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THE MICROSCOPE METHOD USED FOR HERBIVORE DIET ESTIMATES AND BOTANICAL ANALYSIS OF LITTER AND MULCH AT THE PAWNEE SITE

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ABSTRACT

This Technical Report describes the microscope technique used in the identification of plant fragments in herbivore diets, litter, mulch, and other complex plant species mixtures. This report also shows how to estimate the percentage dry weight each species of plant may contribute in complex mixture.

IDENTIFICATION AND QUANTIFICATION OF PLANT FRAGMENTS

The "microtechniques" method is the most accurate for identifying plant material taken from the stomachs of herbivores. This technique was described by Baumgartner and Martin (1939) and the technique was later defined by Dusi (1949). This basic technique has been used in recent years in many studies of the diets of herbivores (Mulkern and Anderson 1959; Ward 1960; Ward and Keith 1962; Johnson 1964; Myers and Vaughan 1964; Bear and Hansen 1966; Hayden 1966; Malechek 1966; Vaughan 1967; Sparks 1968; and Ueckert 1968). A brief discussion of this valuable technique seems appropriate.

Tissues of leaves, stems, and flowers etc. are collected from all plants occurring on the range of the animal whose dietary habits are to be studied. These tissues are partially ground and mounted on glass microscope slides. These slides serve as a reference collection to aid in the identification of material taken from stomachs. The contents of a herbivore's stomach is dried and then ground in a Wiley laboratory mill, usually over a 1 mm screen, to reduce all fragments of plants to a uniform size. Samples are washed over a 200 mesh screen to remove dirt and small fragments of plants. The washed samples from the stomachs, either stained or unstained, are spread evenly and mounted on microscope slides, using Hertwig's solution and Hoyer's solution (Bear and Hansen 1966) or Permount (Hayden 1966). The slides may then be dried in an oven at 60°C for 3 days.

The identification of each plant species, by microscopic techniques, is based on characteristics of epidermal tissues (Davis 1959; Croker 1959; Brusven and Mulkern 1960; Storr 1961). Usually 10 to 80 locations are observed, at 40 to 125 power magnification, on 1 to 5 slides prepared from the contents of 1 animal's stomach. A location is considered as an area of the slide delimited by a field of the microscope at a selected power of magnification. Only those fragments that are recognized as epidermal tissue (other than hair-like structures) are recorded as positive evidence for the presence of a plant species at a location on the slide. Data taken from readings of slides are either expressed as per cent of frequency of each species or as per cent composition of each species. The per cent of frequency (number of locations that the species occurred in out of 100 locations) is most easily and accurately taken for plant species in the sample. Per cent of frequency may be converted to density of particles per location using a table (Table 1) developed by Fracker and Brischle (1944).

The relationship of frequency to density is expressed in the formula $F = 100(1 - e^{-D})$ and the mechanics of the conversion can be seen by using a sample problem, as follows:

If 20 locations were examined on each of 50 slides made from the contents of stomachs of 50 herbivores, taken in the same study area, the conversion of frequency to density is as follows: 50 slides X 20 locations = 1,000 total locations. If plant species A occurred in 700 of these locations the per cent of frequency would be:

$$F = \frac{\text{no. of locations in which species A occurs}}{\text{total no. of locations examined}} \times 100$$

$$F = \frac{700}{1000} \times 100 \qquad F = 70\%$$

Converting F to mean density/1000 locations, we have:

$$70 = 100(1 - e^{-D})$$

$$\frac{70}{100} = (1 - e^{-D})$$

$$0.7 = 1 - e^{-D}$$

$$0.7 - 1.0 = - e^{-D}$$

$$- (- 0.3) = - (- e^{-D})$$

$$0.3 = e^{-D}$$

Now, to find the value of D we look up the X value of $e^{-X} = 0.3$ in a standard mathematical table of "Values of Exponential Functions" and we see that D (or X) equals 1.20.

With a frequency of 70% we have a mean density of 1.20 particles of species A per location, D = 1.20.

There are two requirements that must be met before per cent of frequency can be converted to density (Curtis and McIntosh 1950). The fragments of plants must be distributed randomly over the slide, and the density of particles must be such that the most common species does not occur in more than 86% of the fields of the microscope. Thorough mixing of material and adjustments of the quantity placed on the slides will take care of both these requirements.

Recent technological advances (Sparks and Malechek 1968) have added another dimension to the microscopic technique. Density of particles per location is converted to relative density:

$$\left(\frac{\text{Density of particles of species A}}{\text{Total density of particles of all species}} \times 100\right).$$

The relative density of a species is then used to estimate percent of dry weight of that species in the mixture. Equations of regression that express the relationship between estimated per cent of dry weight (X) and actual per cent of dry weight (Y) were developed for 3 categories of plants: Grasses, forbs, and grass-forb combinations. The ratio between estimated per cent of dry weight (relative density) and actual per cent of dry weight was approximately 1 : 1 for all three categories. Student's (t) test showed there was no significant difference between the equations of regression for grasses and forbs, and that the calculated equations of regression for grasses, forbs and grass-forb combinations were not statistically different from the equation Y = X. Therefore, the percent of dry weight of a mixture can be predicted directly from the relative density.

Storr (1961) and Heady and Van Dyne (1965) reported that specific gravity (weight per unit area) of plant material is not consistent at different stages of maturity nor is it consistent from species to species. The 1 : 1 relationship between estimated per cent of dry weight and actual per cent of dry weight may not be consistent with all species or at all stages of maturity. However, unless the parts of plants in the diet being analyzed are grossly different from those reported by Sparks and Malechek (1968), the added accuracy gained by using an equation of prediction more complicated than Y = X would probably not be worthwhile.

MATERIALS, METHODOLOGY, AND SLIDE MAKING

MATERIALS

Microscope slides . . . . glass, plain, standard size, laboratory grade

Cover slips . . . . . glass, 22 x 40 mm for most animals

glass, 22 x 22 mm for insects

Slide labels

Plastic squeeze bottles with spout (2)

Teasing needles (2)

Spatula with narrow, flexible blade

Sponge

200 mesh screen

Drying oven and racks

Waring blender (1 quart)

Hertwig's solution - clearing agent

270 g chloral hydrate crystals

19 cc 1N HCl

60 cc glycerin

Combine glycerin and HCl, add chloral hydrate crystals, stir until crystals dissolve. The crystals will dissolve faster if the mixture is warmed and agitated in a blender.

Hoyer's solution - mounting medium

200 g chloral hydrate crystals

50 cc water

20 cc glycerin

30 g photo purified gum arabic

Combine glycerin and water, add chloral hydrate crystals, stir until crystals dissolve. Warm the solution and stir in gum arabic. The mixture may be agitated with a blender after the gum arabic is added. Let stand until the solution clears.

METHOD

In the field, samples intended only for diet studies can be frozen, air dried, or preserved in alcohol. Samples intended for energy determinations or chemical analyses, as well as diet study, should be air dried or oven dried (below 70°C) or frozen as soon as possible, and should not be placed in a preservative. Dietary samples from large herbivores (all stomach, fistula, rumen, or fecal material) should be air dried and then ground in a Wiley laboratory mill over a 1 mm (20 mesh) screen before microscope slides are made. Mammals that weigh less than 200 g live weight usually chew their food fine enough so grinding is not necessary. Samples removed from insect crops cannot be ground because of the small quantities involved. Dietary material from these small animals is transferred directly to slides without drying or washing.

Two slides should be prepared from each sample, if possible. Plant fragments should be spread evenly over the slide and should not overlap. At 100 power magnification, there should be about three large fragments per field.

Place approximately 10 cc of ground or blended sample in a 0.1 mm (200 mesh) screen and wash under running water for one minute (Fig. 2). Remove a small amount of the washed material from the screen, with a spatula, and spread near one end of a microscope slide (Fig. 3). Add three or four drops of Hertwig's solution to the wet material on the slide, then carefully boil off most of the Hertwig's by holding the slide above a small alcohol burner (Fig. 4). It is important, at this point, not to char the sample by overheating.

When most of the Hertwig's has boiled off, add enough Hoyer's solution to cover an area about 2/3 as large as a cover slip. With a teasing needle, mix the plant material with the Hoyer's and spread evenly over an area as large as a cover slip (Fig. 5). Place a cover slip on the preparation and heat the slide over the burner until the Hoyer's starts to boil. Immediately wipe the bottom surface of the slide with a cold, damp cloth or sponge to draw air bubbles out of the Hoyer's solution. Press a teasing needle gently on top of the cover slip to squeeze out excess mounting medium and remove any remaining air bubbles. (Very tiny bubbles usually disappear during the drying process and are not detrimental.) Apply a thin ring of Hoyer's solution around the edge of the cover slip, if needed, to form a seal as the slide dries.

Slides are placed flat on racks, in a drying oven at 55°C, for two or three days, or until the Hoyer's solution has hardened, then stored in a dry place. Hoyer's solution forms a permanent mounting medium when hardened, but is soluble in water allowing easy cleaning or reuse of slides.

REFERENCE SLIDES

A collection of all species of plants present at a site should be available for making reference slides. The appropriate slides of leaf, stem, root, flower, and seed should be prepared for each species. The separate parts of each plant are placed in a Waring blender with enough water to at least cover the blades. Less than a teaspoon of plant material is needed and plants may be green or dried.

After one to two minutes at high speed, the contents of the blender are poured into a 0.1 mm mesh screen and washed. Reference slides are made directly from this material, following the same procedure as for dietary samples, but applying more material to the slides.

Woody material may be ground in a Wiley mill before being added to the blender. Very hot water also helps soften tissue and removes plant pigments.

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Table 1. Relations of frequency to density and abundance (for strictly random distributions)

Freq. %	Den. Q	Abun. Q	Freq. %	Den. Q	Abun. Q
1	0.01	1.01	52	0.73	1.41
2	0.02	1.01	53	0.75	1.42
3	0.03	1.02	54	0.77	1.44
4	0.04	1.02	55	0.80	1.45
5	0.05	1.03	56	0.82	1.47
6	0.06	1.03	57	0.84	1.48
7	0.07	1.04	58	0.86	1.49
8	0.08	1.04	59	0.89	1.51
9	0.09	1.05	60	0.91	1.53
10	0.10	1.05	61	0.94	1.54
11	0.11	1.06	62	0.96	1.56
12	0.12	1.06	63	0.99	1.58
13	0.14	1.07	64	1.02	1.60
14	0.15	1.08	65	1.05	1.61
15	0.16	1.08	66	1.08	1.63
16	0.17	1.09	67	1.11	1.65
17	0.18	1.10	68	1.14	1.67
18	0.20	1.10	69	1.17	1.70
19	0.21	1.11	70	1.20	1.72
20	0.22	1.12	71	1.23	1.74
21	0.23	1.12	72	1.27	1.77
22	0.25	1.13	73	1.31	1.80
23	0.26	1.14	74	1.35	1.82
24	0.27	1.14	75	1.39	1.85
25	0.29	1.15	76	1.43	1.88
26	0.30	1.16	77	1.47	1.91
27	0.31	1.17	78	1.51	1.94
28	0.33	1.17	79	1.56	1.98
29	0.34	1.18	80	1.61	2.01
30	0.35	1.19	81	1.66	2.05
31	0.37	1.20	82	1.71	2.09
32	0.38	1.20	83	1.77	2.14
33	0.40	1.21	84	1.83	2.18
34	0.41	1.22	85	1.89	2.23
35	0.43	1.23	86	1.96	2.28
36	0.44	1.24	87	2.04	2.34
37	0.46	1.25	88	2.12	2.41
38	0.48	1.26	89	2.20	2.48
39	0.49	1.27	90	2.30	2.56
40	0.51	1.28	91	2.40	2.64
41	0.52	1.29	92	2.52	2.75
42	0.54	1.30	93	2.66	2.86
43	0.56	1.31	94	2.81	2.99
44	0.58	1.32	95	2.99	3.15
45	0.60	1.33	96	3.22	3.35
46	0.62	1.34	97	3.51	3.62
47	0.63	1.35	98	3.91	3.99
48	0.65	1.36	99	4.60	4.65
49	0.67	1.37	99.5	5.30	5.32
50	0.69	1.38	99.9	6.91	6.91
51	0.71	1.40	100	----	----

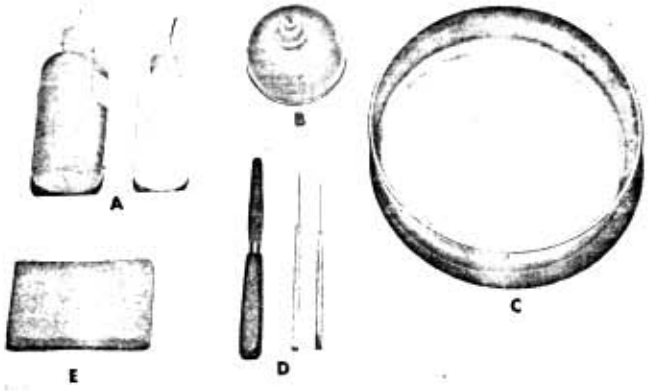

Figure 1. Materials needed: (A) plastic squeeze bottles, (B) alcohol burner, (C) 0.1 mm mesh screen, (D) teasing needles and spatula, (E) sponge.

Figure 2. The ground sample is washed with water to remove dirt and small plant fragments over a 0.1 mm (200 mesh) screen.

Figure 3. A small amount of the sample is placed on a slide. About 3 or 4 identifiable particles per field is the desired density.

Figure 4. Hertwig's solution is boiled off over an alcohol burner.

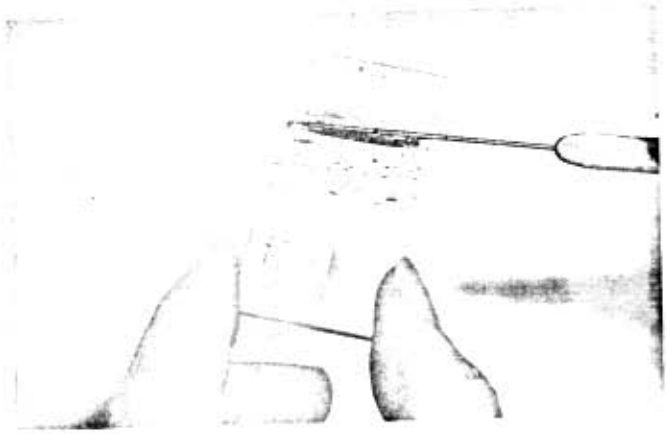

Figure 5. Hoyer's solution is mixed with the sample and spread evenly over the slide.

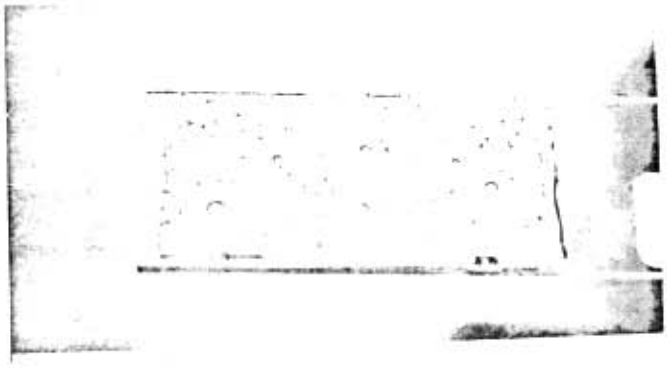

Figure 6. A finished slide showing the desirable approximate density of plant fragments and ring of Hoyer's around the cover slip. The air bubbles may be pressed out with a teasing needle while the slide is cooling after being heated.

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