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NITRATE-N AND PHOSPHATE-P CONCENTRATION IN WINTER WHEAT AT VARYING GROWTH STAGES (1)

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ABSTRACT: Winter wheat *triticum aestivum* L.) was sampled at selected stages of growth from N and P fertility experiments at one location in 1981 and three locations in 1982. Specific growth stages were determined using the Feekes scale. Plants were separated into roots, crowns, and leaves at each growth stage, dried, ground, and analyzed for NO_3-N and PO_4-P.

Crown and leaf NO_3-N at growth stages 4 and 5 were significantly correlated with yield in 1981 at the Stillwater site. Crown and leaf NO_3-N and PO_4-P were also positively correlated with N and P applied at growth stages 4 and 5, respectively.

In contrast to 1981, no moisture stress was present during 1982 and as such contributed to lower tissue concentration of NO_3-N and PO_4-P at all stages of growth. Limited positive correlation between independent crown and leaf PO_4-P and NO_3-N concentration at all growth stages with yield was found in 1982. However, surface response modeling using NO_3-N and PO_4-P in the leaf samples at or before stage 5 with yield provided similar models for both years and locations sampled. Combined location critical levels for NO_3-N and PO_4-P in the leaves at Feekes stage 5 were 2220 and 1843 mg kg^{-1}, respectively. These combined location critical levels (restrictions defined) entered back into site-specific models accounted for 81 to 94% of predicted maximum yields by location. Root NO_3-N and PO_4-P were not affected by treatment and did not

correlate with yield or any other independent tissue variable at any stage of growth, location, or year sampled.

Data from these experiments indicate that sampling for yield prediction models should take place at or before Feekes stage 5. Crown and leaf samples for NO_3-N were both highly correlated with each other at Feekes stage 4 and 5 and were equally effective in predicting yield. This suggests that the entire above-ground portion of the plant can be used for sampling, thus eliminating the need to separate the sample into different plant parts.

INTRODUCTION

Winter wheat yields in the southern Great Plains are often reduced because of rainfall amount and distribution and other environmental factors. Efficient production requires the use of as many diagnostic techniques as possible to aid in management decisions. Fertilizer recommendations based on calibrated soil tests have been used successfully to assess the nutritional status of soils prior to planting. After the crop is planted, plant analysis offers an additional technique that may be useful in making management decisions, providing that a relationship between nutrient concentration and yield can be established. Nutrient deficiencies in plants can often be identified by plant analyses before visual symptoms appear and in some instances early enough to correct the deficiency before a significant yield reduction occurs (6,23).

Nutrient concentrations have been found to vary with plant part and growth stage (7,9). Therefore, critical levels need to be established for specific plant parts at defined growth stages. Nitrate-N in various plant parts have been used to indicate the N status of corn *(Zea mays* L.), wheat *(Triticum aestivum* L.), ryegrass *(Lolium perenne)*, cotton *(Gossypium hirsutum* L.), sugarbeet *(Beta vulgaris* L.), lettuce *(Lactuca sativa)*, radish *(Raphanus sativus)*, and spinach *(Spinacia* oleracea) (11,3,6,8,10,12,14,18,25,26). Nitrogen concentration in leaves has been shown to be influenced by both N supply and rate of plant growth (5). Nitrate-N

is accumulated in small grains during the early portion of the growth cycle and is influenced by N fertilization rates (7,16). However, this accumulation of NO3-NO3-N in the plant may be enhanced by P stress (4), Mn and Mo deficiency, and moisture stress (28,27). Because NO3-N concentration has been found to increase with increased N supply, the possibility of utilizing plant tissue analyses for N fertilizer recommendations exist. Plant tissue NO3-N testing has also been used to detect possible toxicities in research dealing with forage consumption by animals (27).

Plant samples taken at Feekes growth stage 2 to 4 (early to mid-tillering) have been shown to be the most useful in determining whether or not an adequate amount of P fertilizer has been applied (24). However, this same work demonstrated that plant samples taken after Feekes growth stage 6 (jointing) were not useful in diagnosing the P nutrition of the plant because of possible dilution effects. Differences between P fertilization rates were detected early in the sampling season, utilizing P tissue tests in irrigated spring wheat, but the value of the test for P fertilization was limited (8). Whole-plant Kjeldhal N concentration appeared to be a better tissue test when establishing critical levels for wheat versus stem NO3 concentration and crop N uptake (21).

Plant tissue analyses has been suggested to complement soil test information. Accordingly, the objectives of this study were (a) to determine the effect of N and P fertilization on both NO3-N and PO4-P in winter wheat crowns, leaves, and roots at varying stages of growth and (b) to assess the relationship of NO3-N and PO4-P in winter wheat tissue at varying stages of growth with yield.

MATERIALS AND METHODS

Winter wheat (*Triticum aestivum* L.) was sampled at selected stages of growth from one location in 1981 and three locations in 1982. Plots were arranged in a randomized complete block design with four replications at each location. Specific growth stages were determined using the Feekes scale (13). Sampling at all

locations except Haskell in 1982 took place precisely at Feekes growth stages 4, 5, 6, 7, and 10 (4 to 5 leaf stage, pseudo stem strongly erect, second node of the stem formed, boot stage), respectively. Sampling at Haskell included stages 4, 5, 6, 7, and 10. All treatments at this location could not be sampled within the same physiological stage because of the effect fertilization had on growth. A composite sample of 30 plants selected at random outside of the center 3 × 15 m strip used for yield was collected from each plot for NO_3-N and PO_4-P analyses at each stage of growth. The time interval between sampling was not consistent, due to the effect of environmental conditions on plants at the different locations. A summary of soil characteristics at experimental locations is presented in Table 1. The three sites were chosen to obtain a wide native soil fertility range. Similarly, a wide range of treatments from these long-term N-P-K experiments were selected from each site to assess the role of wheat tissue analysis. Following collection, plants were separated into roots, crowns and leaves, dried at 65-70°C, ground to pass a 20-mesh screen, and analyzed for NO_3-N by a modified Milham et al. (15) method which did not include sulfamic acid. PO_4-P was determined by the method of Murphy and Riley (17). Crowns were identified as the portion of the stem 2 cm above the roots at stages 4 and 5. Crown samples at stages 7 and 10 consisted of the 5-cm portion of the stem above the roots. Roots were clipped just below the crown immediately following sampling and were washed in deionized water before being dried and ground.

Fertilizers were applied prior to planting and consisted of ammonium nitrate (34-0-0), concentrated superphosphate (0-20-0), and muriate of potash (0-0-50) as the N, P, and K sources, respectively. A summary of the fertilization and yield response found at each location is presented in Table 2. Wheat, 'TAM 101' was seeded at a rate of 75 kg ha^{-1} in 25-cm rows between late September and early October in both years at all locations. Grain yield was determined by harvesting a 3 × 15 m strip from the center of 6 × 15 m plots, using a self-propelled combine.

TABLE 1. Initial soil characteristics at experimental locations.

Location	Soil Type	Soil Test Index*			
		pH	NO_3–N	P	K
Stillwater	Kirkland silt loam (Pachic Paleustolls)	5.8	13	27	345
Lahoma	Grant silt loam (Udic Argiustolls)	5.3	1	67	666
Haskell	Taloka silt loam (Mollic Albaqualfs)	5.1	1	21	104

*Soil test indexes were determined by pH (1:1) H_2O, NO_3–N specific ion electrode, Bray and Kurtz No. 1 P(1:20) soil:solution ratio and 1 M ammonium acetate extract, respectively.

TABLE 2. Effect of fertilizer on wheat grain yield at three locations.

Trt. #	Stillwater			Lahoma		Haskell	
	Fertilizer N–P–K kg ha^{-1}	Yield, Mg ha^{-1} 1981	1982	Fertilizer N–P–K kg ha^{-1}	Yield, Mg ha^{-1} 1982	Fertilizer N–P–K kg ha^{-1}	Yield, Mg ha^{-1} 1982
1.	0– 0– 0	1.34	1.45	0– 0– 0	1.33	0– 0– 0	0.59
2.	0–30–37	1.18	1.50	0–20–56	1.85	112– 0– 0	0.22
3.	45–30–37	2.17	1.88	22–20–56	2.42	168– 0– 0	0.16
4.	90–30–37	2.36	2.14	45–20–56	2.21	112– 0–111	0.36
5.	134–30–37*	2.76	2.12	67–20–56	2.20	112–20–111	1.38
6.	90– 0–37	2.26	1.97	90–20–56	2.00	112–40–111	1.55
7.	90–15–37	2.69	2.28	112–20–56	1.87	168–60–111	1.81
8.	90–45–37	2.42	2.03	67– 0–56	1.13	168–60–111*	2.06
9.				67–10–56	1.76		
10.				67–30–56	2.27		
11.				67–40–56	2.31		
12.				112–40–56	2.23		
13.				67–30– 0	1.90		

* – N applied in split applications.
Trt. # – Treatment number.

RESULTS AND DISCUSSION

Climate changes in the state of Oklahoma are subject to drastic change for the same location, year to year as well as at different sites within years. Moisture stress conditions, which are known to increase NO_3-N concentration (28), existed throughout the tissue sampling season in 1981. The 1982 year was characterized by an extremely cool spring along with adequate moisture during the sampling months. Due to the wet conditions at harvest in 1982, yields were reduced.

Linear or quadratic responses to N and P fertilization were found for grain yield at all three locations and both years at Stillwater (Table 3). Generally, a linear response must be significant before a quadratic response can be established. However, P rate quadratic at Stillwater in 1981 and N rate quadratic at Lahoma in 1982 were both highly significant ($PR>F <0.01$) above a non-significant linear contrast.

Simple linear regression was used to detect independent correlation between grain yield and plant tissue concentrations of NO_3-N and PO_4-P in the crowns, roots, and leaves. Root NO_3-N and PO_4-P were not correlated with yield at any stage of growth, location, or year sampled and were therefore considered to be a poor response variable. Leaf and crown NO_3-N and PO_4-P at growth stages 7 and 10 were also poorly correlated with yield at all locations and years sampled. Alternatively, significant correlations with yield were found for NO_3-N and PO_4-P in the crowns and leaves at growth stages 4 and 5 at Stillwater in 1981 (r= 0.90**, 0.86** 0.91**, 0.84** 0.53**, 0.64** 0.23^{ns}, 0.44**), respectively (ns, ** = not significant and significant at the 0.01 probability level). Work by Gardner and Jackson (8) demonstrated positive correlation of "stem" NO_3-N (Feekes stage 5) with yield while showing no relationship between yield and "stem" PO_4-P at the same growth stage. Data correlation of NO_3-N and PO_4-P in the crowns and leaves with grain yield in 1982 was markedly reduced. However, a consistent tendency for improved correlation of crown and leaf samples taken at stage 5 versus stage 4 with yield was noted across locations and years sampled. Even

TABLE 3. Analysis of variance and associated contrasts for wheat grain yield at Stillwater, 1981 and 1982, Haskell, 1982 and Lahoma, 1982.

Source of Variation	df		Stillwater 1981	Stillwater 1982	df	Lahoma 1982		df	Haskell 1982	
Treatment	7	TC	**	**	12	TC	**	7	TC	**
N–rate linear	1	(2–5)	**	**	1	(2–7)	NS	1	(1–3)	*
N–rate quadratic	1	(2–5)	**	@	1	(2–7)	**	1	(1–3)	NS
P–rate linear	1	(6,7,4,8)	NS	NS	1	(8–11)	**	1	(4–6)	**
P–rate quadratic	1	(6,7,4,8)	**	@	1	(8–11)	**	1	(4–6)	*
EMS	21		136	220	36		222	21		272
C.V., %			6	12			11			26

**,*,@ – significant at the 0.01, 0.05 and 0.10 probability levels respectively.
NS– not significant. TC – treatment numbers from Table 2 used in contrast. EMS–error mean squares.

though crown and leaf NO_3-N and PO_4-P correlation with yield was reduced in 1982, crown NO_3-N and leaf NO_3-N, and crown PO_4-P and leaf PO_4-P were consistently correlated with each other at Feekes stage 5 at Stillwater in 1981 (0.89**, 0.55**), Stillwater in 1982 (0.90** 0.57**), Haskell in 1982 (0.64**, 0.73**), and Lahoma in 1982 (0.84**, 0.91**), respectively. Both parameters were correlated with each other and demonstrated adequate correlation with yield across locations, which suggests that it is not necessary to separate plant parts (crowns and leaves) and that the entire plant, excluding the roots, should be used. Work conducted in California found that critical stem NO_3-N levels changed with both time and location sampled (19,20). However, the present literature has not thoroughly investigated the use of different plant parts. Although Gardner and Jackson (8) found no correlation between grain yield and stem PO_4-P, they did indicate that leaves might be a better plant part for P tissue analysis, as was found in this experiment. More recent work by Roth et al. (21) demonstrated that whole-plant Kjeldhal-N concentration accounted for more variation in relative yields when compared to stem (part of the plant between the roots and the collar of the first living leaf on each tiller) NO_3-N concentration, but analysis for NO_3-N in the leaves was not considered in their work.

All locations showed a linear increase in NO_3-N in the leaves with increasing N rates at Feekes stage 5 (Table 4). Similarly, either linear or quadratic response to P fertilization for PO_4-P in the leaves at Feekes stage 5 was found at all locations. Although similar responses to N and P fertilization were found for NO_3-N and PO_4-P in the leaves at Feekes stage 4, linear and quadratic correlation with yield were significantly higher at Feekes stage 5.

Surface Response Models: Surface response models by location and all locations combined for wheat grain yield were determined by using SAS (22), RSREG, G3GRID and G3D procedures, containing linear and quadratic terms for NO_3-N and PO_4-P in the leaves at Feekes stage 5 and a linear interaction term. All combinations of NO_3-N and PO_4-P in the crowns, roots, and leaves at stages 4,

TABLE 4. Analysis of variance and associated contrasts for nitrate–N and phosphate–P in leaf samples taken at Feekes stages four and five at Stillwater, 1981 and 1982, Haskell, 1982 and Lahoma, 1982.

Source of Variation	Stillwater 1981					Stillwater 1982					Haskell			Lahoma 1982				
		Stage 4		Stage 5		Stage 4		Stage 5			Stage 5			Stage 4		Stage 5		
	(df)	NL	PL	NL	PL	NL	PL	NL	PL	(df)	NL	PL	(df)	NL	PL	NL	PL	
Treatment	7	**	**	**	@	**	*	**	**	7	**	*	12	NS	**	**	**	
N–Rate L	1	**	NS	**	@	**	NS	**	**	1	**	NS	1	NS	NS	**	NS	
N–Rate Q	1	**	NS	NS	NS	**	NS	**	NS	1	NS	NS	1	NS	NS	NS	NS	
P–Rate L	1	NS	**	NS	NS	*	**	NS	*	1	NS	@	1	NS	**	NS	**	
P–Rate Q	1	**	**	NS	*	NS	NS	*	NS	1	NS	NS	1	*	**	NS	NS	
EMS	12	310	132	301	133	167	67	181	79	21	1052	199	36	204	298	517	258	
C.V.,%		15	7	28	8	41	12	49	16		20	23		16	13	12	12	

@,*,** – significant at the 0.10, 0.05, and 0.01 probability levels respectively.
NS–not significant. EMS– error mean squares.
N–Rate L – Nitrogen Rate Linear, N–Rate Q – Nitrogen Rate Quadratic.
P–Rate L – Phosphorus Rate Linear, P–Rate Q – Phosphorus Rate Quadratic.

5, 7, and 10 were evaluated as stated above. However, regression models were improved by using NO_3-N and PO_4-P in the leaves at Feekes stage 5. Corresponding regression equations, and critical levels to obtain maximum yield are listed in Table 5. All models were significant at the 0.05 probability level and accounted for 37 to 81% of the variability in wheat grain yields. Critical levels for NO_3-N and PO_4-P in the leaves were found at maximums for all individual site equations. However, the magnitude of critical levels generated from the regression models differed by location and year sampled. When data from all locations and years sampled was included in a combined model, critical levels for NO_3-N and PO_4-P in the leaves at Feekes stage 5 were 2221 and 1843 mg kg^{-1}, respectively. Critical NO_3-N levels in wheat taken at Feekes stage 4 in Arizona (8) and in California (G. S. Pettygrove, 1982, personal communication) ranged between 7000 to 12000 mg kg^{-1}. While analytical procedures were consistent, these levels were substantially higher than those found in this experiment, which possibly can be attributed to the drier climates and the high N-fertilization rates at their locations.

As indicated in Table 5, the observed range of concentration for NO_3-N and PO_4-P differed substantially among locations. In order to evaluate critical levels from the combined model within the site specific models, it was necessary to restrict the range of evaluation as to satisfy the following condition:

$$NL_{MIN} <= NL_R <= NL_C <= NL_{MAX} \text{ and } PL_{MIN} <= PL_R <= PL_C <= PL_{MAX}$$

where NL_{MIN}, PL_{MIN}, NL_{MAX}, and PL_{MAX} were the minimum and maximum NO_3-N and PO_4-P values in the leaves observed for each location, respectively, and NL_C and PL_R were the combined location predicted values for NO_3-N and PO_4-P in the leaves, and NL_R and PL_R were the restricted critical levels that met the criteria stated above. This was applied so that the selected critical level according to the defined condition could not utilize values outside of observed location specific data ranges. When the combined location critical values were entered back into the individual location models satisfying the stated conditions, predicted yields were all within 19% of the predicted location maximum yield

TABLE 5. Regression equations, significance and R^2 of NO_3–N and PO_4–P in the leaves at Feekes growth stage 5 versus wheat grain yield at 3 locations in Oklahoma.

Location	Model	R^2	Significance P(F>= f)	Critical Level	Predicted Yield At Critical Levels	Data Range NL_{MIN} NL_{MAX}	Data Range PL_{MIN} PL_{MAX}	[e]Predicted Yield using defined limits for NL_R and PL_R
				mg kg^{-1}	kg ha^{-1}	mg kg^{-1}		kg ha^{-1}
Stillwater 1981	$Y = -2122.64 + 0.237(NL) + 4.242(PL) - 0.000526(NL)^{2**} + 0.000962(PL*NL) - 0.00144(PL)^2$	0.81	0.0001	NL=2255.6 PL=2221.0	2855.3	165 2400	1340 2070	2674.2
Stillwater 1982	$Y = -1400.82 + 0.023(NL) + 11.345(PL)^{@} - 0.000209(NL)^2 + 0.00102(PL*NL) - 0.00974(PL)^2$	0.37	0.0200	NL=1686.8 PL= 670.7	2423.0	99 1700	360 790	2287.9
Haskell 1982	$Y = -4162.08 + 0.617(NL) + 6.925(PL) - 0.000074(NL)^2 - 0.000011(PL*NL) - 0.00227(PL)^2$	0.42	0.0100	NL=4053.4 PL=1517.8	2342.6	2380 7960	610 1360	2071.0
Lahoma 1982	$Y = -1710.64 + 0.014(NL) + 3.310(PL)^{**} - 0.000159(NL)^{2**} + 0.000591(PL*NL)^{*} - 0.00125(PL)^{2**}$	0.41	0.0002	NL=4439.5 PL=2367.8	2238.9	1820 5890	1360 3040	1809.6
ALL LOCATIONS	$Y = 1470.25^{**} - 0.320(NL)^{**} + 1.026(PL)^{**} - 0.00000717(NL)^2 + 0.000191(PL*NL)^{**} - 0.000393(PL)^{2**}$	0.42	0.0001	NL_C=2220.6 PL_C=1843.4	2060.4			

**,*,@ – significant at 0.01, 0.05 and 0.10 probability levels respectively.
Y = Grain Yield, kg ha^{-1}, NL = NO_3–N in leaves, mg kg^{-1}, PL = PO_4–P in leaves, mg kg^{-1}.
NL_C and PL_C – combined location predicted value for NO_3–P and PO_4–P respectively.
NL_{MIN}, NL_{MAX}, PL_{MIN}, PL_{MAX} – minimum and maximum observed values by location for NO_3–N and PO_4–P in the leaves respectively.
[e]Predicted yield using NL_R and PL_R in location specific models where $NL_{MIN} <= NL_R <= NL_C <= NL_{MAX}$ and $PL_{MIN} <= PL_R <= PL_C <= PL_{MAX}$.

using the individual site critical values. This appeared to indicate that the critical values determined from the combined location model were adequate in establishing a site specific maximum yield relationship. Since combined location predicted values for NO_3-N and PO_4-P in the leaves did not fit into all of the location data ranges, limits for NL and PL were needed to confine the determination of maximums within the observed data boundaries. In wet years, concentration is known to be lower and thus critical levels are more difficult to assess. Alternatively, dryer years provide increased concentrations and improved correlation with yield and subsequently result in a defined critical level. Even though the combined location critical levels were in excess of the observed site critical level at Stillwater in 1982, maximum yields would still be obtained using the upper range of the site model. This appears reasonable since response tended to be linear where moisture stress was not present. Interestingly, where concentration levels were higher at Haskell and Lahoma in 1982, the combined location critical levels (NLR and PLR entered into the site models, respectively) were still adequate in predicting site specific maximum yields.

Although various independent variables were evaluated, including individual plot soil tests of NO_3-N and Bray and Kurtz No. 1 P (1:20) on samples taken prior to planting, NO_3-N and PO_4-P in the leaves at Feekes stage 5 provided significant surface response models where critical levels were determined at maximums.

CONCLUSION

The locations used in this experiment represented a wide range in native soil fertility and had varying fertilizer treatments. Root NO_3-N and PO_4-P were not correlated with grain yield at any stage of growth sampled. Sampling for grain yield prediction models was most improved using crown and leaf NO_3-N and PO_4-P at or before Feekes stage 5. Data from this experiment also suggest that plant separation into crowns and leaves was not necessary and that once roots are

removed, the entire plant can be used for tissue tests of NO_3-N and PO_4-P. The simultaneous use of NO_3-N and PO_4-P in the leaves at Feekes stage 5 as a predictor variable for yield was adequate in establishing critical levels across locations and years. Although critical levels for NO_3-N and PO_4-P differed substantially by location, combined critical levels of 2221 and 1843 mg kg^{-1}, respectively, were accurate in predicting near maximum yields at all locations. Ideally, a large data base, including various locations and years, could be used to refine critical levels for NO_3-N and PO_4-P in the leaves for wheat yield prediction. Subsequent research into sidedress response from fertilizer applications after Feekes stage 5 based on an established critical level will be needed. Climatological data will also need to be evaluated relative to response and critical level differences found in this experiment.

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