Petiole Sampling of Sugar Beet Fields in Relation to Their Nitrogen, Phosphorus, Potassium and Sodium Status[1]

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A technique for collecting petiole samples from sugar beet fields for nutrient assay has been used in California for several years (9, 11)[3]. This procedure appears to correlate well with observed growth differences and the results of field experiments, particularly with respect to nitrogen, the principal nutrient limiting yields (10). Aside from the work of Brown (1) in Colorado, there has been little emphasis on the size of sample necessary to estimate with sufficient accuracy the nutrient status of a given field for practical fertilizer recommendations.

With the increasing use of petiole analysis, it seemed desirable to investigate more thoroughly the sources of variation involved in this procedure and, if possible, to improve the overall technique. The procedure used until now to collect petiole samples from commercial fields in California has been described (8). Briefly, it consists of dividing the field to be sampled into equal parts of approximately 10 acres or less; walking across each section at right angles to the rows and collecting 30 to 40 petioles at equally spaced intervals. Fields up to 40 acres in size are represented by four samples each and larger fields by more, each 10 acres being represented by a separate sample.

Procedure

Over a three-year period, 17 different fields were selected for petiole sampling in the following counties: eight in Yolo, five in Monterey and four in Kern. Each field was sampled by collecting individual petioles at uniform intervals while walking across the rows in the middle of a 10-acre area. Such an area would normally constitute a sampling unit from which 30 to 40 petioles would be taken. Each petiole selected for analysis was from a leaf defined by Ulrich (8, 9) as "a youngest mature leaf."

In three of the fields four petioles were collected at each of 25 locations. At each location two of the petioles were taken from the same plant and two taken from another plant located within a few feet of the first. In the remaining fields 25 petioles were taken at equally spaced intervals.

The petioles were bagged separately in the order of their collection, dried, ground and analyzed for nitrate-nitrogen (5, 8), phosphate-phosphorus (8), and sodium. After the plant material had been ashed as previously described (8), the ash was taken up in nitric acid (0.16N, final concentration) and analyzed for potassium and sodium by the lithium internal standard method for a flame photometer (Perkin-Elmer, Model 52A). Two determinations, one on one day and the other on another day, were made on each petiole.

The results of the petiole analyses were analyzed statistically by the usual methods of analysis of variance. The components of variance, variance of a single determination, confidence limits and coefficients of variation

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[3] Numbers in parentheses refer to literature cited.

were determined by the procedures outlined by Brownlee (2) , Cochran and Cox (4) and Snedecor (6) . Specific examples of the calculations are given as footnotes to Table 2.

Results of Intensive Sampling

The components of variance associated with petiole sampling of sugar beet fields, expressed as percentages of the total variation, are given in Table 1 for the nitrate, phosphate, potassium, and sodium determinations of three fields sampled intensively. Of the two main sources of variation in the field and in the laboratory, those in the field, as expected, greatly exceeded the variations in the laboratory. Within the field, surprisingly enough, the variations from petiole to petiole within a beet exceeded those from beet to beet or from location to location, particularly the nitrate determination of field HY. Large variations between petioles within a beet also appeared in the other fields in the nitrate, phosphate, potassium and sodium determinations. Surprising, too, was the fact that in some instances there was no significant component of variation for locations within a field, which indicated that the variations from beet to beet within a location were as great as those of locations within a field.

The components of variance of the chemical analyses of the petiole samples were minute compared to the components of variation within the field. In all instances, except for nitrate-nitrogen in field HY, the analytical variation was less than 2 percent of the total variation, sampling plus analysis. Within the analytical variations the component between dates was significant in most cases, but this constituted less than half the total analytical variation of the nitrate determination, except in field HY where the variation for analysis was exceptionally large. With the possible exception of nitrate analyses made of high-nitrate samples, there is little to be gained from further improvements *in* analytical methods to increase the precision of petiole sampling of sugar beet fields. This conclusion is supported by the results of sampling 14 more fields in Yolo, Monterey and Kern counties in which the variations of field sampling and of laboratory analysis are compared (Tables 2, 3, 4 and 5) .

Since the components of variation between petioles within a beet and between beets within a location proved to be highly significant in all cases, this might imply that the order of selecting two petioles from a single beet or from two adjacent beets could be important in the petiole sampling of sugar beet fields. Once a leaf is selected from a plant, the second leaf selected might conceivably be less desirable for analysis than the first. Similarly, once a beet is selected for sampling within a location, the second beet might differ consistently in some significant manner from the first. Considering these as possibilities, statistical analyses were made of the results of the first petiole compared with the second petiole of each beet, and of the petioles of the first beet with the second beet. These proved to be nonsignificant. Apparently, there is no consistent pattern followed by a field man in selecting the first beet to be sampled or in selecting the first petiole on a beet for analysis. This conclusion, however, may not be true of all field men or of all fields to be sampled.

Table 1.—Components of Variance as Percent of Total Variance for Nitrate, Phosphate, Potassium and Sodium Determinations for Dried Petioles of Recently Matured Leaves.

			Components of variance[2] as percent of total							Total variance[3]
							Analytical			
Field[1]	Constituent	Mean	Between locations (within field) S_L %	Between beets (within location) S_B %	Between petioles (within beets) S_P %	Total between petioles (within field) S_{LBP} %	Between dates S_t %	Residual S_A %	Total between analyses S_{tA} %	S^2_T
H Y	NO_3-N	19,600 (ppm)	18.6[4]	17.5[4]	41.7[5]	77.8[5]	14.9[5]	7.3	22.2	95,500
B Y	NO_3-N	2,227 (ppm)	0	82.2[5]	17.1[5]	99.3[5]	0.0	0.8	0.8	89,495
HD Y	NO_3-N	2,164 (ppm)	45.6[5]	39.8[5]	14.2[4]	99.6[5]	0.08[5]	0.26	0.34	53,007
H Y	PO_4-P	1,918 (ppm)	0	57.3[5]	40.7[5]	97.9[5]	0.5[5]	1.6	2.1	3,779
B Y	PO_4-P	1,011 (ppm)	54.6[5]	15.3[5]	22.2[5]	98.6[5]	0.0	1.4	1.4	1,527
HD Y	PO_4-P	1,848 (ppm)	0	68.6[5]	29.6[5]	98.2[5]	0.5[5]	1.4	1.8	3,173
H Y	K	4.77 (%)	44.7[5]	40.4[5]	13.5[5]	98.4[5]	0.5[5]	1.1	1.6	0.6484
B Y	K	3.06 (%)	36.6[4]	49.0[5]	12.1[4]	97.7[5]	0.6[5]	1.7	2.3	0.6520
HD Y	K	4.44 (%)	28.2[4]	52.5[5]	17.8[5]	98.5[5]	0.0	1.5	1.5	0.6567
H Y	Na	5.11 (%)	55.5[5]	16.7[5]	26.6[5]	98.8[5]	0.2[5]	1.0	1.2	0.9068
B Y	Na	3.67 (%)	0	85.5[5]	13.0[5]	98.6[5]	0.06[4]	1.4	1.4	1.5223
HD Y	Na	1.87 (%)	55.6[5]	26.6[5]	17.0[5]	99.2[5]	0.0	0.8	0.8	0.5310

[1] See Table 2 for field designations.
[2] Total variance values for NO3-N and for PO_4-P have been multiplied by 1/100.
[3] Components of variance were calculated according to procedure by Brownlee (2).
[4] Significant at the 5% level.
[5] Significant at the 1% level.

Results of Extensive Sampling

Although the intensive sampling of three sugar beet fields, involving 2,400 separate chemical analyses, showed conclusively that the bulk of the variation in petiole sampling was in the field rather than in the laboratory, more information about the nature and extent of the field and laboratory variations seemed desirable. For this study a single petiole from each of 25 locations in a field were taken at equidistant intervals across the plant rows in five more fields in Yolo county, five in Monterey county and four in Kern county. Each petiole was analyzed for the same constituent on two different days: nitrate-nitrogen, phosphate-phosphorus, potassium and sodium. The analytical results of these fields along with those of the three original fields intensively sampled are given in Tables 2, 3, 4 and 5.

Nitrate-Nitrogen

The results tabulated in Table 2 give the ranges in nitrate-nitrogen values, the confidence limits of the mean, the components of variance, the gains in precision of different numbers of petioles per sample and times of analysis, the variances of single determinations and the coefficients of variation of each field. Of particular interest in Table 2 is the wide range in nitrate-nitrogen values from field to field and within fields. For example, in field HY the average value is 19,600 ppm., the highest 28,400 and the lowest 13,700. If the critical petiole nitrogen concentration at which a response to nitrogen fertilization may be expected is 1,000 ppm. on a dry basis, then the entire field in HY is amply supplied with nitrogen at the time the petiole samples were taken. Proceeding down the list and judging by the mean values alone, all fields are adequately supplied with nitrogen except field 10Y. Logically, though, nitrogen adequacy would decrease with a decrease in the mean nitrogen values. Thus, mean values of less than 3,000 ppm. nitrate-nitrogen would be considered as approaching the danger line and additional petiole samples should be taken frequently if an adequate supply of nitrogen is to be maintained within the beets at all times. Experience has shown that once the nitrate-nitrogen values fall below a mean value of 1,000 ppm., the beets grow less than those above this value (10) .

Decisions as to the desirability of adding nitrogen to a field of beets must also consider the degree of reliability or confidence which may be placed in mean values obtained by petiole sampling. When nitrate values are determined from samples consisting of 25 petioles and one analysis, the decision to observe or not to observe a field more closely is influenced by the variability of the individual plants in the field. This is definitely true for fields in Kern county where the petiole samples were collected from fields which were obviously spotty as shown by visual observations, by petiole tests *in* the field for nitrate with diphenylamine reagent (7) , and finally by the chemical analysis of the petioles themselves.

The great importance of the individual petiole nitrate values was not fully realized until the laboratory analyses for nitrate were completed and tabulated. Thus, similar mean nitrate values of different fields reflected entirely different distributions of the petiole nitrate values in the field, as in fields 13 K and 7 Y. In field 13 K, which had a mean value of 4,130, 36 percent of the beets sampled were below 1,000 ppm., and field 7 Y, which had a lower mean value, 3,610 ppm., had only 8 percent of the

Table 2.—Nitrate-Nitrogen in Petioles Expressed in Parts Per Million on a Dry Basis for 17 Sugar Beet Fields as Related to the Ranges of Values, Confidence Limits, Components of Variance, Variances of Single Determinations, Gains in Precison and Coefficients of Variation.

Field[1]	Range	Mean	Confidence limits at the 5% level[2] Limits of error: One analysis Petioles per sample 25	One analysis Petioles per sample 50	Two analyses Petioles per sample 25	Two analyses Petioles per sample 50	Components of variance (multiplied by 1/100): Between petioles within field S^2_{LBP}	Analytical: Between dates S^2_t	Analytical: Residual S^2_A	$\frac{S^2_{LBP}}{S^2_T} \times 100$	Variance[2] single analyses x 1/100 petioles 25	Gain in precision: Single analysis petioles[4] 50	Gain in precision: Duplicate analyses petioles[5] 25	C.V.[6]
	ppm.	ppm.	ppm.	ppm.	ppm.	ppm.				%		%	%	%
H Y	28,400—18,700	19,600	±3,050	±2,954	±2,288	±2,155	74,300	14,200[8]	7,000	77.8	24,172	3.2	33.6	7.9
I M	19,800— 8,900	13,606	±2,707	±2,577	±2,058	±1,915	89,300	114[8]	41	85.2	19,070	5.0	29.8	10.2
V M	15,800—10,400	13,110	±1,108	±1,057	± 876	± 788	19,900	0	24	89.2	3,196	6.8	26.5	4.3
VIII Y	22,100— 1,400	9,078	±2,040	±1,544	±1,964	±1,442	231,140	0	1,585	99.3	10,831	32.1	3.9	11.5
IV M	10,900— 1,520	5,350	± 995	± 771	± 943	± 703	51,395	182[8]	338	98.9	2,576	29.1	5.5	9.5
III M	10,900— 520	4,760	±1,177	± 857	±1,159	± 832	84,660	0	219	99.7	3,605	37.3	1.6	12.6
XIII K	19,400— 170	4,130	±2,058	±1,483	±2,038	±1,455	265,048	0	421	99.8	11,023	38.8	1.0	25.4
XIV K	11,400— 150	4,042	±1,295	± 940	±1,278	± 916	103,487	27[7]	201	99.8	4,368	37.8	1.3	16.4
VII Y	8,100— 680	3,614	± 875	± 640	± 859	± 619	46,315	0	140	99.7	1,993	36.7	1.9	12.4
IX Y	8,450— 290	3,529	±1,082	± 783	±1,069	± 765	72,466	0	145	99.8	3,044	38.2	1.2	15.6
II M	7,200— 470	2,984	± 822	± 621	± 793	± 581	57,821	94[8]	152	99.3	1,759	32.4	3.7	14.0
XII K	9,800— 140	2,458	±1,039	± 778	±1,007	± 735	61,810	0	339	99.5	2,811	33.5	3.2	21.6
XI K	9,200— 480	2,271	± 781	± 570	± 768	± 552	37,026	0	106	99.7	1,587	37.0	1.7	17.5
X Y	11,150— 190	2,227	± 848	± 646	± 813	± 600	39,146	0	305	99.2	1,871	31.3	4.3	19.4
IX Y	11,150— 220	2,164	± 939	± 690	± 920	± 664	52,826	45[8]	136	99.6	2,294	35.1	2.1	22.1
VI Y	4,080— 250	1,374	± 458	± 336	± 450	± 324	12,671	14[8]	26	99.7	547	35.3	1.8	17.0
X Y	540— 70	215	± 88	± 80	± 78	± 63	181	0	13	93.3	20	10.2	21.8	21.0

[1] M = Monterey, Y = Yolo, K = Kern County.

[2] Variance of a single determination on a composite of 25 petioles = $S^2_{LBP}/25 + S^2_t + S^2_A$, e.g., 74,300/25 + 14,200 + 7,000 = 24,172; for duplicate analyses on composite of 50 petioles = $74{,}300/50 + \frac{(14{,}200 + 7{,}000)}{2} = 12{,}086$. The values given in the table have been multiplied by 1/100.

[3] Confidence limits = mean ±t (Variance of technique)1/2, *e.g.,* for single analysis on composite of 25 petioles, 19,600 ± 1.96 (24,172)1/2 = 19,600 3,050; for duplicate analyses on composite of 50 petioles, 19,600 + 1.96 (12,086)1/2 = 19,600 + 2,155.

[4] Using the limit of error for single analysis, 25 petioles composited, as 100%; the gain in precision for a 50-petiole sample, one analysis, equals 3,050 - 2,954 x 100 = 103.2% **or** a gain of 3.2%.

[5] Gain in precision for duplicate analyses, 50 petioles, equals 41.4% for all fields.

[6] Coefficient of variation (C. V.) = (Variance for single analysis, 25 petioles composited)1/2 mean x 100.

[7] Significant **at** the 5% level.

[8] Significant at the **1%** level.

beets below 1,000 ppm. Accordingly, for greater precision in judging the nitrogen status of a field of beets, the testing of individual plants or at least observing them closely in the field should be encouraged, particularly in areas sampled for the first time.

A review of the confidence limits of the nitrate means in relation to the number of petioles taken per sample and to the number of times each sample is analyzed is informative. On the average, more precision is gained by taking more petioles in the field rather than by making more analyses of the same sample in the laboratory. Only when the nitrate vaiues are exceptionally high is there an appreciable gain in precision by making duplicate analyses of the same sample. In no case does this gain in precision by more analyses of high-nitrate samples have an effect on the interpretation of the results. Even when the number of petioles taken for a sample is doubled the maximum gain in precision in any one field is less than 40 percent. This improvement in precision, however, occurs mainly *in* the low-nitrate samples when an increase in precision is highly desirable, so more petioles per sample should be taken in the field whenever possible.

Still more important for an accurate evaluation of the nutrient status of a field of beets, however, is the taking of more samples from the same field; for example, one from each quarter section of a field. This yields much more useful information than the collection of 50 petioles per sample with two analyses per sample, because in the taking of more samples per field a measure of the variation of the plants in each field is obtained, an important consideration in the selection of a fertilizer program for a field of sugar beets.

The components of variance found in the 14 fields sampled extensively agree with the findings for the three sugar beet fields sampled intensively. Again, most of the variations are from petiole to petiole in the field and not in the laboratory analyses. The coefficients of variation indicate considerable variation in this statistic from field to field but there is no consistent trend in the values which might offer a better treatment of the results *in* the present sampling technique studies.

Phosphate-Phosphorus

The range in phosphate-phosphorus values of the 17 fields recorded in Table 3 differs just as strikingly as the nitrate values already discussed. If the critical phosphate-phosphorus concentration in the dry petioles is taken to be 750 ppm., then only one field has an average value below this figure, even though all but six fields sampled have some petioles considerably less than this value. If the phosphate-phosphorus value of the 17 fields should have been found at the lower confidence limit for each mean, this would have changed the interpretation of the phosphorus status of only one field; namely, field 8 Y, from a low phosphorus status to one that is below the critical concentration. At the upper confidence limit the single field now below the critical concentration would be just above the critical phosphate-phosphorus concentration. In either case the beets would be classified as low in phosphorus, and methods of raising the phosphorus levels within the plants would be considered carefully, particularly if later samplings should show further declines in phosphorus status.

Deciding whether or not to fertilize a field of beets with phosphorus on the basis of a single sample may not be justified unless the variability

Table 3.—Phosphate-Phosphorus Expressed in Parts Per Milion on a Dry Basis for 17 Sugar Beet Fields as Related to Ranges of Values, Confidence Limits, Components of Variance, Variance of Single Determinations, Gains in Precision and Coefficients of Variation.

Field[1]	Range	Mean	Confidence limits at the 5% level[2] Limits of error: One analysis Petioles per sample 25	One analysis Petioles per sample 50	Two analyses Petioles per sample 25	Two analyses Petioles per sample 50	Gain in precison: Single analysis 50 petioles[4]	Duplicate analyses 25 petioles[5]	Components of variance (multiplied by 1/100): Between petioles within field S_b^2 LRP	Analytical: Between dates S_t^2	Analytical: Residual S_A^2	S_t^2/S_A^2	$\frac{LSD}{T} \times 100$	Variance[6] single analysis x 1/100 25 petioles	C.V.[7]
	ppm.	ppm.	ppm.	ppm.	ppm.	ppm.	%	%					%		%
IV S	3,900— 950	2,003	±296	±229	±282	±210	29.7	5.3	4,595	12[8]	33		99.0	229	7.5
I S	2,970—1,250	1,075	±200	±174	±173	±142	14.9	16.6	1,286	0	53		96.0	104	5.2
H W	3,550— 790	1,918	±295	±242	±268	±208	21.9	9.9	3,701	19[8]	59		97.9	226	7.8
II S	3,070— 750	1,880	±250	±205	±228	±177	22.0	9.6	2,672	9[7]	47		97.9	163	6.8
HD W	3,090— 460	1,848	±265	±215	±243	±187	23.3	9.1	3,115	15[8]	43		98.2	183	7.3
XII K	3,440— 980	1,769	±210	±156	±204	±148	31.3	5.0	2,539	0	13		99.5	114	6.0
III S	2,620— 900	1,765	±346	±323	±274	±245	7.1	26.3	1,992	100[8]	132		89.6	312	10.0
XIII K	2,890— 600	1,572	±251	±182	±248	±177	37.9	1.2	3,892	0	8		99.8	164	8.1
XI K	2,850— 330	1,552	±252	±189	±244	±178	33.3	3.3	3,608	5[8]	15		99.4	165	8.2
V S	2,350— 920	1,523	±159	±126	±148	±112	26.2	7.4	1,216	0	17		98.6	66	5.3
IX W	2,500— 440	1,480	±259	±207	±241	±188	25.0	7.9	3,156	27[8]	22		98.5	175	7.2
X W	2,570— 410	1,175	±281	±233	±255	±199	20.6	10.2	3,287	0	75		97.8	206	12.3
VII W	1,880— 440	1,116	±167	±132	±157	±118	26.5	6.4	1,371	0	18		98.7	73	7.6
VI W	2,190— 430	1,035	±250	±224	±208	±177	11.4	19.8	1,584	48[8]	51		94.1	162	12.3
B W	2,130— 430	1,011	±177	±140	±165	±125	26.4	7.5	1,506	0	30		98.0	81	8.9
VIII W	2,010— 370	844	±187	±141	±181	±133	32.6	3.3	1,978	0	12		99.4	91	11.3
XIV K	1,980— 280	642	±169	±123	±167	±120	37.4	1.2	1,753	0	4		99.8	75	13.4

1 2 3 4 5 6 7 8 For, explanation see Table 2.

of the phosphorus status of the beets within the field is also known. Just as in the nitrate-nitrogen values, similar mean values were observed for different ranges in phosphate values. In field 13 K, with a mean value of 1,572, 12 percent of the petioles were below the critical concentration, while in field 5 M, with an average value of 1,523, no values were below the critical concentration. Thus, the likelihood of an increase in average yield in field 13 K is better than in field 5 M. Again, it should be emphasized that in fields sampled for the first time a measure of the within-field variation should be obtained in order to evaluate the likelihood of response to phosphatic fertilizers. Timing is also important in the successful fertilization of beets, particularly the stage of development of the plants at the time of the deficiency, the duration of the deficiency and the timing of the fertilizer applications.

Increasing the number of petioles taken per sample from 25 to 50 appreciably decreased the limits of error of the mean, while increasing the number of analyses per sample from one to two just slightly decreased these limits. The gain in precision of a 50-petiole sample over a 25-petiole sample was approximately 25 percent, duplicate analyses over a single analysis 9 percent, and for both a 50-petiole sample and duplicate analyses, 41 percent.

The components of variance, just as for nitrate, were made up mostly of variations from petiole to petiole in the 25 locations in the field and not from the variations of analysis in the laboratory, except in field 3 S. Even in this field the component of variation for analysis was only 10.4 percent of the total variation, in contrast with an average variation of 1.7 percent in the remaining fields. The coefficients of variation on the average were considerably less than those of the nitrate determination.

Potassium Analyses

The potassium values of the individual petioles also differed greatly from each other (Table 4). The highest potassium value, 11.44 percent, was observed in field 10Y and the lowest, 0.76 percent, observed in field 4 M. The means of these fields ranged from 7.26 percent to 1.50 percent. If the critical potassium concentration at which a response to potassium fertilization may be expected is tentatively set at 1.00 percent, then none of the fields sampled was below this value. Individual petioles in field 4 M, however, were below the 1.00 percent level in 16 percent of the petioles sampled. If potassium is added to this field and the increased growth in the deficient beets is large enough to give a significant increase in yield for all the beets in the field, deficient and non-deficient, then a higher mean potassium value is indicated at which a response may be obtained under field conditions. Thus, the interpretation of the mean values of field samples could well depend upon the degree of variation of the beets sampled. Critical concentrations of fields of uniform beets would be lower than those of fields with highly irregular beets.

The limits of error of the mean potassium values (Table 4) are so small that no change in the interpretation of the results appears necessary, even if the mean values are taken at the lower confidence limit. At the higher confidence limit the interpretation of the mean values also remains the same since none of the values is below the **1.00** percent level. Again, the

Table 4.—Potassium in Petioles Expressed in Percentages on a Dry Basis for 17 Sugar Beet Fields as Related to Ranges of Values, Conadence Limits, Components of Variance, Variance of Single Determinations, Gains in Precision and Coefficients of Variation.

Field[1]	Range	Confidence limits at the 5% level[5]				Gain in precision		Components of variance (multiplied by 1/100)			S^2 / LBP/S^2_T x 100	Variance[7] single analysis x 1/100	C.V.[8]
		Mean	Limits of error				Single analysis	Duplicate analyses[6]	Between petioles within field	Analytical			
			One analysis Petioles per sample		Two analyses Petioles per sample		50 petioles[4]	25 petioles[3]	S^2 LBP	Between dates S^2 t	Residual S^2 A		25 petioles
	ppm.	ppm.	ppm.	ppm.	ppm.	ppm.	%	%				%		%
X Y	11.44—4.85	7.26	±0.70	±0.55	±0.66	±0.50	27.1	6.6	2.4228	0	0.0303	98.8	0.1272	4.9
XIV K	8.70—4.08	6.55	±0.52	±0.41	±0.49	±0.37	28.0	5.9	1.3979	0.0022[7]	0.0134	98.9	0.0715	4.1
H Y	7.12—3.10	4.77	±0.37	±0.30	±0.34	±0.26	24.4	8.1	0.6380	0.0030[8]	0.0074	98.4	0.0359	4.0
VI Y	6.00—2.80	4.48	±0.42	±0.35	±0.37	±0.30	18.5	12.7	0.6524	0.0091[9]	0.0101	97.1	0.0453	4.8
HD Y	6.85—2.70	4.44	±0.37	±0.30	±0.35	±0.26	24.9	7.5	0.6467	0	0.0098	98.5	0.0359	4.3
VII Y	6.75—3.20	4.31	±0.37	±0.30	±0.33	±0.26	21.5	10.2	0.5613	0	0.0125	97.8	0.0350	4.3
XIII K	6.44—2.87	4.16	±0.46	±0.37	±0.43	±0.33	25.2	7.6	1.0052	0.0024[7]	0.0129	98.5	0.0555	5.7
XII K	6.90—1.58	4.05	±0.49	±0.39	±0.46	±0.35	27.0	6.6	1.2107	0.0037[8]	0.0115	98.8	0.0636	6.2
IX Y	6.36—2.32	4.04	±0.41	±0.31	±0.39	±0.29	31.9	4.1	0.9236	0	0.0066	99.3	0.0435	5.2
II M	4.82—2.78	3.84	±0.24	±0.19	±0.22	±0.17	27.3	6.7	0.2770	0	0.0036	98.7	0.0147	3.2
VIII Y	6.66—2.12	3.77	±0.44	±0.32	±0.43	±0.31	37.6	1.6	1.1963	0	0.0031	99.7	0.0510	6.0
XI K	6.28—1.06	3.09	±0.50	±0.38	±0.49	±0.36	33.2	3.3	1.4359	0.0030[8]	0.0054	99.4	0.0668	8.3
B W	5.25—1.10	3.06	±0.39	±0.33	±0.36	±0.28	20.9	10.7	0.6371	0.0040[9]	0.0109	97.7	0.0404	6.6
III M	4.35—1.42	2.99	±0.34	±0.26	±0.33	±0.24	30.3	4.8	0.6250	0	0.0054	99.1	0.0304	5.8
I M	4.22—1.61	2.71	±0.29	±0.22	±0.27	±0.20	28.3	5.9	0.4140	0	0.0046	98.9	0.0213	5.4
V M	2.50—1.13	1.75	±0.19	±0.16	±0.15	±0.13	12.1	18.6	0.0891	0	0.0053	94.4	0.0089	5.5
IV M	2.42—0.76	1.50	±0.23	±0.19	±0.21	±0.17	21.2	9.9	0.2331	0.0012[8]	0.0038	97.9	0.0145	8.0

12 3 4 5 6 7 8 For explanation see Table 2.

gains in precision are increased by taking more petioles per sample than by making duplicate analyses of either a 25- or 50-petiole sample. Apparently, by using a flame photometer the analysis of the plant material for potassium and for sodium is so precise that little is gained by making more analyses of the same sample. This fact and the low coefficients of variation of a single analysis of a 25-petiole sample again indicate that more useful information about the nutrient status of a field of beets would be gained by analyzing individual petioles or by taking more 25-petiole samples than by taking more petioles per sample.

Sodium Analyses

The recent interest in the sodium content of beets as a means of improving beet varieties, the possible essential nature of sodium as a plant nutrient, and the ease of determining sodium with a flame photometer make the inclusion of this element in the present petiole-sampling technique study highly desirable. The results of these analyses and their statistical evalutaion are given in Table 5. Again, there is a wide range of sodium values in the petioles, 9.00 percent in field 8 Y and only 0.35 percent in field HDY. The sodium means of these fields were as high as 5.60 percent and as low as 1.84 percent.

The confidence limits of the means of all fields are exceptionally narrow and, accordingly, many of the differences between the means are statistically significant even though the biological significance of the values is not known at present. Biologically, there seems to be an inverse relationship between potassium and sodium; for example, the petioles with the highest potassium content (Table 4) have the lowest sodium content (Table 5), and those with the highest sodium content have the lowest potassium content. This inverse relationship is not consistent, however, since the petioles with the third highest potassium content also have the third highest sodium content, showing that there is no simple relationship between potassium and sodium under field conditions.

A review of the components of variance in the field and in the laboratory again points conclusively to the fact that the variations from petiole to petiole in the field are far greater than the variations within the laboratory. Improvements in precision can be made by taking more petioles per sample, but at present more useful information is gained by taking more samples per field instead of taking more petioles per sample.

Discussion

The number of individuals which must be selected at random in order to characterize a population within satisfactory limits of error depends upon the variability of the individuals within the population. In petiole sampling of beet fields, the variability of the petioles is largely composed of three components of variation, of which the variation from beet to beet within the field and between the petioles within the beet are the largest, while the variations between replicated analyses for nitrate, phosphate, potassium and sodium are relatively small. Theoretically, once the overall variability of the petioles from beet to beet within a field has been determined the number of petioles required for a given precision can be calculated readily. Basically, sampling precision increases with the square root of the number of petioles taken per sample.

Table 5.—Sodium in Petioles Expressed in Percentages on a Dry Basis for 17 Sugar Beet Fields as Related to Ranges of Values, Confidence Limits, Components of Variance, Vaiances of Single Determinations, Gains in Precision and Coefficients of Variation.

Field[1]	Range	Mean	Confidence limits at the 5% level[2] Limits of error: One analysis Petioles per sample 25	One analysis Petioles per sample 50	Two analyses Petioles per sample 25	Two analyses Petioles per sample 50	Gain in precision: Single analysis 50 petioles[4]	Gain in precision: Duplicate analyses 25 petioles[5]	Components of variance (multiplied by 1/100): Between petioles within field S^2_{LBF}	Analytical: Between dates S^2_t	Analytical: Residual S^2_A	S^2_{LBF}/S^2_T x 100	Variance[3] single analysis x 1/100 25 petioles	C.V.[6]
	ppm.	ppm.	ppm.	ppm.	ppm.	ppm.	%	%				%		%
VIII Y	9.00—3.40	5.60	±0.73	±0.56	±0.69	±0.51	30.6	4.8	2.8262	0.0049[7]	0.0187	99.2	0.1366	6.6
V M	7.05—4.00	5.37	±0.38	±0.31	±0.35	±0.27	23.5	8.3	0.6524	0	0.0113	98.3	0.6524	3.6
H Y	7.04—3.05	5.11	±0.42	±0.33	±0.40	±0.30	28.0	5.8	0.8965	0.0014[8]	0.0089	98.9	0.0462	4.2
IX Y	7.60—2.88	4.66	±0.53	±0.40	±0.50	±0.37	30.4	4.6	1.4903	0	0.0127	99.2	0.0723	5.8
I M	7.00—3.00	4.34	±0.42	±0.35	±0.39	±0.30	22.0	9.1	0.7834	0	0.0155	98.1	0.0468	5.0
VII Y	6.10—2.76	4.26	±0.35	±0.27	±0.33	±0.25	27.7	6.1	0.6186	0	0.0072	98.8	0.0319	4.2
B Y	7.50—1.05	3.67	±0.56	±0.45	±0.52	±0.40	25.5	7.9	1.5004	0.0009[8]	0.0210	98.6	0.0819	7.8
XI K	6.72—1.84	3.33	±0.56	±0.40	±0.55	±0.40	38.7	1.1	1.9619	0.0017[8]	0.0016	99.8	0.0818	8.6
XII K	6.80—0.96	3.27	±0.58	±0.44	±0.56	±0.41	32.3	3.8	1.8565	0	0.0125	99.3	0.0868	9.0
XIV K	5.70—1.52	3.12	±0.39	±0.29	±0.38	±0.28	35.9	2.2	0.9193	0	0.0034	99.6	0.0402	6.4
IV M	4.46—1.07	3.06	±0.38	±0.29	±0.36	±0.27	30.7	4.7	0.7544	0.0023[8]	0.0041	99.2	0.0366	6.2
XIII K	5.70—1.12	2.82	±0.49	±0.38	±0.47	±0.35	30.2	4.8	1.2881	0	0.0112	99.1	0.0627	8.9
VI Y	4.72—1.30	2.78	±0.34	±0.27	±0.32	±0.24	28.1	5.9	0.5940	0.0027[8]	0.0040	98.9	0.0305	6.3
II M	3.52—1.20	2.37	±0.26	±0.20	±0.25	±0.18	28.9	5.5	0.3508	0	0.0035	99.0	0.0175	5.6
III M	2.50—1.35	1.96	±0.17	±0.15	±0.15	±0.12	14.8	16.3	0.0869	0.0009[7]	0.0032	95.5	0.0076	4.4
HD Y	4.08—0.35	1.87	±0.31	±0.24	±0.30	±0.22	31.8	4.0	0.5269	0	0.0041	99.2	0.0252	8.5
X Y	2.56—0.72	1.84	±0.15	±0.23	±0.14	±0.21	26.4	6.6	0.4184	0	0.0055	98.7	0.0222	8.1

1 2 3 4 5 6 7 8 For explanation see Table 2.

Because of this relationship, the greatest increase *in* precision results from adding the first few petioles to the number of petioles already taken per sample, and thereafter the gain *in* precision is relatively small unless the error variance is large. Even with the large error variances present in petiole sampling of sugar beet fields, the gain in precision of a 50-petiole sample over a 25-petiole sample is on the average only 28.3 percent for nitrate, 25.1 percent for phosphate, 25.8 percent for potassium and 28.5 percent for sodium (Tables 2, 3, 4 and 5) . The gains of a 100-petiole sample over a 50 petiole sample are still less. Thus, when more petioles are to be taken from a field, especially more than 50 petioles, it would be better to take more samples from a field than to take more petioles per sample.

This conclusion contradicts that offered by Brown (1) ; he stresses the need for greater precision per sample rather than the gaining of information about the variability of the nutrient status of a field. By knowing the nutrient variability of the beets in a field, the fertilizer requirements of the crop can be estimated much more accurately than otherwise. In practice this means that higher critical nutrient levels will be observed in highly variable fields and lower critical levels in uniform fields.

Under some conditions the use of critical nutrient concentrations as a guide to the fertilization of sugar beet fields might be improved by using a rapid procedure to estimate the proportion of the plants in the fields below or above the critical nutrient level.

For example, this can be done easily for nitrogen by using the diphenylamine test for nitrate on petioles of recently matured leaves (7) . Whenever 25 percent or more of the field is deficient in nitrogen by this test, i.e. below the critical concentration, nitrogenous fertilizers should be added to the field. This procedure is especially important in very spotty fields such as those observed in Kern county. In field 13 K, as already mentioned, 36 percent of the petiole nitrate values were less than 1,000 ppm., and yet the average value was 4,130 ppm., a figure well above the critical concentration. In another field, 2 S, with an average value of 2,984 ppm., only 12 percent of the samples were less than 1,000 ppm.

Thus, blind adherence to the interpretation of the nutrient status of beet crops based solely on petiole analyses of composite samples without field observations would certainly lead to faulty recommendations in spotty fields. In this particular case field notes included the observation that the pattern of field 13 K was extremely irregular and would require the collection of two distinct samples, one from lush green plants and the other from stunted plants with yellow leaves. The results of the samples taken separately, as shown by an analysis of the individual petioles, would have shown that the samples from the green plants were high in nitrate and the other from the yellow plants much less than 1,000 ppm. of nitrate-nitrogen.

In the case of potassium nutrition field variability, supported by field or laboratory analysis, may be disclosed by the amount of leaf scorch present in the beet field. The amount of necrotic spotting in alfalfa has been found to correlate closely with potassium deficiency in the field (3)-, and a similar procedure-may be found satisfactory for sugar beets whenever areas deficient in potassium are studied intensively. Unfortunately, phosphorus-deficient

plants have no clearly defined deficiency symptoms, except possibly small dark green leaves, which can be used readily to estimate the proportion of phosphorus-deficient plants in a beet field. Fortunately, field and laboratory tests for phosphorus are not difficult to make and can be relied upon for petiole variability studies.

Summary

A detailed study of the technique used in petiole sampling of sugar beet fields for evaluating the nutrient status of beet crops was made on 17 different fields selected over a three-year period in Yolo, Monterey and Kern counties of California.

A statistical analysis of the nitrate, phosphate, potassium and sodium contents of the individual petioles showed that the components of variance between petioles within a beet, between petioles of adjacent beets and between petioles of beets at different locations within a field were large, and furthermore were not related to the order of selecting the petioles within a beet or of beets within a field location. The components of variance for the chemical analyses of the petioles, within and between dates of analysis in the laboratory, were exceedingly small, averaging less than 2.5 percent of the total variance of sampling.

From these observations it seems that the greatest gains in precision are obtained by increasing the number of petioles taken per sample and not by analyzing the same sample more times. Even though the largest gains in precision are made by taking more petioles per sample, the gains are confined mainly to the first few petioles added to the sample. Thus, the average gain for a 50-petiole sample over a 25-petiole sample is only 25 percent, and for more than 50 petioles the gains over a 50-petiole sample are still less. In a further consideration of the sampling problem, particularly in relation to critical nutrient levels, more useful information is obtained by taking more samples per field than by taking more petioles per sample.

The ultimate in sampling procedure is the testing of individual petioles so that the proportion of deficient to non-deficient plants within a field can be estimated accurately. By knowing the proportion of deficient to non-deficient plants within a field, a better evaluation of the nutrient requirements of the crop can be made than by using mean nutrient values alone.

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