AN ABSTRACT OF THE THESIS OF

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Title: Harvest Maturity of Cascade and Willamette Hops.

Abstract approved:

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Thomas H. Shellhammer

Hops *(Humulus lupulus* L.*)* are primarily used to provide specific characteristics to beer, such as bitterness, aroma, flavor, and microbial stability. The chemical composition of hops, relative to how they are used during the brewing process, dictates the expression of these characteristics. Of the raw ingredients that go into making beer, hops are perhaps the most costly. Considerable resources are required to grow quality hops, and therefore, brewers and hop growers alike have a common goal of obtaining the highest quality hops possible. However, quality can be a relative term. While it is commonly agreed upon that high brewing values, such as α-acids and essential oil content, and robust structural integrity are indicators of quality hops, there are many opinions of the ideal aroma.

Changes in the chemical composition of hops during plant maturation are a dynamic process requiring a comprehensive, in-depth chemical and sensory analysis in order to maximize the characteristics of interest to brewers. The complex aroma chemistry associated with hops in beer has been

a confounding variable for the practical brewer, and a deeper understanding of hop aroma development during cultivation is needed.

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 days until harvest. Hop acids did not change appreciably during plant maturation for the period examined, while hop oil content increased hyperbolically to a plateau as the hops aged on the bine. Increases in oil quantity were strongly correlated ($r > 0.90$) with increases in α-pinene, β-pinene, myrcene, limonene, methyl heptanoate, and linalool concentrations. For Cascade, α-pinene, β-pinene, myrcene, limonene, ρ-cymene, caryophyllene, E, β-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 Late and Typical harvests. For Willamette hops, α-pinene, β-pinene, myrcene, limonene, ρ-cymene, and linalool all increased between each time point. Caryophyllene, E β-farnesene, humulene, farnesol and citral all increased from Early harvest to Typical harvest but no difference was observed between Typical and Late.

Clear sensory differences were found between beers brewed with Typical harvest Cascade hops and Late harvest Cascade hops, in terms of difference testing, descriptive analysis and consumer preference tests

Harvest Maturity of Cascade and Willamette Hops

by
Daniel C. Sharp

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

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Daniel C. Sharp, Author

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Dr. Shaun Townsend assisted with experimental design, statistical model development, and hop sample acquisition. Dr. Yanping Qian assisted with preliminary GC-MS analysis of essential oils. Dr. Thomas Shellhammer offered guidance wherever it was needed…which was a lot.

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(1987-2006)
What's up man?

Harvest Maturity of Cascade and Willamette Hops

CHAPTER 1. LITERATURE REVIEW

Hops

The genus *Humulus* of the Cannabaceae family includes three species: *H. japonicas* Siebold & Zucc., *H. yunnanensis* Hu, and *H. lupulus* L.(*1*), better known as hops, which are cultivated in the temperate zones of the northern and southern hemispheres. However, only *H. lupulus* produces glandular trichomes (lupulin glands), which contain the important chemical components prized in brewing for adding flavor, aroma, and stability to beer. The 2011 statistical report prepared by the Hop Growers of America reports that over 28,219 metric tons of hops were sold from the 2010/2011 U.S. harvest(*2*).

Hops are a dioecious perennial climbing plant that when trained clockwise up a climbing support can reach heights in excess of 6 meters. The vegetative growth of hops is dictated by the amount of daylight and begins only after daylight hours exceed 13 hours. The main shoots, called bines, produce axil flowering shoots which in turn eventually develop strobiles, or hop cones, used in brewing. The strobiles consist of bracts and bracteoles connected to a strig and are only found on female plants. It is these bracts and bracteoles that hold the lupulin glands valued by brewers for the attributes they contribute to beer.

Hops in beer

Key chemical compounds extracted from the dried cones either by sophisticated preliminary processing steps, such as supercritical fluid CO_2 extraction, or by their direct addition to beer are responsible for much of the characteristic aroma and flavor of beer. The nature and nuance of these aromas and flavors are dictated in large part by the chemical compounds extracted, and in turn, the chemical composition of the hop from which they were derived. As such, the composition of the hops being used has a direct impact on not only the flavor and aroma of finished beer, but also the quality of finished beer due to the bacteriostatic and storage stability properties of hops. The chemical constituents that contribute to most of the hop bitterness and aroma of beer are found primarily in the resin and essential oil, respectively, of the lupulin glands of hops. The resin portion of hops contains a class of chemical compounds known as α-acids and β-acids, the former being precursors to the principle bittering compounds found in beer(*3*), while the aroma compounds are attributed primarily to the essential oil fraction.

Development and biosynthesis of hop aroma compounds

Essential oils accumulate and are biosynthesized in glandular trichomes located primarily on the bracteoles and leaves. These glandular trichomes are divided into two types based on their morphology; peltate and bulbous. Lupulin glands are primarily the peltate type. Although the details are not clear, lupulin glands increase in size with bud and flower development and

their numbers are most likely established early in leaf development (*4*). The spatial and temporal expressions of phytochemical biosynthetic genes responsible for the biosynthesis of essential oil have yet to be investigated, but are thought to be related to the developmental stages of glandular trichomes. These stages are divided into a growth phase and a biosynthetic secretory phase, prior to entering a storage stage(*5*).

Of the hundreds of compounds found in the complex mixture of hop oil, relatively few are actually formed via biogenesis during hop cone development while the majority are formed via secondary reactions. Terpenes are a diverse class of lipids with more the 20,000 species(*6*) and make up the majority of the essential oils of hops, although not in their entirety. Much of the compositional chemistry the essential oil found in hops is well studied and in-depth reviews are available(*7*)(*8*)(*9*)(*10*)(*9*). The majority of aromatic compounds in hops are derived from a few key parent terpenes and it is thought that they are biosynthesized by the plant as a defense against insects(*11*), while the oxygen-containing terpenes, known as terpenoids, function as membrane constituents, photosynthetic pigments, electron transport carriers, growth substances, and plant hormones. Terpenes contain carbon atoms in multiples of 5 ranging from 10-40 carbon atoms and are composed of isoprene units (C_5H_8) formed through biosynthetic pathways within the plant. While a single isoprene unit is the only hemiterpene, oxygen-containing hemiterpenes, or hemiterpenoids such as isovaleric acid and 3-methyl-2-buten-1-ol, are more

diverse and have can contribute to hop aroma. Monoterpenes (C_{10}) are the product of two isoprene units and include α-pinene, β-pinene, myrcene, ρ-cymene, and limonene among others, while the monoterpenoids include linalool, citronellol, geraniol, and geranyl acetate. Similarly, sesquiterpenes and the oxygen-containing sesquiterpenoids are comprised of 3 isoprene units and include caryophyellene, E, β –farnesene, humulene, farnesol and humulene epoxides. Terpenes or terpenoids larger than C_{15} backbones are not generally found in hop oil or are not considered to be volatile or to contribute directly to aroma due to higher molecular weight.

The biosynthesis of terpenes are formed not from isoprene units directly but rather from 3-methyl-3-butenylpyrophosphate, or isopentenyl pyrophosphate (IPP). Next, IPP is formed via a series of enzyme catalyzed reactions from acetyl-CoA and malonyl-CoA, through the well-studied mevalonate (MVA) and plastidial non-mevalonate (DOXP) pathways(*12*). IPP is isomerized by an enzyme catalyzed reaction with isopentenyl diphophatase-Δ-isomerase to form dimethylally-pyrophosphate (DMAPP)(*13*). Condensation of DMAPP with IPP yields geranyl pyrophosphate (GPP), the parent compound responsible for many montoerpenes, or neryl diphosphate (NDP), the parent compound of cyclic monoterpenes, such as limonene. Reaction of GPP with yet another IPP molecule gives rise to farnesyl pyrophosphate (FPP), the parent molecule for sesquiterpenes and cyclic sesquiterpenes. Elimination of pyrophosphoric acid from FPP yields the sesquiterpene beta-

farnesene. According to Roberts and Stevens(*14*), synthesis of GPP occurs only after resin synthesis is nearly complete and amounts of IPP units are in excess of that needed for resin synthesis.

The distribution of essential oils in hop cones is not uniform and is dependent on the specific tissue. GC-MS analysis of hexane extracts from different plant tissues of Nugget hops at different growth phases showed that myrcene was found exclusively in trichomes(*15*) and increased in content over a period of four weeks after flowering. Linalool, a terpenoid characteristic of many North American aroma type hops, was found mainly in the floral tissue of the hop plant but only in trace amounts. The sesquiterpenes, humulene and caryophyllene, were not specific to trichomes and were found in almost identical ratios in trichomes leaves and flowers, which suggest that they may be formed from the same cyclase enzyme. Although, Okada et. al (2001) (*16*) found that the FPP synthase gene expression was strongest in the lupulin fraction, it was also present in leaf, stem, and non-lupulin fraction of hops(*17*). This suggests that FPP synthase may be responsible for high terpene accumulation in lupulin glands.

Specific genes and catalytic enzymes responsible for the terpene metabolism pathways of essential oil, bitter acids, and prenylflavonoids have been identified in hops. Work by Wang et al. suggests that the genes designated HIMTS2 and HIMTS3 lead to the formation of myrcene in hop trichomes and linalool synthase enzyme in flower tissues respectively. A gene

designated HISTS1 was primarily responsible for humulene and caryophyllene products and were found in leaves, flowers, and lupulin glands. HIMTS1 and HIMST2 levels were highest in trichomes from cones four weeks after flowering(*15*).

Essential Oil Analysis

The composition of essential oil is extremely complex; there are over 450 identified chemical compounds and suggestions that the total number of existing compounds exceeds 1000(*18*). Indeed, as suggested by the sheer number of possible chemical combinations, it has been difficult for hop analysts to provide a simple list of chemical species that can predict the aroma impact of hops on a finished beer due to low sensory detection thresholds in the parts per trillion range, synergistic effects of compounds(*19*) and varying brewing techniques for imparting aroma. In addition, pre-harvest conditions, post-harvest processing and storage conditions and varietal differences influence the composition of the essential oil fraction (*20*) all of which further confounds the complexity of hop aroma analysis and the development of a simple gauge for hop aroma such as the Hop Aroma Unit(*21*). However, many of the compounds found in hop oil exist in quantities well below sensory detection thresholds and therefore may not contribute to the aroma profile of hops, particularly as selectively extracted and diluted by beer.

Early investigations of essential oils by Chapman(*22*) mention analytical work as early as 1822, and imply even earlier research. For in-depth reviews on the aroma chemistry of essential oil from hops and in beer see Sharpe and Laws(*10*), Schönberger and Kostelecky(*8*) and Briggs(*20*), et al. In order to effectively control 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 as hop maturation prior to harvest. Effects on the essential oil composition from different cultivars due to post-harvest aging and storage of hops have been well-studied(*23*) (*24*). Additionally, pre-harvest environmental conditions such as fertilizer treatments have been found to have little influence on varietal uniformity of essential oil composition(*25*), although overall yield may be affected.

The importance of harvest timing in relation to the essential oil fraction of hops was generally noted in 1939 by Rabak (*26*) who commented that “ripe or fully matured hops are characterized by a full and agreeable aroma and an abundance of sticky lupulin.” However, the focus of Rabak’s paper was not the aroma or essential oil properties of the hops but rather the soft resins, specifically the bittering acids. In terms of chemical composition, hop aroma compounds were not characterized with much specificity in regard to principle chemical components and the ripening of hops until the advent of gas chromatography and later work by Howard and Slater(*27*) in 1958. They found that the total oil content of Fuggles hops increased dramatically over a 6-week

ripening period and was attributed primarily to a sixty fold increase in the hydrocarbon fraction containing the four most abundant compounds found in hop oil: myrcene, farnesene, caryophyllene and humulene. Later, work by Murphey and Probasco(*28*) indicated that alpha acid concentration peaked at 22-24% dry matter (typical harvest point), while an increase in essential oil content, attributed to myrcene synthesis, extended well beyond this harvest point which suggested that changes in harvest timing could considerably affect the aroma quality of hops. In regard to harvest timing and its effect on the aroma quality of dry-hopped beers, a recent study(*29*) showed that Hallertau Mittlefrueh hops harvested later in the season and the subsequent beers were rated higher in sensory analysis. Results from these studies warrant an in-depth chemical analysis of hop aroma compounds and their relation to harvest timing.

There is a large body of research attempting to identify important aroma-active hop compounds using various techniques such as solid phase micro extraction (SPME)(*30*),(*31*), solvent extraction(*32*), traditional cohobative hydro-distillation(*21*),(*33*), gas chromatography-olfactory (GC-O)(*34*), and odor dilution techniques (*35*).

Statistical Models

The experimental design of planned agricultural experiments provides many challenges to the researcher. Uncontrollable factors such as weather or

seasonal effects, in addition to the numerous controllable factors make it difficult to properly constrain or account for confounding variables. Therefore it is critical that careful consideration go into the definition of experimental units, the type and levels of factors, and the proper response variables so that the question of interest can be thoroughly and confidently answered.

It is difficult to draw valid and useful conclusions from poorly designed experiments. The time and cost commitments of most studies are substantial, particularly with agricultural experiments due to the time-sensitive logistics, long temporal scales or cycles, and large spatial scales. Well-designed experiments that allow for efficient data analysis and allow for strong and valid conclusions are the result of a thorough understanding of statistical methodologies and a deep knowledge of the field of study.

Of particular importance is the definition of experimental units, their assumed representation of the population for which inferences are made and how treatment factor levels are considered. Fixed effects and random effects are two types of factors that are typically considered in an experimental design. A fixed effect is a factor with predetermined factor levels, from which inferences are made. A fixed model contains only fixed factors, or factors that are predefined and determined or chosen by the researcher. Because the factors are predetermined, or chosen and not randomly sampled, inferences from the model inferences can only be made from the specific factor levels chosen for the study.

A random effect consists of a factor with levels randomly selected (or which represent a random selection) from a population. This lends a greater scope of inference to the researcher and allows an extension of conclusions or inferences to all levels of the population. The use of a model that includes both a fixed and random factor (mixed model) allows the researcher to infer about a larger population subjected to fixed effects. Therefore, inferences can be drawn not only regarding the controllable factor levels used in the experiment, but also regarding the population from which the levels were sampled.

A principle difference between a mixed-model design and fixed-effect design is the way in which effects are tested for significance. The mean squared residual is used as the error term to test between factor levels for fixed effect designs; whereas between factor levels in a mixed model design are tested using error terms based on the co-variation of random sources of variation in the design, or the factors levels themselves. This design, while more conservative, allows for a greater scope of inference than if a farm location were to be considered fixed.

CHAPTER 2. HARVEST MATURITY OF CASADE AND WILLAMETTE HOPS

Introduction

The soft resin, namely the α-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 α-acids are the precursors to the main source of hop-derived bitterness in beer. Pre-harvest conditions, post-harvest processing and varietal factors influence the composition of the essential oil fraction as well as α-acid content (*20*). 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 (*18*). However, many of these compounds exist well below sensory detection threshold quantities and are therefore unlikely to significantly contribute to the aroma profile of hops. 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 controlling for aroma characteristics in hops as a raw ingredient in beer(*34*, *36*, *37*). Although the list of compounds that are likely to contribute to hop aroma is quite long when considering all cultivars, the list becomes somewhat more manageably 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 conditions during cultivation or storage.

It is well known that hops are influenced by factors such as daylight, growing conditions, and post-harvest processing conditions, at that the quality and character they lend to beer are primarily determined before arriving at the brewery. What is not well known is how the stage of maturity or time of harvest influences the aroma/flavor qualities of hops. Therefore, investigations into the cultivation, harvest and post-harvest handling of hops are critical to understanding the agronomic factors that affect hop quality. In order to effectively control the hop aroma in beer, it is important to investigate both extrinsic factors such as processing operations and handling and storage of hops as well as intrinsic factors such hop maturation prior to harvest. The aim of this work was to investigate the effect of hop harvest date on α- and β- acid content and profile, total oil content, and essential oil profile of Cascade and Willamette hops grown in the Willamette Valley during the 2010 and 2011 harvest years. A list of target compounds of interest was generated based on examination of previous studies investigating the contribution of hop aroma compounds in beer (*38*), hop aroma compound analysis (*21*), and preliminary analysis using GC-MS capabilities within the Department of Food Science and Technology at OSU. For practical reasons, the list of compounds selected was refined based on the initial 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 or not 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 due to its simplicity and the practicality of relating results to the standard method used by most hop analysis labs.

Experimental

Hops and Cultivation

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

Three samples (~600-700g undried) of each hop cultivar were randomly obtained at each farm for each of the 3 time-points for the 2010 and 2011harvests (3 samples x 2 cultivars x 2 farms x 3 time-points x 2 years). A total of 72 samples were collected. Samples of hops from the Early and Late time points were hand-picked from the lower, middle and upper side arms 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 commercial kilning. 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.

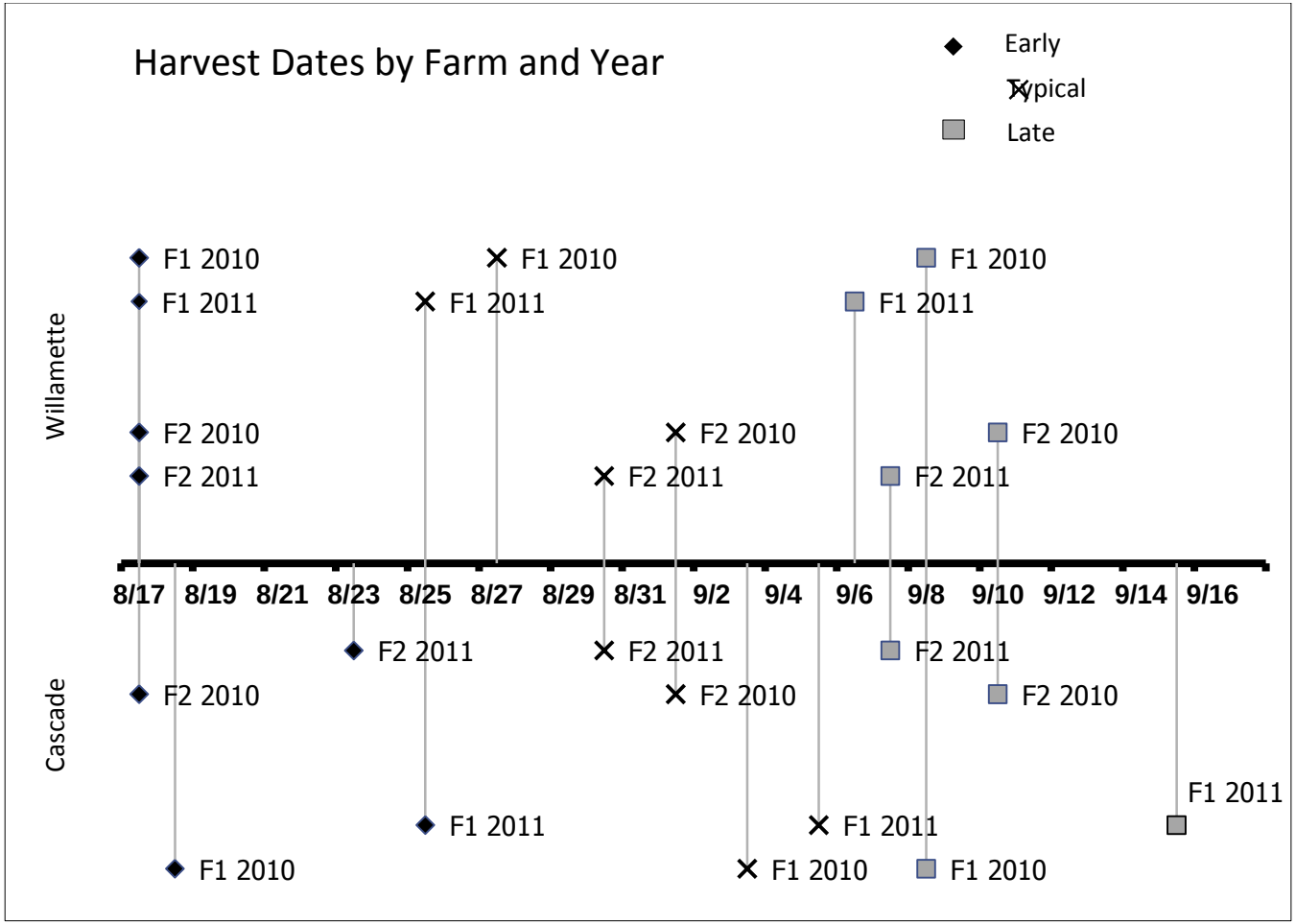

Figure 1: Harvest dates for Willamette and Cascade hops for the 2010 and 2011 growing seasons. Harvest time points Early, Typical, and Late are shown for each cultivar and both locations. Farm I (F1) and Farm II (F2).

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%, 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 preparation and long-term storage. Representative samples were obtained from each mini bale immediately prior to chemical analysis according to the American Society of Brewing Chemists sampling protocol for hops (*39*). 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, α- and β-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. All results were normalized to 8% moisture content prior to statistical analysis.

Moisture

Moisture content was determined according to the ASBC method Hops-4C(*40*) to account for variations in drying. All compositional data were normalized using the moisture data to a “standard” 8% w/w moisture content.

Hop acids measurements

The effect of harvest maturity on the bittering components of hops was determined spectrophotometrically using ASBC method Hops-6(*41*). Hop storage index was measured according to ASBC method Hops-12. Concentrations of α-acids and β-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(*42*). Chromatographic determination was performed using an Agilent 1200 series HPLC system (Boblingen, Germany) equipped with a 100 x 4.6mm Kinetex C18, 2.6 µm column (Phenomenex, Torrance, California, USA) held at a constant temperature of 40ºC. The flow rate was 1.3 ml/min with a 7 µL 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(*43*) 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 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. In this way, increased sample throughput and increased sample preservation was achieved. 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 5ºC 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 ASBC Hops-17(*44*) using a modified temperature program optimized for adequate separation of target compound peaks. A Hewlet 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 60º C held for 1 minute, ramped at a

rate of 3ºC/minute to 175ºC and held for 10 min, then ramped at 3ºC to 230ºC 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 200ºC and 250º C respectively. Quantification of compounds was determined by using an internal standard method with 2-octanol. Area integration reject was set to 1 mV. The compounds of interest analyzed by GC-FID are summarized in Table 1. 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 1).

Table 1: Target odor compounds in Cascade and Willamette hops

Compound Name	Odor Description
ρ-cymene	Solvent, gasoline, citrus(*45*)
α-pinene	Pine(*46*)
Geraniol	floral, citrus, rose-like, flowery(*47*)
Humulene Epoxides*	hay, grassy(*47*)
Limonene	Citrusy(*46*)
Citral	Lemon, bitter(*47*)
Farnesol	Flower Oil (*45*)
Geranyl Acetate	Rose (*45*)
Linalool	Flowery, fruity, floral, citrus, rosewood-like, aniseed, terpenic, rose-like, hoppy(*47*)
Methyl heptanoate	Strong fruity, orris root-like(*46*)
Citronellol	Floral, citrus, rose-like(*47*)
β-pinene	Sharp terpene like pine, coniferous pine(*46*)
E, β –Farnesene	Wood, citrus, sweet(*45*)
Caryophyllene	Woody, spicy, flower, turpentine, clove(*47*)
Humulene	Woody(*35*)
Myrcene	herbs, metallic, resinous, spicy(*47*)

* Purity < 80 %

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 mixed regression of the following model: response = Farm + Harvest + Farm*Harvest + Farm*Year*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 an infinite population of possible levels. In this study, the Farm variable was considered to be a random variable assigned to each hop sample (block). The scope of inference was considered to be hop farms within 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.

Sensory Analysis

Preliminary 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 commercial interest, brewing trials were not conducted using Early harvested hops and therefore sensory analysis was conducted on the Typical and Late harvested samples only.

Sample Preparation

Beers were brewed commercially by an Oregon craft brewery and were identical, aside from each having been brewed with hops harvested at different points in time. In order to highlight hop character, a pale ale hopped to 40 IBU's with 5.0% ABV was used. Beer samples were received from the brewery in 22 L stainless steel kegs and stored at 1° C until testing.

Difference testing

Difference testing 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. Random presentation order was assigned to each panelist. Panelists were asked to identify the sample (Late harvest) that was different from the other two (Typical harvest).

Descriptive analysis

The descriptive sensory analysis consisted of 12 trained panelists, some of whom had been extensively involved with previous 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 previous training sessions for beer aroma with a focus on hop derived aromas; 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 analyze the beers and for descriptive analysis and only analyzed each beer once. There were no repeated measures by any panelist for the descriptive analysis.

Consumer Acceptance

Sixty-two consumers were recruited from the Corvallis community. 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 top 3 most preferred styles of beer. Consumer acceptance of beers hopped with 2010 Typical and Late harvested Cascade hops was carried out at the OSU Sensory Science Lab. 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.

Experimental Design and Statistical Analysis

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, Guleph, Canada. Statistically significant attributes were subjected to post-hoc analysis using Tukey's HSD test at the 95% confidence interval ($P < 0.05$).

Results

Table 3 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 years to account for season to season variation. Values from the dichotomous key proposed by Kenny(*48*) for characteristics which aid in the identification of certain hop cultivars are shown for comparison.

Table 2: 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	Hop Storage Index	α-Acids (% w/w)	β-Acids (% w/w)	α + β (% w/w)	Ratio α/β	Cohumulone (%)	Coluplulone (%)
Cascade	Early	0.202	8.3	6.4	14.6	1.3	35.9	44.6
		(0.007)	(0.9)	(0.9)	(1.3)	(0.2)	(2.6)	(6.9)
	Typical	0.209	9.9	6.6	16.5	1.5	38.9	54.1
		(0.030)	(1.3)	(0.7)	(1.9)	(0.1)	(2.7)	(1.7)
	Late	0.222	8.3	5.5	13.8	1.5	37.9	52.9
		(0.009)	(1.1)	(0.7)	(1.4)	(0.3)	(3.2)	(1.0)
Kenny 1990(*48*)		<0.300	<10.0	--	< 15.0	< 1.2	--	--
Willamette	Early	0.225	5.6	4.0	9.6	1.4	32.7	47.9
		(0.042)	(1.3)	(0.8)	(2.1)	(0.1)	(1.8)	(2.0)
	Typical	0.226	6.3	3.9	10.1	1.6	34.7	45.4
		(0.033)	(0.7)	(0.6)	(1.2)	(0.1)	(2.8)	(9.4)
	Late	0.245	6.4	4.0	10.5	1.6	33.1	50.3
		(0.011)	(1.0)	(0.5)	(1.5)	(0.2)	(2.9)	(2.2)
Kenny 1990(*48*)		<0.300	<10.0	--	<15.0	1.2< x <1.8	< 40.0	--

Standard deviations are shown in parenthesis.

Mixed Model Analysis of Variance

Significant 3-way interactions among time of harvest within season, farm, and harvest year existed for all non-volatile parameters of interest for both Cascade and Willamette hops (Table 3). For all responses a significant 3-way, and in some cases 2-way interaction were found. That is, significant differences were found for H.S.I., α-acids, β-acids, cohumulone contents depending on time of harvest, the originating farm, and the harvest year. Even though Cascade and Willamette were analyzed separately, significant variation in the compounds of interest was observed due to variability contributed by the interactions of time of harvest, farm, and harvest year. These results suggest that differences in year-to-year growing conditions or practices may contribute significant variation to the model, or that substantial differences among dates within a harvest time window contributed to larger sample variation year to year for given time points. However, no significant differences were found regarding the main effects, or regarding the interaction of location and harvest for either cultivar.

Table 3: F-values and significance from Mixed Model ANOVA

	Cascade				Willamette			
Attribute	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
% α-Acids	0.01	5.69	0.47	6.08***	0.91	0.32	0.87	6.53***
% β-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 (*).

Hop Storage Index

Harvest time had a clear influence on the hop storage index (H.S.I.) for both hop varieties. As hops were left on the bine, the H.S.I. of the hop acids in the cones increased (Figure 2). All H.S.I. values were between 0.20 and 0.28. An H.S.I. greater than or equal to 0.30 is generally considered to be an indication of aged hops.

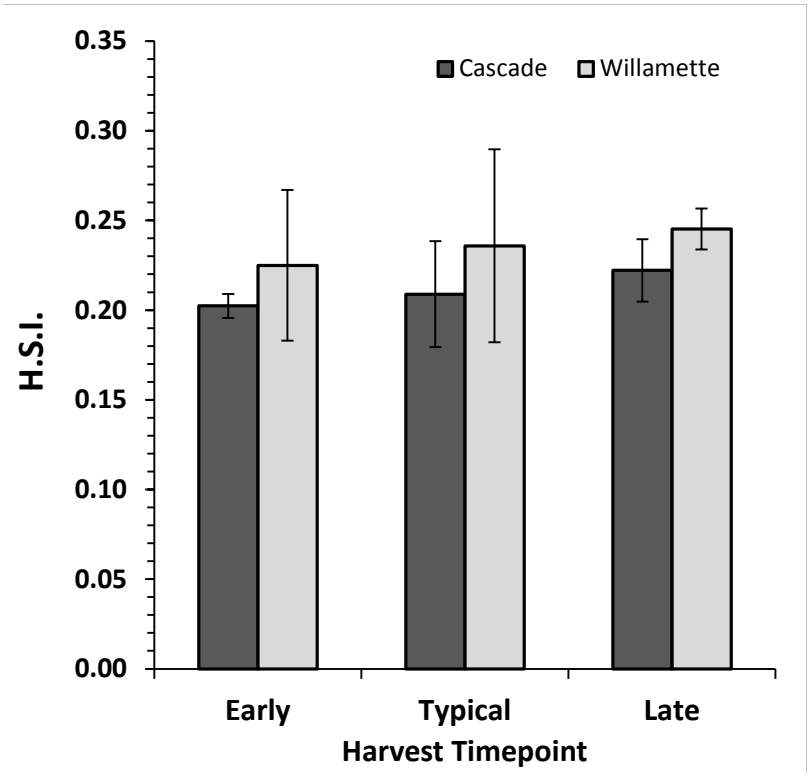

Figure 2: Average hop storage index of Cascade and Willamette hops harvested at different time points. Average of all reps from all farms for 2010 and 2011 harvest years (n=12). Error bars represent standard deviation.

Alpha Acids

Alpha acid percentages are expressed as a per mass basis of hops normalized to 8% moisture. There were no significant main effects for α-acid content, β-acid content or cohumulone content for Cascade or Willamette hops (Table 3 and Figures 3 & 4). However, a farm by time by year interaction (p-values<0.0001) was observed for both hops and all attributes (Table 3). Variation in the data are likely due to 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.

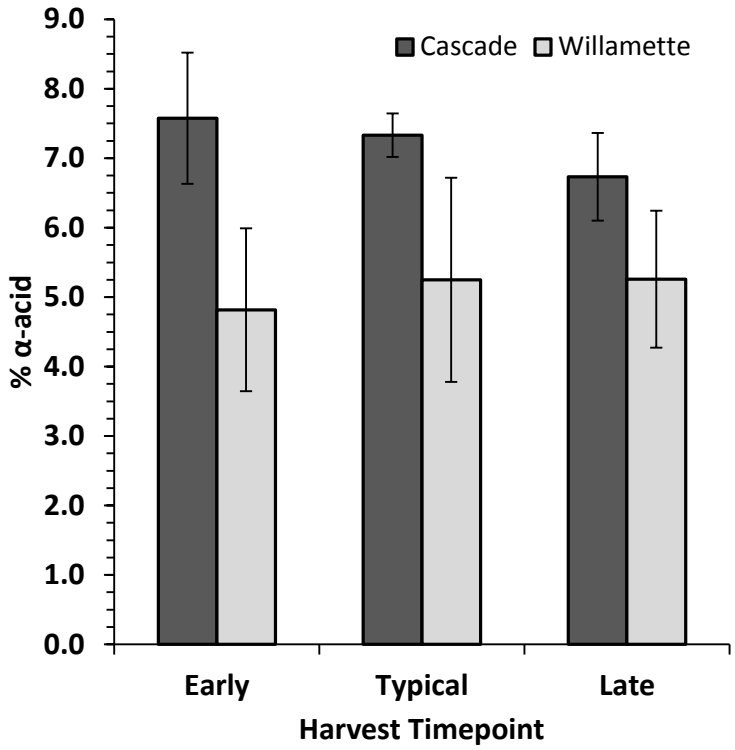

Figure 3: Average α-acid content of Cascade and Willamette hops at different harvest time points Average of all reps from each farm for 2010 and 2011 harvest years (n=12). Error bars represent standard deviation.

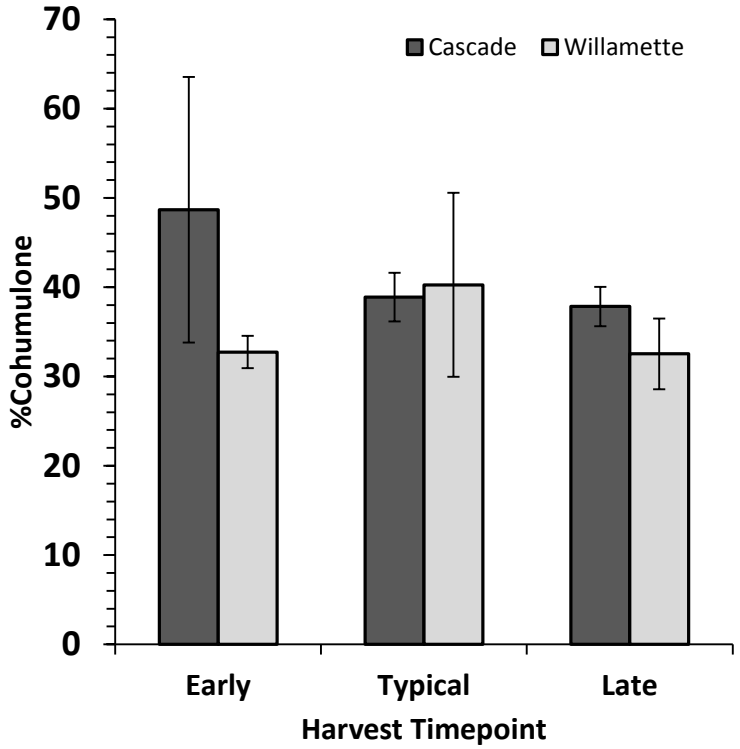

Figure 4: Average cohumulone content of Cascade and Willamette hops harvested at different time points. Average of all reps from all farms for 2010 and 2011 harvest year (n=12). Error bars represent standard deviation.

Table 4: F values and significance by treatments from Mixed Model ANOVA of Essential Oil Components

	Cascade				Willamette			
Attribute	Farm	Harvest	Farm×Harvest	Farm× Harvest× Year	Farm	Harvest	Farm×Harvest	Farm× Harvest× Year
Oil	2.07	150.67***	3.33	32.02***	6.55*	601.65***	0.41	6.62***
α-Pinene	0.02	184.41***	2.90	18.55***	18.84***	627.11***	1.61	6.94***
β-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***
ρ-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, β-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***
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 (*).
Oil components are expressed as mg/g of hops unless noted otherwise

Total Essential Oil

A significant difference of the 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) as shown in Table 4. Early harvested Cascade and Willamette hops had lower average oil content compared to Typical and Late harvested hops (Table 5). However, the oil content decreased for Cascade hops for the later harvest date in 2011 as compared to the later harvest date for 2010 (Figure 5). This may have been due to unrepresentative sampling for 2011 Late harvested Cascade hops from Farm I.

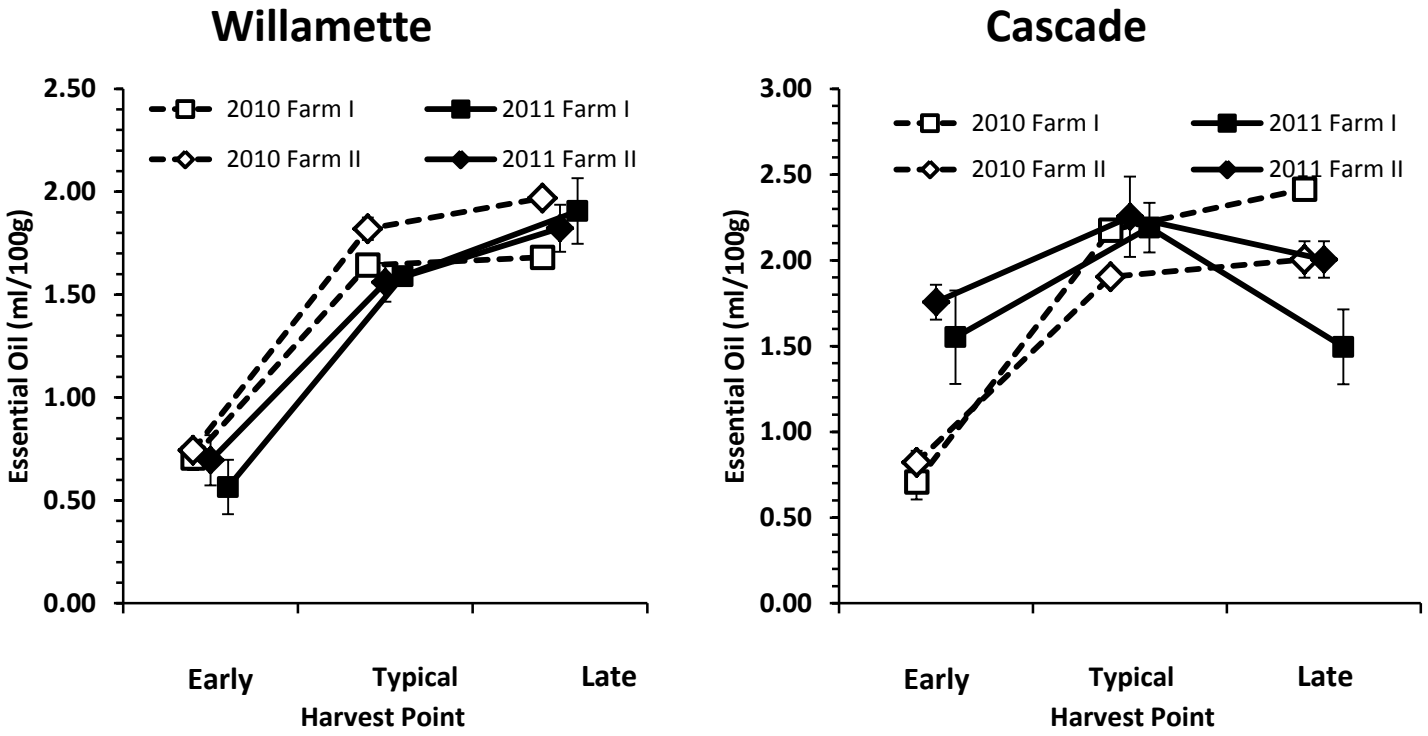

Figure 5: Essential oil content of Willamette (left) and Cascade (right) hops at different harvest points by farm and year. Note, the more pronounced effect of year on Late harvested Cascade hops. Error bars represent standard deviation.

Essential Oil Profile

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% percent moisture (Table 5). Significant main effects, 2-way and 3-way interactions were found for most of the compounds for both Willamette and Cascade hops (Table 4). An increase in oil quantity was strongly correlated ($r>0.90$) with α-pinene, β-pinene, myrcene, limonene, and linalool contents. The 3-way interactions are illustrated for linalool in Figure 6. A similar trend was observed for the montoerpenes that were correlated with oil increase mentioned above. Results from Tukey's HSD ($p < 0.05$) for volatile components by harvest timing are shown in Table 5. For Cascade, α-pinene, β-pinene, myrcene, limonene, ρ-cymene, caryophyllene, E, β-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 Late and Typical harvests. For Willamette hops, α-pinene, β-pinene, myrcene, limonene, ρ-cymene, and linalool all increased between each time point. Caryophyllene, E, β-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.

Additionally, the percentage of farnesene of the total oil and humulene/farnesene ratios are shown. A farnesene content above 1% is considered characteristic of Willamette and Cascade hops while a humulene/farnesene ratio below 3 distinguishes the latter from the former(*48*). A representative GC output of Early and Late harvested Cascade hops (2010) from Farm I is included in the Appendix to illustrate the overall differences observed in this study in terms of essential oil profile.

Table 5: Oil content and profile for Willamette and Cascade hops harvested at three time points.

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

[a,b,c]Means within a row with different letters are significantly different from one another at $p<0.05$ by Tukey's HSD. [1]Volatile compounds are expressed as mg/g of hops adjusted to 8% moisture content.

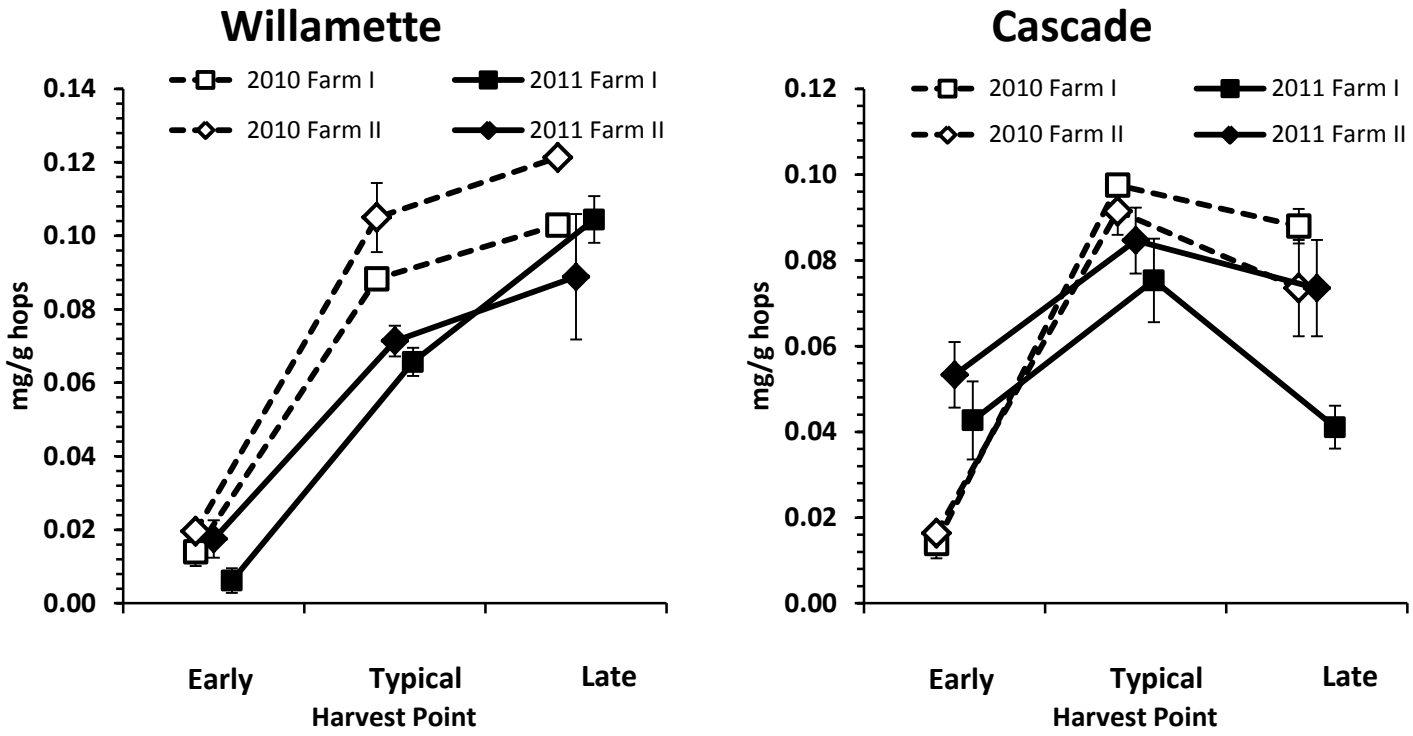

Figure 6. Linalool content of Willamette (left) and Cascade (right) hops from different harvest points by farm and year. Note, the more pronounced effect of year on Late harvested Cascade hops.

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.

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 7). One must not place too much emphasis on this single outcome as it has not been replicated. Sweaty and onion/garlic notes found in the beers made with Typical harvest hops may be attributed to trace sulfur containing compounds(*49*). The higher floral notes found in beer brewed with Late harvested hops may be attributed to higher

Consumer liking

Overall liking, flavor, and sample means plus significance values are provided in Table 6. 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 and as compared to the beer hopped with Late harvest hops (Table 6). Consumer demographics are shown in Appendix D.

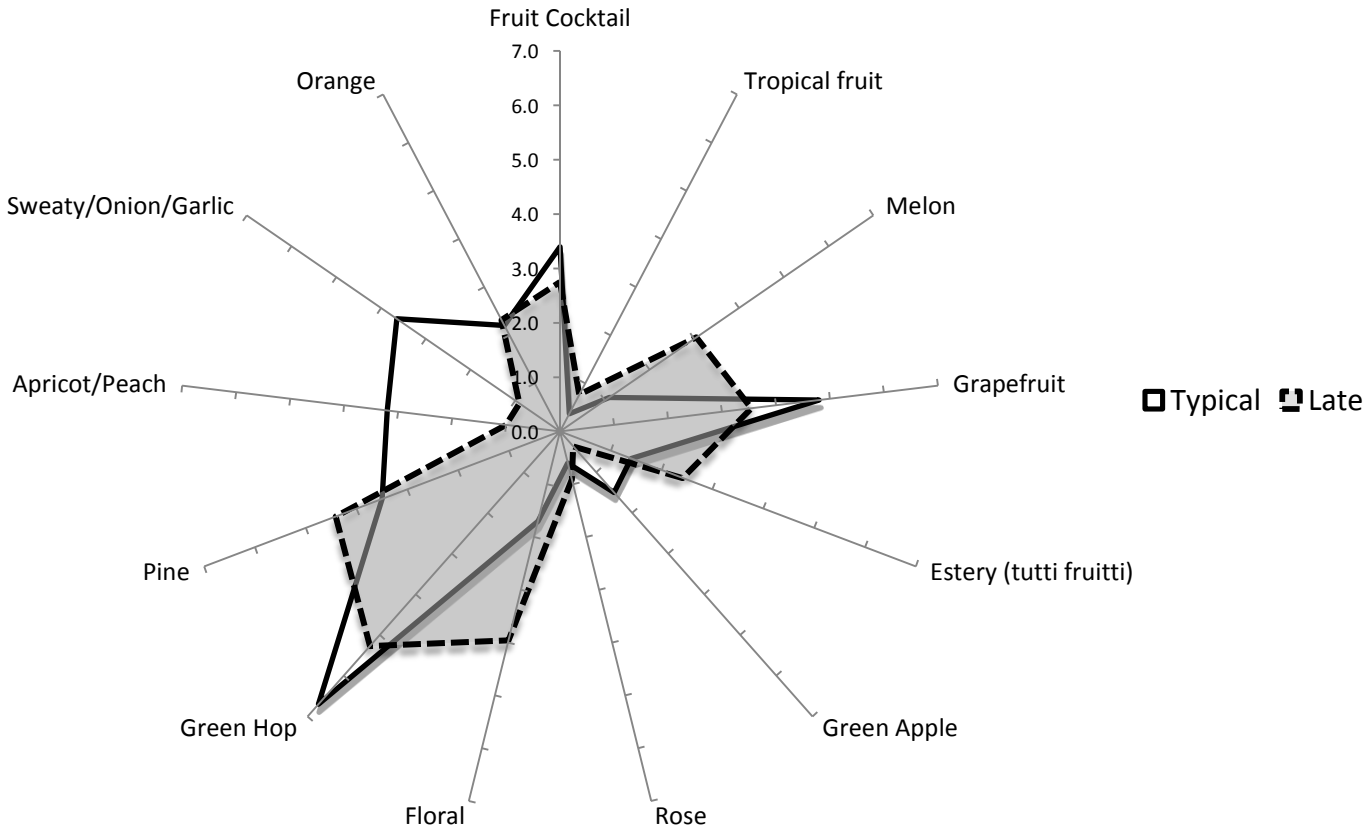

Figure 6: Sensory descriptive data based on one observation of two beers brewed with Cascade hops at Typical and Late harvest dates.

Table 6: Summary data for consumer acceptance testing of Typical and Late Harvest Hopped Beers

Attribute	*Typical*	*Late*
Overall Liking* **	7.11 [a]	6.26 [b]
(SD)	(0.83)	(1.61)
Aroma Liking* **	6.92 [a]	5.82 [b]
(SD)	(1.31)	(1.96)
Flavor Liking **	6.98 [a]	6.23 [b]
(SD)	(1.03)	(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.

Discussion

When it comes to bittering acids, brewers are intimately aware of the variations in the bittering acid content of their hops, and are able to adjust hop dosing to obtain a consistent product, with respect to bitterness intensity, using supplier-provided specifications of the hop acids. However, few quality assurance measures are taken or are available when it comes to correcting for 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 (Figure 1). More importantly, sensory data from beers brewed using Cascade hops from Farm I at Typical and Late harvest dates displayed

significant differences both from descriptive analysis and consumer preference.

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. In general, however, montoerpenes and linalool did increase over time and may contribute to a different aroma profile as other compounds such as humulene, caryophyllene and farnesene had little to no increase. 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 inconsistencies between the sampling intervals across farms, cultivars, and years as shown in 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. Hop Storage Index can be a useful measure of the quality of a hop sample in terms of the bittering acids, but fails to tell the entire story of a hop’s quality from an aromatic perspective. A similar measure for overall quality of hop aroma is has yet to be determined and due to the complexity of hop aroma, a single measure will likely be a useful indicator of aroma properties. Furthermore, hop quality can be a relative term

and uniformly negative quality indicators such as isovaleric acid (cheesy aroma), discoloration, and shattering of hop cones due to late picking or over-drying should be considered. The latter indicator is particularly important since shattering leads to a loss of lupulin glands and ultimately decreased harvest yields per acre of hop.

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. The earlier the harvest date the greener the color of the hop cones. A yellowing and eventual browning was observed corresponding to later harvest dates. These observations could have commercial significance since brewers often use color as an indicator of healthy and/or high quality hops when considering hop purchase.

The importance of how specific compounds affect the sensory characteristics of beer cannot be ignored. It is clear that hop harvest date has a significant effect on beer flavor and which may ultimately be the most representative measure of the effects of harvest timing of hops on beer. 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(*29*). However, differences in hopping

regimes (late hopping vs. dry hopping), hop variety, beer style, and sensory testing methods most likely contribute to the differences. Also, results from a study examining the effects of ageing hops prior brewing showed that both Cascade and Hallertau Mittelfrueh hops benefited from moderate aging to maximize aromas, yet excessive aging lead to more severe losses in aroma than Hallertau Mittelfrueh hops(*23*). Perhaps similar “aging” phenomena occur while hop cones are still on the bine.

Selection of hop oil compounds of interest were based on literature review as well as preliminary examination of GC-MS data. It is recommended that exploratory gas chromatography – olfactory (GC-O) work be carried out to obtain a more comprehensive list of odor impact compounds coupled with mass-spectroscopy for compound identification. However, the complexity of the oil composition data makes interpreting the nature and magnitude of this difference difficult if one examines just the instrumental data.

Conducting more controlled brewing trials on selected hop samples and conducting in-depth sensory research would further aid in identifying important compounds in hops that attribute to the aromatic properties of beer. Non-oil hop constituents may also contribute to hop aroma properties in beer and would further refine and enhance a list of target aroma markers in determining the aromatic contribution of hops to beer.

CHAPTER 3. FUTURE WORK

The results of the 2 years studying the effects of harvest maturity on the chemical composition of the brewing qualities of Cascade and Willamette hops has shown that the number of days to harvest of hops affects its chemical composition and in turn its quality. However, a major drawback in this study was the lack of a quantitative predictor variable other than time. Dry matter data were collected to investigate the relationship between dry matter of the hops at the time of harvest and response variables. Unfortunately, the measurements were greatly affected by relative humidity or residual moisture from rainfall. As such, the results of the dry matter measurement were inconsistent and unrepresentative of the true dry matter of the plant to be useful as predictor variable.

Through consistent sample collecting and measurement procedures a more representative measurement and therefore a potentially better indicator of maturity may be possible. For example, one possible predictor variable may include morphological changes throughout hop plant development

Additionally, more intensive brewing trials and sensory analysis would help elucidate practical impacts of hop maturity on the sensory attributes of beer. Because hop oils are so complex, it is difficult to notice changes in the chemical composition unless careful attention is being paid to target analytes. It is possible that certain chemical changes may go unnoticed if they are not included in the list of target analytes. For this reason, exploratory analysis

using GC-MS technology would be advantageous to help track changes in ripeness. Investigation into other response variables as a function of hop maturity may prove insightful. Recent investigations into the glycoside content of hops suggest these otherwise non-volatile, bound aroma molecules may contribute to beer aroma after fermentation or acid induced hydrolysis during brewing.

Extending the idea of locality and timing of harvest, the design of a multi-year, multi-location study of hops such as in the comparison of the same cultivars would elucidate further the impact of growing conditions on the hops.

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APPENDICES

A. Mean values for Cascade and Willamette Hops from 2010.

	Cascade						Willamette					
	Farm I			Farm II			Farm I			Farm II		
	Early	Typical	Late	Early	Typical	Late	Early	Typical	Late	Early	Typical	Late
α-Acids (%)	7.09	7.41	7.45	6.79	7.67	6.86	5.80	5.48	4.43	4.27	5.90	3.99
Cohumulone (%)	36.74	42.41	40.13	37.35	40.19	35.37	32.96	36.29	36.22	36.81	31.90	31.15
HSI	0.20	0.21	0.23	0.21	0.17	0.22	0.27	0.24	0.26	0.18	0.24	0.19
Oil (ml/100g)	0.71	2.18	2.42	0.82	1.90	2.00	0.79	1.82	1.68	1.64	1.82	0.69
α-Pinene	0.00	0.03	0.03	0.01	0.02	0.02	0.00	0.01	0.01	0.01	0.01	0.00
β-Pinene	0.04	0.21	0.22	0.04	0.18	0.17	0.02	0.11	0.11	0.09	0.11	0.02
Myrcene	2.53	11.74	12.59	2.15	10.47	11.96	1.48	7.16	7.02	6.55	8.03	1.32
Limonene	0.01	0.05	0.06	0.01	0.05	0.05	0.01	0.03	0.03	0.03	0.03	0.01
ρ-cymene	0.00	0.01	0.01	0.00	0.01	0.01	0.00	0.01	0.01	0.00	0.01	0.00
Methyl Hep.	0.04	0.15	0.16	0.05	0.12	0.03	0.05	0.14	0.11	0.12	0.05	0.01
Linalool	0.01	0.10	0.09	0.02	0.09	0.07	0.01	0.10	0.10	0.09	0.09	0.02
Caryophyllene	0.51	0.98	0.86	0.36	1.15	0.67	1.13	1.57	1.32	2.00	1.50	0.88
E, β-farnesene	0.60	1.18	1.13	0.35	1.10	0.97	0.66	0.92	0.90	1.19	1.13	0.60
Humulene	1.54	2.29	2.06	1.21	2.72	1.69	3.24	4.22	3.78	5.46	3.75	2.68
Citral	0.02	0.03	0.03	0.02	0.03	0.06	0.04	0.05	0.04	0.06	0.03	0.03
Geranyl Acetate	0.07	0.10	0.11	0.04	0.17	0.17	0.04	0.05	0.05	0.07	0.00	0.00
Citronellol	0.05	0.26	0.28	0.02	0.22	0.00	0.05	0.07	0.04	0.07	0.00	0.00
Geraniol	0.02	0.02	0.02	0.00	0.02	0.01	0.00	0.02	0.01	0.00	0.02	0.02
Hum Epox 1	0.02	0.01	0.02	0.05	0.01	0.03	0.03	0.04	0.04	0.01	0.02	0.04
Farnesol	0.14	0.10	0.14	0.43	0.08	0.05	0.03	0.08	0.06	0.02	0.04	0.00

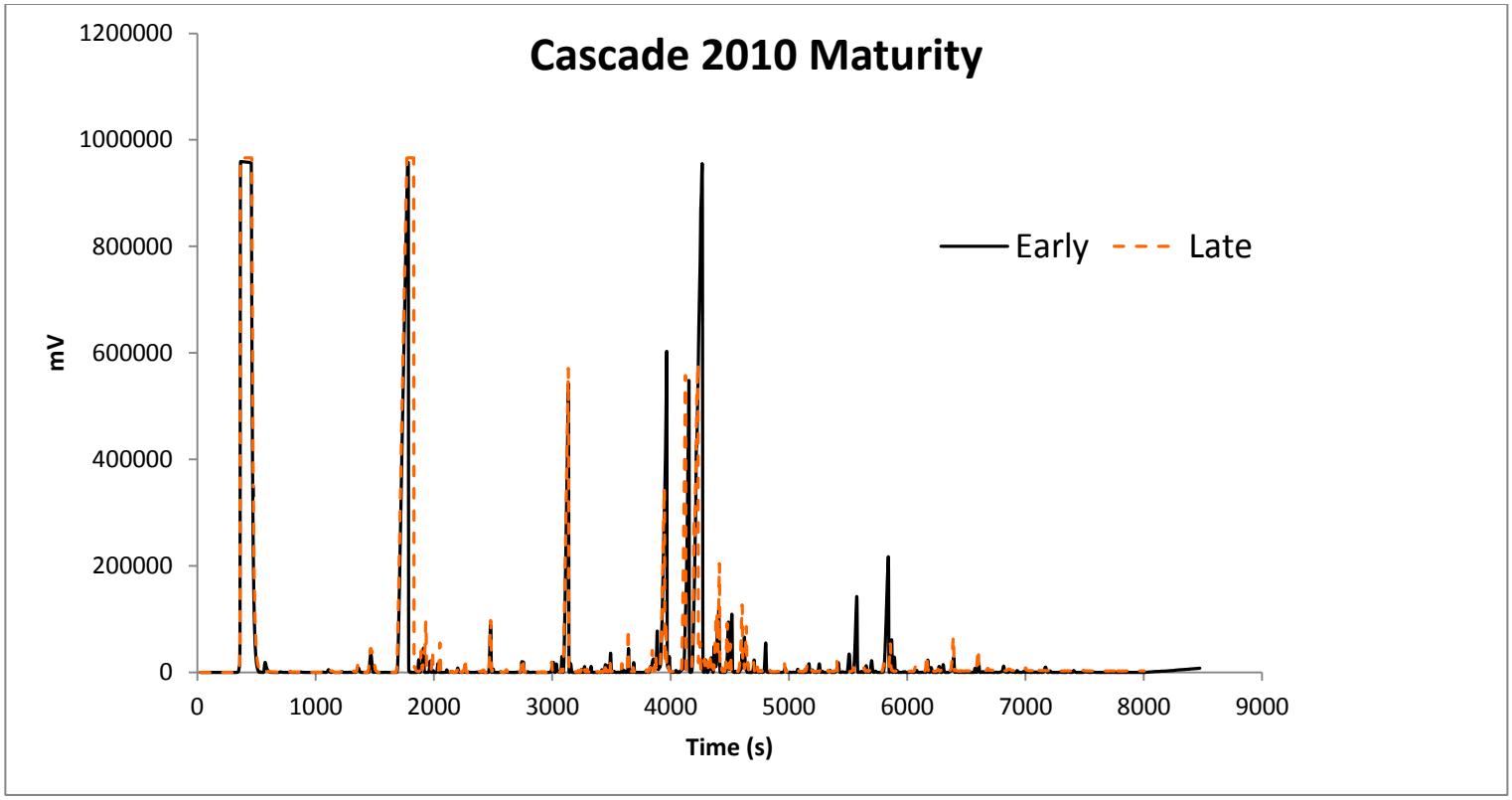
Essential oil components are expressed as mg/g of hops unless noted otherwise.

B: Mean values for Cascade and Willamette Hops from 2011.

	Cascade						Willamette					
	Farm I			Farm II			Farm I			Farm II		
	Early	Typical	Late	Early	Typical	Late	Early	Typical	Late	Early	Typical	Late
α-Acids (%)	8.04	7.17	5.98	8.38	7.09	6.86	3.78	5.52	4.60	3.99	4.10	5.90
Cohumulone (%)	37.08	37.04	34.45	32.61	35.90	35.37	32.07	34.45	30.02	31.15	31.41	31.90
HSI	0.20	0.22	0.21	0.20	0.24	0.22	0.19	0.22	0.24	0.19	0.26	0.24
Oil (ml/100g)	1.55	2.19	1.49	1.76	2.25	2.00	0.56	1.59	1.91	0.69	1.56	1.82
α-Pinene	0.01	0.02	0.02	0.01	0.02	0.02	0.00	0.01	0.01	0.00	0.01	0.01
β-Pinene	0.11	0.17	0.12	0.11	0.17	0.17	0.01	0.07	0.11	0.02	0.07	0.11
Myrcene	7.71	12.44	8.30	6.55	11.17	11.96	0.38	4.89	7.84	1.32	5.07	8.03
Limonene	0.03	0.05	0.03	0.03	0.04	0.05	0.00	0.02	0.03	0.01	0.02	0.03
ρ-cymene	0.01	0.01	0.01	0.01	0.01	0.01	0.00	0.01	0.01	0.00	0.01	0.01
Methyl Hep.	0.02	0.03	0.01	0.02	0.03	0.03	0.00	0.03	0.05	0.01	0.04	0.05
Linalool	0.04	0.08	0.04	0.05	0.08	0.07	0.01	0.07	0.10	0.02	0.07	0.09
Caryophyllene	0.53	0.72	0.50	0.43	0.70	0.67	0.43	1.15	1.10	0.88	1.08	1.50
E, β-farnesene	0.99	1.10	0.68	0.67	0.85	0.97	0.34	0.96	0.98	0.60	0.86	1.13
Humulene	1.56	1.87	1.29	1.39	1.80	1.69	1.61	3.79	3.42	2.68	3.42	3.75
Citral	0.06	0.08	0.06	0.05	0.07	0.06	0.02	0.03	0.03	0.03	0.03	0.03
Geranyl Acetate	0.09	0.20	0.11	0.14	0.22	0.17	0.00	0.00	0.00	0.00	0.00	0.00
Citronellol	0.01	0.01	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Geraniol	0.01	0.02	0.01	0.02	0.02	0.01	0.03	0.05	0.03	0.02	0.05	0.02
Hum Epox	0.06	0.05	0.04	0.23	0.14	0.03	0.18	0.20	0.16	0.04	0.21	0.02
Farnesol	0.12	0.08	0.07	0.42	0.22	0.05	0.00	0.02	0.03	0.00	0.02	0.04

Essential oil components are expressed as mg/g of hops unless noted otherwise.

C. Representative Gas Chromatography Chromatograms of Essential Oils of Early and Late Harvested Cascade Hops.



D. Sensory Demographics

Percent(actual) Demographics			
	All ages	21-29	30-69
Total	100%(62)	89%(55)	11%(7)
Male	69%(43)		
Female	31%(19)