Rapid Determination of Nitrogen Status in Potted Pansy Production©

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Traditional laboratory analysis of tissue samples is time consuming and does not allow for rapid diagnosis of plant nitrogen (N) status and immediate response to plant need. Two experiments were conducted growing Bingo Yellow pansy (*Viola* ×*wittrockiana* 'Bingo Yellow') at N rates ranging from 20 to 160 ppm N. Data were collected to determine the relationship between traditional laboratory analysis of foliar N [(% on a dry weight basis) (FN)] and a rapid test that evaluates nitrate concentration in plant sap (SN) using the Cardy nitrate meter. Pooling data over the two experiments, regression analysis determined that SN levels could be used to predict FN levels with the following equation: log(SN) = 0.47*FN + 1.6 [r^2 = 0.80, n = 134]. Similar to traditional laboratory analysis, SN levels determined with the Cardy nitrate meter could predict N deficiency in the plant prior to the occurrence of visual symptoms.

INTRODUCTION

Potted bedding and garden plant sales recorded $1.02 billion in wholesale value in 2000 (Anonymous, 2001), representing 48% of all bedding and garden plant sales. Pansy (*Viola* sp.) production had a wholesale value of $21.3 million, ranking fourth in popularity among all bedding plants. Nutritional monitoring, especially of nitrogen (N) status, is important for growing quality container plants. Nitrogen deficiency many occur rapidly with annual bedding plants, produced in flats or trade gallon containers. This limits plant growth at a time when maximum growth is critical for spring sales. A reliable method for rapid determination of plant N status in pansy production would benefit growers by allowing for closer monitoring and response to plant fertility need. In container nursery and greenhouse crops, determination of plant N status can be achieved by several methods. Laboratory analysis of tissue samples collected from the crop is a reliable method for determining plant N status. However, this requires the grower to send samples to a laboratory for analysis, which is time consuming and does not allow for rapid diagnosis and immediate response. Other more rapid techniques include using a pour-through method which correlates soluble salt levels in container leachate with available nutrients in the container medium (Yeager et al, 1983). This method offers rapid approximation of plant nutrient status and allows the grower to make frequent fertility adjustments to the crop to maximize growth and marketability. Guidelines for many crops have been determined using this method, however, use of different media, fertilizer sources, and moisture level at the time of measurement will affect final readings and can cause confusion (Kirven, 1986). Also for producing annual bedding plants grown in flats, the pour-through technique is not well defined. In addition, it is generally agreed that

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analyzing both media (soil) and tissue samples provides the most complete information regarding a plant's nutritional status (Smith, 1962).

A device called the Cardy nitrate meter (Horiba Ltd., Kyoto, Japan) has been used to measure nitrate concentration in plant sap for a number of vegetable crops. Sap nitrate concentration in petiole sap has been demonstrated to be a reliable method for measuring plant N status (Coltman, 1988; Kubota et al., 1996; Raynal-Lacroix and Cousin, 1997; Westcott et al., 1993). The Cardy nitrate meter utilizes a nitrate-specific electrode to measure the nitrate concentration in a small sample (0.25 to 0.5 ml) of plant sap. Samples are extracted from the plant by crushing 8 to 12 petioles (usually with a garlic press) and placing the extracted sap on the meter, which will return an instant reading in the range of 1 to 10,000 ppm nitrate. Because of the diverse range of greenhouse and field-grown vegetable crops successfully analyzed with the Cardy nitrate meter, there is reason to believe the devise can be calibrated for use in potted plant production — including pansy.

A procedure that allows for rapid diagnosis of N status in potted plant production would benefit growers by allowing them to accurately and rapidly determine the N status of their crop, and make frequent fertilizer adjustments depending on crop needs. Therefore, the objective of this study was to evaluate the Cardy hand-held sap nitrate meter for determining N status in pansy.

MATERIALS AND METHODS

Experiment 1. Pansy (*Viola* ×*wittrockiana* 'Bingo Yellow') were potted up on 20 Nov. 2000, from 48-cell packs into 20.32-cm (8-inch) azalea pots with a pine bark and peat (3 : 1, v/v) substrate amended per m^3 (yd^3) with 3.0 kg (5 lb) of dolomitic limestone and 0.9 kg (1.5 lb) of Micromax (Scotts Co., Marysville, OH) micronutrients. Plants were grown with liquid feed the first 4 weeks using 160 ppm nitrogen (N) from ammonium nitrate (NH_4NO_3), and 62 ppm phosphorus (P) and 150 ppm potassium (K) from potassium phosphate (K_2HPO_4). On 7 Dec. 2000, uniform plants about 7 cm (2.8 inches) tall and 18 cm (7.1 inches) wide were selected for use in the experiment. Thereafter plants were fertilized with 62 ppm P, 150 ppm K, and either 40, 80, 120, or 160 ppm N. Nitrogen rates were varied to produce plants with an N status that ranged from deficient to excessive. Fertilizer solution was applied to pots individually at a rate of 450 ml (15 oz) per container per irrigation event. Six single plant replicates per treatment were destructively harvested three separate times at 15, 35, and 50 days after initial treatment (DAT). Plants were arranged in a completely randomized design. Data collected included growth index [{G.I.} (height + width + width)/3], flower number, and use of recently matured foliage for the following: SPAD readings (measures chlorophyll content) (Spectrum Technologies, Plainfield, IL), petiole sap nitrate determination using the hand held Cardy nitrate meter, and foliar N (% dry weight basis) using a Leco CN 2000 (LECO Corp., St. Joseph, MI).

Experiment 2. Experiment 2 was conducted similarly to Experiment 1 with a few exceptions. Pansy were potted 10 March 2001 and first fertilizer treatment was applied 2 April 2001. Eight rates of N were used: 20, 40, 60, 80, 100, 120, 140, and 160 ppm N. Plants were harvested 18, 35, and 46 DAT.

RESULTS

Experiment 1. At 14 DAT there were no differences in growth measurements, SPAD readings, or flower number (Table 1). Despite similar growth and appearance

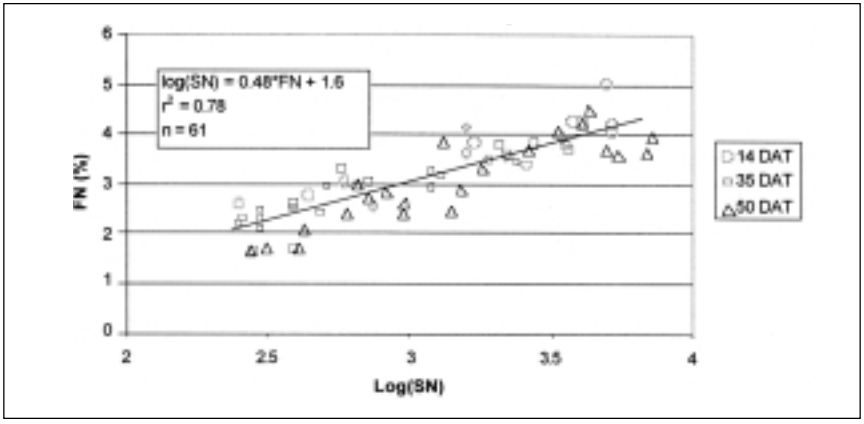

Figure 1. Relationship between FN and SN in pansy harvested at three different dates, Experiment 1.

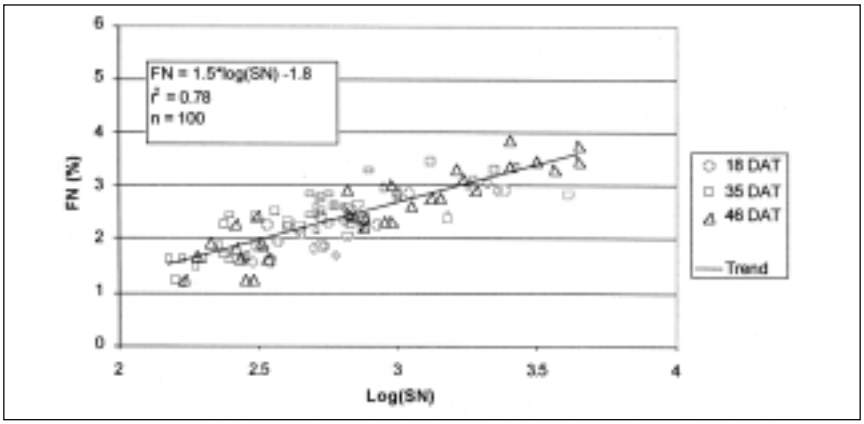

Figure 2. Relationship between FN and SN in pansy harvested at three different dates, Experiment 2.

parameters among plants, petiole sap NO_3^- levels (SN) and foliar nitrogen [(FN) (% total-N on a dry weight basis)] increased linearly with increasing N rate applied. Pansy fertilized with the low N rate (40 ppm N) appeared similar to other plants receiving higher N rates, yet foliar analysis revealed FN levels were lower than those recommended in the literature, 3.4 to 4.2% (Mills and Jones, 1996). These data suggest that foliar analysis (FN) can predict deficient levels of N before physical symptoms of nutrient deficiency occur.

At 35 and 50 DAT all measured parameters increased linearly with increasing N rate applied. The FN levels for plants receiving 40 and 80 ppm N were lower than those recommended in the literature.

The SN data were regressed against FN to determine the nature of their relationship. Regression diagnostics indicated a logarithmic (base 10) transformation of SN data was necessary to homogenize the variance (Neter et al., 1996); actual data are reported (Table 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 for the line was similar at each of the three dates, therefore data from the three dates were pooled for analysis. The FN was regressed against SN to determine the equation for the line of best fit (Fig. 1): log(SN) = 0.48*FN + 1.6 [(r^2 = 0.78, n = 61)]. These data demonstrate a tight relationship between FN and SN, and suggest if one can measure SN concentrations, FN levels can be predicted, and thus the N status of the plant determined.

Experiment 2. When plants were harvested 18 DAT, there were few obvious differences in appearance. Plants were similar in size and flower number, but had a slight increase in foliar color with increasing N rate applied. The FN and SN levels increased linearly with increasing N rate applied. At 35 DAT all measured parameters increased linearly with increasing N rate applied. Data collected 46 DAT was similar to that at 35 DAT, except that flower numbers were similar among all N rates applied (data not shown).

The FN was regressed against SN to determine the equation for the line of best fit (Fig. 2). Similar to Experiment 1, logarithmic transformation of SN was necessary to meet regression assumptions. Fitted equations at 35 and 46 DAT were similar while the equation at 18 DAT differed slightly and had a lower r^2. The fitted equation for 18 DAT was: log(SN) 0.52*FN + 1.7 [r^2 = 0.63, n = 29]. Regression of FN onto SN pooling 35 and 46 DAT data revealed the following equation: log(SN) 0.51*FN + 1.5 (r^2 = 0.78, n = 71). The difference in data at 18 DAT might be explained in part by longevity of the sensor. When plants were harvested 18 DAT, the sensor had been used approximately 200 times in other experiments. The expected life span of a sensor is 200-400 readings, depending on sampling procedures and storage conditions of the meter. While using the sensor at 18 DAT, the authors noticed the meter was difficult to calibrate and had to be corrected several times during the period of data collection. We believe the sensor was at the end of its expected life span, so subsequent readings were conducted with a new sensor. It is our experience that the sensors do not simply stop working, rather they become erratic and difficult to calibrate. We believe this was the cause of the slight difference in response and lower r^2 value at 18 DAT. In the regression analysis the equation for the line of best fit in Experiments 1 and 2 were similar, therefore the data were pooled (except 18 DAT in Experiment 2) to determine a more precise equation (Fig. 3): log(SN) = 0.47*FN

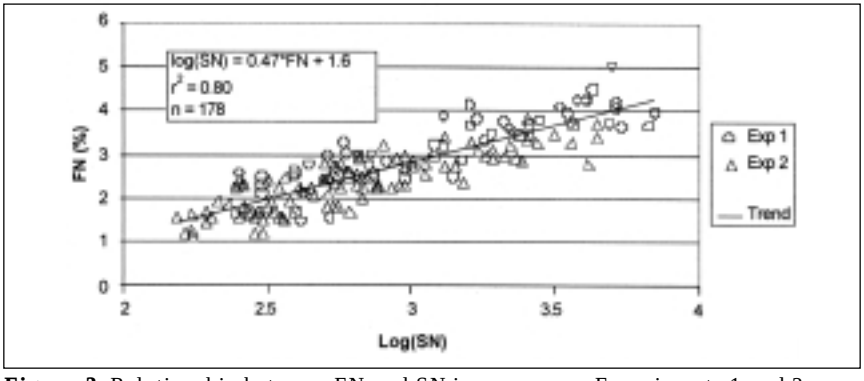

Figure 3. Relationship between FN and SN in pansy over Experiments 1 and 2.

+ 1.6 [r^2 = 0.80, n = 134]. Fonteno et al. (1996) reported FN sufficiency ranges for pansy as being 2.5 to 4.5%. Using these values in the previous equation, SN levels for optimal pansy growth would be 1600 to 5200 ppm NO_3^--N. The upper limit for this range appears high, which was due to the logarithmic relationship between SN and FN (as FN increases, SN increases logarithmically). None of the plants in our experiment exceeded 4.5% FN, thus the upper limit for SN readings cannot be determined conclusively. Hence, values for the lower limit should be reliable for predicting N deficiency in pansy. Generally, N deficiency is the greatest nutritional concern to growers.

Table 1. Pansy response to nitrogen (N) as indicated by flower number, growth index (G.I.), SPAD readings, foliar nitrogen (FN), and sap nitrate (SN) of pansy during Experiment 1.

N rate (ppm)	Flower (no.)	G.I. (cm)	SPAD	FN (%)	SN (ppm)
	14 DAT[z]				
40	2.3	14.9	51.5	2.9 c[y]	534.0 c
80	2.8	15.3	51.5	3.6 b	1960.0 b
120	2.3	16.1	53.1	4.0 ab	2900.0 b
160	2.7	16.1	54.5	4.3 a	4516.7 a
	NS	NS	NS	L***	L***
	35 DAT				
40	4.0 a	15.1 c	44.7 c	2.0 c	354.0 b
80	4.7 a	18.1 b	51.4 b	2.5 b	370.0 b
120	5.3 a	19.7 ab	57.9 a	3.2 a	1230.0 b
160	6.7 a	20.7 a	58.6 a	3.5 a	2450.0 a
	L*	L***	L***	L***	L***
	50 DAT				
40	3.7 b	17.7 c	44.7 c	1.7 d	388.3 c
80	6.0 ab	19.4 bc	52.0 b	2.7 c	778.3 c
120	8.3 a	21.4 ab	59.3 a	3.4 b	2108.3 b
160	9.2 a	22.7 a	62.5 a	3.9 a	5300.0 a
	L**	L***	L***	L***	L***Q***

[z] Days after treatment (DAT).
[y] Means with similar letters are not significantly different (Duncan's Multiple Range Test, $\alpha = 0.05$).

DISCUSSION

Samples used to determine the relationship between FN and SN were collected at 5 different sampling dates from 22 Dec. 2000, through 18 May 2001. Over this period of time, the relationship between FN and SN remained stable despite plants growing at different times of the year and experiencing different temperature and photoperiod exposures. This indicates that the Cardy nitrate meter can provide reliable diagnosis of N status in pansy when used on plants grown throughout the winter and spring seasons. The Cardy sap nitrate meter can be used to rapidly diagnose N status in pansy. Due to the high degree of correlation between FN and SN levels, the Cardy nitrate meter can provide similar information regarding plant N status that traditional laboratory analysis of foliar samples has provided. In addition, similar to traditional laboratory analysis, the Cardy sap nitrate meter can predict deficient levels of N in the plant prior to the occurrence of visual symptoms.

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