Monitoring nitrogen status of vegetable crops and soils for optimal nitrogen management

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Running title: N monitoring of vegetable crops

Abstract

Optimal crop nitrogen (N) management is required to minimize N losses to the environment in vegetable crop production. There are several approaches based on soil and plant monitoring that can assist to improve N management. These include soil monitoring, destructive (tissue N analysis, petiole sap nitrate (NO_3^-) analysis) and non-destructive (optical sensors) crop-based methods, and portable rapid analysis systems. The most promising optical sensors for guiding N management in vegetable production, considering performance and practicality, are chlorophyll meters and canopy reflectance sensors. The crop-based methods are generally sensitive indicators of crop N status in a wide range of vegetable crops. However, they tend to have reduced sensitivity when N supply is excessive. A notable feature of soil monitoring methods (e.g. the Dutch 1:2 soil-water extract method, soil solution monitoring) is that they can detect excess N supply. The combination of crop and soil monitoring will provide vegetable growers with tools to detect crop N deficiency and excess N supply. The selection of the best monitoring approach for a given farm will depend on factors such as crop and farm characteristics, the farmer's technical level, technical support, and economic considerations. Soil and crop monitoring approaches could form part of improved management packages that include Decision Support Systems (DSS), to determine crop N and/or irrigation requirements, and monitoring of soil water status. The use of such packages, when combined with fertigation and drip irrigation, is key for very efficient N management of vegetable crops with reduced N loss to the environment.

Keywords: *chlorophyll*; *reflectance*; *sap analysis*; *soil solution; tissue analysis*; *vegetation indices*

1. Introduction

The high value of vegetable production encourages growers to apply high nitrogen (N) rates and frequent irrigation to ensure high yields (Agostini et al., 2010; Thompson et al., 2017, 2020a). Commonly, N fertilizer and irrigation applications are excessive (Fereres et al., 2003; Thompson et al., 2007, 2020a) contributing to nitrate (NO_3^-) leaching loss (Ramos et al., 2002; Zotarelli et al., 2007) and subsequent NO_3^- accumulation in water bodies (Ju et al., 2007; Pulido-Bosch et al., 2000; Thompson et al., 2020a). Several additional characteristics of vegetable production, such as high cropping intensity and shallow root systems (Thompson et al., 2020a; Thorup-Kristensen and Kirkegaard, 2016) increase the risk of NO_3^- leaching loss.

Public and scientific concerns of environmental impacts have increased political pressure to reduce NO_3^- contamination of water bodies from agriculture. In the European Union (EU), the Nitrates Directive (Council of the European Communities, 1991) and the Water Framework Directive (Council of the European Communities, 2000) require farmers to adopt improved N management practices in areas vulnerable to NO_3^- contamination.

Current commercial N management in vegetable production is largely based on the accumulated experience of growers and advisors, of practices that maximize yield and ensure profitability (Thompson et al., 2007, 2020a). Improved crop N management requires that N fertilizer application should supplement other N sources to ensure that crop N demand is satisfied while avoiding an excessive N supply (Soto et al., 2015; Thompson et al., 2017). Necessary components of optimal N fertilization of vegetable crops are assessment of crop/soil N status to determine if the N supply is deficient, adequate or excessive, assessment of the degree of deficiency or excess, and using these assessments to quantitatively adjust N fertilizer management. Such assessments can be done by monitoring the soil to assess the immediate soil N supply, the crop to assess its N status, or both. Three general N monitoring approaches used with vegetable crops are soil monitoring, assessment of crop N status using destructive methods (i.e. leaf tissue analysis and petiole sap analysis), and assessment of crop N status using non-destructive methods (i.e. optical sensors – both proximal and remote sensors, and electrical impedance spectroscopy). These three general approaches will be reviewed, with a focus on practical methods used on commercial farms, methods with potential for practical use, and methods that have been the subject of recent applied research conducted in a farming context.

2. Soil monitoring for N management

In the context of this review, soil monitoring is the periodic sampling and analysis of soil or soil solution to assess the adequacy of the immediate N supply during a crop. It differs from individual analyses of soil mineral N or NO_3^- conducted as part of N fertilizer recommendation schemes that determine the total amount of fertilizer N required for an individual crop. N fertilizer recommendation schemes for vegetable crops such as the Nmin and KNS are described by Thompson et al. (2017), and in the accompanying article in this Special Issue by Tei et al. (2020). Three soil monitoring methods have been used to assist with N management of vegetable crops, in Europe, being the saturation extract, the Dutch 1:2 soil-water extract method, and soil solution analysis.

2.1 The saturation extract

Solutions from the saturated extract procedure used for soil salinity assessment have been analyzed for NO_3^- concentration to inform of the immediately available N supply (Sonneveld et al., 1990; Sonneveld and Voogt, 2009). Given the time and laboratory requirements to obtain the saturated extract, this is not a practical option for regular monitoring of commercial crops.

2.2 The Dutch 1:2 soil-water extract method

This method is used in The Netherlands to assess root zone soil NO_3^- in commercial, greenhouse-grown vegetable crops that are grown in soil with fertigation. In this system, nutrient solutions are frequently applied. The nutrient solutions used, with soil-grown crops, are similar to those used for soilless crop (Sonneveld and Voogt, 2009), in which N is applied principally as NO_3^-.

Composite soil samples (0–25 cm) are taken regularly (generally monthly) during a crop, and extracted with water (one volume of soil to two volumes of water) (Sonneveld et al., 1990; Sonneveld and Voogt, 2009; Thompson et al., 2017). The relationship of the NO_3^- concentration ($[NO_3^-]$) in the extract solution to target values is used to adjust (if required) the $[NO_3^-]$ of the nutrient solution applied by fertigation. The adjustment procedure is illustrated in Figure 1. When the extract $[NO_3^-]$ is within the range B–C mmol L^{-1}, the standard $[NO_3^-]$ of nutrient solution is maintained (Fig. 1). When the extract $[NO_3^-]$ is progressively less than B mmol L^{-1}, the $[NO_3^-]$ of nutrient solution is progressively increased. When the extract $[NO_3^-]$ is progressively higher than C mmol L^{-1}, the $[NO_3^-]$ of nutrient solution is progressively decreased. Other nutrients and electrical conductivity are managed using the same approach (Sonneveld et al., 1990; Sonneveld and Voogt, 2009). This method is used for N management where all fertilizer N is applied by fertigation by drip or sprinkler irrigation. Nutrient solution composition varies with species and are adjusted for factors such as water quality, cropping stage, and soil type (Sonneveld and Voogt, 2009).

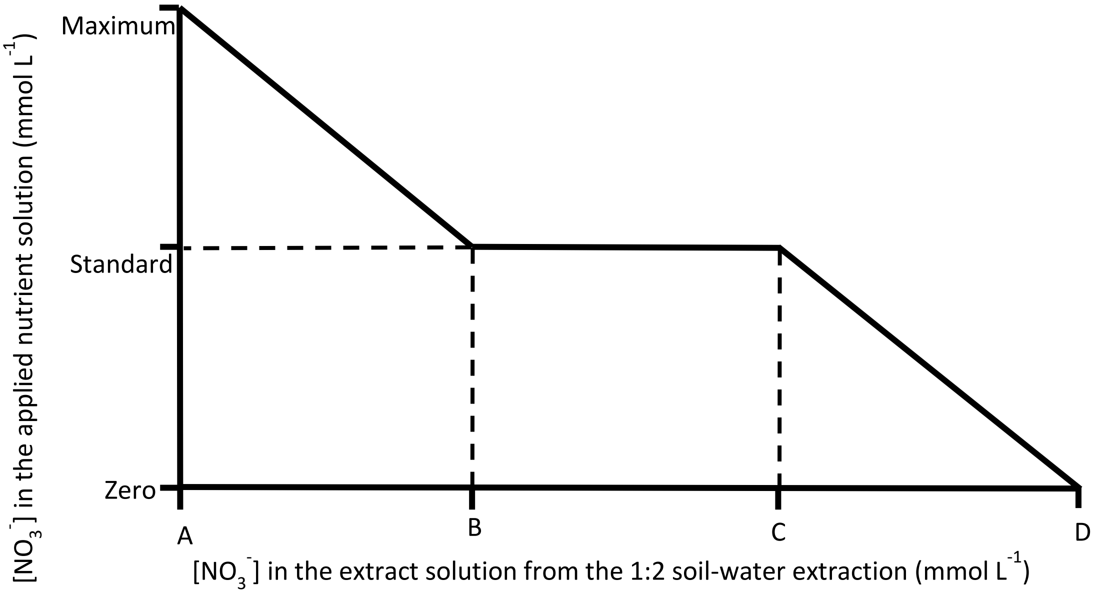

Figure. 1. Diagram explaining the adjustment of the NO_3^- concentration ($[NO_3^-]$) in the applied nutrient solution based on the $[NO_3^-]$ in the extract solution from the 1:2 soil-water extraction. The range B–C in the extract solution is regarded as adequate, for which the applied nutrient solution $[NO_3^-]$ is maintained. The range A–B is regarded as deficient, and the nutrient solution $[NO_3^-]$ is progressively increased as the extract $[NO_3^-]$ tends towards A. The range C–D is regarded as excessive, and the nutrient solution $[NO_3^-]$ is progressively reduced as the extract $[NO_3^-]$ tends towards D. Own preparation based on Sonneveld and Voogt (2009).

With very frequent nutrient addition by fertigation, it is the immediately available nutrients in soil that are of interest. Optimization of frequent nutrient addition requires frequent testing which in turn requires simple and quick procedures to obtain and prepare samples. The use of fresh soil, sampling by volume and the use of a simple ratio, with this method, facilitates rapid sample preparation.

In Dutch commercial practice, 40 cores (2 cm diameter) are taken in each sampled area. In drip irrigated row crops, 50% of the cores are taken 10 cm from a plant and 50% are taken midway between plants in the same row. In sprinkler-irrigated crops such as lettuce or radish, where the complete soil surface is cropped, samples are taken at random. Samples are taken when the soil is at or close to field capacity, avoiding very moist soil immediately after irrigation. Where crops are grown in raised beds, only the beds are sampled. A full description of the use of 1:2 soil-water extract method in commercial Dutch greenhouses is available, in Dutch, in Van den Bos et al. (1999); other descriptions are available in Sonneveld et al. (1990) and Sonneveld and Voogt (2009).

In addition to commercial greenhouse growers in The Netherlands, this method has been adapted to greenhouse conditions in Italy (Incrocci et al., 2017) and Greece (De Kreij et al., 2007). The sufficiency range values determined for crops in Italy are somewhat lower than those used in The Netherlands (Incrocci et al., 2017). In Italy, this method is used by some greenhouse growers in combination with the GreenFert software that facilitates data interpretation (Incrocci et al., 2017). While the method has mostly been used with greenhouse crops, it can be used with open field fertigated crops, given that the reference values are verified/adapted.

2.3 Soil solution analysis

The [NO_3^-] of the soil solution in the root zone can be used to assist with N management of fertigated vegetable crops (Thompson et al., 2017). Like the Dutch 1:2 soil-water extract method, this method informs of the immediately available N in the root zone. Soil solution is sampled regularly (e.g. every 1–4 weeks) during a crop, with ceramic cup suction samplers. The sampler enables periodic sampling of the soil solution from where roots are concentrated, such as from within the drip irrigation bulb (Thompson et al., 2017).

The ceramic cup of the suction sampler is placed within the zone of maximum root density depending on the crop and soil characteristics. In Almeria greenhouses, where the root distribution is generally relatively shallow (Padilla et al., 2017) because of the local soil system (Thompson et al., 2007), the ceramic cup is usually placed at 10–20 cm soil depth, and 8–10 cm from the main stem of the plant.

Ceramic cup suction samplers collect soil solution from the soil volume immediately surrounding the ceramic cup. Consequently, the soil solution sampled with each sampler is a localized point measurement. This enables on-going monitoring of specific locations. However, it can also result in appreciable spatial variability in the measured soil solution [NO_3^-] (Hartz, 2003), particularly where N is applied by combined fertigation and drip irrigation. Through replication and careful selection of representative locations avoiding unrepresentative plants and border areas, and in greenhouses by avoiding zones of rainfall entry, the spatial variability, of drip-irrigated and fertigated crops, can be substantially reduced (Granados et al., 2013; Thompson et al., 2017).

An important practical issue with ceramic cup suction samplers is the limited range of soil matric potentials at which sampling is possible. These samplers are only effective in moist soils with matric potentials in the approximate range of 0 to -50 kPa. The vacuum within the sampler must be more negative than the matric potential of the surrounding soil; the commonly-used manual vacuum pumps apply a maximum vacuum of approximately -60 kPa. Because of the limited soil moisture range, suction samplers are best suited to vegetable crops grown in continually moist soils such as in greenhouses or cool season outdoor crops. In other crops, they are best used soon after irrigation or rainfall. The general use of suction samplers to sample soil solution was reviewed by

Grossmann and Udluft (1991). Recommended sampling procedures for routine practical soil solution NO_3^- concentration monitoring are to apply vacuum, 12–24 hours after the previous irrigation, to allow equilibration of the soil solution, and then to maintain the vacuum for 12–24 hours (Granados et al., 2013; Peña-Fleitas et al., 2015).

Two general approaches are used for data interpretation and N management, (1) the use of absolute limits either as an individual value (sufficiency value) or as a range (sufficiency range), and (2) the use of tendencies. A sufficiency value differentiates between deficient (below the value) and sufficient (above the value); a sufficiency range differentiates between deficient (below the minimum value), sufficient (between the above the minimum and maximum values), and excess (above maximum value).

Identification of absolute limits is challenging because of the interaction of numerous factors (e.g. crop species and phenology, soil characteristics) and spatial variability. Absolute sufficiency values of 4–5 mmol NO_3^- L^{-1} have been proposed (Hartz and Hochmuth, 1996; Thompson et al., 2020b, 2017). Consistently lower values are suggestive of an insufficient N supply. Maximum absolute value of 12–15 mmol NO_3^- L^{-1} have been suggested for greenhouse-grown crops in south-eastern Spain (Granados et al., 2013; Thompson et al., 2020b). Values that are consistently clearly higher than the N concentrations typically applied by fertigation of 10–12 mmol NO_3^- L^{-1} are suggestive of an excessive N supply.

An on-going tendency of increasing soil solution [NO_3^-] is an indicator of excessive N application with fertigated/drip irrigated vegetable crops, particularly where little drainage and therefore NO_3^- leaching occurs (Gallardo et al., 2006; Granados et al., 2013; Peña-Fleitas et al., 2015). This can be seen in Figure 2 with a fertigated tomato receiving very frequent N application (Peña-Fleitas et al., 2015). There was a moderate on-going increase in soil solution [NO_3^-] with an applied nutrient solution of 13 mmol NO_3^- L^{-1}, and a much more rapid on-going increase in soil solution [NO_3^-] with an applied nutrient solution of 22 mmol NO_3^- L^{-1} (Figure 2) (Peña-Fleitas et al., 2015). Conversely, negative tendencies can indicate insufficient N supply. The use of tendencies overcomes the uncertainties associated with spatial variation of point measurements. Dealing with spatial variability is likely to be relatively more important with commercial growers than in research studies because of grower reluctance to have sufficient number (e.g. four) of replicated samplers within a crop.

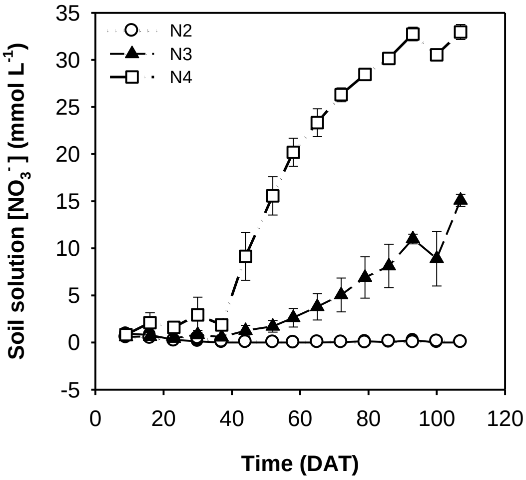

Figure 2. NO_3^- concentration ([NO_3^-]) of root zone soil solution during a fertigated tomato crop grown in a greenhouse in SE Spain. The average N concentrations applied by fertigation/drip irrigation were 5, 13 and 22 mmol L^{-1} for treatments N2, N3 and N4, respectively. Values are means ± SE (n=4). DAT is days after transplanting. Reproduced from Peña-Fleitas et al. 2015. Assessing crop N status of fertigated vegetable crops using plant and soil monitoring techniques. *Annals of Applied Biology* 167: 387-405, published by John Wiley and Sons Ltd., as an open access article under the terms of the Creative Commons Attribution ‐ NonCommercial ‐ NoDerivatives Licence.

Soil solution [NO_3^-] has been used in combination with other methods as part of a prescriptive corrective management approach (Giller et al., 2004) for combined N and irrigation management (Granados et al., 2013; Magán et al., 2019). Both Granados et al. (2013) and Magán et al. (2019) used soil solution [NO_3^-] in combination with Decision Support Systems (DSS) that estimated both crop N and irrigation requirements. Granados et al. (2013) used sufficiency ranges, and Magán et al. (2019) used minimum sufficiency values and tendencies to interpret soil solution [NO_3^-] data. These studies reported reductions in N fertilizer use and NO_3^- leaching of 35–38% and 58–63%, respectively.

Small portable rapid test systems (Parks et al., 2012; Thompson et al., 2009) can be used for on-farm measurement of [NO_3^-] in soil solution samples. These are discussed in section 6. Soil solution obtained with suction samplers can also be used to monitor soil solution electrical conductivity and other nutrients. While much of the research work to data has been conducted in greenhouse soils, soil solution suction samplers can also be used in open field conditions, given that soil moisture conditions are adequate.

2.4. General observations on soil monitoring for N management

A notable feature of the Dutch 1:2 soil-water extract method and the sampling of soil solution [NO_3^-] is that both methods can detect both excessive and deficient N supply. The capacity to detect excess N is influenced by crop management and drainage. Nevertheless, the capacity to detect N excess is an important feature for intensive vegetable production where excessive N supply is common. Given this capacity, soil monitoring methods have an important role in the development of improved N management of vegetable production, either alone or in combination with other methods. They could form part of management packages that include crop monitoring of N status, the use of DSS to determine crop and/or irrigation requirements and monitoring of soil/crop water status.

3. Interpretation of crop N monitoring data

Monitoring of crop N status potentially integrates crop N demand and soil N supply (Schröder et al., 2000). Many of the crop N monitoring approaches that are described in this review enable rapid *in-situ* assessment of crop N status. To provide users with information on crop N status, i.e. to inform of whether a crop N status is deficient or sufficient, either relative or absolute values of monitoring can be used to assess N status. When monitoring measurements deviate from what indicates sufficient crop N status, N fertilizer management should be adjusted. Using a semi-quantitative adjustment approach, this is done by adding more or less N to a previously prepared plan of N fertilizer application (Gianquinto et al., 2004; Thompson et al., 2017). Using a quantitative adjustment approach, algorithms calculate the adjustment to the N fertilizer plan. The following sections describe the use of relative and absolute sufficiency values of crop monitoring measurements.

3.1 Use of relative nitrogen sufficiency indices. Reference plots

A common procedure to interpret crop monitoring measurements is to divide measured values, determined within the crop, by values measured in a well-fertilized, reference plot that has no N limitation. The resulting ratio is known as the Nitrogen Sufficiency Index (NSI) (Debaeke et al., 2006; Piekielek et al., 1995). The underlying concept of the NSI is that monitoring measurements saturate or reach a plateau when there is no N limitation on crop growth. NSI values of <1 indicate N deficiency, and NSI values ≈ 1 indicate N sufficiency. An alternative to the establishment of reference plots is the use of virtual reference plots (Holland and Schepers, 2013), where an area within the field with good growth is assumed not to be N limited and is used for reference measurements.

One of the main advantages of the use of the NSI is that it reduces the influence of factors, other than N, on monitoring measurements. Abiotic and water stress, disease incidence and cultivar may influence monitoring measurements similarly in both the measured area and the reference plot; the use of reference plots isolates the effect of N status of the measured area (Samborski et al., 2009; Thompson et al., 2017).

Reference plots were developed for cereal crops. There are few examples of the use of reference plots in vegetable crops. Westerveld et al. (2004) used an optical sensor (SPAD-502 chlorophyll meter) to aid N fertilization management of cabbage, carrots and onions. N fertilization was applied whenever measurements with proximal sensors fell below 95–97% of values in the reference plot. Similarly, Gianquinto et al. (2010) used a reference plot to guide N fertilization in muskmelon using a chlorophyll meter (see section 5.1.1) which resulted in significantly lower N application.

A limitation of the use of reference plots in fertigated vegetable crops is the requirement for an additional irrigation sector, independent from that of the main crop. Additionally, the work and calculation involved in periodic programming of fertigation would be doubled. For practical reasons, reference plots are not attractive for fertigated commercial vegetable crops.

A consideration with reference plots is the size and number of reference plots required. In general, plot size should be large enough to allow regular measurements on a representative crop area. In fields with homogeneous soil and topography, one representative reference plot is sufficient. However, in heterogeneous fields, one reference plot is required for each identifiable zone of soil and topography.

3.2 Use of absolute sufficiency values of monitoring measurements

The use of absolute sufficiency values of monitoring measurements overcomes the practical limitations of establishing reference plots in fertigated vegetable crops. Also, farmers are not required to calculate sufficiency values. Absolute sufficiency values are made available to farmers and technical advisors through Extension services following determination in research studies.

Two approaches have been used to determine absolute sufficiency values for vegetable crops: (1) the fitting of yield response regression lines, and (2) the use of relationships with an indicator of crop N status. Yield-based sufficiency values are calculated from segmented linear-plateau regression analysis relating relative yield to crop monitoring measurements (Gianquinto et al., 2004; Padilla et al., 2017b). Using the first approach, relative yield is used to standardize yield across years, cultivars, and cropping conditions. Using the second approach, absolute sufficiency values are derived from the relationship between nitrogen nutrition index (NNI), which is an established indicator of crop N status (Lemaire et al., 2008), and the monitoring measurements (Padilla et al., 2015). NNI values of <1 indicate N deficiency, of >1 indicate N excess, and of ≈1 indicate N sufficiency (Lemaire et al., 2008). The NNI is calculated as the ratio between actual crop N content and the critical crop N content, which is the minimum N content necessary for maximum growth (Greenwood et al., 1990). The critical crop N content is obtained from the critical N curve, which is a power function that relates above-ground dry matter production with crop N content (Greenwood et al., 1990). Specific critical N curves are available for some vegetable species, such as tomato (Peña-Fleitas et al., 2015; Tei et al., 2002), sweet pepper (Rodríguez et al., 2020) and cucumber (Padilla et al., 2016). A general critical N curve has been described for C3 crops (Greenwood et al., 1990), and researchers are continually producing critical N curves for more species.

Absolute sufficiency values of monitoring measurements have been related to thermal time (Gianquinto et al., 2010; Padilla et al., 2015) or phenological stages (de Souza et al., 2019; Padilla et al., 2018). Sufficiency values provided for phenological stages facilitate the on-farm use of monitoring approaches because measurements are related to easily-recognizable crop development stages (Padilla et al., 2016). The use of absolute sufficiency values related to crop age (e.g. days after transplanting) has limited applicability, with vegetable crops, because of variations in planting dates, crop cycles, climate, locations etc.

4. Determination of crop N status using destructive methods

4.1. Leaf tissue N analysis

Leaf tissue N analysis refers to the measurement of total N content in leaf blades of the most recently fully expanded leaves. It is a long-established method for monitoring crop N status (Geraldson and Tyler, 1990; Hartz and Hochmuth, 1996). Generally, sufficiency ranges for individual phenological phases of a species are used to interpret results. Sufficiency ranges are available for various vegetable species grown in greenhouse or open field conditions in different regions (Geraldson and Tyler, 1990; Hartz and Hochmuth, 1996; Hochmuth et al., 2015).

As a N monitoring method, leaf tissue N analysis is a relatively insensitive measure of crop N status due to the limited response of leaf N content to short periods of inadequate N supply (Olsen and Lyons, 1994). Additionally, it requires laboratory analysis and there is an inevitable time delay associated with transporting samples, analysis and report preparation. Consequently, this method is not suitable for rapid adjustments of N fertilization required by fertigation, which is being increasingly used in vegetable production. From the farmer’s perspective, the logistics of handling and transporting samples, and the cost of analysis are major disadvantages for regular monitoring (Thompson et al., 2017). Although tissue analysis is limited as a N monitoring approach, multi-element tissue analysis is useful for diagnosis of possible nutritional problems.

4.2. Sap NO_3^- analysis

Sap NO_3^- analysis measures the [NO_3^-] in a solution obtained from conducting tissue (xylem, phloem) plus the apoplastic, cytosolic and vacuolar water of fresh petioles (Hochmuth, 1994). Sap is extracted by squeezing petiole tissue, most commonly by using a domestic garlic press. The sensitivity of petiole sap [NO_3^-] to crop N status has been established for various vegetable crops, such as tomato (Farneselli et al., 2014; Hartz and Bottoms, 2009; Peña-Fleitas et al., 2015), pepper (Olsen and Lyons, 1994), potato (Goffart et al., 2008), lettuce, broccoli and watermelon (Hartz et al., 1993), and onion, cabbage and carrots (Westerveld et al., 2004). Petiole sap [NO_3^-] is appreciably more sensitive to crop N supply than total leaf N content (Olsen and Lyons, 1994).

Generally, petioles are obtained from the most recent fully expanded leaf. To reduce variation between individual plants, it is recommended to collect >25 petioles from different representative plants in a field or plot (Goffart et al., 2008). Recommended protocols should be strictly and consistently followed for leaf selection, time of sampling, petiole removal, petiole handling and storage, sap extraction and storage of sap samples (Farneselli et al., 2006; Goffart et al., 2008; Hochmuth, 2012, 1994; Thompson et al., 2017). Also, sampled crops should not be suffering from water stress or deficiencies of other nutrients (Farneselli et al., 2006; Goffart et al., 2008).

Generally, it was reported that petiole sap NO_3^- concentration declined notably as crops grow (Hartz and Bottoms, 2009; Hochmuth, 2012, 1994). Recommendations were commonly made as sufficiency ranges for phenological phases, with recommended values generally declining as crops developed (Hochmuth, 2012, 1994). However, a number of recent studies with fertigated vegetable crops, receiving very frequent N addition, have reported that petiole sap [NO_3^-] remained relatively constant throughout the crop (Figure 3). This has been observed in tomato (Farneselli et al., 2014; Peña-Fleitas et al., 2015), muskmelon (Peña-Fleitas et al., 2015), pepper (Magán et al., 2019), and cucumber (R.B. Thompson, University of Almeria, unpublished data).

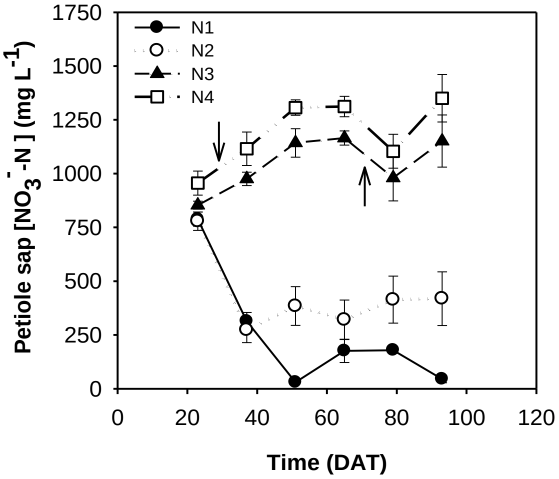

Figure 3. Petiole sap NO_3^- concentration ([NO_3^-]) during a fertigated tomato crop grown in a greenhouse in SE Spain. The average applied N concentration was 1, 5, 13 and 22 mmol L^{-1} for treatments N1, N2, N3 and N4, respectively. Values are means ± SE (n=4). Arrows in each graph indicate the commencement of N treatments (↓) and the day of topping (↑). DAT is days after transplanting. Reproduced from Peña-Fleitas et al. 2015. Assessing crop N status of fertigated vegetable crops using plant and soil monitoring techniques. *Annals of Applied Biology* 167: 387-405, published by John Wiley and Sons Ltd., as an open access article under the terms of the Creative Commons Attribution ‐ NonCommercial ‐ NoDerivatives Licence.

Strong consistent relationships between petiole sap [NO_3^-] and NNI throughout crops were reported for fertigated tomato and muskmelon (Peña-Fleitas et al., 2015), fertigated tomato (Farneselli et al., 2014), and fertigated sweet pepper (R.B. Thompson, University of Almeria, unpublished data). A single linear regression equation described the relationship between petiole sap NO_3^- concentration and NNI for both greenhouse-grown indeterminate tomato in Almeria, Spain, and open field, determinate, processing tomato in Perugia, Italy (Figure 4) (Peña-Fleitas et al., 2015). This single equation covered most of the duration of one greenhouse-grown tomato crop in Almeria and of two open field tomato crops in Perugia. Solving the unique regression equation for NNI = 1 provided a unique sufficiency value, for growth, of 1050 mg $N–NO_3^-$ L^{-1} throughout the tomato crop (Figure 4) (Peña-Fleitas et al., 2015). Similarly, a single relationship was obtained for several fertigated pepper crops (R.B. Thompson, University of Almeria, unpublished data). There are very few reports of relationships between petiole sap [NO_3^-] and NNI in crops other than vegetables. Bélanger et al. (2003) reported linear relationships in potato that shifted with crop growth; however, it was notable that all N was applied at planting.

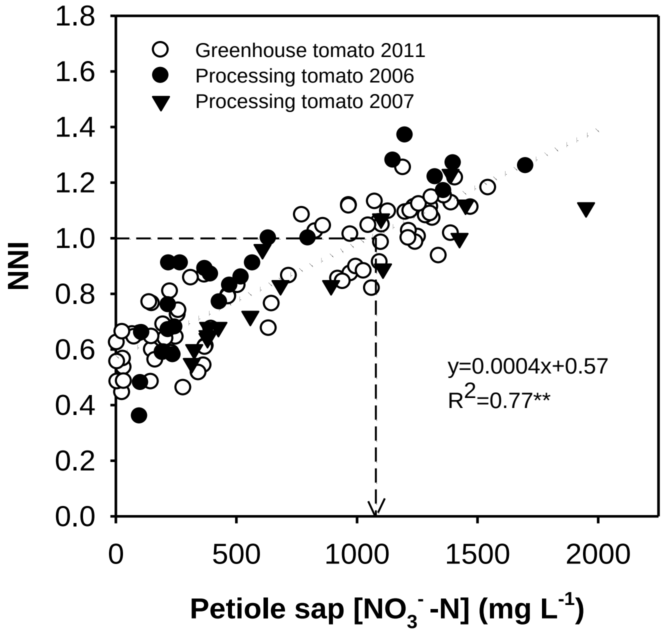

Figure 4. Linear relationship of petiole sap N–NO$_3^-$ concentration to Nitrogen Nutrition Index (NNI) for tomato combining all data collected throughout a greenhouse-grown indeterminate fresh market tomato crop in 2011 (Peña-Fleitas et al., 2015), and two determinate processing tomato crops grown in open fields in 2006 and 2007 (Farneselli et al., 2014). Sap N–NO$_3^-$ concentration was determined by analytical chemistry. The derivation of a general sufficiency value of 1050 mg N–NO$_3^-$ L^{-1} that corresponds to NNI = 1 is shown. Reproduced from Peña-Fleitas et al. 2015. Assessing crop N status of fertigated vegetable crops using plant and soil monitoring techniques. *Annals of Applied Biology* 167: 387-405, published by John Wiley and Sons Ltd., as an open access article under the terms of the Creative Commons Attribution - NonCommercial - NoDerivatives Licence.

Certain aspects of the behaviour of sap [NO$_3^-$] in fertigated vegetable crops, with frequent N addition in recent studies, have differed from that generally observed in earlier studies with more infrequent N application. Generally, in those earlier studies, sap [NO$_3^-$

] declined throughout the crop. It may be that very frequent application of N by combined fertigation and drip irrigation reduces the effects of otherwise influential factors on the tendency of sap NO_3^- concentration during a crop. However, the relative constancy of sap [NO_3^-] in fertigated vegetable crops with frequent N addition has not always been observed. Hartz and Bottoms (2009) reported an appreciable on-going reduction in sap [NO_3^-] of fertigated tomato in California receiving weekly N addition. There is currently insufficient data of fertigated vegetable crops to establish conclusively whether and/or how frequent N addition affects the response of petiole sap [NO_3^-] to crop N status.

Petiole sap NO_3^- analysis is generally sensitive to crop N status of vegetable crops, particularly to N deficiency. This method can provide information on the adequacy of crop N status for a given species within a given region. This requires local field trials to determine/validate sufficiency values. Petiole sap [NO_3^-] values can be influenced by factors such as cultivar, the timing and amount of the previous N application, and rainfall enhancing soil N mineralization (Goffart et al., 2008). Site and variety have been reported to affect sap [NO_3^-] values (Belec et al., 2001; Westerveld et al., 2007). Regular N applications, general crop management practices and similarity of cultivars are likely to improve consistency both within and between regions. It appears that the very frequent N addition of fertigated vegetable crops can reduce the influence of crop management and climatic factors.

While there are indications that petiole sap [NO_3^-] can identify clearly excessive N supply, there are insufficient data to draw firm conclusions. However, it appears that measured sap [NO_3^-] can clearly exceed sufficiency values thereby providing an indication of excessive crop N status.

Sap NO_3^- analysis has been used in commercial farming to assist with crop N management. It has been used by potato farmers in Belgium and The Netherlands (W.

Voogt, Wageningen University and Research, The Netherlands, personal communication). Private companies have been active for a number of years offering sap analysis of various nutrients, including NO_3^-, to assist with nutrient management of commercial horticultural crops, in The Netherlands and in Australia. An interesting adaptation of one of these private companies is the use of blade sap, rather than petiole sap. Magán et al. (2019) used petiole sap NO_3^- concentration as part of a treatment with prescriptive-corrective management (Giller et al., 2004) in which sap $[NO_3^-]$ was consistently notably lower than in a conventionally managed treatment.

Small portable rapid test systems (Parks et al., 2012; Thompson et al., 2009) can be used for rapid on-farm measurement of the NO_3^- concentration in petiole sap. These methods are reviewed in section 6.

5. Determination of crop N status using non-destructive methods

5.1 Optical sensors

Optical sensors provide measurements of optical properties of crops that are indicative of crop N status, thereby indicating N sufficiency or the degree of N deficiency. These sensors do not directly measure N content or N status of crops, but they provide an indirect measurement that is related to actual crop N content or crop N status (Cartelat et al., 2005; Mistele and Schmidhalter, 2008; Padilla et al., 2014). Optical sensors are generally used for proximal sensing, i.e. positioned either in contact or close to the crop (0.4–3.0 m from the crop canopy). Some optical sensors (e.g. spectral radiometers or multispectral cameras) are also used for remote sensing applications, i.e. on unmanned aerial vehicles (drones) or planes. The advantages of the use of optical sensors are that the measurements are made instantly and that the results are very rapidly available (Padilla et al., 2018b). Some optical sensors measure very small areas of leaves (e.g.

chlorophyll meters) whereas others measure relatively large areas of crop canopy through continuous "on-the-go" measurement (Table 1) (Padilla et al., 2018b).

Table 1. Characteristics of some of the most commonly used proximal optical sensors with potential for use for N management of vegetable crops.

Sensor type	**Devices**[†]	**Manufacturer**	**Measurement area**
Chlorophyll meter	SPAD-502	Konica Minolta (Tokyo, Japan)	Leaf
	N-tester	Yara International (Oslo, Norway)	Leaf
	atLEAF+	FT Green LLC (Wilmington, DE, USA)	Leaf
	MC-100 Chlorophyll Concentration Meter	Apogee Instruments Inc. (Logan, UT, USA)	Leaf
	CCM-200 Chlorophyll Content Meter Plus	Opti-Sciences Inc. (Hudson, NH, USA)	Leaf
Reflectance sensor	CropSpec	Topcon Positioning Systems, Inc. (Livermore, CA, USA)	Canopy
	OptRx Crop Sensor	Ag Leader Technology (Ames, IA, USA)	Canopy
	N-sensor ALS	Yara International (Oslo, Norway)	Canopy
	Crop Circle Canopy Sensors	Holland Scientific (Lincoln, NE, USA)	Canopy
	RapidSCAN CS-45	Holland Scientific (Lincoln, NE, USA)	Canopy
	GreenSeeker Sensors	Trimble Inc. (Sunnyvale, CA, USA)	Canopy
Flavonols meter	DUALEX	Force-A (Orsay, France)	Leaf
	MULTIPLEX	Force-A (Orsay, France)	Leaf

[†]Trade or manufacturers' names mentioned are for information only and do not constitute endorsement, recommendation, or exclusion.

5.1.1. Chlorophyll meters

Chlorophyll meters are hand-held optical sensors that estimate chlorophyll content per leaf area. The rationale for using chlorophyll meters for monitoring crop N status is that chlorophyll content is directly related to leaf N content (Evans, 1989; Hatfield et al., 2008). The measured area is generally <10 mm^2; consequently, appreciable replication and consistent measurement protocols are required. The chlorophyll meter output is a dimensionless value that is related to the actual chlorophyll content (Markwell et al., 1995; Monje and Bugbee, 1992; Parry et al., 2014). Most chlorophyll meters measure transmittance of red and near infra-red (NIR) radiation by the leaf. The red radiation is absorbed by chlorophyll and the NIR is mostly transmitted by chlorophyll (Fox and Walthall, 2008). There are currently several commercially available chlorophyll meters (Table 1); the SPAD-502 meter is the most commonly used (Padilla et al., 2018b).

Chlorophyll meter measurements have been used as reliable indicators of leaf N content or crop N status in many vegetable crops, such as tomato (Gianquinto et al., 2006b; Padilla et al., 2015), muskmelon (Gianquinto et al., 2010; Padilla et al., 2014), cucumber (Padilla et al., 2017a), sweet pepper (de Souza et al., 2019), potato (Gianquinto et al., 2004; Olivier et al., 2006) and lettuce (Mendoza-Tafolla et al., 2019). There are reports where chlorophyll meter measurements did not distinguish different N nutrition of tomato (Farneselli et al., 2010; Ulissi et al., 2011) and cucumber (Güler and Büyük, 2007). These contradictory results were likely due to small differences in leaf N content.

Sufficiency values of chlorophyll meter measurements are available for determinate processing tomato (Gianquinto et al., 2004, 2006a), indeterminate fresh-market tomato (Padilla et al., 2018; Padilla et al., 2015), cucumber (Güler and Büyük, 2007; Padilla et al., 2017a), potato (Gianquinto et al., 2003) and sweet pepper (de Souza et al., 2019). In some crops, the sufficiency values determined were relatively constant throughout the crop; therefore, an average sufficiency value could be calculated for the complete crop cycle. In indeterminate tomato, an average value of 54.2 SPAD units was determined (Padilla et al., 2018). In cucumber, sufficiency values of 45.2 SPAD units (Padilla et al., 2017a) and 44.9 SPAD units (Güler and Büyük, 2007) have been recommended for the complete crop cycle. In potato, a sufficiency value of 38.2 SPAD units was recommended for the complete crop cycle (Gianquinto et al., 2003). In contrast, for sweet pepper, there were large differences in SPAD sufficiency values between phenological stages of between 49.7 and 65.2 SPAD units (de Souza et al., 2019). This suggested that a single SPAD sufficiency value cannot be used for a complete sweet pepper crop. These data also demonstrate that each species must be evaluated separately.

Sufficiency values of chlorophyll meter measurements are likely to be affected by cultivar (de Souza et al., 2020; Monostori et al., 2016). Care should be taken when using sufficiency values, determined for a particular cultivar, to other cultivars of the same species.

There is a commonly-held view that chlorophyll meter measurements saturate and are not sensitive at high chlorophyll contents (Fox and Walthall, 2008). The saturation effect is seen as a plateau response of chlorophyll meter measurements to increasingly high chlorophyll contents (Padilla et al., 2018a). Saturation implies that, under these conditions, chlorophyll meters are unable to detect differences in chlorophyll content. Saturation has been reported at relatively high crop N contents in vegetable crops (Goffart et al., 2008). However, numerous studies have not reported saturation responses, in potato (Gianquinto et al., 2004; Majic et al., 2008), tomato (Güler and Büyük, 2007; Padilla et al., 2015) and muskmelon (Padilla et al., 2014). In cucumber (Padilla et al., 2017a) and sweet pepper (de Souza et al., 2019), relatively weak saturation was observed, i.e., asymptotic responses without a clear plateau effect occurred at high chlorophyll content. The available results suggest that the saturation response is not universal in vegetable crops. There are three factors that influence the saturation response at high chlorophyll content. Firstly, the occurrence of and degrees of species-specific luxury N uptake (Thompson et al., 2017). Secondly, leaf chlorophyll content can vary appreciably between species (Padilla et al., 2018b). Thirdly, the saturation response of chlorophyll meters can be influenced by the equations used to calculate the measured value from the radiation transmission measurements of the meters (Padilla et al., 2018a).

There are several published reports in which chlorophyll meter measurements were used to guide N fertilization of vegetable crops. Westerveld et al. (2004) used the SPAD-502 chlorophyll meter to aid N fertilizer management of cabbage, carrots and onions, in Canada. Half of the recommended N fertilization rate was supplied at pre-planting, and the rest was applied as side-dressing when SPAD measurements fell below 95–97% of the value of the highest N rate treatment. Using chlorophyll meter-based fertilization, N application was reduced by 30–45 kg N ha^{-1} compared to farmer practice (Westerveld et al., 2004). With tomato in Italy, the use of chlorophyll meter measurements enabled reductions in N application of 18–45% (Gianquinto et al., 2006b). In this latter case, a procedure for the calculation of chlorophyll meter threshold values was established using data obtained in previous trials from chlorophyll meter measurements and relative tomato yield (see above section 3.2).

A large coordinated project was conducted in Italy, Belgium, Scotland and The Netherlands to guide N fertilization of potato using chlorophyll meters (Gianquinto et al., 2004). This work determined absolute sufficiency values (see above section 3.2) and equations to determine the rate of side dress N required to maximize yield when chlorophyll meter values were below the sufficiency value. In this study, chlorophyll meters identified when, otherwise routine, side-dress N applications were not necessary. The amount of N to apply (N_a) to maximize yield was calculated as follows:

$$N_a\ (\text{kg ha}^{-1}) = [(1 - Y_r) \cdot Y_{max} \cdot N_{crop}] / (\text{NFE} \cdot \text{HI})$$

where Y_r was relative yield corresponding to the chlorophyll meter values measured in the field, Y_{max} was potential yield (kg ha^{-1}) that can be obtained by the crop, N_{crop} was plant N concentration, NFE was N fertilizer efficiency, and HI was harvest index. While some of these terms were easy to determine through crop monitoring (Y_r), grower experience (Y_{max}), or the literature (N_{crop} and HI), NFE estimation was more difficult because of its dependency on numerous variables. Nevertheless, the combined use of chlorophyll meter measurements with this equation reduced N application by 30–60% (Gianquinto et al., 2004). Also in potato, Olivier et al. (2006) developed a practical system to improve crop N management based on the use of a chlorophyll meter (Hydro N Tester; Table 1) sufficiency values and split N applications. The fields were fertilized at planting with 70% of the total N recommendation, the remaining 30% was either applied later or not applied depending on whether chlorophyll meter values were below or above the sufficiency value (Olivier et al., 2006). This strategy saved 30–55 kg N ha^{-1}.

5.1.2. Reflectance sensors

Reflectance sensors provide information on crop N status by measuring radiation reflected from the crop (Hatfield et al., 2008; Ollinger, 2011; Padilla et al., 2018b). Plant tissues absorb approximately 90% of visible radiation (390 to 750 nm) and reflect approximately 50% of NIR (750 to 1300 nm) (Knipling, 1970); reflectance of visible and NIR radiation varies with crop N content (Peñuelas et al., 1994). Reflectance sensors measure crop reflectance, at several wavelengths, which is used to calculate vegetation indices. The most used vegetation indices and their formulae are presented in Table 2. Vegetation indices based on red reflectance (e.g. NDVI, RVI; Table 2) saturate at high chlorophyll contents associated with high N application, whereas vegetation indices based on reflectance in the red edge band (e.g. RENDVI, CCCI; Table 2), centered around 720 nm, do not saturate (Daughtry et al., 2000; Raper and Varco, 2015).

Soil reflectance can confound reflectance measurements, e.g. from top down measurement. Where this may be an issue, there are indices that distinguish vegetation reflectance from soil reflectance (e.g. SAVI; Table 2). Alternatively, positioning the sensor to capture a side-view of the crop minimizes soil reflectance (Padilla et al., 2018b).

Several studies have evaluated the sensitivity of vegetation indices as indicators of crop N status of vegetable crops, such as tomato (Gianquinto et al., 2011; Padilla et al., 2015), muskmelon (Padilla et al., 2014), cucumber (Padilla et al., 2017b; Yang et al., 2010) and broccoli (El-Shikha et al., 2007). The vegetation indices GNDVI and GVI were the most sensitive indicators of crop N status and yield for open field processing tomato (Gianquinto et al., 2011, 2019). NDVI and RVI were the most sensitive indicators of crop N status in greenhouse-grown indeterminate tomato (Padilla et al., 2015). In soil-grown cucumber crops, NDVI and several other vegetation indices were sensitive indicators of crop N status and yield (Padilla et al., 2017b). These results were confirmed by Yang et al. (2010) for leaf N content in hydroponically-grown cucumber. Similar results with NDVI as an indicator of crop N status were observed in muskmelon, another cucurbit crop (Padilla et al., 2014). In broccoli, NDVI was a sensitive indicator of crop N status, but CCCI was more sensitive (El-Shikha et al., 2007).

Table 2. Most used vegetation indices for monitoring crop N status.

Index	**Acronym**	**Equation**	**Author**
Normalized Difference Vegetation Index	NDVI	$\frac{NIR - Red}{NIR + Red}$	Sellers (1985)
Green Normalized Difference Vegetation Index	GNDVI	$\frac{NIR - Green}{NIR + Green}$	Ma et al. (1996)
Red Ratio of Vegetation Index	RVI	$\frac{NIR}{Red}$	Birth and McVey (1968)
Green Ratio of Vegetation Index	GVI	$\frac{NIR}{Green}$	Birth and McVey (1968)
Chlorophyll Index	CI	$\frac{NIR}{Red} - 1$	Gitelson et al. (2003)
Chlorophyll Vegetation Index	CVI	$\frac{NIR}{Green} * \frac{Red}{Green}$	Vincini et al. (2008)
Soil Adjusted Vegetation Index	SAVI	$\frac{NIR - Red}{NIR + Red + L} * (1 + L)$	Huete (1988)
Optimized Soil Adjusted Vegetation Index	OSAVI	$\frac{NIR - Red}{NIR + Red + 0.16}$	Rondeaux et al. (1996)

Red Edge Normalized Difference Vegetation Index	RENDVI	$\frac{NIR - Red\ Edge}{NIR + Red\ Edge}$	Gitelson and Merzlyak (1994)
Canopy Chlorophyll Content Index	CCCI	$\frac{RENDVI - RENDVI_{min}}{RENDVI_{max} - RENDVI_{min}}$	Barnes et al. (2000)

NIR: Near Infrared; L: soil brightness correction factor

A major advantage of canopy reflectance sensors is that they measure a much larger area of the canopy than the leaf-based measurement of chlorophyll meters. In addition, some reflectance sensors (e.g. Crop Circle sensors, Greenseeker; Table 1) make continuous “on-the-go” measurement thereby integrating a large area of crop foliage. These sensors are mounted on tractors or manually supported on lightweight pole systems. There are handheld sensors for making individual spot measurements (e.g. RapidSCAN CS-45, Greenseeker handheld; Table 1); these sensors are generally simpler and cheaper. Reflectance sensors can be passive or active depending on whether they have their own light source. Passive sensors have photodetectors that measure both incident radiation and radiation reflected from the canopy. Active sensors have a light source that emits visible and NIR radiation and photodetectors that measure the reflected radiation (Solari et al., 2008). The main advantage of active sensors over passive sensors is that active sensors can be used under any irradiance conditions (Fitzgerald, 2010; Padilla et al., 2019). For passive reflectance sensors, uniform irradiance conditions are recommended (Oliveira and Scharf, 2014) and measurements must be taken during the central hours of the day (Gianquinto et al., 2019). Active sensors are best suited for on-farm use because their use is not restricted by ambient radiation conditions. An important issue with reflectance sensors for on-farm use is the cost. Some of the more sophisticated sensors can cost >6,000€ in Europe. Simpler sensors are becoming available for <1,000€.

Most of the reflectance sensors listed in Table 1 provide reflectance data of a small number of pre-selected wavelengths (two or three bands). Some sensors (e.g. Greenseeker handheld, RapidSCAN CS-45; Table 1) provide instant measurement of NDVI on LCD screens. Other sensors (i.e. Crop Circle sensors; Table 1) require data logging and data processing; some of these automatically calculate NDVI which can be rapidly downloaded (e.g. Crop Circle ACS-211; Table 1).

Multispectral sensors measure reflectance of 2–10 bands of the electromagnetic spectrum. Hyperspectral sensors provide reflectance measurements across a broad and nearly continuous spectrum that can range between 400 nm and 2500 nm (Jain et al., 2007; Tripodi et al., 2018). Research has been conducted with multi and hyperspectral sensors (Gianquinto et al., 2011; Perry et al., 2012); however, data processing and interpretation is currently too complex for on-farm use (Thompson et al., 2017).

Research on the application of reflectance sensors to guide N fertilization has mostly been conducted with cereals and potato; little work has been conducted with vegetables. A N side-dress system for potato was developed by van Evert et al. (2012) using measurements of the Weighted Difference Vegetation Index (WDVI). The amount of side-dressed N (kg ha^{-1}) was determined as:

$$N_{sidedress} = N_{optimum} - N_{crop}$$

where $N_{optimum}$ was crop N uptake for highest yield (obtained from literature) and N_{crop} was crop N uptake derived from a pre-established relationship between WDVI and crop N uptake. Using this scheme, N savings averaged 44–56 kg N ha^{-1} (23% reduction), while maintaining yield.

For maize, Scharf and Lory (2009) calibrated reflectance measurements to determine the economically optimal side-dress N rate application (EONR). Linear and quadratic regression analysis were used to determine EONR from reflectance measurements. Using these regression equations, reflectance-based fertilization reduced

N fertilizer use by 25% without yield reduction, compared to conventional N management (Scharf et al., 2011).

Complex algorithms that relate vegetation indices to yield and N application rate were developed to guide N fertilizer application to wheat (Berntsen et al., 2006; Thomason et al., 2011). Using a similar algorithm for variable rate N application, Raun et al. (2002) reported that N use efficiency was improved by 15% compared to traditional management with fixed N rates. A generalized algorithm for variable rate N fertilization of both maize and wheat was developed by Solie et al. (2012). The online Sensor Based Nitrogen Rate Calculator, developed by the Oklahoma State University, provides specific N rate recommendations for a wide range of crops based on measurements of the NDVI vegetation index with the GreenSeeker sensor (Table 1).

Some of the commercial sensors listed in Table 1, e.g. N-sensor, GreenSeeker, have their own proprietary algorithms to determine optimum N application rate, for the measured crop area, from canopy reflectance measurements. Generally, these algorithms are not publicly available, nor is information available of the validation process; however, there are exceptions (e.g. Holland and Schepers, 2010). Canopy reflectance sensors are used in commercial farming with various field crops, for variable rate N application and to aid optimal N rate application. As yet, there appears to have been very limited use with commercial vegetable crops.

5.1.3 Fluorescence-based flavonols meters

Flavonols meters are optical sensors that measure relative flavonols content per leaf area (Padilla et al., 2018b; Tremblay et al., 2012) (Table 1). Flavonols are a class of polyphenolic compounds that increase with lower crop N content; therefore, flavonols content is inversely related to chlorophyll content. Flavonols meters provide a dimensionless value that is related to the actual flavonols content (Padilla et al., 2018b; Tremblay et al., 2012).

A major advantage of flavonols meters is that measurements are not influenced by the soil (Tremblay et al., 2012). However, as with chlorophyll meters, the small sampling area measured by flavonols meters requires representative and adequate sampling (Padilla et al., 2018b). Flavonols meters can be used at any time of the day without a significant effect on flavonols measurement (Tremblay et al., 2012). However, flavonols content changes between seasons (Padilla et al., 2016). This is very relevant when comparing absolute measurements of flavonols meters throughout long crop cycles.

There are consistent reports that flavonols meter measurements are sensitive indicators of crop N status. This has been observed in broccoli (Tremblay et al., 2009a), potato (Ben Abdallah et al., 2018), muskmelon (Padilla et al., 2014), cucumber (Padilla et al., 2016) and sweet pepper (R. de Souza, University of Almeria, unpublished data). In a review, Tremblay et al. (2012) highlighted that flavonols meter measurements and the Nitrogen Balance Index (NBI) (Cartelat et al., 2005) were the two most suitable indicators for the assessment of crop N status when using flavonols meters. NBI is the ratio between chlorophyll and flavonols contents.

There are no reports on the use of flavonols meter measurements as tools to guide N fertilizer management in crops. Additionally, the high cost of fluorescence-based flavonols meters (3,000-14,000€ in Europe, depending on the model) makes unattractive to commercial farmers. Until practices are established to aid N fertilizer management and the purchase price is reduced, it is very unlikely that these meters are applicable on commercial farms.

5.2 Electrical impedance spectroscopy

Electrical impedance spectroscopy (EIS) is a technique that measures the impedance, of a material or system, in response to alternating current (AC) applied at a certain potential. The frequency dependence of the impedance can inform of underlying chemical processes, can detect structural characteristics of biological tissue, and can detect changes in the physiological state of biological tissue (Jócsák et al., 2019). Electrical conduction in biological tissues is related to the presence and mobility of ions in cells. Data of electrical properties at various frequency ranges informs of the components and structure of cells/tissues. Consequently, if a change in tissue structure/composition occurs, distinctive impedance spectra can be detected. EIS in lower frequency ranges (10 Hz–1 MHz) is widely applied in biomedical diagnostics, food sciences, and in plant sciences (Jócsák et al., 2019). In plant sciences, the main applications are for root growth estimation, frost hardening capability detection, fruit and vegetable quality measurement, and abiotic and biotic stress detection (Jócsák et al., 2019). The parameters of EIS are also suitable for the estimation of plant nutrient status. Studies on tomato have shown that electrical impedance can be used to detect and diagnose plant nutrition status for phosphorous (Meiqing et al., 2016) and potassium (Jinyang et al., 2016). Muñoz-Huerta et al. (2014) analyzed the electrical impedance response of soilless grown lettuce to different N concentrations in nutrient solution. A strong and positive correlation was observed between plant N content and frequency values, suggesting that electrical impedance may be sensitive to plant N status. For a comprehensive review of the application of electrical impedance measurement on plants, see Jócsák et al. (2019).

6. Use of portable rapid analysis systems

Small portable rapid analysis systems can be used for on-farm measurements of the [NO_3^-] in soil solution (section 2.3) and petiole sap (section 4.2) (Parks et al., 2012; Thompson et al., 2009), thereby providing the grower/advisor with an almost immediate result after sample collection. There are two main groups of rapid analysis systems, NO_3^- specific ion sensitive electrode systems, such as the LAQUAtwin NO_3^- meters (Horiba, Kyoto, Japan), and NO_3^- sensitive test strip readers, such as the RQflex® reflectometer (Merck, Darmstadt, Germany) and Nitrachek reflectometer (Eijkelkamp Agrisearch Equipment, Giesbeek, The Netherlands). Parks et al. (2012) provided a detailed description of these two types of on-farm rapid analysis systems, discussing operation, calibration, measurement range and interferences.

Parks et al. (2012) reported that NO_3^- specific ion sensitive electrode systems tended to overestimate and that they were subject to interference from chloride (Cl^-). Interferences from Cl^- and sulphate (SO_4^{2-}) were reported by Di Goia et al. (2010). From several hundred analyses of nutrient solution, soil solution and sap from different vegetable crops, good agreement was obtained between NO_3^- specific ion sensitive electrode system and laboratory analysis (R.B. Thompson, University of Almeria, unpublished data). However, in this work, there was a tendency for the NO_3^- specific ion sensitive electrode to underestimate sap [NO_3^-] at [NO_3^-] of >6,500 mg L^{-1}, which was overcome by diluting samples. Parks et al. (2012) reported that while accurate results had been reported with NO_3^- sensitive test strip readers, there were limited scientific assessments with plant samples. However, Thompson et al. (2009) obtained accurate results using a NO_3^- sensitive test strip reader with sap samples and soil solution. These authors reported that the limited range of the NO_3^- sensitive test strip reader used (up to 225 mg NO_3^- L^{-1}, RQflex® reflectometer) required dilution of nearly all samples, and that accurate dilution was critically important. Generally, NO_3^- specific ion sensitive electrodes have a much larger working range of $[NO_3^-]$ than NO_3^- sensitive test strip readers.

Further research is required to fully characterize the performance of the currently available rapid analysis systems. Nevertheless, the available information suggests that they can provide reasonably accurate results that are adequate for monitoring used for on-farm decision making (Parks et al., 2012; Thompson et al., 2009). However, results are less accurate than laboratory analysis. Considerable care should be taken, and instructions should be strictly followed. Particular care should be given to handling, cleaning, sample temperature, and dilution, which if not done correctly can introduce errors (Parks et al., 2012; Thompson et al., 2009). Results should be periodically checked against laboratory analysis, and independent standard aqueous solutions should be regularly analyzed to confirm the accuracy in aqueous solutions (Di Gioia et al., 2010).

Rapid analysis systems are available for the measurement of nutrients other than NO_3^-. These systems are test strip readers, ion specific electrodes for specific nutrients or multi ion electrode systems that measure the concentrations of various nutrients. There are few published scientific studies available that have evaluated these systems.

7. Management applications of crop and soil N monitoring in vegetable crops

This article has reviewed different plant and soil monitoring approaches with the capacities to assess crop and soil N status, and to guide N management in vegetable crops. These approaches include soil monitoring, destructive (tissue N analysis, petiole sap NO_3^- analysis) and non-destructive (optical sensors, electrical impedance spectroscopy) crop-based methods, and portable rapid analysis systems for the measurement of $[NO_3^-]$ in solution. These monitoring approaches have been demonstrated to be sensitive indicators of crop N status in a wide range of vegetable crops, and to be useful tools to guide N fertilizer management. In general, the selection of the best monitoring approach for a given farm will depend on factors such as crop and farm characteristics, the farmer's technical level, the support provided, and economic considerations. In scientific terms, the selection of an approach for crop N management should consider the capacity to provide information of crop N status throughout the entire crop cycle or at critical stages. In practical terms, an important issue to consider is the cost and ease of use. The high cost of some optical sensors (i.e. above 3,000€) makes them unaffordable for small vegetable farmers and local enterprises, but it is likely that there will be increasing availability of low-cost sensors, providing an affordable way to monitor crop N status as a basis to adjust in-season N fertilization.

Generally, crop-based methods are sensitive indicators of crop N status in diverse vegetable crops; they are particularly useful to detect N deficiency. However, they and particularly optical sensors have reduced sensitivity to detect excessive N supply. A notable feature of soil monitoring methods (e.g. the Dutch 1:2 soil-water extract method, soil solution monitoring) is that they can detect excess N supply. The combination of crop and soil monitoring methods will provide vegetable growers with tools to detect crop N deficiency and excess N supply. Soil and crop monitoring approaches could form part of improved management packages that include the use of DSSs to determine crop N requirements (see article by Gallardo et al., 2020, in this special issue). In such a prescriptive-corrective management package, soil and crop monitoring measurements would be the bases of corrective adjustments of the prescriptive N fertilizer plan prepared with the DSS. The use of such a package, particularly when combined with fertigation and drip irrigation, will considerably improve N management of vegetable crops resulting in much smaller N losses to the environment.

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Conflicts of Interest

The authors declare no conflict of interest. Trade and manufacturers' names mentioned in this review are for information only, and do not constitute endorsement, recommendation, or exclusion.

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