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PREDICTION OF MINERAL NUTRIENT STATUS OF TREES BY FOLIAR ANALYSIS

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INTRODUCTION

Foliar analysis is a well established method used to assist diagnosis of mineral requirements in agriculture and horticulture. It has also been applied in forestry, but here the method has probably been used less routinely. Reasons for its greater use in agriculture and horticulture may include the high genetic uniformity of the crops, the relative uniformity of the physical and chemical nature of agricultural as compared with forest soils, and the elimination of certain variables, such as moisture stress by irrigation, competition by uniform spacing, and age. The last two sources of variation are also greatly reduced in forest plantations, and it is in plantations that foliar analysis has shown considerable promise. (Leyton, 1958; Will, 1966; Hall and Raupach, 1963; Bevege and Richards, 1972). Furthermore, foliar analysis has proved useful where stand growth has been almost precluded by extreme deficiency of one or more nutrients. For example, in Queensland slash pine (*Pinus elliottii* Engelm.) and loblolly pine (*P. taeda* L.) are grown on P deficient soils, and foliar P levels satisfactorily indicate the necessity for addition of P fertilizer (Bevege and Richards, 1972). By contrast, in eastern Victoria K is a limiting factor, and analysis of foliar K in Monterey pine (*Pinus radiata* D. Don) is suitable for indicating conditions of adequate or

deficient supply, but not conditions of incipient deficiency (Hall and Raupach, 1963, Raupach and Clarke, 1972).

Good success has been obtained with foliar analysis in New Zealand (e.g., Will, 1965, 1966). Usually clearly visible symptoms of ill health exist in the stand and the purpose of foliar analysis is to determine which nutrient is in short supply and causing the symptoms. Consequently the relationship of growth to foliar nutrient concentration does not have to be very precisely defined since differences in foliar nutrient levels between healthy stands and those showing severe stagnation are usually large. The situation in the Pacific Northwest is not generally the same. Douglas fir stands of this region, for example, are not normally severely deficient of any nutrient, although they frequently show some degree of N deficiency (Gessel, 1962). Under these circumstances, when foliar nutrient concentrations seldom decrease to severe deficiency levels, much more precise intrepretation of foliar nutrient concentrations is necessary to predict potential response to fertilizer application. These difficulties with foliar analysis of Douglas fir have led to the conclusion that "results from fertilizer trials with specific tree species under specific soils and climate must be the basis for valid recommendations based on foliar analysis" (Heilman and Gessel, 1963). Work under Scandinavian conditions has resulted in the belief that leaf analysis can supply direct information about the nutritional state of a forest stand, but only indirect information about the nutrient content of the soil (Tamm, 1964a, b). Pronounced deficiency of one nutrient in a forest stand can usually be determined by foliar analysis, but fertilization experiments must be used to determine whether a stand is worth fertilizing.

Similarly, in Britain, much the same ideas have been expressed (Binns and Grayson, 1967). Foliar analysis, which has proved its worth in checked and poorly growing crops, is still not a sufficiently refined method to use with certainty on established, steadily growing crops. More recently it has been suggested (Everard, 1973) that the proper use of foliar analysis is to determine whether any deficiencies exist, ensure that the correct fertilizer is applied, and prevent fertilizer being used wastefully.

Experience suggests that, at present, foliar analysis can be used for identifying the cause of fairly severe deficiency in forest stands, but the existing methods are generally too imprecise to allow quantitative predictions of response to increased nutrient supply. There are exceptions, however, where a single nutrient is the main limiting factor to growth and the use of foliar analysis for growth response prediction is possible.

Perhaps the nutrient status of a forest stand can only be adequately determined by application of several methods such as soil analysis, fertilizer trials, bio-assay, and foliar analysis (e.g., Swan, 1966), but the purpose of the following review is to suggest ways in which foliar analysis alone can be improved as a method of assessing

forest stand nutrient status and predicting probability of response to applied fertilizer.

SAMPLING

Crown class

Trees in a stand can generally be grouped by stem diameter, or crown classes. Such classification offers a basis for stratifying sampling of tissue to be chemically analysed. In attempting to relate growth of red pine (*Pinus resinosa* Ait.) stands to foliar nutrient concentrations three dominant, three co-dominant and three intermediate crown class trees were sampled in every stand (Hoyle and Mader, 1964). Sampling to follow seasonal nutrient changes in yellow birch (*Betula alleghaniensis* Britt.) was confined to three dominants on each site examined (Hoyle, 1965). Alternatively trees were selected for sampling at random from among all diameter classes when studying the effect of N fertilizer on Douglas fir [*Pseudotsuga menziesii* (Mirb.) Franco] stands (Heilman and Gessel, 1963).

Suppressed trees often show higher tissue nutrient concentrations than dominant trees. For example, in a comparison between a dominant and suppressed Corsican pine (*Pinus nigra* var. *calabrica*), needles, branches, and bark of the suppressed tree showed higher concentrations of P, K, and Mg than the dominant tree (Wright and Will, 1958). Similarly, a study of seven red pine indicated average concentrations of foliar nutrients and ash tended to be higher in over-topped than in dominant trees (Madgwick, 1964a).

In an extensive study of crown class effect on foliar nutrient concentration in black spruce (*Picea mariana* Mill. BSP) ash, N, P, and Ca were found to be unaffected (Lowry and Avard, 1968). K, however, was found to have lowest concentrations in dominant trees and increasing concentrations in progressively inferior crown classes. The reverse was true for Mg. Lowry and Avard (1968) concluded that, for practical purposes, dominant and co-dominant trees could be placed into one element concentration group, and intermediate and suppressed trees into a second element concentration group. In Norway spruce (*Picea abies* Karst.) foliar nutrient concentrations were similar for all crown classes, although needle weight and total nutrient content decreased from dominant to suppressed trees (Höhne 1964).

Foliar N levels have been found to decrease from dominant to suppressed Douglas fir trees (Lavender, 1970), and the range of variation about the mean was greater for suppressed than dominant trees.

It has been suggested (Lowry and Avard, 1968) that, when foliar nutrient concentration differences are found to exist between crown classes, the study objectives are used to determine which crown classes are sampled. For example, if the relationship between site class, or site index, and foliar nutrient concentration is to be ex-

TABLE I

TYPE OF NUTRIENT DISTRIBUTION IN CROWN BY SPECIES (SEE FIG. 1)

Species	Fol. Age Yrs.	Authority	Foliar nutrient					
			N	P	K	Ca	Mg	Other
			Type of distribution					
Pinus sylvestris	C*, 1	Wright and Will 1958	II	II	II	II	II	Na I
Pinus sylvestris	C	Leyton and Armson 1955	II	II	II			
Pinus nigra	C, 1	Wright and Will 1958	II	II	II	II	II	Na I
Pinus strobus	C	White 1954			II			
Pinus resinosa	C	White 1954			II			
Pinus resinosa	C, 1, 2	Madgwick 1964a	I	V	V	IV	I	
Pinus taeda		Smith, Switzer and Nelson 1970	II					
Pinus taeda		Wells and Metz 1963	III	II	II	III	III	
Pinus radiata	C, 1	Will 1957	I	III	III	III	I	
Pinus radiata	C, 1, 2	Hall and Raupach 1963	II	II	II			
Picea abies		Strebel 1961	IV	I	V	I	I	
Picea mariana	C	Gagnon 1964	II					
Picea mariana	C	Gagnon and Badoux 1971		II	III	II	II	Fe III
Picea mariana	C	Lowry and Avard 1965	II				II	
Taxus baccata		Nemec 1947			V	III		
Pseudotsuga mensiesii		Lavender and Carmichael 1966	II	II	II	III	II	
Pseudotsuga menziesii	C	Morison 1970	II	I	I	II	II	

*C = current

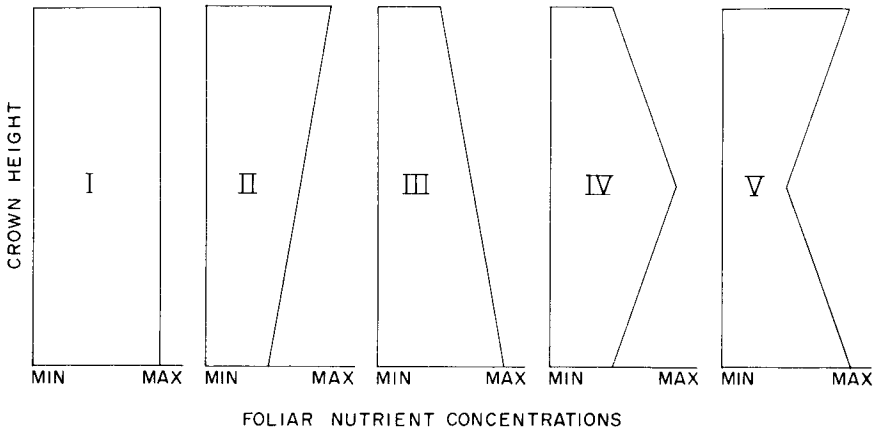

Fig. 1. Types of mineral nutrient distributions within tree crowns (see Table I).

amined, dominants and co-dominants are probably the most satisfactory trees to sample. All crown classes should be representatively sampled, however, if the relationship between stand yield and foliar nutrient concentration is to be examined.

Position in crown (i.e., height and aspect)

A number of different foliar nutrient distribution patterns within the crown have been reported. Although the various distributions shown (Fig. 1) all appear to have been described, the summary of type of distribution by species (Table I) may be misleading. Relatively few investigators have sampled at more than three heights in the crown (usually lower, middle, and upper crown). When only lower and upper crown are sampled (e.g., Gagnon, 1964), it is not possible to decide whether type IV or V distribution exists. Sometimes a number of whorls in the upper crown have been sampled, and none further down (e.g., three uppermost whorls (Leyton and Armson, 1955), ten uppermost whorls (Morison, 1970). Consequently definite information on foliar nutrient distribution within the crown is still relatively inadequate for many species and stand conditions.

While it is quite likely that different species show different nutrient concentration distribution patterns, it is also probable that extent of crown closure affects the pattern. For example, high concentration of nutrients in the lowest whorls of red pine, growing in closed canopy, has been attributed to low photosynthetic rate causing little "nutrient dilution" by new carbohydrate production (White, 1954). On the other hand, black spruce stands are relatively open so that lower branches are well illuminated and tend to show lower nutrient concentrations than upper branches (Gagnon, 1964). The importance of crown closure was shown by loblolly pine (*Pinus*

taeda L.) stands where samples from the upper crown were considered most reliable for comparing trees, since the effect of lower crown position became greater as crown competition increased (Wells and Metz, 1963).

In comparing a rapidly grown red pine stand with a slowly grown red pine stand it was found that effect of crown position was more pronounced in the rapidly grown trees (Madgwick, 1964a). Apparently this was due to greater shading of lower branches, occurring in the rapidly grown stand, resulting in higher concentration of P and K.

Analyses of foliage from upper and lower crowns of white spruce [*Picea glauca* (Moench) Voss], Norway spruce, jack pine (*Pinus banksiana* Lamb), Scots pine (*P. sylvestris* L.), and red pine, presented by Swan (1962), also suggest positional differences in nutrient concentration are greater in vigorous, rapidly growing trees, than in trees of low vigour.

Difference in K availability was thought, by Hall and Raupach (1963), to account for differences in crown distribution of nutrients between Monterey pine from a K deficient site, analysed by them, and Monterey pine from a K rich site (Will, 1957). Similarly, K deficient red pine showed a type II crown distribution of K, but, with higher K availability, type V crown distribution of nutrients was reported (White, 1954).

In higher plants the apical regions exerts control over the remainder of the organism. This is thought to be achieved by production of hormone in the apical region from which it is translocated, setting up concentration gradients within the plant. Evidence from herbaceous species indicates hormone directed transport of mineral nutrients occurs. For example ^{32}P was translocated to sites of exogenously applied auxin in beans (*Phaseolus* spp.) (Davies and Wareing, 1965). Similarly correlative inhibition of lateral buds of flax (*Linum usitatissimum*) was more evident at low than at high levels of N supply indicating that lateral buds, relatively lower in auxin, received less N when competition became acute (Gregory and Veale, 1957). It seems likely that the apical region of a plant will maintain a relatively high nutrient concentration at the expense of distal regions. Consequently the lower crown might be expected to show nutrient status of the whole plant more sensitively than the upper crown, if the argument holds good for trees. It has in fact been suggested that the N, P, and K status of red pine stands could be most sensitively assessed by sampling the lower crown (Madgwick, 1964a).

On the other hand a number of workers have sampled from near the top of the crown since it has been found that tissue nutrient concentrations of upper crown samples are correlated with such factors as tree height (Leyton and Armson, 1955; Hoyle and Mader, 1964), site index (Morison, 1970), and soil nutrient concentrations (Wells, 1965). Mid-crown sampling has been proposed as expressive

of crown means (White, 1954). It has also been shown that analytical results from lower crown samples can be as well correlated with site index as upper crown values (Swan, 1962; Gagnon, 1964).

In practice the sample should be that which has most predictive value for the objective of the study, whether this is response to fertilizer, or prediction of some growth parameter. The most satisfactory crown region to provide this sample can probably only be determined by extensive sampling trials, and it does not seem desirable to confine attention to the upper crown.

A number of workers have restricted their sampling to a particular aspect (e.g., Leyton and Armson, 1955; Hoyle and Mader, 1964; Wells, 1965; Lavender and Carmichael, 1966) presumably to avoid confounding other effects with possible aspect differences. No important variation, however, in K concentration of red and white pine foliage was associated with crown aspect (White, 1954). Similarly aspect was considered unimportant in sampling Norway spruce and Scots pine (Tamm, 1955). Differences in nutrient concentration due to aspect could perhaps be expected when the crown is exposed to uneven competition or shading. Otherwise it may not be an important source of variation.

Type of tissue

The greatest proportion of tissue analyses are done on foliage, as common use of the term "foliar analysis" implies. It has been stated that "the leaf is the focal point of many plant functions and is a relatively sensitive indicator for those mineral elements that directly affect photosynthesis in addition to being a convenient portion of the plant to sample. Up to 50 per cent of the total minerals in a citrus tree may be found in the leaves indicating the importance of this organ for mineral storage and function." (Smith, 1962). It has, however, been suggested that other tissues, e.g., buds or sap, might be more diagnostically suitable for analysis (Leaf, 1970). Twig arginine content was thought to be a more sensitive indicator of loblolly pine N status than total foliar N (Barnes and Bengston, 1968).

Foliage sampled from Valencia orange ramets grown in sand culture for 3-5 months showed N and K concentrations to be the same as those in older trees (Smith *et al.*, 1954). Comparison of concentrations of 12 mineral elements in leaves and fibrous roots showed quantitative differences in all. N, P, and K values in the two organs appeared to parallel one another fairly closely although roots contained lower levels of these elements. Analyses for the other 9 elements showed important discrepancies either in amounts or relation to treatment, between these two organs. Evaluation of leaf, or root, composition alone could lead to different conclusions, with the exception that for study of N, P, or K, alone, any part of the plant might suffice. Most satisfactory indications of nutritional level in the plant would probably be obtained by considering both foliage and rootlets.

An attempt was made to assess mineral nutrient status of *Pinus radiata* in New Zealand by analysing samples of cambium removed at breast height, (Will, 1972[1]), following a similar practice with rubber (*Hevea* sp.) in Malaya (Bolle-Jones, 1957). The procedure was abandoned in favour of foliar analysis due to wide variation and lack of useful correlations in New Zealand. Subsequently foliar analysis superseded cambial analysis with rubber in Malaya.

The chemical composition of xylem sap has been investigated in forest trees (e.g., Barnes, 1963). An important drawback to interpretation of xylem sap analysis is that rate of sap movement is not determined so that no indication of total amount of nutrient being translocated is obtained (Bollard, 1960). Nevertheless xylem sap P level of loblolly pine was increased by P fertilizer treatment, but reduced by N fertilizer treatment if P or K fertilizer was also added (Carter and Larsen, 1965). Effect of N and K fertilizer could be detected as increases in xylem sap N and K levels.

Not surprisingly, large seasonal variation in N, P, K, and Mg content of apple xylem sap was found (Bollard, 1957). The proportion of individual amino acids also changed during the growing season. Furthermore N concentration of apple xylem sap varies with age of shoot from which it is collected, as well as season (Cooper et al., 1972). In April there is an increase from the proximal end of three- year-old wood to the distal end of two-year-old wood, but then a decrease in year old wood. N fertilizer added in October was only detectable as an increase in xylem sap N compounds in the following April.

Tissues other than foliage can provide information on nutrient status, and in a mature forest stand sampling of cambium, or roots, might be more practical than sampling foliage. Relating root samples to specific trees would be difficult, but estimates of root nutrient concentration for a stand might be feasible. The added difficulty of collecting xylem sap is not outweighed by any improvement in diagnostic value. On the limited evidence available, young roots may prove more informative than cambial samples, but effort should probably be devoted to foliage and twigs.

Age of foliage

Older needles are thought to be depleted of nutrients by the growing regions and may therefore be more useful for foliar analysis (Leyton, 1948). This appeared to be true for N, P, and K in red pine, but not for Mg or Ca. The percentage of Ca increased with needle age (Madgwick, 1964a). Two-year-old foliage had higher N and P concentrations than one- or three-year-old foliage in certain Douglas fir samples. (Lavender and Carmichael, 1966). Other samples of this species showed a decreasing N concentration with age and an increas-

[1] G. M. Will. Verbally at Foliar analysis workshop meeting, Olympia, Washington, April, 1972.

ing P concentration with age to three years (Beaton et al., 1965). All reports suggest that Ca concentration increases with age in conifer needles. (e.g., Heiberg and White, 1951; Beaton et al., 1965; Lavender and Carmichael, 1966; Will, 1957; Lowry and Avard, 1965; Wells and Metz, 1963; Hõhne, 1963).

Foliage of current year's growth is normally analysed. It is preferred because of higher correlation between nutrient concentration and shoot length (Leyton and Armson, 1955), site index (Lowry and Avard, 1969), and availability of soil nutrients (Lavender and Carmichael, 1966). It has also been stated (Leyton, 1958) that under N deficient conditions total height is correlated with N status of current needles, but height increment is related to N status of year old needles. Somewhat similarly it has also been suggested that the most suitable age of needles for sampling will depend upon which nutrient is to be studied (Wells and Metz, 1963). In loblolly pine current needles showed P and K differences best, but one-year-old needles showed Ca and Mg differences most clearly. This last point seems to have been little considered. Attention has often been focused primarily on N status of the tree, and current foliage is usually the most sensitive indicator for this. Older foliage could be more informative for other nutrients. Comparison between foliage of different ages might also be informative. In spring N and P concentrations of new Douglas fir shoots decrease more rapidly than N and P concentrations of old shoots (Krueger, 1967). The relative difference in nutrient concentration between old and new shoots may be indicative of nutrient status because translocation from the former to the latter occurs (Tamm, 1955).

Time of year

Nutrient concentration in plant tissue varies with season. N, P, and K concentration in foliage of six hardwoods and Norway spruce decreased rapidly between mid-May and mid-June, and then decreased more slowly until September. By contrast Ca concentration increased between May and September (Mitchell, 1936). This work also showed that decrease in foliar nutrient concentration did not imply reduction in total nutrient content. In fact the reverse was true. Not only Ca, but also N, P, and K total content increased, and the reduction in concentration was due to increase in leaf dry weight.

Foliar N and P concentration in birch (*Betula verrucosa* Ehrh.) was fairly constant from July to September. Foliar K concentration decreased during the same period, whereas Ca concentration increased (Tamm, 1951). Similarly in yellow birch nutrient concentrations decreased in early June and then fluctuated slightly until September, finally decreasing until abscission. The exception to this was Ca, which increased steadily (Hoyle, 1965).

Analysis of petiole, leaf blade, and inflorescence of sycamore (*Acer pseudoplatanus* L.), horse-chestnut (*Aesculus hippocastanum* L.), and beech (*Fagus sylvatica* L.) for 21 macro and micro elements

showed three categories could be defined (Guha and Mitchell, 1966). (*A*) Co, Ni, Fe, V, Ti, Cr, Pb, and Al, showing a fall in leaf concentration early in the season probably due to growth dilution. (*B*) Mn, B, Si, Ca, Sr, Ba, and Mg, showing a continual rise in both concentration and absolute content until late in the season. (*C*) Cu, Mo, Zn, P, K, and Na, showing a gradual fall followed by a period when the concentration remained fairly constant. Category (C) consisted of essential elements, with the exception of Na. Measurement of the concentration of an inessential element in category (*A*), such as Al, might be useful for estimating growth. Ratios of essential elements: Al should then indicate rates of nutrient uptake and could be more informative than straightforward nutrient concentration measurements.

Maximum foliar nutrient levels occurred during July in red pine and decreased throughout the year, becoming quite stable during late winter, with the exception that N showed a minimum during August (White, 1954). Foliar analysis of six Scots pine provenances also showed that N concentration was lower in August than the following February (Gerhold, 1959). In this study seven out of the nine elements analysed had lower levels in August than in February. The exceptions were K and Cu.

N, Ca, and Mg showed a general increase in concentration in Douglas fir foliage from spring to the following winter, but P and K showed a tendency to decrease between fall and winter (Lavender and Carmichael, 1966).

In foliage of black spruce and jack pine it was found, in general, that N, P, K, Ca, and Mg, concentrations reached a maximum in autumn of the first year which was followed by a gradual decline until time of bud expansion in the spring (Lowry and Avard, 1969). An abrupt seasonal low was observed during bud break and the early part of shoot elongation. This low was followed by a rapid rise in concentration until a second maximum was achieved in autumn for the one-year-old needles. Similar patterns for other species have been described in part at least (Wells and Metz, 1963; Miller, 1966b; Krueger, 1967).

It has been pointed out that there is generally a concentration and a content decline of mobile nutrients in foliage during the second half of the growing season due to translocation to new meristematic regions, for next year's growth, and as a result of leaching from foliage by precipitation (Leaf, 1973).

Since the rate of change in concentration of foliar nutrients has generally been found lowest in autumn and winter (thought to be a period of minimum physiological change within the plant), the dormant season is favoured for sampling (Leyton, 1958; Gessel, 1962; Tamm, 1964a; Lavender, 1970). The underlying philosophy seems to be that during dormancy the plant internal nutrient concentration comes into equilibrium with soil nutrients, and so an indication of nutrient availability to the plant is obtained. Sampling during

a period when nutrient concentration is stable has the practical advantage that comparison between different stands are still valid even t hough times of sampling are not exactly the same.

More recently it has been pointed out that "the biological justification for autumnal collection time for foliage from any crown part is questionable. Although this is the period of relative stability of nutrient elements in foliage and is a convenient time to sample, it is a time following translocation of the mobile nutrient elements from the foliage in preparation for next seasons growth and following deposition of the nonmobile elements. Thus the analysis of foliage at this time does not measure nutrient element levels during the physiologically important period of use of these elements in the foliage" (Leaf, 1970).

Study of seasonal foliar nutrient concentration changes in jack pine suggested that the only time when deficiencies of N, P, and K could be detected would be during the summer growing season (Lowry and Avard, 1969).

N concentrations in loblolly pine foliage approached a rather uniform level (0.9 to 1.05%) during the dormant season (Smith et al., 1970). It was considered that this uniformity would mask N differences between areas of different nutrient status if dormant period sampling were carried out. The best time to characterize N differences would be during periods of rapid growth when reserves are depleted and demands for N are large.

Although acknowledging dormant season sampling may result in low sampling error, it has been criticised because "much biological information is sacrificed" (Waring and Youngberg, 1972). This view arose because N level in Douglas fir was found to drop as new foliage expanded, reaching a minimum during late summer. The most appropriate time to sample such foliage was thought to be after new foliage had expanded. The current thought of some workers is that analyses made during the dormant season probably do not give a good indication of nutrient availability during active growth because of subsequent internal redistribution and uptake. A better indication of nutrient status should be obtained by sampling at the period of maximum "nutrient stress", probably, for example, when extension growth is complete.

EFFECT OF EXTERNAL FACTORS

Nutrient availability

Solution cultures, sand cultures, pot tests, and field fertilizer trials show the effect of nutrient availability on foliar nutrient concentration. It is generally found that increasing the supply of one nutrient, when supply of other nutrients is adequate, results in foliar concentration of the nutrient obeying the "law of diminishing returns" (c.f. Leyton, 1956). This has been described mathematically by the Mitscherlich equation (Mitchell, 1934, 1939):

$$N\% = A\,[1 - 10^{-C\,(N+b)}],$$

in which N% is the tissue nutrient concentration, A is the asymptotic

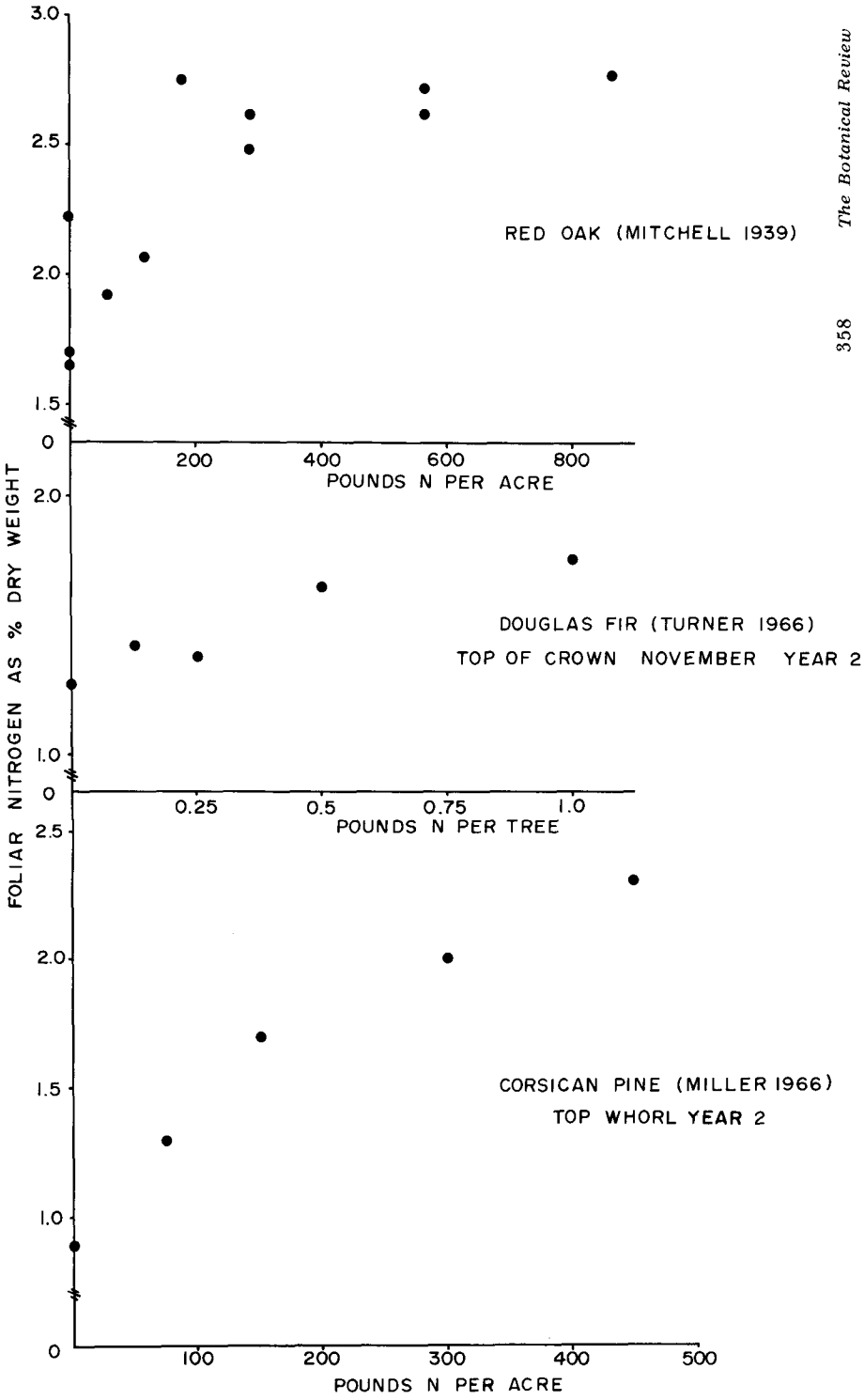

Fig. 2. Examples of relationships between foliar N concentration and N fertilizer applications in forest grown trees.

value of N%, N is the nutrient supply level, b is the N equivalent in the seed, and C is a constant. This equation is closely similar to the monomolecular function (Richards 1959):

$$W = A\,(1 - pe^{-kt}),$$

in which W is plant weight, p and k are constants, e is the base of the natural logarithm, and t is time. The equations are related by

$$kt = CN \log_e 10 \qquad p = 10^{-cb},$$

and their growth rates decline linearly with increase in N% (or W). This means that the rate of increase in foliar nutrient concentration per unit increase in nutrient supply declines linearly with increase in nutrient concentration, and becomes zero at the asymptotic nutrient concentration value. This implies, for example, that the asymptotic nutrient concentration can be simply predicted from two sets of nutrient supply and nutrient concentration values, if the Mitscherlich (or monomolecular) equation is really appropriate.

Sand or solution culture usually provides a well controlled environment in which the effect of changes in one element on foliar nutrient concentration can be examined. Under such conditions operation of the "law of diminishing returns" results in concentration of foliar nutrients being reasonably well related to the logarithm of their supply level when this is relatively low.

Solution culture experiments with Scots pine, Norway spruce, and birch seedlings showed that foliage concentrations of N, P, K, and Mg were more or less linearly related to logarithm of supply level, but Ca concentration appeared to be directly related to increase in Ca level (Ingestad, 1962). Scots pine foliage showed an almost linear increase in N concentration over a wide range of N supply levels (Mitchell, 1934; Boszormenyi, 1958), but white pine foliar N concentration tended to an asymptote with an equivalently high (400 ppm) N supply (Mitchell, 1934). Over a similar range of N supply levels foliar N concentration of loblolly and Virginia pine (*Pinus virginiana* Mill.) was proportional to the logarithm of supply level up to about 100 ppm. Above this foliar N concentration showed a direct linear relationship with supply level up to 400 ppm N (Fowells and Krauss, 1959).

Needle, stem, and root concentrations of N, P, and K appeared generally related to the logarithm of supply level in Douglas fir and Sitka spruce seedlings in sand culture when maximum N and K supplies were 200 ppm and maximum P supply was 30 ppm (van den Driessche, 1969). The same was true for N, P, and K concentrations of spruce, jack pine and hemlock [*Tsuga heterophylla* (Raf.) Sarg.] when maximum supply levels were N: 140 ppm P:62 ppm, and K: 78 ppm (Swan, 1960). Concentrations of N and P in shoots and roots of slash pine (*Pinus elliottii* Engelm.) seedlings in sand culture appeared related to logarithm of supply over part of the lower supply range up to about 125 ppm N and 5 ppm P. Above these levels tissue concentration increased more slowly, reaching an asymptotic value at a supply of 25 ppm in the case of P (McGee, 1963). Shoot K

concentration of this species was linearly related to logarithm of K supply over a range from 5 to 625 ppm.

It is to be expected that, for a particular set of circumstances, increasing supply will result in foliar nutrient concentration reaching an asymptote, and, indeed, application of the "Mitscherlich" equation (Mitchell, 1934; Mitchell and Chandler, 1939) to determine foliar N concentration assumes an asymptotic concentration exists. Furthermore the supply level at which foliar N and K concentration becomes asymptotic is usually considerably above the supply level associated with maximum seedling yield. The increase in tissue nutrient concentration above the maximum yield level, which does not result in further yield increase, is described as "luxury consumption".

The "law of diminishing returns" also governs foliar nutrient concentration response to fertilizer addition in the forest provided conditions over the experimental area are otherwise reasonably uniform (Fig. 2). This response has been noted in greenhouse-grown Douglas fir where N application to the soil caused growth depression when foliar N increased above about 3% (Lavender, 1970). Foliar N concentration of young Douglas fir trees in Oregon increased by about 0.22% for each 100 lb N applied over a foliar N range of 1.3% to 2.3%, but the increase in concentration appeared to be less at the higher levels of supply. Foliar P concentration of Sitka spruce, which ranged from 0.08% to 0.15%, showed a "diminishing returns" relationship to P fertilizer when this was supplied in quantities varying from 0 to 6 ounces per tree (Leyton, 1958).

Plant nutrition can profitably be considered in terms of both intensity and balance (Shear et al., 1946, 1948). Any change in concentration of one element in the plant is usually accompanied by a change in the concentration of the other elements. Such accompanying changes are sometimes called antagonisms, although as Cain (1959) points out, this is not the original sense of the term employed by Osterhout (1907), and interaction seems a more appropriate term. Increase in N supply level generally decreases foliar P and K concentration but may increase Mg concentration (Cain, 1959). An interaction is known to occur between K and Mg so that increasing supply of one nutrient is often associated with a decreasing foliar concentration of the other (Cain, 1955; Swan, 1960). The reason for interactions involving decrease in concentration of nutrients other than the one with an increased supply can often be explained in terms of "dilution". That is, increased supply of one nutrient results in increased dry matter production and the concentration of other nutrients per unit of dry matter is reduced. Other interactions may be of a physiological nature involving changes in translocation (Cain, 1959) or ionic interaction at the root surface or root cell membrane (Lundegardh, 1951).

The possible importance of translocation in affecting foliar nutrient concentrations has been noted in forest trees. When soil Mg

uptake was restricted by spring drought, and pruning had recently reduced the amount of foliage on Monterey pine trees, translocation from remaining foliage to new growth led to severe Mg deficiency symptoms in old foliage (Will, 1968). At onset of dormancy, during a period of little more than two weeks, leaves of *Betula verrucosa* Ehrh. lose one third to one half of their N and P. Loss of K is more gradual and Ca increases on a dry weight basis. These losses are believed due to translocation of the nutrients back to persisting parts of the tree (Tamm, 1951).

Foliar nutrient antagonisms militate against simple interpretation of foliar nutrient concentrations in terms of plant nutrient status. Attempts to rationalise N:P and N:K antagonisms in larch (*Larix leptolepis*) have been made by examining N/P and N/K ratios (van Goor, 1953; Leyton, 1958). In experiments with young Scots pine plants, which received different levels of N and P supply, it was found that good growth was obtained with N/P ratios ranging from 5 to 16 according to the proportion in which the two nutrients were supplied (Boszormenyi, 1958).

Under field conditions the relative supply level of different nutrients varies from place to place. Consequently different interactions can be expected to occur on different sites tending to obscure supply: foliar concentration: yield relationships. Recognition of particular interactions will assist interpretation of foliar analysis data. Detailed models for a number of nutrients could perhaps be developed to advantage, if sufficient data on foliar nutrient concentration interactions were available.

Competition — Moisture stress

It is well known that moisture stress can limit tree growth and there is also evidence that it results in changes in concentrations of foliar nutrients. For example, foliar N concentrations of droughted loblolly pine seedlings were significantly higher than controls at low N treatments (10 and 50 ppm N) (Pharis and Kramer, 1964). The total amount of N per plant was the same, and presumably growth was decreased more than N uptake in these pine seedlings. Similarly white pine seedlings, grown under four nutrient and four irrigation regimes in sand culture, showed decreasing foliar N, P, and K concentrations with increasing irrigation frequency, but foliar Ca and Mg concentrations were unaffected by irrigation treatment (Schomaker, 1969).

Irrigation supplying a total of one inch of water per week during the winter and two inches per week during summer reduced foliar N concentration significantly from 1.07 to 0.99% in 8-year-old slash pine (Barnes and Bengston, 1968). Again, in an experiment in which young plants of loblolly and slash pine were grown with a water table maintained at either 4 inches above mean plot level, at the soil surface, or 4 inches below mean plot level, it was found that foliar N, P, and K concentration tended to be highest in plots with the lowest water table (Walker, 1962).

Foliar N and P concentrations of 70- to 90-year old yellow birch (*Betula lutea* Michx.) was higher in trees growing on drier soil where there was apparently greater moisture stress, but foliar Ca and Mg concentrations, and leaf dry weights were higher in trees growing on poorly drained moist soils (Hoyle, 1965). Moisture stress also increased foliar N and P concentration of eastern cottonwood (*Populus deltoides* Bartr.) (Broadfoot and Farmer, 1969). On the other hand, irrigation of 38 to 40 year old red pine, with 35 cm water per annum, did not affect foliar nutrient concentrations, with the exception that foliar K concentration was significantly increased in one out of three years when analyses were made (Leaf et al., 1970). In the same way foliar P concentration of 23-year-old Douglas fir was increased when 2.5 cm of water was applied each week during summer, but neither N or K concentrations were significantly affected by this treatment (Brix, 1972).

There is no simple rule governing the effect of moisture supply on foliar nutrient concentration. The inconsistent effects may be explained in terms of soil nutrient solubility in some cases. For example, unfertilized slash pine seedlings showed no increase in dry matter production when irrigation was increased from one to four inches per week, but it was found that increased irrigation reduced seedling N and K uptake when these nutrients were supplied in a soluble form, whereas N and K uptake from less soluble supplies tended to increase with higher irrigation levels (Bengston and Voigt, 1962). It can readily be appreciated that a particular soil moisture regime may affect leaching rates of various N fertilizers differently. Nitrate forms tend to be more readily leached than ammonium forms. Consequently foliar nutrient levels could show different interactions between moisture stress and N fertilizer, depending on the form of N in the fertilizer.

Competition — Light

Three month old Scots pine (*Pinus sylvesteris* L.) seedlings grown at five radiation intensities (4%, 11%, 22% 27% and 50% of intensities in the open) showed a decrease in tissue N concentration on media of low N status with increase in radiation intensity (Gast, 1937). On media of high nutrient status tissue N concentration decreased from 4% to 11% radiation, but showed little difference between 11% radiation and the other radiation intensities. There was an indication that tissue P concentration decreased from 22% radiation to 50% radiation on all media. The 50% radiation level was the nominal level for plants exposed in the greenhouse and, as noted by the author, was probably as high as 85% of that in the open at times.

Bottom crown samples obtained from four 12-year-old red pine trees generally gave highest foliar nutrient concentrations (White, 1954). This was attributed to the bottom whorls of live branches being least photosynthetically active, particularly in closed stands, so that nutrient concentration was not "diluted" by carbohydrate synthesis.

Growth of jack pine seedlings, subjected to either short days, or short days with a light interrupted dark period, was greater in the short day, interrupted dark period regime (Giertych and Farrar, 1961). Plants in both day-length regimes also received five levels of N treatment and these significantly affected seedling tissue N concentration. For any N treatment, however, short day plants showed higher tissue N concentrations than the larger plants grown in the interrupted night regimes.

Seedlings, and perhaps foliage, growing relatively slowly can be expected to show higher foliar N, and perhaps other nutrient, concentrations. Since growth is very dependent on light, through photosynthesis, shaded foliage could be expected to show higher nutrient concentrations than otherwise comparable unshaded foliage.

Where competition for moisture and light exists, foliar nutrient concentrations may be higher than if there were no competition. In a dense stand, of course, competition for nutrients may also occur so that the concentrating effect of slow growth, due to competition for water and light is counteracted. Observations of spacing, or crown closure may be of little value for analysing the effect of "competition" on foliar nutrient concentrations until the level of such factors as light, moisture supply, and nutrient supply are known.

Temperature and elevation

Miller (1966b) found a strong statistical correlation of weather factors with foliar nutrient levels of loblolly pine. The weather factors most commonly correlated with foliar nutrient levels were average maximum and average minimum temperatures preceding the sampling date.

One-year-old slash pine seedlings accumulated considerably more ^{32}P when grown at 15/10°C day/night temperature than when grown at 25/20°C, or 35/30°C day night temperatures and 2500 ft-C (McKee, 1972). One would therefore expect that foliar P concentration of slash pine is affected by temperature.

Scots pine growing at 1,875 ft elevation on the Pennines showed chlorosis, although analysis of peat on which it was growing showed levels of available nutrients normally considered adequate at lower elevations (Brown et al., 1964). Foliar analysis indicated growth was mainly limited by lack of K, although some indication of N deficiency was detected. The conclusion was that climate, possibly through a temperature effect, strongly influences nutrient uptake. It was considered reasonable to expect the nutrition of plants growing near their climatic limit to be different from that of plants in a more favourable environment.

It seems likely that temperature would be a useful parameter to assist in explaining annual and elevation discrepancies which are encountered in foliar analysis work.

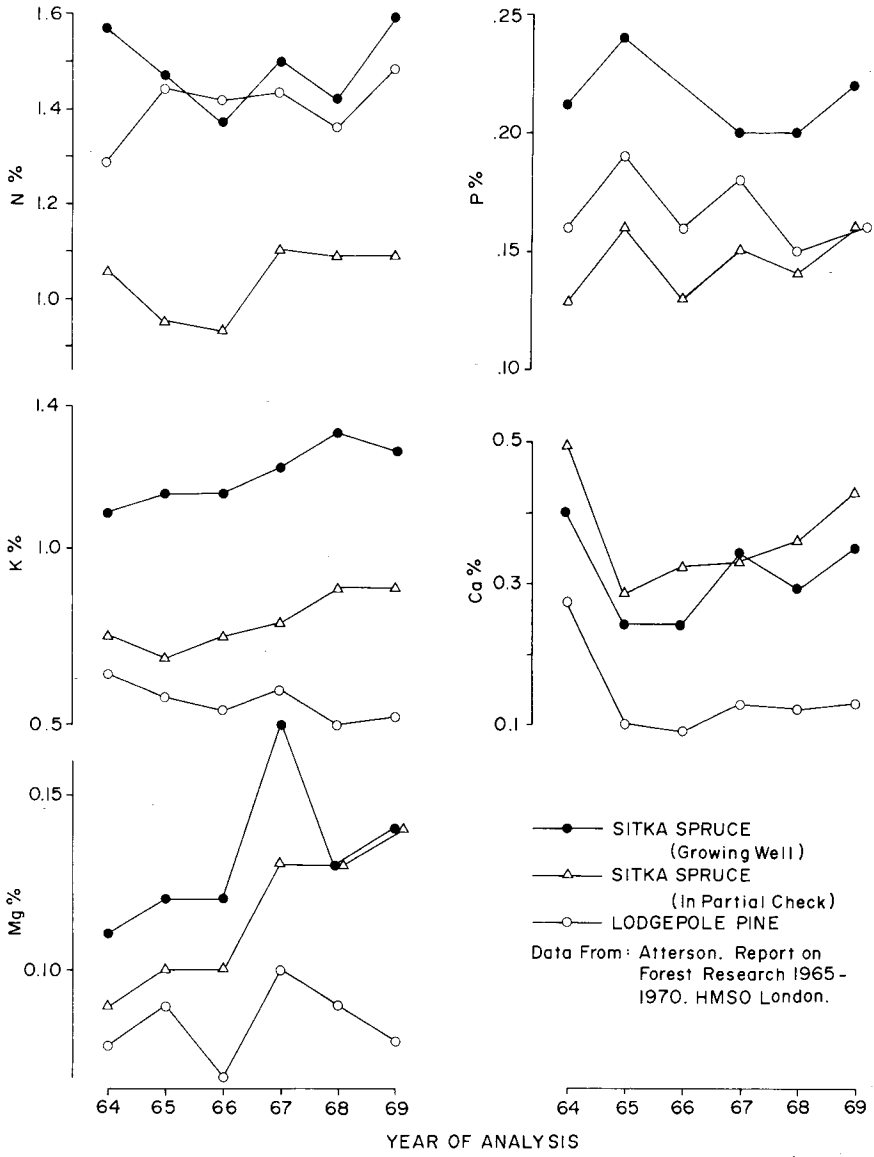

Fig. 3. Annual variation of 5 macro nutrients over 6 years.

ANNUAL VARIATION

Annual variation (variation between the same months in different years) in tissue nutrient levels is to be expected since many of the factors which influence these levels are not constant from year to year.

Annual analyses of current year foliage from 40 red pine trees over a six year period showed significant differences between years in concentrations of N, P, K, Ca, and Mg (Leaf et al., 1970). Furthermore, whereas the highest foliar N concentration occurred in 1968, the highest P concentration occurred in 1965, and the highest K concentration in 1963. It appeared that each nutrient responded differently, in terms of foliar concentration, to conditions of each year. This is also borne out by analyses made on 12 dominant trees of healthy Sitka spruce, partially checked Sitka spruce, and lodgepole pine on 10 sites in Scotland and northern England (Atterson, 1965-1970) (Fig. 3). Mean values showed that differences between years in one nutrient were seldom paralleled by differences in another nutrient. Some comfort can perhaps be drawn from the fact that mean N, P, and K concentrations in healthy Sitka spruce were always higher than in partially checked Sitka spruce. Ca and Mg levels, however, were different in some years and the same in others. In the red pine trees already mentioned (Leaf et al., 1970), foliar K concentrations indicated these trees ranged from severely deficient to nondeficient over the 6 years of sampling, on the basis of published critical levels for the species.

Analysis of foliage from control plots in a Norway spruce fertilizer trial showed substantial variation in nutrient concentration over a 6 year period (Tamm, 1968).

Annual discrepancies in foliar N, P, and K levels of loblolly pine were examined in conjunction with weather data (Miller, 1966b). Foliar nutrient levels were plotted against 17 independent variables which represented measures of precipitation, average maximum and minimum temperature, and the average mean temperature for various periods preceding the sampling date. Average maximum and average minimum temperatures for various periods of two, three, or four weeks showed most correlation with foliar nutrient levels. For example, average minimum temperature for three weeks before sampling and number of days elasping after a rainfall of 0.5 inches, or more, to sampling, jointly explained 72% of the variation in K concentration with time. These data were considered to show the inadvisability of using foliar analysis for site differentation in loblolly pine.

Annual variation of foliar nutrient concentration seems to be of sufficient magnitude (commonly from 15 to 40% of the highest level measured, depending on nutrient) to contribute substantially to the imprecision of foliar analysis as a diagnostic method. Use of appropriate climatic data might eventually reduce this problem since

annual variation may be largely correlated with weather conditions. Certainly collecting of climatic data whenever foliar sampling programmes are undertaken would be valuable.

EFFECT OF PROVENANCE

Occurrence of varietal differences in tissue nutrient concentrations has been established for several agricultural and horticultural crops (Epstein and Jefferies, 1964). One of the earliest observations showing forest tree provenances have different nutrient concentrations was made when Scots pines, from several seed sources, were grown under the same conditions (Langlet, 1936). Young plants of northern provenances were found to contain higher N concentrations than those of southern origin. Foliar analysis of 18-year-old trees of six Scots pine provenances revealed differences between provenances in concentrations of N, Ca, Mg, Fe, and B (Gerhold, 1959). However, ranking of provenances by any of these nutrients varied according to whether analyses were made in August or February. In another Scots pine study tissue levels of N, P, Mg, B, and Na varied significantly between provenances (Steinbeck, 1966). At one site, out of three, it was noticed that slower growing northern provenances tended to accumulate N.

Concentration of N in tissue of 115 day old jack pine (*Pinus banksiana* Lamb.) seedlings varied with degree days at the source of origin (Giertych and Farrar, 1961). Similar results were obtained with 90 day old jack pine seedlings (Mergen and Worrall, 1965), but it was made clear in this instance that the different developmental stage attained by different provenances in this short period probably accounted for many of the nutrient concentration differences. The same probably applies to foliar nutrient differences reported for Norway spruce since, although seedling age is not stated, more than one batch of seedlings was raised in a year (Fober and Giertych, 1971). Differences observed in these experiments possibly will not occur in mature foliage of older trees.

Selected lines of slash pine (*Pinus elliottii* Engelm.) were found to vary in growth rate and needle nutrient concentration, but showed no clear relationship between needle composition and tree growth rate (Pritchett and Goddard, 1967). In a study with five groups of progeny of this species, which were grown at two fertility levels for a year, a significant progeny by fertilizer interaction was found in N and K foliar analysis data (Walker and Hatcher, 1965). Furthermore, a siginficant interaction between needle type (primary and secondary) and provenance occurred in the N data. This latter results emphasises that conclusions about foliar nutrient differences between pine provenances should not be based on primary needle analyses.

Detailed sampling showed distribution of nutrients within the crowns of *Pinus radiata* differed between clones (Forrest and Ovington, 1971). For example, one clone might show a high P concentra-

tion in the top whorl, declining to a low P concentration in the fifth whorl, whereas another clone might show an almost constant P concentration in all whorls. Average concentrations of nutrients in whole crowns showed differences between the six clones examined, but because of the different distribution patterns within crowns, misleading ranking of clones might occur unless average crown concentrations were determined. While emphasising that consistent foliar nutrient differences exist between populations within a species, this work may also imply that nutrient status of the population can not be satisfactorily determined by very restricted sampling within the crown.

One- and two-year seedlings of seven British Columbia provenances of Douglas fir grown on the same site in Lower Austria showed differences in K, Ca, and ash content (Kral, 1965). The lowest, coastal, provenance was highest in foliar K concentration, but lowest in foliar Ca concentration. Comparisons among two-year-old Douglas fir provenances from New Mexico, Colorado, Montana, California, and Vancouver Island, but grown in the same environment, showed significant differences in foliar N, K, Ca, and Mg concentrations, but not P concentration (van den Driessche, 1969). N concentration varied between provenances by as much as 73% and Ca concentration by as much as 175%.

Foliar nutrient concentration differences ranged from 8 to 18% among four Douglas fir provenances from coastal British Columbia (van den Driessche, 1973). Three other coastal Douglas fir provenances were grown at each of three nurseries, and foliar nutrient concentration differences were found to be greatly affected by nursery. For two provenances regressions between height growth and foliar N concentration showed significant differences, although mean N concentrations were similar. This suggested a general species relationship did not exist between shoot length and foliar N concentration so that foliar analysis interpretation should be confined within provenances.

There seems little doubt that foliar nutrient concentration differences exist between provenances, bred lines, and clones of many forest tree species growing under closely comparable conditions. The question then arises as to what extent these foliar concentration differences reflect differences in mineral nutrient status of the provenances, etc. For diagnostic use of foliar analysis it is important to find out if nutrient concentration differences between provenances are related to corresponding yield differences, so that a single response curve applies to the whole species. Should concentration differences and yield differences not correspond this would imply lack of a single foliar nutrient concentration: yield relationship within a species. This could be important when attempting to apply foliar analysis methods to a wide ranging species such as Douglas fir. Possibly, if important population differences in foliar nutrient concentration: yield do exist within a species, it might be possible to

map the various populations and set up critical and deficiency levels appropriate to each population.

SOLUBLE NITROGEN COMPOUNDS

Free amino acids

Non-protein bound amino acids may prove valuable in assessing the nutrient status, in particular the N status, of trees. The effect of mineral nutrition on free amino acid composition of the soluble N fraction in plants has been discussed by Hewitt (1963). Micro-nutrients, as well as macro-nutrients, can have marked effects on proportions of amino acids. Deficiency of a particular nutrient may not have the same effect on free amino acids in different plants, and day length has been shown to interact with nutrient supply.

Increase in the calcium: potassium ratio in *Mentha piperita* caused increases in the ratio of glutamine to glutamic acid and of glutamine as a proportion of total soluble N under both long day and short day conditions (Steward et al., 1959). Ca deficiency was associated with an increased concentration of proline under both long and short days, of asparagine under long days, and of glutamine under short days. K deficiency produced a glutamine: asparagine ratio of about 0.15:1 under short days, and about 3:1 under long days. In the banana (*Musa acuminata* 'Gros Michel') K deficiency resulted in glutamine accumulation before visible symptoms were evident. Glutamine accounted for 24% of total soluble N compounds with severe K deficiency (Freiberg and Steward, 1960).

Arginine

Arginine is considered the most typical and quantitatively important storage compound in apple trees (Oland, 1959), and is also a major storage compound in other fruit trees (Taylor, 1967).It is found in large quantities in storage tissues rich in N, whereas the same tissue may be extremely depleted of arginine after new growth has occurred without external N supply. Arginine is recognized as one of the main forms in which N is stored in a number of plant families (Reuter, 1957). The amides also show the character of storage compounds in apple, but not to the same extent as arginine (Oland, 1959).

Fertilizing slash pine with ammonium nitrate increased free arginine, as well as total free amino acids, and total N of twigs and foliage (Barnes and Bengston, 1968). Relative increases on a percentage basis were in the order: twig arginine (140%) > twig amino acids (40%) > total foliar N (15%) = total twig N (15%). Free arginine level of twigs is evidently more responsive to, and more indicative of, N fertilization treatment than is total N.

Arginine accounts for some 50 to 70% of soluble N in roots, trunks, and stems of grape vines (Kliewer and Cook, 1971), and arginine level of roots and stems was highly correlated with level of N

supply to vines over a three year period. Increase in arginine levels, as a percentage of control, varied from 400 to 600%, depending on tissue and season, whereas total foliar N showed increases of 20 to 75%. The tissue containing most arginine, and therefore most suitable for sampling, was root and stem.

Proline

Moisture stress is known ot result in accumulation of proline in many plants (Kemble and Macpherson, 1954; Barnett and Naylor, 1966). Proline accumulation in wilted leaves is enhanced by high carbohydrate levels since sugars must be oxidized to form proline (Stewart et al., 1966). When bean leaves are kept in darkness to starve them proline is converted to glutamic acid and subsequently metabolized (Stewart, 1972a). Accumulation of free proline stops immediately water stress is removed and the subsequent decrease in proline level is slow when adequate carbohydrate is available, but fast when the carbohydrate level is low (Stewart, 1972b).

Varieties of barley which accumulated larger concentrations of free proline as a result of moisture stress tended to be those which survived extreme water stress more readily and grew more rapidly following stress relief (Singh et al., 1973). Proline accumulation may be a sensitive index of water stress susceptibility, at least for barley.

Proline has been shown to accumulate in two-year old *Cryptomeria japonica* as a result of water stress (Mori et al., 1971).

Putrescine

It is worth pointing out in this section that putrescine, which is not an amino acid, but a diamine, tends to accumulate in a wide variety of plants as a result of K deficiency (Murty et al., 1971). Level of putrescine is inversely related to the level of K in black current, for example, although it may not be a better indicator of K deficiency than visual symptoms in this species.

Seasonal and diurnal variation

Work on bark of apple shoots showed that protein N accumulated from August until mid-winter, and then remained constant until March, when it decreased rapidly after flushing (Tromp, 1970). Soluble N continued accumulating until flushing occurred, when it then decreased. When N supply was low almost all N appeared as protein, but when N supply was high a large soluble N fraction was detected, and in particular arginine level increased. In general, arginine levels, which were highest in bark, increased during autumn and early winter and decreased from mid-April to insignificant levels in late May. It was supposed that arginine is hydrolysed and the released ammonia stored as amides, since amides have been shown to accumulate during this time.

A study conducted on white spruce (Steward and Durzan, 1965) showed that with the onset of bud dormancy free arginine and most

amino acids decreased, but proline accumulated. This was examined in further detail on white spruce saplings (Durzan, 1968a), showing that arginine, which has a lower C/N ratio than most other amino acids, served to store N as shoot elongation ceased (Fig. 4). Arginine level dropped before bud break in spring, when N accumulated as glutamine. In the fall arginine was incorporated into protein and yielded C and N for formation of proline and various guanidino compounds. Application of arginine containing ^{14}C suggested metabolism of arginine and proline were connected via ornithine.

Soluble N, and individual amino acids, expressed in terms of bud and foliage fresh weight, showed a maximum at sunrise, dropping substantially by noon, and then showed a second maximum in the afternoon in white spruce during August (Durzan, 1968b). Concentrations of amino acids showed much less diurnal fluctuation, however, when expressed as a percentage of total soluble N. The free amino acids in buds and leaves consisted primarily of arginine, glutamine, proline, asparagine, and alanine.

Source of nitrogen

Source of N supply has been shown to affect amino acid composition of forest trees. Loblolly pine (*Pinus taeda* L.) seedlings supplied with ammonium N showed higher levels of free amino acids than those supplied with nitrate N, while those supplied with urea were intermediate (Pharis et al., 1964). N source affected levels of arginine, glutamine, asparagine, and proline in particular.

White spruce and jack pine seedlings grown under controlled nutrient conditions (by Swan, 1960) showed differences in amino acid content according to level and source of N supply (Durzan and Steward, 1967). White spruce, which made greater growth on nitrate N, accumulated glutamine on this source. By contrast high levels of arginine and guanidino compounds occurred when spruce was grown on ammonium N. N starvation resulted in an increased level of proline. Jack pine, which made greater growth on ammonium N, showed much lower total soluble N, including arginine, than white spruce plants on this source. On the other hand, jack pine showed relatively high arginine levels when supplied with nitrate N. Arginine became undetectable with severe N starvation.

Nitrate fertilizer stimulated cone production when 13-year-old Douglas fir trees were treated with five levels of either nitrate or ammonium fertilizer (Ebell and McMullan, 1970). Nitrate fertilizer also increased the foliage level of free amino acids, and in particular arginine and guanidino compounds, more than ammonium fertilizer. Amount of cone production and level of arginine appeared similarly related to level of nitrate fertilizer application. Ammonium fertilizer increased the amount of γ-aminobutyric acid.

It seems that nitrate N supply results in accumulation of arginine in both jack pine and Douglas fir, in contrast to loblolly pine and white spruce where greatest arginine accumulation occurs on the ammonium N source.

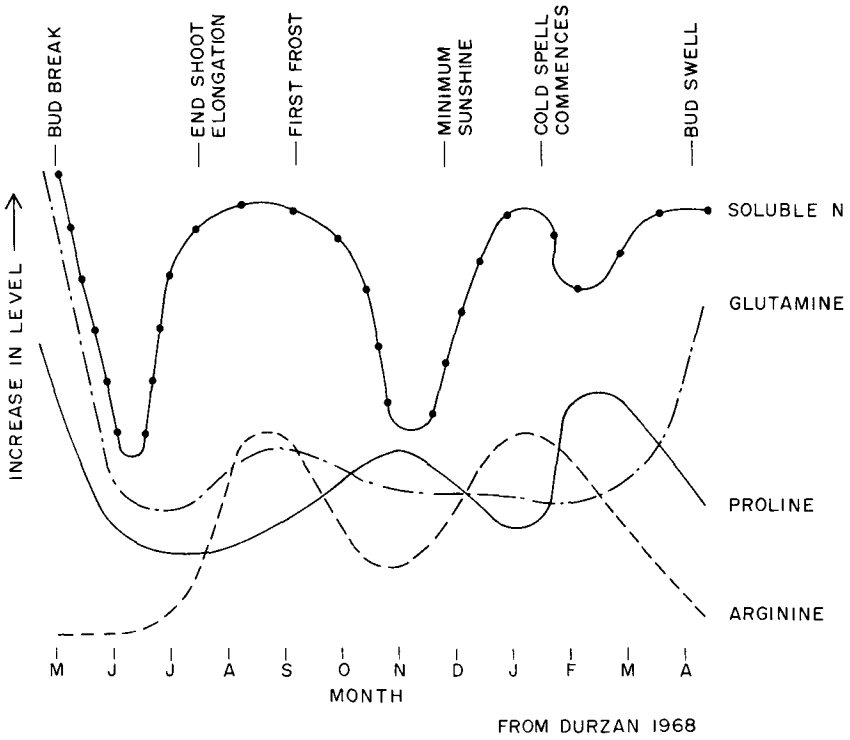

Fig. 4. Relative changes in soluble N and three amino acids in buds and foliage of white spruce saplings during the year.

Amino acids in trees

At least 34 free amino acids were identified from the inner bark of 18-m-tall loblolly pine trees, and there were major amounts of glutamine, asparagine, alanine, serine and valine. (Hodges et al., 1968). Analyses of foliage collected at the end of the growing season showed alanine, γ-aminobutyric acid and tyrosine occurred in relatively large quantities in *Abies balsamea* (L) Mill. and *Picea mariana* (Mill.) BSP (Gagnon, 1966). Proline occurred in substantial quantities in only *P. mariana.*

The free amino acid composition of xylem sap has been investigated in apple trees (e.g., Bollard, 1958; Hill-Cottingham and Bollard, 1965) and in forest trees (e.g., Barnes, 1963; Carter and Larsen, 1965). The more abundant amino acids in xylem sap of forest trees include glutamine, asparagine, citrulline, arginine, aspartic acid and γ-aminobutyric acid. A study of translocation of nitrogen compounds through apple phloem showed asparagine and arginine were the major soluble amino compounds (Tromp and Ovaa, 1971).

Practical considerations

Total soluble nitrogen and total free amino acids show manyfold greater changes in concentration than total N as a result of changes in N supply to trees. Free arginine level seems intimately related with the N status of trees, and is therefore likely to be an informative parameter to measure. Glutamine frequently occurs in large amounts, depending on level and source of N supply, and proline accumulatin may indicate limited growth due to water stress or dormancy. Gas chromatographic procedures to determine amino acids are now becoming practical and can be considered for this application. Alternatively, specific tests are available for a few amino acids (e.g., arginine, proline, guanidino compounds) and total soluble N can be simply determined. A drawback is that all samples must be placed under refrigeration at collection (dry-ice), freeze drying is necessary, and extraction of samples, including removal of extraneous material (phenolic compounds), is lengthy.

A further point to consider is that although a high level of nutrient supply may result in a ten-fold average increase in arginine level, and only a two-fold average increase in N level,the variability in the arginine data may be so much greater that demonstration of a significant increase with arginine is no better than with total N. Nevertheless, estimation of total free amino acids in fresh root and stem material has been used on a field scale for several years to develop fertilizer recommendations for fruit trees (Baxter, 1965). Advantages of the procedure are said to be: large differences between trees of different N status, handling of 50 samples per day, winter sampling, and sampling of roots.

For foresters dealing with 50-m-tall trees, the fact that at least the N status of the tree is probably related to root amino acid concentration may be of considerable practical value.

CHEMICAL ANALYSIS

Sample Preparation

White (1954) demonstrated that pine needle samples that were allowed to air dry underwent a reduction in dry matter attributed to respiration during drying. This resulted in an apparent increase in needle nutrient concentration. To avoid this, tissue samples should be oven dried at 70°C shortly after collection or, when this is not possible in the field, placed under some form of temporary cold storage while in transit.

The handling of samples intended for mineral nutrient analysis is discussed by Humphries (1956). When large quantities of material are collected, sub-sampling may be necessary, contamination must be avoided in all preparatory steps, and if trace elements are to be analysed foliage samples must be washed (in weak soap solution and rinsed, but not to cause leaching) before drying. Dried samples are ground in mechanical mills or with pestle and mortar (to pass a 32

mesh screen), and re-dried prior to ashing. Drying temperature (usually 70 or 80°C) affects final nutrient concentrations in conifers slightly, probably due to volatilization or organic substances, and failure to re-dry before weighing out material for ashing can lead to serious errors due to moisture uptake by stored samples.

Preparation of a large quantity of well mixed standard sample, in which the nutrient concentrations are determined by several replicate analyses, allows checking of methods subsequently. The standard sample can be included after every 30 or 50 analyses when tissue is analysed routinely. Exchange of samples with other laboratories also permits assessment of methods.

Plant material should not be oven dried when analysis of free amino acids is to be made because reactions between carbohydrates and amino acids may be accelerated (Oland, 1959). Samples collected in the field should immediately be packed with dry ice and freeze dried at the earliest opportunity if analysis of organic constituents is planned.

Total nitrogen

Total N is normally determined by some modification of the Kjeldahl procedure (Bremner, 1960; Bremner, 1965). N compounds containing N-N or N-O linkages are not determined by the common procedures, and N is lost by volatilization when the temperature of the digestion exceeds about 400°C. Nitrate is only included in the determination after pre-treatment with reduced iron and dilute acid, or inclusion of salicylic acid (Humphries, 1956). Mercury was found to be the most appropriate catalyst for determining N in woody material, giving close to 100% recovery (Rennie, 1965). Total N may be determined by the Dumas method, but results, particularly for woody material, are likely to be higher than those obtained by the Kjeldahl method (Fornes et al., 1968). Higher values in the Dumas method are probably due to methane interference which can be eliminated by inclusion of Pt in the post heater tube (Stewart et al., 1963, 1964).

Soluble nitrogen

The soluble N of plant tissue consists chiefly of free amino acids and amides, and can be extracted from fresh or freeze dried material using a mixture of methanol, chloroform, and water (MCW) (McMullan, 1971). Alternatively 1N sodium citrate buffer, pH5, containing a few drops of wetting agent may be used (e.g., Baxter 1965). The N content of the extracts can be determined by submitting aliquots to the normal Kjeldahl procedure, or, since most amino acids and amides react quantitatively with ninhydrin (Yemm and Cocking, 1955), a colorimetric determination may be made (Rosen, 1957).

Individual amino acids

Specific colour reagents have been reported for arginine (Gilboe and Williams, 1956) and for creatine, creatinine, arginine, quanidino-

acetic acid, guanidine, and methyl guanidine (van Pilsum et al., 1956). In general these tests are unsatisfactory except in well cleaned plant extracts. The use of polyvinylpyrrolidon, however, allows determination of total mono-substituted guanidino compounds (arginine, guanidine, methyl guanidine, and guanidino acetic acid, guanidino butyric acid) with the Sakguchi reagent in aqueous extract (Smith and Horsewell, 1960).

Fortunately the ninhydrin-proline reaction product absorbs at a different wave length (515 nm) from other ninhydrin-amino acid reaction products (Troll and Lindsley, 1955) and can therefore be used satisfactorily for determining proline in clean extract.

Arginine can be determined using arginase (Anonymous, 1972). Action of the enzyme results in formation of ornithine and urea, and the latter can be estimated colourimetrically after the addition of diacetyl monoxime (Douglas and Bremner, 1970).

Identification and measurement of all amino acids in the soluble N fraction can be achieved by various chromatographic procedures. Two dimensional thin layer chromatography gives good separation (e.g., McMullan, 1971), but is time consuming and not practical for routine analysis of many samples. The recent introduction of automated gas chromatographic machines may allow a greater rate of sample analysis. The extract must be initially cleaned to remove interfering compounds, as in all methods, and the amino acids reacted to form volatile derivatives before being automatically chromatographed.

Mineral constituents other than nitrogen

Analysis of any of the plant tissue constitutuents, P, K, Ca, Mg, Fe, Cu, Mn, Zn, B, Mo, or Al, is normally carried out on ashed sample which has been dissolved in hydrochloric acid. When dry ashing is employed the temperature should not exceed 480° C if no K is to be lost by volatilization (Humphries, 1956). Temperatures of 500° C, however, are recommended as satisfactory (Isaac and Jones, 1972). Wet digestion with perchloric acid has advantages (e.g., Humphries, 1956), but can be hazardous for the analyst.

The concentration of metalic ions in the digest can be most conveniently determined by atomic absorption spectrometery, except perhaps for K, which is readily determined by emmission spectrometery. P can be determined colourimetrically as the yellow phospho-vanado-molybdate yellow complex (Jackson, 1958). S can be oxidized with an oxygen stream in an induction furnace to produce sulphur dioxide which is titrated with iodine (McMullan, 1972). Colourimetric tests are available for B determination (e.g., Chapman and Pratt, 1961).

Rapid tests

Rapid chemical tests seem to have been little used, if at all, on forest tree material. Some reasons for this are probably the difficulty

of expressing sap and the qualititative nature of the data obtained from the older tests. Various extracting solutions,or blending, are now used, however, and more recent tests are quantitative, giving results which show much the same variation as total analyses, and are closely correlated with total analyses (Nicholas, 1957). The rapid methods are advantageous in permitting the handling of many samples of fresh tissue, or sometimes dried tissue (e.g., Baker, 1971), but when sample collection is often difficult and the value of the sample after collection therefore high, it seems logical to obtain the maximum information from it by total analysis.

STATISTICAL ANALYSIS

Number of trees to sample

The precision with which the mean value of any foliar nutrient must be estimated will probably depend on the objective of the investigation. Less precision is likely to be necessary for determining whether a deficiency exists, than would be required for estimating quantitative response to fertilizer. When sampling in connection with nutrient uptake studies of red pine, it was generalized that usually not less than 20 trees, selected from all diameter classes, must be sampled for reliable correlation analyses (Rennie, 1966). Since such an intensive sampling procedure may be "physically overwhelming", however, selection of sample trees of mean basal area was carried out in practice, and it was stated that, for mature red pine, three or four trees gave means, with confidence limits not exceeding more or less than 10 percent, for "several important attributes".

In black spruce no significant differences in variance were found when N, P, K, and Mg concentrations in foliage of different age and crown positions were subjected to a homogeneity of variance test (Lowry and Avard, 1965). This implies that, for this species, it is satisfactory to include needle data of any age or crown position in a statistical analysis. Computations were made to predict sample size necessary to detect significant differences between two means.

Interestingly the lowest predicted sample size occurred in the lower crown of black spruce for N, K, Ca, and Mg concentrations. To detect a difference from the N concentration mean of 5 percent in the upper crown would require sampling 22 trees, in the mid crown 29 trees, and in the lower crown, 7 trees. Estimation of K with equal precision from similar crown positions would require sampling of, respectivel , 217, 258 and 36 trees. It can only be concluded from the data presented (Lowry and Avard, 1965) that estimation of foliar K and Ca concentrations with any degree of precision will generally be prohibited by the large number of samples necessary.

Use of "representative" rather than random samples has been proposed (Morison, 1970) for estimation of foliar N concentration in Douglas fir. Representative trees were chosen as those with average leader length, which invariably excluded trees with extreme foliar N

values, and adequately represented the mean value for the plot. It was also found that correlation coefficients between shoot growth over 5 years and foliar N concentration were higher in representative samples than in random samples. This observation is likely to be unimportant, however, if interest applies to the whole stand rather than to trees with average leader growth.

Analyses of Douglas fir foliage from 40 trees on two soils and in two different years on Vancouver Island (unpublished)[2] provided some information on coefficient of variation for different nutrients.

Soil Year	Hart 1965	Hart 1966	Senton 1965
Nutrient Measured	Coefficient of variation %		
N	10.5	9.9	8.9
P	12.9	19.9	18.2
K	16.8	12.7	19.5
Ca	16.8	24.9	12.9
Mg	22.3	18.6	20.4
S	23.6	20.9	22.5

These coefficients indicate that to detect a 5 percent difference from the mean, with 95% confidence limits, the number of samples required for N is 38, for P is 58, for K is 98, for Ca is 98, for Mg is 174, and for S is 195. The foliage from which these data were obtained came from the top third of the crowns of stands with closed canopy.

Similar computations have been made for estimating foliar N concentration differences, with a magnitude of 5 percent of the mean, in Douglas fir growing in Oregon (Lavender, 1970). The number of dominants required would be 14, the number of co-dominants 24, and the number of suppressed 40. The implication here is that foliar N concentration in dominants varies a lot less than in suppressed trees.

Coefficients of variation for tissue N of Douglas fir growing in Washington have been calculated as 6.7% for needles, 14.2% for live branches, and 14.8% for bark (Heilman and Gessel, 1963). Coefficients for P in the same tissue were, respectively, 14.0%, 15.8%, and 29.2%.

In sampling citrus and coffee foliage for analysis it has been found that careful selection of 25 leaves, according to age and position, allowed adequate estimation of nutrient status within a tree (Smith, 1962). Statistical studies showed variability among leaves on the same tree was the major source of random variation in leaf samples. This means composite samples from several trees of a plot need not

[2] Data provided by Dr. N. Keser, British Columbia Forest Research Division.

contain many more leaves to represent the plot than are required to represent one tree within the plot.

It seems clear that less sampling is necessary for determining N concentration than is required for determining concentration of some of the other nutrients. The number of trees to be sampled can also be reduced if the required mean can be more narrowly defined. For example, it might be reasonable to be concerned that dominants have adequate foliar nutrient levels, since they are the economically important part of the stand, and ignore the remaining co-dominant and suppressed trees. Random sampling in Douglas fir stands will require about 10 samples per plot (or other homogeneous area), from comparable crown positions, to detect foliar N differences $\geqslant$ 10% of the mean. To obtain similar sensitivity for P and K 15 to 20 samples appear necessary. About four times as many samples are required to detect differences $\geqslant$ 5% of the mean with equal (95%) confidence. It seems rather likely that, for practical reasons, it will only be possible to sample sufficiently heavily to detect differences which are at least 10% of the mean. Once samples have been collected there is no objection to bulking them, according to sample areas, for drying and subsequent chemical analysis.

Necessary replication of chemical analysis

In estimation of nutrient levels of foliage, variation due to chemical analysis is generally much less than that caused by biological variation within the stand (e.g., Guha and Mitchell, 1965). Kjeldahl analysis of material from red pine (Rennie, 1965) gave the following results:

Material	N% and standard deviation	Coefficient of variation %	Sample size for an error not exceeding	
			±5%	±10%
Needles	1.047 ± 0.014	1.34	<1	<1
Twigs	0.564 ± 0.008	1.38	<1	<1
Bark (lower bole)	0.196 ± 0.0018	0.92	<1	<1
Dead branches	0.196 ± 0.0059	3.01	<3	<1
Wood (lower stem)	0.063 ± 0.0022	3.49	<4	<1

Coefficients of variation for analytical errors in determining nutrient concentration in foliage of horse-chestnut (*Aesculus hippocastanum* L.), sycamore (*Acer pseudoplatanus* L.) and beech (*Fagus sylvatica* L.) has been given for 21 elements (Guha and Mitchell, 1965). Values for K and Ca, determined by flame photometery, were 3.6% and 2.0%, and the value for P, determined by a vanadomolybdate method, was 2.9%.

In a comparison of analytical methods, designed for application to black currant, strawberry, and apple foliage, coefficients of variation for N, P, K, Ca, and Mg ranged from 0.9% to 3.3% (Bould et al., 1960).

Six bulk foliage samples of several tree species were sent to 29 different laboratories in various countries for analysis (van Goor et al., 1971). In the Douglas fir sample coefficients of variation for five macronutrients were respectively N, 3.1%: P, 10%: K, 6.5%: Ca, 11%: Mg, 14%.

The literature supports the contention that variation between samples within the stand greatly exceeds variation between chemical analyses. Recovery, rather than variability, may be the more important problem in the laboratory, and this must be checked by occasionally analysing samples of known composition.

Methods of expressing nutrient data

Tissue analysis results are normally expressed as a percentage of tissue dry weight. Calculation of total nutrient uptake per plant is possible if the dry weight of the various tissues in the plant is known. This can normally only be done for seedlings, so that results for large trees generally have to be expressed as percentages (but see Ovington, 1957; Ovington and Madgwick, 1959). Lavender (personal communication) has multiplied nutrient concentrations by the weight of foliage produced on 10 selected third whorl branch tips to estimate relative nutrient uptake of Douglas fir trees. There may, however, be an objection to the use of nutrient uptake values calculated from weight of tissues, or the whole plant, if the intention is to correlate growth with nutrient measurements. Under these circumstances nutrient measurements should be independent of growth correlated parameters such as weight (c.f. Tamm, 1964b).

Expression of nutrient concentration in terms of foliage area, rather than foliage dry weight, might be more appropriate because photosynthesis is related more closely to foliage area than weight. Correlation between foliage area and weight exists, however, and it is not clear whether problems of interpretation, due to such factors as "dilution", would be reduced.

It has been suggested that plant growth is a function of two nutritional variables, intensity and balance (Shear et al., 1948). Thus, with a given internal concentration of a particular nutrient the greatest yield is attained when all other nutrients are brought into correct balance with this nutrient. Overall maximum yield is achieved with an optimum intensity and balance of all nutrients. With this hypothesis as a basis it has been common to express nutrient concentration results in terms of ratios. All possible ratios of the five nutrients, N, P, K, Ca, and Mg were calculated for peanuts growing over a wide range of different sites in West Africa (Prevot and Ollagnier, 1954). Maximum yields coincided with foliar N/P ratios around 20. The optimum N/P ratio for Japanese larch seedlings was thought to be about 9 to 12 (van Goor, 1953) and 12.6 to 14.7 for older trees of this species (Leyton, 1958). For Sitka spruce the optimum N/P ratio was thought to be about 10 and the optimum N/K ratio about 1.4 (Leyton, 1958). Scots pine seedlings showed

greatest growth when N/P ratios were in the region of 5 to 16 (Boszormenyi, 1958). The N/P ratio for several stands of Douglas fir was found to be about 5 to 10 for N fertilized trees and 2.6 to 3.6 for unfertilized trees (Heilman and Gessel, 1963) and in Monterey pine the optimum N/P ratio is considered to be about 10 with acceptable limits of 6-16 (Waring, 1972). Leyton (1958) pointed out that in his data both P and K concentrations were negatively correlated with N concentration, so that when N/P or N/K ratios were related to growth N^2 would be similarly related to growth.

Foliar analysis data have also been expressed in terms of ratios of important macronutrients to N (Ingestad, 1966). The values for tree seedlings grown in sand culture are thought to be in the following ranges:

N	P	K	Ca	Mg
100	8-15	50-100	5-10	5-10

It might be supposed that ratios between nutrient elements and an element of no known nutrient significance within the plant, such as Al, could provide an independent basis for assessing concentration of nutrient elements. Entry of the non nutrient element would have to be largely independent of external supply for any advantage to be gained. In fact Al is affected somewhat by external supply level and interacts with P supply level (Humphreys and Truman, 1964, 1972). Consequently this approach may have limited value.

Analysing data

Undoubtedly specific ratios must exist in proteins, amino acids, etc., within the plant, but occurrence of storage compounds and mobility of some nutrients probably accounts for the disappointingly wide range of ratios which are often encountered in healthy crops. Whilst consideration of individual ratios may lead to deductions about physiological processes, it seems that for prediction of yield multiple correlation with individual nutrient concentrations, analysed for linear and quadratic components, is equally satisfactory.

Expression of soluble N compounds, such as amino acids, in terms of total N, will indicate the proportion of free N, which may be more informative than simple expression in terms of tissue dry weight.

The relationship between measure of tree yield, such as height, basal area, volume, or weight, and foliar concentration of a nutrient which is limiting growth, when other nutrients are in adequate supply, is usually one of diminishing returns (Leyton, 1958). The theoretical relationship between yield and nutrient concentration has generally been divided into a region of deficiency or "poverty adjustment", a critical level, and a region of luxury consumption (Barrows,

1959; Richards and Bevege, 1972; Everard, 1973) (Fig. 5). The existence of this relationship in forest trees has often been shown (e.g., Mitchell, 1939; Mitchell and Chandler, 1939; Leyton, 1958; Richards and Bevege, 1969; Ingestad, 1962; Swan, 1972a,b; Everard, 1973). The relationship can be approximated by the Mitscherlich equation (Mitchell, 1939), or by a second degree polynomial (Leyton, 1958). A second degree polynomial was used to show that foliar K concentration accounted for 66% of the variation in red pine leader length in K deficient and fertilized stands (Madgwick, 1964b). In areas of Queensland where growth of slash and loblolly pine was limited by P supply, foliar P concentration, expressed in the form of a second degree polynomial, accounted for 60 to 70% of variation in response, measured as log or basal area increment (Bevege and Richards, 1972).

It has been found (Steenbjerg, 1954; Hewitt, 1957) that when a limiting nutrient, previously in very low supply, is provided to a crop (e.g., cereals) concentration of the nutrient actually decreases further at first. As more nutrient is provided growth and tissue nutrient concentration then increase simultaneously. Consequently it should be borne in mind that the simple Mitscherlich equation or second degree polynomial may not be adequate models for relating response to foliar concentration of a nutrient in limiting supply.

The lowest foliar nutrient concentration associated with near maximum yield is loosely considered to be the critical level (Viets and Hanway, 1957). It is now conventional to define the critical level as the foliar nutrient concentration at which yield attains 90% of the possible maximum (Ulrich, 1952; Richards and Bevege, 1972). Knowing the critical levels for a number of nutrients in theory allows prediction of growth response which could be obtained by appropriately fertilizing the stand. In practice high, intermediate, low and deficiency concentrations have been published for many tree species (e.g., Leaf, 1968) to allow diagnosis of deficiency. Critical values have been published for fewer species (e.g., Richards and Bevege, 1969; Bevege and Richards, 1972; Swan, 1972a, b). It turns out that *diagnosis* of nutrient deficiency in a poorly growing stand by means of foliar analysis can be quite effective, but determination of optimum foliar nutrient concentrations and *prediction* of possible response to fertilizer applications by means of foliar analysis is much more difficult (Leyton, 1958).

For the limited deficiency range of the response/foliar nutrient concentration graph the relationship is essentially linear so that it has been possible to apply linear regression to data falling in this range. When data are treated by multiple regression those foliar nutrients which show a significant and positive contribution to the regression equation are thought to be limiting growth (Letyton, 1956, 1957; Leyton and Armson, 1955). The use of regression tends to avoid the necessity for defining critical levels, and multiple regression reduces the problem of autocorrelation which can occur with simple regres-

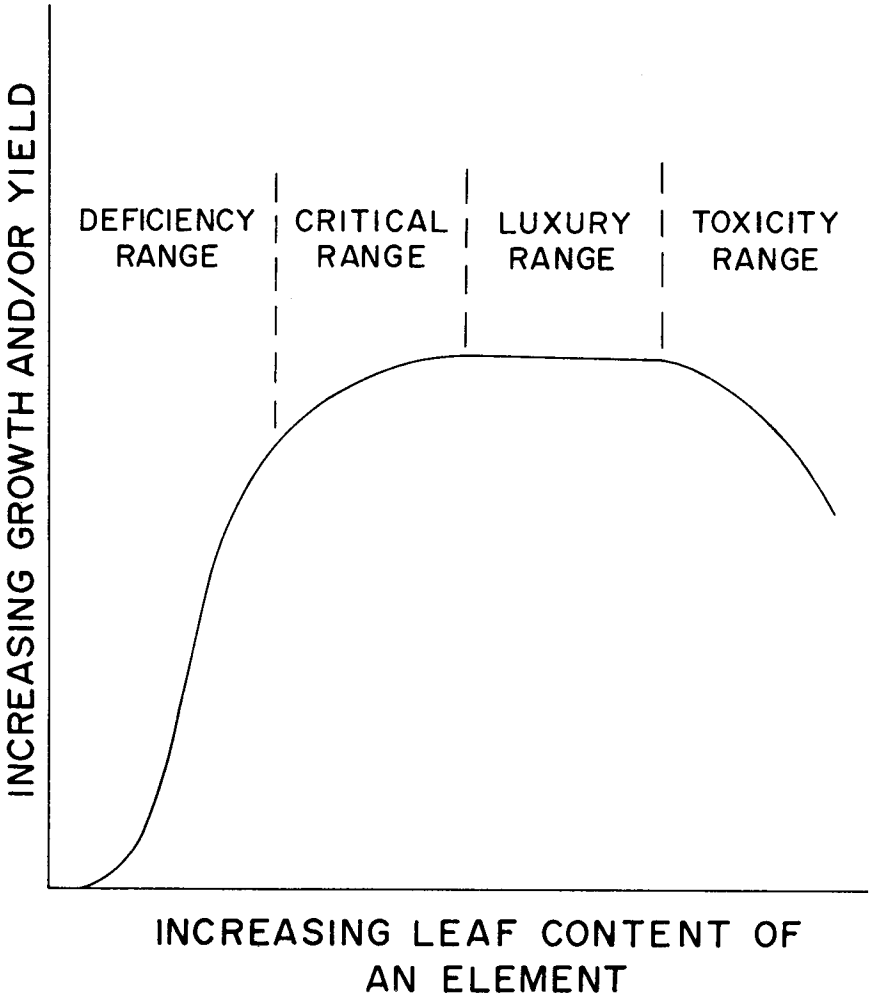

Fig. 5. Relationship between growth and foliar nutrient concentration.

sion. This multiple regression procedure was applied to red pine in Massachusetts with some success, and it was concluded that height growth was related to foliar Ca concentration, and basal area to foliar K concentration (Hoyle and Mader, 1964). The procedure was also examined in K deficient and fertilized red pine stands in New York (Madgwick, 1964b). In this instance K did not contribute significantly to the multiple regressions in 5 cases out of 6, although it was expected to. It was argued that even with multiple regression the

interdependence between concentrations of different foliar nutrients caused bias, depending on which nutrients were actually used in the regression. The choice of response measure also affected the result.

Despite drawbacks the multiple regression approach still seems very practical. For example, it should be possible to decide which nutrient, or nutrients, are limiting growth within the stand, simply by sampling from a number of different trees in the stand, without first laying out fertilizer plots. No doubt attention to adequate sampling is very important and possibly curvilinear, rather than linear, regression could at times prove helpful.

Two other types of treatment which have been used for handling foliar nutrient concentration data are principal component analysis and discriminant analysis. Principal component analysis may reduce the number of independent variables which have to be considered. Results of oil palm N, P, K, Ca, Mg analysis from 9 years of fertilizer plot work showed no clear-cut effect of treatment on nutrient concentrations (Holland, 1967). Two significantly distinguishable vectors (components) accounted for 77% of foliar nutrient variation, however, and were found to have closely similar values when computed for each year separately. Analysis of variance showed fertilizer treatments significantly affected both these vectors. Regression showed mean bunch weight was closely related to one of the vectors. In this case a vector was used to relate treatment to yield where more conventional statistics had failed to show any relationship. Principal component analysis probably has considerable application to interpretation of foliar analyses.

Discriminant analysis has been applied to tissue levels of nutrients in foliage of "green" and "yellow" slash pine (White and Mead, 1971). Fifteen pairs of trees were sampled and N, P, K, Ca, Mg, Cu, Zn, Mn, Fe, and Al determined. Only N and Zn were of significant value in the final discriminant function which classified 90% of the 30 sample trees correctly as green and yellow. The authors emphasize the discriminant value of N and Zn do not necessarily reflect a cause-effect relationship. This is an interesting approach and possibly has some value for diagnostic purposes.

In attempting to relate internal nutrient concentration with growth some sensitive measure of growth must be obtained. This is perhaps more difficult in forest trees than in agricultural crops. Current annual increment may be a better measure of response than total cumulative growth (Raupach, 1967). Basal area increment is considered more sensitive than height increment in southern pines (Richards and Bevege, 1972). Diameter at half height may show considerable response when breast height diameter shows very little response. On the other hand, height increment in spruce is better correlated with N status of year old foliage than current foliage (Leyton, 1958).

Growth of a plant, or stand, does not, of course, depend only on nutrient supply, but upon a number of environmental factors. Fur-

thermore, foliar nutrient concentration, considered as an independent variable, interacts with environmental factors. Consequently a degree of success has been achieved by including other important growth factors with foliar nutrient data in equations designed to predict growth response. In Hawaiian sugar cane crops a number of factors, such as leaf N, temperature, age, light, and various moisture measures have been found important (Clements, 1964). In slash and loblolly pine stands of Southern Queensland 80% of variation in basal area increment was accounted for in terms of P, basal area, site index, and age (Bevege and Richards, 1972). Such equations are a convenient way of taking into account other variables which interact with foliar nutrients. For application of foliar analysis to widespread stands it might be profitable to consider the contribution of such factors as moisture status, temperature, age, competition (basal area or spacing) and soil texture to similar equations.

In summary, there appears considerable scope for development of data handling and interpretation. Fundamental improvement in interpretation is likely to come only from physiology, but improvements at an empirical level should result from appropriate application of existing statistical and mathematical methods.

CONCLUSIONS

Foliar analysis can be used in two different ways. These are: (*1*) to decide which nutrient, or nutrients, are deficient in a stand that is growing poorly, or in check. This is described as diagnostic use, and does not require very precise information about the relationship of foliar nutrient concentrations to growth. Use of foliar analysis in this way is quite likely to be successful using existing methods and knowledge. (*2*) To decide how much response can be expected from application of a fertilizer in any stand. This is described as predictive use and clearly requires much more precise information about the relationship between growth and foliar nutrient concentrations. Further study of the species and environment will undoubtedly be required to use foliar analysis successfully in this way. Examples, of pines in Queensland and sugar cane in Hawaii, suggest this second use may be possible. If it can be realized it will be of considerable assistance in the management of forest stands for maximum production.

The literature review has shown that foliar nutrient concentrations can be affected by many factors other than nutrient supply to the tree. Consequently to accurately interpret foliar analysis these factors must be taken into account, particularly if foliar analysis is to be used predictively. Some of them, such as sample position on the tree, leaf age, etc., can be eliminated by standardization of techniques, as is already done by most workes. Other factors, such as moisture stress, year to year variation, competition effects, and provenance, are largely ignored. Yet it seems that if these other important factors

could be taken into account, perhaps by a stratification procedure, or in multiple regression, greater precision could be achieved with foliar analysis.

This review, though by no means comprehensive, indicates the large amount of research already carried out on application of foliar analysis to forest trees. Whilst showing the importance attached to the technique, it also suggests that further improvements will be relatively difficult to achieve, and that general predictive use of foliar analysis may be a very difficult objective to attain. Consideration of the literature leads one to think that improvements in the precision of foliar analysis could perhaps be made by research into some of the points listed below.

1. Determination of season, or phenological stage of development, together with tissue and position in tree which will give most sensitive indication of nutrient status. Tissue and season most sensitive for one nutrient may not be most sensitive for another.

2. Improvement of estimation of N status by analyzing soluble N compounds. Determination of individual amino acids, notably arginine and glutamine (N storage compounds) and proline (indicative of moisture stress) may be rewarding. Young branches, as well as foliage, should be considered for analysis.

3. Comparison of nutrient concentrations in foliage and twigs of different ages. Translocation from old to new tissue may mean that nutrient status is revealed by differences in concentration between tissue of different ages at certain times of year.

4. Determination of the effect of plant moisture stress, temperature, prevailing light conditions, spacing, soil texture, and other environmental factors on foliar nutrient concentrations. Methods will have to be devised for estimating factors, which are found to be important, in stands to be sampled.

5. Choice of growth response measurement. Which of the measurements: diameter at breast height, diameter at half height, total height, current height growth, current volume increment, are best correlated with the foliar analysis data?

6. Extent of year to year variation in foliar nutrient concentrations. Determination of the climatic, or other, factors causing this variation should make possible appropriate adjustments in interpretation of foliar analysis to reduce the effect of year to year differences.

7. In view of the problem of sampling foliage from dense 100 to 150 feet tall stands the values of phloem and root analyses should be examined. Soluble N concentration in roots may give a satisfactory indication of stand N status.

8. Measurement of some nonessential element concentrations, such as Al, or Ni, could be made to see whether essential: nonessential element ratios have more consistent values than essential: essential element ratios.

9. Expression of nutrient concentration in terms of foliage area rather than dry weight. Surface area may be a more useful basis since

it is the significant measurement unit for photosynthesis and dry matter production.

10. Development of rapid methods for determination of all free amino acids in tissue with a view to studying the relationship between free amino acid levels and mineral nutrient status in the tree.

11. Assessment of the most appropriate statistical procedures for handling foliar analysis data, including investigation of less widely used methods such as principle component analysis.

ZUSAMMENFASSUNG

Die wichtigsten Punkte der vorhergehenden Übersicht sind in dem folgenden Verzeichnis zusammengefasst und können als Hinweise fur weitere wissenschaftliche Versuche betrachtet werden.

1. Ermittlung der Jahreszeit, oder der Wachstumsentwicklung, in Verbindung mit Zellgewebe und Position im Baum, der die stärkste Empfindlichkeit über eine Nährstoffmenge angibt. Eine Kombination von Zellgewebe und Jahreszeit und stärkste Empfindlichkeit für einen gewissen Nährstoff trifft nicht unbedingt fur einen anderen Nährstoff zu.

2. Verbesserungen im Schätzen der N-Menge mit der Anwendung von Analysen mit löslichen N-Verbindungen, ebenso Untersuchungen individueller Amino-Säuren, insbesondere Arginine und Glutamine (N-Ablagerungsverbindungen) und Proline (Wassermangel anzeigend), erscheinen versprechend. Jung-Zweig-und Blattanalysen verdienen Erwägung.

3. Vergleiche zwischen Nährstoffkonzentrationen in altersunterschiedlichen Blättern und Zweigen sollten angestellt werden. Besonders unter Betrachtung von Konzentrationsunterschieden zwischen Zellegeweben verschiedenen Alters für gleiche Jahreszeiten, Verlagerungen von alten zu neuen Zellgeweben, könnten Nährstoffmenge anzeigend sein.

4. Ermittlung der Einflüsse von Wassermangel in der Pflanze, Temperature, vorherrschende Lichtverhältnisse, Stockungsgrad, Böden und andere Standortsfaktoren in Bezug zu Nährstoffkonzentrationen. Messungsmethoden für die Bewertung aller wichtigen und in Ständen vorkommenden Faktoren müssen entwickelt werden.

5. Welche Messungsresultate können am besten mit Blattanalysen correliert werden: Brusthöhendurchmesser, Mittendurchmesser, Gesamthöhe, jährlicher Höhenzuwachs, jährlicher Zuwacks?

6. Ausmass der jährlichen Unterschiede in Nährstoffkonzentrationen in Blättern. Ermittlung der klimatischen oder anderer Faktoren, die Unterschiede hervorrufen sollten, eine ungefähre Angleichung in der Erklärung der Blattanalyse ermöglichen und gleichzeitig jährliche Unterschiede verringern.

7. Wegen der Schwierigkeiten von dichten und 30 bis 50 m hohen Ständen Proben zu erhalten, sollten Phloem und Wurzelunter-

suchungen erwogen werden. Lösliche N-Konzentrationen in Wurzeln mögen zufriedenstellende Hinweise über N-Mengen in Ständen geben.

8. Untersuchungen der Konzentrationen einiger nicht erforderlicher Elemente, wie: Al oder Ni, solten unternommen werden, um zu ermitteln, ob die Verhältniswerte zwischen erforderlichen: nicht-erforderlichen Elementen stärker sind als die der erforderlichen: erforderlichen Elemente.

9. Bericht über die Nährstoffkonzentrationen in Form von Blattoberfläche anstatt des Trockengewichtes. Blattoberfläche scheint eine bessere Basis zu sein, da es ja gleichfalls eine wichtige Masseinheit für die Ermittlung der Photosynthese und Wachstum der Trockensubstanz darstellt.

10. Entwicklung einer Scnellmethode für die Ermittlung aller freien Amino-Säuren in Zellgeweben, um Verhältnisse zwischen den Mengen freier Amino-Säuren und Mineralnährstoffen in Bäumen aufstellen zu können.

11. Ermittlung der geeignesten statistischen Datumsverarbeitungsmethode für die Blattanalysis. Bewertungen sollten weniger angewandte Methoden wie die "Principle component analysis" einschliessen.

(Translated by Karl Rieche)

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