

trouble, as the use of ordinary stopcock grease sometimes results in the presence of interfering substances in the final solutions. Glycerol was used as a stopcock lubricant, and although it produced no interference, it left much to be desired. Although they were not tested in this work, Teflon stopcocks may be more satisfactory.

#### Adsorption and Elution of Plant Extracts

After the columns are washed, 5.0 ml. of plant extract or appropriate ether blanks and standards are introduced. Three separate ether rinses of 3 ml. each are added as the last of the rinses goes into the sand layer; 10 ml. of ether-alcohol is added. Collection of eluate is now begun. As this last portion of solvent passes into the column, the reservoir bulb is filled with the solvent. If a group of columns are being worked, their rates of flow should be made to be approximately equal.

Under the conditions employed, elution of factor A from the column occurred between 140 and 240 ml. of eluate volume (Figure 2). Collecting a somewhat broader range did not result in an increase of interfering impurities; therefore, the fraction was taken routinely from 90 to 290 ml. The rate of movement of factor A on the column was dependent on relative proportions of ether and alcohol in the eluent. Ether and alcohol in a 1 to 1 mixture accomplished elution in the 90- to 160-ml. fraction, but purity was unsatisfactory. Ether alone resulted in prohibitively slow movement of the compound on the column.

#### Determination of Concentration and Purity

The eluates collected from the columns are evaporated to dryness. This may be done under reduced pressure or with the aid of warming and an air stream, but care must be taken to avoid overheating the residue (40° C. is satisfactory). The

**Table I. Recovery of Purified 6-Methoxybenzoxazolinone from Aluminum Oxide Chromatographic Columns**

Amounted Added, $\gamma$	Average Amount Recovered	
	$\gamma$	%
500	460	92
400	340	85
206	182	91
100	93	93
50	41	82
25	23	90

dried residues are taken up in 10.0 ml. of distilled water, with warming. The absorbance of the samples, a reagent blank, and a standard solution of 6-methoxybenzoxazolinone are read in a spectrophotometer against a distilled water blank at wave lengths of 255 and 285  $m\mu$ . From these data, the factor A contents of the samples are calculated. The pure compound shows an absorption minimum at 255  $m\mu$ , and readings of absorbance at this wave length are of value in that they give an indication of the optical purity of the samples. The ultraviolet absorption spectra of a plant extract before and after column purification are shown in Figure 3, where they are compared to the spectrum of recrystallized highly purified 6-methoxybenzoxazolinone. In the unpurified plant sample, interfering substances showed an absorption spectrum with a broad peak between 250 and 270  $m\mu$  which obscured the factor A minimum at 255  $m\mu$ . The column purification gave a satisfactory resolution of the 255- $m\mu$  minimum and the 285- $m\mu$  peak, but not of the absorption peak at 230  $m\mu$ .

The efficiency of the analytical procedure was determined by a series of recovery experiments. In one group of experiments, different amounts of the pure compound were put through the procedure and the percentage recovery determined. Table I shows the results obtained. Recovery experiments in

which known amounts of the pure compound were added to plant extracts prior to column purification were also run. These experiments yielded an average recovery of 91%.

The analytical method described above has been used to estimate the factor A content of different tissues of corn plants of different ages and varieties. Good consistency among replicate samples was obtained. No significant optical interference was encountered in any of the plant samples. Leaves, tassels, stems, leaf sheaths, husks, silks, and pollen samples have been used. Factor A concentrations varied from 3 to 260  $\gamma$  per gram of fresh weight of tissue among the plant parts, varieties, and ages tested.

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## PHOSPHORUS AVAILABILITY

### Solubility Characteristics of Nitric Phosphate Fertilizers in Calcareous Soils, and Comparative Effectiveness in Greenhouse Pot Cultures

NITRIC PHOSPHATE FERTILIZERS are produced by reaction of rock phosphate and nitric acid, either alone or in combination with sulfuric or phosphoric acid. This reaction is followed by ammoniation to neutralize excess acid and to increase the nitrogen content,

and if desired to give a complete fertilizer product, by potassium addition. The cost advantage of the nitric phosphate process is based partly on the action of nitric acid in dissolving rock phosphate and partly on the contribution nitric acid makes to the nitrogen content of the

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final fertilizer grade. The cost of both nitric acid and ammonia used is chargeable to fertilizer nitrogen rather than production of available phosphorus.

The TVA began producing "nitraphosphates" on a pilot plant basis in 1948 (8). The preferred designation of

The availability to plants of phosphorus of some nitric phosphates from France, ammonium phosphate-sulfate, and ammoniated superphosphate added to calcareous soil was compared with the amount of phosphorus released by an equilibrium-extraction technique. The amount of phosphorus obtained by this technique, involving repeated extraction of soil fertilizer mixtures with water at a moisture level of a saturated paste, gave results that correlated directly with that of plant growth and phosphorus uptake by plants. No practical difference in the effectiveness of the phosphorus of nitric phosphates was produced by the phosphonitric and sulfonitric process, ammonium phosphate-sulfate, and ammoniated superphosphate, as measured by growth response and phosphate uptake by two crops. Carbonitric phosphate was significantly less effective than all other sources.

these products has been changed to nitric phosphates. Fertilizers have been produced in Europe for over 25 years by treating rock phosphate with nitric acid. The processes involved are conveniently summarized by Hignett (3) and Walthall and Houston (9). The usual type of processes employed are as follows:

Process I. Rock phosphate + phosphoric acid + nitric acid + ammonia (potassium chloride addition is optional).

Process II. Rock phosphate + sulfuric acid + nitric acid + ammonia (potassium chloride addition is optional).

Process III. Rock phosphate + nitric acid + ammonia + either potassium sulfate or ammonium sulfate.

Process IV. Rock phosphate + nitric acid + ammonia + carbon dioxide (potassium chloride addition is optional).

The phosphorus in nitric phosphates is largely dicalcium phosphate, which has an advantage of being nonhygroscopic and thermostable. The ratio of nitrogen to phosphorus pentoxide in nitric phosphates is relatively high. This is desirable in a fertilizer for western states, but is costly to produce by more conventional methods. The use of the nitric phosphates as fertilizers thus assumes importance from the standpoint of the innovation it is making to the existing structure of the fertilizer industry. A possible disadvantage of the nitric phosphates is that with a high degree of ammoniation the water solubility of the phosphorus fraction is reduced.

Data from field experiments involving nitric phosphates produced experimentally by the TVA have been compiled by Rogers (5). Experiments with corn, wheat, oats, and cotton, conducted on acid soils in six southeastern states, indicate that the phosphorus in nitric phosphates is, as available as that in concentrated superphosphate. Moreover, the nitric phosphates containing nitrogen, phosphate, and potash were as effective as other mixed fertilizers of similar ratios for corn and cotton. Rogers, Ensminger, and Pearson (6) report that these crops do not require phosphorus water solubility greater than 10% in the nitric phosphates.

Low water solubility has been claimed as a factor in reducing phosphorus availability in a limited number of tests in calcareous soils of Iowa and Nebraska (8). The current status of belief for Iowa soils is that water solubility is an important factor in utilization of phosphate by plants (10). In view of these findings and the apparent contradiction in the effectiveness of nitric phosphates with crops on calcareous and acid soils, an investigation was undertaken to explore more fully the behavior of these fertilizers in Arizona soils whose exchange complex is almost wholly occupied by calcium.

The objective of the study was to compare the recovery of phosphorus of three nitric phosphates, ammonium phosphate-sulfate, and ammoniated

superphosphate from calcareous soils by the equilibrium-extraction technique with the uptake by plants using the greenhouse pot-culture technique. The effectiveness of these same five fertilizers along with another nitric phosphate, a complex mix (Potasse Engrais Chimimques process) (7) from France, was compared in calcareous soils using two methods of placement in pots under greenhouse conditions.

### Experimental Procedure

#### Equilibrium-Extraction Technique.

The equilibrium-extraction procedure first described by Fuller and McGeorge (2) was employed to determine the recovery of fertilizer phosphorus added to soils. The various phosphate fertilizers were added to 200-gram samples of calcareous soils at a rate of 50 pounds of phosphorus pentoxide per acre 6-inches. Enough water was added to the soil-fertilizer mixture to make a saturated paste. After it had stood for 2 hours, the paste was placed in a Büchner funnel fitted with filter paper and vacuum was applied to remove the soil solution. The quantity of the clear extract was measured and analyzed for phosphorus by the Dickman and Bray colorimetric method (7). The soil-fertilizer mixtures were then removed from the filter funnel and allowed to air-dry. The soils were again wetted and extracted as before. This process was repeated until phosphorus failed to appear in the filtrate, or until the number of extractions exceeded 25. Samples of soil not receiving phosphorus fertilizer were subjected to the same equilibrium extraction to provide a check sample for calculating the amount of phosphorus derived from the fertilizer. The cumulative amount of phosphorus found in the saturated-paste extracts were then plotted for each soil.

#### Greenhouse Pot-Culture Technique.

The availability of the phosphorus of the fertilizers was compared in a pot test in the greenhouse. One kilogram of soil that had been passed through a 2-mm. sieve was placed in each glazed earthenware pot. The appropriate fertilizer was rolled into the soil at a rate of 50 pounds of phosphorus pentoxide

Table I. Some Chemical Characteristics of Fertilizers Used<sup>a</sup>

Trade Name	Index	P <sub>2</sub> O <sub>5</sub> , %			N, %		Particle Size Ground, %	
		Avail-able	Water soluble	Total	Ammonic	Total	7-60 mesh	60-100 mesh
Phosphonitric	22-22-0	21.65	11.75	21.65	12.74	12.65	21	58
Sulfonitric	16-14-0S	13.55	1.60	13.65	7.18	14.38	20	57
Carbonitric	16-14-0C	14.05	0.20	14.30	7.82	15.94	20	59
Nitric phosphate complex mix	15-14-0M	13.72	2.35	14.46	7.43	14.82	22	60
Ammonium phosphate-sulfate	16-20-0	20.05	18.35	20.25	16.36	16.36	22	50
Ammoniated superphosphate	3-16-0	16.75	11.85	18.30	3.40	...	18	60

<sup>a</sup> Chemical analyses of these fertilizers made by Bureau of Chemistry, California Department of Agriculture, Sacramento, Calif. First four fertilizers obtained from Potasse Engrais Chimimques, Paris, France, and are designated as nitric phosphate. Ammonium phosphate-sulfate came from Consolidated Mining of Canada, and ammoniated superphosphate from Stauffer Chemicals of San Francisco.

per acre 6-inches. Nitrogen in the form of urea was added to bring the rate of nitrogen application to a uniform value of slightly more than 50 pounds of nitrogen per acre 6-inches. Potassium was not included in the fertilizer because it is not a limiting factor in crop production in these soils. Annual rye grass and Earlypack tomatoes were used as test crops. Each fertilizer treatment was replicated three times with each soil. The rye was cut for dry-weight determination and phosphorus analysis (4) 6 weeks after seeding. The tops of tomato plants—15 per pot—were cut at the end of 10 weeks for dry weight and phosphorus analyses.

In a second experiment, two methods of placement were compared—namely, banding and mixing of the fertilizers in the soil. Four kilograms of air-dried soil were placed in glazed earthenware pots with the appropriate fertilizer either as a mix or band placement. The procedure employed in the band applications was to place a single band of the fertilizer, ground to pass a 10-mesh screen, about 3 inches deep in the soil and through the center of the pot. The mixed placement was accomplished by rolling the ground fertilizer with the air-dried soil on paper before potting. The fertilizers were applied to the soil at five rates—0, 50, 100, 200, and 400 pounds of phosphorus pentoxide per acre 6-inches. All soils received an additional 100 pounds of nitrogen per acre, as urea, before they were potted to keep nitrogen from being a limiting factor in growth. Twenty tomato seeds were planted per pot and covered with a thin layer of vermiculite. During the seedling stage the plants were thinned to a uniform number of 15 per pot. After about 8 weeks, the plants were cut at ground level, washed in distilled water, and oven-dried. Analyses of the aerial part were made for dry matter, total phosphorus, and nitrogen according to the procedure given above.

### Materials

**Fertilizers.** Four of the six fertilizers used were nitric phosphates produced in France; the other two were commercial ammonium phosphate-sulfate and ammoniated superphosphate. A description of the fertilizers as shown in Table I indicates that the water solubility of the phosphorus varies considerably. The ratio of the total nitrogen to phosphorus pentoxide is about the same for the nitric phosphates. The value is about 1. The sulfonitric phosphate, 16-14-0S, had an unusually low AOAC water solubility compared with those produced by TVA as reported by Thorne, Johnson, and Seatz (8).

**Soils.** Three soils, low in phosphorus available to plants, were selected from representative agricultural areas in

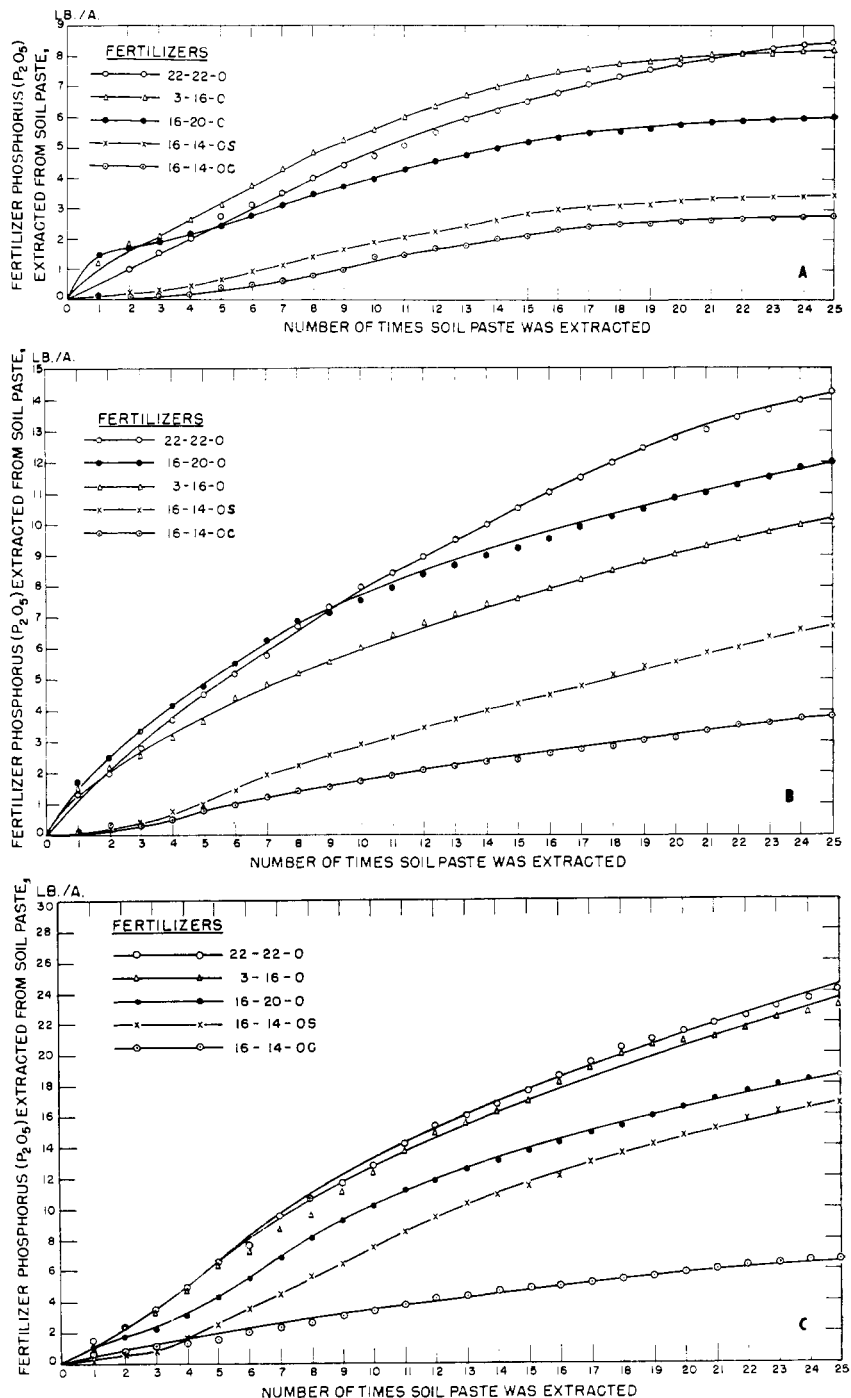


Figure 1. Fertilizer phosphorus found in soil-paste extracts obtained by repeatedly extracting loams treated with different fertilizers at a rate of 50 pounds of phosphorus pentoxide per acre

A. Tucson loam B. Laveen sandy loam C. Mohave clay loam

Arizona: Laveen sandy loam, Tucson loam, and Mohave clay loam. About 9, 2, and 1% calcium carbonate was found in the 0- to 6-inch layer of the three soils, respectively. The pH value of the saturated soil pastes were 7.7, 7.8, and 8.2 for the three soils, respectively. The organic carbon content of the soils is very low, being less than 0.5%. The cation exchange positions are almost wholly occupied by calcium. The cation exchange capacity for the three soils is 6.4, 8.4, 18.5 for the Laveen, Tucson, and Mohave soils, respectively.

### Results and Discussion

**Equilibrium Extraction Study.** The phosphate availability of various fertilizers in three different soils was measured by repeatedly extracting soil, after air drying, with water at a moisture level of a saturated paste. By plotting the cumulative amount of phosphorus found in the saturated paste extracts against the number of extractions, a curve was obtained that gave a characteristic pattern, used in interpreting availability of the phosphate of the fertilizer while in contact with the soil.

**Table II. Uptake of Phosphorus by Rye Grass and Yield of Dry Matter under Greenhouse Conditions from Two Soils as Influenced by Kind of Fertilizer Added**

Fertilizer Added	Oven-Dry Wt. of Rye Grass, <sup>a</sup> G./Pot	P in Plant, %		Total P Taken Up by Grass, γ/Pot
		1st cut	2nd cut	
MOHAVE CLAY LOAM				
None	3.29	0.110	0.067	2.86
Phosphonitric 22-22-0	5.32	0.161	0.145	8.05
Sulfonitric 16-14-0S	4.99	0.160	0.130	7.79
Carbonitric 16-14-0C	3.83	0.130	0.091	4.42
Ammonium phosphate-sulfate 16-20-0	5.02	0.170	0.145	7.88
Ammoniated superphosphate 3-16-0	5.23	0.161	0.142	7.63
L.S.D. 0.05 <sup>b</sup>	1.11	0.053	0.035	3.01
TUCSON LOAM				
None	3.42	0.134	0.132	4.40
Phosphonitric 22-22-0	4.98	0.177	0.159	8.57
Sulfonitric 16-14-0S	4.55	0.162	0.152	7.37
Carbonitric 16-14-0C	4.28	0.147	0.149	6.39
Ammonium phosphate-sulfate 16-20-0	4.58	0.185	0.154	7.51
Ammoniated superphosphate 3-16-0	4.93	0.182	0.153	8.33
L.S.D. 0.05 <sup>b</sup>	N.S.	0.062	0.087	2.17

<sup>a</sup> Sum of two cuttings and mean of three pots.

<sup>b</sup> Calculated for five fertilizers. Unfertilized treatment not included in calculations.

The ability of a fertilizer-treated soil to renew phosphorus in the soil solution is a function of both the fertilizer and soil. The supply of phosphorus to the soil solution is both an intensity and capacity factor. The phosphorus content of any one extract is a measure of the intensity factor and the changes in this intensity factor with successive extracts is a measure of the capacity factor.

The different fertilizers did not supply phosphorus to the soil solution of Tucson loam at the same rate (Figure 1). The

**Table III. Uptake of Phosphorus by Tomatoes and Yield of Tops under Greenhouse Conditions from Two Soils as Influenced by Kind of Fertilizer Added**

Fertilizer Added	Oven-Dry Wt. of Tomatoes, <sup>a</sup> G./Pot	P in Plant, %	Total P Taken Up by Tomatoes, γ/Pot
MOHAVE CLAY LOAM			
None	2.99	0.105	3.14
22-22-0	5.78	0.123	7.11
16-14-0S	5.68	0.117	6.64
16-14-0C	4.78	0.114	5.45
16-20-0	5.68	0.124	7.04
3-16-0	6.47	0.123	8.02
L.S.D. 0.05 <sup>b</sup>	0.95	0.029	1.58
TUCSON LOAM			
None	2.62	0.166	5.48
22-22-0	3.65	0.205	7.48
16-14-0S	3.43	0.182	6.24
16-14-0C	3.44	0.169	5.81
16-20-0	3.47	0.169	5.86
3-16-0	4.20	0.159	6.68
L.S.D. 0.05 <sup>b</sup>	0.79	0.039	1.30

<sup>a</sup> Mean of aerial parts of three replicated pots.

<sup>b</sup> Unfertilized treatment not included in calculations.

least phosphorus was recovered from the nitric phosphates, 16-14-0C, made by Process IV involving carbon dioxide. The most phosphorus was recovered from the soil treated with the nitric phosphate 22-22-0, Process I. There was no great difference in the recovery, however, between the nitric phosphate 22-22-0, ammoniated superphosphate, and ammonium phosphate-sulfate. The amount of AOAC water-soluble phosphorus reported in Table I for these fertilizers was greatest for ammonium phosphate-sulfate, followed by ammoniated superphosphate and phosphonitric fertilizer. Thus the recovery of phosphorus from these three fertilizers when in contact with the soil was not wholly related to the AOAC water-solubility of the fertilizers in the absence of the soil as reported in Table I. The two nitric phosphates, sulfonitric and carbonitric, that had very little AOAC water-soluble phosphorus, yielded the least amount of phosphorus to the soil solutions.

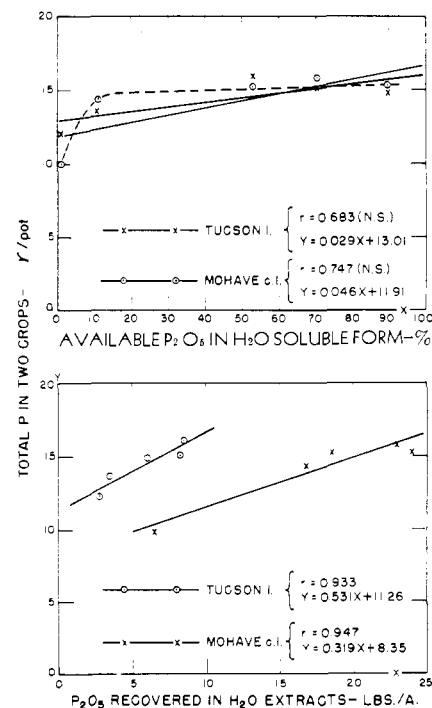
The amount of phosphorus recovered from the fertilizers mixed in Laveen sandy loam and Mohave clay loam was much greater than that from Tucson loam (Figure 1). The difference in the capacity factor between these three soils is statistically significant as interpreted from least-significant-difference (L.S.D.) calculations. About twice as much phosphorus was obtained from the fertilizers in the Laveen as the Tucson soil. More than four times as much phosphorus came into the soil solution from fertilizers mixed in the Mohave than in the Tucson soil (Figure 1).

The relative position of the cumulative curves for the three most soluble fertilizers

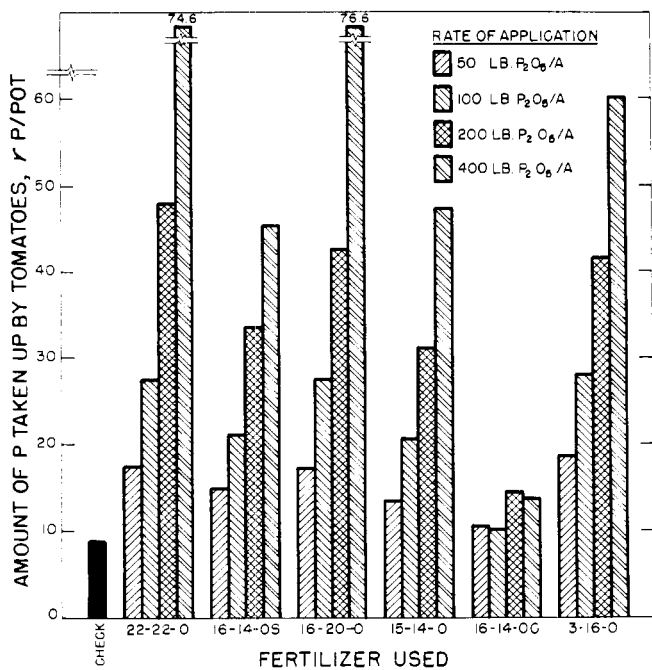
also varied among the soils. Furthermore, the relative amount of phosphate recovered from the sulfonitric, 16-14-0S, compared with 22-22-0, 16-20-0, and 3-16-0 was greater in the Mohave clay loam than in the other two soils. The recovery of sulfonitric in Mohave soil resembled that of 22-22-0 more than that of 16-14-0C. This was not the case with the other two soils.

**Greenhouse Pot-Culture Study.** The effectiveness of the five fertilizers for increasing the yield of rye grass and tomatoes grown on two calcareous soils low in available phosphorus differed little according to the source of phosphate except for that of carbonitric phosphate (Tables II and III). Differences among yields from soils receiving phosphonitric, sulfonitric, ammonium phosphate-sulfate, and ammoniated superphosphate were not significant at the 0.05 level. Yields on soils receiving carbonitric phosphate were significantly lower than those on soils receiving the other fertilizers. All fertilizers, however, increased the yields over those of unfertilized soils.

Although the yields of plant material from soils receiving the four most effective fertilizers were not significantly different, the mean yield figures showed that plant growth response was directly related to the phosphate released to the soil-paste extracts as shown in Figures 1 and 3 for the corresponding soils. For example, the order of decreasing yields of plant materials on the two soils as a result of fertilizer applications was 22-

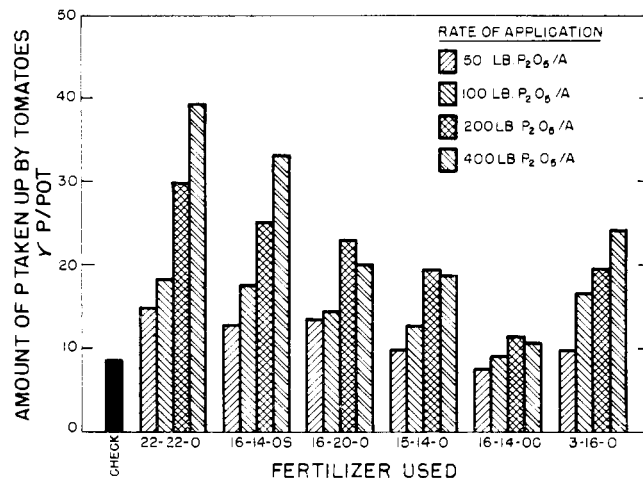


**Figure 2. Relationship between total phosphorus found in two crops, percentage of AOAC available phosphate in fertilizer that is AOAC water soluble, and amount of phosphorus found in soil-fertilizer saturated-paste extracts**



▲ Figure 3. Uptake of phosphorus by tomato plants in Mohave clay loam as influenced by different sources of fertilizers mixed into soil at different rates

▼ Figure 4. Uptake of phosphorus by tomato plants in Mohave clay loam as influenced by different sources of fertilizers banded into soil at different rates



22-0, 3-16-0, 16-20-0, 16-14-0S, 16-14-0C (Tables II and III). The order of phosphorus released as shown for these same soils in Figures 1 and 3 was 3-16-0 > 22-22-0 > 16-20-0 > 16-14-0S > 16-14-0C.

The effectiveness of the five fertilizers as measured by the total uptake from the fertilized soils by the two test crops as shown in Tables II and III was not the same for all sources. Statistically, however, the nitric phosphate 22-22-0 and 16-14-0S, ammonium phosphate-sulfate, and ammoniated superphosphate were not taken up in significantly different amounts at the 5% level. Carbonitric phosphate, though, again was shown to be less effective than that of the other sources.

The effectiveness of the fertilizers, as measured by the total uptake of phosphorus of the two crops, was not well correlated with the amount of AOAC water-soluble phosphorus (see upper diagram in Figure 2). The correlation

coefficient of  $r = 0.747$  and  $r = 0.683$  for Mohave clay loam and Tucson loam, respectively, are not significant at the 5% level. The dashed curved line indicates that when the percentage of AOAC available phosphorus in AOAC water-soluble form exceeds about 11, plant response—as estimated by total phosphorus uptake for both crops—was not measurably influenced by source of the phosphate fertilizer.

The lower diagram of Figure 2 shows that when plant response again is plotted against the values obtained in the laboratory by the equilibrium-extraction technique, the correlation is significant at the 5% level. Correlation coefficients of 0.947 and 0.933 were obtained for the Mohave and Tucson soil, respectively.

**Yield of Dry Matter.** The dry matter obtained from the tomato tops grown on the Mohave clay loam increased as the rate of application increased where the fertilizers were mixed throughout the soil (Table IV). The

plants were small and showed marked phosphate deficiency symptoms in unfertilized soil. The response of tomatoes to the carbonitric phosphate, 16-14-0C, was significantly less than that of other fertilizers at all rates of application (Table IV). The sulfonitric phosphate, 16-14-0S, was significantly less effective at the 5% level than ammonium phosphate-sulfate, 16-14-0, when mixed in the soil. The sulfonitric phosphate, however, was as effective as the other four fertilizers.

The influence of source and rate of fertilizer applications under conditions of band placement on yield of dry matter is shown in Table IV. In general, dry matter production was less when the fertilizers were banded than when mixed with the soil. Only in six of the 24 treatments were yields as great or greater in the banded than in the mixed placements, when comparisons were made for the same fertilizer applied at the same rate. Again, as in the mixed placement, the carbonitric phosphate was significantly less effective than the other fertilizers. The dry matter yield increased directly as the rate of application of fertilizers 22-22-0, 16-14-0S, and 3-16-0 plus urea increased up to the 200-pound level. However, with band placement, increasing the rate of applications of these three fertilizers from an equivalent of 200 pounds of phosphorus pentoxide per acre to 400 pounds per acre decreased yields (Table IV).

**Phosphate Uptake.** The percentage of phosphorus in the tomato tops was lower in plants grown in soils having the fertilizer banded—except for 16-14-0C (Table IV). The source of phosphorus also influenced its concentration in the plant. The lowest concentration was found in plants supplied with fertilizer 16-14-0C. The concentration did not

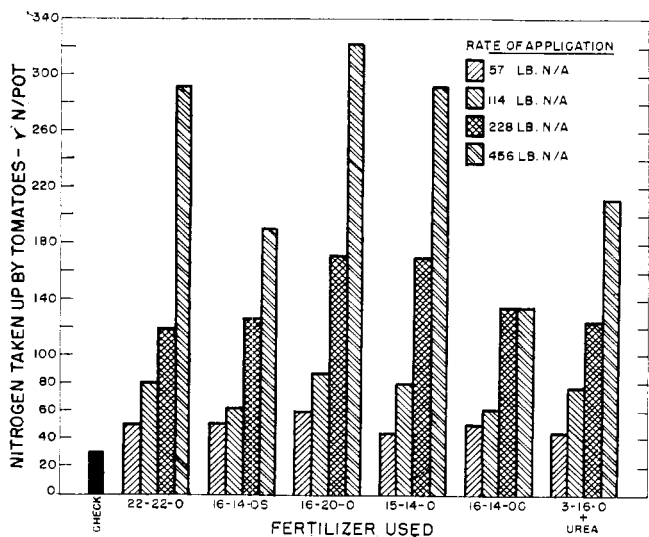


Figure 5. Uptake of nitrogen by tomato plants in Mohave clay loam as influenced by different sources of fertilizer mixed into soil at different rates

**Table IV. Influence of Source and Rate of Application of Fertilizer Mixed and Banded in Mohave Clay Loam on Yield, Nitrogen, and Phosphorus Content of Tomato Tops**

Treatment <sup>b</sup>	Mixed				Banded			
	Phosphorus, <sup>a</sup> lb. P <sub>2</sub> O <sub>5</sub> / acre	Yield oven-dry wt., g./pot	Phosphorus, <sup>c</sup> %	Nitrogen, <sup>c</sup> %	Phosphate, <sup>a</sup> lb. P <sub>2</sub> O <sub>5</sub> / acre	Yield oven-dry wt., g./pot	Phosphorus, <sup>c</sup> %	Nitrogen, <sup>c</sup> %
No fertilizer	0	3.31	0.27	0.74	0	3.28	0.24	0.95
22-22-0	50	7.55	0.23	0.67	50	6.73	0.22	0.60
Phosphonitric phosphate	100	12.47	0.22	0.66	100	11.26	0.16	0.65
	200	17.16	0.28	0.70	200	18.67	0.16	0.75
	400	28.68	0.26	1.02	400	24.59	0.16	1.14
16-14-0S	50	7.16	0.21	0.79	50	5.78	0.22	0.59
Sulfonitric phosphate	100	8.09	0.26	0.76	100	9.72	0.18	0.60
	200	15.29	0.22	0.83	200	17.95	0.14	0.68
	400	22.84	0.20	0.83	400	20.84	0.16	1.08
16-14-0C	50	6.62	0.16	0.81	50	5.04	0.15	0.63
Carbonitric phosphate	100	7.27	0.14	0.87	100	5.96	0.15	1.27
	200	11.15	0.13	1.23	200	7.56	0.15	1.87
	400	11.57	0.12	1.19	400	7.63	0.14	1.95
15-14-0	50	7.10	0.23	0.77	50	5.82	0.17	0.70
Mixed nitric phosphate	100	10.37	0.20	0.78	100	8.48	0.15	0.63
	200	20.81	0.15	0.82	200	16.20	0.12	0.80
	400	26.41	0.18	1.09	400	12.48	0.15	1.65
16-20-0	50	8.67	0.20	0.72	50	6.74	0.20	0.57
Ammonium phosphate-sulfate	100	12.56	0.22	0.69	100	9.46	0.15	0.57
	200	18.58	0.23	0.93	200	17.63	0.13	0.77
	400	30.63	0.25	1.05	400	10.55	0.21	1.08
3-16-0 + urea	50	7.40	0.25	0.64	50	6.81	0.14	0.57
Ammoniated superphosphate + urea	100	11.68	0.24	0.67	100	14.94	0.11	0.72
	200	19.01	0.22	0.77	200	17.57	0.11	0.79
	400	23.46	0.26	0.90	400	23.20	0.11	1.45

<sup>a</sup> L.S.D. at 0.05 indicate no statistical difference between fertilizers at same rate of application except for 16-14-0C, which was less effective than the others.

<sup>b</sup> Nitrogen was added to fertilizers when necessary to give a 8 to 7 nitrogen to phosphorus pentoxide ratio.

<sup>c</sup> Data represent a mean of three pots containing 4 kg. of soil per pot.

exceed 0.2% for plants given this fertilizer.

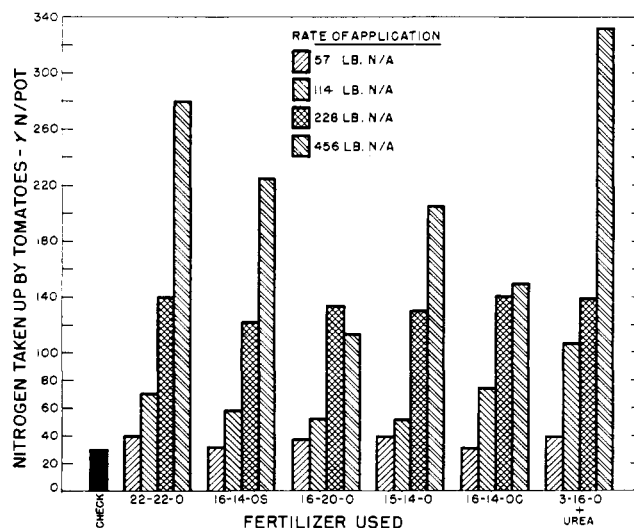
Figure 3 shows that when the fertilizers were mixed into the soil, the total amount of phosphorus taken up was influenced by source as well as rate of application. Except for fertilizer 16-14-0C the total uptake was greater as the rate of application became greater. Total uptake was highest for fertilizer 16-14-0S, and 15-14-0 was a little less than that of 22-22-0 and 16-20-0. Furthermore, 16-14-0S and 15-14-0 appear to be about equal as far as uptake is concerned. Carbonitric phosphate was least available of all sources.

Less phosphorus was taken up by plants from band than mixed placement of fertilizer. The most effective fertilizer source, when band-placed in the soil, was phosphonitric phosphate, 22-22-0 (Figure 4). The fertilizer 16-14-0C again was least available. Nitric phosphate, 15-14-0, was about as available as ammonium phosphate-sulfate, whereas sulfonitric fertilizer appeared to be slightly more available than the ammonium phosphate-sulfate. The phosphorus of band-placed fertilizer, 16-20-0, 15-14-0, and 16-14-0C, was taken in lower amounts at the 400-pound rate than at the 200-pound rate (Figure 4).

**Nitrogen Uptake.** The concentration of nitrogen in the tomato tops

(Tables IV and V) in general increased as the rate of application was increased. The total uptake of nitrogen by tomato plants in the mixed placement series was greatest from fertilizer 16-20-0, and least from fertilizer 16-14-0C (Figure 5). The effectiveness of the nitric phosphate 22-22-0 as evaluated by total nitrogen taken up per pot, was about the same as the 15-14-0 and a little better than 16-14-0S and 3-16-0 plus urea. Uptake from the latter two was about the same at equal rates of application.

The uptake of nitrogen was influenced considerably by method of placement. Figures 5 and 6 show that ammonium phosphate-sulfate was the most effective source as mixed placement and the least effective fertilizer as band placement. Total uptake of nitrogen by the tomato plants was highest, in the banded series, from fertilizers 22-22-0 and 3-16-0 plus urea. Uptake from fertilizer 16-14-0S was almost identical to that from 15-14-0 (Figure 5). These supplied more nitrogen to the tomato plants than ammonium phosphate-sulfate.



**Figure 6. Uptake of nitrogen by tomato plants in Mohave clay loam as influenced by different sources of fertilizer banded into soil at different rates**

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## GRAPEFRUIT PIGMENT DETERMINATION

# Spectrophotometric Methods for Determining Pigmentation—Beta-Carotene and Lycopene—in Ruby Red Grapefruit

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Two methods for the measurement of pigments occurring in colored grapefruit are outlined and the results compared. Method A involves extraction of the sample, separation of the major pigments—lycopene and carotene—on a magnesia-Super Cel column, elution, and spectrophotometric measurements of the separated pigments. By method B, a more rapid but less precise procedure, the pigment is extracted and the absorptivity of the extract determined at 451  $m\mu$  for carotene and 503  $m\mu$  for lycopene. Results of total concentration of the pigments, as determined by simultaneous equations, show method B averages 10.3% higher for lycopene and 16.2% higher for carotene than method A. During a 69-day period, January 26 to April 4, lycopene, in the edible fruit of Ruby Red grapefruit, decreased from 0.29 mg. % to 0.10 mg. %, and carotene from 0.34 mg. % to 0.21 mg. %.

PROBLEMS ASSOCIATED WITH THE COMMERCIAL UTILIZATION of grapefruit are being studied by the U. S. Fruit and Vegetable Products Laboratory, Weslaco, Tex. The majority of the grapefruit trees which are now growing in the Lower Rio Grande Valley are either Ruby Red or Marsh Pink varieties, producing fruit which is colored light red or pink, and containing carotene and lycopene as the principal pigments.

The canning or freezing of juice from colored fruit presents problems in obtaining and standardizing the color of the product. Prior publications (5, 6) have discussed these difficulties and outlined chromatographic and reflectance methods for estimating the pigment content of the pulp and juice from colored grapefruit. Quantitative differences between the two methods of analysis and need for a more rapid chromatographic method for carotene and lycopene determinations stimulated the present work.

Comparisons are made between two methods: method A, a modification of the chromatographic-spectrophotometric method (5); and method B, the spectrophotometric method (6).

Method A is a relatively rapid chromatographic method for quantitatively measuring the carotene and lycopene extracted from the juice, suspended solids, and pulp of the fruit. The hexane-extracted pigments are separated by chromatographing on a magnesia-Super Cel column, eluted, and spectrophotometric measurements made of the pigment intensity of the separate eluates.

Method B is more rapid. The total carotenoid pigments are extracted in the same manner. Spectrophotometric measurements are made directly on the hexane extract at 451 and 503  $m\mu$  and the total pigment is calculated as carotene and lycopene by means of simultaneous equations (6).

### Apparatus and Reagents

**Waring Blender.** The Waring Blender used was equipped with regular and microblender cups.

**Spectrophotometer.** A Beckman Model DU spectrophotometer, with 2- and 5-cm. cells for absorbance, was used in the New Orleans, La., laboratory.

A Cenco-Sheard spectrophotometer was used for tests conducted in the Weslaco, Tex., laboratory. Each instrument was standardized with purified lycopene and carotene.

**Chromatographic Column.** A column 24 mm. in inside diameter and 245 mm. long, with a stem 4 mm. in inside diameter and 140 mm. long was used.

**Chemicals and Reagents.** Westvaco adsorptive powered magnesia, No. 2641 and 2642; Hyflo Super-Cel; commercial hexane, boiling point 66-69° C.; methanol, ACS grade; acetone; sodium sulfate, anhydrous;  $\beta$ -carotene, purified

Table I. Effect of Saponification of Hexane Extract

Extract No.	Nonsaponified Extract, Mg. %		Saponified Extract, Mg. %	
1 Carotene	0.30	0.30	0.29	0.29
1 Lycopene	0.45	0.45	0.43	0.43
2 Carotene	0.16	0.17	0.18	0.18
2 Lycopene	0.21	0.21	0.20	0.19