distinctly curvilinear with 60 pounds, especially from APS. Consequently, yield increases over no applied phosphorus were calculated from the lower three rates only.

Average relative response of wheat forage to the fertilizers in the two tests (Table VIII) decreased as follows: APS > NP-416 > NP-417 > AOSP-415. This is the same order of effectiveness as found by multiple cropping by corn in both greenhouse pot experiments.

Discussion

Results from pot experiment 1 showed conclusively that water solubility of granular phosphate fertilizers is the dominant factor in early growth response by corn. Thus, there is little possibility of measuring differences in crop response to citrate-soluble components of large granules in similar experiments, if the content of water-soluble phosphorus is high. Since relative crop response to water-soluble, and water-insoluble phosphorus components depends upon granule size, different results would be expected with fine granular or nongranular fertilizers, as found by Bouldin, DeMent, and Sample (*1*).

Results from pot experiments 2 and 3 also showed greater response to the granular, highly water-soluble ammonium phosphate sulfate fertilizer. However, with the three fertilizers containing 26 to 28% of their phosphorus in water-soluble forms, crop response to water-insoluble phosphorus in two nitric phosphates (low apatite content) was higher than that in an ammoniated ordinary superphosphate (high apatite content). Response to these four phosphates was closely related to contents of water-soluble plus alkaline citrate phosphorus, but not to water-soluble plus neutral citrate phosphorus (AOAC-available phosphorus). Similar results were found with wheat forage and corn in field experiments.

In contrast, a high correlation was found between available phosphorus in water-insoluble phosphates used in experiment 1, as determined by the neutral and alkaline citrate methods. The high availability of the NPK fertilizers used in experiments 2 and 3 by the neutral citrate method and the poor correlation for available phosphorus by the two methods result largely from the small amount of water-insoluble phosphorus in the gram samples analyzed. The amount of water-insoluble phosphorus per sample varied from about 75 to 225 mg. for the water-insoluble phosphate fractions (Table I), but only from 5 to 41 mg. in the NPK fertilizer samples (Table IV). Terman, Hoffman, and Wright (*7*) have discussed more fully the effect of fertilizer sample size on the available phosphorus content.

Results from this study emphasize the rather close agreement between response to phosphate fertilizers previously found in greenhouse pot experiments and early growth response under field conditions (*5*). Water solubility of the phosphorus is very important under both situations. Results from both field and greenhouse tests also show that content of water-soluble plus alkaline citrate-soluble phosphorus in NPK fertilizers is more closely related to crop response than is water-soluble plus neutral (AOAC) citrate-soluble phosphorus. This agrees with the results obtained by Wright, Lancaster, and Anthony (*8*).

Acknowledgment

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DEFOLIANT RESIDUES

The Microcoulometric Determination of *S,S,S*-Tributyl Phosphorotrithioate in Cottonseed

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In the mechanical harvesting of cotton, it is general practice to apply desiccants or defoliants about 10 days prior to harvest to facilitate harvesting and to reduce the quantity of cotton trash which would otherwise be mixed with the cotton. Two such products which are widely used are DEF (Chemagro Corp.) and Folex, (Virginia Carolina Chemical Corp.).

The active pesticide chemical ingredient in DEF is *S,S,S*-tributyl phosphorotrithioate and in the Folex (Merphos) is *S,S,S*-tributyl phosphorotrithioite.

These products are applied at the rate of 1.5 to 2.0 pounds of active ingredient per acre in the form of a dust or emulsifiable concentrate diluted with water. Detailed directions for use are on the container labels.

Loeffler and MacDougall (*3*) developed a photofluorometric method for the determination of residues of *S,S,S*-tributyl phosphorotrithioate in cottonseed. This method involves hydrolysis to form butyl mercaptan and distillation of the butyl mercaptan into a solution of palladium chelate of 8 - hydroxy - 5 - quinolinesulfonic acid. The mercaptan combines with a portion of the palladium, liberating an equivalent amount of the complexing agent. Addition of magnesium chloride results in the formation of highly fluorescent magnesium chelate whereas the palladium chelate is not fluorescent.

Boyd and Barber (*1*) described a method for the determination of residues of *S,S,S*-tributyl phosphorotrithioite in cottonseed which is based upon hydrolysis and colorimetric determination of the butyl mercaptan.

The purpose of the present investigation was to develop a gas chromato-

A method for the determination of residues of S,S,S-tributyl phosphorotrithioate in cottonseed is based on extraction of the pesticide from cottonseed with a suitable solvent, cleanup of the extract using column chromatography, and determination by gas chromatography. The detection system is based on microcoulometry which will detect submicrogram quantities of sulfur dioxide resulting from the combustion of the chemical after elution from the gas chromatographic column. The method has a sensitivity of about 0.05 p.p.m. S,S,S-tributyl phosphorotrithioate or better, and recovery of 0.05 p.p.m. of this chemical added to untreated cottonseed is 80 to 90%. Untreated samples show no apparent residue. Gas chromatographic studies are reported for S,S,S-tributyl phosphorotrithioate and also for S,S,S-tributyl phosphorotrithioite.

graphic method for the determination of *S,S,S*-tributyl phosphorotrithioate in cottonseed and to apply this method in the analysis of cottonseed from cotton plants that had been defoliated in accordance with the directions for use on the label for the product DEF.

A microcoulometric detection system (Dohrmann Instrument Co., San Carlos, Calif.) connected to a gas chromatograph (Microtek Instruments, Baton Rouge, La.) with a T-200P titration cell was used. This detection system will detect submicrogram quantities of sulfur dioxide resulting from the combustion of a sulfur-containing pesticide after elution from a gas chromatographic column. This gas chromatograph has a removable inlet system which allows very large sample aliquots to be injected, and a venting system which allows solvents and other extraneous material to be vented rather than passing through the detector.

Preliminary investigations using the Beckman GC2 gas chromatograph (Beckman Instrument Co., Fullerton, Calif.) with thermal conductivity detector (Figure 1) and the Aerograph HiFi gas chromatograph (Wilkens Instrument and Research, Walnut Creek, Calif.) with a hydrogen flame ionization detector (Figure 2) indicated that DEF could be eluted from several types of columns.

In general, the conditions tried were 3- to 6-foot aluminum, glass, and stainless steel columns packed with 5% Dow Corning silicone fluid 11, Dow Corning silicone fluid 200, or General Electric SE-30 silicone gum on Chromosorb W (60- to 80-mesh).

Column diameters were $^1/_8$ to $^1/_4$ inch and optimum column temperature was about 225° C. with the block temperature about 20° to 40° higher. Not only did DEF and Merphos have good elution patterns, but the separation of these two compounds was demonstrated. It was then a simple matter to adapt the microcoulometric gas chromatograph to these conditions. For maximum sensitivity, a glass column was ultimately used. This instrument gave a specific, sensitive, and rapid method for the analysis of DEF-treated cottonseed (Figure 3).

Methods and Materials

Reagents. Hexane, redistilled in an all-glass apparatus. Acetonitrile, redistilled in an all-glass apparatus. Sodium sulfate, anhydrous granular. Florisil, Floridin Co., 60 to 100 mesh activated by Floridin Co. at 1200° F., used as is.

Apparatus. Microcoulometric gas chromatograph. Instrument with T-200P titration cell, (Dohrmann Instrument Co., San Carlos, Calif.). A Minneapolis-Honeywell 1-mv. recorder was used to record the signal from the coulometer. Chromatographic columns,

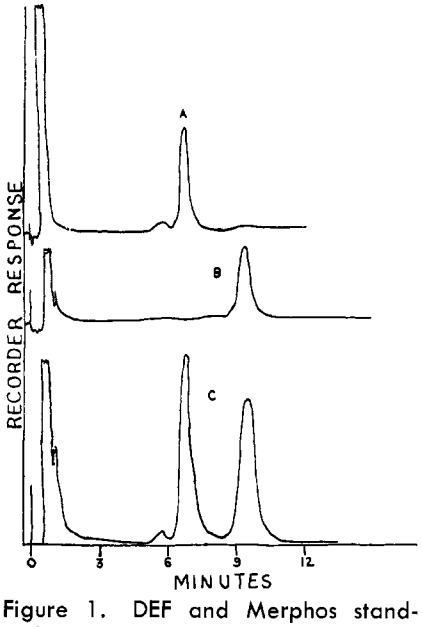

Figure 1. DEF and Merphos standards

Instrument: Beckman GC 2 with a thermal conductivity detector
Column: 6-foot by $^1/_4$-inch aluminum, 5% DC 11 on 60/80 Chromosorb W
Column temperature: 220°C.
Carrier gas: helium, 55 ml. per minute
Sensitivity: attenuation, 1 and 2
A: Merphos 94%, 103.4 μg.
B: DEF 98%, 100 μg.
C: mixture of DEF, 100 μg. and Merphos, 103.4 μg.

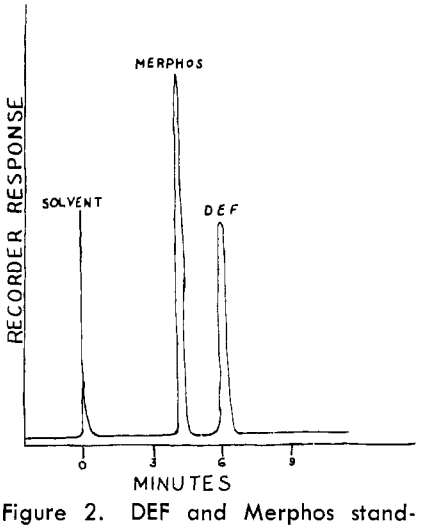

Figure 2. DEF and Merphos standards

Instrument: Aerograph HiFi with hydrogen flame ionization detector
Column: 5 -foot by $^1/_8$-inch stainless steel, 5% SE 30 on 60/80 Chromosorb W
Column temperature: 218°C.
Carrier gas: nitrogen, 16 p.s.i.
Sensitivity: attenuation, 16
Merphos: 94%, 2.8 μg.
DEF: 98%, 2.0 μg.

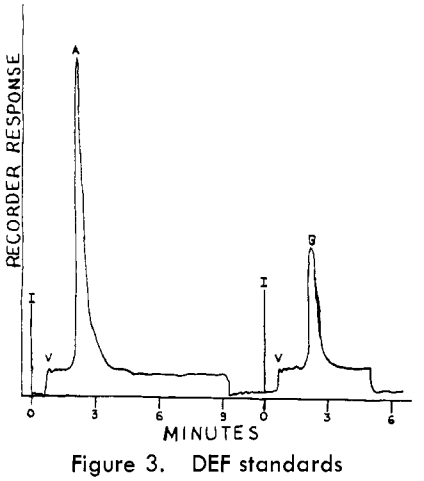

Figure 3. DEF standards

Instrument: Dohrmann microcoulometric gas chromatograph
Column: $3^1/_2$-foot by $^1/_4$-inch glass, 5% DC 11 on 60/80 Chromosorb W
Column temperature: 225°C.
Carrier gas: nitrogen, 100 ml. per minute
Sensitivity: attenuation, 128
I indicates sample injection
V indicates where column vent was closed
A: DEF 98%, 0.7 μg.
B: DEF 98%, 0.3 μg.

30 × 500 mm. with sintered glass plate and Teflon stopcock. Rotary vacuum evaporator and water bath, for evaporation under reduced pressure, Rinco model No. 1007-4IN, or equivalent.

Extraction. Weigh 100 grams of a representative sample of undelinted cotton seed into a 1000-ml. Erlenmeyer flask and add enough hexane to cover the seed. Cover the flask and allow to stand for about 18 hours. Decant the hexane from the seed and rinse the seed with several portions of hexane, adding the rinses to the strip solution. Evaporate the extract to about 100 ml. under a current of air on a steam bath using very low heat.

Cleanup. Prepare a column of Florisil (25 to 30 grams) and top with about 1 inch of anhydrous sodium sulfate. Prewash the column with about 100 ml. of hexane. Transfer the extract to the column and allow to elute until all of the hexane has just entered the top of the column. Wash the flask with 75 ml. of hexane and add this to the column. Discard all hexane eluents. Add 100 ml. of acetonitrile to the column and catch the eluent in a 250-ml. flask. Evaporate the acetonitrile on a rotary evaporator under vacuum. A water bath of about 40° C. may be used to hasten evaporation. Evaporate the acetonitrile just to dryness. Transfer the residue to a graduated, conical centrifuge tube with hexane and further evaporate to 1 or 2 ml. using a current of dry air. Cap the tube and store for gas chromatography.

Gas Chromatography. The gas chromatographic column is glass or stainless steel tubing ($3^1/_2$ feet long and $^1/_4$-inch o.d.) packed with 5% DC 11 silicone grease on 60/80 Chromosorb W (acid washed).

Table I. Residues of DEF on Cottonseed Using the Colorimetric Method

Preharvest Interval, Days	Net Residue, P.P.M.
1	2.8
4	0.35
8	0.15
11	...

Column temperature is 225° C.; injection block temperature, 250° C.; nitrogen carrier gas flow rate, 100 ml. per minute; and the sensitivity range of the coulometer, 128 or 256.

A standard solution containing about 0.1 μg. of DEF per μl. of solution is used. A series of standards, at various concentrations up to about a total of 1 μg. are injected into the instrument to obtain a calibration curve and to calculate retention times. It is advantageous to inject standards throughout the time a series of samples is being run, so that any change in column characteristics, due to extraneous material in the sample, may be compensated for.

The method is sensitive to about 0.02 to 0.05 p.p.m. of DEF. Recoveries of DEF at the 0.05 p.p.m. level were 80 to 90%. The method can be made more sensitive by adjusting the aliquot of material injected into the instrument.

Check samples showed no evidence of DEF, or any other peaks, and therefore no correction is needed for the check samples. The cell and coulometer are sensitive to sulfur, although a slight, raised base line is noted when the vent is closed. This is due to the liberation of iodine by the hot gases entering the cell, and results in a constant titration of the cell and, hence, a raised base line.

Table II. Residues of DEF by the Gas Chromatographic Method

Preharvest Interval[a]	DEF, P.P.M.
1-day check	0
1-day treated	5
4-day check (2)	0,0
4-day treated	0.6
8-day check (2)	0,0
8-day treated (2)	0.14, 0.12
8-day check + 0.05 p.p.m. (2)	0.042(84%), 0.040(80%)
8-day check (2)	0,0
8-day check + 0.05 p.p.m. (1)	0.045(90%)
8-day treated (2)	0.10, 0.10
8-day check + 0.1 p.p.m. (2)	0.08(80%), 0.11(110%)
8-day treated	0.10
11-day check	0
11-day treated	0.03

[a] The numbers in parenthesis refer to the number of determinations.

Results and Discussion

Only cottonseed treated with DEF was used. No Merphos treatments were available. However, it has been reported that in vivo degradation of the thioite (Merphos) results in the thioate (DEF), and the method should be applicable to the detection of both pesticides (*2*).

The bulk of residues, if any, resulting from the use of these chemicals, would be on the lint rather than inside the

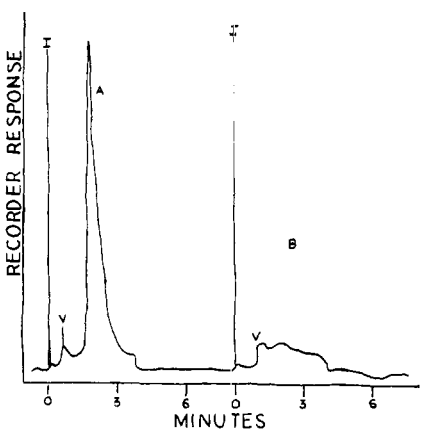

Figure 4. DEF standard and check sample

Instrument: Dohrmann microcoulometric gas chromatograph
Column: $3^1/_2$-foot by $^1/_4$-inch glass, 5% DC 11 on 60/80 Chromosorb W
Column temperature: 225°C.
Carrier gas: nitrogen, 100 ml. per minute
Sensitivity: attenuation, 128
I indicates sample injection
V indicates where column vent was closed
A: standard DEF, 98%; 0.7 μg.
B: check sample, 4-days, aliquot equivalent to 1.25 grams cottonseed injected

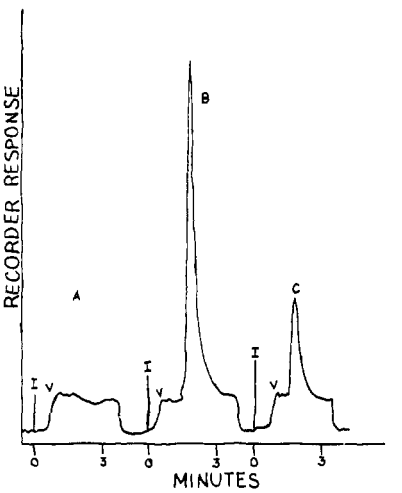

Figure 5. Check–standard DEF–treated sample

Instrument: Dohrmann microcoulometric gas chromatograph
Column: $3^1/_2$-foot by $^1/_4$-inch glass, 5% DC 11 on 60/80 Chromosorb W
Column temperature: 225°C.
Carrier gas: nitrogen, 100 ml. per minute
Sensitivity: attenuation, 128
I indicates sample injection
V indicates where column vent was closed
A: Check sample, 8 days, aliquot equivalent to 1.25 grams cottonseed injected
B: Standard DEF 98%, 0.7 μg.
C: treated sample, 8 days, aliquot equivalent to 1.25 grams cottonseed injected

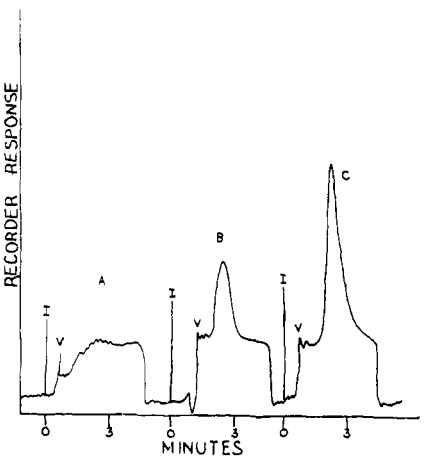

Figure 6. Check–recovery–standard DEF

Instrument: Dohrmann microcoulometric gas chromatograph
Column: $3^1/_2$-foot by $^1/_4$-inch glass, 5% DC 11 on 60/80 Chromosorb W
Column temperature: 225°C.
Carrier gas: nitrogen, 100 ml. per minute
Sensitivity: attenuation, 256
I indicates sample injection
V indicates where column vent was closed
A: check sample, aliquot equivalent to 2.5 grams sample injected
B: check sample plus 0.05 p.p.m. DEF added; found 0.045 p.p.m. or 90% recovery
C: standard DEF 98%, 0.3 μg.

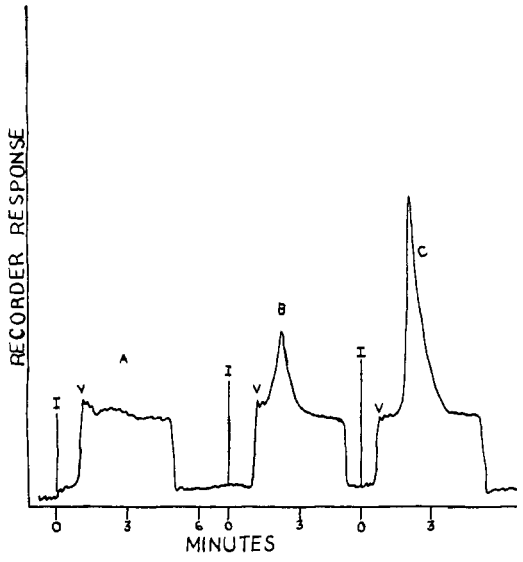

Figure 7. Check–recovery–standard DEF

Instrument: Dohrmann microcoulometric gas chromatograph
Column: $3^1/_2$-foot by $^1/_4$-inch glass, 5% DC 11 on 60/80 Chromosorb W
Column temperature: 225°C.
Carrier gas: nitrogen, 100 ml. per minute
Sensitivity: attenuation, 256
I indicates sample injection
V indicates where column vent was closed
A: check sample, aliquot equivalent to 1.25 grams injected
B: check sample plus 0.10 p.p.m. DEF added; found 0.08 p.p.m. or 80% recovery
C: standard DEF 98%, 0.30 μg.

seed (*1*). All results are based on surface extractions of treated and untreated cottonseed, since previous studies (*2*) suggest that DEF, in an unaltered form, is not translocated within the cotton plant.

All treated samples received treatments of 2 pounds of active ingredient (DEF) per acre. Samples were taken 1, 4, 8, and 11 days after treatment.

The results of these samples, using the colorimetric method (*1*), are shown in Table I. Six check samples were analyzed and the average residues found, calculated as DEF, was 0.15 p.p.m. Recoveries at the 0.1-p.p.m. level, added to untreated cottonseed prior to extraction, were 80% or better.

The same series of samples were analyzed by the gas chromatographic method. The results are given in Table II.

Infrared spectra of DEF before and after gas chromatography indicate that there is no change or breakdown of this material when it is subjected to the conditions of gas chromatography as described in this report. The Aerograph Autoprep (Wilkens Instrument and Research, Walnut Creek, Calif.) was used to collect material eluted from a 4-foot by $^1/_4$-inch stainless steel column at 220° C. containing 20% General Electric SE-30 silicone gum on 60- to 80-mesh Chromosorb W.

The check samples are free from interference and recovery of DEF at the 0.05 p.p.m. level is good (Figures 4 through 7). The method developed is highly specific and sensitive, thanks to the principles of gas chromatography and the microcoulometric detector used in this study.

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