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Critical nutrient concentrations of arable crops

Literature study on the usability of critical concentrations to diagnose nutrient deficiency and/or steer fertiliser application

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Brief summary:

This report discusses the concept “critical nutrient concentrations” in plant tissues and the usefulness for diagnosing nutrient deficiency in crops and/or adjusting fertilisation. In chapter 2, the report gives an overview of factors that influence (critical) nutrient concentrations in plant tissue. Furthermore, chapter 3 describes the methods used for deriving critical nutrient concentrations and chapter 4 gives an overview of published K, Mg, Ca, S, B and Mn concentrations in selected plant organs of potato, onion, sugar beet, wheat, barley, oats, and rye.

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Ministerie van Landbouw,
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Verspreiding

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Contents

Summary

Fertiliser recommendations in the Netherlands are based on soil analysis. Various extraction procedures are used to quantify the availability of plant nutrients in the soil, and based on the amount of available nutrients in the soil an eventual fertiliser application is recommended. Several factors are hindering a proper quantification of nutrient availability based on soil analysis, and for that reason plant analysis might be useful in addition. If plant analysis is used, critical values are needed for the interpretation of nutrient status of plants. That is the reason that NMI and WUR has performed a desk study in order of BO Akkerbouw to investigate the possibilities of plant analysis for the evaluation of the nutrient status of Dutch arable crops.

In chapter 2, background information on the measurements of plant nutrient concentrations and the factors affecting those concentrations is given. The mobility of nutrients differs, which has consequences for nutrient concentrations in various plant parts. N, P, K, Mg, Cl and S are mobile and will be translocated within the plant from old to young leaves in case of deficiency. On the other hand, Ca, B, Fe and Mn are immobile, and will lead to low concentrations in young leaves in case of deficiency. This hampers the selection of one ideal plant part for the evaluation of the status of all nutrients. Moreover environmental factors (such as temperature, soil moisture content, air humidity and radiation), development stage, plant organ and interaction between nutrients affect nutrient concentrations. This should be taken into account when using plant analysis.

Chapter 3 is describing methods that are used to determine critical nutrient concentrations, which is illustrated with some examples. In a lot of cases, critical nutrient concentrations have been determined in short term lab or pot experiments, which was the case with the derivation of a critical Mg concentration in wheat and a critical B concentration in sugar beets. In other situations, the nutrient contents of leaves or in petiole sap during the growing season were related to final crop yield, to derive the optimum nutrient concentration. This was illustrated with examples for K in potato leaves and nitrate in leaf petioles of potato.

In chapter 4 critical nutrient concentrations published in scientific literature and values used by Eurofins-agro are given per crop for K, Mg, Ca, S, B, and Mn. Finally, a discussion and conclusions were given in chapter 5. Conclusions were as follows:

- Measurements in plant tissues may in theory contribute to an improved fertilization advice in comparison with those based on soil sampling only. Information about the nutrient status in plants and soil could lead to a better insight into the nutrient availability and nutrient uptake by plants.
- Critical values of nutrient contents in plant tissue are needed to compare the results of a measurement in plant tissue with the reference set. Retrieving these reference values is difficult because many factors are involved.
- International literature is containing critical values of dry matter tissue analysis for various arable crops. The usability of these mostly international data for Dutch circumstances is limited. For plant sap in arable crops, literature values are lacking except for nitrogen in potato.

- The literature data on critical nutrient values in plant tissue presented in this report can be used as additional indicative diagnostic tool to diagnose the cause of poor crop growth, additional to e.g. soil analysis. It is advised to include the data in the Handboek Bodem en Bemesting.
- We recommend not to invest in the development of new fertilization advice systems based on plant tissue analysis for arable crops. Development of such a system is costly and it is questioned if a useful and simple system can be developed.

1 Introduction

1.1 The motivation for the research

In order of BO Akkerbouw, the branch organisation for Dutch arable agriculture, Nutrient Management Institute (NMI) and Wageningen University & Research Field crops carried out the research described in the forelying report.

In general, fertiliser recommendations in the Netherlands are based on soil analysis. Various extraction procedures are used to quantify the availability of plant nutrients in the soil, and based on the nutrient status of the soil, an eventual application of nutrients with fertilisers is recommended (Handboekbodemenbemesting.nl). Several factors are making it difficult to give a proper quantification of nutrient availability based on soil analysis, of which the following are important:

- The processes affecting nutrient availability are dynamic and depend on environmental factors. This is for example the case with the availability of N, P and S, while mineralisation is playing an important role for the availability of those nutrients, and the amount of nutrients that is coming available during the growing period partly depends on temperature and moisture content, which are not known before the start of the growing season.
- A large spatial heterogeneity in the soil may be a complicating factor in taking a soil sample that is giving a good indication of the amount of nutrients that is available to the plant roots.
- Especially for micronutrients it is difficult to take into account the acquisition mechanisms of plant roots, which may excrete substances (root exudates) that are increasing the availability of nutrients in the soil in the direct vicinity of plant roots, the rhizosphere.

Because of these difficulties, it might be useful to have additional tools for the evaluation of the nutrient supply to plants or crops. Plant analysis might be such a tool, that may be used in addition to or instead of soil analysis. If plant analysis is used, critical values are needed for the interpretation of the nutrient status of plants.

Measuring the nutrient concentrations of leaves can be used to establish whether a crop has a surplus or deficiency of certain nutrients. If the measured concentration of a certain nutrient is below the critical threshold, plant growth will be hampered and appropriate measures may be taken to correct the deficiency and to reduce yield loss.

The Dutch fertilisation manual currently does not contain critical concentration values except for nitrogen (N) for potato, boron (B), magnesium (Mg), and manganese (Mn) for sugar beet, and magnesium and manganese for chicory. This study aims to collect data on critical thresholds for potassium (K), calcium (Ca), sulphur (S), Mg, Mn and B in potato (*Solanum tuberosum*), cereals (wheat, *Triticum aestivum;* barley, *Hordeum vulgare;* oats, *Avena sativa* and rye, *Secale cereal*), onion (*Allium cepa*), and sugar beet (*Beta vulgaris*), so they may be included in the Dutch fertilisation manual. This would allow farmers to monitor their crops more closely and establish whether a crop is deficient in certain nutrients. This will aid in optimising production and more targeted fertilisation.

1.2 Definitions

Some authors prefer to refer to critical nutrient content rather than concentration. In this report, we use the term critical concentration in accordance to Reuter and Robinson (1997) who do this to avoid confusion with the word content meaning the total amount of an element in a plant part. It is also important to note that the definition of optimal yield is not always the same. Optimal yield can refer to the maximum above ground dry matter (DM), the highest seed or tuber DM. While in practice, quality aspects are also important in determining pay-out, think of protein content of grains, starch content of potatoes, or harvestable sugar in beets (Reuter and Robinson 1997). In this literature overview, various definitions of optimal yield could be used for deriving critical nutrient concentrations by various authors. Where available, the used definition of optimal yield will be mentioned.

Some of the terms used in scientific literature on (critical) nutrient concentrations in plant tissue have slightly different meanings in different publications. To reduce confusion caused by terminology, the list below provides the definition of terms used in this report unless there is a specific reference to another definition used by other authors.

Adequate concentration (range)	Nutrient concentration(s) at which no yield reduction due to nutrient deficiency is expected
At-risk concentration range	Nutrient concentration range in which yield loss due to the nutrient being limiting is possible.
Critical concentration	Nutrient concentration below which crop yield is lower than 90% of optimal yield
Deficient concentration (range)	Nutrient concentration(s) at which yields below 90% of optimal yield are to be expected due to low nutrient availability.
Plant analysis	Measuring of nutrient contents in plant sap or plant tissue, where tissue analysis is on dry matter basis.

1.3 Report structure

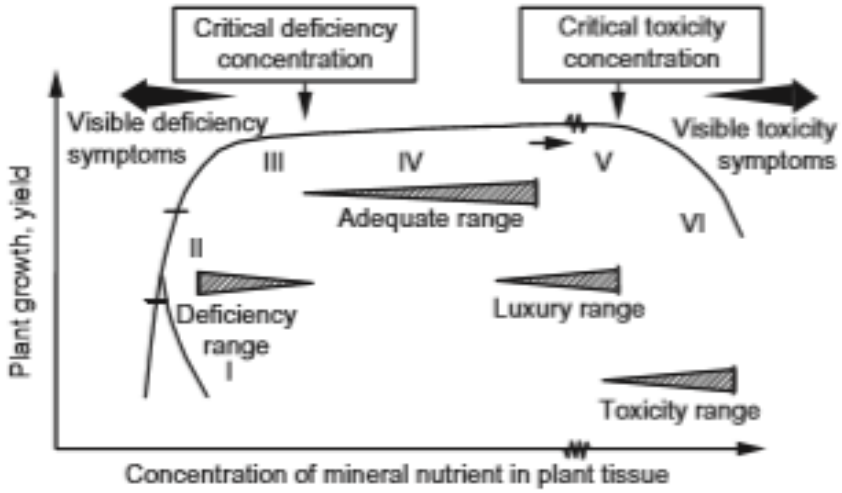
Chapter 2 of this report contains background information on crop nutrient content measurements and the factors that influence crop nutrient concentrations. Chapter 3 is describing methods that are used to determine critical nutrient concentrations, which is illustrated with some examples. In chapter 4 critical nutrient concentrations published in scientific literature and values used by Eurofins-agro are given per crop for K, Mg, Ca, S, B, and Mn.

2 Crop nutrient measurements

2.1 Nutrient concentrations in plants

Fertilisation advice is often based on the nutrient contents of the soil. However, there can be a disparity between measured soil nutrient content and actual plant-available nutrient content. Many methodologies have been developed to measure plant-available nutrients in the soil. Nevertheless, this does not always correspond to plant uptake. In addition to soil analysis, one can measure the nutrients in plant tissues, to indicate whether nutrient availability has been sufficient. This can be done by chemical analysis of plant sap or dry matter samples, or by optical sensors. This study focusses on chemical plant analysis.

Essential nutrients need to be taken up in sufficient quantities to obtain the optimal yield. Chemical diagnosis of plant nutrient status relies on the assumption that the nutrient concentration of a plant part is indicative for its development and eventual yield (Marschner 1995). The idea is that at a certain point in the development of the crop a specific tissue, the leaf, for example, should contain a minimum concentration of a given nutrient to obtain optimal yield. Below this minimum or critical concentration, plant development will suffer, and one will have a reduction in yield. Above the critical value, a mineral concentration range which is sufficient for optimal growth exists (Marschner 1995). Marschner (1995) explains this based on a figure by Jones and Handreck (1967) (Figure 2-1).



	I	II	II	III	IV	V	VI
	Deficient		Low		Adequate	High	Toxic
P (g kg^{-1})	<1.6		1.6–2.5		2.6–5.0	5.1–8.0	>8.0
N (g kg^{-1})	<12.6		12.6–17.0		17.1–25.0	25.1–27.5	>27.5
Mn (mg kg^{-1})	<15		15–20		21–100	101–250	>250

Figure 2-1. Relationship between mineral nutrient concentration in plants and plant growth.

Figure 2-1 also displays a critical toxicity concentration. This is the concentration above which yield is reduced due to toxicity of that nutrient. Nutrient concentrations between the critical deficiency concentration and the critical toxicity concentration are associated with optimal crop growth and yield and are indicated as the adequate range. High nutrient concentrations that are not leading to a decreased growth or yield are indicated as luxury. Many factors affect the critical concentration; therefore, many authors report a critical concentration range in which deficiency may occur. The factors that affect the critical concentration will be discussed below.

2.2 Nutrient mobility

Nutrients in plants are often classified into three categories with regards to their mobility: mobile, immobile, and intermediate. Mobile nutrients that are easily redistributed by phloem[1] are N, P, K, Mg chlorine (Cl), and S. While, Zn, molybdenum (Mo), and copper (Cu) have intermediate mobility. Ca, B, iron (Fe), and Mn, are not mobile in the phloem (Westermann 2005). New plant parts are dependent on xylem[2] transport for supply of immobile nutrients, consequently, nutrient deficiency of Ca, B, Fe, and Mn will be visible in young plant parts but may not be detectable in older parts which can retain adequate nutrient concentrations when deficiency starts (Reuter and Robinson 1997). N, P, K, and Mg can be translocated from old leaves to younger ones quite easily. At low supply of these nutrients, young leaves may receive these nutrients at expense of older leaves, making symptoms of deficiency initially visible in older leaves (Reuter and Robinson 1997). S, Cu, Zn do not readily move from old to young leaves. Only when old leaves senesce do these nutrients translocate to younger leaves (Reuter and Robinson 1997). Mo mobility seems quite variable even when comparing cultivars within the same species (Yu, Hu, and Wang 2002). Nutrients are also not spread equal through plant tissues like blade, petiole, stem and fruiting organs. This has to be taken into account when analysing plant tissue. Petiole tissues are thought to be better in predicting more mobile nutrients as N and Cl in comparison with leaf blade (Bryson and Mills 2014). The differences in mobility of nutrients, hampers the selection of one ideal plant organ for the evaluation of the status of all nutrients. So, a compromise is necessary when selecting a specific plant organ for plant analysis. Often, the youngest mature leaf is used for diagnosis of nutrient deficiencies (see further in 2.5).

Nutrient deficiency can be diagnosed visually, however, once deficiency symptoms have occurred the crop has already suffered yield loss. Ideally, (potential) deficiency is detected at an earlier stage to enable timely intervention. Plant analysis can supposedly be useful to detect or diagnose nutrient deficiency in an early stage before visual symptoms appear.

[1] Dutch: Floëem (bastvaten). Transports nutrients and assimilates (such as sugars) from leaves and storage organs to other plant parts.
[2] Dutch: xylem (houtvaten). Transports water and dissolved nutrients from the roots to the leaves.

Table 2-1. Some symptoms of nutrient deficiencies and where you can find them. After (Marschner 1995).

Plant part	Deficient nutrient	Prevailing visible symptoms
Old and mature leaf blades	N	Uniform chlorosis
	S	Uniform chlorosis
	Mg	Interveinal or blotched chlorosis and or/
	Mn	Interveinal or blotched chlorosis and or/
	K	Tip and marginal scorch necrosis
	B	Tip and marginal scorch necrosis
Young leaf blades and apex	Fe	Uniform chlorosis
	S	Uniform chlorosis
	Zn	Interveinal or blotched chlorosis. or
	Mn	Interveinal or blotched chlorosis
	Ca	Necrosis or chlorosis
	B	Necrosis or chlorosis and-or deformations
	Cu	Necrosis or chlorosis
	Mo	Deformations

For plant sap analyses of nitrogen, diurnal variation must be considered as well. Bryson et al. (2014) and Mc Kerron et al. (1995) reported evidence that the nitrate concentrations in the sap was fluctuating during the time of the day. The presumed reason for this is that light affects the activity of nitrate reductase. In order to avoid differences due to the time of sampling, some laboratories restrict in their protocols a specified time frame in which has to be sampled, e.g. early morning before 10 am.

Mobility of nutrients can also indicate which method of plant analysis is preferred: tissue analysis or sap analysis. Sonneveld (1987) showed that relative cheap plant sap measurements can be used for measuring concentrations of mobile elements as potassium in tomato plants, there was a good correlation with tissue measurements. Correlation of plant sap and tissue analysis for the relative immobile element calcium was much lower than that of mobile elements.

2.3 Environmental factors

Environmental factors such as temperature, soil moisture content, air humidity, and radiation affect transpiration and therefore xylem transport. Decreased xylem flow can temporarily cause deficiency of immobile nutrients in young plant parts. Thus, measuring a deficiency of an immobile nutrient in young plant parts may be more a reflection of unfavourable environmental factors (e.g. dry soil) rather than unavailability of that nutrient. Further, pest and diseases are thought to have an impact on the uptake of nutrients by plants.

Temperature has also been noted to affect tobacco's tolerance for Mn toxicity (Rufty et al. 1979). Higher temperatures make tobacco more tolerant to increased supply and tissue concentration of Mn. Incoming radiation may affect the critical values of nutrients as well as indicated by a study on the effect of shading on the boron requirement of mung beans (Noppakoonwong et al. 1993). Wheat accumulates more Mo if temperatures are higher, deficiency symptoms are more pronounced at low temperatures (Yu et al. 2002). These three examples are no solid proof that temperature and incoming radiation have pronounced effects on the critical values of all nutrients and all crops, they do indicate that such effects may exist. Similar articles are in short supply which could mean that the effect of temperature and radiation on critical concentrations is poorly understood or that there is little reason to believe that they play a very important role.

Atmospheric carbon dioxide (CO_2) concentrations affect the growth rate and potential production of crops, especially for C3 plants such as potato, sugar beet, wheat, and barley. Increased CO_2-concentrations reduces photorespiration. This also has consequences for the critical value of N and P, as less N is tied up in photorespiration and more P is required for the photoreductive cycle (Rogers et al. 1993) (Table 2-2). This may hinder the validity of critical N and P concentrations determined and reported in the 20th century as atmospheric CO_2-concentrations has risen from approximately 354 ppm in 1990 to 411 ppm in 2019 (ESRL GML NOAA 2020). For N in wheat, it is quite certain that elevated CO_2-concentrations decreases N and protein concentration of grains (Bahrami et al. 2017). In potato, differences in nutrient concentration in tubers and leaf tissue have been observed when comparing treatments with ambient air, elevated CO_2-concentrations, and elevated CO_2-concentrations + elevated ozone concentrations. Not for all nutrients the changes were significant; for some, tissue concentrations increased and for others, concentrations decreased. N and protein concentrations in tubers were significantly lower in tubers cultivated under elevated CO_2-concentrations (Fangmeier et al. 2002). Reddy and Zhao (2005) found no changes in K cotton leaf tissue concentration under elevated CO_2-concentrations but did remark that increased growth rates caused by elevated CO_2-concentrations may warrant different fertiliser application. In sugar beet, no significant changes were observed in leaf nutrient concentrations under elevated CO_2-concentrations (Wolf 1998). Wolf did the same experiment with wheat and faba bean. In faba bean he found no effect of CO_2-concentrations on tissue nutrient concentrations (Wolf 1996a). While he found that the minimum leaf N concentration in spring wheat decreased with increased CO_2-concentrations (Wolf 1996b). Besides N and P, there is no reason to suspect that critical nutrient concentrations are affected by CO_2-concentrations . As to N and P, it is hard to say if and how much their critical concentrations will be affected by rising CO_2-concentrations, further studies are required.

Table 2-2. Critical concentrations of nitrogen and phosphorus for cotton and wheat under different atmospheric CO_2 concentrations (Rogers et al. 1993).

Species	CO_2 ($\mu L\ L^{-1}$)	Critical concentration (%)	
		Nitrogen[a]	Phosphorus[a]
Cotton	350	5.1 (0.36)	0.41 (0.83)
	550	4.3 (0.93)	0.53 (0.75)
	900	3.2 (0.49)	0.78 (0.84)
Wheat	350	4.5 (0.83)	0.39 (0.89)
	550	3.8 (0.95)	0.56 (0.98)
	900	3.8 (0.95)	0.53 (0.88)

2.4 Development stage

Uptake of nutrients by plants varies throughout the growing season (Rosen et al. 2014; Marsh and Peterson 1990). Marsh and Peterson demonstrated that Mn concentrations in different organs varied in time and between organs. For N, P and K, nutrient concentrations decline as crop development progresses, this is linked to an increase in structural molecules or dry matter (Marschner 1995). Concentrations considered deficient at the beginning of the season can be excessive at the end of the season (Walworth and Muniz 1993). In wheat, total leaf S and sulphate S concentrations are affected by crop age (Spencer and Freney 1980).

It is often reported that plant N concentrations decline as a potato crop develops (Williams and Maier 1990). The Dutch fertilisation manual takes this into account for its section on potato petiole analysis ("*Sturing N-Bemesting via Nitraatgehalte Bladsteeltjes*" n.d., Figure 2-2).

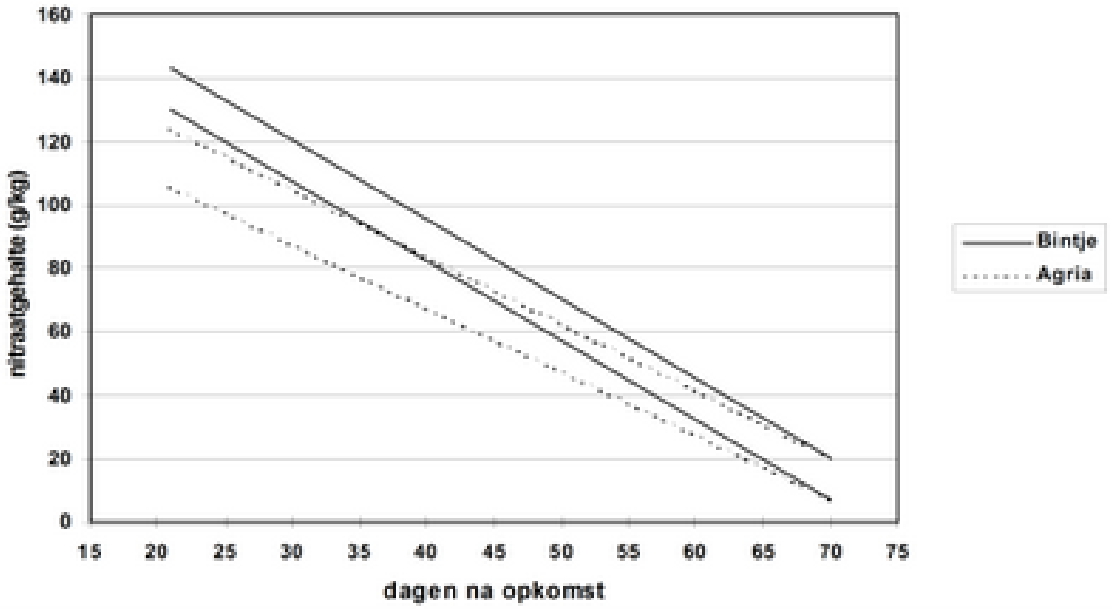


Figure 2-2. Norm (days after emergence) trajectory for nitrate-N concentration (g/kg) of potato petioles for the cultivars Agria and Bintje as included in the Dutch soil and fertilisation manual ("*Sturing N-Bemesting via Nitraatgehalte Bladsteeltjes*" n.d.).

In their study on potato Williams and Maier (1990) remark that some studies use days after planting to indicate the moment of sampling but that this does not accurately reflect the physiological age or development stage of the crop as this is determined by the thermal time of the crop and the accumulated temperature sum. For these reason, Steltenpool and Van Erp (1995) has determined a relationship between temperature sum and accumulative N-uptake for potatoes in the Netherlands. However, such relationships are not derived for critical nutrient concentrations of potatoes, as far as we know. In general, one should be careful using critical values when these are reported based on days after sowing (DAS) or seeding. Luckily, many authors establishing critical values reported the development stage of their cereal crop using Feekes or Zadoks classification or other recognisable development stages (Zadoks et al. 1974; Reuter and Robinson 1997). However, Feekes' scale has been reported as insufficient to account for rapidly changing S concentrations in wheat (Rasmussen et al. 1977).

Critical values reported based on DAS, days after emergence, or days after planting, are less reliable when a growing season is cooler or warmer than the season(s) used to establish the critical value. As in such a case, the development stage of the crops will differ at the same DAS.

Diagnosis and Recommendation Integrated System (DRIS) has been developed to circumvent problems that arise from shifting nutrient concentrations during crop development (Walworth and Sumner 1987). DRIS relies on the assumption that while the absolute concentrations change, the ratios between N, P, and K and Ca and Mg remain roughly constant. However, in young plants ratios can still be quite variable. When determining the nutrient status of a leaf based on a critical value, we assume that the DM concentration of the leaf is stable, so we can assume that our measured concentration nutrient/DM is indicative of a surplus or deficiency of the nutrient (and not of the DM concentration). When one measures a ratio between two nutrients that is higher than the target value, one cannot tell whether this is because nutrient A is in excess or whether nutrient B is deficient. DRIS is a method of ordering a multitude of ratios between nutrients in a way that has meaning, these

results are named DRIS indices (Walworth and Sumner 1987). Walworth and Sumner (1987) list several authors who found DRIS to be more accurate than critical values or critical ranges. They also give an example with data from field trials where DRIS can indicate the most limiting nutrient whereas the other approaches cannot. This does not necessarily mean that DRIS will perform significantly better in Dutch practice. DRIS appears to be a method that gives insight into the ratios between nutrients (see also 3.1.5), which could be of additional value to critical values or ranges of individual nutrients.

2.5 Differences between plant organs

Nutrient concentrations are not uniform within a plant, and the way nutrients are distributed within plants differ between nutrients because of differences in mobility, uptake and function (as has been described in the former paragraph 2.2). Therefore, the critical nutrient concentration differs between plant organs. Walworth and Muniz (1993) point out considerable differences in critical K concentrations between potato leaf blades and petioles (Table 2-3). N concentrations in potato petioles have also been shown to vary between leaves on the same plant (MacKerron et al. 1995). For the choice of organ, one must consider whether the nutrient content of that organ reflects the present nutrient availability to the crop. Organs where nutrients are stored, or from where nutrients cannot be relocated do not achieve this. Generally, leaves are used as these are very metabolically active and have proven suitable for determining the nutrient concentration of both mobile and immobile nutrients, depending on the age of the leaf. Often the most recently matured leaf is used but other plant parts are reported as well such as whole shoot (sugar beet) or petioles (potato) (Reuter and Robinson 1997). Also, a combination of old and young leaves is used (Timmermans and Van der Ven, 2014) to get information about the status of nutrients in leaves of different ages.

Table 2-3. Deficient K concentrations in potato petioles and leaf blades at different stages during the growing season as presented by (James L Walworth and Muniz 1993)*. Walworth and Muniz (1993) include several sources in their table, for clarity, only the values from the same sources are presented here.*

	Petiole deficient (% K)	Leaf-blade deficient (% K)
Early	3.50	9.0
Mid	2.50	7.00
Late	1.50	4.00

2.6 Interactions between nutrients

Further complicating the use of single nutrient tissue analysis is that nutrient concentrations of plants are not fully independent. Therefore, concentrations of other nutrients, higher or lower than in the experiments on which critical concentrations are based, can alter the required concentration for proper metabolism.

Rietra *et al.* (2017) reviewed several interaction mechanisms between essential plant nutrients, differentiating between synergism, antagonism, zero-interaction, and Liebig-synergism (Table 2-4).

Table 2-4. Definitions of synergism, antagonism, zero-interaction, and Liebig-synergism as used by Rietra et al. (2017).

Interaction	Description
Synergism	Nutrient interaction is synergistic where the yield due to the combined application of two nutrients is more than the yield expected on the basis of the effects from the individual applications of the nutrients.
Antagonism	Nutrient interaction is antagonistic where the yield due to the combined application of two nutrients is less than the yield expected on the basis of the effects from the individual applications of the nutrients.
Zero-interaction	Where the yield obtained from a combination of two nutrients is equal to the yield expected on the basis of the individual application of the nutrients, the interaction is said to be zero-interaction.
Liebig-synergism	Typically, in situations where the availability of one nutrient is limiting crop production, the addition of another nutrient shows no effect on yield, whereas addition of both nutrients shows an increased (synergistic) effect. Wallace (1990) introduced the term Liebig-synergism to describe this effect, referring to the Liebig limitation of the first nutrient.

Based on their review of 94 peer reviewed studies investigating the effects of interactions between nutrients on yield, they found that there were 43 cases of synergism (of which 20 Liebig-synergism), 35 cases had zero interaction, and in 17 cases there was an antagonistic relation. It is important to note here that identifications of Liebig-synergism do not reveal an interaction between two nutrients but are cases where the addition of two nutrients improved yield more than the sum of the two fertilisers added individually because addition of one of the nutrients had no effect due to the severe deficiency of the other nutrient. Furthermore, some cases where synergism was identified can be prescribed to the acidifying effect of a fertiliser which reduces unavailable nutrients to a plant-available form.

N can interfere with the relocation of Cu, Zn, and S as these three nutrients are transferred from old to young leaves when the older ones senesce. If deficient N causes senescence of old leaves a deficiency of the other three nutrients (normally visible in young leaves) may be masked as Cu, Zn, and S are relocated from the senescent leaves (Reuter and Robinson 1997).

While not essential for many plants, sodium (Na^+) can partially substitute K^+'s function as osmotic regulator in others, such as sugar beet (Lindhauer, et al., 1990; Wakeel et al. 2011; Wakeel, et al., 2010). In sugar beet, Na^+ also displayed an antagonistic relation with Ca. Notably, substitution of K by Na did not negatively impact the white sugar yield (Wakeel et al. 2010).

A recent report on K, Mg, Ca, N, and Cl interactions in Dutch arable farming concluded that there is an antagonistic relationship between high fertilisation of K on Mg, and Ca uptake but that this does not necessarily cause deficiency if there is enough Mg and Ca in the soil and when the crop is adequately supplied with water (Bussink et al. 2020). Making sure sufficient Mg and Ca is present in the soil can be determined based on soil analysis, plant tissue analysis does not provide additional benefit if - nutrient and water supply are in order.

3 Methods for obtaining critical values

3.1 Introduction

As we have seen in the former chapter, many factors affect critical concentrations: crop species, plant organ (Westermann, et al., 1994), physiological age (Williams and Maier 1990; "*Sturing N-Bemesting via Nitraatgehalte Bladsteeltjes*" n.d.; Spencer and Freney 1980), and nutrient (Marschner 1995; Houba 1973). Researchers have taken different approaches in determining critical concentrations, measuring different organs, at different phenological ages, or in different environments. This can easily be understood because, due to different mobility, selected nutrients have their own ideal plant part for a given crop to determine the critical concentration. This high diversity in methodologies makes it more difficult to interpret the reported critical concentrations and compare them with samples in practice, which are ideally taken from a single plant organ and used to analyse all relevant nutrient concentrations.

From the late 1940s till the early 1990s, a large number of lab, pot and field experiments have been conducted to ascertain critical concentrations of nutrients in crop tissues, expecting that the diagnosis of nutrient deficiency could be used as a tool for fertiliser application and could lead to and increased crop production. In this chapter, some case studies are described in which critical concentrations at tissue dry matter basis have been derived, to give an idea about the methods that are used for obtaining critical nutrient concentrations. These case studies should be considered as examples of the way critical nutrient concentrations are determined in general. In addition, the method used for deriving critical nitrate concentrations in potato petiole sap is also described. This example is described quite extensively, because it is relevant for Dutch arable agriculture and because it is a commercially available tool for adjusting fertiliser application. Furthermore, the larger number of publications on nitrate in potato petiole sap allows for a comparison of methods and definitions used by different authors, which provides input for discussion of critical concentration research in general.

3.2 Case 1: determining critical Mg concentration in wheat

Scott and Robson (1991) studied distribution and retranslocation of Mg in wheat in a solution culture experiment. A summary of their methods is given here to give an impression of methods used to determine a critical concentration.

Wheat seeds (cv. Gamenya) were germinated on white sand and transplanted to solution cultures after 7 days. There were seven levels of Mg solution concentrations and two treatments with discontinued Mg supply to study relocation of Mg from old to young plant parts. Each of the nine treatments had two replicates. Besides Mg in the form of magnesium sulphate, the solution contained other nutrients in sufficient concentrations. Plants were grown in a cooled glasshouse in Australia. Plants were harvested at 24, 25, 35, or 36 days after sowing.

Upon harvest, leaf symptoms (chlorosis and necrosis) were scored as a subjective measure of the affected area of the leaf.

Plant dry matter was digested in a mixture of nitric and perchloric acid. The digest was analysed for Mg by atomic absorption spectrophotometry.

The critical concentrations reported by Scott and Robson (1991) (Figure 3-1) were defined as the minimum concentration in tissue at which maximum weight of shoots was attained. Where the maximum weight of shoots was assumed to be equal to the shoot weight of the treatment with the highest Mg supply (160 µM**)** Note that this is quite different from definitions used by other authors (lowest concentration to obtain 90-100% of optimal yield). A question that might arise is whether these critical concentrations can be used for growing wheat under field conditions (see chapter 3.6).

Figure 3-1. Table from Scott and Robson (1991) *from which they derived critical concentrations (c columns) for different wheat plant organs. YEB = youngest emerged blade. All treatments included two treatments where Mg supply was interrupted in order to study retranslocation of Mg in wheat.*

Table 4. Relationship between the relative shoot weight of wheat at two harvests and the concentration of Mg in several tissues. Dry weights are expressed relative to the yield of the highest Mg solution treatment (160 μM) and the value 'c' is the minimum concentration of Mg ($\mu g\, g^{-1}$) in the tissue at which maximum shoot weight was maintained

Tissue	Constant Mg supply only			All treatments		
	V.A.F.[a] (%)	c ($\mu g\, g^{-1}$)	s.e. of c ($\mu g\, g^{-1}$)	V.A.F. (%)	c ($\mu g\, g^{-1}$)	s.e. of c ($\mu g\, g^{-1}$)
Leaf 1	87	1349	111	83	1351	106
Leaf 2	87	1053	95	85	962	82
Leaf 3	85	1086	93	83	959	70
YEB-2	82	1328	133	74	1258	132
YEB-1	86	955	90	79	734	88
YEB	88	861	76	66	955	138
New growth	77	1005	91	40	1331	255
Sheath 1	69	781	94	60	785	100
Sheath 2	69	700	101	60	728	102
Shoot	88	932	58	65	989	142

[a] Variance accounted for.

3.3 Case 2: determining critical B concentrations in sugar beet

The methods described here are from a study aimed at determining the boron concentration of sugar beet tissues grown in solution with a range of B supply (Vlamis and Ulrich 1971).

Hybrid sugar beet seeds were treated with fungicide and planted in vermiculture in a greenhouse. Irrigation water contained nutrients excluding B. After two weeks the plants were transferred to containers with half strength slightly modified Hoagland solution. The base Hoagland solution had a B content of 0.5 ppm. Boron rates were reduced consecutively by one half for seven treatments until a concentration of 1/128 B in Hoagland was reached. Each treatment had 5 replicates

The plants were grown in a greenhouse from late March till the middle of May. The plants were harvested 7 weeks after transplanting. Upon harvest, leaves were classified as old, mature, or young and the petioles were separated from the leaf blades. Roots were frozen in dry ice for future sugar analysis whilst shoot tissue was dried, weight, and milled.

B in samples was measured by the curcumin method, some material was also analysed by the carmine method for verification. Flame emission spectroscopy was used for other analyses. B concentrations of tissues were plotted against yield (Figure 3-2).

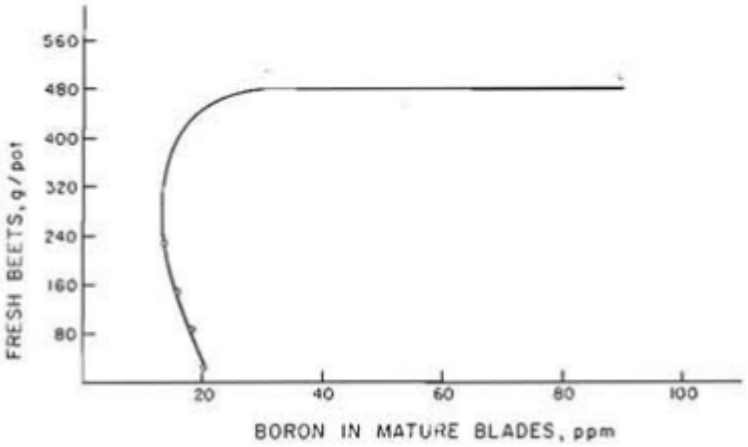


Figure 3-2. Beet root weight plotted in relation to the B concentration of mature leaf blades by (Vlamis and Ulrich 1971).

Based on this plot a critical B concentration for mature leaves was determined at 21 mg/kg DM. Vlamis and Ulrich (1971) remark that this would be a rigid value separating deficiency from adequacy and that it would be more realistic to regard this 21 mg/kg DM as a midpoint of a critical zone of deficiency in the range 15/30 mg B/kg DM.

3.4 Case 3: determining optimal K concentration in potato

To give an idea of the methodology applied in determining critical concentrations in leaves, the methods of (MacKay, et al., 1966) are described here. These authors use the term "optimum" rather than "critical" and define it as "*the elemental concentration in the leaf, above which no further increase in yield could be expected*".

Potato crops of two varieties (Kennebec and Netted gem, both ware potato varieties) were cultivated at 18 sites during three years from 1958 to 1960, five soil series from two soil groups were represented at the sites (MacKay,et al., 1966). Including several sites and years in an experiment required to reduce site and year-specific effects.

From each plot, leaf samples of the uppermost mature leaf of 15-20 plants per plot were collected when approximately 10% of the plants were flowering, about 60-70 days after planting. Petioles and leaves were separated for analysis, dried at approximately 82°C and ground in a Wiley Mill (MacKay, et al., 1966).

Samples were dry ashed at 550°C and dissolved in HCl. K concentration was determined by Beckman DU flame photometer. Nowadays, other methods such as emission spectrometry are available as well (Bryson et al. 2014).

The yield and leaf data were subjected to analysis of variance and the sums of squares for nutrient treatments were divided into their significant polynomial components which were used to determine curves of best fit. The influence of year, variety and soil series were also evaluated (MacKay et al. 1966) (Figure 3-3).

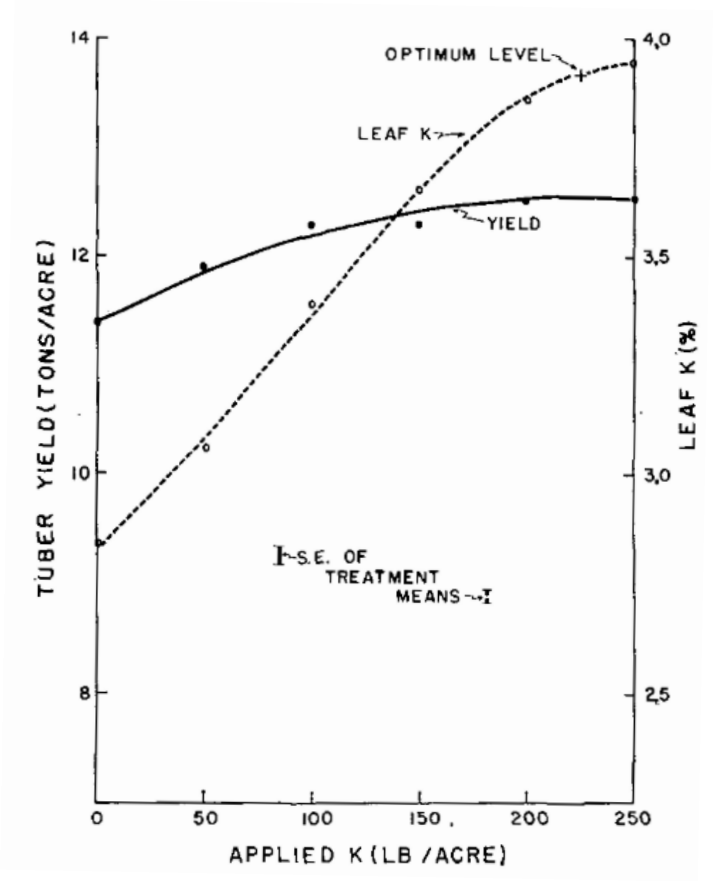


Figure 3-3. Effect of K treatments on tuber yields and K concentration of potato leaves where optimum level corresponds with the K concentration in leaves for optimal yield from (MacKay, MacEachern, and Bishop 1966).

3.5 Case 4: Critical nitrate concentrations in potato petioles

Dry matter nutrient concentration analysis is quite cumbersome as one needs to transport samples to a lab. It can take several days before the results are in, time that a farmer is not always able to spare. Several studies have attempted to develop or use petiole sap analysis methods which can be conducted in the field and are therefore faster. A lot of this research has been done for N in potato.

The underlying principles of petiole sap analysis are much the same as for tissue DM nutrient concentration analysis and therefore also many of the challenges[3] are the same, perhaps even more so. Several workers have published critical values or ranges for nitrate concentrations in petioles or petiole sap from the mid-seventies to the early nineties. MacKerron et al. (1995) are critical of the interpretation of the published values and ranges. Their analysis of the suitability of petiole nitrate concentration in potatoes is interesting but not acutely relevant for dry matter plant analysis of other nutrients. However, their review of literature raises an important question: 'do reported values based on a relation between a nutrient concentration during the growing season and yield have a physiological or agronomic meaning?'

[3] Varying concentrations during the day and development stage, large differences between individual plants in the same field, very sensitive to environmental factors such as water availability

Firstly, they point out that there is no relation between the measured nitrate concentration in the petiole and the crops need for nitrogen at that time. A portion of the measured nitrate is likely to be transported to another organ where it is used at a later moment This undermines the notion that N concentrations at the beginning of the season are supposed to be high and gradually decrease during development for proper plant metabolism. The declining nitrate concentrations regarded as normal or corresponding to optimal growth can also be caused by single application of fertiliser as is common in many experiments that report this phenomenon as opposed to split application. Experiments reporting petiole nitrate concentration where nitrogen is supplied gradually, for example via fertigation, have not been found for potato. In other words: it is not clear whether reported critical or adequate petiole nitrate concentrations have a physiological or an agronomic meaning.

Physiological interpretation of petiole nitrate concentrations

In their guide on petiole analysis assisted nitrogen fertilisation, Jones and Painter (1974) suggest that a minimum petiole nitrate concentration of 4000 ppm[4] is required at all times for optimal growth. Their figure on petiole nitrate concentrations also includes zones of deficient, inadequate, adequate, and excessive nitrate concentration at certain times in the season (Figure 3-4). But they give a slightly different meaning to these zones than authors from more recent publications. The most striking difference with similar figures such as from Williams and Maier, (1990) is the absence of single concentration that defines the deficient zone level (Figure 2-2, Figure 3-5). The adequate zone given by Jones and Painter (1974) are concentrations which, when N fertiliser is applied pre-planting, assure that the petiole nitrate concentration does not fall below the deficiency line. Here it is clear that adequate has an agronomic definition; in the adequate zone, one expects that nitrate petiole concentrations will remain sufficiently high during the entire season to avoid N deficiency at any moment.

[4] Equal to 0.4% nitrate of petiole DM

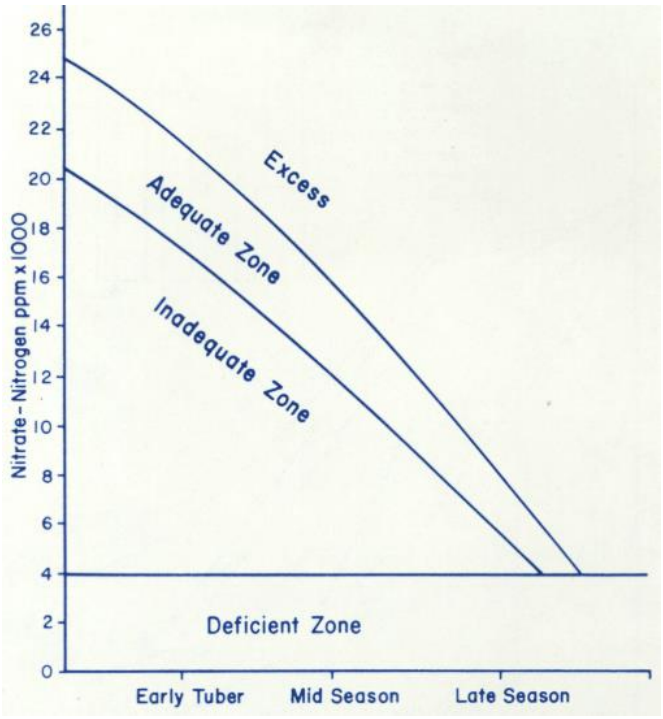


Figure 3-4. Figure from Jones and Painter (1974) illustrating suggested petiole nitrate concentrations during the growing season to avoid nitrate concentrations dropping into the deficient zone at a later point in the growing season.

As pointed out by MacKerron et al. (1995), later authors have wrongly interpreted the concentrations below the adequate zone defined by Jones and Painter (1974) as a zone where petiole nitrate concentration is deficient (Figure 2-1, Figure 3-5). As opposed to concentrations for which one expects N will become yield limiting at some point during the growing season. This is a crucial distinction when one uses analysis of petioles to guide N fertilisation. If one has multiple moments during the growing season on which N fertiliser can be applied instead of supplying all N pre-planting, the aim should not be to obtain adequate nitrate concentrations valid for pre-plant fertilised potatoes but to maintain the petiole nitrate concentration above the deficient concentration level.

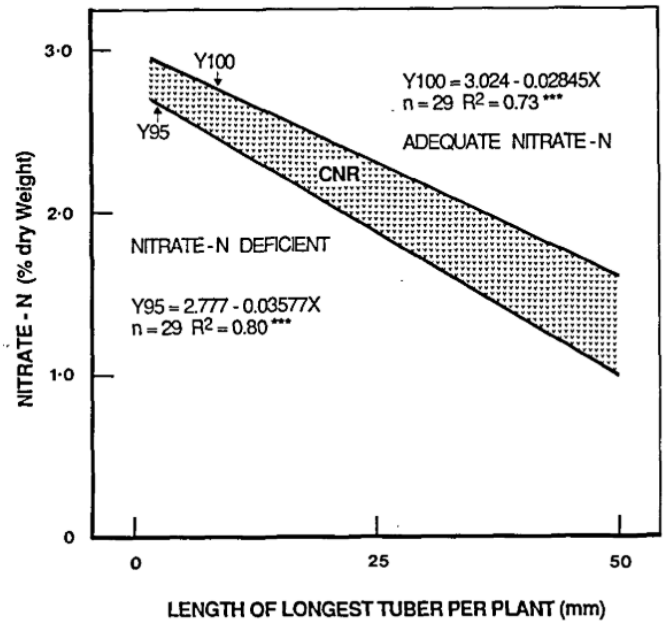


Figure 3-5. Figure from Williams and Maier (1990) with their determination of critical nitrate concentrations for potato petioles.

Such an approach is recommended by Kleinkopf and Westermann (1982) who recommend subsequent fertilisation is given to maintain a petiole nitrate concentration above 15000 ppm[5] (*Figure* 3-6). Exactly how the earlier 4000 ppm deficient concentration level was determined is not clear (Jones and Painter 1974). The 15000 ppm concentration reported by (Kleinkopf and Westermann 1982) is probably based on their experiments which got published three years later (Westermann and Kleinkopf 1985). Based on those experiments they concluded that N fertilisation practices that kept petiole nitrate concentrations over 15000 ppm were also treatments where a desired plant N uptake of 3.7 kg N ha^{-1} day^{-1} was achieved (Westermann and Kleinkopf 1985). This uptake rate was required to prevent relocation of nitrogen and DM from the tops to the tubers.

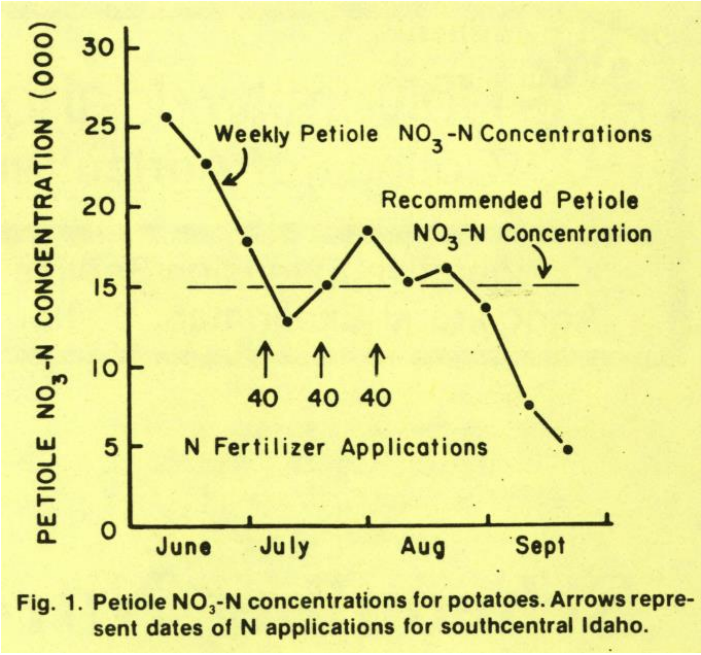


Figure 3-6. Figure from Kleinkopf and Westermann (1982)*. Illustrating their suggestion on how to adjust N fertilisation based on measuring nitrate petiole concentrations.*

Following this logic, in a hypothetical case where N can be supplied continuously, for example by drip irrigation, and petiole nitrate concentration can be measured in real-time, the ideal nitrate concentration would be stable, just above the deficiency line under which optimal metabolism is no longer maintained (Figure 3-7).

[5] Equal to 1.5% nitrate of petiole DM

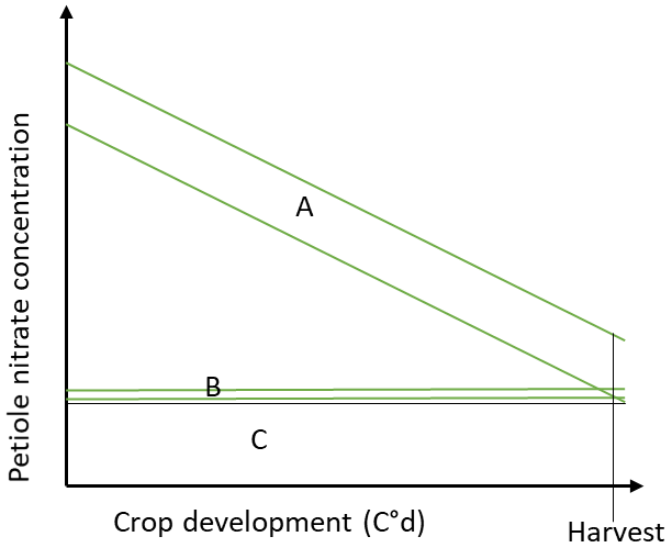


Figure 3-7. Hypothetical petiole nitrate concentrations for optimal potato yield for potatoes fertilised pre-planting (A) and continuously fertilised and measured potatoes (B). Yield declines when petiole nitrate concentration drops into the deficiency zone (C) at any moment during the growing season.

Except for Westermann and Kleinkopf (1985), no other studies give a biological reason why their reported critical petiole nitrate concentration or range is required. Some studies do not even present values while concluding petiole analysis is useful in adjusting fertiliser applications (Vitosh and Silva 1996). For reported critical or adequate concentrations in plant tissues of other nutrients it is also not clear whether they have a physiological or agronomic meaning. Which makes using such values accurately, more difficult.

Potato petiole (sap) nitrate analysis in the Netherlands

The Dutch handbook on soils and fertilisation has taken up information on potato petiole nitrate analysis, but only for the ware potato cultivars "Bintje" and "Agria", norms for starch potato are also included without specifying a cultivar ("*Sturing N-Bemesting via Nitraatgehalte Bladsteeltjes*" n.d.). The data behind the advisory model are described by Van Geel and Brinks (2018). The validation for Bintje reaches back to research by Van Loon and Houwing (1989). The norm values for Agria were obtained from field data on 20 farms in the southwest of the Netherlands.

Excessive N fertilisation of potato can depress yield so with sap analysis the aim is to adjust the last third of the N application to the needs of the crop during the growing season to avoid under or over fertilisation. This handbook does not address whether concentrations below the norm trajectory are acutely deficient (physiological meaning of norm trajectory) or whether at these concentrations there is a risk of deficient N at a later point in the season (agronomic meaning).

Nevertheless, petiole analysis-based fertilisation was one of the methods used by a study group where Dutch farmers applied and compared several methods for guided fertiliser application. The participants indicated that they were motivated to continue collecting petiole samples while doing it collectively as study group. It was concluded that few farmers are likely to apply petiole analysis on their own, as a regular method to steer their N application in potato (van Geel et al. 2012). There is currently no data available about the percentage in which farmers use plant sap methods to estimate the height of a top dressing in the Netherlands. A questionnaire that was carried out in 2011 yielded an implementation rate of 2% of the farmers using a tool for top dressing (e.g. plants ap, dry matter analysis or sensor-based measurements). Among the 2% of the farmers that use it, they stated to use

it on approximately 0-5% of their fields (Smit et all, 2011). Plant sap analysis is used to some extent by the advisory company Delphy in starch potato in the North East of the Netherlands.

3.6 Discussion of methodologies

Growth medium

Two of the three dry matter experiments used a nutrient solution as growth medium (Scott and Robson 1991; Vlamis and Ulrich 1971) in a short term pot experiment. Advantageous to solution culture is that the nutrient supply can be tightly controlled and understood. One can measure plants on a wide spectrum of nutrient supplies that ensures that one has plants that are deficient and ones that are not growth limited. The downside is that results from experiments with a solution culture in a greenhouse do not correspond to the conditions on fields of commercial farms. The third experiment was performed under field conditions, covering a range of soils in the study area (MacKay et al. 1966). However, these soils may not be representative for soils in which potatoes are cultivated worldwide. It is not clear what the consequences are of the varying conditions and the use of different growth media for the usability of critical concentrations in crops cultivated under field conditions.

Cultivar choice

In the experiments on wheat and sugar beet, the researchers used one cultivar. The potato experiment mainly used cv. Kennebec but also included cv. Netted Gem. Use of a single or a limited number of genotypes does not have to pose a problem to obtaining critical concentrations for practical use if there is little difference in critical concentrations between cultivars or if there is little genotypic variation between the most predominantly cultivated cultivars. From these three reports it cannot be inferred whether either of this is the case. So, it is uncertain how applicable their results are to current commercially cultivated varieties.

Besides, these studies are relatively old and originate from the USA and Australia[6]. So, one should be cautious in applying results from these studies to cultivars cultivated in the Netherlands during the 21st century.

For potato petiole analysis it is clear from Figure 2-2 that there are noteworthy differences between cultivars. Many commonly cultivated cultivars are not currently included in the manual while it is known that there exist large differences between cultivars in N-demand. Therefore, Van Geel and Brinks (2018) advised to update the potato petiole advices and include more currently important cultivars. Differences between cultivars can be caused by genotypic variation but can also be related to the earliness of the cultivar when the trajectories are given for days after emergence. Early cultivars experience cooler temperatures early in their development compared to later cultivars.

Definition of concentration in relation to yield

The critical values or c-values reported by (Scott and Robson 1991) are based on the maximum obtained shoot weight after approximately 7 weeks of growth. While lower biomass production can be related to lower nutrient availability, shoot biomass is not the indicator a farmer is interested in. Wheat

[6] Like the majority of the references for reported critical concentration in the plant analysis interpretation manual (Reuter and Robinson 1997).

yield may be more or less sensitive to Mg deficiency than shoot growth, so the critical concentration based on actual yield can be different from the concentrations reported in this experiment.

MacKay et al. (1966) did include tuber yield in their reported critical concentration. Likewise, Vlamis and Ulrich (1971) based themselves on yield. Even taking the quality of the product into account by checking how sucrose content of beets was affected by B supply.

Analysis

In the three dry matter experiments, nutrient concentrations and contents in plant tissue were determined with methods that are currently outdated. Atomic adsorption spectrometry (for Mg), the curcumin method (for B), and flame photometry (for K) are slower, less sensitive, and require more skill than plasma emission spectrometry (Bryson et al. 2014). Which does not mean that the reported concentrations are invalid, just that researchers and commercial labs may use different analytical tools nowadays.

There might be a further impact of the method of drying plant tissues. Beside that the temperatures is thought to have an effect, there are indications that the method of drying has an effect too. The method of drying cannot always be clearly determined from these experiments.

Also plant sap methods are not standardized in extracting the sap and measuring concentrations. A few different procedures of extraction plant sap are described in scientific literature: Sonneveld (1987) extracted plant sap by pressure. De Krey (1996) used for his experiments frozen leaf materials. After thawing, the plant sap was manually retrieved by squeezing the sap out of plant tissue. Some commercially available quick tests describe the use of a garlic press as a method to obtain plant sap.

Suitability of petiole nitrate concentration for determining potato N requirement

Based on a review of several sources MacKerron et al. (1995) concluded that petiole nitrate concentration is a poor predictor to fertiliser response. In other words, one cannot accurately determine the amount of required fertiliser based on petiole nitrate concentrations. Jones and Painter (1974) also state that one cannot infer the amount of fertiliser required by the crop based on petiole nitrate concentrations.

MacKerron et al. (1995) further pointed out that there are no studies that compare treatments where petiole nitrate concentrations are used to adjust fertilisation with control treatments, more recent studies have not done so either (Brink et al. 2002). Van Geel et al. (2012) did report that supplemental N fertilisation steered by petiole analysis could reduce fertiliser use. However, they also found that the method was regarded as too labour intensive and that this method did not always accurately indicate that more fertiliser was required. Therefore, one cannot conclude that adjusting fertilisation based on analysis of petiole (sap), leads to higher yields, a better economic result, or better nutrient use efficiency. To this day, no firm conclusions are drawn on the usefulness of potato petiole nitrate analysis, at best a potential is recognised in scientific literature (Tei et al. 2017). Note that this is also largely the case for other methods of plant analysis.

Environmental factors

Furthermore, the norm trajectory for potato petiole nitrate-N concentration currently in the Dutch soil and fertilisation manual (Figure 2-2), could be improved by basing the trajectory on temperature sum or development stage instead of days after emergence.

4 Critical nutrient concentrations reported in literature

4.1 Introduction

In this chapter a summary is given on the information about K, Ca, Mg, S, Mn, and B currently in the Dutch handbook on soil and fertilisation supplemented with general information on the nutrients' deficiency and toxicity symptoms, their mobility, and on which soils deficiency and toxicity tend to be found based on chapters from the Handbook of plant nutrition (Barker and Pilbeam 2015).

Thereafter, reported nutrient concentrations in plant tissues are presented and discussed per crop. The tables listing nutrient concentrations have black and red values. Black values are based listed in *Plant analysis: an interpretation manual* (Reuter and Robinson 1997) and are reported together with a growth stage, a plant organ, and the type of experiment used to establish the value. This manual is the most comprehensive and up to date collection of nutrient concentrations for a wide variety of crops. Entries of which experimental methods of establishment are unknown, are red values. Additionally, some of the red values are taken from Bryson et al. (2014), who published values used by their commercial laboratory which are based on scientific literature and adapted with their own field data. The distinction between black and red values is made as red values are not reproducible. All reported concentrations are measured from oven dry tissue samples. The data included are for the most commonly used plant organ for each crop; youngest mature leaf (YML) for potato and sugar beet, whole shoot for cereals, unless indicated otherwise.

Most of the reported concentrations are based on experiments from the USA, Canada, and Australia. Thus, these concentrations may not apply to the Dutch context directly, but they may give an impression of what deficient and sufficient concentrations can be expected when looking at Dutch field data.

Critical nutrient concentrations in plant sap of arable crops are not reported in literature except for nitrate in potato petioles. Laboratories that use plant sap analysis for fertilization advice have developed their own reference values which are not publicly available and mostly based on (scientific) experiments but on data gathered in practice. As nitrate is not the focus of this study and that influence of cultivar on the critical nitrogen value is large, no data are presented on critical nitrogen concentrations in potato petioles.

4.2 Nutrient deficiencies in arable crops

Potassium

For potassium, there is an extensive fertiliser recommendation system based on soil analysis which will not be discussed here and can be found online ("*Kali*" n.d.).

The initial symptom of K deficiency is reduced growth, which is hard to identify as this is a rather non-specific symptom. When deficiency becomes more severe, chlorotic and necrotic stipes may form along the leaf margins of older leaves. K deficient plants have trouble forming cuticles which protect against water loss. Therefore, K deficient plants have a lower water use efficiency (Mengel 2015). Mengel (2015) notes that K concentrations of water inside plants such as in vacuoles or cytosol is very stable, for most of the growing season. Therefore, he argues that K concentrations in plant tissue water is a better metric to diagnose K deficiency than dry matter analysis. However, he does not discuss to what degree water stress affects K concentrations or whether tissue water can be measured with comparable accuracy as dry matter. One of the downsides being that dry-out of tissue greatly affects the K concentration of tissue water. Others have argued in favour of measuring K concentrations of plant sap as this would be more physiologically relevant given that K is often in solution rather than solid structure (Leigh and Wyn Jones 1984). Despite these remarks, K concentrations in plant tissue are generally given as a percentage of dry matter.

Magnesium

On clay and marine sand soils, Mg deficiency does not occur very often ("*Magnesium,*" n.d.). Therefore, no specific fertilisation recommendations are currently given for clay and marine sandy soils. However, Mg deficiency may occur in potatoes, which may be exacerbated by a large supply of K. On sandy soils with low amounts of organic matter and low pH, Mg deficiency occurs more frequently. With the right soil fertilisation through mineral or organic fertilisers, Mg deficiency can be prevented. If after Mg fertilisation (mineral or organic), deficiency symptoms still occur, It is recommended to apply foliar fertilisation ("*Magnesium,*" n.d.). If Mg deficiency occurs frequently on a field it is recommended to apply fertiliser before sowing/planting or apply foliar fertilisation. If the Mg concentration of sugar beet is below 250 mg/100 g DM ("*Magnesium,*" n.d.) or 260 mg/100 g DM (Wilting 2016), there is a risk of deficiency. Mg's mobile nature means that deficiency symptoms appear first in older leaves as starch accumulating in the leaves, followed by chlorosis. No clear Mg toxicity symptoms are known. If symptoms appear due to high Mg availability this may be caused by a deficiency of competing cations. Environmental factors that influence transpiration can exaggerate Mg deficiency symptoms as the relocation of Mg is linked to the leaf's transpiration. Factors that influence water uptake can negatively affect Mg supply and therefore increase the likelihood of deficiency symptoms manifesting.

Some studies have been done on the competition for binding sites in the soil between the divalent cations Mg, Zn, Mn, and Cu. However, because these other metals are needed in such small amounts compared to Mg, their toxicity symptoms will likely appear before Mg deficiency symptoms (Merhaut 2015). Bussink *et al.* (2020) discussed the possible antagonistic interaction between K and Mg and conclude that by maintaining ample soil Mg concentrations, Mg deficiency caused by high supply of K can be avoided. They add, however, if the Mg status of the soil is low, high addition of K can worsen Mg deficiency, which is in accordance with the current Dutch advice on Mg fertilisation ("*Magnesium,*" n.d.).

Sulphur

Most sulphur is supplied to a crop from soil processes; the mineral sulphur content left from the previous crop, mineralisation during the growing season, sulphur deposition, sulphur in irrigation water, capillary rise of sulphur-rich groundwater. Another source for sulphur is sulphate containing fertilzers. Nowadays, atmospheric sulphur deposition is much lower than it used to be and does not contribute much anymore. Supply by groundwater or irrigation varies. If a shortage of S is expected based on soil analysis, S can be fertilised before the growing season ("*Zwavel*" n.d.).

Symptoms of S deficiency are enhanced at higher levels of N fertilisation (Haneklaus et al. 2015).

Boron

In the Netherlands, B deficiency occurs on sandy soils with low organic matter content and can more easily occur at very low (<4) or high (>6) pH combined with drought. On clay soils, sugar beets can have B deficiency during a very dry summer. It is currently advised to analyse the soil B content before the growing season for sugar beets and several vegetables to determine if additional B fertilisation is required. B can be fertilised in the soil or as foliar application. Foliar fertilisation should be applied as soon as possible when B deficiency occurs. When B deficiency symptoms are visible it is too late to remediate the deficiency ("*Borium*" n.d.). Deficiency symptoms initially occur on young leaves, as B is very immobile, young tissue depends on xylem transport for supply of sufficient B. In Monocots such as cereals, B accumulates in leaf tips while in dicots B accumulates in leaf margins, B also tends to accumulate in older leaves. These are also the places where toxicity symptoms are first visible. Leaves are the most suitable organ to analyse for boron deficiency as concentrations in the leaves are generally higher compared to other organs. As B is immobile once in the leaves, it is important to sample leaves of the right age. Old leaves may not give an accurate reflection of the current B status of the crop while young leaves may underestimate the available B when little water was available to transport B from the soil to the leaves (Gupta 2015). Therefore the youngest mature leave is typically used. A narrow range between B deficiency and toxicity is often reported, so one should be careful with over fertilisation or using B rich water for irrigation (Gupta 2015). Gupta (2015) further reports that soil organic matter can be a large source of B and that higher pH and clay content can further increase the availability of B from organic matter. This is already acknowledged in the Dutch handbook on soil and fertilisation ("*Borium*" n.d.).

Manganese

Mg deficiency occurs on soils with high pH and/or high OM content with long dry periods. For certain sandy soils,[7] there is no advice based on soil analysis but at soil pH below pH 5.4 there is no chance for Mn deficiency. Between pH>5.4 and pH>6.2 the chance that Mn deficiency will occur increases. Over pH>6.2 Mn deficiency is frequent ("*Mangaan*" n.d.). Above pH 6, soil analysis is unsuitable to measure plant available Mn (Mn-CaCl2) . When this is the case, other analysis methods such as crop analysis can provide insight into the Mn status of the crop. If the soil pH is too high, foliar fertilisation is recommended. For marine clay, Mn deficiency is likely to occur with a soil Mn <60 mg/kg soil (reducible Mn, determined with an extract of ammonium nitrate 1 N hydrochinon) when OM <2.5% or with soil Mn<100 mg/kg soil when OM>2.5% ("*Mangaan*" n.d.). If Mn deficiency is expected, it is recommended to apply foliar fertilisation. Mn deficiency symptoms may also occur during drought, in that case one may want to wait for rain or irrigate. In sugar beet, a Mn concentration of 2.0 mg/100 g DM or higher in the youngest mature leaf is considered sufficient ("*Mangaan*" n.d.).

Mn is immobile but despite being classified as immobile, under the right conditions Mn can be reallocated from roots and shoots. Often, deficiency symptoms are clearly visible when growth is already reduced. Mn deficiency symptoms can easily be confused with those of Fe and Mg. However, Mn deficiency symptoms typically first appear in the youngest fully expanded leaf, while Mg symptoms appear in old leaves (Humphries, et al., 2015). Deficiency is often expressed as diffuse interveinal chlorosis and possible necrotic spots or streaks. Unlike Fe deficiency symptoms, chlorosis caused by Mn deficiency is not uniformly distributed on the affected leaves. Of the crops in this report, sugar beet and oats are reported to be more susceptible to Mn deficiency, while rye hardly is (Humphries et al.2015). On sandy soils with a pH>5.7, manganese deficiency can also occur in potato

[7] Dutch: dekzand en dalgronden

and cereals ("*Mangaan*" n.d.). Differences between cultivars in Mn deficiency sensitivity have been found in barley (Pedas et al. 2008). Potato cultivars differ in sensitivity to foliar Mn fertilisation. Sugar beet is also reported to be more susceptible to Mn toxicity. Mn toxicity expressed differently in different crops and can easily be confused with aluminium toxicity as both tend to occur when crops grow on soils with low pH (Humphries et al. 2015).

Recently, a method to detect Mn deficiency using chlorophyll a fluorescence has been reported (Pedas et al. 2014; Van Maarschalkerweerd and Husted 2015) .

Calcium

Often supplied together with K or P as calcium ammonium nitrate (CAN) or superphosphate. Ca deficiency is very uncommon in arable crops in the Netherlands. Lab analysis may be used to diagnose a possible Ca deficiency but there are no standardised procedures for this ("*Calcium*" n.d.). Although Ca deficiency is rare in the Netherlands, some critical nutrient concentrations will be given.

4.3 Potato

In Table 4-1, reported nutrient concentrations in potato are given in black and red. As has been indicated in the introduction, black values are taken from *Plant analysis: an interpretation manual* (Reuter and Robinson 1997) and are reported together with a growth stage, a plant organ, and the type of experiment used to establish the value. Entries of which experimental methods of establishment are unknown, are given in red. Additionally, some of the red values are taken from Bryson et al. (2014), who published values used by their commercial laboratory which are based on scientific literature and adapted with their own field data.

Table 4-1. Reported critical, adequate and deficient concentrations in potato youngest mature leaves. Early is the period till onset of flowering, mid is from flowering to tuber bulking, late is from tuber bulking to harvest. Red numbers indicate values reported by Reuter and Robinson (1997) of which the type of experiment for establishment is not mentioned or that the values are from (Bryson et al. 2014)*. [1] Definition of critical level is unknown.*

			K (g/100 g DM)	Mg (g/100 g DM)	S (g/100g DM)	Ca (g/100 g DM)	B (mg/kg DM)	Mn (mg/kg DM)
Potato	Early	Critical		0.3		0.8		
		Adequate	4.0-11.5	>0.3 - >0.74 0.50-1.50	0.19-0.36	0.58-1.67 0.8-2.0 0.39-0.59 1.0-2.0 0.6-1.0	>30 25-50	>22 - >61 30-450
		Deficient	<3.0	<0.22		<0.6	<15	<20
	Mid	Critical		0.25		1		
		Adequate	>4 - >7 6-8	>0.25 - >0.78 0.7-1	0.3-0.5 0.2-0.5	1.0-2.5 0.66-1.35 0.92-0.93 1.00-1.13 1.5-2.5	>20 40-70	>30 - >50 30-250
		Deficient	<2.25	<0.20		< <0.7	<10	
	Late	Critical						
		Adequate	>3 - >3.5			>0.20 0.2-0.5	>20	>69 - > 100
		Deficient			<0.12			

Potassium

Deficient K concentrations in the YML dry matter range from <3.0 g/kg DM at early flowering to < 2.25 g/kg DM at tuber bulking. Concentrations of sufficiency vary a bit. For the YML during flowering, most entries report sufficiency between 4 and 6 g/kg DM but one reports 7.0-8.2 while two others have lower ranges of 3.5-5.0 g/kg DM and 3.5-5.5 g/kg DM. Later in the season (100 mm Tuber or Tuber bulking), sufficient YML concentrations are reported somewhat lower from over 3 to over 3.5 g/kg DM.

Magnesium

For potato Reuter and Robinson (1997) have several entries of the youngest mature leaf (YML) during early and late flowering. Based on these reports, deficiency seems to occur when Mg concentration in the YML is around 0.20-0.25 g/kg DM.

For petiole concentrations reported by (Walworth and Muniz 1993), entries vary, deficiency seems to occur when the concentration is below 0.15% before during and after flowering. During the same period, concentrations between 0.30% and 1.00% seem to be sufficient. Whole leaf Mg concentrations where plant growth is expected to respond to fertiliser addition at 0.15-0.25 g/kg DM reported by Walworth and Muniz (1993) was similar to the calculated critical concentration of 0.14 g/kg DM based on a recent meta-analysis (Hauer-Jákli and Tränkner 2019) While the range reported by Walworth and Muniz (1993) is linked to a growth stage (early flowering) the calculated value by Hauer-Jákli and Tränkner (2019) is not.

For many reported tissue concentrations in potato, the reported growth stage is based on the degree of flowering or the size of tubers. This complicates getting an accurate sense of the development stage as not all potato varieties flower and getting an accurate and representative estimate of tuber size is cumbersome. Reporting development stage based on growing degree days would give a more objective and numeric indication of crop development. This would benefit development of tissue nutrient deficiency range curves.

Boron

For potato there are 20 entries with deficient and or sufficient values for B concentrations in Reuter and Robinson (1997), most of them based on experiments. For the youngest mature leaf (YML) concentrations below 15 mg/kg DM has been reported as deficient from early to late flowering and 75 days after emergence. Depending on the development stage, YML B concentrations over 30 (up to tuber bulking) or 20 (after tuber bulking) are reported as sufficient. Concentrations above 50 mg B/kg DM are reported as toxic. Based on these reports, YML B concentrations between 20 and 50 mg/kg DM should be fine but this needs to be cross-checked with data from the Netherlands. No critical values are reported as such. However, since deficiency is reported below 15 mg/kg DM and adequacy is generally reported to be over 30 mg/kg DM, a critical value is likely somewhere in between these values. The toxic concentration of 50 mg-kg DM is reported only twice, it is possible that Dutch cultivars on Dutch soils are more or less tolerant for B toxicity.

Manganese

Only two deficient concentrations are reported for Mn in potato youngest mature leaves. During early flowering concentrations in the YMB below 20 mg/kg DM, is reported as deficient, while during tuber bulking concentrations below <10 mg/kg DM is deficient. During tuber bulking, concentrations in the petiole of the youngest mature leaf can be a little higher <20 mg/kg DM is regarded as deficient while >30 mg/kg DM is reported as adequate. Adequate YML concentrations during flowering range from 22-50 (low) to 37-300 (high). Based on these data, YML concentrations over 30 mg/kg DM are probably sufficient for optimal growth.

4.4 Sugar beet

Ulrich and Hills (1990) state that there is little change in (critical) concentrations in sugar beet during the vegetative growth stage, which extends to most of the sugar beet growing season. They further note that, there is little difference in critical concentrations between commercially available cultivars

Table 4-2. Reported concentrations in youngest mature sugar beet leave.blades. Black numbers are derived from (Vlamis and Ulrich 1971; Reuter and Robinson 1997)*, red numbers are from* (Bryson et al. 2014) *or* (Ulrich and Hills 1990). *Development stage is assumed to matter little after seedling stage as the beet is harvested in the vegetative stage* (Ulrich and Hills 1990). [1]Vlamis and Ulrich (1971) *derived a critical value of 21 mg/kg DM defined as 90% of max mature leaf blade yield. They suggest that the range 15-30 is more realistic.* [2] *Values for whole shoots.*

		K (g/100 g DM)	Mg (g/100 g DM)	S (g/100 g DM)	Ca (g/100 g DM)	B (mg/kg DM)	Mn (mg/kg DM)
Sugar beet	Critical	1		0.75	0.5	21[1] 15-30	15[2] 10
	Adequate	2.0 - 6.0; 1.0 – 6.0	0.25 - 1.00; 0.1-0.25	0.21-0.50	0.4-1.5 0.21-0.50	31 - 200; 35 - 200	30 – 62[2] 26 - 100; 25 – 360
	Deficient	0.3 - 0.6	0.25 - 0.5	0.4-0.75	0.1-0.4	12 - 40	<30[2] 4 - 20;

Potassium

Reuter and Robinson (1997) did not include reports of experimentally determined K concentrations in sugar beet with specified growth stages.

Magnesium

Reuter and Robinson (1997) do not list sugar beet tissue concentrations directly based on experiments but on literature. Most entries also have poorly classified growth stages. There are only three different authors in the sources of which two of them report leaf concentrations. Based on this information it seems that Mg concentration in sugar beet leaves should be above 0.3 g/kg DM

Sulphur

Both Dutch and international studies found that sugar beet does not respond to sulphur fertilisation (IRS, n.d.; "*Zwavel*" n.d.). So, even an observed S deficiency may not be remediated by fertilisation.

Boron

For sugar beet the entries into Reuter and Robinson (1997) are not based on experiments and few indicate the development stage. The majority of entries is based on work by Ulrich. Vlamis and Ulrich (1971) found a critical B concentration of 21 mg/kg DM in mature blades. However, when they compare their value with values reported by others, they suggest a critical range from 15-30 is a better estimate of when deficiency may first occur. In Reuter and Robinson (1997), critical deficiency values are 21 and 27 with general deficient concentrations ranging from 12 to 40 mg B/kg DM in the YMB or YML. Reported sufficient concentrations are over 30.

The value underneath which B deficiency in sugar beet can be expected currently presentend in the Dutch handbook on fertilisation (32 mg/kg DM), corresponds with the literature ("*Borium*" n.d.). This is probably because the value is mostly based on the literature and cross checked with Dutch field data (Wilting 2016).

Manganese

Sufficient Mn concentrations in whole shoots youngest mature blades, and leaves range from 25 - 35 (low) to 62-360 (high). Deficiency is reported for leaf concentrations below 20-25 and shoot concentrations below 30 mg/kg DM. Critical values reported for Mn are lower than the range where deficiency symptoms have been observed (Table 4-2). To avoid Mn deficiency, it seems one should aim to maintain Mn concentrations above 30 mg Mn/kg DM.

4.5 Onion

Table 4-3. Reported critical, adequate and deficient concentrations in onion. WS = whole shoot, YMB = youngest mature blade, TL = tallest leaf. Early is the period from sowing till 1/3 growth. Mid from 1/3 to mid-growth, late from mid growth to maturity. Red numbers indicate values reported by Reuter and Robinson (1997) of which the type of experiment for establishment is not mentioned or from (Bryson et al. 2014)*. [1]Definition of critical concentration unknown.*

			K (g/100 g DM)	Mg (g/100 g DM)	S (g/100 g DM)	Ca (g/100 g DM)	B (mg/kg DM)	Mn (mg/kg DM)
Onion	Early	Critical						
		Adequate	4.18 (WS)	0.47 (WS)		1.6 (WS)		
		Deficient		0.34 (WS)				
	Mid	Critical						
		Adequate	2.5-5.0 (YMB) 4.0 (tallest leaf) 4.00-5.5 (WS)	0.25-0.40 0.30-0.5 (WS)	0.50-1.00 (WS)	1.5-3.5 (YMB) 1.00-2.00 (WS) 1.50-2.20 (WS)	30-45 (YMB) 22-60 (WS) 22-60 (WS)	50-250 (WS)
		Deficient	2.5 (tallest leaf)	0.22-0.24 (WS)	0.30-0.49 (WS)	0.80-0.99 (WS)	18-22 (WS)	
	Late	Critical	1.3 (YMB)[1]					
		Adequate	3.50-5.00 (WS)	0.6-0.8 (YMB) 0.25-0.4 (WS)	0.50-1.00 (WS)	2.2-2.9 (YMB) 1.50-2.20 (WS) 1.00-3.5 (WS)	25-45 (YMB) 25-75 (WS)	55-65 (YMB) 50-250 (WS)
		Deficient			0.30-0.49 (WS)	1.00-1.49 (WS)	20-24 (WS)	

Potassium

In-season nutrient concentrations for onion reported by Reuter and Robinson (1997) are limited. Only one included study reported adequate concentrations for whole shoot, based on field experiments and one study reported an experimentally determined adequate concentration range mid growth for the youngest mature blade (YMB). Reported whole shoot adequate potassium concentrations are 4.18 g/kg DM (2 leaves), 3.48 g/kg DM (4 leaves), and 3.68 g/kg DM (6 leaves). During mid-growth, adequate YMB K concentrations are above 2.5 g/kg DM (Reuter and Robinson 1997).

Magnesium

For onion, Mg concentrations of whole shoots seem to be deficient below 0.25 g/kg DM, but it is not clear how this value was obtained. Reported sufficient whole shoot concentration decrease from 0.47 g/kg DM in the 2-leaf stage to 0.29 g/kg DM in the 6-leave stage. Concentrations in the youngest mature blade are reportedly sufficient between 0.30 g/kg DM and 0.50 g/kg DM during mid growth.

Boron

For onion, Reuter and Robinson (1997) have five entries B concentrations. For the youngest mature blade (YMB) sufficient concentrations during mid-growth range from 30-50 mg B/kg DM. During bulbing 25-45 is reported while concentrations below 20 are classified as low. For the whole shoot from 1/3 growth to maturity sufficient concentrations are between 25 and 60 with slightly higher concentrations of B being sufficient in the second half of development. There is limited information to assess what adequate B concentrations are for onion, the most conservative estimate is that concentrations in the YMB between 30-45 are adequate but the real range is probably somewhat wider.

Manganese

There are only three entries for Mn tissue concentrations in onion. From the reported information, it seems that deficiency occurs when leaf or whole shoot concentration falls below 40 mg/kg DM and is adequate above 50.

4.6 Cereals

Table 4-4. Reported critical, adequate and deficient concentrations in whole shoots of wheat barley and oats. Early is the period from sowing to FS9, just before heading. Mid is from FS10 until FS10.5, just before flowering. Late is from flowering to maturity. Red numbers indicate values reported by Reuter and Robinson (1997) of which the type of experiment for establishment is not mentioned or from (Bryson et al. 2014). *[1]A range of critical values defined as 95% of optimal yield ranging from 4.1 at FS 2 to 2.0 at FS 7 in the early stage 1.45 at FS 10.1 mid stage, and 0.9 at FS 11 at late stage. [2]Critical at 90% max shoot yield. [3] Critical at 90% shoot yield. [4] Definition of critical concentration unknown. [5] Critical at 90% max yield decreasing from 0.24 at 35 days after seeding to 0.08 at 119 days after seeding. [6] Critical at 90% max grain yield*

			K (g/100 g DM)	Mg (g/100 g DM)	S (g/100 g DM)	Ca (g/100 g DM)	B (mg/kg DM)	Mn (mg/kg DM)
Wheat	Early	Critical	4.1 - 2.0[1]		0.3-0.26			11, 35
		Adequate	>4.1 - >2.3	>0.10 - > 0.15	0.15-0.4 0.15-0.40			>35
		Deficient	<3.5 - <1.8					11[2], 35
	Mid	Critical	1.45[1]	0.13, 0.15	0.11-0.18	0.25		
		Adequate	>1.5 1.50-3.00	>0.15 0.15-50.0	0.1-0.15	0.2-0.5 0.2-0.5	>6 - >10 6-10	25-100
		Deficient	<1.25	<0.15	0.06	<0.2	<5	
	Late	Critical	0.9[1]					
		Adequate	>1.0		0.12		43	
		Deficient			0.08			
Barley	Early	Critical				0.34		
		Adequate	>3.8 >3.0	>0.16		0.4-0.7	10 - 15	>17 - >30
		Deficient						<13
	Mid	Critical				0.34		
		Adequate	1.50-3.00	0.15-0.50	0.15-0.4	0.30-1.20	5 - 10 1-5	25-100
			Deficient	<0.15				
Oats	Early	Critical		0.15[4]	0.24-[5]			17.5[6]
		Adequate	4.5-5.8	>0.12 >0.2				>30
		Deficient		<0.07				<15
	Mid	Critical		0.12, 0.18	0.14-0.17[5]			9[6]
		Adequate	>1.5 1.50-3.00	0.15-0.50	0.15-0.4	0.20-0.50	5 - 15 1.5-4	>25 25-100; 40-100
		Deficient	<1.25	<0.15	<0.15		<3.5	<5
	Late	Critical			0.08[5]			
		Adequate						>14
		Deficient						<14
Rye	Early	Critical						
		Adequate	1.90-2.30; 2.7-4.0	0.20-0.60	0.15-0.65	0.2-1	1.5-4; 4-10	20-100
		Deficient	<1.9					

Potassium

For wheat, reported whole shoot concentrations that were based on experiments are given in Table 4-5.

Table 4-5. Experimentally determined whole shoot potassium concentrations (g K/100g DM) of wheat reported in Reuter and Robinson (1997).

Growth stage	Deficient	Critical	Adequate	Country
FS 2	< 3.5	4.1	> 4.1	Australia
FS 3	< 3.0	3.2	> 3.5	Australia
FS 7	<1.8	2.0	> 2.3	Australia
FS 10.1	<1.3	1.5	> 1.6	Australia
FS 10.1			1.5-2.5	Australia
FS 10.1	< 1.25		1.5-3.0	USA
FS 11		0.9	> 1.0	Australia

For barley at tillering, one experimentally determined whole shoot adequate concentration is reported: 3.8-6.2 g/kg DM, similar to the reported values for wheat (FS 2 and FS 3). For the YMB mid-tillering <1.5 g/kg DM is reported as deficient and 2.4-4.0 g/kg DM as adequate. In oats, whole shoot concentration at onset of heading is reportedly deficient <1.25 g/kg DM and adequate at 1.5-3.0 g/kg DM, similar to wheat (FS 10.1). During tillering, concentrations in the YMB below 1.5 g/kg DM are deficient and adequate between 2.4 and 4.0 g/kg DM. For rye, no experimentally derived reports were included.

Magnesium

For the cereals, the whole shoot sufficient Mg concentration is roughly between 0.20 g/kg DM and 0.5 g/kg DM but reports vary, some consider 0.15 g/kg DM as sufficient. Below 0.15 g/kg DM deficiency is expected for most cereals regardless of development stage. For winter wheat, sufficient concentration may be a little below 0.15 g/kg DM. With the collected data it is not possible to estimate a developmental stage-dependent critical value or range for cereals.

Sulphur

Rasmussen et al. (1977) report that the ratio between N and S is a better indicator for S deficiency than S concentrations as there are many factors that influence S tissue concentrations and variation in S concentration between season is large. They further state that Feekes' scale does not provide a sufficiently sensitive measure to monitor changes in S concentration. Concentrations that are sufficient in one year can be deficient in another year. Yet, only for wheat and barley some N/S ratios are reported (Table 4-6). In wheat, the N/S ratio is less sensitive to age, it is suggested to use 15:1 as a critical N/S ratio which can be relaxed a little for older plants (Spencer and Freney 1980). The downside to using ratios between nutrients for diagnostics is that it gives no insight into whether the concentration of one nutrient is very high or that the other nutrients concentration is low. So one may wrongly interpret excess of one nutrient as deficiency of the other (Haneklaus et al. 2015).

Alternatively, sulphate S as a fraction of total S can be used to detect S deficiency in wheat. Spencer and Freney (1980) report that wheat plants should contain more than 13% of total S as sulphate during vegetative growth to avoid growth reduction (Table 4-7).

Table 4-6. Whole shoot wheat and barley N/S ratios reported as deficient, critical, or adequate (Reuter and Robinson 1997).

	Development stage	Deficient	Critical	Adequate
Wheat	FS 4-10	19-33		13-16
	FS 9		19	
	FS 9-10		16.5	
	FS 10.1	21		9-16
Barley	Flowering	25		17

Table 4-7. Whole shoot critical percentage of sulphate S of total S (Reuter and Robinson 1997). *Critical at 90% max yield.*

	Development stage	Critical ratio
Wheat	FS 2 - early joint	13
Oat	335-119 DAS	5

Boron

For the cereals, the majority of entries are based on whole shoot concentrations. Concentrations between 5 and 15 mg B/ kg DM generally are sufficient. Limited reports on deficient concentrations are below 5 or below 3 mg/kg DM in wheat and barley. Some entries report concentrations over 15 as toxic while others report much higher toxicity concentrations of over 50 (in barley, oats, and wheat).

Manganese

According to the data collected in Reuter and Robinson (1997), for wheat, sufficient whole shoot Mn concentrations seem to be a little higher than for the other cereals ranging from 23 to 37 for low sufficient concentrations to 116 for high sufficient concentrations. With deficient or critical values being reported ranging from 6 to 35. In barley, whole shoot and youngest emerged blade Mn concentrations should be sufficient above 25 mg/kg DM. Yield loss seems to occur below concentrations from 10-20 mg/kg DM. In oats, whole shoot Mn concentrations below 5-17.5 mg/kg DM are reported as deficient or critical. The lowest sufficient concentration ranges from 14 to 40 and goes up to about 100 mg/kg DM. For Rye, whole shoot Mg concentrations seem to be sufficient above 20. Only a single deficient report is included for rye at concentrations of 3-13 mg/kg DM.

4.7 Critical nutrient concentrations Eurofins-Agro

The critical nutrient concentrations that are used by Eurofins-Agro for their recommendations in practice are given in table 5.8. These critical concentrations are based on a combination of literature data (mainly from Bryson et al., 2014) and own data (from field experiments and monitoring on farmers' fields). The own data are used for adjusting the critical nutrient concentrations for Mg, B and Mn in potatoes and for the nutrients S, K, Ca, Mg and Mn in onions.

4.8 Critical nutrient concentrations for fertiliser recommendations

Based on the overview of critical nutrient concentrations from scientific literature and the data presented by Eurofins-agro, we propose to use critical nutrient concentrations as a tool for diagnosis of nutrient deficiency in arable crops in the Netherlands (table 5.9). This could be used for the Dutch Handboek Bodem en Bemesting.

Table 5.8. Critical nutrient concentrations of the main arable crops used by Eurofins-Agro (Source: Eurofins-Agro, 2020).

Crop	Plant part	Growth stage	Evaluation class	K (g/ 100 g DM)	Mg (g/100 g DM)	Ca (g/100 g DM)	S (g/100 g DM)	B (mg/kg DM)	Mn (mg/kg DM)
Potato (ware)	Youngest mature leaf	30-60 days after emergence	Adequate	5.0-8.0	0.32-0.52	1.5-2.5	0.2-0.5	24-46	42-195
			Critical	3.0-5.0	0.28-0.32	0.75-1.5	0.1-0.2	22-24	28-42
			Deficient	<3.0	<0.28	<0.75	<0.1	<22	<28
Sugarbeet	Youngest mature leaf		Adequate	3.0-4.5	1.0-1.6	0.8-1.1	0.2-0.5	96-144	156-234
			Critical	1.5-3.0	0.5-1.0	0.3-0.8	0.1-0.2	48-96	50-156
			Deficient	<1.5	<0.5	<0.3	<0.1	<48	<50
Onion	Youngest mature leaf		Adequate	3.2-4.5	0.17-0.23	1.4-2.1	0.56-0.8	22-60	26-79
			Critical	2.8-3.2	0.16-0.17	1.3-1.4	0.5-0.56	11-22	20-26
			Deficient	<2.8	<0.16	<1.3	<0.5	<11	<20
Wheat (winter)	Youngest mature leaf	Period: Jan till June	Adequate	1.5-3.0	0.15-0.5	0.2-0.5	0.15-0.4	6-10	25-100
			Critical	0.75-1.5	0.075-0.15	0.1-0.2	0.075-0.15	3-6	12.5-25
			Deficient	<0.75	<0.075	<0.1	<0.075	<3	<12.5
Barley	Youngest mature leaf		Adequate	1.5-3.0	0.15-0.5	0.3-1.2	0.15-0.4	1-5	25-100
			Critical	0.75-1.5	0.075-0.15	0.15-0.3	0.075-0.15	0.5-1	12.5-25
			Deficient	<0.75	<0.075	<0.15	<0.075	<0.5	<12.5
Rye	Youngest mature leaf		Adequate	1.9-2.3	0.2-0.6	0.2-1.0	0.15-0.65	1.5-4	14-45
			Critical	0.95-1.9	0.1-0.2	0.1-0.2	0.075-0.15	0.8-1.5	7-14
			Deficient	<0.95	<0.1	<0.1	<0.075	<0.8	<7
Oats	Youngest mature leaf		Adequate	1.5-3.0	0.15-0.5	0.2-0.5	0.15-0.4	1.5-4	25-100
			Critical	0.75-1.5	0.075-0.15	0.1-0.2	0.075-0.15	0.8-1.5	12.5-25
			Deficient	<0.75	<0.075	<0.1	<0.075	<0.8	<12.5

Table 5.0. Critical nutrient concentrations in the youngest mature leaf of the main arable crops for Handboek Bodem en bemesting.

Crop	Plant part	Growth stage	Evaluation class	K (g/ 100 g DM)	Mg (g/100 g DM)	Ca (g/100 g DM)	S (g/100 g DM)	B (mg/kg DM)	Mn (mg/kg DM)
Potato (ware)	Youngest mature leaf	30-60 days after emergence	High	>7.0	>0.70	>2.5	>0.5	>50	>250
			Adequate	4.0-7.0	0.3-0.7	1.0-2.5	0.2-0.5	25-50	30-250
			Critical	3.0-4.0	0.28-0.30	0.75-1.0	0.1-0.2	15-25	20-30
			Deficient	<3.0	<0.28	<0.75	<0.1	<15	<20
Sugarbeet	Youngest mature leaf		High	>6.0	>1.0	>1.5	>0.5	>200	>200
			Adequate	2.0-6.0	0.3-1.0	0.4-1.5	0.2-0.5	30-200	30-200
			Critical	0.6-2.0	0.3	0.3-0.4	0.1-0.2	15-30	20-30
			Deficient	<0.6	<0.3	<0.3	<0.1	<15	<20
Onion	Youngest mature leaf		High	>5.0	>0.4	>3.5	>0.8	>60	>250
			Adequate	3.0-5.0	0.25-0.4	1.5-3.5	0.56-0.8	30-60	50-250
			Critical	2.5-3.0	0.25	1.3-1.5	0.5-0.56	11-30	40-50
			Deficient	<2.5	<0.25	<1.3	<0.5	<11	<40
Wheat (winter)	Youngest mature leaf	Period: Jan till June	High	>3.0	>0.5	>0.5	>0.4	>10	>100
			Adequate	1.5-3.0	0.15-0.5	0.2-0.5	0.15-0.4	6-10	25-100
			Critical	1.25-1.5	0.15	0.2	0.06-0.15	3-6	12.5-25
			Deficient	<1.25	<0.15	<0.2	<0.06	<3	<12.5
Barley	Youngest mature leaf		High	>3.0	>0.5	>1.2	>0.4	>5	>100
			Adequate	1.5-3.0	0.15-0.5	0.3-1.2	0.15-0.4	1-5	25-100
			Critical	0.75-1.5	0.075-0.15	0.15-0.3	0.075-0.15	0.5-1	12.5-25
			Deficient	<0.75	<0.075	<0.15	<0.075	<0.5	<12.5
Rye	Youngest mature leaf		High	>3.0	>0.6	>1.0	>0.65	>4	>45
			Adequate	1.5-3.0	0.2-0.6	0.2-1.0	0.15-0.65	1.5-4	14-45
			Critical	1.25-1.5	0.1-0.2	0.1-0.2	0.075-0.15	0.8-1.5	7-14
			Deficient	<1.25	<0.1	<0.1	<0.075	<0.8	<7
Oats	Youngest mature leaf		High	>3.0	>0.5	>0.5	>0.4	>4	>100
			Adequate	1.5-3.0	0.15-0.5	0.2-0.5	0.15-0.4	1.5-4	25-100
			Critical	0.75-1.5	0.075-0.15	0.1-0.2	0.075-0.15	0.8-1.5	12.5-25
			deficient	<0.75	<0.075	<0.1	<0.075	<0.8	<12.5

5 Discussion and conclusions

5.1 Discussion

Plant tissue analysis in combination with soil analysis

Plant tissue analysis is a tool to evaluate if the nutrient supply to crops is sufficient. Plant tissue analysis can be used in addition to soil analysis. It can show if there are actual or upcoming shortages of certain nutrients in the crop. This is complementary to soil analysis which only indicates if nutrients are available in the soil for plant uptake. An example for this is the advice system that was running in the Netherlands in potato with nitrogen in petiole measurements in combination with mineral nitrogen measurements in the soil. However, the system is more costly than measuring nitrogen in potato petiole or in the soil alone and is not available anymore (see paragraph 3.5). Using plant tissue analysis solely without soil analysis is possible but not recommended because of the difficulties in establishing well grounded critical values of nutrients.

Critical values difficult to derive

Plant tissue analysis is difficult to implement as critical values are needed to evaluate nutrient supply. We showed that these critical values are dependent on many factors connected to the crop, the environment, the nutrients in case (including interactions) and the methods of sampling and analysis. This makes it rather difficult to derive a simple scheme of critical values of nutrients per crop on which fertilization advices can be based.

It must be noted that soil analysis has its drawbacks and uncertainties in quantifying the availability of nutrients in the soil for plant uptake. In this case, the environment, the nutrients and the methods of sampling and analysis play a role as well. Overall, soil-based fertilization advices are easier to develop and implement than plant-based advices for arable crops as can be seen in fertilization advices worldwide.

Plant sap analysis versus dry matter plant analysis

Plant sap analysis is easier and cheaper to execute compared to dry matter plant analyses. Retrieving sap from plant organs is less labour intensive compared to drying, milling and grinding of plants. The dry matter method displays the nutritional status of a plant on a longer period and is less sensitive to short term fluctuations. Especially in cases where petioles are used, the plant sap method represents more the actual transport of nutrients than the nutrient status of the plant. Therefore, plant sap is more suited for rather mobile and more bulky nutrients like nitrogen and potassium and less suited for rather immobile nutrients like calcium or micronutrients. In the latter case, dry matter analysis is more appropriate.

Standardization of methods needed

Use of plant tissue analysis can be improved and be made easier when methods are more standardized. Various methods are used both for sampling plants and for measuring the nutrient content of the samples at various moments during crop growth.

Availability and usability of critical values from literature

Literature data were available to derive critical values for dry matter plant tissue analysis and not for plant sap analysis. The usability of these mostly international data, that are often determined in short term lab or pot experiments, for Dutch field conditions remains however questionable. Some reports mention large ranges of sufficiency whereas some reports mention conflicting data. The reason for the disparity between reports is not always obvious which makes the use of these critical values for Dutch circumstances questionable. Therefore, the critical values of plant tissue analysis can be used as an additional diagnostic tool to check the cause of poor crop growth together with other forms of analysis.

Do not develop new fertilization advices based on plant tissue analysis

The critical values available are insufficient to derive fertilization advices for arable crops. We advise also against the development of new fertilization advice systems based on plant tissue analysis. The reference dataset that needs to be build should at least consider factors as cultivar, phenological stage, location and soil conditions. This makes a system difficult to use and costly to develop. Besides we expect that the uncertainties in the system will still be large. If plant nutrient status seems important to consider, we expect that it is more promising to develop sensor-based advice systems as are for nitrogen already available.

5.2 Conclusions and recommendations

- Measurements in plant tissues may in theory contribute to an improved fertilization advice in comparison with those based on soil sampling only. Information about the nutrient status in plants and soil could lead to a better insight into the nutrient availability and nutrient uptake by plants.
- Critical values of nutrient contents in plant tissue are needed to compare the results of a measurement in plant tissue with the reference set. Retrieving these reference values is difficult because many factors are involved.
- International literature is containing critical values of dry matter tissue analysis for various arable crops. The usability of these mostly international data for Dutch circumstances is limited. For plant sap in arable crops, literature values are lacking except for nitrogen in potato.
- The literature data on critical nutrient values in plant tissue presented in this report can be used as additional indicative diagnostic tool to diagnose the cause of poor crop growth, additional to e.g. soil analysis. It is advised to include the data in the Handboek Bodem en Bemesting.
- We recommend not to invest in the development of new fertilization advice systems based on plant tissue analysis for arable crops. Development of such a system is costly and it is questioned if a useful and simple system can be developed. Development of sensor-based plant monitoring systems are expected to be more promising for the evaluation of the nutrient status of nitrogen and other mobile nutrients.

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Annex 1. Plant sap measurements.

Determining the nutrient status with measurements in plant sap

Some processes in the soil like mineralization, denitrification, immobilization, fluctuations in composition of organic manure or leaching make it difficult to know the actual nutrient availability in the soil to a crop. In the situation of upcoming shortage, the grower may want to apply additional nutrients via topdressing. There exist many possible reasons for nutrient deficiencies in crops and some of these factors are complex and interacting with each other (Bergman 1992). Growing conditions like soil moisture and the presence of diseases may also have an effect on the nutrient status of a crop. In the situation of an expected deficiency, farmers may choose from several diagnosis tools during the growing season to estimate the height of a required fertilizer application via topdressing. Some of these methods are nondestructive (e.g. sensor-based tools) while others are based on (destructive) analysis of plant parts. Probably the most widely used tool to determine a suspected nutrient deficiency is tissue analysis. Tissue analysis can be divided into a destructive analysis of the dry matter and plant sap analysis. One of the advantages of analysis of plant sap is the quick and relatively easy method of retrieving sap and the mineral composition can be determined directly in the extracted plant sap. Plant sap analysis can therefore be a relative cost efficient and quick method to determine the nutritional status of a plant in comparison with dry matter analysis.

Beside that is can be used to confirm a suspected nutrient deficient (diagnostic sampling), it is also used as a predictive tool by monitoring the crop through the growing season. Monitoring during the season is used to detect upcoming deficient situations in an early stage so be able to correct in time before yield reductions occur. Plant sap methods were developed for several greenhouse and arable crops.

Sometimes the plant sap methods are used to assess the nutrient status when problems with plant growth occur but most plant sap analysis are used to refine a topdressing advise or in combination with a soil sampling.

What is plantsap analysis?

A plant sap method is a method in which plant sap is being extracted from plant tissues. The nutrients are measured directly in the retrieved plan sap. Depending on the type of analysis, it is possible to determine the amounts of nutrients (N, (NO_{3}-, $NH4^{+}$), P, K, Ca, Mg, S, Na, and micronutrients or ratios' of those nutrients. Some laboratories provide additional pH, electrical conductivity and/ or metabolites like sugar, amino acids or other N-compounds. Not all plant sap laboratories provide all parameters. The actual nutritional status obtained from plant sap can be expressed as content in the sap, fresh weight or as a concentration in dry matter.

Methods of extracting sap from plants

A plant sap analysis is relatively simple. Before it can be analyzed, the sap needs to be extracted from plant tissues. Laboratories use different procedures to extract plant sap. Only a few procedures of extraction plant sap are well described in scientific literature and a few slightly different methods can be distinguished.
Sonneveld (1987) used plant sap results that were obtained from an extraction by pressure. De Krey (1996) used for his experiments frozen leaf materials. After thawing, the plant sap was manually retrieved by squeezing the sap out of plant tissue. Some commercially available quick tests describe the use of a garlic press as a method to obtain plant sap. It is possible that other laboratories have their own, perhaps automatically processed methods of obtaining plant sap.

Methods of measuring in the sap

After extracting, the nutrient content in the sap has to be determined. Commercial laboratories in the Netherlands use specialized equipment like spectrometry (mass- or photo spectrometry). This can in fact be similar or the same equipment as used for detection of nutrients in soil samples. Some self-test kits make use of test strips for one or a few elements or used ion specific sensors/testers.
Several commercial laboratories can perform plant sap analysis. There are multiple commercial test kits available where a grower can extract the plant sap himself and analyze it for a specific element.

Creating a fertilization advice

The next step, after measuring the nutrient status, is to compare the measured values with a reference dataset. The comparison with the reference values indicates whether the nutritional status of the plant is sufficient or insufficient. If the nutrient is insufficient, an advice for a topdressing will be given. The height of an adviced topdressing will be determined based on the intensity of a deficiency. That can be a broad application (with the limitation of a minimum applicable amount of fertilizer to apply) or foliar application.

Biomass
Some plant sap methods use the amount of biomass in their methods. The idea behind it is that plants tend to bulk up certain elements in the leave parts which can give information about the amount of nutrients that are taken up by plants. There is currently no laboratory that use biomass in their models for plant sap. Most sensor-based systems use a vegetation index or another parameter that includes the amount of a biomass in their modelling. The concept of biomass is therefore present in sensor based advisory systems. For plant sap analysis, it is an additional handling at the sampling site and is not very common.

Plantsap analysis in Dutch arable farming

Top dressing N advice in potato based on petiole sap analysis

There is one Dutch advisory model available for nitrate concentration in potato as described on www.handboekbodemenbemesting.nl. The critical values through the season are described on the website of CBAV. The system contains data for the cultivars Bintje and Agria and a generalized advice for starch potatoes. The advised nitrate concentration (norm value) is expressed as days after emergence (figure 1). The norm value can be considered as a reference value for optimal growth. The

data behind the advisory model are described by Van Geel and Brinks (2018). The validation for Bintje reaches back to research by Van Loon and Houwing (1989). The norm values for Agria were obtained from field data on 20 farms in the southwest of the Netherlands. The system contains only an advice for nitrate-N and not for other nutrients. Recently it is advised to update the advices because there are indications that there are more norm values needed for different varieties (Van Geel and Brinks, 2018). This is because of the large amount of varieties with different N-demand.

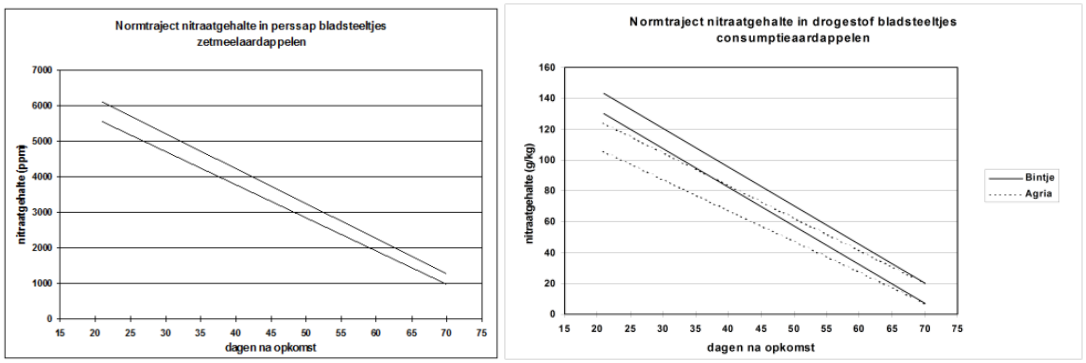

Figure 1. Optimal nitrate concentrations in petioles for the cultivars Bintje and Agria as described on the website of CBAV.

Topdressing N-advise in Brussels sprouts

A top-dressing system has also been developed for Brussels sprouts based on nitrate concentration in plant sap (Vlaswinkel and Van den Berg 2001). This system was developed as an alternative to the N-mineral advice for Brussels sprouts as described on the website of CBAV. The scientific validation is published. Information about a strong correlation between the height of the N-dose and the nitrate concentrations in the petiole for only one cultivar are mentioned. The system was used until 2015 by Altic B.V but is currently in use by Agrocontrol.

Percentage of farmers that use plant sap

Plant sap is one of the methods to determine if a top dressing is needed. There is currently no data available about the percentage in which farmers use plant sap methods to estimate the height of a top dressing in the Netherlands. A questionnaire that was carried out in 2011 yielded an implementation rate of 2% of the farmers using a tool for top dressing (e.g. plant sap, dry matter analysis or sensor-based measurements). Among the 2% of the farmers that use it, they stated to use it on approximately 0-5% of their fields (Smit et all, 2011).

Laboratories and companies in the Netherlands that offer plant sap analysis

There are a few laboratories who supply fertilizer recommendations at the basis of plant sap analysis (?). Market leader is Nova Crop Control. Further laboratories that provide plant sap analysis in the Netherlands are Eurofins Agro Testing and Fertilab. It also occurs that some agricultural advisors make use of the plant sap analysis of one of these laboratories. Some of these advising companies developed an own layout (like QMS from Delphy that use plant sap analysis of Nova Crop Control).

Scientific validation for plant sap methods

Reference values need to be species and variety specific

The key idea behind plant tissue analysis and advisory is a relationship between yield and nutritional status of the plant. Plants need a certain optimal nutrient status to realize optimal yield. If you can detect the nutrient levels in a plant, you can predict the yield level and besides when suboptimal levels are detected, with fertilization the optimal yield level can be secured.
The nutrient concentration in plants however are changing during the growth of a plant. The change is different between elements. The desired concentration at an early stage or later during the season will be different. Nitrate for example will be reduced in plants and will be assimilated; potassium will be stored at sink sources in the plants. It is therefore important to have reference values for each growing stage throughout the season for each element. A further factor that has to be considered is a cultivar specific nutrient demand. In several arable crops, there is a cultivar specific nutrient demand. This makes it more complicated to create a dataset with reference values because it has to be made cultivar specific.

Effect of the age, plant part and position of the leaf

There is an impact by the way tissue is sampled (petiole vs leaf tissue) and the position of the tissue which is sampled in the plant. Nutrients tend to spread unequal through plant tissue and some laboratories compare nutrient concentrations in old and young tissues.
There is thought to be an effect of the age of the leaf. Most sampling instructions of commercial laboratory advise to sample the latest fully-grown leaf. Some laboratories provide an analysis with the comparison between old and young leaves (Timmermans and Van der Ven, 2014) in which they make use of the age of a plant tissue. This type of analysis is done to get information about elements that can be translocated in plants (e.g. nitrogen, potassium, magnesium). Plants are known to relocate mobile elements from older leaves into the growing points of plants. Differences in concentration between old and young leaves are believed to give an impression about the nutrient status. This method also implies that the analysis should be performed in leaf blade sap and not in petiole sap since petiole is mainly composed of conducting vessels and therefore, the transport due to a redistribution in the plant of nutrients cannot be separated from the transport from those nutrients that were taken up by the roots. There is however no scientific validation available about determining nutrient status of plants by comparing old and younger leaves.

Variation during the day (diurnal variation)

Mc Kerron et al., 1995 found evidence that there is variation between the moment of sampling during the time of the day. In their experiments there was evidence that the nitrate concentrations in the sap was fluctuating during the time of the day. The presumed reason for this is that light affects the activity of nitrate reductase. Especially the nitrate concentration seems to be affected by the moment of sampling during the day (Bryson et al., 2014). In order to avoid differences due to the time of sampling, some laboratories restrict in their protocols a specified time frame in which has to be sampled, e.g. early morning before 10 am.

Differences between nutrients

Nutrients are not spread equal through plants tissues as blade, petiole and stem. In general, leaf blades are thought to contain higher amounts of total N, P, Ca, Mg, S, Al, B, Cu, Fe, Mn, Na and Zn (Bryson et al., 2014). Blades are preferred to petioles for evaluating the nutrient status of K, Ca, Mg, S, Na, B, Cu, Mn, Mo, and Zn, whereas petioles are better suited for evaluating nitrate, phosphate, and

chloride (Bryson et al., 2014). Several laboratories provide multi nutrient analysis. In some cases, they provide all necessary macro- and micronutrients.
Sonneveld (1985) conducted greenhouse experiments on two nutrients with a different function and behavior within in plants; calcium and potassium. The objective was to determine the nutritional status for potassium and calcium by comparing leaf tissue analysis versus plant sap. The tomatoes were grown in containers and irrigated with different concentrations of potassium and calcium. In these experiments, the leaf tissue and petioles were sampled and separated for analysis. (Plant sap was extracted by the press method.) The leaf petioles were analyzed by the plant sap method and the blades were analyzed by a destructive method.
The element calcium moves in plants by the xylem vessels to the sites where it is integrated in organic compounds. The floem flow contains relative low calcium and from its function and behavior in plants is to expect that most of the calcium will be incorporated into organic compounds such as cell walls and cell membranes. Potassium on the other hand is almost entirely present in ionic form in plant tissue and only a small amount is bounded to organic tissue. Dry matter testing on the other hand is sensitive to changes in dry matter content. The dry matter content of plants usually increases with a leaves age. Therefore, Sonneveld (1985) expected the plant sap method to have an advantage in testing for the potassium content in plants. Sonneveld presumed that plant sap measurements could give an accurate determination of the K-status since the element remains in ionic form in the plant. The presumed advantage determining K-content in plant sap is that the method is less sensitive to fluctuations in dry matter content of plant tissues. The dry matter method is sensitive to changes in dry matter concentrations and therefore, the potassium concentration will fluctuate due to dilution. Sonneveld (1985) found in his experiments that there was a good correlation between plant sap and dry matter. He further concluded that the plant sap method could be a promising method because of the quick and simple method of extracting sap from plants.
The calcium status in the same experiment was less predictable based on the plant sap method in comparison with the concentration in the dry matter. The experiment of Sonneveld did not yield a critical value and was more to test the power of the dry matter method versus the petiole method. In one of the experiments of Sonneveld (1985) a few further remarks of the Mg and Na content in the plant sap were made. There were indications that the Mg-concentration and the K-concentration were showing an interaction in one of the experiments.

Interaction between plant sap method and specific nutrients

Plant sap provide a snapshot of the actual nutritional status. This snapshot is for some nutrients strongly affected due to differences in functions and behavior of individual nutrients. Some nutrients are quickly being assimilated after uptake while other nutrients are being bulked up in plant parts. Furthermore, some temporary growing conditions like soil moisture content and temperature affect the uptake of certain nutrients causing fluctuations and make it difficult to estimate the actual nutritional status. Especially nutrients like calcium and micronutrients that are not being bulked up in plant sap are more sensitive to fluctuations. Calcium uptake is also low under dry circumstances and measuring calcium in a petiole that displays the actual transport versus a measurement in leaf tissue could lead to a total different conclusion about the nutritional status for calcium. Nutrients that are being bulked up in lager concentrations (e.g. potassium or nitrate N) are likely to be determined more stable because the process of bulking up is an ongoing process. These nutrients are probably also better to be compared between different plant sap methods (using leaf blade or using petiole).

Differences in methods between laboratories

One of the questions that arise when having critical values is the comparability of results from different laboratory with a dataset with different values? The findings in international literature

indicate that plant sap diagnostic methods are not just standardized methods. In fact, it seems that plant sap methods is a collection of different types of analyses. There is an impact by the way tissue is sampled (petiole vs leaf tissue) but also the position of the tissue which is sampled. Nutrients tend to spread unequal through plant tissue and some laboratories compare old and young tissues to conclude about the nutritional status of a plant. Even with a particular analysis, the laboratory might use different methods of extracting and testing. A further complication in creating a reference dataset is that it is also not always known which methodology a laboratory is using.
Evidence for differences between laboratory on leaf tissue form the same source was found by De Kreij (1996). In his experiment he used plant sap methods in a fertilization trial in sweet pepper. Leaf blade with petiole were sampled and send to laboratory of Proefstation Bloemisterij en Glasgroente and to Agrarisch Laboratorium Flevoland for plant sap analysis. The calcium concentration in the plant sap showed no logical fluctuation between treatments. It was therefore concluded that calcium cannot be determined with this method. There were also large differences in the results for calcium between both laboratories. Nitrate and chlorine in the collected tissues that were obtained in the experiment of De Krey tended however to be good determinable with both methods. The results in plant sap were in accordance to the fertilization level of the different treatments for both laboratories. A possible reason that laboratory give different results is the function and behavior of nutrients in plants. Nutrients are not equal spread through the plant and petiole tissues are thought to be better in predicting nitrate and chlorine in comparison with leaf blade (Bryson and Mills 2014).
The leaves were sampled from two places; in the top of the plant and between the second and fourth truss. In both experiments were different plant tissues sampled. In the experiment of Sonneveld, only petioles used whereas De Krey used blade tissue including petiole.

Discussion

Plant sap versus dry matter analysis advantage and disadvantages

Both, plant sap and dry matter analysis can be a diagnostic tool to detect the nutritional status of plants. One of the advantages of plant sap is the relatively easy method of retrieving sap from plants in which directly can be measured. The dry matter is a more labor-intensive method since samples needs drying on an oven. After determination of the dry matter content it requires the milling/ grinding of the samples which makes it a more complex method.
There exist also differences in both methods concerning the power to detect a nutritional deficiency. The dry matter method is believed to display the nutritional status of a plant on a longer period and less sensitive to short term fluctuations. The plants sap method is thought to display the actual transport of nutrients like a snapshot.
The disadvantage that tissue samples need to be send to a laboratory has encouraged the development of various quick tests. The tests are mostly easy to use and quick. Extraction of plant sap can be done with simple devices like a garlic press or commercially available equipment Most of these systems are in particular developed for nitrate.

Plant sap analysis as standalone method or in combination with soil measurements

The plant sap methods described on the CBAV website for potato and brussels sprouts are monitoring tools to detect an upcoming shortage. These systems are relying on a (nearly) optimal nitrogen supply by the farmer. The nitrogen supply can during the growing season be refined by analyzing plant sap Some laboratory also provides another method the combination of a plant sap method in combination with a soil analysis. This can be in combination with the N-mineral

The combination will give a more powerful tool to detect nutrient deficiencies because it is using a measurement in soil and in the plant. The combination of plant sap and soil analysis becomes more interesting when screening for multiple nutrients. For some nutrients it is more useful to use a plant sap method whereas the nutritional status for other elements can be determined by a soil availability analysis.

Conclusions

Plant sap methods can be a useful tool in determining the plants nutrient status for some nutrients as well to detect shortages as a predictive tool to generate fertilization advices. Most laboratory and research consider leaf tissue analysis and plant sap methods complimentary to soil based advisory systems to refine the fertilizer recommendations. The website of CBAV describes a plant sap model for topdressing of nitrogen in potato for Dutch conditions with a scientific validation. Other advises in the Netherlands are based on practical experiences of the individual laboratories. The validation of plant sap models from commercial laboratory in the Netherlands are not public available. These sap analysis provide sometimes all main- and trace elements. But it is also not always obvious if there exist a relationship between yield and a certain parameter because scientific validation is not available.
The advantages of plant sap analyses are:

- quick and relatively easy measurements.
- better insight in plant nutrient status for specific elements (like nitrate and potassium)

The disadvantages of plant sap analyses are

- variability of measurements because of crop, variety, development stage, time in the day, plant part sampled and nutrient
- no standardization between labs
- hardly any scientific validated reference sets available

This literature research unveils that the public available critical values exist for potato and brussels sprouts. There are only critical values for nitrate-N in these crops. Furthermore, there are indications that these critical values in potato are a subject for revision since there exist a wide range of potato varieties with a specific nitrate demand. In order to serve the sector with better critical values, the number of varieties should be expanded, and it could also be possible to develop critical values for the other nutrients. Furthermore, there are factors that have to be taken into account like type of potato and regional differences. To use plant sap analysis with a sufficient scientific validated fertilization advice requires a lot of research. It is expected that this does not give sufficient advantages for farmers to justify the investment. Plant sap analysis can be worthwhile to use to determine nutrient shortages in crops together with soil measurements for some specific elements. It remains also questionable if Dutch farmers will use plant sap on a larger scale. This is currently not the case.

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