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Influence of Cultivation Site on Sesquiterpene Lactone Composition of Forage Chicory (*Cichorium intybus* L.)

JOYCE G. FOSTER,*,† WILLIAM M. CLAPHAM,† DAVID P. BELESKY,† MARIA LABREVEUX,‡,§ MARVIN H. HALL,‡ AND MATT A. SANDERSON||

Appalachian Farming Systems Research Center, Agricultural Research Service, U.S. Department of Agriculture, 1224 Airport Road, Beaver, West Virginia 25813-9423, Department of Agronomy, The Pennsylvania State University, 116 Agricultural Science and Industry Building, University Park, Pennsylvania 16802-3504, and Pasture Systems and Watershed Management Research Unit, Agricultural Research Service, U.S. Department of Agriculture, Curtin Road, Building 3702, University Park, Pennsylvania 16802-3702

The forage potential of chicory (*Cichorium intybus* L.) has not been realized in southern West Virginia (WV) because ruminants are reluctant to consume the herbage. Chicory contains bitter sesquiterpene lactones that can adversely impact palatability. This study was undertaken to determine whether sesquiterpene lactone concentrations in chicory grown in southern WV differ from those in chicory grown in central Pennsylvania (PA) where chicory is grazed readily. Herbage was collected in 1997 and 1998 from cultivars Grasslands Puna (Puna), INIA le Lacerta (Lacerta), and Forage Feast established at research sites near State College, PA, and Beckley, WV. The total concentration of sesquiterpene lactones in WV-grown cultivars was 0.58% (dry matter basis) in Puna, 0.59% in Lacerta, and 0.79% in Forage Feast in 1997 and ranged from 1.03 (Lacerta) to 1.52% (Forage Feast) in 1998. In PA-grown cultivars, sesquiterpene lactones represented 0.16 (Puna), 0.18 (Lacerta), and 0.27% (Forage Feast) of the forage dry matter in 1997 and ranged from 0.32 (Lacerta) to 0.55% (Forage Feast) in 1998. Concentrations of lactucin, lactucopicrin, and total sesquiterpene lactones in Forage Feast exceeded those in the other cultivars grown at the same site. The lowest concentrations of lactucopicrin and total sesquiterpene lactones observed among WV-grown cultivars were higher (2-fold or more) than the highest concentrations present in cultivars grown the same year in PA. Mineral analyses of soils from the two cultivation sites indicate that P availability may influence sesquiterpene lactone composition of chicory herbage. Results provide a foundation for future studies of environmental effects on sesquiterpene lactone composition and palatability of chicory herbage.

KEYWORDS: ***Cichorium intybus* L.; forage chicory; Forage Feast; Lacerta; Puna; sesquiterpene lactones; lactucin; lactucopicrin; 8-deoxylactucin; phosphorus**

INTRODUCTION

Forage chicory is promoted as a valuable, alternative pasture species capable of improving seasonal distribution of high quality herbage (*1*, *2*) and promoting excellent live-weight gains of sheep (*Ovis aries*) (*3*, *4*), cattle (*Bos taurus*) (*5*, *6*), and red deer (*Cervus elaphus*) (*1*, *7*). In New Zealand, lambs grazing on pure stands of Grasslands Puna (Puna) chicory gained 0.24−0.29 kg/day (*3*, *4*). Weight gains by sheep on Puna pastures (0.30−0.35 kg/day) were nearly double those achieved on perennial ryegrass (*Lolium perenne* L.)-white clover (*Trifolium repens* L.) pastures (0.12−0.20 kg/day) (*8*). Liveweight gains reported for cattle grazing on Puna chicory range from 0.6 to 0.9 kg/day (*3*, *5*, 6). Research evaluations of Puna in the United States confirmed the New Zealand claims of good yields, high protein content, and low fiber concentration (*9*−*15*). In grazing studies conducted in Pennsylvania (PA) (*9*), Oklahoma (*10*), Kentucky (*11*, *12*), and northern West Virginia (WV) (*16*), Puna herbage was consumed readily by ruminants. Reid and coworkers (*16*) reported mean average daily gains (0.19 kg/day) and seasonal lamb liveweight gains (918 kg/ha) comparable to those observed in New Zealand. In southern WV, however, lambs refused to eat Puna forage (*17*). Lambs grazing in orchardgrass (*Dactylis glomerata* L.)−Puna chicory pastures lost 24.2 kg/ha during a 44 day midsummer grazing period in 1995, and the total lamb production per hectare was 14% less, as compared to orchardgrass alone, during a 79 day grazing period

* To whom correspondence should be addressed. Tel: 304-256-2809. Fax: 304-256-2921. E-mail: Joyce.Foster@ars.usda.gov.
† Appalachian Farming Systems Research Center, U.S. Department of Agriculture.
‡ The Pennsylvania State University.
§ Current address: Department of Agriculture and Natural Resources, Delaware State University, 1200 North DuPont Highway, Dover, DE 19901.
|| Pasture Systems and Watershed Management Research Unit, U.S. Department of Agriculture.

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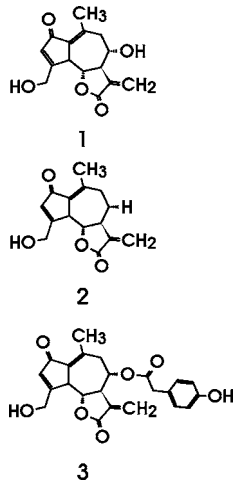

Figure 1. Chemical structures of chicory sesquiterpene lactones: lactucin (**1**), 8-deoxylactucin (**2**), and lactucopicrin (**3**).

in 1996 (*18*). Feral whitetail deer (*Odocoileus virginianus*) discriminated among three forage chicory cultivars grown in southern WV, selecting INIA le Lacerta (Lacerta) and Puna over Forage Feast (*15*). Sheep offered herbage from these three cultivars in two cafeteria trials also discriminated against Forage Feast, even though nutritive quality analyses indicated that the herbages were comparable in fiber composition (287−305 g neutral detergent fiber/kg dry matter; 220−223 g acid detergent fiber/kg dry matter) and protein content (153−162 g/kg dry matter) (*15*). In PA, Labreveux and colleagues (*19*) used the same three cultivars in a summer grazing experiment with beef cow−calf pairs without noting differences in palatability.

Chicory contains the bitter sesquiterpene lactones lactucin (**1**; **Figure 1**), 8-deoxylactucin (**2**), and lactucopicrin (**3**) (*20*−*22*). Our investigations have shown that acceptance of chicory herbage by ruminants is inversely related to total sesquiterpene lactone concentration in general and the concentration of **3** in particular (*23*). The occurrence and concentration of secondary compounds in a plant are determined by genetic factors and influenced by environmental conditions (*24*). Coley and coworkers (*25*) proposed that the nature and quantity of plant constituents that impact herbivory are determined by the resources available and the conditions encountered in the local habitat. This study was therefore undertaken to determine whether sesquiterpene lactone concentrations in chicory cultivars grown in southern WV differ from those in cultivars grown in central PA.

MATERIALS AND METHODS

Plant Growth and Sample Collection. Investigations included the chicory cultivars Lacerta, Puna, and Forage Feast from replicated field plots in central PA and southern WV and from a greenhouse pot study. A randomized complete block design was used when plants were established at each research site. Field plots in WV and PA and the greenhouse experiment were originally independent studies with different objectives, and sample collection and handling procedures varied among the three studies. Specific details related to plant establishment and management and sample collection and processing are provided below. Residual samples from the original studies were used for the current study.

In PA, cultivars were established in a Hagerstown silt loam (fine, mixed, mesic Typic Hapludalfs) soil at The Pennsylvania State University Haller Farm Beef Research Center (40° N, 77° W, 357 m above sea level) near State College. Five plots (12 m × 14 m) of each cultivar were seeded on May 23, 1997, with a plot drill planter using a seeding rate of 4.5 kg/ha. No fertilizer was added at planting. In 1998, plots received 50 kg/ha N as urea on April 10 and again on July 15. On April 10, 1998, 60 kg/ha P and 120 kg/ha K were also applied. Vegetative herbage was harvested on October 3, 1997, and on June 8 and August 24, 1998, from one 0.1 m^2 quadrat cut to ground level with electric shears. Chicory samples, from which contaminating plant species had been removed, were dried for 48 h at 55 °C in a forced-air oven and then ground with a cyclone mill to pass a 1 mm screen. In 1998, samples from only two of the five plots were available for analysis.

In WV, chicory plots were established in 1997 and 1998 in a Gilpin (fine-loamy, mixed mesic Typic Hapludults) soil at a U.S. Department of Agriculture Agricultural Research Service research farm (38° N, 81° W, 850 m above sea level) near Beckley. Replicated plots were hand seeded at a rate of 5 kg/ha. In 1997, plots were 3.7 m × 12.2 m. Seeding was completed on June 25, and no soil amendments were added. Tops from 10 vegetative rosettes were collected randomly from each plot on August 13, 1997. In 1998, 4.3 m × 12.2 m plots were established as described for 1997. Seeding was completed on May 19. Fertilizer (33.6, 29.4, and 55.8 kg/ha N, P, and K, respectively) was applied on May 21 and again on July 2. Herbage was collected on July 16 and July 30, 1998. Chicory leaves were randomly selected from a 1.8 m^2 harvest strip cut with a sickle-bar mower set for a 5 cm stubble height. All WV herbage was frozen immediately in liquid nitrogen, lyophilized, ground to pass a 0.5 mm screen, and stored at −20 °C until analyzed. Samples from only two of the five (1997) or six (1998) replicate plots for each cultivar were available for analysis in this study.

Herbage was collected in 2001 from plots established in WV in 1998 to determine the effect of drying temperature on the sesquiterpene lactone concentration in the dried sample. Leaves from nonbolting plants that had been managed to keep them in the vegetative stage were randomly collected and pooled to give three composite samples for each cultivar. Samples were either freeze dried, oven dried at 70 °C, or air dried at room temperature (25 °C). Dried samples were ground to a 0.5 mm particle size and stored at −20 °C.

In a greenhouse study, chicory cultivars were grown in white, 30 cm, plastic pots (15 pots per cultivar) filled with Pro-Mix BX commercial potting soil (Premier Horticulture, Quakertown, PA) supplemented with 25 g/pot Osmocote (15-9-12 plus micronutrients) time-release fertilizer (Scotts-Sierra Horticultural Products, Marysville, OH) incorporated by hand to a depth of 8 cm. Surface application of the same type and amount of fertilizer was made 60 days after seeds were planted. Each pot was planted with 30−50 seeds; seedlings were thinned to 25 plants per pot 7−10 days after sowing. Pots were maintained in a greenhouse set to provide a minimum night temperature of 13 °C and a maximum day temperature of 25 °C. Supplemental lighting with high-pressure sodium lamps was used to ensure a minimum day length of 12 h. Plants were watered with tap water as needed. Herbage (vegetative rosettes) was harvested from five replicate pots per cultivar 31, 51, and 71 days after sowing. The tissue was frozen in liquid nitrogen, freeze dried, ground (0.5 mm particle size), and stored at −85 °C.

Analytical Procedures. Soils in fields containing chicory plots were sampled to a 15 cm depth prior to plot establishment for the determination of pH and mineral composition. Samples from WV were analyzed by A & L Eastern Agricultural Laboratories, Inc. (Richmond, VA). Samples from PA were analyzed by the Agricultural Analytical Services Laboratory at The Pennsylvania State University.

Plant samples were analyzed for dry matter using procedures of the Association of Official Analytical Chemists (*26*). For other determinations, subsamples were taken as is, and results were converted to a dry matter basis.

The mineral composition of plant samples was determined by inductively coupled plasma emission spectroscopy using a modification of the protocol described by Belesky et al. (*25*). Ground tissue (75 mg) was treated with 1.7 mL of 15.4 M HNO_3, 0.2 mL of 12.1 M HCl, and 0.1 mL of 28.9 M HF. Following microwave digestion, the contents of digestion vessels were transferred quantitatively to tared, 60 mL plastic bottles; 0.1 mL of yttrium standard stock (1 mg/mL) was added to each bottle, and the mass of the bottle contents was adjusted to 10.0 g with analytical grade I water. Reference peach [*Prunus persica* (L.) Batsch] leaf tissue (NIST Standard Reference

Table 1. Sesquiterpene Lactone Composition of Forage Chicory Cultivars Harvested in 1997 and 1998 in Southern WV and Central PA[a]

		sesquiterpene lactone concentration (mg/g of dry matter)			
cultivar	site	lactucin	8-deoxylactucin	lactucopicrin	total
1997					
Lacerta	WV	0.46 (0.00, 8)	3.29 (0.06, 56) a	2.16 (0.03, 36) a	5.92 (0.03) a
	PA	0.42 (0.11, 24)	0.82 (0.12, 46) b	0.53 (0.13, 30) b	1.77 (0.35) b
Puna	WV	0.78 (0.04, 14)	2.74 (0.00, 48) a	2.24 (0.00, 39) a	5.76 (0.04) a
	PA	0.47 (0.04, 30)	0.53 (0.04, 34) b	0.54 (0.04, 35) b	1.55 (0.11) b
Forage	WV	1.58 (0.44, 20)	2.65 (0.01, 34) a	3.68 (0.24, 47) a	7.91 (0.19) a
Feast	PA	1.27 (0.08, 47)	0.43 (0.02, 16) b	1.01 (0.08, 37) b	2.71 (0.17) b
1998					
Lacerta	WV	0.85 (0.25, 8)	3.96 (0.93, 38) a	5.49 (1.09, 53) a	10.30 (2.25) a
	PA	0.94 (0.22, 30)	1.33 (0.15, 42) b	0.91 (0.19, 29) b	3.18 (0.53) b
Puna	WV	1.22 (0.25, 10)	3.78 (1.18, 30) a	7.52 (2.21, 60) a	12.53 (3.60) a
	PA	1.35 (0.20, 35)	1.08 (0.15, 28) b	1.38 (0.25, 36) b	3.81 (0.45) b
Forage	WV	3.65 (0.59, 24) a	2.23 (0.17, 15) a	9.36 (1.26, 61) a	15.24 (2.02) a
Feast	PA	2.87 (0.29, 52) b	0.85 (0.09, 15) b	1.79 (0.18, 32) b	5.51 (0.50) b

[a] For 1997, values for WV-grown cultivars are means for two plots seeded in June and harvested in August. Values for PA-grown cultivars are means for five plots seeded in May and harvested in October. For 1998, values are means for two plots and two harvest dates (WV, July 16 and July 30; PA, June 8 and Aug 24) at each site. Plots in WV were established in May 1998. Plots in PA were established in May 1997. Harvested plants were vegetative rosettes. Within cultivars for each year, concentrations for a sesquiterpene lactone followed by the same letter are not statistically different at $P = 0.05$. For individual sesquiterpene lactones, values in parentheses indicate the standard error of the mean and the percent of total sesquiterpene lactones represented by the associated constituent, respectively. For total sesquiterpene lactones, values in parentheses indicate the standard error of the mean.

Material SRM 1547; U.S. Department of Commerce, National Institute of Standards and Technology, Gaithersburg, MD) was used to standardize the analyses.

Procedures of Tamaki et al. (*28*) were used to extract sesquiterpene lactones from chicory tissues. Briefly, dried tissue was subjected to Soxhlet extraction with methanol and then fractionated using C_{18} Sep-Pak (Waters, Milford, MA) solid-phase extraction cartridges. Fractions containing sesquiterpene lactones were analyzed by gradient reversed-phase high-performance liquid chromatography following the protocol of Sessa et al. (*29*). Chromatography was performed on a 250 mm × 4.6 mm, 5 μm Columbus C_{18} analytical column (Phenomenex, Torrance, CA) using a Perkin-Elmer (Norwalk, CT) series 200 high-performance liquid chromatography system (pump, autosampler, column oven, and diode array detector). Data acquisition and peak quantification were accomplished using Perkin Elmer (Shelton, CT) Nelson TotalChrom software. Sample peaks were identified by comparison of retention times, UV spectra (200−360 nm), and wavelength ratios (A_{257}:A_{228}) with those for authentic compounds. Standards were generously provided by Dr. Mark Henry Bennett, Wye College, University of London (Ashford, Kent) (**1** and **3**) and Dr. Toshio Miyase, University of Shizaoka (Shizaoka, Japan) (**2**). The internal standard, santonin, was purchased from Sigma Chemical Co. (St. Louis, MO). Other chemical reagents and solvents were analytical grade.

Analysis of variance procedures were applied to the various data sets using the MIXED procedure of SAS (*30*, *31*). Sesquiterpene lactone data from field studies were evaluated with a model that included fixed main effects of cultivar, location, year, and the interactions. Random effects were assigned to block, block × location, block × year, and block × cultivar. Mineral data were evaluated with a model that included fixed main effects of cultivar, location, and the interaction. Random effects were assigned to block and block × location. Sesquiterpene lactone data from the tissue-drying temperature study were evaluated with a model that included fixed main effects of cultivar, temperature, and the interaction. Random effects were assigned to block, block × location, and block × temperature. Sesquiterpene lactone data from the greenhouse study were evaluated with a model that included fixed main effects of cultivar, harvest date, and the interaction. Random effects were assigned to block, block × cultivar, and block × harvest date. Bitterness data were evaluated with a model that included fixed main effects of cultivar, location, and the interaction. Random effects were assigned to block and block × location. All models denominator degrees of freedom method was Kenward-Roger. Additional model information for all experiments included the following: covariance structure, variance components; estimation method, restricted maximum likelihood; residual variance method, profile; fixed effects standard error method, Prasad−Rao−Jeske−Kackar−Harville. Differences detected among main effects and interactions were assessed using the least-squares means statement. All tests of significance were made at the 0.05 level of probability.

Temperature and precipitation data for 1998 and long-term means were recorded at the National Weather Service Office (National Oceanographic and Atmospheric Administration) at Beckley, WV (Beckley Raleigh County Memorial Airport, COOP ID 460582; 37°48′ N; 81°07′ W; 763 m above sea level) and State College, PA (COOP ID 368449; 40°48′ N; 77°52′ W; 357 m above sea level).

RESULTS AND DISCUSSION

The sesquiterpene lactone composition of chicory herbage harvested in 1997 from the WV and PA experimental sites is given in **Table 1**. A significant interaction between location and cultivar existed for **2** ($P = 0.05$) and **3** ($P < 0.05$). Concentrations of **2**, **3**, and total sesquiterpene lactones were higher ($P < 0.001$) in WV samples than in PA samples. Within cultivars, concentrations of **1** were similar ($P > 0.05$) in herbage collected from the two locations. Forage Feast exhibited higher ($P < 0.01$) quantities of **1**, **3**, and total sesquiterpene lactones than Puna and Lacerta from the same cultivation site. Amounts of these components in Lacerta and Puna from the same site were not statistically different ($P > 0.05$). Among cultivars from the same site, Lacerta had the highest ($P < 0.01$) concentration of **2**. The concentration of this compound was similar ($P > 0.05$) in Puna and Forage Feast grown at the same location.

Differences in the maturity of plants and age of leaves harvested in August (WV) and October (PA) could be responsible for the differences in sesquiterpene lactone composition of the samples from WV and PA; however, analyses of similarly aged herbage collected during the summer of 1998 from plants established a year apart yielded similar results (**Table 1**). In 1998, the location × cultivar interaction was significant for each of the three sesquiterpene lactones (**1**, $P < 0.05$; **2** and **3**, $P < 0.001$) as well as total sesquiterpene lactones ($P < 0.01$). The cultivars having the highest concentration of each of the sesquiterpene lactones in 1997 also had the highest concentration in 1998. In contrast to 1997, concentrations of **3** and total sesquiterpene lactones in 1998 were greater ($P < 0.001$) in WV Puna than in WV Lacerta. In PA samples, the concentration of

Table 2. Influence of Drying Temperature on Sesquiterpene Lactone Concentrations in Chicory Leaf Tissue[a]

cultivar	drying temp (°C)	sesquiterpene lactone concentration (mg/g of dry matter)			
		lactucin	8-deoxylactucin	lactucopicrin	total
Lacerta	0	0.65 (0.00) b	1.93 (0.00) a	1.35 (0.02) a	3.93 (0.03) a
	25	0.88 (0.03) a	1.39 (0.07) b	1.27 (0.08) a	3.55 (0.18) ab
	70	0.49 (0.01) c	1.71 (0.01) a	0.77 (0.02) b	2.96 (0.04) b
Puna	0	0.80 (0.06) b	2.39 (0.16) a	1.97 (0.16) a	5.15 (0.38) a
	25	1.01 (0.05) a	2.19 (0.07) a	1.99 (0.01) a	5.19 (0.13) a
	70	0.94 (0.01) ab	1.72 (0.01) b	1.06 (0.01) b	3.73 (0.01) b
Forage	0	1.81 b	1.72 b	2.10 b	5.63 b
Feast	25	2.07 (0.08) a	2.07 (0.08) a	2.62 (0.09) a	6.75 (0.24) a
	70	1.52 (0.08) c	1.41 (0.07) b	1.13 (0.05) c	4.06 (0.20) c

[a] Values are means of duplicate samples for each drying temperature except for Forage Feast at 0 °C. The standard error of the mean is given in parentheses. Within cultivars, concentrations for a sesquiterpene lactone followed by the same letter are not statistically different at $P = 0.05$.

3 in Forage Feast was greater ($P < 0.01$) than that in Lacerta but not different ($P > 0.05$) from that in Puna. Concentrations of **3** in PA Puna and PA Lacerta were similar ($P > 0.05$). The concentration of **2** was greater in Lacerta (WV, $P < 0.001$; PA, $P < 0.05$) than Forage Feast. Its concentration in Forage Feast was less than that in Puna in WV samples ($P < 0.001$) but comparable to that in Puna in PA samples ($P > 0.05$). Lacerta and Puna from the same location had similar ($P > 0.05$) concentrations of **2**.

Analysis of the combined data sets from 1997 and 1998 indicated that the year × location interaction was significant for **3** ($P < 0.001$) and total sesquiterpene lactones ($P < 0.05$). The year × cultivar interaction was significant for **1** ($P < 0.01$). For each site, concentrations of **1**, **3**, and total sesquiterpene lactones in 1998 were roughly double ($P < 0.001$) those in 1997 (**Table 1**). Proportions of the individual sesquiterpene lactones in each of the PA-grown cultivars were similar in 1997 and 1998. In WV-grown cultivars, **3** was the dominant sesquiterpene lactone constituent in 1998, and it represented a larger fraction of total sesquiterpene lactones in 1998 (53−61%) than in 1997 (36−47%). The concentration of **2** was comparable ($P > 0.05$) in cultivars harvested in 1997 and 1998. At each location, the percentage of **1** in each cultivar was relatively constant over the 2 years.

When established, chicory plots in WV and PA were not part of a coordinated study. Rather, samples from independent investigations were analyzed to try to identify a chemical basis for the difference in acceptability of chicory herbage grown at the two locations. Chicory samples collected in WV were lyophilized; PA samples were dried at 55 °C. To ascertain the effect of drying temperature on sesquiterpene lactone composition of chicory herbage, analyses were conducted on samples dried at 0, 25, or 70 °C (**Table 2**). Leaves used for these analyses were comparable in age to those collected in 1997 and 1998 but were harvested in 2001 from plants remaining in the 1998 WV plots. Chicory samples from field studies have occasionally been dried at 70 °C, so this temperature was used to obtain a worst case estimation of the loss of sesquiterpene lactones during oven drying. The total sesquiterpene lactone concentration in samples dried at 70 °C was significantly less ($P < 0.05$) than that in samples dried at 0 and 25 °C, reflecting a major decrease (approximately 50%, $P < 0.01$) in the concentration of **3**. If the concentrations of **3** in PA samples (**Table 1**) were corrected for a 50% loss due to drying temperature, they would still be less than those in corresponding tissues produced in WV.

The potential impact of sesquiterpene lactone concentration on palatability of chicory herbage may be magnified by the differences in bitterness of the individual sesquiterpene lactones. Bitterness thresholds (the lowest concentrations, in water, detectable by a human test panel) for **1**, **2**, and **3** are 1.7, 1.1, and 0.5 μg/g, respectively (*21*). These values, converted to relative bitterness factors, can be used to calculate the bitterness contributions of the individual sesquiterpene lactones and the overall bitterness of chicory samples. **Table 3**, derived from 1998 data in **Table 1**, indicates that bitterness of chicory cultivars from PA is not only less than that of WV-grown cultivars but also that the most bitter of the PA cultivars, Forage Feast, is less bitter than the least bitter of the WV cultivars, Lacerta. The greatest contributor to bitterness in all cultivars is **3**. Bitterness detected by humans might not be the same taste sensation in ruminants. Church (*32*) has reported that ruminants also vary in their response to bitterness with cattle and sheep being both less sensitive to and less tolerant of bitterness than deer, which are less sensitive and less tolerant than goats.

Table 3. Bitterness of Forage Chicory Cultivars Due to Sesquiterpene Lactones[a]

cultivar	site	bitterness equivalent (mg/g of dry matter)			
		lactucin	8-deoxylactucin	lactucopicrin	total
Lacerta	WV	0.85 (0.25)	6.10 (1.44) a	18.66 (3.70) a	25.56 (5.36) a
	PA	0.94 (0.22)	2.04 (0.23) b	3.07 (0.66) b	6.06 (1.06) b
Puna	WV	1.22 (0.25)	5.83 (1.82) a	25.50 (7.48) a	32.55 (9.49) a
	PA	1.35 (0.20)	1.66 (0.24) b	4.67 (0.85) b	7.68 (1.09) b
Forage	WV	3.66 (0.59) a	3.43 (0.27) a	31.74 (4.27) a	38.83 (5.12) a
Feast	PA	2.87 (0.29) b	1.31 (0.14) b	6.07 (0.61) b	10.25 (0.94) b

[a] The bitterness equivalent is calculated as the component concentration multiplied by its relative bitterness. Values were determined using 1998 data from **Table 1**; the standard error of the mean is given in parentheses. Relative bitterness (lactucin, 1.00; 8-deoxylactucin, 1.54; and lactucopicrin, 3.40) was calculated using bitterness thresholds (lactucin, 1.7 μg/g; 8-deoxylactucin, 1.1 μg/g; and lactucopicrin, 0.5 μg/g) reported by van Beek et al. (*21*). The threshold value is the lowest concentration, in water, that was detected by a human sensory test panel. Within cultivars, values for a sesquiterpene lactone followed by the same letter are not significantly different at $P = 0.05$.

Support for the hypothesis that the bitterness of sesquiterpene lactones in chicory underlies the avoidance of WV-grown chicory by sheep, and the discrimination among chicory cultivars by whitetail deer (*15*) is provided by the observation that cattle avoid *Vernonia* species and other Compositae, which contain high concentrations of sesquiterpene lactones (*33*). In controlled feeding studies, whitetail deer preferred Tennessee ironweed (*Vernonia flaccidifolia* Small), which lacks sesquiterpene lactones, over giant ironweed [*Vernonia gigantea* (Walt.) Trel.] or Tennessee ironweed coated with the sesquiterpene lactone from giant ironweed (*33*). Cafeteria trials with WV-grown chicory indicated that Forage Feast was the least palatable of the three cultivars (*15*). Sesquiterpene lactone data for 1998 WV samples in **Table 1** represent samples used in those cafeteria

Table 4. Sesquiterpene Lactone Composition of Greenhouse-Grown Forage Chicory Cultivars[a]

cultivar	harvest date	sesquiterpene lactone concentration (mg/g of dry matter)			
		lactucin	8-deoxylactucin	lactucopicrin	total
Lacerta	Feb 15	0.28 (0.05)	1.17 (0.10) ab	0.58 (0.04) b	2.03 (0.13) b
	March 7	0.33 (0.04)	0.73 (0.11) b	0.57 (0.08) b	1.62 (0.23) b
	March 27	0.82 (0.11)	1.45 (0.03) a	1.47 (0.08) a	3.74 (0.19) a
Puna	Feb 15	0.45 (0.05) b	1.76 (0.11) ab	1.00 (0.06) b	3.21 (0.19) b
	March 7	0.65 (0.06) b	1.50 (0.07) b	1.14 (0.07) b	3.29 (0.16) b
	March 27	1.24 (0.12) a	2.15 (0.36) a	2.02 (0.30) a	5.41 (0.74) a
Forage	Feb 15	1.24 (0.10) b	1.36 (0.09)	1.49 (0.13) b	4.10 (0.31) b
Feast	March 7	1.27 (0.18) b	0.89 (0.12)	1.22 (0.16) b	3.39 (0.46) b
	March 27	2.41 (0.54) a	1.19 (0.24)	2.05 (0.41) a	5.65 (1.18) a

[a] Values are means of five replications of each cultivar on each harvest date; the standard error of the mean is given in parentheses. Within cultivars, concentrations for a sesquiterpene lactone followed by the same letter are not statistically different at $P = 0.05$.

trials. These results not only suggest that palatability is linked to the sesquiterpene lactone composition of the herbage but also suggest that acceptability of forage from all cultivars in PA, and possibly other locations within and outside the United States, is due to lower concentrations of sesquiterpene lactones, especially **3**. Sesquiterpene lactone data published by Rees and Harborne (*20*) for a wild chicory accession grown in the United Kingdom are consistent with this conclusion. These researchers reported that sesquiterpene lactone concentrations varied from 0.06 to 0.45% dry weight in leaves with minimal variation in the proportion of the individual sesquiterpene lactones during the season.

Chicory cultivars grown in fertilized artificial potting medium in a greenhouse had sesquiterpene lactone concentrations (**Table 4**) comparable to those in PA-grown cultivars (**Table 1**). The sesquiterpene lactone composition in 4 and 7 week old plants of each cultivar was similar ($P > 0.05$). Higher ($P < 0.05$) concentrations of **3** and total sesquiterpene lactones occurred in 10 week old plants of all cultivars. Elevated concentrations of **1** were also present in 10 week old Puna and Forage Feast plants. These changes in chemical composition could be associated with leaf maturation, but they also paralleled the appearance of signs of nutrient deficiencies in the herbage.

The production of secondary metabolites is a common response of plants to stress (*25*). When available nutrients are not adequate to support the growth potential of a plant, excess resources are diverted to the production of compounds that reduce herbivory (*34*). Differences in sesquiterpene lactone composition of herbage from the same cultivar grown in different locations suggest regulation of sesquiterpene lactone composition by environmental factors. Increased latex production in some plants has been linked to low P levels in the environment (*35*). The location of sesquiterpene lactones in the latex of chicory (*20*) suggests the possibility that soil P availability could impact the sesquiterpene lactone composition of chicory herbage.

Fixation of fertilizer P is a problem commonly associated with Appalachian soils, and low levels of available P are characteristic of many Appalachian hill−land pastures (*36*). Mineral analysis of soils collected from chicory fields prior to establishment of chicory plots indicated that P in the WV soil (15 kg P/ha) occurred at deficiency levels (0−28 kg P/ha) while P in the PA soil (103 kg P/ha) exceeded sufficiency levels (>90 kg P/ha) (*37*). Soil Mg (WV, 668 kg/ha; PA, 298 kg/ha) and K (WV, 228 kg/ha; PA, 560 kg/ha) at the two sites were either adequate or present in surplus amounts (>560 kg Mg/ha >269 kg K/ha). Chicory plots in WV and PA received no supplemental P in 1997, and similar P fertility treatments were applied at the two locations in 1998. Although soil samples were not collected after chicory plots were established, available information suggests that P limitations existed in chicory plots in WV.

Table 5. Nutrient Concentrations in Chicory Cultivars Grown in PA and WV in 1998[a]

nutrient	concentration		statistics
	PA	WV	
P	2.8	3.2	**
K	28.6	36.5	**
Ca	12.8	8.7	***
Mg	2.7	3.3	NS
S	4.1	4.0	NS
Cu	11	8	**
Zn	31	30	NS
B	30	20	***
Fe	171	68	***
Mn	87	33	***

[a] Concentrations of macronutrients (P, K, Ca, Mg, and S) are reported as g/kg dry matter. Concentrations of micronutrients (below dashed line) are reported as mg/kg dry matter. Data for PA are means for samples collected on June 8 and August 24; WV samples were collected on July 16. Asterisks indicate statistical differences between cultivation sites: **, $P < 0.01$; ***, $P < 0.001$. NS, not significant ($P > 0.05$).

Belesky and co-workers (*27*) observed active accumulation of minerals by Puna chicory in field studies conducted in southern WV and concluded that chicory requires high nutrient input to sustain production, especially on soils with marginal fertility. Mineral deficiencies can adversely affect herbage intake by ruminants (*38*). Chicory herbage collected from WV had higher concentrations of P ($P < 0.01$) and K ($P < 0.01$) and lower concentrations of Ca ($P < 0.001$), Cu ($P < 0.01$), B ($P < 0.001$), Fe ($P < 0.001$), and Mn ($P < 0.001$) than corresponding herbage collected in PA (**Table 5**). Concentrations of Mg, S, and Zn were similar ($P > 0.05$) in herbage from the two locations. If mineral levels in the WV chicory plants indicate nutrient stress, the high levels of sesquiterpene lactones in the plants might represent an elevated level of chemical protection against herbivory and a corresponding decrease in palatability to ruminants. Forage Feast (3.2 g P/kg of dry matter) tended to accumulate more P in the herbage than Puna (3.0 g P/kg of dry matter) or Lacerta (2.6 g P/kg of dry matter). This characteristic could be linked to the root−chicory origin of this cultivar (*15*). An increase in the root-to-shoot ratio occurs when growth of plants is limited by a single nutrient (*39*), and proliferation of roots under P deficiency has been demonstrated with several plant species (*40*, *41*). Increases in root mass take resources away from the shoot, slowing herbage production. Protection of the existing herbage is important for plant survival. The three chicory cultivars differ dramatically in root morphology. Forage Feast has a dominant tap root. Puna has a much

smaller tap root while the root system of Lacerta is composed of numerous fine, lateral roots. Higher total concentrations of sesquiterpene lactones in Forage Feast, as compared to Puna and Lacerta (**Table 1**), might reflect a relatively greater proportion of resources being directed to the root of this cultivar. Slower plant growth under P-limiting conditions may be the basis for the higher levels of sesquiterpene lactones in the WV-grown chicory cultivars. Studies to quantify effects of P fertility on the morphological, physiological, and biochemical properties of the individual chicory cultivars are underway.

Peters and colleagues (*42*) reported significant differences in sesquiterpene lactone levels between chicory cultivars used for the production of chicons. They also observed significant effects of N fertilization on sesquiterpene lactone concentrations in the chicons and a significant interaction of cultivar and N fertilization on sesquiterpene lactone level and concluded that sesquiterpene lactone concentrations and the taste of chicons can be influenced by cultivation conditions. Nitrogen treatments were not components of studies from which samples were taken for the current study, but their impact on sesquiterpene lactone concentration in forage chicory needs to be evaluated.

Other biotic or abiotic stresses, including herbivory and attack by pathogens, could cause spikes in sesquiterpene lactone concentrations. Rees and Harborne (*20*) demonstrated that sesquiterpene lactones function as insect-feeding deterrents. No pest or disease problems were noted in the chicory plots in PA or WV.

Climate data for the WV and PA research sites showed that atmospheric temperatures at the two locations were quite similar during June, July, and August of 1998. Temperatures were close to the 30 year mean for the respective study areas. The greatest deviation from normal (+2 °C) occurred in August in PA. Precipitation at the WV site was unusually high (178 vs 98 mm) in June and unusually low (39 vs 86 mm) in August. At the PA site, monthly rainfall was slightly below normal in June (91 vs 103 mm) and July (81 vs 92 mm). August rainfall (84 vs 81 mm) was fairly typical for the month. Sample collections at each site were made during months when precipitation amounts for the month were close to normal. Thus, it is unlikely that atmospheric temperature and soil moisture were responsible for the significant differences in sesquiterpene lactone concentrations in chicory cultivars from the two sites.

Soil and climate conditions in WV and PA can influence concentrations of other chemical and physical properties of chicory herbage. Differences in sugar content or leaf thickness due to carbohydrate accumulation, for example, will ultimately affect palatability of the herbage to ruminants. Nutritive quality analyses of chicory herbage produced in WV in 1997 and 1998 revealed similar concentrations of crude protein, neutral detergent fiber, and acid detergent fiber and similar amino acid composition and in vitro organic matter disappearance in the three cultivars (*15*). Values for each parameter were indicative of a high quality forage. Comparative data for the PA samples are not available. Other secondary compounds such as cichoriin and condensed tannins also function as feeding deterrents (*20*), and environmental regulation of the concentrations of these compounds is also possible. Further investigation is required to gain a full understanding of relationships among environment, plant chemical and physical properties, and palatability of chicory forage. The data reported here provide direction for such studies.

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