

Volatiles from Fresh and Air-Dried Vegetative Tissues of Tall Fescue (*Festuca arundinacea* Schreb.): Relationship to Cattle Preference

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Volatiles from fresh and air-dried forage of eight tall fescue cultivars were collected on Tenax-TA adsorbent and then examined by gas chromatography/mass spectrometry (GC/MS) and GC/flame ionization detector chromatography (GC/FID). Fifty of 52 compounds emitted from fresh forage and 99 of 103 emitted from hay were identified. The same compounds were common to all cultivars, and quantitative differences, within fresh forage or hay, were not dramatic. Fresh forage yielded 12–32 $\mu\text{g/L}$ of volatiles, while dry forage yielded 0.18–0.47 $\mu\text{g/L}$. (*Z*)-3-Hexenyl acetate made up 82% of total emissions from fresh forage but only 0.24% from hay. Green-leaf odor compounds made up 11% in fresh forage and 6% in hay. Previously determined grazing preferences by cattle were related positively to 6-methyl-5-hepten-2-one and negatively to (*Z*)-3-hexenyl propionate and acetic acid, which together made up <0.2% of volatiles from fresh forage.

Keywords: *Festuca arundinacea*; headspace; aroma; preference; palatability; cattle

INTRODUCTION

Grazing animals eat an array of plant species but often prefer some and avoid others (Provenza, 1995). Several factors affect feeding preference, including color (chroma, hue, value), texture (stiffness, toughness), taste (sweetness, saltiness, bitterness, acidity, astringency), and flavor and aroma (Arnold and Hill, 1972). Aroma integrates the impact of volatile compounds released by plants upon the foraging animal's organoleptic sensory system. Scehovic et al. (1985) noted enhanced acceptability by cattle of a low-preference tall fescue (*Festuca arundinacea* Schreb.) when sprayed with juice expressed from a highly preferred Italian ryegrass (*Lolium multiflorum* Lam). However, palatability of Italian ryegrass was reduced when it was sprayed with fescue juice.

Palatability differences may also be found among cultivars within a given species. Shewmaker et al. (1997) reported that cattle exhibited different preferences among eight endophyte-free tall fescue cultivars. Typically, when cattle were introduced to test pastures, they lowered their muzzles into the foliage canopy while moving across plots of various cultivars, occasionally biting off some foliage until finding a plant(s) upon which they continued to graze. This grazing behavior suggested that initial forage quality cues were provided by aromatic or tactile characteristics of plants.

Plants emit volatile compounds, some of which are sensed by animals. Volatiles emitted from some forage plants have been collected and analyzed by a variety of experimental procedures. Emission products have been reported for several forage plants, including orchardgrass (*Dactylis glomerata* L.; Kibe and Kagura, 1976), young oat (*Avena sativa* L.) and wheat (*Triticum aestivum* L.; Buttery et al., 1982, 1985; Hamilton-Kemp and Andersen, 1984, 1986), and red clover (*Trifolium pratense* L.)

leaves (Buttery et al., 1984). Volatiles have also been identified in hay and silage of Italian ryegrass (Aii et al., 1980, 1981a,b, 1985); tall fescue (Scehovic et al., 1985; Tava et al., 1991, 1993, 1995), and *Panicums* (Kami, 1975, 1978; Kibe and Kagura, 1976; Morrison et al., 1986).

Previous research efforts have tried relating forage emissions to cattle preference but were not successful, because quantitative data on animal preference were not available (Tava et al., 1991, 1993). This study (1) identifies and quantitates the volatiles present in both fresh and air-dried tissues of eight tall fescue cultivars and (2) relates previously determined animal preference scores (Shewmaker et al., 1997) to these volatile constituents measured by gas chromatography/mass spectrometry (GC/MS) and GC/flame ionization detection (GC/FID).

MATERIALS AND METHODS

Fescue Cultivars and Tissue Collection. Eight endophyte-free cultivars (Barcel, Kenhy, Kentucky-31, Missouri-96, Mozark, Stargrazer, and breeding lines C-1 and HiMag) (Mayland and Sleper, 1993) were used in this study. Grasses were grown on an irrigated, Portneuf silt loam (Durixerollic calciorthid) soil near Kimberly, ID. Soil fertility and soil water were considered adequate for good forage growth.

Fresh vegetative tillers, about 25-cm lengths, were clipped at an 8-cm stubble height on May 22 (12:30 p.m.) and June 14, 1995 (10:30 a.m.). Leaves were placed in previously well-aerated kraft-paper bags with open tops and kept in subdued fluorescent light at 23 °C. Care was taken not to damage leaves after collection. Within 1 h of harvest, a 100-g subsample of this fresh forage (23% dry matter) was placed in the sample chamber at 23 °C (see below) where volatiles were transported during a 5-min period to the adsorption trap via a 0.5 L/min purified air stream. Dry matter content was determined by water loss upon freeze-drying of the forage samples.

Grass tillers 25 cm in length in vegetative to early boot stage were mechanically harvested at an 8-cm stubble height on July 10 and September 4, 1995. Harvested grass hay was air-dried in the field to 85% dry matter, except that the last 2 days of drying of the second harvest was completed under shelter.

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After air-drying for 6 days, subsamples (140 g average) of hay were placed in the sample chamber at 23 °C and gas sampling was continued as before, except that sampling time was lengthened to 15 min. No hay samples were rained on. Volatile emissions from material harvested May 22 and July 10 were examined by GC/MS, while those from material harvested June 14 and September 4 were submitted to GC/FID examination (see below).

Headspace Sampling Equipment. The sampling chamber consisted of a vertically mounted borosilicate glass tube, 100 cm long \times 5 cm i.d., with O-ring flanges on both ends. Glass caps with glass tube connectors were fitted to each end using Viton O-rings and clamps. Purified air (large activated charcoal filter) was pumped into the chamber bottom using a stainless steel bellows pump throttled at the exit port with a needle valve. Fescue samples were supported with stainless steel screens fitted inside the lower O-ring seal. All plumbing was assembled from stainless steel compression fittings and 0.25 in. o.d. (6.4 mm) Teflon-FEP tubing.

Sampling traps consisted of 0.50 in. o.d. \times 7.0 in. long (13 mm \times 178 mm) stainless steel tubing, containing a 4.5 in. (114 mm) bed of 2.6 g Tenax-TA adsorbent (2,6-diphenyl-*p*-phenylene oxide, 40–60 mesh, Alltech Associates, Deerfield, IL) supported by stainless steel screens. The Tenax was thoroughly extracted with acetone, and then dried, before being packed in the traps. Traps were conditioned before each use by heating to 220–230 °C while maintaining a 15–20 cm³/min flow of purified nitrogen. Conditioned traps were stored in Delrin tubes fitted with threaded caps containing Teflon-faced silicone seals (Supelco TDS tubes, Supelco Inc., Bellefonte, PA). Prepared traps were sent from Albany, CA, to Kimberly, ID, where headspace volatiles were collected, and then the traps were returned for processing and volatile analysis.

Control traps were included to monitor artifacts generated during trap manipulation, sample processing, and analysis. Upon collection of volatiles from tall fescue forage, some control traps were not opened, while others were opened for about 5 s prior to being resealed. All traps were then processed in like manner.

Analytical Instrumentation. Trapped material was transferred from Tenax traps with a Tekmar 6000 AEROTrap desorber and GC-mounted Tekmar CRYOFocusing module. Cryofocused headspace components were then rapidly transferred to the inlet end of a DB-Wax fused silica capillary column (bonded, cross-linked polyethylene glycol type; 60 m \times 0.32 mm i.d.; 0.25- μ m film thickness; J&W Scientific, Folsom, CA) mounted in a Hewlett-Packard 5890 Series II capillary GC. After transfer of the sample into the column, the GC oven was heated at a rate of 4 °C/min from 0 to 225 °C and then held at 225 °C for 5 min. This low starting temperature ensured greater separation of molecules, even though some gas–solid partitioning was expected at the beginning of each run. The exit end of the GC column was connected either to the ion source of a Finnigan 4500 quadrupole mass spectrometer or to a FID on the GC. In MS mode a constant column head pressure of 13.5 psi (93 kPa) was used, and data were acquired and processed with the associated Finnigan SuperINCOS data system. In GC/FID mode a head pressure of 23 psi (160 kPa) was selected, and data were similarly determined and processed with a Hewlett-Packard ChemStation.

Mass Spectrometric Data Processing. Component mass spectra were compared with reference spectra stored in a computer library collection to provide tentative identifications. The library contained the NIH/EPA spectra plus numerous others, many of which were obtained in our Albany laboratory using fully characterized compounds. A commercially available C₅ through C₂₀ *n*-hydrocarbon reference mixture was added to a clean Tenax trap, which was then run through the same desorption–cryofocus–GC/MS sequence, to determine the retention time of each member of the homologous series. This retention time scale was then applied to fescue cultivar headspace runs, and experimental retention indices (RI) of sample components were calculated. Tentative mass spectral identifications were confirmed or rejected by comparison of

these experimental RI values with those obtained with authentic samples of tentatively identified components.

FID Data Processing. Peak area values were determined for all resolved GC peaks. The reference mixture was also submitted to GC/FID conditions, and the resulting scale was used to calculate RI values for GC/FID chromatographic peaks. Corresponding GC/MS (identifications) and GC/FID (peak areas) chromatograms were correlated on the basis of two sets of RI data, providing matches of MS and FID peaks and yielding a peak area value for each component.

FID Response Factor. Some simplifying assumptions were made to allow conversion of FID peak area counts to amounts of components collected and analyzed. FID response factors (*m* units of mass divided by *a* units of peak area) of all components present were assumed to be equal. We also assumed that an appropriate value for this factor could be determined by injecting equal volumes of (*Z*)-3-hexenyl acetate/ethyl ether solutions prepared at several different concentrations into the same GC/FID system, using a cold on-column injector to introduce all of the sample into the column. A response factor of 0.4044 pg/unit area was determined. This factor was used to convert all FID peak area values from the GC/FID headspace runs into corresponding mass amounts.

Preference Scoring and Statistical Analyses. Mean preference scores (Shewmaker et al., 1997) for each cultivar were regressed against the volatile data (Table 1) using SAS's ProcReg and MAXR selection techniques for stepwise multiple regression analysis (Cody and Smith, 1991). Prior to statistical analysis, 0 or 0.1 was assigned to compounds reported as not present or present in trace amounts, respectively. Compounds considered as contaminants were omitted from regression analysis. Regression analysis was chosen over cluster analysis because of easier interpretation.

RESULTS AND DISCUSSION

Fresh Vegetative Plant Material. Table 1 lists headspace components identified in this study. Collectively, they comprised 98–99% of the total FID peak area measured for each cultivar. Total mass of listed components ranged from 12 to 33 μ g/L of headspace. However, no large differences in relative proportions of individual volatiles occurred among eight cultivars. However, there were small differences in *relative* amounts of some measured components. These differences may reflect true differences among cultivars, but they also include experimental error such as variation in tissue damage, variation in time lag between harvest and collection, and variation attributed to field conditions. These errors were minimized, but insufficient replications were conducted to determine the magnitude of error associated with each measured amount.

Two components, (*Z*)-3-hexenyl acetate and (*Z*)-3-hexenol accounted for 81 and 9%, respectively, of emissions from fresh forage. Tava et al. (1993) also reported these two compounds as prominent volatiles from tall fescue, but their relative proportions were reversed (14, 64%) from those in this study. Both compounds are typically released upon damage to a variety of plant tissue (Arey et al., 1991). Although (*Z*)-3-hexenyl acetate was the dominant compound, the amounts were poorly correlated with total emissions from each of the freshly harvested cultivars ($r^2 = 0.52$).

The “green odor” of green leaves arises from eight volatile compounds. Hatanaka (1993) identifies these as four alcohols [hexanol, (*E*)-3-hexenol, (*Z*)-3-hexenol, and (*E*)-2-hexenol] and four aldehydes [hexanal, (*E*)-3-hexenal, (*Z*)-3-hexenal, and (*E*)-2-hexenal], shown in Table 1. (*Z*)-3-Hexenol, at 8.9%, dominated other members in this group. Collectively they made up 11.2% of all volatiles.

Table 1. Headspace Components (Nanograms per Liter) of Freshly Harvested Fescue

compound ^a	cultivar								% composition ^b
	Barcel	C-1	HiMag	Kenhy	Ky-31	Mo-96	Mozark	Stargrazer	
Alcohols									
1-penten-3-ol	236	385	496	1529	339	997	280	337	2.66
(<i>E</i>)-2-pentenol	17	27	24	54	22	53	18	24	0.14
hexanol	8	8	11	14	9	22	8	9	0.05
(<i>E</i>)-3-hexenol	7	3	19	31	16	30	15	11	0.08
(<i>Z</i>)-3-hexenol	1093	1876	1907	3176	1731	3241	958	1404	8.89
(<i>E</i>)-2-hexenol	11	12	11	42	18	20	14	14	0.08
1-octen-3-ol	10	18	10	17	9	10	6	7	0.05
1,5-octadien-3-ol	4	7	6	5	5	5	4	4	0.02
2-ethylhexanol ^c	2	3	2	3	2	3	2	2	0.01
octanol	1	2	2	2	1	5	3	2	0.01
nonanol	1	2	1	2	1	2	4	6	0.01
phenol	2	3	2	2	2	3	2	2	0.01
Aldehydes									
acetaldehyde	15	30	28	47	26	29	13	26	0.12
propanal	4	5	6	13	3	14	4	3	0.03
hexanal	5	11	13	21	9	28	6	7	0.06
(<i>E</i>)-3-hexenal	26	40	63	165	72	309	31	50	0.44
(<i>Z</i>)-3-hexenal	18	46	154	659	93	698	25	24	0.99
(<i>Z</i>)-2-hexenal	11	18	46	72	16	47	13	16	0.14
(<i>E</i>)-2-hexenal	22	34	128	458	93	318	23	25	0.64
nonanal	3	8	3	4	6	5	3	4	0.02
(<i>E,E</i>)-2,4-hexadienal	5	9	10	16	9	16	5	7	0.04
decanal	7	7	6	7	6	6	6	7	0.03
benzaldehyde ^d	8	13	10	5	6	10	6	7	0.04
Ketones									
acetone ^c	13	19	20	29	11	18	17	8	0.08
2-butanone	11	15	6	6	8	10	4	3	0.04
3-pentanone	61	97	49	154	82	83	44	81	0.38
1-penten-3-one	10	27	18	23	8	55	7	22	0.10
6-methyl-5-hepten-2-one	6	10	7	16	10	9	6	9	0.04
acetophenone ^c	3	6	5	3	3	5	4	4	0.02
geranylacetone	4	4	4	2	4	2	2	2	0.01
Esters									
methyl acetate	41	51	45	39	41	38	19	46	0.18
ethyl acetate	4	7	3	5	6	5	4	5	0.02
hexyl acetate	15	39	35	34	23	65	25	26	0.15
((<i>Z</i>)-3-hexenyl formate) ^e	10	26	23	51	15	44	17	18	0.12
(<i>Z</i>)-3-hexenyl acetate	10195	15474	17602	24816	15446	20825	17682	19091	81.50
(<i>E</i>)-2-hexenyl acetate	49	56	64	102	62	81	78	59	0.32
(<i>Z</i>)-3-hexenyl propionate	2	3	1	1	4	5	2	3	0.01
((<i>Z</i>)-oxacyclotridec-10-en-2-one) ^e	3	3	2	2	3	2	3	3	0.01
Acids									
acetic acid	14	16	16	18	15	19	24	19	0.08
benzoic acid ^f	3	5	2	3	3	7	2	3	0.02
Ethers									
2-ethylfuran	15	39	33	41	38	49	29	32	0.16
Halogenated									
dichloromethane ^f	18	20	10	20	23	21	12	23	0.08
chloroform ^c	8	20	11	17	6	41	6	19	0.07
Nitrogen-Containing									
aniline ^c	3	2	3	2	3	7	3	3	0.02
hexamethylenetetramine ^c	3	3	3	2	2	1	1	1	0.01
Sulfur-Containing									
dimethyl sulfide	2	2	2	2	2	1	2	2	0.01
Hydrocarbons									
tridecane	6	17	14	3	9	25	8	7	0.05
pentadecane	tr ^g	1	1	5	2	6	1	1	0.01
toluene ^c	7	13	7	12	6	25	7	9	0.05
limonene	19	31	40	122	27	79	22	27	0.21
Unidentified ^h									
(43, 68, 67, 86, 85, 61, 71, 53, . . .) (128?)	91	120	276	668	227	680	122	179	1.36
(43, 80, 79, 39, 41, 81, 67, 77, . . .)	52	73	71	88	67	82	62	74	0.33
cultivar totals (ng/L)	12184	18766	21331	32630	18650	28161	19664	21777	

^a Listed in order of elution within each compound class. ^b Average values for all cultivars. For calculations "tr" set equal to "0.1".
^c Appears in blank control trap run as well; suspected contaminant. ^d Small portion may be from fescue samples. ^e Parentheses indicate tentative identification by GC/MS only; no RI verification. ^f Likely contaminant. ^g "tr" < 0.5ng/L. ^h See text.

C₁₁ homoterpene 4,8-dimethyl-1,(*E*)3,7-nonatriene has been reported by several authors (Boland and Gäbler, 1989; Turlings et al., 1992) as a plant emission component from damaged fresh plant tissue. This homoterpene (RI = 1302) was not found in fresh forage in our study since GC/MS evidence for its presence was likely masked by the dominant (*Z*)-3-hexenyl acetate peak (RI = 1312).

Several quantitatively minor components listed in Table 1 also appeared in control-trap GC/MS data. These included acetone, chloroform, toluene, benzaldehyde, acetophenone, aniline, and hexamethylenetetramine. Benzaldehyde and acetophenone are known degradation products of Tenax adsorbent, but some benzaldehyde may have been present in fresh forage as well. Benzoic acid was not detected in the control trap sample and was thought to be an artifact, on the basis of parallel dried fescue analyses (see below). Acetone, chloroform, and toluene were probably atmospheric contaminants. The origins of aniline and hexamethylenetetramine were unknown; the latter could have been formed from formaldehyde and ammonia. Dichloromethane, while not appearing in the control trap run, did appear in headspace emissions and was considered a contaminant. 2-Ethylhexanol was very likely a contaminant as well, derived from ubiquitous phthalate diester plasticizer.

The remaining Table 1 entries were typical plant-derived compounds (six-carbon aldehydes and alcohols and corresponding esters of alcohols). Limonene was the only monoterpene hydrocarbon identified. The large-ring lactone, (*Z*)-oxacyclotridec-10-en-2-one, was previously found in a steam distillate of alfalfa leaves and stems (Doss et al., 1989). It was only tentatively identified in the present study, because no authentic sample was available for RI comparison. The mass spectrum of the cultivar component was identical to that of an authentic sample, however.

Two components of intermediate concentration were not identified. The first, at an experimental RI of 1236, had the following mass spectrum: 43 (100.0), 68 (35.8), 41 (27.8), 67 (25.4), 86 (23.0), 57 (20.3), 39 (13.0), 85 (9.5), 53 (8.3), 61 (4.9), 71 (4.8), 40 (4.6) (MW = 128?). The second, at an RI of 1440, had the following mass spectrum: 43 (100.0), 80 (78.6), 79 (62.2), 39 (11.2), 41 (10.6), 81 (6.4), 67 (5.2), 77 (4.7), 53 (3.8), 65 (3.6), 51 (2.8), 52 (2.7) (MW = ?).

Field-Dried Fescue Hay. Headspace components from fescue hay are listed in Table 2. Two features were readily apparent when the data in Tables 1 and 2 were compared. More individual components were present in hay headspace, and total quantity of volatile material was less than from fresh plant material. Total hay emissions ranged from 0.2 to 0.5 $\mu\text{g/L}$, representing a 26–107-fold decrease compared with fresh tissue of individual cultivars. While (*Z*)-3-hexenol and (*Z*)-3-hexenyl acetate composed about 90% of volatiles emitted from fresh forage, they made up only 1.5% of volatiles from dry hay. Green odor compounds that made up 11.2% of fresh-forage volatiles made up 8.5% of hay volatiles. Pentanal was the most abundant volatile in dry hay, making up about 8.8% of total. Compared with volatile groups in fresh tissue, those in hay composed a much lower proportion of esters (82.3, 0.3), a similar proportion of alcohols (10.5, 12.0), and a larger proportion of aldehydes (2.5, 51.4), ketones (5.7, 12.5), acids (0.1, 12.5), and N- and S-containing compounds (0.04, 3.8).

Qualitatively, there were again no major differences among eight cultivars. The same components were found in all headspace samples, except a few trace-level components that could not be detected across all cultivars. Quantitative differences in both *relative* amounts of components within a cultivar and *total* amounts of headspace compounds collected from each cultivar were observed. Nearly all components were present at low concentrations. As with fresh forage, confidence intervals could not be placed on amounts of individual volatiles emitted from each cultivar and the magnitude of experimental variation could not be quantified.

Gas chromatographic peaks representing several components were not completely resolved. Four components, 2-methylfuran, 2-butanone, 2-methylbutanal, and 3-methylbutanal, appeared as a composite peak. By GC/MS, the methylbutanals accounted for most of total peak areas and were present in equal amounts. Estimates of relative contribution of each component to composite peak's total area were applied to yield quantities shown in Table 2. The same approach was used with 3-methylbutyric and 2-methylbutyric acids, which eluted as partly overlapping peaks. Ethanol did not elute cleanly as a discrete peak, but rather appeared over a 3–5-min interval as part of the GC/MS (and presumably GC/FID) background and so could not be quantified.

As noted above, benzaldehyde and acetophenone were both decomposition products of Tenax. Both compounds appeared in all GC/MS runs at roughly the same intensities, so neither can be considered present in tall fescue hay headspace. The same holds true for hexamethylenetetramine and aniline; although their origins cannot be pinpointed, both were likely contaminants. Because benzoic acid appeared in the control trap run, it cannot be classified as a headspace component. Diethyl ether, chloroform, and dichloromethane are obvious contaminants.

Air-drying forage allowed some oxidation of aliphatic aldehydes to corresponding carboxylic acids. A comparison of the listed carboxylic acids and aldehydes reveals that they match fairly well, except for the butanal/butyric acid pair. The latter aldehyde was not identified, perhaps because of GC overlap with another component peak. The question arises whether free acids are present in sun-dried hay or whether they were formed after trapping by air oxidation of corresponding aldehydes in air-filled traps. Because aldehydes present in drying hay had opportunity for oxidation to acids during the drying process, we assume that free acids and aldehydes were present in the headspace of tall fescue hay.

Several unidentified components are listed in Table 2. Their experimental RI values, calculated from GC/MS chromatographic data, are 589, 1169, 1417, and 1427, respectively. Major fragment ions are listed, but relative abundances are not readily estimated, due to partial overlap with other component peaks.

Aroma and Flavor Considerations. A basic assumption in aroma and flavor research is that the aroma impact of an individual component depends on the component's concentration in the sample. Ultimately, the impact depends on the concentration in the air stream reaching the olfactory region *and* the sensitivity of an individual's olfactory system to the compound. Compounds that serve as fixatives, sensitizers, or intensifiers must also be considered. Tables 1 and 2 present information relevant to the first of these vari-

Table 2. Headspace Components (Nanograms per Liter) of Sun-Cured Fescue Hay

compound ^a	cultivar								% composition ^b
	Barcel	C-1	HiMag	Kenhy	Ky-31	Mo-96	Mozark	Stargrazer	
Alcohols									
ethanol ^j									
1-penten-3-ol	24	15	11	10	8	10	10	14	4.46
pentanol	7	7	4	5	3	5	4	5	1.75
(<i>E</i>)-2-pentenol	9	1	1	2	tr ^c	3	2	1	0.83
hexanol	1	tr	tr	tr	tr	1	tr	tr	0.11
(<i>Z</i>)-3-hexenol	4	5	3	4	2	4	3	3	1.22
(<i>E</i>)-2-hexenol	1	tr	tr	tr	tr	tr	tr	tr	0.07
1-octen-3-ol	2	1	1	1	tr	1	1	1	0.35
1,5-octadien-3-ol	2	2	1	1	1	2	2	1	0.52
octanol	1	1	tr	1	tr	1	1	tr	0.23
nonanol	1	tr	tr	1	tr	1	1	tr	0.19
phenol	3	2	2	2	2	2	2	2	0.74
Aldehydes									
acetaldehyde	4	4	3	3	3	3	3	3	1.14
propanal	19	11	9	9	6	10	11	10	3.72
2-methylpropanal	16	11	9	6	5	9	7	8	3.10
2-propenal	3	2	2	2	2	1	2	1	0.66
2-methylpropenal	6	3	3	3	2	2	2	3	1.05
2-methylbutanal ^d	23	22	16	11	8	13	10	14	5.12
3-methylbutanal ^d	23	22	16	11	8	13	10	14	5.12
pentanal	41	35	24	23	15	20	18	26	8.83
(<i>E</i>)-2-butenal	4	3	2	1	1	2	2	1	0.70
hexanal	20	14	10	9	7	11	10	9	3.94
(<i>E</i>)-2-pentenal	4	3	2	2	1	2	2	2	0.79
(<i>E</i>)-3-hexenal	2	1	1	1	tr	1	1	1	0.35
(<i>Z</i>)-3-hexenal	1	tr	tr	tr	tr	tr	tr	tr	0.07
heptanal	9	8	11	10	7	9	10	17	3.54
(<i>Z</i>)-2-hexenal	1	1	tr	tr	tr	1	tr	1	0.19
(<i>E</i>)-2-hexenal	12	11	7	7	4	8	7	7	2.75
octanal	2	2	1	1	1	1	1	2	0.48
(<i>E</i>)-2-heptenal	1	1	1	1	tr	1	1	1	0.31
nonanal	10	5	4	5	4	6	5	3	1.84
(<i>E,E</i>)-2,4-hexadienal	2	1	1	1	1	2	1	1	0.44
(<i>E</i>)-2-octenal	2	tr	tr	tr	tr	tr	tr	tr	0.12
((<i>E,Z</i>)-2,4-heptadienal)	2	3	2	3	2	3	2	3	0.87
furfural	1	1	1	1	1	1	1	1	0.35
decanal	5	2	3	2	2	2	2	2	0.87
(<i>E,E</i>)-2,4-heptadienal	5	3	3	4	3	4	3	3	1.22
benzaldehyde ^e	8	7	5	5	5	6	5	6	2.06
(<i>E</i>)-2-nonenal	1	tr	tr	tr	tr	tr	1	tr	0.11
(<i>E,Z</i>)-2,6-nonadienal	4	2	2	2	1	2	2	2	0.74
β -cyclocitral	2	2	1	1	1	2	1	2	0.52
(<i>E</i>)-2-decenal	2	1	1	1	1	2	1	1	0.44
Ketones									
acetone	17	15	13	8	10	8	10	10	3.98
2-butanone ^d	5	5	4	3	2	3	2	3	1.18
3-buten-2-one	1	tr	1	tr	tr	tr	tr	tr	0.11
2,3-butanedione	3	6	6	6	2	1	4	4	1.40
1-penten-3-one	9	6	5	4	3	3	3	5	1.66
2,3-pentanedione	1	1	1	1	tr	1	1	1	0.31
(6-methylheptan-2-one)	1	tr	tr	tr	tr	tr	tr	tr	0.07
3-hydroxybutan-2-one	tr	tr	tr	tr	tr	tr	tr	tr	0.03
1-octen-3-one	1	1	tr	tr	tr	1	1	1	0.23
2,2,6-trimethylcyclohexanone	2	1	tr	tr	tr	1	tr	1	0.24
2,3-octanedione	2	1	1	1	tr	1	1	1	0.35
6-methyl-5-hepten-2-one	3	3	3	3	2	4	3	2	1.01
3,5,5-trimethyl-2-cyclohexen-1-one	3	2	1	1	1	1	1	1	0.48
camphor	tr	1	1	tr	tr	tr	tr	tr	0.11
((<i>E,Z</i>)-3,5-octadien-2-one)	2	1	1	1	1	1	1	1	0.39
(<i>E,E</i>)-3,5-octadien-2-one	tr	tr	tr	tr	tr	tr	tr	tr	0.03
acetophenone ^f	3	2	1	2	1	2	2	2	0.66
geranylacetone	nd	1	1	1	tr	1	1	1	0.27
β -ionone	nd	tr	tr	tr	tr	tr	tr	tr	0.03
Esters									
ethyl acetate	tr	tr	tr	tr	tr	tr	tr	tr	0.03
(<i>Z</i>)-3-hexenyl acetate	2	1	tr	1	tr	tr	tr	1	0.24
Acids									
acetic acid	12	12	16	13	10	14	8	13	4.29
propionic acid	16	3	3	3	2	3	2	3	1.53
methylpropionic acid	3	2	2	2	1	2	2	1	0.66
butyric acid	3	2	3	2	1	2	2	2	0.74
3-methylbutyric acid ^g	3	3	4	3	2	4	2	4	1.09
2-methylbutyric acid ^g	3	3	4	3	2	4	2	4	1.09
pentanoic acid	3	3	5	4	2	5	3	4	1.27
hexanoic acid	2	1	2	1	1	2	2	1	0.52
benzoic acid ^f	4	3	4	3	3	4	4	4	1.27

Table 2. (Continued)

compound ^a	cultivar								% composition ^b
	Barcel	C-1	HiMag	Kenhy	Ky-31	Mo-96	Mozark	Stargrazer	
Ethers									
diethyl ether ^h	2	1	1	1	1	1	1	1	0.39
2-methylfuran ^d	1	1	1	1	tr	1	tr	1	0.27
2-ethylfuran	3	2	2	2	1	1	1	2	0.61
Halogenated									
dichloromethane ^h	3	3	3	2	1	2	2	2	0.79
chloroform ^h	1	1	1	1	tr	tr	tr	1	0.23
Nitrogen-Containing									
aniline ^f	2	1	1	1	1	1	1	1	0.39
hexamethylenetetramine ^f	18	2	9	7	5	3	2	2	2.10
indole	tr	tr	tr	tr	tr	tr	tr	tr	0.03
Sulfur-Containing									
dimethyl sulfide	4	3	4	5	2	1	2	3	1.05
dimethyl disulfide	1	1	tr	tr	tr	tr	tr	tr	0.11
dimethyl sulfoxide	1	tr	tr	tr	tr	tr	tr	1	0.11
Hydrocarbons									
pentane	4	3	2	2	2	2	2	3	0.87
hexane	3	1	2	1	1	1	1	1	0.48
decane	1	2	1	1	tr	tr	tr	1	0.28
undecane	2	1	1	1	1	1	1	1	0.39
dodecane	5	4	4	2	2	3	2	3	1.09
tridecane	2	1	1	1	1	1	1	1	0.39
tetradecane	1	1	1	1	tr	1	tr	1	0.27
pentadecane	tr	tr	tr	tr	tr	tr	2	tr	0.12
hexadecane	nd	nd	tr	tr	tr	nd	nd	nd	0.01
toluene ^f	2	1	1	1	tr	tr	tr	tr	0.24
<i>p</i> -xylene	1	1	1	tr	tr	1	tr	1	0.23
<i>o</i> -xylene	1	1	tr	tr	tr	tr	tr	tr	0.11
limonene	4	3	2	2	2	3	3	2	0.92
(<i>Z</i>)- β -ocimene	tr	tr	tr	tr	tr	tr	tr	tr	0.03
(<i>E</i>)- β -ocimene	1	tr	tr	tr	tr	tr	tr	1	0.11
4,8-dimethyl-1, <i>E</i> 3,7-nonatriene	1	tr	tr	tr	tr	tr	tr	tr	0.07
Unidentified ⁱ									
(C ₆ H ₁₂)	3	1	1	1	tr	1	1	1	0.40
(69, 41, 55, 109, 123, 83, 67, . . .) (152?)	2	1	1	1	1	1	1	1	0.39
(57, 71, 43, 85, 141, 113, . . .) (branched HC?)	1	1	1	1	tr	1	1	1	0.31
(57, 45, 87, 41, 75, 56, . . .) (2-butoxyethanol?)	3	2	2	2	2	2	2	2	0.74
cultivar totals	467	333	288	255	176	263	230	275	

^a Listed in order of elution within each compound class. ^b Average values for all cultivars. For calculations, "tr" set equal to "0.1". ^c "tr" < 0.50 ng/L. ^d 2-Methylfuran, 2-butanone, 2-methylbutanal, and 3-methylbutanal bands overlap; relative amounts estimated from GC/MS data. ^e Mostly from fescue hay; small portion from Tenax. ^f In blank control trap run as well; suspected contaminant. ^g 2-Methylbutyric and 3-methylbutyric acid bands partially overlap; relative amounts estimated from GC/MS data. ^h Contaminant. ⁱ See text. ^j Not resolved; appeared in GC/MS background over several minute interval.

ables but do not address the second and third. Little if anything is known about chemosensitivity of cattle to individual volatile compounds (Arnold and Hill, 1972; Bate-Smith, 1972). However, S-containing volatiles of several tall fescue seemed to reduce palatability to sheep fed in a trough cafeteria experiment (Scehovic, 1985). Such findings are in keeping with anecdotal information and empirical evidence that sulfur-containing compounds are repellent to herbivores (Beauchamp and Mason, 1991). Cattle may be able to detect very low levels of one or more of the quantitatively minor volatiles in tall fescue, and an animal's feeding preferences might reflect variations in concentration of such minor compounds (Arnold and Hill, 1972).

Researchers have been challenged by the absence of quantitative animal preference information about tested forages. Kami (1975, 1978) stated that qualitative and quantitative differences in aroma constituents seem related to palatability for dairy cattle but did not specify which components he thought to be bioactive or how these components related to palatability.

Tava et al. (1991, 1993, 1995) divided six tall fescue cultivars (including Barcel) into high- or low-palatability groups based only on leaf softness and flexibility. Lacking animal data, they suggested that qualitative

Table 3. Cattle Preference Scores for Tall Fescue Cultivars (*Festuca arundinacea* Schreb.)^a

cultivar	preference score ^b	cultivar	preference score ^b
Kenhy	7.80	Stargrazer	5.10
Kentucky-31	5.78	Barcel	5.28
HiMag	5.48	Missouri-96	4.60
C-1	5.26	Mozark	4.59

^a From Shewmaker et al. (1997). ^b "0" = not eaten, maximum aversion; "10" = all available forage eaten, highly preferred.

and quantitative differences in classes of volatiles, especially the relative presence of the most abundant compounds, may affect animal grazing preference. Others (Arnold and Hill, 1972; Rohan, 1972; Rogers, 1981) report that chemical cues may be present in very small amounts, while certain "abundantly present" compounds contribute little organoleptically, except to provide "fixation and roundness".

Regression of Preference Scores on Volatile Compounds. Quantitative scores for cattle grazing preference were available for tall fescue cultivars used in this study (Shewmaker et al., 1997). Preference scores (Table 3) were regressed against volatile data shown in Table 1. Cattle preference was not related to

Table 4. Stepwise Regression of Grazing Cattle Preference for Tall Fescue Grass Cultivars (Y) As Related to Headspace Emissions of 6-Methyl-5-hepten-2-one [X_{29}], (Z)-3-Hexenyl Propionate [X_{38}], and Acetic Acid [X_{40}] from Freshly Cut Grass^a

	R^2	Prob
$Y = 3.1 + 0.282X_{29}$	0.77	$> F = 0.040$
$Y = 4.0 + 0.271X_{29} - 0.793X_{38}$	0.93	$> F = 0.002$
$Y = 5.2 + 0.265X_{29} - 0.295X_{38} - 0.068X_{40}$	0.97	$> F = 0.002$

^a All emissions in hg/L.

sulfur-containing compounds in this study. Preference was highly correlated with three compounds contained in the fresh forage (Table 1), as shown by the stepwise regressions in Table 4. We infer from these preliminary data that cattle may demonstrate a preference for cultivars having higher levels of 6-methyl-5-hepten-2-one but an aversion to those cultivars containing higher levels of either (Z)-3-hexenyl propionate or acetic acid. That these compounds might serve as effective cues to grazing animals should be further tested. This could be accomplished by spraying combinations of these three volatiles on a given forage and evaluating animal preference for the variously treated diets. We recognize that there are also other cues that grazing animals might use in selecting target plants. Work is also underway to relate preference scores to various carbohydrate, organic acid, mineral, protein, and fiber fractions in each of these tall fescue cultivars. The ultimate goal is to quantitatively describe the cues that ruminant animals use in selecting forage plants.

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