13 Plant Analyses and Interpretation

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Plant analysis historic beginnings are generally attributed to T. de Saussure (1804) following studies by van Helmont, Joseph Priestly, Henry Cavenish, and Antonine Lavoisier. de Saussure showed that the composition of plant ash varied with the part analyzed, with the age of the plant, and with the soil upon which the plant grew. The ash was chiefly composed of alkalis and phosphates. Erasmus Darwin in his 1800 book, *Phytogia: The Philosophy of Agriculture and Gardening* (London, J. Johnson) wrote that both nitrogen (N) and phosphorus (P) were essential components of plants. In 1833, the Fifth Duke of Richmond showed that the value of bone meal fertilizing was due to its P component rather than calcium (Ca), although Justus von Liebig (1852) is generally considered the father of soil fertility. Readers interested in additional historic information should consult Ulrich (1948), Bear (1948), and Russell (1976).

Plant analysis was developed to provide information on the nutrient status of plants. Aldrich (1973) lists seven general uses. These are (i) to diagnose or confirm diagnosis of visible symptoms, (ii) to identify hidden trouble, (iii) to locate areas of incipient deficiencies, (iv) to indicate whether applied nutrients entered the plant, (v) to indicate interactions or antagonisms among nutrients, (vi) at aid understanding internal functioning of nutrient in plants, and (vii) to suggest additional tests to identify the trouble. These are still valid, however plant analysis is increasingly being used to identify potential environmental concerns from overfertilization or toxicities, and nutrient levels in livestock or human diets. Plant analysis is also being used to manage a crop's nutritional status during growth. This real-time use can include the prediction of future nutrient concentrations and seasonal fertilizer applications, as well as plant analysis itself. Potential future monitoring techniques includes nondestructive diagnostic protocols using remote sensing technologies (Hergert, 1998).

Comprehensive reviews of plant analysis as diagnostic tools are given by Aldrich (1973), Munson and Nelson (1973), Jones and Steyn (1973), and Marschner (1986). A recent excellent reference source is a chapter on interpretation of plant analysis by Smith and Loneragan (1997) found in Reuther and Robinson (1997). No single chapter was devoted to this subject in the 1980 ASA-CSSA-SSSA phosphorus publication, although it was partially covered in several chapters, especially that by Ozanne (1980). A brief overview of the principles in-

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volved will be given here followed by recent developments not covered by previous reviews.

Certain elements are classified as essential for plant growth. To be an essential chemical element from the perspective of plant nutrition (i) it must be present for the plant to complete its life cycle; (ii) its metabolic role cannot be replaced by another chemical element; and (iii) it is directly involved in a metabolic process within the plant, either having a direct role in the process or as a compound component involved in the process. Elements meeting these criteria include C, hydrogen (H), oxygen (O), N, P, potassium (K), sulfur (S), magnesium (Mg), Ca, iron (Fe), manganese (Mn), zinc (Zn), copper (Cu), chlorine (Cl), boron (B), molybdenum (Mo), cobalt (Co) and most recently nickel (Ni) (Brown et al., 1987).

Not all essential nutrients are mobile in both the xylem and phloem conductive tissues but P is. The pattern of initial partitioning and distribution, and the rate and extent of cycling and remobilization within the plant varies with the environmental conditions, plant nutrient status, species and state of development. In general, as the P supply becomes limiting, P is transported and retained in the actively growing meristem areas, for example, young leaves and other developing plant parts (Marschner, 1986). As the supply becomes more limiting, it may be translocated from the older leaves. If this occurs sufficiently P deficiency symptoms will be expressed before normal plant senescence. Losses can also occur from the plant's vegetative parts during senescence as the mobile nutrients are solubilized and translocated to the fruiting or storage body before plant maturity.

VISIBLE PHOSPHORUS SYMPTOMS

Visible deficiency symptoms are dependent upon the plant species and the plant's growth stage at onset of deficiency. In general, vegetative and reproductive growth is depressed because protein synthesis is impaired. Phosphorus deficient plants will have a limited root system, thin stems, and smaller leaves. Visible symptoms in the older leaves are often a darkish green color, at times appearing bluish-green. These plants may also appear severely water stressed. Many annual species will have a reddish coloration from an enhanced formation of anthocyanins (red, purple, or brown pigments). Affected leaves senescence prematurely. General plant maturity is delayed because overall growth rate is delayed. Typical P deficiency symptoms can be masked by other nutrient deficiencies or plant disease symptoms. Descriptions of P toxicity symptoms are limited (Jones, 1998; Webb and Loneragan, 1988). These symptoms appear as interveinal chlorosis in younger leaves, necrosis and tip die back occurs in susceptible species, marginal leaf scorch, and shedding of older leaves.

TRADITIONAL APPROACH

Interpretation

Plant analysis is generally defined as the destructive sampling of a plant or plant part, its chemical analysis, and subsequent diagnostic interpretation. This

can include different plant parts, dried or not-dried, soluble or total nutrient analysis, and a range of interpretation techniques. A critical requirement of a successful diagnosis is a reliable relationship between the nutrient concentration in the selected plant part and the growth rate or yield of the plant. Such a relationship is depicted in Fig. 13–1. At a low nutrient concentration, the growth rate or yield is low but it increases rapidly as the concentration increases until the nutrient no longer limits growth. At that concentration, any additional increases do not further increase growth and may eventually reduce growth if the concentration becomes toxic. An appropriate nutrient management program attempts to maintain all essential nutrients in the concentration range needed for optimum growth. The shape of the response curve in Fig. 13–1 is similar to that for the Michaelis-Menten equation describing enzyme kinetics (Epstein, 1972).

The relationship shown in Fig. 13–1 can be obtained from water, sand, or soil culture under greenhouse, growth chamber, or field conditions. The nutrient being studied should be available from a deficient amount to above adequacy. Intervals between nutrient rates should be chosen to fully characterize the response curve, especially the steep portion of the curve and the transitional zone between deficiency and adequacy. Precautions must be taken to ensure that growth is not limited by other factors over the complete range of nutrient concentrations. The dependent variable of this relationship can be plant dry matter production, a harvestible plant part, or the economic yield component. The dependent data are usually normalized and expressed as percentages, especially if they come from field studies. The independent variable, a nutrient, may be expressed on a percentage basis, as an absolute concentration, as the total or soluble portion of the total concentration per plant weight unit, or a total uptake amount per plant. It may be derived from any number of different plant parts or combination of parts taken at dis-

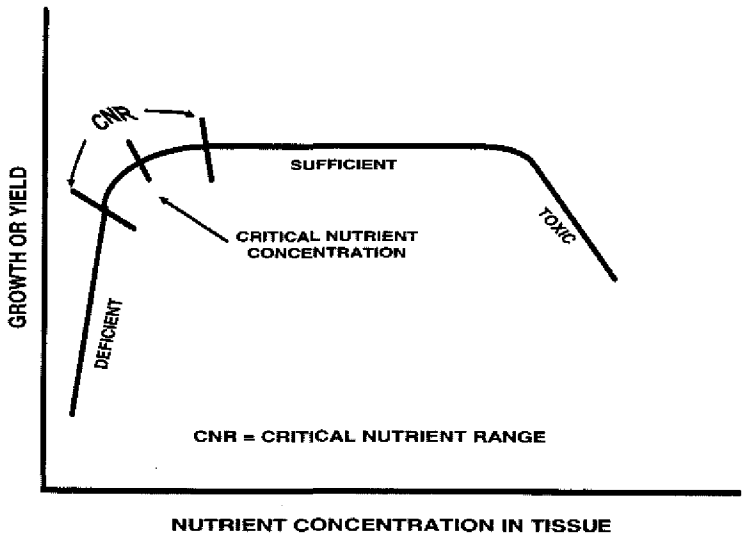

Fig. 13–1. Growth or yield of plants in relation to nutrient concentration in plant tissue.

tinct plant growth stages. If taken at several plant growth stages, a family of curves are usually developed.

After the development of the response curve relationship, two main approaches are used for interpretation. The *critical nutrient concentration* (CNC) is defined as that concentration where growth or yield is 10% less than the maximum, Fig. 13–1 (Ulrich, 1952). It is in the transition zone separating deficiency from sufficiency. It is usually closely associated with a specific plant part and growth stage, and only used for diagnostic purposes. The *critical nutrient range* (CNR) is defined as the range of nutrient concentrations above which the crop is amply supplied and below which the crop is deficient (Dow and Roberts, 1982). The CNR usually defines the range of uncertainty. Its magnitude depends upon the individual relationship, the data available, and the ability to physically and chemically characterize the relationship. It can be depicted as a concentration band that changes with growth so repeated plant samplings during growth are needed to diagnose the changing nutritional status (Fig. 13–2).

The nutrient concentration of the plant tissue can also be interpreted using the *diagnosis and recommendation integrated system,* DRIS (Sumner, 1978a, 1978b; Walworth and Sumner, 1987). This procedure attempts to recognize and quantify antagonisms and synergisms between plant nutrients and emphasizes nutrient balance. Norms are developed for a given crop that are supposedly independent of plant age and applicable across environments, although as originally intended, environmental parameters could be variables. The norms are derived from two populations (high vs. low yielding) which are statistically different for the parameters being evaluated. Since they are strictly empirical, they may or may

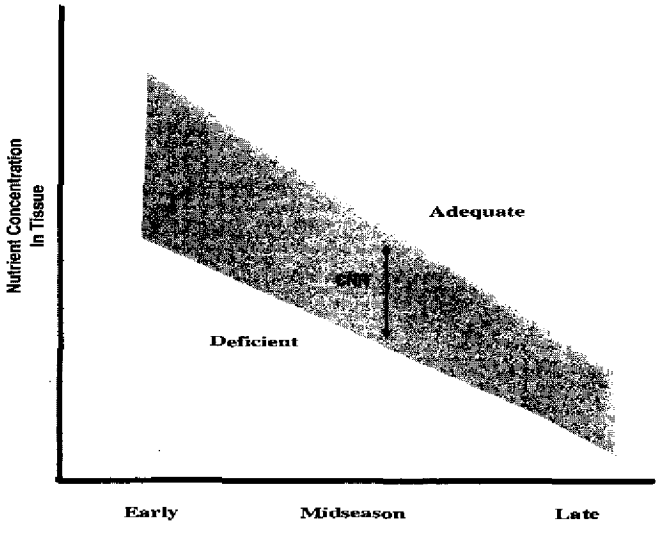

Fig. 13–2. Generalized interpretive guide based on the concept of critical nutrient range (CNR) for tissue sampled at different times though the growing season.

not have a physiological role in the plant. Several studies evaluated the use of DRIS for annual and perennial crops but this technique has not been widely adopted (Hallmark and Beverly, 1991). Relatively recent studies include those reported by Beverly (1993), Hallmark et al. (1990), and Bethlenfalvay et al. (1990) for soybean, and Hartz et al. (1998) for tomato (*Lycopersicon esculentum* Mill.). A somewhat parallel interpretation method is the boundary line development system (Schnug et al., 1996). The Compositional Nutrient Diagnosis (CND) is another approach which is based upon the relationships between the concentration of all nutrients and final yield in survey samples (Parent and Dafir, 1992). It is also empirical, relying on statistical evaluation of the data to establish indices for yield prediction. Additional information on this approach is given by Khiari et al. (2001a, 2001b) and Parent et al. (1994).

Another interpretative approach is a rate-balance approach. This approach especially applies to mobile nutrients and in management systems where fertigation can be practiced. This method relates the nutrient concentration in a plant part to a ratio of the nutrient utilization rate by the whole plant divided by the nutrient utilization rate by the plant part. Relationships may differ for different growth stages. Utilization can be defined as that required for seed development or any other fruiting body, such as storage root, tuber, etc., or the harvested portion of the crop. When the ratio is >1, there is more nutrient uptake than required for the growth of the harvested portion, so nutrients accumulate in the vegetative portions of the plant or are available for additional vegetative growth. When the ratio is less than one, uptake is less than that required for growth and mobile nutrients will be translocated out of the vegetative portions of the plant to the fruiting body. This approach was successfully developed for potato *(Solanum tuberosum* L.) production using total P or soluble PO_4-P in the petiole (Westermann and Kleinkopf, 1985). The approach would not be applicable to nonmobile plant nutrients or in field situations where water may be limiting. As technology continues to improve to allow more intensive management options this approach or similar ones will be developed and adopted by more producers on a greater number of commercial crops.

Plant samples can be analyzed for total nutrient concentration or a portion extractable by water, a weak acid or salt solution. Soluble P was a suitable indicator of the P status of sugar beet (*Beta vulgaris* L.) (Ulrich and Hill, 1990), grapevine (Skinner et al., 1987), and wheat (*Triticum aestivum* L.) (Bollons and Barraclough, 1997), however, total P was preferred for spring wheat (Elliott et al., 1997a, 1997b). There is generally a good relationship between the soluble and total P concentration in petioles (Westermann and Kleinkopf, 1985) but not in plant leaves (Lewis, 1992). Plants partially adapt by modifying their metabolism to enhance inorganic P cycling during P deficiency (Kondracka and Rychter, 1997).

Phosphorus interactions with other chemical elements in soils and plants were reviewed by Adams (1980), Sumner and Farina (1986), and Fageria (2001). In general, the concentrations of other essential nutrients do not significantly affect the interpretation of P concentrations in plant tissue, however adequate or excess concentrations of P can affect the utilization of other essential elements.

Some reported interactions include those with Ca, Zn, Mn, Fe, and Mo (Gianquinto et al., 2000; Heuwinkel et al., 1992; Marschner and Cakmak, 1986; Modi, 2002; Moreira et al., 2001; Saleque et al., 2001; Wallace, 1984; Zhu et al., 2002).

An important factor affecting interpretation and consequently the derived CNC is the choice of model used to define the relationship as shown in Fig. 13–1. Hand-fitted curves should not be used as they may imply a greater degree of accuracy than the data warrant and in addition, they do not provide any measure of variability. Statistical models should estimate both the CNC and its variability. Differences might be large enough to seriously affect management decisions and possibly crop performance. Some methods that attempt to analyze this uncertainty are described by Chen et al. (1997), Mallarino and Blackmer (1992), and Byrne and Drummond (1980).

Sampling Variables

Factors affecting sampling include the plant part sampled, the sampling statistics, sample handling, and the sampling purpose. Plant tissue can be used for both diagnostic and prognostic purposes (Bell, 2000). A diagnostic test is defined by a relationship between a nutrient concentration and a measure of yield at a specific growth stage, indicating only the plant's nutritional status at the point in time when the sample was taken, similar to that shown in Fig. 13–1. A prognostic test is one that attempts to predict the plant's future nutrient status from present and previous concentrations. It usually requires more than one sample since changes occur with time (Lewis et al., 1993). The development of plant analysis for prognostic purposes and its successful application is difficult because of complex interactions in the biological system and unknown future climatic conditions. Carter et al. (1971) and Westermann and Kleinkopf (1985) provide prognostic examples that predict future nutrient concentrations in petioles. Complex simulation models that consider crop growth processes in relationship to climatic conditions and the soil environment may eventually be able to predict nutrient concentrations to use as diagnostic tools (Mendham et al., 1997).

Sources of sampling variability include that within similar-aged tissue on the same plant and that between plants. Increasing the number of individual plants sampled tends to reduce variability errors to more manageable levels. This has to be balanced by the ability to handle larger samples and the amount of time available for taking samples. Systematic variation between individuals doing the sampling may also be a source of variability. All these are in addition to the variability associated with the laboratory's analytical procedure. Limited information is available on the spatial variability of plant nutrient concentrations (Franzen and Peck, 1995). Yu et al. (1999) discussed the importance of phosphorus' spatial variability in the practice of precision agriculture.

Several plant parts may be suitable for nutritional testing. The plant part chosen should be sensitive to nutrient supply, easily identifiable, be related to growth or yield response, have a wide range between deficiency and adequate concentrations, and have a CNC (Ulrich, 1948; Westermann et al., 1994). Examples

of using different plant parts are described by Elliott et al. (1997c), Hoppo et al. (1999), and Knowles et al. (1990). As stated earlier, the nutrient's role in the plant's physiology also affects the appropriate plant part for sampling. For the more mobile nutrients, concentrations will generally be smaller in the older leaves and greater in the younger leaves, while for the immobile nutrients, the reverse occurs. Since leaves are sites of metabolic activity, the concentration of the nutrients used by the activity will generally be low in these tissues, for example, NO_3-N. Leaves are generally better adapted for nutritional monitoring of micronutrients, Ca, Mg, and total N, while petioles and stems are better suited for NO_3-N and soluble concentrations of PO_4-P and K. Several different plant parts may be acceptable as long as a relationship exists between the nutrient concentration and plant growth or yield, and the same plant part is consistently sampled. Normally, plant analysis is used to detect sub-optimum concentration but an attempt was made to evaluate above optimum available soil P concentrations by analyzing different corn (*Zea mays* L.) plant parts (Mallarino, 1995, 1996).

Nutrient concentrations change with plant age. Most nutrient concentrations are highest in the vegetative portions of the plant during early growth and development. Foliage nutrient concentrations that decrease with plant age are N, P, K, Cu, Zn, and S, while Ca, Mg, B, Fe, Cl, and Mn concentrations generally increase. These differences reflect mobility differences within the plant, and the balance between the rate of supply and the use of nutrients by various tissues. Concentrations that are considered sufficient vary from one growth stage to another, emphasizing the need to relate diagnostic standards to phenological growth stage rather than chronological age. It is important to understand these relationships as apparent excess nutrients that accumulate during one growth stage can be important for subsequent growth stages (Liptay and Arevalo, 1998).

Potential diurnal differences exist but can be largely eliminated if sampling time is standardized. Nutrient concentrations in plants under moisture stress or low light (radiation) conditions can be atypical. Low environmental temperatures can reduce root growth and nutrient uptake, especially P during early plant growth and development. There is a tendency for nutrients to accumulate when growth is reduced more than uptake by low temperatures; conversely nutrient concentrations may be lower than normal if growing temperatures are elevated. High soil temperatures accelerate early plant development and can hasten senescence. Plant samples taken 3 to 4 d after a fertilizer application could have nutrient concentrations not indicative of the true nutritional status of the plant. Similarly, tissue concentrations immediately after a foliar nutrient spray may include applied nutrients still on the external surfaces of the tissues.

Genotypic differences may affect the critical nutrient concentration used for diagnostic purposes. Genotypic differences are reported for alfalfa (*Medicago sativa* L.) (James et al., 1995), cotton (*Gossypium hirsutum* L.) (Ahmad et al., 2001), corn (Elliott and Lauchli, 1985), tomato (Coltman et al., 1986; Coltman, 1987), rice (*Oryza sativa* L.) (Hung et al., 1992), wheat (*Triticum aestivum* L.) (Fageria and Baligar, 1999), and common bean (*Phaseolus vulgaris* L.) (Yan et al., 1996) but limited information exists to indicate a direct effect on critical nutrient concentration. An analogous study comparing plant species showed that monocots

were more P efficient than dicots but C4-species were not inherently more P efficient than C3-species (Halsted and Lynch, 1996).

Calibration Process

The overall objective of a calibration effort is to define a nutrient concentration in a plant tissue that accurately reflects the nutritional status of the plant. Plant growth stage, plant part, and total or soluble nutrient concentration should be components of the calibration study. Relationships to develop include those between plant tissue nutrient concentration and nutrient uptake, plant growth or economic yield, and how the plant nutrient concentration is affected by nutrient applications. Consideration should also be given to how the concentration will eventually be used, that is, diagnostic or prognostic. Single articles reporting all the phases of the calibration process are limited so selections from three studies (Hoppo et al., 1999; Knowles, et al., 1990; Mallarino, 1996) will be used to illustrate the process.

In Knowles et al. (1990), the P in the basal stem was compared with leaf tissues at different Feekes growth stages (Large, 1954) as related to the P nutritional status of irrigated durum wheat (*T. turgidum* L.). Soluble P (PO_4-P) was extracted by 2% acetic acid rather than total P concentration (Table 13–1) in the plant parts.

The basal stem PO_4-P concentration increased 1840 mg kg^{-1} from applied P compared with 960 mg kg^{-1} for the upper leaf at GS2. At the GS10 growth stage, this difference was reversed where the increase in the basal stem was 440 mg kg^{-1} compared with 820 mg kg^{-1} for the upper leaf. This suggests that the basal stem might be a better indicator of P applications at early growth stages while the upper leaf was at later growth stages. A comparison of the basal stem with a basal leaf also showed a similar trend. The concentration reversal illustrates the early accumulation of P in the conductive tissues of the young plant which is transferred to the growing point and younger leaves at later growth stages.

Unfortunately, Knowles et al. (1990) did not report total plant P uptake but they did report grain yields. The relationship of the basal stem PO_4-P concentrations at GS2 to relative grain yields is shown in Fig. 13–3. Normally all available

Table 13–1. Effect of phosphorus (P) application on PO_4-P concentration in two Durum wheat plant parts at three growth stages, GS2, GS6 and GS10 (1987–1988) (from Knowles et al., 1990).

	Basal stem PO_4-P			Upper leaf PO_4-P		
P applied	GS2	GS6	GS10	GS2	GS6	GS10
kg ha^{-1}	mg kg^{-1}					
0	1130	1830	400	360	520	1640
20	2080	2290	640	890	710	2030
40	2970	2980	840	1320	1040	2460
LSD(0.05)	180	230	90	130	140	140
Tmt. F	326	78	68	172	42	102

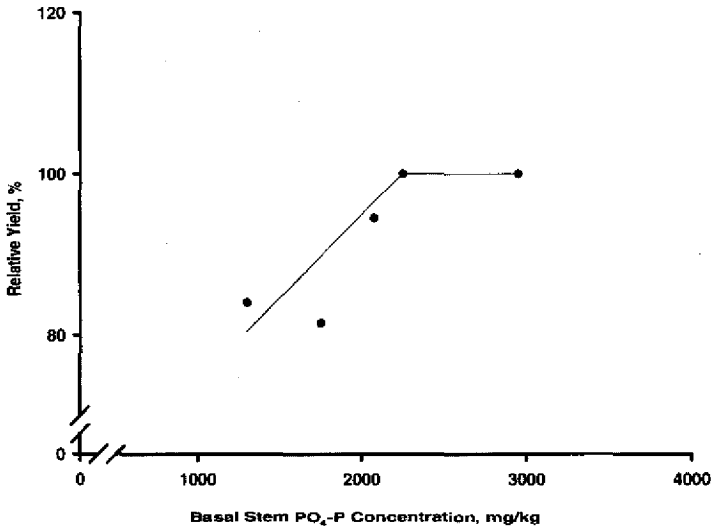

Fig. 13–3. Relationship between basal stem PO_4-P concentration at Feekes GS2 and relative wheat grain yields (adapted from Knowles et al., 1990).

data are used in the relationship, however the authors only gave treatment averages. This plot suggests that about 2000 to 2200 mg kg^{-1} PO_4-P is the critical concentration range in basal stem samples taken at GS2. Grain yield reductions occurred below this concentration while concentrations above this did not increase grain yields. Detecting P deficiency this early in plant development may allow a corrective action to be taken to prevent yield losses but the authors did not report on that hypothesis.

Hoppo et al. (1999) present data showing the relationship between plant tissue concentration and shoot P uptake or yield for barley (*Hordeum vulgare* L.) at different sampling dates or Zadoks growth stages (Zadoks et al., 1974). Plant parts sampled were the whole plants, youngest emerged leaf blade (YEB), and the next oldest leaf blade. The relationships between the P concentration in the YEB and relative shoot yield and applied P are shown in Fig. 13–4. Phosphorus applications increased grain yields at this site. There was a curvilinear effect of the applied P on YEB P concentration at all sampling dates that was much more pronounced in the first two samplings. There were also differences due to P fertilizer application method at the first sampling. Relative shoot dry weight increased as the P concentration in the YEB increased up to about 0.49% P and 0.40% P at Days 42 and 56, respectively. These corresponded to Zadoks 14.1 and 15.1 phenological development. At later sampling dates there was no upper plateau effect. These data suggest that the P concentration in the YEB can be used to determine the P nutritional status of the barley plant under these growing conditions. Since the data shown is for 1 yr and a limited number of sites, additional studies are needed to better define the relationships. At this particular individual study site, the relationship be-

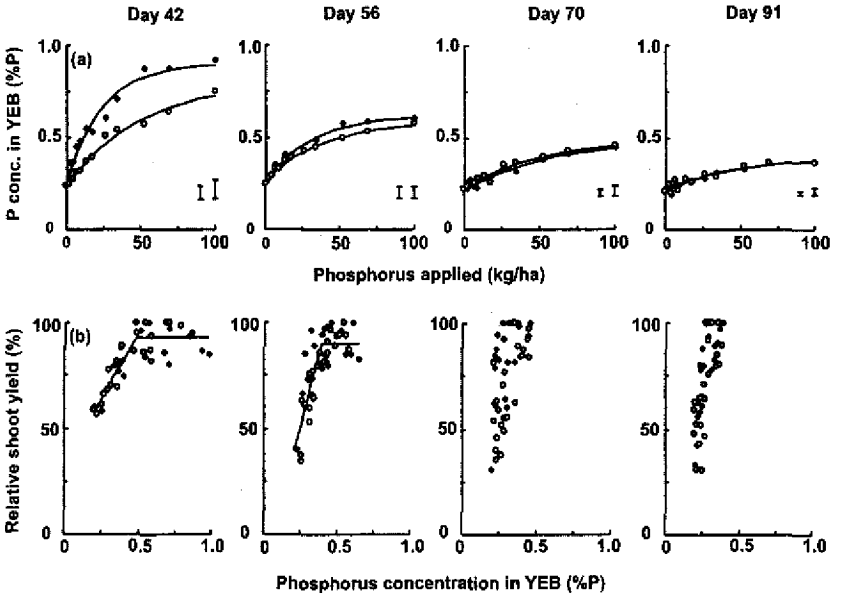

Fig. 13–4. Effect of applied P rate and method of placement with seed (◆) or broadcast prior to sowing (○) on P concentration in YEB (a) and relationship between relative shoot yield and P concentration in YEB (b) at different sampling dates (adopted from Hoppo et al., 1999).

tween grain yield and P concentration in the YEB was poor using a Mitscherlich function.

Mallarino (1996) related the total P concentration in young corn plants, ear-leaf blades at silking, stalks at physiological maturity, and shelled grain to relative yield for 25 field sites in Iowa. The objective of this study was to evaluate the P status of corn in soils testing in the optimum to above-optimum availability range, so not all sites had a significant yield response to P fertilization. The study showed that the P concentration of the young plants and the ear-leaf can be used to identify P deficiency as related to grain yields. The critical concentrations estimated were 3.4 g P kg^{-1} and 2.4 g P kg^{-1} for the young plants and ear-leaf, respectively. Differences in tissue P concentrations among sites were often greater than differences between treatments, so relationships were weakly correlated (Fig. 13–5).

The plant tissue nutrient concentration in the control treatment should also be related to the initial soil nutrient availability, that is, soil test concentration at the field study sites. This comparison relates two independent indicators of nutrient status, so significant relationships would be expected only if both indicators are appropriate indices of nutrient availability. Data given by Mallarino (1996) illustrates this concept (Fig. 13–6) for two soil test procedures. The P concentration of the young plants, ear-leaf, and grain were significantly correlated with available soil P as measured by the two tests. Coefficients of determination were higher for the Olsen than for the Bray-1 method. The Bray-1 underestimated available P in the calcareous soils as explained by the author.

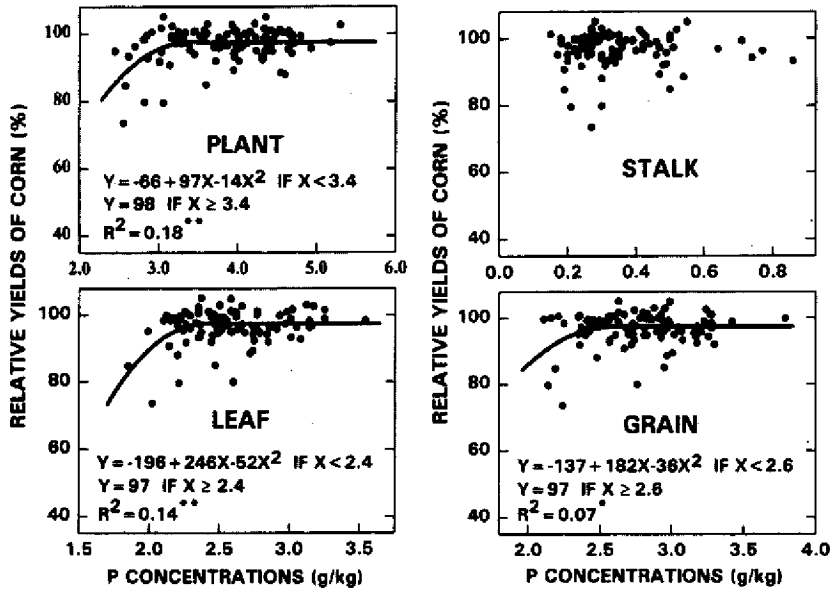

Fig. 13–5. Relationship between phosphorus (P) concentration in four corn tissues and relative grain yields (adopted from Mallarino, 1996).

An example of using plant tissue P nutrient concentrations to predict additional nutritional needs was developed for potato. Westermann and Kleinkopf (1985) described the background information for this procedure. Essentially the P concentration in a designated petiole was related to the ratio of the P changes in the whole plant divided by the P changes in the developing tubers for given time intervals. When this ratio is <1, the plant is taking up less P than needed for tuber growth and P will be translocated from the rest of the plant into the developing tubers; when the ratio is >1, more P is being taken up than needed for tuber growth and consequently, P accumulates in the plant. The petiole P concentration at which the ratio is one was found to be 0.22% P for the Russet Burbank variety.

Petioles are taken at or shortly after tuber initiation on weekly intervals through most of tuber growth. Phosphorus concentrations are plotted on a log scale against time on a linear scale (Fig. 13–7). A line is hand-fitted to the data and extrapolated to estimate petiole P concentrations at future sampling dates. Extrapolation can only be done when concentrations are decreasing. If the extrapolated line remains above the critical petiole P concentration past a predetermined cut-off date before vine kill, then no additional P applications are necessary (Field A, Fig. 13–7). If the line falls below the critical concentration prior to the cut-off date, a P application may be necessary to avoid diseases, deficiency and/or yield reductions (Field B, Fig. 13–7). The extrapolated line can be redrawn or updated each time new samples are obtained. This technique is successfully used in many western U.S. potato production systems.

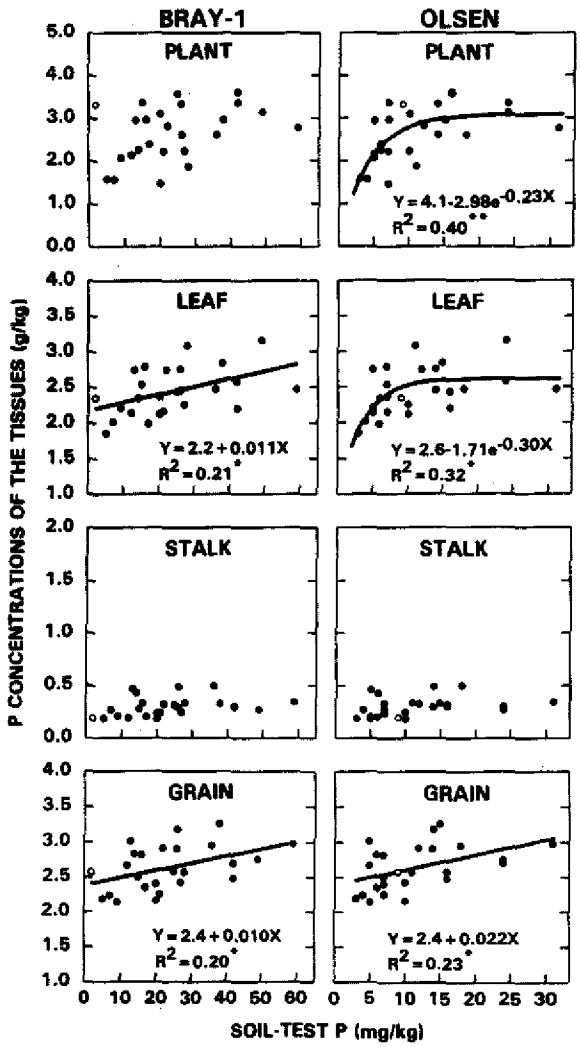

Fig. 13–6. Relationship between phosphorus (P) concentration in four corn tissues and soil test P concentration determined by two methods. Calcareous soil/site identified by (s) (adopted from Mallarino, 1996).

ALTERNATIVE TECHNOLOGIES

Enzyme Diagnosis

Enzymatic methods offer another approach to assessing the mineral nutritional status of plants. These methods are based on the activity of certain enzymes being lower or higher in deficient than normal plant tissue (Osaki et al., 1993; Osuji et al., 1998; Rabe and Lovatt, 1986; Romer et al., 1995; Stewart et al., 2001).

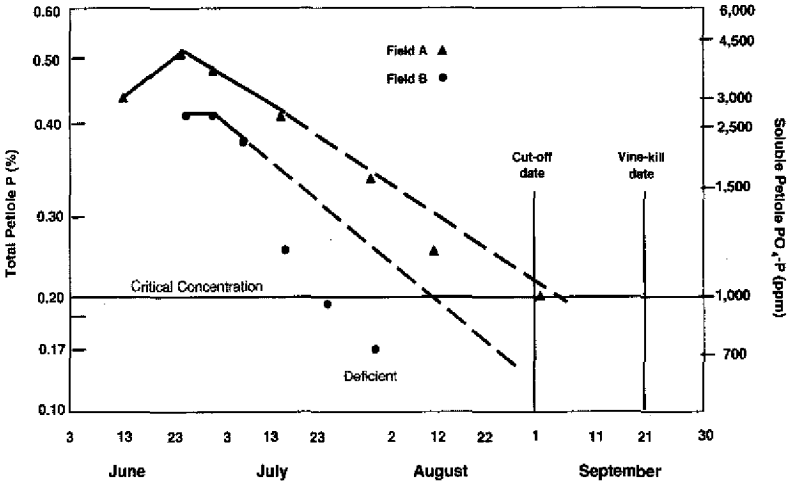

Fig. 13–7. Evaluating mid-season potato plant P status from petiole P concentrations. Petiole P concentrations are plotted on log scale (adopted from Stark and Westermann, 2003).

The actual enzyme activity is determined in the tissue after extraction or the tissue is incubated with the mineral nutrient in question for 1 or 2 d to induce enzyme activity for subsequent analysis. The enzyme activity does not give the actual concentration of the respective nutrient but its magnitude provides an indication of the deficiency. This technique was critically evaluated for several nutrients in citrus plants (Lavon and Goldschmidt, 1999). To replace chemical analysis for diagnostic purposes would depend upon the selectivity of the enzyme analysis, its accuracy, and whether it was sufficiently simple for routine analysis.

Plant acid phosphatase activity has been investigated as an indicator of P deficiency. When P availability is low, phosphatase increases in the leaves to facilitate the availability of bound P in the cytoplasm, while in the roots, phosphatase is excreted to hydrolyze soil organic P compounds at the root surface. Acid phosphatase found in the leaves was found to confirm visual P deficiencies in maize or corn (Elliott and Lauchli, 1986), tomato (Kaya et al., 2000), and wheat (Guthrie et al., 1991). Roots of P-deficient plants also have a higher phosphatase activity (Szabonagy et al., 1987; Ascencio, 1994, 1997; Dracup et al., 1984). Leaf acid phosphates assays were also better indicators of plant P status and yield than inorganic or total P in field grown wheat (McLachian et al., 1987). A somewhat conflicting study reported that the genetic loci for induction of leaf acid phosphatase activity was not associated with the loci conferring P acquisition efficiency or P-use efficiency, thus concluding that leaf acid phosphatase activity did not play a role in the plant's adaptation to P deficiency (Yan et al., 2001).

The increase in phosphatase activity in P deficient plant tissue is an interesting physiological and biochemical phenomenon which might be related to P availability, however the technique is subject to the same limitations and variables for routine diagnostic use as chemical analysis for nutrient concentrations. These include the evaluation of sampling, site, and environmental differences, cultivar,

plant part, and age effects, and a reliable relationship to plant growth or crop yield. The nondestructive nature of this technique and the potential to determine enzyme activity quantitatively using polyclonal antibodies in immunochemical assays for diagnosis of mineral nutrient deficiencies emphasizes this procedure's potential.

Spectral Analysis

Increasing acceptance of site specific management and variable rate fertilizer application technology has stimulated studies to develop procedures and methods to remotely detect and measure soil and plant growth variables (Mulla and Bhatti, 1997; Simmelsgaard and Djurhuus, 1997). When this information is combined with Geographic Information Systems, it allows management decisions to be made on spatial and temporal scales. This emerging technology package as a whole is referred to as precision agriculture (Hergert, 1998; Schepers and Francis, 1998). A significant part of this technology is the need to estimate soil plant nutrient availabilities and plant nutrient sufficiencies using indirect, nondestructive methods (Schepers, 1994). These include remote sensing using photography, multi-spectral images, hyper-spectral images or thermal devices to detect plant stress caused by moisture, heat, or nutrient deficiencies.

The basic level of spectral activity begins with photosynthesis, which is closely linked to chlorophyll activity. Chlorophyll a and b are measured at absorbance wavelengths of 663 and 645 nm, respectively (Gregory, 1971). The visible portion of the white light spectra can be divided into red, orange, yellow, green, blue, and violet (Fig. 13–8). The intensity and color of reflected light are dependent upon the wavelength of the light being absorbed and vice versa. The yellow-green color associated with N deficiency is because the plant absorbs more red light. Plant P deficiencies appear as purple or bluish coloring so the absorbance of green light is increased (Raun et al., 1998). Remote sensors generally detect reflected rather than absorbed light wavelengths.

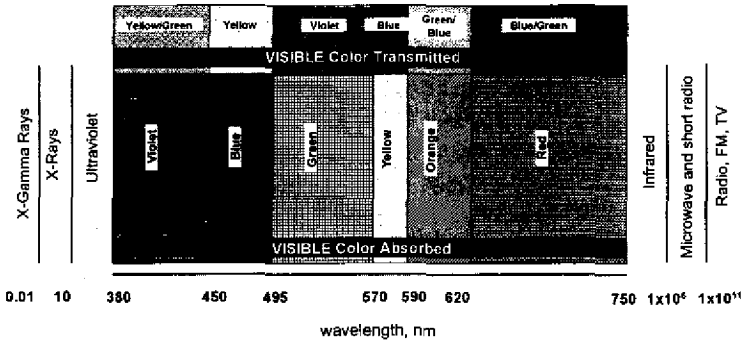

Fig. 13–8. Characteristics of visible and non-visible portions of the light spectra (adopted from Raun et al., 1998).

The characteristics of the plant spectral reflectance and transmittance are functions of leaf geometry, morphology, physiology, and biochemistry. These are also influenced by soil and climatic conditions, and nutrient status (Gates et al., 1965). An excess or deficiency of an essential nutrient may also cause abnormalities in pigmentation, size, and shape of leaves and appearance of other symptoms. Other factors include physiological age, water content, osmotic stress and salinity, pigment composition, and the relation of cell structure upon individual leaf spectra (Al-Abbas et al., 1974). Many interfering factors affecting recommendations based on remote sensor technology have yet to be resolved.

Early studies identified the spectra of normal and nutrient deficient leaves. Nutrient deficiencies of maize caused reduction in leaf chlorophyll content and an alteration of leaf color, reflectivity, and transmittance (Al-Abbas et al., 1974; Evans et al., 1950). A P deficient leaf absorbed less energy in the near infrared (NIR) region (750–1300 nm) than normal plants. Another nondestructive sensing procedure, NIR reflectance spectroscopy was successfully used to determine various quality components in agricultural products (Clark, 1985). This technique had limited success determining the minerals Ca, P, K, and Mg in dried forages (Clark et al., 1987), although with local calibrations, the N, C, and P concentrations of pine needles, *Pinus halepensis*, were accurately predicted (Gillon et al., 1999). These relationships probably indirectly estimate the inorganic nutrients since NIR spectra only responds to rotational and vibrational energies of hydrogen and not inorganic elements. Spectral reflectance changes in growing soybean (*Glycine max* L.) plants included higher reflectance in the green and yellow portions of the electromagnetic spectra for P deficient plants and a shift of the red edge of the chlorophyll absorption band near 680 nm (Milton et al., 1991). Reflectance measurements as a ratio of the green/red percentage and the blue/yellow percentage of a color provided good prediction of N, P, Mg, and Fe status of corn plants (Graeff et al., 2001), although as a diagnostic tool the authors concluded that the relationships between spectral properties, nutrient concentration, and structural changes in the plant tissue are poorly understood.

Field studies are available that describe the use of remote sensing spectra technology to evaluate N deficiencies of growing plants (Blackmer et al., 1994; Filella et al., 1995; Sembiring et al., 1998a, 1998b; Stone et al., 1996; Osborne et al., 2002). Limited studies determined P in growing plants (Osborne et al., 2002; Sembiring et al., 1998a; 1998b). Osborne et al. (2002) evaluated different wavelengths and/or combinations of wavelengths to indicate P deficiency in field grown corn. Spectral radiance measurements were taken at various growth stages in increments from 350 to 1000 nm and correlated with plant N and P concentration, plant biomass, grain N and P concentration, and grain yield. Reflectance in the NIR (730 and 930 nm) and blue regions (440 and 445 nm) predicted early season P stress between growth stages V6 and V8. Late season detection of P stress was not achieved. Grain yield was estimated by reflectance in the NIR region, with the particular wavelength of importance changing with growth stage.

In Sembiring et al. (1998a, 1998b), a wide range of spectral radiance measurements were obtained from field plots, including bands, combination indices, and correlated to forage biomass, and N and P uptake and concentrations. In general, biomass, and N and P uptake could be predicted for bermudagrass (*Cynodon*

dactyon (L.) Pers.) and winter wheat, but no index or combination of wavelengths or indices were related to plant P concentrations. Combinations of wavelengths with indices had better correlations with dependent agronomic variables than single wavelengths. The authors conclude that spectral radiance has the potential to be used for predicting N and P nutritional status but that additional studies are needed to evaluate environmental and sensor instrumentation variables.

EUTROPHICATION

It is beyond the scope of this chapter and book to fully describe the conditions under which nutrients affect the trophic state of water bodies. A wide range of methodologies are used to assess the trophic status of waters and the effects caused by changes to the loading regime (Edwards and Chambers, 2002; Newton and Jarrell, 1999). Factors that affect algal and plant abundance, and biomass accumulation in water are nutrient availabilities, light, substrate for attached plants, time for growth, temperature, grazing pressure, and physical suitability. Each can be controlled by additional factors and processes.

Both P and N or both are widely recognized as the nutrients that drive eutrophication of lakes, rivers, and coastal waters worldwide (Anderson et al., 2002; Correll, 1998; Edwards and Chambers, 2002; Litke, 1999; Smith et al., 1999). In general, P is considered the limiting nutrient for eutrophication in fresh waters (lakes, streams, rivers, reservoirs) while N is the limiting nutrient in coastal waterways. Estuaries are considered transition zones between fresh and brackish water. Lake productivity can be estimated by a simple model developed by Vollenweider (1976). This model predicts algal biomass from total P inputs per unit water surface area, mean water depth, and outflow per unit of water surface area. The idea of an absolute requirement for a minimum amount of P per algae cell was proposed by Droop (1977) and later expanded by Wynne and Rhee (1986).

Water samples for trophic status determination are usually analyzed for reactive and unreactive forms of N and P, and sometimes dissolved oxygen. Phosphorus only occurs in the pentavalent form in aquatic systems. Examples are orthophosphate, pyrophosphate, longer-chain polyphosphates, organic phosphate esters and phosphodiesters, and organic phosphonates. Phosphorus is delivered to aquatic systems as a mixture of dissolved and particulate inputs, each of which can be a complex mixture of different forms of pentavalent P.

It is often desirable to predict whether a water body will have excessive productivity based on the water column concentration of P (Dodds and Welch, 2000). Total P, including particulate P, is measured generally in the water column. Dissolved reactive P can be misleading because of the rapid turnover that can occur. There is also some concern that commonly used chemical and radiochemical techniques overestimate P concentration at low concentrations in lakes when compared with a steady-state radiobioassay (Hudson et al., 2000). Standard methods are available for P analysis in water and wastewater (APHA, 1995). Similar methods for P are found in a recent publication related to agricultural runoff (Pierzynski, 2000). Near-infrared reflectance spectroscopy has also been used to estimate

P in suspended particulate materials in water from lakes of varying trophic status (Malley et al., 1993, 1996).

CONCLUSIONS

Chemical analysis of plant parts is an established diagnostic tool for identifying the nutrient status of plants. A limitation of the traditional approach is that it is largely restricted to the examination of a single nutrient across a space and time continuum. Further progress will only be achieved when efforts are made to identify the more complex relationships and interactions between nutrients, environmental conditions, and soil parameters on plant growth. New and different remote sensor detection systems appear necessary before plant nutrient concentrations or deficiencies can be remotely detected routinely in agricultural crops. Precision agriculture has the potential to be a mechanism to help achieve this advancement.

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