A Revised Method for Determining Phosphate-Phosphorus Levels in Sugar Beet Leaf Petioles[1]

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Knowledge of plant nutrient status during the growing season can be valuable. Early detection of a deficiency sometimes allows it to be corrected that same growing season. Plant analysis also aids in planning the fertilization program the following year.

Johnson and Ulrich (4)[3] developed a test to determine the status of P in the growing sugar beet. They found that the amount of acetic acid soluble $PO_4^{\equiv}$ in the leaf petiole material provided a reliable measure of P status. They have established that a concentration of 750 ppm $PO_4^{\equiv}$-P represents the "critical level."

Johnson and Ulrich determined the $PO_4^{\equiv}$-P content with the phosphomolybdate method using $SnCl_2$ as the reductant. With this method, they have analyzed sugar beet petioles ranging in $PO_4^{\equiv}$-P contents from 100 to 10,000 ppm. Although their method is accurate and precise, it has one serious disadvantage: a lengthy oxidation with H_2O_2 is needed to remove the color of dissolved organic compounds. The subsequent evaporation step to remove excess H_2O_2 also causes the technique to be time consuming. This rather tedious method may have discouraged agronomists from making more frequent use of this technique.

The objectives of the experiment reported here were to determine if the $SnCl_2$ reductant used by Johnson and Ulrich could be replaced with ascorbic acid and if this would allow elimination of the time consuming step of organic matter oxidation and subsequent evaporation of the excess H_2O_2.

Materials and Methods

Samples

Samples used in the method comparison experiments were sugar beet leaf petioles of the most recently matured leaves. They were taken from plots in a P rate experiment in Scottsbluff County, Nebraska. The petioles were oven dried and ground to pass a 1 mm screen.

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[3]Numbers in parentheses refer to literature cited.

Extraction

Extractions were made by adding 100 ml of Johnson and Ulrich acetic acid extractant to 0.4 g of plant sample. These were shaken for 10 minutes on a wrist action shaker and filtered through a NO. 40 Whatman paper. Extractions were made in duplicate.

P-Determination

Two methods were used to determine the $PO_4^{\equiv}$-P concentration of the petioles. Method 1 was Johnson and Ulrich's procedure. This method was used with the $SnCl_2$ reducing agent as described by Johnson and Ulrich and also with the ascorbic acid reducing agent.

Method 2, the proposed substitute, is described below:

1. After extraction, a 5 ml aliquot of the extractant was diluted to 20 ml with distilled water. (This replaces the 2 ml aliquot suggested by Johnson and Ulrich prior to the oxidation step).
2. A 2 ml aliquot was taken from this diluted sample and placed in a test tube.
3. Eight ml of the ascorbic acid working solution (reagents are listed in the next section) was then added to the test tube containing the 2 ml of the diluted sample.
4. After waiting 10 minutes for complete color development, the absorbance was determined at a λ of 880 nm on a Beckman Spectronic 20 and the results compared to a standard curve with a range of 0.5 to 5 ppm P in solution.

Reagents for Method 2 (5)

1. Acid molybdate stock solution. Dissolve 60 g ammonium molybdate, $(NH_4)_6Mo_7O_{24}4H_2O$, in 200 ml of distilled water. If necessary, heat to about 60°C until solution is clear and allow to cool. Dissolve 1.455 g of antimony potassium tartrate in the molybdate solution. Add slowly 700 ml of concentrated sulfuric acid. Cool and dilute to a final volume of 1000 ml. This solution may be blue, but will clear when diluted for use. Store in the dark under refrigeration.
2. Ascorbic acid stock solution. Dissolve 132 g of ascorbic acid in distilled water and dilute to a final volume of 1000 ml. Store in the dark under refrigeration.
3. Prepare a combined molybdate-ascorbic acid working solution each day by adding 25 ml of the acid molybdate stock solution to approximately 600 ml distilled water. Then add 10 ml of the ascorbic acid stock solution and bring to a final volume of 1000 ml. This amount will handle approximately 100 samples with standards and blanks.

Results and Discussion

Methods 1 and 2 are compared in Table 1 and Figure 1. The $SnCl_2$ and the ascorbic acid reducing agents used with method 1 gave essentially

Table 1. Phosphate phosphorus content of sugar beet petioles as determined by methods 1 (Johnson and Ulrich) and 2 (proposed).

Sample No.	Methods 1 Reductant $SnCl_2$	Methods 1 Reductant Ascorbic Acid	Methods 2 Reductant Ascorbic Acid
	ppm		
1	1710	1691	1521
2	2455	2326	2193
3	1958	1912	1758
4	2344	2289	2133
5	2705	2584	2550
6	2294	2200	2076
7	2505	2652	2382
8	2694	2813	2704
9	1613	1597	1457
10	2245	2385	2160
11	2233	2240	2185
12	1377	1235	1194
Average	2178	2160	2026

the same results. The coefficient of determination of $SnCl_2$ vs ascorbic acid in method 1 was $r^2 = 0.96$. Consistency of the results of method 1 with both $SnCl_2$ and ascorbic acid showed that either reductant was adequate. Alexander and Robertson (1,2), Murphy and Riley (6), John (3), Omanwar and Robertson (7), and Watanabe and Olsen (8) have all shown that ascorbic acid is widely suited for different P determination.

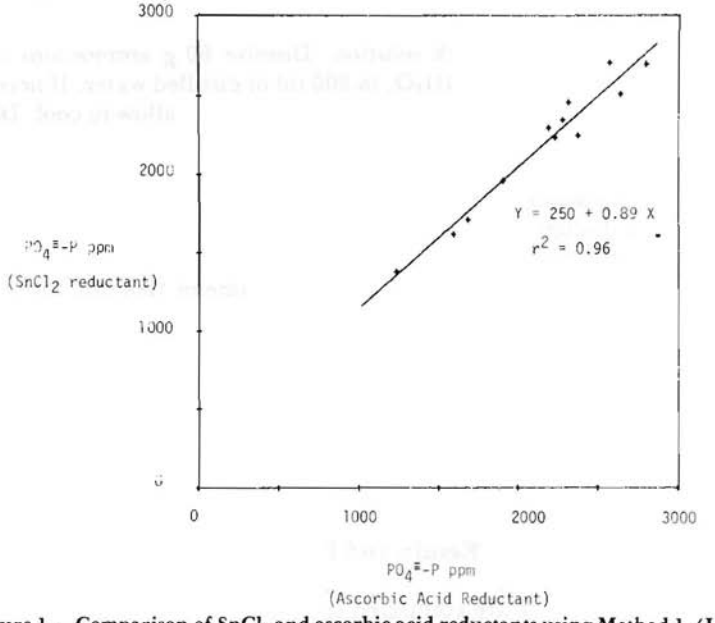

Figure 1.—Comparison of $SnCl_2$ and ascorbic acid reductants using Method 1. (Johnson & Ulrich)

Results from method 2 were slightly lower in most cases than those in method 1 as shown in Table 1. However, the coefficient of determination of method 1 vs method 2 was $r^2 = 0.97$. Figure 2 shows that the relationship between them is essentially 1:1 ($b = 0.92$). This means both methods are measuring essentially the same thing. The slightly lower results with method 2 compared to method 1 may be due to the phosphates released from the organic matter during the oxidation step in method 1. Since method 2 does not contain the oxidation step the organic phosphates are not determined and hence the lower values.

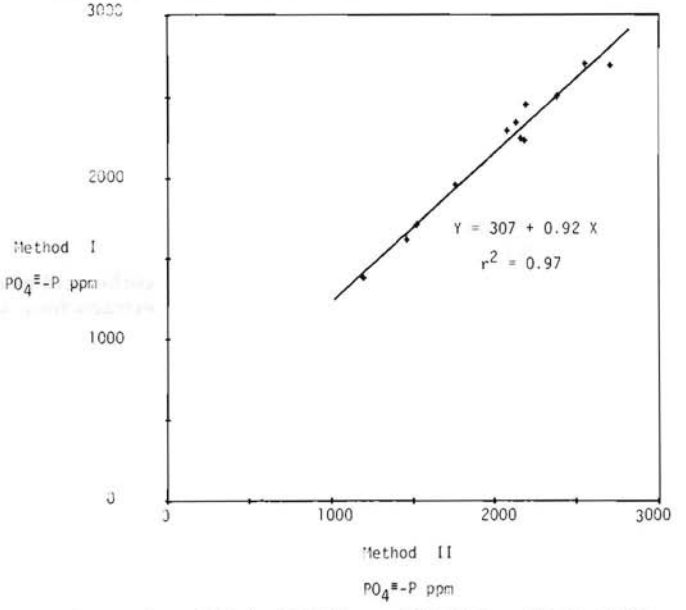

Figure 2.—Comparison of Method 1 (Johnson & Ulrich) and Method 2 (Proposed for determining $PO_4^{\equiv}$ -P levels in sugar beet petioles.

In view of the above results method 2 is proposed as a replacement for method 1. It eliminates the time consuming oxidation and evaporation steps and still determines the $PO_4^{\equiv}$ -P levels accurately. The extraneous colors of the extracts do not affect the results when read at 880 nm as was previously shown by Watanabe and Olsen (8).

By adopting method 2 the time consuming evaporation step is eliminated and the procedure becomes much more routine. About 45-60 minutes can be saved per group (50 samples) and in addition evaporation equipment is not needed. This saving may allow one individual to analyze almost twice as many samples in one day as was possible using method 1.

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