UCLA 12-1342
Conf-8108109--2

LABORATORY OF BIOMEDICA AND ENVIRONMENTAL SCIENCES

UNIVERSITY OF CALIFORNIA
900 VETERAN AVENUE
LOS ANGELES, CALIFORNIA

DOE CONTRACT DE-AC03-76-SF00012

UCLA--12-1342

DE82 005445

LABORATORY OF BIOMEDICAL AND ENVIRONMENTAL SCIENCES

900 VETERAN AVENUE

UNIVERSITY OF CALIFORNIA, LOS ANGELES, CALIFORNIA 90024

These studies were supported by Contract DE-AM03-76-SF00012 between the U.S. Department of Energy and the University of California

Prepared for U.S. Department of Energy under Contract DE-AM03-76-SF00012

MINERAL COMPOSITION OF TWO POPULATIONS OF LEAVES--GREEN AND IRON CHLOROTIC--OF THE SAME AGE ALL FROM THE SAME TREE

J. Procopiou and A. Wallace

C-1.

MINERAL COMPOSITION OF TWO POPULATIONS OF LEAVES --GREEN AND IRON CHLOROTIC-- OF THE SAME AGE ALL FROM THE SAME TREE

KEY WORDS: Iron deficiency, nutrient balance, citrus, lemon, calcareous soil, frequency distribution of elements.

J. Procopiou and A. Wallace
Ministry of Agriculture, Athens, Greece,
Laboratory of Biomedical and Environmental Sciences
University of California, Los Angeles 90024

ABSTRACT

Since carefully washed Fe chlorotic leaves often contain more total Fe on the dry weight basis than do green leaves, a population of leaves of the same age representing chlorotic leaves from each of two lemon trees and green leaves also of the same age and from the same two trees were analyzed individually for mineral elements to determine, especially, the frequency distribution of Fe in the various groups of leaves (n = 47, 48, 71, 48). The chlorotic leaves from one tree had mineral composition typical of lime-induced chlorosis. The chlorotic leaves for this tree were, on the average, higher in P, K and Fe and lower in Ca than the green leaves. For the other tree the chlorotic leaves appeared to be truly Fe deficient; P was not higher in these leaves but the mean K and Ca showed the same pattern as in the first tree. Zinc was higher in the deficient leaves than in the green ones on this tree which can be expected for true Fe deficiency. Mean zinc levels were below the critical levels. Mean manganese was below the critical level for all groups. The coefficient of variation for each element in each group was usually around 30%. Maximum-minimum data indicated that many individual leaves did not fit the patterns just described. Correlation coefficients indicated that most major patterns were consistent in spite of the variability, although there were some differences. The frequency distribution for each of most elements was much like a normal curve with usually a three-fold range for each of the elements. Many of the Fe-deficient leaves had more Fe than some of the green leaves. Analysis of an individual leaf, therefore, cannot result in accurate description of lime-induced chlorosis.

INTRODUCTION

The purpose of this study was to compare the elemental concentration of lemon leaves showing lime-induced chlorosis symptoms with symptomless controls from the same tree and also to see what effects, if any, would iron deficiency have on the leaf content of other nutrient elements in terms of the population. Another purpose was to determine if any nutrient element deficiency not apparent or not certain by visual symptoms was complicating the Fe chlorosis or deficiency.

MATERIALS AND METHODS

Mature lemon trees from Melissi Corinth area of Greece, the center of lemon production of the country were used in this study. The soil contains about 50% $CaCO_3$. From one such tree cv "Maglini" and another cv "Moutsouna" localities "Markaseika" and "Redeika" respectively, 50 to 70 6-month old leaves of each condition from nonbearing shoots were collected on December 17, 1979, washed in liquid detergent and tap water and analyzed by emission spectrography according to Alexander and McAnulty, 1981. Washing was according to Wallace et al. (1980) so that dust elements like Fe and Ti would not complicate results.

RESULTS AND DISCUSSION

On tree 1, the yellow leaves were significantly higher in P, Na, K, Cu, Fe, B, and Al (Table 1). They were significantly lower in Ca, Cr, Ba, and Li. This tree had leaves typical of lime-induced chlorosis (Iljin 1952, Mehrotra et al. 1976). On tree 2, the yellow leaves were significantly lower in P, Ca, Mg, Cu, Fe, Mn, Si, Co, Cr, and Ba. This tree, then was more typical of a true Fe deficiency than of lime-induced chlorosis.

The consistent elements between the two trees were: yellow: high in Na, K, B; low in Ca, Cr, Ba. Other elements were inconsistent. At least for K and Ca, this pattern in both trees is typical of Fe chlorosis although sometimes Ca does not follow this pattern.

The green leaves of tree 1 were on the low side of P adequacy (Embleton et al. 1973). All leaves were low in Mn and most were low enough in Zn to possibly show symptoms. The chlorotic leaves of tree 2 were very likely truly Fe deficient.

Mean, standard deviation, coefficient of variation, maximum and minimum values for some elements in Table 2 indicate typical variability in keeping with normal curves but often the green leaves showed less variability than did yellow chlorotic leaves. Appropriate histograms

TABLE 1

Mineral Element Composition of Elements in Fe Deficient and Green Leaves from Two Lemon Trees.

Group "Tree"		P μg/g	Na μg/g	K %	Ca %	Mg μg/g	Zn μg/g	Cu μg/g
Tree 1	Chlorotic	955	97.6	1.138	3.515	3278	11.5	9.9
	Green	662	20.9	0.712	4.364	3223	10.2	7.9
Tree 2	Chlorotic	1074	217.2	1.451	3.462	4259	18.8	12.8
	Green	1523	62.2	1.078	3.754	4731	11.0	14.9
LSD 0.05	(48)	109	45.1	0.084	0.298	331	1.5	1.6
F Value		82.66	34.46	118.29	16.21	40.20	71.71	28.14

Group "Tree"		Fe μg/g	Mn μg/g	B μg/g	Al μg/g	Si μg/g	Ti μg/g
Tree 1	Chlorotic	104.8	4.4	33.4	101.7	677	1.86
	Green	96.3	5.1	19.4	71.2	671	0.68
Tree 2	Chlorotic	50.0	5.1	34.5	41.2	440	0.028
	Green	64.4	7.4	25.6	35.0	593	0.166
LSD 0.05	(48)	7.1	0.8	3.4	12.1	93.9	-
F Value		118.45	18.52	36.42	52.23	13.58	-

Group "Tree"		Co μg/g	Ni μg/g	Cr μg/g	Sr μg/g	Ba μg/g	Li μg/g
Tree 1	Chlorotic	3.9	3.0	0.3	218	13.2	4.3
	Green	3.6	3.9	0.9	218	15.1	6.7
Tree 2	Chlorotic	2.5	1.6	0.5	206	10.3	9.0
	Green	5.3	4.4	0.6	215	11.9	6.9
LSD 0.05	(48)	0.8	1.0	0.2	N.S.	1.0	1.0
F Value		17.75	14.30	21.62	0.94	38.05	35.08

(Figs 1-4) illustrate the extent of the variation. In Table 3 are data which indicate that skewness and kurtosis of most are within the range of normal curves. Normality would be rejected only in the case of tree 2 for P in the chlorotic group of leaves.

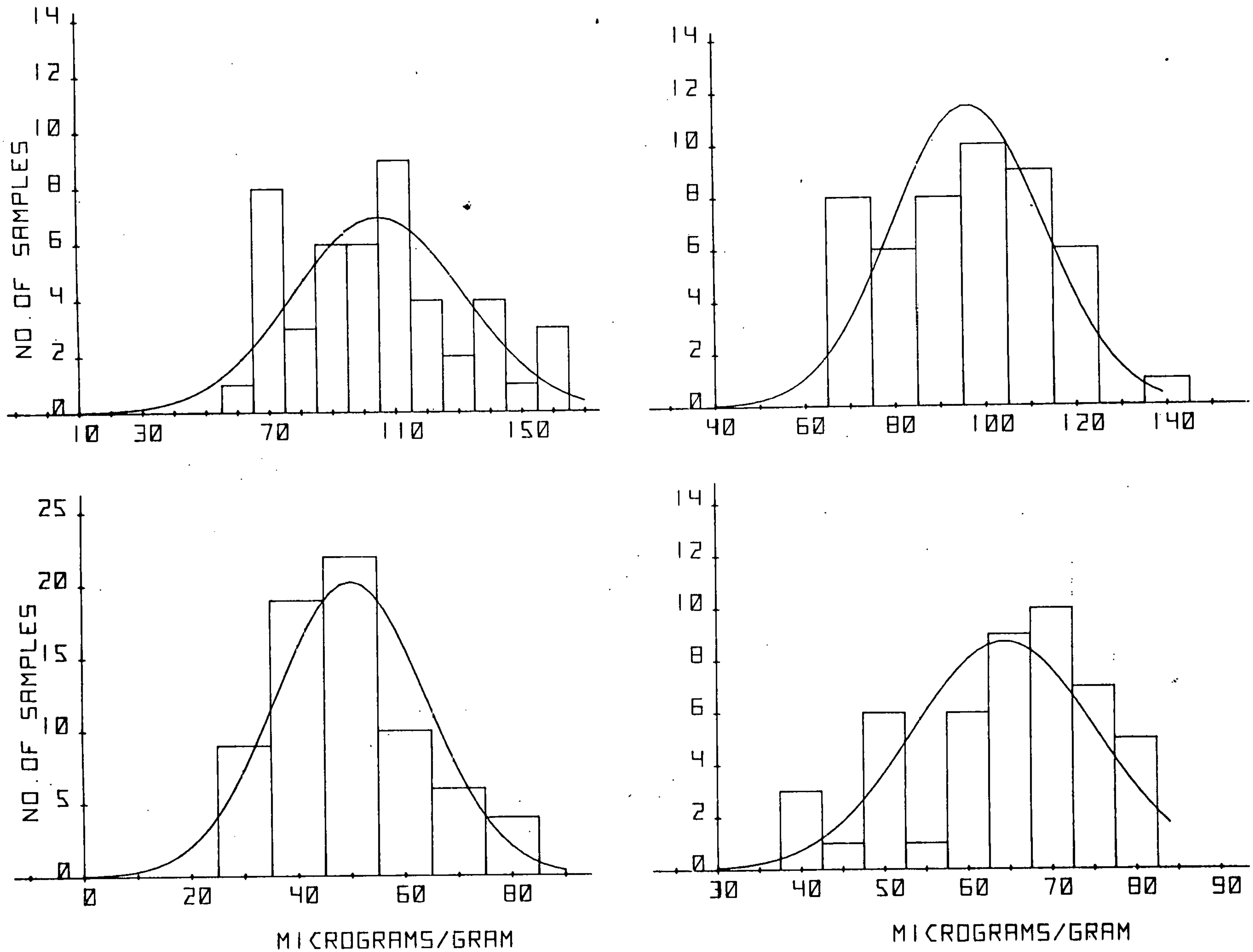
TABLE 2

Mineral Element Composition of Fe Deficient and Green Leaves from Two Different Lemon Trees.

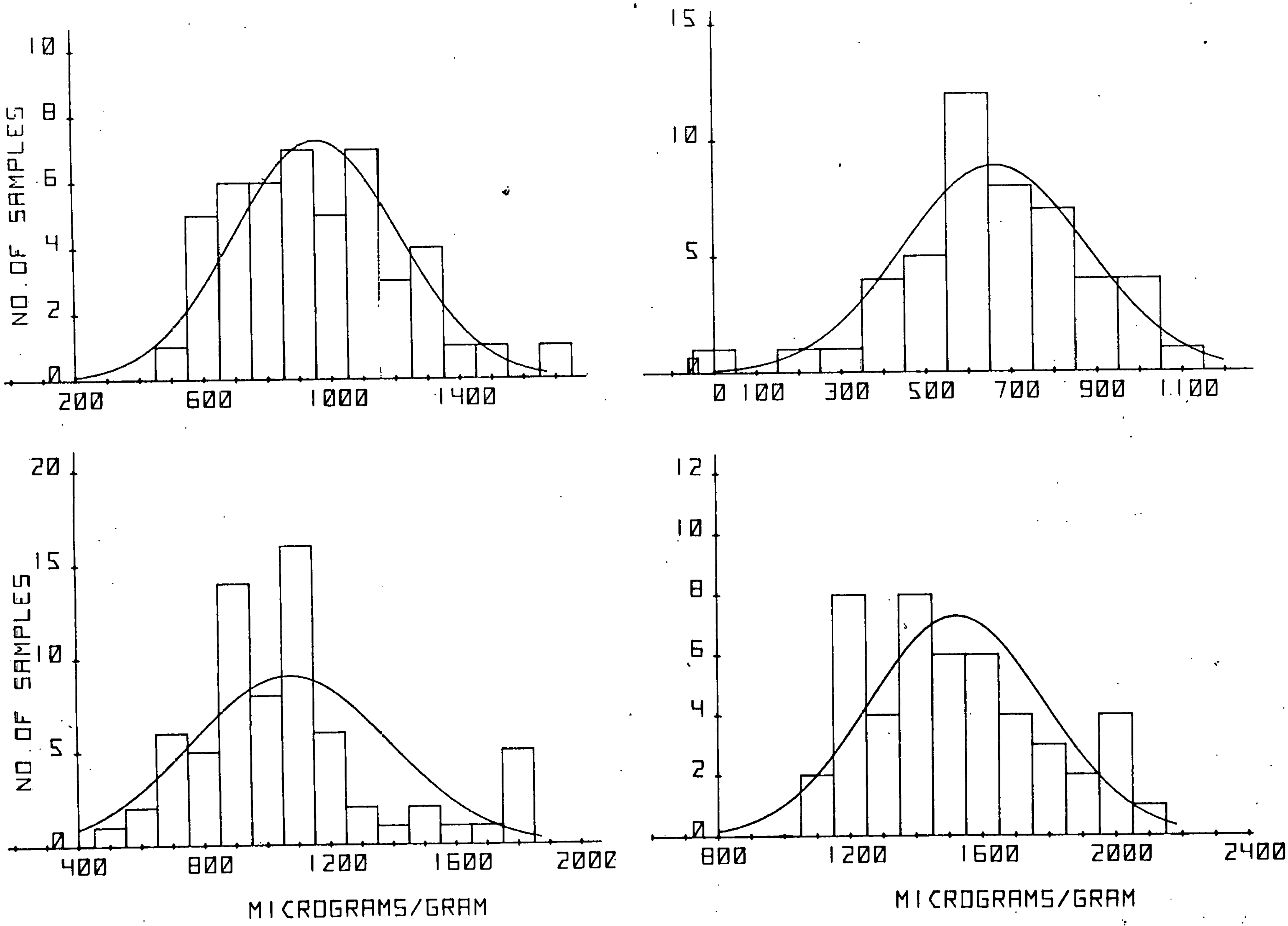
	n	Leaf color	$\bar{m}$	SD	CV %	Maximum	Minimum
			Fe µg/g				
Tree 1	47	yellow	104.8	27.2	26.0	160	56
Tree 1	48	green	96.3	16.8	17.4	138	68
Tree 2	71	yellow	50.0	14.1	28.2	86	25
Tree 2	48	green	64.4	11.1	17.2	82	38
			P µg/g				
Tree 1	47	yellow	955	260	27.2	1676	457
Tree 1	48	green	662	217	32.8	1117	250
Tree 2	71	yellow	1074	315	29.3	1880	543
Tree 2	48	green	1552	266	17.1	2136	1073
			K µg/g				
Tree 1	47	yellow	1.138	0.287	25.2	1.728	0.306
Tree 1	48	green	0.712	0.119	16.7	0.927	0.477
Tree 2	71	yellow	1.451	0.228	15.7	1.984	0.794
Tree 2	48	green	1.078	0.165	15.3	1.422	0.770
			Ca µg/g				
Tree 1	47	yellow	3.515	0.533	15.2	4.894	2.133
Tree 1	48	green	4.364	0.740	17.0	6.515	2.993
Tree 2	71	yellow	3.462	0.829	23.9	5.658	1.603
Tree 2	48	green	3.754	0.763	20.3	5.266	1.987

The histograms indicate that each kind of leaves for each tree can be considered as populations with normal frequency distribution characteristics. Any one leaf from any one group, therefore, would poorly represent the population and if the population were to be characterized, a minimum sample size would be necessary consistent with the formula:

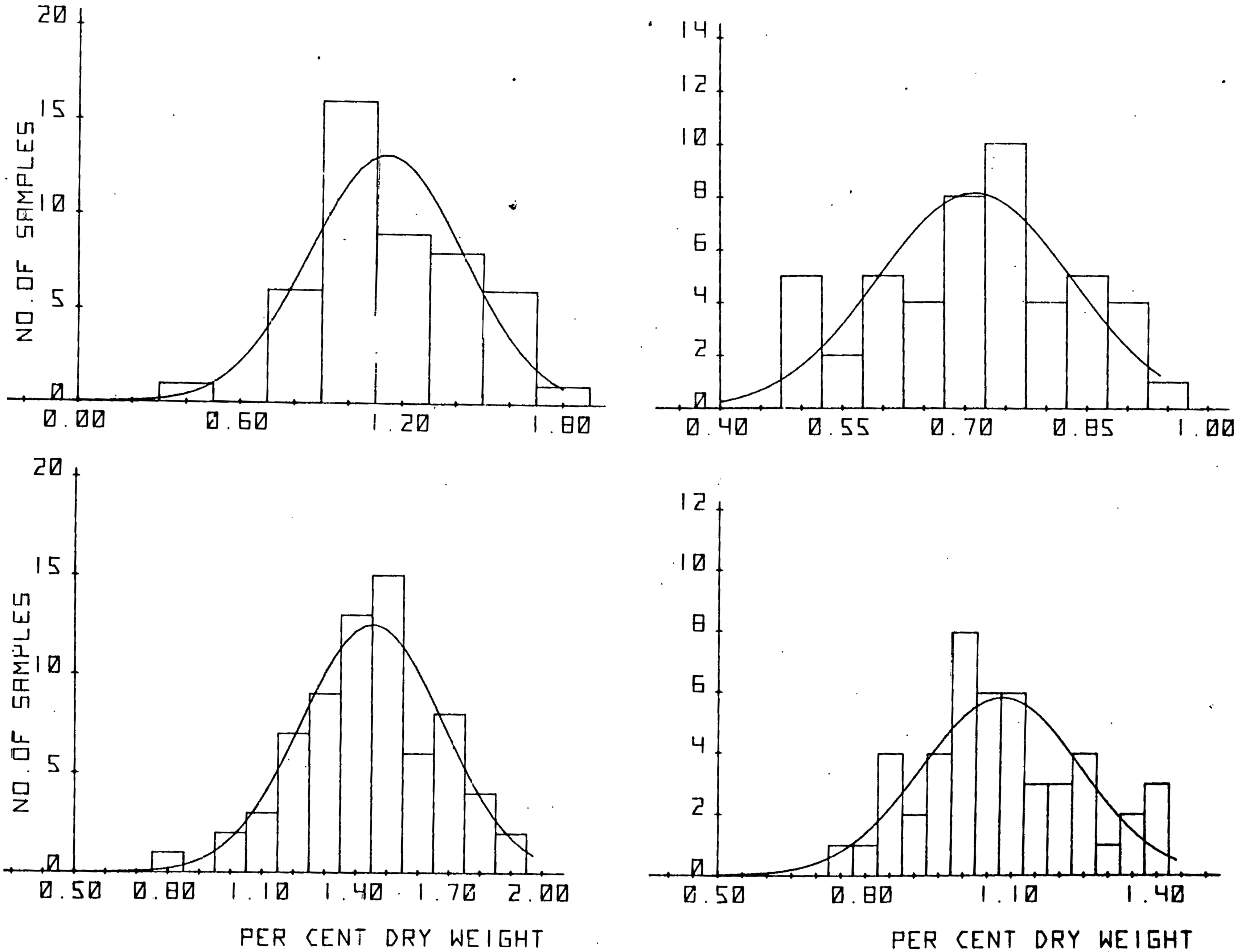
$$n = \frac{t^2 \ CV^2}{d^2}$$

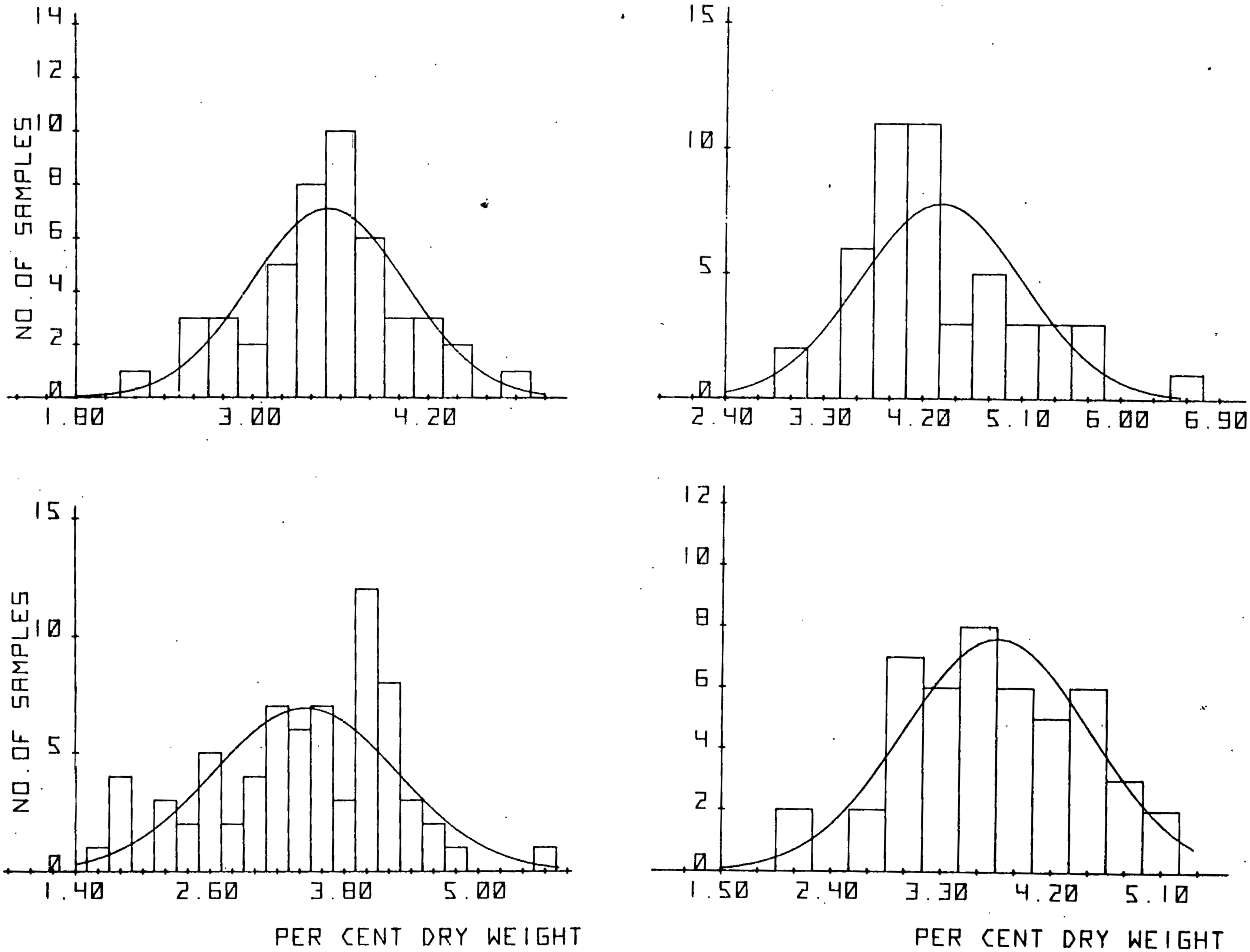


NO. OF SAMPLES
14
12
10
8
6
4
2
0
10
30
70
110
150
14
12
10
8
6
4
2
0
40
60
80
100
120
140
NO. OF SAMPLES
25
20
15
10
5
0
0
20
40
60
80
MICROGRAMS/GRAM
14
12
10
8
6
4
2
0
30
40
50
60
70
80
90
MICROGRAMS/GRAM


NO. OF SAMPLES
MICROGRAMS/GRAM
NO. OF SAMPLES
MICROGRAMS/GRAM
200
600
1000
1400
0 100
300
500
700
900
1,100
400
800
1200
1600
2000
800
1200
1600
2000
2400

Figs. 1 (Top) and 2 (Bottom). Histograms of Fe (top) and P (bottom) concentrations in Fe chlorotic leaves and green leaves from two different lemon trees. For each histogram top are leaves from tree 1; bottom are leaves from tree 2. Left are Fe chlorotic leaves; right are green leaves. Tree 2 shows real iron deficiency; tree 1 is typical of most lime-induced chlorosis; tree 2 is not for either Fe or P.


NO. OF SAMPLES
20
15
10
5
0
0.00
0.60
1.20
1.80
14
12
10
8
6
4
2
0
0.40
0.55
0.70
0.85
1.00
NO. OF SAMPLES
20
15
10
5
0
0.50
0.80
1.10
1.40
1.70
2.00
PER CENT DRY WEIGHT
12
10
8
6
4
2
0
0.50
0.80
1.10
1.40
PER CENT DRY WEIGHT


NO. OF SAMPLES
14
12
10
8
6
4
2
0
1.80
3.00
4.20
15
10
5
0
2.40
3.30
4.20
5.10
6.00
6.90
NO. OF SAMPLES
15
10
5
0
1.40
2.60
3.80
5.00
PER CENT DRY WEIGHT
12
10
8
6
4
2
0
1.50
2.40
3.30
4.20
5.10
PER CENT DRY WEIGHT

Figs. 3 (Top) and 4 (Bottom). Histograms of K (top) and Ca (bottom) concentrations in Fe chlorotic leaves and green leaves from two different lemon trees. For each histogram top are leaves from tree 1; bottom are leaves from tree 2. Left are Fe chlorotic leaves; right are green leaves. Both trees are typical for lime-induced chlorosis for both K and Ca.

TABLE 3

Statistical Evaluation of Frequency Distribution Histograms.
(Figures 1 - 4).

Tree	n	Element	Skewness	Kurtosis "a"	Chi^2
			Yellow		
1	47	P	0.4570	0.8187	probably NS
1	47	K	-0.1531	0.8075	probably NS
1	47	Ca	-0.1396	0.7552	probably NS
1	47	Fe	0.2986	0.8158	probably NS
			Green		
1	48	P	-0.3313	0.7710	probably NS
1	48	K	-0.1499	0.8058	probably NS
1	48	Ca	0.7304	0.7910	probably NS
1	48	Fe	0.0908	0.8490	probably NS
			Yellow		
2*	71	P	0.9826*	0.7281*	S
2	71	K	-0.1297	0.7728	probably NS
2	71	Ca	-0.2853	0.7969	probably NS
2	71	Fe	0.6168	0.7850	probably NS
			Green		
2	48	P	0.4824	0.8252	probably NS
2	48	K	0.3289	0.7988	probably NS
2	48	Ca	-0.0379	0.8258	NS
2	48	Fe	-0.6119	0.8024	probably NS

*Skewness significant at 1%, kurtosis significant at 1%. Probably would reject normality. All others, cannot reject normality.

where t is to value for probability of error (Snedecor 1946), d = 1.96 for P = 0.05 or 2.58 for P = 0.01, CV is coefficient of variation and d is the accuracy desired, in this case as % to coincide with the units of the CV. The value d is usually the difference between two means to be separated.

TABLE 4

Correlation Coefficients for Pairs of Elements in Leaves from the Same Tree With and Without Iron Deficiency Symptoms.

	Tree 1		Tree 2	
	Def.	No Def.	Def.	No Def.
Fe x P	0.129	-0.320	-0.441	-0.352
Fe x K	0.011	-0.022	-0.446	-0.321
Fe x Ca	0.249	0.516	0.558	0.312
P x K	0.458	0.431	0.301	0.365
P x Ca	-0.224	-0.468	-0.413	-0.468
K x Ca	-0.406	-0.096	-0.486	-0.553
Fe x Zn	-0.134	0.163	0.169	0.291
Fe x Mn	0.442	0.396	0.580	-0.036
Fe x Cu	0.411	-0.031	0.547	0.700
Fe x Al	0.804	0.680	0.914	0.813
Fe x Si	0.536	0.465	0.623	0.720
Fe x Ti	0.643	0.414	0.343	-0.345

r needed for P = 0.05 is 0.280 for n = 47.

Part of the correlation matrix is shown in Table 4. It is of interest to note how consistent are the typical lime-induced chlorosis relationships from leaf to leaf with the degree of variability encountered. The P x K correlations were positive and significant for both trees and both deficient and green leaves; those for P and Ca were negative in all cases. All are as expected with chlorosis even though expressed for both green and chlorotic leaves. The Fe and P were negatively correlated in tree 2, the tree showing true Fe deficiency; they were also negatively correlated in the green leaves of tree 1. The correlation coefficients for Fe x K were much like those for Fe x P but neither were significant for tree 1. The K x Ca coefficients were negative as expected although not significant for tree 1 or leaves that were green. This effect may be the result of lack of the chlorosis.

There were some differences in correlation coefficients for Fe x other micronutrients with green and chlorotic leaves. In tree 1, Fe x Ca was positive and significant for chlorotic leaves but not for green leaves. In tree 2 Fe x Mn was positive and significant for Fe deficient leaves but not for green leaves. The Fe and Zn did not show a negative relationship as indicated by the analysis in Table 1. There was a negative relationship for chlorotic leaves of tree 1 but it was not statistically significant.

```
TREE PRINTED OVER CORRELATION MATRIX (SCALED 0-100).
CLUSTERING BY MINIMUM DISTANCE METHOD.
  VARIABLE
NAME      NO.
P     (  2) 42 72/38 52 66 21 41 72 47 44 31 56 41 41 63 46/56/51 37/
NA    (  3) 76/34 27 33 47 42 32 33 58 56 51 61 61 51 50/65/49 45/
K     (  4)/29 29 42 30 37 42 22 45 38 50 52 52 54 42/68/62 41/
CA    (  5) 85 83/82/78/57/68/60 65 62 60 50 56 68/46/57 54/
SR    ( 19) 92/62/70/81/79/52 53 65 54 51 64 65/43/46 44/
BA    ( 20)/56/65/80/73/59 57 72 58 53 71 68/52/44 36/
CR    ( 18)/82/37/56/59 68 47 57 57 44 63/42/61 63/
LI    ( 21)/56/61/59 61 50 51 45 56 70/43/51 49/
MG    (  6)/72/48 35 60 43 45 66 58/36/39 33/
MN    ( 10)/63 69 72 64 57 71 73/47/31 41/
CU    (  8) 90/70 70 64/66 75/64/34 39/
SI    ( 13)/76 80 73/63 72/72/37 47/
FE    (  9) 90 82/66 59/70/43 40/
AL    ( 12) 93/56 56/67/49 50/
TI    ( 14)/50 48/65/49 51/
CO    ( 16) 91/60/22 20/
NI    ( 17)/50/23 29/
B     ( 11)/45 42/
ZN    (  7) 83/
V     ( 15)/
```

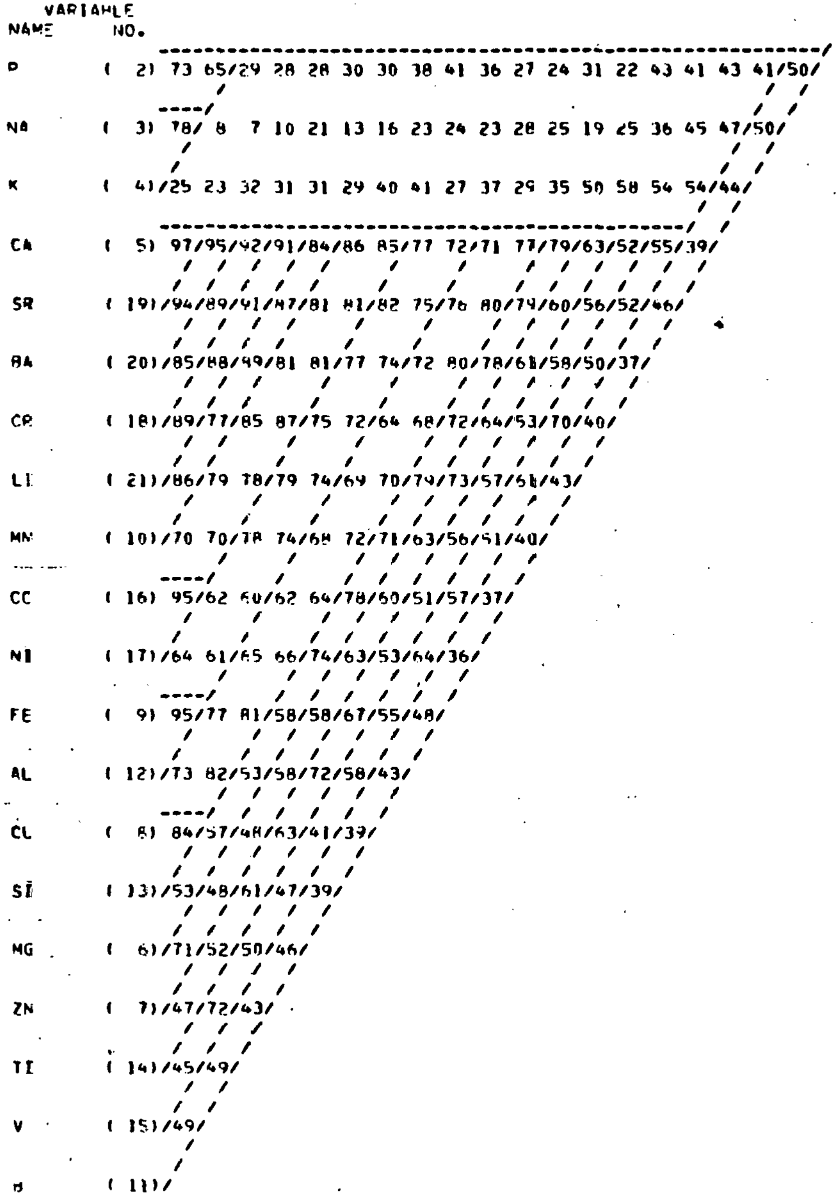
```
NAME      NO.
P     (  2) 71/48/38 46 28 33 21 35 26 30 43 22 49 41 33 54 30 42 55/
K     (  4)/69/59 55 50 48 41 44 45 45 52 45 50 59 57 52 57 56 52/
B     ( 11)/59 60 64 55 57 58 59 65 50 57 58 59 54 45 57 47 36/
NA    (  3) 78 78 54 65 64/42 37 40 55 28 56 71 42 67/49 45/
CU    (  8) 85/48 63 65/29 29 24 42 20 50 63 36 47/45 50/
SI    ( 13)/73 81 73/54 50 46 62 29 57 68 45 64/53 49/
FE    (  9) 83 70/75 74 74 76 66 66 70 69 67/58 43/
AL    ( 12) 90/63 61 54 71 48 64 72 50 63/51 42/
TI    ( 14)/50 52 47 62 45 64 69 45 50/38 39/
CA    (  5) 94/90/88/80/68 64/75/76/62 40/
SR    ( 19)/88/84/87/70 64/75/72/54 31/
BA    ( 20)/83/81/73 68/78/71/52 40/
CR    ( 18)/72/80 82/68/75/47 35/
MG    (  6)/73 60/74/56/44 31/
CO    ( 16) 91/61/53/25 23/
NI    ( 17)/62/67/39 32/
MN    ( 10)/72/63 45/
LI    ( 21)/72 48/
ZN    (  7) 79/
V     ( 15)/
```

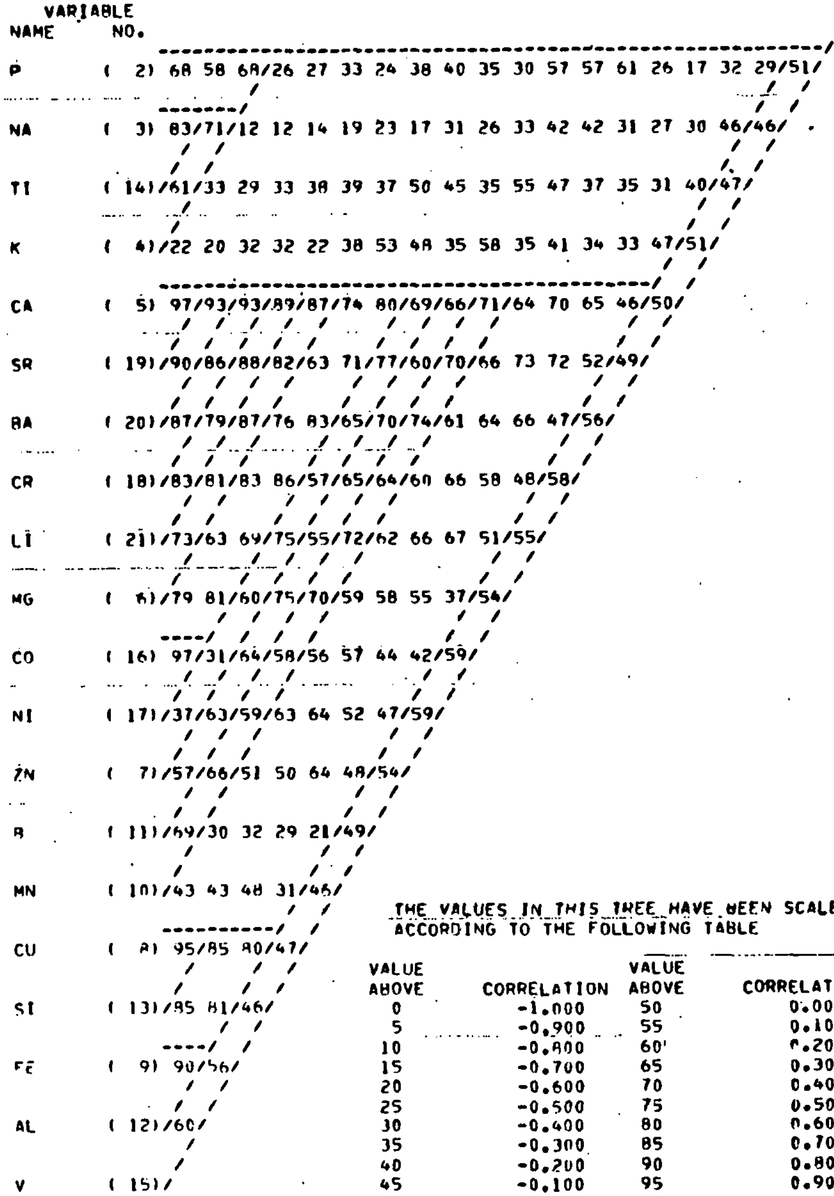
THE VALUES IN THIS TREE HAVE BEEN SCALED ACCORDING TO THE FOLLOWING TABLE

VALUE ABOVE	CORRELATION	VALUE ABOVE	CORRELATION
0	-1.000	50	0.000
5	-0.900	55	0.100
10	-0.800	60	0.200
15	-0.700	65	0.300
20	-0.600	70	0.400
25	-0.500	75	0.500
30	-0.400	80	0.600
35	-0.300	85	0.700
40	-0.200	90	0.800
45	-0.100	95	0.900

Fig. 5. Cluster trees for the Fe-deficient and green leaves of tree 1. Left are Fe-chlorotic leaves; right are green leaves.

TREE PRINTED OVER CORRELATION MATRIX (SCALED 0-100).
CLUSTERING BY MINIMUM DISTANCE METHOD.





THE VALUES IN THIS TREE HAVE BEEN SCALED ACCORDING TO THE FOLLOWING TABLE

VALUE ABOVE	CORRELATION	VALUE ABOVE	CORRELATION
0	-1.000	50	0.000
5	-0.900	55	0.100
10	-0.800	60	0.200
15	-0.700	65	0.300
20	-0.600	70	0.400
25	-0.500	75	0.500
30	-0.400	80	0.600
35	-0.300	85	0.700
40	-0.200	90	0.800
45	-0.100	95	0.900

Fig. 6. Cluster trees for the Fe-deficient and green leaves of tree 2. Left are Fe-chlorotic leaves; right are green leaves.

Except for Fe x Ti in tree 2 for green leaves, there were significant positive correlations for Fe x Al, Fe x Si and Fe x Ti. Possitive correlations among these elements are considered as evidence that leaf washing was inadequate (Wallace et al. 1980). The present leaves were carefully washed but the correlations show nevertheless. The departure of Fe x Ti for one set of leaves may indicate adequate leaf washing. In the previous study cited, acid washing and rubbing of the leaves with cheesecloth did not remove the positive Fe x Al relationship.

It is of considerable interest that some major mineral element relationships that occur for Fe chlorosis (P x K, K x Ca, etc.) obviously hold reasonably well for individual leaves even though the frequency distribution shows 3 or more fold variations in leaf concentrations of individual elements as shown in the histograms.

In Figures 5 and 6 are cluster trees showing possible relationships among elements. The so-called dust elements tend to group together even with washing (Wallace et al. 1980). Some of the relationships shown for correlation coefficients are indicated in the cluster trees.

REFERENCES

Alexander, G. V., and L. T. McAnulty. 1981. Multielement analysis of plant-related tissues and fluids by optical emission spectrometry. Jour. Plant Nutr. 3(1-4):51-59.

Embleton, T. W., W. W. Jones, C. K. Labanauskas, and W. Reuther. 1973. Leaf analysis as a diagnostic tool and guide to fertilization. pp. 183-210, IN: The Citrus Industry, Vol. III, W. Reuther, Ed., Univ. Calif., Riverside, CA.

Iljin, W. S. 1952. Metabolism of plants affected with lime induced chlorosis (calciose). III. Mineral elements. Pl. Soil. 4:11-28.

Mehrotra, S. C., N. K. Mehrotra, S. S. Bisht, and C. P. Sharma. 1976. Resolution of iron chlorosis. Geophytology, 6(2):282-295.

Snedecor, G. W. 1946. Statistical methods. Iowa State College Press, Ames, IA.

Wallace, A., J. Kinnear, J. W. Cha, and E. M. Romney. 1980. Effect of washing procedures on mineral analyses and their cluster analyses for orange leaves. Jour. Plant Nutr. 2(1&2):1-9.