Training Needed for Quantifying Simulated Diets from Fragmented Range Plants

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Abstract

A procedure is described that results in rapid training of observers for microhistological analysis. Observers trained using this procedure were able to evaluate accurately 6 hand-compounded diets comprised of semidesert plant species. The accuracy of microhistological analysis was examined by using the 4 trained observers to evaluate 26 additional hand-compounded diets containing various combinations of 30 different grasses, forbs, and shrubs from semidesert range. The relationship between relative density (estimated percent by weight composition) and actual percent by weight composition was close to unity for species in each forage class individually or in combination. However this relationship would probably have been different if the observers had not used known diets to evaluate their accuracy and make corrections. It is recommended that all technicians using microhistological analysis regularly check their accuracy with hand-compounded diets.

In recent years much of the research concerning range herbivore food habits has been conducted with the microhistological technique first introduced by Baumgartner and Martin (1939). The accuracy of this technique has been demonstrated in 3 studies (Denham 1965, Sparks and Malechek 1968, Vavra and Holechek 1980). Comprehensive reviews of the technique were given by Ward (1970), Theurer et al. (1976), and Holechek et al. (1982b). One of the main problems with the technique is that considerable time may be required to train technicians to use the procedure (Ward 1970). In addition there may be considerable variation in accuracy between technicians once they are trained (Holechek et al. 1982a). On the basis of research conducted by Westoby et al. (1976) it appears that systematic training of observers may be essential if precise and accurate results are to be obtained. The objectives of this research were to develop a procedure that results in rapid training of microhistological observers to test the effectiveness of this procedure, and to evaluate the accuracy of microhistological analysis using the new trained observers.

Methods

In the late summer of 1979 collections were initiated of the primary forage plants found in southcentral New Mexico. Thirty species were selected for experimental use. They included 10 each of grasses, forbs and shrubs (Table 1). Drawings, photographs, microscope slides and keys were developed for separating these species on the basis of epidermal and cellular characteristics. Seventy mixtures with different numbers and amounts of the 30 plant species were hand compounded to represent simulated diets. These diets ranged in complexity from simple mixtures of two highly dissimilar species such as a grass and a shrub to diets comprised of 9 species with various grasses, forbs and shrubs. These mixtures were developed for the purpose of training observers in the use of microhistological analysis, and testing the accuracy of the procedure.

In the late fall of 1979 a step-wise training program was initiated for 4 potential microhistological observers all of whom were graduate or undergraduate students majoring in range or wildlife science. The program was designed so it could be completed within at least 4 weeks. After completion of training the effectiveness of the program was evaluated by providing trainees with slides, drawings, photographs and keys of epidermal material of 9 new plant species (3 each of grasses, forbs, and shrubs). The 4 trainees were instructed to quantify 6 unknown diets containing different numbers and amounts of the 9 plant species with which they had previous familiarity and the 9 new species. A total of 18 species was used since this has been about the maximum number of important dietary species reported in most food habits studies. An additional

Table 1. Semidesert species used in the hand-compounded diets.

Common name	Scientific name
Grasses	
Black grama[1]	*Bouteloua eriopoda*
Blue grama	*Bouteloua gracilis*
Green sprangletop[2]	*Leptochloa dubia*
Hairy grama	*Bouteloua hirsuta*
Mesa dropseed[1]	*Sporobolus flexuosa*
Sideoats grama	*Bouteloua curtipendula*
Silver bluestem[2]	*Bothriochloa saccharoides*
Red threeaw[2]	*Aristida longiseta*
Tobosa grass[1]	*Hilaria mutica*
Vine mesquite	*Panicum obtusum*
Forbs	
Broom snakeweed[2]	*Xanthocephalum sarothrae*
Desert baileya	*Baileya multiradiata*
Faintcrown[2]	*Aphanostephus ramosissimum*
Leatherleaf croton[1]	*Croton corymbulosus*
Peavine[2]	*Astragalus* spp.
Russian thistle[1]	*Salsola kali*
Globemallow[1]	*Sphaeralcea incana*
Spectaclepod	*Dithyrea wizlizenii*
Verbena	*Verbena bipinnatifida*
Wooly paperflower	*Psilostrophe tagetinae*
Shrubs and Trees	
Apache plume[2]	*Fallugia paradoxa*
Fourwing saltbush[1]	*Atriplex canescens*
Honey mesquite[1]	*Prosopis glandulosa*
Longleaf Mormom tea	*Ephedra torreyana*
One-seeded juniper	*Juniperus monosperma*
Soaptree yucca[2]	*Yucca elata*
Tarbush	*Flourensia cernua*
Mountain mahogany[1]	*Cercocarpus breviflorus*
Grey oak	*Quercus grisea*
Winterfat[2]	*Ceratoides lanata*

[1]Used for training and testing observers.
[2]Used only for testing observers.

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observer with 5 years experience was also utilized to quantify the 6 unknown diets. The 5 observers were provided with 10 known diets of different combinations of the 18 species so that correction factors could be developed for later use with the unknown diets if desired.

After the 4 trainees were tested for accuracy, they were given 26 new hand-compounded diets to analyze which contained various combinations of 30 species (Table 2). Botanical composition of the 26 mixtures was unknown to the observers until after sample estimates were recorded. The observers were provided with slides, drawings, photographs, and keys of epidermal material of the 12 new plant species not used in previous testing or training. In addition they were provided with known hand-compounded diets so they could evaluate their accuracy in estimating the new species.

Five slides were prepared for all compounded diets. These slides were prepared by soaking diet material in sodium hydroxide as discussed by Vavra and Holechek (1980) and mounted as discussed by Sparks and Malechek (1968). Slides were prepared so that at least 20 frequency observations were recorded per slide to insure high repeatability between slides as discussed by Holechek and Vavra (1981). Prior to slide preparation all sample material was dried in a forced air oven and then ground through a micro-Wiley mill with a 1-mm screen.

Twenty microscope fields were read for each slide. Only particle fragements were quantified. Hairs, trichomes and very small fragments were disregarded. All unidentifiable fragments in each field were fully observed by moving the microscope slide to allow complete examination. Frequency of occurrence of each species was calculated and converted to relative density, which was used as the percentage weight estimate for each species in the mixture as outlined by Sparks and Malechek (1968).

The accuracy of diet evaluation by observer was calculated using Kulcyznski's formula (Oosting 1956). The similarity value represents the percentage of the estimated mixture identical to the actual mixture. Analysis of variance was used to compare similarity indices between observers. The chi-square test was used to compare observed with expected diets. Ranking of similarity values was conducted with Duncan's multiple range test (Steel and Torrie 1960).

The accuracy of the microhistological technique was evaluated using the 26 mixtures shown in Table 2. Simple correlation and linear regression analysis were used to explore the relationship between estimated percentage dry weight (X) and actual dry weight

Table 2. Estimated means pooled across observers for plant species in the 26 mixtures used to evaluate the accuracy of microhistological analysis.

Species	Mixture number	Actual % composition by weight	Mean estimated % composition by weight	Number of observers	Coefficient of variation
Black grama	1	40	28	3	21
Fourwing saltbush	1	40	40	3	20
Honey mesquite	1	20	32	3	26
Mesa dropseed	2	20	18	4	14
Tobosa grass	2	20	21	4	16
Faintcrown	2	20	18	4	12
Russian thistle	2	20	18	4	15
Fourwing saltbush	2	20	22	4	16
Red threeawn	3	16	16	4	17
Leatherleaf croton	3	33	30	4	21
Russian thistle	3	33	31	4	17
Soaptree yucca	3	17	23	4	15
Soaptree yucca	4	50	50	4	11
Honey mesquite	4	50	50	4	11
Blue grama	5	50	49	4	14
Fourwing saltbush	5	50	51	4	24
Blue grama	6	25	23	1	—
Mesa dropseed	6	25	36	1	—
Red threeawn	6	25	26	1	—
Hairy grama	6	25	15	1	—
Vine mesquite	7	25	38	1	—
Globemallow	7	75	62	1	—
Silver bluestem	8	80	81	3	10
Peavine	8	20	19	3	15
Blue grama	9	60	57	3	19
Mountain mahogany	9	20	17	3	18
Tarbush	9	20	26	3	18
Tobosa grass	10	15	21	4	21
Fourwing saltbush	10	70	64	4	8
Mountain mahogany	10	15	14	4	10
Vine mesquite	11	90	88	2	12
Faintcrown	11	10	12	2	16
Sideoats grama	12	95	97	2	10
Globemallow	12	5	3	2	14
Fourwing saltbush	13	30	40	1	—
Mountain mahogany	13	60	46	1	—
Honey mesquite	13	10	14	1	—
Vine mesquite	14	10	6	1	—
Mountain mahogany	14	90	94	1	—
Black grama	15	11	13	4	19
Tobosa grass	15	11	11	4	16
Mesa dropseed	15	11	12	4	25
Globemallow	15	11	8	4	30
Leatherleaf croton	15	11	11	4	12

Table 2. Continued.

Species	Mixture number	Actual % composition by weight	Mean estimated % composition by weight	Number of observers	Coefficient of variation
Russian thistle	15	11	9	4	14
Honey mesquite	15	11	11	4	19
Mountain mahogany	15	11	9	4	12
Fourwing saltbush	15	11	14	4	37
Silver bluestem	16	11	12	4	17
Green sprangletop	16	11	14	4	24
Sideoats grama	16	11	12	4	31
Globemallow	16	11	12	4	38
Faintcrown	16	11	8	4	24
Peavine	16	11	9	4	13
Tarbush	16	11	10	4	8
Winterfat	16	11	13	4	23
Apache plume	16	11	10	4	9
Verbena	17	50	49	3	10
Broom snakeweed	17	50	51	3	11
Wooly paperflower	18	50	55	1	11
Spectaclepod	18	50	45	1	11
Green sprangletop	19	17	16	3	18
Spectaclepod	19	33	33	3	17
Grey oak	19	33	31	3	16
Wooly paperflower	19	17	20	3	18
Longleaf Mormon tea	20	50	46	2	12
Apache plume	20	50	54	2	14
Hairy grama	21	60	62	3	9
Desert baileya	21	20	17	3	17
One-seeded juniper	21	20	21	3	17
Red threeawn	22	90	86	2	5
Longleaf Mormon tea	22	10	14	2	16
Blue grama	23	30	24	2	23
Grey oak	23	70	76	2	9
Mesa dropseed	24	40	40	1	—
Desert baileya	24	40	32	1	—
Spectaclepod	24	20	28	1	—
Winterfat	25	50	55	2	21
One-seeded juniper	25	50	45	2	23
Green sprangletop	26	25	30	2	24
Wooly paperflower	26	25	20	2	18
Verbena	26	25	24	2	19
Grey oak	26	25	26	2	21

(Y) for grasses, forbs, shrubs, and the 3 forage classes in combination. Variability between observers was expressed using a coefficient of variation as discussed by Steel and Torrie (1960).

Training Procedure

The first phase of the training procedure involved a 2-hour oral description of the technique and its applications. After this trainees were given 3 publications to read which included Sparks and Malechek (1968), Ward (1970) and Holechek et al. (1982b). Sparks and Malechek (1968) provide an illustration of the microhistological technique. Ward (1970) gives a detailed description of procedures involved in microhistological analysis. The paper by Holechek et al. (1982b) considers the various methods for quantifying food habits of range herbivores, and reviews information concerning problems with microhistological analysis. After reading the publications the trainees were quizzed orally over the material. When it was apparent each trainee fully understood the theory, problems, and methodology of the technique, phase two was initiated.

The second phase of the procedure included a laboratory session involving the explanation and demonstration of how to use a microscope and plant epidermal characteristics to quantify herbivore diets. Following a discussion of microscope use, the separation of grasses and grass-like plants from forbs and shrubs was explained. When all trainees demonstrated they could make this distinction, plant epidermal characteristics that allow separation at the genus and species level such as cell shape and size, stomata shape and size, silicious cells, trichomes, and hairs were discussed. Trainees were then required to make actual identification of these plant parts from drawings, photographs, and microscope slides. Next the trainees were given slides, drawings, photographs, and keys of epidermal material of 9 species which included 3 each of grasses, forbs and shrubs. They were told to report back when they could distinguish each species. A trainee had passed this phase when he could identify with 100% accuracy which species occurred on 18 unlabeled slides showing the 9 species.

The third phase of the training program is considered the most important. Each student was given 5 slides for each of 6 diets to evaluate containing different numbers of the 9 species. The species composition of each diet was unknown to the trainee, although they were told that no diet contained more than 4 species. If a student could quantify the 6 diets with an overall accuracy of 90% based on Kulcyznki's formula he was graduated and given 6 complex diets containing 5 to all 9 species. If not he was given a second set of 6 simple diets. No trainee required more than 3 simple diet sets. The primary problem causing initial low accuracy was overestimation of leatherleaf croton *(Croton corymbulosus)* and honey mesquite *(Prosopis glandulosa)*. Croton characteristically fragments into many, very distinct trichomes during sample preparation and, thus, overestimation commonly occurred. Mesquite, in contrast, has cellular characteristics that provide easy identification. Trainees took two approaches to this problem which included (1) quantifying only plant fragments and (2) counting a species present in a field if any identifiable material could be observed and then calculating a correction factor as discussed by Dearden et al. (1975) and Vavra and Holechek (1980) based on known mixtures

Table 3. The mean similarity index between observed and actual diets for the five observers.

	Observer[1,2]				
	1	2	3	4	5
$\bar{X}$ Similarity index	92^{ab}	87^{b}	89^{ab}	94^{a}	93^{a}

[1]Means with different superscripts are significantly different ($P<.05$) using Duncan's multiple range test.
[2]Each mean is an average of six diets.

they had previously evaluated. Both approaches appeared to work. An individual was considered trained when analysis of a complex diet set was accomplished with 90% accuracy. Only one trainee had to read two complex diet sets.

Results and Discussion

None of the observer's estimates of test diets were significantly different ($P>.05$) from expected values when the chi-square test was applied. Only observer 2 had a significantly different ($P<.05$) similarity value than observer 5, who was the control (Table 3). The similarity values between estimated and observed diets for the 5 observers in this study suggest that the training procedure used was effective.

The average coefficient of variation was 17% (Table 2). Data presented by Holechek and Vavra (1981) indicate that examination of 5 slides (100 microscope fields) will estimate with adequate precision (95% confidence, 10% of the mean) those species comprising 30% or more of the diets. Variation in the present study associated with species comprising less than 30% of the hand-compounded diets may be as much the result of an inadequate number of slides as actual variation between observers.

The equations from the present study are quite comparable to the equations of Sparks and Malechek (1968). When all species were used in the regression, the equations from the 2 investigations are nearly identical (Table 4). Coefficients of determination were slightly lower in the present study. However the relationship between estimated dry weight percentages (relative density) and actual dry weight percentage for all 3 forage classes was nearly 1:1.

Several of the species used in this study were collected both when actively growing and when dormant. Both growth stages were used in compounded diets. In many diets mature growth from one species was mixed with immature growth from another. This appears to have had little effect on the results which is consistent with the research of Holechek et al. (1982a).

The shrubs used in this study were accurately estimated. However, research is available showing that some shrubs may undergo considerable destruction of epidermal material during sample preparation (Vavra and Holechek 1980). Westoby et al. (1976) reported that greasewood *(Sarcobatus vermiculatus)* was consistently underestimated using microhistological analysis. However, leafless twigs were used in their study rather than leafy material. Holechek (1982) pointed out that woody plant parts have a much lower amount of identifiable epidermal tissue than those that are green. Only small twigs and leafy material of current years growth were used in the present study. Dearden et al. (1975) found a 1:1 relationship did not exist between estimated and actual values of some shrub and forb species when microhistological analysis was used. These data were obtained from experienced technicians who had not been trained using hand-made mixtures (Hansen, Personal Communication). Correction factors were computed to reduce this problem. Three different studies are available showing that weight per unit area of epidermis is not consistent at different growth stages within species or between species (Storr 1961, Heady and Van Dyne 1965, Theurer 1970). All of these studies strongly suggest considerable caution should be used in applying results obtained from the species used in the present investigation to species found in other areas.

Table 4. Linear regression equations using the model $Y = a + bX$ with their respective coefficients of determination for the relationships between estimated (Y) and observed (X) species values using microhistological analysis.

	a	b	r^2	n[1]
Grasses	1.14	0.97	.96**	31
Forbs	0.76	0.91	.95**	24
Shrubs	2.34	0.96	.95**	28
All species	1.14	0.96	.95**	83
Grasses[2]	−0.57	0.97	.97**	26
Forbs[2]	3.58	0.95	.98**	20
Grasses and forbs[2]	1.20	0.96	.98**	46

**Significant at ($P<.01$)
[1]Actual Values used to develop the equations are shown in Table 2.
[2]Data from Sparks and Malechek (1968).

Without the aid of known hand-compounded diets observers would have probably over-estimated some species and under-estimated others. The authors advocate the training procedures should always be used. Known mixtures should be regularly used by all technicians to evaluate their accuracy and provide for correction if certain species are over-or under-estimated.

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