COMMUN. IN SOIL SCI. PLANT ANAL., 18(5), 543-557 (1987)

# ALTERNATIVE PROCEDURE FOR TOTAL PHOSPHORUS DETERMINATION IN PLANT TISSUE[1/]

Key Words: Tissue analysis, Phosphate-phosphorus, Dilute acid Extractable $PO_4$-P, Zea mays, Triticum aestivum

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ABSTRACT

Maize (*Zea mays* L.) was sampled at five selected stages of growth from two phosphorus (P) fertility experiments in 1983 and 1984 to establish the correlation between two procedures for measuring phosphorus in plant tissue. Sampling took place at the eight leaf, twelve leaf, early silking, early denting and full maturity stages of growth. Tissue samples were analyzed for total P (TP) in a $HNO_3$-$HClO_4$ acid digest and $PO_4$-P in a dilute acid extract (DAEP). The rapid inexpensive DAEP procedure was highly correlated with TP at growth stages prior to full maturity. Limited analyses of wheat tissue at Feekes stage five also showed high positive correlation between TP and DAEP. Slopes for TP-DAEP regression equations (by stage of growth) across locations and

environments were found to be the same. Locations and years did not affect the linear TP-DAEP relationship, but did alter the respective regression equation intercepts. Adjusted intercept regression equations can therefore precisely estimate TP concentration and/or plant uptake from DAEP derived data, which is a more rapid and less expensive procedure.

## INTRODUCTION

Total phosphorus (TP) determinations are necessary for calculating total P uptake in early plant tissue samples, grain and stover. Many different wet or dry ash procedures for oxidizing plant tissues are available to determine TP and/or other elements in the digest[5,6,10,15,17,19]. Modifications of TP procedures have dealt primarily with advantages of reducing agents such as ascorbic acid versus $SnCl_2$ that are used for color development[4,15]. Alternative plant P procedures based on a dilute acid extract[10,13,14] have been used as a reliable index of P nutrition[7,12,15,18,20]. Minor modifications of the initial $CH_3COOH$ acid extractable $PO_4$-P (DAEP) procedure developed by Murphy and Riley[14] have been made for use on plant tissue[11,20].

An alternative water soluble P procedure has been shown to be highly correlated with TP[8]. While DAEP procedures are available that reduce the time, danger and expense that TP methods incur, there are currently no published correlations between DAEP and TP methods. Therefore, the objective of this study was to determine the feasibility of using DAEP as an alternative procedure for TP determinations.

## MATERIALS AND METHODS

Maize (*Zea mays* L.) plant tissue was sampled at selected stages of growth from two locations in 1983 and 1984. An added sampling of wheat (*Triticum aestivum* L.) at Feekes stage five was taken in 1985 (Feekes scale as translated by Croy; Croy, L. I., M.S. Thesis, Oklahoma State University, 1959). Experiments from which samples were taken employed a complete method by source by rate factorial treatment arrangement in a randomized complete block design with 3 replications. Corn plant tissue samples were taken at the eight-leaf stage (8L) (8 whole plants per plot), early silking (ES) (8 ear leaves per plot), early grain denting (ED) (8 ear leaves per plot, and at full maturity (S) (Stover subsample from ground stalks and leaves with the ears removed). In 1984, both locations were also sampled at the 12-leaf stage (12L) (six whole plants per plot). Following collection, tissue samples were dried in a forced air oven at 70 °C and ground to pass a 20-mesh screen. A 0.5 g sample was analyzed for TP by $HNO_3$-$HClO_4$ acid digestion excluding $H_2SO_4$ acid and color was developed by the vanadomolybdate procedure[5,6]. The DAEP in plant tissue was determined in 0.2 g samples by shaking with 50 ml 0.348M (2%) $CH_3COOH$ acid at 25 °C for 30 minutes. Concentration was determined after filtration through Whatman No. 2 paper by the phosphomolybdate colorimetric procedure employed by Murphy and Riley[14]. After analysis of all samples at all locations, groups of samples from each location were selected at random and rerun for TP and DAEP. This data was used as a check in measuring the reproducibility of each procedure.

Linear and quadratic regression equations were determined for growth stage, location, and year using DAEP to predict TP. Differences in slope components (across years and locations) by stage of growth were tested to determine if environments (years) and/or locations affected the TP-DAEP relationship. Prior to testing differences in slopes, residual error terms were tested for heterogeneity of variance.

## RESULTS AND DISCUSSION

Linear and quadratic regression equations were developed and the correlation of DAEP and TP in corn tissue was determined. Data conversions including natural log, log base 10 and inverse functions failed to improve correlation. Quadratic equations were reported where the squared variable was highly significant (Prob > |t| < .01). Regression equations by year, location, and stage of growth are reported in Table 1.

Correlation coefficients (r) of TP-DAEP regression equations were significant at the 0.01 probability level, for all stages of growth, locations and years sampled (Table 1). With few exceptions, slopes by stage of growth for the regression equations across years and locations were not significantly different (Table 2). Slopes of regression equations were also found to be equal when combined over locations and years and had intercepts within the combined regression equation confidence limits (Figs. 1 and 2). Confidence limits (95%) were established for point estimates about the regression lines.

Duplicate sample analysis (Table 1) of both TP and DAEP indicated the error associated with predicting TP from DAEP determination is as much a function of measurement error per respective procedure as it is in prediction of observed values of TP by DAEP data (Table 1).

At early silking (Loup City, 1983 and 1984; Mead, 1983) and 12L stage (Mead, 1984), the relationship between TP and DAEP indicates metabolic plant remobilization of P. If TP values had increased with an increase in DAEP, regression functions would have remained linear. However, this data suggests the increase in ear leaf DAEP at high concentrations must have been internal to the plant. Excess accumulation of P in the leaves, roots, and other vegetative parts is normally translocated to the grain during seed fill. Subsequent depletion of P in the leaves had been shown to take place at this time[9]. It was of interest to note that remobilization of P took place at an earlier stage at Mead in 1984 (quadratic regression equation at the twelve leaf stage) since at early silking, the TP-DAEP regression equation returned to being a linear function (Table 1).



TABLE 1

Regression Equations by Location and Year where TP is Dependent on DAEP.

| Stage | Equation | df | $R^2$ | r | SE |
|---|---|---|---|---|---|
| | **Loup City, 1983** | | | | |
| 8L | $TP=0.5877 + 1.34\ (DAEP)$ | 76 | | 0.89** | 0.22 |
| ES | $TP=0.0819 + 2.48\ (DAEP) - 0.53(DAEP)^2$ | 75 | 0.92** | | 0.18 |
| ED | $TP=0.1771 + 1.50\ (DAEP)$ | 76 | | 0.97** | 0.11 |
| S | $TP=0.1063 + 2.25\ (DAEP)$ | 76 | | 0.68** | 0.12 |
| | **Mead, 1983** | | | | |
| 8L | $TP=0.6040 + 1.55\ (DAEP)$ | 76 | | 0.93** | 0.18 |
| ES | $TP=0.3128 + 2.51\ (DAEP) - 0.56(DAEP)^2$ | 75 | 0.75** | | 0.16 |
| ED | $TP=0.8530 + 1.28\ (DAEP)$ | 76 | | 0.91** | 0.13 |
| S | $TP=0.1993 + 2.45\ (DAEP)$ | 76 | | 0.61** | 0.23 |
| | **Loup City, 1984** | | | | |
| 8L | $TP=-0.8990 + 1.92\ (DAEP)$ | 76 | | 0.93** | 0.28 |
| 12L | $TP= 0.8543 + 1.72\ (DAEP)$ | 76 | | 0.96** | 0.14 |
| ES | $TP= 0.3401 + 2.35\ (DAEP) - 0.59(DAEP)^2$ | 75 | 0.82** | | 0.23 |
| + | $TP= 1.0751 + 0.97\ (DAEP)$ | 76 | | 0.78** | 0.25 |
| ED | $TP= 0.7097 + 1.33\ (DAEP)$ | 76 | | 0.87** | 0.17 |
| S | $TP= 0.2308 + 2.36\ (DAEP)$ | 76 | | 0.65** | 0.15 |
| | **Mead, 1984** | | | | |
| 8L | $TP= 1.5345 + 1.20\ (DAEP)$ | 76 | | 0.86** | 0.22 |
| 12L | $TP=-1.7049 + 5.29\ (DAEP) - 1.35(DAEP)^2$ | 75 | 0.83** | | 0.14 |
| ES | $TP= 0.9511 + 1.47\ (DAEP)$ | 76 | | 0.86** | 0.13 |
| ED | $TP= 1.2643 + 1.08\ (DAEP)$ | 76 | | 0.70** | 0.19 |
| S | $TP= 0.4719 + 2.20\ (DAEP)$ | 76 | | 0.80** | 0.15 |
| | **Imperial, 1985 (wheat)** | | | | |
| F5 | $TP=0.4244 + 2.08\ (DAEP)$ | 46 | | 0.91** | 0.17 |
| Duplicate analysis for procedural accuracy. | | | | | |
| | **Mead, 1983** | | | | |
| ED | $TP_1=0.192 + 0.981\ (TP_2)$ | 76 | | 0.93** | 0.12 |
| | **Imperial, 1985** | | | | |
| F5 | $DAEP_1=0.348 + 0.937\ (DAEP_2)$ | 46 | | 0.82** | 0.10 |

\+ = Linear function at early silking, Loup City, 1984.
8L = Eight leaf stage. 12L=Twelve leaf stage.
ES = Early silking stage. ED=Early denting stage.
S = Stover subsample. F5=Feekes stage 5 (wheat).
** = Significant at 0.01 probability level.
SE = Standard error.

TABLE 2

Effect of Different Locations, Years, and Combining Years on PR > F for Mean Square Error Comparisons and PR > |t| of Regression Coefficients for TP and DAEP Regression Equations for Different Growth Stages of Corn.

| Growth Stage | Year or Location | PR > F | PR > \|t\| Regression Coefficients Linear ($b_1$) | Quadratic ($b_2$) |
|---|---|---|---|---|
| | | Loup City vs. Mead | | |
| 8L | 1983 | NS | NS | |
| ES | | NS | NS | NS |
| ED | | NS | ** | |
| S | | ** | NS | |
| 8L | 1984 | NS | ** | |
| ES | | ** | ** | |
| ED | | NS | NS | |
| S | | NS | NS | |
| | | 1983 vs. 1984 | | |
| 8L | Loup City | NS | ** | |
| ES | | NS | NS | NS |
| ED | | * | NS | |
| S | | NS | NS | |
| 8L | Mead | NS | ** | |
| ES | | NS | * | |
| ED | | ** | NS | |
| S | | ** | NS | |
| | | Loup City vs. Mead (combined over years) | | |
| 8L | | NS | NS | |
| ES | | NS | NS | NS |
| ED | | ** | NS | |
| S | | ** | NS | |
| AS | | NS | ** | |

*, ** = Significant at 0.05 and 0.01 probability level.
NS = Not significant.
8L = Eight leaf stage. ES = Early silking stage.
ED = Early dent stage. S = Stover
PR > F = probability of a greater F when comparing error mean squares between location, years, or locations combined over years.
PR > t = Probability of a greater absolute value of t used to test slopes of various regressions.
AS = All stages combined.



Intercepts for each regression equation were found to vary by year and location for each of the respective stages. Because of the bias associated with changing intercepts, simultaneous solutions were tested for the intercept and TP/DAEP values. Assuming correct slope values (by stage), ten random samples analyzed for both TP and DAEP accurately predicted the TP/DAEP population estimate. The actual number of samples required to estimate the population TP/DAEP value within a 99% confidence interval (CI) increased with advancing stage of growth. However, for all equations tested, no more than 10 samples were required to estimate the TP/DAEP value within the 99% CI. The intercept solution is as follows:

$$TP = bo + b_1\ (DAEP)*$$

$$\frac{TP}{DAEP} = c;\ TP = (DAEP)c$$

$$TP = (DAEP)c = bo + b_1\ (DAEP)$$

$$DAEP\ (c - b_1) = bo$$

* = regression equation having prior estimate of slope.

This analysis holds assuming a true estimate of TP/DAEP is obtained while at the same time having a previously determined estimate of slope. Intercept values for the respective stage of growth can also be determined from a regression equation determined from ten random samples for TP and DAEP.

Combining years, locations, and stages of growth into a TP-DAEP regression equation is not advisable due to marked changes in slopes existent at different sampling times. However, the equation in Fig. 3 does provide an accurate estimate across all parameters of the intercept and slope one could expect to find in corn tissue samples. Correlation of TP and DAEP was improved by combining years, locations, and stages of growth. As the difference between observed values and the population mean increased, the variance about the slope estimate decreased thereby increasing $R^2$.

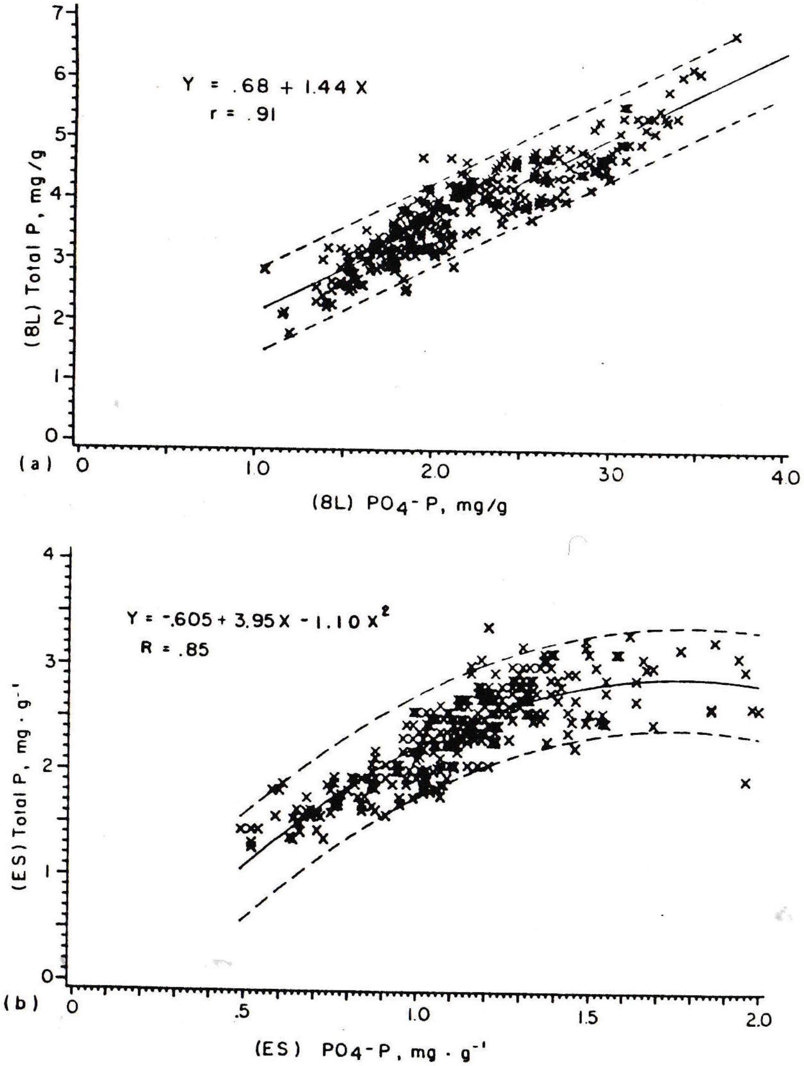


FIG. 1. Data plot and regression equations with 95% confidence belt for predicting TP with DEAP over locations and years for 8L, ES, ED, and S tissue samples.



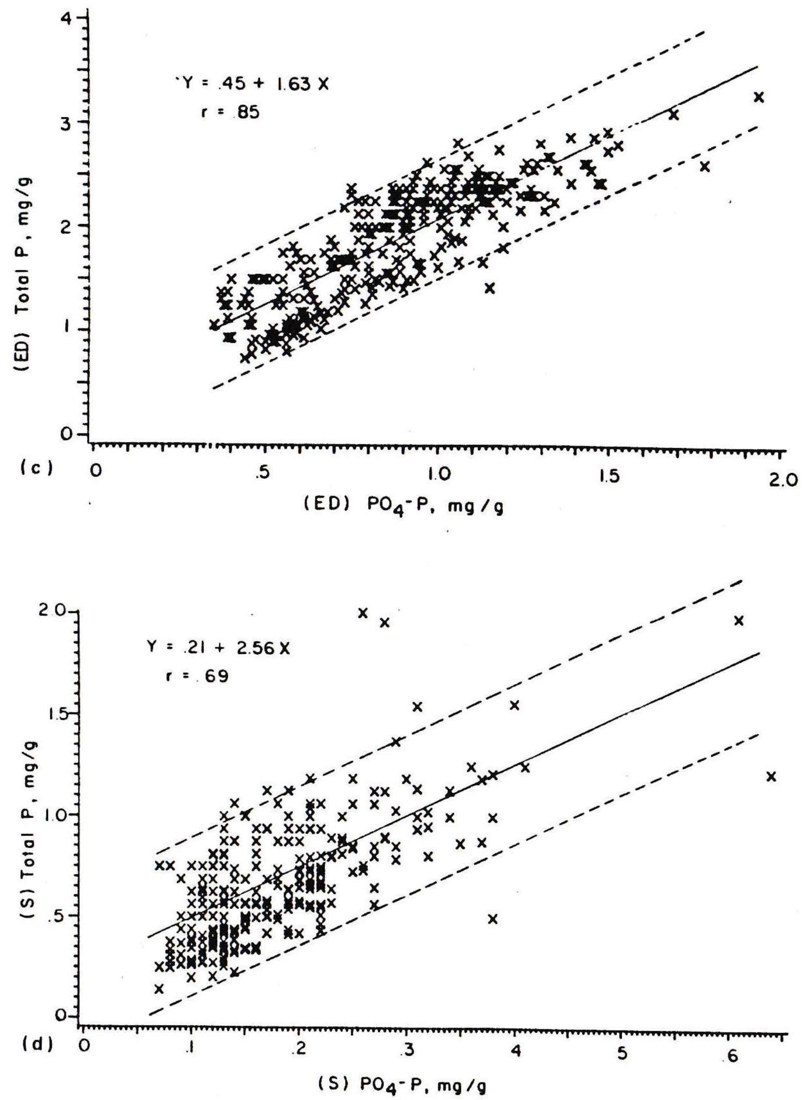


FIG. 1 (continued)

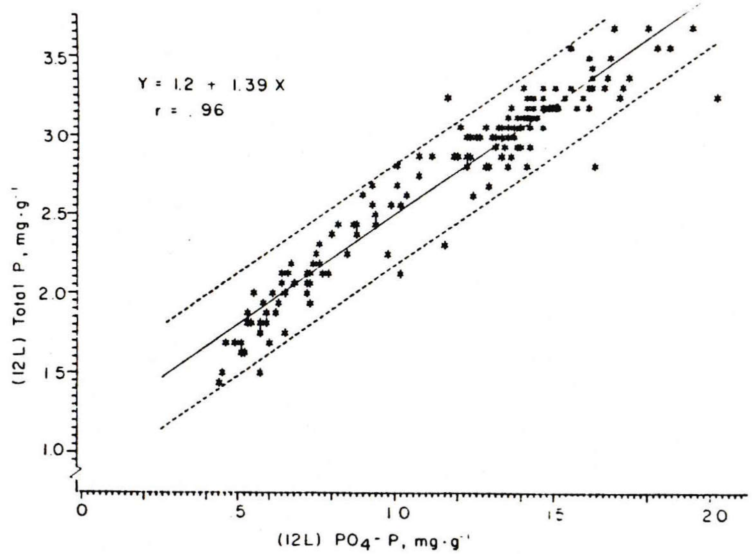


FIG. 2. Data plot and regression equation with 95% confidence belt for predicting TP with DAEP.

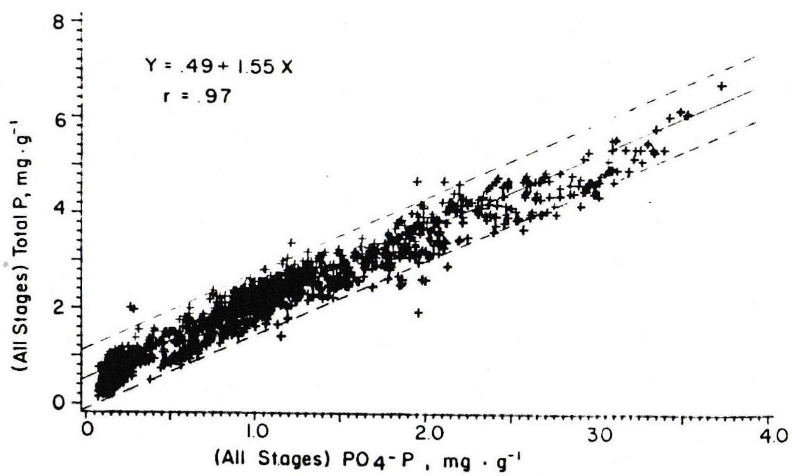


FIG. 3. Data plot and regression equation of TP vs. DAEP over all locations, years, and growth stages.



Results from this experiment also indicate that with advancing stage of growth and a subsequent decrease in concentration, prediction of TP becomes less precise (Fig. 1). Ratios of TP to DAEP (Table 3) were found to increase with time. This appears to demonstrate the efficiency of the plant to both deplete P within the vegetative tissue, while at the same time limiting the amount of immobilized P in non-reproductive parts at maturity. The poorer correlation of stover TP-DAEP regression equations is in part due to the difficulty in acquiring a representative sample for analysis. One sample may have more stalk tissue and less leaf tissue than the next. The stalk portion which is more resistant to weak acid extraction would likely have a higher TP-DAEP ratio where more stalk tissue and less leaf exists. At this stage, the relative amounts of P that have been translocated to reproductive parts are not measured and aid in explaining some of the poorer correlation.

Although only limited tissue samples were taken for wheat, (Triticum aestivum L.) DAEP-TP correlation remained high, suggesting the use of DAEP as a TP index variable for other crops (Table 1).

It was surprising to find that TP-DAEP regression correlation remained high at Mead where no response to applied fertilizer P (data from another experiment) beyond the 8L stage was found. At this location, TP and DAEP concentrations were highest while data ranges were narrowed as compared to Loup City. This appears to indicate precision of DAEP measurements to predict TP while at the same time being unaffected by the magnitude of the range.

Using the DAEP procedure discussed, more than 300 samples can be analyzed per day versus 40 for TP, at one-tenth the chemical cost per sample and without the hazards associated with $HClO_4$ acid. Poor correlation at later stages of growth restricts the use of DAEP to predict TP even though the accuracy in measurement is largely due to sampling error (e.g., stover sub-samples).

When using either DAEP or TP as a dependent variable at a given stage of growth, interpretation of the main effect variables

TABLE 3

Mean Values and Ratios of TP and DAEP in Corn Tissue by Year, Location, and Growth Stage.

| | Mead 1983 | | | Mead 1984 | | | Loup City 1983 | | | Loup City 1984 | | | Loc, + Yr, Avg. | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Stage | TP | DAEP | R | TP | DAEP | R | TP | DAEP | R | TP | DAEP | R | TP | DAEP | R |
| | $mg\ g^{-1}$ | | | $mg\ g^{-1}$ | | | $mg\ g^{-1}$ | | | $mg\ g^{-1}$ | | | $mg\ g^{-1}$ | | |
| 8L | 3.56 | 1.91 | 1.87 | 4.26 | 2.26 | 1.89 | 3.01 | 1.80 | 1.67 | 4.63 | 2.88 | 1.61 | 3.87 | 2.21 | 1.75 |
| ES | 2.31 | 1.19 | 1.94 | 2.79 | 1.23 | 2.26 | 2.09 | 1.09 | 1.92 | 2.08 | 1.04 | 2.01 | 2.32 | 1.14 | 2.04 |
| ED | 2.28 | 1.11 | 2.05 | 2.34 | 0.99 | 2.35 | 1.35 | 0.78 | 1.72 | 1.60 | 0.68 | 2.38 | 1.89 | 0.89 | 2.12 |
| S | 0.77 | 0.23 | 3.33 | 0.88 | 0.19 | 4.71 | 0.43 | 0.15 | 2.96 | 0.54 | 0.13 | 4.26 | 0.65 | 0.17 | 3.79 |
| Avg. | 2.23 | 1.11 | 2.01 | 2.57 | 1.17 | 2.20 | 1.72 | 0.96 | 1.80 | 2.21 | 2.18 | 1.88 | 2.18 | 1.10 | 1.98 |
| 12L | | | | 3.15 | 1.48 | 2.12 | | | | 2.29 | 0.83 | 2.76 | 2.72 | 1.16 | 2.35 |

R = TP/DAEP. 8L = Eight leaf stage. 12L = Twelve leaf stage.

ES = Early silking stage. ED = Early denting stage.

S = Stover subsample. DAEP = Dilute acid extractable phosphorus.

TP = Total phosphorus.



(complete method-source-rate factorial) did not change. Since both measurements were used as an index of P nutrition (data presented in another experiment) this finding helps validate the use of DAEP as an alternative to TP measurements.

## CONCLUSIONS

Tissue analysis for $PO_4$-P using the initial procedure developed by Murphy and Riley was highly correlated with TP at all locations, years, and growth stages prior to full maturity. Slopes for TP-DAEP regression equations (by stage of growth) were not different when tested over locations and/or environments, indicating the existence of a direct TP-DAEP relationship. It is feasible to assume that these correlations, slopes, and intercepts can be established for other crops while at the same time adjusting for intercepts (by environments) by analyzing a limited number of samples for TP and DAEP to establish the TP/DAEP ratio. Where interests lie primarily in plant P nutrition, the DAEP procedure provides a rapid inexpensive method of P analysis. Data from this experiment also showed that plant remobilization of organic P forms took place at or near early silking in corn due to a quadratic DAEP-TP relationship found at both locations and years sampled. High correlation at stages of growth prior to full maturity indicated that one method was as good as the other for assessing the relative P status of the plant which allows the prediction of total P concentration from DAEP derived data.

## ACKNOWLEDGEMENTS

This work was supported in part by the Tennessee Valley Authority and the assistance was greatly appreciated.

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