manufacturing procedures that minimize the formation of the larger inclusions of sampleite. These are less soluble than the finely disseminated copper, which XRD evidence indicates is probably also mainly present as sampleite. If copper OSP is to be used in the manufacture of superphosphates, reverted to dicalcium phosphate with lime or rock phosphate, release of copper may be much slower. Highly acid conditions will not develop in such granules after application to soils so that a soluble copper salt should be added to these fertilizers after reversion has occurred. Similarly the degree of dissolution of sampleite in copper OSP applied to alkaline soils may be lower than is found for acid soils.

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Effect of Phosphorus Fertilization on Phosphorus Constituents in Soybeans

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Two varieties of soybean (Bragg and Punjab-1) grown in pot culture with three levels of phosphorus were studied for their chemical composition and different phosphorus compounds in the seed and its protein fractions. Crude protein, true protein, phosphorus, magnesium, and iron contents of the seed increased under the influence of phosphorus but the amount of nitrogen free extract and calcium decreased slightly. Phytic acid P constituted the major form of P in the seed followed by nucleic acid P and phospholipid P. Inorganic P formed a very small fraction of total P. The compounds containing the maximum amount of total P, phytic acid P, nucleic acid P, and inorganic P were mainly associated with the glycinin fraction of soy proteins. The concentrations of phosphorus compounds in the seed and its protein fractions were enhanced under the influence of soil phosphorus. The association of nucleic acid P with the major component of soy protein suggests their close interrelationship in metabolic activity and some soy proteins may be nucleoproteins.

Grain legumes are known to respond favorably to soil phosphorus. Phosphorus has been reported to influence the protein and phosphorus composition of soybean seed (Bhangoo and Albritton, 1972; Hanway and Weber, 1971). Soybean is a rich source of phosphorus-containing phytin, a calcium and magnesium salt of phytic acid as the main reserve phosphorus compound (Smith and Rackis, 1957; Sobolev, 1962) which has several physiological roles. Phytin is known for its chelating property with mineral elements in reducing their availability (Oberleas, 1973; Rackis, 1974; Lolas and Markakis, 1975). The other phosphorylated compounds are invariably associated in various vital processes in the development of the seed. Nucleic acids are known to play an important role in the formation of seed proteins. It is reported that soybeans contain 1.3% ribonucleic acid suggesting that one or more of the proteins of soybean may be nucleoproteins (DiCarlo et al., 1955). In view of the importance of phosphorylated compounds in the grain legumes, the present investigation was undertaken to study the different phosphorus compounds in the soybean seed and their association with different protein fractions separated by solubility differences. The effect of soil phosphorus on the nutrient composition of seed was also studied.

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MATERIALS AND METHODS

Two varieties of soybean (Bragg and Punjab-1) were raised in pots containing 12 kg of thoroughly mixed soil with a dose of 20, 40, 6, 4, 0.8, 20, 0.2, and 120 kg/ha of nitrogen, potassium, zinc, copper, boron, manganese, molybdenum, and sulfur, respectively, in five replicates in a completely randomized block design. Phosphorus was applied at levels of 0, 56, and 112 kg of P_2O_5/ha. Soil was sandy loam having pH 8.0, 0.23% organic matter, 5.6 kg/ha available P, and 134.4 kg/ha available K. Four plants were

maintained in each pot after germination. The composite samples of mature seeds (112 days after germination) were collected, air dried, ground to a fine powder, and stored in air-tight bottles.

Chemical Analysis. Nitrogen, ash, crude fiber, and ether extract were determined according to AOAC methods (1970). Nonprotein nitrogen was estimated by the method of Kulkarni and Sohonie (1956). Protein nitrogen was obtained by subtracting the value of nonprotein nitrogen from total nitrogen. Crude protein and true protein values were calculated by multiplying the total nitrogen and protein nitrogen values by 6.25, respectively. The percent organic matter was obtained by subtracting percent ash from 100. Nitrogen-free extract was determined by difference (100 – (protein + ash + fat + crude fiber). The energy value was calculated by using the factors 4, 9, and 4 cal/g of nitrogen-free extract plus crude fiber, ether extract, and protein, respectively. Ash was determined by ignition at 550 °C for 6 h in a muffle furnace. The acid-soluble portion obtained after dissolving ash in HCl (1:1) on a water bath at 100 °C was used for the estimation of minerals. Calcium and magnesium were determined using EDTA (Jackson, 1967) and iron was determined by using α,α-dipyridyl (AOAC, 1970). Total P was determined according to King (1932). The method of McComb and McCready (1952) as modified by Kon (1968) was standardized for the estimation of total pectin in soybean seed.

Inorganic P, phytic acid P, and nucleic acid P were extracted from the soybean seed and its protein fractions according to Ogur and Rosen (1950) based on differential solubility in perchloric acid at two different temperatures and acid concentrations. Phospholipids were extracted according to Mecham and Mohammad (1955). Inorganic and phospholipid P were determined colorimetrically according to King (1932) using 1-amino-2-naphthol-4-sulfonic acid. Phytic acid P was estimated according to McCance and Widdowson (1935). Total nucleic acid P was calculated indirectly from the total nucleic acid values assuming that nucleic acids contain 10% P. Total nucleic acids were determined by absorbance at 260 mμ in a UV spectrophotometer by interpolating from a standard curve worked out with purified ribonucleic acid (RNA). Deoxyribonucleic acid P (DNA-P) was estimated from DNA determined by the method of Burton (1956). RNA-P was obtained by difference (total nucleic acid P minus DNA-P). Residual P was calculated by subtracting inorganic, phospholipid, phytic acid, and nucleic acid P from total P.

Proteins were separated by the solubility method (Kapoor and Gupta, 1977) into water (albumin and glycinin), salt (globulin other than glycinin), alcohol (prolamine), and alkali (glutelin) soluble fractions. The water-soluble fraction was further separated by precipitation with 1 N HCl at pH 4.5. The precipitate was taken as glycinin and the supernatant as albumin.

RESULTS AND DISCUSSION

Distribution of Nutrients in Soybean Seed as Influenced by Phosphorus Nutrition. Phosphorus at a level of 56 kg of P_2O_5/ha had a favorable effect on crude protein, true protein, and ash contents of seed (Table I) but 112 kg of P_2O_5/ha did not prove to be beneficial. On the other hand, it decreased the nitrogen-free extract and ether extract. Among the minerals, the concentration of P in the seed was markedly enhanced at both the levels of P nutrition. Magnesium and iron contents increased but the calcium content was depressed. These observations suggest that phosphorus has an impact on the nutritive value of seed in influencing its minerals.

The present findings agree with those of earlier workers (Bhangoo and Albritton, 1972; Hanway and Weber, 1971) who also observed an increase in the nitrogen and phosphorus contents of soybean seed by phosphorus application. Motiramani et al. (1972) reported a reduction in the oil content of soybean under phosphorus nutrition.

Distribution of Phosphorus Compounds in Soybean Seed as Influenced by Phosphorus Nutrition. Phytic acid P formed the major fraction constituting 47–50% of total P followed by nucleic acid P (14–16%) and phospholipid P (13–15%) (Table II). Inorganic P constituted only 9% of total P.

Punjab-1 and Bragg did not differ in their contents of RNA and DNA. The ratio of RNA to DNA was about six in both varieties and did not reflect the genetic features of the soybean varieties. However, the ratio of RNA to DNA in a wheat variety was suggested earlier to represent its genetic feature (Bourdet and Herard, 1960) while other workers (Mihailovic et al., 1964) have reported that such a ratio is affected by agronomical conditions.

The application of 112 kg of P_2O_5/ha markedly enhanced the amount of total P of Bragg and Punjab-I from 556 and 593 to 879 and 983 mg/100 g of seed, respectively, with corresponding increases in the concentrations of different phosphorus compounds. The higher levels of phosphorus compounds in the seed due to phosphorus nutrition are likely to cause an improvement in the seed quality and yield character by affecting the metabolic activity and protein synthesis.

Earlier work mostly relates to phytic acid P in soybean but the data on other phosphorus fractions are meager. Earle and Milner (1938) reported phospholipid P, inorganic P, and phytin P as 11, 4.5, and 71% of total P, respectively. Phytin has been reported as the major phosphorus compound in soybean seed (Smith and Rackis, 1957; Sobolev, 1962).

Distribution of Phosphorus Compounds in Protein Fractions as Influenced by Phosphorus Nutrition. The solubility classes of soybean proteins under the influence of phosphorus have been reported earlier (Kapoor and Gupta, 1977). These solubility classes consisted of 8–10% albumin, 61–63% glycinin, 4–5% globulins other than glycinin, 4–5% prolamine, and 7–9% glutelin of total protein. Albumin and glycinin fractions were studied for the distribution of different phosphorus compounds, whereas the other fractions being comparatively low were analyzed only for total P. Phosphorus compounds were found to be mainly associated with the glycinin fraction. Total P, phytic acid P, nucleic acid P, and inorganic P of the glycinin fraction accounted for 51–58, 57–64, 68–73, and 55–63% of their respective amount of the whole seed (Table III). The corresponding phosphorus compounds in the albumin fraction were quite low. Phytic acid P constituted 50–58% of the total P of the glycinin fraction and 46–49% of the total P of the albumin fraction. Nucleic acid P contributed 20–22% of the total P of the glycinin fraction and 29–34% of the total P of the albumin fraction. The other protein fractions contained a small proportion of the total P of the whole seed. The occurrence of 68–73% of nucleic acid P of the whole seed P with the glycinin fraction suggests that nucleic acids are actively associated with the major component of the protein fraction indicating their close interrelationship in the metabolic activity. The presence of ribonucleic acid in the glycinin fraction of soy protein was also reported earlier (Koshiyama and Iguchi, 1965; Obara and Kimura, 1967; Shutov and Weintraub, 1967). It, therefore, suggests that some

Table I. Distribution of Nutrients in Soybean Seed Influenced by Phosphorus Nutrition (Expressed on a Moisture-Free Basis)

Variety	Levels of P, kg of P_2O_5/ha	Crude protein, %	NPN as % of total N	True protein, %	Ash, %	Org matter, %	Crude fiber, %	Ether extracts, %	N-free extract, %	Energy val., cal/100 g	P, mg/100 g	Ca, mg/100 g	Mg, mg/100 g	Fe, mg/100 g
Bragg	0	39.24	6.88	36.50	5.37	94.63	4.86	22.39	28.14	480	556	440	331	9.86
	56	42.50	7.12	38.56	5.86	94.14	4.73	21.43	25.48	468	701	338	382	11.06
	112	42.76	7.23	39.68	5.79	94.21	5.11	21.29	25.05	472	879	316	390	11.92
Punjab-1	0	39.74	7.00	36.93	5.14	94.86	4.93	21.53	28.66	476	593	402	375	9.10
	56	43.21	7.54	39.12	5.60	94.40	5.04	20.95	25.20	467	815	350	370	10.29
	112	44.56	7.46	41.18	5.51	94.49	5.32	20.85	23.76	468	983	334	405	10.69

Table II. Distribution of Different Phosphorus Fractions in Soybean Seed Influenced by Phosphorus Nutrition (Expressed on Moisture-Free Basis)

Variety	Level of P, kg of P_2O_5/ha	Total P, *a*	Inorg P		Phospholipid P		Phytic acid P		Total nucleic acid P		RNA P		DNA P		Residual P		Recovery, %	RNA, mg/100 g	DNA, mg/100 g	RNA/DNA
			a	*b*	*a*	*b*	*a*	*b*	*a*	*b*	*a*	*b*	*a*	*b*	*a*	*b*				
Bragg	0	556	53	9.5	69	13.4	263	47.3	81	14.6	69	12.4	12	2.2	39	7.0	91.8	690	120	5.8
	56	701	68	9.7	97	13.8	351	50.1	109	15.5	94	13.4	15	2.1	30	4.3	93.4	940	150	6.3
	112	879	84	9.6	134	13.6	429	48.8	143	16.3	123	13.9	19	2.2	34	3.9	92.1	1230	190	6.5
Punjab-I	0	593	53	8.9	91	15.3	289	48.7	82	13.8	70	11.8	12	2.0	41	6.9	93.8	700	120	5.8
	56	815	74	9.1	123	15.1	385	47.2	128	15.7	111	13.6	17	2.1	45	5.5	92.6	1110	170	6.5
	112	983	95	9.7	152	15.5	471	47.9	157	15.9	134	13.6	21	2.1	49	4.9	93.9	1240	210	6.4

[a] mg/100 g of material. [b] As percent of total P.

Table III. Distribution of Phosphorus in Different Protein Fractions of Soybean Seed Influenced by Phosphorus Nutrition (Expressed on a Moisture- and Fat-Free Basis)

Variety	Level of P, kg of P_2O_5/ha	Albumin								Glycinin								Globulin[c] total P		Prolamine total P		Glutelin total P	
		Total P		Phytic acid P		Nucleic acid P		Inorg P		Total P		Phytic acid P		Nucleic acid P		Inorg P							
		a	*b*	*a*	*b*	*a*	*b*	*a*	*b*	*a*	*b*	*a*	*b*	*a*	*b*	*a*	*b*	*a*	*b*	*a*	*b*	*a*	*b*
Bragg	0	41	104	18	45	12	30	7	17	372	1249	217	728	75	252	42	141	33	160	11	46	28	52
	56	53	119	25	56	16	36	8	18	493	1569	270	859	99	315	58	184	37	164	14	62	34	90
	112	61	117	28	53	18	34	10	19	634	1913	310	935	130	392	74	223	41	165	18	72	43	95
Punjab-I	0	39	95	19	46	13	31	5	12	396	1314	224	743	80	265	37	122	35	137	12	57	30	85
	56	48	103	23	49	15	32	6	12	525	1638	290	705	110	343	58	181	45	178	14	57	36	88
	112	64	119	30	56	20	37	9	16	655	1944	329	976	140	415	74	213	54	212	17	75	48	99

[a] mg/100 g of material. [b] mg/100 g protein fraction. [c] Globulin other than glycinin.

soy proteins may belong to the class of nucleoproteins. The higher amount of phytic acid P in the glycinin fraction suggests that it may be forming a complex with soy protein as indicated earlier (DiCarlo et al., 1955). Such a complex is reported to be resistant to digestion by proteolytic enzymes (Barre, 1956).

The different phosphorus compounds in the protein fractions responded favorably to the application of soil phosphorus but the level of 112 kg of P_2O_5/ha did not prove to be beneficial to the phosphorus composition of albumin fraction. The percentage increase in total P, phytic acid P, nucleic acid P, and inorganic P of the glycinin fraction was observed to be 24–25, 18–21, 25–29, and 30, respectively, from the application of 56 kg of P_2O_5/ha and 47–53, 28–31, 55–57, and 54–58, respectively, from an application of 112 kg of P_2O_5/ha. The corresponding values for the albumin fraction were 14, 24, 20, and 5 from 56 kg of P_2O_5/ha. These observations suggest that phosphorus compounds of the glycinin fraction are mostly affected by the phosphorus nutrition, the response being higher in nucleic acid P and inorganic P. The total P of other protein fractions has also responded favorably under the influence of P. O'Dell and Savage (1960) observed that soy protein contained about 0.5% phytic acid P. The acid-precipitated protein was reported to contain 0.5–0.8% P or about 90% of the P extracted from the meal (Smith and Rackis, 1957).

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COMMUNICATIONS

Distribution and Excretion Rates of ^{14}C-Labeled Permethrin Isomers Administered Orally to Four Lactating Goats for 10 Days

Permethrin [3-phenoxybenzyl 3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropanecarboxylate] ^{14}C-labeled isomers were administered orally to four goats. Each goat received one of the following ^{14}C isomers of permethrin daily for 10 days: *trans*-(±)-permethrin labeled in the acid or alcohol moiety or *cis*-(±)-permethrin labeled in the acid or alcohol moiety. Radiocarbon was rapidly excreted in each instance. The major pathway for the elimination of radiocarbon of the cis isomers was the feces (51.7–67.4%), whereas that for the elimination of radiocarbon of the trans isomers was the urine (72.1–79.4%). Recovery in the milk from any treatment was less than 1% of the total radiocarbon dose. Twenty hours after the final dose, detectable levels of radiocarbon were found in most tissues, but none was higher than 0.04 ppm for the trans isomers or 0.25 ppm for the cis isomers.

Permethrin [3-phenoxybenzyl *cis,trans*-(±)-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropanecarboxylate], a new photostable synthetic pyrethroid, has been highly effective in controlling stable flies, *Stomoxys calcitrans* L., and horn flies, *Haematobia irritans* L., when applied as a spray to cattle (Schmidt et al., 1976). Because the prospects for wide use of permethrin on livestock are good, possible residues in meat and milk should be determined. Residues of isomers are of special interest because permethrin is a mixture of trans (FMC 30960) and cis (FMC 45812) isomers.

When the metabolism of permethrin in rats and mice was studied (Elliott et al., 1976; Gaughan et al., 1977), the findings indicated that with [1*R*,trans]- and [1*R*,cis]-acid labeled in the side chain (Cl_2 ^{14}C=CH–) or alcohol labeled at the α-$^{14}CH_2$ of permethrin, their hydrolysis products and metabolites were excreted from the body in a short time and were not retained in the tissues. The current study