

LEAF ANALYSIS
AS A MEANS OF ASSESSING THE
NUTRIENT STATUS OF DECIDUOUS FRUIT TREES AND VINES
IN THE WESTERN CAPE PROVINCE



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November, 1958

THESIS

submitted for the degree of

D.Sc. (Agric.)

in the

Faculty of Agriculture

UNIVERSITY OF STELLENBOSCH

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Agricultural Chemistry

ACKNOWLEDGEMENTS.

The writer wishes to thank the following:

Prof. Dr. R.I. Nel, Director of the Western Province Fruit Research Station, for granting permission to use the facilities and data obtained at this Institute for the purpose of this thesis and for his interest in the work.

Prof. Dr. I. de V. Malherbe, for kindly checking the manuscript and for helpful suggestions.

Dr. W.J. Pienaar and Mr. A.J. Buys and their assistants who carried out the spectrographic and chemical analyses of the leaf samples during the investigation.

Messrs. A.S. Viljoen and P.J.J. Prins, Extension Officers of the W.P.F.R.S. at Worcester and Robertson, for their assistance in selecting suitable vineyards and orchards for leaf sampling in their areas.

His wife, who through her encouragement, interest and many sacrifices, enabled the writer to complete this thesis.

5th Nov. 1958.

RESUME.OBJECTIVE.

High economic production has ever been the aim and aspiration of the agriculturist and no less that of the fruit farmer. In striving towards this aim the latter has for a long time been at a disadvantage with regard to control of his nutritional programme. Even on naturally fertile soil, the question continually arises as to what the correct fertilizer treatment should be to maintain high productivity and how such a decision can be arrived at. A satisfactory answer to these questions could have been obtained from fertilizer trials if it was not such a difficult matter, in view of the extensive and long-term nature of such trials with fruit trees, to establish a sufficient number for each fruit species on different soil types and under different climatic conditions.

Efforts to find a new approach to the problem have turned attention to the plant itself and its chemical make-up as affording the best index of its nutritional requirements. Intensive work in this direction has resulted in the evolution of a new tool in agriculture, the technique of diagnostic leaf analysis or "foliar diagnosis" as originally proposed by Lagatu and Maume in France and Thomas in U.S.A. A review of the literature is presented indicating the prodigious amount of research which has been applied to studies of the relationship between plant response and nutrient supply in terms of plant composition. Agriculturists have been quick to recognize the potentialities of leaf analysis as a practical guide in nutritional problems and advisory services based on foliar analysis have already been established for certain crops overseas.

The experimental basis for formulating such a scheme for deciduous fruit in the Western Cape Province is provided by the factual evidence presented in this thesis.

THE TECHNIQUE.

The technique of diagnostic leaf analysis comprises sampling of leaves, preparation of sample for analysis and the analysis itself followed by interpretation of the analytical results by comparison with previously determined nutritional standards. Numerous factors were found to influence the final composition of the leaf sample as determined by analysis, such that strict adherence to a standardized procedure through all phases of sampling and preparation of leaf samples for analysis is required to eliminate or reduce errors likely to cause misleading interpretations. Experimental data are presented suggesting how the leaf sample should be selected on a tree and how it should be handled, cleaned, dried, ground and stored to reduce sampling and other errors.

The final procedure as adopted eliminates most of the potential sources of experimental error but two unavoidable sources of error remain to be accounted for, that due to tree variation and seasonal effect. The variation in leaf composition from tree to tree was found to be very considerable, so that sampling from a large enough group of trees (6 to 10) to reduce the error involved is essential in order to obtain leaf data which correctly reflects the nutrient status of the portion of the orchard concerned. Secondly, on the grounds of marked consistency found in different fruit species as to seasonal and year to year variation in mineral nutrient concentration, correction factors have been formulated and are suggested as a means of overcoming these sources of error.

THEORETICAL BASIS.

A diagnosis of the nutrient status in terms of the analytical results as finally determined is obtained by comparison of the data with previously established leaf composition standards of reference and by correct interpretation of the deviations from these standards.

(iii)

The theoretical basis for setting up these index values is discussed. The criterion used is based on the concept of Optimum Values which adequately integrates the known relationships between plant response and nutrient supply in terms of internal nutrient concentration. A modification of this concept is proposed to the effect that for maximum growth and yield there exists an optimum range of nutrient concentrations with upper and lower limits for each of the functional elements, and that within this range the interrelationship between the individual nutrient elements is also optimal.

Since no local fertilizer trials with deciduous fruit trees are available and only one for grapes, data from highly productive plants in commercial orchards and vineyards were used to determine the upper and lower limits of the "optimum range", on the following premise. If leaf analysis data are available from a sufficient number of high performance orchards in different localities representing a wide range of nutrient supply and environment, the highest and lowest values obtained may be considered to represent a close approximation of the limits of the range required for optimum performance. It is contended that index values obtained in this way must be of practical value in assessing the nutrient status of fruit trees. It is further postulated that the lower limits for the micro-nutrients and even for magnesium may be justifiably adjusted according to the concentration levels associated with symptom expression.

INDEX VALUES.

The necessary data for determining standards of leaf composition were obtained from leaf analysis surveys of orchards and vineyards and from a grape fertilizer experiment in the Western Cape Province. Visual symptoms of prevailing nutritional disorders are described (supplemented by photographic illustrations) and their relation to leaf composition indicated. Tentative

index values have been determined on the basis indicated for each fruit species, apple, pear, peach, apricot, plum, prune and grapes. These nutritional levels comprise upper and lower limits for the nutrients N, P, K, Ca, Mg, Mn, Fe and Cu, as well as the upper limits for B and Na.

DIAGNOSTIC INTERPRETATIONS.

Assessment of the nutrient status in terms of these index values suggests that many orchards and vineyards in the Western Cape Province, particularly prune, apricot and grapes, are suffering from malnutrition in some form and are likely to show a marked response to nutritional treatment as suggested by foliar diagnosis.

The use of diagnostic leaf analysis constitutes an important advance in dealing with orchard problems in that an immediate decision is possible regarding nutrient status and related aspects such as selection of suitable sites for fertilizer trials and adjustment of the fertilizer programme.

(v)

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I N T R O D U C T I O N

The use of plant analysis has become firmly established as a means of studying the nutrient status of plants. Particular interest has centred on the potentialities of plant analysis as an aid in investigating nutritional disorders and determining the nutrient requirements of crop plants, as may be gathered from the comprehensive review of the subject by Goodall and Gregory (85). Attention has gradually shifted from the whole plant as a subject for analysis by early workers in this field, to the green leaf which in recent years has become the main subject for investigation in view of its sensitivity to change in nutrient supply and the convenience in sampling.

A notable contribution to our knowledge of the fundamental relationship between leaf nutrient content and plant growth has been presented by Lundegardh (118) who claimed that "growth is determined by the concentration of a nutrient in the green parts of a growing plant." After a careful study of the physiological processes governing the mechanism of nutrient absorption and utilization during growth, Lundegardh concluded from pot culture and field experiments that "fundamental plant physiological investigations have shown that leaf analysis, properly handled and granted certain assumptions, provides a usable reflection of plant growth, and that it has an adequate scientific basis as a method for the determination of manurial requirements, since at the same time it reflects the supply of nutrients from the soil."

The practical application of leaf analysis as a means of assessing the nutrient status and nutrient requirements for optimal growth and yield of fruit trees, as with annual plants, depends on how closely nutrient supply and nutrient concentration in the leaf is related to growth or yield response and on how

well this relationship can be reduced to a relatively simple interpretation as for instance by the use of analytical reference values. A further criterion would be that nutritional standards set up on this basis, if they are to be of practical value, should be applicable under a wide range of environmental conditions.

Promising advances along these lines have already been achieved in the form of tentative leaf analysis standards for citrus and for some of the deciduous fruits. Standards for the latter are still sketchy and inadequate, and since the approach is largely empirical their accuracy and general applicability, at least in a particular region, can only be established when sufficient data become available. It is generally accepted that diagnostic leaf analysis provides a most useful if not essential contribution to supplement information obtainable from soil tests and examination of the environmental conditions affecting trees suffering from suboptimal nutrition.

It is proposed in this investigation after reviewing the evidence available on nutrient content of plant tissues and the techniques employed in plant analysis diagnosis, to describe the steps taken to formulate an acceptable leaf analysis technique and to determine standards of reference for diagnosis of the nutrient status of deciduous fruit trees and vines in the Western Cape Province. The nutrient elements to be considered are nitrogen, phosphorus, potassium, calcium, magnesium, iron, manganese, copper, boron and zinc, as well as chlorine and sodium in certain cases.

P A R T I

REVIEW OF LITERATURE

1. FACTORS AFFECTING THE NUTRIENT CONTENT OF PLANT TISSUE.

The nutrient-element content of plant tissue is subject to variation due to differences in supply of nutrient salts from the soil as well as to the nature of the plant and its root system, and the environment in which the plant grows (206). Marked differences in plant composition are brought about by these factors (152, 203) and in order to use plant analysis data correctly for diagnostic purposes it is important to know what influence they have on the relationship between plant nutrient content and yield (191).

1.1 SOIL ENVIRONMENT.

The quantity of available nutrients in the soil which a plant can absorb is limited to the distribution and absorbing capacity of the root system which in turn is influenced by the water supply, aeration, temperature and physical condition of the soil (216, 120). Assuming a favourable root environment, absorption of available plant nutrients takes place at a rate depending on the supply, ion exchange reactions, soil reaction (pH) and competition by other plants if present. Plant composition reflects the net uptake and as such cannot indicate the potential nutrient supply in the soil beyond the reach of the root system nor of that in the root area when other factors, physical or chemical, poor aeration or water depletion, restrict absorption by the roots.

The absolute dependence of plant growth on favourable soil moisture conditions and the serious consequences which follow when plants are subjected to either extreme drought or waterlogged conditions are self evident. In the intermediate range of water supply the availability of nutrients is also

significantly influenced by the moisture content. An increase of the water supply accelerates leaching losses of nitrates, chlorides, calcium, magnesium, potassium and sodium (210). The supply of oxygen is usually reduced, decreasing the rate of respiration and thereby the rate of nutrient absorption. The tendency to create anaerobic conditions promotes reduction processes and accumulation of reduced forms of manganese and iron in acid soil which may have toxic effects. Nitrification is reduced, ^{denitrification sets in} and added nitrogen is assimilated and fixed by anaerobic micro-organisms. According to Boynton, magnesium deficiency in apple is common in wet years (210). Burtch et al found that a high soil moisture level together with low soil temperature is the condition most conducive to the development of iron deficiency chlorosis (39).

Water depletion favours fixation reactions resulting in reduced uptake particularly of potassium and phosphorus and an increase in the magnesium content whereas nitrogen accumulates in the plant owing to reduced rate of growth (210). Lundegardh (118) found that the nitrogen and calcium content of cereal plants tended to increase in a dry season. Boron deficiency symptoms are commonly induced during a drought on soils normally adequately supplied with boron (210, 228).

Alternate wetting and drying of ^{Illite} soils treated with potassium salts causes rapid fixation of potassium in a non-replaceable form whereas little fixation of this kind takes place when the soils are kept continuously moist (210). According to Lilleland and Brown (114), however, potassium absorption by peach trees was only significantly reduced under extreme conditions when soil moisture was below the wilting point for long periods each year.

1.2 SOIL MANAGEMENT.

The nutrient composition of plants is influenced by fertilizer applications to the soil and by changes in cultural practices such as liming, crop rotation, drainage, tillage, irrigation, mulching, cover-cropping and incorporation of organic materials (206, 152). The addition of fertilizers to the soil may or may not increase the leaf content of the added element depending on the level of these nutrient elements and other elements present in the plant. Furthermore the mere addition of an element to the soil is no guarantee that it is being absorbed by the plant as the roots may be incapable of securing it.

Fertilizer application may also affect the leaf concentration of nutrient elements other than those added. The results of numerous fertilizer experiments serve to indicate the general trend of such effects in various fruit and crop plants:-

Heavy nitrogen dressings have been found to decrease the absorption by the plant of P^x (47, 96, 136, 158, 215, 223), K (47, 136, 158, 215, 223), Cu (109, 135) and Zn (158), and to increase that of Mg (47, 96, 158, 215, 223), Ca (47, 135, 158, 223) and Mn (14a). Application of ammonia-N induced Mg deficiency on acid soils whereas nitrate-N increased Mg absorption (135).

An increase of the phosphorus supply has been found to reduce the absorption of N (72, 105, 136), K (72), Fe (22, 61, 65, 80), Cu (16), Zn (16, 215), Mn (23) and to increase that of Ca (72), Mo (135). Reuther et al (157) also found that high phosphate application induced Cu deficiency but that Zn and Mn absorption was increased.

Potassium application reduced the absorption of Mg (46, 96, 135, 136, 158, 215), Ca (46, 96, 135, 136, 145, 158),

^x For the sake of brevity the nutrient elements are designated by their chemical symbols.

Zn (158, 176), Cu (176), B (176). A high K supply usually increases the Fe content (22, 102, 215) but it has also been found to cause Fe deficiency chlorosis in apple and pear (116). Mn absorption was found to be decreased (215) or increased (23) by K application.

A high calcium supply decreased the absorption of N (105), P (105), Mg (105, 135, 136), Mn (176, 215), B (176). Ca application also decreased K absorption (105, 136, 145) but it has also been reported that it increased the K content (135, 139).

Magnesium applications reduce the uptake of Ca (46, 215), K (46), B (176), Mn (176), and increase that of P (132).

The heavy metals Ni, Cu, Co, Cr, Mn and Zn at a high level of supply induce Fe deficiency (39, 61, 96, 159, 184). According to Smith (186) a high Cu, Zn, Mn level in sand cultures increased the uptake of K and decreased that of Ca and Mg in citrus. A high Mn level of supply decreased the absorption of Ca (169, 176) and Mo (109, 135).

Liming through its effect on the soil reaction decreases the availability and absorption of Mn (23, 154), Zn (154, 222), B (154), Cu (154), but increases that of Mo (2, 64), N and P.

Addition of sulphur or accumulation in the soil following S treatment of fruit trees, increases soil acidity and may increase the availability of Mn and Fe (215) and Cu. Boynton and Embleton (33) found that S treatment resulted in lower Ca and Mg levels in apple leaves and that soil Mg was lost more rapidly than K by leaching, resulting in Mg deficiency symptoms. According to Drosdoff and Lagasse (69) the addition of S greatly improved the effectiveness of dolomite in correcting Mg deficiency in tung.

A permanent grass cover crop was found to depress the absorption of N by apple and pear trees whereas that of K and

P was increased (24, 152).

1.3 CLIMATIC CONDITIONS.

Growth and fruit production are directly affected by temperature, light, humidity and rainfall. When growth is improved by favourable weather conditions the plants utilize more nutrients for the enhanced growth and fruiting. The soil may thus fail to meet the nutrient requirements of the crop in a favourable season even though adequate when the growth rate is low (206). As indicated by Nightingale (140), conditions of temperature and light directly affect photosynthetic activity and thereby the rate of assimilation and accumulation of carbohydrates which may affect nutrient concentration in the dry plant material if absorption of nutrient salts does not keep pace with carbohydrate accumulation. Wallace (215, 216) has reported that excessive light may intensify deficiencies of N, Zn, Mg and Fe.

The intensity and distribution of the rainfall through its effect on soil moisture content may influence the availability and absorption of various nutrient salts by plants as indicated in the preceding Section 1.2. Irrigation will of course partly offset the effects which would otherwise have been experienced in a dry season.

Apart from soil moisture relationships, climatic factors are largely beyond the control of man, and their influence on plant composition may possibly limit the applicability of analytical index values to a particular region or to regions with more or less similar climatic conditions. However, Lundegardh (118) claimed that variations in the climatic conditions from year to year do not fundamentally affect absorption and storage of the main nutrient salts in the leaves of oats, wheat and timothy (in Sweden).

1.4 PLANT FACTORS.

Plants of different species and even different varieties of the same species may differ considerably in their nutrient uptake from the same soil (76). Such differences in uptake, frequently observed in varieties exhibiting deficiency symptoms when other varieties of the same species, or the same variety on a different rootstock, grow normally, are related to the kind and extent of root development rather than to differences in nutrient requirements (206). Smith and Wallace (187) suggested that citrus rootstocks may have specific cation-exchange capacities which may explain the differential ability of roots to absorb nutrients and thus account for the observed influences of rootstock on scion composition.

Many workers have noted significant differences in nutrient composition due to

(1) the plant part sampled, which is important in any scheme of diagnostic plant analysis when the whole plant cannot be analysed. In fruit trees the bark, wood, roots, fruit and leaves, and even leaves from different positions on a tree, differ widely in nutrient composition (85). In the case of leaves their morphological position is important in determining which position on the tree is most suitable to provide a reliable reflection of the nutrient status and at the same time be convenient to sample (37, 85).

Thus analytical data designed for the evaluation of comparative nutrient status must be based on a definite plant organ consistently sampled from the same position on the tree.

(2) variety. Significant differences in leaf composition due to variety have been reported for apple (32, 75) pear (152) and citrus (55).

(3) rootstock, in the case of apple trees (50, 84) and citrus (55, 59, 60, 87, 179, 212).

(4) size of crop, in the case of apple (47, 94, 122), peach (114), prune (113) and grapes (58).

(5) stage of development. Numerous investigations have shown that the nutrient composition of plant organs changes markedly in relation to the stage of development of the plant. Such variations are capable of affecting the interpretation of diagnostic leaf analysis (74) unless suitably evaluated as for instance by the use of curves representing the seasonal trends (153), or as is usually done in routine work, by selecting a definite period during the growing season for sampling when the composition is relatively constant.

No significant differences in leaf composition between trees of different ages have been found in the case of apple (75), pear (152), peach (114) or oil palm (37).

2. NUTRIENT ABSORPTION AND DISTRIBUTION IN RELATION TO PLANT COMPOSITION.

Some of the factors affecting nutrient composition of plants have been enumerated in Chapter 1. The processes governing the entry of nutrients into the plant and translocation within the plant may be examined more closely to indicate how they affect nutrient concentrations in the plant.

The apparent ease with which plants grow masks a great number of complex processes many of which are still imperfectly understood. The factors governing absorption of nutrients have been intensively studied and prevailing concepts of ion exchange seem to fit in reasonably well with the observed accumulation of nutrient elements within the plant. Nutrient salts must be dissociated into their respective ions before absorption is possible and it is generally accepted that the main processes of entry into the root is by means of diffusion and ion exchange reactions (including contact ion exchange) at the root surface (120, 165). It is also recognized that ion uptake and accumulation in the roots is closely associated with root respiration (80, 120, 143, 166), and that continued uptake and transport of nutrient salts from the roots, against the concentration gradient and absorption potential (143, 166), can only take place under aerobic conditions favourable to the respiration process (118). The latter is considered to supply positive H-ions for cation exchange and negative HCO_3^- -ions for anion exchange (36).

Two modes of entry of ions at the root surface are recognized (80): (a) passive and reversible processes of diffusion and exchange adsorption, which are consistent with phenomena connected with exudation of ions from the root, and (b) active transport and absorption against the concentration

gradient, an irreversible process. It is postulated that the nutrient ions are carried across the cell walls beneath the root surface by a "carrier" system (77), the ion carrier complexes traversing membranes of limited permeability to free ions. The ions are then irreversibly released from the carriers at the inner surface of the membranes, accumulating in the cytoplasm and vacuole of the cell (166). After accumulation at the root tips, the nutrient ions are partly assimilated and utilized there but the greater portion passes on into the conducting (xylem) tissues of the plant (15), and is transported to the leaves by water movement implemented by root pressure and transpiration (120).

A small portion of the nutrient ions are assimilated in transit to the leaves (15) so that the bulk of the nutrient salts are deposited at the main site of assimilation in the leaves and in the apical primordia. Metabolic use and transpiration are mentioned as two basic factors which influence the direction of movement within the plant (15). A variable proportion of the mineral nutrients in the leaves are redistributed to various parts of the plant depending on age of leaf and development of new tissues and fruit.

2.1 PROCESSES AFFECTING THE EXTERNAL SUPPLY OF NUTRIENTS.

The amount of mineral nutrients that can be taken up by plants varies according to the relative proportions of soluble, exchangeable and fixed forms (133) in the soil, and the rate of mineralization from the solid phase (194) and from organic residues. The available supply in the soil can vary from very low levels which give rise to deficiency effects on plant growth, to very high levels capable of producing toxic effects in the plant.

In addition to the effects of physical condition of the

soil, water relationships and aeration of the soil, the amount of available nutrients that can be absorbed by plants is conditioned by various processes such as cation exchange and precipitation reactions in the soil, cation exchange capacity of the roots and the processes of nutrient uptake.

2.11 CATION EXCHANGE REACTIONS.

The cation exchange capacity of the soil and degree of saturation of the exchange complex to a large extent determine the fertility of a soil. The cation exchange capacity depends on (a) the clay content and type of clay minerals and (b) the humus content of the soil. Three main groups of clay minerals may be distinguished, Montmorillonite, Illite and Kaolinite, with typical exchange capacity ratings of 100, 30 and less than 10 milli-equivalents per 100 grams of colloidal material. According to Malherbe (121) most of the soils in the coastal regions of the Western Cape Province (about 80%) have clay minerals of the Kaolinite group, the remainder belonging to the Illite group, and the arable soils usually are very low in humus content. The soils in this area therefore have an extremely low exchange capacity. Karoo soils on the other hand generally possess a considerable proportion of Montmorillonite minerals which would account for their greater fertility.

Obviously the exchange capacity of soils in which Kaolinite colloids predominate, can be greatly improved by increasing the humus content with its relatively high exchange capacity (about 200 m.e. per 100 gm.). In practice it is exceedingly difficult to build up the humus content in a warm climate by means of green manuring or application of organic material, but evidence has been obtained on a farm in the Elgin area that it is quite feasible under a system of permanent covercropping.

Cation absorption by plants is greatly influenced by the presence and nature of the colloids in the soil where plant roots must compete with the soil colloid for cations (68). Some soils are known to be strongly adsorptive for K (98), Cu (134) and P. The exchangeability of adsorbed cations increase from Na to K, Mg, Ca and finally H, and they tend to be most readily released from the colloid in the same order depending on the degree of saturation of the colloid or on change of concentration of complementary ions following fertilizer additions to the soil (133).

2.12 PRECIPITATION REACTIONS.

Reduction of nutrient availability brought about by chemical precipitation in the soil is mostly related to the nature of the soil reaction (pH). Phosphate is readily precipitated in very acid soil, and some of the micro-nutrients in alkaline soil. The practice of liming greatly influences the availability of soil nutrients through its effect on the soil reaction. The availability of N, P and Mo is improved by liming, whereas that of Fe, B, Zn, Mn and Cu is depressed. Copper may also be rendered less available in acidic peat soil (112). Under these conditions, if the supply of Zn, Mn and Cu is limited, microbial competition may also often give rise to deficiencies.

Manganese in the bivalent form as found in acid soil is available to plants, but if the pH is raised above 6.0 it tends to be converted to higher oxides such as manganic oxide and manganese dioxide which are much less available (112). Gisiger (83) found that Mn is reasonably available in very alkaline soils and considers that Mn, in the intermediate pH range of 6.0 to 7.9 when Mn deficiency usually occurs in plants, is biologically oxidized by soil micro-organisms which reduce its

availability. The harmful effect of reduced boron availability after excessive use of lime in acid soils is ascribed to reduced ability of B to protect the roots against an unfavourable concentration of OH-ions rather than to a precipitation effect.

The so-called "lime-induced chlorosis" of calcareous soils falls in a special category since the observed effects are not confined simply to iron availability and the entire metabolism of the plant may be assumed to be disturbed (99). However, it is well known that Fe availability is depressed by high pH. In very acid soil Fe and Al tend to be precipitated upon addition of soluble phosphates. It has also been shown that Fe may be precipitated, presumably as ferric phosphate, at the root surface of bean plants grown at pH 7.0 in a high P medium, thus reducing further absorption of Fe (15).

The availability and absorption of Fe has been found to be strongly affected by high concentrations in the growth medium of macro-nutrients, Ca, P, K and N (39), K and P (22), K (102), P (61, 65, 80), as well as micro-nutrients (heavy metals), (39, 61, 96). Copper accumulation in orchard soil is considered to be particularly conducive to the development of Fe deficiency (38, 39, 159, 186).

2.13 CATION EXCHANGE CAPACITY OF ROOTS.

Regarding the significance of the C.E.C. of plant roots in plant nutrition, Drake (68) found that plant species differ greatly in their ability to take up cations from the soil and that these differences may in part at least be ascribed to a specific capacity to exchange cations. Plants with high C.E.C. roots, e.g. lettuce, potatoes, tomato, were found to absorb relatively more Ca than K even to the extent of partial exclusion of K (this can be overcome in practice by frequent top dressings of K). Sweetcorn plants on the other hand had low

C.E.C. roots which absorb and take up relatively more K from the soil.

Varying cation exchange capacities of roots has also been suggested as a possible explanation of differences in nutrient uptake by citrus rootstocks (187).

2.14 ENTRY OF NUTRIENT IONS INTO THE ROOT TIP.

The available evidence clearly supports the contention of Lundegardh (118) that ion exchange is the fundamental process in the uptake of nutrient salts. The observed effects of cation exchange reactions in the soil, complementary ion effects, ion antagonism, electrostatic balance of cations and anions, ion exchange at the root surface and the C.E.C. of roots, all seem to fit into a pattern in which ion exchange forms the central or controlling mechanism of absorption. Diffusion also plays a rôle, coupled with exchange adsorption, in the passive absorption of ions as contrasted with active absorption according to the categories proposed by Gauch (80), as mentioned above.

The total absorption of individual ions from an unlimited supply in the soil cannot proceed beyond a certain limit when root injury and other toxicity effects set in. Plants may absorb and tolerate large quantities of certain elements, for instance citrus leaves have been found to contain over 9.0% K in the dry matter (55), the normal content being about 0.5 to 2.0%, and the Mn content (normally not more than about 150 p.p.m.) may rise to more than 10,000 p.p.m. of dry matter in certain plants (215). The tolerance to other elements such as B is very much less. In saline soils the upper limit at which growth becomes affected is set by the rising osmotic pressure of the external medium (71, 224) and by decreasing aeration brought about by deterioration of the soil structure (120).

The mechanism controlling differential and selective absorption of ions by plants is not clear. It is not explained by the process of ion exchange at the root surface (36) although it is evident that the C.E.C. of roots (68) and metabolic demand, as well as adjustment of cation-anion balance to maintain the acid-base equilibrium (204) within the plant, are involved. Mobility of ions influences ion accumulation as shown by Overstreet and Dean (143), who found that K, NO_3 , NH_4 and Cl accumulate rapidly in root tissue, SO_4 and PO_4 less rapidly, and Ca, Mg and Ba much more slowly.

In considering the theory of contact ion exchange, that is, transfer of ions from soil to plant without an intermediate soluble phase, Jenny (101) postulated that in any soil both solution and contact mechanisms will be operating. As far as macro-nutrient cations are concerned the soil solution mechanism would be expected to predominate in sandy soils, whereas in clay soils contact would be the decisive factor. Also, the lower the salt content of the soil solution the greater will be the contribution of contact exchange. For those micro-nutrient cations, including Fe (and Mn), which are largely insoluble at higher pH values, contact exchange may well be the dominant mode of acquisition by roots, although chelation processes presumably may also provide a source of available nutrients under these conditions. Chapman and Rayner (56) thought that citrus trees in the field acquired a portion of their phosphate by contact exchange. Jenny considered that the amount of nutrient ions absorbed is more than can be accounted for by those present in the soil solution.

The theory of contact absorption is contested by Overstreet (143) who, although recognizing that the amount of nutrients present in the soil solution at any given time is inadequate for the nutrition of plants, states that "apparently the soil solution is continuously renewed as it is unlikely that plants

obtain nutrient ions directly from the solid phase by contact feeding."

2.2. PROCESSES AFFECTING THE INTERNAL NUTRIENT CONCENTRATION.

Numerous processes are manifest within plants which determine the variable concentration of nutrients in different organs as found by plant analysis at the time of sampling. Movement and utilization of nutrients subsequent to absorption are influenced by root pressure, transpiration, photosynthesis, assimilation, development of meristematic tissues, redistribution and accumulation.

The importance of enzyme systems in the internal life processes of the plant is recognized and may well prove to be the key to the mechanism controlling nutrient content. Carbohydrate assimilation in the leaves is determined by the salt content as well as by CO_2 concentration, light intensity, water content and chlorophyll content. Through their effect on carbohydrate assimilation the nutrient salts influence the expansion of the assimilating surface and the chemical composition of the leaf (118). But until more is known of the function of mineral nutrients in activating enzyme systems, the significance of nutrient concentration in plant tissues and thus their relationship to growth and fruiting are evaluated empirically by considering the net content of nutrients in so far as they can be determined by chemical analysis.

For our immediate purpose the content and interrelationship of mineral elements in plants may be considered under the headings of mobility of ions, translocation and redistribution, ion equilibrium and rate of growth.

2.21 MOBILITY OF IONS.

The rate of movement of ions in the plant is influenced by differences in apparent ionic diameter or size of ions (118), by interacting and reciprocating effects within the cation-anion equilibrium and by precipitation effects. N, P and K are readily mobile and redistributed from leaves to other parts of the plant (118, 225). The mobility of K appears to be accelerated by the presence of NO_3 (98). Contrary to prevailing opinion, S also appears to be freely mobile as SO_4 -ions, at least as much as P (80). Calcium is relatively immobile and there is no evidence of redistribution of Ca which tends to accumulate in roots and leaves (118). Little Ca is transferred to meristems (15). Magnesium is fairly mobile and accumulates in seeds, migrating from nearby leaves into fruit and inducing a deficiency in the leaves of citrus (225) and apple (130).

The results as to mobility and redistribution of micro-elements are not consistent. Migration of Zn and Mn from the leaf does not seem to be great and they probably migrate more readily from roots and stem than from the leaves (175, 225). According to Lundegardh (118), Cu, Mn and Fe are difficultly mobile. Fe apparently is not transferred from old to young leaves (39) and withdrawal of Fe and B from older leaves does not constitute an important source of Fe and B to meristems (15).

It has been shown that Ca, K and Mg tend to accumulate in newly grown terminal tissues, and P in the bark and wood (50). Large accumulations of Cu may occur in roots without change of leaf Cu (185). Thus plant analysis following differential fertilizer treatment is subject to different interpretations according to the plant part analysed.

Apparently precipitation effects may also be involved in the accumulation of nutrient elements. It is possible that a

high or normal content of Fe, Mn, Zn and B in the presence of a high Ca level does not necessarily signify an adequate supply since a portion may be immobilized in the plant owing to lime-induced effects (164). According to Biddulph (15), precipitation effects, for instance in the case of P and Fe, may occur at the root surface and again in the cells surrounding the xylem and at the xylem extremities. Direct evidence that mineral nutrients may concentrate in veins of leaves and become immobilized has been obtained for Fe using radioactive Fe^{55} . At pH 4.0, Fe may enter the roots unchecked and may be well distributed but at pH 7.0 Fe, if it is not precipitated at the root surface due to high P supply, is precipitated in the veins leaving a deficiency in the mesophyll. Wallihan (218) however found that Fe chlorosis in citrus is reflected by a critical concentration in the leaves and rejected the idea of partial immobilization.

2.22 TRANSLOCATION AND REDISTRIBUTION OF IONS.

Biddulph (15) states that two basic phenomena influence the direction of movement of minerals within a plant: metabolic use and transpiration. These may have a differential magnitude in branches of poor vigour. Weak branches of small diameter also set up frictional resistance to the flow of water resulting in reduced transpiration and decrease of all nutrients in a weak shoot (123).

Nutrient salts deposited in the leaves are by no means static. The continual delivery of minerals via the transpiration stream will result in accumulation in mature leaves in excess of their metabolic needs unless re-exported. P^{32} for instance has been found to move readily into mature leaves yet the P concentration remained below that in apical meristems. Mineral nutrients arriving in young leaves will be metabolized

and continuously exported to new developing leaves and thus incorporated in new protoplasm.

The age of the leaf markedly affects the concentration of mineral elements in the leaf. Three phases in the life history of a single vegetative organ may be considered (225): (a) An initial period of growth (adolescence) in which nutrient ions rapidly accumulate in conjunction with synthesis of new protoplasm. Gregory (225) found that more than 90% of N and P taken up by the developing cereal plant had been accumulated when the dry weight was only 25% of the final weight. (b) A period of constant weight (maturity) after cessation of growth when photosynthesis is the dominant function and carbohydrates are transferred to younger actively growing tissues. (c) A period of decline in weight and of internal disorganization (senescence). The migration of mineral elements from leaves of deciduous plants prior to leaf fall may amount to as much as 90% of the maximum amount of N, P, K, Mg and Fe present (225).

Translocation of nutrients occurs freely in any direction. It has been shown (15) that if the mineral, e.g. P or Fe, is mobile within the phloem tissue, it moves to and supplies actively growing areas at root and stem tips in spite of direct supplies available to them. The most rapid movement is downwards in the phloem but ultimately they move upward again through the xylem. This equalizing mechanism permits growing areas to be supplied when metabolic use is exceeded or when the flow from the roots is impaired depending on solubility and mobility of the mineral elements.

2.23 CATION-ANION BALANCE.

Ion exchange phenomena presuppose independent absorption and transport in the plant of cations and anions (143) so that an electrostatic balance of ions both within the plant and in

the nutrient substrate must be maintained (211). Organic acids increase in the plant when cation exceeds anion absorption, and diminish when anion exceeds cation absorption, thereby preserving the electrostatic equilibrium (204). Hoagland (98) found that when N is supplied in the form of NH_4 a marked decrease in the concentration of organic acids in all parts of the plant takes place to compensate for the acidity produced by the NH_4 -ions; in the nutrient medium also, the solution becomes more acid because NH_4 -ions are removed more rapidly than SO_4 and PO_4 -ions by the roots.

A cation-anion balance in the plant may thus be considered as a fundamental phenomenon. Measurement of total cation and anion absorption by plants however is most difficult particularly in view of the uncertainty as to whether N is absorbed in cationic or anionic form. However, it has been found that cations other than K, Ca, Mg and Na, and anions other than NO_3 , PO_4 , SO_4 and Cl normally constitute only a small proportion of the total so that their omission does not greatly affect the trends observed in cation-anion relationships.

Van Itallie (207) drew attention to an apparent constancy of the sum of the cation equivalents (Ca, Mg, K and Na) per unit weight of dry matter of Italian rye grass in spite of considerable variation in their concentrations in the plant due to differential additions in the nutrient medium. This tendency has also been noted for cations in other plants (209, 234) as well as for anions (110). Other workers showed that the summation values vary with varying supply of nutrient ions (e.g. Ca and K), and with plant species and yield (150, 185, 211, 234).

Wallace (211) summarized the available data on cation and anion equivalents summation values in whole plants for a large number of crop plants. The data indicate that different

species have widely different values and that considerable differences occur within each species, but that high cation summation values tend to be associated with high anion summation values, and low cation with low anion values. The cation-anion ratios were found to be practically constant (110, 211) which is in accordance with the idea of an electrostatic equilibrium of ions in the plant.

The constancy of the cation-anion ratio signifies that reciprocating effects will give rise to varying ionic ratios (209) so that an increase of any cation in the nutrient medium will result in a reduction in absorption of another cation or an increase in uptake of one or more anions. Thus the many observed effects of interaction of ions or ion antagonism in the plant resulting from changes in the rate of supply are in a large measure an expression of mutual replacement of ions operating within the framework of the cation-anion equilibrium. Potassium is the dominant cation controlling cation absorption (234) and NO_3^- the dominant anion in anion absorption (211). Ion equivalent summation values and reciprocating effects vary in different parts of the plant so that an evaluation of leaf composition may be entirely different to that based on root composition or the composition of other plant parts (46, 50, 53, 185).

2.24 RATE OF GROWTH.

The factors discussed so far by no means exhaust the influences which affect the internal nutrient concentration. There is no doubt, as pointed out by Cain (52), that there are many unknown or little understood factors affecting the efficiency with which the plant may utilize mineral nutrients available to the plant root and after absorption.

A most important factor influencing the mineral concen-

tration in the plant is the rate of growth as related to the amount of nutrients present in the plant and translocated to various parts. Since nutrient content is usually expressed in terms of concentration per unit dry weight of tissue, any internal plant factors which change the ratio of dry weight to mineral content of the leaf, irrespective of absorption by the roots, will influence the analytical results. Cain (52) found that about one third of the potassium in the apple tree is located in the fruit at harvest; thus a light fruit crop would permit more K to reach the leaves and give rise to a higher leaf content even without a change in absorption of K by the roots.

Changes in the ratio of dry weight to mineral content are also evident following different rates of nutrient absorption. The elements N, K, Ca and P may be considered as the nutrients chiefly determinative of growth and yield (118). Thus a positive growth response following the increase of one of these nutrients may be accompanied by a decrease of other nutrients entirely due to the expansion of growth. The decrease in percentage content is thus a dilution effect since the total amount of the other nutrients in the plant have not changed or have even increased (50).

A decreasing supply of N sufficient to reduce growth on the other hand may lead to an increased percentage of other nutrients if they continue to enter the plant at the same rate (50). Lundegardh (118) found that the K and P contents of cereal plants was increased by restriction of growth due to N deficiency. Chapman and Brown (55) have also found that N deficiency leads to higher K and P contents in citrus leaves, whereas Broeshart (37) found an increase of P, Ca, Mg and ash contents in oil palm leaves. In the same way, although not always through their effect on amount of growth, deficiencies of practically all nutrient elements have been found to affect

the level of other elements (37, 55, 57, 95).

An enhanced rate of carbon assimilation and accumulation may likewise give rise to dilution effects, such as a lower percentage N, and conversely the percentage N may be higher when carbon accumulation is reduced as in dull weather even with no more N added to the soil (140).

3. CONCEPTS OF PLANT ANALYSIS.

From the foregoing chapters it is evident that a great number of factors operating simultaneously are involved in building up the mineral composition as found by chemical analysis of the plant material. As stated by Ulrich (206), "the nutrient concentration of the plant at any particular moment is an integrated value of all the factors that influenced this concentration up to the time the sample was collected."

3.1 RELATIONSHIP BETWEEN NUTRIENT CONCENTRATION AND PLANT GROWTH.

The internal nutrient concentration has been found to be associated with growth in such a way that growth would be optimal at a certain range of concentrations and suboptimal when the concentration was below or exceeded this range. Lundegardh (118) maintains that numerous experiments have shown unequivocally that the internal concentration level of nutrients reflects their effect on growth.

The use of plant analysis for diagnosis of its nutrient status is based on this relationship between nutrient concentration and plant performance (amount of growth or yield). The feasibility of using the nutrient composition for diagnosis depends on whether the relationship with growth holds irrespective of those factors which influence plant composition and cannot be readily accounted for, such as soil and climatic conditions. Numerous investigations referred to in previous chapters have shown that plant composition can be sufficiently influenced by the plant factors (the plant organ sampled, plant species, variety, rootstock and stage of development) to affect seriously the interpretation of plant analysis. These plant factors can

be allowed for by establishing relationships for a particular variety and plant part sampled at a definite stage of growth. Assuming such a basis it remains to be seen whether the relationship will hold under different conditions of soil and plant environment.

In a critical review of concepts based on the relationship between nutrient supply or nutrient content of the plant and yield (dry matter produced), Macy (119) studied the Law of the Minimum first proposed by Liebig, that plant growth is directly proportional to the supply of the nutrient which is in minimum; the Law of Diminishing Returns, formulated by Mitscherlich, that the increase in yield per unit of limiting nutrient applied is directly proportional to the decrement from the maximum yield; the Minimum Percentage of Wolff; the Sufficiency concept of Pfeiffer, that the sufficiency of a nutrient is a function of its percentage content in the plant; and others.

Realizing the significance of the "sufficiency" idea, Macy proposed a theory of the relationship between the percentage content of a nutrient in a plant and the sufficiency of the nutrient for growth as a measure of the quantitative mineral nutrient requirements of the plant, combining the concepts of Liebig and Mitscherlich. Macy visualized a "critical percentage" of each nutrient in each kind of plant above which there is "luxury consumption" and below which there is "poverty adjustment" which is almost proportional to the deficiency until a "minimum percentage" is reached. Using data from Pfeiffer's work, he presented yield curves for oat plants with increasing N or P supply (Fig. 1), and the corresponding nutrient content - response curves (Fig. 2).

Considering the curve relating yield to internal concentration (Fig. 2), three portions of the curve may be distinguished, (a) the minimum percentage portion where yield rises

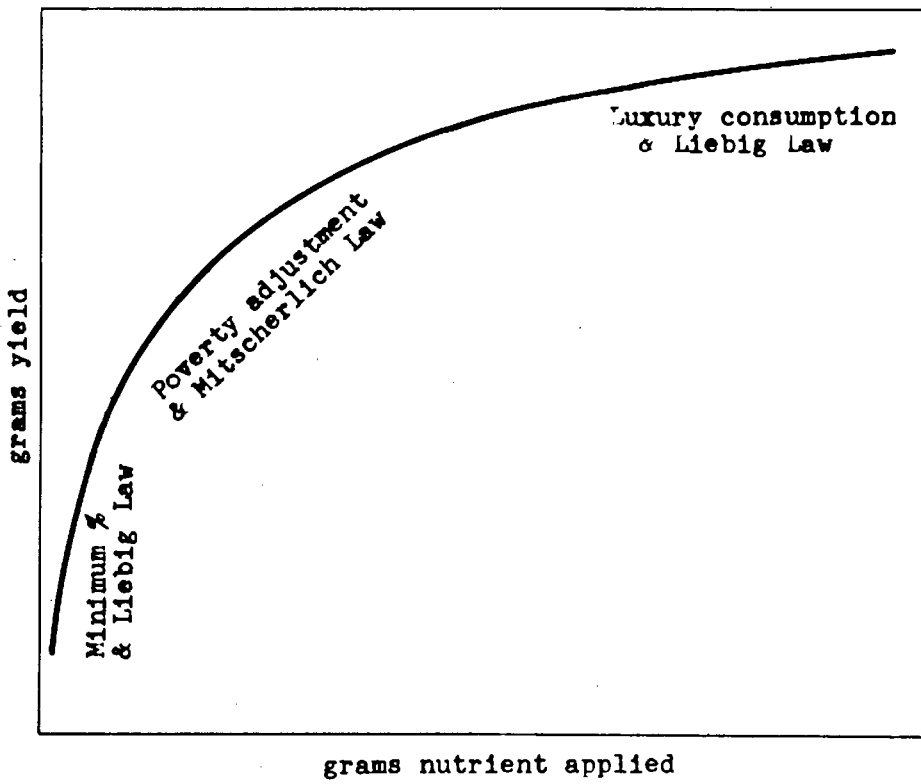


Fig.1

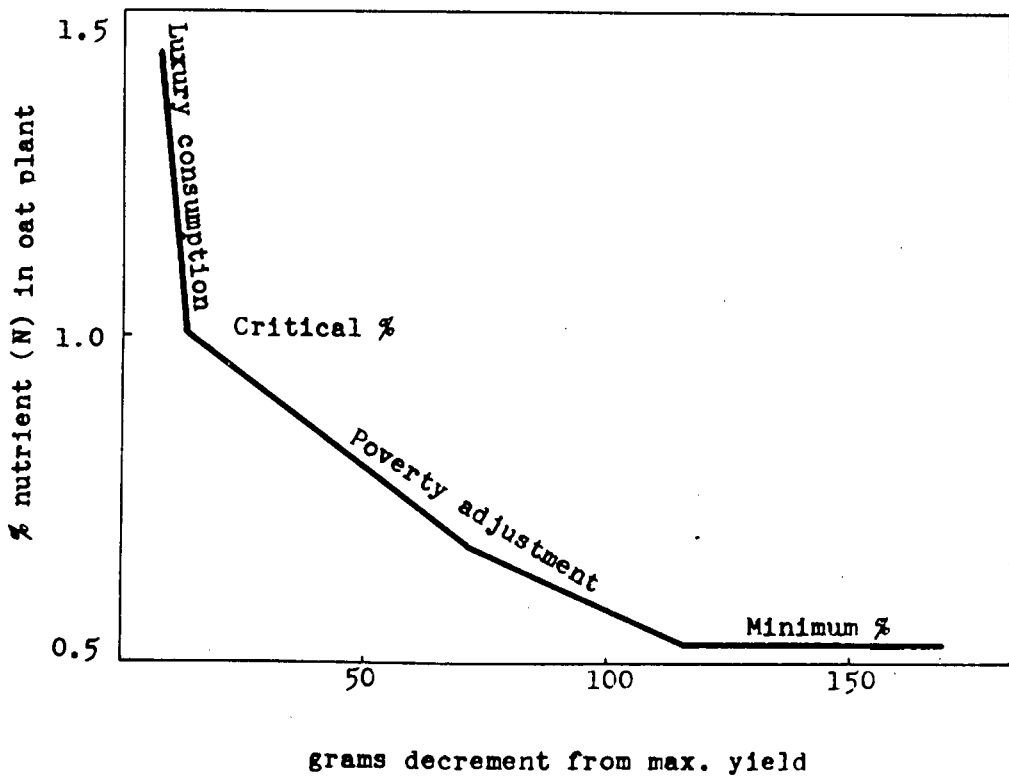


Fig.2

(Adapted from Macy, P. (1936): Plant Physiol. 11, 749-764)

although internal concentration remains constant (Liebig law), (b) poverty adjustment region in which both yield and internal concentration increase, the response decreasing with sufficiency of nutrient (Mitscherlich law), and (c) luxury consumption region in which yield remains constant with rising internal concentration. The transition from (b) to (c) was considered to be a fixed point on the curve designated as the "critical percentage" for each nutrient. Macy presented evidence that when other growth factors such as changing climatic conditions from one year to the other, affect the percentage content of a nutrient in the plant, the sufficiency of the nutrient is likewise affected so that the above relationships still hold.

Steenbjerg (191) drew attention to the possibility that the yield curve, under conditions of low nutrient supply, may be S-shaped (Fig. 3). He found that increasing the Cu supply when at a low level, i.e. below the "point of inflexion", increased yield at a faster rate than the rate of absorption of the element. Plotting Cu content against yield as in Fig. 4, it is evident that the percentage Cu may be at a point on the descending portion of the curve from which it is clear that the same Cu content may correspond to two very different yields. The existence of an S-shaped curve may thus affect the interpretation of chemical plant analysis and must be taken into account when considering the effect of differential fertilizer applications.

In most cases of low nutrient level, however, the relationship between yield and internal concentration can be represented by a point situated on the ascending portion of the curve when yield and internal concentration both increase ("poverty adjustment region" of Macy), even though not necessarily linearly. At very low levels the presence of deficiency symptoms would in any case indicate a potential

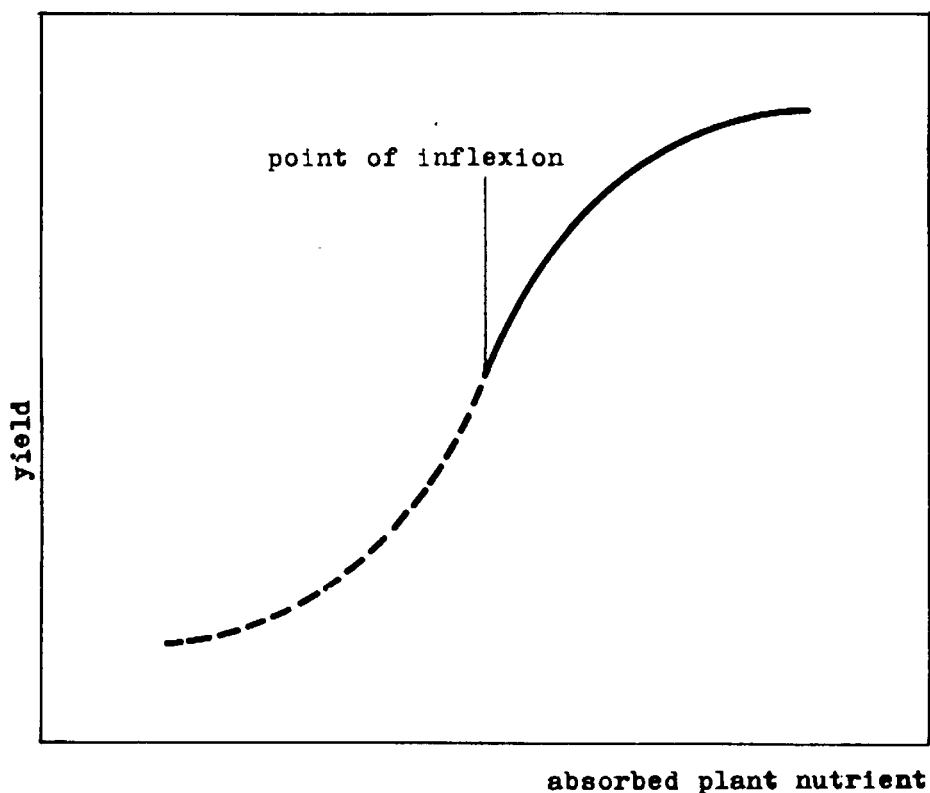


Fig. 3

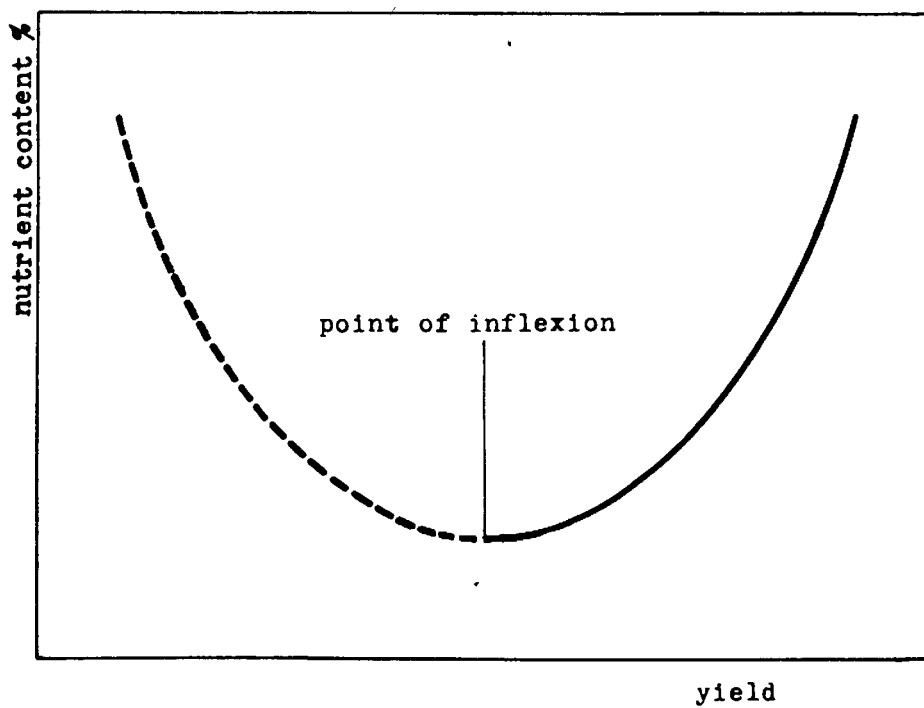


Fig. 4

(Adapted from Steenbjerg, F. (1951): Plant & Soil 3, 97-109)

yield increase if the supply of the limiting nutrient is increased.

3.2 THE RELATIONSHIP AS AFFECTED BY NUTRIENT BALANCE.

The postulated relationships as indicated by Macy are considered to apply when one nutrient factor only is varied, other nutrient factors being held constant. He intimated however that the "critical" and "minimum" percentages of nutrients in plants are not absolutely invariable. Goodall and Gregory (85) subsequently pointed out that in the case of a wide range of combinations of nutrient factors as found in the field, it is clear that the internal nutrient concentration of one only of these nutrients is unlikely to be related to yield over the whole range. "It has been shown that as the level of a nutrient not in ample supply is increased, other factors being held constant, both yield and internal nutrient concentration rise", but the relationship which tends to be linear at low levels will tend to disappear at higher levels depending on interacting factors which come into operation when the nutrient level is no longer limiting.

Lundegardh (118) likewise indicated that for each nutrient element there is a range of values at low concentration, in which a strong positive correlation exists between the nutrient concentration and growth, for instance, at low concentration (deficiency level), the Ca, P and N contents and growth are positively correlated. As the concentration increases into the optimum or super-optimum range, restriction of uptake of other elements come into play. According to Lundegardh, a positive growth response following a concentration increase of one factor may even be accompanied by a decrease in the level of an unchanged element, without prejudicing the postulated relationship between internal concentration and growth.

It is obvious that an evaluation of yield response in terms of the internal concentration of a nutrient must take into account the simultaneous influences of other nutrient factors since, as stated by Shear, Crane and Myers (1976), the ultimate growth expression depends on both concentration and balance of all elements. "All other factors being constant, plant growth and symptom expression are functions of the two variables of nutrition, intensity and balance" ("intensity" being indicated by the total equivalent concentration of all functional elements in the leaf, and "balance" referring to the relative proportions among the essential elements). "At any level of nutritional intensity there exists an optimum balance or proportion among the functional nutrient elements in the leaf at which maximum growth for that intensity level will result. The maximum potential growth and yield for any given plant will be obtained only when the proper balance between all of the nutrients occurs in combination with their optimum intensity." The importance of nutrient balance in optimum nutrition is generally accepted whereas the intensity factor is usually ignored in practical applications to field problems (1976).

Ulrich (1966) combined the concept of critical nutrient levels within the plant with the ideas associated with the theory of limiting values. According to him, "the practicality of plant analysis must be ascertained empirically through comparison of nutrient concentrations of plants restricted in growth to those of plants not so restricted." Thus for any given element and plant part there is a "critical nutrient range" which may be defined as "that range of concentrations at which the growth of the plant is restricted in comparison to that of plants at a higher nutrient level." Response in the field to addition of a nutrient to the soil depends on

whether the nutrient concentration is above or below the critical level; if above there would be no response, if below, the chance of a growth response increases rapidly as concentration decreases, depending on the abundance of other growth factors and on time and duration of the deficiency. As to nutrient balance, Ulrich states that "plants with widely different nutrient composition have similar yields as long as the nutrient concentrations are well above the critical level, but at or below the critical level one element may affect the utilization of another and such a lack of balance is likely to affect plant growth."

3.3 OPTIMAL NUTRIENT CONCENTRATION IN RELATION TO PLANT PERFORMANCE.

Goodall and Gregory (85) postulated that there is an optimum level for each growth factor: "if all external factors including all nutrients except one, are maintained at optimal level, then growth will be a function solely of the available amount of this nutrient, and as the optimum level is approached the maximum possible yield of the plant will be reached." The criterion proposed to define the optimum level was "response to increased uptake of a deficient nutrient by an improvement in development", which in practice would exclude those cases in which increased uptake of a deficient nutrient led to no improvement in development (e.g. Fe by immobilization in the leaf) and those in which the uptake of other nutrients are affected (e.g. by lime and sulphur).

The idea of "optimum values" is also proposed by Wadleigh (209), that "for any given combination of environmental factors there is within a given plant tissue an optimum content and relationship of the mineral nutrients for maximum plant growth, and that deviations from this optimal nutritional status would

be accompanied by decreases from maximum growth." He states further that this theory is "not experimentally verifiable because of the extreme complexity of pertaining factors and because of the evidence that growth response is invariant over a considerable range in content of many of the nutrient elements."

Shear, Crane and Myers (176) believed that "at the present stage of our knowledge it would be futile to set up specific standards of optimum balance and intensity", and that "the significance of any given leaf composition cannot be evaluated intelligently unless the interactions between the elements contributing to that composition are understood and considered." To determine this, data would be required for each crop to enable us to determine accurately the maximum potential economic yield for each crop and the leaf composition which is correlated with that response.

Chapman and Brown (55) concurred with this contention, stating that "it will be difficult if not impossible to lay down a law or principle applicable to all plants which completely describes the relationship of nutrient content to plant performance until more is known of plant growth and the functions and interrelations of nutrient elements."

The general trend in ideas concerning the most suitable criterion for use in leaf analysis diagnosis may be summed up as follows. The original interpretation of a fixed "critical percentage" of nutrient content (Macy), based on the classical concepts of the relationship between nutrient content and yield, has developed into the more recent conception of a "critical nutrient range" which takes into account normal variability and interaction of nutrient elements (Ulrich). Finally, the emphasis has passed from critical levels to "optimum values" (85, 176, 209) which appear to afford the best approach as a basis for in-

terpreting leaf analysis data, according to the information available at the present time.

4. TECHNIQUES OF LEAF ANALYSIS.

Various schemes of leaf analysis based on the concepts discussed above have been proposed for the determination of nutrient requirements of fruit trees. Such practical schemes are naturally dependent on the use of appropriate analytical methods and suitable standards of reference whereby the nutrient composition of trees of unknown nutrient status may be evaluated. The practical application of leaf analysis in this way hinges on the question of the criteria employed in interpreting the analytical data.

4.1 SAMPLING AND ANALYTICAL PROCEDURE.

The procedure to be followed in determining the nutrient status depends partly on the nature of the plant species and the plant part selected for analysis. In view of the effects of translocation on the mineral composition of different plant organs an evaluation of nutrient status based on the composition of whole plants would be ideal as is often done in the case of annuals, but this is hardly feasible with fruit trees. For practical reasons the sample selected for analysis must allow for rapid and convenient collection and at the same time must be suitable for the simultaneous determination of all nutrient elements and serve as a reliable reflection of the nutrient status. In the case of fruit trees the leaves would appear to be the obvious choice, although leaf data do not always provide the best index of the status of all nutrient elements. Analysis of leaf stalks or other plant organs may be more informative (85), but a compromise is often necessary since the simultaneous routine collection and analysis of samples of different plant organs would take up considerably more time.

Apart from the plant organ selected for analysis other factors which may influence leaf composition, such as time of sampling, variety, rootstock, size of crop, climate, must be considered with the object of establishing a standardized procedure which will reduce the error in interpreting leaf analysis data as much as possible.

The necessity for accurate and reliable methods of leaf analysis is obvious. Analytical procedures which are capable of rapid execution without loss of accuracy are preferred and spectrographic methods have contributed a great deal towards this objective. Determination of the total content of nutrient elements has so far been favoured in most of the work on fruit crops. More rapid procedures, such as "tissue tests" i.e. determination of soluble or extractable nutrients present in fresh conducting tissues (74, 137, 162), have been proposed particularly for annual plants (74), but in general these do not permit simultaneous analysis of all nutrient elements, and in any case, reliable tissue test methods are not yet available for the accurate determination of some of the micro-nutrient elements.

4.2 LEAF ANALYSIS STANDARDS.

A prerequisite for the use of leaf analysis for diagnostic purposes is the establishment of standards of reference with which leaf composition data of trees of unknown nutrient status may be compared. Such standards can be established by means of data from fertilizer experiments in the field, from pot culture experiments, from surveys of commercial orchards or from a combination of these. In recent years, one or more of these procedures have been employed by numerous investigators in determining nutrient standards and may be grouped according to the method employed, as follows:

- (i) Fertilizer experiments in the field, 4, 5, 6, 8, 25, 26, 28, 30, 31, 34, 42, 44, 55, 67, 70, 136, 137, 144, 161, 170, 172, 173, 190, 200, 208, 213.
- (ii) Controlled culture experiments, 40, 55, 56, 92, 128, 183.
- (iii) Leaf analysis surveys in commercial orchards, 6, 9, 10, 29, 35, 42, 45, 55, 58, 76, 93, 106, 114, 124, 125, 152, 156, 177, 201, 220.

Rather few of these investigations are supported by tree performance data in relation to differential nutritional treatments which must be considered as the ultimate basis for obtaining data from which analytical standards can be derived. As expressed by Goodall and Gregory (85), "the value of any method for determining fertilizer requirements from data of plant analysis depends upon the accuracy with which the response to fertilizer additions can be forecast." They state further that "it hardly needs stressing that the data collected for selecting a diagnostic technique, whether in artificial culture or in the field, should, if possible, cover the whole range of conditions to which it is intended the technique should subsequently be applicable."

The dissimilarity from conditions in the field will restrict the use of artificial culture methods for determining standard values, particularly for fruit trees.

Leaf analysis data obtained from a series of orchards are useful in establishing the range of nutrient content associated with a particular tree condition or growth characteristic under varying growing conditions, such as the critical nutrient level associated with symptom expression. A further application of the survey technique is designed to define the range of nutrient content of leaves associated with optimum growth and yield, making use of a comprehensive series of high performance

orchards (55). The average nutrient content thus determined cannot be assumed to represent a true optimal value (85, 161), but it constitutes a useful approach in view of the practical limitations pertaining to fertilizer experiments and pot culture work as far as fruit trees are concerned. Further discussion as to the merits of this method will be considered below.

The establishment of fertilizer trials for fruit trees on a large enough scale to provide the data needed would be costly and hardly practicable since differential nutritional treatments covering all of the essential mineral nutrients must be considered, as well as such factors as varietal differences, duplication in many different localities and duration of the trials. With annuals on the other hand it is quite feasible as shown by the innumerable fertilizer experiments which have been and are still being carried out with annual crops. At best, the data from fruit tree fertilizer trials will become available at a slow rate, but their indispensibility in fixing precise critical levels for nutritional requirements and their value in checking index values determined in other ways are recognized (85, 161).

4.3 CRITERIA USED IN DETERMINING INDEX VALUES.

Considerable divergence of opinion exists as to the best way of interpreting the analytical data with respect to evaluation of the nutrient status. Most workers have attempted to establish "critical" levels for each nutrient, expressed either as percentage content of dry plant material or as ratios between two or more elements, and more recently "optimum" or "normal" values have also been employed.

4.31 CRITICAL LEVELS OF NUTRIENT CONTENT.

The idea of critical or threshold values of leaf composition is based on the relationship between percentage nutrient content and yield as conceived by Macy (119). As the concentration of one nutrient is increased, other growth factors being held constant, growth consistently improves throughout the deficiency range although not at a constant rate; increases in yield gradually diminish and disappear as the critical level (threshold optimum) is reached, beyond which luxury consumption takes place. Finally growth and yield deteriorate when the concentration reaches toxic proportions or interferes with absorption of other elements.

Convenient points on the growth curve which can be readily determined are those representing symptom expressions, i.e. the level below which deficiency symptoms appear or above which toxicity symptoms are evident. These levels are often fairly sharply defined and have suggested the use of "limiting" values. However, more often than not, there is a considerable overlap necessitating the use of a range of values for each nutrient in place of a rigid percentage level.

Among the workers who have recorded critical nutrient levels for leaves of fruit trees (deciduous and citrus), the large majority have used visible deficiency or toxicity symptoms as the criterion for determining the standard values: (a) those concerned with macro-nutrient elements, 4, 5, 7, 28, 29, 30, 31, 40, 56, 62, 70, 85, 111, 115, 124, 137, 152, 156, 172, 173, 174, 208, 213, and (b) those concerned with micro-nutrient elements 18, 25, 26, 34, 42, 44, 45, 54, 55, 67, 76, 85, 93, 108, 111, 125, 128, 170, 174, 200, 228.

The threshold level for symptom expression is however often inadequate as a measure of plant performance since a yield response is possible in many cases at a level considerably

higher than that at which deficiency symptoms disappear. Thus data on the yield responses of plants at varying levels of nutrition, as in fertilizer trials, and data on their composition are usually required before standard values of practical significance can be set up (85, 205). On this basis standards have been determined indicating the transition level between deficiency and sufficiency of a nutrient (85) which corresponds to the critical percentage of Macy (119) or the critical nutrient range of Ulrich (206).

Several investigators have presented sets of standards for individual nutrients as for instance Chapman and Brown (55) who specified different levels at which a growth or yield response would be considered (a) highly probable, (b) possible, (c) doubtful and (d) no response. Goodall and Gregory (85) quote other examples of such verbal descriptions.

In general as stated by Goodall and Gregory, it will be unsatisfactory to base conclusions from diagnostic analysis on the data for a single element since the response to an increase of one nutrient has been shown to be dependent on the level of other nutrients. Lundegardh (118) for instance, has shown that the effect of N or K fertilization on oat yield is increased at higher levels of P, and expressed the relationship in the form of a verbal description of the yield increments to be expected with fertilizer treatment at different levels of nutrient content. For instance, a good expectation of a yield increase may be obtained when

K content is 10-20 m.e./100 gm.	and P content < 4.5 m.e./100 gm.
" 20-30 "	" " 4.5-6.5 "
" 30-40 "	" " > 6.5 "

Such a scheme would naturally become involved as more nutrients are considered, and it would be difficult if not impossible to express the simultaneous effects on yield of more than two

nutrients by means of a simple numerical value.

Few such attempts have been made to relate growth and yield of fruit trees with two or more nutrients. The use of standards evaluated for individual nutrients, as is often done, may thus lead to erroneous diagnoses unless suitably qualified by recognition of the characteristic interactions between various elements as indicated by Broeshart (37) and Reuther and Smith (161).

4.32 NUTRIENT RATIOS.

As opposed to or in conjunction with nutritional standards based on percentage concentration of individual nutrient elements, many workers have attempted to establish critical values of various nutrient ratios. In general these have been based on acceptance of the theory of ion antagonism and the belief that the proportion of certain elements in the plant is related more closely to growth and yield than are the individual nutrient concentrations.

A special diagnostic procedure based on leaf analysis which has become known as the "method of foliar diagnosis", was first used in France by Lagatu and Maume, and later in the United States by Thomas (198) and Thomas and Mack (196, 197) and associates. By this method a series of tests on a selected organ of a given crop is made during the growing season to evaluate the nutrient intensity (sum of percentages of N, P and K or Ca, Mg and K) and nutrient quality (the ratio of these elements as percentages of the total milli-equivalents of each set of three). The percentage values are graphically represented as 3-component systems using trilinear ordinates and an equilateral triangle to indicate the course of nutrition during the season.

Criticizing the use of ratios as an index of nutrient status, Goodall and Gregory (85), state that "it is not to be denied

that the ratios of nutrients within the plant may sometimes give useful indications as a supplement to those derived from the actual concentrations, but to use such ratios without consideration of the individual concentration data is in most cases unjustified." Considering the various ratios proposed as criteria for diagnostic purposes, such as P/N, Ca/K or Ca + Mg/K, Fe/Mn, Ca/B, as well as the proportions of N, P, K and K, Ca, Mg proposed by the "foliar diagnosis" school, they came to the following conclusion, that "there is no reason to suppose that ratios in general are likely to be of greater use in diagnostic work than the content of the elements individually. This is not to deny that diagnosis of the nutritional status in respect of one element on a basis of its concentration in the plant may not need modification according to the level found for another element. But the computation of ratios is not in general the best way of making allowance for such effects, and its adoption as a general practice may well obscure relationships which otherwise would be patent."

Many of the observed relationships and interactions between nutrient elements which have inspired the use of nutrient ratios are not fundamental but fortuitous and become apparent only as the result of growth and translocation processes and of mutual replacement of ions within the framework of the cation-anion equilibrium. Cain (50) has shown that changes in the nutrient content of the foliage of apple trees does not necessarily reflect corresponding changes in the shoot tissue nor can they be interpreted always as representing changes in the uptake from the soil. He showed (49) that the total K and total P content of apple leaves was greatly reduced, whereas that of the dormant shoot was increased, by nitrogenous fertilizers.

Cain (50) concluded that the so-called "interactions" are to a large extent misconstrued as indicating ionic antagonisms

or synergisms in the plant or at the absorbing surface of the roots, whereas many of the observed changes in the percentage content of one nutrient ion in the plant tissue induced by addition of another to the nutrient substrate can possibly be explained either by growth dilution or changes in distribution as a result of stimulated metabolic activity and differential rates of translocation. He found with apple trees grown in sand culture that one ion has little if any direct effect on the total absorption of another by the tree although the percentage content of one ion may be decreased by the application of another if its rate of absorption does not keep pace with the enhanced rate of growth stimulated by the added ion. If the addition of one ion caused an increase in dry weight of the plant, there was generally more total absorption of all nutrient ions determined, although some plant parts may show a net loss of some ions. If there was no growth response only the ion applied was absorbed in greater quantity.

Regarding the frequently observed interaction or antagonism between nutrient ions in plant tissue, already referred to (85), Cain found (51) that the apparent relationship between K and Mg in apple trees "is associated entirely within the plant and is in no way related to external supply except as the external supply of one element influences its own absorption by the plant." According to Chapman and Brown (55), little if anything is to be gained, so far as specific K diagnosis is concerned, from the use of nutrient ratios, since all the available evidence indicates that total K on a percentage dry matter basis correlates well with K status. York, et al (233), from alfalfa studies in the greenhouse, came to the same conclusion regarding the reciprocal relationship between Ca and K: "as far as the relation of K supply to growth is concerned, there appears to be little need or justification for considering ratios of these elements when the dominant factor is apparently the absolute amount of K

available to the plant."

The well-known Fe/Mn "antagonism", thought to be due to their respective oxidation-reduction potentials, has likewise been disproved by Hewitt (97). Moreover, Mn apparently is much less effective in producing iron chlorosis than other heavy metals (39, 109), and recent evidence seems to indicate that the rigid reciprocal relationship previously ascribed to the Fe/Mn ratio (81, 189) does not hold in view of results showing independent effects of these elements at both deficiency and excess levels (97, 142, 149). Iron and Mn deficiencies may occur simultaneously in the same plant and toxic effects of excess Mn can readily be distinguished from Fe deficiency (209). Although considerable evidence indicates that Fe chlorosis is caused by a simple deficiency, Gauch (80) quotes several investigations which strongly suggest that chlorotic symptoms are induced by a high level of P, and that both P and Cu may reduce the availability of Fe in the plant and cause chlorosis.

Regarding the reciprocal effects of Ca and B, and Mg and B, Wadleigh (209) contends that "it is doubtful that the various ratios between B and nutrient cations are effective per se in plant metabolism and they only become apparent owing to the differential effect of B and other nutrients upon specific enzyme systems. Wadleigh postulates that "progress in assessing the physiological rôle of the cations will probably continue to develop through cognizance of a concept of balance among the cations; not by calculating mathematical ratios of cations within the gross herbage of the plant, but by assaying the effect of relative cationic activities upon specific enzyme systems within the plant. The latter are fundamental in plant nutrition when one considers that plant growth is largely the resultant of enzymatically controlled energy transfers."

4.33 OPTIMUM VALUES.

In this instance the criterion of nutrient level as related to yield is the optimum value (85, 176, 209) as opposed to critical nutrient levels associated with the onset of deficiency symptoms or yield restriction as discussed in Section 4.31. According to Smith and Taylor (177) the concept of "optimum values" maintains that there is a specific leaf concentration for each of the essential elements which is correlated with optimal response in terms of yield or other characteristics and that these concentrations or optimum values hold over a wide range of soil types and under a variety of climatic conditions. The leaf composition will therefore reflect the potentialities of the desired response. As the optimal nutrient level of each factor, depending on all factors simultaneously, is approached, the maximum possible yield of the plant will be reached (85). If the leaf concentrations are at optimal levels then it must follow that the intensity of nutrition and nutrient element balance also are optimum (177), and thus the concepts of nutrient intensity and balance are also completely accounted for (176).

Broeshart (37) found that the use of critical nutrient levels or interpretations based on nutrient ratios gave erroneous results in the case of the oil palm. Palms with an adequate supply of plant nutrients, whether growing in a light sand, a heavy clay soil or a culture solution, had a "normal" or "optimal" leaf composition associated with maximal growth and production. The optimum values were determined from the results of a large number of fertilizer trials and a sand culture experiment, and were found to be identical for young and old plants.

In addition to fertilizer experiments, leaf analysis of high performance trees, using a comprehensive group of orchards to represent a wide range in nutrient supply, is accepted by

several workers (6, 8, 10, 35, 37, 55, 106, 114, 177, 201, 220) as a useful approach in establishing optimum values of nutrient content, if supported by evidence that such standards have a fixed or unique value (55). In this scheme the mean of the range of concentrations for a sufficient number of orchards with good crop performance is considered by Smith and Taylor (177) to provide an approximate but practical optimum value. If accurately determined for a certain crop, these "optimum values" could be used as standards with which to compare analysis of leaves from plants making unsatisfactory growth, or from those showing deficiency or toxicity symptoms, and fertilizer application could then be made with the aim of raising or lowering each concentration towards that of the optimum.

It is true that, as found by several workers (85, 106, 152 and others), normal growth and yield may occur accompanied by considerable variation in composition as to the general level of nutrients as well as to the relative amounts of the nutrients present in the foliage. Thus the mean value obtained will be susceptible to change depending on the level of fertility and mineral status of the soils occupied by the orchards selected. The influence on the mean value of relatively high concentrations within the luxury consumption range and of relatively low concentrations in the deficiency range will however tend to be diminished by employing the data from a comparatively large number of orchards.

A serious drawback may be the lack of evidence implicit in the data that fertilizer applications to trees, in which the internal nutrient concentration falls below the mean optimum value, will be followed by a yield increase. This is stressed by Goodall and Gregory (85): "until the investigator has data on the yield responses of plants at varying levels of nutrition and on their composition, he is hardly in a position seriously

to consider setting up standard values for internal nutrient concentrations." However, the means of obtaining such direct evidence are limited except in extensive factorially designed fertilizer experiments where the level of one element is varied at constant levels of the other elements, which as already indicated is most difficult to achieve on a sufficiently extensive scale in the case of fruit trees.

It is evident also that the mean optimum value determined will depend in large measure on the basis of selecting the orchard for obtaining the analytical data. Good performance in orchards may be considered to include high quality fruit as well as high yield backed by satisfactory growth vigour of the trees. Such a criterion of economic production will necessarily be arbitrarily fixed as judged by tree records and experience. Healthy tree growth is a necessary prerequisite and trees showing visible symptoms of deficiency or excess are preferably excluded, although good performance as to fruit quality and yield is not always synonymous with the absence of such symptoms. Deficiency symptoms may disappear at a level far short of maximal growth and yield but, on the other hand, they may also be evident at a level well ^{above} ~~beyond~~ the threshold for optimum yield.

High yielding trees have been found to show considerable chlorosis due to Mg deficiency (136), and yield was reduced only when Mg deficiency was severe (141, 183). Mild Mn deficiency symptoms have also been observed in high yielding fruit trees. On the other hand, Chapman and Brown (55) found that a yield response is probable in citrus and that deficiency symptoms are likely to be present when leaf analysis indicates K values below a certain critical level (0.40% D.M.), but there was evidence that under certain conditions fruit size and quality may be influenced by marked increases in K above this level.

Similar results for K have been found in peach (94).

In some cases fruit quality may be the primary consideration. According to Hill, quoted by Bould (27), a marked decrease in quality occurred in Northern Spy fruit from trees with N above 1.9 to 2.0% in dry leaf material, and a similar decrease occurred in the Mc Intosh variety with foliage N above 2.0 to 2.1%. A sharp increase in bitter pit also occurred when leaf N exceeded 2.3%. Weeks, et al (223) found that an increase in leaf N of 0.1% over the range 1.86 to 2.16% (D.M.) caused a decrease of 14% in "fancy grade" fruit (Mc Intosh apple), whereas an increase of 0.1% in leaf K over the range 0.85 to 1.56% gave an increase of 7% in fancy grade fruit.

The status of N and K is also closely associated with quality in pineapples (155). Chapman and Rayner (56) found that raising the P level in citrus leaves decreased fruit quality while increasing yield, and that the best results as to both quality and yield would be achieved by a P level maintained just slightly above the critical deficiency level.

In other cases as with some of the micro-nutrients deficiency levels based on symptom expression may coincide with the level at which maximal potential yield is secured. In this connection the conclusion of Smith (186) may be mentioned that increasing the leaf content of Zn, Mn and Cu above the threshold values for symptom expression was without benefit to citrus trees in sand culture as to vegetative growth and fruiting. The heavy metals seem to be in the same class with Mg in this respect. With Cu and Zn, deficiency results directly in restriction of growth so that in their case the onset of deficiency symptoms marks a critical level which is more definitely defined in relation to yield than in the case of Mg and Mn deficiencies.

It is evident that the use of the mean optimum value may lead to an incorrect diagnosis and that some other criterion

based on considerations of yield, quality or deficiency symptoms is called for to mark the limits of an optimum range of nutrient content since the mean optimum value of a nutrient in any case does not necessarily reflect the minimum requirement for that element, and nutrient levels above or below the mean may equally well be associated with maximum economic production. In applying a standard optimum range instead of a critical percentage level for the purpose of diagnosis, a decision has still to be taken as to whether the percentage content falls within that range so that the necessity for establishing upper and lower limits for the optimum range is evident. These may be fixed according to the frequency distribution of deviates from the mean value (58, 107) or simply the minimum (10, 136) and maximum values obtained in high yielding orchards, but preferably by direct evidence of reduced yield or inferior quality of fruit from the results of carefully performed fertilizer experiments (55) when available.

4.34 INTERPRETATION OF LEAF ANALYSIS DATA ACCORDING TO OPTIMUM VALUES.

Having established the optimum values for the various nutrient elements, it becomes possible to evaluate leaf analysis data from orchards for which a nutrient diagnosis is required. By comparison with the standard values it will be evident which elements need adjustment. However, the deduction that a yield response will follow such adjustment is not always justified in view of the fact that the internal concentration level of one element and the response to an increase of it are often influenced by the concentration level of other elements and such interactions must be borne in mind in assessing the need for adjustments. According to Shear et al (176), the cation-anion ratio is also of significance since any change in the accumulation of cations must be balanced by an equivalent accumulation of anions (the

cation-anion ratio within the leaf being a constant), and many ions, organic and inorganic, are involved in these interactions besides the more common nutrient ions. These workers consider that "the significance of any leaf composition cannot be evaluated intelligently unless the interactions between the elements contributing to that composition are understood and considered. To attempt to alter the nutritional status of a plant without allowing for the interactions which will take place between the elements applied and those already available to or present in the plant may be futile. This may even reduce yield by creating a less favourable balance."

Lundegardh (118) has pointed out that analytical data may be wrongly interpreted when one element is limiting growth. With low concentrations of K and P in the soil, these nutrients may reach high values in the leaves and stems under conditions of N deficiency when plant growth is restricted and the organs remain small in relation to the absorptive area of the roots. Thus low N values although rare under field conditions provide warning against over-valuation of the K and P values.

Chapman and Brown (55) have found that a deficiency of either N, P, Mg, Ca, Zn or Fe was accompanied by a higher level of K in citrus leaves. Thus if the citrus tree is more lacking in some other element than in K, the latter will accumulate in the leaf giving the impression that K is well supplied and that the tree will not respond to K fertilization. This will not invalidate diagnosis by leaf analysis as long as a deficient supply of K will be reflected in percentage leaf K, but it will be difficult to predict what the K status will be when other limiting conditions are corrected.

Reuther and Smith (161) stressed the predominating influence of N in citrus leaf analysis interpretation: "if the probable level of N nutrition is not known by either actual leaf analysis or previous experience it is almost pointless to at-

tempt to classify the status of any of the other nutrients. When the N concentration in leaves is low or deficient P, S and K are likely to be higher and Mg lower than would be the case in the foliage of comparable trees adequately supplied with N. Abnormally high N concentration in leaves is usually associated with low concentrations of P, S and K and sometimes one or more of the heavy metals."

Low K values are nearly always associated with high Ca and Mg values. Deficiencies of Fe, Cu, Zn, Mn and Mo are typically associated with high N and K and low Ca in the leaves. Low B concentration in citrus leaves is likely to be associated with relatively high P and Mg and low K values (161), but in peach Mn and K were found to accumulate in the leaves when B was deficient (57, 95).

Broeshart (37) found that deficiencies of either N, P, K, Ca or Mg in oil palm leaves were accompanied by a decrease or increase in the content of one or more of the other elements. Thus a low content of a particular element in the leaf does not necessarily mean that it is deficient. On the other hand a medium to normal concentration of an element is no guarantee that it is not seriously deficient. He concluded that a satisfactory basis for the diagnosis of deficiencies from leaf analysis data is afforded by recognition of the characteristic deviations from the "normal" leaf composition of the contents of all the elements in the leaf tissue as indicated from the results of a large number of fertilizer experiments. Thus a correct interpretation of the analytical results will be facilitated by a knowledge of these characteristic deviations.

4.4 CONCLUSIONS.

Summing up the above, it appears that the chemical composition of a particular kind of leaf of a particular variety at

a definite stage of growth can be satisfactorily interpreted by comparison with standard optimum ranges based on data from high yielding trees in good performance orchards or fertilizer experiments. The optimum range with upper and lower limits of nutrient content evidently provides a more satisfactory basis for assessing the nutrient status and nutrient requirements than either threshold levels alone or mean optimum values since at the same time it takes account of the normal variation in nutrient content associated with maximum yield. Furthermore, critical ratios of nutrient elements based on leaf analysis are unlikely to be of greater diagnostic value than that provided by percentage content of individual nutrients although it is recognized that the level of other nutrient elements may modify the significance of the analytical data.

In applying diagnostic leaf analysis to problems of nutrition in the field it is necessary to bear in mind for the sake of perspective that in general a reliable estimate of tree response to fertilizer addition may be obtained with the aid of leaf analysis data when used in conjunction with other methods of diagnosis (217) such as visual evaluation of symptoms of deficiency or excess, diagnosis by means of the plant injection technique developed by Roach (163) or by nutrient test sprays, *and particularly* ~~as well as~~ inspection of the root environment and evaluation of pathological factors which may affect tree growth (120, 161, 215). Complete reliance on leaf analysis data may lead to erroneous conclusions and in any case as pointed out by Cain (52), "the mathematical precision with which leaf analysis data are sometimes interpreted for fertilizer requirements is unjustified in the face of the many factors affecting plant response whose influence is unknown or cannot be anticipated or controlled." Cain considers that to achieve the greatest efficiency of diagnostic techniques in forecasting fertilizer needs and response, a

sampling period of at least three years is needed followed by repeat samplings every second or third year. By observing the response to fertilizer treatment suggested by the first leaf analysis, further sampling will provide analytical data which can be more accurately interpreted in terms of adjustment of the rate of fertilizer application.

P A R T II

FORMULATION OF A LEAF SAMPLING
AND
ANALYTICAL PROCEDURE FOR DECIDUOUS FRUITS

1. INTRODUCTION.

It is evident from Part I that a great many factors besides nutrient supply may cause variation in leaf composition and thus influence the interpretation that may be placed on analytical data obtained for the purpose of evaluating the nutrient status of fruit trees.

The first objective in establishing an acceptable leaf analysis technique therefore is to eliminate or reduce to a minimum whatever errors can be avoided. Such errors comprise those which may arise from the human element during the course of selection of samples, time of sampling, preparation of sample for analysis and the analytical determination itself.

Having determined a reliable procedure for collecting samples on a tree and analyzing the leaves, it is possible to evaluate the influence of tree variation and the variation caused by seasonal, varietal and rootstock factors which may introduce errors which are partly unavoidable but which should be clearly recognized before attempting to draw conclusions on nutrient status.

These considerations set the pattern followed by the writer in attempting to develop a suitable technique of leaf analysis which would be applicable under local conditions. The experimental work on this project which was started in 1949 had to be fitted in with other work as time permitted and thus it has taken several years to complete this phase of the work. For the same reason the scope of the investigation had to be narrowed down to the extent that analysis of leaves only was considered.

1.1 PLANT ORGAN FOR ANALYSIS.

It is generally accepted that the leaf is the most suitable organ for analysis in the case of fruit trees (55, 79, 85, 198), as indicated also by Goodall and Gregory (85) in their discussion

on the relative merits of different plant organs for diagnostic analysis.

The organ selected of course would be the one which provides the widest differences in composition at varying levels of nutrition. Thus roots, stems, bark and fruit in turn may supply the most sensitive reflection of supply of a particular nutrient. Ulrich (206) found that petioles gave the best index of K and NO_3 in the grape, whereas K and P in bunch stalks as well as in petioles were found by Piaget (146) and Pienaar (148) to be more sensitive to nutrient supply than in the leaves. Fruit has also been found to be more sensitive as an index of the B status of a tree (89, 126). However, these organs usually provide a less satisfactory index of other nutrient elements, and since a complete picture of the whole nutritional complex is desired as is possible with leaf analysis, the latter is to be preferred. In addition to being suitable for the simultaneous determination of all the nutrient elements the leaves provide a sample which is easily accessible and can be quickly collected.

For the same reason, total analysis of the whole leaf was preferred to the more rapid tissue tests of Emmert (74), Nicholas (137) and others, which at best can have only a limited objective owing to the difficulty of accurately determining micro-nutrients at low concentrations in the sap of conducting tissues, such as stems, petioles and midrib. In the case of grape leaf sampling, considerations of expediency in handling and drying induced the writer to adopt the procedure of removing petioles when sampling, admittedly a concession to convenience.

The analytical results reported in this thesis thus refer without exception to whole-leaf samples in the case of deciduous fruit and to whole-leaf blades with petioles discarded in the case of grape leaves.

1.2 ANALYTICAL PROCEDURE.

There is no need to stress the necessity for the utmost accuracy and precision in analytical procedure. New methods and refinements in analytical technique both chemical and spectrographic, have greatly improved the accuracy and reproducibility of analytical results in recent years but it is all too often taken for granted that a good method is sufficient in itself whereas the best of methods in the hands of incompetent or careless analysts may fail to give reliable results. This of course applies with equal force to the whole sequence of steps which together make up the technique of leaf analysis from sampling to analytical result.

Another important consideration in any leaf analysis scheme is the time factor. In order to expedite analytical determinations several workers have resorted to quick methods based on extraction of plant sap in fresh conducting tissues (74, 137, 162). Apart from the fact that some of the functional elements, particularly micro-elements at deficiency levels, cannot be determined with sufficient accuracy in this way, the nutrients present in plant sap at best reflect only what is available at the time of sampling and thus may be subject to variation due to wide fluctuations in the soil as shown later for soluble N fractions (Table 15). This may be an advantage in the case of annuals when considering the flow of nutrients which the plant can obtain from the soil (73) at the time of analysis. With perennials, total analysis of the whole leaf determines the nutrients assimilated in the leaf tissue in addition to the soluble inflow, providing a reflection of a summation of the effects of environment over a long period and thus a more reliable index of the nutritional potential.

The analytical data presented herein, accordingly, are based entirely on total quantitative analysis of dried leaf tissue. Results are expressed as a percentage or parts per million of the oven-dry material which is considered to be the most suitable basis,

in preference to a fresh weight basis or to total nutrient content on an absolute basis which are unduly subject to variation in weight and size of leaf. The analytical methods employed at the W.P. Fruit Research Station in obtaining the data presented in this thesis were selected for accuracy and reproducibility of results, and in this selection the rapidity of carrying out determinations was an important consideration for obvious reasons.

1.3 METHODS OF ANALYSIS.

The writer was responsible for the experimental work reported herein and all phases of leaf sampling as well as preparation of leaf samples for analysis but not for the actual analytical determinations which were carried out by his colleagues in the analytical branch.

The elements Ca, Mg, K, Na, Fe, Mn and Cu were determined spectrographically and N, P, B and Cl by chemical methods. The spectrographic analyses were carried out by Dr. W.J. Pienaar, using the Hilger Littrow Quartz Spectrograph according to methods developed and perfected by him (147, 148). The percentage standard error for each element was found to be less than 6%.

The chemical determinations were carried out under the direction of Mr. A.J. Buys, the procedure briefly being as follows: Total N was determined according to a modification of the A.O.A.C. Kjeldahl method distilling into a saturated boric acid solution and using methylene blue indicator in the final titration with standard H_2SO_4 . For P, B and Cl the dry material was ashed with magnesium acetate and aliquots of the H_2SO_4 extract used for the determination of (a) P, according to the molybdenum-blue colorimetric method with hydroquinone or ammonium vanadate as reducing agent, using the Evelyn photoelectric colorimeter, (b) B, by addition of quinalizarin reagent and colorimetric measurement of the yellow colour in the Evelyn, and (c) Cl, by precipitating with $AgNO_3$ and titrating with KCNS. The percentage standard error

found for N and P was less than 3%, and for B and Cl about 10%.

As regards Zn determinations, which were recently begun using a polarographic method, no analytical data are presented here since there has not been sufficient time to test the accuracy of the results obtained.

Steyn (193) claims relatively high precision with the colorimetric and flame photometric methods used in analyzing citrus leaf samples. His percentage analytical error was calculated from data for 16 parallel determinations on different portions of a well mixed sample, evidently in consecutive aliquots. The reproducibility values in the analytical work of Pienaar (147, 148) and Buys (private communication) mentioned above, refer to precision in a broader sense embracing repetition of determinations over long periods of a year or more, and using different sets of standards and reagents; thus a much more exacting test and correspondingly more appropriate as a test of precision.

1.4 STATISTICAL TREATMENT OF DATA.

The statistical variability of data, significance of differences and analysis of variance were determined according to standard formula and methods as described by Saunders and Rayner (168) and Love (117).

2. PREPARATION OF SAMPLE FOR ANALYSIS.

Since the dried leaf material serves as the starting point for analytical determinations, factors which may cause a loss or gain in dry weight and thus influence the ratio of mineral content to dry material, must be considered in order to reduce the experimental error as far as possible.

Leaf contamination is a serious hazard capable of causing completely misleading analytical results particularly in the case of the micro-nutrients, and must obviously be avoided or eliminated.

2.1 CLEANING OF FRESH SAMPLES.

The removal of surface contamination is essential when dealing with the micro-nutrient content of leaves since a very small amount of contamination may cause large errors in analytical results. The main sources of extraneous deposits are dust and spray residues. The chief element in dust contamination likely to affect the analytical results is probably Fe, but Cu contamination of citrus leaves has been reported (193) and other micro-nutrients and Ca may also be present depending on the origin of the dust and the proximity to industrial works (85).

Quite recently, failure to recognize the contribution of Fe in dust to over-estimation of the nutrient-content, led to what may well be a doubtful concept of Fe immobilization in leaf tissue (218).

2.11 DUST DEPOSITS.

Removal of the dust film on leaves can be readily accomplished by washing or by wiping the leaf surface with damp muslin or cheesecloth. The latter procedure is unduly time-consuming and washing can be more satisfactorily carried out in practice when large

numbers of samples are involved. The possibility that washing may leach out some of the mineral constituents (85) has been investigated but no evidence of leaching losses occurred when the leaves were immersed for a short time (91, 138, 195). Washing with distilled water alone has been found inadequate to remove insoluble contaminants and recently various detergent solutions with or without dilute HCl have come into general use for cleaning leaf samples for analysis (34, 125, 138, 161, 180, 193, 195, 218).

Analytical results at Stellenbosch prior to 1953 occasionally indicated abnormally high Fe values. Leaf samples at that time were thoroughly washed in water only but the high Fe values obtained raised the suspicion that dust contamination was responsible and that the washing procedure was inadequate to remove all traces of dust. Accordingly the effect of including a liquid detergent, Agral LN, in the washing procedure was investigated.

Identical midshoot leaf samples from Kakamas peach and Wemmershoek apple trees were collected on 6/2/53 at the University farm, Welgevallen, at Stellenbosch, and treated as follows prior to drying and analysis:

- (A). Not washed.
- (B). Washed in 3 changes of tapwater.
- (C). Washed in a 0.1% solution of Agral LN followed by washing in 4 changes of tapwater.

The analytical results presented in Table 1 show certain significant effects of washing procedure as determined statistically by analysis of variance. The differences in Cl (peach) and B (apple), although significant at the .05 probability level, may be ascribed to analytical errors since they bear no relation to the conditions of the experiment. Both apple and peach samples show a pronounced and significant decrease in apparent Fe content after washing in tapwater and the Fe values are consistently further reduced when Agral was used. The high Fe values obtained in analyzing the unwashed leaves thus clearly indicated the presence of dust

**TABLE 1. - EFFECT OF WASHING PROCEDURES A, B AND C ON COMPOSITION OF PEACH AND APPLE LEAVES (6/2/53).
AVERAGE OF DUPLICATE SAMPLES, EXPRESSED AS PERCENTAGE OR PPM ON DRY WEIGHT BASIS.**

	N %	P %	K %	Ca %	Mg %	Mn ppm	Fe ppm	Cu ppm	B ppm	Na %	Cl %
<u>Peach</u>											
(A)	3.10	.132	2.63	2.13	.52	59	320	10.1	36	.042	.14
(B)	3.06	.129	2.58	2.19	.49	59	172	11.2	40	.041	.12
(C)	3.09	.129	2.40	2.33	.49	56	137	10.0	34	.038	.09
S.D. .05*							36				.03
<u>Apple</u>											
(A)	2.18	.177	2.32	1.57	.39	85	621	11.0	43	.039	.18
(B)	2.28	.179	2.36	1.62	.38	73	213	10.3	56	.033	.20
(C)	2.33	.169	2.36	1.46	.39	75	133	11.3	48	.032	.16
S.D. .05*							95		12		

*Significant difference at the .05 probability level.
**Decimal values are presented without the usual zero.

**TABLE 2. - EFFECT OF WASHING TREATMENTS B, D AND E ON COMPOSITION OF PEACH AND APPLE LEAVES (24/4/53).
AVERAGE OF DUPLICATE SAMPLES, EXPRESSED ON DRY WEIGHT BASIS.**

	N %	P %	K %	Ca %	Mg %	Mn ppm	Fe ppm	Cu ppm	B ppm	Na %	Cl %
<u>Peach</u>											
(B)	2.51	.113	1.71	2.94	.66	90	162	8.4	30	.039	.23
(D)	2.52	.115	1.76	2.82	.70	91	128	9.7	28	.037	.24
(E)	2.62	.116	1.74	2.78	.77	92	149	8.4	28	.039	.24
S.D. .05							32				
<u>Apple</u>											
(B)	1.69	.138	1.84	1.83	.47	71	278	11	38	.052	.28
(D)	1.68	.133	1.76	1.62	.49	68	216	14	35	.044	.26
(E)	1.68	.130	1.90	1.65	.49	67	255	12	40	.047	.29

contamination. Washing with the detergent was evidently also more efficient than washing with tapwater only and the Fe values obtained must therefore be much nearer to the true Fe content of the leaves.

Another detergent Teepol 410 (a sodium higher alkyl sulphate) which had come into general use as a spreader for spraying purposes and as a liquid soap, proved to be equally effective in removing dust deposits from leaves, as indicated in Table 2. The data were obtained from identical midshoot leaf samples collected at the University Farm on 24/4/53 and washed as follows:

- (B). 3 changes of tapwater.
- (D). Once in 0.15% Teepol solution, then 4 changes of tapwater.
- (E). Once in a solution containing 0.15% Teepol and 1% HCl, then 4 changes of tapwater.

The further addition of 1% HCl, frequently used to remove spray deposits, did not appear to provide any additional advantage over Teepol by itself and was actually less effective in removing Fe contamination. The Na content of the leaves was not affected by the presence of Na as a constituent of Teepol, so that washing once with a solution of 0.15% Teepol in tapwater followed by rinsing in tapwater seemed to be completely satisfactory.

Since leaf samples for analysis usually consist of about 120 leaves it was found convenient to wash each sample by hand in a large glass basin, 4 inches deep and 10 inches in diameter. Stirring by hand was adequate and obviates scrubbing with a handbrush as done by Smith et al (180). The sample is immersed in 3 litres of tapwater containing 5 ml. of Teepol and well agitated by hand for 3 minutes after which the soap solution is decanted off. Tapwater is introduced, stirring all the time and decanting as soon as the basin is full. Rinsing is repeated 2 or 3 times and the leaves finally shaken to remove excess water before inserting the sample in a clean paper bag and transferring to the drying oven.

The tapwater originally employed in washing leaf samples was relatively pure with an exceedingly low conductivity rating equivalent to that of distilled water. Since 1953 however when a new storage dam was inaugurated by the municipality, the conductivity of the water was found to show a higher rating and it was considered advisable to wind up the washing process with a final rinsing in distilled water even though tests on 9/2/54 and 11/3/54 failed to show any discrepancy in the analytical results which may have been associated with the use of tapwater only. As indicated in Table 3, the Ca and Cl values which are most likely to be affected by a change of water supply, do not show any difference due to rinsing in distilled water.

TABLE 3. - EFFECT OF RINSING IN DISTILLED WATER (D) AS COMPARED WITH TAPWATER (T) AFTER WASHING WITH TEEPOL, ON COMPOSITION OF PEACH (9/2/54) AND APPLE (11/3/54) LEAVES. AVERAGE OF REPLICATE SAMPLES EXPRESSED ON DRY WEIGHT BASIS.

	N %	P %	K %	Ca %	Mg %	Mn ppm	Fe ppm	Cu ppm	B ppm	Na %	Cl %
<u>Peach</u>											
(T)	2.86	.120	2.51	2.14	.43	183	120	6.7	31	.022	.12
(D)	2.76	.120	2.53	2.14	.43	187	123	7.2	32	.019	.12
<u>Apple</u>											
(T)	1.91	.110	2.07	1.24	.34	192	247	7.3	35	.034	.22
(D)	1.92	.113	2.01	1.24	.33	188	267	7.6	36	.030	.23

2.12 SPRAY RESIDUES.

Foliage sprays commonly used as fungicides, insecticides and nutrient sprays often contain mineral elements such as Zn, Mn, Cu,

Fe, and even N and P. Analysis of sprayed leaves will thus reflect that portion which presumably has been absorbed into the leaf cells as well as the residue adhering to the surface which is not removed by washing, as shown by the following assays:

Kakamas (24/10/50) and Boland (16/1/51) peach leaves with a normal content of less than 20 p.p.m. Mn gave values of 310 and 274 p.p.m. respectively after spraying with $MnSO_4$. Bosc pear leaves (29/10/51) analyzed after spraying with a Cu fungicide gave a Cu value of 240 p.p.m. as compared with 8 p.p.m. in the case of unsprayed leaves. Boland peach leaves (18/1/56) with a content of 6 p.p.m. Cu in unsprayed leaves, gave a value of 148 p.p.m. Cu two months after spraying with Cu and Zn. Waltham Cross grape leaves (18/11/54) sprayed with Zn, Mn and Cu gave values of 518 p.p.m. Mn and 288 p.p.m. Cu. The usual washing procedure removed the greater part of the spray residue as shown by the Cu values for Elberta peach leaves (7/12/55); leaves sprayed with Cu, not washed 350 p.p.m., after washing 92 p.p.m., as compared with unsprayed leaves, 17 p.p.m.

Just how much of the element found by analysis after washing is actually absorbed and active in leaf metabolism is difficult to determine.

In contrast to the micro-elements, N values are proportionately much less increased by foliage sprays containing N, as indicated by data for Early Dawn peach leaves sprayed with 0.5% and 1.0% urea solutions. Midshoot leaf samples were collected at the University farm, Stellenbosch, on 9/11/49 before applying the sprays on the same day. Subsequent samples were taken in the same positions on the trees over a period of 16 days. All samples were washed in tapwater prior to analysis. The results for total N are shown in Table 4.

TABLE 4. - TOTAL N CONTENT OF PEACH LEAVES BEFORE AND AFTER SPRAYING WITH UREA. PERCENTAGE ON DRY WEIGHT BASIS.

Date	Unsprayed	0.5% urea	1.0% urea
9.11.49	3.8	3.8	3.9
10.11.49	3.7	4.0	4.5
11.11.49	4.0	4.1	4.3
14.11.49	3.8	3.9	4.1
17.11.49	3.8	3.8	4.0
21.11.49	3.7	3.8	3.8
25.11.49	3.8	3.6	3.9

Spraying leaves which contained 3.9% N resulted in a maximum value of 4.5% N, 24 hours after application of 1.0% urea. Since the increment disappeared after 12 days there is no evidence here of a persistent residue effect due to the urea sprays. Even if a N residue amounting to a hypothetical N value of 500 p.p.m. did persist, it would not measurably affect leaf N values in the normal range of 20,000 to 40,000 p.p.m. Matlock and Childers (123) also found that the N content was not affected by N spray deposits.

It may be concluded that analytical values for micro-elements in leaves which have been sprayed with mixtures containing them are not trustworthy however thoroughly the leaves have been washed. Taylor (195) found that wiping leaves individually was superior to washing with a detergent to remove Fe spray residues from the leaves. Washing in an acidulated solution with or without a detergent is also fairly effective (34, 91, 161, 180, 219). Nevertheless, it is generally accepted that the portion of the spray

remaining in the leaf even after the best cleaning procedure is not all absorbed (34, 125, 177, 195, 219). According to Wallihan and Herschberg (219), there is a strong fixation of Zn on the leaf surface which cannot be completely removed by washing. Thus, as stated by Taylor (195), it appears that little credence can be placed in an analysis for any element which has been included in spray materials using the cleaning procedures employed at the present time. There is no evidence to doubt this standpoint in so far as the micro-elements are concerned, and accordingly analytical results in this thesis for such elements which have been applied in sprays prior to sampling are omitted altogether.

2.2 HANDLING OF FRESH LEAF SAMPLES.

During routine sampling work, a delay in drying the leaves is often unavoidable when collected at a distance from the laboratory. During the delay, they may become wilted and even desiccated before they can be cleaned and dried. In order to determine the effect of such delays, identical midshoot leaves from Kakamas peach and Wemmershoek apple trees were collected at the University farm, Welgevallen, on 9/2/54 and 11/3/54 respectively, and kept at room temperature for different periods before washing and drying. As indicated in Table 5, other factors having been kept constant, a delay of up to 5 days when the leaves were practically air-dry, had no measurable effect, as determined statistically by analysis of variance, on the nutrient content except Fe.

TABLE 5. - EFFECT OF DELAY IN WASHING ON COMPOSITION OF PEACH AND APPLE LEAVES. AVERAGE OF REPLICATE SAMPLES, EXPRESSED ON DRY WEIGHT BASIS.

Delay	N %	P %	K %	Ca %	Mg %	Mn ppm	Fe ppm	Cu ppm	B ppm	Na %	Cl %
<u>Peach</u>											
Direct	2.85	.117	2.71	2.00	.430	214	126	6.9	31	.017	.13
24 hrs.	2.86	.120	2.51	2.14	.433	183	120	6.7	31	.022	.12
5 days	2.90	.121	2.52	2.22	.455	212	228	8.1	31	.020	.12
S.D. .05							35				
<u>Apple</u>											
Direct	1.93	.119	2.11	1.21	.340	197	222	7.1	40	.029	.23
24 hrs.	1.92	.112	2.01	1.24	.330	189	267	7.6	36	.030	.23
5 days	1.93	.112	1.99	1.21	.345	204	316	8.1	34	.032	.17
S.D. .05							67				

The marked apparent increase in Fe content with delayed washing may be explained, as discussed in Section 2.11, as being due to incomplete removal of Fe-containing dust from the leaves which after 4 or 5 days were comparatively dry, preventing thorough cleansing of the leaf surfaces.

There was no evidence here of a general apparent increase in nutrient content which as found by Smith (178), Goodall and Gregory (85) and Steyn (193) may result from dry weight losses through respiration prior to drying. As a precaution however, leaf samples collected at some distance from the laboratory should be kept cool and preferably refrigerated to reduce the rate of respiration. This would also tend to retard desiccation of the leaves which in any case must be prevented, as the results show.

2.3 USE OF PAPER BAG CONTAINERS.

It is necessary at this stage to point out that brown paper bags have been used throughout this investigation not only to convey fresh leaf samples to the laboratory but also to hold the cleaned samples during the drying process. After washing and final rinsing in distilled water the last drops are shaken off and the leaves placed in clean paper bags which are then immediately transferred to the drying oven. Quick transfer after washing was considered necessary to prevent loss of mineral substances by leaching.

It was observed that even after shaking off most of the distilled water and quickly transferring to the oven, some moisture collected in drops on the leaves and ran down to the bottom of the paper bag. After removal from the oven the paper was usually found to be slightly stained, suggesting the possibility of extrusion of cell sap from the heated leaf tissue. Since the discoloration might conceivably include mineral substances which may have leached out of the leaves during the initial stages of drying, this point was investigated by drying leaf samples in paper bags and in porcelain dishes. Identical midshoot leaf samples were collected from Kakamas peach trees at the University farm and treated in duplicate as follows:-

- (A) Samples washed as usual in Teepol solution and rinsed in distilled water, shaking off the last drops and transferring immediately in porcelain dishes to the drying oven.
- (B) As for (A) using paper bag containers.
- (C) Samples placed in paper bags without shaking off drops of water and left for 3 hours at room temperature before transferring to the oven.

With regard to Treatment (C), it was observed that considerable

run-off occurred in the paper bags staining the whole of the base, whereas only a few small stains resulted from Treatment (B).

The analytical results for Treatments (A) and (B) presented in Table 6 are practically identical, indicating that the composition of the samples, as regards the nutrient elements at any rate, was not altered by using paper containers according to the routine procedure.

TABLE 6. - COMPOSITION OF PEACH LEAVES
AS AFFECTED BY TREATMENTS A,
B AND C (SEE TEXT). DRY
WEIGHT BASIS.

Nutrient	(A)	(B)	(C)
N %	2.71	2.73	2.77
P "	.113	.111	.113
K "	1.70	1.72	1.72
Ca "	2.39	2.35	2.41
Mg "	.46	.47	.46
Na "	.023	.022	.024
Mn ppm	76	74	75
Fe "	172	176	203
Cu "	5.2	5.1	5.5
B "	30	25	50

Regarding Treatment (C), the extensive run-off and staining of the paper prior to drying did not decrease the concentration of K and Na which of all nutrient elements are most likely to be lost by leaching. Presumably some organic substance does leach out from the leaves to produce the dark coloured stains since an empty paper bag soaked in water developed no more than a pale brown stain after drying in the oven.

However, the most striking result of Treatment (C) was the marked increase in the Fe and particularly in the B values as compared with the standard Treatment (B). The higher B values for Treatment (C) can only be ascribed to contamination from the paper bags used after washing, which according to a recent report may contain a certain amount of B. Winsor (226) drew attention to the fact that B is used in modern paper manufacture and that it also forms an ingredient of the adhesives used in making paper bags. He found that soil samples, when damp and dried in paper bags, picked up 100 to 800% of their content of B from that present in the paper.

The possibility of such B contamination is a potential risk if wet leaves are kept in contact with brown paper for some time before drying as in Treatment (C). The increase in Fe content may conceivably have been picked up in the oven from the metal shelf through the moist paper. In view of these results it has evidently been a wise precaution to shake off the drops thoroughly from the leaves and immediately transferring to the oven although the original purpose was to prevent leaching from the leaves and not contamination from the paper. The procedure of conveying fresh leaf samples to the laboratory in paper bags may introduce a further hazard although sampling conditions rarely involve moist or wet leaves which would exclude the possibility of contamination.

A substitute for brown paper containers such as cloth bags for fresh samples and muslin for drying, as used by Steyn (193), is considered advisable even though no evidence of contamination resulted from the existing procedure. As a further precaution the metal shelves in the drying oven should be covered with blotting paper.

2.4 DRYING OF LEAF SAMPLES.

In routine analytical work it is of great advantage to convert the fresh sample material into a stable dry condition which would be unaffected by enzyme action and be suitable for storage until such time when the samples can be conveniently analyzed. The accuracy of analytical data expressed as a percentage of the dry material is directly influenced by the dry weight determination; the term "dry weight" implying moisture-free material. In practice the moisture is usually removed by some form of heating but experience has shown that it is most difficult to remove the last traces of moisture without some caramelization and even decomposition of the plant material. Thus the "dry weight" may vary to the extent that moisture may be incompletely removed due to inadequate heating or that some loss of weight (decomposition) may occur due to excessive heating. This will be reflected in a lower or higher percentage content of the nutrient elements.

During the course of analytical work on orange pulp and rind in 1934 the writer found a much higher loss in weight when drying at 100° C than at 70° and 50° C. Moreover both materials continued to lose weight over a period of 5 days of drying at 100° C, suggesting thermal decomposition. Samples dried at 70° C however showed relatively little change in weight after 24 hours of drying, the loss in weight for rind increasing gradually from 65.0% after 24 hours drying to 65.5% after 5 days, and that for pulp increasing from 86.6% to 87.1% over the same period. Drying samples at 50° C required an unduly long period to attain a fairly constant dry weight.

Consequently when the work on leaf analysis was commenced in 1949 it was assumed that a drying treatment of 2 days at 70° C, consistently applied, would provide a satisfactory dry weight basis for routine analysis. Since a convection type of electrical oven was used, this was modified to the extent of holding the

oven temperature at 90° to 100° C for a short time, usually about 30 minutes, to drive off excess moisture at the beginning of the drying period when a large batch of samples had to be dealt with.

A further precaution observed in ensuring a reliable dry weight basis was that, since dried material showed signs of stickiness during the grinding process, leaf powder samples were redried overnight (18 hours) at 70° C as a regular practice prior to analysis.

Reference to the literature on the subject of drying techniques, indicates that most workers favour a drying temperature of 60° to 70° C, but the only critical study made until now appears to be that recently presented by Steyn (193) in a comprehensive investigation of the errors involved in the various steps from sampling to analysis of citrus and pineapple leaves. According to Steyn, the object in drying plant material for analysis must be to apply a sufficiently high temperature to remove moisture and to destroy enzymes but not high enough to induce appreciable thermal decomposition. Steyn found that thermal decomposition becomes increasingly predominant over moisture loss at temperatures above 50° C. Although citrus leaf samples can be satisfactorily dried at 50° C in a forced draught oven the dried material may not be stable since some enzymes will only be destroyed at a temperature above 60° C as shown by further loss in weight during storage of citrus samples which had been dried at 50° C. Steyn finally adopted 65° C as the drying temperature since after drying at this temperature there was no evidence of enzymatic activity and thermal decomposition was probably less than 1%. He also found that citrus leaf powder picked up 3 to 5% moisture during the grinding process and that redrying was therefore necessary. Since leaf powder proved to be much more susceptible to thermal decomposition than the fresh material and

lost weight rapidly at 105° C, he adopted the procedure of drying leaf powder at 65° C for 24 hours.

The leaf drying procedure as used in the present investigation for deciduous fruit is thus in close agreement with that proposed by Steyn for citrus leaves, the only material difference being a slightly higher drying temperature, namely 70° C, for both fresh material and leaf powder. The drying treatments as to temperature and duration of drying, were consistently applied as a standardized procedure and as such may be expected to provide a strictly comparable dry weight basis.

2.5 GRINDING OF DRIED MATERIAL.

The dried leaves must be reduced to a uniform powder to provide fully representative aliquot samples for analysis. Considering the large amount of material which had to be handled, mechanical grinding was imperative and a Wiley Intermediate mill was used at first, grinding to a fineness sufficient to pass the 40 mesh sieve. The error due to metal contamination particularly Fe and Cu from the use of the Wiley mill was considered to be insignificant since there was practically no difference in mineral composition between identical apple leaf samples ground in the Wiley mill and in a porcelain mortar as shown in Table 7.

TABLE 7. - EFFECT OF GRINDING DRY APPLE LEAVES IN A WILEY MILL (A) AND IN A PORCELAIN MORTAR (B) ON THEIR COMPOSITION.

	N %	P %	K %	Ca %	Mg %	Mn ppm	Fe ppm	Cu ppm	B ppm	Na %	Cl %
(A)	2.45	.099	1.27	.90	.33	27	64	4.0	35	.044	.12
	2.53	.093	1.27	.85	.32	28	67	4.4	30	.042	.16
(B)	2.44	.095	1.17	.93	.33	25	66	3.7	31	.045	.10
	2.42	.098	1.20	.97	.33	26	64	4.0	32	.044	.12

Very rarely, however, occasional high Cu values occurred during the analytical work which Dr. Pienaar (private communication) found to be due to fragments of copper wire present in the leaf sample which evidently had broken loose from a sieve, so that as a precaution sieves were frequently replaced. Further evidence showed that samples ground in a porcelain ball mill contained slightly less Fe than the same sample after regrinding

in the Wiley mill, so that the possibility cannot be excluded that leaf powder may pick up traces of metal from knife blades and sieves during the grinding process when using a Wiley mill.

In order to eliminate possible error from this source all leaf samples have for some time been ground solely in a porcelain ball mill. This precaution is in accordance with the experience of Goodall and Gregory (85) and Steyn (193) who cite cases of serious Fe and Cu contamination from the Wiley mill. Steyn could find no difference in composition of citrus leaf samples ground in an agate ball mill as compared with an agate mortar and pestle.

2.6 STORAGE OF LEAF POWDER.

The final stage of leaf sample preparation consists of transferring the oven-dried material to air-tight glass containers, grinding the contents and storing in cupboards until the analytical determinations can be carried out. It is generally assumed that such sterilized moisture-free leaf powder may be stored indefinitely without change of composition. Steyn (193) however produced evidence indicating that citrus and pineapple leaf powder was subject to decomposition resulting in appreciable loss of N. He found that citrus leaf powder lost 1.2% of its N after two months storage and that this loss gradually increased to 10% after 5 months storage. On the basis of these results he concluded that citrus leaf powder cannot be safely stored for longer than two months prior to analysis.

This point was checked at Stellenbosch by repeating N determinations on half a dozen samples which had been stored for longer than six years. The samples were selected at random from different fruit species as indicated in Table 8. The data show a small but consistent decrease from the original values, confirming the finding that leaf powder loses N during storage although not to the extent as found by Steyn.

TABLE 8. - EFFECT OF STORAGE ON N CONTENT OF LEAF POWDER.

Sample No	Variety	Original analysis		Repeat analysis		% Decrease
		Date	% N (D.M.)	Date	% N (D.M.)	
588	Apple	7/2/51	2.33	12/8/58	2.30	1.3
773	Pear	22/4/52	2.06	"	2.00	2.9
651	Peach	30/1/52	2.82	"	2.63	6.7
689	Apricot	25/4/52	2.02	"	1.95	3.5
745	Prune	26/3/52	2.34	"	2.14	8.5
683	Orange	21/3/52	2.24	"	2.20	1.8
Mean						4.1

The average reduction in N content over the six-year period amounted to 4.1%. Accordingly, the N loss over a period of six months, which is normally ample to complete the analytical determinations, may be expected to be considerably less and unlikely to influence the interpretation of results.

Furthermore, there was no evidence of change in the content of K, Ca, Mg, Fe, Mn, Cu and Na for several samples originally analyzed in 1955 which Dr. Pienaar (private communication) happened to re-analyse two years later, in 1957, so that the dry weight evidently did not change during this time

There appears to be no reason to question the validity of analytical data for samples which have been stored prior to analysis according to the existing procedure but as a precaution it is evidently desirable that N determinations should be carried out without undue delay.

2.7 PROCEDURE ADOPTED.

Leaf samples are collected in clean brown paper bags or preferably cloth bags and transferred to the laboratory as quickly as possible. A delay of a few days necessitated by sampling at some distance from the laboratory is no disadvantage provided the samples are kept cool and refrigerated if possible.

The fresh leaves are cleaned by washing with a detergent and water. A glass basin with a diameter of 10 inches and 4 inches deep has been found satisfactory for handling samples of 100 to 120 leaves. Each sample in turn is agitated by hand for 3 minutes in 3 litres of a solution of 0.15% Teepol 410, the liquid is decanted off and the sample rinsed in two changes of tapwater and finally in distilled water.

After the final rinse, drops are shaken off as thoroughly as possible, the sample is placed in a clean paper or preferably muslin bag and transferred directly into an electric drying oven. When a large batch of samples are washed the drying temperature is held at about 90° C until the last sample is ready when the temperature is allowed to settle down to a constant level at 70° C. The drying period is 48 hours for a convection-type oven and 24 hours for a forced-draught oven.

The dried leaves on removal from the oven are transferred to airtight glass containers, ground in a porcelain ball mill and stored in a dark cupboard. The leaf powder is re-dried at 70° C for 18 hours before weighing out aliquots for analysis. Nitrogen determinations should be carried out without undue delay.

Analytical values for micro-nutrients obtained by analysis of leaves previously sprayed with mixtures containing them, are not admissible as data for nutrient status evaluation.

3. SELECTION OF THE LEAF SAMPLE.

Since leaves vary considerably in composition from one position to another on a tree, it is necessary to select comparable samples the composition of which is not influenced by positional effects which may prejudice the interpretation of the analytical data when used for diagnosis of the nutrient status.

The influence of sampling at different times during the day and of the personal factor in sampling will also be considered in this chapter.

3.1 POSITION OF LEAF ON SHOOT.

Leaves on shoots of deciduous fruit trees are readily accessible whatever the species, and as such are convenient to collect. Leaf composition, however, varies according to the position of the leaves on a shoot as found in the case of Elberta peach leaves picked on the same day at Tulbagh (2/3/49) from the tip, middle and base of shoots (Table 9).

TABLE 9. - COMPOSITION OF LEAVES FROM DIFFERENT POSITIONS ON ELBERTA SHOOTS, EXPRESSED AS % OF DRY MATERIAL.

Sample	N	P	K
Terminal leaves	1.95	.201	1.47
Midshoot "	1.70	.181	1.81
Basal "	1.65	.172	2.23

The analytical data in Table 9 show a gradient in N and P content increasing from basal to terminal leaves while K shows a marked trend in the opposite direction. Frear et al (79)

found similar gradients in peach shoot leaves. According to McClung and Lott (126), N, Mg, Zn and Cu showed a consistent gradient decreasing from terminal to basal leaves; Ca, Fe and Al were lower and B higher in terminal than in basal and midshoot leaves; P and Mn were not measurably different.

These differences may be expected to be reflected to a varying degree in the composition of random samples. In Table 10 the analytical values for random leaf samples, collected from 3 Kakamas peach trees and 2 individual Golden Delicious apple trees at the University farm, are compared with corresponding values for midshoot leaf samples from the same trees. Marked differences in composition are shown as to most of the nutrient elements. It is obvious that variation in random samples may occur in any direction depending on the proportion of leaves which may happen to be included from different positions. This can be avoided by selecting only leaves from a particular position on the shoots.

In the sampling study by Frear et al (79) it was found that the basal leaves were most suitable for estimating the level of K supply available to the trees, but that terminal leaves furnished the highest degree of correlation between leaf K and potash application. Goodall (84) thought that the spur leaves were superior to basal shoot leaves for the diagnosis of K deficiency in apple trees. He also studied the suitability of basal, middle and apical spur leaves and basal shoot leaves but could find no evidence for choosing one type of leaf rather than another for diagnosis of Ca, Mg, K, Mn and Fe status.

Shoot leaves, however, should be preferred to spur leaves if only for the reason that spur leaves may be hard to find on certain kinds of fruit trees and on young trees. Moreover, shoot growth is continuous over a portion of the season and is more likely to provide sound leaves suitable for analysis. During the period of active growth, shoots also provide new

TABLE 10. - COMPOSITION OF MIDSHOOT (M) AND RANDOM (R) LEAF
SAMPLES FROM THE SAME TREES (19/2/52). DRY
WEIGHT BASIS.

Nutrient	Peach		Apple A		Apple B		Max. % Diff.
	M	R	M	R	M	R	
N %	2.94	2.90	2.36	2.38	2.46	2.38	3
P %	.120	.126	.089	.125	.114	.105	40
K %	3.01	2.86	1.70	1.65	2.10	2.22	6
Ca %	2.20	1.83	1.26	1.70	.96	1.16	35
Mg %	.44	.49	.15	.23	.23	.30	53
Na %	.036	.033	.040	.033	.029	.024	18
Cl %	.18	.16	.17	.27	.13	.12	59
Mn ppm	63	54	70	86	60	61	23
Fe ppm	227	193	190	158	140	144	17
Cu ppm	23	13	21	27	16	18	43
B ppm	24	25	22	28	26	25	27

leaves of the same physiological age. Leaf growth on spurs is more nearly determinate and thus subject to aging and damage which may complicate sampling later in the season.

As pointed out by Goodall and Gregory (85), basal or terminal leaves may provide better indices of deficiency depending on the mobility of the nutrient in the plant. Thus for the highly mobile K and to a lesser extent N and P, the older leaves would be preferable, whereas for Ca and B which are not at all readily remobilized the younger leaves would provide a better reflection of a deficiency.

Selection of basal or apical leaves may involve sampling leaves which are severely chlorotic or necrotic. Young terminal leaves are invariably more severely affected with chlorosis or necrosis due to Zn, Cu or Fe deficiency, with dwarfing due to Zn deficiency or

with necrosis due to Na or Cl toxicity, whereas basal leaves may be severely chlorotic or necrotic as a result of Mn, Mg or K deficiency. The following cases illustrate the impression obtained from analyzing such samples as compared with midshoot samples on the same tree (Table 11):

- (A) Ohenimuri apple leaves (Stellenbosch, 2/3/55), small and chlorotic varying from midshoot to terminal leaves on shoots showing Zn deficiency symptoms.
- (B) Golden Delicious apple leaves (Stellenbosch, 11/3/54) from base of shoots, all affected with chlorosis due to Mg deficiency.
- (C) W.W. Pearmain apple leaves (Ceres, 25/1/56), all severely affected with marginal necrosis due presumably to Mg deficiency. (This sample was not washed which accounts for its relatively high Fe value.)

The analytical results in Table 11 show that the content of several nutrients in affected leaves differs widely from that of midshoot leaves, although some but certainly not more than a small part of this difference may be ascribed to positional effect. A more definite diagnosis of the deficiency concerned is furnished by the relatively low values for Mg, but it is evident also that the level of other nutrients in the affected leaves is unduly disturbed so that the composition of the midshoot leaves would more correctly reflect the general nutritional condition of the trees in question. Goodall (84), comparing the mineral content of scorched and healthy leaves from the same apple tree, found Mg and Mn to be significantly lower in severely scorched leaves and considered that the latter should be avoided in sampling. He found no significant difference in composition between slightly scorched and healthy leaves.

TABLE 11. - COMPOSITION OF MIDSHOOT LEAVES (M) COMPARED WITH THAT OF LEAVES SHOWING SEVERE SYMPTOMS OF NUTRIENT DEFICIENCIES (A, B AND C) ON THE SAME TREES. DRY WEIGHT BASIS.

Nutrient	Ohenimuri		G. Delicious		W.W. Pearmain	
	M	A	M	B	M	C
N %	2.32	1.97	1.92	1.65	1.82	1.60
P %	.101	.064	.119	.114	.103	.081
K "	1.49	1.22	1.35	1.57	3.16	3.33
Ca "	.88	.78	.99	1.09	.48	.22
Mg "	.39	.38	.21	.15	.33	.11
Na "	.021	.031	.029	.037	.006	.034
Cl "	.14	.15	.17	.23	.07	.09
Mn ppm	36	20	259	331	29	14
Fe "	75	72	126	145	44	123
Cu "	4.4	3.1	4.9	4.6	4.7	5.2
B "	29	30	26	30	47	54

It may be concluded that it will be difficult to find a single position for sampling which will be optimal in all respects. Basal or apical leaves on shoots may be preferable for deficiency diagnosis of particular nutrients but such leaves are also more likely to reflect a nutrient content differing from the general nutritional condition of the tree. Midshoot leaves would thus appear to be the best choice and more likely to provide a suitable sample under adverse conditions for studying the status of all nutrients. Since a single sampling position had to be selected for the present investigation, the writer decided to concentrate on midshoot leaf samples.

In collecting samples, trees and leaves showing mechanical

damage or pathological symptoms were consistently avoided. Thomas et al (199), in studying the composition of diseased and healthy leaves from the same peach tree infected with *Bacterium pruni*, found that the concentration of N, P, K, Ca and Mg was significantly lower in diseased leaves, indicating a rate of metabolism similar to that of leaves undergoing the normal process of senescence. Boynton and Compton (32) also state that any conditions resulting in inability of the root system or conducting tissue to function normally will be likely to reduce the K, Mg and N content in fruit tree leaves.

The above considerations apply to leaf samples from deciduous fruit trees. In the case of grape vines, leaf position was not specifically investigated. As a tentative procedure based on practical considerations of ease of sampling, the basal leaves were provisionally selected as the sample for analysis since intertwining of canes and the practice of topping of shoots would complicate satisfactory sampling in other positions, whereas basal leaves provide a definite position where comparable samples can be conveniently picked. Actually the first normal sized leaf not higher than the fifth node ^{from} ~~at~~ the base of fruiting shoots, is selected on each of two or three branches per vine, collecting about 30 to 40 leaves per sample.

3.2 SELECTION OF SHOOTS ON TREE.

A certain amount of variation in composition of midshoot leaves may arise depending on the position of the shoots from which the leaf sample is collected. A comparison was made of terminal shoots on one-year old wood with shoots arising from older wood which usually are more vigorous and may be referred to as "vegetative" shoots. Midshoot leaf samples were collected from terminal and vegetative shoots on the same trees, namely Royal apricot (2/1/53), Alma apple (15/1/53) and Bon Chretien pear (30/1/53), at the University farm, Stellenbosch.

TABLE 12. - COMPOSITION OF MIDSHOOT LEAVES ON TERMINAL SHOOTS (T) AS COMPARED WITH THAT ON VEGETATIVE SHOOTS (V) ON THE SAME TREES. DRY WEIGHT BASIS.

Nutrient	Apricot		Apple		Pear		Max. % Diff.
	T	V	T	V	T	V	
N %	3.33	3.11	2.58	2.44	2.63	2.48	7
P "	.145	.158	.172	.288	.140	.147	67
K "	3.43	3.83	2.07	2.60	1.52	1.78	25
Ca "	1.35	1.48	1.58	1.59	1.36	1.30	10
Mg "	.37	.39	.28	.25	.30	.27	11
Na "	.025	.025	.038	.037	.036	.033	8
Cl. "	.15	.14	.22	.16	.10	.11	27
Mn ppm	54	59	86	83	99	82	17
Fe "	110	115	174	173	135	151	12
Cu "	6.3	5.7	8.3	8.0	12	12	9
B "	35	37	35	36	26	31	19

The analytical results in Table 12 show reasonably good agreement in some of the nutrient values as between the two sets

of samples, but wide differences also occur such as for P, K and Cl in the apple leaf samples, indicating that the values for vegetative shoots deviate sufficiently from those for terminal shoots to bias interpretation of the results. Consequently it was decided to avoid vegetative shoots as well as excessively vigorous and weak terminal shoots when collecting leaf samples. In this connection it may be mentioned that Matlock and Childers (123) found that spur leaves from weakly growing spurs also contained less Ca, K and Mn than did leaves from spurs of good vigour.

To represent the nutritional condition of the tree as a whole, a leaf sample should obviously be drawn uniformly from shoots around the periphery of the tree. The practice was further adopted of taking the sample more or less at shoulder height to avoid possible gradients from top to bottom.

In studying such positional effects in relation to composition of citrus leaves, Chapman and Brown (55) compared samples from different positions around the tree but found no difference in composition except that the Ca concentration tended to be a little higher in leaves picked on the South and West sides. Bathurst (6) produced evidence showing that the N content of leaves on the northern aspect was significantly higher than that on the South side. Chapman and Brown also found that leaves from the top of the tree were somewhat lower in N, P and K and slightly higher in Ca and Mg than were leaves from the middle and lower parts of the tree.

These findings emphasize the necessity of collecting samples at regular intervals around the tree and only at a definite height, the middle part around the periphery of the tree evidently being the most convenient.

3.3 EFFECT OF FRUIT CROP ON LEAF COMPOSITION.

Since K and B (89) and possibly other nutrients accumulate in fruit it is quite possible that the size of the crop on a tree may influence the concentration of nutrients in the leaves.

Evidence in this connection was produced by Lilleland (113). Leaf data from paired adjacent prune trees, one of which was completely defruited, showed that as the season advances the difference in P levels becomes increasingly greater. Early season leaf levels of 0.22 and 0.21% become 0.25% and 0.13% for non-bearing and bearing trees, respectively.

Lilleland and Brown (114) found that the K content of leaves on heavily bearing peach trees was lower than on defruited trees. By defruiting the trees they showed that with practically no difference in leaf K at the beginning of the season the non-fruiting trees show an ever-increasing leaf level as the season advances while the bearing trees show a decrease. May values of 1.12% and 1.18% become 0.98% and 1.66% in August for bearing and non-bearing trees, respectively. In their survey of leaf K in California peach orchards they also found that several of the orchards with highest leaf K had the lightest crops.

According to McClung (126) the fruit crop had little effect on the nutrient composition of peach leaves but K was lower and Ca and Mg slightly higher in leaves from trees with a crop. Havis and Gilkenson (94) found that heavy pruning usually increased leaf K in peach.

In the case of citrus, Chapman and Brown (55) could find no difference in K content of leaves from fruit bearing twigs as compared with non-fruit bearing twigs. However the concentrations of Mg, K, N and P in leaves from bearing trees were all slightly lower than those in leaves of trees from which all fruit had been removed. They considered that the differences were not large enough to affect the interpretation of the results.

In studying biennial bearing in Miller's Seedling apple, Mason (122) found highly significant differences in the composition of leaf samples from the terminal portion of shoots, taken at the same time from fruiting and non-fruiting trees. The N, P, Ca, Mg and Mn contents were all higher in leaves of trees in their "on" year but there was no significant difference in the Fe or K content.

Regarding grapes, Cook (58) reported that several of the highest yielding vineyards showed increasingly lower P levels in the leaves as the season advanced, and lower yielding vineyards had higher P values.

The evidence on the whole thus indicates that leaf composition will be influenced by size of crop on the trees sampled and when there is a tendency to biennial bearing, but there is no agreement in the work reported above as to a consistent effect of size of crop on concentration of individual nutrients.

The only contribution the writer can offer on this subject is that occasional pronounced differences in leaf composition were found when vegetative shoots were compared with terminal shoots around the tree, the latter invariably being fruit bearing shoots (Table 12). On the other hand, removal of the fully matured crop did not appear to alter the seasonal nutrient trends in Kakamas peach leaves (Fig. 5).

Until further data become available it will have to be recognized that variation in yield may have a potential influence on leaf composition and that when considering the nutrient status of trees varying markedly from the average in production, it cannot at present be predicted exactly to what extent the analytical values will be biased.

3.4 DIURNAL VARIATION.

Since the rate of carbon assimilation changes according to the light intensity during the day (140), the nutrient content may likewise vary in relation to changes in the dry weight of the leaves. As indicated by Goodall and Gregory (85), the concentration of N and some of the mineral elements has been found to fluctuate during the day but most of these variations have been observed as occurring in the plant sap. In a recent investigation Steyn (193) produced evidence of a small increase in nutrient concentration of lemon leaves from 7 A.M. to midday. The percentage content of N, P, K and Ca increased by 4.3, 2.5, 4.0 and 2.5% respectively.

As no data on diurnal variation in the nutrient content of the leaves of deciduous fruit trees were available, this question was investigated by the writer at Stellenbosch. Comparable mid-shoot leaf samples were collected from the same shoots at three different times, 9 A.M., midday and 5 P.M., which would more than cover the period during which sampling would normally be carried out. Samples were obtained at the University Farm for each of the sampling times, from

- (A) Kakamas peach trees on 15/1/53
- (B) " " " " 6/3/53
- (C) Alma apple trees on 15/1/53
- (D) Granny Smith apple trees on 6/3/53.

The analytical values agree very closely, as indicated in Table 13, and statistical treatment of the results by analysis of variance showed that there were no significant differences due to time of sampling during the day. Nor was there any evidence of a general trend towards higher values from 9 A.M. to 12 noon similar to those found by Steyn (193), except perhaps for K which increased slightly in three of the sampling groups. Comparison of the means indicates that the differences for the

TABLE 13. - COMPOSITION OF COMPARABLE PEACH (A AND B) AND APPLE (C AND D) MIDSHOOT LEAF SAMPLES COLLECTED AT DIFFERENT TIMES DURING THE DAY. DRY WEIGHT BASIS.

Variety	Nutrient	9 A.M.	Noon	5 P.M.	Nutrient	9 A.M.	Noon	5 P.M.
A	N %	3.33	3.28	3.28	P %	.156	.155	.158
B		2.97	2.80	2.99		.111	.111	.119
C		2.56	2.48	2.44		.173	.162	.156
D		2.10	2.09	2.13		.097	.104	.100
Mean		2.74	2.66	2.71		.134	.133	.133
A	K %	2.68	2.86	2.51	Ca %	1.83	1.83	1.84
B		2.70	2.86	2.71		2.26	2.32	2.43
C		2.07	1.95	2.16		1.65	1.59	1.56
D		1.79	1.95	1.86		.82	.87	.93
Mean		2.31	2.41	2.31		1.64	1.65	1.69
A	Mg %	.42	.45	.41	Na %	.027	.023	.025
B		.52	.55	.53		.025	.027	.026
C		.27	.25	.26		.040	.037	.038
D		.22	.24	.26		.031	.032	.035
Mean		.36	.37	.37		.031	.030	.031
A	Mn ppm	53	55	53	Fe ppm	146	146	137
B		66	72	67		193	192	234
C		85	81	86		181	172	161
D		60	62	64		190	224	211
Mean		66	68	68		178	184	186
A	Cu ppm	7.0	6.8	6.7	B ppm	30	29	28
B		7.1	7.1	7.1		56	55	55
C		9.0	8.0	9.0		35	38	32
D		6.2	6.7	6.4		32	32	30
Mean		7.3	7.2	7.3		38	38	36

other nutrients were negligible so that the not very conclusive trend for K may be merely due to chance. Accordingly, it may be concluded from this evidence that sampling can be safely carried out at any time between 9 A.M. and 5 P.M. without prejudicing the interpretation of results.

3.5 SAMPLER ERROR.

The leaf samples from which the analytical data reported in this thesis have been obtained, were all collected personally by the writer. Duplicate samples taken over a considerable period have consistently shown very close agreement in analytical values. In fact the values usually varied so little that taking duplicate samples as a regular routine was abandoned for reasons of economy and saving of time.

As an example of the reproducibility in analytical results obtained by successive sampling of the same trees, the values presented in Table 13 may be referred to. Midshoot samples were collected at 9 A.M., midday and 5 P.M. but since the results showed no significant differences it may be concluded that besides indicating no significant diurnal variation in composition there was also no significant difference between the triplicate samples when collected by one person as in this case.

There is evidence, however, that significant errors may be introduced by allowing other persons to collect samples (6). Comparison of deviations in composition of midshoot leaf samples collected from the same trees by the writer and a colleague serves to confirm that even a standardized system of sampling may be subject to personal errors. Four parallel sets of composite samples were obtained from replicated fertilizer plots in an experimental block of W.W. Pearmain apple trees at Ceres (24/4/56). The analytical results in Table 14 indicate that the samples collected by sampler (B) differed quite considerably in composition from those collected by the writer (A). The mean percentage deviation calculated by averaging the deviations from the A values for each group of trees as a percentage of the mean of the (A) values, vary from 1.6% to 13.8%. In comparison the mean percentage deviation in composition of triplicate samples collected by the writer at Stellenbosch (from Table 13) showed

TABLE 14. - COMPARISON OF DEVIATIONS IN THE COMPOSITION OF MIDSHOOT APPLE LEAF SAMPLES COLLECTED FROM THE SAME GROUPS OF TREES BY SAMPLERS A AND B WITH THOSE IN PEACH SAMPLES COLLECTED BY SAMPLER A (FROM TABLE 13). DRY WEIGHT BASIS.

Nutrient	Sampler	Group 1	Group 2	Group 3	Group 4	Mean % Deviation	
						Groups 1 to 4	Vars. A to D (Table 13)
N %	A	1.79	1.77	1.75	1.78	1.6	1.5
	B	1.84	1.81	1.75	1.76		
P "	A	.156	.160	.184	.181	8.4	2.5
	B	.164	.164	.211	.199		
K "	A	1.70	1.57	1.62	2.08	4.7	3.4
	B	1.58	1.50	1.56	2.00		
Ca "	A	1.58	1.45	1.58	1.41	5.3	2.3
	B	1.55	1.49	1.41	1.49		
Mg "	A	.22	.24	.23	.16	8.2	3.5
	B	.20	.23	.24	.19		
Na "	A	.017	.016	.015	.013	8.6	3.9
	B	.016	.017	.013	.012		
Mn ppm	A	27	31	31	27	13.8	2.5
	B	32	34	34	32		
Fe "	A	69	70	67	63	7.8	5.4
	B	75	73	76	66		
Cu "	A	3.9	4.0	3.4	3.9	3.9	3.2
	B	3.9	4.4	3.5	4.0		
B "	A	43	55	60	63	10.1	4.4
	B	36	55	71	58		

much better agreement, varying from 1.5% to only 5.4%, as indicated in the last column of Table 14.

In addition to the overall deviation noted, several nutrients in (B) samples were consistently higher or lower in composition than in (A) samples for all the groups, such that the differences in K, Mn and Fe values were even statistically significant as

calculated by applying Student's t-test to the data. This is rather surprising since it would appear to be comparatively easy to select midshoot leaves around the trees. It is clear that persons entrusted with sampling must be carefully briefed to avoid or minimize error due to personal factors.

3.6 PROCEDURE ADOPTED.

In view of the factors considered above which are capable of influencing leaf composition when selecting leaf samples, it is essential that a standardized procedure should be consistently followed to eliminate errors from this source or to reduce them to a minimum.

The procedure adopted is as follows. In the case of fruit trees, select midshoot leaves on terminal, usually fruit bearing, shoots of average length and vigour, situated within easy reach around the periphery of the tree and more or less at shoulder height. The sample should be taken uniformly around each tree from current shoots borne on one-year old wood, avoiding vegetative shoots. In the case of vines, the first normal sized leaf below the fifth node ^{from} ~~at~~ the base of fruiting shoots is selected.

The sample may be collected at any time during the day from 9 A.M. to 5 P.M. All samples should be collected by the same person unless assisted by a helper who is thoroughly conversant with the sampling procedure and has been previously trained by the regular sampler.

The actual leaves selected should be free from disease, insect or mechanical damage. The presence of mild symptoms of nutritional deficiencies is no disadvantage but severely scorched leaves should be avoided. The sample should be selected only from shoots on branches of uniform vigour. Trees or vines showing root or trunk injury must be avoided. Samples from trees which have abnormally small crops should be marked for special consideration when interpreting the results.

4. TIME OF SAMPLING.

Experience has shown that leaf composition varies during the course of a season and also from one season to the next. The magnitude of such variation may be considerable, and completely misleading interpretations could be placed on analytical data depending on the time of sampling as shown in the following investigation.

Seasonal data on leaf composition were obtained from samples collected in an orchard and vineyard at Welgevallen, the University farm at Stellenbosch. The orchard consists of different varieties and fruit species planted in unreplicated blocks adjacent to each other, in 1919, on an alluvial sandy loam derived from Table Mountain Sandstone, granite and Malmesbury Shale. The vineyard also consists of different varieties, planted in 1933, but is located at some distance from the orchard on a brown hillside loam derived from Malmesbury Shale.

Both orchard and vineyard soils are fairly deep and well drained. They have a good waterholding capacity and are not irrigated. According to Greenstein (86), the phosphate content was relatively low as well as the base exchange capacity. General orchard practices have been applied as under commercial conditions.

The fertilizer programme for the orchard since 1949 consisted of an annual application of 600 lbs. of rock phosphate (Langfos) per morgen in autumn before planting lupins as a green manuring crop which is supplemented in spring with a dressing of 200 lbs. of ammonium sulphate per morgen. The vineyard received the same nitrogen treatment annually in spring but lupins, fertilized with 400 lbs. of fertilizer mixture H, were grown in one row and compost applied in the next, alternating each year.

Soil data obtained during the 1950/51 season are presented in Table 15 to indicate seasonal variation in certain soil factors. Representative composite first and second foot soil samples were periodically collected in selected areas in the orchard and vineyard, using a Veihmeyer sampling tube. The nitrate- and ammonia-nitrogen contents were determined in the soil extracts using Morgan's sodium acetate mixture, the electrical resistance of moistened samples being measured with a Leeds and Northrupp Ohmmeter, and the pH of 1:1 soil water suspensions with a Beckman pH meter.

According to the data, as supplied by the analytical section of the W.P. Fruit Research Station, the ammonification process showed a steadily increasing rate of activity until mid-December after which the ammonia-N concentration gradually dropped to a low level. The nitrate-N values in both soils remained fairly constant at a low level until November or December after which a higher level prevailed until April. Nitrification was evidently promoted by the higher temperatures in summer so that soil moisture content must have been adequate for bacterial activity during this period. The rate of evolution of soluble N corresponds roughly to that found later by Fourie (78) in Bien Donne soils.

The concentration of soluble salts in the two soils as indicated by the electrical resistance readings showed considerable variation but no seasonal trend. The soil reaction also remained fairly constant except for a substantial increase in pH values late in the season. The values obtained indicate that the orchard soil was slightly acid, whereas the surface soil in the vineyard was practically neutral in reaction.

TABLE 15. - NITRATE AND AMMONIA-NITROGEN, ELECTRICAL RESISTANCE
AND pH OF SOIL SAMPLES FROM WELGEVALLEN, STELLEN-
BOSCH.

Date	Sample depth	Orchard site				Vineyard site			
		NO ₃ -N ppm	NH ₄ -N ppm	Resist. ohms.	pH	NO ₃ -N ppm	NH ₄ -N ppm	Resist. ohms.	pH
18.10.50	1-12"	5.9	11.5	1700	5.8	8.7	9.8	1500	6.9
	12-24"	4.3	10.6	3200	5.1	6.1	12.5	1300	5.9
25.10.50	1-12"	6.1	15.0	1400	5.8	4.4	13.5	1200	6.4
	12-24"	3.6	14.0	2700	5.1	2.9	12.5	1600	5.7
1.11.50	1-12"	5.5	18.4	1900	6.0	8.1	17.8	1300	6.9
	12-24"	2.6	18.9	3400	5.4	2.6	15.5	2100	5.9
8.11.50	1-12"	23.8	21.4	1000	5.9	7.7	18.9	1100	6.5
	12-24"	7.3	16.6	2800	5.2	4.7	16.3	2000	5.9
15.11.50	1-12"	8.9	15.5	1000	5.8	8.0	12.8	1000	6.8
	12-24"	1.7	14.6	3500	5.1	2.6	14.6	1600	6.1
13.12.50	1-12"	28.8	29.0	800	6.0	12.1	31.4	900	6.6
	12-24"	9.4	29.8	1900	5.3	7.8	28.2	1200	5.9
10. 1.51	1-12"	17.5	22.8	1100	5.8	18.8	24.0	900	6.8
	12-24"	6.1	24.0	2900	5.2	5.3	21.5	1900	5.9
7. 2.51	1-12"	26.5	20.4	1000	5.7	12.1	22.8	1000	6.7
	12-24"	8.2	18.7	2700	5.7	6.6	20.9	1400	6.0
7. 3.51	1-12"	30.5	5.5	1100	5.8	9.2	4.6	1100	6.7
	12-24"	7.6	4.6	3200	5.6	4.7	5.5	1400	6.1
4. 4.51	1-12"	29.5	8.2	700	5.9	13.5	7.8	900	6.9
	12-24"	11.1	6.9	1900	5.5	34.0	6.2	1000	6.4

4.1 SEASONAL CHANGES IN KAKAMAS PEACH LEAVES.

Midshoot leaves were collected at weekly intervals throughout the 1949/50 season on 4 Kakamas peach trees at Welgevallen. The number of leaves was recorded and percentage dry weight determined for each sample to provide data on leaf weight, total amount of nutrient per leaf and percentage content on the dry weight basis, as indicated in Table 16. The weekly data were condensed by averaging the values for each 4 consecutive sampling dates, entering each group average under the mid-date for the sampling period.

It is evident that considerable variation in dry weight of leaves and nutrient content occurred during the season, and that the change in percentage dry weight obscures the apparent trends in actual nutrient content when expressed as percentages on oven-dry material basis.

In order to illustrate the proportional differences between the trends for the various elements, a log transformation of the values was employed in drawing the curves shown in Figure 5 after first converting the actual values to a percentage of the data as obtained on 14/11/49.

4.11 ABSOLUTE CONTENT.

Considering first the absolute amount of nutrient per leaf, it is evident that leaf N remained fairly constant throughout the season from November to June. This may be interpreted as indicating that the amount of N translocated to other parts practically balances the amount entering the leaf. The curve shows very little change in direction, in contrast to the marked fluctuations in available nitrogen in the soil by ammonification and nitrification, particularly in November and December, as was indicated in Table 15.

TABLE 16. - COMPOSITION OF MIDSHOOT PEACH LEAVES, EXPRESSED AS WEIGHT OF NUTRIENT PER LEAF AND WEIGHT OF NUTRIENT PER 100 GRAMS OF DRY MATERIAL (% D.M.).

Date	Leaf Wt. mgm	N mgm	P mgm	K mgm	Ca mgm	Mg mgm	Mn µg
14.11.49	152	6.47	.458	4.79	2.14	.44	10.1
12.12.49	205	7.32	.458	6.57	3.03	.59	13.3
9. 1.50	228	7.27	.362	7.27	3.90	.76	15.7
6. 2.50	257	7.28	.340	7.71	4.61	.96	19.6
6. 3.50	249	6.77	.303	6.72	5.19	1.00	20.6
3. 4.50	269	6.42	.300	5.97	5.81	1.08	23.2
1. 5.50	269	6.38	.328	4.86	6.21	1.13	23.6
29. 5.50	269	6.48	.369	4.53	6.77	1.16	22.9

	Dry Wt. %	N %	P %	K %	Ca %	Mg %	Mn ppm
14.11.49	31.5	4.28	.305	3.13	1.40	.290	66.8
12.12.49	34.1	3.57	.223	3.21	1.48	.288	65.0
9. 1.50	36.3	3.20	.159	3.20	1.71	.333	69.0
6. 1.50	39.3	2.83	.132	2.99	1.79	.373	76.0
6. 2.50	40.4	2.72	.122	2.70	2.08	.402	83.8
3. 4.50	41.6	2.38	.111	2.22	2.16	.401	86.0
1. 5.50	41.3	2.38	.121	1.81	2.31	.419	87.8
29. 5.50	41.5	2.41	.137	1.69	2.52	.430	85.3

P is used up and translocated more rapidly than the amount entering the leaf until May when accumulation sets in. K accumulates fairly rapidly until February after which it moves out of the leaves at a more rapid rate than the rate of entry. Ca, Mg and Mn accumulate consistently throughout the season.

Midshoot leaves maintain a fairly consistent physiological age while the shoots increase in length, in this case until the

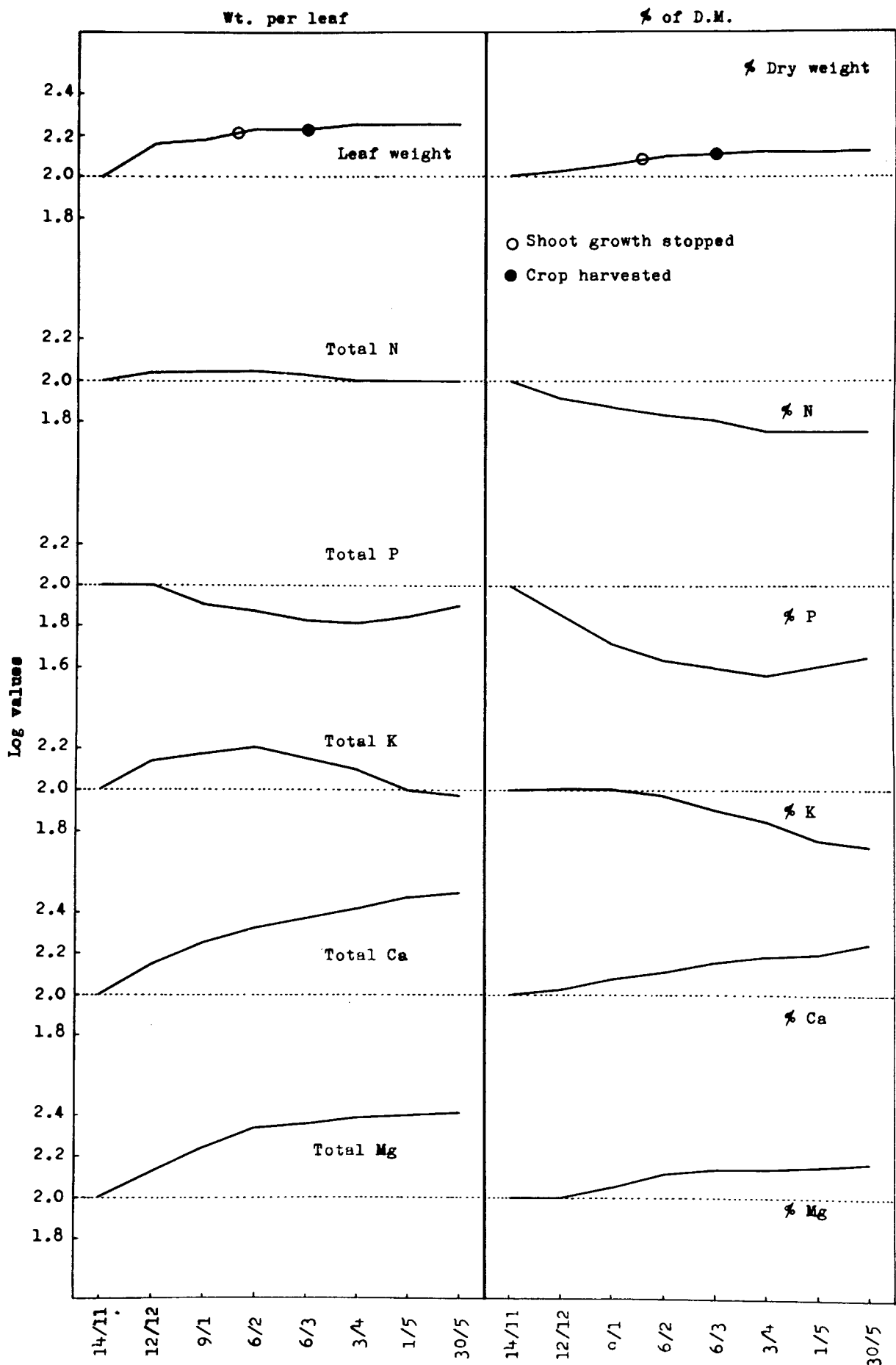


Fig. 5. Seasonal variation in nutrient content of neach leaves, expressed on a log transformation basis in relation to the November (14/11) data.

end of January when shoot growth ceased, so that up to the latter stage no major physiological change in the tree, except that due to tree growth and fruit development, would be expected to influence the nutrient composition. After cessation of shoot growth the midshoot leaves are subject to aging processes which would tend to increase the K concentration and decrease that of N and P (see Table 9), even though nutrient supply in the soil does not change. The results, however, showed no change in the K, N and P trends after shoot growth ceased, nor was there any indication of an increased rate of nutrient accumulation in the leaves after the crop was harvested in the beginning of March. The late season accumulation of P in May to June does not appear to be associated with removal of crop.

The curves thus appear to reflect characteristic changes in nutrient content subject to normal processes of movement to the leaves, assimilation and accumulation in the leaves and translocation to other parts. The absence of change following cessation of shoot growth and removal of fruit serves to support the selection of midshoot leaves for diagnostic analysis.

4.12 PERCENTAGE CONTENT.

Since nutrient content in diagnostic leaf analysis is invariably and more conveniently expressed in terms of weight of nutrient per 100 grammes of dry leaf material, that is, as a percentage on a dry weight basis, it must be recognized that the seasonal trends determined on this basis must differ from the "per leaf" trends in accordance with the change in percentage dry weight of leaves occurring during the season. The "percentage" curves as shown in Figure 5 obviously differ markedly from the "per leaf" curves and as such obscure the actual changes occurring in the leaf. This is no disadvantage as long as the characteristic "percentage" curves for the various nutrients are

not interpreted as true changes in actual nutrient content.

Due to the increase in dry weight, the percentage values for some of the nutrients (N and K) now show a fairly consistent decline during the season, whereas others (Ca, Mg, Mn) show a relatively small gradient as compared with the "per leaf" curves. The percentage shows a much steeper decline but also increases at the end of the season in accordance with the "per leaf" values.

The seasonal trends for most nutrients are evidently of sufficient magnitude to render leaf analysis data useless for diagnostic purposes unless reference is made to the time of sampling. For instance, N and P values obtained by analyzing samples collected in December may be erroneously construed as indicating an adequate nutritional status if compared with standard values which have been determined from the nutrient content in February.

4.2 SEASONAL TRENDS IN PERCENTAGE NUTRIENT CONTENT.

In order to test the consistency of the trends as found for Kakamas in 1949/50, and if possible to establish standard gradients for future diagnostic interpretations, seasonal variations were investigated in the case of a number of varieties during successive seasons from 1950 to 1953.

Midshoot leaf samples were consistently collected from the same trees (basal leaves in the case of grape vines) at the University farm, throughout each season or until such time as defoliation or other factors terminated sampling. Early leaf fall occurred in the case of apricot probably owing to drier soil conditions in the apricot block, whereas some of the peach varieties suffered from early infestation of rust, and severe wind damage invariably carried away most of the midshoot plum leaves each January.

The samples were analyzed for N, P, K, Ca, Mg, Mn, Cu and B, but the Cu and B data are incomplete since these elements were not determined prior to 1951, and the apple and pear samples of 1951 were contaminated with copper fungicide spray residue (see Section 2.12). Iron data are not submitted since the samples at that time were not adequately washed to remove dust contamination (see Section 2.11).

The analytical results showed relatively more variability early in the season and again towards the end of the season so that only the data for the four-month period from December to March will be considered here. Data for weekly and fortnightly samples were pooled so as to present a uniform series each year for five sampling dates, viz. December 4, January 3, January 31, February 26 and March 26. The actual sampling dates coincided within a day or two in each year. No attempt is made to relate the sampling dates to actual stage of development for individual varieties which in fact showed little variation from year to

year, nor to that for different varieties which of course varies a great deal:

The varieties considered and their reference letters as used in Figures 6 to 13 are as follows:-

Peach	:	K	Kakamas
		E	Early Dawn
		B	Babcock
		G	Goldmine
Apple	:	A	Alma
		S	Granny Smith
		D	Golden Delicious
Pear	:	BC	Bon Chretien
Plum	:	SR	Santa Rosa
Apricot	:	R	Royal
Grape	:	W	Waltham Cross on rootstock 1202
		H	White Hanepoot on 420A.

The results are presented graphically in Figures 6 to 13, using the same scale for each nutrient in turn to facilitate comparisons, and grouping the data according to variety and to season. As already mentioned the data from which the graphs have been constructed are derived from average or single determinations from single plots and thus lack evidence of statistical significance. It is contended, however, that since samples were drawn from the same trees at each sampling date, which as has been shown in Section 3.5, does not involve an appreciable experimental error, the data so obtained are directly comparable, and, since a considerable number of comparisons are available, may be expected to provide a reliable reflection of seasonal variations, though not of varietal differences.

The conclusions may be considered under two headings, that concerning variation during the season for which all the data including that provided by different varieties are applicable, and that concerning only the variation from year to year.

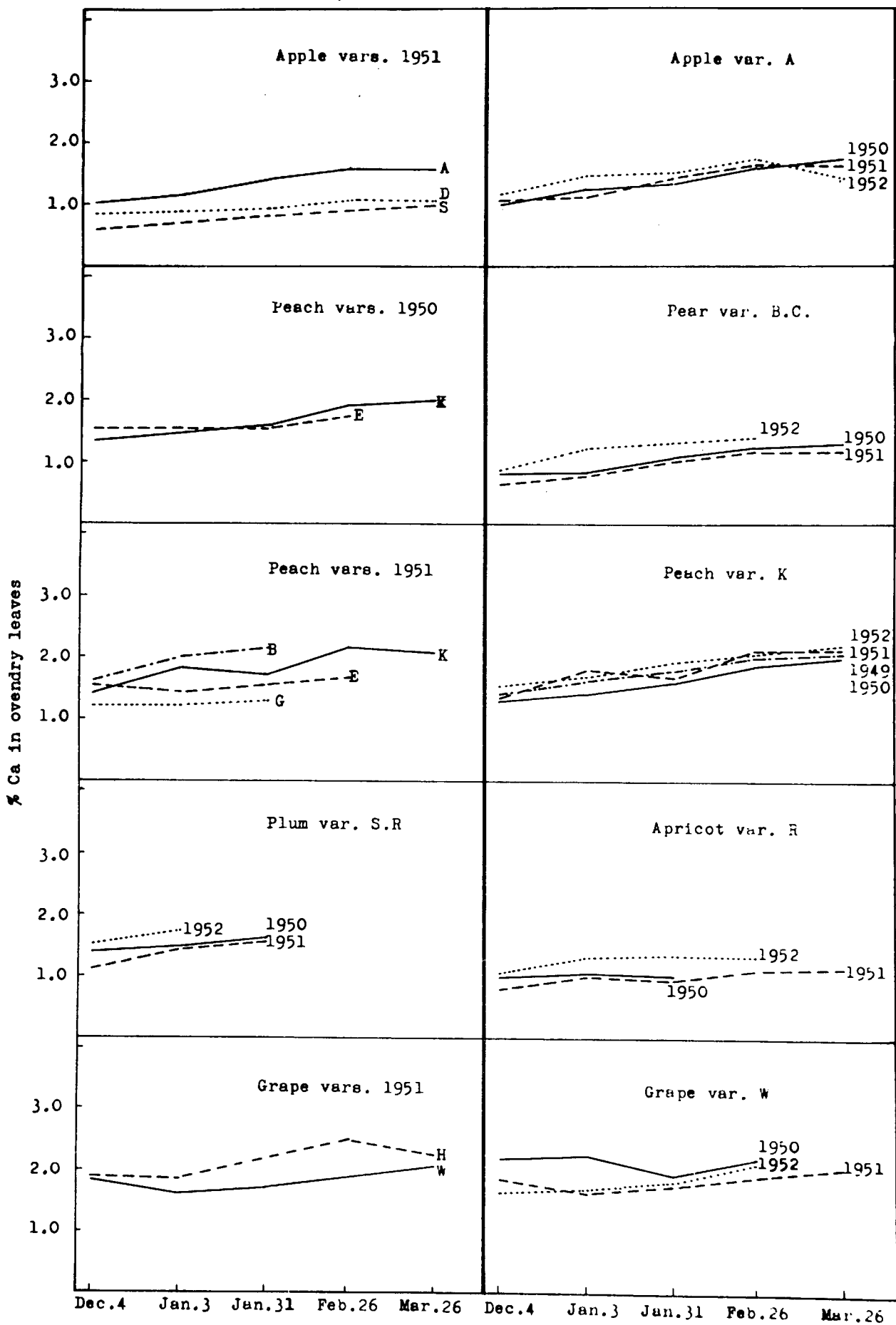


Fig. 8 Seasonal trends in leaf concentration of calcium

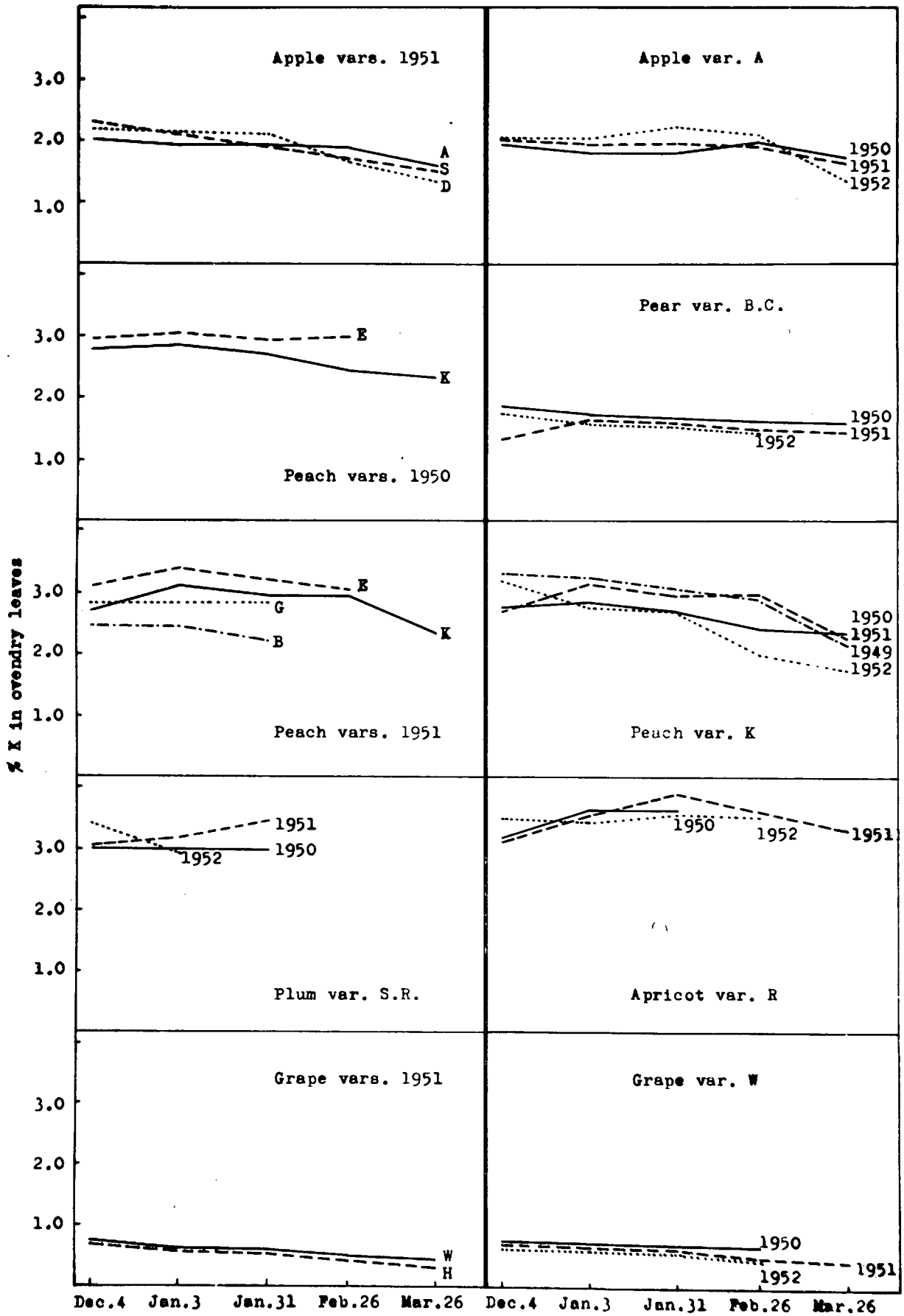


Fig. 9 Seasonal trends in leaf concentration of potassium

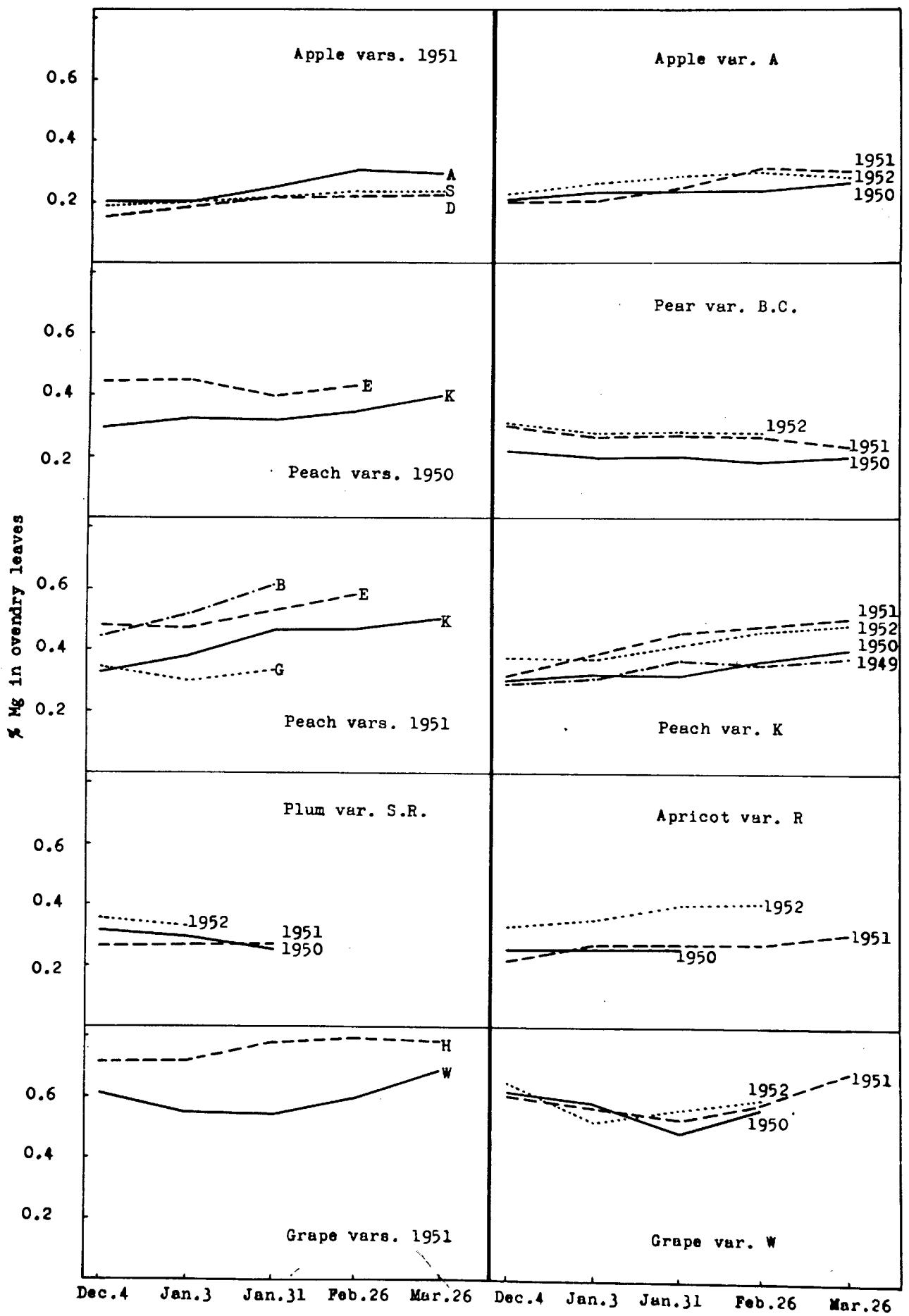


Fig. 10 Seasonal trends in leaf concentration of magnesium

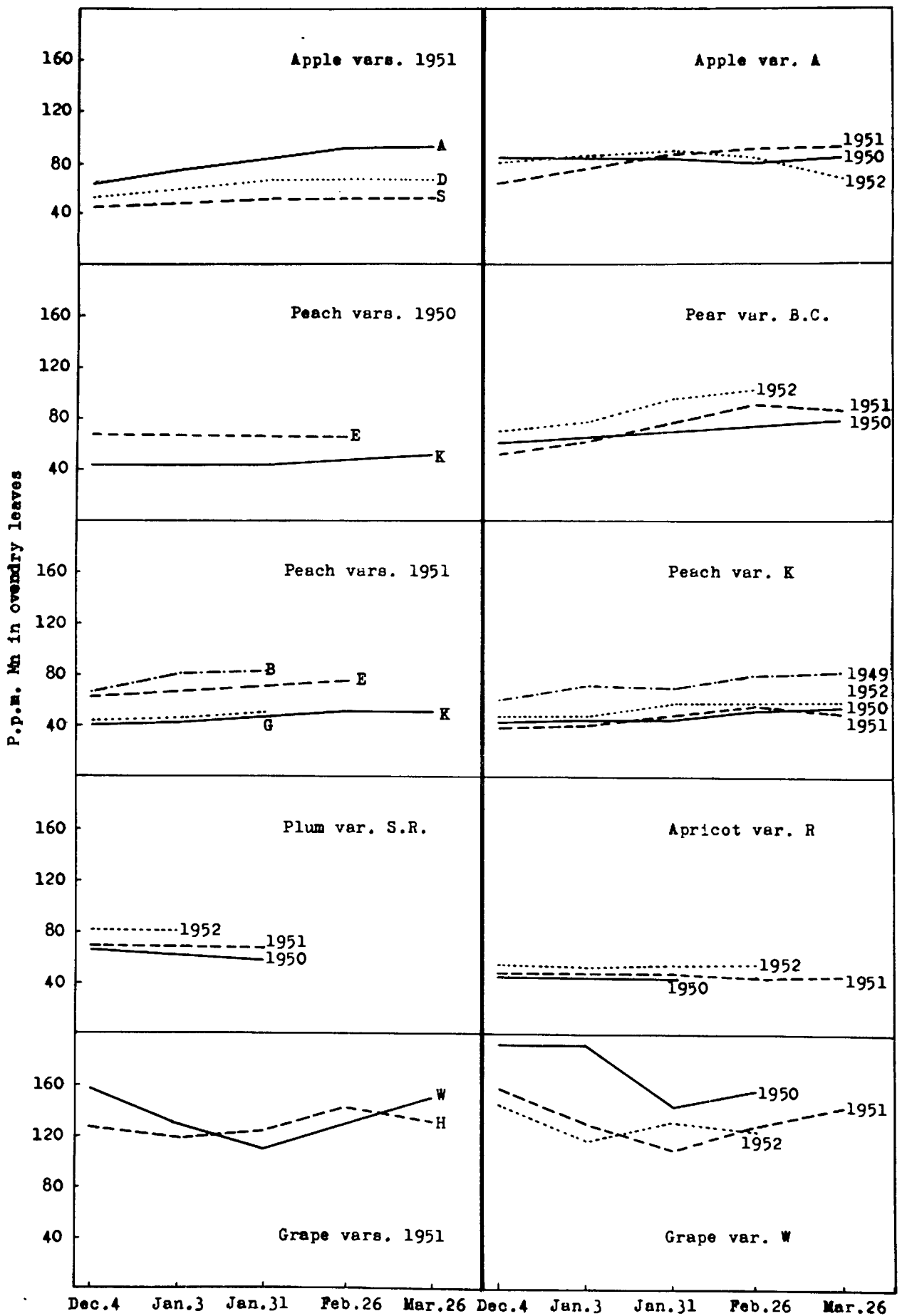


Fig. 11 Seasonal trends in leaf concentration of manganese

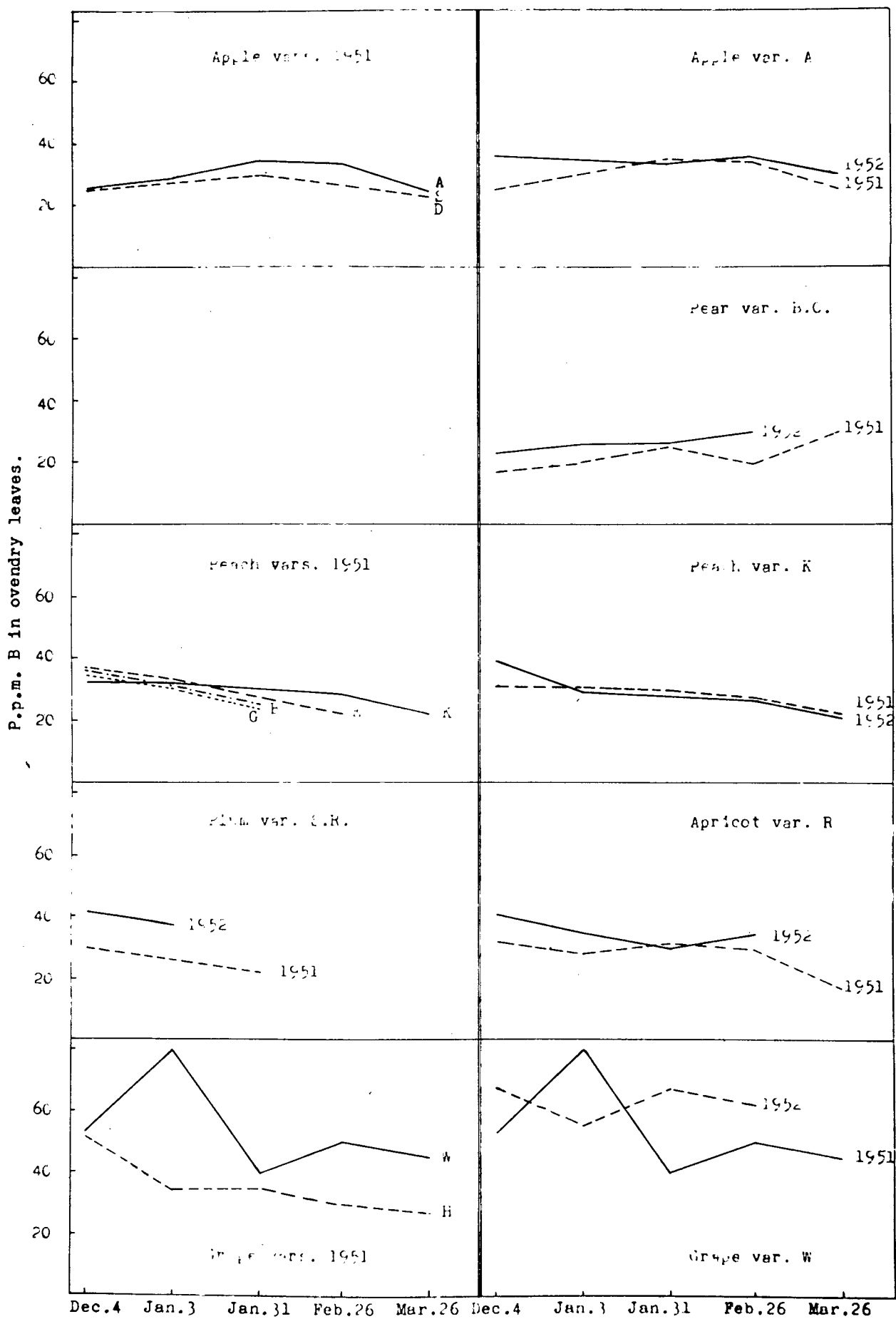


Fig. 12 Seasonal trends in leaf concentration of boron

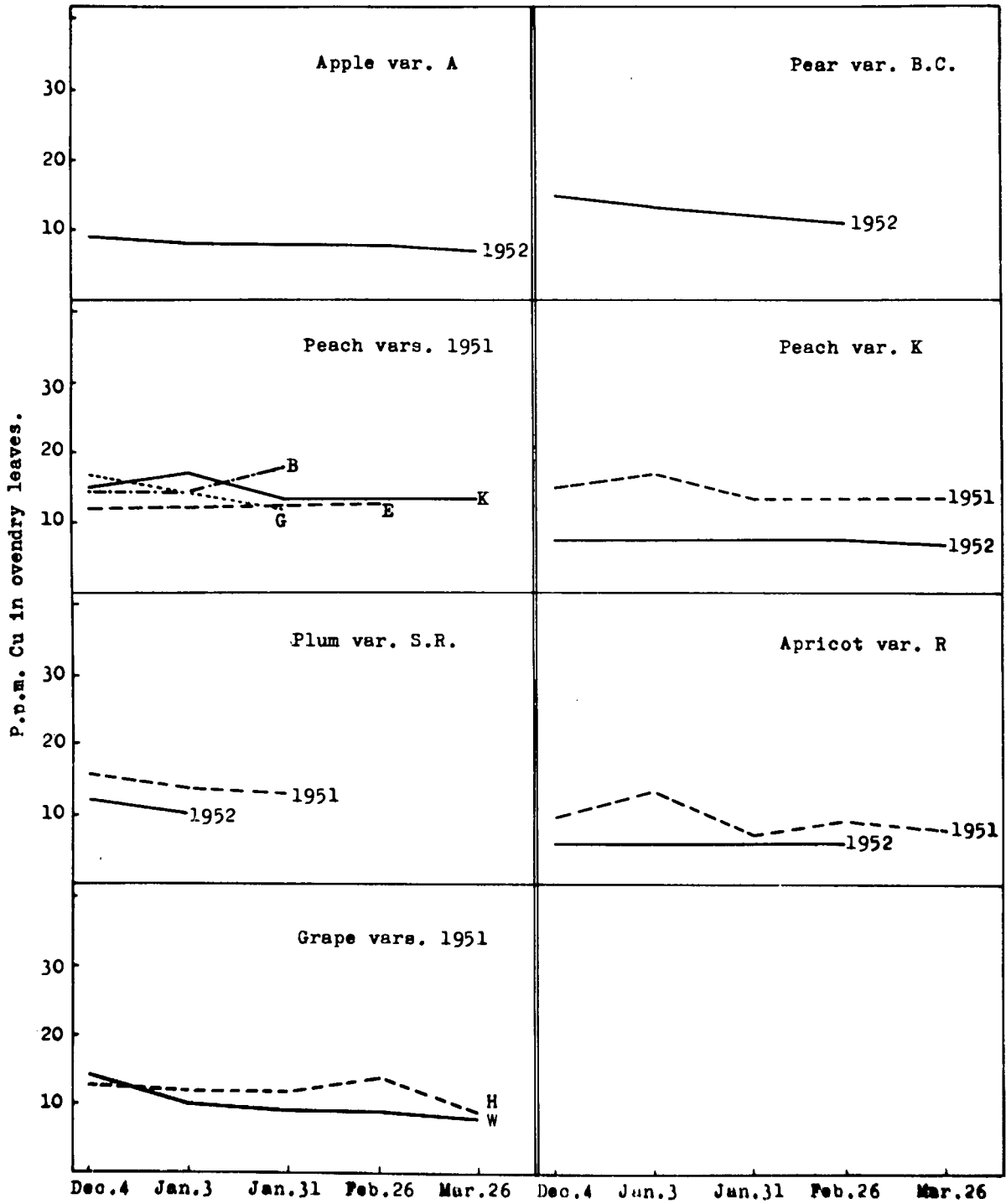


Fig. 13 Seasonal trends in leaf concentration of copper

4.21 SEASONAL VARIATION.

Reference to the curves shows that there is a distinct and consistent downward trend in the N and P values (Figures 6 and 7), and an equally distinct upward trend in Ca (Figure 8). The K curves (Figure 9) in general show a slight downward tendency with the possibility of a small initial rise before levelling off and then declining; the K curves for plum and apricot do not provide a definite picture owing to insufficient data for February and March. The Mg curves (Figure 10) show a slight upward tendency except in the case of plum and pear. The Mn and B curves (Figures 11 and 12) for grapes show wide fluctuations lacking a definite trend; the Mn curves for the other fruit species with the exception of that for plum show a consistent though slight upward tendency, while the B curves show relatively little change and lack consistency. The Cu curves (Figure 13) on the whole show a tendency to fall a little at the start before levelling off.

In studying these curves in relation to selection of a suitable stage during the season for routine collection of samples for diagnostic analysis, it is evident that since the P values fall rather sharply and some of the K values tend to rise between December 4 and January 3, this period should preferably be avoided. Furthermore, apricot and plum leaf samples are often difficult to find after February owing to leaf fall, so that the best time of sampling would appear to fall between beginning January and end February. In order to arrive at a numerical estimate of seasonal variation during this period, the means of the values for January 3, January 31 and February 26 for all varieties and seasons were calculated, as shown in Table 17, grouping deciduous fruit trees and grape vines separately.

TABLE 17. - SEASONAL VALUES CALCULATED AS A GENERAL MEAN FOR ALL VARIETIES AND SEASONS.

Nutrient element	Jan. 3	Jan. 31	Febr. 26	% Diff. from Jan. 31 value	
				Jan. 3	Febr. 26
<u>Peach, apple, pear, apricot</u>					
N %	2.96	2.78	2.45	+ 6	- 12
P %	.168	.144	.127	+ 17	- 12
K %	2.53	2.53	2.39	0	- 6
Cu ppm	10.7	8.8	8.8	+ 21	0
Ca %	1.34	1.45	1.64	- 8	+ 13
Mg %	.303	.325	.341	- 7	+ 5
Mn ppm	63	69	72	- 9	+ 4
B ppm	29	29	29	0	0
<u>Grape</u>					
N %	1.86	1.82	1.50	+ 2	- 18
P %	.167	.137	.107	+ 22	- 22
K %	.67	.64	.52	+ 5	- 19
Cu ppm	11	11	11	0	0
Ca %	1.84	1.90	2.22	- 3	+ 17
Mg %	.60	.60	.70	0	+ 17

The mathematical means indicate substantially the same trends as deduced from the individual curves, N, P, K and Cu showing a downward gradient and Ca, Mg and Mn an upward gradient whereas B remains constant. In view of the variability in the Mn and B values for grapes no definite trends for these elements can be presented in the case of grapes.

Taking the January 31 samples as a basis for comparison, it is evident that the values for samples collected four weeks earlier or later may differ by as much as 20%. On the whole, the values deviated more in February than in January.

It is obvious that considerable error will be incurred by using analytical values without reference to what may be termed the characteristic seasonal drift in percentage nutrient concentration unless sampling is consistently carried out on a particular date each year. In practice, collection of a large number of samples must necessarily be spread over a considerable period so that a correction factor or reference curves will have to be used to eliminate or reduce this source of error. The gradients found are not linear and apply to fruit trees growing at Stellenbosch. More intensive sampling during the two month period as well as data for other fruit growing districts are required before reliable reference curves for each nutrient and fruit species can be established.

Discussion.

Numerous references in the literature indicate general agreement that seasonal variation must be taken into account when interpreting leaf analysis data for both deciduous and citrus fruit varieties (47, 52, 55, 58, 70, 74, 84, 94, 103, 104, 114, 122, 126, 153, 161, 167, 177, 181, 182).

The information concerning deciduous fruit varieties, however, is far from complete and certain discrepancies as to the direction of change have been reported. The percentage N has consistently been found to decrease during the season (32, 58, 122, 126, 153, 167, 177). The percentage P also decreased (58, 122, 126, 153, 167, 177) but Proebsting and Brown (153) found that apricots showed a distinct increase in P which is at variance with the consistent downward trend found for apricots

in the present investigation (Figure 7). Calcium increased consistently and Mg also (32, 47, 122, 126, 153, 167) but a decrease of Mg in apple has also been reported (177).

According to Smith and Taylor (177) B, Zn and Cu values decrease and Mn and Fe increase. Similar trends for these elements were found by McClung and Lott (126) except that B increased. Epstein (76) found no definite trend for Mn in basal peach leaves, whereas in the present investigation Mn in midshoot leaves increased during the season and B remained constant.

The decrease in K (32, 47, 58, 122, 126, 153, 167, 177) and the initial rise before levelling off and decreasing as reported for apricot and peach by Proebsting and Brown (153), is consistent with the results obtained in the present investigation. Havis and Gilkeson (94) reported a rather disturbing relationship with level of K supply in peach. They found that leaf K decreased when the rate of K fertilization was low but that it increased throughout the season when there was no crop and the level of K supply to the soil was high.

These results appear to indicate that seasonal trends may vary in different countries and even in different varieties growing under the same conditions. A definite decision as to whether specific trends are typical under all conditions and as to what factors influence them does not seem possible until more data become available, but in the meantime a series of observations in a particular area, such as those obtained at Stellenbosch, may tentatively be considered to represent the trends applicable to that area.

Regarding the possible modifying effect of level of nutrition, Jones and Parker (104) came to the conclusion that this has little effect on the seasonal changes in mineral composition of orange leaves, and considered the seasonal trends obtained

for N, P, K, Ca, Mg and Na, as typical under the climatic conditions prevailing in the coastal valleys of Southern California. Reuther and Smith (161) also concluded that most nutrient elements have distinctive overall seasonal trends of concentration in citrus leaves, which are not fundamentally altered by soil, climate or cultural factors but may be displaced upward or downward in response to the level of supply.

It appears, however, from the data of Proebsting and Brown (153) and those obtained by the writer, that inconsistencies are more likely to occur in the early part of the season owing to varying climatic conditions which may affect rate of growth and thus the ratio of nutrient to dry weight, whereas later in the season, for instance during January and February when weather conditions are generally more stable, the trends in nutrient concentration are more likely to be consistent. In the northern hemisphere, the midsummer months, July and August, are also considered to be the most suitable months for leaf sampling (32, 52, 114, 126, 167).

Cain (52) found that a single sample taken during the two-week period following cessation of terminal growth was quite satisfactory for routine diagnostic work, thus eliminating the need for seasonal curves. Goodall and Gregory (85) also implied that a constant stage of development should be considered as a basis for recurrent analysis and not a constant sampling date. However, the data in Figures 5 to 13 do not indicate any definite change in direction of seasonal trends following cessation of either terminal growth or removal of fruit, so that sampling during the relatively stable months of January and February seems justifiable, irrespective of the actual stage of development, even though this period may not always coincide with the optimum stage when, as pointed out by Goodall and Gregory, the proportional difference in nutrient content is greatest and most likely to give a correct forecast of a yield increase.

4.22 YEAR TO YEAR VARIATION.

Reporting on seasonal trends in citrus, Reuther and Smith (161) stated that the climatic conditions such as rainfall and temperature prevailing during the season, affect leaf composition directly and also indirectly through accumulation or depletion of carbohydrates which sometimes appreciably changes the ratio of mineral constituents to dry matter. They concluded that these variations in leaf composition from season to season were generally not large enough to cause major changes in nutrient status classification except in cases that lie in the range between deficiency and adequacy. Chapman and Brown (55) found fairly wide K fluctuations from one year to the next in citrus leaves from trees not deficient in K, whereas fairly uniform values were found under conditions where K deficiency was at a constant level.

Wide variations of up to 20% and more have been found in deciduous fruits (70, 114, 153) but the widest differences in successive years appear to accompany samples collected either early or late in the season whereas the midseason period particularly under a uniform system of fertilization may not be subject to as wide variations.

The Stellenbosch results indicate considerable variation from one season to the next even during the midseason period. To obtain some idea of these differences the data for January 3, January 31 and February 26 were averaged to eliminate within-season variation and the means expressed as percentages of the 1952 values, as indicated in Tables 18 and 19. These mean values show wide differences for some fruit species, the maximum difference varying from 16% for N (apple) to 46% for B (plum), and even greater differences occurred in some of the yearly values for individual sampling dates.

A feature of these variations is that with a few exceptions the 1951 and 1953 values for fruit tree species are fairly

TABLE 18. - YEARLY VARIATION IN COMPOSITION OF MIDSHOOT LEAVES OF FRUIT TREE SPECIES, EXPRESSED AS PERCENTAGES OF THE 1952 VALUES.

		Peach	Plum	Apricot	Apple	Pear	Mean % Diff. from 1952 values
N	1951	98	96	91	84	102	- 6
	1952	100	100	100	100	100	
	1953	102	93	100	91	104	- 2
P	1951	107	126	113	108	127	+ 16
	1952	100	100	100	100	100	
	1953	97	122	95	106	118	+ 7
K	1951	92	91	96	96	106	- 4
	1952	100	100	100	100	100	
	1953	83	92	93	110	97	- 5
Ca	1951	97	103	105	101	104	+ 2
	1952	100	100	100	100	100	
	1953	99	122	138	116	133	+ 21
Mg	1951	80	100	94	90	75	- 12
	1952	100	100	100	100	100	
	1953	94	121	143	110	107	+ 15
Mn	1951	97	87	95	96	91	- 7
	1952	100	100	100	100	100	
	1953	113	119	120	100	121	+ 15
B	1951	-	-	-	-	-	-
	1952	100	100	100	100	100	
	1953	92	146	113	105	125	+ 16

TABLE 19. - YEARLY VARIATION IN COMPOSITION OF BASAL LEAVES OF GRAPE VINES, EXPRESSED AS PERCENTAGES OF THE 1952 VALUES.

	N	P	K	Ca	Mg	Mn	B
1951	99	134	116	122	99	135	-
1952	100	100	100	100	100	100	100
1953	101	123	104	106	103	102	106

consistently either higher or lower than those for 1952, so that the data may be condensed by determining the overall mean percentage differences from the 1952 values to provide a general evaluation of seasonal effect (see last column in Table 18).

Evidently large seasonal differences occurred which in the case of Ca, Mg, Mn and B, could not have been caused by fertilizer applications since phosphate fertilizer containing Ca was applied at the same rate each year and the other nutrients were not used. The reason for the differences must therefore be ascribed to the differential effects of changing environmental conditions which may modify availability of nutrients and absorption capacity of roots. Reference to weather records at the University farm, Welgevallen, indicated that although the mean summer temperatures showed little change from year to year, the amount of rainfall during the midsummer months showed wide differences, as indicated in Table 20. Since irrigation was not applied, the soil moisture content was evidently at a lower level in the 1951/52 season.

TABLE 20. - TOTAL MONTHLY RAINFALL (INCHES) AT WELGEVALLEN.

	1950/51	1951/52	1952/53
November	3.17	2.91	4.19
December	2.44	.04	.35
January	1.39	.05	.42
February	.14	.26	.06

Assuming that moist soil conditions favour the efficiency of P absorption (70) and that a higher rainfall may lead to loss of N by leaching (78), these conditions may account for the higher P and lower N leaf values found in the 1951 and to a less extent

in the 1953 samples.

Mg is also fairly readily lost by leaching whereas moister soil conditions favour the availability of Mn and B, which may account for the lower Mg content in 1951 and the higher Mn and B values in 1953, respectively. The Mg and Ca values in 1953 and the Mn value in 1951, however, show changes in the opposite direction.

Thus, although some of the differences seem to conform to a pattern, it would be impossible to predict how seasonal factors will influence the content of all nutrient elements in the leaf. The magnitude of the seasonal differences observed were such that diagnostic interpretations are bound to be seriously affected.

The deviations shown by the grape leaf data for some of the nutrient elements correspond to those shown by the fruit tree data, but marked discrepancies also occur particularly in the K and Mn values, probably on account of differences in nutrient supply and in absorption capacity of the plant species.

CONCLUSION.

There appears to be no reason to doubt that considerable yearly variation in nutrient content does occur even under a uniform system of annual fertilizer applications. Some of the deviation may be ascribed to analytical error, but climatic factors may largely be held responsible for differences in nutrient absorption and growth, and this would undoubtedly influence leaf composition. Differences in nutrient content of up to 20% from one year to the next cannot be considered as exceptional, and fully bears out the conclusion of Proebsting and Brown (1953) that "rigid general standards of adequacy or deficiency based on a single sample have little validity in the face of the variability in response found in different seasons", unless properly taken into account.

The variation occurring during a particular season of course, may be accounted for by seasonal reference curves since typical seasonal gradients in nutrient content for each fruit species have been found to be quite consistent from year to year. Appropriate procedures of allowing for these sources of variation are suggested in Part III (Section 2.4).

5. VARIETAL AND ROOTSTOCK EFFECTS.

5.1 PLANT SPECIES.

That different plant genera vary as to their mineral requirements and composition is understandable but even the more closely related species of one genus such as different species of fruit trees show large differences in capacity for absorption and accumulation of nutrient elements. Associated with their capacity for differential uptake of nutrients, fruit species also differ as to their critical or optimum leaf nutrient levels. Different fruit species may thus require different nutrient standards as has been shown by investigations on both citrus (161) and deciduous fruit trees (32). This does not exclude the possibility that different fruit species may show the same response at a common critical level of a particular nutrient. For instance, in the case of Mn content it was found (76) that the critical minimum level tended to be about the same for all fruit species studied.

5.2 VARIETAL DIFFERENCES.

There appears to be considerable evidence of differences in leaf composition between varieties but in this case there is also strong evidence that these differences are relatively small and unlikely to affect interpretations based on comparison with standards determined for varieties of the same species.

Chapman and Brown (55) found that the composition of leaves is definitely affected by rootstock and variety; the ability of the plant to secure adequate K or other nutrients from a soil may be influenced by variety, rootstock and various scion-root combinations. However, results showed that the critical nutrient level for K in citrus leaves is constant and that this value

holds irrespective of rootstock or variety. The same views as to varietal differences were proposed by Reuther and Smith (161) in connection with citrus varieties, Ulrich (206) for grapes, Lilleland and Brown (114) and Emmert (75) for peach varieties, and others (85).

Goodall and Gregory (85), in their review of earlier work, state that most investigators agree that varietal differences in nutrient content are usually relatively small and that since they represent simply differing ability to absorb nutrients from the substrate in question and not differing reaction to a given internal concentration, standard values may apply without modification according to variety.

5.3 ROOTSTOCK EFFECT.

Significant differences in leaf composition due to the influence of the rootstock on the scion have been found by many investigators (50, 55, 59, 60, 84, 87, 161, 179, 187, 212). Some of these differences are probably associated with differences in root distribution (203) enabling some rootstocks to absorb a greater amount of nutrients than others. At least some of the differences are related to the ability of various rootstocks to differentially absorb nutrients from the soil (212). Smith and Wallace (187) pointed out that rootstocks may have specific cation-exchange capacities, and the differential ability of roots to absorb nutrients may thus account for some of the observed influences of rootstock on scion composition.

Evidence of such rootstock effects was obtained from an experimental pear orchard established for the purpose of determining the influence of various pear and quince rootstocks on performance of Bon Chretien pear trees. The orchard is located on a deep alluvial acid loamy sand at Bien Donn e, the experimental farm of the W.P. Fruit Research Station. The trees were planted in 1943 in randomized blocks with 3 replications, each plot consisting of 5 trees. Pruning, spraying and cultural practices were applied uniformly throughout the orchard each year.

The rootstocks used and data on yield and volume of growth, according to data for 1957 and 1958, supplied by the Pomology Section, are indicated in Table 21. The yield and tree volume data, arranged in order of total yield per tree, indicate that yield is associated quite definitely with growth vigour, the lower yielding trees being also smaller in size.

Leaf samples were collected from each plot on 4/3/57, consisting of 100 midshoot leaves, that is, 20 leaves from each of 5 trees per plot. They were washed, dried, ground and analyzed according to the standard procedure. The analytical

TABLE 21. - AVERAGE YIELD AND TREE VOLUME OF BON CHRETIEN
PEAR TREES ON DIFFERENT ROOTSTOCKS.

Rootstock		Av. yield per tree. (lbs.)	Tree volume. (cu. metres)
B 12	M pear selection	320	113
B 10	Kieffer C selection	290	110
B 15	Tol II pear selection	270	137
B 4	EP 4162 pear selection	260	83
B 14	W Pear A selection	250	78
A	Quince A seedling	240	76
B	Quince B selection	150	65
B 9	Kieffer B selection	135	52
B 13	W Pear A selection	105	37
C	Quince B with intermediate B. Hardy stock	95	47

results, presented in Table 22, were statistically evaluated by analysis of variance for randomized blocks. As indicated, highly significant differences, even at a probability level of .001, occur in all nutrients except P, Fe and B. These differences occur between the quince and pear groups as well as between rootstocks of the same species.

If the differences, as found, are interpreted as reflecting not only the differing ability of the various rootstocks to absorb nutrients from the soil, it remains to be seen to what extent growth and yield are associated with the internal nutrient content as found by leaf analysis.

Referring to the tree performance data in Table 21 and the leaf analysis data in Table 22, both arranged in order of yield per tree, it will be seen that the trees with the poorest performance (B, B9, B13 and C) also have the lowest leaf contents

TABLE 22. - MEAN COMPOSITION OF MIDSHOOT PEAR LEAVES ON DIFFERENT ROOTSTOCKS. DRY WEIGHT BASIS.

Root-stock	N %	P %	K %	Ca %	Mg %	Na %	Mn ppm	Fe ppm	Cu ppm	B ppm
B12	2.16	.136	1.80	1.24	.16	.009	162	148	8.7	72
B10	2.02	.118	1.49	1.43	.20	.009	60	98	7.0	65
B15	2.12	.112	1.51	1.48	.20	.011	91	106	10.0	73
B4	2.09	.122	1.60	.98	.30	.012	228	119	8.3	72
B14	2.07	.124	1.48	1.05	.24	.010	131	134	7.0	75
A	2.18	.124	1.57	1.01	.20	.010	198	114	8.3	73
B	1.89	.106	1.92	.87	.18	.011	90	117	5.7	59
B9	1.79	.100	1.48	1.35	.25	.011	72	114	6.5	46
B13	1.56	.104	1.24	.73	.29	.051	92	112	6.1	70
C	1.72	.104	1.56	.92	.23	.016	104	127	5.1	58
S.D. at .05*	.18	N.S.D.	.28	.16	.05	.010	60	N.S.D.	1.4	N.S.D.
S.D. at .001	.33	N.S.D.	.53	.29	.09	.018	112	N.S.D.	2.6	N.S.D.

* Significant Difference at .05 and .001 probability levels.

of one or more of the nutrients N, P, K and Ca. If the low yield and poor growth are taken as evidence of near-deficiency effects, the concentrations of these nutrients may also be considered as lying at or near the critical minimum level. If so, it must be concluded that certain rootstocks had the effect of reducing nutrient content in scion leaves to deficiency levels whereas other rootstock scion combinations provided good performance trees with leaf nutrient contents at a higher level. There is an exception in the case of Mg, however, in that the best rootstock for growth, B12, had the lowest Mg content in the scion leaves.

The results demonstrate that great differences in nutrient content as well as in growth and yield can be caused through the

influence of rootstock on scion characteristics. Such wide differences may not occur in practice since the pear seedling rootstocks used commercially never show the marked scion differences in growth and yield which occur in the experimental block under discussion. However, considerable variation in composition may be expected as a result of the variability inherent in seedling stock as commonly used in nurseries. Unfortunately, there are no means of avoiding this source of variation, but that due to the rootstock species at any rate may be eliminated by grouping together varieties grafted on a common rootstock species, such as plum, apricot and prune which are commonly grafted on either peach or plum stock.

Considering the main question of adequacy and evaluation of nutrient content in relation to growth determination, the existence of variation in leaf composition caused by rootstock effects is actually irrelevant, although of interest as regards the potential utilization of the available nutrient supply in the soil. The essential point is whether the nutrient content, which represents the resultant of all factors influencing absorption, is associated with a particular reaction in the plant.

In view of the statement by Goodall and Gregory (85) that since varietal differences represent simply differing ability to absorb nutrients from the substrate in question and not differing reaction to a given internal concentration, it may be argued that the same applies to rootstock differences and that index values may thus also apply without modification according to rootstock. This contention is supported by the evidence presented above for pear rootstocks and is in accordance with results for K in citrus which led Chapman and Brown (55) to conclude that the critical nutrient level for K in citrus leaves holds irrespective of rootstock.

5.4 CONCLUSIONS.

Considering its bearing on diagnostic work, it is clear from the evidence that standards of reference for leaf composition cannot be expected to apply more widely than within a particular species of fruit tree. Reports on varietal differences all indicate that standard values will apply without modification according to variety within a given species, so that orchards can be grouped simply according to species such as apple, peach, apricot, etc.

The kind of rootstock used is bound to influence the efficiency of absorption and nutrient content by virtue of the variability in the stock used by nurseries at the present time, so that it will be difficult to classify orchards in this respect. Seedling stock in any case cannot be traced and the variability from this source is unavoidable. With plum, prune and apricot a distinction between peach and Marianna plum rootstocks may be of value in at least eliminating the variation contributed by these rootstock species. However since it may reasonably be assumed that differences in leaf composition caused by the rootstock do not affect the reaction of the plant to internal concentration, the rootstock effect may be ignored when considering the adequacy of the nutrient levels as found in the leaves.

6. SAMPLING IN THE ORCHARD.

Having determined how to select a leaf sample on a tree with the minimum of error, the next step is to define in what manner a sample must be collected to give a true reflection of the nutrient status of the group of trees for which a diagnosis is required. Since soil variation directly affects leaf composition, the question of orchard representation is not considered at this juncture, only tree variation as may occur in a comparatively small group of trees covering a limited soil area which may be assumed to be uniform.

In studying the variation in K content of peach leaves, Lilleland and Brown (114) found considerable variation from tree to tree, even between adjacent trees which appeared to be uniform in growth and on a uniform soil. They considered that such variability between trees may be of common occurrence. They found that averages for 10 trees showed satisfactory agreement and considered a sample from 10 trees sufficiently accurate for a foliar survey. Chapman and Brown (55) later adopted the procedure of collecting 15 to 20 leaves from each of 5 to 10 representative trees which they thought sufficed to give a reliable reflection of K status in the particular part of the citrus orchard.

Reuther and Smith (161) considered that the trees sampled should be reasonably uniform and either in a compact group or grouped according to a particular tree condition. They reported that samples of 10 leaves per tree from 5 trees are adequate for most elements (although only N, P and K were considered), and that analysis of such samples will approximate the composition of the entire population of leaves in a block of about 60 to 120 trees with a standard error for a single sample of about 5 to 10%. Bathurst (6) recommended picking 10 to 16 leaves from each of 2 percent of the trees when determining N and P status.

When the present investigation was started, the writer proceeded on the assumption that a sample of 12 to 20 leaves per tree from a group of 10 to 6 trees, that is 120 leaves per sample, would adequately reflect the nutrient status of the particular part of the orchard concerned. Twenty leaves seemed from practical considerations to be the minimum to represent all branches around a large tree and 12 for small trees. Samples were, of course, consistently collected from midshoot leaves on shoots of average length, borne on one-year old wood and well distributed around the periphery of the tree at about shoulder height.

6.1 PEACH ORCHARD

In an attempt to gauge the efficiency of the sampling procedure, a group of 12 Elberta peach trees, growing in a row adjacent to each other in an experimental orchard at Bien Donn , were individually sampled on 7/2/56, collecting 100 leaves per tree. The 15 year old trees were reasonably uniform in growth, size and yield, and showed mild symptoms of Zn deficiency. The soil is a uniform deep alluvial loamy sand, rather high in available Mn.

The leaf composition data are presented in Table 23, together with the percentage Standard Error as calculated for each nutrient.

TABLE 23. - LEAF COMPOSITION DATA OF INDIVIDUAL PEACH TREES GROWING IN A ROW ADJACENT TO EACH OTHER (BIEN DONN ). DRY WEIGHT BASIS.

Tree	N %	P %	K %	Ca %	Mg %	Na %	Cl %	Mn ppm	Fe ppm	Cu ppm	B ppm
1	3.04	.135	1.77	1.82	.42	.013	.07	180	129	4.7	99
2	2.88	.138	1.98	1.62	.34	.011	.07	191	139	5.2	65
3	2.86	.138	2.07	1.42	.34	.013	.07	196	142	6.5	80
4	2.84	.141	1.74	1.48	.32	.013	.06	172	153	5.7	86
5	2.92	.151	2.05	1.36	.32	.014	.07	138	126	6.4	81
6	3.18	.143	2.38	1.53	.32	.012	.09	136	140	6.5	65
7	2.99	.140	2.07	2.06	.39	.016	.10	125	142	6.9	58
8	2.92	.139	1.62	2.02	.39	.013	.12	93	123	5.4	74
9	2.92	.141	2.08	1.51	.30	.011	.08	79	142	5.7	66
10	3.16	.155	1.95	1.81	.34	.010	.08	97	128	5.9	81
11	3.18	.137	1.83	2.42	.43	.012	.10	135	146	6.4	84
12	2.91	.126	1.58	2.40	.52	.013	.09	152	141	6.4	96
Mean	2.98	.140	1.93	1.79	.37	.013	.08	141	138	6.0	78
% SE	4.2	5.3	11.8	20.6	17.2	12.5	20.8	27.4	6.6	25.1	16.3

The percentage dry weight data (not shown in Table 23) were relatively constant, the percentage S.E., namely 2.4%, indicating that variability in percentage dry weight evidently could not have accounted for the considerable variation in composition from tree

to tree which amounted to over 20% for Ca and Mn. Even when allowance is made for analytical error considerable variation remains.

It was clear that a fairly large number of trees would have to be sampled in order to obtain a composite sample which would correctly represent the nutrient status of this group. Assuming that the mean calculated from the data for 12 trees is a correct estimate of the composition of the whole block of 50 trees, it is possible to determine statistically the number of trees that should be sampled within a certain degree of precision. An appropriate formula based on consideration of the significance of differences between means, appears to be that of Paterson as described by Love (117), as follows:

$$n = \frac{t \times \sqrt{2} \times \text{S.E.}}{D} \quad \text{or} \quad \frac{2t^2 \times \text{S.E.}^2}{D^2}$$

where n = no. of replicates, or trees in this case.

D = difference that is desired to be measured.

t = reading from table of t values for a desired probability and the no. of degrees of freedom from which S.E. was determined.

S.E. = standard error of a single observation.

Employing this formula and substituting percentage S.E. and percentage difference for the actual values, the number of trees required for sampling to give mean values differing from the "true" mean by 10% and 20% at the 0.05 level of significance was calculated for each nutrient as indicated in Table 24. The values obtained show that a relatively large number of trees must be sampled to ensure that the composition does not differ by more than 10% from that representing the "true" mean, in fact only N, P and Fe will be accurately reflected to within 10% by a composite sample from less than the number actually sampled. The variation

TABLE 24. - NUMBER OF PEACH TREES TO SAMPLE TO SHOW SIGNIFICANT DIFFERENCES (D) OF 10% AND 20% AT A PROBABILITY LEVEL OF 0.05, AS CALCULATED FROM TABLE 23.

Nutrient	% S.E.	D. = 10%	D. = 20%
N	4.2	2	1
P	5.3	3	1
K	11.8	14	4
Ca	20.6	41	11
Mg	17.2	29	8
Na	12.5	16	4
Cl	20.8	42	11
Mn	27.4	73	19
Fe	6.6	5	2
Cu	25.1	61	16
B	16.3	26	7
Mean	15.3	28	7

for several elements is such that differences of as much as 20% from the true mean are possible when using composite samples from less than 10 trees.

6.2 APPLE ORCHARD.

A projected Zn Mn experiment in a high-yielding Ohenimuri apple orchard at Elgin provided the opportunity of examining the variability in leaf composition of 20 two-tree plots distributed over the experimental block of 10 x 25 trees. The 24 year old trees were comparatively uniform in growth, size and yield. The plots were selected on the basis of a uniform intensity of Zn and Mn deficiency symptoms. The soil is a brown Bokkeveld loam overlying gravel. Leaf samples comprising 50 midshoot leaves from each of two trees per plot were collected on 26.2.57, cleaned, dried and analyzed according to the standard procedure.

The analytical results are tabulated in Table 25 which also shows the means and percentage S.E. as calculated from the data for each plot. Further statistical treatment was employed as with the peach data, to determine the number of plots required to show significant differences of 10% and 20% (Table 26).

The results show that with the variability occurring in the apple block, a composite sample must be drawn from all the plots actually sampled, in the case of several nutrients, if it is required to provide a mean value which does not differ significantly from the "true" mean by more than 10%. This situation corresponds fairly closely with that found in the case of the peach data. The variability, probably owing to soil variation, is even greater than in the peach Block since 6 plots (12 trees) are required to represent all the nutrients to within 20% of the Block mean as compared with 7 trees in the case of the peach.

TABLE 25. - LEAF COMPOSITION DATA FOR TWO-TREE PLOTS DISTRIBUTED OVER AN EXPERIMENTAL BLOCK OF APPLE TREES (ELGIN). DRY WEIGHT BASIS.

Plot	N %	P %	K %	Ca %	Mg %	Na %	Mn ppm	Fe ppm	Cu ppm	B ppm
1	1.92	.116	1.40	1.44	.35	.014	11.0	67	3.1	35
2	2.05	.123	1.14	1.47	.41	.014	8.3	82	3.4	33
3	1.97	.147	1.05	1.36	.38	.019	8.3	84	4.1	39
4	1.98	.163	1.37	1.36	.29	.017	9.6	72	3.9	41
5	2.08	.183	1.30	1.35	.33	.018	8.9	74	4.4	41
6	2.05	.120	1.50	1.06	.29	.017	8.4	87	3.2	42
7	2.08	.135	1.47	1.29	.32	.016	7.4	84	3.4	37
8	2.03	.127	2.02	.96	.29	.019	7.8	95	3.1	45
9	2.01	.124	1.30	1.09	.31	.019	6.5	78	3.1	33
10	2.06	.147	1.24	1.25	.30	.019	7.0	73	4.0	35
11	2.01	.131	1.15	1.47	.35	.017	7.1	79	3.7	39
12	2.03	.112	1.50	.98	.31	.013	6.2	74	2.9	31
13	2.02	.126	1.45	1.24	.37	.016	7.0	86	3.4	33
14	2.03	.126	1.27	1.24	.28	.019	8.8	79	3.5	37
15	1.97	.129	1.40	1.09	.28	.015	7.3	74	2.9	41
16	2.09	.124	2.25	.81	.29	.016	6.8	82	2.8	37
17	2.00	.131	1.72	.98	.38	.018	6.1	102	3.2	66
18	1.98	.135	1.40	1.23	.30	.016	8.6	72	3.1	60
19	2.09	.126	1.60	.86	.23	.011	6.4	73	2.6	62
20	2.17	.125	1.51	1.16	.29	.015	8.5	85	2.9	56
Mean	2.03	.133	1.45	1.18	.32	.016	7.8	80	3.3	42
% SE	2.8	12.5	19.8	16.8	13.8	13.8	16.1	10.6	14.1	24.8

TABLE 26. - NUMBER OF TWO-TREE PLOTS TO SAMPLE TO SHOW SIGNIFICANT DIFFERENCES (D) OF 10% AND 20% AT A PROBABILITY LEVEL OF 0.05, AS CALCULATED FROM TABLE 25.

Nutrient	% S.E.	D. = 10%	D. = 20%
N	2.8	1	1
P	12.5	14	4
K	19.8	35	9
Ca	16.8	25	7
Mg	13.8	17	5
Na	13.8	17	5
Mn	16.1	23	6
Fe	10.6	10	3
Cu	14.1	18	5
B	24.8	54	14
Mean	14.5	22	6

6.3 COMPARISON OF PEACH AND APPLE DATA.

The data for peach and apple may now be used to estimate the error involved in following the procedure of drawing samples from 6 to 10 trees as used in the present investigation as well as from for instance 20 trees, which in the case of the apple data would correspond to 3, 5 and 10 plots respectively. A difference between the sample mean and the true mean to be significant at the 0.05 probability level, can be calculated from Paterson's formula, since n is known, as follows:

$$D = \sqrt{\frac{2t^2 \times S.E.^2}{n}}$$

The two sets of data obtained in this way are given in Table 27 for comparison.

TABLE 27. - PERCENTAGE DIFFERENCE FROM THE "TRUE" MEAN PEACH AND APPLE LEAF COMPOSITION, AT THE 0.05 PROBABILITY LEVEL, WHEN COMPOSITE SAMPLES ARE DRAWN FROM 6, 10 OR 20 TREES.

Nutrient	Peach			Apple		
	6 Trees	10 Trees	20 Trees	6 Trees	10 Trees	20 Trees
N	6	5	3	5	4	3
P	7	6	4	21	17	12
K	15	12	8	34	27	19
Ca	27	21	14	29	23	16
Mg	22	17	12	24	19	13
Na	16	13	9	24	19	13
Cl	27	21	15	-	-	-
Mn	35	27	19	28	22	15
Fe	9	7	5	18	14	10
Cu	32	25	17	24	19	13
B	21	17	11	34	27	23
Mean	20	16	10	24	19	13

The data show that wide differences must be allowed for most nutrient elements when samples are drawn from 6 or 10 trees. Individual nutrients show evidence of different degrees of variability for peach as compared with apple, due evidently to differential response in the two situations. The average error involved for all nutrients amounts to 20% for peach and 24% for apple when 6 trees are sampled. These values of course include analytical errors which have not been deducted, and in the case of the apple data soil variation will account for some error due to distribution of plots as indicated by the larger differences for the same number of trees sampled as compared with those for the compact group of peach trees.

On the basis of these results therefore, the error due to analysis and tree variation may be such that differences between leaf analyses which are to be compared with each other, must exceed 20% in order to be significant when samples are drawn from 6 trees. If 20 trees per sample were used, the precision would increase to the extent that differences exceeding about 10% would be significant.

6.4 DISCUSSION.

In a recent study of tree variation in citrus orchards, Steyn (193) found a considerable sampling error of about the same magnitude as found above, from which he concluded that sampling only a small percentage of trees in an orchard may lead to entirely faulty interpretations as regards some of the nutrients, particularly if the orchard is a poor one.

Steyn set out first to determine how many leaves must be picked from a single tree to reflect the "true" values of the 10 nutrient elements in those leaves falling in the sampling category. Comparing the analytical values for sets of 25, 100 and 675 leaves he found that a 25 leaf sample will represent most of the elements to within 10% of the true values in a tree.

In his study on tree variation, Steyn selected a group of outwardly homogenous trees and of average size in each of 3 orchards, good, average and poor. The analytical results for individual trees in each group were treated statistically to determine the number of trees which should be sampled in each to represent the various elements within a certain degree of precision. The formula used was

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

which is identical to that of Paterson, as quoted by Love (117), used for the peach and apple data above.

In his calculations, Steyn adjusted the Coefficient of Variation (% S.E.) for each element by subtracting the analytical variance found in his analytical determinations from the variance as found in each block of trees. His values for analytical error were exceptionally low and did not materially alter the magnitude of the total error. Steyn's data indicating the sampling error found for each element and the minimum number of trees to be sampled to represent the various elements to within 10% and 20%

of the mean values at a probability of 19 to 1, are reproduced in Table 28.

TABLE 28. - DATA ON CITRUS ACCORDING TO STEYN (193) FROM HIS TABLES 28, 30, 32 AND 33, INDICATING THE % S.E. AND THE MINIMUM NUMBER OF TREES TO SAMPLE IN BLOCKS A, B AND C FOR A GIVEN DEGREE OF REPRESENTATION AT THE 5% POINT.

Element	Block A			Block B			Block C		
	% S.E.	D = 10%	D = 20%	% S.E.	D = 10%	D = 20%	% S.E.	D = 10%	D = 20%
N	5.3	3	1	2.2	1	1	7.0	6	2
P	3.6	2	1	6.8	5	2	5.4	4	1
K	15.8	23	6	10.8	11	3	25.2	71	18
Ca	7.2	5	2	8.1	6	2	11.6	16	4
Mg	18.3	31	8	23.5	51	13	71.4	574	143
Na	27.1	67	17	11.4	12	3	15.8	28	7
Fe	8.0	6	2	19.2	34	9	10.3	12	3
Mn	16.9	26	7	11.4	12	3	6.1	5	2
Zn	20.0	37	10	7.9	6	2	23.2	61	16
Cu	18.3	31	8	13.6	17	5	8.5	9	3
Mean	14.1	24	7	11.5	16	5	18.5	79	20

Block A comprised 16 large, high-yielding 40 year old orange trees showing Zn deficiency symptoms, Block B consisted of 16 medium sized, high-yielding 18 year old trees showing Mg deficiency symptoms, and Block C, 8 poor, low-yielding 25 year old trees affected with Mg, Cu and Zn deficiency symptoms.

As the data (Table 28) show, the variability in the poor orchard is relatively much greater than in Blocks A and B, due,

however, mainly to the abnormally high value for one element, Mg, the analysis of which would seem to be suspect. The other nutrients in Block C on the whole show a degree of variability of much the same magnitude as in Blocks A and B, though differing individually. Steyn argues that when the concentration of an element was at a deficient level the variation tends to be exceptionally large. This does not seem to apply in all cases since Block C was also deficient in Cu and yet showed only moderate variation as to this element. Moreover, the Elgin apple orchard was seriously deficient in Mn (Table 25) and yet showed only a moderate degree of variation in Mn content.

Comparing the tree variation as found in the peach (Table 24) and apple (Table 26) orchards with that in the citrus orchards (Table 28), it is evident that these orchards have much in common as to variability in leaf composition. If this is a feature of orchards in general, as appears to be the case, much greater differences must be attributed to tree variation than is generally supposed and sampling from a small number of trees for diagnostic purposes is open to criticism. Statistical treatment showed that this source of error can be reduced by employing a larger number of trees, preferably not less than 10, when collecting samples for analysis, in order to obtain a reliable estimate of the nutritional condition of the trees in a particular locality. Sampling from 20 trees will provide even better representation subject to the condition that they comprise a compact group homogenous as to growth characteristics and on a uniform soil.

In practice however sampling from as many as 20 trees is cumbersome and time-consuming since the selection of a compact group of homogenous trees becomes more difficult as the number increases while at the same time augmenting the magnitude of soil variation. In view of these circumstances then, sampling from 10 homogenous trees in a compact group and on a uniform soil would seem to be

the more reliable course to follow in diagnostic work. The size of the sample likewise should be limited to fit in with an efficient system of washing, drying and storage. Samples of 100 to 150 leaves can be conveniently handled so that 10 to 15 leaves from each of 10 trees, carefully selected to represent all the branches around each tree, would be required. Larger samples from each of a larger number of trees may be more representative but a compromise is evidently necessary to avoid errors imposed by too unwieldly a procedure.

6.5 CONCLUSION.

For the sake of perspective it may be pointed out that the existence of relatively large differences in leaf composition from tree to tree, which appears to be a characteristic feature of fruit trees, does not invalidate conclusions drawn from leaf analysis designed to identify a particular nutritional disorder or to evaluate the response to fertilizer applications in experimental procedure. In the former case tree variation is irrelevant, and in the latter, analysis of variance applied to a factorial layout will account for tree variation since variance is based on the behaviour of all the trees in the experimental block.

The question of tree variability however becomes of paramount importance in interpretation of analytical results obtained for the purpose of evaluating the nutrient status of orchards when the leaf composition of a group of trees is to be compared with a previously determined standard composition. It is essential, then, to employ data which correctly reflects the nutritional condition of the majority of the trees concerned, which can be achieved, as the results show, only by avoiding sampling from too small a number of trees. As indicated above 10 trees per sample will be adequate. A smaller number may sometimes be justifiable but less than 6 trees per sample is likely to provide quite misleading results.

Furthermore, the results obtained for the trees sampled cannot be expected to apply more widely than to the particular locality, and by no means to a large orchard. Owing to soil variation and differential response in various parts of an orchard, composite samples from trees scattered throughout the orchard are bound to provide average values liable to obscure specific nutritional disorders the evaluation of which is necessary to predict what treatment will prevent further deterioration. Steyn (193) pro-

posed that orchards should be divided into small uniform blocks, sampling at least 20% of the trees in each block. Such intensive sampling will be difficult to achieve in practice except in exceptional cases. A reasonable approach in advisory work would seem to be an evaluation of the nutrient status in one or two problem localities selected according to a particular soil or growth condition while recognizing that not all parts of the orchard will respond equally to treatment based on the sample data.

P A R T I I I

DETERMINATION OF LEAF NUTRIENT STANDARDS

1. INTRODUCTION.

The various steps which constitute the technique of diagnostic leaf analysis include sampling and preparation of sample for analysis which have been dealt with in Part II, then the actual chemical determinations followed by interpretation of the analytical results by comparison with previously determined nutritional standards.

For the purpose in view, namely diagnosis of the nutrient status of trees in commercial orchards, it is necessary to have some criterion by which a decision as to the adequacy or not of the leaf composition as found by analysis can be reached. Such a standard of reference must be capable of indicating the level or range of nutrient values at which maximum growth and yield may be expected.

From theoretical considerations, as discussed in Chapter 3 of Part I, it is clear that the relationship between yield or growth and internal nutrient concentration which may be linear at low levels will be influenced at higher levels by the concentration of other nutrients through metabolic interactions which come into play when the nutrient level is no longer limiting. Individual nutrients thus cannot be considered independently as entities in relation to response. The ultimate growth expression depends on both concentration and balance of all the functional elements so that maximum potential growth and yield is possible only when the concentration of each nutrient as well as the inter-relationship between nutrients are both optimal (176, 198).

Ulrich (206) proposed the term "critical nutrient range" to indicate the range of concentrations at which the growth of the plant is restricted in comparison with that of plants at a higher nutrient level; at or below this level one element may affect the utilization of another and such a lack of balance is likely to

affect plant growth. This idea developed into the more appropriate concept of "optimum values" as postulated particularly by Goodall and Gregory (85), Wadleigh (209) and Smith and Taylor (177). According to the latter, the concept of "optimum values" maintains that there is a specific leaf concentration for each of the essential elements which is correlated with optimal response in terms of yield or other characteristics, and that these concentrations or optimum values hold over a wide range of soil types and under a variety of climatic conditions. The leaf composition will therefore reflect the potentialities of the desired response. As the optimal nutrient level of each factor, depending on all factors simultaneously, is approached the maximum possible yield of the plant will be reached.

Experience has shown that high performance in fruit trees is accompanied by considerable variation in leaf composition as to both concentration and interrelationships between nutrients. Accordingly, if considered realistically the concept of "optimum values" should have a broader meaning than that originally proposed and interpreted by Smith and Taylor (177) and others as a specific level or narrow range of values representing the ideal composition. If nutrient concentrations above or below this optimum value are also associated with maximum performance, it is evidently of greater value in diagnostic work to have information on the limits of the whole range in composition which may be associated with the maximum production potential.

Considering the "optimum range" as now visualized, it is evident that it may include relatively high concentrations approaching luxury consumption which however will be no disadvantage since as long as maximum production is possible the nutrient balance or interrelationship between nutrients must still be favourable. Nutrient values at the lower limit of the range associated with maximum production must likewise be considered adequate. Under

these conditions the ratios between nutrient elements may vary considerably as for instance between a nutrient (Mg) at the lower limit and another (K) near the upper limit, and vice versa. According to the concept however the lowest value in the "optimum range" will be above the level at which its concentration in relation to an excess of another will result in unfavourable metabolic interactions which may have an adverse effect on growth. According to Ulrich (206), growth is adversely affected through unfavourable utilization of one nutrient as influenced by another only when its concentration is at or below the "critical level", or lower limit of the optimum range in the present case.

In accordance with the idea of an optimum range in nutrient concentration, representing or associated with high performance, it is necessary that the upper and lower limits of the range be established for the purpose of diagnostic comparisons. The critical percentage content associated with the appearance of deficiency symptoms may be assumed to mark the lower limit for Mg and the micro-nutrients since as discussed in Section 4.33 of Part I (page 47) there is at present not sufficient evidence to indicate that performance of fruit trees is improved by these nutrients at concentrations higher than the level associated with the disappearance of visible deficiency symptoms, except perhaps in the case of B in grapes (170). But in the case of N, P, K and Ca it is known that these nutrients definitely influence yield and quality of fruit at concentrations well above the threshold level for symptom expression. For these elements therefore the lower limit ^{of the optimum range} must represent the threshold level for maximum production, that is, the level above which no further improvement in performance due to increased nutrient supply would be likely to occur.

In similar vein it may be contended that the upper limit for both macro- and micro-elements would be marked by the level

at which luxury consumption begins to disturb the nutrient balance by interfering with the absorption and utilization of other elements, or when the concentration reaches toxic proportions, as reflected by deterioration in growth and yield of fruit.

The most reliable method of establishing these limits is by means of a large number of factorially designed fertilizer experiments in different localities, through which data can be obtained on yield response and internal nutrient concentration at different levels of supply of one nutrient at constant (adequate) levels of all the other functional elements. Recent work by the French workers, Prevot and Ollagnier (151), demonstrates what has been achieved in connection with groundnuts and palms in Tropical Africa. They showed that diagnoses based on the interrelationships between macro-nutrients may lead to a more complete interpretation of leaf analysis data as provided in such factorial experiments than by the use of critical levels of individual elements alone. As already indicated such data from a sufficient number of factorial experiments are not available for deciduous fruit in South Africa and are not likely to be for a long time.

Another approach is by considering the leaf composition of trees known to have a record of high performance as to yield and quality of fruit. If leaf analysis data are available from a large number of such orchards in different localities representing a wide range of nutrient supply, the highest and lowest values may be considered to represent a close approximation of the limits required for maximum production. Such data can be obtained from a leaf analysis survey of high producing orchards. The lower limit of the optimum range, as indicated above, will be subject to adjustment on the basis of critical threshold levels for deficiency symptoms which may be determined from a survey covering localities varying in fertility and including poorly

nourished orchards. If the appropriate surveys are comprehensive enough, a sufficient number of low and high values will be available from which reliable index values may be deduced for future diagnostic interpretations.

In accordance with these considerations, the required data have been sought on the basis of extensive leaf analysis surveys, firstly for the purpose of fixing threshold levels of symptom expression and secondly, for determining the limits of the optimum range.

After reviewing the technique of leaf analysis (already discussed in detail in Part II), a description will be given in the following pages of the visual symptoms of nutritional disorders, followed by presentation of leaf analysis data from surveys in relation to symptom expression and to high performance, and finally consideration of the data for determination of index values.

2. TECHNIQUE OF LEAF ANALYSIS FOR NUTRITIONAL DIAGNOSIS.

The procedure followed in obtaining data for determination of nutrient standards and which will be applicable also for determining the nutrient status of trees of unknown nutrition, may be briefly reviewed. The experimental evidence as to sampling and other errors as discussed in Part II emphasize the necessity for attention to detail and for particular care in following a standardized procedure which will eliminate or minimize errors likely to cause erroneous results and misleading interpretations. The procedure considered most likely to accomplish this, in addition to reliable methods of analysis, was adopted as follows:-

2.1 SAMPLING IN THE ORCHARD.

This subject has been discussed in Chapter 6 of Part II (pages 122 to 137). Each sample must consist of 100 to 150 midshoot leaves selected according to a particular tree condition from a compact group of 6 to 10 trees in the orchard under consideration. The trees must be uniform as to growth vigour and size and on a uniform soil.

In practice sampling was varied according to the size of the trees, ranging from 10 - 15 leaves from each of 10 small trees to 20 - 25 leaves from each of 6 large trees.

In the case of grape vines two basal leaves on fruiting shoots from each of 15 - 20 adjacent vines were collected for each sample.

2.2 SELECTION OF LEAF SAMPLE.

The procedure for selecting leaves on each tree has been described in Section 3.6 of Part II (page 95).

2.3 PREPARATION OF FRESH SAMPLE FOR ANALYSIS.

The particulars regarding cleaning, drying, grinding and storage of leaf samples are as stated in Section 2.7 of Part II (page 79).

2.4 TIME OF SAMPLING.

The experimental evidence presented in Chapter 4 of Part II (pp. 96 to 121) indicated that marked seasonal and year to year variation in nutrient concentration must be taken into account when using leaf analysis data for diagnostic purposes. It was found that leaf composition appeared to be most stable during the months January and February, and that leaf sampling is best carried out during this period. Even then, substantial differences in concentration occurred from January 3 to February 26 and from one year to the next.

(a) Within season variation.

The effect of seasonal variation in analytical values must be considered since it would be impossible to collect all samples on a particular day or even within the space of a week or two. Seasonal gradients in nutrient content as determined for each fruit species were found to be quite consistent in successive years so that the variation during a particular season can be simply corrected by reference to the typical seasonal curves applicable to each nutrient. The gradients for apple, pear, peach, apricot, plum and prune were found to correspond fairly well and may be grouped together, leaving grapes with its own set of correction curves.

This means that analytical data for samples collected at any time during January and February are corrected for seasonal effect by adjustment to a specified sampling date which may be conveniently

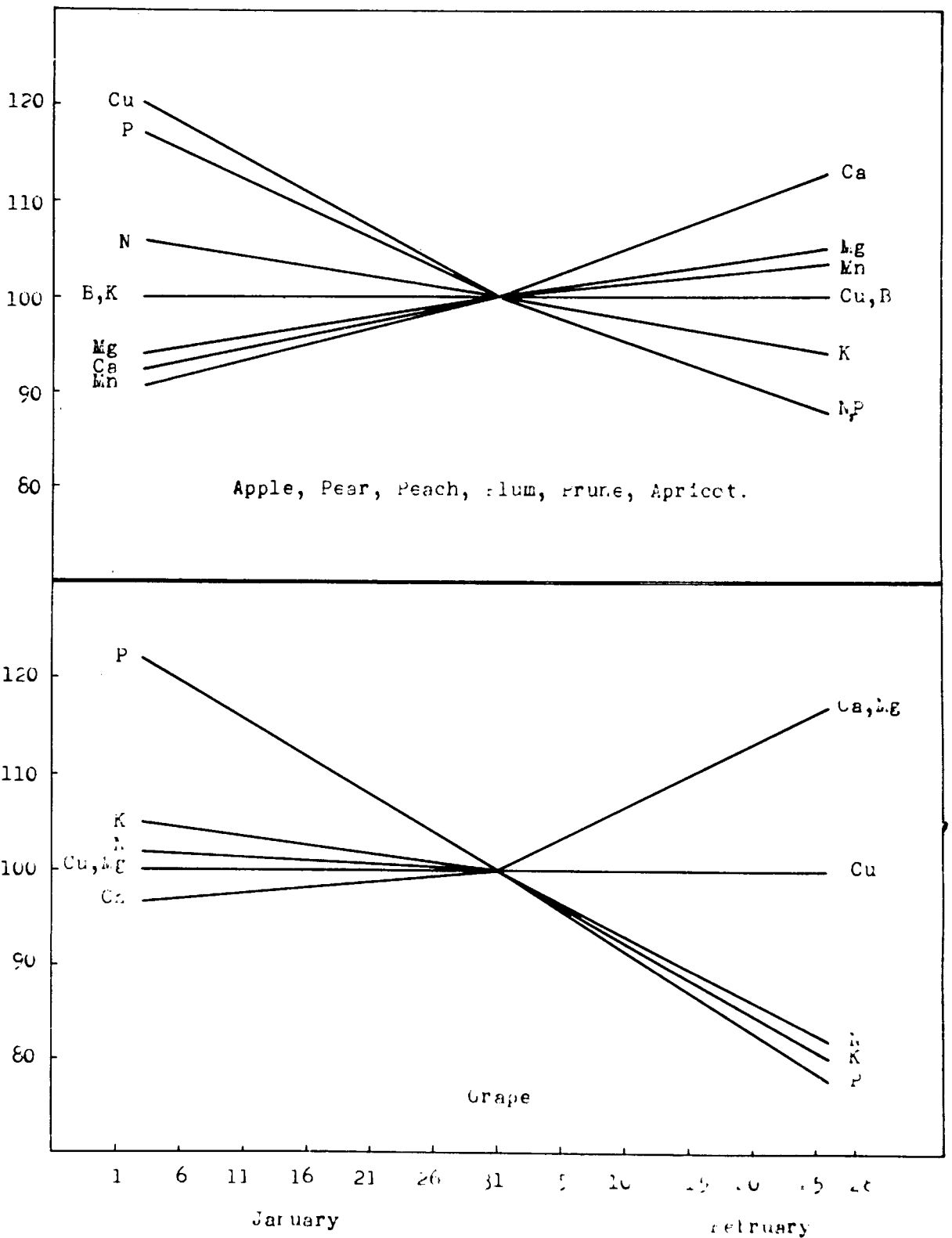


Fig. 14. Reference curves for adjustment of seasonal variation on the basis of percentage difference from January 31 values. (from data in Table 10).

fixed at January 31 using the correction curves presented in Figure 14. These curves represent the percentage deviation in relation to the values obtained for January 31 (from data in Table 17 on page 106). The correction for sampling dates other than January 31, then, is obtained by multiplying the analytical value for each nutrient by 100 and dividing by the appropriate percentage value from Figure 14.

There is evidence that the shape of the seasonal curves may vary in regions differing widely in climatic conditions so that reference curves should be drawn up for distinctive regions. Furthermore, since the curves are not linear over the two-month period, sampling at more frequent intervals than was the case in obtaining the data presented in Table 17, will improve the reliability of adjustments. Until further refinements can be effected, however, the use of the available data in Table 17 can be expected to reduce seasonal error sufficiently to justify corrections on this provisional basis.

(b) Year to year variation.

Differences in nutrient content of as much as 20% from one year to the next were found for trees receiving a uniform system of annual fertilizer applications. As discussed in Section 4.22 of Part II (page 112), these differences arise mainly as the result of changing environmental conditions which modify the availability of nutrients and the absorption capacity of the roots. A change in the fertilizer programme will of course differentially influence the nutrient content apart from the general fluctuation due to climate.

Although nutrient status diagnosis by comparison with standard index values in a particular year is not affected by year to year differences, the nutrient status may be erroneously considered adequate in one year when a high level prevails whereas

it may be at a marginal or inadequate level in the next.

The writer proposes to overcome this source of variation by means of reference data obtained each year whereby standard index values for each nutrient can be adjusted to the level applicable to any particular year. Permanent reference plots for each fruit species will be marked off in different localities according to the main climatic regions and leaf samples collected each year from the same trees. By limiting the reference plots to high performance orchards where a change in fertilizer regimen is unlikely to be necessary, the change in nutrient content occurring in successive years may be assumed to represent that due specifically to climatic factors.

2.5 VARIETAL GROUPING.

As indicated in Chapter 5 of Part II (page 121), for purposes of nutritional diagnosis, standards of leaf composition will apply without modification according to variety or rootstock. For the purpose of determining nutrient standards therefore varieties of the same species may be grouped together irrespective of rootstock.

3. NUTRITIONAL LEVELS ASSOCIATED WITH SYMPTOM EXPRESSION.

Extreme deficiencies and excesses of essential nutrients in plants are characterized by specific abnormal growth manifestations depending on the physiological functions of the nutrient. The symptoms are usually sufficiently distinctive to identify the nature of the disorder. Symptom expression thus provides a most convenient criterion for spotting serious nutritional disorders and this forms the basis of the well known technique of visual diagnosis of malnutrition. Although by no means infallible it makes possible diagnosis at a glance and as such has been found invaluable in practice, particularly for fruit trees.

Certain conditions may restrict the use of visual diagnosis. Symptoms of nutritional disorders may sometimes be confused with other physiological or pathological manifestations or they may not be easily identifiable when deficiency symptoms of one element resemble those of another. For instance, a definite decision is often difficult in the case of deficiencies of K and Mg and excess of Na or Cl. With multiple deficiencies the symptoms of one may be masked by those of another since the most deficient element tends to dominate symptom expression. The writer has often found that correction of Zn deficiency symptoms by Zn treatment was followed by appearance of Mn deficiency symptoms and vice versa. Such plants invariably show a deficient level of Mn by leaf analysis. On the other hand, symptoms of two or more deficiencies frequently occur simultaneously on the same tree such as those due to Zn and Mn, or Zn and Mg, or Zn, Mn and Mg or Zn, Mn and Fe, as observed in the Western Cape Province.

Nevertheless, by far the majority of cases permit a definite diagnosis at least in so far as Mg and the trace elements are concerned, as repeatedly substantiated by the writer through leaf analysis and response to treatment. Symptoms of N, P, K and Ca

deficiencies as a rule are not very much in evidence owing to the relatively high levels of supply maintained in most commercial orchards by regular applications of fertilizers containing them. Phosphorus and Ca deficiencies in any case cannot be satisfactorily identified on the basis of visual symptoms and those of N and K only when acute.

In general, symptom expression is reflected in leaf composition, deficiency symptoms appearing at a certain low concentration and toxicity symptoms at a certain high concentration of the nutrient concerned. The transition points tend to correspond to more or less definite concentration levels but are not fixed in relation to nutrient content so that lack of symptoms is no guarantee that the plant is not seriously deficient. When dealing with a large number of suboptimal orchards it is possible to correlate leaf nutrient concentration with symptom expression and fix a nutrient level below which deficiency symptoms will occur more often than not and above which they are unlikely to occur very often.

As intimated in the Introduction to Part III (page 141), this threshold level for symptom expression in the case of Mg and the trace elements may provisionally be considered to coincide with the level at which maximum economic production is possible.

A brief description of the visual symptoms of malnutrition are given below, indicating only the more prominent features which in the opinion of the writer may be most helpful as a basis for identification purposes. The symptoms encountered in South African orchards have already been recorded by the writer (14, 14a). A somewhat more comprehensive account is presented here, supplemented by the photographic illustrations of symptoms found in the Western Cape Province and by reference to other sources as indicated. All of the essential nutrient elements are considered except S and Mo since there is no local evidence which

would suggest that the supply or leaf content of these nutrients constitutes a nutritional problem at present.

3.1 DEFICIENCY SYMPTOMS.

NITROGEN.

Common symptoms are lack of green colour and restriction of growth. Leaves are smaller than usual, yellowish green and ultimately show yellow, red or purple tints. The older leaves are affected first and drop prematurely. Shoots are short, thin and few. Blossoming, fruit set and yield are reduced. Fruits remain small, become highly flushed and have good flavour and keeping quality (63, 129, 202, 216).

Because of its rapid renewal growth, peach is particularly susceptible to N deficiency in spring, in spite of heavy dressings of N fertilizers. This was strikingly illustrated a few years ago in the Outeniqua Pass area where heavy rain fell early in October leaching out the available N applied earlier. Marked yellowing and red spotting of the older leaves was observed in samples submitted for diagnosis. On another occasion young Early Dawn peach trees at Banhoek exhibited yellowish foliage and marked purplish tinting on the older leaves (the N content of ovendry midshoot leaves, as adjusted to January 31, was found to be 2.11%).

The effects of N deficiency are insidious and unless acute as in neglected orchards, may escape positive diagnosis, so that symptom expression cannot be regarded as a reliable index of N status.

Since quality and colour of fruit are closely associated with the level of N supply, a common practice with grape growers in certain areas is to restrict the supply of N with the object of inducing early ripening particularly of Waltham Cross grapes. In such vineyards, as well as in heavily bearing orchards, pale green and even greenish yellow foliage is frequently encountered at the picking stage.

PHOSPHORUS.

A characteristic feature of P deficiency is the dark green colour of the foliage and the tendency of the leaves to develop purple or bronze tints especially in cool weather. Fruits may be highly flushed but unlike those subject to N deficiency they have a green ground colour, high acidity and poor keeping quality (216).

Apart from the abovementioned features the deficiency effects of P closely resemble those due to N deficiency, resulting in restriction of growth, blossoming and fruiting (63, 129, 202, 216). Thus distinctive symptoms that will accurately identify P deficiency in fruit trees are limited.

Symptoms are probably more common in young trees in which the reserve supply of P is still small. Only two positive cases of P deficiency have been encountered by the writer, in both cases young peach trees growing in acid sandy soil at George and Plettenberg Bay.

POTASSIUM.

Marginal scorch of the older leaves is characteristic of K deficiency in fruit trees. The scorch may vary from necrotic spotting as reported for peach and grape (63, 216) to a well defined necrosis of the leaf margin, coloured black in pears and brown or gray in other fruits. Necrosis may be preceded or accompanied by yellowing or interveinal chlorosis as in prune (Plates 1 and 2), peach, plum and apple (216).

Potassium is readily mobile so that linear growth is not much affected at first although twigs are slender, but an acute deficiency results in stunted growth and die-back of shoots and branches. The first leaves to be affected are those on the lower half of shoots in midsummer and even if severely scorched they show little tendency to drop. An acute deficiency in peach may cause leaf necrosis on emerging growth in spring. Another early symptom in peach is crinkling and rolling of the leaves. Fruit bud formation is restricted and fruit tends to be small and poorly coloured (63, 88, 127, 129, 202, 216).

Typical marginal scorch due to K deficiency resembles and may be confused with heat and drought scorch and "brak" injury.

PLATE 1

K deficiency. Chlorosis and scorch of prune leaves, var. D'Agen (Ceres, 14/3/56)



PLATE 2

K deficiency. Chlorosis and scorch of prune leaves, var. D'Agen (Laingsburg, 18/2/54)

MAGNESIUM.

Moderate to severe symptoms of Mg deficiency have been encountered in South African apples, prunes and grapes (Plates 3, 4 and 5). The deficiency produces a distinctive form of leaf chlorosis arising as a pale green discolouration near the leaf margin which changes to bright yellow (or red in the case of certain grape varieties) until a broad marginal zone is affected extending inwards between the main veins. Chlorosis appears in midsummer and develops first on the older leaves at the base of current shoots or on spurs, spreading until all the foliage is affected. Chlorosis is often accompanied or followed by marginal scorch. Fruiting generally does not show signs of deteriorating until vigour and shoot growth have been markedly reduced.

Chlorosis in apple invariably follows the pattern as illustrated, having been observed in White Winter Pearmain, Golden Delicious, Starking and Granny Smith. The central purple tinting and brown necrosis found on Mg deficient leaves of Cox Orange Pippin and Lane's Prince Albert (131, 216) have not been encountered in apple varieties grown in South Africa.

No marked tendency of leaves affected with either chlorosis or necrosis to drop early has been observed locally but this appears to be a common feature in other countries (63, 129, 130, 131, 172, 216).

Magnesium deficiency symptoms have rarely been encountered in pear, peach and plum in South Africa. The symptoms found on peach and pear trees follow the usual pattern of marginal chlorosis and necrosis (Plates 6 and 8) but in pear central interveinal chlorosis and necrosis may also occur (Plate 7).

PLATE 3

Mg deficiency.

Chlorosis of grape leaves, var. Waltham Cross
(left) and Barlinka (right) (Bien Donne, 2/3/53)



PLATE 4

Mg deficiency.

Chlorosis of apple leaves, var. W.W. Pearmain
(Ceres, 17/3/53)

PLATE 5

Mg deficiency. Chlorosis of prune leaves, var. President
(Banhoek, 19/2/54)



PLATE 6

Mg deficiency. Marginal chlorosis and scorch of peach leaves,
var. King Edward (Bien Donne, 3/3/54)

PLATE 7

Mg deficiency. Central chlorosis and necrosis of pear leaves, var. Orange Bergamotte (Bien Donne, 3/3/54)

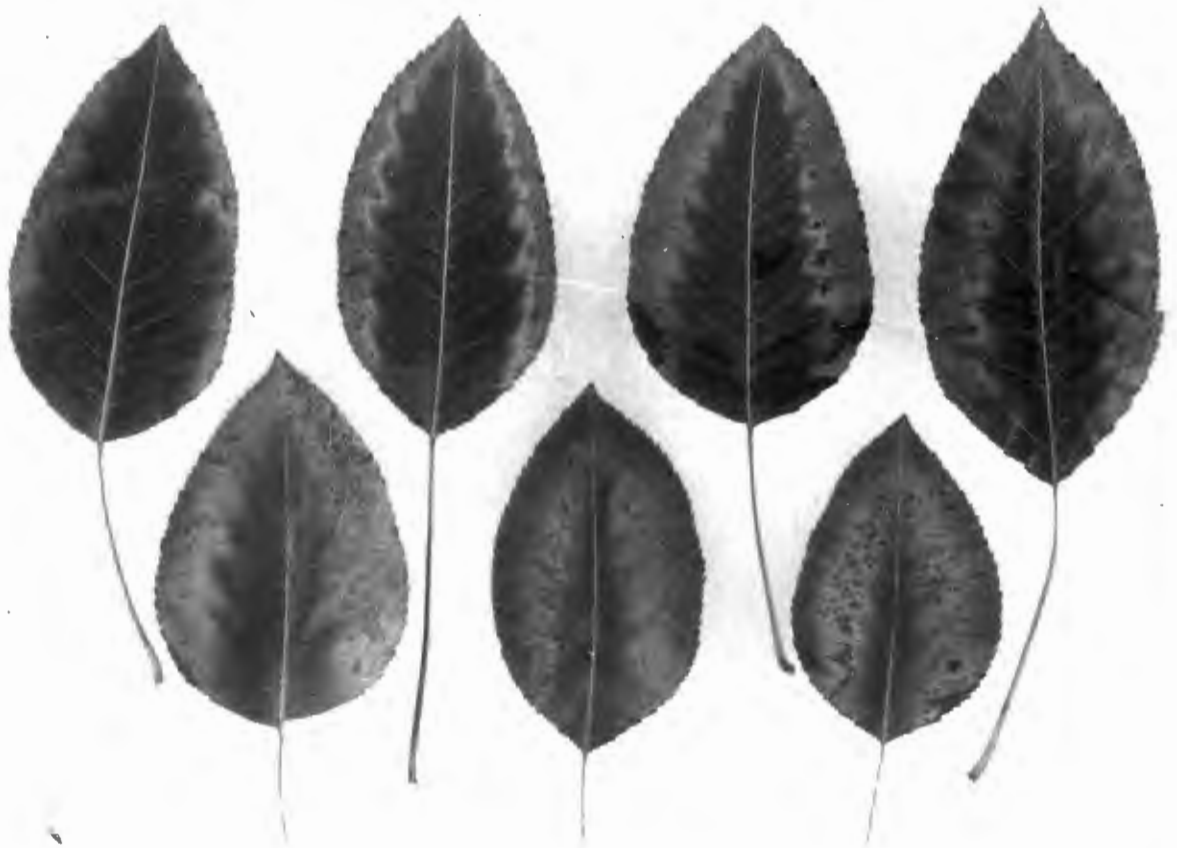


PLATE 8

Mg deficiency. Chlorosis of pear leaves, var. Louis Bonne (Bien Donne, 3/3/54)

CALCIUM.

Calcium deficiency results in breakdown of the meristematic tissues in both root and stem (129), the most prominent feature being death of the growing points of terminal shoots followed by scorching of tip leaves and die-back. The most pronounced effect is injury to root tips resulting in abnormally short and thick roots. This restricts growth and may be associated with tip and marginal necrosis of terminal leaves followed by die-back (63, 88, 129, 216).

Due to lack of mobility of Ca in the plant during the growing season, tip growth of young trees is particularly susceptible to Ca deficiency. Owing to liberation during the dormant season of insoluble Ca stored in mature tissues, older trees rarely show breakdown at the growing tips (63).

ZINC.

"Little leaf" is probably the most distinctive symptom of Zn deficiency. New growth in spring is checked in one or more terminal parts of the tree, shoots fail to elongate or have short internodes, and the leaves remain small and narrow and usually chlorotic between the main veins. Rosettes of small chlorotic leaves are also common in peach and apple. Terminal leaves on current shoots may develop interveinal chlorosis and in the case of peach, wavy margins (Plates 9 to 14).

Fruit buds on affected twigs usually fail to develop and if they do the fruits remain small; in peach and apricot they may be pointed and flattened (Plate 9). The stunted branches are prematurely defoliated but generally survive until winter when they die back to a varying extent.

The above symptoms of Zn deficiency correspond to those recorded elsewhere (11, 13, 21, 25, 43, 63, 129, 202, 216, 221). In the case of grapes a widened petiole sinus is another characteristic feature of Zn deficiency if associated with chlorosis and dwarfing of terminal leaves (58).

PLATE 9

Zn deficiency.

"Little leaf" and malformed peach fruit, var. Duke of York. Normal shoot and fruit on left (Banhoek, 12/12/54)



PLATE 10

Zn deficiency.

"Little leaf" and leaf chlorosis on peach branch var. Kakamas (Banhoek, 23/12/52)

PLATE 11

Zn deficiency. "Little leaf" on apple shoots, var. Ohenimuri
(Elgin, 7/12/53)



PLATE 12

Zn deficiency. "Little leaf" and chlorosis of grape leaves
(Simondium, 28/11/50)

PLATE 13

Zn deficiency.

"Little leaf" and terminal die-back on apricot branch, var. Royal (La Motte, 22/11/52)



PLATE 14

Zn deficiency.

"Little leaf" of plum shoots, var. Methley (Elsenburg, 4/11/53)

MANGANESE.

Interveinal chlorosis of the leaves is the main feature of Mn deficiency in all fruits (Plates 15 to 20) except pears in which case the leaves turn greenish yellow, as in N deficiency, without much evidence of a chlorosis pattern. Chlorosis invariably appears first in the older fully expanded leaves at the base of current shoots, and on spurs (Plate 17). If the deficiency is acute, all the leaves become chlorotic and somewhat reduced in size, shoot growth is restricted, and premature defoliation and die-back of terminals may occur. Fruiting is not usually directly affected unless the deficiency is serious in which case fruit buds may be devitalized to such an extent that blossoming and fruit set are sometimes markedly reduced.

According to overseas reports Mn chlorosis of peach and apricot leaves is often accompanied by necrotic spots which may fall out (202, 229). Otherwise the symptoms reported (21, 129, 202, 216, 229) are as described above.

PLATE 15

Mn deficiency. Chlorosis of peach leaves, var. Duke of York
(Banhoek, 30/11/51)



PLATE 16

Mn deficiency. Chlorosis of apple leaves, var. Ohenimuri. Normal
leaf on left (Elgin, 20/11/50)

PLATE 17

Mn deficiency.

Chlorosis of older leaves on plum branch, var. Santa Rosa. Normal leaves at tip of shoot (Banhoek, 4/11/53)



PLATE 18

Mn deficiency.

Chlorosis of plum leaves, var. Santa Rosa. Normal leaf on right (Banhoek, 30/11/51)

PLATE 19

Mn deficiency. Chlorosis of apricot leaves, var. Royal
(Paarl, 29/11/51)

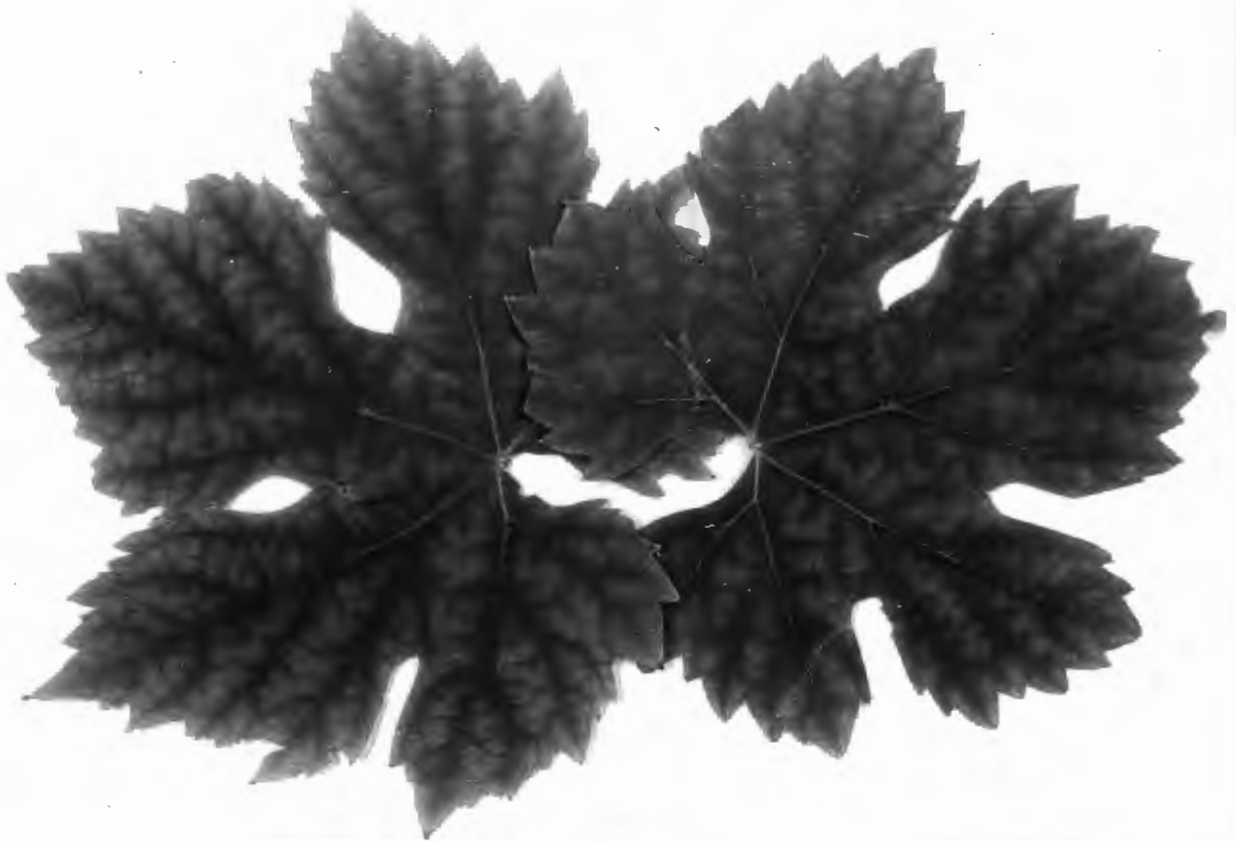


PLATE 20

Mn deficiency. Chlorosis of grape leaves, var. Steen
(Stellenbosch, 21/1/53)

IRON.

As with Mn deficiency, leaf chlorosis is the most outstanding feature of Fe deficiency but unlike the former the first signs of chlorosis always appear in the young terminal leaves of current shoots, eventually spreading to older leaves (Plates 21 to 23). The chlorosis pattern resembles a network of green veins on a yellowing background but severely chlorotic leaves become totally bleached, followed by more or less necrosis and shedding. Although the young terminal leaves are first affected, the terminal growing points of shoots remain active for a time so that there may be fair extension growth on chlorotic trees, but growth vigour ultimately deteriorates, leaf size is reduced, shoot growth is arrested, fruiting deteriorates and shoots or branches die back.

Essentially the same symptoms have been found to occur in other countries. In severe cases, fruiting is reported to be seriously affected, flowering being restricted and fruits reduced in size and highly coloured (11, 63, 129, 202, 216).

Grape vines appear to be far less susceptible to Fe deficiency than stone and pome fruits.

PLATE 21

Fe deficiency.

Chlorosis of peach leaves, var. Kakamas
(Robertson, 5/11/53)



PLATE 22

Fe deficiency.

Chlorosis of peach leaves, var. Kakamas. Greener
leaves at base of shoots (Robertson, 22/12/54)

PLATE 23

Fe deficiency, Chlorosis of apricot leaves on terminal portions of shoots, var. Royal (Robertson, 22/12/54)



PLATE 24

Cu deficiency, Withering of apical leaves and tips of apple shoots, var. Golden Delicious (Piketberg, 31/3/56)

COPPER.

The only deciduous fruits known to be affected with visual symptoms of Cu deficiency in South Africa at present are apple and pear. Leaf symptoms usually appear early in summer in the form of a necrosis of the terminal leaves on actively growing current shoots; in pears the leaf tips turn black whereas in apples reddish necrotic spots may appear in the leaves prior to tip and marginal scorch (Plates 24 and 27). This is followed by a certain amount of defoliation as the shoots die back in summer, the withered portion becoming characteristically curved to one side. These effects have suggested the descriptive terms "summer die-back" and "wither tip".

The bark on the older wood tends to become necrotic and deeply fissured. This rough bark condition has consistently been observed in both apple and pear orchards affected with summer die-back symptoms (Plates 25, 26 and 28).

Similar symptoms in apple and pear have been reported locally (3) and overseas (26, 129, 221). In the case of peach, plum and apricot, die-back of growing tips with rosette and multiple bud development as well as a variable amount of interveinal chlorosis in terminal leaves have been reported (3, 11, 26, 129, 216).

PLATE 25

Cu deficiency. Bark necrosis and "wither tip" symptoms on apple branch, var. Golden Delicious. (Piketberg, 25/4/5)



PLATE 26

Cu deficiency. Bark necrosis of apple branches, var. Winter Banana (Langkloof, 6/2/56)

PLATE 27

Cu deficiency. Tip scorch of apical pear leaves, var. Bon Chretien (Langkloof, 24/2/55)

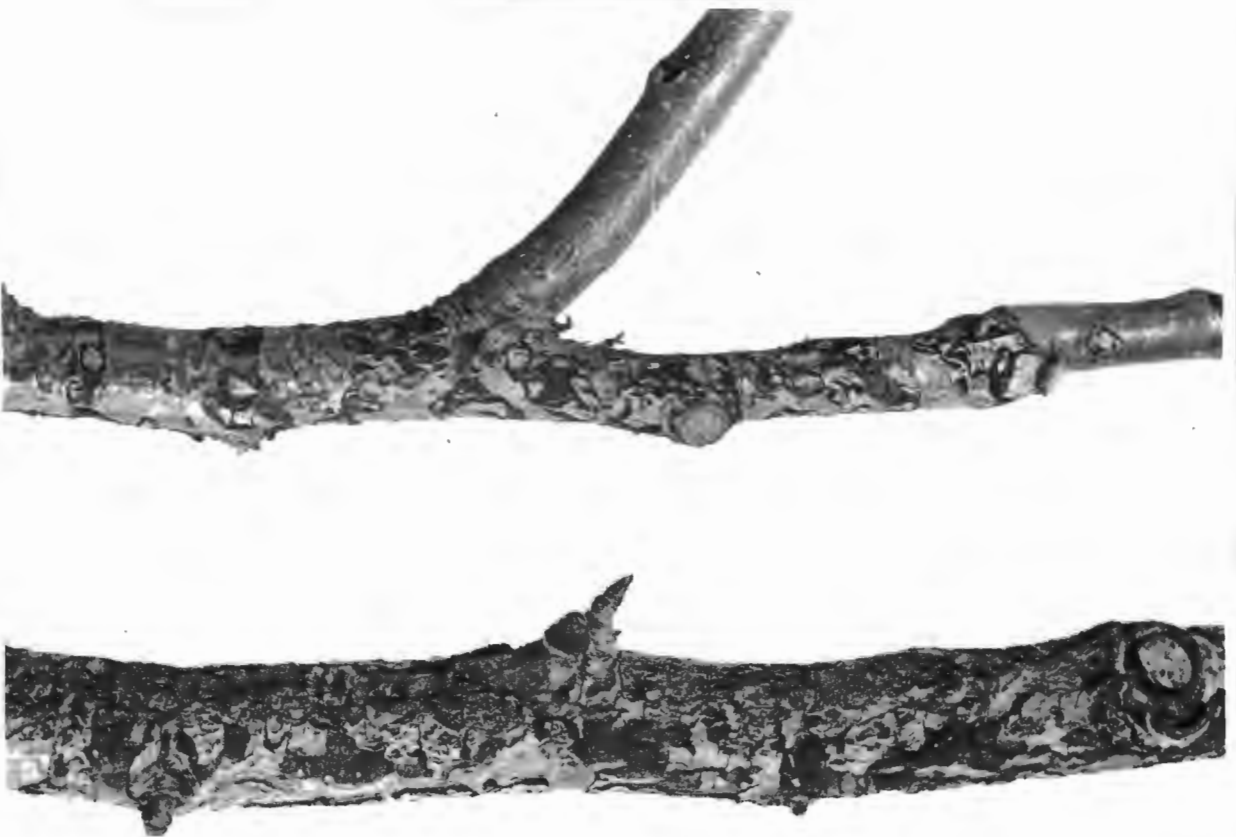


PLATE 28

Cu deficiency. Bark necrosis of pear branches, var. Beurre Hardy (Ceres, 21/7/55)

BORON.

According to overseas reports, fruit symptoms are probably the most distinctive feature of B deficiency in apple (12, 21, 43, 63, 129, 216, 221), pear (21, 129, 221), apricot (21, 129, 221, 230), plum (21) and prune (93): of these fruits, apple trees appear to be most susceptible to B deficiency, the fruit developing distinctive symptoms known as drought spot (external cork) or corky core (internal cork) which are reported to be more prevalent after a spell of hot dry weather. In apricot the deficiency causes brown spotting in the fruit flesh.

Boron is not stored in plant tissues nor transferred to regions of new growth so that interruption of supply may induce symptoms at any time during the season.

Young growing tissues may be affected, giving rise to death of growing points and various growth abnormalities in the different fruit species, such as dwarfing, chlorosis and thickening of terminal leaves with smooth margins, and excessive branching, rosetting and die-back (63, 128, 129, 192, 216, 230). Excessive wilting of flowers shortly after bloom ("blossom blast") has also been ascribed to B deficiency (228).

Bark symptoms may also occur in apple and pear in the form of blisters (apple "measles") and large necrotic cracked areas (67, 129, 221).

In grapes the growing tips are affected first resulting in stunted new growth in spring. This is accompanied by chlorosis and twisting or cupping of leaves toward the underside, and shortening of apical internodes (63, 88, 129, 170). Flower clusters may be malformed and fail to set fruit (100, 170), or accentuated millierandage (100, 221). Late season growth of vines seldom develops deficiency symptoms (170).

Regarding South African fruit, symptoms resembling some of those described above have occasionally been observed but no

evidence of specific B deficiency symptoms, supported by leaf analysis data or response to treatment, have yet been established.

3.2 TOXICITY SYMPTOMS.

According to Wallace (216), injury to plants may come about through direct injury to protoplasm, which is more prevalent with trace elements, or indirectly through excess of one element which may induce a deficiency of another and ultimately result in a deranged metabolism.

Direct toxic effects have occasionally been observed in the Western Cape Province as the result of excess of saline salts, and Mn toxicity has also been encountered. Boron toxicity symptoms have not been recognized as yet but are described below in view of the relatively high concentrations sometimes found in the leaves.

MANGANESE TOXICITY.

Manganese toxicity in peach and prune occurs near Tulbagh on acid manganiferous soil causing direct toxic effects in the form of dark necrotic areas in the bark (Plate 30) and death of buds and die-back. This is usually accompanied by strong pink colouration of the bark on young peach shoots. A certain amount of leaf chlorosis (Plate 29) resembling that due to a Mn deficiency may be associated with bark symptoms. Induced Fe deficiency effects have not been observed.

BORON TOXICITY.

Toxic effects of an excess of B in apple fruit has been described as browning of the flesh, increase of watercore, leaf mottling and root injury.

In peach, apricot and plum, toxic effects result in necrotic areas in the leaf along the midrib and small cankers on the stem and back of the midrib and petiole, also die-back and defoliation

PLATE 29

Mn toxicity. Chlorosis of peach leaves, var. Kakamas
(Tulbagh, 16/5/51)



PLATE 30

Mn toxicity. Necrosis of peach bark, var. Elberta (Tulbagh,
16/5/51)

of tips of current season branches and malformed fruit (11, 128, 230). In apricot in sand culture greatly enlarged nodes on terminal portions were a striking feature, associated with shortening of internodes. In peach, blossoming may be delayed and reduced (11, 80) and the fruit malformed and split (80).

SALT EXCESS (BRAK).

According to Lilleland (115), deciduous fruit trees have been found to be very sensitive to saline salts particularly Na salts of Cl , SO_4 , HCO_3 and CO_3 . Leaf scorch is the most prominent leaf symptom appearing at the leaf tip and extending along the leaf margin. In apricot, marginal scorch results in cupping of the affected leaves. Scorch effects are associated with root injury so that young trees usually are not affected while the roots are shallow.

Leaf scorch due to excess of either Na or Cl (brak) commonly occurs in orchards in the Karoo but several cases have also been encountered in the Koue Bokkeveld and other parts of the Western Cape Province.

3.3 LEAF ANALYSIS SURVEY IN THE WESTERN CAPE PROVINCE.

A useful method of determining critical levels of nutrient content associated with symptom expression is by direct correlation of leaf analysis data with the incidence of visual symptoms. As such it must be based on observations and records from a large number of orchards in different areas and on soils varying in nutrient supply. In this way deficiency and toxicity levels applicable to the fruit growing region may be examined more closely and at the same time a cross-section is obtained of nutrient concentrations prevailing in the region concerned.

During the course of investigational and advisory work since 1950 the writer had the opportunity of visiting a large number of farms in the Western Cape Province. As a rule leaf samples were collected and records taken of visual symptoms and other particulars. From the accumulated records it is now possible to assemble data indicating the range of nutrient concentrations in mid-shoot leaves (or basal leaves in the case of grapes) and the values associated with symptom expression.

All the functional elements, except S and Mo, are considered as well as Na and Cl which are of significance in relation to toxic saline effects. The analytical data for Zn, however, are not available as yet. In order to ensure a comparable basis for samples collected at different times during the season the original data have been adjusted to a common date namely January 31 by the use of correction curves for seasonal trends as indicated in Section 2.4 (page 146). Analytical data derived from samples contaminated with spray residues containing trace elements have been rejected, as well as early Fe data for samples inadequately washed at that time (Section 2.11, Part II: page 60). The fruit varieties considered comprise only commercial types belonging to the following species: apple (*Prunus malus*), pear *Pyrus communis*), peach (*Prunus persica*), apricot (*Prunus amagdalidis*)

plum (*Prunus salicina*), prune (*Prunus domestica*) and grape (*Vitis vinifera*).

RESULTS.

The available data are presented in Tables 29 to 35, in the form of a frequency distribution of the analytical values (represented as the midpoint of each class interval) as found for each nutrient element and each fruit species. The incidence of orchards showing symptoms is indicated by the numbers in brackets which refer strictly only to cases where typical symptoms clearly identifying the particular disorder were in evidence.

The manner of presentation of the data provides direct evidence of critical levels as well as a reflection of the range of nutrient concentrations occurring in the Western Cape Province. The highest and lowest values associated with the occurrence of symptoms, where sufficient data are available, may be considered as a reasonable estimate of the threshold values for deficiency and toxicity symptoms, respectively. The data thus provide evidence of deficiency levels for Mg, Mn, Cu, Fe and toxicity levels for Mn, Na, Cl in the particular fruits concerned. Index values suggested on the basis of these levels are recorded later in Tables 40 to 46.

The data also reflect the general status of macro- as well as micro-nutrients in orchards and vineyards in the Cape. Evidently many of the extreme values fall in the deficiency or toxicity categories or are indicative of lack of balance. As such they provide a useful guide as to which orchards and vineyards may be most profitably investigated with a view to improvement of the nutritional conditions.

TABLE 29. - APPLE. LEAF COMPOSITION IN RELATION TO NUMBER OF ORCHARDS NOT SHOWING SYMPTOMS AND THOSE SHOWING SYMPTOMS (IN BRACKETS).

Content	No.	Content	No.	Content	No.	Content	No.
<u>N</u> %		<u>P</u> %		<u>K</u> %		<u>Ca</u> %	
1.2	1	.08	3	.4	1	.4	5
1.4	0	.10	10	.6	3	.6	8
1.6	1	.12	10	.8	10	.8	12
1.8	7	.14	25	1.0	7	1.0	42
2.0	5	.16	23	1.2	18	1.2	17
2.2	23	.18	24	1.4	20	1.4	13
2.4	32	.20	7	1.6	19	1.6	6
2.6	23	.22	1	1.8	7	1.8	1
2.8	12	.24	0	2.0	9	2.0	1
3.0	2	.26	1	2.4	7	2.2	1
		.28	0	3.0	4		
		.30	1	3.6	1		
	<u>106</u>		<u>105</u>		<u>106</u>		<u>106</u>
<u>Mg</u> %		<u>Na</u> %		<u>Cl</u> %			
.05	0 (1)	.005	6	.02	7		
.10	0 (1)	.01	25	.06	11		
.15	1 (2)	.02	32	.10	8		
.20	0 (4)	.03	8	.15	11		
.25	11 (3)	.04	10	.20	2		
.30	15 (3)	.06	6	.25	0 (1)		
.35	21 (3)	.08	1	.30	0 (1)		
.40	13	.12	3	.40	1 (1)		
.45	14	.17	1	.50	0 (2)		
.50	11	.19	0 (1)	.60	0		
.70	2	.27	0 (1)	.90	0 (1)		
.90	1	.65	0 (1)				
	<u>89 (17)</u>		<u>92 (3)</u>		<u>40 (6)</u>		
<u>Mn</u> ppm		<u>Fe</u> ppm		<u>Cu</u> ppm		<u>B</u> ppm	
5	0 (4)	40	3	2.0	1 (1)	15	1
10	1 (7)	50	4	2.4	1 (2)	20	2
15	3 (2)	60	6	2.8	3 (2)	25	6
20	5 (3)	70	11	3.2	7 (1)	30	6
25	10	80	21	3.6	3 (1)	40	23
30	9 (1)	90	14	4.0	13	50	21
40	15	100	4	5.0	24	60	17
60	9	110	2	6.0	13	70	8
80	6	120	8	7.0	10	80	4
100	2	140	8	8.0	5	90	2
120	2	180	3	10.0	5	100	1
150	1	220	1	12.0	2	115	1
	<u>63 (17)</u>		<u>85</u>		<u>87 (7)</u>		<u>92</u>

TABLE 30. - PEAR. LEAF COMPOSITION IN RELATION TO NUMBER OF ORCHARDS NOT SHOWING SYMPTOMS AND THOSE SHOWING SYMPTOMS (IN BRACKETS).

Content	No.	Content	No.	Content	No.	Content	No.
<u>N</u> %		<u>P</u> %		<u>K</u> %		<u>Ca</u> %	
1.2	0	.08	5	.6	2	.6	2
1.4	1	.10	15	.8	11	.8	5
1.6	2	.12	17	1.0	13	1.0	14
1.8	9	.14	11	1.2	15	1.2	18
2.0	12	.16	7	1.4	7	1.4	18
2.2	16	.18	2	1.6	4	1.6	2
2.4	15	.20	1	1.8	1	1.8	0
2.6	3	.22	0	2.0	3	2.0	0
2.8	2	.24	1	2.2	0	2.2	1
3.0	0	.26	0	2.4	2		
		.28	0	2.6	2		
		.30	1				
	<u>60</u>		<u>60</u>		<u>60</u>		<u>60</u>
<u>Mg</u> %		<u>Na</u> %		<u>Cl</u> %			
.06	0 (1)	.005	3	.02	1		
.14	1 (1)	.01	6	.04	6		
.18	0 (1)	.015	3	.06	4		
.22	2	.02	8	.08	2		
.26	4	.025	9	.10	4		
.30	9	.03	5	.12	1		
.34	11	.035	4	.14	0		
.38	11	.04	3	.16	1		
.42	8	.045	2				
.46	8	.05	3				
.56	2	.06	5				
.76	1	.07	2				
	<u>57 (3)</u>		<u>53</u>		<u>19</u>		
<u>Mn</u> ppm		<u>Fe</u> ppm		<u>Cu</u> ppm		<u>B</u> ppm	
5	0 (4)	50	1	2	0 (1)	15	1
10	1 (1)	60	4	3	1	20	3
15	0	70	4	5	2	25	7
20	2	80	14	6	1	30	11
25	1 (1)	90	5	7	5	40	6
30	6	100	8	8	16	50	8
35	6	120	4	9	8	60	4
40	10	140	2	10	5	70	7
60	7	160	3	11	3	80	2
90	6	180	0	12	7	100	1
120	2	200	1	14	2	140	2
150	1	240	1	20	1	180	1
	<u>42 (6)</u>		<u>47</u>		<u>51 (1)</u>		<u>53</u>

TABLE 31. - PEACH. LEAF COMPOSITION IN RELATION TO NUMBER OF ORCHARDS NOT SHOWING SYMPTOMS AND THOSE SHOWING SYMPTOMS (IN BRACKETS).

Content	No.	Content	No.	Content	No.	Content	No.
<u>N</u> %		<u>P</u> %		<u>K</u> %		<u>Ca</u> %	
1.8	0 (1)	.06	1	.6	3 (1)	.4	1
2.0	3 (1)	.08	2	.8	4	.7	3
2.2	5 (1)	.10	10	1.0	1	1.0	11
2.4	7	.12	31	1.4	22	1.3	33
2.6	14	.14	37	1.8	25	1.6	36
2.8	22	.16	44	2.2	30	1.9	28
3.0	23	.18	19	2.6	36	2.2	14
3.2	34	.20	7	3.0	11	2.5	18
3.4	21	.22	2	3.2	11	2.8	7
3.6	18	.24	1	3.5	8	3.1	1
4.0	3	.26	0	3.8	2	3.4	2
4.4	1	.30	1	4.0	1	4.2	1
	<u>151 (3)</u>		<u>155</u>		<u>154 (1)</u>		<u>155</u>
<u>Mg</u> %		<u>Na</u> %		<u>Cl</u> %			
.2	2 (1)	.005	4	.01	2		
.3	10 (1)	.01	54	.02	5		
.4	25	.015	29	.04	9		
.5	29	.02	9	.06	15		
.6	37	.025	8	.08	7		
.7	17	.03	9	.10	5		
.8	12	.04	1	.15	4		
.9	8	.05	0	.20	6		
1.0	9	.07	1	.30	4		
1.1	2	.08	2	.40	1		
1.2	1	.17	2	.50	0 (1)		
1.4	1			.60	1		
	<u>153 (2)</u>		<u>119</u>		<u>59 (1)</u>		
<u>Mn</u> ppm		<u>Fe</u> ppm		<u>Cu</u> ppm		<u>B</u> ppm	
10	2 (27)	40	0 (2)	2.5	1	20	4
20	5 (9)	60	3 (3)	3.0	3	25	6
30	15 (5)	80	22 (3)	3.5	10	30	14
40	26 (1)	100	25 (2)	4.0	6	35	11
60	19	120	22	4.5	14	40	16
100	10	140	10	5.0	23	50	11
150	9	160	1	6.0	30	60	19
200	2	180	2	7.0	13	70	15
250	2	200	1	8.0	5	80	12
300	2	220	1	9.0	4	90	5
350	1	240	1	12.0	7	100	2
400	0 (1)			16.0	2	115	2
	<u>93 (43)</u>		<u>88 (10)</u>		<u>118</u>		<u>117</u>

TABLE 32. - APRICOT. LEAF COMPOSITION IN RELATION TO NUMBER OF ORCHARDS NOT SHOWING SYMPTOMS AND THOSE SHOWING SYMPTOMS (IN BRACKETS).

Content	No.	Content	No.	Content	No.	Content	No.
<u>N</u> %		<u>P</u> %		<u>K</u> %		<u>Ca</u> %	
1.0	1	.04	1	.8	0 (1)	.4	3
1.3	1	.06	2	1.4	1	.6	1
1.6	3	.08	4	1.8	2	.8	11
1.9	10	.10	12	2.2	7	1.0	9
2.2	14	.12	15	2.6	8	1.2	13
2.5	13	.15	16	3.0	19	1.4	12
2.8	7	.18	7	3.4	6	1.6	6
3.1	3	.21	7	3.8	10	1.8	4
3.4	4	.24	1	4.2	6	2.1	4
3.7	0	.27	1	4.6	5	2.5	3
4.0	1	.30	1	5.0	1	3.5	0
		.33	1	5.4	1	4.5	1
	<u>57</u>		<u>68</u>		<u>66 (1)</u>		<u>67</u>
<u>Mg</u> %		<u>Na</u> %		<u>Cl</u> %			
.20	0 (1)	.005	7	.02	2		
.25	4	.01	13	.04	6		
.30	3	.02	6	.06	3		
.35	4	.03	9	.08	2		
.40	3	.04	4	.10	5		
.45	11	.05	1	.15	4		
.50	7	.06	0	.20	3		
.55	3	.07	0	.25	1		
.60	10	.08	0	.30	2		
.70	14	.09	2	.40	1		
.80	4			.50	1		
.90	4			.60	0 (1)		
	<u>67 (1)</u>		<u>42</u>		<u>30 (1)</u>		
<u>Mn</u> ppm		<u>Fe</u> ppm		<u>Cu</u> ppm		<u>B</u> ppm	
5	0 (8)	30	0 (1)	2	1	15	1
10	0 (4)	40	3	3	7	20	1
15	1 (4)	50	1	4	6	25	1
20	3 (2)	60	3 (1)	5	6	30	4
25	9	70	2 (1)	6	5	35	3
30	5 (2)	80	4 (1)	7	3	40	8
40	11	90	1 (1)	8	4	45	4
70	6	100	2	9	4	50	3
100	8	120	3	10	1	55	4
150	0	140	2	11	1	60	0
200	2	160	1	13	3	70	3
250	1	180	1	15	1	80	2
	<u>46 (20)</u>		<u>23 (5)</u>		<u>42</u>		<u>34</u>

TABLE 33. - PLUM. LEAF COMPOSITION IN RELATION TO NUMBER OF ORCHARDS NOT SHOWING SYMPTOMS AND THOSE SHOWING SYMPTOMS (IN BRACKETS).

Content	No.	Content	No.	Content	No.	Content	No.
<u>N</u> %		<u>P</u> %		<u>K</u> %		<u>Ca</u> %	
1.2	1	.06	1	1.2	1	.6	1
1.4	0	.08	0	1.4	0	.8	1
1.6	0	.10	6	2.0	2	1.0	1
1.8	0	.12	10	2.2	4	1.2	2
2.0	1	.14	6	2.4	4	1.4	5
2.2	4	.16	2	2.6	6	1.6	3
2.4	7	.18	2	2.8	1	1.8	5
2.6	11	.20	1	3.0	6	2.0	2
2.8	4			3.2	2	2.2	3
3.0	1			3.4	2	2.4	3
				3.6	0	2.7	2
				3.8	1	3.0	1
	<u>29</u>		<u>28</u>		<u>29</u>		<u>29</u>
<u>Mg</u> %		<u>Na</u> %		<u>Cl</u> %			
.15	1	.008	1	.01	1		
.20	1	.010	8	.03	2		
.25	2	.015	8	.05	1		
.35	2	.020	1	.07	1		
.45	6	.03	3	.09	1		
.50	4	.04	0	.10	3		
.55	7	.05	0	.20	0		
.60	2	.06	3	.30	0		
.65	3	.07	1	.40	1		
.80	1	.08	0				
		.10	0				
		.15	±				
	<u>29</u>		<u>26</u>		<u>10</u>		
<u>Mn</u> ppm		<u>Fe</u> ppm		<u>Cu</u> ppm		<u>B</u> ppm	
10	0 (4)	60	1	2.0	1	25	2
15	0 (1)	70	1	2.5	1	30	0
20	0 (1)	80	3	3.0	3	35	3
25	1	90	2	3.5	1	40	7
30	2	100	3	4.0	4	50	6
40	3	110	3	5.0	6	60	2
60	3	120	1	6.0	7	70	1
80	1	140	3	7.0	1	80	1
100	2	180	1	8.0	0	90	2
130	1	200	2	12.0	2	100	0
180	1	230	2			140	1
630	0 (1)	260	2				
	<u>14 (7)</u>		<u>24</u>		<u>26</u>		<u>25</u>

TABLE 34. - PRUNE. LEAF COMPOSITION IN RELATION TO NUMBER OF ORCHARDS NOT SHOWING SYMPTOMS AND THOSE SHOWING SYMPTOMS (IN BRACKETS).

Content	No.	Content	No.	Content	No.	Content	No.
<u>N</u> %		<u>P</u> %		<u>K</u> %		<u>Ca</u> %	
1.2	1	.06	1	.2	0 (2)	.4	1
1.4	3	.08	1	.4	0 (4)	.6	3
1.6	2	.10	4	.6	0 (1)	.8	4
1.8	6	.12	7	.8	0	1.0	13
2.0	2	.14	7	1.0	1 (1)	1.2	11
2.2	11	.16	12	1.3	3	1.4	4
2.4	13	.19	6	1.6	8	1.6	6
2.6	4	.22	4	1.9	5	1.8	1
2.8	4	.25	1	2.2	6	2.2	2
		.28	1	2.6	11	2.4	1
		.31	2	3.0	3	2.6	1
				3.4	1		
	<u>46</u>		<u>46</u>		<u>38 (8)</u>		<u>47</u>
<u>Mg</u> %		<u>Na</u> %		<u>Cl</u> %			
.20	0 (1)	.005	1	.02	4		
.25	4 (2)	.010	5	.04	4		
.30	2 (1)	.015	10	.06	3		
.35	5	.020	7	.08	2		
.40	4	.025	5	.10	5		
.45	4	.03	3	.12	1		
.50	10	.04	0	.14	4		
.6	8	.05	2	.16	0		
.7	2	.06	2	.18	2		
.8	2	.07	2	.20	1		
1.0	1	.08	0	.22	1		
1.2	1	.09	1				
	<u>43 (4)</u>		<u>38</u>		<u>27</u>		
<u>Mn</u> ppm		<u>Fe</u> ppm		<u>Cu</u> ppm		<u>B</u> ppm	
15	1	40	1	2.0	0	20	3
20	5	50	2	2.5	1	25	2
25	2	60	9	3.0	2	30	2
30	2 (1)	70	7	3.5	2	35	6
35	3	80	5	4.0	3	40	5
40	3	90	2	5	5	50	7
50	6	100	1	6	12	60	4
60	7	110	2	7	6	70	4
80	8	130	3	8	3	80	2
150	4	170	1	9	0	90	1
250	1	220	1	10	1	100	1
350	1						
	<u>43 (1)</u>		<u>34</u>		<u>35</u>		<u>37</u>

TABLE 35. - GRAPE. LEAF COMPOSITION IN RELATION TO NUMBER OF VINEYARDS NOT SHOWING SYMPTOMS AND THOSE SHOWING SYMPTOMS (IN BRACKETS)

Content	No.	Content	No.	Content	No.	Content	No.
<u>N</u> %		<u>P</u> %		<u>K</u> %		<u>Ca</u> %	
1.0	0	.04	1	.4	5	.2	1
1.2	1	.06	1	.6	17	.8	1
1.4	14 (1)	.08	9	.8	16	1.0	3
1.6	19 (4)	.10	8	1.0	18	1.2	2
1.8	13	.12	17	1.2	11	1.4	5
2.0	18	.14	16	1.4	8	1.6	12
2.4	8	.17	13	1.6	3	2.0	23
2.8	4	.20	8	1.8	3	2.4	24
3.2	1	.25	4	2.0	2	2.8	7
3.6	1	.30	3	2.2	0	3.2	4
		.40	1	2.4	0	3.6	1
		.45	1	2.6	1	4.0	1
	<u>79 (5)</u>		<u>82</u>		<u>84</u>		<u>84</u>
<u>Mg</u> %		<u>Na</u> %		<u>Cl</u> %			
.06	0 (1)	.01	5	.02	5		
.10	1 (6)	.02	14	.04	8		
.14	1 (3)	.03	15	.06	1		
.18	3 (3)	.04	4	.08	3		
.22	15	.05	9	.10	16		
.30	21 (1)	.06	4	.20	7		
.40	14	.07	8	.30	4		
.50	8	.08	4	.40	0		
.60	5	.10	4	.50	2		
.70	1	.12	5	.60	2		
.80	0	.16	1				
.90	1	.20	1				
	<u>70 (14)</u>		<u>74</u>		<u>48</u>		
<u>Mn</u> ppm		<u>Fe</u> ppm		<u>Cu</u> ppm		<u>B</u> ppm	
5	0 (3)	50	1	3	4	15	1
10	0 (6)	60	1	4	5	20	3
15	1 (1)	70	8	5	9	25	0
20	1 (1)	80	6	6	8	30	6
25	1	90	9	8	5	40	14
30	3	100	9	10	0	50	19
70	17	120	5	12	1	60	9
150	15	140	2	14	5	70	8
250	6	160	1	16	1	80	6
450	4	200	1	18	0	90	2
750	4	220	2	20	1	120	4
950	1	240	1			150	1
	<u>53 (11)</u>		<u>46</u>		<u>39</u>		<u>63</u>

1. NUTRITIONAL LEVELS ASSOCIATED WITH OPTIMUM GROWTH AND YIELD.

The use of high performance trees in providing leaf analysis data for the purpose of establishing nutrient standards evidently demands a definition of the characteristics which must be considered when selecting such trees. High yield is a necessary requirement but the capacity of the tree to maintain high production over a number of years is equally important. The ability to produce heavy crops regularly can only be achieved if growth is sufficiently vigorous to sustain high productivity. Furthermore, quality of fruit and keeping quality must also be taken into account. In some cases fruit quality may be the primary consideration, depending on specific requirements as demanded by the canning trade or export market.

The criterion used in selecting trees for the purpose in view, therefore, may best be described by the term "economic production", comprising high yield, satisfactory vigour and good quality fruit. In deciding what yield may be considered as good, the writer was guided by yield records for each fruit based on the 1955 orchard survey in the fruit growing areas (66). Yields of double the average for the Cape Fruit Industry was used as the minimum requirement for the high production category, only trees bearing consistently above this level being **considered**. The level of production in pounds (lbs.) per tree on this basis was as follows:- apple and pear, 200 lbs.; canning peaches, 150, and dessert, 100; apricot and prune, 100; plum, 60; and grapes $1\frac{1}{2}$ boxes (15 lbs.) per vine (on sloping trellis with average spacing). As a further guide the age of the trees was also taken into account. It is generally considered that apple and pear trees are capable of maximum production from the age of 15 to 35 years; peach, apricot, plum and prune from 9 to 16, and grapes from 5 to 25 years.

Vigour was judged by observation in the orchard and considered adequate if sufficient new growth was in evidence. As to fruit quality, the decision had to be confined as to whether the fruit was sound and of good appearance and size, since it was impossible to apply further tests for keeping quality and canning requirements.

With these criteria in mind the writer made a systematic survey in the main fruit producing areas of as many orchards as possible during January and February, 1958, selecting those which conformed to the required standard of vigorous growth, high yield and good quality fruit. With regard to grapes, the investigation was confined to the Hex River Valley which is the largest producing area for table grapes. Trees showing symptoms of nutritional disorders, insect or disease infestation were avoided, as well as localities associated with abnormal growing conditions, such as shallow soil, brak and poor drainage, so as to ensure that leaf composition would not be influenced by factors other than nutritional.

Only the most important commercial varieties were considered, selecting those best adapted to conditions in each district, irrespective of soil type, as follows:-

- Apple : Ohenimuri, Golden Delicious, Starking, White Winter Pearmain, Rome Beauty and Granny Smith.
- Pear : Bon Chretien and Packham's Triumph.
- Peach : Kakamas, Goosen, Elberta, Peregrine, Boland and Early Dawn.
- Apricot : Royal on both peach and Marianna stock.
- Plum : Santa Rosa, Gaviota and Kelsey, mostly on Marianna stock.
- Prune : D'Agen on peach and Marianna.
- Grape : Barlinka and Waltham Cross.

A total of 138 orchards and 17 vineyards were selected for this study. The distribution of the sampling sites according to fruit species and district is indicated in Table 36.

TABLE 36. - NUMBER OF SAMPLING SITES ACCORDING TO FRUIT SPECIES AND DISTRICT.

District	Apple	Pear	Peach	Apricot	Plum	Prune	Grape
Elgin	27	5	3				
Somerset West		3	2				
Stellenbosch		3	1		4		
Barhoek		1			2		
Villiersdorp	4		1				
Fransch Hoek			6		4		
Groot Drakenstein		6	5	1			
Paarl			3				
Wellington				3			
Koue Bokkeveld	7	3	2			1	
Ceres	1	4	3		1	1	
Tulbagh			3			3	
Wolseley		1	1	1	1		
Worcester			3	2			
Robertson			4				
Montagu			4				
Barrydale	1	1	3			3	
Hex River Valley							17
Total	40	27	44	7	12	8	17

In carrying out the survey it was found that a few of the best orchards showed mild symptoms of Mn and Fe deficiencies.

Since these orchards were capable of high performance over a number of years the presence of mild symptoms could not be interpreted as meaning that the macro-nutrients were out of balance and accordingly were not rejected for the purpose of determining index values for macro elements.

Leaf samples were collected from a compact group of trees in each orchard in accordance with the procedure described in Chapter 2. All relevant factors were recorded including age, growth, yield, soil type, fertilizer and cultural treatments, nutrient sprays applied and date of sampling.

4.1 RESULTS OF THE SURVEY.

The analytical results are presented in Tables 37 and 38, indicating the lowest and highest values found according to the survey which as suggested in Chapter 1, are considered to represent the lower and upper limits of the postulated optimum range. The validity of this deduction is dependent on data being available from a sufficient number of sampling sites covering a wide range of nutrient supply. In the case of apricot, plum and prune, the number was inadequate and accordingly the values obtained for these fruits cannot be regarded as altogether representative. The Mn and Cu values for grapes are also not representative owing to the reduced number available after rejecting data from samples which had been sprayed with these elements.

Comparison of data for the various fruits indicate considerable differences in the respective leaf nutrient levels (particularly marked in the case of K and Ca) which may be assumed to reflect the particular requirements of each fruit species for economic production. The mean values differ according to fruit species but may also be influenced by the soil fertility potential applying to the particular fruit. They are of significance in

TABLE 37. - LEAF COMPOSITION ASSOCIATED WITH GOOD PERFORMANCE ORCHARDS.

Nutrient element	Fruit	No. of orchards	Leaf Composition (%)			
			Low	High	Mean	% S.E.
N	Apple	40	2.21	3.02	2.52	7
	Pear	27	2.01	2.83	2.37	9
	Peach	44	2.01	3.71	2.99	15
	Apricot	7	1.68	2.66	2.20	-
	Plum	12	2.30	2.98	2.61	-
	Prune	8	2.31	2.84	2.51	-
	Grape	17	1.49	2.34	1.91	14
P	Apple	40	.125	.212	.157	13
	Pear	27	.101	.172	.131	15
	Peach	44	.120	.188	.151	10
	Apricot	7	.109	.202	.135	-
	Plum	12	.114	.210	.140	-
	Prune	8	.157	.303	.195	-
	Grape	17	.128	.400	.214	44
K	Apple	40	.51	2.07	1.32	25
	Pear	27	.70	1.86	1.12	24
	Peach	44	.74	3.23	2.12	24
	Apricot	7	2.33	3.50	2.85	-
	Plum	12	2.23	3.03	2.63	-
	Prune	8	1.21	2.63	1.90	-
	Grape	17	.56	1.60	.96	31
Ca	Apple	40	.72	1.56	1.11	19
	Pear	27	.89	1.52	1.21	14
	Peach	44	1.16	3.50	2.06	27
	Apricot	7	1.13	1.71	1.32	-
	Plum	12	1.30	2.62	1.95	-
	Prune	8	1.13	2.18	1.66	-
	Grape	17	1.62	2.36	2.06	11
Mg	Apple	40	.21	.57	.36	24
	Pear	27	.24	.53	.36	20
	Peach	44	.36	1.08	.63	34
	Apricot	7	.46	.69	.58	-
	Plum	12	.43	.59	.50	-
	Prune	8	.42	.61	.51	-
	Grape	17	.17	.62	.31	36

TABLE 38. - LEAF COMPOSITION ASSOCIATED WITH GOOD PERFORMANCE ORCHARDS.

Nutrient element	Fruit	No. of orchards	Leaf Composition (ppm)			
			Low	High	Mean	% S.E.
Mn	Apple	24	11	126	35	72
	Pear	19	22	93	49	38
	Peach	34	18	134	47	55
	Apricot	6	28	79	48	-
	Plum	6	32	134	75	-
	Prune	7	19	72	51	-
	Grape	9	51	274	115	-
Fe	Apple	40	54	222	108	35
	Pear	27	59	232	103	39
	Peach	44	39	241	117	33
	Apricot	7	44	125	79	-
	Plum	12	64	266	157	-
	Prune	8	61	136	86	-
	Grape	17	53	167	99	25
Cu	Apple	40	3.3	8.9	5.3	2
	Pear	27	5.3	12.0	8.4	25
	Peach	44	3.1	6.9	5.2	20
	Apricot	7	2.2	5.7	3.9	-
	Plum	12	3.6	6.0	4.7	-
	Prune	7	4.4	7.3	5.9	-
	Grape	6	4.6	27.0	14.4	-
B	Apple	40	36	115	56	30
	Pear	27	29	180	61	40
	Peach	44	29	120	61	32
	Apricot	7	32	70	50	-
	Plum	12	41	142	65	-
	Prune	8	39	76	61	-
	Grape	17	36	95	56	32
Na	Apple	40	70	480	190	48
	Pear	27	50	680	310	60
	Peach	44	70	210	110	28
	Apricot	7	70	120	100	-
	Plum	12	80	300	140	-
	Prune	8	130	680	290	-
	Grape	17	80	1210	390	82

that they may be considered to approximate the ideal composition (Chapter 1, page 140) both as to concentration and nutrient balance in the areas concerned.

As may be expected from samples drawn from such a wide range of growing conditions, the data for each nutrient show considerable variability as indicated by the percentage Standard Error (not calculated for apricot, plum and prune). The magnitude of this variability in concentration tends to be greater with some of the trace elements particularly Mn and Na owing to luxury consumption.

4.2 SUPPORTING EVIDENCE FROM A GRAPE FERTILIZER TRIAL.

Data from fertilizer experiments are fundamental in providing the ultimate basis on which index values should be established. The only fertilizer trial available which may supply such evidence is one concerning table grapes conducted at Bien Donné, the experimental farm of the Western Province Fruit Research Station. The yield and leaf composition data for 1951, when the experiment had been in operation for twelve years, may be considered. The analytical values for N, P, K, Ca and Mg in composite samples of basal leaves of the two varieties, Barlinka and Waltham Cross, are presented in Table 39. These values represent the leaf composition at harvest after adjustment for seasonal effect.

The data in Table 39 are presented in order of mean yield per vine calculated from data (unpublished) supplied by Mr. P.E. le R. van Niekerk. It is evident from Table 39 and other data on quality of fruit (146) that only the Barlinka plots treated with N₃P₂K₂ and N₃K₂ fertilizer mixtures (No. 1 and 2) exceeded the minimum standard of yield and quality (1½ boxes per vine) as adopted for selecting good performance vineyards (described above). The leaf composition of these

TABLE 39. - LEAF COMPOSITION AND YIELD DATA FROM FERTILIZER PLOTS AND OTHER VINEYARDS.

No.	Variety and source	Yield (boxes per vine)	N %	P %	K %	Ca %	Mg %
<u>Barlinka Fert. Expt.</u>							
1	Treatment N3P2K2	2.1	2.44	.155	1.56	2.24	.190
2	" N3 K2	2.0	2.37	.147	1.47	2.17	.149
3	" N3P2	1.6	2.43	.147	.72	2.58	.248
4	" N1 K2	1.5	2.24	.146	1.50	2.46	.142
5	" N1P2K2	1.5	2.22	.150	1.74	2.51	.118
6	" N3	1.5	2.45	.143	.67	2.89	.250
7	" N1	1.3	2.24	.143	.64	2.91	.227
8	" N1P2	1.2	2.34	.152	.73	2.51	.255
	Signift. Diff. at .05	.5					
<u>Waltham Fert. Expt.</u>							
9	Treatment N3P2K2	.6	1.64	.110	1.26	2.40	.094
10	" N3 K2	.4	1.60	.112	1.19	2.09	.069
11	" N1 K2	.3	1.47	.105	1.33	1.96	.069
12	" N1P2K2	.3	1.44	.110	1.38	2.30	.060
13	" N1P2	.2	1.58	.113	.47	2.04	.141
14	" N3P2	.2	1.80	.120	.49	3.11	.157
15	" N1	.2	1.67	.111	.45	2.24	.187
16	" N3	.2	1.76	.112	.45	2.27	.181
	Signift. Diff. at .05	.2					
17	Waltham, Hex Valley	1.0	1.60	.130	.72	2.28	.22
18	" "	1.0	1.82	.132	.60	2.76	.21
19	" "	1.0	1.49	.134	1.24	.96	.33
20	" , Paarl	1.0	1.48	.113	1.04	2.46	.19
21	" , Stellenbosch	4.0	1.48	.122	1.01	1.81	.21
22	" "	4.0	1.50	.114	1.04	1.65	.24
23	Barlinka, Hex Valley	.5	2.14	.176	1.20	1.97	.51

vines must therefore be considered optimal, and that from the other treatments sub-optimal. Under the conditions of the experiment it is evident that high performance was possible only through a high level of supply of N and K fertilizers so that the low K values for No. 6, 7 and 8 indicate an outright deficiency of K. The poorer quality and yield of No.3 may likewise be ascribed to K inadequacy. The critical sufficiency level for leaf K accordingly must lie above 0.73%. In view of this

evidence, the lower limit at 0.56% K found in the survey (Table 37) is evidently too low and may provisionally be raised to 0.80%.

The relatively poor performance of No.4 and 5 is due either to inadequate N supply (which is not clearly reflected by the leaf values), to Mg deficiency or to poor nutrient balance. Both No.4 and 5 showed marked symptoms of Mg deficiency as recorded by the writer in a previous publication (14a), but so did the good performance No.2. Thus although the low Mg values indicate the need for supplementing the supply of Mg, the low status in itself did not prevent high performance. The K and Ca values of No.4 and 5 could be interpreted as suggesting that they may be out of balance in that both values are rather high. This deduction is supported by the respective upper limits found for good performance vineyards (Table 37). A diagnosis based on the leaf values for No.4 and 5 would indicate the need for improving the Mg status; this can be adjusted most effectively by supplying Mg, and also N which is known to increase absorption and thus indirectly also satisfy the need for N application as indicated by the yield data.

The Waltham Cross plots irrespective of fertilizer treatment failed to reach the stipulated standard of performance, suggesting that some factor other than nutrient supply was effective in reducing the yielding capacity of this variety as compared with the far superior performance of Barlinka under the same conditions. The poor yields of Waltham Cross are adequately reflected by the acutely subnormal K and Mg values as well as the relatively low range of N values.

4.3 CONSIDERATION OF A FEW MISCELLANEOUS VINEYARDS.

At the time of the survey in the Hex Valley, the writer happened to investigate a number of vineyards which were either less productive or situated in other districts. The composition of

leaf samples from these vineyards (No.17 to 23 at foot of Table 39) provides the opportunity of checking the nutritional levels found above.

The moderate performance of No.17 and 18 would be attributable to inadequate K supply as indicated by the low K values (lower than the suggested limit of 0.80%). The low yield of No.19 would probably be due to low Ca supply in view of the subnormal Ca value. No.20 appears to have a normal range in concentration of all the nutrients except Mn (not shown in Table 39) which was found to be 11 p.p.m., the vines showing marked deficiency symptoms. The high yields of No.21 and 22 (even though favoured by wider spacing and high trellising) should be associated with analytical values in the optimum range as is actually the case. No.23, however, although its leaf data, including the micro-nutrient status, fall in the optimum range, produced a very low yield. In this case some other factor is evidently responsible necessitating a re-examination of non-nutritional factors such as soil environment and root development.

CONCLUSION.

The use of the nutritional levels suggested by the survey and grape fertilizer trial thus permit a reasonably complete interpretation of the analytical results in the majority of the cases described. Evidence that response will follow fertilizer applications predicted on the basis of such diagnosis is provided by the data given in connection with the grape fertilizer experiment.

5. NUTRITIONAL LEVELS, AS DETERMINED, COMPARED WITH CRITICAL VALUES REPORTED IN THE LITERATURE.

Critical levels of nutrient content may be expected to correspond fairly well in different countries, particularly those associated with symptom expression and with upper and lower limits of the optimum range; theoretically they should hold irrespective of soil type and climatic conditions. Mean "optimum values" may differ more substantially depending on the fertility potential prevailing in the particular fruit producing region.

A comparison of the values for each fruit species as determined in Chapters 3 and 4 of Part III with critical levels reported from other countries is presented in Tables 40 to 46. All available sources from the literature have been employed which permit comparison on a common basis. The values given all refer to midshoot leaf samples (or basal leaves in the case of grapes), collected at or near the end of January (or end of July in the northern hemisphere) and are based on the results of fertilizer experiments, sand culture work and orchard surveys.

The analytical categories tabulated in the tables refer, firstly, to nutrient levels associated with the onset of symptom expression, indicating the levels below or above which symptoms of deficiency or toxicity will occur more often than not. The "critical sufficiency level" denotes the concentration below which an increase in supply of the nutrient concerned is likely to be accompanied by a yield increase. The only reference to upper and lower limits of the "optimum range" was derived from data recorded in conjunction with "mean optimum values" by two investigators (106, 220).

According to the available sources of information direct comparison with local data is practically limited to the deficiency-symptoms category as found for Mg, Mn and Cu. Although

there is good agreement in certain cases, the threshold levels as reported for Mg and Mn deficiency symptoms in apple for instance, are generally lower and that for Cu higher than the levels suggested by the local data.

The B data suggest the interesting possibility that the B status of local orchards may be reasonably adequate with a tendency towards high levels approaching toxicity in some cases, as compared with overseas standards.

The respective values shown in Tables 40 to 46 correspond in a general way but it is evident that many of the reported index values differ considerably from one source to another. This would serve to support the general conviction that the use of index values at present should be restricted to the particular region where they have been worked out until such time as standardization of the technique of leaf analysis has reached the stage of perfection and uniformity necessary to permit a critical evaluation of index values determined in different countries.

TABLE 40. - APPLE. NUTRIENT LEVELS FROM DATA IN TABLES (T) 29, 37, 38, COMPARED WITH INDEX VALUES REPORTED IN THE LITERATURE (REFERENCE NUMBERS IN BRACKETS).

Element	Threshold for Deficiency Symptoms	Critical Sufficiency Level	Optimum Level		Critical Upper or Toxic Level
			Low	High	
N %	- 1.90(174)	- 1.50(220) 1.85(32,85)	2.21(T37) 1.69(106) 1.24(220)	3.02(T37) 2.84(106) 2.69(220)	- 2.00(32)
P %	- .17(137)	- .10(220,232)	.125(T37) .09(106) .11(220)	.212(T37) .75(106) .39(220)	-
K %	- .7-.8(7,32,206,213), 1.0(28,70,85,137,156)	- .68(201).75(85) 1.0(9,220),1.5(7)	.51(T37) .60(106) .61(220)	2.07(T37) 2.82(106) 2.50(220)	-
Ca %	- .70(213)	- 1.0(220)	.72(T37) .69(106) .74(220)	1.56(T37) 2.67(106) 2.42(220)	- 4.0(232)
Mg %	.35(T29) .15-.2(9,29,32,70,231,232), .21-.23(30,137,190,213), .47(174).	- .14(201) .20(85,220)	.21(T37) .28(106) .10(220)	.57(T37) .75(106) .50(220)	- 1.5(232)
Mn ppm	30(T29) 5(85),16(227) 25(76),30(34)	-	11(T38) 38(106) 23(220)	126(T38) 200(106) 280(220)	-
Fe ppm	-	-	54(T38) 40(106) 66(220)	222(T38) 630(106)	-
Cu ppm	3.6(T29) 4.0(85,174) 5.0(26)	- 4.0(85)	3.3(T38) 3.0(106) 13.0(220)	8.0(T38) 100(106)	-
B ppm	- 15(45),18(44),23(67) 25(85),26(174)	- 25(220)	36(T38) 10(106) 14(220)	115(T38) 150(106) 46(220)	- 143(214) 200(44)
Zn ppm	- 10(25),14(200),54(54)	-	- 14(220)	- 102(220)	-
Na %	-	-	.007(T38)	.048(T38)	.2(T29) .5(115,232)
Cl %	-	-	-	-	.25(T29) .5(232)

TABLE 41. - PEAR. NUTRIENT LEVELS FROM DATA IN TABLES (T) 30, 37, 38, COMPARED WITH INDEX VALUES REPORTED IN THE LITERATURE (REFERENCE NUMBERS IN BRACKETS).

Element	Threshold for Deficiency Symptoms	Critical Sufficiency Level	Optimum Level		Critical Upper or Toxic Level
			Low	High	
N %	-	-	2.01(T37) 2.13(106)	2.83(T37) 2.75(106)	-
P %	-	.10(232)	.101(T37) .11(106)	.172(T37) .16(106)	-
K %	.43(85).50 (232)	-	.70(T37) .80(106)	1.86(T37) 2.16(106)	-
Ca %	-	-	.89(T37) 1.18(106)	1.52(T37) 3.0(106)	4.0(232)
Mg %	.20(T30) .05(174).20 (232)	-	.24(T37) .32(106)	.53(T37) .42(106)	1.5(232)
Mn ppm	25(T30) 25(76)	-	22(T38) 68(106)	93(T38) 220(106)	-
Fe ppm	-	-	59(T38) 28(106)	232(T38) 240(106)	-
Cu ppm	2.2(T30) 5(26)6.7(85)	4(85)	5.3(T38) 5(106)	12.0(T38) 100(106)	-
B ppm	- 1(228)5(108)	-	29(T38) 10(106)	180(T38) 43(106)	-
Zn ppm	- 10(20,25)71 (111)	-	-	-	-
Na %	-	-	.005(T38)	.068(T38)	.5(115,232)
Cl %	-	-	-	-	.5(232)

TABLE 42. - PEACH. NUTRIENT LEVELS FROM DATA IN TABLES (T) 31, 37, 38, COMPARED WITH INDEX VALUES REPORTED IN THE LITERATURE (REFERENCE NUMBERS IN BRACKETS).

Element	Threshold for Deficiency Symptoms	Critical Sufficiency Level	Optimum Level		Critical Upper or Toxic Level
			Low	High	
N %	2.2(T31)	-	2.01(T37) 3.43(106)	3.71(T37) 4.60(106)	-
P %	.111(208)	.1(232).11(85)	.120(T37) .092(106)	.188(T37) .72(106)	-
K %	.65(T31) .3-1.0(4,5, 32,62,206, 232) 1.95(85)	- 1.0(114) 1.5(85,232)	.74(T37) .76(106) .60(114)	3.23(T37) 2.35(106) 3.4(114)	-
Ca %	-	-	1.16(T37) 1.06(106)	3.50(T37) 2.71(106)	- 4.0(232)
Mg %	.30(T31) .19(124).20 (232)	-	.36(T37) .41(106)	1.08(T37) 1.45(106)	- 1.5(232)
Mn ppm	40(T31) 11-19(76,85, 227) 30(34)	-	18(T38) 17(106)	134(T38) 270(106)	400(T31)
Fe ppm	100(T31)	-	39(T38) 31(106)	241(T38) 540(106)	-
Cu ppm	-	-	3.1(T38) 4(106)	6.9(T38) 30(106)	-
B ppm	- 10-20(128, 174,230)	-	29(T38) 12(106)	120(T38) 150(106)	- 80-90(80, 128,174) 168(230)
Zn ppm	- 7.5-18(18, 85,125)	-	-	-	-
Na %	-	-	.007(T38)	.021(T38)	- 1.0(174)
Cl %	-	-	-	-	.5(T31)

TABLE 43. - APRICOT. NUTRIENT LEVELS FROM DATA IN TABLES (T) 32, 37, 38, COMPARED WITH INDEX VALUES REPORTED IN THE LITERATURE (REFERENCE NUMBERS IN BRACKETS).

Element	Threshold for Deficiency Symptoms	Critical Sufficiency Level	Optimum Level		Critical Upper or Toxic Level
			Low	High	
N %	-	-	1.68(T37)	2.66(T37)	-
P %	-	-	.109(T37)	.202(T37)	-
K %	.82(T32) 1.0(232)	2.0(232)	2.33(T37)	3.50(T37)	-
Ca %	-	-	1.13(T37)	1.71(T37)	4.0(232)
Mg %	.22(T32) .20(232)	-	.46(T37)	.69(T37)	1.5(232)
Mn ppm	30(T32) 10(19)	-	28(T38)	79(T38)	-
Fe ppm	90(T32)	-	44(T38)	125(T38)	-
Cu ppm	-	-	2.2(T38)	5.7(T38)	-
B ppm	7(85)27(230) 50(42)	-	32(T38)	70(T38)	82(85) .94(230)
Zn ppm	30(85)	-	-	-	-
Na %	-	-	.007(T38)	.012(T38)	1.0(174)
Cl %	-	-	-	-	.6(T32) 0.5(232) 1.0(40)

TABLE 44. - PLUM. NUTRIENT LEVELS FROM DATA IN TABLES (T) 33, 37, 38, COMPARED WITH INDEX VALUES REPORTED IN THE LITERATURE (REFERENCE NUMBERS IN BRACKETS).

Element	Threshold for Deficiency Symptoms	Critical Sufficiency Level	Optimum Level		Critical Upper or Toxic Level
			Low	High	
N %	- 1.18(174)	-	2.30(T37)	2.98(T37)	-
P %	-	.10(232)	.114(T37)	.210(T37)	-
K %	- .68(206).75 (32) 1.82(85)	1.5(85)	2.23(T37)	3.03(T37)	-
Ca %	-	-	1.30(T37)	2.62(T37)	- 4.0(232)
Mg %	- .14(174).20 (232)	-	.43(T37)	.59(T37)	- 1.5(232)
Mn ppm	20(T33) 15(214)	-	32(T38)	134(T38)	630(T33)
Fe ppm	-	-	64(T38)	266(T38)	-
Cu ppm	- 2.9(3) 4(85)	-	3.6(T38)	6.0(T38)	-
B ppm	-	-	41(T38)	142(T38)	- 176(85)
Zn ppm	-	-	-	-	-
Na %	-	-	.008(T38)	.030(T38)	- .5(115,232) .7(40)
Cl %	-	-	-	-	- .5(232) .6(40)

TABLE 45. - PRUNE. NUTRIENT LEVELS FROM DATA IN TABLES (T) 34, 37, 38, COMPARED WITH INDEX VALUES REPORTED IN THE LITERATURE (REFERENCE NUMBERS IN BRACKETS).

Element	Threshold for Deficiency Symptoms	Critical Sufficiency Level	Optimum Level		Critical Upper or Toxic Level
			Low	High	
N %	-	-	2.31(T37)	2.84(T37)	-
P %	-	.10(232)	.157(T37)	.303(T37)	-
K %	1.0(T34) 1.0(232)2.0 (28)	- 1.0(114) 1.5(232)	1.21(T37)	2.63(T37)	-
Ca %	-	-	1.13(T37)	2.18(T37)	- 4.0(232)
Mg %	.30(T34) .20(232)	-	.42(T37)	.61(T37)	- 1.5(232)
Mn ppm	30(T34) 25(76)	-	19(T38)	72(T38)	-
Fe ppm	-	-	61(T38)	136(T38)	-
Cu ppm	-	-	4.4(T38)	7.3(T38)	-
B ppm	- 10(230)25(93)	-	39(T38)	76(T38)	- 60(230) 90-240(92)
Zn ppm	-	-	-	-	-
Na %	-	-	.013(T38)	.068(T38)	- .5(115, 232) .7(40)
Cl %	-	-	-	-	- .5(232) .6(40)

TABLE 46. - GRAPE. NUTRIENT LEVELS FROM DATA IN TABLES (T) 35, 37, 38, COMPARED WITH INDEX VALUES REPORTED IN THE LITERATURE (REFERENCE NUMBERS IN BRACKETS).

Element	Threshold for Deficiency Symptoms	Critical Sufficiency Level	Optimum Level		Critical Upper or Toxic Level
			Low	High	
N %	1.6(T35)	- 1.5(85)	1.49(T37)	2.34(T37)	-
P %	-	- .19(85)	.128(T37)	.400(T37)	-
K %	- .50(232).55 (206) .59(85).68 (31)	- 1.0(85,232)	.80(T39)	1.60(T37)	-
Ca %	-	-	1.62(T37)	2.36(T37)	-
Mg %	.30(T35) .18(173).22 (172)	-	.17(T37)	.62(T37)	-
Mn ppm	20(T35)	-	51(T38)	274(T38)	>1000(T35)
Fe ppm	-	-	53(T38)	167(T38)	-
Cu ppm	- 5.4(85,111)	- 4(85)	4.6(T38)	27(T38)	-
B ppm	- 25(171)86 (85)	-	36(T38)	95(T38)	- 900(85) 1000(111)
Zn ppm	-	-	-	-	-
Na %	-	-	.008(T38)	.121(T38)	-
Cl %	-	-	-	-	- .5(40) 1.07(85)

6. TENTATIVE INDEX VALUES FOR WESTERN CAPE PROVINCE.

The nutrient levels indicated by the data presented in Chapters 3 and 4 of Part III are suggested as tentative standards of leaf composition for the Western Cape Province. They are summarized in Table 47 as upper and lower limits of the "optimum range" (from data in Chapter 4) with the lower limits adjusted according to the level associated with the onset of visual deficiency symptoms (from Chapter 3).

Minor adjustments within the limits of the optimum range may be considered valid and are applied in a few cases where such a step may lead to an improved estimate of the index values. For instance in some cases the lower limit values (Tables 37, 38) are shown to be too low in relation to the threshold level for symptom expression (Section 3.3), such that K, Mg and Mn in apple have been raised from 0.51, 0.21, 11 to 0.8, 0.3, 25 respectively.

Except for rounding off the upper limit values for Mn, Fe and Cu, adjustment to levels beyond the limits of the optimum range have not been considered since the interrelationship between nutrients would then cease to be optimal (which is a condition of the concept relating to the optimum range).

It will be noted that the upper limits for Na, Mn, Fe, Cu and B (Cl and Zn data not yet available) are based on the data from high performance orchards and not on the level at which toxicity symptoms may become evident. Trees with leaf concentrations in the intermediate range may of course be found capable of high performance as in the case of B. In contrast to most of the other elements, the Fe data do not provide clear evidence of distinct threshold levels.

Sodium and Cl are of interest chiefly in relation to their potential toxicity effects. In the case of apple, toxicity

became evident at lower concentrations (from 0.2%) than reported values would appear to indicate.

Some of the lower-limit B values have been adjusted according to the reported values in view of the absence of definite deficiency symptoms in South Africa. Values borrowed from the literature (in brackets) apply to Zn (local data not available in time), B deficiency and toxic levels of the saline elements Na and Cl.

TABLE 47. - TENTATIVE INDEX VALUES FOR MIDSHOOT LEAVES (BASAL IN THE CASE OF GRAPES) ON 31 JANUARY,
INDICATING THE LIMITS OF THE OPTIMUM RANGE AND TOXICITY LEVELS (VALUES DERIVED FROM THE
LITERATURE IN BRACKETS)

	Nutrient Level	Apple	Pear	Peach	Apricot	Plum	Prune	Grape
N %	Low High	2.0 3.0	2.0 2.8	2.2 3.8	1.8 2.8	2.2 3.0	2.2 2.8	1.6 2.4
P %	Low High	.12 .22	.10 .18	.12 .20	.11 .20	.11 .20	.14 .30	.12 .40
K %	Low High	.8 2.2	.7 2.0	.8 3.2	2.0 3.6	2.0 3.2	1.2 2.8	.8 1.6
Ca %	Low High	.7 1.6	.8 1.6	1.2 3.5	1.1 1.8	1.2 2.6	1.1 2.2	1.6 2.4
Mg %	Low High	.30 .60	.25 .60	.35 1.10	.25 .70	.30 .60	.30 .60	.20 .60
Mn ppm	Low High Toxic	25 140 -	25 100 -	30 140 400	30 100 -	25 140 600	30 100 -	20 300 >1000
Fe ppm	Low High	60 240	60 240	60 240	60 140	60 240	60 140	60 180
Cu ppm	Low High	3.5 20	3.5 20	3.0 20	2.5 20	3.0 20	3.0 20	3.0 30
B ppm	Low High Toxic	(25) 120 (140)	(25) 180 -	(20) 120 (80)	(25) 70 (80)	(25) 140 (180)	(25) 80 (60)	(25) 100 (900)
Zn ppm	Low High	(15) (100)	(10) -	(18) -	(30) -	- -	- -	- -
Na %	High Toxic	.05 .20	.07 (.5)	.02 (1.0)	.02 (1.0)	.03 (.5)	.07 (.5)	.12 -
Cl %	High Toxic	.15 .30	.12 (.50)	.10 (.50)	.20 .60	.10 (.50)	.14 (.50)	.25 (.50)

7. CONCLUSION.

In a final analysis of the arguments and data presented, it may be claimed that a reasonable basis has been employed for setting up reference standards of leaf composition and that these provisional index values may be expected to serve as a useful guide in diagnosis of nutritional problems.

Proof of the validity of the index values obtained must be sought in applying the ultimate test provided by fertilizer experiments or otherwise simply by trial and error. Some evidence that adjustment of the nutrient supply forecast on the basis of deviations from these index values will result in a yield response is provided by the data in Table 39 (page 180) in connection with the grape fertilizer experiment. A diagnostic leaf analysis advisory service would need to operate on the basis of repeated samplings, diagnoses and adjustments in successive years, and such a scheme would provide evidence of its efficiency in due course. The value of a diagnostic decision will depend largely on the accuracy with which the analytical results are interpreted in terms of the deviations from the optimum range and the characteristic effects which may result from interactions between nutrient elements.

Some idea of the nutrient status of orchards and vineyards in the Western Cape Province may be gathered by considering how many of those visited do or do not conform to the optimum composition as laid down for high performance. The proportion falling either below or above the limits of the optimum range is indicated on a percentage basis in Table 48. It is clear that in terms of the proposed index values, every kind of fruit is subject in varying degrees to some form of malnutrition whether deficiency, luxury consumption or lack of balance. Where 20% or more of the orchards are affected (those underlined in Table 48) the situation becomes a serious matter such

TABLE 48. - PERCENTAGE OF ORCHARDS AND VINEYARDS IN WESTERN CAPE PROVINCE WHICH FALL BELOW THE LOWER LIMIT OF THE PROPOSED OPTIMUM RANGE (THOSE BEYOND THE UPPER LIMIT IN BRACKETS).

	Apple	Pear	Peach	Apricot	Plum	Prune	Grape
N	10 (0)	<u>30</u> (0)	5 (3)	14 (19)	14 (0)	<u>41</u> (0)	<u>32</u> (12)
P	17 (2)	<u>20</u> (7)	18 (5)	<u>28</u> (13)	<u>25</u> (0)	<u>35</u> (2)	<u>33</u> (1)
K	8 (11)	3 (8)	4 (10)	6 (<u>35</u>)	7 (14)	<u>20</u> (9)	<u>36</u> (8)
Ca	12 (6)	8 (3)	13 (1)	<u>35</u> (12)	14 (10)	<u>45</u> (6)	<u>21</u> (<u>30</u>)
Mg	<u>30</u> (3)	12 (2)	9 (2)	4 (<u>22</u>)	14 (17)	17 (<u>21</u>)	<u>21</u> (6)
Mn	<u>38</u> (1)	19 (6)	<u>39</u> (10)	<u>52</u> (11)	<u>29</u> (9)	<u>20</u> (13)	19 (14)
Fe	12 (0)	6 (0)	5 (0)	<u>25</u> (7)	0 (8)	<u>20</u> (6)	2 (9)
Cu	<u>20</u> (0)	4 (0)	2 (0)	2 (0)	16 (0)	6 (0)	5 (0)
B	6 (0)	13 (0)	2 (0)	6 (9)	4 (0)	11 (8)	6 (8)

that most fruits would appear to require urgent attention as to the status of several nutrients, particularly in the case of prunes, apricot and grapes.

It is interesting to note that P still figures largely as a deficiency in spite of extensive use of heavy P fertilizer dressings. Apricot is seriously deficient in Ca, at the same time showing relatively high K and Mg levels; two conditions which are evidently interrelated. Manganese deficiency is evidently a major problem and so also is Zn deficiency judging from the widespread incidence of deficiency symptoms. By overseas standards the B status would appear to be reasonably adequate in most orchards but evidently requires attention in some cases.

Many of these deviations from the optimum represent true nutritional disorders capable of correction on the lines suggested by the foliar diagnosis. A few may not show the expected

response indicated since although subnormal nutrient levels in the leaf indicates inability to obtain enough of the nutrient, they do not indicate the presence of other factors which may be acting in a way to prevent the plant from utilizing a possibly available supply of the nutrient concerned. This serves to emphasize the need, as already stated, for employing different methods when approaching an orchard problem and by elimination of other factors seek to determine whether it is purely nutritional.

In conclusion it may be pointed out that the index values presented are not by any means final, and that further refinements in the technique and adjustments to the provisional standards are contemplated. Furthermore, it is realized that the approach used in endeavouring to establish index values, that is, on the basis of good performance and symptom expression, can serve only as a preliminary step in characterizing growth response in terms of leaf analysis. The need for more intensive studies of the relationship between internal nutrient concentration and growth response to fertilizer additions is obvious. Such data can be obtained through extensive factorial experiments and may lead to a far more complete interpretation of leaf analysis data than is possible at present.

In any case the immediate advantage which can be derived from diagnostic leaf analysis is that a direct decision is possible in most cases regarding

- (a) identification of doubtful symptoms,
- (b) impending deficiencies when symptoms are absent,
- (c) lack of nutrient balance and toxic concentrations,
- (d) evidence as to where fertilizer trials may be most advantageously carried out, and
- (e) adjustment of the fertilizer programme.

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