Rapid Determination of Nitrogen Status in Annual Vinca[1]

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Abstract

Three experiments were conducted to develop a method for rapid determination of nitrogen (N) status in 'Pacifica White' vinca (*Catharanthus roseus* (L.) G. Don). In experiment 1, N was applied to potted vinca at 40, 80, 120, or 160 ppm N to produce plants ranging from N deficient to N sufficient. Data were collected 14, 28, and 42 days after treatment (DAT) and included flower number, growth index [(height + width + width) ÷ 3], and from recently matured foliage the following parameters: SPAD-502 readings, petiole sap nitrate (SN) concentration, and total-N (percent of dry weight) (FN). Experiment 2 was similar with the exception that N rates applied were 30, 60, 90, 120, 150, or 180 ppm N, and data were collected 14, 29, and 42 DAT. In experiment 3, N was applied at 0, 90, or 180 ppm N and data were collected 0, 4, 7, 10, and 14 DAT. In experiments 1 and 2, SPAD readings were poorly correlated to FN (correlation coefficients ranged from 0.35 to 0.88) and the relationship between FN and SPAD readings changed at different collection dates. Petiole sap nitrate concentration determined by a Cardy nitrate meter was highly correlated throughout the three experiments. Above 380 ppm SN, the relationship between FN and SN was determined to be: SN/1000 = 2.3 × FN – 4.8 (r^2 = 0.73, n = 104). In experiment 3, FN and SN readings were able to detect N deficiency in vinca by 4 DAT, despite lack of visual symptoms until 14 DAT. SPAD readings were not suitable for predicting FN and plant N status. SN was a reliable predictor of FN and thus could be used to rapidly determine plant N status.

Index words: nitrate, landscape, bedding plants, Cardy nitrate meter, nutritional monitoring, plant diagnosis.

Significance to the Nursery Industry

One method for increasing nitrogen (N) use efficiency (NUE) is to apply fertilizers based on plant need. By improving NUE, landscape professionals can reduce N leaching. Applying fertilizer based on plant need requires a method for rapid determination of plant N status. Currently, the most reliable method for determining plant N status is by foliar analysis in a laboratory. This method may take one to two weeks to obtain results and thus does not allow for immediate response to plant need. Data herein indicate that foliar analysis of vinca is highly correlated to sap nitrate levels determined rapidly with the Cardy nitrate meter (Horiba Ltd., Kyoto, Japan). Foliar N (FN) (percent of dry weight) determined with a LECO CN 2000 (LECO Corp., St. Joseph, MI) can be predicted by sap nitrate levels (SN) determined with a Cardy nitrate meter. FN sufficiency range for vinca is 3 to 5%, based on these values, SN sufficiency range for optimal vinca growth would be 2100 to 6700 ppm NO_3^-. Landscape professionals can use SN readings to rapidly and accurately diagnose the N status of their crop, instead of sending samples to a laboratory for analysis. Rapidly determining plant N status will allow landscape professionals to more accurately apply N based on plant need thus improving NUE and minimizing N loss.

Introduction

Nitrogen (N) has several fates when applied to the landscape. Nitrogen use efficiency (NUE) is the percentage of applied N absorbed by the plant. Nitrogen can also be immobilized, volatilized, or leached from plant roots and potentially into groundwater (14). Nitrate-N is mobile in soil, but even when applied as ammonium-N (or urea-N), nitrification to nitrate-N is rapid and thus N is still prone to leaching.

Nitrogen leaching into groundwater is a major concern, and has prompted many researchers to develop methods of fertilization that reduce N leaching and maximize NUE. Research with agronomic crops has shown that several methods of fertilization reduce N leaching including use of controlled-release fertilizers (CRFs) (6, 7), split application of fertilizers (25), and reduced irrigation rates (27). These are all practices that can be adapted to landscape bedding plants, although research is necessary to develop best management practices (BMPs) specific to conditions in urban landscapes that address which CRF, irrigation method, and fertilizer timing scheme minimize N loss. While this would provide landscape professionals and homeowners valuable information for reducing N contamination of groundwater, it is difficult to develop an all-inclusive fertilizer scheme that works for the vastly different soil types, slopes, mulches, irrigation systems, and bedding plant species encountered in urban landscapes.

Applying fertilizer based on soil N levels has been shown to be effective in reducing N leaching. Sogbedji et al. (26) reported that N applied based on a pre-sidedress nitrate test resulted in reduced N leaching while maintaining corn (*Zea mays*) yields. However, soil analysis requires samples to be sent to laboratories for analysis, a timely procedure (? 1 to 2 weeks). Slow turnaround times for receiving results does not allow for rapid diagnosis and immediate response to plant need.

Applying N based on some measured plant response could also increase NUE, improve plant growth, and reduce N leaching. Coltman (4) reported that applying N to tomato (*Lycopersicon esculentum*) based on maintenance of plant sap nitrate levels resulted in less N use over the growing season, thus improving plant NUE. Peng et al. (21) demonstrated that fertilizer management in rice (*Oryza sativa*) based on chlorophyll meter (SPAD) readings was able to produce similar yields with lower N rates and greater NUE. Simi-

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larly, Blackmer and Schepers (3) showed SPAD meters were able to detect N deficiency in corn, and supplemental N applications based on SPAD readings were able to prevent yield losses. Rose et al. (23) reported that poinsettia (*Euphorbia pulcherrima*) fertilized with a constant rate (liquid feed) compared to a variable rate (applied N was linked to N accumulation pattern of the crop) resulted in plants with similar shoot N concentrations, size, and quality, although plants fertilized with the variable N rate required 41% less in N applications, and thus had greater N recovery rates. Magdoff et al. (13) demonstrated that corn had a greater probability of response to N fertilizer additions if decisions were based on nitrate tests compared to the traditional recommendation based on estimates derived from cropping history, yield goal, soil type, and manure management information provided by farmers. In addition, Magdoff et al. (13) indicated that for this methodology to work effectively, rapid analysis or turnaround time in the laboratory was needed. A method adaptable for use by landscape professionals for determining N status in landscape plants would allow more efficient fertilizer use and less N loss in container production and landscapes.

Nitrate concentration in plant sap as a measure of plant N status has been shown to be reliable for many crops. The Cardy nitrate meter (Horiba Ltd., Kyoto, Japan) is portable and can rapidly determine nitrate concentration in plant sap over a range of 1 to 10,000 ppm nitrate. This methodology has been evaluated and proven reliable for use in pansy (*Viola wittrockiana*) (2), potato (*Solanum tuberosum*) (24), cauliflower (*Brassica oleracea* Botrytis Group) (12), broccoli (*Brassica oleracea* Italica Group) (11), and cereal forages (5).

A procedure that allows for rapid determination of N status in landscape bedding plants would enable landscape professionals to make more accurate fertilizer applications based on plant need. This could improve NUE, and thus minimize N leaching. Therefore, the objective of this study was to evaluate the Cardy nitrate meter for rapid determination of N status in annual vinca (*Catharanthus roseus*).

Material and Methods

Experiment 1. 'Pacifica White' vinca were transplanted January 8, 2001, from 48-cell packs into 20 cm (8 in) azalea pots with a pinebark:peat (3:1 by vol) medium amended per cu m (cu yd) with 3.0 kg (5 lb) dolomitic limestone and 0.9 kg (1.5 lb) Micromax (Scotts Co., Marysville, OH) micronutrient fertilizer. Annual vinca was used because of its popularity, ranking 10th in bedding plants sold in 1999 (18). Plants were grown in a double layer polyethylene covered greenhouse under natural photoperiod and irradiance, and heater/vent set points of 24/29C (75/85F). With two of every three irrigation events, plants were fertigated with a solution containing 160 ppm N from ammonium nitrate (NH_4NO_3), 62 ppm phosphorus (P) and 150 ppm potassium (K) from potassium phosphate (K_2HPO_4). Plants were watered with tap water at every third irrigation. On February 8, 2001, uniform plants about 12 cm (4.7 in) tall and 21 cm (8.3 in) wide were selected from a larger group for use in the experiment. Plants were fertilized with 62 ppm P, 150 ppm K, and either 50, 100, 150, or 200 ppm N using salts and frequencies mentioned previously. Nitrogen treatments were designed to produce plants with N status ranging from deficient to excessive. Fertilizer salts were mixed in plastic containers and applied slop culture at a rate of about 500 ml (16.7 oz) per container per irrigation event.

Six single plant replicates were destructively harvested three separate dates (14, 23, and 42 days after initial treatment (DAT)). Plants were arranged in a completely randomized design. Data collected included flower number and growth index [(height + width + width) ÷ 3]. Recently matured foliage was used for SPAD-502 readings, petiole/midrib sap nitrate determination, and foliar N (percent dry weight basis). Because vinca petioles are small [1 to 2 mm in length (0.039 to 0.078 in)], 19.1 mm (0.75 in) of the midrib was used by removing the leaf blade (as recommended by the manufacturer). For petiole sap nitrate determination, petiole/midribs (hereafter referred to as petioles) of recently matured leaves were excised and placed into a modified garlic press (Spectrum Technologies, Plainfield, IL) and crushed to exude petiole sap. Sap was absorbed onto sample paper (Spectrum Technologies) which was placed onto the sensor of a Cardy nitrate meter. Recently matured foliage was also collected, rinsed with deionized water, dried 72 h in a forced air oven, and ground for laboratory determination of total-N (%) using a LECO CN 2000 carbon and N analyzer (LECO Corp., St. Joseph, MI). Other reports examining reliability of petiole sap nitrate tests for predicting N status of crops compared SN determined in the field to laboratory analysis of petiole nitrate concentration (dry weight basis) (8, 11, 24). In each instance the relationship was linear. This type of correlation is suitable for agronomic and/or vegetable crops for which yield has been calibrated to nitrate content of petioles,

Table 1. Response of 'Pacifica White' vinca to four N rates (Expt. 1).

N rate (ppm)	Flower no.	G.I.[z] (cm)	SPAD	SN[y] (ppm)	FN[x] (%)
			14 DAT[w]		
50	16	23.6	41.8	828	2.6
100	18	23.9	44.9	1370	2.8
150	21	25.2	46.2	3167	3.5
200	18	25.2	47.1	4367	3.8
	NS[v]	NS	L**	L***	L***
			28 DAT		
50	19	23.4	40.0	395	2.3
100	19	27.8	45.1	625	2.3
150	26	29.6	48.3	1590	2.7
200	26	27.2	47.7	2417	3.2
	NS	L*Q**	L***Q**	L***	L***
			42 DAT		
50	24	27.1	34.1	502	2.5
100	33	30.6	38.6	1117	2.8
150	44	31.3	42.4	2483	3.0
200	48	31.8	50.1	3450	3.3
	L***	L**	L***	L***	L**

[z]Growth index: (height + width + width) ÷ 3.

[y]Sap nitrate concentration.

[x]Foliar nitrogen (% of dry weight).

[w]Days after treatment.

[v]NS, *, **, or *** Nonsignificant, linear (L), or quadratic (Q) response at *P* ? 0.05, 0.01, or 0.001, respectively.

that is, SN levels determined in the field can be used to predict nitrate-N on a dry weight basis and thus plant N status. However, with landscape bedding plants, where comparatively less research has been done analyzing different plant parts for determining nutrient status, the most common and reliable measure of plant N status has been total-N determination of recently matured foliage (19). Therefore, correlations were made between petiole SN and total-N of recently matured foliage.

Experiment 2. Experiment 2 was conducted to validate results of experiment 1, and was conducted similarly with the following exceptions. Vinca were potted April 10, 2001, and the first fertilizer treatment was applied May 13, 2001, when plants were 14 cm (5.5 in) tall and 20 cm (8.9 in) wide. Plants were grown under natural photoperiod and irradiance, and the thermostat was set at heater/vent setpoints of 27/32C (80/90F) [although temperatures often reached as high as 32C (100F)]. Six rates of N were used: 30, 60, 90, 120, 150, or 180 ppm N. Plants were harvested 14, 29, and 42 DAT.

Experiment 3. The objective of this experiment was to determine how rapidly FN, SN, and SPAD levels from vinca respond to low N availability. It was conducted similarly to experiment 1, with the following exceptions. On June 17, 2001, plants were potted and fertilized with 180 ppm N, 62 ppm P, and 150 ppm K from salts mentioned previously. On July 6, 2001, and thereafter, plants were fertilized with either 0, 90, or 180 ppm N. Plants were harvested 0, 4, 7, 10, and 14 DAT. Plants were ? 26 cm (10 in) tall and 39 cm (15 in) wide at the time of initial treatment application.

Data for all experiments were analyzed with regression, non-linear regression, and correlation analysis.

Results and Discussion

Experiment 1. At 14 DAT, flower number and growth index were similar for all plants regardless of N rate (Table 1). SPAD meters measure foliar chlorophyll content, and thus plant 'greenness' (19). While regression indicated that chlorophyll content increased linearly with increasing N rate, differences were small and not visually apparent, that is, all plants appeared similar. FN and SN increased linearly with increasing N rate. FN sufficiency range for vinca is 3 to 5% (9), and throughout the rest of this paper, plants with FN values lower than these will be considered N deficient. While plants appeared similar, those receiving ? 100 ppm N were N deficient, indicating that FN can be used to diagnose N deficiency before the onset of visual symptoms. Other researchers have reported detection of N deficiency with an analytical measure of plant N content before the onset of visual symptoms (1, 10). SN also increased linearly with increasing N rate and was highly correlated to FN (r = 0.95) (Table 2), indicating SN could be used to predict FN, and thus N status in vinca. This has been demonstrated with other crops (2), although most work has correlated SN concentration of fresh tissue with nitrate-N on a dry weight basis (discussed previously).

By 28 DAT, plants had responded visually to N treatments. While there was no significant rate response, plants receiving ? 100 ppm N had fewer flowers than those receiving ? 150 ppm N (determined with contrast analysis, not presented), and growth index and SPAD readings increased quadratically with increasing N rate. FN and SN increased linearly

Table 2. Correlation matrix[z] for measured parameters of 'Pacifica White' vinca (Exp. 1).

	N Rate (ppm)	SPAD	SN[y] (ppm)	FN[x] (%)
	14 DAT[w]			
N Rate (ppm)	1.00			
SPAD	0.67	1.00		
SN (ppm)	0.91	0.61	1.00	
FN (%)	0.89	0.50	0.95	1.00
	28 DAT			
N Rate (ppm)	1.00			
SPAD	0.73	1.00		
SN (ppm)	0.85	0.60	1.00	
FN (%)	0.82	0.59	0.93	1.00
	42 DAT			
N Rate (ppm)	1.00			
SPAD	0.81	1.00		
SN (ppm)	0.90	0.58	1.00	
FN (%)	0.60	0.35	0.74	1.00

[z]Matrix of numbers representing Pearson's correlation coefficient (r) between row and column parameters.

[y]Sap nitrate concentration.

[x]Foliar nitrogen (% of dry weight).

[w]Days after treatment.

with increasing N rate, and again were highly correlated (r = 0.93). At 42 DAT, all measured parameters increased linearly with increasing N rate.

A plot of the data indicated a linear relationship between FN and SN (Fig. 1). The date at which the data were collected was entered into the model to determine if there were differences in response among the three collection dates. Regression analysis indicated the slope and intercept for the line were similar at all three dates, so the data were pooled for analysis. FN regressed against SN revealed the following equation for the line of best fit: SN/1000 = 2.4 × FN – 5.2 (r^2 = 0.82, n = 62) (Fig. 1). A 95% confidence interval for the slope of the line of best fit was [2.1, 2.7].

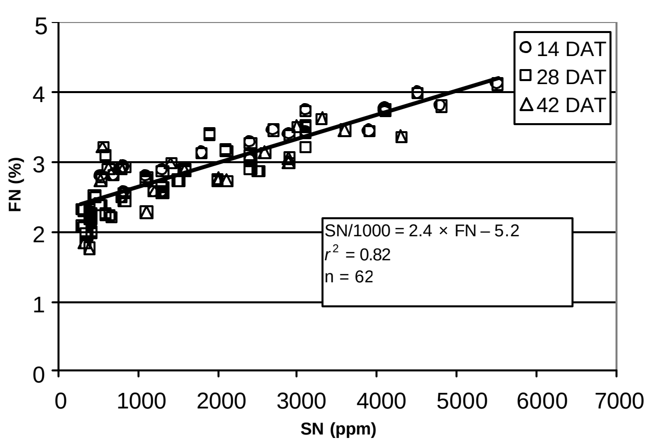

Fig. 1. Relationship between foliar nitrogen (FN) and petiole sap nitrate concentration (SN) of 'Pacifica White' vinca harvested at three dates (Expt. 1).

SPAD data were also regressed against FN to determine the nature of their relationship (Fig. 2). The relationship between FN and SPAD at 14 and 28 DAT was similar with a significant, though weak, relationship (r = 0.50 and 0.59, respectively). Although SPAD readings increased linearly (quadratically 28 DAT) with increasing N rate (Table 1), and thus could be used to indicate N deficiency, the relationship between SPAD and FN was not consistent enough to develop reliable calibrations for field use. At 42 DAT, there was no relationship between FN and SPAD readings (P = 0.34 for H_0: $?_1 = 0$). SPAD meters were unreliable predictors of FN throughout this experiment, and thus would not be useful for diagnosing N deficiency in vinca.

Experiment 2. Trends in plant response to N rate were similar to experiment 1, so for brevity, these data will not be presented. Because plants in experiment 2 received lower N rates compared to experiment 1 (30 to 180 ppm N compared to 50 to 200 ppm N, respectively), SN and FN levels were generally lower throughout the experiment. This provided additional information about the relationship between FN and SN towards the lower end of SN values (Fig. 3). There are two distinct regions on the graph, 1) below ? 340 ppm SN there is no relationship between FN and SN, that is, FN cannot be expressed as a function of SN (data points appear to form a vertical line); and 2) above ? 340 ppm SN, FN increases linearly with increasing SN. Nitrate is a prominent storage form of N in plants (14, 15), and petioles serve as storage tissue for nitrate. This could explain the nature of the relationship between FN and SN in region 1. Under N deficiency, nitrate is mobilized from storage in petioles and utilized for production of metabolites. Observed increases in FN with no increase in SN (region 1) is likely a result of increases in the organic-N fraction, with no increase in stored nitrate-N. When there is sufficient available N, increases in absorbed N result in increasing stored nitrate-N in petioles (region 2). Millard and Catt (16) reported increasing fertilizer N from 0 to 25 g/m^2 (0 to 0.17 oz/yd^2) resulted in excess N accumulation in leaves, principally in the form of nitrate and protein; and Millard and MacKerron (17) reported nitrate depletion in leaves of non-fertilized white potato. Nonlinear piece-wise regression analysis was used to determine the break-point (point of shift from region 1 to region 2) (20), and the relationship between FN and SN beyond the break-point. The relationship below the break-point is trivial since all SN levels below this were associated with N deficient plants (FN < 3%). Above the break-point, the relationship between FN and SN was linear and highly correlated (Fig. 3). Similar to experiment 1, the date at which data were collected did not affect the relationship between FN and SN, therefore the data were pooled to determine the equation for the line of best fit (above the break-point): SN/1000 = 2.2 × FN – 4.8 (r^2 = 0.74, n = 42). A 95% confidence interval for the slope of the line of best fit was [1.9, 2.6].

Because confidence intervals for the estimated slopes and y-intercepts (data not presented) overlapped for the two experiments, the data were pooled to calculate a comprehensive equation for the line of best fit: SN/1000 = 2.3 × FN – 4.8 (r^2 = 0.73, n = 104) (Fig. 4). Fonteno et al. (9) reported FN sufficiency ranges of 3 to 5% for vinca. Using these values in the previous equation, SN levels for optimal vinca growth would be 2100 to 6700 ppm NO_3^-. Our data and observations agree with the calculated lower limit for this range

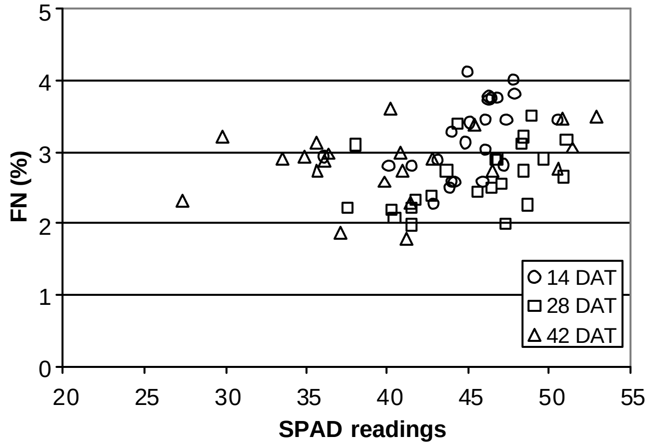

Fig. 2. Relationship between foliar nitrogen (FN) and SPAD readings of 'Pacifica White' vinca (Expt. 1).

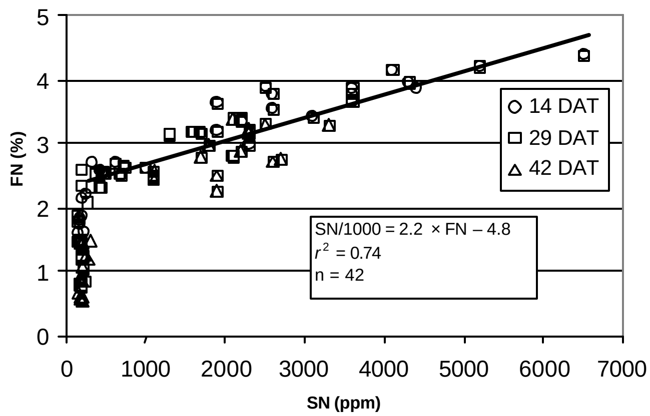

Fig. 3. Relationship between foliar nitrogen (FN) and petiole sap nitrate concentration (SN) of 'Pacifica White' vinca harvested at three dates (Expt. 2).

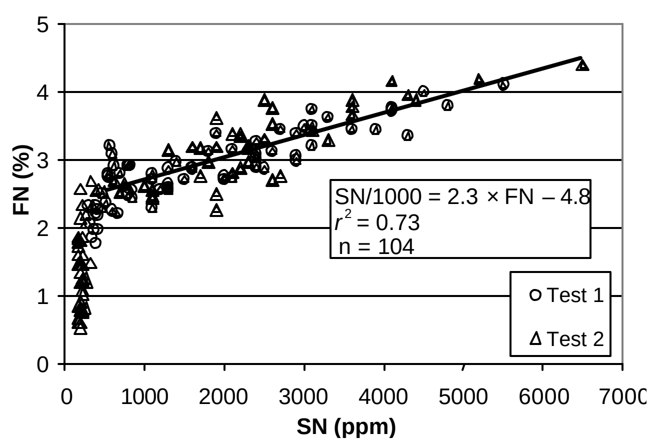

Fig. 4. Relationship between foliar nitrogen (FN) and petiole sap nitrate concentration (SN) of 'Pacifica White' vinca over Expts. 1 and 2.

Table 3. Effect of N rate on 'Pacifica White' vinca (Expt. 3).

N rate (ppm)	Flower no.	G.I.[z] (cm)	SPAD	SN[y] (ppm)	FN[x] (%)
			4 DAT[w]		
0	58	39.1	49.6	472	2.5
90	37	40.8	52.3	1460	3.4
180	55	40.6	49.7	2640	3.8
	Q**[v]	NS	NS	L***	L***
			7 DAT		
0	32	.	53.1	170	2.0
90	46	.	52.4	518	2.8
180	44	.	52.8	3980	3.8
	NS		NS	L***Q***	L***
			10 DAT		
0	43	35.3	47.0	176	1.3
90	57	38.8	49.5	312	2.4
180	64	39.4	50.3	3460	3.7
	L*	L**	NS	L***Q***	L***
			14 DAT		
0	38	38.2	42.8	207	1.2
90	69	42.3	47.3	213	2.1
180	79	46.3	48.8	2900	3.3
	L**	L**	L***	L***Q**	L***

[z]Growth index: (height + width + width) ÷ 3.

[y]Sap nitrate concentration.

[x]Foliar nitrogen (% of dry weight).

[w]Days after treatment.

[v]NS, *, **, or *** Nonsignificant, linear (L), or quadratic (Q) response at *P* ? 0.05, 0.01, or 0.001, respectively.

(2100 ppm NO_3^-). However, there were no plants that displayed symptoms of excessive N in this experiment, so we cannot be sure the upper limit (6700 ppm NO_3^-) is accurate. Nonetheless, landscape professionals are typically concerned with N deficiency.

Experiment 3. Prior to application of N treatments, plants had SN levels of 1980 ppm and FN of 3.5%. At 4 DAT, all plants were similar in size and chlorophyll content (SPAD) (Table 3). There was a quadratic response in flower number with increasing N rate, though this trend was transient and not observed 3 days later (7 DAT). As early as 4 DAT, plants receiving 0 ppm N were N deficient. FN and SN readings increased linearly with increasing N rate, indicating that these parameters respond soon after only low N levels are available to the plant. Nitrate is mobile within vinca, and petioles are sites of storage and transport of nitrate. It is not surprising that lower levels of expressed SN were observed shortly after available N to the plant was reduced. However, it is surprising that FN levels responded so rapidly. Mills and Jones (19) have described petiole SN tests as revealing more of the current relationship of nutrient supply to leaves, while traditional whole tissue analyses provide a more long-term historical perspective on nutrient supply, uptake, and transport prior to sampling. Our data contradict this in that FN (whole tissue analysis) responded immediately to lower levels of available N (lower N rates applied). Our results concur with Quijada et al. (22) who reported N content in different plant parts of tomato declined by day 4 after receiving 0 meq N. By 10 DAT, juvenile expanding foliage on plants receiving 0 ppm N was noticeably lighter in color than those receiving 90 or 180 ppm N. Because recently matured foliage was used for SPAD readings, these data were similar for plants regardless of N rate applied. By 14 DAT, plants fertilized with 0 ppm N were noticeably lighter in color throughout the plant compared to others, and consequently, chlorophyll content responded by increasing linearly with increasing N rate. These data demonstrate that N deficiency occurs before onset of visual symptoms, and that use of visual symptoms is a poor method for plant nutritional monitoring.

FN was regressed against SN to determine the nature of their relationship immediately after imposed N deficiency: $SN/1000 = 2.4 \times FN - 5.9$ ($r^2 = 0.70$, n = 36) (data not presented). A 95% confidence interval for the slope of the line of best fit was [1.9, 3.0] which overlaps those obtained in experiments 1 and 2. Thus, the relationship between FN and SN was validated in a third study conducted even later in the year.

In summary, data herein demonstrate the Cardy nitrate meter can rapidly diagnose N deficiency in annual vinca. Due to the high degree of correlation with FN levels, SN in vinca petioles can be used to predict FN levels, and thus plant N status. In addition, because SN levels respond within days to changes in applied N, the Cardy nitrate meter can predict deficient levels of N prior to the occurrence of visual symptoms in the plant. This methodology can be useful to growers and landscape professionals for applying fertilizers based on vinca need, and thus minimizing N leaching.

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