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A Comparison of Rapid Potentiometric and Colorimetric Methods for Measuring Tissue Nitrate Concentrations in Leafy Green Vegetables

Kristy A. Ott-Borrelli^{1,3}, Richard T. Koenig¹, and Carol A. Miles²

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SUMMARY. Leafy green vegetables such as lettuce (*Lactuca sativa*), Asian greens (*Brassica* spp.) and spinach (*Spinacia oleracea*) have a tendency to accumulate high concentrations of potentially harmful nitrate-nitrogen (NO₃-N). It would be advantageous for growers to have rapid and inexpensive methods to accurately measure plant tissue NO₃-N to make fertility and harvest management decisions for these crops. This study compared fresh sap expressed from whole leaves and analyzed with a Cardy meter with the analysis of dry leaf tissue extracts analyzed with a benchtop ion selective electrode (ISE) and an automated colorimetric method for determining NO₃-N concentration. Results from ISE and colorimetric analysis of the same dry leaf tissue extracts had a strong relationship ($r^2 = 0.92$). The ISE was relatively easy to operate and affordable, suggesting it is an adequate substitute for automated colorimetric analysis of dry plant tissue extracts. Results of fresh whole leaf sap analyzed with the Cardy meter showed a poor relationship with dry leaf tissue extracted and analyzed using the ISE ($r^2 = 0.25$) or with colorimetric analysis ($r^2 = 0.21$). When fresh whole leaf sap was diluted 1:1 with aluminum sulfate [Al₂(SO₄)₃] to adjust for potential matrix effects, there was still a relatively poor relationship ($r^2 = 0.41$) between the diluted sap samples analyzed with a Cardy meter and the dry leaf tissue extracted and analyzed with the ISE. When the same dry leaf tissue extracts were analyzed with the Cardy meter and the ISE, the results related well ($r^2 = 0.96$). As a result of tissue processing and/or instrument differences, Cardy meter analysis of sap expressed from whole leaves was not comparable to ISE or colorimetric analyses of dry leaf tissue extracts for leafy green vegetables.

Leafy green vegetables can accumulate high concentrations of nitrate-nitrogen (NO₃-N) that are potentially harmful if consumed by humans (Blom-Zandstra, 1989; Corre and Breimer, 1979; Maynard et al., 1976; Roorda van Eysinga, 1984). Nitrate-N concentration in leafy greens can fluctuate diurnally because it is often inversely related to light intensity (Muramoto, 1999;

Reinink, 1991; Steingrover et al., 1986; Steingrover and Ratering, 1986). As a result of rapid NO₃-N

fluctuation, it would be convenient for growers to be able to quickly monitor concentrations to optimize fertilization and harvest timing. Although conventional means of measuring plant tissue NO₃-N are accurate, well accepted, and reliable, they often require sophisticated equipment and trained technicians and can be time-consuming, expensive, and impractical outside of a laboratory setting.

Many laboratories are interested in analytical methodology capable of overcoming the inconveniences and high costs of conventional methods such as sequential or flow injection analysis (Lin et al., 2005). Potentiometric methods involve the use of ion selective electrodes (ISEs) to measure ion concentrations in plant tissue and soil extracts and are relatively inexpensive, easy to operate, and rapid (Watson and Isaac, 1990). These advantages of potentiometry have led to the development of NO₃-N ISEs that expedite analytical procedures (Lin et al., 2005). An Orion 93-07 NO₃-N ion-selective electrode and Orion 90-02 double-junction reference electrode (Thermo Fisher Scientific, Waltham, MA) gave accurate and precise NO₃-N measurements of vegetable tissue extracts (Consalter et al., 1992) and in situ measurements of soil NO₃-N concentration (Thottan et al., 1994). Lin et al. (2005) analyzed solutions of mineral water and vegetable extracts with two ISEs and a spectrophotometric method and found that the potentiometric methods were both precise and accurate with no significant difference between the two methods at the 95% confidence level.

Relatively inexpensive and portable ISE nutrient monitoring devices such as the Cardy NO₃-N meter (Horiba Instruments, Irvine, CA, and Spectrum Technologies, Plainfield, IL) are capable of measuring fresh plant sap NO₃-N levels directly. Several authors have shown that measurements made with the Cardy meter using nondiluted plant sap were well correlated to conventional methods

Units

To convert U.S. to SI, multiply by	U.S. unit	SI unit	To convert SI to U.S., multiply by
29.5735	fl oz	mL	0.0338
28.3495	Oz	g	0.0353
1	Ppm	mg·kg ⁻¹	1
1	Ppm	mg·L ⁻¹	1
(°F - 32) ÷ 1.8	°F	°C	(1.8 × °C) + 32

of $\text{NO}_3\text{-N}$ analysis (Altland et al., 2002, 2003; Hartz et al., 1993; Westcott et al., 1998). However, others found Cardy meter $\text{NO}_3\text{-N}$ values were consistently higher than values derived from conventional analysis methods unless sap was diluted to account for ionic strength effects and specific ion interferences (Davenport and Jabro, 2001; Errebhi et al., 1998; Hochmuth, 1994; Kubota et al., 1996; Rosen et al., 1996; Westcott et al., 1993). Although several of these authors suggest that dilution may be necessary to improve accuracy, most still found adequate relationships between conventional methods of tissue analysis and fresh sap analysis with the Cardy meter (Errebhi et al., 1998; Kubota et al., 1996; Rosen et al., 1996; Westcott et al., 1993, 1998).

The objective of this study was to compare the analysis of fresh leaf sap using a Cardy portable NO_3 meter with dry leaf tissue extracts analyzed using ISE and colorimetric methods to determine if rapid, less expensive tissue processing and analysis methods can substitute for more laborious procedures requiring expensive instrumentation for quality assessment in leafy green vegetables.

Materials and methods

Samples for this study were taken from a larger experiment in which 24 varieties of lettuce, Asian greens, and spinach were harvested three times at two locations during winter (Ott, 2007). Comparisons between methods of analysis described subsequently used different subsample groups of plants taken from this larger experiment. Because the purpose of this study was to compare methods of

tissue processing and analysis, the effects of location, growing conditions, and variety on tissue $\text{NO}_3\text{-N}$ concentration are not discussed here.

After harvest, all fresh plant samples were rinsed with distilled water to remove soil and other debris from the leaves. Two to four outermost leaves, including petioles, from lettuce, Asian greens, and spinach were selected for processing and $\text{NO}_3\text{-N}$ analyses as described subsequently. Because the objective of this study involved quality assessment of leafy green vegetables for human consumption, whole leaves were used in the analyses instead of petiole tissue, which would have been more relevant for fertility analyses in these crops. Leaf samples intended for dry tissue extraction and analysis were weighed before and after drying for 48 h at 80 °C. Leaf samples intended for fresh sap analysis were processed immediately after rinsing.

DRY LEAF TISSUE PROCESSING. Oven-dry leaf samples were ground with a mortar and pestle and placed in plastic vials for storage. For dry extraction, 0.4 g of tissue was transferred to a 50-mL vial to which 40 mL of 0.025 M $\text{Al}_2(\text{SO}_4)_3$ extracting solution was added (Baker and Smith, 1969; Heanes, 1982). If the sample did not have 0.4 g of tissue, the maximum amount available was weighed and the weight was recorded. The vial was capped and shaken for 30 min. The solution was filtered through a Whatman #42 filter paper into clean vials and refrigerated (Heanes, 1982).

ION SELECTIVE ELECTRODE ANALYSIS. All dry leaf tissue extracts ($n = 544$) were analyzed using an Orion 720A+ meter coupled with an Orion Ionplus NO_3 ISE (Thermo Fisher Scientific). The ISE was calibrated using $\text{NO}_3\text{-N}$ standards at concentrations of 1.4, 14, and 140 $\text{mg}\cdot\text{L}^{-1}$ prepared using a 0.1 M NO_3 stock solution (Thermo Fisher Scientific) in a 0.025 M $\text{Al}_2(\text{SO}_4)_3$ matrix. Each analyte was brought to room temperature before analysis. Approximately 30 mL of solution was poured into a 50-mL beaker and stirred using a magnetic bar and stir plate. The ISE was placed directly into the solution and a reading recorded after the meter stabilized. The ISE was checked for drift by reading the 14 $\text{mg}\cdot\text{L}^{-1}$ $\text{NO}_3\text{-N}$ standard every six samples. If the value deviated from

the standard by greater than 10%, the meter was recalibrated.

COLORIMETRIC ANALYSIS. A subset ($n = 183$) of dried leaf tissue extracts prepared for ISE analysis was selected for colorimetric analysis using a continuous flow auto analyzer (Quick Chem FIA+ 8000 Series; Lachat Instruments, Milwaukee, WI). This instrument uses a cadmium column to reduce NO_3 to nitrite (NO_2) followed by analysis of the NO_2 using the modified Griess-Ilosvay method at a wavelength of 540 nm (Mulvaney, 1996). Extract samples were diluted before analysis to obtain concentrations within the standard curve of the instrument.

FRESH LEAF TISSUE PROCESSING AND ANALYSIS. A Cardy NO_3 meter (Horiba Instruments and Spectrum Technologies) was used to analyze samples of fresh and diluted leaf sap and a subset of dry plant tissue extract samples prepared as described previously for ISE analysis. Direct analysis of fresh sap with the Cardy meter was performed on selected plants ($n = 352$) that were also processed for dry plant tissue extracts and analyzed with the ISE and/or colorimetric procedures. From each plant, two outermost leaves, including petioles, were placed into a fresh tissue press. The intent was to express two samples (≈ 0.5 mL each) of fresh sap directly onto the Cardy meter electrode and to average the two readings. Frequently, delicate plant tissue would pass through the press and sufficient sap could be obtained for only one reading.

Personnel operated separate meters according to instructions described in the operation manual (Spectrum Technologies, 1997). The Cardy meter was calibrated with $\text{NO}_3\text{-N}$ standards of 20 and 450 $\text{mg}\cdot\text{L}^{-1}$ in a deionized water matrix, provided by the manufacturer, and recalibrated every three to five samples to prevent drift. The manual recommends taking a reading after 30 to 45 s. However, the meter did not stabilize for ≈ 2 to 5 min, which is when measurements were recorded. The sensor was replaced after ≈ 200 samples or as necessary when the meter failed to calibrate.

Selected samples ($n = 50$) of the dry leaf tissue extract prepared for ISE and colorimetric analysis were also analyzed with the Cardy meter. The

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¹Department of Crop and Soil Sciences, Washington State University, P.O. Box 646420, Pullman, WA 99164-6420

²Department of Horticulture and Landscape Architecture, Washington State University, Northwestern Research and Extension Center, Mount Vernon, WA 98273-4768

³Corresponding author. E-mail: kristy_ott@wsu.edu.

Cardy meter was calibrated with two of the three ISE $\text{NO}_3\text{-N}$ standards: 14 and 140 $\text{mg}\cdot\text{L}^{-1}$ in the 0.025 M $\text{Al}_2(\text{SO}_4)_3$ matrix. In addition, fresh sap samples ($n = 25$) were diluted with $\text{Al}_2(\text{SO}_4)_3$ and analyzed for $\text{NO}_3\text{-N}$ concentration with the Cardy meter to compare with dry tissue extracts of the same plants analyzed with the ISE. Diluted samples were prepared by placing two leaves into a fresh tissue press and expressing sap into a weigh boat. Fresh sap (0.5 mL) was transferred by pipette and mixed with 0.5 mL of 0.025 M $\text{Al}_2(\text{SO}_4)_3$ in a separate weigh boat. Two separate samples (0.5 mL each) of the diluted solution were transferred to the electrode of the Cardy meter and results averaged. The average dilute sap value was multiplied by two to account for the dilution factor.

CALCULATIONS. Nitrate-N readings derived from the ISE and colorimetric analyses of dry leaf tissue extracts were adjusted using the relative water content (RWC) of the subsamples to the same units as the Cardy meter (milligrams per liter of sap) for direct comparison. Fresh tissue $\text{NO}_3\text{-N}$ concentrations were calculated by multiplying the $\text{NO}_3\text{-N}$ concentration in the dry tissue extract by the ratio of the volume of $\text{Al}_2(\text{SO}_4)_3$ solution per kilogram of ground tissue used in the extraction process and then dividing by the RWC of each plant sample (liters per kilogram of dry tissue).

STATISTICS. Simple linear regression analysis was conducted using Sigma PlotTM software (Systat Software, San Jose, CA) to estimate the coefficient of determination (r^2), y-intercept, and slope of the regression line. Confidence intervals for slope and y-intercept parameters in the regression equations were calculated from SES obtained in regression analyses (Statistix 7.0; Analytical Software, Tallahassee, FL) and t-values obtained from an online calculator (National Institute of Standards and Technology, 2006). Confidence intervals were used to determine if values for the slope were different from one and y-intercept different from zero.

Results and discussion

COLORIMETRIC AND ION SELECTIVE ELECTRODE ANALYSIS. There was strong relationship between colorimetric and ISE analyses of the

same dry tissue extracts [$r^2 = 0.92$ (Fig. 1)]. However, concentrations determined using the ISE were consistently higher than for colorimetric analysis. The slope and y-intercept of the regression line between the two methods were significantly higher than 1 and 0, respectively (Fig. 1). Deviation of the slope from 1 could be attributable in part to nine samples with $\text{NO}_3\text{-N}$ concentrations above the highest calibration standard for the ISE (140 $\text{mg}\cdot\text{L}^{-1}$). Readings obtained beyond these points may not be as accurate as those that fall within the calibration range if the calibration is not linear beyond the range of the standards. Removing values greater than 140 $\text{mg}\cdot\text{L}^{-1}$ only slightly altered the regression analysis ($r^2 = 0.94$, $y = 1.20x + 4.45$), indicating that $\text{NO}_3\text{-N}$ values above the calibration range were not responsible for differences between the analytical methods. The higher ISE $\text{NO}_3\text{-N}$ concentrations could be caused by the calibration standards themselves. When the ISE standards were analyzed using the colorimetric method, the $\text{NO}_3\text{-N}$ concentrations were 1.5, 12.9, and 125 $\text{mg}\cdot\text{L}^{-1}$ compared with prepared concentrations

of 1.4, 14, and 140 $\text{mg}\cdot\text{L}^{-1}$, respectively. The lower colorimetric assay concentrations of the ISE standards would result in ISE values that were higher than the colorimetric values and would explain the results in Figure 1. An alternative explanation is that the standards against which the colorimetric instrument is calibrated are not accurate. Standards for both ISE and colorimetric instruments are prepared from stock solutions and are subject to dilution and other errors during preparation. Although values from the ISE were higher than those returned by the colorimetric procedure, the relative simplicity and low cost of the ISE, coupled with the high coefficient of determination between the ISE and colorimetric methods, suggests that the ISE could be an adequate substitute for automated colorimetric methods of dry leaf tissue extract analysis for $\text{NO}_3\text{-N}$. Paul and Carlson (1968) found that results from a $\text{NO}_3\text{-N}$ ion-specific electrode were similar with those obtained by the phenoldisulfonic acid colorimetric method, and Consalter et al. (1992) found that an Orion 93-07 $\text{NO}_3\text{-N}$ ISE coupled with an Orion 90-02 double junction reference electrode

Fig. 1. Comparison of leafy green vegetable (lettuce, Asian greens, and spinach) nitrate-nitrogen ($\text{NO}_3\text{-N}$) concentrations from dry whole leaf tissue extracts using colorimetric analysis and an ion selective electrode (ISE). The 95% confidence intervals for regression line parameters are: 1.17 to 1.29 for the slope and 1.15 to 9.75 for the y-intercept (1 $\text{mg}\cdot\text{L}^{-1} = 1$ ppm).

accurately recovered $\text{NO}_3\text{-N}$ from a variety of vegetable species.

CARDY METER COMPARISONS. There was a poor relationship between fresh leaf sap $\text{NO}_3\text{-N}$ concentration determined with a Cardy meter and dry leaf tissue extracts analyzed with either the ISE [$r^2 = 0.25$ (Fig. 2)] or the colorimetric [$r^2 = 0.21$ (Fig. 2)] procedures and expressed on a sap concentration (milligrams per liter) basis. Analysis of fresh leaf sap with the Cardy meter produced values that were consistently higher than dry tissue extracts of the same plants analyzed with either the ISE or colorimetric procedures.

Results of this study suggest that the extraction and analysis of fresh leaf sap with a Cardy meter is not comparable to procedures in which dry leaf tissue is extracted and analyzed with ISE or colorimetric procedures to determine $\text{NO}_3\text{-N}$ concentrations. Other authors found that analysis of fresh plant sap with a Cardy meter produced values that were consistently higher than more standard analytical methods (Davenport and Jabro, 2001; Errebhi et al., 1998; Rosen et al., 1996; Westcott et al., 1993). For

example, Errebhi et al. (1998) found that potato (*Solanum tuberosum*) sap $\text{NO}_3\text{-N}$ concentrations from a Cardy meter were an average of $50 \text{ mg}\cdot\text{L}^{-1}$ higher than sap concentrations measured with another instrument. However, these authors noted that the Cardy meter was a quick and useful tool with strong precision ($r^2 = 0.91$) when comparing fresh plant sap with dry tissue NO_3 concentrations. Davenport and Jabro (2001) found that the Cardy meter produced values 10 to 1000 times higher than conventional analysis when soil slurry samples were analyzed for $\text{NO}_3\text{-N}$ and concluded that the Cardy meter is not a suitable tool for analyzing soil slurries.

Several authors found that Cardy meter analysis of fresh plant sap was well correlated with several methods of dry tissue extract and analysis for $\text{NO}_3\text{-N}$ and suggested the Cardy could provide rapid, precise $\text{NO}_3\text{-N}$ measurements for a variety of crops (Altland et al., 2002, 2003; Hartz et al., 1993; Kubota et al., 1996; Rosen et al., 1996; Westcott et al., 1993, 1998). Authors note that it may be necessary to dilute samples that are

beyond the calibration range of the meter (Hochmuth, 1994; Rosen et al., 1996; Westcott et al., 1993), although Rosen et al. (1996) found a linear relationship between methods of analysis up to $2000 \text{ mg}\cdot\text{L}^{-1}$ $\text{NO}_3\text{-N}$, suggesting that the linear range of the Cardy meter extends well beyond the high standard concentration. In the present study, the majority of fresh plant sap samples had $\text{NO}_3\text{-N}$ concentrations above the high standard of $450 \text{ mg}\cdot\text{L}^{-1}$. However, relationships between fresh sap analyzed with the Cardy meter and dry tissue extracts analyzed with the ISE or colorimetric procedures were poor at sap concentrations $450 \text{ mg}\cdot\text{L}^{-1}$ $\text{NO}_3\text{-N}$ or less (Fig. 2).

It is possible that we introduced errors when the dry leaf tissue extract values from the ISE and colorimetric analyses were adjusted arithmetically to fresh sap concentrations using the moisture content determined for each individual sample. Vitosh and Silva (1994) found a highly significant linear correlation between fresh plant sap and dry plant tissue $\text{NO}_3\text{-N}$ concentrations using a portable HACH One pH/ISE meter (Hach, Loveland, CO). Other researchers found adequate relationship between non-diluted plant sap analyzed with a Cardy meter and petiole $\text{NO}_3\text{-N}$ expressed on a dry weight basis (Altland et al., 2002, 2003; Errebhi et al., 1998; Hartz et al., 1993; Hochmuth, 1994; Kubota et al., 1996; Westcott et al., 1993, 1998). To determine whether errors in tissue water content determination affected the comparisons in this study, fresh sap $\text{NO}_3\text{-N}$ concentrations (milligrams per liter of sap) determined from the Cardy meter were compared with dry plant extract $\text{NO}_3\text{-N}$ concentrations (milligrams per kilogram of dry tissue) determined with the ISE for data in Figure 2 ($r^2 = 0.30$, $y = 5.29x + 1612$). The poor relationship indicates that the problem between the two methods likely lies with tissue extraction and/or analysis and not with the mathematical conversion of dry tissue to fresh sap concentrations.

Previous studies used similar plant materials when comparing the Cardy meter with other methods (Errebhi et al., 1998; Hartz et al., 1993; Kubota et al., 1996; Rosen et al., 1996; Vitosh and Silva, 1994; Westcott et al., 1993). The use of

Fig. 2. Comparison of leafy green vegetable (lettuce, Asian greens, and spinach) nitrate-nitrogen ($\text{NO}_3\text{-N}$) concentrations from dry whole leaf tissue extracts using colorimetric analysis and an ion selective electrode (ISE) with analysis of whole leaf fresh sap using a Cardy meter. The 95% confidence intervals for the regression line parameters of the Cardy meter versus colorimetric comparison are: 0.25 to 1.33 for the slope and -621 to 430 for the y-intercept. The regression line parameters of the Cardy meter versus ISE comparison are: 0.33 to 0.73 for the slope and -104 to 253 for the y-intercept ($1 \text{ mg}\cdot\text{L}^{-1} = 1 \text{ ppm}$).

different plant tissue sampling methods (sap expressed from whole leaves versus whole leaf dry extract) is a possible source of error in this study. Nitrate-N concentration is higher in the petioles of leafy greens than in the leaf blades (Barker et al., 1974; Breimer, 1982; Maynard et al., 1976; Muramoto, 1999; Olday et al., 1976). Although we sampled the whole leaf in both fresh sap and dry tissue procedures, the majority of fresh sap likely came from the petiole because this tissue was more succulent than the leaf blade. Drying and processing the whole leaf may result in more dilute $\text{NO}_3\text{-N}$ concentrations compared with the analysis of sap expressed from fresh leaf samples.

When a subset of fresh leaf sap samples was diluted with $\text{Al}_2(\text{SO}_4)_3$ and analyzed with the Cardy meter, there was still a relatively poor relationship with dry plant tissue extracts analyzed with the ISE ($r^2 = 0.41$), and slope and y-intercept values differed significantly from 1 and 0, respectively (Fig. 3). This suggests that dilution of sap with an ionic strength adjuster before analysis with the Cardy meter was either not responsible for the poor relationship between methods or that a 1:1 dilution is not sufficient to account for ionic strength interferences. A comparison of dry tissue extract $\text{NO}_3\text{-N}$ (y-axis) to fresh plant sap $\text{NO}_3\text{-N}$ (x-axis) showed an x-intercept value of $322 \text{ mg}\cdot\text{L}^{-1}$, indicating that at low or zero values of plant dry matter $\text{NO}_3\text{-N}$, the Cardy meter was still indicating the presence of $\text{NO}_3\text{-N}$ (Westcott et al., 1993). These authors attribute the high $\text{NO}_3\text{-N}$ values of the Cardy meter to anion interferences and suggest that the addition of $\text{Al}_2(\text{SO}_4)_3$ is necessary to suppress these interferences. Kubota et al. (1996) also attribute differing results between dry petiole and petiole sap $\text{NO}_3\text{-N}$ measurement methods to ion interference in the absence of $\text{Al}_2(\text{SO}_4)_3$ in fresh sap analyses.

There was a strong relationship between instruments when the same dry plant tissue extracts were analyzed with the Cardy meter and ISE [$r^2 = 0.96$ (Fig. 4)]. The slope and y-intercept did not differ from 1 and 0, respectively (Fig. 4). This strong relationship is attributed to the use of the same plant materials and extract procedures for this method. The

Fig. 3. Comparison of leafy green vegetable (lettuce, Asian greens, and spinach) nitrate–nitrogen ($\text{NO}_3\text{-N}$) concentrations from dry whole leaf tissue extracts using an ion selective electrode (ISE) with rapid analysis of fresh plant sap diluted with 0.025 M aluminum sulfate using a Cardy meter. The 95% confidence interval for regression line parameters are: 0.31 to 0.95 for the slope and 92 to 445 for the y-intercept ($1 \text{ mg}\cdot\text{L}^{-1} = 1 \text{ ppm}$).

Fig. 4. Comparison of leafy green vegetable (lettuce, Asian greens, and spinach) nitrate–nitrogen ($\text{NO}_3\text{-N}$) concentrations from dry whole leaf tissue extracts using an ion selective electrode (ISE) and a Cardy meter. The 95% confidence intervals for regression line parameters are: 0.34 to 1.36 for the slope and -6.27 to 11.25 for the y-intercept ($1 \text{ mg}\cdot\text{L}^{-1} = 1 \text{ ppm}$).

strong relationship between the Cardy meter and ISE analyses of the same dry plant tissue extracts suggests that the Cardy meter could substitute

for ISE or colorimetric analysis of dry tissue extracted with $\text{Al}_2(\text{SO}_4)_3$. The drying, grinding, and extracting of dry plant tissues with $\text{Al}_2(\text{SO}_4)_3$

negates many of the purported benefits of the Cardy meter as a tool for rapid analysis of fresh plant sap and would not be feasible in a field setting for leafy green vegetables.

Summary and conclusions

Ion selective electrodes are accurate, easy to use, and less expensive than flow injection colorimetric instruments, making them an appealing substitute for analyzing large number samples for $\text{NO}_3\text{-N}$. However, in this study, rapid analysis of fresh leaf sap with a Cardy NO_3 meter did not compare well to dry leaf tissue samples extracted and analyzed with an ISE or colorimetric procedure. Other researchers found that the Cardy meter can accurately measure $\text{NO}_3\text{-N}$ concentration in the tissue of other species, but it did not provide acceptable results for the leafy green vegetables included in this study. We were unable to determine if this was the result of a difference in tissue processing method (fresh leaf sap versus dry leaf tissue extract) or the analytical instruments used.

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