

# Testing Petiole Sap for Nitrate and Potassium: A Comparison of Several Analytical Procedures

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**Abstract.** An important aspect of establishing critical sap nutrient concentrations for diagnostic purposes is to determine the accuracy of the analytical method used. We compared a Cardy flat membrane NO<sub>3</sub> electrode, a Hach portable NO<sub>3</sub> electrode, and a Wescan N analyzer for their ability to determine NO<sub>3</sub> concentrations in sap of potato (*Solanum tuberosum* L.) petioles. The Hach and Wescan instruments require diluted sap, while nondiluted sap can be used with the Cardy. Nitrate-N concentrations in nondiluted petiole sap measured with the Cardy electrode were 90 to 120 mg·L<sup>-1</sup> higher than the other two methods. Using sap diluted with 0.075 M aluminum sulfate tended to lower Cardy NO<sub>3</sub> readings to concentrations closer to the other methods, but made the procedure more complicated for practical use. We also compared a Cardy K electrode with flame emission spectroscopy for determining K concentrations in sap. Using nondiluted sap with the Cardy procedure resulted in K concentrations 200 to 2500 mg·L<sup>-1</sup> lower than those determined by flame emission, depending on K concentration of the sap. Diluting sap with 0.075 M aluminum sulfate or deionized water for use with the Cardy electrode resulted in K concentrations similar to those determined by flame emission.

Among the recent techniques for N and K management in vegetable crops has been the use of petiole sap analysis to determine supplemental fertilizer needs (Hochmuth, 1994; Prasad and Spiers, 1984; Scaife and Turner, 1984). Sap tests to determine nutrient status of crops have been used to a limited degree since the 1920s (Hochmuth, 1994; Jones et al., 1991). Until recently, however, these tests have been considered semi-quantitative, at best. Within the past 15 years, advances have been made in determining sap NO<sub>3</sub> and K in various crops using Merck (Darmstadt, Germany) EM Quant test strips (Coltman, 1987a, 1987b; Coltman and Riede, 1992; Prasad and Spiers, 1984). Critical values for NO<sub>3</sub> levels using the EM Quant strips in tomatoes (*Lycopersicon esculentum* Mill.) (Coltman, 1988; Huett and Rose, 1988) and potatoes (Williams and Maier, 1990) have been established. According to Williams and Maier (1990), EM Quant strips require dilution of the sap and a portable reflectometer to obtain accurate readings and strips have to be periodically checked for variability between and within vials.

Ion-specific electrodes have been available for many years; however, portability has

always been a problem for a quick test application. Scaife and Stevens (1983) compared EM Quant strips with electrode methods and found that NO<sub>3</sub> concentrations obtained with the test strips were lower than those obtained with the electrode. In that study, however, differences in sample preparation may have contributed to differences in the results. Both the Hach Company (Loveland, Colo.) and Horiba Instruments (Kyoto, Japan) have commercially introduced portable NO<sub>3</sub> electrodes capable of determining NO<sub>3</sub> in plant sap. Horiba Instruments has also introduced a portable flat membrane K electrode. The Hach electrode performs in a similar manner to conventional electrodes in that the sample must be diluted to obtain a reading. Vitosh and Silva (1994) have shown that sap NO<sub>3</sub> determined by the Hach NO<sub>3</sub> electrode is highly correlated with petiole NO<sub>3</sub> expressed on a dry weight basis.

The electrode introduced by Horiba Instruments, called the “Cardy meter,” has a flat membrane capable of providing a reading for either NO<sub>3</sub> or K concentration in nondiluted sap. Researchers using the Cardy NO<sub>3</sub> electrode with nondiluted sap have also shown that sap NO<sub>3</sub> is correlated to petiole NO<sub>3</sub> expressed on a dry weight basis (Hartz et al., 1993; Hochmuth, 1994; Westcott et al., 1993). The Cardy K meter has been used to establish sufficiency levels of K in petiole sap for eggplant (*Solanum melongena* L.) production (Hochmuth et al., 1993).

While concentrations of NO<sub>3</sub> and K are readily obtained using the electrode methods discussed above, no studies have been conducted to determine the accuracy of the measurements taken. Knowing whether the values obtained with the different techniques are the

“true” values or if they are unique to the instrument or methodology is important when establishing critical values. If the instrument used is precise but not accurate, then the critical values reported would be useful only to those using that particular instrument. However, if the instrument used provides accurate and precise values, then the methods or instruments used to establish critical values are not a significant factor, giving those critical values wider application.

The overall objectives of our experiments were to evaluate the accuracy and performance of 1) the Hach and Cardy NO<sub>3</sub> electrodes for determining NO<sub>3</sub> in petiole sap and 2) the Cardy K electrode for determining K in petiole sap.

## Materials and Methods

**Nitrate.** A 4-year field study on irrigated ‘Russet Burbank’ potatoes was used to obtain sap for testing the NO<sub>3</sub> electrodes. The study was established at the Sand Plain Research Farm at Becker, Minn., on a Hubbard loamy sand (Udic Haploboroll). Ten treatments comprised of four N rates as urea or ammonium nitrate ranging from 0 to 270 kg·ha<sup>-1</sup> at various times of application were tested. Each treatment was replicated four times in a randomized complete-block design. Fifteen to 30 recently matured leaves (fourth or fifth from terminal) were collected from each plot at 7- to 14-day intervals from mid-June until mid-August. A total of 240 samples were collected each year. Leaflets were immediately stripped in the field and the petioles were placed in a plastic bag in a cooler. Within 6 h of collection, petioles were crushed with a Hach press to express the sap. Expressed sap was stored frozen in plastic vials until all analytical procedures could be performed. From a practical standpoint, freezing is not recommended, but because of the many samples to be processed in this study, freezing was necessary to preserve the sap. Freezing did not significantly affect sap NO<sub>3</sub> concentrations (data not presented). After thawing, sap was mixed in the vial, pulp was allowed to settle, and sap was decanted into a clean vial for subsequent NO<sub>3</sub> determinations.

Two instruments designed for NO<sub>3</sub> sap tests were compared: the Hach NO<sub>3</sub> electrode and the Horiba/Cardy NO<sub>3</sub> electrode. Nitrate also was determined conductimetrically using a Wescan (nonportable) N analyzer. This instrument was chosen because it is not sensitive to the same interferences often observed with NO<sub>3</sub> electrodes. All NO<sub>3</sub> standards were prepared with sodium nitrate.

For the Hach sap test, 1 mL of expressed sap was mixed with 25 mL of a 0.075 M aluminum sulfate [Al<sub>2</sub>(SO<sub>4</sub>)<sub>3</sub>·(H<sub>2</sub>O)<sub>18</sub>] solution. The electrode was immersed in the solution and an mV reading was recorded. Standards with NO<sub>3</sub>-N at 10 and 100 mg·L<sup>-1</sup>, prepared with 0.075 M aluminum sulfate background, were used to calibrate the instrument. Unknown readings were related to NO<sub>3</sub>-N concentration (activity) in solution using the Nernst equation (Skoog and West, 1976).

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For the Cardy sap test, a small volume ( $\approx 0.1$  mL) of nondiluted sap was placed directly on the electrode membrane and a direct reading of  $\text{NO}_3\text{-N}$  was recorded. The Cardy meter has three scales depending upon the concentration of the sample. Concentrations of  $\text{NO}_3\text{-N}$  between 0 and 100 can be read to the nearest  $1 \text{ mg}\cdot\text{L}^{-1}$ , those between 100 and 1000 are read to the nearest  $10 \text{ mg}\cdot\text{L}^{-1}$ , and those between 1000 and 10000 are read to the nearest  $100 \text{ mg}\cdot\text{L}^{-1}$ . The instrument was calibrated with  $\text{NO}_3\text{-N}$  standards at 34 and  $450 \text{ mg}\cdot\text{L}^{-1}$  with a deionized water background, similar to standards prepared by Horiba Instruments. Although many sap values were above the  $450 \text{ mg}\cdot\text{L}^{-1}$  range, the slope above this range is linear up to  $2000 \text{ mg}\cdot\text{L}^{-1}$  (Horiba Instruments, personal communication). The electrode membrane (sensor) was replaced with a new membrane at the beginning of each growing season.

For the Wescan sap test, 1 mL of sap was mixed with deionized water to a volume of 100 mL. Nitrate-N in solution was determined conductimetrically after reduction to  $\text{NH}_3$  using granular zinc (Carlson, 1986; Carlson et al., 1990). Readings from diluted solutions were related to  $\text{NO}_3\text{-N}$  in the sap using a standard curve. Standards ranged from 0 to  $28 \text{ mg}\cdot\text{L}^{-1}$  in a deionized water background.

The standard addition technique was used to evaluate the accuracy of each method. Frozen sap collected over 1 year was thawed, bulked into one large sample, and then mixed thoroughly. Standard additions of  $\text{NO}_3\text{-N}$  at 200, 400, 600, 800, and  $1000 \mu\text{g}\cdot\text{mL}^{-1}$  sap were made by weighing the required amount of sodium nitrate in a 25-mL volumetric flask and adding enough sap to bring up to volume. All  $\text{NO}_3$  determinations were made in triplicate using the procedures described above. An additional dilution procedure was included for the Cardy  $\text{NO}_3$  determinations. For the dilution, 0.5 mL of sap was mixed with 2 mL  $0.075 \text{ M}$  aluminum sulfate solution.  $\text{NO}_3\text{-N}$  standards of 34 and  $450 \text{ mg}\cdot\text{L}^{-1}$  also were prepared with aluminum sulfate. A small volume of the mixture was placed directly on the electrode and a concentration reading was recorded.

**Potassium.** A 2-year field study on irrigated 'Russet Burbank' potatoes was used to obtain sap for determining K concentrations. The study was established at the Sand Plain Research Farm on a Hubbard loamy sand. Nine treatments comprised of five K rates as potassium chloride or potassium nitrate ranging from 0 to  $300 \text{ kg}\cdot\text{ha}^{-1}$  at various times of application were tested. Each treatment was replicated four times. Petiole and sap collection were similar to that described for the  $\text{NO}_3$  study.

Potassium determination using the Cardy electrode was compared to conventional methods using an atomic absorption spectrophotometer in the flame emission mode (model 3030B; Perkin Elmer, Norwalk, Conn.). Nondiluted and diluted sap preparations also were compared using the Cardy electrode. All K standards were prepared with potassium chloride.

For the nondiluted sap preparations, the Cardy electrode was calibrated using two stan-

dard K solutions, 150 and  $2000 \text{ mg}\cdot\text{L}^{-1}$ , in a deionized water background. A few drops of nondiluted sap were placed on the electrode membrane and a direct reading of K concentration was recorded. As with the  $\text{NO}_3$  electrode, the K electrode has three scales, depending on concentration of the sample. Accuracy of each scale for K is the same as described for  $\text{NO}_3$ . For the diluted preparations, 1 mL of sap was diluted to a volume of 10 mL with  $0.075 \text{ M}$  aluminum sulfate solution. Standards with K at 150 and  $2000 \text{ mg}\cdot\text{L}^{-1}$ ,

prepared with aluminum sulfate, were used to calibrate the electrode. A few drops of the diluted solution were placed on the electrode membrane and a direct reading of K was recorded. For K determination using the spectrophotometer, sap was diluted 1:100 with water and Cs at  $1 \text{ g}\cdot\text{L}^{-1}$  was added to suppress ionization. The emission mode set at  $669.9 \text{ nm}$  was used to determine K concentration in solution. Standards ranged from 0 to  $80 \text{ mg}\cdot\text{L}^{-1}$  in a deionized water background with Cs.

As with  $\text{NO}_3$ , the standard addition tech-

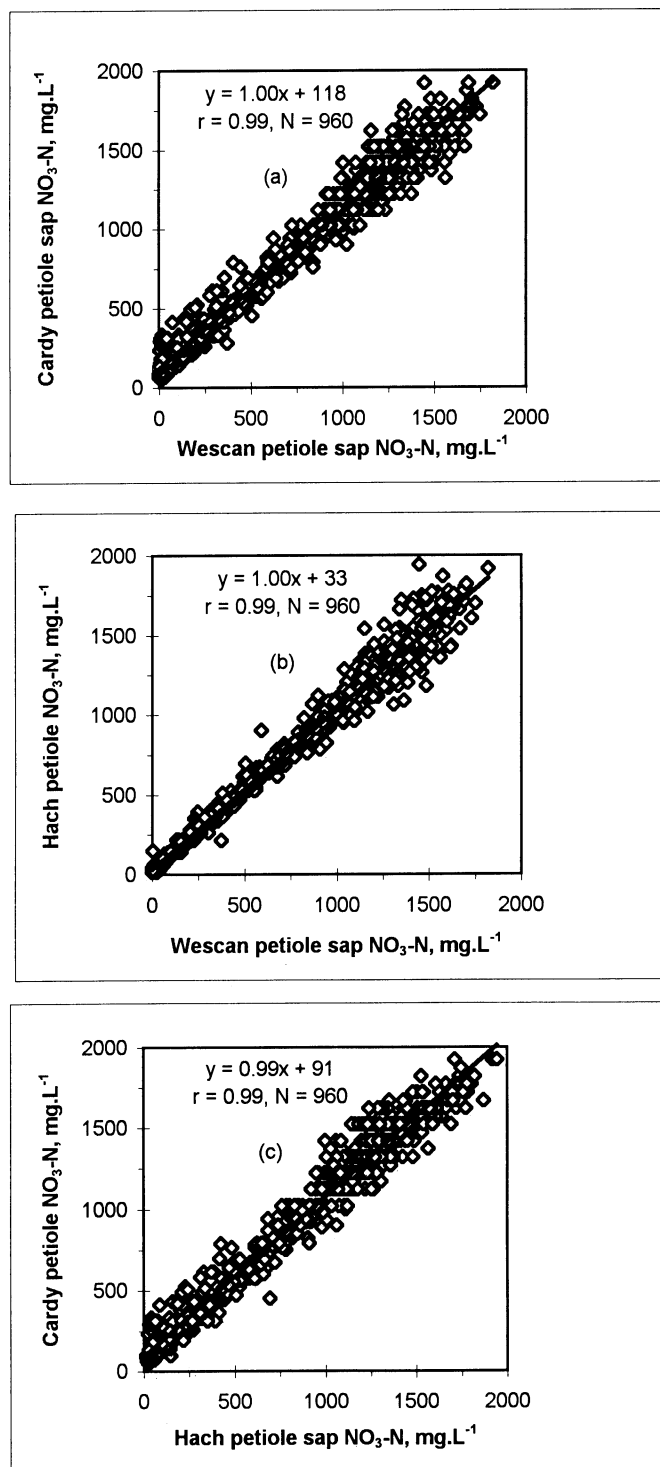


Fig. 1. Correlation between the various analytical techniques used for nitrate determination in petiole sap: a = Wescan vs. Cardy; b = Wescan vs. Hach; c = Hach vs. Cardy.

nique was used to evaluate the accuracy of each method. The same procedure was used to obtain a bulked sap sample. Standard K additions of 400, 800, 1200, 1600, and 2000  $\mu\text{g}\cdot\text{mL}^{-1}$  sap were made by weighing the required amount of potassium chloride in a 25-mL volumetric flask and adding enough sap to bring to volume. All K determinations were made in triplicate using the procedures described above. An additional dilution procedure was included for the Cardy procedure determinations. For the dilution, 1 mL of sap was mixed with 10 mL water. K standards of 150 and 2000  $\text{mg}\cdot\text{L}^{-1}$  in a deionized water background were used for calibration. A small volume of the mixture was placed directly on the electrode membrane and a concentration reading was recorded.

**Statistical analyses.** For both studies, linear regression using the least squares method was used to generate the relationships among the various methods (Steele and Torrie, 1980). Differences among slopes and intercepts were determined using 95% confidence intervals.

## Results and Discussion

Tuber yield increased quadratically with N and K fertilizer (data not presented). In these studies sap concentrations of  $\text{NO}_3$  and K spanned from below to above the optimum range.

**Nitrate.** A series of correlations among the three methods for sap  $\text{NO}_3$  determination are based on 4 years of data covering a wide range of sampling dates and N fertilizer treatments (Fig. 1a-c). All three methods were highly correlated. Slopes of the regression lines comparing the methods were not significantly different from unity ( $P < 0.05$ ). The intercepts were all significantly different from 0 and from each other ( $P < 0.05$ ). These results suggest that the lines are parallel over the concentration ranges covered and the effects of method on  $\text{NO}_3$ -N determination are estimated by differences in the intercept. Therefore, sap  $\text{NO}_3$ -N concentrations measured with the Cardy meter were 118  $\text{mg}\cdot\text{L}^{-1}$  higher than those for the Wescan and 91  $\text{mg}\cdot\text{L}^{-1}$  higher than for the Hach. The Hach measurements were slightly higher (33  $\text{mg}\cdot\text{L}^{-1}$ ) than those determined by the Wescan.

Recovery of  $\text{NO}_3$  added to bulk sap ranged from 99% to 104% for the Wescan method, 100% to 105% for the Hach, 100% to 106% for the Cardy nondiluted sap preparation, and 96% to 108% for the Cardy diluted sap preparation (Table 1). The zero standard errors associated with Cardy readings from nondiluted sap are due to the fact that when  $\text{NO}_3$ -N is above 1000  $\text{mg}\cdot\text{L}^{-1}$ , the meter is accurate to the nearest 100  $\text{mg}\cdot\text{L}^{-1}$ . Therefore, the  $\text{NO}_3$ -N concentrations can vary by as much as  $\pm 50$   $\text{mg}\cdot\text{L}^{-1}$  and the reading on the Cardy meter would be the same. Differences between the Wescan values and the Cardy nondiluted values are similar to those found in the 4-year study. Hach values were higher than expected but still lower than those for Cardy nondiluted sap preparation. Diluting the sap for the Cardy method generally resulted in lower  $\text{NO}_3$ -N

Table 1. Recovery of nitrate added to potato petiole sap by four analytical techniques.

Nitrate added ( $\text{mg}\cdot\text{L}^{-1}$ )	Method							
	Cardy, nondiluted		Cardy, diluted		Hach		Wescan	
	Found ( $\text{mg}\cdot\text{L}^{-1}$ )	Recovered (%)	Found ( $\text{mg}\cdot\text{L}^{-1}$ )	Recovered (%)	Found ( $\text{mg}\cdot\text{L}^{-1}$ )	Recovered (%)	Found ( $\text{mg}\cdot\text{L}^{-1}$ )	Recovered (%)
0	900 $\pm$ 0 <sup>a</sup>	---	875 $\pm$ 14	---	882 $\pm$ 17	---	800 $\pm$ 5	---
200	1100 $\pm$ 0	100	1067 $\pm$ 17	96	1092 $\pm$ 8	105	1008 $\pm$ 1	104
400	1300 $\pm$ 0	100	1283 $\pm$ 8	102	1282 $\pm$ 3	100	1217 $\pm$ 12	104
600	1533 $\pm$ 34	106	1525 $\pm$ 14	108	1497 $\pm$ 2	103	1398 $\pm$ 11	100
800	1733 $\pm$ 34	104	1717 $\pm$ 8	105	1700 $\pm$ 6	102	1594 $\pm$ 12	99
1000	1933 $\pm$ 34	103	1900 $\pm$ 29	103	1922 $\pm$ 2	104	1801 $\pm$ 17	100

<sup>a</sup>mean  $\pm$  standard error

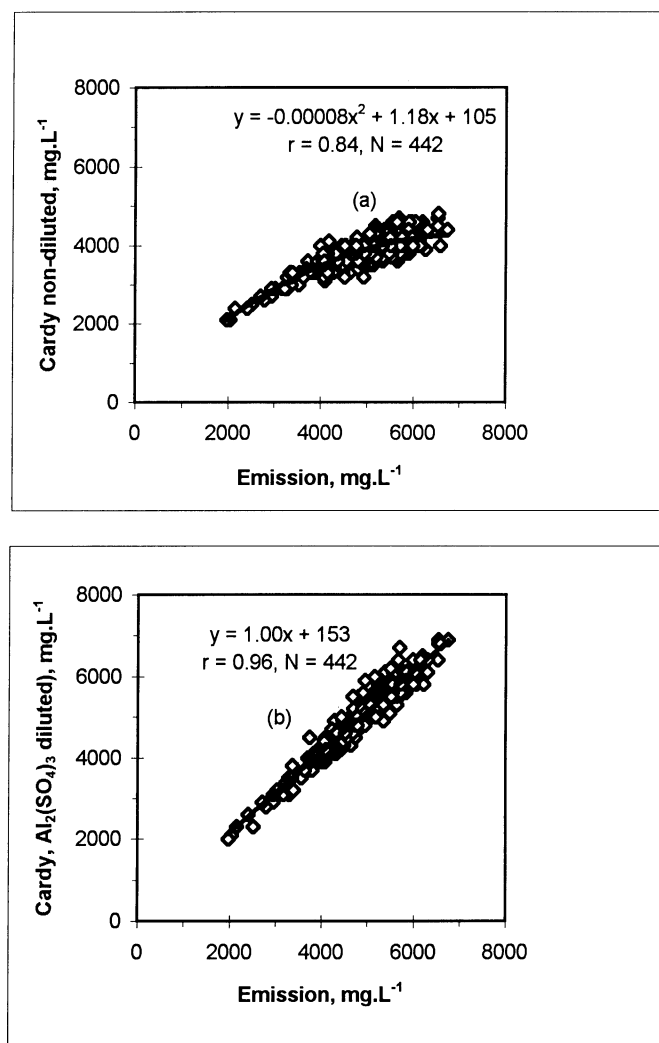


Fig. 2. Correlation between the various analytical techniques used for potassium determination in petiole sap: (a) Emission vs. Cardy, nondiluted sap; (b) Emission vs. Cardy, sap diluted with 0.075 M aluminum sulfate.

concentrations than nondiluted sap. These results support the conclusion that using nondiluted sap will, on average, result in slightly elevated values. The question of whether dilution is worth the extra effort needs to be considered when deciding on a method of sap  $\text{NO}_3$ -N determination. If accurate sap  $\text{NO}_3$ -N readings are desired, then we recommend use of diluted sap. However, this added step may introduce error and makes the procedure less convenient for field use. The overall recovery of  $\text{NO}_3$  using nondiluted sap was in an acceptable range, suggesting that the extra effort of diluting may not be necessary.

**Potassium.** The results with the Cardy K electrode were not as encouraging as with the  $\text{NO}_3$  electrodes. Nondiluted sap K concentrations determined by the Cardy meter were quadratically correlated with diluted sap K concentrations determined by flame emission (Fig. 2a). In the range of K concentrations studied, use of nondiluted sap was useful for determining sap K concentrations in the range of 2000 to 3000  $\text{mg}\cdot\text{L}^{-1}$ . However, use of nondiluted sap underestimated K concentrations ( $\text{mg}\cdot\text{L}^{-1}$ ) by 250 to 1000 when sap K concentrations were 3500 to 5000 and by 1000 to 2500 when they were 5000 to 7000. Diluted

sap K concentration measured by the Cardy meter corresponded much more closely to the K concentrations determined by flame emission (Fig. 2b), although the Cardy meter consistently overestimated K concentrations by  $\approx 150 \text{ mg}\cdot\text{L}^{-1}$  within the sap K concentration range studied. While the intercept of  $150 \text{ mg}\cdot\text{L}^{-1}$  was statistically different from zero ( $P > 0.05$ ), the error over the range of sap K concentrations studied ranged from 2% to 8%.

Hochmuth (1994) reported petiole sap K sufficiency ranges of 2500 to 5000  $\text{mg}\cdot\text{L}^{-1}$  depending on potato growth stage using the Cardy meter with nondiluted sap. Our K concentrations for potato petiole sap ranged from 2000 to 5000  $\text{mg}\cdot\text{L}^{-1}$  (Fig. 2) for nondiluted sap, but the flame emission data showed that the actual values ranged from 2000 to 7000  $\text{mg}\cdot\text{L}^{-1}$ . The nondiluted values may still be useful, but only for establishing a relative scale for the Cardy meter.

Standard addition of K to bulk sap resulted in poor recovery of K (69% to 92%) when nondiluted sap was used, with lowest recovery at the higher rate of standard addition (Table 2). Recovery of K added to bulk sap ranged from 98% to 110% for flame emission when sap was diluted with water, 96% to 106% for the Cardy K electrode when sap was diluted with 0.075 M aluminum sulfate, and 92% to 108% for the Cardy K electrode when sap was diluted with water. At concentrations  $< 3000 \text{ mg}\cdot\text{L}^{-1}$ , dilution may not be necessary; however, for potato petioles where sap K concentrations are often  $> 3000 \text{ mg}\cdot\text{L}^{-1}$ , the sap should be diluted to obtain an accurate K concentration reading.

The higher recovery of K with the Cardy electrode when sap was diluted, either with water or 0.075 M aluminum sulfate, may, in part, be the result of a reduction in K activity due to the high ionic strength of nondiluted sap (Skoog and West, 1976). In dilute solutions ( $< 0.01 \text{ M}$ ), the concentration is about equal to the activity, assuming unity for the activity coefficient. As the ionic strength increases, the activity coefficient decreases, which, in turn, will decrease activity. However, the Cardy  $\text{NO}_3$  electrode tended to result in slightly higher  $\text{NO}_3$  concentrations in nondiluted sap compared to diluted sap. If ionic strength were causing the decrease in activity,  $\text{NO}_3$  should have been affected as well, since the molar concentrations of  $\text{NO}_3$  (0.07 to 0.14 M) were similar to the molar concentrations of K (0.08 to 0.18 M). Reasons why the Cardy  $\text{NO}_3$  meter response was not the same as the Cardy K

Table 2. Recovery of potassium added to potato petiole sap by four analytical techniques.

Potassium added ( $\text{mg}\cdot\text{L}^{-1}$ )	Method							
	Cardy nondiluted		Cardy diluted in water		Cardy diluted in $\text{Al}_2(\text{SO}_4)_3$		Emission	
	Found ( $\text{mg}\cdot\text{L}^{-1}$ )	Recovered (%)	Found ( $\text{mg}\cdot\text{L}^{-1}$ )	Recovered (%)	Found ( $\text{mg}\cdot\text{L}^{-1}$ )	Recovered (%)	Found ( $\text{mg}\cdot\text{L}^{-1}$ )	Recovered (%)
0	$3967 \pm 33^z$	---	$5567 \pm 59$	---	$5467 \pm 30$	---	$5512 \pm 34$	---
400	$4333 \pm 66$	92	$6000 \pm 0$	108	$5850 \pm 48$	96	$5904 \pm 21$	98
800	$4600 \pm 0$	79	$6300 \pm 0$	92	$6317 \pm 43$	106	$6298 \pm 28$	98
1200	$4800 \pm 0$	69	$6767 \pm 70$	100	$6650 \pm 48$	99	$6699 \pm 23$	99
1600	$5100 \pm 58$	71	$7167 \pm 30$	100	$7150 \pm 70$	105	$7279 \pm 48$	110
2000	$5367 \pm 66$	70	$7550 \pm 32$	99	$7567 \pm 47$	105	$7501 \pm 38$	99

<sup>z</sup>mean  $\pm$  standard error.

meter response can not be determined from this study.

### Conclusions

In conclusion, the quick tests used to determine  $\text{NO}_3$  and K in petiole sap can provide accurate readings when diluted sap is used. Testing of nondiluted sap with the Cardy  $\text{NO}_3$  meter can result in  $\text{NO}_3$  concentrations slightly higher compared to methods using dilution. However, for practical purposes, the error involved with using nondiluted sap does not appear to be large enough to warrant the added effort of dilution. For the K Cardy meter, use of nondiluted sap can result in K concentrations 200 to 2500  $\text{mg}\cdot\text{L}^{-1}$  lower compared to diluted sap, with greatest discrepancy at higher K sap concentrations. Dilution of the sap is, therefore, necessary to determine accurate sap K concentrations.

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