Title: Petiole sap nitrate concentration to assess crop nitrogen status of greenhouse sweet pepper

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Abstract

Vegetable production requires improved nitrogen (N) management practices. Monitoring petiole sap nitrate concentration ([NO_3^-–N]) is a simple and cheap method to evaluate crop N status. The sensitivity of petiole sap [NO_3^-–N] to assess crop N status of sweet pepper was evaluated. Three sweet pepper crops were grown in different cropping seasons, each with an autumn-winter growing period. The crops commenced in 2014, 2016, and 2017. Combined fertigation and drip irrigation frequently applied (every 1–4 days) complete nutrient solution throughout each crop. The crops were grown in a greenhouse in soil. Five N treatments as N concentrations were applied throughout each crop: N1 (2.0–2.4 mmol L^{-1}); N2 (5.3–6.2 mmol L^{-1}); N3 (9.7–12.6 mmol L^{-1}); N4 (13.1–16.1 mmol L^{-1}); N5 (16.7–20.0 mmol L^{-1}). These corresponded to very deficient, deficient, conventional, excessive and very excessive N supply. Petiole sap [NO_3^-–N] was determined every 1–2 weeks and related to Nitrogen Nutrition Index (NNI), which was used as an indicator of crop N status. For each of the N treatments in each crop, petiole sap [NO_3^-–N] was relatively constant throughout the crop. The relationship between petiole sap [NO_3^-–N] and NNI, for pooled data from the three pepper crops, was described by (a) the polynomial equation $\mathrm{NNI} = -1.10\mathrm{E}-07 \times Sap^2 + 0.000473 \times Sap + 0.5514$ with an R^2 of 0.84, and (b) the segmented linear equations $\mathrm{NNI} = 0.00034 \times Sap + 0.572$ and NNI = 1.04, with an R^2 of 0.83. Sufficiency values for maximum growth of sweet pepper were obtained by (a) solving the polynomial equation for NNI = 1.0, and (b) using the intercept value of the horizontal line of the segmented linear regression. The corresponding sufficiency values for the duration of a complete crop cycle were 1441 and 1367 mg NO_3^-–N L^{-1}, respectively. A sufficiency value of 1400 mg NO_3^-–N L^{-1} was rounded-off and suggested for the duration of a complete crop cycle of greenhouse-grown sweet pepper in SE Spain. The relationships between petiole sap [NO_3^-–N] and NNI, and the derived sufficiency values for the flowering and early fruit growth, and harvest phenological stages were similar to those determined for the entire crop.

Petiole sap [NO_3^-–N] is a sensitive and effective method to monitor crop N status of sweet pepper.

Keywords: *Capsicum annuum* L*; crop N status; N fertilizer management; NNI; petiole sap analysis; sufficiency values; vegetable crop.*

1. Introduction

Intensive vegetable production in plastic greenhouses and large plastic tunnels is an important industry throughout the Mediterranean Basin (Pardossi et al., 2004). On the southeast (SE) coast of Spain, vegetable production takes place in 42,000 ha of plastic greenhouses (Valera et al., 2016), of which 32,000 ha are in Almeria province (Junta de Andalucía., 2019a). In the greenhouses of Almeria, 90% of cropping is in soil (García et al., 2016). The crops grown in soil are produced with combined fertigation and drip irrigation, with which N is frequently applied in nutrient solution throughout the crop (Thompson et al., 2007, 2017a, 2020a).

For intensive production of greenhouse vegetable crops, excessive amounts of mineral nitrogen (N) fertilizer are commonly applied (Soto et al., 2015; Thompson et al., 2007). Additionally, appreciable amounts of N are supplied by the soil as mineral N or through N mineralization from organic N in soil organic matter and/or applied manure (Granados et al., 2013; Jadoski et al., 2013; Thompson et al., 2007). The consequent excessive N supply (Jadoski et al., 2013) often results in appreciable nitrate (NO_3^-) leaching loss (Gallardo et al., 2006; Granados et al., 2013; Thompson et al., 2013, 2020a). These N losses have resulted in NO_3^- contamination of associated groundwater and the eutrophication of adjoining natural surface water bodies (Thompson et al., 2020a, 2020b). In Almeria, there is substantial NO_3^- contamination of aquifers from greenhouse vegetable production (Pulido-Bosch et al., 2018).

Given the extent of aquifer NO_3^- contamination, nearly all of the greenhouse growing areas of Almeria have been identified as being Nitrate Vulnerable Zones (NVZ) (BOJA., 2015) as required by the European Union (EU) Nitrate Directive (Anonymous., 1991). Being in NVZs, vegetable growers in Almeria greenhouses are required to adopt management practices that appreciably improve crop N management to considerably decrease aquifer NO_3^- contamination.

The management of N of soil-grown vegetable crops inside greenhouses in Almeria is complicated by the likely occurrence of appreciable quantities of N supplied by the soil as residual soil mineral N (Granados et al., 2013; Thompson et al., 2020a) and mineralized N from periodic manure applications (Thompson et al., 2020a, 2007). Generally, growers apply standard concentrations of N, throughout crops, without taking into account N supplied by the soil, which can be substantial (Jadoski et al., 2013; Thompson et al., 2020a, 2007).

Monitoring approaches that provide assessment of crop N status will greatly assist growers, in this and other systems, to make decisions and adjustments to optimize crop N management (Thompson et al., 2017b). Petiole sap nitrate concentration ($[NO_3^-{-}N]$) is a sensitive indicator of crop N status of various vegetable species (Farneselli et al., 2014; Goffart et al., 2008; Hochmuth, 1994). It is substantially more responsive to crop N nutrition than leaf total N analysis (Majić et al., 2008; Olsen and Lyons, 1994). Petiole sap $[NO_3^-{-}N]$ is particularly sensitive to changes in crop N status (Goffart et al., 2008; Olfs et al., 2005; Villeneuve et al., 2002). This makes it an interesting option for vegetable production in greenhouses in Almeria where frequent N application would enable rapid correction of detected deficient crop N status.

A recent study with drip irrigated/fertigated tomato, receiving frequent N application, suggested that there was a strong and consistent linear relationship, throughout the crop, between petiole sap $[NO_3^-{-}N]$ and crop N status, measured as Nitrogen Nutrition Index (NNI) (Peña-Fleitas et al., 2015). The NNI is an established quantitative indicator of crop N status (Lemaire et al., 2008; Ziadi et al., 2010); it is the ratio between the actual crop N concentration and the critical N concentration of the crop (i.e. the minimum crop N concentration required for maximum growth; Greenwood et al., 1990). An NNI value of one indicates N sufficiency for growth (dry matter production, values <1 indicate N deficiency, and values >1 indicate N excess; Lemaire et al., 2008).

Sweet pepper is an important crop in Almeria greenhouses (Valera et al., 2016). Approximately, 10,000 ha of the greenhouse surface area is cropped annually with sweet pepper (Junta de Andalucía, 2019b). Given the considerable importance of pepper in this system, and the severity of the N problem of this system, it is necessary to develop tools that will improve the management of N of sweet pepper crops.

Petiole sap [NO_3^-–N] is an approach that could be used to assess sweet pepper N status within this vegetable production system. The general sensitivity of sweet pepper petiole sap [NO_3^-–N] to N supply was demonstrated by Olsen and Lyons, (1994). The critical N curve developed by Rodríguez et al., (2020a) enables the calculation of NNI values that facilitate quantitative evaluation of the capacity of petiole sap [NO_3^-–N] to indicate crop N status and for the derivation of sufficiency values for crop growth.

The objectives of the present work were for sweet pepper, grown in soil in a greenhouse, to: (i) evaluate the response of petiole sap [NO_3^-–N] to different nutrient solution N concentrations applied by fertigation, (ii) assess the use of petiole sap [NO_3^-–N] to evaluate crop N status, and (iii) derive sufficiency values of petiole sap [NO_3^-–N] for maximum crop growth for the duration of the crop, and also for three different phenological stages.

2. Material and methods

2.1. Experimental site

Three sweet pepper (*Capsicum annuum* L. cv. “Melchor”) crops, each in a different autumn-winter growing season, were grown in a greenhouse at the research farm of the University of Almeria, Retamar, Almeria, SE Spain (36°51’ N, 2°16’ W). Cropping conditions were equivalent to local commercial production. The crops were grown in an artificial soil, known locally as "enarenado" that is characteristic in the region (Thompson et al., 2007; Gázquez et al., 2017).

The soil consisted of sandy loamy soil of natural origin covered with a 30 cm layer of imported loam textured soil, with 10 cm layer of a fine gravel (mostly 2–5 mm diameter) placed on the imported soil as a mulch (Padilla et al., 2014). Some relevant physical and chemical characteristics (0–10 cm), from a 2011 analysis, are soil pH (1:2.5, soil:water) of 8.2, bulk density (Mg m^{-3}) of 1.2, total N of 0.20%, and organic C of 2.8%. More details of the soil are available in Padilla et al., (2014) and Soto et al., (2014).

The greenhouse had a multi-tunnel type structure (Valera et al., 2016) with walls of polycarbonate. The roof was low-density polyethylene (LDPE), tri-laminated film (200-µm thick) that had approximately 60% transmittance of Photosynthetically Active Radiation (PAR) (Padilla et al., 2014). The greenhouse was passively ventilated through flap roof windows and lateral side panels. The orientation of the greenhouse was east-west, and that of the crop rows was north-south. The area that was cropped was 1327 m^2.

The irrigation system was above-ground drip tape. The tape was organized in pairs of lines with a separation 0.8 m between lines in each pair, and a 1.2 m separation between pairs of lines. There was a 0.5 m separation between emitters in each drip line. The discharge rate of the drip emitters was 3 L h^{-1}, and the coefficient of uniformity of the irrigation system was >95%. Fertigation was used to apply mineral fertilizer.

The greenhouse was arranged as 24 experimental plots of 6 × 6 m; twenty plots were used. In every plot there were three paired lines of drip tape, with 12 emitters in each line. Individual plots were hydraulically separated from one another by vertically positioning polyethylene film (250 µm thick) to 30 cm soil depth. The plants were located 6 cm to the side of an emitter. Plant density was 2 plants m^{-2}, for a total of 72 plants in each plot.

2.2. Pepper crops and N treatments

The three sweet pepper crops were grown in different autumn-winter growing seasons, in 2014–2015 (2014 crop), 2016–2017 (2016 crop) and 2017–2018 (2017 crop). The 2014 crop was from 12 August 2014 to 29 January 2015 (170 days), the 2016 crop from 19 July 2016 to 24 March 2017 (248 days), and the 2017 crop from 21 July 2017 to 20 February 2018 (214 days) (Table 1). Plants were transplanted as five-week-old seedlings. Following transplanting, seedlings were irrigated with water (<0.04 mmol N L^{-1}) from the first day after transplanting (DAT) until the N treatments started. The same three pepper crops were used in the studies of Rodríguez et al., (2020a, 2020b).

Table 1. General information of the three pepper crops, and of each N treatment. Provided are the dates of the crops, and of the N treatments expressed as the average nutrient solution N concentration, and total quantity of N applied. Total dry matter production (DMP) and total yield (TY; fresh weight) data are presented.

Crop year	Date of transplanting	Date end of the crop (duration)	N treatment[a]	[N] in nutrient solution (mmol L^{-1})[b]	Total N applied (kg N ha^{-1})[c]	DMP (t ha^{-1})	TY (t ha^{-1})
2014	12/08/2014	29/01/2015 (170 days)	N1	2.4	64	5.7	38.7
			N2	6.2	189	7.9	52.2
			N3	12.6	516	8.6	52.9
			N4	16.1	804	9.7	51.1
			N5	20.0	990	9.3	46.4
2016	19/07/2016	24/03/2017 (248 days)	N1	2.0	88	8.8	67.2
			N2	5.3	302	12.6	86.4
			N3	9.7	561	15.2	91.5
			N4	13.5	1052	14.4	94.2
			N5	17.7	1320	13.6	89.7
2017	21/07/2017	20/02/2018 (214 days)	N1	2.0	86	5.1	33.3
			N2	5.7	304	9.3	54.4
			N3	9.7	519	10.5	61.0
			N4	13.1	870	12.6	65.1
			N5	16.7	1198	12.6	68.9

[a] N1: very N deficient; N2: N deficient; N3: conventional N; N4: excessive N; N5: very excessive N.

[b] For the period in which the N treatments were applied.

[c] For the complete cropping cycle.

Five treatments of different N concentration were applied to each crop. They were applied in each irrigation event, throughout the crops, from 1, 9 and 10 DAT in the 2014, 2016 and 2017 crops, respectively. The different N treatments were: very N deficient (N1), N deficient (N2), conventional N management (N3), excessive N (N4) and very excessive N (N5). There were minor differences between the N concentrations applied as equivalent treatments in the three pepper crops (Table 1). The total quantities of mineral N applied are provided in Table 1. More than 90% of applied mineral N was in the form of NO_3^-, the rest as ammonium (NH_4^+).

Other than the different N concentrations, the nutrient solution supplied all other macro and micronutrients to ensure that they were not limiting. The composition of the nutrient solution applied to the N3 treatments in the three pepper crops are presented in Supplementary Table 1.

Irrigation was conducted to maintain the soil matric potential of the root zone in the range −10 to −30 kPa. Soil matric potential was measured with one tensiometer (Irrometer, Co., Riverside, CA, USA) per plot, at 0.15 m depth. Irrigation frequency was one irrigation every 1–4 days, with irrigation being more frequent in warm conditions. To reduce the build-up of soil salinity, additional irrigation as nutrient solution or water was applied, to certain treatments during the crops, for short periods. These additional irrigations are described in Rodríguez et al., (2020b).

Local crop management practices were used; crop management practices in this system are described in detail by Valera et al. (2016). For the three pepper crops in the current study, nylon cords were placed horizontally along the crop rows to physically support the crops. This is a local system known as “enfajado”. To control high temperature in the greenhouse, the plastic cladding was white-washed with applications of $CaCO_3$ suspension, prior to transplanting the seedlings. The whitewash was removed by hosing in early Autumn. Otherwise, climate control was based on the opening and closing of side panels and roof windows. Pest and pathogen control was mostly with biological control; when necessary, chemical agents were used. To prevent insect entry, all side panels and roof windows had fine mesh, and entry was through chambers with doors on each side. All crop management operations, apart from irrigation and fertilizer application, such as transplanting, pest treatments, pruning, harvesting, etc., were conducted manually.

2.3. Petiole sap [NO_3^-–N] measurements

Petiole sap [NO_3^-–N] was determined every week in the 2014 crop, and every two weeks in the 2016 and 2017 crops. Sap measurements commenced at 21, 22 and 19 DAT for the 2014, 2016 and 2017 crops, respectively, and continued throughout the crops.

For each sampling of petiole sap [NO_3^-–N], the most recently fully expanded leaf was removed from sixteen different plants, in each replicate plot, between 07:00 and 09:00 h on each sampling date. Immediately after sampling, each sampled leaf was placed in a sealed plastic bag, from which air was pressed, and which was then placed in a chilled cooler box. At the completion of sampling, petioles were separated from leaf blades in a laboratory adjacent to the greenhouse. The petioles were then immediately placed in individual sealed plastic bags from which air was pressed out. The bags were then placed in a chilled cooler box in which they were promptly transported to a laboratory at the University of Almeria (UAL).

In the laboratory at UAL, they were stored at 5°C prior to being cut into 1 cm long sections that were immediately pressed with a domestic garlic press. The extracted liquid was diluted (1:10) and centrifuged at 1900 g (4500 rpm) for 15 minutes, at a temperature of 4°C. The [NO_3^-–N] was measured with a SAN++ segmented flow analyzer (Skalar Analytical B.V., Breda, The Netherlands). Analysis was conducted within 6 h after sampling the petioles.

2.4. Determination of crop dry matter production, crop N concentration, crop N uptake, and yield

Crop above-ground dry matter production (DMP) was measured at approximately three weekly intervals. In each replicate plot, one complete and representative plant was removed, which was separated into stem, leaf, and fruit material. For each biomass component (leaf, stem, fruit), dry matter content was assessed by oven-drying at 65°C until constant weight. The dry matter of the transplanted seedlings was determined in 100 seedlings. The amount of dry matter of pruned material was assessed at each pruning in each crop, from eight selected marked plants in each plot. At the end of the crop, the final biomass was determined by removing and measuring the fresh weight of the eight marked plants. The fractions of leaf, stem, and fruit were assessed in two representative plants. The dry matter contents of these components were determined by oven-drying at 65°C, until constant weight.

In all biomass samplings, the dry matter mass of each biomass component was calculated as the product of dry matter percentage and fresh weight. Total DMP at each biomass sampling, was the sum of the mass of dry matter of leaf, stem and fruit, plus the dry matter mass of all previously-sampled harvested fruit and pruned material.

Sub-samples of leaf, stem and fruit from all biomass samplings, and of pruned material and of harvested fruit, from each plot, were ground in a knife mill. The total N concentration (%N) of all sub-samples was analyzed with a Rapid N elemental analyzer system (Elementar Analysensysteme GmbH, Hanau, Germany). The mass of N in each plant component was the product of the dry matter mass and the N concentration.

For all biomass samplings, total crop N uptake (kg N ha^{-1}), in each plot, was the sum of N in all components (leaf, stem, immature fruit, previously pruned material and previously harvested fruit). Total crop N concentration (%N) for each plot was total crop N uptake divided by total DMP.

Fresh fruit were regularly harvested from the same eight marked plants, in each plot, used to collect pruned material. For harvested fruit from each plot, fresh and dry weights were assessed. Harvests took place every 7–14 days. They commenced at 98, 101 and 110 DAT for the 2014, 2016 and 2017 crops, respectively. There were seven, sixteen and eleven harvests, respectively. Total yield, for each treatment in each crop, was the sum of all harvested fresh fruit.

2.5. Determination of crop Nitrogen Nutrition Index (NNI)

To relate petiole sap [NO_3^-–N] values to NNI, NNI was estimated for each date of petiole sap sampling. NNI was calculated as:

$$NNI = \frac{N_{act}}{N_c}, \quad (1)$$

where N_{act} is the actual crop N concentration and N_c is the critical crop N concentration. N_{act} values were obtained by interpolating from measured crop N concentration values, which were determined approximately every 21 days, for each treatment, during each of the three individual pepper crops, as described in section 2.4. The N_c values, for each date of petiole sap sampling, were obtained using the critical N curve $\%Nc = 4.71 \times DMP^{-0.22}$ for greenhouse-grown sweet pepper (Rodríguez et al., 2020a). This critical N curve was derived from the same three pepper crops that were used in the present work. The DMP values used to derive N_c values, for each treatment in each crop, were interpolated from measured DMP values from the periodic biomass samplings.

2.6. Data analysis

2.6.1. Relationship between crop NNI and petiole sap [NO_3^-–N]

NNI was used as an indicator of crop N status. For each date of sap sampling, the corresponding NNI values were interpolated, for each N treatment. The relationships between NNI and petiole sap [NO_3^-–N] were examined using regression analysis. Two different types of regression were used to compare the relationship, being: (i) polynomial regression, and (ii) segmented linear regression analysis.

The polynomial regression was defined by the equation $\mathrm{NNI} = a \times Sap^2 + b \times Sap + c$; where a, b and c are the quadratic, linear and independent parameters determined by the equation, and Sap is the petiole sap [NO_3^-–N] (mg L^{-1}). The regression equations were fitted, and the coefficient of determination (R^2) and standard error (SE) values were obtained using CurveExpert Professional 2.2.0 software (D. Hyams, Madison, AL, USA).

Segmented linear regression analysis consisted of fitting two linear regression lines, a sloped lined described by $\mathrm{NNI} = a \times Sap + b\ (if\ Sap < Sap_0)$ and a horizontal line described by $\mathrm{NNI} = c\ (if\ Sap \geq Sap_0)$, where a is the slope of the sloped segment, b and c are intercept values, and Sap_0 is the petiole sap [NO_3^-–N] value where the two lines intersect. The sloped line implies a linear increase in NNI with increasing petiole sap [NO_3^-–N]. The horizontal line implies that NNI remains constant at higher petiole sap [NO_3^-–N]. The value Sap_0 is the petiole sap [NO_3^-–N] that maximizes NNI, below which NNI is reduced, and above which NNI is constant. The segmented line analysis was conducted using RStudio2 software (RStudio Inc., Boston, MA, USA).

2.6.2. Determination of integrated petiole sap [NO_3^-–N] and NNI values

For each pepper crop, integrated values of petiole sap [NO_3^-–N] ($\mathrm{Sap}i$) and NNI values ($\mathrm{NNI}i$) were calculated as single integrated values, for each treatment, to evaluate how different N treatments affected petiole sap [NO_3^-–N] for the complete crop and for each phenological stage. Integrated values, for each treatment, were calculated as: $\mathrm{NNI}i = \sum\ (\mathrm{NNI}_m\ \times d_m\,/D)$ and $\mathrm{Sap}i = \sum\ (\mathrm{Sap}_m\ \times d_m\,/D)$, where NNI_m and Sap_m were the values at each date of sap measurement, d_m was the number of days since the previous measurement (from two consecutive measurements), and D was the total number of days from first to the last measurement. For the initial measurement, for which there was no previous measurement, a 7–day interval was used for the 2014 crop, and a 14–day interval for the 2016 and 2017 crops, which was consistent with the sampling frequency.

For the calculation of the integrated values, when the beginning of a phenological stage occurred between two sap sampling dates, the number of days to the first sap sampling, in the stage, was considered. Similarly, when the end of the phenological stage occurred between two sampling dates, the number of days from the last sap sampling, in the stage, was considered.

Three different phenological stages were examined, being (i) vegetative, (ii) flowering and early fruit growth, and (iii) harvest. The vegetative stage started at transplanting and continued until the commencement of flowering, it corresponsed to 0 to 41 DAT in the three cropping cycles. The flowering and early fruit growth stage started at the commencement of flowering (at 42 DAT in the three crops) and continued until the first fruit harvest at 97, 100 and 109 DAT in the 2014, 2016 and 2017 crops, respectively. The harvest stage, which was the longest stage, commenced with the initial harvest of fruit and continued until the end of the crop, being 98–170, 101–248, and 110–214 DAT, in the 2014, 2016 and 2017 crops, respectively.

Treatment effects on integrated NNI and integrated petiole sap [NO_3^-–N] values were evaluated with analysis of variance (ANOVA) and least significant difference (LSD) tests. ANOVA was conducted following verification of assumptions of equal variance and normality. The LSD test compared multiple means when treatment effects were significant at $P < 0.05$. ANOVA results will be indicated as: no significant difference at $P \geq 0.05$ (ns), significant at $P < 0.05$ (*), very significant at $P < 0.01$ (**), and highly significant at $P < 0.001$ (***). The Statistica 13 software (TIBCO Software Inc., Palo Alto, CA, USA) was used for these statistical analyses.

2.6.3. Determination of petiole sap [NO_3^-–N] sufficiency values for maximum growth

Sufficiency values were obtained from the relationships between petiole sap [NO_3^-–N] and NNI. The calculation of sufficiency values for maximum growth (i.e. of DMP) was determined using two approaches: (1) by solving the polynomial equation that related NNI to petiole sap for $\mathrm{NNI} = 1.0$, and (2) by determining the intersection of the sloped and horizontal lines of the segmented line analysis, i.e. the Sap_0 value defined in section 2.6.1.

Using both approaches, sufficiency values were derived for the duration of each of the three pepper crops, and for each phenological stage of each crop. Also, using both approaches with pooled data from throughout the three crops, a single sufficiency value was determined for sweet pepper, and single sufficiency values were determined for each of three phenological stages, which were the vegetative stage, the combined flowering and early fruit growth stage, and the harvest stage.

3. Results

3.1. Effect of N on petiole sap [NO_3^-–N] of sweet pepper crops

3.1.1. Petiole sap [NO_3^-–N] throughout the crops

Petiole sap [NO_3^-–N] was influenced by the N treatments, at all sampling dates, in each of the 2014, 2016 and 2017 pepper crops (Fig. 1). With very few exceptions, there were consistent differences in petiole sap [NO_3^-–N] between N treatments, with the order N5 > N4 > N3 > N2 > N1 being maintained throughout each of the three pepper crops (Fig. 1). Petiole sap [NO_3^-–N] for each treatment was relatively constant throughout each crop, particularly after the first 40–60 days when some fluctuation occurred in some treatments. There were consistent appreciable differences between the N1, N2 and N3 treatments in each of the three pepper crops. The average differences, between petiole sap [NO_3^-–N] of treatments N1 and N2 and between treatments N2 and N3, were 475 and 979 mg NO_3^-–N L^{-1}, respectively (Fig. 1). The differences between the N4 and N3 treatments were generally consistently notable in each of the three pepper crops; the average difference being 348 mg NO_3^-–N L^{-1}. There were generally small, but consistent, differences between the N4 and N5 treatments; the average difference in the three pepper crops being 103 mg NO_3^-–N L^{-1}.

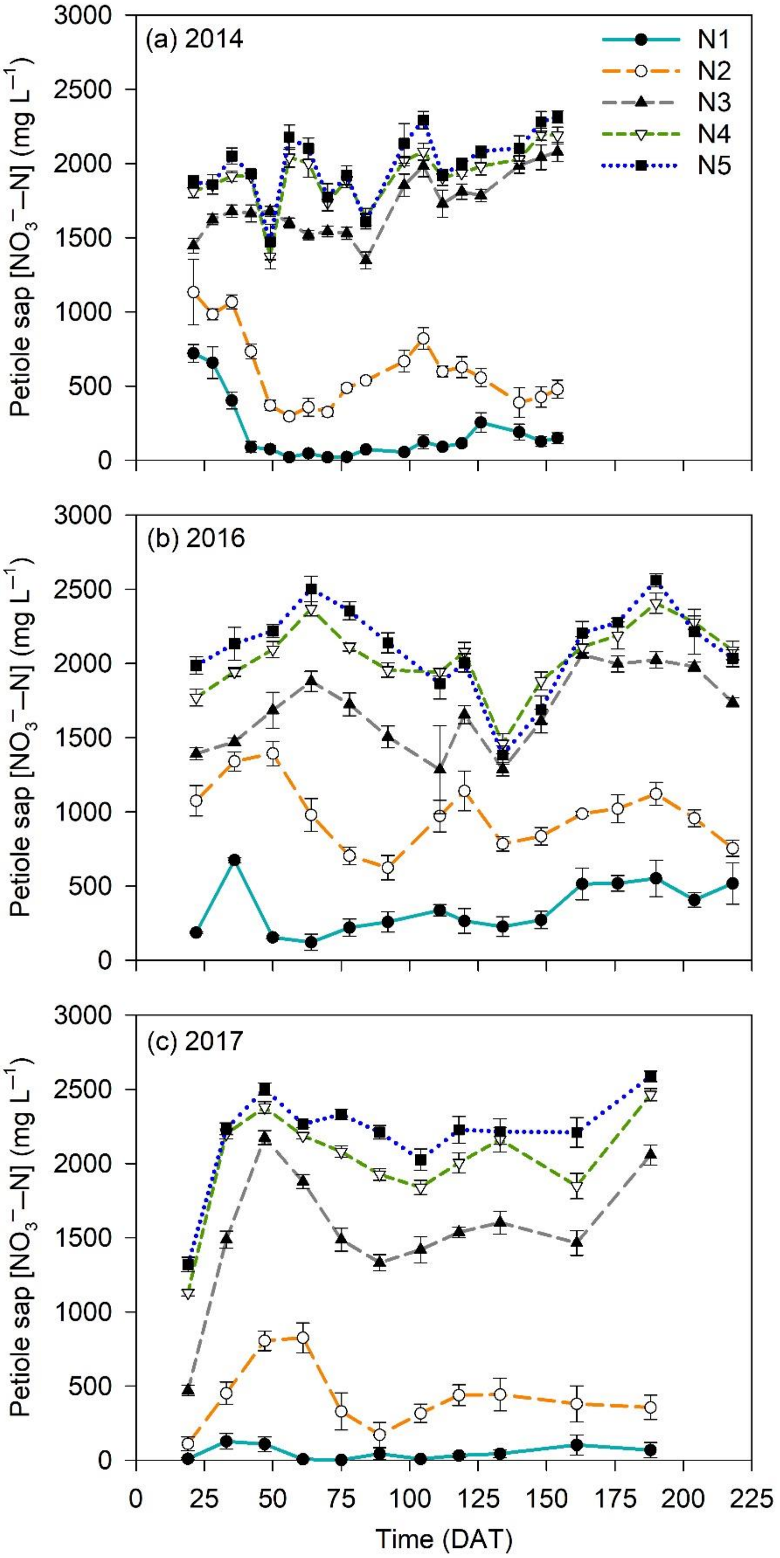

Fig. 1. Effect of increasing N concentration in nutrient solution, on petiole sap [NO_3^-–N] values throughout the (a) 2014, (b) 2016 and (c) 2017 pepper crops. The data presented are means (*n* = 4) ± standard error (SE).

3.1.2. Integrated petiole sap [NO_3^-–N] values

For the integrated petiole sap [NO_3^-–N] ($\mathrm{Sap}i$) values, there were the following significant differences ($P < 0.05$) between N treatments for the duration of the crop and in each of the three phenological stages, for each of the 2014, 2016 and 2017 crops: N4 > N3 > N2 > N1 (Table 2). The N5 treatment had significantly ($P < 0.05$) higher integrated petiole sap [NO_3^-–N] values than the N4 treatment for the duration of the 2014 and 2017 crops, and in five of the nine phenological stages evaluated in the three pepper crops (Table 2).

Table 2. Integrated petiole sap [NO_3^-–N] ($\mathrm{Sap}i$) (mg L^{-1}) values for each treatment, the entire crop and phenological stage for the 2014, 2106 and 2017 crops. Different letters indicate significant differences ($P < 0.05$) between treatments in each crop, using LSD. A summary of the significance of the N treatments in the ANOVA is presented, with highly significant differences indicated by $P < 0.001$ (***). Values are means (n = 4) ± standard error (SE).

Crop	Treatment	$\mathrm{Sap}i$ [NO_3^-–N] (mg L^{-1})			
			Phenological Stage		
		Entire crop	Vegetative	Flowering and early fruit growth	Harvest
2014	N1 (Very N deficient)	172 ± 23 a	481 ± 61 a	47 ± 4 a	149 ± 29 a
	N2 (N deficient)	595 ± 22 b	988 ± 72 b	465 ± 15 b	537 ± 49 b
	N3 (Conventional)	1736 ± 19 c	1600 ± 31 c	1611 ± 25 c	1922 ± 24 c
	N4 (Excessive N)	1918 ± 25 d	1873 ± 26 d	1837 ± 39 d	2042 ± 13 d
	N5 (Very excessive N)	2006 ± 25 e	1928 ± 28 d	1911 ± 57 d	2136 ± 31 e
	Significance	***	***	***	***
2016	N1 (Very N deficient)	350 ± 35 a	389 ± 10 a	211 ± 39 a	408 ± 61 a
	N2 (N deficient)	957 ± 23 b	1121 ± 117 b	892 ± 54 b	944 ± 16 b
	N3 (Conventional)	1687 ± 34 c	1470 ± 31 c	1644 ± 56 c	1752 ± 45 c
	N4 (Excessive N)	2044 ± 12 d	1892 ± 33 d	2110 ± 15 d	2049 ± 26 d
	N5 (Very excessive N)	2101 ± 34 d	2084 ± 68 e	2251 ± 36 e	2030 ± 48 d
	Significance	***	***	***	***
2017	N1 (Very N deficient)	56 ± 24 a	78 ± 26 a	25 ± 12 a	72 ± 41 a
	N2 (N deficient)	413 ± 49 b	398 ± 59 b	446 ± 81 b	391 ± 87 b
	N3 (Conventional)	1568 ± 31 c	1245 ± 32 c	1585 ± 49 c	1701 ± 59 c
	N4 (Excessive N)	2038 ± 32 d	1823 ± 23 d	2037 ± 16 d	2135 ± 58 d
	N5 (Very excessive N)	2222 ± 41 e	1938 ± 30 e	2233 ± 36 e	2341 ± 70 d
	Significance	***	***	***	***

3.2. Effect of N on NNI of sweet pepper crops

3.2.1. NNI throughout the crops

NNI was influenced by the N treatments, at all sampling dates, in the 2014, 2016 and 2017 pepper crops (Fig. 2). In general, there were consistent differences, in the order of N5 = N4 > N3 > N2 > N1, throughout the three pepper crops (Fig. 2). Despite some fluctuations in a minority of treatments, the general tendency was of relatively constant NNI values for all treatments. There were generally consistent and clear differences in NNI between the N1, N2 and N3 treatments in each of the three pepper crops. In general, there were relatively small and consistent differences in NNI between the N3 and N4 treatments. The NNI values of the N4 and N5 treatments were generally very similar.

NNI values of the N1 and N2 treatments were mostly in the ranges of 0.47–0.88, and 0.68–0.99, respectively (Fig. 2). Those of the N3 treatment were consistently close to 1.0, and those of the N4 and N5 treatments were generally 0.92–1.14.

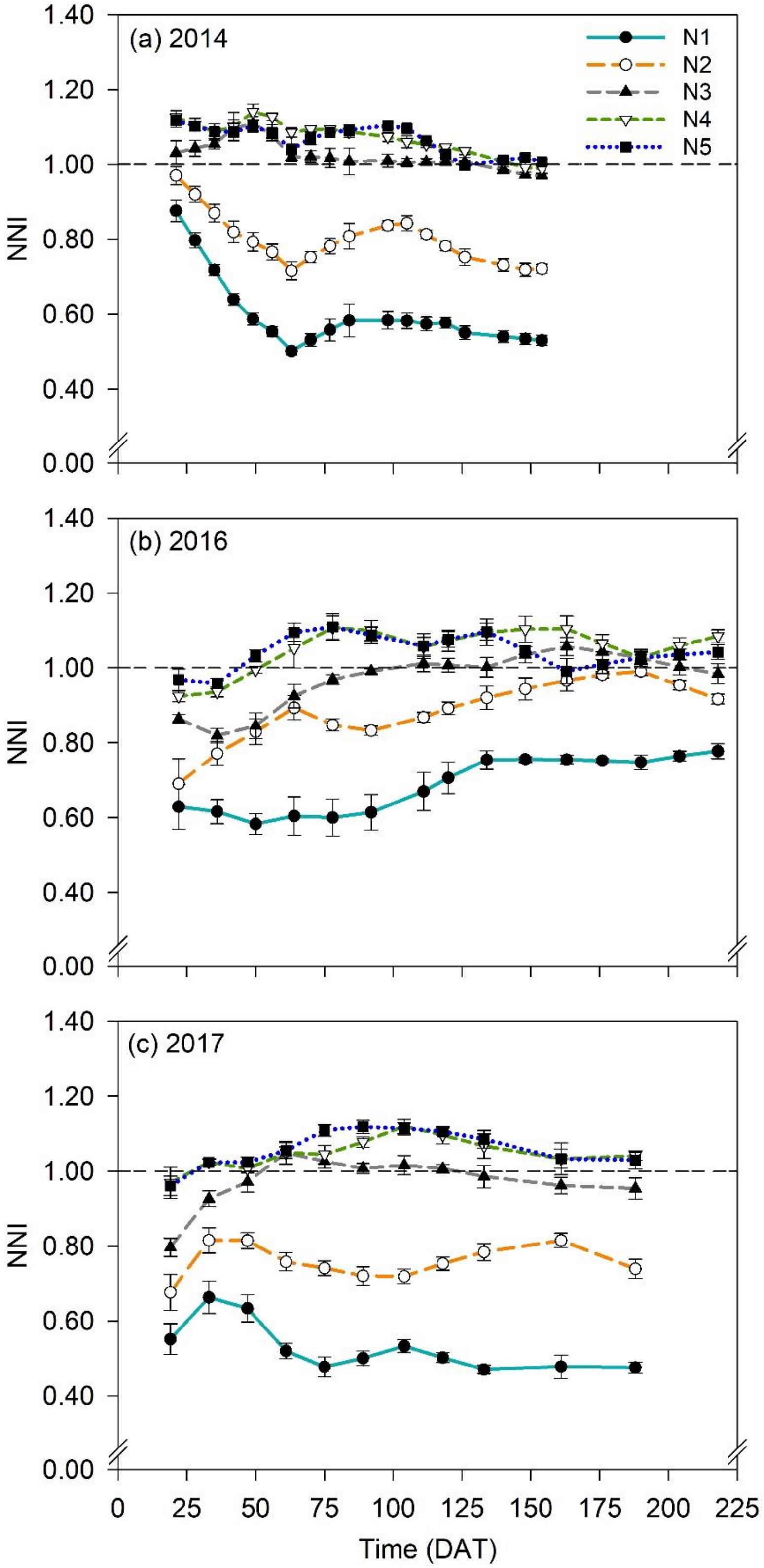

Fig. 2. NNI values throughout the (a) 2014, (b) 2016, and (c) 2017 pepper crops. Presented values are means (*n* = 4) ± standard error (SE). The horizontal dotted line represents NNI = 1.0. Modified from de Souza et al. (2019). The use of chlorophyll meters to assess crop N status and derivation of sufficiency values for sweet pepper. Sensors 19, 2949, published by MDPI and distributed as open access under the Creative Commons Attribution (CC BY) license.

3.2.2. Effect of N on integrated NNI values

For the integrated NNI ($\mathrm{NNI}i$) values, there were the following significant differences (P <0.05) between treatments for the duration of the crop and in each phenological stage, for each of the 2014, 2016 and 2017 pepper crops: N5 = N4 > N3 > N2 > N1 (Table 3). There were no significant differences (P <0.05) in $\mathrm{NNI}i$ values between the N5 and N4 treatments.

Table 3. Integrated Nitrogen Nutrition Index ($\mathrm{NNI}i$) values for each treatment for the complete crop and for phenological stage for the 2014, 2106 and 2017 crops. Different letters indicate significant differences (P <0.05) between means in each crop year according to LSD. A summary of the significance of the N treatments in the ANOVA is presented, with highly significant differences indicated by P <0.001 (***). Values are means (*n* = 4) ± standard error (SE).

Crop	Treatment	Entire crop	Phenological Stage		
			Vegetative	Flowering and early fruit growth	Harvest
2014	N1 (Very N deficient)	0.60 ± 0.01 a	0.76 ± 0.02 a	0.56 ± 0.01 a	0.55 ± 0.02 a
	N2 (N deficient)	0.80 ± 0.01 b	0.90 ± 0.02 b	0.79 ± 0.01 b	0.76 ± 0.01 b
	N3 (Conventional)	1.02 ± 0.01 c	1.06 ± 0.02 c	1.04 ± 0.02 c	0.99 ± 0.00 c
	N4 (Excessive N)	1.07 ± 0.01 d	1.11 ± 0.02 d	1.10 ± 0.01 d	1.03 ± 0.01 d
	N5 (Very excessive N)	1.07 ± 0.00 d	1.10 ± 0.01 d	1.08 ± 0.01 d	1.03 ± 0.00 d
	Significance	***	***	***	***
2016	N1 (Very N deficient)	0.69 ± 0.02 a	0.62 ± 0.04 a	0.61 ± 0.04 a	0.75 ± 0.01 a
	N2 (N deficient)	0.89 ± 0.02 b	0.76 ± 0.04 b	0.85 ± 0.02 b	0.94 ± 0.01 b
	N3 (Conventional)	0.97 ± 0.02 c	0.84 ± 0.02 c	0.94 ± 0.02 c	1.02 ± 0.02 c
	N4 (Excessive N)	1.05 ± 0.02 d	0.94 ± 0.01 d	1.07 ± 0.03 d	1.07 ± 0.02 d
	N5 (Very excessive N)	1.04 ± 0.01 d	0.97± 0.02 d	1.08 ± 0.02 d	1.04 ± 0.01 cd
	Significance	***	***	***	***
2017	N1 (Very N deficient)	0.53 ± 0.01 a	0.60 ± 0.03 a	0.51 ± 0.01 a	0.50 ± 0.03 a
	N2 (N deficient)	0.76 ± 0.01 b	0.76 ± 0.03 b	0.74 ± 0.01 b	0.78 ± 0.01 b
	N3 (Conventional)	0.97 ± 0.01 c	0.89 ± 0.02 c	1.02 ± 0.01 c	0.97 ± 0.02 c
	N4 (Excessive N)	1.05 ± 0.01 d	1.00 ± 0.02 d	1.07 ± 0.01 d	1.05 ± 0.01 d
	N5 (Very excessive N)	1.06 ± 0.01 d	1.00 ± 0.01 d	1.09 ± 0.01 d	1.05 ± 0.02 d
	Significance	***	***	***	***

3.3. Relationship between NNI and petiole sap [NO_3^-–N] for entire crop

Nitrogen nutrition index (NNI) was strongly related to petiole sap [NO_3^-–N] in the three pepper crops. Using pooled data from the duration of the three crops, the relationship between NNI and sap [NO_3^-–N] was best described by (a) a polynomial regression of $\mathrm{NNI} = -1.10\mathrm{E}-07 \times Sap^2 + 0.000473 \times \ Sap + 0.5514$ with an R^2 of 0.84 (Fig. 3; Table 4a), and (b) a segmented linear regression of $\mathrm{NNI} = 0.00034 \times Sap + 0.572\ (if\ Sap < Sap_0)$ and $\mathrm{NNI} = 1.04\ (if\ Sap \geq Sap_0)$ with an R^2 of 0.83 (Fig. 3; Table 4b). In the segmented linear regression, the horizontal linear regression corresponds to the "maximum NNI value".

The sufficiency value for maximum DMP, derived from the polynomial equation that corresponded to an NNI value of 1.0, was 1441 mg NO_3^-–N L^{-1} (Fig. 3; Table 4a). The sufficiency value for maximum DMP, determined as the intersection of the inclined linear and horizontal segments (Sap_0 in Fig. 3), was 1367 mg NO_3^-–N L^{-1} (Fig. 3; Table 4b). The sufficiency values for maximum DMP calculated using the two approaches were very similar, the difference being only 74 mg NO_3^-–N L^{-1}.

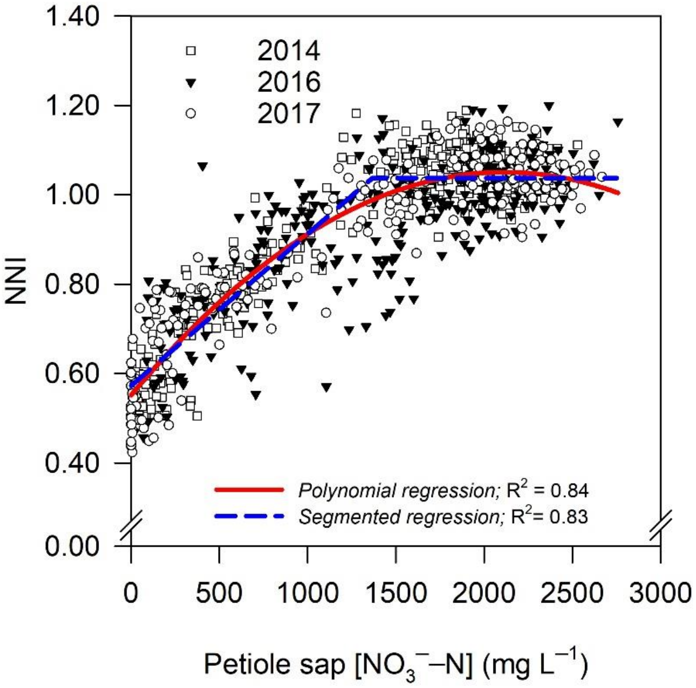

Fig. 3. Relationship between N nutrition index (NNI) and petiole sap [NO_3^-–N] for pooled data from the three different pepper crops. The red line is the polynomial regression of $\mathrm{NNI} = -1.10\mathrm{E}-07 \times Sap^2 + 0.000473 \times Sap + 0.5514$ (R^2 = 0.84) and the blue dotted lines are the linear segmented regression described by the sloped line of $\mathrm{NNI} = 0.00034 \times Sap + 0.572\ (if\ Sap < Sap_0)$ and the horizontal line described by $\mathrm{NNI} = 1.04\ (if\ Sap \geq Sap_0)$ (R^2 = 0.83). The values are from five different N treatments with four replications from the 2014, 2016 and 2017 pepper crops (*n* = 878); *n* is the number of data points.

For the duration of each of the three individual pepper crops, individual polynomial equations described the relationship between NNI and petiole sap [NO_3^-–N] with R^2 values of 0.91, 0.66 and 0.89 for the 2014, 2016 and 2017 crops, respectively (Table 4a). Using these three polynomial equations, sufficiency values for maximum DMP, estimated by solving for NNI = 1.0, were 1256, 1706 and 1415 mg NO_3^-–N L^{-1} for the 2014, 2016 and 2017 crops, respectively (Table 4a).

The segmented linear regression relationships derived between NNI and petiole sap [NO_3^-–N] had R^2 values of 0.91, 0.64 and 0.88 for the 2014, 2016 and 2017 crops, respectively (Table 4b). The sufficiency values for maximum DMP determined from the segmented linear regression analysis (Sap_0), were 1278, 1875 and 1380 mg NO_3^-–N L^{-1} for 2014, 2016 and 2017, respectively. The maximum NNI values, corresponding to the horizontal line segment, were 1.05 for the 2014 crop, and 1.04 for the 2016 and 2017 crops (Table 4b).

The polynomial equations and the equations of the segmented linear regression analysis were similar for the duration of each of the three pepper crops, and for the pooled data set from the three pepper crops (Tables 4a and 4b). The DMP sufficiency values calculated, for each of the three pepper crop, using the polynomial equation and the segmented linear regression analysis were similar (Tables 4a and 4b). The differences between the sufficiency values determined using the two statistical approaches were 22, 169 and 35 mg NO_3^-–N L^{-1} for the 2014, 2016 and 2017 crops, respectively.

There were very strong relationships between NNI and petiole sap [NO_3^-–N], using either polynomial equations or segmented linear regression relationships (R^2 of 0.88–0.91; Tables 4a and 4b), in the 2014 and 2017 crops. For both types of relationship, the relationships were noticeably weaker in the 2016 crop (R^2 of 0.64–0.66; Tables 4a and 4b).

Table 4 (a). Polynomial regression analysis relating NNI to petiole sap [NO_3^-–N] for 2014 (*n* = 359), 2016 (*n* = 299) and 2017 (*n* = 220) pepper crops, and using pooled data for the duration of the three crops (*n* = 878); *n* is the number of data points. The fitted equations and coefficients of determination (R^2) are shown. SE is standard error of the estimation. The values of petiole sap [NO_3^-–N] (mg L^{-1}) that correspond to NNI values of 1.0 are presented.

Crop year	Polynomial equation	Petiole sap [NO_3^-–N] (mg L^{-1}) for NNI = 1.0	R^2	SE
2014	NNI = $-1.50E-07 \times Sap^2 + 0.000567 \times Sap + 0.5248$	1256	0.91	0.06
2016	NNI = $-6.60E-08 \times Sap^2 + 0.000340 \times Sap + 0.6109$	1706	0.66	0.09
2017	NNI = $-1.10E-07 \times Sap^2 + 0.000475 \times Sap + 0.5469$	1415	0.89	0.07
Combined crops	NNI = $-1.10E-07 \times Sap^2 + 0.000473 \times Sap + 0.5514$	1441	0.84	0.08

Table 4 (b). Segmented linear regression analysis relating NNI to petiole sap [NO_3^-–N] for 2014 (*n* = 359), 2016 (*n* = 299) and 2017 (*n* = 220) pepper crops, and using pooled data for the duration of the three crops (*n* = 878); *n* is the number of data points. The fitted equations and coefficients of determination (R^2) are shown. SE is standard error of the estimation. The two linear equations of the segmented analysis are presented as (i) the sloped equation and (ii) the horizontal equation, which is presented as the maximum NNI value. The values of petiole sap [NO_3^-–N] (mg L^{-1}) that correspond to Sap_0, which is the petiole sap [NO_3^-–N] value associated with the maximum NNI value, are presented.

Crop year	Inclined linear equation of segmented linear regression	Maximum NNI value	Sap_0 value (mg NO_3^-–N L^{-1})	R^2	SE
2014	NNI = $0.00039 \times Sap + 0.551$	1.05	1278	0.91	0.06
2016	NNI = $0.00020 \times Sap + 0.660$	1.04	1875	0.64	0.09
2017	NNI = $0.00035 \times Sap + 0.561$	1.04	1380	0.88	0.07
Combined crops	NNI = $0.00034 \times Sap + 0.572$	1.04	1367	0.83	0.08

3.4. Relationships between NNI and petiole sap [NO_3^-–N], and derivation of sufficiency values, for each phenological stage

Polynomial equations and segmented linear regression equations were derived for each of the three phenological stages of vegetative, flowering and early fruit growth, and harvest, using pooled data, for each phenological stage, of the 2014, 2016 and 2017 crops (Tables 5a and 5b). For the polynomial equations for the vegetative, flowering and early fruit growth, and harvest stages, using pooled data from the three crops, the R^2 values were 0.66, 0.89, 0.86, respectively

(Table 5a). For the segmented regression analyses, the respective R^2 values were 0.67, 0.88 and 0.86 (Table 5b).

Sufficiency values for maximum DMP were derived for the three phenological stages using the pooled data set from the three crops. Sufficiency values corresponding to NNI values of one, derived from the polynomial equations, were 1747, 1327 and 1403 mg NO_3^-–N L^{-1} for the vegetative, flowering and early fruit growth and harvest stages, respectively (Table 5a). Using the segmented linear regression analysis, the sufficiency values (i.e. Sap_0 values) were 1817, 1335 and 1230 mg NO_3^-–N L^{-1} for the vegetative, flowering and early fruit growth, and harvest stages, respectively (Table 5b). The maximum NNI values were 1.03–1.05 (Table 5b).

The estimated sufficiency values for maximum DMP for each phenological stage, calculated with the two different approaches were very similar (Tables 5a and 5b). The differences between equivalent sufficiency values calculated with the two methods were 70, 8 and 173 mg NO_3^-–N L^{-1} for the vegetative, flowering and early fruit growth and harvest stages, respectively. Using both approaches, the sufficiency values for the vegetative stage were appreciably higher than the other two stages, which were very similar to one another (Tables 5a and 5b).

Table 5 (a). Polynomial regression analysis relating NNI to petiole sap [NO_3^-–N] for vegetative (*n* = 139), flowering and early fruit growth (*n* = 320) and harvest (*n* = 419) stages using combined data from the 2014, 2016, and 2017 pepper crops; *n* is the number of data points. The fitted equations and coefficients of determination (R^2) are shown. SE is the standard error of the estimation. The values of petiole sap [NO_3^-–N] (mg L^{-1}) that correspond to NNI values of 1.0 are presented.

Phenological stage	Polynomial equation	Petiole sap [NO_3^-–N] (mg L^{-1}) for NNI = 1.0	R^2	SE
Vegetative	NNI = −5.90E−08 × Sap^2 + 0.000333 × Sap + 0.5992	1747	0.66	0.09
Flowering and early fruit growth	NNI = −1.20E−07 × Sap^2 + 0.000504 × Sap + 0.5435	1327	0.89	0.07
Harvest	NNI = −1.24E−07 × Sap^2 + 0.000498 × Sap + 0.5452	1403	0.86	0.07

Table 5 (b). Segmented linear regression analysis relating NNI to petiole sap [NO_3^-–N] for vegetative (n = 139), flowering and early fruit growth (n = 320) and harvest (n = 419) stages using combined data from the 2014, 2016, and 2017 pepper crops; *n* is the number of data points. The fitted equations and coefficients of determination (R^2) are shown. SE is the standard error of the estimation. The two linear equations of the segmented analysis are presented as (i) the sloped equation, and (ii) the horizontal equation, which is presented as the maximum NNI value. The values of petiole sap [NO_3^-–N] (mg L^{-1}) that correspond to maximum NNI (i.e. Sap_0 value) are presented.

Phenological stage	Inclined linear equation of segmented linear regression	Maximum NNI value	Sap_0 value (mg NO_3^-–N L^{-1})	R^2	SE
Vegetative	NNI = 0.00022 × Sap + 0.630	1.03	1817	0.67	0.09
Flowering and early fruit growth	NNI = 0.00037 × Sap + 0.560	1.05	1335	0.88	0.07
Harvest	NNI = 0.00038 × Sap + 0.559	1.03	1230	0.86	0.07

4. Discussion

4.1. Petiole sap [NO_3^-–N] during the crop

In general, in each of the three sweet pepper crops, petiole sap [NO_3^-–N] for a given treatment, remained relatively constant throughout the crop. In some cases, during the first 40–60 DAT there were fluctuations attributable to the establishment of the N treatments. Relative constancy of petiole sap [NO_3^-–N] throughout the duration of a crop has been observed with greenhouse-grown pepper (Magán et al., 2019), tomato (Peña-Fleitas et al., 2015), and muskmelon (Peña-Fleitas et al., 2015) crops, and in open field tomato (Farneselli et al., 2010), that all received frequent N addition (at least every week) through combined fertigation and drip irrigation.

Numerous studies have reported on-going reductions in petiole sap [NO_3^-–N] during vegetable crops e.g. in cabbage, onion, carrot (Westerveld et al., 2004) and during potato crops (Vitosh and Silva, 1996; Zhang et al., 1996). The recommended petiole sap [NO_3^-–N] values for numerous vegetable species declined with on-going phenological development (Hochmuth, 1994, 2012).

Generally, in studies where petiole sap [NO_3^-–N] declined during the crop, there were a small number (e.g. 2–3) of N fertilizer applications; commonly, a pre-plant application and 1–2 sidedress applications (Vitosh and Silva, 1996; Westerveld et al., 2004). In these studies, an appreciable portion of the fertilizer N was applied prior to planting. In the current study, for the conventional N treatments (N3) that supplied sufficient N, there were 72, 108, and 94 separate N fertilizer applications throughout the 2014, 2016 and 2017 crops, respectively. The respective average amounts and ranges of N applied in each application were 7.1 (1.9–21.1), 5.2 (1.2–11.9), and 5.6 (0.6–18.1) kg N ha^{-1}. In the 2014, 2016 and 2017 crops, 83, 99 and 94%, respectively, of the individual N applications each supplied <10 kg N ha^{-1}. The larger N applications were generally because of larger nutrient solution applications to control soil salinity as described in section 2.2.

It appears that very frequent small applications of N (mostly <10 kg N ha^{-1}, every 1–4 days) through combined fertigation and drip irrigation systems contribute to relatively constant petiole sap [NO_3^-–N] throughout the crop. The application of N throughout each crop was very strongly related to dry matter production. The relationship between cumulative N addition (kg N ha^{-1}) and cumulative dry matter production (t ha^{-1}), throughout the three crops, considered together, was described by the linear regresion equation $y = 39.6x + 37.2$ with $R^2 = 0.93$.

Hochmuth (1994) reported that petiole sap [NO_3^-–N] of greenhouse crops tended to decrease less when compared to crops grown in open field conditions. The indeterminate growth of many greenhouse-grown vegetable crops, with the constant growth of new shoot material may be a contributing factor to the relatively constant petiole sap [NO_3^-–N] values observed with greenhouse-grown vegetable crops.

4.2. Relationship of petiole sap [NO_3^-–N] to crop NNI

The strong asymptotic relationships between petiole sap [NO_3^-–N] and NNI, for pooled data from throughout the three sweet pepper crops, and throughout each of the three crops considered separately, were described by either polynomial or segmented linear regression equations. A very small number of previous studies have established linear relationships between petiole sap [NO_3^-–N] and NNI (Bélanger et al., 2003; Peña-Fleitas et al., 2015). Peña-Fleitas et al., (2015) obtained R^2 values of 0.77 for combined data of three tomato crops, and also for a muskmelon crop. Bélanger et al., (2003) obtained R^2 values of 0.29–0.62 for three different periods of two potato cultivars. Other studies have reported that petiole sap [NO_3^-–N] was strongly related to apparent crop N status in various vegetable species such as broccoli (Villeneuve et al., 2002), potato (Poljak et al., 2008), cauliflower (Kubota et al., 1996), and processing tomato (Farneselli et al., 2010).

The steep linear relationships between petiole sap [NO_3^-–N] and NNI at NNI values of <1 indicated that petiole sap [NO_3^-–N] is a sensitive measure of crop N deficiency in sweet pepper. The plateau response at NNI values close to 1.0 demonstrated that sap [NO_3^-–N] continued to accumulate, with increasing N supply, even though there was very limited luxury uptake of N (Rodríguez et al., 2020a). This suggests that maximum values of a sufficiency range could be identfied, above which petiole sap [NO_3^-–N] could be used to identify excessive N supply. It appears that the relationships between NNI and petiole sap [NO_3^-–N] are asymptotic in species with little luxury N uptake, and that relationships may be linear where luxury N uptake occurs (e.g. Peña-Fleitas et al., 2015). There appears to be variation between vegetable species in the ocurrence and degree of luxury N uptake (Rahn et al., 2010; Thompson et al., 2017b).

For both the polynomial and segmented linear analyses, the equation coefficients and R^2 values were very similar both for the flowering and early fruit growth stage, and for the harvest stage. The R^2 values for both types of analysis in these two stages were very high, being 0.88–0.91. In contrast, for the earlier vegetative stage, the equation coefficients were notably different, and the R^2 values were clearly lower. These differences, between the earlier vegetative stage and the two later phenological stages, are likely due to the relatively large fluctuations in petiole sap [NO_3^-–N] during the earlier vegetative stage, when the N treatments were being established. The equation coefficients for the flowering and early fruit growth stage and for the harvest stage, for the three crops considered together, were very similar to the equivalent values for the duration of the 2014 and 2017 crops, for each type of equation. The similarity of the equation coefficients and the R^2 values for these two phenological phases, and for the 2014 and 2017 crops, demonstrates the strength and consistency of these relationships throughout the crops and between different crops.

4.3. Petiole sap [NO_3^-–N] sufficiency values

Using the polynomial relationship for the duration of the crop cycle of the three crops considered together, a sufficiency value of 1441 mg NO_3^-–N L^{-1} was derived for a NNI = 1.0, that is the minimum petiole sap [NO_3^-–N] for maximum dry matter production. Using the segmented linear regression relationship, the sufficiency value was 1367 mg NO_3^-–N L^{-1}, which corresponded to the maximum NNI value of 1.04 for the duration of the crop cycle. Using both types of equations, the sufficiency values derived for the duration of the individual 2014 and 2017 crops were very similar. The complete crop cycle sufficiency values derived for the individual 2016 crop, using both approaches were notably higher being 1706 and 1875 mg NO_3^-–N L^{-1}. The appreciably lower R^2 values for the 2016 crop, and the clear differences in equation coefficients, compared to both the 2014 and 2017 crops, suggest that the equations and whole crop sufficiency values of the 2016 crop were anomalous. Considering these observations, a rounded-off sufficiency value, for growth (i.e. maximum dry matter production), of 1400 mg NO_3^-–N L^{-1} is proposed for greenhouse-grown sweet pepper in SE Spain. This sufficiency value may be applicable for the greenhouse-grown sweet pepper throughout the Mediterranean Basin; however, validation is recommended for use outside of SE Spain.

This proposed whole crop sufficiency value is consistent with the sufficiency values reported by Hochmuth (1994, 2012) for outdoor pepper of 1400–1600 mg NO_3^-–N L^{-1} for the first two stages of first flower buds and first open flowers. However, as previously stated, the sufficiency values proposed by Hochmuth (1994, 2012) declined as the crop developed.

Using polynomial and segmented linear regression analysis, the sufficiency values derived for the combined flowering and early fruit growth stage were 1327 mg and 1335 mg NO_3^-–N L^{-1}, and for the harvest stage were 1403 and 1230 mg NO_3^-–N L^{-1}. These values are very similar to proposed whole crop sufficiency value of 1400 mg NO_3^-–N L^{-1}, confirming the constancy of the the sufficiency value throughout the crop. As previously explained, the vegetative stage was anomalous because of the establishment of the N treatments. The proposed sufficiency value derived in the current study is for dry matter production, i.e. growth. Further work is required to determine whether this value also applies to yield.

4.4. General observations

Possible effects of different cropping systems and of cultivar will need to be considered when using petiole sap [NO_3^-–N] as a management tool. Peña-Fleitas et al. (2015) described the relationship of petiole sap [NO_3^-–N] to NNI, and the derived sufficiency value, being very similar for two different types of tomato grown in different conditions. Future work is required to examine how factors such as crop type and cultivar, production system and location affect the relationships and suggested sufficiency value reported here for sweet pepper.

5. Conclusions

The strong relationship between petiole sap [NO_3^-–N] and NNI suggests that petiole sap [NO_3^-–N] can be used to improve N management of greenhouse-grown sweet pepper. In each N treatment of the three pepper crops, which received frequent N application, petiole sap [NO_3^-–N] was relatively constant throughout the crops. Using the pooled data from the three cropping cycles, a sufficiency value for maximum crop growth of 1400 mg NO_3^-–N L^{-1} was derived for an entire crop. For two of the three different phenological stages (for the flowering and early fruit growth stage, and the harvest stage) the derived sufficiency values were similar to the derived sufficiency value for the duration of the crop. This sufficiency value can be a guide to vegetable growers and advisors to achieve optimal N fertilization. However, in each region and cropping system, verification is recommended. Petiole sap [NO_3^-–N] analysis is a practical and effective tool to assess crop N status of sweet pepper grown in greenhouses.

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