Leaf Analysis in Citrus: Interpretation Tools

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Abstract

Occurrence of nutrient constraints is as old as history of citrus cultivation. Identifying multiple nutrient constraints has always perplexed the nutritionists due to lack of consistency in response to fertilization when executed in field. Such a discrepancy in diagnostic interpretation has emerged on account of a variety of interpretation tools made under usage. For example, when a leaf analysis dataset is subjected to different interpretation tools, highly overlapping diagnoses are obtained. Analysis of different tools used in leaf analysis data interpretation further warranted the necessity of working out the cultivar-specific nutrient diagnostic norms. Large number of interpretation tools have been put forward to interpret the leaf analysis data. Late DRIS (Diagnosis and Recommendation Integrated System), initially developed for rubber crop, found its great utility to many of the annual and perennial crops, citrus being one of them. Work done in India with reference to different citrus cultivars suggested a strong cultivar-specific dependence of the diagnoses that agreed with field diagnoses. Use of DRIS-based diagnoses provided replicating outcome under diverse soil types. The detailed account of different leaf analysis interpretation tools is further discussed.

Keywords

Leaf analysis • Interpretation tools • Critical nutrient concept • Nutrient concentration range • Nutrient balance • Crop logging • Boundary line concept • DRIS

5.1 Background Information

Perennial crops are quite different from annual crops in their nutritional requirement due to their plant size, density, rate of growth and rooting pattern, and phenomenon of bud differentiation and its relationship with the yield during the following season/year. Determination of the nutritional needs of fruit trees must be made prior to the renewed growth or the determination of potential yield. To ensure high economic productivity and to sustain the available soil nutrient status at a desirable level, correct doses of manures, biofertilizers, and chemical fertilizers must be applied, based on the use of reliable diagnostic tools. Considering energy, economy, and environment, it is imperative that manures, biofertilizers, and chemical fertilizers be used efficiently. The best diagnostic tool is one that recommends nutrient application in a direct economic response of the fruit crop. Diagnostic tools are designed to avoid nutrient shortage or excess, and if used properly, no decrease in fruit production or quality should occur. Leaf analysis seems to be the best method for identifying the need for application of nutrients.

The interpretation of leaf analysis is based on the premise that there is a significant biological relationship between the elemental content in leaf, plant growth, and fruit yield with a purpose to predict fertilizer requirement depending upon site characteristics. This is popularly known as ‘critical value

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approach' widely applied in citrus orchards with considerable success. These relationships normally reflect a sigmoidal response curve on which two critical values can be identified. These values for each nutrient are the value below and above which plant performance is reduced (Terblanche and Du Plessis 1992).

5.2 History of Leaf Analysis

Justus von Liebig, the German chemist (1803–1873), was the first to have an opinion that growth of plants was proportional to the amount of mineral substance available in the fertilizer and developed the "law of minimum" which states that the "growth of plants is limited by the nutrient present in the smallest quantity." As it can be seen in *The Book of the Rothamsted Experiments (1905)*, Hall (2009) envisaged plant analysis as a method for estimating the nutrient content of soils.

Leaf analysis is based on the premise that "the plant behaviour is related to concentrations of essential minerals in leaf tissue" (Smith 1966). Leaf analysis, as a method for assessing the nutrient requirements of crops, is based on the assumption that "within certain limits, there is a positive relation between doses of the nutrient supplied, leaf nutrient content and yield and/or quality" (Smith op. cit.). Nutrient supply in 1 year may have a major effect on both fruit tree nutrition and crop production in subsequent years as the plant responds to direct and residual soil fertility. The application of the same dose year after year is thus irrelevant (Bhargava and Chadha 1993). The crop, through leaf analysis, informs the growers that the power of his soil to supply the nutrient has not kept pace with the nutrient requirements of the crop.

The diagnosis of the nutritional status of citrus, based on the chemical analysis of some of its organs, has been generalized since the middle of the past century. Among the first and most complete works published on this subject, Chapman (1961) refers those from Thorpe, in 1868, from Wolf, in 1871–1880, from Ricciardi, in 1880, from Boschi, in 1895, and from Olivieri and Guerrieri, in 1895. Still, according to Chapman (op. cit.), in 1931, Barnette and his collaborators published the results from the chemical analysis of adult grapefruit trees, not only the chemical composition of the different parts of the trees but also its weight.

Based on the fact that the leaves have an active "assimilatory" function, being the "synthetic laboratory" that controls the plant nutrition, in the first half of the past century, Thomas (1945) referred that through the leaf analysis, it is possible to control the nutritional status of the plants. In face of that, the majority of the work done in this subject has incurred mainly in the leaves. However, more recently, several authors defend the analysis of other plant organs, such as flowers (Pestana et al. 2001) or fruits (Lacertosa et al. 2001), but there are not yet reference values or even consensus about the preference for one of those organs as the most adequate base for the management of the fertilization in the following cultural cycle or the correction of nutritional unbalances in the same year.

According to Bould (1984), leaf analysis is based on the following four assumptions: (1) leaf is the main site of plant metabolism; (2). changes in nutrient supply are reflected on the composition of the index tissue such as leaf or its petiole; (3) changes are more pronounced at certain stages of development than at others; and (4) concentration of the nutrients in the leaf at the specific growth stage is related to the performance of the crop.

For some authors, with the exception of N, that is slightly different for the various commercial species (Embleton et al. 1978), the chemical composition of the citrus is identical (Hanlon et al. 1995; Davies and Albrigo 1998). However, other authors, as Legaz et al. (1995) and Dias et al. (2002), indicate different reference values for N, P, and K for the various citrus species. Besides the species, the concentration of the nutrients in the leaves is dependent on, among other factors, the age, the type, and the localization of the leaves (Smith 1966; Embleton et al. 1978; Legaz et al. 1995; Davies and Albrigo 1998; Carranca 1999).

The concentration of the different nutrients is more stable in 4–7-month-old leaves and that is why this is the most adequate period referred for leaf sampling (Smith 1966; Embleton et al. 1978; Hanlon et al. 1995; Legaz et al. 1995; Davies and Albrigo 1998; Carranca 1999; Correia 2000). On the other hand, the presence of flowers or small growing fruits, in a branch, diminishes significantly the leaf concentration of the nutrients, as they are translocated to those organs, once the leaves, besides its photosynthetic function, also act as a storage organ (Smith 1966; Embleton et al. 1978; Legaz et al. 1995; Davies and Albrigo 1998; Carranca 1999). In fact, the nutrient concentration in nonfruiting shoot leaves seems to be the best indicator of the nutritional status of the tree, better than fruiting shoot leaves, since those are the branches that will carry the flowers and subsequently the fruits on the following year (Embleton et al. 1978; Legaz et al. 1995; Carranca 1999). According to Davies and Albrigo (1998), with the exception of South Africa (where the leaves for chemical analysis are sampled from fruiting shoots), in most part of the citrus production regions of the world, leaf samples are collected in nonfruiting shoots from spring flushes at 4–7 month old.

For foliar chemical analysis, Swietlik (1996), Alva and Tucker (1999), Carranca (1999), and Dias et al. (2002) recommend, per 2–4 ha, the sampling of 8–10 nonfruiting shoot leaves per tree, collected from the four quadrants of the canopy, in 15–20 trees randomly chosen and representative of the general status of the orchard. The diagnosis of the

Table 5.1 Reference values for leaf nutrients concentration, in nonfruiting shoot leaves, from the spring flushes (5–7 month old), for mature 'Valencia Late' orange trees

Nutrient	Deficient	Low	Optimum	High	Excess
N (g kg^{-1})	<22	22–23	24–26	27–28	>28
P (g kg^{-1})	<0.9	0.9–1.1	1.2–1.6	1.7–2.9	>3
K (g kg^{-1})	<4	4.0–6.9	7–10.9	11–20	>23?
Ca (g kg^{-1})	<16	16–29	30–55	56–69	>70?
Mg (g kg^{-1})	<1.6	1.6–2.5	2.6–6.0	7.0–11	>12?
B (mg kg^{-1})	<21	21–30	31–100	101–260	>260
Cu (mg kg^{-1})	<3.6	3.6–4.9	5–16	17–22?	>22?
Fe (mg kg^{-1})	<36	36–59	60–120	130–200?	>250?
Mn (mg kg^{-1})	<16	16–24	25–200	300–500?	>1,000?
Mo (mg kg^{-1})	<0.06	0.06–0.09	0.1–3.0	4–100	>100?
Zn (mg kg^{-1})	<16	16–24	25–100	110–200	>300

Adapted from Embleton et al. (1978)

Table 5.2 Reference values for leaf nutrients concentration, in nonfruiting shoot leaves, from the spring flushes (4–6 month old), for mature citrus trees

Nutrient	Deficient	Low	Optimum	High	Excess
N (g kg^{-1})	<22	22–24	25–27	28–30	>30
P (g kg^{-1})	<0.9	0.9–1.1	1.2–1.6	1.7–3.0	>3.0
K (g kg^{-1})	<7	7–11	12–17	18–24	>24
Ca (g kg^{-1})	<15	15–29	30–49	50–70	>70
Mg (g kg^{-1})	<2.0	2.0–2.9	3.0–4.9	5.0–7.0	>7.0
B (mg kg^{-1})	<20	20–35	36–100	101–200	>200
Cu (mg kg^{-1})	<3	3–4	5–16	17–20	>20
Fe (mg kg^{-1})	<35	35–59	60–120	121–200	>200
Mn (mg kg^{-1})	<17	17–24	25–100	101–300	>300
Mo (mg kg^{-1})	<0.05	0.06–0.09	0.1–1.0	2.0–5.0	>5.0
Zn (mg kg^{-1})	<17	17–24	25–100	101–300	>300

Adapted from Hanlon et al. (1995)

nutritional status of the trees must be made comparing the results from leaf chemical analysis with the reference values. However, these values must be used as a general indication, when compared with the results from the chemical analysis of citrus leaf samples, once, as already referred, may exist differences among rootstocks, cultivars, tree age, and phase of the growth cycle. The results of leaf analysis in young trees, for example, must be scrutinized very carefully, since the concentration of Fe and Zn is lower in these leaves (Swietlik 1996) and the content of N and K is higher, in relation to full production trees (Smith 1966; Swietlik 1996).

Most of the reference values of leaf analysis, existing on the bibliography, were established based on the relation between the concentration of the several leaf elements and the development of the plant, standardized through experimental studies on fertilization adequately delineated (Terblanche and Du Plessis 1992; Hanlon et al. 1995) for orchards at full production. Through the analysis of the results obtained in an experimental study with young Navel orange trees, Thompson et al. (2003) proposed that N concentration in young tree leaves, when adequately supplied with this element, should be around 28 g N kg^{-1} dry weight instead of 25 g N kg^{-1} dry weight, which is the value established by Embleton et al. (1978) as the optimum for adult trees.

In a long-term study (16 years) carried out in Portugal including 38 orange orchards of different varieties, aiming the establishment of critical levels for the several nutrients contained in the leaves, based on a correlation with some fruit quality indexes, Fragoso et al. (1990) observed that in a general way, the values were within the intervals adopted by Embleton et al. (1978) for mature 'Valencia Late' orange trees (Table 5.1), concluding that will be adequate to adopt them as reference values for orange trees in plain production.

More recently, Hanlon et al. (1995) published an actualization of the reference values for leaf content of nonfruiting shoot leaves for mature citrus trees (Table 5.2). These values were confirmed in the work of Alva and Tucker (1999) and are generally those adopted in Florida. In a recent publication (Kallsen 2002), where reference values of nutrients for nonfruiting shoot leaves in adult orange trees are available, it is possible to endorse the critical values (Table 5.1) that were considered uncertain by Embleton et al. (1978). The comparison between Tables 5.1 and 5.2 reveals some differences, being the greater for K, for which the values considered by Embleton et al. (1978), in any of the ranges, are lower than those considered by Hanlon et al. (1995). In relation to Mg, on the contrary, the values considered by Embleton et al. (1978) for the ranges optimum, high, and excessive are higher than those considered by Hanlon et al. (1995). Maximum limits for the elements that Embleton et al. (1978) considered uncertain (Table 5.1) were already established (Table 5.2), with a clear difference in case of the Mo.

In Portugal (Fragoso et al. 1990), as well as in Spain (Legaz et al. 1995; Agusti 2000), the tables from Embleton et al. (1978), based on values obtained in California, are the most adequate, probably due to the climatic identity. In any case, these values were established for full production trees, and there are no reference values for young nonbearing trees when the chemical composition of the plant is different from the mature tree (Smith 1966; Swietlik 1996).

5.2.1 Reference Values for N Confirmed by Foliar Analysis, from Planting till Full Production

As it was observed by Menino (2005) in a field experiment with ‘Lane Late’ oranges planted in a sandy soil located at Algarve (South of Portugal), during the first 4 years after transplanting the N leaf concentration, although increasing with increasing N rates, decreased from the first till the fourth year, suggesting that the optimum concentration values for leaf N content, for young nonbearing trees, with a vigorous growth, should not be defined, in a general way, by a unique value for all of the years (28 g N kg^{-1} dry weight, as suggested by Weinert et al. (2002)) but by a series of decreasing values till the optimum recommended for mature trees (25 g N kg^{-1} dry weight, according to Embleton et al. (1978)). Assuming that the mean values of leaf N concentration for all treatments correspond to the adequate nutritional status of the trees, the optimum leaf N concentration obtained in the experiment carried out by Menino (2005), expressed as g N kg^{-1} dry weight, could be evaluated in accordance with the following logarithmic adjustment:

$$N = 34.7 - 7.4 \times \log_{10}(x), \text{ with an } r^2 = 0.84 \text{ for } p \leq 0.029,$$

as it is shown in Fig. 5.1. According to this logarithmic adjustment, the reference values for leaf N concentration, during the first years in the field, would be the following:

34.7 g N kg^{-1} dry weight, for the first year; 32.5 g N kg^{-1} dry weight, for the second year; 31.2 g N kg^{-1} dry weight, for the third year; 30.2 g N kg^{-1} dry weight, for the fourth year; and 29.5 g N kg^{-1} dry weight, for the fifth year.

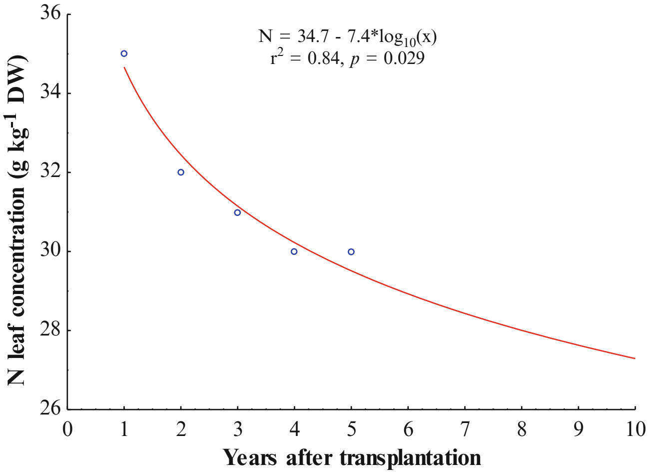

Fig. 5.1 Logarithmic adjustment for the mean N leaf concentration in ‘Lane Late’ orange trees, in the first 5 years after planting, with the estimation of values for the following 5 years

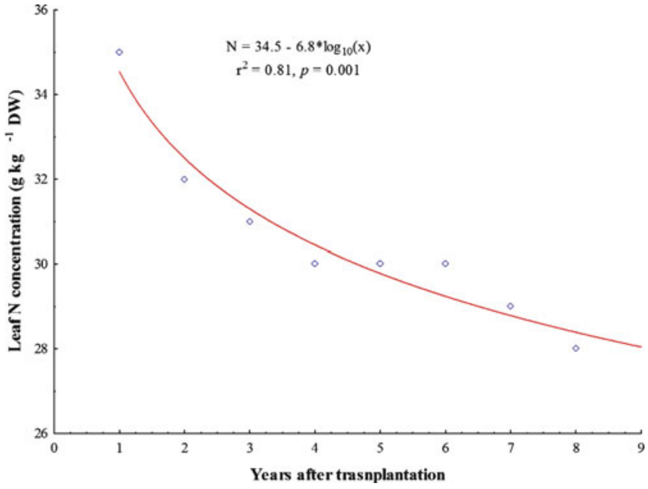

Fig. 5.2 Logarithmic adjustment for the mean N leaf concentration in ‘Lane Late’ orange trees, in the first 8 years after planting

Nevertheless, according to the abovementioned adjustment (Fig. 5.1), the critical value suggested by Weinert et al. (2002) for young trees (28 g N kg^{-1} dry weight) would only be reached in the eighth year, which means that in the referred experimental conditions, the values were always much higher than the optimum. On the other hand, the optimum value referred by Embleton et al. (1978) for mature trees (between 24 and 26 g N kg^{-1} dry weight) would only be reached in the 15th year.

In the prosecution of the mentioned study (Menino op. cit.) till the eighth year (Menino et al. 2008), the logarithmic adjustment (Fig. 5.2) maintains very close to the previous, with

$$N = 34.5 - 6.8 \times \log_{10}(x), \text{ with an } r^2 = 0.81 \text{ for } p \leq 0.001,$$

as it is illustrated in Fig. 5.2. According to this logarithmic adjustment, the reference values for leaf N concentration, during the first years after transplanting in the field, would be the following:

34.5 g N kg^{-1} dry weight, for the first year; 32.5 g N kg^{-1} dry weight, for the second year; 31.3 g N kg^{-1} dry weight, for the third year; 30.4 g N kg^{-1} dry weight, for the fourth year; 29.7 g N kg^{-1} dry weight, for the fifth year; 29.2 g N kg^{-1} dry weight, for the sixth year; 28.8 g N kg^{-1} dry weight, for the seventh year; and 28.4 g N kg^{-1} dry weight, for the eighth year.

These values reveal a straight adjustment with those calculated earlier, for the first 5 years, and a perfect convergence for the reference values suggested by Weinert et al. (2002) for mature trees in full production.

5.3 Major Breakthroughs

A variety of interpretation tools (IT) have shown their application in leaf analysis of citrus. These are: critical nutrient concentration (Terblanche and Du Plessis 1992; Srivastava et al. 1999); nutrient concentration range (Parent and Dafir 1992); nutrient balance using factorial method (Cantarella et al. 1992), Kenworthy's balance index (Kenworthy 1973), Moller-Nielson balance concept (Moller Nielson and Friis-Nielson 1976b); crop logging (Abaev 1977); and boundary line concept (Walworth et al. 1986) – all suggesting only single value concentration and diagnosis and recommendation integrated system (DRIS) which considers the nutrient ratio (Walworth and Sumner 1987; Beverly 1987). The utility of these ITs in the past faced many limitations, especially in the context of alternative to identify the nutrient constraint at any growth stages during the season and, therefore, diagnoses found application only to a specified growth stage due to strong influence of leaf age.

Of different ITs, DRIS is claimed to have certain advantages over other conventionally used ITs (Malavolta et al. 1993; Li et al. 1999). The working premises of DRIS (Mourão Filho 2004) "are based on: (a) the ratios among nutrients are frequently better indicators of nutrient deficiencies than isolated concentration values; (b) some nutrient ratios are more important or significant than others; (c) maximum yields are only reached when important nutrient ratios are near the ideal or optimum values, which are obtained from high yielding-selected populations; (d) as a consequence of the stated in (c), the variance of an important nutrient ratio is smaller in a high yielding (reference population) than in a low yielding population, and" "the relations of significant nutrient ratios of high and low yielding populations can be used in the selection of significant nutrient ratios; (e) the DRIS indices can be calculated individually, for each nutrient, using the average nutrient ratio deviation obtained from the comparison with the optimum value of a given nutrient ratio, hence, as pointed by Jones (1981) and Walworth and Sumner (1987), the ideal value of the DRIS index for each nutrient should be zero." The efforts in the past have successfully established the DRIS norms for 'Valencia' orange in USA (Beverly et al. 1984; Wallace 1990), South Africa (Woods and Villiers 1992), Venezuela (Rodriguez et al. 1997), Brazil (Mourão Filho and Azevedo 2003); 'Verna' lemon in Spain (Cerda et al. 1995); 'Sicilian' lemon in Italy (Creste 1996) and 'Pera' sweet orange in Brazil (Creste and Grassi Filho 1998); acid lime (Varalakshmi and Bhargava 1998), 'Kinnow' mandarin (Hundal and Arora 2001), 'Nagpur' mandarin, 'Khasi' mandarin, and 'Mosambi' sweet orange in India (Srivastava et al. 2001; Srivastava and Singh 2003c, 2006).

Almost any conclusion can be drawn from the earlier attempts on the development of leaf nutrient diagnostics in countries like Argentina (Perez 1996), Australia (Jorgensen and Price 1978), Brazil (Quaggio et al. 1998), China (Koto et al. 1990), France (Marchal et al. 1978), India (Chahill et al. 1991; Srivastava et al. 1999; Srivastava and Singh 2002), Italy (Dettori et al. 1996), Japan (Terblanche and Du Plessis 1992), Turkey (Saatci and Mur 2000), Spain (Hellin et al. 1988), Costa Rica (Alvardo et al. 1994), USA (Chapman 1949; Koo et al. 1984; Swietlik 1996), employing a variety of diagnostic methods using different aged index leaves from fruiting as well as nonfruiting terminals. Such efforts have generated differential diagnostic capabilities in the absence of uniformity in guidelines used in diagnosing the nutrient constraints, e.g., a different set of optimum values are obtained when specific leaf analysis data are subject to contrasting ITs like multivariate quadratic regression analysis (MQRA) or diagnosis and recommendation integrated system (DRIS) using some commercial citrus cultivars of India (Table 5.3). DRIS-derived values were further very close to original values from high-performance elite orchards than values obtained from MQRA (Wallace 1990; Woods and Villiers 1992; Srivastava and Singh 2003c). Alves and Mourão Filho (2005) observed that conventional sufficient range approach (SRA) and DRIS were in agreement for nutritional diagnosis of K, while other nutrients like Cu, Mn, and Fe were diagnosed as deficient by DRIS and classified adequate to high by SRA in 'Valencia' sweet orange orchards on three different rootstocks in São Paulo, Brazil.

Arguments are often put forward to support the view that deficient, optimum, or excessive levels of nutrient concentration cannot be determined by means of absolute figures (critical levels) due to great deal of variation in vegetative activity. Hence, a new concept of the above evolutionary balance of bioelements and the critical area based on the links between nutrients and the balance of all the bioelements was proposed by Carpena-Artés (1978). According to this concept, the leaf level of any nutrient for a given moment is determined by the difference between the amount of nutrient which has reached the leaf and, from there, the amount transported to other plant organs. Hence, four diagnostic criteria of deficiency area,

Table 5.3 Optimum leaf nutrient levels for three commercial citrus cultivars of India using two most common ITs

	NM		MSO		KM	
Nutrients	MQRA	DRIS	MQRA	DRIS	MQRA	DRIS
N (%)	2.2–2.4	1.7–2.8	2.4–2.5	2.0–2.6	2.2–2.5	2.0–2.6
P (%)	0.07–0.10	0.09–0.15	0.13–0.15	0.09–0.17	0.10–0.11	0.09–0.10
K (%)	1.2–1.6	1.0–2.6	1.6–2.3	1.3–1.7	1.9–2.1	0.99–1.9
Ca (%)	1.3–1.5	1.8–3.3	2.6–3.2	1.7–3.0	2.1–2.3	2.0–2.5
Mg (%)	0.48–0.67	0.43–0.92	0.32–0.49	0.32–0.39	0.28–0.38	0.24–0.48
Fe (ppm)	110–132	75–113	132–148	70–137	148–180	85–249
Mn (ppm)	49–43	55–85	52–112	42–87	72–85	42–87.6
Cu (ppm)	8–14	10–18	7–10	7–16	10–19	2–14
Zn (ppm)	18–30	14–30	25–43	12–29	24–39	16–27
Yield (kg tree^{-1})	40–54	47–117	87–95	77–138	45–62	32–56

Adapted from Srivastava et al. (1999) and Srivastava and Singh (2003c)
NM 'Nagpur' mandarin, *MSO* 'Mosambi' sweet orange, *KM* 'Khasi' mandarin

critical area, normal area, and excess area were suggested for determining nutritional requirement.

Du Plessis and Koen (1992) advocated different leaf nutrient norms, based on climatic zones. The norms derived for the small fruit area, of cool climate, like Nelspruit in Mpumalanga province of South Africa (2.0–2.4% N, 0.95–1.50% K, and N/K ratio of 1.6–2.2) were different of those for the large fruit size area of Citrusdal in the Western Cape province (2.1–2.7% N, 0.70–0.90% K and N/K ratio of 3.0–4.5), having warm climate, with other nutrients like P (0.11–0.16%), Ca (3.5–5.5%), and Mg (0.30–0.55%) showing no significant difference. Most of the leaf nutrient diagnostics have, therefore, failed to find any universal applicability when tested over space and time under varying conditions. A cultivar-specific nutrient standard to suit regional growing conditions and comparing norms developed by different sampling methods appears to be the best approach. Conversion factors to relate norms that are developed for nonfruiting terminals with those for fruiting terminals and vice versa would be useful.

5.3.1 Critical Nutrient Concept

Over the past century, the pendulum of analytical diagnosis has swung from soil analysis to plant analysis. The outstanding contribution of Lundegardh (1951) has provided the usefulness of leaf analysis convincingly. Lagatu and Maume (1926) were the first to adopt the new approach to which they termed "Diagnostic Foliar." Macy (1936) introduced the concept of "critical nutrient percentage" in leaf dry matter and visualized three ranges or portions of a curve relating plant response to concentration percentages, namely the following: (1) a narrow minimal percentage range where both response and internal concentration remain fairly stable, (2) a poverty adjustment range where both response and internal concentration rise, and (3) a luxury consumption range where response is hardly noticed and concentration increases.

A critical nutrient level as "the range of concentrations at which growth of the plant are restricted in comparison with that of plants of higher nutrient level" was postulated by Ulrich (1948). This approach is based on the establishment of relationships between the concentration of different nutrients in the leaves and plant performance, with the sigmoidal response curve where two critical limits can be identified. These values for each element are respectively the values below and above which the plant growth is reduced (Terblanche and Du Plessis 1992). The interpretation of results of leaf analysis is based on the concept of critical values defined by Ulrich (1948) as the range of concentrations at which the growth of the plant is restricted in comparison to that of plants at a higher nutrient level. The results showed that plants with widely different nutrient consumption give similar yields as long as these nutrient concentrations are well above the critical level. Lundegardh (1951) described the physiological basis of leaf analysis and named assimilating plant leaf as "Central Laboratory of Nutrition." The leaf analysis became more widespread in 1955 when Kenworthy offered a service to fruit growers in Michigan for monitoring nutrient levels of their vineyards and orchards. In India, the first Leaf Analysis Laboratory was established in the Indian Institute of Horticultural Research at Hessaraghatta, Bangalore, in 1980 to conduct research and provide leaf analysis service to growers in India.

In order to make the best commercial use of leaf analysis, one must know the relative importance of the nutritional problems existing in a given orchard as well as the potential individual benefit or adverse effects of the nutrient's application. As early as Ulrich (1952) discussed the physiological basis for assessing the nutrient requirement for plants, the foundation on which the interpretation of leaf analysis was established.

The inability of the leaf analysis approach to deal adequately with the variation in nutrient concentration on a dry matter basis with age is probably its greatest disadvantage. To overcome this inconvenience, three approaches have been anticipated: in the first, sets of critical values for different stages of growth were proposed (Geraldson et al. 1973; Tserling 1974); in the second, the accumulation of dry matter with age is monitored in order to correct the nutrient concentration for increasing dry matter (Melsted et al. 1969); and in the third, sufficiency ranges were advanced such that the lower limit represents roughly the critical level while the upper is set at a value corresponding to an unusually high or toxic concentration (Small and Ohlrogge 1973). Although sufficiency ranges are purported to improve flexibility in diagnosis, they in fact decrease diagnostic precision because the limits are often too wide. Few of these attempts to improve the critical value approach have met with great success.

Critical value approach was established by Cate and Nelson (1965): the leaf analysis data are divided into two or more classes for the purpose of making the nutrient recommendation for a potential yield and fruit quality. However, the basis of defining different classes, i.e., deficient, very low, low, optimum, high, excessive, and/or toxic, is often subjective or arbitrary. A number of methods are available for setting the class limits. The procedure is to split the data into two groups, using the successive critical levels to ascertain that the particular critical level, which will maximize the overall predictive ability (R^2) with the means of two groups (classes) as the predictor value. An example for using the data, which is believed to be typical of this kind of problem, is presented. Several continuous correlation models can also be fitted to the same data. However, none gave as high as R^2 as a single low high split according to the procedure described by Bhargava and Singh (2001). The subsequent procedure is followed to work out the critical limit of a nutrient in an index tissue.

1. Correlation factor (CF) is calculated as $(\mathrm{GT})^2/n$.
2. Calculation of the total sum of squares (TSS) $(X_1 + X_2 + X_3 + X_4 + \ldots X_n) - \mathrm{CF}$.
3. Calculation of SSQ1 = $(X_1 + X_2) - \mathrm{CF}_1$ where $\mathrm{CF}_1 = (X_1 + X_2)_2/2$.
4. Calculation of the $\mathrm{SSQ}_2 = (X_3 + X_4 + X_5 + X_n)/2 - \mathrm{CF}_2$ where $\mathrm{CF}_2 = (X_3 + X_4 + \ldots X_n)_2/n$
5. $\mathrm{TSS} = (\mathrm{SSQ}_1 + \mathrm{SSQ}_2) = r$.
6. $R_2 = r \times 100/\mathrm{TSS}$.

According to the above technique, the orchards are divided in two groups: one in which is expected to have a relatively large response to a particular nutrient and another one in which is expected to have little or no response, assuming that the other nutrients were present in adequate amounts. A dividing line between the two categories might be determined approximately by a graphical technique in which the vertical and the horizontal lines are superimposed on the scatter diagrams so as to maximize the number of points in positive quadrants. The horizontal line is 90% probability line, and the vertical line is so drawn that the maximum points of the scatter diagram are on two positive quadrants. The vertical line, which is determined by the eye judgment, is known as critical levels.

Page and Martin (1964) suggested critical limit of leaf K as 0.28–0.44%. Bar-Akiva et al. (1967) recommended 0.07% as critical limit of leaf P content for Persian lime. Magnitskii and Takidze (1972) suggested critical level of 0.19% P and 1.6% K in mandarin trees in calcareous and podzolic soils of Georgia. Wang (1985) suggested critical limit of Ca, Mg, and Zn as mandarin grown on red earth in China. Aso (1967) suggested critical limit of less than 1.7% N as critical limit in Tucuman, Argentina. Bar-Akiva and Lavon (1968) recommended critical limit of leaf P as 0.075% for grapefruit. Primo et al. (1969) suggested critical limit of B, Mn, Fe, and Zn as less than 30, 18, 60, and 19 ppm, respectively, for sweet orange. While Ishihara et al. (1972) suggested critical limit based on various plant organs and accordingly, critical limit of Cu was found as 4.0, 3.0, 3.8, and 10.0 ppm for leaves, shoots, fruits, and fine roots, respectively. Rodriguez and Gallo (1961) observed the critical level of N, P, K, Ca, and Mg as 2.20%, 0.12%, 1.00%, 3.00%, and 0.30%, respectively. Coetzee (1980) suggested critical limit of K as 0.70–0.80% for Valencia orange.

In Brazil, critical level of N and K was observed as 2.66% and 1.87%, respectively, for foliar K diagnosis. In other studies, leaf K content of 1.0–1.7% has been suggested as optimum K concentration and 0.87% as critical level in 6–7-month-old leaves from fruit-bearing terminals of 'Valencia' orange (Rodriguez and Gallo 1961). In South Africa, critical limit of leaf K was observed as 0.9% in 7–9-month-old leaves from fruits bearing terminals and leaf K content of 0.55–0.80% associated with a N/K ratio of 3.3–4.1 for maximum production and fruit size of Valencia' orange in Citrusdal area (Du Plessis 1977). Studies conducted on 'Washington Navel' oranges in Australia indicated critical limit for leaf and juice K as 0.4% (0.80–1.10% optimum) and 11,314–1,373 ppm (1,424–1,575 ppm optimum), respectively, and suggested that fertilizer recommendations can be made on the basis of undigested juice K content (Gallasch et al. 1984). Shimizu and Morii (1985) suggested critical limit of Mg, Mn, Zn, and B as less than 0.13%, less than 16 ppm, less than 16 ppm, and 9–16 ppm, respectively, for 'Satsuma' mandarin.

Liu et al. (1984) suggested critical limit for Zn, Mg, and Ca as less than 25 ppm, 0.30%, and 3.0%, respectively, for major citrus species, viz., *Citrus reticulata, Citrus tankan, Citrus sinensis, Citrus tangerina,* and *Citrus grandis* of Fujian province (China). Singh and Tripathi (1985) suggested 20 ppm leaf Zn as a critical limit for distinguish chlorotic sweet orange from healthy trees, in Agra region of Uttar Pradesh.

5.3.2 Critical Nutrient Range

Evidence shows that plant with somewhat different nutrient concentration, well above the critical level, and changes in nutrient balance adversely affect the growth and productivity of the plant. The use of critical nutrient range (CNR) was, therefore, suggested by Dow and Robert (1981) rather than critical nutrient concentration (CNC).

There is generally a good relationship between concentration of the nutrients with the growth and yield. CNR is defined as the range of nutrient, at a specified growth stage, above the upper limit of which we are reasonably confident that the crop is amply supported and below the lower limit of which we are reasonably confident that the crop is deficient in the nutrient. It seems more practical to deal with critical concentration range rather than single concentration limit.

5.3.3 Nutrient Balance

5.3.3.1 Prevot's Factorial Method

Based on a considerable amount of research and experience with tropical crops, Prevot and Ollagnier (1961) developed a system based on Liebig's law of minimum, which takes nutrient balance, synergisms, and antagonisms into account using factorial experiments. The effect of increasing levels of one or several factors is calibrated, keeping all other conditions constant. From these calibrations, it is possible to determine the relative proportions of nutrient for balanced nutrition. In fact, the major problem with this approach is that there may be interactions between the factors being varied and those kept constant (Prevot and Ollagnier 1961). Although it is a definite improvement over the single factor approach used in the critical value system, it is unable to take into account simultaneously the many nutrient factors affecting growth, as is done in the DRIS approach (Bhargava and Chadha 1988).

5.3.3.2 Kenworthy's Balance Index

The Kenworthy's balance index (Kenworthy 1973) is calculated by the following procedure:

1. If a simple value (X) is smaller than standard values (S), then the balance index is calculated by:
   $B = (P + I)$
   $P = (X/S) \times 100$
   $I = (100 - P) \times (V/100)$
   where B = balance index; I = influence of variation; P = per cent of standard; V = coefficient of variation; S = standard value; X = value of sample under diagrams.
2. If sample value (X) is larger than the standards value (S), then calculation will be:
   $B = (P - I)$
   $P = (X/S) \times 100$
   $I = (100 - P) \times (V/100)$

These calculations tend to move the percent value toward a balance index of 100, based on the coefficient of variation for each nutrient.

Balance index has been used by Awasthi et al. (1979) to work out judicious nutrient doses for apple in Himachal Pradesh. The concept of nutrient balance is possible to understand when the composition values are converted into percent of the standard values. Therefore, a way of adjusting percent of the standard values is needed to account for normal variation. This may be done with the use of coefficients of variation for normal plants to develop a balance index. Awasthi et al. (1979) transformed the balance index into nutrient status of apples as indicated below:

Shortage – 17–50% index; below normal – 50–83% index; normal or optimum – 83–117% index; above normal – 117–150% index; excessive – 150–183% index.

Fertilizer treatments are not suggested until nutrient value approaches the level below which the tree performance may be reduced. In the case of N deficiency, applications may be increased approximately by the same percentage by which the balance index falls short of 100. A method was devised to convert the observed values into balance indexes that would eliminate these discrepancies of diagnosis. Mean of "normal range" is taken as 100. The coefficient of variation for each nutrient was selected as a means of adjusting the balance index according to variability in composition associated with normal plants. The method involved calculations that could adjust the deviations from 100 or standard values toward 100 in accordance to the coefficient of variation. If the sample value was below the standard value, the influence of variability was added to the percentage of standard to obtain the balance index. If the sample value was above the standard value, the influence of variability was subtracted from the percentage of standard.

5.3.3.3 Moller-Nielson Balance Concept

A diagnostic system, which attempted to address problems associated with physiological age and nutrient interactions, was proposed by Moller Nielson and Friis-Nielson (1976b) as follows:

- A series of curves are first generated from the nutrient response experiments, which relate nutrient concentration and accumulation of dry matter. The relationship of N concentration and dry plant mass at different times is determined from the trials and varied levels of added N. Similarly, those of P concentration and dry matter are worked out from P response experiments. With the aid of these curves, individual plant samples are collated with a standard plant mass. While this procedure theoretically eliminates variations in plant composition, due to stage of maturity, there is little evidence to suggest that the relationship of nutrient concentration and an increase of dry plant mass during aging is universal.

- At a second step of Moller Nielson's diagnostic method, standard nutrient values are derived through analysis of data from the factorial-designed fertilizer experiments. A boundary line approach is used to determine the optimal nutrient concentration. Only the uppermost points at each foliar nutrient concentration are used to draw the boundary line, which was called the "'pure'-effect nutrient" (Moller Nielson and Friis-Nielson 1976a).
- In the third step, boundary line curves for plots of interacting nutrients are used to determine the optimum levels of the other nutrients at the existing level of most limit nutrient, as done at the second step.
- The final step in this process consists of the calculations of the amount of the most limiting nutrient to be applied and its effect on the status of other nutrients. The method of calculating the required amount of nutrients is arbitrary, based on yield, level, plant uptake, soil fertility status, soil reactions, availability of irrigation water, and climate. After this, recommendations are made for the supplement of nutrients.

Moller Nielson's diagnostic system represents an innovative attempt to overcome two major problems in foliar diagnosis, i.e., effect of physiological maturity and nutrient interactions. On foliar composition and plant performance, however, several questions remain unanswered about the applicability of some of the relationships. Unfortunately, the amount of data required for accurately defining these relationships and the factors, which affect them, is extremely large which it is a serious obstacle to the widespread adoption of the system. Due to the requirement of extremely large data, it has not been used on a large scale. However, it has great potential for the proper diagnosis of problems associated with nutrition.

5.3.4 Crop Logging

Crop log may be defined as the manipulation of the biotic and abiotic factors relying on the information gleaned from the growing crop. In crop logging, the maximum frequency and thorough sampling are followed in 2–5 weeks. Leaf blade is normally used for the estimation of nitrogen, phosphorus, and potash. Root system is used for the information about the soil toxicities. Where salinity is a problem, water levels and electrical conductivity both are determined. All such data form the part of crop log. The sample collected may be used to monitor the nutritional status of an individual crop during its development, i.e., crop logging, therefore, ensures that its nutrient requirements are being met satisfactorily (Clements 1961; Gartell et al. 1979). Abaev (1977) suggested various level of N, P, K according to critical growth stages as: 2.1–2.3% N, 0.22% P_2O_5, and 1.8% K_2O at flowering; 2.4–2.7% N, 0.25–0.28% P_2O_5, and 1.85–2.0% K_2O at fruit formation; and 2.1–2.3% N, 0.25% P_2O_5, and 1.7–1.8% K_2O at fruit ripening stage, for lemon grown in Western Georgia.

5.3.5 Boundary Line Concept

A new approach to study the crop productivity has been developed by Webb (1972) in which the performance of the best, in the sample examined, is taken as a standard against which to judge the remainder, on the assumption that there are reasons other than chance which account for the inferior performance by a part of the population. The line defining the best performance in the population lies at the edge of any body of data, hence the name Boundary Line, and occurs wherever a cause-effect relationship between two variables exists. As stated by Walworth et al. (1986), "whether utilizing a critical value or a nutrient balance system such as the DRIS for interpreting plant tissue composition, determination of accurate optima is of paramount importance." In this same article, two procedures for determining such optima (within the Boundary Line Approach) are proposed, "one using the mean of a high yielding population, the second establishing yield maxima at all nutrient values." When the best performance can be quantified, the overall deficiency in yield, due to inferior performance, can be assessed. When it is allied to the knowledge of the components of yield, the position of the boundary line can be used to direct attention to the phase of growth most likely to respond to better management (Walworth et al. 1986; Bhargava and Sumner 1987).

Researchers have reasoned that if a unique relationship between a single growth factor and crop yield or quality can be defined, then optimizing that factor should permit the best crop performance. As a result, the literature is replete with regression relationships between such parameters as plant and soil analyses data and crop yield. These have often been used to establish critical values for diagnostic purposes. Unfortunately, most of relationships are developed under conditions where only one, two or, sometimes, three nutrients are tested at two or three levels. Consequently, the relationships so determined are specific to the condition unique to the experiment(s) involved and often do not hold valid under all conditions (Andrew 1968).

Nutritionists have attempted to identify and quantify the factors that are closely related to plant performance. If the expected relationship between growth factors and yield/quality can be defined (through techniques as, e.g., analysis of variance, correlation, and regression equations between plant analysis and crop yield), then optimizing those factors should permit the best crop performance. Using mean values of leaf analysis data of high-yielding population (Kenworthy 1973;

Cate and Nelson 1965; Schaffer et al. 1987), critical limits have been fixed for diagnostic purpose in horticultural crops, including citrus. Because the effect of a particular growth factor may change under varying conditions, due to interactions with other factors, critical values established in this way are not unique or universally applicable. This is quite obvious by wide variation in critical values published in literature (Chapman 1967; Kenworthy 1973; Cook and Wheeler 1978; Bhargava and Chadha 1988; Embleton et al. 1973). Use of sufficiency range rather than single value as critical limit (Dow and Robert 1981; Munsen and Nelson 1973; Bhargava and Chadha 1988) will alleviate this problem, although the dynamic nature of the relationship between mineral nutrition and dry matter over time is certainly responsible for some of the variations that exist.

Percentage yield (Nelson and Anderson 1977) has often been used in an attempt to overcome some of these difficulties. However, combining yields from different years or sites in this way largely ignores the complexity of the relationship between plant growth and environment. In the absence of identification and quantification of parameters affecting plant growth, regression approach is of limited value in interpolating to unknown situations and will simply remain on a posteriori approach to organizing data. The boundary line approach defines yields that may occur under a given set of conditions and can be used to determine plant tissue optima, offering an alternative to the conventional critical value system. In addition, the maximum yield that is possible at any given compositional value may be predicted from boundary lines. A comparison of the optima determined via the boundary line approach and those estimated by the mean of the high-yielding population revealed extremely small differences indicating that either method is acceptable for estimating these parameters (Walworth et al. 1986).

If one consciously sets about varying controllable growth factors as much as possible at many locations, a bank of observations that represent the variability encountered in the real world can be generated. This can also be achieved by sampling the variability that occurs naturally in a given crop industry under all conditions where that crop is produced. A scatter diagram of yield plotted against a plant growth factor for such data usually peaks at the optimum level of that particular growth factor. It became possible to develop a set of norms from such data bank, i.e., quantification of a growth factor to maximum yield level, that should be diagnostically precise and more universally applicable (Walworth et al. 1986). This concept is equally applicable to the critical value system, diagnosis, and recommendation integrated system and also to nutrient sufficiency level. The boundary line should be useful in diagnostic work in that the maximum possible yield consistent with any growth factor could be determined. The specificity of regression relationships of this type is due to the unique characteristics of the large number of plant growth factors existent in the individual plots for the particular growing season that produced the data. In different years, the regression equations may be different because other factors or interactions with other factors become more important.

Once a boundary line has been defined, it is a simple matter to locate the apex of that line, which corresponds to the optimal level of the growth factor in question. Alternatively, optima can be estimated by averaging the values of all observations if the population of observations is distributed normally. This method is essentially that used to calculate foliar norms for use in DRIS. Kenworthy (1967, 1973) used a similar technique to develop standard nutritional values for diagnosis of fruit tree foliage.

5.3.6 Diagnosis and Recommendation Integrated System

Development of soil-plant nutrient diagnostics has been the popular area of investigation, world over using a variety of diagnostic tools. A majority of the studies concentrated mainly on sweet orange cultivars. The scope of conventional diagnosis is limited due to strong influence of leaf age. The critical nutrient concentration and sufficiency range limit, developed by using index leaves as interpretation tools, provide little time in the growing season for fertilizer application to be really effective. Therefore, the currently available diagnostic methods are applicable only to narrowly specified developmental stage of crop. In this regard, it is difficult to draw any conclusion from the earlier attempts in several countries (Australia (Jorgensen and Price 1978), China (Wu et al. 1998), Turkey (Saatci and Mur 2000), Spain (Hellin et al. 1988), Costa Rica (Alvardo et al. 1994), USA (Swietlik 1996), Chile (Razeto et al. 1988), and various parts of India (Sharma and Mahajan 1990; Chundawat et al. 1990; Srivastava and Singh 2003b, 2004b)), employing a variety of diagnostic methods and using different aged index leaves from fruiting as well as nonfruiting terminals, amounting to many discrepancies in the diagnostic capabilities in the absence of any commonality in guidelines used in diagnosing the nutrient constraints. The orchards, hence, continue to produce suboptimally due to erroneous identification of the nutrient deficiency that made it further difficult to match the expanding gap emerged from the amount of nutrients added to that of annual demand with orchard age. The nutrient diagnostics available for commercial cultivars (e.g., 'Valencia' and 'Navel' (Perez 1996), 'Nagpur' mandarin (Srivastava et al. 2001; Srivastava and Singh 2003a), 'Satsuma' mandarin (Koto et al. 1990), 'Mosambi' sweet orange (Srivastava and Singh 2003b, 2004a)) have not found their universal applicability and often lacked severely in reproducibility when applied under different contrasting growing conditions because of distinct regional differences.

One of the major drawbacks of quantifying nutrient elements in terms of concentration on total leaf dry weight (DW) is that an increase in the leaf DW (as a result of sugar, starch, or other nutrient accumulation) will reduce the concentration of the nutrient for the same weight (dilution effect) with the opposite effect (concentration effect) when DW decreases. "A nutrient concentration which changes due to changing DW could then lead to inaccurate interpretation of nutrients results when comparing with standard published values or thresholds" (Schumann 2009). For this reason, in such efforts, the type of shoot, period of sampling, and number of leaves collectively affect the leaf sample and, therefore, the analytical result for nutrient concentration.

Besides physiological causes (e.g., Moreno et al. (1996) compared the DRIS indexes with standard methods to evaluate the effectiveness of DRIS in diagnosis Fe-chlorosis), also pathogenic effects have been reported as interfering in dilution/concentration effects. This is the case for the Huanglongbing (HLB) infection in citrus leaves where one of the recognized symptoms is a dramatic accumulation of starch. In diseased tissues, accumulations of starch capable of increasing the total leaf DW by nearly 50% have been recorded. Consequently, most leaf nutrient concentrations show apparent decline in HLB-infected blotchy-mottled leaves due to dilution by the added weight of accumulated starch. For example, in a replicated study of ten HLB-infected and ten healthy Hamlin orange trees, the sulfur concentration in symptomatic blotchy-mottled leaves was 13% lower than in asymptomatic leaves from the HLB trees or the healthy trees. Analysis of the data with DRIS revealed that the amounts of sulfur in the different leaf samples were not significantly different. The same conclusion was reached by converting the sulfur concentration data to a leaf area basis (milligrams per square meter). It was concluded that the sulfur "deficiency" in blotchy-mottled leaves was false and likely caused by the accumulation of starch (Schumann 2009).

Bias from undesirable nutrient dilution or concentration effects, due to uncontrollable changes in leaf tissue DW, is noticeably diminished when using nutrient interpretation with DRIS, since this method calculates ratios of nutrient concentrations, expressed as a fraction of DW, which being common to all of them is annulated. There are variants of the DRIS computations that can estimate an index of the leaf dry weight.

DRIS diagnoses generally agreed the diagnoses made by the sufficiency range method, with the advantage that DRIS reflected nutrient balance and identified the order in which nutrients are likely to become limiting. DRIS reflected changes in nutrient concentrations due to alternate-bearing or crop-load effects and agreed with the sufficiency range method when concentration changes were sufficient to affect this method (Beverly et al. 1984).

Diagnosis and recommendation integrated system (DRIS), although firstly developed for rubber trees (Beaufils 1973), is claimed to have certain advantages over other conventional interpretation tools (Beverly 1987; Malavolta et al. 1993; Li et al. 1999). DRIS method expresses results of plant nutritional diagnosis through indexes, which represent, in a continuous numeric scale, the effect of each nutrient in the nutritional balance of the plant. DRIS diagnoses generally agree with diagnoses made by the sufficiency range method, but with some additional advantages that DRIS reflects the nutrient balance (fluctuates narrowly across different crop developmental stage), identifies the order in which nutrients are responsible for limiting the fruit yield, and its ability to make diagnosis at any stage of crop development. These merits impart DRIS to be able to identify nutrient constraint early in crop growth and allow sufficient time for remediation of identified problem right in the same season of crop (Walworth and Sumner 1987). The efforts in the past have successfully established the DRIS norms for different citrus cultivars (Sumner 1977; Beverly et al. 1984; Varalakshmi and Bhargava 1998; Hundal and Arora 2001).

Several model modifications have been proposed to increase accuracy in the nutritional diagnosis for several crops. The calculation of the nutrient ratio functions is made according to one of three methods, namely the following: (1) the original method proposed by Beaufils (1973), (2) the Jones (1981) method, and (3) the Beaufils (1973) method, modified by Elwali and Gascho (1984). Although these nutrient function ratio calculation methods have been evaluated in some researches, there is not yet a clear definition for the best recommendation. The three methods applied to rubber trees revealed that Beaufils (1973) and Elwali and Gascho (1984) procedures presented similar results, and that Jones (1981) procedure showed dependence on the nutrient ratio (Bataglia and Santos 1990). In some citrus databases, the Beaufils (1973) method highlighted nutritional deficiencies, the Jones (1981) method had advantage for presenting more simple calculation and larger statistical formality, and the Elwali and Gascho (1984) method showed lesser interpretation errors (Santos 1997).

According to Beverly (1991), there are two ways for the second and last stage of DRIS indexes calculation (the function sum involving each nutrient), namely DRIS (Beaufils 1973) and M-DRIS (Hallmark et al. 1987; Walworth et al. 1986). The original DRIS method just uses the nutrient ratio functions. On the other hand, the M-DRIS method, a variation and expansion of original DRIS, foresees dry matter inclusion in the indexes calculation. The expressions are identical to the ordinarily used; however, in this case, the dry matter is treated as an additional constituent, and a new index is calculated, in the same way as for the other plant constituents. In fact, dry matter is, essentially, the sum of the concentration of three nutrients usually ignored in nutritional considerations:

C, H, and O. That additional index is the dry matter mass index, a good indicator of the sampled tissue maturity regarding the standard.

5.3.6.1 Brief Developments

DRIS has shown its application in both annual crops, viz., lettuce (Sanchez et al. 1991), tomato (Caron and Parent 1989), potato (Parent et al. 1994a), onion (Caldwell et al. 1994), cucumber (Mayfield et al. 2002), and carrot (Parent et al. 1994b) as well as perennial crops, viz., apple (Goh and Malakouti 1992), grapes (Bhargava and Raghupathi 1995), pecan (Beverly and Worley 1992), peach (Sanz 1999), mango (Schaffer et al. 1988), mango (Raghupathi and Bhargava 1999), pomegranate (Raghupathi and Bhargava 1998), banana (Angeles et al. 1993), sapota (Appa Rao et al. 2006), litchi (Hundal and Arora 1996), and papaya (Bowen 1992) with equally reproducing results. To overcome perceived weakness in the traditional DRIS approach to tissue nutrient analysis interpretation, three revisions and two new methods were applied to data for oranges cv Valencia. Use of logarithmic transformation, population parameters, and a single calculation method removed systematic errors, simplified the diagnostic method, and extended its applicability. The two new methods are individual nutrient concentrations, rather than the ratios. The changes produce diagnoses similar to those given by DRIS or the sufficiency range approach, but result in better recommendations, judging from a yield response test (Beverly 1987). The below described is the detail account of developments that have taken place with regard to application of DRIS in citrus.

5.3.6.2 Steps Involved for DRIS Norms

DRIS technique consists of describing the nutrient status of high-yielding populations, and to identifying variations from those conditions in unknown samples. The observations were divided into high- and low-yielding subpopulations, using 50 kg tree^{-1} (averaged yield level usually obtained at growers' field) as cut-off yield level to separate the subpopulations. For the two subpopulations, the mean (x^{-}), standard deviation, and variance (S) were calculated for each nutrient concentration as well as all the ratios between nutrient concentrations (N/P, N/K, P/K, etc.). A variance ratio (S^2 for low-yielding population/S^2 for high-yielding population) was calculated for each nutrient concentration, and of two ratios involving each pair of nutrients, finally selecting the one with the larger variance ratio. The mean and coefficient of variation (CV) values in the high-yielding population for the selected ratios were used for calculating DRIS indexes. The nutrient with the most negative index is considered the most deficient and most limiting to fruit yield and *mutatis mutandis* in the opposite case.

The following procedure as initially developed by Beaufils (1973) and modified by Bhargava (2002) was used through a PC-based program for the development of DRIS norms, comprising: (1) definition of the parameters to be improved and the factors likely to affect them, (2) collection of all the reliable data available from the fields and experimental plots, (3) study of the relationship between yield and available nutrients in soil, (4) establishment of the relationship between yield and leaf nutrient composition, using the following steps: (a) each internal plant parameter is expressed in so many forms as possible (e.g., N/DM, N/P, P/N, N × P); (b) the whole population is divided into a number of subgroups based on the economic optimum; (c) the mean of each subpopulation is calculated for the various forms of expression; (d) if necessary, class interval limits between the average and the outstanding yields are readjusted so that the means of below average populations remain comparable; (e) chi-square test is performed to know if the populations confirm a normal distribution; (f) the variance ratios between the yield of subpopulations for all the forms of expressions are calculated together with the coefficient of variation; (g) the forms of expressions, for which significant variance ratios were obtained and essentially the same mean values for the population were selected in expression with common nutrient; and (h) the following equations were developed for the calculation of DRIS indexes based on leaf analysis:

1. N = 1/9 [f (N/P) + f (N/K) + f (N/Ca) + f (N/Mg) + f (N/Fe) + f (N/Mn) + f(N/Cu) + f (N/Zn)]

$$\text{where } f(\mathrm{N/P}) = \left(\frac{\mathrm{N/P}}{n/p} - 1\right)\left(\frac{1{,}000}{\mathrm{CV}}\right) \text{ when } \mathrm{N/P} > n/p,$$

for example,

$$\text{and } \left(1 - \frac{n/p}{\mathrm{N/P}}\right)\left(\frac{1{,}000}{\mathrm{CV}}\right) \text{ when } \mathrm{N/P} > n/p,$$

where N/P is the actual value of the ratio of N and P in the plant under diagnosis, *n*/*p* the value of the norm (the mean value of high-yielding orchards), and CV the coefficient of variation for population of high-yielding orchards.

1. P = 1/9 [-f (N/P) + f (P/K) + f (P/Ca) + f (P/Mg) + (P/Fe) + f (P/Mn) + f (P/Cu) + f(P/Zn)]
2. K = 1/9 [-f (N/K) + (K/P) + (K/Ca) + (K/Mg) + (K/Fe) + (K/Mn) + (K/Cu) + (K/Zn)]
3. Ca= 1/9 [-f (N/Ca) – f(P/Ca) – f(K/Ca) + f (Ca/Mg) + f (Ca/Fe) + f (Ca/Mn) + f (Ca/Cu) + f (Ca/Zn)]
4. Mg = 1/9 [-f (N/Mg) – f(P/Mg) – (K/Mg) – f(Ca/Mg) + f (Mg/Fe) + f (Mg/Mn) + f (Mg/Cu) + Mg/Zn)]
5. Fe = 1/9 [-f (N/Fe) – f (P/ Fe) – f (K/Fe) – f (Ca/ Fe) – f (Mg/Fe) + f (Fe/ Mn) + f (Fe/Cu) + f (Fe/ Zn)
6. Mn = 1/9 [-f (N/Mn) – f (P/Mn) – f (K/Mn) – f (Ca/Mn) – f (Mg/Mn) -f (Fe/Mn) + f(Mn/Cu) + f (Mn/Zn)]
7. Cu = 1/9 [-f (N/Cu) – f (P/Cu) – f (K/Cu) – f (Ca/Cu) – f (Mg/Cu) – f (Fe/Cu) – f(Mn/Cu) + f (Cu/Zn)]
8. Zn = 1/9 [-f (N/Zn) – f (P/Zn) – f (K/Zn) – f (Ca/Zn) – f (Mg/Zn) – f (Fe/Zn) – f (Mn/Zn) – f (Cu/Zn)]

The norms for classification of nutrients in leaves are derived using the mean of high-yielding orchards as the mean for optimum. The range for optimum is the value derived from −4/3 to +4/3 standard deviation from mean. The range for low was obtained by calculating −4/3 to −8/3 standard deviation from mean, and the value −8/3 standard deviation below mean was considered deficient. The value above +4/3 standard deviation from mean was considered as an excess (Bhargava 2002).

5.3.6.3 Leaf Analysis-Based Norms

Methods for nutritional diagnosis using leaf analysis consist of critical value, the SRA, and DRIS. The last method, since it uses the balancing concept (relationship among nutrients), might be more precise in detection of nutritional disorders.

Early studies on DRIS were carried out at California (USA) by Beverly et al. (1984) when preliminary reference values were derived for nutritional diagnosis of N, P, K, Ca, and Mg for 'Valencia' sweet orange. These values were also used for subsequent comparisons with the SRA and, overall, both methods presented similar results. However, the DRIS diagnosis was affected by the sample tissue type and maturation, and the indexes reflected the nutrient concentration change related to the yield alteration or to the presence of fruits in the shoots at sampling time. The DRIS indexes were in agreement with the SRA diagnosis, only when changes in nutrient concentration significantly affected the second method.

In a subsequent work, Beverly (1987) suggested three modifications on the DRIS method and proposed two new methods for nutritional diagnosis for 'Valencia' sweet orange. The logarithmic transformation, the use of standard populations, and the adoption of a unique calculus procedure are modifications introduced to avoid systematic errors and simplify the diagnosis method, broadening its application. The two new suggested methods were based on individual plant nutrient concentrations instead of nutrient ratios. The diagnosis resulted similar to the one obtained by DRIS or SRA, but provided more precise recommendations when evaluated by field tests. After this work, new researches involving data collecting during five more years revealed that SRA could be more advantageous than DRIS for 'Valencia' sweet orange (Beverly 1992). The author compared SRA, DRIS, and three modifications of DRIS. The SRA showed efficacy (not presenting false diagnosis) for N and P diagnosis status in 75% and 90% of the cases, respectively, compared to 50%, or less, obtained by the other methods.

The most advantageous of the three available procedures for the DRIS indexes calculations was that proposed by Jones (1981) which calculated the DRIS norms for N, P, K, Ca, Mg, and S, using a reference subpopulation with productivity equal or superior to 120 kg tree^{-1}. All tested methods showed efficacy for K diagnosis.

Wallace (1990) carried out studies on DRIS for 'Valencia' sweet orange, from the established by Beverly et al. (1984), investigating several N, P, and K ratios and interactions. This author observed a 23% yield increase in response to K supply which amount to a 69% increase when N and P were also added. DRIS reveals to be an effective method for nutritional diagnosis in this study. Woods and Villiers (1992), in a research work developed at South Africa, obtained well-succeeded DRIS results for 'Valencia' sweet orange, in disagreement with the results reported by Beverly (1992). Those authors observed good correlation between yield (kg tree^{-1}) and fruit quality (fruit mass; g), with DRIS indexes derived from 1,700 observations. The results were compared with the conventional diagnosis method. The DRIS norms were also evaluated in fertilization experiments, and the increase in yield and fruit quality (fruit mass) was consistent with DRIS diagnosis.

To develop DRIS norms for 'Verna' lemon nutritional diagnosis, research was carried out by Cerda et al. (1995) at Murcia and Alicante (Spain). The adopted reference population presented yield equal to above 125 kg tree^{-1}. The DRIS determinations were influenced by the rootstock/scion combination and leaf sampling period. The results of diagnosis agreed with those obtained by the SRA only when the analyzed leaves came from the same period of sampling than the ones for the DRIS norms. Under salinity conditions, DRIS was not effective in detecting, if the cause of nutrient deficiency, that is, whether the nutrient unbalance was due to high salinity or fertilization deficiency. Results obtained in hydroponics were used to establish a data bank for DRIS indexes calculation for several citrus rootstock/scion combinations in Spain (Moreno et al. 1996). Useful reference values were determined for Fe availability evaluation and its influence in the nutrition of studied citrus rootstock/scion combinations, under sufficient and deficient Fe supply. A lemon scion budded on *Citrus macrophylla* rootstock showed less Fe-chlorosis deficiency symptoms compared to the same lemon budded on sour orange. *Citrus volkameriana* induced higher Fe-deficiency tolerance than Cleopatra mandarin when used as rootstocks combined with sweet orange scions.

DRIS norms were developed for 'Valencia' sweet orange for a plant population with different plant ages, on various rootstocks, at several regions for the four most important citrus-producing states of Venezuela (Rodriguez et al. 1997). The reference population was obtained through the selection of the 20% most productive plants. The values obtained were comparable to the previously determined, as referred in the literature. The authors concluded that DRIS method might be a low cost, timesaving, and trustful alternative for the development of nutritional diagnosis norms.

In Brazil, there is a paucity of publications on DRIS method investigation, especially in fruit crops. Apart from the research carried out in other species, as, for instance, in banana (e.g., Teixeira et al. 2002), few studies are reported in citrus. Bataglia (1989) was probably the first author to report the application of this method for citrus nutritional diagnosis and indicated DRIS as an alternative diagnosis method, pointing out the need of using it together with other diagnoses criteria. Creste (1996) reported the first DRIS evaluation by comparison with the SRA in groves of Brazil, studying 'Siciliano' lemon. Data were obtained from the analysis of leaves of fruit branches of different plant ages and rootstocks, collected in several harvesting years. The reference population was derived from plants with productivity greater than 80 t ha^{-1}. After the DRIS norm calculations, the method was evaluated under field conditions. DRIS showed to be more advantageous over the SRA, mainly because it was able to discriminate the nutrient importance order of deficiency or excess. Santos (1997) evaluated the DRIS method using results of leaf analysis derived from a series of field experiments with N, P, K fertilization in commercial groves of the State of São Paulo. This author obtained superior results with the DRIS compared to SRA, for detecting yield limitation by nutrient deficiency. Mourão Filho and Azevedo (2003) established DRIS norms for the 'Valencia' sweet orange budded on Rangpur lime, 'Caipira' sweet orange, and *Poncirus trifoliata* rootstocks. The nutritional balance and indexes calculated by the derived norms were highly correlated with yield for the rootstock/scion combinations, from what it was inferred that DRIS norms might be applicable always that leaf sampling is collected from nonbearing fruit branches of irrigated-plant groves.

DRIS indexes developed for different citrus cultivars in India predicted optimum value of different nutrients as: 1.70–2.81% N, 0.09–0.17% P, 0.96–2.59% K, 1.73–3.43% Ca, 0.24–0.92% Mg, 69.5–249.0 ppm Fe, 21.0–87.6 ppm Mn, 2.13–17.6 ppm Cu, and 11.6–50.0 ppm Zn, in relation to fruit yield of 31.6–37.9 kg tree^{-1} and 15.7–19.4 kg tree^{-1} for mandarins and acid lime, respectively (Table 5.4).

Nutrient diagnostics popularly used in Australia differ widely as per the diversity in citrus-growing regions. Jorgensen and Price (1978) suggested leaf nutrient norms for central coastal areas of Queensland which recommended optimum limit of different nutrients reading 2.4–2.6% N, 0.14–0.16% P, 0.9–1.2% K, 3.0–6.0% Ca, 0.23–0.60% Mg, 60–120 ppm Fe, 25–100 ppm Mn, 5–10 ppm Cu, and 25–100 ppm Zn, while Gallasch and Pfeiler (1988) developed a comprehensive leaf nutrient standard for Riverland District of Victoria and Sunraysia District of New South Wales (Australia) which suggested optimum limit of 2.4–2.7% N, 0.14–0.17% P, 0.70–1.49% K, 50–129 ppm Fe, 6–15 ppm Cu, and 25–60% Zn ppm.

These limits turn out to be widely different in China using optimum values measuring 3.0–3.5% N, 0.15–0.18% P, 1.0–1.6% K, 2.5–5.0% Ca, 0.30–0.60% Mg, 50–120 ppm Fe, 25–100 ppm Mn, 4–100 ppm Cu, and 25–100 ppm Zn for Satsuma mandarin grown on quaternary red earth (Alfisols) using third leaf from vegetative terminals (Wang 1985). On the other hand, the leaf nutrient standards have been developed for citrus belts (concentrated in seven provinces) of contrasting climates (the cool and warm regions separately) and fruit sizes (small and large) in South Africa (Du Plessis and Koen 1992).

Table 5.4 Leaf nutrient indexes (derived from DRIS-based analysis) for different commercial citrus cultivars of India

	Indexes		
Nutrients	Low	Optimum	High
Nagpur mandarin (*Citrus reticulata* Blanco)			
N (%)	1.12–1.69	1.70–2.81	2.82–3.38
P (%)	0.06–0.08	0.09–0.15	0.16–0.19
K (%)	0.22–1.01	1.02–2.59	2.60–3.38
Fe (ppm)	55.6–74.8	74.9–113.4	113.5–132.7
Mn (ppm)	40.2–54.7	54.8–84.6	84.2–98.7
Cu (ppm)	5.9–9.7	9.8–17.6	17.7–21.5
Zn (ppm)	5.5–13.5	13.6–29.6	29.7–37.7
Yield (kg tree^{-1})	12.9–47.6	47.7–117.2	117.3–152.1
Khasi mandarin (*Citrus reticulata* Blanco)			
N (%)	1.67–1.96	1.97–2.56	2.57–2.85
P (%)	0.06–0.08	0.09–0.10	0.11–0.13
K (%)	0.52–0.98	0.99–1.93	1.94–2.40
Fe (ppm)	22.6–84.5	84.6–249.0	249.1–331.3
Mn (ppm)	18.6–41.5	41.6–87.6	87.7–110.6
Cu (ppm)	1.83–2.12	2.13–14.4	14.5–20.6
Zn (ppm)	11.1–16.2	16.3–26.6	26.7–31.8
Yield (kg tree^{-1})	19.1–31.5	31.6–56.3	56.4–68.8
Mosambi sweet orange (*Citrus sinensis* Osbeck)			
N (%)	1.28–1.97	1.98–2.57	2.58–2.68
P (%)	0.050–0.090	0.091–0.17	0.18–0.21
K (%)	1.12–1.32	1.33–1.72	1.73–1.92
Ca (%)	1.09–1.72	1.73–2.98	2.99–3.62
Mg (%)	0.13–0.31	0.32–0.69	0.70–0.87
Fe (ppm)	25.9–69.4	69.5–137.1	137.2–200.1
Mn (ppm)	29.7–42.1	42.2–87.0	87.1–159.5
Cu (ppm)	2.0–6.5	6.6–15.8	15.9–20.5
Zn (ppm)	9.0–11.5	11.6–28.7	28.8–37.3
Yield (kg tree^{-1})	45.9–76.5	76.6–137.9	138.0–168.5
'Sathgudi' sweet orange (*Citrus sinensis* Osbeck)			
N (%)	1.32–2.00	2.01–2.42	2.43–2.60
P (%)	0.09–0.10	0.11–0.13	0.14–0.16
K (%)	0.72–1.11	1.12–1.82	1.83–2.01
Fe (ppm)	22.2–53.4	53.5–82.2	82.2–110.8
Mn (ppm)	18.2–48.6	48.7–79.3	79.4–116.2
Cu (ppm)	1.2–3.6	3.7–8.9	9.0–14.6
Zn (ppm)	10.2–16.4	16.5–23.2	23.3–35.8
Yield (kg tree^{-1})			

Adapted from Srivastava and Shyam Singh (2004a, 2008) and Varalakshmi and Bhargava (1998)

It remains to be seen that the diagnostic norms derived from specific index leaves and orchards, categorized into

Table 5.5 Leaf analysis-based DRIS indexes for identifying nutrient constraints in different citrus cultivars

'Nagpur' mandarin ($n=57$)	Nutrients found deficient and low ($n=27$)				Nutrients found high and excess ($n=30$)						Yield (kg tree^{-1})
	Zn	P	N	Fe	Cu	Mn	Mg	K	Ca		
Conc. (mg kg^{-1a})	9.2	0.06	1.56	68.3	19.2	91.6	0.92	2.62	3.34		32
DRIS indexes	−166	−60	−28	−20	16	42	55	63	98		
'Mosambi' sweet orange ($n=60$)	Nutrients found deficient and low ($n=32$)				Nutrients found high and excess ($n=28$)						Yield (kg tree^{-1})
	N	Zn	K	P	Mg	B	Ca	Mo	Fe	Cu	
Conc. (mg kg^{-1a})	1.28	9.1	1.14	0.08	0.70	28.2	3.01	1.1	138.1	18.1	39
DRIS indexes	−185	−111	−82	−58	38	40	48	74	92	144	
'Khasi' mandarin ($n=108$)	Nutrients found deficient and low ($n=68$)						Nutrients found high and excess ($n=40$)				Yield (kg tree^{-1})
	Zn	P	Ca	N	Mg	Cu	K	Mn	Fe		
Conc.(mg kg^{-1a})	10.5	0.06	1.66	1.60	0.18	1.9	1.98	94.2	268.1		22
DRIS indexes	−201	−101	−91	−86	−78	−42	104	219	276		

Adapted from Srivastava et al. (2007)

[a] Values of N, P, K, Ca, and Mg are given in %

deficient or optimum in different nutrients on the basis of nutrient concentration, have the same utility as that of norms developed through leaves sampled at other crop developmental stages (leaving the index sampling period) in order to make DRIS a more flexible monitoring tool without affecting the production at any crop stage. The overriding influence of physiography has a definite impact in dictating the relationship between the nutrient composition of index leaves, nutrient composition of soil, and the time of fruit maturity, when compared with the orchard conditions of valley versus hill slopes or floodplains versus hill slopes (Gualiya and Zonn 1990). Irrespective of such physiographical divergence, DRIS norms developed in one specific region may be applied to another region, if the elemental composition of high-yielding orchards is nearly identical, with normal skewness-free distribution of data.

5.3.6.4 Identification of Nutrient Constraints and Their Frequency Distribution

DRIS indexes are expressed by positive or negative values, which that the referred nutrient is in excess or deficiency range, respectively. The closer to zero are the indexes for all the nutrients. Occurrence of single or multiple nutrient deficiencies in citrus orchards is reported from all the six continents (Srivastava and Singh 2003a). These deficiencies are if not addressed in time through suitable diagnostic norms; the orchards coupled with reduced longevity continue to impart recurrent loss in production and imbalances the production economics. Works done for different commercial citrus cultivars in India showed nutrient deficiencies of Zn, P, N, and Fe due to their negative values in decreasing order (Table 5.5) using leaf analysis data. While, other nutrients, viz., Cu, Mn, Mg, K, and Ca with increasing positive indexes were observed in high to excess limit. A large positive nutrient index (more negative an index, the more lacking is the nutrient) indicates that the corresponding nutrient is present in relatively excessive quantity. Using the progressive nutrient diagnosis, if the first limiting factor Zn is corrected by its supply, the next nutrient that will limit the yield is P. Further, if Zn and P are satisfied, the next limiting nutrient is N followed by Fe (Table 5.5).

The frequency distribution of nutrient constraints as diagnosed through DRIS-based plant-soil nutrient diagnostics demonstrated a good complementarity between leaf and soil analysis. This was further evident from different correlation coefficient values between leaf and soil analysis values for N ($r=0.624$ $p=0.01$), P ($r=0.412$ $p=0.01$), K ($r=0.212$ $p=0.05$), Ca ($r=0.123$, nonsignificant), Mg ($r=0.181$, nonsignificant), Fe($r=0.416$ $p=0.01$), Mn ($r=0.512$ $p=0.01$), Cu ($r=0.458$ $p=0.01$), and Zn ($r=0.583$ $p=0.01$). The earlier studies have shown that nutritional problems of citrus orchards are better identified with the combined use of leaf and soil analysis than either of the two alone (Jorgensen and Price 1978; Srivastava et al. 2001). The nutrient Zn was estimated low to deficient (63.4–72.8%) followed by N (52.3–66.3%), K (28.3–35.3%), P (28.1–31.3%), and Fe (28.2–29.8%) irrespective of test methods used. Worldwide, Zn is claimed to be the single most frequently limiting nutrient impairing with the sustainable citrus production (Srivastava and Singh 2004a). These nutrient constraints laid the basis for fertilization to maximize the yield, and subsequently verify the ability of DRIS indexes in identifying the nutritional problems existing actually under the field conditions.

The utility of DRIS-based data is often questioned due to less dynamic in nature over a growth period limited by many interacting cofactors. This has resulted in limited work carried out in this direction. The studies undertaken with respect to commercial citrus cultivars in India demonstrated that mean DRIS indexes emerging deficient to low level of organic carbon, Zn, P, Fe, N, and K due to their negative values in decreasing order.

5.3.6.5 Validation of DRIS Indexes

Crop response studies are considered as the most reliable method of establishing the nutrient constraints occurring in the field. In an effort to validate DRIS norms in Nagpur mandarin through a fertilizer response experiment, various DRIS-based fertilizer treatments produced a significant response on both leaf nutrient composition and fruit yield (Table 5.6). The treatment (N, P, K, Zn, Fe) showed a significantly higher fruit yield (31.6 kg tree^{-1}) over other treatments such as $N_0P_1K_1Zn_1Fe_1$ (21.5 kg tree^{-1}), $N_1P_0K_1Zn_1Fe$ (23.2 kg tree^{-1}), and $N_1P_1K_0Zn_1Fe_1$ (22.2 kg tree^{-1}), supporting as an evidence to deficiency of N, P, and K, respectively. Such a fertilizer response became more evident from the changes in leaf nutrient concentration. The –N, –P, and –K respective treatments registered 1.89% N, 0.06% P, and 0.70% K, significantly lower over 2.04% N, 0.11% P, and 0.86% K.

The yield was further maximized to 43.3 kg tree^{-1} with an additional increment of N ($N_2P_3K_2Zn_1Fe_1$) registering over comparatively lower N rate ($N_1P_3K_2Zn_1Fe_1$) 36.4 kg tree^{-1}. Increasing K rate from K_2($N_2P_3K_2Zn_1Fe_1$) to K_4($N_2P_3K_4Zn_1Fe_1$) was associated with corresponding increase in leaf K from 1.19% to 1.54% imparting a corresponding increase in fruit yield from 43.3 to 58.8 kg tree^{-1}. The fruit yield was comparatively higher, 38.9 kg tree^{-1} with treatment containing Zn (N_1P_2 $K_2Zn_1Fe_1$) against only 32.1 kg tree^{-1} with no Zn treatment ($N_1P_2K_2Zn_0Fe_1$). Likewise, absence of Fe treatment ($N_1P_1K_1Zn_1Fe_0$) showed a lower fruit yield of 25.8 kg tree^{-1} against 31.6 kg tree^{-1} with presence of Fe treatment ($N_1P_1K_1Zn_1Fe_1$), establishing the deficiency of both Zn as well as Fe.

DRIS indexes for various nutrients demonstrated remarkable changes under different treatments, e.g., $N_1P_1K_1Zn_1Fe_1$ treatment registered much lower index −20 versus −52 with treatment $N_0P_1K_1Zn_1Fe_1$ for N, −38 with $N_1P_0K_1Zn_1Fe_1$ versus −08 with $N_1P_1K_1Zn_1Fe_1$ for P, and −43 with $N_1P_1K_0Zn_1Fe_1$ versus −35 with $N_1P_1K_1Zn_1Fe_1$ for K. The K indexes reduced from −43 with $N_1P_1K_0Zn_1Fe_1$ to as much as −04 with $N_2P_3K_4Zn_1Fe_1$ indicating that each additional level of K brought down the negative index of K. Both the micronutrients, Zn and Fe, registered a negative DRIS index as −18 and −20 with N_1P_2 K_2 $Zn_0Fe_{1,}$ and $N_1P_1K_1Zn_1Fe_0$, respectively, which significantly improved with those treatments supplying both the nutrients. It is important to recognize that an individual nutrient is not necessarily present in optimum concentration, if its indexes are equal to zero. A measure of total nutritional balance in a plant is indicated by the sum of nutrient indexes irrespective of sign. Increasing the nutrient doses reduced the sum of indexes from 164 ($N_0P_1K_1Zn_1Fe_1$) to as low as 38 ($N_2P_3K_4Z_1Fe_1$) which registered the highest yield (Table 5.6). The relationship between nutrient balance and yield thus became more visible. The fruit yield decreased substantially by increasing the sum of indexes ($r = -0.729$, $p = 0.01$) irrespective of their sign indicating that higher fruit yield is not necessarily obtained with large sum of indexes. Applying nutrient ratios instead of the isolated concentration values of each nutrient in the interpretation of leaf analysis, Mourão Filho and Azevedo (2003) reported a high correlation between the DRIS-based nutritional balance and fruit yield of 'Valencia' sweet orange orchards of São Paulo, Brazil.

5.4 Conclusion and Future Research

Often, the established standard sampling period many times occurs too late in the growing season so that fertilizer application will not be effective to correct the nutritional problem or may not match the sudden symptoms of a nutritional disorder when the producer mostly need the information (Walworth and Sumner 1987). To overcome this problem, there is a need for precise definition, of the sampling time, and important maturation stages of specific cultivar/variety. In addition to these limitations, little research has been developed to determine the influence of the cultivars in the nutrient concentration in a given maturation or developmental stage. Finally, factors that affect the tissue aging rate might also influence the relation between nutrient concentration and maturation. An option for these diagnostic methods is proposed through the DRIS (Beaufils 1973), which defined that, in general, nitrogen, phosphorus, and potassium concentrations decrease with tissue maturation. Therefore, the ratios N/P, N/K, and P/K (or reciprocal ratios) should remain constant. In the same way, because the concentrations of Ca and Mg generally increase with maturation, quotients between these nutrients (Ca/Mg or Mg/Ca) should result in constant values. Moreover, the product of two nutrients, with concentrations running in opposite directions with the time (N × Ca, for example), also should remain constant.

There are controversies regarding calculation procedures for the norms and DRIS indexes. One of the main questions is about the method application validation and the data universe that the norms are expected or supposed to represent. Most research results have indicated that the more specific is the universe for DRIS norms derivation, the more effective the method application is.

The criteria for the reference subpopulation definition also demand further studies and are, to a certain extent, specifically adjusted for each situation. In this way, DRIS norms should be developed for specific conditions, in which all factors to be correlated with yield or quality (or any other variable), to

Table 5.6 Progressive diagnosis with corresponding leaf nutrient concentration and fruit yield of Nagpur mandarin grown on Typic Haplustert soil type

Treatments					Leaf nutrient composition							DRIS indexes							Sum of indexes irrespective of sign	Mean fruit yield (kg tree^{-1})
N	P	K	Zn	Fe	N	P	K	Fe	Mn	Cu	Zn	N	P	K	Fe	Mn	Cu	Zn		
					(%)			(ppm)												
0	1	1	1	1	1.89	0.08	1.02	87.6	76.8	5.9	20.8	−52	−18	−12	42	18	20	02	164	21.5
1	0	1	1	1	1.97	0.06	0.86	89.6	49.1	6.5	17.9	−26	−38	−14	32	25	13	08	156	23.2
1	1	0	1	1	1.91	0.09	0.70	84.6	49.4	5.6	20.0	−28	−08	−43	42	20	11	06	158	22.2
1	1	1	1	1	2.04	0.11	0.86	88.5	48.6	5.9	19.7	−20	−07	−35	28	20	10	04	144	31.6
1	1	1	1	0	2.08	0.11	0.92	70.2	42.6	6.7	20.1	−06	−04	−28	−20	30	26	04	116	25.8
1	2	2	0	1	2.12	0.12	1.09	80.6	43.5	6.0	16.0	−14	−02	−20	18	20	16	−18	108	32.1
1	2	2	1	1	2.10	0.11	1.12	78.4	44.2	5.2	20.1	−20	−01	−14	10	17	10	−02	74	38.9
1	3	2	1	1	2.06	0.12	1.20	76.0	42.1	5.0	19.6	−18	05	−14	10	15	11	−04	72	36.4
2	3	2	1	1	2.26	0.13	1.19	82.4	50.6	6.7	19.8	−14	04	−13	08	10	09	−04	62	43.3
2	3	3	1	1	2.26	0.11	1.35	78.2	48.1	5.1	21.0	−12	03	−08	08	08	04	−03	46	49.3
2	3	4	1	1	2.32	0.11	1.54	86.2	48.7	5.5	27.0	−12	04	−04	06	06	03	−03	38	58.8
LSD ($p = 0.05$)					0.16	0.01	0.14	6.8	NS	NS	2.5									5.2

Adapted from Srivastava and Singh (2008) attain specific objectives, are known *per se*: cultivar, climate, soil and crop management, productivity, etc.

Finally, it is highlighted that researches, both in a worldwide basis, on DRIS method utilization are still in developing stage. Further investigations are necessary, on the identification and isolation of factors that significantly affect productivity under several citrus cultivar-specific management production systems, since DRIS reflects changes in nutrients concentration, due to alternate-bearing or crop-load effects in addition to age and type of tissue sampled.

DRIS-based nutrient diagnostic norms with further expansion under diverse applications to different critical growth stages of crop could prove an effective decision support system to address multiple nutrient constraints in order of decreasing or increasing influence on yield. A different nutrient diagnostic is required for prebearing to bearing orchards, and probably according to crop growth stages, since nutrients undergo a definite redistribution as per nutrient demand by developing fruits and supply form underground root system. Citrus decline is now a threatening problem in the world; application of DRIS holds a great promise in order to prioritize the impact of different nutritional disorders.

DRIS has further proven to be a good precision tool in extrapolating the nutrients level in relation to higher yield targets which have found a genuine validation through soil/leaf analysis values – crop response studies under long-term multilocation experiments. Studies of late have demonstrated good utility of DRIS for soil test studies, which will be very useful in predicting the soil test values-leaf nutrient interaction more gainfully to harness the consistency in production pattern of perennial crop like citrus. DRIS is warranted to be expanded to some nonessential nutrients like Na and Si.

Diagnosis of nutrient constraints based on DRIS analysis showed a good agreement between leaf and soil analysis data. All the nutrient constraints identified through original orchard data analysis further indicated a significant field response on fruit yield and improvement in respective nutrient concentration in leaves. These observations lend strong support for utility of DRIS in identification and management of nutrient constraints in citrus.

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