AN ABSTRACT OF THE THESIS OF

Karli R. Van Simaeys for the degree of Master of Science in Food Science and Technology presented on December 15, 2020.

Title: Examining Environmental and Genetic Influences on the Brewing Performance of Hops (*Humulus lupulus*) and Barley (*Horduem vulgare*).

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Thomas H. Shellhammer

In short, the brewing process uses hot water to extract fermentable sugars from malt to make a wort that is bittered by hops and finally fermented by yeast to produce beer. The four key ingredients in brewing are malt, water, hops, and yeast. Malt is perhaps the key ingredient, as it contains starches and protein as well as the enzymes required to break them down and is the source of fermentable extract that is ultimately converted to beer during an alcoholic fermentation.

Hops, the cones of the female *Humulus lupulus* plant, are used for both bittering beer and providing aromatic qualities. Hops are grown in various regions globally and differences in soil, weather, climate, disease pressure, and grower practices provide inherent variation among them. This variation in hop growing conditions leads to differences in final hop quality, which growers have begun to focus on in recent years. This relationship, called terroir or regional identity, examines how the quality of agricultural products relate to their place of origin. It encompasses the soil, weather and climate, topography of the growing location, and to a certain degree the influences of grower management in response to these influences. Specified growing regions have been established for other agricultural products, such as wine grapes, coffee, cocoa, and tea. Anecdotal knowledge and published research show early evidence of regional identity affecting hops. Several studies have shown differences in hop chemistry and sensory qualities among samples grown in different locations. However, more work is needed to

understand the extent of regional variation and to determine which factors drives differences among hops grown in different locations.

To assess the potential effect of regional identity on hops, three varieties were harvested from a total of eleven commercial fields managed by a single hop grower within the Oregon Willamette Valley. Weather, climate, management practices, and soil data were collected for each site. Multiple Factor Analysis of these data sets showed evidence of a location effect. As expected, chemical analysis showed sample groupings by variety but variation within each variety demonstrated the effect of growing location. Sensory evaluation showed significant differences between samples within the same variety. Variation within field and between fields of the same variety provide early evidence of regional influences on hop qualities.

Seeds from the barley plant, *Hordeum vulgare*, are unsuitable for brewing until they are transformed via controlled germination and kilning during the malting process. While malting is a significant driver of malt flavor, recent work shows that other factors, such as genetics, may also influence flavor. To evaluate the contributions of barley genotype to beer flavor, two independent sets of barley germplasm were evaluated. Pedigree, quality of malt and beer, and beer metabolomic profiles were compared within and between the two sets. Sensory attributes of malt hot steeps and lager beers were evaluated, and distinct but subtle differences were reported. Distinct metabolomic profiles, attributable to barley genotype, were detected. In conclusion, metabolite variation observed is a direct result of genetic differences that lead to differential chemical responses within the malting and brewing processes, thus affecting flavor.

Examining Environmental and Genetic Influences on the Brewing Performance of Hops (*Humulus lupulus*) and Barley (*Horduem vulgare*)

by
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APPROVED:

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Dean of the Graduate School

I understand that my thesis will become part of the permanent collection of Oregon State University libraries. My signature below authorizes release of my thesis to any reader upon request.

Karli R. Van Simaeys, Author

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Finally, I want to say how grateful I am for my family and friends. I would not be here without your encouragement and support. Thank you for everything.

CONTRIBUTION OF AUTHORS

For Chapter 2, several authors contributed to the project. My contributions included gathering data, executing sensory analysis, performing all statistical analysis and interpretation. Andy Gallagher performed soil mapping, soil coring, and interpretation. Garrett Weaver assisted with site selection, gathering farm management data, and project guidance. Arnbjørn Stokholm assisted with harvest and performed hop chemistry analysis. Dr. Tom Shellhammer conceived the project concept and identified research goals, acquired funding, contributed to project planning, data interpretation and manuscript reviewing and editing.

For Chapter 3, the work was divided into three sections, malt production and genetics, brewing and sensory, and metabolomics. My contributions to the project were executing laboratory panel sensory testing and analyzing and interpreting the results. My contributions will be included in Chapter 3, while the full publication will be attached as an appendix. Sarah Windes, Scott Fisk, and Dr. Patrick Hayes developed the experimental varieties and were responsible for malting. Harmonie Bettenhausen and Dr. Adam Heuberger performed metabolomics and analysis. Jeff Clawson brewed the experimental beers. Dr. Juyun Lim and Sue Queisser contributed the consumer sensory data. Dr. Tom Shellhammer provided guidance with data interpretation and contributed to manuscript review and editing.

TABLE OF CONTENTS

TABLE OF CONTENTS (Continued)

LIST OF FIGURES

LIST OF FIGURES (Continued)

LIST OF TABLES

LIST OF APPENDIX FIGURES

LIST OF APPENDIX TABLES

DEDICATION

To my parents, I couldn't have done this without your support and guidance.

CHAPTER 1: A review of the brewing process, its key ingredients, and their production

Brewing Process

In short, the brewing process uses hot water to extract fermentable sugars from malt to make a wort that is bittered by hops and finally fermented by yeast to produce beer.[1] The four key ingredients in brewing are malt, water, hops, and yeast. Malt is perhaps the key ingredient in beer, as it is both the source of starch and protein as well as the source of enzymes required to break them down. The brewing process (Figure 1) begins by milling the malt, which produces a coarse grist and exposes the starch to the enzymes from the endosperm. Mashing is then performed with hot water to extract malt starch and convert it to fermentable sugars via malt enzymes, alpha and beta amylase. The liquid containing the solubilized malt extracts is called wort, which is then separated from the spent grain. The husk from the malt can be used as a filtering aid in this process.

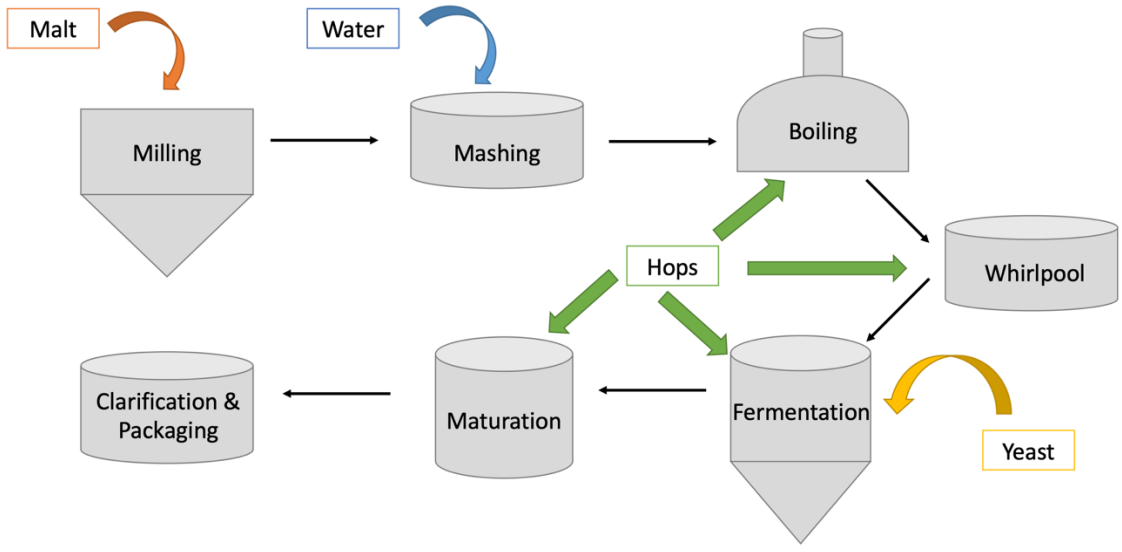

Figure 1: Summary of brewing process and points of ingredient addition.

The wort is then boiled to achieve many goals, including sterilization of the wort and removal of proteins which can produce hazy beers. It is also used to increase the color and evaporate excess water from the wort. Additionally, boiling is the first phase in which hops can be added, though when added at this stage hops will lose their aroma volatiles. Hop additions during kettle boil, serves the purpose of bittering the beer. Alpha acids, known as humulones, from hops isomerize during the hot boil to form bitter and soluble iso-alpha acids, or iso-humulones. Two other sources of bitterness from hops come from the oxidation of both beta acids (lupulones) and alpha acids (humulones) to hulupones and humulinones, respectively.[2] Though these bittering compounds are extracted during the boil, they are formed during storage of hops. Once all of the goals of the boil are met, the wort is clarified of trub and spent hop material, and then cooled down.

Yeast is then pitched into the aerated wort and fermentation begins to take place. Hops can also be added on the "cold side" of the brewing process with the goal of adding hop aroma. This process, called dry-hopping, is the cold extraction of hop essential oils from hops into fermenting or fermented beer. Hop oils contain the desired aromatic compounds which can be classified into two major fractions, hydrocarbons and oxygenated compounds. A third, minor fraction is the sulfur compounds which are present in low concentrations but are highly aromatic. Once the hop oil is extracted and fermentation is complete, the beer goes through aging and conditioning processes followed by clarification and packaging of the final product.

From Barley to Malt

Barley, or *Hordeum vulgare*, is an annual cereal grain that is planted in either fall or spring. Its seeds, or kernels, are harvested and later made suitable for the brewing via the malting process. The kernel contains the endosperm, holding the important starches and proteins, and the husk, which is an important filtering aid in brewing. Barley kernels must go through a modification, or changes in physical, chemical, and biological properties, to make malt which is ready for brewing. As harvested, barley lacks enzymes, flavor, and color, is difficult to mill, and low in amino acids. The goal of the malting process is to address all of these shortcomings.

Malting is made up of three major steps: steeping, germination, and kilning. In the field, the kernels dry (approximately 10-12% moisture) prior to harvest, so to initiate malting the kernels must be soaked in water (10 to 15 °C) for 40-50 hours, in a process called steeping. Hydration of the kernel provides a pathway for biochemical reactions to occur. Once the target moisture, usually 42-46%, is reached the kernels are removed from the water and the germination process can begin.

The barley is moved to a germination chamber, which is designed to allow even flow of cool, humid air through the bed of germinating barley. They also allow for rotation of the kernels to prevent root entanglement, and a way to release carbon dioxide and input oxygen. Germination typically takes 3 to 5 days. During germination, the embryo releases gibberellins, which stimulate the aleurone layer to produce and release the modification enzymes. Enzymes begin to break down endosperm cell walls which contain beta-glucan. This allows access to starch and protein within the cells and is also

important for reducing wort viscosity, and in turn filtration times, during mash separation and beer filtration. A small amount of starch is degraded by enzymes to feed the embryo. Additionally, some protein is broken down to yield amino acids which support growth of the embryo in malting and also yeast health during fermentation in brewing. Once sufficiently modified, the green malt is then kilned.

Kilning, the process of blowing warm, dry air through the grain, is used to reduce moisture content of the malt down to 3-5%. At first, low heat is used to drastically reduce moisture without degrading important enzymes. Then high heat is applied for final moisture reduction and flavor development. Maillard reaction products from highly kilned malts are responsible for toasty or roasted flavors and darker colors. Kilning is easily adjusted to produce malts with various colors and flavors, which are used by brewers to impart different color and flavor qualities in beer. Additionally, diastatic power, the measure of alpha and beta amylase activity, is an important consideration in kilning because of their temperature sensitivity. Pale malts are low in color and high in diastatic power, while highly kilned malts are darker and lower in diastatic power.

While kilning is the most obvious source of variation in malt flavor, recent work shows that other factors must be taken into account, such as barley genetics. The goal of barley production has, in general, been to achieve high levels of consistency. However, with the rise of the premiumization of beer, some craft brewers are looking for more interesting or novel malt flavor. One important factor being explored in depth is the role of genetics in flavor.[3] In two papers published in 2017, Herb et al. explored relationships between barley variety, location, and modification on the sensory properties of resulting

test beers.[4, 5] Their work demonstrated significant effects of both variety and growing location on the sensory properties of nano scale brews. The work also gave evidence to support the role of genetics in malt flavor. In their second paper, they further explored how the degree of modification can affect flavor.[4] They showed significant differences in sensory attributes due to genetics, degree of modification, and the interaction of the two factors. Clearly, there is more to malt flavor and its contributions to beer than just kilning. The origins of malt flavor most likely can be tied to how barley genetics influence the development of flavor precursors, such as the barley's amino acid spectrum, which result in malt flavor following kilning.

Hops

Hops, or the cones of the female *Humulus lupulus* plant, are used for both bittering beer and providing aromatic qualities. In recent years, a shift in popularity in the United States from bittering hops to aroma hops has occurred as an outcome of the rising prominence of hop-forward beers. For example, the India Pale Ale, has a characteristic high hop aroma and is made with 10 - 20 times the amount of hops as an American light lager style. In order to keep up with this trend in the craft beer industry, the overall acreage of hops fields grown per year has increased by 12,232 hectares from 2010 to 2020 and the percentage of aroma hop acreage has increased roughly 4 fold as well.[6] Over the past 10 to 15 years, hop breeders have focused on the aromatic potential of the hops as opposed to being nearly solely focused on hop acids production.

Hop is a climbing bine, which grows from a perennial rootstock. During the growing season, the bine grows upward and reaches a height between 16 and 26 feet depending on variety and trellis system. However, some dwarf varieties exist which do not grow as tall. Only the cones are of brewing value, as their lupulin glands contain the oil and resin that is extracted during brewing. Hop plants start to grow during the spring, but first growth is often cut back to reduce disease pressure in the field.[7] Additionally, pruning gives a more even start across the field, in turn providing a more consistent harvest window and thus more consistent hop quality. Hop plant growth is supported by a trellis system. The bines grow up a string and eventually reach the top wire. As bines grow taller, lateral offshoots form and eventually cone formation begins. Once the cones are mature, they are harvested between late August and early October in northern hemisphere. Bines are cut down and transported to picking facility. Here hop cones are picked off of the bine and the bine and leaf material are removed and separated from each other. The green hops are kilned, which is the process of drying the hops to approximately 10% moisture to achieve shelf stability. Cones are placed evenly in a kiln bed and hot air is forced through the bed from below. Once dried, they are cooled and baled or further processed into other hop products.

Hops are grown in varying conditions in many locations throughout the world, with the three largest growing areas in the Unites States, Germany, and the Czech Republic.[6] Hops are primarily grown within the 35 and 55 latitudes both north and south of the equator. Within the United States, 96% of hops are grown in the Pacific Northwest, with 13% in the Oregon.[8, 9] In Oregon, the majority of commercials farms are located

within latitudes 44.5 and 45.5 North. The primary growing region of Oregon is the Willamette Valley, which runs north to south. It is located between the Cascade mountain range to the west and the Coast range, also known as the Pacific Coast range, located to the east.

Several key requirements exist for locating where to plant hops for commercial production -long daylength in the summer (16 daylight hours) and cold winters. Provided those can be met, hops can grow in a variety of climates and soil types.[10] Variation in soil, weather, climate, and disease pressure provide inherent variation between growing regions. While hops can grow in different soil types, they require soil with sufficient depth to develop a deep root system and moisture from the soil, but do not grow well if the soil is waterlogged.[11] Within the Willamette Valley growing region there are two broad types of soil: Missoula flood silts and river alluvium soil from the Willamette River.[12] As for weather, sufficient spring rain, followed by hot, sunny days in the summer is required for hop growth.[13] Additionally, current prevailing knowledge states that off season temperatures of less than 40 °F are required in order for vernalization to occur.[13] However, recent evidence showed that winter vernalization is not required for good hop yield or quality.[14] In the Willamette Valley, spring is cool and wet and the summer is warm and dry.[15] On average, precipitation ranges between 35 and 80 inches per year but varies by elevation.[15] In addition to location, soil, and weather differences, hop yards can be greatly affected by pest and disease pressure such as insects, downy mildew (caused by *Pseudoperonospora humuli*), powdery mildew (caused by *Podosphaera macularis*), and viruses. To achieve the best yield and highest quality, growers control the amount

and timing of fertilizer, insecticides, fungicides, and irrigation. Additionally, the timing of training, pruning, and harvest can be altered to some degree thus impacting timing of harvest maturity. These variations in grower practices will affect the final product.[16] Other considerations for hop quality outcomes include harvest timing and post-harvest practices. The maturity of cones at harvest has been shown to affect hop quality and resulting beer aroma quality.[17, 18] Kilning has been shown to have a potential effect on hop quality.[19] In summary, many sources of variation exist which can create differences in hop growing conditions and final hop quality.

Regional Identity

Preliminary and anecdotal evidence from within the hop growing community suggests that hop quality may be affected by the location which they are grown. This concept of regional identity, also called terroir, examines how the quality of agricultural products relate to their place of origin which encompasses the soil, weather and climate, and topography of the growing location. Though fairly new to hops, this concept has been used to establish specified growing regions for other agricultural products, such as wine, coffee, cocoa, and tea. For tea, geographical indications (GI) have been established for Darjeeling and Kangra tea of India and Boseong green tea of South Korea among others.[20, 21] The GI established for Boseong tea was shown to improve tea quality and increase price, and increase tourism to the region, to the benefit of local tea farmers.[21] The concept of terroir has also been discovered in coffee beans, driving the consumer to desire single-origin beans. One example from Costa Rica demonstrated that beans from

two different regions had significant effects on sensory attributes.[22] One location had more floral aromas, while the other had more chocolate aromas. Within each region there was some variation due to altitude and slope exposure. Possibly the most well-known product influenced by terroir is wine, where the concept is well-established.[23-25] Throughout the world various growing region designations have been established such as the American Viticultural Areas (AVA) in the United States, and Appellation d'Origine Contrôlée (AOC) or "designations of origin" in France. Wine grape terroir research provides a helpful framework for conducting research on hops, which is in early stages of research.

Early evidence of regional identity applying to hops comes from anecdotal knowledge and previously published research. Several Oregon hop growers have reported that some brewers have preferences for which hop lots they purchase every year from individual farms. While hop aroma consistency may be more important for large breweries, smaller craft brewers are excited by the potential differences within the same variety.[26] Even though anecdotal evidence is beneficial in garnering interest in hop terroir, scientific studies seek to understand if the concept applies to hops. Hop regional identity studies began as early as 1997, in a study that compared Tettnanger, Saaz, Hallertau, and Fuggle hops grown across the world.[27] This study and several more recent studies have shown some evidence of the existence of hop chemistry differences between samples grown in different locations.[27-31] While demonstrating chemical differences between hops is important, the resulting sensory attributes are likely to be more important to the brewer and consumer. A few studies have examined sensory differences of different hop lots by

brewing single-hopped IPAs.[28, 31, 32] In the study by Forster and Gahr, Cascade and Comet samples from Germany and Washington State were evaluated.[31] While there were important differences in the chemistry between the two, only small differences were found in the resulting sensory of the beers. On the other hand, Van Holle, et al. showed that hop aromas of beers made from Amarillo hops grown in Idaho and Washington were noticeably different in addition to differences in chemistry.[28] Barry, et al. showed sensory differences in hop grinds and benchtop dry-hopped beers for four varieties in two different locations.[32] Unfortunately, all three of these studies did not examine the differences in growing environments between the various locations. While sensory and chemistry properties can differ due to location effects, the variation between locations is unclear. A study during the 2018 harvest year at Oregon State University sought to address this question with the assistance of Coleman Agriculture, the largest hop producer in Oregon.[33] Two hop varieties, Centennial and Sterling, were collected from two and three different fields, respectively. Data was collected on farm management, soil characterization, weather, and climate to understand what could be driving differences between samples. Post-harvest, the hop chemistry of the samples was analyzed. Hop samples were subsequently brewed with and evaluated using sensory analysis methods. Though the sample size was small, there was initial evidence to show that these hops were affected by regional differences. Further work is needed to confirm these findings with a larger sample set and better understand how management and environmental effects alter hop quality.

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CHAPTER 2 – Examining regional differences of new American aroma hops grown in the Willamette Valley, Oregon

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Abstract

In order to assess the potential effect of regional identity on hops, three American aroma hop varieties (Mosaic®, Simcoe® and Strata®) were harvested from a total of eleven commercial fields within the Willamette Valley, Oregon. Within each field, hops were harvested from two to five distinct sites. Weather, climate, and management data were collected for each field and the soil was characterized at each site sampled. Hops from each site were analyzed for total oil content, alpha and beta acids, and 24 aroma compounds. Single-field IPAs were brewed using composite samples from each field and assessed via sensory evaluation. Multiple Factor Analysis revealed groupings by field location when run on all of the variables. Discrimination and descriptive sensory testing showed significant differences between samples within the same variety. Variation within field and between fields of the same variety show preliminary evidence of a regional identity for hops grown in the Willamette Valley, Oregon.

Introduction

As hop-forward beers have become prominent in the United States, the use of aroma hops has become central to craft brewing. The India Pale Ale, the most notable hop-forward style, has characteristic high hop aroma and is made with 10 - 20 times the amount of hops as an American light lager style. In order to keep up with the growing popularity of highly hopped beers, the overall acreage of hops fields grown per year has increased by 12,232 hectares from 2010 to 2020.[1] This increase also resulted in a dramatic shift away from bittering hops towards the production of aroma hops. Not only

are brewers increasing their hop use, but they are also interested in new and aromatically distinct hop varieties. Consequently, hop breeders and growers have focused on the aromatic potential of hops and their contribution to beer flavor. With this new interest also comes a focus on how growing conditions, including location, may influence hop aroma qualities.

Hops, *Humulus lupulus*, are grown under varying conditions in many different locations across the globe. Worldwide, the top three largest hop growing areas are the Unites States, Germany, and the Czech Republic.[1] Within the United States, 96% of hops are grown in the Pacific Northwest (Washington, Oregon and Idaho).[2] Hop growing regions are primarily located within the 35 and 55 latitudes both north and south of the equator. This provides hop plants with the necessary 16 daylight hours during peak growing season.[3] Beyond latitude, differences in geographical locations bring inherent variation in soil, weather, climate, and disease pressure. Hop plants can grow in many different soil types; however, they require soil with sufficient depth to develop a deep root system.[4] They require moisture from the soil, but do not grow well if the soil is waterlogged.[4] Hops require moisture in the spring, followed by hot, sunny days in the summer.[5] The current prevailing knowledge states that in order for vernalization to occur, they require temperatures of less than 40 °F (4.5°C) for one to two months.[5] However, one recent study showed evidence that winter vernalization is not required for good hop yield or quality.[6] Pest and disease pressure can come from insects, downy mildew (caused by *Pseudoperonospora humuli*), powdery mildew (caused by *Podosphaera macularis*), and viruses. Growers can vary the amount, type and frequency of fertilizer, insecticides, fungicides, and irrigation used to achieve the best yield and quality. Additionally, the timing of training, pruning, and harvest can be altered. All of these different grower management practices are believed to affect the final product.[7] Harvest maturity has also been shown to play an important role in hop quality and resulting beer aroma quality.[8, 9] Post-harvest practices, such as kilning, have recently been shown to have an effect on hop quality.[10] In summary, geographical differences, inherent field

variation, and management philosophy can create different hop growing conditions that may influence hop aromatic qualities.

Within the United States, roughly 13% of hops were grown in Oregon in 2019.[11] The majority of commercial farms are located within latitudes 44.5 and 45.5 North (Figure 1) in the Willamette Valley, which runs north to south between the Cascade mountain range to the east and the Oregon Coast Range, located to the west. Two broad groups of soil exist in the Willamette Valley hopyards: one group formed in Missoula flood silts and the other group formed in river alluvium from the Willamette River.[12] The weather in the Willamette Valley is cool and wet during the spring and warm and dry in the summer.[13] Precipitation varies by elevation, but on average is between 35 and 60 inches (90 and 152 cm) per year.[13] Nevertheless, nearly all hops in Oregon are irrigated to some degree during the summer months.

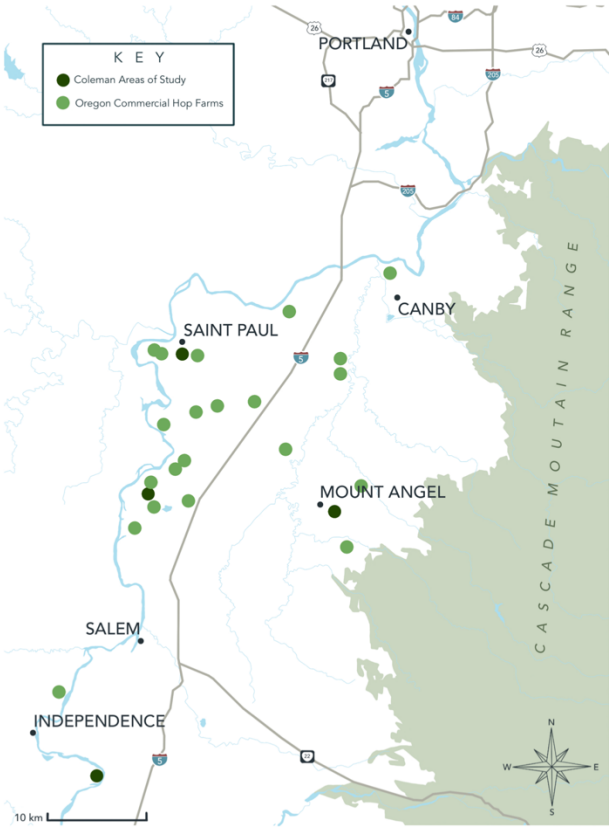

Figure 1: Map of Oregon commercial hop growing region within the Willamette Valley

Recently, some hop growers have begun to focus on the growing environment unique to their fields and its effects on hop aromatic properties. This concept, called regional identity, or terroir, examines how the qualities of agricultural products relate to their place of origin. It encompasses the soil, weather and climate, and topography of the growing location. Terroir has been used to establish specified growing regions for other agricultural products, such as wine grapes, coffee, cocoa, and tea. The concept of terroir is well-established in the winemaking industry.[14-16] Regional identity has been used to establish American Viticultural Areas (AVA) in the United States, Appellation d'Origine Contrôlée (AOC) or "designation of origin" in France, and in other countries around the world. The concept of terroir in wine grapes provides a helpful framework for conducting research into hop terroir, although one must keep in mind that the grape analogy only goes so far when examining hops.

While wine grape terroir has been well-researched, the question of regional identity in hops is in early stages of research. Anecdotal knowledge and previously published research show early evidence of regional identity concept applying to hops. Several hop growers have reported that brewers repeatedly seek out specific hop lots grown on specific locations year after year. Perhaps the first study comparing varieties grown in different locations was published in 1997, which compared Tettnanger, Saaz, Hallertau, and Fuggle hops grown in the USA, Australia, and Europe.[17] Several studies have shown some evidence of the existence of hop chemistry differences between samples grown in different locations.[17-21] Several studies focused on different growing regions with one country, such as the United States and Italy.[18-20] Other studies with broader scope made comparisons between countries.[17, 21] Additionally, a few studies have examined sensory differences using hopped IPAs.[18, 21, 22] This study seeks to build off a 2018 pilot trial with Coleman Agriculture in which two hop varieties, Centennial and Sterling, were collected from two and three different fields, respectively.[23] While the sample size was small, there was initial evidence to show that these hops may have been affected by terroir.

The goal of this work was to identify whether regional differences exist between fields grown by the same hop farmer, Coleman Agriculture, within the Willamette Valley. Understanding regional identity is beneficial to the hop growers and hop farmers and of interest to consumers. Hop growers would be better able to understand what makes each of their farms unique, thus allowing them to tailor their farming practices to maximize their hops aromatic potential. Additionally, brewers are already making distinctions on labels to indicate what hop varieties are used in their beers. If regionality becomes important, brewers can further engage their consumers by listing place of origin information on labels. Consumers are already interested in learning more about hop varieties, and they would likely be drawn to beers with more information regarding where hops are grown. The understanding of terroir in hops has the potential to benefit growers, brewers, and consumers, but it must first be backed by sound scientific methods as demonstrated. This work builds on the 2018 pilot study, by looking at three varieties, instead of two, and including more fields in the study.[23] One goal of the 2019 study was to describe the soil landscape setting and classify the soils accurately within Willamette Valley hop yards. Another goal was to collect soil samples for laboratory analysis from a set of georeferenced sample points with documented soil classification to use in analysis with hop data. We aim to understand how each of the variables, including weather, climate, soil, management, and chemistry, are interrelated. These relationships can give us insight into the regional identity of hops.

Materials & Materials

Identification, Description, and Characterization of Locations of Farms

This study was performed collaboratively with Coleman Agriculture, the largest hop grower in Oregon, who grow hops in three distinct regions within the Willamette Valley: St. Paul/Gervais, Mt. Angel, and Independence (Fig. 1). Three hop varieties grown in eleven fields total were selected for this study, including three Mosaic® (HBC 369), five Simcoe® (#PP12213), and three Strata® (OR91331) fields (Table 1).[24-26] Hop fields were selected to represent two broad soil groups: soils formed in Missoula flood

silts (informally referred to as terrace soil) and soils formed in river alluvium. Alluvial soils tended to occur within the Willamette River floodplain, with the exception of a portion of field 86 which was in the floodplain of a tributary of the Abiqua Creek. Within each field, multiple sites (from 2 to 5) were sampled in order to provide data on variation within the field which can be compared to between field variation. These sites were not randomly chosen, rather they were selected to represent the various soil series present within each field. In addition to soil type, other factors considered when selecting fields included, location within the valley, planned future production of the yard, historically average vigor/yields, mature age of yard and historical popularity/ uniqueness of lot quality. GPS coordinates were used to harvest specific bines within the hop plots of interest. The hop yard sites used in this study contained hops that were on average five years old with the youngest yards being three years and the oldest at six years at the time of the harvest.

Table 1: Experimental design of the eleven fields; Dry matter for each hop sample taken at harvest

Hop Variety	Field Information	Nearest Town														
		Mt. Angel					Independence					St. Paul				Keizer
Mosaic	**Field ID** (Field Size)	**86** (9.27 ha)					**33** (7.65 ha)					**44** (7.24 ha)				
	Harvest Date	9/10/19					9/12/19					9/12/19				
	Site	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5
	Dry Matter %	26.0	23.4	24.1	23.7	23.2	NR	NR	NR	NR	NR	24.3	25.4	24.8	24.0	23.5
Simcoe	**Field ID** (Field Size)	**82** (11.65 ha)					**23** (11.49 ha)					**42** (5.22 ha)				
	Harvest Date	8/26/19					8/30/19					9/3/19				
	Site	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5
	Dry Matter	30	28	29	31	26	25	25	25	27	26	31.0	29	30	30	27
	Field ID (Field Size)	**83** (2.91 ha)										**9** (8.70 ha)				
	Harvest Date	8/26/19										9/3/19				
	Site	1	2	3	4	5						1	2	3	4	5
	Dry Matter %	26	28	28	28	29						28	27.0	28	29	28
Strata	**Field ID** (Field Size)						**49** (3.08 ha)									**73** (3.36 ha)
	Harvest Date						8/22/19									8/22/19
	Site						1		2		3					1	2
	Dry Matter %						23.3		22.8		27.1					30.1	27.0
	Field ID (Field Size)						**50** (10.95 ha)									
	Harvest Date						8/22/19									
	Site						1	2	3	4	5					
	Dry Matter %						25	25	27	26	28					

Homeplace Farm (St. Paul and Gervais, OR)

The Homeplace farm is made up of fields near St. Paul and Gervais, OR totaling about 900 acres (~365 ha). Most of the Willamette Valley hop producers have farms in this area (Fig 1). The four fields studied were Aunt Dora 9 (Simcoe®), Williams 44 (Mosaic®), Grassman 42 (Simcoe®), and Goulet 73 (Strata®). The Goulet field is 6 miles from the Williams and Grassman fields, and 9 miles from the Aunt Dora field. For our purposes, the St. Paul and Gervais fields are viewed as belonging to the same growing area.

Alluvial Farm (Independence, OR)

Named after the alluvial plain on which the farm sits, the Coleman Alluvial farm is the southernmost and largest contiguous hop farm in the state of Oregon. Located south of Independence, OR, this farm raises 16 different varieties of hops on roughly 1,000 acres (~404 ha). The farm is one of the last remnants of a long history of hop production in the area, stretching back to the early 20th century, when it was known as the hop capital of the world. The farm is located in the Willamette River flood plain, with some fields located closer to the river and some farther to the west. The four fields studied were Alluvial 33 (Mosaic®), Alluvial 23 (Simcoe®), Alluvial 49 (Strata®), and Alluvial 50 (Strata®).

Mt. Angel Farm (Mt. Angel, OR)

The Mt. Angel farm is located 15 miles (24 km) east of the Homeplace farm and 23 miles (37km) northeast of the Alluvial farm in Mt. Angel, OR. The farm is spread over three blocks totaling nearly 300 acres (121 ha). It is located along the base of Mount Angel, a 485 ft high butte that protrudes from the Willamette Valley. The three fields studied were Mt. Angel 86 (Mosaic®), Mt. Angel 82 (Simcoe®), and Mt. Angel 83 (Simcoe®).

Soil Mapping, Physical Properties, and Soil Nutrient Measurements

Site selection

Soil classification and soil mapping were performed by Red Hill Soils, Certified Soil Classifier, Corvallis, Oregon. Sample sites for hops and soils were selected to capture the soil variability within the hopyard block using a combination of digital terrain data, existing USDA National Resource Conservation Service (NRCS) soil maps, and historical aerial photography of previous crops. These preselected sample points were located on the ground using GPS. Three strings of hops were harvested at each of these points in summer 2019 and soils were sampled at these points in the winter of 2020 (Figures 2 & 3).

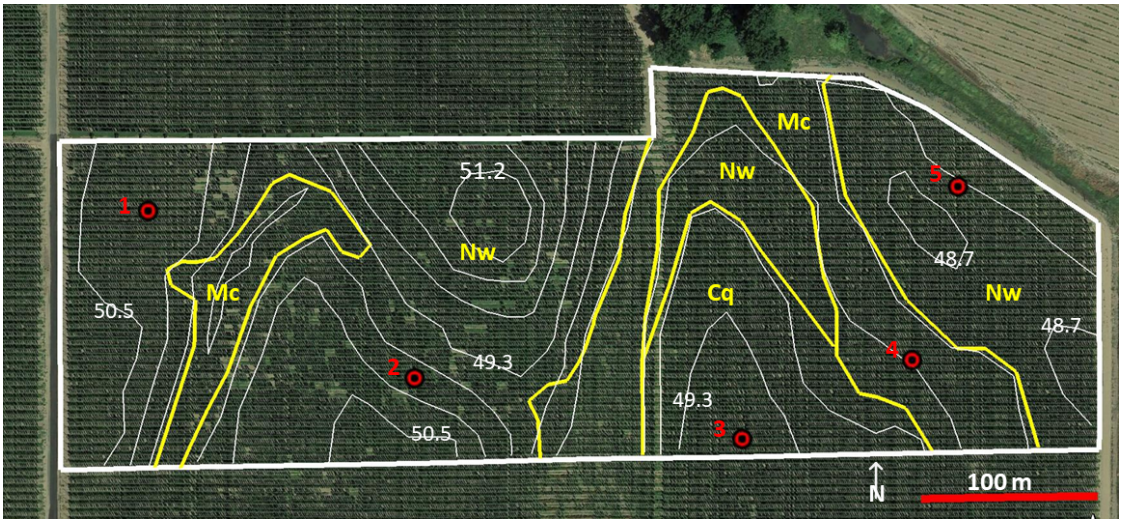

Figure 2: Soil map and sampling sites for Alluvial 33 Mosaic® field (MOS – All 33). Thin white lines indicate elevation change (0.6 m/2 ft contours), yellow lines represent approximate boundaries of soil series, and red dots show location of soil cores. Here, Cq = Cloquato, Mc = McBee, Nw = Newberg.

Soil Sample Coring

Soil pedons were described and classified from 76 mm diameter cores sampled with a bucket auger to 1.5 m depth. Soil profiles were described using standard USDA methods and USDA Soil Taxonomy to classify soils to the series level.[27-29] Soils were hand textured to estimate USDA soil texture, and percent sand, silt, and clay. Available water holding capacity (AWHC) was estimated based on soil texture, soil structure

pedotransfer functions and National Cooperative Soil Survey laboratory data for water retention when available.

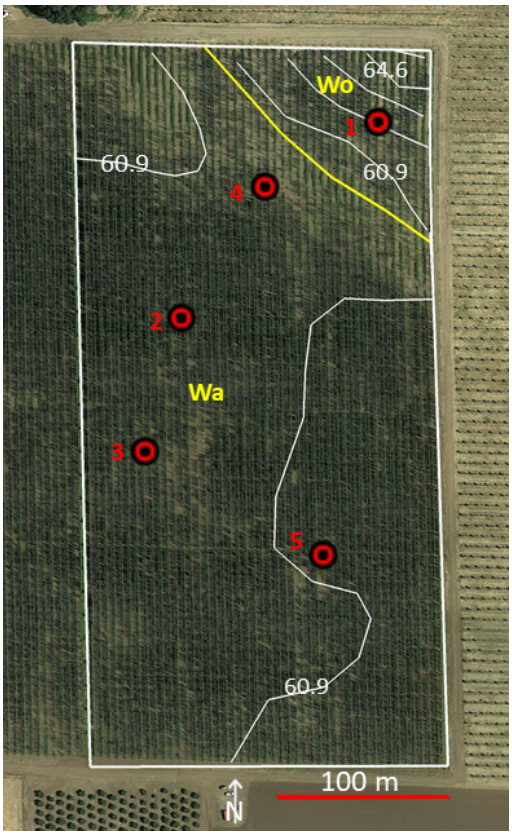

Figure 3: Soil map and sampling sites for Mt. Angel 86 Mosaic® field (MOS – MA 86). Thin white lines indicate elevation change (0.6 m/2 ft contours), yellow lines represent approximate boundaries of soil series, and red dots show location of soil cores. Here, Wa = Waldo, Wo = Woodburn.

Climate and Weather Data

Climate and weather data were acquired from the PRISM Climate Group, using the GPS coordinates of the centroid of each field in the study.[30] The PRISM Climate Group, maintained by the Northwest Alliance for Computational Science and Engineering, provides high spatial resolution modeled climate and weather data. Climate data (based on 30-year averages, 1981–2010) consisted of annual mean minimum, maximum, and mean temperatures and cumulative precipitation. The 2019 weather data

collected from the PRISM database consisted of mean minimum, mean maximum, cumulative precipitation, mean diurnal flux as calculated from the first day of hop training to the harvest date for each field. Additionally, growing degree day accumulation (base temperature 41 ºF/5 ºC) was calculated from minimum and maximum temperatures for the period from training to harvest. However, if daily minimums fell below 41 ºF (5 °C) they were set to 41 ºF, and if they exceeded 86 ºF (30 °C), they were set to 86 ºF. Once these adjustments were made, the average of the daily minimum and maximum was taken from which the base 41 ºF was subtracted. The daily values were then summed over the growing season (from training to harvest). Winter temperatures are also important for hop vernalization.[5] Therefore, winter average temperature was calculated from December 1st to February 28th for each field.

Farm Management Practices

Approach to Management

Each yard in the study was managed for commercial production in accordance with Coleman Agriculture's best practices. The approach to management was similar at all locations, with slight adjustments made based on the soil type, variety, and soil nutrient levels of each yard. Pruning/ training dates, fertilization rate, irrigation and pest management were the primary management variables included in the study. All yards in the study were chemically pruned in March and April to remove the previous year's growth and allow new shoots to emerge. Newly emerged shoots were then trained onto the 18ft (5.5m) strings in May. Growing season fertilization was carried out primarily through drip irrigation systems, however supplemental foliar applications were applied as needed. The need for foliar fertilizer applications was determined by leaf petiole testing during the growing season. This was usually done to compensate for micronutrient deficiencies in specific areas of a field. One common use for foliar spraying was in response to yellowing leaves, which can indicate a nutrient deficiency.

Every yard received five liquid nitrogen applications from June- July. Dry fertilizer was spread in the spring as needed according to soil nutrient testing taken in the

fall. All yards were amended with the previous year's hop-compost in the fall and spring. Soil moisture probes and on-site weather stations were used to manage irrigation timing. Irrigation schedules from yard to yard varied based on local weather and soil type, however the typical program consisted of a five-hour set every one to two days, starting in the spring and ending in the fall. The type, amount, and frequency of pesticide applications were based on reports from trained field scouts. The field scouts tracked pest and disease pressure in each field on a biweekly basis and reported their findings to the farm managers who confirmed the diagnoses and decided how each field would be treated. Coleman Agriculture participates in the Salmon Safe program and limit the type and amounts of pesticides used to minimize environmental effects. During the growing season, yards were tilled every 3-4 weeks to reduce weed pressure. After harvest, a cover crop blend of spring barley (*Hordeum vulgare*), white clover (*Trifolium repens*), and lacy phacelia (*Phacelia tanacetifolia*) was planted to reduce soil erosion and capture nutrients over the winter.

Management Data Collection

Data were collected from Coleman Agriculture on key management practices. Both dosage and frequency of nutrient applications were documented for nitrogen, phosphorous, potassium, sulfur, calcium, magnesium, zinc, copper, manganese, boron and iron. Additionally, fungicide and insecticide applications frequency and dosage were documented. Training date, chemical pruning date, and commercial harvest dates were recorded.

Harvest & Postharvest Practices

Harvest Date and Maturity

Harvest timing depends on a number of factors, including historic precedents, weather, farm logistics, and hop maturity.[23] Hop maturity has not yet been well-defined, though many growers use dry matter (DM) testing to assess harvest readiness. Research sample harvest timing was left to the discretion of Coleman Agriculture, based on the

commercial harvest date for each field. Commercial harvest can vary up to one-week from farm to farm depending on maturity levels and logistics. Maturity levels are determined by dry matter testing in combination with tactile, visual, and smell assessments performed by farm managers. Harvest logistics are a function of the picking/kilning facilities process rate, acreage, and the target harvest date for each variety. For these reasons, determining the commercial harvest timing for a yard can be a challenge and requires careful planning to effectively balance of all of these variables. Therefore, the timing of the commercial harvest served as a guide for the experimental sample harvest. Sample bines, three strings of bines per each within-field site, were hand harvested roughly 24 to 48 hours before the entire field was commercially harvested. The fields were harvested between August 22nd and September 12th, 2019. Dry matter (DM) samples were collected by hand-picking a selection of hops from different bines and along different vertical positions of each bine prior to running the bines through the OSU Wolf picker (Model 140, WOLF Anlagen-Technik GmbH, Geisenfeld, Germany). Dry matter measurements were performed by coarsely grinding a sample of green hop cones, drying the sample for 2 hours in a drying oven at 100°C, and recording weights before and after drying. Dry matter was expressed as percent by weight dry basis. Each field was sampled for DM on the day it was harvested, with the exception of Mosaic® Alluvial 33, which was mistakenly not sampled.

Kilning and Storing Processes

Following picking, green cones were placed in labeled onion sacks and transported to the Coleman Alluvial Farm (30 min drive north of Corvallis, OR) where they were placed in commercial kilns (32 x 32 ft) covered with loose hops that were being dried at the time. Drying was carried out with an air-on temperature of 135 ºF (57 ºC) with a duration determined by the kiln operator whereby the bed reached a target final moisture of 8-10%. Typical drying times range from 8 to 12 h depending on variety with which the sampled hops were dried. Once dry, the hops were moved to a cooling room for 12-24 hours, and then delivered to Oregon State University (OSU). Upon arrival at

OSU, the hops were placed in high-barrier packaging and vacuum-sealed with a nitrogen gas backflush. Packaged hops were stored at -10°F (-23°C) until analysis or brewing. On average, the combined 3-5 sites within each field yielded 16 lb (7 kg) of whole cone hops.

Hop Chemical Analysis

A 150g of homogenized, whole cone hop sample of each combination of variety, field, and field site was coarsely ground into grist to prepare for chemical analysis using a meat grinder (Cabela's Inc. carnivore model #541555 (1-1/2 HP)). Hops were analyzed in the OSU Shellhammer Lab following ASBC Methods for moisture content (Hops-4A), total hop acids by spectrophotometry (Hops-6A), hop storage index (Hops-12) and total hop acids by HPLC (Hops-14). Total oil content was measured by hydrodistillation (Hops-13), and the oil was collected in 2.5mL amber vials with foil-lined closures.[31-35] After filling with oil, the amber vials were flushed with nitrogen. Hop oil was stored at -20°C until subsequent compositional analysis. Oil composition by capillary gas chromatography-flame ionization detection (Hops-17) was then used to characterize the oil by examining 24 terpenes, terpene alcohols, and esters.[36] The selected compounds are important for hop aroma quality.[37-39]

Hop essential oil terpene analysis - reagents and standards

The following 18 hop volatiles were the focus of this study and were chosen based on preliminary research and relevance in literature: isovaleric acid, beta-pinene, beta-myrcene, 3-carene, methyl heptanoate, rho-cymene, limonene, linalool, geranial, neral, alpha-terpineol, nerol, geraniol, geranyl acetate, alpha humulene, caryophyllene, geranyl isobutyrate, and caryophyllene oxide. All chemical standards were purchased from Sigma Aldrich (St. Louis, MO, USA) and were >95% purity. 2-octanol was purchased from Alfa Aesar (Heysham, England). HPLC-grade (98.5% purity) hexanes were purchased from EMD Millipore (Darmstadt, Germany).

Hop essential oil terpene analysis

Hydrodistillation was performed to determine the total oil content of the homogenized hop grist using ASBC Hops-13.[35] Post-distillation, hop oil was collected in 2.5mL amber vials with foil-lined closures. After filling with oil, the amber vials were flushed with nitrogen. Hop oil was stored at -11°C until subsequent compositional analysis. Hop oil compositional analysis was performed using a HP 5890 gas chromatograph with a Flame ionization detector (FID) and HP 7673 Autosampler under modified conditions from ASBC Hops-17.[36] A 10% by volume hop oil solution was prepared by adding 100 uL sample of hop oil to 900 uL of 1% 2-octanol in hexanes standard. Hop oils were diluted to 1% with the 2-octanol/hexane solution in screw top glass vials. 1-µL of the diluted hop oil was directly injected into the injection port held at 200°C and operating in split mode (1:50) using the septum purge option. The analytical column was a 30m x 250 um x 0.25 Supelcowax column (Supelco) and ultra-pure nitrogen was used as the carrier gas (constant flow rate, 1 ml/min). The temperature program was modified to optimize adequate separation of target compound peaks. The modified temperature program was: 60°C held for 1 min, ramped to 175°C at 3°C per minute, held at 175°C for 10 minutes, ramped to 230°C at 3°C/min, and held at 230°C for 10 minutes. The FID temperature was 250°C. Quantification of compounds was determined by using an internal standard of 2-octanol as outlined in ASBC Hops-17.[36] Area integration reject was set to 1 mV. The target analyte concentrations in hop oil were then standardized on a per-mass basis using the total oil content determined during hydrodistillation.

Brewing

Ten single-hop India pale ales were brewed on an Esau and Hueber 2.5hl brewing system (Esau & Huber GmbH, Schrobenhausen, Germany) using 100% pale lager malt (Rahr Premium Pilsner) targeting 14.8 ºP original gravity. It was not logistically possible to create on beer for each site, so one beer for each field was brewed, with the exception of Mosaic® Alluvial 33, which was unavailable for brewing. Composite hop samples

from all of the sites within each field were created by taken blending equal parts (by weight) of each site within the field. This mirrors commercial practices, where hops from a whole lot are homogenized before being sold. There was not enough experimental hop material from the Strata® Goulet 73 field, so it was combined with a commercial sample from the same field. Consequently, the dry-hop material consisted of 590g from each of the two sites and 340 g of the commercially harvested sample, and the boil and whirlpool were entirely commercial samples. Hops were added at the beginning of a 60 min boil (weight determined by target of 34 IBU), at whirlpool (300 g/hL), and post-fermentation dry hopping (800 g/hL). Beers were fermented at 20 ºC (68 ºF) with ale yeast (Wyeast 1056 California Ale) for 6 days, during which acetolactate decarboxylase (Maturex®, Novozymes, Denmark) was added to aid in speeding diacetyl reduction. The beer was dry hopped near the end of fermentation when there was approximately 1–3 ºP of remaining fermentable extract or roughly 72 hours after pitching yeast. The tank was then roused every 24 hours for 72 hours. Roughly 144 hours after yeast pitching, the spent hop material and yeast were removed from the fermenter cone and the beer was monitored for diacetyl and acetaldehyde. Once diacetyl dropped below 35 ppb, beers were cooled to 0ºC (34 ºF) and rough filtered (Pall HS6000). Beer was stored in 1/2 barrel (58 L) stainless steel kegs at 1–2 ºC with 12 psi of CO_2 overpressure until sampling.

Sensory Evaluation

Discrimination Testing

The goal of sensory evaluation was to determine whether there was a perceivable difference between beers hopped with samples from different fields. Comparisons were made only between beers hopped with the same variety using orthonasal aroma evaluation only. In August 2020, the beers were evaluated using multiple triangle tests in order to determine if a significant difference could be perceived between samples, following ASBC method - Sensory Analysis 7.[40] Approval for this work was granted by Institutional Review Board at Oregon State University. The panel consisted of 15 people (10 F, 5 M; 23 - 56 years old), most of whom had prior experience performing sensory

analyses on beer and other beverages. Each test was replicated three times, for a maximum of 45 evaluations. There were eleven different triangle tests, each of which was replicated three times. The order of the 33 total triangle tests was randomized and performed across seven days to avoid panelist fatigue. All panelists assessed the samples in the same room, which was free of aromas and temperature controlled to 20 °C. During the testing sessions, panelists were given ~60 mL of beer in a 300 mL glass covered with a plastic lid. The beer was served from two 8-head draft systems operating at 4 °C and at 13 psi (Micro Matic, Northridge, CA). Beer was dispensed into a 48-oz pitcher, then poured into blind coded sample glasses ~1 hour before the start of testing, capped with a plastic lid and allowed to warm to ~20 °C. Each beer sample was given different three-digit blind codes. Both the order of the triangle tests and sample evaluation order within each test was randomized for each panelist. Panelists were instructed to take a two-minute break between each triangle test. All sensory data were collected via Qualtrics Software (Provo, UT).

Descriptive Testing

Upon determining that significant differences existed among hops from different fields within each variety, the ten beers were evaluated for qualitative differences using a Check All That Apply (CATA) approach.[41] The panel consisted of 17 people (11 F, 6 M; 21 - 56 years old), most of whom had prior experience performing sensory analyses on beer and other beverages. Each test was replicated two times on separate days in October 2020. The ballot consisted of 19 aroma attributes for hoppy beers. The sample preparation and data collection followed that of the discrimination testing described above.

Statistical Analysis

All data were analyzed using XLSTAT base and sensory packages (Addinsoft, New York, NY). General summary statistics were performed for the chemistry and weather variables. The chemistry variables were reduced by applying correlation analysis

and knowledge of which analytes are most important for hop quality. The chemistry data were analyzed using Principal Component Analysis for the reduced chemistry set of 22 variables. Agglomerative Hierarchical Clustering was used to determine grouping of samples based on chemistry analytes. Additionally, Analysis of Variance (ANOVA) was used to evaluate both between variety and within variety variation for each analyte. For between variety and within variety analyses, "variety" and "field" were considered fixed effects, respectively.

Multiple Factor Analysis (MFA) was used to analyze the soil variables, including parent material and soil characterization variables. For the evaluation of all of the variables together, MFA plots were used with each table of variables (weather/climate, soil characteristics, parent material, management, fungicide/insecticide applications), with chemistry analytes as supplemental to the analysis. Once MFA was performed, factor scores for each site within a field were then averaged to create a new plot comparing fields.

For the sensory data, the Discrimination Test Analysis was used to determine if each triangle test performed was significant. Correspondence Analysis plots were created using the CATA frequency data. Additionally, the significance of each attribute was assessed using Cochran's Q test. Each panelist was identified as separate for their two replications.

Results and Discussion

Comparing growing sites based on weather/climate, soil differences, and management

Weather and Climate

Many possible variables exist to quantify and characterize regional and subregional weather and climate, and 10 indices were selected based on guidance from an OSU agronomist (Supplemental Table 1). Furthermore, all data came from a single source, the PRISM database rather than relying on on-farm data collection and thereby eliminating potential errors due to differences in instrument calibrations and lack of

sufficient sensor density to capture the spatial variation. Weather indices were calculated for the hop growing season determined as the time spanning the hop training date (the first 2 weeks in May) to harvest date (late August to mid-September) for each variety and for every individual field in this study. In contrast, the climate was characterized by examining 30-year averages for the full calendar year, not just the growing season. Given the relatively close proximity or the four clusters of field locations in the study (Figure 1), it is not surprising to see relatively little differences in climate data across these sites. One could argue that this study examined subregional differences since the Willamette Valley could easily be considered its own region in comparison to other major growing regions in the Pacific Northwest. Future studies will expand their geographic scope and will therefore display more variation in weather and climate. The 30-year average, minimum, and maximum temperatures were all less than 0.5 degree (°C) different across all of the fields, which was to be expected given the proximity of the fields to one another. The 30-year annual average precipitation was highest for the Mt. Angel farm (112 cm) and lowest for the Home Place Farm (104 cm).

Turning to the 2019 growing season, the Alluvial farm had the most precipitation (10.9 cm) while Home Place had the least (8.6 cm), which is in contrast to the annual 30-year climate data. The growing season precipitation had the largest variation between the fields in this study. Studies have shown that precipitation early in the growing season increases alpha acids.[42] Additionally, it should be pointed out that all fields were irrigated throughout the growing season. Growing degree days (GDD) and average highs/low during the growing season were used to characterize heat units in each field and were important in differentiating between fields. The Alluvial farm was significantly cooler than the rest, while the warmest field was Aunt Dora. The Alluvial 50 field had 449 less GDD than the highest, which was the Williams/Grassman fields 44/42 cluster. Heat indices are important for hop quality as increases in growing season temperature are associated with decreases in alpha acids.[42, 43] This was shown to be particularly important during the cone maturation phase.[44, 45] The farm with the most diurnal flux between daytime highs and nighttime lows was Alluvial, because it sits on the edge of a

geographical feature known as the Van Duzer corridor where cooler summertime coastal breezes blow eastward through a natural break in the Oregon Coastal Range and cool this part of the Willamette Valley.[46] Mt. Angel was the least affected by this localized weather pattern because it was farthest east. The measure of diurnal flux is important in berry development of wine grapes and may have some importance with hops.[47, 48]

Management variation

Fertilization levels are determined on a yard-by-yard basis, primarily based on soil nutrient levels, however, irrigation water mineral content, and farm logistics may have been factors in the 2019 study as well. The differences in soils between the farming locations in the study likely account for most of the differences in fertilization level. Although not looked at in this study, mineral content from the irrigation water source is also considered to a smaller degree when choosing a fertilizer program because of its interaction with the soil's ability to take up nutrients. All three farms draw from different water sources that have different mineral make ups which may have contributed to some of the differences between study sites. Lastly farm logistics influence how yards are fertilized. Multiple yards are often run off a single irrigation pump site. It is more efficient to have one large tank to hold fertilizer at each pump site than multiple tanks with slightly different fertilizer mixes which results in similar treatment of yards running off the same pump. Some of the yards within the study were run off the same pump, which may have led to more similarity in fertilizer application within each farming location.

Some of the major factors affecting spray frequency include proximity to other yards, program type, and a grower's appetite for risk. Some mildews as well as insects can be easily spread via wind, dust, or other proximity related factors. With this in mind, managers may treat hop yards located near other yards more aggressively, especially if the other yards are managed by other growers who are not as risk averse. The Home Place yards in the 2019 study are located in the largest hop growing region in Oregon, near the town of St. Paul. Similarly, the Mt. Angel farm, is nearby to a few other hop

growers as well. This likely contributed to a higher risk of infestation or infection and may have resulted in more occurrences or proactive treatments at those locations. The Alluvial farm, on the other hand, is relatively isolated and does not share the same level risk of infection via proximity to other growers. Another contributing factor to spray frequency is program type. Some treatments call for lighter doses with more applications where others call for a heavier dose with fewer applications. The program depends on product used, pest type, and severity. Each yard in the study was treated with a program based on its specific need. Closer proximity of yards within the same farm likely led to similar pest issues and therefore similar pesticide programs and spray frequency. Lastly, a farm manager's tolerance for crop damage risk can affect the number of preemptive applications. While the three farms in this study were owned by Coleman Agriculture, and thus managed with a common, overarching philosophy, the day-to-day operations at each farm was carried out by different individuals thus the personal aversion to risk held by each manger could have influenced the number of sprays applied.

Though management techniques are not necessarily in the traditional definition of terroir, they nonetheless influence terroir. Soil, weather and proximity to other crops (similar or different) require different management interventions and thus management cannot be entirely removed from the regional influences on product characteristics. And thus, they play a confounding role in looking at differences between fields. Management techniques are a way for the grower to maximize field productivity and quality. Fertilizer, fungicide, and insecticide application needs tell us about what the field is lacking.

While fertilizers, fungicides, and pesticides are used to promote healthy plant growth, they may be responsible for altering hop aroma quality. A 2019 study showed that altering nitrogen dosage and timing had an effect on total oil, and alpha and beta acids. However, across multiple years and experiments, sensory differences were inconsistent.[7] Another 2010 study on the cultivar Cascade showed that copper sulfate fertilizer usage affected thiol content in both hops and beers.[49] The use of copper sulfate decreased 4MMP but increased 3MH in cones and in the resulting beers 3MHA and 3MH increased but 4MMP decreased. The fields in the present study had at most 1.0 lbs./acre

copper fertilization per field, which makes this effect likely lower with these samples. Thiols are important contributors to the aroma of Mosaic®, however hop samples in the present study were not analyzed for thiol content. More work is needed to determine the effects of fertilizers on hop chemistry and resulting aroma quality.

Soil variation

The sites investigated fall into three main soil groups that may be useful in defining hop terroir in the Willamette Valley. The first soil group formed in recent river alluvium of the Willamette River. These soils are strongly associated with one another on the Ingram geomorphic surface, which is the higher flood plain of the Willamette River and its major tributaries. The Alluvial Farm (fields 23, 33, 49, and 50) and Goulet Farm (field 73) are in this group. The second group is the clayey alluvium of a tributary stream at Mount Angel field 86. The third group is the soils formed in Ice-Age flood silts represented by the soils of the Homeplace Farms (fields 9, 42 and 44) and two of the three Mount Angel fields (82 and 83) (Supplemental Figure 1 & Supplemental Table 2).

Four parent material types were defined for the soils in this study. Two parent material types are in the Willamette River alluvium soils. Parent Material 1 is coarse river alluvium that is loamy sand and sandy loam. Parent Material 2 is silty and loamy river alluvium. Parent material 3 is the clayey alluvium at Mount Angel field 86, and Parent Material 4 is the Ice-Age Flood silts of the Homeplace farms.

The soils of the Willamette floodplain are predominantly Mollisols (Haploxerolls), which are characterized by a thick dark surface, high base saturation and weak development in which most of the vertical variability is a result of sedimentation as opposed to soil formation. These soils are flooded occasionally, and deposition of new sediment keeps the soils in a young developmental stage. The Pilchuck series (Dystric Xeropsammenmts) are the sandiest soils in this floodplain group, which includes Alluvial field 49. Geologically the sediments of the Willamette flood plain are from the Cascades and the Coast Range provenance of the Willamette River Basin.

Soil variability on the floodplain is driven by soil texture and stratification of materials of different particle size distribution. The Newberg (all Alluvial fields) and Pilchuck (Alluvial 49) are coarser textured with lower available water holding (AWHC), lower cation exchange capacity (CEC) and lower fertility. The Cloquato (Alluvial 23, 33, 50 and Goulet 73) and McBee (Alluvial 33) soils are siltier and are texturally silt loams and have higher AWHC. Chehalis (Alluvial 50 and Goulet 73) has slightly more clay (silty clay loam) and slightly higher CEC.

While also formed in recent alluvium, the soils in the Mosaic® 86 field at Mount Angel are quite distinct from the other alluvial soils in the study because the Waldo series soils in this field formed in clayey alluvium of a tributary to the Willamette River (Abiqua creek). Waldo soils (Vertic Endoaquolls) are clayey and poorly drained, and the subsoil clay is smectitic mineralogy giving the soils the property of higher CEC, higher shrink-swell and a very slowly permeable subsoil. Waldo soils also have lower native productivity than the other alluvial soils in this study.

The second major soil group formed in silty sediments of the Ice Age Floods. Known as Missoula Flood Silts and Willamette Silts these are glaciolacustrine silts deposited by a series of cataclysmic floods over several thousand years at the end of the last glacial period. The floods inundated the Willamette Valley to about 122 m m.s.l. elevation. This is a very important marker deposit in the Willamette Valley since these silty sediments are largely exotic to the valley. Soils formed in silty deposits that were carried in a floodwater torrent with a jumble of sediment, gravel, boulders, and ice that flowed from Glacial Lake Missoula to the Willamette Valley.

The soils that formed on the Ice-Age Flood Silts are on an older surface called the Senecal geomorphic surface. Soils from the Homeplace (Williams 44, Grassman 42, Aunt Dora 9) and the upper parts of Mount Angel fields formed in these Ice-Age Flood silts. These sites are topographically above the Willamette River floodplain on broad terraces home to the soil association Willamette (Pachic Ultic Argixerolls), Woodburn (Aquultic Argixerolls), Amity (Argiaquic Xeric Argialbolls), Dayton (Vertic Albaqualfs), and Concord (Typic Endoaqualfs) soil series (well drained, moderately well drained,

somewhat poorly drained, and poorly drained in that order). They are on a stable landform of the valley terrace and have had enough time to form dark surface layers of mollisols and the clayey enriched subsoil horizon called an argillic horizon. These soils are more weathered than the floodplain soils and there is more vertical change from the surface to the subsoil in terms of texture, base saturation, and cation exchange capacity. However, compared to the soils of the foothills on the perimeter of the Willamette Valley the Missoula Flood soils are younger, less developed, and more fertile.

There are important differences in the soils that derive from the parent material type, their topographic position, and the soil morphology. These properties include the available water holding capacity (AWHC), the cation exchange capacity (CEC), and native productivity. These soil properties can affect hop growth and indirectly affect the role that crop management contributes to the terroir. Another important driver of soil variability on these soils is the depth to seasonal water table and the length of subsoil wetness in winter. This is closely related to topography and wetter soils tend to be in swales. This difference in natural drainage is especially a driver of variability within the soils formed on Ice-Age Flood Silt and in the clayey Waldo soils.

Hop Chemistry Differences Among the Sites

The chemical analyses of the three varieties revealed groupings by variety, as was expected. A Principal Components Analysis (PCA) was performed on the chemistry variables, which showed first two axes contained 67.3% of the variation. The F1 axis (49.3% of the variation) was mostly driven by the oil components. The F2 axis (18.0%) was driven by alpha-acid and dry matter. Agglomerative Hierarchical Clustering showed three groupings were present, representative of the three varieties, with the exception of Mosaic® Mt. Angel 86 (MOS-MA 86), which was grouped with the Simcoe® samples (data not shown). To facilitate visualization, the factor scores coming from the PCA of the individual sites were averaged within each field (Supplemental Figure 2). For all but two of the analytes, the ANOVA showed that there were significant differences ($p < 0.05$) between the three varieties (Supplemental Table 3). Within these three hop

varieties, Mosaic® distinguished itself by having the highest total oil and correspondingly higher myrcene, limonene, and rho-cymene levels. Simcoe® was somewhat similar to Mosaic®, but it had the highest alpha acids and nerol levels. By contrast, Strata® was considerably different from the other two varieties in its chemistry as it had the highest beta acids but generally the lowest hop oil components. It was particularly low in geraniol and citral but much higher than Mosaic® and Simcoe® for alpha humulene, (e)-beta-caryophyllene, and geranyl isobutyrate.

Within each variety, statistically significant variation among different fields existed, giving evidence of regional identity. Within the Mosaic and Simcoe varieties, only two and five analytes were not significantly different between fields, respectively (Supplemental Table 3). However, within the Strata variety only eight of the twenty-two analytes were significant in discriminating between fields. One must keep in mind that the hop chemistry focused on terpenes, terpene alcohols and esters and did not examine sulfur-containing compounds such as thiols.

In general, Mt. Angel fields were lower in aromatic compounds while the Alluvial fields were higher within their own varieties. Focusing just on the Mosaic® fields, Alluvial 33 had the highest levels of hop oil aroma compounds with the exception of isovaleric acid and myrcene. Total oil content was nearly identical for Alluvial 33 and Williams 44, though Alluvial 33 had higher levels of alpha acids and Williams 44 had higher levels of beta acids. Mt. Angel 86 was the lowest in all aroma compounds, alpha acids, beta acids, and total oil. Turning to the Simcoe® fields, Grassman 42 had the highest total oil content, while Mt. Angel 82 had the highest alpha and beta acid content. Interestingly, Mt. Angel 83 field usually had the lowest amount of hop volatiles. The highest concentrations were not consistently found in one field, though Aunt Dora 9 had more maximum concentrations of compounds than the rest of the fields. Finally, for the Strata® samples, Alluvial 49 had the highest concentration of aroma compounds, with the exception of citral 2 and caryophyllene oxide. Alluvial 49 was also highest in total oil content, although Alluvial 50 was highest in alpha acids and Goulet 73 was highest in beta acids. Goulet 73 was the lowest for almost all of the aroma compounds. Variation in

chemistry is important as it is well established that hop oil composition, which is made up of hundreds of compounds, is responsible for hop aroma.[37-39, 50]

Relationships among soil, weather, and management variables – evidence of regional identity

Multiple Factor Analysis was performed on each data table (weather/climate, soil, parent material, fungicide/insecticide applications, and management) with the chemistry data set as supplemental to the analysis (Supplemental Figure 3). The labelling nomenclature within the supplemental figures includes the site location within each field, thus MOS-MA 86/1 refers to Mosaic® grown in Mt. Angel on field 86 at site #1. To improve visual clarity and allow easier comparisons among fields, the factor scores for all sites within a field were averaged to create a new plot to compare fields (Figure 4). It is important to note that both weather and management data were the same for the whole field, thus helping to group the sites within the field together. However, the soil samples were taken at each site within the field, allowing for differentiation between them. Weather and parent material (PM) variables contributed highly to the F1and F2 axes while soil parent material contributed to the F3 axis. The weather variables were most closely aligned with the final MFA (RV coefficient of 0.861). When the F1 (38.76% of the variation) and F2 (20.35% of the variation) axes are plotted against one another, the hop samples did not group by variety but for the most part by location, further evidence of regional differences. The Mt. Angel 86 samples were in a different group than the Mt. Angel 82 and 83 samples with the exception of MOS - MA 86/1, which was due to similarities of soil parent material between this field site and the MA 82 & 83 fields for site 1 while the rest of field 86 was alluvial soil with high clay content. The Goulet field fell between Alluvial and Aunt Dora/Grassman/Williams groupings. Soil composition and heat units drove differences along the F1 axis, with clay soils and higher GDD on the right and sandy soils and lower GDD on the left. Precipitation and nitrogen fertilization drove differences along the F2 axis.

Observing the relationship between F1 and F3 helps explain more of the variation within the data set (12.50% of the variation explained by F3), particularly for the Strata® hop fields. In this plot (Figure 4), the Grassman/Williams/Aunt Dora complex of fields and Mt. Angel fields merged together along F3, while considerable separation occurred within the Alluvial fields due primarily to differences in the soil parent material. Parent material 2, which was loamy to silty textured recent alluvium, was at the top of the graph and included all of the STT-All 50 sites, STT-Gou 73 sites, MOS-All 33 (field sites 3&4), and SIM-All 23/2. Parent material 1, which was coarse textured recent alluvium, was at the bottom and included all of the STT-All 49 sites, MOS-All 33 (field sites 1,2, & 5), and SIM-All 23 (field sites 1,3,4, & 5). Parent materials 3 and 4, clayey recent alluvium and glaciolacustrine silts, respectively, made up the rest of the sites, and these separated primarily along F2.

To summarize, the Mt. Angel fields experienced higher heat units in the 2019 growing season, were higher in clay, were derived from soil parent material 4 - ice age flood silts, the exception being Field 86 where sites 2-5 which were derived from soil parent material 3 (clayey alluvium) and had moderate fungal disease pressure. The Grassman/Williams/Aunt Dora complex of fields had warmer temperatures and less precipitation for the growing season and the highest available water holding capacity of the soils. These sites also received the highest nitrogen and lowest sulfur and phosphorous fertilization rates. This subregional location was also experiencing most fungal disease and insect pressures given the greatest number of fungicide and insecticide applications. The Alluvial fields were the coolest and had higher precipitation, experienced more diurnal flux due to the Van Duzer corridor effect, had the highest phosphorous fertilization and were sandier on average because of the river alluvium soil and the proximity to the Willamette River. The soils at both the Alluvial farm and the Goulet field 73 were formed from Willamette River alluvium.

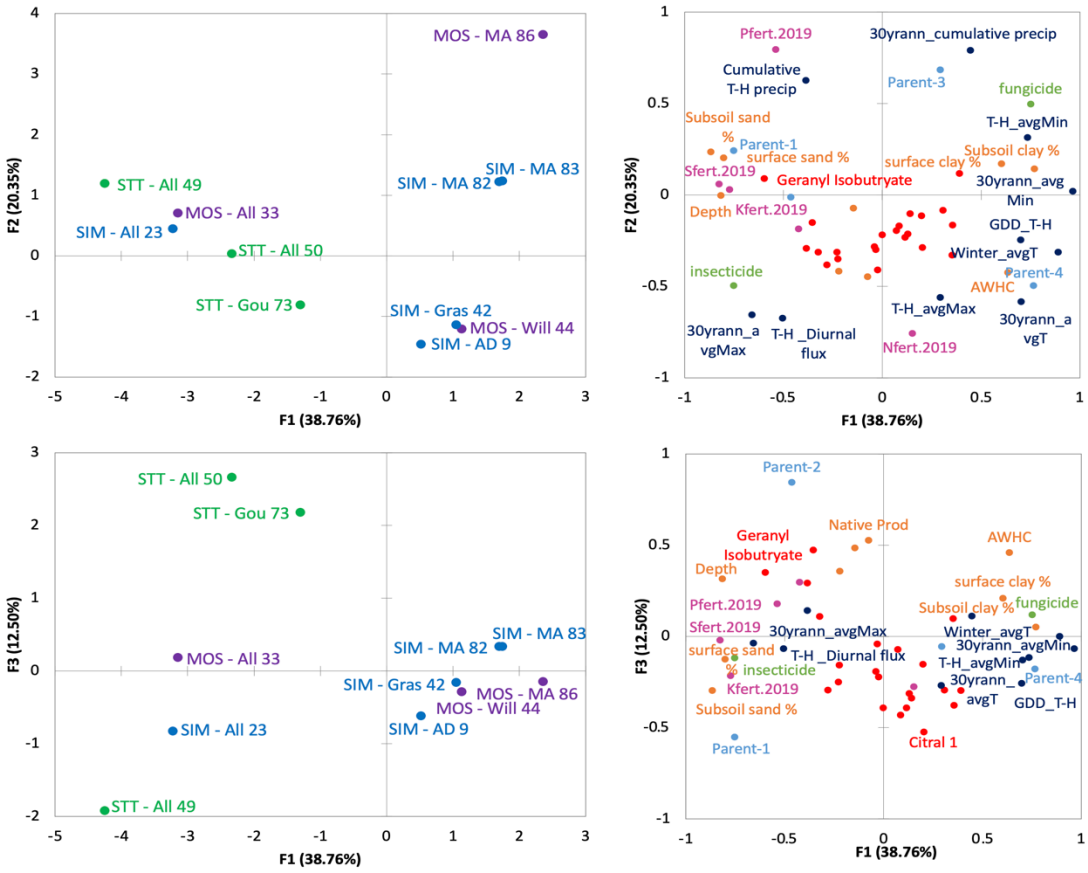

Figure 4: Multiple Factor Analysis for all variables; Top left – F1 versus F2, averaged sites within field; Top right – display of variables along F1 and F2 axes; Bottom left – F1 versus F3, averaged sites within field; Bottom right – display of variables along F1 and F3 axes. On left, samples are colored by variety (Strata® = green, Simcoe® = blue, Mosaic® = purple). On right, variables are colored by data set (Parent material = light blue, soil = orange, fungicide/insecticide = green, fertilizers = pink, weather/climate = dark blue, chemistry = red)

Sensory Discrimination and CATA Results

To determine if perceivable differences existed between fields of the same variety, ten single-hop, single-field beers were prepared and pairwise discrimination testing within hop variety using triangle tests was performed. For the Simcoe® beers, there were too many samples to carry out all pairwise comparisons of each field. Preliminary evaluation suggested that Mt. Angel 82 and 83 were not different from one

another so the Mt. Angel 82 site was not compared to the rest of the samples during testing. Triangle testing confirmed that Mt. Angel 83 and 82 were not significantly different from one another. Perceivable differences existed between Mt. Angel 83 and Grassman 42, Alluvial 23 and Aunt Dora 9, and Alluvial 23 and Grassman 42. The difference between Grassman 42 and Aunt Dora 9 was borderline significant (Table 2). For the Mosaic® beers, Williams 44 and Mt. Angel 86 were significantly different from one another. For the Strata® beers, Alluvial 49 was significantly different from both Alluvial 50 and Goulet 73; however, Alluvial 50 and Goulet 73 were not significantly different from one another. Once these perceivable differences were established, samples were then evaluated for qualitative differences using a Check All That Apply (CATA) method. These differences correspond, in large part, to the spatial separation observed in the chemistry PCA biplots (Supplemental Figure 2).

Table 2: Results of discrimination tests performed on ten beers

	Simcoe Triangle Tests							Strata Triangle Tests			Mosaic Triangle Test
Samples compared	SIM 83	SIM 9	SIM 83	SIM 23	SIM 23	SIM 42	SIM 83	STT 49	STT 49	STT 50	MOS 44
	SIM 23	SIM 83	SIM 42	SIM 9	SIM 42	SIM 9	SIM 82	STT 50	STT 73	STT 73	MOS 86
# correct	14	17	23	25	21	18	18	24	29	17	22
Evaluations	40	40	40	38	38	38	40	45	45	45	45
% correct	35.00	42.50	57.50	65.79	55.26	47.37	45.00	53.33	64.44	37.78	48.89
p-value	0.470	0.144	**0.001**	**< 0.0001**	**0.004**	0.051	0.083	**0.004**	**< 0.0001**	0.313	**0.022**

From a sensory point of view, the three hops used in this study are somewhat similar in aromatic qualities in that they were all described using *citrus* and *tropical fruit* descriptors, which were used the most frequently, 183 and 182 times in total, respectively (Supplemental Table 4). The descriptive panel generally grouped the three varieties as three clusters, with the exception of STT-All 49 (Figure 5). Although *citrus* and *tropical fruit* were frequently used, they did not significantly discriminate among the samples (Cochran's Q test) (Supplemental Table 4). However, seven attributes were significant ($p<0.05$) in differentiating the samples: *sweaty/stinky*, *fruity*, *berry*, *cannabis*, *onion/garlic*, *pomme*, *vegetal*. Additionally, there appeared to be a correlation between *onion/garlic*, *vegetal*, and *cannabis*. For the Strata® beers, the Goulet 73 and Alluvial 50 were similar and Alluvial 49 was different from both of them, which matched the results

from the discrimination test and the chemistry results. In fact, the Strata® from Alluvial 49 was more similar to the Mosaic® samples. Goulet 73 and Alluvial 50 were both associated with fruity characteristics, though Goulet 73 had more *berry* and Alluvial 50 had more *overripe fruit*. Alluvial 49, on the other hand, was more *sweaty/stinky*, *onion/garlic*, and *vegetal*. The two Mosaic® beers were grouped together but were differentiated by some attributes. Williams 44 was more *vegetal* and *cannabis* and Mt. Angel 86 was more *citrus* and *resinous*. The Simcoe® beers were grouped together and were generally higher in *citrus* frequency than the other two varieties. They were also generally lower in *stinky/sweaty*, *onion/garlic*, and *vegetal* character. Some of the Simcoe® samples had some *resinous* character. Though the fields are very close geographically, Mt. Angel 82 tended to have more *citrus* and *tropical* than Mt. Angel 83, while Mt. Angel 83 had more *cannabis*. Aunt Dora 9 had the highest *resinous* and *tropical* quality of the Simcoe® hops. Grassman 42 had more fruity characteristics that were described by *pomme* and *melon,* in addition to the common *citrus* and *tropical* characteristics shared by others. Alluvial 23 was described by *overripe fruit*, *floral*, and no *onion/garlic*.

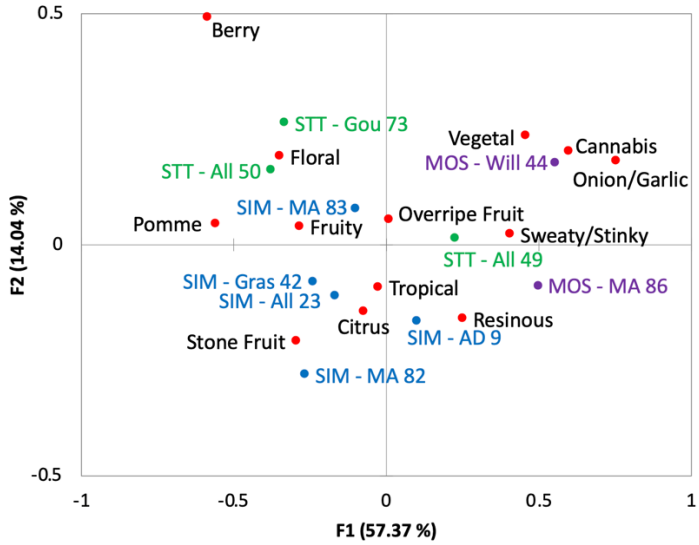

Figure 5: Correspondence Analysis of key attributes from CATA testing. Samples colored by variety (Strata® = green, Simcoe® = blue, Mosaic® = purple). Attributes shown as red circles.

Observing variation in chemistry and sensory outcomes within variety

The PCA plot of the chemistry analytes did not match the MFA of all variables because variety has a stronger influence on chemistry than growing environment. As is well-established in plant breeding, phenotypic expression relies on both genotype and environmental factors. Here, variation of chemical composition within variety seems to be driven by the environmental factors. This finding confirms that of other studies which have shown that chemistry differences exist when one variety is grown in different locations.[18, 20, 21] Additionally, the differences in aroma perceived in these beers is in agreement with a 2017 study which demonstrated the same sensory variation in beers made with Amarillo hops grown in different regions.[18] For the Strata® samples, Goulet 73 and Alluvial 50 were more similar to each other and Alluvial 49 was different by observing all of the environmental factors as well as the chemistry and sensory outcomes (see variation along F3 in Figure 4). The Alluvial 49 field was a different parent material, on average much sandier, and very close to the Willamette river. For the Mosaic® fields, the Alluvial 33 and Williams 44 were more similar chemically, but all three fields were different when all factors were considered. This was driven by their growth in soils with three different parent materials. These differences led to a significant difference in beer aroma between Williams 44 and Mt. Angel 86. Within the Simcoe® variety, the MFA showed similarities between the adjoining Mt. Angel fields 83 and 82, as expected, and they were also had similar chemical makeup. Additionally, the Grassman 42 and Aunt Dora 9 fields were similar in both chemistry and MFA, despite their locations more than 7 km apart. The Alluvial 23 field, on average, stood alone in chemical makeup and MFA. While there were some significant differences between pairwise comparison of the Simcoe® beers, the CATA analysis showed that the differences were nuanced and did not always match discrimination analysis or MFA results. The differences observed among the various fields as visualized in the MFA biplots, along with the differences perceived via sensory evaluation offer initial evidence of regional differences in hop qualities.

Confounding variables and other considerations

There are several other considerations that should be accounted for in evaluating regional identity of hops. It is possible that the influence of regional identity or the environmental factors, will vary from variety to variety. Newer hop varieties, including Strata®, which was initially bred in 2009, are bred for consistency when grown in various locations. Also, some varieties may experience true-to-type issues over time, though this problem is usually minimal with proprietary varieties.

Some confounding variables with the current study should also be acknowledged. Due to the logistical challenges with harvest, the samples were kilned in commercial driers at the same farm (Alluvial) along with other varieties, some of which were not part of this study. While all samples were kilned at the same temperature, the drying times varied depending on what other varieties the samples were being dried with, as each variety dries at its own rate. The average final moisture content of the hops was 9.5%, which is an industry standard. One exception was Simcoe® Alluvial Field 23, which averaged 17% moisture.

To fully understand the role of terroir in hop growing, other considerations should be accounted for in future work that the current research was unable to include. These considerations include but are not limited to irrigation amount and water quality, soil chemistry and microbiology, bine age, and viral pressure. In the current study, fields were chosen to avoid viral pressure, as it is known that viruses can alter hop quality.[51] Another significant consideration in all hop studies is hop maturity at harvest.[8, 52] Harvest decision making is a complex process influenced by and determined by a number of difference factors as explained in the materials and methods section. While dry matter is not the only way to characterize maturity, it is a widely used metric in the American hop growing community. The dry matters at harvest for Simcoe® averaged 27.9%, for Mosaic® averaged 24.2% and for Strata® averaged 26.4%. More research is needed to identify better indices of quantifying hop cone maturity and determining harvest readiness.

The concept of terroir can be evaluated by a long list of potential measurable variables. Nevertheless, certain hop varieties may already be planted in specific locations due to the experiential knowledge of hop growers. Learning from past successes and failures may have already guided their planting decisions. The goal of this work is not to identify the "best" places to grow hops, nor to identify the "best" hop. Rather it is focused on investigating how growing locations differ and how these differences are expressed in hops. Ideally, the information presented here along with future work can be used as a way to aide decision making by hop growers and brewers.

Conclusion

Three hop varieties, (Mosaic®, Simcoe® and Strata®) grown in eleven fields in the Willamette Valley, Oregon were sampled and analyzed from two to five sites within each field. Analysis of the chemical makeup of the hops showed that they grouped by variety, but between field variation existed within the variety at the same magnitude or greater than the within-field variation for many fields. There were small differences between fields for the weather variables due to their relatively close location to one another. However, the farm with the most precipitation during growing season was Alluvial and the fields with the most GDD were Grassman 42 and Williams 44, which are located next to each other. From the management data collected from each field, Grassman 42 had most fungicide and insecticide applications. For the fertilizer applications the Williams 44 field received the most nitrogen, Mt. Angel 86 the most phosphorous, Alluvial 23 the most potassium, and Alluvial 33 the most sulfur. Four distinct soil Parent Materials (PM) were found within the eleven fields, three of them being river alluvium and one from Ice Age flood silts. Some of the fields had two different PMs within them, creating variation between sites. Soil characterization showed that soils were highly variable, particularly in their composition of sand, silt and clay. The sandiest soil on average was Alluvial 49 and the most clayey soil on average was Mt. Angel 86.

Multiple Factor Analysis was performed on all of the data sets, with the chemistry variables being labeled as supplemental to the other variables. The MFA showed that samples grouped by their location instead of by variety. Variables that displayed the most variation were sandy versus clayey along the F1 axis, precipitation and temperature ranges along the F2 axis, and Parent Materials 1 & 2 along the F3 axis.

Both discrimination and descriptive sensory analysis were performed on beers hopped with a single-field composite sample. Discrimination testing showed perceivable differences between fields within each variety. For the Strata® beers there were significant differences between Alluvial 49 and Alluvial 50, and Alluvial 49 and Goulet 73. The two Mosaic® beers, Williams 44 and Mt. Angel 86 were significantly different. Of the Simcoe® beers evaluated, there were significant differences between Alluvial 23 and Aunt Dora 9, Alluvial 23 and Grassman 42, and Mt. Angel 83 and Grassman 42. Additionally, during CATA testing there were significant differences in the usage of the attributes *sweaty/stinky*, *fruity*, *berry*, *cannabis*, *onion/garlic*, *pomme*, and *vegetal* between samples.

In summary, the analysis presented shows evidence of regional identity effect on hops. As expected, the chemical analysis showed sample groupings by variety. Although variety had the largest influence on chemical makeup, variation existed within each variety which could be associated with differences in soil, weather and management. Observing chemical differences based on where the samples were grown suggests regional identity. Additionally, sensory differences within variety were exemplified by the panelist's ability to discriminate within a variety in manners that supported the chemical differences. Further research will seek to expand the scope of the research to growing regions outside of the Willamette Valley. Furthermore, additional harvest years must be studied along with additional regional factors in order to expand our knowledge of how growing regions may influence hops' aromatic qualities.

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Supplemental Material

Tables

Supplemental Table 1: Explanation of variables used in analysis

Hop Chemistry	Explanation	Management	Explanation
Total oil	mL of oil per 100 g of oil via distillation adjusted to 8% moisture content	**Nfert.2019**	Nitrogen fertilization rate; lbs/acre
% Alpha	Alpha Acids; Calculated at 8% MC by UV	**Pfert.2019**	Phosphorous fertilization rate; lbs/acre
% Beta	Beta Acids; Calculated at 8% MC by UV	**Kfert.2019**	Potassium fertilization rate; lbs/acre
DM	Dry Matter; 1 - ((sample weight - dry sample weight)/sample weight)) * 100	**Sfert.2019**	Sulfur fertilization rate; lbs/acre
Iso Valeric Acid	Aromatic compounds; mg per 100 g of hops	**yield**	Hops harvested on each field; bales/acre
B-pinene		**fungicide**	Number of days fungicides were applied
Myrcene		**insecticide**	Number of days insecticides were applied
3-carene			
Methyl Heptanoate			
Rho-cyme			
Limonene			
Linalool			
Citral 1			
Citral 2			
Alpha terpeniol			
Nerol			
Geraniol			
Geranyl Acetate			
Alpha humulene			
E-Beta Caryophyllene			
Geranyl Isobutryate			
Caryophyllene oxide			

Weather and Climate	Explanation	Soil	Explanation
Winter_avgT	Winter Average Temperature; Calculated from Dec 1st to Feb 28th	**Surface thickness**	Indicates development of the soil; Measured in inches
GDD_T-H	Growing Degree Days; SUM ((daily max T - daily minT/2) - 41) from training to harvest. Min temp corrected to 41; mac temp corrected to 86.	**Surface sand %**	Measure of soil surface texture (0-8 inch depth); %sand + % clay + %silt = 100%
T-H_avgMin	Average minimum temperature from training to harvest; Shows the cooling of the field	**Surface clay %**	Measure of soil surface texture (0-8 inch depth); %sand + %clay + %silt = 100%
T-H_avgMax	Average maximum temperature from training to harvest	**Subsoil sand %**	Measure of subsoil texture (24-36 inch depth); %sand + % clay + %silt = 100%
T-H _Diurnal flux	Diurnal flux; Daily max temp minus daily min temp averaged daily from training to harvest	**Subsoil clay %**	Measure of subsoil texture (24-36 inch depth); %sand + % clay + %silt = 100%
Cumulative T-H precip	Cumulative precipitation from training to harvest; measurement of rainfall during plant growth (inches)	**Depth**	Depth to seasonal water table (in inches); If above 60, written as 60
30 yr annual_avgMin	30 year annual averaged minimum temperatures	**AWHC**	Available Water Holding Capacity; Estimate in inches based on texture & soil series
30 yr annual_avgT	30 year annual averaged temperatures	**Native Prod**	Native Productivity; Score out of 100 - based on soil type
30 yr annual_avgMax	30 year annual averaged maximum temperatures	**Max Prod**	Maximum Productivity; Native productivity adjusted for farm practices
30 yr annual_cumulative precip	30 year annual averaged cumulative precipitation; Total snow and rain (in inches) averaged over 30 years	**Parent material**	1 - coarse textured recent alluvium 2 - loamy to silty textured recent alluvium 3 - clayey recent alluvium 4 - glaciolacustrine silts

Supplemental Table 2: Soil series present in each of the fields

		Willamette River Alluvium							Ice-Age Flood Silts			
		Soil Series							Soil Series			
Farm block	**Hop Variety**	**Cloquato**	**Chehalis**	**McBee**	**Newberg**	**Pilchuck**	**Wapato**	**Waldo**	**Woodburn**	**Amity**	**Santiam**	**Concord/Dayton**
Alluvial 23	Simcoe	x			x		x					
Alluvial 33	Mosaic	x		x	x							
Alluvial 49	Strata				x	x						
Alluvial 50	Strata	x	x		x							
Goulet 73	Strata	x	x									
Mt Angel 86	Mosaic							x	x			x
Mt Angel 82	Simcoe								x	x	x	x
Mt Angel 83	Simcoe								x	x		
Williams 44	Mosaic								x	x		x
Grassman 42	Simcoe								x	x		
Aunt Dora 9	Simcoe								x	x		x

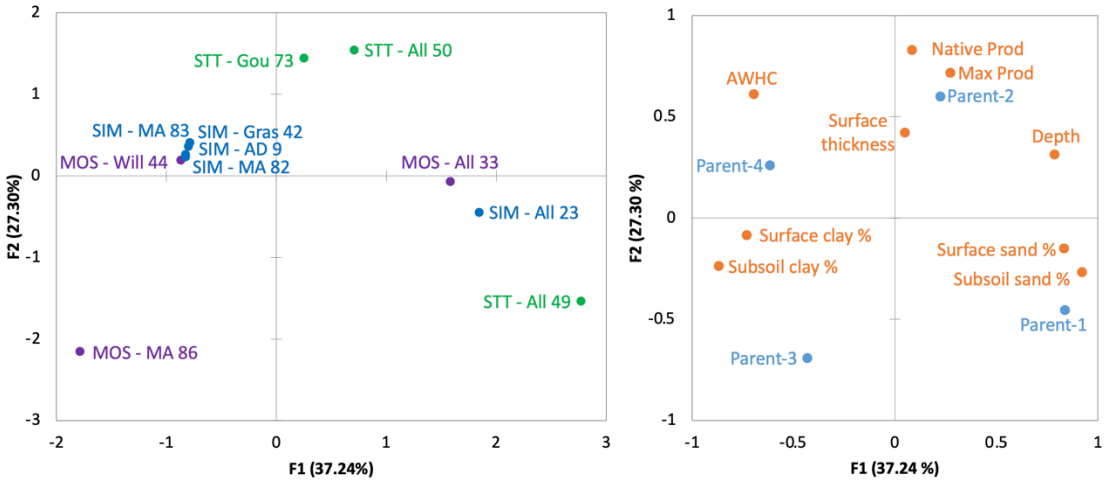
Supplemental Table 3: F-values produced by Analysis of Variance of chemical analytes between varieties and between fields within each variety. Bolded F-values indicate significant treatment effect for the analyte ($p < 0.05$).

		Field effect within variety		
Analytes	**Variety**	**Mosaic**	**Simcoe**	**Strata**
Total oil (ml/100g)	**5.8**	**9.9**	**3.8**	3.5
Alpha (%w/w)	**16.8**	**10.1**	2.0	0.5
Beta (%w/w)	**78.5**	**26.0**	**7.7**	2.7
Iso Valeric Acid	0.0	**8.6**	**3.1**	2.4
B-pinene	**37.2**	**6.3**	**4.0**	**14.3**
Myrcene	**48.3**	**10.0**	**5.0**	2.7
3-carene	**27.6**	**25.5**	**3.0**	**6.9**
Methyl Heptanoate	1.4	**11.8**	**9.2**	3.7
Rho-cyme	**49.6**	**10.3**	**5.2**	3.6
Limonene	**53.3**	**7.0**	2.6	2.3
Linalool	**6.2**	**14.2**	**7.3**	2.3
Citral 1	**53.9**	**4.8**	**6.4**	**12.7**
Citral 2	**43.3**	**6.3**	**4.7**	0.3
Alpha terpeniol	**152.0**	**6.9**	2.5	1.1
Nerol	**69.9**	**12.6**	1.0	0.8
Geraniol	**140.7**	**12.5**	**5.4**	**7.7**
Geranyl Acetate	**94.5**	1.9	2.0	**6.2**
Alpha humulene	**87.8**	**15.0**	**5.7**	**10.2**
E-Beta Car	**82.5**	**14.8**	**4.5**	**9.7**
Geranyl Isobutryate	**783.5**	**26.5**	**7.8**	**20.6**
Car oxide	**20.7**	**13.5**	**4.2**	2.2
DM Average*	**22.4**	0.2	**6.7**	3.5

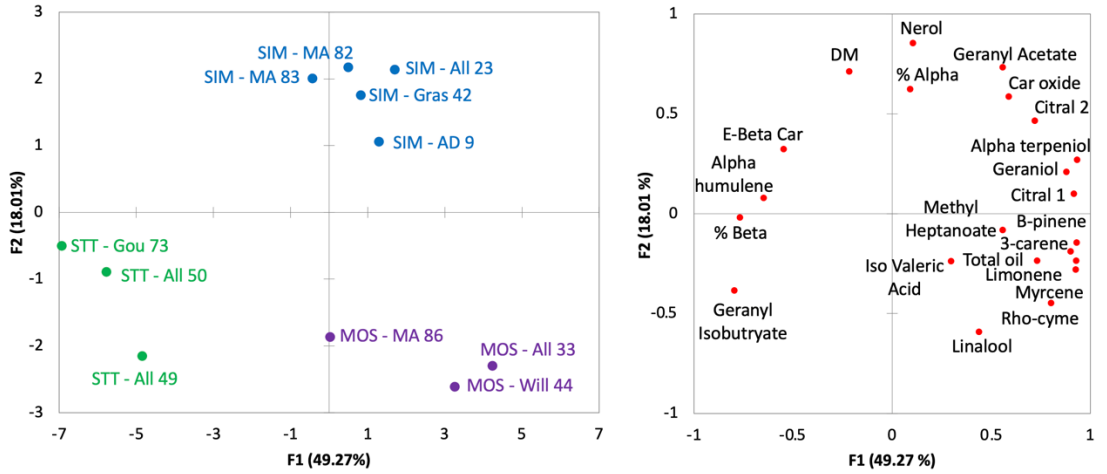
Supplemental Table 4: Heat Map of frequency of Check All That Apply attributes with dark red attributes being most frequently used; Attributes highlighted in yellow indicate significant discriminators by Cochran's Q test; Thick box indicates attributes used in Correspondence Analysis

Attribute	MOS - Will 44	MOS - MA 86	SIM - MA 82	SIM - MA 83	SIM - AD 9	SIM - Gras 42	SIM - All 23	STT - All 49	STT - All 50	STT - Gou 73	Total Usage	% of Most Used Attribute
Citrus	13	20	24	20	18	18	21	18	15	16	183	100
Tropical	16	22	19	13	22	19	19	16	19	17	182	99
Sweaty/Stinky	19	17	7	6	10	8	6	18	8	5	104	57
Fruity	7	3	11	9	8	14	8	10	12	12	94	51
Overripe Fruit	11	11	4	5	8	12	11	7	14	6	89	49
Resinous	8	11	6	7	11	5	6	7	2	5	68	37
Stone Fruit	3	2	11	4	7	6	8	7	7	6	61	33
Floral	4	3	4	6	5	5	8	1	8	10	54	30
Berry	1	0	3	6	2	3	3	4	9	11	42	23
Cannabis	11	7	1	6	6	1	2	4	0	4	42	23
Onion/Garlic	10	10	1	3	3	2	0	7	2	2	40	22
Pomme	1	0	5	4	1	7	2	3	6	4	33	18
Vegetal	7	2	1	1	1	1	4	5	1	2	25	14
Herbaceous	6	2	3	4	7	5	3	6	5	5	46	25
Melon	1	1	5	4	3	5	3	5	2	5	34	19
Spicy	4	4	2	1	4	2	3	3	4	5	32	17
Grassy	3	2	6	6	2	1	3	2	1	1	27	15
Sweet Aromatic	4	4	0	2	1	4	1	2	1	2	21	11
Earthy	0	1	1	0	2	2	1	2	1	2	12	7

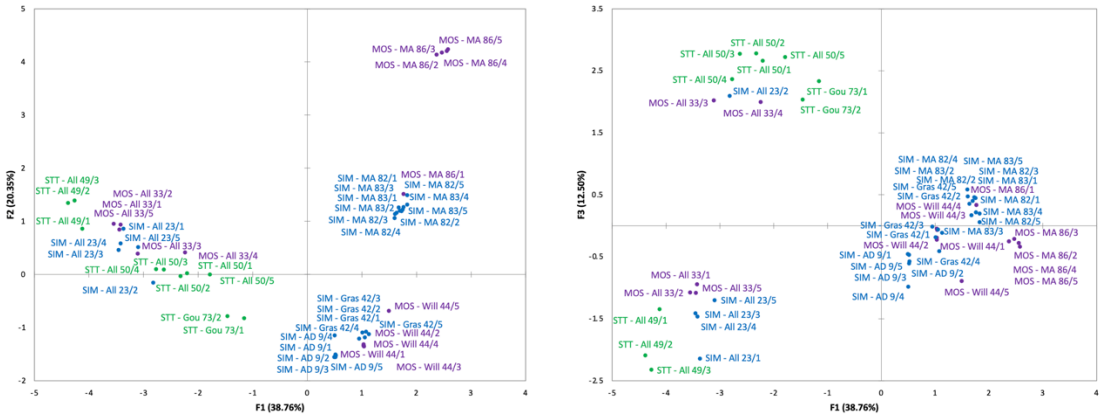
Figures



Supplemental Figure 1: Multiple Factor Analysis of soil characterization and parent material; Plot on left displays field grouping; Plot on right shows how variables are related to one another and each of the fields. On left, samples are colored by variety (Strata® = green, Simcoe® = blue, Mosaic® = purple). On right, variables are colored by data set (Parent material = light blue, soil = orange)



Supplemental Figure 2: Principal Component Analysis of chemistry analytes; Plot on left displays averaged value of sites within each field; Plot on right shows how variables are related to one another and each of the fields. On left, samples are colored by variety (Strata® = green, Simcoe® = blue, Mosaic® = purple). On right, chemistry variables indicated by red circles



Supplemental Figure 3: Multiple Factor Analysis of all variables for each site; Plot on left displays F1 axis versus F2 axis; Plot on right displays F1 axis versus F3 axis. Samples are colored by variety (Strata® = green, Simcoe® = blue, Mosaic® = purple).

Chapter 3 - Comprehensive analysis of different contemporary barley genotypes enhances and expands the scope of barley contributions to beer flavor

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Chapter 3 – Excerpt from Comprehensive analysis of different contemporary barley genotypes enhances and expands the scope of barley contributions to beer flavor

Introduction

This publication was a large, multi-laboratory project which was effectively divided into three sections, malt production and genetics, brewing and sensory, and metabolomics. The overarching goal of the project was to understand the contributions of different barley genotypes to beer flavor. Sarah Windes, Scott Fisk, and Dr. Patrick Hayes developed the experimental varieties and were responsible for malting. Harmonie Bettenhausen and Dr. Adam Heuberger performed metabolomics and analysis. Jeff Clawson brewed the experimental beers. Dr. Juyun Lim and Sue Queisser contributed the consumer sensory data. Dr. Tom Shellhammer provided guidance with data interpretation.

As co-first author, my contributions to the project were the execution of laboratory panel sensory testing, analysis of the data, and interpretation of the results. These contributions are included here, while the full publication is attached as an appendix. The sensory analysis sought to characterize both malt steep and beer aroma and flavor for each of the malts. Additionally, the potential of hot steep malt sensory evaluation as an economical, effective tool for assessing barley/malt impacts on beer flavor was investigated.

Sensory Methodology

Beer sensory

A beer sensory pipeline was performed as described in Bettenhausen et al. (2020) [3], and two types of sensory studies were conducted 1) a consumer panel and 2) a laboratory panel.

The consumer panel testing was performed in collaboration with the Oregon State University Center for Sensory & Consumer Behavior Research (http://agsci-labs.oregonstate.edu/sensoryresearch/). WRC beers were tested in August 2019 while NP beers were tested in January 2020. The procedures were performed as described by

Bettenhausen et al. (2020) [3] and detailed in Supplemental File 3. Briefly, participants (WRC n = 152; NP n = 155) were asked to answer a series of questions per beer, including 1) overall liking (scale from 1-9), 2) Check All That Apply (CATA) for sensory characteristics, 3) "ideal lager" attributes, and 4) demographics.

The laboratory panel testing was performed in collaboration with the OSU Brewing Science Lab in October 2019. Thirteen panelists (6 M, 7 F; 22 - 55 years old), who had prior experience on beer and wine descriptive analysis sensory panels, were trained over three separate sessions with the beers in question using the Projective Mapping with Ultra Flash Profiling sensory method [7,8] and detailed in Supplemental File 4. WRC beers and NP beers were assessed for sensory attributes on two separate days, with each beer being presented in duplicate (WRC n=10; NP n=8). During each testing session, panelists assessed the orthonasal aroma and flavor by mouth of the beer in two separate tests, with new blind codes for the samples.

Hot steep malt sensory

Sensory analysis was performed on liquid extract produced from hot steeps of all malts in the experiment, prepared in accordance with ASBC Methods of Analysis – Sensory Analysis 14 [6]. Descriptive data were collected using Projective Mapping (PM) combined with Ultra Flash Profiling [7,8]. Due to changes in panelist availability between the beer and hot steep malt sensory analyses, a new laboratory panel was recruited and trained over four, one-hour sessions, detailed in Supplemental File 5. This 15-member panel (8 M, 7 F; 23-68 years old) consisted of some of the same members as the beer sensory panel, but also included some new members, most of which had prior experience performing sensory analyses on other foods such as wine. Laboratory panel testing was performed in collaboration with the OSU Brewing Science Lab in March 2020. Malt hot steeps from five WRC malts and four NP malts were assessed for sensory attributes on separate days. During each testing session, panelists assessed both the orthonasal aroma and the flavor by mouth of the malt hot steeps in two separate tests. Half the panel carried out the orthonasal testing session followed by a five-minute break and then the flavor

session, while the other half of the panel proceeded in the opposite order. Unique blind codes were used for each test, and the serving order was randomized for each panelist. The WRC malt hot steep sessions were carried out with 15 panelists held over two days, while the NP malt hot steep session was carried out with ten panelists on a single day.

Sensory data analysis

All sensory data were collected via Compusense Cloud Software (Version 20.0.7404.31336, Guelph, Ontario, Canada). Projective Mapping combined with Ultra Flash Profiling provides both attribute counts and coordinate data for each sample evaluated. Coordinate data was analyzed using XLSTAT Multiple Factor Analysis (MFA) (Addinsoft, New York, NY). Individual MFA plots for aroma and flavor were created for both WRC and NP sample sets in both beer and malt hot steeps. Attribute data was processed in order to combine specific descriptors under the more broad descriptors, in accordance with the Base Malt Flavor Map (Supplemental File 6). Post processing, descriptor data were then analyzed by Correspondence Analysis (CA) in XLSTAT. Attributes were ranked according to frequency of use summed across all of the samples in the set. As there is no standard cutoff for attribute inclusion, it is up to the researcher to determine the appropriate threshold [8]. In this case, the cutoff was set in order to display pertinent attributes, while filtering out attributes that do not help further explain the relationship between the samples. Those attributes that were used at a rate of at least 45% of the most frequently used attribute were included in the CA plot for the laboratory panel beer aroma sensory data. For the malt hot steeps, aroma and flavor CA plots were created individually before being combined and plotted together with the attributes used being those that were used by the overall panel with a frequency of >25% of that of the most frequently used top attribute.

Results

Sensory characteristics for malt hot steeps

Projective Mapping was used to evaluate both aroma and flavor attributes of malt hot steeps made from the WRC (15 panelists) and NP (10 panelists) samples. In each sample set, one malt was randomly selected to be presented as a duplicate. For the WRC malts, Flavia was replicated giving six total malt hot steep samples. Based on aroma evaluation only, panelists grouped duplicates closely together, implying perceived similarities between them, and dissimilarities between other samples. During the flavor evaluation, the Flavia duplicates were not placed as close to one another. *Thin body* was the only mouthfeel attribute used frequently enough to be plotted. Coordinate data from aroma evaluation showed that Thunder and Violetta were different from the other samples (Supplemental Figure 3). During the aroma evaluation, *grainy* was used consistently among the samples but showed more variable usage during flavor evaluation (Figure 1). In both aroma and flavor evaluations, *grassy* had a large variation in usage among the samples, with Calypso being described as *grassy* most frequently. Additionally, Calypso's aroma was described by *vegetal*, while its flavor was described by *cracker*. Both Flavia samples were high in *grassy*, and on average were high in *earthy*. Thunder and Violetta were each much lower in *grassy* than the rest of the samples. Thunder was consistently described by *sweet aromatic, breakfast cereal*, and *sweet bread*. Violetta was also more closely associated with *bread*. Descriptors used for Wintmalt varied between aroma and flavor, but *grassy* was used in both.

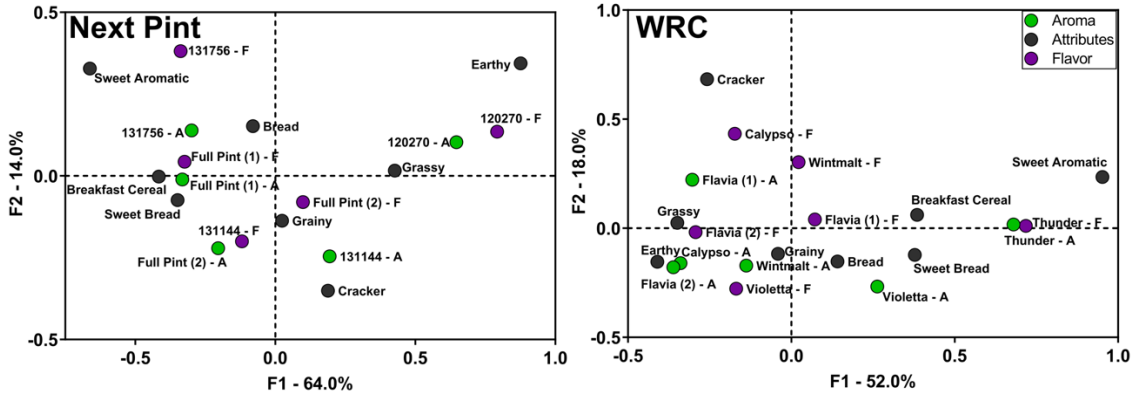


Figure 1. Correspondence Analysis from hot steep Projective Mapping (left pane: Western Rivers Conservancy samples, right pane: Next Pint samples). "1" and "2" designates duplicate observations of the same samples with different blind codes. CA plots show which attributes (black squares) are used to describe the samples (indicated by green and purple circles). Samples that are close together are described similarly, while samples far apart were described differently. Both Aroma and Flavor evaluations are plotted together with the top eight most frequently used attributes.

For the NP malts, Full Pint was replicated, giving five malt hot steep samples. The coordinate data showed similar configurations between aroma and flavor evaluations, with the exception of a Full Pint duplication moving positions (aroma data shown in Supplemental Figure 3, flavor data not shown). In both the MFA and CA plots, DH120270 appeared distinct from the other malt steep samples. *Grainy* was the most used descriptor for the NP aroma and flavor evaluations and was not helpful in the discrimination of samples, hence its location near the center of the samples (Figure 1). There were large differences in usage across samples for *grassy* in both flavor and aroma, and *sweet aromatic* via aroma only (attribute count data not shown for concision). Additionally, *sweet bread, earthy*, and *breakfast cereal* highlighted the differences between the samples during the flavor evaluation. In both aroma and flavor evaluations, Full Pint was described by *breakfast cereal*, with the exception of one Full Pint flavor replication. DH120270 was the most unique sample of the group and was highly *grassy* and *earthy* across both evaluations. DH131144 and DH131756 were both described

attributes within the *bread* category, though DH131144 was described with *cracker* and DH131756 with *sweet aromatic*.

Beer sensory – laboratory panel

Projective Mapping was used to assess both aroma and flavor attributes of the WRC (13 panelists) and NP (10 panelists) beers in duplicate (10 and 8 beers per set, respectively). Multifactor Analysis (MFA) plots of the WRC aroma coordinate data showed separation of the duplicates, which indicates that differences between the beers were subtle (Supplemental Figure 4). This pattern was also present in the coordinate data from the WRC flavor test, with the exception of Calypso and Violetta duplicates, which were closer together (data not shown). Correspondence Analysis (CA) with attribute data showed Calypso duplicates were close together and were described by *fruity* and *floral* in aroma (Figure 2), and *fruity* in flavor (data not shown for concision). Aroma attribute data showed differences between duplicates for the other 4 beer samples. *Fruity* was the most commonly used descriptor for this sample set, while *earthy, grainy*, and *floral* helped discriminate the samples from one another. Additionally, the flavor data showed Flavia duplicates were similar and described by *grainy* and *grassy*. Wintmalt duplicates were close together and described by *sweet aromatic*, *floral* and *vegetal*. On average, Violetta duplicates were higher in *dough* and *sweet bread* than the other samples, which did not match its description by orthonasal evaluation. Thunder duplicates showed differences in use of *sweet bread* and *sweet aromatic* between them. In summary, there were inconsistencies in describing the WRC samples and with grouping the duplicate beer samples.

The MFA plots for the NP aroma sample set (8 beers) showed that, with the exception of DH131756, the duplicates are placed closely together, indicating that they were perceived as similar by the panel (Supplemental Figure 4). In the plot for the NP flavor sample set, DH131756 and DH131144 duplicates were mixed together, indicating that panelists were confusing these four beer samples. For both aroma and flavor evaluation, *grainy* was the most frequently used attribute for the sample set and thus

unhelpful for discriminating samples (Figure 2). In both aroma and flavor, both *sweet bread* and *vegetal* had high variation in usage frequency between the samples (attribute count data not shown). DH120270 was described by *grassy* via orthonasal evaluation but was described by *vegetal* via taste evaluation (flavor data not shown). In both the aroma and flavor evaluations, the duplicates for DH131144 varied somewhat. In general, they were described with both *sweet aromatic* or *sweet bread*, as well as *dough*, *pasta,* or *cracker*. Although there were differences between the DH131756 duplicates they were both high in *fruity* in the aroma evaluation, and high in *sweet aromatic* in the flavor evaluation. Full Pint duplicates varied in their attribute counts for various descriptors but was consistently associated with *dough* in both aroma and flavor. Overall, duplicates were more similarly described for the NP sample set than the WRC sample set, indicating that there were greater differences between samples within the NP set.

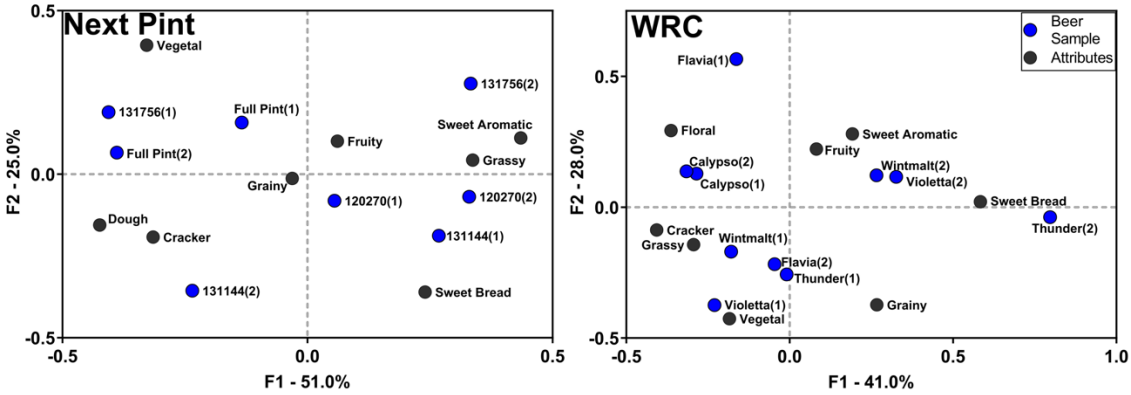


Figure 2. Correspondence Analysis of top 8 most used aroma attributes from beer Projective Mapping with Laboratory Panel (right pane: Next Pint beers; left pane: Western Rivers Conservancy beers). 1 and 2 designates duplicate observations of the same samples with different blind codes. CA demonstrates which aroma attributes (indicated by black squares) are used to describe the beer samples (indicated by blue circles).

Discussion

Sensory attributes of malt hot steeps and beer, and their relationships

Hot steep malt sensory

Prior to the establishment of the hot steep malt sensory method, Congress worts were used for sensory evaluation of malt samples [31]. Since its development, the hot steep malt sensory evaluation method has piqued the interest of the brewing and malting industries to improve analysis of malt sensory and predict beer sensory for malts of interest [5,6]. It is helpful when only a small quantity of malt is available and is more convenient than making beer. The predictive ability of this method, though much more rapid than brewing, has yet to be fully understood. With the analysis pipeline implemented in this research, we can identify relationships of hot steep malt sensory with other traits. However, determining if relationships are causal and predictive will require further experiments.

Within the WRC set, Thunder and Calypso were standout samples for hot steep malt sensory. The former was higher in *sweet bread* and *sweet aromatic* for both aroma and flavor while the latter was *grassy* and *vegetal* in aroma and *cracker* in flavor. Considering the other varieties in this set, Thunder and Violetta were lower in *grassy* thus separating them from the other samples. DH120270 was a standout sample within the NP set. In both the aroma and flavor evaluations, it was consistently described by panelists as more *grassy* and *earthy* than the other samples. Malt analytics provide clues that Thunder was more modified than Calypso, thus leading to differences in hot steep malt sensory. While it seems likely that the *sweet bread* and *sweet aromatic* descriptors for malt hot steeps are attributable to the higher enzyme profiles of Thunder, DH131144, and DH131756, further research is necessary. The basis of the *grassy* profile for Calypso is not obvious, however in the case of DH120270, it could be ascribed to under-modification. Given this line's Maris Otter heritage, this may be a question for further research. From a plant breeding perspective, the poor modification of DH120270 and its *grassy* and *earthy* profile in the hot steep malt sensory would be grounds for not advancing it on to brewing and beer sensory. In this sense, evaluations using hot steep

malt sensory could be a tool in variety selection. In order to assess its value for the malting and brewing industries, the key question remains "is hot steep malt flavor predictive of beer flavor"? The current research provides some insights into this relationship, but further experiments will be required. Within the current experiment, the connection between malt and beer sensory is best explored using the laboratory panel data, given the commonality of protocol and lexicon.

Laboratory beer sensory

The laboratory beer sensory panel had some difficulty matching duplicates within the WRC set to one another, with the exception of Calypso. However, differences in sensory attributes were still perceived among the beer samples. This pattern suggests that stringent selection for commercial potential led to barleys that, despite differences in malt and beer analytics, produced beers that are only subtly different in sensory profiles. The nuanced differences may result from inconsistencies in malt-modification (Table 2) [1]. There is evidence to show that undermodified malts may result in higher *grassy* qualities [3]. In the NP set, duplicates were more similarly described for both aroma and flavor, indicating that panelists not only found differences among the beers but that these differences could be identified with consistency. This consistency of difference may be due to the more limited selection and validation for malting and brewing properties of the NP set, as compared to the WRC set. DH120270 duplicates were closely grouped, with consistent *grassy* aroma and *vegetal* flavors. This could be due to the lower malt modification of DH120270, leading to *grassy* and *earthy* flavors [3], compared to the other NP samples. DH131756, DH131144, and Full Pint had similar malt analytical profiles, which may be one reason why there was less distinction in flavor profiles among the beers made from these malts.

Comparing beer and hot steep malt sensory

While beer samples were all duplicated, only one malt hot steep sample per set was duplicated. Therefore, there was only one measurement of panelist consistency for

the malt hot steep evaluations. While mashing and steeping processes mirror one another, it is important to note that mashing takes place at a higher temperature for a longer time than steeping. A commercial mashing operation thus converts more starch to fermentable sugar and reduces proteins to smaller polypeptides. Both of these variables can impact flavor and mouthfeel [32]. It is clear that the differences among beers were more subtle and nuanced than those of the malt hot steeps. For example, once the malt was brewed into beer, the *grassy* characteristic of DH120270 decreased, making it more similar to the profiles of the other NP samples. The standout samples for the malt hot steeps, DH120270 (*grassy*) and Thunder (*sweet bread* and *aromatic*), were less noticeably different in the beer sensory evaluation. Observing patterns of descriptor usage across the two sensory methods can give us insights into the connection between the two. Both *grassy* and *grainy* were used more in malt hot steep characterization than beer characterization. *Floral* was used only once in the description of malt hot steep aroma but became an important attribute for beer sensory. Similarly, *fruity* was used infrequently to describe malt hot steep samples but very frequently to describe the resulting beers. *Floral* and *fruity* aromas were likely present in beer due to the addition of hops and the production of esters by yeast during fermentation [33,34]. Nonetheless, some attributes were stable across both malt hot steep and beer sensory. For example, Thunder retained its *sweet bread* quality from malt hot steep to beer. Results from this study indicate that hot steep malt sensory profiles are more distinct than those of their resulting beers. It is important to note that beer sensory profiles will also be influenced by fermentation byproducts and interactions with hops. More evidence is needed to make further conclusions about the predictive ability of the hot steep malt method.

Comparing consumer and laboratory beer sensory

Differences in lexicon, panel size, methodology (including panel training), and goals preclude directly comparing the sensory results from laboratory panel and consumer panels. Nevertheless, both panels identified differences in beer flavor within the WRC set; in particular, the consumer panel identified *citrus, floral, hoppy*, and *sweet*

as the differentiating attributes within the set. For the laboratory panel, *dough, sweet bread fruity,* and *floral* were key attributes that differentiated the finished beer samples. It is important to note that a set of lexicons were preselected and provided to consumers to describe each beer sample due to panelists lacking specific sensory training. The lexicon provided to consumers had fewer attributes related to the *bread* category, while adding more options that fell under *sweet aromatic* (*caramel*, *honey*). Beers brewed from Violetta and Calypso – at opposite ends of the overall liking spectrum – had very similar malt and beer analytics, suggesting that these objective measures are not necessarily predictive of hedonic assessment. This finding also indicates that there can be differences in beer flavor, attributable to barley variety, in the relatively small number of commercially available winter two-row malting barley varieties.

In contrast to the WRC set, no significant differences were found in overall liking of NP beers evaluated by the consumer panel. However, both laboratory and consumer panels coincided in differentiating DH120270 from other samples: *lighter* and *thin/watery* by the consumer panel and *grassy* by the laboratory panel. DH120270, therefore, is consistently different from the other selections and the Full Pint check, indicating that this experimental variety could have been eliminated at the malt analysis stage, with no need to go on to the expense of malt and beer sensory. In a commercial application, the lack of significant differences in liking between DH131756 and DH131144 indicates that either of them could potentially be selected to replace Full Pint without an adverse consumer perception of beer flavor. The decision could be based primarily on agronomics and malt analytics. The latter, while not necessarily predictive of beer flavor in this research, can be key in variety approval and malt sales.

Conclusions

This study contributed to the body of knowledge by examining the effects of more and different barley genotypes on beer flavor. The current results support our previous findings that barley genotype does lead to differences in flavor profiles of lager beer. Two sets of barley germplasm 1) commercially available winter barleys and 2) Full Pint

and three advanced progeny breeding lines were found to have distinct, subtle differences that contributed to nuanced flavor profiles of both malt hot steeps and finished lager beer. Variations between and among barley germplasm sets were greatest for malt analytics, and this variation declined for beer analytics and then again for sensory profiling. Consumer and laboratory panels detected differences in sensory attributes of beer and malt hot steeps, but the basis of these differences was not always obvious. It is important to emphasize, in this context, that the descriptors and preferences reported are applicable only to these research beers and should not be taken as representative of the specific barley varieties and/or selections and their production environments.

Nonetheless, the research findings support the value of sensory assessments of pilot and commercial-scale beers of potential and new varieties. While common practice in the final stages of the variety recommendation and/or adoption processes, brewing and sensory assessment may also have value earlier in the variety development pipeline. Sensory assessments can continue to play an important role for defect elimination and can be expanded to include discovery of new flavor opportunities. In the case of the WRC set, a variety with acceptable malt and beer analytics was not favored by the sensory panels while a variety with less favorable malt and beer analytics was acceptable. In the case of the NP set, one potential variety could be eliminated based on flavor as well as on poor malting and brewing quality attributes. The remaining two selections were not appreciably different in sensory profile from the reference variety, which simplifies the variety selection process to decisions based on agronomics, malting quality, and/or beer quality.

All measures and procedures used in this research have value in guiding decisions regarding variety selection, but none were directly predictive of another. For example, malt analytics can guide maltster decisions on what barley varieties are likely to produce consistent malt using existing malting protocols in order to meet brewers' expectations. Additionally, while exploring the ability of hot steep malts as an economical and efficient predictive tool for beer flavor profiles, there were some attributes that were stable across both beer and hot steep malt sensory analysis. Hot steep malt sensory profiles were found

to be more distinct than those of their resulting beers. The current research provides some insights into this relationship, but other experiments are justified in order to define the basis of this relationship: the hot steep malt sensory may provide a useful common language for maltsters and brewers.

References

See Appendix 1

Supplemental Material

Supplemental File 4

Beer Sensory – Laboratory Panel

A laboratory panel consisting of 13 people (6 M, 7 F; 22 - 55 years old) with prior experience in sensory analysis was trained over 3 separate training sessions. The first day of training consisted of familiarization of the panel to the lexicon presented on the Base Malt Flavor Map (https://www.draughtlab.com/flavormaps) using appropriate aroma references, which lasted approximately 1 hour. The second day of training consisted of a two-hour training session. During the first hour, the panelists performed a blind identification task in which the aroma references from day one were presented, and the panelist was requested to identify the aroma using the flavor map. There was then an open-ended discussion about the flavor map and aroma references. For the second hour, the panel was given examples of malts and resulting malt steeps to evaluate, while referring to the flavor map. On the final day of training, the panelists practiced using the Projective Mapping method with a subset of the beers to be evaluated during the testing sessions.

During the testing sessions, panelists were given ~60 mL of beer in a 300 mL glass covered with a plastic lid. The beer was served from two 8-head draft systems operating at 4 °C and at 13 psi (Micro Matic, Northridge, CA). Beer was dispensed into a 48-oz pitcher, then poured into blind coded sample glasses ~1 hour before the start of testing, capped with a plastic lid and allowed to warm to room temperature. Each beer sample was presented in duplicate, each with different three-digit blind codes, giving 10 WRC and 8 NP samples.

Panelists were given a 28 by 22-inch sheet of paper, on which they were instructed to place their samples based on similarity (close together) or dissimilarity (far apart). Additionally, they identified the presence of sensory attributes using the Base Malt Flavor Map, which was available to them during testing, although they could also add any attributes they saw fit. Panelists recorded their responses on the paper as well as on Chromebook tablets using Compusense software (Guelph, Ontario, Canada). For each of

these sessions, Compusense was also used to randomly assign the serving order of samples for each panelist. The panelists evaluated WRC and NP samples separately on two different days. For each sample set, the panelists performed two tests, orthonasal aroma and flavor by mouth evaluation. The order of these two tests was randomly assigned to the panelists. Panelists were given new samples, with newly randomized blind codes for both of the tests.

Supplemental File 5

Malt Steep Sensory – Laboratory Panel

The laboratory panel, which consisted of 15 people (8 M, 7 F; 23 - 68 years old), was recruited and trained over 4, one-hour training sessions. Over the course of the training sessions, the panelists were shown the Base Malt Flavor Map along with food references to build a familiar sensory lexicon for the most salient attributes in hot steeped malt. The panel was also given examples of commercial base malts and asked to begin characterizing them with the attributes shown on the map. Discussion was guided by panelist responses via a Qualtrics survey (Provo, UT). During subsequent sessions, the panel was given some examples of the malt samples to be evaluated during testing. Additionally, the panel was given malt steep samples to evaluate, using both orthonasal aroma and flavor by mouth descriptors. Once the panel was comfortable with the lexicon, they were given a day to practice using the Projective Mapping method.

During the testing sessions, panelists were given ~35 mL of malt steep samples in a 300 mL glass covered with a plastic lid. The malt steep samples were prepared within 4 hours prior to testing using the protocol described in ASBC MOA – Sensory Analysis 14. The samples were kept at room temperature in a sealed jar until the testing session began and were poured into glasses roughly 20 minutes prior to evaluation. Testing methodology followed that of the beer sensory evaluation. Panelists followed the Projective Mapping procedure described earlier and were instructed to use at least 3 attributes to describe each sample. For each of the WRC and NP sets, one malt sample was presented in duplicate, so the panel evaluated 6 WRC samples and 5 NP samples.

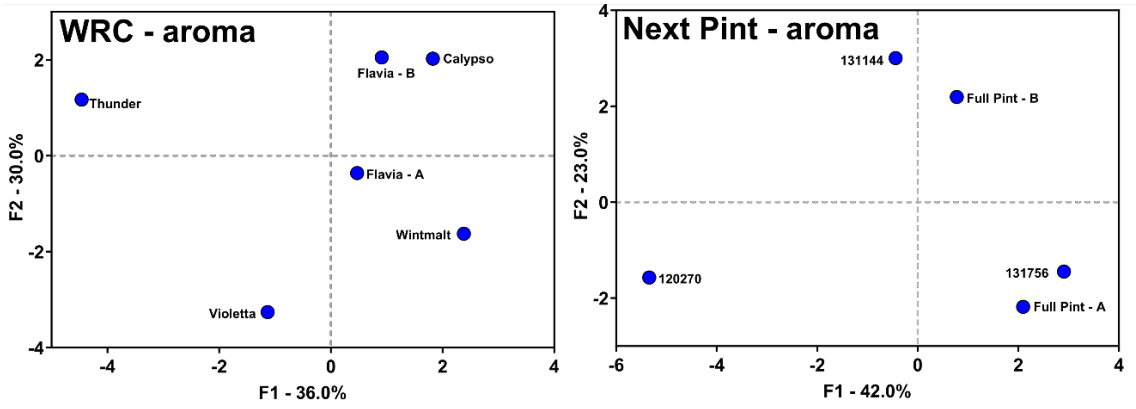
Supplemental File 6

Sensory Lexicon

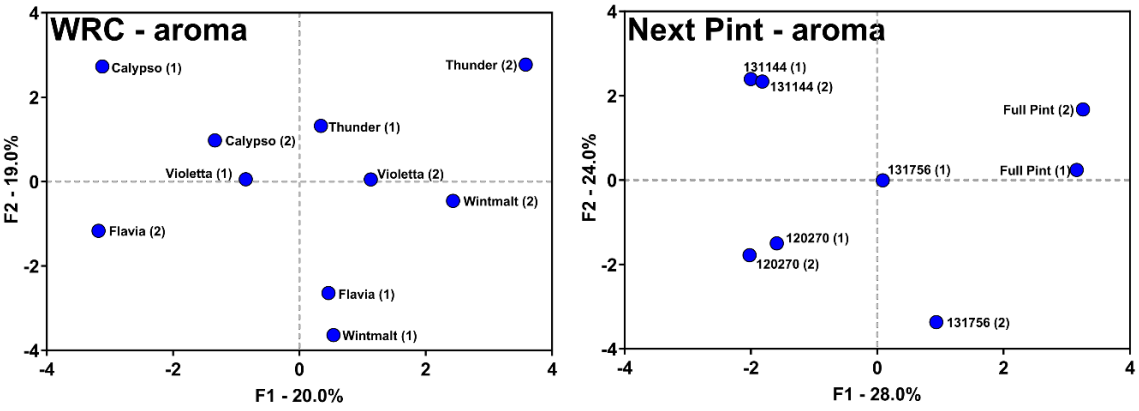
Most frequently used attributes and their descriptions used for both beer and malt steep laboratory panels

Attribute	Description/Examples
Bread	Toast, biscuit, pretzel, flour
Breakfast cereal	Grape Nuts®, Cheerios®, Bran Flakes®
Cracker	Oyster Crackers, saltines
Dough	Yeasty, PlayDoh®
Earthy	Barnyard, soil, pond water, dirt
Floral	Linalool/geraniol, clover, dandelion
Fruity	Melon, apple, citrus
Grainy	Raw barley, oats
Grassy	Green tea, black tea, hay
Sweet aromatic	Honey, caramel, toffee
Sweet bread	Graham cracker, sugar cookie
Vegetal	Corn, DMS, green vegetables

Supplemental Figures



Supplemental Figure 3: Hot Steep Malt Sensory. Multifactor Analysis of coordinate data from Hot Steep Projective Mapping of Aroma (left pane: Western Rivers Conservancy; right pane: Next Pint). One malt in each set, Flavia in the WRC set and Full Pint in the NP set, were chosen to serve as an internal replicate, as designated by 1 and 2 below. Evaluating how close the replicates are to one another allows us to understand how well the panelists could identify differences and similarities between the samples.



Supplemental Figure 4: Beer Sensory. Multifactor Analysis of coordinate data from beer Projective Mapping of Aroma (left pane: Western Rivers Conservancy; right pane: Next Pint). Each beer sample was replicated, as designated by 1 and 2, to provide duplicate observations of the same samples. Evaluating how close the replicates are to one another allows us to understand how well the panelists could identify differences and similarities between the samples.

Future Work

In Chapter 2, the analysis of three hop varieties provided evidence of a regional identity effect on hops grown in the Willamette Valley, Oregon. While the concept of terroir can be evaluated by a long list of potential measurable variables, in this study weather, climate, soil characterization, parent material, and management practices were all analyzed. However, to fully understand the role of terroir in hop growing, other considerations should be accounted for in future studies. These considerations include but are not limited to irrigation amount and water quality, soil chemistry and microbiology, bine age, and viral pressure. Future work should include these additional variables to better understand environmental effects on hop quality. Another significant consideration in all hop studies is hop maturity at harvest. While dry matter is not the only way to characterize maturity, it is a widely used metric in the American hop growing community. Future research should seek to identify better indices of quantifying hop cone maturity and determining harvest readiness.

Further research will seek to expand the scope to growing regions outside of the Willamette Valley and include additional harvest years. Regional identity studies should continue to focus on investigating how growing locations differ and how these differences are expressed in hops all the while reiterating that these studies do not seek to identify the “best” places to grow hops or to identify the “best” hop. The information presented here along with future work can be used as a way to aide decision making by hop growers and brewers.

In Chapter 3, different barley genotypes lead to variation in flavor profiles of lager beer, which supported previous findings. The current research provides some insights into this relationship between hot steep and beer sensory, but future work should seek to explore their relationship further. The hot steep malt sensory may provide a useful common language for maltsters and brewers. Additionally, metabolomics can provide insights into the chemical basis of specific sensory descriptors and consumer preference. Further research is needed to connect metabolites to genes, giving barley breeders will

have additional targets for selection. Future work should seek to study additional barley genotypes, different malts of the same varieties, and different beer styles.

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Appendix 1

Comprehensive analysis of different contemporary barley genotypes enhances and expands the scope of barley contributions to beer flavor

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Abstract

Recent research has demonstrated contributions of barley genotype to beer flavor based on the progeny of a cross between an heirloom and a more contemporary barley variety. To advance this line of research, the current study used two independent sets of barley germplasm to address the contributions of different barley genotypes to beer flavor. Pedigree, quality of malt and beer, and beer metabolomic profiles were compared within and between the two sets. Utilizing both laboratory and consumer panels, differences in sensory attributes of malt hot steeps and lager beers that are attributable to barley genotype were investigated. Genotype, in this context, is defined in the broadest sense to include experimental germplasm and released varieties. Results concur with previous studies: the two sets of barley germplasm were found to have, both within and between, distinct but subtle differences in flavor profiles of malt hot steeps and finished lager beers. Distinct metabolomic profiles, attributable to barley genotype, were detected. Further, covariation of metabolomic profiles and sensory attributes were identified using data from both sensory panels. These observations lead to the conclusion that the variable metabolites observed among the two sets of barley germplasm are a direct result of genetic differences that lead to differential chemical responses within the malting and brewing processes.

Introduction

Malted barley is the primary source of fermentable sugars used to ferment most beers. Until recently, barley contributions to beer flavor were mostly attributed to Maillard Reaction Products (MRPs) developed during malt kilning and the interactions of malts with hops. However, recent research exploring the relationship between genetic variation of barley and beer flavor has shown that genotype does impact beer flavor [1,2,3]. Genotype, in this context, is defined in the broadest sense to include experimental germplasm and released varieties. The degree of malt modification and growing environment were also determined to impact the sensory characteristics of beer, based on a large number of nano-brews, malt analytics, and a research sensory panel [1,2]. Bettenhausen et al. (2020) [3] carried this research a step further with *i*) larger, pilot scale malts and beers, *ii)* brewery, consumer, and laboratory sensory panels, and *iii*) measurement of volatile and non-volatile metabolites.

The interactions between malt chemistry traits and genotypes have been demonstrated to contribute unique beer flavor characteristics. Genetic differences and resulting metabolite composition differences lead to variation in the amount and composition of precursor amino acids and saccharides within the barley kernel. Through the process of malting, these precursors have the potential for biochemical reactions during germination to produce metabolites and MRPs vital for flavor characteristics. Our previous research on the contributions of barley to beer flavor was based on the progeny of a cross between an heirloom (Golden Promise) and a more contemporary barley variety (Full Pint) with a unique malting quality profile [1,2,3,4]. By expanding the scope of the evaluated germplasm, the current study addresses the next question: what are the contributions of other, different, and contemporary barley genotypes to beer flavor?

To address this question, two different sets of barleys were chosen: 1) winter two-row commercially available malting varieties and 2) spring two-row potential varieties with Full Pint as one parent and varieties other than Golden Promise as the other parent. Pedigree, malt quality, beer quality, sensory attributes, and metabolomic profiles were compared within and between the two sets. The commercially available varieties were grown near Condon, Oregon in collaboration with the Western Rivers Conservancy

(WRC; http://www.westernrivers.org/) within the framework of a project designed to enhance riparian habitat around the John Day River and its tributaries. The acquisition of the Rattray Ranch, historically used to produce dryland winter wheat, allowed for assessing the potential for winter malting barley as an alternative crop. Strips of four commercially available barley varieties were embedded within a commercial field of Wintmalt. The second set was derived from the Next Pint (NP) project, a collaboration between Mecca Grade Estate Malt (MGEM; https://www.meccagrade.com/) and Oregon State University to develop a variety to replace Full Pint, the current MGEM estate variety. Three advanced lines and Full Pint were grown, with irrigation, near Madras, Oregon at MGEM facilities.

The two sets of barley lines followed an experimental pipeline similar to that described in Bettenhausen et al. (2020) [3]. Briefly, each line underwent *i*) pilot scale malting and brewing, *ii*) quality analysis of malts and beers, *iii*) sensory analysis of beer by a trained laboratory panel and a consumer panel, and *iv*) metabolomic profiling of finished beer. In addition, sensory analysis of malt hot steeps was conducted. Since its development, the hot steep malt sensory evaluation method has piqued the interest of brewing and malting industries as an improved approach to evaluate malt sensory, as well as predict beer sensory characteristics derived from malts [5,6]. Though widely used and discussed, there are few formal comparisons of hot steep malt and beer sensory. Therefore, the potential of hot steep malt sensory evaluation as an economical, effective tool for assessing barley/malt impacts on beer flavor was investigated. The current study advances research examining contributions of barley genotype to sensory characteristics of malt and finished beer.

Materials and Methods

Plant material

Two independent sets of barley germplasm were used in this experiment, designated WRC set and NP set (Table 1). The WRC set included five released cultivars all of which are two-row winter growth habit types, four of European origin and one

developed at Oregon State University (https://barleyworld.org/). Three of the five cultivars are approved by the American Malting Barley Association (AMBA) (Wintmalt, Thunder, Violetta; https://ambainc.org/2020-amba-recommended-malting-barley-varieties/). The NP set included three advanced lines and a Full Pint "check", all of which are two-row spring growth habit types developed by the Oregon State University barley breeding program. None of the barleys in the NP set are on the AMBA approved list. The three advanced lines were bred and selected over three years of testing from a larger set of 126 doubled haploid progeny derived from crosses with Full Pint.

The WRC set was grown at the Rattray Ranch, near Condon, Oregon (45°14′8″N 120°11′6″W). Briefly, the varieties were planted in the fall of 2017 and harvested in the summer of 2018. No irrigation was applied, as is standard practice in this summer-fallow dryland production area. Each variety, except Wintmalt, was grown in in 1.6 ha strip. The strips were embedded in a 197 ha field of Wintmalt. The strips were planted, maintained, and harvested using commercial equipment. The NP set was grown at the Klann Farm, near Madras, Oregon (44°46'29.3"N 121°10'17.0"W). Briefly, the three advanced lines were planted in the spring of 2018 in 0.05 ha strips. Irrigation was applied following regular practices. The strips were embedded in a commercial field of wheat. The strips were planted and harvested using OSU Barley Project research equipment. Full Pint grain was sourced from an adjoining field managed by Oregon State University. Additional details on growing the WRC and NP sets, including agronomic practices, are provided in Supplemental File 1.

Malting and malt quality

Approximately 230 kg subsamples of grain were obtained for each of the barley lines in the WRC and NP sets. Each barley line was malted independently in 90 kg batches, using the OSU mini-malter (https://barleyworld.org/), as previously utilized by Bettenhausen et al. (2020) [3]. Steeping conditions were the same for both sets and supplemental moisture was provided during the first day of germination by spraying if required. In order to optimize modification of the grain, the WRC set had a target

moisture of 46% and the target for the NP set ranged from 45-51% based on results from micro-malting. Both sets were germinated for four days (WRC at 16°C and NP at 18°C) and had identical kilning conditions. Detailed malt protocols are available in Supplemental File 2. Malt quality analyses were conducted by the Hartwick Center for Craft Food & Beverage (https://www.hartwick.edu/about-us/centers-institutes/center-for-craft-food-and-beverage/) following standard ASBC testing methods [3,4]. The malting quality traits (and results) are shown in Table 2.

Table 1: Pedigree and developer or provider of barley lines per project/set: Western Rivers Conservancy (WRC) and Next Pint (NP). Pedigree based on breeding annotated method mother/father. DH, doubled haploid, experimental barley selection that has not been released.

Project /set	Variety/selection	Pedigree	Developer/Provider
WRC	Wintmalt	(Opal*3087/96, F1)*(8751/Magie)	Ackermann Saatzucht GmbH & Co. KG
	Thunder	Wintmalt/Charles	Oregon State University
	Violetta	Opal x Br 2324b616	Saatzucht Josef Breun GmbH & Co.
	Flavia	(((Carrrero * NIKS.2230) * Aquarelle) * Metaxa) * Wintmalt	Ackermann Saatzucht GmbH & Co. KG
	Calypso	Sunbeam/Suzuka	Limagrain Cereal Seeds
NP	DH131756	Violetta/Full Pint	Oregon State University
	DH131144	Full Pint/Violetta	Oregon State University
	DH120270	Maris Otter/Full Pint	Oregon State University
	Full Pint	Orca/Harrington	Oregon State University

Brewing

Using an Esau and Hueber 2.5hl brewery at Oregon State University (OSU), lager beers were prepared in collaboration with the OSU Brewing Science Lab. Each malt variety/selection was mashed and brewed separately in two different batches 1) WRC malts in May 2019, 2) NP malts in July 2019, yielding 1.2hL each of German Pilsener-style, malt-forward lager. The brewing recipe and protocol were adapted from a single-malt, lager protocol supplied by Rahr Malting intended to emphasize malt forward characteristics and achieve a drinkable, “commercial style” lager. Key ingredients were

the neutral yeast (Bohemian Lager Strain 2124, Wyeast Labs), hop extracts (Isohop, John I. Haas, Inc.) and hop pellets (Kazbek hops, Brewers Supply Group). The brewing protocol was similar to Bettenhausen et al. (2020) [3] but with modifications, and the full protocol is provided in Supplemental File 2. Analysis of the beer was performed by the OSU Brewing Science Lab as described in Table 3.

Table 2: Malt quality of barley lines per project/set. All-malt and Adjunct malt criteria are based on parameters suggested by American Malting Barley Association (https://ambainc.org/wp-content/uploads/2019/10/Malting_Barley_Breeding_Guidelines_June_2019.pdf) Color is measured using Standard Reference Method (SRM); SP, soluble protein; TP, total protein; S/T, soluble/total percentage of protein; FAN, free amino nitrogen; DP, diastatic power in degree Lintner; AA, alpha amylase.

Project /set	Variety	Moisture %	Friability %	Extract %	Color °SRM	β-glucan mg/L	SP %	TP %	S/T %	FAN mg/L	DP °L	AADU	Filtration Time	Clarity	pH
WRC	Wintmalt	4.6	91.2	80.3	1.56	128	3.78	10	37.8	123	102	43.4	normal	hazy	6.07
	Thunder	4.8	97.0	83.9	1.97	58	4.89	9.1	53.7	202	124	78.7	normal	clear	5.91
	Violetta	4.6	95.2	80.3	1.69	29	3.89	9.5	40.9	141	113	40.2	normal	clear	6.06
	Flavia	4.6	96.8	80.0	1.57	33	3.64	9.2	39.6	127	111	44.1	normal	clear	6.06
	Calypso	4.3	99.2	81.3	1.73	31	3.83	8.8	43.5	150	114	46.6	normal	clear	6.04
NP	DH131756	4.6	82.5	82.5	1.94	77	5.8	13.8	42	237	163	70.2	normal	clear	5.83
	DH131144	4.7	84.7	81.4	2.22	38	5.62	12.2	46.1	236	174	83.9	normal	clear	5.98
	DH120270	4.5	72.1	78.5	1.41	272	4.35	13.1	33.2	150	161	58.5	normal	clear	5.98
	Full Pint	4.7	69.4	82.9	1.84	110	5.32	12.9	41.2	220	208	91.9	normal	clear	5.99
Adjunct Malt Criteria		NA	NA	> 81%	0.812-1.27	< 100	4.8 - 5.6 %	≦ 13 %	40 - 47 %	> 210	> 140	> 50	NA	NA	NA
All-malt Criteria		NA	NA	> 81%	0.812-1.42	< 100	< 5.3 %	≦ 12 %	38 - 45 %	140 - 190	110-150	40 - 70	NA	NA	NA

Table 3: Beer quality of barley lines per project/set. From beer produced from each malt; ABV, alcohol by volume; OG, Original Gravity of wort (ºP, Degrees Plato); RE, real extract, based on attenuation of wort; AE, apparent extract, RDF, real degree of fermentation; Color, based on EBC method; IBU, international bittering units based on dissolved solids. German Pilsener guidelines provided by the Brewers Association (https://www.brewersassociation.org/edu/brewers-association-beer-style-guidelines/#Lager%20Styles).

Project/set	Sample Name	ABV (%)	OG (°P)	RE (%w/w)	AE (°P)	Color (EBC)	RDF (%)	IBU
WRC	Wintmalt	5.12	12.14	4.38	2.52	3.79	65.44	22.94
	Thunder	5.41	12.05	3.82	1.87	4.01	69.64	23.6
	Violetta	5.42	12.27	4.04	2.09	3.17	68.47	20.74
	Flavia	5.40	12.31	4.11	2.16	3.16	68.03	21.35
	Calypso	5.31	12.06	3.99	2.07	4.09	68.29	23.88
NP	DH131756	5.21	12.08	4.18	2.30	6.57	66.85	21.11
	DH131144	5.34	12.12	4.01	2.08	7.89	68.33	23.94
	DH120270	4.99	11.70	4.11	2.30	4.72	66.29	22.33
	Full Pint	5.10	11.64	3.86	2.01	6.21	68.17	22.1
BA Guidelines	German Pilsener	4.6-5.3	11.0-12.9	NA	NA	3-4	NA	25-50

Beer sensory

A beer sensory pipeline was performed as described in Bettenhausen et al. (2020) [3], and two types of sensory studies were conducted 1) a consumer panel and 2) a laboratory panel.

The consumer panel testing was performed in collaboration with the Oregon State University Center for Sensory & Consumer Behavior Research (http://agsci-labs.oregonstate.edu/sensoryresearch/). WRC beers were tested in August 2019 while NP beers were tested in January 2020. The procedures were performed as described by Bettenhausen et al. (2020) [3] and detailed in Supplemental File 3. Briefly, participants

(WRC n = 152; NP n = 155) were asked to answer a series of questions per beer, including 1) overall liking (scale from 1-9), 2) Check All That Apply (CATA) for sensory characteristics, 3) "ideal lager" attributes, and 4) demographics.

The laboratory panel testing was performed in collaboration with the OSU Brewing Science Lab in October 2019. Thirteen panelists (6 M, 7 F; 22 - 55 years old), who had prior experience on beer and wine descriptive analysis sensory panels, were trained over three separate sessions with the beers in question using the Projective Mapping with Ultra Flash Profiling sensory method [7,8] and detailed in Supplemental File 4. WRC beers and NP beers were assessed for sensory attributes on two separate days, with each beer being presented in duplicate (WRC n=10; NP n=8). During each testing session, panelists assessed the orthonasal aroma and flavor by mouth of the beer in two separate tests, with new blind codes for the samples.

Hot steep malt sensory

Sensory analysis was performed on liquid extract produced from hot steeps of all malts in the experiment, prepared in accordance with ASBC Methods of Analysis – Sensory Analysis 14 [6]. Descriptive data were collected using Projective Mapping (PM) combined with Ultra Flash Profiling [7,8]. Due to changes in panelist availability between the beer and hot steep malt sensory analyses, a new laboratory panel was recruited and trained over four, one-hour sessions, detailed in Supplemental File 5. This 15-member panel (8 M, 7 F; 23-68 years old) consisted of some of the same members as the beer sensory panel, but also included some new members, most of which had prior experience performing sensory analyses on other foods such as wine. Laboratory panel testing was performed in collaboration with the OSU Brewing Science Lab in March 2020. Malt hot steeps from five WRC malts and four NP malts were assessed for sensory attributes on separate days. During each testing session, panelists assessed both the orthonasal aroma and the flavor by mouth of the malt hot steeps in two separate tests. Half the panel carried out the orthonasal testing session followed by a five-minute break and then the flavor session, while the other half of the panel proceeded in the opposite order. Unique blind

codes were used for each test, and the serving order was randomized for each panelist. The WRC malt hot steep sessions were carried out with 15 panelists held over two days, while the NP malt hot steep session was carried out with ten panelists on a single day.

Sensory data analysis

All sensory data were collected via Compusense Cloud Software (Version 20.0.7404.31336, Guelph, Ontario, Canada). Projective Mapping combined with Ultra Flash Profiling provides both attribute counts and coordinate data for each sample evaluated. Coordinate data was analyzed using XLSTAT Multiple Factor Analysis (MFA) (Addinsoft, New York, NY). Individual MFA plots for aroma and flavor were created for both WRC and NP sample sets in both beer and malt hot steeps. Attribute data was processed in order to combine specific descriptors under the more broad descriptors, in accordance with the Base Malt Flavor Map (Supplemental File 6). Post processing, descriptor data were then analyzed by Correspondence Analysis (CA) in XLSTAT. Attributes were ranked according to frequency of use summed across all of the samples in the set. As there is no standard cutoff for attribute inclusion, it is up to the researcher to determine the appropriate threshold [8]. In this case, the cutoff was set in order to display pertinent attributes, while filtering out attributes that do not help further explain the relationship between the samples. Those attributes that were used at a rate of at least 45% of the most frequently used attribute were included in the CA plot for the laboratory panel beer aroma sensory data. For the malt hot steeps, aroma and flavor CA plots were created individually before being combined and plotted together with the attributes used being those that were used by the overall panel with a frequency of >25% of that of the most frequently used top attribute.

Detection of the metabolome in beer

Volatile metabolites in beer were detected using a non-targeted metabolomics approach. The methods included analysis of volatiles using headspace solid-phase microextraction gas chromatography-mass spectrometry (HS/SPME-GC-MS) with

methods as previously described [3] and detailed in Supplemental File 7. Briefly, mass spectra from the MS platform was converted to the .cdf file format and processed and annotated using the workflow described in Bettenhausen et al. [3,4]. Metabolite quantities were established as previously described [4]. Briefly, each sample resulted in a matrix of molecular features (defined by retention time and mass (m/z)) generated using XCMS software in R v. 3.2.4 [9]. Mass spectra were deconvoluted using the RamClust algorithm [10] and normalized to total ion current (TIC); the relative abundance and variance of each molecular feature was determined by the mean area of the pooled quality control (QC) injection. Volatile metabolites were annotated by spectral matching in RamSearch software [11] to an in-house database of ~1,500 compounds and to external and theoretical databases including NIST v14 (http://www.nist.gov), Metlin [12], Golm Metabolome Database [13], MSFinder software (v. 3.26, RIKEN Center for Sustainable Resource Science, Yokohama, Kanagawa, Japan) [14,15], Human Metabolome Database (HMDB) [16], and FooDB [17]; Spectra were also evaluated using the findMAIN function of the interpretMSSpectrum R package [18] and chemical ontologies were established using HMDB and the ClassyFire package in R [19].

Statistics (metabolomics)

Volatile metabolite abundances for each dataset (WRC and NP) were compared independently. Principal Components Analysis (PCA) was conducted on unit-variance (UV) scaled metabolites and sensory traits from each panel with SIMCA software v. 15 (Sartorius Stedim Biotech, Umea, Sweden) [20]. Respective sensory attributes of each independent sensory panel were integrated with the volatile metabolites for further multivariate analysis. Orthogonal projection to latent squares (OPLS) analysis was conducted for the WRC set on two PCA-reduced and UV-scaled components for sensory (one for the Violetta/Calypso trend, a second component for the Thunder/Wintmalt trend) and the 130 UV-scaled volatile metabolites. OPLS analysis was conducted for the NP set on two PCA-reduced and UV-scaled components for sensory (one for the Full Pint/DH120270 trend, a second component for the DH131144/DH131756 trend) and 160

UV-scaled volatile metabolites, both with SIMCA software. The 20 sensory attributes from the consumer panel (y) were regressed on the UV-scaled metabolite data (x). Predictive power (Q^2) was determined via cross-validation, by which the data was divided into seven parts and 1/7th of the data was removed, and the model was built on the remaining 6/7th of data remaining, and the removed 1/7th of data are predicted from the model. Heat maps were created after z-transformation of the metabolite data. The resulting z-scores were converted into colors and grouped using hierarchical clustering on the Spearman's rank correlation (r_s) between metabolite and sensory trait values [21].

Results

Barley, malting quality, and beer quality associated with barley genetics

As shown in Table 1, and in greater detail in Supplemental Figure 1, there were genetic relationships among the barley varieties/selections used in this study. Varieties were selected based on logistical constraints: the WRC set chosen from commercially available winter malting barleys with sufficient seed availability; the NP set chosen within the scope of work of the project with Mecca Grade Estate Malt. In the WRC set of winter growth habit two-row varieties, Opal is a parent shared by Wintmalt and Violetta. Wintmalt, in turn is a parent shared by Thunder and Flavia. Calypso does not have Wintmalt or Opal in its pedigree. Both of its parents have Puffin in their pedigrees, and Puffin has Maris Otter in its pedigree. Thunder has Charles, the first North American two-row malting barley approved by AMBA, as its other parent. Thunder is unique in this set in having European and North American parentage. The NP set, comprised of spring growth habit two-row experimental varieties and the variety Full Pint, has an unusual genetic structure in that the three selections are derived from "wide" crosses between European winter two-rows (Violetta and Maris Otter) and a North American two-row (Full Pint). Two of the selections, DH131144 and DH131756, are sisters derived from the cross of Full Pint x Violetta; Violetta was the male parent of the former and female parent of the latter. In this set, DH120270 is unique in having Maris Otter as a parent. Violetta and Maris Otter are, therefore, genetic commonalities between the WRC and NP sets.

There were notable similarities and some key differences in malting quality within and between the WRC and NP sets (Table 2), using the AMBA specifications for adjunct and all-malt quality. Within the WRC, all varieties were highly friable. Calypso, Flavia, and Violetta were well-modified and the most similar to each other. They met most criteria for the all-malt specifications but were too low in free amino nitrogen (FAN), diastatic power (DP), and alpha-amylase (AA) for the adjunct specifications. Wintmalt was the least modified of the set, with the highest beta-glucan and lowest S/T (soluble/total protein), not meeting all-malt or adjunct criteria. Thunder was the most modified and notable for its high extract, FAN, AA, and S/T. Entries within the NP set came closest to meeting adjunct criteria, rather than all-malt criteria. DH131756 and DH131144 were well-modified and met most if not all AMBA adjunct specifications. DH120270 was under-modified, with low friability, high ß-glucan, lower extract, S/T, FAN, DP, and AA. It did not meet all-malt or adjunct criteria. Full Pint was less modified than DH131756 and DH131144, with lower friability and higher ß-glucan. It met AMBA adjunct specifications for most criteria but was slightly over specifications for ß-glucan and total protein (TP). Comparisons between the two sets show that the WRC malts were more friable and - except for Thunder - had lower extracts, TP, FAN, DP, and AA than the NP set. Overall, Calypso came closest to meeting the all-malt criteria and DH131144 met all the criteria for adjunct malting.

All beers fell within range for German lager-style, Pilsener beer guidelines – except for color and ABV, as described by the Brewers Association Beer Style Guidelines (https://www.brewersassociation.org/edu/brewers-association-beer-style-guidelines/#Lager%20Styles). All the NP beers were darker in color and fell outside of the style guidelines. The IBU values were similar for all beers, but below the BA guidelines for this beer style (Table 3). With each set of malts (WRC and NP), Wintmalt and DH120270 had the lowest alcohol by volume (ABV) and real degrees of fermentation (RDF), respectively, while Thunder and DH131144 had the highest. Compared collectively across both data sets, ABV ranged from 4.99 – 5.42% while RDF ranged from 65.44 – 69.64%.

Sensory characteristics for malt hot steeps

Projective Mapping was used to evaluate both aroma and flavor attributes of malt hot steeps made from the WRC (15 panelists) and NP (10 panelists) samples. In each sample set, one malt was randomly selected to be presented as a duplicate. For the WRC malts, Flavia was replicated giving six total malt hot steep samples. Based on aroma evaluation only, panelists grouped duplicates closely together, implying perceived similarities between them, and dissimilarities between other samples. During the flavor evaluation, the Flavia duplicates were not placed as close to one another. *Thin body* was the only mouthfeel attribute used frequently enough to be plotted. Coordinate data from aroma evaluation showed that Thunder and Violetta were different from the other samples (Supplemental Figure 3). During the aroma evaluation, *grainy* was used consistently among the samples but showed more variable usage during flavor evaluation (Figure 1). In both aroma and flavor evaluations, *grassy* had a large variation in usage among the samples, with Calypso being described as *grassy* most frequently. Additionally, Calypso's aroma was described by *vegetal*, while its flavor was described by *cracker*. Both Flavia samples were high in *grassy*, and on average were high in *earthy*. Thunder and Violetta were each much lower in *grassy* than the rest of the samples. Thunder was consistently described by *sweet aromatic, breakfast cereal*, and *sweet bread*. Violetta was also more closely associated with *bread*. Descriptors used for Wintmalt varied between aroma and flavor, but *grassy* was used in both.

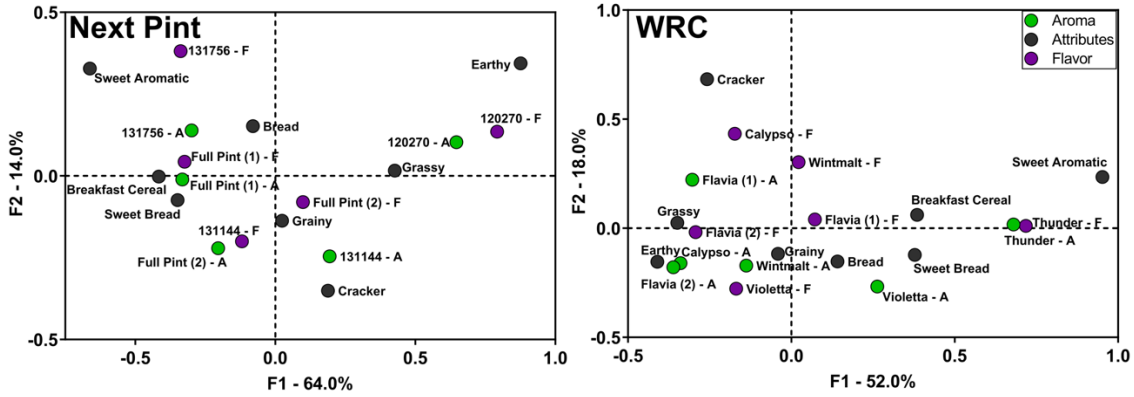

Figure 1. Correspondence Analysis from hot steep Projective Mapping (left pane: Western Rivers Conservancy samples, right pane: Next Pint samples). "1" and "2" designates duplicate observations of the same samples with different blind codes. CA plots show which attributes (black squares) are used to describe the samples (indicated by green and purple circles). Samples that are close together are described similarly, while samples far apart were described differently. Both Aroma and Flavor evaluations are plotted together with the top eight most frequently used attributes.

For the NP malts, Full Pint was replicated, giving five malt hot steep samples. The coordinate data showed similar configurations between aroma and flavor evaluations, with the exception of a Full Pint duplication moving positions (aroma data shown in Supplemental Figure 3, flavor data not shown). In both the MFA and CA plots, DH120270 appeared distinct from the other malt steep samples. *Grainy* was the most used descriptor for the NP aroma and flavor evaluations and was not helpful in the discrimination of samples, hence its location near the center of the samples (Figure 1). There were large differences in usage across samples for *grassy* in both flavor and aroma, and *sweet aromatic* via aroma only (attribute count data not shown for concision). Additionally, *sweet bread, earthy*, and *breakfast cereal* highlighted the differences between the samples during the flavor evaluation. In both aroma and flavor evaluations, Full Pint was described by *breakfast cereal*, with the exception of one Full Pint flavor replication. DH120270 was the most unique sample of the group and was highly *grassy* and *earthy* across both evaluations. DH131144 and DH131756 were both described

attributes within the *bread* category, though DH131144 was described with *cracker* and DH131756 with *sweet aromatic*.

Beer sensory – consumer panel

The consumer panel noted differences in flavor between the WRC beers, but these were not significant. Violetta was liked more than Calypso (Tukey's Post Hoc HSD test $p=0.06$; Supplemental Table 1A). Consumers were able to distinguish significant differences in attributes *citrus, floral, hoppy*, and *sweet* between the five WRC beers (Cochran's Q test, $p<0.05$, Supplemental Table 2A). Thunder was significantly less *citrus* than the other four varieties, more *hoppy* than Violetta, and more *toasted* than Wintmalt; Violetta was found to be significantly more *sweet* and *floral* than Calypso, Flavia, and Wintmalt, and more *refreshing* than Calypso; And Wintmalt and Violetta were significantly more *crisp* than Thunder (McNamara's multiple pairwise comparison, $p<0.05$, Supplemental Table 3A).

There were no significant differences in "Overall Liking" for the NP beers (ANOVA, $p= 0.72$; Supplemental Table 1B). However, consumers were able to distinguish significant differences in the *bitter* attribute between the four beers (Cochran's Q test, $p<0.05$, Supplemental Table 2B). Full Pint was found to be significantly less bitter than DH120270; DH120270 was found to be significantly more *light* in mouthfeel than DH131756; and DH131144 and more *thin/watery* than either DH131756 and Full Pint (McNamara's multiple pairwise comparison, $p<0.05$, Supplemental Table 3B).

Consumer panelists identified important attributes for an "ideal lager" from the list of common descriptors given in the CATA. *Crisp* and *refreshing* were selected as key attributes for an "ideal lager" in both the WRC and NP sets. *Citrus* and *light* were also selected as key attributes for the WRC and NP sets, respectively (Supplemental Figure 2A and B).

Beer sensory – laboratory panel

Projective Mapping was used to assess both aroma and flavor attributes of the WRC (13 panelists) and NP (10 panelists) beers in duplicate (10 and 8 beers per set, respectively). Multifactor Analysis (MFA) plots of the WRC aroma coordinate data showed separation of the duplicates, which indicates that differences between the beers were subtle (Supplemental Figure 4). This pattern was also present in the coordinate data from the WRC flavor test, with the exception of Calypso and Violetta duplicates, which were closer together (data not shown). Correspondence Analysis (CA) with attribute data showed Calypso duplicates were close together and were described by *fruity* and *floral* in aroma (Figure 2), and *fruity* in flavor (data not shown for concision). Aroma attribute data showed differences between duplicates for the other 4 beer samples. *Fruity* was the most commonly used descriptor for this sample set, while *earthy, grainy*, and *floral* helped discriminate the samples from one another. Additionally, the flavor data showed Flavia duplicates were similar and described by *grainy* and *grassy*. Wintmalt duplicates were close together and described by *sweet aromatic*, *floral* and *vegetal*. On average, Violetta duplicates were higher in *dough* and *sweet bread* than the other samples, which did not match its description by orthonasal evaluation. Thunder duplicates showed differences in use of *sweet bread* and *sweet aromatic* between them. In summary, there were inconsistencies in describing the WRC samples and with grouping the duplicate beer samples.

The MFA plots for the NP aroma sample set (8 beers) showed that, with the exception of DH131756, the duplicates are placed closely together, indicating that they were perceived as similar by the panel (Supplemental Figure 4). In the plot for the NP flavor sample set, DH131756 and DH131144 duplicates were mixed together, indicating that panelists were confusing these four beer samples. For both aroma and flavor evaluation, *grainy* was the most frequently used attribute for the sample set and thus unhelpful for discriminating samples (Figure 2). In both aroma and flavor, both *sweet bread* and *vegetal* had high variation in usage frequency between the samples (attribute count data not shown). DH120270 was described by *grassy* via orthonasal evaluation but

was described by *vegetal* via taste evaluation (flavor data not shown). In both the aroma and flavor evaluations, the duplicates for DH131144 varied somewhat. In general, they were described with both *sweet aromatic* or *sweet bread*, as well as *dough*, *pasta,* or *cracker*. Although there were differences between the DH131756 duplicates they were both high in *fruity* in the aroma evaluation, and high in *sweet aromatic* in the flavor evaluation. Full Pint duplicates varied in their attribute counts for various descriptors but was consistently associated with *dough* in both aroma and flavor. Overall, duplicates were more similarly described for the NP sample set than the WRC sample set, indicating that there were greater differences between samples within the NP set.

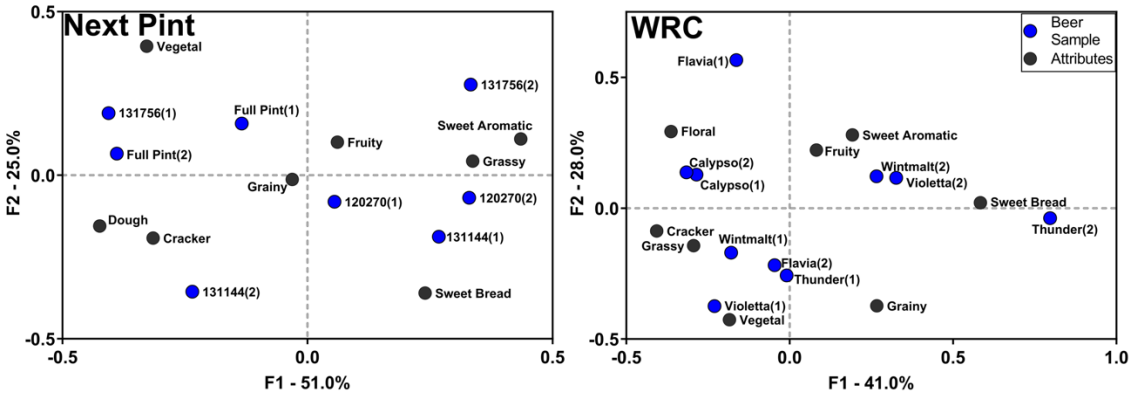

Figure 2. Correspondence Analysis of top 8 most used aroma attributes from beer Projective Mapping with Laboratory Panel (right pane: Next Pint beers; left pane: Western Rivers Conservancy beers). 1 and 2 designates duplicate observations of the same samples with different blind codes. CA demonstrates which aroma attributes (indicated by black squares) are used to describe the beer samples (indicated by blue circles).

Metabolomics

Metabolite variation among beers within the WRC and NP sets

From HS/SPME-GC-MS, 1,342 metabolites were detected and 130 were annotated within the WRC set and within the NP set, 676 metabolites were detected and 160 were annotated (Figure 3). Volatile beer metabolites were annotated and assigned to a super and sub-class based on chemical ontology (Tables 4, 5). Classes of metabolites varied between WRC and NP datasets (Figure 3 A,B).

PCA was conducted on the 130 volatile compounds with the five WRC beers and this demonstrates that variation was attributed to the *barley variety* (Figure 4A). PCA generated three principal components and was able to explain 86.6% of the variation in the data for the WRC varieties. In this scores plot, PC1 (39.8%) explained the separation between Wintmalt, Flavia, and Violetta vs. Thunder and Calypso. The loadings plot (Figure 4B) of volatile metabolites attributed to these WRC varieties did not explain any trends among the varieties.

PCA was conducted on the 160 volatile compounds detected in the NP set resulting in three principal components (Figure 4C) which explained 87.0% of the variation in the data for the three selections and Full Pint. In this scores plot, PC1 (61.4%) explained the separation between DH120270 and DH131756 vs. DH131144 and Full Pint. The loadings plot (Figure 4B) of volatile metabolites attributed to these varieties demonstrates a high content of lipids (fatty acid esters), terpenoids, and organoheterocyclic compounds (potential MRPs), specifically for DH120270.

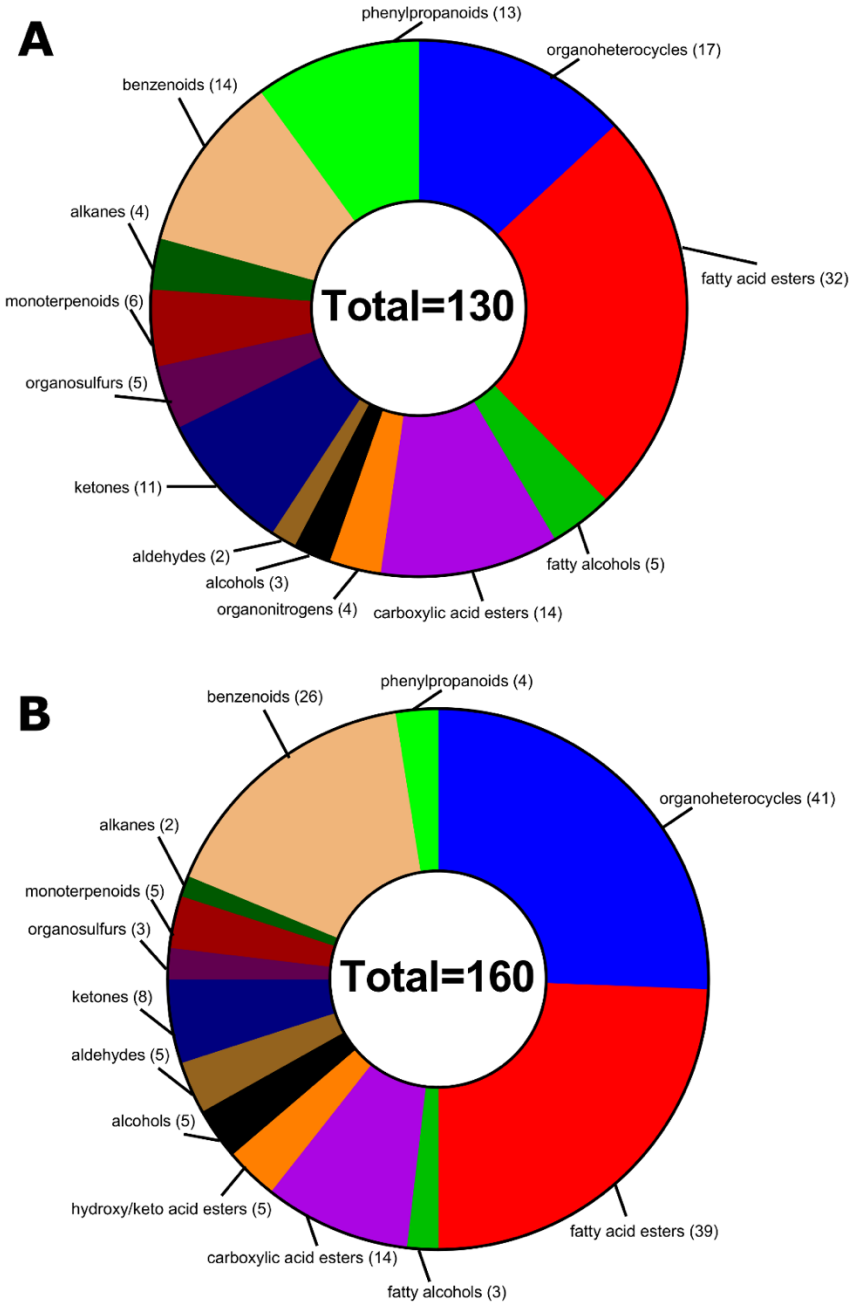

Figure 3. Annotated beer metabolites and the corresponding chemical classes for WRC and NP datasets. A total of 130 and 160 metabolites were annotated for (A) WRC and (B) NP, respectively. Pie charts display metabolites, by broad class (black text).

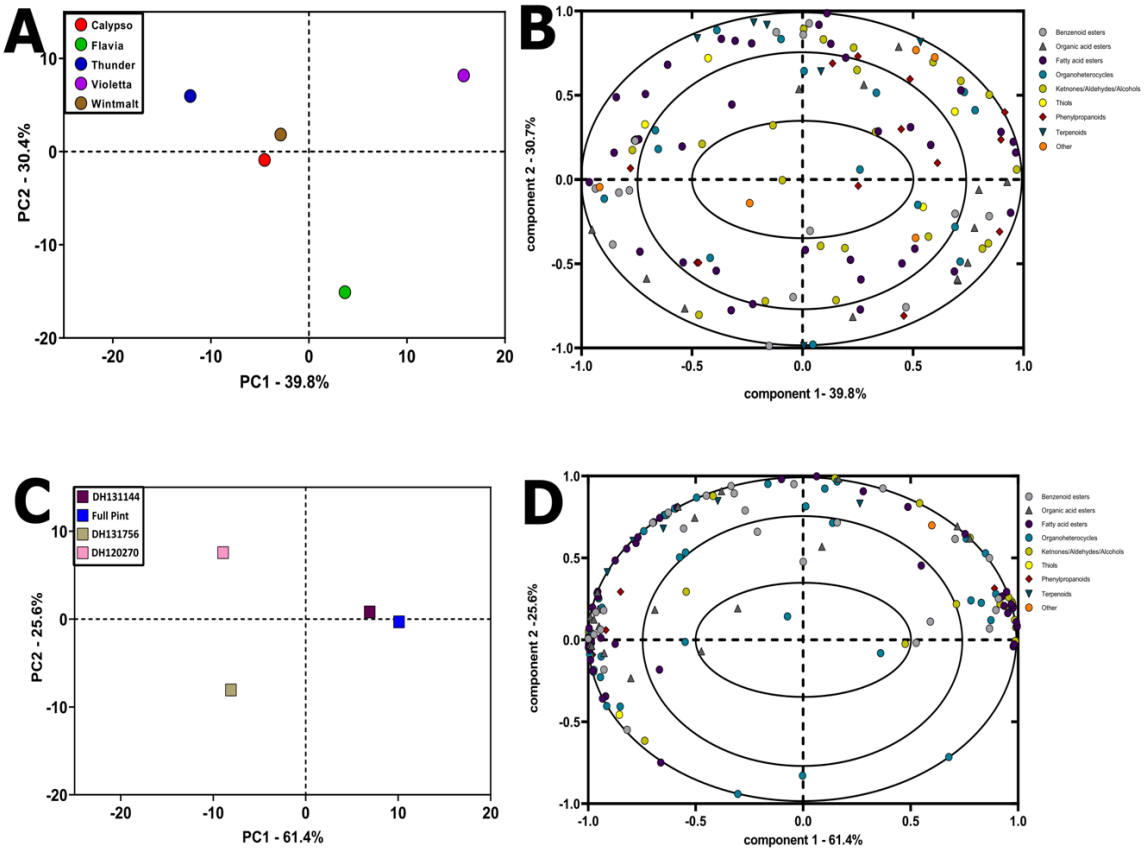

Figure 4. Principal component analysis (PCA) of beer metabolites of the 9 beers from WRC and NP, performed on the annotated metabolites for those datasets. PCA scores plots were produced based analysis of the 130 and 160 volatile metabolites, respectively (A) PC1 and PC2 for WRC and (B) corresponding correlation-scaled loadings plot, (C) PC1 and PC2 for NP and (D) corresponding correlation-scaled loadings plot. Loadings were colored according to broad sensory trait.

OPLS modeling

To investigate relationships between the beer volatiles and each of the beer descriptors from the consumer panel (Figure 5A,B), an orthogonal projection to latent structures (OPLS) model was developed for two sensory attribute principal components (correlation-scaled PC1 scores for Violetta and traits such as *crisp, overall liking, refreshing, citrus,* and *floral,* with orthogonally correlated traits such as *astringent, bitter* (associated with Calypso) and correlation-scaled PC2 scores for *hoppy, honey,* and

toasted (such as are associated with Thunder). The OPLS algorithm for the WRC set resulted in one predictive and two orthogonal component that explained 76.8% of the variation, with a predictive power of Q^2 = 98.8% to support that the model was not over-fit. Metabolites were considered to be associated to the "Violetta" trend if the correlation-scaled Component 1 loading > |0.75| and < |0.25| for the correlation-scaled orthogonal component (Figures 5A and C, Table 4 (presented at end)). Furthermore, the OPLS algorithm which regressed PC2 scores resulted in one predictive and two orthogonal components that explained 76.4% of the variation with a predictive power of Q^2 = 94.8%. The metabolites associated with the "Thunder" trend (correlation-scaled PC2 scores) were subject to the thresholds previously mentioned (Figure 5E, Table 4).

For the NP set, an OPLS model was developed for two sensory attribute principal components (correlation-scaled PC1 scores for Full Pint and traits such as *toasted, molasses, caramel,* and *honey* with orthogonally correlated traits such as *citrus, bitter* (associated with DH120270) and correlation-scaled PC2 scores for *malty* and *non-tropical fruity* (such as are associated with DH131144) (Figures 5B and D, Table 5 (presented at end)). The OPLS algorithm for the NP set resulted in one predictive and one orthogonal component that explained 81.9% of the variation, with a predictive power of Q^2 = 65.0% to support that the model was not over-fit). Metabolites were considered to be associated to the "Full Pint" trend if the correlation-scaled Component 1 loading > |0.75| and < |0.25| for the correlation-scaled orthogonal component (Table 4). Furthermore, the OPLS algorithm which regressed PC2 scores resulted in one predictive and two orthogonal components that explained 74.4% of the variation with a predictive power of Q^2 = 96.9% (Figure 5F). The metabolites associated with the "DH131144" trend (correlation-scaled PC2 scores) were subject to the thresholds previously mentioned (Figure 5F). A SIMCA 'distance to model' function was applied to characterize the metabolites with the largest contribution to explaining the variation in significantly different sensory traits. The data indicate associations with organic acid esters, fatty acid esters, and benzoic acids, which are known classes of aroma compounds.

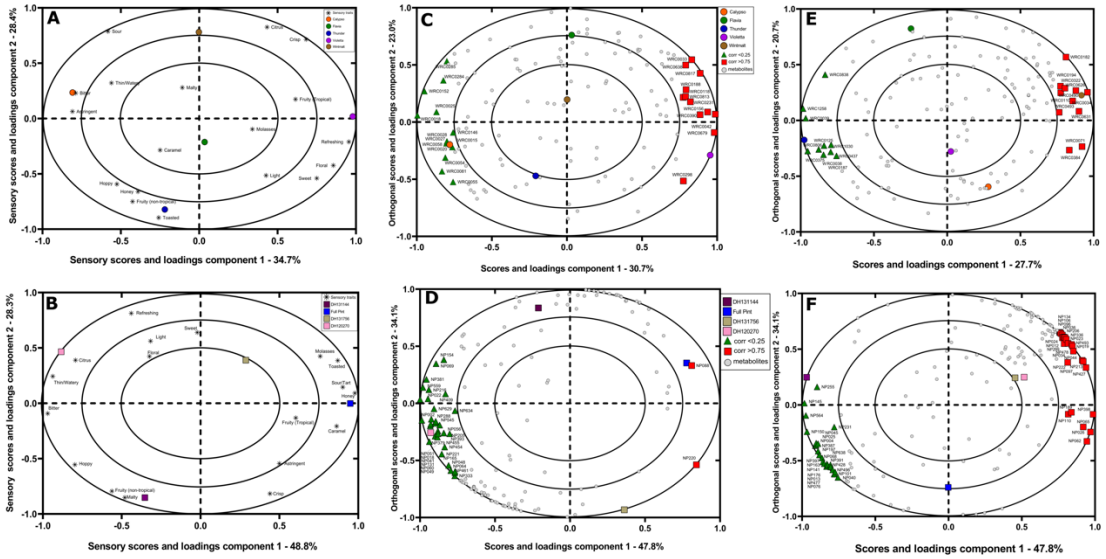

Figure 5. Multivariate association of beer metabolites with consumer panel sensory traits. PCA was performed on data for 14 sensory traits quantified for the 12 malt hot steeps (A) WRC PCA scores and correlation-scaled loadings biplot based on consumer panel data. (B) NP PCA scores and correlation-scaled loadings biplot based on consumer panel data. The association between beer metabolites and consumer panel sensory traits was evaluated with orthogonal projection to latent structures (OPLS) and performed on 130 and 160 volatile metabolites, respectively and PC1 scores from sensory analysis. Data is plotted as a biplot for correlation scaled scores (circles colored as per maltster; samples) and loadings (red squares for corr > |0.75| loadings; squares for orthogonal corr < |0.25 loadings; grey circles for metabolites which did not meet the threshold of loading corr) (C) WRC OPLS scores and loadings plot for regression against PC1 scores; (D) NP OPLS scores and loadings plot for regression against PC1 scores, (E) WRC OPLS scores and loadings plot for regression against PC2 scores; (F) NP OPLS scores and loadings plot for regression against PC2 scores. Notations for metabolites displayed as meeting the threshold are in Tables 4 and 5, respectively.

The sensory/chemistry which characterizes the "Violetta Trend" demonstrates co-variation of Violetta with traits such as *crisp, overall liking, refreshing, citrus,* and *floral,* but displays a negative association with traits such as *astringent, bitter* (associated with Calypso) (Figure 5A and C). The metabolites that are associated with this trend (correlations greater than 0.5 for Component 1, and less than 0.5 for Component 2) are noted in Table 4 (WRC) and 5 (NP) of sensory/volatiles. Metabolites that were positively correlated with attributes covarying with Violetta included benzenoids (4), fatty acid

esters (5), organic acids (7), coumarins (2), ketones (2), and varying other classes. Two of the most correlated metabolites were an hydroxycinnamic acid (putatively identified as chicoric acid, WRC0679) which may impart a woody and nutty flavor (however, there are three other phenylpropanoids that are highly correlated, as well) and isomaltose (WRC0156, fatty acyl glycoside/oligosaccharide) which may contribute to sweetness, isopentyl acetate (WRC0390, banana, fruity). Other fatty acid esters and organic acid esters also had higher rates of correlation and have been associated to not only light, fruity flavors, but also to floral, refreshing flavors. Negative correlations included compounds of many of the same classes, but included many metabolites putatively identified as Maillard Reaction Products (such as WRC08606, Ethanoic acid ester; furans, pyrazines, pyrans).

The sensory/chemistry cluster along OPLS Component 1 demonstrates co-variation of Full Pint and traits such as *crisp*, *fruity (tropical)*, and *sour/tart* to a lesser extent, *honey, caramel, toasted, astringent,* and *molasses,* and co-variation of DH131144 with both *fruity (tropical)* and *fruity (non-tropical).* By contrast, they are negatively correlated with *sweet, refreshing,* and *bitter* (Figure 5B and D, Table 5). DH120270 demonstrates co-variation with *bitter* and *thin/watery*. The metabolites that are associated with this trend (correlations greater than 0.5 for Component 1, and less than 0.5 for Component 2) are noted in Table 5 and Figure 5D. Metabolites that were positively correlated with attributes co-varying with DH131144consisted of fatty acid esters (6) which are known volatiles related to fruity (tropical and non-tropical) attributes, specifically, diethyl maleate (NP477), ethyl hexanoate (NP025), a pentanoic acid ester (NP145), methyl caprylate (NP197), 10-undecenoic acid ester (NP013), and ethyl decanoate (NP021). Other classes which co-vary with DH131144 include benzenoids (benzoic acid esters, 4), organoheterocyclic compounds (potential MRPs, 9), and others. The heterocycles of note include 5-methylquinoxaline (NP150), known to contribute to Maillard-related attributes (coffee, roasted), and a thiophene (NP564), which can be attributed to garlic or onion flavors or aromas. Full Pint had a similar profile, with many similar co-varying metabolites. Three metabolites of note include: one fatty acid ester,

ethyl hexanoate -like (NP027), known to contribute many tropical and non-tropical attributes, some of which were found in DH131144, octyl benzoate, a benzoic acid ester (NP035), which can contribute lemon balm, and 2,6-dimethylbenzenethiol (NP565), a thiophene, which can contribute Maillard-type attributes, such as meaty, roasted, and sulfur. DH131756, which contained the most abundant metabolite profile, co-varied with the consumer panel sensory attributes *sweet, refreshing,* and *molasses.* Metabolites which contributed to this are heterocyclic compounds (9), fatty acid esters (9), organic acid esters (4), benzenoids (2), and others. Fatty acid esters of note were ethyl-9-decenoate (NP006), decyl propionate (NP047), and methyl caprylate -likes (NP026, NP019) which all are known to contribute to sweeter, more complex, fruity attributes. Vanillylmandelic acid, a benzenoid (phenol, NP011) can contribute to sweet and vanilla attributes; ethyl lactate, an organic acid ester, can contribute to butterscotch, fruity, and tart flavors. DH120270 had a unique profile, co-varying with *light, thin/watery, floral, citrus,* and *bitter* sensory attributes. Metabolite classes included heterocyclic compounds (15), fatty acid esters and terpenoids (11), organic acid esters (6), and others. Two heterocyclic compounds of note are 4-methylpyridine (a pyridine, NP629), known for tea and fig properties, and 5-methylquinoxaline, known for roasted properties. There are many metabolites which are known to have phenolic and bitter sensory properties that may contribute to the co-variation with *bitter,* assessed by the consumer panel and with the *cracker* and *sweet aromatic* assessed by the laboratory panel. Examples of these metabolites include 2-phenyl-2 butenal (NP146), a phenylacetaldehyde known to contribute a bitter, black tea note and 2-methoxy-4-vinylphenol (NP381), recognized for the contribution of clove, smoky, and spicy attributes.

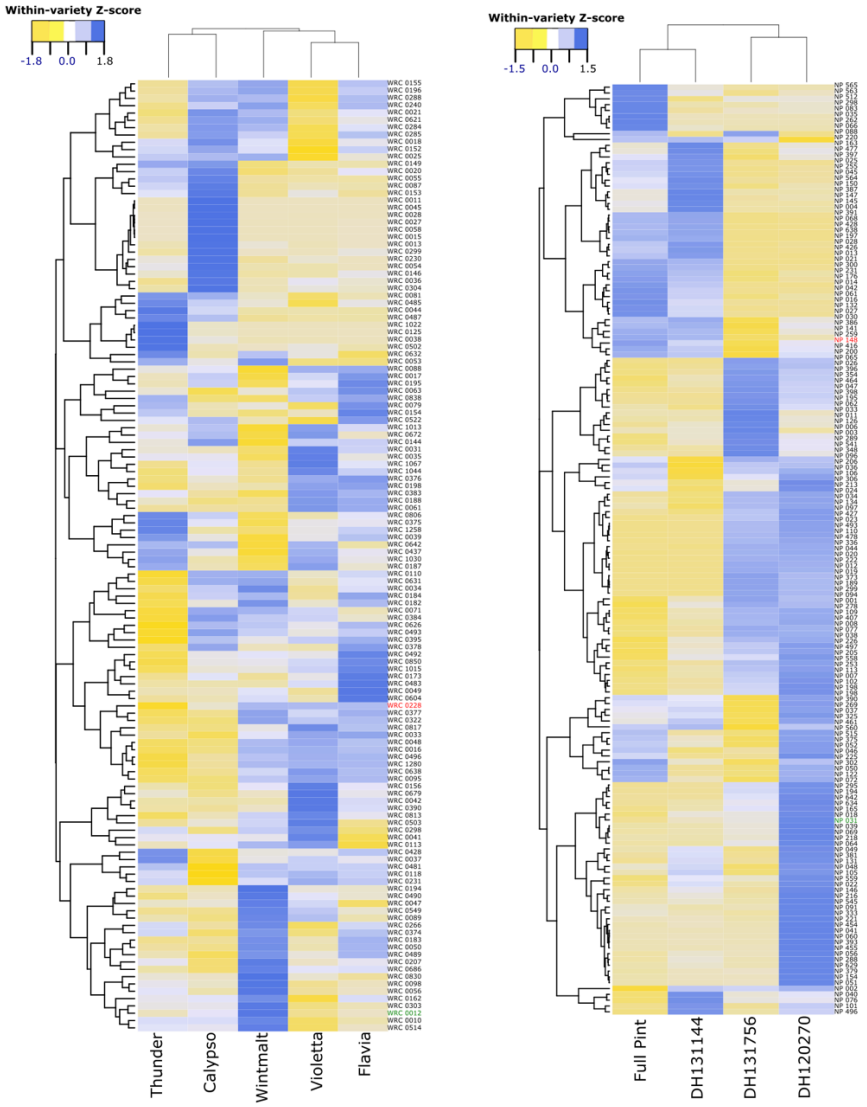

Figure 6. Univariate analysis of volatile metabolite variation among the 9 beers. Prior to heatmapping, volatile metabolite data were normalized within each variety via z-transformation normalized peak area - mean/standard deviation of total peak area of each metabolite).The resulting z-scores were converted into colors and grouped using hierarchical clustering on the Spearman's rank correlation (r^s) between metabolite and sensory trait values. Heat maps with hierarchical clustering were built within for (A) WRC dataset (B) NP dataset. The color in each cell represents the z-transformed abundances of the averaged replicates (n = 2) per beer sample. Z-transformation was based on the mean abundance and standard deviation of the metabolite across all samples. Metabolites in heatmaps are cross-referenced in Tables 3, 4, and Supplemental Tables.

Other trends among chemical classes

The data were evaluated to determine if broad trends of metabolite classes could distinguish each of the beers within the sets: specifically, for lipids (to include fatty acid ester formation), nitrogenous compounds, organic acids, and phenolics. Metabolite abundances were z-transformed to express the data as a profile within a variety, therefore a range in color denotes range in variation of a compound class within a variety, with very blue (high) or very yellow (low) indicate proportions of a metabolite's contribution to the profile (Figure 6 A,B, Supplemental Tables 4,5).

The heatmap for the WRC beers showed Calypso had a unique profile, abundant in alkanes, alkenes, and benzoic acid esters that were not abundant in the other four varieties, also being more abundant in prenol lipids (terpenoids) including linalool (WRC0071), p-methan-1-ol (WRC1030), alpha-cadinol (WRC0284), alpha-cuebene (WRC0196), and geraniol (WRC0182). These metabolites have been associated, in literature, not with *bitter* and *astringent* sensory attributes, as denoted from the sensory panel, but with the *grassy* and *vegetal* (among other attributes noted in the literature, such as floral, citrus, and menthol) noted in the aroma factor analysis from the laboratory panel [22,23,24]. Calypso was also abundant in a class of organoheterocycles known as "quinolines," which have been shown to be attributed to a tea-like flavor (bitter, astringent) in the literature [17]. Among the five beers, there were no trends among lipids/fatty acid esters, as they were equally distributed. The nitrogenous compounds shared by Wintmalt and Flavia included 42-diethoaminoethanol (WRC0626), pyridine-like compounds (WRC0374, WRC0493, WRC0489) which may contribute to or overpower the other sensory attribute of *citrus* and instead contribute to the *malty* seen in the consumer panel and *breakfast cereal, bready*, and *earthy* attributes from the laboratory panel. Organic acids predominate Violetta, and to a lesser degree, Thunder (Figure 6, Supplemental Table 4). One organic acid ester, triethyl citrate (WRC0375), which is known to contribute to vinous and non-tropical fruity attributes, is seen to covary with Thunder and the *fruity (non-tropical)* sensory attribute from the consumer

panel, as well as the *sweet aromatic* attribute from the laboratory panel. The organic acids most unique to Violetta included acetic acid ester (WRC0035), triethyl citrate (WRC0047), ethyl propanoate (WRC0194), isopentyl acetate (WRC0390), 4-isopropylphenylacetic acid (WRC0638), dimethyl malonate (WRC0384), and heptyl-2-methylpropanoate (WRC0188) (Supplemental Table 5). Violetta, Wintmalt, and Flavia displayed negative correlations with the prevalent benzenoid class which was shown to covary with Calypso. This class included 1,2-benzenedicarboxylic acid ester (WRC0153), known to be associated with almond, floral, herbal, green, and more phenolic attributes, 4-hydroxybenzyl alcohol (WRC 0481), and benzaldehyde (WRC1013), associated with more almond, bitter attributes.

The heatmap for the NP beer set displays trends between Full Pint/DH131144, and within certain classes between DH131756/DH120270, although DH120270 again was recognized as having the most unique profile (Figure 6, Supplemental Table 4). The trends between Full Pint and DH131144 include higher abundances of aldehydes and ketones such as 2-nonen-4-one (NP428), 1-hexene (NP255), and 1-pentanol (an alcohol, NP132). Full Pint and DH131144 also shared many abundant fatty acid esters, noted in the previous section. Trends within the organic acid ester class occurred between DH131756 and DH120270, including many -likes of acetic acid, keto acids, and an acetamide of note (NP097) which, in literature has been known to contribute a mousy attribute.

Metabolomics: considering both sets of beers

To assess the Next Pint and WRC beers together, PCA and OPLS was performed on all nine beers (Figure 7). Only metabolites which were annotated and shared among all varieties were included in the analysis, abundances were unit variance normalized. Four principal components were able to explain 94.8% of the data. PC1 (68%) and PC2 (16.6%) were able to explain significant variation among these data (Figure 7). The differences may be attributable to “environment” (i.e. two completely different locations, one dryland, the other irrigated); genetic relationships (i.e. Full Pint as a parent of all NP

lines and no WRC lines); growth habit (one set winter and the other spring); degree of selection (one set commercially available, the other set comprised of three advanced experimental varieties and the "control"); and/or to the higher abundance of metabolites in the WRC set (Figure 7).

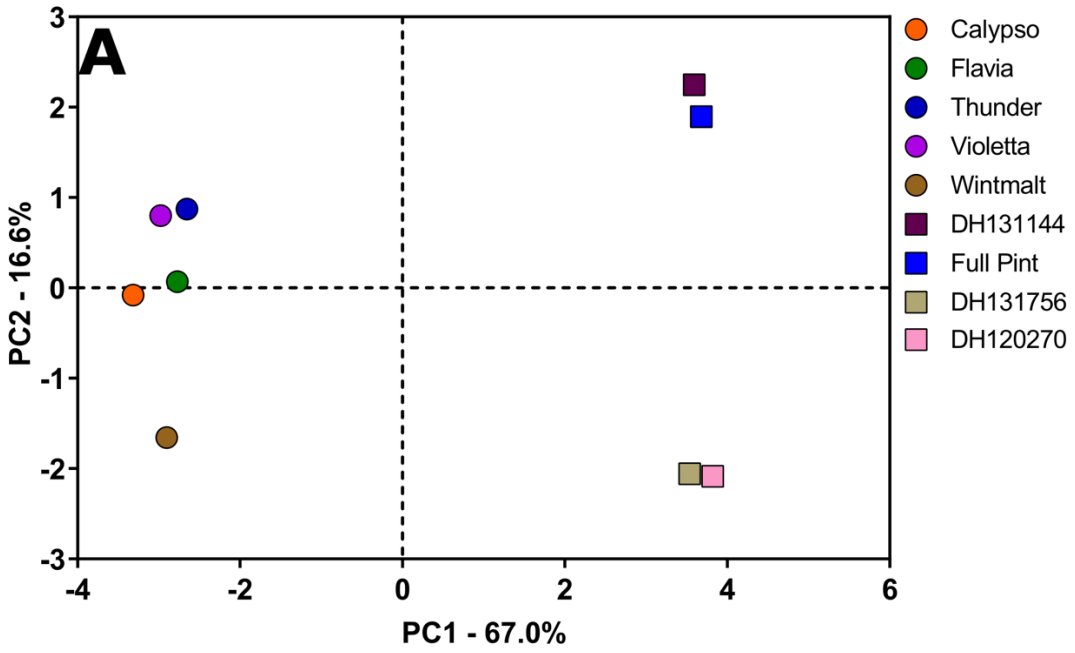

Figure 7. Principal component analysis (PCA) of beer metabolites of the 9 beers from WRC and NP, combined, performed on the annotated metabolites for those datasets. PCA scores plots were produced based on analysis of 290 metabolites.

Discussion

Barley, malting quality, and beyond

The barleys used for this research form two distinct groups categorized by three factors that may confound the data: growth habit, commercial status, and production environment. The WRC set is comprised of winter growth habit, commercially available varieties grown under dryland conditions while the NP set is comprised of spring growth habit experimental selections and a "check", grown under irrigated conditions. Although

the two sets were treated identically through brewing, beer and malt hot steep sensory, and beer metabolomics, these treatments occurred at different time points. Therefore, it is necessary to discuss the results of each set separately. However, there are commonalities between sets that merit some further discussion and integration, both *inter se* and with prior research.

The first commonality is genetic relatedness. Violetta, a member of the WRC set, is also a parent of two members of the NP set (DH131756 and DH131144). Violetta is the female parent in one cross and the male parent in the other, which could have some bearing on the flavor differences between the two sister lines: in Angiosperms, organelles show maternal inheritance: therefore, the chloroplast and/or mitochondrial genomes these two selections could be genetically different and those polymorphisms could lead to flavor differences. However, most phenotypes of commercial importance in barley studied to date (e.g. agronomic and malting quality traits) show nuclear, rather than cytoplasmic, inheritance [25]. In this regard, it is not surprising that these two doubled haploid siblings could have contrasting malting quality and other downstream phenotypes based on contributions from the nuclear genome only.

Exploring pedigree records provides insight to possible genetic contributions to beer flavor and malt quality. Tracing further back in the pedigree chart (Supplemental Figure 1) shows many genotypes in this experiment sharing notable malting varieties, such as Hanna (Czech - Haná) and Spratt, in their pedigrees. Haná originates in Moravia (present day Czech Republic) and was used in the development of Pilsner beer in the 1840s. The spread of Pilsner and Pilsner-style beer in the late 19th century and Haná's reputation for agronomic, malting, and beer quality led it to be used in many breeding programs and it factors in the pedigree of many contemporary malting barleys. Spratt is well known as the parent of iconic British malting variety Spratt-Archer, which was lauded for its vastly improved agronomics and adaptability for the time [26]. Spratt-Archer was widely grown in the middle 20th century and figures into the pedigrees of other iconic varieties such as Maris Otter [27]. Klages is a notable American variety that fits in the pedigrees of six of the experimental genotypes, including all of the NP set. It was the

dominant malting variety grown in the Pacific Northwest in the 1970s and 1980s and was the 2-row variety adopted by many early craft brewing companies. Maris Otter, an heirloom variety from the United Kingdom with a reputation for providing a unique flavor profile [28], is a direct parent of one NP member (DH120270) and also figures in the pedigree of one WRC member (Calypso) [29]. Full Pint, the "check" in the NP set and a parent of all three experimental varieties in the NP set, was chosen as a parent of the Oregon Promise due its reputed flavor profile, as described by Bettenhausen et al. (2020) [3] and Herb et al. [1,2]. Other varieties of note that contribute to the pedigrees in this experiment: European landraces Criewener 403, Pflugs Intensiv, Bavaria, and Danubia (all nine lines); Isaria, Kenia, and Gull (all nine lines); and Puffin and Malta (missing from Full Pint and DH120270).

While pedigree doesn't provide the full picture of the genetic relationships between these nine barleys, it is valuable in showing common and different ancestries that may explain some of the phenotypic flavor contrasts. A systematic investigation of flavors contributed by notable varieties in these pedigrees, coupled with genome profiling, is warranted. In order to increase the efficiency of such an undertaking, DNA fingerprinting of the nine genotypes featured in the current research is underway. This information, coupled with the QTL mapping of flavor that is also underway in the Oregon Promise population, could identify specific alleles associated with specific metabolites. These alleles and metabolites could then be traced back through pedigrees to identify specific genotypes for grain production, malting, and brewing.

Capitalizing on this genetic relatedness to identify the genetic drivers of differences in quality parameters, flavor, and metabolic profiles will be the topic of a future paper - where sample size is larger and complete genotype data are available. At this point, however, specific differences and commonalities between the two sets can be pointed out that relate to variety and therefore impact on one of the questions driving this research: "do barley genotypes contribute to beer flavor?" These differences and commonalities will be highlighted during this Discussion, which will proceed

sequentially by feature (e.g. malt analysis, sensory analyses, metabolomics) but progressively integrating results for each trait and its impacts on other traits.

Malting quality specifications are key metrics for barley variety release. Within the WRC set, the lower degree of modification of Wintmalt and higher degree of modification and enzyme-related trait values for Thunder were notable. Both varieties are on the AMBA recommended variety list, which requires thorough vetting for quality and brewery performance. Although every effort was made to produce optimum malts for all varieties, for reasons unknown Wintmalt did not achieve target specifications in this project. Lastly, the NP set had overall higher grain protein, which may have affected downstream flavor, sensory, and metabolite composition. The impact of grain protein on beer quality parameters is known [30], but the specific impact of protein across different genotypes is outside the scope of this paper. Field sites in this study were managed for supplemental nitrogen per their respective standard operating procedures. Research on field nitrogen applications and impact on grain protein, malt quality, and flavor is ongoing.

Sensory attributes of malt hot steeps and beer, and their relationships

Hot steep malt sensory

Prior to the establishment of the hot steep malt sensory method, Congress worts were used for sensory evaluation of malt samples [31]. Since its development, the hot steep malt sensory evaluation method has piqued the interest of the brewing and malting industries to improve analysis of malt sensory and predict beer sensory for malts of interest [5,6]. It is helpful when only a small quantity of malt is available and is more convenient than making beer. The predictive ability of this method, though much more rapid than brewing, has yet to be fully understood. With the analysis pipeline implemented in this research, we can identify relationships of hot steep malt sensory with other traits. However, determining if relationships are causal and predictive will require further experiments.

Within the WRC set, Thunder and Calypso were standout samples for hot steep malt sensory. The former was higher in *sweet bread* and *sweet aromatic* for both aroma and flavor while the latter was *grassy* and *vegetal* in aroma and *cracker* in flavor. Considering the other varieties in this set, Thunder and Violetta were lower in *grassy* thus separating them from the other samples. DH120270 was a standout sample within the NP set. In both the aroma and flavor evaluations, it was consistently described by panelists as more *grassy* and *earthy* than the other samples. Malt analytics provide clues that Thunder was more modified than Calypso, thus leading to differences in hot steep malt sensory. While it seems likely that the *sweet bread* and *sweet aromatic* descriptors for malt hot steeps are attributable to the higher enzyme profiles of Thunder, DH131144, and DH131756, further research is necessary. The basis of the *grassy* profile for Calypso is not obvious, however in the case of DH120270, it could be ascribed to under-modification. Given this line's Maris Otter heritage, this may be a question for further research. From a plant breeding perspective, the poor modification of DH120270 and its *grassy* and *earthy* profile in the hot steep malt sensory would be grounds for not advancing it on to brewing and beer sensory. In this sense, evaluations using hot steep malt sensory could be a tool in variety selection. In order to assess its value for the malting and brewing industries, the key question remains "is hot steep malt flavor predictive of beer flavor"? The current research provides some insights into this relationship, but further experiments will be required. Within the current experiment, the connection between malt and beer sensory is best explored using the laboratory panel data, given the commonality of protocol and lexicon.

Laboratory beer sensory

The laboratory beer sensory panel had some difficulty matching duplicates within the WRC set to one another, with the exception of Calypso. However, differences in sensory attributes were still perceived among the beer samples. This pattern suggests that stringent selection for commercial potential led to barleys that, despite differences in malt and beer analytics, produced beers that are only subtly different in sensory profiles. The

nuanced differences may result from inconsistencies in malt-modification (Table 2) [1]. There is evidence to show that undermodified malts may result in higher *grassy* qualities [3]. In the NP set, duplicates were more similarly described for both aroma and flavor, indicating that panelists not only found differences among the beers but that these differences could be identified with consistency. This consistency of difference may be due to the more limited selection and validation for malting and brewing properties of the NP set, as compared to the WRC set. DH120270 duplicates were closely grouped, with consistent *grassy* aroma and *vegetal* flavors. This could be due to the lower malt modification of DH120270, leading to *grassy* and *earthy* flavors [3], compared to the other NP samples. DH131756, DH131144, and Full Pint had similar malt analytical profiles, which may be one reason why there was less distinction in flavor profiles among the beers made from these malts.

Comparing beer and hot steep malt sensory

While beer samples were all duplicated, only one malt hot steep sample per set was duplicated. Therefore, there was only one measurement of panelist consistency for the malt hot steep evaluations. While mashing and steeping processes mirror one another, it is important to note that mashing takes place at a higher temperature for a longer time than steeping. A commercial mashing operation thus converts more starch to fermentable sugar and reduces proteins to smaller polypeptides. Both of these variables can impact flavor and mouthfeel [32]. It is clear that the differences among beers were more subtle and nuanced than those of the malt hot steeps. For example, once the malt was brewed into beer, the *grassy* characteristic of DH120270 decreased, making it more similar to the profiles of the other NP samples. The standout samples for the malt hot steeps, DH120270 (*grassy*) and Thunder (*sweet bread* and *aromatic*), were less noticeably different in the beer sensory evaluation. Observing patterns of descriptor usage across the two sensory methods can give us insights into the connection between the two. Both *grassy* and *grainy* were used more in malt hot steep characterization than beer characterization. *Floral* was used only once in the description of malt hot steep aroma but

became an important attribute for beer sensory. Similarly, *fruity* was used infrequently to describe malt hot steep samples but very frequently to describe the resulting beers. *Floral* and *fruity* aromas were likely present in beer due to the addition of hops and the production of esters by yeast during fermentation [33,34]. Nonetheless, some attributes were stable across both malt hot steep and beer sensory. For example, Thunder retained its *sweet bread* quality from malt hot steep to beer. Results from this study indicate that hot steep malt sensory profiles are more distinct than those of their resulting beers. It is important to note that beer sensory profiles will also be influenced by fermentation byproducts and interactions with hops. More evidence is needed to make further conclusions about the predictive ability of the hot steep malt method.

Comparing consumer and laboratory beer sensory

Differences in lexicon, panel size, methodology (including panel training), and goals preclude directly comparing the sensory results from laboratory panel and consumer panels. Nevertheless, both panels identified differences in beer flavor within the WRC set; in particular, the consumer panel identified *citrus, floral, hoppy*, and *sweet* as the differentiating attributes within the set. For the laboratory panel, *dough, sweet bread fruity,* and *floral* were key attributes that differentiated the finished beer samples. It is important to note that a set of lexicons were preselected and provided to consumers to describe each beer sample due to panelists lacking specific sensory training. The lexicon provided to consumers had fewer attributes related to the *bread* category, while adding more options that fell under *sweet aromatic* (*caramel*, *honey*). Beers brewed from Violetta and Calypso – at opposite ends of the overall liking spectrum – had very similar malt and beer analytics, suggesting that these objective measures are not necessarily predictive of hedonic assessment. This finding also indicates that there can be differences in beer flavor, attributable to barley variety, in the relatively small number of commercially available winter two-row malting barley varieties.

In contrast to the WRC set, no significant differences were found in overall liking of NP beers evaluated by the consumer panel. However, both laboratory and consumer

panels coincided in differentiating DH120270 from other samples: *lighter* and *thin/watery* by the consumer panel and *grassy* by the laboratory panel. DH120270, therefore, is consistently different from the other selections and the Full Pint check, indicating that this experimental variety could have been eliminated at the malt analysis stage, with no need to go on to the expense of malt and beer sensory. In a commercial application, the lack of significant differences in liking between DH131756 and DH131144 indicates that either of them could potentially be selected to replace Full Pint without an adverse consumer perception of beer flavor. The decision could be based primarily on agronomics and malt analytics. The latter, while not necessarily predictive of beer flavor in this research, can be key in variety approval and malt sales.

Beer metabolomics: connecting chemistry with sensory analysis and analytics

Metabolomics and sensory

Of the WRC beers, Violetta produced the beer with the highest score for *overall liking* in the consumer sensory panel, encompassing previously described desirable traits for a lager – namely *refreshing, crisp, citrus, sweet,* and *light* [3]. This variety had reduced MRPs and a unique profile of fatty acid esters (Figures 3, 6). Calypso, unique in pedigree, similar to the other varieties in malt and beer analysis, and a standout in hot steep malt sensory and beer sensory, had a unique chemical profile. It also had the lowest *likeability* score of the WRC beers in the consumer sensory panel. Because the PCA revealed separation of the WRC varieties that did not match any of the similarity groupings according to malting quality, beer analytics, or laboratory/consumer sensory, we looked to specific variety:metabolite associations.

The stringent selection applied to varieties during breeding and commercialization – which may not have included consumer sensory assessment - may have led to minor differences in volatile compounds, including an increase in compounds that convey *bitter* or *astringent.* As noted in the results, Calypso was more abundant in prenol lipids (terpenoids) and in a class of organoheterocycles known as “quinolines,” which are associated with a tea-like flavor (bitter, astringent) [17].

There were no trends among lipids/fatty acid esters among the five varieties, as the lipid/fatty acid ester class (acetate esters) was generally equally distributed. The medium-chain fatty acid ethyl esters (ethyl hexanoate and ethyl octanoate), however, co-varied with Calypso (Figures 5, 6) [35,36]. The nitrogenous compounds shared by Wintmalt (less modified malt) and Flavia (well-modified malt) included 2-mercapto-2-diethylaminoethanol (WRC0626) and pyridine-like compounds (WRC0374, WRC0493, WRC0489) which may contribute to, or overpower, the sensory attribute of *citrus* and instead contribute to *malty* noted by the consumer panel and the *breakfast cereal, bready*, and *earthy* attributes identified by the laboratory panel. Organic acids predominate in Violetta, and to a lesser degree, Thunder (Figure 6, Table 4). An organic acid ester, triethyl citrate (WRC0375), which is known to contribute to vinous and non-tropical fruity attributes, co-varied with Thunder and the *fruity (non-tropical)* sensory attribute from the consumer panel, as well as the *sweet aromatic* attribute from the laboratory panel. The organic acids most unique to Violetta included acetic acid ester (WRC0035), triethyl citrate (WRC0047), ethyl propanoate (WRC0194), isopentyl acetate (WRC0390), 4-isopropylphenylacetic acid (WRC0638), dimethyl malonate (WRC0384), and heptyl-2-methylpropanoate (WRC0188) (Table 5). Violetta, Wintmalt, and Flavia had negative correlations with the prevalent benzenoid class, which covaried with Calypso. This class included 1,2-benzenedicarboxylic acid ester (WRC0153), known to be associated with almond, floral, herbal, green, and more phenolic attributes; 4-hydroxybenzyl alcohol (WRC0481); and benzaldehyde (WRC1013), which is associated with more almond, bitter attributes.

In the NP set, Full Pint and DH131144 had higher abundances of aldehydes and ketones – such as 2-nonen-4-one (NP428), 1-hexene (NP255), and 1-pentanol (an alcohol, NP132) – and they shared many abundant fatty acid esters. Although Full Pint, DH131144, and DH131756 were similar in sensory attributes, DH131756 and DH120270 shared many -likes of acetic acid, keto acids, and an acetamide of note (NP097) which is noted in literature to contribute a mousy attribute. There are many metabolites that are known to have phenolic and bitter sensory properties that may contribute to the co-

variation with *bitter* in DH120270*,* identified by the consumer panel and with the *cracker* and *sweet aromatic* assessed by the laboratory panel. Examples of these metabolites include 2-phenyl-2 butenal (NP146), a phenylacetaldehyde known to contribute a bitter, black tea note and 2-methoxy-4-vinylphenol (NP381), recognized for the contribution of clove, smoky, and spicy attributes.

Given the distinctiveness of the WRC and NP germplasm sets in terms of growth habit, production environment, and commercialization status, the causes of similarities and differences are confounded, but notable. Some of these differences could be attributed to genetic relatedness: e.g. Full Pint is unique to the NP set as a member and as a parent. When DNA fingerprint data are available for the WRC and NP sets, causal effects based on genetic differences may be identifiable. The WRC varieties, as a group, contained fewer organoheterocycles (potential MRPs) than the NP varieties (Figure 3). As discussed in Bettenhausen et. Al. (2020) [3], MRPs play a major role in beer flavor. Two metabolites, furfural and 2-pentylfuran belong to the class of organoheterocycles known as furans, furfural serving as a precursor to 2-pentylfuran, which contributes fruity, grassy flavors (NP148 and WRC0228, Figure 6, denoted in red text). All varieties contained this furan, but normalized abundances differed among all varieties. Lower abundances of MRP in the WRC may be related to the lower grain protein, overall. Since degree of modification involves protein breakdown (through protease activity), incomplete modification would leave these varieties lacking in components for the Maillard Reaction (proteins, saccharides) [37]. In the NP set there were fewer instances of phenylpropanoids (a class including cinnamic acid esters and coumarins) and more benzenoids (phenols, benzoic acid esters) than in the WRC set. Phenolic compounds are formed via the shikimate pathway and are known to contribute to more *bitter* and *astringent* attributes, such as those found in DH120270. Fatty acid esters, especially ethyl dodecanoate, (WRC0012 and NP031, denoted in Heat Map (HMap) in green text) were present in DH120270 and Wintmalt. Abundances of ethyl dodecanoate in other varieties were well below the amounts in Wintmalt and DH120270. These two genotypes were also the least modified (Table 2) and differed the most for beer analytics. The

development of these fatty acid esters, through esterification of ethanol with fatty acids, is crucial for development of flavors, but the lipids that are present in each variety (type and amount) may play a role in how much of that flavor is developed and at what rate. The presence of these compounds (ethyl octanoate, ethyl-9-decenoate, n-decanoic acid) in conjunction with the low MRP/organoheterocycle profile of WRC suggests not only that these compounds contribute to desirable attributes associated with Violetta, but that they could also contribute to off-flavors during aging [38,39,40].

Metabolomics, malting quality, and beer analytics

Wintmalt met the fewest malt quality specifications of the WRC set (Table 2) yet produced an acceptable beer by consumer panel standards and no negative attributes were noted by the laboratory panel. Violetta and Flavia were noted as having more complex flavor profiles; this is potentially due to variable (on the edge of acceptability) S/T, total protein, and FAN levels (Table 2), leaving less for the development of Maillard reactions products (MRPs) to create *roasted* and *caramel* attributes from degraded protein and saccharides [37,38]. The lack of MRP attribute creation leaves more room for lipid conversion into fatty acid esters and therefore the potential for lighter *fruity, floral* attributes to be perceived. Thunder, which had the highest diastatic power and lowest RDF, produced a beer that was perceived as more *crisp* and *dry*, with no residual sweetness and showed co-variation with the *caramel, honey, toasted,* and *non-tropical fruity* from the consumer panel and *sweet bread* and *sweet aromatic* from the laboratory panel. The higher FAN in Thunder, as opposed to the level found in Violetta, may be a source of MRPs, and thus be an indicator of potential flavors in beer. The lighter flavors expressed by Violetta may be linked to the greater concentrations of fatty acid esters, which are described as *sweet*, *fruity*, and *floral* [41]. The lower degree of modification of Wintmalt and DH120270 could produce beers with *grassy* attributes due to the presence of acetaldehyde, hexanal, hexanol and general “greenness” of the malt [41]. Furthermore, under-modified malt tends to produce less extract during mashing and therefore lower than target ethanol concentrations after fermentation. The lower level of modification

combined with low diastatic power of Wintmalt were likely reasons for it producing the lowest RDF in the study (Tables 2 & 3). Wintmalt and DH120270 also had the haziest wort, which may have been due to either low modification or high molecular weight beta-glucans, these in turn could lead to possible unintentional flavor outcomes. Full Pint and DH131144 were chemically the most similar of the NP varieties despite differences in two malt quality parameters linked to endosperm modification - friability and beta-glucan. The NP set as a group was less friable than the WRC set, averaging 77% versus 96% (Table 2). Nonetheless, there were no significant differences in the brewhouse yield between the two sets of malt ($t = 0.494$, $p = 0.318$ for one-sided t-test).

Conclusions

This study contributed to the body of knowledge by examining the effects of more and different barley genotypes on beer flavor. The current results support our previous findings that barley genotype does lead to differences in flavor profiles of lager beer. Two sets of barley germplasm 1) commercially available winter barleys and 2) Full Pint and three advanced progeny breeding lines were found to have distinct, subtle differences that contributed to nuanced flavor profiles of both malt hot steeps and finished lager beer. Variations between and among barley germplasm sets were greatest for malt analytics, and this variation declined for beer analytics and then again for sensory profiling. Consumer and laboratory panels detected differences in sensory attributes of beer and malt hot steeps, but the basis of these differences was not always obvious. It is important to emphasize, in this context, that the descriptors and preferences reported are applicable only to these research beers and should not be taken as representative of the specific barley varieties and/or selections and their production environments.

Nonetheless, the research findings support the value of sensory assessments of pilot and commercial-scale beers of potential and new varieties. While common practice in the final stages of the variety recommendation and/or adoption processes, brewing and sensory assessment may also have value earlier in the variety development pipeline. Sensory assessments can continue to play an important role for defect elimination and

can be expanded to include discovery of new flavor opportunities. In the case of the WRC set, a variety with acceptable malt and beer analytics was not favored by the sensory panels while a variety with less favorable malt and beer analytics was acceptable. In the case of the NP set, one potential variety could be eliminated based on flavor as well as on poor malting and brewing quality attributes. The remaining two selections were not appreciably different in sensory profile from the reference variety, which simplifies the variety selection process to decisions based on agronomics, malting quality, and/or beer quality.

All measures and procedures used in this research have value in guiding decisions regarding variety selection, but none were directly predictive of another. For example, malt analytics can guide maltster decisions on what barley varieties are likely to produce consistent malt using existing malting protocols in order to meet brewers' expectations. Additionally, while exploring the ability of hot steep malts as an economical and efficient predictive tool for beer flavor profiles, there were some attributes that were stable across both beer and hot steep malt sensory analysis. Hot steep malt sensory profiles were found to be more distinct than those of their resulting beers. The current research provides some insights into this relationship, but other experiments are justified in order to define the basis of this relationship: the hot steep malt sensory may provide a useful common language for maltsters and brewers. Moreover, metabolomics can provide insights into the chemical basis of specific sensory descriptors and consumer preference. Distinct metabolomic profiles were detected within and between germplasm sets which were attributable to variety. Covariation of metabolomic profiles and sensory attributes was identified in both panels. These observations lead to the conclusion that the variable metabolites observed among the two sets of barley germplasms are a direct result of genetic differences that lead to differential responses within the malting and brewing processes. When metabolites are connected to genes, barley breeders will have additional targets for selection in order to meet target, or novel, beer flavor profiles. Until then, the new knowledge generated by this research can be capitalized upon by extending it to

additional barley genotypes, different malts of the same varieties, and different beer styles.

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Oversized Tables

Table 4: WRC metabolite data

Code	Class	Subclass	Metabolite	Sensory (Lit)[a]	PC1 Correlation - scaled loadings[b]	PC2 Correlation - scaled loadings[b]	Pvals (FDR adjusted)[c]
WRC04090	alkaloids	alkaloids	piperine	animal, pepper	0.26	0.91	0.71
WRC10013	benzenoids	benzaldehydes	benzaldehyde	almond, bitter, burnt sugar, cherry, sweet	0.74	-0.34	0.62
WRC04087		benzamines	benzoic acid, 2-amino-4-methyl-	NA	-0.80	0.09	0.57
WRC04037		benzenoids	1-phenyl-2-pentanol	earthy, green, mild, sweet	-0.83	-0.41	0.87
WRC01018			4-hydroxybenzyl alcohol	astringent	0.79	-0.12	< 0.05
WRC00058			benzamide, 4-ethyl-n-butyl-n-tetradecyl-	NA	-0.55	-0.13	< 0.05
WRC03003			n,n-dimethyl-3-methylaniline	NA	-0.61	0.40	0.93
WRC00031			phenylethyl alcohol	alcoholic	-0.03	-0.35	0.69
WRC01053		benzoic acid esters	1,2-benzenedicarboxylic acid, butyl 2-methylpropyl ester	almond, floral, herb, lettuce, phenolic, prune, sweet, wintergreen	-0.51	0.61	0.02
WRC02040			benzoic acid, 3-amino-	bitter	-0.49	0.54	0.78
WRC00081			m-anisic acid, cyclobutyl ester	NA	0.26	-0.84	0.43
WRC04085			phenoxyacetic acid	sour, sweet	0.66	-0.23	0.75
WRC04081			4-hydroxybenzyl alcohol	almond, bitter, coconut, fruity, sweet	-0.74	-0.55	< 0.05
WRC01095		phenols	phenol	phenolic	-0.62	-0.64	0.78
WRC01062		xylenes	2-thiophenecarboxaldehyde	NA	0.20	-0.45	0.99

Code	Class	Subclass	Metabolite	Sensory (Lit)[a]	PC1 Correlation - scaled loadings[b]	PC2 Correlation - scaled loadings[b]	Pvals (FDR adjusted)[c]
WRC0632	hydrocarbons	alkanes	pentane	alkanes	-0.65	-0.36	0.60
WRC0298		hydrocarbons	(+/-)-n,n-dimethyl menthyl succinamide	cool, minty	0.14	0.59	0.35
WRC0087			2-butene, 2-methyl-	NA	0.78	0.06	0.03
WRC0183			3-octen-1-yne	NA	0.01	0.07	0.48
WRC0549	lipids	Fatty acids/fatty acid esters	2-hexenyl valerate	apple, banana, cognac, fruity, green, pineapple	-0.66	0.38	0.42
WRC0017			3-methylbutyl octanoate	coconut, fruity, green, pineapple, soapy, sweet	-0.68	0.27	0.73
WRC0038			3-methylbutyl octanoate	coconut, fruity, green, pineapple, soapy, sweet	-0.50	0.68	0.83
WRC1280			butanedioic acid ester	apple, apricot, chocolate, cooked, cranberry, fruty, grape, musty	0.71	0.54	0.50
WRC0395			cis-3-hexenyl 3-methylbutanoate	apple, fresh, fruity, green, pineapple, tropical	0.03	-0.39	0.61
WRC0042			decanoic acid, 2-methylbutyl ester	apple, brandy, fruity, grape, pear, sweet, waxy	-1.00	0.08	0.56
WRC0054			dodecanedioic acid ester	clean, floral, soapy, sweet	-0.66	0.70	0.00
WRC0016			ethyl 9-decenoate	fatty, fruity, green, soapy, waxy	-0.87	0.17	0.36
WRC0012			ethyl dodecanoate	clean, floral, soapy, sweet	-0.77	0.17	0.99
WRC0010			ethyl nonanoate - like	fruity, rose, rum, tropical, wine	0.83	0.05	0.91
WRC0021			ethyl nonanoate - like 1	fruity, rose, rum, tropical, wine	-0.33	0.88	0.18
WRC0034			ethyl nonanoate - like 2	fruity, rose, rum, tropical, wine	-0.45	0.35	0.72
WRC0037			ethyl nonanoate - like 3	fruity, rose, rum, tropical, wine	0.51	-0.62	0.10
WRC0838			hexanedioic acid ester	NA	-0.34	-0.80	0.31

Code	Class	Subclass	Metabolite	Sensory (Lit)[a]	PC1 Correlation - scaled loadings[b]	PC2 Correlation - scaled loadings[b]	Pvals (FDR adjusted)[c]
WRC1258			hexanoic acid, 2-ethyl-, 1,1-dimethylethyl ester	apple peel, banana, fruity, pineapple, sweet	-0.07	-0.97	0.55
WRC0044			hexanoic acid, ethyl ester	apple peel, banana, fruity, green, pineapple, sweet	0.89	-0.04	0.84
WRC0055			hexanoic acid, ethyl ester	apple peel, banana, fruity, green, pineapple, sweet	-0.60	-0.61	0.05
WRC0850			hexyl butyrate	apple, apple peel, fruity, green, soapy, sweet, waxy	-0.72	0.23	0.88
WRC0502			methyl stearate	oily, waxy	-0.44	0.13	0.65
WRC0428			methylglutaric acid	NA	-0.81	-0.01	0.11
WRC0025			n-capric acid isobutyl ester	green, herbal, aldehydic, orange, sweet, vegetable	-0.76	-0.45	0.55
WRC0018			n-decanoic acid	apple, brandy, fruity, grape, pear, sweet, waxy	-0.13	0.46	0.14
WRC0056			octadecanoic acid, 2-(2-hydroxyethoxy)ethyl ester	fatty, waxy	-0.26	0.56	0.58
WRC0053			octanoic acid, 3-methylbutyl ester	coconut, fruity, green, pineapple, soapy, sweet	-0.95	0.00	0.88
WRC0039			pentadecanoic acid ester	NA	-0.75	0.44	0.85
WRC0033			pentadecanoic acid, ethyl ester	NA	0.13	0.56	0.19
WRC0048			pentanoic acid, 3-methyl-, ethyl ester	apple, fruity, green, nutty, pineapple, sweet	0.37	-0.64	0.34
WRC0152			picolinyl 2,5-octadecadienoate	NA	0.13	0.42	0.34
WRC1067			propionic acid, ethyl ester	fruity, grape, juicy, pineapple, rum, sweet	0.07	-0.61	0.99
WRC0011			stearic acid	NA	0.56	0.71	< 0.05

Code	Class	Subclass	Metabolite	Sensory (Lit)[a]	PC1 Correlation - scaled loadings[b]	PC2 Correlation - scaled loadings[b]	Pvals (FDR adjusted)[c]
WRC0098			tetradecanoic acid, ethyl ester	ether, soapy, sweet, violet, waxy	0.41	-0.83	0.74
WRC0036		fatty alcohols	1,2-hexanediol	NA	0.25	0.62	< 0.05
WRC0288			5-hexenol	green	0.60	0.12	0.44
WRC0028			octadecane-1,2-diol	NA	0.39	0.14	< 0.05
WRC0045			octadecane-1,2-diol	NA	0.03	-0.98	< 0.05
WRC1044		fatty amides	butyramide	nutty	0.49	0.70	1.00
WRC0638	organic acids	carboxylic acid esters	4-isopropylphenylacetic acid	cumin	0.58	-0.41	0.44
WRC0035			acetic acid, 2-phenylethyl ester	acidic, vinegar	0.20	0.68	0.94
WRC0149			acetic acid, hydroxy-, ethyl ester	vinegar, acetic	0.67	-0.24	0.21
WRC0679			chicoric acid	NA	-0.64	-0.53	0.39
WRC0063			cyclohexane carboxylic acid, hexyl ester	NA	0.79	-0.31	0.17
WRC0384			dimethyl malonate	fruity	0.14	0.80	0.23
WRC0188			heptyl 2-methylpropanoate	apple, apricot, cherry, floral, fruity, grape, green, orange, pear, raspberry	-0.18	-0.95	0.37
WRC0390			isopentyl acetate	banana, bitter, fruity, solvent, sweet	-0.08	0.83	0.49
WRC0813			methoxyphenylacetic acid	NA	0.94	0.03	0.64
WRC0194			propanoic acid, ethyl ester	fruity, grape, juicy, pinapple, tropical, rum, sweet	0.79	0.41	0.64
WRC0047			triethyl citrate	acidic	0.98	-0.14	0.80
WRC0375			triethyl citrate	fruity, wine	-0.43	-0.88	0.99

Code	Class	Subclass	Metabolite	Sensory (Lit)[a]	PC1 Correlation - scaled loadings[b]	PC2 Correlation - scaled loadings[b]	Pvals (FDR adjusted)[c]
WRC08006		thioesters	ethanethioic acid, s-(1-methylethyl) ester	coffee, fruity, garlic, meaty, onion, sulfur	0.77	0.56	0.75
WRC00061	organoheterocycles	benzodiazines	quinoxaline	NA	-0.80	0.11	0.51
WRC03004		benzopyrans	9h-xanthene-9-carboxylic acid 4-iodo-phenyl ester	NA	-0.76	-0.40	< 0.05
WRC02999		furanones	2,5-dimethyl-4-(1-pyrrolidinyl)-3(2h)-furanone	cereal	-0.78	0.11	< 0.05
WRC02228		furans	2-pentylfuran	butter, green bean	0.70	-0.18	0.75
WRC00020		indoles	1h-indole	NA	0.38	0.51	0.09
WRC01887		lactones	4-hydroxybutanoic acid lactone	NA	-0.18	-0.38	0.64
WRC04883		lactones	5-methyl-delta-valerolactone	herbal, sweet	0.49	-0.76	0.61
WRC06221		pyridines	2-methyl-5-(methylthio) pyrazine	NA	0.63	0.07	0.23
WRC01113			2-pyridinecarboxaldehyde	orange, beer	0.47	0.72	0.99
WRC03374			3-acetoxypyridine	NA	-0.67	0.30	0.28
WRC01998			3-butenoic acid, 2-oxo-4-phenyl-	caramel, green, radish, sweet, walnut	-0.56	0.26	0.69
WRC05514			3-pyridinecarboxamide	NA	-0.04	0.10	0.95
WRC04893			4-pyridinecarboxylic acid	NA	0.34	-0.16	0.28
WRC04889			pyridine	amine, fishy, putrid, rancid, sour	0.34	0.40	0.21
WRC01144		pyrimidines	6-amino-4-phenyl-1h-quinazolin-2-one	NA	-0.21	0.76	0.25

Code	Class	Subclass	Metabolite	Sensory (Lit)[a]	PC1 Correlation - scaled loadings[b]	PC2 Correlation - scaled loadings[b]	Pvals (FDR adjusted)[c]
WRC00015		quinolines	4,8-dimethylquinoline	tea	-0.54	0.44	< 0.05
WRC00027			quinoline	tea	-0.72	0.68	< 0.05
WRC06026	organonitrogen compounds	amines	2-diethylaminoethanol	NA	-0.10	0.02	0.53
WRC02031		aminoalcohols	ethanol, 2-mercapto-	meaty, sulfur	-0.76	0.04	< 0.05
WRC01446		aminoxides	trimethylamine n-oxide	NA	0.82	-0.25	< 0.05
WRC00049		monoalkylamines	1,2-diamino-2-methylpropane		-0.04	0.78	0.69
WRC03777	organooxygen compounds	alcohols	1,3-propanediol	bitter	0.47	0.14	0.42
WRC00095			1-pentanol	balsam, balsamic, fusel, oil, sweet, vanilla	0.13	0.52	0.33
WRC00079			2,3-butanediol	buttery, creamy, fruit, fruity, onion	-0.34	-0.51	0.63
WRC04992		sugar alcohols	maltitol	NA	0.63	0.62	0.91
WRC06672		aldehydes	5-hydroxymethyl-2-furancarboxaldehyde	caramel, cardboard, musty, waxy	0.32	-0.54	0.55
WRC01154			pyrrole-2-carboxaldehyde	ethereal	0.48	0.70	0.59
WRC00041		alkenes	1,2-dimethoxy-ethene	NA	-0.05	0.86	0.98
WRC00050		cyclic ketones	5h-inden-5-one, 1,2,3,3a,4,7a-hexahydro-7a-methyl-, trans-	NA	-0.01	-0.69	0.40
WRC06686		enals	2-butenal, 3-methyl-	almond, cherry, fruity, nutty, sweet	0.99	0.13	0.29
WRC05522			2-propenal	almond, cherry	-0.24	0.69	0.54
WRC06642			2-propenal - like	almond, cherry	0.53	-0.06	0.56

Code	Class	Subclass	Metabolite	Sensory (Lit)[a]	PC1 Correlation - scaled loadings[b]	PC2 Correlation - scaled loadings[b]	Pvals (FDR adjusted)[c]
WRC01110		ketones	2,4,6-tri-isopropylacetophenone	NA	0.49	0.63	0.43
WRC03376			5-methyl-3-hexen-2-one	berry, cheese, sweet	-0.38	0.28	0.79
WRC01184			benzyl ethyl ketone	tea	-0.74	-0.24	0.76
WRC06031			p-pentylacetophenone	NA	-0.33	0.90	0.41
WRC03378		monosaccharide phosphates	.alpha.-d-mannose 1-phosphate	NA	0.02	-0.61	0.35
WRC01156		o-glycosyl compounds	isomaltose	sweet	0.21	-0.40	0.33
WRC00088			hydroxylamine, o-methyl-	NA	0.43	0.59	0.99
WRC00089	organosulfur compounds	sulfonyls	methyl methanethiosulfonate	sulfur	-0.72	0.40	0.37
WRC00013		thiols	1-propene-1-thiol	sulfur	0.62	0.60	< 0.05
WRC02085			3-mercapto-3-methyl-1-butanol	meat, meat broth, roasted, spicy, sweet, vegetable	-0.80	0.39	0.25
WRC05003			ethanethiol	sulfur	0.73	0.45	0.80
WRC06004	phenylpropanoids	chalcones	2,2',4'-trihydroxychalcone	bitter	-0.27	-0.79	0.63
WRC10022		cinnamaldehydes	3-(4-methylphenyl)-2-propenal	cinnamon, spicy	0.04	-0.03	0.72
WRC02030		cinnamic acid esters	isoamyl cinnamate	cocoa, floral, musty, orchid	0.26	0.35	< 0.05
WRC10015		coumarin glycosides	7-diethylaminocoumarin		-0.64	0.02	0.79
WRC03383		coumarins	3,4-dihydro-2h-1-benzopyran-2-one	almond, cinnamon, coconut, coumarin, creamy, herbal, sweet, tobacco	-0.31	0.23	0.52
WRC04096			3-hydroxycoumarin	NA	0.32	0.77	0.52
WRC08017			7-methoxycoumarin-4-acetic acid	NA	0.66	-0.56	0.36

Code	Class	Subclass	Metabolite	Sensory (Lit)[a]	PC1 Correlation - scaled loadings[b]	PC2 Correlation - scaled loadings[b]	Pvals (FDR adjusted)[c]
WRC01125		curcuminoids	curcumin	NA	0.42	0.72	0.73
WRC01173		flavonoids	quercetin 3'-methyl ether	NA	0.02	0.20	0.96
WRC08030			kaempferol 3-o-rutinoside	NA	0.89	0.18	0.71
WRC02007			quercetin 3,5,7,3',4'-pentamethyl ether	orange, oregano	0.03	0.58	0.29
WRC02266		hydroxycinnamic acid esters	trans-ferulic acid	NA	0.35	0.40	0.53
WRC03322		phenols	phenol	NA	-0.54	-0.72	0.32
WRC00071	prenol lipids	monoterpenoids	linalool	citrus, floral, green, lavender, lemon, orange, sweet	-0.02	0.92	0.37
WRC10030			p-menthan-1-ol	NA	-0.66	0.56	0.60
WRC01182			trans-geranic acid methyl ester	tea	0.08	0.81	0.82
WRC02284		sesquiterpenoids	alpha-cadinol	herb, woody	-0.68	0.59	0.22
WRC01196			alpha-cubebene	herbal	-0.82	0.52	0.51
WRC01155			epicubenol	NA	0.43	-0.84	0.57

a=Predicted flavor attribute based on information in FooDb [17]; NA=No flavor information found.
b= Correlation-scaled loadings examine the strength and direction of the relationship between the metabolite(s) and the sensory component (*X*) metabolites shown are those which met the threshold for this analysis, $|< 0.75|$.
c= From ANOVA supporting variation among the n = 5 beers

Table 5: NP metabolite data

Code	Class	Subclass	Metabolite	Sensory (Lit)[a]	PC1 Correlation-scaled loadings[b]	PC2 Correlation-scaled loadings[b]	Pvals (FDR adjusted)[c]
NP163	alkane	alkane	18-methyl-nonadecane-1,2-diol	alkane, bland	-0.30	-0.94	0.82
NP110	benzenoids	benzaldehydes	benzaldehyde-like	almond, bitter, burnt sugar, cherry, sweet	-0.69	-0.67	0.32
NP225			benzaldehyde-like	almond, bitter, burnt sugar, cherry, sweet	-0.92	-0.10	0.12
NP496		benzenoids	1-(3,4-dimethylphenoxy)-4-(3,4-dimethylphenylsulfonyl)benzene	benzene	-0.58	0.71	0.80
NP034			2-phenylethanol	bitter, floral, honey, lilac, rose, spice	-0.93	0.24	0.31
NP105		benzoic acid esters	1,2-benzenedicarboxylic acid, butyl 2-methylpropyl ester	almond, floral, herb, lettuce, phenolic, prune, sweet, wintergreen	0.13	-0.69	0.18
NP397			4-methoxybenzyl phenylacetate	anise, balsam, honey, woody	0.66	-0.17	0.82
NP083			allyl benzoate	berry, cherry, floral, sweet	-0.96	0.09	0.76
NP045			amyl salicylate	azalea, chocolate, clover, floral, green, herbal, sweet	-0.28	0.61	0.54
NP046			benzamide-like	bitter	0.62	0.05	0.23
NP041			butyl salicylate	clover, bitter, harsh	-0.32	0.59	< 0.05
NP390			ethyl benzoate	anise, balsam, banaba, berry, bitter, cherry, cranberry, fruit, grape, minty, musty, sweet	-0.52	0.81	0.29
NP035			octyl benzoate	lemon balm, balsam, fruity	-0.52	0.52	0.66
NP226			phenylacetate	flower, honey	-0.14	0.32	0.24
NP113			salicylic acid ester	azalea, chocolate,	0.69	-0.02	0.20

Code	Class	Subclass	Metabolite	Sensory (Lit)[a]	PC1 Correlation-scaled loadings[b]	PC2 Correlation-scaled loadings[b]	Pvals (FDR adjusted)[c]
NP354			4-hydroxybenzoic acid	clover, floral, green, herbal, sweet nutty, phenolic	-0.83	0.49	0.73
NP298			benzoic acid-like	bitter	-0.76	0.57	0.77
					-0.20	-0.93	
NP146		phenlyacetaldehydes	(e)-2-phenyl-2-butenal	phenolic, black tea	0.66	0.15	< 0.05
NP407		phenols	1,2-benzenediol	NA	-0.57	-0.04	0.35
NP122			2-ethylphenol	coffee	0.20	0.62	0.44
NP381			2-methoxy-4-vinylphenol	clove, curry, peanut, smoky, spicy	-0.78	0.54	0.09
NP091			phenol-like	phenolic, bitter	-0.84	0.44	< 0.05
NP221			phenol-like	phenolic, bitter	-0.86	0.48	< 0.05
NP379			phenol-like	phenolic, bitter	-0.43	0.89	< 0.05
NP348			vanillylmandelic acid	sweet, vanilla	0.08	-0.99	0.88
NP565		thiophenols	2,6-dimethylbenzenethiol	meaty, metallic, phenolic, roasted, sulfurous	-0.72	0.67	0.63
NP062	dithioles	1,2-dithioles	dithiole-like	sulfur	0.06	0.77	0.90
NP068	hydrocabons	alkanes	2-methylheptane	NA	0.34	-0.87	0.45
NP013	lipids	fatty acid esters	10-undecenoic acid, ethyl ester	clean, cognac, creamy, fruity, musty, soapy, waxy	-0.71	0.73	0.43
NP642			2-butenoic acid, phenyl ester	caramel, green, radish, sweet, walnut	0.27	-0.94	0.07
NP014			3-methylbutyl octanoate	coconut, fruity, green, pineapple, soapy, sweet	-0.90	0.39	0.45
NP398			3-nonenoate	fruity, green, melon, pear, watermelon	-0.74	0.70	0.76
NP416			butanoic acid, butyl ester	apple, banana, berry, fruity, peach, pear, pineapple, sweet	-0.80	0.61	0.98
NP024			decanoic acid ester	citrus, fatty, rancid, sour	-0.13	0.75	0.32

Code	Class	Subclass	Metabolite	Sensory (Lit)[a]	PC1 Correlation-scaled loadings[b]	PC2 Correlation-scaled loadings[b]	Pvals (FDR adjusted)[c]
NP047			decyl propionate	cognac, ether, fatty, fruity, rum	0.30	0.49	0.80
NP375			diethyl decanedioate	fruity, melon, quince, wine	0.02	-0.51	0.28
NP477			diethyl maleate	banana	-0.39	0.81	0.90
NP011			ethyl 9-decenoate	fatty, fruity, green, soapy, waxy	0.46	-0.76	0.81
NP033			ethyl 9-decenoate	fatty, fruity, green, soapy, waxy	-0.44	0.19	0.72
NP012			ethyl decanoate -like 1	apple, brandy, fruity, grape, pear, sweet, waxy	-0.88	0.51	0.46
NP021			ethyl decanoate -like 2	apple, brandy, fruity, grape, pear, sweet, waxy	0.23	0.54	0.43
NP031			ethyl dodecanoate	clean, floral, soapy, sweet	-0.35	-0.92	< 0.05
NP061			ethyl nonanoate -like 1	fruity, rose, rum, tropical, wine	-0.80	0.63	0.59
NP020			ethyl nonanoate -like 2	fruity, rose, rum, tropical, wine	-0.77	-0.05	0.41
NP016			ethyl nonanoate -like 3	fruity, rose, rum, tropical, wine	-0.23	0.79	0.50
NP044			ethyl nonanoate -like 4	fruity, rose, rum, tropical, wine	0.69	-0.09	0.39
NP096			ethyl propionate -like 1	fruity, grape, juicy, pineapple, rum, sweet	-0.85	0.49	0.65
NP295			ethyl propionate -like 2	fruity, grape, juicy, pineapple, rum, sweet	-0.87	-0.05	0.11
NP325			glutaric acid ester	NA	-0.53	0.81	0.19
NP165			glutaric acid, 2-ethylphenyl decyl ester	NA	0.17	-0.98	0.07
NP065			heptanoic acid, ethyl ester -like 1	berry, floral, fruit, green, sweet, waxy	0.62	-0.48	0.64
NP066			heptanoic acid, ethyl ester -like 2	berry, floral, fruit, green, sweet, waxy	-0.45	0.87	0.64

Code	Class	Subclass	Metabolite	Sensory (Lit)[a]	PC1 Correlation-scaled loadings[b]	PC2 Correlation-scaled loadings[b]	Pvals (FDR adjusted)[c]
NP051			hexadecanoic acid, ethyl ester	balsam, creamy, fruity, milky	0.49	-0.53	< 0.05
NP048			hexanoic acid, ethyl ester -like 1	apple peel, banana, fruity, green, pineapple, sweet	-0.44	0.82	0.16
NP023			hexanoic acid, ethyl ester -like 2	apple peel, banana, fruity, green, pineapple, sweet	0.63	-0.44	0.30
NP025			hexanoic acid, ethyl ester -like 3	apple peel, banana, fruity, green, pineapple, sweet	-0.47	0.80	0.47
NP027			hexanoic acid, ethyl ester -like 4	apple peel, banana, fruity, green, pineapple, sweet	-0.85	0.49	0.48
NP302			isopropyl 2-methylbutanoate	ethereal, fruity, green, pineapple, sweet, tropical	0.38	-0.85	0.65
NP197			methyl caprylate -like 1	green, herbal, aldehydic, orange, sweet, vegetable	-0.30	0.81	0.43
NP019			methyl caprylate -like 2	green, herbal, aldehydic, orange, sweet, vegetable	0.56	-0.61	0.56
NP026			methyl caprylate -like 3	green, herbal, aldehydic, orange, sweet, vegetable	-0.30	0.76	0.60
NP154			octadecanoic acid, 17-methyl-, methyl ester	fatty, waxy	0.21	-0.95	< 0.05
NP028			pentadecanoic acid, ethyl ester	NA	-0.04	-1.00	0.48
NP018			pentanoic acid ester	fruity	-0.40	0.81	0.06
NP145			pentanoic acid, 2,4-dimethyl-, methyl ester	apple, fruity, green, nutty, pineapple, sweet	-0.92	0.43	0.56
NP222			pentanoic acid, 2-methyl	apple, berry, fruity, hazelnut, tropical	-0.02	0.02	0.45

Code	Class	Subclass	Metabolite	Sensory (Lit)[a]	PC1 Correlation-scaled loadings[b]	PC2 Correlation-scaled loadings[b]	Pvals (FDR adjusted)[c]
NP218			tetradecanoic acid, ethyl ester	ether, soapy, sweet, violet, waxy	-0.13	0.71	< 0.05
NP194		fatty alcohols	1,2-hexanediol	NA	0.10	0.99	0.11
NP064			2-nonen-1-ol	cardboard	-0.72	0.71	< 0.05
NP288			cis-4-decenol	fatty, fruity, waxy	-0.82	0.56	< 0.05
NP097	organic acids	carboximidic acid esters	acetamide	mousy	-0.99	0.14	0.23
NP007		carboxylic acid esters	3-mercaptohexyl acetate	floral, fruity, passion fruit, pear, tropical	-0.58	-0.68	0.17
					0.00	-0.79	
NP558			3-mercaptopropionic acid	roasted, sulfurous	-0.70	0.71	0.27
NP253			acetic acid, 2-methylphenyl ester	vinegar, acetic	-0.62	0.73	0.27
NP037			acetic acid, 2-phenylethyl ester	vinegar, acetic	-0.54	0.84	0.27
NP038			acetic acid, methyl ester	vinegar, acetic	-0.48	0.74	0.38
NP077			acetic acid-like	vinegar, acetic	-0.67	0.67	0.44
NP206			acetic acid-like	vinegar, acetic	-0.72	-0.16	0.59
NP200			ethyl acetate	anise, balsam, ethereal, fruity, green, pineapple, sweet	0.14	0.95	0.91
NP003			ethyl lactate	butter, butterscotch, fruity, tart	-0.67	0.53	0.94
NP216			fumarate	NA	-0.07	-0.53	< 0.05
NP101			oxalic acid ester	NA	-0.90	0.34	0.75
NP040			1-butanol, 2-methyl	banana, fruity, juicy, overripe fruit, peanut, sweet	-0.54	0.49	0.97
NP030			isopentyl acetate	banana, bitter, fruity, solvent, sweet	-0.47	0.73	0.83
NP022		hydroxy acids	beta-hydroxypyruvic acid	cabbage, sour, radish	-0.85	0.45	0.10
NP141			ethyl 2-(methylthio)acetate	apricot, citrus, earthy, floral, fruity, green, herbaceous, meaty, nutty	-0.02	0.46	0.81
NP001			ethyl (±)-3-hydroxybutyrate	NA	-0.82	0.04	0.56

Code	Class	Subclass	Metabolite	Sensory (Lit)[a]	PC1 Correlation-scaled loadings[b]	PC2 Correlation-scaled loadings[b]	Pvals (FDR adjusted)[c]
NP008			hydroxybutyric acid	NA	-0.71	-0.67	0.46
NP002			malic acid	NA	-0.55	0.73	0.50
NP454		keto acids	ketobutyric acid	NA	0.32	-0.88	< 0.05
NP056		benzodiazines	5-methylquinoxaline-;like	burnt, coffee, corn, nutty, roasted, toasted	-0.31	0.81	< 0.05
NP150			5-methylquinoxaline-;like	burnt, coffee, corn, nutty, roasted, toasted	0.86	0.43	0.64
NP213		benzopyrans	3,4-dihydro-6-methoxy-2,2-dimethyl-2h-1-benzopyran-4-ol	mushroom	-0.60	0.65	0.14
NP036			4-methylene-3,4-dihydroisocoumarin	NA	-0.10	-0.98	0.44
NP220		benzothiazoles	benzothiazole	coffee, gasoline, meat, nutty, rubber, sulfur, vegetable	-0.07	0.74	< 0.05
NP195		furanones	2(5h)-furanone, 5-methyl-5-phenyl-	NA	-0.78	0.64	0.82
NP387			5-methyl-3(2h)-furanone	NA	-0.44	0.78	0.60
NP198		furans	2-furoic acid ester	fruity, fungal, mushroom, sweet, tobacco	0.67	0.30	0.13
NP148			2-pentylfuran	NA	0.01	-1.00	0.97
NP259			3,4-furandicarboxylic acid	maillard	0.27	-0.74	0.65
NP464		heteroaromatic compounds	2-(methoxymethyl)furan	coffee, roasted	-0.78	-0.26	0.77
NP393			2-(methylthiomethyl)furan	garlic, horseradish, onion, sulfur, vegetable	-0.42	0.84	< 0.05
NP076			2,5-dimethyl-3-(methylthio)furan	coffee, roasted	-0.87	0.39	0.96
NP563			2-propylthiophene	NA	-0.43	0.62	0.72
NP049			5-ethyl-(3h)-furan-2-one	spice	-0.35	0.35	0.09
NP289			dimethyl furan	onion	-0.15	0.34	0.98
NP052			furfuryl ethyl ether-like	coffee, roasted	-0.06	0.98	0.24
NP069			furfuryl ethyl ether-like	coffee, roasted	-0.88	0.42	< 0.05
NP497			furfuryl ethyl ether-like	coffee, roasted	0.45	-0.75	0.29

Code	Class	Subclass	Metabolite	Sensory (Lit)[a]	PC1 Correlation-scaled loadings[b]	PC2 Correlation-scaled loadings[b]	Pvals (FDR adjusted)[c]
NP564			thiophene	garlic, onion	0.79	-0.59	0.55
NP545		isocoumarans	isobenzofuranone-like	celery, herbal	-0.50	0.19	< 0.05
NP515		lactones	6-butyloxan-2-one	coconut, coumarin, milky, sweet	-0.83	0.57	0.27
NP006		purines	hypoxanthine	NA	-0.76	0.52	0.57
NP306			purine-like	maillard	-0.04	0.50	0.08
NP102		pyrazines	isopropyl pyrazine	green, honey, minty, nutty	-0.21	0.64	0.14
NP541			pyridine-4-carboxylic acid, 2,2,6,6-tetramethyl-4-oxo-1-piperidinyl ester	NA	-0.52	-0.70	0.98
NP088		pyrazoles	3-nonyl-1h-pyrazole	NA	-0.94	0.10	0.43
NP336		pyridines	3-butenoic acid	NA	-0.84	0.49	0.40
NP629			4-methylpyridine	tea, fig	0.16	-0.43	< 0.05
NP189			4-vinylpyridine	tea	0.39	0.35	0.55
NP050			4-vinylpyridine-like	tea	-0.73	0.70	0.43
NP300			5-methoxypyrimidine	NA	0.07	0.63	0.46
NP278		pyrimidines	2,4-diamino-5,6-dihydroxypyrimidine	NA	-0.22	0.72	0.38
NP512		pyrrolidines	2-pyrrolidinone	NA	-0.89	0.42	0.81
NP461		pyrrolines	3-acetyl-1h-pyrroline	NA	-0.04	-0.98	0.31
NP391			1-(4-methyl-1h-pyrazol-1-yl)ethanone	bread, nut, walnut	0.55	-0.22	0.46
NP396		quinolines	4,8-dimethylquinoline	tea	-0.79	0.45	0.71
NP094		thiazolidines	4,4-dimethyl-thiazolidine	NA	0.11	-0.98	0.59
NP333	organooxygen compounds	alcohols	1-(2-furyl)-3-buten-1-ol	fruity, sweet	0.45	-0.66	< 0.05
NP132			1-pentanol	balsam, balsamic, fusel, oil, sweet, vanilla	0.59	-0.48	0.51
NP147			2,3-butanediol	buttery, creamy, fruit, fruity, onion	-0.50	0.80	0.64
NP262			2-buten-1-ol	NA	0.58	-0.53	0.67
NP455			shikimate	NA	-0.19	0.90	< 0.05
NP427		aldehydes	2-methyl-2-heptenal -like 1	almond, fatty, fresh, green, pungent,	0.69	-0.01	0.22

Code	Class	Subclass	Metabolite	Sensory (Lit)[a]	PC1 Correlation-scaled loadings[b]	PC2 Correlation-scaled loadings[b]	Pvals (FDR adjusted)[c]
				soap, vegetable			
NP560			2-methyl-2-heptenal -like 2	almond, fatty, fresh, green, pungent, soap, vegetable	-0.61	0.78	0.69
NP426			5-hydroxymethyl-2-furancarboxaldehyde	caramel, cardboard, musty, waxy, fatty	-0.39	-0.53	0.47
NP386			5-methyl-2-furancarboxaldehyde	almond, burnt sugar, caramel, maple, spice	0.35	-0.88	0.94
NP493			nonanal	citrus, fatty, fishy, fresh, grapefruit, lime, orange peel	0.33	-0.89	0.30
NP126		aryl alkyl ketones	2-acetylfuran	almond, balsam, beef, caramel, cocoa, coffee, peanut, potato, sweet	0.21	-0.96	0.79
NP478		carbonyl compounds	1-phenyl-1-pentanone	balsam, valerian	-0.84	0.48	0.34
NP042			2,5-dihydroxybenzaldehyde	NA	-0.14	-0.98	0.49
NP106			2-acetyl-3-(1-methyl-2-pyrrolyl)-1,4-benzenediol	bread, nut, walnut	0.16	0.44	0.30
NP255			1-hexene	caraway, celery, green, pepper, rooty, spicy	-0.05	-0.72	0.53
NP176		ethers	1-hexene, 4-methyl-	earthy, green, leafy, mushroom, violet	-0.66	-0.07	0.54
NP638		ketones	2-nonen-4-one	fruity	-0.54	0.80	0.42
NP428			3-penten-2-one	acetone, fishy, fruity, phenolic	-0.83	0.50	0.44
NP060			9-heptadecanone	NA	-0.31	0.81	< 0.05
NP269		sugar alcohols	galactitol	NA	0.40	-0.67	0.25
NP231	organosulfur compounds	thioethers	3-(methylthio)thiophene	NA	-0.32	0.82	0.58
NP373		thiols	3-mercapto-3-methyl-1-butanol	meat broth, roasted, spicy, sweet, vegetable	-0.37	0.93	0.54
NP299	phenylpropanoids	chalcones	2,4-dihydroxychalcone	NA	-0.07	-0.99	0.52

Code	Class	Subclass	Metabolite	Sensory (Lit)[a]	PC1 Correlation-scaled loadings[b]	PC2 Correlation-scaled loadings[b]	Pvals (FDR adjusted)[c]
NP109		cinnamic acid esters	1-(m-methoxycinnamoyl)pyrrolidine	NA	-0.88	0.42	0.39
NP134			propyl cinnamate	amber, musty, vine	-0.60	0.64	0.33
NP205			ferulic acid	NA	-0.25	0.16	0.19
NP004		flavonoids	epicatechin	NA	-0.81	0.62	0.58
NP072	prenol lipids	monoterpenoids	4-isopropylbenzoic acid	NA	-0.98	0.25	0.41
NP131			alpha-terpineol	anise, citrus, floral, lilac, mint, oil, pine, terpene, woody	-0.98	0.08	0.10
NP634			citral	citrus, lemon, mint	-0.82	0.57	< 0.05
NP039			linalool	citrus, floral, green, lavender, lemon, orange, sweet			< 0.05
NP559			p-menthan-2-one	herbal, minty, spearmint	-0.98	0.25	0.09

a=Predicted flavor attribute based on information in FooDb [17]; NA=No flavor information found.
b= Correlation-scaled loadings examine the strength and direction of the relationship between the metabolite(s) and the sensory component (*X*) metabolites shown are those which met the threshold for this analysis, |< 0.75|.
c= From ANOVA supporting variation among the n = 4 beers

Supplemental Material
Supplemental File 1

Field History

Western Rivers Conservancy (WRC): Rattray Ranch (acquired by the Western Rivers Conservancy) is in a dryland production area receiving an average annual rainfall of 8-10" (254 mm) (Gilliam County). Historically the ranch has grown soft white winter wheat every other year in a summer fallow winter wheat system, averaging yields of ~30-40 bu/acre (~2500 kg/ha) and grain protein of ~9%. Prior to growing malting barley for the 2017-18 year, the ground was allowed to lie fallow after growing a winter wheat crop in 2015-16. Glyphosate was applied to the ground in early spring of 2017, followed by three later applications of glyphosate, one which included dicamba in a tank mix. Winter barley was seeded from the 16th to the 18th of October 2017. The test plots were seeded on the 16th with a target rate of 56 kg/ha. The fertilizer used was solution 32 mixed at a 5 to 1 ratio with sulfur. The target application rate was 93 L/ha (13 kg/ha nitrogen). In the spring of 2018 a combination of broad spectrum herbicides (Patriot, 7.3 ml/ha, Treaty, 22 ml/ha, and LV6, 511 ml/ha), was applied to the barley on the 24th and 25th of April 2018. Test strips and surrounding Wintmalt field was harvested July 11, 2018. An aliquoted sample of ~500lbs (226.8kg) of each variety was collected in tote sacks for the purposes of further research by the Barley Project at Oregon State University.

Next Pint (NP): Mecca Grade Estate Malt is a dryland production area located in Madras, Oregon (45°14′8″N 120°11′6″W) receiving an average annual rainfall of 10-12" (304mm). The three advanced barley breeding lines and Full Pint were planted on April 24, 2018 within a field on rotation with soft white winter wheat. Fertilizer rates of 21% nitrogen, 11% phosphorus, 11% potassium, and 7% sulfur where applied. One application of pesticide was applied on May 24, 2018: 13 oz/ac (950ml/ha) Huskie, 0.5 oz/ac (36.5ml/ha) Affinity, 16.4 oz/ac (1198.5ml/ha) Axial, 3 oz/ac (219ml/ha) Headline (fungicide), and 6 oz/ac (438.5ml/ha) DC4 (adjuvant). Irrigation was applied at a minimum average of 1.25" (31.75mm)/week throughout the growing phase. Full Pint and the three advanced selections were harvested August 8, 2018 and aliquoted samples were

collected for the purposes of further research by the Barley Project at Oregon State University.

Supplemental File 2

Malting Protocol

Next Pint:

Malting conditions were the same for all genotypes in the set except for supplemental moisture added during the first day of germination. Supplemental moisture was provided by spraying in order to reach target moisture levels ranging from 45-51% based on results from micro-malting.

Steeping Cycle:

10 hrs Wet (12 hrs Air) 10 hrs Wet (10 hrs Air) 6.5 hrs Wet @ 16°C

Germination Conditions:

96 Hours @ 18°C

Kilning Conditions (air on)

12 hrs @ 50°C, 3 hrs @ 60°C, 3 hrs @ 65°C, 2 hrs @ 70°C, 2 hrs @ 80°C, 4 hrs @ 90°C

Kilning Conditions (grain bed)

12 hrs @ 46°C, 3 hrs @ 57°C, 3 hrs @ 63°C, 2 hrs @ 67°C, 2 hrs @ 74°C, 4 hrs @ 81°C

Genotype	Steep-Out Moisture (%)	24 Hour Germ Moisture (%)	Germ-Out Moisture (%)
Full Pint	43.67	47.32	45.19
DH131756	45.04	45.24	41.94
DH131144	44.8	45.77	42.69
DH120270	43.38	51.09	46.98

Western Rivers Conservancy:

Malting conditions were the same for all genotypes in the set except for supplemental moisture added during the first day of germination. Supplemental moisture was provided by spraying in order to reach a target moisture level of 46% for all genotypes.

Steeping Cycle:

10 hrs Wet (12 hrs Air) 10 hrs Wet (10 hrs Air) 6.5 hrs Wet @ 14°C

Germination Conditions:

96 Hours @ 16°C

Kilning Conditions (air on)

12 hrs @ 50°C, 3 hrs @ 60°C, 3 hrs @ 65°C, 2 hrs @ 70°C, 2 hrs @ 80°C, 4 hrs @ 90°C

Kilning Conditions (grain bed)

12 hrs @ 46°C, 3 hrs @ 57°C, 3 hrs @ 63°C, 2 hrs @ 67°C, 2 hrs @ 74°C, 4 hrs @ 81°C

Genotype	Steep-Out Moisture (%)	24 Hour Germ Moisture (%)	Germ-Out Moisture (%)
Wintmalt	43.98	46.35	44.54
Thunder	45.12	45.52	42.12
Violetta	44.44	45.77	43.9
Flavia	44.14	48.78	46.24
Calypso	44.39	47.78	46.53

Base Malt Research Lager Protocol

Mash:

42.28 kg malt milled with 131.1 liters of strike water for a mash temperature of 50°C. Mash held at 50°C for 5 minutes then ramped at 1°C/min to 62°C. Held at 62°C for 20 minutes then ramped at 1°C/min to 72°C. Held at 72°C for 20 minutes then ramped at 1°C/min to 78°C then pumped over to lauter tun.

Lauter:

10 minute rest after pump-over, 5 minute cloudy wort recirculation. 55 liters first wort #1 collected in kettle, 180l/h runoff speed, 65 liters first wort #2 collected at same rate with rakes at 2% rotation speed and height of 40mm. 1st sparging of 68 liters with pump at 1,200 l/h and rakes at 5% rotation and 1mm height.

40 liters additional wort collected in 2nd lautering with no rakes. 2nd sparging of 46 liters with pump at 1,200 l/h, rakes at 2% rotation speed and 70mm height. 40 more liters of wort collected in 3rd sparging with rakes at 2% rotation and 50mm height. Total of 220 liters of wort collected into the kettle with final runnings of 1.8-2.0°P.

Kettle:

Kettle volume of 220 liters @~13.1-13.3°P, added water to bring starting volume to 274 liters. Boiled for 60 minutes with calandria temperature at 104°C and pump speed of 2,500 l/h. Added 22.3 mls of Isohop (John I. Haas) hop extract at 5 minutes into boil. At 30 minutes into boil, added 12.1g Whirlfloc G (BSG) and 25.5g Yeast X (BSG) dissolved into 2 liters hot wort. At 50 minutes into boil added 103.3g Kazbek hops (BSG) (Czech). Final gravity at end of boil 11.8-12.0°P

Fermentation/cellar:

228-233 liters of cast wort was pitched with 5.6 kg yeast slurry (7.0E+08 cells/ml), 2124 Bohemian Lager strain (Wyeast Labs) (Belgian). Fermentation was carried out at 12°C for 72 hours then temperature was dropped 0.7°P per day until 10°P was reached. Beers were held at 10°P until they passed VDK, then dropped over five days to 0°C and lagered for 5 to 6 weeks. Beers were filtered through a 40x40 plate filter equipped with Seitz HS200 (Pall) filter pads at 4-8µm Relative Retention Rating. Beers were carbonated to approximately 2.7 volumes of CO2.

Supplemental File 3

Beer Sensory – Consumer Panel

Subjects:

152 participants (81 female, 71 male, 22+ years) were recruited from Corvallis, OR and surrounding communities based on results from an online survey. All participants consumed lagers/pilsners at least 2 to 3 times per month, had no food allergies, had a valid driver's license and did not work in the brewing industry or study/conduct research in fermentation science.

Samples:

All samples used for this study were prepared in the Oregon State University pilot brewery as described earlier and were stored in and dispense from stainless steel 20L keg via an 8-head mobile draft system (Micro Matic, Northridge, CA) operating at 4°C.

Testing Procedure:

Consumers participated in one testing session in the sensory testing facility at Oregon State University. Upon arrival, consumers gave written informed consent before participating in the test. A computerized test was given to all consumers in testing booths. All samples were served by licensed servers. 2-ounce samples in glasses were presented in a serial monadic fashion. Panelists were first asked an Overall Liking question. Next, panelists were asked to re-taste the beer and select from a list the best descriptors for that particular beer using CATA (Check All That Apply). The descriptors were separated into three columns: taste, aroma/flavor and mouthfeel. After trying each sample, panelists were provided with unsalted crackers and spring water to cleanse the palate while they waited 2 minutes for the next sample. After all 5 samples had been tested, panelists were asked to imagine their ideal lager and choose the best descriptors. The selections from that question were piped to the next question where they were then asked to rank the top 3 qualities of their ideal lager. Finally, panelists were asked to give their age and gender. 3 digit numeric blinding codes were used for all samples. All sample presentation orders

were randomized and balanced. Consumers were compensated for their time with a $10 gift card.

Data Analysis:
Liking data were analyzed using ANOVA with Tukey's Post Hoc HSD test. CATA data were analyzed using both Cochran's Q test and McNemara's multiple pairwise comparison. Principal components analysis and all other data analyses were performed using Compusense Cloud (Guelph, Ontario, Canada).

Supplemental File 4

Beer Sensory – Laboratory Panel

A laboratory panel consisting of 13 people (6 M, 7 F; 22 - 55 years old) with prior experience in sensory analysis was trained over 3 separate training sessions. The first day of training consisted of familiarization of the panel to the lexicon presented on the Base Malt Flavor Map (https://www.draughtlab.com/flavormaps) using appropriate aroma references, which lasted approximately 1 hour. The second day of training consisted of a two-hour training session. During the first hour, the panelists performed a blind identification task in which the aroma references from day one were presented, and the panelist was requested to identify the aroma using the flavor map. There was then an open-ended discussion about the flavor map and aroma references. For the second hour, the panel was given examples of malts and resulting malt steeps to evaluate, while referring to the flavor map. On the final day of training, the panelists practiced using the Projective Mapping method with a subset of the beers to be evaluated during the testing sessions.

During the testing sessions, panelists were given ~60 mL of beer in a 300 mL glass covered with a plastic lid. The beer was served from two 8-head draft systems operating at 4 °C and at 13 psi (Micro Matic, Northridge, CA). Beer was dispensed into a 48-oz pitcher, then poured into blind coded sample glasses ~1 hour before the start of testing, capped with a plastic lid and allowed to warm to room temperature. Each beer sample

was presented in duplicate, each with different three-digit blind codes, giving 10 WRC and 8 NP samples.

Panelists were given a 28 by 22-inch sheet of paper, on which they were instructed to place their samples based on similarity (close together) or dissimilarity (far apart). Additionally, they identified the presence of sensory attributes using the Base Malt Flavor Map, which was available to them during testing, although they could also add any attributes they saw fit. Panelists recorded their responses on the paper as well as on Chromebook tablets using Compusense software (Guelph, Ontario, Canada). For each of these sessions, Compusense was also used to randomly assign the serving order of samples for each panelist. The panelists evaluated WRC and NP samples separately on two different days. For each sample set, the panelists performed two tests, orthonasal aroma and flavor by mouth evaluation. The order of these two tests was randomly assigned to the panelists. Panelists were given new samples, with newly randomized blind codes for both of the tests.

Supplemental File 5

Malt Steep Sensory – Laboratory Panel

The laboratory panel, which consisted of 15 people (8 M, 7 F; 23 - 68 years old), was recruited and trained over 4, one-hour training sessions. Over the course of the training sessions, the panelists were shown the Base Malt Flavor Map along with food references to build a familiar sensory lexicon for the most salient attributes in hot steeped malt. The panel was also given examples of commercial base malts and asked to begin characterizing them with the attributes shown on the map. Discussion was guided by panelist responses via a Qualtrics survey (Provo, UT). During subsequent sessions, the panel was given some examples of the malt samples to be evaluated during testing. Additionally, the panel was given malt steep samples to evaluate, using both orthonasal aroma and flavor by mouth descriptors. Once the panel was comfortable with the lexicon, they were given a day to practice using the Projective Mapping method.

During the testing sessions, panelists were given ~35 mL of malt steep samples in a 300 mL glass covered with a plastic lid. The malt steep samples were prepared within 4 hours prior to testing using the protocol described in ASBC MOA – Sensory Analysis 14. The samples were kept at room temperature in a sealed jar until the testing session began and were poured into glasses roughly 20 minutes prior to evaluation. Testing methodology followed that of the beer sensory evaluation. Panelists followed the Projective Mapping procedure described earlier and were instructed to use at least 3 attributes to describe each sample. For each of the WRC and NP sets, one malt sample was presented in duplicate, so the panel evaluated 6 WRC samples and 5 NP samples.

Supplemental File 6

Sensory Lexicon

Most frequently used attributes and their descriptions used for both beer and malt steep laboratory panels

Attribute	Description/Examples
Bread	Toast, biscuit, pretzel, flour
Breakfast cereal	Grape Nuts®, Cheerios®, Bran Flakes®
Cracker	Oyster Crackers, saltines
Dough	Yeasty, PlayDoh®
Earthy	Barnyard, soil, pond water, dirt
Floral	Linalool/geraniol, clover, dandelion
Fruity	Melon, apple, citrus
Grainy	Raw barley, oats
Grassy	Green tea, black tea, hay
Sweet aromatic	Honey, caramel, toffee
Sweet bread	Graham cracker, sugar cookie
Vegetal	Corn, DMS, green vegetables

Supplemental File 7

SPME/GC-MS Method for HB-708 11/22/19 JA

Previously submitted samples (beer samples in 10-mL SPME vials) were quantitatively transferred to 20-mL SPME vials. For instrumental analysis, the samples were first incubated at 65°C for 5 min, and then the headspace volatiles were extracted at the same temperature by a SPME fiber (DVB/PDMS/CAR 50/30 µm, Stableflex, Sigma-Aldrich) for 20 min, and injected into a DBWAXUI column (30 m x 0.25 mm x 0.25 µm, Agilent) in a Trace1310 GC (Thermo) coupled to an ISQLT MS (Thermo). SPME fiber desorbed at injection port (250°C) for 3 min, and then at fiber conditioning port (270°C) for 5 min. GC inlet was operated under splitless mode during fiber desorption. The oven program started at 40°C for 4 min, ramped to 240°C at a rate of 5°C/min, and a final hold at 240°C for 0.5 min. Data were acquired under electron impact mode, with full scan of 40-500 amu at a rate of 5 scans/second. Transfer line and source temperatures were held at 250°C. Samples were not provided in replicates. One pooled QC was analyzed at the end of the run.

Supplemental Table 1 A and B: Consumer Panel (hedonics) data showing overall liking for WRC and NP beers. Summary of mean liking values (SE), Tukey's HSD and p-values. Samples means with different letters within a row are significantly different from one another at $p < 0.05$ by Tukey's HSD test.

A.

Question						HSD	
Overall Liking	6.13ab	5.95b	6.27ab	6.36a	6.18ab	0.39	0.06
(SD)	(1.62)	(1.53)	(1.57)	(1.58)	(1.61)		

B.

Question					HSD	
Overall Liking	6.34a	6.38a	6.35a	6.23a	0.37	0.72

Supplemental Table 2 A (WRC) and B (NP): Consumer Panel summary of citation rates for all attributes. Citation rate is the percentage that an attribute was selected as a descriptor. Citation rates within a row that do not share a letter are significantly different (Cochran's Q test (alpha = 0.05)).

A. WRC

Word						P-value[2]
Astringent	0.21a	0.22a	0.16a	0.16a	0.20a	0.41
Bitter	0.54a	0.54a	0.52a	0.47a	0.56a	0.39
Caramel	0.09a	0.10a	0.07a	0.09a	0.07a	0.66
Citrus	0.21b	0.33a	0.30a	0.38a	0.36a	**<0.001**
Crisp	0.49a	0.51a	0.54a	0.60a	0.61a	0.10
Floral	0.28ab	0.22b	0.27b	0.36a	0.22b	**0.01**
Fruity (non-tropical)	0.18a	0.18a	0.18a	0.15a	0.12a	0.40
Fruit (Tropical)	0.06a	0.04a	0.08a	0.07a	0.08a	0.47
Honey	0.20a	0.18a	0.20a	0.17a	0.18a	0.94
Hoppy	0.36a	0.28ab	0.32ab	0.23b	0.29ab	**0.04**
Light	0.73a	0.64a	0.74a	0.70a	0.70a	0.22
Malty	0.32a	0.29a	0.28a	0.29a	0.34a	0.55
Molasses	0.02a	0.03a	0.01a	0.04a	0.01a	0.35
Other	0.08a	0.07a	0.10a	0.05a	0.07a	0.33
Refreshing	0.44a	0.37a	0.43a	0.49a	0.43a	0.25
Sour/Tart	0.15a	0.20a	0.17a	0.15a	0.20a	0.44
Sweet	0.42ab	0.28c	0.33bc	0.45a	0.32c	**<0.001**

B. NP

Word					P-value[2]
Astringent	0.22a	0.22a	0.19a	0.21a	0.86
Bitter	0.48ab	0.54ab	0.58a	0.45b	**0.04**
Caramel	0.1a	0.09a	0.06a	0.1a	0.36
Citrus	0.36a	0.34a	0.37a	0.3a	0.48
Crisp	0.57a	0.58a	0.57a	0.58a	0.99
Floral	0.26a	0.27a	0.34a	0.31a	0.31
Fruity (non-tropical)	0.14a	0.18a	0.15a	0.14a	0.70
Fruity (Tropical)	0.05a	0.06a	0.06a	0.09a	0.48
Honey	0.26a	0.23a	0.21a	0.28a	0.35
Hoppy	0.3a	0.35a	0.32a	0.27a	0.28
Light	0.67a	0.66a	0.77a	0.72a	0.06
Malty	0.28a	0.35a	0.3a	0.29a	0.41
Molasses	0.06a	0.03a	0.03a	0.05a	0.25
Other	0.1a	0.09a	0.08a	0.1a	0.97
Refreshing	0.45a	0.41a	0.45a	0.41a	0.68
Sour/Tart	0.21a	0.19a	0.19a	0.27a	0.15
Sweet	0.43a	0.35a	0.38a	0.35a	0.40

Supplemental Table 3 A (WRC) and B (NP): Summary of significant p-values for McNamara's multiple pairwise comparisons.

A. WRC

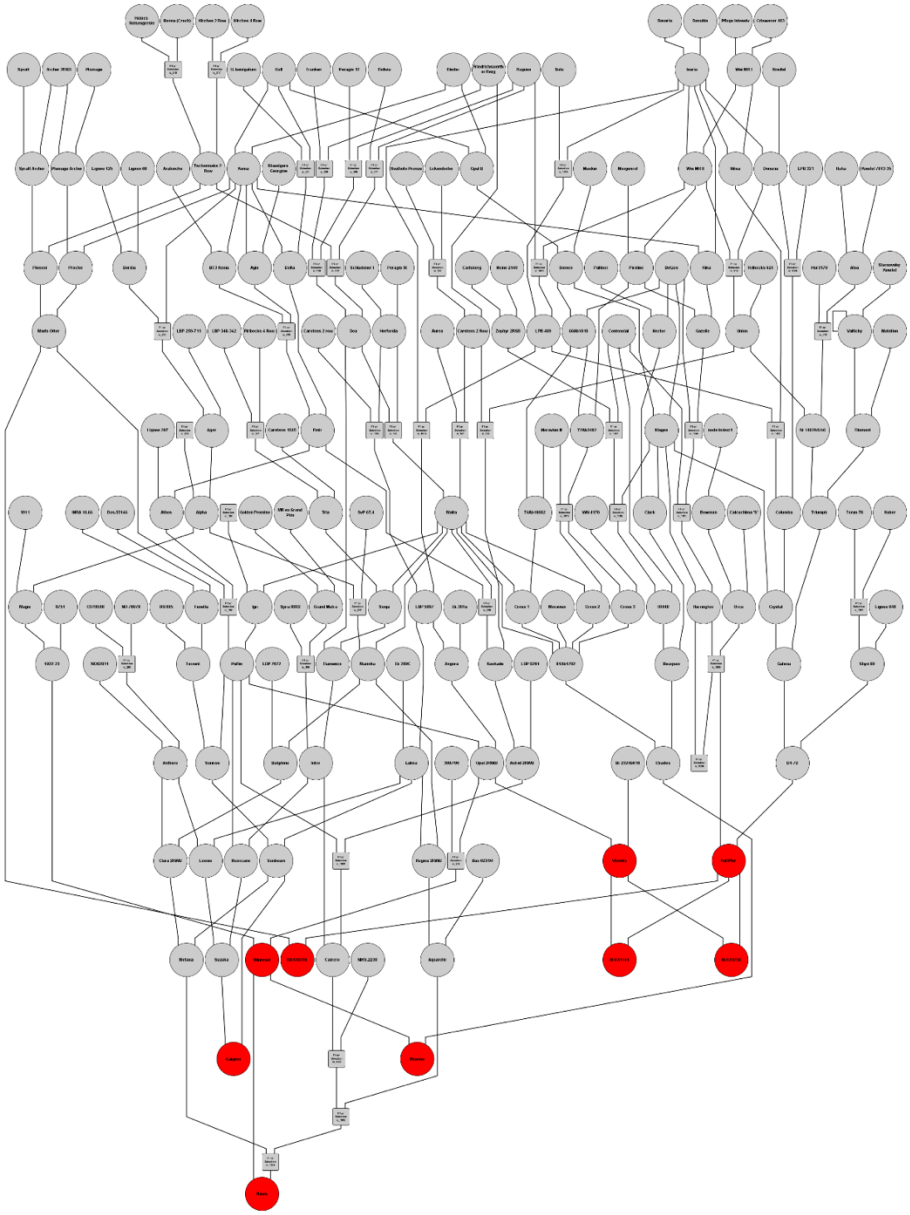
Word	Thunder vs Calypso	Thunder vs Flavia	Thunder vs Violetta	Thunder vs Wintmalt	Calypso vs Flavia	Calypso vs Violetta	Calypso vs Wintmalt	Flavia vs Violetta	Violetta vs Wintmalt
Astringent									
Bitter									
Caramel									
Citrus	**	*	***	***					
Crisp			*	*					
Floral						***		*	**
Fruity - Non Tropical									
Fruity - Tropical									
Honey									
Hoppy			**						
Light									
Malty									
Molasses									
Other									
Refreshing						*			
Sour/Tart									
Sweet	**			*		***		**	*
Thin/Watery									
Toasted				*					

* p ≤ 0.05
** p ≤ 0.01
*** p ≤ 0.001

B. NP

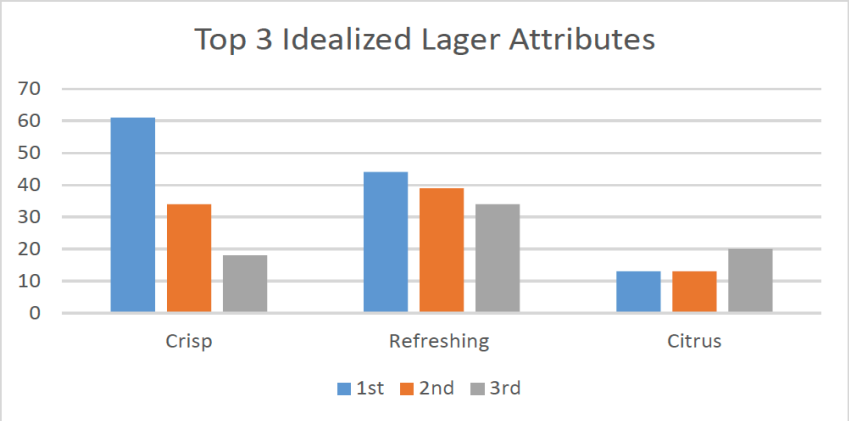
Word	DH131756 vs DH131144	DH131756 vs DH120270	DH131756 vs FULL PINT	DH131144 vs DH120270	DH131144 vs FULL PINT	DH120270 vs FULL PINT
Astringent						
Bitter						**
Caramel						
Citrus						
Crisp						
Floral						
Fruity - Non Tropical						
Fruity - Tropical						
Honey						
Hoppy						
Light		*		*		
Malty						
Molasses						
Other						
Refreshing						
Sour/Tart						
Sweet						
Thin/Watery		*				*
Toasted						

* p ≤ 0.05
** p ≤ 0.01

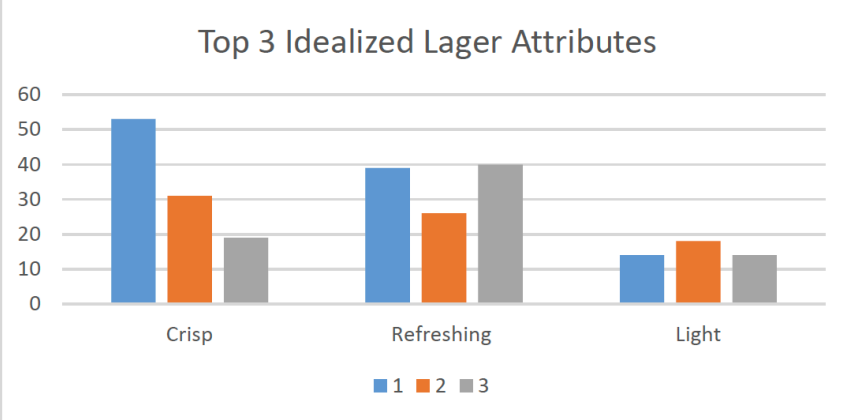


Supplemental Figure 1. Pedigrees of the barleys comprising the Western Rivers Conservancy and Next Pint sets. For enhanced visibility an interactive complete pedigree file is available at https://barleyworld.org/flavor

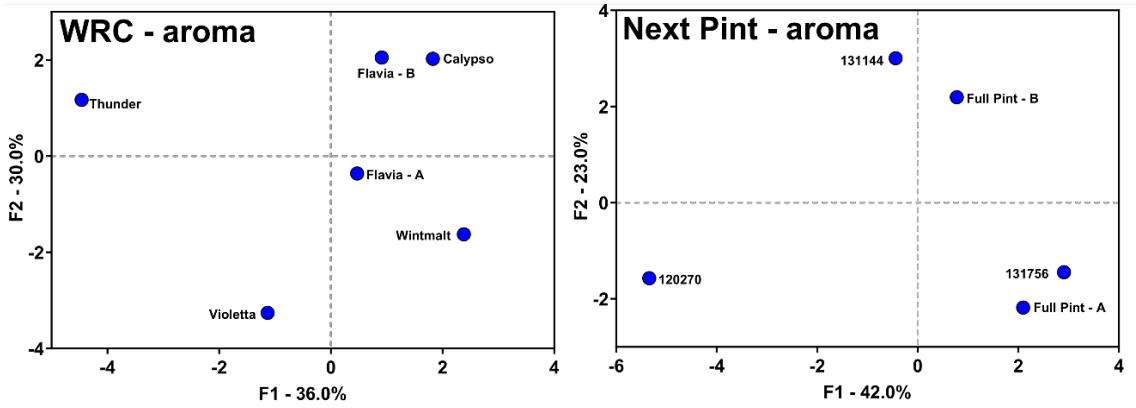
A. WRC



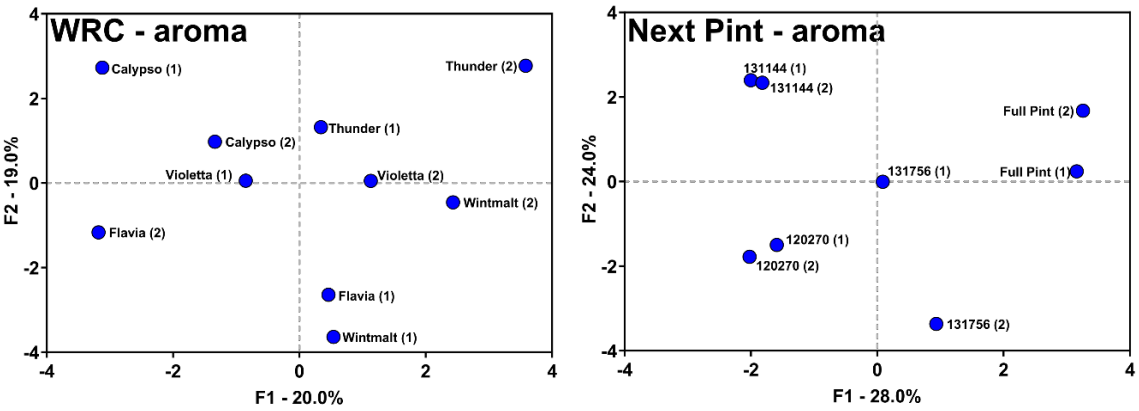
B. NP



Supplemental Figure 2 A and B: Consumer Panel data showing top three rated attributes for an Ideal Lager.



Supplemental Figure 3: Hot Steep Malt Sensory. Multifactor Analysis of coordinate data from Hot Steep Projective Mapping of Aroma (left pane: Western Rivers Conservancy; right pane: Next Pint). One malt in each set, Flavia in the WRC set and Full Pint in the NP set, were chosen to serve as an internal replicate, as designated by 1 and 2 below. Evaluating how close the replicates are to one another allows us to understand how well the panelists could identify differences and similarities between the samples.



Supplemental Figure 4: Beer Sensory. Multifactor Analysis of coordinate data from beer Projective Mapping of Aroma (left pane: Western Rivers Conservancy; right pane: Next Pint). Each beer sample was replicated, as designated by 1 and 2, to provide duplicate observations of the same samples. Evaluating how close the replicates are to one another allows us to understand how well the panelists could identify differences and similarities between the samples.

Supplemental Table 4 (provided in excel, if requested) – Raw metabolomics abundance data of NP set, Heatmap z-scores.

Supplemental Table 5 (provided in excel, if requested) – Raw metabolomics abundance data of WRC set, Heatmap z-scores.