Departamento de Agronomía

Escuela Superior de Ingeniería

Universidad de Almería

Monitoring with proximal optical sensors to optimize N management of greenhouse vegetable crops

Ph.D. Dissertation

Stefani Romina de Souza Ibarra

Almería, septiembre 2020

Monitoring with proximal optical sensors to optimize N management of greenhouse vegetable crops

Ph.D. Dissertation

Monitorización con sensores ópticos proximales para optimizar el manejo del N en cultivos hortícolas bajo invernadero

Tesis Doctoral

Stefani Romina de Souza Ibarra

Almería, septiembre 2020

Autorización de los Directores

Esta tesis, "Monitorización con sensores ópticos proximales para optimizar el manejo del N en cultivos hortícolas bajo invernadero", se presenta para aspirar al título de Doctor con Mención Internacional por la Universidad de Almería de:

Stefani Romina de Souza Ibarra

Esta tesis se realizó en el Departamento de Agronomía de la Universidad de Almería, dentro del programa de doctorado "Agricultura Protegida", en la línea de investigación "Control Climático y Gestión del Agua en Agricultura Protegida".

Almería, junio de 2020

Francisco Manuel Padilla Ruiz	Rodney B. Thompson
Director	Co-director

Acknowledgements

First of all, I would like to thank my supervisors Paco Padilla and Rodney Thompson, and my tutor Marisa Gallardo, for giving me the opportunity to accomplish this work in their research group, for their support and follow-up and for trusting me. In particular, I would like to thank Paco Padilla for his tireless help and collaboration throughout the process of data analysis and preparation of this work.

I would also like to thank Tere Peña, who helped and guided me in the field and in the laboratory in obtaining the experimental data where I learned a lot.

Special thanks go to all the UAL-ANECOOP staff, especially to Paco Palmero and Pepe, for their great dedication and contribution.

I am thankful to Urs Schmidhalter and his group for hosting me at the Technical University of Munich and making me feel a member of the group during my research stay.

I acknowledge with thanks the Ministry of Economy and Competitiveness of Spain for funding the project, funded by the AGL2015-67076-R grant, in which this thesis was conducted, as well as the FPI grant (BES-2016-076706) awarded to me to conduct my doctoral studies, which also included funding for the international research stay at the Technical University of Munich.

Finally, I would like to express my gratitude to my family for their unconditional support throughout this process. Finally, I thank my friends and co-workers who, in one way or another, have supported this effort.

Agradecimientos

En primer lugar, agradecer a mis directores de tesis Paco Padilla y Rodney Thompson, y a mi tutora Marisa Gallardo, por haberme dado la oportunidad de realizar este trabajo en su equipo, por su apoyo y seguimiento y por haber confiado en mí. En especial a Paco Padilla por su incansable ayuda y colaboración en todo el proceso de procesamiento de datos y elaboración de este trabajo.

También quisiera expresar mis agradecimientos a Tere Peña quién me ayudó y guio en la etapa de obtención de datos en campo y laboratorio, donde he aprendido mucho.

Agradecer a todo el personal de la finca UAL-ANECOOP, especialmente a Paco Palmero y Pepe por sus grandes aportes.

Agradecer a Urs Schmidhalter y todo su equipo en la Universidad Técnica de Munich por haberme recibido y haberme hecho sentir parte del grupo durante mi estancia de investigación.

También dar las gracias al Ministerio de Economía y Competitividad de España por el financiamiento del proyecto AGL2015-67076-R en el cual estuvo enmarcada esta tesis, así como por otorgarme una beca FPI (BES-2016-076706) para conducir mis estudios doctorales la cual incluyó también el financiamiento de la estancia internacional.

También quisiera expresar todo mi agradecimiento a mi familia, por su apoyo incondicional a lo largo de este trayecto. Por último, dar las gracias a mis amigos y compañeros de trabajo que, de un modo u otro, han respaldado este esfuerzo.

Table of contents

Scientific contributions during the predoctoral stage

Published JCR articles included in the doctoral thesis

de Souza, R., Grasso, R., Teresa Peña-Fleitas, M., Gallardo, M., Thompson, R.B., Padilla, F.M., 2020. Effect of cultivar on chlorophyll meter and canopy reflectance measurements in cucumber. *Sensors* 20(2): 509. https://doi.org/10.3390/s20020509. JCR impact factor: 3.031. Quartile: Q1.

de Souza, R., Peña-fleitas, M.T., Thompson, R.B., Gallardo, M., Grasso, R., Padilla, F.M., 2019. The use of chlorophyll meters to assess crop N status and derivation of sufficiency values for sweet pepper. *Sensors* 19(13): 2949. https://doi.org/doi:10.3390/s19132949. JCR impact factor: 3.031. Quartile: Q1.

Padilla, F.M., **de Souza, R.,** Peña-Fleitas, M.T., Grasso, R., Gallardo, M., Thompson, R.B., 2019. Influence of time of day on measurement with chlorophyll meters and canopy reflectance sensors of different crop N status. *Precision Agriculture* 20(6): 1087-1106. https://doi.org/10.1007/s11119-019-09641-1. JCR impact factor: 3. 356. Quartile: Q1.

Other published JCR articles not included in the doctoral thesis

de Souza, R., Peña-fleitas, M.T., Thompson, R.B., Gallardo, M., Padilla, F.M., 2020. Assessing Performance of Vegetation Indices to Estimate Nitrogen Nutrition Index in Pepper. *Remote Sensing*. 12, 1–19. https://doi.org/10.3390/rs12050763. JCR impact factor: 4.118. Quartile: Q1.

Gallardo, M., Padilla, F.M., Peña-Fleitas, M.T., **de Souza, R.,** Rodríguez, A., Thompson, R.B., 2020. Crop response of greenhouse soil-grown cucumber to total available N in a Nitrate Vulnerable Zone. *European Journal of Agronomy* 114: 125993. https://doi.org/10.1016/j.eja.2019.125993. JCR impact factor: 3.384. Quartile: Q1.

Padilla, F.M., **de Souza, R.,** Peña-Fleitas, M.T., Gallardo, M., Giménez, C., Thompson, R.B., 2018. Different responses of various chlorophyll meters to increasing nitrogen supply in sweet pepper. *Frontiers in Plant Science* 9: 1752. https://doi.org/10.3389/fpls.2018.01752. JCR impact factor: 4.106. Quartile: Q1.

Padilla, F.M., Gallardo, M., Peña-Fleitas, M.T., **de Souza, R.,** Thompson, R.B., 2018. Proximal optical sensors for nitrogen management of vegetable crops: A review. *Sensors* 18: 2083. https://doi.org/10.3390/s18072083. JCR impact factor: 3.031. Quartile: Q1.

Proceedings and conference communications

de Souza, R., Grasso, R., Peña-Fleitas, M.T., Gallardo, M., Thompson, R.B., Padilla, F.M. 2019. Efecto del cultivar en las medidas de medidores de clorofila e índices de vegetación en pepino., in: II Congreso Jóvenes Investigadores Ciencias Agroalimentarias de la Universidad de Almería. 17 October 2019. Almería, Spain pp. 75–78. Type of presentation: oral.

Padilla, F.M., **de Souza, R.,** Peña-Fleitas, M.T., Gallardo, M., Gimenez, C., Thompson, R.B. 2019. Diferente respuesta de saturación de varios medidores de clorofila a niveles crecientes de nitrógeno., in: VII Jornadas del Grupo de Fertilización de la Sociedad Española de Ciencias Hortícolas "Una fertilización más eficiente es posible". 23 and 24 January 2019. Valencia, Spain. Type of presentation: oral

de Souza, R., Thompson, R.B., Padilla., F.M. 2018. Use of optical sensors for nitrogen management. Application in horticulture. In: Reunión final de la Red SIRENA. 6 and 7 November 2018. Universidad Politécnica de Madrid. Spain. Type of presentation: oral.

de Souza, R., Giménez, C., Thompson, R.B., Padilla., F.M. 2018. Assessment of various chlorophyll meters to estimate leaf chlorophyll content in sweet pepper. In: EUVRIN 2nd Workshop Fertilization and Irrigation. 13 and 14 September 2018. Bleiswijk, The Netherlands. Type of presentation: oral.

de Souza, R., Giménez, C., Thompson, R.B., Padilla, F.M. 2017. Presentación de resultados del ensayo Comparación de medidores de clorofila para estimar el contenido de clorofila en pimiento. In: Jornada técnica de la Red SIRENA-Plan Nacional I+D+i de

Excelencia: Uso de sensores para la estimación del estado nutricional de los cultivos. 13 and 14 November 2017. Córdoba, Spain. Type of presentation: oral.

Padilla, F.M., Peña-Fleitas, M.T., **de Souza, R.,** Gallardo, M., Giménez, C., Thompson, R. 2017. Sensitivity of different chlorophyll meters to estimate leaf chlorophyll in sweet pepper. In: ECPA 2017 - 11th European Conference on Precision Agriculture. 16 to 20 July 2017. John McIntyre Centre, University of Edinburgh, UK. Type of presentation: oral.

1. Summary

1.1. Summary

Intensive vegetable systems are commonly associated with appreciable nitrate contamination of water bodies, as occurs in the greenhouse-based system of southeast (SE) Spain. This contamination is associated to the nitrogen (N) fertilizer and irrigation applications used to ensure high levels of production. To reduce this contamination, optimal N management is required to reduce the N losses to the environment and improve N use efficiency. There are several tools that can be used to assess crop N status and which could improve the N use efficiency. The use of these tools will provide vegetable growers with the ability to match the N supply to crop N demand throughout the growing season. An effective and rapid means to assess crop N status is the use of proximal optical sensors. These sensors do not directly measure N content in plant tissue, but provide measurements of optical properties that are indicative of crop N status. There are three main groups: chlorophyll meters, reflectance sensors, and fluorescence-based flavonols meters. These tools have demonstrated their capacity to assess N crop status in field crops, but relatively few studies have been conducted in greenhouse-based vegetable crops. This thesis has evaluated the capacity of chlorophyll meters and several vegetation indices calculated from canopy reflectance sensors to assess crop N status in sweet pepper and cucumber crops grown in soil in greenhouse in Almería, in SE Spain.

The experimental work was conducted in a greenhouse located at the Experimental Station of the University of Almeria, very similar to those used for commercial production of Almería. In this thesis, the data used were collected in three sweet pepper crops (*Capsicum annuum* 'Melchor') and a cucumber crop (*Cucumis sativus* L.), grown with fertigation and planted in an *enarenado* soil. The sweet pepper crops were subjected to five N treatments applied in each irrigation throughout the crop, ranging from a very deficient to very excessive N supply. The cucumber crop used three different cultivars that were subjected to three N treatments ranging from a very deficient to very excessive N supply. Periodic biomass samplings were taken throughout the crops and the N content of the crops was determined. The critical N curve derived for sweet pepper in this work was used to calculate the nitrogen nutrition index (NNI), which was used as a

measure of crop N status. In the cucumber crop, periodic leaf samples were collected and leaf N content was analysed as a proxy of crop N content.

Different measurements with optical sensors were conducted periodically throughout the crops. Three chlorophyll meters (SPAD-502, atLEAF+, and MC-100) and two canopy reflectance sensors (GreenSeeker handheld and Crop Circle ACS-470) were used. Their sensitivity to assess crop N status and the consistency of measured values throughout the crops were evaluated. The sensitivity of chlorophyll meters to assess crop N status was analysed for four phenological stages (vegetative, flowering, early fruit growth and harvest) in the three sweet pepper crops, and sufficiency values for maximum growth were calculated for the same phenological stages. The influence of the time of day in optical sensor measurements was evaluated by measuring at 9:00, 12:00, 15:00 and 18:00 h solar time, in one sweet pepper crop. The effect of cultivar on optical sensor measurements was evaluated with three cucumber cultivars, 'Strategos', 'Pradera' and 'Mitre'.

In general, the research conducted in this thesis has shown that chlorophyll meter measurements were strongly related to crop N status (NNI) in all phenological stages of the three sweet pepper crops, indicating that these measurements are good indicators of the crop N status of pepper. The sufficiency values determined for each phenological stage for SPAD-502 and atLEAF+, were very consistent between the different years. This demonstrated the potential for using these meters with sufficiency values to improve the N management of commercial sweet pepper crops. For the MC-100 there was only one crop; so, it was not possible to assess the consistency of sufficiency values between crops.

For the chlorophyll meters SPAD-502 and MC-100, there were significant differences between the cultivars of cucumber, particularly when N supply was sufficient and excessive, but not when N supply was deficient. However, there were no consistent differences between cultivars in vegetation indices evaluated (NDVI, GNDI, RVI, and GVI) which were measured with the canopy reflectance sensor Crop Circle ACS 470. The optical measurements made with both chlorophyll meters and canopy reflectance sensors, were strongly and linearly related to leaf N content in each of the three cucumber cultivars. Cultivar had a significant effect on the relationship between leaf N content and chlorophyll meter measurements, but not on the relationships between leaf N content and canopy reflectance vegetation indices. The lack of a consistent effect of cultivar, on the relationship with leaf N content, suggests that a unique equation to estimate leaf N content from vegetation indices can be applied to all three cultivars. This unique equation, however, may not be applied for chlorophyll meter measurements because of the significant cultivar effect that was detected.

Time of day had a slight effect on relative chlorophyll measurements made with the SPAD-502 meter, only in the very deficient N treatment, suggesting that the effects of time of day on SPAD-502 readings were related to crop N status. Regarding the MC-100 meter, there was a slight increase in the values in the measurements made at 15:00 and at 18:00 hours solar time, compared to measurements at 9:00, regardless of the N treatment. The NDVI index, measured both with the GreenSeeker handheld and the Crop Circle ACS- 470, the GNDVI and the GVI, measured with the Crop Circle, were not affected by time of day in any of the N treatments of this study. These results show that these sensors and indices can be used with confidence at any time of the day.

Overall, the results of this thesis show the potential of optical sensors measurements (chlorophyll meters and canopy reflectance sensors) to assess crop N status in sweet pepper and cucumber. The sufficiency values of chlorophyll meters calculated for sweet pepper (‘Melchor’), were consistent throughout three crops, which demonstrated the potential for using these meters to improve the N management of commercial sweet pepper crops. The effect of cultivar observed with cucumber for different chlorophyll meters suggests that care should be taken when using sufficiency values that were determined for a particular cultivar, on different cultivars. For different cultivars, normalization procedures may be needed in these latter cases. On the contrary, the lack of differences in vegetation indices (NDVI, GNDI, RVI, and GVI) between these cultivars, suggests that they could be used to assess crop N status in cucumber. Regarding the lack of consistent effect of time of measurement on optical sensors measurements, results showed that these sensors and indices can be used with confidence at any time of the day.

1.2. Resumen

La producción intensiva de hortalizas bajo invernadero se asocia comúnmente con una apreciable contaminación por nitrato de los cuerpos de agua. Esta situación puede deberse a la gran cantidad de fertilizante nitrogenados y la aplicación de agua utilizados para garantizar altos niveles de producción. Por esto, la gestión óptima del nitrógeno (N) es esencial para reducir las pérdidas al medio ambiente y mejorar la eficiencia en el uso del N. Existen varias herramientas que pueden usarse para evaluar el estado de N del cultivo y que podrían mejorar la eficiencia del uso del N. Estas herramientas deben tener la capacidad de igualar el suministro con la demanda de N del cultivo durante la temporada de crecimiento. Una forma eficaz y rápida para evaluar el estado de N del cultivo es mediante el uso de sensores ópticos proximales. Estos sensores no miden directamente el contenido de N en el tejido vegetal, pero proporcionan mediciones de las propiedades ópticas que son indicativas del estado de N del cultivo. Hay tres grupos principales: medidores de clorofila, sensores de reflectancia y medidores de flavonoles basados en fluorescencia. Estas herramientas han demostrado su capacidad para evaluar el estado de N del cultivo en cultivos a campo, pero relativamente pocos estudios se han realizado en cultivos hortícolas en invernadero. Esta tesis ha evaluado la capacidad de los medidores de clorofila y varios índices de vegetación calculados a partir de sensores de reflectancia para evaluar el estado de N del cultivo en cultivos de pimiento y pepino cultivados en suelo bajo invernadero en Almería (sureste (SE) España).

El trabajo experimental se realizó en un invernadero ubicado en la Estación Experimental de la Universidad de Almería, muy similar a los utilizados para la producción comercial en el sureste de España. En esta tesis, los datos utilizados se tomaron en tres cultivos de pimiento (*Capsicum annuum* 'Melchor') y un cultivo de pepino (*Cucumis sativus* L.), cultivados con fertirriego y plantados en suelo enarenado. Los cultivos de pimiento se sometieron a cinco tratamientos de N aplicados en cada riego durante todo el cultivo, que fue desde un suministro de N muy deficiente a muy excesivo. En el cultivo de pepino se usaron tres cultivares diferentes los cuales fueron sometidos a tres tratamientos con N, desde un suministro de N muy deficiente a uno muy excesivo. Se tomaron muestras periódicas de biomasa en todos los cultivos y se

realizó un análisis del contenido de N del cultivo. La curva de N crítica derivada para el pimiento en el grupo de investigación se utilizó para calcular el índice de nutrición de nitrógeno (NNI), como una medida del estado de N del cultivo. En el cultivo de pepino, se recolectaron muestras periódicas de hojas y se analizó el contenido de N de la hoja como una medida aproximada del contenido de N del cultivo.

Periódicamente se realizaron mediciones con sensores ópticos a lo largo de los cultivos. Se utilizaron tres medidores de clorofila (SPAD-502, atLEAF + y MC-100) y dos sensores de reflectancia del dosel (GreenSeeker handheld y Crop Circle ACS-470). Se evaluó su sensibilidad para evaluar el estado de N del cultivo y la estabilidad a lo largo de diferentes horas de medición. En pepino, se evaluó el efecto de diferentes cultivares en las mediciones de los sensores ópticos. En los tres cultivos de pimiento, se analizó la sensibilidad de los medidores de clorofila para evaluar el estado de N del cultivo en cuatro etapas fenológicas (vegetativa, floración, crecimiento temprano de la fruta y cosecha) y se calcularon valores de suficiencia para el crecimiento máximo en las mismas etapas. La influencia de la hora del día en las mediciones de los sensores ópticos se evaluó midiendo a las 9:00, 12:00, 15:00 y 18:00 h, hora solar, en un cultivo de pimiento. El efecto del cultivar en las mediciones de los sensores ópticos se evaluó en tres cultivares de pepino, 'Strategos', 'Pradera' y 'Mitre'.

En general, la investigación realizada en esta tesis ha demostrado que en las cuatro etapas fenológicas de los tres cultivos de pimiento las mediciones de los medidores de clorofila estaban fuertemente relacionadas con el estado de N del cultivo (NNI), lo que indica que estas mediciones son buenos indicadores del estado de N del cultivo en pimiento. Los valores de suficiencia determinados para cada etapa fenológica para SPAD-502 y atLEAF +, fueron muy consistentes entre los diferentes años. Esto demuestra el potencial para usar estos medidores con valores de suficiencia para mejorar el manejo de N de los cultivos comerciales de pimiento. En el caso del MC-100 solo había un cultivo; entonces, no fue posible evaluar la consistencia de los valores de suficiencia entre cultivos.

En cuanto a los medidores de clorofila SPAD-502 y MC-100, hubo diferencias significativas entre los cultivares en las mediciones, particularmente cuando el suministro de N fue suficiente y excesivo, pero no cuando el suministro de N fue deficiente. Sin embargo, no hubo diferencias consistentes entre los cultivares en los índices de vegetación evaluados (NDVI, GNDI, RVI y GVI) y medidos con el sensor de reflectancia del dosel Crop Circle ACS 470. Las mediciones ópticas tomadas tanto con medidores de clorofila como con sensores de reflectancia del dosel, estuvieron fuerte y linealmente relacionado con el contenido de N de la hoja en cada uno de los tres cultivares. El cultivar tuvo un efecto significativo en la relación entre el contenido de N de la hoja y las mediciones de los medidores de clorofila, pero no en las relaciones entre el contenido de N de la hoja y los índices de vegetación de reflectancia del dosel. La falta de un efecto consistente del cultivar, en la relación con el contenido de N de la hoja, sugiere que una única ecuación para estimar el contenido de N de la hoja a partir de los índices de vegetación puede aplicarse a los tres cultivares. Sin embargo, esta ecuación única no puede aplicarse para las mediciones de los medidores de clorofila debido al significativo efecto del cultivar detectado.

La hora del día tuvo un ligero efecto en las mediciones realizadas con el medidor SPAD-502, solo en el tratamiento muy deficitario de N, lo que sugiere que el efecto de la hora del día en las lecturas del SPAD-502 estaban relacionadas con el estado de N del cultivo. Con respecto al medidor MC-100, se encontró un ligero aumento en los valores en las mediciones realizadas a las 15:00 y a las 18:00 horas, en comparación con las mediciones realizadas a las 9:00, independientemente del tratamiento de N. El índice NDVI, medido tanto con el sensor de mano GreenSeeker como con el Crop Circle ACS-470 y los índices GNDVI y GVI, medidos con el Crop Circle, no se vieron afectados estadísticamente por la hora del día en ninguno de los tratamientos de N en este estudio. Estos resultados muestran que estos sensores e índices pueden usarse con confianza en cualquier momento del día.

En general, los resultados de esta tesis muestran el potencial de las mediciones de los sensores ópticos (medidores de clorofila y sensores de reflectancia del dosel) para evaluar el estado de N del cultivo en pimiento y pepino. Los valores de suficiencia de los medidores de clorofila calculados para el pimiento (’Melchor’) fueron consistentes entre los tres cultivos, lo que demuestra de alguna manera el potencial para usar estos

medidores para mejorar el manejo de N de los cultivos comerciales de pimiento. El efecto del cultivar encontrado en el cultivo de pepino en las mediciones de los medidores de clorofila sugiere que se debe tener cuidado al usar valores de suficiencia, determinados para un cultivar en particular, en cultivares no probados; procedimientos de normalización pueden ser necesarios en estos casos. Por el contrario, la falta de diferencias en los índices de vegetación (NDVI, GNDI, RVI y GVI) entre estos cultivares sugiere que podrían usarse para evaluar el estado de N del cultivo en pepino. Con respecto al efecto de la hora de medición en las mediciones con sensores ópticos, los resultados mostraron que estos sensores e índices se pueden usar en campo con confianza en cualquier momento del día.

2. Introduction

Nitrogen (N) is one of the most important nutrients for plant growth (Azcón-Bieto and Talón, 2013). N is used by plants for manufacturing amino acids, proteins and other assimilates needed for plant growth and dry matter production (Burns, 2006; Gauder et al., 2010). Because of its great importance for plant growth, N is applied as fertilizer in large amounts to ensure high crop (Ju et al., 2006; Thompson et al., 2017, 2007).

In protected vegetable production, costs of fertilization are relatively low in comparison to other agricultural inputs (Sonneveld and Voogt, 2009); as a result, vegetable farmers do not feel strong economic pressure reduce N fertilizer application (Sonneveld and Voogt, 2009). Consequently, the amounts of N fertilizer applied to vegetable crops often appreciably exceed crop requirements (Thompson et al., 2017; Zotarelli et al., 2009). The excess N is susceptible to nitrate (NO_3^-) leaching loss (Thompson et al., 2007; Zotarelli et al., 2009), and to subsequent environmental problems as N contamination of aquifers, surface water bodies (Padilla et al., 2018c; Sonneveld and Voogt, 2009) and eutrophication (Congreves and Van Eerd, 2015; Padilla et al., 2018b). Excessive N fertiliser application of vegetable crops is also associated with enhanced emissions of the greenhouse gas nitrous oxide (N_2O) (Bouwman et al., 2002).

The combination of excessive N supply (Ju et al., 2006; Thompson et al., 2007) with excessive irrigation (Fereres et al., 2003) are commonly used in vegetable production systems (Thompson et al., 2007), which has caused environmental problems such as nitrate leaching and therefore N contamination of aquifers and surface water bodies. Nitrate contamination of water bodies has been reported for diverse agricultural regions of the world (Padilla et al., 2018b), such as southeastern (SE) Spain (Pulido-Bosch et al., 2000), SE United States (Zotarelli et al., 2009) and China (Cui et al., 2011; Ju et al., 2006; Zhang et al., 1996). One of the keys to reducing contamination of water by nitrate leaching is to define clearly crop water and nutrient requirements, and to match them with the supply to the crop, both in time and amount (Meisinger et al., 2008; Thompson et al., 2017; Zhu et al., 2005). An optimal N supply is important to secure high and profitable production of field crops and high-quality horticultural products (Samborski et al., 2009). In the case of vegetable production systems, fertigation systems are commonly used (Thompson et al., 2017); these combine fertilizer application with irrigation (Hartz and Hochmuth, 1996). Fertigation systems provide the technical capacity for precise timing, uniform distribution of fertilizers, and the frequent application of small amounts of fertilizers (Hartz and Hochmuth, 1996). To fully exploit this technical capacity, tools are required that assess crop N status. Assessing crop N status enables determination of whether the crop N content is sufficient for optimal growth and yield, and whether adjustments in fertilization need to be made. In vegetable crops with fertigation, ideal N management would involve rapid and frequent assessment of crop N status, enabling rapid adjustment of the N being applied (Thompson et al., 2018).

Different soil and crop monitoring approaches have been developed to assist with the N management of vegetable crops (Thompson et al., 2017), such as methods based on soil analysis, plant analysis, and the use of proximal optical sensors (Fox and Walthall, 2008; Thompson et al., 2017). The methods based on soil and plant analyses are labour-intensive, time-consuming, and generally are not suitable to characterize the temporal and spatial variability of crop N status (Mistele and Schmidhalter, 2008). Additionally, these procedures do not provide results quickly for field crops which is required to have timely fertilizer recommendations (Gauder et al., 2010; Gianquinto et al., 2011a).

The use of proximal optical sensors for monitoring crop N status is a promising methodology (Gianquinto et al., 2011b). These sensors are a group of tools that provide effective, rapid, and non-destructive assessment of crop N status, in the field (Padilla et al., 2018c; Thompson et al., 2017). Proximal optical sensors are a form of remote sensing in which the sensors are positioned either in contact with or close to the crop (Thompson et al., 2017). These sensors do not directly measure N content in plant tissue (Fox and Walthall, 2008; Samborski et al., 2009; Thompson et al., 2017), but provide measurements of optical properties that are indicative of crop N status, thereby indicating N sufficiency or the degree of N deficiency (Thompson et al., 2017). Some sensors are limited to individual spot measurements, while others have continuous on-the-go capabilities that enable large representative surface areas of foliage to be measured (Padilla et al., 2018c). Several reviews have evaluated the potential of these tools for crop monitoring and N management in cereal crops such as corn and wheat (Fox and Walthall, 2008; Meisinger et al., 2008; Samborski et al., 2009) and in vegetable crops (Gianquinto et al., 2011a; Padilla et al., 2018c; Thompson et al., 2017).

Proximal optical sensors, suitable for crop N management applications, can be considered as belonging to three groups, (1) chlorophyll meters, (2) reflectance sensors, and (3) fluorescence-based flavonols meters. This doctoral thesis has focused on groups one and two, but other research activities carried out during my doctoral work have also included group three.

Chlorophyll meters are small, hand-held, clip-on optical sensors (Thompson et al., 2017), that estimate the relative chlorophyll content per unit of leaf surface area (Padilla et al., 2018c), measuring the absorbance and transmittance of radiation of two light wavelengths by the leaf (peaks approx. 650 and 940 nm) (Gianquinto et al., 2004; Tremblay, 2013). Chlorophyll absorbs red radiation (650 nm) and transmits most of the near infra-red (940 nm) radiation, which is influenced by leaf thickness, leaf age, leaf structure, among several parameters (Fox and Walthall, 2008; Jones and Vaughan, 2010; Padilla et al., 2018c). Absorbance of red radiation increases with chlorophyll content, resulting in higher chlorophyll meter values (Hu et al., 2011; Schepers et al., 1996). Given that the chlorophyll content is accepted as an indicator of N availability (Samborski et al., 2009), these measurements can be used to assess crop N status (Gianquinto et al., 2004; Tremblay, 2013).

There are currently several commercially available sensors, including the SPAD-502 (Konica-Minolta, Japan), atLEAF+ sensor (FT Green LLC, Wilmington, DE, USA), and MC-100 chlorophyll meter (Apogee Instruments, Inc., Logan, UT, USA). There are small differences between these meters in the wavelengths used. The SPAD-502 measures absorbance at 650 nm (red) and 940 nm (near-infrared (NIR)), the atLEAF+ at 660 nm and 940 nm, and the MC-100 at 653 nm and 931 nm (de Souza et al., 2019). These three meters calculate a dimensionless numerical value, which is related to chlorophyll content (Padilla et al., 2018a). For each individual measurement, the measured area is generally <10 mm^2 (Padilla et al., 2018c). Consequently, there is a requirement for appreciable replication e.g. 20–40 measurements on different plants per field or experimental treatment, and for strict measurement protocols (e.g. leaf selection, position on leaf) (Thompson et al., 2017). It is recommended that measurements be made on the most recent fully expanded and well-lit leaf, between the stalk (stem) and leaf tip, midway between the margin and mid-rib, on the adaxial (top) side of the leaf (Padilla et al., 2018c). These instruments are very easy to use, do not require any analytical skills, and perform measurements instantaneously, without further steps (Gianquinto et al., 2004).

The other group of optical sensors widely used for N management are the reflectance sensors. In this group of sensors, the radiation collected by the sensing device is radiation reflected from the crop canopy (Tremblay, 2013). These sensors assess crop N status by measuring the reflection of two or more specific wavelengths of radiation from crop foliage (Ollinger, 2011). Spectral reflectance wavelengths useful for N assessment are chosen because of their sensitivity to the changes of chlorophyll status, foliage density, percent vegetation cover, and biomass that accompany N stress (Fox and Walthall, 2008). The individual wavelengths commonly used are green, red, far-red (visible), and NIR, which then are used in mathematical equations to calculate vegetation indices (Thompson et al., 2017). Crop canopy absorbs a large proportion of incident radiation in the visible wavelengths, and absorbs relatively little radiation in the infrared (Jones and Vaughan, 2010). The degree of absorbance and reflectance in the visible and NIR portions of the spectrum varies with crop N content (Padilla et al., 2018c), with N-deficient crops generally reflecting more visible and reflecting less NIR than N-sufficient crops canopy (Schepers et al., 1996). One of the main advantages of these sensors is that each individual measurement can integrate a large area of crop canopy (Schepers et al., 1996; Thompson et al., 2017).

There are two types of sensors regarding the light source used. Passive sensors use sunlight as the light source, in contrast to active sensors, which possess their own light-emitting units (Winterhalter et al., 2013). Active sensor measurements are independent of irradiation conditions (Erdle et al., 2011; Fitzgerald et al., 2010; Padilla et al., 2019).

Examples of active sensors are the various Crop Circle and GreenSeeker sensors (Padilla et al., 2018c).

Optical sensor measurements do not directly indicate crop N status, so interpretation is required (Padilla et al., 2018c). One approach to assess crop N status is the use of sufficiency index (Thompson et al., 2017). This approach is based on the use of a fully fertilized reference plot or strip in the same field (Tremblay et al., 2011). The sufficiency index is calculated by dividing the measured values of the crop by those from the fully fertilized plot and provides a relative value that indicates the degree of N deficiency (Thompson et al., 2017). This approach compensates for factors other than N status that could affect optical sensor measurements such as abiotic and water stress, disease incidence and cultivar (Samborski et al., 2009). The sufficiency index is widely accepted for cereal crops (Samborski et al., 2009; Zhu et al., 2011). However, this approach is considered to be impractical for commercial fertigated vegetable crops (Padilla et al., 2018c), given the additional cost of having separate fertigation sectors for reference plots (Padilla et al., 2018a; Padilla et al., 2014).

Another approach to interpret the optical sensors measurements, for the assessment of crop N status, is the use of absolute sufficiency values based on direct measurement (de Souza et al., 2019). This approach has been demonstrated to be useful in vegetable crops (Thompson et al., 2017). The sufficiency value is an absolute value, below which the crop is N deficient and responds to additional N fertilizer, and above which yield is not affected (Gianquinto et al., 2004) and the immediate N supply may be excessive (Thompson et al., 2017). To determine the crop N status and to relate it to optical sensors measurements, the nitrogen nutrition index (NNI) (Lemaire et al., 2008) has been proposed. The NNI is a ratio of actual crop N (Na) to critical N status (Nc). Values greater than one indicate luxury consumption of N while those less than one indicate an N deficit crop (Fitzgerald et al., 2010). Sufficiency values of optical sensors measurements (chlorophyll meters and vegetation indices) are derived from the relationship between crop NNI and optical sensors measurements by solving the relationship for NNI = 1 (Padilla et al., 2017a).

Considerable research has demonstrated the capacity of proximal optical sensors to assess crop N status in various field crops, mostly in cereals such as rice (Cao et al., 2013; Wakiyama, 2016), maize (Blackmer and Schepers, 1995; Graeff and Claupein, 2003) and wheat (Bronson et al., 2017; Samborski et al., 2016; Ziadi et al., 2010). Additionally, the capacity of proximal optical sensors to assess crop N status has been evaluated in vegetable crops such as potato (Gianquinto et al., 2004), tomato (Gianquinto et al., 2006; Padilla et al., 2015), cucumber (Güler and Büyük, 2007; Padilla et al., 2017b), muskmelon (Padilla et al., 2014), and sweet pepper (de Souza et al., 2019). Most of these studies assessed the crop N status for a particular cultivar of a given species. However, if sufficiency values are determined for a given cultivar of one species, these may vary with different cultivars (Padilla et al., 2018c; Thompson et al., 2017). Assessing whether cultivar has a significant effect on optical sensor measurements is an important practical consideration in vegetable production. New cultivars are continuously being introduced into the market.

Limited research has shown that differences between cultivars could affect chlorophyll meter sensor measurements. In wheat, Monostori et al., (2016) and Hoel, (2003) working with chlorophyll meters, reported that cultivar had a notable effect on the relationship between chlorophyll meter readings and grain yield. Similar results were also found in rice, in the relationship between SPAD-502 measurements and leaf N content (Peng et al., 1993). In tomato, there were significant differences in chlorophyll meter measurements between one cultivar and four others (Sandoval-Villa et al., 2000). Finally, in cucumber, (de Souza et al., 2020) found significant differences between three cultivars in measurements of the chlorophyll metersSPAD-502 and MC-100.

The effect of cultivar on vegetation indices calculated with reflectance sensors has been less studied. The few reported studies have examined mainly the NDVI vegetation index. The NDVI was able to differentiate different cultivars at different growth stages in wheat (Sultana et al., 2014). In vegetable crops, the effect of cucumber cultivars on four vegetation indices was assessed (de Souza et al., 2020).

The practical use of optical sensors to assist with N fertilization on farm requires that measurements are accurate and reproducible on time (Martínez and Guiamet, 2004), regardless of the conditions at the time of measurements. To enable comparison of sequences of measurements taken throughout a crop, time of day and the irradiance conditions should have a negligible effect on optical sensor measurements (Padilla et al., 2018c). Changes in irradiance can cause light-dependent chloroplast movements that can affect leaf absorbance of radiation (Kasahara et al., 2002) and consequently of chlorophyll meter values (Hoel and Solhaug, 1998). Xiong et al., (2015) found that diurnal variation of SPAD values was dependent on N supply, with lower SPAD values at midday in the treatments with low N supply. Regarding active canopy reflectance sensors that are fitted with their own light-emitting units, it is assumed that measurements are independent of varying irradiation conditions (Winterhalter et al., 2013). However, there are studies indicating that active reflectance sensors can also be influenced by the time of day (Kim et al., 2012; Teixeira Crusiol et al., 2017).

2.1. References

Azcón-Bieto, J., Talón, M., 2013. Fundamentos de fisiología vegetal, McGrawHill. https://doi.org/10.1017/CBO9781107415324.004

Blackmer, T.M., Schepers, J.S., 1995. Use of a chlorophyll meter to monitor nitrogen status and schedule fertigation for corn. J. Prod. Agric. 8, 56–60.

Bouwman, A.F., Boumans, L.J.M., Batjes, N.H., 2002. Emissions of N 2 O and NO from fertilized fields: Summary of available measurement data . Global Biogeochem. Cycles 16, 6-1-6–13. https://doi.org/10.1029/2001gb001811

Bronson, K.F., White, J.W., Conley, M.M., Hunsaker, D.J., Thorp, K.R., French, A.N., Mackey, B.E., Holland, K.H., 2017. Active optical sensors in irrigated durum wheat: nitrogen and water effects. Agron. J. 109, 1060–1071. https://doi.org/10.2134/agronj2016.07.0390

Burns, I.G., 2006. Assessing N fertiliser requirements and the reliability of different recommendation systems. Acta Hortic. 700, 35–48. https://doi.org/10.17660/ActaHortic.2006.700.2

Cao, Q., Miao, Y., Wang, H., Huang, S., Cheng, S., Khosla, R., Jiang, R., 2013. Non-destructive estimation of rice plant nitrogen status with Crop Circle multispectral active canopy sensor. F. Crop. Res. 154, 133–144.

https://doi.org/10.1016/j.fcr.2013.08.005

Congreves, K.A., Van Eerd, L.L., 2015. Nitrogen cycling and management in intensive horticultural systems. Nutr. Cycl. Agroecosystems 102, 299–318. https://doi.org/10.1007/s10705-015-9704-7

Cui, M., Sun, X., Hu, C., Di, H.J., Tan, Q., Zhao, C., 2011. Effective mitigation of nitrate leaching and nitrous oxide emissions in intensive vegetable production systems using a nitrification inhibitor, dicyandiamide. J. Soils Sediments 11, 722–730. https://doi.org/10.1007/s11368-011-0357-0

de Souza, R., Grasso, R., Teresa Peña-Fleitas, M., Gallardo, M., Thompson, R.B., Padilla, F.M., 2020. Effect of Cultivar on Chlorophyll Meter and Canopy Reflectance Measurements in Cucumber. Sensors 20, 509. https://doi.org/10.3390/s20020509

de Souza, R., Peña-fleitas, M.T., Thompson, R.B., Gallardo, M., Grasso, R., Padilla, F.M., 2019. The Use of Chlorophyll Meters to Assess Crop N Status and Derivation of Sufficiency Values for Sweet Pepper. Sensors 19, 1–20. https://doi.org/doi:10.3390/s19132949

Erdle, K., Mistele, B., Schmidhalter, U., 2011. Comparison of active and passive spectral sensors in discriminating biomass parameters and nitrogen status in wheat cultivars. F. Crop. Res. 124, 74–84. https://doi.org/http://dx.doi.org/10.1016/j.fcr.2011.06.007

Fereres, E., Goldhamer, D.A., Parsons, L.R., 2003. Irrigation water management of horticultural crops. HortScience 38, 1036–1042. https://doi.org/10.21273/hortsci.38.5.1036

Fitzgerald, G.J., Rodriguez, D., O'Leary, G., 2010. Measuring and predicting canopy nitrogen nutrition in wheat using a spectral index-The canopy chlorophyll content index (CCCI). F. Crop. Res. 116, 318–324.

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

Gauder, M., Pfenning, J., Liebig, H.P., Graeff, S., Claupein, W., 2010. Relations between optical properties and N status of tomato leaves. Acta Hortic. 852, 247–252.

Gianquinto, G., Goffart, J.P., Olivier, M., Guarda, G., Colauzzi, M., Dalla Costa, L., Delle Vedove, G., Vos, J., Mackerron, D.K.L., 2004. The use of hand-held chlorophyll meters as a tool to assess the nitrogen status and to guide nitrogen fertilization of potato crop. Potato Res. 47, 35–80.

Gianquinto, G., Orsini, F., Fecondini, M., Mezzetti, M., Sambo, P., Bona, S., 2011a. A methodological approach for defining spectral indices for assessing tomato nitrogen status and yield. Eur. J. Agron. 35, 135–143. https://doi.org/10.1016/j.eja.2011.05.005

Gianquinto, G., Orsini, F., Sambo, P., Paino D'Urzo, M., 2011b. The Use of Diagnostic Optical Tools to Assess Nitrogen Status and to Guide Fertilization of Vegetables. Horttechnology 21, 287–292.

Gianquinto, G., Sambo, P., Orsini, F., Sciortino, M., Forte, V., 2006. Optical tools, a suitable means to reduce nitrogen use in fertigated tomato crop. HortScience 41, 982.

Graeff, S., Claupein, W., 2003. Quantifying nitrogen status of corn (Zea mays L.) in the field by reflectance measurements. Eur. J. Agron. 19, 611–618.

Güler, S., Büyük, G., 2007. Relationships among chlorophyll-meter reading value, leaf N and yield of cucumber and tomatoes. Acta Hortic. 729, 307–311.

Hartz, T.K., Hochmuth, G.J., 1996. Fertility management of drip-irrigated vegetables. Horttechnology 6, 168–172.

Hoel, B.O., 2003. Chlorophyll meter readings in winter wheat: Cultivar differences and prediction of grain protein content. Acta Agric. Scand. Sect. B Soil Plant Sci. 52, 147–157. https://doi.org/10.1080/090647103100004843

Hoel, B.O., Solhaug, K.A., 1998. Effect of irradiance on chlorophyll estimation with the Minolta SPAD-502 leaf chlorophyll meter. Ann. Bot. 82, 389–392.

Hu, J., He, D., Yang, P., 2011. Study on plant nutrition indicator using leaf spectral transmittance for nitrogen detection. Adv. Inf. Commun. Technol. 347, 504–513.

Jones, H.G., Vaughan, R.A., 2010. Remote sensing of vegetation. Oxford University Press, New York, USA.

Ju, X.T., Kou, C.L., Zhang, F.S., Christie, P., 2006. Nitrogen balance and groundwater nitrate contamination: Comparison among three intensive cropping systems on the North China Plain. Environ. Pollut. 143, 117–125. https://doi.org/10.1016/j.envpol.2005.11.005

Kasahara, M., Kagawa, T., Olkawa, K., Suetsugu, N., Miyao, M., Wada, M., 2002. Chlomplast avoidance movement reduces photodamage in plants. Nature 420, 829–832. https://doi.org/10.1038/nature01213

Kim, Y., Glenn, D.M., Park, J., Ngugi, H.K., Lehman, B.L., 2012. Characteristics of active spectral sensor for plant sensing. Trans. ASABE 55, 293–301.

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. Eur. J. Agron. 28, 614–624.

Martínez, D.E., Guiamet, J.J., 2004. Distortion of the SPAD 502 chlorophyll meter readings by changes in irradiance and leaf water status. Agronomie 24, 41–46.

Meisinger, J.J., Schepers, J.S., Raun, W.R., 2008. Crop Nitrogen Requirement and Fertilization. Am. Soc. Agron. Crop Sci. Soc. Am. Soil Sci. Soc. Am. 563–612. https://doi.org/10.2134/agronmonogr49

Mistele, B., Schmidhalter, U., 2008. Estimating the nitrogen nutrition index using spectral canopy reflectance measurements. Eur. J. Agron. 29, 184–190.

Monostori, I., Árendás, T., Hoffman, B., Galiba, G., Gierczik, K., Szira, F., Vágújfalvi, A., 2016. Relationship between SPAD value and grain yield can be affected by cultivar, environment and soil nitrogen content in wheat. Euphytica 211, 103–112. https://doi.org/10.1007/s10681-016-1741-z

Ollinger, S. V, 2011. Sources of variability in canopy reflectance and the convergent properties of plants. New Phytol. 189, 375–394.

Padilla, F.M., de Souza, R., Peña-Fleitas, M.T., Gallardo, M., Giménez, C., Thompson, R.B., 2018a. Different responses of various chlorophyll meters to increasing nitrogen supply in sweet pepper. Front. Plant Sci. 9, 1752. https://doi.org/10.3389/fpls.2018.01752

Padilla, F.M., de Souza, R., Peña-Fleitas, M.T., Grasso, R., Gallardo, M., Thompson, R.B., 2019. Influence of time of day on measurement with chlorophyll meters and canopy reflectance sensors of different crop N status. Precis. Agric. https://doi.org/10.1007/s11119-019-09641-1

Padilla, F.M., Gallardo, M., Manzano-Agugliaro, F., 2018b. Global trends in nitrate leaching research in the 1960–2017 period. Sci. Total Environ. 643, 400–413. https://doi.org/10.1016/j.scitotenv.2018.06.215

Padilla, F.M., Gallardo, M., Peña-Fleitas, M.T., de Souza, R., Thompson, R.B., 2018c. Proximal Optical Sensors for Nitrogen Management of Vegetable Crops: A Review. Sensors 18, 2083. https://doi.org/10.3390/s18072083

Padilla, F.M., Peña-Fleitas, M.T., Gallardo, M., Giménez, C., Thompson, R.B., 2017a. Derivation of sufficiency values of a chlorophyll meter to estimate cucumber nitrogen status and yield. Comput. Electron. Agric. 141, 54–64. https://doi.org/10.1016/j.compag.2017.07.005

Padilla, F.M., Peña-Fleitas, M.T., Gallardo, M., Thompson, R.B., 2017b. Determination of sufficiency values of canopy reflectance vegetation indices for maximum growth and yield of cucumber. Eur. J. Agron. 84. https://doi.org/10.1016/j.eja.2016.12.007

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. Ann. Appl. Biol. 166, 271–285. https://doi.org/10.1111/aab.12181

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. Eur. J. Agron. 58, 39–52. https://doi.org/10.1016/j.eja.2014.04.006

Peng, S., Garcia, F. V., Laza, R.C., Cassman, K.G., 1993. Adjustment for specific leaf weight improves chlorophyll meter's estimate of rice leaf nitrogen concentration. Agron. J. 85, 987–990. https://doi.org/10.2134/agronj1993.00021962008500050005x

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 Dalias (SE - Spain). Environ. Geol. 39, 791–799. https://doi.org/10.1007/s002540050495

Samborski, S.M., Gozdowski, D., Stępień, M., Walsh, O.S., Leszczyńska, E., 2016. On-farm evaluation of an active optical sensor performance for variable nitrogen application in winter wheat. Eur. J. Agron. 74, 56–67. https://doi.org/http://dx.doi.org/10.1016/j.eja.2015.11.020

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

Sandoval-Villa, M., Guertal, E.A., Wood, C.W., 2000. Tomato leaf chlorophyll meter readings as affected by variety, nitrogen form, and nighttime nutrient solution strength. J. Plant Nutr. 23, 649–661.

Schepers, J.S., Blackmer, T.M., Wilhelm, W.W., Resende, M., 1996. Transmittance and reflectance measurements of corn leaves from plants with different nitrogen and water supply. J. Plant Physiol. 148, 523–529.

Sonneveld, C., Voogt, W., 2009. Plant Nutrition of Greenhouse Crops. Springer. https://doi.org/10.1007/978-90-481-2532-6

Sultana, S.R., Ali, A., Ahmad, A., Mubeen, M., Zia-Ul-Haq, M., Ahmad, S., Ercisli, S., Jaafar, H.Z.E., 2014. Normalized difference vegetation index as a tool for wheat yield estimation: A case study from Faisalabad, Pakistan. Sci. World J. 2014, 8. https://doi.org/10.1155/2014/725326

Teixeira Crusiol, L.G., Corrêa Carvalho, J.F., Ribeiro Sibaldelli, R.N., Neiverth, W., Do Rio, A., Ferreira, L.C., de Oliveira Procópio, S., Mertz-Henning, L.M., Lima Nepomuceno, A., Neumaier, N., Bouças Farias, J.R., Crusiol, L.G.T., Carvalho, J.D.F.C., Sibaldelli, R.N.R., Neiverth, W., Do Rio, A., Ferreira, L.C., Procópio, S.D.O., Mertz-Henning, L.M., Nepomuceno, A.L., Neumaier, N., Farias, J.R.B., 2017. NDVI variation according to the time of measurement, sampling size, positioning of sensor and water regime in different soybean cultivars. Precis. Agric. 18, 470–490. https://doi.org/10.1007/s11119-016-9465-6

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. Agric. Water Manag. 89, 261–274. https://doi.org/10.1016/J.AGWAT.2007.01.013

Thompson, R.B., Tremblay, N., Fink, M., Gallardo, M., Padilla, F.M., 2017. Tools and strategies for sustainable nitrogen fertilisation of vegetable crops, in: Tei, F., Nicola, S., Benincasa, P. (Eds.), Advances in Research on Fertilization Management in Vegetable Crops. Springer, Heidelberg, Germany, pp. 11–63. https://doi.org/in press

Thompson, R.B., Voogt, W., Incrocci, L., Fink, M., de Neve, S., 2018. Strategies for optimal fertiliser management of vegetable crops in Europe. Acta Hortic. 1192, 129–140. https://doi.org/10.17660/ActaHortic.2018.1192.15

Tremblay, N., 2013. Sensing technologies in horticulture: Options and challenges. Chron. Horticult. 53, 10–13.

Tremblay, N., Fallon, E., Ziadi, N., 2011. Sensing of crop nitrogen status: Opportunities, tools, limitations, and supporting information requirements. Horttechnology 21, 274–281.

Wakiyama, Y., 2016. The relationship between SPAD values and leaf blade chlorophyll content throughout the rice development cycle. Japan Agric. Res. Q. 50, 329–334.

Winterhalter, L., Mistele, B., Schmidhalter, U., 2013. Evaluation of active and passive sensor systems in the field to phenotype maize hybrids with high-throughput. F. Crop. Res. 154, 236–245. https://doi.org/http://dx.doi.org/10.1016/j.fcr.2013.09.006

Xiong, D., Chen, J., Yu, T., Gao, W., Ling, X., Li, Y., Peng, S., Huang, J., 2015. SPAD-based leaf nitrogen estimation is impacted by environmental factors and crop leaf characteristics. Sci. Rep. 5, 13389. https://doi.org/10.1038/srep13389

Zhang, W.L., Tian, Z.X., Zhang, N., Li, X.Q., 1996. Nitrate pollution of groundwater in northern China. Agric. Ecosyst. Environ. 59, 223–231. https://doi.org/10.1016/0167-8809(96)01052-3

Zhu, J., Tremblay, N., Liang, Y., 2011. A corn nitrogen status indicator less affected by soil water content. Agron. J. 103, 890–898.

Zhu, J.H., Li, X.L., Christie, P., Li, J.L., 2005. Environmental implications of low nitrogen use efficiency in excessively fertilized hot pepper (Capsicum frutescens L.) cropping systems. Agric. Ecosyst. Environ. 111, 70–80. https://doi.org/10.1016/j.agee.2005.04.025

Ziadi, N., Bélanger, G., Claessens, A., Lefebvre, L., Tremblay, N., Cambouris, A.N., Nolin, M.C., Parent, L.E., 2010. Plant-based diagnostic tools for evaluating wheat nitrogen status. Crop Sci. 50, 2580–2590.

Zotarelli, L., Dukes, M.D., Scholberg, J.M.S., Muñoz-Carpena, R., Icerman, J., 2009. Tomato nitrogen accumulation and fertilizer use efficiency on a sandy soil, as affected by nitrogen rate and irrigation scheduling. Agric. Water Manag. 96, 1247–1258. https://doi.org/10.1016/j.agwat.2009.03.019

3. Objectives

The main objective of this thesis was to evaluate and compare the available and most promising proximal optical sensors for on-farm monitoring of crop N status, in vegetable crops grown under greenhouse conditions. For this objective, it was necessary to evaluate these sensors in terms of sensitivity, consistency of the relation to crop N status, reproducibility, ease of use, and practical suitability as tools for on-farm management. The specific objectives of this thesis were:

1. Evaluate the sensitivity of three different chlorophyll meters, and various vegetation indices calculated from canopy reflectance sensors, to assess crop N status in sweet pepper and cucumber crops.
2. Calculate sufficiency values for each chlorophyll meter for maximum crop growth for four different phenological stages in sweet pepper.
3. Evaluate the effects of cucumber cultivar on chlorophyll meter measurements, and vegetation indices measured with a canopy reflectance sensor, and to assess how cultivar differences could affect the relationship between leaf N content and optical sensors measurements.
4. Assess the effects of time of day on measurements of chlorophyll meters and canopy reflectance sensors.

4. Chapter one: The use of chlorophyll meters to assess crop N status and derivation of sufficiency values for sweet pepper

de Souza, R., Peña-Fleitas, M.T., Thompson, R.B., Gallardo, M., Grasso, R., Padilla, F.M. 2019. The use of chlorophyll meters to assess crop N status and derivation of sufficiency values for sweet pepper. *Sensors* 19(13): 2949.

4.1. Abstract

Chlorophyll meters are promising tools for improving the nitrogen (N) management of vegetable crops. To facilitate on-farm use of these meters, sufficiency values that identify deficient and sufficient crop N status are required. This work evaluated the ability of three chlorophyll meters (SPAD-502, atLEAF+, and MC-100) to assess crop N status in sweet pepper. It also determined sufficiency values for optimal N nutrition for each meter for pepper. The experimental work was conducted in a greenhouse, in Almería, Spain, very similar to those used for commercial production, in three different crops grown with fertigation. In each crop, there were five treatments of different N concentration in the nutrient solution, applied in each irrigation, ranging from a very deficient to very excessive N supply. In general, chlorophyll meter measurements were strongly related to crop N status in all phenological stages of the three crops, indicating that these measurements are good indicators of the crop N status of pepper. Sufficiency values determined for each meter for the four major phenological stages were consistent between the three crops. This demonstrated the potential for using these meters with sufficiency values to improve the N management of commercial sweet pepper crops.

Keywords: atLEAF; CCI; greenhouse; horticulture; nitrogen nutrition index; proximal optical sensors; SPAD; vegetable crops

4.2. Introduction

To optimize nitrogen (N) fertilizer application, it is necessary to match the N supply to the N demand (Meisinger et al., 2008; Monostori et al., 2016b). A potentially very effective approach would be the rapid and frequent on-farm assessment of crop N status that permits rapid adjustment of the N supply (Gianquinto et al., 2004; Padilla et al., 2015; Thompson et al., 2017). Proximal optical sensors are a broad group of non-destructive monitoring tools that can be used to assess crop N status (Fox and Walthall, 2008; Padilla et al., 2018c; Thompson et al., 2017). One particularly promising group of proximal optical sensors are leaf chlorophyll meters.

Chlorophyll meters are relatively simple proximal optical sensors that indirectly assess relative leaf chlorophyll content by measuring the differential absorbance and transmittance of different radiation wavelengths by the leaf (Gianquinto et al., 2004; Khoddamzadeh and Dunn, 2016; Padilla et al., 2018c). Given that leaf chlorophyll content is usually related to crop N content (Fox and Walthall, 2008; Mastalerczuk et al., 2017; Schepers et al., 1996), these measurements can be used to assess crop N status (Gianquinto et al., 2004; Padilla et al., 2018c). Three commercially-available meters, with different characteristics, such as the wavelengths used, are the SPAD-502 meter (Konica-Minolta, Tokyo, Japan), atLEAF+ sensor (FT Green LLC, Wilmington, DE, USA), and MC-100 chlorophyll meter (Apogee Instruments, Inc., Logan, UT, USA) (Padilla et al., 2018a, 2018c; Thompson et al., 2017). The SPAD-502 measures absorbance at 650 nm (red) and 940 nm (NIR), the atLEAF+ at 660 nm and 940 nm, and the MC-100 at 653 nm and 931 nm. Using the two absorbance values, these three meters calculate a dimensionless numerical value, which is related to chlorophyll content (Padilla et al., 2018a). There are also differences in price between these meters; the atLEAF+ sensor is almost 10 times cheaper than the SPAD-502 and the MC-100 meters. The major practical advantages of chlorophyll meters as indicators of crop N status are that they are easy to use, do not require any particular training, and they make measurements very rapidly, with no or very little data processing (Gianquinto et al., 2004; Minotti et al., 1994; Padilla et al., 2018c; Padilla et al., 2015).

Chlorophyll meter measurements do not directly indicate crop N status, so interpretation is required (Padilla et al., 2018c). Two broad approaches have been proposed to interpret chlorophyll meter measurements to assess crop N status. One approach is the use of so-called "reference plots" (Westerveld et al., 2004; Zhu et al., 2011b). This approach divides the measured values of the crop by those from a well-fertilized reference plot that has no N limitation (Tremblay et al., 2011). This is considered to isolate the effect of relative N status from other confounding factors that are common to both areas (Samborski et al., 2009b), which could greatly facilitate the adoption of chlorophyll meters on farms. However, this approach is considered to be impractical for commercial fertigated vegetable crops, given: (1) the additional cost of having separate fertigation sectors for reference plots, and (2) the implicit assumption of sensor saturation may not apply when luxury N uptake occurs, as has been reported for some vegetable species (Padilla et al., 2018a; Thompson et al., 2017).

Another approach to interpret chlorophyll meter measurements, for the assessment of crop N status, is the use of absolute sufficiency values based on direct measurement. The sufficiency value is an absolute value, below which the crop is deficient and responds to additional N fertilizer (Gianquinto et al., 2004; Padilla et al., 2017a), and above which yield is not affected (Gianquinto et al., 2004) and the immediate N supply may be excessive (Thompson et al., 2017). Sufficiency values provide information on whether adjustments in N fertilization are required when absolute measurements deviate from sufficiency values (Olivier et al., 2006).

To determine sufficiency values, the nitrogen nutrition index (*NNI*) can be used (Padilla et al., 2018c; Zhu et al., 2011b). The *NNI* is an effective and established indicator of crop N status (Lemaire et al., 2008) that relates the actual crop N content to 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 $NNI = 1$ correspond to optimal N nutrition (Lemaire et al., 2008). Sufficiency values of chlorophyll meter measurements are derived from the relationship between crop *NNI* and chlorophyll meter measurements by solving the relationship for $NNI = 1$ (Padilla et al., 2018c; Padilla et al., 2017a). Chlorophyll meter sufficiency values have been determined for fresh tomato (Padilla et al., 2018; Padilla et al., 2015), and cucumber (Güler and Büyük, 2007; Padilla et al., 2017a). Sufficiency values are not available for most vegetable species, including important crops such as sweet pepper. Additionally, sufficiency values should be determined for specific agricultural systems and regions.

In Southeast (SE) Spain, the greenhouse-based intensive vegetable production system consists of approximately 40,000 ha of relatively simple plastic greenhouses, most of which are concentrated in the province of Almeria (Castilla and Hernandez, 2005; Junta de Andalucía, 2016). Nitrate (NO_3^-) leaching from this system (Thompson et al., 2007) is associated with considerable aquifer NO_3^- contamination (Pulido-Bosch et al., 2000). Frequent monitoring of these fertigated vegetable crops with chlorophyll meters is a promising approach to optimize crop N management, which would reduce N fertilizer use, thereby contributing to reduced aquifer NO_3^- contamination. In Almeria, sweet pepper is either the most or second most important crop, depending on the year, occupying approximately 8000 ha each year (Valera et al., 2017). Globally, sweet pepper is grown on 1.9 million hectares (FAOSTAT, 2019).

The objectives of the present work were: (i) to evaluate the sensitivity of three different chlorophyll meters to assess the crop N status of sweet pepper crops, and (ii) to calculate sufficiency values for each chlorophyll meter for maximum crop growth for four different phenological stages. This work was conducted in three different sweet pepper crops grown in different cropping years (2014–2015, 2016–2017, and 2017–2018) in a greenhouse. In each crop there was five different N treatments, ranging from very deficient to very excessive.

4.3. Materials and Methods

4.3.1. Experimental Site

Three sweet pepper (*Capsicum annuum,* cultivar 'Melchor') crops were grown in soil in a plastic greenhouse, in conditions similar to commercial greenhouse vegetable production in SE Spain (Valera et al., 2017). The experimental work was conducted at the Experimental Station of the University of Almeria (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 (lateral side panels and flap roof windows), and an east–west orientation, with crop rows aligned north–south. The cropping area was 1300 m^2. The crops were grown in an "enarenado" soil, typical of those used for soil-grown greenhouse production in Almería (Thompson et al., 2007). A more detailed description of the soil used is available in Padilla et al. (Padilla et al., 2014).

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 of lines, 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 two emitters m^{-2}. The greenhouse was organized into 24 plots, measuring 6 m × 6 m; 20 plots were used in the current study. There were five N treatments with four replicate plots per treatment, arranged in a randomized block design. Each plot contained three paired lines of plants (six lines of plants in total), with 12 plants in each line, separated by a 0.5 m spacing. One plant was positioned 60 mm from and immediately adjacent to each dripper, giving a plant density of two plants m^{-2} and 72 plants per replicate plot. Sheets of polyethylene film (250 µm thickness) buried up to 30 cm depth acted as a hydraulic barrier between plots (Padilla et al., 2016).

4.3.2. Experimental Design

The three sweet pepper crops were grown in different years. The first crop, in 2014–2015 (“the 2014 crop”) was transplanted on 12 August 2014 and ended on 29 January 2015 (cropping period of 170 days). The second crop, “the 2016 crop”, was transplanted on 19 July 2016 and ended on 24 March 2017 (cropping period of 248 days). The third crop, “the 2017 crop”, was transplanted on 21 July 2017 and ended on 20 February 2018 (cropping period of 214 days). The three crops were transplanted as 35-day old seedlings using the same cultivar.

In each crop, there were five treatments of different N concentration in the nutrient solution, applied by fertigation throughout the crops. In the 2014 crop, the N treatments commenced one day after transplanting (DAT), in the 2016 crop at nine DAT, and in the 2017 crop at 10 DAT. Plants were irrigated with water only (<0.04 mmol N L^{-1}) prior to commencing the N treatments. The N treatments were applied in every irrigation until the end of the crops. In each crop, the N treatments were very deficient (N1), deficient (N2), conventional (N3), excessive (N4), and very excessive (N5). The average mineral N (NO_3^-–N + NH_4^+–N) concentrations (mmol L^{-1}), applied in the nutrient solution, and the amounts (kg ha^{-1}) of N applied in each N treatment in each crop are presented in Table 1. For all treatments, N was applied mostly as nitrate (NO_3^-), the rest as ammonium (NH_4^+); on average 88% of the N was applied as NO_3^-. All other nutrients were applied in the nutrient solution to ensure they were not limiting.

The crops were managed following local practice. The crops were physically supported using a system of nylon cords placed horizontally along the side of the crop. Irrigation was scheduled to maintain soil matric potential (SMP) in the root zone, at 12 cm deep, within −15 to −25 kPa; one tensiometer (Irrometer, Co., Riverside, CA, USA) per plot was used to measure SMP. High temperature within the greenhouse was controlled by white-washing the plastic cladding with $CaCO_3$ suspension.

Table 1. Mineral N (NO_3^-–N + NH_4^+–N) concentration in the nutrient solution and mineral N amount applied in fertigation in the three sweet pepper crops.

	2014		**2016**		**2017**	
N treatment	**N concentration (mmol L^{-1})**	**N amount (kg ha^{-1})**	**N concentration (mmol L^{-1})**	**N amount (kg ha^{-1})**	**N concentration (mmol L^{-1})**	**N amount (kg ha^{-1})**
N1—Very deficient	2.4	64	2.0	88	2.0	86
N2—Deficient	6.2	189	5.3	302	5.7	304
N3—Conventional	12.6	516	9.7	561	9.7	519
N4—Excessive	16.1	804	13.5	1052	13.1	870
N5—Very excessive	20.0	990	17.7	1320	16.7	1198

4.3.3. Chlorophyll Meter Measurements

Chlorophyll meter measurements commenced on 27 (15 DAT), 18 (25 DAT), and 11 (21 DAT) August for the 2014, 2016, and 2017 crops, respectively. In the 2014 crop, measurements were made every seven days, and in the 2016 and 2017 crops every 14 days. In the three crops, measurements were made until the end of the crop. Three different leaf-clip chlorophyll meters were used, the SPAD-502 meter (Konica Minolta, Inc., Tokyo, Japan), the atLEAF+ meter (FT Green LLC, Wilmington, DE, USA), and the MC-100 Chlorophyll Concentration Meter (Apogee Instruments, Inc., Logan, UT, USA). The respective measurement values are SPAD units, atLEAF units, and chlorophyll content index (CCI). The SPAD-502 meter was used in each of the three crops (2014, 2016, and 2017). The atLEAF+ meter was used in the 2016 and 2017 crops. The MC-100 meter was used only in the 2017 crop. The areas measured in each measurement were 6 mm^2 for the SPAD-502, 13 mm^2 for the atLEAF+, and 63.6 mm^2 for the MC-100.

Measurements were made on one leaf of each of the 16 marked plants in each replicate plot. The value for each replicate plot was the mean of the 16 individual measurements. They were made at the same time of day (8:00 to 10:00 solar time), before irrigation/fertigation. Measurements were made on each plant on the most recently fully expanded and well-lit leaf, on the distal part of the adaxial side of the leaf, midway between the margin and the mid-rib of the leaf. Measurement was made by clipping the sensor onto the leaf. Leaves with physical damage or with condensed water were not measured, alternative plants being selected.

4.3.4. Determination of Crop Nitrogen Nutrition Index

The critical N curve derived for greenhouse-grown sweet pepper, $Nc = 4.488 \cdot DMP^{-0.196}$ (A. Rodríguez and R.B. Thompson, University of Almeria, personal communication), where DMP is dry matter production, was used to calculate the nitrogen nutrition index (*NNI*) as a measure of crop N status.

The *NNI* was calculated as:

$$NNI = \frac{Nact}{Nc}, \tag{1}$$

where *Nact* is the measured N content of the crop and *Nc* is the critical N content obtained from the critical N curve for each treatment for each biomass sampling date. *NNI* values for each day of chlorophyll meter measurement were calculated by interpolating DMP and crop N content values between the two biomass samplings on either side of the measurement date. Above-ground dry matter production 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 the dry matter contents determined by oven-drying representative sub-samples at 65 °C until a constant weight was reached. Fruit production and pruning was determined throughout the crop, in eight selected plants in each replicate plot. Representative samples of leaves, stems, and fruit from each biomass sampling, from each replicate plot, were ground sequentially in knife and ball mills. The total N content (%N) of each sample was determined using a Dumas-type elemental analyzer system (model Rapid N, Elementar, Analysensysteme GmbH, Hanau, Germany). The mass of N in each relevant component was calculated from the %N and the corresponding mass of dry matter. Total crop N uptake in each replicate plot, at each biomass sampling, was the sum of N in all relevant components. Crop N content (%N) for each biomass sampling was calculated, for each replicate plot, as crop N uptake divided by DMP.

4.3.5. Data Analysis

To account for differences in planting dates that occur in vegetable crops, and to facilitate the use and interpretation of chlorophyll meters in practical farming, measurements and analyses were based on phenological stage rather than on days after transplanting. Because of the frequent measurements with chlorophyll meters during the pepper cycle, there were several dates of measurements within each phenological stage. To integrate the various dates of measurement to provide a unique value for each phenological stage, integrated values of each chlorophyll meter measurement (*SPADi*, *atLEAF*i, and *CCIi*) and for the crop nitrogen nutrition index (*NNIi*), were calculated for each phenological stage. These integrated values were calculated as:

$$\textit{Integrated value} = 1/D \times \sum(V \times \mathrm{d}s), \tag{2}$$

where D was the total number of days of each phenological stage, V was the value measured at each measurement date, and ds was the interval between two successive measurement dates (values of each measurement date were pondered by the time elapsed between two consecutive measurements). Four major phenological stages were considered: (i) vegetative, (ii) flowering, (iii) early fruit growth, and (iv) harvest. The vegetative stage was from transplanting to the beginning of flowering. The flowering stage was from the beginning of flowering until fruit set. Early fruit growth was from fruit set until fruit maturation. The harvest stage commenced with the first fruit harvest and ended when the crop finished, this was the longest of the four phenological stages.

To evaluate the sensitivity of *SPADi*, *atLEAF*i, and *CCIi*, to estimate crop *NNIi*, regression analyses were performed for each phenological stage. Four types of regression equations (linear, quadratic, power, and exponential) were considered, and the best equation was selected using the Akaike information criterion (Akaike, 1974), which represents the best compromise between the goodness of fit and the complexity of a model. These regression analyses were performed for each phenological stage in each crop, and for each crop in its entirety. Additionally, where there was more than one crop in which a particular chlorophyll meter was used (SPAD, atLEAF+), these regression analyses were conducted on: (a) pooled data for each phenological stage from the different crops, and (b) composite whole crop data from the different crops. The CurveExpert Professional® 2.2.0 software (Daniel G. Hyams) was used for these regression analyses.

Sufficiency values of chlorophyll meter measurements for maximum crop growth were derived for each phenological stage from the relationship between integrated chlorophyll meter measurements and *NNIi*. The approach of Padilla et al. (Padilla et al., 2015) was used, in which the Akaike Information Criterion (AIC) best-fit equations that related chlorophyll meter measurements to *NNI* were solved for *NNI* = 1, which is the value of *NNI* that represents the optimal N nutrition for maximum growth. Sufficiency values of chlorophyll meter measurements were calculated for: (a) each phenological stage for each crop considered separately, (b) each whole crop, (c) each phenological stage for multiple crops, and (d) the whole crop using data from multiple crops.

4.4. Results

4.4.1. Effects of N Treatments on the Nitrogen Nutrition Index

In general, throughout the three crops (the 2014, 2016, and 2017 crops), *NNI* was consistently clearly less than one in the N1 and N2 treatments (Figure 1). The exception was the 2016 crop, where the N2 treatment had *NNI* values close to one in the second half of the crop (Figure 1b). In the three crops evaluated, N4 and N5 treatments had values higher than one for most of the crop. The N3 treatment in the three crops had *NNI* values that were consistently close to one for most of the crop (Figure 1).

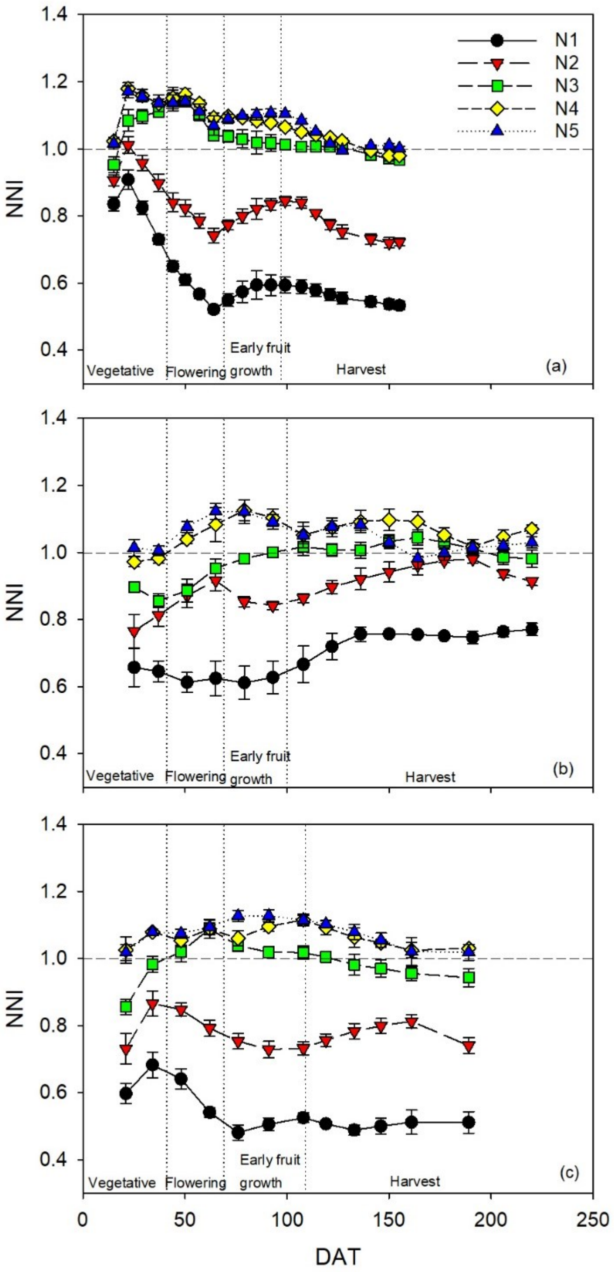

Figure 1. Temporal dynamics of the nitrogen nutrition index (NNI) for the sweet pepper (*Capsicum annuum*) crops in the (a) 2014, (b) 2016, and (c) 2017 crops, subjected to five different N treatments with four repetitions. Values are means (n = 4) ± standard error (±SE). DAT is days after transplanting. Vertical dotted lines represent the different phenological stages; the horizontal dotted line indicates NNI = 1.

In each of the three crops, there were significant differences in integrated *NNI* (*NNIi*) values between the N1, N2, N3, and N4 treatments in the vegetative phenological stage (Table 2). There were no significant differences in *NNIi* between the N4 and N5 treatments in the vegetative stage in the three crops (Table 2). Generally, the statistical results from comparing the *NNIi* values of the N treatments were very similar for the flowering, early fruit growth, and harvest phenological stages, in each of the three crops (Table 2). There were some exceptions, mostly when the *NNIi* values of the N3 treatment were not significantly different to those of the N4 treatment (Table 2).

Table 2. Integrated nitrogen nutrition index (NNIi) values for each N treatment within each phenological stage in each of the three sweet pepper (*Capsicum annuum*) crops. Different lower-case letters (a-d) show significant differences between N treatments within each phenological stage and crop, after the least significant difference (LSD) post-hoc test of ANOVA. p-value < 0.001. Values are means (n = 4) ± standard error (SE). Each crop was subjected to five different N treatments with four repetitions.

Phenological stage	**Treatment**	**2014 crop**	**2016 crop**	**2017 crop**
Vegetative	N1	0.82 ± 0.02^{a}	0.65 ± 0.04^{a}	0.64 ± 0.03^{a}
	N2	0.94 ± 0.02^{b}	0.79 ± 0.04^{b}	0.80 ± 0.03^{b}
	N3	1.06 ± 0.02^{c}	0.88 ± 0.02^{c}	0.92 ± 0.02^{c}
	N4	1.12 ± 0.02^{d}	0.98 ± 0.01^{d}	1.05 ± 0.02^{d}
	N5	1.12 ± 0.01^{d}	1.01 ± 0.02^{d}	1.05 ± 0.02^{d}
Flowering	N1	0.59 ± 0.01^{a}	0.62 ± 0.04^{a}	0.59 ± 0.02^{a}
	N2	0.80 ± 0.02^{b}	0.89 ± 0.03^{b}	0.82 ± 0.02^{b}
	N3	1.11 ± 0.02^{c}	0.92 ± 0.03^{b}	1.05 ± 0.03^{c}
	N4	1.13 ± 0.01^{c}	1.06 ± 0.03^{c}	1.07 ± 0.02^{c}
	N5	1.11 ± 0.02^{c}	1.10 ± 0.02^{c}	1.08 ± 0.02^{c}
Early fruit growth	N1	0.58 ± 0.03^{a}	0.62 ± 0.05^{a}	0.51 ± 0.02^{a}
	N2	0.81 ± 0.02^{b}	0.85 ± 0.01^{b}	0.74 ± 0.02^{b}
	N3	1.03 ± 0.03^{c}	0.99 ± 0.01^{c}	1.02 ± 0.01^{c}
	N4	1.09 ± 0.01^{cd}	1.11 ± 0.03^{d}	1.09 ± 0.01^{d}
	N5	1.10 ± 0.00^{d}	1.11 ± 0.03^{d}	1.12 ± 0.02^{d}
Harvest	N1	0.56 ± 0.02^{a}	0.74 ± 0.01^{a}	0.51 ± 0.02^{a}
	N2	0.77 ± 0.01^{b}	0.93 ± 0.01^{b}	0.77 ± 0.01^{b}
	N3	0.99 ± 0.00^{c}	1.01 ± 0.02^{c}	0.96 ± 0.02^{c}
	N4	1.02 ± 0.01^{cd}	1.07 ± 0.02^{d}	1.05 ± 0.01^{d}
	N5	1.03 ± 0.00^{d}	1.03 ± 0.02^{cd}	1.05 ± 0.02^{d}

4.4.2. Effects of N Treatments on Chlorophyll Meters Measurements

The temporal dynamics of measurements with the three chlorophyll meters (SPAD-502, atLEAF+, and MC-100) throughout the crops were very similar, regardless of the chlorophyll meter (Figure 2). Generally, treatment N1 had the lowest values, treatment

N2 was lower than treatments N3, N4, and N5, treatments N4 and N5 were the highest and were very similar, and treatment N3 was often intermediate between treatments N2 and N4. At times, values from treatments N3, N4, and N5 were all similar.

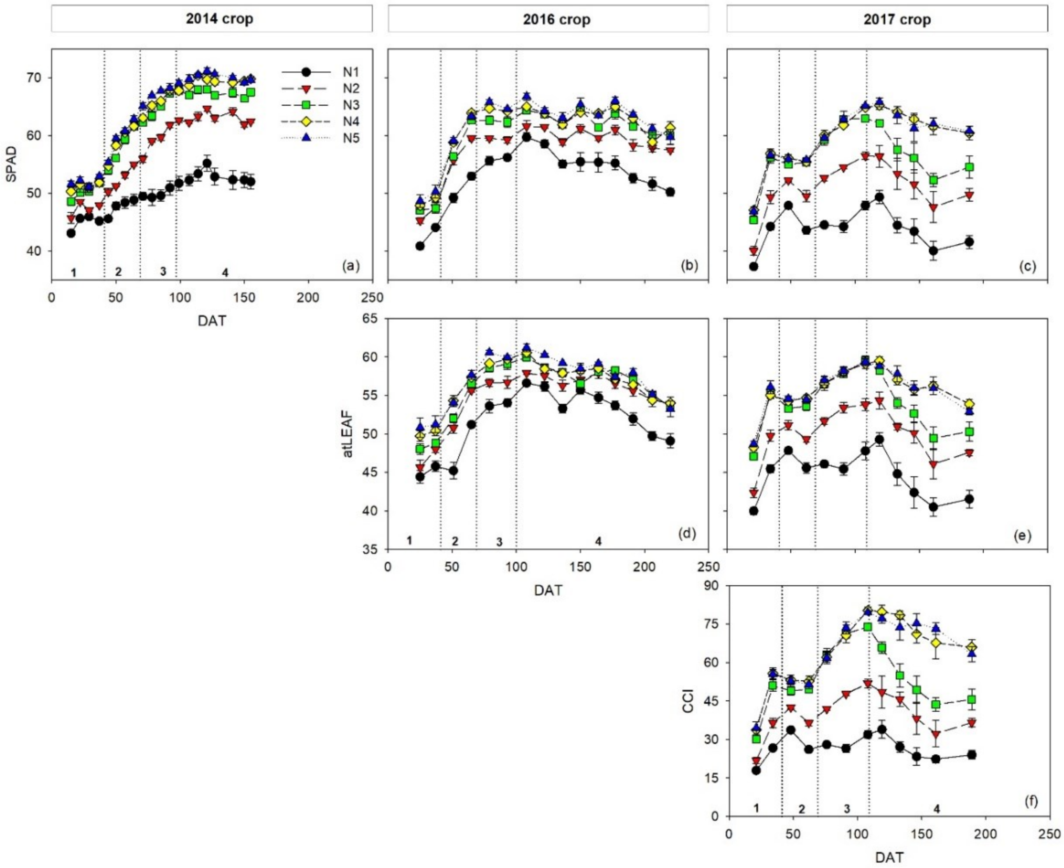

Figure 2. Temporal dynamics of chlorophyll meters measurements of SPAD values **(a,b,c)**, atLEAF values **(d,e)**, and CCI values **(f)**, for the sweet pepper (*Capsicum annuum*) crops subjected to five different N treatments with four repetitions. Vertical dotted lines and numbers represent the different phenological stages: 1—vegetative, 2—flowering, 3—early fruit growth, 4—harvest. Values are means (n = 4) ± standard error (SE). DAT is days after transplanting.

In each of the three crops (2014, 2016, and 2017) there were generally significant differences in integrated SPAD (*SPADi*) values between the N1, N2, and N3 treatments in each phenological stage (vegetative, flowering, early fruit growth, and harvest) (Table 3). There were generally no significant differences in *SPADi* values between the N3 and

N4 treatments. In all of the phenological stages of crops, there were no significant differences in *SPADi* between the N4 and N5 treatments (Table 3).

Table 3. Integrated SPAD (SPADi) values for each N treatment within each phenological stage in each of the three sweet pepper (*Capsicum annuum*) crops. Different lower-case letters (a-d) show significant differences between N treatments within each phenological stage and crop, after LSD post-hoc test of ANOVA. *p*-value < 0.001. Values are means (n = 4) ± standard error (SE). Each crop was subjected to five different N treatments with four repetitions.

Phenological stage	Treatment	2014 crop	2016 crop	2017 crop
Vegetative	N1	45.0 ± 0.1^{a}	42.5 ± 0.2^{a}	40.8 ± 0.2^{a}
	N2	47.3 ± 0.3^{b}	46.4 ± 0.8^{b}	44.7 ± 0.9^{b}
	N3	50.2 ± 0.4^{c}	47.3 ± 0.7^{bc}	50.8 ± 0.7^{c}
	N4	51.2 ± 0.6^{cd}	48.6 ± 0.6^{bc}	52.0 ± 0.4^{c}
	N5	52.0 ± 0.5^{d}	49.5 ± 1.1^{c}	51.8 ± 0.7^{c}
Flowering	N1	47.7 ± 0.7^{a}	51.1 ± 0.7^{a}	45.8 ± 0.5^{a}
	N2	52.5 ± 0.1^{b}	57.6 ± 0.4^{b}	50.9 ± 0.4^{b}
	N3	57.9 ± 0.5^{c}	59.5 ± 0.5^{c}	55.2 ± 0.5^{c}
	N4	58.8 ± 0.2^{cd}	61.4 ± 0.4^{d}	55.8 ± 0.6^{c}
	N5	59.6 ± 039^{d}	61.3 ± 0.4^{d}	55.8 ± 0.3^{c}
Early fruit growth	N1	49.8 ± 1.0^{a}	55.9 ± 0.6^{a}	45.7 ± 0.5^{a}
	N2	59.1 ± 0.3^{b}	59.4 ± 0.4^{b}	54.7 ± 0.2^{b}
	N3	64.5 ± 0.4^{c}	62.5 ± 0.7^{c}	61.8 ± 0.7^{c}
	N4	65.5 ± 0.4^{cd}	64.5 ± 0.2^{d}	62.4 ± 0.5^{c}
	N5	67.0 ± 0.4^{d}	65.2 ± 0.3^{d}	62.7 ± 0.4^{c}
Harvest	N1	52.7 ± 1.2^{a}	54.9 ± 0.7^{a}	43.2 ± 0.8^{a}
	N2	63.2 ± 0.3^{b}	59.7 ± 0.3^{b}	51.2 ± 1.9^{b}
	N3	67.4 ± 0.3^{c}	62.4 ± 0.2^{c}	55.9 ± 1.6^{c}
	N4	69.3 ± 0.2^{d}	63.0 ± 0.4^{cd}	62.3 ± 0.6^{d}
	N5	70.0 ± 0.3^{d}	63.7 ± 0.4^{d}	62.3 ± 1.0^{d}

Regarding the atLEAF+ meter, there were significant differences in integrated atLEAF (*atLEAF*i) values between the N1 and N2 treatments in all phenological stages in the 2016 and 2017 crops (Table 4). However, for the 2016 crop in most phenological stages there were no significant differences in *atLEAF*i values between the N2 and N3, but there were significant differences between these treatments in the 2017 crop. For both crops there were no significant differences between the N3 and N4 treatments, and between the N4 and N5 treatments in most phenological stages (Table 4).

Table 4. Integrated atLEAF (*atLEAF*i) values for each N treatment within each phenological stage in the two sweet pepper (*Capsicum annuum*) crops. Different lower-case letters (a-d) show significant differences between treatments within each phenological stage and crop, after LSD post-hoc test of ANOVA. *p*-value < 0.001. Values are means (n = 4) ± standard error (SE). Each crop was subjected to five different N treatments with four repetitions.

Phenological stage	Treatment	2016 crop	2017 crop
Vegetative	N1	45.1 ± 0.6^{a}	42.7 ± 0.3^{a}
	N2	46.8 ± 0.6^{ab}	46.1 ± 0.6^{b}
	N3	48.4 ± 0.4^{bc}	51.3 ± 0.4^{c}
	N4	50.1 ± 0.7^{cd}	51.6 ± 0.4^{c}
	N5	51.0 ± 1.1^{d}	52.4 ± 0.6^{c}
Flowering	N1	48.2 ± 0.6^{a}	46.7 ± 0.4^{a}
	N2	53.2 ± 0.3^{b}	50.2 ± 0.4^{b}
	N3	54.3 ± 0.2^{b}	53.5 ± 0.3^{c}
	N4	55.8 ± 0.3^{c}	54.5 ± 0.3^{d}
	N5	55.9 ± 0.2^{c}	54.5 ± 0.2^{d}
Early fruit growth	N1	53.8 ± 0.6^{a}	46.5 ± 0.7^{a}
	N2	56.7 ± 0.6^{b}	53.0 ± 0.4^{b}
	N3	58.8 ± 0.6^{c}	58.1 ± 0.4^{c}
	N4	59.4 ± 0.1^{cd}	58.0 ± 0.6^{c}
	N5	60.3 ± 0.2^{d}	58.2 ± 0.2^{c}
Harvest	N1	53.4 ± 0.3^{a}	43.1 ± 0.8^{a}
	N2	56.3 ± 0.4^{b}	49.2 ± 0.8^{b}
	N3	57.2 ± 0.2^{bc}	52.2 ± 0.8^{c}
	N4	57.3 ± 0.2^{c}	55.9 ± 0.4^{d}
	N5	58.0 ± 0.2^{c}	55.6 ± 0.4^{d}

For integrated CCI values (*CCIi*) measured with the MC-100 meter in the 2017 crop, there were significant differences in *CCIi* between N1 and N2 treatments, and between N2 and N3 treatments (Table 5). There were no significant differences in *CCIi* between the N4 and N5 treatments (Table 5).

Table 5.. Integrated CCI (CCIi) values for each N treatment within each phenological stage in the 2017 sweet pepper (*Capsicum annuum*) crop. Different lower-case letters (a-d) show significant differences between N treatments within each phenological stage, after LSD post-hoc test of ANOVA. p-value < 0.001. Values are means (n = 4) ± standard error (SE). The crop was subjected to five different N treatments with four repetitions.

Phenological stage	**Treatment**	***CCIi***
Vegetative	N1	22.3 ± 0.5^{a}
	N2	29.1 ± 1.5^{b}
	N3	40.5 ± 1.4^{c}
	N4	44.8 ± 1.2^{c}
	N5	45.0 ± 2.0^{c}
Flowering	N1	29.9 ± 0.8^{a}
	N2	39.5 ± 0.7^{b}
	N3	49.2 ± 0.8^{c}
	N4	52.9 ± 1.8^{d}
	N5	52.2 ± 1.3^{cd}
Early fruit growth	N1	29.0 ± 1.0^{a}
	N2	47.5 ± 0.8^{b}
	N3	69.7 ± 2.1^{c}
	N4	71.6 ± 1.1^{c}
	N5	72.2 ± 1.4^{c}
Harvest	N1	25.5 ± 1.1^{a}
	N2	39.2 ± 3.4^{b}
	N3	50.2 ± 3.2^{c}
	N4	71.1 ± 2.4^{d}
	N5	70.7 ± 2.5^{d}

4.4.3. Relationships between Integrated Chlorophyll Meters Measurements and Integrated NNI

Relationships between SPADi and NNIi values for each phenological stage in each of the three crops had coefficients of determination (R2) of 0.89–0.97, 0.80–0.88, and 0.86–0.96 for the 2014, 2016, and 2017 crops, respectively (Table 6). When averaged for the duration of each crop, the R2 values of the 2014, 2016, and 2017 crops were 0.93, 0.86, and 0.90, respectively (Table 6). Generally, in each of the three crops, the relationships between SPADi and NNIi values had similar R2 values for the different phenological stages (Table 6). Combining the three crops together, the R2 values of the four phenological stages ranged from 0.64 (harvest) to 0.85 (early fruit growth), with an average R2 value of 0.76 across the four phenological stages (Table 6). There was no evidence of saturation of SPAD values at higher NNI values in any of the four phenological stages in any of the three crops (Figure 3). Regression analysis showed that SPAD values increased when NNI values exceeded the optimal value, for instance, for crop growth.

Table 6. Coefficients of determination (R^2) of regressions between integrated SPAD (*SPADi*) values and integrated nitrogen nutrition index (*NNIi*) for each phenological stage in each of the three sweet pepper (*Capsicum annuum*) crops independently, and for the three crops together. Each crop was subjected to five different N treatments with four repetitions. According to the Akaike information criterion, the best-fit regression model (exponential, linear, power, quadratic, and natural logarithm) is shown. Also, the fitted equation and standard error of the estimate (SEE) are presented. All regressions were highly significant at *p*-value <0.001. N is the number of data points of regressions.

Crop	Phenological stage	Regression	Equation	R^2	SEE (±*NNIi*)	N
2014	Vegetative	Quadratic	$NNIi = -0.004 \cdot SPADi^2 + 0.4324 \cdot SPADi - 10.5$	0.89	0.04	20
	Flowering	Linear	$NNIi = 0.047\ SPADi - 1.6315$	0.95	0.05	20
	Early fruit growth	Exponential	$NNIi = 0.087e^{0.038 \cdot SPADi}$	0.92	0.05	20
	Harvest	Exponential	$NNIi = 0.082e^{0.0362 \cdot SPADi}$	0.97	0.04	20
2016	Vegetative	Natural Logarithm	$NNIi = 2.127 \cdot \log(SPADi) - 7.319$	0.80	0.07	20
	Flowering	Linear	$NNIi = 0.043 \cdot SPADi - 1.5753$	0.88	0.07	20
	Early fruit growth	Natural Logarithm	$NNIi = 3.079 \cdot \log(SPADi) - 11.741$	0.88	0.07	20
	Harvest	Natural Logarithm	$NNIi = 1.981 \cdot \log(SPADi) - 7.1744$	0.86	0.05	20
2017	Vegetative	Natural Logarithm	$NNIi = 1.517 \cdot \log(SPADi) - 4.9752$	0.86	0.07	20
	Flowering	Natural Logarithm	$NNIi = 2.379 \cdot \log(SPADi) - 8.5002$	0.92	0.06	20
	Early fruit growth	Exponential	$NNIi = 0.064 \cdot e^{0.0453 \cdot SPADi}$	0.96	0.06	20
	Harvest	Natural Logarithm	$NNIi = 1.349 \cdot \log(SPADi) - 4.523$	0.87	0.08	20
2014 + 2016 + 2017	Vegetative	Quadratic	$NNIi = -0.002 \cdot SPADi^2 + 0.1935 \cdot SPADi - 4.57$	0.82	0.07	60
	Flowering	Quadratic	$NNIi = -0.002 \cdot SPADi^2 + 0.2031 \cdot SPADi - 5.5115$	0.73	0.11	60

Early fruit growth	Power	$NNIi = 0.00007 \cdot SPADi^{2.3116}$	0.85	0.08	60
Harvest	Natural Logarithm	$NNIi = 1.125 \cdot \log(SPADi) - 3.6978$	0.64	0.11	60

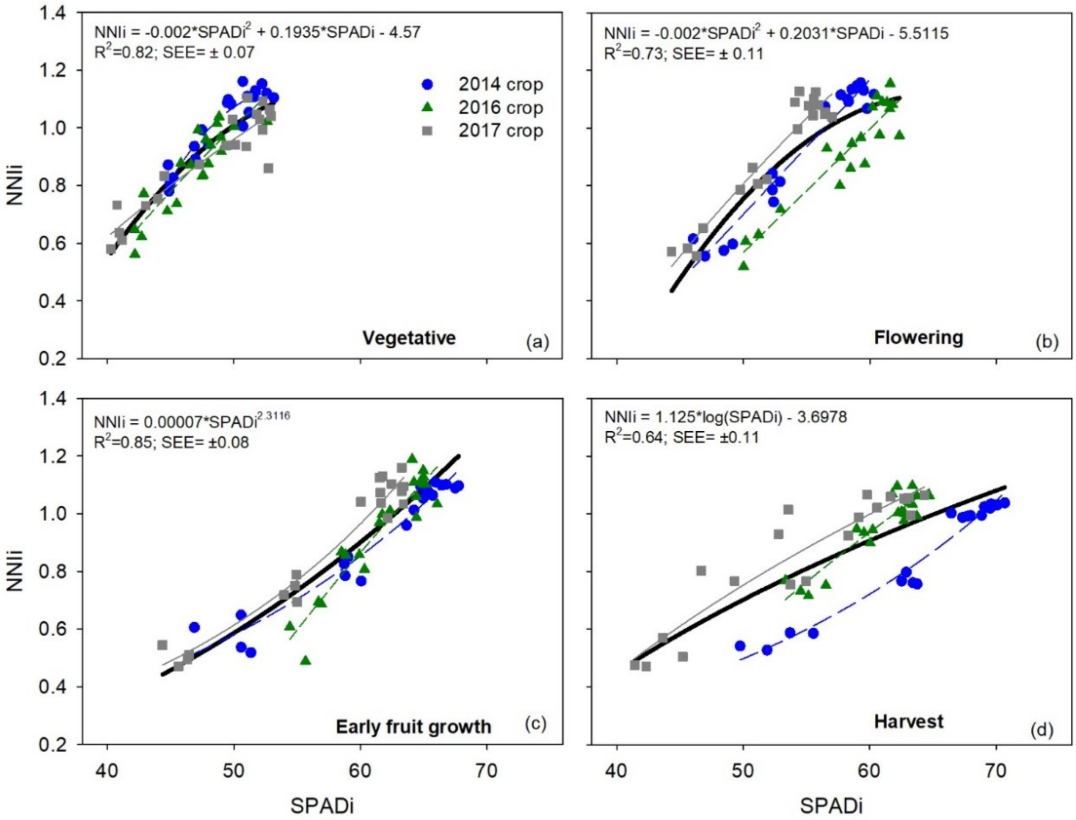

Figure 3. Relationships between integrated SPAD (*SPADi*) values and the integrated crop nitrogen nutrition index (*NNIi*) for each phenological stage in each of the three sweet pepper (*Capsicum annuum*) crops. Each crop was subjected to five different N treatments with four repetitions. The bold black line and the equation represent the adjustment for the combined dataset of the three crops together. Results of regression for each crop separately are in Table 6.

For the atLEAF+ meter, relationships between *atLEAF*i values and *NNIi* values for each phenological stage, in each of the crops, had R^2 values of 0.74 to 0.94 (Table 7). Averaged for each crop, R^2 values were 0.81 and 0.90 for the 2016 and 2017 crops, respectively (Table 7). There were no appreciable differences between the four phenological stages within each crop (Table 7). Combining the data of the two crops, the R^2 values of the four phenological stages ranged from 0.77 (harvest) to 0.83 (flowering), with an average R^2 value for the two entire crops of 0.80 (Table 7). There was no appreciable saturation of atLEAF values at higher *NNI* values in any of the four phenological stages in the two crops (Figure 4).

Table 7. Coefficients of determination (R^2) of regressions between integrated atLEAF (*atLEAFi*) values and integrated nitrogen nutrition index (*NNIi*) for each phenological stage in each of the two sweet pepper (*Capsicum annuum*) crops independently and for the two crops together. Each crop was subjected to five different N treatments with four repetitions. According to the Akaike information criterion, the best-fit regression model (exponential, linear, power, quadratic, and natural logarithm) is shown. Also, it is presented the fitted equation and standard error of the estimate (SEE). All regressions were highly significant, with p-value < 0.001. N is the number of data points of regression.

Crop	Phenological stage	Model	Equation	R^2	SEE (±*NNIi*)	N
2016	Vegetative	Natural Logarithm	$NNIi = 2.333 \cdot \log(atLEAFi) - 8.1826$	0.74	0.08	20
	Flowering	Linear	$NNIi = 0.057 \cdot atLEAFi - 2.1224$	0.85	0.07	20
	Early fruit growth	Natural Logarithm	$NNIi = 4.042 \cdot \log(atLEAFi) - 15.458$	0.84	0.08	20
	Harvest	Natural Logarithm	$NNIi = 3.561 \cdot \log(atLEAFi) - 13.406$	0.81	0.06	20
2017	Vegetative	Natural Logarithm	$NNIi = 1.841 \cdot \log(atLEAFi) - 6.2617$	0.84	0.07	20
	Flowering	Natural Logarithm	$NNIi = 3.117 \cdot \log(atLEAFi) – 11*.38$	0.94	0.05	20
	Early fruit growth	Exponential	$NNIi = 0.026 \cdot e^{0.0637 \cdot atLEAFi}$	0.93	0.07	20
	Harvest	Natural Logarithm	$NNIi = 1.996 \cdot \log(atLEAFi) - 6.9817$	0.90	0.07	20
2016 + 2017	Vegetative	Natural Logarithm	$NNIi = 1.988 \cdot \log(atLEAFi) - 6.8381$	0.79	0.07	40
	Flowering	Natural Logarithm	$NNIi = 2.828 \cdot \log(atLEAFi) - 10.284$	0.83	0.08	40
	Early fruit growth	Exponential	$NNIi = 0.027 \cdot e^{0.0617 \cdot atLEAFi}$	0.82	0.10	40
	Harvest	Natural Logarithm	$NNIi = 1.724 \cdot \log(atLEAFi) - 5.9541$	0.77	0.09	40

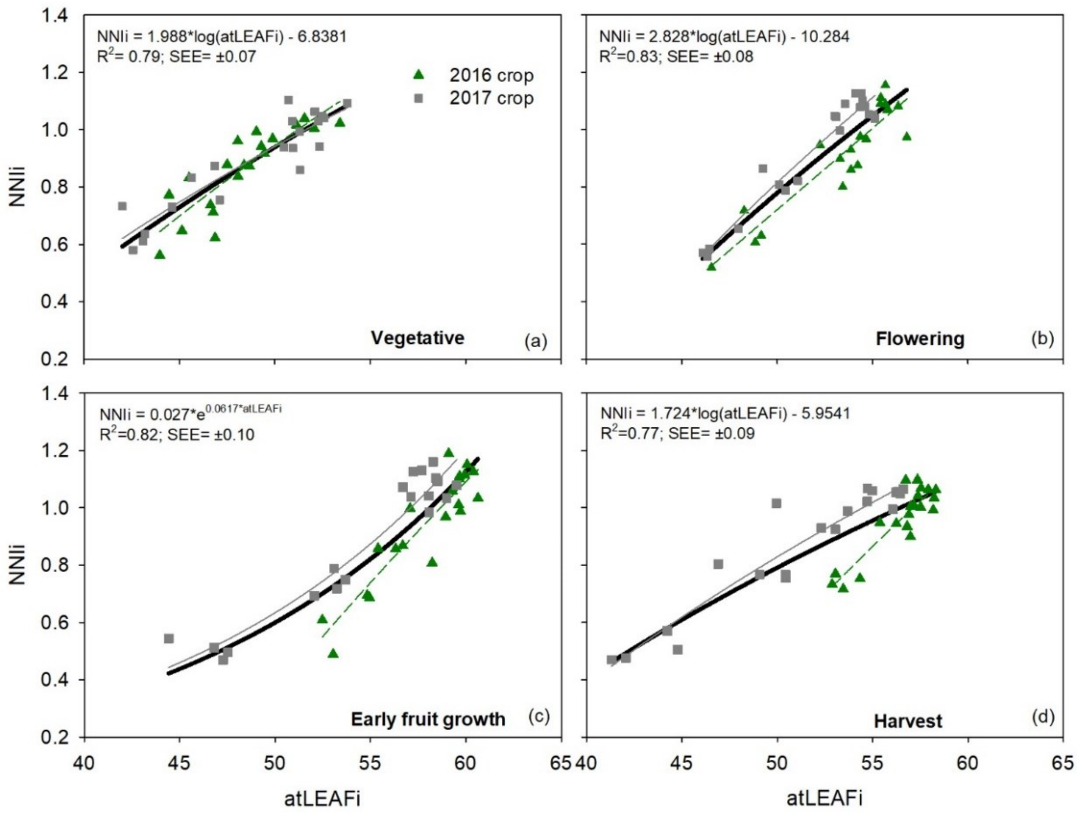

Figure 4. Relationships between integrated atLEAF (*atLEAFi*) values and the integrated crop nitrogen nutrition index (*NNIi*) for each phenological stage in each of the two sweet pepper (*Capsicum annuum*) crops. Each crop was subjected to five different N treatments with four repetitions. The bold black line and the equation represent the adjustment for the combined dataset of the two crops together. Results of regression for each crop separately are in Table 7.

Relationships between *CCIi* and *NNIi* values for each phenological stage of the 2017 crop had R^2 values of 0.87–0.96 (Table 8). The lowest R^2 value was observed in both the vegetative and harvest stages, and the highest value in the early fruit growth stage. The average R^2 value for all four phenological stages was 0.91 (Table 8). There was no indication of saturation of *CCIi* values at higher *NNI* values in any of the four phenological stages (Figure 5).

Table 8. Coefficients of determination (R^2) of regressions between integrated chlorophyll content index (*CCIi*) values and the integrated nitrogen nutrition index (*NNIi*) for each phenological stage in a sweet pepper (*Capsicum annuum*) in 2017. The crop was subjected to five different N treatments with four repetitions. According to the Akaike information criterion, the best-fit regression model (exponential, linear, power, quadratic, and natural logarithm) is shown. Also, it is presented the fitted equation and standard error of the estimate (SEE). All regressions were highly significant, with *p*-value < 0.001. N is the number of data points of regression.

Phenological stage	Model	Equation	R^2	SEE (±*NNIi*)	N
Vegetative	Natural Logarithm	$NNIi = 0.537 \cdot \log(CCIi) - 1.0186$	0.87	0.06	20
Flowering	Quadratic	$NNIi = -0.001 \cdot CCIi^2 + 0.0683 \cdot CCIi - 0.9521$	0.94	0.05	20
Early fruit growth	Linear	$NNIi = 0.014 \cdot CCIi + 0.1119$	0.96	0.05	20
Harvest	Natural Logarithm	$NNIi = 0.487 \cdot \log(CCIi) - 1.0157$	0.87	0.08	20

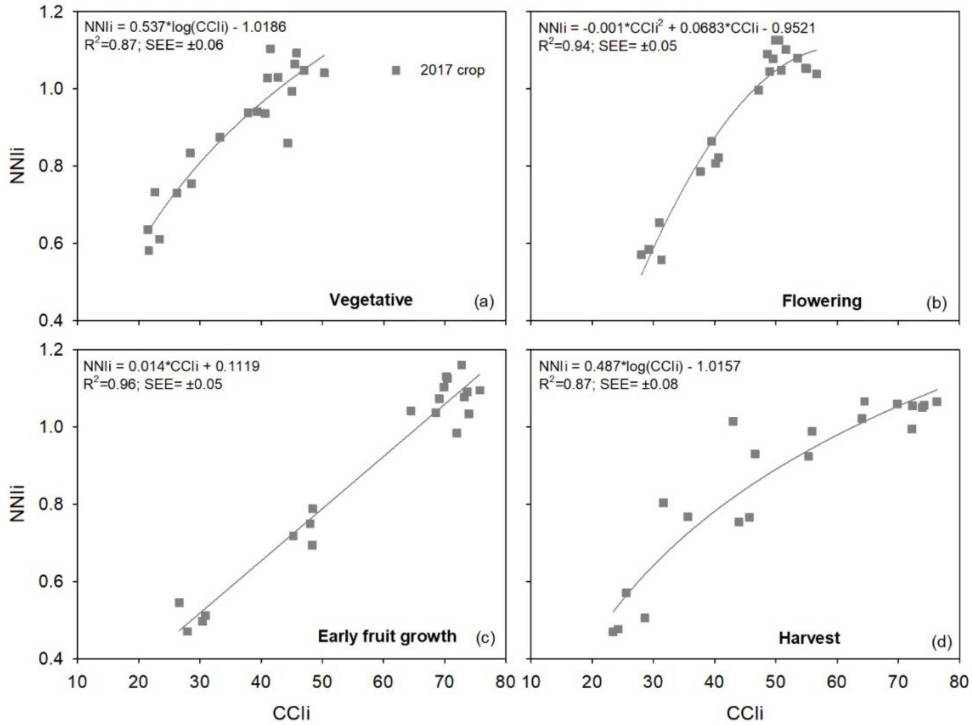

Figure 5. Relationships between integrated chlorophyll content index (*CCIi*) values and the integrated crop nitrogen nutrition index (*NNIi*) for each phenological stage in a sweet pepper (*Capsicum annuum*) crop in 2017. The crop was subjected to five different N treatments with four repetitions. Results of regression are in Table 8.

4.4.4. Sufficiency Values of Chlorophyll Meters Measurements

The sufficiency values for each phenological stage of each chlorophyll meter for maximum crop growth were derived, from the relationship between the integrated chlorophyll meter measurements of a given phenological stage and integrated *NNI* value of that phenological stage. Sufficiency values for the three chlorophyll meters for each of the four phenological stages in each of the three crops, and when the three crops were considered together are presented in Table 9.

SPAD sufficiency values in the vegetative stage were lower than for the other three phenological stages in each of the three crops. The average value for the vegetative stage of the three crops considered together was 49.7 ± 2.3 SPAD units. Sufficiency values for the flowering stage were intermediate between the vegetative and the early fruit growth and harvest stages, which were similar. The average sufficiency value for the flowering stage of the three crops considered together was 56.6 ± 4.6 SPAD units. SPAD sufficiency values for the early fruit growth and harvest stages were similar in the 2016 and 2017 crops, the average value for both stages for both crops was 61.4 ± 0.6 SPAD units. In the 2014 crop, sufficiency values of these two phenological stages were slightly higher, with the average value for both stages being 66.5 ± 2.4 SPAD units. The SPAD sufficiency values for the early fruit growth and harvest phenological stages of the three crops, considered together, were 62.7 ± 2.3 and 65.2 ± 6.3 SPAD units, respectively (Table 9). Averaged across all four phenological stages and the three crops, the single SPAD sufficiency value for the entire crop was 58.6 ± 3.5 SPAD units.

Sufficiency atLEAF values were lowest in the vegetative stage, intermediate in the flowering stage, and highest in the early fruit growth and harvest stages, for both crops (Table 9). Sufficiency atLEAF values for each phenological phase averaged for the two crops considered together ranged between 51.6 ± 1.9 atLEAF units (vegetative stage) and 58.1 ± 1.5 atLEAF units (early fruit growth stage) (Table 9). Averaged across all four phenological stages and the two crops, the atLEAF sufficiency value for the entire crop was 54.9 ± 0.8 atLEAF units.

Sufficiency values of CCI, measured with the MC-100 meter in the 2017 crop, were lowest in the vegetative stage (42.9 ± 4.9), intermediate in the flowering stage (46.5 ± 3.4), and highest in the early fruit growth and harvest stages (average value for the two stages of 64.3 ± 1.5) (Table 9). Averaged across all four phenological stages, the CCI sufficiency value for the entire crop was 54.5 ± 5.7.

The relative differences in sufficiency values between phenological stages were notably larger for CCI than for SPAD and atLEAF. In the 2017 crop, which was the only crop in which all three chlorophyll meters were used, relative differences in the sufficiency values between the flowering and vegetative stages were 5.4% for SPAD, 2.8% for atLEAF, and 7.7% for CCI. The respective relative differences in the sufficiency values between the early fruit growth and flowering stages were 10.9% (SPAD), 7.0% (atLEAF), and 29.3% (CCI).

Table 9. Sufficiency values of SPAD, atLEAF, and CCI, in each of the four phenological stages, for individual sweet pepper (*Capsicum annuum*) crops and for all years combined. Values are means ± standard error (SE).

Crop	Phenological stage	SPAD	atLEAF	CCI
2014	Vegetative	48.1 ± 1.0	—	—
	Flowering	56.4 ± 1.1		
	Early fruit growth	64.1 ± 1.4		
	Harvest	68.8 ± 0.1		
2016	Vegetative	49.9 ± 1.5	51.2 ± 1.7	—
	Flowering	60.1 ± 1.5	54.9 ± 1.3	
	Early fruit growth	62.7 ± 1.4	58.7 ± 1.2	
	Harvest	61.9 ± 1.5	57.1 ± 0.9	
2017	Vegetative	51.3 ± 2.2	51.6 ± 1.9	42.9 ± 4.9
	Flowering	54.2 ± 1.3	53.1 ± 0.8	46.5 ± 3.4
	Early fruit growth	60.8 ± 1.2	57.1 ± 1.1	65.7 ± 4.0
	Harvest	60.1 ± 3.5	54.5 ± 1.9	62.8 ± 10.1
All years	Vegetative	49.7 ± 2.3	51.6 ± 1.9	—
	Flowering	56.6 ± 4.6	54.1 ± 1.5	
	Early fruit growth	62.7 ± 2.3	58.1 ± 1.5	
	Harvest	65.2 ± 6.3	56.5 ± 2.9	

4.5. Discussion

Integrated measurements of the three chlorophyll meters (SPAD-502, atLEAF+, and MC-100) were very strongly related to integrated *NNI* for: (a) each of the four phenological stages (vegetative, flowering, early fruit growth, and harvest) of each pepper crop, (b) each crop considered in its entirety, (c) individual phenological stage, using composite data for all crops in which measurements were made, and (d) single values for the entirety of the crop, for the crops in which measurements were made. These results demonstrate that the three chlorophyll meters provided good estimations of the crop N status of sweet pepper. This is in agreement with studies that reported strong relationships between chlorophyll meter measurements and crop N status, in various horticultural (Padilla et al., 2017a, 2015; Wu et al., 2012) and cereal crops (Cartelat et al., 2005; Prost and Jeuffroy, 2007; Zhao et al., 2018).

Considering the four individual phenological stages, the strongest relationships between the three integrated chlorophyll meter measurements and *NNIi* were obtained in the flowering and early fruit growth stages, which occurred in the middle of the crops, for individual crops, and for when data was combined from multiple crops. Similarly, the strongest relationship between chlorophyll meter measurements and leaf N concentration occurred in the middle of the growing season in potatoes (Gianquinto et al., 2004). In the present study, there were also strong relationships at the beginning (in the vegetative stage) and at the end of the crop (in the harvest stage), but with slightly lower R^2 values than in the flowering and early fruit growth stages. The strong relationship in the vegetative stage of sweet pepper, in this study, contrasts with the results for cucumber in a previous study, where there was a weak relationship between SPAD measurements and *NNI* in the vegetative stage (Padilla et al., 2017a), which was attributed to limited differentiation of the N treatments at the beginning of that crop (Padilla et al., 2017a). In the current study, the high R^2 values between the integrated chlorophyll meters measurements and *NNIi* in each of the four phenological stages, regardless of year and chlorophyll meter, demonstrated the robust ability of chlorophyll meter measurements to be used as indicators of the crop N status of sweet pepper.

For the three chlorophyll meters, there was no evidence of saturation when relating measurements to *NNIi* in any of the four phenological stages and in the different crops. Regression analysis showed that *SPADi*, *atLEAFi*, and *CCIi* values increased when *NNIi* values exceeded the optimal value for the crop growth of one. Saturation of SPAD-502 and atLEAF+ measurements at high chlorophyll contents, which are associated with high crop N contents has been often reported (Cartelat et al., 2005; Novichonok et al., 2016; Padilla et al., 2018a). However, saturation of chlorophyll meter measurements does not always occur at higher crop N contents (Gianquinto et al., 2004; Padilla et al., 2015), as it depends on whether leaf chlorophyll contents are sufficiently high to cause saturation (Padilla et al., 2018a). None of the three chlorophyll meters evaluated were able to differentiate between the N4 and N5 treatments. This was not due to a saturation response of the chlorophyll meters, but rather was due to the similar crop N status of these two treatments, as indicated by the very similar *NNIi* values. The *NNIi* values of treatments N4 and N5 were not significantly different for any of the three crops.

Regarding the calculation of sufficiency values of the SPAD-502 meter, there were only small differences in sufficiency values for each phenological stage between the three different crops. Similarly, there were only small differences between sufficiency values for individual crops and the corresponding sufficiency value for the combined crop data set, for a given phenological stage. These data indicate that the sufficiency values determined for each phenological stage were very consistent between the three different years, and that SPAD sufficiency values obtained with the combined dataset are representative of the three crops.

The relative constancy of SPAD sufficiency values over time can be assessed by comparing the sufficiency values of the different phenological stages, using the combined data of the three crops. The relative difference between the sufficiency values of the vegetative and flowering stages was 12.2%, between flowering and early fruit growth was 9.7%, and between the early fruit growth and harvest stages was 3.8%. The differences between the sufficiency values for successive stages diminished as the crop grew. This was attributed to the temporal dynamics of chlorophyll meter values (Figure 2) because SPAD measurements increased in the early part of the crops and reached relatively stable values midway through the crops. There was a large difference in SPAD sufficiency values between the harvest stage (last phenological stage of the crop) and the vegetative stage (first phenological stage of the crop) of 15.5 SPAD units, the relative difference being 23.4%. This large difference during the crop suggests that a single SPAD sufficiency value cannot be used for a whole sweet pepper crop. In contrast, single SPAD sufficiency values for a whole crop have been proposed for cucumber (Güler and Büyük, 2007; Padilla et al., 2017a) and grapevine (Zoran G. Cerovic et al., 2015).

The SPAD sufficiency values derived for sweet pepper, in the present study, are generally higher than reported for other horticultural crops; the highest sufficiency value obtained in the present work was 64.0 ± 1.3 SPAD units. In indeterminate tomato, the average sufficiency value for the complete crop cycle was 54.2 SPAD units (F.M. Padilla et al., 2018). In cucumber, sufficiency values have been recommended for the whole crop of 45.2 SPAD units (Padilla et al., 2017a) and 44.9 SPAD units (Güler and Büyük, 2007). In potato, a whole crop sufficiency value of 38.2 SPAD units was recommended (Gianquinto et al., 2003). The appreciably higher sufficiency values for the SPAD-502 meter for sweet pepper in the present work can be explained by the very high leaf chlorophyll content of sweet pepper (Padilla et al., 2018a). In a study with 22 common crop species, sweet pepper had the highest leaf chlorophyll concentration, which was double that of maize (Parry et al., 2014).

The performance of the atLEAF+ and MC-100 meters was similar to the SPAD-502 meter in terms of sufficiency values. With both the atLEAF+ and MC-100 meters, the lowest sufficiency values were in the vegetative stage, and the highest in the early fruit growth and harvest stages. As with the SPAD, these temporal variations were associated with the dynamics of chlorophyll meter measurements throughout the crop, which initially increased and then were relatively constant in the second half of the crop. For the atLEAF+ meter, there were only small differences in the sufficiency value, for each of the four phenological stages, between the 2016 and 2017 crops. This indicates that the atLEAF sufficiency values determined were consistent between the two crops. It also demonstrates that the sufficiency values calculated using the combined data set of the two crops are representative of both crops. For the MC-100 meter, this is one of the first studies to provide sufficiency values of CCI. With this chlorophyll meter in the current study, there was only one crop; so, it was not possible to assess the consistency of sufficiency values between crops. The relative differences in sufficiency values between each of the four phenological stages, for the atLEAF+ sensor and the MC-100 meter, were calculated to assess the consistency of sufficiency values for each chlorophyll meter over time. The atLEAF+ meter had the narrowest range in sufficiency values, with the difference between the early fruit growth stage (maximum sufficiency value) and the vegetative stage (minimum sufficiency value) being 11.2%. The largest range of sufficiency values was with the MC-100 meter, where the relative difference between the maximum sufficiency value (early fruit growth stage) and the minimum sufficiency value (vegetative stage) was 34.0%.

Following the evaluation and the derivation of sufficiency values, chlorophyll meters could be used to frequently assess the crop N status of fertigated pepper crops that frequently receive N by regular drip irrigation. In greenhouses in SE Spain, N and other nutrients are applied every one to four days in each irrigation. Sufficiency values are required for practical real time monitoring of crop N status, using chlorophyll meters. Frequent effective monitoring of crop N status will enable rapid correction of crop N status by adjusting mineral N fertilizer application when chlorophyll meter measurements deviate from sufficiency values (Padilla et al., 2015), thereby ensuring optimal N nutrition. This will also reduce excessive "insurance" N applications that are applied to avoid the risk of N deficiency. In crops grown with fertigation systems, where N is applied in every irrigation, adjustment in N fertilization can be made very soon after such deviations are detected (Thompson et al., 2017). The results obtained in this study may be applied to sweet pepper crops grown in greenhouses; for sweet pepper crops grown outdoors, further research is required to validate these sufficiency values.

Overall, the results of this study show the potential of chlorophyll meters for monitoring crop N status and to assist with N fertilizer management of sweet pepper. The strong relationship between integrated chlorophyll meter measurements and NNIi for each phenological stage of each crop, when considered separately and as a combined dataset from different crops, demonstrated the consistency and robustness of chlorophyll meter measurements as indicators of crop N status. The sufficiency values calculated for chlorophyll meter measurements in each phenological stage and their consistency throughout crops showed the potential for the sufficiency values to be used in commercial farming to achieve improved N management of sweet pepper crops.

4.6. References

Akaike, H., 1974. A new look at the statistical model identification. IEEE Trans. Automat. Contr. 19, 716–723.

Cartelat, A., Cerovic, Z.G., Goulas, Y., Meyer, S., Lelarge, C., Prioul, J.L., Barbottin, A., Jeuffroy, M.H., Gate, P., Agati, G., Moya, I., 2005. Optically assessed contents of leaf polyphenolics and chlorophyll as indicators of nitrogen deficiency in wheat (Triticum aestivum L.). F. Crop. Res. 91, 35–49.

Castilla, N., Hernandez, J., 2005. The Plastic Greenhouse Industry of Spain. Chron. Horticult. 45, 15–20.

Cerovic, Z.G., Ghozlen, N. Ben, Milhade, C., Obert, M., Debuisson, S., Le Moigne, M., 2015. Nondestructive Diagnostic Test for Nitrogen Nutrition of Grapevine (Vitis vinifera L.) Based on Dualex Leaf-Clip Measurements in the Field. J. Agric. Food Chem. 63, 3669–3680.

FAOSTAT, 2019. FAO [WWW Document]. URL http://www.fao.org/faostat/en/#data/QC (accessed 4.11.19).

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

Gianquinto, G., Goffart, J.P., Olivier, M., Guarda, G., Colauzzi, M., Dalla Costa, L., Delle Vedove, G., Vos, J., Mackerron, D.K.L., 2004. The use of hand-held chlorophyll meters as a tool to assess the nitrogen status and to guide nitrogen fertilization of potato crop. Potato Res. 47, 35–80.

Gianquinto, G., Sambo, P., Bona, S., 2003. The use of SPAD-502 chlorophyll meter for dynamically optimising the nitrogen supply in potato crop: a methodological approach. Acta Hortic. 627, 217–224.

Greenwood, D., Lemaire, G., Gosse, G., Cruz, P., Draycott, A., Neeteson, J., 1990. Decline in Percentage N of C3 and C4 Crops with Increasing Plant Mass. Ann. Bot. 66, 425–

436.

Güler, S., Büyük, G., 2007. Relationships among chlorophyll-meter reading value, leaf N and yield of cucumber and tomatoes. Acta Hortic. 729, 307–311.

Junta de Andalucía, 2016. Cartografía de invernaderos en el litoral de Andalucía Oriental. Año 2016 21.

Khoddamzadeh, A.A., Dunn, B.L., 2016. Application of optical sensors for nitrogen management in Chrysanthemum. HortScience 51, 915–920.

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. Eur. J. Agron. 28, 614–624.

Mastalerczuk, G., Borawska-Jarmułowicz, B., Kalaji, H.M., Dąbrowski, P., Paderewski, J., 2017. Gas-exchange parameters and morphological features of festulolium (Festulolium braunii K. Richert A. Camus) in response to nitrogen dosage. Photosynthetica 55, 20–30.

Meisinger, J.J., Schepers, J.S., Raun, W.R., 2008. Crop Nitrogen Requirement and Fertilization. Am. Soc. Agron. Crop Sci. Soc. Am. Soil Sci. Soc. Am. 563–612.

Minotti, P.L., Halseth, D.E., Sieczka, J.B., 1994. Field chlorophyll measurements to assess the nitrogen status of potato varieties. HortScience 29, 1497–1500.

Monostori, I., Árendás, T., Hoffman, B., Galiba, G., Gierczik, K., Szira, F., Vágújfalvi, A., 2016. Relationship between SPAD value and grain yield can be affected by cultivar, environment and soil nitrogen content in wheat. Euphytica 211, 103–112.

Novichonok, E. V, Novichonok, A.O., Kurbatova, J.A., Markovskaya, E.F., 2016. Use of the atLEAF+ chlorophyll meter for a nondestructive estimate of chlorophyll content. Photosynthetica 54, 130–137.

Olivier, M., Goffart, J.P., Ledent, J.F., 2006. Threshold value for chlorophyll meter as decision tool for nitrogen management of potato. Agron. J. 98, 496–506.

Padilla, Francisco M., de Souza, R., Peña-Fleitas, M.T., Gallardo, M., Giménez, C., Thompson, R.B., 2018a. Different responses of various chlorophyll meters to increasing nitrogen supply in sweet pepper. Front. Plant Sci. 9, 1752.

Padilla, Francisco M., Gallardo, M., Peña-Fleitas, M.T., de Souza, R., Thompson, R.B., 2018b. Proximal Optical Sensors for Nitrogen Management of Vegetable Crops: A Review. Sensors 18, 2083.

Padilla, F.M., Peña-Fleitas, M.T., Gallardo, M., Giménez, C., Thompson, R.B., 2017. Derivation of sufficiency values of a chlorophyll meter to estimate cucumber nitrogen status and yield. Comput. Electron. Agric. 141, 54–64.

Padilla, F.M., Peña-Fleitas, M.T., Gallardo, M., Thompson, R.B., 2016. Proximal optical sensing of cucumber crop N status using chlorophyll fluorescence indices. Eur. J. Agron. 73, 83–97.

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. Ann. Appl. Biol. 166, 271–285.

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. Eur. J. Agron. 58, 39–52.

Padilla, F.M., Thompson, R.B., Peña-Fleitas, M.T., Gallardo, M., 2018. Reference values for phenological phases of chlorophyll meter readings and reflectance indices for optimal N nutrition of fertigated tomato. Acta Hortic. 1192, 65–72.

Parry, C., Blonquist, J.M., Bugbee, B., 2014. In situ measurement of leaf chlorophyll concentration: analysis of the optical/absolute relationship. Plant. Cell Environ. 37, 2508–2520.

Prost, L., Jeuffroy, M.H., 2007. Replacing the nitrogen nutrition index by the chlorophyll meter to assess wheat N status. Agron. Sustain. Dev. 27, 321–330.

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 Dalias (SE - Spain). Environ. Geol. 39, 791–799.

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

Schepers, J.S., Blackmer, T.M., Wilhelm, W.W., Resende, M., 1996. Transmittance and reflectance measurements of corn leaves from plants with different nitrogen and water supply. J. Plant Physiol. 148, 523–529.

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. Agric. Water Manag. 89, 261–274.

Thompson, R.B., Tremblay, N., Fink, M., Gallardo, M., Padilla, F.M., 2017. Tools and strategies for sustainable nitrogen fertilisation of vegetable crops, in: Tei, F., Nicola, S., Benincasa, P. (Eds.), Advances in Research on Fertilization Management in

Vegetable Crops. Springer, Heidelberg, Germany, pp. 11–63.

Tremblay, N., Fallon, E., Ziadi, N., 2011. Sensing of crop nitrogen status: Opportunities, tools, limitations, and supporting information requirements. Horttechnology 21, 274–281.

Valera, D.L., Belmonte, L.J., Molina-Aiz, F.D., López, A., Camacho, F., 2017. The greenhouses of Almería, Spain: Technological analysis and profitability, in: Acta Horticulturae. pp. 219–226.

Westerveld, S.M., McKeown, A.W., Scott-Dupree, C.D., McDonald, M.R., 2004. Assessment of chlorophyll and nitrate meters as field tissue nitrogen tests for cabbage, onions, and carrots. Horttechnology 14, 179–188.

Wu, X., Guo, J., Zhao, C., Chen, L., Zhang, Y., Fang, Z., Yang, Y.Y., 2012. Research and application of non-destructive testing diagnosis technology of tomato. Sens. Lett. 10, 666–669.

Zhao, B., Zhang, J., Yang, Q., Ata-Ul-Karim, S.T., Zhang, Y., Ning, D., Duan, A., Qin, A., Xiao, J., Liu, Zhandong, Liu, Zugui, 2018. Simple Assessment of Nitrogen Nutrition Index in Summer Maize by Using Chlorophyll Meter Readings. Front. Plant Sci. 9, 1–13.

Zhu, J., Tremblay, N., Liang, Y., 2011. A corn nitrogen status indicator less affected by soil water content. Agron. J. 103, 890–898.

5. Chapter two: Effect of cultivar on chlorophyll meter and canopy reflectance measurements in cucumber

de Souza, R., Grasso, R., Teresa Peña-Fleitas, M., Gallardo, M., Thompson, R.B., Padilla, F.M. 2020. Effect of cultivar on chlorophyll meter and canopy reflectance measurements in cucumber. *Sensors* 20(2): 509.

5.1. Abstract

Optical sensors can be used to assess crop N status to assist with N fertilizer management. Differences between cultivars may affect optical sensor measurement. Cultivar effects on measurements made with the SPAD-502 (Soil Plant Analysis Development) meter and the MC-100 (Chlorophyll Concentration Meter), and of several vegetation indices measured with the Crop Circle ACS470 canopy reflectance sensor, were assessed. A cucumber (*Cucumis sativus* L.) crop was grown in a greenhouse, with three cultivars. Each cultivar received three N treatments, of increasing N concentration, being deficient (N1), sufficient (N2) and excessive (N3). There were significant differences between cultivars in the measurements made with both chlorophyll meters, particularly when N supply was sufficient and excessive (N2 and N3 treatments, respectively). There were no consistent differences between cultivars in vegetation indices. Optical sensor measurements were strongly linearly related to leaf N content in each of the three cultivars. The lack of a consistent effect of cultivar on the relationship with leaf N content suggests that a unique equation to estimate leaf N content from vegetation indices can be applied to all three cultivars. Results of chlorophyll meter measurements suggest that care should be taken when using sufficiency values, determined for a particular cultivar.

Keywords: genotype; greenhouse; leaf nitrogen; proximal optical sensors; vegetation index

5.2. Introduction

In intensive vegetable production, large applications of nitrogen (N) fertilizer are used to ensure high yields (Ju et al., 2006; Thompson et al., 2007). The amounts of N applied often appreciably exceed crop requirements; the excess N is susceptible to nitrate (NO_3^-) leaching (Thompson et al., 2007; Zotarelli et al., 2009), and to subsequent N contamination of aquifers and surface water bodies (Meisinger et al., 2008; Padilla et al., 2018c). Nitrate contamination of aquifers and surface water bodies, from intensive vegetable production, has been reported for diverse regions, such as southeast Spain (Pulido-Bosch et al., 2000), southeast United States (Zotarelli et al., 2009) and China (Cui et al., 2011; Ju et al., 2006).

For optimal management of N in intensive crop and vegetable production, with minimal N loss to the environment, it is necessary to match N supply to crop N demand (Thompson et al., 2017). Assessment of crop N status informs of the immediate balance between N supply and demand (Schröder et al., 2000; Thompson et al., 2017). An effective and rapid means to assess crop N status is through the use of proximal optical sensors (Fox and Walthall, 2008; Padilla et al., 2018c; Thompson et al., 2017). Chlorophyll meters have been extensively researched and are used commercially to assess crop N status because their measurements of relative leaf chlorophyll content are generally strongly related to leaf N content, which reflects crop N status (Fox and Walthall, 2008; Padilla et al., 2018c; Samborski et al., 2009b; Schepers et al., 1996). Chlorophyll meters make non-destructive measurements of relative leaf chlorophyll content by measuring the absorbance and transmittance of radiation of two light wavelengths by the leaf. Chlorophyll absorbs red radiation and transmits most of the near infra-red (NIR) radiation, which is influenced by leaf thickness, among several parameters (Fox and Walthall, 2008; Padilla et al., 2018c; Schepers et al., 1996). Absorbance of red radiation increases with chlorophyll content, resulting in higher chlorophyll meter values (Padilla et al., 2018c; Schepers et al., 1996). Chlorophyll meters are well suited for on-farm use because they are easy to operate, do not require any particular training, and make measurements quickly (Gianquinto et al., 2004; Padilla et al., 2018c). Given these characteristics, chlorophyll meters are useful practical tools for assessing crop N status to identify required adjustments in N fertilizer application to ensure optical crop N status (Padilla et al., 2018c).

Canopy reflectance sensors can be used in commercial farming to determine crop N fertilizer requirements, and for variable rate N fertilizer application (Fox and Walthall, 2008; Samborski et al., 2009b). These sensors assess crop N status by measuring the reflection of two or more specific wavelengths of radiation from crop foliage (Ollinger, 2011). Visible and near-infrared wavelengths are used (Fox and Walthall, 2008; Padilla et al., 2018c). The reflectance of the measured wavelengths is entered into mathematical equations to derive vegetation indices. Numerous vegetation indices are available, depending on the wavelengths and formula used. Vegetation indices have been reviewed by Bannari et al. (Bannari et al., 1995), Ollinger (Ollinger, 2011) and Hatfield and Prueger (Hatfield and Prueger, 2010), who described the appropriate applications of the various indices. The most widely-used vegetation index is the normalized difference vegetation index (NDVI) (Padilla et al., 2018c; Sultana et al., 2014). Proximal canopy reflectance sensors are a form of remote sensing in which sensors are placed close to the crop; the distance ranging from several centimeters to several meters from the canopy (Padilla et al., 2018c). Reflectance sensors detect crop responses that are sensitive to crop N status, such as leaf chlorophyll, foliage greenness, foliage density and biomass (Fox and Walthall, 2008). The advantage of reflectance measurements is that they can integrate a substantially larger surface area of the crop than single leaf measurements made with a chlorophyll meter (Schepers et al., 1996; Thompson et al., 2017).

Considerable research has demonstrated the capacity of proximal optical sensors to assess crop N status in various field crops, mostly in cereals such as rice (Wakiyama, 2016), maize (Blackmer and Schepers, 1995) and wheat (Debaeke et al., 2006; Mistele and Schmidhalter, 2008; Ziadi et al., 2010). Additionally, their capacity to assess crop N status has been evaluated in diverse horticultural crops such as potato (Gianquinto et al., 2003; Olivier et al., 2006), tomato (Gianquinto et al., 2011; Güler and Büyük, 2007; Padilla et al., 2015), cucumber (Padilla et al., 2017b) and muskmelon (Padilla et al., 2014). Most of the research with proximal optical sensors to assess crop N status has been with a specific cultivar of a given species. Few reports have examined how differences between cultivars affect optical sensor measurements.

Working with wheat, Monostori et al. (Monostori et al., 2016b), reported that cultivar had a notable effect on the relationship between chlorophyll meter (SPAD-502) readings and grain yield. Similar results with wheat were obtained by Hoel (Hoel, 2003) using the Hydro N-Tester chlorophyll meter. In rice, the relationship between SPAD-502 measurements and leaf N content differed markedly with genotype (Peng et al., 1993). In tomato, Sandoval-Villa et al. (Sandoval-Villa et al., 2000b) reported significant differences in chlorophyll meter measurements in one cultivar compared to four others, but not amongst the other four cultivars.

Few studies have examined how cultivar influences measurements made with canopy reflectance sensors; the few reported studies have examined only the NDVI index. The NDVI was able to differentiate different cultivars at different growth stages in wheat (Sultana et al., 2014). With wheat also, Samborski et al. (Samborski et al., 2015) obtained statistically significant differences in NDVI between cultivars in one growth stage. Available reports suggest that cultivar effects on reflectance measurements can occur in cereal crops. We are unaware of published relevant information for vegetable crops.

Understanding cultivar effects on optical sensors such as chlorophyll meters and canopy reflectance sensors is fundamental for the use of these sensors in commercial farming. New cultivars are continually being introduced into commercial production; sometimes, there are notable phenotypic differences between cultivars. For a given species, it is necessary to identify if and to what extent cultivar affects optical sensor measurement. Secondly, if such effects are appreciable, procedures will need to be developed to deal with them when using optical sensors for crop N management.

The objectives of the present work were (1) to evaluate the effects of cucumber cultivar on chlorophyll meter measurements and vegetation indices measured with a canopy reflectance sensor, and (2) to assess how differences in cultivars affect the relationship between leaf N content and optical sensors measurements. Optical sensors measurements and their relationships with leaf N content were compared for three cucumber cultivars grown in a greenhouse, with three different N treatments.

5.3. Materials and Methods

5.3.1. Experimental Site

A cucumber (*Cucumis sativus* L.) crop was grown in soil in a greenhouse in conditions very similar to those of commercial greenhouse vegetable production in southeast (SE) Spain. The crop was grown in a multi-tunnel greenhouse at the Experimental Station of the University of Almería, located in Retamar, Almería, SE Spain (36°51′51′′N, 2°16′56′′W and 92 m elevation; a detailed description of the greenhouse is provided by Padilla et al. (Padilla et al., 2017b). The crop was grown in an "enarenado" soil typical of those used for soil-grown greenhouse production in Almería. More information on the soil used is provided by Padilla et al. (Padilla et al., 2014). A general description of "enarenado" soil is given by Thompson et al. (Thompson et al., 2007).

The cropping area was 1300 m^2, the crop rows were aligned north–south in paired lines. The greenhouse was divided in 12 plots of 12 m × 6 m each. Each plot contained six paired lines of plants, with 24 plants per line; the distance between plants in each line was 0.5 m. Separation between lines within a paired line was 0.8 m and the distance between adjacent paired lines was 1.2 m, giving a plant density of 2 plants m^{-2} and 144 plants per replicate plot. Sheets of polyethylene film (250 μm thickness) buried to 30 cm depth acted as a hydraulic barrier between plots (Padilla et al., 2016).

Above-ground drip irrigation was used. There was one emitter per plant, each emitter had a discharge rate of 3 L h^{-1}. All mineral fertilizer was applied through the drip irrigation system by fertigation. Complete nutrient solution was applied in each irrigation. Irrigation/fertigation occurred every 1–2 days depending on crop demand.

5.3.2. Experimental Design

The experiment was carried out in 2018, the crop was transplanted on 24 April and ended on 3 July, being grown for 70 days after transplanting (DAT). The crop was transplanted as 21-day old seedlings.

Three different cucumber cultivars, 'Strategos' (Syngenta International AG, Basel, Switzerland), 'Pradera' (Rijk Zwaan Zaadteelt en Zaadhandel B.V., De Lier, The Netherlands) and 'Mitre' (Semillas Fitó, Barcelona, Spain) were grown. The three cultivars were planted in each experimental plot, with one paired line (i.e., two lines per plot) of plants being planted with each cultivar. In each plot, there were three paired lines, one of each cultivar. The position of the paired lines of each cultivar in each plot was randomized.

There were three different N treatments that were applied to each of the cultivars. The N treatments were applied as different N concentration in the nutrient solution applied by fertigation. There were four replicated plots per treatment. The plots were organized in a randomized block design. The intended N treatments were very deficient (N1), sufficient (N2) and excessive (N3).

Before transplanting, a series of large irrigations were applied, in total 402 mm, to leach residual NO_3^- from the soil root zone and to homogenize the soil within the different plots. At the moment of transplanting, the mean soil mineral N content in the 0–60 cm depth (excluding gravel mulch) was 24, 34 and 63 kg N ha^{-1} in the N1, N2 and N3 treatments, respectively.

The average mineral N ($N–NO_3^- + N–NH_4^+$) concentrations applied in the nutrient solution were 2.4, 8.5 and 14.8 mmol L^{-1}, for the deficient, sufficient and excessive N treatments, respectively. During the first four days after transplanting, the plants were irrigated with water only (0.1 mmol N L^{-1}) and during the next four days, all three treatments received a common nutrient solution of 1.0 mmol N L^{-1}. Differential N treatments began nine days after transplanting and continued until the end of the crop. Regardless of the treatment, most N was applied as a NO_3^- (91% of applied N) and the rest as NH_4^+. All other nutrients were applied in the nutrient solution to ensure they were not limiting.

General crop management followed standard local practice; the crops were periodically pruned and were supported by nylon cord guides. Irrigation was scheduled to maintain the soil matric potential (SMP) in the root zone, at 15 cm depth between −10 and −30 kPa. One tensiometer (Irrometer, Co., Riverside, CA, USA) was installed in each plot to measure SMP (Padilla et al., 2016). Topping (the removal of the apical shoot to arrest stem elongation) was conducted on 46 DAT.

5.3.3. Optical Sensors Measurements

Optical measurements of relative leaf chlorophyll content were made with two hand-held leaf-clip sensors, the SPAD-502 (Minolta Camera Co. Ltd., Tokyo, Japan) and the MC-100 Chlorophyll Concentration Meter (Apogee Instruments, Inc., Logan, UT, USA). For individual measurements, the SPAD-502 measures a leaf surface area of 6 mm^2 and the MC-100 an area of 63.6 mm^2. The SPAD-502 measures absorbance at 650 nm (red) and 940 nm (NIR), and the MC-100 at 653 nm and 931 nm. Measurements with both sensors were made by clipping the sensor onto the leaf.

Measurements with chlorophyll meters commenced at 22 DAT. Measurements were then made weekly until the end of the crop and were made on seven dates. Measurements were made on each of eight marked plants, of each cultivar, in each replicate plot. They were made at the same time (8:00–10:00 solar time), before irrigation/fertigation was applied. On each plant on each measurement date, one measurement was made on the most recently fully expanded and well-lit leaf, on the distal part of the adaxial side of the leaf, midway between the margin and the mid-rib of the leaf, consistent with the protocol developed by Padilla et al. (Padilla et al., 2014; F.M. Padilla et al., 2018). 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 of the selected plants (Padilla et al., 2014).

Measurements of canopy reflectance were made with the Crop Circle ACS-470 sensor (Holland Scientific Inc., Lincoln, NE, USA), which is an active proximal canopy reflectance sensor (Solari et al., 2008b). Filters were selected to measure reflectance at 550 nm (green), 670 nm (red) and 760 nm (near-infrared, NIR). The sensor was held vertically parallel to the crop rows, facing the upper part of the foliage at a 45 cm horizontal distance giving a field of view on the foliage surface of 26 cm (height) × 5 cm (width) (Padilla et al., 2014). The sensor was positioned so that the top of the field of view was level with the most recently fully expanded leaf, in accordance with the protocol developed by Padilla et al. (Padilla et al., 2015, 2014) in greenhouse-grown vertically supported crops. Measurements were always made at the same time each day (10:00–11:00 solar time). They commenced once the crop had sufficient height to enable measurement considering the 26 cm height of the field of view, at 29 DAT. Measurements continued weekly until the end of the crop, for a total of six measurement dates. In each replicate plot, four measurement passes of 4 m were made, for each cultivar, at walking speed (approx. at 1.5 km h^{-1}). There were ten measurements per second, giving approximately 400 individual measurements per plot. Reflectance data of each wavelength were stored in a portable GeoScout GLS-400 data logger (Holland Scientific, Inc., Lincoln, NE, USA) and subsequently processed.

From each individual reading, four vegetation indices were calculated based on the reflectance values of individual wavelengths. The individual index values from each reading were then averaged to provide a single value for the measurement in each replicate plot. The indices were: (i) normalized difference vegetation index (NDVI) (Sellers, 1985), (ii) the normalized difference vegetation index on greenness (GNDVI) (Ma et al., 1996), which is a variation on NDVI using the green wavelength, (iii) the red ratio vegetation index (RVI) (Birth and McVey, 1968) and (iv) green ratio vegetation index (GVI) (Birth and McVey, 1968). These indices are among the reflectance indices of vegetation most commonly used to evaluate crop N status (Fitzgerald, 2010; Fox and Walthall, 2008; Hatfield et al., 2008; Padilla et al., 2018c; Samborski et al., 2009b).

5.3.4. Leaf N Content

On each date of measurement with optical sensors, eight plants per cultivar and replicate plot were selected, and the most recently fully expanded leaf was removed for determination of total N content (%N). Measurement of leaf N content is a long established method for assessment of crop N status of vegetable crops (Thompson et al., 2017). The removed leaves were placed in a paper bag and oven dried at 65 °C until constant weight. Petioles were discarded. Dry material was ground sequentially in knife and ball mills. The total N content (%N) of each sample was determined using a Dumas-type elemental analyzer system (model Rapid N, Elementar, Analysen systeme GmbH, Hanau, Germany).

5.3.5. Cultivar Characterization

To characterize the three cultivars, measurements of crop height (level of the gravel mulch to top leaf) were made immediately before topping, at 46 DAT, in eight plants per cultivar. Leaf color analysis was performed on eight of the latest fully expanded leaves of each cultivar in each replicate plot. A colorimeter (Minolta Chroma Meter CR-400, Konica Minolta, Osaka, Japan) was used, providing CIE 1931 color space coordinates (i.e., luminance (Y), chromatic coordinate x and chromatic coordinate y). For determination of leaf area index (LAI), a destructive sampling was conducted in which all leaves from a randomly selected plant per cultivar and replicate plot were removed at 45 DAT. After excision, leaves were kept refrigerated in zip-lock plastic bags and immediately taken to the laboratory. Total leaf area was measured with an area meter (LI-3100C; LI-COR, Inc., Lincoln, NE, USA). LAI was calculated by dividing total leaf area by sampled soil area.

5.3.6. Statistical Analysis

For measurements conducted one time during the crop, such as LAI, crop height and leaf color, factorial analysis of variance (ANOVA) was performed to the test the effects of N treatments and cultivars on the measured variables. For measurements taken several times during the crop, such as leaf N content and optical sensor measurements, repeated-measure analysis of variance (RM-ANOVA) were conducted to test the effects of N treatments, cultivars and time on measured variables. Homogeneity of variances was checked prior to ANOVA analysis and variables were transformed if ANOVA assumptions were not met. The IBM SPSS 25 software program (IBM Corporation, Armonk, NY, USA) was used.

Linear regressions between leaf N content (dependent variable) and optical sensor measurement (independent variable) were evaluated for each cultivar and date of measurement separately. Coefficient of determination (R^2), standard error of the estimate (SSE), probability (p-value), slope and intercept, were calculated using the IBM SPSS 25 software.

To compare the effect of cultivar on the relationship between leaf N content and optical sensor measurement, the methodology used by ArchMiller et al. (ArchMiller and Samuelson, 2016) was used. Firstly, the relationship between leaf N content and optical sensor measurement for the three cultivars together was established, for chlorophyll and canopy reflectance sensor measurements. This regression equation was called "reduced regression":

$$\text{Leaf N content} = \text{a} + \text{b x (Optical sensor measurement)}, \tag{3}$$

where a and b are the intercept and slope of the regression, respectively. Secondly, the change in linear regression between leaf N content and optical sensor measurement of the reduced regression calculated in Equation (1), and linear regression between leaf N content and optical sensor measurement of each of the three cultivars separately, was analyzed with the sum of squares reduction test (F-statistic), for each date of measurement, using the equation:

$$\text{F} - \text{statistic} = \frac{(\text{SSEred} - \text{SSEcultivar})/(\text{dfred} - \text{dfcultivar})}{\text{SSEcultivar}/\text{dfcultivar}}, \tag{4}$$

where SSEred and SSEcultivar are the error sum of squares and dfred and dfcultivar are the degrees of freedom, of the reduced and each cultivar regression, respectively. Each cultivar regression had individual a and b parameters. To analyze if the reduced regression was different from the cultivar regression, the F-statistic was used to calculate the p-value. p-values ≤ 0.05 indicate that the reduced regression was statistically different from the cultivar regression, thus indicating a significant effect on cultivar on the relationship between leaf N content and optical sensor measurements.

5.4. Results

5.4.1. Cultivars Characterization

Crop height was not significantly different between cultivars ($p > 0.05$). However, there were statistical differences between cultivars in LAI, luminance and chromatic coordinates x,y ($p < 0.05$) (Table S1 and Figure S1); 'Strategos' had significantly higher LAI, luminance and x,y coordinate values than 'Pradera' and 'Mitre' (Table 1).

Table 1. Averages of the three N treatments of leaf area index (LAI), crop height, luminance (Y), coordinate x and coordinate y for each cultivar of cucumber (*Cucumis sativus* L.) crop grown in 2018. Values are means ± standard error. There were twelve measurements of each parameter for each cultivar, three for each N treatment. Different lower-case letters (a-c) show significant differences between cultivars.

Cultivar	LAI	Crop height (m)	Luminance (Y)	Coordinate x	Coordinate y
'Strategos'	5.68 ± 0.69^{a}	1.75 ± 0.11^{a}	10.47 ± 0.72^{a}	0.331 ± 0.003^{a}	0.401 ± 0.007^{a}
'Pradera'	5.20 ± 0.74^{b}	1.71 ± 0.12^{a}	9.57 ± 0.84^{b}	$0.330 \pm 0.003^{a,b}$	0.396 ± 0.008^{b}
'Mitre'	4.98 ± 0.70^{b}	1.72 ± 0.11^{a}	8.94 ± 0.70^{c}	0.328 ± 0.003^{b}	0.390 ± 0.007^{c}

5.4.2. Differences in leaf N content between cultivars

There were significant differences between cultivars in leaf N content values depending on N treatment and time (RM-ANOVA, $p < 0.05$; Table S2). In the N1 treatment, 'Strategos' had significantly higher leaf N content than 'Pradera' and 'Mitre' throughout most of the crop. 'Pradera' had the lowest leaf N content, but it was not significantly lower than 'Mitre' (Figure 1a). Average leaf N content in the N1 treatment for the whole crop cycle was 2.35% ± 0.05%, 2.08% ± 0.04% and 1.97% ± 0.09%, for 'Strategos', 'Mitre' and 'Pradera', respectively.

In the N2 treatment, 'Strategos' had the highest leaf N content, 'Pradera' the lowest and 'Mitre' had an intermediate leaf N content (Figure 1b). Average leaf N contents for the N2 treatment for whole crop cycle were 4.59% ± 0.07%, 4.33% ± 0.04% and 4.12% ± 0.10%, for 'Strategos', 'Mitre' and 'Pradera', respectively.

In the N3 treatment, there were no clear differences between cultivars in leaf N content (Figure 1c). Average leaf N content in the N3 treatment for the whole crop was 5.11% ± 0.05%, 5.17% ± 0.03% and 4.95% ± 0.05%, for 'Strategos', 'Mitre' and 'Pradera', respectively.

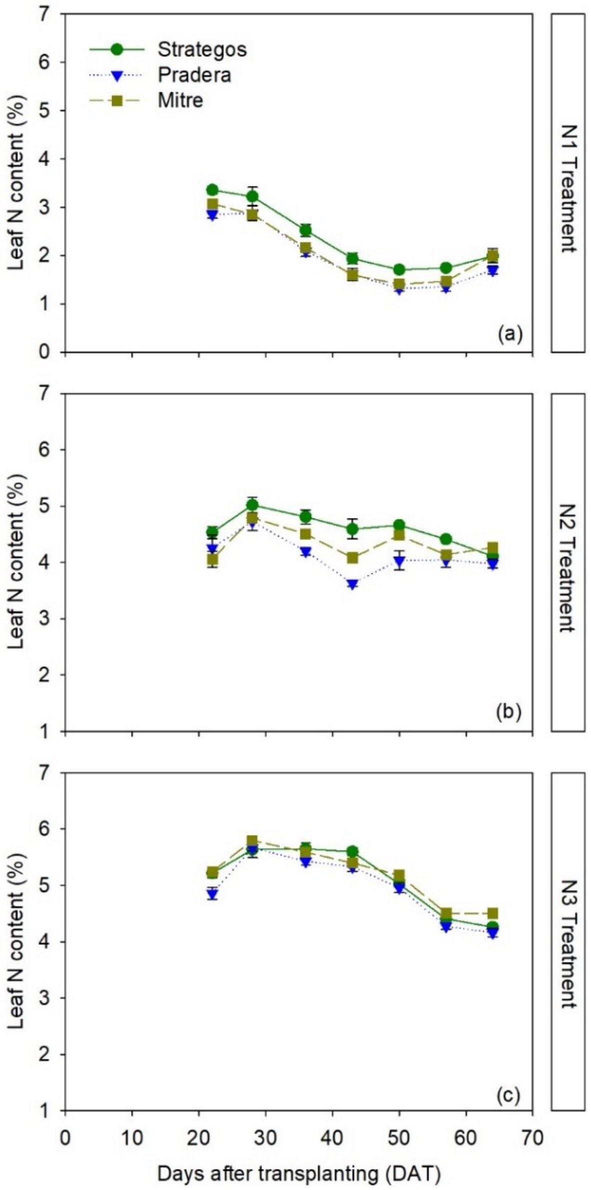

Figure 1. Temporal dynamics of leaf N content (%) of three cultivars of cucumber (*Cucumis sativus* L. 'Strategos', 'Pradera' and 'Mitre') under three N treatments (N1 (panel **a**), N2 (panel **b**) and N3 (panel **c**)). Values are means ± SE.

5.4.3. Chlorophyll Meter Measurements

The RM-ANOVA indicated significant differences between cultivars in chlorophyll meter measurements, depending on N treatment and time, both for the SPAD-502 meter (RM-ANOVA, $p < 0.001$) and for the MC-100 meter (RM-ANOVA, $p < 0.001$; Table S3). Generally, in all treatments 'Mitre' was the cultivar with the highest SPAD values, 'Strategos' had the lowest SPAD values, and 'Pradera' was intermediate. The average differences in SPAD values throughout the crop, considering the three N treatments, were the following: 'Mitre' was 3.7 ± 1.0 SPAD units higher than 'Pradera', and 'Pradera' was 2.6 ± 1.1 SPAD units higher than 'Strategos'. Expressed as percentages, these differences were 8.1% and 6.2%, respectively.

For the N1 treatment, there were no significant differences between the three cultivars throughout the crop (Figure 2a). In the N2 and N3 treatments, SPAD values of 'Mitre' were statistically significantly higher than those of 'Pradera' and 'Strategos'. In N2 treatment, SPAD values of 'Pradera' were consistently statistically higher than those of 'Strategos' (Figure 2b).

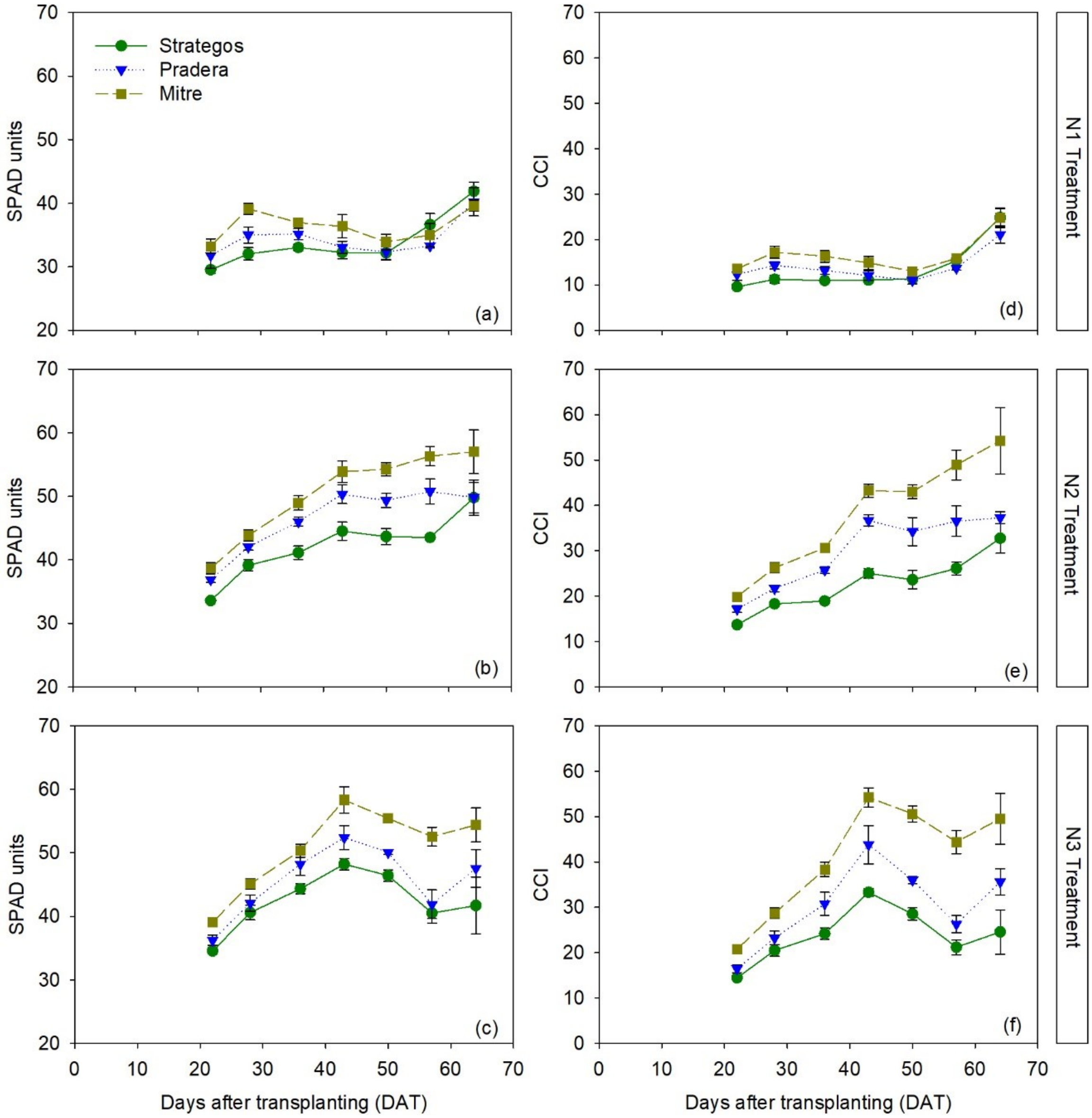

Figure 2. Temporal dynamics of SPAD (panels **a–c**) and chlorophyll content index (CCI) measurements (panels **d–f**) of three cultivars of cucumber (*Cucumis sativus* L. 'Strategos', 'Pradera' and 'Mitre') under three N treatments (N1, N2 and N3). Values are means ± SE.

For measurements with the MC-100 meter, 'Mitre' had significantly higher chlorophyll content index (CCI) values than 'Pradera' and 'Strategos' in the N2 and N3 treatments (Figure 2e,f). In the N1 treatment, there were no statistical differences (Figure 2c). For each of the three N treatments, 'Mitre' had the highest CCI values, 'Strategos' the lowest and 'Pradera' was intermediate. Averaged throughout the crop and for the three N treatments, 'Mitre' had CCI values that were 7.1 ± 2.4 CCI units higher than 'Pradera' and 'Pradera' was 4.7 ± 2.2 CCI units higher than 'Strategos'. In percentage terms, these values corresponded to differences of 22.3% and 19.1%, respectively.

5.4.4. Canopy Reflectance Measurements

There was a similar dynamics of red and green reflectance throughout most of the crop cycle, regardless of the N treatment (Figure 3). Reflectance of both red and green bands increased in the second half of the crop, particularly in N2 and N3 treatments (Figure 3).

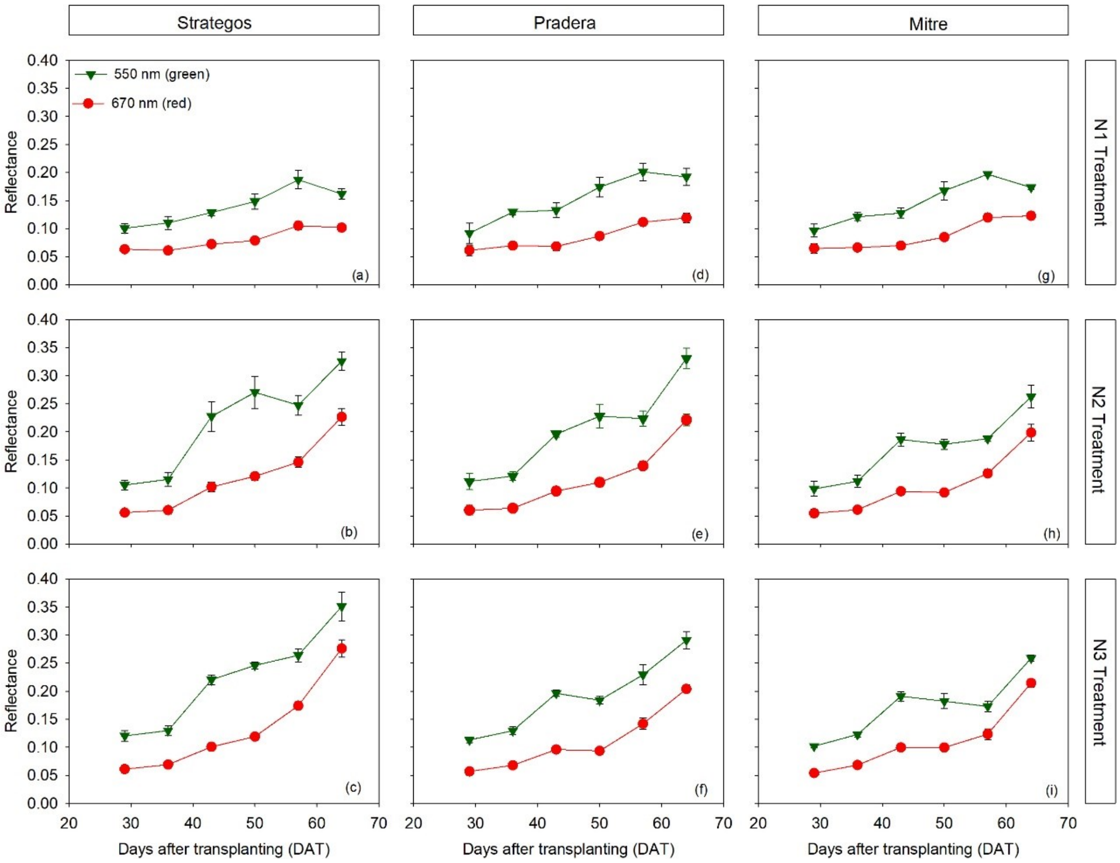

Figure 3. Temporal dynamics of red and green reflectance of three cultivars of cucumber (*Cucumis sativus* L. 'Strategos' (panels **a**-**c**), 'Pradera' (panels **d**-**f**) and 'Mitre' (panels **g**-**i**)) under three N treatments (N1, N2 and N3). Values are means ± SE.

There were differences between cultivars in NDVI only in the N1 treatment (Table S4). In N2 and N3 treatments, there were no significant differences between the three cultivars (Figure 4b,c). Similar results were found for RVI (Figure S2a–c). In the N1 treatment, 'Strategos' and 'Pradera' had statistically comparable NDVI values but 'Strategos' was significantly different to 'Mitre', being, in two measurements date, superior than 'Mitre' and in the other two, lower than 'Mitre' (Figure 4a). Overall, the average differences in NDVI and GVI values between 'Strategos' and 'Pradera' with 'Mitre' in the N1 treatment were 0.003 ± 0.001 and 0.13 ± 0.014, respectively; expressed as percentage, these average differences were 0.43% and 4.3%, respectively.

For GVI, in N2 treatment during most of the crop, there were statistical differences between 'Strategos' and the other two cultivars, with 'Strategos' having the lowest values. In the N3 treatment, there were significant differences after 50 DAT, when 'Pradera' and 'Mitre' had statistically higher GVI values than 'Strategos' (Figure 4d–f). There were inconsistent differences between cultivars for GNDVI (Figure S2d–f).

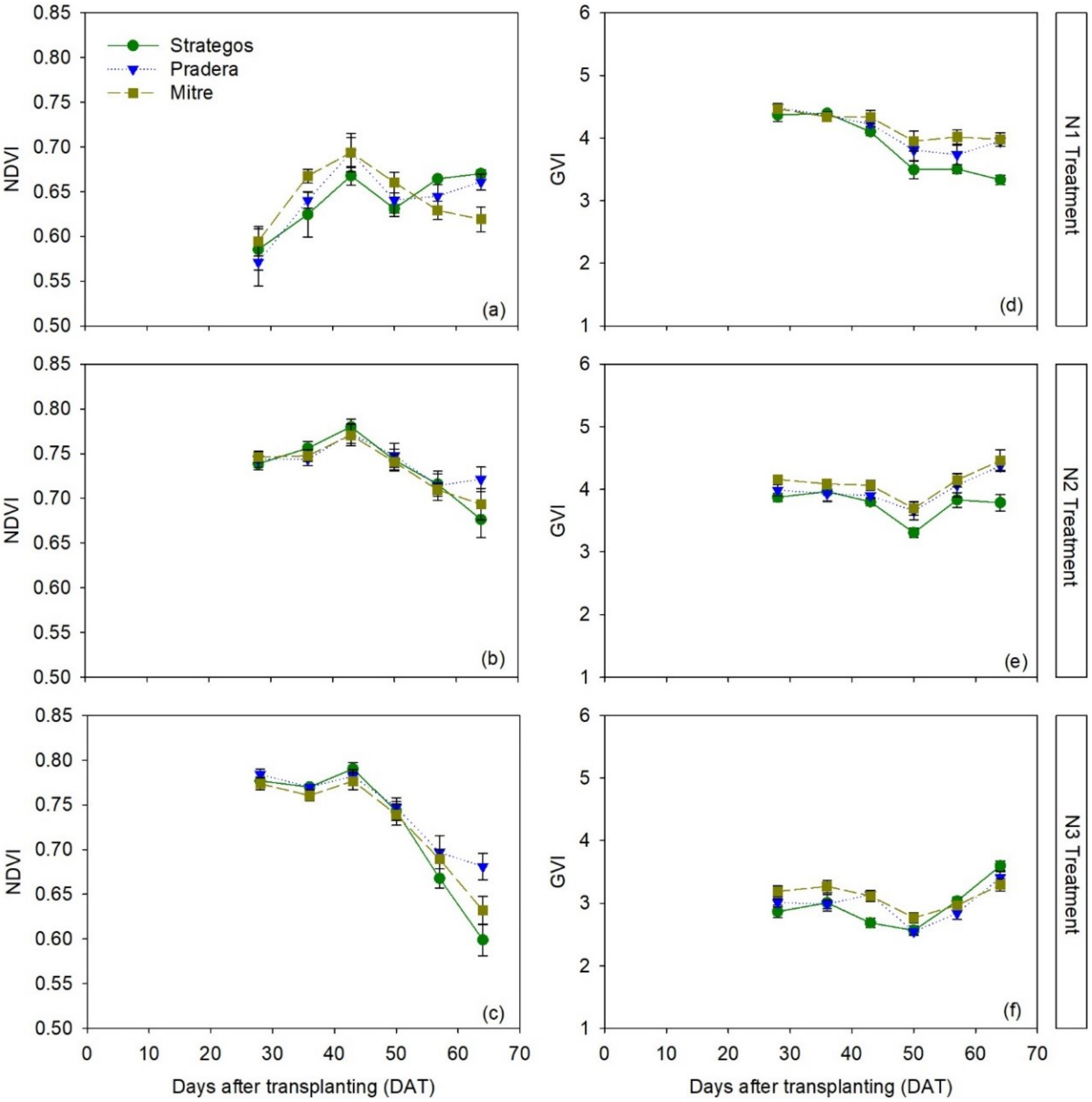

Figure 4. Temporal dynamics of normalized difference vegetation index (NDVI) (panels **a-c**) and green ratio vegetation index (GVI) (panels **d-f**) measurements of three cultivars of cucumber (*Cucumis sativus* L. 'Strategos', 'Pradera' and 'Mitre') under three N treatments (N1, N2 and N3). Values are means ± SE.

5.4.5. Relationships Between Optical Sensor Measurements and Leaf N Content

Most of the linear regressions between leaf N content and optical sensor measurements (from chlorophyll meters and the canopy reflectance sensor), for individual measurement dates, were significant for the three cultivars (Figures 5–8). On most measurement dates, R^2 values of the linear regressions were strong or very strong

(R^2 of 0.80–0.98; Table S5). For the SPAD-502, the average R^2 values of linear regressions, from all measurement dates, were 0.81 ± 0.07, 0.65 ± 0.08 and 0.79 ± 0.06 for 'Strategos', 'Pradera' and 'Mitre', respectively. For CCI, the respective average R^2 values were 0.83 ± 0.05, 0.74 ± 0.06 and 0.84 ± 0.06. For NDVI, they were 0.85 ± 0.04, 0.72 ± 0.08 and 0.78 ± 0.05, and for GVI were 0.83 ± 0.04, 0.82 ± 0.06 and 0.83 ± 0.05.

For the SPAD-502 (Figure 5), the F-statistic analysis showed that each of the three cultivars had statistically the same linear regression as the reduced regression at 36, 57 and 64 DAT (Table 2), indicating no cultivar effect on the relationship between leaf N content and SPAD measurements in three out of seven measurement dates. 'Strategos' had statistically different regressions than the reduced regression at 22, 29 and 43 DAT, and 'Mitre' had statistically different regression than the reduced regression at 50 DAT, indicating a significant cultivar effect on the relationship between leaf N content and SPAD measurements in four out of seven measurement dates (Table 2). By contrast, the regression of 'Pradera' was statistically similar to the reduced regression for all measurement dates.

The F-statistic analysis showed that the reduced regression between leaf N content and measurements of the MC-100 was statistically comparable to the individual regressions for each cultivar at 64 DAT, indicating no cultivar effect on the relationship between leaf N content and MC-100 measurements in one of seven measurement dates (Figure 6). 'Strategos' and 'Mitre' had significantly different regressions to the reduced regression at 22, 29 and 43 DAT, and at 36, 43, 50 and 57 DAT, respectively (Table 2). The regression of 'Pradera' was statistically similar to the reduced regression on all measurement dates (Table 2).

For canopy reflectance vegetation indices, the relationship between leaf N content and NDVI was statistically comparable between the reduced regression for all three cultivars and each of the individual regressions for each of the three cultivars for all measurement dates (Table 2), indicating no significant cultivar effect on the relationship between leaf N content and NDVI (Figure 7). Very similar behavior to that of NDVI occurred with RVI and GNDVI (Table S6 and Figures S3 and S4). Overall, the results of the F-statistic analysis for GVI were very similar to those of NDVI (Figure 8), without significant differences between the reduced regression for all three cultivars together and the individual regression for each cultivar, on five out of six measurement dates (Table 2). Regressions for ‘Strategos’ and ‘Pradera’ were statistically different to the reduced regression on 43 and 29 DAT, respectively (Table 2).

Table 2. *p*-values of the F-statistic analysis comparing the relationship between leaf N content and optical sensor measurements between the reduced regression for all three cultivars together and the regression of each cultivar of cucumber (*Cucumis sativus* L.) separately. Numbers in bold show significant differences ($p < 0.05$) between the reduced regression and the cultivar regression.

DAT	SPAD			CCI			NDVI			GVI		
	‘Strategos’	**‘Pradera’**	**‘Mitre’**	**‘Strategos’**	**‘Pradera’**	**‘Mitre’**	**‘Strategos’**	**‘Pradera’**	**‘Mitre’**	**‘Strategos’**	**‘Pradera’**	**‘Mitre’**
22	**0.005**	0.350	0.262	**0.009**	0.281	0.177						
29	**0.046**	0.281	0.215	**0.046**	0.201	0.131	0.262	0.409	0.281	0.350	**0.019**	0.166
36	0.070	0.139	0.098	0.057	0.083	**0.001**	0.245	0.262	0.377	0.350	0.189	0.229
43	**0.048**	0.201	0.078	**0.015**	0.103	**0.001**	0.147	0.409	0.444	**0.041**	0.087	0.189
50	0.116	0.131	**0.034**	0.123	0.123	**<0.001**	0.098	0.409	0.350	0.201	0.281	0.215
57	0.377	0.324	0.078	0.229	0.262	**0.032**	0.444	0.377	0.177	0.324	0.377	0.166
64	0.484	0.302	0.147	0.484	0.054	0.229	0.281	0.281	0.324	0.444	0.166	0.215

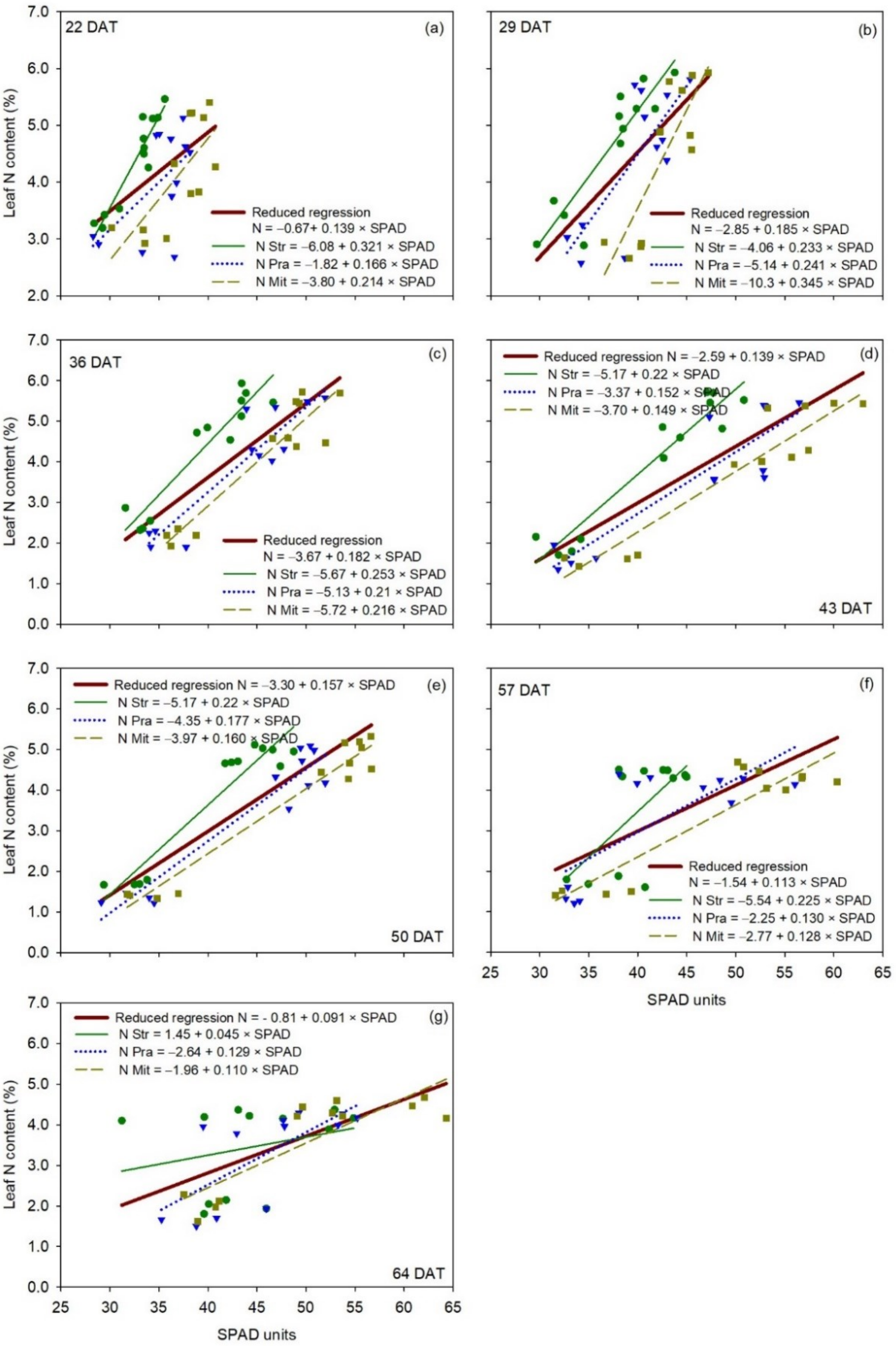

Figure 5. Linear regression between SPAD measurements (independent variable) and leaf N content (dependent variable) for each cultivar of cucumber (*Cucumis sativus* L.), for each measurement date (panels **a**-**g**). The reduced regression is a regression with data of all three cultivars together. DAT is days after transplanting. Str, 'Strategos', Pra, 'Pradera', Mit, 'Mitre'.

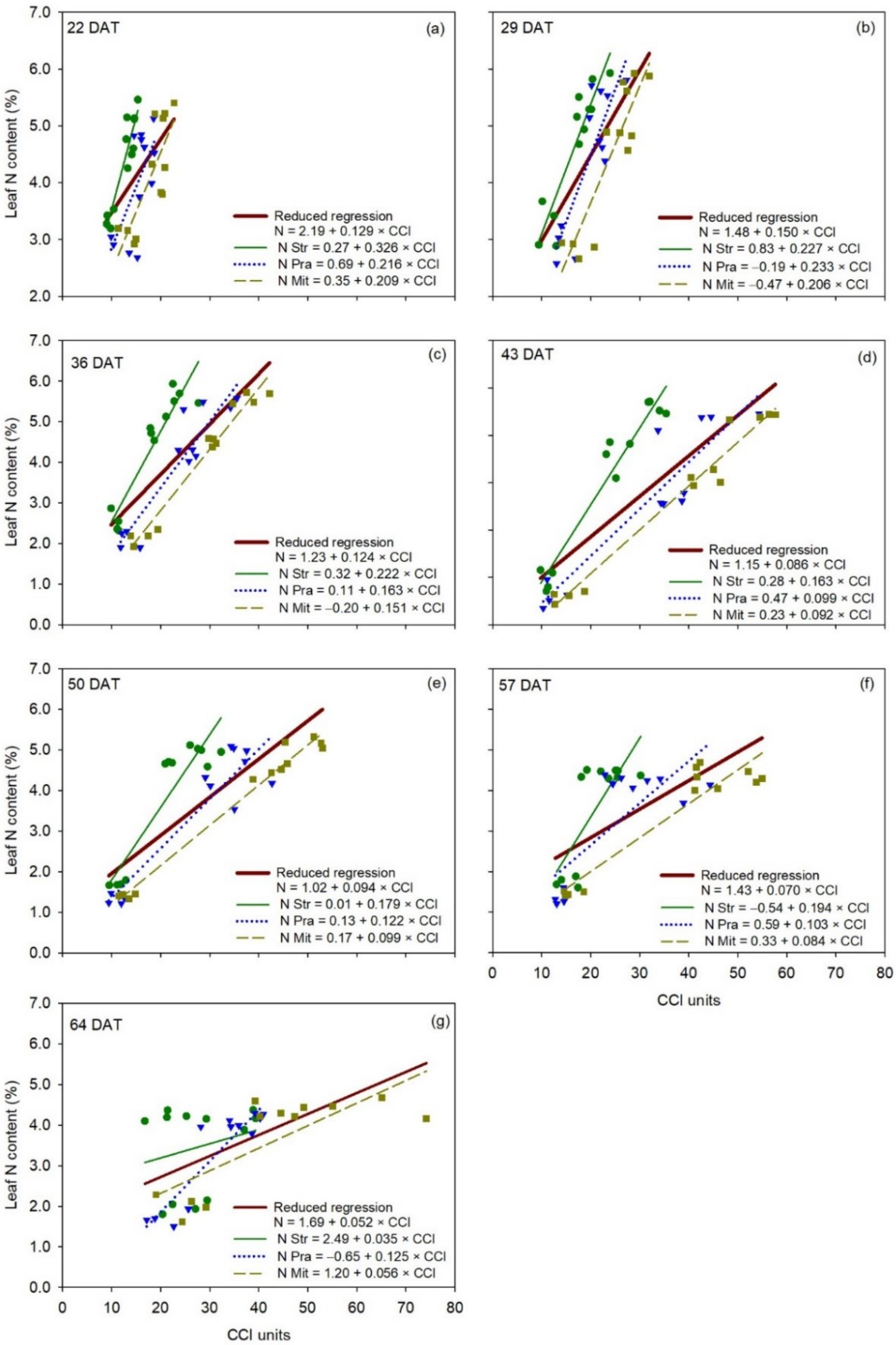

Figure 6. Linear regression between CCI measurements (independent variable) and leaf N content (dependent variable) for each cultivar of cucumber (*Cucumis sativus* L.), for each measurement date (panels **a-g**). The reduced regression is a regression with data of all three cultivars together. DAT is days after transplanting. Str, 'Strategos', Pra, 'Pradera', Mit, 'Mitre'.

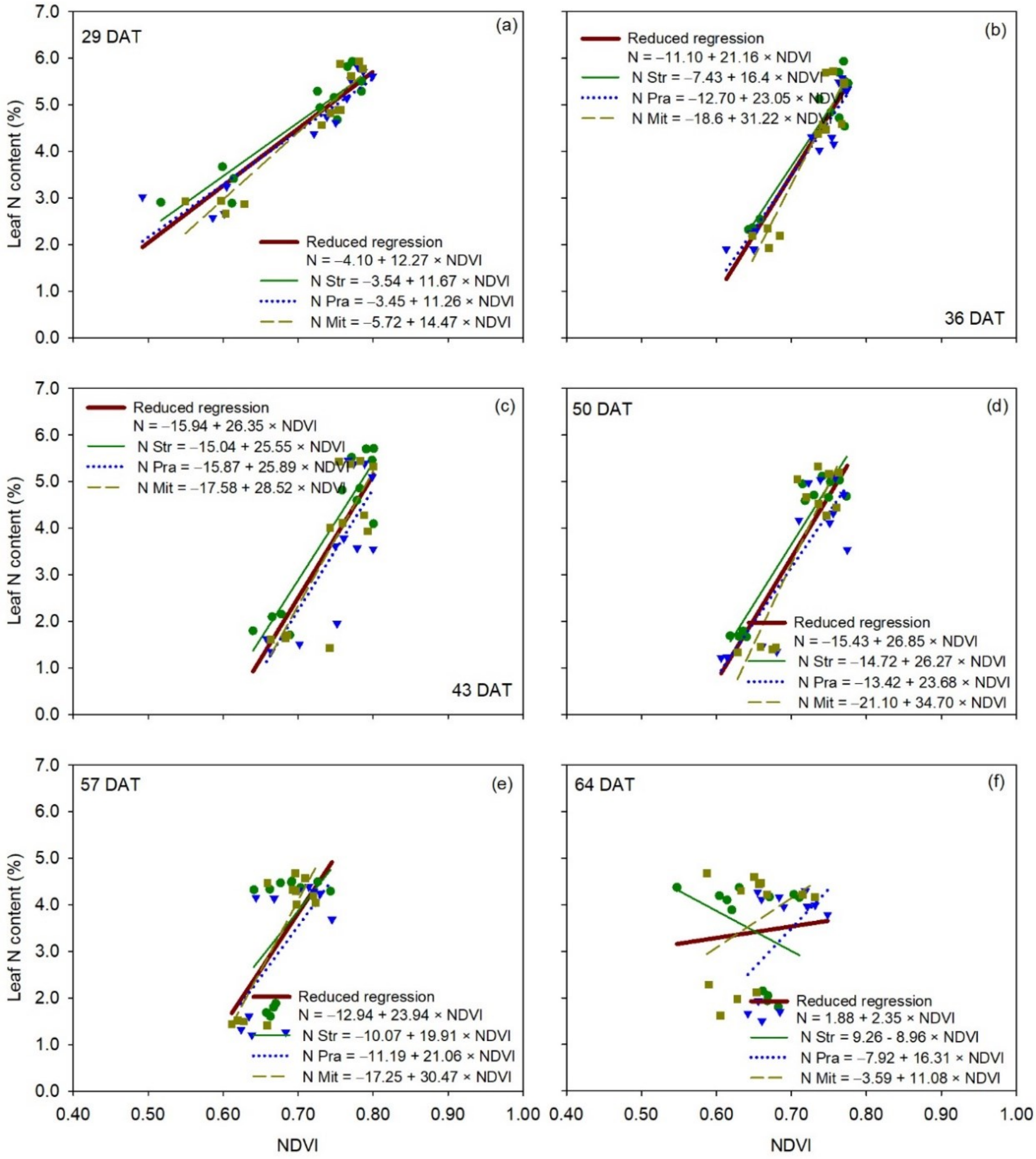

Figure 7. Linear regression between NDVI measurements (independent variable) and leaf N content (dependent variable) for each cultivar of cucumber (*Cucumis sativus* L.), for each measurement date (panels **a**-**f**). The reduced regression is a regression with data of all three cultivars together. DAT is days after transplanting. Str, 'Strategos', Pra, 'Pradera', Mit, 'Mitre'

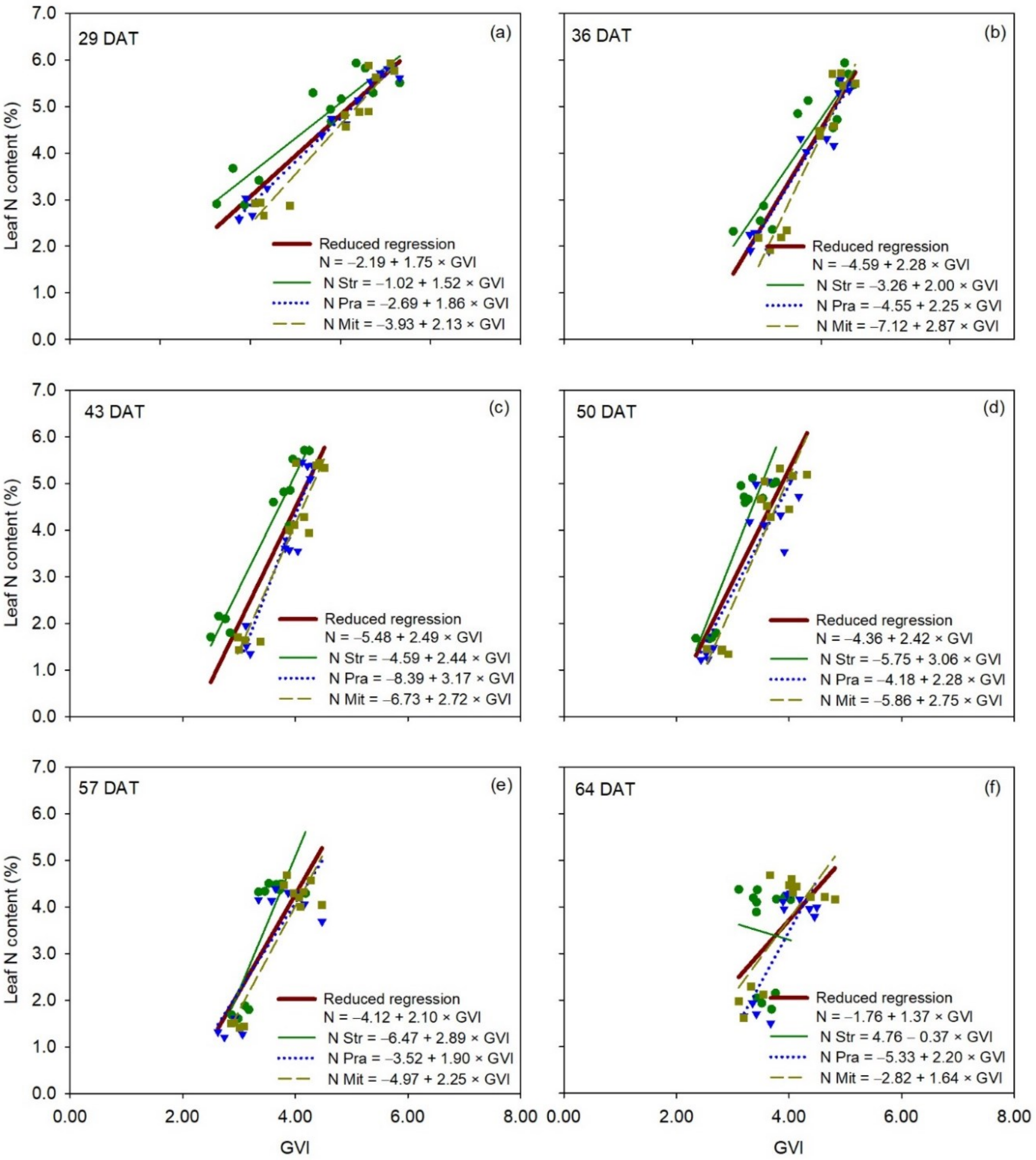

Figure 8. Linear regression between GVI measurements (independent variable) and leaf N content (dependent variable) for each cultivar of cucumber (*Cucumis sativus* L.), for each measurement date (panels **a**-**f**). The reduced regression is a regression with data of all three cultivars together. DAT is days after transplanting. Str, ‘Strategos’, Pra, ‘Pradera’, Mit, ‘Mitre’.

5.5. Discussion

There were differences between cultivars, for equivalent N treatments, of measurements made with the SPAD-502 and MC-100 chlorophyll meters, when the N supply was sufficient and excessive (N2 and N3 treatments), but not when the N supply was deficient (N1 treatment). There are previous reports of cultivar notably affecting SPAD measurements in wheat (Monostori et al., 2016b) and rice (Peng et al., 1993). For the vegetation indices measured with the Crop Circle ACS-470 reflectance sensor, there were no consistent significant differences between cultivars.

The general similarities, for the three cultivars, in the slopes of the linear relationships between sensor measurements and leaf N content, for the three optical sensors, indicated that the sensitivity of the two chlorophyll meters and the canopy reflectance sensor was not affected by cultivar. However, there were significant differences in relationships between the reduced regression for all three cultivars considered together and the regressions for individual cultivars, particularly for chlorophyll meters. This indicated a significant cultivar effect on the relationship between leaf N content and optical sensor measurements. It suggested that a unique regression equation to estimate leaf N content from sensor measurement could not be used for each of the three cucumber cultivars examined in the present work. These results are subsequently discussed more fully.

5.5.1. Assessment of Cultivar Effects on Optical Sensor Measurements

With both chlorophyll meters, there were consistent differences in measurements between the three cultivars, mainly between 'Mitre' and 'Strategos', with 'Pradera' being intermediate. These differences between cultivars were most apparent in the sufficient and excessive N treatments (N2 and N3). These results are consistent with previous work with other species where cultivar effects on SPAD measurements were more pronounced at higher N supply, in rice (Yuan et al., 2016b), potato (Minotti et al., 1994) and tomato (Sandoval-Villa et al., 2000b). There are no previous reports evaluating cultivar effects on measurements made with the MC-100 chlorophyll meter.

In the present work, the use of two different chlorophyll meters enabled the relative effect of cultivar on the two sensors to be compared. The differences in measurement between cultivars were appreciably larger with the MC-100 compared to the SPAD-502. For example, in the N3 treatment, the average relative difference between 'Mitre' and 'Strategos' was 42% with the MC-100 meter, and 17% with the SPAD-502 meter. The relative differences between 'Mitre' and 'Strategos' cultivars were slightly lower than the relative differences in measurements between the N1 and N2 treatments and appreciably larger than those between the N2 and N3 treatments. These results contradict the observation of Hoel (Hoel, 2003) that the soil N availability affected chlorophyll meter readings more than cultivar, growth stage and other nutrients in wheat.

The cultivar effect observed with chlorophyll meters, in the current study, has implications for the use of absolute sufficiency values, of chlorophyll meter measurements, as indicators of optimal crop N status. Sufficiency values (also known as reference or threshold values) being those that distinguish between deficiency (below the value) and sufficiency (above the value) (Thompson et al., 2017). Monostori et al. (Monostori et al., 2016b) reported for wheat that SPAD values should be calibrated for each cultivar to obtain more accurate N diagnosis and yield prediction. The present work and previous research (Liu et al., 2017; Zhao et al., 2016) suggest that in order to use absolute sufficiency values, regardless of the cultivar, that procedures to normalize absolute chlorophyll meter measurements should be developed.

The relative differences between cultivars in vegetation indices measured with Crop Circle ACS-470 sensor were much smaller and less consistent than occurred with chlorophyll meter measurement. For NDVI, statistical differences between cultivars were detected in N1, but not in N2 and N3 treatments, which was the opposite to what was observed with chlorophyll meters. Cultivar differences in NDVI were reported by Sultana et al. (Sultana et al., 2014) who observed significant differences in NDVI between wheat cultivars under four different nitrogen levels. Similar results for geranium (*Pelargonium × hortorum*) were reported by Wang et al. (Wang et al., 2012). The lack of consistent differences in the N1 treatment, in the present work, may be due to the limited vegetative growth of this treatment, the lack of continuity of vegetative cover may have influenced canopy reflectance. Padilla et al. (Padilla et al., 2018c), Wang et al. (Wang et al., 2012) and Johansen and Tømmervik (Johansen and Tømmervik, 2014), reported that NDVI is susceptible to measurement error caused by background reflectance when the canopy is not sufficiently closed. Comparing the LAI between the different cultivars, 'Strategos' had the highest LAI values, but this did not influence vegetation indices; the values of vegetation indices were generally comparable, in statistical terms, between cultivars. This suggests that not only the quantity of leaves has an influence on reflectance measurements but also other plant characteristics such as the angle position of leaves (Fox and Walthall, 2008).

A factor that affected canopy reflectance measurements in the final stages of the crops, in the present study (after 50 DAT), was foliar damage due to fungal infection of powdery mildew (*Pseudoperonospora cubensis*), which marked an appreciable portion of the leaves with yellow spots, mostly in the N2 and N3 treatments. This foliar damage could have influenced the decrease in reflectance indices in the three cultivars, which was most apparent in the cultivar 'Strategos'. This was consistent with the relatively large increase in reflectance of the red and green bands towards the end of the crop. Similar results were found in soybean, where a decline in NDVI was strongly related to foliar damage (Hikishima et al., 2011).

Considering the entire data set, of canopy reflectance measurement, in the current study, the vegetation indices using the green wavelength (GNDVI and GVI) were more sensitive than the red indices (NDVI and RVI) for detecting cultivar differences. This is in agreement with Padilla et al. (Padilla et al., 2017b), where the GNDVI and GVI indices were the most sensitive vegetation indices for estimating both crop nitrogen nutrition index (NNI) and yield in cucumber. With processing tomato, green vegetation indices were also more sensitive than red vegetation indices for estimating leaf N content (G Gianquinto et al., 2011). Loss of sensitivity of red vegetation indices related to saturation of reflectance in the red region at high leaf area index (LAI) values has been reported in different field crop species such as wheat, soybean and maize (Gitelson, 2004). However, in the present study, differential saturation of the red and green bands during the crop was not observed (Figure 4a–f).

5.5.2. Relationships Between Optical Sensor Measurements and Leaf N Content

The strong relationships between chlorophyll meter measurements (both SPAD-502 and MC-100) and leaf N content indicated that these measurements were good indicators of leaf N content, for the cultivars examined. These results are consistent with previous research in which chlorophyll meter measurements were strongly related to leaf N content (Castelli and Contillo, 2009; Esfahani et al., 2008; Padilla et al., 2018c). Comparing chlorophyll meter measurements with the parameters measured with the colorimeter, the results were apparently contradictory. 'Strategos' was the cultivar with lowest chlorophyll meter measurements while having the highest luminance and chromatic coordinates. It may be that higher luminance measured with the colorimeter in 'Strategos' is indicative of lower light absorption and higher light transmittance and reflectance, as indicated by the lower chlorophyll meter measurements and vegetation indices values of this cultivar.

Similarly, the generally strong relationships, between NDVI, RVI, GNDVI and GVI, and leaf N content, indicated that these vegetation indices are effective indicators of leaf N content in cucumber, for the cultivars examined. Padilla et al. (Padilla et al., 2017b) reported that these vegetation indices were good estimators of crop N status in cucumber. Previous studies in tomato and geranium have reported strong relationship between vegetation indices such as NDVI and GNDVI with leaf N content (G Gianquinto et al., 2011; Johansen and Tømmervik, 2014).

Significant differences were found between the reduced regression for all three cultivars considered together and the individual regressions for 'Strategos' and 'Mitre' considered separately, with the SPAD-502 and MC-100, for most measurement dates. This indicated a significant cultivar effect on the relationships between chlorophyll meter measurement and leaf N. Consequently, it appears that it is not feasible to use a unique equation for the three cultivars to estimate leaf N content from chlorophyll meter measurements. These results imply that procedures to normalize differences between cultivars should be developed in order to use absolute sufficiency values developed for a given species.

For canopy reflectance, the lack of significant differences between the reduced regression for all three cultivars together and the regressions for each of the three cultivars separately, for most measurement dates, indicated that there was not a significant cultivar effect on the relationship between leaf N content and vegetation indices in cucumber. This suggested that a single regression equation could be used to estimate leaf N content, for the three cultivars, for measurements of NDVI, GNDVI, RVI and GVI.

5.6. Conclusions

Cultivar had an effect on SPAD-502 and MC-100 chlorophyll meter measurements when the N supply was adequate and excessive. For the red band based vegetation indices (NDVI and RVI) measured with the Crop Circle ACS470 sensor, there was no effect of cultivar, regardless of N applied. For the green band based vegetation indices (GNDVI and GVI), there was a cultivar effect, mainly with 'Strategos', which indicated it is not possible to use a unique sufficiency value for the three cultivars. Cultivar had a significant effect on the relationship between leaf N content and chlorophyll meter measurements, but not on the relationships between leaf N content and canopy reflectance vegetation indices. The lack of a consistent effect of cultivar, on the relationship with leaf N content, suggests that a unique equation to estimate leaf N content from vegetation indices can be applied to all three cultivars. This unique equation, however, may not be applied for chlorophyll meter measurements because of the significant cultivar effect detected in the present study.

5.7. References

ArchMiller, A.A., Samuelson, L.J., 2016. Intra-annual variation of soil respiration across four heterogeneous longleaf pine forests in the southeastern United States. For. Ecol. Manage. 359, 370–380.

Bannari, A., Morin, D., Bonn, F., Huete, A.R., 1995. A review of vegetation indices. Remote Sens. Rev. 13, 95–120.

Birth, G.S., McVey, G.R., 1968. Measuring the color of growing turf with a reflectance spectrophotometer. Agron. J. 60, 640–643.

Blackmer, T.M., Schepers, J.S., 1995. Use of a chlorophyll meter to monitor nitrogen status and schedule fertigation for corn. J. Prod. Agric. 8, 56–60.

Castelli, F., Contillo, R., 2009. Using a Chlorophyll Meter to Evaluate the Nitrogen Leaf Content in Flue-Cured Tobacco (Nicotiana tabacum L.). Ital. J. Agron. 4, 3–11.

Cui, M., Sun, X., Hu, C., Di, H.J., Tan, Q., Zhao, C., 2011. Effective mitigation of nitrate leaching and nitrous oxide emissions in intensive vegetable production systems using a nitrification inhibitor, dicyandiamide. J. Soils Sediments 11, 722–730.

Debaeke, P., Rouet, P., Justes, E., 2006. Relationship between the normalized SPAD index and the nitrogen nutrition index: Application to durum wheat. J. Plant Nutr. 29, 75–92.

Esfahani, M., Abbasi, H.R.A., Rabiei, B., Kavousi, M., 2008. Improvement of nitrogen management in rice paddy fields using chlorophyll meter (SPAD). Paddy Water Environ. 6, 181–188.

Fitzgerald, G.J., 2010. Characterizing vegetation indices derived from active and passive sensors. Int. J. Remote Sens. 31, 4335–4348.

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

Gianquinto, G., Goffart, J.P., Olivier, M., Guarda, G., Colauzzi, M., Dalla Costa, L., Delle Vedove, G., Vos, J., Mackerron, D.K.L., 2004. The use of hand-held chlorophyll meters as a tool to assess the nitrogen status and to guide nitrogen fertilization of potato crop. Potato Res. 47, 35–80.

Gianquinto, G., Orsini, F., Fecondini, M., Mezzetti, M., Sambo, P., Bona, S., 2011. A methodological approach for defining spectral indices for assessing tomato nitrogen status and yield. Eur. J. Agron. 35, 135–143.

Gianquinto, G., Sambo, P., Bona, S., 2003. The use of SPAD-502 chlorophyll meter for dynamically optimising the nitrogen supply in potato crop: a methodological approach. Acta Hortic. 627, 217–224.

Gitelson, A., 2004. Wide Dynamic Range Vegetation Index for Remote Quantification of Biophysical Characteristics of Vegetation. J. Plant Physiol. 161, 165–173.

Güler, S., Büyük, G., 2007. Relationships among chlorophyll-meter reading value, leaf N and yield of cucumber and tomatoes. Acta Hortic. 729, 307–311.

Hatfield, J.L., Gitelson, A.A., Schepers, J.S., Walthall, C.L., 2008. Application of spectral remote sensing for agronomic decisions. Agron. J. 100, S117–S131.

Hatfield, J.L., Prueger, J.H., 2010. Value of using different vegetative indices to quantify agricultural crop characteristics at different growth stages under varying management practices. Remote Sens. 2, 562–578.

Hikishima, M., Giovanetti Canteri, M., Godoy, C. V, Koga, L.J., da Silva, A.J., 2011. Quantificação de danos e relações entre severidade, medidas de refletância e produtividade no patossistema ferrugem asiática da soja. Trop. Plant Pathol. 35, 96–103.

Hoel, B.O., 2003. Chlorophyll meter readings in winter wheat: Cultivar differences and prediction of grain protein content. Acta Agric. Scand. Sect. B Soil Plant Sci. 52, 147–157.

Johansen, B., Tømmervik, H., 2014. The relationship between phytomass, NDVI and vegetationcommunities on Svalbard. Int. J. Appl. Earth Obs. Geoinf. 27, 20–30.

Ju, X.T., Kou, C.L., Zhang, F.S., Christie, P., 2006. Nitrogen balance and groundwater nitrate contamination: Comparison among three intensive cropping systems on the North China Plain. Environ. Pollut. 143, 117–125.

Liu, X., Ferguson, R.B., Zheng, H., Cao, Q., Tian, Y., Cao, W., Zhu, Y., 2017. Using an active-optical sensor to develop an optimal NDVI dynamic model for high-yield rice production (Yangtze, China). Sensors (Switzerland) 17, 1–13.

Ma, B.L., Morrison, M.J., Dwyer, L.M., 1996. Canopy light reflectance and field greenness to assess nitrogen fertilization and yield of maize. Agron. J. 88, 915–920.

Meisinger, J.J., Schepers, J.S., Raun, W.R., 2008. Crop Nitrogen Requirement and Fertilization. Am. Soc. Agron. Crop Sci. Soc. Am. Soil Sci. Soc. Am. 563–612.

Minotti, P.L., Halseth, D.E., Sieczka, J.B., 1994. Field chlorophyll measurements to assess the nitrogen status of potato varieties. HortScience 29, 1497–1500.

Mistele, B., Schmidhalter, U., 2008. Estimating the nitrogen nutrition index using spectral canopy reflectance measurements. Eur. J. Agron. 29, 184–190.

Monostori, I., Árendás, T., Hoffman, B., Galiba, G., Gierczik, K., Szira, F., Vágújfalvi, A., 2016. Relationship between SPAD value and grain yield can be affected by cultivar, environment and soil nitrogen content in wheat. Euphytica 211, 103–112.

Olivier, M., Goffart, J.P., Ledent, J.F., 2006. Threshold value for chlorophyll meter as decision tool for nitrogen management of potato. Agron. J. 98, 496–506.

Ollinger, S. V, 2011. Sources of variability in canopy reflectance and the convergent properties of plants. New Phytol. 189, 375–394.

Padilla, Francisco M., Gallardo, M., Peña-Fleitas, M.T., de Souza, R., Thompson, R.B., 2018. Proximal Optical Sensors for Nitrogen Management of Vegetable Crops: A Review. Sensors 18, 2083.

Padilla, F.M., Peña-Fleitas, M.T., Gallardo, M., Thompson, R.B., 2017. Determination of sufficiency values of canopy reflectance vegetation indices for maximum growth and yield of cucumber. Eur. J. Agron. 84.

Padilla, F.M., Peña-Fleitas, M.T., Gallardo, M., Thompson, R.B., 2016. Proximal optical sensing of cucumber crop N status using chlorophyll fluorescence indices. Eur. J. Agron. 73, 83–97.

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. Ann. Appl. Biol. 166, 271–285.

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. Eur. J. Agron. 58, 39–52.

Padilla, F.M., Thompson, R.B., Peña-Fleitas, M.T., Gallardo, M., 2018. Reference values for phenological phases of chlorophyll meter readings and reflectance indices for optimal N nutrition of fertigated tomato. Acta Hortic. 1192, 65–72.

Peng, S., Garcia, F. V., Laza, R.C., Cassman, K.G., 1993. Adjustment for specific leaf weight improves chlorophyll meter’s estimate of rice leaf nitrogen concentration. Agron. J. 85, 987–990.

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 Dalias (SE - Spain). Environ. Geol. 39, 791–799.

Samborski, S.M., Gozdowski, D., Walsh, O.S., Lamb, D.W., Stępień, M., Gacek, E.S., Drzazga, T., 2015. Winter wheat genotype effect on canopy reflectance: Implications for using NDVI for in-season nitrogen topdressing recommendations. Agron. J. 107, 2097–2106.

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

Sandoval-Villa, M., Guertal, E.A., Wood, C.W., 2000. Tomato leaf chlorophyll meter readings as affected by variety, nitrogen form, and nighttime nutrient solution strength. J. Plant Nutr. 23, 649–661.

Schepers, J.S., Blackmer, T.M., Wilhelm, W.W., Resende, M., 1996. Transmittance and reflectance measurements of corn leaves from plants with different nitrogen and water supply. J. Plant Physiol. 148, 523–529.

Schröder, J.J., Neeteson, J.J., Oenema, O., Struik, P.C., 2000. Does the crop or the soil indicate how to save nitrogen in maize production? Reviewing the state of the art. F. Crop. Res. 66, 151–164.

Sellers, P.J., 1985. Canopy reflectance, photosynthesis and transpiration. Int. J. Remote Sens. 6, 1335–1372.

Solari, F., Shanahan, J., Ferguson, R., Schepers, J., Gitelson, A., 2008. Active sensor reflectance measurements of corn nitrogen status and yield potential. Agron. J. 100, 571–579.

Sultana, S.R., Ali, A., Ahmad, A., Mubeen, M., Zia-Ul-Haq, M., Ahmad, S., Ercisli, S., Jaafar, H.Z.E., 2014. Normalized difference vegetation index as a tool for wheat yield estimation: A case study from Faisalabad, Pakistan. Sci. World J. 2014, 8.

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. Agric. Water Manag. 89, 261–274.

Thompson, R.B., Tremblay, N., Fink, M., Gallardo, M., Padilla, F.M., 2017. Tools and strategies for sustainable nitrogen fertilisation of vegetable crops, in: Tei, F., Nicola, S., Benincasa, P. (Eds.), Advances in Research on Fertilization Management in Vegetable Crops. Springer, Heidelberg, Germany, pp. 11–63.

Wakiyama, Y., 2016. The relationship between SPAD values and leaf blade chlorophyll content throughout the rice development cycle. Japan Agric. Res. Q. 50, 329–334.

Wang, Y.W., Mao, P.S., Dunn, B.L., Arnall, D.B., 2012. Use of an active canopy sensor and SPAD chlorophyll meter to quantify geranium nitrogen status. HortScience 47, 45–50.

Yuan, Z., Ata-Ul-Karim, S.T., Cao, Q., Lu, Z., Cao, W., Zhu, Y., Liu, X., 2016. Indicators for diagnosing nitrogen status of rice based on chlorophyll meter readings. F. Crop. Res. 185, 12–20.

Zhao, B., Liu, Z.Z., Ata-Ul-Karim, S.T., Xiao, J., Liu, Z.Z., Qi, A., Ning, D., Nan, J., Duan, A., 2016. Rapid and nondestructive estimation of the nitrogen nutrition index in winter barley using chlorophyll measurements. F. Crop. Res. 185, 59–68.

Ziadi, N., Bélanger, G., Claessens, A., Lefebvre, L., Tremblay, N., Cambouris, A.N., Nolin, M.C., Parent, L.E., 2010. Plant-based diagnostic tools for evaluating wheat nitrogen status. Crop Sci. 50, 2580–2590.

Zotarelli, L., Dukes, M.D., Scholberg, J.M.S., Muñoz-Carpena, R., Icerman, J., 2009. Tomato nitrogen accumulation and fertilizer use efficiency on a sandy soil, as affected by nitrogen rate and irrigation scheduling. Agric. Water Manag. 96, 1247–1258.

6. Chapter three: Influence of time of day on measurement with chlorophyll meters and canopy reflectance sensors of different crop N status

Padilla, F.M., de Souza, R., Peña-Fleitas, M.T., Grasso, R., Gallardo, M., Thompson, R.B. 2019. Influence of time of day on measurement with chlorophyll meters and canopy reflectance sensors of different crop N status. *Precision Agriculture* 20(6): 1087-1106.

6.1. Abstract

Optical sensors are a promising approach for assessing nitrogen (N) status of vegetable crops. However, their potential may be undermined if time of day influences measurements. This study evaluated the effects of time of day and N addition on measurements, made with two chlorophyll meters, SPAD-502 and MC-100, and two active canopy reflectance sensors, GreenSeeker handheld and Crop Circle ACS-470. Three treatments (N1, deficient, N2, conventional, and N3, excessive N application) of N concentration in the nutrient solution were applied by fertigation throughout a sweet pepper crop grown in soil in a greenhouse. Time of day of 12:00 and 15:00 hours had an effect on measurements made with the SPAD-502, but only in the N1 treatment, suggesting that the effects of time of day were related to crop N status. This effect was slight, being 1.7±0.02 SPAD units lower at 12:00 and 15:00 hours compared to at 9:00 hours (relative decrease of 3.6%). For the MC-100, a slight increase in Chlorophyll Content Index (CCI) values of 3.3±0.1 units (relative increase of 6.3%) was observed at 15:00 and 18:00 hours, relative to CCI values at 9:00 hour, regardless of N treatment. The time of day effect on chlorophyll meters appears to be negligible in relation to the wide range of values measured in greenhouse-grown sweet pepper. Normalized Difference Vegetation Index (NDVI), measured both with the GreenSeeker and Crop Circle, and Green Normalized Difference Vegetation Index (GNDVI), measured with the Crop Circle, were not affected by time of day in any of the N treatments, showing that these sensors and indices can be used with confidence at any time of the day.

Keywords: irradiance; optical sensors; solar radiation; sweet pepper; vegetable crops; vegetation indices

6.2. Introduction

Nitrogen (N) fertilizer is applied in large amounts in vegetable production to ensure high yields (Meisinger et al., 2008; Neeteson, 1994). Generally, only a minor part of applied N is recovered by crops, and the excess N is susceptible to loss to the environment where it is associated with various environmental problems (Cameron et al., 2013; Padilla et al., 2018b; Soto et al., 2015). Optimal N fertilization of vegetable crops, in respect to both quantity and timing, would benefit appreciably from accurate assessment of crop N status (Thompson et al., 2017). Traditional approaches to vegetable crop N management have been fertilizer recommendation schemes based on soil, and also on plant analysis (Fox and Walthall, 2008; Schröder et al., 2000; Thompson et al., 2017). While these procedures are useful, the analyses are time-consuming, and commonly the time to obtain laboratory results prevents timely adjustments of fertilizer applications (Gianquinto et al. 2011).

Proximal optical sensors are a promising approach for rapid and periodic assessment of crop N status (Padilla et al., 2018c; Usha and Singh, 2013). Proximal optical sensors are a form of sensing in which the sensors are positioned either in contact with or close to the crop (Padilla et al., 2018c; Thompson et al., 2017). They can be used any time during the growth cycle or at certain phenological stages on commercial farms, require limited labor, and can be integrated with fertilizer decision making procedures (Gianquinto et al. 2011). Optical sensors do not directly measure the N content in plant tissue, but provide assessment of either (a) indicator compounds, such as measurement with chlorophyll meters, or (b) indices of radiation characteristics, such as measurement with canopy reflectance sensors (Padilla et al., 2018c; Samborski et al., 2009). Both types of assessment are sensitive to crop N status (Fox and Walthall, 2008; Samborski et al., 2009; Tremblay et al., 2012).

Chlorophyll meters indirectly estimate relative chlorophyll content per unit of leaf surface area (Monje and Bugbee, 1992; Padilla et al., 2018a, 2018c). All chlorophyll meters are generally easy to use, do not require any specific training of users, and make measurements very rapidly with very little data processing (Gianquinto et al., 2004). Given their ease of use, small size and relatively low cost, chlorophyll meters are very well-suited for on-farm use to provide rapid assessment of vegetable crop N status (Padilla et al., 2018c). However, given the relatively small sampling area, appreciable repetition and strict sampling protocols have been developed for their practical use (Gianquinto et al., 2011b; Padilla et al., 2015; Samborski et al., 2009).

Canopy reflectance sensors provide information on crop N status by measuring specific wavelengths of radiation absorbed and reflected from crop foliage (Hatfield et al., 2008; Ollinger, 2011; Peñuelas et al., 1994). Being proximal optical sensors, they are positioned relatively close to the crop, e.g. 0.4–3.0 m from the crop canopy (Padilla et al., 2018c). The wavelengths used commonly correspond to four narrow bands centered around 675 nm (maximum absorption of red), 905 nm (maximum reflection of near infrared (NIR)), 720 nm (mid portion of the red-edge range), and 550 nm (maximum reflectance of green) (Gianquinto et al. 2011; Read et al. 2002; Thenkabail et al. 2002). To increase the sensitivity to specific biophysical characteristics and reduce variability, spectral vegetation indices combining spectral reflectance from 2–3 wavelengths are commonly used (Bannari et al., 1995; Scotford and Miller, 2005). Canopy reflectance sensors are classified as passive or active sensors depending on whether they have their own light source. For passive reflectance sensors, uniform irradiance conditions are highly recommended (Oliveira and Scharf, 2014). An intended advantage of active reflectance sensors is that there may be no special requirement for uniform irradiance conditions (Solari et al., 2008).

The use of absolute sufficiency values for chlorophyll meters measurements (Gianquinto et al., 2006, 2004; Güler et al., 2006; Padilla et al., 2017a, 2015) and of vegetation indices, measured with canopy reflectance sensors (Padilla et al., 2017b, 2015), have been proposed as practical procedures to assist with N management of vegetable crops (Padilla et al., 2018c; Thompson et al., 2017). However, the potential value of absolute sufficiency values for crop N management may be reduced if environmental conditions influence measured values (Thompson et al., 2017). Practical use of optical sensors to assist with N fertilization requires that measurements are precise and reproducible throughout the day regardless of environmental conditions (Martínez and Guiamet, 2004; Oliveira and Scharf, 2014).

Time of day and underlying changes in solar irradiance can influence chlorophyll meter measurements (Hoel and Solhaug, 1998; Martínez and Guiamet, 2004; Xiong et al., 2015). Higher values of chlorophyll meters have been measured at lower irradiance conditions, in winter wheat (Hoel and Solhaug, 1998; Martínez and Guiamet, 2004), rice (Xiong et al., 2015), tobacco (Nauš et al., 2010) and soybean (Xiong et al., 2015). The influence of irradiance on chlorophyll meter measurements may be species-specific, depending on species adaptation to high irradiance (Mamrutha et al., 2017). For instance, measurements of various chlorophyll meters were not significantly influenced by diurnal variation in irradiance in six spring wheat genotypes (Mamrutha et al., 2017).

Active canopy reflectance sensors are intended to be able to measure at any time of day regardless of variations in solar irradiance (Fitzgerald, 2010; Kipp et al., 2014; Solari et al., 2008). However, there are studies indicating that active sensors can be influenced by the time of day (Kim et al., 2012; Oliveira and Scharf, 2014; Teixeira Crusiol et al., 2017). Lower NDVI values were reported at solar noon, compared to early morning or late in the day, in soybean (Teixeira Crusiol et al., 2017), turfgrass (Kim et al., 2012) and cotton (Oliveira and Scharf, 2014).

Standardization of time of day and irradiance conditions when making optical sensor measurements is recommended for comparison of sequences on a given crop (Padilla et al., 2018c). The general recommendation is for optical sensor measurements to be made at the same time of day under clear skies (Gianquinto et al., 2004). In commercial farming, restricting measurements, to such conditions, would be practically difficult where farm work has to be scheduled considering numerous management activities. For the practical use of optical sensors, it is necessary to understand the influence of time of day on optical sensor measurement.

The establishment within a crop of a well-fertilized plot without N limitations, usually called the reference plot, has been widely suggested as a way to reduce time of day effects on sensor measurements (Holland and Schepers, 2013; Zhu et al., 2011). In this approach, optical sensor measurements made in the crop are divided by measurements taken in the reference plot, the result being called Nitrogen Sufficiency Index (NSI) (Debaeke et al., 2006; Piekielek et al., 1995). The influence of factors other than N on optical sensor measurements are minimized as effects will be very similar in both the measured crop and the reference plot. However, the use of reference plots in fertigated horticultural crops is impractical for technical reasons (Padilla et al., 2018c), and nearly all studies with optical sensors in fertigated vegetable crops have used absolute values of (Padilla et al., 2018c).

The objectives of this study were to assess the effects of time of day on measurements of chlorophyll meters and active canopy reflectance sensors. Sweet pepper was chosen because of its high chlorophyll content (Parry et al., 2014) ensuring a wide range of measurement values.

Three treatments of increasing N concentration in the nutrient solution were applied throughout the crop by fertigation. There was a very deficient N treatment, a conventional N management treatment, that was regarded as being close to optimal, and a very excessive N treatment.

6.3. Materials and Methods

6.3.1. Experimental Site

A sweet pepper (*Capsicum annuum* 'Melchor') crop was grown in soil in a plastic greenhouse. 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 (lateral side panels and flap roof windows), and an east-west orientation, with crop rows aligned north-south. The cropping area was 1300 m^2.

The soil was an artificial layered "enarenado" soil (Thompson et al., 2007), consisting of a 300 mm layer of imported silty loam texture soil placed over the original loam soil and a 100 mm layer of fine gravel (mostly 2–5 mm diameter) placed on the imported soil as a mulch. At greenhouse construction in July 2007, 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).

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; 12 plots were used in the current study. There were three N treatments with four replicate plots per treatment, arranged in a randomized block design. Each plot contained three paired lines of plants (six lines of plants in total), with 12 plants in each line with a 0.5 m spacing. Separation between the two lines that made the paired line of plants was 0.8 m, and separation between two paired lines was 1.2 m. One plant was positioned 60 mm 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 wide path along its east-west axis; there were two complete blocks in each of the northern and southern side of the greenhouse. There were border areas along the edges of the greenhouse.

6.3.2. Sweet pepper crop

The sweet pepper crop was grown with a summer-winter growing cycle in 2017–2018. The crop was grown from transplanted five-week-old seedlings, from 21 July 2017 to 20 February 2018 (cropping period of 214 days).

There were three treatments of different N concentration in the nutrient solution, applied by fertigation, that commenced 10 days after transplant (DAT). The treatments were applied in every irrigation throughout the crop. The N treatments were very N deficient (N1), conventional N management (N2), and very excessive N (N3), according to the N concentration in the applied nutrient solution. The average applied N concentrations in N1, N2 and N3 treatments during the crop were 2.0, 9.7 and 17.1 mmol N L^{-1}, respectively. Considering the total irrigation volume per treatment, the total applied N amounts in N1, N2 and N3 treatments were 86, 519 and 1198 kg N ha^{-1}, respectively. Other than N, complete nutrient solutions were applied to all three 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^-), the rest as ammonium (NH_4^+).

Plants were managed following local practice. The crop was physically supported using a system of nylon cords placed vertically and horizontally along the side of the crop. Irrigation was scheduled to maintain soil matric potential (SMP) in the root zone, at 120 mm depth, within -15 to -25 kPa; one tensiometer (Irrometer, Co., Riverside, Ca, USA) per plot was used to measure SMP. High temperature within the greenhouse was controlled by white-washing the plastic cladding with a $CaCO_3$ suspension, eight days before transplanting (0.5 kg L^{-1}) and 34 days after transplanting (0.40 kg L^{-1}). The white-washing was removed 68 days after transplanting.

6.3.3. Optical sensor measurements

Optical sensor measurements commenced on 27 September 2017 and were repeated every 17 days, on average, throughout most of the crop, until 12 January 2018, for a total of six measurement dates: 27 September (68 DAT), 10 October (81 DAT), 26 October (97 DAT), 9 November (111 DAT), 5 December (137 DAT) and 12 January (175 DAT). Optical sensor measurements were made, each day of measurement, at 9:00, 12:00, 15:00 and 18:00 hours solar time; these times are hereafter referred to as early morning, midday, afternoon and evening, respectively.

Measurements of relative chlorophyll content were made with two leaf-clip chlorophyll meters, the SPAD-502 meter (Konica Minolta, Inc., Tokyo, Japan) and the MC-100 Chlorophyll Concentration Meter (Apogee Instruments, Inc., Logan UT, USA). The measurement areas of each meter were 6 mm^2 for the SPAD-502 and 63.6 mm^2 for the MC-100. The two meters determine the relative content of chlorophyll by measuring light absorbance in the red and NIR; the SPAD-502 measures absorbance at 650 nm (red) and 940 nm (NIR), and the MC-100 at 653 nm and 931 nm. Using the two absorbance values, the meters calculate a dimensionless numerical value which is related to the chlorophyll content (Padilla et al., 2018a). The equations employed by SPAD-502 meter to calculate the numerical value are confidential (Parry et al., 2014). The MC-100 calculates a ratio between transmission of radiation at 931 nm divided by transmission of radiation at 653 nm, the result being called the Chlorophyll Content Index (CCI). Measurement output for the SPAD-502 meter are SPAD units and for the MC-100 meter are CCI values.

Individual measurements with each meter were made on eight different plants in each of the four replicate plots of the three N treatments. These plants were located in the two central lines of plants in each plot; four plants were measured per line. 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. Leaves with physical damage or with condensed water were not measured, alternative plants were selected.

Measurements of crop canopy reflectance were made with the GreenSeeker handheld sensor (Trimble Navigation Limited, Sunnyvale, CA, USA) and the Crop Circle ACS-470 sensor (Holland Scientific Inc., Lincoln, NE, USA). These are active canopy reflectance sensor fitted with their own polychromatic light sources (Solari et al., 2008). Both sensors were held vertically parallel to the crop rows, having a side view of the upper part of the foliage. The sensors were positioned so that the top of the field of view was level with the most recently fully expanded leaf. Measurements commenced once the crop had sufficient height to avoid sensing of soil reflectance, considering the field of view of each sensor.

Measurements with the GreenSeeker handheld sensor were made at 600 mm horizontal distance, giving an oval field of view with a height of ≈250 mm. One-shot measurements per plant were made by placing the sensor in front of each of eight plants in each replicate plot, the four plants were in each of the two inner lines of plants in each plot. The value for each plot was the average of the eight measurements. This sensor calculates the Normalized Difference Vegetation Index (NDVI; Table 1) by measuring crop reflectance at two wavelengths, 780 nm (NIR) and 660 nm (red).

Canopy reflectance measurements with the Crop Circle ACS-470 sensor were made at a 450 mm horizontal distance, giving a field of view of ≈260 (vertical) × 50 (horizontal) mm. In each measurement, two passes consisting of a 4 m pass in each of the two inner lines of plants were made at walking speed (approx. at 1.5 km h^{-1}). Ten measurements were made per second, giving approximately 200 individual measurements per plot. The filters selected to measure reflectance were 550 nm (green), 670 nm (red) and 760 nm (NIR). Reflectance data of each wavelength were stored in a portable GeoScout GLS-400 datalogger (Holland Scientific, Inc.) and subsequently processed. From each individual measurement, vegetation indices were calculated based on the reflectance of each wavelength (Table 1); the values of the vegetation index for individual measurements were averaged to provide an average vegetation index value for each replicate plot.

Table 1. Vegetation indices calculated from canopy reflectance measurements made with the Crop Circle ACS-470 sensor in a sweet pepper crop grown in a greenhouse.

Vegetation Index	Abbreviation	Equation	Reference
Normalized Difference Vegetation Index	NDVI	$\frac{NIR - Red}{NIR + Red}$	Sellers (1985)
Green Normalized Difference Vegetation Index	GNDVI	$\frac{NIR - Green}{NIR + Green}$	Gitelson et al. (1996)
Red Ratio Vegetation Index	RVI	$\frac{NIR}{Red}$	Birth and McVey (1968)
Green Ratio Vegetation Index	GVI	$\frac{NIR}{Green}$	Birth and McVey (1968)

NIR (Near infrared), 760 nm; Red, 670 nm; Green, 550 nm

6.3.4. Air temperature, solar radiation and photosynthetically active radiation (PAR)

Air temperature in the greenhouse was measured with a ventilated aspirated psychcrometer (model 1.1130, Thies Clima, Göttingen, Germany) and solar radiation with a pyranometer (model SKS 1110, Skye Instruments, Llandrindod Wells, Wales, UK). Both devices were placed in a shadow-free location of the greenhouse over the crop canopy. All data were recorded and stored using a data logger (CR10X, Campbell Scientific, Inc., Logan, UT, USA).

The photosynthetically active radiation (PAR) over the crop canopy was measured at the time of optical sensor measurements, using a linear quantum sensor (model LP-80, Decagon Devices Inc., Pullman, WA, USA). Each PAR value consisted of the average of two measurements, taken at the beginning and at the end of optical sensor measurements each time. Measurements were made by placing the linear quantum sensor horizontally over a line of plants located in the center of each replicate plot per treatment.

6.3.5. Data analysis and statistics

Exploratory repeated-measure analysis of variance (RM-ANOVA) were conducted to test the effect of time of day (9:00, 12:00, 15:00 and 18:00 hours), N treatment (N1, N2 and N3), date of measurement (68, 81, 97, 111, 137 and 175 DAT), and their two- and three-term interactions, on optical sensor measurements, using date of measurement as a within-subjects factor, and time of day, N treatment and block as between-subjects factors (Tables S1 and S2). The results of these analyses showed that the three-term Nitrogen x Time x Date interaction was not significant at $p \geq 0.68$ for all optical sensor measurements (Tables S1 and S2), and the two-term Time x Date interaction was not significant at $p \geq 0.39$ for all optical sensor measurements but RVI (Tables S1 and S2). These generally not significant two- and three-term interactions showed that the effects of time of day on optical sensor measurements were independent of the date of measurement. Given these results, and to provide a clear representation of the data, integrated values of optical sensor measurements, which integrated data from the six measurement dates into a unique value, were calculated following Padilla et al. (2017b).

Integrated values (IV) of PAR and optical sensor data, for the six sampling dates, were obtained by calculating a weighted average value for each replicate plot, for each time of day. The IV was calculated as an average of the individual values (iv) of each measurement date pondered by the time elapsed between two consecutive measurements (Padilla et al., 2017b), as:

$$\mathrm{IV} = \Sigma \mathrm{ivm} \cdot \mathrm{dm}/\mathrm{D}, \qquad (5)$$

where iv_m was the individual value at each date of measurement m, d_m was the number of days since the previous measurement, and D was the total number of days from first to last measurement. On the first date of measurement, when there was no preceding measurement, a seventeen-day interval was assumed because that was the average frequency throughout most of the crop.

To represent the variation in PAR and optical sensor measurements during the day, the relative changes in measurements made at 12:00, 15:00 and 18:00 hours to those made at 9:00 hour were calculated as percentages.

Significant effects of time of day (9:00, 12:00, 15:00 and 18:00 hours) and N treatment (N1, N2 and N3), and their interaction, on integrated PAR and integrated optical sensor measurements were evaluated by RM-ANOVA, using time of day as a within-subjects factor, and N treatment and block as between-subjects factors.

When necessary to meet the homoscedasticity assumption of ANOVA, transformed values were used. The statistical package IBM SPSS 22 (IBM Corporation, Armonk, NY, USA) was used. Significant differences were considered to occur at $p<0.05$, followed by LSD pair-wise comparison tests.

6.4. Results

6.4.1. Temperature, solar radiation and PAR

On most measurement dates, solar radiation in the greenhouse increased to maximum values at 14:00–15:00 hours, and then rapidly declined to negligible or small values by 18:00 (Figure 1). During the daylight period, on all measurement dates, air temperature in the greenhouse, increased to maximum values at 12:00–15:00 hours, after which it generally declined appreciably (Figure 1).

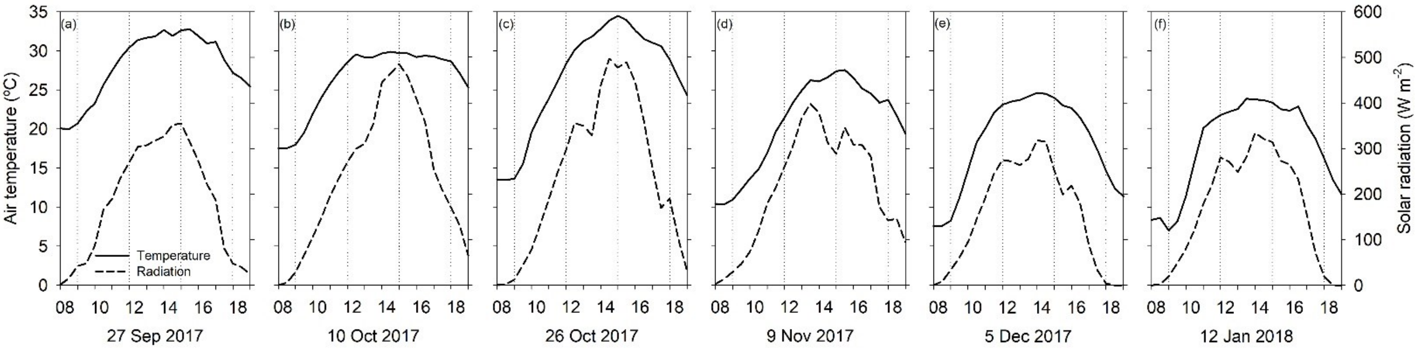

Figure 1. Diurnal evolution of air temperature (ºC; solid line), on main y-axis, and solar radiation (W m-2; dotted line), on secondary y-axis, in the greenhouse, on the six dates of measurement. Vertical dotted lines show the time of day with optical sensor measurements, i.e. 9:00, 12:00, 15:00 and 18:00 hours.

During the daylight period, integrated PAR significantly increased ($\text{RM-ANOVA}_{\text{Time}}$ F=325.9, P<0.001) reaching a maximum value at 15:00 hour (Figure 2). Integrated PAR at 18:00 hour was not statistically different to that at 9:00 (Figure 2). Similar integrated PAR levels were measured regardless of N treatment ($\text{RM-ANOVA}_{\text{N}}$ F=1.3, P=0.333).

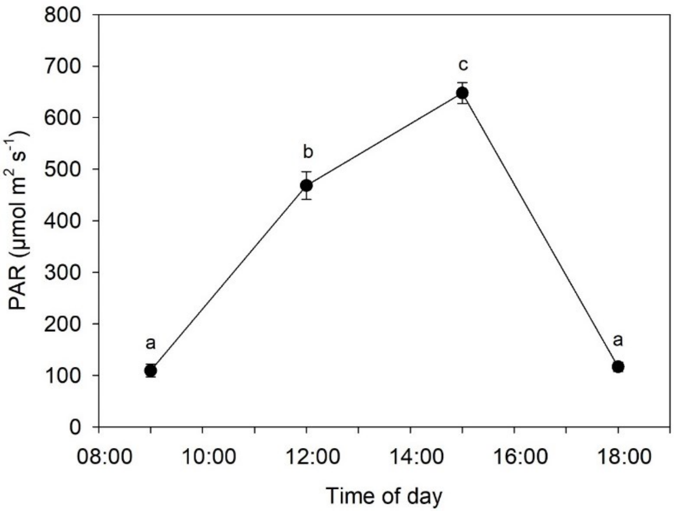

Figure 2. Diurnal evolution of integrated photosynthetically active radiation (PAR) over a sweet pepper crop grown in greenhouse. Different lower-case letters show significant differences, at $p<0.05$, between time of day, after LSD posthoc tests. Data have been pooled over the three different N treatments. Error bars show standard error.

6.4.2. Effect of nitrogen on chlorophyll meter and canopy reflectance measurements

Measurements of both chlorophyll meters were very significantly affected by N treatment (RM-ANOVA$_{N}$, $p<0.001$), regardless of time of day (RM-ANOVA$_{\mathrm{Time \times N}}$, $P>0.09$ for both meters). Chlorophyll meter measurements significantly increased with N addition; there were very significant differences from N1 to N2 treatments, and significant differences between the N2 and N3 treatments (Figure 3).

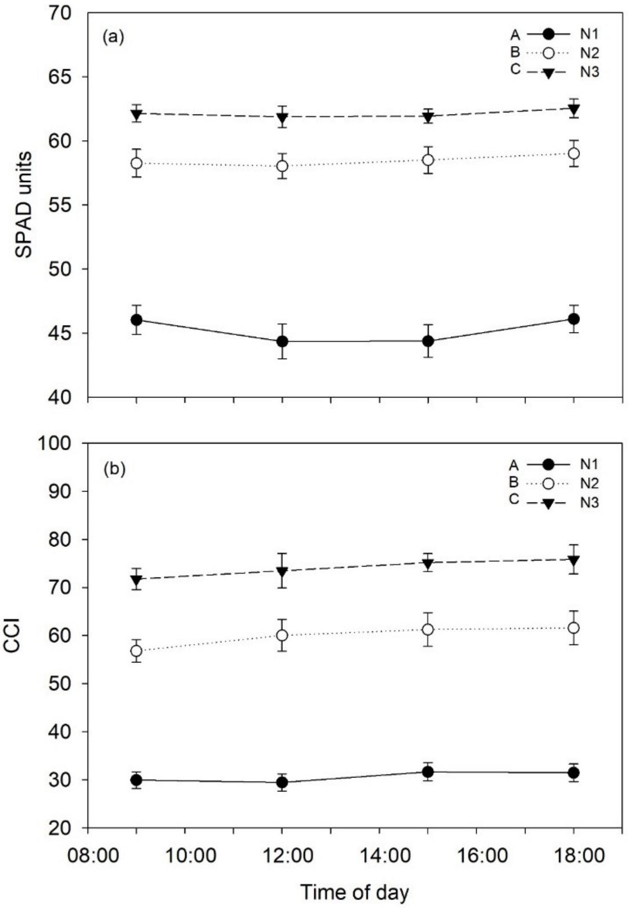

Figure 3. Diurnal evolution of integrated SPAD (a) and CCI values (b), in a sweet pepper crop grown in greenhouse. Different upper-case letters show significant differences, at p<0.05, between N treatments, regardless of time of day, after LSD posthoc tests. Error bars show standard error. CCI is Chlorophyll Concentration Index, measured with a MC-100 meter.

Nitrogen treatment had a very strong effect on (a) NDVI values, measured with both the GreenSeeker handheld sensor (Figure 4a) and the Crop Circle ACS-470 sensor (Figure 4b), and (b) values of three other vegetation indices measured with the Crop Circle sensor, i.e. GNDVI, RVI and GVI (Figure 4c–e). This N effect (RM-ANOVA$_{N}$, P<0.001 in all vegetation indices) occurred regardless of the time of day (RM-ANOVA$_{Time \times N}$, P>0.05 in all vegetation indices). Values of all four vegetation indices increased significantly (P<0.05 in all cases) with N addition from the N1 to N2 and N3 treatments. This effect was consistent for all four times of day (Figure 4).

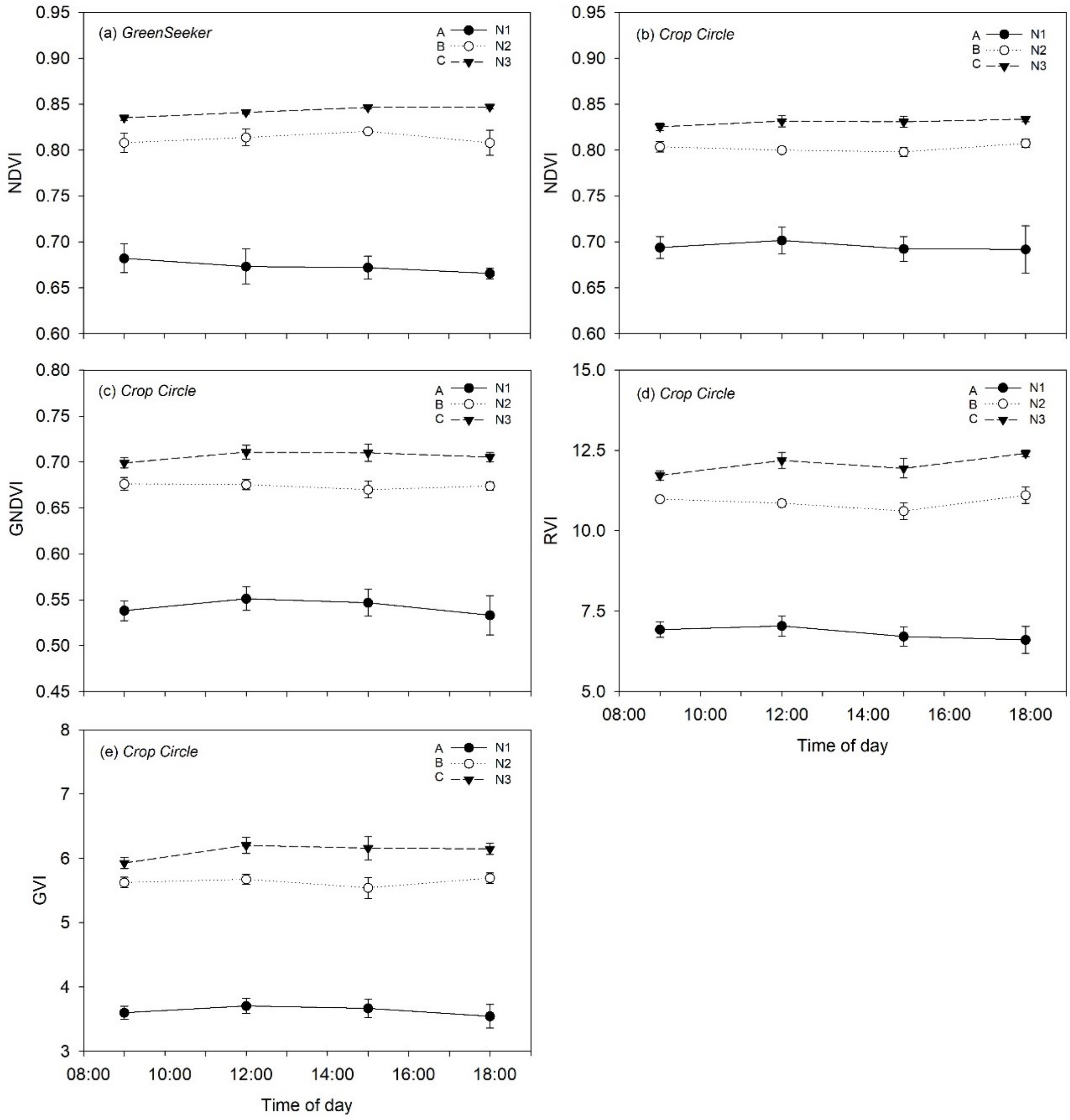

Figure 4. Diurnal evolution of integrated NDVI values, measured with the GreenSeeker handheld (a) and with the Crop Circle ACS-470 (b), and of integrated GNDVI (c), RVI (d) and GVI (e) values, measured with the Crop Circle ACS-470, in a sweet pepper crop grown in greenhouse. Different upper-case letters show significant differences, at $P<0.05$, between N treatments, regardless of time of day. Error bars show standard error.

6.4.3. Effect of time of day on chlorophyll meters

With the SPAD-502 meter, there was a significant Time x Nitrogen interaction (Table 2), indicating that time of day had a significant effect on SPAD-502 measurements depending on the N treatment. In the N1 treatment, SPAD-502 values were significantly lower at 12:00 and 15:00 hours than at 9:00 hour, while SPAD-502 values at 18:00 hour were statistically comparable to those at 9:00 hour (Figure 5a). The reduction of SPAD-502 values at 12:00 and 15:00 hours was, on average, 3.62±0.03% relative to SPAD values at 9:00 hour; this percentage corresponded to a decrease in absolute SPAD-502 values that was, on average, 1.67±0.02 SPAD units. In the N2 and N3 treatments, SPAD-502 values did not change significantly during the day (Figure 5a).

Table 2. Repeated-measures analysis of variance (RM-ANOVA) testing the effect of nitrogen treatment and time of day on change in integrated SPAD-502 and CCI values, relative to 9:00 hour, in a sweet pepper crop grown in a greenhouse.

Effect	**df**	**SPAD units**		**CCI**	
		F	**p**	**F**	**p**
Block	3	0.81	0.533	0.55	0.666
Nitrogen (N)	2	0.34	0.725	0.09	0.917
Time (T)	3	9.29	<0.001	17.57	<0.001
N x T	6	3.31	0.023	1.66	0.189
Error	18				

With the MC-100 meter, CCI values changed statistically during the day, regardless of the N treatment (RM-ANOVA$T_{\text{Time x N}}$ P=0.189; Table 2). On average, CCI values were significantly higher at 15:00 (6.1%) and 18:00 (6.4%) hours than CCI values measured at 9:00 hour (Figure 5b), for the three N treatments. The increase in CCI values at 15:00 and 18:00 hours corresponded to an absolute increase in CCI values of 3.2 and 3.5 units, respectively, relative to measurements at 9:00 hour. CCI values measured at 12:00 were statistically comparable to those at 9:00 hour (Figure 5b).

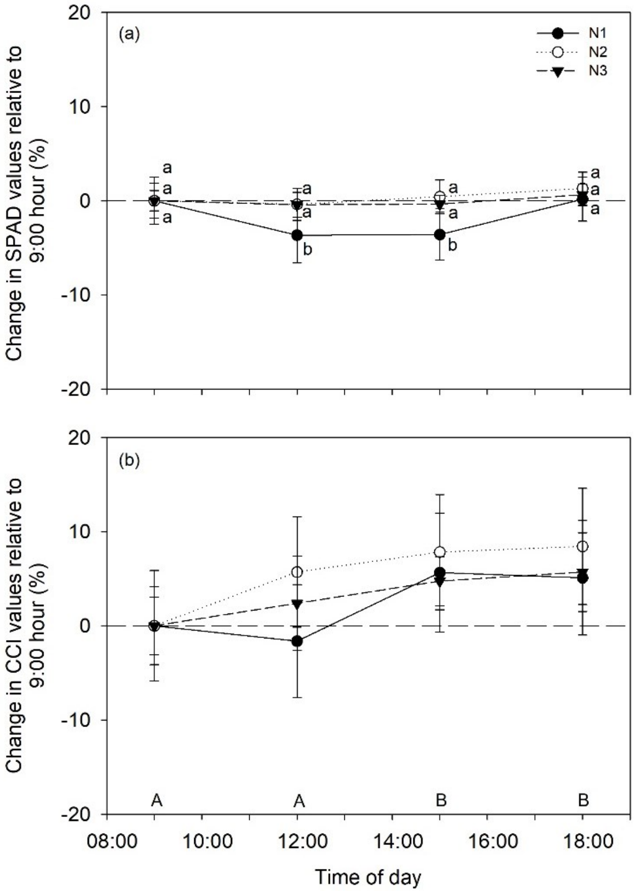

Figure 5. Change in integrated SPAD (a) and CCI values (b) relative to 9:00 hour (%), in a sweet pepper crop grown in greenhouse. In (a), different lower-case letters show significant differences, at p<0.05, between time of day within each N treatment, after LSD posthoc tests. In (b), different upper-case letters on x-axis show significant differences between time of day, regardless of N treatment. Error bars show standard error.

6.4.4. Effect of time of day on canopy reflectance sensors

Values of NDVI, measured both with the GreenSeeker handheld sensor and the Crop Circle ACS-470 sensor, and of GNDVI and GVI, measured with the Crop Circle sensor (Figure 6), were not affected by time of day (RM-ANOVA$_{\text{Time}}$ P>0.1 in all cases; Table 3). However, for GVI, the time of the day effect was marginal at P=0.057 (Table 3). There was no interaction between time of day and N treatment in NDVI, GNDVI and GVI (P>0.1 in all cases; Table 3). Values of RVI, measured with the Crop Circle ACS-470 sensor, statistically changed during the day depending on N treatment (RM-ANOVA$_{\text{Time}}$

x N P=0.008; Table 3). In the N1 treatment, RVI values at 18:00 hour were significantly 4.6% less than values at 9:00 hour (Figure 6d). This percentage corresponded to an absolute decrease in RVI values of 0.32 units. RVI values measured at 12:00 and 15:00 hours were statistically comparable to RVI values at 9:00 hour. In the N3 treatment, RVI values at 12:00 and 18:00 hours were significantly 4.0 and 5.8% higher, respectively, than RVI values measured at 9:00 hour. These percentages corresponded to an increase in absolute RVI values of 0.47 and 0.68 units, respectively. RVI values measured at 15:00 hour were statistically comparable to RVI values at 9:00 hour (Figure 6d). In the N2 treatment, there was no significant variation of RVI values during the day (Figure 6d).

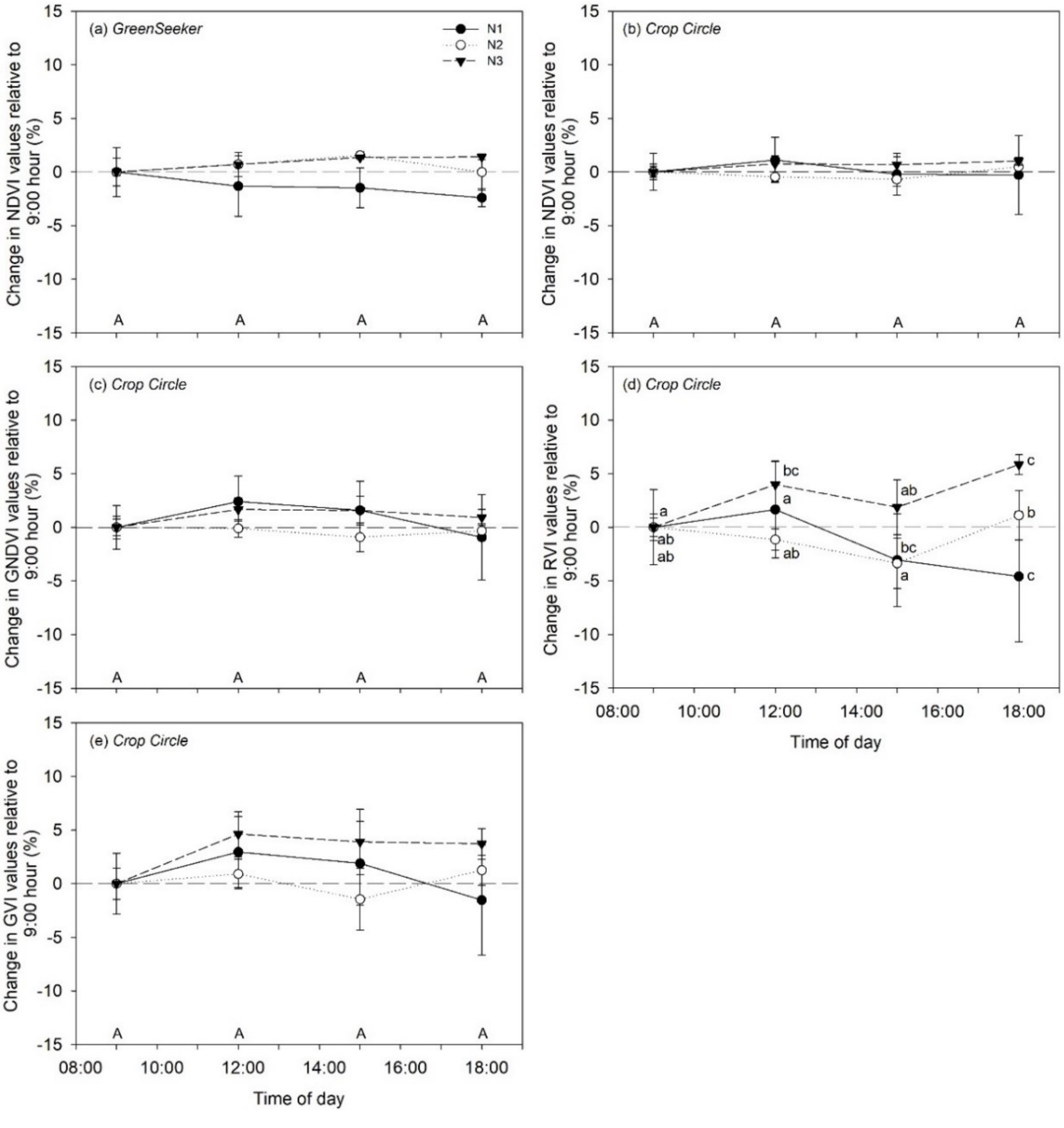

Figure 6. Change in integrated NDVI values, measured with the GreenSeeker handheld sensor (a) and with the Crop Circle ACS-470 sensor (b), and of integrated GNDVI (c),

RVI (d) and GVI (e) values, measured with the Crop Circle sensor, relative to 9:00 hour (%), in a sweet pepper crop grown in greenhouse. In (a), (b), (c) and (e), same upper-case letters on the x-axis show not significant differences, at P<0.05, between time of day, regardless of N treatment. In (d), different lower-case letters show significant differences between time of day within each N treatment. Error bars show standard error.

Table 3. Repeated-measures analysis of variance (RM-ANOVA) testing the effect of nitrogen treatment and time of day on change in integrated NDVI values, measured with the GreenSeeker handheld sensor and with the Crop Circle ACS-470 sensor, and integrated GNDVI, RVI and GVI values, measured with the Crop Circle sensor, relative to 9:00 hour, in a sweet pepper crop grown in a greenhouse.

Effect	df	NDVI GreenSeeker		NDVI Crop Circle		GNDVI Crop Circle		RVI Crop Circle		GVI Crop Circle	
		F	P	F	p	F	p	F	p	F	P
Block	3	0.44	0.733	1.11	0.417	0.80	0.538	0.96	0.471	0.58	0.650
Nitrogen (N)	2	0.86	0.469	0.09	0.916	0.17	0.848	0.69	0.536	0.30	0.751
Time (T)	3	0.31	0.816	0.36	0.780	2.47	0.095	3.26	0.046	3.01	0.057
N x T	6	0.78	0.599	0.52	0.790	1.51	0.231	4.27	0.008	2.11	0.102
Error	18										

6.5. Discussion

6.5.1. Effect of nitrogen on chlorophyll meter and canopy reflectance measurements

Measurements of both chlorophyll meters, the SPAD-502 and MC-100, and the vegetation indices measured with the GreenSeeker handheld sensor (i.e. NDVI) and the Crop Circle ACS-470 sensor (i.e. NDVI, GNDVI, RVI and GVI), were all responsive to N treatments in sweet pepper. All chlorophyll meter measurements and vegetation indices increased with N addition and were significantly different between the three N treatments. These observations demonstrate that the two chlorophyll meters and two reflectance sensors can be used to differentiate very different N nutrition in sweet pepper, such as the very excessive N treatment (N3) from the conventional N treatment (N2). This is notable, because proximal optical sensors have been reported to lose sensitivity and have a tendency for saturation responses under conditions of high N application (Padilla et al., 2018c, 2018a; Thompson et al., 2017).

The results of this study with sweet pepper are in agreement with numerous studies that reported that chlorophyll meters can be used to differentiate N nutrition and to assess crop N status in vegetable 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), cucumber (Padilla et al., 2017a), sweet pepper (Parry et al., 2014), and potato (Gianquinto et al., 2004; Olivier et al., 2006). Regarding the canopy reflectance sensors, the capacity of the vegetation indices to differentiate N nutrition in sweet pepper, observed in the current study, is consistent with results with processing tomato (Gianquinto et al. 2011a), indeterminate tomato for fresh consumption (Padilla et al. 2015), hydroponically-grown (Yang et al., 2010) and soil-grown (Padilla et al. 2017b) cucumber, and in muskmelon (Padilla et al. 2014).

6.5.2. Effect of time of day on chlorophyll meters

The present study found that measurements with the SPAD-502 meter in the N1 treatment were significantly lower at the time of maximum solar radiation and PAR, compared to early morning and evening measurements. Measurements in the evening were comparable to those made early morning, despite that the air temperature was appreciably higher in the evening (Figure 1). These observations suggest that solar radiation had a statistically significant effect on relative chlorophyll measurements made with the SPAD-502 meter in greenhouse-grown sweet pepper, which is consistent with previous studies (Hoel and Solhaug, 1998; Martínez and Guiamet, 2004; Xiong et al., 2015). This effect is believed to be caused by modifications in chlorophyll distribution within leaves during the day which can be attributed to changes in chloroplast arrangement in response to variations in solar irradiance (Nauš et al., 2010). Therefore, chlorophyll meter readings can be influenced by irradiance conditions before and during measurement (Hoel and Solhaug, 1998; Williams et al., 2003). Under low irradiance, most chloroplasts are positioned along the top and bottom walls of the cells, perpendicular to the incident light (face position) to maximize light absorption. When exposed to high irradiance, they are more likely to be found along the sides of the cells to prevent light damage (Nauš et al., 2010; Williams et al., 2003). Movement to the high-irradiance position is sometimes called ‘avoidance movement’, because the chloroplasts move out of the most direct light, while movement to the low-irradiance position is often called ‘accumulation’, because chloroplasts accumulate to increase light absorption (Williams et al., 2003).

The decline of SPAD-502 values at midday in the N1 treatment was, in absolute terms, slight, being on average 1.7±0.02 SPAD units. From a practical perspective, this decline is negligible considering the range of SPAD units measured in the three N treatments of this study. SPAD-502 measurements were 13.2 and 16.9 SPAD units higher in both the conventional N2 and very excessive N3 treatments, respectively, compared to measurements in the very deficient N1 treatment. This range of SPAD readings is much larger than the diurnal decline of SPAD-502 measurements observed in the current study. Therefore, it is not strictly necessary that measurements with this sensor, in greenhouse-grown sweet pepper, are made at a particular time of the day. However, following a sampling protocol within a restricted time period is recommended to maximize homogeneity of measurement (Padilla et al., 2018c).

The magnitude of the time of day effect on SPAD-502 measurements in the N1 treatment, in the present study, was notably less than reported in previous studies. The 3.6% decline of SPAD values detected at midday in this study contrasted with the reported decreases of 5–8% in winter wheat (Hoel and Solhaug, 1998; Martínez and Guiamet, 2004), 13% in rice (Xiong et al., 2015), 15% in *Oxalis acetosella* (Hoel and Solhaug, 1998), 16% in tobacco (Nauš et al., 2010), and 28% in soybean (Xiong et al., 2015). The influence of solar irradiance on chlorophyll meter measurements may be a species-specific effect that depends on species adaptation to high irradiance (Mamrutha et al., 2017; Xiong et al., 2015). The slight irradiance effect in sweet pepper may be due to adaptation of this species to high irradiance. Additionally, solar radiation values in the greenhouse where the study was conducted are typically 20–60% (depending on whitewashing) of external values (Padilla et al., 2016, 2015, 2014). These values are well within the ranges recorded in commercial plastic greenhouses of the area (Castilla, 2013; Valera-Martínez et al., 2016). Without the greenhouse plastic cover, the relative effect of time of day on SPAD-502 readings may have been higher.

Unlike the very deficient N1 treatment, there was no time of day effect on SPAD-502 values in the conventional N2 and very excessive N3 treatments. A very similar finding was also reported for rice and soybean by Xiong et al. (2015). These authors reported that there was no significant diurnal variation in SPAD-502 measurements of plants supplemented with N, but that SPAD measurements of plants that did not receive supplemental N were significantly lower at midday. Xiong et al. (2015) suggested that chloroplasts enlarged under high N conditions, occupying almost the entire cell space, which inhibited chloroplast movement at midday. For the sweet pepper crop, in the present study, data are not available of chloroplast size or chloroplast cell coverage. The work by Xiong et al. (2015) and the findings of the present study suggest that the effects of time of day on SPAD readings are related to crop N status.

For the MC-100 meter, CCI values measured at 9:00 and 12:00 hours were comparable, and relatively higher values were measured at 15:00 and 18:00 hours. These findings are not consistent with the decline of SPAD-502 readings at midday observed in the present study and other studies (Hoel and Solhaug, 1998; Martínez and Guiamet, 2004; Nauš et al., 2010; Xiong et al., 2015). To our knowledge, this is first report of differential response to time of day between two chlorophyll meters, in this case, the SPAD-502 and the MC-100 meters. Previously, Mamrutha et al. (2017) found that response of various chlorophyll meters (SPAD-502, atLEAF+ and CCM-200) to solar radiation was equal in six spring wheat genotypes. We cannot explain the difference between the SPAD-502 and the MC-100 meters observed in the present study. Measurements with the two meters were made immediately one after the other, on the same location of the same leaf. In addition, the two sensors measure red and NIR radiation absorbance at very similar wavelengths, i.e. at 650 nm and 940 nm in SPAD-502 meter, and at 653 nm and 931 nm in the MC-100 meter. It is difficult to explain the difference results between the two sensors. The major difference between them is that the measurement area of the SPAD-502 meter is considerably smaller than that of the MC-100 meter (i.e. 6 *versus* 64 mm^2); however, it is difficult to understand how this would cause the observed differences in measurement.

The increase of CCI values in the afternoon and evening was, on average, 6.2±0.2% relative to CCI values in early morning and midday. In absolute terms, this represented an increase in CCI values of 3.3±0.1 units. This increase can be considered small from a practical point of view, as the range of CCI values measured between the three N treatments of this study was considerably larger. CCI values measured in the conventional N2 and very excessive N3 treatments were 30.0 and 43.9 units higher, respectively, than those measured in the very deficient N1 treatment, and CCI values measured in the very excessive N3 treatment were 14.1 units higher than those measured in the conventional N2 treatment. The difference in measured CCI values between the three N treatments was well above the increase of CCI values in the afternoon and evening. Therefore, given these results, it is not justified that measurements with this sensor, with sweet pepper grown in greenhouse, be made at a particular time of the day. However, as with all optical sensors, a consistent sampling protocol, including the range of measurement times, is recommended to enhance homogeneity of measurement (Padilla et al., 2018c).

6.5.3. Effect of time of day on canopy reflectance sensors

Values of NDVI, measured both with the GreenSeeker handheld and the Crop Circle ACS-470, and of GNDVI and GVI, measured with the Crop Circle, were not affected statistically by time of day in any of the N treatments of this study. These results support the long-stated assumption that active canopy reflectance sensors can be used under any irradiance conditions without alterations in vegetation indices measured (Solari et al., 2008). Various previous studies with wheat and cotton have reported that there were not time of day or irradiance effects on canopy reflectance measurements (Fitzgerald, 2010; Kipp et al., 2014; Oliveira and Scharf, 2014).

Since both the GreenSeeker handheld and the Crop Circle ACS-470 sensors are active sensors with their own light source, time of day effects on these sensors were expected to occur only if they were able to detect the changes in the distribution of leaf chlorophyll that occurred during the day, as discussed for chlorophyll meters. However, unlike chlorophyll meters that measure a very localized area of a leaf, the area of measurement of these reflectance sensors is much larger and integrates not only leaves but also stems, fruits and air gaps within the crop structure. It is reasonable that sensors that integrate different tissues and air gaps have reduced sensitivity to detect changes in chlorophyll distribution that occur during the day mostly at the leaf level.

There are reports of slight effects of solar irradiance on measurements of active canopy reflectance sensors, particularly the GreenSeeker handheld sensor. Several studies reported effects in turfgrass (Kim et al., 2012), cotton (Oliveira and Scharf, 2014) and soybean cultivars (Teixeira Crusiol et al., 2017). Although the literature suggests that measurements of the GreenSeeker handheld sensor are more sensitive to changes in solar radiation during the day than active Crop Circle sensors; in the present study, no irradiance effect on GreenSeeker handheld measurements was observed.

In the present study, the behavior of simple ratio vegetation indices, such as RVI and GVI, in relation to time of day effects tended to differ from the normalized vegetation indices measured with the Crop Circle ACS-470, i.e. NDVI and GNDVI. While NDVI and GNDVI were not affected by time of day, GVI was marginally affected and RVI was significantly affected but these effects were contingent on the N treatment. In particular, in the very deficient N1 treatment, RVI values were nearly 5% lower in the evening than those measured in early morning. In the very excessive N3 treatment, RVI values were approximately 5% higher at midday and in the evening compared to early morning. These results are inconclusive since contrary time of day effects were observed in the N1 and N3 treatments. Given that the variation in RVI values during the day was only 5%, it may be that the effects observed in this vegetation index were due to measurement variability. In fact, normalized indices such NDVI and GNDVI, unlike simple ratio indices such as RVI and GVI, enable compensation for effects of non-uniform illumination, i.e. different amounts of incoming light and varying illumination angles (Bannari et al., 1995; Jones and Vaughan, 2010). These are often the underlying factors behind aspect and topography effects in remote sensing (Jones and Vaughan, 2010; Mason, 2004). In our study in a greenhouse crop, the uneven distribution, inclination and orientation of foliage in the sweet pepper foliage may have resulted in similar effects to those of topography and aspect that are sometimes observed in remote sensing, leading to larger variability and inconsistent results with RVI.

6.6. Conclusions

Overall, this study showed that measurements of the SPAD-502 and MC-100 chlorophyll meters, and vegetation indices measured with the GreenSeeker handheld sensor (i.e. NDVI) and Crop Circle ACS-470 sensor (i.e. NDVI, GNDVI, RVI and GVI), were responsive to N treatments and can be used to differentiate appreciably different N nutrition in sweet pepper.

Time of day, particularly midday and the afternoon, had an effect on relative chlorophyll measurements made with the SPAD-502 meter, but only in the very deficient N1 treatment. This suggested that the effects of time of day on SPAD-502 readings were related to crop N status. Nevertheless, this effect was slight and of little practical relevance for sweet pepper in greenhouse. For the MC-100 meter, a slight increase in CCI values was obtained in measurements made in the afternoon and the evening, compared to early morning measurements, regardless of the N treatment. This time of day effect on the MC-100 meter measurements is small for practical use on farm of this meter with greenhouse-grown sweet pepper.

Values of NDVI, measured both with the GreenSeeker handheld and the Crop Circle ACS-470, and of GNDVI, measured with the Crop Circle, were not affected by time of day in any of the N treatments of this study. These results support the assumption that these two active canopy reflectance sensors can be used under any radiation conditions without alterations in the vegetation indices measured.

6.7. References

Bannari, A., Morin, D., Bonn, F., Huete, A.R., 1995. A review of vegetation indices. Remote Sensing Reviews. 13, 95–120.

Birth, G.S., McVey, G.R., 1968. Measuring the color of growing turf with a reflectance spectrophotometer. Agronomy Journal. 60, 640–643.

Cameron, K.C., Di, H.J., Moir, J.L., 2013. Nitrogen losses from the soil/plant system: a review. Annals of Applied Biology 162, 145–173.

Castilla, N., 2013. Greenhouse technology and management, 2nd ed. Oxfordshire, UK: CABI.

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.

Fitzgerald, G.J., 2010. Characterizing vegetation indices derived from active and passive sensors. International Journal of Remote Sensing 31, 4335–4348.

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

Gianquinto, G., Goffart, J.P., Olivier, M., Guarda, G., Colauzzi, M., Dalla Costa, L., et al. 2004. The use of hand-held chlorophyll meters as a tool to assess the nitrogen status and to guide nitrogen fertilization of potato crop. Potato Research 47, 35–80.

Gianquinto, G., Orsini, F., Fecondini, M., Mezzetti, M., Sambo, P., Bona, S., 2011a. A methodological approach for defining spectral indices for assessing tomato nitrogen status and yield. European Journal of Agronomy 35, 135–143.

Gianquinto, G., Orsini, F., Sambo, P., Paino D'Urzo, M., 2011b. The use of diagnostic optical tools to assess nitrogen status and to guide fertilization of vegetables. HortTechnology 21, 287–292.

Gianquinto, G., Sambo, P., Borsato, D., 2006. Determination of SPAD threshold values for the optimisation of nitrogen supply in processing tomato. Acta Horticulturae 700, 159–166.

Gitelson, A.A., Kaufman, Y.J., Merzlyak, M.N., 1996. Use of a green channel in remote sensing of global vegetation from EOS- MODIS. Remote Sensing of Environment 58, 289–298.

Güler, S., Ibrikci, H., Büyük, G., 2006. Effects of different nitrogen rates on yield and leaf nutrient contents of drip-fertigated and greenhouse-grown cucumber. Asian Journal of Plant Sciences 5, 657–662.

Hatfield, J.L., Gitelson, A.A., Schepers, J.S., Walthall, C.L., 2008. Application of spectral remote sensing for agronomic decisions. Agronomy Journal 100, S117–S131.

Hoel, B.O., Solhaug, K.A., 1998. Effect of irradiance on chlorophyll estimation with the Minolta SPAD-502 leaf chlorophyll meter. Annals of Botany 82, 389–392.

Holland, K.H., Schepers, J.S., 2013. Use of a virtual-reference concept to interpret active crop canopy sensor data. Precision Agriculture 14, 71–85.

Jones, H.G., Vaughan, R.A., 2010. Remote sensing of vegetation. New York, USA: Oxford University Press.

Kim, Y., Glenn, D.M., Park, J., Ngugi, H.K., Lehman, B.L., 2012. Characteristics of active spectral sensor for plant sensing. Transactions of the ASABE 55, 293–301.

Kipp, S., Mistele, B., Schmidhalter, U., 2014. The performance of active spectral reflectance sensors as influenced by measuring distance, device temperature and light intensity. Computers and Electronics in Agriculture 100, 24–33.

Mamrutha, H.M., Sharma, D., Sumanth Kumar, K., Venkatesh, K., Tiwari, V., Sharma, I., 2017. Influence of diurnal irradiance variation on chlorophyll values in wheat: A comparative study using different chlorophyll meters. National Academy Science Letters 40, 221–224.

Martínez, D.E., Guiamet, J.J., 2004. Distortion of the SPAD 502 chlorophyll meter readings by changes in irradiance and leaf water status. Agronomie 24, 41–46.

Mason, I., 2004. Remote sensing of the Earth’s climate system, in: Harra, L.K., Mason, K.O. (Eds.), Space Science. London, UK: Imperial Collegue Press (distributed by World Scientific Publisihing Co), pp. 13–72.

Meisinger, J.J., Schepers, J.S., Raun, W.R., 2008. Crop nitrogen requirement and fertilization, in: Schepers, J.S., Raun, W.R. (Eds.), Nitrogen in Agricultural Systems, Agronomy Monograph No. 49. Madison, WI, USA: American Society of Agronomy, Crop Science Society of America, Soil Science Society of America, pp. 563–612.

Monje, O.A., Bugbee, B., 1992. Inherent limitations of nondestructive chlorophyll meters: a comparison of two types of meters. HortScience 27, 69–71.

Nauš, J., Prokopová, J., Řebíček, J., Špundová, M., 2010. SPAD chlorophyll meter reading can be pronouncedly affected by chloroplast movement. Photosynthesis Reserarch 105, 265–271.

Neeteson, J.J., 1994. Nitrogen management for intensively grown arable crops and field vegetables, in: Bacon, P. (Ed.), Nitrogen Fertilization and the Environment. New York, USA: Marcel Dekker, pp. 295–325.

Oliveira, L.F., Scharf, P.C., 2014. Diurnal variability in reflectance measurements from cotton. Crop Science 54, 1769–1781.

Ollinger, S. V, 2011. Sources of variability in canopy reflectance and the convergent properties of plants. New Phytologist 189, 375–394.

Padilla, F.M., de Souza, R., Peña-Fleitas, M.T., Gallardo, M., Giménez, C., Thompson, R.B., 2018a. Different responses of various chlorophyll meters to increasing nitrogen supply in sweet pepper. Frontiers in Plant Science 9, 1752.

Padilla, F.M., Gallardo, M., Manzano-Agugliaro, F., 2018b. Global trends in nitrate leaching research in the 1960–2017 period. Science of the Total Environment 643, 400–413.

Padilla, F.M., Gallardo, M., Peña-Fleitas, M.T., de Souza, R., Thompson, R.B., 2018c. Proximal Optical Sensors for Nitrogen Management of Vegetable Crops: A Review. Sensors 18, 2083.

Padilla, F.M., Peña-Fleitas, M.T., Gallardo, M., Giménez, C., Thompson, R.B., 2017a. Derivation of sufficiency values of a chlorophyll meter to estimate cucumber nitrogen status and yield. Computers and Electronics in Agriculture 141, 54–64.

Padilla, F.M., Peña-Fleitas, M.T., Gallardo, M., Thompson, R.B., 2017b. Determination of sufficiency values of canopy reflectance vegetation indices for maximum growth and yield of cucumber. European Journal of Agronomy 84, 1–15.

Padilla, F.M., Peña-Fleitas, M.T., Gallardo, M., Thompson, R.B., 2016. Proximal optical sensing of cucumber crop N status using chlorophyll fluorescence indices. European Journal of Agronomy 73, 83–97.

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.

Padilla, F.M., Teresa Peña-Fleitas, M., 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.

Parry, C., Blonquist, J.M., Bugbee, B., 2014. In situ measurement of leaf chlorophyll concentration: analysis of the optical/absolute relationship. Plant, Cell and Environment 37, 2508–2520.

Peñuelas, J., Gamon, J.A., Fredeen, A.L., Merino, J., Field, C.B., 1994. Reflectance indices associated with physiological changes in nitrogen- and water-limited sunflower leaves. Remote Sensing of Environment 48, 135–146.

Piekielek, W.P., Fox, R.H., Toth, J.D., Macneal, K.E., 1995. Use of a chlorophyll meter at the early dent stage of corn to evaluate nitrogen sufficiency. Agronomy Journal 87, 403–408.

Read, J.J., Tarpley, L., McKinion, J.M., Reddy, K.R., 2002. Narrow-waveband reflectance ratios for remote estimation of nitrogen status in cotton. Journal of Environmental Quality 31, 1442–1452.

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.

Schröder, J.J., Neeteson, J.J., Oenema, O., Struik, P.C., 2000. Does the crop or the soil indicate how to save nitrogen in maize production?: Reviewing the state of the art. Field Crops Research 66, 151–164.

Scotford, I.M., Miller, P.C.H., 2005. Applications of spectral reflectance techniques in northern European cereal production: A review. Biosystems Engineering. 90, 235–250.

Sellers, P.J., 1985. Canopy reflectance, photosynthesis and transpiration. International Journal of Remote Sensing 6, 1335–1372.

Solari, F., Shanahan, J., Ferguson, R., Schepers, J., Gitelson, A., 2008. Active sensor reflectance measurements of corn nitrogen status and yield potential. Agronomy Journal 100, 571–579.

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 and Environment 200, 62–70.

Teixeira Crusiol, L.G., Corrêa Carvalho, J.F., Ribeiro Sibaldelli, R.N., Neiverth, W., Do Rio, A., Ferreira, L.C., et al. 2017. NDVI variation according to the time of measurement, sampling size, positioning of sensor and water regime in different soybean cultivars. Precision Agriculture 18, 470–490.

Thenkabail, P.S., Smith, R.B., De Pauw, E., 2002. Evaluation of narrowband and broadband vegetation indices for determining optimal hyperspectral wavebands for agricultural crop characterization. Photogrammetric Engineering and Remote Sensing 68, 607–621.

Thompson, R.B., Martinez-Gaitan, C., Gallardo, M., Gimenez, C., Fernandez, 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 and Water Management 89, 261–274.

Thompson, R.B., Tremblay, N., Fink, M., Gallardo, M., Padilla, F.M., 2017. Tools and strategies for sustainable nitrogen fertilisation of vegetable crops, in: Tei, F., Nicola, S., Benincasa, P. (Eds.), Advances in Research on Fertilization Management in Vegetable Crops. Heidelberg, Germany: Springer, pp. 11–63.

Tremblay, N., Wang, Z., Cerovic, Z.G., 2012. Sensing crop nitrogen status with fluorescence indicators. A review. Agronomy for Sustainable Development 32, 451–464.

Usha, K., Singh, B., 2013. Potential applications of remote sensing in horticulture—A review. Scientia Horticulturae 153, 71–83.

Valera-Martínez, D.L., Belmonte-Ureña, L.J., Molina-Aiz, F.D., López-Martínez, A., 2016. Greenhouse agriculture in Almeria. A comprehensive techno-economic analysis, Serie Económica 27. Almeria, Spain: Cajamar Caja Rural.

Williams, W.E., Gorton, H.L., Witiak, S.M., 2003. Chloroplast movements in the field. Plant, Cell and Environment 26, 2005–2014.

Xiong, D., Chen, J., Yu, T., Gao, W., Ling, X., Li, Y., et al. 2015. SPAD-based leaf nitrogen estimation is impacted by environmental factors and crop leaf characteristics. Scientific Reports 5, 13389.

Yang, W., Li, M., Nick, S., 2010. Estimating nitrogen content of cucumber leaves based on NIR spectroscopy. Sensor Letters 8, 145–150.

Zhu, J., Tremblay, N., Liang, Y., 2011. A corn nitrogen status indicator less affected by soil water content. Agronomy Journal 103, 890–898.

7. Discussion

This thesis evaluated (a) the sensitivity of chlorophyll meters to assess crop N status of sweet pepper, (b) the effect of cultivar on chlorophyll meter and canopy reflectance measurements in cucumber, and (c) the effect of time of day on chlorophyll meter and canopy reflectance measurements. All the studies were conducted under field conditions in a plastic greenhouse similar to those of commercial vegetable production in SE Spain.

The sensitivity of chlorophyll meters to assess crop N status was evaluated in three sweet pepper crops. For ease of interpretation and simplification of numerous bi-weekly measurements made during a long crop cycle, results were integrated to provide a unique value for each phenological stage (vegetative, flowering, early fruit growth, and harvest), for each crop and for the three crops combined. Measurements with the three chlorophyll meters evaluated (SPAD-502, atLEAF+, and MC-100) were very strongly related to NNI for each of the four phenological stages of each pepper crop, and for individual phenological stage using composite data from all crops. These results demonstrate that the three chlorophyll meters evaluated provided good estimates of crop N status of sweet pepper. This is in agreement with studies that reported strong relationships between chlorophyll meter measurements and crop N status, in various horticultural crops (Padilla et al., 2017a; Wu et al., 2012) and cereal crops (Cartelat et al., 2005; Prost and Jeuffroy, 2007).

Within the four phenological stages, the strongest relationships between the three integrated chlorophyll meter measurements and NNI were obtained in the flowering and early fruit growth stages. The generally high R^2 values between measurements, with the chlorophyll meters, and NNI in each of the four phenological stages, demonstrated the ability of chlorophyll meter measurements to be used as indicators of crop N status throughout sweet pepper crops.

Sufficiency values derived for the SPAD-502 were very consistent for each phenological stage, in the three different crops. These data show the potential of the use of sufficiency values to improve N management of sweet pepper crops in commercial farming. There were large differences in SPAD sufficiency values between the harvest and vegetative stages, of 15.5 SPAD units; the relative difference being 23.4%. This large difference indicates that is not possible to use a unique sufficiency value for the entire crop cycle of sweet pepper as was proposed for cucumber (Güler and Büyük, 2007; Padilla et al., 2017a) and grapevine (Cerovic et al., 2015).

The variation between phenological stages of sufficiency values derived for the atLEAF+ and MC-100 meters was similar to that of the SPAD-502 meter. For the atLEAF+ and MC-100 meters, the lowest sufficiency values were in the vegetative stage, and the highest in the harvest stage. For atLEAF+ meter, there were data of two sweet pepper crops. Differences in atLEAF sufficiency values within each phenological stage between the two crops were small. This indicates that the atLEAF sufficiency values determined were consistent between the two crops. For the MC-100 meter, it was not possible to evaluate the consistency of sufficiency values between crops because there was only data from one crop.

Differences between cucumber cultivars of chlorophyll meter measurements, made with the SPAD-502 and MC-100 meters, were observed when the N supply was sufficient and excessive (N2 and N3 treatments), but not when the N supply was deficient (N1 treatment). These findings are in agreement with previous work where cultivar effects on SPAD measurements were more pronounced at higher N supply (Minotti et al., 1994; Yuan et al., 2016a). These results have implications for the use of absolute sufficiency values, of chlorophyll meter measurements, as indicators of optimal crop N status. This finding suggests that it may not be possible to use a unique sufficiency value for different cultivars of the same species. The use of procedures to normalize absolute chlorophyll meter measurements to deal with possible cultivar effects should be developed (Zhao et al., 2016). Also, care should be taken when using absolute sufficiency values for chlorophyll meter measurements for a cultivar different to that used to obtain the absolute sufficiency values. Where normalization procedures are not used, the absolute sufficiency values should be verified or adjusted following testing.

In the vegetation indices measured with the Crop Circle ACS-470 canopy reflectance sensor, the differences between cultivars were much smaller and less consistent than occurred with the two chlorophyll meters.

For each of the cucumber cultivars examined, both chlorophyll meter measurements and vegetation indices had strong relationships with leaf N content, indicating that these measurements were good cultivar specific indicators of leaf N content. However, there was a statistically significant cultivar effect on the relationships between chlorophyll meter measurement and leaf N. These results show that it is not feasible to use a unique equation for the three cucumber cultivars to estimate leaf N content from chlorophyll meter measurements. These differences were not found for vegetation indices, for which a single regression equation could be used to estimate leaf N content, for the three cultivars, for measurements of NDVI, GNDVI, RVI and GVI.

Regarding the effect of time of day on optical sensors measurements, there was a statistically significant effect with chlorophyll meters. However, this was of minor relevance for practical on-farm use. The significant effect on relative chlorophyll measurements made with the SPAD-502 coincides with results of previous work (Hoel and Solhaug, 1998; Xiong et al., 2015). In practical terms, the time of day effect on SPAD measurements is negligible considering the range of SPAD units measured in the present study. Regarding the vegetation indices measured with the GreenSeeker handheld (NDVI) and Crop Circle ACS- 470 (NDVI, GNDVI, RVI and GVI) sensors, these were not affected by time of day in any of the N treatments of this study. These results are consistent with the assumption that active canopy reflectance sensors can be used under any irradiance conditions without alterations in the vegetation indices measured (Solari et al., 2008a).

Overall, the results of the present thesis show that both chlorophyll meters (SPAD-502, MC-100, and atLEAF+) and several vegetation indices, measured with Crop Circle ACS-470 and GreenSeeker handheld sensors, are tools that provided good estimates of crop N status of sweet pepper and cucumber crops. These observations show that these chlorophyll meters and reflectance sensors can be used to identify very different N nutrition in the crops evaluated. The work with different cucumber cultivars showed that cultivar had an effect on chlorophyll meter measurements, and on the relationship between chlorophyll meter measurements and leaf N content. These findings indicate that it is not possible to use a unique sufficiency value and a common equation to estimate leaf N content for the three cucumber cultivars evaluated. They also suggest that considerable care should be taken when using absolute sufficiency values for chlorophyll meters that were derived with one cultivar for any vegetable species. The lack of a consistent effect of cultivar on canopy reflectance vegetation indices suggests that a unique equation to estimate leaf N content from vegetation indices can be applied to all three cucumber cultivars. In practical terms, generally, measurements of chlorophyll meters (SPAD-502 and MC-100) and vegetation indices (NDVI and GNDVI) were not appreciably affected by time of day, showing that these sensors and indices can be used at any time of the day.

7.1. References

Cartelat, A., Cerovic, Z.G., Goulas, Y., Meyer, S., Lelarge, C., Prioul, J.L., Barbottin, A., Jeuffroy, M.H., Gate, P., Agati, G., Moya, I., 2005. Optically assessed contents of leaf polyphenolics and chlorophyll as indicators of nitrogen deficiency in wheat (Triticum aestivum L.). F. Crop. Res. 91, 35–49.

Cerovic, Z.G., Ghozlen, N.B., Milhade, C., Obert, M., Debuisson, S., Le Moigne, M., Ben Ghozlen, N., Milhade, C., Obert, M., Debuisson, S., Le Moigne, M., 2015. Nondestructive diagnostic test for nitrogen nutrition of grapevine (Vitis vinifera L.) based on Dualex leaf-clip measurements in the field. J. Agric. Food Chem. 63, 3669–3680.

Güler, S., Büyük, G., 2007. Relationships among chlorophyll-meter reading value, leaf N and yield of cucumber and tomatoes. Acta Hortic. 729, 307–311.

Hoel, B.O., Solhaug, K.A., 1998. Effect of irradiance on chlorophyll estimation with the Minolta SPAD-502 leaf chlorophyll meter. Ann. Bot. 82, 389–392.

Minotti, P.L., Halseth, D.E., Sieczka, J.B., 1994. Field chlorophyll measurements to assess the nitrogen status of potato varieties. HortScience 29, 1497–1500.

Padilla, F.M., Peña-Fleitas, M.T., Gallardo, M., Giménez, C., Thompson, R.B., 2017. Derivation of sufficiency values of a chlorophyll meter to estimate cucumber nitrogen status and yield. Comput. Electron. Agric. 141, 54–64.

Prost, L., Jeuffroy, M.H., 2007. Replacing the nitrogen nutrition index by the chlorophyll meter to assess wheat N status. Agron. Sustain. Dev. 27, 321–330.

Solari, F., Shanahan, J., Ferguson, R., Schepers, J., Gitelson, A., 2008. Active sensor reflectance measurements of corn nitrogen status and yield potential. Agron. J. 100, 571–579.

Wu, X., Guo, J., Zhao, C., Chen, L., Zhang, Y., Fang, Z., Yang, Y.Y., 2012. Research and application of non-destructive testing diagnosis technology of tomato. Sens. Lett. 10, 666–669.

Xiong, D., Chen, J., Yu, T., Gao, W., Ling, X., Li, Y., Peng, S., Huang, J., 2015. SPAD-based leaf nitrogen estimation is impacted by environmental factors and crop leaf characteristics. Sci. Rep. 5, 13389.

Yuan, Z., Ata-Ul-Karim, S.T., Cao, Q., Lu, Z., Cao, W., Zhu, Y., Liu, X., 2016. Indicators for diagnosing nitrogen status of rice based on chlorophyll meter readings. F. Crop. Res. 185, 12–20.

Zhao, B., Liu, Z.Z., Ata-Ul-Karim, S.T., Xiao, J., Liu, Z.Z., Qi, A., Ning, D., Nan, J., Duan, A., 2016. Rapid and nondestructive estimation of the nitrogen nutrition index in winter barley using chlorophyll measurements. F. Crop. Res. 185, 59–68.

8. Conclusions

8.1. Chapter one

de Souza, R., Peña-fleitas, M.T., Thompson, R.B., Gallardo, M., Grasso, R., Padilla, F.M. 2019. The use of chlorophyll meters to assess crop N status and derivation of sufficiency values for sweet pepper. *Sensors* 19(13): 2949.

- There is potential for chlorophyll meters to monitor crop N status and to assist with N fertilizer management of sweet pepper.

- There were strong relationships between chlorophyll meter measurements and NNI for each phenological stage of sweet pepper crops, when each crop was considered separately, when three crops were considered as a combined data set. This demonstrated the consistency and robustness of chlorophyll meter measurements as indicators of crop N status.

- The sufficiency values calculated for chlorophyll meter measurements in each phenological stage and their consistency for a given phenological stage between different crops showed the potential for the sufficiency values to be used in commercial farming to achieve improved N management of sweet pepper crops.

8.2. Chapter two

de Souza, R., Grasso, R., Teresa Peña-Fleitas, M., Gallardo, M., Thompson, R.B., Padilla, F.M. 2020. Effect of cultivar on chlorophyll meter and canopy reflectance measurements in cucumber. *Sensors* 20(2): 509.

- Cultivar had a significant effect on SPAD-502 and MC-100 chlorophyll meter measurements when N supply was adequate and excessive.
- For the red band-based vegetation indices (NDVI and RVI) measured with the Crop Circle ACS470 sensor, there was no effect of cultivar, regardless of N applied.
- For the green band-based vegetation indices (GNDVI and GVI), there was a cultivar effect, mainly with the variety ‘Strategos’.

- Cultivar had a significant effect on the relationship between leaf N content and chlorophyll meter measurements, but not on the relationships between leaf N content and canopy reflectance vegetation indices.
- The lack of a consistent effect of cultivar, on the relationship with leaf N content, suggests that a unique equation to estimate leaf N content from vegetation indices can be applied to all three cultivars. However, a unique equation cannot be applied for chlorophyll meter measurements because of the significant cultivar effect detected in the present study.

8.3. Chapter three:

Padilla, F.M., de Souza, R., Peña-Fleitas, M.T., Grasso, R., Gallardo, M., Thompson, R.B. 2019. Influence of time of day on measurement with chlorophyll meters and canopy reflectance sensors of different crop N status. *Precision Agriculture* 20(6): 1087-1106.

- The measurements of the SPAD-502 and MC-100 chlorophyll meters, and vegetation indices measured with the GreenSeeker handheld sensor (i.e. NDVI) and Crop Circle ACS-470 sensor (i.e. NDVI, GNDVI, RVI and GVI), were responsive to N treatments and can be used to differentiate appreciably different N nutrition in sweet pepper.
- Time of day, particularly midday and the afternoon, had an effect on chlorophyll measurements made with the SPAD-502 meter, but only in the very deficient N treatment. This suggested that the effects of time of day on SPAD-502 readings were related to crop N status. Nevertheless, this effect was slight and of little practical relevance for commercial sweet pepper crops grown in greenhouse.
- For the MC-100 meter, a slight increase in CCI values was obtained in measurements made in the afternoon and the evening, compared to early morning measurements, regardless of the N treatment. This time of day effect on the MC-100 meter measurements is very small in the context of practical on-farm use with greenhouse-grown sweet pepper.

- Values of NDVI, measured both with the GreenSeeker handheld and the Crop Circle ACS-470 sensors, and of GNDVI, measured with the Crop Circle sensor, were not affected by time of day in any of the N treatments of this study. These results support the assumption that these two active canopy reflectance sensors can be used under any irradiance conditions without alterations in the vegetation indices measured.

9. Supplementary materials

9.1. Chapter two. Effect of cultivar on chlorophyll meter and canopy reflectance measurements in cucumber

9.1.1. Supplementary Tables

Table S1: Analysis of variance testing the effect of cultivar and nitrogen on crop leaf area index (LAI), crop height, luminance (Y) and chromatic coordinates xy of CIE 1931 color space, of a cucumber (*Cucumis sativus* L.) crop.

		LAI		**Crop height**		**Luminance (Y)**		**Coordinate x**		**Coordinate y**	
Effect	**df**	F	P	**F**	**P**	**F**	**P**	**F**	**P**	**F**	**P**
Block	3	1.13	0.356	2.48	0.085	1.60	0.215	1.02	0.403	0.68	0.573
Cultivar (C)	2	6.32	0.006	0.65	0.532	22.32	<0.001	7.44	0.003	11.38	<0.001
Nitrogen (N)	2	373.40	<0.001	272.85	<0.001	347.55	<0.001	504.19	<0.001	372.69	<0.001
C x N	4	1.10	0.379	1.61	0.204	1.98	0.130	2.21	0.10	2.26	0.092
Error	24										

Table S2: Analysis of variance testing the effect of cultivar, nitrogen and time, on leaf N content of cucumber (*Cucumis sativus* L.) crop.

		F	**P**
Block	3	3.46	0.032
Cultivar (C)	2	29.40	<0.001
Nitrogen (N)	2	2441.02	<0.001
C x N	4	3.73	0.017
Error	24		
Time (T)	6	257.75	<0.001
T x C	12	3.71	<0.001
T x N	12	73.01	<0.001
T x C x N	24	1.88	<0.013
Error	144		

Table S3: Analysis of variance testing the effect of cultivar, nitrogen and time, on SPAD and CCI measurements of cucumber (*Cucumis sativus* L.) crop.

		SPAD		**CCI**	
Effect	**df**	**F**	**P**	**F**	**P**
Block	3	14.12	<0.001	8.55	<0.001
Cultivar (C)	2	72.75	<0.001	110.37	<0.001
Nitrogen (N)	2	310.88	<0.001	277.20	<0.001
C x N	4	7.34	<0.001	15.58	<0.001
Error	24				
Time (T)	6	111.07	<0.001	134.15	<0.001
T x C	12	1.62	0.091	5.72	<0.001
T x N	12	23.47	<0.001	30.94	<0.001
T x C x N	24	3.01	<0.001	3.05	<0.001
Error	144				

Table S4: Analysis of variance testing the effect of cultivar, nitrogen and time, on NDVI, GNDVI, RVI and GVI measurements of cucumber (*Cucumis sativus* L.) crop.

		NDVI		**GNDVI**		**RVI**		**GVI**	
	df	**F**	**P**	**F**	**P**	**F**	**P**	**F**	**P**
Block	3	28.52	<0.001	5.36	0.006	30.01	<0.001	6.28	0.003
Cultivar (C)	2	3.30	0.054	10.46	<0.001	4.71	0.019	18.25	<0.001
Nitrogen (N)	2	469.37	<0.001	442.93	<0.001	375.34	<0.001	331.41	<0.001
C x N	4	1.52	0.228	1.98	0.129	0.84	0.516	1.43	0.256
Error	24								
Time (T)	5	89.51	<0.001	61.28	<0.001	197.22	<0.001	67.18	<0.001
T x C	10	3.08	0.002	2.93	0.003	2.41	<0.012	2.58	0.007
T x N	10	42.44	<0.001	27.92	<0.001	70.81	<0.001	28.47	<0.001
T x C x N	20	2.96	<0.001	3.42	<0.001	4.57	<0.001	4.54	<0.001
Error	120								

Table S5: Coefficients of determination (R^2) and standard error of the estimate (SEE) of linear regression between each optical sensor measurements (independent variable) and leaf N content (dependent variable) for each cultivar of cucumber (*Cucumis sativus* L.). DAT is days after transplanting. Symbols close to R^2 values show significance of linear regression (ns, not significant at $p \geq 0.05$; *, $p<0.05$; **, $p<0.01$; ***, $p<0.001$).

Sensor / Index	DAT	'Strategos'		'Pradera'		'Mitre'	
		R^2	±SEE	R^2	±SEE	R^2	±SEE
SPAD	22	0.89***	0.29	0.35*	0.78	0.53**	0.68
	29	0.83***	0.48	0.60**	0.82	0.71***	0.73
	36	0.89***	0.50	0.84***	0.60	0.88***	0.54
	43	0.92***	0.50	0.81***	0.73	0.90***	0.55
	50	0.91***	0.50	0.91***	0.52	0.96***	0.38
	57	0.44*	1.03	0.57**	0.96	0.84***	0.60
	64	0.08ns	1.10	0.50*	0.87	0.73***	0.65
CCI	22	0.86***	0.32	0.45*	0.72	0.64**	0.59
	29	0.85***	0.46	0.72***	0.68	0.81***	0.58
	36	0.88***	0.51	0.87***	0.56	0.96***	0.30
	43	0.94***	0.42	0.86***	0.62	0.97***	0.30
	50	0.85***	0.63	0.86***	0.63	0.98***	0.25
	57	0.56**	0.87	0.61**	0.91	0.88***	0.51
	64	0.06 ns	1.11	0.82***	0.52	0.61**	0.78
NDVI	29	0.87***	0.41	0.86***	0.48	0.90***	0.43
	36	0.91***	0.74	0.93***	0.42	0.88***	0.55
	43	0.86***	0.64	0.64**	1.01	0.65**	1.02
	50	0.91***	0.50	0.73***	0.89	0.77***	0.86
	57	0.21 ns	1.23	0.45*	1.09	0.71***	0.81
	64	0.14 ns	1.06	0.25 ns	1.07	0.16 ns	1.15
GVI	29	0.85***	0.45	0.98***	0.20	0.94***	0.34
	36	0.85***	0.57	0.92***	0.44	0.91***	0.47
	43	0.95***	0.39	0.92***	0.46	0.89***	0.58
	50	0.83***	0.68	0.80***	0.77	0.84***	0.71
	57	0.68***	0.78	0.68**	0.83	0.84***	0.59
	64	0.01 ns	1.14	0.65**	0.73	0.58**	0.81
RVI	29	0.88***	0.41	0.94***	0.32	0.95***	0.31
	36	0.87***	0.52	0.89***	0.51	0.84***	0.64
	43	0.86***	0.64	0.61**	1.04	0.74***	0.89
	50	0.81***	0.71	0.64**	1.03	0.72***	0.95
	57	0.31 ns	1.15	0.45*	1.08	0.70***	0.81
	64	0.01 ns	1.14	0.38*	0.97	0.27 ns	1.07
GNDVI	29	0.89***	0.39	0.96***	0.25	0.92***	0.38
	36	0.91***	0.44	0.94***	0.36	0.90***	0.50
	43	0.92***	0.48	0.87***	0.60	0.90***	0.55

	50	0.79***	0.75	0.86***	0.64	0.90***	0.57
	57	0.73***	0.71	0.76***	0.72	0.90***	0.46
	64	0.05 ns	1.11	0.42*	0.94	0.65**	0.75

Table S6: P-values of the F-statistic analysis comparing the relationship between leaf N content and optical sensor measurements between the reduced regression for all three cultivars together and the regression of each cultivar of cucumber (*Cucumis sativus* L.) separately.

DAT	RVI			GNDVI		
	‘Strategos’	‘Pradera’	‘Mitre’	‘Strategos’	‘Pradera’	‘Mitre’
29	0.377	0.215	0.201	0.281	0.066	0.262
36	0.281	0.262	0.409	0.302	0.189	0.409
43	0.139	0.444	0.324	0.157	0.281	0.229
50	0.166	0.409	0.350	0.350	0.245	0.177
57	0.409	0.377	0.189	0.377	0.377	0.123
64	0.350	0.245	0.324	0.377	0.262	0.131

9.1.2. Supplementary Figures

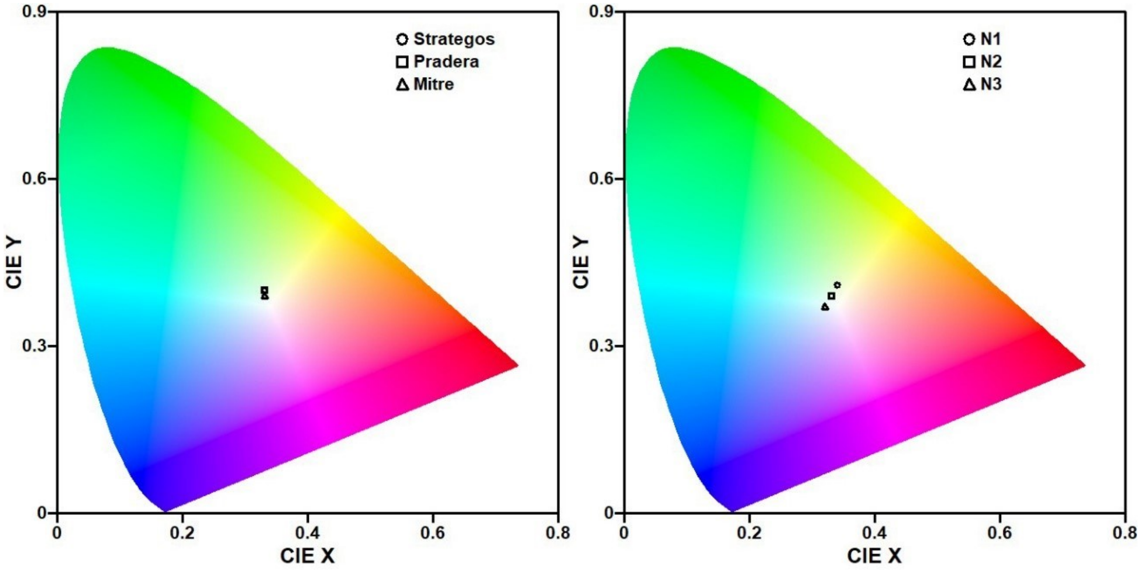

Figure S1. Chromatic differences (x coordinate and y coordinate of CIE 1931 color space) between (a) cultivars, when pooling over the three N treatments, and between (b) N treatments, when pooling over the three cultivars, of a cucumber (*Cucumis sativus* L.) crop under three N treatments. Values are means ± SE. Pooling was possible because of not significant Cultivar x Nitrogen interaction for x and y coordinates (see Table 1).

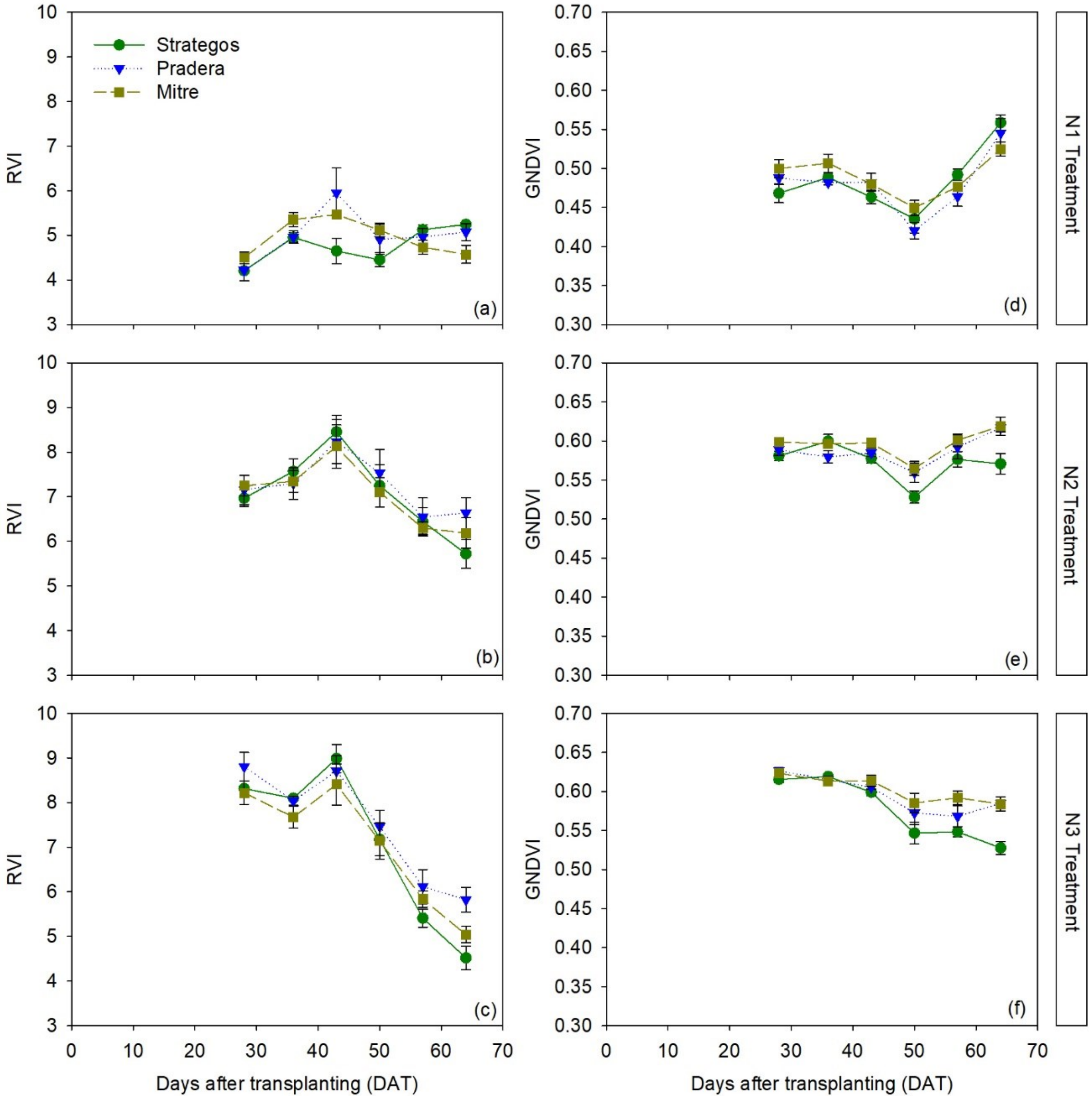

Figure S2. Temporal dynamics of RVI (panels **a**-**c**) and GNDVI (panels **d**-**f**) measurements of three cultivars of cucumber (*Cucumis sativus* L. 'Strategos', 'Pradera' and 'Mitre') under three N treatments (N1, N2 and N3). Values are means ± SE.

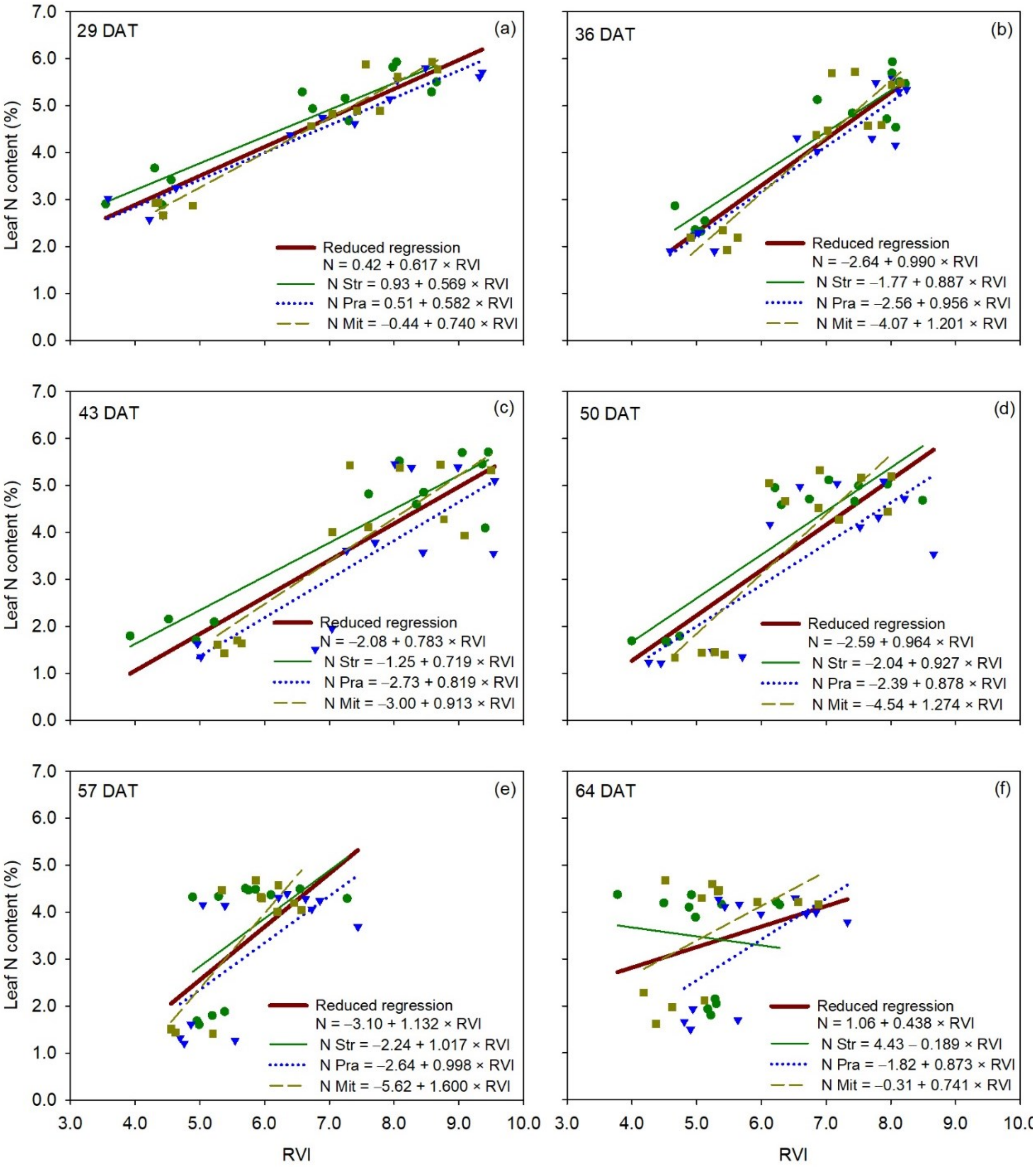

Figure S3. Linear regression between RVI measurements (independent variable) and leaf N content (dependent variable) for each cultivar of cucumber (*Cucumis sativus* L.), for each measurements date (Panels **a**-**f**). The reduced regression is a regression with data of all three cultivars together. DAT is days after transplanting. Str, 'Strategos', Pra, 'Pradera', Mit, 'Mitre'.

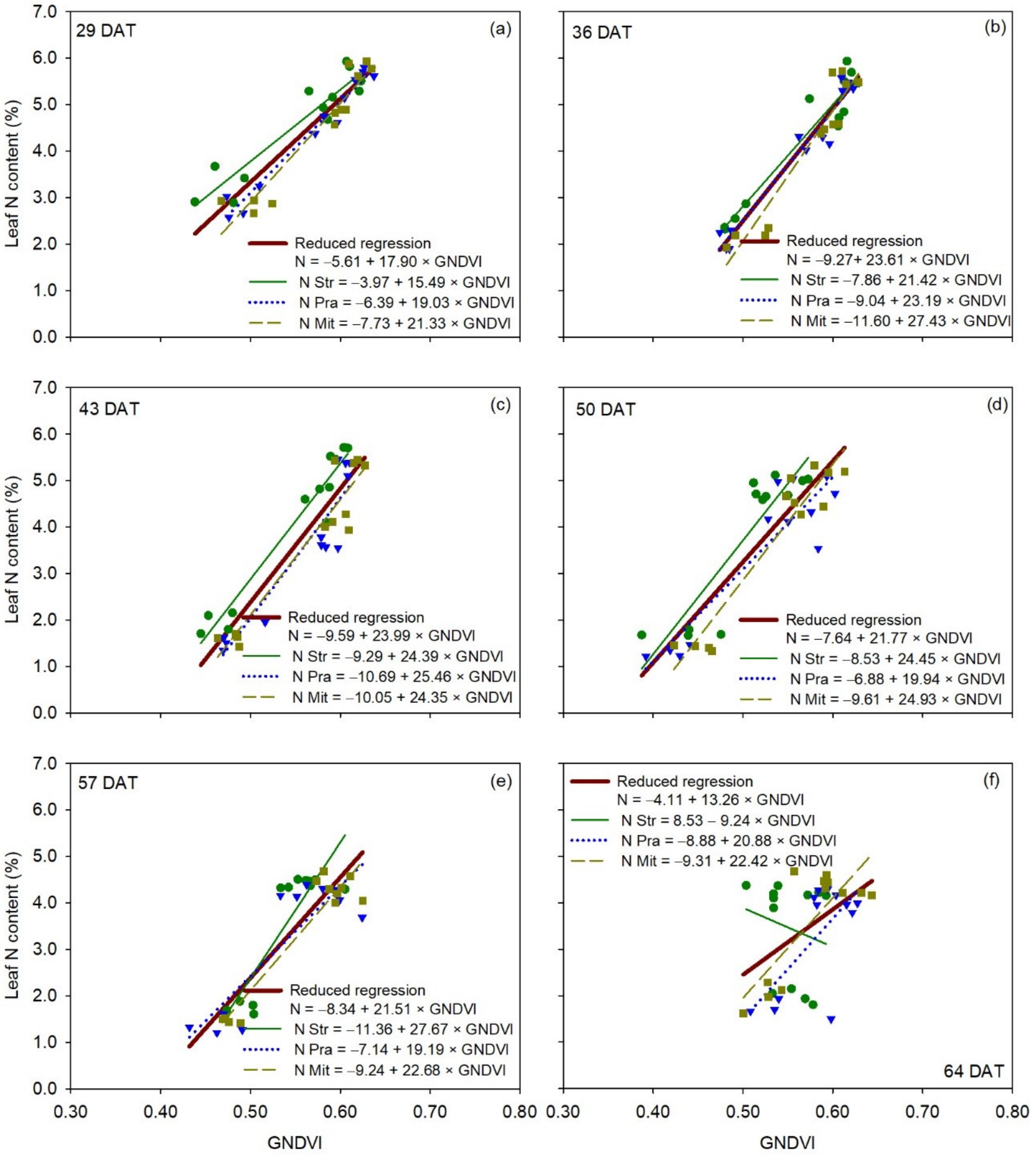

Figure S4. Linear regression between GNDVI measurements (independent variable) and leaf N content (dependent variable) for each cultivar of cucumber (*Cucumis sativus* L.), for each measurements date (Panels **a**-**f**). The reduced regression is a regression with data of all three cultivars together. DAT is days after transplanting. Str, ‘Strategos’, Pra, ‘Pradera’, Mit, ‘Mitre’.

9.2. Chapter three. Influence of time of day on measurement with chlorophyll meters and canopy reflectance sensors of different crop N status

9.2.1. Supplementary Tables

Table S1. Repeated-measures analysis of variance (RM-ANOVA) testing the effect of nitrogen treatment, time of day and date of measurement, on SPAD-502 units and CCI values in a sweet pepper crop grown in a greenhouse.

Effect	**df**	**SPAD**		**CCI**	
		F	**p**	**F**	**p**
Block	3	4.76	0.007	4.59	0.009
Nitrogen (N)	2	594.34	<0.001	557.47	<0.001
Time (T)	3	1.34	0.279	2.34	0.091
N x T	6	0.21	0.970	0.13	0.991
Error	33				
Date (D)	5	84.37	<0.001	91.46	<0.001
D x N	10	14.03	<0.001	24.65	<0.001
D x T	15	0.25	0.998	0.79	0.69
D x N x T	30	0.67	0.904	0.50	0.99
Error	165				

Table S2. Repeated-measures analysis of variance (RM-ANOVA) testing the effect of nitrogen treatment, time of day and date of measurement, on NDVI values, measured with the GreenSeeker handheld sensor and with the Crop Circle ACS-470 sensor, and GNDVI, RVI and GVI values, measured with the Crop Circle sensor, in a sweet pepper crop grown in a greenhouse.

Effect	df	NDVI Greenseeker		NDVI Crop Circle		GDVI Crop Circle		RVI Crop Circle		GVI Crop Circle	
		F	p	F	p	F	p	F	p		
Block	3	1.41	0.258	4.57	0.009	3.39	0.029	6.48	0.001	2.82	0.054
Nitrogen (N)	2	394.88	<0.001	241.61	<0.001	414.09	<0.001	776.49	<0.001	746.10	<0.001
Time (T)	3	0.47	0.702	0.21	0.889	1.14	0.346	0.49	0.690	2.92	0.050
N x T	6	0.45	0.842	0.09	0.997	0.11	0.994	0.47	0.829	0.20	0.975
Error	33										
Date (D)	5	571.22	<0.001	896.27	<0.001	1032.33	<0.001	1743.17	<0.001	1083.71	<0.001
D x N	10	113.66	<0.001	67.53	<0.001	43.22	<0.001	39.90	<0.001	40.61	<0.001
D x T	15	1.05	0.407	1.07	0.389	0.93	0.521	2.49	0.006	1.01	0.444
D x N x T	30	0.64	0.923	0.52	0.969	0.51	0.971	0.84	0.682	0.71	0.839
Error	165										

10. List of Tables and Figures

10.1. List of tables

Chapter one: The use of chlorophyll meters to assess crop N status and derivation of sufficiency values for sweet pepper

Chapter two: Effect of cultivar on chlorophyll meter and canopy reflectance measurements in cucumber

Chapter three: Influence of time of day on measurement with chlorophyll meters and canopy reflectance sensors of different crop N status

10.2. List of figures

Chapter one: The use of chlorophyll meters to assess crop N status and derivation of sufficiency values for sweet pepper

Chapter two: Effect of cultivar on chlorophyll meter and canopy reflectance measurements in cucumber

Chapter three: Influence of time of day on measurement with chlorophyll meters and canopy reflectance sensors of different crop N status