ENVIRONMENTAL INFLUENCE ON HOP ALPHA ACID CONTENT IN SONOMA COUNTY

By:

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Introduction

The research objective of this paper is to examine whether certain environmental factors influence the amount of alpha acids in a hop, *Humulus lupulus*. The method involves soil sampling and testing for correlations between soil nutrient uptake rates and alpha acid percentages. Under examination for this study is seven different hop plants planted by individual local hop farmers of the Northern California Hop Growers Alliance, headquartered in Sonoma County, California. Each hop plant analyzed are of the Cascade hop variety and were grown in five different locations across Sonoma County. Two of the hop plants were planted in 2015, while the remaining five hop plants were planted in 2016. The farms of each hop plant are located at Sonoma (BiRite Farms), Cloverdale (Eric's Farm), Healdsburg (Fogbelt Farm), and Sebastopol (Warm Spring Wind Farm and Redwood Hill Farm).

Soil samples are taken at each farm to analyze how soil nutrient supply rates impact the production of alpha acids on a hop plant. Plant nutrient uptake rates were measured using Plant Root Simulator (PRS) probes, provided by Western Ag Innovations. Data on alpha acid percentages for each hop sample were quantified by the Chemistry Department of Sonoma State University. Linear regression is then used to test for correlations between soil nutrient supply rates with the alpha acid levels pertaining to each hop plant. Linear regression is also used to estimate alpha acid to nutrient supply response rate to compare differences between post- and pre-growing nutrient supply rates in the soil. Geographic features at each farm, such as slope aspect, are also examined to understand the microclimate at each farm. These locational properties are compared to understand possible differences in hop development based on their respective location. This paper attempts to examine the relationship between the environment and alpha acid production in hops.

Literature Review

Overview of Hops

The hop plant, *Humulus lupulus*, is a member of the *Cannabinaceae* plant family (Koplin, 2008). Hops are perennial deciduous plants that die in the winter and rapidly grow bines in the spring (Hops Industry, 2018). They are robust and have a permanent rootstock, which can survive for over 30 years (Godin, 2017). Female hops are a necessary ingredient in beer-crafting (Sirrine, 2010). A single hop specifically refers to a flower cone on a hop plant (Godin, 2017). These cones are used in the brewing process because they contain lupulin glands that are filled with resins and essential oils, which all contain properties that are favored for distinct taste profiles in beer (Koplin, 2008). These chemical compounds include alpha acids, beta acids, and essential oils (Godin, 2017). The overall taste, aroma, and perceived bitterness of beer are determined by the combination of these chemical compounds (Kneen, 2017). Hops high in alpha acids are used for bittering while hops low in alpha acid content are used for flavoring and aroma (LaShell, 2014). Alpha acid levels are unique to each hop variety and are variable depending on annual growing conditions (Eyck, Gehring, 2015). The Cascade hop variety on average has an alpha acid content of five to seven percent (Carter, et al. 2017, Darby, 2011). Alpha acid percentage is an important quality parameter for marketing in the hops industry and is expressed as a percentage of the weight of a hop cone at 10% moisture (Pavlovic et al. 2012). Alpha acid percentage is also used to determine a finished beer's International Bittering Units (IBU). Although hops are grown across the nation, Washington, Oregon, and Idaho are currently the dominant regions of hop production in the United States (Sirrine, 2010). Understanding what affects the production of alpha acids on a hop plant is vital information to hop farmers, brewers, and consumers.

Climate Suitability

Hop plants are indigenous to the temperate regions of the Northern Hemisphere and are now typically farmed in both hemispheres between the 35th and 55th latitudes (Sirine, 2010). Hops require ample moisture in the spring followed by warm summers (Hiller, Gingrich, Haunold, 2017). During their growing season, which spans from June to August, hops prefer an average temperature of approximately 12.78 to 18.89 ^{o}C and summers with day lengths of at least 15 hours for more direct sunlight. They also have a chilling requirement—about one to two months of winter temperatures below 4.4^{o}C —and they need a minimum of 120 frost free days for flowering development (Kneen, 2017). This list of growing conditions makes Sonoma County a suitable location for hop farming. Sonoma County is located at 38 degrees north of the equator, has an average summer temperature of 15 ^{o}C, an average winter temperature of 9.56 ^{o}C, and on average receives a maximum of about 14.833 hours of sunlight daily in mid-June, which is only slightly lower than the preferred at least 15 hours of sunlight daily for hop plants (WRCC, 2016, Snyder et al. 2008). Sonoma County also has a Mediterranean dry-summer climate, which means that Sonoma County experiences nearly all its annual precipitation in the winter months (Chistopherson, 2010). This is beneficial to hop plants because dry weather towards the end of the growing season decreases the possibility of mildew or aphid attacks (Morehead, Vossen, 2017).

Slope aspect contributes to the microclimate of a location. Equatorial-facing slopes tend to receive much more solar radiation on average than nonequatorial-facing slopes (Warren, 2010).

Consequentially, in the northern hemisphere south-facing slopes tend to have a warmer and dryer microclimate. This relationship between slope aspect and solar insolation becomes more extreme with higher latitudes. Deciduous crops growing on south-facing slopes bloom earlier and thus have a higher probability of being afflicted by freeze damage (Snyder, 2000). There has not been any research done on the effects of slope aspect directly affecting hop plant maturation nor alpha acid production in hops.

Soil Nutrient Demand and Amendments

According to most of the literature on ideal hop plant soil conditions, the important soil nutrients for hop farming include the following: nitrogen (N), phosphorus (P), potassium (K), magnesium (Mg), calcium (Ca), boron (B), zinc (Zn), manganese (Mn), and sulfur (S) (Darby, 2011). Quality hop plants with a high yield largely result from having sufficient nutrients in the soil (Gingrich, 2017). The nutrient requirement of a hop plant can be measured in the hop nutrient uptake (Gingrich, Hart, Christensen, 1994). For instance, hops are typically stored and purchased in 200-pound bales (Eyck, Gehring, 2015). After the first year on average, an 8- to 10-bale/acre cone yield can take up 100 to 150 lbs/acre of N, 20 to 30 lbs/acre of P, and 80 to 150 lbs/acre of K (Darby, 2011). For farmers, annual nutrient amendments applied to the soil must replace the nutrients removed by the hop plants to maximize continued yield.

There is limited research linking the quantities of soil nutrients directly to the production of alpha acid percentages. However, there are some known effects of soil nutrient imbalances to a hop plant that may indirectly influence the production of alpha acid levels (Shapiro, 2017). For example, excessive Ca can induce Mg and K deficiencies, zinc deficiencies result in poor cone production, and excessive nitrogen can reduce cone alpha acid levels (Gent, et al. 2018). Nutrient deficiencies are shown in the hop plant, but it is unclear exactly what concentrations of each nutrient will cause nutrient imbalances. For instance, low K levels can produce weakened bines and low N levels can cause yellowing in the leaves of the hop plant.

Analyzing Environmental Influence of Alpha Acid Content in Hops

Similar studies on hop plants have been conducted in the Northwest and Northeast regions of the United States, and in Slovenia (Darby, 2011, Pavlovic et al. 2012); no similar studies in California have been identified thus far. In Slovenia, a correlational study from 1994-2009 was conducted comparing the weather parameters temperature, sunlight, precipitation, and relative humidity on Aurora hops (Pavlovic et al. 2012). This study found that different combinations of these weather parameters occurring at different time periods in the growing season can be linked to alpha acid production. Specifically, this study found high correlations of these weather parameters significantly impacting alpha acid growth during the 25th to 32nd week of the year. Another study in Slovenia attempted to create a mathematical model for predicting the hop alpha acid content, using the sum of effective temperatures and rainfalls from the second germination after spring pruning until technological maturity of hop cones (Srečec et al. 2013). However, the results from this paper were inconclusive.

Hop research in the United States has mainly been conducted in Washington, Oregon, Vermont, and Colorado. In these studies, soil testing or foliar analysis was conducted to measure soil nutrient availability to choose appropriate fertilizer amendments to improve the soil for hop growing (Brown, 2018). Performing soil tests on a hop-yard is a useful procedure to measure exactly what nutrients are contained in the soil. However, there is limited literature about preferred soil nutrients in hop farming directly relating to optimal production of alpha acids in hops.

There is no known literature or previous studies that have attempted to correlate soil nutrient uptake rates with alpha acid percentages in hops. In general, correlational studies are important because they are a useful tool in identifying casual relationships between two quantitative variables (Hale, 2011). Correlations do not imply causation, but causation does imply correlation. Thus, correlational studies help to rule out certain hypothesis and act as a precursor step to conducting more conclusive experimental methods.

Methods

In the Field at Each Farm

To analyze soil nutrient supply rates, a representative soil sample was taken around each individual hop plant at their respective farm. Using a soil sampler probe and a random stratified sampling method, soil samples were taken at each corner of each square foot surrounding the plant, starting with the hop plant directly in the center of the first measured square foot (McFarland, 2013). This was done to obtain a reasonable representative sample of the soil around the plant. This resulted in taking approximately twenty sample cores around each plant. Many soil cores were taken to reduce variation in soil nutrients that the hop plant uptakes. Each core spanned from 6 to 12 inches deep into the soil, since the roots of a full-grown hop plant may penetrate about 15 inches deep into the soil (Carter, et al. 2011). Each core was then immediately dropped and stored in a plastic five-quart bucket.

Each bucket was then labeled with the name of its representative hop plant and then brought back to the Geography lab at Sonoma State University for soil testing. While at each farm, qualitative observations of slope aspect and steepness were recorded.

In the Lab: PRS® Probe Analysis

To analyze soil nutrients in each sample bucket, Plant Root Simulator (PRS) probes were used. These PRS® probes are effective at imitating the roots of a plant to quantify the nutrient supply rates (PRS Technology, 2018). The PRS® probes are ion exchange resin membranes held in plastic supports that are easily inserted into soil to measure ion supply in situ with minimal disturbance. While buried in soil, they attract and absorb the cations and anions contained in the soil. Nutrient supply rates are not exactly an effective measure of soil nutrient availability for a given volume, however, they provide a more accurate representation of actual nutrient supply to the plant. The probes were provided as a grant for this undergraduate research project by Western Ag Innovations, a private agronomic research company headquartered in Saskatoon, Saskatchewan, Canada.

The procedure used to analyze soil with the PRS® probes is titled the "Saturated Paste PRS® Probe Sandwich Method," and is used to provide a standardized measure of soil ion supply rate for reference soils that have been dried and ground. First, each bucket of soil is mixed thoroughly to guarantee an even distribution of soil nutrients in our sample. Then 100 mL of deionized water for every 300 g of soil is gradually and uniformly added until ideal conditions of saturation. The soil and water are then left to equilibrate for 60 minutes. After this, the four PRS® probes for each soil sample are placed on wax paper. Then, approximately 5 mL of saturated soil is applied to the membrane of each side of the probes. Waxed paper is then used to press or "sandwich" the saturated soil slabs to ensure there is good membrane to soil contact. The four PRS® probes with each sample are then incubated for 3 hours. In this time, the PRS® probes collectively tested nutrient uptake for the following nutrients in the soil: nitrate (NO_3-), ammonium (NH_4+), K, P, Ca, Mg, S, Zn, B, Mn, cadmium (Cd), aluminum (Al), iron (Fe), lead (Pb), and copper (Cu).

After 3 hours, the probes were cleansed thoroughly with deionized water and a hard-bristled brush. After properly cleaning the probes for each sample, they were directly moved and stored in Ziploc bags with labels pertaining to their respective farm location. The PRS® probes were then shipped back to Western Ag Innovations for analysis. The soil test results containing the nutrient supply rates were then reported back to me in an Excel spreadsheet via email.

Testing for Correlations

Simple linear regression is used to find evidence of soil nutrient uptake impacting the production of alpha acids in hops. Using a Microsoft Excel, linear regression was used to test for correlations between nutrient supply rates and maximum alpha acid percentages. This was done with nutrient supply rates as the predictor variable and alpha acid percentages as the response variable. Each nutrient was analyzed separately to see if any individual nutrient was associated with alpha acid percentages. The maximum alpha acid percentages were calculated using an original high-performance liquid chromatography method developed in the Chemistry Department at Sonoma State University.

Two data points of maximum alpha acid percentage were removed from the statistical analysis portion of this paper. Redwood Hill Farm's sample points were removed because there was an unknown complication in quantifying the alpha acid content of his hops that resulted in significantly lower alpha acid percentages. This data point was treated as an outlier and was removed from the analysis. Eric's Farm's 2015 sample points on hop alpha acid percentages were never quantified because the samples were never obtained from their source. This resulted in five reliable sample points for analysis.

Results

The results from each iteration of soil sampling is organized into Figure 1 below.

			PRS(tm)-probe supply rate (micro grams/10cm^2/burial length)													
Sample	**NO3-N**	**NH4-N**	**Ca**	**Mg**	**K**	**P**	**Fe**	**Mn**	**Cu**	**Zn**	**B**	**S**	**Pb**	**Al**	**Cd**	**MaxAA%**
April 4 2017																
Redwood Hill Farm	131	2	566	162	362	13.1	1.4	1.8	0.3	0.8	0.2	32	0	5.3	0	4.89
Warm Spring Wind Farm	85	1	639	177	120	5.8	1.1	1.8	0	0.8	0	26	0.3	3.5	0	7.51
BiRite Farms	58	0	871	236	52	7.4	2	1.1	0.1	1.1	0.6	53	0	9	0	6.78
Eric's Farm	6	0	311	83	41	1.3	0.9	0.1	0.1	0.3	0.4	8	0.1	5.8	0	5.4
Fogbelt Farm	190	1	1405	150	119	1.4	2.1	6.1	0.8	0.8	1.4	482	0.1	10.1	0	6.23
BiRite Farms (2015)	31	0	477	169	66	2.1	1.1	6	0	0.6	0.1	38	0	3.1	0	5.97
Eric's Farm (2015)	25	0	446	73	42	2.2	1.2	0.2	0.1	0.3	0.7	6	0	6.9	0	N/A
Septmember 2 2017																
Redwood Hill Farm	44	7	406	100	186	8.7	2	2.8	0.5	0.7	0.9	35	0.1	7	0	4.89
Warm Spring Wind Farm	30	3	1253	313	97	7.2	3.3	3.9	0.3	1.3	1	312	0.9	8.6	0	7.51
BiRite Farms	34	1	918	223	53	6	2.5	12.3	0.2	1.4	0.4	53	0.1	8.1	0	6.78
Eric's Farm	26	1	503	90	36	0.9	0.8	2.1	0.7	0.1	0.1	21	0.1	2	0	5.4
Fogbelt Farm	190	4	1200	174	162	0.6	3.1	16	1.7	2.4	0.2	527	0.2	4.8	0	6.23
BiRite Farms (2015)	14	3	610	155	66	5.1	1.2	11.1	0.3	1.3	0.2	47	0.1	2.9	0	5.97
Eric's Farm (2015)	82	0	732	111	39	1	1.2	5.1	1.2	0.3	0.3	40	0	5.6	0	N/A

Figure 1: The nutrient supply rate is the net rate of nutrient ion adsorption by the PRS® probe. The units are expressed as the weight of nutrient adsorbed per surface area of ion-exchange membrane over time (micrograms of nutrient/10cm^2 ion-exchange membrane surface area/time of burial). The time of burial was three hours. Maximum alpha acid percentage (MaxAA%) for each sample of hops was calculated using an HPLC procedure. The units are expressed as the percent weight of alpha acid content out of the total weight of the hop at 10% moisture (% w/w).

In Microsoft Excel, the alpha acid content of each hop sample was correlated with their respective soil sample nutrient supply rates from September of 2017. Each element was examined individually. This was done to see what relationship each individual soil nutrient has on the production of alpha acid content on a hop plant. For example, the strongest correlation found was with Mg and nutrient supply rates from the post-growing season soil samples, which produced a correlation coefficient value of 0.997. This can be seen below in figure 2.

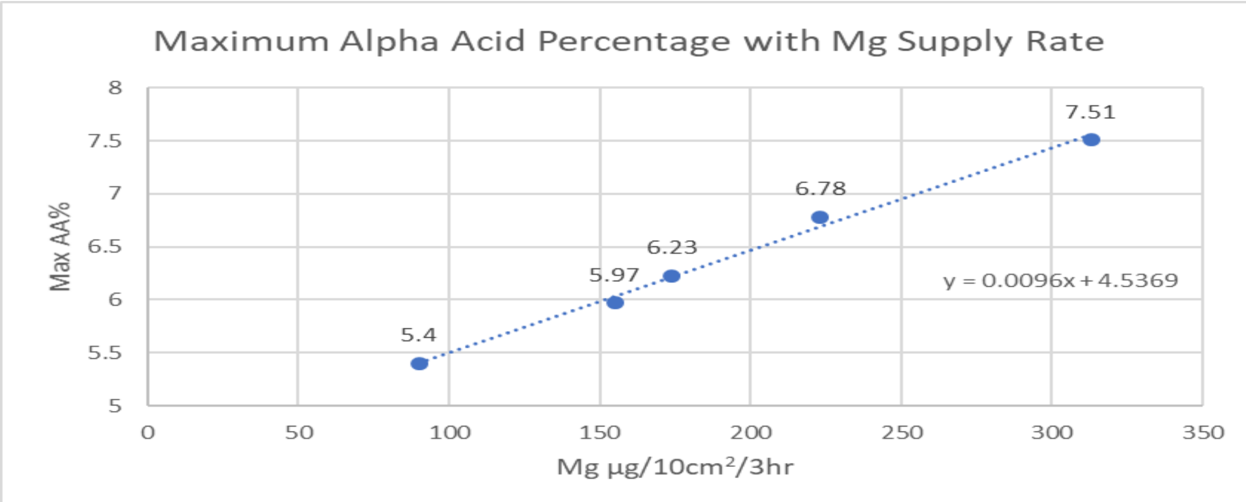

Figure 2: Example scatterplot of soil nutrient uptake rate and alpha acid percentage with a trendline.

Alpha acid response rate was also calculated from the regression analysis. The slope of each regression line equation gives an estimation of alpha acid percentage growth per nutrient supply rate unit. For instance, based on our sample, the alpha acid response rate for Mg is approximately 0.0096. This means for every increase of one $\mu g/10cm^2/3hr$ of Mg, we predict an increase in alpha acid content of 0.0096 percent. The alpha acid response rates for soil nutrients with higher correlations can be seen below in figure 3 which compares the post- and pre-growing season alpha acid response rates.

	Post-Growing Season (Sept 2)		Pre-Growing Season (Apr 4)	
Soil Nutrient	**acid:nutrient response rate**	**Correlation Coefficient**	**acid:nutrient response rate**	**Correlation Coefficient**
Mg	0.0096	0.997	0.0131	0.900
Al	0.2566	0.952	0.0709	0.279
B	2.0527	0.927	0.0976	0.068
Fe	0.5939	0.831	0.5939	0.819
Ca	0.0019	0.799	0.0006	0.296
Pb	1.8254	0.793	-3.9167	0.596
P	0.2052	0.772	0.1698	0.594
Zn	0.3951	0.401	2.15	0.788
S	0.0013	0.360	-0.00009	0.022
K	0.0046	0.282	-0.0038	0.176

Figure 3: Table showing the alpha acid response rate for post- and pre- growing season soil nutrient supply rates. The table shows the highest correlation coefficients of soil nutrient supply rate associated with alpha acid percentage calculated from our post-growing season soil samples.

Discussion

The results to the regression analysis in this study provide inconclusive results on the relationship between nutrient supply rates and alpha acid percentages. One reason for this is that this analysis was done with a small sample size of five data points. A much larger sample size is required to produce accurate and reliable results of how nutrient supply rates are associated with alpha acid production in a hop. The small sample size was a result of a lack of time, money, and resources to take more soil samples. To conduct this analysis effectively, more samples should be taken. For instance, taking soil samples twice a month at each farm throughout the growing season would provide enough data points for a more reliable analysis. Furthermore, taking soil and hop samples from more than one hop plant of the same age at each farm would provide additional data points. Looking at pre-growing season soil conditions likely does not accurately represent the amount of nutrients absorbed by the hop plant. This is because farmers make amendments to their soil throughout the growing season, which was not effectively captured by the PRS probes. Thus, it would likely be more effective to take multiple sets of soil samples late-July to September to gather a larger sample size, account for fertilizer amendments, and account for the time-period of peak alpha acid growth. Furthermore, it could be interesting to apply this same correlational study in a multiple regression analysis. Here one could test for correlations using combinations of different nutrient supply rates in one model, using interaction terms and a second-degree model.

Another limitation of this analysis is that there are a few features of soil composition impacting soil nutrient availability and hop maturation that were not being tested. This includes the cation exchange capacity (CEC), pH level, and soil type. The CEC of soil is important to test because this indicates the nutrient holding capability of a soil (Brown, 2018). This property of soil depends on soil type. Normally, the greater the clay content and organic content of the soil, the higher the CEC of a soil is. The pH level of the soil can also impact the CEC value. The pH level also impacts soil nutrient availability in the soil, and vice-versa. This implies that there is a dynamic relationship between soil type, CEC, pH level, and nutrient availability, all of which have an influence on alpha acid production in hops. The absence of testing for the CEC value, pH level, and soil type limits the reliability of this analysis. Future studies should include the CEC value, pH level, and soil type in their analysis to produce more meaningful and clear results.

Specific geographic differences at each farm may have had an impact on each hop plant. For instance, Eric’s farm is in Healdsburg while BiRite Farm is in Sonoma. Eric’s farm is located at a higher elevation and on a steeper slope that is facing south-west, while BiRite Farm is located on flat land. Each farm is located along the 38th latitude line. While the farms do not have significant latitudinal differences, they have differences in the average temperature, precipitation, relative humidity, amount of surrounding vegetation, soil type, slope aspect, slope steepness, irrigation, styles of farming, and different uses and types of home-made fertilizers. These variables were not accurately measured. A weather station installed at each farm could properly collect and record these data. Geographical parameters like these could have impacted the hops in specific ways. For future studies, it would be ideal to control these variables. Environmental quantities like averages of temperature, precipitation, or relative humidity could be used in a multivariate regression analysis to attempt to create a more meaningful statistical model. This would include using interaction terms and using a second degree model.

Conclusion

In conclusion, this project is an attempt to examine the association between environmental features and alpha acid production. Furthermore, this paper attempted to analyze the relationship between the variables soil nutrient supply rates and alpha acid percentages in hops. Extensive research needs to be conducted on the relationship between environmental parameters and alpha acid production. This knowledge would be valuable to hop farmers who want to precisely grow Cascade hop plants.

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