Derivation of sufficiency values of a chlorophyll meter to estimate cucumber nitrogen status and yield

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Abstract

Chlorophyll meters are a promising approach for monitoring crop N status of intensively-produced vegetable crops. To do so effectively, it is fundamental that the nature and strength of the relationships between chlorophyll measurement and actual crop N status and yield, throughout the whole crop cycle be evaluated. Another fundamental requirement for the practical use of chlorophyll meters for crop N monitoring is the availability of sufficiency values or ranges, for a given crop, that indicate N deficiency (below the value) or sufficiency (above the value). The SPAD-502 meter was evaluated as an estimator of crop N status and yield in cucumber, and sufficiency values of SPAD measurements were derived for maximum crop growth and for yield. Two crops were grown in soil in a greenhouse in Almeria (SE Spain), in different cropping cycles, in Autumn 2013 (from early September to late November) and in Spring 2014 (from early March to late May). Chlorophyll measurements were made on a weekly basis throughout the crop cycle and relationships were established between measurements and the Nitrogen Nutrition Index (NNI), i.e. the ratio between actual and critical crop N contents, and yield. Sufficiency values for maximum growth were based on the relationships with NNI and sufficiency values for maximum yield were based on linear-plateau relationships with yield. Relationships of SPAD measurements with crop nitrogen nutrition index (NNI) and yield had average coefficients of determination (R^2) of 0.59 and 0.55, respectively, for most of the Autumn crop, and R^2 values of 0.84 and 0.83, respectively, for most of the Spring crop. Relationships were weak in the initial vegetative phase and were stronger in the subsequent reproductive and harvest phases. Generally, there were small differences between the sufficiency values for maximum growth and those for yield, and between the three phenological phases. The average sufficiency value for all phenological phases for both maximum growth and maximum yield was 45.2±0.7 SPAD units. This study showed that measurements of the SPAD-502 meter can be used as reliable estimators of crop N status and yield throughout most of the cycle of cucumber in Autumn and Spring growing seasons. The development and application of sufficiency values for maximum growth and maximum yield may improve N management of fertigated cucumber crops with frequent N application, by providing information for adjustments in N fertilization when SPAD measurements deviate from sufficiency values.

Keywords: vegetable crop; fertilization; N management; proximal optical sensor; NNI; SPAD; *Cucumis sativus*

Abbreviations:

AIC, Akaike Information Criterion; ANOVA, analysis of variance; Chl, Chlorophyll; CM, chlorophyll meter; DAT, days after transplanting; N, nitrogen; NNI, nitrogen nutrition index; R^2, coefficient of determination; SEE, standard error of the estimate.

1. Introduction

Intensive vegetable crops are characterized by large applications of mineral nitrogen (N) fertilizer (Neeteson, 1994; Thompson et al., 2007). Commonly, the N supply from mineral fertilizer and other sources considerably exceeds crop N requirements (Ju et al., 2006; Soto et al., 2015) which can result in substantial nitrate (NO_3^-) leaching loss (Gallardo et al., 2006; Zotarelli et al., 2007). Optimal N management that meets crop requirements while reducing N loss to the environment is required (Cameron et al., 2013).

Optimal N management requires that the rate and timing of N supply match crop N demand (Gebbers and Adamchuk, 2010; Meisinger et al., 2008). An effective approach to achieve this is accurate and rapid on-farm assessment of crop N status (Fox and Walthall, 2008; Padilla et al., 2014; Thompson et al., 2015). A promising method is non-destructive crop N monitoring using proximal optical sensors (Fox and Walthall, 2008; Samborski et al., 2009; Usha and Singh, 2013). These sensors can provide indirect measurements of indicator compounds which are sensitive to crop N status (Fox and Walthall, 2008; Samborski et al., 2009; Tremblay et al., 2012). Chlorophyll (Chl) is one such compound; leaf Chl content is strongly influenced by plant N content (Cartelat et al., 2005; Samborski et al., 2009; Schepers et al., 1996). Leaf Chl can be measured indirectly with hand-held chlorophyll meters (CMs). CM measurements have been shown to be positively related to crop N content and to be sensitive to differential N nutrition in vegetable crops (Gianquinto et al., 2006; Padilla et al., 2014; Padilla et al., 2015).

A fundamental requirement for the practical use of CMs for N fertilizer management is the development of sufficiency values that indicate N deficiency (below the value) or sufficiency (above the value). Sufficiency values for different monitoring approaches have been derived based on yield response functions (Fox and Walthall, 2008; Gianquinto et al., 2004; Ordoñez et al., 2015) or from measurements of crop N status (Padilla et al., 2015; Peña-Fleitas et al., 2015). Generally, yield-based sufficiency values are derived from linear-plateau segmented regression analysis (Gianquinto et al., 2004; Ordoñez et al., 2015). This analysis assumes a linear increase in yield with increasing values of the monitored index until yield is maximized after which yield remains constant with higher index values. The breakpoint of this relationship indicates the sufficiency value of the index value for maximum yield. This regression model has been used to calculate sufficiency values of CM measurements in processing tomato (Gianquinto et al., 2004; Gianquinto et al., 2006) and of the specific leaf N content in maize (DeBruin et al., 2013; Ordoñez et al., 2015).

Nitrogen status-based sufficiency values can be derived from the relationships between crop Nitrogen Nutrition Index (NNI) and measured index values by solving the mathematical functions of the relationships for NNI=1. The NNI is an effective and established indicator of crop N status (Lemaire and Gastal, 1997; Mistele and Schmidhalter, 2008; Ziadi et al., 2010) and is calculated as the ratio between actual crop N content and the critical crop N content (i.e. the minimum N content necessary to achieve maximum growth) (Greenwood et al., 1990). Values of NNI=1 correspond to optimal N nutrition (Lemaire and Gastal, 1997). The NNI approach has been used to derive sufficiency values, for maximum growth, of vegetation indices of canopy reflectance and CM measurements (Padilla et al., 2015), and of petiole sap NO_3^- concentration in tomato (Peña-Fleitas et al., 2015).

The greenhouse-based intensive vegetable production system of southeastern (SE) Spain consists of 38,000 ha of relatively simple plastic greenhouses of which the majority are concentrated in the province of Almeria (Castilla and Hernández, 2005; Junta de Andalucia, 2013). Cucumber is one of the most important crops in Almeria, occupying approximately 7,000 ha each year (Reche-Mármol, 2011). Nitrate leaching loss from this system has caused considerable NO_3^- contamination of underlying aquifers (Pulido-Bosch et al., 2000; Thompson et al., 2007). Monitoring cucumber crop N status with CMs is a promising approach to optimize N management.

The objectives of the present work were: i) to evaluate the effectiveness of CM measurements to estimate crop N status and yield, and ii) to derive sufficiency values of CM measurements for maximum crop growth and yield.

2. Materials and methods

2.1. Experimental site

Two cucumber (*Cucumis sativus* ‘Strategos’) crops were grown in soil in a greenhouse under similar conditions to those of commercial vegetable crops in SE Spain. This vegetable production system uses low to medium technology plastic greenhouses very similar to those used throughout the Mediterranean Basin (Pardossi et al., 2004). The experimental work was conducted at the Experimental Station of the University of Almeria (SE Spain, 36° 51’ N, 2° 16’ W and 92 m elevation). The greenhouse had polycarbonate walls and a roof of low density polyethylene (LDPE) tri-laminated film (200 µm thickness) with transmittance to photosynthetically active radiation (PAR) of approximately 60%. It had no heating or artificial light, had passive ventilation (two 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” soil, typical of the region (Thompson et al., 2007), consisting 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. 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 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 soil, prior to adding the gravel layer, consistent with established local practice (Thompson et al., 2007). A detailed description of the soil is provided in Padilla et al. (2016).

Above-ground drip irrigation was used for combined irrigation and mineral fertilizer application. 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 emitters had a discharge rate of 3 L h^{-1}.

The greenhouse was organized into a total of 24 plots, measuring 6 m x 6 m; 20 plots were used in the current study. Sheets of polyethylene film (250 µm thickness) buried to 30 cm depth acted as a hydraulic barrier between plots. This depth was estimated as sufficient to isolate plots given the rooting depth of the crop and the low volumes of irrigation applied by frequent drip irrigation. There were five N treatments with four replicate plots per treatment, arranged in a randomized block design. Each plot 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 N treatment in the northern and southern sides. There were border areas along the edges of the greenhouse.

2.2. Cucumber crops and N treatments

Two cucumber crops were grown in contrasting seasons, the first crop with an Autumn cycle in 2013 (hereafter, Autumn crop) and the second with a Spring cycle in 2014 (hereafter, Spring crop); see Padilla et al. (2016) for details of climatic conditions. In SE Spain, cucumber is grown in both of these seasonal cycles (Reche-Mármol, 2011). The Autumn crop was grown from 5 September to 22 November 2013 (cycle of 78 days); the

Spring crop from 4 March to 28 May 2014 (cycle of 85 days). Both crops were grown from transplanted four week-old seedlings.

There were five treatments of different N concentrations in the nutrient solution applied by fertigation. Once the treatments commenced, they were applied in every irrigation throughout each crop. The different N treatments were applied by fertigation from 15 DAT in the Autumn crop and from 4 DAT in the Spring crop. The N treatments were very N deficient (N1), N deficient (N2), conventional N management (N3), excessive N (N4) and very excessive N (N5), according to the N concentration in the applied nutrient solution. There were slight differences in the applied N concentration between the Autumn and Spring for equivalent treatments. The average applied N concentration in N1, N2, N3, N4 and N5 treatments was 1, 5, 12, 16 and 20 mmol L^{-1}, respectively, for the Autumn crop, and 1, 6, 14, 18 and 21 mmol N L^{-1} for the Spring crop. Considering the applied irrigation volume, the corresponding total amounts of N applied throughout the crops were 12, 62, 218, 280 and 380 kg N ha^{-1} in the Autumn crop, and 16, 109, 344, 472 and 565 kg N ha^{-1} in the Spring crop. Other than N, complete nutrient solutions were applied to all five treatments to ensure that the other macro, secondary and micro-nutrients were not limiting. For all treatments, most N was applied as nitrate (NO_3^-) (92 % of applied N), the rest as ammonium (NH_4^+).

Plants were managed following local practice, being supported by nylon cord guides and being periodically pruned. Irrigation was scheduled to maintain soil matric potential (SMP) in the root zone, at 15 cm depth, within -10 to -30 kPa; one tensiometer (Irrometer, Co., Riverside, Ca, USA) per plot was used to measure SMP. Topping (the removal of the main apical shoot to arrest stem elongation) was conducted at 35 DAT in the Autumn crop, and at 66 DAT in the Spring crop, according to local practice. High temperature within the greenhouse was controlled by white-washing the plastic cladding with a $CaCO_3$ suspension, nine days before transplanting of the Autumn crop (0.30 kg L^{-1})

and on 34 DAT in the Spring crop (0.18 kg L^{-1}). Average levels of midday photosynthetically active radiation (PAR) were 550–800 µmol m^{-2} s^{-1} in the Autumn crop, and 650–1200 µmol m^{-2} s^{-1} in the Spring crop.

2.3. Determination of crop NNI

Above-ground dry matter production (DMP) during the crop was measured by periodic biomass sampling (approximately every 14 days), by removing one complete and representative plant in each replicate plot. The dry matter content of each plant component (stem, leaf and fruit) was determined by oven-drying all the material at 65°C until constant weight. At transplanting, DMP was determined in 100 seedlings. Eight selected plants marked in each replicate plot were used for obtaining pruned material; pruned dry matter was determined as described previously. All mature fruit, of the same eight plants marked for pruning, were harvested periodically and fresh and dry weight determined. All fresh material of each component (stem, leaf and un-harvested fruit) was weighed, and the dry matter percentage was then determined by oven-drying representative sub-samples (approximately 20% of fresh weight) at 65°C until constant weight. Dry matter of sampled component was calculated by multiplying the fresh weight by the dry matter percentage, and then summing the mass of dry matter of the three components. Total DMP, at each biomass sampling was calculated as the sum of dry matter of leaf, shoot and fruit on that sampling date plus all previously sampled pruned material and harvested fruit. Pruned material (mostly young leaf and secondary stem) and harvested fruit accounted for 3% and 46% of total DMP, respectively.

Representative sub-samples of leaf, stem, and fruit from each biomass sampling, pruned material, and harvested fruit from each replicate plot were ground sequentially in knife and ball mills. Total N content (%) of each sub-sample was determined using a

Dumas-type elemental analyzer system (model Rapid N, Elementar, Analysensysteme GmbH, Hanau, Germany). The mass of N in each plant organ was calculated from the %N of the sub-sample and corresponding dry matter of the sample. Above-ground total crop N uptake (kg N ha^{-1}) in each replicate plot, at each biomass sampling, was the sum of N in all relevant components including previous pruned material and harvested fruit as was done for the calculation of total DMP. Total crop N content (%N) was calculated, for each replicate plot, as total crop N uptake divided by total DMP.

The critical N curve derived by Padilla et al. (2016) for greenhouse-grown cucumber ($Nc = 4.85 \cdot DMP^{-0.263}$) was used to calculate Nitrogen Nutrition Index (NNI) values for a given time (Lemaire and Gastal, 1997). NNI values were calculated as the ratio between the total and the critical crop N content (*Nc*) for each treatment for each biomass sampling date. NNI values for each day of the crop were calculated by interpolating dry matter production and crop N content values between the previous and subsequent biomass samplings.

2.4. Crop yield

Yield was measured by periodic harvesting all mature fruit collected from a marked area of 4 m^{-2} that contained eight selected plants (four plants per two paired lines) in each replicate plot. These were the same plants as used to measure pruned material. There were six harvests in the Autumn crop at 49, 54, 60, 64, 70 and 77 DAT, and eight in the Spring crop at 57, 63, 66, 70, 73, 77, 80 and 84 DAT. In each harvest, fruits were separated into marketable and non-marketable categories according to EU marketing regulations (CE 717/2001), and the fresh weight of each was measured; both categories were summed for total yield. Crop yield was the cumulative yield of all harvests for each crop.

2.5. Chlorophyll meter measurements

CM measurements were made with the hand-held, leaf-clip SPAD-502 sensor (Soil Plant Analysis Development, Minolta Camera Co. Ltd., Japan). Measurements commenced at 19 DAT (Autumn crop) and 22 DAT (Spring crop), after the beginning of differential N treatments, and were repeated weekly until the end of each crop. Measurements were always made at the same time each day (7:00 to 9:00 solar time) before fertigation. Individual CM measurements were made on 16 different marked plants in each replicate plot, being four plants located in each of the four central lines of plants in each plot. One measurement per plant was made on the most recently fully expanded and well-lit leaf, on the distal part of the adaxial (top) side of the leaf, midway between the margin and the mid-rib of the leaf. The value for each plot was the average of the 16 individual measurements. All measured plants were visually representative of the replicate plot. Plants in side rows and in border areas were not measured. Leaves with physical damage or with condensed water were not measured, alternative plants being selected. After topping and the associated cessation of new leaf production, measurements were made on the same leaf.

2.6. Extractable chlorophyll content

On two dates of the Spring crop, 56 and 70 DAT, one leaf disk (5.6 mm diameter) was collected in six different plants in each replicate plot using a metal ring, after which the leaf disk was immediately frozen. Leaves and sampling areas were similar to those for SPAD measurements. Leaf Chl was extracted with 80% aqueous acetone solvent following Porra et al. (1989). Each leaf disk was ground with 7.5 mL of the solvent in a homogenizer (IKA T25 digital ULTRA-TURRAX, IKA-Werke GmbH & Co. KG, Staufen, Germany). The homogenate was centrifuged at 2500 rpm for ten minutes (NAHITA model 2655, AUXILAB S.L., Beriain, Spain) and the supernatant was transferred to another tube and the volume was adjusted to 15 mL by addition of the solvent. This extract was diluted 1:8 and the absorbance measured at 646.5 nm, 663.5 nm and 750 nm with a spectrophotometer (Zuzi model 4201/20, AUXILAB S.L.) which had been zeroed and auto-calibrated. The concentration of Chl *a*+*b* was calculated using the equation Chl *a*+*b* = $17.76 \cdot A^{646.6} + 7.34 \cdot A^{663.6}$, where A is the absorbance measurements at the indicated wavelengths that have the absorbance at 750 nm subtracted, as described by Porra et al. (1989). Absorbance was measured at 646.5 nm and 663.5 nm instead of at 646.6 nm and 663.6 as in the procedure of Porra et al. (1989) because of the resolution of the spectrophotometer (0.5 nm). The Chl content of each replicated plot was the average of the six individual leaf disks collected per plot.

2.7. Data analysis

2.7.1. Effects of N treatments on NNI, yield and SPAD measurements

Differences in NNI and in SPAD measurements between N treatments were evaluated by repeated-measures analysis of variance (RM-ANOVA), using time as a within-subjects factor and N treatment as a between-subjects factor. Significant differences between N treatments were determined at $p<0.05$. When necessary to meet the Mauchly's sphericity assumption, Huyn-Feldt (epsilon >0.75) or Greenhouse-Geisser (epsilon <0.75) adjusted degrees of freedom were used (Girden, 1992). Differences in total yield at the end of the crops were separately evaluated for each crop by analysis of variance (ANOVA). When necessary, data were transformed to meet ANOVA assumptions. The IBM SPSS 22 software (IBM Corporation, Armonk, NY, USA) was used.

2.7.2. Relationship between extractable chlorophyll content and SPAD measurements

For the two dates of extractable Chl measurements, regression analyses were conducted to evaluate the nature of the relationship between Chl content and SPAD measurements. Four regression models (linear, quadratic, power and exponential) were considered and the best model, for the two dates of measurement together, was selected using the Akaike Information Criterion (AIC; (Akaike, 1974)), which represents the best compromise between highest goodness of fit and smallest model complexity. The CurveExpert Professional® 2.2.0 software (Daniel G. Hyams) was used to compare models and to retrieve the coefficient of determination (R^2) of the selected model.

2.7.3. Evaluation of SPAD measurements to estimate crop NNI and yield

For each date of SPAD measurement, regression analyses were conducted to evaluate the effectiveness of SPAD measurements to estimate crop NNI and yield. For relationships with NNI, four regression models (linear, quadratic, power and exponential) were considered and the best model for each measurement date was selected using the Akaike Information Criterion (Akaike, 1974). For relationships with yield, segmented linear regression analysis was conducted as this model effectively describes yield responses to N addition (Gianquinto et al., 2011; Ordoñez et al., 2015). A segmented regression consists of two regression lines, an inclined segment described by the equation $y=ax+b$ (if $x<x_0$), and a horizontal segment described by the equation $y=c$ (if $x\geq x_0$), where a is the slope of the inclined segment and b and c are intercepts. The first segment implies a linear increase in yield with increases in N addition, and therefore with SPAD measurements, and the second segment implies that the yield is not increasing at higher N addition and therefore is constant at higher SPAD values. The software GraphPad Prism 6.01 (GraphPad Software, Inc., La Jolla, CA, USA) was used to conduct segmented regression analysis and to obtain values of R^2, SEE and the intersection of the relationship (x_0).

In addition to regression analysis for each date of measurement, the relationship between SPAD measurements with NNI and with yield were examined for phenological phases, by pooling the relevant weekly datasets within each phenological phase of each crop. Three major phenological phases were examined: i) vegetative, ii) reproductive, and iii) harvest period. The vegetative phase was from transplant to the beginning of flowering (defined as an average 5 flowers per plant), which was 0–28 DAT in the Autumn crop and 0–36 DAT in the Spring crop. The reproductive phase included flowering and fruit maturation, which was 29–48 DAT in the Autumn crop and 37–57 DAT in the Spring crop. The harvest phase commenced with the first fruit harvest and ended when the crop finished; the harvest phase being 49–78 DAT in the Autumn crop and 58–84 DAT in the Spring crop. The relationships for phenological phases were also developed for the two crops together; the Akaike Information Criterion was used to obtain the optimal regression model as described previously.

In all cases, regression models were classified, according to R^2 values, as very strong ($R^2 \geq 0.85$), strong ($0.85 > R^2 \geq 0.7$), moderate ($0.7 > R^2 \geq 0.5$), weak ($0.5 > R^2 \geq 0.2$) and very weak ($R^2 < 0.2$).

2.8. Derivation of SPAD sufficiency values for maximum growth and yield

Sufficiency values of SPAD measurements for maximum crop growth and yield were derived, respectively, from the relationships of SPAD measurements to NNI and to yield. For determining sufficiency values for maximum crop growth, the approach of Padilla et al. (2015) was used, in which the mathematical functions relating SPAD measurements to NNI were solved for NNI=1. For yield, the abscissa of the intersection of the two lines of segmented regression (x_0) indicated the value of the SPAD measurement that maximized yield. Sufficiency values were derived independently, for each crop, for each date of measurement and for phenological phases (vegetative, reproductive and harvest). Sufficiency values were also derived for phenological phases for the two crops considered together. Sufficiency values for phenological phases were determined on the data set obtained by grouping all data from the measurements conducted within a given phenological phase, both for individual crops and both crop together. Average values for the entire crop, for the Autumn and Spring crops, were obtained by 1) averaging the sufficiency values from all individual measurements and 2) averaging values from the phenological phases. An average unique value for both crops was calculated by averaging the two average values, calculated from values for phenological phases, of both crops.

3. Results

3.1. Effects of N treatments on NNI, yield and SPAD measurements

Generally, throughout both crops, N1 and N2 treatments had NNI values consistently less than one, whereas N4 and N5 had NNI values greater than one; N3 had NNI values slightly less than one in the Autumn crop and close to one or slightly higher in the Spring crop (Figure 1a,b). Comparing the Spring to the Autumn crop, the N1 treatment had relatively lower NNI values and the N5 had relatively higher NNI values. There were no significant differences between NNI values of the N4 and N5 treatments in either of the two crops.

Yield increased with N addition in both crops reaching a maximum in the N3 treatment, after which yield was relatively constant (Figure 1c,d). In both crops, there were no significant differences in yield between the N3, N4 and N5 treatments.

SPAD measurements generally increased with N addition from N1 to N5; however, there were no significant differences between the N4 and N5 treatments throughout the two crops (Figure 1e,f). SPAD measurements were generally comparable between both crops for equivalent treatments; however, the Spring crop had slightly higher SPAD values in N5 and slightly lower values in N1 compared to the equivalent treatments in the Autumn crop. Topping had a noticeable effect on the temporal dynamics of SPAD measurements particularly in the Spring crop; values dropped immediately after topping at 66 DAT, but thereafter steadily increased.

3.2. Relationship between extractable chlorophyll content and SPAD measurements

Extractable Chl content was positively and strongly related to SPAD measurements with an almost linear relationship (Figure 2). The AIC best fit model was a quadratic equation with a R^2 value of 0.90; however, the linear regression was similarly strong with a R^2 value of 0.89. SPAD measurements increased almost linearly from approximately 35 to 55 SPAD units with increases in Chl content of approximately 20 to 45 µg cm^{-2}, with no indication of saturation or a plateau of SPAD measurements at higher Chl contents.

3.3. Evaluation of the relationship of SPAD to crop NNI and yield for individual dates of measurement

Relationships of SPAD measurements with crop NNI for each measurement date had coefficients of determination (R^2) of 0.04–0.88 in the Autumn crop (average R^2 of 0.59, period 19–76 DAT) and of 0.62–0.97 in the Spring crop (average R^2 of 0.84, period 22–78 DAT) (Table 1). In the Autumn crop, relationships were very weak to weak (R^2 of 0.04–0.38) on the first three dates of measurement (19, 27 and 34 DAT); subsequently R^2 values increased appreciably being above 0.70 from 41 DAT to the end of the crop (Table 1). When excluding the first value of 19 DAT and the first three values for 19, 27 and 34 DAT, the average R^2 values for the Autumn crop were 0.66 and 0.78, respectively.

Coefficients of determination from the segmented regression analysis between SPAD measurements and crop yield for each date of measurement were in the range 0.14–0.79 for the Autumn crop (average R^2 of 0.55, period 19–76 DAT) and 0.74–0.88 in the Spring crop (average R^2 of 0.83, period 22–78 DAT). In the Autumn crop, relationships were very weak or weak (R^2 of 0.14–0.42) in the first three dates of measurement (19, 27 and 34 DAT) and were moderate or strong (R^2 of 0.59–0.79) thereafter (Table 2).

3.4. Evaluation of the phenological relationships of SPAD to crop NNI and yield

For the vegetative phase, relationships of SPAD measurements with crop NNI were very weak (Autumn crop, R^2 of 0.11) and weak (Spring crop, R^2 of 0.42) considering each crop separately, and were also weak when the two crops were considered together (R^2 of 0.21) (Table 3, Figure 3a). The relationships improved appreciably for the reproductive and harvest phases, when the two crops were considered independently and when they were combined (Table 3). For the reproductive phase, there was a moderate relationship in the Autumn crop (R^2 of 0.61) and a very strong relationship in the Spring crop (R^2 of 0.92) (Figure 3b); the relationship was strong when the two crops were considered together (R^2 of 0.77). For the harvest phase, the relationship was strong for the Autumn crop (R^2 of 0.71), and moderate for the Spring crop (R^2 of 0.66) and for the combination of both crops together (R^2 of 0.55) (Figure 3c).

For relationships of SPAD measurements with crop yield, very weak relationships were obtained for the vegetative phase in the Autumn crop (R^2 of 0.04) and for the two crops combined (R^2 of 0.17) (Table 4, Figure 4a). A moderate relationship was obtained for the Spring crop (R^2 of 0.64). The relationships improved for the reproductive and harvest phases (Table 4). In these two phases, moderate relationships were obtained in the Autumn crop (R^2 of 0.50 for the reproductive and 0.62 for the harvest) and for the two crops together (R^2 of 0.65 and 0.60, respectively); relationships were strong for the Spring crop in both phases (R^2 of 0.84 and 0.74, respectively) (Figure 4b,c). The slopes of the inclined segment of segmented regressions were steeper in the reproductive than in the harvest phase, particularly in the Autumn crop and in the two crops together, showing higher sensitivity of crop yield to changes in SPAD values in the reproductive than in the harvest phase. The slopes were steeper in the Spring than in the Autumn crop, indicating higher sensitivity in the Spring crop.

3.5. SPAD sufficiency values for maximum growth and yield

For each measurement date, SPAD sufficiency values for maximum growth were 42.9–49.7 SPAD units in the Autumn crop (average value for all measurement dates of 46.7±0.8) and 40.2–49.9 SPAD units in the Spring crop (average value for all dates of 44.7±1.3) (Table 5). In the Autumn crop, sufficiency values for maximum yield were 35.1–53.1 SPAD units (average value for all measurement dates of 45.5±1.8); in the Spring crop, sufficiency values were 36.4–44.7 SPAD units (average value for all dates of 41.8±0.8) (Table 5). SPAD sufficiency values for maximum yield were comparable to the sufficiency values for growth for the Autumn crop, and they were slightly lower than those for maximum growth in the Spring crop.

For individual phenological phases, SPAD sufficiency values for both crops considered together, for both maximum growth and yield, were generally intermediate to the equivalent sufficiency values, for each phenological phase, calculated for each crop (Table 5). For the two crops combined, sufficiency values for maximum growth were 47.5, 46.9 and 44.9 SPAD units, for the vegetative, reproductive and harvest phases, respectively; the average value for all three phenological phases was 46.4±0.8. For maximum yield, the sufficiency values for the two crops combined were slightly lower being 44.0, 42.8 and 44.9 SPAD units, for the vegetative, reproductive and harvest phases, respectively, giving an average sufficiency value for the entire crop (considering both crops together) of 43.9±0.6 SPAD units. The average sufficiency value for the entire crop for both maximum growth and maximum yield was 45.2±0.7 SPAD units.

4. Discussion

4.1. Evaluation of relationships of SPAD measurements to crop NNI and yield

Strong and very strong relationships were obtained between SPAD measurements and crop NNI, and between SPAD measurements and yield, for most dates of individual measurement in the two cucumber crops grown in different growing seasons. These results are consistent with those of other studies that reported similarly strong relationships between CM measurements and crop N status and/or yield in horticultural crops such as fresh tomato (Padilla et al., 2015; Wu et al., 2012), processing tomato (Farneselli et al., 2010; Gianquinto et al., 2006), muskmelon (Padilla et al., 2014) and potato (Gianquinto et al., 2004; Olivier et al., 2006).

When considering individual phenological phases, there were strong relationships of SPAD measurements with crop NNI and with yield for reproductive and harvest phases, for each of two crops when considered separately and together. These strong relationships in most phenological phases, regardless of growing season, demonstrated the robust ability of SPAD measurements to be indicators of crop NNI and yield, which is consistent with the strong relationships reported by Wu et al. (2012) between SPAD measurements and leaf N content in individual growth stages of indeterminate tomato.

There were some individual measurements where SPAD measurements were poorly related to crop NNI and yield during the early and latter parts of the crops. In the early part of the crops, it is likely that uncertainties with the initial adjustments to the fertigation system to establish the N treatments influenced the small young plants. Relatively weak relationships in the final measurement dates were presumably associated with mottling and discoloration that occurred in the measured leaves during the final weeks of the crop. Previous research with indeterminate tomato (Padilla et al., 2015) also reported weaker relationships in the final part of the crop cycle.

There was no evidence of saturation of SPAD measurements when related to crop NNI values in either of the two crops; regression analysis showed that SPAD measurements increased when NNI values exceeded the optimal value for crop growth of 1 (Figure 3). There is a commonly-held view that CM measurements saturate at higher crop N contents associated with excessive N fertilization (Fox and Walthall, 2008). However, in addition to the present study, the saturation effect at higher crop N contents has not been observed in numerous field studies with crops (Blackmer et al., 1994; Gianquinto et al., 2004; Padilla et al., 2015; Schepers et al., 1996). It has been shown that the relationship of CM measurements to extractable leaf Chl content plateaus off at high leaf Chl contents (Cartelat et al., 2005; Markwell et al., 1995; Monje and Bugbee, 1992), but concentrations of leaf Chl sufficiently high to saturate CM measurements may not occur under field conditions with crop species, even with clearly excessive N fertilization. In the present study, the relationship of CM measurements to extractable leaf Chl content was nearly linear over the entire range of measured Chl contents. The range of measured Chl contents in the present study (approximately 20–45 µg cm^{-2}) was appreciably narrower that that reported by Monje and Bugbee (1992) (approximately 0–70 µg cm^{-2}) and Cartelat et al. (2005) (approximately 20–70 µg cm^{-2}) where saturation of CM measurements was reported.

While there was no saturation of SPAD measurements in relation to NNI or extractable chlorophyll content, there was no difference in SPAD measurements between treatments N4 and N5, indicating a plateau effect in relation to applied N. It appears that in both these treatments that a similar degree of luxury N uptake occurred as suggested by the similar NNI values of treatments N4 and N5, and that within the range of crop N content determined, as suggested by NNI data, that SPAD measurements were related to crop N content.

4.2. Evaluation of phenological relationships

For each crop, the establishment of phenological relationships enabled a substantial reduction in the number of relationships, from nine weekly individual relationships to one relationship for each phenological phase. It is notable that SPAD measurements were strongly related to crop NNI and yield in the reproductive and harvest phases when using the combined dataset, for each phase, from the two crops. This demonstrated that common relationships can be applied to both Autumn and Spring crop cycles. Relationships for phenological phases and the derivation of associated sufficiency values can facilitate the use of CMs and other monitoring devices because measurements are associated to easily-recognizable crop development phases, compared to chronological age (Padilla et al., 2015). The use of chronological age has the limitation that it does not consider differences in crop development caused by different growing conditions of each crop cycle, being mainly irradiance and temperature. However, a disadvantage of the use of phenological relationships is the weaker R^2 values, compared to those of individual measurement dates, as observed in the current study and by Padilla et al. (2016); this is a common consequence of the larger data point dispersion associated with pooling data from different dates of measurement.

The three phenological phases were relevant for using SPAD measurements to obtain maximum yield particularly in the Spring crop. In the Autumn crop and in the two crops combined, the slope of the inclined segment of segmented regression between SPAD measurements and yield was steeper in the reproductive and harvest phases than in the vegetative phase, indicating higher sensitivity of crop yield to changes in SPAD values during the reproductive and harvest phases. In the Spring crop, the highest sensitivity of yield to SPAD values (i.e. highest slope of the inclined segment of segmented regression) was observed in the vegetative phase, followed by lower sensitivity in the reproductive and harvest phases. The large difference in sensitivity in the vegetative phase between the Autumn and Spring crops, is likely attributable to issues associated with the establishment of the differential N concentration treatments in the Autumn crop, that was referred to previously. The high sensitivity in the vegetative phase and subsequent phases of the Spring crop suggests that there is potential to use the SPAD meter throughout a cucumber crop and to use it to make early adjustments to N nutrition.

4.3. SPAD sufficiency values

For each date of measurement, there were generally small differences between sufficiency values of SPAD measurements for maximum growth and yield for each crop. Similarly, there were generally small differences between sufficiency values for the Autumn and the Spring crop, for similar dates of measurement. These results suggest that either set of sufficiency values (maximum growth or maximum yield) may be used for optimal N management of cucumber crops.

Sufficiency values for each phenological phase for the combined sets of the two crops combined were similar to one another, and in general, were intermediate to the sufficiency values derived for each phenological phase for each crop, suggesting that the values derived for the two crops together could be used for both cropping seasons. The maximum difference between sufficiency values for maximum growth obtained for each of the three individual phenological phases with the combined data set for both crops was 2.6 SPAD units, and the maximum difference between the equivalent sufficiency values for maximum yield was 2.1 SPAD units. Given the ranges of SPAD units measured over the two crop cycles, these differences are relatively small, suggesting that the average sufficiency value for the three phases combined can be used for the entire crop cycle. The calculated unique sufficiency value for the entire crop for both maximum growth and maximum yield of 45.2±0.7 SPAD units is consistent with the sufficiency value of 44.9 SPAD units reported by Güler and Büyük (2007) for cucumber.

Frequent monitoring with the SPAD meter in combination with sufficiency values provides a method for rapid correction of non-optimal crop N status for maximum growth and yield by providing information of required adjustments to mineral N fertilizer application. Where N is applied in most irrigations using combined drip irrigation and fertigation systems (Granados et al., 2013; Thompson et al., 2007), monitoring with the SPAD meter will enable any required adjustments to the N supply to be made very soon after measurement, given the availability of relevant sufficiency values (Thompson et al., 2017). The SPAD sensor has several attractive practical characteristics: measurements can be made easily, quickly and frequently throughout a crop, the results are rapidly available, and there are no time delays and logistical issues as with methods involving laboratory analysis. However, further research is required to validate the sufficiency values, reported in the current work, in different cucumber crops and cultivars.

5. Conclusions

This study showed that chlorophyll measurements of the SPAD-502 sensor can be used as reliable estimators of crop N status and yield throughout most of the cycle of cucumber crops grown in Autumn and Spring growing seasons. SPAD measurements were consistent between the two different cropping seasons, which enabled the development of sufficiency values for maximum growth and maximum yield that were applicable to cucumber crops regardless of the cropping season. The use of sufficiency values for phenological phases of the crop may improve N management of frequently fertigated cucumber crops, by providing information on adjustments in N fertilization that are required when SPAD measurements deviate from sufficiency values.

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TABLES AND FIGURES

Table 1. Relationships of SPAD measurements with crop Nitrogen Nutrition Index (NNI) for each individual measurement date of each of the two cucumber crops. Coefficient of determination (R^2) and standard error of the estimate (SEE) of the best regression model (Akaike Information Criterion) are shown. DAT is days after transplanting. *n*=20 in each individual day.

Crop	Phenological phase	DAT	Model	R^2	SEE
Autumn	Vegetative	19	Power	0.04	0.08
		27	Exponential	0.25	0.11
	Reproductive	34	Quadratic	0.38	0.14
		41	Quadratic	0.78	0.10
		48	Power	0.88	0.10
	Harvest	55	Exponential	0.71	0.16
		62	Power	0.79	0.13
		69	Power	0.75	0.14
		76	Power	0.77	0.12
Spring	Vegetative	22	Power	0.83	0.04
		29	Quadratic	0.75	0.05
		36	Quadratic	0.90	0.05
	Reproductive	43	Linear	0.91	0.07
		50	Linear	0.95	0.07
		57	Quadratic	0.97	0.07
	Harvest	65	Linear	0.89	0.13
		71	Quadratic	0.72	0.21
		78	Linear	0.62	0.25

Table 2. Relationships of SPAD measurements with crop yield for each individual measurement date of each of the two cucumber crops. Coefficient of determination (R^2) and standard error of the estimate (SEE) of segmented regressions are shown. DAT is days after transplanting. *n*=20 in each individual day.

Crop	Phenological phase	DAT	R^2	SEE
Autumn	Vegetative	19	0.14	1.71
		27	0.42	1.41
	Reproductive	34	0.29	1.56
		41	0.79	0.84
		48	0.77	0.89
	Harvest	55	0.66	1.07
		62	0.70	1.01
		69	0.59	1.18
		76	0.60	1.17
Spring	Vegetative	22	0.81	1.59
		29	0.74	1.83
		36	0.85	1.40
	Reproductive	43	0.88	1.24
		50	0.87	1.33
		57	0.88	1.25
	Harvest	65	0.85	1.40
		71	0.83	1.47
		78	0.79	1.66

Table 3. Relationships of SPAD measurements with crop NNI, for each phenological phase, for each crop when considered independently and with the combined dataset of the two crops. Coefficient of determination (R^2), standard error of the estimate (SEE), number of data points in regression (*n*) and equation of the best regression model (Akaike Information Criterion) are shown.

Crop	Phenological phase	Model	R^2	SEE	*n*	Equation
Autumn	Vegetative	Quadratic	0.11	0.08	40	$NNI=0.002\cdot SPAD^2-0.139\cdot SPAD+3.387$
	Reproductive	Quadratic	0.61	0.13	60	$NNI=-0.002\cdot SPAD^2+0.192\cdot SPAD-4.033$
	Harvest	Power	0.71	0.15	80	$NNI=0.001\cdot SPAD^{1.810}$
Spring	Vegetative	Quadratic	0.42	0.09	60	$NNI=-0.001\cdot SPAD^2+0.116\cdot SPAD-1.768$
	Reproductive	Linear	0.92	0.09	60	$NNI=0.046\cdot SPAD-1.240$
	Harvest	Quadratic	0.66	0.22	60	$NNI=-0.003\cdot SPAD^2+0.285\cdot SPAD-6.072$
Autumn+Spring	Vegetative	Exponential	0.21	0.12	100	$NNI=\cdot 0.536e^{SPAD\cdot 0.013}$
	Reproductive	Quadratic	0.77	0.12	120	$NNI=-0.001\cdot SPAD^2+0.151\cdot SPAD-3.341$
	Harvest	Quadratic	0.55	0.20	140	$NNI=-0.001\cdot SPAD^2+0.167\cdot SPAD-3.485$

Table 4. Relationships of SPAD measurements with crop yield, for each phenological phase, for each crop when considered independently and with the combined dataset of the two crops. Coefficient of determination (R^2), standard error of the estimate (SEE), number of data points in regression (*n*) and equation of the segmented regression are shown.

Crop	Phenological phase	R^2	SEE	*n*	Equation
Autumn	Vegetative	0.04	1.72	40	Yield=0.523·SPAD-11.980
	Reproductive	0.50	1.24	60	Yield=0.523·SPAD-11.980
	Harvest	0.62	1.07	80	Yield=0.277·SPAD-2.977
Spring	Vegetative	0.64	2.06	60	Yield=1.795·SPAD-58.380
	Reproductive	0.84	1.39	60	Yield=0.950·SPAD-31.580
	Harvest	0.74	1.74	60	Yield=0.912·SPAD-28.390
Autumn+Spring	Vegetative	0.17	2.65	100	Yield=0.289·SPAD-2.019
	Reproductive	0.65	1.62	120	Yield=0.936·SPAD-29.430
	Harvest	0.60	1.66	140	Yield=0.546·SPAD-14.110

Table 5. Sufficiency values of SPAD measurements for maximum growth and maximum yield for each individual measurement date of each crop, and for the three phenological phases for each crop when considered independently and with the combined dataset of the two crops. DAT is days after transplanting. Averages ± standard error are shown.

Crop	DAT	Sufficiency values for individual days of measurement		Phenological phase	Sufficiency values for phenological phases	
		Growth	Yield		Growth	Yield
Autumn	19	44.3	35.1	Vegetative	46.2	38.4
	27	46.5	41.6			
	34	42.9	42.2	Reproductive	46.1	43.5
	41	44.4	42.8			
	48	48.7	48.7			
	55	49.1	50.1	Harvest	48.6	49.8
	62	49.7	53.1			
	69	48.3	49.0			
	76	46.7	46.6			
	Average	46.7±0.8	45.5±1.8	Average	47.0±0.8	43.9±3.3
Spring	22	41.3	40.9	Vegetative	44.4	38.2
	29	40.2	36.4			
	36	46.8	41.4			
	43	49.3	43.4	Reproductive	48.9	44.3
	50	49.9	44.7			
	57	46.4	43.3			
	65	40.6	40.4	Harvest	41.8	42.2
	71	42.0	43.4			
	78	46.2	42.4			
	Average	44.7±1.3	41.8±0.8	Average	45.0±2.1	41.6±1.8
Autumn+Spring				Vegetative	47.5	44.0
				Reproductive	46.9	42.8
				Harvest	44.9	44.9
				Average	46.4±0.8	43.9±0.6
					45.2±0.7	

Figure 1. Temporal dynamics of (a,b) the nitrogen nutrition index (NNI), (c,d) cumulative yield, and (e,f,) SPAD measurements during the two cucumber crops subjected to five different N treatments. Patterned boxes on the x-axis indicate the vegetative, reproductive and harvest phases. Arrows on the x-axis mark the date of topping. The dashed horizontal line in panels a) and b) shows a NNI value of 1. Values are means ± standard errors. DAT: days after transplanting.

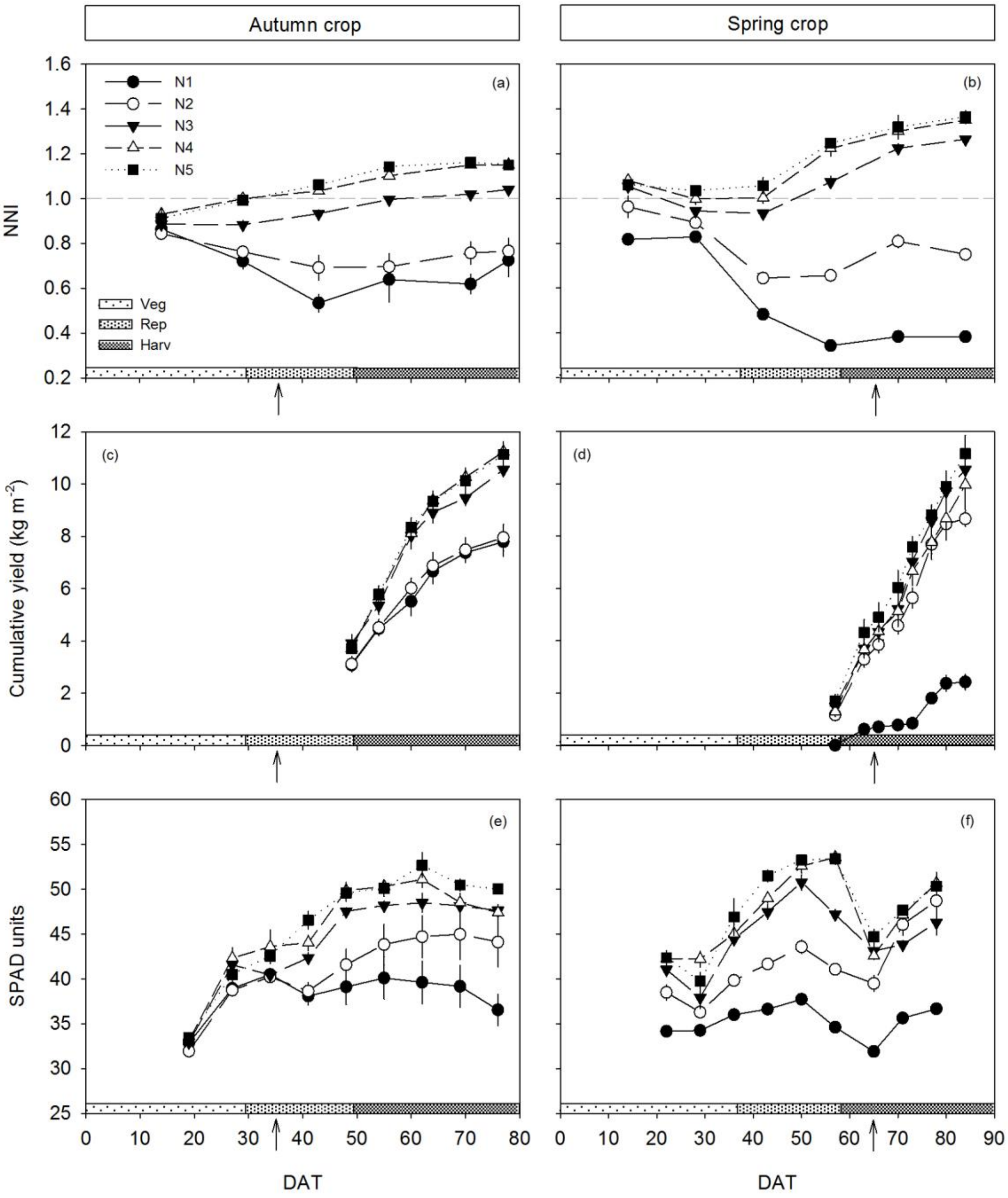

Figure 2. Relationship between extractable chlorophyll content and SPAD measurements combining two separate sampling days of the Spring crop (56 and 70 DAT). Coefficient of determination (R^2) and equation of quadratic regression are shown.

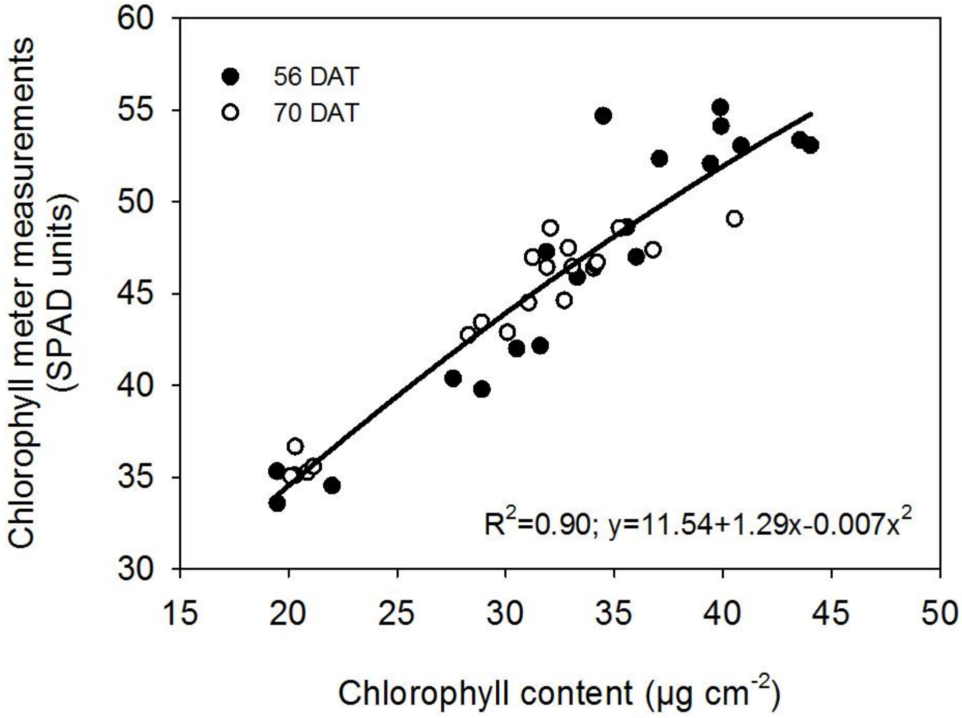

Figure 3. Relationships of SPAD measurements with crop NNI, for each phenological phase, for each crop independently (dashed grey lines) and for the combined dataset of the two crops (solid black lines). The coefficient of determination (R^2) of the best regression model is shown. Each data point represents the average of 16 SPAD measurements performed in each of four replicated plots for each of the five N treatments in a given day of measurement.

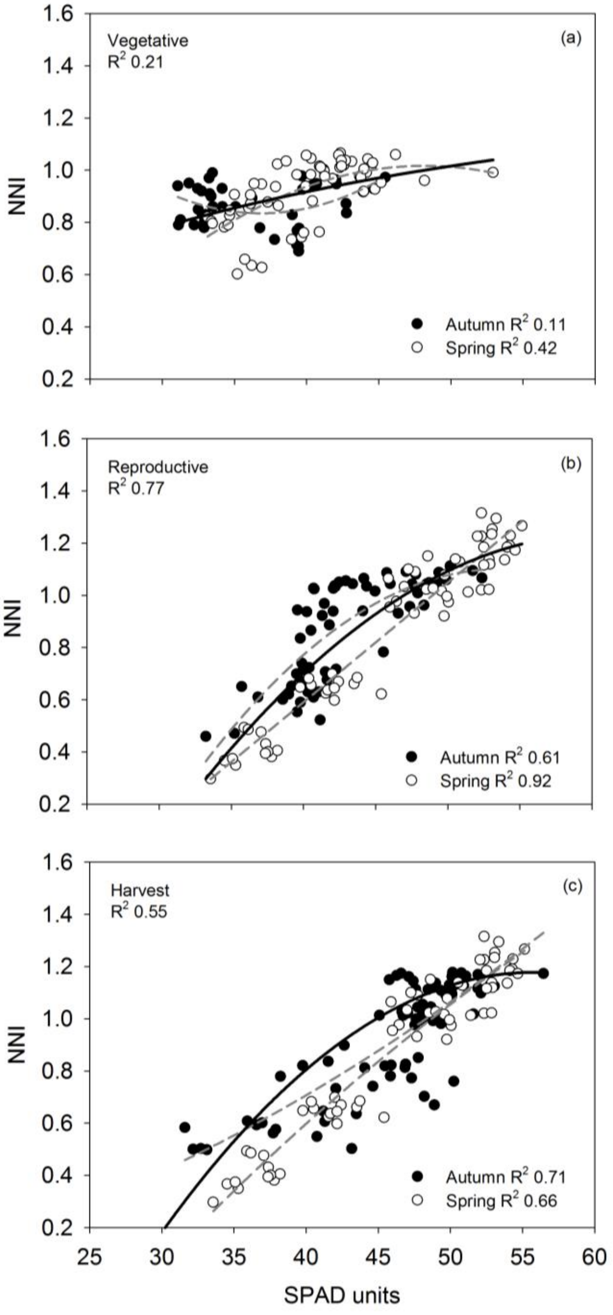

Figure 4. Relationships of SPAD measurements with crop yield, for each phenological phase, for each crop independently (dashed grey lines) and for the combined dataset of the two crops (solid black lines). The coefficient of determination (R^2) of the segmented regression is shown. Each data point represents the average of 16 SPAD measurements performed in each of four replicated plots for each of the five N treatments in a given day of measurement.