Reprinted from the AGRONOMY JOURNAL
Vol. 49:190–193, 1957

The Constituent Differential Method of Estimating Species Composition in Mixed Hay[1]

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SYNOPSIS

A method is presented for determining the species composition by weight of a two-component forage mixture when the two components contain different concentrations of a given constituent. It consists of measuring the constituent concentration of a large sample from each plot and the species components from small samples taken at random from plots treated alike. Species composition of the large sample is then computed using appropriate formulae. The use of dry matter, calcium and crude protein to estimate botanical composition was investigated. The method was found to be more efficient than hand separation.

IN FORAGE crop investigation it is often necessary to determine the vegetative composition of a forage mixture by weight, in order to evaluate properly the effect of treatment or environment on the composition of the mixture. Hand separation of a mixture sample into its component species has been the accepted standard method in such investigations. Hand separation, however, is objectionable not only because of the cost but also because of the limited time for separation before species in the mixture become unidentifiable. Several rapid techniques have been proposed to obviate the necessity of hand separation (6, 7, 10). These methods have shown considerable variation and in many instances do not give an accurate estimate of the botanical composition (2, 9). Thus, an investigator is faced with the choice between laborious hand separation or one of several rapid methods of questionable reliability.

One solution to the problem is the use of a double, or two stage, sampling technique as described by Wilm et al. (11), Cochran (3) and other investigators. By this technique a large sample with an easily measured attribute is taken from the mixture. Then a small sample with a difficultly measured attribute which is highly correlated to the easily measured attribute is taken at random from the large sample. As pointed out by Wilm et al. (11) the efficiency of a double sampling procedure depends upon the relative cost of measuring the attributes in question and on the degree of correlation between the two attributes.

This paper reports the development of a relationship which was used in a double sampling technique for estimating the botanical composition of a two-component forage mixture. It is based on the concentration of an easily measured constituent of the mixture and the difference in concentration between one species component and the other with respect to the same constituent. Although the fundamental relationship upon which the proposed method is based has been used in other fields, it has not been adapted to forage investigations (1, 5, 8).

In this paper the word "component" refers to species or species groups composing the vegetative composition and the word "constituent" refers to the attribute such as oven dry matter, crude protein, or calcium content in an herbage sample.

EXPERIMENTAL PROCEDURE

Theory

The relationship used in the proposed method may be derived for two situations, one in which the concentration of the constituent in question is measured in the dry forage, and the other in which the constituent is measured in the green forage. The derivation of the relationship to be used in the dry-weight situation may be illustrated with a mixture of clover and grass using calcium as the measured constituent.

Let: X = percent clover in oven dry mixture, H = percent Ca in the oven dry mixture, L = percent Ca in the oven dry clover, G = percent Ca in the oven dry grass. When X = O, H = G and when X = 100, H = L.

Under these restrictions the linear relationship between X, H, L, and G may be shown to be:

$$\frac{X - O}{H - G} = \frac{100 - O}{L - G} \text{ or } X = \frac{100\,(H - G)}{(L - G)} \qquad [1]$$

Thus, if the Ca content of the grass (G) and the clover (L) is known or can be estimated from a small, hand separated sample of the mixture, the clover content, and hence the grass content, can be estimated from the Ca content of the entire mixture.

In a similar manner the relationship between a constituent measured in the green forage and the botanical composition of the dry forage may be derived. This may be illustrated using dry matter itself as the constituent under consideration. Let: Y = percent green legume in the green mixture, X = percent dry legume in the dry mixture, H = percent dry matter in the green mixture, G = percent dry matter in the green grass, L = percent dry matter in the green clover.

Then, from formula [1]: $Y = \frac{100\,(H - G)}{(L - G)}$, and the percent of dry legume, X, in the dry mixture may be obtained from

$$X = (L/H)\ Y, \text{ or } X = \frac{100\,(H - G)\,(L)}{(L - G)\,(H)} \qquad [2]$$

Materials and Methods

A series of sampling studies were initiated, on a native flood meadow in southeastern Oregon. The vegetation studied was a mixture of a native annual clover (*Trifolium variegatum*) and a group of grasses and grass like species, hereafter referred to as "grass". The grass like species were mainly rush (*Juncus* spp.) and sedge (*Carex* spp.). The yield and quality of hay from this native vegetation can be improved through proper fertilization and management (4).

Experiment A: To determine the degree of sampling necessary to characterize the botanical composition of a plot, ten 2 × 2 samples were taken adjacent to each other through the length of a 20-foot plot and ten 2-inch strips 20 feet long were clipped

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[1] Contribution from the Squaw Butte–Harney Range and Livestock Experiment Station, Burns, Oregon, which is jointly operated by the A.R.S., U.S.D.A. and Oregon Agr. Exp. Sta., Oregon State College, Corvallis, Ore. Technical paper No. 1003. Received October 8, 1956.

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from the center of another plot. In both cases the samples were hand separated into their component species, and the components oven dried.

Experiment B: To compare hand separation values with computed values of botanical composition, a series of ten 2-inch by 20-foot samples was clipped from one of the more uniform plots in the area. Each sample was hand separated and the dry matter content of each of the separates was determined. The dry matter content of the mixture of each sample was obtained by compilation of separate data. Botanical composition of each sample was then computed using the average dry matter concentration in the "grass" and legume from the ten samples and the dry matter concentration in the hay in each sample. The values thus obtained were then compared with the composition as obtained by hand separation.

Experiment C: In order to estimate the uniformity of concentration of calcium, crude protein and dry matter in the "grass" and the legume components, a series of random samples of each was taken. These samples were also used to evaluate the difference between the "grass" and the legume with respect to these constituents. The samples were hand separated and oven dried. Dry matter, calcium, and crude protein were determined on the species components in each sample.

Experiment D: To determine the degree of sampling necessary to obtain a reliable estimate of the constituent concentration in the mixture, 3 strips 3 feet wide and 20 feet long were clipped. The hay on each strip was raked and sampled by taking twenty samples, each consisting of 10 handfuls taken at random from each yield sample. Dry matter content of each sample was determined.

EXPERIMENTAL RESULTS

The botanical composition of the area under consideration was extremely variable. Clover composition ranged from 27 to 55% in the samples taken from the area. With this variation thirteen 2 × 2 foot samples or three 2-inch by 20-foot samples were required to estimate the botanical composition on a single plot within 5% of the mean.

In table 1 the actual clover composition of ten samples of forage may be compared with the computed composition. The correlation between the actual and the computed values is high ($r = .96$). Under these conditions a double sampling procedure (3) using the constituent differential method would be as efficient as hand separation even if the cost of hand separation were as little as 1.6 times the cost of oven drying.

Crude protein, calcium and dry matter values obtained from random samples are presented in table 2. As judged by the magnitude of the coefficient of variation (C.V.)

Table 1.—Percent dry matter in clover, grass and mixed hay, and actual and computed percent clover in ten random 2 in. × 20 ft. samples.

Sample No.	Oven Dry Matter			Clover	
	Clover	Grass	Mixed Hay	Actual*	Computed†
	%	%	%	%	%
1	20.0	33.5	28.0	29.0	26.4
2	19.5	34.0	28.0	28.8	26.4
3	19.5	34.0	29.5	20.5	18.1
4	19.0	33.5	31.5	8.3	8.3
5	19.0	34.0	27.5	29.9	29.3
6	19.0	33.0	26.5	33.2	35.6
7	19.5	33.0	27.0	32.1	32.4
8	19.0	33.0	27.0	30.1	32.4
9	19.5	33.5	27.0	33.5	32.4
10	19.0	32.5	27.5	25.6	29.3
Ave.	19.3	33.4		C.V. 28.2	30.3

* Determined by hand separation.
† Computed from Equation [2] using $L = 19.3\%$, $G = 33.4\%$, $H =$ dry matter in mixed hay from each sample.

each of the three constituents is relatively uniform from one sample to the next. It will also be noted that the difference in concentration between the grass and the legume, upon which the accuracy of the method largely depends, is quite uniform for each constituent. Therefore, any of the three constituents could be used.

The size of sample necessary to measure the dry matter, crude protein, or calcium content of the yield sample within 1% of the mean was found to be 20 random handfuls, weighing about 1,000 g. The sample size of each species component should also be about 1,000 g. when weighed to the nearest gram.

Upon considering the results of these studies a double sampling technique was developed whereby botanical composition could be estimated with maximum accuracy at a minimum cost. This technique consists of sampling in the following manner: *(1)* The usual yield strip is clipped from the experimental plot. The concentration of the hay constituent considered is subsequently determined from this yield sample. *(2)* A small random sample of each botanical component is taken from each series of plots treated alike. These samples are retained for the determination of constituent concentration. *(3)* Concentration values are then used along with the appropriate value from the hay sample in either equation [1] or [2] to compute the botanical composition of each plot.

DISCUSSION

Several points should be considered when applying the proposed method to the estimation of botanical composition in a forage experiment. The accuracy in estimating botanical composition in a yield sample is characterized by the correlation between calculated and true composition values. This correlation is affected, to a considerable ex-

Table 2.—Actual oven-dry-matter, crude protein, and calcium contents of legume and "grass" samples taken from random plots of mixed hay.

Sample No.	Legume	Grass	Difference
	%	%	%
	Oven-dry matter		
1	15.4	31.0	15.6
2	15.5	29.8	14.3
3	15.0	30.8	15.8
4	14.7	30.5	15.8
Mean	15.2	30.5	15.3
C.V.	2.4%	1.7%	4.7%
	Crude Protein		
1	18.3	07.5	10.8
2	17.8	07.6	10.2
3	19.7	07.0	12.7
4	18.7	06.8	11.9
Mean	18.6	07.2	11.4
C.V.	4.3%	5.4%	9.8%
	Calcium		
1	1.82	0.42	1.42
2	1.70	0.33	1.37
3	1.67	0.35	1.32
4	1.73	0.30	1.43
Mean	1.73	0.34	1.39
C.V.	3.8%	4.6%	3.6%

tent, by the magnitude of the difference in constituent concentration between the species, by the uniformity of concentration within a species, and by the size of sample used to estimate constituent concentration in the mixture.

It is suggested that the absolute difference in concentration be accurate to 2 significant digits. All of the constituents used in this study conform to this suggestion. The greater the relative difference between species, the more accurate will be the estimate of botanical composition. Thus, from table 2, dry matter would give the most accurate estimates, followed in order by calcium and crude protein, provided that all three may be sampled with equal precision.

As shown in table 2, the 3 constituents studied were relatively uniform in both species. Hence a single sample could be used to estimate the constituent concentration in each species growing over a rather large area. This may not always be the case. If treatments are expected to affect the concentration of the constituent considered, separate samples should be taken from each treatment. Where treatments do not affect concentration, it is likely that adequate estimates of the constituent concentration within the separate species may be obtained by separating a single composite sample from each replication.

The constituent concentration in a yield sample of hay is almost exclusively the result of vegetative composition. Therefore, the sub-samples taken to determine constituent concentration must be botanically representative of the hay mixture throughout the yield strip for an accurate determination of vegetative composition. Under the conditions of this study a sample consisting of about 20 handfuls and weighing about 1,000 g. gave an estimate of concentration of oven dry matter within 1% of the mean concentration. A sample of this size is also required to determine the mean concentration of crude protein and calcium. A sample of about 1,000 g. is normally taken from each plot for dry matter determination in the course of experimental work, and therefore represents no extra cost or labor.

The relative efficiency of the proposed technique as compared to hand separation is measured by the correlation between calculated and hand separated values, and by the relative cost of the two methods. As indicated previously the proposed technique is superior to hand separation if dry matter is used as the constituent. The cost of hand separating a small representative sample is the only expense involved in addition to that required to estimate the dry matter yield of the plot.

Although dry matter appears to be a satisfactory constituent upon which to base the constituent differential method of botanical composition estimation, it possesses some disadvantages. Speed is essential to avoid error from moisture loss during sampling. In addition, such other factors as precipitation, time of day, and humidity may also introduce error. Both calcium and protein have distinct advantages over dry matter in that speed is not a factor in handling the sample, and time of day and climatic conditions are not so likely to influence them. The only disadvantage in using these constituents is that chemical determinations are not always a part of the planned experimental procedure, and would incur additional cost. In general, chemical constituents will be more satisfactory than dry matter percentages.

Computation of composition values is easily performed when data are arranged as illustrated in table 3 for simulated crude protein values.

Table 3.—Tabular arrangement of constituent concentrations for the computation of legume composition.

Crude Protein			H − G	L − G	$X - 100 \frac{H-G}{L-G}$
Legume (L)	Grass (G)	Hay (H)			
%	%	%			%
19.6	7.6	13.6	6.0	12.0	50
19.6	7.6	8.6	1.0	12.0	8
7.6	19.6	13.6	−6.0	−12.0	50
7.6	19.6	8.6	−11.0	−12.0	92

SUMMARY AND CONCLUSIONS

A method is presented for determining the species composition by weight of a two component forage mixture when the two components contain different concentrations of a given constituent. The method consists of measuring the constituent concentration of a large sample from each plot and of the two species components from a small sample taken at random from plots treated alike. The species composition of the large sample is then computed using one of the following formulae:

$$X = 100 \frac{(H - G)}{(L - G)} \qquad [1]$$

Where X = percent legume composition on a dry weight basis, H = percent of the measured constituent in the oven dry sample of "Hay", G = percent of the measured constituent in the oven dry sample of "Grass", and L = percent of the measured constituent in the oven dry sample of "Legume".

$$\text{Or } X = 100 \frac{(H - G)(L)}{(L - G)(H)} \qquad [2]$$

Where X = percent legume (on a dry weight basis) in the green sample, H = percent dry matter in the green sample of "Hay", G = percent dry matter in the green sample of "Grass", and L = percent dry matter in the green sample of "Legume".

The use of dry matter, calcium, and crude protein to estimate botanical composition was investigated and the necessary requirements for using other constituents were discussed.

It was concluded that, at least where dry matter is the measured constituent, the method is more efficient than hand separation.

LITERATURE CITED

1. AHLGREN, H. L. A comparison of methods used in evaluating the results of pasture research. Jour. Amer. Soc. Agron. 39:240–260. 1947.
2. ARNY, A. C., and SCHMID, A. R. A study of the inclined point quadrat method of botanical analysis of pasture mixtures. Jour. Amer. Soc. Agron. 34: 238–247. 1942.
3. COCHRAN, W. G. Sampling techniques. John Wiley and Sons, Inc., New York, N. Y. 1953.
4. COOPER, CLEE S. More mountain meadow hay with fertilizer. Ore. Agr. Exp. Sta. Bul. 550. 1955.
5. DAVIES, W. Methods of pasture analysis and fodder sampling. Welsh Plant Breeding Sta. Rpt. No. 1. 1931.
6. LEASURE, J. K. Determining the species composition of swards. Jour. Amer. Soc. Agron. 41:204–206. 1949.
7. LEVY, B. E., and MADDEN, E. A. The point method of pasture analysis. New Zealand Jour. Agr. 46:267–279. 1933.

8. Report of Joint Committee of A.S.A., A.D.S.A. and A.S.R.M. Pasture and Range Techniques. Jour. Amer. Soc. Agron. 44:39–50. 1952.
9. SPRAGUE, V. G., and MYERS, W. M. A comparative study of methods for determining yields of Kentucky bluegrass and white clover when grown in association. Jour. Amer. Soc. Agron. 37:370–377. 1945.
10. TINNEY, F. W., AAMODT, O. S., and AHLGREN, H. L. Preliminary report of a study of methods used in botanical analysis of pasture swards. Jour. Amer. Soc. Agron. 29: 835–840. 1937.
11. WILM, H. G., COSTELLO, D. F., and KLIPPLE, G. E. Estimating forage yield by the double sampling method. Jour. Amer. Soc. Agron. 36:194–203. 1944.