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Characterization of normalized difference vegetation index of eight poinsettia (*Euphorbia pulcherrima* L.) cultivars during bract color development

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Abstract

Normalized difference vegetative index (NDVI) values are vegetative indices, calculated from active canopy sensor measurements using the reflectance values for red and near-infrared. Use of NDVI sensors offers the possibility of rapid, non-destructive readings that correlate with plant properties such as plant biomass and plant nutrition when plants are green, however, the affects of color on NDVI sensor readings has not be investigated. Poinsettia cultivars 'Enduring Marble', 'Ice Punch', 'Winter Rose Early Red', 'Prestige Red', 'Prestige Maroon', 'Peterstar White', 'Maren', and 'Orange Spice' were analyzed by an NDVI sensor starting four weeks after transplanting and through bract color development. The results indicated that cultivar effect, time effect and the interaction of cultivar and time on NDVI value were significant ($P < 0.0001$). An increase of NDVI value occurred from initial measuring date, and reached the greatest value (ranged from 0.718 to 0.837) between week 6 and week 7 for all cultivars except 'Orange Spice' and 'Winter Rose Early Red'. From the peak readings, all NDVI values in tested cultivars declined significantly, especially after bract coloration. This result demonstrated an inverse relationship with increased bract coloration and reduced NDVI readings, so use of NDVI readings to detect N deficiencies should be before bract coloration in poinsettias. However, the NDVI sensor could be used to monitor bract color development. Because the effects of cultivar, time, and the interaction between cultivar and time were significant, standards using a pocket NDVI sensor in specific cultivars during determined growth stages may need to be established at each growing facility.

Key words: *Euphorbia pulcherrima*, NDVI, bract color, greenhouse

Introduction

Poinsettia (*Euphorbia pulcherrima* L.) is one of the most important ornamental flowering plants in the United States. Greenhouse production of colorful (red, marble, pink, white, orange, and multi-colored) poinsettia cultivars occurs in the fall and plants are usually scheduled for sale in early December (Kannangara and Hansson, 1998). Fertilization, particularly nitrogen (N), is an important component to meet production standards and scheduling. Therefore, in recent years, N recommendations have been reevaluated to meet the needs of the crop for both economic and environmental concerns (Turner and Jund, 1994).

Since N is a dominant nutrient affecting plant chlorophyll content (Moorby and Besford, 1983), measuring chlorophyll content can be a useful index to assess the growth status and leaf N level of a plant (Seemann *et al.*, 1987). It was found that vegetative indices based on reciprocal reflectance in narrow spectral bands at 550 nm and 700 nm from near distance by reflectance spectroscopy are directly proportional to leaf chlorophyll content for a variety of plants without anthocyanin pigmentation (Gitelson and Merzlyak, 1997). Ground-based remote sensing of plant chlorophyll content and concentration offers the possibility to rapidly estimate crop N status and plant quality, under field and greenhouse production. Because the reflectance and absorption in green from 530 to 590 nm and in the red edge wavelength around 700 nm are more sensitive to moderate to high chlorophyll content, many mathematical indices have been developed according to these spectral bands and used to estimate chlorophyll content in the leaves of various plant species (Gitelson and Merzlyak, 1996; Gitelson *et al.*, 2003). A Normalized Difference Vegetation Index (NDVI) is one of these indices, which typically uses two different sets of wavelengths of light, the red light, generally between 660 to 720 nm, is a 'index wavelength' and strongly absorbed by chlorophyll; the near-infrared light, typically between 750 to 900 nm, is a 'reference wavelength' that is used to adjust for differences in leaf structure. Since most of the hand-held chlorophyll meters are based on a single index value, they are designed or determined to be sensitive to desired plant attributes and functions (*i.e.*, color, biomass, N status, and fluorescence) using specific wavelengths. Compared with chlorophyll meters, estimation of other pigments like carotenoid and anthocyanin contents remains more difficult (Sims and Gamon, 2002).

GreenSeeker™ technology is portable and uses an active lighting optical sensor with high intensity light emitting diodes (LED's) that emit light at 660 nm (red) and 780 nm (NIR) wavelengths. This technology is widely accepted in large scale agronomic field production of corn (*Zea mays* L.), wheat (*Triticum aestivum* L.), and cotton (*Gossypium hirsutum* L.) (Carrillo, 2006; Clay *et al.*, 2006; Eitel *et al.*, 2008), and has been investigated in turfgrass (Baghzouz *et al.*, 2007; Bell *et al.*, 2004; Xiong *et al.*, 2007). Recently, a prototype handheld pocket sensor for NDVI measurements was developed at Oklahoma State University (Crain *et al.*, 2012). The prototype handheld pocket sensor was developed to provide similar readings at a tenth of the cost of the original GreenSeeker™ technology, and can be used to easily measure a single plant or multiple plants in a greenhouse setting. Wang *et al.* (2012a, 2012b) used the prototype handheld NDVI sensor, to show that NDVI values can be used to estimate N status in geraniums when at a non-flowering state. Martin *et al.* (2007) and Raun *et al.* (2005) reported reduced NDVI values associated with yellowing of corn tassels, however, there has been little work performed using indices such as NDVI on crops or plants which are not completely green in nature. In this experiment, NDVI values were measured weekly for eight poinsettia cultivars developing various bract colors. The objective of this study was to investigate the effects of different bract coloration changes on NDVI values to see if color affects NDVI readings.

Materials and methods

Plant material: Three early cultivars including 'Enduring Marble', 'Ice Punch' and 'Winter Rose Early Red', and three mid season cultivars 'Prestige Red', 'PeterStar White' and 'Maren' poinsettia cuttings were received from Esbenshade's Greenhouse, Inc. (Fleetwood, PA) on 17 August 2011. One mid season cultivar 'Prestige Maroon' and one late season cultivar 'Orange Spice' poinsettia cuttings were received from Paul Ecke Ranch (Encinitas, CA) on 16 August 2011. Based on the descriptions of Paul Ecke Ranch poinsettia products, cultivars were classified as early (8-8.5 weeks for bract coloration), mid (8.5-9 weeks for bract coloration) and late (9-9.5 weeks for bract coloration) season groups. All cuttings were rooted in wedge-shaped Oasis® foam plugs prior to shipping, and were placed under intermittent mist until transplanting. Cuttings were transplanted into standard 15.24 cm Elite Azalea Pots (ITML Horticultural Products, Middlefield, OH) filled with Metro Mix 702 media (SunGro Horticulture Distribution Inc., Bellevue, WA) on 24 August 2011. Poinsettias were grown in the Oklahoma State University teaching greenhouses in Stillwater, and were spaced 30.5 cm apart.

Growth conditions: No supplemental lighting was used, but the plants received a photosynthetic photon flux density (PPFD) range of 500-900 μmol m^{-2} s^{-1} sunlight at noon. Greenhouse temperatures were set at 21°C during the day and 16 °C during the night. Plants were watered as needed with non-pressure compensated drip emitters (Chapin Watermatics Inc. Watertown, NY) until water ran through the pots allowing ~20% leaching. Plants were fertigated at each watering with 200 mg L^{-1} 20N-4.4P-16.6K (Jack's Professional® General Purpose acidic fertilizer, J.R. Peters Inc., Allentown, PA). On 14 September 2011, all poinsettias were pinched to six nodes. The pH and EC of the growing media were recorded weekly using the pour through method and averaged 6.20 and 1.9 dS m^{-1}, respectively. On 22 September 2011, cultivars were treated with 902 g magnesium sulfate, 114 g Soluble Trace Element Mix (J.R. Peters Inc., Allentown, PA) and 0.5 g molybdenum. TriStar WPS 70 (Cleary Chemicals Company, Dayton, NJ) and Banrot® (The Scotts Company, Maysville, OH) were applied on 9 September 2011 and again six weeks later. Poinsettias were treated with a mixture of 2 g L^{-1} calcium chloride mixed with 30 mL of a Spread-It (Parkway Research Corp., Houston, TX) sticker spreader occurring weekly starting on 17 November 2011.

Data collection: Starting on 24 September 2011, 30 individual plants per cultivar were scanned for NDVI readings weekly (total of 10 repeated-reading dates) using a prototype NDVI pocket sensor (Crain *et al.*, 2012). The prototype NDVI pocket sensor calculates NDVI values using red light at 660 nm (Red) and near infrared at 780 nm (NIR), both spectral bands characterized by ~25 nm Full Width at Half Maximum (FWHM). During measurements, the sensor was placed 45 cm above the plant canopy, giving the sensor a circular field of view with a diameter of ~11.85 cm. Flower bract color was noted for each cultivar. Percentage of canopy bract coloration was recorded using the Ecke Poinsettia Bract Meter (http://www.ecke.com/poinsettias/bractmeter/) as a reference.

Data processing and analysis: The curves for the mean NDVI values of each cultivar across 10 repeated-measures dates were graphed using Microsoft Excel. The experimental design was one factor with 10 repeated-measures and was analyzed using multivariate analysis of variance (MANOVA) (von Ende, 2001). The sources of variation were divided into between-subjects (cultivar) and within-subject effects. The latter includes the within-subject main effect (time) and its interaction with the between-subjects factor (cultivar×time). Here the total number of samples (N) was 240, the number of between-subjects treatment levels (M) was eight and the number of dependent variable (measuring date, k) was 10, N-M far larger than k+9, this condition satisfied the constraints recommended by Maxwell and Delaney (1990) for doing MANOVA for one within-subject design. The multivariate comparisons of the NDVI values among eight cultivars for 10 weeks were followed by profile analysis with SAS procedures provided by von Ende (2001). Data was analyzed using SAS software (version 9.2; SAS Institute, Cary, NC).

Results and discussion

Fig. 1 showed changes of NDVI values for eight cultivars from week 4 (vegetative stage) to week 13 (bract color development). For 'Winter Rose Early Red', the highest NDVI value of 0.769 was measured initially on week 4 (24 September) due to a relatively larger leaves surface area and higher N concentration in the leaves then decreased thereafter while color development increased. An increase of NDVI values occurred from the initial measuring date, and reached the greatest value (ranging from 0.718 to 0.837) on week 6 (8 October) for other cultivars except 'Orange Spice'. 'Orange Spice' exhibited its highest NDVI value of 0.755 on week 7 (15 October), one week later than most of the tested cultivars.

By profile analysis of MANOVA over the 10 NDVI rating dates, the results indicated that time effect, and the interaction of cultivar and time on NDVI value were significant (P < 0.0001). A significant difference in the changes in the NDVI value due to cultivar occurred for each individual contrasts (Table 1). The NDVI values differed significantly among cultivars. Plant growth stage also affected NDVI significantly, and the NDVI rank of cultivars differed with plant growth stage at the time of measurement. Using orthogonal polynomial, only linear, quadratic, and cubic trends were analyzed. Both cultivar and

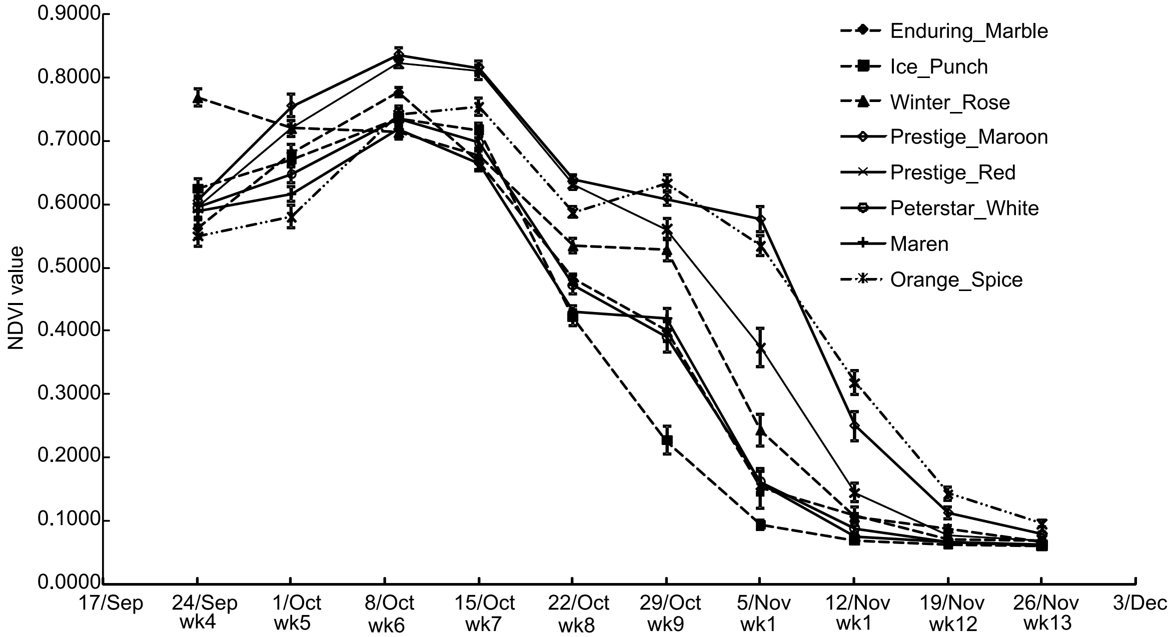

Fig. 1. Normalized difference vegetative index (NDVI) for eight poinsettia cultivars before and after bract coloration grown in Stillwater, OK in 2011

Table 1. MANOVA on each of the contrasts of within-subject factor over time for NDVI values collected on eight different poinsettia cultivars

Source	df	MS	*F*	*P>F*
Contrast variable: week 5 - week 4				
Mean	1	0.954	71.47	<.0001
Cultivar	7	0.127	9.54	<.0001
Error	232	0.013		
Contrast variable: week 6 - week 5				
Mean	1	1.782	221.43	<.0001
Cultivar	7	0.065	8.13	<.0001
Error	232	0.008		
Contrast variable: week 7 - week 6				
Mean	1	0.296	40.21	<.0001
Cultivar	7	0.040	5.49	<.0001
Error	232	0.007		
Contrast variable: week 8 - week 7				
Mean	1	9.667	1573.28	<.0001
Cultivar	7	0.071	11.61	<.0001
Error	232	0.006		
Contrast variable: week 9 - week 8				
Mean	1	0.680	59.42	<.0001
Cultivar	7	0.157	13.67	<.0001
Error	232	0.011		
Contrast variable: week 10 - week 9				
Mean	1	8.208	450.03	<.0001
Cultivar	7	0.235	12.90	<.0001
Error	232	0.018		
Contrast variable: week 11 - week 10				
Mean	1	4.811	315.96	<.0001
Cultivar	7	0.339	22.28	<.0001
Error	232	0.015		
Contrast variable: week 12 - week 11				
Mean	1	0.835	175.87	<.0001
Cultivar	7	0.123	25.90	<.0001
Error	232	0.005		
Contrast variable: week 13 - week 12				
Mean	1	0.059	53.98	<.0001
Cultivar	7	0.008	7.67	<.0001
Error	232	0.001		

time (mean) had significant linear, quadratic, and cubic responses of NDVI during the experiment (Table 2). Goreta *et al.* (2008) reported an increase and significant differences in plant canopy diameter among potted poinsettias cultivars after transplanting, which would explain the trends.

Under the same N application rate, NDVI ratings of most tested cultivars increased with increasing plant size in this study. In addition, NDVI value also changed with growth stage and plant canopy color. In previous studies in potted geranium (*Pelargonium* ×*hortorum* L.H. Bailey), NDVI ratings on a single pot increased substantially along with plant growth and expansion of plant size, and tended to detect variation in N status as the plant canopy reached adequate coverage (Wang *et al.*, 2012a, 2012b). Nitrogen requirement in poinsettias follow a pattern of low but increasing need in the vegetative growth stage, maximum in the inductive stage, and decreasing through bract development (Rose *et al.*, 1994; Scoggins and Mills (1998). The decreasing NDVI rating between week 7 and week 8 in this study coincided with N requirement and N content changes in poinsettias.

In this study, bract coloration began before or during week 8 for all cultivars except ‘Prestige Maroon’ and ‘Orange Spice’. In the early maturity group, bract coloration in ‘Ice Punch’ and ‘Winter

Table 2. Individual ANOVAs for first-, second- and third-order orthogonal polynomials for poinsettia NDVI values

Source	df	MS	*F*	*P>F*
Contrast variable: first order-linear model				
Mean	1	131.294	25994.8	<.0001
Cultivar	7	0.231	45.66	<.0001
Error	232	0.013		
Contrast variable: second order-quadratic				
Mean	1	9.751	1454.89	<.0001
Cultivar	7	0.607	90.53	<.0001
Error	232	0.008		
Contrast variable: third order-cubic				
Mean	1	12.697	2227.66	<.0001
Cultivar	7	0.211	37.10	<.0001
Error	232	0.006		

Table 3. Development of percent of colored bracts for tested poinsettia cultivars grown in Stillwater, OK in 2011

Cultivar	Bract color	Flower	Bract canopy color (%)[a]					
		maturity type	Oct. 22 wk8	Oct. 29 wk9	Nov. 5 wk10	Nov. 12 wk11	Nov. 19 wk12	Nov. 26 wk13
Enduring Marble	Yellow/pink	Early	5-10	15-20	30-40	50-60	70-80	85-95
Ice Punch	Pink/white	Early	30-40	50-60	70-80	80-90	95-100	95-100
Winter Rose Early Red	Red	Early	20-30	40-50	70-80	80-90	95-100	100
Prestige Maroon	Dark red	Mid	0	5-10	30-40	50-60	70-80	85-95
Prestige Red	Red	Mid	5-10	15-20	30-40	45-55	60-70	85-95
Peterstar White	White	Mid	10-20	30-40	50-60	60-70	70-80	85-95
Maren	Pink	Mid	5-10	15-20	30-40	50-60	70-80	85-95
Orange Spice	Orange	Late	0	0	5-10	10-15	20-30	40-60

[a]A single group value was reported at each recording date and based on Ecke Poinsettia Bract Meter™ based on percent of canopy showing color.

Rose Early Red' initiated earlier than 'Enduring Marble' (Table 3). In the mid maturity category, bract coloration of 'Prestige Maroon' changed slightly later than the other three cultivars in the same group. For all mid maturity cultivars and 'Enduring Marble', a cultivar in the early maturing group, 85 to 95% of complete bract coloration was present on the last measuring date (Table 3). Whereas, a late maturing cultivar, 'Orange Spice' showed a very late initiation of bract coloration and at most 60% of bracts showed coloration at the end of this experiment (Table 3). Results indicated that maturity classes can be subjective, and depends a lot on production practices. Staby and Kofranek (1979) also indicated that light and temperature can affect development and maturity in poinsettia.

When comparing bract coloration with NDVI, except for 'Winter Rose Early Red', a significant decrease in NDVI values occurred approximately one week earlier in the other seven tested cultivars. This may have been due to slight pigment changes on leaves unfolding or a decrease in chlorophyll content prior to first bract coloration. Rose *et al.* (1994) reported at bract development stage, the mean leaf N concentration was 5% under a constant N application rate of 200 mg L^{-1}, whereas N concentration in the bracts ranged between 3 to 4%.

Since effects of cultivar, time, and the interaction between cultivar and time were significant on indirect chlorophyll content measurements, standards for detecting nutrient deficiency using a pocket NDVI sensor in specific cultivars during determined growth stages may need to be established at each growing facility. The best time for detection of nutrient deficiencies using the pocket NDVI sensor would be in the vegetative stage, because as seen in Figure 1, any color other than green will cause NDVI values to decrease as color development increase ultimately affect the reliability of quantifying nutrient status in a plant. Although use of an NDVI sensor during flowering for nutrient studies would be limited, this study did show that an NDVI sensor may have applications for continuously monitoring bract color development in poinsettias.

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