Chapter 5

Plant Analysis

Renato de Mello Prado and Gustavo Caione

Additional information is available at the end of the chapter

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1. Introduction

Several tools are available to evaluate the nutritional state of plants. Plant analysis is an efficient one since it uses the plant itself as a nutrient extractor. Thus, it complements soil chemical analysis and makes it possible to predict nutritional disorders before the appearance of visual symptoms in the plant tissue. However, it is necessary to integrate both techniques, chemical analysis of plants and chemical and physical analysis of soil, besides visual diagnosis to maximize fertilization efficiency in terms of cost and prevention of environmental damage.

Adequate fertilization avoids damage to the environment by reducing soil acids, water euthrophy, pollution of the phreatic zone and area salinization. Furthermore, efficient fertilizer handling is fundamental in any productive system, especially in the recent decades, due to increased cost, scarcity of some nutrient sources and consumer insistence for high quality products.

Precise analytic methods only are not sufficient to an adequate fertilization handling. A competent professional having theoretical and practical experience and knowledge about the various factors involved in the production chain, like interactions "soil-plant-environment- handling, is also an absolute requirement.

By plant analysis it is possible, among others, to determine culture nutrient needs and exportation, identify nutritional deficiencies that produce similar symptoms, evaluate nutritional states, help in the managing of fertilization programs and diagnose about levels of nutrients in diverse plant organs. Several procedure, direct and indirect, are available to achieve these aims. This chapter will emphasize the main methods utilized in the diagnosis of the nutritional state of plants, like chemical foliar analysis, biochemical tests, measurements of leaf green color and visual observation. The linked information in the present chapter

were obtained from an extensive literature review and also were inserted professional experiences of the authors.

Again, it is emphasized that efficient foliar diagnosis includes all procedures starting from correct field sampling to adequate laboratory analysis.

2. Factors that affect nutritional diagnosis

It is important to know the main factors that interfere in the diagnosis of plant nutritional status, so that confident analytic results are obtained and compared to pre-established literature standards. The composition of the vegetal tissue reflects the interaction of factors acting up to the moment the samples are collected for analysis.

Initially, it is necessary to exclude biotic and abiotic factors that affect nutrient concentrations in plants. Among these should be considered, lack or excess of water, high or low temperatures winds, pests and illnesses, compacted or poorly plowed soils, mechanical damage and herbicide toxicity [1]. These and other factors may produce deficiency symptoms in the plant by preventing absorption and/or translocation of nutrients. In such cases symptoms of deficiency are only eliminated by removing the stress factors.

Table 1 shows some of the factors directly involved in the appearance of real or apparent symptoms, which are similar and confound typical deficiency and toxic patterns.

Factors	Cause
Biotic	Occurrence of illnesses and/or pests causing damage to the aerial or root systems in the plant and inducing symptoms similar to mineral deficiencies. Natural leaf senescence produces color changes. It is a tightly regulated process involving the coordinated expression of specific genes and hormonal participation, mainly cytokinins and ethylene in sequential events and mechanisms that are not well known [2].
Abiotic	Extreme environmental conditions (temperature, drought, floods, strong winds), especially in the 10 to 15 days before sampling and recent removal of weeds. Inadequate application of products or interaction of products like chemical fertilizers, organic matter, fungicides, insecticides herbicides, antibiotics, growth regulators or foliar fertilizers, which could prevent absorption of a nutrient and/or simulate deficiency symptoms. Inadequate physical and/or chemical soil conditions like poor manipulation, erosion, sharp slopes, excess aluminum, iron or manganese, low levels of available nutrients. Culture practices inductive of plant abnormal symptoms, like poor irrigation, addition of organic matter not completely digested, intense pruning and deep soil harrowing.

Table 1. Effects of biotic and abiotic factors, that may directly or indirectly induce typical deficiency or toxicity patterns. (Adapted from [1]).

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To exclude the factors in Table 1, the professional must know the interactions "plant-environment-soil – farming activity" before proceeding to sampling.

3. Criteria for plant sampling

In this item, sampling criteria will be discussed, in terms of leaf analysis, although it should be emphasized that these principles are also applied in visual diagnosis and indirect methods.

Sampling is a fundamental step in the outcome of foliar analysis. Poor or inadequate sampling compromises all available recommendations. Results quality and precision are directly dependent on the procedure. This is a critical step since nutrients concentrations are not the same in all plant parts, and may differ according to age and variety. Foliar analysis results will only be useful and representative of the culture if sampling is correctly performed.

Some criteria are similar to the ones employed in soil sampling and follow basic procedures [3].

1. Cultures should be divided in plots not bigger than 10 ha, having uniformity in age, variety, spacing, soil and manipulations.
2. In each plot, the indicated leaves from the desired cultures are collected in a zigzag direction.
3. Preferably, collections should be made between 7 and 11 AM, more than 24 hours after a rain
4. At least 20 leaves must be collected from each plot and mixed before being sent to the laboratory.
5. All samples must be packed in clean unused paper bags to avoid contamination.
6. Samples are identified by tags corresponding to each plot
7. Samples should be immediately sent to the laboratory. When this is not possible the material must be kept in an isolated container, fitted with a 150w lamp during 72hours, for initial drying.
8. Sampling must never be conducted after fertilization or spraying. In these cases, collection of samples is made 30 days later to avoid foreign residues.
9. Leaf samples are sent to the Foliar Analysis Laboratory after complying with the rules described.

Additional important details are:

- damaged or abnormal looking leaves must not be collected unless this is caused by nutritional problems
- soil-contaminated samples should be avoided and also the ones collected from plants situated close to roads or entrance pathways.

- sample collector must make sure hands are clean.
- samples packed in open and perforated paper bags sent to the laboratory two days after collection do not need decontamination and previous field drying procedures. If this is not possible, samples could be treated as already described or a) washed successively in clean water, 0.1% detergent solution, clean water followed by drying in a 70ºC oven or in a sunny environment before being sent to the laboratory and b) samples packed in polyethylene bags may be kept at low temperatures (2 to -4ºC) for a maximum of 72 hours.

Recent matured leaves are the usual plant organ analyzed but eventually stem pieces or branches may be used. In leaves, analysis may be performed in the whole structure or only in specific parts like the lamina or the petiole. In some cases, like in sugar cane, the leaf midrib is removed when foliar diagnosis is desired. In perennial cultures, like coffee or citrus, leaf composition may vary by the presence or absence of fruit in branches. In general, recent matured and physiologically active leaves are the plant organs, which better reflect the nutritional status. They respond more readily to variations in nutrient supply and are, thus, better qualified as samples.

Concerning the number of samples, it must be enough to reduce variability and be representative of the plant population. In rare occasions, dry material in each sample must exceed 10g (100 to 200g fresh green tissue for most species), but this indicates that different number of samples may be necessary for particular needs of cultures and soils. On average, it is considered that 20 single units would be sufficient to compose a sample [1].

4. Recommendations about foliar sampling and adequate nutritional levels in some plant species

To evaluate nutritional status the sample, one plant, a set of plants or a previously determined plant part, must be compared to a standard, which consists of a set of nutritionally "healthy" plants. A plant is considered "healthy" when all its tissues show nutrients in adequate quantities and proportions, it is able to attain high productivity and it looks like the specimens found in very productive cultures. However, the reference culture must be as close as possible of the culture to be sampled and analyzed, and it should be a true representative of the peculiar soil-climate characteristics as well as of type of handling and ecological zoning. The reference may have the best productivity but the comparison must be with the same genetic material under the same handling regime and the sampling must follow the same procedures for normal and problem plants.

It is important to establish which plant part it is going to be analyzed in the best period since composition of different parts is not the same and nutrient concentrations also vary according to growth stage.

The previously established physiological stage of the comparison standard must be kept if it is available, or else if this is not existent the start of the reproductive stage should be preferred being a period of the highest nutrient concentration. Thus, if a deficiency is detected, it can still be corrected and it will not compromise or minimize the productivity of the next crop.

The sampling must follow recommendations, as discussed above, to produce reliable analytical results that will be compared to a standard. The analytical results should be produced by a competent laboratory engaged in a constant quality control program.

Publications by several authors, [4], [5], [6], [7], [8], [9], [10], [11], report previously defined plant organs, sample numbers and the sampling period for diverse cultures. But to utilize such data as a standard it is necessary to be careful about the physiological age of each plant and leaf as stated by the author.

Table 2 shows that there is not a standard method of sampling for all cultures. Furthermore, adequate levels of nutrients vary according to different authors emphasizing the care that should be taken to always consider the same author when following a method of collection and in comparing the adequate nutrient levels. It should be noted that adequate levels of chlorine were not described and only some gave values for molibdenium.

Culture	Plant organ; number of samples and period of sampling	Adequate dosages		References
		Macronutrients (g kg^{-1})	Micronutrients (mg kg^{-1})	
	Fruits			
Avocado	50 leaves (1/plant) for each homogeneous plot Type of leaf: leaves 5 to7 months old, recently expanded from medium height crowns. February to March.	N - 16-20 P - 0,8-2,5 K - 7-20 Ca - 10-30 Mg - 2,5-8,0 S - 2,0-6,0	B - 50-100 Cu - 5-15 Fe - 50-200 Mn - 30-100 Mo - 0,05-1,0 Zn - 30-100	[4]
Pineapple	50 leaves (1/plant) for each homogeneous plot. Type of leaf: recently matured "D" (generally the 4th leaf from the apex), soon before floral induction. Cut leaves in pieces of 1 cm wide, eliminating the basal portion without chlorophyll. Homogenize and separate about 200 g to be sent to the laboratory.	N - 15-17 P - 0,8-1,2 K - 22-30 Ca - 8-12 Mg - 3-4 S - 2-6	B - 20-40 Cu - 5-10 Fe - 100-200 Mn - 50-200 Mo - Zn - 5-15	[6]
Acerola Barbados Cherry	50 leaves (1/plant) for each homogeneous plot. Type of leaf: to sample the 4 sides of the plant, for young leaves totally expanded from fructifying branches.	N - 20-24 P - 0,8-1,2 K - 15-20 Ca - 15-25 Mg - 1,5-2,5 S - 4-6	B - 25-100 Cu - 5-15 Fe - 50-100 Mn - 15-50 Mo - Zn - 30-50	[4]
Banana	25 leaves (1/plant) for each homogeneous plot less than 4ha. Tree it is recommended to sample the third	N - 27-36 P - 1,6-2,7 K - 32-54	B - 10-25 Cu - 6-30 Fe - 80-360	[4, 5]

Culture	Plant organ; number of samples and period of sampling	Adequate dosages		References
		Macronutrients (g kg^{-1})	Micronutrients (mg kg^{-1})	
	leaf from the apex when the inflorescence shows all the uncovered female bunches (without bracts) and not more than three male flower bunches. Collect 10 to 25 cm of the internal median part of the limb and eliminate the central rib. For varieties: Nanica, Nanicão e Grande Naine,under irrigation regimens.	Ca - 6,6-12 Mg - 2,7-6 S - 1,6-3	Mn- 200-1800 Mo - Zn - 20-50	
Banana	30 leaves (1/plant) for each homogeneous plot. Type of leaf: the 5-10 cm central part of the 3rd leaf from the inflorescence, eliminating the central rib and the peripheral halves.	N - 27-36 P - 1,8-2,7 K - 35-54 Ca - 3-12 Mg - 3-6 S – 2,5-8	B - 10-25 Cu - 6-30 Fe - 80-360 Mn- 200-2000 Mo - Zn - 20-50	[6]
Banana	25 leaves (1/plant) for each homogeneous plot less than 4ha. For the banana tree it is recommended to sample the third leaf from the apex, when the inflorescence shows all the uncovered female bunches (without bracts) and not more of three male flower bunches. Collect 10 a 25 cm of the internal median part of the limb, and eliminate the central rib. For varieties: Prata, Anã, under irrigation regimens	N - 25-29 P - 1,5-1,9 K - 27-35 Ca - 4,5-7,5 Mg - 2,4-4,0 S - 1,7-2	B - 25-32 Cu - 2,6-8,8 Fe - 72-157 Mn - 173-630 Mo - Zn - 14-25	[11]
Orange	100 leaves (4leaves/tree), for each homogeneous plot. Type of leaf: 3rd leaf from the fruit. Leaf born in the spring, 6 months old, in branches with fruit 2 to 4cm in diameters.	N - 23-27 P - 1,2-1,6 K - 10-15 Ca - 35-45 Mg - 2,5-4,0 S - 2-3	B - 36-100 Cu - 4-10 Fe - 50-120 Mn - 35-300 Mo - 0,1-1,0 Zn - 25 - 100	[6]
Fig	100 leaves (4 leaves/tree) for each homogeneous plot. Type of leaf: recently matured and totally expanded leaf, in the middle portion of a branch, 3 months after sprouting.	N - 10-25 P - 1,0-3,0 K - 10-30 Ca - 30-50 Mg - 7,5-10 S - 1,5-3,0	B - 30-75 Cu - 2-10 Fe - 100-300 Mn - 100-350 Mo - Zn - 50-90	[4]

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Culture	Plant organ; number of samples and period of sampling	Adequate dosages		References
		Macronutrients (g kg^{-1})	Micronutrients (mg kg^{-1})	
Guava c.v. Paluma	30 leaves (1/plant) for each homogeneous plot. Type of leaf: 3rd pair of leaves, recently matured (with petiole) from branch extremities, collected in the period of full bloom in the culture.	N - 20-23 P - 1,4-1,8 K - 14-17 Ca - 7-11 Mg - 3,4-4 S - 2,5-3,5	B - 20-25 Cu - 20-40 Fe - 60-90 Mn - 40-80 Mo - Zn - 25-35	[7]
Mango	80 leaves (4/tree) for each homogeneous plot. Type of leaf: middle leaves in branches with flowers in the extremities from the last vegetative flux. Thus, during florescence.	N - 12-14 P - 0,8-1,6 K - 5-10 Ca - 20-35 Mg - 2,5-5 S - 0,8-1,8	B - 50-100 Cu - 10-50 Fe - 50-200 Mn - 50-100 Mo - Zn - 20-40	[6]
Apple	100 leaves (4 a 8/plant) for each homogeneous plot. Type of leaf: recently matured and totally expanded.	N - 19-26 P - 1,4-4 K - 15-20 Ca - 12-16 Mg - 2,5-4 S - 2-4	B - 25-50 Cu - 6-50 Fe - 50-300 Mn - 25-200 Mo - 0,1-1,0 Zn - 20-100	[6]
Papaya	15 petioles of young leaves, totally expanded. (1/tree) for each homogeneous plot. When leaves are mature (17th to 20thleaves from the apex), with a visible axially set flower.	N - 10-25 P - 2,2-4 K - 33-55 Ca - 10-30 Mg - 4-12 S -	B - 20-30 Cu - 4-10 Fe - 25-100 Mn - 20-150 Mo - Zn - 15-40	[6]
Passion fruit	20 laves (1/tree) for each homogeneous plot. Type of leaf: 3rd or 4thleaf, from the apex of non-shaded branches. (As an alternative, collect a leaf with an axially located floral bud soon to be opened). Autumn.	N - 43-55(33-43) P - 2,3-2,7(1,2-2,1) K - 20-30(22-27) Ca - 9-25(12-16) Mg - 1,9-2,4(2,5-3,1) S - 3,2-4	B - 40-100 Cu - 10-15 Fe - 120-200 Mn - 40-250 Mo - 1,0-1,2 Zn - 25-60	[4]
Peach	100 leaves (4/tree) for each homogeneous plot. Type of leaf: recently matured and totally expanded.	N - 30-35 P - 1,4-2,5 K - 20-30 Ca - 18-27 Mg - 3-8 S - 1,5-3	B - 20-60 Cu - 5-16 Fe - 100-250 Mn - 40-160 Mo - Zn - 20-50	[4]
Grape	100 leaves (1/tree) for each homogeneous plot.	N - 30-35 P - 2,4-2,9	B - 45-53 Cu - 18-22	[6]

Culture	Plant organ; number of samples and period of sampling	Adequate dosages		References
		Macronutrients (g kg^{-1})	Micronutrients (mg kg^{-1})	
	Type of leaf: the youngest, recently matured, from branch apices.	K - 15-20 Ca - 13-18 Mg - 4,8-5,3 S - 3,3-3,8	Fe - 97-105 Mn - 67-73 Mo - Zn - 30-35	
		Cereals		
Corn	30 leaves/ha, of a homogenous plot showing female inflorescence (hair). Type of leaf: leaf obsta and below the corn ear	N - 27,5-32,5 P - 2,5-3,5 K - 17,5-22,5 Ca - 2,5-4 Mg - 2,5-4 S - 1,5-2	B - 15-20 Cu - 6-20 Fe - 50-250 Mn - 50-150 Mo - 0,15-0,2 Zn - 15-50	[8]
Sorghum	30 leaves/ha of a homogeneous plot at the start of tillering. Type of leaf: median	N - 13-15 P - 4,0-8,0 K - 25-30 Ca - 4-6 Mg - 4-6 S - 0,8-1	B – 20 Cu - 10 Fe - 200 Mn - 100 Mo - Zn – 20	[8]
		Forest species		
Eucalyptus	18 leaves/ha of a homogeneous plot, in Summer- Autumn. Type of leaf: recently matured primary branches in the superior third of the plant.	N - 14-16 P - 1-1,2 K - 10-12 Ca - 8-12 Mg - 4-5 S - 1,5-2	B- 40-50 Cu - 8-10 Fe - 150-200 Mn - 100-600 Mo - 0,5-1 Zn - 40-60	[8]
Pinus	18 leaves/ha of a homogeneous plot in Summer-Autumn. Type of leaf: Recently matured , primary	N - 12-13 P- 1,4-1,6 K - 10-11 Ca - 3-5 Mg - 1,5-2 S - 1,4-1,6	B - 20-30 Cu - 5-8 Fe - 50-100 Mn - 200-300 Mo - 0,1-0,3 Zn- 34-40	[8]
		Oilseeds		
Peanut	30 leaves/haof a homogeneous plot at the start of flowering. Type of leaf: 4th leaf of the main stalk from the basis (1ª = above the cotyledon air ebrabches).	N – 40 P - 2 K - 15 Ca - 20 Mg - 3 S - 2,5	B - 140-180 Cu - Fe - Mn - 110-440 Mo - 0,13-1,39 Zn -	[8]
Sunflower	30 leaves/ha of a homogeneous plot at the start of flowering.	N - 33-35 P - 4-7	B - 50-70 Cu - 30-50	[8]

Culture	Plant organ; number of samples and period of sampling	Adequate dosages		References
		Macronutrients (g kg^{-1})	Micronutrients (mg kg^{-1})	
	Type of leaf: leaves of the upper third.	K - 20-24 Ca -17-22 Mg - 9-11 S - 5-7	Fe - 150-300 Mn - 300-600 Mo - Zn - 70-140	
Soybean	30 leaves/ha of a homogeneous plot at the end of flowering. Type of leaf: first matured leaf from the branch end, excluding the petiole. General lythea 3rdleaf	N - 45-55 P - 2,6-5,0 K - 17-25 Ca - 4-2 Mg - 3-10 S - 2,5	B - 21-55 Cu - 10-30 Fe - 51-350 Mn - 21-100 Mo - Zn - 21-50	[8]
---------- **Saccharine** ----------				
Sugarcane (Plant)	20-30 leaves/ha of a homogeneous plot Type of leaf: leaf +3; leaf +1 = with the first ligula (=membranous outgrowth at the junction between the leaf blade and the sheath). Median third excluded the main rib	N - 19-21 P - 2-2,4 K - 11-13 Ca - 8-10 Mg - 2-3 S - 2,5-3	B - 15-50 Cu - 8-10 Fe - 200-500 Mn -100-250 Mo - 0,15-0,3 Zn - 25-30	[8]
Sugarcane (Ratoon)	20-30leaves/ha of a homogeneous plot, 4 month after sprouting. Type of leaf: leaf +3; leaf +1 = with first ligula (=membranous out growth at the junction between the leaf blade and the sheath). Median third excluded the main rib.	N - 20-22 P - 1,8-2 K - 13-15 Ca - 5-7 Mg - 2-2,5 S - 2,5-3	B - Cu - 8-10 Fe - 80-150 Mn - 50-125 Mo - Zn - 25-30	[8]
---------- **Vegetable crops** ----------				
Potato	30 leaves/ha of a homogeneous plot, in the middle of the cycle, 30-45 days after emergence. Type of leaf: Petiole of the 4^{ty} leaf from the tip.	N – 30 P - 3,5 K - 50 Ca - 20 Mg - 7,5 S - 3,5	B - 40-50 Cu - 5-8 Fe- 800-1000 Mn - Mo - Zn -	[8]
Onion	40 tip leaves/ha of a homogeneous plot at the middle of the cycle. Type of leaf: the highest one.	N – 40 P - 3 K - 40 Ca - 4 Mg - 4 S – 7	B - Cu - Fe - Mn - Mo - Zn -	[8]
Tomato	40 leaves/ha of a homogeneous plot in full flowering, or first ripe fruit;	N – 30 P - 3,5	B - 50-70 Cu - 10-15	[8]

Culture	Plant organ; number of samples and period of sampling	Adequate dosages		References
		Macronutrients (g kg^{-1})	Micronutrients (mg kg^{-1})	
	Type of leaf: 4th from the tip.	K - 40 Ca - 14-18 Mg - 4 S – 3	Fe - 500-700 Mn - 250-400 Mo - 0,3-0,5 Zn - 60-70	
---------- Stimulants ----------				
Coffee	At least 30 days after the 2nd fertilizer portion or after one foliar spraying, in the pinhead phase, that is, before grain filling (December) sample the 3rd or 4th pair of leaves from the apex of productive branches, located in the plant median portion. Collect two pairs of leaves in both sides of the row in a total of 25 plants /homogeneous area sampled (100 leaves/ sample).	N - 29-32 P – 1,2-1,6 K - 18-22 Ca - 10-13 Mg - 3,1-4,5 S - 1,5-2	B - 40-80 Cu - 8-16 Fe - 70-180 Mn - 50-200 Mo - 0,1-0,2 Zn - 10-20	[9]
Cocoa tree	18 leaves/ha of a homogeneous plot in the Summer. Type of leaf: 3rd leaf from the tip, mature in plants half-shade.	N - 19-23 P - 1,5-1,8 K - 17-20 Ca - 9-12 Mg - 4-7 S - 1,7-2	B - 30-40 Cu - 10-15 Fe - 150-200 Mn - 150-200 Mo - 0,5-1,0 Zn - 50-70	[8]

Table 2. Procedures for leaf collection and ranges considered adequate of macro and micro nutrients contents in some cultures.

The table shows how important it is to follow the same recommendation (standard) for sampling and after the comparison of results. Collection mistakes that lead to wrong diagnostics and recommendations are common. It is emphasized that the main factors responsible for different nutritional levels in plants are:1- plant age; 2- organ analyzed; 3-type of plant (species, variety, graft/stock, crown);4- period of the year; 5- method of sample cleaning, extraction and quantification of nutrients; 6- water percentage in soil (for nutrients determined in the sap); 7- time of day (for nutrients determined in the sap);8- inadequate production of dry matter from the plant due to isolated or interative soil, climate, genotypic or human imperfections [1].

5. Preparation of vegetal material for analysis

In the laboratory the collected plant material is decontaminated (only the fresh non-dried material), dried, ground, the residual humidity is determined followed by weighing, nu-

trients quantification and results expressed. More details about the stages in plant analysis, including the determination of macro and micro nutrients is reported in [12] and [8].

5.1. Sample decontamination

According to [12], when only macro nutrients are to be determined in samples washing may be plain, to eliminate gross contaminations like dust. Just shaking the sample under tap water and rinsing with distilled water will be enough but the procedure must be fast to avoid the loss of soluble elements. Procedures are more elaborate when determination of microelements is contemplated. In this case the samples must be successively washed in tap water, dilute non -ionic detergent (0.1%, v/v), distilled water to remove detergent, 0.1M HCl, distilled water and finally deionized water. With samples highly soiled the battery of solvents must be changed as necessary. To avoid loss of soluble inorganic constituents the washing stages must not take more than 30 seconds.

Contaminations by pesticides and foliar fertilizers (especially when applied with surfactants in the spraying mixture) are difficult to remove by washing. Collection of samples in these cases must be carefully overseen.

5.2. Drying

Drying of samples must be as fast as possible to minimize biological and chemical alterations. After eliminating excess water, samples packed in paper bags are dried in 65 to 70ºC ovens fitted with devices for forced air circulation [12]. According to [1] temperatures must be higher, 70 to 80ºC to avoid putrefaction especially if samples are too close together. Samples should be kept in the oven till constant weight, which will be attained after 48 to 72 hours, depending on the vegetal material.

5.3. Grinding

Mills provided with stainless steel or plastic chambers are recommended to grind the vegetal material to reduce to a minimum contamination by micronutrients like Fe and Cu. Grinding is necessary to homogenize samples for analytical determinations and it must produce material that can be sieved through 1 to 20 mesh when using Wiley type mills. When alternating grinding of different samples mills are cleaned by brushing with 70% alcohol between procedures.

5.4. Chemical analysis: extraction and determination of nutrients

Chemical quantification of nutrients is the next step in the diagnosis of the nutritional status of a foliar sample. Several factors are involved in the choice among the different analytical methods available for this purpose. Some of them are: safety (hazard or toxicity), equipment available, type of element to be determined, precision and accuracy, period of time taken by analysis, limit of detection and cost [13].

In the laboratory the sample will be submitted to the following procedures: weighing, preparation of the extract and element determination (Figure 1).

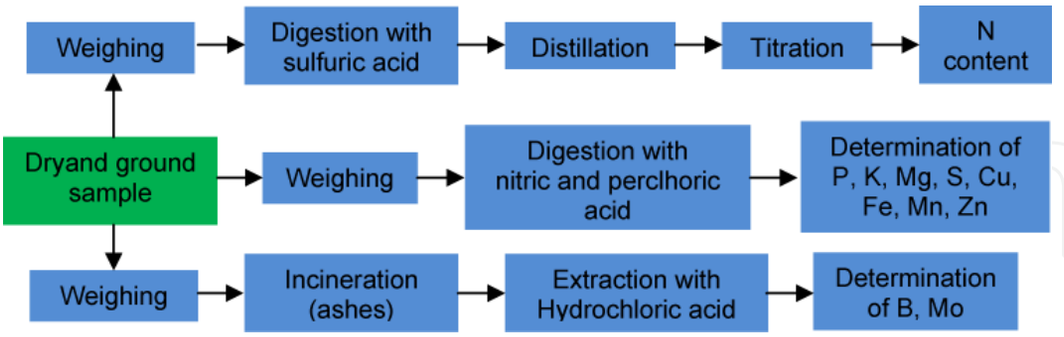

Figure 1. Simplified schematic procedure of foliar analysis to be conducted in a plant nutrition laboratory.

6. Interpretation of analytical results

Results are interpreted by comparing the concentration values of each element in the sample with the respective standard or a value considered optimal.

The foliar chemical analysis may be expressed in different methods, the most used are: 1- in the single variable methods only one of the elements is selected and the results are expressed by the deviation of the optimum percentage, the critical level and the sufficiency range; 2- the relation between the concentration values of nutrients is the basis for the double variable method named DRIS (Diagnosis and Recommendation Integrated System), or 3- the multivariable method named NCD (Nutritional Composition Diagnosis).

The flow chart in Figure 2 shows all the steps involved in foliar diagnosis starting with sampling up to the results obtained.

6.1. Sufficiency range

Most cultures do not have a single definite point for optimal production but a range of nutrient concentrations. Thus, it is adequate to recommend degrees of fertilization to keep nutrients slightly above the critical level, but included in the sufficiency range [14]. However, both have limitations the critical level by its precise character and the sufficiency range for lack of precision due to very wide limits.

The use of the sufficiency range is an attempt to extend a single optimal point into an optimal range and to make sure that at its highest level the culture is adequately supplied and at the lowest level it is so deficient that production will be negatively affected [1]. Generally, the sufficiency range corresponds to 90-100% of maximal production [15]. Also, the lowest limit of the sufficiency range will be the minimal critical point and superior limit the toxic critical point [1].

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The ratio, foliar concentration and production is characterized by different ranges or zones (Figure 3), which should be discussed as detailed by [16].

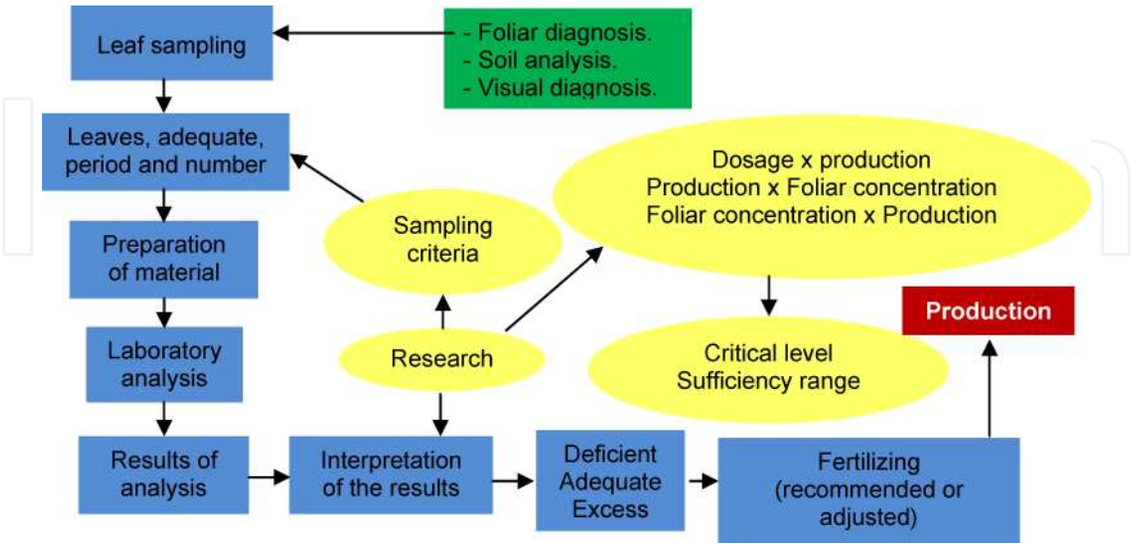

Figure 2. Flow chart for evaluation of the nutritional status of plants and its expansions according to the critical level and sufficiency range.

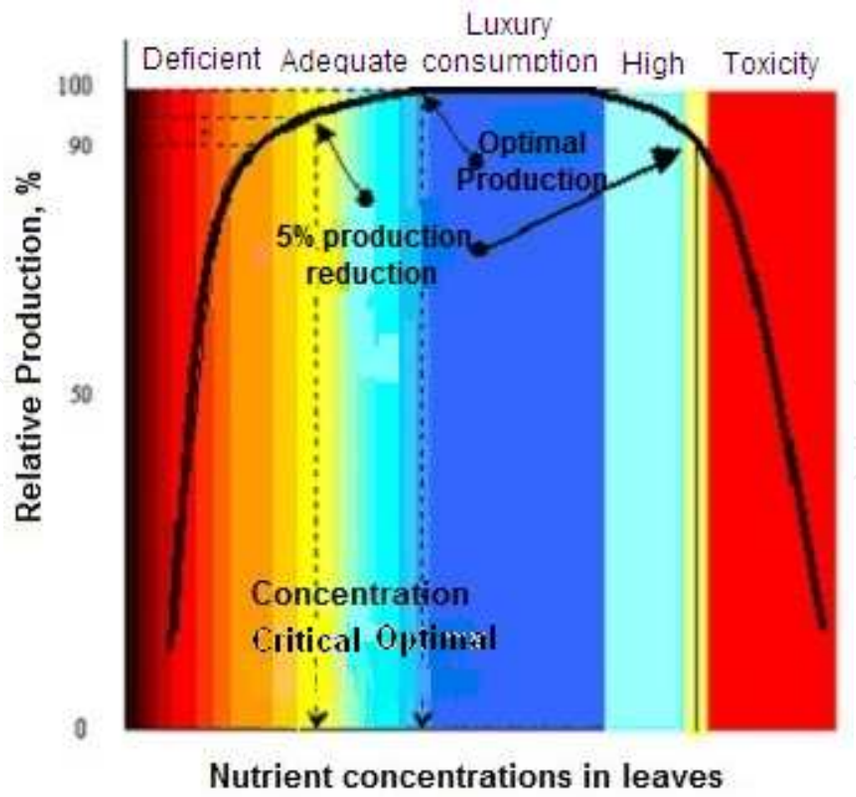

Figure 3. Relation of nutrient concentration and relative production.

1. In the deficient range or zone the symptoms are visible and occur in soils (or substrates) very deficient in an element due to insufficient dosages. In these conditions the response in production of dry matter is high, the element concentration is not increased and it may even be diluted. The nutrient dilution effect caused by organic matter formation is known as the Steembjerg effect. When the concentration of a plant nutrient is set in this range it is considered deficient.

2. In the transition range or zone, deficiency symptoms are not visible (disguised hunger) but there is a direct relation between nutrient foliar concentration of and production. When the nutrient concentration allows an average of 80 to 95% of the maximum production, this level corresponds to the critical level. The relation of nutrient concentrations and maximal production (100%) is seen in soils (or substrates) with slight deficiency and with lower responses in growth and production when the nutrient is applied. In these conditions the increases in foliar concentrations are proportional to growth and production, that is, greater absorption is compensated by increasing organic material. A nutrient concentration in this range, considered between the critical level and maximal production is interpreted as adequate.

3. In the luxury consumption range or zone, increasing element concentration does not increase production. This is observed in non-deficient soils receiving element dosages.

Although plant tissues show absorption of the increased nutrient concentrations this is not expressed in increased growth. Thus, the element concentration in this range, which corresponds to maximal or optimal production and it is below the toxicity critical point, is considered to be high.

4. The toxicity range or zone starts when increased nutrient concentrations significantly reduce production. Reductions of 5% up to 20% indicate toxic levels. The condition is observed in soils (or substrates) with excess nutrients receiving additional dosages that are absorbed as shown by increased tissue concentrations but expressed in decreased growth and/or imbalance in relation to other nutrients.

The critical level of deficiency is a factor largely employed in research and it corresponds to an optimal nutrient concentration. Below it the growth index (production or quality) is significantly decreased and above it, production represents poor economics.

After attaining maximal production, increased nutrient concentrations will not result in growth but in plant "luxury consumption". During this period nutrients accumulate in cell vacuoles and may be gradually liberated to supply eventual plant nutritional necessities. As already stated nutrient concentrations above the level of luxury consumption can lead to decreased production and characterize the toxicity range.

Interpretation of foliar nutrient concentrations based on the critical level and the sufficiency range is made directly by comparison with standard values. The plant nutritional status (deficiency, sufficiency, luxury consumption) is defined independently for each element by the range of values found for the sample. However, the plant mineral composition is the result of its adaptation to an environment under the action of several limiting factors. Lack of con-

sideration of well-known and documented interactions between elements is severely criticized in these methods [17, 18].

6.2. Deviation from the percentage optimum

The deviation from the percentage optimum (DPO) is an improvement of the critical level method [19]. It evaluates each nutrient concentration in relation to the optimum value (median of the sufficiency range) by the expression: DPO= [(Cx100/C_R)-100] where C is an element concentration in the sample dry matter and C_R it is the optimal concentration for the same conditions (culture, tissue analyzed, manipulation, plant development stage etc.). In the absence of the sufficiency range the critical level is taken as the optimum value.

This is a procedure not common in the literature but it permits the evaluation of the nutritional status of the plant and the arrangement of the elements as a function of the degree of deficiency. However, the limitation order is not representative because element interactions are not considered and the conventional table is still used.

6.3. Diagnosis and Recommendation Integrated System (DRIS)

DRIS is an alternative to the conventional method for the determination of the nutritional status of a plant [20]. It considers nutrient interactions in the diagnostic process, which is conducted by the combination of all the relations in the form of ratios [20] or products [21]. In this technique indexes, which express nutrient equilibrium in a plant or culture are calculated for each one, as a function of concentration ratios of each element and the total and compared in groups of two to other ratios considered standard or norms obtained in a population of highly productive plants.

Foliar diagnosis, in this method, aims to adjust fertilization, so far only recommended by soil fertility and culture productivity, by additional production gains and correction of deficiencies. It also makes possible the management other nutrient availabilities, possibly reducing them and permitting an equilibrated fertilization, in view of the culture nutritional necessities.

6.4. Diagnosis of nutritional composition (DNC)

The method relates nutrient concentrations in a multivariable form, as a function of ratios of each nutrient concentration and the geometrical mean of the nutritional composition of the sampled tissue [23]. The method is not widely used although it deals with relations between all elements analyzed.

DNC and DRIS are independent calibration methods, since use of double or multi variable methods minimizes non controlled effects of accumulated biomass, in contrast to the critical level, which needs calibration assays conducted in places and different years, and maintain control on other production factors (including other nutrients) and on a supply adequate to full plant development [24].

However, it is important to emphasize that all methods that interpret foliar analysis results are based on analysis of nutrient concentrations in plant dry matter. Thus, all procedures described in the previous topics (excluding biotic and abiotic factors that may interfere in the collection, preparation and analysis of sample and results) should be well conducted, since no analytical or interpretative method will correct mistakes in these steps.

7. Visual diagnosis

Visual plant nutrition diagnosis aims a detailed characterization of deficiency or toxicity symptoms in a plant-problem and compare them to standard patterns of deficiency or toxicity described in the literature.

To use this diagnosis it is necessary to make sure that the problem is caused by deficiency or excess of a nutrient, and not by pests and other diseases that may "masque" the problem by producing similar symptoms.

The symptoms caused by nutritional disorder generally have the following characteristics:

1. Dispersion- nutritional problems usually occur in the fields in a homogeneous form. In cases of pest/diseases the occurrence may be limited to isolated plants or dense growth. Nutritional deficiencies rarely appear only in some plants.

2. Simetry- nutritional disorders usually occur symmetrically in leaves while phytopathogenic or insect injuries provoke asymmetrical symptoms with the exception of the ones caused by viruses, which translocate though the whole plant and may produce foliar symptoms similar to nutritional deficiency.

3. Gradient- in a plant or branch the symptoms appear in a gradient, becoming more severe going from old to young leaves or in reverse, according to the element mobility in the plant.

In visual diagnosis symptoms of deficiency/excess may vary in cultures. Generally, deficiency signs start in older leaves for the easily distributed elements and in new leaves and shoots for elements of lower redistribution. The signs may be visualized in roots, like in conditions of Al toxicity, which induces ill-formed roots, thick and short. Visual symptoms of nutritional deficiency may be grouped in six categories: a) reduced growth; b) uniform chlorosis or leaf spots; c) interrib chlorosis; d) necrosis; e) red color; f) deformities.

The visual diagnosis method allows for fast identification of deficiencies or excesses with consequent correction of fertilization. However, it is a limited method criticized by some authors as described in [17].

- In the field the plant may suffer from interfering agents (pests and pathogens) that mimetize nutritional deficiency symptoms, as already stated.

- Deficiency symptoms may be different from the ones described in the literature or specialized publications. For example, symptoms may be light instead of the severe ones described.
- Element deficiency signs may be different according to element and culture. Zn deficiency in fruit trees is expressed by smaller leaves and in corn cultures, new leaves are bleached.
- Deficiency symptoms may be similar for different nutrients.
- Certain deficiencies may reduce production without plant symptoms.
- Deficiencies of two or more nutrients prevents identification.
- Excess of one nutrient may be mistakenly taken as the deficiency of another one.
- Adequate visual diagnosis must be conducted by technicians with significant experience in cultures of the region.
- Visual diagnosis does not quantify neither the deficiency level nor the excessive one.

Furthermore, when the nutritional disorder is acute and visual symptoms of deficiency or excess are obvious and able to be differentiated a significant part of production (around 40-50%) may have been already compromised by a series of irreversible injuries to the physiology of the plant. Thus, visual diagnosis should not be used as a rule but only as complement.

8. Other methods

Foliar diagnosis is a direct evaluation method that utilizes nutrient concentrations in plant tissues as an indicator of nutritional status. However, indirect methods exist and are useful. When a deficient nutrient is part of an organic component or activates an enzymic activity this can be indirectly expressed. For example N deficiency may be shown by low chlorophyll levels or low activity of nitrate reductase. A description of biochemical tests that may be employed to evaluate plant nutritional status has been reported in [8]. For N, reductase and glutamine synthetase activity, amide N and asparagine; for P, fructose-1,6-diphosphate and photosynthesis ; phosphatase activity; for K,amide concentrations; free amino-acids; for Mn, peroxidases and a/b chlorophyll ratios; for B, ATP-ase activity; for Zn, ribonuclease, carbonic anhydrase, arginine concentration. In the case of P other studies indicate that P_i in vacuole cells may indicate the nutritional status of the plant [25, 26]. These are additional tools to evaluate plant nutrition, which are not commonly used because some of the tests require special methods of sampling, storage and complex analytical procedures and costly equipment. Other methods, specifically for N, evaluate the index of green color by a portable device called chlorophyll meter. This index is strongly correlated to the chlorophyll concentration in leaves and N nutritional status of the plant.

9. Final considerations

Plant analysis is a fundamental tool for nutritional diagnoses in cultures. The technique permits control of the nutritional equilibrium in cultures, reduction of costs and avoids environmental impact though rational use of fertilizers and consequent gains in production and profit. The main difficulties in the procedure, leaf sampling and interpretation of analysis results but these are improving as time goes by, becoming safer, economical fast and precise. The non-standardized sampling techniques diverge among author preferences but are intensely researched and improved by recommendations in comparative studies between samples and standard. Results interpretation is mainly by the critical level and sufficiency range. Alternative methods, like DRIS and DNC have been proposed but their use is still incipient.

Efficient fertilization calls for equal consideration and care to all phases of the process as plant sampling in the fields, laboratory analysis but mostly it should conducted by competent and experienced professionals In addition it is recommended the integrated utilization of techniques, that is, chemical analysis must be complemented by visual diagnosis so that, fertilization is efficient, economically profitable but safe to the environment.

Author details

Renato de Mello Prado* and Gustavo Caione*

*Address all correspondence to: rmprado@fcav.unesp.br

*Address all correspondence to: gustavocaione@agronomo.eng.br

UNESP (Universidade Estadual Paulista), Jaboticabal, SP, Brazil

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