house production as long as it is readily available and of reasonable quality. In arid regions, where there is likely to be severe competition between industrial, domestic and agricultural users for available water supplies, the position of protected horticulture is outstanding.

The results suggest that significant yield improvements in the future are likely to result from control of plant water potential, and that increases in efficiency of water utilization will require additional information on energy relationships between the plant and its aerial environment in the greenhouse.

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Table 3); a figure that assumes there was no transmission loss through the greenhouse cover, and all energy was utilized in evapotranspiration. It appears logical to assume that advective sensible heat and large vegetational surface areas were important in influencing water loss.

Comparison of Sampling Methods for the Determination of Seasonal Changes in the Nutrient Content of Apple Leaves[1]

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Abstract. In studies of seasonal trends in apple leaf nutrients, 5 different seasonal leaf sampling methods using the same shoots of young and old apple trees have been compared with a sampling of mid-shoot leaves on different shoots each sampling date to determine whether there would be any differences in leaf P, K, Ca and Mg trends. Sampling methods resulted in significant interaction with the check only in data for leaf concentration of Ca. The methods which were compared with the check had average seasonal leaf concentrations of P, K, Ca and Mg which differed significantly from the check in a few instances. They also produced slightly to considerably lower coefficients of variation than the check in 78 of 100 possible comparisons. This makes such methods very desirable as alternative sampling procedures where they do not otherwise vary from the standard.

INTRODUCTION

IN determining the seasonal trend in concentration of inorganic nutrients in leaves of young apple trees, it may be necessary to take leaf samples repeatedly from the same terminal shoots. The same situation may exist with older trees fruiting freely from terminal buds. No direct information is available showing the best sequence for sampling such shoots or, whether there is any difference resulting from the seasonal pattern of leaf sampling.

A number of papers have presented results from which one would infer that manner of leaf sampling sequentially on the same shoots might make a difference in the seasonal curve of nutrient concentration. Beyers (2) and Lilleland and Brown (6) showed that certain inorganic nutrients varied in concentration in peach leaves at different positions on a shoot on a single date. Myers and Brunstetter (8) obtained similar information in an analysis of tung leaves. Presumably by utilizing different shoots on each sampling date, Drosdoff (3) working with tung and Emmert (4,5) with apple determined that the relative seasonal change in nutrient concentration of leaves at different positions on a shoot depends on the element and, in the latter reference, on the season. Mason (7) sampled, and chemically analyzed, leaves from Malling VII whips in a nursery row. Different, randomly-selected, whips were used on each sampling date. His results, which are presented in considerable detail, show that relative changes through the season developed in 7 elements at different, precisely defined leaf positions on the shoots.

Rogers et al. (9) departed from the usual procedure of sampling leaves on different shoots on successive dates. Their technique consisted of punching disks from the same leaves at each sampling time. These authors stated that this manner of sampling gave results very similar to the usual sampling method. However, it required numerous terminals because of the small amount of tissue obtained on each date from each shoot.

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It was the purpose of the present study to determine whether results obtained by repeated sampling of leaves from the same shoot on successive dates would differ from those involving the usual procedure of sampling different shoots on successive dates. Of particular interest was whether different reference points for beginning, and patterns for sequential sampling on the shoot would result in different seasonal curves of leaf nutrient concentration than the standard.

Materials and Methods

In 1961 and 1962, initially 1-year-old 'Royal Red Delicious' (hereafter, in this paper, called simply 'Red Delicious') and 'Golden Delicious' apple trees were used for a serial leaf sampling study. Also, in 1961 only, approximately 25-year-old 'Red Delicious' trees were selected in an orchard a mile away for the same study. Two-tree plots in the young orchard were necessary to obtain sufficient leaf material, whereas single tree plots were adequate in the mature orchard.

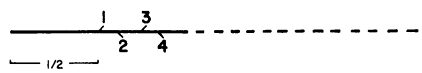
A randomized complete block design was used in allocating treatments to plots. Treatments were replicated 8 times for each of the 2 varieties and 2 years in the younger orchard and 6 times for the single variety the one year in the older orchard. Randomization of treatments was performed independently in the 5 situations. All units were sampled at 2-week intervals through the season; thus the design for each of the 5 situations was that of a split plot in time (10).

Five methods of serial leaf sampling were compared with what is considered a standard method in each of the 5 parts of the study. Each method, except the check, will be identified throughout this paper solely by the symbols preceding the descriptions. The symbol, or symbols, enclosed in parentheses defines the starting point of the sampling procedure. The remaining symbol, or symbols, indicates whether subsequent samples were taken from positions toward the base (B), the tip (T), randomly (R) or at mid-shoot (M). The leaf sampling methods were as follows:[2]

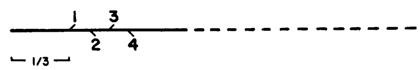
[2]Solid portions of lines represent shoot length at initial sampling on the shoot. Dashed portions represent subsequent shoot extension. Bold numbers indicate the sequence in time of leaf sampling.

(M)R—Initial leaf taken at mid-shoot. Subsequent leaves were next adjacent taken randomly toward base or tip of same shoots. The leaf to be detached was determined by flipping a coin. This selection procedure was applied to each shoot on each sampling date.

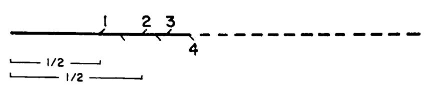
(M)T—Initial leaf taken at mid-shoot. Subsequent leaves sampled were next adjacent toward tip of same shoots.



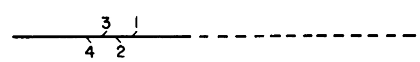
(1/3B)T—Initial leaf taken one-third of distance from base to tip of shoot. Subsequent leaves to be removed were next adjacent toward tip on same shoots.



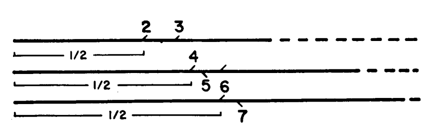
(M)M-T—Initial leaf taken at mid-shoot. Subsequent leaves were detached at mid-point until terminal growth ceased and then at next adjacent leaf toward tip.



(1/3T)B—Initial leaf taken one-third of distance from tip to base. Subsequent leaves taken were next adjacent toward base.



(M)—Check, or standard, method. This simulated the commonly used method of sampling at the midpoint on different shoots of each sampling date.



Each leaf sample consisted of 8 leaves per young tree or 16 leaves per plot, and 20 leaves per mature tree. On the first sampling date for the young trees the average of the leaf nutrient concentrations found for methods (M)R, (M)T and (M)M-T was used as the initial value for method (M). Subsequently, on each pair of successive sampling dates, different 2-tree plots in each replicate were used in obtaining mid-shoot leaves by this method for determining nutrient concentration.

Leaves were dried at 75°C for 24 hr a forced draft oven and ground in a Wiley Intermediate mill to pass a 40-mesh screen.

Chemical analyses of each sample were made for P, K, and Mg by the method of Toth et al. (11) and for Ca by a method used by the Plant Chemistry Unit of the Canada Department of Agriculture (14). Periodically analyses were run in duplicate to check on reproducibility. Analytical results are expressed as per cent dry weight (%DW).

The data were analyzed statistically for each of the 5 situations as outlined by Steel and Torrie for split plots in time (10).

A prime objective of the study was to compare the seasonal trend of inorganic nutrients in leaves as measured by each of the new methods with that measured by the standard method. Hence, in cases where methods by dates interactions were significant the analyses were broken down further to permit testing the non-orthogonal set of contrasts indicated below:

Source	df
Methods × dates	(m-1) (d-1)
Standard vs. (M)R × dates	d-1
Standard vs. (M)T × dates	d-1
Standard vs. (1/3B)T × dates	d-1
Standard vs. (M)M-T × dates	d-1
Standard vs. (1/3T)B × dates	d-1
error (b)	(r-1) (m-1) (d-1)

The symbols, r, m, and d are the number of replicates, methods and dates, respectively.

In computing LSD's for comparing 2 methods on a given date the following formula was used to obtain the standard errors:

$$S_{\bar{d}} = \sqrt{2[E_a + (d\text{-}1)\ E_b]/rd}$$

in which E_a and E_b are the error mean squares for testing methods and methods × dates respectively.

This standard error involves 2 error mean squares; thus the correct number of degrees of freedom for obtaining the tabular "t" value to be included in the LSD must be approximated. A method presented by Anderson and Bancroft (1) was used.

A logical criterion of "goodness" of a leaf sampling technique is that the coefficient of variation (CV) of the data obtained by the method be minimal. To compare, within nutrient elements, the sampling methods by this criterion, a separate analysis of variance was made of data obtained by each method of sampling in each of the 5 situations. These analyses are outlined as follows:

Source	df
Replicates	(r-1)
Dates	(d-1)
error	(r-1) (d-1)

The error mean square (EMS) was used to compute the CV. Thus:

$$CV\ (\%) = 100\sqrt{EMS}\ /\ \overline{M}$$

in which $\overline{M}$ equals the method mean.

Results and Discussion

Comparison of trends in concentration of leaf nutrients resulting from sampling methods. Since one intent of this study was to find an acceptable substitute for the check, or standard, leaf sampling method as an indicator of seasonal nutrient trends in special categories of trees, all comparisons are made only with that method. In order to show which method, or methods, differed through the season from the standard, the statistical technique for examining interactions outlined previously was used.

On this basis, no P, K or Mg curve for any leaf sampling method differed significantly from the curve obtained by the standard method of sampling.

The variance for methods by dates in the overall analysis was significant in several of the groups of data for these elements. This indicates that 2 or more of the leaf sampling methods introduced for comparison with the check differed from each other, but not from the check method.

The curve resulting from plotting the data for leaf concentration of Ca for a season by method (1/3T)B differed significantly from the check, or standard, curve in 3 of the 5 sets of data.

In leaves from 'Golden Delicious' trees sampled by method (1/3T)B in 1961, Ca is lower in the early part and higher in the later part of the sampling period than in leaves taken by the check method (Fig. 1A). The change in the relation of the values on the curves to each other occurs at about the middle of the sampling period.

In curves depicting the change in Ca concentration of leaves from mature 'Red Delicious' trees in 1961 (Fig. 1C) and young 'Red Delicious' trees in 1962 (Fig. 1D) the same relative trends occur from sampling by method (1/3T)B and the check. As with the 1961 data for 'Golden Delicious', the curves for mature 'Red Delicious' in 1961 cross at about the middle of the sampling period (Fig. 1C). However, in young 'Red Delicious' in 1962 the lines cross much earlier in the sampling period and generally continue to diverge thereafter.

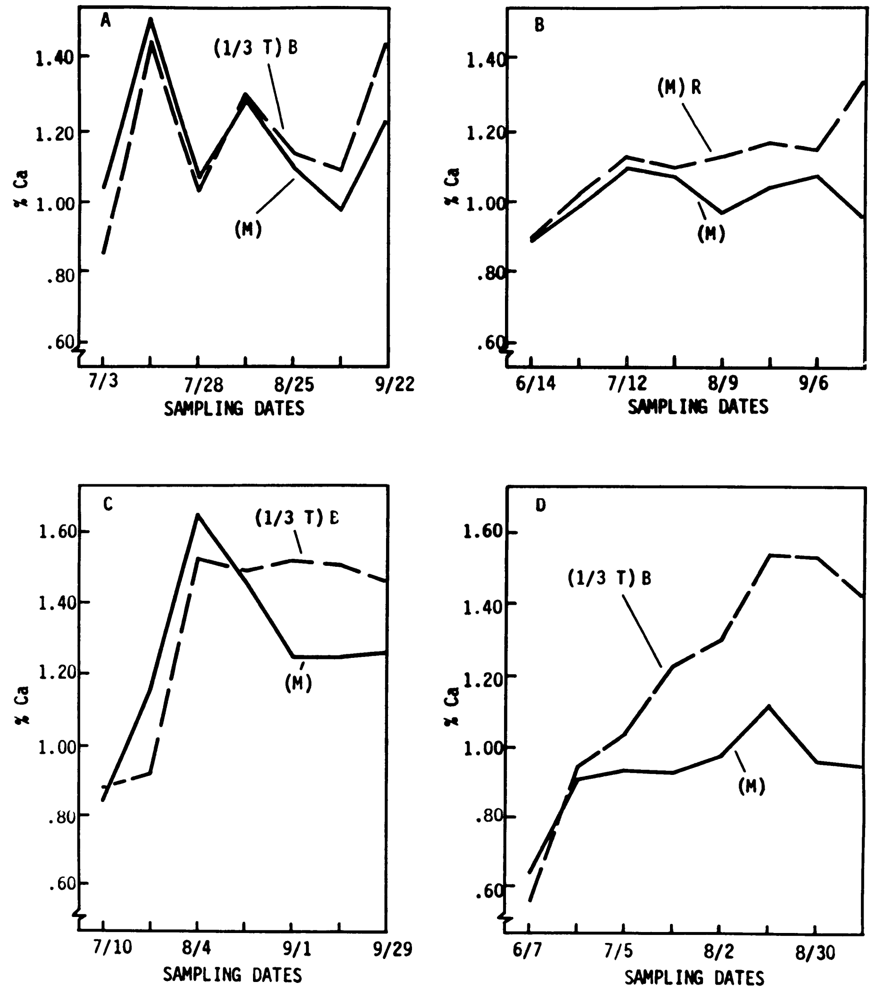


Fig. 1. Comparison of trends in leaf concentration of Ca (% DW) through a season by check sampling method, (M), and by methods which differed significantly from the check. (A), Young 'Golden Delicious' trees—1961. (B), Young 'Golden Delicious' trees—1962. (C), Mature 'Red Delicious' trees—1961. (D), Young 'Red Delicious' trees—1962.

In the data for 'Golden Delicious' for 1962, the variance attributable to method (1/3T)B in a comparison with the check is almost significant at the 5% level of confidence. The curve for method (1/3T)B in this instance bears the same relationship to the curve for the check as it does in the sets of data presented.

In 'Golden Delicious' trees in 1962, a significant methods by date interaction occurred in which sampling by method (M)R resulted in a curve differing from that of the check method (Fig. 1B). During the early part of the sampling period, method (M)R showed a slightly higher Ca concentration in leaves. Beginning in early August the difference became greater. This situation continued to the end of the sampling period.

The Ca curve resulting from sampling by method (M)R on 'Golden Delicious' in 1962 (Fig. 1B) is in a similar relationship to the curve for the check method as is the curve obtained by sampling by method (1/3T)B in 1962 (Fig. 1D). The Ca curves for 1961 (Fig. 1A and 1C) also lie in a similar relationship during the later part of the sampling period. The relationship between the pair of curves in each of the 4 graphs is similar to the extent that the difference between them is invariably greater in the later than in the early part of the sampling period and the standard curve always registers the lower values toward the end of the period.

The divergence in the 2 curves in each graph (Fig. 1, A,B,C,D) reflects the fact that leaf samples obtained by the check method on each successive date were at an increasingly greater distance distally from the sampling position of the other 2 methods under comparison. The Ca content of apple leaves throughout the growing season has been shown to be progressively higher in leaves as one samples from the tip toward the base of a shoot (7).

The results obtained in this study would be accentuated or diminished as the rate and extent of terminal growth varied in relation to the leaf sampling pattern. Table 1 shows the relative position on the shoot of the last leaf taken by 4 sampling methods during 1962. The last leaves sampled by methods (M)T, (1/3B)T and (M)M-T were taken nearer the tips on 'Golden Delicious' than on 'Red Delicious'. Hence, the terminal growth was less on the former than on the latter variety. The considerable variation in terminal growth is indicated by the range of variation in the positions of sampling the last leaves by the 4 methods.

Table 1. Position of last leaf sampled (as per cent of distance from base to tip) by sampling methods—1962, on young 'Red Delicious' and 'Golden Delicious' apple trees.

Method	Red Delicious		Golden Delicious	
	Average	Range	Average	Range
(M)T..........	50	40–95	65	45–95
(1/3B)T........	45	30–70	55	40–90
(M)M-T........	60	45–75	70	55–100
(1/3T)B........	10	0–20	10	0–20

It is noteworthy that the spread between the curves for the (1/3T)B and check methods is greater for 'Red Delicious' (Figs. 1C and 1D) than for 'Golden Delicious' data (Fig. 1A). This suggests the possibility that a greater tip-leaf to base-leaf gradient in Ca may develop during the season in 'Red Delicious' than in 'Golden Delicious'. Since the ultimate leaf sampling position (Table 1) was, on the average, not as near the shoot tip on 'Red Delicious' as on 'Golden Delicious', the greater difference in the spread between the curves for (1/3T)B and check methods for 'Red Delicious' than for 'Golden Delicious' might have been even greater had sampling occurred nearer the tip on the former variety.

Ca concentration in apple leaves fluctuated much more sharply from one sampling date to the next in 1961 (Fig. 1A and 1C) than in 1962 (Fig. 1B and 1D). It is believed that this can be explained by the much heavier and more widely fluctuating rainfall in 1961 than in 1962. Monthly rainfall for the period of June through September varied from 1.85 inches in September to 15.02 inches in August, 1961, whereas the range in 1962 was from 2.90 inches in August to 7.07 inches in June. Tukey et al. (12) showed an approximately 12-fold increase over a 24-hr period in the accumulation of radioactive Ca in water which had been collected after running off squash leaves. This suggests the possibility of considerable loss of Ca from leaves under conditions of rather sustained rainfall such as occurred in August, 1961.

Method averages for season. A difference in the averages of sampling dates for the methods for a season would not indicate anything about the relative slope of idealized linear curves for the methods. It would, however, show that one method might have a consistent tendency to produce higher or lower values than another method.

Table 2 presents the method averages for all sampling dates for leaf concentration of P, K, Mg and Ca. Averages which are significantly different from the check are underlined.

All other sampling methods produced higher leaf P averages in young 'Golden Delicious' trees in 1961 than did the check method. In the nonsignificant data, which are not presented, there was no tendency for the methods being compared with the check to result in lower seasonal leaf P averages.

Table 2. Seasonal averages of elements (% dry wt. basis) in leaves of immature apple trees sampled serially through the season by 6 methods.

Method	Golden Delicious 1961			Red Delicious 1962
	P	K	Mg	Ca
(M)R..............	0.18	1.18	0.32	1.00
(M)T..............	0.17	1.16	0.33	0.97
(1/3B)T............	0.19	1.25	0.29	1.17
(M)M-T...........	0.17	1.30	0.30	0.87
(1/3T)B............	0.17	1.27	0.31	1.19
(M) (Ck)..........	0.16	1.28	0.34	0.92
LSD (.05)..........	0.01	0.10	0.03	0.13

Sampling by methods (M)R and (M)T resulted in lower leaf K contents in 'Golden Delicious' for the 1961 season (Table 2). There were also differences in the 1961 data (not presented) for mature 'Red Delicious'. However, none of the later differences related to the check method. Throughout the 5 sets of data obtained comparing these sampling methods, method (M)R has been consistent in yielding lower seasonal leaf K values than the check method.

Only on young 'Red Delicious' in 1962 did patterns of leaf sampling other than the check method produce different seasonal leaf Ca averages (Table 2). Methods (1/3B)T and (1/3T)B resulted in significantly higher leaf Ca concentrations. This relative situation tended to occur in all of the other sets of data, not presented, except with method (1/3B)T on 'Golden Delicious' in 1962. Though the data are not significant, method (M)M-T invariably resulted in slightly lower, and method (M)R in somewhat higher, seasonal averages.

'Golden Delicious' leaves sampled by methods (1/3B)T, (M)M-T and (1/3T)B had lower Mg contents for 1961 than did leaves obtained by the check method (Table 2). This relationship was consistent, though not significant, for methods (1/3B)T and (1/3T)B in the remaining 4 sets of data, but not for method (M)M-T.

Dates without respect to methods. Comparing date averages, all sets of leaf analysis data for the elements P, K, Ca and Mg are significant, except the data for Mg in leaves from young 'Golden Delicious' in 1961. These data are not presented since they are not pertinent to the objectives of this experiment.

Coefficients of variation. Tables 3, 4, 5 and 6 present, respectively, the coefficients of variation for leaf P, K, Ca and Mg concentration by the various methods of leaf sampling for each of the 5 parts of the experiment.

In general, no pattern of difference exists in the magnitude of the coefficients of variation for leaf concentration of P, K, Ca and Mg between varieties or years. One would expect that compositing the leaves from 2 tree plots, as was done in 1961, would reduce variation. There is, however,

Table 3. Coefficients of variation (%) from P data (% DW) from 'Red Delicious' and 'Golden Delicious' apple leaves sampled serially through the season by 6 methods.

Method	1961			1962	
	Mature	Immature		Immature	
	Red	Red	Golden	Red	Golden
(M)R........	15.8	16.2	21.5	18.7	7.3
(M)T........	19.0	16.3	20.4	18.8	8.4
(1/3B)T......	15.2	15.2	19.0	22.6	6.8
(M)M-T.....	18.8	14.3	18.6	21.5	9.1
(1/3T)B......	17.0	13.8	15.6	17.8	7.7
(M) Check....	18.0	16.6	20.8	21.6	8.0

Table 4. Coefficients of variation (%) from K data (% DW) from 'Red Delicious' and 'Golden Delicious' apple leaves sampled serially through the season by 6 methods.

Method	1961			1962	
	Mature	Immature		Immature	
	Red	Red	Golden	Red	Golden
(M)R........	11.8	12.1	11.3	12.5	11.6
(M)T........	11.1	12.7	14.2	15.6	12.3
(1/3B)T......	11.7	16.0	13.0	14.8	13.3
(M)M-T.....	11.1	17.3	11.3	16.8	21.1
(1/3T)B......	15.3	14.7	12.0	15.9	12.6
(M) Check....	13.4	22.9	13.3	22.3	30.9

no consistent effect associated with this procedure.

The groups of coefficients for P and Mg are, generally, larger than those for K and Ca. This is not in agreement with the relative magnitude of the coefficients of variation for these elements as found by Walker and Mason (13) in a nutritional survey of apple leaves from North Carolina orchards. In that study, the greater variation occurred in leaf concentration of Ca and Mg.

In 78 of 100 comparisons (Tables 3 thru 6), the coefficient of variation is smaller from methods involving the continuous leaf sampling of the same shoots on the same trees rather than the same position on different shoots on the same tree or on adjacent trees (check method). The check method did not result in the lowest coefficient of variation in any set of data. All other sampling methods had at least 2 cases in which they were lowest in per cent variation. Method (1/3B)T was lowest in 8 instances. This may reflect the greater maturity of the leaves selected each time by this method.

Conclusions

As a matter of simplification, it would be desirable if one method of successively sampling leaves on the same shoots were satisfactory for characterizing the seasonal trends of all nutrient elements for which chemical analyses were run. The results in this study show that whether this can be achieved will depend upon the number of criteria of similarity needed to be, or desired to be, applied.

Table 5. Coefficients of variation (%) from Ca data (% DW) from 'Red Delicious' and 'Golden Delicious' apple leaves sampled serially through the season by 6 methods.

Method	1961			1962	
	Mature	Immature		Immature	
	Red	Red	Golden	Red	Golden
(M)R	18.6	16.5	16.1	14.6	12.1
(M)T	28.8	12.4	11.1	13.9	8.0
(1/3B)T	16.8	12.1	10.4	11.2	7.0
(M)M-T	13.6	16.9	14.3	9.8	9.3
(1/3T)B	21.7	12.3	15.1	10.7	9.8
(M) Check	16.4	15.8	16.2	15.5	10.7

Table 6. Coefficients of variation (%) from Mg data (% DW) from 'Red Delicious' and 'Golden Delicious' apple leaves sampled serially through the season by 6 methods.

Method	1961			1962	
	Mature	Immature		Immature	
	Red	Red	Golden	Red	Golden
(M)R	16.6	29.6	28.4	17.6	20.4
(M)T	14.2	32.6	19.0	20.0	18.9
(1/3B)T	14.0	35.4	21.8	16.4	16.9
(M)M-T	15.2	33.8	20.6	17.6	20.4
(1/3T)B	15.1	29.2	23.1	18.9	18.8
(M) Check	17.5	30.5	20.6	19.8	21.8

The primary criterion for selecting a single shoot method of sampling must be the similarity of seasonal trend in nutrient content to the check, or standard, method. Using only this criterion, methods (M)T, (1/3B)T and (M)M-T would all be equally satisfactory as alternative sampling methods for characterizing seasonal trends in P, K, Ca, and Mg in leaves of young or heavily-bearing, apple trees. Methods (M)R and (1/3T)B are excluded because they produce seasonal trend lines for leaf Ca which differ from those for the check method.

If the average leaf level of the 4 elements for a season were an important consideration, none of the single shoot methods would be a satisfactory choice as a substitute for the check method. If the average seasonal content of only 1 of the 4 elements determined in this study were being examined, methods (1/3B)T and (M)M-T for K, (M)T for Mg, (M)M-T and (M)T for Ca would be suitable alternative methods. Possibly methods (M)T and (M)M-T would also be satisfactory in characterizing leaf P. Although they produce different averages from the check method, the difference is slight and they are acceptable by other criteria.

A third criterion which it is possible to apply is the coefficient of variation. The alternative methods of leaf sampling are mostly superior to the check method in the frequency of occurrence of lower coefficients of variation. But, because the magnitude of the difference is mostly rather small, little basis is afforded by this criterion for making a choice between methods.

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