Title: Effect of cultivar on measurements of nitrate concentration in petiole sap and leaf N content in greenhouse soil-grown cucumber, melon, and sweet pepper crops

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Abstract

Excessive N fertilizer applications in intensive vegetable production in soil is commonly associated with appreciable N losses causing negative environmental impact. Measuring petiole sap [NO_3^-–N] and leaf N content (%) are simple and practical monitoring methods to assess crop N status for improving N fertilizer management. The effect of cultivar on petiole sap [NO_3^-–N] and leaf N content was evaluated. One cucumber, two melon, and two sweet pepper crops were grown in different cropping periods, with three cultivars in each crop. Three N treatments, deficient (N1), sufficient (N2) and excessive (N3) N supply, were applied by combined fertigation with drip irrigation. For a given N supply, there were often significant differences between cultivars in petiole sap [NO_3^-–N] and leaf N content in cucumber, the two melon crops and one pepper crop. This was, particularly so with the sufficient (N2) and excessive (N3) N supply. In the cucumber and two melon crops, there were consistent differences in petiole sap [NO_3^-–N] between cultivars in two or three of the different N treatments. In some crops, very little petiole sap [NO_3^-–N] was measured with deficient (N1) N supply. In the two pepper crops, the differences between cultivars were less clear than with cucumber and melon. In general, for the three species examined petiole sap [NO_3^-–N] was subject to more consistent and larger effects between cultivars, than was leaf N. Average differences between cultivars in petiole sap [NO_3^-–N] of 200–450 mg NO_3^-–N L^{-1} were observed during periods of 4–6 weeks in cucumber and melon. The differences between different cultivars of the same species in petiole sap [NO_3^-–N] and leaf N content when receiving the same N supply has implications for the practical applications of these methods for monitoring crop N status.

Keywords: *Capsicum annuum* L.; *Cucumis melo* L.; *Cucumis sativus* L.; *analysis; N fertilizer management; sufficiency values; vegetable crops*

1. Introduction

In intensive vegetable production, applications of nitrogen (N) fertilizer generally appreciably exceed crop requirements (Ju et al., 2009; Min et al., 2011; Soto et al., 2015; Thompson et al., 2007). Excessive N application and the resultant N losses associated with various serious environmental problems (Di and Cameron, 2002; Grizzetti et al., 2011). Nitrate (NO_3^-) leaching is a major contributor to two of the major environmental issues associated with intensive horticultural systems, being NO_3^- contamination of underlying aquifers and eutrophication of surface water bodies (Thompson et al., 2020).

Approximately 200,000 ha of plastic greenhouses are used for intensive horticultural production in the Mediterranean Basin, where 90% of the crops are grown in soil (Incrocci et al., 2020; Pardossi et al., 2004). 42,000 ha of these greenhouse are concentrated in south-eastern (SE) Spain (Valera et al., 2016), with 32,000 ha located in the province of Almeria. Due to extensive and substantial aquifer NO_3^- contamination, most of the areas in Almeria where greenhouses are concentrated have been declared "Nitrate Vulnerable Zones" (NVZ) (BOJA., 2020a) in accordance with the Nitrates Directive of the European Union (Anonymous., 1991). Having been declared NVZs, these areas are required to adopt improved crop N management practices (Anonymous., 1991; BOJA., 2020b).

A very effective general approach to N management of soil-grown vegetable crops in greenhouses is prescriptive-corrective management (Thompson et al., 2017a; Thompson et al., 2017b). Prescriptive management being the preparation of a crop- and site-specific plan that meets the expected N requirements of an individual crop (Gallardo et al., 2020; Granados et al., 2007; Thompson et al., 2017a). Corrective N management is the regular use of monitoring approaches that enable adjustments to the N supply to ensure constant optimal crop N status (Granados et al., 2007; Padilla et al., 2020; Thompson et al., 2017a). Prescriptive-corrective management considerably reduces NO_3^- leaching loss and N use (Granados et al., 2007; Magán et al., 2019), and can result in very high recoveries of applied N (Martínez-Gaitán et al., 2020), of greenhouse-grown vegetable crops.

Two relatively simple methods to directly and regularly monitor the N status of vegetable crops, for corrective N management, are (1) analysis of NO_3^- concentration ($[NO_3^-]$) in petiole sap, and (2) the determination of leaf N content (as %N) (Thompson et al., 2017b; Padilla et al., 2020). With these two methods, compared to optical sensors, there is no need for a large initial investment and data interpretation is straightforward, unlike with more technological approaches such as optical sensors (Thompson et al., 2017b). Petiole sap $[NO_3^-]$ can be rapidly and accurately measured on the farm with small, relatively cheap analytical systems (Parks et al., 2012; Peña-Fleitas et al., 2021). Leaf N analysis requires that samples be sent to an analytical laboratory.

Measurement of leaf N content (%N) is a traditional and established method for monitoring crop N status (Geraldson and Tyler, 1990; Hartz and Hochmuth, 1996). Being an established method, it has the advantage that reference values, for data interpretation, are commonly available (e.g. Casas and Casas, 1999; Geraldson and Tyler, 1990; Hartz and Hochmuth, 1996). While useful for assessing overall crop N status (Geraldson and Tyler, 1990; Hartz and Hochmuth, 1996; Thompson et al., 2017b); when compared to petiole sap $[NO_3^-]$, it is a less sensitive indicator of crop N status (Peña-Fleitas et al., 2015) and less sensitive to changes in crop N management (Majić et al., 2008; Olsen and Lyons, 1994).

Petiole sap $[NO_3^-]$ analysis has been demonstrated to be a very sensitive indicator of crop N status in numerous fruit vegetable crops (Farneselli et al., 2014; Goffart et al., 2008; Hochmuth, 2012; Rodríguez et al., 2021), root vegetable crops (Westerveld et al., 2007), leafy vegetables lettuce (Matthäus and Gysi, 2001; Parks et al., 2012), brassica vegetable crops (Altamimi et al., 2013; Kubota et al., 1996; Westerveld et al., 2004) and potato (Majić et al., 2008; Zhang et al., 1996). In tomato and pepper, petiole sap $[NO_3^-]$ has been strongly related to

Nitrogen Nutrition Index (NNI) (Peña-Fleitas et al., 2015; Rodríguez et al., 2021), which is an established indicator of crop N status (Lemaire et al., 2008). NNI relates actual crop N content to the critical crop N content (Greenwood et al., 1990; Lemaire et al., 2008).

Sufficiency values for petiole sap [NO_3^-] are available in scientific and technical literature for different crop species (Hochmuth, 2012, 1994). The available sufficiency are species-specific. Hochmuth (1994, 2012) distinguished between sufficiency values for open field and greenhouse vegetable crops (Hochmuth, 2012; Thompson et al., 2017b). However, very few studies have evaluated cultivar effects on petiole sap [NO_3^-] for different crop species. Studies on carrot (Westerveld et al., 2007) and potato (Bélanger et al., 2003; Goffart et al., 2008; Waterer, 1997) suggested that petiole sap [NO_3^-] may be affected by cultivar The available sufficiency values for leaf N content are species specific, we are unaware of any studies that have reported cultivar effects on leaf N content (Geraldson and Tyler, 1990; Hartz and Hochmuth, 1996).

In vegetable production regions, large numbers of cultivars available for a given species and new cultivars are constantly being introduced. Considering the increasing pressure to improve crop N management, it is essential that possible cultivar effects on crop monitoring for improved N management be assessed. It is essential to know, if such effects occur and if they do, if they are sufficiently large to require different sufficiency values for certain cultivars, or if they are relatively unimportant.

In Almeria province, approximately 12,000, 5,000, and 2,000 ha of sweet pepper (*Capsicum annuum* L), cucumber (*Cucumis sativus* L.) and melon (*Cucumis melo* L.) are grown annually in greenhouses (Junta de Andalucía, 2020). For each of these species, in this system, there are numerous cultivars. We are unaware of any studies that have assessed whether cultivar effects affect petiole sap [NO_3^-] and leaf N of these species.

The objectives of the present work were for different cultivars of cucumber, melon and pepper grown, in soil in greenhouses in Almeria, to: (i) evaluate the effect of cultivar on petiole sap [NO_3^-], and (ii) evaluate the effect of cultivar on leaf N content.

2. Materials and methods

2.1. Experimental site

A cucumber (*Cucumis sativus* L.) crop, two melon (*Cucumis melo* L.) crops and two sweet pepper (*Capsicum annuum* L.) crops were grown in two greenhouses, similar to those used for local commercial production (Valera et al., 2016), at the Experimental Station of the University of Almeria located in Retamar in southeastern (SE) Spain (36°51' N, 2°16' W and 92 m elevation). The cucumber, two melon crops and one sweet pepper crop were grown in greenhouse 1, and one sweet pepper crop in greenhouse 2.

Greenhouse 1 had a multi-tunnel structure of galvanized steel with polycarbonate walls (Padilla et al. (2014), and greenhouse 2 was a ‘raspa y amagado’ type, characterized by several modules each with a symmetrical ridged roof with low angles (Valera et al., 2016). Both greenhouses had low-density polyethylene roofs of tri-laminated film (200 µm thickness) with transmittance to photosynthetically active radiation (PAR) of approximately 60%. The greenhouses had passive ventilation (lateral side panels and flap roof windows) and east-west orientation.

The crops were all grown in soil, under conditions very similar to those of commercial vegetable production used in the area. The soil in greenhouse 1 was an artificial “enarenado” soil that is typical of the region (Gázquez et al., 2017; Thompson et al., 2007). It consisted of a 30 cm layer of imported silty loam textured soil placed over the original loam soil and a 10 cm layer of fine gravel (mostly 2–5 mm diameter) placed on the imported soil as a mulch. Relevant characteristics of the imported soil (0–10 cm) of greenhouse 1 were: pH of 8.2 (1:2.5, soil:water), bulk density of 1.5 Mg m^{-3}, 0.2% total N and 2.8% organic C (Padilla et al., 2014; Soto et al., 2014). The soil of greenhouse 2 was different to that of greenhouse 1 in that sand had been mixed into the profile at greenhouse construction in 2007, and there was no sand mulch. The texture of 0–20 cm was sandy loam. Relevant characteristics of the 0–20 cm soil layer were: pH of 8.0 (1:2.5, soil:water), bulk density of 1.5 Mg m^{-3}, 0.1% total N and 0.6% organic C. In greenhouse 2, a layer of black plastic was used as mulch.

Combined drip irrigation and fertigation was used in all crops. In each irrigation, complete nutrient solutions were applied via fertigation every 1–4 days, according to crop demand. The nutrient solutions were prepared using mineral fertilizers. Irrigation was more frequent during warm periods and less frequent during cooler periods.

In both greenhouses, drip tape was distributed in paired lines with 0.8 m spacing between lines within each pair, 1.2 m spacing between adjacent pairs of lines, and 0.5 m spacing between drip emitters within drip lines, giving a density of 2 emitters m^{-2}. Each emitter had a discharge of 3 L h^{-1}. Individual plants were immediately adjacent (approximately 8 cm) to each emitter, giving a plant spacing of 2 plants m^{-2}. The uniformity coefficient measured at the start of each crop was >95%.

In greenhouse 1, there were 12 experimental plots of 12 m (width) x 6 m (length) each, with crop lines aligned north-south. Each plot had six paired lines of plants, with 24 plants per paired line and 144 plants in total, allocating 48 plants per cultivar. Between the plots, sheets of polyethylene film (250 µm thick) were buried to 30 cm deep as a hydraulic barrier (Padilla et al., 2016). The total cropped area was 1327 m^2 including the border area. In greenhouse 2, there were 12 experimental plots of 6 m (width) x 7 m (length) each, with crop lines aligned north-south. Each plot had six paired lines of plants, with 28 plants per paired line and 84 plants in total, allocating 48 plants per cultivar. The total cropped area was approximately 1700 m^2 including the border area.

2.2. Experimental crops and treatments

Five different experimental crop were grown each at a different time. Each experimental crop consisted of one crop, with three cultivars and three N treatments. There were one cucumber crop, two melon crops and two sweet pepper crops. The cucumber crop was grown from 24 April to 3 July 2018, the first melon crop from 27 February to 11 June 2020, the first pepper crop from 22 July 2020 to 28 January 2021, the second melon crop from 26 February to 08 June 2021, and the second pepper crop from 22 July 2021 to 9 January 2022 (Table 1).

For each crop, in each greenhouse, the experimental area was divided into three irrigated sectors, each with four plots per N treatment, arranged in a randomized block design. Before transplanting each crop in greenhouse 1, a series of abundant irrigation volumes were applied to leach residual mineral salts and to homogenize residual NO_3^- and electrical conductivity in the soil profile between plots. As a result, there was always <80 kg mineral N ha^{-1} in the first 40 cm (excluding sand mulch) at transplanting of all crops in this greenhouse. However, in greenhouse 2, used for pepper 21, there was an unexpected delay in preparing the fertigation system, and there was insufficient time to sufficient abundant irrigation, prior to transplanting the purchased seedlings, to substantially reduce the residual mineral N. The amount of residual N in first 40 cm, at transplanting, was 290–346 kg mineral N ha^{-1}.

The cucumber, two melon and two pepper crops were transplanted 21–37 days after seeding. The dates of transplanting and duration of the crops are given in Table 1. During the first days after transplanting (DAT), seedlings of the three crops were irrigated with water (<0.04 mmol N L^{-1}) until the different N treatments commenced at 9, 0 and 6 DAT in the cucumber, melon and pepper crops, respectively.

The three cucumber cultivars were Dutch type: 'Strategos' (Syngenta International AG, Basel, Switzerland), 'Pradera' (Rijk Zwaan Zaadteelt en Zaadhandel B.V., De Lier, The Netherlands) and 'Mitre' (Semillas Fitó, Barcelona, Spain). For melon 20, the cultivars were cantaloupe type: 'Tezac' (Seminis, Inc., Bayer AG, Leverkusen, Germany), 'Magiar' (Nunhems, BASF SE, Ludwigshafen, Germany), 'Jacobo' (Semillas Fitó) and 'Bosito' (Seminis, Inc.). For melon 21, the variety ‘Tezac’ was replaced by the closely-related derivative variety ‘Bosito’ (Seminis, Inc.); both 'Magiar' and 'Jacobo' were also used in this crop. In the two pepper crops, two pepper cultivars were California type: 'Melchor' (Zeraim Iberica, Syngenta Crop Protection AG, Basel, Switzerland) and 'Machado' (Hazera Seeds Ltd., Limagrain Group, Saint Beauzire, France) and one pepper cultivar was Lamuyo type: 'CLX PLRJ731' (De Ruiter). In this work, Lamuyo 'CLX PLRJ731' (HM. Clause SAS, La Motte, Portes-lès-Valence, France) will be referred to as another cultivar.

For each cultivar of the different crops, three treatments of different N concentrations were applied via fertigation throughout the crop cycle. The N treatments were very deficient N (N1), moderately deficient N (N2) and excessive N (N3) (Table 1). The N2 treatments were borderline sufficient/deficient N. The total amounts of irrigation and N applied to each treatment are presented in table 1. Most of the mineral N was applied as NO_3^- (92% of applied N), the rest as ammonium (NH_4^+). The other nutrients were applied in the nutrient solution in sufficient concentrations to ensure that they did not limit crop growth.

Table 1. General information of the cucumber, two melon and two pepper crops, and N treatments. Information included are dates of transplanting and end of crops (and duration), N concentrations applied in the nutrient solution, total amount of mineral N applied, and total irrigation applied. The N treatments were N1: very deficient; N2: sufficient; N3: excessive; the actual N concentrations are provided in Table 2. The total amounts of N applied were for the period in which the N treatments were applied. The total irrigation volume is for the duration of the crop.

Crop	Date of transplanting (DD/MM/YYYY)	Date end of the crop (DD/MM/YYYY) (duration)	N Treatment[a]	[N] in the nutrient solution (mmol L^{-1})	Total N applied (kg N ha^{-1})	Total irrigation volume (mm)
[a]Cucumber 18	24/04/2018	03/07/2018	N1	2.4	38	114
(One cucumber crop)		(70 days)	N2	8.5	302	253
			N3	14.9	514	247
[a]Melon 20	27/02/2020	11/06/2020	N1	2.7	65	173
(1st melon crop)		(105 days)	N2	8.3	309	265
			N3	14.0	542	276
[a]Pepper 20	22/07/2020	28/01/2021	N1	2.2	66	217
(1st pepper crop)		(190 days)	N2	8.4	428	363
			N3	14.2	704	353
[a]Melon 21	26/02/2021	08/06/2021	N1	2.6	60	162
(2nd melon crop)		(102 days)	N2	8.0	243	217
			N3	14.5	540	266
[b]Pepper 21	22/07/2021	04/01/2022	N1	1.9	70	262
(2nd pepper crop)		(166 days)	N2	8.2	337	295
			N3	14.2	615	309

[a] Grown in greenhouse 1, which is described in Materials and Methods.

[b] Grown in greenhouse 2, which is described in Materials and Methods.

Irrigation was applied to maintain the soil matric potential in the root zone at 15 cm depth, between −10 and −30 kPa; one tensiometer (Irrometer, Co., Riverside, CA, USA) was used per plot. High temperature within the greenhouse was controlled by white-washing the plastic cladding of the greenhouse with applications of $CaCO_3$ suspensions, reducing the PAR to 15–50%.

All crops were managed following local practices, as used in commercial greenhouse vegetable production. The cucumber and melon plants were physically supported using a system of nylon cords on vertical guides, and plants were pruned periodically to maintain an open canopy. The apical bud of the main stem of the cucumber plants was removed at 46 DAT, and of both melon crops on 54 DAT. In both pepper crops, a local physical support system known as "enfajado" was used, which consists of the horizontal placement of a series nylon cords at height increments of approximately 10 cm along the side of the crop. In pepper 20, flowers and recently set fruit were removed up to the first branching (cross) of the stem on 17 DAT, and leaves below this point were removed on 104 DAT. There were no prunings in pepper 21.

2.3. Measurements

2.3.1. Petiole sap

Nitrate concentration ([NO_3^-–N]) in petiole sap was determined every week in the cucumber and melon crops, and every two weeks in the pepper crops. Sap measurements commenced at 22 DAT in cucumber, at 20 DAT in melon 20 and pepper 20, at 24 DAT in melon 21 and at 26 DAT in pepper 21, and continued throughout the crops. Cucumber, melon 20, pepper 20, melon 21 and pepper 21 were sampled a total of 7, 9, 10, 9 and 8 times respectively. Petiole sap [NO_3^-–N] was always measured in petioles from the most recently fully expanded leaf. The leaves were removed from eight plants in each replicate plot in the cucumber and melon crops, and from 12 plants in the pepper crops. Leaves were collected between 08:00 and 09:00 h on each sampling date.

Immediately after sampling, petioles and leaf blades were separated. The petioles from each plot were placed in a sealed plastic bag, from which air was pressed, and were then immediately placed in a chilled cooler box. Immediately after all petioles were collected, they were transported a laboratory at the University of Almeria (UAL); the journey time was 20 min. In the laboratory at UAL, the petioles were stored at 5°C. The petioles were then cut into 1 cm long sections that were immediately pressed with a manual garlic press. A sub-sample of the extracted sap was diluted, at a dilution factor of 1:5 for cucumber and 1:10 for melon and pepper. The diluted samples were 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.

2.3.2. Leaf N content (%)

At each measurement date, the leaf blade separated from the petiole as described in the section 2.3.1 was used to determine the N content (%N). They leaf blades were placed in paper bags and dried in an oven at 65°C until constant weight. The dry material was ground sequentially with a knife mill and a ball mill (model MM-200, Retsch GmbH, Haan, Germany). The N content (%N) of each sample was determined using an elemental analyzer system (model Rapid N, Elementar Analysensysteme GmbH, Hanau, Germany).

2.3.3. Dry matter production and total yield

Dry Matter Production (DMP) and total yield were determined for each cultivar in each crop. For each cultivar, all the material removed at each pruning during the crop cycle was collected from eight marked plants within each replicate plot, and the dry matter content of the material was assessed by oven-drying at 65°C until constant weight. From the same eight marked plants, fruit was periodically harvested. In the cucumber crop, harvests took place every 3–4 days, starting at 45 DAT; a total of eight harvests were made. In melon 20, two harvests were made, at 96 and 104 DAT. In melon 21, one harvest was made at 101 DAT. In pepper 20, harvests took place every 8–29 days, starting at 98 DAT; there were a total of six harvests. In pepper 21, harvests took place every 14–30 days, starting at 90 DAT; there were a total of four harvests. The total yield at the end of each crop for each cultivar was calculated as the sum of all fruit harvested from all harvests, including fruits that were not considered commercial due to size or imperfections (Table 2).

At the end of all crops, the DMP was measured by removing two representative plants of the eight marked plants for each cultivar in each replicate plot. The two sampled plants were then separated into biomass component (i.e. leaves, stems and unharvested fruits). The dry matter content of these components was measured by drying representative fresh samples of leaves, stems and fruit to constant weight at 65°C. The DMP of each cultivar at the end of each crop was determined as the sum of the amount of dry matter of the leaves and stem at the final biomass sampling, total fruit production, and of all pruned shoot material (Table 2).

Table 2. Total dry matter production (DMP) and total yield (TY; fresh weight) for each cultivar of the different cucumber, melon, and pepper cultivars.

Cucumber crop			Melon 20 crop			Pepper 20 crop			Melon 21 crop			Pepper 21 crop		
Cultivar	(t ha^{-1})		Cultivar	(t ha^{-1})		Cultivar	(t ha^{-1})		Cultivar	(t ha^{-1})		Cultivar	(t ha^{-1})	
	DMP	TY		DMP	TY		DMP	TY		DMP	TY		DMP	TY
'Strategos'			'Tezac'			'Melchor'			'Bosito'			'Melchor'		
N1	2.4	20	N1	6.6	54	N1	4.6	35	N1	2.8	25	N1	7.4	54
N2	5.6	79	N2	9.5	79	N2	11.9	74	N2	6.5	70	N2	9.0	59
N3	5.3	77	N3	11.3	83	N3	12.3	82	N3	8.6	79	N3	9.4	56
'Pradera'			'Magiar'			'Machado'			'Magiar'			'Machado'		
N1	2.1	19	N1	5.3	39	N1	4.7	39	N1	2.3	18	N1	7.6	57
N2	5.3	79	N2	9.8	67	N2	11.5	83	N2	7.2	72	N2	7.7	62
N3	5.9	82	N3	11.6	77	N3	11.3	81	N3	9.0	74	N3	7.1	57
'Mitre'			'Jacobo'			Lamuyo			'Jacobo'			Lamuyo		
N1	2.1	18	N1	6.6	36	N1	4.5	30	N1	2.5	18	N1	6.5	56
N2	5.7	86	N2	9.6	60	N2	11.5	92	N2	7.0	55	N2	8.1	68
N3	5.7	75	N3	10.6	62	N3	10.4	82	N3	8.0	61	N3	7.7	66

2.4. Statistical data analysis

As sap measurements and leaf N content measurements were taken periodically during the crop, repeated-measure analysis of variance (RM−ANOVA) was used to examine the effects of N treatments, cultivars, and time on the measured variables. The RM−ANOVA was conducted following verification of assumptions of equal variance and normality. The LSD test compared multiple means when treatment effects were significant at $P < 0.05$.

The results of RM−ANOVA are presented as: no significant difference at $P \geq 0.05$ (ns), significant at $P < 0.05$ (*), highly 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 statistical analysis.

3. Results

3.1. Effect of N on petiole sap [NO_3^-–N] in different cucumber, melon, and sweet pepper cultivars

3.1.1. Cucumber cultivars

The RM−ANOVA indicated that during the cucumber crop there were statistically significant differences between the cultivars 'Strategos', 'Pradera' and 'Mitre' in petiole sap [NO_3^-–N] with respect to N treatment and time (RM−ANOVA; T × C × N, $P < 0.05$) (Table 3; Fig. 1).

Table 3. Results of repeated-measure analysis of variance (RM–ANOVA) testing the effect of cultivar, N treatments and time on the measurements of petiole sap [NO_3^-–N] in cucumber crop. Significant effects at *P* <0.05 are show in bold, d. f are degrees of freedom, F is the Fisher value of ANOVA and *P* is the probability value.

Effect	d. f	Petiole sap [NO_3^-–N]	
		F	*P*
Block	3	1.38	0.273
Cultivar (C)	2	24.15	**<0.001**
Nitrogen (N)	2	1068.06	**<0.001**
C × N	4	5.36	**0.003**
Error	24		
Time (T)	6	85.26	**<0.001**
T × C	12	5.62	**<0.001**
T × N	12	26.89	**<0.001**
T × C × N	24	2.70	**<0.001**
Error	144		

The three N treatments clearly affected petiole sap [NO_3^-–N] in the cucumber cultivars 'Strategos', 'Pradera' and 'Mitre' (Fig. 1a,b,c). In the N1 treatment, the petiole sap [NO_3^-–N] was consistently zero or very close to zero (Fig. 1a). For all cultivars, petiole sap [NO_3^-–N] was progressively higher in the N2 and N3 treatments (Fig. 1). It was consistently higher in the N3 compared to N2 treatment (Fig. 1 b,c). Generally, petiole sap [NO_3^-–N] remained relatively constant, with some small fluctuations, until 50 DAT, in the N2 and N3 treatments, after which it increased notably (Fig. 1b,c).

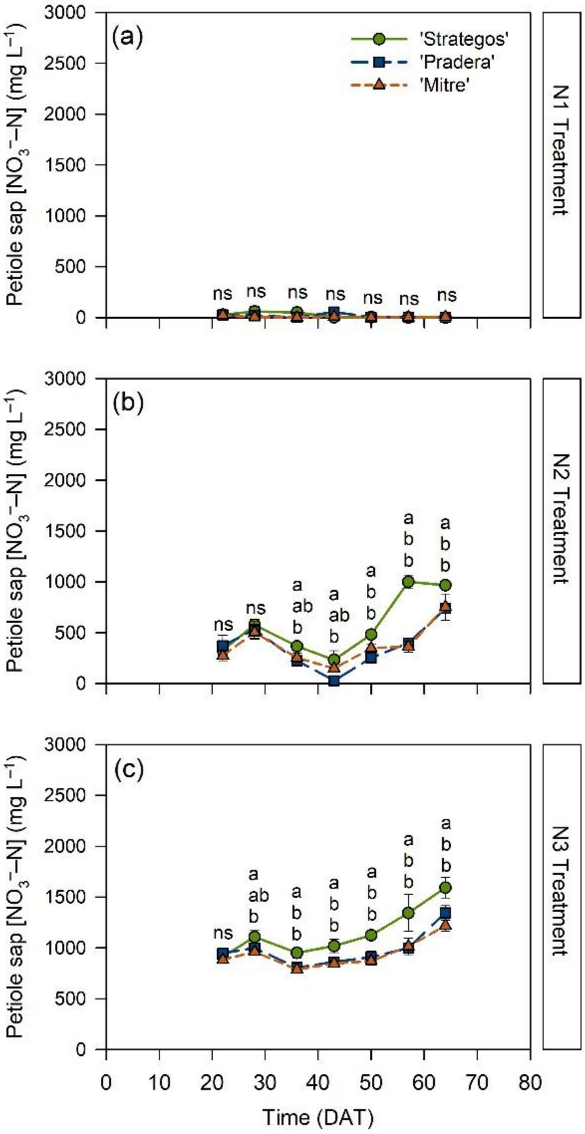

Fig. 1. Evolution of petiole sap [NO_3^-–N] of three cucumber cultivars ('Strategos', 'Pradera' and 'Mitre') under three N treatments (N1, N2 and N3) grown in greenhouse. Values are means (*n*=4) ± standard error (SE); *n* is the number of data.

In the N2 treatment, until approximately 28 DAT, there were no significant differences between cultivars (Fig. 1b). Thereafter, the cultivar 'Strategos' consistently had significantly higher petiole sap [NO_3^-–N] than the cultivar 'Mitre' (Fig. 1b). From 50 DAT on, 'Strategos' had significantly higher values than both 'Mitre' and 'Pradera' (Fig. 1b). Petiole sap [NO_3^-–N] measured in 'Pradera' and 'Mitre' was consistently very similar in the N2 treatment (Fig. 1b).

In the N3 treatment, the cultivar 'Strategos' consistently had significantly higher values of petiole sap [NO_3^-–N] than 'Mitre' from 28 DAT on, and significantly higher values than both 'Pradera' and 'Mitre' from 36 DAT on (Fig. 1c). There no statistically significant differences between the cultivars 'Pradera' and 'Mitre' throughout the crop. Throughout the crop, the average petiole sap values for 'Strategos', 'Pradera' and 'Mitre' (Fig. 1c), in the N3 treatment, were 1150, 981 and 940 mg NO_3^-–N L^{-1}, respectively. In treatment N3, the average value of 'Strategos' was approximately 15% and 18% higher than 'Pradera' and 'Mitre' respectively. During the period of 36–64 DAT, the average difference between 'Strategos' and 'Mitre' was 259 NO_3^-–N L^{-1}, which was 27% of the average value of 'Mitre' during this period.

3.1.2. Melon cultivars

During the melon 20 and melon 21 crops, there were statistically significant differences between the cultivars 'Tezac', 'Magiar' and 'Jacobo', and the cultivars 'Bosito', 'Magiar' and 'Jacobo' in petiole sap [NO_3^-–N] with respect to N treatment and time (RM–ANOVA; T × C × N, P <0.05) (Table 4; Fig. 2).

Table 4. Results of repeated-measure analysis of variance (RM–ANOVA) testing the effect of cultivar, N treatments and time on the measurements of petiole sap [NO_3^-–N] in two melon crops. Significant effects at P <0.05 are show in bold, d. f are degrees of freedom, F is the Fisher value of ANOVA and P is the probability value.

Effect	d. f	Petiole sap [NO_3^-–N]			
		Melon 2020		Melon 2021	
		F	*P*	F	*P*
Block	3	2.21	0.112	6.31	**0.003**
Cultivar (C)	2	21.91	**<0.001**	40.71	**<0.001**
Nitrogen (N)	2	353.29	**<0.001**	966.57	**<0.001**
C × N	4	0.87	0.491	12.69	**<0.001**
Error	24				
Time (T)	8	101.93	**<0.001**	61.77	**<0.001**
T × C	16	2.06	**0.01**	1.96	**0.02**
T × N	16	23.37	**<0.001**	21.63	**<0.001**
T × C × N	32	3.25	**<0.001**	3.17	**<0.001**
Error	192				

Melon 20

There were consistent differences in petiole sap [NO_3^-–N] between N1, N2 and N3 treatments, for each of the three cultivars examined in melon 20 (Fig. 2). The ranges of petiole sap [NO_3^-–N] were progressively higher with increasing applied N, for each cultivar (Fig. 3).

In treatment N1, petiole sap [NO_3^-–N] had a decreasing trend until 48 DAT; thereafter it remained relatively constant at values that close to zero Fig. 2a). Petiole sap [NO_3^-–N] in treatments N2 and N3, for each cultivar, remained relatively constant, with some fluctuations (Fig. 2b,c). There was a general tendency, the three N treatments, for the cultivar 'Tezac' to have lower petiole sap [NO_3^-–N] than the cultivars 'Magiar' and 'Jacobo', which were generally similar. Comparing the two melon crops, for equivalent combinations of cultivar and N treatments, petiole sap [NO_3^-–N] was generally moderately less in the melon 21 crop (Fig. 2).

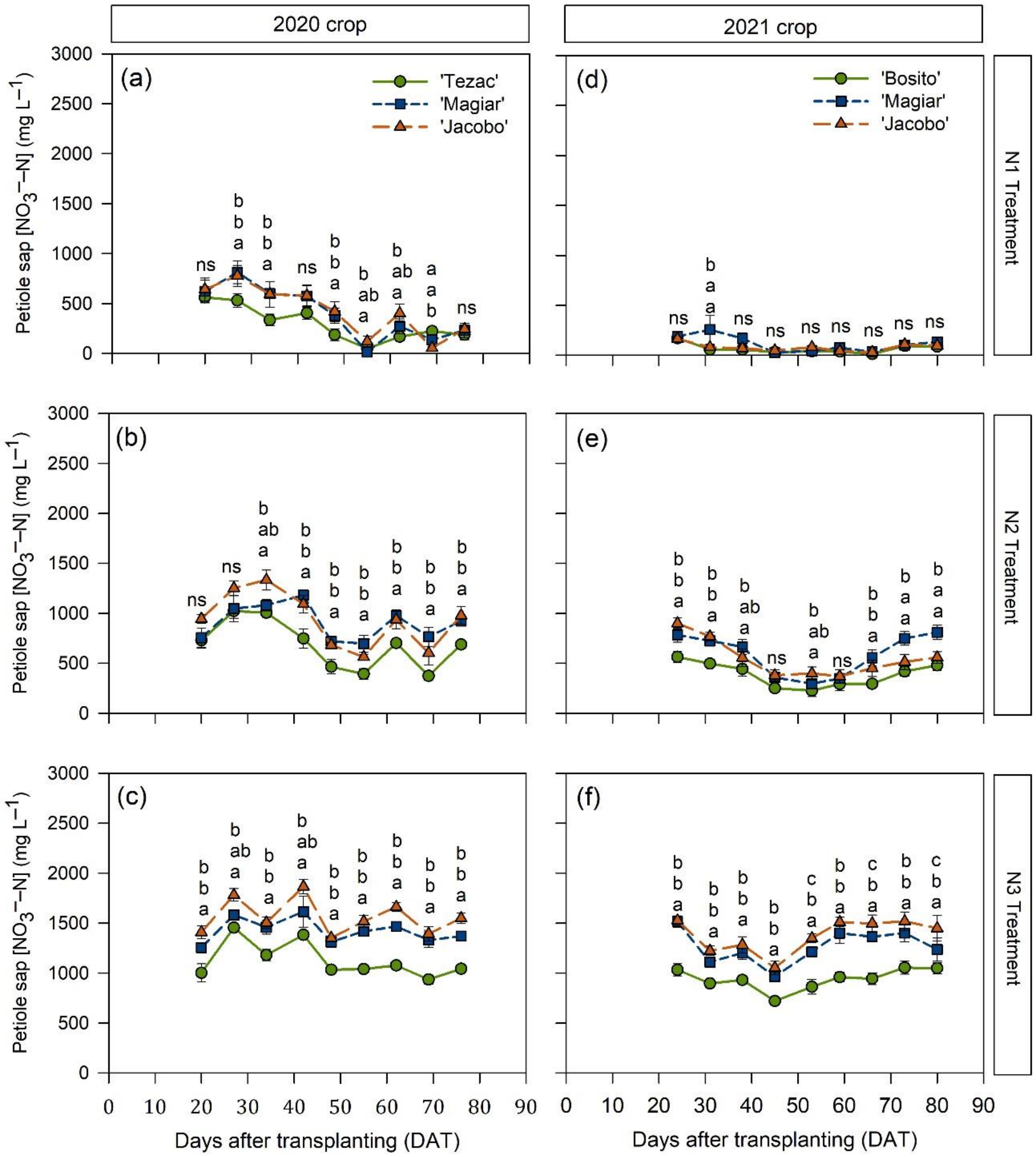

Fig. 2. Evolution of petiole sap [NO_3^-–N] of three melon 20 cultivars ('Tezac', 'Magiar' and 'Jacobo') and three melon 21 cultivars ('Bosito', 'Magiar' and 'Jacobo') under three N treatments (N1, N2 and N3) grown in greenhouse. Values are means (*n*=4) ± standard error (SE); *n* is the number of data.

Generally, in treatment N1 of melon 20, the cultivar 'Tezac' had consistently lower values than 'Magiar' and 'Jacobo' until 55 DAT, after which all cultivars were close to zero (Fig. 2a). On three of the first six sampling dates, values for 'Tezac' were significantly less than in the other two varieties, which were generally similar (Fig. 2a). For the N1 treatment, average values for the crop for the cultivars 'Tezac', 'Magiar' and 'Jacobo' were 295, 405 and 426 mg NO_3^-–N L^{-1} (Fig. 2a). Petiole sap [NO_3^-–N] of the cultivar 'Tezac' was on average 26% and 31% less than in 'Magiar' and 'Jacobo', respectively.

In the N2 treatment, from 34 DAT on, the cultivar 'Tezac' consistently had significantly less petiole sap [NO_3^-–N] than the other two cultivars, which were generally very similar (Fig. 2b). It also had significantly less than 'Jacobo' on 27 DAT (Fig. 2b). Average values during the crop for 'Tezac', 'Magiar' and 'Jacobo' were 681, 905 and 932 mg NO_3^-–N L^{-1}, respectively (Fig. 2b). Petiole sap [NO_3^-–N] of the cultivar 'Tezac' was on average 24% and 27% less than 'Magiar' and 'Jacobo', respectively. From 42 DAT until the last measurement on 76 DAT, there was an appreciable and consistent difference between 'Tezac' and both 'Magiar' and 'Jacobo'. During this period, the average difference between 'Tezac' and and 'Jacobo' was 247 NO_3^-–N L^{-1}, which was 44% of the average value for 'Tezac' during this period. The average difference between 'Tezac' and ‘Magiar' during this period was 315 NO_3^-–N L^{-1}, which was 56% of the average value for 'Tezac'.

In the N3 treatment, the cultivar 'Tezac' had significantly less petiole sap [NO_3^-–N] than both 'Magiar' and 'Jacobo' on seven of the nine sampling dates (Fig. 2c). On average, throughout the crop, petiole sap [NO_3^-–N] was 1127, 1422 and 1559 mg NO_3^-–N L^{-1} in 'Tezac', 'Magiar' and 'Jacobo', respectively, (Fig. 2c). During the crop, petiole sap [NO_3^-–N] of cultivar 'Tezac' was on average 19% and 28% less than 'Magiar' and 'Jacobo', respectively, relative to 'Tezac'. On the last four sampling dates, between 55 and 76 DAT, these differences were most pronounced. During this period, 'Tezac' had 372–507 mg NO_3^-–N L^{-1} less than the other two varieties; the average difference was 36 and 49% in 'Magiar' and 'Jacobo' relative to 'Tezac' (Fig. 2c).

Melon 21

In melon 21, petiole sap [NO_3^-–N] in the N1 treatment throughout the crop was generally very low throughout the crop (Fig. 2d). Petiole sap [NO_3^-–N] was consistently higher in the N3 compared to the N2 treatments for the three cultivars (Fig. 2e,f). In each of the N2 and N3 treatments, petiole sap [NO_3^-–N] values for each cultivar were relatively constant with some fluctuations (Fig. 2e,f).

In N2 treatment, 'Bosito' had significantly less petiole sap [NO_3^-–N] than the other two cultivars at 24, 31 and 66 DAT. On the last two sampling dates, 'Magiar' had significantly higher values than the other two cultivars (Fig. 2e). For the N2 treatment, average values throughout the crop for 'Bosito', 'Magiar' and 'Jacobo' were 385, 588 and 544 mg NO_3^-–N L^{-1}, respectively (Fig. 2e). On average, petiole sap [NO_3^-–N] of the cultivars 'Magiar' and 'Jacobo' were, respectively, 35% and 29% more than 'Bosito'. The differences between 'Bosito' and 'Magiar' were most pronounced in the periods 24–32 DAT and 66–80 DAT.

In the N3 treatment of melon 21, the cultivar 'Bosito' consistently had significantly less petiole sap [NO_3^-–N] than 'Magiar' and 'Jacobo' on all sampling dates throughout the crop (Fig. 2e). Also, in treatment N3, 'Jacobo' consistently had higher values than 'Magiar'; these differences were significant on three sampling dates (Fig. 2e). Throughout the crop, for the N3 treatments in melon 21, petiole sap [NO_3^-–N] was 939, 1267 and 1378 mg NO_3^-–N L^{-1} in 'Bosito', 'Magiar' and 'Jacobo', respectively, throughout the crop (Fig. 2f). On average, petiole sap [NO3––N] of the cultivars 'Magiar' and 'Jacobo' were, respectively, 35% and 47% more than 'Bosito'.

3.1.3. Sweet pepper cultivars

During the pepper 20 crop, statistically significant differences were observed between the cultivars 'Melchor', 'Machado' and Lamuyo in petiole sap [NO_3^-–N] depending on N treatment and time (RM–ANOVA; T × C × N, $P < 0.05$) (Table 5; Fig. 3 a,b,c). During pepper 21, there were significant differences on N treatments in petiole sap [NO_3^-–N] depending on time (RM–ANOVA; T × N, $P < 0.05$) (Table 5; Fig. 3d,e,f).

Table 5. Results of repeated-measure analysis of variance (RM–ANOVA) testing the effect of cultivar, N treatments and time on the measurements of petiole sap [NO_3^-–N] in two pepper crops. Significant effects at *P* <0.05 are show in bold, d. f are degrees of freedom, F is the Fisher value of ANOVA and *P* is the probability value.

Effect	Petiole sap [NO_3^-–N]					
	Pepper 2020			Pepper 2021		
	d. f	F	*P*	d. f	F	*P*
Cultivar (C)	2	15.40	**< 0.001**	2	8.724	**0.002**
Nitrogen (N)	2	4316.72	**< 0.001**	2	1193.971	**< 0.001**
C × N	4	5.45	**0.003**	4	1.057	0.4
Error	26			23		
Time (T)	9	50.94	**< 0.001**	7	65.180	**< 0.001**
T × C	18	6.13	**< 0.001**	14	1.866	**0.03**
T × N	18	19.36	**< 0.001**	14	3.878	**< 0.001**
T × C × N	36	2.37	**< 0.001**	28	0.881	0.6
Error	234			161		

Pepper 20

In the pepper 20 crop there were clear differences in petiole sap [NO_3^-–N] between the N1, N2 and N3 treatments, for each cultivar. (Fig. 3a,b,c). Petiole sap [NO_3^-–N] in the N2 and N3 treatment remained relatively constant for each throughout the crop with some fluctuations (Fig. 3b,c).

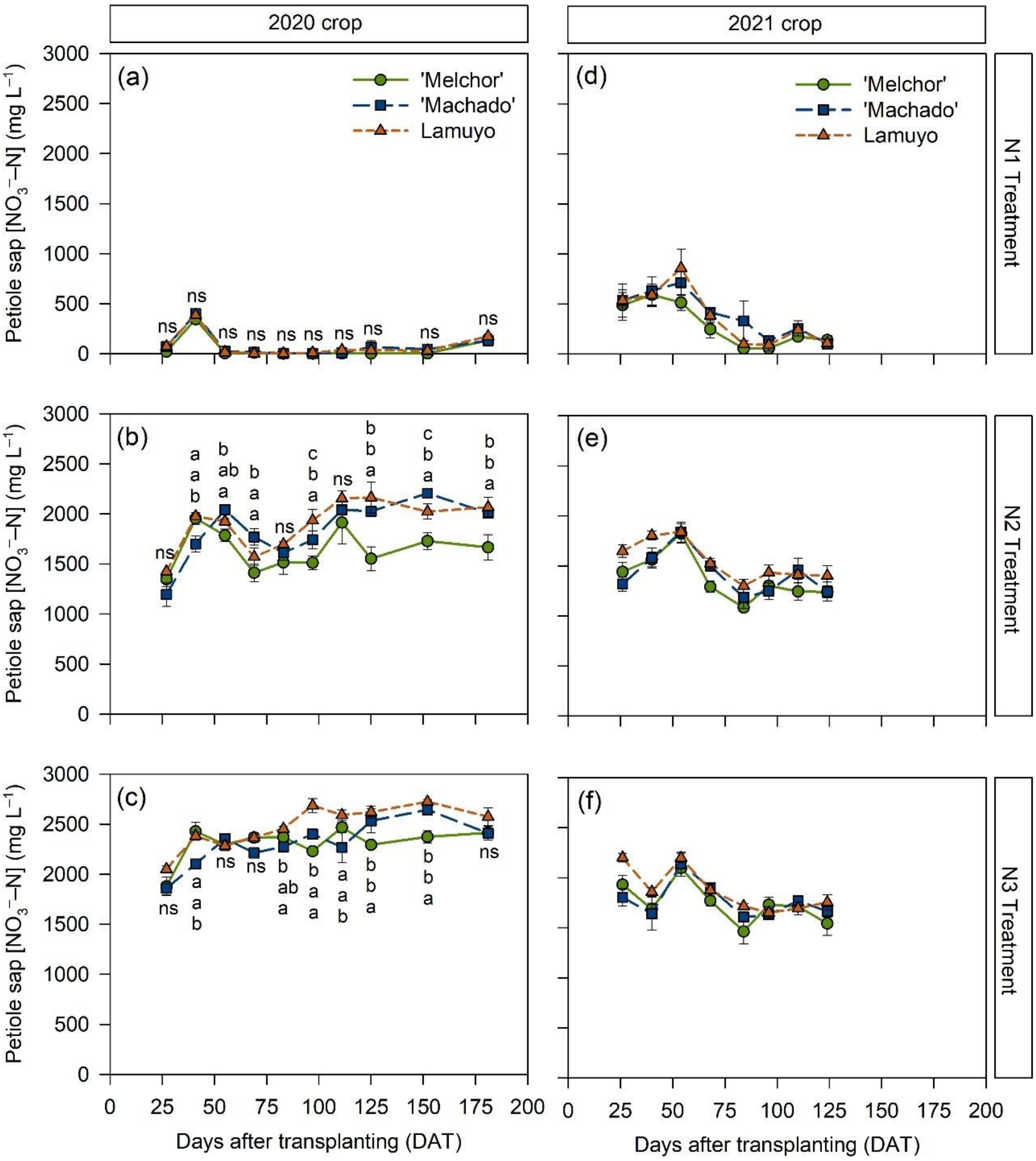

Fig 3. Evolution of petiole sap [NO$_3^-$–N] of three pepper 20 and pepper 21 cultivars ('Melchor', 'Machado' and Lamuyo) under three N treatments (N1, N2 and N3) grown in greenhouse. Values are means (*n*=4) ± standard error (SE); *n* is the number of data. In panels (d), (e) and (f) of the pepper 21 crop, there were no significant differences between any of the cultivars.

In the N1 treatment, petiole sap [NO$_3^-$–N] was zero or very close to zero throughout the crop (Fig. 3a). In treatment N2, there were either no or inconsistent differences between cultivars until 111 DAT (Fig. 3b). The cultivar 'Melchor' had significantly less petiole sap [NO$_3^-$–N] than the cultivars 'Machado' and Lamuyo on 97 DAT, and from 125 DAT until the end of the crop (Fig. 3b). The value for Melchor was significantly lower than that of Machado on five of seven sampling dates from 69 DAT (Fig. 3b). The average values throughout the crop, for the N2 treatment, for 'Melchor', 'Machado' and Lamuyo were 1639, 1834 and 1893 mg NO_3^-–N L^{-1}, respectively (Fig. 3b). The average values of 'Machado' and Lamuyo were 12 and 6% more that of 'Melchor'.

In the N3 treatment, there were either no or inconsistent differences until 125 DAT (Fig. 3c). The cultivar 'Melchor' was significantly less than 'Machado' and Lamuyo on two consecutive sampling dates on 125 and 152 DAT (Fig. 3c). On average, during the entire crop, petiole sap [NO_3^-–N] was 2312, 2306 and 2473 mg NO_3^-–N L^{-1} in 'Melchor', 'Machado' and Lamuyo, respectively (Fig. 3c).

Pepper 21

In pepper 21, 'Melchor', 'Machado' and Lamuyo showed very similar values in petiole sap [NO_3^-–N] throughout the crop in each of the N treatments (Fig. 3 d,e,f). No clear cultivar effects were apparent. Presumably, the high background of soil mineral N at the start of crop substantially reduced the effects of the N treatments and the cultivar (Fig. 3 e,f,g), compared to the pepper 20 crop (Fig. 3 a,b,c).

3.2. Effect of N on leaf N content (%N) in different cucumber, melon, and sweet pepper cultivars

3.2.1. Cucumber cultivars

During cucumber crop there were statistically significant differences between the cultivars 'Strategos', 'Pradera' and 'Mitre' in leaf N content (%) depending on N treatment and time (RM–ANOVA; T × C × N, $P < 0.05$) (Table 6; Fig. 4).

Table 6. Results of repeated-measure analysis of variance (RM–ANOVA) testing the effect of cultivar, N treatments and time on the measurements of leaf N content (%) in cucumber crop. Significant effects at *P* <0.05 are show in bold, d. f are degrees of freedom, F is the Fisher value of ANOVA and *P* is the probability value.

Effect	d. f	Leaf N content (%N)	
		F	*P*
Block	3	3.46	0.032
Cultivar (C)	2	29.40	**<0.001**
Nitrogen (N)	2	2441.02	**<0.001**
C × N	4	3.73	**0.02**
Error	24		
Time (T)	6	257.75	**<0.001**
T × C	12	3.71	**<0.001**
T × N	12	73.01	**<0.001**
T × C × N	24	1.88	**0.01**
Error	144	3.46	

Leaf N content (%N) in cucumber cultivars 'Strategos', 'Pradera' and 'Mitre' was affected by the different N concentrations applied in N1, N2 and N3 treatments (Fig. 4). For each cultivar, the leaf N content was generally higher with higher rates of applied N. Leaf N content decreased during the crop in the N1 treatment (Fig. 4a). In the N2 treatment, there was a on overall slight downward tendency during the crop (Fig. 4b). In the N3 treatment, leaf N content was relatively constant until 43 DAT, after which it declined (Fig. 4c).

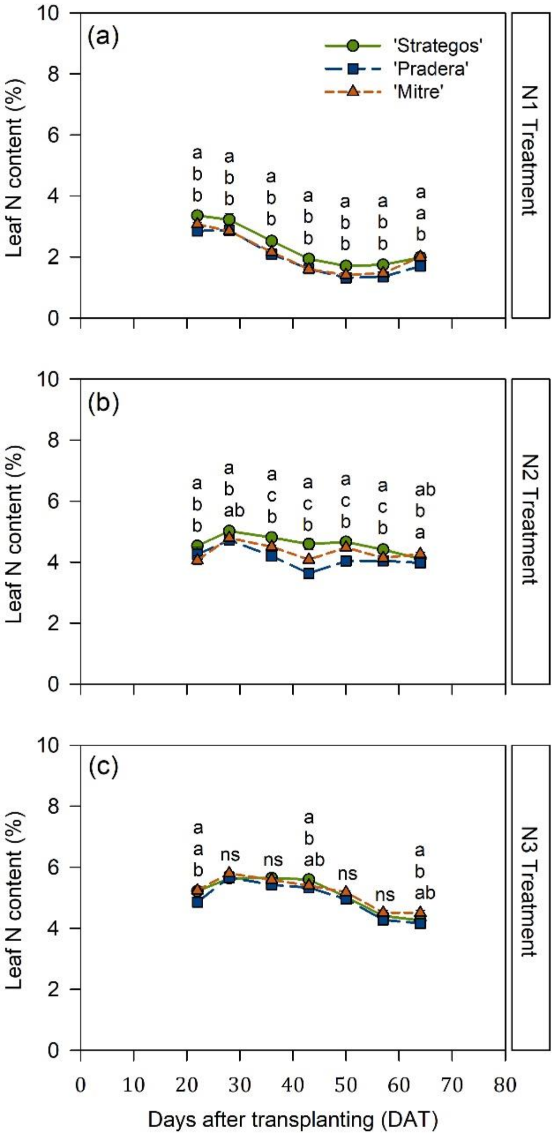

Fig. 4. Evolution of leaf N content (%N) of three cucumber cultivars ('Strategos', 'Pradera' and 'Mitre') under three N treatments (N1, N2 and N3) grown in greenhouse. Values are means (*n*=4) ± standard error (SE); *n* is the number of data. Figure were modified from de Souza et al. (2020). Effect of cultivar on chlorophyll meter and canopy reflectance measurements in cucumber. Sensors 20: 509, published by MDPI and distributed as open access under the Creative Commons Attribution (CC BY) licence.

In treatment N1, the leaf N content of the cultivar 'Strategos' was significantly higher than 'Pradera' and 'Mitre' throughout the crop (Fig. 4a). Values for 'Pradera' and 'Mitre' were consistently very similar. On average throughout the crop, the leaf N content of 'Strategos' was 2.4%, exceeding 'Pradera' and 'Mitre' by 0.3% (Fig. 4a). In treatment N2, the leaf N content of

'Strategos' was significantly higher than 'Pradera' throughout the crop, and was significantly higher than 'Mitre' on four of the seven sampling dates (Fig. 4b). 'Mitre' was significantly higher than 'Pradera' on four of the seven dates (Fig. 4b). Average values for the N2 treatment throughout the crop were 'Strategos', 'Pradera' and 'Mitre' were 4.6, 4.1 and 4.3%, respectively (Fig. 4b). In treatment N3 during the crop, the cultivars generally had very similar leaf N content values (Fig. 4c).

3.2.2. Melon cultivars

Statistically significant differences in leaf N content were observed between the cultivars 'Tezac', 'Magiar' and 'Jacobo' in melon 20, and the cultivars 'Bosito', 'Magiar' and 'Jacobo' in melon 21, depending on N treatment and time (RM–ANOVA; T × C × N, $P < 0.05$) (Table 7; Fig. 5).

Table 7. Results of repeated-measure analysis of variance (RM–ANOVA) testing the effect of cultivar, N treatments and time on the measurements of leaf N content (%) in two melon crops. Significant effects at $P < 0.05$ are show in bold, d. f are degrees of freedom, F is the Fisher value of ANOVA and *P* is the probability value.

Effect	d. f	Leaf N content (%N)			
		Melon 2020		Melon 2021	
		F	*P*	F	*P*
Block	3	2.21	0.112	3.32	**0.04**
Cultivar (C)	2	15.62	**<0.001**	78.08	**<0.001**
Nitrogen (N)	2	302.54	**<0.001**	820.73	**<0.001**
C × N	4	0.12	0.974	2.49	0.070
Error	24				
Time (T)	8	269.93	**<0.001**	162.86	**<0.001**
T × C	16	6.27	**<0.001**	4.06	**<0.001**
T × N	16	24.02	**<0.001**	18.95	**<0.001**
T × C × N	32	1.98	**0.003**	1.55	**0.038**
Error	192				

Melon 20

Leaf N content increased in all cultivars with increasing N supply (Fig 5). In the N1, N2 and N3 treatments, leaf N content maintained a similar decreasing trend in the different cultivars during the crop (Fig. 5a,b,c).

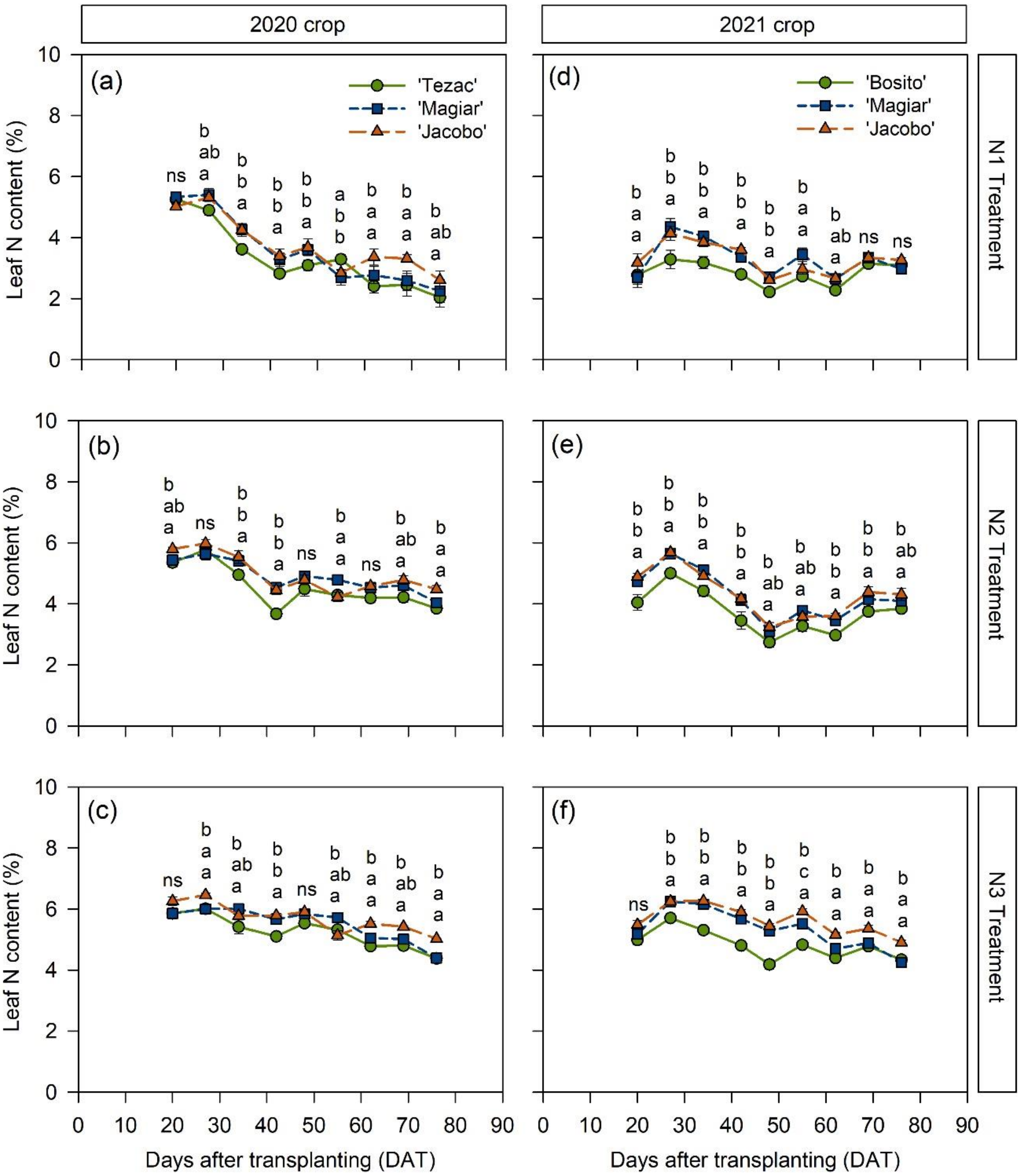

Fig. 5. Evolution of leaf N content (%N) of three melon 20 cultivars ('Tezac', 'Magiar' and 'Jacobo') and three melon 21 cultivars ('Bosito', 'Magiar' and 'Jacobo') under three N treatments (N1, N2 and N3) grown in greenhouse. Values are means (*n*=4) ± standard error (SE); *n* is the number of data.

In the N1 treatment, leaf N content of the cultivar 'Tezac' was generally lower than of 'Magiar' and 'Jacobo' (Fig. 5a). It was significantly lower than 'Magiar' and 'Jacobo' on three consecutive dates between 34 and 48 DAT (Fig. 5a). Additionally, in Tezac, it was significantly lower than in 'Jacobo' on three consecutive dates between 62 and 76 DAT, towards the end of the crop (Fig. 5a). In the N1 treatment, average values for 'Tezac', 'Magiar' and 'Jacobo', for the entire crop, were 3.3, 3.6 and 3.8% respectively (Fig. 5a). Between 27 and 55 DAT, the leaf N content of 'Tezac' was consistently 0.3–0.4% less than in 'Magiar' and 'Jacobo', respectively (Fig. 5a).

In the N2 and N3 treatments, there was a general tendency for 'Tezac' to have lower leaf N content than 'Magiar' and 'Jacobo', which generally had similar values (Fig. 5b,c). However, the difference with both 'Magiar' and 'Jacobo' was significant on only two dates in the N2 treatment (Fig. 5b), and on one date in the N3 treatment (Fig. 5c). 'Tezac' was significantly less than 'Jacobo' on three additional dates in the N2 treatment (Fig. 5b), and on six additional dates in the N3 treatment (Fig. 5c). In the N2 treatment, average values for 'Tezac', 'Magiar' and 'Jacobo' were 4.5, 4.9 and 5.0% (Fig. 5b). In the N3 treatment, average values for 'Tezac', 'Magiar' and 'Jacobo' were 5.2, 5.5 and 5.7% (Fig. 5c), respectively. Averaged over the crop, the difference between ‘Tezac’ and ‘Jacobo’ was 0.5% in both the N2 and N3 treatments.

Melon 21

Leaf N content (%) of the cultivar 'Bosito' in the N1 treatment was generally less in 'Magiar' and 'Jacobo' mainly at the first part of the crop (Fig. 5d). 'Bosito' was significantly lower than the other cultivars on four of the seven sampling dates in three consecutive dates between 31 and 53 DAT (Fig. 5d). In the N1 treatment, average values for 'Bosito', 'Magiar' and 'Jacobo', for the entire crop, were 2.8, 3.3 and 3.3% respectively (Fig. 5d).

In the N2 and N3 treatments, 'Bosito' generally had lower leaf N content than the other cultivars, and leaf N contents of 'Magiar' and 'Jacobo' were generally similar (Fig. 5e,f). In treatment N2, 'Bosito' generally had lower leaf N content than the other cultivars; on five of the nine sampling dates, the differences were significant. In the N2 treatment, average values for 'Bosito', 'Magiar' and 'Jacobo' were 3.7, 4.2 and 4.3% (Fig. 5e). In the N3 treatment, average values for 'Bosito', 'Magiar' and 'Jacobo' were 4.8, 5.3 and 5.6% (Fig. 5f). The differences between ‘Bosito’ and the other two cultivars were significant in the four samplings between 59 and 80 DAT (Fig. 5f).

3.2.3. Sweet pepper cultivars

During pepper 20, statistically significant differences were observed between the cultivars 'Melchor', 'Machado' and Lamuyo in leaf N content depending on N treatment and time (RM–ANOVA; T × C × N, *P* <0.05) (Table 8; Fig. 6a,b,c).

Table 8. Results of repeated-measure analysis of variance (RM–ANOVA) testing the effect of cultivar, N treatments and time on the measurements of leaf N content (%) in two pepper crops. Significant effects at *P* <0.05 are show in bold, d. f are degrees of freedom, F is the Fisher value of ANOVA and *P* is the probability value.

Effect	Leaf N content (%N)					
	Pepper 2020			Pepper 2021		
	d. f	F	*P*	d. f	F	*P*
Cultivar (C)	2	12.84	**< 0.001**	2	3.50	**< 0.05**
Nitrogen (N)	2	2421.31	**< 0.001**	2	176.30	**< 0.001**
C × N	4	0.89	0.485	4	0.64	0.637
Error	26			23		
Time (T)	9	281.51	**< 0.001**	7	297.53	**< 0.001**
T × C	18	6.65	**< 0.001**	14	7.03	**< 0.001**
T × N	18	39.93	**< 0.001**	14	2.05	**< 0.05**
T × C × N	36	3.10	**< 0.001**	28	1.02	0.4
Error	234			161		

Pepper 20

Leaf N content in the three cultivars decreased in the N1 treatment until 69 DAT, and then remained constant (Fig. 6a). In treatments N2 and N3, leaf N content maintained a similar trend in all cultivars, decreasing during the entire crop (Fig. 6 b,c).

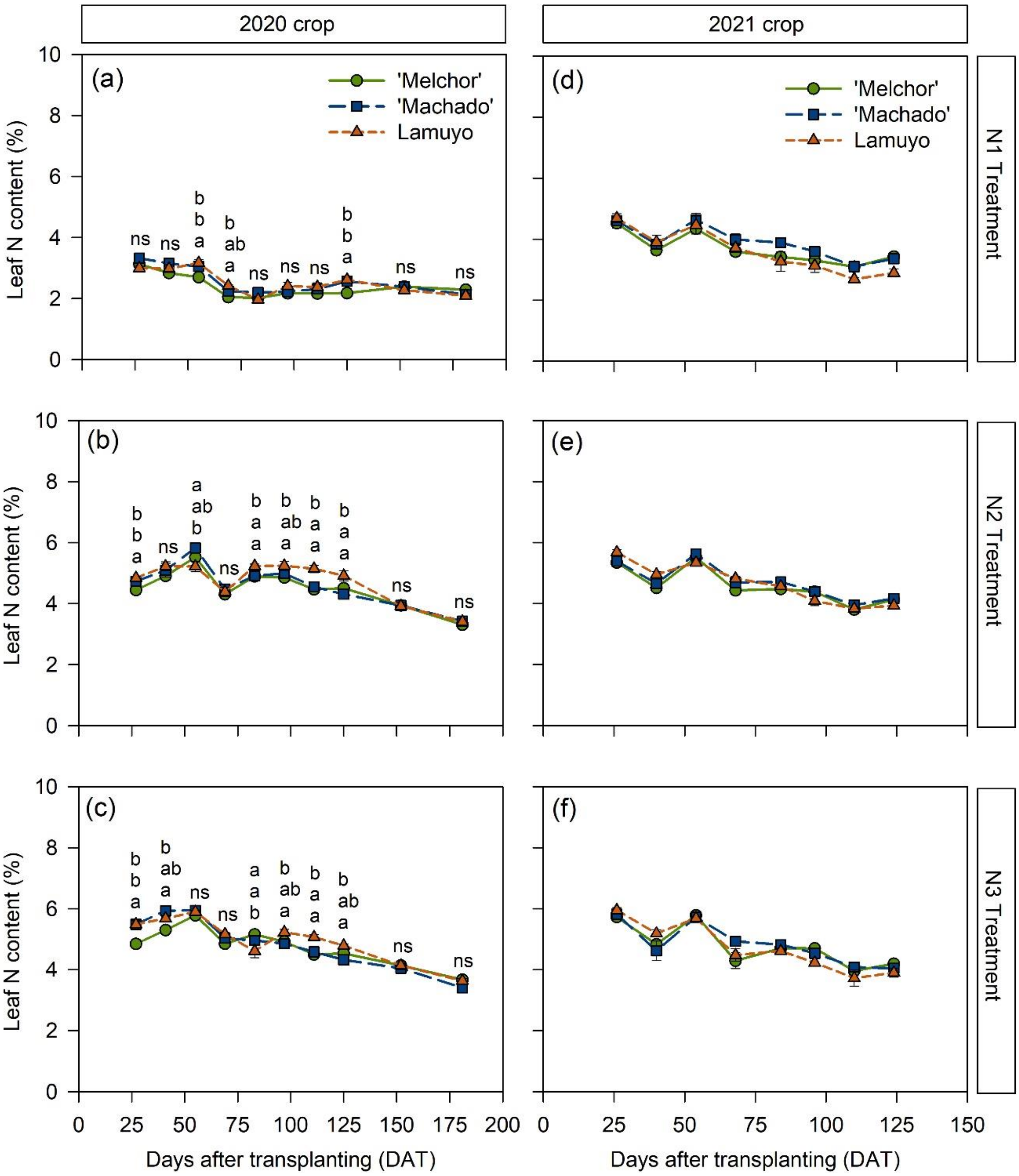

Fig. 6. Evolution of leaf N content (%N) of three pepper 20 and pepper 21 cultivars ('Melchor', 'Machado' and Lamuyo) under three N treatments (N1, N2 and N3) grown in greenhouse. Values are means (*n*=4) ± standard error (SE); *n* is the number of data. In panels (d), (e) and (f) of the pepper 21 crop, there were no significant differences between any of the cultivars.

In treatments N1, the values for the three cultivars were generally similar (Fig. 6a). The average values of 'Melchor', 'Machado' and Lamuyo throughout the crop, in the N1 treatment, were 2.4, 2.6 and 2.5% respectively (Fig. 6a). In treatments N2 and N3, there was a general tendency for Lamuyo to have higher leaf N than 'Melchor' and 'Machado', which were generally similar (Fig. 6b,c). This effect was most pronounced during the period 69–125 DAT, when on a number of dates, values for Lamuyo were significantly higher than in one or both of the other two cultivars in N2 and N3 (Fig. 6b,c). In the preceding and subsequent periods, the differences between cultivars were either not significant or were inconsistent (Fig 6b,c). In the N2 treatment, the average values for 'Melchor', 'Machado' and Lamuyo were 4.5, 4.6 and 4.8% (Fig. 6b), and in the N3 treatment were 4.8, 4.9 and 5.0%, respectively (Fig. 6 c).

Pepper 21

In each of the N treatments (Fig. 6 d,e,f), no clear cultivar effects were apparent. 'Melchor', 'Machado' and Lamuyo showed very similar values in leaf N content.

3.3. Relationships between leaf N and petiole sap [NO_3^-–N]

The relationships between leaf N and petiole sap [NO_3^-–N] were evaluated, using pooled data from the three cultivars, for each crop (Table 9). In the cucumber and two peppers crops, data associated with petiole sap [NO_3^-–N] of <100 mg NO_3^-–N L^{-1} were excluded. In each of the cucumber, two melon and two pepper crops, there were highly significant ($P<0.001$) linear relationships between leaf N and petiole sap [NO_3^-–N] (Table 9). For the two melon crop, the relationships were very similar (Table 9). Also, for the two pepper crops, the relationships were very similar. The two cucurbit species, cucumber and melon, had very similar slope values (0.016–0.019) which were double that of pepper (0.008–0.009) (Table 9). Within each individual crop, the slope and intercept values for the three different cultivars were very similar (data not presented).

The strong relationships between leaf N and petiole sap [NO_3^-–N] demonstrated that both parameters reflected crop N status. The slope values of 0.008–0.019 demonstrated that petiole sap [NO_3^-–N] was much more than leaf N content to crop N status.

Table 9. Linear regression relationships between leaf N content (%N) and petiole sap [NO_3^-–N] (mg NO_3^-–N L^{-1}) for each of the cucumber, two melon and two pepper crops. The equations, coefficient of determination (R^2), values and number of samples (*n*) are presented for each crop. For each crop, data from the three cultivars examined were pooled. In the cucumber and two pepper crops, data associated with petiole sap [NO_3^-–N] of <100 mg mg NO_3^-–N L^{-1} were excluded. *** signifies the relation was very highly significant at P <0.001.

Crop	Equation	R^2	*n*
Cucumber	$y = 0.0016x + 3.9$	0.60***	117
Melon 20	$y = 0.0019x + 3.0$	0.64***	324
Melon 21	$y = 0.0018x + 3.1$	0.67***	324
Pepper 20	$y = 0.0008x + 3.3$	0.44***	199
Pepper 21	$y = 0.0009x + 3.3$	0.49***	266

4. Discussion

4.1. Cultivar response of petiole sap [NO_3^-–N]

In the cucumber, two melon and one of the pepper crops, in the current study, the general effects of different N treatments on petiole sap [NO_3^-–N] were similar for all cultivars. The pepper 21 crop was an exception in that there were no clear differences between the N2 and N3 treatments. Generally, for each cultivar in each N treatment, the petiole sap [NO_3^-–N] remained relatively constant throughout the crop. Exceptions were the increase in the N2 and N3 treatments of cucumber, and the decreases in N1 of melon 20 and pepper 21, which are discussed subsequently.

In all crops, apart from pepper 21, there were periods with consistent differences in petiole sap [NO_3^-–N] between cultivars within the N2 and N3 treatments. These periods were for several weeks, generally in the latter part of the crop (cucumber, pepper 20), or for the duration of the crop (melon 20 and 21). In the N1 treatments of the cucumber, melon 21 and pepper 20 crops, there was very little or no differences between cultivars because most [NO_3^-–N] values were very close to zero. The combination of the low applied N concentration and leaching soil residual NO_3^- prior to cropping resulted in a strong N deficiency that severely limited accumulation of NO_3^- in sap. In the melon 20 and pepper 21 crops, [NO_3^-–N] in relatively low concentrations was present in sap in the first weeks, after which it then declined to negligible values. This was presumably due to the presence and subsequent decline of soil NO_3^- in the immediate root zone. In the N1 treatment in melon 20, the cultivar 'Tezac' had consistently less NO_3^- than the other varieties until all values declined to near zero values.

In cucumber, in the N2 and N3 treatments, the cultivar 'Strategos' consistently had notably higher petiole sap [NO_3^-–N] than 'Pradera' and 'Mitre', which were consistently very similar. There was a tendency for petiole sap [NO_3^-–N] to increase towards the end of the cucumber crop, which was not observed with two melon and two pepper crops. In the T2 treatment, this may have followed a period of acute N deficiency when crop N demand was very high. In the N3 treatment, it may have been due to an excessive N supply following a period of high N demand.

In both the melon 20 and melon 21 crops, the results regarding cultivar differences were very similar. The cultivar 'Tezac' in 2020 and its progeny cultivar ‘Bosito’ in 2021 both consistently had appreciably lower petiole sap [NO_3^-–N] than 'Magiar' and 'Jacobo', which were generally very similar, in the N2 and N3 treatments in both crops. In the two melon crops, there was a notable similarity of results in the two crops. Petiole sap [NO_3^-–N] values for each cultivar in each N treatment and their tendencies with time were very similar in both crops.

In pepper 20, the cultivar 'Melchor' generally had lower petiole sap [NO_3^-–N] than 'Machado' and Lamuyo, in the N2 treatment. These data clearly demonstrated that there were consistent differences between varieties in cucumber, muskmelon and sweet pepper. The only crop in which there were no consistent statistically significant difference between cultivars in N2 and/or N3 treatments was pepper 21. This can be explained by the fact that much of the residual soil NO_3^- was not leached prior to transplanting; the higher overall N supply presumably masked cultivar differences.

The percentage differences between cultivars within a given N treatment were sometimes considerable. Average differences for the duration of a crop, of 200–330 mg NO_3^-–N L^{-1} were observed in cucumber and melon. In the melon 20 crop, an average difference between cultivars of 450 mg NO_3^-–N L^{-1} was observed during a seven week period. In this crop, during 6–7 weeks, the largest difference between cultivars was equivalent to 29–36 of the value of the cultivar with the lowest values.

Such differences have implications for use of reference values to interpret petiole sap [NO_3^-–N] data of these species. In other vegetable species, consistent differences in petiole sap [NO_3^-–N] have been observed when receiving the same N supply, by Waterer (1997) in potato and by Westerveld et al., (2007) in carrot. The data of the current study and of Waterer (1997) in and Westerveld et al., (2007) suggest that, ideally, studies should be conducted to develop sufficiency values for popular cultivars or at least for the most important breeding lines. It is suggested that currently available sufficiency values developed for individual species (e.g. Hochmuth, 2012, 1994) can be used as guides. However, care should be taken when considering absolute sufficiency values. Additionally, it is suggested that attention be paid to tendencies, particularly with continually fertigated vegetable crops (Padilla et al., 2020; Thompson et al., 2017a). It appears in fertigated vegetable crops receiving very frequent N addition that petiole sap [NO_3^-–N] remains relatively constant (Farneselli et al., 2014; Peña-Fleitas et al., 2015; Rodríguez et al., 2021), and that changes in slope can indicate a change in crop N status (Padilla et al., 2020; Thompson et al., 2017a). Using petiole sap [NO_3^-–N] in combination with soil N monitoring such as the [NO_3^-] in the soil solution (Rodríguez et al., 2020) provides a more comprehensive assessment of N status of the plant-soil system (Padilla et al., 2020; Thompson et al., 2017a).

4.2. Cultivar response on leaf N content (%N)

For leaf N content in cucumber, melon 20 and melon 21 there were consistent significant differences between cultivars for a given N treatment. Between some pairs of cultivars, average differences for a given N treatment, throughout the crop, were notable being 0.3–0.8% N. The differences in leaf N content between cultivars for the five crops of the three species examined in the present study were relatively smaller and less consistent than the differences in petiole sap [NO_3^-–N] between the same cultivars.

The relatively appreciably larger effects of cultivar on petiole sap [NO_3^-–N] than on leaf N content is consistent with the observations that (1) that petiole sap [NO_3^-–N] is a more sensitive indicator of crop N status in vegetable crops (Farneselli et al., 2014; Goffart et al., 2008), and (2) that it is more sensitive to changes in the N status of the crop than leaf N content (Majić et al., 2008; Olfs et al., 2005; Olsen and Lyons, 1994; Villeneuve et al., 2002).

5. Conclusions

Cultivar influenced petiole sap [NO_3^-–N] and leaf N content of greenhouse vegetable crops. In melon and cucumber, the differences in petiole sap [NO_3^-–N] between some cultivars receiving the same N supply were consistently appreciable. Also, with these species, some cultivars consistently had very similar petiole sap [NO_3^-–N] when receiving the same N supply. The observed differences in petiole sap [NO_3^-–N] were sufficiently large to have implications for the use of species-specific sufficiency values of petiole sap [NO_3^-–N]. Differences between cultivars in leaf N content when receiving the same N supply were relatively small and inconsistent compared to those for petiole sap [NO_3^-–N]. This work confirmed the sensitivity of petiole sap [NO_3^-–N] to crop N supply. However, it also demonstrated that cultivar can appreciably affect petiole sap [NO_3^-–N], and that this may have implications for practical crop N management using petiole sap [NO_3^-–N].

CRediT authorship contribution statement

A. Rodriguez: Conceptualization, Methodology, Research, Data curation and analysis, Writing-Original draft preparation, Reviewing and editing. **M.T. Peña-Fleitas:** Methodology, Research, Data curation and analysis. **F. Padilla:** Funding acquisition, Data curation and analysis. **M. Gallardo:** Writing-Reviewing and editing. **R.B. Thompson:** Funding acquisition, Conceptualization, Methodology, Data analysis, Reviewing and editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

This research was funded by project RTI2018-099429-B-100 of the National Research Plan of the Spanish Ministerio de Ciencia e Innovación (MICINN) of the Spanish national government. Alejandra Rodríguez received assistance from the University of Costa Rica (UCR contract OAICE-180-2017).

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