Standardization of the Wheat Stem Tissue NO_3-N Test Procedure

Tim Knowles, Thomas Doerge and Mike Ottman

ABSTRACT

Current University of Arizona recommendations require periodic stem NO_3-N tests to determine nitrogen (N) fertility status of wheat crops. Lack of data on the importance of sample handling techniques, plant part selection, grinding criteria and extraction conditions have resulted in a reluctance by some growers and laboratory operators to utilize this test procedure. A laboratory study was carried out to examine factors important in wheat stem tissue analysis for NO_3-N. Sample handling, fineness of tissue grinding, and different extraction ratios were examined to determine their effects on NO_3-N recovery. Detailed partitioning of wheat plants at the 3-4 leaf, joint and boot growth stages was conducted to document which plant part is the best indicator of the N status of wheat. Optimal recovery of tissue NO_3-N existed for stem tissue separated immediately in the field and dried within 8 hours; stem tissue ground to 30 mesh or less and extracted for at least 30 minutes; and when using a sample size of 0.1000 g, in conjunction with 25 ml of extractant (i.e. 1:250 plant tissue to extractant ratio). Partitioning data confirmed current University of Arizona wheat tissue sampling guidelines which suggest sampling of the basal portion of the stem tissue.

INTRODUCTION

Nitrogen (N) is the nutrient most often limiting wheat production in Arizona. The current University of Arizona recommendations require periodic NO_3-N analyses. Therefore, many local laboratories run routine NO_3-N tissue tests. A lack of data detailing sampling and analytical procedures has caused concern over the accuracy of the wheat stem NO_3-N test.

The factors affecting standardization of the tissue test include selecting the plant part to be sampled, sample handling, grinding technique, selecting tissue/extractant ratios and choosing a determination method. The specific ion electrode is recognized as an accurate, rapid and inexpensive method of determining NO_3-N in plant tissue. Nonetheless, an evaluation of the preceding factors is needed to optimize the accuracy of laboratory tissue NO_3-N analyses for wheat.

A laboratory study was carried out to examine stem tissue NO_3-N analysis procedures of wheat grown in N fertility trials at Maricopa Agricultural Center during the 1986-87 growing season. The study had the following objectives: 1) to evaluate the effects of several sample handling techniques on NO_3-N determinations; 2) to examine the effects of fineness of sample grinding and plant tissue: extractant ratios on NO_3-N recoveries; and 3) to examine differences in the NO_3-N content of various wheat plant parts throughout the season.

MATERIALS AND METHODS

SAMPLE HANDLING STUDY

A fertility trial with "Aldura" durum wheat was conducted on a Casa Grande sandy loam to evaluate sample handling techniques and NO_3-N partitioning. Individual plots were 10 x 10 feet; fertilizer N levels of 0, 250, and 500 lbs. N/A were applied in a randomized complete block design with three replications. Sixty percent of the required N as ammonium sulfate was applied prior to planting and was disked into the surface six inches of soil. The remaining 40% was broadcast as urea immediately prior to the 5-6 leaf irrigation.

Tissue samples consisting of below ground stems from 40 randomly selected plants were obtained at the 3-4 leaf stage and were handled according to the methods shown in Table 1. Samples were ground to pass a 30 mesh screen and analyzed for NO_3-N content with a specific ion electrode using 0.1000 gm plant tissue which was extracted with 25 ml of 0.5 M $Al_2(SO_4)_3$ buffer for 30 minutes.

Table 1. Sample handling techniques imposed on Aldura wheat plants grown on a Casa Grande s.l. soil receiving various amounts of nitrogen fertilizer.

Treatment	Sample Handling Technique
1	Separate stems immediately in the field and dry* immediately
2	Separate stems immediately in the field, dry after 8 hours at 181C
3	Separate stems after 4 hours at 18±C and dry
4	Separate stems after 24 hours at 18±C and dry
5	Separate stems after 72 hours at 18±C and dry
6	Separate stems after 72 hours at 0±C and dry

* All samples were dried at 60±C

WHEAT NITRATE PARTITIONING STUDY

Whole Aldura wheat plant samples taken at the 3-4 leaf, joint and boot growth stages were partitioned into the following plants parts: 1) stem between ground level and the seed (below ground stem); 2) first two inches of stem at approximately ground level; 3) second two inches of stem above ground level; 4) leaves; and 5) shoots which included all remaining tissues. Plants were sampled from the same field experiment described in the section above for the Sample Handling Study which included N fertilizer application rates of 0, 250 and 500 lbs. N/A replicated three times in a randomized complete block design. Forty, twenty and ten plants were sampled at the 3-4 leaf, joint and boot stages of growth, respectively.

Tissue NO_3-N was determined with a specific ion electrode using 0.1000 g plant tissue ground to pass a 30 mesh screen and extracted with 25 ml of 0.5 M $Al_2(SO_4)_3$ buffer for 30 minutes.

SAMPLE GRINDING STUDY

A laboratory study was carried out on basal wheat stem tissue known to contain about 3800 ppm NO_3-N. The bulk stem sample was dried, coarse-ground and homogenized. Sub-samples, ground using a small Wiley mill to pass through 20, 30, 40 and 60 mesh screens, were extracted in triplicate using shaking times of 5, 15, 30 and 120 min. on a gyroratary shaker. The method used for NO_3-N determination is the same as that listed above for the Sample Handling Study.

PLANT TISSUE: EXTRACTANT RATIO STUDY

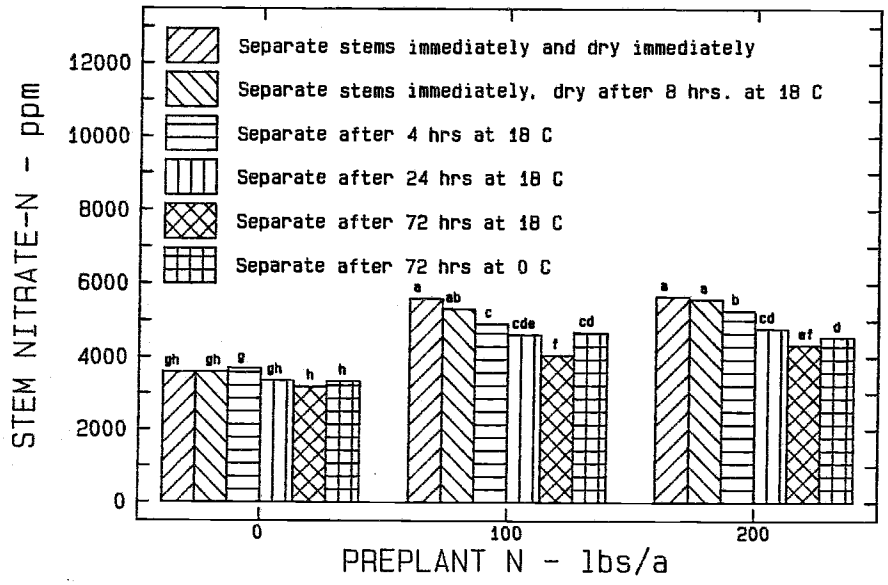
Five Aldura wheat stem samples known to contain a range of tissue NO_3-N from 300-9000 ppm were ground to pass a 30 mesh screen and extracted for 30 minutes to evaluate the effects of sample size used in conjunction with 25 ml 0.5M Al_2 $(SO4)3$ buffer extractant on NO_3-N recovery. The sample sizes used were 0.1000, 0.2000, and 0.3000 gms which resulted in extraction ratios of 1:250, 1:125 and 1:83.3, respectively. Three replications for each treatment were used in the determination of NO_3-N using a specific ion electrode.

RESULTS AND DISCUSSION

SAMPLE HANDLING STUDY

Figure 1 shows the effect of sample handling on wheat stem NO_3-N at the 3-4 leaf growth stage. Maximum NO_3-N levels were obtained when stems were separated from the wheat plant immediately following sampling and dried within 8 hours. Significant decreases in NO_3-N recovery occurred when stems were separated after 4 or more hours of storage at 18IC. Refrigerating whole plant samples seemed to prolong the permissible storage time after which significant losses of NO_3-N would be expected to occur. Nonethless, immediate separation of stem tissue is preferable to refrigeration of whole plant samples for separation at a later time.

Figure 1. Quantities of NO_3-N recovered from below ground stem tissue of Aldura wheat subjected to six sample handling techniques which was sampled at the 3-4 leaf stage and had received varying amounts of preplant N fertilizer.



WHEAT NITRATE PARTITIONING STUDY

Figure 2 shows the NO_3-N content of various plant parts at the 3-4 leaf, joint and boot stages of growth. Highest NO_3-N values indicating accumulation of NO_3-N occurred in below ground stem tissue for samples taken at the 3-4 leaf stage, and the first two inches of stem at approximately ground level for the remaining dates. All of the plant parts examined responded to fertilizer N applications.

Stem NO_3-N decreases as distance above ground level increases in wheat as shown in Figure 3 for a plant partitioned at the boot growth stage. Even newly mature heads and flag leaves show lower NO_3-N contents that lower leaves. We speculate that this is due to significant nitrate reductase activity located in chloroplasts, primarily in leaf tissue but also in wheat stems. Wheat leaf midribs have also been shown to be quite low in NO_3-N (B.R. Gardner, unpublished data). Partitioning data for NO_3-N confirm the currently recommended wheat stem sampling guidelines of the University of Arizona.

Figure 2. Quantities of NO_3-N recovered from various plant parts of Aldura wheat sampled at the 3-4 leaf (a), joint (b) and boot (c) growth stages which had received varying amounts of N fertilizer.

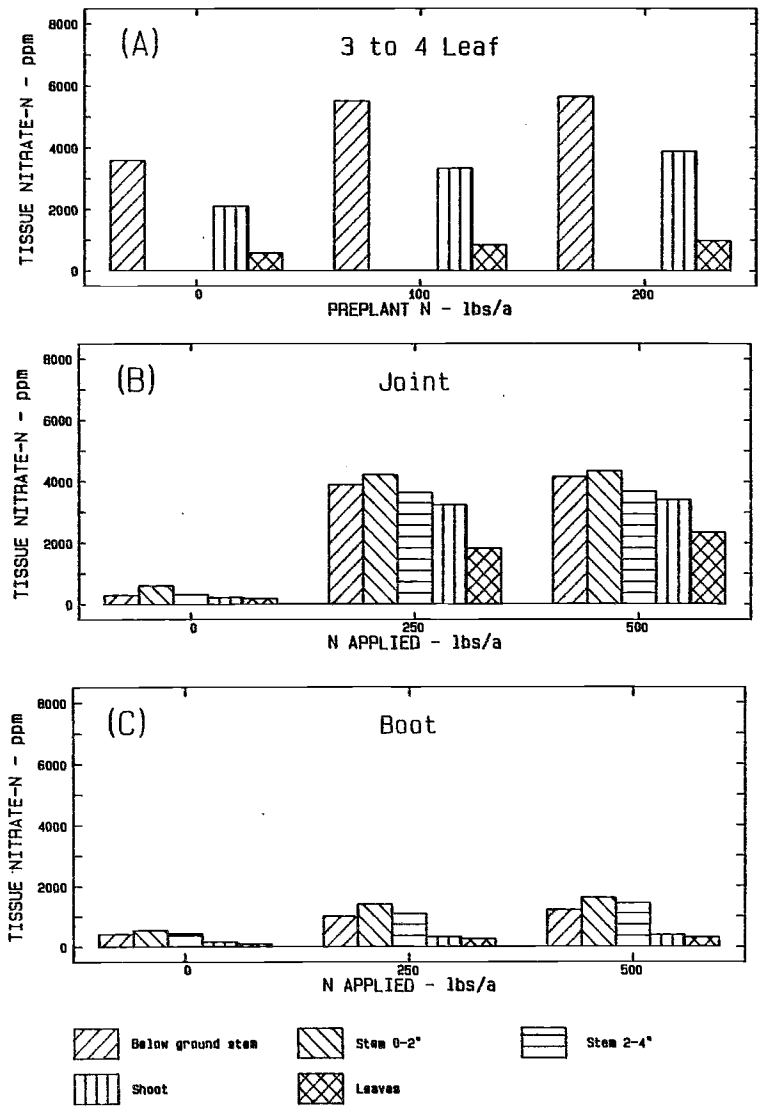
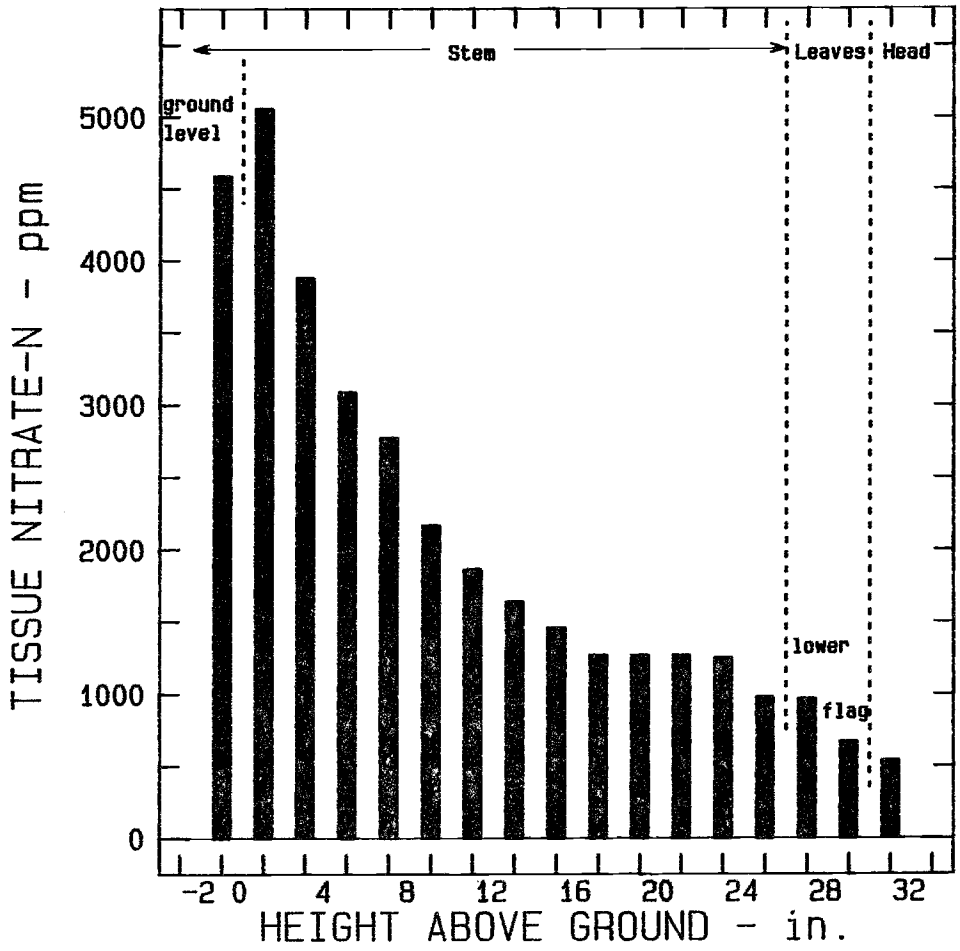


Figure 3. Levels of NO_3-N measured in various plant parts of Aldura wheat sampled at the boot growth stage.



SAMPLE GRINDING STUDY

Table 2 shows the effects of sample grinding on wheat stem NO_3-N recovery. Larger sample particle sizes required longer extraction times for maximal NO_3-N recovery. This was accomplished by passing tissue through a 20 mesh screen and shaking for 120 min., a 30 mesh screen for 30 min., or a 40 or 60 mesh screen for 15 min. Poor recoveries were obtained for all grinding treatments with a 5 min. shaking time. An additional factor, that of operator time, exists for sample processing since grinding tissue samples to pass finer screen sizes requires considerably more time per sample.

Table 2. Percent NO_3-N recovery from wheat stem tissue as affected by different sample grinding techniques and varying extraction periods.

Extraction Time	Screen Mesh Size			
	20	30	40	60
	% of NO3-N Recovery			
5	87.1 e*	89.7 cd	90.8 bc	91.6 bc
15	88.6 de	92.6 b	98.4 a	99.5 a
30	97.6 a	98.4 a	99.7 a	100 a
120	100 a	100 a	100 a	100 a

* numbers followed by the same letter are not significantly different at the 5% level according to the SNK method.

PLANT TISSUE: EXTRACTANT RATIO STUDY

Table 3 shows the effect of different extraction ratios on NO_3-N recovery. This was achieved by varying tissue sample size while keeping extractant volume constant at 25 ml. Optimal extraction rates were obtained using 0.1000 g plant tissue or a plant tissue: extractant ratio of 1:250. Maximum NO_3-N recoveries were obtained using 0.2000 and 0.3000 g tissue testing below 1800 ppm NO_3-N. Recoveries decreased with larger tissue sample sizes when stem levels exceeded 3800 ppm due to incomplete extraction. This indicates that the tendency of analysts to prefer larger sample sizes to increase analytic precision may at times decrease accuracy.

Table 3. Percent NO_3-N recovery from wheat stem tissue as affected by stem NO_3-N content and plant tissue: extractant ratio.

Sample Weight	Tissue:Extractant Ratio	Tissue NO3-N (ppm)				
		300	1800	3800	4300	9000
gm	w/v	% of NO3-N Recovery				
0.1000	1:250	100 a*	100 a	100 a	100 a	100 a
0.2000	1:125	93.5 a	100 a	94.4 b	87.9 b	94.1 b
0.3000	1:83.3	90.3 a	100 a	94.2 b	88.4 b	89.7 c

* numbers within the same column followed by the same letter are not significantly different at the 5% level according to the SNK method.