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RESEARCH ARTICLE

Assessing crop N status of fertigated vegetable crops using plant and soil monitoring techniques

M.T. Peña-Fleitas[1,2], M. Gallardo[1,2], R.B. Thompson[1,2], M. Farneselli[3] & F.M. Padilla[1,2]

1 Department of Agronomy, University of Almeria, Almería, Spain
2 BITAL Research Centre for Agricultural and Food Biotechnology, University of Almeria, Almería, Spain
3 Department of Agricultural Food and Environmental Sciences, University of Perugia, Perugia, Italy

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Correspondence
Dr R. Thompson, Department of Agronomy, University of Almeria, Carretera de Sacramento s/n, 04120 La Cañada de San Urbano, Almería, Spain. Email: rodney@ual.es

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Abstract

Evaluation of crop N status will assist optimal N management of intensive vegetable production. Simple procedures for monitoring crop N status such as petiole sap [NO_3^-–N], leaf N content and soil solution [NO_3^-] were evaluated with indeterminate tomato and muskmelon. Their sensitivity to assess crop N status throughout each crop was evaluated using linear regression analysis against nitrogen nutrition index (NNI) and crop N content. NNI is the ratio between the actual and the critical crop N contents (critical N content is the minimum N content necessary to achieve maximum growth), and is an established indicator of crop N status. Nutrient solutions with four different N concentrations (treatments N1–N4) were applied throughout each crop. Average applied N concentrations were 1, 5, 13 and 22 mmol L^{-1} in tomato, and 2, 7, 13 and 21 mmol L^{-1} in muskmelon. Respective rates of N were 23, 147, 421 and 672 kg N ha^{-1} in tomato, and 28, 124, 245 and 380 kg N ha^{-1} in muskmelon. For each N treatment in each crop, petiole sap [NO_3^-–N] was relatively constant throughout the crop. During both crops, there were very significant ($P < 0.001$) linear relationships between both petiole sap [NO_3^-–N] and leaf N content with NNI and with crop N content. In indeterminate tomato, petiole sap [NO_3^-–N] was very strongly linearly related to NNI ($R^2 = 0.88–0.95$, $P < 0.001$) with very similar slope and intercept values on all dates. Very similar relationships were obtained from published data of processing tomato. A single linear regression ($R^2 = 0.77$, $P < 0.001$) described the relationship between sap [NO_3^-–N] and NNI for both indeterminate and processing tomato, each grown under very different conditions. A single sap [NO_3^-–N] sufficiency value of 1050 mg N L^{-1} was subsequently derived for optimal crop N nutrition (at NNI = 1) of tomato grown under different conditions. In muskmelon, petiole sap [NO_3^-–N] was strongly linearly related to NNI ($R^2 = 0.75 - 0.88$, $P < 0.001$) with very similar slope and intercept values for much of the crop (44–72 DAT, days after transplanting). A single linear relationship between sap [NO_3^-–N] and NNI ($R^2 = 0.77$, $P < 0.001$) was derived for this period, but sap sufficiency values could not be derived for muskmelon as NNI values were >1. Relationships between petiole sap [NO_3^-–N] with crop N content, and leaf N content with both NNI and crop N content had variable slopes and intercept values during the indeterminate tomato and the muskmelon crops. Soil solution [NO_3^-] in the root zone was not a sensitive indicator of crop N status. Of the three systems examined for monitoring crop/soil N status, petiole sap [NO_3^-–N] is suggested to be the most useful because of its sensitivity to crop N status and because it can be rapidly analysed on the farm.

Introduction

Excessive N supply and consequent nitrate (NO_3^-) leaching loss commonly occur with intensive vegetable production, as reported in diverse regions such as south-eastern (SE) Spain (Pulido-Bosch *et al.*, 2000; Gallardo *et al.*, 2006), central Italy (Benincasa *et al.*, 2011), Florida, USA (Zotarelli *et al.*, 2007), and China (Min *et al.*, 2011). There is a pressing requirement to improve the N management of vegetable crops. Assessment of crop or soil N status, throughout a crop or at key times, to detect N deficiency or excess, and to permit subsequent corrective management, has been proposed as an approach to improve N management (Thompson *et al.*, 2013). Such assessment would be particularly useful for vegetable crops grown, in soil, with combined drip irrigation and fertigation where the common practice of frequent N application provides the opportunity for rapid corrective management.

Various approaches have been considered for assessing crop N status, ranging from relatively simple methods based on analysis of the total N or NO_3^- content of plant tissue, hereafter referred to as traditional methods, to sophisticated remote sensing approaches such as the use of proximal optical sensors (Fox & Walthall, 2008; Samborski *et al.*, 2009; Thompson *et al.*, 2013; Padilla *et al.*, 2014, 2015). Traditional approaches include the determination of the NO_3^- concentration ($[NO_3^-]$) of petiole sap (Hochmuth, 1994), the total N content of leaf tissue (Geraldson & Tyler, 1990), and the $[NO_3^-]$ of the soil solution (Hartz & Hochmuth, 1996). For growers, the use of these traditional methods on the farm is relatively simple, and they have no large initial purchase cost as is the case with many proximal optical sensors.

Petiole sap NO_3^- analysis measures the $[NO_3^-$–N] in conducting tissue, and is considered to be a sensitive indicator that reflects crop N status at the time of sampling (Burt *et al.*, 1995; Goffart *et al.*, 2008). The sensitivity of sap $[NO_3^-$–N] to crop N status has been demonstrated in various vegetable crops, including processing tomato (Prasad & Spiers, 1984, 1985; Fontes & Ronchi, 2002; Farneselli *et al.*, 2014), pepper (Olsen & Lyons, 1994) and potato (Zhang *et al.*, 1996). The use of small rapid analysis systems enables on-farm measurement of sap $[NO_3^-$–N] to be made immediately after sampling (Hartz *et al.*, 1993; Farneselli *et al.*, 2006; Thompson *et al.*, 2009), thereby providing an immediate *in-situ* assessment of crop N status.

Measurement of leaf N content is a long established method of plant analysis (Geraldson & Tyler, 1990; Burt *et al.*, 1995; Goffart *et al.*, 2008). Most commonly, the most recently fully expanded leaf is sampled. As for soil testing approaches, monitoring of the soil solution $[NO_3^-]$ has been suggested to be a monitoring approach suitable for greenhouse soils (Sonneveld *et al.*, 1990; Sonneveld & Voogt, 2009). Recent studies in the greenhouse-based vegetable production system of SE Spain have demonstrated that controlling the $[NO_3^-]$ of soil solution, sampled with ceramic cup soil solution suction samplers, can reduce N fertiliser use and NO_3^- leaching loss (Gallardo *et al.*, 2006; Granados *et al.*, 2013). As with sap $[NO_3^-$–N], analysis of soil solution can be conducted on-farm using rapid analysis systems (Thompson *et al.*, 2009). Sufficiency values of soil solution $[NO_3^-]$ of $>5\,mmol\,L^{-1}$ have been recommended for vegetable crops in California (Burt *et al.*, 1995; Hartz & Hochmuth, 1996) and are used in commercial farming practice in Israel (S. Kramer, Israeli Foreign Ministry, personal communication). Granados *et al.* (2013) suggested a sufficiency range of $8–12\,mmol\,L^{-1}$. The suggested sufficiency values for soil solution $[NO_3^-]$ are generic values independent of species. Moreover, proposed sufficiency values have generally been based on observations related to production (Sonneveld & Voogt, 2009; Granados *et al.*, 2013). Their sensitivity to crop N status has yet to be evaluated.

The sensitivity of sap $[NO_3^-$–N] and of leaf N content to assess crop N status has been reported to be influenced by factors such as species, growing conditions and timing of N fertiliser application (Goffart *et al.*, 2008). It has been suggested that the sensitivity of these methods and sufficiency values need to be determined for each combination of species and cropping system (Goffart *et al.*, 2008). However, inter-site comparisons have not yet been conducted. Demonstration of shared relationships to crop N status and of shared sufficiency values for a given species under different cropping conditions (sites, cropping system) would have important practical implications.

To evaluate a monitoring technique that assesses crop N status, it is necessary to relate measurements from that technique to an indicator of crop N status. The nitrogen nutrition index (NNI) is an effective and established indicator of crop N status (Lemaire *et al.*, 2008). The NNI is the ratio between actual crop N content and the critical crop N content (i.e. the minimum N content necessary to achieve maximum growth of a crop) (Greenwood *et al.*, 1990). Values of NNI of <1 indicate N deficiency, values of >1 indicate N excess, and values of ≈1 indicate N sufficiency (Lemaire *et al.*, 2008). The determination of NNI values requires a critical nitrogen curve (CNC) (Greenwood *et al.*, 1990) specific to the given species and cropping conditions. The use of NNI is an established approach to evaluate the effectiveness of monitoring approaches to assess crop N status (Lemaire *et al.*, 2008; Ziadi *et al.*, 2008; Errecart *et al.*, 2012; Farneselli *et al.*, 2014).

There are an estimated 170,000 ha of Mediterranean-style plastic greenhouses within the Mediterranean Basin (Pardossi *et al.*, 2004), of which the largest concentration

is the 27,000 ha in the province of Almeria, SE Spain (Castilla *et al.*, 2004; Pardossi *et al.*, 2004).

Indeterminate tomato and muskmelon are important crops in the greenhouse-based vegetable production system of Almeria, SE Spain (Valera-Martínez *et al.*, 2014). Depending on prices, indeterminate tomato is either the most or second most important vegetable crop (along with sweet pepper) in this horticultural system (Valera-Martínez *et al.*, 2014). Nearly 8,700 ha are dedicated annually to this crop, which is commonly grown from August to February (autumn–winter cycle) or from February/March to June (spring cycle). Muskmelon is an important spring–summer crop being grown on 3,800 ha each year. It is most commonly grown with short cropping cycles of 3–4 months from February/March to June (Valera-Martínez *et al.*, 2014).

Simple easy to use, monitoring techniques such as determination of petiole sap [NO_3^-–N], leaf N content and soil solution [NO_3^-] may contribute to improved crop N management of tomato and muskmelon in this and other vegetable production systems. Very little information is available regarding the sensitivity of these techniques with indeterminate fresh-market tomato and muskmelon crops grown in greenhouses within the Mediterranean region. The sensitivity of petiole sap [NO_3^-–N] and leaf N content to assess crop N status of processing tomato was recently evaluated in open field conditions (Farneselli *et al.*, 2014). However, the different phenology of indeterminate fresh market tomato may cause appreciably different growth and N uptake patterns to that of processing tomato which may influence the relationship of crop N status to petiole sap [NO_3^-–N]. For leaf N analysis and soil solution [NO_3^-], there are very few published studies assessing the sensitivity of these methods to assess crop N status of indeterminate tomato and muskmelon.

Few studies of these three monitoring techniques have been conducted with very frequent N application that occurs with combined fertigation and drip irrigation systems. The timing of fractionated N applications can influence petiole sap [NO_3^-–N] (Goffart *et al.*, 2008). Therefore, there is a requirement to examine the relationship of petiole sap [NO_3^-–N] to crop N status under conditions of frequent N application that may reduce the effects of fractionated N fertiliser application on petiole sap [NO_3^-–N].

The objectives of the present study were: (a) to evaluate the use of petiole sap [NO_3^-–N], leaf N content and soil solution [NO_3^-] to assess crop N status throughout fertigated crops of indeterminate tomato and muskmelon, using the NNI and crop N content, (b) to compare the use of petiole sap [NO_3^-–N] to assess crop N status in indeterminate, fresh-market, greenhouse-grown tomato in SE Spain with its use in determinate, processing, tomato grown in open field conditions in central Italy, and (c) to suggest sufficiency values for petiole sap [NO_3^-–N] for crop N sufficiency.

Materials and methods

Experimental site

A tomato (*Solanum lycopersicum* L. 'Ramyle') and a muskmelon (*Cucumis melo* L. 'Tezac') crop were grown in soil in a representative plastic greenhouse at the Experimental Station of the University of Almería, located in Retamar, Almería, SE Spain (36°51′N, 2°16′W and 92 m elevation). The greenhouse had a multi-span structure of galvanised steel with polycarbonate walls and a roof of low density polyethylene (LDPE) tri-laminated film (200-μm thickness). It had no heating, and had passive ventilation (lateral side panels and flap roof windows) and an east–west orientation, with crop rows aligned north–south. The cropping area was 1,327 m^2. The soil was an artificial layered 'enarenado' typical of the region (Thompson *et al.*, 2007), consisting of a 30 cm layer of imported silty loam soil placed over the original silty loam soil and a 10 cm layer of fine gravel (mostly 2–5 mm diameter) placed on the imported soil as a mulch. At greenhouse construction in July 2007, 5 cm of sand was mixed into the surface of the original soil to improve infiltration prior to adding the layer of imported silty loam soil, and 200 $m^3\ ha^{-1}$ of sheep manure (63% dry matter, 1.7% total N content and 0.7 $t\ m^{-3}$ density) was mixed into the top layer of the imported silty loam soil, prior to adding the gravel layer, consistent with established local practice (Céspedes López *et al.*, 2009). More details of soil properties are presented in Soto *et al.* (2014).

Above-ground drip irrigation was used for combined irrigation and mineral fertiliser application (i.e. fertigation). Drip tape was arranged 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 an emitter density of 2 emitters m^{-2}. The compensating emitters had a discharge rate of 3 $L\ h^{-1}$.

The greenhouse had different fertigation sectors, with four replicate plots per sector, arranged in a randomised block design. Each plot measured 6 × 6 m and contained three paired lines of drip tape with 12 drip emitters in each line. One plant was positioned 6 cm from and immediately adjacent to each dripper, giving a plant density of 2 plants m^{-2} and 72 plants per replicate plot. The greenhouse was divided longitudinally into northern and southern plots by a 2 m path along its east–west axis, with two plots of each fertigation sector in the northern and southern sectors. There were border areas along the edges of the greenhouse.

Crops and experimental treatments

The indeterminate tomato crop was transplanted as 6-week-old seedlings on 14 March 2011 and grown until 14 July 2011 (122 days from transplant to end); the muskmelon was transplanted as 5-week-old seedlings on 19 April 2010 and grown until 6 July 2010 (78 days from transplant to end). Before transplanting, a series of large irrigations (total volumes of 510 and 400 mm for tomato and muskmelon, respectively) were applied to leach residual soil NO_3^- and to homogenise the soil profile between sectors. The soil mineral N content to 60 cm depth at transplanting was on average 15 kg N ha^{-1} for all plots in muskmelon and in N1 to N3 plots in tomato, and 66 kg N ha^{-1} in N4 plots of tomato. For the first 6–8 days after transplanting, seedlings were irrigated with water (0.7 mmol N L^{-1}). Following local practices, a nutrient solution of 4.5 and 2.3 mmol N L^{-1} for tomato and muskmelon, respectively, was applied from 9 DAT in tomato and 7 DAT in muskmelon until the commencement of the experimental treatments on 28 and 23 DAT in tomato and muskmelon, respectively. The amounts of mineral N applied during the post-transplanting irrigation and establishment phases were 0.1 kg N ha^{-1} and 4.3 kg N ha^{-1} in tomato and 0.7 and 5.3 kg N ha^{-1} in muskmelon, respectively. Once initiated, the N treatments were continued until the end of the crops.

In each crop, four experimental treatments were applied consisting of four different N concentrations in the nutrient solution applied by fertigation in every irrigation. The intended N treatments ranged from very deficient N to excessive N according to the N concentration in the applied nutrient solution. In tomato, the average applied N concentrations (NO_3^-–N + NH_4^+–N) during the treatment period (28–122 DAT) were 1.1, 5.2, 13.4 and 21.7 mmol L^{-1} for the very N deficient (N1), N deficient (N2), conventional N management (N3) and excessive N (N4) treatments, respectively. In muskmelon, average applied N concentrations (NO_3^-–N + NH_4^+–N) during the treatment period (23–78 DAT) were 1.6, 6.9, 13.4 and 20.9 mmol L^{-1} for the intended very N deficient (N1), intended N deficient (N2), conventional N management (N3) and excessive N (N4) treatment, respectively. The N treatments were based on varying the NO_3^- concentration; the ammonium (NH_4^+) concentration was 0.3–0.5 mmol L^{-1}. Other than N, complete nutrient solutions were applied, to ensure that all other nutrients were not limiting. The total amounts of mineral N applied during the treatment period were in tomato: 23, 147, 421 and 672 kg N ha^{-1} for N1, N2, N3 and N4, respectively (Fig. 1a), and in muskmelon were: 28, 124, 245 and 380 kg N ha^{-1} for N1, N2, N3 and N4, respectively (Fig. 1b). The volume of each individual irrigation in each

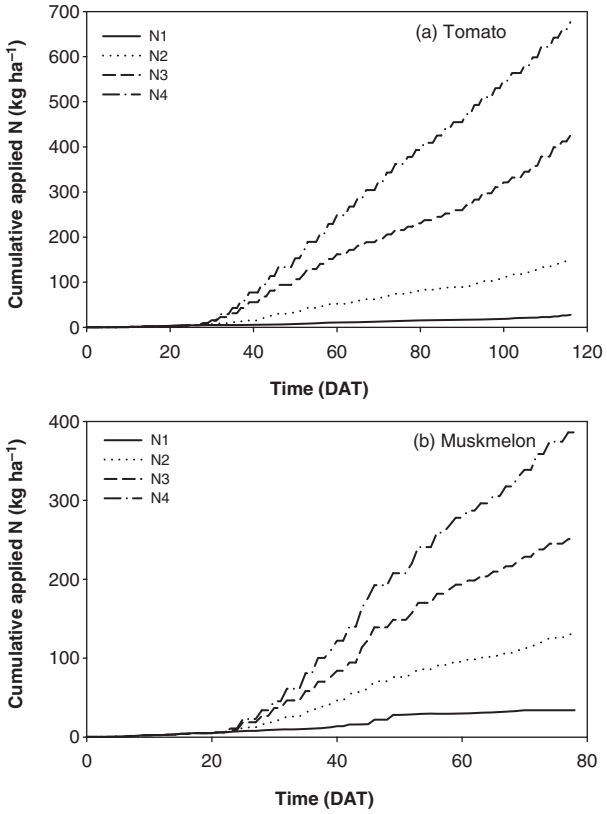

Figure 1 Cumulative applied N (kg N ha^{-1}) in four different N treatments throughout the greenhouse-grown (a) indeterminate tomato and (b) muskmelon crops. The treatments consisted of four N concentrations (NO_3^-–N + NH_4^+–N) in the nutrient solution applied by fertigation every 1–3 days from 28 and 23 DAT in tomato and muskmelon, respectively, until the end of the crop. Average applied N concentration from the commencement of experimental N treatments to the end of the crop was 1, 5, 13 and 22 mmol L^{-1} in tomato and 2, 7, 13 and 21 mmol L^{-1} in muskmelon for treatments N1, N2, N3 and N4, respectively.

treatment was measured with volume metres. Twice per week, two replicate samples of applied nutrient solutions for each treatment were collected from separate emitters. The concentrations of NO_3^- and NH_4^+ in these samples of nutrient solution were analysed with an automatic continuous segmented flow analyser (model SAN++, Skalar Analytical B.V., Breda, The Netherlands).

The crops were managed following local practices, being periodically pruned, harvested and supported by nylon cord guides to maintain an open canopy structure. Irrigation was scheduled to maintain the soil matric potential in the root zone, at 12 cm depth (from the surface of the imported soil layer) within −15 to −40 kPa; one tensiometer (Irrometer, Co., Riverside, CA, USA) per plot was used to measure soil matric potential. Irrigation was applied every 1–3 days, with irrigation being more frequent during warmer periods and less frequent

during cooler periods. Topping (the removal of the apical shoot to arrest stem elongation) was conducted in tomato at 72 DAT when there were eight trusses per plant and in muskmelon at 39 DAT when there were eight fruits per plant in all treatments. Pollination was conducted by introducing, into the greenhouse, bumblebees and bees for the tomato and muskmelon crops, respectively. High temperature within the greenhouse was controlled by white-washing the plastic cladding with applications of 0.10–0.25 kg L^{-1} of $CaCO_3$ suspension. In tomato, there were three applications, at 25, 50 and 73 DAT after which the white-washing was maintained until the end of the crop. In muskmelon, there was an application of 0.15 kg L^{-1} of $CaCO_3$ suspension on 29 DAT which was maintained until the end of the crop.

Measurements

Petiole sap [NO_3^-–N] and leaf N content

The [NO_3^-–N] in petiole sap and leaf N content were determined every two weeks throughout the indeterminate tomato and muskmelon crops. In the indeterminate tomato crop, these measurements were from 23 DAT, prior to the commencement of the different N treatments on 28 DAT, until the end of the crop. In muskmelon, measurements were made from 30 DAT, shortly after commencement of the different N treatments on 23 DAT, until the end of the crop.

For each determination of petiole sap [NO_3^-–N] and leaf N content, the most recently fully expanded leaf from each of six different plants in each replicate plot were removed. After topping (removal of plant apical tissue), equivalent leaves on all plants were sampled from the upper part of the canopy. Upon sampling, leaves were enclosed in sealed plastic bags, from which air had been squeezed, and were immediately refrigerated before processing in the laboratory within 6 h of sampling. Petioles were removed from each leaf, cut into 1 cm long pieces and then squeezed with a manual garlic press. The resulting juice from the six petioles of each replicate plot was diluted and filtered before analysis of [NO_3^-–N] with an automatic continuous segmented flow analyser (model SAN++, Skalar Analytical B.V.). The leaf blades were oven dried at 65°C until constant weight and ground sequentially in a knife mill and ball mill. The total N content of each sample consisting of six leaf blades from each replicate plot was determined using a Dumas-type elemental analyser system (model Rapid N, Elementar Analysensysteme GmbH, Hanau, Germany).

Soil solution sampling

At the beginning of the two crops, two replicate soil solution suction samplers (model 1900 L12, Soilmoisture Equipment Co., Santa Barbara, CA, USA) were installed at 12 cm depth in each replicate plot of each treatment. Depths are in relation to the surface of the imported soil layer. The soil solution suction samplers were installed at 8 cm from the plant and 5 cm from the emitter line. At weekly intervals, samples of soil solution were collected by applying vacuum (−70 kPa) for 24 h prior to sample collection; no irrigation/application of nutrient solution was made during the 24 h period of sample collection and during the 24 h prior to the application of vacuum. The [NO_3^-] and [NH_4^+] were analysed using an automatic continuous segmented flow analyser (model SAN++, Skalar Analytical B.V.). The [NH_4^+] was always negligible.

Determination of crop dry matter production and N content

Above-ground dry matter production (DMP) during the crop was measured by periodic biomass sampling (approximately every 14 days), by removing two complete plants in each replicate plot. All fresh material of each biomass component (stem, leaf and fruit) was weighed and dry matter content determined by oven-drying representative sub-samples (approximately 20% of fresh weight) at 65°C until constant weight. DMP for each biomass sampling was calculated by multiplying the fresh weight and dry matter percentage of each component, and then summing the mass of dry matter of the three biomass components. At transplanting, dry matter mass was determined for 100 seedlings. At each pruning during the crop, pruned dry matter mass was determined in leaf and shoot material as described previously, from eight marked plants in each replicate plot; the same eight plants were used for all prunings. Fruit production was collected from the same eight marked plants. In tomato, mature red fruits were successively harvested; at each of the four harvests of red fruit, fresh and dry weights were determined. In muskmelon, all mature fruit was harvested once on 78 DAT; fresh and dry weights were determined. The final biomass sampling at the end of each crop was conducted using the same eight plants; total fresh weight was measured and the percentages of fresh weight as leaf, stem and un-harvested fruit were determined on two of the eight plants; dry matter content was determined as described previously.

DMP, at each biomass sampling, was considered as being either (a) total DMP or (b) standing biomass DMP. Total DMP was calculated as the sum of dry matter mass of leaf, shoot and fruit on that sampling date plus all previously sampled pruned material and harvested fruit, which accounted for 42–48% of total DMP at the end of the tomato crop. Standing biomass DMP

represented the dry matter present in the greenhouse at a particular time as leaf, stem and fruit and did not include previously removed pruned or harvested fruit material. Final total DMP was the sum of dry matter of leaf, shoot and un-harvested fruit on that date, and of all previously harvested fruit and pruned material.

Representative samples of leaves, stems and fruit from each biomass sampling, and of pruned material and harvested fruit, from each replicate plot, were ground sequentially in knife and ball mills. Total N content (%) of each sample was determined using a Dumas-type elemental analyser system (model Rapid N, Elementar Analysensysteme GmbH). The mass of N in each relevant component was calculated from the %N and corresponding mass of dry matter.

As with DMP, crop N uptake ($kg\,N\,ha^{-1}$) was considered as being either (a) total crop N uptake or (b) standing biomass crop N uptake. Mean values (for total and standing biomass) of crop N uptake and DMP for each biomass sampling date were determined for each treatment from the four replicate plots. Standard errors (SE) of means were calculated. Crop N content (%N) for each biomass sampling was calculated, for each treatment, as crop N uptake divided by DMP. Crop N content was calculated as both: (a) total crop N content or (b) standing biomass crop N content. The former was the N content (%N) of all biomass produced to a certain date; the latter was the N content (%N) of N present in above-ground plant material on that date. Standing crop N content (%N) values were used for evaluating the sap and leaf N measurements in terms of crop N content. Interpolated values of crop N content (both total and standing biomass) were derived for dates of sap and leaf N measurement that were intermediate to biomass sampling dates.

Evaluation of sensitivity of plant measurements to detect crop N status

The crop N status was evaluated through the use of the NNI. NNI values were calculated for each treatment as the ratio between (a) the measured or interpolated total crop N content and (b) the critical N content (Lemaire *et al.*, 2008). For indeterminate tomato, the critical N curve (CNC) of Padilla *et al.* (2015) of $\%Nc = 4.87 \times DMP^{-0.329}$ was used. This curve was derived from total crop DMP and total crop N content; total crop signifies that the DMP and crop N content measurements at each biomass sampling also included previously removed fruit and pruned material. For muskmelon, the curve of $\%Nc = 5.16 \times DMP^{-0.63}$ reported by de Freitas Fogaça *et al.* (2008) was used to calculate NNI values.

Statistics

For each date of petiole sap [NO_3^-–N] and leaf N determination, linear regression analysis was conducted to evaluate the relationships between (a) each of these determinations and (b) both standing crop N content (as %N) and NNI. Standing crop N content was used for the direct evaluation of sap and leaf N measurements because it was a measure of the N present in above-ground biomass at the time of sampling. NNI was based on total crop N content following the established and widely accepted protocol (Bélanger *et al.*, 1992; Debaeke *et al.*, 2006; Lemaire *et al.*, 2008; Mistele & Schmidhalter, 2008; Ziadi *et al.*, 2008). It was used as a measure of crop N status to which sap and leaf N data could be related. The coefficient of determination (R^2) was used to quantify the precision of sap and leaf N measurements to estimate standing crop N content or NNI. *P*-values of the fitted model were used to identify statistically significant relationships.

In addition to exploring relationships for each individual date of measurement, data were combined from various dates to establish general relationships between (a) petiole sap [NO_3^-–N] and NNI throughout the indeterminate tomato crop and (b) petiole sap [NO_3^-–N] and NNI for much of the muskmelon crop.

For determinate processing tomato grown in open field conditions, data of Farneselli *et al.* (2014) were re-analysed using linear regression analysis, with exclusion of data from the first and final sampling dates in each year, to obtain linear relationships of petiole sap [NO_3^-–N] to NNI for various samplings throughout each of the two individual crops from 2006 and 2007. The excluded data were from the first sampling date at 30 DAT in both the 2006 and 2007 crops, and from last sampling dates at 84 DAT in 2006 and at 71 and 84 DAT in 2007. The excluded data represented samplings conducted very soon after treatments were established and samplings made at the end of a determinate crop cycle. In the study of Farneselli *et al.* (2014), petiole sap [NO_3^-–N] was measured every 2 weeks; for each individual sampling date, petiole sap [NO_3^-–N] was related to NNI values which were determined using the critical N curve for processing tomato developed by Tei *et al.* (2002). Farneselli *et al.* (2014) performed correlation analysis between petiole sap [NO_3^-–N] and NNI. In the current study, linear regression analysis was conducted and a general linear equation was derived for processing tomato using data from the 2006 and 2007 crops of Farneselli *et al.* (2014).

The linear regression equations for indeterminate fresh market tomato grown in a greenhouse in SE Spain and for determinate processing tomato grown in open fields in central Italy were compared to assess whether there were

statistically significant differences in slope and intercept values. Two relationships were considered sufficiently similar if intercept and slope values were not significantly different at $P < 0.01$ using the comparison of regression lines module of Statgraphics Centurion XVI (StatPoint Technologies Inc., Warrenton, VA, USA). In this latter case, we used probability at $P < 0.01$ to maximise integration of comparable relationships. Where two individual equations were sufficiently similar to meet these criteria, a composite equation that integrated the datasets of those individual equations was derived. Subsequently, a linear regression analysis was performed by combining data from indeterminate fresh market tomato (this study) and the determinate processing tomato crops, excluding the first and final samplings in processing tomato (Farneselli *et al.*, 2014) as mentioned previously.

All statistical analyses were conducted with Statgraphics Centurion XVI.

Results

Critical N determination in indeterminate crops

Given the indeterminate nature of the tomato crop and the periodic fruit harvests, an issue was whether to use a CNC based on total crop biomass (including previously harvested fruit and previously pruned material), or a CNC based on standing biomass, that considered only the above-ground plant material removed at biomass samplings (and which excluded previous fruit harvests and pruned material). A CNC of $\%Nc = 4.74 \times DMP^{-0.355}$ was derived for standing crop DMP and standing crop N content (Fig. 2a), using the same data set used by Padilla *et al.* (2015) to determine the CNC for total crop biomass and total crop N content. The CNCs for indeterminate tomato derived from both total crop and standing crop data were similar, with differences in the derived $\%Nc$ value for a given DMP of only 0.13–0.19% N, for DMP values between 1 and 7 Mg ha^{-1}. The CNC derived from standing crop biomass was more similar to the CNC of $\%Nc = 4.53 \times DMP^{-0.327}$ reported for determinate processing tomato by Tei *et al.* (2002) (Fig.2a) which like standing biomass in the current indeterminate tomato, did not experience pruning and intermediate harvests.

NNI values, obtained using both the CNCs for total crop and standing crop biomass, were compared using integrated NNI values (NNI*i*, Sadras & Lemaire (2014), Fig. 2b). NNI*i* values are the weighted average of NNI values for a treatment throughout a crop. The NNI*i* values were slightly higher when calculated based on total DMP than on standing DMP; however, the differences in absolute NNI*i* values were negligible, being on average only 0.04 (Fig. 2b). The relationships of NNI*i* values to relative

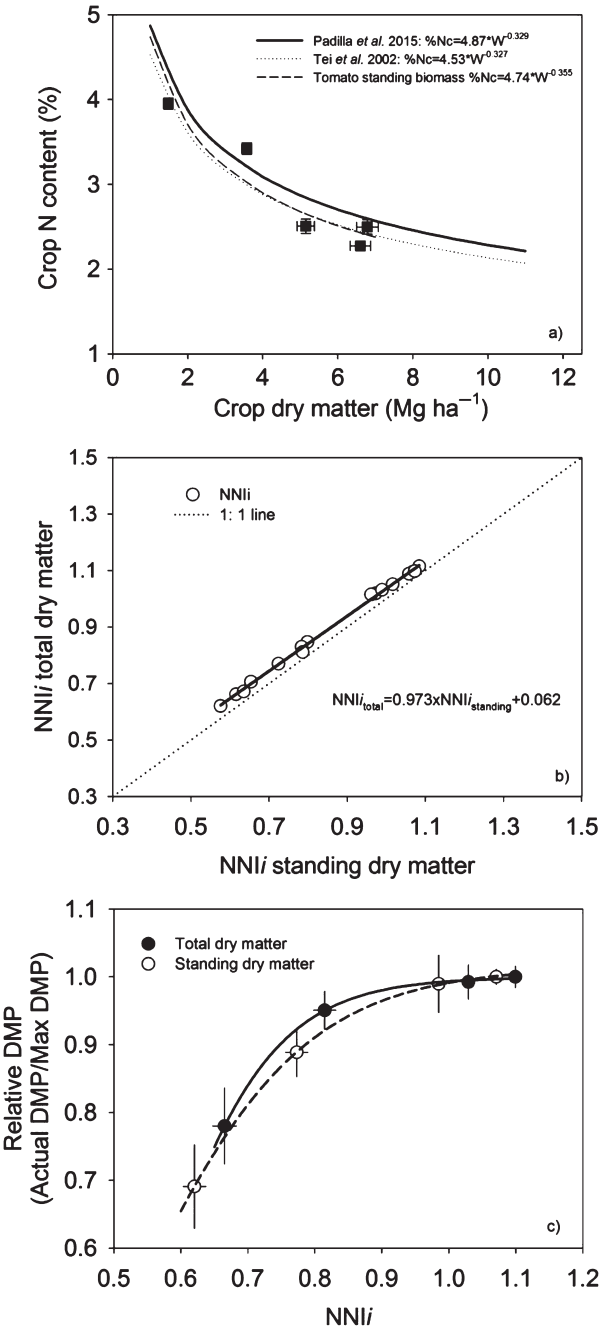

Figure 2 (a) Critical N dilution curve for indeterminate tomato (bold line) using total crop DMP and total crop N content from Padilla *et al.* (2015). Values are means ± SE. Also presented are the critical N dilution using standing crop DMP and standing crop N content derived from the Padilla *et al.* (2015) dataset for indeterminate tomato (dashed line), and the critical N dilution curve of Tei *et al.* (2002) in processing tomato (dotted line). (b) Relationship (solid line) between integrated nitrogen nutrition index (NNI*i*, open dots) values for standing crop dry matter and for total crop dry matter, for individual replicate plots. The 1:1 relationship is shown for reference (dotted line). (c) Relationships between NNI*i* and relative DMP, calculated for total crop dry matter (full symbols) and for standing crop dry matter (open symbols). Values are means ± SE.

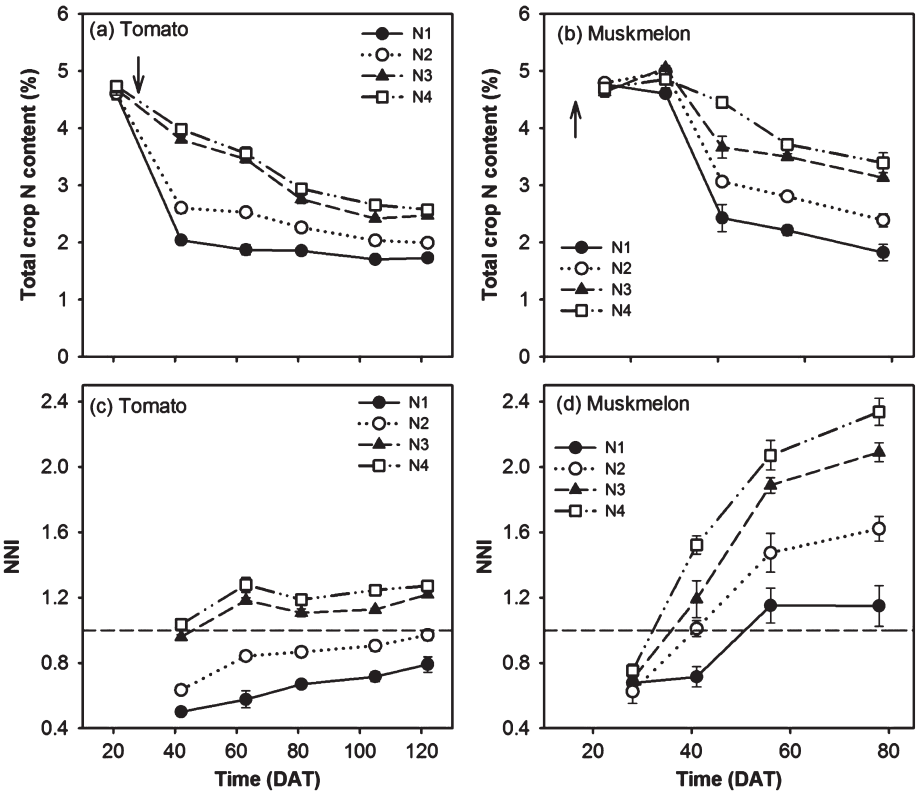

Figure 3 Total crop N content (%) of above-ground biomass in the greenhouse-grown (a) indeterminate tomato and (b) muskmelon crops, with four different N fertigation treatments, and nitrogen nutrition index (NNI) values for the indeterminate tomato crop (c) and for the muskmelon crop (d). Days of commencement of N treatments are indicated with arrows in panel (a) and (b). Values are means ± SE ($n = 4$).

DMP (calculated as the ratio between actual DMP to maximum DMP) for both total and standing dry matter were similar (Fig. 2c), in both cases a relative DMP value of 1 occurred with a NNI*i* value of approximately 1.

The similarities in the CNC curves, the NNI*i* values and the relationships between NNI*i* and DMP, for total and standing crop data, indicated that NNI values determined with either the total or standing crop CNC curves could be used to characterise crop N status of the indeterminate tomato crop. We chose to use that of the total crop because it included harvested fruit in a similar manner to CNC curves for determinate crops.

Crop N content and crop N status during crops

In both crops, there were consistent differences in measured total crop N content between the four N treatments (Fig. 3a and Fig. 3b). In indeterminate tomato, differences between treatments occurred from the first biomass sampling after the application of the treatments at 42 DAT, until the end of the crop (Fig. 3a). In muskmelon, following the commencement of the N treatments (at 23 DAT), differences in measured crop N content between treatments were consistent from 41 DAT onwards (Fig. 3b). In both crops, there were appreciable differences in total crop N content between treatments N1 and N2, and between N2 and N3 (Fig. 3a and Fig. 3b). Treatments N3 and N4 had similar total crop N contents throughout both crops. Total crop N content was positively related to applied N concentration, in both crops. The final crop N content of the N1, N2 and N3 treatments were, on average 59, 73 and 95%, respectively, that of the N4 treatment in tomato, and 56, 71, 89% that of the N4 treatment in muskmelon.

Similar to the measured crop N content, in tomato there were large consistent differences in NNI between treatments from 42 DAT until the end of the crop (Fig. 3c), and in muskmelon there were large consistent differences in NNI between treatments from 41 DAT onwards (Fig. 3d). In tomato, treatments N1 and N2 had NNI values of <1 throughout the crop, and treatments N3 and N4 consistently had NNI values >1 (Fig. 3c). Average NNI values in tomato throughout the period 42–122 DAT were 0.65, 0.84, 1.12 and 1.20 for treatments N1 to N4, respectively. In muskmelon, on DAT 28 all treatments had NNI values <1; thereafter, with the exception of treatment N1

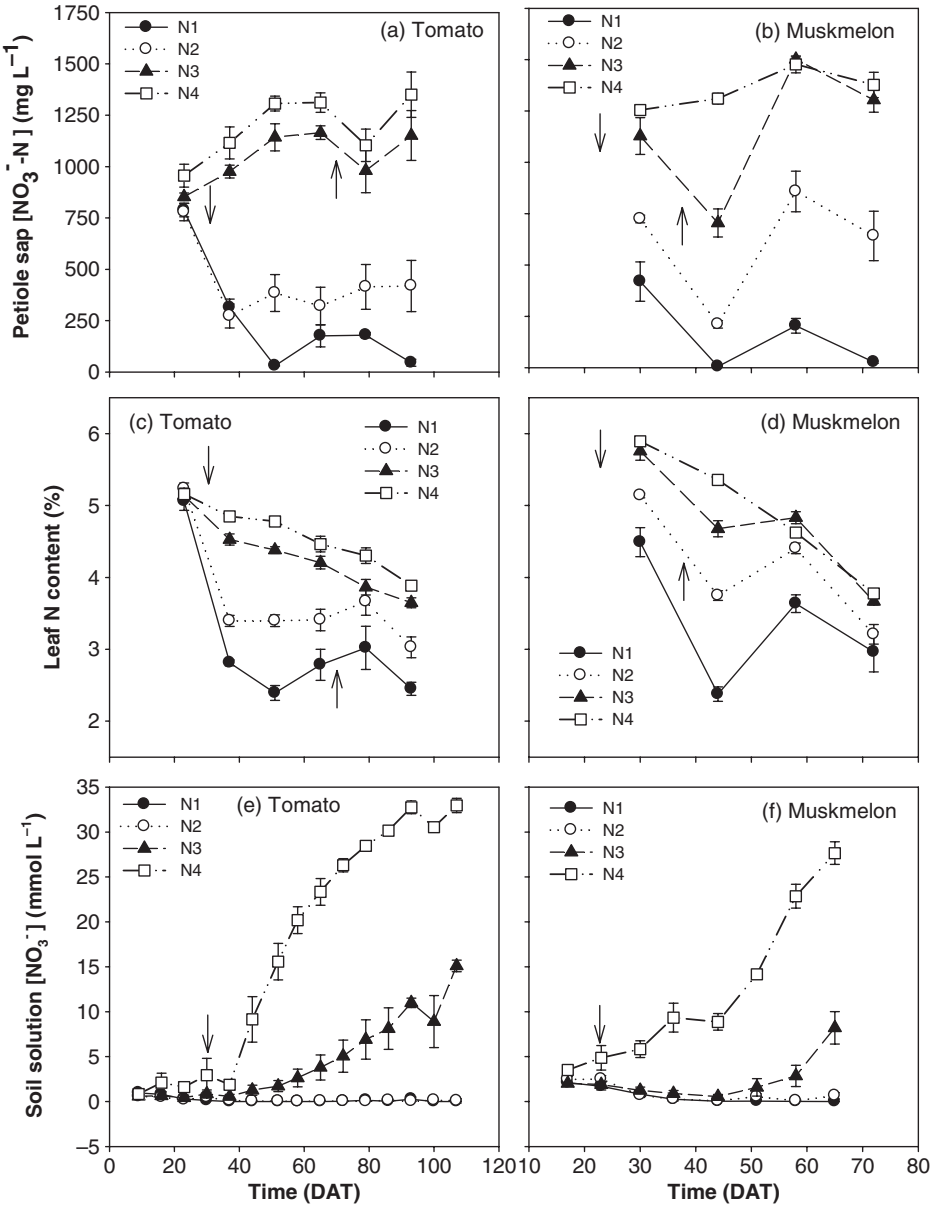

Figure 4 Values of petiole sap [NO_3^-–N] throughout the greenhouse-grown (a) indeterminate tomato and (b) muskmelon crops, of leaf N content throughout the greenhouse-grown (c) indeterminate tomato and (d) muskmelon crops, and of soil solution [NO_3^-] throughout the greenhouse-grown (e) indeterminate tomato and (f) muskmelon crops. Values are means ± SE ($n = 4$). Arrows in each graph indicate the commencement of N treatments (↓) and the day of topping (↑).

on 41 DAT, all NNI values were >1 (Fig. 3d). Average values of NNI for the period 41–78 DAT were 1.00, 1.37, 1.72 and 1.98 for treatments N1 to N4, respectively.

Petiole sap [NO_3^-–N], leaf N content and soil solution [NO_3^-] over time

In the indeterminate fresh-market tomato crop at 23 DAT, prior to the commencement of the N treatments (at 28 DAT), petiole sap [NO_3^-–N] was similar in all treatments (Fig. 4a). Immediately following the commencement of the treatments until 51 DAT, sap [NO_3^-–N] increased in treatments N3 and N4 and decreased sharply in treatments N1 and N2. From 37 DAT onwards, petiole sap [NO_3^-–N] for each treatment were within relatively constant ranges, with some fluctuation, and showed consistent relative differences between N treatments in the order of N4 > N3 > N2 > N1 (Fig. 4a). Throughout

the period when the N treatments were applied in the indeterminate tomato crop (28 DAT onwards), average values of petiole sap [NO_3^-–N] were 149, 362, 1082 and 1238 mg NO_3^-–N L^{-1} for treatments N1, N2, N3 and N4, respectively (Fig. 4a). Following topping on 72 DAT, petiole sap [NO_3^-–N] decreased in the subsequent sampling (at 79 DAT) in treatments N3 and N4, followed by recovery to values that were similar to those previously obtained during the period 51–65 DAT.

In the muskmelon crop, petiole sap [NO_3^-–N] maintained a consistent pattern of N4 ≈ N3 > N2 > N1 throughout the crop (Fig. 4b). In general, each treatment consistently maintained values within a narrow range throughout the muskmelon crop, albeit with some appreciable fluctuations in treatments N2 and N3 which are discussed subsequently. Treatment N4 had slightly higher values than N3 on 30 and 72 DAT and relatively much higher values on 44 DAT. In treatment N1 and particularly in treatments N2 and N3 in the sampling immediately after plant topping (at 39 DAT), there was a sharp decrease in petiole sap [NO_3^-–N] followed by a subsequent increase. The average values of petiole sap [NO_3^-–N] throughout the N treatment period (30–72 DAT) of the muskmelon crop were 164, 609, 1160 and 1355 mg NO_3^-–N L^{-1} for treatments N1, N2, N3 and N4, respectively.

In the indeterminate tomato and muskmelon crops there was a general tendency for leaf N content to decrease during the crop, with some fluctuations in treatments N1 and N2 in tomato (Fig. 4c) and in treatments N1, N2 and N3 in muskmelon (Fig. 4d). In both crops, relative differences between treatments were very similar to those observed for petiole sap [NO_3^-–N] (Fig. 4a and Fig. 4b). Following the commencement of the treatments, there was a consistent, moderate decrease in leaf N content in treatments N3 and N4. In treatments N1 and N2, of tomato, there was an initial rapid decline followed by relatively constant values (Fig. 4c). In tomato, average values of leaf N content, during the treatment period, were 2.7, 3.4, 4.1 and 4.5% for treatments N1, N2, N3 and N4, respectively. In muskmelon, leaf N content (Fig. 4d) showed the same fluctuations throughout the crop as petiole sap [NO_3^-–N] (Fig. 4b). Values of leaf N content were very similar in treatments N3 and N4 on the last two sampling dates (Fig. 4d). In muskmelon, average values of leaf N content, during the treatment period, were 3.4, 4.1, 4.7 and 4.9% for treatments N1, N2, N3 and N4, respectively.

The dynamics of soil solution [NO_3^-] during the season, at 12 cm depth in the root zone near to the plant, and the relative differences between treatments were similar in the indeterminate tomato (Fig. 4e) and muskmelon crops (Fig. 4f). In each crop, soil solution [NO_3^-] was very similarly low in all treatments during the establishment period (application of water only and then a dilute nutrient solution). In treatments N1 and N2, throughout both crops, soil solution [NO_3^-], in the root zone, remained consistently close to zero (Fig. 4e and Fig. 4f). In treatment N4 in both crops, soil solution [NO_3^-] increased rapidly and consistently throughout the crops reaching values of 33 and 28 mmol L^{-1} by the end of the crops, in tomato and muskmelon, respectively. In treatment N3 of tomato, soil solution [NO_3^-] increased slowly throughout the crop, reaching final values of 15 mmol L^{-1} (Fig. 4e). In treatment N3 of muskmelon, soil solution [NO_3^-] was similar to that of treatments N1 and N2 until 50 DAT after which it steadily increased reaching final values of 8 mmol L^{-1} (Fig. 4f).

Relationship of petiole sap [NO_3^-–N] to crop N status

In both the indeterminate tomato and muskmelon crops, statistically significant ($P < 0.001$) linear relationships of petiole sap [NO_3^-–N] with standing crop N content were obtained for each day of measurement during the periods in which the crops received the different N treatments (Table 1; Fig. 5a and Fig. 5b). The relationships were very strong throughout the tomato crop with R^2 values of 0.88–0.95 ($P < 0.001$), and throughout the muskmelon crop with R^2 values of 0.85–0.91 ($P < 0.001$), with the exceptions of 79 DAT in tomato ($R^2 = 0.75$, $P < 0.001$) and 30 DAT in muskmelon ($R^2 = 0.66$, $P < 0.001$; Table 1).

Petiole sap [NO_3^-–N] was strongly linearly related to NNI for each day of measurement throughout the indeterminate tomato crop (Table 1; Fig. 5c), and to NNI for much of the muskmelon crop (Table 1; Fig. 5d). In indeterminate tomato, the individual linear relationships of petiole sap [NO_3^-–N] with NNI for each date were statistically significant at $P < 0.001$ with R^2 values of 0.85–0.95 (Table 1). In muskmelon, the individual linear relationships of petiole sap [NO_3^-–N] with NNI for each date were statistically significant at $P < 0.001$ with R^2 values of 0.75–0.88 for all dates, with the exception of the first measurement date of 30 DAT ($R^2 = 0.17$, $P > 0.1$) (Table 1).

In tomato, the slope of the linear relationship between petiole sap [NO_3^-–N] and standing crop N content decreased appreciably with time (Fig. 5a). In contrast, the slope of the relationship between petiole sap [NO_3^-–N] and NNI for indeterminate tomato was relatively constant for all individual measurement dates, being 0.0003–0.0005 (Table 1). The intercept values for the relationship between petiole sap [NO_3^-–N] and NNI for indeterminate tomato were also relatively constant for all individual sampling dates, being 0.50–0.67 (Table 1). A single linear relationship $y = 0.0004x + 0.56$ with R^2 of 0.81 and $P < 0.001$ described the relationship between petiole sap

Table 1 Linear regression analysis relating petiole sap [NO_3^-–N] to standing crop N content (%) and to nitrogen nutrition index (NNI) in the greenhouse-grown tomato and the muskmelon crops for each day of measurement ($n = 16$)

	Relationship with standing crop N Content				Relationship with NNI		
	DAT	Slope	Intercept	R^2	Slope	Intercept	R^2
Tomato							
Petiole sap [NO_3^-–N]	37	0.0015	2.45	0.88***	0.0004	0.51	0.88***
	51	0.0014	1.92	0.95***	0.0004	0.50	0.95***
	65	0.0013	1.74	0.93***	0.0005	0.52	0.90***
	79	0.0007	1.79	0.75***	0.0004	0.65	0.85***
	93	0.0006	1.74	0.90***	0.0003	0.67	0.91***
Leaf N content	37	0.90	−0.18	0.88***	0.21	−0.10	0.88***
	51	0.82	−0.14	0.96***	0.25	−0.13	0.96***
	65	0.90	−0.57	0.87***	0.33	−0.34	0.84***
	79	0.54	0.33	0.58***	0.26	−0.08	0.57***
	93	0.60	0.22	0.88***	0.33	−0.13	0.95***
Muskmelon							
Petiole sap [NO_3^-–N]	30	0.0007	3.86	0.66***	0.00012	0.71	0.17^{ns}
	44	0.0014	2.42	0.85***	0.00060	0.91	0.84***
	58	0.0012	1.83	0.91***	0.00061	1.04	0.75***
	72	0.0006	1.70	0.90***	0.00077	1.13	0.88***
Leaf N content	30	0.45	2.11	0.71***	0.19	−0.13	0.36*
	44	0.66	0.58	0.88***	0.36	−0.13	0.93***
	58	1.34	−2.84	0.85***	0.51	−0.16	0.88***
	72	1.38	−1.98	0.71***	0.66	−0.25	0.93***

***Statistically significant at $P < 0.001$; *statistically significant at $P < 0.05$; ns $P > 0.05$.

[NO_3^-–N] and NNI for the five sampling dates of the greenhouse-grown indeterminate tomato crop (Table 3).

Re-analysis of data of Farneselli *et al.* (2014), with linear regression analysis, for field-grown processing tomato in Italy showed strong and statistically significant ($P < 0.05$) linear relationships between petiole sap [NO_3^-–N] and NNI for all individual sampling dates in the 2006 and 2007 crops (Table 2; Fig. 5e and Fig. 5f). On 7 of 10 sampling dates, these relationships were significant at $P < 0.01$ or better (Table 2). Linear regression analysis of all data in each of the 2006 and 2007 crops produced a general linear relationship for each year; both were statistically significant at $P < 0.001$ with R^2 values of 0.32–0.36 (Table 3).

For processing tomato, the slopes of the linear relationship between petiole sap [NO_3^-–N] and NNI for the last sampling date in 2006 and for the last two sampling dates in 2007 were appreciably different from the other sampling dates in each year (Table 2; Fig. 5e and Fig. 5f). This was presumably related to the cessation of N application towards the end of these crops and that these crops were determinate crops in the phase of fruit ripening. Similarly, linear relationships of the first sampling date in each year (30 DAT) were appreciably different from subsequent relationships, which suggest that there may be a different relationship during crop establishment. Also, that all NNI values at 30 DAT were considerably <1 in both 2006 and 2007, and in most cases were <0.6 (Fig. 5e and Fig. 5f) suggested appreciable N deficiency in all treatments on 30 DAT in both years. For the period of 42–71 DAT in 2006, the linear regression $y = 0.0005x + 0.59$ described the data set ($R^2 = 0.80$, $P < 0.001$; Table 3); and for the period 42–57 DAT in 2007, the linear regression $y = 0.0004x + 0.53$ described the data set ($R^2 = 0.80$, $P < 0.001$; Table 3). These two sets were combined to form a single equation for processing tomato of $y = 0.0004x + 0.59$ ($R^2 = 0.70$, $P < 0.001$; Table 3).

The single equation describing the relationship between petiole sap [NO_3^-–N] and NNI for processing tomato grown in open field conditions in central Italy was compared with the equivalent equation for fresh market indeterminate tomato grown in a greenhouse in SE Spain, described previously. The slopes and intercepts of the two equations were not statistically different at $P < 0.01$. Combining the data of the fresh market indeterminate tomato crop and the field-grown, processing determinate tomato crops of both 2006 and 2007 ($n = 120$), a general linear relationship relating petiole sap [NO_3^-–N] (x) to NNI (y) of $y = 0.0004x + 0.57$ was derived with a R^2 of 0.77 which was statistically significant at $P < 0.001$ (Table 3; Fig. 6). This equation is for entire tomato crops excluding the initial establishment and final fruit production phases in determinate crops. From this general relationship for tomato, a threshold value for optimal crop N nutrition of tomato of 1048 mg NO_3^-–N L^{-1} was derived as the petiole sap [NO_3^-–N]

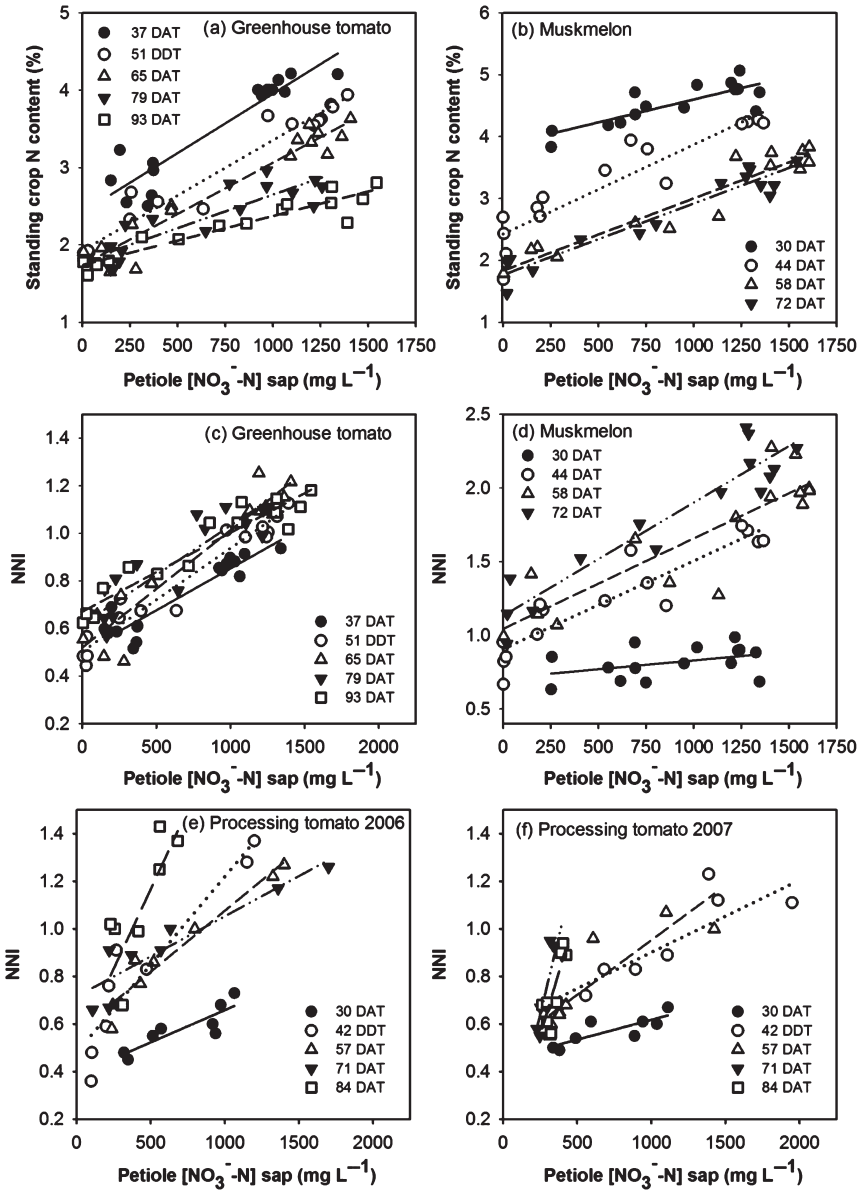

Figure 5 Linear relationships of petiole sap [NO_3^-–N] to standing crop N content (%N) in the (a) indeterminate tomato and (b) muskmelon crops, of (c) petiole sap [NO_3^-–N] to nitrogen nutrition index (NNI) in the indeterminate tomato crop, (d) petiole sap [NO_3^-–N] to NNI in the muskmelon crop, and of re-analysed data of Farneselli *et al.* (2014) of petiole sap [NO_3^-–N] to NNI in field processing tomato in (e) 2006 and (f) 2007. The R^2, slope, and intercept values, and significance of the linear regressions are presented in Tables 1 and 2.

corresponding to NNI = 1 (Fig. 6), this was rounded out to 1050 mg NO_3^-–N L^{-1}. This threshold value does not include the early establishment and final fruit production phases in determinate crops.

In muskmelon, apart from the first sampling at 30 DAT, linear relationships between petiole sap [NO_3^-–N] and NNI were very similar to one another in terms of slope and intercept (Fig. 5d). A single linear relationship of $y = 0.0007x + 0.9971$ ($R^2 = 0.77$, $P < 0.001$) described the relationship for the period 44–72 DAT. Unlike tomato, sap sufficiency values could not be derived for muskmelon from this single relationship between petiole sap [NO_3^-–N] and NNI because NNI values were in most cases >1.

Relationship of leaf N content to crop N status

In greenhouse-grown indeterminate tomato and muskmelon, the individual relationships between leaf

Table 2 Linear regressions relating petiole sap [NO_3^-–N] to nitrogen nutrition index (NNI) for field processing tomato in Italy (Farneselli *et al.*, 2014). Regressions were conducted for each day of measurement for data of 2006 and 2007 crops ($n = 8$).

	2006			2007		
DAT	Slope	Intercept	R^2	Slope	Intercept	R^2
30	0.0003	0.39	0.76***	0.0002	0.45	0.68*
42	0.0007	0.48	0.87***	0.0003	0.57	0.79**
57	0.0005	0.56	0.94***	0.0004	0.50	0.76**
71	0.0003	0.72	0.86**	0.0025	0.014	0.73**
84	0.0013	0.50	0.62*	0.0020	0.05	0.65*

***Statistically significant at $P < 0.001$; **statistically significant at $P < 0.01$; *statistically significant at $P < 0.05$.

Table 3 Linear regression analysis relating petiole sap [NO_3^-–N] to nitrogen nutrition index (NNI) for greenhouse tomato and field processing tomato in 2006 and 2007 (Farneselli *et al.*, 2014) including all data and also when excluding DAT 30 and 84 in 2006 and DAT 30, 71 and 84 in 2007. Finally, a general regression was conducted combining data of the greenhouse tomato and processing tomato from 2006 and 2007 excluding the days previously mentioned.

Data	*n*	Slope	Intercept	R^2
Greenhouse tomato 2011	80	0.0004	0.56	0.81***
Farneselli *et al.* (2014) processing tomato 2006	40	0.0004	0.63	0.32***
Farneselli *et al.* (2014) processing tomato 2007	40	0.0003	0.58	0.36***
Farneselli *et al.* (2014) 2006 excluding 2 days	24	0.0005	0.59	0.80***
Farneselli *et al.* (2014) 2007 excluding 3 days	16	0.0004	0.53	0.80***
Farneselli *et al.* (2014) 2006 exc. 2 days and 2007 exc. 3 days	40	0.0004	0.59	0.70***
Combination of greenhouse and processing tomato	120	0.0004	0.57	0.77***

***Statistically significant at $P < 0.001$.

N content and standing crop N content for each day of measurements were statistically significant at $P < 0.001$ with R^2 values of 0.88–0.96 in indeterminate tomato and of 0.71–0.88 in muskmelon, indicating very strong relationships (Table 1; Fig. 7a and Fig. 7b). An exception was the relationship for 79 DAT ($R^2 = 0.58$) in indeterminate tomato (Table 1). In indeterminate tomato, the slope of the relationship between leaf and standing crop N content tended to decrease moderately with time while intercept values were consistently close to zero (Fig. 7a). In muskmelon, the slope tended to increase with time and there were appreciable differences in intercept values (Fig. 7b). In both crops, a given leaf N content tended to be associated with a progressively lower standing crop N content as the crop grew due to a dilution effect in older tissue.

Statistically significant linear relationships were obtained between leaf N content and NNI for tomato with R^2 values of 0.84–0.96, with the exception of 79 DAT ($R^2 = 0.57$, $P < 0.05$) (Table 1; Fig. 7c). Statistically significant linear relationships were obtained between leaf N content and NNI for muskmelon with R^2 values of 0.88–0.93, with the exception of 30 DAT ($R^2 = 0.36$, $P < 0.05$) (Table 1; Fig. 7d). In indeterminate tomato, the slopes of the linear relationships between leaf N content and NNI were relatively constant and there was some moderate variation in intercept values (Table 1, Fig. 7c). In muskmelon, slopes tended to increase with time and there was appreciable variation in intercept values (Table 1, Fig. 7d).

Relationship of soil solution [NO_3^-] to crop N status

There was a slightly positive linear relationship between soil solution [NO_3^-] and crop N status in the indeterminate tomato crop ($R^2 = 0.33$, $P < 0.001$, Fig. 8a). This relationship ($y = 0.005x + 0.886$) had a nearly horizontal slope (Fig. 8a), indicating that NNI values hardly changed with increasing values of soil solution [NO_3^-]. For muskmelon, the relationship was described by a sigmoidal function ($R^2 = 0.66$, $P < 0.001$, Fig. 8b); there was a dispersion of NNI values at soil solution [NO_3^-] values close to zero and a tendency for NNI values to increase slightly across the range of higher soil solution [NO_3] values (Fig. 8b).

Discussion

The very strong relationships between (a) petiole sap [NO_3^-–N] and NNI in indeterminate tomato and (b) between petiole sap [NO_3^-–N] and NNI in muskmelon, throughout much of the growing seasons of both crops, suggested that in these crops that sap analysis was a good indicator of crop N status. Sap [NO_3^-–N] has been reported as being a sensitive indicator of crop N status in diverse vegetable crops such as processing tomato (Farneselli *et al.*, 2014), pepper (Olsen & Lyons, 1994), and broccoli (Belec *et al.*, 2001).

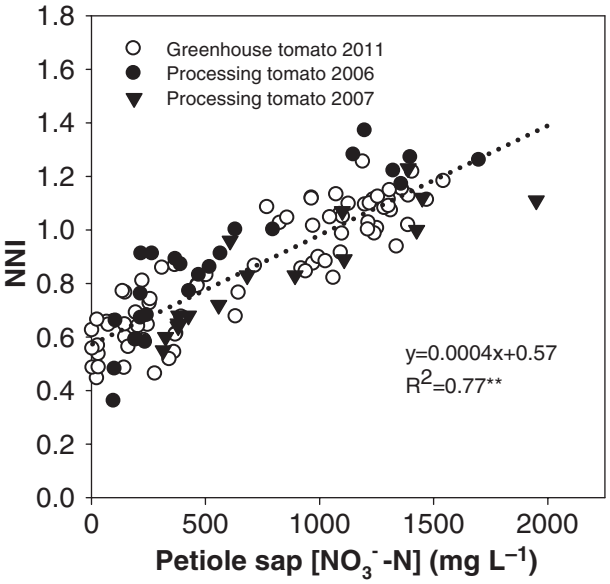

Figure 6 Linear relationship of petiole sap [NO_3^-–N] to nitrogen nutrition index (NNI) for tomato combining all data from greenhouse-grown indeterminate tomato (present study) and the two years of data of determinate processing tomato grown in open fields of Farneselli *et al.* (2014). Data excluded in processing tomato were from the first sampling date at 30 DAT in both the 2006 and 2007 crops and the last sampling dates of 84 DAT in 2006 and of 71 and 84 DAT in 2007.

Throughout the indeterminate tomato crop, an individual linear regression equation described the relationship between sap [NO_3^-–N] and NNI. Similarly, an individual linear regression equation described the relationship between sap [NO_3^-–N] and NNI throughout much of the muskmelon crop. The linear relationship for fertigated indeterminate tomato grown in a greenhouse in Spain was very similar to that derived for the period of maximum growth of fertigated determinate processing tomato grown in open fields in Italy. This enabled the derivation of a general linear relationship between sap [NO_3^-–N] and NNI for both indeterminate and processing tomato grown with fertigation. This general linear relationship was for the entire indeterminate tomato crop and for the period of maximum growth of processing tomato which excluded the initial establishment period and the final period of fruit maturity when leaf ageing presumably affected the relationship. The general linear relationship enabled estimation of a sufficiency value for fertigated tomato of ≈1050 mg NO_3^-–N L^{-1} which corresponded to an NNI value of one.

It has been commonly reported that petiole sap [NO_3^-–N] of vegetable crops declines continuously throughout a crop (Prasad & Spiers, 1985; Hochmuth, 1994; Goffart *et al.*, 2008). The indeterminate character of this tomato crop may have influenced the constancy of sap [NO_3^-–N]; generally, studies with determinate processing tomato have reported that sap [NO_3^-–N] declined as the crop matures (Prasad & Spiers, 1985; Hochmuth, 1994). Hochmuth (1994) reported that petiole sap [NO_3^-–N] declined less in tomato grown in greenhouse conditions than in open field conditions. The data of the current study and Hochmuth (1994) suggest that petiole sap [NO_3^-–N] may be more constant throughout greenhouse-grown crops. Continuous N application by fertigation may also have contributed to sap [NO_3^-–N] being more stable throughout the indeterminate tomato and the muskmelon crops.

The derivation of a general sufficiency value for tomato contrasts observations with carrot (Westerveld *et al.*, 2007) and broccoli (Belec *et al.*, 2001) that standard sufficiency values could not be established for a species because of variability among years, cultivars and soil types. It may be that the regular application of N by combined fertigation and drip irrigation reduces the effects of otherwise influential factors on the relationship between sap [NO_3^-–N] and NNI.

The sufficiency value for sap [NO_3^-–N] of tomato, derived in the current work, of 1050 mg L^{-1} is generally consistent with sufficiency values for tomato in the literature. Prasad & Spiers (1985) reported, for open field tomato, that sufficiency values were initially 900–1300 mg NO_3^-–N L^{-1} and were lower later in the crop. Reported sufficiency ranges for greenhouse tomato in Florida were 1000–1200 mg NO_3^-–N L^{-1} from transplant to second cluster, 800–1000 mg L^{-1} from second to fifth cluster and 700–900 mg L^{-1} for the harvest season (Hochmuth, 1994). For open field tomato, Hochmuth (1994) reported similar initial values but with a more rapid decline to relatively lower values.

In muskmelon, a single linear relationship between sap [NO_3^-–N] and NNI described the data on three of four measuring dates. The exception was the first sampling date of 30 DAT as occurred with both of the processing tomato crops (Fig. 5e and Fig. 5f); these data suggest that there may be an initial establishment period when another relationship may be required. In the muskmelon crop, the intercept values of NNI of the general linear equation and the individual equations for each date were all close to one, and the highest NNI values of 2.0–2.3 were appreciably larger than the NNI values determined for either indeterminate or processing tomato. These observations suggest that there is some uncertainty regarding the coefficients of the strong linear relationship that described all data from three of the four sampling dates for muskmelon. Given this uncertainty, it was not possible to derive a threshold value for muskmelon. Although the linear relationships between sap [NO_3^-–N] and NNI for muskmelon cannot

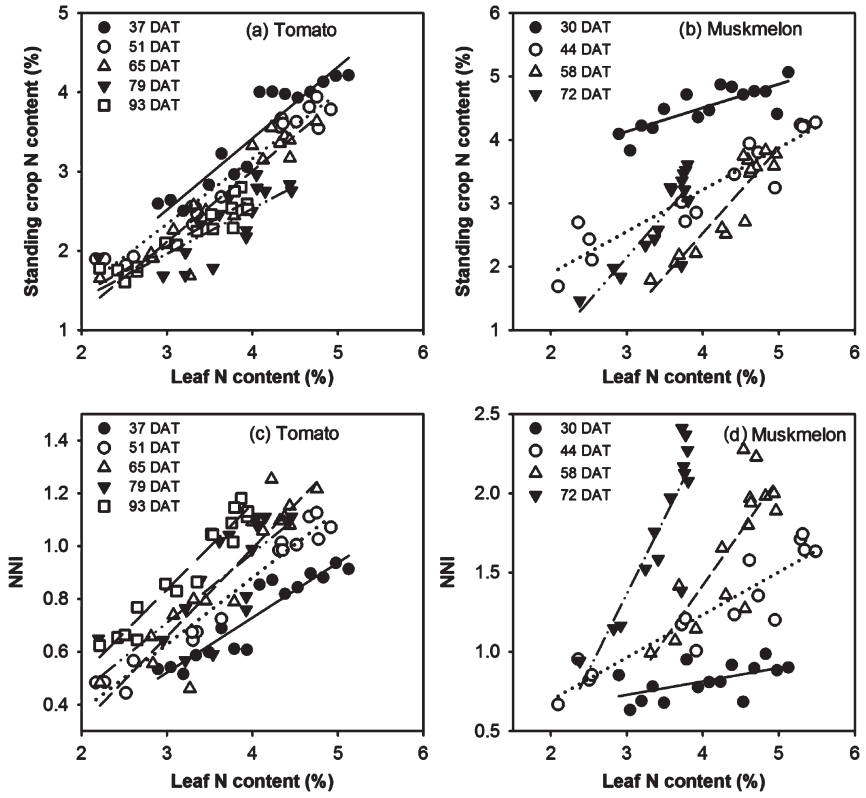

Figure 7 Linear relationships of leaf N content (%N) to standing crop N content (%N) in greenhouse-grown (a) indeterminate tomato and (b) muskmelon crops, of (c) leaf N content (%) to nitrogen nutrition index (NNI) in the indeterminate tomato crop and of (d) leaf N content (%) to NNI in greenhouse-grown muskmelon. The R^2, slope and intercept values and significance of linear regressions are presented in Table 1.

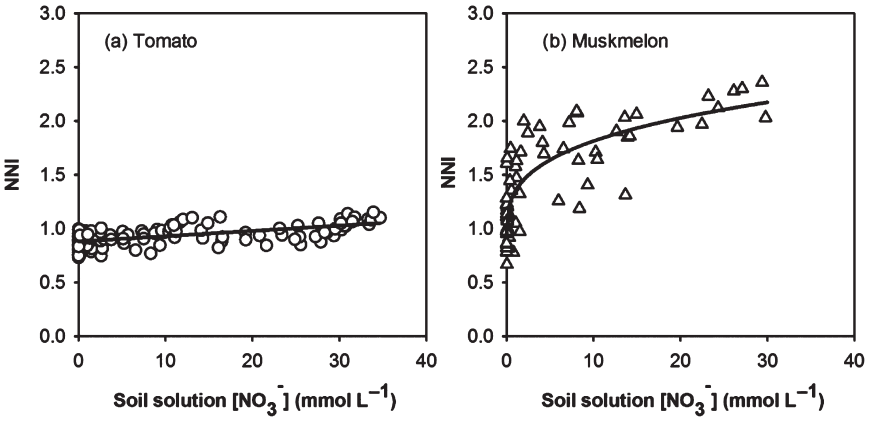

Figure 8 Nitrogen nutrition index (NNI) values plotted against soil solution [NO_3^-] for all determinations made throughout the crop for (a) indeterminate tomato and (b) muskmelon. The fitted lines are described in the text of the Results section.

be regarded as being fully quantitative, the consistent strong linear relationships on three of four measuring dates indicated that sap [NO_3^-–N] is strongly and consistently related to muskmelon crop N status.

The very strong relationships, for individual measurement dates, in (a) the indeterminate tomato crop between leaf N and both NNI and crop N content, and (b) in the muskmelon crop between leaf N and both NNI and crop N content, demonstrated that leaf N content was also a sensitive indicator of crop N status. This agrees with previous work with drip-irrigated processing tomato where leaf N content was strongly related to whole plant

N content (Hartz & Bottoms, 2009). The lack of constancy in the relationship of leaf N content to standing crop N content in both the indeterminate tomato and the muskmelon crops is likely to be due to several factors such as the relatively larger progressive dilution of N in lower shaded leaves compared to upper leaves, ageing of the upper leaves following topping, pruning, particularly of lower leaves in tomato, and different degrees of translocation of N from lower leaves between treatments over time.

Olsen & Lyons (1994) observed that leaf N content was well related to crop N status of pepper; however, they reported that sap [NO_3^-–N] was much more sensitive than leaf N content to assess crop N status. In the present study, the strength (R^2 values) of the relationships of both petiole sap [NO_3^-–N] and leaf N content with crop N status were similar indicating that they were similarly good indicators of crop N status.

Given that with sap analysis, a single linear relationship described the relationship with NNI for all or much of three individual tomato crops whereas several different equations were required for leaf N content for a single crop, petiole sap appears to be a more suitable parameter for practical use. Olsen & Lyons (1994) commented that because sap NO_3^-–N is extracted from conducting tissue rather than structural tissue it is a more direct measurement of current N supply compared to leaf N content which could remain constant for short periods, even though the crop was experiencing a notable short-term deficiency or excess of N. Additionally, sap [NO_3^-–N] can be measured on the farm using rapid analysis systems (Thompson *et al.*, 2009; Parks *et al.*, 2012), whereas leaf N requires laboratory analysis. For these various reasons, it is suggested that sap [NO_3^-–N] is preferable to leaf N analysis because it enables rapid and sensitive assessment of crop N status of fertigated tomato and muskmelon crops.

Indeterminate tomato like some other vegetable crops grown in greenhouses has a growth pattern characterised by the lack of a clear distinction between vegetative and reproductive phases, with simultaneous production of vegetative and reproductive organs. Topping (i.e. the elimination of the apical meristem) is conducted in spring-grown tomato and muskmelon in the middle part of the cropping season to stop production of new fruit clusters and to facilitate the ripening of existing fruits. In both the indeterminate tomato and muskmelon crops of the present study, a sharp decrease in petiole sap [NO_3^-–N] and leaf N content occurred immediately after topping, and then increased subsequently. Removal of the apical meristem may induce changes in the distribution of assimilates and nutrients within the plant, which may affect transport of N within conducting tissue such as of sap NO_3^-–N in petioles. Topping may have affected the relationships of leaf N content with standing crop N content and NNI, as the sampled leaves after topping were older and likely had a lower N content than the most recently fully expanded leaves sampled before topping.

Soil solution [NO_3^-] in the root zone was not an effective method to determine crop N status in either of the crops. In indeterminate tomato, soil solution [NO_3^-] was very insensitive to crop NNI, as indicated by the slope of 0.005 (very close to 0) of the linear relationships with NNI. For muskmelon, given the sigmoidal nature of the relationship between soil solution [NO_3^-] and NNI, soil solution [NO_3^-] was considered to be unsuitable as an indicator of crop N status.

Crop N uptake was 160 kg N ha^{-1} for the N1 treatment in tomato (Soto *et al.*, 2015) and 119 kg N ha^{-1} for this same treatment in muskmelon (Padilla *et al.*, 2014). Given that (a) crop N uptake was larger than the amount of applied N in the N1 treatment (27 and 34 kg N ha^{-1} for tomato and muskmelon, respectively), and (b) soil solution [NO_3^-] in the dripper area was consistently close to zero, it is clear that crops were able to obtain appreciable amounts of N from sources other than the mineral fertiliser N applied by fertigation. In these two crops, N mineralised from a large manure application at greenhouse construction was an appreciable source of N; the estimated average daily N mineralisation rates were 0.99 kg N ha^{-1} day^{-1} during the tomato crop (Soto *et al.*, 2015) and 1.06 kg N ha^{-1} day^{-1} during the muskmelon crop (unpublished data). The N mineralised from manure would have been distributed horizontally throughout the greenhouse soil. Presumably, mineralised N within the dripper area, where there was a proliferation of roots, would have been rapidly absorbed soon after mineralisation. The lateral roots that extended outside of the dripper area in both crops would also have absorbed mineralised N. Minimum threshold values could not be established for soil solution [NO_3^-] sampled within the localised bulb of soil water and nutrients supplied through the drip emitters, because of (a) the rapid depletion of [NO_3^-] within this zone, and (b) that appreciable amounts of N from sources other than mineral fertiliser were made available to the crop from outside of this zone.

In previous work in the same vegetable production system (Gallardo *et al.*, 2006; Granados *et al.*, 2013), soil solution [NO_3^-] in the immediate root zone of pepper crops, with autumn–winter growing cycles, was maintained within recommended ranges for slower growing, autumn–winter cycle crops. Maintaining soil solution [NO_3^-] within recommended ranges appears to be appreciably more difficult with faster-growing spring crops such as muskmelon where more rapid depletion of soil solution [NO_3^-] can occur, than with autumn–winter grown crops. The on-going increases in soil solution

$[NO_3^-]$ in treatments receiving higher N concentrations, in the present study, were presumably associated with accumulation of soil mineral N, suggesting that increasing soil solution $[NO_3^-]$ is indicative of excessive N application. The current results suggest soil solution $[NO_3^-]$ within the main root zone is not a reliable indicator of crop N status, as no consistent soil solution $[NO_3^-]$ value could be associated with deficient (NNI < 1.0) or adequate (NNI ≈ 1) crop N status in tomato, nor with excessive N consumption (NNI > 1.0) in muskmelon.

Of the three systems of monitoring crop/soil N status examined in the present work, both petiole sap $[NO_3^-–N]$ and leaf N content were sensitive to crop N status of tomato and muskmelon. Given its sensitivity to crop N status and the various practical advantages described previously, petiole sap $[NO_3^-–N]$ is suggested to be an effective and practical method for monitoring crop N status of tomato and muskmelon. Considerable care needs to be taken when extracting, handling and analysing plant sap to ensure viable results (Hochmuth, 1994).

Further work is required to verify the petiole sap sufficiency value for tomato suggested in the present work, and to establish sufficiency values for muskmelon with treatments that include NNI values of <1. To establish such treatments, the relation between N supply and crop N demand (at critical N status) is the determining factor (Lemaire & Gastal, 2009). In the current study, total N supply to muskmelon from N mineralised from manure (1.06 kg N ha^{-1} day^{-1}) and from N applied by fertigation (0.55 kg N ha^{-1} day^{-1} during for 41–78 DAT) was similar to the crop N demand (at critical N status) in the N1 treatment (1.10 kg N ha^{-1} day^{-1} for 41–78 DAT) which resulted in the intended very N deficient N1 treatment being an N sufficient treatment (NNI ≈ 1). In tomato, which had a much higher crop N demand (1.97 kg N ha^{-1} day^{-1} for the N1 treatment, for 42–122 DAT), total N supply from N mineralised from manure (0.99 kg N ha^{-1} day^{-1}) and from N applied by fertigation (0.25 kg N ha^{-1} day^{-1} during 42–122 DAT) was less than the crop N demand (at critical N status) in the N1 treatment, and consequently the NNI values were <1.

Conclusions

This study demonstrated that both, petiole sap $[NO_3^-–N]$ and leaf N content are sensitive indicators of crop N status in greenhouse-grown indeterminate tomato and muskmelon. As a practical method to assess crop N status, petiole sap $[NO_3^-–N]$ is preferable to leaf N content as it: (a) assesses crop N status at the time of sampling and (b) can be analysed with on-farm quick tests. In indeterminate tomato, the relationships between petiole sap $[NO_3^-–N]$ and NNI were very similar throughout the crop enabling derivation of a single relationship throughout a crop.

Combining two years of data of processing tomato grown in open fields in Italy, a very similar linear relationship between petiole sap $[NO_3^-–N]$ and NNI was obtained to that of indeterminate tomato. The two linear relationships between petiole sap $[NO_3^-–N]$ and NNI for indeterminate and processing tomato were not significantly different ($P < 0.01$) with respect to slope and intercept. Consequently, a general relationship for tomato between petiole sap $[NO_3^-–N]$ and NNI was derived. From this general relationship, a sufficiency value of sap $[NO_3^-–N]$ for optimal N nutrition (i.e. NNI = 1) of 1050 mg L^{-1} was derived for both indeterminate and processing tomato. This sufficiency value applied for the duration of the indeterminate crop and until fruit maturation of the determinate processing tomato crops. For muskmelon, a single linear relationship between petiole sap $[NO_3^-–N]$ and NNI described data on three of the four sampling dates, but sap sufficiency values could not be derived for muskmelon as NNI values were >1. Soil solution $[NO_3^-]$ was not a sensitive indicator of crop N status.

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References

Bélanger G., Gastal F., Lemaire G. (1992) Growth analysis of a tall fescue sward fertilized with different rates of nitrogen. *Crop Science*, **32**, 1371–1376.

Belec C., Villeneuve S., Coulombe J., Tremblay N. (2001) Influence of nitrogen fertilization on yield, hollow stem incidence and sap nitrate concentration in broccoli. *Canadian Journal of Plant Sciences*, **81**, 765–772.

Benincasa P., Guiducci M., Tei F. (2011) The nitrogen use efficiency: Meaning and sources of variation-case studies on three vegetable crops in central Italy. *HortTechnology*, **21**, 266–273.

Burt C., O'Connor K., Ruehr T. (1995) *Fertigation*. San Luis Obisbo, CA: Irrigation Training and Research Center, California Polytechnic State University.

Castilla, N., Hernández, J., Abou-Hadid, A.F. (2004) Strategic crop and greenhouse management in mild winter climate areas. *Acta Horticulturae*, **633**, 183–196.

Céspedes López A.J., García García M.C., Pérez-Parra J.J., Cuadrado Gómez I.M. (2009) *Caracterización de la Explotación Hortícola Protegida de Almería*. Huércal de Almería, Spain: Fundación para la Investigación Agraria en la Provincia de Almería.

Debaeke P., Rouet P., Justes E. (2006) Relationship between the normalized SPAD index and the nitrogen nutrition index: application to durum wheat. *Journal of Plant Nutrition*, **29**, 75–92.

Errecart P.M., Agnusdei M.G., Lattanzi F.A., Marino M.A. (2012) Leaf nitrogen concentration and chlorophyll meter readings as predictors of tall fescue nitrogen nutrition status. *Field Crops Research*, **129**, 46–58.

Farneselli M., Simonne E.H., Studstill D.W., Tei F. (2006) Washing and/or cutting petioles reduces nitrate nitrogen and potassium sap concentrations in vegetables. *Journal of Plant Nutrition*, **29**, 1975–1982.

Farneselli M., Tei F., Simonne E. (2014) Reliability of petiole sap test for N nutritional status assessing in processing tomato. *Journal of Plant Nutrition*, **37**, 270–278.

Fontes P.C.R., Ronchi C.P. (2002) Critical values of nitrogen indices in tomato plants grown in soil and nutrient solution determined by different statistical procedures. *Pesquisa Agropecuária Brasileira*, **37**, 1421–1429.

Fox R.H., Walthall C.L. (2008) Crop monitoring technologies to assess nitrogen status. In *Nitrogen in Agricultural Systems, Agronomy Monograph No. 49*, pp. 647–674. Eds J.S. Schepers and W.R. Raun. Madison, WI: American Society of Agronomy, Crop Science Society of America, Soil Science Society of America.

de Freitas Fogaça M.A., Luiz Andriolo J., dos Santos Godoi R., Peixoto de Barros C.A., Janisch D.I., Braz Vaz M.A. (2008) Nitrogen critical dilution curve for the muskmelon crop. *Ciencia Rural*, **38**, 345–350.

Gallardo, M., Thompson, R.B., Fernández, M.D., López-Toral, J. (2006) Effect of applied N concentration in a fertigated vegetable crop on soil solution nitrate and nitrate leaching loss. *Acta Horticulturae*, **700**, 221–224.

Geraldson C.M., Tyler K.B. (1990) Plant analysis as an aid in fertilizing vegetable crops. In *Soil Testing and Plant Analysis*. 3rd edn, pp. 549–562. Ed. R.L. Westerman. Madison, WI: Soil Science Society of America.

Goffart J., Olivier M., Frankinet M. (2008) Potato crop nitrogen status assessment to improve N fertilization management and efficiency: Past–Present–Future. *Potato Research*, **51**, 355–383.

Granados M.R., Thompson R.B., Fernández M.D., Martínez-Gaitán C., Gallardo M. (2013) Prescriptive–corrective nitrogen and irrigation management of fertigated and drip-irrigated vegetable crops using modeling and monitoring approaches. *Agricultural Water Management*, **119**, 121–134.

Greenwood D., Lemaire G., Gosse G., Cruz P., Draycott A., Neeteson J.J. (1990) Decline in percentage N of C3 and C4 crops with increasing plant mass. *Annals of Botany*, **66**, 425–436.

Hartz T.K., Bottoms T.G. (2009) Nitrogen requirements of drip-irrigated processing tomatoes. *HortScience*, **44**, 1988–1993.

Hartz T.K., Hochmuth G.J. (1996) Fertility Management of drip-irrigated vegetables. *HortTechnology*, **6**, 168–172.

Hartz T.K., Smith R.F., LeStrange M., Schulbach K.F. (1993) On-farm monitoring of soil and crop nitrogen status by nitrate-selective electrode. *Communications in Soil Science and Plant Analysis*, **24**, 2607–2615.

Hochmuth G.J. (1994) Efficiency ranges for nitrate-nitrogen and potassium for vegetable petiole sap quick tests. *HortTechnology*, **4**, 218–222.

Lemaire, G., Gastal, F. (2009) Quantifying crop responses to nitrogen efficiency and avenues to improve nitrogen use efficiency. In *Crop Physiology*, pp. 171–211. Eds V.O. Sadras, D. Calderini. Academic Press: San Diego, USA.

Lemaire G., Jeuffroy M.H., Gastal F. (2008) Diagnosis tool for plant and crop N status in vegetative stage. Theory and practices for crop N management. *European Journal of Agronomy*, **28**, 614–624.

Min J., Zhao X., Shi W.M., Xing G.X., Zhu Z.L. (2011) Nitrogen balance and loss in a greenhouse vegetable system in Southeastern China. *Pedosphere*, **21**, 464–472.

Mistele B., Schmidhalter U. (2008) Estimating the nitrogen nutrition index using spectral canopy reflectance measurements. *European Journal of Agronomy*, **29**, 184–190.

Olsen J.K., Lyons D.J. (1994) Petiole sap nitrate is better than total nitrogen in dried leaf for indicating nitrogen status and yield responsiveness of capsicum in subtropical Australia. *Australian Journal of Experimental Agriculture*, **34**, 835–843.

Padilla F.M., Peña-Fleitas M.T., Gallardo M., Thompson R.B. (2014) Evaluation of optical sensor measurements of canopy reflectance and of leaf flavonols and chlorophyll contents to assess crop nitrogen status of muskmelon. *European Journal of Agronomy*, **58**, 39–52.

Padilla F.M., Peña-Fleitas M.T., Gallardo M., Thompson R.B. (2015) Threshold values of canopy reflectance indices and chlorophyll meter readings for optimal nitrogen nutrition of tomato. *Annals of Applied Biology*, **166**, 271–285.

Pardossi A., Tognoni F., Incrocci L. (2004) Mediterranean Greenhouse Technology. *Chronica Horticulturae*, **44**, 28–34.

Parks S.E., Irving D.E., Milham P.J. (2012) A critical evaluation of on-farm rapid tests for measuring nitrate in leafy vegetables. *Scientia Horticulturae*, **134**, 1–6.

Prasad M., Spiers T.M. (1984) Evaluation of a rapid method for plant sap nitrate analysis. *Communications in Soil Science & Plant Analysis*, **15**, 673–679.

Prasad M., Spiers T.M. (1985) A rapid nitrate sap test for outdoor tomatoes. *Scientia Horticulturae*, **25**, 211–215.

Pulido-Bosch A., Bensi S., Molina L., Vallejos A., Calaforra J.M., Pulido-Leboeuf P. (2000) Nitrates as indicators of aquifer interconnection. Application to the Campo de Dalías (SE - Spain). *Environmental Geology*, **39**, 791–799.

Sadras V.O., Lemaire G. (2014) Quantifying crop nitrogen status for comparisons of agronomic practices and genotypes. *Field Crops Research*, **164**, 54–64.

Samborski S.M., Tremblay N., Fallon E. (2009) Strategies to make use of plant sensors-based diagnostic information for nitrogen recommendations. *Agronomy Journal*, **101**, 800–816.

Sonneveld C., Voogt W. (2009) *Plant Nutrition of Greenhouse Crops*. Dordrecht, The Netherlands: Springer.

Sonneveld C., van den Ende J., de Bes S. (1990) Estimating the chemical composition of soil solutions by obtaining saturation extracts or specific 1:2 by volume extracts. *Plant and Soil*, **122**, 169–175.

Soto F., Gallardo M., Giménez C., Peña-Fleitas T., Thompson R.B. (2014) Simulation of tomato growth, water and N dynamics using the EU-Rotate_N model in Mediterranean greenhouses with drip irrigation and fertigation. *Agricultural Water Management*, **132**, 46–59.

Soto F., Gallardo M., Thompson R.B., Peña-Fleitas M.T., Padilla F.M. (2015) Consideration of total available N supply reduces N fertilizer requirement and potential for nitrate leaching loss in tomato production. *Agriculture, Ecosystems & Environment*, **200**, 62–70.

Tei F., Benincasa P., Guiducci M. (2002) Critical nitrogen concentration in processing tomato. *European Journal of Agronomy*, **18**, 45–55.

Thompson R.B., Martínez-Gaitan C., Gallardo M., Giménez C., Fernández M.D. (2007) Identification of irrigation and N management practices that contribute to nitrate leaching loss from an intensive vegetable production system by use of a comprehensive survey. *Agricultural Water Management*, **89**, 261–274.

Thompson R.B., Gallardo M., Joya M., Segovia C., Martínez-Gaitán C., Granados M.R. (2009) Evaluation of rapid analysis systems for on-farm nitrate analysis in vegetable cropping. *Spanish Journal of Agricultural Research*, **7**, 200–211.

Thompson R.B., Padilla F.M., Gallardo M., Peña Fleitas M.T. (2013) Improved nitrogen management practices for vegetable production. In *Proceedings of NUTRIHORT Conference*, pp. 16–22. Eds K. D'Haene, B. Vandecasteele, R. De Vis, S. Crappé, D. Callens, E. Mechant, G. Hofman, S. De Neve. Belgium: Ghent.

Valera-Martínez L., Belmonte-Ureña L.J., Molina-Aiz F.D., López-Martínez A. (2014) *Los invernaderos de Almería. Análisis de su tecnología y rentabilidad*. Almeria, Spain: Cajamar Caja Rural.

Westerveld S.M., McDonald M.R., McKeown A.W. (2007) Establishment of critical sap and soil nitrate concentrations using a Cardy nitrate meter for two carrot cultivars grown on organic and mineral soil. *Communications in Soil Science and Plant Analysis*, **38**, 1911–1925.

Zhang H., Smeal D., Arnold R.N., Gregory E.J. (1996) Potato nitrogen management by monitoring petiole nitrate level. *Journal of Plant Nutrition*, **19**, 1405–1412.

Ziadi N., Brassard M., Bélanger G., Claessens A., Tremblay N., Cambouris A.N., Nolin M.C., Parent L.É. (2008) Chlorophyll measurements and nitrogen nutrition index for the evaluation of corn nitrogen status. *Agronomy Journal*, **100**, 1264–1273.

Zotarelli L., Scholberg J.M., Dukes M.D., Muñoz-Carpena R. (2007) Monitoring of nitrate leaching in sandy soils: comparison of three methods. *Journal of Environmental Quality*, **36**, 953–962.