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THE TRACE AND MAJOR ELEMENT COMPOSITION OF THE LEAVES OF SOME DECIDUOUS TREES

II. SEASONAL CHANGES

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INTRODUCTION

While soil examination is valuable for indicating the nutrient status of agricultural land, tissue analysis is often used as a means of studying the nutritional status of trees and plantation crops. Interpretation of the results of tissue analysis requires not only an appreciation of the complex biochemical and physiological functions of the inorganic constituents, but also a knowledge of seasonal changes in composition.

Arnon [1], Gauch [12], Hewitt [15], and Stiles [32], in reviewing published work, report some interchangeability of the functions of different elements in plants. Antagonism between different elements has been reported by Hoagland [16], Olsen [23 24], Lundegårdh [20] and others, while Cain [8] has shown that antagonism reported in leaves can often be ascribed to translocation and distribution factors rather than absorption or uptake by the plants. Biddulph [4] observed that symptoms of malnutrition are as often due to failures in the distribution system as in the initial absorption. Thus analysis of leaves or other organs cannot be considered to be an unequivocal indicator of nutritional status. Translocation of inorganic elements in plants is itself a complex subject. After Stout and Hoagland [33] provided direct evidence that upward movement of inorganic elements occurs through the xylem, important studies on translocation, mostly using radioactive isotopes, have been reported by Biddulph and Markle [5], Biddulph [4], Robertson [28 29], Langston [18] and Bollard [6]. The mechanism is far from being completely understood.

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In an attempt to throw some light on the uptake and distribution of inorganic elements by plants, an investigation on the seasonal variation in composition of leaf and petiole samples collected from a number of mature deciduous trees was carried out during the season of 1959. Some samples of inflorescence were also examined. The effect of sampling height on such composition was also studied. The results of these investigations are reported in this paper, while the details of the materials and methods, together with the findings of some preliminary investigations. such as sampling error, contamination problems and reproducibility of analytical methods, prerequisite to the present study, were given in Part I (Guha and Mitchell [14]). The object has been to advance the knowledge of factors related to the assessment of nutritional status by tissue analysis, not to consider the plant physiological aspects of the findings.

As indicated by Deleano and Bordeianu [10], Mitchell [21], Olsen [23], and Kramer and Kozlowski [17], many of the studies on alleged translocation of nutrients to and from the leaves based on concentration alone are of doubtful value because changes in dry weight of the leaves during the season cause fluctuations in concentration. Both concentration and absolute amounts have therefore been considered in this investigation. In all tables presented here, results are given for the weight of 1000 leaves or leaf blades, enabling absolute amounts to be calculated. Unless otherwise stated, all results refer to samples taken near the bottom of the crown.

SEASONAL CHANGES

Studies of seasonal variations were made on three sycamore and horse-chestnut and nine beech trees from the locations detailed in Part I. They were sampled regularly during the growing season, but as space is limited and different trees of the same species gave essentially similar results, generally only one tree of each species, on a granitic soil at Craigiebuckler, Aberdeen, is considered here. Full results have been recorded by Guha [13] elsewhere. Figures 1 to 21 present graphically the seasonal changes in concentration (v. also Table 4 of Part I) and absolute amount of 21 different elements in leaf blades of Sycamore No. 1 and Horse-chestnut No. 1 and leaves of Beech No. 1. Table 1 presents results for petiole, leaf blade, and inflorescence from a second sycamore tree (No. 2); result of samplings from Sycamore No. 3, Horse-chestnut No. 3 and

TABLE 1

Composition of petiole (P), leaf blade (LB) and inflorescence (INF) of Sycamore No. 2 from Craigiebuckler
Expressed as concentration in ppm or per cent of oven-dry material

Date of sampling		13 May	15 June	3 July	30 July	24 Aug	23 Sept	26 Oct
Wt. (g) of 1000	P	23	54	61	81	64	70	56
	LB	108	252	280	364	306	323	287
	INF	302	416	708	1604	1312	1277	—
No. of INF per 1000 LB		71	142	124	101	112	108	—
Wt. (g) of INF per 1000 LB		21	59	88	162	147	138	—
Wt. (g) of INF per 1000 g of LB		198	234	312	443	484	428	—
Co, ppm	P	0.09	0.05	0.06	0.05	0.05	0.09	0.10
	LB	0.23	0.15	0.27	0.18	0.30	0.54	0.53
	INF	0.28	0.22	0.11	0.11	0.10	0.16	—
Ni, ppm	P	0.93	0.56	0.60	0.48	0.42	0.51	0.36
	LB	1.58	0.78	1.10	0.78	1.01	1.56	1.64
	INF	2.06	1.34	1.07	1.06	1.04	1.23	—
Fe, ppm	P	63	56	64	48	53	93	95
	LB	218	185	309	208	347	628	617
	INF	254	165	104	86	69	151	—
V, ppm	P	0.19	0.20	0.20	0.18	0.22	0.38	0.44
	LB	0.83	0.96	1.32	0.97	2.85	3.04	3.85
	INF	1.23	1.07	0.48	0.38	0.39	0.74	—
Ti, ppm	P	5.5	5.9	5.7	4.4	5.9	8.7	9.5
	LB	20	22	37	24	68	89	113
	INF	32	22	13	9	9	21	—
Cr, ppm	P	0.20	0.16	0.13	0.16	0.19	0.29	0.47
	LB	0.69	0.56	0.78	0.81	2.10	1.67	2.86
	INF	0.83	0.66	0.30	0.26	0.27	0.53	—
Pb, ppm	P	0.9	0.6	1.1	0.8	1.0	1.3	1.4
	LB	3.0	0.9	1.8	1.8	1.3	3.1	2.0
	INF	2.3	1.1	0.9	0.5	0.4	1.0	—
Al, ppm	P	92	125	108	80	115	152	161
	LB	289	206	201	207	490	789	762
	INF	283	294	185	128	136	227	—
Mn, ppm	P	48	42	62	71	77	96	147
	LB	53	69	87	100	84	118	168
	INF	48	37	40	55	59	68	—

Cont.

TABLE 1 (*Continued*)

Date of sampling		13 May	15 June	3 July	30 July	24 Aug	23 Sept	26 Oct
B, ppm	P	24	22	24	31	29	29	32
	LB	18	21	28	45	58	73	76
	INF	46	19	14	21	21	29	—
Si, %	P	0.02	0.03	0.02	0.02	0.03	0.06	0.03
	LB	0.12	0.28	0.45	0.49	0.65	0.88	0.88
	INF	0.10	0.08	0.06	0.06	0.07	0.11	—
Ca, %	P	0.91	1.74	1.93	2.38	2.51	2.90	3.89
	LB	0.20	1.21	1.38	1.59	1.91	2.08	2.59
	INF	0.16	0.41	0.32	0.46	0.52	0.98	—
Sr, ppm	P	52	54	99	71	75	78	91
	LB	9	26	31	41	36	48	50
	INF	8	18	19	21	29	37	—
Ba, ppm	P	79	94	127	113	102	118	171
	LB	18	50	62	60	65	90	88
	INF	19	38	35	43	54	69	—
Mg, %	P	0.31	0.26	0.26	0.30	0.36	0.40	0.61
	LB	0.20	0.25	0.27	0.25	0.30	0.29	0.33
	INF	0.24	0.18	0.14	0.14	0.17	0.20	—
Cu, ppm	P	14.4	12.3	10.0	10.3	9.6	8.7	2.8
	LB	15.8	8.8	9.2	8.5	7.0	7.4	7.1
	INF	22.0	9.3	8.4	9.7	10.6	14.8	—
Mo, ppm	P	0.40	0.30	0.25	0.18	0.13	0.10	0.07
	LB	0.21	0.08	0.10	0.05	0.10	0.14	0.15
	INF	0.23	0.36	0.19	0.16	0.16	0.18	—
Zn, ppm	P	45	55	58	69	75	76	94
	LB	39	33	40	41	14	33	—
	INF	58	19	28	28	24	31	—
P, %	P	0.64	0.34	0.26	0.23	0.22	0.22	0.10
	LB	0.60	0.23	0.20	0.19	0.19	0.19	0.11
	INF	0.70	0.36	0.27	0.26	0.28	0.33	—
K, %	P	4.32	3.05	3.37	2.92	2.55	2.97	1.92
	LB	2.16	1.16	1.17	1.11	1.13	1.37	1.13
	INF	2.94	2.16	1.94	1.84	1.95	2.14	—
Na, %	P	0.03	0.07	0.10	0.08	0.12	0.14	0.19
	LB	0.08	0.02	0.03	0.02	0.02	0.02	0.05
	INF	0.10	0.05	0.04	0.03	0.04	0.05	—

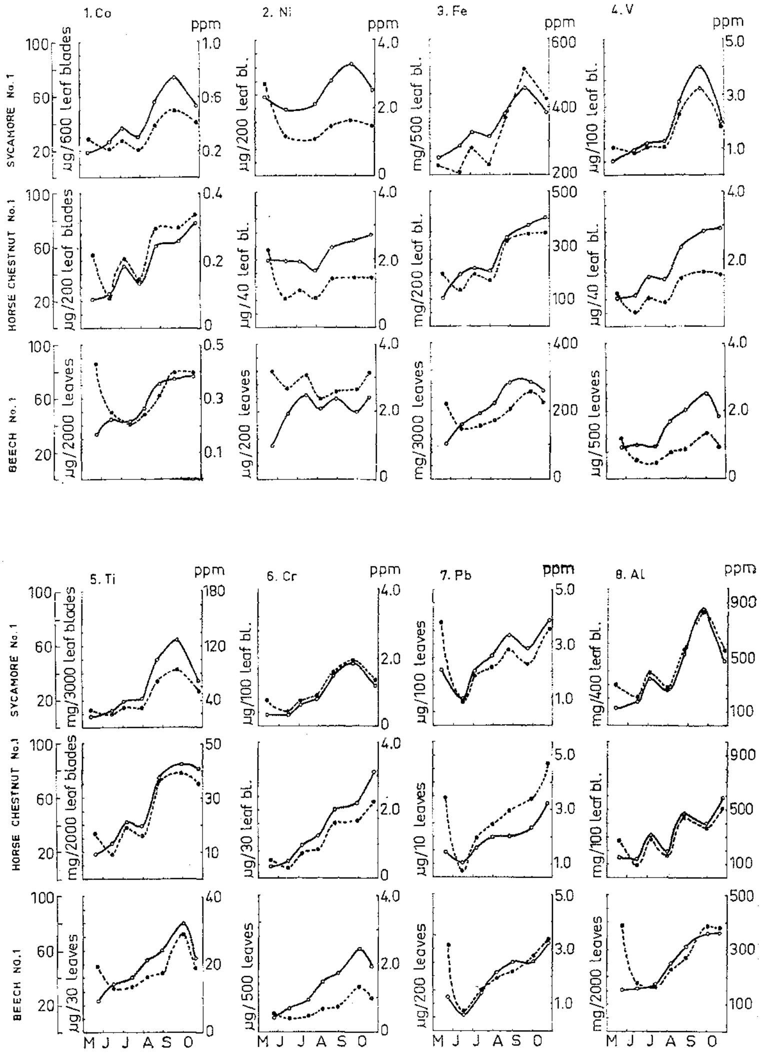
Beech No. 3 are reported in Table 2, which includes results for different sampling heights. The 21 elements could be divided on the basis of seasonal variation into three groups which are discussed in turn for leaf and inflorescence.

Leaf or leaf blade

Group A: Co, Ni, Fe, V, Ti, Cr, Pb and Al. None of the elements of this Group except Fe are normally considered to be essential to plants, though there have been reports claiming Co to be beneficial or essential (Bolle-Jones and Mallikarjuneswara [7], Reisenauer [26]).

From mid-May to mid-June, the period following emergence from the bud, the concentrations of the elements of this Group (Fig. 1–8) fell markedly. For instance, in leaf blade of sycamore, Ni fell from 2.72 to 1.18 ppm, and Cr from 0.76 to 0.41 ppm; in leaf blade of horse-chestnut, Co from 0.22 to 0.09 ppm, and Al from 273 to 92 ppm; in beech leaves, Fe from 220 to 147 ppm, V from 1.18 to 0.55 ppm, and Ti from 19.6 to 13.0 ppm. Figures 1 to 8 indicate that, unlike concentration, the absolute amounts of all but one of these elements per unit number of leaves did not fall, but rose or remained more or less constant over this period, during which the young leaves were undergoing vigorous vegetative growth, as is evident from the rapid increase of dry weight of the leaves (Part I, Table 4). The fall in concentration is therefore obviously not the result of any loss or back transport from the leaves, but of dilution due to the increase in dry weight. Only Pb behaved differently; both concentration and absolute amount fell during this initial period: the reason for this is being studied further. The abnormally high dry weight of the June horse-chestnut leaf blade sample (not confirmed by the other trees of this species) is unexplained.

From the middle of June until the end of September there is no marked change in dry weight, the curves for the changes in concentration and absolute amount in consequence following similar courses, the relative positions and slopes of which depend on the arbitrary ordinate scales. During this period both concentrations and absolute amounts of all elements of this group increased. The maximum concentrations of Co, Ni, Fe, V, Ti, Cr, and Al in leaf blade of sycamore all occurred in the September samples, being



Figs. 1–8

Figs. 1—21. Seasonal variation in content of leaves or leaf blades.

○———○———○ contents, in absolute amounts (µg or mg) per unit number of leaves.

• — — • — — • content, in concentration (ppm or per cent)

0.50 ppm Co, 1.64 ppm Ni, 510 ppm Fe, 3.20 ppm V, 85.9 ppm Ti, 1.86 ppm Cr, and 837 ppm Al. The increase in the Pb-content continued during October, the maximum concentration, 3.6 ppm, being found in late October. The minimum concentrations all occurred in the mid-June samples. Similar end of season trends are shown in the results for beech.

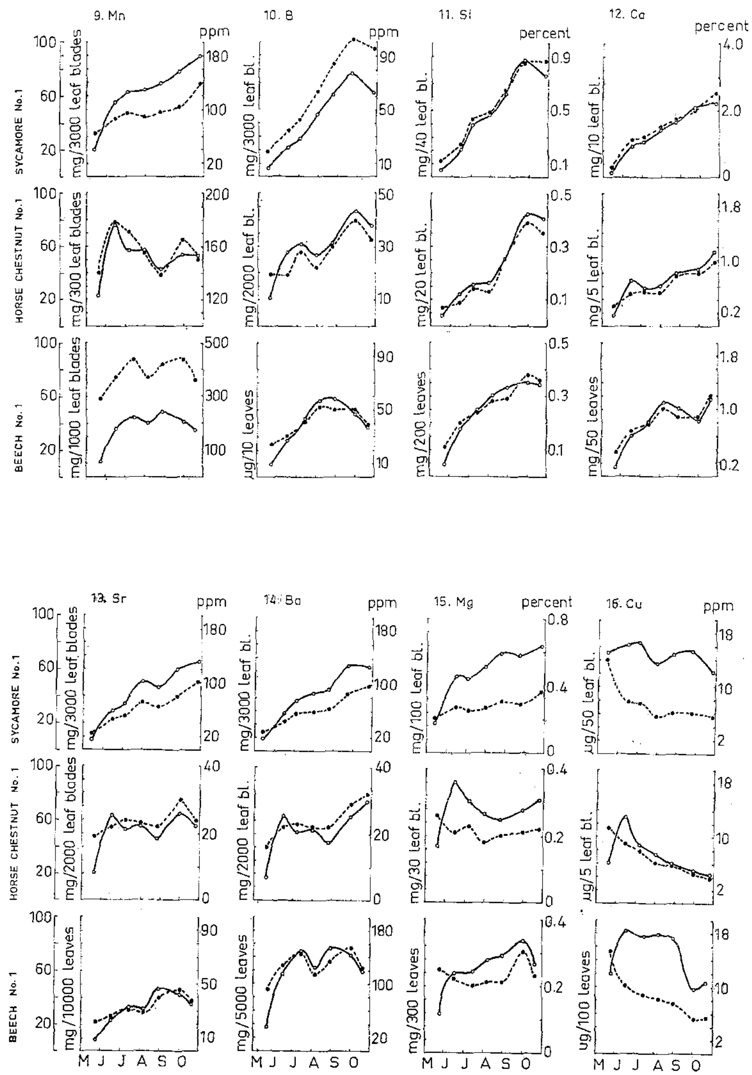
After reaching the maximum levels at the end of September, both the concentrations and absolute amounts of all elements, except Pb, in these 2 species either showed a marked fall or tended to level off. This decrease suggests a back translocation from the leaves prior to leaf fall. It was not detected in the particular horse-chestnut for which the results are presented, but was visible for some elements (V, Ti, and Cr) in the other two horse-chestnut trees studied (*v.* Table 2). At the date of the final sampling the leaves of Horse-chestnut No. 1 were greener than those of the other trees, and it is possible that they had not reached the required stage of senescence. The leaves of the sycamore were fully senescent and ready to fall while those of the beech were about 5 per cent yellow. On the other hand, in Sycamore No. 2 reported in Table 1, whose leaves were still unwithered on 26th October, no evidence of back transport is apparent. Thus, while there is some evidence for translocation from leaves back to the tree when the leaves become senescent, the period of such translocation appears to be quite short and readily missed, since the leaves may fall soon after becoming senescent. As the leaves fell during a period of strong winds it unfortunately proved impossible to obtain samples of fallen leaves.

No reports on seasonal variation in the contents of many of the elements in this group have been found in the literature. Deleano and Andreesco [9] found that the absolute amount of Fe in leaves of *Salix fragilis* increased for about 50 days at the beginning of the season, followed by a period of about 100 days when the absolute amount remained almost constant, and finally, during the last 60 days the absolute amount deceased by about 30 per cent. This is in agreement with the results of the present investigation in so far as the initial increase in absolute amount and the final decrease before the leaf fall are concerned. But in none of the fifteen trees of the present study did the absolute amount of Fe in leaves remain unaltered during the middle of the season. In agreement with the present observations, Olsen [23] reported a gradual increase in the Fe-content of beech leaves at this time, but did not find a decrease in the amount towards the end of the season, possibly due to the difficulties of sampling at the appropriate time.

Group B: Mn, B, Si, Ca, Sr, Ba, and Mg. Two trace elements, Mn and B, recognized to be essential to plants, and Si, which has proved to be similar in behaviour to B, are included in this group, together with Ca, Sr, Ba, and Mg, two essential major elements and two non-essential trace elements. None of these elements shows an obvious concentration inflexion point following the period of rapid leaf growth, a characteristic feature of the Group-A elements. They generally show a continual increase in concentration until near the end of the season.

In spite of the rapid increase in dry weight during the May-June period, the concentrations of all seven elements (Fig. 9–15) did not fall, but increased. In leaf blade of sycamore, the increases in concentration were, for Mn from 64 to 87 ppm, for B from 18 to 34 ppm, and for Si from 0.12 to 0.25 per cent, for Ca from 0.31 to 1.12 per cent, for Sr from 24 to 46 ppm, for Ba from 28 to 45 ppm and for Mg from 0.21 to 0.27 per cent. The corresponding increases in absolute amounts per one thousand leaf blades were, for Mn from 6.9 to 18.3 mg, for B from 1.9 to 7.1 mg, for Si from 0.12 to 0.52 g, for Ca 0.33 to 2.35 g, for Sr 2.6 to 9.7 mg, for Ba from 3.0 to 9.5 mg and for Mg from 223 to 573 mg. The supply of these elements to the leaves was, therefore, very fast at the stage of rapid leaf growth, the absolute amounts in the leaves increasing faster than the dry-weight increase. At the middle of June the absolute amounts present per leaf were at least three times as high as the corresponding amounts found at the middle of May. The Group-A elements did not show such a large increase.

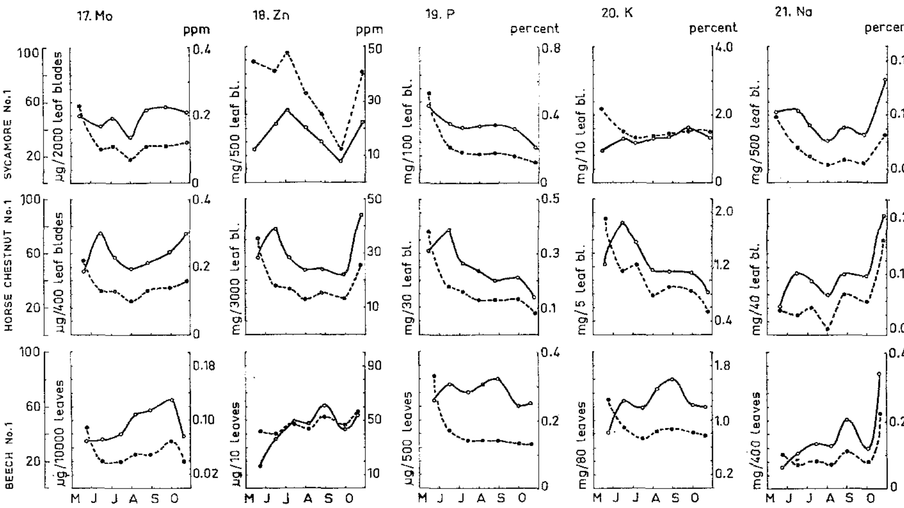
The increase in contents of leaves, both as concentration and absolute amount, is maintained at least until the middle of July, except for a few elements in horse-chestnut, which show an intermediate June maximum. The contents of Si, Ca, and Sr continue to increase steadily in all three species until the end of September; Mn, B, Ba, and Mg show a steady increase only in sycamore. During October, a tendency for a slight back translocation from the leaves of all elements except Ca is apparent. This is occasionally not



Figs. 9–16

(For legends see Fig. 1–8)

Corrigendum: Fig. 9 Beech No. 1 'leaf blades' should read *leaves*



Figs. 17–21

observed in sycamore. Horse-chestnut shows it for 4 elements of this group, suggesting that the back translocation may possibly occur sooner with some elements than with others.

Figure 15 shows that the concentration of Mg in leaves of all the trees displayed very little variation during the whole season, being almost always within the 0.20–0.30 per cent range. Accordingly, the absolute amount, with a rapid increase at the beginning and then a very slow increase up to the end of the season, more or less followed the dry weight changes of the leaves, possibly suggesting some direct correlation between total Mg and an organic constituent. Olsen [23] found the Mg-concentration in beech leaves to rise from leafing until the end of May, whereafter it remained relatively constant until the fall of the leaves.

Except for Mg, the minimum values of Group B elements both in concentration and absolute amount, occur at the beginning of the season. The respective maximum values, which for Si and B could be as high as ten times and for Mg less than twice the minimum values, occur towards the end of the season just before the leaves become senescent.

It has generally been found by other workers that the Si-content increases with age of leaves (Deleano and Bordeianu [10], Leyton [19] and Olsen [23]). Deleano and Bordeianu reported a back translocation from the foliage in autumn. These reports are in agreement with the results of the present investigation. For Mn and B, reports in the literature are few. Bertrand and Rosenblatt [2] and Bertrand and Silberstein [3] observed that, for both elements, the maximum concentration in leaves of various tree species generally occurred during the period of leaf development, with some species showing a second maximum later in the season and others a progressive decrease. Smith, Reuther, and Specht [31] found that in citrus leaves B- and Mn-concentrations were lowest in very young leaves. These observations on concentration are in line with the present findings.

From many published reports reviewed by Leyton [19], the Ca-content of leaves generally appears to increase with age. Reports on seasonal variation in Sr- and Ba-contents of leaves have not been found, although there has been much work on Sr-contents from the radioactive fallout aspect. That Sr and Ba, but not Mg, behave like Ca, is of interest in the the light of the observations of Epstein and Leggett [11] who, in studying the specificity of carriers in active transport of mineral elements, found that Ca^{++}, Sr^{++} and Ba^{++} compete for identical binding sites, but Mg^{++} does not.

Group C: Cu, Mo, Zn, P, K and Na. This group includes three essential trace elements, two major plant nutrients and Na, which although not considered essential, is claimed partially to replace K, particularly in deficiency conditions (Richards and Berner [27] and Pirson [25]). The concentrations of all except Zn and Na (Fig. 16–21) are maximal when the leaves are immature and show a pronounced fall from mid-May to mid-June during rapid growth of the leaves. Then follows a period until about the end of September when the concentrations continue to fall very slowly or remain more or less constant. Like the Group-A elements there is an initial fall in concentration, but unlike them there is no tendency for this fall be made up after the rapid increase in dry weight of leaves has ceased. The similarity in the trends for K and P is very marked.

The curves for absolute leaf contents show that almost the total supply of Cu, Mo, Zn, P, and K to the leaves takes place early in the season. The first sampling of Sycamore No. 1 was possibly too late to record the large increase in absolute amount seen for most of the Group-C elements in the other species between the first and second samplings.

The maximum absolute amounts are generally found in the mid-June samples, the minimum contents, both in absolute amount and concentration being found at the end of the season. In the Group-A elements, on the other hand, further supply occurs after mid-June and both concentration and absolute amount increase as if to make up the fall in concentration during leaf growth, although it has not been established that such a requirement in fact exists.

For Zn and Na the three species of trees behave somewhat differently. In Sycamore No. 1, the Zn-concentration does not change appreciably until July or August, when a sharp fall continues until the end of September. Sycamore No. 2 (Table 1) displays a similar fall in August. In horse-chestnut, the Zn-fall takes place in late May and early June, followed by a period until October during which the concentration remains more or less constant. In all beech trees, the concentrations of Na and Zn were found to remain more or less constant until the end of September. Towards the end of the season, unlike other elements of the group, but like Pb, the Zn- and Na-concentrations increased sharply in all leaves irrespective of species and site, being least pronounced for Zn in beech at Craigie-buckler. In sycamore, the September and October leaf blade samples contained 12 and 41 ppm Zn and 0.03 and 0.07 per cent Na respectively.

The absolute amount of Zn increased until the leaves neared full development. In sycamore and horse chestnut, the absolute amount then fell steadily until the end of September, and similar falls occurred with Na. This fall in the middle of the season was absent in beech. In October, as with concentration, the absolute amounts of Zn and Na increased sharply in all trees, indicating a transference to the leaf, unlike what happened with all other elements so far considered, except Pb.

Smith, Reuther and Specht [31] reported the changes in concentration of Cu, Zn, B, and Mn in citrus leaves over a period of about sixteen months. In young leaves the results are in agreement with the present observations. Because of the evergreen nature of citrus trees, results for the mature leaves are not comparable. Leyton [19] from a review of earlier reports on forest trees, Olsen [23] for beech and Smith and Reuther [30] for citrus, have shown that the maximum concentrations of P and K occur in young leaves. This is in agreement with the present observations. At the end of the season, Deleano and Andreesco [9] and Deleano and Bordeianu [10] observed a back translocation of P and K in horse-chestnut and Salix leaves, but Olsen [23] did not find this in beech leaves.

The above observations suggest that seasonal variations in content of different elements in leaves appear to be related to the physiological importance of the elements. Most of the non-essential elements occur in Group A and behave similarly, though certain of the Group A elements are now known to be able to perform some functions of the essential trace elements, while Fe and possibly Co are essential. The essential elements, both trace and major, fall generally into two readily distinguishable groups, both of which differ from Group A in not displaying a marked concentration inflection point early in the season. Groups B and C also contain four elements, Si, Sr, Ba, and Na, whose essentiality has not been fully demonstrated.

2. Inflorescence

In Part I, in the course of investigations on sampling procedures, the effect of analysing the leaf with and without separating petioles was studied by comparing the proportional dry weights of petiole, leaf blade, and whole leaf and their contents of inorganic elements. The concentrations of the elements in sycamore inflorencences, which are presented in Table 1, are now compared with those in petiole and leaf blade: the changes during the season are also discussed. Insufficient inflorescences developed on the horse-chestnuts chosen to enable seasonal sampling to be made.

At the beginning of the season, when the inflorescence comprised only the flowers and tender stalks, the composition of the inflorescence was, for almost all elements, similar to that of leaf blade, while the composition of petiole was often considerably higher or lower, in line with the findings in Part I. This was particularly marked for Co, Fe, V, Ti, Cr, Al, Si, Ca, Sr, Ba, Mo, and K, the last five showing high petiole contents. For example, the iron contents in leaf blade and inflorescence were 218 and 254 ppm respectively, whereas that in petiole was only 63 ppm. For Ca, the concentrations in leaf blade and inflorescence were 0.20 and 0.16 per cent, as compared to 0.91 per cent in petiole. The concentrations of the other elements studied were similar in all the three organs.

Later, as the inflorescences matured and the seeds were formed, the Co, Ni, Fe, V, Ti, Cr, Pb, and Mo concentrations in inflorescence began to fall. The fall continued until about the end of August, whereafter, towards the end of the growing season, the concentration of these elements again showed some increase. Iron, for instance, fell from 254 ppm to 96 ppm and then increased again to 151 ppm. This behaviour of the inflorescence is somewhat, though not exactly, similar to that of leaf blade, where the increase starts earlier, generally towards the end of June. For P and K the concentrations in inflorescence also fall during the season, but no marked increase towards the end of the season is observed. For Ca, Sr, and Ba, on the other hand, the concentration in inflorescence, as in leaf blade, increases as the season progresses. This increase is less marked in inflorescence than in leaf blade. For example, Ca-content in inflorescence rose steadily from 0.16 to 0.98 per cent: in leaf blade the increase was from 0.20 to 2.59 per cent. Towards the end of the season the weight ratio of leaf blade to inflorescence was about 2 : 1.

From the dry weight changes and the changes in concentrations it is apparent that the absolute amounts of all elements in inflorescence increased with age, as the proportional increase in dry weight is much greater than the decrease in concentration. The dry weight of the inflorescence increased from 302 g per 1000 inflorescences at the first sampling, to over four times this value, when the inflorescences were mature in August. For the elements Co, Ni, Fe, V, Ti, Cr, Al, B, and Zn, the concentration during this period fell only to one-half to one-third. The absolute amounts of these elements in leaf blade and whole leaf also increased during this period. For the elements Ca, Sr, Ba, and Mg, the absolute amounts increased in all the three organs throughout the season.

For the elements Cu, P, and K, for which evidence of some back translocation from the leaves to the trees was reported above, the absolute amounts in inflorescence increased during the season; but they cannot be inter-related as the back translocation from the leaves occurred at the end of the season when there were no inflorescences left on the tree.

It therefore appears that in both their contents of inorganic

TABLE 2

Effect of sampling height on composition of leaf blades or whole leaves from Craigiebuckler
Samples from bottom (B), Middle (M) or top (T) of crown
Expressed as concentration in ppm or per cent of oven-dry material

Plant and plant part		Sycamore no. 3 Leaf blade				Horse chestnut no. 3 Leaf blade					Beech no. 3 Whole leaf				
Date of sampling		14 May	3 July	24 Aug	23 Sept	19 May	6 July	25 Aug	29 Sept	26 Oct	21 May	14 July	29 Aug	1 Oct	20 Oct
Wt. (g) of	B	215	435	514	551	361	701	1049	876	1058	38	76	82	94	75
1000 leaves	M	184	480	530	550	412	1490	1410	1740	1760	45	116	123	112	109
or blades	T	190	489	593	602	780	1720	2200	2190	2950	43	132	148	137	125
Co, ppm	B	0.40	0.34	0.37	0.59	0.36	0.22	0.35	0.45	0.43	0.28	0.13	0.17	0.24	0.19
	M	0.30	0.29	0.32	0.54	0.30	0.16	0.32	0.38	0.40	0.18	0.05	0.09	0.14	0.13
	T	0.18	0.16	0.13	0.25	0.24	0.14	0.21	0.20	0.23	0.15	0.05	0.10	0.18	0.15
Ni, ppm	B	2.05	1.13	1.26	1.82	2.74	1.00	1.26	1.56	1.57	2.54	1.50	1.35	1.43	1.34
	M	1.88	1.12	1.16	1.72	2.42	0.72	1.11	1.35	1.52	2.05	1.00	0.80	0.93	1.06
	T	1.52	0.71	0.57	0.94	1.91	0.66	0.80	0.68	0.87	2.10	1.00	0.90	1.18	1.04
Fe, ppm	B	308	272	328	450	274	276	403	506	462	251	133	161	197	159
	M	253	262	307	438	246	236	351	443	432	174	68	91	130	116
	T	178	152	165	242	206	215	294	286	353	135	72	105	176	120
V, ppm	B	1.50	1.35	1.90	2.85	1.26	1.22	1.61	2.57	1.78	1.34	0.49	0.88	1.17	0.80
	M	1.20	1.32	1.95	2.55	1.03	0.81	1.69	2.08	1.56	0.89	0.15	0.38	0.64	0.45
	T	0.71	0.57	0.74	1.17	0.79	0.64	0.79	1.04	0.89	0.57	0.21	0.41	0.81	0.50
Ti, ppm	B	35	35	51	67	36	30	42	66	37	24	10	13	20	13
	M	28	32	48	51	22	20	43	49	35	17	4	7	12	9
	T	15	13	19	25	17	18	20	26	20	12	4	7	16	8
Cr, ppm	B	0.89	0.80	1.47	1.64	0.71	0.97	1.65	2.38	1.81	0.59	0.36	0.61	1.01	0.75
	M	0.76	0.71	1.50	1.50	0.61	0.71	2.08	2.03	2.02	0.42	0.21	0.42	0.88	0.64
	T	0.47	0.44	0.64	0.82	0.61	0.61	1.31	1.40	0.96	0.23	0.23	0.56	1.25	0.71
Pb, ppm	T	2.7	1.9	2.7	3.2	4.7	3.3	5.6	4.5	8.1	4.0	2.0	2.1	2.6	3.2
	M	2.9	1.8	2.7	3.5	3.5	2.2	2.6	3.8	6.0	2.6	1.3	1.5	2.1	2.3
	T	2.9	1.6	1.9	2.6	3.6	2.0	2.4	2.5	3.3	1.4	1.3	1.7	2.7	3.0
Al, ppm	B	408	461	546	560	358	382	521	807	690	328	140	174	254	251
	M	251	426	552	652	241	266	456	639	597	228	94	147	211	207
	T	212	214	238	284	270	185	298	340	313	197	72	120	190	212
Mn, ppm	B	55	45	56	63	161	168	179	158	186	240	293	274	337	220
	M	51	48	52	58	146	199	185	226	160	259	194	250	280	242
	T	43	42	36	49	145	210	128	134	115	194	216	236	274	212

Cont.

TABLE 2 (*Continued*)

Plant and plant part		Sycamore no. 3 Leaf blade				Horse chestnut no. 3 Leaf blade					Beech no. 3 Whole leaf				
Date of sampling		14 May	3 July	24 Aug	23 Sept	19 May	6 July	25 Aug	29 Sept	26 Oct	21 May	14 July	29 Aug	1 Oct	20 Oct
B, ppm	B	27	41	65	76	16	28	24	26	25	26	38	40	33	26
	M	27	46	65	84	16	22	23	25	26	28	37	37	33	25
	T	30	47	68	86	15	15	14	16	16	25	36	36	35	24
Si, %	B	0.22	0.35	0.61	0.77	0.12	0.22	0.38	0.50	0.61	0.16	0.26	0.31	0.37	0.43
	M	0.16	0.35	0.60	0.66	0.09	0.20	0.33	0.42	0.55	0.13	0.19	0.24	0.33	0.40
	T	0.15	0.33	0.61	0.74	0.11	0.16	0.27	0.29	0.32	0.09	0.22	0.26	0.33	0.38
Ca, %	B	0.46	1.11	1.86	2.27	0.29	0.74	0.94	1.01	1.17	0.43	0.79	0.88	1.10	0.88
	M	0.45	1.15	1.83	2.36	0.29	0.74	1.03	1.05	1.27	0.41	0.47	0.66	0.82	0.76
	T	0.36	0.95	1.72	2.36	0.32	0.70	0.86	1.21	1.05	0.38	0.47	0.55	0.59	0.51
Sr, ppm	B	21	27	47	58	12	20	22	25	30	18	20	24	31	20
	M	22	31	44	67	15	20	24	29	25	18	13	17	22	19
	T	18	24	40	61	14	23	26	29	27	15	14	16	18	17
Ba, ppm	B	23	22	32	36	12	16	16	20	24	83	112	97	117	91
	M	23	24	29	43	14	19	20	26	25	85	72	67	90	81
	T	17	20	23	33	13	18	21	27	27	70	69	62	70	73
Mg, %	B	0.22	0.18	0.22	0.22	0.31	0.31	0.29	0.28	0.28	0.20	0.20	0.19	0.23	0.19
	M	0.23	0.18	0.20	0.23	0.28	0.30	0.29	0.23	0.29	0.22	0.13	0.15	0.18	0.17
	T	0.19	0.14	0.15	0.16	0.29	0.26	0.22	0.17	0.22	0.23	0.13	0.13	0.13	0.14
Cu, ppm	B	15.5	8.2	8.0	7.2	15.6	9.6	7.2	5.9	5.5	13.9	6.9	6.0	4.7	4.2
	M	15.8	8.9	7.4	7.6	16.5	8.3	6.5	5.5	5.0	13.0	4.9	4.4	3.4	3.7
	T	15.1	8.9	6.7	5.7	13.5	8.2	4.6	4.1	3.1	12.3	5.1	4.2	3.5	3.3
Mo, ppm	B	0.32	0.11	0.10	0.14	0.49	0.28	0.26	0.30	0.33	0.16	0.09	0.08	0.09	0.07
	M	0.42	0.12	0.11	0.13	0.83	0.43	0.31	0.33	0.32	0.12	0.06	0.06	0.06	0.05
	T	0.31	0.10	0.07	0.08	0.50	0.44	0.33	0.25	0.28	0.10	0.06	0.06	0.08	0.05
Zn, ppm	B	31.4	18.7	22.5	22.9	38.0	16.5	15.1	10.4	32.3	33.2	25.5	29.5	30.4	35.1
	M	35.4	23.2	17.7	22.7	42.3	15.9	9.1	12.3	35.4	29.0	21.1	23.1	26.5	32.8
	T	30.5	27.3	20.5	20.8	36.5	13.3	11.9	9.1	20.4	32.5	19.2	23.4	20.5	25.4
P, %	B	0.40	0.16	0.14	0.14	0.59	0.24	0.18	0.17	0.13	0.35	0.18	0.17	0.20	0.22
	M	0.43	0.15	0.13	0.13	0.54	0.22	0.14	0.11	0.10	0.35	0.14	0.13	0.18	0.26
	T	0.42	0.17	0.14	0.13	0.52	0.19	0.13	0.17	0.08	0.39	0.14	0.15	0.20	0.28
K, %	B	1.97	1.06	1.08	1.21	2.32	1.63	1.29	1.27	0.80	1.46	0.94	0.84	0.70	0.64
	M	2.09	1.02	1.04	1.21	2.20	1.38	1.06	0.98	0.52	1.38	0.75	0.74	0.62	0.59
	T	2.23	0.97	1.00	0.97	2.02	1.24	0.87	0.79	0.46	1.52	0.73	0.74	0.53	0.50
Na, %	B	0.07	0.04	0.03	0.03	0.13	0.10	0.17	0.14	0.20	0.15	0.12	0.15	0.19	0.28
	M	0.08	0.04	0.03	0.03	0.09	0.09	0.18	0.15	0.22	0.10	0.07	0.14	0.16	0.22
	T	0.06	0.03	0.01	0.02	0.09	0.08	0.11	0.14	0.19	0.10	0.10	0.15	0.22	0.24

elements and their seasonal variation, the behaviour of inflorescences of sycamore follows that of leaf blades rather than petioles. There is no evidence of transfer from the leaf to the inflorescence at any stage.

VARIATION WITH SAMPLING HEIGHT

In forest trees, appreciable differences in the foliar content of inorganic elements can occur with variation in height of the sampling position (Leyton [19]). White [34] observed considerable variation in the K-concentration in pine needles collected from different whorls of 12-year-old trees. The effect of sampling height may be related to the processes effecting translocation; and in this respect a deciduous species of annual growth habit may differ considerably from an evergreen coniferous species.

The results presented in Table 2 indicate the magnitude of the effect of sampling height on composition. The elements appear to fall into groups similar to those found for seasonal changes, in particular the elements of Group A differ markedly from Groups B and C which can be considered together. Results are available for four or five sampling dates throughout the growing season. Only concentration results are presented: absolute contents can readily be calculated from the dry weight values.

Group A: Co, Ni, Fe, V, Ti, Cr, Pb and Al. The feature of this group is that the concentrations are always considerably lower at the top than at the bottom of the tree. This is observed in different degree in all three species of trees, and was found also in petioles and inflorescences (Guha [13]). Over the whole season, the concentration in the bottom samples was generally around twice as much as that in the top samples in sycamore, with rather smaller differences in horse-chestnut and beech for a few of the elements.

In sycamore, the average dry weight of a leaf was only some 10 per cent higher at the top than at the bottom, and was not enough to explain by dilution the lower concentration at the top. In horse-chestnut the increase in dry weight of two to three times was greater than the decrease in concentration. That the effect is not related to dilution alone is confirmed by the fact that the relative decrease at the top was not the same for all elements. Although the dilution effect cannot be completely ignored, the results suggest a physiological preference for a higher concentration of the elements of Group A in the leaves at the bottom than in those at the top of the trees.

Groups B and C: Mo, Zn, Cu, Mn, B, Si, Ca, Sr, Ba, Mg, P, K and Na: The first six elements show only slight (seldom more than 20 per cent), if any, reduction in concentration with height. In May, there was little significant difference between the bottom and top samples with respect to concentration. After the leaves have matured, a somewhat lower concentration and a higher dry weight is generally found in the top samples. The fall in concentration is small and less than could be accounted for by the dilution factor, suggesting a compensating increased supply to the upper leaves. In horse-chestnut leaf blades, where dry weight difference was greatest, the average weights over the season per 1000 blades of the bottom and top samples were 809 g and 1968 g respectively, but average concentrations were as close as 0.33 and 0.36 ppm Mo, 22.4 and 18.2 ppm Zn, 8,76 and 6.68 ppm Cu, 170 and 146 ppm Mn, 24 and 15 ppm B, and 0.37 and 0.23 per cent Si. It therefore appears that these six elements are more readily transported upwards than the elements in Group A. Compared with Group A, the supply of these elements to the plant parts sampled is more uniform at all heights.

In sycamore and horse-chestnut, the concentrations of Ca, Sr, and Ba were fairly constant at all heights. Increased dry weight at the top, most marked in horse chestnut leaf blade, does not seem to have affected the concentration. The avarage values for the bottom and top leaf blades of sycamore are respectively 1.42 and 1.35 per cent Ca, 38 and 36 ppm Sr, and 28 and 23 ppm Ba; corresponding values for horse-chestnut leaf blades are 0.83 and 0.83 per cent Ca, 21 and 24 ppm Sr, and 18 and 21 ppm Ba. In beech leaves, on the other hand, the concentrations of Ca, Sr and Ba show a definite fall with height. This is observed almost throughout the season, but is more apparent towards the end. The average concentrations for the bottom and top leaves are respectively 0.81 and 0.50 per cent Ca, 23 and 16 ppm Sr, and 101 and 69 ppm Ba.

The Mg-concentration, in almost all cases, falls with height; the fall, however, is always quite small, seldom exceeding 20 per cent, much less than in Group A.

At the beginning of the season, P- and K-levels were similar at all heights. In sycamore and beech, this uniformity in concentration persisted throughout the season. In some beech samples, the concentration of P was even slightly higher at the top than at the bottom. In horse-chestnut, a fall in concentration with height was observed later in the season. The very marked increase in dry weight from bottom to top later in the season may in part explain this, as the supply of these elements appears to stop soon after the leaves are fully developed. No marked effect related to sampling height was observed in the Na-content of the samples.

The results suggest that, in the species studied, only for Group A elements is the proper selection of sampling height of great significance, as two- to three-fold variation in concentration may occur between extreme sampling heights. In the other groups the variations seldom exceed 20–30 per cent. It is interesting to recall that in the duplicate horse–chestnut leaf blade samples taken on 6th July which, as mentioned in Part I, showed a marked difference in dry weight, it was the Group-A elements which showed the greatest analytical differences. Some of the essential elements showed no significant differences.

DISCUSSION AND CONCLUSIONS

The foregoing results help to throw more light on the movement of elements in trees. It is known (Biddulph [4]) that in the phloem Ca is immobile, P is very mobile, whereas the mobility of Fe is determined by several factors including Fe-content, P-content and pH of the growing medium. It is also known that mobile elements, such as P, are not accumulated in mature leaves, but are transferred via the phloem and then through xylem to the growing parts. Elements such as Ca, on the other hand, which are not freely mobilized in the phloem cannot be so transported, but gradually accumulate in mature leaves. It has been seen in the present investigation that Ca, Sr, Ba, B, Si, and almost all the non-essential metals were likewise accumulated in mature leaves, suggesting that these elements are not easily mobilized in the phloem. The elements P, K, Zn, Cu, Na, and Mg, on the other hand, did not show accumulation in mature leaves, although Zn and Na showed a sudden increase at senescence. It is possible that, like P, these elements are mobilized from the mature leaves via the phloem and then through the xylem, to the younger tissues near the apical meristems.

The above observations on mobility are almost always supported when considered from another aspect, the transport of the elements to different heights in the crown. It was observed that the supply of most of the Group-A elements (the non-essential heavy metals) and in beech also Ca, Ba and Sr to the leaves was smaller at the top than at the bottom of the tree, whereas the supply of P, K, Zn, Cu, Na, Mn, and Mg was such as to give almost uniform concentrations at all heights. It is interesting to note that the dilution factor due to increase in dry weight, irrespective of when and where it occurs, always affects the concentration of the Group-A elements only, but not of the Group-B or -C elements.

In the behaviour of the various elements there is little direct correlation with the properties associated with periodic classification or with chemical affinity. No factor other than biological requirement would appear to explain the similar trends exhibited by K and P or by B and Si. Only occasionally, as with Ca and Sr, does chemical similarity appear to be a relevant factor in overriding biological effects.

There would appear to be some mechanism operative at the time of development of senescence whereby there is a tendency for a movement of many of the elements out of the leaf back into the tree. There is no increase in petiole content at this stage. Marked exceptions to this trend are Pb, Zn and Na, which accumulate in the leaf at this stage. A similar increase in Pb content at senescence has been observed in pasture herbage leaves, and it is possible that some very significant alteration in transport conditions or membrane equilibrium may occur at this time. The absolute amounts concerned are about 2000 times as great for Na and 20 times as great for Zn as for Pb, the amounts being of the order of 2 mg Na, 20 μg Zn and 1 μg Pb per gram of dry leaf.

Inspection, in Fig. 1–21, of the variations in concentration during the period June to September, the season normally chosen for diagnostic sampling, indicates that this includes for most elements a spell of quite rapid change involving a several-fold increase or decrease in concentration. Absolute amounts in the leaf are seldom used for diagnostic purposes. During this period the least variable elements are probably Mo, Mg, P, K and Na, although the last two show appreciable variation in horse-chestnut. For the other elements it is doubtful if the content is sufficiently constant for ad hoc samples taken during this period to be used diagnostically. Early and late in the season the variations appear to make precisely controlled sampling unfeasible for all elements. Recourse to good and poor comparison samples is possibly preferable in such instances, but there is always the possibility that a nutritional abnormality may alter the rate of development and so give discordant results with elements which are not concerned with the disorder.

The findings possibly suggest some factors relevant to the fertilisation programme of plantation crops and to remedial measures designed to overcome deficiencies. Thus it would appear that, at least in healthy trees, the total leaf content of such elements as Cu, Mo, Zn, P and K has been supplied to the leaf when or soon after it leaves the bud and the efficacy of late supplementation by soil addition may be questionable. This aspect could be less important in the essential elements Fe, Mn, B, Ca, and Mg, which show a steady increase in the leaf throughout the season. This would also apply to such elements as Si, Sr, Ba and those Group-A elements for which no requirement has so far been established. Before definite conclusions can be drawn, information is needed regarding the rate of root uptake from the soil and transfer from the root through the trunk and branches to the green parts.

SUMMARY

The contents of 21 trace and major constitutents in the leaves or leaf blades of three species of deciduous trees, sycamore, horse-chestnut and beech, have been studied at regular intervals throughout the season. The various elements can be grouped into three categories: (A) Co, Ni, Fe, V, Ti, Cr, Pb and Al, (B) Mn, B, Si, Ca, Sr, Ba, and Mg, and (C) Cu, Mo, Zn, P, K, and Na. The Group-A elements, including most of those whose essentiality has not been established, together with Fe, show a fall in concentration in the leaf early in the season, probably as a result of dilution due to increase in dry weight as the absolute amount per leaf rises slightly, followed by steady rise until senescence, when a further fall occurs. In Group B, in which Si Sr, and Ba are considered non-essential, there is a continual rise both in concentration and absolute content until late in the season. In Group C, all the elements of which, except possibly Na, are essential, there is generally a gradual fall followed by a period when the content remains relatively constant, although Na and Zn, together with Pb from Group A, show an unexpected increase at senescence.

Group-A elements show a decrease in concentration with height, which can only partly be explained by dilution due to increase in dry weight with height, while Group-B and -C elements are relatively constant at all heights. Results are also presented for some petioles and inflorescences. There does not appear to be any systematic transfer from leaf to inflorescence at any stage of growth. The only elements whose contents could be used systematically for diagnostic purposes appear to be those in Group C, during the mid-season period of constant concentration.

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REFERENCES

1 Arnon, D. I., Growth and function as criteria in determining the essential nature of inorganic nutrients. *In:* Mineral Nutrition of Plants, ed. Truog, 313–342. Madison: Univ. of Wisconsin Press (1951).

2 Bertrand, G. and Rosenblatt, M., Recherches sur les variations de la teneur en manganese des feuilles avec l'age. Ann. Inst. Pasteur **36**, 494–501 (1922).

3 Bertrand, G. and Silberstein, L., Sur les variations de la teneur en bore des feuilles avec l'age. Ann. Inst. Pasteur **64**, 87–89 (1940).

4 Biddulph, O., The translocation of minerals in plants. *In:* Mineral nutrition of Plants, ed. Truog, 261–278. Madison: Univ. of Wisconsin Press (1951).

5 Biddulph, O. and Markle, J., Translocation of radio-phosphorus in the phloem of the cotton plant. Am. J. Botany **31**, 65–70 (1944).

6 Bollard, E. G., Transport in the Xylem. Annu. Rev. Plant Physiol. **11**, 141–166 (1960).

7 Bolle-Jones, E. W. and Mallikarjuneswara, V. R., The beneficial effect of cobalt on the growth of the rubber plant (*Hevea brasiliensis*). Nature (Lond.) **179**, 738–739 (1957).

8 Cain, J. C., Observations on antagonistic effects in leaf analysis. *In:* Mineral Nitrotion of Trees – A Symposium, Duke University: School of Forestry, Bull. **15**, 63–70 (1959).

9 Deleano, N. T. and Andreesco, M. I., Beiträge zum Studium der Rolle und Wirkungsweise der Mineral- und organischen Stoffe im Pflanzenleben. I. Der quantitative Stoffwechsel der Mineral- und organischen Substanze in den *Salix fragilis* – Blättern während ihrer Entwicklung. Beiträge Biol. Pflanzen **19**, 249–286 (1932).

10 Deleano, N. T. and Bordeianu, C., Beiträge zum Studium der Rolle und Wirkungsweise der Mineral- und organischen Stoffe im Pflanzenleben. II. Der quantitative Stoffwechsel der Mineral- und organischen Substanzen in der Blättern und geschalten samen von Aesculus hippo-castanum während ihrer Entwicklung. Beiträge Biol. Pflanzen **20**, 179–197 (1933).

11 Epstein, E. and Leggett, J. E., The absorption of alkaline earth cations by barley roots: Kinetics and mechanism. Am. J. Botany **41**, 785–791 (1954).

12 Gauch, H. G., Mineral nutrition of plants. Annu. Rev. Plant Physiol. **8**, 31–64 (1957).

13 Guha, M. M., The study of the trace-element uptake of deciduous trees. Ph. D. Thesis, Aberdeen University (1961).

14 Guha, M. M. and Mitchell, R. L., The trace and major element composition of the leaves of some deciduous trees I. Sampling techniques. Plant and Soil **23**, 323–338 (1965).

15 Hewitt, E. J., The role of mineral elements in the activity of plant enzyme systems. *In:* Encyclopedia of Plant Physiology, ed. Ruhland **IV**, 427–470 (1958).
16 Hoagland, D. R., Lectures on the Inorganic Nutrition of Plants. The Chronica Botanica Co., Watham, Mass. (1944).
17 Kramer, P. J. and Kozlowski, T. T., Physiology of Trees, 248–259. McGraw-Hill Book Co. Inc., New York (1960).
18 Langston, R., Distribution patterns of radioisotopes in plants. Proc. Am. Soc. Hort. Sci. **68**, 370–376 (1956).
19 Leyton, L., Mineral nutrient relationships of forest trees. Forestry. Abstr. **9**, 399–408 (1948).
20 Lundegårdh, H., Leaf Analysis, Hilger and Watts Ltd., Lond., translated by R. L. Mitchell (1951).
21 Mitchell, H. L., Trends in the nitrogen, phosphorus, potassium and calcium content of the leaves of some forest trees during the growing season. Black Rock For. Paper **1** (6), (1936).
22 Olsen, C., Water culture experiments with higher green plants in nutrient solutions having different concentrations of calcium. Compt. Rend. Lab. Carlsberg Ser. Chim. **24**, 69–97 (1942).
23 Olsen, C., The mineral, nitrogen and sugar content of beech leaves and beech leaf sap at various times. Compt. Rend Lab. Carlsberg Ser. Chim. **26**, 197–230 (1948).
24 Olsen, C., The significance of concentration for the rate of ion-adsorption by higher plants in water culture. Compt. Rend. Lab. Carlsberg Ser. Chim. **27**, 291–306 (1950).
25 Pirson, A., Functional aspects of mineral nutrition of green plants. Annu. Rev. Pl. Physiol. **6**, 71–114 (1955).
26 Reisenauer, H. M., Co in N fixation by a legume. Nature, (Lond.) **186**, 375–376 (1960).
27 Richards, F. J. and Berner, E. Jr., Physiological studies in plant nutrition. XVII. A general survey of the free amino acids of barley as affected by mineral nutrition with special reference to potassium supply. Ann. Botany (Lond.) N.S. **18**, 15–33 (1954).
28 Robertson, R. N., Mechanism of absorption and transport of inorganic nutrients in plants. Ann. Rev. Pl. Physiol. **2**, 1–24 (1951).
29 Robertson, R. N., The uptake of minerals. *In:* Encyclopedia of Plant Physiology, ed. Ruhland **IV**, 243–279 (1958).
30 Smith, P. F. and Reuther, W., Seasonal changes in Valencia orange trees. I. Changes in leaf dry weight, ash, and macro-nutrient elements. Proc. Am. Soc. Hort. Sci. **55**, 61–72 (1950).
31 Smith, P. F., Reuther, W. and Specht, A. W., Seasonal changes in Valencia orange trees. II. Changes in micro-elements, sodium and carbohydrates in leaves. Proc. Am. Soc. Hort. Sci. **59**, 31–35 (1952).
32 Stiles, W., Essential micro-(trace-) elements and other elements. *In:* Encyclopedia of Plant Physiology, ed. Ruhland **IV**, 558–614 (1958).
33 Stout, P. R., and Hoagland D. R., Uptake and lateral movement of salt in certain plants as indicated by radioactive isotopes of potassium, sodium and phosphorus absorbed by roots. Am. J. Botany **26**, 320–324 (1939).
34 White, D. P., Variation in the nitrogen, phosphorus and potassium contents of pine needles with season, crown position, and sample treatment. Soil Sci. Soc. Am. proc. **18**. 326–330 (1954).