Rapid Determination of Nitrate Nitrogen in Sugarbeets with the Specific Ion Electrode[1]

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Nitrogen fertilization for sugarbeets should be as accurate as possible because of the close inverse relation between nitrogen fertility and sucrose content (3,7,8,9)[3]. Determining the optimum nitrogen fertility level by soil tests and past cropping histories is not entirely reliable (3). Consequently, plant tissue analysis for nitrate-nitrogen (NO_3-N) frequently is used to supplement soil tests to guide fertilization and harvesting schedules (3). A "critical" NO_3-N concentration of approximately 1000 ppm on the dry petiole weight basis is desirable 4 to 6 weeks before harvest (7). A yield response to additional nitrogen can be expected if the NO_3-N in the petioles falls below this level; the earlier in the season the critical level is reached, the more probable the yield response. Also, a delay in harvest may be recommended under some conditions if the petiole nitrate remains above the critical level.

The success of using petiole analysis for fertility and harvest planning to a large extent depends upon rapid and accurate analysis so that the results can be obtained in a minimum of time. Nitrate-N in plant material usually is determined colorimetrically by the phenoldisulfonic acid (PDA) method (4). After obtaining an extract from the dried and ground plant material, about eight separate operations are necessary to develop the color for the NO_3-N determination. These operations involve considerable time. A less complicated, but accurate procedure for determining NO_3-N in plant material would substantially contribute to the routine use of plant analysis for fertility and harvest planning. The objective of the study was to determine the adaptability and accuracy of the nitrate specific ion electrode for plant nitrate analysis.

Materials and Methods

Plant preparation

Sugarbeet petiole, leaf, or root samples were dried in a forced-draft oven at 65°C and ground to pass a 40-mesh screen in a stainless steel equipped Wiley mill.

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[3] Numbers in parentheses refer to literature cited.

Phenoldisulfonic acid procedure

The PDA procedure described by Johnson and Ulrich (4) was used to determine NO_3-N colorimetrically. Nitrate was determined with a Coleman, Jr. Model 620 spectrophotometer.

Electrode procedure

Equipment: An expanded-scale pH meter equipped with a nitrate specific ion electrode and a reference electrode was used.

An expanded-scale pH meter, preferably a research model, is necessary for accurate work in this procedure. An Orion Model 801 digital pH meter with the Orion nitrate ion electrode, Model 92-07, and a Corning calomel reference electrode were used. Equipment with equivalent features should produce satisfactory results.

Instrument calibration: Some electrode "drift" occurs with the NO_3-N specific ion electrode. Therefore, it is necessary to calibrate the procedure carefully. The method of calibration and the range in NO_3 concentration will depend upon the particular potentiometer (pH-meter) used. The calibration procedure can be developed from the instrument instruction manual. Generally, the instrument is recalibrated for each reading as the standard curve is constructed. Recalibration between each separate "unknown" determination generally is not necessary. With the equipment in this laboratory, little drift occurred if the instrument was recalibrated to a reference standard after four or five readings of unknowns.

Some time must be allowed for the electrodes to equilibrate with the solution. An exact time for the equilibration improves the reproducibility of the results. For the procedure reported here, the NO_3-N and reference electrodes were placed in the standard or extract solution and shaken gently for exactly 30 seconds; then the millivolt reading was recorded exactly 1 minute after the shaking was stopped.

Since electrode potentials are affected by temperature changes, standard and unknown solutions should be within 1 or 2°C. If the room temperature varies more than ± 2°C from the temperature at which the calibration curve is made, a new standard curve may be required unless a slope correction device is built into the potentiometer.

Reagents: 1) Nitrate extracting solution — 0.0122 *M* Ag_2SO_4 (3.5 g Ag_2SO_4 dissolved and diluted to 1 liter with deionized or distilled water), 2) Stock NO_3-N solution — 1000 ppm NO_3-N in 0.0112 *M* Ag_2SO_4 solution, and 3) NO_3-N standard calibration solutions — 1 to 1000 ppm NO_3-N in 0.0112 *M* Ag_2SO_4.

Procedure: Weigh 1.00 g of ground plant material, transfer

to a 125 ml Erlenmeyer flask, and add 50 ml of extracting solution. Shake for 30 minutes and filter through No. 40 Whatman paper or centrifuge at 2000 rpm. Less shaking time produces inconsistent results. The millivolt reading is determined by the same procedure as for the nitrate standards during calibration. Nitrate-N concentrations of the unknowns are read directly from the standard curve.

Interferences: The NO_3-electrode has a high degree of specificity for the NO_3 ion, but it is subject to interference from a number of anions (5). The relative order of magnitude of interference of the main inorganic anions which may be extracted from plant material is bicarbonate > chloride > sulfate. Bicarbonate contents of the extracts of petioles used in this study were minor. In the proposed procedure, chloride in the plant material is precipitated as AgCl during extraction with Ag_2SO_4, thus eliminating the interference. The Ag^+ in 50 ml of extracting solution will precipitate the Cl^- in a 1-g sample containing about 4% chloride. The normal plant chloride content is generally less than 2%. Sulfate-S in the plant material is obscured by the high concentration in the extracting solution.

Paul and Carlson (6) reported that excess Ag^+ in the extract, which apparently had interacted with the calomel electrode, caused a drift in the readings accompanied by significantly low results. Drift, although annoying, is compensated for by operating in a "relative millivolt" mode with the potentiometer used in this study.

Organic staining of the electrode exchanger disk reduces the life of the disk to about a month. The addition of about 0.5 g of a decolorizing carbon to the plant sample during extraction to clarify the solution produced approximately 5% low results. The addition of decolorizing carbon in NO_3-N standards produced significantly lower results.

Experimental Methods

To assess the adaptability and accuracy of the electrode for plant analyses, a series of samples was analyzed by the PDA and electrode procedures. In one series of determinations sugarbeet petiole, leaf samples, and root samples representing a wide range in NO_3-N concentration were analyzed in quadruplicate for NO_3-N by the two procedures. Nitrate-nitrogen was determined in duplicate in the extracts by each procedure. In another non-replicated extraction series, NO_3-N was determined on a large number of petiole samples, again by both procedures.

Correlation and regression analyses of average NO_3-N contents were used to evaluate the two procedures. Error variances of

the replicated samples were statistically analyzed according to the procedure of Blaedel and Meloche (1).

Results and Discussion

Preparation of standard nitrate curve for electrode procedure: A standard curve was prepared for NO_3-N in .0112 *M* Ag_2So_4 solutions. When the NO_3-N in standard solutions was plotted against EMF (millivolts) on semi-log paper, a near linear relationship was obtained over a concentration range from 1 to 1000 ppm NO_3-N (Figure 1). There was a slight downward curvature at very low concentrations and a slight upward curvature in the range of 250-1000 ppm. If Ag_2SO_4 was omitted from the standards, the standard curve showed greater deviation from linearity in the 1-10 ppm range.

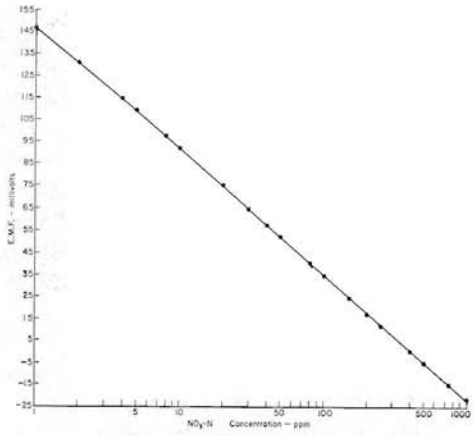

Figure 1.—Standard NO_3-N curve in 0.0112 *M* Ag_2SO_4 at 24°C.

Determination of NO_3-N in sugarbeets: Nitrate-nitrogen in petioles and leaves was determined by the electrode and PDA procedures. The NO_3-N determined with the electrode procedure was correlated with values obtained with the PDA procedure over a range in concentration in the plant material from about 100 to 22,000 ppm. The relationship between the two procedures (Figure 2) has an r^2 value of 0.998. The slope of the regression line is only slightly different from unity, thus showing close agreement between the two procedures. Statistical analysis of the quadruplicate samples revealed no significant difference between the means of the two procedures at the 5% probability level.

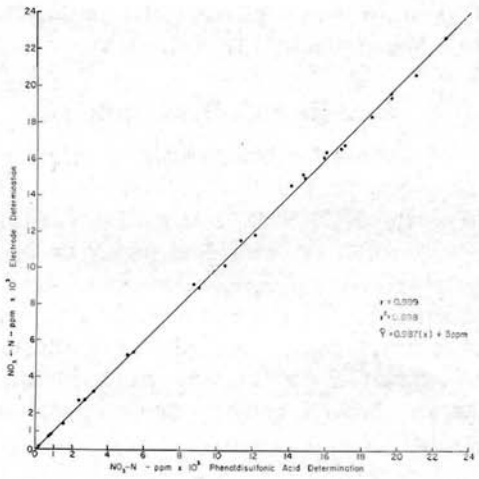

Figure 2.—The correlation between NO_3-N determined in sugarbeet petioles and leaves by electrode and phenoldisulfonic acid procedures.

Error variance was calculated to determine the relative precision of the two methods. A highly significant difference (1% probability level) was found between the error variances of the two procedures. The relative standard deviation between the two procedures is shown in Figure 3 as the coefficient of variation. With the exception of one point, the coefficient of variation for the electrode procedure was lower over the whole NO_3-N range.

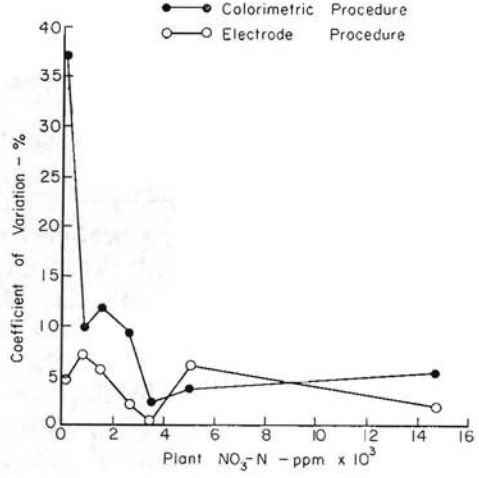

Figure 3.—The coefficient of variation in NO_3-N determined by electrode and phenoldisulfonic acid procedures for various levels of plant NO_3-N.

This indicates better precision with the electrode than with the PDA procedure. The coefficient of variation for the PDA method is quite high at low NO_3-N concentrations.

Analysis of sugarbeet roots for NO_3-N by the two procedures did not agree as well as did the results for leaves and petioles. Nitrate-nitrogen by the electrode procedure was consistently lower than by the PDA procedure. The higher nitrate content obtained with the PDA procedure for roots appears to be related to partial oxidation of amines during the peroxide treatment. It was found that standard solutions of glutamine increased in nitrate concentration approximately fourfold during peroxide digestion. Higher NO_3-N also is obtained with the electrode after digesting the root extracts in peroxide. For example, the average NO_3-N from quadruplicate samples of a sugarbeet root was 387 ppm with the PDA method and 253 ppm with the electrode method. After a partial peroxide digestion, the NO_3-N determined with the electrode was 332 ppm, an increase of 79 ppm. Amino acid contents of roots are reported to be considerably higher in roots than in leaves or petioles (2).

The electrode procedure was used routinely for NO_3-N analyses of petiole and leaf samples for 1968 and subsequent experiments. Approximately twice as many samples per day can be analyzed with the electrode as with the phenoldisulfonic acid procedure under comparable laboratory conditions. It should also be noted that the size of plant sample to be extracted has to be reduced when the plant material contains high concentrations of chloride in order to get complete precipitation as AgCl.

Summary

A specific ion electrode procedure was investigated as a rapid method for determining NO_3-N in sugarbeet petioles, leaves, and roots. The results show that precision is greater for the electrode procedure than for the phenoldisulfonic acid colorimetric procedure for petiole and leaf analysis. The results indicate greater accuracy of the electrode method for NO_3-N content of roots, because the partial oxidation of amines during the digestion step appears to cause high results by the phenoldisulfonic acid procedure.

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