Potassium did not decrease the magnesium content of the leaves. In the first season's samples and in the first samples taken, the second year, the magnesium content increased significantly as the rate of application of nitrogen increased. In the second samples, the second year, the magnesium followed a pattern which was the inverse of the yield pattern, probably determined by the amount removed by the fruit, which in turn was related to the nitrogen application. It seems probable that the increase in the magnesium from the first to the second sampling from the low nitrogen plots resulted from the top-dressing of urea. There was no evidence of a constant cation sum.

Both too little and too much nitrogen obscured the value of phosphorus and potassium. The yield reduction resulting from the application of 300 pounds of nitrogen per acre can be only the result of direct injury. The plants were not extremely vegetative, there was no large accumulation of nitrogen in the leaves, and there was no significant interference with the absorption of any element determined.

The results in the last sampling show that the pattern of the yields was set during the early part of the growth of the plants, whereas the composition of the leaves reflected the available nutrient supply at the time of sampling. The reaction of the fertilizer salts with the soil played a very significant role, particularly in the case of magnesium. Soil tests using acid ammonium acetate as extractant did not provide a useful index of the available nutrients in this Rockdale soil.

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PLANT ANALYSES

Rapid and Accurate Automatic Titration Method for Determination of Calcium and Magnesium in Plant Material with EDTA Titrant

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A method is described for the determination of calcium and magnesium in plant materials which combines rapid precipitation and extraction techniques to eliminate interferences and automatic titrations to eliminate subjective evaluation of the end points. The method is more rapid than conventional methods and as accurate. After wet digestion of the plant samples, the total time for determination of both calcium and magnesium is about 15 minutes, including separations and titrations.

A NUMBER OF METHODS have been proposed for the determination of calcium and magnesium in plant material using disodium (ethylenedinitrilo)-tetraacetate (EDTA) as titrant and metal indicators for visual end point detection. One difficulty encountered in these methods is the presence of iron, manganese, aluminum, copper, and phosphate in amounts sufficient to interfere with both titrations. Many procedures for the elimination of these interfering ions have been presented (*1, 2, 4, 7, 8, 10, 11*). Investigation showed that the removal of phosphate by precipitation with zirconium nitrate (*8*) and extraction of the heavy metals with carbon tetrachloride as the diethyldithiocarbamate complexes (*4*), provided a simple, rapid, and good separation of interferences.

Another difficulty often encountered with EDTA titrations is the visual end point detection. This problem can be eliminated for calcium and magnesium titrations by using the Sargent Spectro-Electro titrator, whereby titrations can be automatically and precisely terminated at their end points (*6*). Calcium and magnesium in plant material are determined by automatic titrations after the interferences are removed by the relatively rapid precipitation and extraction techniques. An integrated, rapid, accurate, and easy to follow procedure incorporating the separation and automatic titration techniques is described.

The automatic titrations are both completed in a few minutes, and the results check closely with the values assigned to four standard fruit tree leaf samples: apple, cherry, citrus, and peach (*5*).

Apparatus and Reagents

Connect the Sargent-Malmstadt Spectro-Electro automatic titrator (E. H. Sargent Co., Chicago, Ill.) (*9*) and Teflon stopcock burets as previously reported (*6*).

STANDARD CALCIUM SOLUTION. Dry primary standard calcium carbonate (Mallinckrodt Chemical Co.) in an oven

Table I. Automatic Titration Results for Standard Plant Samples

Sample	Present, % Ca	Present, % Mg	Found, % Ca	Found, % Mg	Difference, % Ca	Difference, % Mg
Alfalfa	1.41	0.25	1.40	0.27	−0.01	+0.02
			1.40	0.28	−0.01	+0.03
			1.38	0.25	−0.03	0.00
Apple	1.17 ± 0.04	0.36 ± 0.003	1.19	0.34	+0.02	−0.02
			1.17	0.36	0.00	0.00
Cherry	2.95 ± 0.07	0.92 ± 0.038	2.93	0.95	−0.02	+0.03
			2.93	0.95	−0.02	+0.03
Citrus	3.86 ± 0.13	0.33 ± 0.014	3.74	0.32	−0.12	−0.01
Peach	1.98 ± 0.04	0.53 ± 0.012	2.03	0.53	+0.05	0.00

for 3 to 4 hours at 110° C. Transfer exactly 1.0009 grams to a 1-liter volumetric flask with about 200 ml. of deionized water. Add 20 to 22 ml. of 1*M* hydrochloric acid to the flask, and heat on a hot plate to 70° C. until the complete solution and evolution of carbon dioxide. After cooling to room temperature, fill the flask to the mark with deionized water—1 ml. contains 0.4008 mg. of calcium.

EDTA Solution. Dissolve approximately 3.73 grams of disodium (ethylenedinitrilo)tetraacetate dihydrate in distilled water and dilute to 1 liter.

Sodium Diethyldithiocarbamate Solution. Prepare just before use a 1% w./v. aqueous solution.

Zirconium Nitrate Solution, 1% w./v. Suspend 1 gram of zirconium nitrate in water, add a few drops of concentrated nitric acid, warm until dissolved, then filter and make up to 100 ml. with distilled water.

Prepare indicators, buffer, 1*N* sodium hydroxide, and triethanolamine as described (*6*).

Procedure

Preparation of Automatic Titrator (*6*). Switch the Spectro-Electro titrator to the Spectro position. Throw the polarity switch to position 2, turn the filter wheel to the 650-mμ position, insert the auxiliary ultraviolet cutoff filter, and set the pegs in the base to position the 50-ml. beakers. Connect the small stirrer, delivery tip, and suitable buret and set the titrant delivery rate at about 2.5 to 3 ml. per minute. The same conditions are adequate for both end points.

Standardization of Titrant. Pipet a 5-ml. aliquot of the standard calcium solution into a 50-ml. beaker, dilute to 25 ml. with deionized water, add enough 1*N* sodium hydroxide to bring the pH to 13 (test with pH paper), add 1 to 2 drops of Calcon indicator, insert the beaker in the automatic Spectro titrator, push the start button to begin the stirring and the delivery of titrant, and read the buret after automatic termination at the end point.

Preparation of Plant Material Sample. Weigh approximately 1 gram of a representative dried sample into a 250-ml. beaker, add 10 ml. of concentrated nitric acid, and mix it thoroughly with the sample. Add 10 ml. of distilled water and then 10 ml. of perchloric acid (70%). Cover the beaker with a watch glass supported on glass hooks and boil the contents gently on a sand bath until all the organic matter is oxidized and the solution becomes straw yellow. Remove the beaker and let it cool. Rinse the watch glass and sides of the beaker with a small amount of distilled water, remove the glass, place the beaker on the sand bath, and evaporate the perchloric acid to dryness below its boiling point. Remove the beaker and let it cool. Take the residue up in 25 ml. of approximately 1*N* hydrochloric acid and heat below the boiling point on the sand bath for 15 minutes and then make up to volume with deionized water in a 50-ml. volumetric flask. If silica is present, filter the solution (*3*).

Pipet a 10-ml. aliquot of the dissolved sample into a 15-ml. centrifuge tube, add 5 drops of 1% zirconium nitrate solution, mix thoroughly, and place the centrifuge tube in boiling water for 3 minutes to aid the flocculation of the precipitate. Centrifuge at about 2000 r.p.m. for 2 to 3 minutes. Pour the supernatant liquid into a separatory funnel, wash the residue with a few milliliters of cold water, recentrifuge for 1 minute, and add the supernatant liquid to the solution in the separatory funnel. Add 1 ml. of a 1% sodium diethyldithiocarbamate solution, shake the separatory funnel, and add 10 to 15 ml. of carbon tetrachloride. Transfer the aqueous solution into a 25-ml. volumetric flask, wash the funnel with deionized water, and make up the solution to volume with distilled water. Use this solution for the calcium and magnesium titrations.

Titration of Total Calcium and Magnesium. Pipet a 10-ml. aliquot of the prepared sample into a 50-ml. beaker and dilute up to 25 ml. with deionized water. Add 1 ml. of triethanolamine, 2 ml. of the ammonium hydroxide–ammonium chloride buffer solution, insert the beaker into the titrator, and start the stirrer to mix the masking agent thoroughly with the sample. Add 2 drops of the Eriochrome Black T indicator solution and titrate by pushing the start button on the derivative control unit. Read the buret after the automatic termination at the end point. The color change is from wine-red to blue.

Table II. Reproducibility of Complete Procedure for Calcium and Magnesium in Plant Materials

	Found,[a] % Ca	Found,[a] % Mg
	1.48	0.52
	1.50	0.49
	1.49	0.49
	1.49	0.49
	1.53	0.49
	1.50	0.50
	1.49	0.50
	1.49	0.52
	1.50	0.50
	1.49	0.51
Av.	1.49	0.50
Std. dev.	0.01	0.01

[a] Sample contained 1.50% calcium, 0.50% magnesium, 0.9% phosphate, 12 p.p.m. copper, 150 p.p.m. iron, 50 p.p.m. manganese, 12 p.p.m. aluminum, and 40 p.p.m. zinc.

Titration of Calcium. Pipet another 10-ml. aliquot of the prepared sample into a 50-ml. beaker and dilute to about 25 ml. with deionized water. Add 1 ml. of triethanolamine and enough 1*N* sodium hydroxide to adjust the pH of the solution to 13 (use Hydrion paper), insert the beaker into the titrator, and start the stirring. Add 1 to 2 drops of the Calcon indicator solution, and push the start button on the titrator to titrate with standard EDTA solution until the automatic termination at the end point. The color change is from pink to blue. If large amounts of magnesium hydroxide precipitate, the final color may be pinkish blue.

Magnesium. Calculate magnesium by subtracting the volume of EDTA used for the calcium titration from the volume used in the total calcium and magnesium titration. A blank of 0.03 ml. is also subtracted from the remaining volume.

Calculations

When the sample, after wet digestion, is diluted to 50 ml., 10 ml. of this solution are diluted to 25 ml. after the removal of interfering ions, and a 10-ml. aliquot of the latter solution is taken for each titration. Calcium and magnesium can be calculated as follows:

$$\frac{A \times B \times 1.25}{C} = \% \text{ Ca}$$

$$\frac{D \times [E - B - 0.03] \times 1.25}{C} = \% \text{ Mg}$$

where A = mg. of calcium per ml. of EDTA solution [this is equal to 2.004 divided by milliliters of EDTA used in the standardization of the titrant]. B = ml. of EDTA solution in the calcium titration. C = weight of the dry sample in grams. D = mg. of magnesium per ml. of EDTA solution [this is equal to 1.216 divided by milliliters of EDTA used in the standardization of the titrant]. E = ml. of EDTA solution used in the total calcium and magnesium titration.

Results and Discussion

Four standard fruit tree leaf samples of different origin—apple, cherry, citrus, peach—and a standard alfalfa sample were analyzed to establish the validity of the method. The results obtained show the precision and accuracy of the procedure (Table I). Obtained values for calcium and magnesium were within the range given for the standard sample values, except for the calcium content of the peach sample. However, the reported values obtained by chemical, flame, and spectrographic methods for the calcium content of this sample are 1.94, 2.02, and 2.04, respectively (*5*).

To check the reproducibility of the proposed complete procedure—after the wet ashing—separations and automatic titrations were carried out for 10 equal aliquots of a synthetic sample (Table II).

The delivery rate of the titrant is adjusted at about 2.5 to 3 ml. per minute —both for the standardization of the titrant and for the determination of calcium and magnesium—to provide a signal from the Spectro titrator which is sufficient to terminate the titrations automatically at their end points. This can be done with either a suitable capillary delivery tip or by adjustment of the Teflon stopcock and pinch-off valve. At this delivery rate no correction is necessary for calcium, but a blank of 0.03 ml. is applied when Eriochrome Black T is used as indicator, because of a slight but reproducible overshooting of the equivalence point.

The phosphate (PO_4) in plant material ranges from about 0.4 to 1.3%. Five drops of a 1% zirconium nitrate solution were best suited for the removal of phosphates, within the above range, from the recommended 10-ml. aliquot solution containing approximately 0.2 gram of the original dry sample.

Larger amounts of zirconium nitrate should be avoided—except in samples much richer in phosphate—because the remaining free zirconium interferes with the sharp color change at the end point in both titrations, particularly in calcium determination, and thus may impede the automatic termination of the titration.

Although a double extraction with carbon tetrachloride is recommended in the procedure, the first extraction was usually sufficient for removal of iron and manganese. The addition of triethanolamine prior to the titration masks traces of iron, manganese, copper, and aluminum which may remain in solution to block the indicator. However, in most cases, the removal of the interfering ions was complete and the addition of triethanolamine was not necessary.

A 1% freshly prepared aqueous solution of sodium diethyldithiocarbamate is used instead of the solid salt to avoid precipitation of calcium and magnesium with the probable excess of the salt. Larger aliquots of the sample can be taken for titration in larger beakers. A 10-ml. aliquot—corresponding to about 0.08 gram of the dry sample—is recommended for each titration, because of the smaller quantities of reagents and titrant consumed and the shorter titration time. The accurate results obtained justify this consideration.

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GIBBERELLINS ANALYSIS

Infrared Determination of Gibberellins

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A simple infrared method designed for the determination of mixtures of gibberellic acid and of gibberellin A is described. The method is based on the determination of the absorbances at 12.86 and at 10.85 microns of a 15% solution of the sample in pyridine. These bands are characteristic of gibberellic acid and gibberellin A, respectively.

THE GREAT INTEREST in the plant hormones of the gibberellin group (*8*) justifies efforts to develop analytical methods for determination of the components in mixtures—at least four have been described. The Japanese workers have named these gibberellins A_1, A_2, A_3, and A_4. Gibberellic acid is considered equivalent to gibberellin A_3 and gibberellin A and A_1 are considered equivalent. The terms gibberellic acid (or gibberellin X) and gibberellin A are more commonly used in this country. This paper is concerned primarily with determination of gibberellic acid (gibberellin X, gibberellin A_3) and gibberellin A (A_1) in mixtures. Samples of A_2 and A_4 have not been available for analysis by this method.

Gibberellic acid and gibberellin A have been separated by paper chromatography by Bird and Pugh (*4*) and by Varner, Hargie, and Schenck (*10*). These methods are suitable for qualitative analysis, but cannot detect small amounts of one in the presence of large amounts of the other component. The radioisotopic method of Baumgartner *et al.* (*3*) has been used for quantitative analysis.