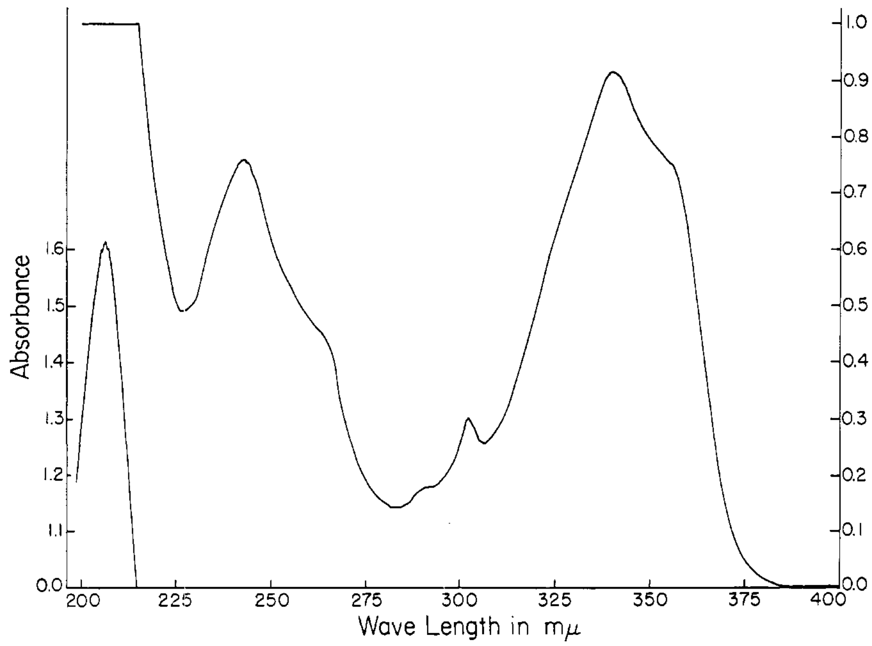

Figure 2. Ultraviolet absorption spectrum of crystalline coumestrol in methanol

Table III. Comparison of Estrogenic Activity of Ladino Clover Estrogen with Genistein

Substance Fed	*Amount per Mouse, Mg.*	*No. of Mice*	*Mouse Uterine Weight after 7 Days, Mg.*
Normal control	0	5	10
Ladino clover estrogen	0.50	5	34
	0.75	5	61
Genistein	10	2	18
	15	5	44

fraction collector as 11 to 24 contained a single compound that was a monomethyl ether of the estrogen—melting point, 278° C.

Analysis of monomethylether: Calculated for $C_{15}H_7O_4(OCH_3)$: C, 68.1; H, 3.54; and OMe, 11.1%. Found: C, 68.1; H, 4.00; and OMe, 11.0%. The tubes numbered 36 to 79 contained the dimethyl ether melting point, 198° C.

Analysis of dimethyl ether: Calculated for $C_{15}H_6O_3(OCH_3)_2$: C, 68.9; H, 4.05; and OMe, 20.9%. Found: C, 68.9; H, 4.52; and OMe, 19.3%. These results indicate two free hydroxyl groups in the compound.

Absorption Spectrum. The ultraviolet absorption spectrum of coumestrol in methanol is presented in Figure 2. As coumestrol has strong ultraviolet absorption peaks at 208, 243, and 343 mμ, the possibility that the compound is an isoflavone similar to genistein, biochanin A, or formononetin was considered unlikely, because isoflavones generally show no prominent absorption peaks above 300 mμ. These latter compounds are the only known estrogens previously isolated from forage plants.

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PHOSPHORUS AVAILABILITY

Effect of Particle Size on Availability to Plants of Phosphorus in Phosphate Rock from Various Sources

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FOR THE YEAR ENDING June 30, 1955, 605,000 tons of ground phosphate rock were marketed in the United States and territories to use as a fertilizer medium for direct application (*5*). The main ore-producing areas are located in the southeastern and western United States. In a recent experiment, Armiger and Fried (*1*) compared the relative agro-

Phosphate rocks ground to a −325-size class exerted more influence on yields and phosphorus uptake by alfalfa and buckwheat plants than did the −100-, −100 + 150-, −150 + 325-size classes. The −100-mesh size was only slightly less effective than the −325-class fraction. Fine grinding did not affect all sources alike. Relative uptake and relative *A* values gave similar residual characterizations. Fine grinding was not as important as the nature of a phosphate rock in determining its agronomic effectiveness.

nomic effectiveness of rocks from these regions and of rocks from several foreign sources. Caro and Hill (*2*) studied the physical and chemical properties of these same materials and evaluated the relative effectiveness of each material as a source of phosphorus. The results of the two investigations were closely correlated and showed that the various phosphate rocks differ markedly in their effectiveness.

The above investigations compared phosphate rocks of only one particle size. The present investigation compares several different mesh sizes of various phosphate rocks in order to determine if particle size influences agronomic effectiveness.

Experimental Procedure

The effectiveness of different mesh sizes of the various rocks was measured on alfalfa grown in the greenhouse in No. 10 cans. Five harvests were taken over a period of 211 days. Following the last alfalfa harvest the remaining roots and crowns were removed, and the soil was sieved through an 8-mesh screen. The soil from a single can was halved and placed in No. 2 cans. In one series, buckwheat was grown to test the residual phosphorus by plant yield; in the other, the soils were treated with a radioactive resin phosphate that contained the equivalent of 20 pounds of phosphate per acre as superphosphate, and buckwheat was grown in order to determine *A* values (*3*, *4*) as a measure of residual phosphorus.

Phosphate Rocks. The phosphate rocks selected for this work are representative of ores produced in the southeastern United States. Previous tests (*1*) have shown Florida pebble and South Carolina land rock to be the more effective sources of phosphorus for alfalfa and buckwheat. Tennessee brown rock and Virginia apatite have very low quantities of available phosphorus for plant growth. These rocks are included in this test to determine if finer grinding will increase their effectiveness as a source of phosphorus.

Phosphate rocks of foreign origin are represented by deposits from North Africa and the Curaçao Island. The North African deposit of Gafsa Tunis rock is an ore with a high fluorine content. Curaçao Island ore represents a low fluorine content material. Both materials are more effective in supplying phosphorus to plants than any of the domestic sources.

Size Classes. Four mesh fractions were selected for the test: −100 + 150, −150 + 325, −325 and −100. Caro and Hill (*2*, p. 684) have described the preparation of these various particle sizes. The quantity of the −325-mesh fraction contained within the −100-mesh composite of each material is as follows: Tunis rock, 10%; Curaçao rock, 28%; Florida pebble, 35%; South Carolina rock, 41%; Tennessee brown rock, 19%; and Virginia apatite, 57%.

Physical and Chemical Properties. The total phosphate and the citrate-soluble phosphate content of each mesh size of the rocks are shown in Table I. Separating a phosphate rock into mechanical fractions, in general, yields products that differ chemically from the composite. There is a wide variation in total phosphate content of the various materials, but only slight differences between the corresponding mesh sizes of each material. The range is from a low of 26.4% for South Carolina land rock to a high of 40.7% for Virginia apatite. Tunis rock has the highest citrate solubility and Virginia apatite the lowest. Fineness of grinding seems to exert slightly more influence on the solubility of the poorer materials.

Phosphate Treatments. All phosphate rock materials were applied at the rate of 640 pounds of total phosphate per acre. Superphosphate was applied at five rates: 40, 80, 160, 320, and 640 pounds of total phosphate per acre in order to establish phosphorus uptake curves for the various soils. All phosphorus carriers were mixed throughout the soil.

Basal Treatments. Ammonium nitrate was applied to all cultures at the rate of 100 pounds of nitrogen per acre and potassium chloride at the rate of 200 pounds of potassium oxide per acre. Basal treatments were applied in solution form. The soils were not limed. An additional 50 pounds of nitrogen per acre as ammonium nitrate was added to the cultures after a growth period o 85 days, and an additional 100 pounds of potassium oxide as the chloride after 107 days.

The residual phosphate rock pots received 50 pounds of nitrogen per acre as ammonium nitrate and 100 pounds of potassium oxide per acre as potassium chloride 7 days after seeding the buckwheat.

Soils. Three soils of different origin were selected for these studies: Crosby silt loam, pH 5.8; Elliott silt loam, pH 5.8; and Cecil sandy loam, pH 5.6.

The choice of fairly acid soils was made in order to create as near as possible an ideal environment for the utilization of phosphorus by plants from phosphate rock.

Crops. Alfalfa (Ranger variety) was selected as the indicator crop for a long term experiment. The alfalfa was seeded on April 11, 1955, and the plants were

Table I. Physical and Chemical Properties of the Phosphate Rocks at Various Particle Sizes

		Mechanical Fraction −100 + 150 Mesh		Mechanical Fraction −150 + 325 Mesh		Mechanical Fraction −325 Mesh		Composite −100 Mesh	
Lab. No.	Phosphorus Source	Total P_2O_5, %	Citrate-sol. P_2O_5, % of total	Total P_2O_5, %	Citrate-sol. P_2O_5, % of total	Total P_2O_5, %	Citrate-sol. P_2O_5, % of total	Total P_2O_5, %	Citrate-sol. P_2O_5, % of total
1551	Tunis phosphate rock	29.9	16.4	28.8	16.5	26.5	18.9	29.9	20.0
985	Curaçao phosphate rock	37.9	11.9	38.2	12.0	37.5	14.6	37.6	14.8
910	Florida land pebble	30.9	10.4	30.7	10.5	31.8	14.4	31.7	11.1
1484	So. Carolina land rock	27.1	10.0	26.4	14.7	29.0	15.7	27.6	17.2
907	Tennessee brown rock	35.0	5.1	34.5	5.1	33.7	7.1	34.6	6.1
1295	Virginia apatite	40.3	1.5	41.4	5.8	40.6	6.4	40.7	7.6

repeatedly thinned until the final stand of seven plants per pot was attained on April 24. The plants were cut 1.5 inches above the soil at intervals of 61, 85, 107, 139, and 211 days after emergence. The plant material was oven-dried, and phosphorus determinations were made from the dry plant material from the first and fourth harvests.

In the residual test, buckwheat was selected as the test crop inasmuch as it is known to utilize phosphorus effectively from phosphate rock. The buckwheat was seeded April 26, 1956, at the rate of 10 seeds per pot with subsequent thinning to five plants per pot on May 4. The plants were harvested when in full bloom on May 28. The plant material was oven-dried and phosphorus determinations were made on the dry plant material.

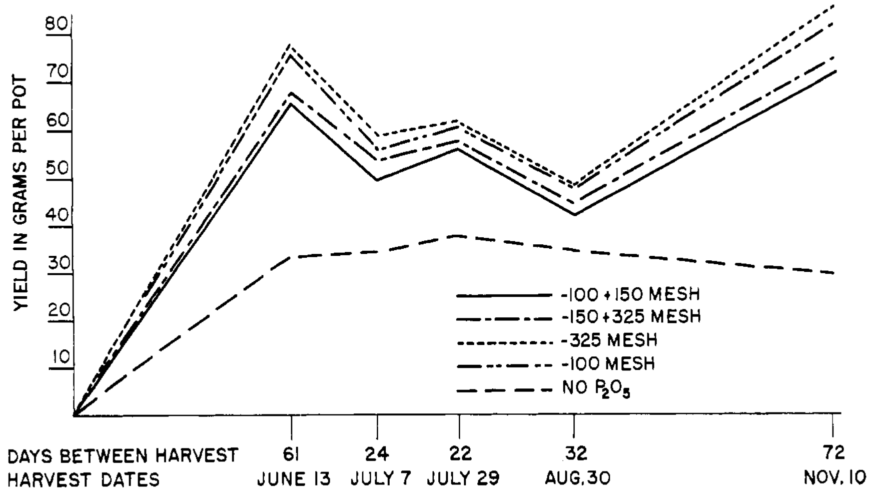

Figure 1. Effect of fineness of grinding of phosphate rock on yields of alfalfa. Each mesh size represents the accumulated averages of six sources of phosphate rock

Results and Discussion

Yield Data. Data plotted in Figure 1 indicate that fineness of grinding of phosphate rock does influence alfalfa yields. An accumulated total yield of alfalfa from three soils and six phosphate rock sources shows that a fineness of −325 mesh gives the largest yields at each of five harvests. A composite of −100 mesh fineness is only slightly less productive than the −325-mesh fraction. The close parallel between the two is in accord with results attained in laboratory tests (*2*).

The −100 + 105-sized fraction is the poorest mesh size, exhibiting about the same difference from the −150 + 325-mesh size as is shown by the difference between the −325-sized fraction and the −100-mesh rock. The differences between mesh sizes are relatively constant for the first four harvests, but appear to increase at the fifth harvest. Excellent plant growth response is noted for all size classes when compared with the no phosphorus check.

The relative effectiveness of the various −325-mesh phosphate rock materials, on a scale where superphosphate applied at equivalent rates equals 100, were: Tunis rock, 110.08; Curaçao rock, 103.50; Florida pebble, 94.09; South Carolina land rock, 105.47; Tenessee brown rock, 75.90; and Virginia apatite, 54.02.

Phosphorus Uptake Data. Findings based on the yield data are substantiated by the phosphorus uptake data from all harvests. Combined data from the three soils for the first and fourth harvests of alfalfa are plotted in Figures 2 and 3 (mesh sizes for Figures 3 to 7 are indicated by legend in Figure 2), against a scale on which the uptake by −325-mesh Tunis rock equals 100% (uptakes in Figures 2 to 7 are expressed on this relative basis). At the first harvest, the −325-fraction of Tunis rock had a significantly greater uptake than the −100-

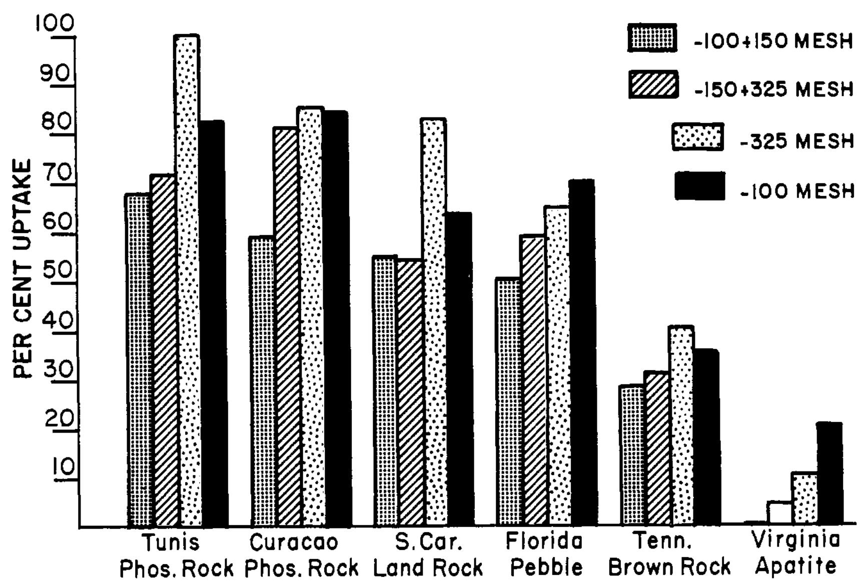

Figure 2. Effect of phosphate rock source and particle size on phosphorus uptake by alfalfa at the first harvest

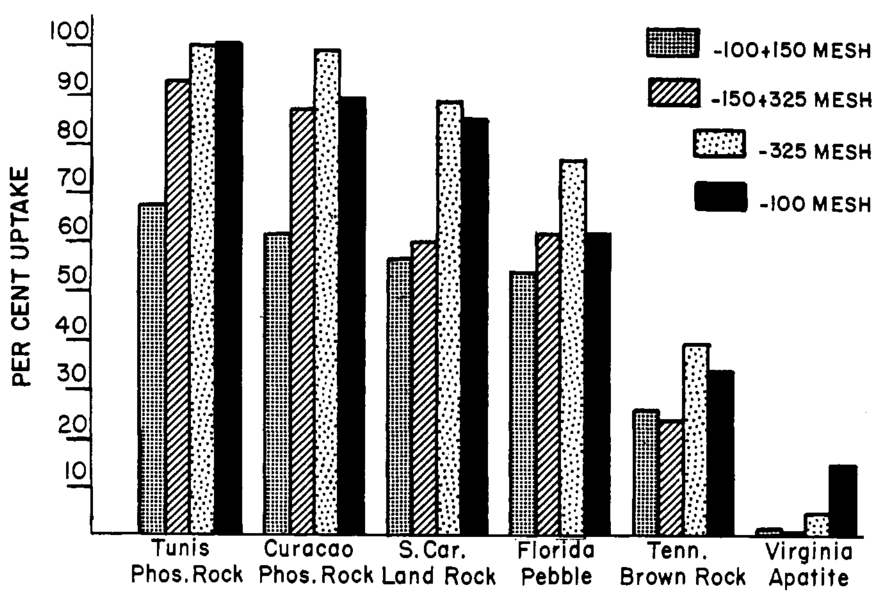

Figure 3. Effect of phosphate rock source and particle size on phosphorus uptake by alfalfa at the fourth harvest

mesh rock and both the −100 + 150- and −150 + 325-mesh size classes. The Florida pebble and Virginia apatite data indicate superior performance from the −100-mesh size in relation to the −325-mesh fraction, but in both instances the differences do not approach significance. South Carolina land rock, at the fineness of −325-mesh, shows highly significant increases over all other mesh sizes.

At the fourth harvest (Figure 3), the phosphorus uptake by alfalfa from the Tunis rock at the −100-mesh size is practically the same as that from the −325-mesh size, thus indicating that, with length of time, the −100-mesh size probably supplies as much phosphorus as the −325-mesh fraction. Differences obtained with the −100-mesh size of Tunis rock were greater than those obtained from the −100-mesh + 150-mesh sizes. The uptakes obtained by the −325-sized fraction from Curaçao rock, Florida pebble, South Carolina rock, and Tennessee brown rock are larger than from any other sized fraction. The most highly significant differences found at this harvest are the −325-mesh sizes of Curaçao rock and South Carolina land rock over the −100 + 150-sized fractions of each. Virginia apatite is an extremely poor source of phosphorus, but the −100-mesh size does show an approximate 10% increase over the −325-mesh size fraction.

The uptake data also confirm the results of a previous experiment comparing various sources of phosphate rock (*7*). Thus both foreign sources were, in general, more effective than the domestic sources. Of the domestic sources the South Carolina land rock was most effective, the Florida pebble next, and the Tennessee brown rock and Virginia apatite were the poorest.

The relative effectiveness of the various −325-mesh phosphate rock materials, on a scale where superphosphate applied at equivalent rates equals 100, were: Tunis rock, 99.43; Curaçao rock, 94.30; Florida pebble, 78.02; South Carolina land rock, 88.80; Tennessee brown rock, 57.20; and Virginia apatite, 34.94.

Residual Test: Uptakes. Phosphorus uptake by buckwheat plants utilizing residual phosphorus as supplied by various size fractions of phosphate rock have shown a decidedly similar pattern of results to those obtained from a previous crop of alfalfa at the first harvest. Uptake data from the residual test, plotted in Figure 4, indicate that interaction of materials and mesh sizes are more evident between the residual data and that of the fourth harvest of alfalfa than that shown by the first harvest of alfalfa.

The largest variation between the residual test and the first and fourth harvest of alfalfa seems to be in the difference between the −325-mesh and −100-mesh

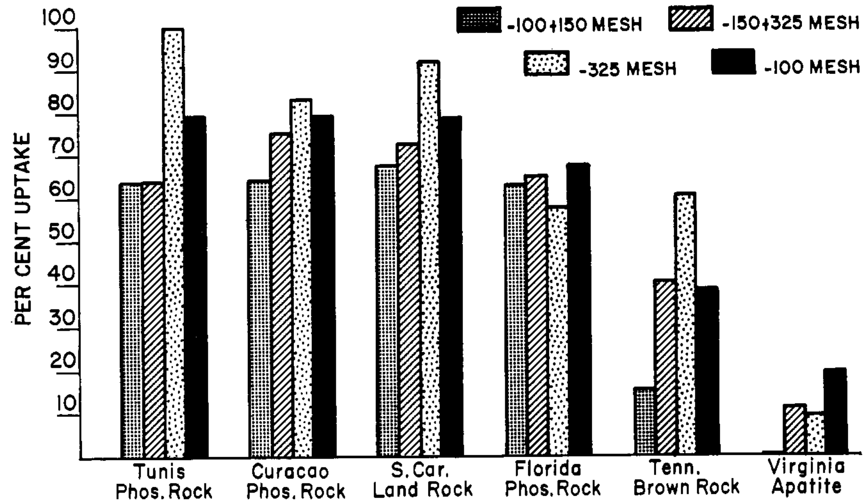

Figure 4. Residual effect of phosphate rock particle size on phosphorus uptake by buckwheat

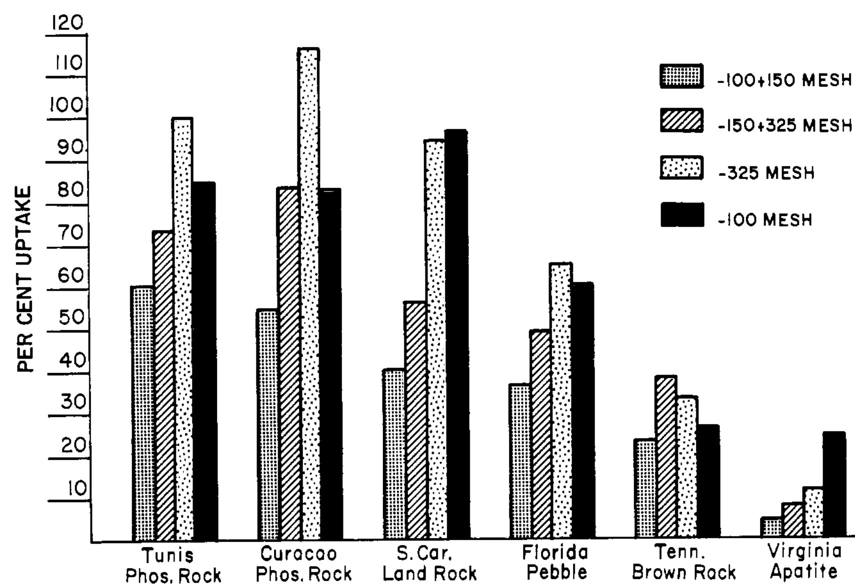

Figure 5. Residual effect of phosphate rock particle size on phosphorus uptake by buckwheat as determined by the *A* value technique

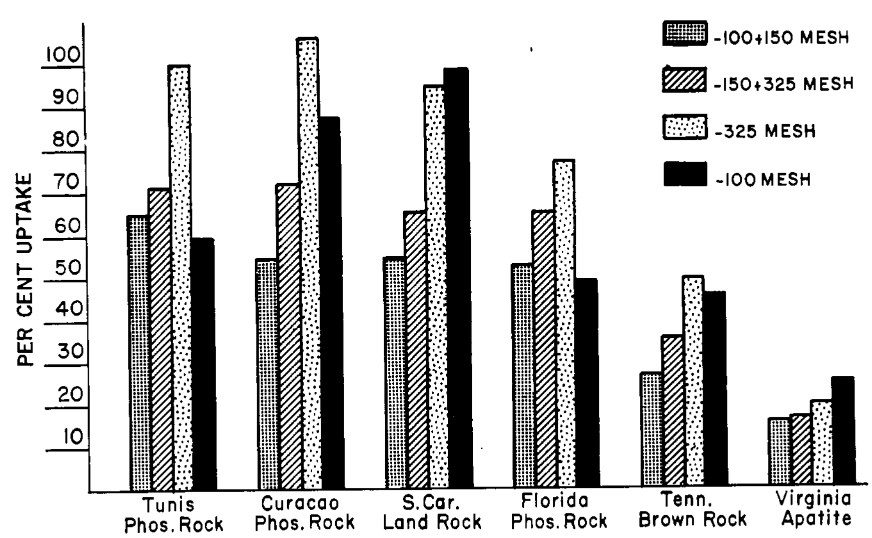

Figure 6. Residual effect of phosphate rock particle size on phosphorus uptake by buckwheat from six phosphate rock sources

as in the first harvest of alfalfa. Tunis rock, −325-mesh size, shows an approximate 20% increase over the −100-mesh size in the residual test. The data from the fourth harvest of alfalfa, however, show the two size classes to be practically comparable in uptake for the Tunis rock. Various mesh sizes of Curaçao rock at the first harvest of alfalfa and the residual test are more nearly in accord than is the fourth harvest of alfalfa with the residual test. All data relative to South Carolina rock seems to be comparable for both alfalfa harvests and the residual test. Probably the most pronounced change has taken place between the various mesh fractions of Tennessee brown rock in the residual test. Here increases favoring the −325-mesh size have increased to as much as 45% over the −100-mesh size fraction.

***A* Value Measurements.** A residual test was made using an adaptation of the *A* value technique as a direct measure of residual value (*3*). These results (Figure 5) indicate that the residual agronomic value of the various mesh sizes of the phosphate rocks is similar to their initial agronomic value at the various alfalfa harvests. The phosphorus uptake data from the pots containing radioactive phosphorus can also be utilized to measure relative residual value of the various particle sizes in the more conventional manner. The results of this calculation are presented in Figure 6.

Figure 7. Residual effect of phosphate rock particle size on phosphorus uptake by buckwheat as determined by the *A* value technique

The two methods of evaluating particle size importance are nearly comparable. If anything, the *A* value seems to accentuate the differences between the various sizes of like materials.

The use of the *A* value to determine soil interaction effects on phosphorus source and particle size is shown in Figure 7. A very definite pattern of results is shown by this data. None of the soils show a variation of more than approximately 15% for any one given particle size. There is excellent agreement between the three soils in regard to phosphorus uptake by the various particle sizes.

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FEED SUPPLEMENTS PRODUCTION

Microbiological Production of Beta-Carotene in Shaken Flasks

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When appropriate plus and minus strains of various members of the Choanephoraceae were grown together in a grain-based medium in shaken flasks, carotene production was increased four- to fivefold over that obtained with unmated strains. Production of carotene by mated cultures was further enhanced by the addition to the medium of vegetable oils, detergent, and β-ionone. Chromatographic analysis showed that 75% of the pigments produced was all-*trans*-β-carotene.

Cartenoid-containing materials are incorporated into feeds for poultry and livestock to provide a source of vitamin A and to impart a desirable color to the skin, fat, and other tissues of the animals. From the standpoint of feed efficiency, a high-potency carotene supplement that is relatively low in crude fiber would be advantageous for use in formulating mixed feeds. The well-known synthetic powers of certain groups of microorganisms prompted a search for those capable of synthesizing carotene in practical amounts.

Barnett, Lilly, and Krause (*2*), Plempel (*5*), and Hesseltine and Anderson (*3*) demonstrated that mating of appropriate heterothallic strains of various members of the order Mucorales increased production of carotene within the mycelium. Further investigations were undertaken at this laboratory to ascertain the effects of certain adjuncts on the yield of carotene produced and to determine the problems involved in developing a practical fermentation process using the mating technique.