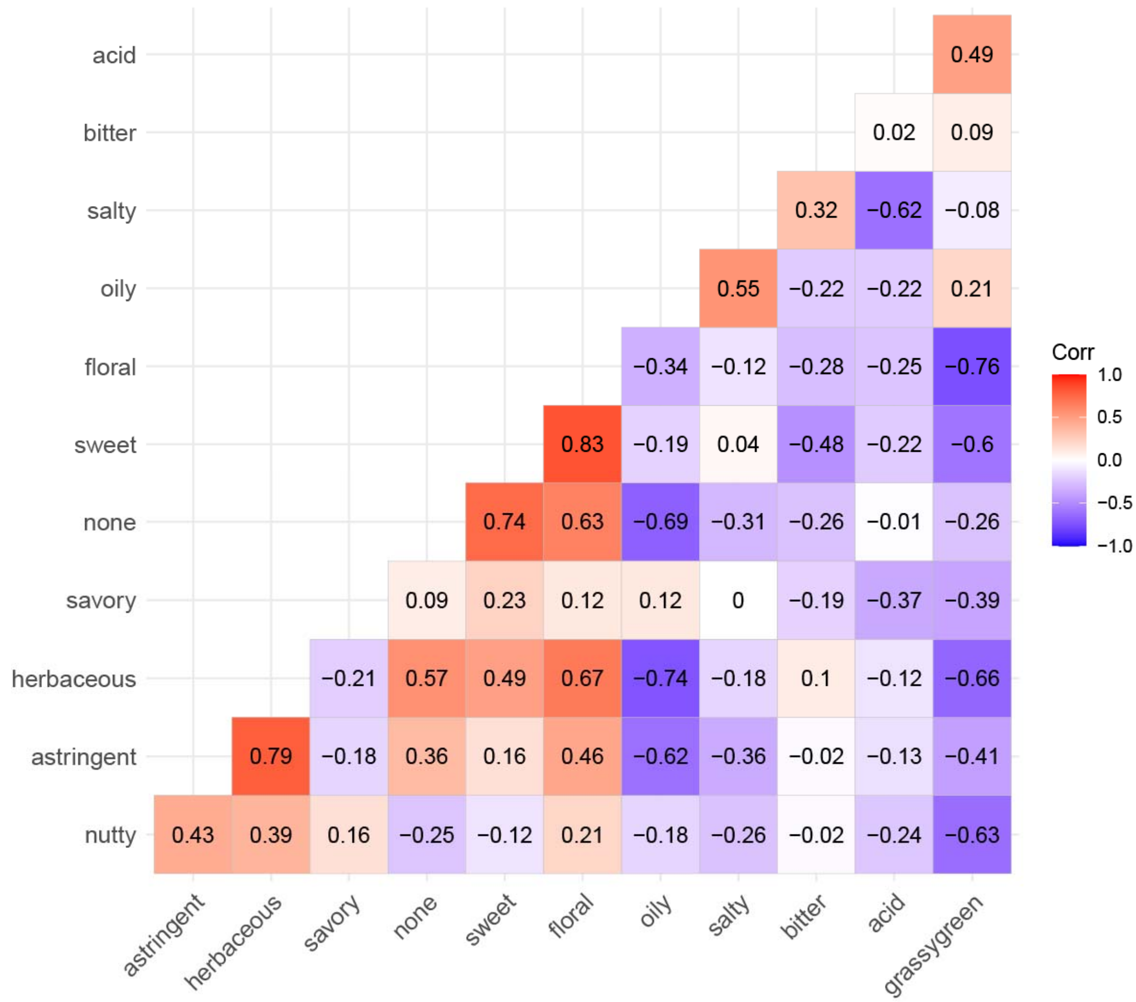


Fig. 4.7a-d Texture Descriptors Negatively Correlated with Increasing Acceptability
Correlation coefficients reported for cultivars combined; no statistically significant cultivar effects detected between hedonic acceptability and descriptors detected by panelists. Watery vs Acceptability: r = -0.91 (a), Stringy vs Acceptability r = -0.46 (b), Dry vs Acceptability: r = -0.10 (c), Mushy vs Acceptability: r = -0.16 (d).

Fig. 4.8 Texture Check All That Apply (CATA) Correlations

Fig. 4.9 Flavor Check All That Apply (CATA) Correlations



Flavor correlations (Fig. 4.9) present a more complex analysis due to the greater number of CATA flavor descriptors compared to texture descriptors, and the variability in descriptor sets across different sampling years (Table 4.4). Despite this complexity, it is evident that the descriptor "Grassy green" exhibited the strongest overall negative association with all other descriptors, except for the mixed response descriptors "Oily" and "Acid". Interestingly, the descriptor "Bitter" showed a more neutral correlation with other descriptors. Conversely, "Oily" demonstrated the strongest negative correlation with “Herbaceous” and “Astringent”, as well as with the “None” (no response) category. These findings suggest two distinct pathways through which fruit can be perceived as less acceptable: one characterized by “Oily” and the other by “Grassy green”. This understanding would have “Grassy green” occupy a similar sensory space as “Herbaceous” or “Floral”, but with stronger negative correlations to acceptability.

Duo-Trio: Duo-trio tests were conducted in both years, with a more extensive range of cultivars tested in the second year (Table 4.5). In the first year, panelists generally did not perform above chance in detecting differences between ‘Eugenin’, ‘Flavia’, and ‘Hass’; out of 22 tests conducted from October 2021 through May 2022, panelists performed above chance in only 3 tests. However, in the second year, panelists were able to distinguish between these three cultivars in five out of nine tests across three harvests. Additionally, in the 2022-2023 duo-trio tests, ‘Hass’ was compared with various other UC breeding program varieties. Panelists consistently identified differences between ‘Hass’ and these varieties, as well as among the varieties themselves when ‘Hass’ was not included. These results highlight both the degree of similarity among these somaclonal varieties and their discernible differences.

Table 4.5 Duo-Trio Panel Data, October 2021-August 2023

Comparison	Harvest	N	Correct	Incorrect	d'	p-value	Significant at 0.5
Hass vs Flavia	October 2021	20	11	9	0.76	0.41	NO
Hass vs Eugenin	October 2021	20	8	12	0	0.87	NO
Flavia vs Eugenin	October 2021	20	10	10	0	0.59	NO
Hass vs Flavia	November 2021	20	12	8	1.12	0.25	NO
Hass vs Eugenin	November 2021	25	14	11	0.84	0.35	NO
Flavia vs Eugenin	November 2021	24	16	8	1.52	0.08	NO
Hass vs Flavia	December 2021	25	14	11	0.84	0.35	NO
Hass vs Eugenin	December 2021	20	10	10	0	0.59	NO
Flavia vs Eugenin	December 2021	20	9	11	0	0.75	NO
Hass vs Flavia	January 2022	20	13	7	1.42	0.13	NO
Hass vs Eugenin	January 2022	20	11	9	0.76	0.41	NO
Flavia vs Eugenin	January 2022	20	12	8	1.12	0.25	NO
Hass vs Flavia[a]	February 2022	20	16	4	2.36	0.01	YES
Hass vs Eugenin	February 2022	14	7	7	0	0.6	NO
Flavia vs Eugenin	February 2022	20	7	13	0	0.94	NO
Hass vs Flavia	March 2022	18	12	6	1.52	0.12	NO
Hass vs Eugenin	March 2022	21	13	8	1.23	0.19	NO
Flavia vs Eugenin	March 2022	19	9	10	0	0.68	NO
Hass vs Flavia	April 2022	20	14	6	1.72	0.06	YES
Hass vs Eugenin	April 2022	20	15	5	2.02	0.02	YES
Flavia vs Eugenin	April 2022	20	9	11	0	0.75	NO
Hass vs Eugenin	May 2022	20	10	10	0	0.59	NO
Hass vs Flavia	October 2022	20	9	11	0	0.75	NO
Hass vs Eugenin	October 2022	20	10	10	0	0.59	NO
Flavia vs Eugenin	October 2022	20	16	4	2.36	0.01	YES
Hass vs Flavia	November 2022	20	13	7	1.42	0.13	YES
Hass vs Eugenin	November 2022	20	14	6	1.72	0.06	YES
Flavia vs Eugenin	November 2022	20	9	11	0	0.75	NO
Hass vs Flavia	March 2023	20	10	10	0	0.59	NO
Hass vs Eugenin	March 2023	20	11	9	0.76	0.41	YES
Flavia vs Eugenin	March 2023	20	12	8	1.12	0.25	YES
Hass vs UC V02[b]	January 2023	16	13	3	2.45	0.01	YES
UC V02 vs UC V03	January 2023	20	15	5	2.02	0.02	YES
Hass vs BL516	May 2023	19	14	5	1.94	0.03	YES
UC V01 vs Hass	May 2023	20	12	8	1.12	0.25	YES
UC V01 vs BL516	May 2023	20	12	8	1.12	0.25	YES
UC V01 vs BL516	June 2023	20	14	6	1.72	0.06	YES
UC V01 vs Hass	June 2023	19	13	6	1.62	0.08	YES
Hass vs BL516	June 2023	20	14	6	1.72	0.06	YES
BL516 vs Hass	August 2023	20	15	5	2.02	0.02	YES
BL516 vs UC V01	August 2023	20	15	5	2.02	0.02	YES
UC V01 vs Hass	August 2023	20	15	5	2.02	0.02	YES

[a]shaded rows represent panelists discerning a difference between cultivars with statistical significance determined by a T-test with Cohen's d. [b]Cultivars UCV01, UCV02, UC Vo3 and BL516 are distant progeny cultivars of Hass and are included to demonstrate that panelists can consistently find a difference between related cultivars much more readily than they can between cultivars that arise from putative somatic mutations.

Volatiles and E Tongue: The volatiles and E Tongue analysis were conducted exclusively during the first year, 2021-2022 (Fig. 4.10, Fig. 4.11, Fig. 4.12a-b, Table 4.6a-b). The E Tongue was able to differentiate between the three cultivars in terms of Umami, but not in richness. Unfortunately, the E Tongue operates as a “black box,” making it impossible to quantify its qualitative ratings. Notably, the detected difference in Umami appears to be concentrated around the ‘Hass’ fruit collected on January 10, 2022, as this is where ‘Hass’ ratings diverge and significantly dip below all other ratings, whether analyzed by cultivar or by harvest date.

Fig. 4.10 Etongue Richness Ratings

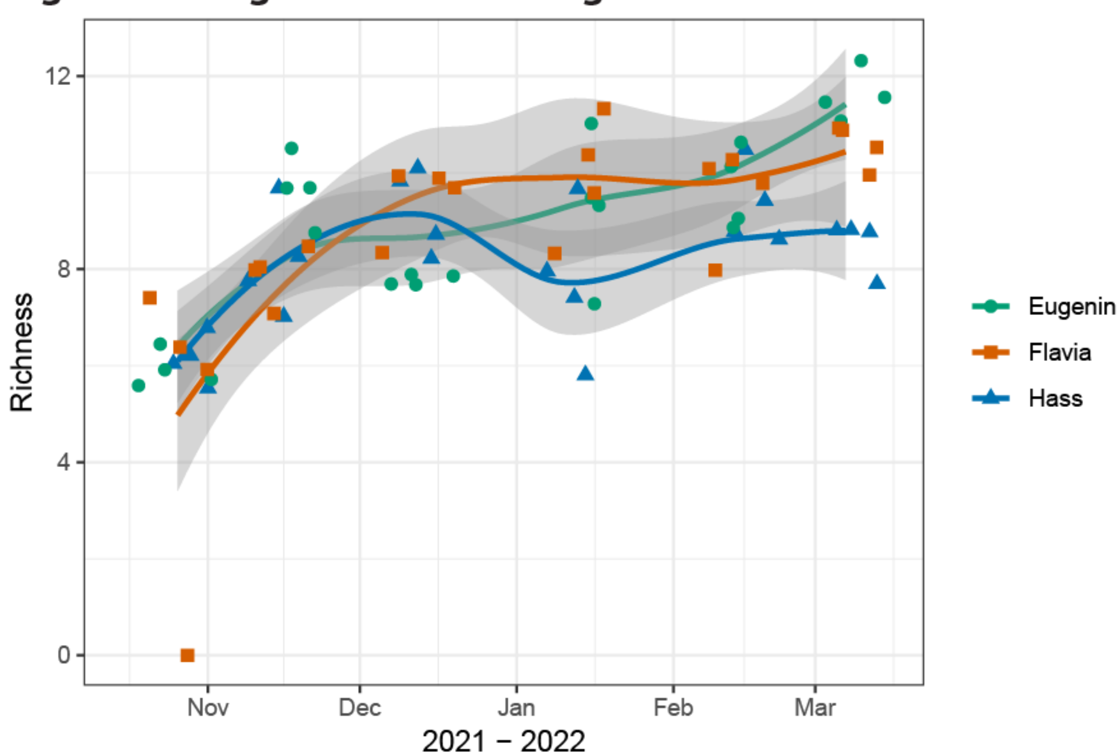
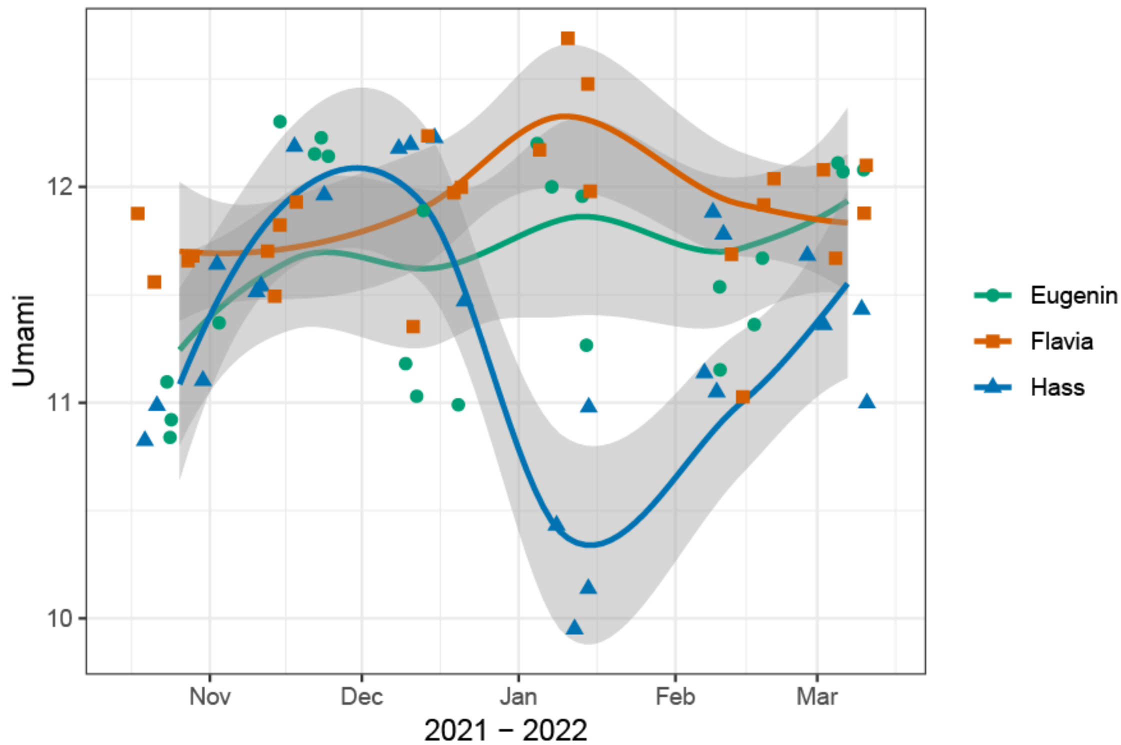


Fig. 4.11 Etongue Umami Ratings



There was no statistically significant correlation between the three cultivars and any of the volatile profiles detected. However, volatile trends generally followed those reported in previous research(Hausch et al., 2020; Obenland et al., 2012), with high levels of hexanal and, to a lesser extent, E-2-hexenal in the early season likely contributing to “green” or “grassy” notes for panelists in the October and November taste panels (Fig.4.12a-b). Notably, the November 2021 scores for 'Eugenin' fruit were strikingly different from those of the other two cultivars in that month, as well as from the 'Eugenin' scores in October and December (Table 4.6a-b).

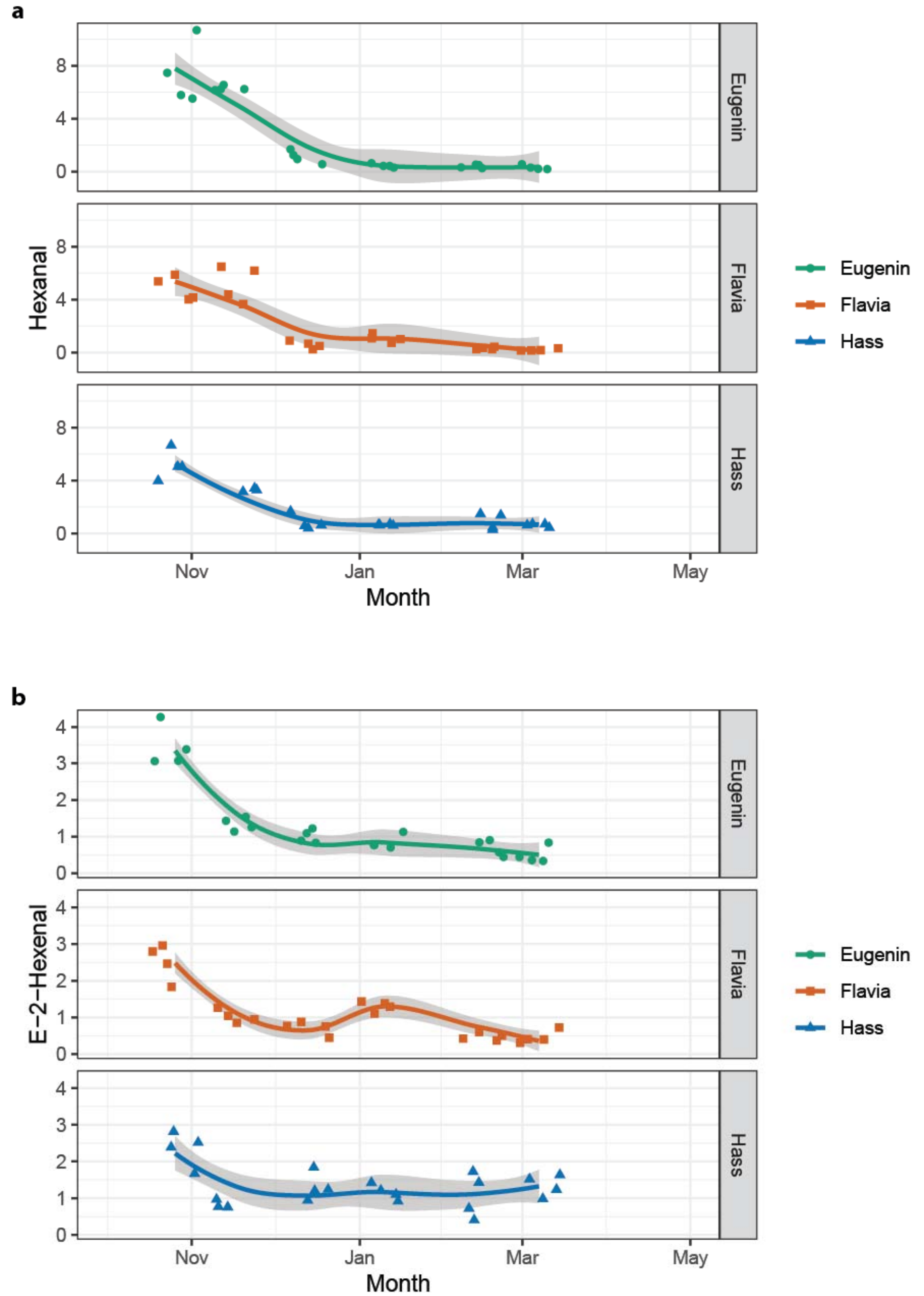


Fig. 4.12a-b Hexanal or E-2-Hexenal Levels by Cultivar, October 2021-March 2022
Hexanal (a) or E-2-Hexenal (b) over the sampling period in the first year of sensory panels. No significant differences between cultivars detected; all followed same general trend of decreasing volumes of these grassy leaf volatiles detected as the fruit increases in maturity over the season.

Table 4.6a Volatiles Detected, October 2021 - March 2022

Identity	CV	October	November	December	January	February	March
2-decenal	Eugenin	0.0032	0.7338	n.d.	0.0007	0.0069	0.0011
	Flavia	0.0075	0.0025	n.d.	n.d.	n.d.	n.d.
	Hass	0.0361	0.0026	0.0028	0.0014	n.d.	0.0052
heptanal	Eugenin	0.0284	0.8009	0.0077	0.0100	0.0066	0.0032
	Flavia	0.0270	0.0192	0.0070	0.0087	0.0039	0.0027
	Hass	0.0421	0.0249	0.0064	0.0079	0.0067	0.0089
hexanoic acid	Eugenin	0.0164	0.9797	0.0041	0.0018	0.0100	0.0050
	Flavia	0.0186	0.0119	0.0015	0.0034	0.0034	0.0034
	Hass	0.0497	0.0098	0.0020	0.0044	0.0041	0.0101
2-decenal	Eugenin	0.0032	0.7338	n.d.	0.0007	0.0069	0.0011
	Flavia	0.0075	0.0025	n.d.	n.d.	n.d.	n.d.
	Hass	0.0361	0.0026	0.0028	0.0014	n.d.	0.0052
heptanol	Eugenin	0.0037	0.0712	0.0016	n.d.	0.0016	0.0014
	Flavia	0.0028	0.0040	0.0013	0.0009	0.0012	0.0021
	Hass	0.0074	0.0033	0.0018	0.0012	0.0008	0.0022
octanal	Eugenin	0.0196	1.9886	0.0025	0.0046	0.0072	0.0023
	Flavia	0.0164	0.0112	0.0026	0.0036	0.0011	0.0018
	Hass	0.0456	0.0135	0.0033	0.0050	0.0029	0.0094
decanal	Eugenin	0.0027	0.0517	n.d.	n.d.	0.0002	0.0001
	Flavia	0.0027	0.0022	n.d.	n.d.	n.d.	n.d.
	Hass	0.0043	0.0023	0.0002	n.d.	0.0002	n.d.
nonanal	Eugenin	0.0306	1.3643	0.0070	0.0070	0.0123	0.0098
	Flavia	0.0289	0.0256	0.0055	0.0069	0.0048	0.0071
	Hass	0.0620	0.0347	0.0072	0.0096	0.0070	0.0163
octanol	Eugenin	0.0069	0.1268	0.0016	0.0014	0.0026	0.0016
	Flavia	0.0047	0.0046	0.0017	0.0017	0.0012	0.0004
	Hass	0.0115	0.0048	0.0022	0.0016	0.0016	0.0010
pentanal	Eugenin	0.0401	0.1493	0.0272	0.0351	0.0411	0.0216
	Flavia	0.0511	0.0350	0.0191	0.0409	0.0362	0.0349
	Hass	0.0287	0.0408	0.0351	0.0380	0.0308	0.0499
E-2-heptenal	Eugenin	0.0797	0.1866	0.0226	0.0238	0.0358	0.0265
	Flavia	0.0798	0.0378	0.0196	0.0291	0.0279	0.0263
	Hass	0.0927	0.0355	0.0286	0.0259	0.0308	0.0343
propanal	Eugenin	0.0010	0.0042	0.0001	n.d.	n.d.	n.d.
	Flavia	0.0007	0.0012	0.0003	0.0002	n.d.	n.d.
	Hass	0.0011	0.0005	n.d.	n.d.	n.d.	n.d.
2-pentyl-furan	Eugenin	0.0542	0.0913	0.0388	0.0312	0.0320	0.0300
	Flavia	0.0397	0.0377	0.0283	0.0335	0.0319	0.0337
	Hass	0.0297	0.0246	0.0349	0.0334	0.0335	0.0319
butanal	Eugenin	0.0009	0.0066	0.0013	0.0014	0.0016	0.0015
	Flavia	0.0012	0.0003	0.0017	0.0022	n.d.	n.d.
	Hass	0.0010	n.d.	0.0032	0.0027	0.0023	0.0027
trans-alpha-bergamotene	Eugenin	0.0007	0.0720	0.0003	n.d.	n.d.	n.d.
	Flavia	0.0004	0.0031	n.d.	n.d.	n.d.	n.d.
	Hass	0.0006	0.0091	n.d.	n.d.	n.d.	n.d.

Table 4.6b Volatiles Detected, October 2021 - March 2022

Identity	CV	October	November	December	January	February	March
methyl acetate	Eugenin	0.0029	0.0418	0.0039	0.0048	0.0059	0.0025
	Flavia	0.0041	0.0060	0.0053	0.0050	0.0054	0.0031
	Hass	0.0038	0.0102	0.0090	0.0051	0.0069	0.0079
trans-caryophyllene	Eugenin	0.0020	0.0915	0.0005	0.0002	n.d.	n.d.
	Flavia	0.0026	0.0080	n.d.	n.d.	n.d.	n.d.
	Hass	0.0020	0.0335	0.0007	0.0002	0.0004	n.d.
p-cymene	Eugenin	0.0044	0.0129	0.0024	0.0016	0.0017	0.0012
	Flavia	0.0029	0.0025	0.0016	0.0021	0.0017	0.0016
	Hass	0.0026	0.0112	0.0014	0.0021	0.0020	0.0022
limonene	Eugenin	0.1054	0.6393	0.1092	0.1087	0.0777	0.0822
	Flavia	0.0838	0.0750	0.0813	0.0744	0.0901	0.0961
	Hass	0.0601	1.6668	0.0792	0.1032	0.1363	0.0743
1-pentanol	Eugenin	0.0068	0.0155	0.0104	0.0085	0.0079	0.0074
	Flavia	0.0049	0.0177	0.0110	0.0070	0.0138	0.0062
	Hass	0.0126	0.0167	0.0069	0.0108	0.0102	0.0054
2,3-octanedione	Eugenin	0.0127	0.0155	0.0015	0.0011	0.0016	0.0004
	Flavia	0.0109	0.0108	0.0019	0.0022	0.0010	0.0007
	Hass	0.0140	0.0075	0.0015	0.0020	0.0019	0.0012
hexanal	Eugenin	7.3657	6.2930	1.1143	0.4513	0.4034	0.3193
	Flavia	4.8607	5.1801	0.5784	1.0726	0.3208	0.2031
	Hass	5.1779	3.2800	0.8172	0.6493	0.8966	0.6201
3-octen-2-one	Eugenin	0.0102	0.0082	0.0004	0.0001	n.d.	0.0002
	Flavia	0.0153	0.0044	0.0003	0.0004	n.d.	n.d.
	Hass	0.0151	0.0028	0.0005	n.d.	n.d.	n.d.
E-2-hexenal	Eugenin	3.4433	1.3414	1.0100	0.8422	0.6923	0.4947
	Flavia	2.5133	1.0279	0.7116	1.3039	0.4745	0.4601
	Hass	2.3476	0.8313	1.3063	1.1633	1.0699	1.3431
2-propanone	Eugenin	0.0257	0.0088	0.0059	0.0081	0.0081	0.0102
	Flavia	0.0253	0.0379	0.0056	0.0054	0.0098	0.0107
	Hass	0.0195	0.0071	0.0062	0.0073	0.0092	0.0107
E-2-hexen-1-ol	Eugenin	0.0783	0.0683	0.0164	0.0104	0.0123	0.0121
	Flavia	0.0455	0.0550	0.0083	0.0159	0.0111	0.0077
	Hass	0.1800	0.0265	0.0214	0.0175	0.0173	0.0155
hexanol	Eugenin	0.0338	0.0348	0.0004	n.d.	0.0002	0.0011
	Flavia	0.0108	0.0401	0.0003	0.0004	0.0002	n.d.
	Hass	0.1834	0.0219	0.0023	n.d.	0.0012	n.d.
Z-3-hexenol	Eugenin	0.1066	0.0914	0.0002	n.d.	n.d.	0.0003
	Flavia	0.0322	0.1533	n.d.	0.0003	0.0003	n.d.
	Hass	0.1172	0.1696	0.0030	n.d.	0.0002	n.d.
sabinene	Eugenin	0.0075	0.0061	0.0044	0.0153	0.0152	0.0141
	Flavia	0.0076	0.0149	0.0134	0.0145	0.0151	0.0130
	Hass	0.0060	0.0154	0.0141	0.0106	0.0177	0.0108
B-myrcene	Eugenin	0.1862	0.1150	0.1655	0.1393	0.1405	0.1383
	Flavia	0.1480	0.1556	0.1247	0.1430	0.1487	0.1552
	Hass	0.1215	0.1278	0.1511	0.1481	0.1474	0.1439
unknown	Eugenin	0.0387	0.0123	0.0322	0.0159	0.0128	0.0061
	Flavia	0.0378	0.0164	0.0118	0.0295	0.0072	0.0054
	Hass	0.0464	0.0137	0.0204	0.0289	0.0200	0.0146

Correlations among the various volatiles followed a general trend, with most aldehydes, ranging from three-carbon propanal to ten-carbon decanal, showing strong associations with each other and with related alcohols such as octanol and heptanol. Interestingly, the six-carbon volatile hexenal was less strongly correlated with this aldehyde group. Hexanal and its related compounds—E-2-hexenal, E-2-hexen-1-ol, Z-3-hexenol, and hexanol—also exhibited weak or no correlation with the larger group of aldehydes. However, these six-carbon species were relatively well correlated with each other and with 2,3-octanedione and 3-octene-2-one. Previous research on avocado volatiles and a web search indicate that this group of related compounds is associated with "green" or "grassy" odors and flavors (Table 4.7). It is likely that this "grassy" grouping of volatiles is prominent in early-season fruit and becomes less prevalent later in the season as other aldehydes dominate. This is supported by Table 4.6a-b, which show that while many volatiles remain relatively constant throughout the season, the "green" or "grassy"-associated volatiles decline rapidly after the initial panels in 2021. Additionally, the pair of monoterpenoids, β-myrcene and sabinene, were most negatively correlated with almost all other volatiles. Unlike other terpenoids such as limonene and p-cymene, β-myrcene and sabinene were either negatively correlated or not correlated with the large group of non-hexenal-related aldehydes.

Fig. 4.13 Detected Volatiles Correlation Plot

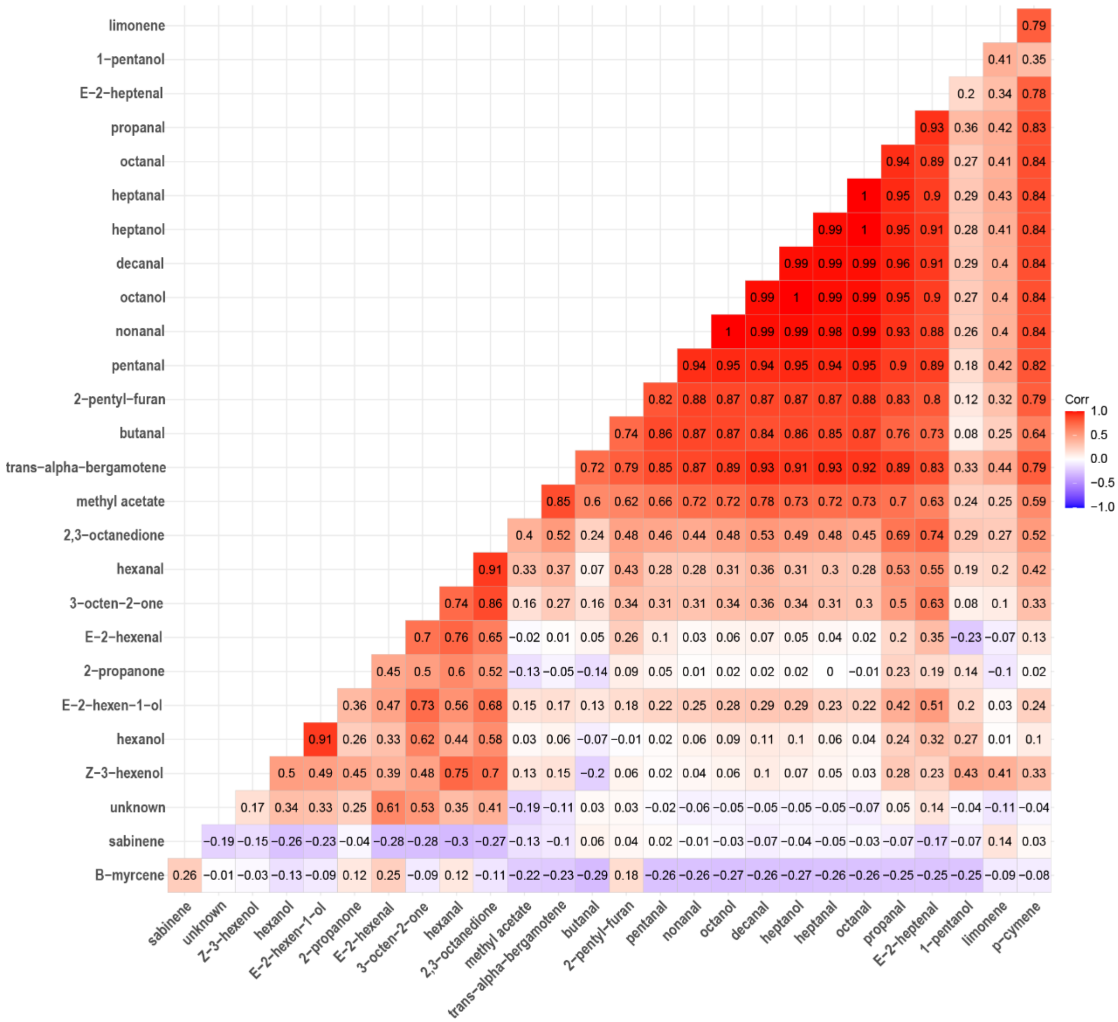


Table 4.7 Descriptors and Attributes of Detected Volatiles Encountered in Literature or From Selected Websites Online

Chemical name	Descriptors/Attributes
E-2-hexenal	Fruit/sour candy[a]; Strong fruity, green, vegetable-like aroma[c]
hexenal	Grassy[a]; Fresh, green[b]
E-2-heptenal	Cheese biscuits, crackers[a]; Green, fruity[b]; Pungent green, somewhat fatty aroma[c]
decanal	Oily/oxidized[a]; Floral-fatty odor[c]; Citrus odor[c]; Similar to that of an orange peel[c]; Penetrating, sweet, waxy, floral, citrus, pronounced fatty odor that develops a floral character on dilution[c]; Fatty, citrus-like odor[c]; Sharp, orange flavor[c]; Floral, fried, orange peel, penetrating, tallow[d]
nonanal	Cucumber/fatty[a]; Orange-rose odor[c]; Floral, waxy, green[c]; Fat, floral, green, lemon[d]
2-pentylfuran	Rancid[a]; Fruity aroma[c]; Butter, floral, fruit, green bean[d]
octanal	Grain/fatty[a]; Strong, fruity odor[c]; Fatty, citrus, honey odor on dilution[c]; Pungent odor; citrus-like on dilution[c]; Taste characteristics at 25 ppm: aldehyde, green with a peely citrus orange note[c]; Citrus, fat, green, oil, pungent[d]
B-myrcene	Musty, wet soil[b]; Pleasant[c]; Terpene odor[c]; Sweet, citrus[c]; Balsamic, fruit, geranium, herb, must[d]
methyl acetate	Ether, sweet[b]; Pleasant odor[c]; Fragrant, fruity odor[c]; Fleeting, fruity taste[c]; Ester, green[d]
2, 3-octanedione	Fruity nutty aroma[c]; green[d]
2 propanone	Fruity odor[c];Characteristic odor; Pungent, sweetish taste[c]; Pungent[d]
p-cymene	Sweetish aromatic odor[c]; When pure, has a weak citrus odor[c]; Mild pleasant odor[c]; Citrus, Fresh, Solvent[d]
propanal	Suffocating, fruity[c]; Characteristic odor similar to acetaldehyde[c]; Pungent, unpleasant[c]; Choking odor[c]; Floral, pungent, solvent[d]
butanal	Characteristic, pungent, aldehyde odor[c]; Banana, green, pungent[d]
heptanal	Fatty, pungent odor[c]; Penetrating fruity odor[c]; Fatty taste[c]; Citrus, fat, green, nut[d]
limonene	Pleasant lemon-like[c]; Sweet, citrus taste[c]; Citrus, mint[d]
1 pentanol	Characteristic fusel-like odor[c]; Mild odor[c]; Burning taste[c]; Balsamic, fruit, green, pungent, yeast[d]
hexanol	Characteristic, sweet alcohol, pleasant[c]; Fatty, fruity[c]; Aromatic flavor[c]; Banana, flower, grass, herb[d]
Z 3 hexenol	Powerful grassy-green odor[c]; Strong odor resembling that of isoamyl alcohol, approaching the odor of green leaves when highly dilute[c]; Characteristic odor of freshly cut grass[c]; Green, grassy, melon rind-like with a pungent freshness[c]; Fresh, green, raw fruity with a pungent depth[c]; Grass, green fruit, green leaf, herb, unripe banana[d]
E 2 hexen 1 ol	Strong, fruity-green aroma[c]; Blue cheese, vegetable[d]
3 octen 2 one	Pleasant odor[c]; Earthy, fruity blueberry note[c]
heptanol	Fragrant[c]; Faint, aromatic, fatty[c]; Pungent, spicy taste[c]
octanol	Fresh orange rose odor[c]; Penetrating aromatic odor[c]; Oily, sweet, slightly herbaceous taste[c]; Bitter almond, burnt matches, fat, floral[d]
trans alpha bergamotene	Fruity[d]
pentanal	Powerful, acrid, pungent odor[c]; Strong, acrid, pungent odor[c]; Warm, slightly fruity, & nut-like at low levels[c]; Pleasant, chocolate aroma & taste[c]; ... It has sharp, penetrating flavor[c]; Almond, bitter, malt, oil, pungent[d]
hexanal	Characteristic fruity odor (on dilution)[c]; Strong, green grass odorc; Sharp, aldehyde odor[c]; Characeteristic fruity taste (on dilution)[c]; Green, woody, vegetative, apple, grassy, citrus and orange with a fresh lingering aftertaste[c]; Apple, fat, fresh, green, oil[d]
sabinene	one of the chemical compounds that contributes to the spiciness of black pepper and is a major constituent of carrot seed oil. It also occurs in tea tree oil at a low concentration. It is also present in the essential oil obtained from nutmeg, Laurus nobilis, and Clausena anisata[e]

Odor and flavor descriptors sourced from Obenland et al 2012[a] and Hausch et al 2020[b] were generated on an Agilent 6890N with a sniff port. Additionally, descriptors are reported here from the websites https://pubchem.ncbi.nlm.nih.gov/[c], https://www.femaflavor.org/[d], and https://www.ebi.ac.uk/chebi/init.do[e]

Unfortunately, the equipment necessary to conduct these analyses was not accessible in the second year. Therefore, it is not possible to speculate on whether these findings would differ in another year, as observed with the taste panel and analytical fruit quality results.

Discussion

A key difference between 'Flavia' (and 'Eugenin') and 'Hass' is the purported earlier ripening and eating acceptability of the former compared to the latter. The patent application for 'Flavia' highlights earlier dry weight accumulation as a key distinction from 'Hass'(Schiappacasse Macchiavello, 2014). However, there is significant variance in dry weight data across different environments and cultivars, suggesting that this metric may not be the most reliable or sole indicator of eating quality or acceptability.

When comparing other metrics to eating quality or sensory attributes, similar issues arise with the available methods and measurements. Notably, differences between hedonic acceptability scores are often small, necessitating a large and carefully structured panel of participants. As panelists gained experience and increased exposure to the avocados in the trial, their ability to discern differences between cultivars improved. Despite this, linking panelists' Check-All-That-Apply (CATA) ratings to detected and identified volatile organic compounds remains challenging, as much of the existing sensory chemistry research is difficult to apply to avocado mesocarp. The most detectable signal in this attribute data was the presence of green leafy volatiles across cultivars early in the season, which correlated with less favorable hedonic ratings. Conversely, correlating positive hedonic ratings with volatiles proved more elusive in this subtle-tasting, lipid-rich fruit(Hausch et al., 2020; Pedreschi et al., 2019).

An additional issue arises with using ‘Hass’ as a control or benchmark. For new cultivars derived from somatic mutations of the ‘Hass’ lineage, this may not pose a problem. However, duo-trio data (Table 4.5) indicate that non-trained consumer panelists can easily distinguish between ‘Hass’ and other cultivars. Often, these other cultivars have similar hedonic acceptability to ‘Hass’ despite being easily distinguishable. This raises the question: when comparing a given fruit to ‘Hass’, is the fruit’s eating quality being measured objectively, or is it being assessed based on its similarity to ‘Hass’ in specific categories? For instance, nuttiness is a descriptor frequently applied to ‘Hass’ avocados and positively correlates with hedonic acceptability, but this correlation is weaker in non-‘Hass’ avocados(Asensio and Arpaia, 2023). All CATA attributes must be considered in relation to the acceptability of the fruit being tested, but this is challenging as it is impossible to test fruit from the same location sampled in different months within the same panel.

Another notable finding is the correlation plot of volatiles (Fig. 4.13), where volatiles containing a six-carbon chain are correlated separately from other volatiles. Many of these volatiles are classified as Green Leafy Volatiles (GLVs) and are synthesized from polyunsaturated fatty acids linoleic and linolenic in the plastids(Picazo-Aragonés et al., 2020). This suggests either a more active plastidial system in the lipidome of early developing fruit or an increasingly active system outside the plastids in the endoplasmic reticulum or other regions of the lipid body as the fruit matures. Additionally, avocado mesocarp idioblasts, which are another (plastidial) part of the fruit’s lipid storage system, remain poorly understood in terms of their differentiation over the course of fruit development, despite documented structural aspects(Platt and Thomson, 1992; Platt-Aloia and Thomson, 1981; Yang et al., 2018). Idioblasts’ internal lipid biosynthesis pathways could be a further unstudied wrinkle in understanding the formation of the avocado mesocarp lipid body. Furthermore, the dynamic changes in the lipidome during fruit maturation, particularly in relation to the activity of plastidial and extraplastidial systems, warrant more detailed investigation to elucidate their roles in volatile compound synthesis and overall fruit development.

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Conclusion

There are two fundamental aspects of a successfully maturing avocado fruit: the accumulation of non-water (primarily lipid-based) components to the point of ultimate maturation, and the fruit remaining attached to the tree long enough to reach its intended composition. In California, two basic ecotypes are considered: the rapidly maturing *drymifolia* and the long-hanging *guatemalensis*. The 'Hass' avocado combines the best of both worlds, reaching perceived maturity faster than a traditional *guatemalensis* type while persisting on the tree much longer than the typical *drymifolia*, providing farmers with a wide window for harvesting.

An issue in perception arises from sampling to determine a commodity threshold value, which is not necessarily biologically or evolutionarily based. The mechanisms behind the commodity fruit reaching this threshold are not fully understood. Additionally, the focus on reaching this commodity threshold has guided past sampling efforts, with dry weights generally considered only when the fruit is in a relatively ambiguous developmental state towards the end of its time on the tree, biasing the reported data. There has been no consideration of the factors midway between flowering/fruit set and this later ambiguous phase, which set up the later dry weight profile trends. By starting fatty acid profiling in August (roughly 120-150 days after flowering), only the end of the early fruit development phase is captured, midway through a period of summer environmental factors that differ significantly between sites. Sampling earlier is challenging due to the smaller size and different physical makeup of the fruit. It is almost like comparing two completely different types of fruit rather than two different developmental phases of the same fruit. Additionally, there may not be enough mesocarp in the small summer fruit/fruitlet to collect a sample of meaningful size.

Tracking fruit from flowering or early fruitlet to fully mature fruit ready for harvest has been time-consuming and impractical due to high fruit drop and differences in sample mass. This process is interesting biologically and physiologically but not currently valuable to farmers or consumers who primarily want to know when a fruit is ready to pick, sell, or eat. However, identifying an earlier environmental effect that informs the timing of the later arriving maturity metric could provide a valuable tool for farmers, handlers, and consumers. It would also aid in predicting a tighter range for the maturity index of a given cultivar's fruit in new or novel environments, whether these represent separate geographic locations or climate change-driven differences in a given location. Much work remains to be done, with significant practical applications beyond the strictly scientific understanding of the process.

From a sensory science perspective, it is unnecessary to imagine a length or line segment of the fruit development timeline that represents "perfect maturity". The timeline of acceptability or favorable eating conditions incorporates elements beyond the evolutionary tract of fruit development, as human taste and preferences influence the scoring of a physiological process that evolved without their input. Acceptability can be viewed as a separate floating bar above the actual timeline of fruit maturation rather than as a segment between too early and too late (Fig. 5.1). The relationship between these segments differs with different people involved in deciding what is acceptable and with different avocado variety's inherent traits overlapping between immature and overmature. Acceptability exists on a separate scale or plane from that of fruit physiology; they have strong interaction but need to be understood as separately operating realms.

Fruit traits associated with immaturity include improper or incomplete softening, overly green or grassy volatile profiles, a tendency for the mesocarp to be perceived as watery (likely due to an inability to properly emulsify), shriveling, and failure of the peel/epicarp to separate easily from the mesocarp, along with specific postharvest physiological issues. Traits associated with overmature fruit include a dry, pasty, doughy, and/or oily taste or texture, rancidity, a dry and crumbling or brittle peel that clings to the stylar end of the mesocarp, and a separate set of postharvest physiological issues from those of immature fruit.

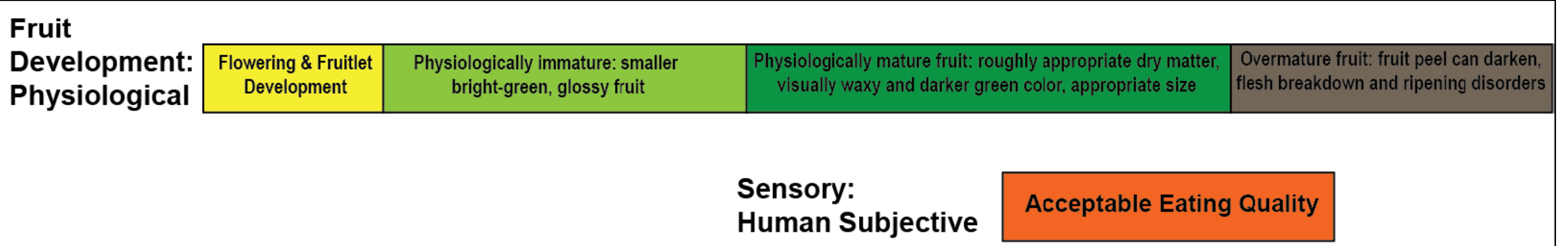

Fig. 5.1 Visualizing Acceptable Eating Quality vs. Fruit Physiological Maturity
Although fruit development and maturation can be thought of as existing on a physiological continuum, the sensory aspect is on a separate plane not least because it contains significant interaction with human subjective experience. This Acceptable Eating Quality plane can differ in spatial location and size in relation to the Physiological Fruit Development plane's stages depending on not only the cultivar of avocado, preharvest growing conditions, and postharvest handling, but also on the subjective perception of the panelist or person tasting the fruit or sample. Thus, some fruit will be considered more acceptable with a low dry weight or oil content, a more watery unstructured mesocarp, and some visual shriveling of the peel whereas another fruit will score an equal acceptability with a very high dry weight content, a drier more structured texture to the mesocarp, and a drier, brittle peel. Volatile profiles are also important to consider and, as an example, the focus on Hass' "nuttiness" as a positive trait to search for in other cultivars may lead them to be perceived as less acceptable when relevant "nuttiness" volatiles are used as a correlating metric to quantify eating quality.

It may be posited that hedonic acceptability cannot be directly detected solely through the examination of fruit physiological traits, whether individually or collectively, as hedonic acceptability is a conjunction of fruit physiology and human sensory experience. Nonetheless, a collection of physiological traits can provide significant insights into whether a given fruit would be considered acceptable by a specific audience or panel. The challenge lies in determining whether the selected physiological traits are appropriate for all possible cultivars or environments.

Despite these considerations, 'Eugenin' and 'Flavia' exhibit fruit qualities sufficiently similar to 'Hass' that 'Hass' metrics can generally be applied. Adjusting the 20.8% minimum maturity dry weight threshold may or may not be necessary for 'Flavia', as with an adequate sample size, 'Flavia' can achieve this minimum dry weight earlier than 'Hass'. Conversely, the California industry's separate minimum fruit mass metric, which allows a 'Hass' fruit to bypass dry weight testing requirements earlier in the year, may be applicable to 'Flavia' and 'Eugenin', potentially permitting an earlier release date than would otherwise be allowed through dry matter percentage alone.

From a physiological perspective, the significant difference in palmitoleic profiles between 'Eugenin' and 'Hass' warrants further investigation. This monounsaturated desaturation pathway, distinct from the oleic pathway, is poorly researched. Continued observation of this difference in future trials could enhance our understanding of the fatty acid desaturase pathways in avocados. Additionally, the differences in polyunsaturated fatty acid profiles between the two sites, Lindcove Research and Extension Center (LREC) and South Coast Research and Extension

Center (SCREC), may shed light on the distinct roles of plastidial and endoplasmic reticulum fatty acid biosynthesis pathways in the early development of avocado mesocarp. Given the importance of lipid accumulation in the avocado mesocarp, it is unsurprising that lipid biosynthetic pathways are highly regulated in the fruit, indicating a rich area for further research.

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