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## **Effect of Harvest Maturity on the Chemical Composition of Cascade and Willamette Hops**

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### **ABSTRACT**

Considerable expertise is required to grow high-quality hops, and brewers and hop growers alike have a common goal of obtaining the highest quality hops possible. Change in the chemical composition of hops during plant maturation is a dynamic process requiring a comprehensive chemical and sensory analysis in order to maximize the characteristics of interest to brewers. The effect of harvest date, location, and cultivar on key chemical components of Willamette and Cascade hops was investigated for the 2010 and 2011 growing seasons. Hops were harvested at 3 time points (Early, Typical, and Late), within a 3-week interval from 2 different farms in the Willamette Valley, Oregon. A split-plot experimental design for each cultivar was used; each farm represented a main plot and harvest years were designated as subplots. American Society of Brewing Chemist standard methods of analysis were used to measure moisture content, hop acids and their homologs, hop storage index, total essential oil content and volatile profile by GC-FID. Additionally, difference testing, descriptive analysis, and consumer acceptance testing was conducted using beers brewed with either Typical or Late harvested Cascade hops from the 2010 harvest year. The response of analytes was dependent on the cultivar being examined, its location within the Willamette Valley, as well as timing of harvest. Hop acids did not change appreciably during plant maturation for the period examined,

while hop oil content increased. Increases in oil quantity were strongly correlated ( $r > 0.90$ ) with increases in  $\alpha$ -pinene,  $\beta$ -pinene, myrcene, limonene, methyl heptanoate, and linalool concentrations. Clear sensory differences were found between beers brewed with Typical and Late harvested Cascade hops using triangle testing, consumer preference testing, and descriptive analysis.

**Keywords:** Aroma, Essential Oil, GC-FID, Hop Acids, Terpenes

## INTRODUCTION

Much of the characteristic aroma and flavor of beer can be attributed to the combination of key chemical compounds extracted from either fresh or dried hop cones. Extraction and dissolution of these compounds into beer can be achieved by sophisticated preliminary processing steps, such as the addition of supercritical CO<sub>2</sub> extracted hop material, or by simply adding hops (pelletized or whole) during the brewing process. The nature and nuance of hop-derived aromas and flavors is dictated in large part by the chemical compounds extracted, and in turn, the chemical composition of the hops from which they were derived. As such, the composition of hops has a direct impact on not only the flavor and aroma of finished beer, but also the storage stability of finished beer. The soft resin, namely the  $\alpha$ -acids, and the essential oil fraction stored in the glandular trichomes (lupulin glands) of hops make the predominant contributions to the aroma and flavor of beer. Much of the hoppy aroma in beer is attributed to the essential oil fraction while  $\alpha$ -acids are the precursors to the main source of hop-derived bitterness in beer. Additionally, it has been shown that pre-harvest conditions (5), post-harvest processing (9) and varietal factors influence the composition of the essential oil fraction as well

as  $\alpha$ -acid content(6) . The composition of the essential oil of hops is an extremely complex mixture with over 450 identified compounds and suggestions that over 1000 chemical compounds may exist (9). Much work has been done in attempting to identify important odor active hop compounds in both processed hops and beer to gain a better understanding of how the aroma characteristics in hops as a raw ingredient influence the flavor of beer (7, 17, 20, 25). Many of these compounds exist well below sensory detection threshold quantities and yet still may significantly contribute to the aroma profile of hops either through synergistic effects with other compounds (27)or biotransformation during fermentation (10, 11, 12, 15, 28) into more odor active compounds. Although the list of compounds that are likely to contribute to hop aroma is quite long when considering all cultivars, the list becomes somewhat smaller when each cultivar is considered individually, as each cultivar has its own unique essential oil profile. Additionally, a smaller set of compounds may serve as an indicator of change within a cultivar due to environmental or management conditions during cultivation or storage (19).

In order to effectively control the hop aroma in beer, it is important to investigate both extrinsic factors such as processing operations, handling and storage of hops as well as intrinsic factors such hop maturation prior to harvest. It is well known that hops are influenced by factors such as daylight, growing conditions, and post-harvest processing conditions, and that much of the quality and character they can potentially lend to beer is determined before arriving at the brewery (5,9). What is not well known is how the maturity stage or harvest timing influences the aroma and flavor qualities of hops. The detailed account of hop terpene biosynthesis by Wang et al. (29) provides helpful insight into the biosynthetic and enzymatic pathways of principle hop terpenes associated with approximate developmental stages, but it does not address the changes of other key aroma compounds and how they relate to harvest timing beyond 4 weeks after onset

of flowering. Therefore, investigation of cultivation, harvest and post-harvest handling of hops is critical to understanding the agronomic factors that affect hop quality. The aim of this work was to investigate the effect of hop harvest date on  $\alpha$ - and  $\beta$ - acid content and profile, total essential oil content, and essential oil profile of two important hop varieties, 'Cascade' and 'Willamette'. Results are from samples obtained during the 2010 and 2011 harvests. A list of target compounds of interest was generated based on examination of previous studies investigating the contribution of hop aroma compounds in beer (18), hop aroma compound analysis (22), and preliminary analysis using GC-MS capabilities within the Department of Food Science and Technology at Oregon State University (data not shown). For practical reasons, the list of target analytes was refined based on the concentration in the hops and the maximum concentrations likely to be found in beer brewed with these hops. Bittering acid content was also monitored to examine whether bittering acid concentrations were affected as a result of changes in harvest timing. For the purpose of this study, traditional cohobative hydro-distillation was used for its simplicity and practicality of relating results to standard methods used by most hop analysis labs.

## **EXPERIMENTAL**

### **Hops and Cultivation**

Willamette and Cascade hops from the 2010 and 2011 growing seasons were harvested from two commercial farms located at approximately the same latitude (N 44.850° - 45.001°) on the east and west sides of the Willamette Valley in Oregon, USA. Hops were sampled approximately 10-14 days prior to the projected commercial harvest date, on the commercial harvest date, 5-10 days after the commercial harvest and designated as either Early, Typical, or Late harvest, respectively (Figure 1). Differences between dates and within-harvest time points

for each year and farm were due to the discrepancy between the predicted Typical harvest dates, from which the Early time points were determined, and the actual Typical harvest dates.

On both farms, plants were managed during the growing season using typical hop production practices for the Willamette Valley. Downy mildew (*Pseudoperonospora humuli* Miyabe & Takah.) G.W. Wilson control measures included the use of a copper-based fungicide on each farm with application dates ranging from mid-May to mid-June. Previously, Kishimoto et al. (16) have suggested that copper-based fungicides might affect the accumulation of some hop chemical compounds.

Three samples (~600-700g fresh, not dried) of each hop cultivar were randomly obtained at each of the two farms for each of the three time-points in the 2010 and 2011 harvests for a total of 72 samples. Samples of hops from the Early and Late time points were hand-picked from sidearms located in the lower, middle and upper canopy of randomly selected plants growing in well-established commercial hop yards at each farm. Samples from the Typical harvests were collected directly from on-farm hop picking machinery just prior to commercial kilning. Late-harvested samples, except for late-harvested Cascade from Farm I in 2011, were taken from a group of plants hanging at the end of one row. Samples from the 2011 Late-harvested Cascade from Farm I were harvested from the remnant, untrained ground shoots due to all of the bines being mistakenly harvested during the Typical commercial harvest date. These samples may be unrepresentative of commercially harvested hops for that time point.

### **Sample Preparation and Storage**

Immediately after harvest, samples were dried at 49 °C in a forced air oven for 12 hours to a moisture content of approximately 8% (w/w), packaged into “mini-bales” weighing approximately 500 grams and stored in clear plastic bags at ~5° C for no more than 2 days prior

to analysis or packaging for long-term storage. Representative samples were obtained from each mini bale prior to chemical analysis according to the American Society of Brewing Chemists sampling protocol for hops (2). Unused hops were packaged in plastic dual layer foil pouches, flushed with nitrogen, vacuum-sealed, and stored at -20° C.

### **Chemical Analysis**

Each dried sample was analyzed for moisture, hop storage index,  $\alpha$ - and  $\beta$ -acid content, cohumulone and colupulone content, total essential oil volume, and essential oil profile determination by gas chromatography using American Society of Brewing Chemists Standard Methods of Analysis (2) and modified in some cases as described below. All results were normalized to 8% (w/w) moisture content prior to statistical analysis. Dry matter content was also measured (data not shown) for each sample but displayed such high variation and sensitivity to uncontrollable factors that it was not a practical indicator of maturity and ultimately not used in this study.

### **Hop acids measurements**

Hop storage index was measured according to ASBC method Hops-12 (2). Concentrations of  $\alpha$ -acids and  $\beta$ -acids, as well as cohumulone and colupulone percentages, were measured using HPLC. Extraction, dilution, identification and quantitation techniques of bittering acids were performed according to ASBC Hops-14 (2). Chromatographic determination was performed using an Agilent 1200 series HPLC system (Boblingen, Germany) equipped with a 100 x 4.6mm Kinetex C18, 2.6  $\mu$ m column (Phenomenex, Torrance, California, USA) held at a constant temperature of 40°C. The flow rate was 1.3 ml/min with a 7  $\mu$ L injection volume. Three mobile phases were used for separation. Mobile phase A was 100% water, mobile phase B was 75% methanol, 24% water, and 1 % phosphoric acid and mobile phase C was 100% methanol. Initially, elution began with 10% of mobile phase A and 90% B for the first 8 min, followed by a

gradient of 100% mobile phase C for 5 minutes which was then followed by another gradient back to 10% mobile phase A and 90% mobile phase B for an additional 5 minutes and then held for 7 minutes (total run time = 25 minutes). The ASBC International Calibration Extract (ICE-3) was used as a standard for peak identification and quantitation.

### **Essential oils**

Total essential oil content of hop samples was determined by cohobative hydro-distillation according to ASBC method Hops -13 (2) using a modified sample preparation method. Instead of grinding the sample using a food chopper as recommended by the ASBC standard method of analysis, 100-110 grams of whole dried hops were blended for 30 seconds with 1.5 liters of cold deionized water using a 3.8 liter stainless steel blender (Waring CB15) and transferred quantitatively using an additional 1.5 liters of deionized water to a 5000 ml round bottom boiling flask. After 4 hours of distillation, oil samples were cooled to room temperature in the receiver before the volume of the oil fraction was measured, which was then collected and stored at 5°C in 4 ml glass vials purged with nitrogen gas and capped with foil lined screw-top caps until GC-FID analysis.

### **Hop oil composition**

Chromatographic separation of hop oil components was performed according to the standard method (2), but with a modified temperature program optimized for adequate separation of target compound peaks. A Hewlett Packard 5890 GC-FID with a HP 7673A auto-sampler was used for sample injection. Compounds were separated on a 30 m x 0.25 mm I.D. fused silica capillary Supelcowax 10 column (Supelco) with a 0.5 µm film thickness. The modified temperature program started at 60° C held for 1 minute, ramped at a rate of 3°C/minute to 175°C and held for 10 min, then ramped at 3°C to 230°C and held for 10 min. A split ratio of 1:50 was used with a carrier gas of pure nitrogen at a flow rate of 1 ml/min. The injector temperature and

FID temperature were 200°C and 250° C respectively. Quantification of compounds was determined by using an internal standard of 2-octanol as outlined in ASBC Hops-7 (2). Area integration reject was set to 1 mV. The compounds of interest analyzed by GC-FID are summarized in Table I. Analytical standards used for peak identification were obtained through Sigma-Aldrich and were of >95% purity unless noted otherwise. Characteristic aroma descriptions of each compound are shown for each standard (Table I).

### **Experimental Design and Statistical Analysis**

A split-plot experimental design for each cultivar was used; each farm represented a main plot and harvest years were designated as subplots. Statistical analysis was performed using a general linear regression of the following mixed model:  $\text{response} = \text{Farm} + \text{Harvest} + \text{Farm} * \text{Harvest} + \text{Farm} * \text{Year} * \text{Time}$  with Farm being assigned as a random factor. Mixed models are used for the statistical analysis of experimental designs which include both a fixed (assigned) variable and a categorical predictor variable that can be considered a random effect; a classification that assumes the levels of the variable have been randomly selected from a population of possible levels. In this study, the Farm variable was considered to be a random variable assigned to each hop sample. The scope of inference was considered to be Cascade and Willamette hops grown in the Oregon Willamette Valley.

All summary statistics, analysis of variance and post-hoc multiple comparisons tests for instrumental data were generated using SAS/STAT software, Version 9.2 of the SAS system software for Windows (Copyright 2002-2008 by SAS Institute Inc., Cary, NC, USA).

For sensory testing, data collection and analysis of variance was conducted on the sample means for overall liking, aroma liking, and flavor liking using Compusense 5.0®, version 4.6,

Guelph, Canada. Statistically significant attributes were subjected to post-hoc analysis using Tukey's HSD test at the 95% confidence interval.

### **Sensory Analysis**

By coincidence, 25 hL brewing trials were being conducted independently by a local brewery investigating the effects of hop harvest timing on beers. Although not part of the initial experimental design, the opportunity to perform sensory testing was not ignored. Difference testing, consumer acceptance and descriptive analysis was carried out on beers made with Cascade hops from the 2010 Typical and Late harvest periods from Farm I. Because the Early harvested samples displayed brewing values that were quite low and not of practical commercial interest, brewing trials were not conducted using Early harvested hops. It should be noted that hops used for brewing trials were sampled separately from those used for chemical analysis but in a manner consistent to that described in the methods section.

### **Brewing and Sample Preparation**

Beers were brewed commercially by an Oregon brewery with every attempt to produce identical beers, aside from the effects of each having been brewed with Cascade hops harvested at different points. In order to highlight hop character, a pale ale style beer (5.25% ABV, 15 SRM) hopped to 32 IBU's was brewed. Hop dosages consisted of a 75 minute kettle boil addition and a 30 minute kettle boil addition each dosed at 1.45 grams hops/liter wort followed by a whirlpool late hop addition of 5.50 g/L. Wort was fermented using a British ale type yeast at 18 C to ending apparent gravities of 2.70°P. Filtered and carbonated beer samples were received from the brewery in 22 L stainless steel kegs and stored at 1° C until testing. Beer analyses such as color, gravity, IBUs, and alcohol were conducted onsite by brewery staff. Due to limitations, all sensory testing was performed on a single batch of each beer although a replicated study design would have been ideal.

## **Difference testing**

Difference testing of beer samples was performed using triangle tests to determine if a noticeably significant difference existed. Panelists (n=18) consisted of untrained Oregon State University Brewing Science students. Each panelist was assigned a random presentation order and asked to identify the sample (Late harvest) that was different from the other two (Typical harvest).

## **Consumer Acceptance**

Consumer acceptance of beers hopped with either 2010 Typical or Late harvested Cascade hops was carried out at the OSU Sensory Science Lab. Sixty-two consumers were recruited from the local community (Corvallis, Oregon). Prospective consumers were screened on the following criteria: 1) between the ages of 21 and 55; 2) consume beer at least once per month; and 3) indicated that pale ale style beers were within their top 3 most preferred styles of beer. Fifty five (89%) of the consumers were between 21 and 29 years old while 17 (11%) were between 30 and 55 years old. Forty three (69%) were male and the remaining 19 (31%) were female. Each consumer received a 75 ml sample of each beer for evaluation and asked to rate acceptance for overall liking, aroma, and flavor using a 9-point hedonic scale; Rating Scale: 1=dislike extremely, 2=dislike very much, 3=dislike moderately, 4=dislike slightly, 5=neither like or dislike, 6=like slightly, 7=like moderately, 8=like very much, and 9=like extremely. Samples were served in clear 300 ml glasses covered with a clear plastic odorless lid to minimize aroma loss. Each glass was identified by a three-digit random number. Samples were served to each consumer in monadic order and the first sample served was removed before the consumer received the second sample. Serving order was randomized so that approximately 50% of the consumers evaluated the Typical sample first and 50% of the consumers evaluated the Late

sample first. Saltine crackers and water were provided to panelists for palette cleansing between samples.

### **Descriptive analysis**

Descriptive sensory analysis was performed using 12 trained panelists who had been extensively involved with recent sensory work regarding beer evaluations. Samples were allowed to equilibrate to room temperature (20° C) during evaluation to maximize aroma. The descriptive ballot was based on 13 descriptive terms developed by the panel during preliminary consensus based training sessions for beer aroma with a focus on hop derived aromas and contained the following descriptive attributes; fruit cocktail, tropical fruit, melon, grapefruit, estery, green apple, rose, floral, green hop, pine, apricot/peach, sweaty/onion/garlic, and orange. Panelists met only once to assess the beers and examined each beer once. There were no repeated measures by any panelist for descriptive analysis.

## **RESULTS**

### **Hop Acids and HSI**

Table II shows the summary of hop acid data for hops from the 2010 and 2011 crop years harvested at different time points. Values were averaged across all reps from both years to account for season to season variation. For reference, an H.S.I. greater than or equal to 0.30 is generally considered to be an indication of aged hops and it should be noted that all values were between 0.20 and 0.28. Alpha-acid and  $\beta$ -acid percentages are expressed as a per mass basis of hops normalized to 8% moisture. Cohumulone percentages are expressed on a per mass basis of  $\alpha$ - acids and normalized to 8% moisture.

There were no significant main effects for H.S.I.,  $\alpha$ -acid content,  $\beta$ -acid content or cohumulone content for Cascade or Willamette hops (Table III). However, a 3-way Farm by

Harvest by Year interaction ( $p$ -value $<0.0001$ ) was observed for both hops on all attributes except H.S.I. values for Willamette hops (Table III). That is, significant differences were found for H.S.I.,  $\alpha$ -acids,  $\beta$ -acids, and cohumulone contents depending on time of harvest, the originating farm, and the harvest year. These results suggest that differences in year-to-year growing conditions or practices may contribute significant variation to hop chemical content. Other explanations for variation may be attributed to different and irregular sampling dates within a given harvest window, the inherent variation among experimental units associated with random sampling in a large hop yard and, to a lesser extent, inability to exactly reproduce treatment conditions (harvest dates) from one experimental unit to another over seasons and years. For future work it is recommended to sample on a regular and consistent schedule for all hop yards and cultivars being examined.

### **Total Essential Oil and Composition**

Averages and results from Tukey's HSD ( $p < 0.05$ ) for essential oil and volatile components by harvest timing are shown in Table IV. A significant difference in total oil content of Willamette and Cascade hops was found between harvest time points ( $p$ -value  $< 0.0001$ ) with a significant 3-way interaction of time, farm, and year ( $p$ -value  $< 0.0003$ ) and main effect of harvest timing as shown in Table V and illustrated in figure 2. Early harvested Cascade and Willamette hops had lower average oil content compared to Typical and Late harvested hops (Table IV).

An increase in oil quantity was strongly correlated ( $r > 0.90$ ) with  $\alpha$ -pinene,  $\beta$ -pinene, myrcene, limonene, and linalool contents (data not shown). The percentage of farnesene of the total oil and humulene/farnesene ratios (Table IV) are shown for comparison to the dichotomous key proposed by Kenny (14). Farnesene content above 1% was considered characteristic of Willamette and Cascade hops while a humulene/farnesene ratio below 3 distinguishes the latter

from the former (14) and agrees with the results found here. With the exception of the farnesene percentage and humulene/caryophyllene ratio, concentrations of compounds are expressed as milligrams of compound per gram of hops that have been normalized to 8% (w/w) moisture (Table IV). A similar trend was observed for the monoterpenes that were correlated with oil increase mentioned above. Significant main effects, 2-way and 3-way interactions were found for most of the compounds for both Willamette and Cascade hops (Table V).

For Cascade,  $\alpha$ -pinene,  $\beta$ -pinene, myrcene, limonene,  $\rho$ -cymene, caryophyllene, E,  $\beta$ -farnesene, and humulene all increased from Early to Typical points but no increase was observed between the Typical and Late time point. Linalool and methyl heptanoate increased between each time point while citral and humulene epoxide differed between Early harvest and Late harvest, but not between Early and Typical or Typical and Late harvests. For Willamette hops,  $\alpha$ -pinene,  $\beta$ -pinene, myrcene, limonene,  $\rho$ -cymene, and linalool all increased between each time point. Caryophyllene, E,  $\beta$ -farnesene, humulene, farnesol and citral all increased from Early harvest to Typical harvest but no difference was observed between Typical and Late. Geraniol peaked at Typical harvest date.

### **Sensory Difference Testing**

Fourteen out of eighteen panelists were able to correctly identify the different sample in a triangle test indicating that a significant sensory difference was observed ( $p$ -value < 0.001) between beers prepared with Typical and Late harvested Cascade hops. This result prompted a consumer acceptance study and descriptive analysis.

### **Consumer Acceptance**

Mean scores, standard deviations and results from post-hoc tests for overall liking, flavor, and aroma plus significance values are provided in Table VI. Results indicate a significantly higher overall liking ( $p=0.0002$ ), aroma liking ( $p=0.0004$ ), and flavor liking ( $p=0.0019$ ) for the

beer hopped with Typical harvest Cascade hops compared to beer hopped with Late harvest hops (Table VI).

### **Descriptive Analysis**

The Typical harvest date for Cascade hops resulted in a beer with significantly higher apple, apricot/peach, and sweaty/onion/garlic notes while the Late had higher melon and floral notes ( $p < 0.05$ ) (Figure 3). Sweaty and onion/garlic notes found in the beers made with Typical harvest hops may be attributed to trace sulfur containing compounds (24). Interestingly, the panel found higher floral notes in beers brewed with Late harvested hops despite the lack of instrumental data showing the increase of aroma compounds typically associated with floral aroma. This could be due to lack of replication in descriptive testing or an increase in a compound(s) not investigated here.

### **DISCUSSION**

Although increased oil compound concentrations were observed at later harvest dates, there is no clear evidence that one specific compound increased more than others, or that one specific compound is a marker for increased observable differences for later harvest dates as observed by Murphey and Probasco (21). In general, however, monoterpene hydrocarbons and linalool did increase over time and may contribute to a different aroma profile. These results partially corroborate findings by Howard and Slater (13) who observed an initial increase in the oil content and hydrocarbon fraction (myrcene, humulene, caryophyllene) of Fuggles hops over a 6 week harvest period with an eventual decrease in oil at the latest harvest date, but no significant increase in the oxygenated fraction. In the present study it was shown that alpha acids don't change significantly over the harvest dates investigated for Willamette and Cascade hops. Similar results were observed in previous studies for Willamette hops but increases were observed in other varieties (4, 21) suggesting that the optimal harvest timing for maximum alpha

contents is dependent on variety. Also, the cohumulone proportions did not increase for either hop variety as observed in Fuggles hops by Howard and Slater (13). The 2010 hop growing season was not considered a “normal” growing season; growers reported that harvest dates were about 2 weeks later than usual and there was substantial rainfall during commercial harvest times. Significant variation may have also been artificially included in the model due to sampling technique and inconsistencies among the sampling intervals across farms, cultivars, and years (Figure 1).

While increased oil volumes may be desirable for aroma type hops, other properties of the hops should be considered at each harvest time point to determine the overall quality of the hops. For example, HSI can be a useful measure of hop quality in terms of the bittering acids, but provides little information regarding hop quality from an aromatic perspective, other than a non-specific indication of possible age related aroma compounds. Refractive index and UV spectra measurements of hop oil have not proved to be indicative of quality or composition, although decreasing R.I. values have been correlated to later harvested hops (30). A similar measure for overall hop aroma quality has yet to be determined and due to the complexity of hop aroma, a single measure will not likely be a useful indicator of aroma properties. Furthermore, hop quality is a complex term and uniformly negative quality indicators such as isovaleric acid (cheesy aroma), cone discoloration, and hop cone shattering due to late picking or over-drying should also be considered. Shattering is particularly important since it leads to a loss of lupulin glands (i.e. reduced brewing quality) and decreased harvest yields.

Quality indices such as shatter and discoloration were not quantified in this study, however, it was noted that later harvested hops had a higher tendency to shatter or break apart during processing. Furthermore, variation in hop cone color was noticeable across the three

harvest dates. Cones harvested early were greener than hop cones harvested late. A yellowing and eventual browning was observed at the later harvest dates. It should be noted that although discolored hops are often used for extracted hop production and are not necessarily a definite index of poor quality, these observations may have commercial significance since brewers often use color as an indicator of healthy and/or high-quality hops at market.

Hop harvest timing is typically determined by cone dry matter content as an indicator of overall plant maturity. While cone dry matter content is helpful in gauging hop harvest timing and was used successfully by Murphey and Probasco (21), sampling technique, plant water status, disease and pest pressure, and presence of seeds can lead to erroneous estimates of plant growth stage and harvest readiness. In the present study, we noted inconsistencies in dry matter determinations across samples, farms for both years of the study. Days in which hops were harvested during or shortly after it had rained yield erroneous dry matter estimates using standard drying techniques. Separately, it should be noted that samples were obtained throughout the plant canopy, and is likely that some immature cones were inadvertently collected from bines that had self-trained well after the normal spring training date. Collecting all of the samples from the upper canopy would probably have made for more consistent dry matter determinations (Gene Probasco, Personal Communication). Additionally, more homogeneously equivalent samples representative of the plot may have been obtained from quantitatively combined subsamples of mechanically harvested hop cones.

It is clear that hop harvest date has a significant effect on beer flavor, and this management factor may significantly impact beer flavor and aroma. Interestingly, the consistently higher consumer acceptance ratings for beers brewed with Typical harvested hops over Late harvested Cascade hops contradict sensory results from a previous study using

Hallertau Mittelfrueh hops harvested at different time points (4). However, differences from these results are likely due to differences in hopping regimes (late kettle hopping vs. dry hopping), hop variety, beer style, regional preferences and sensory testing methods. Results from an earlier study examining the effects of aging hops prior to brewing(19) showed that both Cascade and Hallertau Mittelfrueh hops benefited from moderate post-harvest aging to maximize aromas. However, the same study showed that excessive aging led to a more dramatic aroma loss in Cascade hops than in Hallertau Mittelfrueh hops (19). Perhaps similar “aging” phenomena occur while hop cones are still on the bine and lead to beers with a lower consumer acceptance when made with Cascade hops but not with Hallertau Mittelfrueh hops.

Brewers are intimately aware of the variable bittering acid content of their hops and are able to adjust hop dosing based upon a chemistry analysis to obtain a consistent product. However, few quality assurance measures are available for adjusting hopping rates due to variations in hop aroma profiles. As illustrated here, a statistically significant difference in total essential oil was observed in hops over a 2-3 week harvest period, and in some cases, significant changes were observed in less than 1 week. Results from both instrumental and sensory analysis indicate that more thorough and consistent monitoring of hop aroma chemical composition may be needed to ensure consistency during brewing.

For this study, the selection of hop oil compounds of interest was based on previous reports as well as preliminary examination of GC-MS data. However, because hop-derived aroma is variety dependent, a variety-specific list of target analytes for a given harvest time might be helpful for brewers. While the results presented here clearly point to compositional changes in hop chemistry that ultimately affect beer character, the challenge of determining the optimal harvest timing for certain hop varieties, after considering various management factors,

lies primarily with brewer preference, and ultimately, with consumers. Furthermore, despite much advancement, a deeper understanding of the compositional variation between hop varieties is needed such as investigations of sulfur containing compounds, polyphenols, and flavor/aroma precursors in hops and their relation to hoppy aroma in beer. Therefore, data obtained through sensory analysis techniques and correlated to instrumental results used to define quality parameters would ultimately provide the most relevant measures of hop aroma.

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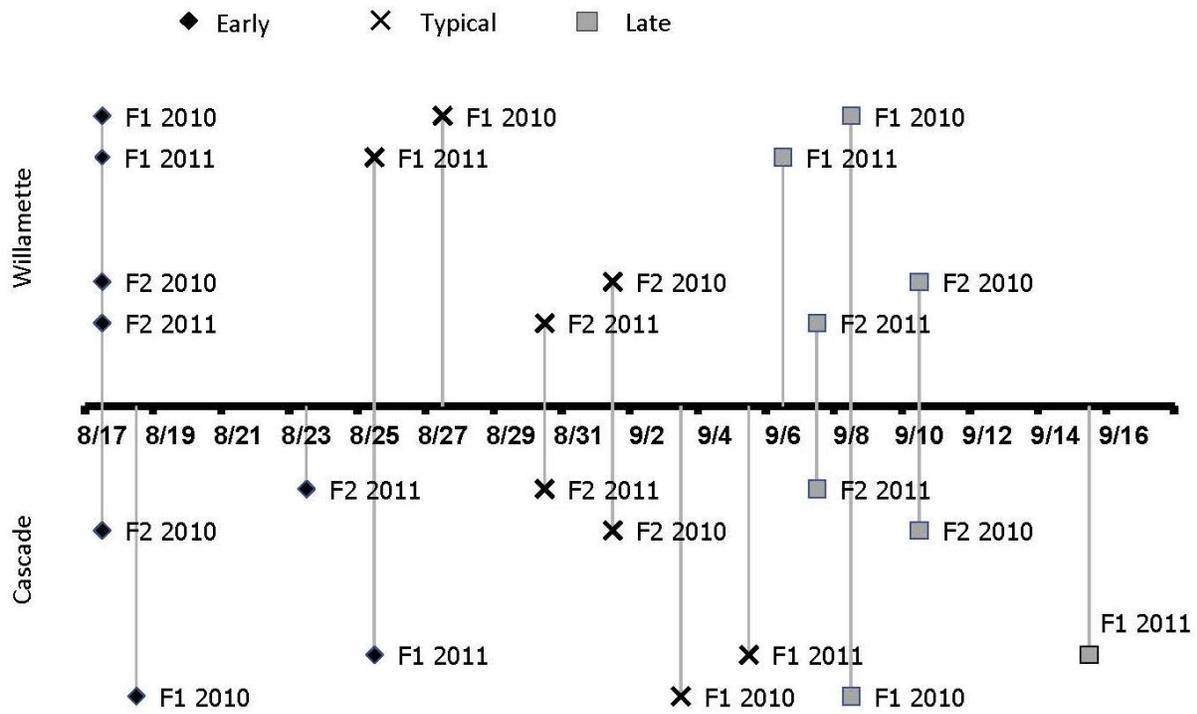


Figure 1: Harvest dates for Willamette and Cascade hops for the 2010 and 2011 growing seasons. Harvest time points Early, Typical, and Late are shown for each cultivar and both locations. Farm I (F1) and Farm II (F2).

Table I: Target odor compounds in Cascade and Willamette hops

Compound Name	Odor Description
p-cymene	Solvent, gasoline, citrus(1)
$\alpha$ -pinene	Pine(8)
Geraniol	Floral, citrus, rose-like, flowery(3)
Humulene Epoxides*	Hay, grassy(3)
Limonene	Citrusy(8)
Citral	Lemon, bitter(3)
Farnesol	Flower Oil (1)
Geranyl Acetate	Rose (1)
Linalool	Flowery, fruity, floral, citrus, rose-like(3)
Methyl heptanoate	Strong fruity, orris root-like(8)
Citronellol	Floral, citrus, rose-like(3)
$\beta$ -pinene	Sharp terpene like pine, coniferous pine(8)
E, $\beta$ -Farnesene	Wood, citrus, sweet(1)
Caryophyllene	Woody, spicy, flower, turpentine, clove(3)
Humulene	Woody(26)
Myrcene	Herbs, metallic, resinous, spicy(3)

\* Purity < 80 %

Table II: Summary by harvest time point averages of hop samples from the 2010 and 2011 crops from Farm I and Farm II for hop acid characteristics

Cultivar	Harvest	H.S.I.	$\alpha$ -Acids (% w/w)	$\beta$ -Acids (% w/w)	Cohumulone (%)
Cascade	Early	0.202	8.3	6.4	35.9
		(0.007)	(0.9)	(0.9)	(2.6)
	Typical	0.209	9.9	6.6	38.9
		(0.030)	(1.3)	(0.7)	(2.7)
	Late	0.222	8.3	5.5	37.9
		(0.009)	(1.1)	(0.7)	(3.2)
Willamette	Early	0.225	5.6	4.0	32.7
		(0.042)	(1.3)	(0.8)	(1.8)
	Typical	0.226	6.3	3.9	34.7
		(0.033)	(0.7)	(0.6)	(2.8)
	Late	0.245	6.4	4.0	33.1
		(0.011)	(1.0)	(0.5)	(2.9)

Standard deviations are shown in parenthesis.

Table III: F-values and significance from Mixed Model ANOVA

Attribute	Cascade				Willamette			
	Farm	Harvest	FarmxHarvest	Farmx Harvestx Year	Farm	Harvest	FarmxHarvest	Farmx Harvestx Year
H.S.I.	0.54	5.37	0.25	5.83***	0.2	0.45	1.35	7.04
% $\alpha$ -Acids	0.01	5.69	0.47	6.08***	0.91	0.32	0.87	6.53***
% $\beta$ -Acids	0.059	3.682	1.826	2.296***	0.317	0.084	1.261	2.732*
% Cohum	0.76	3.16	0.24	23.26***	0.53	3.47	0.19	6.95***

Significance levels are indicated for F values at the 99.9% confidence level (\*\*\*), 99.0% level (\*\*), and the 95.0% level (\*)

Table IV: Oil content and profile for Willamette and Cascade hops harvested at three time points

Attribute	Cascade			Willamette		
	Early	Typical	Late	Early	Typical	Late
Oil (ml/100g)	1.21 <sup>b</sup>	2.13 <sup>a</sup>	1.98 <sup>a</sup>	0.70 <sup>c</sup>	1.65 <sup>b</sup>	1.81 <sup>a</sup>
$\alpha$ -Pinene <sup>1</sup>	0.01 <sup>b</sup>	0.02 <sup>a</sup>	0.02 <sup>a</sup>	0.00 <sup>c</sup>	0.01 <sup>b</sup>	0.01 <sup>a</sup>
$\beta$ -Pinene	0.07 <sup>b</sup>	0.18 <sup>a</sup>	0.17 <sup>a</sup>	0.02 <sup>c</sup>	0.09 <sup>b</sup>	0.11 <sup>a</sup>
Myrcene	4.73 <sup>b</sup>	11.46 <sup>a</sup>	11.21 <sup>a</sup>	1.18 <sup>c</sup>	5.91 <sup>b</sup>	7.73 <sup>a</sup>
Limonene	0.02 <sup>b</sup>	0.05 <sup>a</sup>	0.05 <sup>a</sup>	0.01 <sup>c</sup>	0.02 <sup>b</sup>	0.03 <sup>a</sup>
$p$ -cymene	0.01 <sup>b</sup>	0.01 <sup>a</sup>	0.01 <sup>a</sup>	0.00 <sup>c</sup>	0.01 <sup>b</sup>	0.01 <sup>a</sup>
Methyl Hep.	0.03 <sup>c</sup>	0.08 <sup>b</sup>	0.06 <sup>a</sup>	0.03 <sup>c</sup>	0.08 <sup>b</sup>	0.06 <sup>a</sup>
Linalool	0.03 <sup>c</sup>	0.09 <sup>b</sup>	0.07 <sup>a</sup>	0.01 <sup>c</sup>	0.08 <sup>b</sup>	0.10 <sup>a</sup>
Caryophyllene	0.40 <sup>b</sup>	0.89 <sup>a</sup>	0.67 <sup>a</sup>	0.91 <sup>b</sup>	1.45 <sup>a</sup>	1.35 <sup>a</sup>
E, $\beta$ -farnesene	0.65 <sup>b</sup>	1.06 <sup>a</sup>	0.93 <sup>a</sup>	0.54 <sup>b</sup>	0.98 <sup>a</sup>	1.04 <sup>a</sup>
Humulene	1.42 <sup>b</sup>	2.17 <sup>a</sup>	1.68 <sup>a</sup>	2.66 <sup>b</sup>	4.22 <sup>a</sup>	3.68 <sup>a</sup>
Citral	0.03 <sup>b</sup>	0.06 <sup>a</sup>	0.05 <sup>a,b</sup>	0.03 <sup>b</sup>	0.04 <sup>a</sup>	0.03 <sup>a</sup>
Geranyl Acetate	0.09 <sup>a</sup>	0.17 <sup>a</sup>	0.14 <sup>a</sup>	0.02 <sup>a</sup>	0.03 <sup>a</sup>	0.01 <sup>a</sup>
Citronellol	0.02 <sup>b</sup>	0.12 <sup>b</sup>	0.07 <sup>b</sup>	0.02 <sup>a</sup>	0.03 <sup>a</sup>	0.01 <sup>a</sup>
Geraniol	0.01 <sup>a</sup>	0.02 <sup>a</sup>	0.01 <sup>a</sup>	0.01 <sup>b</sup>	0.03 <sup>a</sup>	0.02 <sup>b</sup>
Hum. Epox. 1	0.09 <sup>a</sup>	0.05 <sup>a,b</sup>	0.03 <sup>b</sup>	0.07 <sup>a</sup>	0.11 <sup>a</sup>	0.06 <sup>a</sup>
Farnesol	0.02 <sup>a</sup>	0.02 <sup>a</sup>	0.02 <sup>a</sup>	0.01 <sup>b</sup>	0.03 <sup>a</sup>	0.04 <sup>a</sup>
Farnesene %	8.6 <sup>a</sup>	6.4 <sup>b</sup>	6.1 <sup>b</sup>	9.5 <sup>a</sup>	7.3 <sup>b</sup>	6.6 <sup>b</sup>
H/F ratio	2.39 <sup>a</sup>	2.06 <sup>b</sup>	2.04 <sup>b</sup>	4.98 <sup>a</sup>	4.28 <sup>b</sup>	3.91 <sup>b</sup>

<sup>a,b,c</sup>Means within a row with different letters are significantly different from one another at  $p < 0.05$

by Tukey's HSD. <sup>1</sup>Volatile compounds are expressed as mg/g of hops adjusted to 8% moisture content.

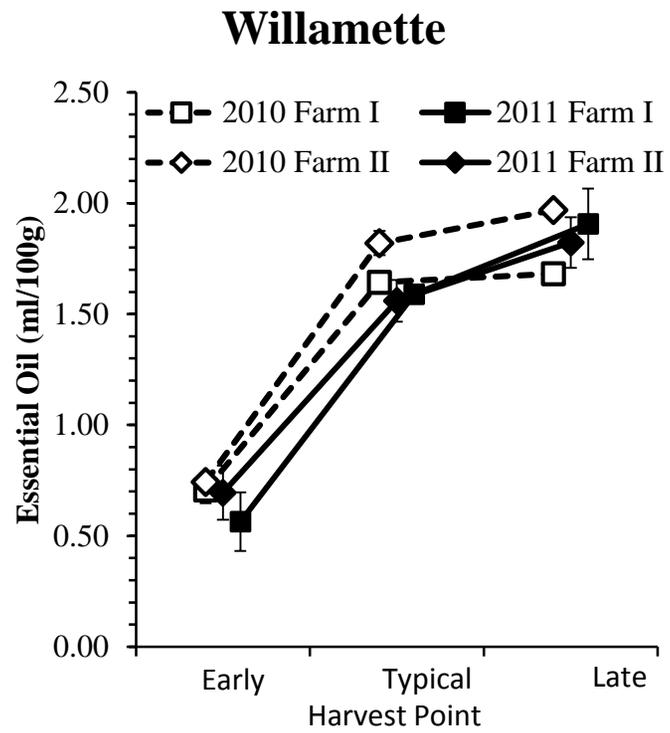
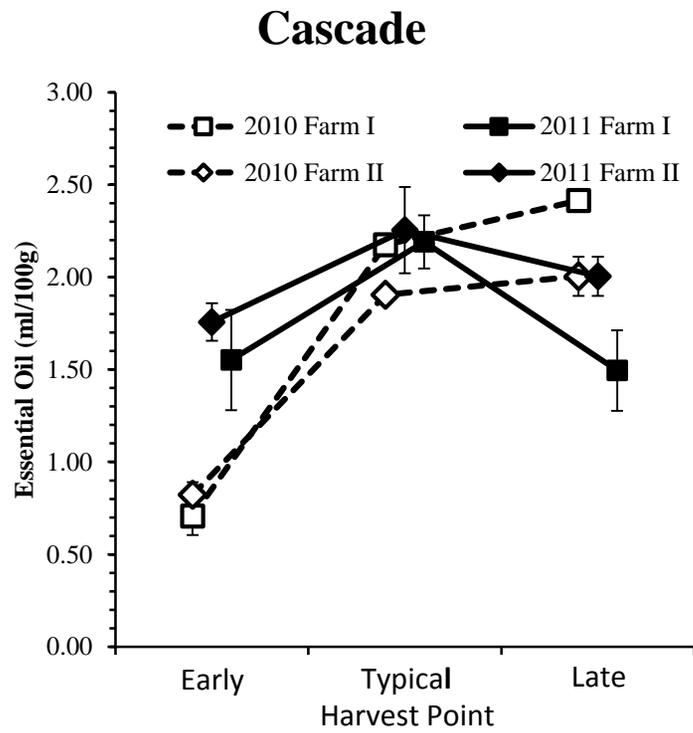


Figure 2: Essential oil

content of Willamette (left) and Cascade (right) hops at different harvest points by farm and year. Note, the more pronounced effect of year on Late harvested Cascade hops. Error bars represent standard deviation.

Table V: F-values and significance by treatments from Mixed Model ANOVA of Essential Oil Components

Attribute	Cascade				Willamette			
	Farm	Harvest	Farm x Harvest	Farm x Harvest x Year	Farm	Harvest	Farm x Harvest	Farm x Harvest x Year
Oil	2.07	150.67***	3.33	32.02***	6.55*	601.65***	0.41	6.62***
$\alpha$ -Pinene	0.02	184.41***	2.90	18.55***	18.84***	627.11***	1.61	6.94***
$\beta$ -Pinene	0.02	257.43***	4.00*	30.42***	7.71*	453.52***	0.4	9.17***
Myrcene	0.63	136.86***	3.96*	13.31***	2.89	173.16***	0.05	2.85*
Limonene	0.01	158.39***	2.64	21.37***	7.06*	380.51***	0.06	6.02***
$\rho$ -cymene	15.23***	11.24***	0.29	5.32**	2.74	206.97***	2.04	5.16**
Methyl Hep.	1.23	664.14***	10.54***	914.50***	68.33***	985.69***	16.66***	627.83***
Linalool	24.02***	232***	10.02***	32.72***	10.01**	616.79***	1.77	16.22***
Caryophyllene	1.95	28.8***	4.84*	7.79***	1.89	14***	4.41*	5.3**
E, $\beta$ -farnesene	5.22*	23.45***	5.10*	5.81***	0.00	25.69***	2.58	1.68
Humulene	2.23	18.01***	4.81*	7.08***	0.01	18.19***	3.6*	3.89**
Citral	0.46	7.35*	0.39	11.48***	2.7	11.15**	4.37**	16***
Geranyl Acetate	12.55**	25.38***	1.06	6.53***	1.02	1.32	7.23**	85.87***
Citronellol	6.17*	141.89***	0.15	184.80***	0.18	0.79	0.34	9.34***
Geraniol	0.08	0.94	1.21	3.98**	0.79	18.94***	1.63	18.53***
Hum Epox	9.90**	5.15*	3.79*	6.26***	0.17	0.193	0.1981	0.0018
Farnesol	0.76	2.9	0.24	10.91***	8.03**	20.28***	4.44*	8.83***
E, $\beta$ -farnesene %	33.10***	11.47***	16.64***	3.96***	1.25	9.51*	1.16	2.92*
H/C ratio	38.36***	0.52	1.16	17.41***	0.029	3.39	0.17	5.25**

Significance levels are indicated for F values at the 99.9% confidence level (\*\*\*), 99.0% level (\*\*), and the 95.0% level (\*).

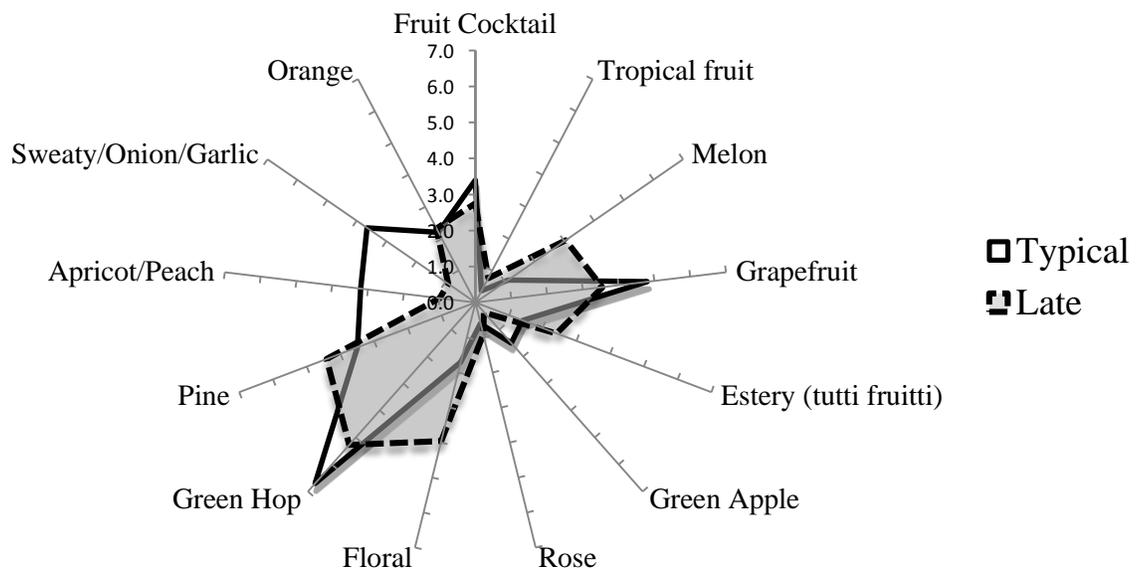


Figure 3: Sensory descriptive data based on one observation of two beers brewed with Cascade hops at Typical and Late harvest dates.

Table VI: Summary data of consumer acceptance scores for Typical and Late Harvest Beers

<b>Attribute</b>	<b>Typical</b>	<b>Late</b>
<b>Overall Liking***</b>	7.11 <sup>a</sup> (0.83)	6.26 <sup>b</sup> (1.61)
<b>Aroma Liking***</b>	6.92 <sup>a</sup> (1.31)	5.82 <sup>b</sup> (1.96)
<b>Flavor Liking**</b>	6.98 <sup>a</sup> (1.03)	6.23 <sup>b</sup> (1.68)

\*\*, \*\*\*Attribute Significant at  $p < 0.01$ , and  $0.001$ , respectively. Means within a row with different letters are significantly different from one another at  $p < 0.05$  by Tukey's HSD. Standard deviations are shown in parentheses below means. Scale: 1 = dislike extremely, 9 = like extremely. Standard deviations are shown within parenthesis.