Evaluation of Pesticide Residues from Conventional, Organic, and Nontreated Hops on Conventionally Hopped, Late-Hopped, and Wet-Hopped Beers

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ABSTRACT

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This study was conducted to determine whether beers produced with late (i.e., after kettle-boil) additions of hops *Humulus lupulus* cultivars Cascade or Chinook (either dry hopped with whole or pelletized hops or wet hopped with green hops) resulted in an increase in pesticide residue levels between hopping regimes compared with beers hopped at the traditional kettle-boil stage. Hops were grown under conventional, organic, and untreated pesticide regimes, which are described. Test beers were brewed under controlled conditions and divided into four treatments based on timing of hop introduction, each of which was performed with green ("wet") conventionally treated hops, dried whole hop conventionally treated hop cones, dried whole organically treated hop cones, dried whole untreated hop cones, and hop pellets formed from conventionally treated hops. The major finding was that only two pesticides (bifenazate and boscalid) were detected in the beers at above the level of quantification that could be analyzed statistically, and these detections were orders of magnitude below levels with any health or legal ramifications. The hop production specifications, brewing and treatment regimens, and analytical methodologies are detailed and findings discussed.

Keywords: Dry hopping, Hops, Pesticide residues, Wet hopping

Consumer demand for specialty and nontraditional beers continues to grow. Beers with a pronounced hop taste have become extremely popular among consumers. To meet this demand, breweries worldwide have been increasing their use of hops and modifying their hopping regimes. Hops are increasingly being added later in the brewing process (i.e., after the kettle-boil stage, at which hops continue to be added to provide bitterness), and an increasing number of breweries are producing wet-hopped products (i.e., introducing green, undried, high-moisture hops to the brewing process). Concurrently, consumer concern for food safety is at an all-time high. Interest in organic, sustainable, and low-input agricultural products is keen, and scrutiny of pesticide residue levels in agricultural commodities and end products continues to increase. Craft brewers are proactively responding to their selective consumer demands for sustainably produced beers.

We sought to determine whether late addition of hops impacted pesticide residue levels in beer. We sought also to determine whether pesticide residue levels in wet-hopped beers produced with undried hop cones were consistent with beers made with dried hops.

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Previous studies to quantify pesticide residues in beer have been conducted by using dried hops treated with conventional pesticides and added during the kettle-boil stage (2,4). Our study produced beers by using late additions of dried (whole and pelletized) hops and of green hops, representing conventional, organic, and untreated hops, and compared them with beers using additions of dried hops during the traditional kettle-boil stage.

EXPERIMENTAL

Hop Production

Hop production plots were established in 2013 in a 3-year-old Cascade hop research yard at Washington State University's Irrigated Agriculture Research and Extension Center (WSU-IAREC) in Prosser, WA, U.S.A. Onto these plots we applied a series of pesticide treatments, using the maximum label rates that potentially would have been applied by a conventional hop farmer in high pest pressure conditions. Consequently, although these applications were likely greater than those in a standard conventional commercially produced block, they were within the realm of possibility. Herbicides applied included preemergent conventional flat-fan boom applications of norflurazon and trifluralin, and four applications of carfentrazone-ethyl as a basal defoliant. Fungicides were applied by air blast sprayer and included two applications of dimethomorph, three applications of boscalid plus pyraclostrobin, one application of mefenoxam, one application of copper hydroxide, one application of myclobutanil, four applications of quinoxyfen, and three applications of triflumizole. One application of the insecticide imidacloprid was applied via chemigation through the drip irrigation system for aphid control, and one application each of the miticides etoxazole and bifenazate was applied by airblast sprayer for spider mite control. Hops originating from this block within our research hop yards will be referred to as "conventional," and subsequently all the beers brewed from hops originating from this block will be referred to as "conventional" as well.

Commercial organically produced Chinook hops were obtained from a grower certified organic by the Washington State Department of Agriculture and located near Moxee, WA, U.S.A. Pesticide and nutrient spray records were obtained from the grower. Insecticidal and miticidal compounds applied by airblast sprayer included one application of pyrethrins (plus saponin as an adjuvant), four applications of potassium salts of fatty acids (soap), and three applications of *Isaria fumosorosea* Apopka strain 97 (plus saponin as an adjuvant). Fungicidal compounds included one application of extract of *Reynoutria sachalinensis* and two applications of *Chromobacterium subtsugae.* No herbicidal compounds were applied. These hops and the beers that were brewed from these hops are referred to as "organic."

Concurrent to the commercial and organic hop production, an 8-year-old Cascade block at WSU-IAREC was rehabilitated. This block of hops had been used in a cover crop study that had been discontinued and therefore had not been strung for several years. Consistent irrigation was applied to this block, and organically acceptable methods were used to enrich the soil nutrients in 2012 and 2013. No pesticides had been purposefully applied to this block of hops in over 6 years. Hops harvested from this block and beers subsequently brewed with these hops are referred to as "nontreated."

Hop Harvest and Handling

Hops were harvested from the conventional and nontreated blocks grown at WSU-IAREC. Aliquots of fresh (wet, green) hops were frozen at –18°C for use in wet hopping beers. The rest of the hops were dried down to 8–10% moisture in a forced-air, propane-heated research hop kiln. These dried hops were then pressed into 454 g bricks and stored in a walk-in cooler at –18°C. Samples from the green hop aliquots, the 454 g hop blocks, and the organically produced hops were sent to the IR-4/Trace Analytical Laboratory (TAL) in Davis, CA, U.S.A., for pesticide residue analysis.

In November 2013, hops were removed from the freezer and allowed to thaw to room temperature. These hops were then pelletized in our research pelletizer and vacuum packed in plastic wrap with a Seal-a-Meal food packing device. These pelletized hops were subsequently returned to our walk-in freezer at –18°C for later use in our brewing process.

Analysis of Residues in Hops

Wet and dried hop samples were received frozen at TAL in October 2013. The hop samples were chopped in the presence of dry ice to generate a homogeneous sample suitable for analysis. In April 2014, aliquots of the samples (0.5 g of dried hop and 2.0 g of wet hop) were placed into 50 mL polypropylene centrifuge tubes, and 15 mL of acetonitrile was added. The tubes were capped and vigorously shaken for 1 min at 1,500 rpm with a Geno/Grinder 2010 shaker. The sample extract was transferred to a polymeric solid-phase extraction cartridge for cleanup. The resulting extract was concentrated, redissolved in a mixture of 10 mM ammonium acetate/methanol, and analyzed by liquid chromatography–tandem mass spectrometry (LC-MS/MS) (1).

Methodology for Residue Trial Brewing

In the winter of 2013–2014, four batches of beer were brewed per brew day. Prior to boiling, 102 L of sweet wort was prepared and divided evenly between two 68 L Blichmann Boilermaker brew kettles. The adjusted original gravity of the wort was 1.062 ± 0.003, which was fermented down to a final gravity of 1.011 ± 0.002, for a final estimated alcohol content by volume of 6.68 ± 0.52%. Hot tap water (34–38 L), at approximately 60°C, was added to each pot. Two 30.3 L pouches of William's American Light liquid malt extract were stirred into each pot, with extract residue rinsed out of each pouch with additional 60°C water and added to the pot. Additional hot water was added to each pot to bring the volume up to 51 L. The contents of the two pots were mixed by pouring sweet wort back and forth with a 5 L plastic pitcher, with approximately 11 L at a time being transferred from one kettle to the other, followed by stirring, and then transferring approximately 23 L of this mixture back to the first kettle. This process was repeated several times to allow for thorough mixing.

The mixed kettle was divided between two 30 L Boilermaker brew kettles. Each 25.5 L batch of wort was boiled for a total of 75 min, with temperature monitored to keep both pots boiling at the same pace. The postboil wort volume was approximately 19 L per batch. A Blichmann HopBlocker system with a built-in thermometer was used in each 30 L brew kettle to prevent hop material (when hops were added) from blocking taps and tubing.

After the boil, the wort was stirred continuously for 10 min to simulate a whirlpool step and then allowed to settle for 5 min. Wort was drained from the kettle and sent through a Blichmann Therminator counter-flow chiller to cool it below 26.6°C. A sample was taken immediately after chilling to measure the original gravity. After chilling, pure oxygen was bubbled into the wort at approximately 0.35 kg/cm^2 for 1 min. Two packs of Fermentis Safale US-05 yeast were added to the wort and swirled briefly to mix in the yeast before the fermenter was sealed.

Beers were ale-fermented at temperatures typically ranging from 21 to 25.5°C in Cornelius-style soda kegs. Primary fermentation lasted 7 days, at which time the wort was transferred to a sanitized, CO_2-purged keg using CO_2 pressure. After an additional 7 days, the secondary fermenter keg was placed in a 4.4°C refrigerator for several hours to clarify the beer before transfer into a sanitized, CO_2-purged serving keg. Primary fermenters were modified by cutting off the last 4 cm of the dip tube to account for the settling of yeast sediment at the bottom of the keg. Secondary fermenters were similarly modified, with the last 2 cm of dip tube removed.

Test beers were divided into four treatments with different hopping regimens. In treatment 1, hops were added to the wort 15 min after the start of boil and boiled for 60 min. In treatment 2, hops were added 70 min after the start of boil and boiled for 5 min. In treatment 3, hops were added at flameout, immediately after the end of the boil. For treatment 4, hops were not added to the brew kettle but were added to the secondary fermenting container immediately before racking. Aliquots of beer from each of these brews were then shipped to TAL for analysis. One sample was taken per replicate. Each subfermenter was a replicate; therefore, each was sampled.

Each of these four treatments was performed with green (wet) conventionally treated hops, dried whole hop conventionally treated hop cones, dried whole organically treated hop cones, dried whole untreated hop cones, and hop pellets formed from conventionally treated hops. Rates for each brewing method and hopping regime are detailed in Table I.

Analysis of Residues in Beer

Beer samples were received frozen at the TAL between June and August 2014. Prior to analysis, the beer samples were thawed and mixed thoroughly. Aliquots of beer (20 mL) were transferred into 50 mL polypropylene centrifuge tubes, and 4 g of magnesium sul-

TABLE I
Hopping Rates by Brewing Method and Type of Hop

Brewing method	Wet hops[z]	Dry hops	Pelletized hops
60 min boil	350 g (0.2985 g/hL)	70 g (0.597 g/hL)	70 g (0.597 g/hL)
5 min boil	189.2 g (0.1613 g/hL)	36.6 g (0.312 g/hL)	36.6 g (0.312 g/hL)
Flameout	365.8 g (0.3118 g/hL)	73.2 g (0.624 g/hL)	73.2 g (0.624 g/hL)
Dry or wet at 7 days	365.8 g (0.3118 g/hL)	73.2 g (0.624 g/hL)	73.2 g (0.624 g/hL)
No hops	0 g	0 g	0 g

[z] Green hops used in wet-hopping are typically five times heavier than dried hops. For this wet-hopping method, green hops were added to the beer rather than flowing the beer through a green hop "tea bag."

fate, 1 g of sodium chloride, and 5 mL of 1% acetic acid in acetonitrile were added. The tubes were capped and vigorously shaken for 1 min at 1,500 rpm with a Geno/Grinder 2010 shaker. Beer samples were placed into a centrifuge for 5 min at 4,000 rpm. The resulting upper layer of acetonitrile was sampled and diluted with a mixture of 10 mM ammonium acetate/methanol prior to analysis by LC-MS/MS. The methodology is described in the first part this manuscript series, which appears in the same volume (3).

RESULTS AND DISCUSSION

Pesticide Residues Detected in Hops

Of the pesticides applied to the conventionally treated hops, residues were detected for six, as detailed in Table II. All pesticide residues detected were substantially below tolerances (maximum residue levels) established by the U.S. Environmental Protection Agency. As expected, conventional pesticides were not detected on the organic hops, with the exception of a trace (below the analytical level of quantification) of boscalid on the wet hops. This trace was likely the result of inadvertent spray drift from nearby conventionally produced crops. Although no pesticides were purposefully applied to the nontreated hops, low-level detections were made of bifenazate (trace) and etoxazole (trace) on the wet hops and of boscalid (0.16 μg/g), spirotetramat (trace), and etoxazole (0.062 μg/g) on the dry hops. These detections were attributed to inadvertent drift of pesticides applied to a nearby wine grape vineyard (cv. Chardonnay), which was anecdotally verified by obtaining the spray records of the vineyard and correlating them with products detected.

TABLE II
Pesticide Residues Detected on Conventionally Treated Hops (μg/g)[z]

Chemical	Wet hops	Dry hops	U.S. tolerance
Imidacloprid	ND	ND	6
Mefenoxam	ND	ND	4
Boscalid	1.75	6.46	35
Dimethomorph	ND	ND	60
Bifenazate	4.56	8.62	15
Spirotetramat	ND	ND	10
Carfentrazone-ethyl	ND	ND	0.1
Pyraclostrobin	0.545	1.36	23
Triflumizole	0.127	0.191	11
Quinoxyfen	0.246	0.389	3
Etoxazole	0.070	0.185	7

[z] Nondetection (ND) indicates that no residues were detected at levels above the analytical detection ability. Limit of detection = 0.0225 μg/g, and limit of quantitation = 0.05 μg/g.

Pesticide Residues Detected in Beer

In the final analysis for pesticide residues present in beer, only two pesticides that were detected above the level of quantitation could be analyzed statistically. These were bifenazate and boscalid. The results of this analysis are detailed in Table III. The contribution of residues of boscalid and bifenazate by dry hopping with conventional hops was consistent between using dried whole-cone and dried pelletized hops. Trends within hopping regimes in beers brewed with dry whole-cone or dry pelletized hops indicate that boiling time reduces boscalid and bifenazate residue levels, but this was only statistically significant ($P < 0.05$) in the conventional whole-cone beers. A similar trend was observed with wet hopping with timing at 7 days after boil during fermentation contributing the most boscalid and bifenazate residues detected in beers.

In the magnitude of pesticide residue tests performed on beers brewed with no hops added, it was determined that the extract contributed a small amount of boscalid. The affected values were corrected in the results presented in Table III.

TABLE III
Pesticide Residues of Boscalid and Bifenazate Detected in Beers (ng/mL)[u]

Hop source	Brewing technique	Boscalid	Bifenazate
Untreated	60 min boil	ND	ND
Untreated	5 min boil	ND	ND
Untreated	Flameout	ND	0.06 ± 0.06
Untreated	Dry hop at 7 days[v]	ND	ND
Organic	60 min boil	0.21 ± 0.21[w]	ND
Organic	5 min boil	0.37 ± 0.37	ND
Organic	Flameout	0.39 ± 0.39	ND
Organic	Dry hop at 7 days	0.74 ± 0.74	ND
Conventional dry[x]	60 min boil	6.83 ± 0.87	7.30 ± 0.97a
Conventional dry	5 min boil	5.79 ± 1.07	6.10 ± 0.82a
Conventional dry	Flameout	8.60 ± 0.58	7.77 ± 0.83ab
Conventional dry	Dry hop at 7 days	8.96 ± 0.90	9.93 ± 0.68b
Conventional pellet[y]	60 min boil	5.95 ± 1.51	7.35 ± 0.87
Conventional pellet	5 min boil	4.86 ± 0.88	6.05 ± 0.86
Conventional pellet	Flameout	8.28 ± 0.85	6.83 ± 1.00
Conventional pellet	Dry hop at 7 days	8.32 ± 1.53	8.41 ± 0.67
Conventional wet[z]	60 min boil	8.28 ± 1.91a	10.30 ± 1.81a
Conventional wet	5 min boil	8.67 ± 2.31a	11.07 ± 2.28a
Conventional wet	Flameout	11.11 ± 2.19ab	11.77 ± 1.98a
Conventional wet	Wet hop at 7 days	17.27 ± 3.84b	22.48 ± 4.24b

[u] Mean squares from the ANOVA were 100.2 and 64.9 for boscalid and bifenazate, respectively, with degrees of freedom (df) at 19 and 64 and error terms 7.3 and 6.6, respectively. Analysis of variance mean square values for the quantity of boscalid are corrected for the 2.46 ng/g that were detected in beers brewed with extract A. Limit of detection = 0.225 ng/mL, and limit of quantitation = 0.5 ng/mL. ND = not detected.

[v] After 7 days of primary fermentation without hops, hops were added and remained for 7 days during secondary fermentation.

[w] All values are in nanograms per gram (ng/g).

[x] One-way ANOVA of brewing technique within conventional dry hops: df = 3,12, $F = 2.92$, and $P > 0.05$ for boscalid and $F = 3.68$ and $P < 0.05$ for bifenazate. Means of residues detected in beers not followed by a common letter are significantly different at $P < 0.05$.

[y] One-way ANOVA of brewing technique within conventional pelletized hops: df = 3,12, $F = 3.64$, and $P > 0.05$ for boscalid and $F = 1.96$ and $P > 0.05$ for bifenazate.

[z] One-way ANOVA of brewing technique within conventional wet hops: df = 3,12, $F = 3.41$, and $P < 0.05$ for boscalid and $F = 4.35$ and $P < 0.05$ for bifenazate. Means of residues detected in beers brewed with a common hop source not followed by a common letter are significantly different at $P < 0.05$.

In beers brewed with both conventional dry hops and wet hops there were several trace detections of imidacloprid and pyraclostrobin, but these were below the level of detection that could be analyzed statistically.

CONCLUSIONS

Wet hopping with the hops grown under the conventional pesticide treatment regime consistently made the greatest contribution of boscalid and bifenazate to the beers compared with beer brewed with the conventionally treated dry or conventionally treated pelletized hops. However, in all cases detections were far below levels with any health or legal ramifications. Wet hopping is not a traditional method for hopping, and it is used seasonally in a small minority of beers.

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