# NON-DESTRUCTIVE MEASUREMENT OF PERENNIAL CROP N STATUS 

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#### Abstract

Little research has been conducted to evaluate the use on hand-held leaf meters as tools to assess plant N status in perennial crops. In this study, using replicated N rate plots in place for up to four years, we used two hand-held leaf meters (the Minolta SPAD meter and the Field Spec CM1000) to monitor tissue N status in apple and one meter (CM1000 only) in wine grape. In both crops, relationships were found between leaf meter readings and both fertilizer N rate as well as tissue N concentration. For both crops, differentiation between 0 N rate and high N rates were apparent, but low and medium N rates were often not statistically different from the zero and high rates. In apple, the data suggest that a minimum sample size of 24 leaves is required to assess plant N status, suggesting that a larger sample size is needed before guidelines for these meters can be developed.


## INTRODUCTION

Measuring perennial crop N status for adjustments in N during the growing season is typically conducted by using plant tissue sampling. This requires delivering a sample to a test lab, sample preparation and analysis, and reporting the results. It is rare for the results to be reported any sooner than three days after sampling.

The Minolta SPAD meter (http://konicaminolta.com/products/instruments/spad/index.html) uses light transmission in the near infrared region to estimate chlorophyll concentration. Previous research using SPAD to estimate N availability in apple (Neilsen et al., 1995) showed a significant relationship between N rate and SPAD measurements for four varieties of apple. The FieldScout CM1000 (CM1000, Spectrum Technologies, Plainfield, Ill.) is a newer handheld chlorophyll meter that measures leaf surface reflectance in the red and near infrared wavelengths to exploit the unique differential reflectance properties of green leaves. The CM1000 measurements are analogous to the Normalized Difference Vegetation Index (NDVI; Rouse et al., 1973) used extensively in remote sensing of crop characteristics.

The objectives of our study were to assess the utility of leaf spectral reflectance measurement in assessing plant N status in apple (Malus domestica Borkh.) and wine grape (Vitis vinifera L.) and to compare the CM1000 to the SPAD for relation to N fertilizer management.

## METHODS

Apple: Replicated N fertilizer rate plots were established in 2002 in a commercial 'Fuji' apple block near Mattawa, WA (latitude $46^{\circ} 40^{\prime} \mathrm{N}$ and longitude $119^{\circ} 50^{\prime} \mathrm{W}$ ) in a Winchester sand (mixed, mesic, Xeric, Torripsamment). Each plot was 10 trees long by 2 rows (N-S orientation) wide, with four border trees between each plot. N was applied in a single
application during the dormant period (Dec to March) as granular urea banded (6'wide vegetation free strip) in the rows at 4 rates: control ( $0 \mathrm{lbs} / \mathrm{A}$ ), 0.5 rate ( $17.5 \mathrm{lbs} / \mathrm{A}$ ), 1.0 rate ( 35 lbs/A), and 2.0 rate ( $70 \mathrm{lbs} / \mathrm{A}$ ). Plots were fertilized from 2002 through 2005.

To assess plant N status, 15 leaves were collected from two plants in each row (total of 4 trees per plot), using the $3^{\text {rd }}$ tree from the N and S end of the plot. Leaf position for tissue collection was tree mid=height (approximately 4' high) on both E and W exposures. Leaf meter readings with both the SPAD and CM1000 were collected immediately upon leaf removal. Samples were collected early season (May), mid season (June and/or July), and late season (August). Leaf tissue was dried $\left(140^{\circ} \mathrm{F}\right)$ for 24 hours, ground, and analyzed for total N by dry combustion (Bremner, 1996) using a LECO CNS analyzer (St. Joseph, MI.).

Grape: Replicated N fertilizer rate plots were established in 2003 in four commercial vineyard sites. Both 'Merlot' and 'Riesling' blocks were used on Quincy fine sand (mixed, mesic Xeric Torripsamments) near Paterson, WA (latitude $45^{\circ} 55^{\prime} \mathrm{N}$ and longitude $119^{\circ} 36^{\prime} \mathrm{W}$ ) and Warden silt loam (coarse-silty, mixed, superactive, mesic Xeric Haplocambids) near Prosser, WA (latitude $46^{\circ} 17^{\prime} \mathrm{N}$ and longitude $119^{\circ} 38^{\prime} \mathrm{W}$ ). In 2004, a third Merlot vineyard block was added to the study in the Paterson location, also on Quincy sand. Plots were 1 row wide and 10 vines long with 4 vines between each plot. Nitrogen was applied as UAN 32 pipetted mid-set in the well below each drip emitter. The N 4 rates were 0 , low, medium, or high using $0,10,20$, or $30 \mathrm{lbs} / \mathrm{A}$ on the silt loam soil and $0,20,40$, and $60 \mathrm{lbs} / \mathrm{A}$ on the sand. The total application was split across 4 timings based on plant phenology: bloom, fruit set, veraison, and post harvest.

Leaf tissues were collected approximately 1 week after fertilizer application from the center eight vines using the leaf opposite the basal cluster at bloom and the fifth flat leaf at fruit set and veraison. In each plot, the CM1000 was used to measure leaf reflectance on one leaf from the first, fourth and eighth vines (3 leaves per plot). Tissues were dried and ground as per apple and leaf blade total N analyzed using the same dry combustion technique. Leaf petiole tissue was extracted with distilled water (1:100 w/v) and analyzed for NO3-N colorimetrically (Mulvaney, 1996) using a flow injected ion analyzer (O-I Analytical, College Station, TX).

Data Analysis: Analysis of variance was determined using PC SAS Proc GLM (SAS Institute, Cary, NC) for N rate effects and time of sampling on tissue N concentration and leaf meter reading values. For grape tissue, Proc FREQ was used to determine the distribution of the data in ranges related to N rate treatments.

## RESULTS AND DISCUSSION

Leaf Sensors for Nitrogen Estimation in Apple
To assess how well single individual leaf meter measurements can be used to predict nitrogen status for surrounding trees, we compared estimates of applied nitrogen based on SPAD and CM1000 measurements with the actual applied N for each plot. General linear models were fitted to predict applied nitrogen based on the SPAD and CM1000 measurements across all measurement dates and averaged by plot.

$$
\begin{aligned}
& \text { N Applied }{ }_{\text {SPAD }}=-434.2 * \text { SPAD }+13.5 \\
& \mathrm{r}^{2}=0.87 ; \mathrm{N}=16 \\
& \text { N Applied }^{\wedge} \text { CM1000 }=-330.2 * \text { CM1000 }+2.0 \\
& \mathrm{r}^{2}=0.76 ; \mathrm{N}=16
\end{aligned}
$$

These global parameters were then applied to individual leaf measurements of the SPAD and CM1000 sensors on each measurement date to estimate the applied N . The absolute difference between the estimated and applied nitrogen were calculated, and the average of the
differences was calculated for each measurement date. The same approach was used to generate differences between measured leaf Total N and the Total N estimated with the following global equations:

$$
\begin{aligned}
& \text { Leaf Total } \mathrm{N}^{\wedge}{ }_{\text {SPAD }}=0.04167^{*} \text { SPAD }+0.4239 \\
& \mathrm{r}^{2}=0.85 ; \mathrm{N}=16 \\
& \text { Leaf Total } \mathrm{N}^{\wedge}{ }_{\text {CM1000 }}=0.006230 * \mathrm{CM} 1000+0.7520 \\
& \mathrm{r}^{2}=0.69 ; \mathrm{N}=16
\end{aligned}
$$

Results for the CM1000 and SPAD measurements to estimate applied N are shown in Table 1. The single leaf differences between the estimated and actual N applications for the CM1000 are in the range of approximately $40-60 \mathrm{lbs} / \mathrm{A}$. For SPAD, the range is approximately $50-80 \mathrm{lbs} / \mathrm{A}$ for individual leaves. Taking the average of the three leaves per tree reduced the differences for both CM1000 (to 30-50 lbs/A) and SPAD (to 30-70 lbs/A), however, these average differences are still quite large relative to the range of nitrogen application treatments between $0 \%$ and $100 \%$ of the grower application rate ( $40 \mathrm{lbs} / \mathrm{A}$ ). While there was a difference in the number of leaves sampled, the time of season did not appear to have any impact on the differences between the estimated and actual applied nitrogen values. Results for the estimation of leaf N were similar. The estimates improve with increasing the number of leaves used from one to three. Also, there was no time of season effect of estimating the leaf N .

Table 1. Differences between N Values Estimated by Leaf Meters and Actual Applied

|  |  | Average Difference Between Estimated and Applied N <br> (lbs/A) |  |  |  |
| :---: | :---: | :---: | :---: | :---: | :---: |
|  |  | By Individual Leaves |  | Average of Three Leaves per <br> Tree |  |
| YEAR, <br> Day of <br> Year | Time of <br> Season | CM1000 | SPAD | CM1000 | SPAD |
| 2002,199 | Mid | 55.0 | 58.0 | 43.8 | 45.4 |
| 2002,227 | Late | 56.7 | 55.8 | 47.7 | 40.3 |
| 2003,119 | Early | 50.4 | 62.3 | 37.9 | 52.8 |
| 2003,176 | Mid | 41.5 | 56.7 | 32.4 | 39.3 |
| 2003,210 | Mid | 41.7 | 77.6 | 35.0 | 68.9 |
| 2003,245 | Late | 54.5 | 69.8 | 45.8 | 51.8 |
| 2004,153 | Early | 43.3 | 49.0 | 33.4 | 38.8 |
| 2004,181 | Mid | 58.6 | 54.2 | 43.7 | 43.2 |
| 2004,209 | Mid | 48.7 | 64.0 | 31.9 | 45.8 |
| 2004,239 | Late | 53.3 | 59.3 | 39.9 | 39.2 |
| 2005,146 | Early | 44.6 | 43.7 | 41.1 | 32.9 |
| 2005,181 | Mid | 49.1 | 47.3 | 40.9 | 33.4 |
| 2005,207 | Mid | 40.0 | 51.5 | 29.2 | 34.2 |
| 2005,238 | Late | 43.4 | 59.5 | 35.3 | 40.3 |

To better characterize how estimates of applied N and leaf N improved with the number of leaves sampled, the absolute differences between the measured and estimated values were determined for three additional sample sizes: by plot, by two plots of the same treatment combined, and by treatment. Combined with the values by leaf and by tree as described above,
these differences represent sample sizes of $1,3,12,24$, and 48 leaves. The fifteen values representing the average differences calculated for each date were averaged again by sample size. The resulting relationship between sample size and estimated versus actual application rate is shown in Figure 1. As the number of leaves used to estimate the nitrogen application increases, the difference between the estimated and actual amounts appears to achieve a minimum near $20 \mathrm{lbs} / \mathrm{A}$ as the number of leaves used approaches 50. In comparison, note that the treatment levels differ by 20 to $40 \mathrm{lbs} / \mathrm{A}$. The differences between measured and estimated leaf N also decrease asymptotically. With both meters, the improvement seems to diminish sharply as the number of leaves approaches 24 , resulting in a difference between the measured and estimated values of $0.23 \%$. In comparison, the standard deviations of leaf N by treatments ranged from 0.20 to $0.31 \%$. For both the CM1000 and SPAD measurements, the minimum values appear to approach the standard deviation of the leaf N measurements within treatment, suggesting a limit based on the uncertainty of the foliar chemistry.


Figure 1. The nitrogen estimation accuracy versus number of leaves measured with the SPAD and CM1000 meters. The accuracy was determined as the difference with applied N (top) and measured leaf total N (bottom). For reference, N treatments (rates applied) were $0,17.5,35$, and $70 \mathrm{lbs} / \mathrm{A}$. Average leaf N (\%) for the 4 treatments was 1.77, 1.82, 1.86, and 2.00. The range in standard deviations (within each treatment) for leaf N was 0.20 to $0.31 \%$.

Non-Destructive N assessment in Grape:
Across all years of study, at 4 of the 5 vineyard blocks, both petiole $\mathrm{NO}_{3}-\mathrm{N}$ and blade total N were related to N rate (Table 2). The Merlot in vineyard 3 and Riesling in vineyard 1 also showed significant differences in $\mathrm{NO}_{3}-\mathrm{N}$ and blade total N concentrations with time of season (Table 3).

Table 2: Average leaf N concentrations in Merlot and Riesling vineyards in response to different N fertilizer rates. The number following the vineyard site number ( $\mathrm{V} \#$ ) indicates number of seasons with complete trial results. Values followed by the same letter are not statistically different.

|  |  | Merlot |  |  | Riesling |  |
| :--- | :---: | :---: | :---: | :---: | :---: | :---: |
|  | N Rate | V1 (2) | V2 (3) | V3 (3) | V1 (3) | V2 (4) |
| Petiole <br> NO $_{3}-\mathrm{N}$ <br> $(\mathrm{ppm})$ | None | 133 b | 2731 a | 32 b | 108 c | 884 b |
|  | Low | 281 a | 2499 a | 25 b | 157 bc | 1015 b |
|  | Medium | 345 a | 2653 a | 51 b | 193 b | 1023 b |
|  | High | 408 a | 2534 a | 113 a | 270 a | 1215 a |
| Blade <br> Total N <br> $(\%)$ | None | 2.74 b | 3.49 a | 2.62 c | 2.73 b | 2.79 b |
|  | Low | 2.80 ab | 3.48 a | 2.68 b | 2.82 a | 3.01 ab |
|  | Medium | 2.82 ab | 3.47 a | 2.75 ab | 2.80 ab | 3.01 ab |
|  | High | 2.85 a | 3.50 a | 2.85 a | 2.78 ab | 3.30 a |

Table 3: Average tissue N concentrations by time of season across 3 and 4 growing seasons, respectively, for four different N fertilizer rates on Merlot and Riesling. Values followed by the same letter are not statistically different.

| Time of Season | Petiole $\mathrm{NO}_{3}-\mathrm{N}(\mathrm{ppm})$ |  | Blade total N(\%) |  |
| :--- | :---: | :---: | :---: | :---: |
|  | Merlot | Riesling | Merlot | Riesling |
| Bloom | 31.05 b | 856 b | 2.59 c | 3.05 b |
| Fruit Set | 37.55 b | 1610 a | 2.85 a | 3.10 a |
| Veraison | 95.26 a | 882 b | 2.74 b | 2.85 c |

Statistical analysis of leaf reflectance data collected using the hand held leaf reflectance meter (CM1000) showed differences in meter readings by year, variety and N rate, but there was no interaction between N rate and year. Additionally, there was not significant difference in leaf meter readings and time of sampling. Thus, the data from all three years were combined and evaluated in comparison with zero, low, medium, or high N rate.

Figure 2 shows the percentage of all data from each N rate plotted against leaf meter reading values. As with tissue N data, the peaks of the zero and high N rate occurred at different values (131-150, 151-170, respectively), but zero, low and medium were not clearly different. An average value for each N rate was developed based on the majority ( $60-80 \%$ ) of the CM1000 values for each N rate. These were determined to be 149, 156,160, and 168 for zero, low, medium and high N rates (Fig. 2), suggesting that a vineyard with average leaf meter reading values between approximately 155 - 165 would have sufficient plant $N$ but lower or higher values may indicate a shortage or excess of N . Please note, these ranges are preliminary.

## SUMMARY

Overall, both the SPAD meter and CM1000 showed potential for use to assess plant N status in apple and the CM1000 in grape. The limitations of the data from the current study appear to be related to sample size. The data from apple suggests a sample of at least 24 leaves per area for adequate assessment of N status. More detailed results and discussion on this project are available (Perry and Davenport, 200X). Further field validation on both apple and grape will help in elucidating guidelines for use of these handheld meters.


Figure 2. The percentage of 3 years data in ranges of leaf reflectance readings (meter readings, X axis) taken with the CM1000 at each of 4 N rates (zero, low, medium, high). The value in parenthesis after the N rate in the legend is the weighted average value for leaf reflectance under that treatment.

## REFERENCES

Bremner, J.M. 1996. Nitrogen - Total. p. 1085-1122 In J. M. Bigham (ed.) Methods of Soil Analysis, Part 3 - Chemical Methods, SSSA Book Series \#5, Madison, WI.
Mulvaney, R.L. 1996. Nitrogen - Inorganic. p. 1123-1184 In J.M. Bigham (ed.) Methods of Soil Analysis, Part 3 - Chemical Methods, SSSA Book Series \#5, Madison, WI.
Neilsen, D.; Hogue, E.J.; Neilsen, G.H.; Parchomchuk, P. 1995. Using SPAD-502 values to assess the nitrogen status of apple trees. HortScience 30: 508-512.
Perry, E.M., and J.R. Davenport. 200X. Spectral and spatial differences in response of vegetation indices to nitrogen treatments on apple. Computers and Electronics in Agriculture. In Review.
Rouse, J.W., Haas, R.H, Schell, J.A., Deering, D.W., Harlan, J.C., 1974. Monitoring the vernal advancements and retrogradation of natural vegetation. NASA/GSFC final report. Greenbelt, MD, USA. 371pp.

