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Use of optical sensors to monitor *Gaillardia* Foug. nitrogen status

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Abstract

Greenhouse production of *Gaillardia* is becoming increasingly popular for potted production due to growing interests in drought tolerant plant material. The objective of this study was to see if nondestructive handheld sensors could be used to monitor nitrogen (N) status in *Gaillardia aristata* 'Arizona Apricot'. Topdressed fertilizer treatments of 0, 4, 8, 10, or 12 g of controlled release fertilizer (CRF) 16N-3.9P-10K were added to greenhouse grown plants. Individual plants were scanned from 10 pots per treatment for Normalized Difference Vegetative Index (NDVI) and Soil-Plant Analyses Development (SPAD) over eight different sampling dates starting 7 days after fertilizer treatment application (DAT). Height, width, leaf N concentration, and number of panicles were also recorded. Linear, cubic, and quadratic trends were seen for NDVI and SPAD. Plant height was greatest in the 10 g treatment, but was not different than any other treatment. Plant width was greatest in the 12 g treatment, but was not different from the 4 g and 10 g treatments. Number of panicles was highest in the 12 g treatment, but was not different from the 10 g fertilizer treatment. Neither sensor showed correlations with leaf N concentration 7 DAT; however, the NDVI sensor showed the earliest correlation with leaf N concentration starting 14 DAT. Both sensors were correlated with each other at 35, 42, and 56 DAT. Results from this study indicate that 10 g CRF was sufficient for plant growth and flowering. Both sensors can be used to predict N status in potted *Gaillardia*; however, consistency in sample collection and sampling time may be necessary to correlate values with N status.

Key words: Blanket flower, fertilizer, plant quality, greenhouse, NDVI, SPAD

Introduction

The genus *Gaillardia* Foug., popularly known as blanket flower, is a member of the family Asteraceae and is native to much of the United States (Danielson, 2005). There are at least 13 species of blanket flower with some species having several varieties and cultivars (USDA, 2014). Several species, including *Gaillardia aristata* Pursh, are commonly utilized in native seed mixes for diversity and rehabilitation of disturbed native plant colonies (Winslow, 2011). *Gaillardia* is suitable for use as an ornamental wild flower in low maintenance or naturalistic landscapes because of early blooming, compact growth habit, exceptional branching, and heat and drought tolerance. The use of wild flower perennials in the landscape has increased in recent years, and to meet this demand nurseries have begun producing plants in container production (Derr, 1994). Proper fertilization, particularly nitrogen (N), is one of the decisive factors influencing growth and flowering of perennial plants cultivated in containers. Nitrogen deficiency reduces stem elongation, leaf area, leaf or canopy photosynthesis, dry matter accumulation, and leaf chlorophyll content (Bar-Tal *et al.,* 2001; Zhao *et al.,* 2003), while excessive application of N usually increases input cost and can reduce plant and water quality. Excessive fertilizer application in commercial greenhouses can result in unacceptably high N levels in the soil under greenhouses (McAvoy, 1994). Fertilizer runoff from greenhouses can contaminate ground and surface water (James and van Iersel, 2001). Therefore, the application of N based fertilizers on crops is critical.

To date, little work has been done on fertilization requirements for perennials (Kessler, 2013). Fertilization recommendation of *G. aristata*, based on results from trials conducted under Central European conditions, is 130 to 150 mg L^{-1} N weekly in the form of a complete balanced fertilizer with 3 kg m^3 controlled release fertilizer (CRF) in the substrate (Benary, 2013). In a plant propagation protocol, Wick *et al.* (2008) fertilized *G. aristata* plants with 20N-8.7P-16.6K constant liquid fertilizer (CLF) at 100 mg L^{-1} weekly until fall and with 10N-8.7P-16.6K CLF at 200 mg L^{-1} in the fall. However, these studies were not designed to identify the optimum N concentration for *G. aristata* production. Gadagi *et al.* (2004) reported that *Gaillardia pulchella* Foug., which is an annual or short-lived perennial species, requires a high level of N (150 kg ha^{-1}). Awchar *et al.* (2010) found that a higher dose of N (200 kg ha^{-1}) resulted in increased plant height, width, primary branching, flowering, and seed yield of *G. pulchella.* In a study by Shafi *et al.* (2002), *G. pulchella* was tallest in treatments having 30N-4.4P-8.3K g m^{-2}, had maximum number of flowers per plant with 20N-4.4P-8.3K g m^{-2} and showed maximum survival of 97% in plants treated with 25N-4.4P-8.3K g m^{-2}. GrowerFacts (2008) recommended that *Gaillardia* × *grandiflora* Van Houtte should be fertilized at 175 to 225 mg L^{-1} N using predominately nitrate-form fertilizer with low phosphorus and high potassium or 100 to 175 mg L^{-1} N for constant liquid feed (CLF). An alternative system would be to use controlled release fertilizers (CRF), which are considered more efficient for container production because they generally improve foliar color and plant size compared to CLF (Altland *et al.,* 2002; Hershey and

Paul, 1982), while reducing sudden loss of nutrients and runoff (Cox, 1985; Morvant *et al.,* 2001). Pilon (2005) stated that *G. aristata* 'Arizona Sun' requires light to moderate fertilization and grows well under constant CLF programs with rates of 50 to 100 mg L^{-1} nitrates with each watering or 150 to 200 mg L^{-1} at every other watering or CRF incorporated at a rate equivalent to 453.6 g of N per yard of growing medium.

Nitrogen requirement varies with plant age and type for ornamental plants grown in a greenhouse. Three types of monitoring approaches, plant-based, soil-based, and reflectance-based measurements are commonly used to diagnose N deficiency and determine N requirements of crops (Wang *et al.,* 2012b). Existing methods of soil and plant analysis can be time-consuming, slow, and expensive, thus having the ability to generate instant, nondestructive sampling techniques is of interest to growers (Link and Reusch, 2006). A Soil-Plant Analyses Development (SPAD) chlorophyll meter is a hand-held, self-calibrating, and nondestructive lightweight device used to calculate the amount of chlorophyll present in plant leaves. Meter records optical density measurement at 650 nm and 940 nm wavelengths, converts them into digital signals, and then into a SPAD value (Rodriguez and Miller, 2000). Leaf N and SPAD were found to be strongly correlated for several horticultural crops (Westerveld *et al.,* 2003; Zanin and Sambo, 2006), yet poorly correlated in other species (Martín *et al.,* 2007).

Use of vegetative indices technology like Normalized Difference Vegetation Index (NDVI) to estimate N status is widely used in large-scale agronomic field production, but has seen limited use in potted horticultural production in a greenhouse. Recently, Wang *et al.,* (2012a; 2012b) showed that NDVI and SPAD can be used to easily measure a single plant or multiple green-leafed geraniums in a greenhouse setting during the vegetative stage. The NDVI sensor produces a NDVI value using a self-illuminated (active sensor) light source in the red (660 nm) and near infrared wavelengths (780 nm). The objective of this study was to determine if SPAD and NDVI readings could be used to monitor N status in *Gaillardia* and to see if the sensors were correlated to leaf N and each other.

Materials and methods

Plant material and growth conditions: On 6 March 2012, 288 cell tray plugs (2 to 4 leaves) of *G. aristata* 'Arizona Apricot' were obtained from Park Seed Co. (Greenwood, SC). Plugs were transplanted into standard (15.2 cm diameter and 1.35 L volume) pots with ~0.35 kg Sun GR Metro-Mix media (Sun Gro Horticulture, Bellevue, WA) the next day. A single plant was placed in each pot and plants were grown in the Department of Horticulture and Landscape Architecture Research Greenhouses at Stillwater, OK under natural photoperiods. Temperature was set at 18°C/21°C day/night with a maximum photosynthetic photon flux density (PPFD) range of 450 to 1,400 μmol m^{-2} s^{-1}at 1200 HR. 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) during establishment. On 4 April, 2012, fertilizer treatments of 0, 4, 8, 10, or 12 g of 16N-3.9P-10K (Osmocote® Plus, The Scotts Co., Marysville, OH) were applied on the surface of each pot, and tap water was then used during irrigations. Pots were drip irrigated at a rate that allowed media saturation and ~20% leaching. Nitrogen treatments were designed to produce plants with N status ranging from deficient to excessive.

NDVI, SPAD, leaf N concentration and plant growth: Individual plants were scanned from 10 random pots per treatment for NDVI and SPAD every 7 or 14 days starting 7 days after fertilizer application (DAT). Developed panicles were removed 30 DAT. During measurements, the prototype NDVI sensor (Crain *et al.,* 2012) was placed 45 cm above the plant canopy, giving the sensor a circular field of view with a diameter of ~11.85 cm. For each pot, SPAD measurements were taken from four mature leaves from the middle to upper level of the plant using the middle of the leaf not including the midrib. Leaf foliar analysis consisted of collecting the leaves used for SPAD readings with 10 pots bulked per treatment for average total N concentration per treatment each week. At the last rating date, measurements on plant height (taken from the top of the pot to the highest vegetative point), width (average of two perpendicular measurements), and number of panicles were recorded.

Statistics: The experiment, consisting of five treatments was replicated 50 times with single pot replications, thereby giving a total of 250 pots. Pots were arranged in a completely randomized design (CRD). Continuous response variables of NDVI, SPAD, height, width, and number of panicles were analyzed using generalized linear mixed models methods. When significant, N means were compared using post-hoc least significant difference (LSD) methods additionally. Linear and quadratic trends in fertilizer rates and across time were evaluated for each response variable. Correlation analysis of fertilizer rate, NDVI, SPAD, and leaf N concentration was also computed. All tests of significance were performed at the ($P < 0.05$) level. Data analysis was generated using SAS/STAT software, Version 9.3 (SAS Institute Inc., Cary, NC).

Results and discussion

NDVI, SPAD, and leaf N concentration: NDVI values ranged from 0.5 to 0.8 and increased with increasing N rates, in agreement with Wang *et. al.* (2004), except at 7 DAT (Table 1). *Gaillardia* are generally low to medium nutrient requiring plants (Pilon, 2005), and thus plants may not have developed N deficiency during the early sampling dates due to being fertigated during establishment prior to the start of the study. Also, the NDVI sensor will show more accurate values after the plants canopy has reached an appropriate growth to completely cover the sensor field of view. As reported by Gao (1996), NDVI sensors are not able to remove background soil reflectance effects completely. With increasing N rates, linear, cubic, and quadratic trends for NDVI were seen (Table 1). Within sampling dates, a cubic trend was seen at 21 DAT, a quadratic trend at 56 DAT, and all other sampling dates showed linear trends. This was consistent with Wang *et al.* (2012a) who reported linear trends for NDVI values in geranium. Across sampling times, the linear and quadratic trends were significant except for the 4 g fertilizer treatment, which could be attributed to N utilization. Smith (1986) states that nutritional status influences both distribution and remobilization of nutrients, which would therefore subsequently affect nutrient content and growth. At 14 DAT, NDVI values were not significantly different among plants receiving N fertilizer, but

Table 1. Normalized Difference Vegetation Index (NDVI) measurements on *Gaillardia* 'Arizona Apricot' with different rates of fertilizer at eight dates after fertilizer treatment (DAT) using a controlled release fertilizer

Fertilizer rate (g)	7 DAT	14 DAT	21 DAT	28 DAT	35 DAT	42 DAT	56 DAT	70 DAT	
0	0.635a[z]	0.612b	0.606b	0.632c	0.611d	0.548c	0.499c	0.564d	L**[y]
4	0.604a	0.629a	0.598b	0.701b	0.700bc	0.661b	0.687b	0.650c	NS
8	0.618a	0.668a	0.715a	0.739ab	0.773ab	0.758a	0.759ab	0.754a	L**Q**
10	0.659a	0.650a	0.727a	0.774a	0.766ab	0.723ab	0.702ab	0.738b	Q*
12	0.612a	0.663a	0.727a	0.756ab	0.793a	0.747ab	0.764a	0.820a	L***Q*
	-------	L*[x]	L***C*	L***	L***	L***	L***Q**	L***	

[z]Mean separation within columns using Fisher's protected least significant difference at $P \leq 0.05$. Means (n=10) within a column with the same letter are not significantly different from one another.
[y]NS,*, **, ***Linear (L), or quadratic (Q) response across weeks either not significant, $P \leq 0.05$, $P \leq 0.001$, or $P \leq 0.0001$, respectively.
[x]NS,*, **, ***Linear (L), or quadratic (Q), or cubic (C) response within sampling dates either not significant, $P \leq 0.05$, $P \leq 0.001$, or $P \leq 0.0001$, respectively.

were different from the control. At 70 DAT, 8 g and 12 g NDVI values were higher than the 10 g treatment, which could have been the result of data collection at height above the canopy can affect readings or nutrient uptake and utilization.

SPAD values ranged from 27.1 to 54.3 and increased progressively with N rates, are in agreement with Wang *et al.* (2012a; 2012b), Zhu *et al.* (2012), Turner and Jund (1994), and Piekielek and Fox (1992). Values showed linear and cubic trends within sampling dates except for 14 DAT (Table 2). Schepers *et al*. (1992) and Tremblay *et al*. (2011) reported that chlorophyll reading reach a plateau at moderate N supply, and thus had adequate nutrient content from fertilizer applied during establishment. SPAD values showed linear responses for all treatments across sampling dates except 8 g, which showed a quadratic response across sampling dates. This was consistent with Wang *et al.* (2012a) who also noted quadratic responses for SPAD values in geranium. Soil-Plant Analyses Development values were able to differentiate fertilizer treatments clearly as early as 28 DAT (Table 2). Murdock *et al.* (1997) also showed that N added prior to SPAD readings took around three weeks to fully affect chlorophyll development and corresponding SPAD readings in wheat (*Triticum aestivum* L.).

Leaf N ranged from 1.2% to 4.6% of dry leaf mass across all samples (Table 3). Leaf N concentration generally increased in response to N fertilizer rates and decreased over time (Table 3). The decrease in leaf N over time or with increasing biomass can be attributed to a decrease in total plant N associated with photosynthesis in relation to the need of N for structural and storage constituents (Bélanger and Gastal, 2000).

Plant height, plant width, and number of panicles: Plant height was not influenced by N fertilizer rates (Table 4). Plants receiving 0 g N had reduced widths compared to any treatment with N. The 12 g treatment produced the largest widths, but the difference was not significant than the 4 or 10 g treatment. Plants receiving 12 g N had the greatest number of panicles, but did not differ significantly from plants receiving 4 g or 10 g N (Table 4). The fact that leaf area, N supply, and production of panicles are all related, supports work by Reis *et al.* (2014) that found that N supply can affect plant growth and productivity by altering leaf area and photosynthetic rates. Based on height, width, and number

Table 2. Readings of a SPAD meter on *Gaillardia* 'Arizona Apricot' with different rates of fertilizer at eight dates after fertilizer treatment (DAT) using a controlled release fertilizer

Fertilizer rate (g)	7 DAT	14 DAT	21 DAT	28 DAT	35 DAT	42 DAT	56 DAT	70 DAT	
0	50.42a[z]	48.45b	40.49c	41.52d	39.60d	40.98b	27.13d	28.93c	L***[y]
4	49.61a	48.24b	48.27b	46.16c	47.97bc	47.17ab	43.14c	39.47b	L**
8	51.28a	54.29a	52.44ab	50.70ab	51.56ab	47.34ab	45.69bc	37.23b	L***Q*
10	52.79a	50.11ab	52.16ab	47.30bc	50.52ab	50.01a	42.21c	42.07b	L***
12	52.68a	50.51ab	53.93a	52.51a	54.27a	51.90a	50.51ab	45.11ab	L***
	-------	NS[x]	L***	L***	L***	L**	L***C*	L**	

[z]Mean separation within columns using Fisher's protected least significant difference at $P \leq 0.05$. Means (n=10) within a column with the same letter are not significantly different from one another.
[y]NS,*, **, ***Linear (L) or quadratic (Q) response across weeks either not significant, $P \leq 0.05$, $P \leq 0.001$, or $P \leq 0.0001$, respectively.
[x]NS,*, **, ***Linear (L), or quadratic (Q), or cubic (C) response within sampling dates either not significant, $P \leq 0.05$, $P \leq 0.001$, or $P \leq 0.0001$, respectively.

Table 3. Leaf nitrogen concentration for *Gaillardia* 'Arizona Apricot' reported as (g kg^{-1} DM) for eight dates after fertilizer treatment (DAT) using a controlled release fertilizer

Fertilizer rate (g)	7 DAT	14 DAT	21 DAT	28 DAT	35 DAT	42 DAT	56 DAT	70 DAT
0	39.8[z]	26.4	22.7	24.8	20.8	16.2	12.1	12.3
4	39.7	27.7	29.0	26.1	23.0	22.4	16.5	16.0
8	41.8	34.4	33.3	33.3	28.2	25.8	23.5	26.6
10	41.8	33.8	35.2	29.4	32.5	28.6	21.8	25.8
12	45.6	36.8	36.2	34.1	29.6	30.1	25.3	25.9

[z]Values are from combining five mature leaves and no petioles from each of 10 different pots per treatment for a single composite sample.

Table 4. Response of *Gaillardia* 'Arizona Apricot' to five fertilizer rates of controlled release fertilizer 70 days after initial fertilizer treatment (DAT)

Fertilizer rate (g)	Height (cm)	Width (cm)	Number of panicles
0	29.26a[z]	19.56c	2.12c
4	30.53a	24.62ab	2.05c
8	32.30a	23.35b	2.35bc
10	32.35a	24.30ab	2.51ab
12	30.01a	25.13a	2.62a

[z]Mean separation within columns using Fisher's protected least significant difference at $P \leq 0.05$. Means (n=10) within a column with the same letter are not significantly different from one another.

of panicles, 10 g CRF performed the best, which is a similar rate used by Derr (1994) in *G. aristata* grown in 4 L pots fertilized with 17N-6P-12K at 9 g per pot.

Relationships between sensors, fertilizer rates, and leaf N: There was a positive correlation between N fertilizer rates and leaf N concentration as well as between NDVI and leaf N concentration at all sampling dates except at 7 DAT and 28 DAT (Table 5). This can likely be explained by soil reflectance at 7 DAT before the plants filled the pot, and color reflectance from developing flowers that were removed at 30 DAT. Across all sampling dates, the relationship between leaf N and NDVI values was not significant ($P < 0.05$), while there was a correlation between N rates and NDVI (r = 0.728). Correlation between SPAD and leaf N concentration was significant at 21, 28, 42, and 56 DAT. Neilsen *et al*. (1995) reported that sampling time affected, whether or not SPAD reading were correlated with leaf N concentration in apples (*Malus domestica* Mill.). Readings for NDVI were correlated to SPAD only at 35, 42, and 56 DAT. The relationship between SPAD and leaf N concentration or N rates was strong though not always significant at each sampling dates; however, when combined across all sampling dates the relationships between SPAD and leaf N or N rates were significant at $P \leq 0.001$ (r = 0.798 and r = 0.604, respectively). Wang *et al.* (2012b) also reported significant correlations between NDVI and SPAD when combined across all sampling dates for two different geranium cultivars.

Results indicate that both sensors can be used to correlate values with N status in *Gaillardia* depending on sampling time. Initially, SPAD and NDVI readings were low during the vegetative stage, and increased during the flowering stage at 35 DAT, then decreased thereafter. Sandoval-Villa *et al.* (2002) reported a similar trend for greenhouse grown tomatoes using a SPAD chlorophyll meter. Although NDVI and SPAD values were correlated with leaf N on most sampling dates, factors other than leaf N can affect plant growth and quality. Growers should establish critical SPAD and NDVI values under specific environmental conditions, growth stages, planting dates, and fertilizer source (Takebe and Yoneyama, 1989; Fontes and de Araujo, 2006; Tremblay *et al.,* 2011; Wang *et al.,* 2012b). If not used for in season fertilizer recommendations, these sensors could also be used to track crop production consistency from one year to the next. The 10 g CRF was sufficient for growth and flowering in this study, but to develop fertilization guidelines, further research is needed evaluating different production practices and additional cultivars.

Table 5. Coefficients of determination (R^2) matrix for measured sensor parameters, fertilizer rate, and leaf nitrogen concentration for *Gaillardia* 'Arizona Apricot'

	NDVI	SPAD	Leaf N (g kg^{-1} DM)
	7 DAT		
Fertilizer Rate (g)	0.053[z]	0.845	0.852
NDVI		0.492	-0.113
SPAD			0.795
	14 DAT		
Fertilizer Rate (g)	0.897*	0.504	0.961**
NDVI		0.821	0.949*
SPAD			0.670
	21 DAT		
Fertilizer Rate (g)	0.914*	0.953*	0.987**
NDVI		0.834	0.890*
SPAD			0.984**
	28 DAT		
Fertilizer Rate (g)	0.953*	0.896*	0.869
NDVI		0.813	0.756
SPAD			0.955*
	35 DAT		
Fertilizer Rate (g)	0.968**	0.945*	0.926*
NDVI		0.985**	0.886*
SPAD			0.808
	42 DAT		
Fertilizer Rate (g)	0.917*	0.954*	0.993***
NDVI		0.880*	0.929*
SPAD			0.976**
	56 DAT		
Fertilizer Rate (g)	0.872	0.871	0.965**
NDVI		0.980**	0.924*
SPAD			0.900*
	70 DAT		
Fertilizer Rate (g)	0.978**	0.902*	0.938*
NDVI		0.865	0.938*
SPAD			0.736

[z]Representing Pearson's correlation coefficient (r). *, **, *** significance at $P \leq 0.05$, $P \leq 0.01$, or $P \leq 0.001$ level, respectively.

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