Chapter 9
Evaluating Plant Potassium Status

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Abstract Several methods exist for evaluating plant nutritional status. Looking for visual deficiency symptoms is perhaps the simplest approach, but once symptoms appear, crop performance has already been compromised. Several other techniques have been developed. All of them require correlation studies to provide plant performance interpretations. Reflectance is a remote sensing technique that detects changes in light energy reflected by plant tissue. It has proven successful in detecting nutrient deficiencies but does not yet have the ability to discriminate among more than one deficiency. Chemical assays of leaf tissue, known as tissue tests, require destructive sampling but are the standard against which other assessments are compared. Sufficiency ranges provide concentrations of each nutrient that are considered adequate for crop growth and development. They consider nutrients in isolation. Other approaches have been developed to consider how the concentration of one nutrient in tissue impacts the concentrations of other nutrients. These approaches strive to develop guidelines for maintaining nutrient balance within the plant. All approaches require large data sets for interpretation.

Ideally, a diagnostic test, whether of the soil or plant, meets four requirements: (1) it is easy and inexpensive to perform; (2) it definitively identifies a potassium (K) deficiency; (3) it allows farmers time to respond; and (4) it leads to interventions with high probabilities of success. Soil tests have been widely used and have been useful for assessing plant-available K, but they do have diagnostic limitations. As Hall (1905) stated over 100 years ago:

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> One of the main problems placed before the agricultural chemist is the estimation of the requirements of a given soil for specific manures.... For various reasons the obvious method of determining the proportions of nitrogen, phosphoric acid, and potash in the soil fails in many cases to give the required information.... Hence from time to time attempts have been made to attack the problem from another side and to use the living plant as an analytical agent. The scheme is to take a particular plant grown upon the soil in question, and determine in its ash the proportions of constituents like phosphoric acid and potash. Any deviations from the normal in these proportions may then be taken as indicating deficiency or excess of the same constituent in the soil and therefore the need or otherwise for specific manuring in that direction.

Hall's statement elucidates the shortcomings of a soil test as the sole guide for K management and captures the rationale for adding plant measurements to the suite of nutritional assessments. This chapter provides an overview of the many "attempts...to use the living plant as an analytical agent."

9.1 Visual Symptoms of Potassium Deficiency

When K is deficient, several processes are impaired (Marschner 2002). Low K inhibits enzyme activation, making plants more susceptible to fungal attack. Impaired stomatal activity results in poor control over gas exchange, impairing photosynthesis and water control, making plants more susceptible to stresses from drought, frost, water uptake, and soil salinity. Low K also impairs proton (H^+) exchange across the thylakoid membranes in chloroplasts, resulting in worsening symptoms under higher light intensity (Marschner and Cakmak 1989). Transport of photosynthates can also be impaired, resulting in a buildup of sugars and a reduction in protein and starch synthesis, lowering the plant's dry weight. Impaired lignification of vascular bundles may result in weaker stalks and increased lodging. Potassium is an abundant cation (K^+) found in the cytoplasm, providing cell turgidity and rigidity by maintaining the osmotic potential. The K^+ concentration can be anywhere between 10 and 200 mM and in some cases as high as 500 mM in guard cells and pulvini of Fabaceae family member species. Lack of K results in reduced cell size and number, causing reduced growth and affecting nyctinasty in the Fabaceae family. Because K is mobile in the plant, it can be remobilized from older tissue to younger tissue when uptake from the soil is insufficient; therefore, visual symptoms generally occur first on older tissue, often the most recently matured.

Learning to recognize visual symptoms of deficiency requires training to become familiar with symptoms that can be crop specific (Table 9.1). Potassium deficiency may first appear as deep green plants with shorter and fewer internodes and smaller leaves, followed by the rapid development of necrotic spots along the margins and across leaf blades of recently matured leaves. In most cases, necrotic lesions begin without prior chlorotic lesions. In some cases, chlorosis develops in the tissue surrounding necrotic spots as the necrosis enlarges in advanced stages, or as necrosis is followed by chlorosis on recently matured and maturing leaves.

Table 9.1 Descriptions of visual symptoms of K deficiency for several horticultural crops

Crop	Description
Brassica oleracea L. (broccoli)	Internodes of maturing and recently matured leaves develop purplish concentric circles with green, normal-looking tissues in the center. Gradually, the undersides of these leaves' petioles shrivel, followed by the development of irregular sunken light green concentric circles with normal islands on tissues (Fig. 9.1a). The lesions progress further (Fig. 9.1b–d) with severe shriveling of petioles and the spreading of purplish pigmentation on the internodes. Eventually, necrosis develops across the entire lamina.
Cucumis sativus L. (cucumber)	In the early stages of appearance, the leaf area is reduced (Fig. 9.2a, b). As symptoms worsen, leaves appear deeper green (Fig. 9.2c), and lesions of grayish necrosis develop on recently matured leaves and on matured leaves below them (Fig. 9.2d).
Spinacia oleracea L. (spinach)	Lesions of light greenish, sunken, irregular necrotic spots develop on recently matured leaves (Fig. 9.3a). The lesions rapidly coalesce and progress to distinct whitish necrotic spots on interveinal tissue on the acropetal leaf area (Fig. 9.3b, c).
Cucurbita pepo L. (zucchini squash)	In the early stages, leaves may appear darker green (Fig. 9.4a). Necrotic spots of light whitish pin-head sized lesions develop on interveinal tissue across the leaf blades closer to the primary veins (Fig. 9.4b), which then enlarge to irregular sunken necrotic spots and eventually into large necrotic spots of 0.5–1.0 cm (Fig. 9.4c).
Lactuca sativa L. (romaine lettuce)	Lesions of dark grayish irregular sunken necrotic spots begin at the acropetal area of recently matured leaves (Fig. 9.5a). These necrotic lesions rapidly coalesce into large irregular necrotic patches randomly across the lamina (Fig. 9.5b, c).
Carica papaya L. (papaya)	Necrosis begins as greenish-gray patches just inside the leaflet margins of recently matured leaves and rapidly progresses to large necrotic areas and the tissue collapses along the terminal margins (Fig. 9.6a, b). As patches increase in size and number toward the leaf base, areas between the necrotic patches turn chlorotic and the leaves eventually abscise (Fig. 9.6c).
Solanum tuberosum L. (potato)	Plants initially appear deeper green and compact due to fewer nodes and shorter internodes (Fig. 9.7a). Further K deprivation results in minor puckering and crinkling of leaves, with slight cupping of recently matured leaves (Fig. 9.7b). Plant growth is reduced. These symptoms progress rapidly to severe puckering and reduced leaf size (Fig. 9.7c). Randomly spaced necrotic areas first appear on the abaxial leaflet surface and along the secondary and tertiary veins of outermost leaflets of recently matured leaves. The symptoms gradually progress to the adjacent leaflets and then to the remaining leaflets. As the deficiency becomes severe, dark necrotic spots develop on the adaxial surface of maturing leaves and necrotic areas enlarge.
Arachis hypogaea L. (Peanut)	Recently matured/matured leaves initially appear slightly darker green (Fig. 9.8a). As the K deprivation extends, lesions of light grayish irregular sunken necrotic spots begin to develop across the lamina (Fig. 9.8b). The lesions rapidly progress to distinct brownish necrotic spots surrounded by chlorotic tissues (Fig. 9.8c).

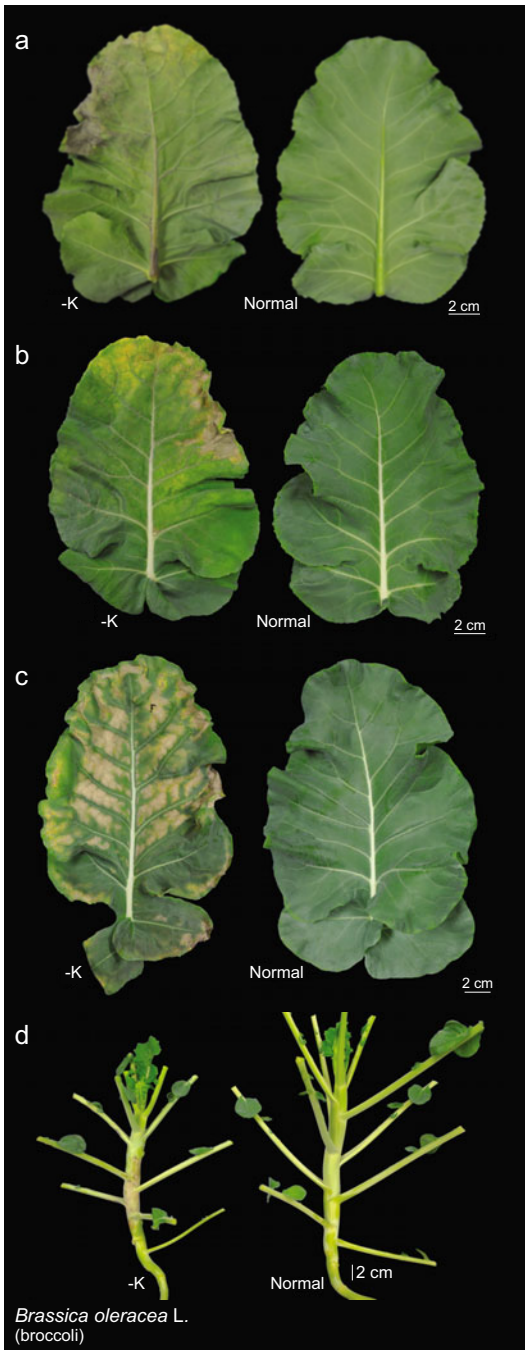

Fig. 9.1 Potassium deficiency symptoms exhibited on *Brassica oleracea* L. (broccoli). For a description, see Table 9.1

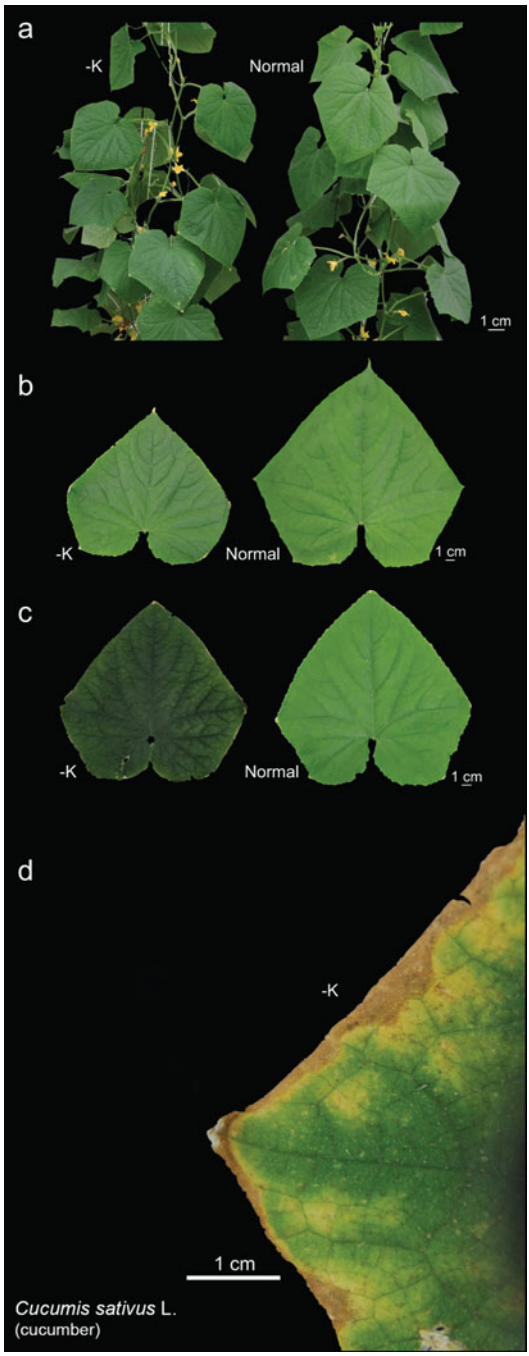

Fig. 9.2 Potassium deficiency symptoms exhibited on *Cucumis sativus* L. (cucumber). For a description, see Table 9.1

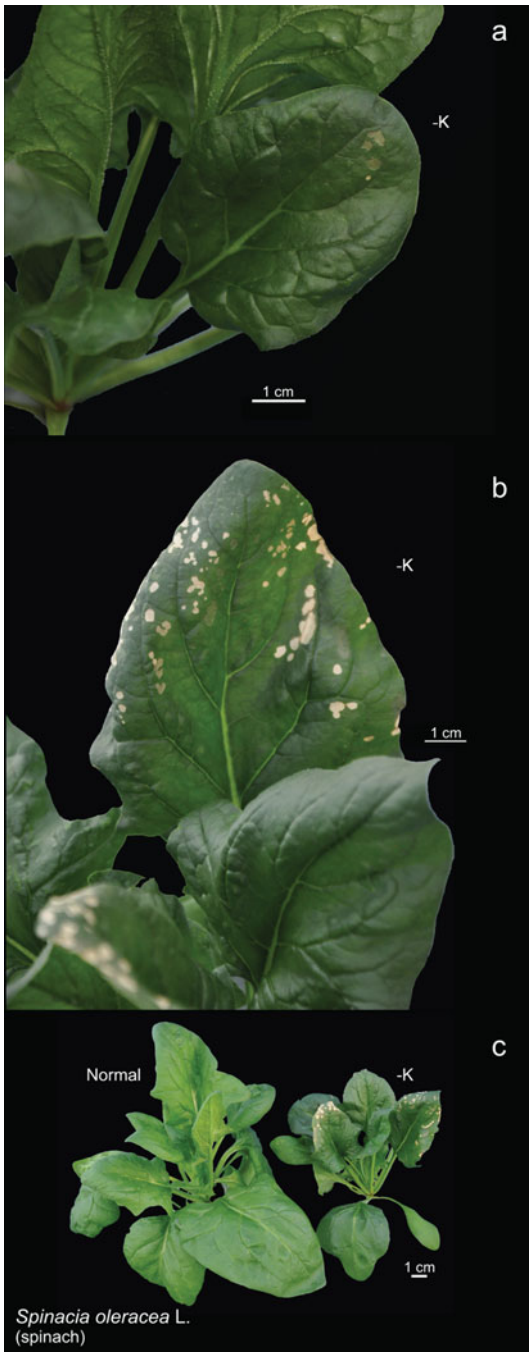

Fig. 9.3 Potassium deficiency symptoms exhibited on *Spinacia oleracea* L. (spinach). For a description, see Table 9.1

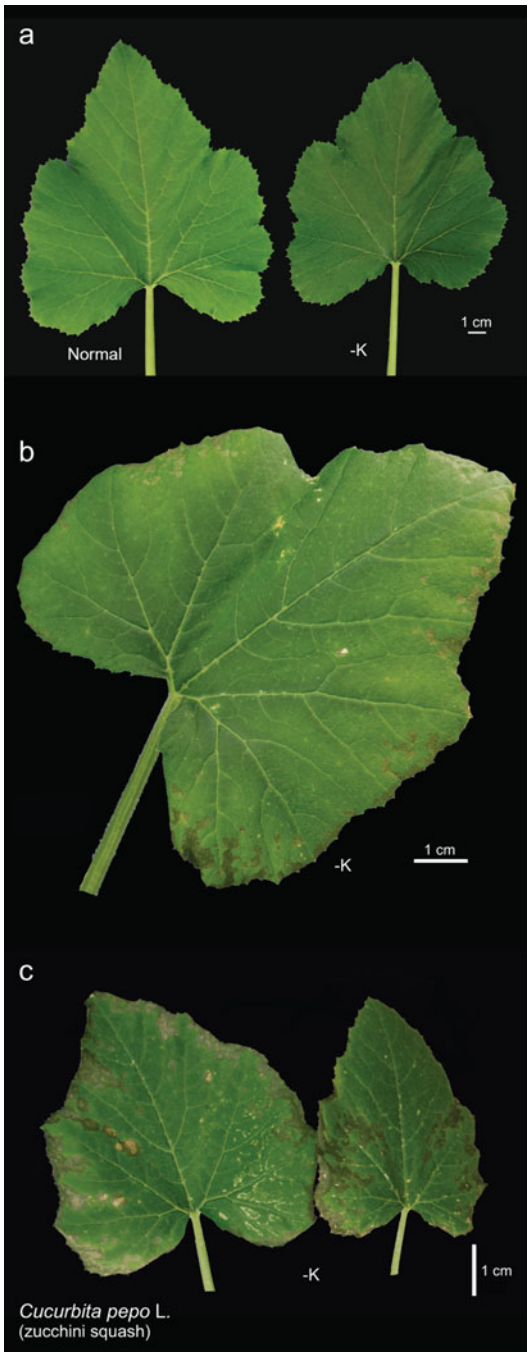

Fig. 9.4 Potassium deficiency symptoms exhibited on *Cucurbita pepo* L. (zucchini squash). For a description, see Table 9.1

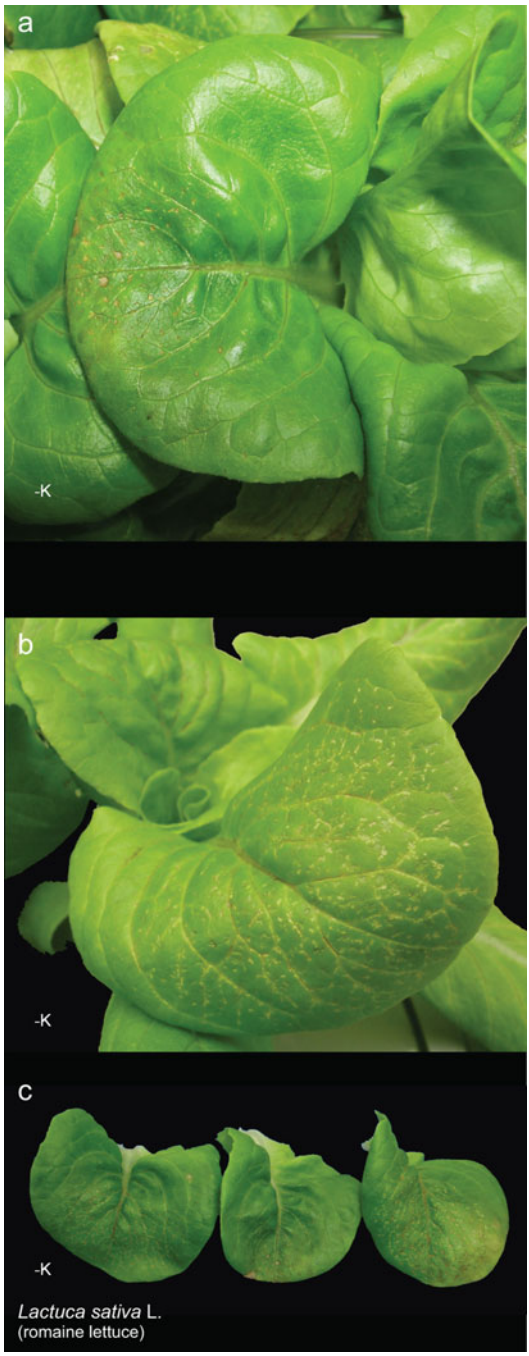

Fig. 9.5 Potassium deficiency symptoms exhibited on *Lactuca sativa* L. (romaine lettuce). For a description, see Table 9.1

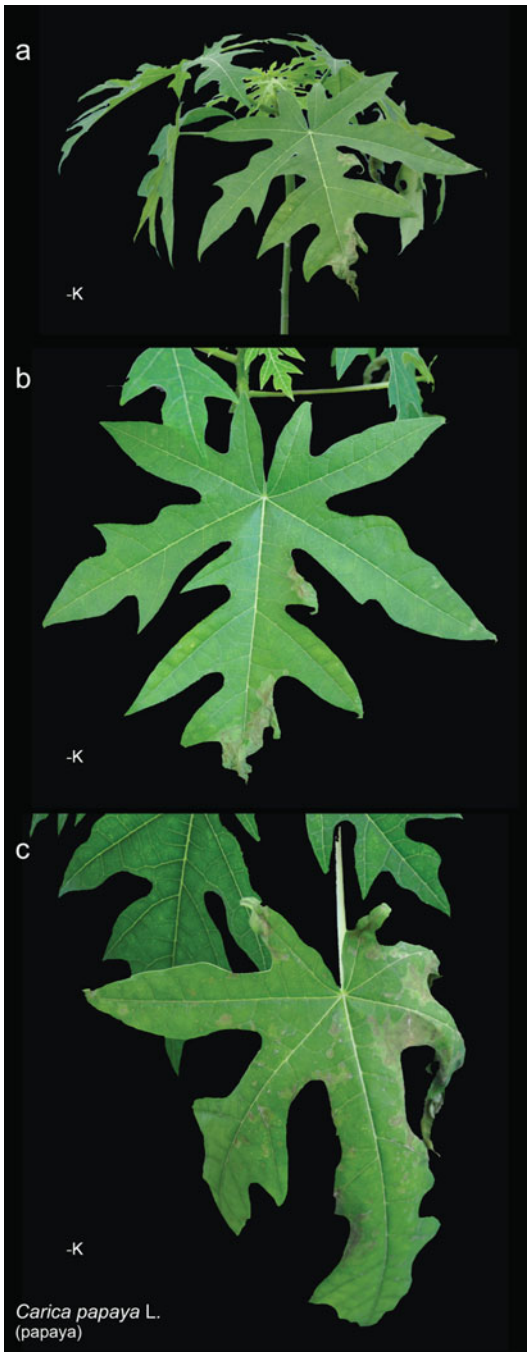

Fig. 9.6 Potassium deficiency symptoms exhibited on *Carica papaya* L. (papaya). For a description, see Table 9.1

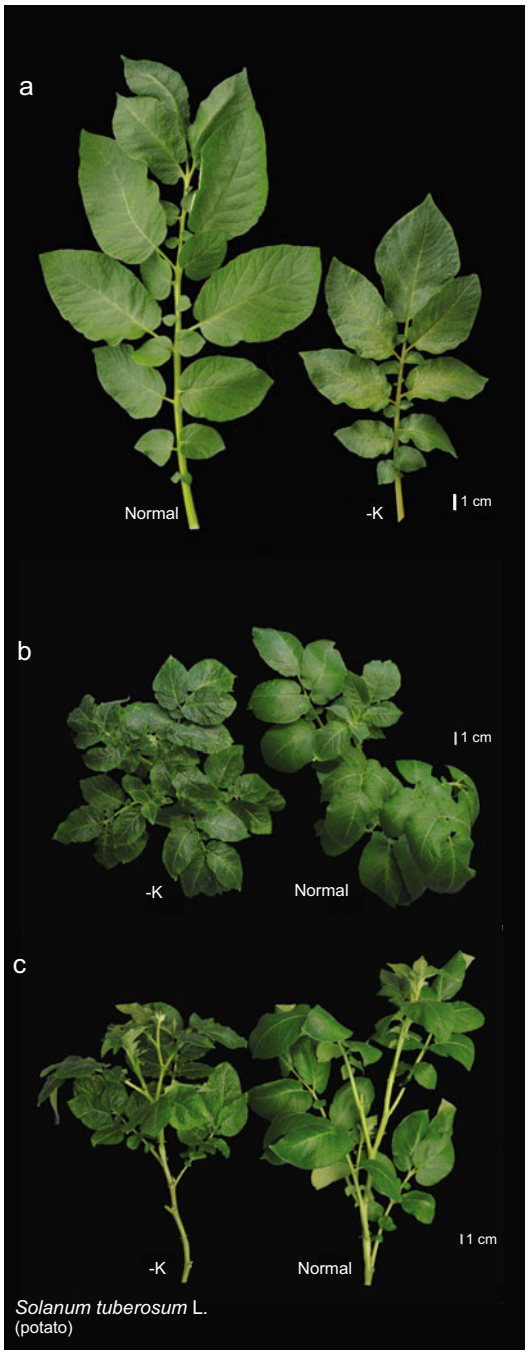

Fig. 9.7 Potassium deficiency symptoms exhibited on *Solanum tuberosum* L. (potato). For a description, see Table 9.1

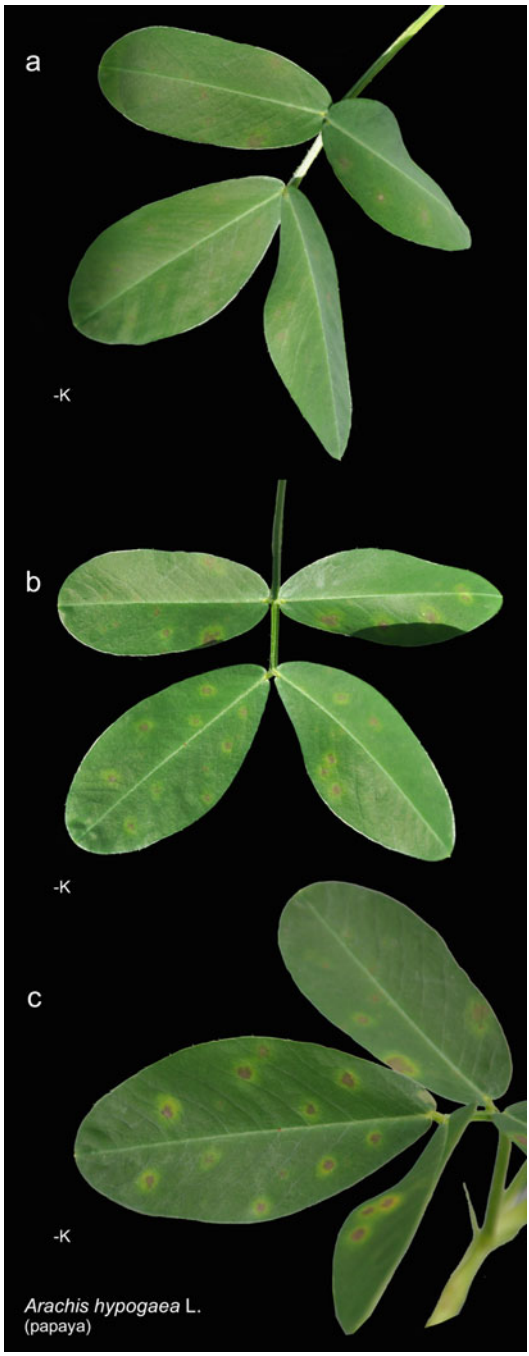

Fig. 9.8 Potassium deficiency symptoms exhibited on *Arachis hypogaea* L. (peanut). For a description, see Table 9.1

Nutrient deficiency symptoms can be used as a rapid diagnostic tool for identifying factors that might limit crop yield and quality. Deficiency symptoms are not the ideal approach for dealing with nutritional shortages, because by the time symptoms are visible, plant productivity has already been impaired by the period of malnutrition preceding the appearance of symptoms, a condition called "hidden hunger."

9.2 Light Reflectance

Potassium deficiency can cause changes in both individual plant organs and, collectively, the crop canopy. When leaf tissue becomes chlorotic or necrotic, it no longer reflects light the same way it did when it was healthy. Visibly, leaves change from green to yellow or brown, but changes also occur outside the visible spectrum, particularly at the nexus of visible and infrared wavelengths.

When incident energy hits the surface of a leaf or other plant organ, it is either absorbed, reflected, or transmitted through the tissue. Several types of detectors can measure, over a range of wavelengths, the energy not absorbed by the plant. Under controlled conditions, positioning both the light source and the energy detector on the same side of the plant tissue measures reflected energy. Positioning the light source behind the tissue and the detector in front of the tissue measures transmitted energy. In spectral analysis, the reflectance is the reflected energy expressed as a percentage of the incoming energy. To calculate this percentage, the energy of the light source must be known. Some instruments provide their own light source of known energy. Instruments without a light source require reference materials with a known reflectance, such as a white panel (Albayrak et al. 2011). A higher reflectance means a lower energy absorbance. Because energy measurements do not require contact with plant tissue, they are considered a type of "remote sensing." For agricultural uses, energy detectors have been mounted on tractors, airplanes, unmanned aerial vehicles, and satellites.

Figure 9.9 is an example of a reflectance spectrum. Reflectance (%) is plotted over a range of wavelengths (nm). Reflectance in the visible spectrum, 400–700 nm, is less than reflectance in the infrared spectrum (700 nm–1 mm). Chlorophylls, carotenoids, and anthocyanins absorb light in the visible spectrum (Knipling 1970), and combined they absorb more energy in the blue and red wavelengths and less in the green wavelengths (Shull 1929). The longer wavelength infrared light is not absorbed by the aforementioned phytochemicals, resulting in a rapid increase in reflectance at the end of the visible and the beginning of the infrared spectrum, termed the "red edge" (Horler et al. 1983). Farther into the longer wavelength range of the infrared spectrum (not shown in Fig. 9.9) several compounds in the plant absorb energy. Among these are protein, oil, starch, lignin, cellulose, water, and sugar (Curran 1989). Additionally, water absorbs energy across both visible and infrared spectra.

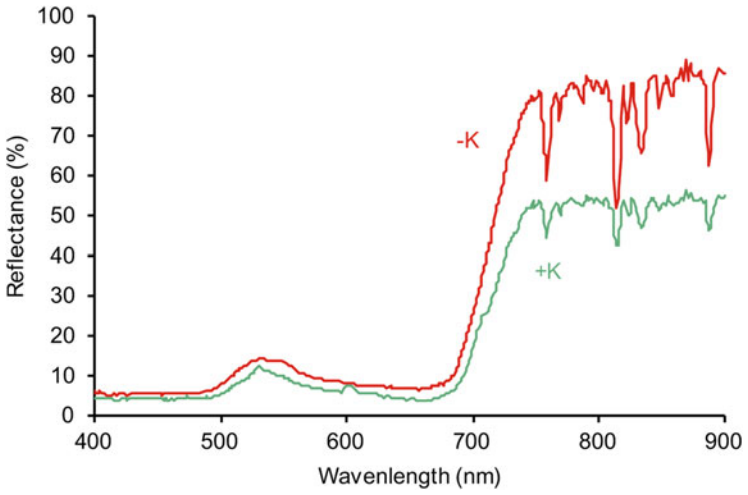

Fig. 9.9 Reflectance spectra for wheat (*Triticum aestivum* L.) without K (−K) and with K (+K), and all other nutrients at sufficient levels. (adapted from Ayala-Silva and Beyl 2005)

A red edge at lower wavelength is associated with K deficiency. Figure 9.9 shows two spectra for wheat (*Triticum aestivum* L.), one where K was added to the nutrient solution (+K) and the other where K was omitted (−K) (Ayala-Silva and Beyl 2005). The K deficiency resulted in a red edge at shorter wavelengths and increased reflectance in the visible range (400–700 nm). When tissue yellows or becomes necrotic due to nutritional deficiencies or other damage, less chlorophyll is available to absorb energy in the visible spectrum, and reflectance increases (Shull 1929). Also, when tissue dries, it typically reflects more energy (Woolley 1971). A confounding factor in analyzing shifts in the red edge is that as plant tissue ages, the red edge shifts to longer wavelengths (Horler et al. 1983; Milton et al. 1991). Therefore, a red edge occurring at shorter wavelengths under K deficiency could be interpreted as an "...inhibition of the normal shift to longer wavelengths" (Milton et al. 1991).

Spectral analysis usually involves creating indexes that are diagnostic of plant nutritional status. Commonly used metrics of nutritional status are K concentration in dry tissue [g K (kg dry matter)$^{-1}$] and total K accumulation in plant biomass (kg K ha^{-1}). Indexes are created from the combinations of wavelength reflectance measurements that correlate most strongly with K concentration or K accumulation. In remote sensing applications, spectra are the result not only of the absorption of light by the crop canopy but also absorption by water and other compounds in the atmosphere and in the soil not covered by the canopy.

Vegetative indexes are calculated by a normalization technique that uses ratios of wavelength combinations. The normalized difference vegetation index (NDVI) is a commonly used ratio. It is a combination of reflectance in the near-infrared spectrum (R_{NIR}) and reflectance in the red region (R_{red}). Specifically, NDVI = ($R_{NIR} - R_{red}$)/

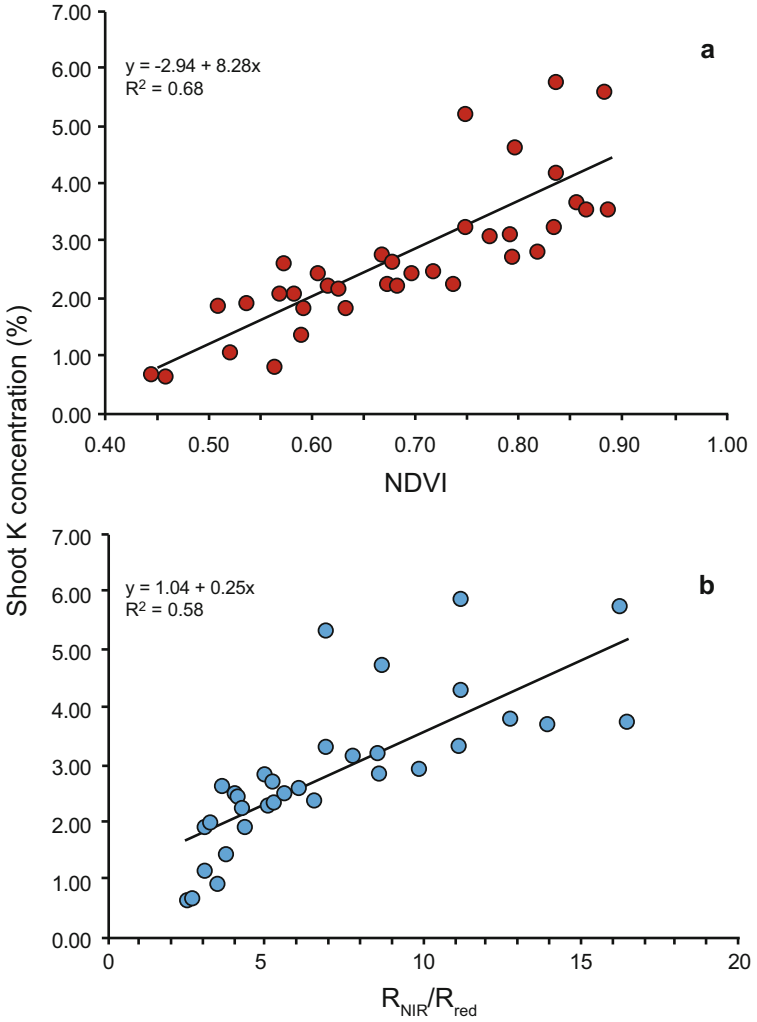

Fig. 9.10 Correlation of *Vicia villosa* Roth (hairy vetch or winter vetch) shoot tissue K concentration with (**a**) the normalized difference vegetative index (NDVI) and (**b**) the ratio of near-infrared reflectance to reflectance in the red region (R_{NIR}/R_{red}). (adapted from Albayrak et al. 2011)

($R_{NIR} + R_{red}$) (Tarr et al. 2005). Another often used index is the simple ratio R_{NIR}/R_{red} (Birth and McVey 1968).

For example, Albayrak et al. (2011) correlated NDVI and the simple ratio to leaf K concentrations of shoot tissues of three vetch (*Vicia*) species. Figure 9.10 shows the correlations with both indexes for *Vicia villosa* Roth, which was representative of the correlations of the other two *Vicia* species. The NDVI correlation was somewhat better than that of the simple ratio. Many other vegetative indices have been tested using tissue K concentration: green-to-red and near-infrared-to-green ratios (Gómez-

Casero et al. 2007); green normalized difference vegetation index (GNDVI), soil adjusted vegetation index (SAVI), optimized soil adjusted vegetation index (OSAVI), N_870_1450 and N_1645_1715 (Mahajan et al. 2014; Pimstein et al. 2011); P_1080_1460, S_660_1260, and S_660_1080 (Mahajan et al. 2014); vegetation vigor index (VVI) (Noori and Panda 2016); the GM Index, the Vogelmann Index (VOG), the Green Leaf Index (GLI), the normalized difference index (NDI), and a simple ratio RI (Anderson et al. 2016); and the re-normalized difference vegetation index (RDVI) (Guo et al. 2017). Other normalization procedures are continuum removal and band depth (BD) normalization (Kokaly and Clark 1999); continuum-removed derivative reflectance (CRDR), the normalized band depth ratio (BDR), and the normalized band depth index (NBDI) (Mutanga et al. 2004).

Statistical techniques are commonly used to create multivariate models of wavelengths that account for the most variation in either tissue K concentration or total K accumulation. The approach is to develop a statistical model with one set of data, termed a calibration or training data set, then test how well that model predicts either K concentration or total K accumulation in another data set, termed the validation, prediction, or test data set (Geladi and Kowalski 1986). The most widely used statistical technique has been stepwise regression; however, uninformed use of this approach can lead to overfitting, making a model "...unlikely to be replicated if the experiment is repeated" (Mutanga et al. 2004). Another criticism of stepwise regression has been that the wavelengths most highly correlated with plant chemical content may not relate to known absorption features of those chemicals (Curran et al. 1992). To address concerns of overfitting, researchers are turning to other techniques to build models, such as partial least squares (PLS) regression (Geladi and Kowalski 1986), kernel partial least squares regression (KPLSR), and support vector regression (SVR) (Pullanagari et al. 2016).

The major difficulty that has yet to be overcome is the inability of reflectance to discriminate among more than one deficient element. Predictive models have been built for individual nutrients, but not for combinations of nutrients. Fridgen and Varco (2004) conducted a study with a factorial combination of nitrogen (N) and K fertilizer application rates applied to cotton (*Gossypium hirsutum* L.). They built a predictive model for leaf N content using partial least squares regression. When K was limiting, the model performed poorly, as measured by the correlation between predicted and observed N content ($r^2 = 0.06$); however, when K was adequate, the model performed markedly better ($r^2 = 0.70$). From their experiment examining several isolated nutrient deficiencies, Pacumbaba and Beyl (2011) concluded that spectral analysis could detect nutrient stress but was not able yet to identify which nutrient was causing the stress.

An additional consideration is when to take reflectance measurements. Early detection of K deficiency is the goal. As discussed in the previous section, K deficiency can progress rapidly from "hidden hunger" to complete tissue necrosis. Creating diagnostic interpretations of reflectance spectra needs to consider the dynamic nature of K deficiency during the plant's lifecycle.

9.3 Plant Tissue Chemical Content

The most widely adopted determinations of plant tissue K content are those performed in the laboratory on tissue samples collected from the field. Farmers or consultants gather samples of specific tissue at specific growth stages (Jones 1998). After the laboratory receives the samples, technicians dry them, typically in a forced-air oven, then grind them to reduce particle size. A small subsample of the ground material is weighed out and digested in an acid solution (Campbell and Plank 1998; Hanlon 1998). The quantity of K in an aliquot of the digestate is determined using either an atomic absorption spectrophotometer (Hanlon 1998) or an inductively coupled plasma atomic emission spectrometer (Isaac and Johnson 1998). Although laboratory determination of K content is the scientific standard, it is not available everywhere, since it requires capital to set up the laboratory, an appropriate business plan to sustain laboratory operations, trained personnel, well-developed infrastructure to transport samples and maintain equipment, and quality assurance and quality control procedures to ensure results are accurate and precise.

For meaningful interpretation, tissue test results must be correlated to crop performance. The stronger the correlation, the more useful tissue analyses become as a diagnostic tool. Yield has traditionally been the performance metric of universal interest; however, crop quality and plant health can be just as important, depending on the crop and the requirements for marketable yield. The objective of correlations is to identify test levels associated with K sufficiency and balanced nutrition.

Balance considers concentrations of other nutrients in plant tissues. The tissue concentration of K is influenced by the supply of other cations such as ammonium, magnesium (Mg), calcium (Ca), iron, manganese, copper, and zinc. The growing environment also plays an important role in the determination of the critical level of K, since tissue K concentrations are influenced by factors such as incident radiation, light intensity, air and soil temperature, soil moisture, etc. The demand for K also changes during the season, as discussed in Chap. 1, with increases in many species during the reproductive stage, especially during flowering and fruiting. The following sections discuss approaches that have been developed to create diagnostic criteria.

9.3.1 Sufficiency Ranges (SR)

Macy (1936) formulated many of the concepts used today for interpreting tissue test correlations. He assembled data from studies that added or subtracted a given nutrient from the plant-available supply and measured changes in biomass production and nutrient concentration in plant tissue. He defined yield response as the decrease in any given yield from the maximum yield. He plotted those yield decreases against the associated tissue nutrient concentrations to create correlations like the one in Fig. 9.11. His approach considered one nutrient at a time and assumed levels of other nutrients were adequate. He defined “poverty adjustment” as the

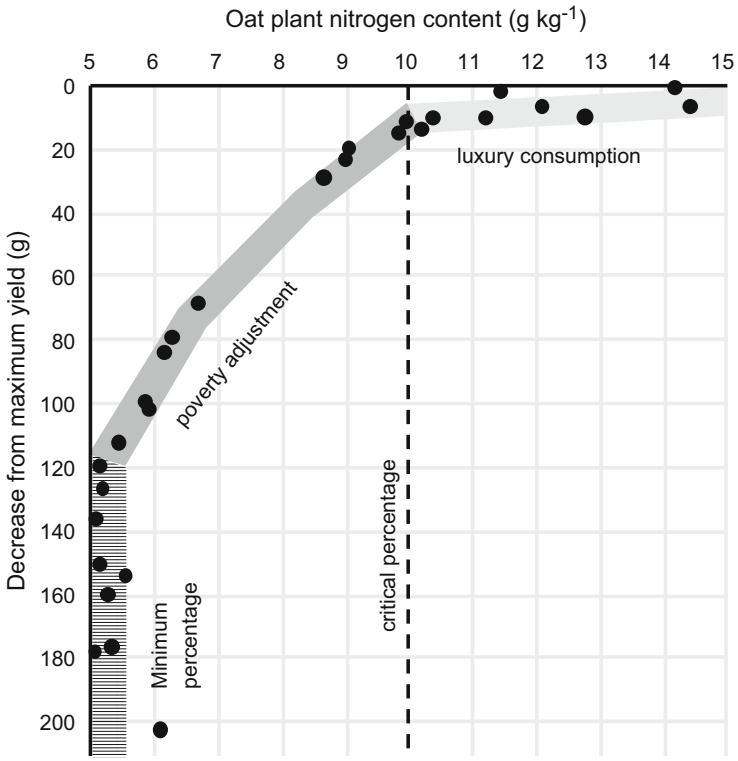

Fig. 9.11 Interpretation of oat (*Avena sativa* L.) tissue test correlation data into three categories: minimum percentage, poverty adjustment, and luxury consumption, the latter two divided by the critical percentage. (Macy 1936)

range where biomass yield climbed toward maximum yield as tissue nutrient concentration increased. At higher nutrient concentrations, biomass no longer increased, a range he termed "luxury consumption." He then defined the "critical percentage" as the tissue concentration dividing poverty adjustment from luxury consumption. He also observed that a range of the largest yield decreases occurred within a relatively narrow range of low nutrient concentrations. He termed this range "minimum percentage." As Macy's concept was adopted by others, relative yield or actual yield was used as the dependent variable rather than the decrease from maximum yield. Relative yield is the ratio of observed yield to maximum yield. Research has been going on for decades on numerous crops to develop correlations between tissue concentrations and crop yields, and studies are periodically summarized in reviews such as Hardy et al. (1967) and Westerman (1990). While correlations of yield or relative yield with tissue concentrations provide the basis for diagnosing a K deficiency, they do not indicate how much K needs to be applied to rectify the deficiency. Calibration experiments, like those discussed in Chap. 1, provide that kind of information.

The selection of models fit to correlation data influences the determination of critical level or range. As an example, we compare models fit to data correlating maize (*Zea mays* L.) yield to K concentration in the leaf blade opposite and below the ear, sometimes called the "sixth leaf" or the "ear leaf." Tyner (1947) modeled his ear leaf correlation data set using actual yields and a linear model. His data did not have a clear "flex point" where yield no longer increased with K concentration (no luxury consumption). He set the critical concentration at the concentration where only two higher yielding observations at lower concentrations fell outside the standard error interval. Stammer and Mallarino (2018), correlating leaf concentrations to maize and soybean (*Glycine max* (L.) Merr.) relative yields, defined a critical range, rather than a single level, from two different least squares statistical models fit to the same data. The inflection of the linear-plateau model set the lower limit of the range and the plateau of the quadratic-plateau model set the upper limit. In addition to those already mentioned, other statistical models commonly used in nutrient response or correlation studies are: linear segmented models (Anderson and Nelson 1975); quadratic, exponential, and square root functions (Cerrato and Blackmer 1990; Mombiela et al. 1981); and the "Cate-Nelson" procedure (Cate and Nelson 1971).

Empirical data comprising tissue test correlations are gathered under specific conditions. When conditions change, interpretations may also change. For instance, Kovács and Vyn (2017) correlated yields and ear leaf K concentrations of modern maize hybrids and found that the lower limit of the sufficiency range in existing interpretations was too low. Their more recently collected data revealed that a higher range in tissue K concentrations needed to be recommended to farmers. This example demonstrates that tissue test correlations must be reevaluated when current practices or growing conditions differ from the historical ones upon which the recommendations were based. Unfortunately, funds for conducting such research are usually sparse and only sporadically available. Unmet funding needs have limited scientists' ability to update data sets in a timely manner, resulting in interpretations and recommendations that may reflect older cropping practices, genetics, and climatic conditions.

Tissue K concentration is affected by factors other than the quantity of plant-available K in soil. Over 100 years ago, Hall (1905) noted that environmental factors can alter nutrient concentrations as much as variations in the K content of the soil. To quiet the noise from other factors, Mallarino and Hagashi (2008) used relative rather than absolute tissue concentrations. In their field experiments, they calculated relative concentrations for a given season at a given site (site-year) by dividing tissue K concentrations from unfertilized plots by the tissue K concentration of the highest treatment mean. They then aggregated data across all site-years and examined the relationship between soil test level and either absolute or relative tissue concentration. Relative tissue concentrations were better related to changes in soil test levels than absolute concentrations; however, they did not examine the relationship between relative concentration and relative yield, likely because only small yield responses to K fertilization occurred in the study.

Table 9.2 Interpretations of leaf K concentrations of mature Valencia and navel orange (*Citrus sinensis* (L.) Osbeck) leaves. (Embleton et al. 1978)

	Leaf K concentration
Interpretation	g K (kg dry matter)$^{-1}$
Deficient	<4.0
Low	4.0–6.9
Optimum	7.0–10.9
High	11.0–20.0
Excess	>23.0

Although correlation studies are essential, they do not completely define the process used to develop diagnostic criteria for tissue tests. To quote Melsted et al. (1969):

> Critical plant composition values can seldom be derived through a single carefully designed experiment. Rather they evolve through hundreds of fertility trials and the resulting thousands of plant analyses. Slight variations in plant composition may result through differences in individual plants, in plant varieties, in seasonal changes, in nutrient levels, in nutrient ratios, and in various other factors. There is no good way to evaluate or balance some of these differences except through personal judgement.

To elucidate the process of deriving tissue test interpretations for production settings, we explore an example from the citrus industry in California. We begin with the current recommendations. Embleton et al. (1978) published the interpretations for leaf tissue K concentration reproduced in Table 9.2 which are still used today. Farmers and consultants compare their own test results with those in the table to assess the K status of their orange trees. The interpretations are valid for specific plant tissue: 5–7-month-old, spring cycle, terminal leaves from nonfruiting, and nonflushing shoots that are 0.9–1.5 m above the ground. These interpretations apply to orange (*Citrus sinensis* (L.) Osbeck), grapefruit (*Citrus paradisi* Macfad.), and lemon (*Citrus limon* (L.) Osbeck). To reach the desired number of fruit per tree, farmers manage fertilizer K to keep tissue tests in the optimum range (7.0–10.9 g kg^{-1}). To attain the desired fruit size and quality, a higher concentration in the range of 10.0–12 g kg^{-1} is recommended. Although not shown in Table 9.2, ranges are provided for other nutrients too. The presentation of interpretation ranges for individual nutrients with associated descriptions of tissue at a given range of positions on the plant and an associated age range is representative of guidelines in use for many crops and nutrients (Mills and Jones 1996; Shear and Faust 2011; Uchida 2000). Commonly, the optimum range is called the "sufficiency range." Baldock and Schulte (1996) classified sufficiency ranges as an independent nutrient index (INI), because they represent the sufficiency of one nutrient independently of other nutrients.

The tissue K interpretations in Table 9.2 are a result of a long period of work. We explore the process followed to create the entries in that table. Doing so reveals the many steps required to create tissue test interpretations.

Southern California farmers' desire for evidence-based management practices for oranges sparked the long process of creating tissue test interpretations. The Citrus Experiment Station was established at Riverside, California in 1907. Field trials

were also started that year. Research summaries published in 1922 (Vaile 1922) and 1942 (Parker and Batchelor 1942) concluded that N was the limiting nutrient for Valencia and navel oranges, not K; however, Chapman and Brown (1943) stated that despite the lack of experimental evidence, many farmers continued to believe that K was necessary to sustain fruit yield and quality.

Chapman and his colleagues began investigating K nutrition more closely. Their initial work focused on characterizing the effects of K deficiency, sufficiency, and excess on the growth and fruit production of Valencia and navel orange trees (Chapman and Brown 1943; Chapman et al. 1947). To do this, they initially conducted greenhouse studies using nutrient solutions with and without K to compare differences in leaf tissue concentrations as well as visual deficiency symptoms. They also conducted "controlled-culture experiments" on 4-year old trees growing in large (700 L) pots outdoors under ambient conditions and fed with nutrient solutions with varying levels of K (3–7 ppm K, 30–40 ppm K, and 300–400 ppm K) (Chapman et al. 1947). They noted that in the early stages of K deficiency, trees showed only faint symptoms and set fruit normally, but at harvest, their fruit was much smaller. Although the K-deficient trees did not exhibit clear visual symptoms, the K concentrations in their leaves were much lower than those where K was sufficient. Driven by their concern that "incipient" K deficiencies likely went unnoticed in commercial orchards (termed "hidden hunger" earlier in this chapter), Chapman and Brown (1950) set up a series of investigations to determine if the K concentration in leaves could serve as a diagnostic test.

Although they had been analyzing leaves in their work, Chapman and Brown (1950) were not sure leaves were the best tissue for diagnosing tree nutrient status. They took samples from a long-term experiment where navel orange trees had been growing for over 13 years on differentially fertilized plots. Given the long time they had to respond to differences in fertility, trees on these plots were the most likely to exhibit clear differences in tissue K concentration. Unfortunately, the effects of K could not be isolated, since S was applied with K. They sampled: leaves; small, "pencil-sized" terminal shoots (twigs); blossoms; immature fruit; and mature fruit. An excerpt from their results is in Table 9.3. Comparing the pair of treatments without K (N and N + P) to treatment with K (N + P + K + S and manure) showed that leaves were the plant organs most sensitive to changes in K nutritional status. In a separate study, Chapman and Brown (1950) also tested petioles and found they offered no advantage over leaves. Foundational work of others on a variety of crops, summarized by Ulrich (1952), showed that nutrient concentrations in vegetative organs of plants were often more sensitive to changes in soil nutrient supply than those in fruits, seeds, or tubers.

Chapman and Brown (1950) investigated the best time to sample leaf tissue. Under southern California conditions, orange trees, which are evergreen, have three cycles of new leaf growth (flushes) during the following approximate times of the year: April (first or spring flush), June (second flush), and August through September (third flush). Experiments examining the time of sampling revealed that K concentrations generally decreased in leaves as they aged, a phenomenon noted by many other researchers working on many other crops, for example, Thomas (1937)

Table 9.3 Tissue K concentrations in various organs of navel orange trees (*Citrus sinensis* (L.) Osbeck) growing for over 13 years on differentially fertilized plots in a long-term experiment. (Chapman and Brown 1950)

	Twig bark	Twig wood	Leaves	Blossoms	Immature fruit	Mature fruit
Treatment[a]	g K (kg dry matter)$^{-1}$				g K (kg fresh weight)$^{-1}$	
N	3.9	1.2	6.5	12.9	2.5	1.4
N + P	3.3	1.5	6.8	12.9	2.4	1.4
N + P + K + S	2.1	1.1	10.7	12.6	2.6	1.5
Manure	3.8	2.0	13.2	15.5	3.1	1.4

[a]N = nitrogen applied as urea; P = phosphorus applied as triple superphosphate; K + S = potassium and sulfur co-applied as potassium sulfate; manure = dairy manure

studying potato leaves, Bell et al. (1987) examining soybean leaves, and Xue et al. (2016) analyzing rice (*Oryza sativa* L.) leaves. Using data from the "controlled-culture experiments," Chapman and Brown (1950) determined that K concentrations in 12-month-old leaves taken from shoots that had been spring flush, fruit-bearing terminal shoots the previous year had the lowest K concentration (Fig. 9.12). They posited that by 12 months, some of the K in these older leaves had translocated to newly developing tissue. The K concentration in the older leaves had the clearest separation between deficient, slightly deficient, and ample K nutrition; however, accurately identifying these leaves in commercial orchards was questionable. Also, sampling at this leaf age was limited to a short period in the spring. Both of these practical limitations led to recommendations for sampling younger leaves, 4–7 months old, located immediately behind the fruit on spring flush, terminal fruiting shoots (Chapman 1949, 1960; Chapman and Brown 1950; Embleton et al. 1962). These leaves were more readily identifiable and could be sampled over a several-month period, giving them practical value as a diagnostic test.

Chapman and Brown (1950) conducted additional studies to provide further details on sampling leaves. They examined how high to sample on the tree and found that leaf K concentrations at the top (3.0–4.9 m) were slightly lower than other positions. Samples taken at 1.5–3.0 m were no different than those at 0.6–1.5 m. The lowest height was easiest to reach and that height has carried through to the current recommendation of 0.9–1.5 m. Other investigations by Chapman and Brown (1950) showed that leaf K concentrations were relatively constant on various sides of the tree. Large and small leaves were also similar in concentration. Chapman and Brown (1950) summarized their own as well as other scientists' data, including international data sets, and published the first set of interpretations.

In later work, Jones et al. (1955) and Embleton et al. (1962) found that sampling the youngest fully expanded leaf from nonfruiting, rather than fruiting, terminal shoots resulted in more reliable interpretations. The current recommendation in California is to sample 5–7-month-old, spring cycle, terminal leaves from nonfruiting, and nonflushing shoots (Embleton et al. 1978). That approach has proven so reliable that it is used by the citrus industry worldwide.

In developing sufficiency ranges, both Chapman and Embleton gathered data from farmers' orchards. They surveyed leaf concentrations in orchards of

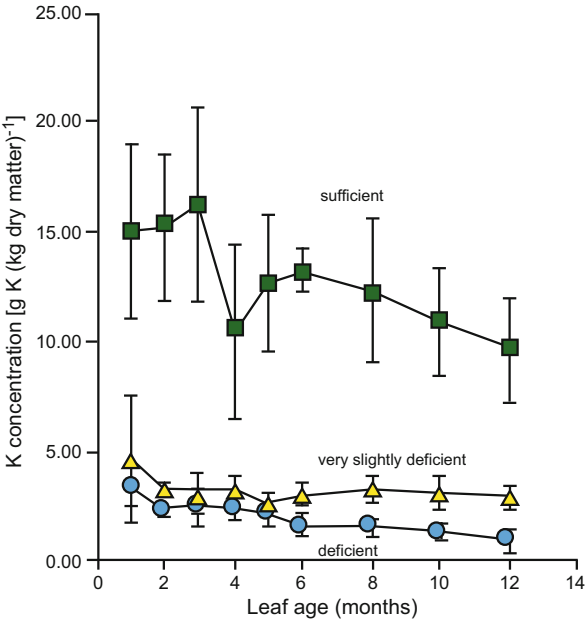

Fig. 9.12 Temporal changes in Valencia and navel orange (*Citrus sinensis* (L.) Osbeck) leaves taken from spring flush, fruit-bearing terminals, based on data in Table 6, Chapman and Brown (1950), and experiment descriptions in Chapman et al. (1947). Error bars are ±2 standard deviations

top-producing farmers. Additionally, Embleton worked with growers whose orchards were deficient in one or more nutrients and established fertilizer rate trials there. Stemming from such practical research, Embleton et al. (1974) stated that one of the reasons for setting 7.0 g kg^{-1} as the threshold between "low" and "optimum" was their experience that it was difficult to increase leaf K concentration once it fell below this level. Also, Embleton et al. (1978) looked beyond the number of fruit per tree and examined other quality parameters, such as fruit size and quality. On-farm research, on-farm surveys, on-farm experience, and goals for not only yield but also crop quality helped define the interpretations used today.

An important observation by Chapman and Brown (1950) was that K concentrations in leaves were influenced by the concentrations of other nutrients and vice versa. Table 9.4 shows that when K was deficient, the leaf concentrations of N, Ca, and Mg increased. Additionally, when the other listed nutrients were deficient, the concentration of K in leaves increased.

The implication of the findings in Table 9.4 is that accurate interpretations of adequate and higher K concentrations depend upon knowing the concentrations of other nutrients. Higher K concentrations considered in isolation could be misinterpreted as sufficient when other nutrients are deficient. Chapman and Brown (1950) concluded that low leaf K concentrations were diagnostic and would not produce a false positive for K deficiency. However, when other nutrients were limiting, higher K concentrations could produce a false negative for K deficiency. The possibilities for misdiagnosis have led to ongoing research on how to consider more than one nutrient at a time when interpreting tissue analyses.

Table 9.4 Directional changes in nutrient concentrations of orange (*Citrus sinensis* (L.) Osbeck) leaves when other nutrients are deficient. (adapted from Chapman and Brown 1950)

Nutrient	Directional change in K leaf concentration due to a deficiency in the nutrient in the first column	Directional change in leaf concentration of the element in the first column due to a deficiency in K[a]
Nitrogen	Increase	Increase
Phosphorus	Increase	?
Calcium	Increase	Increase
Magnesium	Increase	Increase
Sulfur	Increase	?
Boron	Increase	?
Iron	Increase	?
Zinc	Increase	?

[a]? indicates the direction is not known with certainty

9.3.2 *Diagnosis and Recommendation Integrated System (DRIS)*

The importance of nutrient interactions on tissue concentrations was an early observation. Hall (1905) noted that the total quantity of sodium (Na) and K, summed together, was relatively constant but the cation that contributed more to the sum was the one in greater supply in the soil. He observed that,

> Any abundance of soda acts as a diluent and reduces the proportion of potash in the mangel ash, even though the plant may have an excess of potash available. In consequence the normal proportion of potash in the ash of the mangel will vary with factors other than the potash content of the soil. . .

The most well-known approach for considering multiple nutrients is the Diagnosis and Recommendation Integrated System (DRIS), developed by Beaufils (1973) in South Africa from his work on maize and rubber trees. The objective of DRIS is to rank nutrients in their order of sufficiency to one another. The rank reveals which nutrients are most limiting and which are least, relative to each other. The rank does not, however, indicate whether a crop will respond favorably to fertilization if the lowest-ranked nutrient or nutrients are added (Sumner 1990). Baldock and Schulte (1996) classified DRIS as a dependent nutrient index (DNI), because it considers two or more nutrients in its interpretations.

DRIS begins by assembling all available data, typically hundreds to thousands of records, into a database. The minimum data required for each record are yield and the tissue nutrient concentrations associated with that yield. This large data set is parsed into at least two subpopulations based on yield: low and high. Often, the yield delineating the subpopulations is chosen from experience. For example, Sumner (1977a) set the delineating soybean grain yield at 2.6 Mg ha^{-1}.

The next step is to create parameters, which are ratios of two nutrient concentrations. Continuing with the soybean example, Sumner (1977a) created the following parameters for the nutrients N, phosphorus (P), and K: N/P, N/K, and K/P

Table 9.5 An example of DRIS parameters and norms for N, P, K concentrations in soybean (*Glycine max* (L.) Merr.) leaves derived from 879 observations in the low-yielding subpopulation and 366 observations in the high-yielding subpopulation. (excerpted from Sumner 1977a)

	High-yielding subpopulation					
Parameter	Mean or "norm"[a]	Standard deviation (sd)	Coefficient of variation (CV) (%)	Variance	Variance in the low-yielding subpopulation	Ratio of variances (low/high)[b]
N/P	13.77	2.72	20	7.40	19.89	2.69**
N/K	2.43	0.50	21	0.25	1.88	7.52**
K/P	5.97	1.47	25	2.16	6.81	3.15**

[a]Because units of concentrations ratios are unity, units of all descriptive statistics except the coefficient of variation are also unity. The means of each parameter from the high-yielding subpopulation are the DRIS "norms"
[b]** Indicates the variance ratio is highly statistically significant

(Table 9.5). He calculated descriptive statistics for each one: mean, variance, standard deviation (sd), and coefficient of variation (CV). Beaufils (1973) advised ensuring the distribution of each parameter in each yield subpopulation be normally distributed. None of the statistics for the parameters have units, since nutrient concentrations are expressed as ratios of one another. In the soybean example, Sumner calculated statistics for N/P, N/K, and K/P within each yield subpopulation. Table 9.5 shows all of these statistics for the high yield subpopulation but displays only the variance for the low-yielding one. To determine if a parameter should be included, Sumner (1977a) calculated the ratio of the low-yielding variance to the high-yielding variance. The assumption was that higher yields were less variable than lower yields. If the variance ratio was statistically significant, that parameter was included. Beaufils and Sumner (1976) interpreted significance to mean that, "...a relationship between yield and...plant composition is possible and that this relationship...can be exploited to suit diagnostic purposes." Table 9.5 shows that the variance ratios of all three parameters were highly statistically significant, so he included all of them.

In DRIS, the means of each parameter from the high-yielding subpopulation are "norms." When the yield is normally distributed across levels of a parameter, the parameter mean is associated with the highest yields in the high yield subpopulation. Walworth et al. (1986) noted that high-yielding subpopulations were less likely to be skewed and more likely to follow the normal distribution, providing an additional reason for using them when creating norms. Norms are the standards used for interpreting tissue concentrations from individual data sets and represent the target levels of each parameter. The DRIS norms are the basis for ordering nutrients according to their sufficiency in the plant. There are two ways of creating this ranking: (1) graphically using a DRIS chart or (2) numerically using DRIS indexes.

9.3.2.1 DRIS Chart

The DRIS chart (Fig. 9.13) ranks three nutrients qualitatively. When more than three nutrients are analyzed, the user interprets multiple DRIS charts simultaneously, as outlined in Beaufils and Sumner (1976). In the soybean example, the chart is composed of three axes, one for each parameter. The arrowheads on each axis indicate the direction of the increase in each parameter. The intersection of the three axes is the norm for each parameter (the "norms" in Table 9.5): 13.77 for N/P, 2.43 for N/K, and 5.97 for K/P. The inner circle has a radius of (2/3) × sd for each parameter (Sumner 1977a). For example, the radius of the circle for the N/P line is (2/3) × 2.72 = 1.813. Adding and subtracting that value from the N/P norm (13.77) calculates the two intersection points of the inner circle on the N/P line: 13.77 + 1.813 = 15.6 and 13.77–1.813 = 12.0. The outer circle has a radius of (4/3) × sd. Calculations are repeated for the other two lines to find their intersection points with both circles. The arrows next to the element symbols denote the balance of the nutrients on either side of a given axis. Keeping with the N/P axis, the inner circle represents nutrient balance between N and P, denoted by the horizontal arrows next to each nutrient. Within this circle, tissue concentrations are close to norms and are therefore associated with the highest yields in the high yield subpopulation. Moving upward on the N/P axis to the zone between the two concentric circles, the level of N begins to become too high, denoted by the upward slanted arrow, and the level of P starts to become too low, denoted by the downward slanted arrow. This is a transition zone. Moving still farther upward outside the second circle is nutrient imbalance. N is too high (arrow straight up) and P is too low (arrow straight down). An N/P ratio outside the second circle is associated with the lowest yields in the high

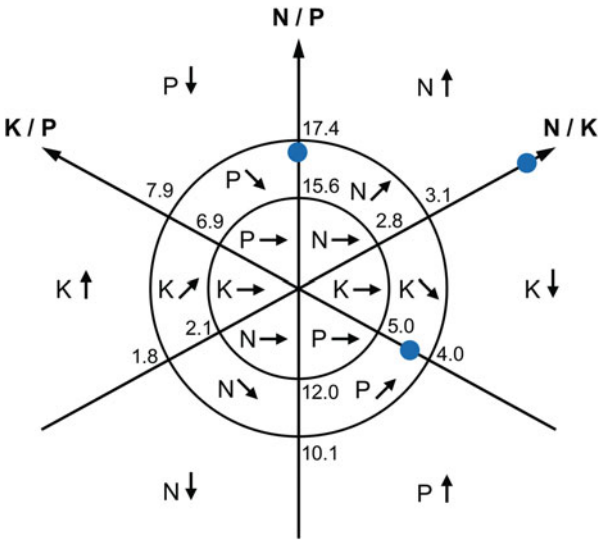

Fig. 9.13 DRIS chart for the parameters N/P, N/K, and K/P. The radius of the inner circle is (2/3) × sd and that of the outer circle is (4/3) × sd. The blue points are the values of the parameters for the second entry (92 days after emergence) in Table 9.6: N/P = 17.00; N/K = 3.86; K/P = 4.40

yield subpopulation. Imbalances between N and P move in the opposite direction downward from the center on the N/P axis, with P being too high and N being too low outside the second circle on the bottom of the N/P axis. Similar interpretations exist for the other nutrients on the other two axes (N/K and K/P).

DRIS charts like that in Fig. 9.13 rank three nutrients from most deficient to least deficient. For example, Sumner (1977a) applied the soybean DRIS norms in Table 9.5 to soybean tissue concentrations he interpolated from a study by Hanway and Weber (1971) (Table 9.6). The general method for using a DRIS chart was best

Table 9.6 Soybean (*Glycine max* (L.) Merr.) leaf tissue nutrient concentrations from Hanway and Weber (1971) as interpolated by Sumner (1977a), concentration ratios, and rank in order of most yield-limiting to least yield-limiting

	N[a]	P	K	Parameters			
Days after emergence	g (kg dry matter)$^{-1}$			N/P	N/K	K/P	Rank[b]
73	64.5	4.2	17.5	15.36	3.69	4.17	K > P ≈ N
92	42.5	2.5	11.0	17.00	3.86	4.40	K > P > N
102	29.0	2.0	9.5	14.50	3.05	4.75	K > P ≈ N

[a]Tissue data are from the top leaves, above node 14

[b]Rank differs slightly from that published by Sumner (1977a) who used computational methods rather than the DRIS chart to rank nutrients

described by Sumner (1977b). We apply that method to the soybean tissue concentrations listed in the second entry in Table 9.6 (92 days after emergence): 42.5 g N kg^{-1}, 2.5 g P kg^{-1}, and 11.0 g K kg^{-1}.

First, we calculate tissue nutrient concentration ratios to create parameters that match the axes in Fig. 9.13, shown in columns 5–7 in Table 9.6: N/P = 17.00, N/K = 3.86, and K/P = 4.40.

Next, we locate 17.00 along the N/P axis (top leftmost point in Fig. 9.13) and see that it falls between the inner and outer circles. We then find the nutrients and arrows to the left and right of the axis where the point is located. To the left of the N/P axis is a P\ and to the right is an N/. In the typeset used in this chapter, "\" denotes an arrow slanted downward and "/" denotes an arrow slanted upward. For each nutrient, we tally only the horizontal or downward-facing arrows. We record a "\" next to P, as shown in the first step of the tally in Table 9.7.

Next, we locate N/K = 3.86 along the N/K axis, which falls outside the outer circle (point in the upper right of Fig. 9.13). To the right of the N/K axis outside the outer circle is a K↓ and to the left of the N/K axis is an N↑. We record only the downward-facing arrow and add it to K in the second step of the tally in Table 9.7. The \ arrow next to P from the first step is copied down into the second step.

Next, we locate K/P = 4.40 along the K/P axis, which falls between the inner and outer circles (the point in the lower right in Fig. 9.13). To the right of this axis next to the point is a K\ and to the left of this axis is a P/. We record only the downward slanted arrow next to K in step 3 in Table 9.7, again copying all arrows from the previous step.

Table 9.7 Ranking of the N, P, and K tissue concentrations listed in Table 9.6 for the entry associated with samples taken 92 days after soybean (*Glycine max* (L.) Merr.) emergence

Progressive steps in the tally	Parameter interpreted	Parameter value	Nutrient with a horizontal or downward arrow[a]	Tally of horizontal and downward arrows[b]
1	N/P	17.00	P\	N P\ K
2	N/K	3.86	K↓	N P\ K↓
3	K/P	4.40	K\	N P\ K↓\
Nutrient not assigned an arrow	N	→		N→ P\ K↓\
Final ranking (from most limiting to least yield-limiting)				K > P > N

[a]An arrow slanting downward is denoted by "\" in the typeset of this chapter. These arrows are read from the DRIS chart in Fig. 9.13

[b]Downward slanting arrows are given a lower ranking (less limiting) than straight downward arrows (more limiting), so in order of most to least yield-limiting, the combination "↓\" denotes a more limiting nutrient than "\" alone

By convention, after all, three parameters have been read from the DRIS chart, the nutrient not yet assigned an arrow receives a horizontal one (→), which we have added in the fourth entry in Table 9.7.

The last step is to order the nutrients from most limiting to the least limiting. Straight downward arrows are more yield-limiting than slanted downward arrows. In the example in Table 9.7, K with both a ↓ and a "\" is more limiting than P with only a "\." Finally, N is the least limiting since it has no downward arrows. The final ranking, from most to least yield-limiting is therefore: K > P > N. Repeating this process for the other two entries in Table 9.6 (73 and 102 days after emergence) fills out the remaining rankings for the earlier sampled and later sampled soybean tissue. We see that in each entry in Table 9.6, K is identified as the most limiting nutrient, with P possibly the next limiting, and N the least limiting.

9.3.2.2 DRIS Indexes

DRIS indexes rank multiple nutrients quantitatively. Nutrient indexes combine functions of each parameter. For the soybean example, three functions exist: *f*(N/P), *f*(N/K), *f*(K/P) (Sumner 1977a). Each function (not displayed here) is composed of (1) the distance a particular parameter is from its norm, (2) a weighting factor, and (3) a sensitivity coefficient that is simply a multiple of 10. The distance is negative when the parameter is less than its norm and positive when the parameter is greater than its norm. The distance is zero when the parameter equals its norm. Distances are on a continuous scale. The weight in the function is the inverse of the coefficient of variation (CV) associated with the parameter norm. The DRIS index for a particular nutrient is an average of the functions containing that nutrient. Keeping to the three nutrients N, P, and K, their respective indexes are:

$$\begin{aligned} \text{N Index} &= I_{\text{N}} = [f(\text{N/P}) + f(\text{N/K})]/2 \\ \text{P Index} &= I_{\text{P}} = [-f(\text{N/P}) - f(\text{K/P})]/2 \\ \text{K Index} &= I_{\text{K}} = [f(\text{K/P}) - f(\text{N/K})]/2 \end{aligned} \tag{9.1}$$

Because weights are in the functions, the average that calculates an index is a weighted average. The index calculation assigns a negative sign to a function that has the nutrient of interest in the denominator and a positive sign to a function with the nutrient of interest in the numerator. Therefore, for the K index, *f*(N/K) is negative and *f*(K/P) is positive. Indexes provide a continuous scale for ranking nutrients as opposed to the DRIS chart which provides only qualitative rankings.

When Sumner (1977a) used indexes rather than a chart to rank the data in Table 9.6, his ranking was K > P > N, and this ranking was the same across all plant ages. This is notable, since the actual concentrations of N, P, and K in tissue decreased over time in Table 9.6. Sumner considered the stability of rankings with plant age one of the major contributions of DRIS to tissue test interpretations. Interpretations for sufficiency ranges, discussed previously, are for specific growth stages. Sumner (1990) argued that the ranking stability arose from the use of tissue concentration ratios. Nutrient concentrations are normally reported on a dry matter basis, such as g K (kg dry matter)$^{-1}$. In ratios of two concentrations, such as K/N, the dry matter units cancel each other, making the ratios less sensitive to changes in dry matter content as tissue ages.

When setting up nutrient ratios, Sumner (1990) advised accounting for how nutrient concentrations of each element changed as tissue aged. The most consistent ranking of nutrients over time occurred when all nutrient concentrations in the analysis changed in the same direction, for instance becoming less concentrated, as is the case for N, P, and K. However, some nutrients increase in concentration at later growth stages, like Ca in sugarcane (*Saccharum officinarum* L.) leaf tissue (Sumner 1990). Taking the inverse (1/Ca) of concentrations that increase with tissue age shifts their direction to match those that decrease. So instead of using a ratio of N/Ca, one would instead use a ratio of N/(1/Ca), which results in the product N × Ca. Creating such consistency in directional change produced much more consistent rankings across tissue ages (Sumner 1990), and increased the accuracy of predicting yield responses (Hallmark et al. 1988). In addition, Hallmark et al. (1988) observed that not taking the inverse of Ca concentration resulted in Ca often appearing in rankings as the most limiting nutrient.

Jones (1981) suggested two modifications to the DRIS procedure. DRIS used one function when a parameter was greater than its norm and another function when a parameter was less than its norm. Jones (1981) pointed out that the functions did not weight parameter values equivalently. The difference in weighting biased the indexes. In his first modification, Jones advocated for a single functional form that weighted variances equivalently regardless of how a parameter value compared to its norm (Eq. 9.2). In that equation, the parameter function $f(p_{ij})$ is equal to the value of parameter j in a given tissue sample i (p_{ij}) minus the norm of that parameter (M_j) divided by the standard deviation of the norm of that parameter (sd_j). Both M_j and sd_j are calculated using the high yield subpopulation. The weight in Eq. (9.2) is $1/\text{sd}_j$.

The equation calculates negative values when the parameter is less than its norm, positive values when it is greater, and zero when the two are equal. In later work, DRIS adopted Jones' single equation as the parameter function (Hallmark et al. 1987).

$$f(p_{ij}) = (p_{ij} - M_j)/\mathrm{sd}_j \tag{9.2}$$

Jones (1981) also questioned the use of only variance ratios, like those in Table 9.5, to select which parameters to include. He observed that parameter means between high and low-yielding subpopulations could be significantly different even though their variances were not. In his second modification, he argued for testing means in addition to variances for selecting parameters. He also noted that more parameters would be statistically selected as the number of observations in the yield subpopulations increased.

One of the assumptions of the DRIS system was that yield distributions across levels of any ratio were normally distributed. Beverly (1987a, b) demonstrated that in some cases, those ratios were positively skewed. Lack of normality created different norms for the same ratio when the numerator and denominator in that ratio were switched. Taking the natural log of the concentration ratio corrected this problem. The log transformation was the major modification. That change also led to a simplification of the index calculation.

Elwali and Gascho (1984) created the nutrient balance index (NBI) for DRIS, also termed by some the nutrient imbalance index (NII). It is the sum of the absolute values of all of the nutrient indexes. For N, P, and K the NBI is:

$$\mathrm{NBI} = | I_{\mathrm{N}} | + | I_{\mathrm{P}} | + | I_{\mathrm{K}} | \tag{9.3}$$

The closer the nutrient balance index is to 0, the more balanced are the nutrient concentrations. The NBI does not indicate which nutrients are out of balance. It indicates only the overall magnitude of imbalance.

9.3.3 The Modified DRIS System (M-DRIS)

DRIS, as originally created, did not have a method for predicting the probability of crop response to applications of nutrients that it ranked as most yield-limiting. Researchers have tested several methods targeted at fulfilling this need. Jones (1981) observed in his sugarcane data set that negative, rather than positive, index values more accurately identified when crops would respond. Walworth et al. (1986), working with alfalfa (*Medicago sativa* L.), proposed including an index for dry matter (DM) when ranking nutrients. The DM index served as an internal standard. Nutrients with indexes less than the DM index were likely yield-limiting and alfalfa was more likely to respond to additions of those nutrients. Alfalfa performed better when applying all nutrients with indexes less than the DM index compared to applying only the nutrient with the most negative index. Walworth

(1986) did provide the caveat that using the DM index as a delineation level for crop response might work best for crops where total DM production is desired, like forage crops. Hallmark et al. (1987) included DM as a factor when diagnosing nutrient balance in soybean leaves. They confirmed that including DM improved predictions of crop response for grain crops too and that such improvements were not limited to only forage crops.

Including DM as a factor is what differentiates the modified DRIS (M-DRIS) from DRIS. Additionally, M-DRIS uses the single-parameter function (Eq. 9.2) proposed by Jones (1981). As Walworth et al. (1986) noted, including DM as a factor made M-DRIS more susceptible to changes in plant age than DRIS.

9.3.4 Plant Analysis with Standardized Scores (PASS)

Baldock and Schulte (1996) developed the Plant Analysis with Standardized Scores (PASS) system to combine the respective strengths of sufficiency range and DRIS interpretations. They did this to better relate indexes to probabilities of crop response. The PASS system consists of two sections. The PASS Dependent Nutrient Index (PASS DNI) is an interpretation based on DRIS. The PASS Independent Nutrient Index (PASS INI) is an interpretation based on sufficiency ranges.

PASS DNI creates indexes for nutrient ratios. It uses the Jones (1981) parameter function in Eq. (9.2). Like DRIS, the PASS DNI for a given nutrient is the average of all parameter functions using nutrient ratios containing that nutrient. Unlike DRIS, PASS DNI includes only "common response nutrients." To be classified as a common response nutrient: (a) the crop must have a high requirement of that nutrient, and (b) the correlation of the concentrations of that nutrient to the yield responses of that crop must have a well-defined critical concentration. Failure to meet both of these criteria categorize a nutrient as a "rare response nutrient."

PASS INI is an index for nutrient concentrations, not nutrient ratios. It uses the critical concentration (C_j) as the reference for its values. The function that transforms nutrient concentration to the PASS INI is based on Eq. (9.2). Instead of using the parameter mean (M_j), Baldock and Schulte substituted the critical concentration plus one standard deviation ($C_j + \mathrm{sd}_j$) based on the assumption that $M_j \approx (C_j + \mathrm{sd}_j)$. They also introduced a sensitivity coefficient by multiplying the numerator in Eq. (9.2) by 10:

$$\text{PASS INI}_{ij} = 10\left[p_{ij} - \left(C_j + \mathrm{sd}_j\right)\right]/\mathrm{sd}_j$$

which simplifies to

$$\text{PASS INI}_{ij} = \left[10\left(p_{ij} - C_j\right)/\mathrm{sd}_j\right] - 10 \tag{9.4}$$

According to Eq. (9.4), when a sample nutrient concentration is equal to the critical concentration, PASS INI equals −10. Values between or equal to −10 and 10 are sufficient, which is equivalent to the range $M_j \pm \text{sd}_j$. Index values less than −10 are deficient. The more negative an index becomes, relative to −10, the greater the chances become that a crop will respond to an application of that nutrient. Index values greater than 10 indicate that the nutrient concentration is high and that crops are not likely to respond to additions of that nutrient. PASS INI uses the same division of nutrients as PASS DNI (common response and rare response).

PASS uses three interpretation categories: (1) probable nutrient deficiency, (2) slightly possible nutrient deficiency, and (3) nutrient sufficiency. PASS places common response nutrients with PASS INIs below −10 in the "probably nutrient deficient" category. It places rare response nutrients with PASS INIs below −10 in the "slightly possible nutrient deficiency" category. Also into this category go nutrients that have both PASS INIs less than zero and PASS INI and PASS DNI sums that are less than −10. Into the third category, "nutrient sufficiency," go all nutrients not already in the first two categories.

When making fertilizer decisions, a combination of PASS INI and PASS DNI is helpful. PASS INI identifies nutrients that are yield-limiting. If more than one nutrient is yield-limiting, PASS DNIs order them in a rank from most yield-limiting to least. This ranking helps prioritize which nutrients to apply.

To simplify interpretations and give greater weight to PASS INI values that were more negative, Baldock and Schulte (1996) created the PASS yield index (PASS YI). The PASS YI reassigns all values greater than −10 to zero, since no crop response is expected at those index levels. All values less than −10 are squared, giving exponentially greater weight to diminishing tissue concentrations. The PASS YI subtotals all of the squared values across all nutrients within each group: both common and rare response nutrients. The subtotal of the sums of squares in the common response group is multiplied by 2 to give it more weight than the subtotal of the sums of squares of the rare response group. After the multiplication, the two subtotals are added to calculate the PASS YI across all nutrients. Because PASS YI uses squared values, its minimum is zero. That minimum value means all nutrients considered are at or above their respective critical concentrations and no crop response to any of them is likely. With no restrictions from nutrient limitations, yields are expected to be higher. Conversely, as PASS YI values become greater, more nutrient restrictions exist and yields will likely be lower unless nutrients are applied.

The PASS approach has not been tested by researchers to the extent DRIS has been. We were able to locate only two relatively recent studies that compared PASS to other methods of interpretation (Simón et al. 2013; Urricariet et al. 2004). Both showed PASS to be a promising diagnostic approach.

9.3.5 *Compositional Nutrient Diagnosis (CND)*

Compositional nutrient diagnosis (CND) arose from analysis techniques developed for geological sediment compositions (Aitchison 1982, 1983). Compositions are made up of individual compounds. Each compound comprises a given percent of the total composition. The percentages of all compounds add up to 100%. Traditional statistical approaches assume that levels of factors are unbounded; however, in compositions, there is an upper bound (100%) to the level of any one factor. In addition, if the percentage of one compound increases, the percentage of at least one other compound must decrease. Therefore, compositions are not independent. Finally, compositional data are not normally distributed, as Beverly (1987a, b) pointed out when working with DRIS. Consequently, compositions require their own statistical approaches.

Parent and Dafir (1992) adapted the work of Aitchison (1982, 1983) to plant tissue nutrient compositions. They termed their approach "compositional nutrient diagnosis" or CND. Parent created two approaches to CND: (1) one that uses centered log ratios (CND-*clr*) and (2) one that uses isomeric log ratios (CND-*ilr*). Both approaches examine the interactions of nutrients in plant tissue. Compositional nutrient diagnosis falls under Baldock and Schulte's (1996) classification as a DNI. CND-*clr* was developed first and shared many characteristics with DRIS (Parent and Dafir 1992). Both CND-*clr* and DRIS analyze all possible combinations of nutrients first, leaving the user to interpret the results afterward. CND-*ilr* was developed to provide the user the ability to incorporate knowledge of nutrient interactions into the analysis ahead of time (Parent 2011). Therefore CND-*ilr* allows users to test for certain interactions in a given sample.

9.3.5.1 CND-*clr*

CND-*clr* examines all possible interactions of one nutrient concentration with all other measured concentrations (Parent and Dafir 1992). In this regard, it is fundamentally different than the DRIS approach which considers the interaction of only two nutrient concentrations at a time. Parameters in CND-*clr* differ from those in DRIS. Interaction parameters formed from N, P, and K in DRIS are nutrient concentrations ratios like N/P, N/K, and K/P. In CND-*clr*, interaction parameters for N, P, and K are logarithms of ratios, like $\log[N/(N \times P \times K \times R)^{1/4}]$, $\log[P/(N \times P \times K \times R)^{1/4}]$, and $\log[K/(N \times P \times K \times R)^{1/4}]$. The denominator in all three CND-*clr* parameters is the same and is the geometric mean of all measured nutrient concentrations and the "filling-up value" R. The filling-up value is the percent remaining after summing all of the nutrient concentrations: $R = 100 - (N + P + K)$. The filling-up value is always an additional term in the geometric mean. The general formula for the interaction of any one nutrient with all other elements is:

$$V_{ij} = \log\left[x_{ij}/G_i\right] \tag{9.5}$$

where V_{ij} is the parameter for nutrient j in sample i, x_{ij} is the concentration of nutrient j in sample i, and G_i is the geometric mean of the following concentrations: nutrient j in sample i (x_{ij}); the filling-up parameter R; and the concentrations of all other nutrients in sample i.

The filling-up parameter and the logarithmic function come from considering leaf tissue to be a closed compositional system (Parent and Dafir 1992). The percentages of all compounds and elements in tissue must add up to 100%. Increasing the percentage of one element decreases the percentage of at least one other element or compound. Adjustments in composition do not have to occur with other elements that are measured. They may occur with any number of compounds not measured but included in the filling-up value, resulting in a lower value for R.

Just as in DRIS, CND-*clr* computes indexes for each nutrient (Parent and Dafir 1992). The equation CND-*clr* uses to calculate indexes is similar to the equation DRIS uses (Eq. 9.2) to calculate parameter functions:

$$I_{ij} = \left(V_{ij} - V_j^*\right)/\mathrm{sd}_j^* \tag{9.6}$$

where I_{ij} is the CND-clr index for nutrient j in a given tissue sample i, V_{ij} is the CND-*clr* parameter for nutrient j in sample i (Eq. 9.5), V_j^* is the CND-*clr* norm for nutrient j (the average V_j of the high-yielding subpopulation), and sd_j^* is the standard deviation of V_j in the high yield subpopulation. In DRIS, the yield level separating high and low-yielding subpopulations is usually chosen from experience. For CND-*clr*, Khiari et al. (2001) developed a statistical method to separate those populations. In DRIS, Eq. (9.2) calculates parameter values for each ratio of two nutrients. Since a given nutrient may be in more than two ratios, DRIS requires Eq. (9.1) to combine all ratios containing that nutrient. In CND-*clr*, an equation like Eq. (9.1) is not needed, since each nutrient has only one log ratio (Eq. 9.5). Just like in DRIS, CND-*clr* indexes classify nutrients in order of their limitation (Parent et al. 1994a), with more negative values indicating greater deficiency and more positive values indicating greater excess.

Akin to the DRIS NBI (Eq. 9.3), CND-*clr* provides quantitative evaluation of overall nutrient balance. Two metrics have been developed for this purpose. The first one developed was a Euclidian distance d (Parent et al. 1994a, b). The greater the value of d, the more overall nutrient imbalance exists. The second metric developed was the CND r^2 value, defined as the sum of the squares of all CND-*clr* indexes for a particular sample (Khiari et al. 2001). The farther CND r^2 is from zero, the greater the nutrient imbalance.

For practical use in production settings, a tool has been developed to implement CND-*clr* analyses for guava (Rozane et al. 2012). Users enter nutrient concentrations as received from an analytical laboratory, and the tool returns CND-*clr* indexes for each nutrient, presented in three ways: numerically, displayed in a radar chart, and displayed in a bar chart. The tool also provides the associated CND r^2 value.

9.3.5.2 CND-*ilr*

CND-*ilr* compares two user-specified subsets of nutrients. The user selects these groups ahead of analysis. Table 9.8 provides an example of how a user can create a subset (Parent 2011). The first row (Sample) is the concentration of nutrients in a leaf tissue sample. The second through sixth rows are sets that define the groups to compare. Each set examines two groups. In a given set, all nutrients with a 1 are in one group and all nutrients with a −1 are in the other group. By assigning 1s and −1s, users can group nutrients in meaningful ways. The number of sets is equal to one less than the number of components of the composition, including the filling-up value *R*. Because the composition is made up of six components (N, P, K, Ca, Mg, and *R*), 5 sets can be compared.

After creating subsets, the next step is to calculate isometric log ratio (ilr) coordinates for each set *i*. The general formula is:

$$\mathrm{ilr}_i = [(r \times s)/(r + s)]^{1/2} \times \ln[\mathrm{g}(x+)/\mathrm{g}(x-)] \tag{9.7}$$

where ilr_i is the ilr coordinate of set *i*, *r* is the number of components assigned a 1 in set *i*, *s* is the number of components assigned a −1 in set *i*, g(*x*+) is the geometric mean of the percentages of components assigned a 1 in set *i*, and g(*x*−) is the geometric mean of the percentages of components assigned a −1 in set *i*. For example, for set 4 in Table 9.8, K versus Ca + Mg, the ilr coordinate for the sample (ilr_4) is $[(1 \times 2)/(1 + 2)]^{1/2} \times \ln[(2.64)/(1.15 \times 0.11)^{1/2}] = 1.637$. According to Parent (2011), the average ilr coordinate for this set in the high-yielding subpopulation is $\mathrm{ilr}_4^* = 1.154$. The difference $\mathrm{ilr}_4 - \mathrm{ilr}_4^*$ determines how far the ilr of sample set 4 is from that of the same set in the high-yielding subpopulation: 1.637–1.154 = 0.483. This difference is the second highest of the sets and indicates that K is out of balance with Ca + Mg (set 4, Table 9.8). The difference that was greater belonged to set 5, which showed that Ca was out of balance with Mg. Because it had a greater difference, Ca and Mg were more out of balance than

Table 9.8 Orthogonal partitions of N, P, K, Ca, Mg concentrations, and *R* (the filling-up value) nutrient concentrations subgroups for an apple (*Malus domestica* Borkh.) leaf analysis. (Parent 2011)

	Components						
	N	P	K	Ca	Mg	*R*	Interpretation
	Concentration (%)						
Sample:	2.50	0.22	2.64	1.15	0.11	93.38	
	Orthogonal partitions						
Set 1:	1	1	1	1	1	−1	Nutrients versus filling-up value
Set 2:	1	1	−1	−1	−1	0	Anions versus cations
Set 3:	1	−1	0	0	0	0	N versus P
Set 4:	0	0	1	−1	−1	0	K versus Ca + Mg
Set 5:	0	0	0	1	−1	0	Ca versus Mg

were K and Ca + Mg. Parent (2011) concluded that the Mg supply needed to be increased and K fertilization needed to be either decreased or halted. The power of CND-*ilr* is the ability to test specific nutrient combinations for their relative balance.

9.3.6 Multiple Regression Approaches

Statistical approaches, like multiple regression, are another avenue for considering more than one nutrient at a time when evaluating nutrient concentrations in plant tissue. Lissbrant et al. (2010) used a combination of cluster analysis, logistic regression, and concentration ratios like that in DRIS to predict alfalfa yield in an experiment examining various rates of applied P and K. Cluster analysis classified crop yield into groups, then those groups were further classified as "acceptable" (high and medium-high-yielding groups) or "unacceptable" (all lower-yielding groups). To best predict acceptable or unacceptable performance for May cuttings, binary logistic regression identified a combination of tissue P, K, and the K/P ratio as model factors. For the later June cutting, the regression model included only tissue K and the K/P ratio. Other statistical approaches have also been developed, like multiple regression using combinations of concentrations of multiple nutrients (Martinez et al. 2003; Srivastava et al. 2001).

9.3.7 Metabolite Profiles

Potassium-deficient plants have different metabolite profiles than plants with sufficient K. For instance, shoot tissue of K-deficient *Arabidopsis* exhibited higher concentrations of the soluble sugars sucrose, glucose, and fructose; higher concentrations of several basic or neutral amino acids; and lower concentrations of the acid amino acids glutamic acid and aspartic acid (Armengaud et al. 2009). In their review, Amtmann et al. (2008) concluded that while increased concentrations of soluble sugars, organic acids, and amino acids were often observed in tissue from K-deficient plants, there was great variability and not all crops showed increases. Some crops, in fact, showed decreases in some of these same metabolites. A particularly insightful observation by Armengaud et al. (2009) was that the observed changes in metabolite concentrations, "...were not always related to tissue K content." This demonstrates the shortcomings of using tissue K concentration as the sole metric of K deficiency. While metabolite profiles have the potential to be diagnostic of K deficiency, there is much yet to be learned before they can be incorporated into fertilizer recommendations and on-farm decision-making.

9.3.8 Potassium Content in Plant Sap

Another approach to diagnosing K status is to analyze the K content of plant sap. Sap is extracted by pressing plant tissue with tools such as a pliers (Syltie et al. 1972), a garlic press (Gangaiah et al. 2016), or a press attached to a syringe (Burns and Hutsby 1984). Freshly sampled petioles and leaf midribs are the typical samples. Hand-held analytical equipment makes in-field testing possible. Hand-held meters with ion-selective electrodes provide quantitative evaluations of the K concentration in the sap. Also available are test strips or spot tests that react the sap with reagents to create a color. The user compares that color with standardized colors representing different K concentrations. Unless the color matches perfectly with that of a standard, the user must interpolate concentrations between two adjacent color standards (Burns and Hutsby 1984). With test strips or spot tests, there is a limited range of detection, and the user cannot infer values beyond either end of that range (Syltie et al. 1972).

When sampling sap, it is important to recognize that its chemical composition changes throughout the day. Meitern et al. (2017) sampled branches of hybrid aspen (*Populus tremula* L. x *Populus tremuloides* Michx.) saplings. They found that the K concentration in xylem sap increased quickly after 6:00 am, plateaued at 12:00 pm to 3:00 pm, then decreased again. These changes started after dawn as photon flux density increased, air temperature increased, and relative humidity decreased. For this reason, diagnostic interpretations need to specify the times of day for collecting samples.

Like tissue K concentration, sap K concentration also changes with organ and age. Age is usually specified by position on the plant. For instance, the "uppermost" leaf specifies the youngest leaf. Burns (1992) observed that when K supply was cut off from actively growing lettuce (*Lactuca sativa* L.), sap K concentration dropped more quickly in the youngest leaf than in the older ones. This meant the youngest leaf was more sensitive to changes in K supply, making it a good choice for diagnosis. Further support for sampling young tissue was provided by Vruegdenhil and Koot-Gronsveld (1989) when they observed that the K concentration in the sap was highest in the uppermost fully developed leaf of castor bean (*Ricinus communis* L.).

Creating diagnostic interpretations for plant sap K concentrations requires correlations to yield or other metrics of crop performance, like those previously discussed for tissue K concentration. As an example, Hochmuth et al. (1993) grew eggplant (*Solanum melongena* L.) on a K deficient soil and applied incremental rates of K fertilizer. Several times during the season they used a hand-held ion-specific electrode to measure the K concentration in plant sap extracted from petioles of the youngest fully expanded leaves. At the same time, they also collected whole leaf samples, including petioles, and analyzed them for K concentration with traditional laboratory techniques. Third, they measured total marketable eggplant yield, summed over the yields of various market grades throughout the season. They determined critical plant sap K concentrations and confirmed sap analysis as a viable

Table 9.9 Sufficiency ranges for sap K concentration in petioles of the most recently matured leaves and K concentration in the most recently matured, whole leaves of various vegetable crops sampled during the day from 9:00 to 16:00. (excerpted from Hochmuth 1994a)

		Petiole sap K concentration	Whole leaf K concentration
Crop[a]	Growth stage	mg K L^{-1}	g kg^{-1}
Eggplant	First fruit (5 cm long)	4500–5000	35–50
Pepper	First open flowers	3000–3200	45–50
Potato	First open flowers	4500–5000	30–50
Tomato (field)	First open flowers	3500–4000	35–40
Watermelon	Vines 15 cm long	4000–5000	35–40

[a]Eggplant (*Solanum melongena* L.); pepper (*Capsicum* spp.), potato (*Solanum tuberosum* L.), tomato (*Solanum lycopersicum* L.), watermelon (*Citrullus lanatus* (Thunb.) Matsum. & Nakai)

diagnostic tool. In a later review of 10 years of his and his colleagues' research, Hochmuth (1994a) published interpretations of sap K concentration for other vegetable crops, along with tissue K concentration (Table 9.9). Those interpretations are used in nutrient management guidance for growers (Hochmuth 1994b).

Some studies focus on correlating sap K concentration to leaf K concentration and do not include correlations to yield. This is often done in exploratory studies examining new analytical methods (Gangaiah et al. 2016; He et al. 1998; Iseki et al. 2017). Eventually, however, each new method must be correlated to yield or some metric of crop performance.

9.4 Conclusions

We have reviewed a number of ways scientists have determined the nutritional status of plants. Visual symptoms detect moderate to severe deficiencies where plant metabolism has already been irreversibly and negatively impacted. Measuring light reflectance is non-invasive and non-destructive, but to date, methods have not uncovered spectral combinations specific to K nutritional status. Tissue sampling is destructive but has been the most diagnostic approach to date. Sufficiency ranges consider nutrients in isolation and do not account for nutrient interactions. Interpretation methods that do account for interactions are DRIS, M-DRIS, PASS, CND-*clr*, CND-*ilr*, and multifactor statistical models. Metabolite profiles show promise, but more research is needed to determine if the level of certain metabolites or their interactions with other components are diagnostic of K deficiency. Sap analysis provides rapid results while in the field. Interpretation of results has been so far limited to sufficiency ranges. Sap analysis results can be highly variable because of diurnal fluctuations in K content.

Moving forward, an important theme throughout all of the tissue sampling interpretations is the value of large, crop-specific data sets composed of nutrient

contents and associated yield and quality levels. At the least, such a data set must centralize data from as many high-yielding production settings as possible. Large data sets representing high-yielding and/or high-quality crops have been used for creating sufficiency ranges as well as norms for DRIS, M-DRIS, PASS, CND-*clr,* and CND-*ilr*. Indeed, this was Beufils (1973) original vision. He saw the need for large, multinational databases that contained large amounts of meta-data for each yield observation. He divided these meta-data into two categories: (1) "external characters" comprised of soil properties, climatic conditions, and farming practices, and (2) "internal characters" comprised of data on the chemical and physical characteristics of various plant organs, including nutrient concentration. In his vision, data could come from farmers' fields or controlled experiments. Data from both sources would be merged and used to create norms. Further, querying large databases rich in meta-data could potentially guide a user to enough relevant studies to develop quantifiable recommendations, such as rates of specific K sources to apply, to rectify any given nutrient deficiency. While many isolated databases have been developed, there is a lot more to be done, both in centralization as well as in completeness of meta-data, to realize a vision Beaufils had decades ago but which is just as relevant today.

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