NOTES & UNIQUE PHENOMENA

Using a Nitrate Specific Ion Electrode to Determine Stalk Nitrate–Nitrogen Concentration

Wallace W. Wilhelm,* S. L. Arnold, and James S. Schepers

ABSTRACT

The end-of-season stalk NO₃ test has been used to determine N sufficiency in corn (Zea mays L.). Nitrate concentration is commonly determined with flow-injection analysis (FIA), which is accurate but uses hazardous chemicals and is time-consuming. Use of a simpler method of NO₃ determination, such as the NO₃ specific ion electrode (SIE), may save time and costs, and reduce hazards. The objective of this study was to compare estimates of stalk NO₃ concentration by FIA and NO₃ SIE. For FIA, NO₃ was extracted with 2 M KCl, and the extract was filtered before analysis. For SIE, NO₃ was extracted with 0.04M (NH₄)₂SO₄, and the extract was analyzed without filtration. The slope of the linear regression between concentrations estimated by SIE and FIA did not differ from 1.0. Use of the NO₃ SIE, compared with FIA, reduces costs, sample processing, and use of hazardous chemicals.

The end-of-season corn stalk NO₃ test was proposed and advocated by Binford et al. (1990) as a method of determining if excessive or insufficient N was available to the corn crop during the latter part of the season. In the test, 20-cm segments of corn stalks (between 10 and 30 cm above the soil) are collected from several plants (=10), dried, ground, and analyzed for NO₃–N. Nitrate N concentrations less than about 700 mg kg⁻¹ plant tissue indicate that N limited grain yield; NO₃–N concentrations above 2000 mg kg⁻¹ indicate that excessive amounts of N were available to the crop (Binford et al., 1992). Other researchers have evaluated the proposed test and concur that when end-of-season stalk NO₃ concentrations are great (>2000 mg kg⁻¹), excessive levels of N were available to the crop (Varvel et al., 1997). These studies suggest that the end-of-season corn stalk NO₃ test can be used as a postmortem to determine if yield-limiting or excessive N was present. Historical knowledge of crop N need may be used by producers to guide future fertilizer-N management, thereby improving profitability and reducing environmental degradation.

In the initial publications on use of the end-of-season stalk NO₃ test, Binford et al. (1990, 1992) reported using the MgO–Devarda alloy steam-distillation procedure (Keeney and Nelson, 1982) and the Lachat¹ flow-injection procedure (Lachat Instruments, Milwaukee; Method 12-107-04-1-B) to determine NO₃ concentration in aliquots of filtered extracts prepared by shaking known weights of ground stalk material for 30 min in 100 mL of 2 M KCl. Though accurate, these analytical procedures are expensive, time-consuming, and employ hazardous chemicals (strong acids and bases and Cd).

Given that the goal of the stalk NO₃ test is to determine if stalk NO₃–N concentrations are less than 700 mg kg⁻¹ or greater than 2000 mg kg⁻¹, it seems logical that a somewhat less accurate procedure could provide essentially the same information, with the possibility of saving time and laboratory resources and avoiding safety and environmental hazard issues. A candidate procedure that is less expensive and less time-consuming, but may be less accurate, is the use of a NO₃ SIE. The object of this study was to compare stalk NO₃ concentration determined by the flow-injection method and NO₃ SIE techniques.

MATERIALS AND METHODS

Shortly after physiological maturity, stalk samples were collected from 10 corn plants in a crop sequence × inbred line × N rate experiment initiated to determine the optimum rate of N fertilizer application for hybrid seed production fields (Wilhelm and Johnson, 1997). Twenty-two (Table 1) of these samples were selected for use in this study to compare methods of determining stalk NO₃ concentration. Samples were selected a priori to represent the range of treatment combinations in the study, and therefore were assumed to provide samples covering the range of stalk NO₃ concentrations found in producers’ fields.

Stalk segments were 10 to 20 cm in length and came from the base of the stalk, from 0 to 25 cm above the soil surface. At sampling time, all plants in a 3.1-m segment of row were cut at the soil surface and moved to the field edge. Ten of these plants were selected at random and a stalk segment was taken from each. Each stalk segment was composed of one node and one internode (Fig. 1). Individuals collecting the samples estimated the fraction of total length of internode between the lowest node and the cut end of the stalk on each sampled plant. The length of internode above the lowest node needed to represent the complement of the fraction below the node was estimated and the stalk cut at that point. In the example shown in Fig. 1, about 0.3 of the internode below the lowest node remained on the stalk as it was removed from the field. To collect the equivalent of one internode, 0.7 of the internode above the lowest node was estimated and the

¹ Mention of commercial products in this paper is solely to provide specific information for the reader. It does not constitute endorsement by the USDA-ARS or University of Nebraska’s Agricultural Research Division or other products that may be suitable.

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stalk cut at that point. In so doing, each stalk segment was composed of one node and one internode, but part of the internode portion of the sample came from the internode below the node and part from the internode above the node. This sampling procedure was used so that differences in NO₃ concentration between node and internode tissue and differences in length of internodes would not influence estimates of the stalk NO₃ concentration. Stalk segments were dried at about 60°C and ground with a Wiley mill to pass a 2-mm screen before extraction and NO₃ analysis.

In this paper we will use the term FIA to mean the automated procedure for NO₃ analysis defined by Lachat Instruments (Milwaukee, WI; Method 12-107-04-1-B). This procedure is a modification of the Griess-Ilosvay method (Keeney and Nelson, 1982). Nitrate was extracted by shaking a 0.25-g sample of ground stalk tissue for 30 min with 100 mL of 2 M KCl. Extraction media were filtered through Whatman No. 1 paper before analysis with the flow-injection procedure.

For the NO₃ SIE method, 0.25 g of stalk tissue was shaken with 50 mL of 0.04 M (NH₄)₂SO₄ for 30 min. This extraction medium was chosen because it is one of many possible weak salt solutions that could be used to extract NO₃ from plant tissue and is the solution used in the outer chamber of the reference electrode. If water were used as the extraction medium, equal parts of extractant and 0.08 M (NH₄)₂SO₄ would be combined to determine NO₃ concentration with the NO₃ SIE. By using 0.04 M (NH₄)₂SO₄, the need to filter the media was also eliminated, because the electrode could be placed directly into the extraction medium to determine NO₃ concentration. Reference and NO₃ SIE (Orion Research, Boston) were placed directly into the agitating extraction media and electrometer readings observed. Readings were recorded after sequential additions of 1-mL aliquots of NO₃ interference suppressor [0.0378 M (Al₂(SO₄)₃), 0.0109 M Ag₂SO₄, 0.0257 M H₂SNO₃, and 0.0210 M H₃BO₃] produced no change in meter output. Several ions can influence the accuracy of NO₃ concentration estimates made with NO₃ SIE. The NO₃ interference suppressor was used to eliminate interference from organic anions (aluminum sulfate), halogens, cyanide and sulfide ions (silver sulfate), nitrite (sulfamic acid), and carbonate and bicarbonate ions (boric acid; Orion Research, 1980).

For both analytical methods, NO₃-N concentration in stalk tissue was calculated from a standard curve (NO₃-N on log scale) developed from known standards ranging in NO₃-N concentration from 0 to 20 mg kg⁻¹. For the FIA, standards were prepared in 2 M KCl; for the NO₃ SIE, in 0.04 M (NH₄)₂SO₄. Analysis of variance, regression analysis, and t-tests were used to determine if the two methods differed in

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<th>Inbred†</th>
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<td>Corn-soybean rotation</td>
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† Source of inbreds: FR1075 from Illinois Foundation Seed, Champaign, IL; R03 and P38 from Pioneer Hi-Bred International, Johnston, IA.
‡ N rate, kg ha⁻¹.

Fig. 1. Diagrammatic description of the method used to collect stalk segments composed of one node and approximately one internode. (An internode may be composed of a portion of the internodes below and above the node.)
RESULTS AND DISCUSSION

To be useful as an alternative method for assessing stalk NO$_3$ concentration, the NO$_3$ SIE method must have two characteristics. First, mean values must be similar to those found by methods assumed to be the standard (FIA). Secondly, estimates of NO$_3$ concentration must be repeatable.

We will address the second question first. Though we expected FIA to provide more precision than the NO$_3$ SIE, mean standard deviations (3 extractions and analyses on each of 22 samples) for the two methods were similar; 37.5 mg NO$_3$-N kg$^{-1}$ for FIA and 44.3 mg NO$_3$-N kg$^{-1}$ for the NO$_3$ SIE. Sample NO$_3$-N concentrations ranged from about 100 to 5300 mg kg$^{-1}$. These standard deviations values may seem large; however, when they were converted to coefficients of variation and expressed as percent of the mean, the precision of both methods was very acceptable (1.5% for FIA and 1.8% for NO$_3$ SIE). Visual examination of the relationship between standard deviations and means (Fig. 2) appears to show a stronger association between these parameters for the NO$_3$ SIE than for FIA. However, when linear correlation coefficients were computed the reverse was found: For the NO$_3$ SIE method, $r = 0.52$ ($P = 0.0141$, $n = 22$); for the FIA method, ($r = 0.72$, $P = 0.0002$, $n = 22$). This apparent contradiction was caused by the strong influence of five samples that showed very little variation with the NO$_3$ SIE (i.e., the five points falling on the x-axis in Fig. 2). When these points were removed, results of the correlation analysis agreed with our visual assessment. The recalculated correlation coefficient for the NO$_3$ SIE method was $r = 0.98$ ($P < 0.0001$, $n = 17$). The reason for several points having no variation is largely an artifact of the use of a digital electrometer to measure output from the NO$_3$ SIE. The meter cannot display very small differences between samples. Therefore, the meter readout was the same for all samples and the variation was calculated to be zero. The purpose of the stalk NO$_3$ test is to determine if NO$_3$-N concentrations are less than 700 mg kg$^{-1}$ or greater than 2000 mg kg$^{-1}$. Therefore, the inability to detect small differences between samples and a strong correlation between the mean and standard deviation of measurements (undesirable characteristics for analytical procedures) have little bearing on the usefulness of the technique.

To the first question: Are NO$_3$-N concentration estimates with the NO$_3$ SIE similar to those from the standard method (FIA)? Slope of the linear fit of NO$_3$ SIE estimates of stalk NO$_3$-N concentrations to those estimated with FIA was not different from 1.0 ($t = 1.25$ (NS), $\alpha = 0.05$, df = 20; Fig. 3). In addition, the $t$-test of the paired analyses indicated no bias in the estimates ($t = 0.074$ (NS), $\alpha = 0.05$, $n = 22$). Analysis of variance of stalk NO$_3$-N concentrations measured by FIA and NO$_3$ SIE indicated the two methods differed (flow injection, 2419 mg NO$_3$-N kg$^{-1}$; NO$_3$ SIE, 2467 mg NO$_3$-N kg$^{-1}$; $P < 0.001$). Though these means were different, the NO$_3$ SIE estimate is less than 2% greater than the estimate from FIA. When a difference of less than 2% is found to be significant, the results more reflect the precision of both methods than a lack of accuracy in either. These results indicate that, although absolute NO$_3$ concentration determined by the two methods may differ slightly, the relative values and their rank will be similar. Results certainly indicate that the NO$_3$ SIE can repeatedly, and reliably, be used to determine if NO$_3$-N concentrations of samples are less than 700 mg kg$^{-1}$ or greater than 2000 mg kg$^{-1}$.

In conclusion, these data indicate that stalk NO$_3$-N concentration estimated by the two methods may differ slightly. The strong relationship between results produced by the methods indicates that any discrepancy between methods would be small and within the requirements for the end-of-season stalk NO$_3$ test. In addition, savings in terms of equipment costs and time for sample preparation could be substantial. Use of hazardous
chemicals is also eliminated: There is no need for strong acids and bases, nor for the carcinogen Cd.

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REFERENCES


