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Comparative chemical attributes of native North American hop, *Humulus lupulus* var. *lupuloides* E. Small

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Abstract

The genetic diversity of 159 representative genotypes of native hop (*Humulus lupulus* var. *lupuloides* E. Small, Cannabaceae) from 34 selected populations was assessed by relative magnitudes and ranges of alpha acids (AA), beta acids (BA), and the cohumulone (CoH) component of alpha acids, with reference to temporal changes between 1989–1990 and 2001, and to the same attributes in American and European hop cultivars, principally *H. lupulus* var. *lupulus* L. Chemical profiles of these genotypes were generated by high pressure liquid chromatography (HPLC) of methanol extracts from their processed samples (cones). The alpha ratio (AR, alpha acids / alpha + beta acids) measured the degree to which alpha acids predominated in cone extracts. Synchronous ranges of AR and CoH were also selected for graphic portrayals of native hop genotypic diversity. Cones sampled and analyzed from eight populations that were accessible in both 1989 and 2001 were distinct in chemical attributes, indicating a succession of genotypes, and suggesting temporal cycling of *H. lupulus* var.*lupuloides* germplasm. The principal distinctions between the two sub-species were a markedly higher proportion of CoH (38–88% vs. 19–41%) in alpha acids of *H. l.* var. *lupuloides*, and generally higher concentrations of AA in cultivars of both American and European commercial hop cultivars, predominantly *H. lupulus* var. *lupulus*. All of the 159 native hop genotypes also contained detectable levels of xanthohumol and xanthogalenol, prenylflavonoids recently reported to have mammalian anti-cancer activity. Some native genotypes had previously exhibited natural repellence of insect and mite pests; thus *H. lupulus* var. *lupuloides* germplasm offers a diverse resource of underutilized and yet undefined biochemicals.
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Keywords: Hop; *Humulus lupulus* var. *lupuloides*; Cannabaceae; Genotypes; Chemotypes; Resins; Lupulin; HPLC; Alpha and beta bitter acids; Alpha ratio; Cohumulone; Xanthohumol; Xanthogalenol; Prenylflavonoids

1. Introduction

A native North American hop, *Humulus lupulus* L. var. *lupuloides* E. Small, Cannabaceae, as implied by its name, is more closely related to European hop (*H. lupulus* var. *lupulus*) than the two other North American sub-species, *H. lupulus* var. *neomexicanus* Nelson and Cockerell and *H. l.* var.*pubescens* E. Small (E. Small, 1978, 1980, 1981). A recent characterization of this native hop in upper mid-western North America (Hampton et al., 2001), indicates that these native plants comprise valuable gene sources for improving commercial hop cultivars and as unutilized biochemical (biopesticidal) sources of insect repellency factors, described for other plant species by Devakumar and Parmar (1993). Natural and selected genotypes of native hop repelled chewing insects (*Melanoplus spp.*, subfamily *Plusiinae*), and also repelled sucking plant pests including the two-spotted mite (*Tetranychus urticae* Koch) and the hop aphid (*Phorodon humuli* Schrank). The cones of one genotype of this taxonomic variety also resisted natural bio-degradation for two years (Hampton et al., 2001). Current preliminary evidence also indicates that this sub-species contains genes resistant to numerous pathotypes of powdery mildew [*Sphaerotheca humuli* DC. (Burr.)] that threaten hop production, world-wide (personal communication, Anton Lutz, Hop Research Institute, Huell, Germany).

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The first recorded use of *H. lupulus* var. *lupuloides* for breeding purposes (i.e., new cultivar development) was that of E.S. Salmon (1906–1951), Wye College, Kent, England (Salmon and Amos, 1908; Salmon, 1917, 1934, 1938) in the use of "English-grown American" (Barth et al., 1994), also called "Manitoba Seedling", from which were derived many of the world's most significant past and present high-alpha hop cultivars.

Hops (i.e., the female inflorescenses, or cones, or strobili), historically, have been used for brewing, as a preservative and for improving flavor (Barth et al., 1994; Neve, 1996). The chemical components of hops (Neve, 1996) were gradually elucidated, including early work at the Carlsberg Laboratory, Copenhagen, and brewing trials in many places, including East Malling, Kent, England (Ford, 1932). The essential components of mature cones are glandular trichomes, referred to as lupulin, that contain resins, oils and polyphenols. These constituents contribute to the flavor and aroma of beer. Xanthohumol and xanthogalenol, of recent interest because of mammalian anti-cancer activity (Buhler et al., 1999; Henderson et al., 1998; Miranda et al., 1999), also constituents of hop resins, were traced to specific hop origins and characterized by Stevens et al. (2000).

For the sake of relevance to research and to the hop and brewing industries, individual hop plants in this paper are designated as genotypes and as chemotypes, the latter based on their content of alpha acids, beta acids, and the cohumulone component of alpha acids, i.e., the basis for plant comparisons within and between hop populations, 1989 and 2001, and between these plants and representative American and European hop cultivars.

2. Results

2.1. Chemical traits of 50 native hop genotypes, 1989, relative to hop cultivars

Chemical distinctions among fifty 1989 native hop genotypes from 10 populations (Table 1) are shown as variations in % alpha acids (AA), % beta acids (BA), alpha ratio (% alpha acids/% alpha + % beta acids) (AR), and as % cohumulone (CoH), the percentage of cohumulone comprising alpha acids, relative to those in commercial hop varieties.

Maximal percentages of AA among these genotypes occurred at Mohall-W (7.33), Indian Head-E (7.05), and at Northgate-E (6.51). Maximal percentages of BA occurred at Oxbow-S (6.26), Mohall-W (6.09), and Indian Head-E (6.08). Maximal values of AR in these populations ranged from 67 (Mohall-W) to 38 (Oxbow-S) and those of CoH from 87 (Burlington-N) to 54 (Logan-N). Corresponding values for hop cultivars (Table 1), American and European, respectively, were 14.60 and 12.20 (AA), 6.90 and 6.30 (BA), 78 and 72 (AR), and 53 and 40 (CoH). CoH quantities among American cultivars ranged from 19 to 53 and from 22 to 40 among the selected European cultivars (see Table 1 footnote regarding the cultivar Talisman). Values of coefficients of variation (cv) were comparable for native genotypes and hop cultivars (i.e., greater AA and BA values than AR and CoH; with similar ranges).

2.2. Chemical traits of 109 native hop genotypes, 2001

The levels of chemicals detected among 109 Year 2001 native hop genotypes from 24 populations (Table 2) were generally less than in those of 1989. Maximal percentages of AA among genotypes sampled in 2001 occurred at Qu'Appelle-2 (5.65), White Earth-S (5.18), and Grenfell-N (4.51). Maximal percentages of BA occurred at Souris-E2 (5.10), Glen Ewen-S (4.98), and White Earth-S (4.92). Values of AR in these populations ranged from 18 (Oakville-W) to 65 (Northgate-E) and those of CoH from 88 (Grenfell-N) to 38 (Minot-E). Minimal values of all attributes were markedly lower for 2001 than for 1989 samples.

In comparing 1989 and 2001 data, AA values for native hop genotypes ranged from 7.33% (Mohall-W) to 1.13% (Oxbow-S, 1989) and from 5.65% (Qu'Appelle-2) to 0.22% (Burlington-N2, 2001) (Tables 1 and 2), whereas those of 34 commercial hop cultivars ranged from 14.60 to 3.70% (Table 1). Likewise, the percentage of beta acids for native genotypes (both 1989 and 2001) and hop cultivars ranged, respectively, from 6.26 to 0.68% and 6.90 to 2.50% . And the ranges of AR values of native genotypes and hop cultivars were 67–18% and 78–43%, respectively; counterpart contrasts in CoH values were 88–38% vs. 41–19%, except for the cultivar 'Talisman' (see Table 1 footnote). The values of attribute cv's ranged slightly higher in 2001 samples than in 1989 samples, particularly cv values for AA.

2.3. Attribute comparisons, 1989 and 2001 samples from eight populations

There were consistent distinctions in attribute mean-values among genotypes of eight native hop populations available for sampling in both 1989 and 2001 (Table 3; see footnote). With only two exceptions in 32 1989 and 2001 data comparisons (% Cohumulone, Souris-E and Logan-N, 2001), there were higher average values of the four chemical attributes in genotypes of the 1989 populations; in some cases, by factors of >3-fold. Such contrasts indicate a temporal chemotypic / genetic shift, and suggest cyclic trends of genotypes and plant-chemical concentrations associated with natural and/or land-use factors affecting habitats occupied by *H. lupulus* var. *lupuloides* (Hampton et al., 2001).

Table 1
Chemical attributes of cone extracts from North American native hop plants, *Humulus lupulus* var. *lupuloides*, 1989

Name of population	No. of plants	% Alpha acids				% Beta acids				Alpha ratio[b]				% Cohumulone[c]			
		Min	Max	Mean	10×[a] cv	Min	Max	Mean	10× cv	Min	Max	Mean	10× CV	Min	Max	Mean	10× cv
Souris-E	1 (1)	0[d]	0	2.9	0	0	0	2.2	0	0	0	68	0	0	0	54	0
Logan-N	2 (2, 3)	5.12	5.52	5.34	0.49	3.88	4.01	3.94	0.23	56	59	57.5	0.37	46	54	50.0	1.13
Minot-E	8 (4–11)	0.86	4.93	2.85	5.19	1.09	2.93	2.48	3.79	38	65	51.8	1.92	40	61	51.6	1.43
Burlington-N	8 (12–21)[e]	0.86	4.00	2.48	4.15	1.27	3.64	2.50	3.12	32	60	48.5	1.90	49	87	59.4	2.08
Mohall-W	7 (22–28)	1.57	7.33	4.62	4.42	1.73	6.09	3.72	3.95	38	67	54.3	1.95	51	67	57.0	0.95
Northgate-E	10 (29–38)	1.34	6.51	3.70	4.24	1.80	4.83	2.63	3.42	43	63	57.3	1.23	58	77	67.2	0.80
Glen Ewen-S	1 (39)	0	0	3.5	0	0	0	3.8	0	0	0	47	0	0	0	60	0
Oxbow-S	6 (40–45)	1.13	3.62	2.04	6.03	1.98	6.26	3.64	5.22	30	38	35.2	0.81	45	74	54.2	2.56
Midale-W	1 (46)	0	0	6.1	0	0	0	3.3	0	0	0	65	0	0	0	56	0
Indian Head-E	6 (47–52)	3.09	7.05	4.50	3.22	3.05	6.08	4.21	4.22	42	55	51.3	0.97	62	72	67.8	0.52
Total	50																
Averages of means				1.20	5.57	3.80	2.11	4.83	3.24	39.9	58.1	53.6	1.31	50.1	70.3	57.7	1.35
Averages of cv × 10					3.96				3.42								
Am. Hop Cultivars	14	6.10[f]	14.60	10.08	3.12	3.10	6.90	4.81	2.64	51	78	66.7	1.14	19	53[f]	35	2.51
Eur. Hop Cultivars	20	3.70	12.20	6.98	3.65	2.50	6.30	4.22	2.87	43	72	62.1	1.36	22	40	27.6	2.09
Averages of cv × 10					3.38				2.76				1.25				2.30

American hop cultivars include: Cascade, Centennial, Chinook, Cluster, Columbia, Comet, Eroica, Galena, Horizon, Mt. Hood, Nugget, Olympic, Talisman, and Willamette. European hop cultivars include: Bramling, Brewer's Gold, Bullion, Fuggle, Hallertauer, Hersbrucker, Huller Bitter, Landhopfen, Northern Brewer, Perle, Pride of Kent, Progress, Record, Saazer, Spalter, Styrian, Tettnanger, Wye Challenger, and Wye Target.

[a] cv = Coefficient of variation = standard deviation / mean of attribute values; cv is a measure of sampled-plant diversity, allowing comparisons of attributes of differing numeric ranges. The multiple, C × 10, simplifies comparisons of diversity among chemical attributes within and among plant populations, and between native genotypes and hop cultivars.

[b] Alpha ratio = alpha acids / alpha + beta acids.

[c] % Cohumulone = the proportion of cohumulone in total alpha acids, i.e., relative to humulone + adhumulone.

[d] Only a single plant in each of three populations produced the number of cones required for analysis; the value for each is entered as the sample mean.

[e] Cone-sample numbers 14 and 16 were damaged; thus were discarded.

[f] Note that maximal values of alpha acids in native hop-plant cones approximate minimal alpha acid values for American hop cultivars. Conversely, minimal values for cohumulone (CoH) in cones of native hop plants approximate the maximal CoH values of all European and most American hop cultivars. The 53% CoH content of the American hop cultivar Talisman was the highest of any hop selection considered acceptable for brewing, i.e., 41% CoH (e.g., Cluster, Comet, Eroica) has generally been an upper limit. Most cultivars containing >35% CoH included native North American hop in their parentage.

Table 2
Chemical attributes of cone extracts from North American native hop plants, *Humulus lupulus* var. *lupuloides*, 2001

Name of population	No. of plants	% Alpha acids				% Beta acids				Alpha ratio[b]				% Cohumulone[c]			
		Min	Max	Mean	10×[a] cv	Min	Max	Mean	10× cv	Min	Max	Mean	10× cv	Min	Max	Mean	10× cv
Logan-N	5 (1–5)	0.53	2.10	1.23	5.04	0.82	3.33	1.84	5.27	37	43	34.0	0.61	44	67	52.4	1.88
Minot-E	6(6–11)	0.40	2.02	1.07	4.86	0.97	2.21	1.64	3.23	28	51	38.5	2.56	38	53	48.2	1.09
Burlington-N	7(12–18)	0.43	1.58	0.88	5.45	0.77	2.91	1.63	4.05	22	47	34.0	2.73	51	61	55.1	0.56
Burlington-N2	6(19–24)	0.22	1.83	0.81	6.91	1.21	2.54	1.52	3.88	21	42	32.3	2.18	40	60	48.5	1.66
White Earth-S	5 (25–29)	1.32	5.18	2.98	5.27	1.53	4.92	2.94	4.83	45	53	49.6	0.63	55	61	58.0	0.42
WhiteEarth-S2	6(30–35)	0.40	2.47	1.47	5.31	0.78	2.44	1.92	3.12	34	52	41.2	1.90	47	53	49.3	0.42
Little Knife	5(36–40)	0.59	2.71	1.34	6.04	0.85	1.91	1.24	3.31	36	59	49.6	1.78	45	59	51.8	1.15
Stanton-W	1 (41)	0	0	1.4	0	0	0	1.8	0	0	0	44	0	0	0	60	0
NorthGate-E	6(42–47)	0.38	2.17	1.07	5.89	0.88	1.75	1.28	2.19	24	65	43.0	2.52	46	58	53.7	0.99
GlenEwen-S	6(48–53)	0.44	3.20	1.54	5.65	1.53	4.98	2.31	4.11	23	50	38.7	2.57	53	62	56.7	0.68
Oxbow-S	6(54–59)	0.33	3.29	1.24	9.03	1.00	4.30	2.27	5.24	25	58	41.0	2.87	48	74	58.7	1.71
Indian Head-E	3 (60–62)	0.64	1.19	0.89	3.15	0.76	1.52	1.13	3.36	35	51	44.0	1.86	53	59	56.3	0.54
Bridge-2S	3 (63–65)	1.20	3.05	2.10	4.43	0.93	2.81	1.81	5.25	52	56	54.3	0.38	48	67	55.0	0.90
2nd Bridge-N	1(66)	0	0	1.1	0	0	0	2.18	0	0	0	42	0	0	0	57	0
Pheasant Creek-W	3(67–69)	1.21	1.71	1.42	1.83	1.14	2.36	1.86	3.44	39	51	44.0	1.42	45	57	52.7	1.26
Bridge3	2(70,71)	0.78	1.24	1.01	3.27	0.97	1.11	1.04	0.96	45	53	40.0	1.16	48	60	54,0	1.57
Qu'Appelle-2	5 (72–76)	0.58	5.65	2.06	9.90	1.75	3.99	2.62	3.17	25	59	38.0	3.36	39	55	48.2	1.35
Qu'Appelle-3	6(77–82)	0.40	3.77	1.28	9.92	0.68	2.66	1.26	6.27	35	59	46.2	1.69	42	78	59.7	2.21
Grenfell-N	6(83–88)	2.33	4.51	3.30	2.20	1.81	4.65	2.66	3.83	49	59	56.0	0.64	73	88	78.5	0.73
Melville-S	5(89–93)	0.73	3.63	1.91	7.17	0.69	3.23	1.62	6.36	45	64	52.0	1.37	47	84	65.6	2.14
Crooked Lake-W	3 (94–96)	0.53	1.06	0.72	4.03	1.03	2.06	1.45	3.72	32	34	33.3	0.35	46	53	50.7	0.80
Oakville-W	6(97–102)	0.35	1.37	0.72	5.42	0.80	2.69	1.62	4.32	18	35	30.5	2.12	47	65	54.8	1.07
Carroll-S	1(103)	0	0	1.9	0	0	0	1.7	0	0	0	53	0	0	0	50	0
Souris-E2	6(104–109)	1.24	3.62	2.23	4.62	1.33	5.10	2.93	5.84	39	52	44.7	1.06	49	65	55.8	1.31
Total	109																
Averages of means		0.72	2.73	1.51		1.06	3.02	1.84		33.8	52.0	42.7		47.8	63.8	55.4	
Averages of cv × 10					5.49				4.08				1.70				1.16

[a] cv = Coefficient of variation = standard deviation / mean of attribute values; cv is a measure of sampled-plant diversity, allowing comparisons of attributes of differing numeric ranges. The multiple, C × 10, simplifies comparisons of diversity among chemical attributes within and among plant populations, and between native genotypes and hop cultivars.

[b] Alpha ratio = alpha acids / alpha + beta acids.

[c] % Cohumulone = the proportion of cohumulone in total alpha acids, i.e., relative to humulone + adhumulone.

2.4. AR and CoH data points, 1989 and 2001, inter- and intra-population chemotypes

Graphic arrays of AR and CoH data points, 1989 and 2001, visually illustrate the phenomenon of phytochemical (genetic) relatedness or diversity among the 159 native plant genotypes compared (Figs. 1 and 2). Interactions between AR and CoH values among 34 populations in contiguous regions illustrate measured ranges of chemotype diversity within and between populations.

These arrays distinguished 156 of the 159 genotypes, excepting plant numbers 42 and 43 (Fig. 1) and 9 and 16 and 94 and 95 (Fig. 2) (enclosed by ovals), connoting genetic affinity within or between (i.e., 9 and 16) populations. In each of these cases, however, non-identity was indicated by numeric distinctions of alternate attributes, as follows. Cones from genotypes 42 and 43 (Fig. 1) produced distinct AA and BA values, respectively, of 1.13 and 1.98 vs. 1.46 and 2.62 and genotypes 94 and 95 (Fig. 2) produced AA and BA values, respectively, of 0.53 and 1.03, vs. 1.06 and 2.06. Likewise, cones from genotypes 9 and 16 (Fig. 2) produced AA and BA values, respectively, of 0.99 and 2.21, vs. 0.59 and 1.31. Inadvertent sampling of cones from the same genotype was precluded either by marked phenotypic distinctions between plants/cones or by a 5-m separation of sampled plants (Hampton et al., 2001).

Groups of shaded sample numbers (10 in 1989; 20 in 2001) represent chemotype resemblances within populations. Also shown are many examples of chemotype similarities of samples from distant populations, e.g., cones from 1989 genotype No's 5 and 46, from Minot-E and Midale-W. The latter demonstrates chemical traits shared by the sub-species, across populations. Note similar examples from 2001 genotypes 29 and 109, from White Earth-S and Stanton-W, as well as that of other genotype numbers barely outside shaded areas (Figs. 1 and 2), but from distinct populations. In essence, Figs. 1 and 2 exemplify both native hop-plant relatedness within populations and conversely the chemotypic (genetic) diversity among plants sampled from 34 populations.

2.5. Prenylflavanoids, xanthohumol and xanthogalenol, in 2001 samples

Cones sampled in 2001 were also analyzed for the presence of xanthohumol and xanthogalenol. All 109 cone samples contained detectable quantities of both chemicals. The ranges of each, respectively, were 0.17–0.02% and 0.17–0.01%. The samples of Stevens et al. (2000) from North Dakota (N_0 and N_7) are most likely representative of *H. lupulus* var. *lupuloides* and fall within the "majority-tested" category (containing 4′-*O*-methylchalcones), inclusive of all native North American genotypes tested by them.

3. Discussion

The geographic area explored since 1982 and sampled in these studies spans major portions of the Souris River and Qu'Appelle River watersheds and lesser portions of the Missouri River and Assiniboine River watersheds, comprising a riparian distance of some 920 km. Sampling of populations for definitive informational development was contingent on survival and stability of plant numbers per study site, as well as the percentage

Table 3
Attribute changes per population, between 1989 and 2001, illustrating genetic shifts during plant replacements by gametic reproduction, i.e., seeds

Name of population	Mean values of chemical attributes							
	% Alpha acid		% Beta acid		Alpha ratio		% Cohumulone	
	1989	2001	1989	2001	1989	2001	1989	2001
Souris-E[a]	2.93	2.23	2.16	2.93	68	44.7	54	55.8
Logan-N	5.34	1.23	3.94	1.84	57.5	34.0	50.0	52.4
Minot-E	2.85	1.07	2.48	1.64	51.8	38.5	51.6	48.2
Burlington-N	2.48	0.88	2.50	1.63	48.5	34.0	59.4	55.1
Northgate-E	3.70	1.07	2.63	1.28	57.3	43.0	67.2	53.7
Glen Ewen-S	3.46	1.54	3.85	2.31	47	38.7	60	56.7
Oxbow-S	2.04	1.24	3.64	2.27	35.2	41.0	54.2	58.7
Indian Head-E[a]	4.50	3.15	4.21	1.13	51.3	44.0	67.8	56.3
Average of means	3.29	1.55	3.18	1.88	52.08	39.74	58.02	54.61
Difference in means, 1989 and 2001	1.70		1.30		12.34		3.41	
Average change (%) per attribute	51.7		40.9		23.7		5.88	

[a] At both of these locations, populations sampled in 1989 had not survived in 2001. Alternate populations/samples (1.8 km downstream from that of 1989 Souris-E, and 70 m upstream from that of 1989 Indian Head-E) are thus presented for 2001.

of cone-bearing plants available per year (rarely >10%), per site. Consequently, multiple visits over decades of time were required for quasi-morphotypic or chemotypic characterizations of this native *H. lupulus* sub-species.

Genotypic shifts over time had been observed by the first author since 1982, as losses of individual plants and year-to-year changes in extant plants and their cone morphologies. Such changes were difficult to effectively document and to precisely portray. It is fortunate, therefore, that this temporal variance can be quantitated and represented as measurements of recognized *Humulus*-chemical constituents.

These data and related information suggest that our arrays of chemical attributes from 34 populations may approximate the breadth of *H. lupulus* var. *lupuloides* diversity. We encourage, and look forward to, further studies of genetic and biochemical diversity for this sub-species.

Priority challenges for future information development of *H. lupulus* var. *lupuloides* include (a) the isolation, purification, chemical characterization and synthesis of chemical components that repel chewing and sucking insect pests (Hampton et al., 2001; Devakumar and Parmar, 1993), (b) extend and compare genotypic and chemical characterizations among all three North American *Humulus lupulus* sub-species and (c) examine sub-species intergradations in probable zones of sub-species convergence.

4. Experimental

4.1. Exploration and selection of populations and plant genotypes, 1982–2001

Incidence of *H. lupulus* var. *lupuloides* populations was generally sporadic over large watershed areas, with greatest abundance and diversity among riparian ecosystems of the Souris and Qu'Appelle rivers and their tributaries. Study sites were selected for investigation based on habitat access, evidence of population survival and fruitfulness, and wherever possible for plant- and

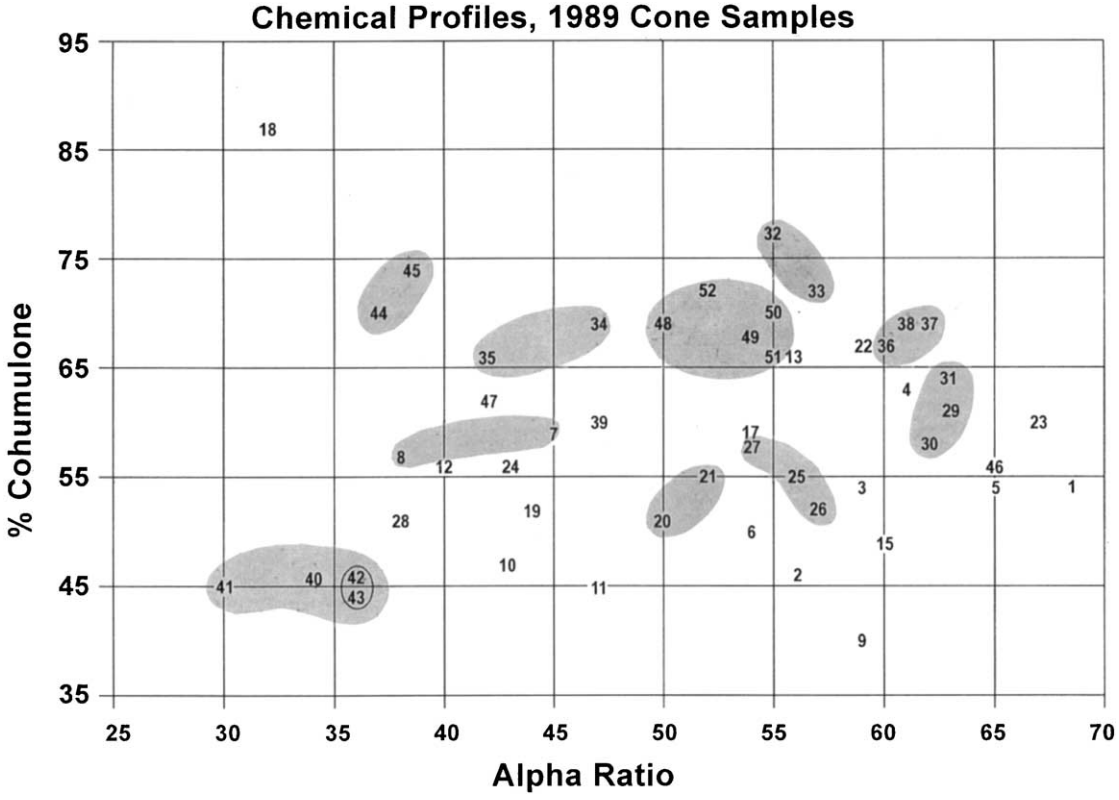

Fig. 1. Chemical profiles of 1989 cone samples. Distribution of AR vs. CoH data points from 50 cone samples representing 10 populations of *Humulus lupulus* var. *lupuloides* sampled and analyzed in 1989 (samples 14 or 16 rejected). Alpha Ratio ranged from 30 (Plant 41) to, 68 (Plant 1) and% cohumulone ranged from 40 (Plant 9) to 87 (Plant 18). Shaded areas denote plant chemotype clusters, within populations. Populations were: Souris-E, Sample No. 1; Logan-N, Nos. 2 and 3; Minot-E, 4–11; Burlington-N, 12–21 (excluding Samples 14 and 16); Mohall-W, 22–28; Northgate-E, 29–38; Glen Ewen-S, 39; Oxbow-S, 40–45; Midale-W, 46; and Indian Head-N, 47–52 (Ref. Table 1). Populations at Northgate-E and Oxbow-S are depicted as three and two distinct chemotype clusters, respectively. The oval enclosure of samples 42 and 43 indicates identical values of alpha ratio (36) and % Cohumulone (45); however, the plants/cones were distinct by % alpha acids (1.13 vs. 1.46) and % beta acids (1.98 vs. 2.62).

cone-type diversity. Though some populations survived throughout this period, individual plants usually survived no more than five seasons, and often only 2 or 3, depending upon habitat conditions, including climatic, soil-moisture, biospheric, or land-use detriments.

Cones were typically collected in mid-August, for optimal lupulin development and yield. Collected cones were stored loosely in paper bags, in ice chests, until express-shipped to the hop chemistry laboratory, Oregon State University.

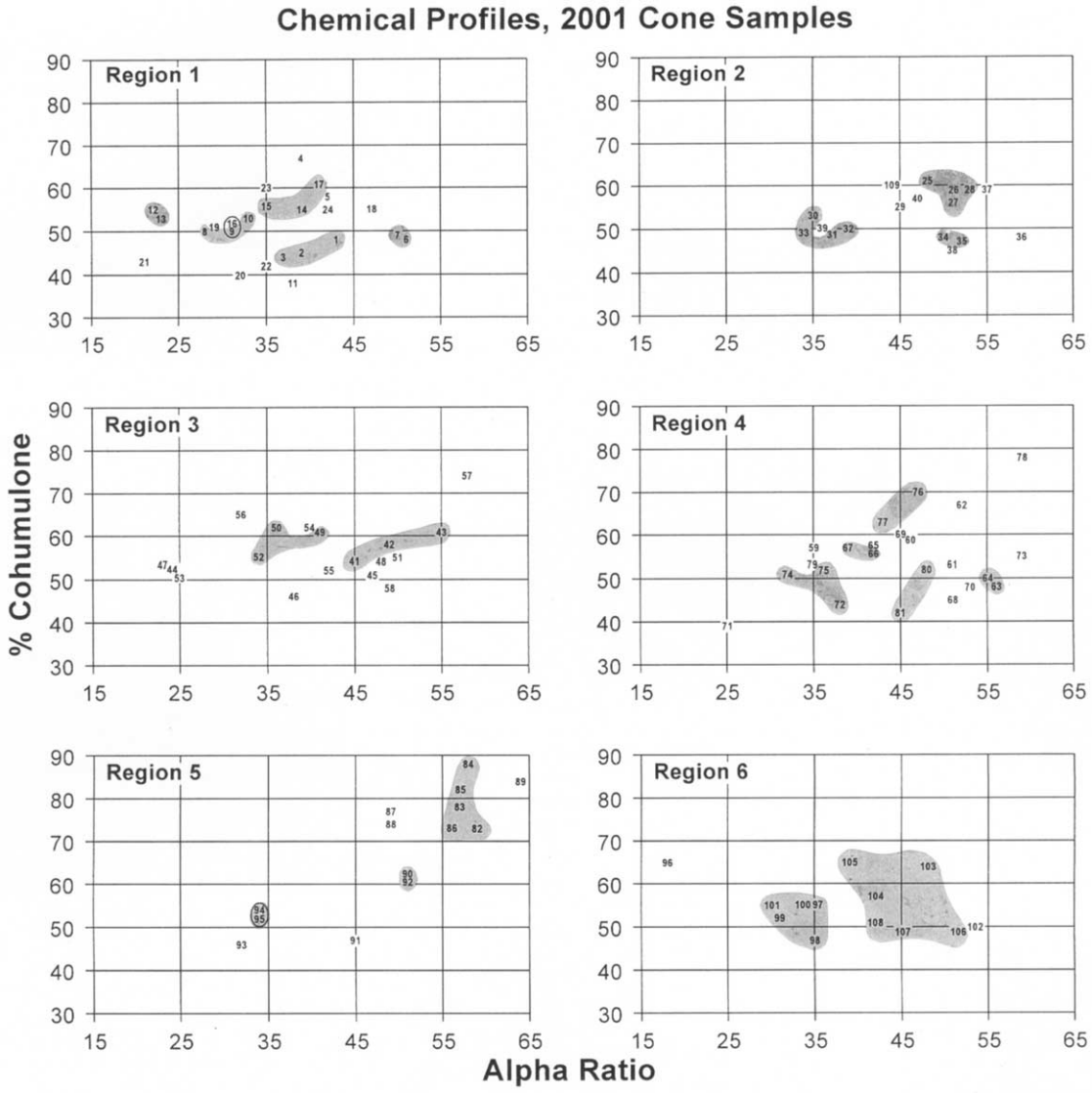

Fig. 2. Chemical profiles of Year 2001 cone samples. Distribution of AR vs. CoH data points from 109 cone samples representing 24 populations of *Humulus lupulus* var. *lupuloides*, sampled and analyzed in 2001. Alpha ratios ranged from 18 (Plant 96, Region 6) to 64 (Plant 89, Region 5), and% cohumulone ranged from 38 (Plant 11, Region 1) to 88 (Plant 84, Region 5). Shaded areas denote chemotype similarities within populations. Region 1 represents four populations: Logan-N (Sample Nos. 1–5; see Table 2), Minot-E (6–11), Burlington-N (12–18), and Burlington-N2 (19–24) of the mid-Souris River watershed (Minot, ND area). Region 2 represents four populations: White Earth-S (25–29), White Earth-S2 (30–35), Little Knife 36–40), and Stanton (109), of the Missouri River watershed (Stanley and Stanton, ND areas). Region 3 represents three populations: Northgate-E (41–46), Glen Ewen-S (47–52), and Oxbow-S (53–58) of the upper Souris River watershed (ND-Saskatchewan boundary area). Region 4 represents 7 populations: Indian Head-N (59–61), Bridge-2S (62–64), 2nd Bridge-N (65), Pheasant Creek-W (66–68), Bridge 3 (69–70), 2 Qu'Appelle (71–75), and 3 Qu'Appelle (76–81) of the mid-Qu'Appelle River watershed (SE Saskatchewan area). Region 5 represents three populations: Grenfell-N (82–87), Melville-S (88–92), and Crooked Lake-W (93–95), downstream 22 km from the east-most Qu'Appelle River population of Region 4. Region 6 represents Oakville-W (96–101) of the Assinaboine River watershed, below its confluence with the Souris River, plus two populations of the lowermost portion of the Souris River, Carroll-S (102) and Souris-E2 (103–108)(Ref. Table 2). The populations at Minot-E (Region 1) and at White Earth-S2 (Region 2) both generated two diverse chemotype clusters. The oval enclosure of Nos. 9 and 16 indicate identical values of AR and CoH (31 and 51), and of Nos. 94 and 95, with identical values of AR and CoH (34 and 53); however, the two sets of plants were distinct by AA content (0.99 vs. 0.59) and (0.53 vs. 1.06), respectively, and by BA content (2.21 vs. 1.31) and (1.03 vs. 2.06). Note sample 109 (Stanton), included in Region 2, Missouri River watershed.

4.2. Sample processing

Green cone samples were received in the laboratory, placed into small paper bags (No. 2 or 3) and kept in frozen storage until processed. For analysis, frozen samples were dried at 50 °C for 4–6 h, with constant air flow. After equilibration to room temp. and humidity, the bags were weighed. The cones were transferred to a 100 ml Waring Blender cup and each paper bag, minus cones, was re-weighed to obtain the cone weight. MeOH (50 ml) was added to the blender cup and the sample was extracted for 2 min at maximum blender speed. The MeOH extract was vacuum filtered through a regenerated cellulose filter (Alltech, #656117, 0.45 μm pore size) and diluted 1–10 in MeOH for HPLC analysis.

4.3. Sample analysis

HPLC separation of constituents was performed using a 15 cm × 3.1 mm 5u Nucleosil RP C18 column with 0.6 ml/min and MeOH:water:phosphoric acid (85:17:0.25) at 50 °C. These conditions optimize separation of humulone + adhumulone, colupulone, and lupulone + adlupulone, xanthohumol, and xanthogalenol. The ASBC (American Society of Brewing Chemists) standard extract ICE-2 was used to calibrate alpha acids (cohumulone, humulone + adhumulone) and beta acids (colupulone, lupulone + adlupulone). Purified xanthohumol from J. Fred Stevens, Chemistry Department, Oregon State University was used to quantitate xanthohumol and xanthogalenol.

4.4. Data analysis

Laboratory data are presented as comparisons of ranges and means of four selected chemical attributes (Tables 1–3). Scatter graphs were prepared from values of AR and CoH, to visually portray cone chemistry heterogeneity for each of 159 native hop genotypes.

Data in Tables 1 and 2 were analyzed by use of coefficients of variation (cv), a statistic not providing levels of statistical significance but that, instead, validated variance comparisons among numeric values of markedly different ranges (i.e., those precluding comparative use of standard deviations). The *t*-test for probability estimates of significance was applied in differentiating chemical attribute averages per population, i.e., populations available for sampling, in both 1989 and 2001 (Table 3). However, the largest distinctions of attributes between the 2 years were those of AA at Logan-N, and none were significant at $P = 0.10$.

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