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ABSTRACT

A multi-year field study was conducted in Oregon and Washington to evaluate the influence of nitrogen fertilization rate and timing on cone quality and nitrate accumulation in cones. The impact of nitrogen rate on cone yield, levels of hop acids, total oil content, color, and nitrate level were year dependent. However, when data were aggregated over years and analyzed using a mixed effect model, α-acids, β-acids, and total oil volume decreased linearly with increasing nitrogen rate; while cone color, expressed as the degree of greenness of cones, and nitrate content of cones increased linearly with nitrogen rate. Yield was not improved with the highest nitrogen rate. In one of four studies, panelists used triangle tests to evaluate hop aroma of ground hop cones and detected a difference among treatments. The α- and β-acids decreased and nitrate concentration increased when nitrogen was applied after bloom. One harvest showed that fertilizer timing led to differences in the aroma of the hop cones although this difference was within the standard aroma variation for the variety. Overall, this research indicates that applying the lowest feasible nitrogen rate and ceasing nitrogen applications before or at bloom may optimize certain cone quality factors while minimizing nitrate accumulation.

Introduction

In the Pacific Northwest, hop is a specialty crop that is grown on more than 22,000 hectares. The majority of the industry is located in Washington, where approximately 16,000 hectares were harvested in 2018, followed by Oregon with 3,000 hectares, and Idaho with 3,000 hectares.[1] Due to the rapid growth pattern of hop plants, the crop requires relatively large amounts of nitrogen during the spring.[2,3] Nitrogen (N) requirements are reported to range between 150 to 225 kilograms per hectare applied annually for optimal production.[3] Hop plants are well known for efficiently utilizing and retaining large quantities of nitrogen, which can lead to nitrate accumulation when excess nitrogen is not utilized for vegetative growth.[3–6] Nitrogen fertility may influence yield, arthropod pests, disease, cone aroma and quality, cone chemistry, cone color, and nitrate accumulation in the cones; although, some of these effects are not well quantified.[3,7–10] Likens and Nickerson[11] found that excessive nitrogen application (448 kg/ha) reduced α-acids and total oil, but did not influence oil composition.

There has long been a concern that elevated levels of nitrate and nitrite in a diet can lead to increased risk of gastrointestinal cancer and, in infants, methemoglobinemia or "blue baby syndrome." Most dietary nitrates come from vegetables, fruits, and cured meats, with approximately 80% of all dietary nitrogen originating from vegetables.[12–15] However,

beer is also a potential dietary source of nitrate. Because nitrate is water soluble, as hopping rate increases, as does the potential for nitrate content of beer.[4,5,16] Currently, there is not an established maximum residue level for nitrate in beer in the United States; therefore, drinking water standards are often used, even if these standards may not be appropriate.[4] Nitrate levels in some beers have been found to be below the maximum residue level (MRL) limit for Europe[5] of 50 mg/L but above the recommended EPA drinking water standard of 10 mg/L.[4,17] A study in Germany found several batches of beer that had been dry-hopped with pellets or powder to be above 50 mg/L of nitrate[4] and Mitter and Cocuzza[5] and Kaltner et al.[16] both state that as hopping rate increases so does the nitrate level in beer. Some literature indicates that there is a nearly 100% transfer rate of nitrate from hop material to wort and beer.[4] However, there is no evidence that increased nitrate in hops is detrimental to the brewing process or human health.

There is motivation to understand how nitrogen fertility practices in the field influence multiple aspects of cone quality, yield, and nitrate accumulation. The research presented herein provides data from seven location-years collected from three hop cultivars investigating how cone yield, chemical, and quality metrics of cones, and beer quality change in response to varied nitrogen fertilizer rates and application times.

Experimental

Experimental design of field studies

Replicated field plots of hop plants were established in Oregon and Washington to determine if the rate or timing of nitrogen fertilizer application provoked changes in cone yield or brewing characteristics. Note that the treatments evaluated were not intended to identify an optimum rate or application time of nitrogen fertilization, but rather to describe the dose-response relationship between the response variables and nitrogen fertilization treatments. Note also that nitrogen rate and application time can be variable across fields and varieties.

Nitrogen rate studies

Studies evaluating nitrogen rate were conducted in Washington during 2015 and 2016 in a commercial yard of cultivar Tomahawk, and in Oregon from 2014 to 2018 in experimental plots of cultivar Willamette. A general nitrogen (N) fertilization recommendation for commercial hop plants is between 150 to 225 kilograms per hectare applied annually.[3] In the studies in Washington, three different nitrogen rates, 90 kg/ha, 179 kg/ha, and 269 kg/ha, were evaluated for the influence on the various response variables described in the following section to understand dose-response relationships. The hop yard was planted to Tomahawk in 2008 on a 4.3 m wide row spacing and 1 m between each hill (plant), allowing for 2,197 hills per ha under a 5.5 m trellis. Thirty-four kilograms per hectare (2015) and 86 kg/ha (2016) of ammonium nitrate nitrogen was banded in late winter to early spring across the whole field. The yard was irrigated by surface drip irrigation and the remaining nitrogen, necessary to reach the overall desired rates, was injected through the drip on bi-weekly intervals as urea ammonium nitrate (32-0-0) or calcium ammonium nitrate (21-0-0). A plot consisted of at least three rows that ran the length of the yard, with each plot at least 0.4 hectare in size. Each nitrogen rate treatment was replicated four times in a randomized complete block design. The same treatments were applied to the plots in 2016. All other management inputs and decisions, including disease and arthropod pest control, were made according to standard production practices by the cooperating grower.

Similar experiments were established in an experimental hop yard located at the Oregon State University Vegetable Research Farm near Corvallis, Oregon. The experimental hop yard was planted in 2005 to cultivar Willamette with plants arranged on a 2.1-m grid pattern and under a 5.5-m trellis. Because this was a non-commercial field, a lower rate of nitrogen, 45 kg/ha, also was included to collect data on an exceptionally low nitrogen rate. This rate is well below commercial recommendations, but was included to better characterize the dose-response relationship between nitrogen and hop yield and quality factors of interest. In all years, an application of 16-16-16 fertilizer was broadcast-applied to the entire field during mid-April, delivering a total of 45 kg/ha of nitrogen, 20 kg/ha of P, and 37 kg/ha of K. The remaining nitrogen, 0, 45 kg/ha, 135 kg/ha, or 224 kg/ha, was banded over each hill in two equal applications as urea (46-0-0) in mid-May and mid-June. Each plant was irrigated using a garden hose to dissolve the fertilizer and the hop yard cultivated immediately afterward to fully incorporate the fertilizer into the soil and minimize ammonia volatilization. Plots were established in a randomized complete block design with each plot replicated four times. The individual plots consisted of 24 plants, separated by at least one row of plants that did not receive any additional nitrogen after mid-April. Irrigation was supplied by a surface drip system and herbicides were applied according to standard production practices in Oregon.[18] These treatments were applied in each year from 2014 to 2018. Only data from 2015, 2016, and 2018 are reported in this paper because during 2017 there was a large outbreak of two-spotted spider mites that confounded results for that year.

Nitrogen timing studies

In 2017 and 2018, a separate study was conducted to evaluate the impact of nitrogen timing on cone quality factors by applying a single rate of nitrogen at three different times. The study was conducted in a commercial yard of Simcoe® planted in 2016 near Toppenish, Washington. During late winter, 112 kg/ha (2017) and 42 kg/ha (2018) was banded across the whole field as ammonium thiosulfate and mono-ammonium phosphate to provide initial nutrients as per standard practices. The remainder of the total nitrogen delivered was applied weekly via drip irrigation over a six-week period, with the timing of the initial application varying by approximately two weeks to simulate an early timing (May 15 to July 1), standard timing (June 1 to July 15), and late timing (June 15 to August 1). In the northern hemisphere, hop plants typically bloom near early to mid-July when the day length is between 15 and 16 hours.[3] The nitrogen application times were designed to apply the majority of the nitrogen prior to bloom (early), before and during bloom (standard), and well after bloom (late). On days when fertilizer was applied, plots not receiving fertilizer received the same duration of irrigation to avoid confounding effects from varying amounts of water. The total amount of nitrogen applied was 224 kg/ha in 2017 and 168 kg/ha in 2018 based on the request of the cooperating grower. A plot consisted of four rows running the length of the yard. Each treatment was replicated seven times in a randomized complete block design. As with the nitrogen rate study, standard production practices were followed by the cooperating grower for all other inputs.

Cone sample preparation and storage

Cones were harvested from 8 to 16 plants per replicate plot using a small hop picking machine. Promptly after harvest, all cone samples were dried to a moisture content of approximately 8% (w/w), either in a commercial kiln or a small, forced air electric dryer. Dried cones were pressed into approximately 0.5 kg 'mini-bales' and held at ~3 °C for

no more than 48 h. Mini-bales were then packaged for long-term storage in high-barrier foil bags purged with nitrogen prior to vacuum sealing; the samples were maintained at −10 °C until chemical analyses and sensory testing took place.

Chemical analysis

Hop cones were analyzed for moisture content, percentage of α- and β- acids, hop storage index (HSI), and total essential oil content using the ASBC standard methods of analyses unless otherwise noted.

Dry matter and overall cone color were evaluated for each hop sample in all years according to Gent et al.[19] In brief, dry matter was calculated by dividing the fresh cone weight by the dry weight and multiplying by 100 to get a percentage. Approximately 50 g of fresh cones were collected from each plot in duplicate and samples were then averaged to obtain one dry matter estimate per plot. Color was visually assessed by one individual using a 10-point ordinal scale.[20]

Bittering acid measurements

Total α-acids, β-acids, and HSI were measured using ASBC method Hops-6A[21] using a Shimadzu PharmaSpec UV-1700 spectrophotometer, Shimadzu Corporation (Columbia, MD). Briefly, 5 g of ground hops were extracted in 100 mL of toluene for 30 min. Five mL of the clarified toluene extract was added to 100 mL of alkaline methanol. The absorbance of this solution was then determined at 275, 325, and 355 nm. HSI is a measure of hop oxidation (or % humulones lost) and is the ratio of the absorbance maximum of hop oxidation products (275 nm) to the absorbance maximum of α-acids (325 nm).

Essential oils

Hydrodistillation was performed to determine the total oil content of the hop cones using ASBC Hops-13.[21] In brief, ~105 g of coarsely ground hops was boiled in 3 L of distilled water for 3 h. Post-distillation, hop oil was collected in 2.5 mL amber vials with foil-lined closures. After the oil was collected the amber vials were flushed with nitrogen. Hop oil was stored at −20 °C until subsequent compositional analysis.

Nitrate accumulation

Nitrate accumulation was evaluated on a Lachat Instrument using a cadmium reduction flow injection protocol[22] in a laboratory at the USDA Forage Seed and Cereal Research Unit or the Central Analytical Lab at Oregon State University. In brief, samples were dried to completion (0% moisture), ground to a fine powder, and then covered with 30 mL of a 2 M KCL solution for 30 min on a shaker set to 250 rpm. After shaking, the samples were filtered using a Whatman #1 filter and the filtrate was analyzed for nitrate concentration.

In addition to testing cones at harvest, in the nitrogen timing studies during 2017 and 2018, developing cones were also sampled mid-July and mid-August to quantify when nitrate begins to accumulate in the reproductive structure. Samples were collected from 10 plants at three canopy heights of approximately 2, 3, and 5 meters and combined to make one sample per plant. Plant samples were then completely dried and stored in paper bags until processing. Samples were analyzed for nitrate as previously described.

Sensory testing of whole cone hop aroma

To determine if there were perceptible differences in aroma due to nitrogen rate or timing, difference testing using triangle testing was performed as outlined in ASBC Sensory Analysis 7.[21] Hop material was ground using a small kitchen blender to rupture lupulin glands and mimic hop powder before pelletizing. Panelists consisted of untrained Oregon State University Brewing Science students and faculty familiar with analyzing hop flavors. Samples were blind coded with a random three-digit number and presented to panelists in a randomized order. Panelists were asked to evaluate the orthonasal aroma of the samples and identify which sample was different from the other two. Panelists performed a series of three triangle tests in order to compare three nitrogen rates (90 kg/ha vs. 269 kg/ha, 90 kg/ha vs. 179 kg/ha, and 179 kg/ha vs. 269 kg/ha), and the nitrogen application times (early vs. late, early vs. standard, standard vs. late).

The early, standard, and late nitrogen application treatments were further analyzed to understand the variation in aroma due to the nitrogen treatments relative to the typical variation seen on a commercial farm. To do this, whole cone hops from each of the three nitrogen treatments were evaluated amongst three other Simcoe® lots collected from different hop yards on the same farm. Sixty-one panelists that were familiar with evaluating hop aroma were recruited from among attendees at the 62nd American Hop Convention in Palm Desert, CA in January 2018 and asked to assess the aroma characteristics of the six samples of Simcoe® cones using a check-all-that-apply (CATA) ballot. Again, the hops were ground using a small kitchen blender and approximately 10 g was placed into a 100 mL cup with a lid. Panelists were given tablet computers with a list of 45 attributes encompassing hop aroma and were instructed to select all the attributes that best described the orthonasal aromas of the six samples. The samples were presented to the panelists in a random order and with a random three-digit code identifier.

Sensory testing of hop aroma in beer

To evaluate the differences in the hop aroma imparted to beer from the Tomahawk cones harvested during 2015 and 2016, single-hop pale base beers were prepared. In brief, the wort for these beers was prepared using a grist of 100% pale ale malt (Great Western Malting, Vancouver, WA, U.S.A) at a starting extract concentration of 14.8°P, utilizing a single infusion mash (68.8°C). The mineral content of the brewing

water was adjusted using 18.0 mg/L of $CaCl_2$ and 38 mg/L of $CaSO_4$.

In both years, hops from the high and low nitrogen application (90 kg/ha of nitrogen and 269 kg/ha of nitrogen, respectively) were used to produce beer. For each beer, kettle hops were added at the start of a 60 min boil at a rate of 0.53 g hop/L wort (targeting ∼35 mg/L of iso-α-acid). Whirlpool hop treatments were performed at 2 g hop/L wort after boiling and held for approximately 25 min at 100°C. Fermentation was carried out with Wyeast 1056 ale yeast at 20.4°C. After the diacetyl rest, the tank temperature was reduced to 15.5°C and the yeast was removed. The green beer was dry-hopped at a rate of 4 g hop/L by adding coarsely ground hops to the fermentation vessels and held at 15.5°C for 24 h. After 24 h, the temperature was dropped to 1.1°C and held for another 24 h. The green beer was then filtered to stop the dry-hopping process with a plate and frame filter using diatomaceous earth impregnated cellulose pads (HS2000, Pall Corporation, Port Washington, NY, U.S.A.). Dissolved oxygen (DO) was monitored during filtration using an Orbisphere 3100 Portable Oxygen Analyzer (Hach, Loveland, CO). Bright beer was not collected until the DO was below 80 μg/L. After the DO was within specification, bright, filtered beer was collected in a closed 19.6 L stainless steel keg with sufficient backpressure to reduce foaming. Between each filter run of the two batches of beer, filter pads were exchanged to prevent carry-over. Filtered beer was stored at 2°C and under CO_2 overpressure (83 kPa) until sensory evaluation.

Discrimination testing using triangle testing was performed on these beers. Panelists were presented with three triangle tests. Within each triangle test, there were three samples; two of the samples were the same and one of the samples was different. The panelists were instructed to taste the samples and select the odd sample. For each of the three sets of duplicates, the design of the triangle test ensured an equal frequency of appearance of each duplicate as the "odd" sample. The serving order within each of the triangle tests was also randomized. The dry-hopped beer was dispensed from the keg into a pitcher, which was used to pour ∼60 mL of beer into 300-mL sample glasses, which were covered with plastic lids and coded with randomized 3-digit numbers. The beer was allowed to warm to room temperature before sensory analysis.

Statistical analyses

Yield, oil content, percentage of α- and β-acids, percent dry matter, color, and nitrate accumulation were analyzed by year and study in general linear mixed effect models using the GLIMMIX procedure in SAS version 9.4 (SAS Institute, Cary, NC, U.S.A.). The response distribution was specified as Gaussian and the "identity" link function was utilized in all analyses. Denominator degrees of freedom were determined using a general Kenward-Roger approximation. In these analyses, nitrogen treatment (rate or timing) was a fixed classification effect and block was a random factor in the models. To aggregate data from the same cultivar over years, mixed effect models were fit to the data by considering nitrogen rate

a fixed, continuous effect (for all variables except yield), and year, block nested with year, and year × treatment random effects. This is similar to the analysis used for a multiple-location experiment for a mixed effect regression.[23] When covariance parameter estimates were zero for a random effect, these terms were removed from the model and the model refit. To visualize year-to-year variability in the data, simple linear regressions were plotted along with the results of the combined analyses. A similar analysis was conducted for the nitrogen timing study, but nitrogen timing treatments were considered classification variables (i.e., a mixed effect ANOVA was conducted) because the treatments were applied over a period of time and did not have a natural continuous form as dose rate.

Nitrate data collected over time from the sample hop plants in the nitrogen timing study was analyzed as a repeated measures analysis.[23] The fixed effects were nitrogen timing treatment, sampling date, and their interaction. Random effects were block and an explicit R-side random term for each subject (treatment × block combination) to account for potential correlation of residuals. Multiple covariance structures were investigated and the most parsimonious covariance structure that best described the data was selected by minimizing the Akaike information criterion. Analyses were conducted using the GLIMMIX procedure in SAS version 9.4.

Data from the triangle tests that were evaluating aroma were analyzed using a binomial distribution in Microsoft Excel[24] to determine whether the proportion of correct responses were greater than expected random chance. Data from the check-all-that-apply (CATA) evaluations consisted of frequencies or use for individual attributes. These attributes were ranked from most used to least used and the bottom 15% were removed from further analyses. The remaining data were analyzed using Chi-square tests with a single degree of freedom to determine if the frequencies of individual attributes varied between the nitrogen timing treatments; analyses were conducted using Excel.[24] A CATA analysis module, which includes principle component analysis, was conducted on the same, reduced data set using XLSTAT 2017 (Addinsoft, New York, NY, U.S.A.).

Results

Nitrogen rate studies

In Tomahawk, all response variables showed year-to-year variation when years were analyzed individually (Table 1). Yield, total oil content, and nitrate levels were the only variables to be significantly affected in every year. Yield and nitrate content tended to increase as nitrogen rate increased, although the highest nitrogen rate did not always lead to the greatest yield. In both years, there was a trend for decreasing α-acids and total oil content with an increase in nitrogen rate, but the differences were not always statistically significant. Percentage of β-acids showed no significant or consistent trend in either year. Cone color showed an increasing trend for greener color as the nitrogen rate increased in both years, but this effect was only significant in 2016

Table 1. Nitrogen rate results.[a]

Nitrogen rate (kg/ha)	Yield (kg/string)	Oil[b]	α-acids (%)[c]	β-acids (%)[c]	Dry matter (%)	Cone color[d]	Nitrate (ppm)
2015 Tomahawk							
90	0.61a	4.1a	19.7a	5.7	26.4	5.5	898c
179	0.69b	3.8b	19.0b	5.6	25.9	6.5	1734b
269	0.66ab	3.8ab	18.6b	5.6	26.0	6.0	2793a
P-value	0.068	0.056	0.006	0.659	0.537	0.274	0.0003
2016 Tomahawk							
90	0.55a	3.6a	18.8a	5.3	26.0	4.8b	570b
179	0.65b	3.4b	18.8a	5.5	26.8	5.3a	1830a
269	0.72c	3.4b	18.5a	5.5	26.6	6.3a	2336a
P-value	0.001	0.038	0.739	0.724	0.611	0.064	0.0167
2015 Willamette							
45	0.21a	1.7	6.1	3.8a	25.1	7.3b	915b
90	0.25b	1.7	6.4	3.8a	24.9	7.5b	1309b
179	0.30c	1.7	5.4	3.6b	25.5	7.5b	2184a
269	0.32c	1.5	5.4	3.5b	26.0	8.5a	2742a
P-value	0.0001	0.173	0.296	0.0005	0.778	0.022	0.0019
2016 Willamette							
45	0.30a	1.2	4.8	3.4a	23.1	5.8	708c
90	0.31a	1.1	4.9	3.2ab	22.6	5.8	974c
179	0.41ab	1.2	4.7	3.1b	23.2	6.0	2047b
269	0.37ab	1.1	4.5	3.1ab	21.9	6.8	3455a
P-value	0.019	0.541	0.194	0.144	0.701	0.292	0.0005
2018 Willamette							
45	0.19d	2.0a	4.8	3.4a	22.2	5.8b	281c
90	0.25c	1.9b	4.8	3.4a	22.0	6.0b	405c
179	0.28b	1.78bc	4.5	3.2ab	21.4	6.8a	1083b
269	0.30a	1.7c	4.4	3.0b	21.6	7.0a	2170a
P-value	<0.0001	0.0051	0.144	0.063	0.215	0.0008	<0.0001

[a]Variables were analyzed by year in general linear mixed effect models. Means with different letters are statistically different at $P = 0.05$.
[b]Total oil is reported as mL of oil per 100 g of hop material adjusted to 8% moisture.
[c]The α- and β-acids were determined by ASBC spectrophotometric methods.
[d]Cone color was rated on a 1 to 10 scale, where 10 is the best possible color.

(Table 1). Percentage of dry matter of cones was independent of nitrogen rate in both years (Table 1).

When data were analyzed over years, there was enough statistical power to detect significant relationships between all response variables except yield. Yield data combined over years revealed a trend for higher yields with the two higher nitrogen rates ($P = 0.136$, Figure 1). Yield was 14 to 16% higher with the 179 and 269 kg/ha rates when compared to the 90 kg/ha rate ($P = 0.0648$). Levels of α-acids, total oil content, nitrate, and cone color were all significantly related to nitrogen rate (Figures 1 and 2; $P \leq 0.0132$). The α-acids and total oil decreased as the nitrogen rate increased and were related to nitrogen rate via the equations:

$$\alpha\text{-acids } (\%) = 22.0548 + -0.00545 \text{ (kg/ha of nitrogen)}$$

$$\text{total oil (ml/100g)} = 4.4026 + -0.00173$$

$$\text{(kg/ha of nitrogen)}$$

Cone color and nitrate content increased with nitrogen rate and were related by the equations:

$$\text{cone color} = 5.278 + 0.00701 \text{ (kg/ha of nitrogen)}$$

$$\text{nitrate (ppm)} = -160.90 + 10.36 \text{ (kg/ha of nitrogen)}$$

Slope and intercept terms were significantly different than zero for all of these models ($P \leq 0.0223$).

The β-acid levels were independent of nitrogen rate when data was aggregated over years. The β-acid levels were related to nitrogen rate by the equation:

$$\beta\text{-acids } (\%) = 5.5081 + 0.000260 \text{ (kg/ha nitrogen)},$$

The intercept was significantly different than zero ($P \leq 0.0001$); however, the slope was not ($P = 0.7511$).

Similarly, in Willamette, there was year-to-year variation in all response variables (Table 1). Yield and nitrate accumulation were the only variables to be significantly affected by nitrogen rate in every year. Yield and nitrate tended to increase with nitrogen rate, but as with studies in Tomahawk, the highest nitrogen rate did not always lead to significantly greater yield or nitrate content. In general, in a given year there were often trends for lower levels of hop acids and oil content with increasing nitrogen rate that were in some instances significantly different, although typically these differences were insignificant. Cone color was improved significantly with increasing nitrogen rate in two of the three years. The percentage of dry matter of cones was independent of nitrogen rate in both years (Table 1).

When the data were aggregated and analyzed over all three years, there was adequate statistical power to detect a relationship between nitrogen rate and yield (Figure 1; $P = 0.0033$). Yield was 20 to 27% greater with nitrogen rates of 179 or 269 kg/ha as compared to lower rates. However, yield was similar between these two rates. Levels of hop acids, oil content, cone color, and nitrate levels were
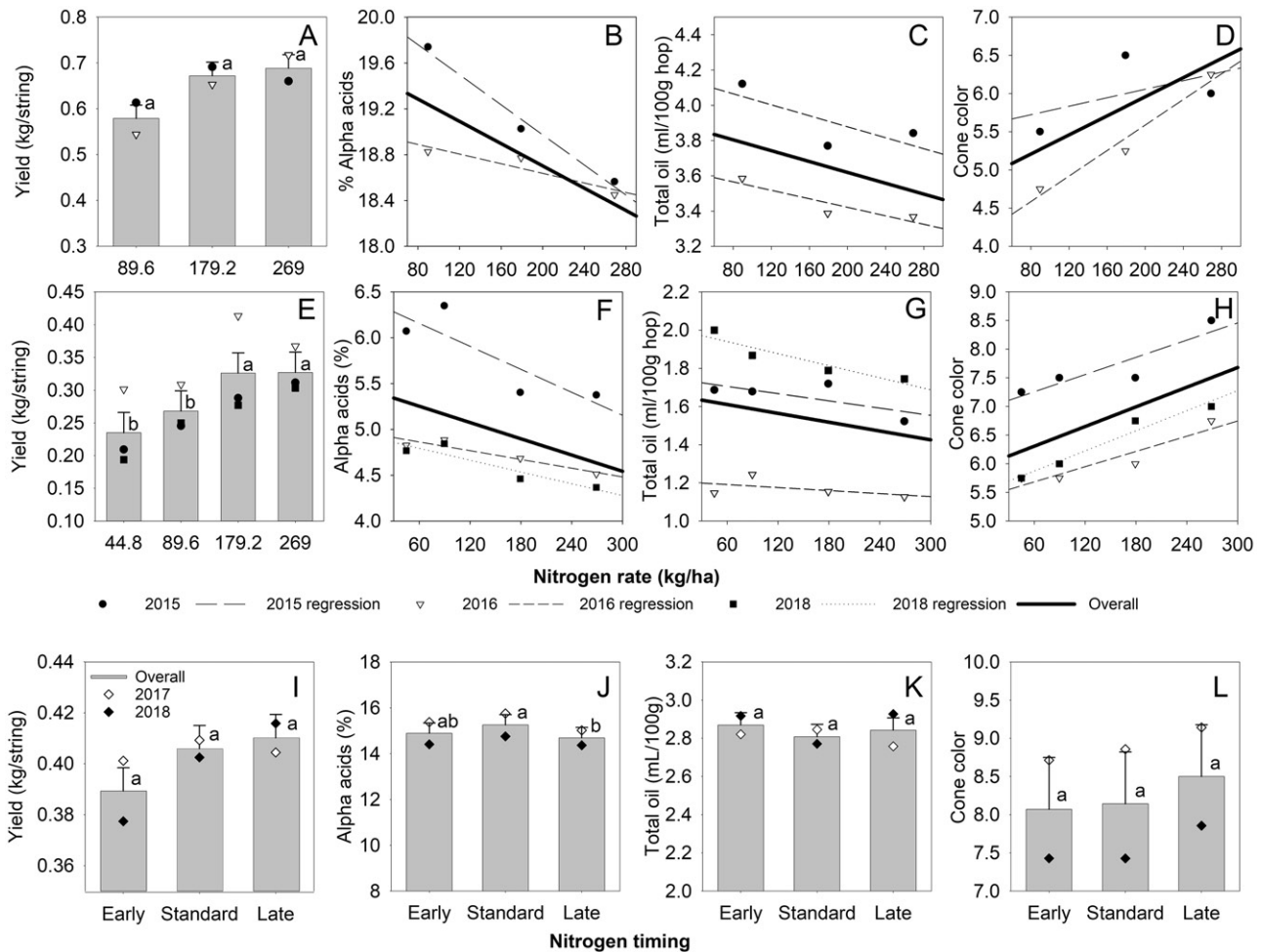
Figure 1. Cone yield A, E, and I; α-acids B, F, and J; total oil C, G, and K; and cone color D, H, and L. Panels A–D is data from cv. Tomahawk, E–H is data from cv. Willamette, and I–L is data from cv. Simcoe®. To aggregated data from the same cultivar over years, mixed effect models were fit to the data by considering nitrogen rate a fixed, continuous effect (for all variables except yield) and year, replication within year, and year × treatment random factors. For nitrogen timing studies, nitrogen timing treatments were considered classification variables. Means with different letters are statistically different at $P = 0.05$. Error bars indicate standard errors.

significantly related to nitrogen rate (Figures 1 and 2; $P \leq 0.0044$). The hop acids and oil content decreased with nitrogen rate, being described by the equations:

$$\alpha\text{-acids} \ (\%) \ = \ 6.0884 \ + \ -0.00332 \ (\text{kg/ha of nitrogen})$$

$$\beta\text{-acids} \ (\%) \ = \ 3.5718 \ + \ -0.00150 \ (\text{kg/ha of nitrogen})$$

$$\text{total oil} \ (\text{ml/100g}) \ = \ 1.8570 \ + \\ -0.00086 \ (\text{kg/ha of nitrogen}).$$

In contrast, cone color and nitrate content were positively related to nitrogen rate. The equations relating these variables were:

$$\text{cone color} \ = \ 6.6864 + \ 0.006412 \ (\text{kg/ha of nitrogen})$$

$$\text{nitrate} \ (\text{ppm}) \ = \ 105.82 \ + \ 9.6968 \ (\text{kg/ha of nitrogen})$$

The slope and intercept terms were significantly different from 0 in all of these models except for the intercept term for nitrate (P always $\leq$ 0.0132; $P = 0.7022$ nitrate intercept).

Nitrogen timing studies

As with the nitrogen rate studies, most responses were not significantly affected by nitrogen timing consistently in the year-by-year analysis (Table 2). During 2017, the percentage of β-acids and percentage of dry-matter both decreased as nitrogen application timing was delayed; whereas, nitrate levels increased. Only yield was significantly affected by nitrogen timing treatments in 2018 ($P = 0.073$; Table 2).

When aggregated over both 2017 and 2018, yield, oil content, and cone color were similar and independent of nitrogen timing ($P \geq 0.139$; Figure 1). Hop acids were influenced by nitrogen timing. The α-acids were reduced 4% between the standard and late nitrogen timing treatments ($P = 0.0314$). The β-acids decreased 4% with later application timing of nitrogen ($P = 0.0168$; Supplemental Figure 1). Nitrate content of cones increased linearly as nitrogen application time was delayed ($P = 0.0376$; Figure 2).
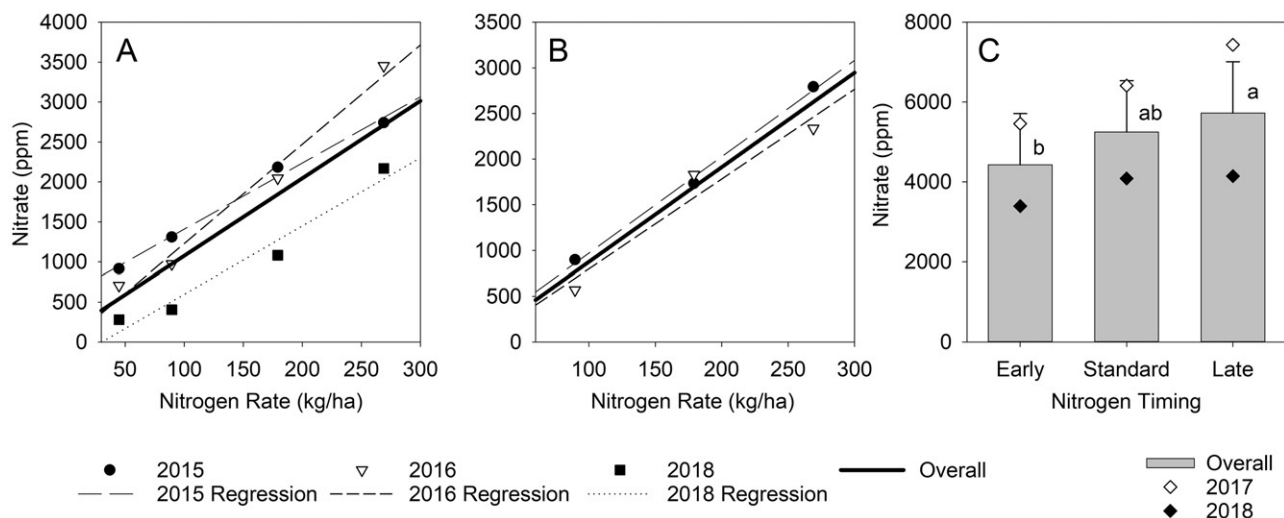
Figure 2. Nitrate accumulation in cones. In C, means with the same letter are not significantly different according to a mixed effect model ($P = 0.05$). A is cv. Willamette, B is cv. Tomahawk, and C is cv. Simcoe®.

Table 2. Nitrogen timing results.[a]

Nitrogen timing	Yield (kg/string)	Oil[b]	α-acids (%)[c]	β-acids (%)[c]	Dry matter (%)	Cone color[d]	Nitrate (ppm)
2017 Simcoe®							
Early	0.40	2.9	15.4ab	4.2a	23.8a	8.7	5460a
Standard	0.41	2.8	15.8a	4.1ab	22.8b	8.9	6409ab
Late	0.40	2.8	15.0b	4.0b	22.7b	9.1	7350b
P-value	0.844	0.823	0.116	0.052	0.032	0.449	0.0206
2018 Simcoe®							
Early	0.38b	2.9	14.4	3.9	22.6	7.4	3394
Standard	0.40ab	2.8	14.8	3.9	22.9	7.4	4088
Late	0.42a	2.9	14.4	3.8	22.9	7.9	4146
P-value	0.073	0.278	0.208	0.3007	0.883	0.317	0.4493

[a]All variables were analyzed by year in general linear mixed effect models. Means with different letters are statistically different at $P = 0.05$.
[b]Total oil is reported as mL of oil per 100 g of hop material adjusted to 8% moisture.
[c]The α-and β-acids were determined by ASBC spectrophotometric methods.
[d]Cone color was rated on a 1 to 10 scale, where 10 is the best possible color.

When nitrate content of the developing cones was analyzed over time, there was a significant effect of sampling date in 2017 and 2018 ($P < 0.0001$) that was independent of timing of the nitrogen application ($P = 0.1822$ in 2017; $P = 0.4748$ in 2018) and the interaction of fertilizer timing $\times$ sampling date ($P = 0.1126$ in 2017; $P = 0.2730$ in 2018). In 2017, nitrate levels increased over time and were significantly different on all three sampling dates. In 2018, nitrate levels were significantly greater on the last two sampling dates as compared to the first (Supplemental Figure 2).

Sensory testing of whole cone hop aroma

Panelists were able to differentiate hop samples based on nitrogen rate in only one of the four panels. The untrained panel in 2016 (n = 35) was able to differentiate cones of Tomahawk that received 90 kg/ha from the 179 kg/ha nitrogen rates ($P = 0.001$; Table 3) and the 176.9 kg/ha rate from the 269 kg/ha rate ($P = 0.003$; Table 3). For the nitrogen timing study, only the 2017 panel was able to significantly differentiate between cones receiving different treatments. The panel (n = 25) was able differentiate the early application

from the standard application time ($P = 0.046$; Table 3) of the Simcoe® samples.

Based on the check-all-that-apply (CATA) approach, aroma descriptors did not significantly vary among the three nitrogen treatments. For descriptors checked by at least 10% of panelists, there was a tendency for cones receiving the early nitrogen treatment to be described as stone fruit (17.5% of panelists) as compared to the late treatment (8.8% of panelists; Chi-square = 1.67; $P = 0.198$). Similarly, cones from the standard timing treatment were less often (10.5% of panelists) described as herbal/tea as compared to the other timing treatments (24.5% of panelists; Chi-square = 3.2; $P = 0.074$). PCA plots with five axes explained 100% of the variation in the samples, with 76.3% of the variation explained by the first two axes (Figure 3). Qualitatively, the variation in aroma due to nitrogen treatment was limited in PCA axis 2 and overall within the variation observed between lots harvested from the same farm. In general, there was more variation across the three samples of typical nitrogen timing obtained from various plots on the same farm than there was within the nitrogen timing samples. Thus, nitrogen application timing had a relatively minor impact on hop cone aroma characteristics.

Sensory testing of hop aroma in beer

In triangle tests of beer brewed using the Tomahawk cones from the rate studies in both 2015 and 2016, the untrained panelists differentiated beers based on the nitrogen rate treatments more often than by chance (Table 4; $P \leq 0.0001$ 2015; $P = 0.047$ 2016).

Table 3. Triangle test results of hop aroma assessment of ground cone samples.

Treatment comparison[a]	Number of panelists	Correct	Proportion correct	P-value[b]
2015 Tomahawk				
90 vs. 179	39	12	0.31	0.734
179 vs. 269	39	12	0.31	0.734
269 vs. 90	39	17	0.44	0.174
2016 Tomahawk				
90 vs. 179	35	21	0.60	0.001
179 vs. 269	35	20	0.57	0.003
269 vs. 90	35	14	0.40	0.403
2015 Willamette				
90 vs. 179	35	17	0.44	0.174
179 vs. 269	35	11	0.28	0.497
269 vs. 90	35	17	0.44	0.174
2016 Willamette				
90 vs. 179	35	12	0.34	0.905
179 vs. 269	35	9	0.26	0.339
269 vs. 90	35	13	0.37	0.633
Simcoe® 2017				
Early vs. Late	25	11	0.44	0.089
Early vs. Standard	25	12	0.48	0.046
Standard vs. Late	25	8	0.32	0.315
2018 Simcoe®				
Early vs. Late	29	11	0.38	0.182
Early vs. Standard	29	8	0.28	0.401
Standard vs. Late	29	8	0.28	0.401

[a]Treatment comparison is either nitrogen rate in kg/ha or application time. Panelists evaluated hop samples for an overall difference in aroma.
[b]Data were analyzed by comparing the proportion of correct response to expectations under a binomial distribution.

Discussion

This research establishes that nitrogen fertilization practices influence multiple aspects of hop cone yield and quality. These impacts are multifaceted and varied depending on the cone quality variable examined. Although general expectations for crop responses were identified, cultivar and year-to-year variation were substantial. Therefore, identifying an optimum rate and timing of nitrogen fertilizer is nuanced and a single optimum may not exist.

Because trellised areas for growing hops are finite, growers tend to apply ample amounts of nitrogen fertilizer to help maximize yield. Yield varied substantially among treatments depending on the year of observation and in three of the five location years, the highest yield was observed with the highest nitrogen rate evaluated. Interpretation of this data requires caution because in Oregon in 2015 and 2018, spring growth was substantially delayed due to lack of winter chilling (2015 study) and severe defoliation of plants in 2017 from spider mites (2018 study). In Washington in 2016, spring regrowth of plants also was severely delayed due to abnormally warm winter weather. The intermediate rate (179 kg/ha) was not

Table 4. Triangle test results evaluating taste of single hop beers made with Tomahawk cones from different nitrogen rate treatments.

Treatment comparison[a]	Number of panelists	Correct	Proportion correct	P-value[b]
2015				
269 vs. 90 kg/ha	40	34	0.85	<0.0001
2016				
269 vs. 90 kg/ha	43	20	0.47	0.0470

[a]Treatment comparison is nitrogen rate in kg/ha. Panelists evaluated beer samples for an overall difference in taste.
[b]Data were analyzed by comparing the proportion of correct response to expectations under a binomial distribution.
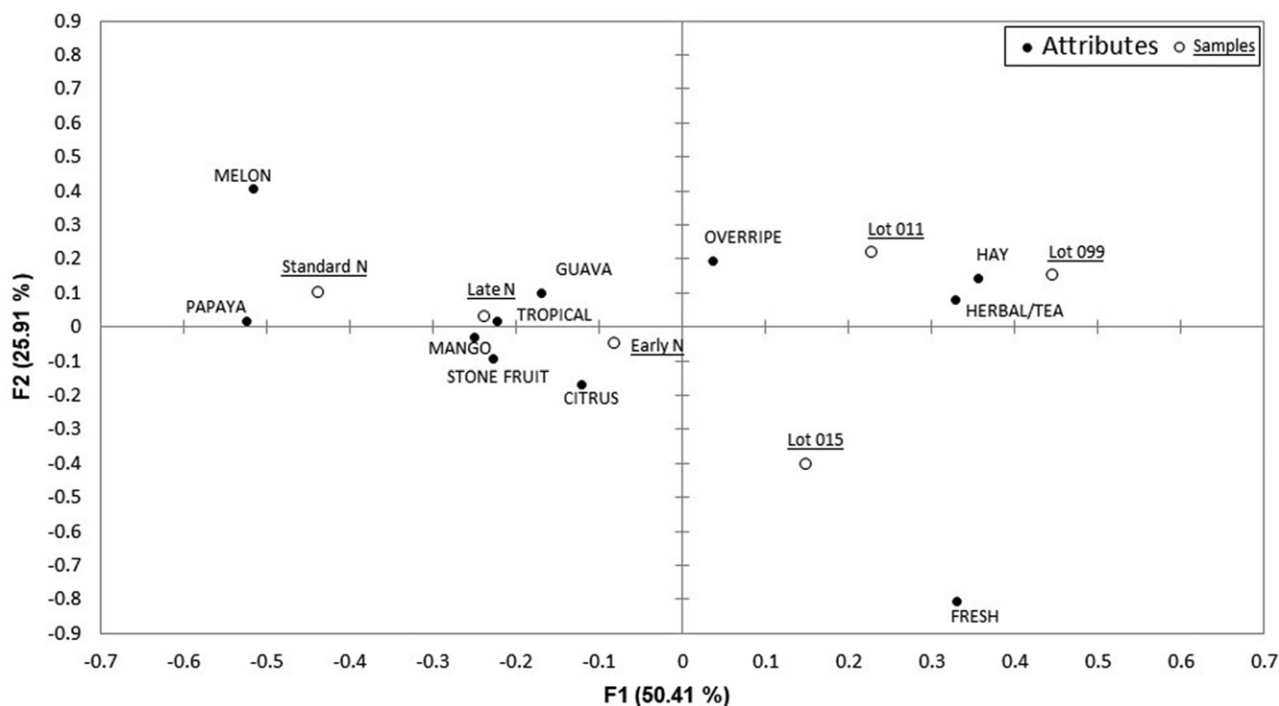

Figure 3. Principal component analysis results from the check-all-that-apply (CATA) sensory evaluation of ground Simcoe® hop cones.

significantly different from 269 kg/ha in the other years when spring growth was more typical. Thus, these data suggest that very high rates of nitrogen have the largest impact on yield when growing conditions are suboptimal in spring.

The data indicate a negative association between percent of bittering-acids and total oil with nitrogen rates that is consistent across two cultivars. Based on this study, α-acids could decrease by 0.44% in a cultivar similar to Tomahawk if 269 kg/ha of nitrogen is applied as opposed to 179 kg/ha. This reduction is not trivial for growers when crops are contracted based on α-acids yield. Likewise, this reduction is not trivial for brewers who tightly manage beer quality to keep their brand consistent. Similarly, total oil could be reduced by 0.14 mL/100 g hop. For both α-acids and total oil, inter-annual variation was of greater magnitude than the effect of nitrogen fertilization rate or timing. However, the analyses presented here considered year and year × treatment interactions as random factors. Thus, while year-to-year variation may be of greater magnitude than nitrogen fertilization practices, in any given year α-acids and total oil still are expected to decrease in response to increasing nitrogen fertilization rate. Although not reported here due to space constraints, individual constituents in the oil profile were also quantified. Similarly to Likens and Nickerson,[11] a consistent effect of nitrogen rate or timing on the quantity of the 20 individual compounds was not discerned.

Not unexpectedly, cone color increased on average by 0.5 of a point between either 90 kg/ha and 179 kg/ha or 179 kg/ha and 269 kg/ha. It is well known that nitrogen deficiency leads to a general yellowing of leaves and certain plant organs,[25] including hop leaves and cones. The contribution of the current study is in quantifying the magnitude of the response as the dose and timing of nitrogen fertilizer was varied. Furthermore, the present study also highlights that hop selection based in part on cone color may lead a brewer to inadvertently select lots that may be higher in nitrate as compared to other lots.

All of these measurements of cone attributes integrate multiple factors that influence the values observed at harvest. Diseases, wind/mechanical damage, and heat and sun intensity can all alter the color of hop cones. The sub-processes that alter, for instance, cone color cannot be differentiated from how nitrogen changes cone color in the absence of other environmental effects based on our method of analysis. Nonetheless, there was a relationship between cone color and nitrogen rate. These studies were not designed to evaluate specific mechanisms within hop plants that lead to the observed responses, but rather to provide a descriptive summary of the probable integrated effect of nitrogen on the desired brewing characteristics.

Aside from influencing cone chemistry, altering the nitrogen rate may influence overall cone aroma quality. Aroma was evaluated for an overall difference, not for a change in any one characteristic. This is again evaluating the integrated effect of different nitrogen rates and not changes in cone aroma due solely to direct effects of the treatments.

Similar to cone aroma, there is also a potential change in sensory characteristics of beer made from hops fertilized with varying nitrogen rates. Two untrained panels differentiated beer that had been brewed with cones from the 90 kg/ha rate from those brewed with the 269 kg/ha rate. It is important to recall here the experimental design of the brewing studies. In both 2015 and 2016, hopping rate was measured by weight of hops, not quantity of α-acids and in both years nitrogen rate did influence, to varying degrees, the percent of α-acids. Since the beer samples were evaluated for an over-all difference, and not a difference in a specific characteristic, the difference in α-acids and subsequent bitterness may have influenced this outcome. Regardless, there was a detectable difference in the beer when the hop plants received a different rate of nitrogen fertilizer. Therefore, brewer awareness of potential changes in sensorial attributes of hops and beer due to nitrogen fertility is warranted.

Altering nitrogen application time appears to have less influence on overall yield and cone quality than altering nitrogen rate. While the data indicate year-to-year variation and only a few significant effects when analyzed over years, these results are still valuable for hop growers and brewers. The lack of a statistically significant change suggests that altering the nitrogen application time does not have a large effect on overall hop chemistry and quality. This holds true for cone aroma as well. In only one out of six triangle tests, was the untrained panel able to correctly differentiate the different nitrogen timing treatments. Thus, any aroma differences were subtle enough that the panels could not detect them. Indeed, differences in cone aroma due to nitrogen timing appear to be within the intra-annual variation that may be observed among hop lots produced on the same farm. Although the panels assembled were relatively large, the panelists used for these studies likely influenced the results. Trained panelists may have detected differences in aroma characteristics more sensitively than the untrained panelists in the present study. However, even with this caveat, the results still point to nitrogen fertilization having only a small effect on aroma characteristics.

Our data show that independent of year, growing region (state), or cultivar, there is a linear relationship between the rate of nitrogen that is applied and the nitrate accumulation in cones. Therefore, growers should be motivated to apply the minimum amount of nitrogen necessary for their production situation and brewers should encourage this decision. However, as found in this study, this quantity of nitrogen may be year-dependent and difficult to define precisely. Further, later applied nitrogen (after bloom in this study) also increased nitrate levels in cones. Nitrate is stored in plant tissue when the plant has excess nitrogen and no way to use it,[25] accumulating in sinks such as reproductive structures. For a hop plant, the cones are both the reproductive structures and the product that possess the valuable brewing compounds.[3] Higher rates and later timing of nitrogen tend to lead to more nitrate accumulation in cones; this is likely a reflection of having a surplus of nitrogen available to the plant.[25] Our data from sampling the developing cones further support these results in that nitrate levels increased over time with cone development. These results indicate that the date on which cones are sampled

will have an impact on the level of nitrate, suggesting that harvest timing may influence levels of nitrate. Other cone quality factors, such as total oil and chemistry values, likely will determine when hops are harvested.[3,26] Nonetheless, variation in harvest timing may explain some of the variation in nitrate levels within the same variety and even within the same farm.

Although these studies focused on cone quality factors of most importance to brewers in this research, nitrogen fertilization is known to influence other aspects of hop production, such as soil-borne diseases[27–29] and the abundance of some arthropod pests.[30–33] These results, along with other knowledge of how high nitrogen rates can negatively influence pest pressure in hop production,[27–30] indicate that a higher nitrogen rate will not consistently result in a higher quality hop and in fact may expose growers and brewers to greater risk of quality defects and higher pesticide usage. Thus, growers and brewers alike should find motivation for reducing nitrogen rates and avoiding late season applications when feasible.

Conclusions

With seven location years of data, it can be concluded that nitrogen fertility can influence cone quality by altering the percent of hop acids, total oil, overall yield, and cone color. However, yearly variation caused by environmental factors and higher nitrogen rates may increase yield in certain situations, particularly when suboptimal growth conditions exist during the spring. Moderating nitrogen rates and avoiding late-season applications in the field should help to reduce both nitrate levels in harvested hops and improve several quality factors important to brewers and growers.

Acknowledgments

Funding

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