AN ABSTRACT OF THE DISSERTATION OF

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Title: Investigating the Quality Dynamics of American Aroma Hops Intended for Dry-Hopping Beer

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Historically, brewers have used dry-hopping (a cold extraction of nonvolatile and volatile chemicals from hops into fermenting or finished beer) to increase the microbial stability and shelf life of their beer. As hoppy beer styles have gained in popularity over the last decade (2007-2017), the objective of dry-hopping has turned to imparting hop aroma and flavor to beer while minimizing bitterness extraction. To extract hop aroma into beer, brewers have been using extreme hopping rates (sometimes > 0.7 kg/hL, equivalent to more than 18 lb/US bbl), which are mostly driven by increases in dry-hopping rates. These addition rates may be unsustainable from an agronomic perspective, potentially wasteful due to beer losses, and suboptimal at efficiently extracting aroma from hops.

Therefore, the extraction efficiencies of a number of key hop volatile and nonvolatile constituents related to hoppy beer aroma and flavor were investigated over a range of commercially relevant Cascade dry-hopping rates (0, 0.2, 0.4, 0.8, and 1.6 kg/hL). It was determined that adding more hops during dry-hopping did not simply lead to increased aroma intensity but also changes aroma quality in the finished beer. Dry-

hopping rates >0.8 kg/hL had hop aromas that were more herbal/tea in quality than citrus. To maintain a more balanced hop aroma quality, the use of a static dry-hopping rate between 0.4 and 0.8 kg/hL was suggested. Also, using dry- hopping rates >0.8 kg/hL lead to diminishing returns in terms of increasing hop aroma and is an inefficient use of raw material.

From 2007-2017, Cascade and Centennial hops were the most commercially important aroma varieties to the American hop and craft brewing industries. They were very popular with US (and global) brewers because of the unique aroma and flavor they impart to hop-forward beer styles, especially during dry-hopping. However, there is no scientifically-validated method to predict beer aroma intensity and quality during dry-hopping. Many brewers rely a hop's total oil content as a measure of its aroma potential, but to date the connection between total oil content and a hop's aromatic intensity has not been proven. Additionally, the variation that exists in the hop volatile profiles and dry-hop aroma potential within these important commercial hop varieties over a given harvest year is not documented.

Over the 2014, 2015, and 2016 hop harvests a large sample of Cascade (n=51) and Centennial (n=33) hops were procured from farms throughout the Pacific Northwest (WA, ID and OR). Within each of these harvest years, significant differences were observed in the hop volatile chemical profiles and the aroma intensities/qualities that these hops attributed to beer. These results indicate that at the same static dry-hopping rate of 3.86 g/L, there were significant and measurable differences in the aroma intensity as well as the quality of aroma attributed to beer from different commercially available Cascade and Centennial samples from the same harvest year. In agreement with prior

research, it was also determined that total oil content (mL oil/100g hop) did not serve as an effective predictor of dry-hop aroma performance in beer. Instead, the concentration (mg/ 100g hop) of specific hop volatiles in hydrodistilled hop oil (geraniol for Cascade and β-pinene for Centennial) served as superior indicators of dry-hop aroma performance.

Strategies both on the farm and in the brewery were investigated as ways to promote or modify aroma quality and intensity during dry hopping. On the farm, the impact of harvest maturity on Cascade quality and dry-hop aroma potential was evaluated using a unique weekly sampling protocol, whereby, 5-6 samples were collected from the same location within a commercial hopyard over three consecutive harvest years. For this specific hopyard, hop aroma intensity (OHAI) and citrus quality attributed to the beer during dry-hopping increased as a function of harvest date. Total hop essential oil content and a number of different hop essential oil volatiles (notably geraniol) displayed a significant positive trend with harvest date. For the first time, concentrations of thiol precursors (mainly S-3-(hexan-1-ol)-l-cysteine) were observed to decrease over harvest, while the concentrations of free thiols (mainly 3-mercaptohexanol) increased. Taken together these findings suggests that for brewers to best utilize Cascade hops, early harvested hops might be better for bittering or kettle/whirlpool additions, while later harvested hops might be better for dry-hopping or aroma additions.

In the brewery, a sensory directed study on beers dry-hopped with Cascade, Centennial, and Chinook was used to evaluate the qualitative changes in the aroma of dry-hopped beers when these hops were used individually and in different blended combinations for dry-hopping. Blending hops as opposed to dry-hopping with single varieties produced the most intense aromas. In addition, specific blends of hops were

found to achieve similar aroma qualities to single varieties. Therefore, by utilizing hop blends brewers may be able to make substitutions when faced with shortages due to cost and/or quality while maintaining similar aroma profiles.

Overall, the results from these studies provide hop breeders with aromatic quality and metabolite targets for creating new / replacement hop varieties that have similar aroma profiles to these important American varieties. Growers benefit by being able to fine tune growing and post-harvest processing conditions to promote the concentrations of these hop volatiles in these varieties. Finally, this research will help brewers maximize the efficiency of aroma extraction during dry-hopping and guide the development of more sustainable techniques to better utilize this raw ingredient, improve beer quality, and obtain consistent hoppy aroma in beer.

Investigating the Quality Dynamics of American Aroma Hops Intended for Dry-Hopping Beer

by
Scott R. Lafontaine

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APPROVED:

Major Professor, representing Food Science and Technology

Head of the Department of Food Science and Technology

Dean of the Graduate School

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

Scott R. Lafontaine, Author

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CONTRIBUTION OF AUTHORS

Dr. Dan Vollmer performed the brewing trails and sensory analyses for the 2014 Cascade samples for Chapters 3 and 5. Dr. Clifford Pereira ran the multiple regression analysis for Chapter 3 and designed the efficient resolvable incomplete block design used for presenting the samples over multiple sessions throughout sensory analyses in Chapters 4 and 6. Scott Varnum harvested the weekly hop samples used for Chapter 5. Nyséos (Dr. Laurent Dagan, Stéphane Delpech, Dr. Aurélie Roland) performed free thiol and thiol precursor analyses on beer and hops for Chapter 5. Dr. Toru Kishimoto performed thiol analyses on beer for Chapter 5.

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LIST OF ABBREVIATIONS

3MH	*3-mercaptohexanol*
3MHA	*3-mercaptohexylacetate*
4MMP	*4-methyl-4-mercaptopentan-2-one*
ABV	*Alcohol by Volume*
AICC	*Small-sample-size corrected version of Akaike information criterion*
ANOVA	*Analysis of variance*
ASBC	*American Society of Brewing Chemists*
CIC	*Character impact compound*
Cys3MH	*3-S-cysteinylhexan-1-ol*
Cys4MMP	*4-S-cysteinyl-4-methylpentan-2-one*
DO	*Dissolved oxygen*
Fisher's LSD	*Least Significant Difference*
G3MH	*3-S-glutathionylhexan-1-ol*
G4MMP	*4-S-glutathionyl-4-methylpentan-2-one*
GC-MS	*Gas chromatography–mass spectrometry*
H.S.I.	*Hop storage index*
HACP	*Hop aroma component profile*
IBU	*International bitterness units*
IPA	*India pale ale*
MOA	*Methods of analysis*
OHAI	*Overall hop aroma intensity*
OSU	*Oregon State University*
PCA	*Principal component analysis*
PRESS	*Predicted residual error sum of squares*
RE	*Residual extract*
SBC	*Schwarz Bayesian Criteria*
SPME	*Solid-phase microextraction*
Tukey’s HSD	*honestly significant difference*

“Smelly”

Hops. Aromatic.

Tropical, citrus, herbal

|Beautiful canvas|

Dedicated to Llewyn. Be prepared to work hard, know that the universe always has other life plans for you (be receptive to them), and enjoy the adventure.

Chapter 1. Introduction/Background

1.1 Rise of the India Pale Ale (IPA) and its impact on the hop and beer industry

Historically hop was as minor ingredient in beer but still had a large impact on beer flavor, appearance and stability.[1] Beers were often kettle hopped by brewers to impart bitterness and noble hop aroma and bitterness ranged from 15-50 international bitterness units (IBU).[2] Over the past 10-15 years, due to the rise in demand and production of hop-forward beer styles, such as the IPA, the American craft beer market has changed the way hops are utilized in the brewing process. These beers have much more intensely hoppy chemical and sensory profiles than historical styles, and a recent survey of commercial American IPAs found that these beers can range in IBU from 30 - >100.[3]

In 2017, the overall beer market was valued at $111.4 billion with craft beer accounting for 23% ($26 billion).[4] Over a ten year period (2007-2017) the number of breweries in the United States has increased 320% (Figure 1A). Over that same timeframe craft beer production increased 218% and currently accounts for 12.7% of the overall beer market (in terms of production).[4] In 2017, India Pale Ales (IPAs) accounted 20.3% of the overall craft sales (~$6 billion).[5] From 2008 to 2017, it is estimated that craft IPA production increased 976% (based on beers produced for off premise sales).[6-7] However, this is probably a low estimate of the overall increase in production for this style over this timeframe. It is likely that IPA production has increased by a much larger amount when accounting for on premise IPA production as well as IPA production from large multi-national non-independent brewers. It is clear from these statistics that hop-forward beer styles have a significant economic impact on the brewing industry.

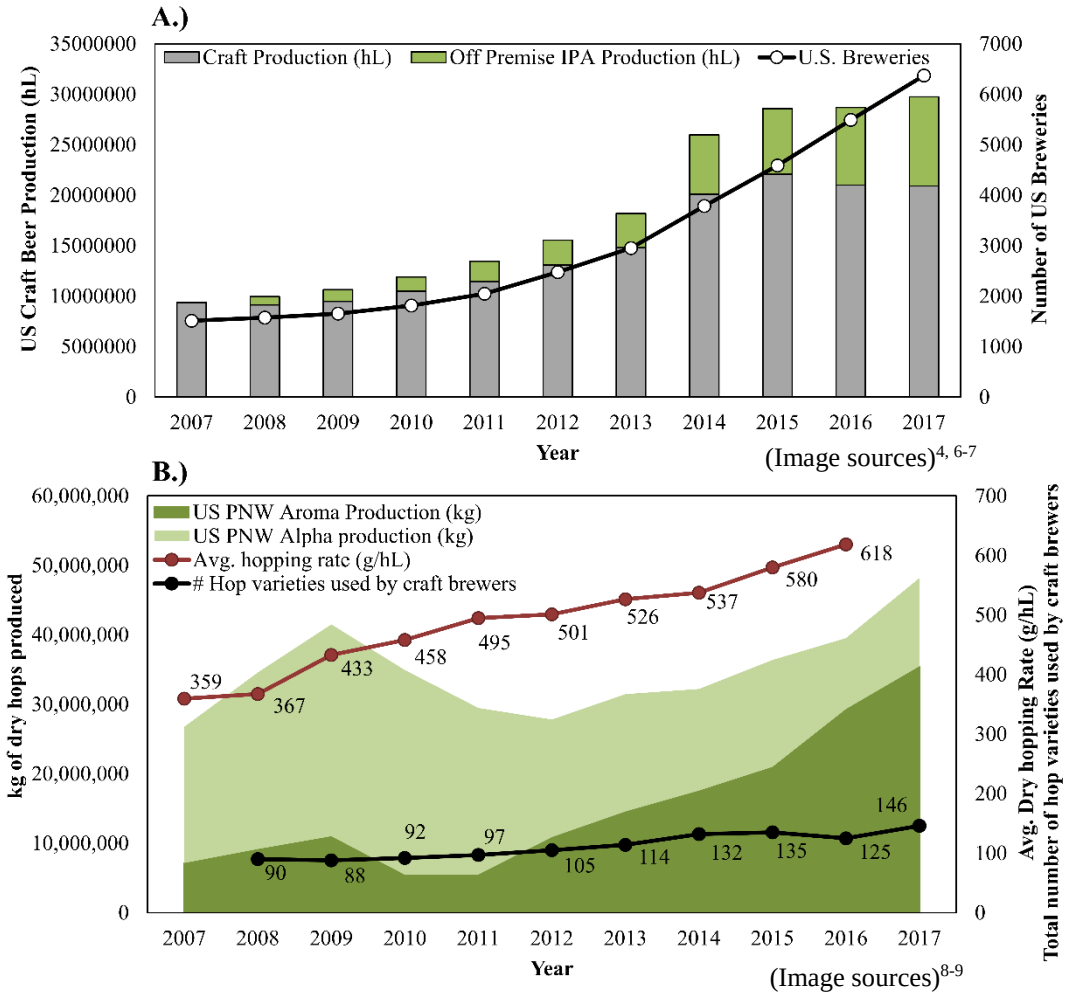
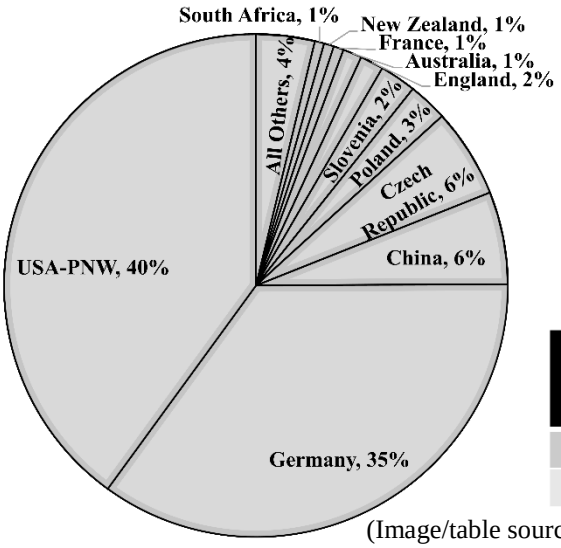
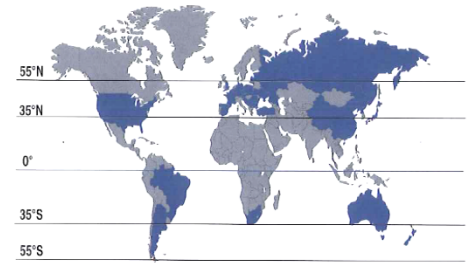


Figure 1. A. The increase in the number of breweries in the US and growth of production for Craft beer and India Pale Ales from 2007 to 2017. **B.** Shifting of hop production in the Pacific Northwest from alpha to aroma varieties as a function of the average US craft brewer self-reported hopping rate and total number of hops used by US craft brewers from 2007 to 2017.

Not surprising, this growth in production of IPA has had a profound impact on the global hop industry. The average self-reported hopping rate of craft brewers increased 72% from 2007 to 2016 (Figure 1B).[9] Hop-forward beers use ~700% more hop than used produce domestic premium brands such as American light lagers (Figure 2),[1] which still make up a majority of the American beer industry (roughly 38% based on sales).[10] US craft brewers are also using a diverse selection of hop varieties (over 140 in 2017) and the self-reported total number of hop varieties used by US craft brewers increased 62% from 2008-2017 (Figure 1B).[9] A majority of the varieties used by brewers to produce IPAs, are classified as aroma varieties (generally defined as hops not intended as a feedstock for α-acid production).[11]

From 2007-2017, global hop production has increased ~29% and the percentages of aroma to alpha varieties produced has shifted from 44:56 to 61:39 (Figure 2).[12-13] The main global producers of hop are the U.S.A. (mainly in the Pacific Northwest (PNW) Washington, Idaho, and Oregon) and Germany. In comparison to the PNW, over this timeframe, Germany saw only relatively small changes in production and shifts in total aroma vs alpha hops. However, from 2007-2017 hop production has increased by 73% in the PNW and the percentages of aroma to alpha varieties produced have almost flipped from 27:73 to 71:29 (Figure 1B).[8] Of the top nine varieties produced in 2017 in the PNW six were aroma type varieties (Figure 2). From 2007-2017, unquestionably the most important of these have been the public aroma varieties Cascade, Centennial, and Chinook. In 2017, these three varieties made up 10% of the global hops produced in 2017, while in 2007 they made up only 1.7%.[8, 12]

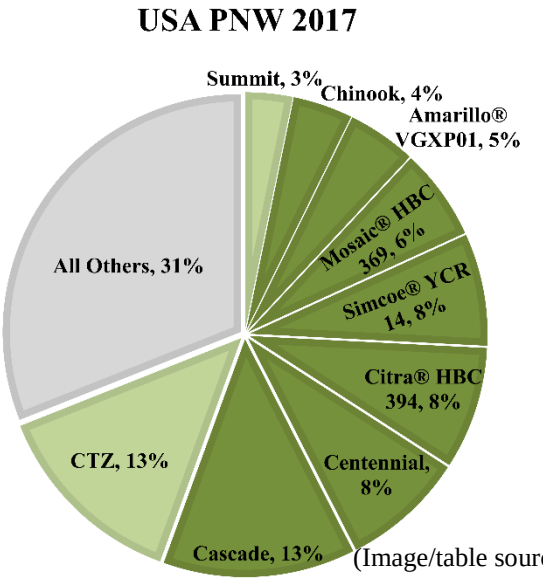




(Image source)[2]

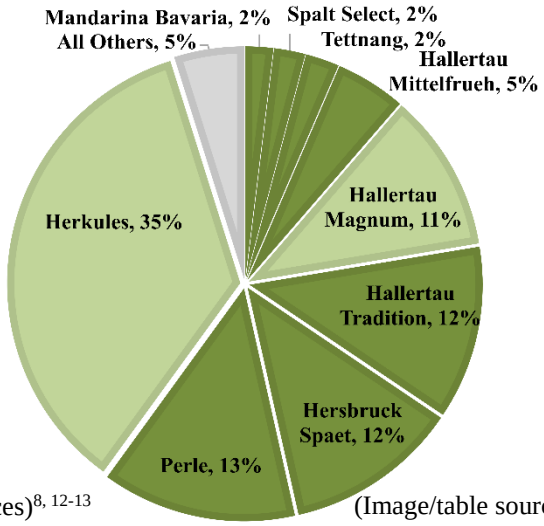
Year	Total Production (kg)	Aroma Percent of Production	Alpha Percent of Production
2007	91,583,816	44%	56%
2017	118,400,763	61%	39%

(Image/table sources)[12-13]



(Image/table sources)[8, 12-13]

Year	Total Production (kg)	Aroma Percent of Production	Alpha Percent of Production
2007	27,330,645	27%	73%
2017	47,339,905	71%	29%



(Image/table sources)[12-13]

Year	Total Production (kg)	Aroma Percent of Production	Alpha Percent of Production
2007	32,136,936	58%	42%
2017	41,555,917	49%	51%

Figure 2. Snapshot of the major trends in the global hop industry, the Pacific Northwest, and Germany from 2007 to 2017

It is clear that the demand of American aroma varieties has grown considerably from 2007-2017. Currently, the pricing model for aroma hops is based mainly on α-acid percentage and organoleptic evaluation (appearance, rub & sniff evaluations). However, the factors that dictate the brewing value of hops is dependent on how a brewer uses hops throughout the brewing process. This dissertation examines hop quality through this lens. It should be noted that this review is not intended to be an in-depth evaluation of the brewing process. There are many texts that provide excellent overviews of this industrial process. Lewis and Young[14] and Briggs et al.[15] are two examples.

1.2 Turn down bitterness and turn up aroma – The shift in how hops are utilized in 21st century

Of the three *Humulus* species in the *Cannabaceae* family, only *H. Lupulus* has value to brewers because the inflorescence of mature hop plants (hop cones) contain glandular trichomes (lupulin glands) that produce essential compounds responsible for beer flavor, aroma, and stability (Figure 3).[16] Although hops have been used to influence the microbial[17] and flavor stability [18] of beer for centuries, the main function of hops is to add aroma (blue) and flavor (red) to beer (Figure 3). From a flavor perspective, over the twentieth century, brewers were mainly focused on how to utilize hop and hop products based on bitterness potential. Verzele and De Keukeleire[19] have extensively reviewed the chemistry of hop bittering acids. The main source of bitterness in hops is determined by concentrations of humulones (α-acids), which range from roughly 2-17 w/w% (Figure 3).

While humulones are not very soluble or bitter,[20] they undergo a heat catalyzed isomerization to form iso-humulones which are considered the primary drivers of hop

Major components influencing flavor in beer	Concentration (% w/w)
α-acids [2]	2-17
Humulinones [21-22]	0.1-0.5
Polyphenols/ Tannins [2]	3-6
Hulupones [22]	0.05, 0.5
β-acids [2]	2-10
Essential Oil [2]	0.5-4.0
Aroma Precursors (Thiol precursors [23], aglycons [24], geranyl esters [25])	0.013-0.053 (0.006-0.002, 0.0004-0.015, 0.003-0.036)
Monosaccharides (glucose, fructose) [26]	2-4 (0.38-0.55,0.32-0.44)
Organic Acids (succinic, malic, citric) [26]	?
Metal ions (Fe, Mn, Zn, and Cu) [27]	0.03-0.06
Lipids and fatty acids [2]	1-5
Hop Enzymes [28-29]	?
Overall importance for hop derived bitterness	Overall importance for hop derived aroma

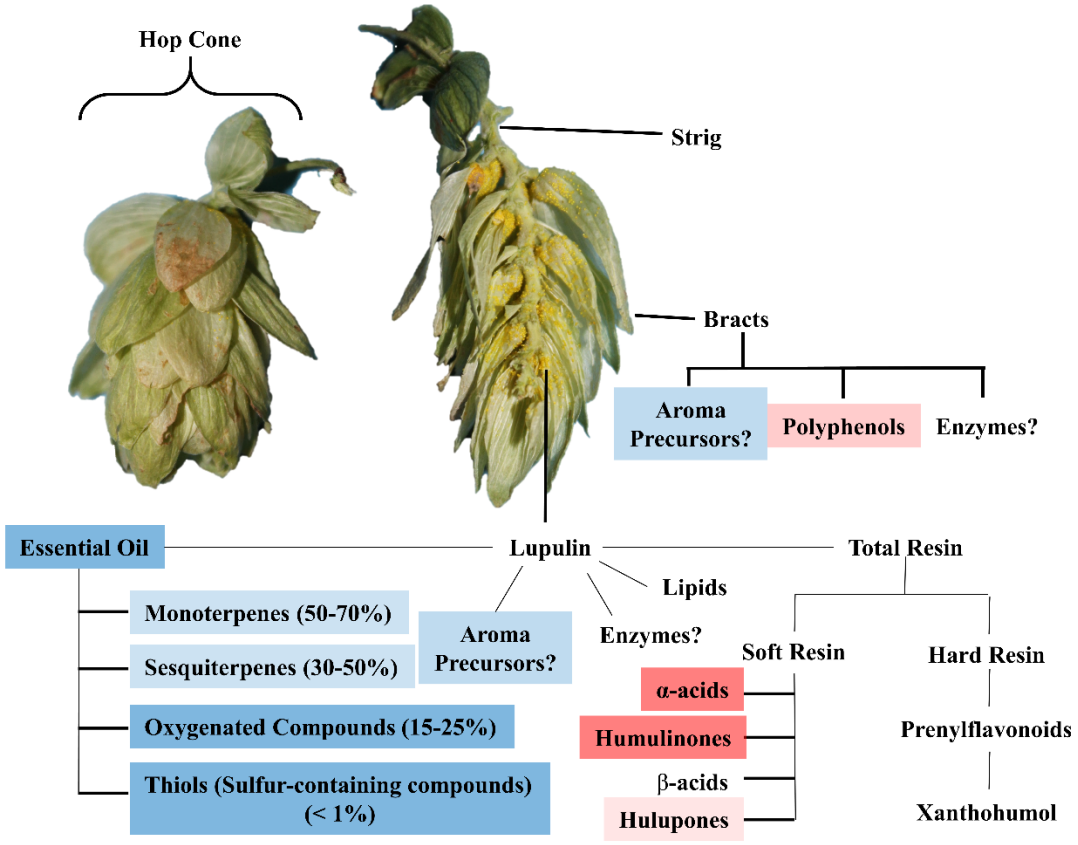


Figure 3. Factors impacting hoppy beer aroma and flavor. Anatomy and composition of the hop cone adapted from Benitez et al.[2] and Biendl et al.[1]

derived beer bitterness.[3, 30-31] Interestingly, iso-humulones have also been recently identified as potential compounds for the prevention and treatment of (chronic) liver disease.[32] Depending on the pH of wort (pH ~5.2) or beer (pH ~4.2-4.8) iso-humulones are also 28-250x more soluble than humulones.[1] While a number of factors can impact the isomerization of humulones (such as the type of hop or hop product, pH, freshness of the hop product, strength and composition of the wort and the hopping rate) time and temperature are the main drivers.[1]

When using whole cone hops or pelletized hops, bitterness utilization is mainly adjusted by modifying the timing of hop additions throughout the brewing process, the temperature at which hops are added, and the contact time (Figure 4). Throughout the twentieth century, the most popular technique employed to add hops during the brewing process was during wort boiling or "kettle-hopping". If hops are added at the beginning of boiling the expected utilization for whole cone hops and pellets range from 24-40% for a 60 minute addition (depending on the kettle design, temperature and wort strength).[1, 33-34] The use of hop products in the kettle, such as pre-isomerized extract, can achieve utilizations of hop derived bitterness of up to 70 %.[35]

$$\text{Hop utilization \%} = \frac{\text{Isohumoulones in wort/beer } (\frac{\text{g}}{\text{hl}})}{\text{Humulones added to wort } (\frac{\text{g}}{\text{hl}})} \times 100$$

Although, oxygenated terpenoids[36-40] and oxygenated sesquiterpenes[41] have been shown to be important for kettle-hop aroma and lead to descriptors such as 'floral' and 'spicy'. Typically, beers that have only been kettle-hopped have very low hop aroma because much of the aroma volatiles are lost during this early addition to volatilization, oxidation or mechanical removal.[1]

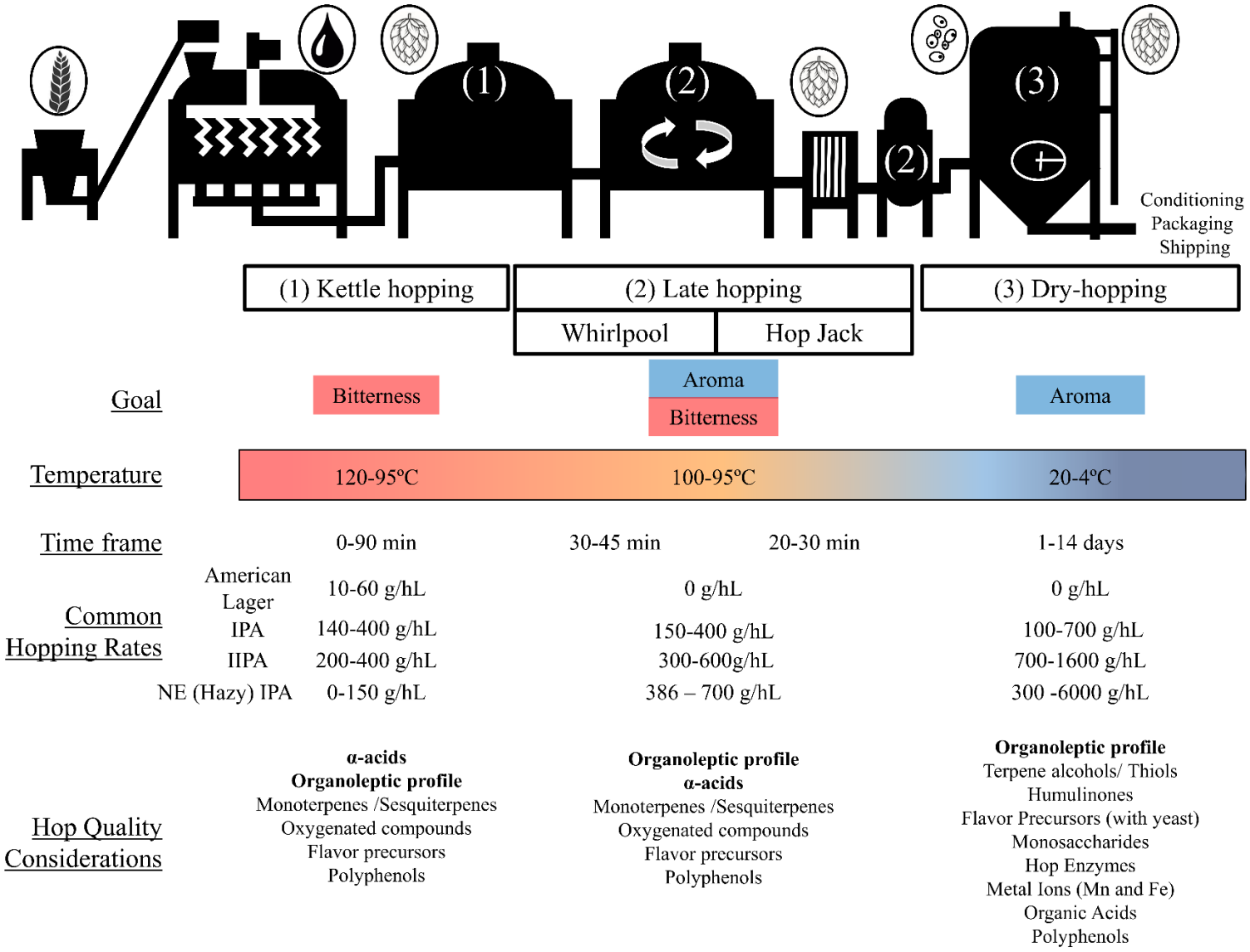


Figure 4. Overview of the three main hop additions used by brewers, the estimated hopping rates for different beer styles,[1, 34] and hop quality considerations for each hop addition

As hops are added later, at the end of boiling (late-hopping) or at lower temperatures in the whirlpool (whirlpool hopping) or hop back (hop back hopping) less utilization occurs (5-20%) and more of the aroma volatiles are able to be retained.[1, 33] To further drive down utilization some brewers also cool wort on the way the whirlpool separator or hop jack (75-95°C). These additions allow brewers to impart hop aroma while reducing the hop's bitter contribution. Therefore concentrations of hop volatiles[36, 39-40, 42-43] and aroma precursors, such as thiol[23] and geraniol[44] precursors, are important considerations of hop quality for these additions as they have an impact on the final beer aroma. Particularly, if precursors are added prior to primary fermentation, the bound volatiles can be liberated by yeast enzymatic activity during fermentation and lead to increased aroma.[23-24, 45-46] Although, hop cultivar has little impact on the aroma imparted from kettle additions.[40] Cultivar has a significant impact on the aroma imparted to beer from late or whirlpool hopped additions. Beer hopped with Hallertau Mittelfrüh during these additions was characterized as 'noble', 'spicy' and 'floral'.[38, 40, 47] However, beer hopped with Simcoe during late or whirlpool additions are more 'citrusy' and 'tropical fruit' in character.[40]

During the recent 5 - 10 years, brewers have been brewing beer styles with very intense hop aromas. To achieve this, brewers have turned to "dry-hopping" additions. Dry-hopping is defined as the cold extraction (4-20°C) of non-volatile and volatile chemicals from hops into an alcoholic solution (Figure 4).[48] While dry-hopping was used historically by brewers to increase both the microbial and flavor stability of beer, the average dry-hopping rates that U.S. brewers are using to produce hop-forward beers are almost 2x greater than historical rates reported.[21, 34, 49-50] Due to these extreme rates the

hop aroma imparted to beer during dry-hopping is distinctly different than the other hop additions and has been classified as ‘tropical’, ‘citrusy’, ‘pine’, ‘dank’ depending on the hop variety being used.[42, 44, 51]

While studies have shown that there may be overlap in the volatiles that are important for both late- and dry- hop additions,[40, 52-53] attempts to define harvest indicators of hop aroma potential for hops intended for dry-hop additions have been inconclusive. This is because there are a number of different dry-hopping techniques and parameters that influence the extraction rate of hop volatiles such as varietal differences,[53] temperature,[54] static vs dynamic extraction systems,[55] scale,[56] contact time,[57] and yeast interactions/bio transformations.[58]

It should be mentioned that there are other hopping additions that brewers employ such as mash hopping or first-wort hopping, although there is some evidence that these additions might have only small effects on flavor stability.[59] The impact of these additions on final beer flavor in hoppy beer is likely negligible as compared to the contributions of kettle-, whirlpool- and dry- hop additions on beer flavor (for equivalent hopping rates).

1.3 What is in hop oil that is influencing hop aroma in beer?

In general, the total essential oil fraction of hops, ranging from 0.5 - 4.0 w/w % depending on the variety, is recognized as the main source of hop aroma (Figure 3). Recently, Rettberg et al.[47] published a comprehensive review on the current understanding of the drivers of hop aroma and their subsequent analysis in hops and beer. Historically, the main analytical indicator that the brewing industry has relied on to gauge the aroma intensity and quality of hops has been total oil content. However, Vollmer et al.[4] recently observed that total oil content is not an effective indicator of hop aroma

potential during dry-hopping and suggested that the composition of hop essential oil might be more important.

The composition of hop essential oil is estimated to be made up of over 1000 compounds.[48] The volatile chemicals that compose hop essential oil, which have been shown to be important for beer flavor, can be split into three general groups: hydrocarbons (mainly monoterpenes and sesquiterpenes), oxygenated compounds, and sulfur containing compounds (Figure 3).[47-48] There are numerous studies highlighting the compositional differences that exist between the volatile fractions of different hop varieties[23, 36, 43-44, 60-61] as well as the impact that hop maturity[16, 62-65] has on the development of the volatile fraction.

Monoterpenes and sesquiterpenes make up 70-80% of hop essential oil depending on the variety and have been characterized by a number of studies to be important for raw hop aroma (Figure 3).[66-68] The work by Wang, et al. [69] provides great insight into the early developmental biosynthetic and enzymatic pathways that drive terpene synthesis in hops (although only 4 weeks after onset of flowering) and found that β-myrcene originates from geranyl pyrophosphate, β-caryophyllene, α-humulene, β-farnesne originate from farnesyl pyrophosphate, and limonene originates from neryl diphosphate.

However, due to their physical chemical properties monoterpenes and sesquiterpenes are not typically not found in concentrations above their detection thresholds (β-myrcene = 350 μg/L, α-humulene = 450 μg/L, and β-caryophyllene = 230 μg/L)[70] and thus are unlikely contributors to dry-hop aroma. If hops are stored in aerobic conditions, β-myrcene can undergo autoxidation forming cyclic products (such as α-pinene and β- pinene) and monoterpene oxides (such as linalool, geraniol, geranial and

neral).[47] Some of these volatiles have been found in beer at or above their reported detection thresholds.[36] Oxygenated derivatives of sesquiterpenes, such as the humulene epoxides and caryophyllene oxide, have also been shown to increase during wort boiling[71] and hop oxidation[37, 68, 72]. While the concentration of these oxygenated derivatives has been shown to correlate with increases in noble or kettle hop aroma[71] the impact they have on dry-hop aroma is unclear.

Numerous studies have highlighted the importance of monoterpene oxides (particularly, linalool, geraniol, nerol, and α-terpineol) on "kettle", "whirlpool" and "dry" hop aroma in beer.[36, 39-40, 43, 52-53, 73-76] Other oxygenated compounds have also been identified as possible contributors on hop varietal aroma such as methyl heptanoate[25] and isobutyl isobutyrate[53]. Though, linalool in particular is regarded as one of the most important hop volatiles and has been recognized as the first hop volatile to be measured above its threshold even when just "kettle" hopping.[1] The detection thresholds of the two stereoisomers of linalool, R-linalool and S-linalool, are 3 μg/L[77] and 180 μg/L[74] respectively. Hops contain ~94% of R-linalool (depending on the variety) and dry-hopping has been shown to have little impact on the isomeric ratio of linalool that is transferred to beer.[25] However, wort boiling and storage have been shown to have a greater impact on isomerization of R-linalool to S-linalool.[74]

Overall, identifying the contribution of specific monoterpene oxides to beer aroma has proved extremely complicated. Although a number of these volatiles exist in hops[68, 78], throughout the brewing process, fermentation, and storage of beer, many reactions (Figure 5) and physical processes can change their concentrations. If hops are added prior

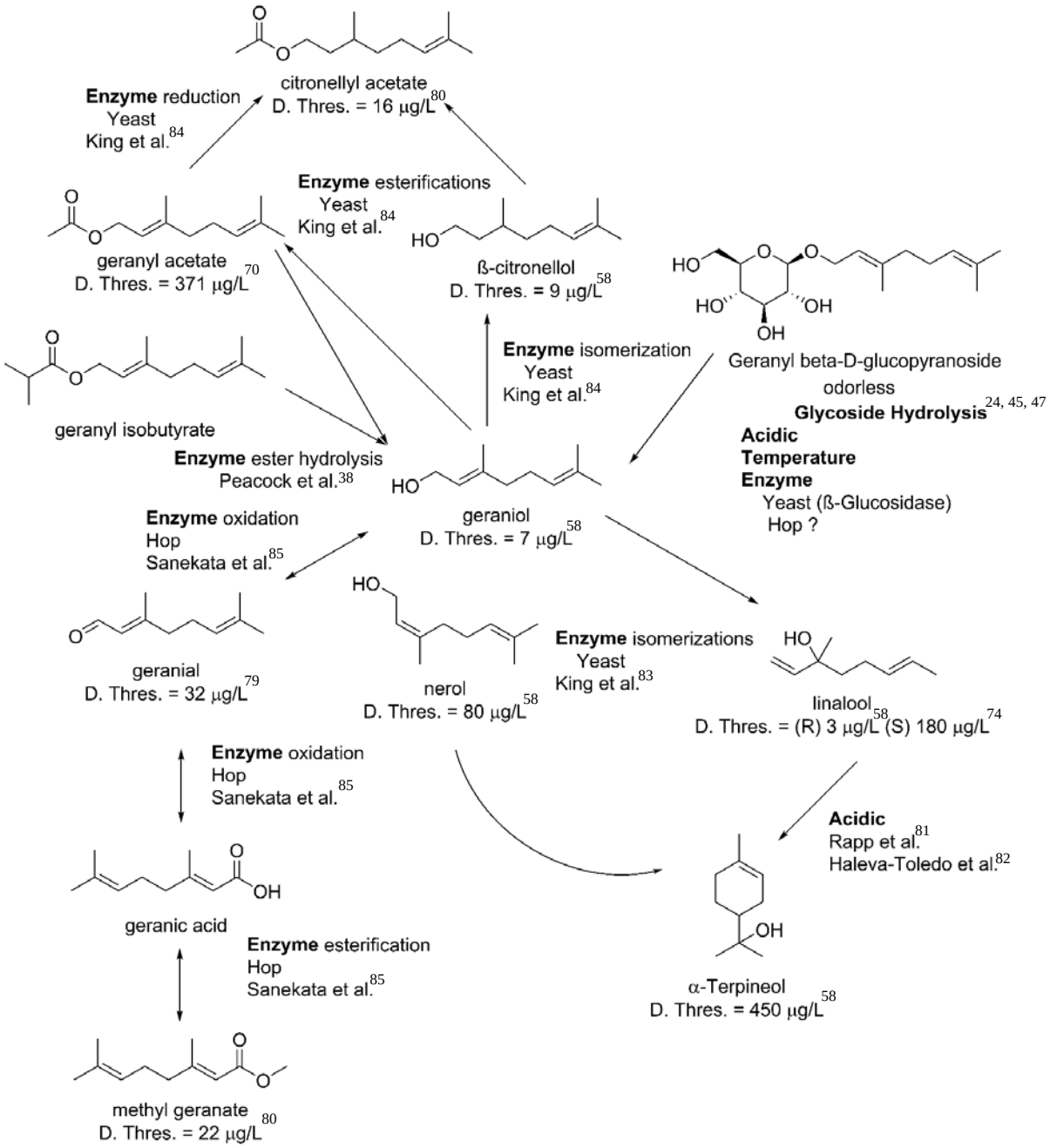


Figure 5. Reported detection thresholds and reactions that are associated with geraniol biosynthesis in hops, yeast enzymatic activity during fermentation, or influenced by environmental conditions (i.e. temperature and pH) (D. threshold - detection thresholds in water and beer)

to fermentation or in the presence of yeast, depending on the yeast variety and conditions of fermentation (i.e. temperature) these volatiles can undergo a number of different biotransformations. For example, Peacock et al.[38] showed that geranyl esters (ex. geranyl acetate and geranyl isobutyrate) can be hydrolyzed to form geraniol. However, King et al.[84] has postulated that some yeast varieties might esterify geraniol back to geranyl acetate and possibly reduce geranyl acetate to citronellyl acetate. Another study by King et al.[83] proposed that yeast can also isomerize geraniol to linalool and that both linalool and nerol can be isomerized to α-terpineol. Under acidic conditions in both model wine[81] and citrus fruit[82, 86] solutions linalool has also been shown to isomerize to α-terpineol, which might impact the flavor stability of hoppy aroma throughout distribution and storage.

Aglycons (particularly, geraniol, linalool, α-terpineol) can also be liberated from odorless glycosides via enzymatic activity, acidic conditions, or high temperatures.[24, 45, 47] However, Sharp et al.[45] and Cibaka et al.[24] recently showed that hop glycosides likely have only a minor contribution on the overall hoppy aroma in beer. Also recently, Sanekata et al.[85] proposed that hop enzymatic activity is responsible for the formation of high concentrations of geranic acid in Sorachi Ace. When considering the detection thresholds of these different volatiles (Figure 5) it is apparent that shifts in their concentrations can have profound impacts on hop aroma quality in beer.

To maximize hop aroma potential, one way brewers could utilize hop volatile and precursor concentrations is to decide how to use different hop varieties for different hop additions. For example, Takoi et al.[44] recently identified two potential groupings; ‘geraniol-rich hops’ (such as Motueka, Bravo, Cascade, Citra, Mosaic, Sorachi Ace, etc.)

and ‘geraniol precursor dominant hops’ (such as Vic Secret, Comet, Hallertau Blanc, Polaris, Amarillo, and Summit). By adding geraniol precursor dominant hops prior to or during fermentation in “kettle”, “whirlpool”, or “dry” hop additions, yeast can convert higher threshold volatiles into lower threshold volatiles. However, geraniol-rich hops might be better suited for dry-hopping post fermentation as there is higher concentrations of potent volatiles to extract. This approach was supported by the observations by Lafontine et al.[61], which found that Cascade dry-hop quality was positively described by geraniol concentrations.

Due to the extremely low odor detection thresholds of thiols (ng/L)[36, 87-88] minor changes in thiol concentrations can have a large impact on beer aroma. However, due to the very low concentrations of thiols in beer (ng/L) and in hops (ng/g), the complexity in measuring sulfur containing compounds has limited much of the work performed on these analytes until relatively recently. Some sulfur containing compounds (such as, dimethyldisulphide, S-methylthio-isovalerate, and S-methylthiohexanoate)[87] have been related to unpleasant aromas in hops such as “onion” or “garlic”. Though, the influential positive role of polyfunctional thiols (mainly 4-methyl-4-mercaptopentan-2-one (4MMP), 3-mercaptohexylacetate (3MHA), and 3-mercaptohexanol (3MH)) in hop and beer aroma has been highlighted in several papers.[89-94] Thiols can exist in hops as thiol precursors and as free thiols.[23, 93]

Recently, Roland, et al. [23] suggested that the form (free vs bound) thiols that exist in hops may influence how a brewer should use hops throughout the brewing process to maximize their potential. Similar to using the ratio of free geraniol to geraniol precursor concentrations to time hop additions proposed by Takoi et al.[44], the study by Roland et al.

recommended that hops with higher thiol precursor concentrations (i.e. Saaz, Hallertau Perle, and Calypso) should be used in “kettle” and “whirlpool” additions to maximize their potential. This is because the free thiols can be liberated from precursors during fermentation via yeast β-lyase activity (Figure 6).[46] Hops with higher free thiol (i.e. Bravo, Citra, Hallertau Cascade, and Simcoe) concentrations should be used for dry-hopping as they contain greater quantities of free volatiles that can be extracted during dry-hopping. However, thiols are highly reactive and as shown in wine,[95] oxidation reactions can lead to the formation of odorless disulfides throughout shelf life thereby reducing the impact of thiols on hoppy beer flavor[93]. This again highlights the difficulty around trying to define general analytical markers of aroma hop quality and shows that the timing of hop additions during the brewing process is a major consideration.

If hops are added during active fermentation, physical processes also have a significant impact on hop volatile concentrations and significant losses of monoterpene oxides and monoterpenes have been shown to occur due to the stripping effects of CO_2, adsorption onto yeast cells, and/or the partitioning into beer foam which leads to significant losses of hop aroma.[84] Throughout storage in bottles, crown liners also have been shown to lead to significant adsorption of hop volatiles.[96-97] These factors should be considered when trying to decide an appropriate time to dry-hop and/or promote the shelf-life stability of hop aroma in packaged beers.

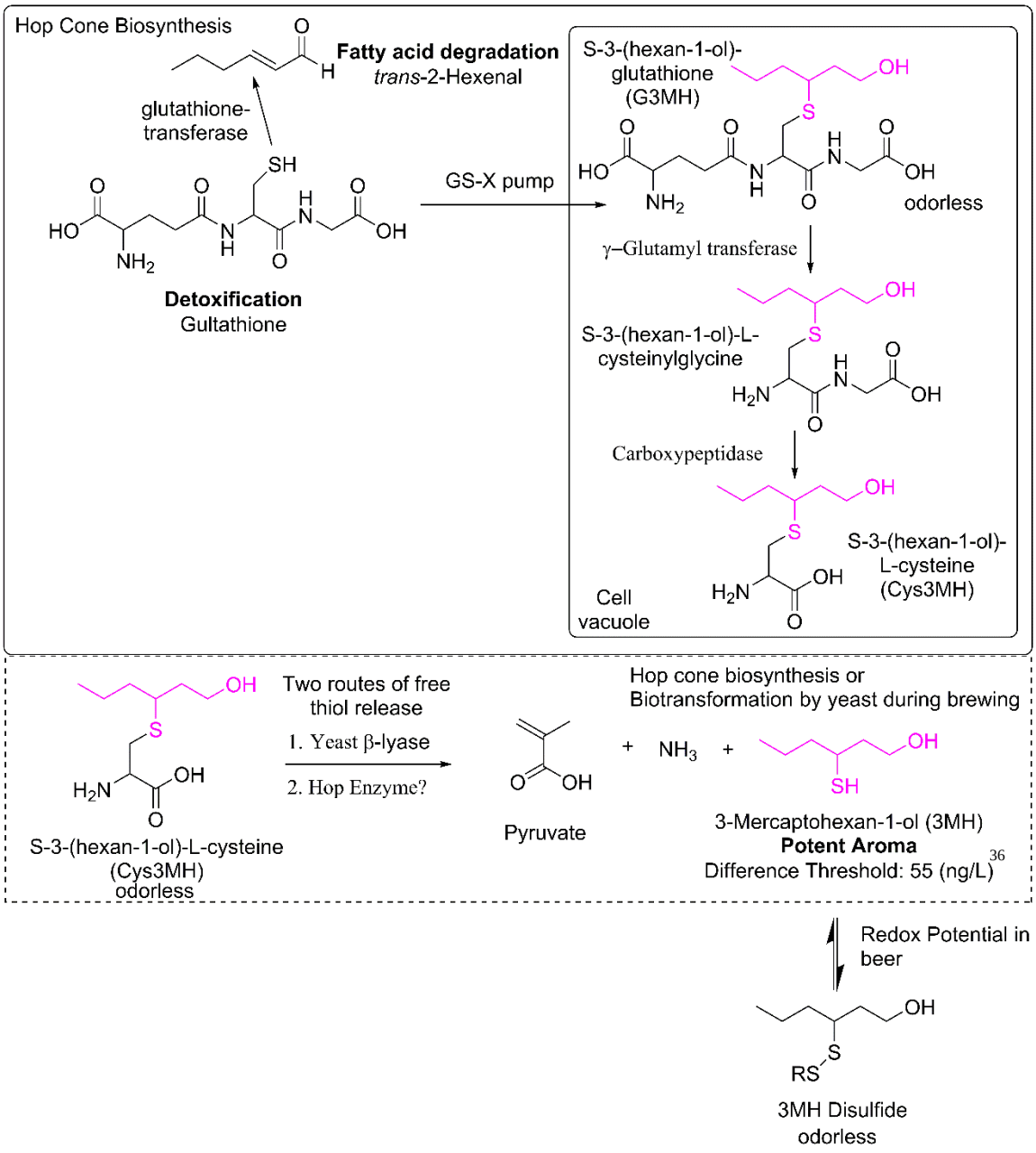


Figure 6. Possible thiol biosynthesis in hops based on the proposed biosynthesis by Kobayashi et al.[98] and Wüst[99] in wine grapes, release of thiols from precursors via yeast β-lyase activity[46], and redox potential of thiols during beer storage[95]

Some of the first attempts to link hop volatiles in hydrodistilled oil to changes in beer aroma were performed by Nickerson et al.[78] and Engel et al. [100]. These studies developed the hop aroma component profile (HACP) specifically for late- and dry-hopped beers. The HACP was comprised of 22 volatiles found in hydrodistilled hop oil that were thought to be important for hoppy beer flavor. While their approach was unique, the low sample size in these studies (n=3) made it difficult to identify grower or brewery adoptable relationships between hop volatile concentrations and hop aroma in beer. These studies also did not address the amount of variation that exists between the aroma potential of different commercial samples from the same variety.

Although considerable research has been performed on investigating extraction rates of hop volatiles into beer under different parameters,[40, 53, 101] few studies[102-103] have considered the amount of chemical variation that exists within single hop varieties and even fewer[61, 104] have considered the variation in the aroma intensity and quality attributed to beer during dry-hopping for a given hop variety. Recently, Lafontaine et al.[61] showed that dry-hopping at a rate of 3.86 g/L led to significant and measurable differences in the aroma intensities and qualities attributed to beer from different commercially available Cascade and Centennial hops procured from within the same harvest year. This same study suggested that hop volatiles (geraniol for Cascade and β-pinene for Centennial) serve as superior indicators of dry-hop aroma quality as compared to total oil. As the drivers of dry-hop aroma are better understood the impact of agricultural factors on aroma hop quality should be reevaluated.

1.4 Agricultural factors that influence hop quality

While the monoterpene/ sesquiterpene, thiol, oxygenated terpenoid are largely driven by varietal differences,[23, 36, 43-44, 60-61] agricultural factors have been shown to have a significant impact on hop quality. Due to day length requirements (vegetative growth only proceeds after daylight hours >13hr) the top ten commercial hop growing regions exist between 35º and 55º latitude in both hemispheres (Figure 2).[2] Hops are perennials and if well maintained, rootstock can produce for up to 25 years. However, rootstock age and health has been shown to have an impact on hop quality.[105]

Soil nutrients also have an impact on yields and quality.[1] Therefore, it is essential for farmers to fertilize and maintain an optimal nutrient balance. Specifically, nitrogen is one of the key nutrients that dictates plant health and is applied usually in the form of urea or calcium ammonium nitrate at a rate of 45-68 kg (100-150 lbs) of nitrogen per acre per year.[1, 106] However, this application should be carefully monitored because it can have a direct impact on disease pressure as well as hop chemistry.[107] Nitrate levels in hop cones have been reported to be ~4410-9900 mg/kg depending on the variety and are impacted by fertilizer application rates.[108] This same study found that extraction rates of nitrates were ~75% during dry-hopping. Therefore, at high dry-hopping rates the impact of nitrates should be considered. Interestingly, a number of studies[109-112] have highlighted the role of nitrates in increasing blood oxygen uptake and Bailey et al.[109] observed that an increased dietary NO_3^- intake has the potential to enhance exercise tolerance during longer term endurance exercise. Elevated nitrate concentrations in hop forward beers should be investigated as a reason linking beer consumption to exercise.[113]

In general, after winter, old growth and the first shoots are pruned (either chemically or mechanically) to prevent disease as well as synchronize growth. In the spring as new shoots emerge, the strongest (2-3) are trained onto wire or twine supported by a trellis structure. These shoots have hooked hairs which allow the bine to climb and at peak conditions can grow up to 30cm (~1ft) in length a day.[1] Although some commercial dwarf varieties exist, in general, in the PNW these trellis structures used to grow hops are generally to 5.5-7.0 m (18-23 ft) (Figure 7A). Lateral branches emerge from the bine and as the day length shortens from July to August in the PNW, hop cones develop on these branches.

Although, climatic conditions and regional differences have been shown to impact hop quality.[102-103] Growing factors (such as ripening time) and processing factors can be controlled by famers and have a significant impact on the development of secondary metabolites essential for the brewing process. Historically, farmers have used dry matter content of green cones and the concentrations of humulones (α-acids) contained in the soft resins of hops to determine when to harvest.[65] With the exception of some hop varieties containing high amounts of humulones, the concentrations of humulones peak at ~20-22% dry-matter.[65] Therefore, the bittering potential of hops is reached fairly early in the harvest window for most varieties.[62-65, 114]

However, total oil content and hop volatile concentrations are significantly impacted by ripening time.[62-65, 114] Recent studies Bailey et al. [62], (on Hallertauer Mittelfrüh), Sharp et al. [114] (on Cascade and Willamette), Lafontaine et al. (on Cascade) and Matsui et al.[64] (on Saaz) have shown that on the bine ripening time has a significant impact on the monoterpene and terpene alcohol development of hops as well as their

Figure 7. From bine to bale - Highlighting the significant steps of hop harvest. **A.** Fully grown hop bine ready to be harvested **B.** Harvested hops in a commercial kiln **C.** Hop piles conditioning **D.** Baled hops on their way to cold storage.

potential to influence beer aroma. In general, these studies showed that later picked hops have significantly higher total oil contents, higher concentrations monoterpene oxides as well as free thiols and imparted more intense aromas to beers. This highlights that later picked hops might be better utilized in dry-hopping additions, while earlier harvested hops might be better suited for kettle and whirlpool hop additions.

Very few studies have considered the agricultural factors which influence the development of polyfunctional thiols and sulfur containing compounds in hops. Most of the studies that have identified these compounds in hops and beer have largely focused on the impact of hop variety,[23, 60, 89, 92] and there has been very little work done to investigate the impact of hop harvest factors on the concentrations of sulfur containing components. Kishimoto, et al. [90] showed that 4MMP concentrations in hop varieties (i.e. Perle and Nugget) grown with copper-containing fungicides in Germany had reduced concentrations as compared to the same varieties grown without those fungicides in the U.S. Kammhuber, et al. [87] (on Cascade, Mandarina Bavaria, Hallertau Blanc, Huell Melon and Polaris) identified some sulfur analytes (dimethyldisulphide 2, *S*-methylthio-isovalerate 5, and *S*-methylthiohexanoate) that might be responsible for the onion garlic note (largely perceived as negative on hop quality) in late harvested hops. However, this study did not consider the impact of harvest maturity and of these analytes on beer aroma.

Recently, Lafontaine et al.[63] was the first to show that thiol precursors are significantly negatively correlated with the length of ripening time and dry matter content, while free thiols were positively correlated (Figure 8).[63] The development of

these analytes during on-bine maturation in hops was opposite to that found for grapes, where the concentrations of thiol precursors reached a maximum later in the harvest window. [98, 115] The following biosynthesis for *S*-cysteine conjugates of 3MH and 4MMP was proposed by Kobayashi et al.[98] and Wüst[99] in wine grapes (Figure 6). A glutathione-cysteine conjugate of a polyfunctional thiol is created when glutathione transferase reacts with glutathione and an unsaturated α, ß-unsaturated carbonyl compound. The GS-X glutathione conjugate pump then actively transports this glutathione-cysteine conjugate into the cell vacuole, where a peptidase in the vacuole further cleaves the glutathione moiety and yields the specific *S*-cysteine conjugate.

Prior to the study by Lafontaine et al. the only proposed release of thiols from these cysteine-conjugates in hops and beer was thought to be due to yeast β-lyase activity during fermentation.[46] Identifying this genetic pathway could be a useful tool for hop breeders and may help explain some of the varietal differences observed in the concentrations of free thiols and thiol precursors. Results from this study provided additional evidence that timing of harvest should be dictated on how brewers intend to use hops throughout the brewing process. Later harvested hops with >25-26 % dry matter had higher concentrations of free thiols and would be better suited for dry-hopping. On the other hand, hops with high thiol precursor concentrations should be added to the kettle or whirlpool because peak humulones concentrations have been developed and these additions occur before primary fermentation. Therefore, yeast β-lyase activity might liberate the free thiol from the precursor during fermentation and lead to increased aroma in beer. It is important to note that as dry-matter increases so does the propensity for hop

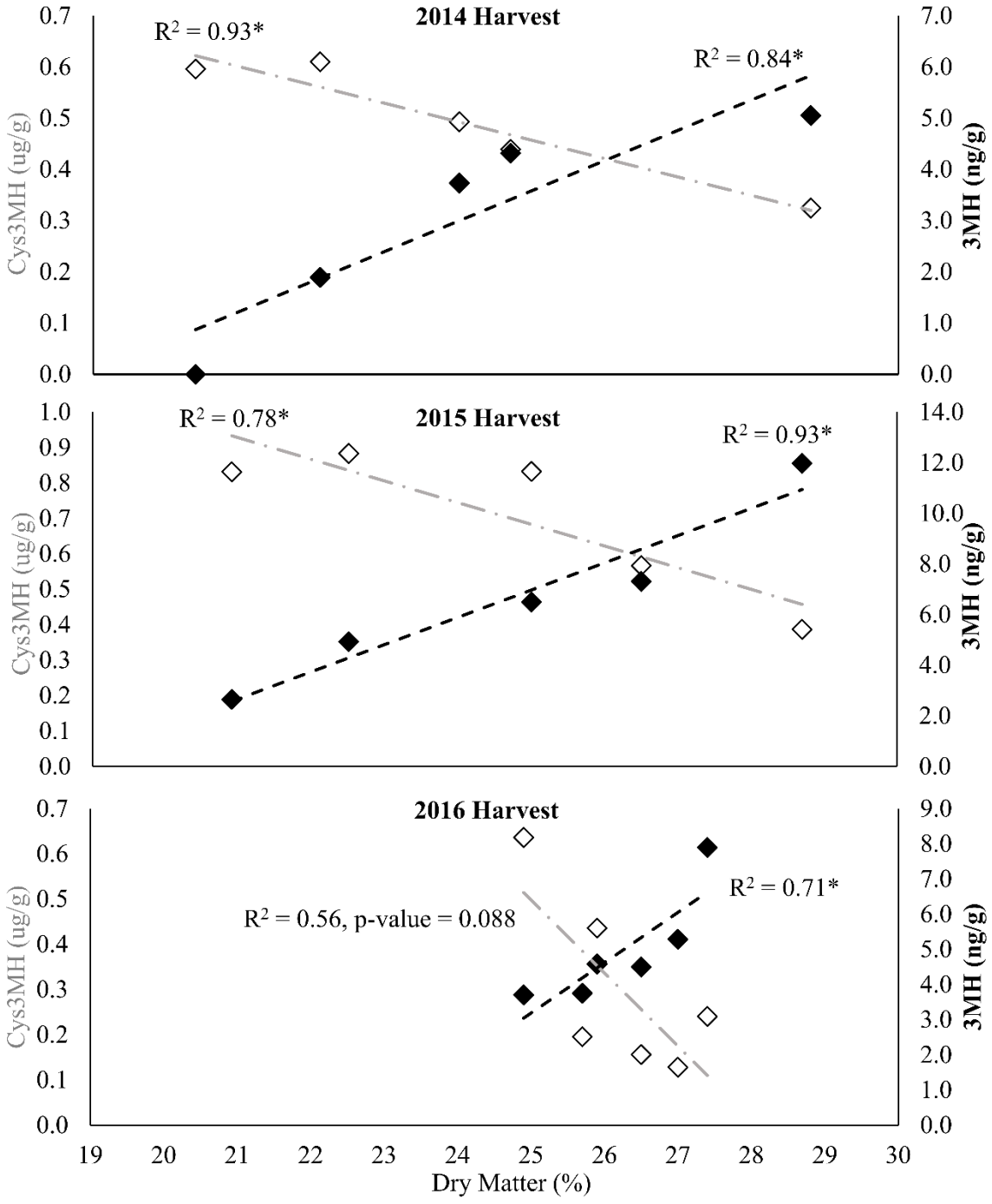


Figure 8. Dry matter (%) vs the Cys3MH (µg/g) (◇) and 3MH (ng/g) (◆) concentrations. *Pearson correlation coefficient significantly different than 0, p-value < 0.05

cones to shatter during harvesting, which can significantly impact yield. It is ultimately up to the grower and brewer to set realistic quality specifications.

Typically, hops are harvested from the first or second week in August until the end of September (Figure 9). Hop varieties ripen at different rates therefore harvest timing is a function of variety. As the need for aroma varieties has increased over the past decade there are fewer early and late maturing varieties. In the PNW, many of the sought-after aroma varieties mature around the same time (mid-September) which might cause varieties that are less commercially important to be harvested outside of their optimal window. As highlighted previously, to define optimal maturity windows it is essential to understand how hop quality changes during ripening and couple that to how a brewer intends to use hops in his/her brewing process (i.e. kettle, whirlpool, or dry-hopping).

Hop bines are removed from the field either manually by cutting the bottom and top of the bin or by using specialized combines (known as bottom and top cutters). These hop bines are placed on trucks and delivered to the harvesting facility where hop cones are separated from the plant material using mechanical sorting techniques such as dribble belts, fans and screens. The green hops are ~70-80 % w/w moisture and need to be dried to below ~10% shortly after picking to prevent degradation (Figure 7B). Fast throughput from picking through drying is necessary given the narrow harvest maturity window for hops. Therefore, growers seek to remove as much of the water from the hops as fast as possible to reach a target moisture content of ~10% post conditioning [116]. Several key

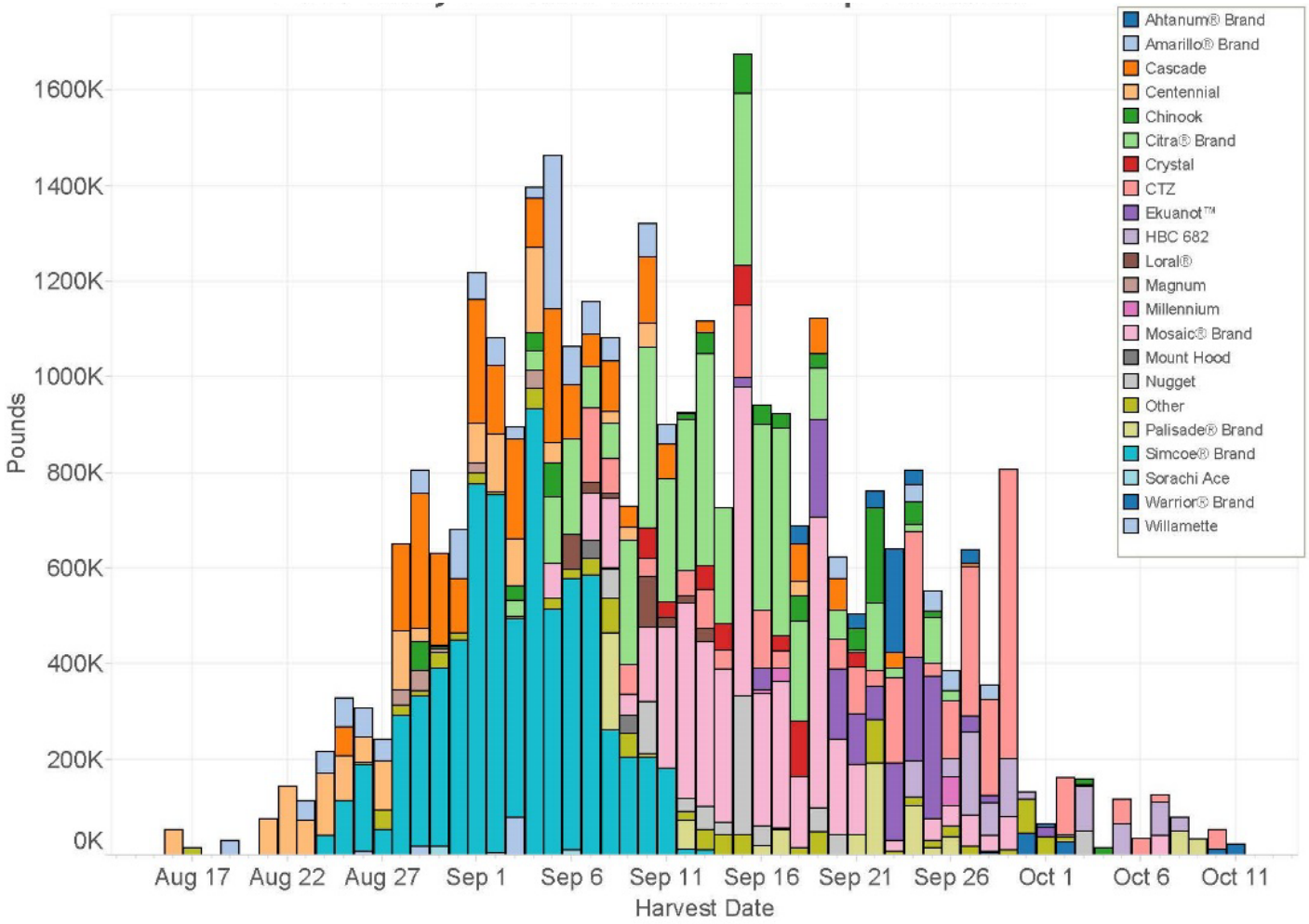


Figure 9. Overview of timing and quantity of hop varieties harvested during the 2017 harvest in the PNW. Graph provided by Yakima Chief Hops ©[117]

parameters are important to consider in order to preserve quality during kilning. For example, to prevent stewing/cooking the hops, temperatures must not be too high (i.e. < ~170°F) and air flow through the bed must not be too slow. To prevent shattering or damage to the cone structure, hops must also not be kilned for too long.[118]

Interestingly, most of the current hop kiln conditions were established when the main hop quality indicator was the concentrations of humulones. As the drivers of aroma hop quality are better defined it is necessary to reevaluate these factors to make sure optimal aroma hops are being produced. In the PNW, a majority of the hops are dried in deep (28-36") beds with forced-air convection using temperatures between 54-66°C (130-150ºF) for ~7-10 hr.[119] Due to the depth of the bed there is often a moisture gradient that leads to inhomogeneity in moisture content across a lot of hops.

To redistribute the remaining moisture content throughout the entire lot, post-kilning hops are conditioned in large piles (Figure 7C) at 20-24°C and 58-65% relative humidity.[1] After conditioning, hops are compressed into 90-140 kg (200-300 lb) rectangular bales and enclosed in synthetic burlap. Bulk densities above 150 kg/m^3 should be avoided in bales as levels above this amount have the potential to lead to crushed lupulin glands and reduced hop quality.[1] Post baling hops are transported (Figure 7D) to refrigerated storage (1-5ºC) until downstream processing (pelletizing as subsequent extraction) can occur. Storage conditions have a significant impact on hop quality and hops should be stored cold and in oxygen free environments as much as possible.[1]

The focus of this review has been focused on examining factors that influence whole cone or pelletized hop quality. However, there are numerous texts which cover the production, uses, and benefits of downstream hop products (such as preisomerized

extract, reduced iso-humulones, distilled hop oil, concentrated lupulin powder, super critical CO_2 extract, etc.)[1-2, 35, 120-122] These products do not only provide benefits for adding unique flavors, enhancing foam, and/or improving the beer stability, they also reduce inhomogeneity and increase the oxidative stability of hops.

1.5 The unintended consequences of dry-hopping: What is going on with the non-volatile fraction?

While the main function of dry-hopping is to add aroma to beer, a lot of unintended changes occur to the non-volatile fraction of hoppy beers at the extreme hopping rates that brewers are using. Although, iso-humolones have been identified as the main driver of bitterness in "kettle-hopped" beers, the oxidation products of humulones (called humulinones) have been identified as important drivers of bitterness in dry-hopped beers.[3, 22, 123] Humulinones have been shown to be ~2/3 as bitter as iso-humulones[124] and are present in freshly baled, aged, or improperly stored hops at varying levels (0.1-0.5 w/w %) (Figure 3)[22, 54]. Even though the mechanism of humulinone formation is still unknown, the oxidation of humulones in aerobic storage can occur as quickly as a few days and is likely to be the main driver of humulinone formation.[22] Pelletization has also been shown to increase concentrations of humulinones by ~0.2 w/w%.[22] Due to their increased polarity, humulinones are highly soluble in beer and 75-90% will dissolve in 1-2 days of dry-hopping.[21-22] Consequently, the bitterness contribution of humulinones is an important (and typically underestimated) quality consideration for hops destined for dry-hopping. Recently, Hahn et al.[3] developed an analyte specific methodology based on concentrations of iso-humulones and humulinones using high performance liquid chromatography (HPLC) which can be used to adjust beer bitterness during "dry-hopping".

Lupulones (β-acids) are structurally quite similar to humulones and they are often found in hops in similar concentrations as humulones.[1] Yet due to their very low solubility, they are not found in clarified hoppy beer in high concentrations, typically less than 1 mg/L. Their oxidation products (called hulupones) can be found in hops but are ~5x lower in concentration than humulinones (although this difference is likely dependent on variety).[22] In beer, hulupones are not found in high concentrations and do not correlate with bitterness in hoppy beers.[3, 22] Nevertheless, the conventional wisdom is that hulupones are a contributor to beer bitterness in lager beer.[1] This is because concentrations of hulupones in beer have been found to be directly proportional to the duration of time hops are boiled in wort. It is more likely that "kettle additions" and the subsequent oxidation of beta acids significantly impact the concentrations of hulupones.

Recently, Wietstock et al. found humulones suppressed oxidative reactions[59] and are capable of forming complexes with prooxidants (Cu^{2+} and Fe^{2+} ions)[125] but were not effective at complexing with manganese. However, Porter et al. observed that although hops contain more iron than manganese (Figure 3), proportionately more leaching of manganese occurs during dry-hopping and the concentrations of manganese extracted during dry-hopping are sufficient to cause the formation of reactive oxygen species. Interestingly, Reyes et al.[126] observed that the elemental composition of hops was connected with growing region, possibly suggesting that hops from different regions could have a different ROS forming potential. Although humulones have the potential to have a significant impact on some prooxidant concentrations, more work is needed to understand the impact of the hop elemental composition on dry-hop beer flavor stability.

Hops contain ~3-6 % w/w of polyphenolic material.[2] The comprehensive review by Aron and Shellhammer[127] highlights the impact of polyphenols on beer haze, flavor stability, and bitterness principally in lager beer. While polyphenols have been observed to impact bitterness in kettle hopped beers[1, 127], recent studies by Hahn et al.[3] and Parkin et al.[128] suggest that they might only be minor contributors to dry-hop beer bitterness as compared to humulinones and iso-humulones. However, these compounds are often regarded for their antioxidant activity and have shown to have significant beneficial bioactivities that may have positive impacts on human physiological functions.[1] Particularly, xanthohumol has shown potential as an anti-cancer[1] and anti-hyperlipidemic agent[129].

A number of studies have shown that dry-hopping has a direct impact on beer pH.[21-22, 54] The reported pH for lager beer is around 4.2, while the reported pH for hoppy beer is ~4.8.[3] Recently, Lafontaine and Shellhammer[21] observed a linear increase in the pH value ~0.14 pH for every 386 g hop added/ hL beer during dry-hopping. Maye et al. [22] observed a similar pH value increase when dry-hopping with both Cascade hop pellets and spent CO_2 extracted hop powder and has suggested that the increase in the pH value may be a result of something in the vegetative material. Although the cause of this pH increase has not been identified, concentrations of tri- and di-protic acids (such as succinic [pKa_1 = 4.2, pKa_2=5.6][130], malic [pKa_1 = 3.40, pKa_2=5.2][130] and citric [pKa_1 = 3.13, pKa_2=4.76, and pKa_3=6.39][131]) have been measured in fresh hops[26] and the impact of hop organic acid composition on pH should be investigated. The increase in the pH value may lead to an improved flavor stability of dry-hopped beers by driving the formation of less reactive oxygen radicals[132-133] and has been shown to reduce the

perception of malt derived staling aldehydes (i.e. trans-2-nonenal (card board like aroma) and methional (potato)) during aging[134].

Real extract (RE, w/w%) has also observed to linearly increase as a function of dry-hopping rate by Lafontaine and Shellhammer, ~0.07 w/w% for every 386 g hop /hL beer. Monosaccharides have been shown to comprise ~2 w/w% of hop cones (Figure 4) [49] with 0.38-0.55% fructose, 0.32-0.44% glucose, and 0.10-0.57% saccharose as well as small amounts of raffinose, stachyose and some pentosans.[26] Recently the dextrin reducing power of hop enzymes has been identified as a potential consumer safety consideration in bottle conditioned dry-hopped beers.[28-29] During dry-hopping, hop enzymes are extracted into beer and have the ability to breakdown unfermentable beer dextrins into fermentable mono- and di- saccharides (maltose, fructose and glucose). In dry-hopped beers that are bottle conditioned with active yeast or bacteria both the increase in fermentable sugar and enzymatic activity from hops should be considered, as these factors can lead unexpected refermentations in the bottle which influence diacetyl concentrations as well as lead package over pressurization. High kiln temperatures have recently been identified as a promising way to mitigate this activity without sacrificing hop aroma quality.[135]

1.6 Just when you think you understand something

The constant innovation of beer styles by U.S craft brewers will continue to uncover interesting things. For example, Maye et al.[136] recently showed that due to the extreme dry-hopping rates used in New England IPAs (NEIPAs) (Figure 4) these beers contain relatively high concentrations of non-polar hop compounds, such as β-myrcene, humulones, xanthohumol and lupulones. NEIPAs have high protein concentrations and

Maye speculated that the high level of protein (mainly from brewing adjuncts such as, oat syrup solids) may serve as an emulsifier for these non-polar analytes thereby keeping them dispersed in beer. It is not clear from the Maye study how these components impact the flavor and stability of this beer style, but this finding serves as great example that it is always important to ground observations and decisions involving hop quality on the type matrix or beer style and most importantly the type of additions that are being used to add hops to the brewing process. Simply put, there is not a one size fits all model to hop aroma quality and as we better understand hop chemistry it is important to contextualize our understanding based on practical application.

Chapter 2. Justification and objectives of this dissertation

Brewing scientists have been trying to identify how hop chemistry impacts beer aroma performance for decades. The approach that twenty-first century craft brewers take in using hops has created a new reason and urgency to understand and predict hop aroma intensity and quality in beer. Brewers are using significantly more hops, >5 lb/US bbl, and adding them later in the brewing process, using a technique called dry-hopping. While progress has been made in identifying the drivers of kettle hop aroma, the drivers of dry-hop aroma remained unclear. Therefore, a reproducible dry-hopping protocol,[137] which mitigated the impact of in-process oxidation and yeast effects, was used to gain some further understanding of the dynamics and drivers of dry-hop aroma (Figure 1). The results of these experiments were published or are in review in four international peer-reviewed journals. Briefly, the body of the dissertation is split into roughly three segments:

- Understanding the impact of dry-hopping rate on beer aroma intensity and quality
- Identifying the drivers of dry-hop aroma quality in hops
- Investigating ways to promote dry-hop aroma intensity and quality either on the farm or in the brewery.

Because the drivers of hop aroma were not understood, varying the hopping amount was one of the easier variables for brewers to manipulate during the brewing process. The underlying assumption was that adding more hop material to beer via dry-hopping would lead to more hop aroma. However, the impact of dry-hopping rate on the overall hop aroma intensity and quality of dry-hopped beer was not fully investigated. **Publication A** was used to evaluate the impact of dry- hopping rate on hop aroma

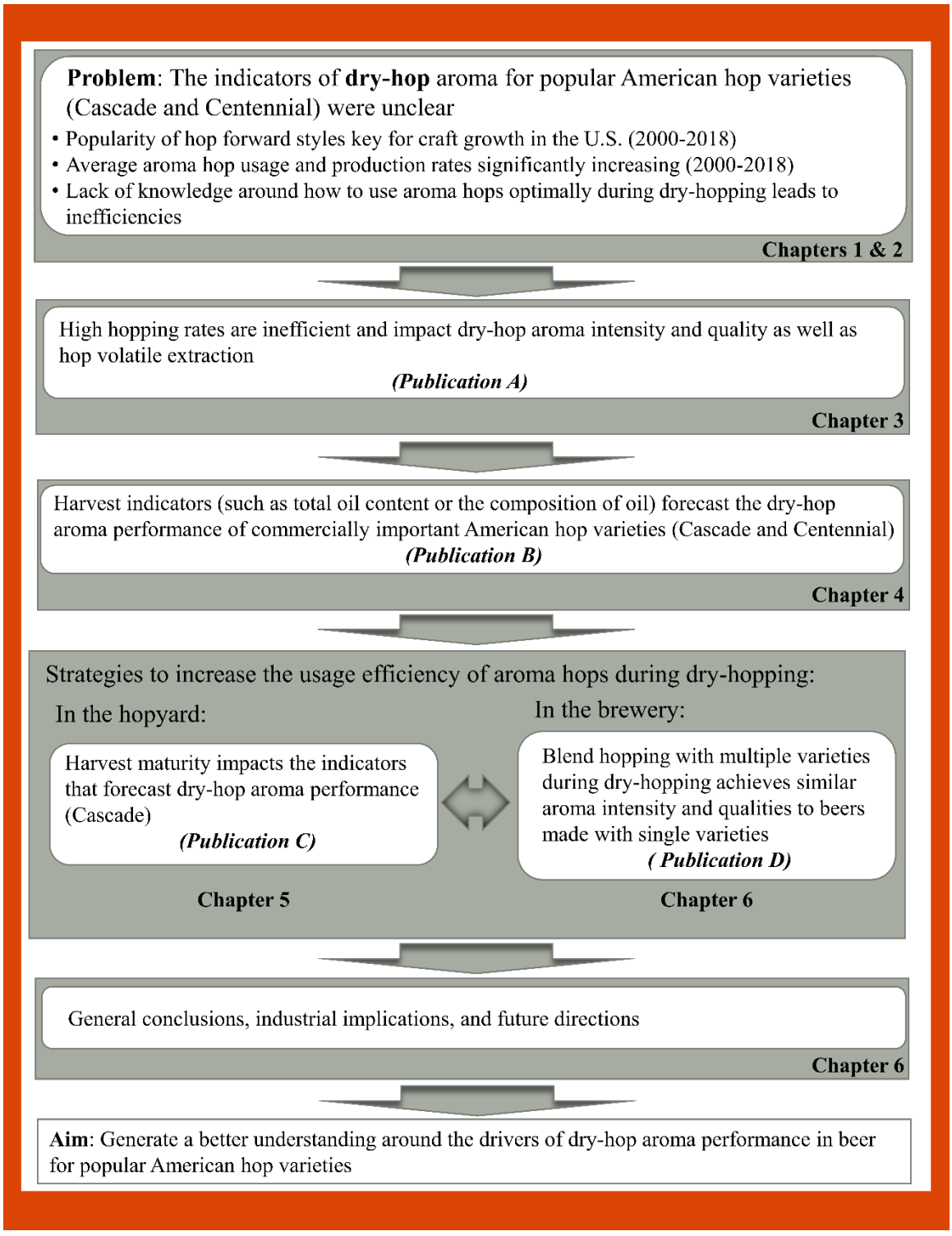


Figure 1. General overview of the dissertation and research hypotheses for each of the different publications.

intensity and quality in beer as well as the extraction efficiencies for several key volatile and nonvolatile hop constituents.

The primary goal of dry-hopping is to add intense aroma to beer. However, the main the predictors of dry-hop aroma potential in hops remains undefined, resulting in a problem when trying to have quantifiable quality targets to direct the purchasing and use of aroma hops as well as create consistent hoppy aroma on a lot-to-lot basis. Vollmer and Shellhammer[104] found that one of the main analytical indicators used by the brewing industry (total oil content) was not predictive of dry-hop aroma potential. Thus, **Publication B** was utilized to identify superior indicators of dry-hop aroma potential using components of hydrodistilled hop oil.

As predictors of dry-hop aroma are better understood, strategies can be employed both agriculturally and/or in the brewery to better utilize aroma hops. **Publication C** was used to investigate the impact of harvest maturity on dry-hop aroma intensity and quality as well as the impact on the development of key analytical markers of hop quality. The results from this publication show that farmers can adjust harvest timing based on how brewers intend to use hops throughout the brewing process to maximize their potential.

Using hop blends as opposed to single varieties is common strategy that brewers employ to create hoppy beers. However, this technique has not been systemically investigated as way to produce consistent hoppy beer aroma and promote hop aroma extraction. Consequently, **Publication D** was used to investigate the impact of dry-hopping with blends of Cascade, Centennial and Chinook on hop aroma intensity and quality in beer. The results from this study indicate that hop blends might serve an effective strategy for brewers to create consistent dry-hop aroma on a lot-to-lot basis.

Chapter 3. Publication A

Impact of static dry-hopping rate on the sensory and analytical profiles of beer

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Highlights:

- Increasing static dry-hopping rates leads to:
  - More intense aroma intensities that are more herbal/tea in quality than citrus
  - Decreasing extraction rates of hop aroma volatiles
  - Increases in pH, residual extract, bitterness units
- Static dry-hopping rates between 400 and 800 g/hL lead to more balanced hop aroma quality
- Spent dry-hops potentially have significant brewing value (likely to contain high concentrations of humulones, monoterpenes, and sesquiterpenes)

Summary:

The impact dry-hopping rate on the overall hop aroma intensity and quality of dry-hopped beer as well as on the extraction efficiencies of hop constituents that are considered important for hoppy aroma in beer was uncertain. This manuscript outlines the extraction efficiencies for several key volatile and nonvolatile constituents of hops that have been shown to be important for beer flavor over a range of commercially relevant Cascade dry-hopping rates (0, 200, 400, 800, and 1600 g/hL). The relationship between dry-hopping rate and the sensorial and analytical characteristics of the finished beer found in this study was not linear and had an optimal range between 400-800 g/hL. Brewers can use these findings to help gauge their efficiency of aroma extraction from the hops they are using during dry-hopping and hopefully guides the development of more sustainable techniques to better utilize this raw ingredient, improve beer quality, and obtain consistent hoppy beer flavor.

Abstract

Dry-hopping is a technique that has been used by brewers to increase the hop aroma and flavour of beer for centuries. Throughout the twenty first century, dry-hopping has become an increasingly popular method among craft brewers to impart intense hoppy aroma and flavour to beer. Many US craft brewers use extremely high dry-hop dosing rates of up to 2200 g/hL and this is both unsustainable and potentially wasteful. This study examines the impact of dry-hopping rate on the sensorial and analytical characteristics of dry-hopped beers. An unhopped pale beer was statically dry-hopped with whole cone Cascade from the 2015 harvest over a broad range of dry-hopping rates (200–1600 g/hL) in replicated, pilot scale (80 L) aliquots. Trained panellists using descriptive analysis scaled the overall and qualitative hop aroma intensity of these beers, as well as the unhopped base beer. Instrumental analysis was used to measure the levels of hop volatile and non-volatile extraction between the treatments. The relationship between dry-hopping rate and the sensorial and analytical characteristics of the finished beer was not linear and, based on the extraction efficiencies of select hop volatiles, had an ideal range between 400 and 800 g/hL.

Introduction

Dry-hopping has been defined as the cold extraction of non-volatile and volatile chemicals from hops into an alcoholic solution[1] and has been a technique used by brewers to increase both the microbial[2] and flavour stability[3] of beer. As hoppy beer styles have risen in popularity with consumers over the twenty first century[4], craft brewers have turned to dry-hopping as a way to enhance beer aroma and flavour. To achieve intense hop aromas and flavours there are a number of factors that brewers can

modify during dry-hopping, such as static vs dynamic extraction[5], the presence/absence of yeast[6, 7], different temperatures[8, 9] and varying hopping amounts[8].

Historically, static dry-hopping of cask beer was performed over a period of weeks[10]. However, current industrial static dry-hopping timeframes occur over a few days in large cylindroconical vessels. Using a 2 hL pilot-scale system, Wolfe[5] showed that after static dry-hopping (at 386 g hop/hL beer) for 6 h, the majority of selected key hop volatiles were extracted from hops and after 24 h the extraction peaked for these hop volatiles for both static and stirred/dynamic extractions. In the same study, extraction rates of key hop volatiles were found to vary based on the hop format during dynamic and static dry-hopping. Pelletised hops increased the extraction of linalool and geraniol during static dry-hopping by ~20%. Dynamic dry-hopping also promoted the extraction of hop volatiles for both whole cone and pelletised hops and increased the overall aroma intensity of dry-hopped beer. However, the bitterness intensity, bitterness duration and astringency of dynamically dry-hopped beers also increased owing to the elevated extraction of polyphenols and humulinones. Due to these unintended flavour consequences and the ease of implementation, static dry-hopping is often preferred to dynamic dry-hopping in the industry.

In terms of adding flavour to beer, varying the hopping amount is one of the easier levers to change during the brewing process. The underlying assumption is that adding more hop material to beer via dry-hopping will lead to more aroma and flavour. Around the 1890s, dry-hopping rates in the UK ranged from 65 to 274 g/hL[11]. In the 1960s, dry-hopping rates for British beers were reported to be ~4.3 g/hL for low gravity draught beer and ~138.6 g/hL for high gravity beers[10]. Current industrial hopping rates in

the USA on average range from 500 to 800 g/hL; however, it is not difficult to find beers that have been dry-hopped at rates as high as 2200 g/hL. These extreme cases are both unsustainable from an agronomic perspective and potentially wasteful. Although it has been suggested that maximum hop flavour is achieved when dry-hopping with ~500 g/hL[1], there have been few studies that have explored how dry-hopping rate specifically impacts beer aroma/flavour and the extraction of hop constituents.

The goals of this study were to (a) scale the changes in hop aroma intensity and quality for 'unhopped' beer dry-hopped statically with ground whole cone cascade at five different rates, 0, 200, 386, 800 and 1600 g/hL and (b) examine the impact and changes in extraction efficiencies on the non-volatile and volatile constituents over these dry-hopping rates.

Materials and methods

Experimental design

Five beers (including the 'unhopped' control) were prepared by statically dry-hopping an 'unhopped' beer with ground, whole cone Cascade hops from a single harvest lot by varying the dry-hopping rate at 200, 386, 800 and 1600 g/hL. Descriptive sensory analysis was used to scale the aroma intensity and quality of these beers. Non-volatile and volatile chemical analyses were performed on the hops used for dry-hopping and on the finished beers to determine the extraction efficiencies of hop derived aroma and flavour compounds into beer.

Hop collection

A 10 lb (4.5 kg) mini-bale from a single lot of whole cone Cascade hops was collected after harvest in 2015 from Crosby Hop Farms (Woodburn, OR, USA). Upon

arrival at Oregon State University the whole-cone hops were repackaged in high-barrier foil pouches, purged and sealed with nitrogen, and stored cold (−20°C) until dry-hopping and chemical analysis.

'Unhopped' beer production

'Unhopped' beer was prepared on a commercial scale by a regional brewing operation in Portland, Oregon. The 'unhopped' wort was prepared with 86% pale two-row, 13.5% Caramel 10°L and 0.5% Caramel 120°L malt (Great Western, Vancouver, WA, USA) to a starting concentration of 11.3°P. Fermentation was performed using a Scottish ale yeast (Wyeast 1728) at 19.4–20°C. Following fermentation, a kieselguhr filter was used to clarify the green beer and remove yeast. Post filtration iso-humulones (IsoHop, John I Haas, Yakima, WA, USA) were added at a concentration of 18 mg/L. This resulted in ~55 hL of a 19.8 BU, 4.75% ABV 'unhopped' base beer. The beer was carbonated and packaged into 60 L stainless steel kegs, shipped to Oregon State University and held at 2°C until dry-hopping.

Dry-hopping protocol and hop preparation

The dry-hopping process reported by Vollmer et al.[12] was used to reduce the variation between treatments on the pilot scale. In brief, 24 h prior to hop addition the 'unhopped' beer was removed from the cooler at 4°C and allowed to warm to ~15°C. For each treatment, 40 L of beer was transferred into two modified 60 L stainless kegs with a 4 inch stainless steel opening fitted with a standard Sankey D-system coupler and modified spear (Sabco, Toledo, OH, USA). To achieve the 200, 386, 800 and 1600 g hop/hL unhopped beer treatment rates, the whole cone hops were ground into a hop grist which was divided by mass into two mesh bags (EcoBag, Ossining, NY, USA). These

bags were stored inside high barrier pouches flushed with nitrogen until dry-hopping. For each dry-hop treatment, the two kegs filled with 40 L beer were temporarily de-pressurised and opened under a stream of low-pressure carbon dioxide. Simultaneously, the high-barrier pouch bag was opened and the mesh bag containing ground hop grist was added to the beer. After the addition, the headspace was flushed with CO_2 and purged. The kegs were inverted three times to ensure proper mixing.

After 24 h of dry-hopping the beer was filtered to stop the dry-hopping process. The average temperature of dry-hopping ranged from 13.3 to 15°C. During filtration the two kegs were blended via a three-way fitting prior to entering a plate and frame filter using diatomaceous earth impregnated cellulose pads (HS2000, Pall Corporation, Port Washington, NY, USA)[13]. Dissolved oxygen (DO) was monitored during filtration using an Orbisphere 3100 Portable Oxygen Analyser (Hach, Loveland, CO, USA). Bright beer was not collected until the DO was <110 μg/L. When in specification for DO, bright, filtered beer was collected in a closed 19.6 L stainless steel keg with sufficient backpressure to reduce foaming. Between each filter run, filter pads were exchanged to prevent carry over. Directly after filtration the DO was measured and the bright beer tank DO concentration was recorded. Filtered beer was stored at 2°C and under CO_2 overpressure (11–12 psi) until sensory evaluation. To minimise artefacts and scalping in the crown liner owing to packaging in glass bottles[14, 15], all beer for this experiment was kept in 19.6 L kegs and served directly from two eight-head draught systems (Micro Matic, Northridge, CA, USA) throughout the sensory and instrumental data collection periods.

Sensory descriptive analysis

Thirteen trained panellists were used to scale orthonasal aroma of the treatments and were selected based on previous experience (11 males and two females; 25–66 years old). Four intensive training sessions were completed in advance of data collection. During these sessions panellists were trained using external reference samples and the actual experimental treatments to develop a relevant lexicon of sensory attributes and a scale that best explained the differences in the samples. Based on discussion from these training sessions and prior results[13], the final ballot included the attributes: Overall Hop Aroma Intensity (OHAI), Citrus and Herbal/tea evaluated on a 0–15 point scale. Previous work in our laboratory used a broader array of descriptors to describe Cascade hop aroma including Resinous/Hop oil, Green and Tropical Fruit plus OHAI, Citrus and Herbal/tea. However, the quality attributes that described the most variation for the Cascade dry-hop aroma were OHAI, Citrus and Herbal/tea. Therefore, these attributes were used to characterise the changes in Cascade hop aroma in the present study. During each session, the panellists had access to five external reference samples, three of which were the experimental treatments (i.e. the unhopped control, plus 386 g/hL and 1600 g/hL dry-hop treatments) and two of which were commercial, hoppy beers (Hop Valley Citrus Mistress and Ballast Point Grapefruit Sculpin). These five beers had sensory descriptors with intensity scores assigned by consensus during training, and their purpose was serve as anchors for the 0–15 point intensity scale (Table 1).

Table 1. Reference standards with intensity scores used in descriptive analysis panels.

Attributes	**Unhopped Control**	**386 g/hL**	**1600 g/hL**	**Citrus Mistress**	**Grapefruit Sculpin**
OHAI[a]	0	8-9	14-15	7-8	14-15
Citrus	0	7-8	5-6	6-7	13-14
Herbal/Tea	0	5-6	12-13	6-7	1-2

a. OHAI = Overall Hop Aroma Intensity

The four dry-hop dosage treatments and the unhopped control were evaluated randomly amongst 28 beers dry-hopped at 386 g/hL with different lots of Cascade as part of a separate study. Over the course of 16 sessions, the 13 panellists evaluated all samples four times. An efficient resolvable incomplete block design was used to create a presentation order for the samples across four replications (SAS, Cary, NC, USA). Four sessions were needed per replication to evaluate all the hopped samples (three sessions of eight samples and one session of nine samples). Panellists were given ~60 mL of dry-hopped beer in a 300 mL glass covered with a plastic lid. Beer was served from two eight-head draft systems (Micro Matic, Northridge, CA, USA) into pitchers at ~1.5°C and at 12 psi. Beer was poured into sample glasses ~1 h before the start of testing and allowed to warm to room temperature. Panellist responses were collected on Chromebook tablets using Qualtrics (Provo, UT, USA). For each session, Qualtrics was also used to randomly assign the serving order of samples for each panellist.

Beer and hop analysis

Simultaneous with the hop sampling for the dry-hopping, a ~150 g portion of the homogenised hop grist was taken for chemical analysis. All beer was stored in 19.6 L kegs at ~1°C until analysis.

Non-volatile analysis reagents and standards

Octyl alcohol was obtained from Sigma-Aldrich Chemical Co. (St Louis, MO, USA). HPLC-grade methanol was obtained from VWR International, BDH analytical (West Chester, PA, USA). Hydrochloric acid, 2,2,4-trimethylpentane and phosphoric acid were obtained from Avantor performance materials (Center Valley, PA). DCHA-Iso ICS-I3 and international calibration extract ICE-3 standards were obtained from ASBC. Humulinone standards were produced[16] and DCHA-humulinones standards were obtained through Robert Smith from S.S. Steiner Inc.

Non-volatile beer and hop analysis

Total humulones, lupulones and hop storage index were measured and calculated using ASBC Hops – 6[17]. The concentrations of hop acids in hops and beer samples were analysed using ASBC methods Hops – 14 and Beer – 23E under modified HPLC conditions[17]. The modified HPLC analysis was performed on an Agilent 1200 HPLC. Prior to analysis beer was degassed via filtration through GHP Acrodisc® 13 mm, Pall Corporation (East Hills, NY, USA) syringe filters. Analysis was performed using a 2.6 μm EVO C-18 100 Å 100 × 4.6 mm LC column (Phenomenex, Torrance, CA, USA) held at 40°C. A 7 μL aliquot of each beer sample was injected and the elution was carried out using a flow rate of 1.6 mL/min. The solvent gradient was as follows: 10% solvent A (reagent water)–90% solvent C (90% 75% MeOH, 24% H2O, 1% H3PO4) held for 5 min, then changed to 100% solvent D (100% MeOH) over 5 min and held for 2 min, then returned to 10% solvent A–90% solvent C over 2 min, for a total run time of 14 min. Based on absorbance maximum of each hop acid, the absorbance of iso-humulones and humulinones were measured at 275 nm and that of humulones was measured at 314 nm[16].

Bitterness units were measured according to ASBC methods of analysis Beer – 23A[17]. Spectrophotometric analysis for bitterness units were carried out using a Shimadzu PharmaSpec UV-1700 spectrophotometer, Shimadzu Corporation (Columbia, MD, USA). Residual extract and pH were analysed using an Anton-Paar Alcolyser with supporting pH module (Anton Paar USA, Ashland, VA, USA).

Volatile analysis reagents and standards

β-Myrcene, linalool, geraniol, citral, methyl geranate, geranyl acetate, 4-octanol, terpinen-4-ol, α-terpineol, nerol, β-caryophyllene, α-humulene and β-farnesene were obtained from Sigma-Aldrich (St Louis, MO, USA). 2-Octanol was obtained through Alfa Asear (Haverhill, MA, USA). Hexanes purchased from J.T. Baker (Center Valley, PA, USA) were redistilled to remove impurities before analysis. Sodium chloride was purchased from EMD Millipore (Billerisa, MA, USA).

Hop volatile analysis

Hydrodistillation was performed to determine the total oil content of the homogenised hop grist using ASBC Hops – 13[17]. Post-distillation, hop oil was collected in 2.5 mL amber vials with foil-lined closures. After filling with oil the amber vials were flushed with nitrogen. Hop oil was stored at −20°C until subsequent compositional analysis.

Hop oil compositional analysis was performed using an HP 6890 gas chromatograph with an Agilent 5972a mass spectrometer (GC–MS) under modified conditions from ASBC Hops – 17[17]. In brief, a 1% 2-octanol (8190 mg/L) solution was prepared in reagent-grade hexane. Hop oils were diluted to 10% with the 1% 2-octanol–hexane solution in crimped glass vials. A 1 μL aliquot 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 30 m × 250 μm × 0.25 μm Zebron ZB-1 MS (Phenomenex, Torrance, CA, USA) and ultra-pure helium was used as the carrier gas (a constant flow rate, 1.4 mL/min). The following temperature programme was used: 50°C held for 1 min; 50–180°C (2°C/min), held for 10 min; 180–200°C (3°C/min); and 250°C held for 5 min. The auxiliary line and mass spectrometer were operated at 280 and ~180°C respectively. The mass spectrometer was operated using electron-impact mode at 70 eV and in full-scan mode set up to detect ions with mass-to-charge ratios (m/z) of 30–350. Four-point calibration curves (50, 100, 400, and 800 mg/L) were created for all target analytes. For high concentration target analytes (β-myrcene, α-humulene, β-caryophyllene, β-farnesene) three additional calibration points were added (1000, 5000 and 9000 mg/L). Target analytes were quantified using the following ions for each analyte: m/z 41 (geranial), m/z 45 (2-octanol), m/z 59 (α-terpineol), m/z 69 (β-farnesene, geraniol, nerol, methyl geranate, and geranyl acetate), m/z 71 (terpinen-4-ol and linalool) and m/z 93 (β-Myrcene, β-caryophyllene and α-humulene). The target analyte concentrations in hop oil were standardised on a per-mass basis using the total oil content determined during hydrodistillation.

Beer volatile analysis

Headspace solid phase microextraction was performed on the dry-hop treatments using a 1 cm 24 gauge divinylbenzene–carboxen–polydimethylsiloxane Stableflex fibre with 30/50 μm coating thickness (Supelco, Bellefonte, PA, USA)[6, 18]. An 9 mL aliquot of each sample was placed into a 20 mL screw-top amber vial with 3 g sodium chloride. 4-Octanol (911 μg/L) was used as an internal standard and added to each vial. A

MultiPurpose autosampler (MPS2; Gerstel, Mülheim, Germany) was used for pre-incubation, stirring, extraction and injection. Samples were pre-incubated for 15 min at 30°C and adsorbed by piercing the vial septa and exposing the fibre to the headspace for 45 min with agitation. After adsorption, the fibre was desorbed into the GC sample inlet (splitless mode, 250°C) for 10 min. The analytical column was a 30 m × 250 μm × 0.25 μm Zebron ZB- 1 MS (Phenomenex, Torrance, CA, USA) and ultra-pure helium was used as the carrier gas (at constant pressure, 11 psi). The following temperature programme was used: 50°C held for 1 min; 50–250°C (5°C/min); held for 11 min; and 250°C, held for 5 min. The auxiliary line and mass spectrometer were operated at 280 and 180°C respectively. The mass spectrometer was operated using electron-impact mode at 70 eV and in full-scan mode set up to detect ions with a mass-to-charge ratio (m/z) of 30–350. Three-point calibration curves (40,100, and 200 μg/L) were created for all target analytes. Calibration curves were made in a model beer solution (5% v/v ethanol) and were prepared using the methodology previously described above. Target analytes were quantified using the following ions for each analyte: m/z 55 (4-octanol), m/z 59 (α-terpineol), m/z 69 (β-farnesene, geraniol, nerol, methyl geranate, geranial and geranyl acetate), m/z 71 (terpinen-4-ol and linalool) and m/z 93 (β-myrcene, β-caryophyllene and α-humulene).

Statistical analysis

Two-way analysis of variance with a mixed model (including the factors panellist, sample, and replication as well as corresponding two-way interactions), Pearson correlation analysis, multiple comparison analysis (Tukey's HSD), principle component analysis and graphical construction were carried out using XLstat 2017 (Addinsoft, New

York, NY, USA). These tests and graphs were used to gauge the panel and panellist effectiveness in generating descriptive data, evaluate the significant differences in aroma quality and intensity among the dry-hopping treatments, and assess the associations between the collected chemical and sensory data.

Results and discussion

Descriptive analysis: panellist/panel evaluation

Each panellist was evaluated on their performance to discriminate differences among the treatments on at least one of the sensory attributes, their ability to replicate among all sessions and their lack of interactions. Any panellist that failed these three criteria were removed from further analyses. Three of the 13 original panellists were removed from the dataset. The resultant dataset included 40 observations per attribute, per sample.

Two-way ANOVA with a mixed model was performed on the attributes using the remaining 10 panellist (Table 2). Significant sample effects were observed across the attributes and a significant panellist × sample effect was observed for OHAI. The significant panellist × sample effect is common in sensory analysis and indicates that there were slight differences in the ways the panellist scaled OHAI[19]. No significant effect of replication or interactions between panellist and replication or between sample and replication were observed. This indicates the panellists could effectively replicate their attribute scaling for the samples across the four replications and that the ratings provided for the attributes for a given panellist did not depend on replication. Although there are inconsistencies among the group of panellists with scaling OHAI, individually the panellist results demonstrate consistent ratings across the sensory attributes. The least

square means and results from Tukey's HSD ($p < 0.05$) for the sensory attributes from the descriptive analysis panel on the dry-hop treatments are summarised in Table 3.

Table 2. Mixed model analysis of variance on the sensory attributes.

			OHAI		Herbal/ Tea		Citrus	
Source	**Type**	**DF**	**F-Statistic**	**P-value**	**F-Statistic**	**P-value**	**F-Statistic**	**P-value**
Sample	Fixed	4	41.7	**< 0.0001**	34.1	**< 0.0001**	28.3	**< 0.0001**
Panelist	Random	9	1.2	0.358	1.5	0.237	1.8	0.138
Rep	Fixed	3	0.4	0.721	0.5	0.690	0.7	0.582
Sample*Panelist	Random	36	2.1	**0.002**	1.4	0.079	1.1	0.295
Sample*Rep	Fixed	12	0.8	0.683	0.5	0.895	0.8	0.630
Panelist*Rep	Random	27	0.7	0.835	0.8	0.680	1.3	0.147
Error		108						

Values in **bold** indicate p-value < 0.05

Table 3. Summary of least squared means for the sensory attributes resulting from descriptive analysis.

	Dry-hop rate (g/hL)				
Sensory Attributes	0	200	386	800	1600
OHAI	3.0^{e}	6.3^{d}	8.1^{c}	10.4^{b}	12.3^{a}
Herbal/Tea	2.5^{d}	4.3^{c}	5.7^{c}	7.4^{b}	10.4^{a}
Citrus	1.9^{c}	4.4^{b}	$5.8^{a,b}$	7.1^{a}	7.0^{a}

Letters indicate statistically significant groupings (Tukey's HSD tests p-value < 0.05)

Descriptive analysis: aroma intensity and quality response to hop dosage

It was hypothesised that the greater the concentration of hops used for dry-hopping was, the higher the overall hop aroma intensity would be. Significant ($p < 0.05$) positive Pearson correlation coefficients (r) were observed between dry-hopping rate and the sensory attributes OHAI (0.960) and Herbal/tea (0.994), indicating that as the dry-hop rate increased so did the values of these attributes. However, when examining dry-hop rate vs overall hop aroma intensity, a nonlinear relationship between dry-hopping rate treatments and the sensory attributes was observed (Fig. 1). Although there were five statistically significant groupings for OHAI, indicating that the overall intensity increased over the dry-hopping treatments, the 1600 g/hL appeared to yield diminishing returns. In

fact, there was no significant difference in Citrus intensity between the two highest hopping rates. When considering the hop quality sensory attributes over the dry-hopping treatments it can be seen that the aroma quality changed over the treatments. At the low dry-hopping rates the Citrus and Herbal/tea attributes appeared to increase at similar rates, but the high dry-hopping rate (1600 g/hL) overall hop aroma character was predominately Herbal/tea. This is evidence that using very high hopping rates may not necessarily result in amplification of just hop aroma intensity and that the quality of the hop aroma will change as a function of hopping rate.

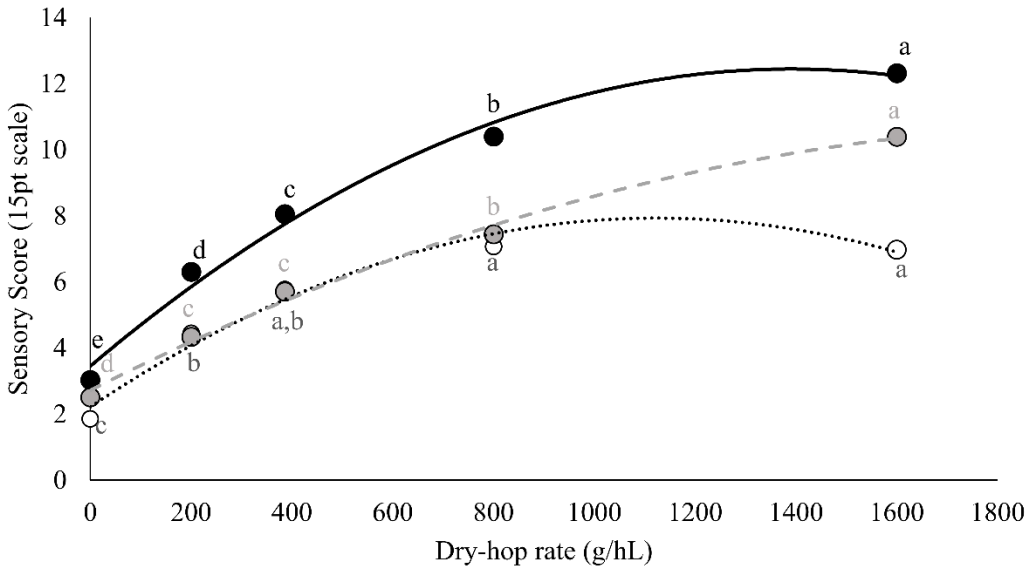

Figure 1. Mean values of overall hop aroma intensity (OHAI; black circles), Citrus (white circles) and Herbal/tea (gray circles) sensory attributes vs dry-hopping rate. Letters associated with the markers in the figure indicate statistically significant groupings (Tukey's HSD tests p-value < 0.05).

Steven's power law has been used previously to describe olfaction and the relationship between odourant concentration and aroma intensity[20]. The log–log plot of the sensory attributes vs the dry-hopping rate (Fig. 2) shows that both OHAI and the

Herbal/tea quality are described by Steven's power law. The exponents n measured for OHAI (n = 0.35) and Herbal/tea (n = 0.30) are similar to those found in literature for single hop constituents 21. For each of these attributes, n was <1, which indicates that the exponent is compressive and that aroma intensity was increasing slowly as the dry-hop rate increased. The Citrus quality did not follow the Steven's power law and this could be due to suppression of this quality by the Herbal/tea quality or by its reaching a solubility limit.

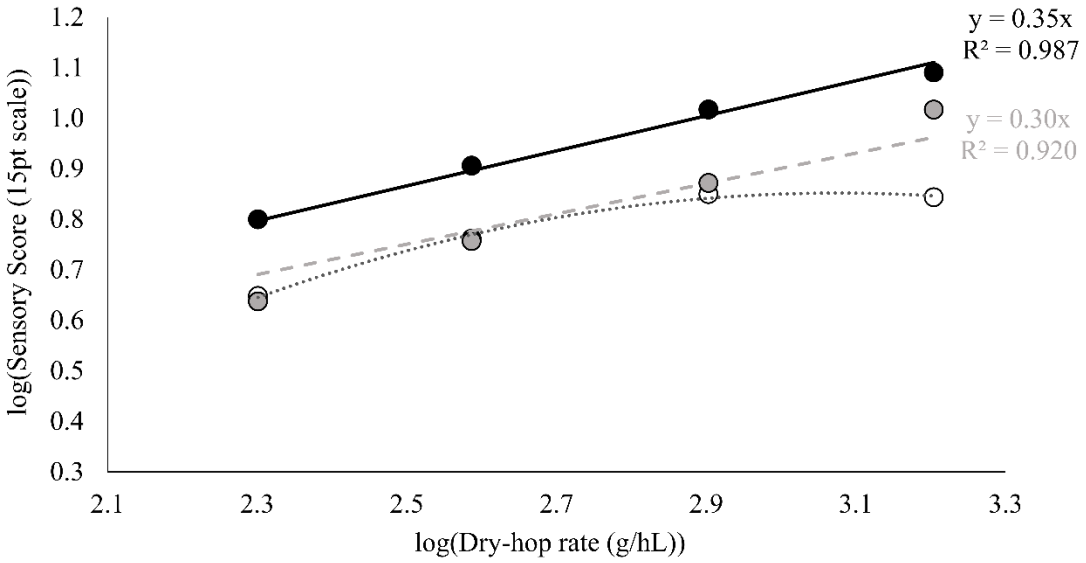

Figure 2. Logarithmic values of the mean values for OHAI (black circles), citrus (white circles) and herbal/tea (gray circles) sensory attributes vs the corresponding logarithmic dry-hopping rate.

Hop dosage and hop volatile extraction

The measured volatile components in the hops used for dry-hopping and the impact that dry-hop rate had on selected hop volatiles in beer were examined (Table 4). Significant ($p < 0.05$) positive Pearson correlation coefficients (r) were observed for β-caryophyllene (0.964), α-humulene (0.963), terpinen-4-ol (0.971), α-terpineol (0.973),

linalool (0.994), nerol (0.985), geraniol (0.982) and geranial (0.954), indicating that as the dry-hopping rate increased, so did the values of these analytes in beer (Fig. 3).

Table 4. 2015 harvest Cascade hop[a] and beer volatile chemistry over the dry-hopping rate treatments.

Target Analytes	**Hop volatile analysis (mg/100g)[b,c]**	**Beer volatile analysis (μg/L)[d]**				
		Dry-hop rate (g/hL)				
		0[c]	200[e]	386[e]	800[e]	1600[f]
β-myrcene	729.4	0.9	41.1	35.2	56.9	20.5
β-caryophyllene	95.4	n.d.	n.d.	0.2	0.4	2.3
α-humulene	184.8	n.d.	0.6	0.4	0.9	2.8
β-farnesene	47.9	n.d.	0.5	0.5	0.5	2.1
Terpinen-4-ol	n.d.	n.d.	1.8	3.6	3.9	7.0
α-terpineol	n.d.	n.d.	9.0	10.7	11.4	13.7
Linalool	8.4	n.d.	38.3	53.9	71.1	104.3
Nerol	0.7	n.d.	0.8	0.8	1.1	1.6
Geraniol	6.9	n.d.	17.8	21.4	27.8	34.3
Geranial	0.4	n.d.	0.5	0.5	13.1	19.5
Methyl geranate	0.4	1.3	4.7	3.3	4.4	0.7
Geranyl acetate	21.2	n.d.	7.0	5.2	5.9	1.5

a Total oil content = 2.0 (mL/100 g). Measured using ASBC MOA Hops – 13 17.
b Analysed using under modified GC/MS conditions based on ASBC MOA Hops – 17[17]. Analytes are reported in mg/100 g hops.
c Analysed using under modified GC/MS conditions based on published methodology 6, 18. Analytes are reported in μg/L and are blank corrected.
d Based on one instrumental run.
e Average of four instrumental runs.
f Average of two instrumental runs.
n.d., Not detected.

Dry hopping at the lowest rate, 200 g/hL, led to the concentrations of the terpene alcohols, linalool, geraniol and nerol being above their reported difference threshold values in beer[22]. The extraction rates of these analytes decreased with increased dry-hopping rate (Fig. 3). At 200 g/hL only ~23, ~13 and ~6% of the total amounts of linalool, geraniol and nerol were extracted from the hops into the beer during dry-hopping, while at 1600 g/hL even less (~7, ~3 and ~1%) was extracted for each of these analytes respectively. Wolfe[5] observed similar peak extraction rates when statically dry-hopping (at 386 g/hL) with whole cone hops for linalool and geraniol to be ~29 and 70%

respectively. Although the contact time was much longer (~4 weeks) and the technique for hop addition during dry-hopping differed significantly from this study, Forster et al.[23] reported extraction rates of linalool and geraniol during dry-hopping to be ~100 and 50–100% respectively. It is expected that the static dry-hopping technique used in this study led to the observed reduced extraction rates of terpene alcohols. However, these low extraction rates indicate that hop volatiles may not be fully extracted from hops during static dry-hop events and potentially more aroma can be extracted from hops used for dry-hopping.

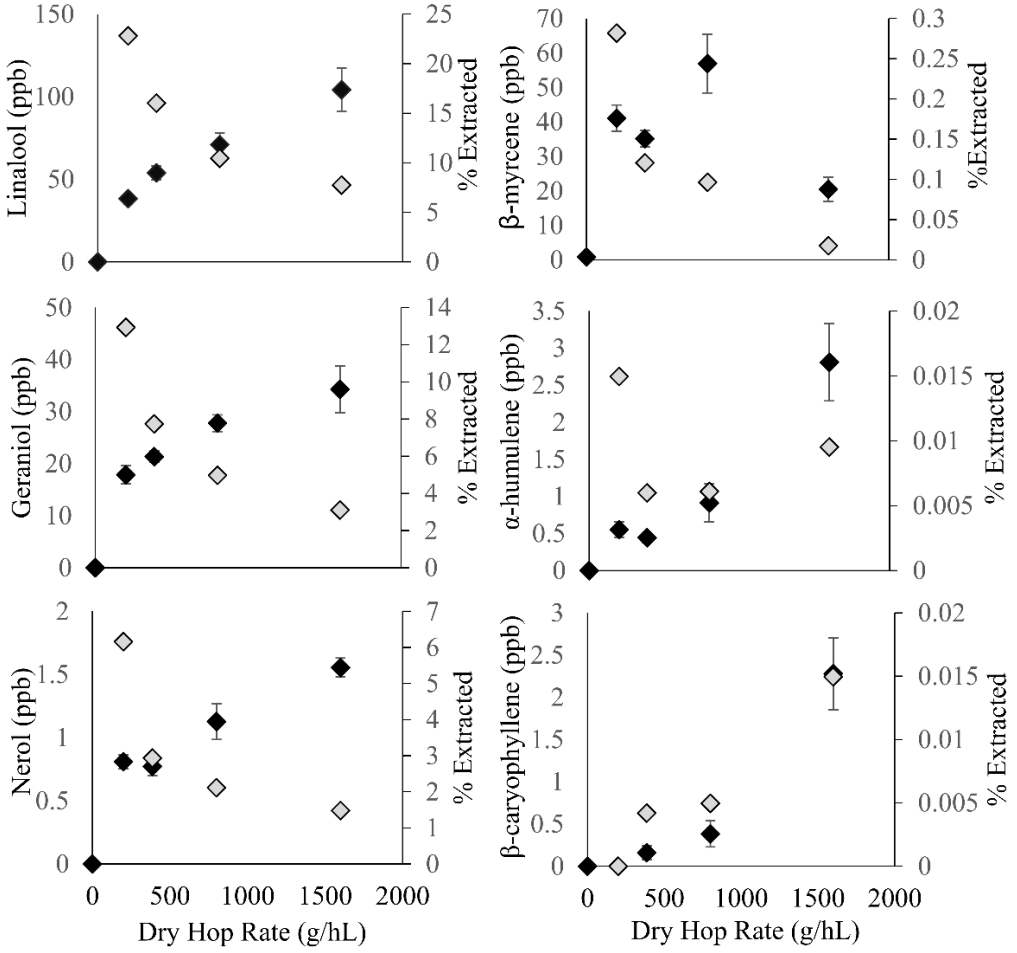

Figure 3. Comparison of mean concentrations (black diamonds, µg/L) and extraction rate from hops into beer (gray diamonds, %) for selected hop volatiles across all dry-hopping rates. Error bars represent one standard error within instrumental replicates (n = 2–4).

Very low extraction rates (< 1%) for β-myrcene, β-caryophyllene, α-humulene and β-farnesene were observed. Similar extraction rates (0.3–2.6%) for these analytes were observed in a number of studies[23]. The physical–chemical properties of these analytes make them insoluble in beer and therefore they are not extracted to an appreciable degree during dry-hopping. It is unlikely, unless at extreme dry-hopping rates, that these analytes play much of a role in the sensory perception of dry-hopped beer.

Terpinen-4-ol and α-terpineol were not found to be present above the detection limit in the hop oil or the 'unhopped' beer but were found to be present in the dry-hopped beer. There is evidence that these analytes can appear in beer via degradation[24, 25] or enzymatic[7, 26] transformation of other hop volatiles such as linalool. In this study dry-hopping occurred in the absence of yeast; therefore it is likely that these analytes are degradation products.

Methyl geranate and geranyl acetate were both found to be present in the 'unhopped' beer. Although non-significant Pearson correlation coefficients (r) were measured, as dry-hop rate increased the concentrations of methyl geranate ($r = -0.854$, p-value = 0.15) and geranyl acetate ($r = -0.920$, p-value = 0.08) decreased. It has been previously reported by Forster et al.[27] that trace levels of geranyl acetate have been observed in dry-hopped beers and that it can be hydrolysed to geraniol. There is evidence that geranyl acetate esterase is commonly present in plant species[28] and has been shown to regulate the level of geraniol in lemongrass[29]. One explanation for the decrease in geranyl acetate concertation as the dry-hopping rate increases is that hops may contain geranyl acetate esterase that could convert geranyl acetate to geraniol during dry-

hopping. There is also evidence that methyl geranate may be converted into geranic acid. Therefore it is possible that the reduction in concentration of these analytes may be a result of hop-derived enzymes extracted from the plant material during dry-hopping.

Hop dosage impact on BU and non-volatile chemistry

The measured non-volatile components in the hop material used for dry-hopping and the impact of dry-hop rate on the non-volatile beer profile was also reviewed (Table 5). As the dry-hopping rate increased, a rise in the bitterness units and humulinone concentration was observed (Fig. 4). Extraction of humulinones during dry-hopping has previously been associated with an increase in bitterness units[30]. Interestingly, as the dry-hopping rate increased, the extraction rate of humulinones from the hops into the beer decreased: 200 g/hL (113%), 386 g/hL (76%), 800 g/hL (74%), and 1600 g/hL (47%). After five days dry-hopping, Maye et al.[31] observed similar extraction rates of humulinones from Centennial hop pellets: 200 g/hL (98%), 386 g/hL (91%) and 800 g/hL (87-88%). The extraction rate of humulone from the hops into beer was very low over the dry-hopping treatments: 386 g/hL (2%), 800 g/hL (1%) and 1600 g/hL (1%). Other studies have also shown that the extraction of α-acids during dry-hopping was low and roughly 4–6%[23]. No change was observed in the iso-humulone concentration over the dry-hopping treatments. However, at concentrations ≥50 mg/L iso-humulone, a decrease in iso-humulone concentration should be expected as the dry-hopping rate increases[31, 32].

Owing to the amount of hop material used at the high dry-hopping rates and the static dry-hopping technique used in this study, it is likely that the decreased humulinone extraction at the high dry-hopping resulted from the hops not being homogenously dispersed in solution and the increased hop solids load. These factors may have led to

poor mass transfer and decreased diffusion rates of the humulinones out of the plant material and into the beer. This phenomenon may also occur in commercial dry-hopping where there are high hop solid concentrations and similar beer-to-hop solid ratios.

Table 5. 2015 harvest Cascade hop[a] and beer non-volatile chemistry over the dry-hopping rates.

Target Analytes	**Hop nonvolatile analysis (% w/w)**	**Beer nonvolatile analysis (mg/L)[c]**				
		Dry-hop rate (g/hL)				
		0	200	386	800	1600
Humulones	5.5[b]	0.0	0.0	4.0	5.7	9.0
Humulinones	0.1[b]	0.0	2.5	3.3	6.5	8.2
Iso-humulones		16.4	16.7	17.0	16.5	16.4
BU		17.0	19.4	21.0	25.0	26.0
pH[d]		4.11	4.20	4.25	4.33	4.50
Real Extract[d] (w/w%)		3.16	3.19	3.23	3.28	3.51

BU, Bitterness units; n.d., Not detected.
a Hop storage index, 0.381. Measured using ASBC MOA Hops – 6[17].
b Measured using modified conditions of ASBC MOA Hops – 14[17]. Analytes are reported as w/w %
c Measured using modified conditions of ASBC MOA Beer – 23E[17]. Analytes are reported as mg/L.
d Measured using an Anton Paar Alcolyzer with supporting pH module.

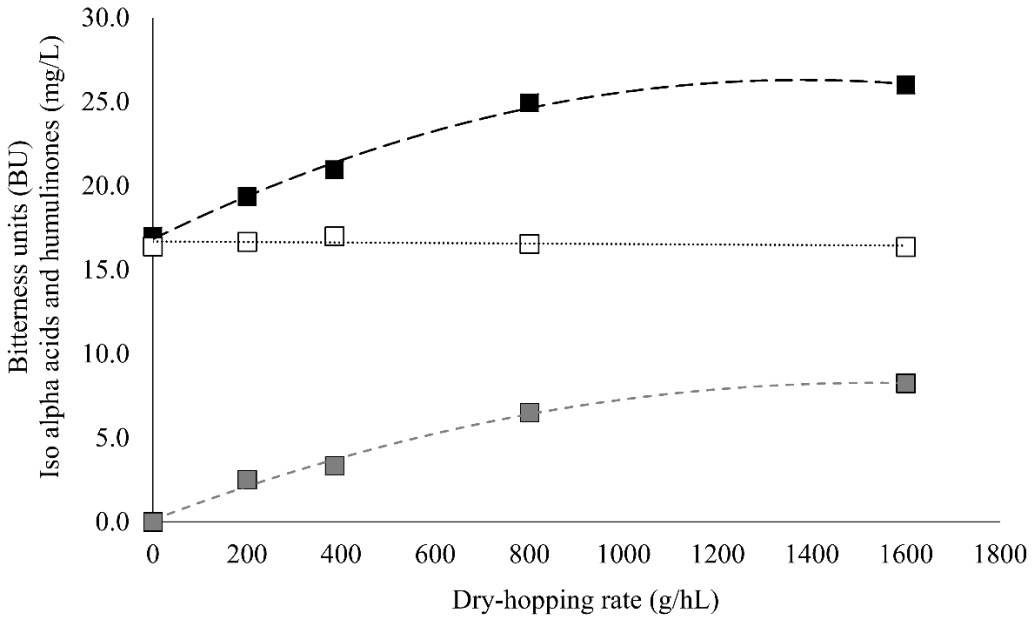

Figure 4. Hopping rate influences final beer bitterness units (BU) (black squares), iso-α-acids (mg/L) (white squares) and humulinones (mg/L) (gray squares).

It was also observed that the rise in BU across the dry-hopping rates was slightly more than the sum of iso-humulone and humulinone concentrations in the dry-hopping treatments. Although polyphenols were not measured in this study, it is expected that the extraction of hop polyphenols at the higher dry-hopping rates may have led to this deviation. Hahn et al.[33] recently observed that the perception of bitterness intensity of hoppy beers is associated with primarily humulinone and iso-humulone concentration. Therefore, although the sensory bitterness of these dry-hopped beers was not evaluated, it is clear that dry-hopping rate has a direct impact on the concentration of analytes that are important for the perception of bitterness in beer.

A linear increase in pH (~0.14 pH for every 386 g/hL) was observed over the dry-hopping treatments (Table 5). This has also been reported in the literature and seems independent of both hop variety and beer style. Maye et al.[31] observed a similar pH value increase when dry-hopping with both Cascade hop pellets and spent CO2-extracted hop powder, and has suggested that the increase in the pH value may be a result of the vegetative material. This increase in the pH value may lead to an improved flavour stability of dry-hopped beers by driving the formation of less reactive oxygen radicals[21,34] and has been shown to reduce the flavour perception of both trans-2-nonenal (cardboard-like aroma) and methional (potato) during aging[35].

Real extract (RE, %w/w) was also observed to linearly increase as a function of dry-hopping rate (~0.07 %w/w) for every 386 g/hL (Table 5). It has been shown that there are numerous sugars in hops[36] accounting for ~2% w/w of hop cones[10] with 0.38–0.55% fructose, 0.32–0.44% glucose and 0.10–0.57% sucrose as well as small amounts of raffinose, stachyose and pentosans[36]. This implies that the increase in RE is due to the

addition of hops and not a result of the measurement technique. In dry-hopped beers that are bottle conditioned with yeast or bacteria this increase in fermentable sugar from hops should be considered along with the enzymatic/reducing power of hops. These factors may impact secondary fermentation in bottle which could influence diacetyl concentration and package over pressurisation, the former being a quality issue and the latter being a serious consumer safety risk.

Conclusions/industrial considerations

Adding more hops by static dry-hopping does not simply lead to increased aroma intensity but also changes aroma quality in the finished beer. Dry-hopping rates >800 g/hL lead to hop aromas that were more herbal/tea in quality than citrus. To maintain a more balanced hop aroma quality this study suggests using a static dry-hopping rate between 400 and 800 g/hL. Using dry-hopping rates >800 g/hL leads to diminishing returns in terms of increasing hop aroma and is an inefficient use of raw material. Although work needs to be done to evaluate what is left in hops post dry-hopping, there is evidence that most of the analytes (humulinones) that impact bitterness perception are extracted from hops during dry-hopping (~75%), but that there are still hop volatiles left in the spent hop material. In addition, there are also a considerable amount of humulone left in the spent dry-hop material. Therefore, this spent dry-hop material could potentially have use elsewhere in the brewing process.

It is expected that the low extraction rates of terpene alcohols observed in this study are a result of the static dry-hopping technique used. These extraction rates may be impacted by tank/dry-hopping dynamics such as tank or extraction environment dimensions as well as hop particle settling velocity and concentration in the dry-hopping

vessel. Therefore, it is important to consider the beer-to-solids ratio within the vessel during dry-hopping as this may have an impact on the extraction of analytes that impart hoppy flavour. It is hypothesised that extraction during static dry-hopping may be promoted through multiple static dry-hopping events or gentle agitation. However, agitation has been shown to change the quality of the hop aroma extracted to more of a herbal/grassy character and promote the extraction of polyphenols, which may increase the astringency of beer[5]. Ultimately it is up to the brewer to decide which dry-hopping technique promotes the best usage of hops and achieves the desired sensory profile. It is expected that understanding how static dry-hopping rates impact aroma quality and intensity will help promote environmentally and economically sustainable brewing practices.

Acknowledgments

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Chapter 4. Publication B

The Effectiveness of Hop Volatile Markers for Forecasting Dry-hop Aroma Intensity and Quality of Cascade and Centennial Hops

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Highlights:

- At the same dry-hopping rate (3.86 g/L), whole cone Cascade and Centennial hops from different lots led to significantly different aroma intensities and qualities in beer
- Concentrations of hop oil volatiles, geraniol (for Cascade) and β-pinene (for Centennial), are better predictors of dry-hop aroma potential than total oil content
- Harvest year has a significant impact on aroma hop quality

Summary:

The indicators of aroma hop quality which forecast a hop's dry-hop aroma performance and the variation (both chemically and sensorially) that exists within Cascade and Centennial samples was unclear. 84 Cascade and Centennial hop samples were obtained and evaluated over three consecutive harvest years. It was determined that total oil content was not an effective predictor of dry-hop aroma performance in beer. Furthermore, two hop volatiles (geraniol for Cascade and β-pinene for Centennial) were identified as better indicators at predicting dry-hop aroma quality as compared to total oil content. Brewers can use this information to guide their current hop selections and recipes, growers can use these findings to fine-tune growing, harvesting and kilning conditions, and hop breeders gain effective chemistry targets for creating new hop varieties.

Abstract

Eighty-four individual hop samples were gathered over three harvest years to determine chemical factors in hops that serve as indicators of a hop's aroma potential during dry-hopping. Two public American hop varieties that are important to U.S. hop farmers and used by craft brewers globally, Cascade (n = 51) and Centennial (n = 33), were evaluated. Using a constant dry-hopping rate (3.8 g/L), significantly different aroma intensities and qualities were observed across the various samples of hops within each cultivar. Multiple linear regression analysis based on the concentrations of 16 hop oil analytes identified geraniol to be more effective than total oil content in predicting Cascade aroma quality and intensity in dry-hopped beer. Centennial hops differed from Cascade in that β-pinene was identified as being a more improved indicator of dry-hop aroma as compared to total oil content. In each hop variety, the single hop volatiles explained approximately 50% of the variation in the sensory qualities of the dry-hopped beer, while total hop oil content explained less than 30% of the same variation. These results suggest that the dry-hop aroma potential of different hop varieties is predicted by different hop volatiles and that total oil content is not the best indicator of a hop's dry-hop aroma intensity or quality.

Introduction

The demand for aroma hops has drastically changed over the last decade [3]. Craft brewers, and now large brewing operations, are purchasing greater quantities of hops to support brewing hop-forward and “craft” style brands. Since 2007, the top two public American hop varieties grown in the U.S. and used by brewers have been Cascade and Centennial [3]. Currently, the pricing model for these hops is based to some extent on visual and aromatic quality (appearance, rub & sniff evaluations), but principally upon on a weight basis.

Dry-hopping is a brewing practice generally recognized as a cold extraction of hops in fermented or partially fermented beer [42]. The main objective of late/whirlpool-hopping and dry-hopping is to add intense hop aroma to beer with minimal bitterness [10]. Currently, the main analytical indicator that the brewing industry relies on to gauge the aroma intensity and quality of hops is total oil content. However, *Vollmer* et al. [55] recently observed that total oil content is not a great indicator of hop aroma potential during dry-hopping and suggested that the composition of hop essential oil might be more important. While a number of hop distributors report concentrations of select volatiles in hydrodistilled oil as metrics of aroma hop quality, it still remains unclear which of these volatiles actually serve as indicators of a hop aroma intensity and quality in dry-hopped beer. If the function of adding hops to beer is primarily to impart aroma during dry-hopping (as opposed to bitterness), then pricing based on different indicator(s) in hops for hop aroma intensity and quality performance in dry-hopped beer could be useful. Furthermore, these indicators may be hop variety-dependent due to the complexity of hop aroma.

Hop oil consists of hundreds of unique compounds [17, 45]. While a number of studies have investigated the key volatiles that define the aroma of hops and hop essential oil [9, 46, 52], the complexity of the brewing process and hop oil has made it challenging to establish a list of volatiles that can serve as indicators or predictors of hoppy aroma in beer [39]. The perception of hop aroma can be influenced by synergistic or masking effects that occur in mixtures of hop volatiles and within the beer itself [11, 48]. The aroma intensity and quality that hops attribute to beer depends on both the timing of hop additions throughout the brewing process as well as the influence of individual hop varieties. This is because chemical profiles between varieties are unique and hop volatiles experience differences in extraction rates, removal processes and reactions when they are added during the kettle boil, whirlpool, and/or during fermentation or post-fermentation (i.e. dry-hopping) [6, 17, 21, 23, 28, 36, 37, 43, 49-51, 53]. Therefore, defining indicators of hop aroma quality depends on how the brewer plans to use hops. Hops intended for dry-hopping might have different quality specifications than hops used in kettle/ whirlpool additions.

Past research has been heavily focused on the aroma impact of hop volatiles that are transferred during kettle or late hop additions [17, 23, 36, 50, 51]. The aroma imparted to beer as a result of kettle additions has been described as “noble”, “floral”, and “spicy” [39] because the hop volatiles that remain at levels above their detection thresholds are the oxygenated terpene [35] and sesquiterpenoid [37] fractions along with some other chemical classes [17, 23, 51]. Nevertheless, a main function of kettle hopping is to add bitterness to beer. As a result humulone concentrations, which are the precursors

of iso-humulones (the main drivers of hop derived beer bitterness), serve as the main quality index for hops intended for kettle additions.

For late and whirlpool hop additions, the contact time with hot wort is much shorter and the amounts of hops used are considerably higher. Due to the shorter contact time and reduced temperatures, there is less potential for humulones to isomerize to bitter-tasting iso-humulones [18]. Thus, brewers use whirlpool hopping as a way to impart hop aroma while reducing the hop's bitter contribution. Therefore, concentrations of hop volatiles and aroma precursors, such as thiol precursors [40] and geraniol precursors [47] are important to consider. Particularly, if aroma precursors are added prior to primary fermentation, the bound volatile can be liberated by yeast enzymatic activity during fermentation and lead to increases in beer aroma perception [43].

However, by adding hops to fermenting or fermented beer (i.e. dry-hopping), brewers can further increase hop aroma intensity without adding any iso-humulone bitterness. While studies have shown that there may be overlap in the volatiles that are important for both late- and dry- hop additions [28, 43, 50]. attempts to define harvest indicators of hop aroma potential for hops intended for dry-hop additions have been inconclusive. This is because there are a number of different dry-hopping techniques and parameters that influence the extraction rate of hop volatiles such as varietal differences [50], temperature [34], static vs dynamic extraction systems [56], scale [41], contact time [4], and yeast interactions/biotransformations [49]. The aroma quality that dry-hopping imparts to beer is different than late- and whirlpool- hopping and has been described as "citrusy", "piney" and "resinous" suggesting the importance of other aroma compounds [39].

Nickerson et al. [32] and *Engel* et al. [53] developed the hop aroma component profile (HACP) specifically for late- and dry-hopped beers. The HACP was comprised of 22 analytes found in hydrodistilled hop oil that were thought to be important for hoppy beer flavor. The HACP was developed to adjust late- or dry- hopping rates based on volatile concentrations in hydrodistilled hop oil at harvest or during storage to achieve a greater level of consistency of hop aroma in beer. While their approach was unique, the low sample size (n = 3) made it difficult to identify the individual components' significance in impacting hop aroma perception in beer or address the amount of variation that existed within single cultivars of hops. There is also the potential that different markers of hop oil composition can be responsible for the hop aroma imparted to beer for different varieties of hops. Although considerable research has been performed on investigating extraction rates of hop volatiles into beer under different parameters [6, 43, 50], few studies [7, 54] have considered the amount of chemical variation that exists within single hop varieties and none have considered the variation in the aroma intensity and quality attributed to beer during dry-hopping for a given hop variety, which prevents these studies have making conclusive predictions about which oil constituents in hops determine dry-hop aroma performance of these varieties in beer.

There is a potentially tremendous benefit to brewers, hop growers, and breeders in identifying chemical (and other) indicators that are indicative of high or low overall hop aroma intensity and quality in finished dry-hopped beer. A number of harvest and post-harvest factors have been shown to change the composition of hop oil such as nutrient or growing conditions [7, 54], hop cone ripening time [2, 25, 29, 44], kilning conditions [24], and storage conditions [52]. Therefore, identifying indicators of aroma quality could

help farmers adjust growing practices to promote and/or retain important hop volatile development and aid brewers in modifying or developing brewing strategies to best utilize their aroma hops.

For that reason, a reproducible and static pilot scale dry-hopping approach [55] was used to evaluate a large sample size of Cascade and Centennial samples over multiple harvest years. The primary objective of this project was to determine whether the total oil content of hops or an individual/combination of 16 hop oil volatiles could be used as indicators of hop aroma intensity and quality in dry-hopped beer. The goals of this study were to identify indicators of dry-hop aroma quality for Cascade and Centennial and to evaluate the variation in hop chemistry and dry-hop aroma that exists within these important varieties across multiple harvest years.

Materials and Methods

Experimental design

Over the 2014, 2015, and 2016 harvest years 84 hop samples were obtained via donations from farmers and hop dealers encompassing two American varieties that are widely used by craft brewers for dry-hopping;[3] Cascade (n = 51) and Centennial (n = 33) (Tables 1, 2 and S1, S1 see p. 101). Whole cone hops were received in the form of brewer's cuts (a 500–700 g compressed portion of a large (100 kg) hop bale) or bale cores directly from the farmer. Cascade hops were obtained following the harvest in 2014 and 2015, while Centennial hops were obtained after the 2015 and 2016 harvests. The samples were collected from different farms throughout the Pacific Northwest (in WA, OR, and ID). Upon arrival at Oregon State University, hops were placed in high barrier flexible pouches, flushed with nitrogen, sealed, and stored frozen (-20°C) for up to 5

Table 1. Overview of select harvest data for the 2014, 2015 and 2016 Cascade hops.

Sample ID	Farm State	Farm (coded)	Harvest Date	Harvest Year	Total Oil[a] (ml/100g)
CAS_06_14	WA	1	9/1	14	1.00
CAS_07_14	WA	1	9/1	14	1.70
CAS_10_14	WA	1	9/2	14	1.50
CAS_11_14	WA	1	9/2	14	0.90
CAS_13_14	WA	1	9/9	14	1.70
CAS_15_14	WA	1	9/10	14	1.70
CAS_16_14	WA	1	9/11	14	1.70
CAS_17_14	WA	1	9/13	14	1.90
CAS_18_14	WA	2	8/14	14	0.70
CAS_20_14	WA	2	8/21	14	1.00
CAS_21_14	WA	2	8/27	14	1.20
CAS_22_14	WA	2	9/12	14	2.00
CAS_24_14	WA	2	9/22	14	1.75
CAS_01_14	WA	3	8/20	14	0.60
CAS_14_14	WA	3	9/9	14	1.20
CAS_02_14	OR	4	8/23	14	0.70
CAS_04_14	OR	4	8/28	14	1.70
CAS_12_14	OR	4	9/2	14	1.00
CAS_03_14	OR	6	8/26	14	1.40
CAS_05_14	OR	6	9/1	14	1.10
CAS_08_14	OR	6	9/2	14	1.80
CAS_09_14	OR	6	9/2	14	1.30
CAS_28_15	WA	1	9/7	15	1.37
CAS_27_15	WA	1	9/5	15	0.60
CAS_12_15	WA	2	8/11	15	0.47
CAS_11_15	WA	2	8/18	15	1.03
CAS_10_15	WA	2	8/25	15	1.53
CAS_13_15	WA	2	9/2	15	1.48
CAS_14_15	WA	2	9/9	15	2.59
CAS_01_15	OR	4	9/6	15	1.69
CAS_02_15	OR	4	8/25	15	1.43
CAS_03_15	OR	4	.	15	1.19
CAS_05_15	WA	5	9/8	15	1.02
CAS_04_15	WA	5	9/8	15	0.81
CAS_07_15	ID	7	8/30	15	0.70
CAS_06_15	ID	7	9/8	15	0.91
CAS_08_15	OR	8	9/4	15	1.48
CAS_24_15	WA	9	9/1	15	0.65
CAS_21_15	ID	10	8/29	15	0.61
CAS_29_15	WA	11	.	15	1.42
CAS_26_15	WA	12	9/2	15	0.90
CAS_25_15	OR	13	8/22	15	0.82
CAS_09_15	ID	14	.	15	0.77
CAS_16_15	WA	15	9/3	15	1.08
CAS_15_15	WA	16	.	15	1.19
CAS_17_15	OR	17	.	15	1.15
CAS_18_15	WA	18	.	15	1.71
CAS_20_15	WA	19	.	15	1.27
CAS_19_15	WA	20	8/28	15	0.79
CAS_23_15	WA	21	9/1	15	1.20
CAS_22_15	ID	24	8/31	15	0.62

[a]Total oil at the time of dry-hopping. Colored by farm.

Table 2. Overview of select harvest data for the 2015 and 2016 Centennial hops.

Sample ID	Farm State	Farm (coded)	Harvest Date	Harvest Year	Total Oil[a] (ml/100g)
Cent_09_15	WA	1	8/31	15	1.97
Cent_05_15	OR	4	8/22	15	1.78
Cent_06_15	OR	4	8/26	15	1.98
Cent_07_15	OR	4	8/20	15	1.75
Cent_08_15	OR	4	.	15	1.40
Cent_10_15	WA	5	8/30	15	1.05
Cent_11_15	WA	5	9/6	15	2.06
Cent_02_15	WA	21	8/23	15	1.77
Cent_04_15	OR	22	8/20	15	1.97
Cent_12_15	ID	30	.	15	1.94
Cent_01_15	WA	38	8/21	15	1.22
Cent_03_15	OR	39	8/18	15	1.89
Cent_04_16	WA	5	9/3	16	1.62
Cent_05_16	WA	5	9/4	16	2.15
Cent_08_16	WA	5	9/2	16	1.35
Cent_02_16	WA	11	8/29	16	1.81
Cent_10_16	WA	11	9/1	16	1.66
Cent_21_16	WA	12	8/31	16	1.95
Cent_13_16	ID	14	9/11	16	2.29
Cent_17_16	ID	14	9/10	16	2.00
Cent_01_16	WA	21	8/24	16	1.50
Cent_09_16	WA	29	9/8	16	2.19
Cent_07_16	OR	31	8/26	16	1.05
Cent_15_16	WA	32	9/1	16	1.44
Cent_03_16	WA	35	8/24	16	1.29
Cent_16_16	WA	36	9/6	16	2.12
Cent_19_16	WA	37	8/24	16	1.61
Cent_11_16	WA	38	8/25	16	1.39
Cent_18_16	OR	39	8/24	16	1.36
Cent_12_16	OR	40	8/31	16	1.74
Cent_06_16	WA	41	9/13	16	2.27
Cent_14_16	WA	41	9/20	16	2.51
Cent_20_16	WA	41	9/6	16	2.16

[a]Total oil at the time of dry-hopping

months until they were used for dry-hopping on a pilot 40 L scale and chemically analyzed.

Sensory descriptive analysis performed by a trained panel was used to evaluate the hop aroma intensity and quality of these dry-hopped beers. Panel performance was evaluated using two-way analysis of variance with a mixed model (including the factors panelist, sample, and replication as well as corresponding two-way interactions). Internal process replicates were performed by dry-hopping randomly selected hop lots twice. These internal process replicates were evaluated using discrimination tests (triangle tests) to ensure that the differences observed among the treatments was not due to the dry-hopping process but rather to the differences in dry-hopped treatments.

Hydrodistillation was used to collect total oil contents on the day each dry-hopping event occurred. GC-FID and GC-MS were used to characterize 16 target hop volatiles that comprised the hydrodistilled oil. Multiple linear regression was used to identify salient aroma hop chemistry indicators (total oil and 16 selected hop volatile concentrations) that could predict hop aroma intensity and quality in beer. Additional statistical analysis approaches were used to group the dry-hopping treatments based on their sensorial or chemical similarities.

Unhopped beer production

To evaluate the dry-hop aroma of the different hop samples, an unhopped beer was prepared by commercial breweries in Portland: Craft Brew Alliance for the 2014 Cascade harvest samples and Bridgeport Brewing for the 2015 Cascade harvest samples. The unhopped wort was prepared with 86% pale two row, 13.5% Caramel 10°L, and 0.5% Caramel 120°L malt (Great Western, Vancouver, WA). The starting extract

concentrations to evaluate the 2014 and 2015 Cascade harvest samples were 10.9°P and 11.3°P, respectively. Fermentation was carried out with Wyeast 1056 ale yeast at 18-19°C for the 2014 Cascade harvest samples and Wyeast 1728 at 19-20°C was used for the 2015 Cascade harvest samples. Following fermentation and post clarification, iso-humulones (IsoHop, John I Haas, Yakima, WA) were added at a target concentration of 18 mg/L. This resulted in ~40 hL of a 15.0 BU, 4.5% ABV unhopped base beer for the 2014 Cascade harvest samples and ~55 hL of a 20.0 BU, 4.8% ABV unhopped base beer for the 2015 Cascade harvest samples.

The starting extract concentrations for the 2015 and 2016 Centennial harvest samples were 10.7°P and 11.1°P, respectively. For these dry-hopping treatments fermentation was carried out with BridgePort Brewing Company's house yeast strain at 19-20°C. Following fermentation and post clarification, iso-humulones (IsoHop, John I Haas, Yakima, WA) were added at a target concentration of 18 mg/L. This resulted in ~46 hL of a 19.7 BU, 4.4% ABV unhopped base beer to evaluate the 2015 Centennial harvest samples and ~52 hL of a 19.0 BU, 4.4% ABV unhopped base beer to evaluate the 2016 Centennial harvest samples. Beer was carbonated and packaged into 60L stainless steel kegs, shipped to Oregon State University, and held at 4°C until dry-hopping.

Dry-hopping protocol and hop preparation

The dry-hopping process established by *Vollmer* et al. [55] has been shown to be reproducible on a pilot scale. In brief, 24 hours prior to hop addition, the unhopped beer was removed from the cooler at 4°C and allowed to warm for approximately 24 hours to 15°C. For each treatment, 40 L of warmed beer was transferred into each of two modified 60 L stainless kegs with a 10.2 cm stainless steel opening fitted with a standard Sankey

D-system coupler and modified spear (Sabco, Toledo, OH, U.S.A.). A dry hopping rate of 386 g hop /hL of beer was used for each of the treatments. The whole cone hops were coarsely ground into a hop grist which was divided up by mass into two mesh bags (EcoBag, Ossining, NY). These bags were stored inside high barrier pouches flushed with N_2 until the dry-hopping event. For each dry-hop treatment, the two kegs filled with 40 L beer were temporarily de-pressurized and opened under a stream of low pressure CO2. Simultaneously, the high barrier pouch bag was opened and the mesh bag containing ground hop grist was added to the beer. After the addition, the headspace was flushed with CO_2 and purged. After purging, the kegs were inverted three times to ensure proper mixing.

After 24 hours of dry-hopping, the beer was filtered to stop the dry-hopping process. The average temperature of the dry-hopping events ranged from 13.3-15°C. Dry-hopping was stopped after 24 hr because prior work by *Wolfe* et al. [57] showed that the extraction of key hop volatiles occurred within 24 hr during dry-hopping. During filtration the two kegs were blended via a three-way fitting prior to entering a plate and frame filter using diatomaceous earth impregnated cellulose pads (HS2000, Pall Corporation, Port Washington, NY, U.S.A.) [55]. Dissolved oxygen (DO) was monitored during filtration using an Orbisphere 3100 Portable Oxygen Analyzer (Hach, Loveland, CO). Bright beer was not collected until DO was below 110 µg/L. After DO was within specification, bright, filtered beer was collected in a closed 19.6 L stainless steel keg with sufficient backpressure to reduce foaming. Between each filter run, filter pads were exchanged to prevent carry-over of beer from one treatment to the next. Filtered beer was stored at 2°C and under CO_2 overpressure (83 kPa) until sensory evaluation.

Sensory: Discrimination testing of internal process replicates

Discrimination testing was performed on the internal process replicates to examine dry-hopping process variation within treatments. The replicates were evaluated by panels of self-identified craft beer drinkers (Table S2, see p. 102). Panelists were presented with four triangle tests, the first of which was a warm up. Within each triangle test there were three samples; two of the samples were the same and one of the samples was different. Based only on the orthonasal aroma of the sample, the panelists were instructed to select the odd sample for each of the four triangle tests. For each of the 3 sets of duplicates, the design of the triangle test ensured an equal frequency of appearance of each duplicate as the "odd" sample. The serving order within each triangle tests was also randomized. The dry-hopped beer was dispensed from the keg into a pitcher, which was used to pour ~60 mL of beer into 300 mL sample glasses coded with randomized 3-digit numbers, which were covered with plastic lids. The beer was allowed to warm to room temperature before sensory analysis. Each station was used ~2 times over the course of 2 hrs.

Sensory: Descriptive analysis

To evaluate the sensory qualities of the 2014, 2015, and 2016 harvest samples, 4 descriptive analysis panels were used to quantify perceived hop intensity and quality of the dry-hopped beers. The general approach used trained panelists to scale only the orthonasal aroma of the beer treatments. Panelists were selected based on previous experience with evaluating hoppy beer flavor.

Intensive training sessions using commercial beer and a random set of blind coded dry-hop treatments were completed in advance of data collection to develop a relevant

lexicon of sensory attributes, establish a scale that best explained the differences in the samples, and to train panelists to use external reference samples as anchors for these most salient attributes. During each session, the panelists had access to external reference samples that had sensory descriptors with intensity scores assigned by consensus during training, and their purpose was to serve as anchors for the 0-15 point intensity scale. The external references and descriptive attributes used to evaluate the different harvest samples are outlined in Table S3 (see p. 103)and included the following descriptors: Overall Hop Aroma Intensity (OHAI), Citrus, and Herbal/Tea for both cultivars and additionally just for Centennial, Tropical/Catty, Tropical/Fruity, and Pine/Resinous/Dank. These sensory descriptors were not meant to encompass the entire sensory impression of the beer but just the aromatic impact of each hop to the base beer. Due to the seasonal nature of commercial beer production and panel feedback, the same commercial beers and rankings were unable to be used throughout the entire three years of the study. This change in references could have impacted how the panelists were assessing the beers on a year to year basis but is not expected to have had a major impact on the trends observed in the results. More in-depth details of each descriptive analysis panel, including the differences in how the descriptive analysis panels were carried over the different harvest years, can be found in the supporting information.

Hop chemical analysis

Concurrent with the hop sampling for the dry-hopping, approximately 150 g of the homogenized hop grist was taken for chemical analysis.

Hop essential oil analysis - reagents and standards

β-Myrcene, β-pinene, linalool, geraniol, citral, limonene, geranyl acetate, α-pinene, nerol, isobutyl isobutyrate, methyl heptanoate, β-caryophyllene, α-humulene, β-farnesene, and caryophyllene oxide were obtained from Sigma-Aldrich (St. Louis, MO). 2-Octanol was obtained through Alfa Asear (Haverhill, MA). Hexanes purchased from J.T. Baker (Center Valley, PA) were redistilled to remove impurities before analysis. Sodium chloride was purchased from EMD Millipore (Billerisa, MA).

Hop essential oil analysis

At the time of dry-hopping, hydrodistillation was performed to determine the total oil content of the homogenized hop grist using ASBC Hops-13.[1] 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 -20°C until subsequent compositional analysis.

In 2014, hop oil compositional analysis was performed under modified conditions from ASBC Hops-17 [1]. In 2015 and 2016, hop oil compositional analysis was performed using previously published methodology [27] using a HP 6890 gas chromatograph with an Agilent 5972a mass spectrometer (GC-MS) under modified conditions from ASBC Hops-17. In brief, a 1% 2-octanol (8190 ppm) solution was prepared in reagent grade hexane. Hop oils were diluted to 10% with the 1% 2-octanol/hexane solution in crimped 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 (1:20) using the septum purge option. The analytical column was a 30m x 250 µm x 0.25 µm Zebron ZB-1 MS (Phenomenex, Torrance, CA) and ultra-pure helium was used as the carrier gas (a

constant flow rate, 1.4 ml/min). The following temperature program was used: 50°C hold for 1 min, 50-180°C (2°C/min) hold for 10 minutes, 180-200°C (3°C/min) and 250°C hold for 5 minutes. The auxiliary line and mass spectrometer were operated at 280 and ~180°C respectively. The mass spectrometer was operated using electron-impact mode at 70 eV and set up to detect ions with a mass-to-charge ratio (m/z) of 30–350. 4-point calibration curves (50, 100, 400, and 800 ppm) were created for all target analytes. For high concentration target analytes (β-myrcene, α-humulene, β-caryophyllene, and β-farnesene) three additional calibration points were added (1000, 5000, and 9000 ppm). Target analytes were quantified using the following ions for each analyte: m/z 41 (geranial), m/z 45 (2-octanol), m/z 69 (β-farnesene, geraniol, nerol, neral, and geranyl acetate), m/z 71 (isobutyl isobutyrate and linalool), m/z 74 (methyl heptanoate), m/z 79 (caryophyllene oxide), and m/z 93 (α-pinene, β-pinene, β-Myrcene, β-caryophyllene, and α-humulene). The target analyte concentrations in hop oil were then standardized on a per-mass basis using the total oil content determined during hydrodistillation.

Statistical Analysis

Two-way analysis of variance with a mixed model (including the factors panelist, sample, and replication as well as corresponding two-way interactions), multiple comparison analysis (Fisher's LSD), and graphical construction were carried out using XLSTAT 2017 (Addinsoft, New York, NY). Two tailed t-tests using $\alpha = 0.05$ were carried out using JMP Pro 12 (Buckinghamshire, England). These tests and graphical outputs were used to gauge the panel and panelist effectiveness in generating descriptive data, evaluate the significant differences in aroma quality and intensity among the dry-

hopping treatments, and assess the associations between the chemical and sensory data collected.

Multiple linear regression was performed on the chemical and sensory data to identify chemical predictors of sensory intensity and quality. Model selection was conducted using the GLMSELECT procedure in SAS version 9.4 (TS1M3). Stepwise forward selection was used with sixteen hop volatiles and total oil as factors of interest in the context of a 2nd-order response surface type model (linear and quadratic in each factor as well as linear-by-linear interaction). Because of the small sample size relative to the potential number of predictors, three strategies were employed to prevent overfitting of the data. First, a model hierarchy requirement was included (quadratic terms could only enter the model when the linear term was already present in the model and a linear-by-linear interaction term could enter the model only when the two individual linear terms were already present). Second, multiple methods of selection were used (SBC, AICC and Press) to look for predictors selected by all 3 methods. Third, bootstrap resampling followed by model selection with SBC was conducted (n >= 100 resamples) to verify that predictors were selected in a large proportion of the varying bootstrap samples.

Results and Discussion

Discrimination testing: Evaluating internal process replicates

Discrimination testing on the internal process replicates found no difference between the internal process replicates (Table S2, see p. 102), confirming that the pilot dry-hopping process was reproducible and had a negligible impact on the dry-hop aroma

within the same treatments. For descriptive analysis testing one of the internal replicates was randomly selected as the observation for that hop treatment.

Descriptive analysis: Assessing the dry-hop aroma intensities and qualities of beer dry-hopped with Cascade and Centennial

The impact of the hop treatments on the sensory intensity and quality of the dry-hopped beer was evaluated via two-way ANOVAs with mixed models (Table S4 and S5, see pp. 108-109). This outcome demonstrated the broad and significant range of aromatic intensities and qualities that can occur within a single cultivar of hops depending on where the hop was grown, how it was grown, and when and how it was picked and dried. Significant panelist × sample effects were observed for some of the attributes and this interaction indicates that there were slight differences in the way the panelists scaled these attributes [31]. Significant panelist × rep interactions were also observed for some of the hop aroma quality attributes (mainly Herbal/Tea) and this interaction indicates that from one session to another, panelist(s) scores were not consistent for all the products. This interaction mainly occurred because panelist(s) misidentified the unhopped beer (control) during at least one session. The F-values for all significant interactions were substantially lower than those for the sample and panelist effects and, with these few exceptions, the panelists could effectively replicate their attribute scaling for the samples across all replications thereby demonstrating generalized consistency throughout each of the descriptive analysis panels.

The least squared means and results from Fisher's LSD ($p < 0.05$) multiple comparisons for the sensory attributes from the descriptive analysis panels were summarized (Table S6 & S7, see pp. 110-111). Fisher's LSD tests were chosen as the mean comparison technique instead of a more conservative method, such as Tukey's

HSD tests, to highlight the potential differences that exist between the dry-hop aroma profiles of the treatments. Over the four panels, although the unhopped base was identified by panelists to have some aroma, it was not grouped with any of the dry-hopped treatments for any of the aroma attributes.

For Cascade, Overall Hop Aroma Intensity (OHAI) was significantly correlated with citrus quality for the 2015 samples but not for the 2014 samples (Tables S8 and S9, see pp. 112-113). An early harvest sample in 2014 (CAS_01_14, 8/20/14) attributed a high aroma intensity to beer that was mainly Herbal in quality, and this single point disrupted the OHAI-Citrus correlation for 2014. Therefore, differences in citrus quality, as opposed to OHAI, were used to compare the Cascade dry-hop treatments over the two harvest years. The average Citrus scores for the highest LSD groupings were 1.7x and 1.3x higher over the 2014 and 2015 harvest years respectively when compared to the lowest Citrus LSD groupings (Table S6, see p. 110). Although there was no significant difference (two-tailed t-test, p-value = 0.94) in the OHAI ratings between the two harvest years. The dry-hop treatments in 2015 were rated significantly higher in both Herbal and Citrus (two-tailed t-test, p-value < 0.001) than the dry-hop treatments from 2014. As stated previously, this could be due to changes in hop chemistry as a function of harvest year or changes in the descriptive analysis panels. Previous research has also shown that Cascade dry-hop quality can change between harvest years [7].

For Centennial, OHAI was significantly correlated with both Citrus and Tropical/Catty over the two harvest years (Table S10, see p. 114). With the exception of Tropical/Catty, which was scored higher in the 2015 samples (two-tailed t-test, p-value = 0.01), there were no significant differences (two-tailed t-test, p-value = 0.14) observed

over the two harvest years between the sensory ratings. When compared to the lowest OHAI LSD groupings, the average OHAI scores for the highest LSD groupings were 1.4x and 1.8x higher in OHAI for the 2015 and 2016 harvest years respectively (Table S7, see p. 111).

These results highlight that at the same static dry-hopping rate of 3.86 g/L there are significant and measurable differences in the aroma intensities and qualities attributed to beer from different commercially available Cascade and Centennial samples procured from within the same harvest year. Understanding what drives these differences will help create strategies to produce higher quality aroma hops and more consistent dry-hopped beer.

Chemical analyses: Comparing hop variety and harvest year

The samples of Cascade and Centennial hops used in this study represented a wide range of total oil contents (Table 1 & 2) as well as concentrations of the 16 hop volatiles (Tables S11-S13, see pp. 115-117), and the variation was visible both within and between the different harvest years. When comparing the entire data sets between the two varieties the Centennial samples had significantly higher total oil contents as well as concentrations of many of the hop volatiles (two-tailed t-test, p-value < 0.05). This was expected, and in fact Centennial is sometimes anecdotally referred to as “super Cascade” within the brewing industry. Nonetheless, Cascade had the highest concentrations of geranyl acetate and β-farnesene (two-tailed t-test, p-value < 0.0001). β-Farnesene has been shown to be a marker compound of Cascade and was not detected in Centennial [19, 44]. Both α-pinene and β-myrcene concentrations were similar in Cascade and Centennial

(two-tailed t-test, p-value = 0.30 and 0.46, respectively). Other studies have also shown that hop essential oil composition is varietal specific [17, 19, 44].

When comparing the total oil content and the concentrations of the 16 hop volatiles in each variety between the two harvest years, significantly higher total oils and concentrations of β-myrcene, linalool, nerol, neral, geraniol, geranial, β-caryophyllene, α-humulene, β-farnesene, and caryophyllene oxide were observed in the 2014 Cascade samples as compared to 2015 (two-tailed t-test, p-value < 0.03).While, significantly higher concentrations of geranyl acetate, limonene, methyl heptanoate, α-pinene and isobutyl isobutyrate were observed in the 2015 Cascade harvest samples (two-tailed t-test, p-value < 0.002) and concentrations of β-pinene were not different between the harvest years (two-tailed t-test, p-value = 0.16). For Centennial there was no difference observed in total oil, α-humulene, nerol, neral, β-caryophyllene, and linalool between the harvest years (two-tailed t-test, p-value =0.17). However, concentrations of β-myrcene, methyl heptanoate, geraniol, limonene, α-pinene, β-pinene, and isobutyl isobutyrate were higher in the 2015 samples (two-tailed t-test, p-value < 0.03). Concentrations of geranyl acetate, geranial, and caryophyllene oxide were higher in the 2016 samples as compared to 2015 (two-tailed t-test, p-value < 0.0001). These observations are in agreement with *Forster* et al. [7] who also showed that oil composition can vary within single varieties between harvest years.

Notable, while many of the hop volatiles were positively correlated with one another (Tables S8-S10, see pp. 112-114), caryophyllene oxide was often negatively correlated with most of the hop volatiles regardless of the cultivar. These trends are in

agreement with *Nielsen* et al. [33] who hypothesized caryophyllene oxide to be a marker of hop oxidation during post-harvest processing.

It is clear that harvest year had a very pronounced impact on the dry-hop aroma quality/intensity and chemical characteristics of the hop lots, especially for the Cascade. The climate in the Pacific Northwest over these harvest years might explain this observation since 2015 was unusually dry and hot compared to 2014 and 2016 [13-15]. In addition, prior research has identified trends between growing regions and hop chemistry [22, 54]. In this study and in agreement with *Forster* et al. [7], growing regions/ terroir did not seem to explain the observed differences in hop lot chemistry or dry-hop aroma sensory (data not shown). However, there were some significant correlations observed between harvest date and the volatile concentrations in hop oil, total oil contents, and dry-hop aroma potential [25]. This indicates that harvest maturity may have more of an influence on dry-hop aroma quality and intensity as well as chemistry than growing region. These observations are indirectly supported by a number of published studies [12, 16, 29, 38, 44].

Multiple linear regression modeling - identifying indicators of hop aroma intensity and quality in Cascade hops

Model selection was performed in SAS GLMSELECT using total oil content and the concentrations of the 16 hop volatiles (including linear, quadratic, and linear-by-linear interactions). The data for the Cascade samples were modeled on a harvest year basis due to the significant year effects in both the chemistry and sensory results and the sample sizes for the two harvest years of Cascade (n = 22 for 2014 and n = 29 for 2015). A key assumption of model selection via multiple linear regression is that the data are treated as independent observations. This was considered a possible issue for the samples

from the 2014 harvest because multiple samples were obtained from the same farms and fewer farms were represented in the sample set as compared to the 2015 data set. Therefore, multiple linear model selection of the Cascade hops began with the 2015 harvest year because it encompassed the most extensive and diverse samples originating from 20 unique farms throughout Washington, Oregon, and Idaho (Tables 1 and S1, S1 see p. 101).

Multiple linear regression modeling was applied to the 2015 harvest data multiple times using the different selection criteria (SBC, AICC and Press) to predict OHAI. The most important (and in nearly all cases the sole) predictor of OHAI was geraniol. This single-component model fit OHAI relatively well ($R^2 = 0.56$) for the 2015 harvest samples and was selected over 15x more than the next most frequently selected model identified via resampling. However, when using the 2014 harvest data no predictors entered the model for OHAI. As mentioned previously, this result is likely due to the early harvest sample (Cas_01_14) which had a very high OHAI impression but was dominated by Herbal/Tea aroma as opposed to Citrus. The dry-hop aroma quality of Cascade has recently been shown to vary from Herbal to Citrus during ripening [25]. indicating that citrus quality may serve as an indicator of dry-hop aroma development for Cascade.

Therefore, dry-hop citrus quality was modeled using the same approach on the 2015 samples. Again, the only predictor that was selected with all 3 selection criteria was linear in geraniol. This simple linear model described Citrus relatively well ($R^2 = 0.50$). When using SBC for selection, linear in geraniol came into 69% of the models (it was the predictor with the highest frequency). For comparison linear in total oil content was

selected in only 5% of the models. Geranial was identified as a candidate for future investigation because it entered at least one selection method, but not all. Interestingly, total oil content did not enter any of the models as a predictor. Comparing the linear model in geraniol ($R^2 = 0.50$) to the linear model in total oil content ($R^2 = 0.24$), it is evident that geraniol describes more of the variation for dry-hop citrus quality (Figure 1, C and D). Furthermore, an outlier sample with a very high total oil content (total oil = 2.59) was very influential in the relationship between total oil content and citrus quality (Figure 1 D). If this sample were removed from the dataset the slope and R^2 between total oil and citrus quality would decrease considerably. Using multiple regression with both geraniol and total oil in the model shows there is still strong evidence for a linear in geraniol effect even after total oil is already in the model ($p = 0.0011$), but there is no evidence of any predictive ability for total oil with geraniol already in the model ($p = 0.56$) (Table 3).

Similar to 2015, performing model selection for the 2014 Cascade harvest found that linear in geraniol was the only predictor selected by all 3 selection methods. Linear in geraniol described citrus quality ($R^2 = 0.44$) much better than total oil ($R^2 = 0.07$) (Figure 1, A and B). Using SBC for selection linear in Geraniol came into the model for 91% of the samples (the highest). No other predictor came into > 60% of the samples. Again, for comparison linear in total oil content was selected in only 15% of the samples.

It is evident (Figure 1, A and C) that slopes between geraniol and Citrus were different between these two harvest years, indicating a significant year effect. Thus, despite having similar geraniol concentrations over the two years, the hops produced different citrus intensities. This could be a function of hop chemistry or differences in

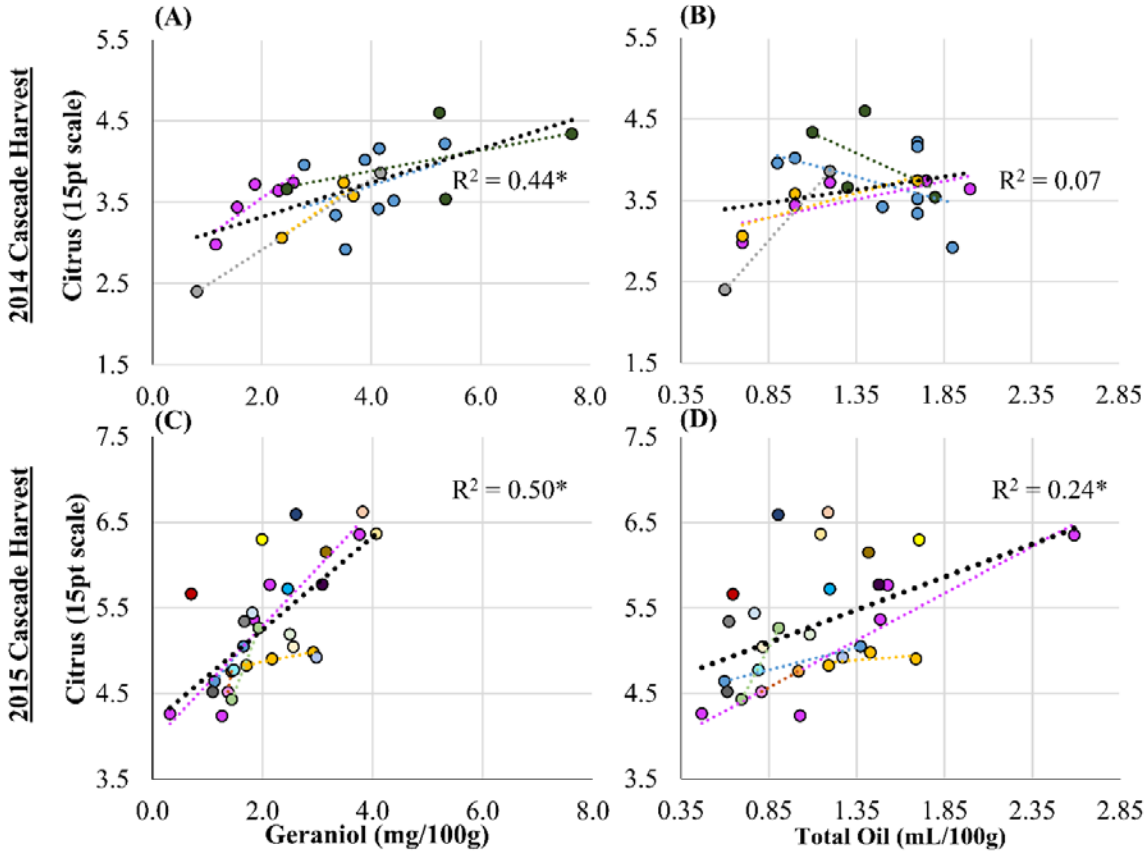

Figure 1. Comparing the relationships between dry-hop citrus quality and hop quality factors (Geraniol concentration in hydrodistilled hop oil (mg/100g) (**A** and **C**) and total oil content (mL/100g) (**B** and **D)**) for the 2014 and 2015 **Cascade** hops.

Table 3. Multiple regression parameter results for the **2015 Cascade** hops highlighting the importance of Geraniol concentration in hydrodistilled hop oil (mg/100g) compared to total oil content (mL/100g) as an indicator of Citrus dry-hop aroma quality.

Parameter Estimates					
Variable	**DF**	**Parameter Estimate**	**Standard Error**	**t Value**	**Pr > |t|**
Intercept	1	4.073	0.281	14.49	<.0001
Geraniol	1	0.499	0.136	3.68	0.0011
Total Oil	1	0.163	0.280	0.58	0.5658

how the panel scaled citrus quality over the two harvest years. This makes it challenging to assign hard boundaries around what makes an optimal Cascade for dry-hopping based on geraniol concentrations. However, the geraniol concentrations (mg/100g) for the four lowest citrus samples in both the 2014 and 2015 harvests ranged from 0.8-3.5 and 0.3-1.4, respectively. While the geraniol concentrations (mg/100g) for the four highest scored citrus samples in both 2014 and 2015 harvests ranged from 4.2-7.7 and 2.6-4.1, respectively. Despite being broad, these ranges may serve as a good starting place to guide organoleptic evaluations of Cascade hops on a year-to-year basis.

When considering Pearson correlations between citrus quality and the 16 hop volatiles over the two harvest years (Tables S8 and S9, see pp. 112-113), geraniol had the highest correlations with citrus quality over the two harvest years. Notably, other hop volatiles often associated with dry-hop flavor, such as β-myrcene (which often comprises ~50% of Cascade hop oil), were not highly correlated with Cascade dry-hop aroma quality. This observation is in agreement with other studies [26] and it is hypothesized that the physical–chemical properties of these analytes make them insoluble in beer and therefore they are not extracted to an appreciable degree during dry-hopping in clarified beer. However, recently concentrations of these volatiles have been shown to be elevated in hazy hop forward beers [30].

The significance of geraniol as an indicator of Cascade aroma in beer is supported by work of *Peacock* et al. [35] which highlighted the importance of geraniol in describing the specific “kettle-hop” and floral hop aroma of Cascade as compared to European hop varieties. However, as stated previously kettle hopping presents an entirely different set of extraction conditions/kinetics as well as oxidation/biotransformation reactions for hop

volatiles as compared to dry-hopping. Recently, *Takoi* et al.[47] identified Cascade as a 'geraniol rich hop' indicating that Cascade has high levels of free geraniol and *Vollmer* et al. [28] identified geraniol as a charter impact compound for dry-hop beer flavor. One should also keep in mind that in the presence of yeast geraniol may be transformed to other compounds such as citronellol [20]. In the present study dry-hopping was performed in the absence of yeast. While it is evident geraniol is not the only driver of Cascade aroma quality, these results offer evidence that geraniol is a better than total oil at gauging the aromatic intensity of Cascade hops used for dry-hopping.

Multiple linear regression modeling -Identifying indicators of hop aroma quality in Centennial hops

When performing model selection on Centennial, the data were combined for 2015 and 2016 due to the smaller sample sizes (n = 12 and n = 21 respectively). To incorporate possible differences between years, harvest year was included in the model selection process as a classification variable to allow there to be both additive year effects and year-by-predictor interactions.

For citrus quality, the only predictor that came into the model for every model selection method was linear in β-pinene (R^2 = 0.45). Caryophyllene oxide was identified as a candidate for future investigation because it entered at least one selection method, but not all. When resampling with SBC for model selection, linear in β-pinene came into the model for 81% of the samples (the highest percentage of any predictor). By comparison, total oil content was selected for the model in only 24% of the samples. Comparing linear in β-pinene (R^2 = 0.46) to linear in total oil content (R^2 = 0.29), it is evident that β-pinene describes more of the variation for dry-hop citrus quality in the Centennial hop data (Figure 2). Multiple regression with both β-pinene and total oil in

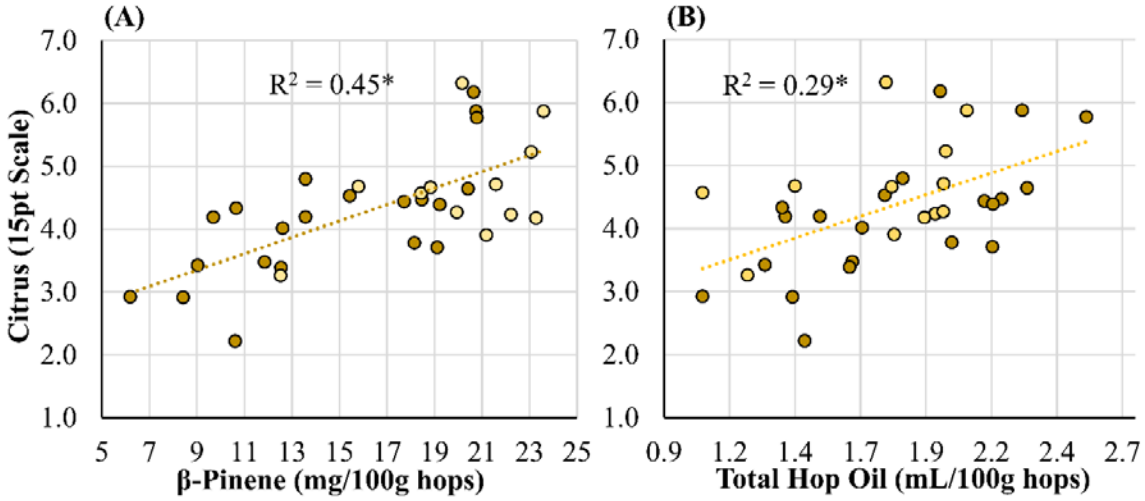

Figure 2. Comparing the relationships between dry-hop citrus quality with **A.** β-pinene concentrations in hydrodistilled hop oil (mg/100g) and **B.** total oil content (mL/100g) for the 2015 (light yellow) and 2016 (dark yellow) **Centennial** hops.

Table 4. Multiple regression parameter results for the **2015 and 2016 Centennial** hops highlighting the importance of β-pinene concentration in hydrodistilled hop oil (mg/100g) compared to total oil content (mL/100g) as an indicator of citrus dry-hop aroma quality.

Parameter Estimates					
Variable	**DF**	**Parameter Estimate**	**Standard Error**	**t Value**	**Pr > |t|**
Intercept	1	1.993	0.618	3.22	0.0030
β-pinene	1	0.118	0.039	3.05	0.0047
Total Oil	1	0.219	0.511	0.43	0.6711

the model shows that once β-pinene is in the model, there is no evidence of any predictive ability for total oil (p=0.67). Conversely with total oil in the model, there is still strong evidence for a linear in β-pinene effect (p=0.0047) (Table 4).

The β-pinene concentrations (mg/100g) for the four lowest citrus samples over the 2015 and 2016 harvests ranged from 6.2-12.5, while the four highest ranged from 19.9-23.6. This shows that the highest rated citrus samples had approximately twice as much β-pinene as the lowest citrus samples. These ranges, while not absolute, provide an initial guide to the relative magnitude of β-pinene on the organoleptic evaluations of Centennial hops.

Recently, *Takoi* et al. showed that β-pinene was found in relatively high concentrations in Centennial and Citra hops, but was not found to be transferred into beer during dry-hopping at high rates [5]. This is evidence that β-pinene might not be the compound that is directly responsible for the hop aroma impression of dry-hopped beer. In the present study we do not attempt to characterize citrus quality by measuring the hop volatiles in beer. Rather, the goal was to examine the composition of hops and hop oil and identify a marker or markers useful to brewers for estimating their aroma performance in beer. While there was a significant correlation between total oil and OHAI, total oil did not enter any of the statistical models as a predictor for any of the sensory descriptors. Furthermore, total oil was less effective than β-pinene for describing Centennial dry-hop citrus quality (Figure 2).

Conclusions/ Industrial Considerations

The objectives of this study were to examine the composition of hops and hop oil with the goal of identifying a marker or markers in hops that are useful to breeders,

growers, and brewers for estimating dry-hop aroma performance in beer. From the results, it is clear that a significant amount of variation in both hop chemistry and dry-hop aroma potential exists within Cascade and Centennial hops within a single harvest year and across multiple harvest years. When comparing the results of multiple linear regression modeling over the three harvest years, total oil was never selected as a predictor of hop aroma intensity for either Cascade or Centennial. These results support those of *Vollmer* et al. [55] and suggest that a hop's total hop oil content may not serve as the best indicator of its dry-hop aroma potential. Specific hop volatile components, namely geraniol for Cascade and β-pinene for Centennial, were identified as statistically relevant for forecasting dry-hop aroma quality. These results suggest that the markers of dry-hop aroma are varietal-dependent. Although these single volatiles only describe approximately 50% of variation in the dry-hop citrus quality these varieties display in beer, they offer improvement over total oil content which explains less than 30% of the variation. It is important to point that in the present study dry-hopping was performed in the absence of yeast. In the case of dry-hopping in the presence of yeast, biotransformation reactions should be considered as they have the potential to modify the aromatic quality and intensity contributions of hop volatiles [49].

It is clear there are other hop volatiles that may add additional ability to forecast a hop's aroma potential during dry-hopping. For instance, there is increasing evidence that polyfunctional thiols, which were not considered in this study, are important for dry-hop beer flavor [8, 22, 40, 46, 48]. Future studies should investigate the variation of these volatiles within single varieties at harvest and evaluate if they play a role in predicting that dry-hop aroma of hops in beer. Looking beyond just hop aroma, recent studies have

shown that humulinones (as a result of hop acid oxidation) can contribute significantly to beer bitterness in hop forward beers [10]. Therefore, concentrations of humulinones should also be considered as a quality metric for hops destined for dry-hopping as they directly impact beer flavor.

Interestingly, total oil content did not serve as a good predictor of hop aroma intensity in dry-hopped beer. And in some instance, there existed a negative correlation between total oil content and overall hop aroma intensity (Figure 1). By comparison, these negative correlations were not observed between geraniol and overall hop aroma intensity. One possible explanation for this observation is that post-harvest processing factors (kilning, baling, etc.) have a greater impact on total oil content than geraniol. Given that a majority of hop oil (>50%) is made up of hydrocarbons, such as β-myrcene, β- caryophyllene, and α-humulene, which are less aromatically important than the terpene alcohols and esters for dry-hop aroma, their loss during post-harvest processing and kilning may have less of an impact on dry-hop aroma potential than losses in geraniol. Future work should investigate the impact of post-harvest processing, such as kilning on hop chemistry and dry-hop aroma potential in beer.

Results from this study offer brewers and growers insight on how best to use analytical information that is already being collected on hops. Hop companies routinely measure geraniol and β-pinene, along with other hop volatiles, in addition to total oil. These results suggest that a hop's total oil content is a poor indicator for forecasting a hop's aroma potential for dry-hopping and that these hop volatiles (geraniol for Cascade and β-pinene for Centennial) may be more important to consider. When examined from the brewer's or hop grower's quality control perspective, the concentrations of geraniol

for Cascade and β-pinene for Centennial could be used to guide organoleptic evaluations (color, rub-and-sniff, etc.) when assessing hop aroma quality on a year-to-year basis and as a way to generate unbiased data for selecting hops destined for dry-hopping. For instance, high geraniol Cascade or high β-pinene Centennial hops might be better suited for dry hopping, while those containing lower amounts of these volatiles might be better suited for kettle or whirlpool hopping. Concentrations of these hop volatiles might also serve as potential targets for hop breeders who are trying to develop higher yielding and more disease resistant replacements with similar aroma profiles to these popular American varieties. Finally, this information is also relevant to growers who can fine-tune harvest timing or post-harvest processing parameters to promote the production of these hop volatiles [25].

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Supporting Information

Table S1. Overview of the Cascade and Centennial hop samples procured from hop distributors following the 2014, 2015 and 2016 harvests.

Cultivar		Cascade		Centennial	
Harvest Year		14	15	15	16
Region	Farm	(n)	(n)	(n)	(n)
WA	1	8	2	1	.
	2	5	5	.	.
	3	2	.	.	.
	5	.	2	2	3
	9	.	1	.	.
	11	.	1	.	2
	12	.	1	.	1
	15	.	1	.	.
	16	.	1	.	.
	18	.	1	.	.
	19	.	1	.	.
	20	.	1	.	.
	21	.	1	1	1
	29	.	.	.	1
	32	.	.	.	1
	35	.	.	.	1
	36	.	.	.	1
	37	.	.	.	1
	38	.	.	1	1
	41	.	.	.	3
Total		15	18	5	16
OR	4	3	3	4	.
	6	4	.	.	.
	8	.	1	.	.
	13	.	1	.	.
	17	.	1	.	.
	22	.	.	1	.
	31	.	.	.	1
	39	.	.	1	1
	40	.	.	.	1
Total		7	6	6	3
ID	7	.	2	.	.
	10	.	1	.	.
	14	.	1	.	2
	24	.	1	.	.
	30	.	.	1	.
Total		.	5	1	2
Overall Total		22	29	12	21

Table S2. Discrimination (Triangle) test results of internal dry-hopping process replicates.

2015 Cascade internal dry-hopping process replicates						
Triangle Tests	**Number of hoppy beer consumers**	**Number of females**	**Age range**	**Number of correct responses**	**Z-value**	**p-value**
Cas_11_15_1 vs Cas_11_15_2	54	20	23-66	19	0.14	0.44
Cas_10_15_1 vs Cas_10_15_2	54	20	23-66	15	-0.99	0.16
Cas_13_15_1 vs Cas_13_15_2	54	20	23-66	24	1.56	0.06
Cas_14_15_1 vs Cas_14_15_2	54	20	23-66	21	0.71	0.24
2015 Centennial internal dry-hopping process replicates						
Cent_12_15_1 vs Cent_12_15_2	40	17	21-66	13	-0.28	0.39
2016 Centennial internal dry-hopping process replicates						
Cent_7_16_1 vs Cent_7_16_2	43	17	21-66	14	-0.12	0.45

Table S3. Sensory reference standards with intensity scores used in descriptive analysis panels over the different harvest years.

	OSU beer%						**Commercial beer**						
Attributes	**Unhopped Control**	**386 g/hL**	**1600 g/hL**	**100% Chinook**	**100% Centennial**	**100% Cascade**	**Hop Valley Sir Orange-A-Lot**	**Ballast Point Grapefruit Sculpin**	**Hop Valley Citrus Mistress**	**Sierra Nevada Pale Ale**	**Ballast Point Pineapple Sculpin**	**10-Barrel Joe IPA**	**Founders All Day IPA**
Cascade 2014 Harvest Descriptive Analysis Anchors													
OHAI*	.	.	.	.	.	.	8	15	.	.	.	.	.
Cascade 2015 Harvest Descriptive Analysis Anchors													
OHAI	0	8-9	14-15	.	.	.	.	14-15	7-8	.	.	.	.
Citrus	0	7-8	5-6	.	.	.	.	13-14	6-7	.	.	.	.
Herbal/Tea	0	5-6	12-13	.	.	.	.	1-2	6-7	.	.	.	.
Centennial 2015 Harvest Descriptive Analysis Anchors													
OHAI	0	.	.	6	9	8	.	.	.	7	10-11	14-15	.
Citrus	0	.	.	2	7	8	.	.	.	6	6	5-6	.
Herbal/Tea	0	.	.	3	4-5	6	.	.	.	5	2	1	.
Tropical/Catty	0	.	.	4-5	2-3	3	.	.	.	3	4	9-10	.
Tropical/ Fruity	0-1	.	.	2-3	5-6	3	.	.	.	4	7-8	4-5	.
Pine/ Resinous/ Dank	0	.	.	1	2	2	.	.	.	2	4	4	.
Centennial 2016 Harvest Descriptive Analysis Anchors													
OHAI	0	.	.	.	.	.	.	†	.	5-6	.	†	12
Citrus	0	.	.	.	.	.	.	11	.	3	.	5-6	6-7
Herbal/Tea	0	.	.	.	.	.	.	.	.	4	.	1	5
Tropical/Catty	0	.	.	.	.	.	.	.	.	1	.	9-10	3-4
Tropical/ Fruity	0-1	.	.	.	.	.	.	7-8	.	1	.	4-5	2-3
Pine/ Resinous/ Dank	0	.	.	.	.	.	.	.	.	2	.	4	7-8

*OHAI = Overall Hop Aroma Intensity (.) did not measure

†Did not scale OHAI for this external reference standard

%These pilot beers were made in the Oregon State University pilot brewery. They served as external references alongside the commercial beers so that the panelists could anchor their attribute scaling during the descriptive analysis panels. The scores for these beers were defined by the panelists during the training sessions.

Sensory analyses protocols and panel/panelist validation

Panelists were given ~60 mL of dry-hopped beer in a 300mL glass covered with a plastic lid. For the 2014 Cascade harvest samples beer was packaged and served from bottles that had been warmed to room temperature for 35-45 min. For the rest of the study beer was served from two 8-head draft systems operating at at ~1°C and at 82.7 kPa (Micro Matic, Northridge, CA). Beer was poured into sample glasses ~1 hour before the start of testing, capped with a plastic lid, and allowed to warm to room temperature. For the 2014 Cascade harvest samples panelist responses were collected on paper ballots. For the rest of the study panelist responses were collected on Chromebook tablets using Qualtrics (Provo, UT). For each of these sessions, Qualtrics was also used to randomly assign the serving order of samples for each panelist.

Descriptive Analysis – Cascade 2014 Harvest

23 dry-hopped beers (22 different hop lots (dry-hopped at 3.8 g/L) and one unhopped control) were evaluated by a trained panel experienced with assessing hop forward beer aroma. The panel was comprised of 11 trained panelists (9 males and 2 females; 25-65 yrs. old). Three intensive training sessions were completed in advance of data collection. Based on discussion from these training sessions the final ballot included the attributes: Overall Hop Aroma Intensity (OHAI), Citrus, Herbal, Resinous/hop oil, Tropical Fruit to be evaluated on a 0-15 point scale. Over the course of 15 sessions, the panelists evaluated all of the samples five times in a randomized fashion. 10 samples were evaluated per session and the presentation order was blocked by replication and randomized for each panelist.

Descriptive Analysis – Cascade 2015 Harvest

30 dry-hopped beers (29 different hop lots (dry-hopped at 3.8 g/L) and one unhopped control) were evaluated by a trained panel experienced with assessing hop forward beer aroma. The panel was comprised of 13 trained panelists (11 males and 2 females; 25-66 yrs. old). Four intensive training sessions were completed in advance of data collection. Based on discussion from these training sessions and the results from the 2014 Cascade harvest panel, the final ballot included the attributes: Overall Hop Aroma Intensity (OHAI), Citrus, and Herbal/Tea to be evaluated on a 0-15 point scale. An efficient resolvable incomplete block design was used to create a presentation order for the samples across four replications (SAS, Cary, NC). Over the course of 20 sessions, the 13 panelists evaluated all the samples five times in a randomized fashion. The first replication (i.e. sensory block) was used to familiarize the panelists with the samples and the testing environment. Because of the large number of treatments, it took the panelists four sessions (3 sessions of 8 samples and 1 session of 9 samples) to evaluate all the hopped samples per replication.

Descriptive Analysis – Centennial 2015 Harvest

13 dry-hopped beers (12 different hop lots and one unhopped control) were evaluated by 15 trained panelists experienced in evaluating hop forward beer aroma (11 males and 4 females; 25-66 yrs old). Four intensive training sessions were completed in advance of data collection. Based on discussion from these training sessions the final ballot included the attributes: OHAI, Citrus, Herbal/Tea, Pine/Resinous/Dank, Tropical/Fruity, and Tropical/Catty to be evaluated on a on a 0-15 point scale. An efficient resolvable incomplete block design was used to create a presentation order for

the samples across four replications (SAS, Cary, NC). Unlike the 2014 and 2015 Cascade harvest descriptive analysis panels the unhopped control was nested into each session. Over the course of 10 sessions, the 15 panelists evaluated all the samples five times in a randomized fashion. The first replication was used to familiarize the panelists with the samples and the testing environment. It took the panelists 2 sessions, of 7 samples, to experience all the hopped samples per replication.

Descriptive Analysis –Centennial 2016 Harvest

12 trained panelists (9 males and 3 females; 21-55 yrs old) were used to evaluate the 2016 Centennial harvest samples. 22 dry-hopped beers (21 different hop lots and 1 unhopped control) were evaluated. Four intensive training sessions were completed in advance of data collection. Based on discussion from these training sessions the final ballot included the attributes: OHAI, Citrus, Herbal/Tea, Pine/Resinous/Dank, Tropical/Fruity, and Tropical/Catty to be evaluated on a on a 0-15 point scale. To evaluate the Centennial samples an efficient resolvable incomplete block design was used to create a presentation order for the samples across four replications (SAS, Cary, NC). The unhopped control was nested into each session. It took 3 sessions of 8 samples to experience all the treatments per replication. Over the course of 15 sessions, the 15 panelists evaluated all the Centennial samples five times in a randomized fashion. The first 2 replications were used to familiarize the panelists with the samples.

Descriptive Analysis – Panelist/ panel evaluation

Following each descriptive analysis panel, every panelist was evaluated on their performance based upon their ability to discriminate differences among the dry-hop treatments on at least one of the sensory attributes, replicate among all sessions, and their

lack of interactions. Any panelists that failed these three criteria were removed from further analyses.

For the 2014 Cascade harvest samples 1 panelist of the original 11 panelists was removed from the data set resulting in 50 observations per attribute, per sample. For the 2015 Cascade harvest samples, 3 panelists of the original 13 panelists were removed from the data set resulting in 40 observations per attribute, per sample. For the 2015 Centennial harvest samples, 5 panelists of the original 15 panelists were removed from the data sets resulting 40 observations per attribute, per sample. For the 2016 Centennial harvest samples 5 panelists of the original 12 panelists were removed from the data sets resulting in 21 observations per attribute, per sample.

Table S4. Mixed model analysis of variance of the sensory attributes for the descriptive analysis panels over the harvest years for **Cascade** treatments.

2014 Cascade Mixed Model ANOVA								
			OHAI		**Citrus**		**Herbal/ Tea**	
Source	**Type**	**DF**	**F**	**P-value**	**F**	**P-value**	**F**	**P-value**
Sample	Fixed	22	8.7	**< 0.0001**	3.6	**< 0.0001**	8.9	**< 0.0001**
Panelist	Random	9	22.6	**< 0.0001**	29.0	**< 0.0001**	10.8	**< 0.0001**
Rep	Fixed	4	1.3	0.289	0.9	0.496	1.1	0.375
Sample*Panelist	Random	198	2.3	**< 0.0001**	1.9	**< 0.0001**	2.1	**< 0.0001**
Sample*Rep	Fixed	88	1.2	0.146	1.0	0.431	1.1	0.213
Panelist*Rep	Random	36	0.8	0.819	1.6	**0.016**	1.7	**0.009**
Error		792						
2015 Cascade Mixed Model ANOVA								
			OHAI		**Citrus**		**Herbal/ Tea**	
Source	**Type**	**DF**	**F**	**P-value**	**F**	**P-value**	**F**	**P-value**
Sample	Fixed	29	6.8	**< 0.0001**	4.4	**< 0.0001**	3.9	**< 0.0001**
Panelist	Random	9	24.6	**< 0.0001**	20.9	**< 0.0001**	28.3	**< 0.0001**
Rep	Fixed	3	0.2	0.874	0.5	0.659	0.2	0.903
Sample*Panelist	Random	261	1.5	**< 0.0001**	1.5	**< 0.0001**	1.3	**0.007**
Sample*Rep	Fixed	87	1.0	0.451	0.8	0.903	1.3	**0.032**
Panelist*Rep	Random	27	1.3	0.134	1.1	0.328	1.5	**0.041**
Error		783						

Values in **bold** indicate p-value < 0.05

Table S5. Mixed model analysis of variance of the sensory attributes for the descriptive analysis panels over the harvest years for **Centennial** treatments.

2015 Centennial Mixed Model ANOVA														
			OHAI		**Citrus**		**Tropical/ Catty**		**Tropical/ Fruity**		**Pine/Resinous/ Dank**		**Herbal/ Tea**	
Source	**Type**	**DF**	**F**	**P-value**	**F**	**P-value**	**F**	**P-value**	**F**	**P-value**	**F**	**P-value**	**F**	**P-value**
Sample	Fixed	12	41.1	**< 0.0001**	27.6	**< 0.0001**	11.5	**< 0.0001**	8.2	**< 0.0001**	13.0	**< 0.0001**	16.9	**< 0.0001**
Panelist	Random	9	1.5	0.201	4.8	**0.001**	4.5	**0.001**	7.0	**< 0.0001**	10.9	**< 0.0001**	9.4	**< 0.0001**
Rep	Fixed	3	1.0	0.419	0.8	0.504	0.6	0.598	0.9	0.458	0.5	0.671	0.7	0.579
Sample*Panelist	Random	108	1.4	**0.026**	1.3	0.056	1.4	**0.013**	1.9	**< 0.0001**	1.4	**0.008**	2.1	**< 0.0001**
Sample*Rep	Fixed	36	0.9	0.611	1.3	0.134	1.2	0.226	1.1	0.350	0.9	0.647	1.2	0.255
Panelist*Rep	Random	27	1.3	0.176	1.9	**0.005**	0.6	0.913	1.6	**0.038**	2.5	**< 0.0001**	1.4	0.113
Error		364												
2016 Centennial Mixed Model ANOVA														
Source	**Type**	**DF**	**F**	**P-value**	**F**	**P-value**	**F**	**P-value**	**F**	**P-value**	**F**	**P-value**	**F**	**P-value**
Sample	Fixed	21	18.6	**< 0.0001**	11.8	**< 0.0001**	9.0	**< 0.0001**	6.3	**< 0.0001**	9.9	**< 0.0001**	7.3	**< 0.0001**
Panelist	Random	6	9.8	**< 0.0001**	21.7	**< 0.0001**	15.9	**< 0.0001**	18.9	**< 0.0001**	29.	**< 0.0001**	6.2	**0.000**
Rep	Fixed	2	1.3	0.308	1.6	0.243	0.6	0.547	2.8	0.100	1.2	0.319	0.03	0.974
Sample*Panelist	Random	126	2.4	**< 0.0001**	2.8	**< 0.0001**	2.2	**< 0.0001**	1.8	**< 0.0001**	2.4	**< 0.0001**	2.9	**< 0.0001**
Sample*Rep	Fixed	42	1.5	**0.024**	1.7	**0.007**	1.9	**0.002**	1.2	0.187	1.4	**0.046**	1.0	0.515
Panelist*Rep	Random	12	1.1	0.363	2.2	**0.010**	1.8	**0.047**	1.1	0.338	1.3	0.243	2.9	**0.001**
Error		294												

Values in **bold** indicate p-value < 0.05

Table S6. Sensory attributes of the Cascade 2014 and 2015 dry-hop treatments sorted by increasing Citrus quality.

Sample ID	OHAI	Citrus	Herbal	Sample ID	OHAI	Citrus	Herbal/Tea
"Unhopped" base	2.8 [m]	1.2 [h]	1.4 [h]	"Unhopped" base	3.0 [h]	1.9 [i]	2.5 [h]
CAS_01_14	9.0 [abc]	2.4 [g]	7.2 [a]	CAS_11_15	7.2 [efg]	4.2 [h]	5.3 [cdefg]
CAS_02_14	6.1 [l]	2.9 [fg]	2.4 [g]	CAS_12_15	6.7 [g]	4.3 [h]	5.0 [efg]
CAS_03_14	7.5 [efghi]	3.0 [fg]	3.6 [bcd]	CAS_07_15	6.7 [g]	4.4 [gh]	4.7 [fg]
CAS_04_14	6.6 [ijkl]	3.1 [efg]	2.9 [defg]	CAS_21_15	7.0 [fg]	4.5 [gh]	5.1 [defg]
CAS_05_14	6.5 [jkl]	3.3 [def]	2.9 [defg]	CAS_04_15	7.3 [efg]	4.5 [gh]	5.4 [cdefg]
CAS_08_14	6.2 [kl]	3.4 [cdef]	2.4 [g]	CAS_27_15	6.6 [g]	4.6 [fgh]	4.5 [g]
CAS_07_14	7.9 [defgh]	3.4 [cdef]	3.6 [bcde]	CAS_19_15	6.7 [g]	4.8 [efgh]	4.9 [efg]
CAS_06_14	7.1 [hijk]	3.5 [bcdef]	2.9 [defg]	CAS_03_15	7.6 [cdefg]	4.8 [efgh]	5.5 [cdefg]
CAS_09_14	6.2 [kl]	3.5 [bcdef]	2.3 [g]	CAS_05_15	7.0 [fg]	4.8 [efgh]	4.8 [efg]
CAS_10_14	7.3 [fghij]	3.6 [bcdef]	2.8 [efg]	CAS_01_15	7.0 [g]	4.9 [efgh]	5.2 [defg]
CAS_11_14	8.7 [abcd]	3.6 [bcdef]	4.1 [bc]	CAS_20_15	7.4 [defg]	4.9 [efgh]	5.1 [defg]
CAS_12_14	7.3 [fghij]	3.7 [bcdef]	2.8 [fg]	CAS_25_15	7.6 [cdefg]	5.0 [efgh]	5.6 [cdefg]
CAS_13_14	8.1 [bcdef]	3.7 [bcdef]	3.4 [cdef]	CAS_02_15	7.3 [defg]	5.0 [efgh]	5.2 [defg]
CAS_14_14	8.1 [cdefg]	3.7 [bcdef]	2.8 [efg]	CAS_28_15	7.3 [defg]	5.1 [efgh]	5.1 [defg]
CAS_15_14	8.9 [abc]	3.7 [bcdef]	4.4 [b]	CAS_16_15	7.3 [efg]	5.2 [defgh]	4.7 [efg]
CAS_16_14	6.9 [hijkl]	3.9 [abcde]	3.1 [defg]	CAS_06_15	7.5 [defg]	5.3 [cdefgh]	5.5 [cdefg]
CAS_17_14	7.1 [ghijk]	4.0 [abcd]	2.4 [g]	CAS_22_15	7.4 [defg]	5.3 [bcdefg]	5.1 [defg]
CAS_18_14	7.3 [fghij]	4.0 [abcd]	2.5 [g]	CAS_09_15	7.4 [defg]	5.4 [bcdefg]	4.7 [efg]
CAS_20_14	9.0 [ab]	4.2 [abc]	3.1 [defg]	CAS_13_15	7.6 [cdefg]	5.4 [bcdefg]	5.1 [defg]
CAS_21_14	8.5 [bcde]	4.2 [abcd]	3.0 [defg]	CAS_23_15	8.0 [bcdef]	5.7 [abcde]	5.4 [cdefg]
CAS_22_14	8.1 [bcdef]	4.3 [ab]	3.1 [defg]	CAS_24_15	7.4 [defg]	5.7 [abcdef]	5.4 [cdefg]
CAS_24_14	9.5 [a]	4.6 [a]	4.0 [bc]	CAS_10_15	8.5 [abc]	5.8 [abcde]	6.0 [cde]
				CAS_08_15	8.1 [bcde]	5.8 [abcde]	5.7 [cdef]
				CAS_29_15	8.8 [ab]	6.2 [abcd]	6.3 [abc]
				CAS_18_15	8.1 [bcde]	6.3 [abc]	5.3 [cdefg]
				CAS_17_15	9.0 [ab]	6.4 [ab]	7.1 [a]
				CAS_14_15	8.3 [abcd]	6.4 [ab]	5.6 [cdef]
				CAS_26_15	9.2 [a]	6.6 [a]	6.9 [ab]
				CAS_15_15	9.0 [ab]	6.6 [a]	5.5 [cdefg]

Mean scores.

Letters in brackets indicate statistically significant groupings within each descriptor (Fisher's LSD tests, p-value < 0.05).

Table S7. Sensory attributes of the Centennial 2015 and 2016 dry-hop treatments sorted by increasing overall hop aroma intensity (OHAI).

Sample ID	OHAI	Citrus	Herbal/Tea	Tropical/Catty	Tropical/Fruity	Pine/Resinous/Dank
"Unhopped" base	1.3 [h]	0.6 [f]	0.5 [e]	0.5 [d]	0.8 [e]	0.2 [e]
Cent_01_15	5.4 [g]	3.5 [e]	2.9 [d]	2.0 [c]	2.5 [d]	1.2 [d]
Cent_05_15	6.3 [fg]	3.9 [de]	3.2 [cd]	2.9 [b]	2.9 [cd]	1.9 [bc]
Cent_08_15	6.6 [ef]	4.3 [cd]	3.7 [bc]	2.5 [bc]	3.2 [bcd]	1.8 [c]
Cent_04_15	6.8 [def]	4.5 [bcd]	3.5 [bcd]	2.7 [bc]	2.9 [cd]	2.4 [ab]
Cent_10_15	7.0 [cdef]	4.5 [bcd]	3.6 [bc]	2.6 [bc]	3.0 [cd]	1.9 [bc]
Cent_02_15	7.0 [cdef]	4.5 [bcd]	3.4 [cd]	2.7 [bc]	3.0 [cd]	2.2 [abc]
Cent_12_15	7.2 [bcde]	5.0 [abc]	3.8 [bc]	2.7 [bc]	3.4 [abc]	2.2 [abc]
Cent_03_15	7.4 [bcde]	4.5 [bcd]	3.5 [bc]	3.0 [b]	3.4 [abc]	1.9 [bc]
Cent_06_15	7.7 [bcd]	5.1 [ab]	3.8 [bc]	2.9 [b]	3.9 [ab]	2.4 [ab]
Cent_07_15	7.9 [abc]	5.4 [a]	3.7 [bc]	2.8 [b]	4.2 [a]	2.2 [abc]
Cent_09_15	8.1 [ab]	5.1 [abc]	4.1 [ab]	3.7 [a]	3.6 [abc]	2.5 [ab]
Cent_11_15	8.8 [a]	5.2 [ab]	4.8 [a]	3.9 [a]	3.5 [abc]	2.6 [a]
"Unhopped" base	0.6 [j]	0.1 [j]	0.5 [j]	0.4 [h]	0.6 [h]	0.1 [j]
Cent_15_16	4.4 [i]	2.2 [i]	2.8 [i]	1.6 [g]	1.8 [g]	2.1 [i]
Cent_11_16	5.3 [hi]	2.9 [hi]	3.1 [ghi]	1.7 [fg]	2.2 [efg]	2.3 [hi]
Cent_7_16	5.4 [hi]	2.9 [hi]	3.5 [defghi]	1.6 [g]	2.3 [efg]	2.6 [ghi]
Cent_3_16	5.8 [gh]	3.4 [fgh]	3.4 [efghi]	1.9 [efg]	2.1 [fg]	3.0 [efghi]
Cent_17_16	5.9 [fgh]	3.8 [cdefgh]	3.1 [hi]	2.1 [cdedfg]	2.9 [ef]	2.8 [fghi]
Cent_4_16	6.0 [efgh]	3.5 [efgh]	3.2 [fghi]	2.0 [defg]	2.4 [efg]	3.1 [efgh]
Cent_5_16	6.0 [efgh]	3.7 [defgh]	3.2 [fghi]	2.0 [defg]	2.6 [efg]	2.8 [fghi]
Cent_10_16	6.1 [defgh]	4.0 [cdefg]	3.3 [fghi]	2.2 [cdedfg]	2.9 [ef]	3.5 [defg]
Cent_19_16	6.1 [defgh]	3.4 [gh]	3.8 [cdefgh]	2.4 [bcde]	2.2 [efg]	2.8 [fghi]
Cent_20_16	6.6 [cdefg]	4.4 [cdefg]	3.7 [cdefghi]	2.2 [cdedfg]	3.0 [cdef]	3.3 [efg]
Cent_16_16	6.7 [cdefg]	4.4 [cdef]	4.0 [bcdefgh]	2.6 [bcd]	3.1 [bcde]	3.6 [def]
Cent_18_16	6.7 [cdefg]	4.2 [cdefg]	3.7 [cdefghi]	2.4 [bcde]	3.0 [bcdef]	3.0 [efghi]
Cent_1_16	6.8 [cdefg]	4.2 [cdefg]	4.3 [abcde]	2.4 [bcde]	2.9 [def]	3.6 [def]
Cent_12_16	6.9 [cdefg]	4.5 [cd]	3.7 [cdefghi]	2.3 [cde]	3.0 [bcdef]	3.9 [cde]
Cent_8_16	7.0 [cdef]	4.3 [cdefg]	4.5 [abcd]	2.3 [cdef]	2.9 [ef]	3.6 [defg]
Cent_9_16	7.2 [cde]	4.5 [cde]	4.0 [bcdefg]	2.1 [defg]	3.0 [bcdef]	3.5 [defg]
Cent_13_16	7.3 [bcd]	4.6 [cd]	3.8 [cdefgh]	2.8 [bc]	4.1 [a]	3.3 [efg]
Cent_2_16	7.6 [bc]	4.8 [bc]	4.1 [bcdef]	2.6 [bcd]	3.0 [bcdef]	4.5 [bcd]
Cent_6_16	8.5 [ab]	5.9 [a]	4.5 [abc]	3.0 [b]	3.8 [abcd]	5.4 [ab]
Cent_14_16	9.3 [a]	5.8 [ab]	5.1 [a]	3.8 [a]	3.9 [abc]	4.8 [abc]
Cent_21_16	9.3 [a]	6.2 [a]	4.9 [ab]	3.7 [a]	4.0 [ab]	5.6 [a]

Mean scores.

Letters in brackets indicate statistically significant groupings within each descriptor (Fisher's LSD tests, p-value < 0.05).. OHAI=overall hop aroma intensity

Table S8. Pearson Correlation Coefficients for 2014 harvest Cascade (n=22).

Variables	OHAI	Citrus	Herbal	Total Oil	Isobutyl Isobutyrate	α-Pinene	β-Pinene	β-myrcene	Methyl Heptanoate	Limonene	Linalool	Nerol	Neral	Geraniol	Geranial	Geranyl acetate	β-caryophyllene	α-humulene	β-farnesene	Caryophyllene Oxide
OHAI	**1.00**																			
Citrus	0.37	**1.00**																		
Herbal	**0.65**	-0.37	**1.00**																	
Total Oil	-0.03	0.27	-0.28	**1.00**																
Isobutyl Isobutyrate	-0.02	0.27	-0.24	**0.88**	**1.00**															
α-Pinene	0.04	0.24	-0.22	**0.96**	**0.91**	**1.00**														
β-Pinene	0.06	0.25	-0.19	**0.97**	**0.90**	**0.99**	**1.00**													
β-myrcene	0.05	0.25	-0.20	**0.97**	**0.89**	**0.98**	**1.00**	**1.00**												
Methyl Heptanoate	-0.03	0.24	-0.27	**0.89**	**0.91**	**0.93**	**0.91**	**0.89**	**1.00**											
Limonene	-0.02	0.20	-0.20	**0.91**	**0.86**	**0.95**	**0.94**	**0.93**	**0.92**	**1.00**										
Linalool	-0.14	0.40	**-0.43**	**0.81**	**0.90**	**0.82**	**0.78**	**0.77**	**0.89**	**0.79**	**1.00**									
Nerol	-0.13	**0.50**	**-0.52**	**0.72**	**0.69**	**0.68**	**0.67**	**0.68**	**0.75**	**0.66**	**0.84**	**1.00**								
Neral	0.06	0.18	-0.14	**0.92**	**0.94**	**0.93**	**0.93**	**0.93**	**0.90**	**0.88**	**0.80**	**0.56**	**1.00**							
Geraniol	-0.05	**0.66**	-0.42	0.37	**0.44**	0.30	0.29	0.30	0.38	0.24	**0.66**	**0.68**	0.27	**1.00**						
Geranial	-0.29	0.15	-0.30	0.12	0.02	0.08	0.07	0.06	0.26	0.18	0.32	**0.59**	-0.07	0.40	**1.00**					
Geranyl acetate	-0.23	0.00	-0.31	**0.77**	**0.83**	**0.84**	**0.80**	**0.79**	**0.79**	**0.75**	**0.76**	**0.52**	**0.80**	0.25	0.05	**1.00**				
β-caryophyllene	-0.17	**0.44**	**-0.49**	**0.74**	**0.68**	**0.71**	**0.71**	**0.72**	**0.77**	**0.73**	**0.81**	**0.97**	**0.59**	**0.60**	**0.61**	**0.54**	**1.00**			
α-humulene	-0.18	0.41	**-0.48**	**0.71**	**0.65**	**0.69**	**0.69**	**0.70**	**0.75**	**0.70**	**0.78**	**0.96**	**0.54**	**0.58**	**0.65**	**0.52**	**0.99**	**1.00**		
β-farnesene	-0.32	**0.55**	**-0.67**	**0.55**	**0.51**	**0.46**	**0.46**	**0.48**	**0.53**	**0.47**	**0.71**	**0.91**	0.37	**0.75**	**0.59**	0.36	**0.88**	**0.85**	**1.00**	
Caryophyllene Oxide	0.08	0.08	-0.21	-0.15	-0.13	-0.10	-0.14	-0.17	-0.03	-0.16	-0.11	-0.11	-0.14	0.04	-0.17	-0.13	-0.20	-0.18	-0.17	**1.00**

*Values in **bold** are different from 0 with a significance level alpha=0.05*

Table S9. Pearson Correlation Coefficients for 2015 harvest Cascade (n=29).

Variables	OHAI	Citrus	Herbal	Total Oil	Isobutyl Isobutyrate	α-Pinene	β-Pinene	β-myrcene	Methyl Heptanoate	Limonene	Linalool	Nerol	Neral	Geraniol	Geranial	Geranyl acetate	β-caryophyllene	α-humulene	β-farnesene	Caryophyllene Oxide
OHAI	**1.00**																			
Citrus	**0.90**	**1.00**																		
Herbal	**0.85**	**0.67**	**1.00**																	
Total Oil	**0.45**	**0.49**	0.27	**1.00**																
Isobutyl Isobutyrate	**0.45**	**0.53**	0.22	**0.97**	**1.00**															
α-Pinene	**0.43**	**0.48**	0.25	**1.00**	**0.98**	**1.00**														
β-Pinene	**0.46**	**0.50**	0.28	**0.99**	**0.97**	**0.99**	**1.00**													
β-myrcene	**0.40**	**0.46**	0.21	**0.98**	**0.97**	**0.99**	**0.99**	**1.00**												
Methyl Heptanoate	**0.43**	**0.49**	0.25	**0.99**	**0.98**	**0.99**	**0.98**	**0.99**	**1.00**											
Limonene	**0.46**	**0.50**	0.28	**1.00**	**0.98**	**1.00**	**0.99**	**0.99**	**0.99**	**1.00**										
Linalool	**0.55**	**0.60**	0.35	**0.91**	**0.90**	**0.91**	**0.91**	**0.88**	**0.90**	**0.91**	**1.00**									
Nerol	0.35	**0.45**	0.19	**0.81**	**0.85**	**0.83**	**0.81**	**0.85**	**0.87**	**0.82**	**0.76**	**1.00**								
Neral	0.04	0.10	-0.07	**0.45**	**0.47**	**0.45**	**0.40**	**0.44**	**0.49**	**0.44**	**0.46**	**0.57**	**1.00**							
Geraniol	**0.75**	**0.71**	**0.57**	**0.62**	**0.61**	**0.60**	**0.62**	**0.56**	**0.60**	**0.62**	**0.80**	**0.54**	0.28	**1.00**						
Geranial	**0.62**	**0.62**	**0.61**	**0.60**	**0.56**	**0.60**	**0.62**	**0.56**	**0.60**	**0.61**	**0.67**	**0.48**	0.33	**0.62**	**1.00**					
Geranyl acetate	**0.52**	**0.57**	0.34	**0.78**	**0.78**	**0.76**	**0.73**	**0.71**	**0.77**	**0.76**	**0.80**	**0.72**	**0.52**	**0.75**	**0.58**	**1.00**				
β-caryophyllene	**0.41**	**0.42**	0.31	**0.86**	**0.76**	**0.85**	**0.83**	**0.80**	**0.84**	**0.84**	**0.81**	**0.70**	**0.44**	**0.59**	**0.63**	**0.78**	**1.00**			
α-humulene	0.33	0.34	0.27	**0.66**	**0.57**	**0.66**	**0.63**	**0.62**	**0.67**	**0.65**	**0.60**	**0.62**	**0.37**	**0.46**	**0.53**	**0.66**	**0.93**	**1.00**		
β-farnesene	0.11	0.17	0.02	**0.63**	**0.56**	**0.62**	**0.56**	**0.59**	**0.64**	**0.60**	**0.54**	**0.63**	**0.45**	0.33	**0.43**	**0.73**	**0.80**	**0.85**	**1.00**	
Caryophyllene Oxide	0.13	0.12	0.13	-0.26	-0.29	-0.28	-0.30	-0.33	-0.26	-0.27	-0.23	-0.18	-0.23	0.04	-0.08	0.03	0.01	0.24	0.19	**1.00**

*Values in **bold** are different from 0 with a significance level alpha=0.05*

Table S10: Combined 2015 and 2016 Centennial Harvest Pearson Correlation Coefficients (n=33).

Variables	OHAI	Citrus	Herbal	T/Catty	T/ Fruity	P/ R/D	Total Oil	Isobutyl Isobutyrate	α-Pinene	β-Pinene	β-myrcene	Methyl Heptanoate	Limonene	Linalool	Nerol	Neral	Geraniol	Geranial	Geranyl acetate	β-caryophyllene	α-humulene	Caryophyllene Oxide
OHAI	**1.00**																					
Citrus	**0.95**	**1.00**																				
Herbal	**0.83**	**0.72**	**1.00**																			
T/Catty	**0.84**	**0.78**	**0.56**	**1.00**																		
T/ Fruity	**0.86**	**0.87**	**0.71**	**0.67**	**1.00**																	
P/ R/D	**0.54**	**0.48**	**0.65**	0.22	**0.36**	**1.00**																
Total Oil	**0.55**	**0.54**	0.31	**0.58**	**0.46**	**0.44**	**1.00**															
Isobutyl Isobutyrate	**0.46**	**0.53**	0.13	**0.69**	**0.53**	**-0.35**	**0.35**	**1.00**														
α-Pinene	0.30	**0.35**	0.01	**0.59**	0.31	**-0.55**	0.10	**0.92**	**1.00**													
β-Pinene	**0.63**	**0.67**	0.32	**0.75**	**0.61**	0.04	**0.74**	**0.83**	**0.67**	**1.00**												
β-myrcene	**0.54**	**0.56**	0.24	**0.71**	**0.47**	-0.09	**0.66**	**0.85**	**0.74**	**0.93**	**1.00**											
Methyl Heptanoate	**0.46**	**0.50**	0.22	**0.68**	**0.38**	-0.31	0.22	**0.86**	**0.91**	**0.71**	**0.78**	**1.00**										
Limonene	**0.45**	**0.50**	0.15	**0.69**	**0.43**	-0.34	**0.36**	**0.93**	**0.94**	**0.84**	**0.87**	**0.92**	**1.00**									
Linalool	**0.38**	**0.38**	0.16	**0.42**	0.23	0.33	**0.65**	0.18	0.06	**0.47**	0.31	0.26	0.24	**1.00**								
Nerol	0.28	0.32	0.06	0.33	0.20	0.10	0.26	0.21	0.13	0.25	0.10	0.27	0.21	**0.67**	**1.00**							
Neral	0.01	0.07	-0.06	-0.04	0.09	0.21	0.16	-0.18	-0.28	-0.10	-0.27	-0.20	-0.22	**0.47**	**0.61**	**1.00**						
Geraniol	0.12	0.28	-0.23	0.34	0.18	**-0.47**	0.12	**0.65**	**0.66**	**0.46**	**0.44**	**0.64**	**0.62**	**0.35**	**0.53**	0.14	**1.00**					
Geranial	-0.05	-0.13	0.13	-0.28	-0.13	**0.64**	0.18	**-0.69**	**-0.81**	**-0.37**	**-0.48**	**-0.69**	**-0.66**	0.33	0.15	**0.37**	**-0.55**	**1.00**				
Geranyl acetate	0.20	0.13	0.17	0.00	0.12	**0.65**	**0.46**	**-0.39**	**-0.62**	-0.08	-0.19	**-0.50**	**-0.42**	**0.42**	0.33	**0.49**	-0.33	**0.80**	**1.00**			
β-caryophyllene	0.27	0.23	0.10	**0.43**	0.18	-0.11	0.24	**0.37**	**0.41**	**0.41**	0.32	**0.42**	**0.49**	**0.47**	**0.53**	0.28	**0.41**	-0.04	0.11	**1.00**		
α-humulene	0.14	0.14	-0.07	0.31	0.15	-0.26	0.12	**0.38**	**0.41**	0.30	0.23	**0.36**	**0.43**	**0.38**	**0.55**	**0.40**	**0.52**	-0.12	0.07	**0.94**	**1.00**	
Caryophyllene Oxide	-0.02	-0.08	0.11	-0.24	-0.07	**0.50**	-0.05	**-0.62**	**-0.64**	**-0.42**	**-0.60**	**-0.56**	**-0.56**	0.26	0.20	**0.47**	**-0.37**	**0.70**	**0.60**	0.00	-0.03	**1.00**

Values in **bold** are different from 0 with a significance level alpha=0.05. Pine/resinous/dank-P/R/D. Tropical-T/Catty & T/Fruity

Table S11. Concentrations of 16 volatiles in hydrodistilled hop oil for the 2014 Cascade samples

Sample ID	Isobutyl Isobutyrate	α-Pinene	β-Pinene	β-myrcene	Methyl Heptanoate	Limonene	Linalool	Nerol	Neral	Geraniol	Geranial	Geranyl acetate	β-caryophyllene	α-humulene	β-farnesene	Caryophyllene Oxide
CAS_01_14	0.24	0.26	2.36	151	0.22	0.73	1.20	1.02	0.00	0.81	0.13	0.07	43.4	125	26.8	1.49
CAS_02_14	0.52	0.45	3.78	222	0.89	1.20	4.95	1.93	0.00	2.36	0.13	0.12	66.7	188	70.2	5.73
CAS_03_14	2.15	1.36	12.3	815	1.90	4.75	10.4	3.06	6.76	5.25	0.09	0.26	109	265	117	2.40
CAS_04_14	1.83	1.90	15.9	1010	2.59	6.17	11.1	3.94	5.60	3.50	0.93	0.38	136	341	156	3.31
CAS_05_14	0.52	0.61	5.66	411	1.09	1.78	6.77	3.25	0.00	7.67	0.84	0.00	110	277	156	5.26
CAS_06_14	0.49	0.64	5.89	432	0.81	1.86	6.00	2.91	0.00	3.88	0.66	0.00	105	262	147	1.71
CAS_07_14	1.28	1.55	13.2	888	1.46	5.04	8.39	2.60	5.54	3.35	0.33	0.47	97.0	241	114	1.75
CAS_08_14	2.20	1.97	17.0	1100	3.34	6.44	14.1	3.96	9.32	5.36	1.18	0.74	146	353	151	2.01
CAS_09_14	1.46	1.41	10.8	651	2.17	4.54	8.12	1.95	7.57	2.46	0.13	0.36	72.1	174	73.8	4.88
CAS_10_14	1.90	1.55	13.6	934	1.84	4.20	9.32	3.06	6.89	4.13	0.38	0.72	107	266	140	1.69
CAS_11_14	0.30	0.27	3.26	244	0.43	1.13	3.40	2.42	0.00	2.77	0.38	0.00	91.6	213	132	2.40
CAS_12_14	1.02	0.65	6.33	403	0.68	1.77	4.95	1.94	2.16	3.68	0.21	0.34	69.8	189	89.4	4.19
CAS_13_14	1.92	2.08	17.2	1130	2.01	4.37	10.9	3.25	7.54	5.35	0.10	0.67	110	265	126	4.11
CAS_14_14	1.22	1.25	11.3	738	1.49	3.31	7.10	2.23	3.48	4.17	0.36	0.40	87.9	237	108	2.90
CAS_15_14	2.10	1.90	16.7	1110	2.54	5.05	10.5	3.21	9.39	4.15	0.14	0.57	110	275	136	3.29
CAS_16_14	1.86	1.89	16.4	1140	2.33	7.08	10.5	3.04	8.41	4.41	0.43	0.56	124	301	144	1.93
CAS_17_14	2.18	1.78	15.5	1100	2.43	4.85	9.82	3.08	9.08	3.53	0.15	0.59	111	268	131	4.26
CAS_18_14	0.28	0.57	4.65	286	0.48	1.43	1.96	2.14	0.00	1.16	0.36	0.16	88.8	232	82.6	3.76
CAS_20_14	0.33	0.75	7.11	483	0.91	2.07	2.96	2.09	0.00	1.55	0.38	0.28	81.2	222	74.7	2.72
CAS_21_14	0.60	1.10	9.43	592	1.31	3.52	4.51	2.12	2.37	1.87	0.34	0.31	82.6	220	76.9	5.09
CAS_22_14	2.08	2.40	22.2	1520	2.89	8.40	8.05	3.17	9.46	2.30	0.33	0.52	120	294	118	3.55
CAS_24_14	1.28	1.62	16.3	1120	1.63	4.24	5.55	2.34	8.83	2.58	0.13	0.21	93.1	241	82.8	2.43

Concentrations (mg/100g) of the hop volatiles are heat mapped: green cells represent the highest concentration and red cells represent the lowest concentrations

Table S12. Concentrations of 16 volatiles in hydrodistilled hop oil for the 2015 Cascade samples

Sample ID	Isobutyl Isobutyrate	α-Pinene	β-Pinene	β-myrcene	Methyl Heptanoate	Limonene	Linalool	Nerol	Neral	Geraniol	Geranial	Geranyl acetate	β-caryophyllene	α-humulene	β-farnesene	Caryophyllene Oxide
CAS_01_15	8.56	6.64	15.4	572	8.45	9.09	5.17	0.69	0.00	2.17	0.25	19.4	81.2	152	62.2	0.00
CAS_02_15	6.19	5.25	11.1	402	7.26	7.32	5.94	0.60	0.15	2.92	0.22	17.9	83.3	166	61.3	0.00
CAS_03_15	5.77	4.30	9.67	359	5.57	6.54	1.82	0.31	0.00	1.71	0.20	14.2	53.5	110	40.5	0.44
CAS_04_15	3.57	2.78	4.60	165	3.89	3.94	1.42	0.44	0.12	1.37	0.14	11.1	44.0	93.9	40.9	0.00
CAS_05_15	4.67	3.66	7.18	283	5.28	5.23	2.84	0.54	0.00	1.44	0.15	11.8	51.2	109	47.3	0.00
CAS_06_15	3.83	3.49	7.91	236	4.68	4.74	2.15	0.32	0.03	1.93	0.35	9.13	54.2	111	35.3	0.13
CAS_07_15	2.74	2.37	4.28	134	3.10	3.45	1.86	0.25	0.00	1.44	0.14	12.0	59.5	156	61.5	2.76
CAS_08_15	7.15	5.39	11.5	430	7.48	8.10	3.88	0.61	0.07	3.09	0.15	18.1	71.0	156	37.1	1.54
CAS_09_15	4.56	2.71	5.38	215	3.46	3.95	2.19	0.10	0.00	1.82	0.17	6.95	33.8	76.5	28.5	0.00
CAS_10_15	6.36	5.72	13.7	509	7.42	8.43	4.15	0.30	0.00	2.13	0.32	10.4	77.3	167	47.3	0.00
CAS_11_15	4.09	3.76	7.99	275	4.95	5.39	1.79	0.22	0.00	1.26	0.13	4.97	60.4	137	36.5	1.72
CAS_12_15	1.78	1.56	2.49	69.6	2.01	2.27	0.49	0.00	0.00	0.32	0.03	0.58	30.8	79.8	14.2	1.12
CAS_13_15	6.51	5.69	13.6	521	7.72	8.27	4.26	0.38	0.00	1.84	0.25	11.2	76.9	163	49.5	0.00
CAS_14_15	14.3	10.5	25.8	1130	15.7	15.2	8.58	2.14	0.17	3.76	0.39	24.3	108	241	85.9	0.00
CAS_15_15	6.84	4.41	10.8	363	5.98	6.55	4.59	0.69	0.00	3.82	0.12	16.2	53.6	103	29.5	1.36
CAS_16_15	5.12	3.90	8.04	279	5.35	5.64	3.51	0.29	0.16	2.50	0.26	17.8	72.5	165	60.5	1.64
CAS_17_15	5.46	4.11	9.42	314	5.64	6.41	5.25	0.45	0.00	4.07	0.40	13.9	56.0	114	34.7	1.97
CAS_18_15	9.84	6.62	15.5	579	9.15	9.71	5.78	0.69	0.08	1.99	0.32	17.0	69.1	132	44.7	0.00
CAS_19_15	3.18	2.78	4.99	167	3.73	3.99	1.68	0.36	0.00	1.48	0.17	8.68	60.3	160	53.9	1.28
CAS_20_15	5.61	4.81	12.1	383	6.26	7.22	4.80	0.38	0.03	2.98	0.29	10.9	63.1	128	35.2	0.59
CAS_21_15	2.57	2.20	4.21	119	2.81	3.01	1.45	0.19	0.00	1.09	0.06	5.36	50.5	133	38.0	2.93
CAS_22_15	2.51	2.18	4.36	124	2.85	3.22	1.75	0.28	0.03	1.67	0.23	6.17	53.6	127	25.0	4.02
CAS_23_15	5.29	4.23	8.50	270	5.91	6.19	2.99	0.37	0.00	2.46	0.21	17.6	72.7	182	74.9	9.44
CAS_24_15	2.70	2.24	4.09	125	2.97	3.15	0.94	0.16	0.00	0.70	0.10	5.80	38.4	78.7	26.4	1.03
CAS_25_15	4.06	3.04	6.85	201	4.00	4.32	2.96	0.22	0.03	2.56	0.18	12.3	48.0	104	27.6	1.03
CAS_26_15	3.84	3.20	6.71	205	4.73	4.70	3.12	0.60	0.04	2.61	0.41	17.3	72.1	187	54.6	3.67
CAS_27_15	2.64	2.11	3.59	129	2.84	2.92	1.42	0.21	0.00	1.13	0.09	6.20	29.5	63.2	26.4	1.41
CAS_28_15	7.34	5.17	11.5	436	7.39	7.58	3.92	0.72	0.14	1.66	0.30	13.8	59.6	121	50.1	0.30
CAS_29_15	6.81	5.29	12.6	403	6.93	7.69	5.08	0.28	0.00	3.16	0.32	15.1	73.8	143	37.1	1.20

Concentrations (mg/100g) of the hop volatiles are heat mapped: green cells represent the highest concentration and red cells represent the lowest concentrations

Table S13. Concentrations of 16 volatiles in hydrodistilled hop oil for the 2015 and 2016 Centennial samples

Sample ID	Isobutyl Isobutyrate	α-Pinene	β-Pinene	β-myrcene	Methyl Heptanoate	Limonene	Linalool	Nerol	Neral	Geraniol	Geranial	Geranyl acetate	β-caryophyllene	α-humulene	β-farnesene	Caryophyllene Oxide
Cent_01_15	5.31	5.06	12.5	450	6.22	6.89	6.88	0.88	0.15	56.2	0.87	0.00	72.6	141	n.d	0.00
Cent_02_15	8.82	6.96	18.8	637	11.4	10.9	14.0	6.25	5.34	97.5	2.70	0.77	154	347	n.d	7.22
Cent_03_15	10.6	7.94	23.3	850	11.6	11.3	13.2	4.32	0.87	88.3	2.04	0.24	90.5	152	n.d	0.00
Cent_04_15	12.3	8.30	21.6	751	12.4	12.3	14.5	8.38	2.17	143	2.55	1.15	138	291	n.d	3.19
Cent_05_15	10.4	7.23	21.2	649	10.7	11.0	13.9	5.57	2.08	82.6	2.82	0.90	123	238	n.d	9.44
Cent_06_15	11.9	8.27	23.1	721	12.2	11.9	14.1	4.06	1.88	74.6	3.05	0.34	106	197	n.d	3.90
Cent_07_15	10.2	7.17	20.2	629	10.5	11.0	13.7	6.61	2.23	114	3.48	1.27	141	296	n.d	6.62
Cent_08_15	7.76	5.61	15.8	428	7.53	8.10	9.46	4.56	1.68	82.1	3.05	0.32	118	246	n.d	8.60
Cent_09_15	9.87	7.57	19.9	823	12.2	11.5	10.3	4.16	0.58	70.3	1.75	0.32	110	211	n.d	0.59
Cent_10_15	9.68	7.42	18.4	675	11.3	11.4	6.20	3.59	0.18	62.4	0.95	0.25	118	223	n.d	0.00
Cent_11_15	12.4	8.68	23.6	940	12.9	12.8	8.89	3.22	0.88	34.4	1.67	1.25	96.1	167	n.d	0.00
Cent_12_15	9.62	8.34	22.2	774	11.7	13.3	14.6	5.00	1.01	52.7	10.2	1.15	208	377	n.d	7.02
Cent_01_16	3.25	0.93	13.6	331	4.85	4.66	12.0	4.02	1.95	43.4	11.6	1.18	93.6	174	n.d	17.4
Cent_02_16	2.62	0.70	13.6	384	3.98	5.32	12.8	4.31	2.48	40.8	11.0	1.96	113	201	n.d	27.0
Cent_03_16	0.72	0.60	9.04	266	2.96	2.88	9.17	2.93	1.43	27.2	6.60	0.90	94.4	168	n.d	13.0
Cent_04_16	1.92	0.80	11.9	400	3.02	4.75	10.7	3.26	1.47	41.5	11.2	1.36	91.1	174	n.d	13.1
Cent_05_16	4.59	1.91	19.1	559	2.04	7.80	12.9	4.49	2.02	45.9	11.2	1.58	97.0	168	n.d	10.3
Cent_06_16	6.03	1.40	20.8	698	7.53	6.66	13.1	3.63	1.74	58.0	10.7	1.63	93.7	151	n.d	6.27
Cent_07_16	1.04	0.38	6.20	211	2.43	2.55	6.68	3.88	1.35	34.6	7.42	0.80	81.6	165	n.d	5.01
Cent_08_16	2.21	0.59	10.7	353	2.47	4.26	8.69	2.59	1.64	34.6	9.29	0.95	75.1	143	n.d	10.5
Cent_09_16	3.48	0.89	18.5	615	6.86	7.22	14.0	5.99	2.27	46.3	11.2	1.81	108	197	n.d	14.1
Cent_10_16	2.06	0.95	12.6	365	3.57	5.57	12.8	4.80	2.55	47.3	13.6	1.76	105	198	n.d	16.6
Cent_11_16	0.93	0.45	8.42	298	2.93	3.34	10.6	3.99	1.81	60.7	9.37	1.52	117	239	n.d	9.06
Cent_12_16	5.57	1.37	15.4	401	6.32	5.18	15.6	7.09	3.15	55.4	12.3	1.80	112	230	n.d	12.0
Cent_13_16	7.67	1.46	20.4	649	1.59	6.28	10.9	3.85	2.57	50.3	11.0	2.15	119	261	n.d	10.5
Cent_14_16	6.75	1.87	20.8	772	8.29	7.51	15.7	5.08	1.84	45.8	10.2	1.81	111	186	n.d	4.85
Cent_15_16	1.54	0.57	10.6	348	3.12	3.72	11.4	4.21	2.53	41.8	7.98	1.34	105	216	n.d	11.3
Cent_16_16	3.06	1.18	17.7	496	4.93	6.54	10.6	4.66	2.59	47.5	8.29	1.58	118	205	n.d	9.04
Cent_17_16	6.78	1.46	18.2	548	1.10	5.32	9.73	3.42	2.13	41.5	7.97	1.49	104	226	n.d	4.31
Cent_18_16	3.10	0.64	9.69	250	3.35	3.22	11.6	7.17	3.56	46.8	13.3	1.61	99.1	194	n.d	14.1
Cent_19_16	1.29	0.84	12.5	361	4.21	4.43	12.9	5.46	2.73	51.2	10.9	1.43	132	236	n.d	9.03
Cent_20_16	3.58	1.64	19.2	664	5.50	6.74	15.3	4.30	1.57	64.1	10.4	1.16	110	202	n.d	6.63
Cent_21_16	3.91	1.79	20.7	500	5.52	6.82	14.1	6.37	2.27	45.7	9.11	1.37	126	225	n.d	15.1

Concentrations (mg/100g) of the hop volatiles are heat mapped: green cells represent the highest concentration and red cells represent the lowest concentrations (n.d) - not detected

Chapter 5. Publication C

Impact of harvest maturity on the aroma characteristics and chemistry of Cascade hops used for dry-hopping.

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Highlights:

- Harvest maturity has a significant impact on the aroma of Cascade hops.
- Citrusy aroma in Cascade hops increases with harvest maturity.
- Later harvested Cascade hops have higher total oils and aroma volatiles.
- As Cascade hops mature on bine, bound thiols decrease while free thiols increase.
- Later harvested Cascade hops are likely to be better suited for dry-hopping.

Summary:

To date there have been few studies that have investigated the impact of harvest maturity on hop quality and further its influence on beer performance. Only one of these studies has focused on the impact of harvest maturity on Cascade aroma/flavor in kettle and late hopped beer.[114] Along with being the first multi-year study to evaluate the impact of ripening (or harvest maturity) on Cascade dry-hop aroma performance, this is the first time the impact of harvest timing on free thiols and thiol precursors in hops (and more broadly flowers) has been reported. This along with the other chemical analyses performed make this manuscript a very comprehensive study on how harvest maturity influences aroma hop quality. The findings in this manuscript suggest that to maximize the aroma/ flavor potential of hops, harvest timing should be adjusted based on how brewers ultimately plan to use hops throughout the brewing process. These results give growers a guide so they might better predict the impact of harvest timing on hop quality and in turn on how brewers intend to use their hops throughout the brewing process.

Abstract

The impact of ripening on the dry-hop aroma potential and chemical development of Cascade hops is not well understood. Therefore, 5–6 weekly hop samples were collected over the 2014, 2015 and 2016 harvests. Concentrations of humulones did not change as a function of harvest date, while total hop essential oil content displayed significant positive trends. Concentrations of thiol precursors decreased over harvest while concentrations of free thiols increased. These weekly samples were used to dry-hop an unhopped base beer. Overall hop aroma intensity and citrus quality attributed to beer during dry-hopping increased as a function of harvest date. These results suggest that for brewers to maximize the efficiency of hop usage, early harvested Cascades might be better for bittering, while, later harvested Cascades might be better for dry-hopping or aroma additions because they attributed more intense citrusy aromas to beer and had higher concentrations of free thiols and terpene alcohols.

1. Introduction

The chemical constituents extracted from hops (Humulus lupulus L.) during the brewing process impart aroma and flavor to beer, as well as increase microbial and flavor stability. Therefore, the chemical composition of hops and the factors that drive the changes in hop chemical composition during hop production are important considerations for brewers and hop growers.

Historically, the main consideration around hop quality for brewers has been focused on the bittering potential of hops, which is mainly driven by the concentration of humulones (α-acids) contained in the soft resins of hops (Verzele & De Keukeleire, 1991). While humulones are not directly responsible for leading to beer bitterness, they are isomerized to iso-humulones

(the main drivers of bitterness in beer) when hops are added to the kettle during wort boiling. A number of studies have shown that pre- and post-harvest factors as well as on the bine ripening time can influence the concentrations of humulones in hops (Bailey, Schönberger, Drexler, Gahr, Newman, Pöschl, et al., 2009; Howard and Tatchell, 1956, Matsui et al., 2016, Probasco and Murphey, 1996, Sharp et al., 2014).

In contrast to traditional kettle hopping, brewers wishing to increase hop aroma without adding hop bitterness are turning to dry-hopping, a brewing practice generally recognized as a cold extraction of hops in fermented or partially fermented beer (Schönberger & Kostelecky, 2011). Recently, Hahn, Lafontaine, Pereira, and Shellhammer (2018) observed that in dry-hopped and hop-forward beers the oxidized forms of humulones, humulinones, were also main contributors to beer bitterness. Thus, the concentration of humulinones in hops, which is primarily driven by post-harvest processing or storage conditions (Maye, Smith, & Leker, 2016), should also be considered as a driver of hop quality from a bitterness potential perspective.

In general, the main objective of late/whirlpool- and dry- hopping is to add intense hop aroma to beer without imparting much bitterness. The current thought within the brewing community is that a hop's total essential oils content is a predictor of its aroma intensity and quality. Several studies have observed that both pre- and post-harvest factors as well as on the bine ripening time can influence the total essential oil concentration in hops (Bailey et al., 2009, Howard and Slater, 1958, Matsui et al., 2016, Probasco and Murphey, 1996, Sharp et al., 2014). However, Vollmer and Shellhammer (2016) observed that total essential oil is not a great predictor of hop aroma potential during dry-hopping and suggested that the composition of that essential oil might be more important. Further, work has shown that geraniol is a key component

for Cascade's aroma (Lafontaine, Pereira, Vollmer, & Shellhammer, Unpublished Data). Therefore, the harvest factors influencing the composition of hop essential oil may be the main drivers of hop aroma quality.

The composition of hop essential oil is estimated to be made up of over 1000 compounds (Schönberger & Kostelecky, 2011). The volatiles that compose hop essential oil, which have been shown to be important for beer flavor, can be split into three general groups: hydrocarbons (monoterpenes), oxygenated compounds (terpene alcohols), and sulfur-containing compounds (Rettberg et al., 2018, Schönberger and Kostelecky, 2011). Historically, much of the focus on the harvest factors that influence hop oil composition has been on the development of mono- and sesqui- terpenes (mainly β-myrcene, α-humulene, and β-caryophyllene) because they can comprise up to 80% of the essential oil of certain varieties. The work by Wang, Tian, Aziz, Broun, Dai, He, et al. (2008) provides great insight into the early developmental biosynthetic and enzymatic pathways that drive terpene synthesis in hops (although only 4 weeks after onset of flowering). However, the work by Wang et al. does not investigate the development of other essential aroma analytes important for beer flavor and is limited in describing hops harvested later in the commercial harvest window. Recent studies by Bailey, et al. (2009), (on Hallertauer Mittelfrüh), Sharp, et al. (2014) (on Cascade and Willamette), and Matsui, et al. (2016) (on Saaz) have shown that on the bine ripening time has a significant impact on the monoterpene and terpene alcohol development of hops as well as their potential to influence beer aroma. However, aside from the work performed by Matsui, et al. (2016) these studies were limited only to one or two harvest years and do not consider the impact of harvest maturity on sulfur containing compounds.

Due to their extremely low concentrations in beer (ng/L) and in hops (ng/g), the complexity in measuring sulfur containing compounds has limited much of the work performed on these analytes until relatively recently. Numerous studies have identified the presence of thiol precursors and free polyfunctional thiols, mainly 4-methyl-4-mercaptopentan-2-one (4MMP), 3-mercaptohexyl acetate (3MHA), and 3-mercapto-1-hexanol (3MH) in hops and beer (Gros et al., 2011, Kishimoto et al., 2008, Reglitz and Steinhaus, 2017; Roland, Viel, Reillon, Delpech, Boivin, Schneider, et al., 2016; Takoi, Degueil, Shinkaruk, Thibon, Maeda, Ito, et al., 2009). The impact of these compounds on beer flavor and aroma is dependent on their concentrations in beer. Generally, it has been determined that these compounds attribute distinct aromas to beer such as black currant, tropical fruit, and/or catty qualities. Most of the studies that have identified these compounds in hops and beer have largely focused on the impact of hop variety, and there has been very little work done to investigate the impact of hop harvest factors on the concentrations of sulfur-containing components. Kishimoto, et al. (2008) showed that 4MMP concentrations in hop varieties (i.e. Perle and Nugget) grown with copper-containing fungicides in Germany had reduced concentrations as compared to the same varieties grown without those fungicides in the U.S. Kammhuber, Hundhammer, and Weihrauch (2017) (on Cascade, Mandarina Bavaria, Hallertau Blanc, Huell Melon and Polaris) identified some sulfur analytes (dimethyl disulfide, S-methylthioisovalerate, and S-methylthiohexanoate) that might be responsible for the onion garlic note (largely perceived as negative on hop quality) in late harvested hops. However, this study did not consider the impact of harvest maturity and of these analytes on beer aroma.

Recently, Roland, Delpech, and Dagan (2017) suggested that the thiol potency of hops (free thiols vs thiol precursor concentrations) might dictate when/how a brewer should add hops into the brewing process to maximize their value and achieve consistent beer flavor. In Sauvignon Blanc wine grapes, thiol precursors (cysteinylated (3-S-cysteinylhexan-1-ol (Cys3MH) and 4-S-cysteinyl-4-methylpentan-2-one (Cys4MMP)) and glutathionylated precursors (3-S-glutathionylhexan-1-ol (G3MH) and 4-S-glutathionyl-4-methylpentan-2-one (G4MMP)) have been shown to be impacted by harvest maturity and ripening (Kobayashi, Takase, Suzuki, Tanzawa, Takata, Fujita, et al., 2011; Roland, Vialaret, Razungles, Rigou, & Schneider, 2010). Therefore, this study serves as the first examination into how the thiol precursor and free thiol concentrations are impacted by the harvest maturity of hops.

The goals of this study were to quantify how hop chemical composition (humulones, monoterpenes, sesquiterpenes, terpene alcohols, free thiols, and thiol precursors) changes throughout a commercial harvest window, using hops sampled from the same plot over three harvest years; to evaluate how hop maturity impacts the quality and intensity of aroma that is attributed to beer during dry-hopping; and to consider the role of polyfunctional thiols (4MMP, 3MH and 3MHA) in beer flavor.

2. Materials and Methods

2.1. Experimental design

Cascade hops were harvested during the commercial harvest at five to six weekly time points over the 2014 (5 treatments), 2015 (5 treatments), and 2016 (6 treatments) harvests (Table 1). In total, 16 dry-hopped beers were prepared by statically dry-hopping an unhopped beer with ground, whole cone hops shortly (5–8 months) after harvest. Descriptive sensory analysis was

used to scale the aroma intensity and quality of these dry-hopped beers as well as the three "unhopped" beer bases used. The impact of harvest maturity on the chemicals that drive the quality and intensity of hop flavor and aroma in beer were investigated. Both non-volatile (humulones and thiol precursor Cys3MH, Cys4MMP, G3MH, G4MMP) and volatile chemical analyses (monoterpenes, sesquiterpenes, and thiols) were performed on the hop samples used for dry-hopping. The impact of hop polyfunctional thiols on beer thiol concentrations was evaluated by measuring the concentrations of thiols in the dry-hopped beers made with the 2014 and 2016 samples.

Table 1. Basic Hop Quality Harvest Data.

Harvest Date	Dry matter (%)	Humulones (%)	Lupulones (%)	H.S.I.[$]	Total Essential Oil (ml/100g)
8/14/2014	20.4	5.0	8.3	0.212	0.70
8/21/2014	22.1	4.9	8.5	0.253	1.00
8/27/2014	24	5.2	8.2	0.219	1.20
9/12/2014	24.7	4.4	6.85	0.226	2.00
9/22/2014	28.8	5.0	6.0	0.216	1.75
Pearson's r	**0.955**	-0.310	**-0.964**	-0.211	**0.925**
8/11/2015	20.9	4.6	7.3	0.216	0.47
8/18/2015	22.5	5.12	7.62	0.219	1.03
8/25/2015	25	5.79	8	0.239	1.53
9/2/2015	26.5	5.16	7.56	0.236	1.48
9/9/2015	28.7	4.81	6.82	0.208	2.59
Pearson's r	**0.996**	0.144	-0.381	0.006	**0.946**
8/23/2016	24.9	5.06	5.81	0.256	0.76
8/29/2016	25.9	5.26	6.31	0.261	0.86
9/5/2016	25.7	5.45	7.11	0.277	1.07
9/12/2016	26.5	5.02	6.15	0.286	0.92
9/20/2016	27.4	5.12	6.27	0.284	1.29
9/28/2016	27	5.48	6.72	0.289	2.52
Pearson's r	**0.914**	0.342	0.391	**0.925**	**0.832**

*Pearson's r calculated between the harvest date and the given hop quality measurements. Values in bold are significant (p-value < 0.05)
[$]H.S.I. – Hop Storage Index

2.2. Hop collection

A unique harvest maturity sampling protocol was performed at a commercial hop farm in Yakima, WA and yielded 5–6 weekly time points for each of the 2014, 2015 and 2016 commercial harvest windows (Table 1). The whole cone Cascade samples from this farm were collected from a small area (42 hills, 98 strings, covering a two-row section) within a commercial field. Although there were significant differences in the climate between the harvest years, in general, harvest started when dry matter content was ~20%. However, processing constraints limited the ability to pick early harvest samples in 2016 and harvest collection was started at ~24% dry matter content. Typically, Cascades are harvested commercially from 24–26% dry matter content. The soil type in this field was Ashue loam with a 0–2% slope and plant spacing of 1.1 × 4.3 m. In 2014 and 2015, 63.5 kg of nitrogen was applied through irrigation using 32–0–0, and in 2016, 56.7 kg of nitrogen was applied through irrigation using 12–3–3–3.8 (sulfur). A border row around this small area was used to protect from wind and other elements. During harvest, ~15 strings were randomly harvested from 15 different hills to ensure that within a weekly sample two strings were never harvested from one hill. At the time of harvest these small samples were kilned to approximately 10% moisture on a pilot-scale electric dryer (62.8 °C), packaged, and shipped to Oregon State University. Upon arrival at Oregon State University, all hops samples were repackaged in high-barrier foil pouches, purged with nitrogen, vacuum sealed, and stored cold (−20 °C) until dry-hopping and chemical analysis.

2.3. Unhopped beer production

To evaluate the dry-hop aroma of the different hop samples, an unhopped beer was prepared by commercial breweries in Portland (Craft Brew Alliance) for the 2014 harvest samples and Bridgeport Brewing for the 2015 and 2016 harvest samples. The unhopped wort

was prepared with 86% pale two row, 13.5% Caramel 10°L and 0.5% Caramel 120°L malt (Great Western, Vancouver, WA). The starting extract concentrations to evaluate the 2014, 2015, and 2016 harvest samples were 10.9°P, 11.3°P, and 11.1°P, respectively. Fermentation was carried out with Wyeast 1056 ale yeast at 16.7–18.9 °C for the 2014 harvest samples, Wyeast 1728 at 19.4–20 °C was used for the 2015 harvest samples and BridgePort Brewing Company's house yeast strain at 19.4–20 °C was used for the 2016 harvest samples. Following fermentation and clarification, iso-alpha acids (IsoHop, John I Haas, Yakima, WA) were added at a target concentration of 18 mg/L. This resulted in ∼40 hL of a 15.4 BU, 4.5% ABV "unhopped" base beer for the 2014 harvest samples, ∼55 hL of a 19.8 BU, 4.75% ABV "unhopped" base beer for the 2015 harvest samples and ∼52 hL of a 19.0 BU, 4.37% ABV "unhopped" base beer for the 2016 harvest samples. Beer was carbonated and packaged into 60-L stainless kegs, shipped to Oregon State University and held at 2 °C until dry-hopping.

2.4. Dry-hopping protocol and hop preparation

The dry-hopping process established by Vollmer and Shellhammer (2016) has been shown to be reproducible on a pilot scale. In brief, 24 hours prior to hop addition, the unhopped beer was removed from the cooler at 4 °C and allowed to warm for approximately 24 hours to 15 °C. For each treatment, 40 L of warmed beer was transferred into two modified 60-L stainless kegs with a 10.2-cm stainless steel opening fitted with a standard Sankey D-system coupler and modified spear (Sabco, Toledo, OH). The hop treatments were dry-hopped at 386 g hop/hL unhopped beer. The whole cone hops were coarsely ground into a hop grist, which was divided up by mass into two mesh bags (EcoBag, Ossining, NY). These bags were stored inside high barrier pouches flushed with N_2 until the dry-hopping event. For each dry-hop treatment, the two kegs filled with 40 L beer were temporarily de-pressurized and opened under a stream of low

pressure CO_2. Simultaneously, the high barrier pouch bag was opened and the mesh bag containing ground hop grist was added to the beer. After the addition, the headspace was flushed with CO_2 and purged. After purging, the kegs were inverted three times to ensure proper mixing.

After 24 hours of dry-hopping, the beer was filtered to stop the dry-hopping process. The average temperature of the dry-hopping events ranged from 13.3–15 °C. Dry-hopping was stopped after 24 h because prior work by Wolfe, Qian, and Shellhammer (2012) showed that the extraction of key hop volatiles occurred within 24 h during dry-hopping. During filtration the two kegs were blended via a three-way fitting, prior to entering a plate and frame filter using diatomaceous earth impregnated cellulose pads (HS2000; Pall Corporation, Port Washington, NY) (Vollmer & Shellhammer, 2016). Dissolved oxygen (DO) was monitored during filtration using an Orbisphere 3100 Portable Oxygen Analyzer (Hach, Loveland, CO). Bright beer was not collected until DO was below 110 µg/L. After DO was within specification, bright, filtered beer was collected in a closed 19.6-L stainless steel keg with sufficient back-pressure to reduce foaming. Between each filter run, filter pads were exchanged to prevent carry-over. Filtered beer was stored at 2 °C and under CO_2 overpressure (83 kPa) until sensory evaluation.

2.5. Sensory: Descriptive analysis

To evaluate the sensory qualities of the 2014, 2015, and 2016 harvest samples, 3 descriptive analysis panels were used to quantify perceived hop intensity and quality of the dry-hopped beers. The general approach used trained panelists, who were selected based on previous experience and relevance, to scale only the orthonasal aroma of the beer treatments. Intensive training sessions on commercial samples (Sup. Table 1) and a random set of blind coded dry-hop treatments for each of the harvest years were completed in advance of data collection to develop a relevant lexicon of sensory attributes, establish a scale that best explained the differences in the

samples, and to train panelists to use external reference samples as anchors for the most salient attributes. During each session, the panelists had access to external reference samples that had sensory descriptors with intensity scores assigned by consensus during training, and their purpose was to serve as anchors for the 0–15 point intensity scale. The external references and attributes used to evaluate the different harvest samples are outlined in Supplementary Table 1. Due to the seasonal nature of commercial beer production and panel feedback, the same commercial beers and rankings were unable to be used throughout the entire three years of the study. This change in references could have impacted how the panelists were assessing the beers on a year to year basis but is not expected to have had a major impact on the results observed. Panelists were given ~60 mL of dry-hopped beer in a 300-mL glass covered with a plastic lid. For the 2014 Cascade harvest samples beer was packaged and served from bottles that had been warmed to room temperature for 35–45 min. For the rest of the harvest samples beer was served from two 8-head draft systems (Micro Matic, Northridge, CA) into pitchers at ~1 °C and at 82.7 kPa. Beer was poured into sample glasses ~1 hour before the start of testing and allowed to warm to room temperature. For the 2014 Cascade harvest samples panelist responses were collected on paper ballots. For the other harvest samples panelist responses were collected on Chromebook tablets using Qualtrics (Provo, UT). For each of these sessions, Qualtrics was also used to randomly assign the serving order of samples for each panelist. More in-depth details of each descriptive analysis panel, including the differences in how the descriptive analysis panels were carried over the different harvest years, can be found in the supplementary information.

2.6. Beer and hop chemical analysis

Concurrent with the hop sampling for the dry-hopping, approximately 150 g of the homogenized hop grist were taken for chemical analysis. Beer was stored (< 4 months) in bottles in 2014 and in 19.6-L kegs in 2015 and 2016 at ∼1 °C until analysis.

2.*7. Non-volatile hop analysis*

During harvest, % dry matter of the hop cones was determined by drying ∼100 g at 56 °C for 12–14 hours. % dry matter was determined by the following formula: (dry cone weight/green cone weight)*100 = dry matter. The total concentration of humulones and lupulones as well as hop storage index (H.S.I.) were determined by ASBC – 6A α-and β-Acids in Hops by Spectrophotometry (ASBC Methods of Analysis). Briefly, 5 g of ground hops were extracted in 100 mL of toluene for 30 min. This extract was then centrifuged and 5 mL of the clarified toluene extract were added to 100 mL of alkaline methanol. The absorbance of this solution was then determined at 275, 325, and 355 nm. H.S.I. is a measure of hop oxidation (or % humulones lost) and is the ratio of the absorbance maximum of hop oxidation products (275 nm) to the absorbance maximum of humulones (325 nm).

2.8. Hop essential oil analysis- reagents and standards

β-Myrcene, β-pinene, linalool, geraniol, citral, limonene, geranyl acetate, α-pinene, nerol, isobutyl isobutyrate, methyl heptanoate, β-caryophyllene, α-humulene, β-farnesene, and caryophyllene oxide were obtained from Sigma-Aldrich (St. Louis, MO). 2-Octanol was obtained through Alfa Aesar (Haverhill, MA). Hexanes purchased from J.T. Baker (Center Valley, PA) were redistilled to remove impurities before analysis.

2.9. Hop essential oil analysis

At the time of dry-hopping, hydrodistillation was performed to determine the total oil content of the homogenized hop grist using ASBC Hops-13 (ASBC Methods of Analysis). In brief, ~105 g of coarsely ground hops were boiled in 3 L of distilled water for 3 h. Post-distillation, the total oil content was recorded and the hop oil was collected in 2.5-mL amber vials with foil-lined closures. After filling with oil, the amber vials were flushed with nitrogen. Hop oil was stored at –20 °C until subsequent compositional analysis.

In 2014, hop oil compositional analysis was performed under modified conditions from ASBC Hops-17 (ASBC Methods of Analysis). In 2015 and 2016 hop oil compositional analysis was performed using previously published methodology (Lafontaine and Shellhammer, 2018, Sharp et al., 2017) using a HP 6890 gas chromatograph with an Agilent 5972a mass spectrometer (GC-MS) under modified conditions from ASBC Hops-17. In brief, a 1% 2-octanol (8190 ppm) solution was prepared in reagent-grade hexane. Hop oils were diluted to 10% with the 1% 2-octanol/hexane solution in crimped glass vials. A 1-µL aliquot of the diluted hop oil was directly injected into the injection port held at 200 °C and operating in split mode (1:20). The analytical column was a 30 m × 250 µm × 0.25 µm Zebron ZB-1 MS (Phenomenex, Torrance, CA) and ultra-pure helium was used as the carrier gas (a constant flow rate, 1.4 ml/min). The following temperature program was used: 50 °C hold for 1 min, 50–180 °C (2 °C/min) hold for 10 minutes, 180–200°C (3 °C/min) and 250 °C hold for 5 minutes. The auxiliary line and mass spectrometer were operated at 280 and ~180°C respectively. The mass spectrometer was operated using electron impact mode at 70 eV and set up to detect ions with a mass-to-charge ratio (m/z) of 30–350. Four-point calibration curves (50, 100, 400, and 800 ppm) were created for all target analytes. For high concentration target analytes (β-myrcene, α-humulene, β-caryophyllene, and

β-farnesene) three additional calibration points were added (1000, 5000, and 9000 ppm). Target analytes were quantified using the following ions for each analyte: m/z 41 (geranial), m/z 45 (2-octanol), m/z 69 (β-farnesene, geraniol, nerol, neral, and geranyl acetate), m/z 71 (isobutyl isobutyrate and linalool), m/z 74 (methyl heptanoate), m/z 79 (caryophyllene oxide) and m/z 93 (α-pinene, β-pinene, β-myrcene, β-caryophyllene, and α-humulene). The target analyte concentrations in hop oil were then standardized on a per-mass basis using the total oil content determined during hydrodistillation. A total ion chromatogram is provided in the supplementary information (Sup. Figure 1).

2.10. Free thiol and thiol precursor analysis in hops and beers- reagents and standards

The following reagents and standards were used for free thiol and thiol precursor measurements performed by Nyseos in hops and beers. All analytical solvents were purchased from Biosolve (Dieuze, France) and analytical reagents were purchased from Merck (Saint Quentin Fallavier, France). 3MH and 3MHA were purchased from Merck (Saint Quentin Fallavier, France), whereas the other analytical standards were synthesized by Nyseos according to previously published methods (Fedrizzi et al., 2009, Roland et al., 2010). Briefly, thiol precursors were synthesized by a Michael addition of glutathione or Boc-Cys-OH on either hexenal or mesityl oxide to afford the corresponding glutathionylated and cysteinylated precursors of 3MH and 4MMP, respectively. For the labeled analytical standards, hexyn-1-ol was deuterated using Lindlar's catalyst then oxidized under mild conditions with manganese dioxide to afford hexenal-d2 (Roland, Schneider, Le Guernevé, Razungles, & Cavelier, 2010), that was directly used to synthesize G3MH-d2 and Cys3MH-d2. The labeled mesityl oxide was purchased from Merck (Saint Quentin Fallavier, France) and used to synthesize G4MMP-d6 and Cys4MMP-d6.

The following reagents and standards were used for free thiol measurements in beer performed by Asahi Brewing Company Ltd. (Moriya, Ibaraki Prefecture, Japan). 4MMP, 3MH, and 3MHA were purchased from Penta Manufacturing Co. (Livingston, NJ). d10-4-methyl-4-mercapto-2-pentanone (d10-4MMP) was purchased from aromaLAB AG (Freising, Germany). d2-3-mercapto-1-hexanol (d2-3MH) was purchased from NARD Institute, Ltd. (Hyogo, Japan). ETP and tris(hydroxymethyl)aminomethane hydrochloride (Tris-HCl) solution (1 M, pH 9.0) were purchased from Wako Pure Chemicals (Osaka, Japan). Sodium hydroxide (NaOH) solution (1 N) and ethanol (pesticide residue analysis grade) were purchased from Kanto Kagaku (Tokyo, Japan).

2.11. Free thiol and thiol precursor analysis in hops

Free thiol and thiol precursor analysis was performed by Nyseos (Montpellier, France) on ground hops. Thiol precursors were analyzed in hops by stable isotope dilution assay (SIDA) and nanoLC-MS/MS as previously reported (Roland et al., 2010, Roland et al., 2016). In brief, ground hops (200 mg) were extracted for 1 h at room temperature in an ethanolic mixture (11% ethanol; 4 g/L of tartaric acid; pH = 3.5), centrifuged, and supernatant was spiked with labeled internal standards (G3MH-d2, Cys3MH-d2, Cys4MMP-d6, and G4MMP-d6) before being analyzed by nanoLC-MS/MS under multiple reaction monitoring mode (MRM). The LOD, LOQ and MS/MS conditions for the methodology were reported previously (Roland, et al., 2016) and the repeatability statistics were reported previously (Roland, et al., 2010). The limits of detection (LODs) for free thiols were 0.1–0.5 µg/kg and for thiol precursors were 0.5–19 µg/kg. Chromatograms of the free and thiol precursors are provided in the supplementary information (Supplementary Figure 2, Figure 3).

2.12. Free thiol analysis in beer

Free thiols (4MMP, 3MH, and 3MHA) in the dry-hopped beer made from the 2014 hop samples were measured by Asahi Brewing Company Ltd. (Moriya, Ibaraki Prefecture, Japan) using stir bar sorptive extraction with in situ derivatization (der−SBSE) using ethyl propiolate (ETP), followed by thermal desorption and gas chromatography−tandem mass spectrometry (TD−GC−MS/MS) with selected reaction monitoring (SRM) mode using published methodology (Ochiai, Sasamoto, & Kishimoto, 2015). In brief, beer samples were adjusted to pH 9 using NaOH solution (1 M). A total of 10 mL of sample containing 35 mM ethyl propiolate (ETP), internal standards (20 ng/L d10-4MMP and 200 ng/L d2-3MH), and the PDMS stir bar was transferred to 20-mL headspace vials. The vial was sealed with the metal screw cap, and the PDMS stir bar was first stirred at room temperature (25 °C) for 10 min at 500 rpm for the ETP derivatization step (Herbst-Johnstone, Piano, Duhamel, Barker, & Fedrizzi, 2013). After 10 min of stirring, 30% NaCl was added and SBSE was performed for 180 min while stirring at 1500 rpm. The stir bars were thermally desorbed by programming the thermal desorption unit (TDU) from 30 °C (held for 0.5 min) to 200 °C (held for 3 min) at 720 °C/min with 50 mL/min desorption flow. Desorbed compounds were focused at 10 °C on a liner packed with quartz wool in the Peltier-cooled PTV inlet for subsequent TD−GC−QQQ−MS analysis. The column temperature for the DB-Wax was programmed from 100 °C (held for 3 min) to 250 °C (held for 11 min) at 10 °C/min. The QQQ−MS was operated in three acquisition modes: (1) scan mode at a mass range of m/z 29−500, (2) product ion scan mode at a mass range of m/z 29−500, and (3) SRM mode with the selected transitions (precursor to product ion). Transitions of the analytes, LODs, LOQs, and repeatability statistics are listed in (Ochiai, et al., 2015). The limits of detection (LODs) ranged from 0.19 to 27 ng/L.

Free thiols (4MMP, 3MH, and 3MHA as well as 3MH disulfides) in the dry-hopped beer made from the 2016 hop samples were measured by Nyseos using published methodology. Both 3MH and 3MHA were analyzed in beer by stable isotope dilution assay and nanoLC-MS/MS as previously described (Fedrizzi et al., 2009, Roland et al., 2016). Briefly, beer sample (1 mL) was spiked with internal standards (3MH-d2 and 3MHA-d5) and then derivatized using ammonium bicarbonate buffer (1 M, 300 µL) and N-phenylmaleimide solution (25 mM; 120 µL). After quenching with ice acetic acid (200 µL), samples were purified by SPE (Bond Elut Plexa Cartridge, 200 mg), and then analyzed by nanoLC-MS/MS in MRM mode as previously detailed (Fedrizzi et al., 2009, Roland et al., 2016). The limits of detection (LODs) ranged from 0.9−2.8 ng/L. For the 3MH disulfides, the reduced form was measured as previously described (Roland, et al., 2016). Chromatograms of the free thiols and 3MH disulfides are provided in the supplementary information (Sup. Figure 2).

2.13. Statistical Analysis

Two-way analysis of variance with a mixed model (including the factors panelist, sample, and replication, as well as corresponding two-way interactions), Pearson correlation analysis, multiple comparison analysis (Tukey's HSD), principal component analysis and graphical construction were carried out using XLSTAT 2017 (Addinsoft, New York, NY). These tests and graphical outputs were used to gauge the panel and panelist effectiveness in generating descriptive data, evaluate the significant differences in aroma quality and intensity among the dry-hopping treatments, and assess the associations between the chemical and sensory data collected.

3. Results and Discussion

3.1. Descriptive analysis: panelist/panel evaluation and the impact of harvest maturity on dry-hop aroma intensity and quality

Following each descriptive analysis panel over the three harvest years, each panelist was evaluated on their performance based upon their ability to discriminate differences between the dry-hop treatments and unhopped control within each harvest year on at least one of the sensory attributes, replicate among all sessions, and lack of interactions. Any panelists that failed these three criteria were removed from further analyses. One panelist was removed from the 2014 panel, one panelist was removed from the 2015 panel and four panelists were removed from the 2016 panel, resulting in descriptive analysis panels that contained 10, 10, and 8 panelists, respectively, over the three harvest years.

Two-way ANOVA using a mixed model was performed on the attributes for each of the remaining panelists (Sup. Table 2). In general, significant sample effects were observed across the attributes and indicated that the panelists were able to detect significant differences between the samples. Significant panelist × sample effects were also observed for most of the attributes (mainly Overall Hop Aroma Intensity (OHAI), Citrus and Herbal/Tea). Significant panelist × sample effects are common in sensory analysis and indicate that there were slight differences in the way the panelists scaled those attributes (Meilgaard, Carr, & Civille, 2007). No significant effect of replication or interactions between panelist and replication or between sample and replication were observed for the OHAI and Citrus attributes. Minor significant interactions were observed, primarily the Sample × Rep effect on the Herbal/Tea attribute for the 2015 panel and the Panelist × Rep effect for the Herbal/Tea and Tropical/Catty attributes for the 2016 panel. These interactions indicate that from one session to another, the panelist scores were not consistent for all the products. With these few exceptions, the panelists could effectively

replicate their attribute scaling for most of the qualitative descriptors across all replications for each of the samples. Furthermore, the responses provided for a given panelist did not depend on replication.

The least squared means and results from Tukey's HSD ($p < 0.05$) for the sensory attributes from the descriptive analysis panel on the dry-hop treatments were summarized (Table 2). In general, OHAI and Citrus flavor increased as a function of harvest maturity.

Table 2. Summary of least squared means for the sensory attributes resulting from descriptive analysis as a function of harvest maturity.

Harvest Date	OHAI	Citrus	Herbal	Tropical Fruit	Resinous/Hop Oil	Tropical/Catty
8/14/2014	7.5 b	3.0 a	3.6 ab	3.4 a	3.2 b	-
8/21/2014	7.9 ab	3.4 a	3.6	4.2 a	3.4 b	-
8/27/2014	8.1 ab	3.7 a	2.8 b	3.8 a	4.2 ab	-
9/12/2014	8.7 ab	3.6 a	4.1 a	4.9 a	3.6 b	-
9/22/2014	8.9 a	3.7 a	4.4 a	3.8 a	4.9 a	-
Pearson's r	**0.990**	0.758	0.678	0.421	0.711	
Harvest Date	**OHAI**	**Citrus**	**Herbal/Tea**			
8/11/2015	6.7 b	4.3 b	5.0 a	-	-	-
8/18/2015	7.2 b	4.2 b	5.3 a	-	-	-
8/25/2015	8.5 a	5.8 a	6.0 a	-	-	-
9/2/2015	7.6 ab	5.4 a	5.1 a	-	-	-
9/9/2015	8.3 a	6.4 a	5.6 a	-	-	-
Pearson's r	0.744	**0.888**	0.368			
Harvest Date	**OHAI**	**Citrus**	**Herbal/Tea**	**Tropical/Fruity**	**Pine/Resinous/Dank**	**Tropical/Catty**
8/23/2016	4.3 d	2.1 e	2.3 d	1.5 d	1.2 d	0.5 c
8/29/2016	4.6 d	2.7 de	2.8 bc	1.7 d	1.5 cd	1.1 b
9/5/2016	6.8 b	4.3 b	3.5 ab	2.5 b	2.5 b	1.1 b
9/12/2016	6 bc	3.5 bc	3.2 bc	2.3 bc	2.0 bc	1.3 b
9/20/2016	5.7 c	3.4 cd	3.1 bc	1.9 cd	1.6 cd	0.9 bc
9/28/2016	9.4 a	6.1 a	4.0 a	3.2 a	3.6 a	2.3 a
Pearson's r	**0.818**	**0.817**	**0.828**	0.766	0.726	0.766

Letters indicate statistically significant groupings (Tukey's HSD tests p-value < 0.05) (-) did not measure

*Pearson's r calculated between the harvest date and the given sensory attribute. Values in bold are significant (p-value < 0.05)

This result suggests that that hops picked later in the harvest window attributed significantly more aroma to beer during dry-hopping and that the quality of this aroma was primarily perceived to be citrusy. Similar observations were made by Bailey, et al. (2009) in beers that were kettle, whirlpool, and dry- hopped with Hallertauer Mittelfrüh of different harvest maturities from the same location. In that study, beers made with the later picked Hallertauer Mittelfrüh had higher hoppy aroma and flavor intensities. In the study by Bailey et al., the beers made with later picked Hallertauer Mittelfrüh were also rated better for aroma and flavor using a modified German Agricultural Society (DLG) tasting scheme. Matsui, et al. (2016) found that beers that were kettle and whirlpool hopped with later harvested Saaz had stronger hoppy aroma intensities. Inui, et al. (2016) also found that harvest timing had a significant impact on the floral, citrusy, and fruity aroma characteristics that Saaz hops attributed to hop teas. However, in these studies, hoppy aroma intensity and quality was also dependent on farm location. This indicates that growing conditions and/or post-harvest processing conditions also have a significant impact on hoppy aroma potential.

Sharp, et al. (2014) observed that beers that were kettle hopped (at 1.45 g/hl) and whirlpool hopped (at 5.5 g/hL) with Cascade hops harvested at a typical time on a commercial farm had higher overall likings in a consumer panel when compared to beers made with later picked Cascade. The later picked Cascades in this study attributed higher pine, melon, and floral notes. It is important to note that this study did not utilize dry-hopping, and for reasons that will become apparent later in the discussion, early, or typical harvested Cascade may be better suited for kettle and whirlpool hopping than dry-hopping.

3.2. Influence of harvest maturity on concentrations of humulones and lupulones, total essential oil and % dry matter

Most commercial hop farmers use percent dry matter as an indicator of when to harvest. In general, commercial farms in the USA typically start to harvest Cascade at ~24–25% dry matter. Over the three harvest years, dry matter increased as a function of harvest maturity as expected (Table 1). Similar observations were made by Probasco and Murphey (1996), albeit in different hop varieties (Mt. Hood, Nugget, Galena, and Willamette). This increase in dry matter has a direct impact on a farmer's yield. Therefore, from a grower's perspective, it is key to maximize dry matter without sacrificing cone quality, structure, and/or the pickability of hops. It has been estimated that for every 1% increase in dry matter the increase in yield will be ~ 90 lb/acre on a variety averaging 2000 lb/acre (or ~100.8 kg/ha on a variety averaging 2240 kg/ha) (Probasco & Murphey, 1996).

When considering the development of the non-volatile fraction and the analytes that impact the bittering potential of hops during harvest, in general, concentrations of humulones and lupulones as well as H.S.I. (Table 1) were not dependent on the date harvested throughout the commercial harvest window. The concentration of these compounds plateaued prior to the harvest window and stayed roughly constant throughout harvest. Similar findings were made by Sharp, et al. (2014) (in Cascade) and have been seen in other varieties as well (Howard and Tatchell, 1956, Matsui et al., 2016). Other factors, such as the year-to-year growing conditions, are considered to have a larger impact on the concentrations of humulones as compared to on the bine ripening. Also, the optimal harvest timing window for the concentrations of humulones is varietal specific and some hop varieties have been shown to increase concentrations of humulones over harvest with on the bine ripening (Bailey et al., 2009, Probasco and Murphey, 1996).

Historically, total essential oil content has been viewed as an indicator of hop aroma potential in beer. During cone production, the development of total essential oil is delayed in relation to the production of hop acids and has been shown to increase over the commercial harvest window for a number of varieties (Howard and Slater, 1958, Howard and Tatchell, 1956, Matsui et al., 2016, Sharp et al., 2014). Differences from this trend are expected to be a result of the post-harvest processing (for instance kilning parameters) along with storage conditions.

Over the three harvest years, total essential oil content significantly increased as a function of harvest date (Table 1). Recently, Vollmer and Shellhammer (2016) showed that total essential oil content is not an effective predictor of hop aroma potential and suggested that individual components of hop essential oil might yield a better predictor of hop aroma potential. Therefore, consideration of the development of the different hop volatiles throughout the harvest window is important. Although OHAI and Citrus quality increased as total essential oil content increased over the three harvest years (Figure 1), it is likely that the compositional development of this oil is leading to the sensorial changes observed among the samples to a greater degree than total oil content.

3.3. Effect of harvest maturity on the composition of hop essential oil

Although there are some slight differences between the three harvest years, in general, 11 of the 16 hop volatiles (isobutyl isobutyrate, α-pinene, β-pinene, β-myrcene, methyl heptanoate, limonene, linalool, neral, geraniol, β-caryophyllene, and α-humulene) increased with on the bine ripening time over harvest (Sup. Table 3). Similar to other studies (Howard and Slater, 1958, Sharp et al., 2014), the major hydrocarbon fraction (β-myrcene, β-caryophyllene, and α-humulene) significantly increased over harvest. Although these compounds make up a significant portion of hop essential oil (>50%), their physicochemical properties make them unlikely

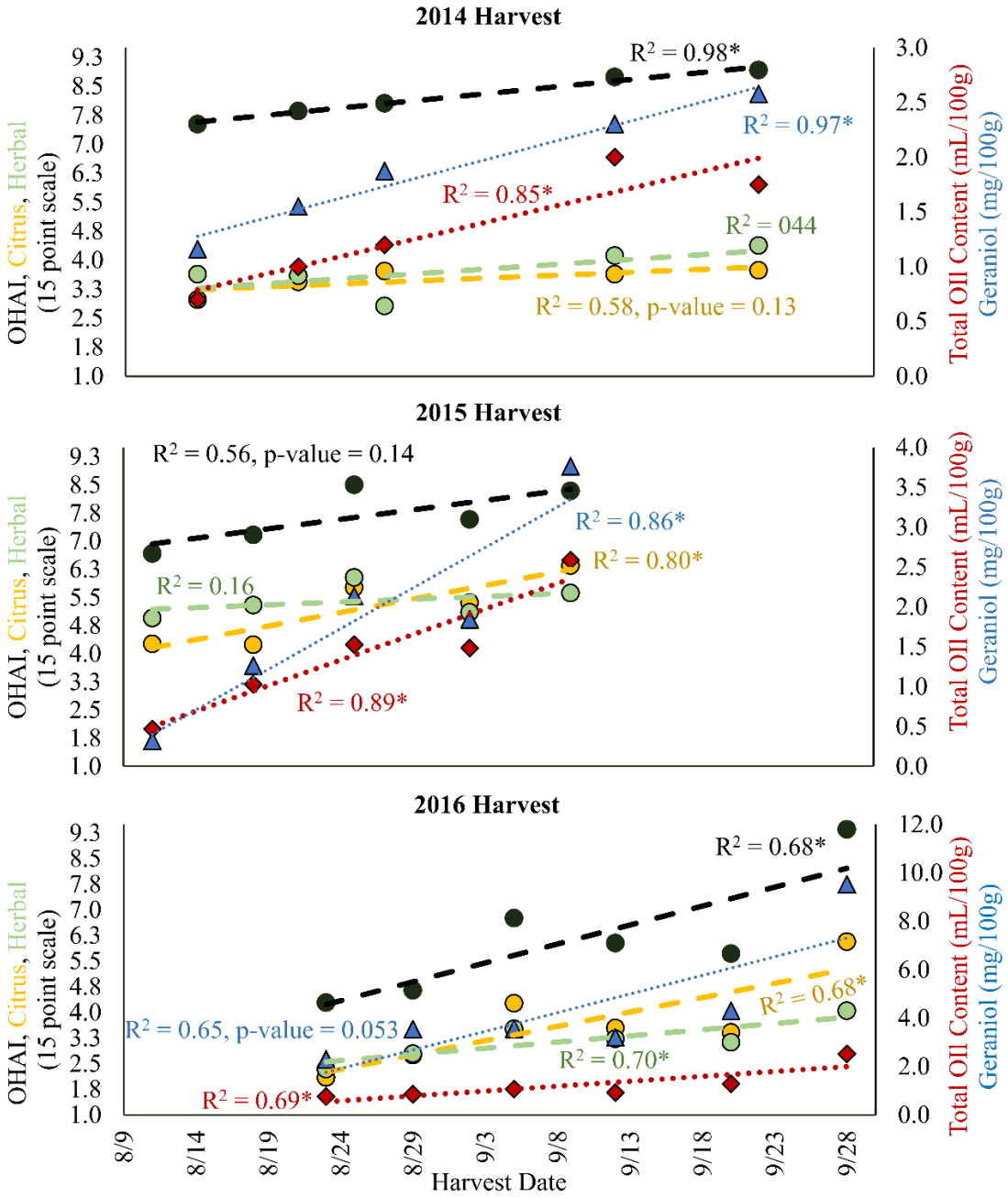

Figure 1. The impact of harvest maturity on Cascade quality as described by total oil content (mL/100g) (◆), geraniol (mg/100 g hop) (blue triangle) as well as dry-hop aroma intensity (●) and dry-hop aroma quality (Citrus (●) and Herbal (●)) over the 2014 (n=5), 2015 (n=5), and 2016 (n=6) harvests. *Pearson correlation coefficient significantly different than 0, p-value < 0.05. OHAI = Overall Hop Aroma Intensity.

contributors to beer flavor (Rettberg, et al., 2018). Although these analytes are easy to measure and may help distinguish between varieties (Probasco & Murphey, 1996), they are of little importance to predicting beer flavor during dry-hopping.

There is evidence that suggests monoterpene alcohols play a significant role in hoppy beer flavor (Inui et al., 2013, Kishimoto et al., 2006, Lafontaine and Shellhammer, 2018, Lafontaine et al., 2018; Takoi, Itoga, Koie, Kosugi, Shimase, Katayama, et al., 2010). Over the three harvest years, the monoterpene alcohols, geraniol and linalool, were found to significantly increase with on the bine ripening time during harvest. Similarly, Sharp, et al. (2014) observed that linalool concentrations increased between early, typical and late harvest Cascades, but that geraniol concentrations peaked in typical harvested Cascades. When considering other hop varieties, Bailey, et al. (2009) (on Willamette) and Matsui, et al. (2016) (on Saaz) found that linalool and geraniol concentrations significantly increased with on the bine ripening time. Again, although there were some differences between the harvest years, OHAI and Citrus quality were also positively correlated to geraniol concentrations (Figure 1). These observations highlight the importance of harvest maturity in producing the highest quality Cascades for dry-hopping. Yet harvest maturity/timing must be balanced against the risk of hop cones shattering during harvest and/or post-harvest processing. There is a limit to how late a farmer can pick hops without suffering a dramatic decrease in harvest yield and/or lupulin loss (Sharp, et al., 2014).

3.4. Impact of harvest maturity on thiol precursor and free thiol concentrations

The influential role that polyfunctional thiols (3MH, 3MHA, and 4MMP) have on beer aroma has been highlighted in several papers (Gros et al., 2011, Kishimoto et al., 2008, Kishimoto et al., 2008, Reglitz and Steinhaus, 2017, Roland et al., 2016, Takoi et al., 2009). These thiols can exist in hops as thiol precursors and as free thiols (Roland et al., 2017, Roland et

al., 2016). The form thiols exist in hops may influence how a brewer uses those hops during the brewing process to maximize their potential. Hops with higher thiol precursor concentrations are recommend for use in the kettle and whirlpool, as thiols can be liberated from these precursors during fermentation via yeast β-lyase activity. Hops with higher free thiol concentrations should be used for dry-hopping as they contain greater quantities of free volatiles that can be extracted during this cold extraction process.

Unfortunately, there is no published data on how these analytes develop in hops (or more broadly in flowers) during harvest and how this might impact beer aroma. In general, over the three harvest years it was observed that free thiol concentrations (mainly 3MH) significantly increased with harvest date (Sup. Table 4) and were significantly positively correlated to OHAI and Citrus quality for each of the three harvest years. Notably, 3MHA was not detected in the ground hop material, which is supported by (Kishimoto, et al., 2008), but was quantified in distilled hop oil (data not shown). It should be noted that the extraction of hop essential oil via hydrodistillation has been shown to lead to artifact formation in the compositional analysis of essential oils (N. Rettberg, Thörner, & Garbe, 2012). In comparison, thiol precursors (mainly Cys3MH) significantly decreased as a function of harvest date (Sup. Table 5). In general, the concentrations of thiol precursors were also negatively correlated to OHAI and Citrus quality over the three harvest years. Although the concentrations of free thiols and thiol precursors differed between harvest years, it is clear, at least on this farm, that early harvest Cascade hops had higher thiol precursor concentrations and were better suited for kettle and/or whirlpool additions, while later harvested Cascade hops were higher in free thiol concentrations and might be better suited for dry-hopping additions.

Interestingly, the development of these analytes during on bine maturation in hops was opposite that found for Sauvignon Blanc grapes, where the concentrations of thiol precursors reached a maximum later in the harvest window (Kobayashi et al., 2011, Roland et al., 2010). This suggests that the development of these thiol precursors differs between the maturation of flowers and fruits. Interestingly, the concentrations of cysteinylated 3MH were significantly negatively correlated to dry matter in hops, while the concentrations of free 3MH were positively correlated to dry matter (Figure 2). Although the concentrations of these analytes were different over the three harvest years for similar dry matter content, later harvested Cascades (with >25–26 % dry matter) would be better suited for dry-hopping because there was a higher amount of terpene alcohols and free thiols. Conversely, earlier harvested Cascades (dry matter content 20–24%) contained high thiol precursor concentrations and should be added to the kettle or whirlpool. This is because these additions occur before primary fermentation and would allow yeast β-lyase activity to liberate the free thiol from the precursor during fermentation and lead to increased aroma in beer.

The following biosynthesis for S-cysteine conjugates of 3MH and 4MMP was proposed by Wüst in Sauvignon Blanc grapes (Kammhuber, et al., 2017). A glutathione-cysteine conjugate of a polyfunctional thiol is created when glutathione transferase reacts with glutathione and an unsaturated α, ß-unsaturated carbonyl compound. The GS-X glutathione conjugate pump then actively transports this glutathione–cysteine conjugate into the cell vacuole, where a peptidase in the vacuole further cleaves the glutathione moiety and yields the specific S-cysteine conjugate. In beer the only proposed release of thiols from these cysteine-conjugates is believed to be a result of yeast β-lyase activity during fermentation, although it is possible that enzymes derived from molds grown on the surface of hops could have impacted free thiol and thiol precursor

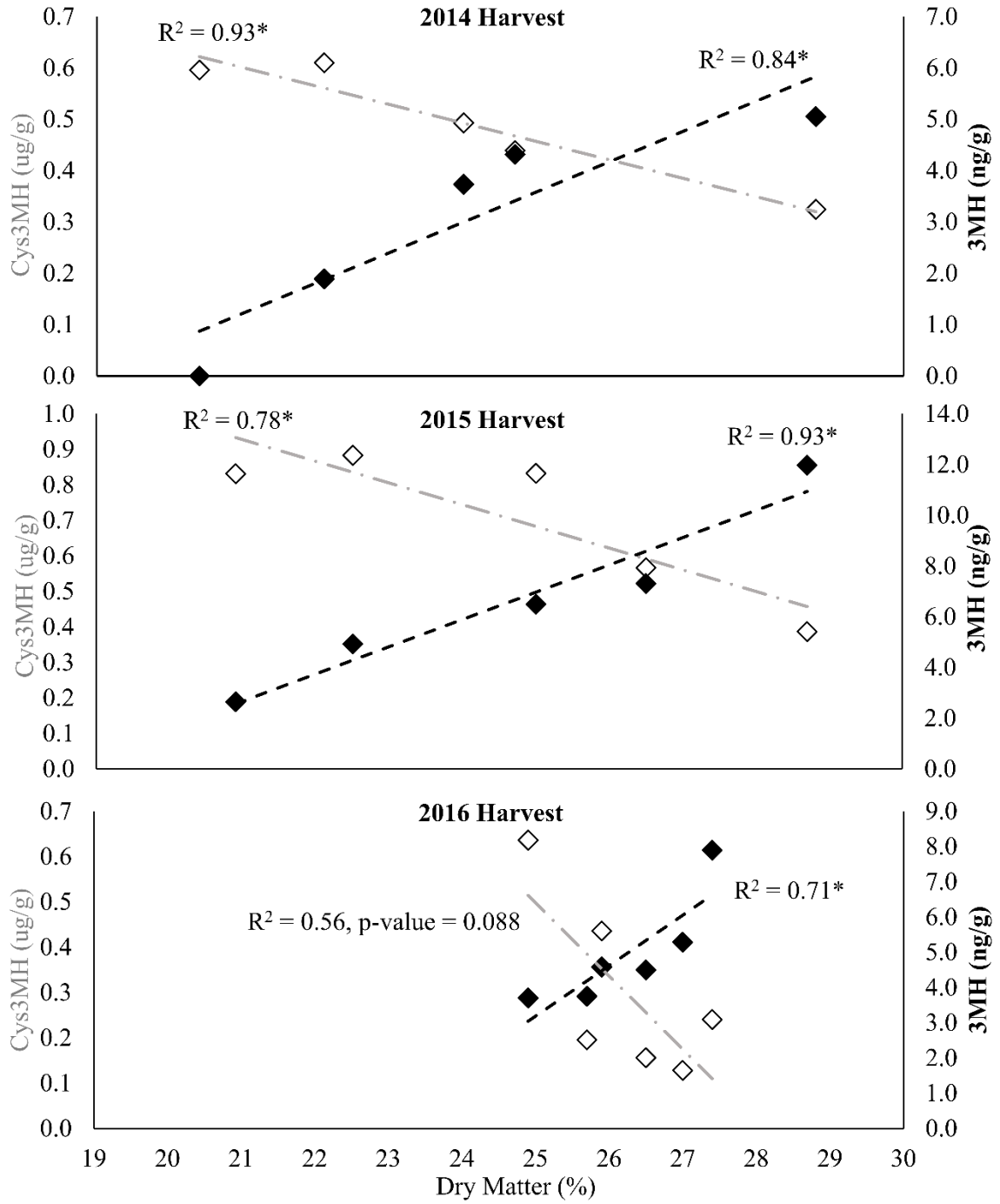

Figure 2. Dry matter (%) vs the Cys3MH (µg/g) (◇) and 3MH (ng/g) (◆) concentrations. *Pearson correlation coefficient significantly different than 0, p-value < 0.05.

concentrations. Due to the significant rise in concentration of free thiols and decrease in thiol precursor concentrations throughout ripening over the three harvest years, it is more likely that there may be an enzymatic pathway in hops which drives the conversion of 3MH thiol precursors to free 3MH. Identifying this pathway could be a useful tool for hop breeders and may help explain some of the varietal differences observed in the concentrations of these analytes.

In grapes, concentrations of 4 MMP precursors are found to be equally distributed between the berry skin and pulp, while the precursors of 3MH are detected at concentrations 8× higher in the berry skin as compared to in the pulp (Peyrot des Gachons, C., Tominaga, T., & Dubourdieu, D. , 2002, Roland et al., 2011). Due to the non-selective thiol analysis approach used in this study (i.e. analysis of ground hop cones), the location of the thiol precursors and free thiols within the hop cone (i.e. lupulin gland, strig, and/or bract) is unclear. However, the location of these analytes within the cone could have an influence on their concentration during the post-harvest processing of whole hops into concentrated lupulin powders, pellets, and extracts. Further examination of the occurrence of these analytes within the hop cone is of importance to the hop processing industry.

3.5. Investigating the influence of harvest maturity on hop quality using a multivariate approach

Principal component analysis (PCA) was performed on the correlation (n−1) matrix of the mean sensory scores for the dry-hop treatments, % dry hop matter, total oil content, as well as the concentrations of humulone, lupulone, free thiols, thiol precursors, and essential oil components (Figure 3). The first three principal components explained 78.8% of the variation within the data set, with PC1 accounting for 39.8% and describing

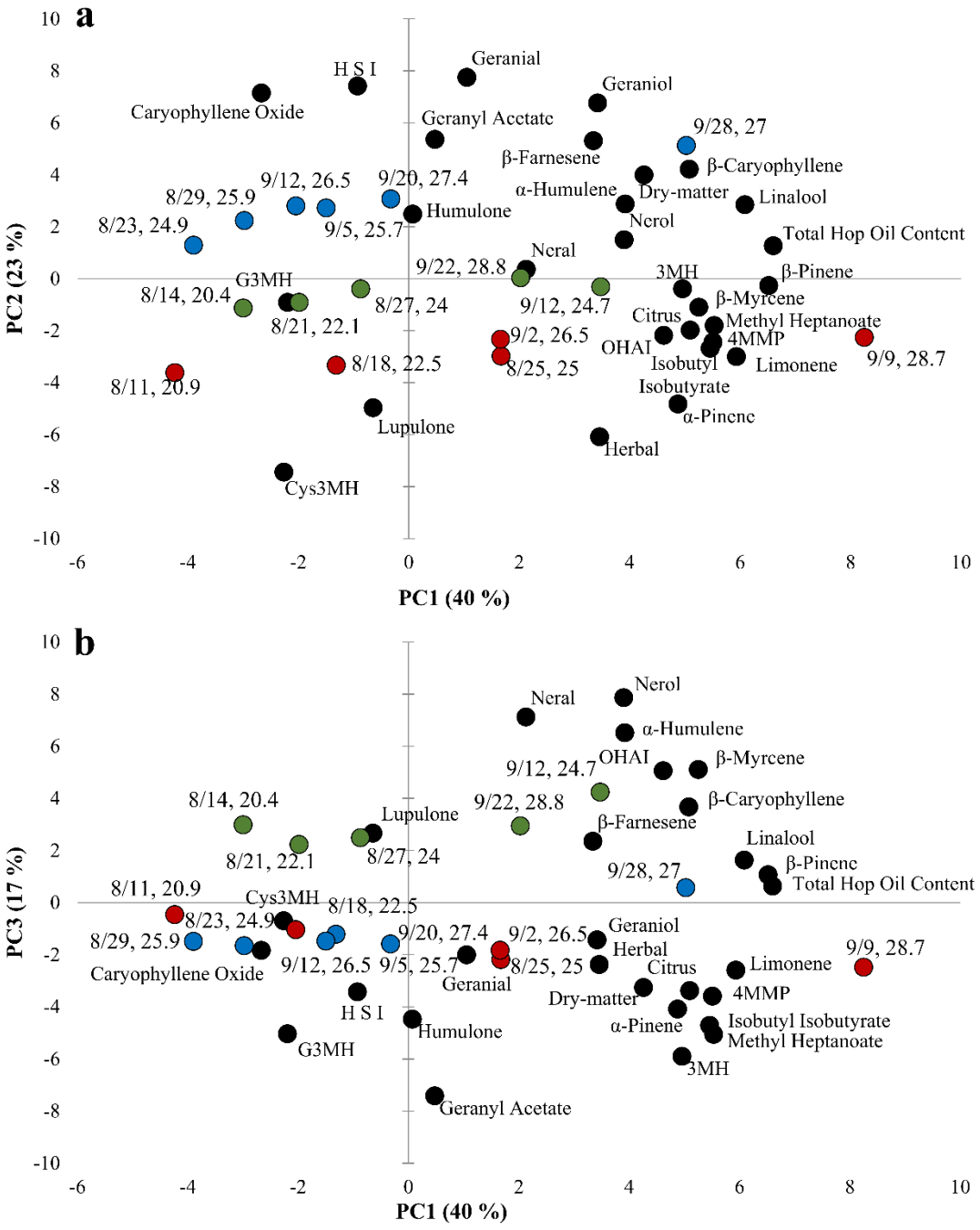

Figure 3: Principle Component Analysis (type - pearson correlation) of the mean scores of the sensory attributes and hop quality chemical analyses (●) among the (16) dry-hop harvest treatments over the 2014 (●), 2015 (●), and 2016 (●) harvests. (a) biplot of PC1 & PC2 explaining 63% of the variation in the data (b) biplot of PC1 & PC3 displaying an additional 17% of the variation in the data set. The treatment codes represent the (harvest date, dry-matter %).

the harvest date, PC2 accounting for 22.4%, and PC3 accounting for 16.6%. Moving from left to right in the biplots across PC1 shows that as hops were harvested later, the beer sensory attributes, dry matter, total oil content, a majority of the hop essential oil volatiles, and the free thiol concentrations increased. Concentrations of thiol precursors followed an opposite trend. There are also trends between the harvest years, with the samples from 2016 occurring at the top of the biplot (PC2 in Figure 3a), followed by the 2014 samples, then the 2015 samples. It is not surprising that different growing seasons led to Cascade hops that were chemically different and attributed different aroma profiles during dry-hopping. Similar observations were made in (Forster and Gahr., A. , 2014, Van Holle et al., 2017). Although there were significant differences between the harvest years, harvest maturity and on the bine ripening time had the largest impact on the development of a majority of the hop volatiles as well as the aroma intensity and quality that the hops attributed to beer during dry-hopping.

3.6. Hop thiol concentrations influencing beer thiol concentrations

The concentrations of 4MMP, 3MHA, and 3MH were considered in the dry-hopped beer (Table 3). As discussed previously, there are several studies that highlight the importance of terpene alcohols in hoppy beer flavor (Inui et al., 2013, Kishimoto et al., 2006, Lafontaine et al., 2018, Takoi et al., 2010). Although no 3MHA was detected in the ground hop material, detectable concentrations of 3MHA were found in the dry-hopped beer. Observations by Kishimoto, et al. (2008) were similar and demonstrated that 3MHA increased during fermentation, and they proposed that 3MHA release could be yeast strain dependent and beers with higher 3MHA should have higher aroma. However, in this study, only clarified and fully attenuated beer was dry-hopped at ~386 g

Table 3. Impact of harvest maturity on free beer thiol concentrations.

Harvest Date	Dry Matter (%)	3MH (ng/L)	3MH Disulfides (ng/L)	4MMP (ng/L)	3MHA (ng/L)
8/14/2014	20.4	124.6	-	6.7	3.8
8/21/2014	22.1	97.9	-	7.6	3.3
8/27/2014	24	107.9	-	10.4	13.8
9/12/2014	24.7	141.2	-	5.8	9.3
9/22/2014	28.8	108.5	-	6.8	15.3
Pearson's r with harvest date		0.187		-0.306	0.755 (p=0.14)
Pearson's r with dry matter		-0.069		-0.083	0.857 (p=0.06)
8/23/2016	24.9	72.9	186.4	n.d.	2.1
8/29/2016	25.9	66.5	179.4	n.d.	3.1
9/5/2016	25.7	79.7	119.7	n.d.	3.2
9/12/2016	26.5	83.7	184.5	n.d.	4.8
9/20/2016	27.4	80.3	170.2	n.d.	4.9
9/28/2016	27	74.7	124.2	n.d.	4.8
Pearson's r with harvest date		0.447	-0.481		**0.916***
Pearson's r with dry matter		0.401	-0.208		**0.952***

* Values in bold are significant (p-value < 0.05) (-) did not measure (n.d.) not detected

hop/hL with the different harvest samples. Therefore, the potential of biotransformation by yeast was not expected and it is possible that something other than yeast, such as hop-derived enzymes, may also drive the conversion of 3MH to 3MHA during dry-hopping. While no strong correlations were found between the beer sensory scores and the concentrations of 3MHA in beer, the threshold for 3MHA (5 ng/L) is 10× lower than that of 3MH (55 ng/L) (Kishimoto, et al., 2008), suggesting that concentrations of 3MHA should have a higher impact on beer aroma.

No clear trends were observed between the concentrations of 4MMP or 3MH in beer with harvest date, % dry matter, or the beer sensory attributes. The concentrations of

disulfide-bound 3MH was also investigated as a potential aroma reservoir in beer, as it has been highlighted as an important reservoir for wine aroma (Roland, et al., 2016). These compounds are an important indicator of the oxidation state for 3MH and it was observed that 60% of the total 3MH was oxidized in the dry-hopped beers (Table 3). Although the impact of 3MH disulfides on overall beer aroma remains unclear, this indicates that even under relatively low dissolved oxygen conditions during dry-hopping (< 110 µg/L), oxidation always occurs and can lead to challenges when trying to identify the impact of polyfunctional thiols on beer flavor. To fully elucidate the impact of polyfunctional thiols on beer flavor, one must evaluate the concentrations of thiol disulfides, thiol precursors and free thiols in relation to each other. This is because there are a number of possible avenues that may influence the impact thiols have on beer flavor such as the direct extraction of free thiols from hops during dry-hopping, the chemical release of free thiols from thiol precursors during dry hopping (i.e. Strecker degradation of dicarbonyls (Tran, Cibaka, & Collin, 2015)), the possible liberation of free thiols from thiol precursors due to residual hop enzymes during dry-hopping, and/ or the oxidation of free thiols into thiol disulfides during dry-hopping and beer storage.

4. Conclusions/ Industrial Considerations

It was observed that overall hop aroma intensity (OHAI) and Citrus quality attributed to beer during dry-hopping increased as a function of harvest date, indicating that later picked Cascades tended to produce dry-hopped beers with higher overall hop aroma intensities that were primarily citrusy in quality. The development of humulones did not change as a function of harvest date. However, total essential oil content displayed a significant positive trend with the harvest date. At an individual component

level, a number of different hop volatiles were positively correlated with the harvest date. Most notably geraniol concentrations increased significantly with harvest maturity, and the latest harvested hops had ~2×, ~12×, and ~ 4× more geraniol than the early harvested samples in 2014, 2015, and 2016, respectively. This study is the first to report how the concentrations of thiol precursors (Cys3MH, G3MH, Cys4MMP, G4MMP) and free thiols (4MMP, 3MHA, and 3MH) in hops are impacted by harvest maturity. Concentrations of thiol precursors decreased over harvest and the concentrations of free thiols increased. Three years of data from this plot indicates that later-picked Cascades had higher total oils, higher geraniol concentrations, lower thiol precursors, higher free thiol concentrations and attributed more intense dry-hop aroma than earlier picked hops.

In general, these results suggest that hops harvested later in the harvest window (dry matter content >26%) might be better suited for use in dry-hopping because they attribute the highest and most citrusy aroma to beer. This is because later harvested Cascades had the highest concentrations of most of the hop essential oil volatiles and free thiols (mainly 3MH) available to be extracted during dry-hopping. Conversely, early harvested Cascades (dry matter content 20–24%) were higher in thiol precursor concentrations (mainly Cys3MH) and might be better suited for use in the kettle/whirlpool hop additions because bitterness potential has fully developed (i.e. peak humulone concentrations reached) and these additions occur before primary fermentation. Therefore, yeast β-lyase activity may liberate the thiols from these precursors during fermentation to increase the aroma perception of beer.

It is important to note that this study is limited to Cascade hops grown on one farm. There are several studies that have shown that hop quality can vary significantly as

a function of harvest timing, harvest location and hop variety (Bailey et al., 2009, Forster and Gahr., A. , 2014, Van Holle et al., 2017). Therefore, future work should explore the impact of harvest maturity for Cascade hops grown on other farms, in addition to examining other hop varieties that have been shown to have high concentrations of thiol precursors, such as Saaz, or free thiols, such as Citra. Investigating the maturity effect on the concentrations of free and bound thiols in these varieties may help identify the genetic pathways that make these varieties unique from a thiol perspective and also prevent off flavors (such as onion garlic notes) from forming due to other sulfur-related analytes in later harvested hops. Future studies should also investigate the impact of harvest maturity on the development of terpene glycosides, as this may explain some of the increase in geraniol and linalool during harvest.

Due to the nature of commercial hop harvesting, it is possible that the same variety of hop will be picked at different times during the harvest window because of brewer preferences, processing limitations, competing optimal maturity windows with other hop varieties, etc. Understanding how to maximize the brewing potential of hops allows growers to target hop quality based upon how a brewer plans to use hops. However, practical constraints still need to be considered. Hop shattering is a complication that stems from harvesting overly mature hops with high dry matter. While very mature hops might be desirable from a dry-hopping perspective, these mature hops could be a challenge for growers to process. It is up to the hop grower and brewer to set practical and commercially achievable targets for hop quality.

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5. Supplementary Information

Sensory analyses protocols and panel/panelist validation

Descriptive Analysis - Cascade 2014 Harvest

The 5 dry-hopped beers made from the samples collected from the 2014 harvest were evaluated among 27 dry-hopped beers that were made in a similar fashion (22 different hop lots (dry-hopped at 3.8 g/L), three internal process replicates and one unhopped control). The panel was comprised of 11 trained panelists (9 males and 2 females; 25-65 yrs. old). Three intensive training sessions were completed in advance of data collection. Based on discussion from these training sessions the final ballot included the attributes: Overall Hop Aroma Intensity (OHAI), Citrus, Herbal, Resinous/hop oil, Tropical Fruit to be evaluated on a 0-15 point scale. Over the course of 15 sessions, the panelists evaluated all of the samples five times in a randomized fashion. 10 samples were evaluated per session and the presentation order was blocked by replication.

Descriptive Analysis - Cascade 2015 Harvest

The 5 dry-hopped beers made from the samples collected from the 2015 harvest were evaluated among 33 dry-hopped beers that were made in a similar fashion (29 different hop lots (dry-hopped at 3.8 g/L), three dry-hop reference standards made with the same hop lot (2 g/l, 8 g/l and 16 g/l) and one unhopped control). The panel was comprised of 13 trained panelists (11 males and 2 females; 25-66 yrs. old). Four intensive training sessions were completed in advance of data collection. Based on discussion from these training sessions and the results from the 2014 Cascade harvest panel, the final ballot included the attributes: Overall Hop Aroma Intensity (OHAI), Citrus, and Herbal/Tea to be evaluated on a 0-15 point scale. An efficient resolvable incomplete

block design was used to create a presentation order for the samples across four replications (SAS, Cary, NC). Over the course of 20 sessions, the 13 panelists evaluated all the samples five times in a randomized fashion. The first replication (i.e. sensory block) was used to familiarize the panelists with the samples and the testing environment. Because of the large number of treatments, it took the panelists four sessions (3 sessions of 8 samples and 1 session of 9 samples) to evaluate all the hopped samples per replication.

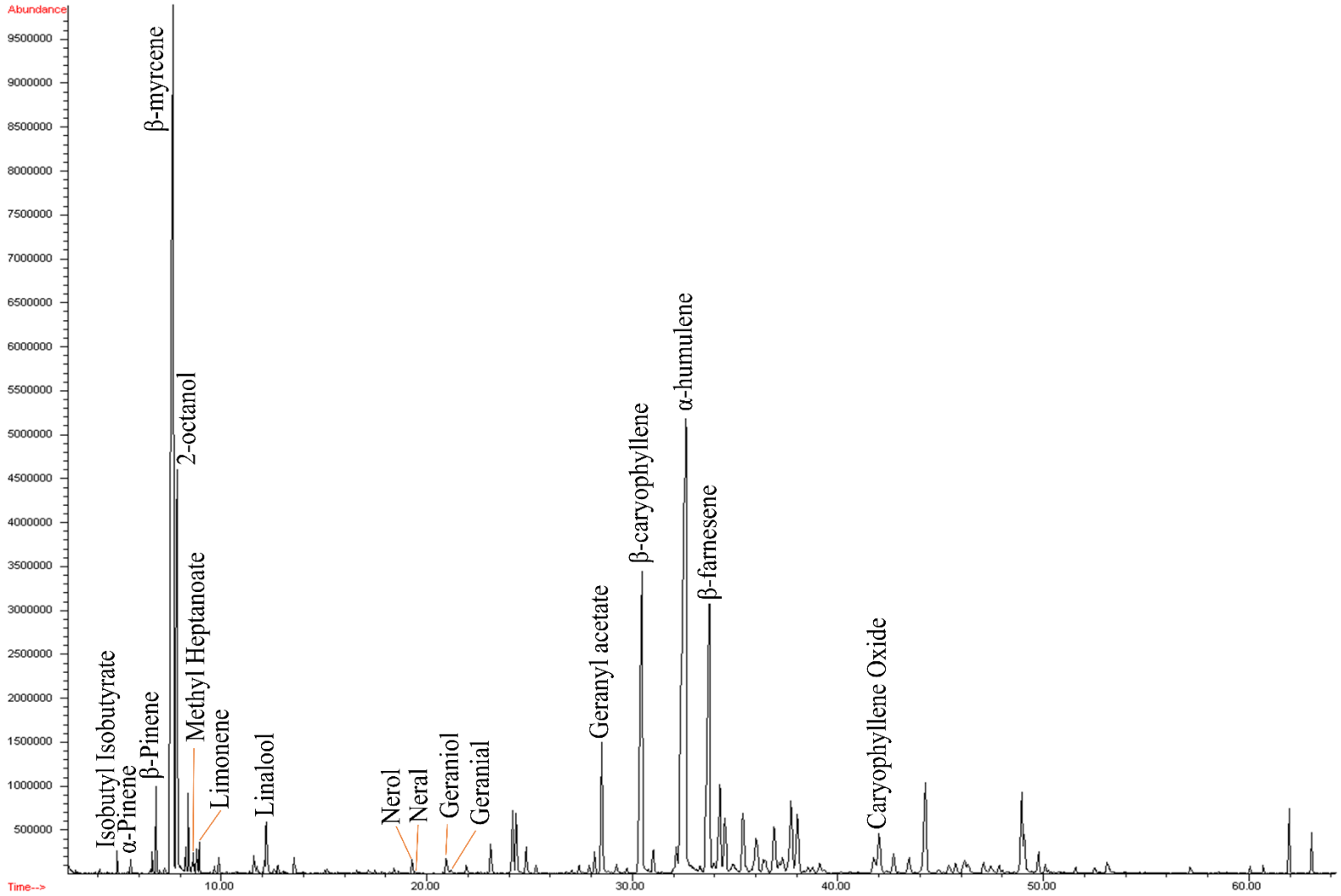
Descriptive Analysis - Cascade 2016 Harvest

The 6 samples collected from the 2016 harvest were evaluated by 12 trained panelists (9 males and 3 females; 21-55 yrs old). Seven samples (6 different hop lots, and 1 unhopped control) were evaluated. Four intensive training sessions were completed in advance of data collection. Based on discussion from these training sessions the final ballot included the attributes: OHAI, Citrus, Herbal/Tea, Pine/Resinous/Dank, Tropical/Fruity, and Tropical/Catty to be evaluated on a on a 0-15 point scale. It took the panelists 1 session of 7 samples to experience all the Cascade samples per replication. To ensure the repeatability of the panelist responses, 9 replications were used to evaluate the Cascade harvest samples. The first three replications were used to familiarize the panelists with the samples and the testing environment, and the final six replications were used for data analysis.

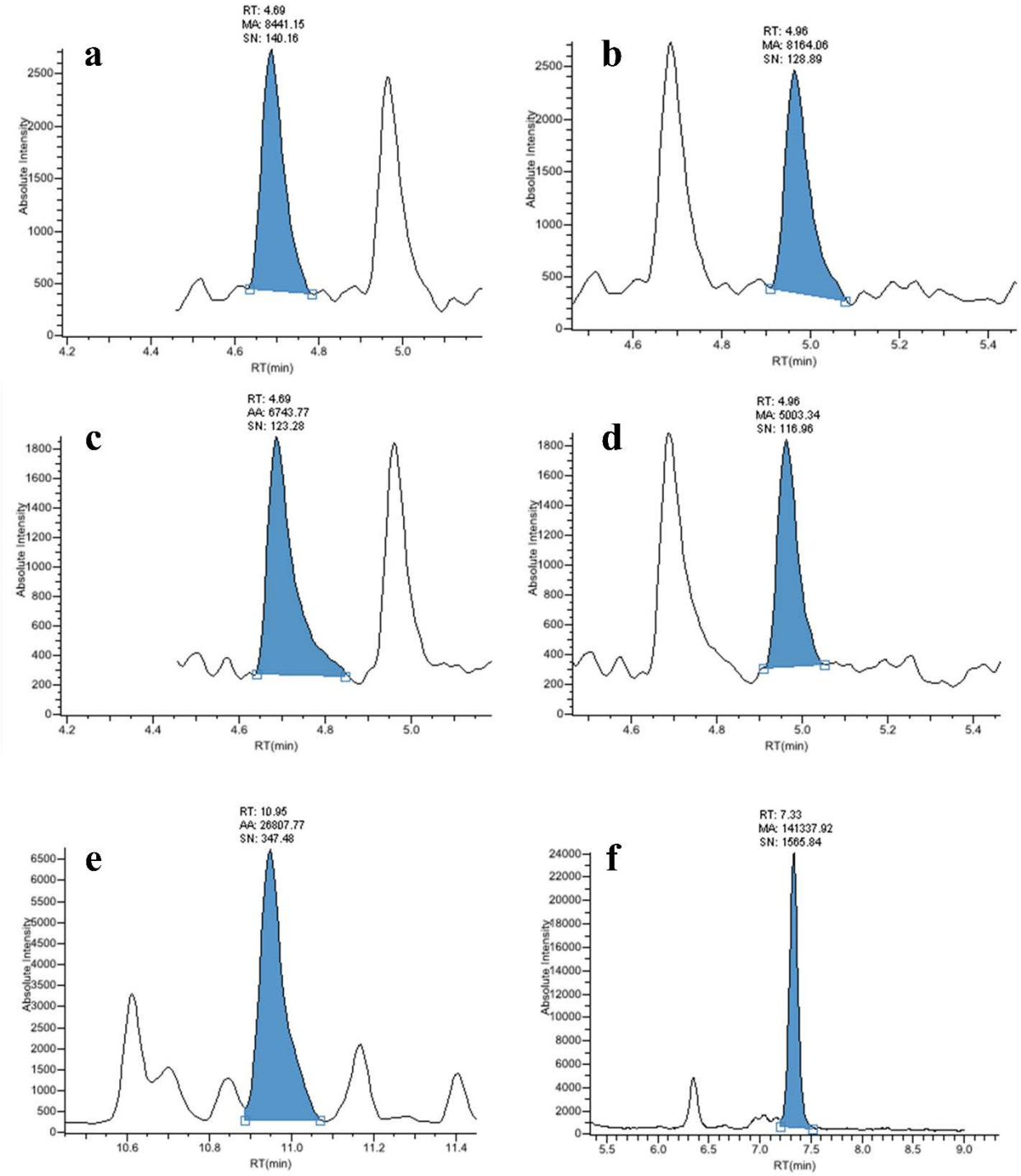
Sup. Table 1. Reference standards with intensity scores used in descriptive analysis panels.

Attributes	**Unhopped Control**	**386 g/hL**	**1600 g/hL**	**Hop Valley Sir Orange-A-Lot**	**Ballast Point Grapefruit Sculpin**	**Hop Valley Citrus Mistress**	**Sierra Nevada Pale Ale**	**10-Barrel Joe IPA**	**Founders All Day IPA**
Cascade 2014 Harvest									
OHAI	-	-	-	8	15				
Cascade 2015 Harvest									
OHAI	0	8-9	14-15	-	14-15	7-8	-	-	-
Citrus	0	7-8	5-6	-	13-14	6-7	-	-	-
Herbal/Tea	0	5-6	12-13	-	1-2	6-7	-	-	-
Cascade 2016 Harvest									
OHAI	0	-	-	-	Did not consider for OHAI	-	5-6	Did not consider for OHAI	12
Citrus	0	-	-	-	11	-	3	5-6	6-7
Herbal/Tea	0	-	-	-	-	-	4	1	5
Tropical/Catty	0	-	-	-	-	-	1	9-10	3-4
Tropical/ Fruity	0-1	-	-	-	7-8	-	1	4-5	2-3
Pine/ Resinous/ Dank	0	-	-	-	-	-	2	4	7-8

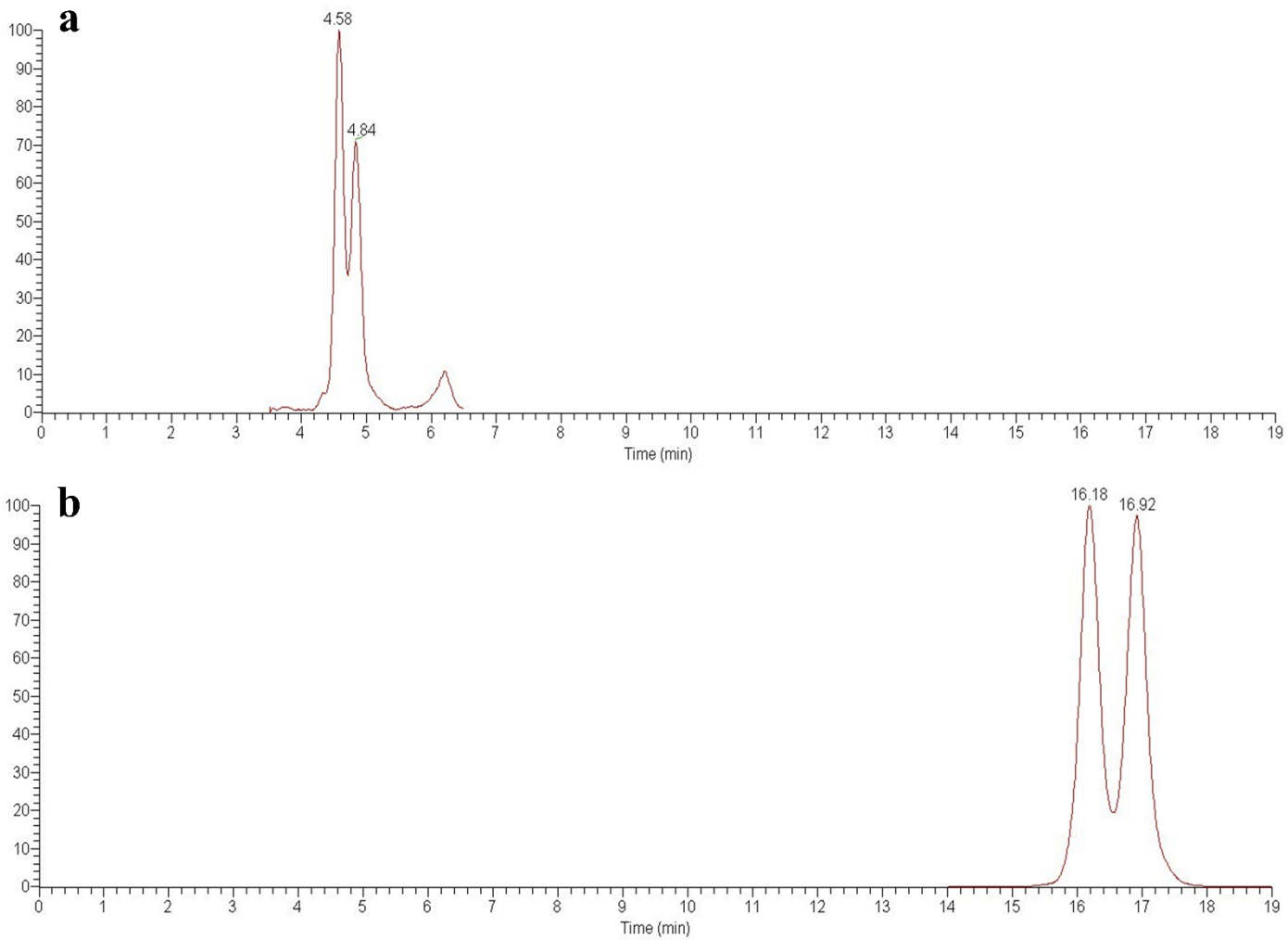
*OHAI = Overall Hop Aroma Intensity (-) did not measure



Sup. Figure 1. Total ion chromatogram and identification of select hop volatiles in hydrodistilled oil obtained by GC-MS.



Sup. Figure 2. Chromatograms of 3MH diastereomers (a and b), 3MH-disulfide diastereomers (c and d), 3MHA (e) and 4MMP (f) in hops and beer.



Sup. Figure 3. Chromatograms of Cys3MH (a) and G3MH (b) in hops.

Sup. Table 2. Mixed model analysis of variance on the sensory attributes.

2014 Cascade Mixed Model ANOVA

			OHAI		Citrus		Herbal/ Tea		Tropical/ Fruity		Pine/Resinous/ Dank		Tropical/Catty	
Source	**Type**	**DF**	**F**	**P-value**	**F**	**P-value**	**F**	**P-value**	**F**	**P-value**	**F**	**P-value**	**F**	**P-value**
Sample	Fixed	5	17.9	**< 0.0001**	6.8	**< 0.0001**	5.4	**0.001**	6.1	**0.000**	11.5	**< 0.0001**	-	-
Panelist	Random	9	4.5	**0.001**	15.2	**< 0.0001**	2.5	**0.028**	2.2	**0.041**	5.0	**0.000**	-	-
Rep	Fixed	4	0.3	0.868	1.2	0.337	0.5	0.739	0.1	0.986	0.8	0.509	-	-
Sample*Panelist	Random	45	2.7	**< 0.0001**	2.0	**0.001**	2.7	**< 0.0001**	2.4	**< 0.0001**	1.9	**0.001**	-	-
Sample*Rep	Fixed	20	1.2	0.291	1.0	0.501	1.4	0.111	1.2	0.264	1.1	0.377	-	-
Panelist*Rep	Random	36	0.8	0.786	0.6	0.950	0.8	0.775	1.4	0.076	1.03	0.428	-	-
Error		180												

2015 Cascade Mixed Model ANOVA

Source	**Type**	**DF**	**F**	**P-value**	**F**	**P-value**	**F**	**P-value**	**F**	**P-value**	**F**	**P-value**	**F**	**P-value**
Sample	Fixed	5	19.2	**< 0.0001**	13.2	**< 0.0001**	6.9	**< 0.0001**	-	-	-	-	-	-
Panelist	Random	9	6.3	**0.000**	4.3	**0.004**	5.3	**0.000**	-	-	-	-	-	-
Rep	Fixed	3	0.5	0.690	0.1	0.937	0.3	0.837	-	-	-	-	-	-
Sample*Panelist	Random	45	1.4	**0.083**	1.3	0.136	1.7	**0.010**	-	-	-	-	-	-
Sample*Rep	Fixed	15	1.2	0.280	0.7	0.825	1.9	**0.030**	-	-	-	-	-	-
Panelist*Rep	Random	27	0.9	0.664	0.8	0.728	1.1	0.364	-	-	-	-	-	-
Error		135												

2016 Cascade Mixed Model ANOVA

Source	**Type**	**DF**	**F**	**P-value**	**F**	**P-value**	**F**	**P-value**	**F**	**P-value**	**F**	**P-value**	**F**	**P-value**
Sample	Fixed	6	22.4	**< 0.0001**	14.5	**< 0.0001**	12.2	**< 0.0001**	12.3	**< 0.0001**	4.6	**0.001**	7.0	**< 0.0001**
Panelist	Random	7	2.7	**0.026**	8.6	**< 0.0001**	5.9	**< 0.0001**	7.9	**< 0.0001**	7.4	**< 0.0001**	2.9	**0.012**
Rep	Fixed	5	1.0	0.443	1.7	0.151	0.6	0.700	1.6	0.199	0.8	0.533	0.5	0.788
Sample*Panelist	Random	42	2.5	**< 0.0001**	2.7	**< 0.0001**	2.2	**0.000**	1.1	0.277	6.8	**< 0.0001**	2.8	**< 0.0001**
Sample*Rep	Fixed	30	1.0	0.507	1.4	0.100	1.2	0.262	1.3	0.183	0.9	0.598	1.1	0.370
Panelist*Rep	Random	35	1.0	0.717	0.8	0.776	1.5	**0.039**	1.1	0.388	1.4	0.070	2.5	**< 0.0001**
Error		210												

Values in bold indicate p-value < 0.05 (-) did not measure

Sup. Table 3. Harvest maturity and its impact on select hop volatiles (mg/100g) in essential oil.

Harvest Date	Isobutyl Isobutyrate	α-Pinene	β-Pinene	β-Myrcene	Methyl Heptanoate	Limonene	Linalool	Nerol	Neral	Geraniol	Geranial	Geranyl Acetate	β-Caryophyllene	α-Humulene	β-Farnesene	Caryophyllene Oxide
8/14/2014	0.3	0.6	4.6	286.0	0.5	1.4	2.0	2.1	0.0	1.2	0.4	0.2	88.7	232.0	82.6	3.8
8/21/2014	0.3	0.8	7.1	482.7	0.9	2.1	3.0	2.1	0.0	1.5	0.4	0.3	81.2	222.2	74.7	2.7
8/27/2014	0.6	1.1	9.4	592.2	1.3	3.5	4.5	2.1	2.4	1.9	0.3	0.3	82.6	219.9	76.9	5.1
9/12/2014	2.1	2.4	22.2	1523.1	2.9	8.4	8.0	3.2	9.5	2.3	0.3	0.5	120.0	294.1	117.7	3.6
9/22/2014	1.3	1.6	16.3	1118.6	1.6	4.2	5.5	2.3	8.8	2.6	0.1	0.2	93.1	241.0	82.8	2.4
Pearson's r	0.814	0.820	**0.867**	**0.871**	0.744	0.701	0.814	0.565	**0.951**	**0.985**	-0.829	0.368	0.552	0.544	0.457	-0.388
8/11/2015	1.8	1.6	2.5	69.6	2.0	2.3	0.5	0.0	0.0	0.3	0.0	0.6	30.8	79.8	14.2	1.1
8/18/2015	4.1	3.8	8.0	275.0	4.9	5.4	1.8	0.2	0.0	1.3	0.1	5.0	60.4	136.9	36.5	1.7
8/25/2015	6.4	5.7	13.7	508.8	7.4	8.4	4.1	0.3	0.0	2.1	0.3	10.4	77.3	166.7	47.3	0.0
9/2/2015	6.5	5.7	13.6	520.7	7.7	8.3	4.3	0.4	0.0	1.8	0.2	11.2	76.9	163.3	49.5	0.0
9/9/2015	14.3	10.5	25.8	1126.5	15.7	15.2	8.6	2.1	0.2	3.8	0.4	24.3	108.6	241.4	85.9	0.0
Pearson's r	**0.918**	**0.944**	**0.949**	**0.937**	**0.932**	**0.944**	**0.951**	0.807	0.707	**0.925**	**0.909**	**0.946**	**0.954**	**0.941**	**0.947**	-0.775
8/23/2016	0.4	0.3	4.8	154.9	1.3	1.7	2.1	0.2	0.3	2.3	1.0	18.2	69.1	174.4	60.4	8.6
8/29/2016	0.5	0.4	5.8	169.1	1.9	2.3	2.8	0.3	0.3	3.5	1.4	25.3	68.9	165.8	58.9	12.3
9/5/2016	1.2	0.7	8.8	233.7	3.0	3.3	3.2	0.4	0.4	3.6	1.8	31.7	83.0	184.8	69.0	14.5
9/12/2016	0.6	0.4	6.1	202.1	1.5	2.3	2.7	0.4	0.4	3.2	1.4	29.5	82.8	189.7	69.3	9.7
9/20/2016	2.3	0.8	10.5	326.9	3.3	3.9	6.2	0.5	0.6	4.3	1.7	31.9	84.9	187.9	80.4	9.4
9/28/2016	5.8	1.5	17.2	602.5	8.1	5.6	9.1	3.2	0.7	9.5	3.8	16.1	137.2	244.6	382.6	6.2
Pearson's r	**0.840**	**0.851**	**0.871**	**0.870**	**0.793**	**0.882**	**0.892**	0.737	**0.957**	**0.805**	0.789	0.013	**0.838**	**0.834**	0.721	-0.504

*Pearson's r calculated between the harvest date and hop essential oil components. Values in **bold** are significant (p-value < 0.06

Sup. Table 4. Impact of harvest maturity on the free thiol concentrations in dried, ground hops.

Harvest Date	3MH (ng/g)	4MMP (ng/g)	3MHA (ng/g)	∑ Free thiols (ng/g)
8/14/2014	nd	0.2	nd	0.2
8/21/2014	1.9	0.9	nd	2.8
8/27/2014	3.7	0.7	nd	4.5
9/12/2014	4.3	1.9	nd	6.2
9/22/2014	5.1	1.5	nd	6.6
Pearson's r	**0.915**	**0.893**	**-**	**0.936**
8/11/2015	2.6	0.5	nd	3.1
8/18/2015	4.9	1.7	nd	6.6
8/25/2015	6.5	1.9	nd	8.4
9/2/2015	7.3	1.8	nd	9.1
9/9/2015	12.0	3.4	nd	15.4
Pearson's r	**0.960**	**0.902**	**-**	**0.956**
8/23/2016	3.7	1.1	nd	4.8
8/29/2016	4.6	1.0	nd	5.6
9/5/2016	3.8	1.1	nd	4.8
9/12/2016	4.5	0.9	nd	5.4
9/20/2016	7.9	1.1	nd	9.0
9/28/2016	5.3	1.2	nd	6.5
Pearson's r	0.640	0.475		0.651

*Pearson’s r calculated between the harvest date and free thiols. Values in **bold** are significant (p-value < 0.05)

Sup. Table 5. Impact of harvest maturity on the thiol precursor concentrations in dried, ground hops.

Harvest Date	Cys3MH (ug/g)	G3MH (ug/g)	Cys4MMP (ug/g)	G4MMP (ug/g)	∑ bound thiols (ug/g)
8/14/2014	0.6	5.5	nd	nd	14.6
8/21/2014	0.6	8.6	nd	nd	14.1
8/27/2014	0.5	3.2	nd	nd	11.5
9/12/2014	0.4	11.3	nd	nd	16.5
9/22/2014	0.3	5.0	nd	nd	11.0
Pearson's r	**-0.960**	0.161			-0.240
8/11/2015	0.8	12	nd	nd	17.2
8/18/2015	0.9	13	nd	nd	19.3
8/25/2015	0.8	14	nd	nd	19.8
9/2/2015	0.6	11	nd	nd	15.5
9/9/2015	0.4	9	nd	nd	11.8
Pearson's r	**-0.897**	-0.703			-0.729
8/23/2016	0.6	25	nd	nd	35.1
8/29/2016	0.4	14	nd	nd	22.1
9/5/2016	0.2	11	nd	nd	16.9
9/12/2016	0.2	9	nd	nd	13.1
9/20/2016	0.2	8	nd	nd	12.7
9/28/2016	0.1	6	nd	nd	9.0
Pearson's r	**-0.831**	**-0.877**			**-0.902**

*Pearson's r calculated between the harvest date and thiol precursors. Values in **bold** are significant (p-value < 0.05)

Cys3MH - 3-*S*-cysteinylhexan-1-ol; G3MH - 3-*S*-glutathionylhexan-1-ol; Cys4MMP - 4-*S*-cysteinyl-4-methylpentan-2-one; G4MMP- 4-*S*-glutathionyl-4-methylpentan-2-one

Chapter 6. Publication D

Sensory Directed Mixture Study of Beers Dry-Hopped with Cascade, Centennial, and Chinook

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Highlights:

- Dry-hopping with single hop varieties, Cascade, Chinook, and Centennial, achieved unique varietal aroma profiles in beer.
- Using specific blends of Cascade, Chinook, and Centennial during dry-hopping accomplished similar aroma intensities and qualities to single varietal dry-hop treatments.
- The use of hop blends during dry-hopping increased aroma perception and promoted volatile extraction.

Summary:

Cascade, Chinook, and Centennial are the top three American public aroma hop varieties that are ubiquitously used by U.S. craft brewers, singly or in blends, for dry-hopping. Recently, Takoi et al.[88] showed that using blends of hops during dry-hopping promotes synergy among hop aroma compounds and maximizes the sensory perception of certain beer attributes, such as tropical and citrus character. To investigate the impact of hop blends on the dry-hop aroma profiles of these American varieties, sixteen beers were created with different blends of ground whole cone Cascade, Centennial, and Chinook by utilizing a 4th degree simplex-lattice mixture-design. Outcomes highlighted the benefits of blending hops to produce more intense aromas as compared to dry hopping with single varieties. Some combinations of hop blends achieved similar aroma profiles to single varieties, which might help brewers make substitutions when faced with shortages due to cost and/or quality. These results should help brewers better utilize hops, improve beer quality, and obtain consistent by hoppy beer aroma.

Abstract

American craft beer style and flavor is often driven by the unique qualities of American hops. Cascade, Chinook, and Centennial hops are used prominently for dry-hopping singly and/or in blends to impart an intense hoppy aroma to beer. A sensory directed dry-hopping mixture study was performed to understand the contribution that each of these hops make to beer aroma. Utilizing a 4th degree simplex-lattice mixture-design, sixteen beers were prepared (including an "unhopped" control) by dry-hopping a common "unhopped" base beer with different blends of ground whole cone hops made from the three hop cultivars. The treatments were evaluated by trained panelists using descriptive analysis, where the response variables used by the panel encompassed the sensory attributes that described the unique aromatic features of these three hops, (i.e., citrus, tropical/fruity, tropical/catty, and herbal). Using these outputs, the sensory contributions of each individual cultivar, as well as mixtures of the cultivars, were examined on a per attribute basis. These results can be used to select combinations or blends of the three hops for use during dry-hopping that provide similar or dissimilar overall aroma intensity and quality in dry-hopped beer.

Introduction

The sensory perception of beer is based on a number of factors, which make predicting the aroma and flavor of beer complex. Synergistic, antagonistic, and masking effects have been shown to impact the sensory perception of mixtures of volatile components important for beer aroma.[1] It has been observed that the coexistence of the hop volatiles linalool, geraniol, and β-citronellol can increase the sensory perception of citrus character in model solutions[2] and hopped beer.[3] Controlling hop aroma in beer

requires an understanding of the important hop-derived components that are transferred from the hops into beer and how these components interact with one another to impact sensory perception.

Many craft brewers use dry-hopping as a technique to create an intense hop aroma in finished beer.[4] Cascade, Chinook, and Centennial are American hop varieties that are ubiquitously used, singly or in blends, for dry-hopping.[5] In-depth flavor analysis of beer dry-hopped with each of these hop varieties has shown that each of these hop varieties has unique aroma compounds (i.e. character impact compounds [CICs]) that are important for the aroma profile of each of these hops.[6, 7] Although a number of the CICs were unique to each hop cultivar, some of the CICs were important for all three cultivars, albeit to differing degrees. Most likely these compounds occur in different concentrations in finished dry-hop beer due to the amount of these compounds in the hop material (intra and inter cultivar differences) and the amount of hop material added. Recently Takoi et al.[3] observed that using blends of hops during dry-hopping could promote synergy among hop aroma compounds and maximize the sensory perception of certain beer attributes, such as tropical and citrus character.

A sensory-directed mixture study was performed to understand the contribution that Cascade, Chinook, and Centennial make to dry-hopped beer aroma both singly and in combination. The two objectives of this study were (1) to utilize a 4th degree simplex-lattice mixture-design[8, 9] to combine these three hop varieties in different proportions for dry-hopping and to evaluate the qualitative changes in the resultant beers using descriptive analysis with trained panelists and (2) to understand how these combinations drive particular sensory characteristics in dry-hopped beer.

Experimental

Experimental design

In total, 19 beers (15 blends, 3 internal process replicates, and an "unhopped" control) were prepared using a 4th degree simplex-lattice mixture-design to create blends that varied in the amount of whole cone Chinook, Centennial, and Cascade hops. An "unhopped" pale ale was dry-hopped with these blends at a rate of 3.86 g/L (1 lb/US barrel) (Table 1). The internal process replicates were prepared by performing the dry-hopping procedure twice for each of the 100% (single) cultivar treatments. Discrimination testing was used to evaluate these internal process replicates to ensure the dry-hopping process was reproducible. Descriptive sensory analysis was then used to scale the aroma intensity and quality of the 16 unique treatments. Volatile chemical analyses were performed on the treatment beers to confirm the analytes that may be important for describing the dry-hop aroma these cultivars transmit to beer.

Table 1. The fourth degree simplex-lattice mixture-design of dry-hop treatments from blends of ground whole cone Chinook, Centennial, and Cascade that were used to dry-hop an "unhopped" pale base beer at a rate of 3.86 g/L (1 lb/US barrel).

Dry-hop blending treatments	% Cascade	% Chinook	% Centennial
1-rep 1	100	0	0
1-rep 2	100	0	0
2	75	25	0
3	75	0	25
4	50	50	0
5	50	25	25
6	50	0	50
7	25	75	0
8	25	50	25
9	25	25	50
10	25	0	75
11-rep 1	0	100	0
11-rep 2	0	100	0
12	0	75	25
13	0	50	50
14	0	25	75
15-rep 1	0	0	100
15-rep 2	0	0	100
16	0	0	0

Hop collection

4.5 kg minibales from single lots of whole cone Cascade, Chinook, and Centennial hops were collected after harvest in 2015 courtesy of Crosby hop farms (Woodburn, OR, U.S.A.). Upon arrival at Oregon State University, the hops were repackaged in high barrier foil pouches, purged of air using nitrogen, sealed and stored cold (−20 °C) until dry-hopping and chemical analysis. The total essential oil and compositional analysis of these hops at the time of dry-hopping are shown in Table 2.

"Unhopped" beer production

"Unhopped" beer was prepared on a commercial scale by a regional brewery (BridgePort Brewery, Portland, OR, U.S.A.). Wort was prepared using a single temperature infusion mash of 86% pale two row, 13.5% Caramel 10°L and 0.5% Caramel 120°L malt (Great Western, Vancouver, WA, U.S.A.) to a starting concentration of 10.7°P. Fermentation was carried out using Bridgeport Brewing company's house ale strain at 19.4–20 °C. Following fermentation, a kieselguhr filter was used to clarify the green beer and remove yeast. Post filtration, iso-humulones (IsoHop, John I Haas, Yakima, WA, U.S.A.) were added at concentration of 18 mg/L.

This resulted in ~46 hL of a 19.7 BU, 4.38% ABV "unhopped" base beer. Beer was carbonated and packaged into 60-L stainless kegs, shipped to Oregon State University, and held at 2 °C until dry-hopping.

Table 2. Summary of average mean scores for the sensory attributes resulting from descriptive analysis on the dry-hop blending treatments sorted by increasing overall hop aroma intensity.[a]

	Target Analytes	**β-myrcene**	**β-caryophyllene**	**α-humulene**	**β-farnesene**	**Terpinen-4-ol**	**α-terpineol**	**Linalool**	**Nerol**	**Geraniol**	**Geranial**	**Methyl geranate**	**Geraniol Acetate**	**Caryophyllene Oxide**	**Sum of Analytes Measured**
Hop Variety	Total Oil [b] (ml/100g)	Hop volatile analysis (mg/100g)[c, d]													
Cascade	1.0	209.7	98.7	262.1	100.2	n.d.	n.d.	3.7	1.0	4.5	0.4	9.6	26.6	9.5	726.0
Chinook	1.8	366.9	245.0	552.4	n.d.	n.d.	n.d.	6.1	3.6	65.5	1.1	30.8	0.5	3.5	1275.4
Centennial	1.9	665.2	182.4	347.2	n.d.	n.d.	n.d.	15.2	5.2	125.3	3.2	101.2	1.0	6.2	1452.1
Dry-hop treatment	Blend Code	Beer volatile analysis (µg/L)[e, f]													
16	0:0:0	0.9	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	1.3	n.d.	n.d.	2.2
11-rep 1	0:100:0	4.3	n.d.	0.1	0.3	4.2	12.0	43.2	3.6	44.8	1.6	4.3	1.0	3.1	122.5
13	0:50:50	54.8	n.d.	0.6	0.9	8.5	25.7	85.6	7.1	82.6	2.3	30.7	1.7	3.1	303.6
10	25:0:75	18.0	n.d.	0.4	0.5	3.9	15.7	40.2	3.5	36.6	1.0	19.0	1.8	1.3	141.9
1-rep 1	100:0:0	2.0	n.d.	n.d.	n.d.	3.3	8.4	23.9	1.8	18.6	0.5	n.d.	1.2	1.2	60.9
4	50:50:0	57.1	0.4	1.0	1.2	6.0	26.6	82.1	5.5	66.4	2.5	10.3	4.8	4.5	268.4
5	50:25:25	227.6	0.5	4.3	2.5	7.8	37.3	56.0	6.4	84.6	1.9	39.1	6.4	3.6	478.0
12	0:75:25	58.6	n.d.	0.8	0.6	7.5	24.8	82.6	6.1	78.3	2.3	28.4	2.2	3.9	296.1
7	25:75:0	33.5	0.1	0.6	0.6	5.7	23.4	66.9	4.0	67.4	2.0	11.3	3.1	4.8	223.4
3	75:0:25	57.2	0.4	0.8	0.7	7.0	32.3	82.9	5.0	56.4	1.7	25.9	5.8	2.4	278.5
2	75:25:0	36.6	0.3	0.8	0.9	5.2	27.0	54.7	3.1	45.8	1.0	6.0	4.3	2.9	188.6
8	25:50:25	35.4	n.d.	0.4	0.5	3.8	12.1	49.8	3.8	46.1	1.7	17.1	2.2	2.6	175.5
14	0:25:75	57.6	n.d.	0.4	1.0	11.7	43.8	132.7	11.2	127.6	2.4	48.4	2.2	3.5	442.5
15-rep 2	0:0:100	11.5	n.d.	0.4	0.7	5.4	17.9	86.8	8.5	117.2	1.8	40.5	1.3	2.0	294.0
6	50:0:50	290.2	1.0	5.1	1.7	14.1	44.6	139.4	11.3	144.0	2.9	74.3	8.0	3.0	739.6
9	25:25:50	371.5	2.7	8.0	2.4	16.3	41.1	157.8	12.5	181.4	6.0	88.1	6.4	4.6	898.8

[a] The treatment blending codes are represented as %Cascade:%Chinook:%Centennial.

[b] Measured using ASBC MOA Hops-13[16].

[c] Analyzed using under modified GC/MS conditions based on ASBC MOA Hops-17 [16]. Analytes are reported in mg/100 g hops.

[d] Average of 3 instrumental runs.

[e] Analyzed using under modified GC/MS conditions based on published methodology [16, 17]. Analytes are reported in µg/L and are blank corrected.

[f] Average of 2 instrumental runs.

n.d., not detected.

Dry-hopping protocol and hop preparation

The 19 treatments were prepared in a randomized order using a dry-hopping process established previously by Vollmer and Shellhammer.[10] In brief, 24 h prior to hop addition the "unhopped" beer was removed from the cooler at 4 °C and allowed to warm to approximately 15 °C. For each treatment, 40 L of warmed beer was transferred aseptically into two modified 60 L stainless steel beer kegs each with a 4″ stainless steel opening fitted with a standard Sankey D-system coupler and modified spear (Sabco, Toledo, OH, U.S.A.). To prepare the hop blends, the whole cone hops were ground into a hop grist, which was divided up by mass into two mesh bags (EcoBag, Ossining, NY, U.S.A.). These bags were stored inside high barrier pouches flushed with N_2 until the dry-hopping event. For each dry-hop treatment, the two kegs filled with 40 L beer were temporarily de-pressurized and opened under a stream of low pressure CO_2. Simultaneously, the high barrier pouch was opened and the mesh bag containing ground hop grist was added to the beer. After the addition, the headspace was flushed with CO_2 and purged.

After 24 h of dry-hopping the beer was filtered to stop the dry-hopping process. The average temperature of the dry-hopping events ranged from 13.3 to 15 °C (56–59 °F). Dry-hopping was stopped after 24 h because prior work by Wolfe et al.[11, 12] showed that the extraction of key hop volatiles occured within 24 h during dry-hopping. The two kegs were blended via a three-way fitting and filtered using a plate and frame filter containing impregnated cellulose pads (HS2000, Pall Corporation, Port Washington, NY, U.S.A.).[13] Dissolved oxygen (DO) was monitored during filtration using an Orbisphere 3100 Portable Oxygen Analyzer (Hach, Loveland, CO, U.S.A.).

Bright beer was not collected until the DO was below 110 μg/L After the DO was within specification, filtered beer was collected in a closed 1/6 bbl stainless steel keg with sufficient backpressure to reduce foaming. Between each filter run, filter pads were exchanged to prevent carry-over. Directly after filtration, the DO of the bright beer was measured and recorded. Filtered beer was stored at 2 °C and under CO_2 overpressure (76–83 kPa) until sensory evaluation. To minimize artifacts from packaging in glass bottles, such as DO pick up and potential aroma scalping via crown liner material,[14, 15] all beer for this experiment was kept in the 19.6 L (1/6 US bbl) kegs at ∼1 °C. To perform sensory and analytical analysis beer was served directly from these kegs using two 8-head draft systems (Micro Matic, Northridge, CA, U.S.A.).

Sensory: Discrimination testing of internal process replicates

Discrimination testing was performed on the internal process replicates for each of the 100% (single) cultivar treatments to examine dry-hopping process variation within treatments. The replicates were evaluated by a panel of 40 craft beer drinkers (23 males and 17 females, 21–66 years of age). Panelists were presented with four triangle tests, the first of which was a warm up. Within each triangle test, there were three samples; two of the samples were the same and one of the samples was different. Based only on the orthonasal aroma of the sample, the panelists were instructed to select the odd sample for each of the four triangle tests. For each of the three sets of duplicates, the design of the triangle test ensured an equal frequency of appearance of each duplicate as the “odd” sample. The serving order within each triangle test was also randomized. The dry-hopped beer was dispensed from the keg into a pitcher, which was used to pour ∼60 mL of beer into 300-mL sample glasses coded with a 3-digit random number. After the beer was

poured the glass was covered with a plastic lid and the beer was allowed to warm to room temperature before sensory analysis. Each station was used ~2 times over the course of 2 h.

Sensory: Descriptive analysis

Sixteen trained panelists were used to scale only the orthonasal aroma of the treatments and they were selected based on previous experience and relevance (12 males and 4 females; 21–66 years of age). Five training sessions were completed in advance of data collection. During these sessions panelists were trained using external reference samples and the actual experimental treatments to develop a relevant lexicon of sensory attributes and a scale that best explained the differences in the samples. Based on discussion from these training sessions and prior results,[13] the final ballot included the attributes: Overall Hop Aroma Intensity (OHAI), Citrus, Tropical/Catty, Tropical/Fruity, Pine/Resinous/Dank, and Herbal/Tea to be evaluated on a 0–15 point scale. During each session, the panelists had access to seven external reference samples, four of which were treatment beers (unhopped control, 100% Centennial, 100% Chinook and 100% Cascade) and three of which were commercial, hoppy beers (Sierra Nevada Pale Ale, 10 Barrel Joe IPA and Ballast Point Pineapple Sculpin). These five beers had sensory descriptors with intensity scores assigned by consensus during training, and their purpose was to serve as anchors for the 0–15 point intensity scale (Table 3).

Over the course of eight sessions, the 16 panelists evaluated all of the samples four times. The presentation order throughout the study was randomized and blocked by replication and panelist, and two sessions were needed per replication to evaluate all the samples (two sessions of eight samples). An efficient resolvable incomplete block design

was used to create a presentation order for the samples within each of the four replications (SAS, Cary, NC, U.S.A.). Panelists were given ~60 mL of dry-hopped beer in a 300-mL glass covered with a plastic lid. Beer was served from two eight-head draft systems (Micro Matic, Northridge, CA, U.S.A.) into pitchers at ~1 °C and at 83 kPa. Beer was poured into sample glasses ~1 h before the start of testing and allowed to warm to room temperature. Panelist responses were collected on Chromebook tablets using Qualtrics (Provo, UT, U.S.A.). For each session, Qualtrics was also used to randomly assign the serving order of samples for each panelist

Table 3. Reference standards with intensity scores used in descriptive analysis panels.

Attributes	**Unhopped Control**	**100% Chinook**	**100% Centennial**	**100% Cascade**	**Sierra Nevada Pale Ale**	**Ballast Point Pineapple Sculpin**	**10-Barrel Joe IPA**
OHAI[a]	0	6	9	8	7	10-11	14-15
Citrus	0	2	7	8	6	6	5-6
Herbal/Tea	0	3	4-5	6	5	2	1
Tropical/Catty	0	4-5	2-3	3	3	4	9-10
Tropical/ Fruity	0-1	2-3	5-6	3	4	7-8	4-5
Pine/ Resinous/ Dank	0	1	2	2	2	4	4

a. OHAI = Overall Hop Aroma Intensity

Volatile analysis reagents and standards

β-myrcene, linalool, geraniol, citral, methyl geranate, geranyl acetate, 4-octanol, terpinen-4-ol, α-terpineol, nerol, *β*-caryophyllene, *α*-humulene, and *β*-farnesene were obtained from Sigma-Aldrich (St. Louis, MO, U.S.A.). The 2-octanol was obtained from Alfa Asear (Haverhill, MA, U.S.A.). Hexanes were purchased from J.T. Baker (Center Valley, PA, U.S.A.) and were redistilled to remove impurities prior to analysis. Sodium chloride was purchased from EMD Millipore (Billerisa, MA, U.S.A.).

Hop volatile analysis

Hydrodistillation was performed to determine the total oil content of the homogenized hop grist using ASBC Hops-13.[16] Post-distillation, hop oil was collected in 2.5-mL amber vials with foil-lined closures. After filling with oil, the amber vials were flushed with N2. Hop oil was stored at −20 °C until compositional analysis.

Hop oil compositional analysis was performed using a HP 6890 gas chromatograph with an Agilent 5972a mass spectrometer (GC-MS) under modified conditions from ASBC Hops-17.[16] In brief, a 1% 2-octanol (8190 mg/L) solution was prepared in reagent grade hexane. Hop oils were diluted to 10% with the 1% 2-octanol/hexane solution in a crimped glass vials. A 1-µL aliquot 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 30 m × 250 µm × 0.25 µm Zebron ZB-1 MS (Phenomenex, Torrance, CA, U.S.A.) and ultra-pure helium was used as the carrier gas (a constant flow rate, 1.4 mL/min). The following temperature program was used: 50 °C hold for 1 min, 50–180 °C (2 °C/min) hold for 10 min, 180–200 °C (3 °C/min), and 250 °C hold for 5 min. The auxiliary line and mass spectrometer were operated at 280 and ∼180 °C, respectively. The mass spectrometer was operated using electron-impact mode at 70 eV and in full scan mode set up to detect ions with a mass-to-charge ratio (m/z) of 30–350. Four-point calibration curves (50, 100, 400, and 800 mg/L) were created for all target analytes. For high concentration target analytes (β-myrcene, α-humulene β-caryophyllene, β-farnesene) three additional calibration points were added (1000, 5000, and 9000 mg/L). Target analytes were quantified using the following ions for each analyte: m/z 41 (geranial), m/z 45 (2-octanol), m/z 59 (α-

terpineol), m/z 69 (β-farnesene, geraniol, nerol, methyl geranate, and geranyl acetate), m/z 71 (terpinen-4-ol and linalool), and m/z 93 (β-Myrcene, β-caryophyllene, and α-humulene). The target analyte concentrations in hop oil were then standardized on a per-mass basis using the total oil content determined during hydrodistillation.

Beer volatile analysis

Headspace-Solid Phase Micro Extraction (HS-SPME) was performed on the dry-hop treatments using a 1 cm 24-gauge divinylbenzene/carboxen/polydimethylsiloxane (DVB/CAR/PDMS) Stableflex fiber with 30/50 µm coating thickness (Supelco, Bellefonte, PA, U.S.A.).[16, 17] An 8 mL sample of each was placed into a 20-mL screw top amber vial with 3 g sodium chloride. The compound 4-octanol (911 µg/L) was used as an internal standard and added to each vial. A MultiPurpose auto sampler (MPS2; Gerstel, Mülheim, Germany) was used for pre-incubation, stirring, extraction, and injection. Samples were preincubated for 15 min at 30 °C and adsorbed by piercing the vial septa and exposing the fiber to the headspace for 45 min with agitation. After adsorption, the fiber was desorbed into the GC sample inlet (splitless mode, 250 °C) for 10 min. The analytical column was a 30 m × 250 µm × 0.25 µm Zebron ZB-1 MS (Phenomenex, Torrance, CA, U.S.A.) and ultrapure helium was used as the carrier gas (at constant pressure, 73 kPa). The following temperature program was used: 50 °C hold for 1 min, 50–250 °C (5 °C/min) hold for 11 min and 250 °C hold for 5 min. The auxiliary line and mass spectrometer were operated at 280 and 180 °C respectively. The mass spectrometer was operated using electron-impact mode at 70 eV and in full scan mode set up to detect ions with a mass-to-charge ratio (m/z) of 30–350. Three point calibration curves (40,100, and 200 µg/L) were created for all target analytes. Calibration curves

were made in a model beer solution (5% v/v ethanol) and were prepared using the methodology previously described. Target analytes were quantified using the following ions for each analyte: m/z 55 (4-octanol), m/z 59 (α-terpineol), m/z 69 (β-farnesene, geraniol, nerol, methyl geranate, geranial, and geranyl acetate), m/z 71 (terpinen-4-ol and linalool), and m/z 93 (β-myrcene, β-caryophyllene, and α-humulene).

Statistical analysis

The Z tests on proportions were used to evaluate the sensory discrimination tests on the internal process replicates. Two-way analysis of variance with a mixed model (including the factors panelist, sample, and replication as well as corresponding two-way interactions), multiple comparison analysis (Fisher's LSD), hierarchical cluster analysis, principle component analysis (PCA), and graphical constructions were carried out using XLstat 2017 (Addinsoft, New York, NY, U.S.A.). These tests and graphs were used to gauge the panel and panelist effectiveness in generating descriptive data, evaluate the significant differences in aroma quality and intensity among the dry-hopping treatments, and assess the associations between the chemical and sensory data collected. When performing statistical analysis on the data generated from the beer volatile analysis, all nondetected values were treated as zero values.

Results and discussion

Discrimination testing: Evaluating internal process replicates

Discrimination testing on the internal process replicates for each of 100% cultivar treatments yielded no significant differences (Table 4). This indicated that any process variation during dry-hopping had a negligible impact on the dry-hop aroma within the same treatment and therefore any differences observed among the treatments were not

due to processing variation. For descriptive analysis testing, only one of the replicates for each of the 100% cultivar treatments was evaluated and it was randomly selected.

Table 4. Triangle test results of dry-hopping process replicates.[a]

Triangle Tests	number of correct responses	Z-value	p-value
100% Centennial (15-rep 1 vs 15-rep 2)	13	-0.28	0.39
100% Cascade (1-rep 1 vs 1-rep 2)	18	1.40	0.08
100% Chinook (11-rep 1 vs 11-rep2)	16	0.73	0.23

[a] The panel was comprised of 40 hoppy beer consumers; 17 females and 23 males with ages 21–66.

Descriptive analysis: panelist/panel evaluation

Each panelist was evaluated on their performance to discriminate differences among the treatments on at least one of the sensory attributes, their ability to replicate among all sessions, and their lack of interactions. Any panelists that failed these three criteria were removed from further analyses. Seven of the 16 original panelists were removed from the data set. The resultant data set included 36 observations per attribute, per sample.

Two-way ANOVA with a mixed model was performed on the attributes using the remaining 9 panelists (Table 5). Significant ($P < 0.05$) sample effects were observed across the attributes. Significant ($P < 0.05$) panelist effects were observed for all of the attributes. Panelist effects are expected in descriptive analysis because panelists tend to express their perceptions on the same sample using different parts of the scale. Overall it is the relative differences in their ratings, and not the absolute value of their ratings, that is important.[18]

Table 5. Mixed model analysis of variance of the sensory attributes

			OHAI		Citrus		Tropical/ Catty		Tropical/ Fruity		Pine/Resinous/ Dank		Herbal/ Tea	
Source	**Type**	**DF**	**F**	**P-value[a]**	**F**	**P-value[a]**	**F**	**P-value[a]**	**F**	**P-value[a]**	**F**	**P-value[a]**	**F**	**P-value[a]**
Sample	Fixed	15	13.7	**< 0.0001**	10.4	**< 0.0001**	3.7	**< 0.0001**	6.1	**< 0.0001**	3.4	**< 0.0001**	4.0	**< 0.0001**
Panelist	Random	8	7.3	**< 0.0001**	17.4	**< 0.0001**	12.2	**< 0.0001**	6.5	**< 0.0001**	26.2	**< 0.0001**	7.4	**< 0.0001**
Rep	Fixed	3	1.6	0.218	0.5	0.697	1.7	0.194	0.8	0.513	0.2	0.872	0.3	0.797
Sample*Panelist	Random	120	1.4	**0.014**	1.2	0.083	1.2	0.107	1.3	**0.041**	1.2	0.134	1.3	**0.031**
Sample*Rep	Fixed	45	1.2	0.208	1.0	0.419	0.9	0.638	1.0	0.529	0.9	0.595	0.8	0.811
Panelist*Rep	Random	24	1.3	0.143	1.4	0.116	0.8	0.759	0.8	0.707	1.5	0.077	2.2	**0.001**
Error		360												

[a] Values in bold indicate p-value <0.05. DF, degrees of freedom.

Significant panelist × sample effects were observed for OHAI, Herbal/Tea and Tropical/Fruity. Significant panelist × sample effects are common in sensory analysis and indicate that there were slight differences in the way the panelists scaled these attributes.[18] With the exception of a significant ($P < 0.05$) panelist × replication effect observed for Herbal/Tea, no significant effects of replication or interactions between panelist and replication or between sample and replication were observed. This indicates the panelists could effectively replicate their attribute scaling for the samples across the four replications and that the ratings provided for the attributes for a given panelist did not depend on replication.

The mean values for the sensory attributes and results of the Fisher's LSD tests on these attributes were summarized and sorted by OHAI (Table 6). Fisher's LSD tests were chosen as the mean comparisons technique instead of a more conservative method, such as Tukey's HSD tests, to highlight the potential differences that exist between the dry-hop aroma profiles of the treatments. The greatest amount of variation was found in the OHAI and Citrus attributes and the least in the Tropical/Fruit and Herbal/Tea attributes. For all of the attributes, the "unhopped" blank was not grouped with any of the dry-hop blending treatments. Interestingly, the panelists perceived the 50:0:50 and 25:25:50 blends of Cascade, Chinook, and Centennial to be the most intense in OHAI and Citrus. Similarly, Takoi et al.[3] observed that the coexistence monoterpene alcohols (linalool and geraniol) that can occur when dry-hopping with blends of hops in comparison with single-hopped control beers increased average Citrus scores and created drastically different flavor profiles. The coexistence of polyfunctional thiols and monoterpene alcohols has also been shown to increase the aroma perception of blending treatments.[3]

Therefore, it is possible that these interactions were responsible for the increased aroma perception of the blended dry-hop treatments.

Table 6. Summary of mean scores for the sensory attributes of the dry-hop blending treatments sorted by increasing overall hop aroma intensity (OHAI).[a]

Dry-hop Treatment	Blend Code	OHAI	Citrus	Herbal/Tea	Tropical /Catty	Tropical /Fruity	Pine/Resinous/ Dank
16	0:0:0	2.6 [f]	1.2 [f]	1.3 [c]	1.4 [c]	1.2 [e]	0.8 [d]
11-rep 1	0:100:0	6.4 [e]	3.9 [e]	2.8 [b]	3.5 [ab]	3.3 [d]	2.2 [abc]
13	0:50:50	6.6 [de]	4.2 [de]	2.9 [ab]	3.7 [ab]	3.5 [cd]	2.2 [abc]
10	25:0:75	6.7 [de]	4.1 [e]	2.9 [b]	3.7 [ab]	3.4 [cd]	2.0 [abc]
1-rep 1	100:0:0	6.8 [cde]	4.6 [abcde]	3.4 [ab]	3.1 [ab]	3.8 [bcd]	1.9 [bc]
4	50:50:0	6.9 [cde]	4.2 [de]	3.3 [ab]	3.6 [ab]	3.3 [d]	1.9 [bc]
5	50:25:25	7.0 [bcde]	4.7 [abcde]	3.3 [ab]	3.1 [b]	4.2 [abc]	2.1 [abc]
12	0:75:25	7.0 [bcde]	4.4 [cde]	3.1 [ab]	4.1 [a]	3.2 [d]	2.4 [ab]
7	25:75:0	7.1 [bcde]	4.6 [abcde]	2.8 [b]	3.8 [ab]	4.4 [ab]	2.2 [abc]
3	75:0:25	7.3 [bcd]	4.9 [abcd]	3.4 [ab]	3.8 [ab]	4.3 [abc]	2.5 [ab]
2	75:25:0	7.4 [abcd]	5.1 [abc]	3.2 [ab]	3.8 [ab]	3.8 [bcd]	1.8 [c]
8	25:50:25	7.4 [abcd]	5.0 [abcd]	3.3 [ab]	3.2 [b]	4.2 [abc]	2.1 [abc]
14	0:25:75	7.6 [abc]	4.6 [bcde]	3.2 [ab]	4.0 [ab]	4.5 [ab]	2.2 [abc]
15-rep 2	0:0:100	7.6 [abc]	5.3 [ab]	3.2 [ab]	3.5 [ab]	4.9 [a]	2.1 [abc]
6	50:0:50	7.8 [ab]	5.3 [ab]	3.5 [a]	4.1 [a]	4.5 [ab]	2.5 [a]
9	25:25:50	8.1 [a]	5.4 [a]	3.5 [ab]	4.3 [a]	4.5 [ab]	2.5 [ab]

[a] The treatment blending codes are represented as %Cascade:%Chinook:%Centennial.
[b] Letters in brackets indicate statistically significant groupings within each descriptor (Fisher's LSD tests P value <0.05).

Multivariate analysis of sensory data

Hierarchical cluster analysis and PCA have been shown to be successful data dimension reduction techniques involving the sensory and chemical analyses of beer[19, 20] and other carbonated beverages.[21, 22] Three clusters were formed when performing agglomerative hierarchical clustering using the Euclidean distance for the dissimilarly scale and Ward's method as the agglomeration method (Figure 1). The 100% Cascade, Chinook, and Centennial dry-hopping treatments were sorted into three different clusters. This suggests that dry-hopping with each of these cultivars individually leads to beers with different hop aroma intensities and qualities. This observation is emphasized if the Ward clusters are overlaid onto a ternary plot (Figure 2). Previous work has shown that

each of these cultivars has distinct character impact compounds that define the dry-hop aroma in beer for these cultivars.[7] However, it was also observed that in combination, blends of these three hops may lead to dry-hop aroma profiles that are similar in quality and intensity to the 100% Cascade, Chinook, and Centennial dry-hopping treatments. In general, the cluster in blue was defined by the 100% Chinook treatment, which could also be built from blends of Centennial and Chinook. The cluster in green was defined by the 100% Cascade treatment, along with of blends of Cascade, Centennial, and Chinook. The cluster in red was defined by the 100% Centennial treatment and included blends made with Cascade and Centennial.

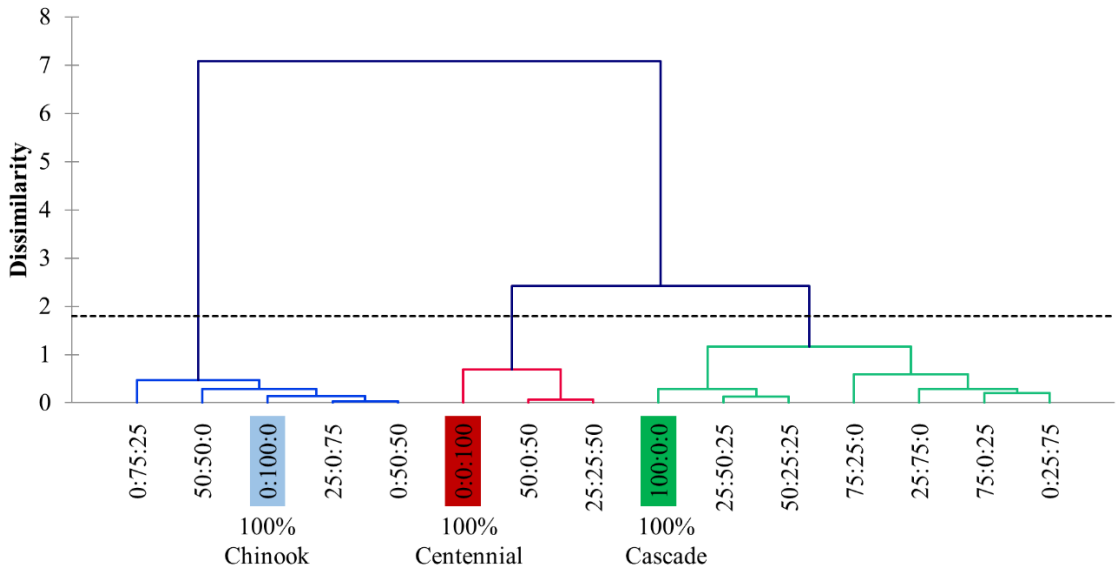

Figure 1. Agglomerative hierarchical clustering based on the sensory attributes using the Euclidean distance for the dissimilarly scale and Ward's method for agglomeration. The dotted line represents the automatic truncation option based on entropy. The treatment blending codes are represented as %Cascade; %Chinook; %Centennial.

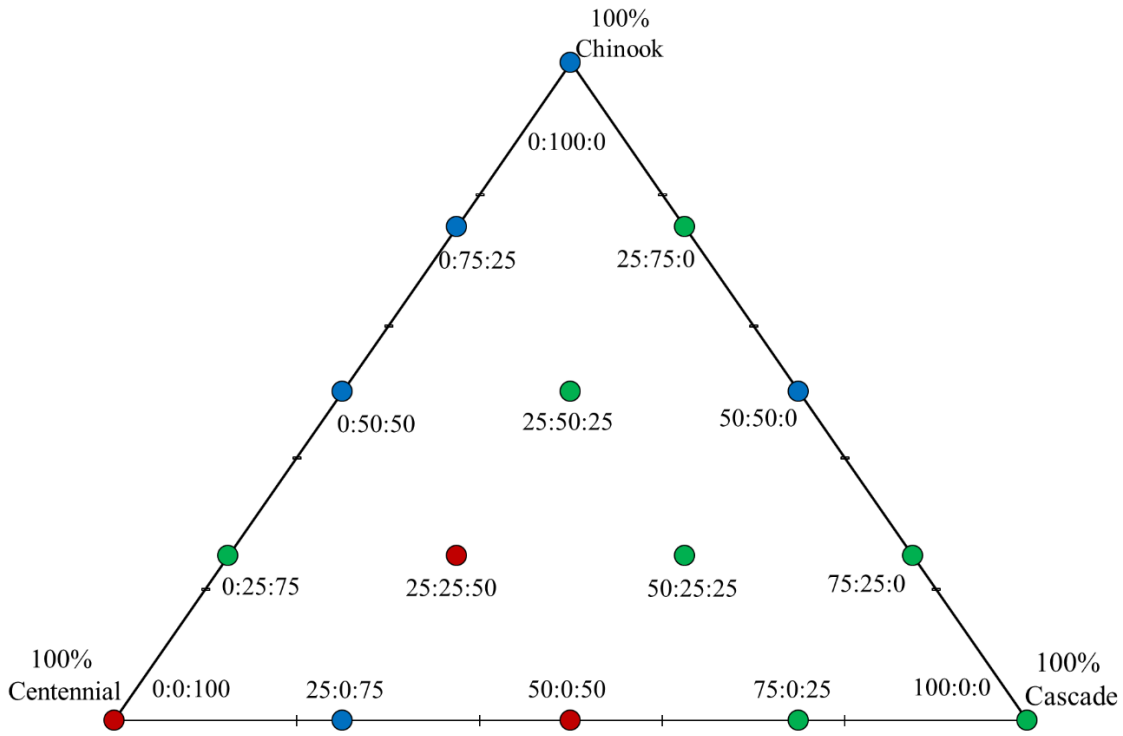

Figure 2. Ternary plot colored by the agglomerative hierarchical Ward clusters (red, blue and green circles). The treatment blending codes are represented as %Cascade; %Chinook; %Centennial.

PCA was performed on the covariance (n-1) matrix of the mean sensory scores for the dry-hop treatments and the resulting biplots were colored based on the agglomerative hierarchical Ward clusters (Figure 3). Overall, the first three principal components explained 95% of the variation within the data set, with PC1 accounting for ~72% and described variation in OHAI and Citrus qualities and to a lesser degree Tropical/Fruity aroma. PC2 accounted for ~16% and described variation in Tropical/Catty, and PC3 accounted for ~7% and described variation in Herbal/Tea. Each of the of the three Ward clusters highlights the aroma profiles observed for the single-cultivar dry-hopping treatments and the corresponding blending treatments that produce similar dry-hop aroma profiles.

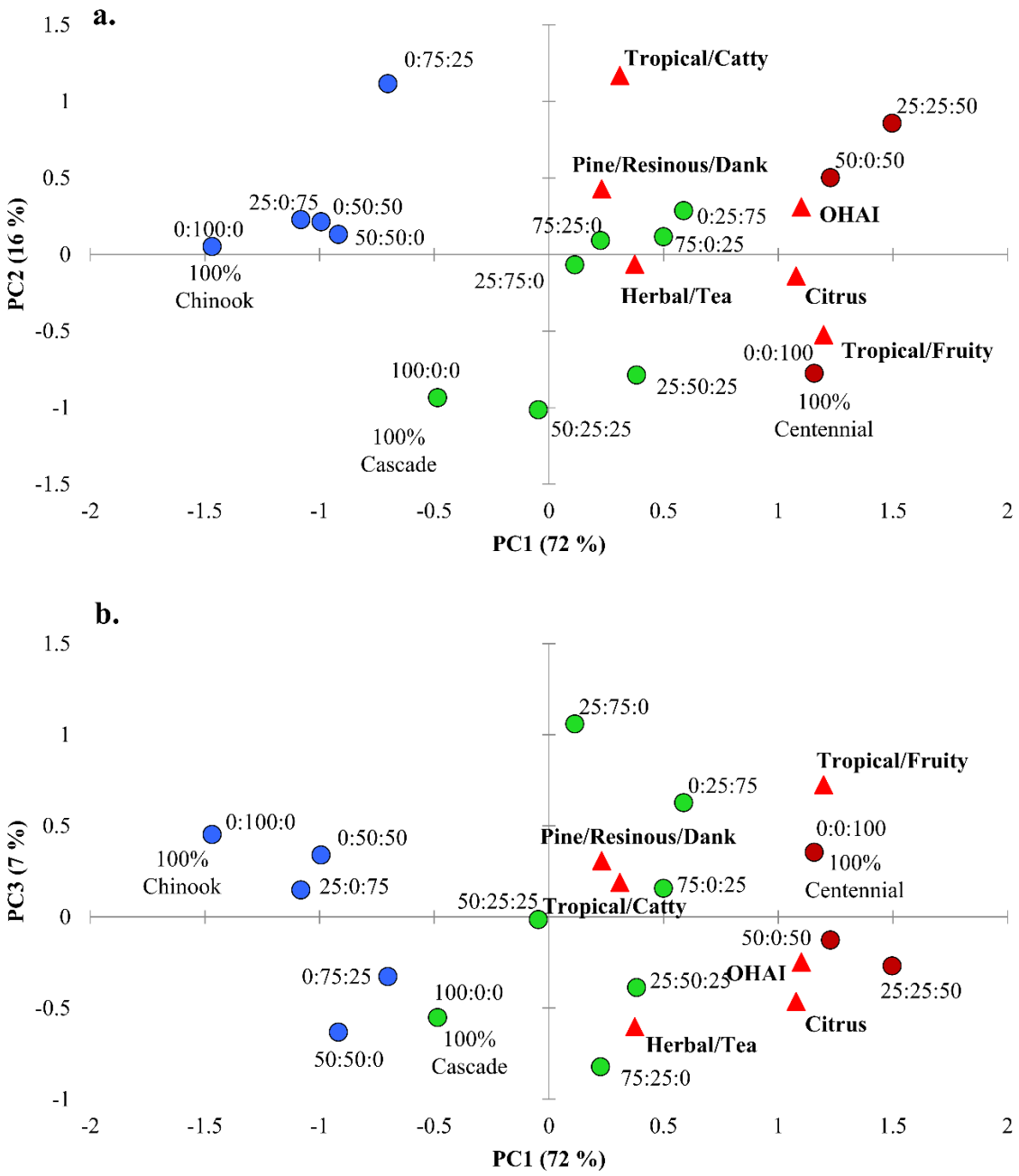

Figure 3. Principle Component Analysis biplots of the mean scores of the sensory attributes (red triangles) among the (16) dry-hop blending treatments colored by the agglomerative hierarchical Ward clusters (red, blue and green circles). (a) biplot of PC1 & PC2 explaining 88% of the variation in the data (b) biplot of PC1 & PC3 displaying an additional 7% of the variation in the data set. The treatment blending codes are represented as %Cascade; %Chinook; %Centennial.

The cluster in blue, which included the 100% Chinook treatment, was perceived to be the lowest in overall hop aroma intensity but was highlighted by the Tropical/Catty and Pine/Resinous/Dank attributes. Modest and negative Pearson correlation coefficients were observed between % Chinook and the sensory attributes Citrus ($r = -0.51$, $p = 0.53$), Herbal/Tea ($r = -0.57$, $p = 0.26$) and Tropical/Fruity ($r = -0.47$, $p = 0.76$). This indicates that as the percentage of Chinook increased, the perceived value of these attributes decreased. The cluster in green, which included the 100% Cascade treatment, was perceived to be between Chinook and Centennial in terms of overall hop aroma intensity and was primarily defined by the Herbal/Tea and Citrus attributes. As the % Cascade increased, the perceived Herbal/Tea attribute increased significantly ($r = 0.51$, $P = 0.53$). The cluster in red, which included the 100% Centennial treatment, was perceived to be the highest in overall hop aroma intensity and was primarily defined by the Tropical/Fruity and Citrus attributes. As the % Centennial increased, the perceived Tropical/Fruity attribute increased ($r = 0.46$, $P = 0.087$).

Using binary and tertiary blends of the three hops increased the hop aroma intensity above that from a single-hop treatment and this effect was observable in all three treatment clusters in the PCA biplot (Figure 3a). Notice, within each cluster, the single-hop treatment sits in the lower left-hand region and as other hops are blended in, the data cluster moves upward and toward the right, which indicates greater aroma intensity. This was the case even in the most intense variety in the study, Centennial (0:0:100), where blends with Cascade (50:0:50) or Cascade and Chinook (25:25:50) produced more intensely hoppy beers. This indicates that using blends of hops during

dry-hopping might increase the aroma potential of hops as compared to using single varieties and could be a way to reduce hopping rates while maintaining high aroma intensities.

Chemical analysis of select hop volatiles in hops with gas chromatography/mass spectrometry (GC/MS) and dry-hop treatments with solid-phase microextraction- gas chromatography/mass spectrometry (SPME-GC/MS)

The measured volatile components in the hops used for dry-hopping and the impact the different blending treatments had on selected hop volatiles in beer were examined (Table 2). Hop volatile concentrations and the perceived overall hop aroma intensity were significantly positively correlated ($r = 0.533–0.744$, P values = < 0.001–0.41) for all of the hop volatiles with the exception of caryophyllene oxide. Surprisingly, there appeared to be a synergistic effect of dry-hopping with blends of hops on the concentration of hop volatiles, which was highlighted when comparing the concentrations of the blends to the single cultivar treatments. It is unclear what caused this effect. There was no yeast present in the dry-hopping treatments; therefore, it is not attributable to yeast biotransformation. However, this does not rule out the possibility of a hop-derived enzyme catalyzed biotransformation.

Significant positive Pearson correlation coefficients were observed between % Centennial and linalool ($r = 0.464$, $P = 0.082$), geraniol ($r = 0.550$, $P = 0.034$), methyl geranate ($r = 0.558$, $P = 0.021$), and nerol ($r = 0.615$, $P = 0.015$), indicating that as the percentage of Centennial increased in the dry-hopping treatments, the concentrations of these hop volatiles increased. Previous work has identified linalool, geraniol, and methyl geranate as character impact compounds for describing Centennial dry-hop beer.[7]

While none of the hop volatiles were individually correlated to the % Cascade, a significant positive correlation was observed between the ratio of linalool/geraniol and % Cascade, suggesting that the amount of Cascade in the dry-hop blends had an impact on this ratio indicating the importance of these analytes for Cascade dry-hop aroma in beer. These hop volatiles were also identified as character impact compounds for Cascade dry-hop beer[7] and highlighted as key drivers of Cascade aroma in numerous other studies[23–26] further highlighting the importance of these analytes in describing Cascade hop aroma and flavor in beer.

Caryophyllene oxide was significantly positively correlated with % Chinook ($r = 0.557$, $P = 0.024$). While the concentration of caryophyllene oxide was the lowest in Chinook, the concentration of β-caryophyllene was the highest of the three hop varieties. Although dissolved oxygen was monitored during processing and was relatively low (<110 μg/L post filtration), it is speculated that β-caryophyllene could potentially oxidize to caryophyllene oxide during dry-hopping and thus might be a marker for oxidation during dry-hopping with Chinook.

A limitation of the analytical analysis in this study is that polyfunctional thiols were not measured or considered. There are a number of studies[3, 27–30] that have emphasized the importance of these analytes in describing hop aroma in beer. In future studies, these analytes should be considered to have a possible impact on dry-hop flavor, especially when dry-hopping with blends of hops.

Conclusions

This study demonstrated that it is possible to achieve similar aroma profiles when dry-hopping beer with varying blends of Cascade, Chinook, and Centennial hops and that

some of these blends may achieve an aroma profile similar to a single variety. Using blends of hops during dry-hopping has obvious benefits and promotes both the increase in perceived aroma intensity and quality as well as the increase in hop volatile extraction in dry-hopped beer. By utilizing a blending approach for dry-hopping, the brewer is able to make substitutions when faced with shortages due to cost and/or quality. While only hop aroma was evaluated in this study, dry-hopping can also impact bitterness. Therefore, the humulone content and age of the hops that will be blended for dry-hopping should be considered. These factors have a direct effect on the humulinone concentration of the hops and will subsequently modify the bitterness profile of beer.[31–33]

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Disclosure statement

No potential conflict of interest was reported by the authors.

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Chapter 7. General conclusions, industrial implications, and future directions

It is apparent that there are many challenges in trying to define analytical markers of dry-hop quality for aroma hops. Hop aroma is complex and should always be considered in relationship to the method(s) used to add hops throughout the brewing process. The static dry-hopping method[137] used in this dissertation was not meant to define all dry-hopping situations but instead provided a reproducible way to examine different dry-hopping treatments. Combining this production technique with reliable sensory and chemical approaches allowed for a novel in-depth evaluation of the underlying chemistry impacting dry-hop aroma.

It is clear from the results from Publication A that simply increasing static dry-hopping rates does not lead to a linear increase in hoppy aroma. Rather, at very high levels one observes a point of diminishing returns where large increases in hopping rates results in modest or negligible increases in hoppy aroma. Furthermore, the aromatic quality of hopping at very high rates is different than at lower rates, with higher rates leading to more herbal and less citrus qualities for Cascade hops. Future investigations should probe other dry-hopping techniques that promote specific, desired aroma profiles and extraction efficiencies of prominent hop volatiles, for instance gentle agitation or multiple dry-hopping events (i.e. double or triple dry-hopping). Based on the low concentrations of humulones, monoterpenes, and sesquiterpenes extracted into beer during dry-hopping, recently supported by Hauser et al.[138], there is potentially brewing value left in this spent material. Future work should investigate the potential of reusing this material to create a more sustainable process.

The results from Publication D indicate that blends of hops can create consistent dry-hop aroma on a lot-to-lot basis. Interestingly, the dry-hop treatments made with blends of hops were able to achieve similar aroma qualities and intensities to treatments made with single varieties. Therefore, by utilizing a blending approach for dry-hopping, the brewer is able to make substitutions when faced with shortages due to cost and/or quality. The blended treatments also were perceived higher in aroma intensity and quality, in addition to having greater concentrations of hop volatiles. Further work should explore the synergy between different blends of hop varieties during dry-hopping that promote aroma extraction and increase dry-hop aroma in beer. The interaction between malt type, yeast type, and hop type should also be investigated throughout the shelf-life of dry-hopped beer to develop tactics to improve the flavor stability of this style.

A major question that the brewing industry should address is how to evaluate hop aroma in a more objective manner to provide concrete data for decisions regarding the growing, selling, and purchasing of aroma hops. The current use of organoleptic evaluations (i.e. smell, appearance/color, …etc.) of hops to make purchasing decisions between different aroma hop lots is inefficient for trying to define quantifiable targets that a grower can use to guide changes in processing or growing conditions to improve hop quality.

While the rub-and-sniff technique for evaluating hops has been shown to be sufficient for identifying hops with severe defects,[126] there is little evidence that brewers can use this approach to identify hops that lead to superior aroma performance in beer. This is because a majority of the aroma of hops (50-80%) is made up of monoterpenes and sesquiterpenes, which have been shown to have a minimal impact on the aroma of

dry-hopped beer. From the standpoint of quality assurance, organoleptic evaluations place a lot of faith on the “subjective” opinions of individuals assessing raw material quality on a year-to-year basis. Companies responsible for evaluating aroma hop quality should establish strategies such that multiple individuals understand the targets of aroma hop quality for that company. This ensures that understanding on the targets of hop aroma quality do not necessarily lay in the hands of a single individual for that company.

However, for organoleptic evaluations of hops to be relevant for beer, individuals must evaluate hops in a meaningful way and must be trained to evaluate the volatiles that actually influence beer aroma based on how the brewer intends to use the hop throughout the brewing process. While not the primary focus of this dissertation, the technique employed to create and evaluate the different dry-hopping treatments in unhopped beer allowed for the unbiased comparison of the factors that influenced dry-hop aroma between the treatments evaluated. Further work should be carried out to evaluate the effectiveness of ASBC MOA Sensory Analysis – 15 (Hop Tea Sensory Method) as a way to evaluate and compare the dry-hop aroma quality of multiple lots of aroma hops. Using this approach may be a more beneficial way to evaluate the differences in extractable water-soluble volatiles between lots, which are particularly important for dry-hop aroma.

The quality targets defined Publication B (geraniol for Cascade and β-pinene for Centennial) that predicted the aroma potential for these varieties during dry-hopping can hypothetically be used to categorize these varieties into low and high aroma potential groupings much better than using total oil content. This type of strategy could help guide organoleptic evaluations on a year-to-year basis to yield more consistent dry-hop aroma and beer quality. Having targets that are predictive of dry-hop aroma potential may allow

brewers to tune dry-hopping recipes based on these volatiles to better control the lot-to-lot variation on hop aroma and/or provide a tool that processors can use to help decide which lots should be blended during pelletizing or post-harvest processing. These hop volatiles are also measurable targets of hop quality that breeders or growers can use to produce higher quality aroma hops.

As discussed in Publication C, harvest timing has a direct influence on the dry-hop aroma intensity, quality, and geraniol concentrations in Cascade. Therefore, geraniol and β-pinene concentrations might serve as maturity indicators of dry-hop quality during ripening. Future work should validate whether these volatiles can be used to forecast dry-hop aroma quality by evaluating lots both high and low in the concentrations of these volatiles. The volatiles that predicted the aroma of Cascade and Centennial were different, suggesting that the dry-hop aroma potential of different varieties is not explained by the same volatile(s). The efficiency of other methods such as non-targeted metabolomics should be investigated as a way to identify the drivers of aroma quality in other commercially important hop varieties.

As the drivers of dry-hop aroma are better understood, a serious re-investigation of the impact of post-harvest processing (particularly kilning) on aroma hop quality is needed. Most of the research investigating the impact of kilning parameters on hop quality was performed between the 1950s and 1970s. Based on this work, recommendations for optimal kiln temperatures (130°F-150°F) and air speeds (0.5 ft/s – 1 ft/s) were established for the large, single tier deep bed kilns typically used in the U.S.[116, 118, 139-141] However given that lager beer was the main beer style being produced at the time, the principal hop quality parameter during this time was preserving the

concentrations of humulones with little attention paid to aromatic quality. In these studies, brewing trials were also rarely performed alongside the chemical evaluation of hops. Therefore, it is hard to assess the direct impact these different kilning parameters have on hop aroma quality in beer.

More recently, Nielsen et al. [119] evaluated the effects of air temperature and flow rate in two aroma hop varietals (Cascade and Citra) using two hop kilns located in the Yakima Valley operating at 130°F and 150°F. They found that hops dried at lower temperatures with higher flow rates produced hops that were more preferred by brewers via blind rub-and-sniff evaluations. However, the brewing performance of these treatments was not evaluated, making it hard to gauge the influences of these factors directly on dry-hop aroma.

Recently, the enzymatic dextrin reducing power of hops has also been identified as a potential safety concern in dry-hopped bottle conditioned beers. During dry-hopping, hop enzymes are also extracted into beer and have the ability to breakdown unfermentable beer dextrins into fermentable mono- and di- saccharides (glucose and maltose). In bottle conditioned beers these fermentable sugars can lead to a refermentation resulting in significant package overpressurizations.[29] Lafontaine et al.[135] performed pilot scale kilning trails on Amarillo hops and found that hops kilned at 116°F and 170°F attributed similar aroma intensities to beer during dry-hopping but the hops kilned at the higher temperature dried to 8% moisture 5x faster and lead to 2x less maltose production. This indicates that higher temperature kilning might be used to speed up processing and reduce this safety concern without sacrificing aroma hop quality. This would allow more hops to be picked closer to their optimal maturity windows. While

promising, this study was only performed on the pilot scale and much larger replicated study is currently underway to further evaluate the impact of kilning on aroma hop quality.

Ultimately beer is not a static system. Molecular constituents in beer are subject to various reactions (oxidation, biotransformation, etc.) that impact their concentrations. This makes it challenging to track and define specific constituents that are directly responsible for impacting beer aroma. It is critical for studies investigating hop aroma to be performed with reproducible brewing practices and with an understanding of the commercially relevant intricacies that impact beer aroma and stability, for instance the impact of hop addition timing, hop variety, presence and type of yeast strain, concentration of dissolved oxygen, etc. Historical research on hop aroma must also be viewed through this lens so that the findings of these studies can be contextualized in a meaningful way as to not overstate their meaning. It is up to the grower and brewer to establish a great working relationships to define achievable aroma hop quality targets that results in consistently exceptional hoppy beer. However, it is most important to consider the relevancy of all of these findings on the consumer preference of the final product. At the end of the day the factors which influence the purchasing preference of the consumer are what really matter.

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APPENDIX 1– ACADEMIC ACHIEVEMENTS

List of publications

Publications in international peer-reviewed journals related to this Dissertation

Publication A

Lafontaine, S. R.; Shellhammer, T. H.* Impact of static dry-hopping rate on the sensory and analytical profiles of beer. *Journal of the Institute of Brewing*. **2018,** *124* (4), 434-442. DOI: https://doi.org/10.1002/jib.517

Publication B

Lafontaine, S. R.*, Pereira, C.B., Vollmer, D.M. and Shellhammer, T.H. The effectiveness of hop volatile markers for forecasting dry-hop aroma intensity and quality of Cascade and Centennial hops. *BrewingScience.* **2018**, *71* (Nov/Dec). DOI: https://doi.org/10.23763/BRSC18-19LAFONTAINE

Publication C

Lafontaine, S*; Varnum, S., Roland, A., Delpech, S., Dagan, L., Vollmer, D., Kishimoto, T., and Shellhammer, T. Impact of harvest maturity on the aroma characteristics and chemistry of Cascade hops used for dry-hopping. *Food Chemistry*, **2018**, *278*, 228-239. DOI: https://doi.org/10.1016/j.foodchem.2018.10.148.

Publication D

Lafontaine, S. R.; Shellhammer, T. H.* 2018: Sensory Directed Mixture Study of Beers Dry-Hopped with Cascade, Centennial, and Chinook, *Journal of the American Society of Brewing Chemists*, **2018**, *76* (3), 199-208. DOI: 10.1080/03610470.2018.1487747

Further publications in peer-reviewed journals

Hauser, D. G., **Lafontaine, S. R**., Shellhammer, T. H. (In Review). The extraction efficiency of hop volatiles and non-volatiles during dry-hopping. *Journal of the American Society of Brewing Chemists*

Lafontaine, S. R., Vollmer, D.M. and Shellhammer, T.H.* (2018): Aroma Extract Dilution Analysis of Beers Dry-Hopped with Cascade, Chinook, and Centennial, *Journal of the American Society of Brewing Chemists*, **2018**, *76* (3), 190-198. DOI: 10.1080/03610470.2018.1487746

Hahn, C., **Lafontaine, S. R.**, Pereira, C.B., and Shellhammer, T.H.* 2018: Evaluation of the nonvolatile chemistry affecting the sensory bitterness intensity of highly hopped beers. *Journal of Agricultural and Food Chemistry* **2018** *66* (13), 3505-3513 DOI**:** 10.1021/acs.jafc.7b05784

Contributions to other non-peer reviewed technical reports

Bulletins and technical reports

Shellhammer, T.H., **Lafontaine, S.R**., and Pereira, C. 2017. Identifying aroma hop quality factors that predict hops aroma/ quality in dry-hopped beers. Technical report to the Hop Research Council. 31 pages.

Shellhammer, T.H., **Lafontaine, S.R.**, Vollmer, D., and Pereira, C. 2016. Identifying brewing qualities which aid hop breeding: Examining hop oil content and its influence on dry-hop aroma in beer. Technical report to the Hop Research Council. 28 pages.

Conference contributions at professional meetings, symposia, and conferences

1. **Lafontaine, S.**, Roland, A., Delpech, S., Dagan, L., T., and Shellhammer, T. Impact of harvest maturity on the aroma characteristics and chemistry of Cascade hops during dry-hopping. 6th International Young Scientist Symposium on Malting, Brewing and Distilling. Bitburg, Germany. September 12-14, 2018. *Poster Presentation.* ***(recognized as best poster)***

2. **Lafontaine S.**, Hauser, D., Foster, R., Donaldson, J., Gamache, D., Shellhammer, T. Impact of kiln temperatures on the aroma and enzymatic potential of hops during dry-hopping. EBC Symposium "Recent Advances in Hop Science." Nuremberg, Germany. September 10th, 2018. *Oral Presentation*.

3. **Lafontaine, S. R**.; Shellhammer, T. H. Examination of factors that predict the dry-hop aroma performance of hops in beer. Brewing Summit. San Diego, CA. August 14th, 2018. *Oral Presentation.*

4. **Lafontaine, S. R**., Sharp, D., Shellhammer, T. H. Dry-Hopping Beer to Achieve Consistent Flavor. Oral Presentation. Craft Brewers Conference. Nashville, TN. May 2nd, 2018. *Oral Presentation.*

5. **Lafontaine, S. R**., Vollmer, D.M. and Shellhammer, T.H**.** 2018. Understanding the dry hop aroma of American hop varieties. 2018 American Hop Convention/Hop Research Council Winter Meeting, Palm Desert, CA. January 23 – 26, 2018. *Poster presentation.*

6. **Lafontaine, S. R.**; Shellhammer, T. "Examination of factors that predict the dry-hop aroma performance of hops in beer" Winter Hop Research Council Meeting, Palm Desert, CA. Jan 24, 2018. *Oral Presentation.*

7. **Lafontaine, S. R.**; Shellhammer, T. "Examination of factors that predict the dry-hop aroma performance of hops in beer" Summer Hop Research Council Meeting, Corvallis, OR. July 28, 2017. *Oral Presentation.*

8. Iskra, A. E., **Lafontaine, S. R.**, Phillips, C., Shellhammer, T. H., Trippe, K., Twomey, M. C. Woods, J. L., Gent. 2017, D. H. The Multifaceted Influence of Nitrogen Fertility on Hops in the Pacific Northwest. 2nd International Brewers Symposium on Hop Aroma and Flavor in Beer, Corvallis, OR. July 25 – 28. *Poster presentation*

9. **Lafontaine, S. R**., Vollmer, D.M. and Shellhammer, T.H**.** 2017. Understanding the dry hop aroma of American hop varieties. 2nd International Brewers Symposium on Hop Aroma and Flavor in Beer, Corvallis, OR. July 25 – 28. *Poster presentation* ***(runner up for best poster)***

10. **Lafontaine, S. R.** and Shellhammer, T.H. 2017. Evaluating hop chemistry and its contribution to hop aroma intensity in dry-hopped beer. American Society of Brewing Chemists Annual Meeting, Fort Myers, FL. June 4 – 7. *Oral presentation (4).*
11. Shellhammer, T.H. and **Lafontaine, S. R.** 2017. Sensory directed mixture study of beers dry-hopped with Cascade, Centennial, and Chinook. American Society of Brewing Chemists Annual Meeting, Fort Myers, FL. June 4 – 7. *Oral presentation* (31).
12. Hahn, C., **Lafontaine, S. R.** and Shellhammer, T.H. 2017. A comprehensive evaluation of the nonvolatile chemistry affecting the bitterness intensity of highly hopped beers. American Society of Brewing Chemists Annual Meeting, Fort Myers, FL. June 4 – 7. *Oral presentation (18).*
13. **Lafontaine, S. R.**, and Shellhammer, T.H. 2017. Understanding the impact hopping rate has on the aroma quality and intensity of dry hopped beers. 36th Congress of the European Brewing Convention, Ljubljana, Slovenia. May 14 - 18. *Oral presentation #18*
14. **Lafontaine, S. R.**, Hahn, C. and Shellhammer, T.H. 2017. Insight into the American IPA. A deconstruction of America's popular beer style. 36th Congress of the European Brewing Convention, Ljubljana, Slovenia, May 14 - 18. *Poster presentation #082*
15. **Lafontaine, S. R.**; Shellhammer, T. "Examination of raw material factors in hops that contribute to their dry hop aroma performance in beer" Winter Hop Research Council Meeting, Bend, OR. Jan 18, 2017. *Oral Presentation.*
16. **Lafontaine, S. R.**, Wietstock, P. and Shellhammer, T.H. 2016. Update: Solid Phase Extraction of Isomerized Alpha Acids in Beer and Subsequent Spectrophotometric Measurement. World Brewing Congress, Denver, CO. August 13-17. *Poster presentation #85*
17. Hahn, C., **Lafontaine, S. R.** and Shellhammer, T.H. 2016. A holistic examination of beer bitterness. World Brewing Congress, Denver, CO. August 13-17. *Oral presentation #65*
18. Iskra, A. I., **Lafontaine, S. R.**, Phillips, C., Shellhammer, T.H.**,** Trippe, K., Twomey, M., Woods, J., and Gent, D. 2016. Nitrogen fertilization increases powdery mildew, arthropod pests, and nitrate accumulation in hops. APS Pacific Division Meeting, La Conner, WA. June 28-30. *Poster presentation.*
19. **Lafontaine, S. R.** and Shellhammer, T.H. 2016. Identifying unique drivers of hop aroma in beers dry-hopped with Cascade, Centennial, and Chinook. Young Scientists Symposium, Chico, CA. April 21-23. *Oral presentation.*
20. Barnette, B. **Lafontaine, S.R.** and Shellhammer, T.H. 2016. Determination of method variability in steam distillation of hop essential oils by gas chromatography-flame ionization detection of oxygenated terpenoids. Young Scientists Symposium, Chico, CA. April 21-23. *Poster presentation.*
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APPENDIX 2– SAS code used to develop sensory analysis serving orders and process data

SAS Code used for sensory panels by Dr. Cliff Pereira to generate an efficient incomplete block design for assigning beers to sessions within each full replication.

The method used is described in Pereira and Tobias (2015)[142] which is available at

http://support.sas.com/resources/papers/proceedings15/3148-2015.pdf

The macro %RBDEval (which is used to evaluate the design) is available to copy and paste at:

http://support.sas.com/rnd/app/qc/examples/OPTEX/sas.html

Example where beers need to be assigned to sessions: Studying 10 beers overall, but want to have only 5 beers presented per sensory session, so that it takes 2 sessions to have a complete replicate (every beer evaluated exactly once). There will be 4 replicates for a total of 8 sessions. The design goal is to assign the beers to the sessions within each replicate, so that, over the 4 replicates, pairs of beers occur together in the same session in a reasonably balanced way. (If one were just to assign the 10 beers randomly to the sessions within each replicate, one can get an unfortunate randomization, such that over all 4 replicates some pairs of beers occur together in sessions considerably more often than other pairs of beers. The method shown below avoids such highly unbalanced randomizations.)

Treatments (beers) = 10 = t

Session (block) size = 5 = k

Replicates = 4 = r

The design setting can be written as a triplicate (t, k, r) = (10, 5, 4) with every t/k= 10/5=2 sessions being a complete replicate. Such a design is called "resolvable" as explained in Pereira and Tobias (2015)).

A key parameter for an incomplete design setting like this is lambda = r*(k-1)/(t-1) where r = number of reps, k = block size and t = number of treatments. Lambda is the average number of times that pairs of treatments occur together in the same incomplete block (session). For this design lambda = 4*(4)/10 = 1.6. The fact that lambda is NOT an integer, tells us that no balanced incomplete block (BIB) design exists for the setting of 10 treatments, incomplete blocks of size 5 and 4 complete replicates. PROC OPTEX can be used to get an efficient (reasonably-balanced) incomplete block design for any such settings.

BEGIN EXAMPLE SAS CODE (Text between /* and */ are comments)

```
/*  NOTE:  Assuming all panelists at a session will see the same 5
beers, which means that panelists do not come into the design part.
Here just deciding which 5 beers to present at each session.
Treatments (beers) = 10
Replicates = 4
```

```
Session size = 5
*/

/* Create and check treatment structure data set */

data trt;
   do trt = 1 to 10;
      output;
   end;
run;
proc print data=trt;
run;

/* Create and check rep and session structure data set */

data Setup;
   do Rep = 1 to 4;
      do Session = 1 to  2;
          do entry = 1 to 5;
            output;
         end;
      end;
   end;
   run;
proc print data=setup;
  run;


/* size of design = 4 reps X 10 beers = 40. Divide size by 10 (40/10 =
4) to get 2nd prior within model statement below (see Pereira and
Tobias (2015))

Create quick design with the default 10 random starts to show that PROC
OPTEX is finding resolvable designs when using second prior value of 4
*/

proc optex data=trt coding=orthcan seed=85417;
   class trt;
   model trt;
   block design=Setup;
   class Rep Session;
   model Rep, Session(Rep) / prior=0,4;
   output out=Design1;
run;

/* Run macro to evaluate the quick design */

%RBDEval(Design1,trt,Rep,Session);

/*  Output from macro

                 trt Efficiency for Rep
                 and Session-within-Rep

Evaluation             D        A       BD
Rep             100.0000 100.0000 100.0000
Session(Rep)     87.7570  86.5649  98.7266
```

```
Rep line:  100's tell you design is Resolvable (every treatment occurs
once in each rep -- each rep is a complete block)  See Pereira and
Tobias (2015)


Session(Rep) line:  Last number in row under BD is most important.  It
tells you that this design is 98.7% as D-efficient as an upper bound
which cannot be achieved (because such a design would need to be a BIB
which does not exist for this setting).  So this is an efficient design
for the setting.  If a design exists that is more efficient, then such
a design could at most be only very slightly more efficient than the
one that was found by PROC OPTEX.
*/
/*
ONE CAN STOP AT THIS POINT and use the design in the data set Design1.
Or one can increase the number of random starts (NITER) to some higher
number such as 10000 to see if the design can be improved upon by
allowing PROC OPTEX to do considerably more searching in the design
space
*/
proc optex data=trt coding=orthcan seed=45378;
   class trt;
   model trt;
   block design=Setup niter=10000 keep=10;
   class Rep Session;
   model Rep, Session(Rep) / prior=0,4;
   output out=Design2;
run;


%RBDEval(Design2,trt,Rep,Session);

/*  Output from macro
                  trt Efficiency for Rep
                  and Session-within-Rep

Evaluation             D        A       BD
Rep             100.0000 100.0000 100.0000
Session(Rep)     87.7570  86.5649  98.7266

Same efficiency as with the default niter=10 --
 design2 with niter=10000 did not improve the efficiency over the quick
design using just the 10 random starts (iterations).
 */
/* EXAMINING THE DESIGN WITH SIMPLE TABLES
/*
Tabulate the design to show the complete reps  (every beer should occur
once in each rep)
*/
proc tabulate data=design2;
  class rep trt;
  table rep,trt;
  run;

/* Tabulate design to show sessions within reps */
proc tabulate data=design2;
```

```
  class rep trt session;
  table rep*session,trt;
  run;

/* Can export design to a choice of file type such as an Excel file */
```
END EXAMPLE SAS CODE

SAS Code used by Dr. Cliff Pereira to perform second-order multiple linear regression model selection.

See Methods section 2.10 Statistical Analysis. To avoid overfitting three strategies were employed:

1) hierarchy option, 2) required agreement on predictors for three methods of selection (SBC, AICC and Press) and 3) bootstrap resampling followed by model selection with SBC to verify that predictors were selected in a large proportion of the bootstrap samples.

BEGIN SAS CODE

```
/*  OHAI response prediction with Cascade 2015 data and 17 potential
predictors (16 hop volatiles and total oil)*/

/* MODEL SELECTION with hierarchy option.  Repeat with model statement
option select=aicc and then again with select=press to see what
predictors are common to all three selection methods.  */

proc glmselect data=casc15 plots=all;
     effect order2 = polynomial(toil isobIsobutyrate APinene BPinene
Myrcene MHeptanoate rlimonene Linalool Nerol Neral Geraniol Geraneal
GAcetate Caryoph AHumulene Farnesene  Caryophox/degree=2);
model ohai = order2 /selection=stepwise(select=sbc)
orderselect hierarchy=single stb;
run;

/* BOOTSTRAP RESAMPLING using "modelaverage" statement with model
selection by SBC.  Primary output of interest is the percentage of
samples where each effect is in the selected model (effect selection
percentage table).*/

proc glmselect data=casc15 plots=all;
     effect order2 = polynomial(toil isobIsobutyrate APinene BPinene
Myrcene MHeptanoate rlimonene Linalool Nerol Neral Geraniol Geraneal
GAcetate Caryoph AHumulene Farnesene  Caryophox/degree=2);
model ohai = order2 /selection=stepwise(select=sbc)
orderselect hierarchy=single stb;
modelAverage tables=(EffectSelectPct(all) ParmEst(all));
run;
```

END SAS CODE