Bulletin 821

• Compositional and Physiological • Responses of the Cotton Plant • to the Systemic Insecticides • Schradan and Demeton

September 1955

TEXAS AGRICULTURAL EXPERIMENT STATION

R. D. LEWIS, DIRECTOR, COLLEGE STATION, TEXAS

SUMMARY

Current systemic insecticides are organophosphorus compounds which, when applied to a portion of a plant, are distributed throughout the plant and kill insects feeding on any part.

Cotton plants grown in solution cultures containing schradan accumulated the insecticide in successively lower concentrations in leaves, roots, bolls, petioles and stems. Relatively low concentrations of schradan stimulated vegetative development but higher concentrations were phytotoxic to both vegetative and fruiting activity.

Increased concentrations of chlorophyll and carotenoid pigments were directly correlated with schradan treatment. It is suggested that this additional photosynthetic potential was related to increases in plant dry weights which occurred with the non-phytotoxic concentrations.

As the nitrogen level in the nutrient solution was decreased, schradan was found to accumulate in the plant in significantly larger quantities. A similar but weaker trend was noted when the phosphorus level was varied. The level of external potassium had little or no effect on schradan absorption.

Plants grown in phosphorus-deficient solutions made significantly greater growth with than without added schradan in non-phytotoxic concentrations. Marginal leaf burning characterizing the low phosphorus plants was not evident on those receiving schradan.

Neither schradan nor demeton, when applied to cotton as a foliar spray or when the former was absorbed by the roots, affected the viability of the seed produced. The schradan-treated plants produced seed which tended to be higher in protein and lower in oil than the untreated plants, while the demeton-treated plants produced seed which were higher in oil and lower in protein. There was no effect on phosphorus accumulation.

Young bolls contained only 1 percent of the total schradan accumulated by the cotton plant, and there was no evidence of increased accumulation through the addition of boron and sucrose. Embryos from plants grown continuously on schradan contained 1 to 10 p.p.m. of the insecticide. Attempts to determine the demeton in treated cotton plants by the cholinesterase inhibition method were not successful because of the presence of a natural inhibitor in the boll, possibly gossypol. A 5 to 12-fold enhancement of schradan was found after passage through the plant when measured by cholinesterase activity.

Soluble nitrogen in the plant increased as the schradan concentration in the nutrient solution was increased, but there was no effect on the protein fraction. At the highest level of insecticide used, there was a slight increase in phosphorus and a highly significant reduction in total sugars. The effect of schradan on starch, which was increased substantially by the two higher concentrations, appeared limited to the root tissues.

CONTENTS

Compositional and Physiological Responses of the Cotton Plant to the Systemic Insecticides Schradan and Demeton [1]

JOSEPH HACSKAYLO and DAVID R. ERGLE*

A SYSTEMIC INSECTICIDE IS A COMPOUND WHICH is absorbed and translocated by actively growing plants in quantities sufficient to kill insects feeding on a part remote from the point of application. These materials not only have a residual effect, but they also make toxic to insects portions of the plant heretofore left unprotected by the conventional sprays and dusts. Since growing plants are continually producing new growth, insect protection is afforded the new growth by systemic action from the older treated plant parts.

Schradan and demeton are the only systemic insecticides approved for use on food crops at the present time; others are being tested for possible release as safe insecticides. There are many materials which may serve as excellent systemic insecticides, but their high mammalian toxicity properties eliminate them as plant protection chemicals.

Most of the early studies concerning these materials were associated mainly with the biochemical and toxicological problems relating to their use. Very little work has been done to establish what influence these materials have on the physiology of plants in general.

The purpose of this investigation was to obtain a broad spectrum of the growth and physiological responses of the cotton plant to schradan and demeton, with most emphasis being placed on the former.

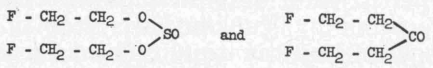
REVIEW OF LITERATURE

History of Development

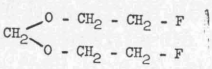
Although the subject of systemic insecticides appears to be relatively new, attempts to produce systemic effects in plants from chemicals actually date back to the 17th century. The techniques commonly employed previous to the 1930's consisted of either introducing chemicals into the translocation stream by hypodermic injection or by filling bored holes in tree trunks with the desired chemicals. The substances used were usually heavy metal complexes and phenolic derivatives. Since few favorable results were obtained, these methods for insect control were not generally accepted. Craighead and St. George (6) gave an excellent review of this subject up to 1938.

Neiswander and Norris (27) reported that sodium selenate was toxic to mites and aphids when present in plants upon which these insects were feeding. This method of insect control also was eliminated since selenium, even at relatively low concentrations, was found to be very toxic to higher animals.

A major stimulus in the development of systemic insecticides came in 1935 when Schrader (33), working in Germany, noted that the esters of B-fluoro-ethyl alcohol had strong contact insecticidal action. These compounds have the formulae:

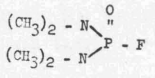


Additional studies by Schrader and Kuekenthal (cited in 33) showed that the methylals of B-fluoro-ethyl alcohol could penetrate actively growing plants and remain unchanged for long periods of time in the plant's system. The formula for this compound is:



Since all of the foregoing compounds proved to be very toxic to warm-blooded animals, their use as systemic insecticides was abandoned.

Another important compound synthesized was fluoro-phosphoric-acid-di-dimethylamide in 1941. The formula is:



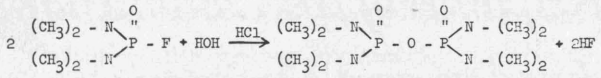
Remarkable systemic properties were noted for this material. However, its toxic properties partially eliminated its use as a plant protection chemical.

Since the mode of action of these materials was obscure at that time, Schrader was interested in determining whether fluorine was responsible for the toxic properties exhibited by these compounds. The above compound was placed in an aqueous solution of HCl in an effort to replace

[1] Adapted from a dissertation in plant physiology submitted to the faculty of the Graduate School of the A&M College of Texas in partial fulfillment of the requirements for the degree of doctor of philosophy.

*Plant physiologists, Entomology Research Branch and Field Crops Research Branch, Agricultural Research Service, U. S. Department of Agriculture, and Department of Plant Physiology and Pathology, Texas Agricultural Experiment Station, College Station, Texas.

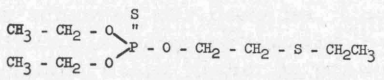
the fluorine atom, and the following reaction ensued:



The new compound proved to be octamethyl pyrophosphoramide (OMPA, Pestox, schradan).

This chemical not only had excellent systemic properties but has recently been approved for use on certain crop plants, including cotton, to control spider mites and aphids.

Still another important systemic formulated was the diethyl thiophosphoric acid ester of ethyl thioglycol ether. This compound was marketed under the name of Systox (demeton) and has the proposed formula:



Absorption by Plants

Absorption of systemics by plants occurs through leaves, stems, roots and seed. Reynolds (31) and Ripper (32) presented excellent reviews on this subject.

Leaves

The penetration of systemics into leaves was shown to occur by Kuekenthal (cited in 33) and all subsequent investigators. The rate of uptake varies with the age, type of plant and environmental factors. Heath and Llewellyn (cited in 32) found that visible light and near infra-red greatly increases the uptake of systemic insecticides, perhaps being associated with carbohydrate content of the plant. David (7) reported that 69 percent of the schradan applied to leaves of bean was absorbed in 14 hours, and Metcalf and March (24) found that 50 percent enter citrus leaves in 24-48 hours.

Stems

Application of hanane to the bark of coffee trees was shown by Bond (1) to be very effective in controlling mealy bugs. Jeppson (21) reported very effective control of aphids and mites on citrus by trunk applications of schradan. Metcalf and March (24) state that application of schradan to the base of orange seedlings was much more effective than solution culture treatments.

Roots

The plant root system is capable of readily absorbing systemic insecticides from soil or solution culture. Casida *et al.* (3) reported that the pea plant absorbs significantly greater amounts of schradan from phosphorus deficient nutrients. Metcalf and March (24) grew lemon seedlings in solution culture containing 360 p.p.m. schradan and found that the distribution of the insecticide in leaves, stems and roots was 69, 20 and 11 percent, respectively. These and many other investigators have shown that systemics absorbed by the roots are translocated to all parts of the plant.

Seed

Ivy *et al.* (18) showed that cotton seed absorb schradan in sufficient quantities to provide mite and aphid protection for several weeks after planting. Other investigators confirmed these findings.

Translocation

When schradan was applied to the soil, Wedding and Metcalf (37) found that the systemic moved to the younger leaves of the valentine bean. The rate of upward movement in the stem was measured and found to be approximately 20 cm. per hour.

By the use of girdling experiments, Wedding (38) reported that the movement of P^{32} Systox (demeton) in the lemon plant was principally in the phloem when applied to the stem. When the labeled Systox was applied above the girdle, very little downward movement was detected, while considerable upward movement resulted. When Systox was applied below the girdle, very little upward or downward movement resulted. This indicates that the phloem is the chief avenue of transport when stem applications are made. The rate of movement in the lemon plant was estimated to be 2.5 cm. per hour.

Metcalf and Reynolds (cited in 31) sprayed Acala cotton with P^{32} Systox and OMPA (schradan) and found that after 15 days, 2.3 percent of the Systox and 19 to 24 percent of the OMPA was translocated into the new growth. On root crops such as sugar beets and carrots, large amounts of OMPA were translocated into the fleshy taproots, while Systox remained in the aerial portions of the plants.

Metcalf (25) reported that cotton plants sprayed with P^{32} schradan at the rate of 1 pound per acre accumulated 167 p.p.m. schradan in the cottonseed cake and 109 p.p.m. in the raw oil 43 days after application. This indicates that considerable amounts of this material are translocated to the seed.

Mode of Action of Schradan

In pure form, schradan is not toxic to mammals or insects. When applied to the skin or administered orally, a subsequently formed metabolic intermediate has been shown to be very toxic to mammals (8). Estimates have indicated that 280 to 560 mg. would be lethal to man; however, daily doses of 25 mg. have been administered to humans for 3 weeks for treatment of *Myasthenia gravis*, giving beneficial results and producing no pronounced toxic symptoms.

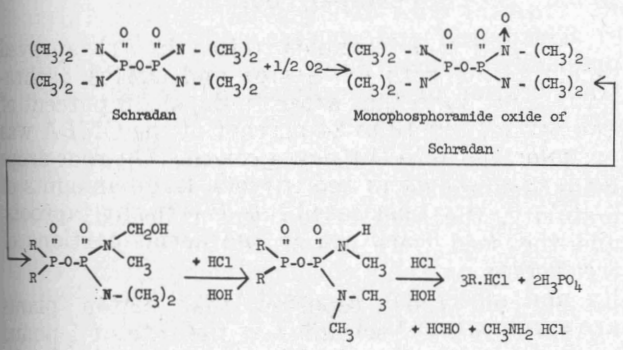
In animals, schradan is metabolized in the liver to produce an active intermediate which causes the inactivation of cholinesterase. As a result, acetylcholine accumulates and a stimula-

tion of the para-sympathetic nervous system ensues, which, if severe enough, causes death of the animal.

Since some insects are not susceptible to schradan poisoning, it has been postulated that resistant insects lack the enzyme system to convert schradan to an active metabolite. It was reported (28) that the fore-gut, mid-gut, hind-gut, fat body, nerve tissue and cuticle of a resistant cockroach could convert schradan, consequently the above hypothesis should be amended. O'Brien and Spencer (28) proposed that resistant insects convert most of the OMPA in the fat body, consequently little ever reaches the nerve cord unchanged. The relative inactivity of the converted schradan may be due to the inability of the metabolized material to penetrate the membrane which invests the nervous system. The half life of monochloro-schradan is 40 minutes and if the schradan metabolite is similar, its half life may be of the same order.

Plants likewise are capable of converting schradan to an anti-cholinesterase agent, which, according to Casida *et al.* (5), is identical to the animal and insect metabolite. Since plants have no nervous system, no toxic response similar to that found in mammals resulted from schradan.

The proposed plan (5) for the production of the active intermediate is as follows:



This theory proposes that the phosphoramide oxide selectively combines with cholinesterase resulting in inactivation of the enzyme.

It also was reported by Casida *et al.* (2) that schradan is capable of inhibiting chrymotrypsin.

ANALYTICAL METHODS

Schradan

An adaptation of the method employed by Casida *et al.* (3) was used for the chemical determination of schradan. Approximately 0.5 gm. of dried ground plant material was placed in a mortar containing 10 ml. of distilled water and ground with pure quartz sand. After the plant sample had been thoroughly macerated, the water extract was decanted into a 50 ml. volumetric flask. The extraction procedure was repeated three times. The resulting plant extract and residue was made to volume and centrifuged. Suitable aliquots were made to 19 ml., to which was added 1 ml. of a 2 normal solution of NaOH, and hydrolyzed by heating in a water bath at 80° C. for 30 minutes. The hydrolysate was cooled and decanted into a separatory funnel containing 30 ml. of chloroform and the mixture shaken for 1 minute. After standing for a few minutes, the chloroform layer was collected in a 40 ml. centrifuge tube and centrifuged for 5 minutes at 2,000 r.p.m. Twenty ml. of the chloroform was introduced into a 12-inch pyrex test tube containing several glass beads and evaporated nearly to dryness in a water bath under the hood. The residue was wet ashed with diluted H_2SO_4 and H_2O_2. The phosphorus content was determined and the amount present multiplied by 7.87 to give the amount of schradan present in the original aliquot.

Cholinesterase activity, using a purified enzyme preparation (acetylcholinesterase, manufactured by Winthrop-Stearns, Inc.) was measured manometrically by using the Warburg apparatus as outlined by Casida *et al.* (3).

Carbohydrates

Determinations of the carbohydrate fractions were by methods given in detail by Eaton and Rigler (9).

Nitrogen

Total nitrogen was determined by the semi-micro Kjeldahl method (14). Soluble nitrogen was determined similarly and included all of the nitrogenous compounds extractable from dried plant tissue with water at 80° C. Insoluble nitrogen was obtained by the difference of the above two fractions. Nitrate nitrogen present in the water soluble fraction was estimated colorimetrically by the phenoldisulfonic acid method (14).

Oil

Suitable quantities of seed were ground to pass a 20-mesh screen in a Wiley mill and dried in an oven at 80° C. for 24 hours. The material was weighed then introduced into an alundum thimble and extracted in a Soxhlet apparatus for 24 hours with petroleum ether. The petroleum ether was evaporated, the residual oil weighed and the percent composition calculated.

Other Constituents

Boron was determined colorimetrically as outlined by Hatcher and Wilcox (16). A modified Wolf procedure was followed for the determination of phosphorus as outlined by Hall (14).

Potassium determinations were made as follows: 1 gram of dried plant material was ashed in a muffle furnace for 1 hour at 250° C., followed by 4 hours at 550° C. Five ml. of HCl and 5 ml. of distilled water were added to the ash after cooling to room temperature. The solution was then brought to a volume of 50 ml. and filtered through number 12 Watman filter paper. The potassium content was determined with the Beck-

man flame photometer as outlined in Instrumental Methods of Analysis (39).

Chloroplast Pigments

The chlorophylls and carotenoids were determined quantitatively on fresh leaves as given by Loomis and Schull (22) and Shertz (34, 35). Absorption spectra also were determined on the extracted chlorophylls and carotenoids by the Beckman spectrophotometer.

CULTURE OF PLANTS

Nutrient Solution

The basal nutrient solution used during these investigations contained millimolar concentrations of salts as follows: 6 $Ca(NO_3)_2.4\ H_2O$, 4 KNO_3, 1 KH_2PO_4, 1 KCl, 2 $MgSO_4.7H_2O$, and 1 NaCl. The following trace elements in parts per million also were supplied: 5 p.p.m. boron, 0.5 p.p.m. manganese, 0.05 p.p.m. zinc, 0.01 p.p.m. copper and 5 p.p.m. iron as sodium sequestrian.

Solution Culture

Five-gallon glazed earthenware jars were fitted with removable wooden lids containing ten 1-inch holes, through which the plant root systems were suspended into the nutrient solutions. Absorbent cotton was packed around the stem in each hole to support the plants. A continuous supply of air was forced through gas-diffuser stones to insure adequate aeration in the root zone. The pH of the culture solution was maintained at 5.8 throughout the growth of the plants by additions of nitric acid.

INSECTICIDES USED

The schradan used throughout this work was manufactured by the Monsanto Chemical Company and has the following composition:

Active ingredients	90 percent
Octamethyl pyrophosphoramide	70 percent
Related organic phosphates	20 percent
Inert ingredients	10 percent

Technical grade demeton (Systox), the other insecticide used in this series of investigations, was produced by the Chemagro Corporation.

EXPERIMENTAL PROCEDURE

Foliar Applications of Schradan and Demeton and Their Influence on Seed Properties and Chemical Composition

Since dementon and schradan can persist in plants up to 3 and 6 weeks, respectively, it would be beneficial to determine (a) their translocation to, and persistence in, the cotton seed, and (b) their effect on seed properties and chemical composition. The following experiment was, therefore, conducted to investigate these effects and was performed in the greenhouse during the fall of 1953.

Five groups of Stoneville 2B cotton plants consisting of four plants per group were grown in 3-gallon glazed jars containing fertile Houston Black clay. Beginning with the appearance of the first flower, four of the groups were sprayed weekly from September through December with one of the following:

1. 0.2 percent schradan
2. 0.2 percent schradan plus 2 percent sucrose and 40 p.p.m. boron
3. 0.1 percent demeton
4. 0.1 percent demeton plus 2 percent sucrose and 40 p.p.m. boron
5. Untreated

The boron and sucrose additions were used primarily to determine whether they enhanced translocation of the systemics from the leaves to the bolls, as had been observed with 2,4-D (26).

The spray applications were discontinued when dehiscence of the first boll occurred. At maturity, the seed cotton was collected, ginned and the seed were examined for effects of the treatments on their chemical composition, viability and insecticide accumulation. These results are summarized in Table 1.

Seed Index

The seed index is defined as the weight in grams of 100 seeds. Both schradan and demeton reduced the weight of the seed as compared with the seed of the untreated plants. The incorpor-

TABLE 1. SEED INDEX AND CHEMICAL COMPOSITION OF WHOLE SEED AND SEED FRACTIONS FROM COTTON PLANTS SPRAYED WITH DEMETON AND SCHRADAN ALONE, AND IN COMBINATION WITH SUCROSE AND BORON

Treatment	Seed index (weight of 100) gms.			Oil, %		Protein (NH_3 x 5.13) %		Phosphorus, %		Carbohydrates, %	Boron, p.p.m.
	Seed coats	Embryos	Whole seed	Whole seed	Embryos	Whole seed	Embryos	Whole seed	Embryos	Embryos	Embryos
	Data expressed on a dry weight basis										
Control	4.8	6.4	11.2	22.9	35.6	26.4	38.7	0.668	1.066	6.69	17.99
Schradan	4.4	5.6	10.0	21.6	34.1	27.1	40.2	.698	1.050	5.73	16.46
Schradan plus boron and sucrose	4.2	5.3	9.5	25.0	39.0	22.5	32.6	.688	1.021	7.28	17.24
Demeton	4.4	5.4	9.8	24.4	38.0	24.4	36.2	.694	1.046	6.96	16.61
Demeton plus boron and sucrose	4.3	5.1	9.4	25.1	39.9	21.7	32.9	.603	.950	8.31	16.14

ation of sucrose and boron into the sprays caused a further decrease. To determine whether this reduction was in the seed coat, embryo, or both, indices were obtained on these seed fractions. Where a reduction in the weight of the whole seed resulted, there also was a corresponding reduction in the weights of both the seed coats and embryos.

Germination

One hundred seed obtained from plants in each treatment were planted in flats of sand in the greenhouse. Germination counts made 2 weeks later indicated that there was no significant influence of treatment on seed viability. Germination percentages were: control, 98; schradan, 100; schradan plus sucrose and boron, 94; demeton, 94; and demeton plus sucrose and boron, 96.

Oil

Schradan alone caused a slight reduction in oil content of the seed but demeton increased the oil from 35.6 (embryo basis) to 38.0 percent. Addition of sucose and boron to each of the insecticide sprays caused an increase in oil content; both, as compared wih the controls and systemics alone.

Protein

Schradan increased slightly the protein content of both the whole seed and embryo, but demeton caused a decrease. In each instance, the addition of sucrose and boron to the sprays resulted in protein reduction, as compared with both the control and insecticides alone.

Carbohydrates

The plants sprayed with demeton plus sucrose and boron produced seed which contained the highest carbohydrate level, while those sprayed with schradan plus sucrose and boron were next. Demeton alone caused a slight increase and schradan alone caused a slight decrease over the controls.

Phosphorus and Boron

A decrease in total seed phosphorus was found in the demeton plus sucrose and boron treated plants, while the other treatments remained essentially the same. There was no significant influence of treatment on boron accumulation in the seeds.

Residual Toxicity and Insecticide Content

Seed from each of the treatments were germinated in flats of sand and the seedlings infested with cotton aphids, *Aphis gossypii* Glov. and spider mites, *Tetranychus tumidus* Banks. None of the seedlings was toxic to the test insects. Chemical tests for schradan also were negative.

Persistence and Distribution of Schradan

As previously mentioned, schradan-treated plants may be toxic to aphids and mites for periods up to 6 weeks. Since the length of residual toxicity is inadequately defined, an experiment was performed in the greenhouse to furnish information concerning the exact length of time required to render non-toxic to mites and aphids a known initial amount of this insecticide, when present in a vigorously growing cotton plant.

TABLE 2. SCHRADAN CONTENT OF 28-DAY COTTON PLANTS GROWN IN SOLUTION CULTURE

Schradan in nutrient solution	Leaves		Stems		Roots		Entire plant
	Per gram dry wt.	Per total dry wt.	Per gram dry wt.	Per total dry wt.	Per gram dry wt.	Per total dry wt.	
P.p.m.	All values expressed in mg. per gram dry weight						
0	0.00	0.00	0.00	0.00	0.00	0.00	0.00
10	0.09	0.12	0.04	0.03	0.05	0.02	0.17
100	0.97	1.21	0.13	0.08	0.40	0.18	1.47
1000	8.73	9.16	1.08	0.55	4.59	1.61	11.32

Cotton seed of the Empire variety were planted in metal flats containing sterilized builders sand on April 17, 1954. On May 1, 1954, 10 seedlings were transplanted into each of twenty 5-gallon glazed earthenware jars containing nutrient solution. The plants were then separated into four groups consisting of 5 jars each (50 plants per group). Two weeks later, schradan was added to the solutions of each group to produce treatments consisting of 0, 10, 100 and 1,000 p.p.m.

After the plants had remained in the treated solutions for 7 days, three plants from each jar per treatment were selected at random, partitioned into leaves, stems and roots, and each fraction weighed. Harvests were made in three replications. The plant portions were then placed in a forced draft oven and dried for 24 hours at 78° C. The dried material was weighed and ground in a Wiley mill to pass an 80-mesh screen, then stored in a stoppered bottle.

The total schradan content was determined on the harvested plant material and the amount present per plant calculated. Table 2 shows that the plants grown in 0, 10, 100 and 1,000 p.p.m. schradan contained 0.00, 0.17, 1.47 and 11.32 mg. per plant, respectively. Table 3 shows that approximately 80 percent of the schradan absorbed through the root system by young cotton plants accumulates in the leaves, 5 to 16 percent in the stems and 12 to 14 percent in the roots.

Concurrently with the plant harvest, one plant from each of four replicates per treatment was removed, the root system thoroughly washed with

TABLE 3. SCHRADAN DISTRIBUTION IN 28-DAY COTTON PLANTS GROWN IN SOLUTION CULTURE

Schradan in nutrient solution	Leaves		Stems		Roots	
	Total dry wt.	Schradan distribution	Total dry wt.	Schradan distribution	Total dry wt.	Schradan distribution
Gms.	P.p.m.	%	Gms.	%	Gms.	%
0	1.28	0.00	0.68	0.00	0.45	0.00
10	1.27	72.05	0.69	16.14	0.42	11.80
100	1.25	82.30	0.64	5.45	0.45	12.35
1000	1.05	80.93	0.51	5.87	0.35	14.20

TABLE 4. PERSISTENCE OF SCHRADAN IN THE COTTON PLANT AS MEASURED BY THE NUMBER OF DAYS REQUIRED FOR COTTON APHID POPULATION TO INCREASE

Days	Schradan treatment (p.p.m.) 0 Aphid count	0 Percent mortality	10 Aphid count	10 Percent mortality	100 Aphid count	100 Percent mortality	1000 Aphid count	1000 Percent mortality
0	112	0.00	113	0.00	111	0.00	117	0.00
3	114	1.79[1]	81	28.32	38	65.77	0	100.00
6	267	138.39[1]	135	19.46[1]	2(93)[2]	98.19	0(103)[2]	100.00
10	972	767.85[1]	481	334.51[1]	70	26.31	0(99)[2]	100.00
14	[3]		1617	1130.97[1]	226	137.89[1]	46	53.54
17	[3]		[3]		1125	1084.20[1]	185	76.76[1]
20	[3]		[3]		[3]		366	269.69[1]
24	[3]		[3]		[3]		1190	1202.20[1]

[1] Percent increase.
[2] Reinfestation.
[3] Complete infestation.

de-ionized water, and transplanted into a 5-gallon glazed earthenware jar containing only nutrient solution. Two plants in each treatment were infested with cotton aphids, *Aphis gossypii* Glov. and the other two infested with spider mites, *Tetranychus tumidus* Banks. Since these plants were taken from the same substrate as the plants harvested and analyzed for schradan, it was assumed that the plants used in the persistence study had the same insecticide content as the harvested plants.

As the insecticide content of the plants increased, insect protection was found to extend over a longe period of time. The maximum time limit for aphid and mite protection was found to be approximately 18 and 14 days, respectively (Tables 4 and 5).

To determine the distribution of schradan in cotton plants having young bolls, the following experiment was performed in the greenhouse during the summer of 1954.

Two groups of Empire cotton plants were grown in solution culture as described earlier in this section, except that only one plant was cultured in each jar. Enough schradan was added to produce a treatment consisting of 100 p.p.m. On the ninth week, the plants were harvested and separated into old leaves, young leaves, petioles, flowers plus bracts, bolls plus bracts (1 to 10 days old), stems and roots. The fresh weight, dry weight and schradan content were determined on all of these fractions.

When schradan was expressed as mg. per gm. of dry weight, the insecticide accumulated in successively lower amounts in old leaves, young leaves, roots, flowers plus bracts, bolls plus bracts, petioles and stems, Table 6.

Approximately 80 percent of the total schradan absorbed was found in the leaves. The old leaves contained more of the insecticide than the young leaves when expressed either as mg. per gm. of dry weight or as percent distribution. The roots contained the next highest amount of the insecticide in terms of total dry weight. Schradan accumulated in slightly smaller amounts in the fruiting fractions than in the woody por-

TABLE 6. DISTRIBUTION OF SCHRADAN IN 9-WEEK-OLD COTTON GROWN IN NUTRIENT SOLUTIONS CONTAINING 100 P.P.M. SCHRADAN

Plant Part	Schradan Per gram dry wt.	Schradan Per total dry wt.	Schradan distribution
	Mg.	Mg.	%
Old leaves	0.52	12.80	65.95
Young leaves	0.35	3.77	19.43
Roots	0.15	1.79	9.20
Flowers plus bracts	0.12	0.22	1.12
Bolls plus bracts (1 to 10 days)	0.12	0.19	0.98
Petioles	0.05	0.35	1.82
Stems	0.04	0.29	1.15
Total		19.41	

TABLE 5. PERSISTENCE OF SCHRADAN IN THE COTTON PLANT AS MEASURED BY THE NUMBER OF DAYS REQUIRED FOR SPIDER MITE POPULATION TO INCREASE

Days	Schradan treatment (p.p.m.) 0 Spider mite count	0 Percent mortality	10 Spider mite count	10 Percent mortality	100 Spider mite count	100 Percent mortality	1000 Spider mite count	1000 Percent mortality
0	75	0.00	50	0.00	48	0.00	49	0.00
3	94	12.53[1]	46	8.00	41	14.58	3	93.88
6	171	128.00[1]	55	1.00[1]	33	31.25	2(52)[2]	95.92
10	438	484.00[1]	108	116.00[1]	152	216.66[1]	771	24.07[1]
14	1210	1513.33[1]	880	1760.00[1]	1025	2035.41[1]	92	88.88[1]
17	[3]		[3]		[3]		304	562.96[1]
20	[3]		[3]		[3]		1223	2164.14[1]
24	[3]		[3]		[3]		[3]	

[1] Percent increase.
[2] Reinfestation.
[3] Complete infestation.

tions when expressed on a percent distribution basis.

Influence of Schradan on Growth and Development

Empire cotton seed was planted in quartz sand contained in 16 tall four-gallon glazed earthenware jars on April 24, 1954. Nutrient solutions were supplied to each of the jars. On May 15, 2 weeks after germination, the plants were thinned to one per jar and the remaining plants separated into four groups consisting of four jars each. Each group was then supplied with two liters of nutrient solution containing either 0, 10, 100 or 1,000 p.p.m. schradan. The solutions were changed weekly. Supplementary de-ionized water was added to the cultures as needed to maintain sufficient moisture conditions.

The flowers were tagged and dated at anthesis, while the bolls were tagged and dated at dehiscence. The tags from shed bolls were collected daily and recorded. The experiment was terminated on August 27, 1954, and the seed cotton harvested, ginned, and its fiber and seed properties determined.

The plants grown on the 0 and 10 p.p.m. treatments showed no noticeable phytotoxic symptoms, while those on 100 p.p.m. schradan developed necrotic flecking and slight cupping of the lower leaves. The terminal leaves of the plants grown in cultures containing 1,000 p.p.m. schradan were dark green and appeared to be healthy. Marginal and interveinal necrosis, accompanied by considerable cupping of the lower leaves, occurred after the first week on plants at this level of schradan. After the second week, severe necrosis and abscission of the lower-most leaves continually occurred.

The effects of schradan supply upon its content in the foliage, vegetative and reproductive characters, and on dry-weight production, are given in Table 7.

At the two higher schradan levels, there was a decrease in the number of flowers produced and an increase in the number of leaves abscised. Plants on the highest treatment level developed more nodes but shorter main stems. An increase in dry weight was apparent over the control plants in those grown in 10 and 100 p.p.m. schradan and a decided decrease was evidenced in

TABLE 7. INFLUENCE OF SCHRADAN SUPPLY ON LEAF INSECTICIDE CONTENT, REPRODUCTIVE CHARACTERS, AND DRY-WEIGHT PRODUCTION

Schradan content and plant character	Schradan in nutrient solution (p.p.m.)			
	0	10	100	1000
Mg. schradan in leaves	0.00	2.13	13.19	41.16
No. flowers produced	33.7	33.5	30.2	12.7
No. leaves abscised	12.0	9.2	12.7	21.2
No. main stem nodes	22.7	24.0	23.7	26.7
Height of main stem (cm.)	111.5	115.7	116.5	97.2
Dry weight (gms.)	89.7	117.6[1]	105.2	36.8[2]

[1] Significant at 5% level.
[2] Significant at 1% level.

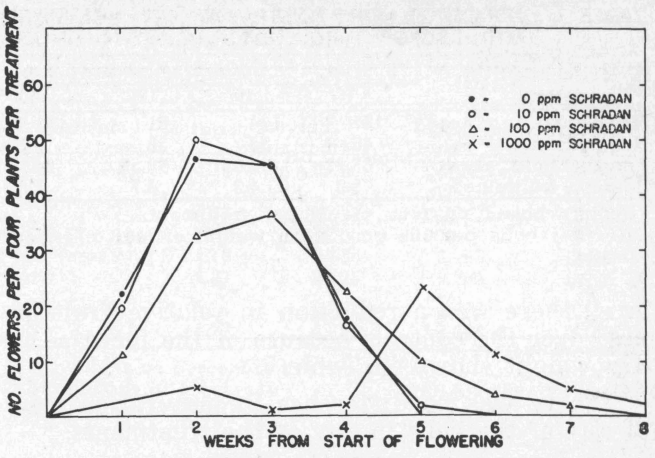


Figure 1. Influence of schradan on flower production and flowering time in cotton.

the 1,000 p.p.m. plants. The total schradan content of the leaves was found to be correlated with the supply.

Figure 1 shows that flower production was not only reduced at the two higher levels, but also was extended over a longer period of time. There was no difference between the 0 and 10 p.p.m. plants in flower production or flowering date.

Since cotton usually produces flowers until a full fruit load is set, the bolls on the 0 and 10 p.p.m. plants were located on the lower-most fruiting branches, while those on the 100 p.p.m. plants were distributed on fruiting branches along the entire main stem axis (Figure 2). An average of only one boll per plant developed on those grown on the highest insecticide treatment (1,000 p.p.m.).

Not only were there fewer flowers produced and bolls set on the 100, and 1,000 p.p.m. treatment levels, but there also was an increase in the percent boll shed (Table 8). Relative fruitfulness, as measured by Eaton (10), was inversely correlated with schradan supply. Even though there were more bolls produced on the 10 p.p.m.

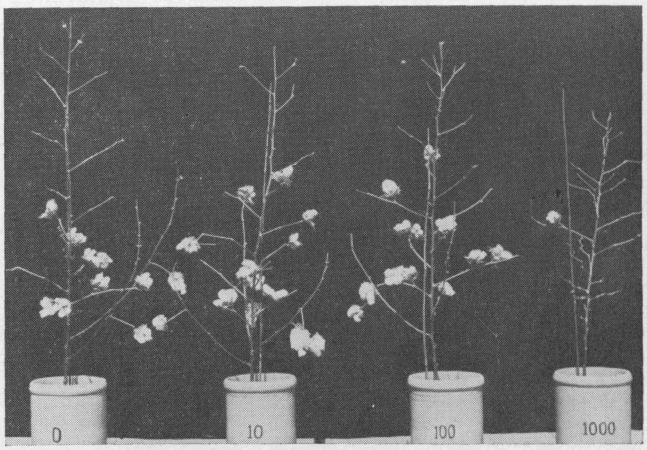


Figure 2. Fruiting activity of cotton grown on 0, 10, 100 and 1,000 p.p.m. schradan.

TABLE 8. INFLUENCE OF SCHRADAN ON SHEDDING AND RELATIVE FRUITFULNESS OF COTTON[1]

Reproductive character	Schradan in nutrient solution (p.p.m.)			
	0	10	100	1000
No. flowers produced	135	134	121	51
No. bolls set	46	50	38	3
Percent bolls shed	65.92	62.69	68.59	94.12
Relative fruitfulness[2]	5.3	4.3	3.7	1.0

[1] Results based on four plants per treatment.
[2] Number bolls per 100 gms. fresh weight of leaf and stem tissue.

level, there was a reduction in relative fruitfulness over the controls because of the increase in dry weight shown in Table 7.

There was no difference in maturation time of any of the bolls in any of the treatments.

Total seed cotton and the seed cotton per boll were reduced as the level of schradan in the nutrient solutions increased (Table 9). There was no apparent influence of schradan on the seed index, but the lint index increased slightly with increasing treatment up to the 100 p.p.m. level. The lint index at 1,000 p.p.m. was below the other treatment levels, but higher than the controls.

TABLE 9. INFLUENCE OF SCHRADAN ON THE YIELD OF SEED COTTON AND THE SEED AND LINT INDICES

Item	Schradan in nutrient solution (p.p.m.)			
	0	10	100	1000
	Values as means per plant			
Total seed cotton, gms.	56.50	54.10	40.30	4.30
Seed cotton per boll, gms.	4.89	4.33	4.13	4.27
Seed index[1]	14.90	14.70	15.00	14.40[2]
Lint index[3]	5.42	5.79	5.98	5.63[2]

[1] Weight in grams of 100 seed.
[2] Results based on 64 seed.
[3] Weight in grams of lint per 100 seed.

The fiber data (Table 10) show a uniform trend toward a reduction in the length and fineness by schradan at the 100 and 1,000 p.p.m. level.

The oil, phosphorus, protein and schradan contents of the seed embryos obtained from plants grown in each of the treatment levels were determined. Table 11 shows that oil decreases and protein increases as the treatment level increases up to 100 p.pm. The oil content of the embryos in the 1,000 p.p.m. treatment level was essentially the same as the 100 p.p.m., however, the amount of protein in the 1,000 level was less than

TABLE 10. INFLUENCE OF SCHRADAN ON FIBER PROPERTIES OF COTTON

Schradan in nutrient solution	Fiber property			
	Length		Tensil strength (100 lb.per sq. in.)	Fineness (micrograms per inch)
	Upper half mean (in.)	Mean (in.)		
P.p.m.	— — — Values as means per plant — — —			
0	1.08	0.87	94	3.45
10	1.08	0.87	95	3.70
100	1.06	0.85	94	4.00
1000	0.95	0.78	93	—[1]

[1] Insufficient cotton for test.

the 100, but more than the 10 p.p.m. treatment. No consistent trend was found in the phosphorus content even though a slight increase in schradan was detected in the oil-free residue as the treatment level increased.

Nutritional Factors Associated with the Absorption and Accumulation of Schradan

It has been reported (3) that increasing levels of phosphorus in the substrate cause corresponding reductions in the amounts of schradan absorbed through the root system of the pea plant. Since this work involved using levels up to 10 times the amount commonly employed in nutrient solutions, an experiment was designed to determine whether the various nitrogen, phosphorus and potassium levels more commonly used would influence the insecticide uptake by the cotton plant.

TABLE 11. OIL, PHOSPHORUS, PROTEIN AND SCHRADAN CONTENT OF COTTON SEED EMBRYOS OBTAINED FROM PLANTS GROWN IN SAND CULTURES SUPPLIED WITH NUTRIENT SOLUTION CONTAINING VARYING AMOUNTS OF SCHRADAN[1]

Schradan in nutrient solution	Oil	Phosphorus	Protein (NH_3 x 5.13)	Schradan (oil-free residue)
P.p.m.	%	%	%	P.p.m.
0	36.4	1.145	34.3	0
10	35.5	1.118	35.4	1.3
100	34.9	1.167	36.7	4.8
1000	34.8	1.156	35.9	9.6

[1] Values expressed on a dry-weight basis.

On June 8, 1954, 1-week-old Empire cotton seedlings were transplanted to solution cultures containing three levels of N, P and K (Table 12). Each treatment contained 200 p.p.m. schradan. The plants were harvested on June 22, 1954.

TABLE 12. COMPOSITION OF NUTRIENT SOLUTIONS AS MILLIMOLES AND MILLIEQUIVALENTS PER LITER[1]

Element		Total milli-equivalents of N, P or K	Millimoles								
			$Ca(NO_3)_2$	KNO_3	$CaCl_2$	KH_2PO_4	KCl	$NaNO_3$	NaH_2PO_4	$MgSO_4$	NaCl
Nitrogen	high	16.0	6.0	4.0		1.0	1.0			2.0	1.0
	med.	4.0		4.0	6.0	1.0	1.0			2.0	1.0
	low	1.0		1.0	6.0	1.0	4.0			2.0	1.0
Phosphorus	high	1.00	6.0	4.0		1.00	1.00			2.0	1.0
	med.	0.25	6.0	4.0		0.25	1.65			2.0	1.0
	low	0.0625	6.0	4.0		0.06	1.94			2.0	1.0
Potassium	high	6.000	6.0	4.0		1.0	1.000			2.0	1.0
	med.	1.500	6.0			1.0	0.500	4.0		2.0	1.0
	low	0.375	6.0				0.375	4.0	1.0	2.0	1.0

[1] Plus: 5 p.p.m. boron; 0.5 p.p.m. manganese; 0.05 p.p.m. zinc; 0.01 p.p.m. copper; 5 p.p.m. iron as sodium sequestrian; and 200 p.p.m. schradan.

The plants grown in the high N-P-K solutions were growing vigorously at the termination of the experiment and produced the greatest amount of dry weight per plant (Table 13). The plants grown on the medium and low nitrogen, phosphorus and potassium series produced less growth as the corresponding elemental content decreased. Mottling was evident on leaves of the low and medium nitrogen plants; the leaves of the plants grown on medium and low phosphorus and potassium remained dark green.

Chemical analyses for nitrogen, phosphorus and potassium were made on plants harvested from each series. The amounts present in the plants were correlated with the external elemental supply (Table 14). As the nitrogen supply decreased, there occurred an increase in schradan content when expressed as mg. per gm. of dry weight. A similar but weaker trend was noted when the phosphorus supply was varied. The level of external potassium had little or no effect on schradan absorption.

Nutritive Value of Schradan Phosphorus

Since schradan contains 21.7 percent phosphorus, an experiment was devised to determine whether any of the phosphorus liberated from the metabolized schradan in the plant can be utilized in growth.

Cotton seedlings were transplanted into nutrient solutions containing the following treatments: complete nutrient solution (31 p.p.m. P), nutrient solution minus P, and minus P nutrient solution plus schradan equivalent to the phosphorus content of the plus P solution (31 p.p.m.).

The plants were harvested 27 days later and fractioned into leaves, stems and roots. The dry weight and total phosphorus content of each tissue fraction was determined.

The control plants were growing vigorously at the termination of the experiment. Marginal burning of the leaves was evident on the minus P plants, but did not occur on the schradan-treated plants.

As measured by the amount of dry weight produced, the plants grown in the schradan solutions produced less growth than the controls, but more than the minus P plants (Table 15). Analyses for total phosphorus established the presence of a greater amount of phosphorus in the schradan-treated plants than in the minus P plants. However, the schradan-treated plants contained much less phosphorus than the controls.

TABLE 13. HEIGHT, FRESH AND DRY WEIGHT AND PERCENT MOISTURE OF 28-DAY-OLD COTTON PLANTS GROWN IN SOLUTION CULTURES FOR 21 DAYS[1]

Treatment	Height	Fresh weight	Dry weight	Moisture
	Cm.	Gms.	Gms.	%
High N-P-K	19.5	9.17	0.89	90.29
Medium N	19.4	8.42	0.78	90.73
Low N	17.6	7.09	0.65	90.83
Medium P	20.1	8.90	0.84	90.56
Low P	19.8	8.36	0.79	90.55
Medium K	20.2	8.52	0.81	90.49
Low K	19.1	7.15	0.71	90.07

[1] All values as means per plant.

TABLE 14. NITROGEN, PHOSPHORUS, POTASSIUM AND SCHRADAN CONTENTS OF 28-DAY-OLD COTTON PLANTS GROWN IN SOLUTION CULTURES FOR 21 DAYS[1]

Treatment	Nitrogen	phorus Phos-	Potassium	Schradan
High N-P-K	4.19	0.686	3.125	0.160
Medium N	3.82	0.662	3.295	0.168[2]
Low N	3.59	0.650	3.195	0.186[3]
Medium P	4.18	0.654	3.195	0.167
Low P	4.27	0.525	3.280	0.170
Medium K	4.14	0.694	3.175	0.164
Low K	4.20	0.695	2.392	0.165

[1] All values as percent of dry weight.
[2] Significant at 5% level.
[3] Significant at 1% level.

Metabolic Intoxification of Schradan

Hall *et al.* (12) found that chloroform extracts of bean plants treated with schradan were 700 times more effective in inhibiting cholinesterase activity than the original insecticide. Casida *et al.* (3) reported that pea plants produce an 18 times enhancement of schradan as measured by cholinesterase activity. Zeid and Cutcomp (41), however, were unable to corroborate these findings. De Petri-Tonelli and March (30) and Ivy *et al.* (18) using insect bioassay found no evidence of intoxification of schradan in the bean and cotton plant, respectively, and concluded that these plants merely serve as carriers for the insecticide.

Since the reports on this matter are controversial, an experiment similar to the one performed by Casida *et al.* (3) on the pea plant was conducted with the cotton plant.

On September 9, 1954, 1-week-old Empire seedlings were transplanted into four 5-gallon glazed jars containing nutrient solutions. On September 20, 1954, schradan was added to each jar to produce treatment of 0, 10, 100 and 1,000 p.p.m. One week later, one plant from each treatment was removed, washed with de-ionized water, and macerated in a mortar containing Ringers buffer solution and a little quartz sand. The brei was

TABLE 15. DRY WEIGHTS AND PHOSPHORUS CONTENTS OF 34-DAY-OLD COTTON PLANTS AFTER 27 DAYS ON PLUS AND MINUS P AND SCHRADAN[1]

Plant part	High P		Low P		Schradan	
	Dry weight	P	Dry weight	P	Dry weight	P
	Gms.	%	Gms.	%	Gms.	%
Leaves	1.78	1.438	0.48	0.149	0.74	0.219
Stems, petioles	.89	1.216	.10	.123	.22	.152
Root	.83	1.441	.32	.125	.46	.180
Entire plant	3.50	4.095	.90	.397	1.42	.551

[1] All values expressed on a dry-weight basis.

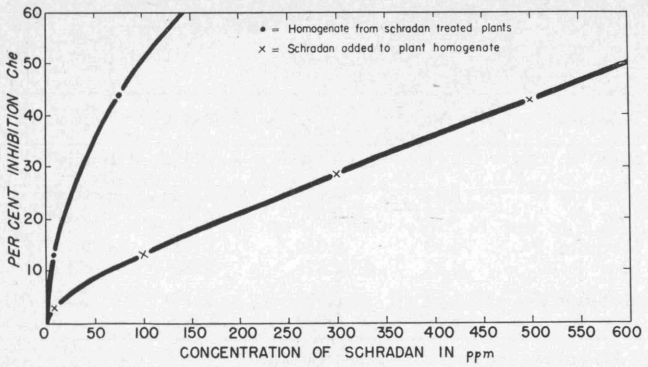


Figure 3. Metabolic intoxification of schradan as measured by cholinesterase (Che.) activity before and after passage through the cotton plant.

strained through a muslin cloth and the tissue-free homogenate was made to 50 ml. with the buffer solution. Schradan was added to similar untreated plant homogenates to produce concentrations of 0, 10, 100, 300 and 500 p.p.m. Chemical and anti-cholinesterase activity determinations were made on all of the plant extracts and the data plotted (Figure 3).

It was shown by chemical analysis that the homogenates from plants grown on 10, 100 and 1,000 p.p.m. schradan contained 2, 8 and 86 p.p.m. of the insecticide when diluted to 50 ml., and caused 4.8, 13.5 and 44.3 percent inhibition of cholinesterase activity, respectively. On the other hand, the schradan added to the untreated plant homogenates in the amounts of 10, 100, 300 and 500 p.p.m. inhibited the enzyme by 3.6, 14.5, 28.6 and 43.1 percent, respectively.

Since 2 p.p.m. schradan from the treated plant homogenate produced a 4.8 percent inhibition and a 3.6 percent inhibition resulted in the untreated plant homogenate to which 10 p.p.m. schradan were added, the former was approximately 12 times as effective in inhibiting cholinesterase activity. At the highest concentration used, 86 p.p.m. of the insecticide in the treated plant homogenate produced approximately the same amount of enzyme inhibition as 500 p.p.m. that were added to the untreated plant homogenate. These results indicate the formation of a cholinesterase inhibitor within the cotton plant which is more effective than schradan *per se*.

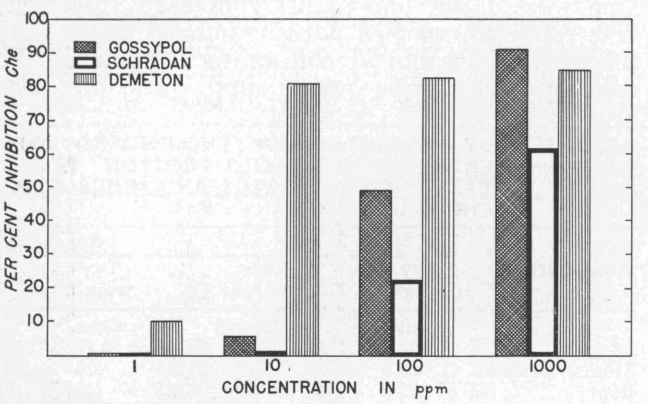


Figure 4. IN VITRO inhibition of cholinesterase activity by schradan, demeton and gossypol.

Attempts to Determine Residual Insecticides in the Developing Boll by Cholinesterase Activity Measurements

The results in the section on Metabolic Intoxification of Schradan have shown that a measure of residual schradan in young cotton plants could be obtained by using the enzyme cholinesterase. Since no suitable chemical method for the determination of demeton has been devised, it was thought that the use of this enzyme also might provide a measure of accumulation of both insecticides. A preliminary field experiment was performed in the spring and summer of 1954 to determine whether demeton and schradan accumulated in the developing boll in measurable quantities as determined by cholinesterase activity.

On June 29, 1 week after flowering had started, field-grown cotton plants in 30-foot rows were sprayed with 800 ml. of one of the following insecticide treatments: schradan 0.2 percent (70 percent) ; demeton 0.1 percent (50 percent) ; and control (untreated).

Two days after spraying, previously tagged bolls, 2 to 8 days old, were collected, washed and frozen. About 1 week later, the bolls were thawed and the sap expressed in a Carved hydraulic press. A portion of the sap was added to an acetylcholine-cholinesterase system and the enzymatic activity determined manometrically.

Natural inhibitors present in the bolls were found to impair the cholinesterase activity so drastically that no conclusions on the accumulation of schradan or demeton could be deduced.

Since gossypol may be present in the bolls in amounts up to 1.2 percent (11), it seemed of interest to know what its influence would be on the cholinesterase enzyme system.

Solutions containing 0, 10, 100 and 1,000 p.p.m. of gossypol, demeton and schradan were prepared and the inhibition of the enzyme system determined on each material.

Demeton caused a 10 percent inhibition of cholinesterase at 1 p.p.m. and 84 percent inhibition at 10 p.p.m. (Figure 4). Schradan, however, produced only a 23 percent inhibition at 100 p.p.m. and 62 percent at 1,000 p.p.m. Gossypol also inhibited cholinesterase, being 6 perceent at 10 p.p.m. and 93 percent at 1,000 p.p.m. When the percent inhibition was plotted against the log of the concentration, a straight line relationship existed between concentrations of 1 to 10, 10 to 500 and 10 to 1,000 p.p.m. for demeton, schradan and gossypol, respectively.

Chloroplast Pigments

During the course of this project, it was noticed that plants treated with schradan appeared to produce greener leaves than those of the un-

TABLE 16. DRY WEIGHTS AND CONCENTRATIONS OF CHLOROPHYLL AND CAROTENOIDS IN LEAVES OF PLANTS GROWN 15 DAYS IN NUTRIENT SOLUTIONS WITH 0, 10, 100 OR 1,000 P.P.M. OF SCHRADAN

Schradan, p.p.m.	Chlorophyll		Carotenoids	Dry weight per 10 plants, gms.
	%	Millimoles[1]	%	
	Results of dry-weight basis			
0	0.97	1.08	0.37	22.3
10	1.03	1.15	.44	26.5
100	1.09	1.22	.43	25.1
1000	1.22	1.36	.50	18.0

[1] Chlorophyll a ($C_{55}H_{72}O_5N_4Mg$).

treated plants. Since an increase in dry weight results in plants grown in non-toxic concentrations of this insecticide, an increase in chlorophyll and photosynthesis was indicated. With the foregoing in mind, chlorophyll and the associated carotenoid pigments were determined.

The chlorophylls and carotenoids were determined on fresh leaves which were obtained from plants grown as reported in the section on Persistence and Distribution of Schradan.

As the treatment level increased, there was an increase in chlorophyll content of the leaves; however the percent of carotenoids in the 100 p.p.m. level was slightly less than in the 10 p.p.m., but greater than the controls (Table 16).

Since it was clear that an increase in chlorophyll resulted with increasing schradan treatment, absorption spectra were determined on these two pigments to establish their identity definitely.

Figures 5 and 6 show that the characteristic peaks of chlorophylls and the carotenoids, respectively, were obtained. Two additional peaks were found, one at 460 mu, and the other at 590 mu, in the 1,000 p.p.m. chlorophyll curve.

Influence of Schradan on the Chemical Composition

Various chemical fractions were determined on the dried plant material which had been collected and stored in stoppered bottles as described

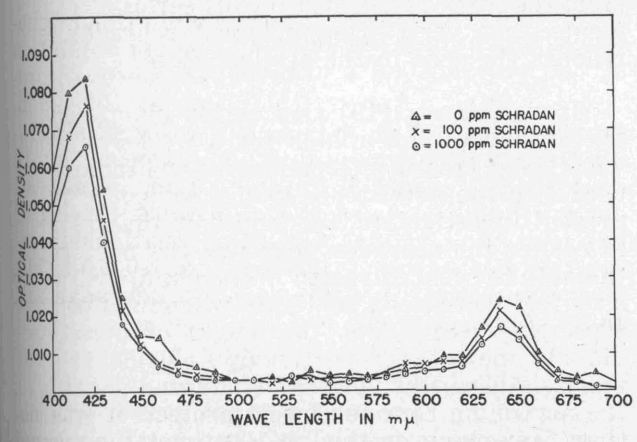


Figure 5. Absorption spectra of the saponified chlorophylls extracted from plants treated with schradan.

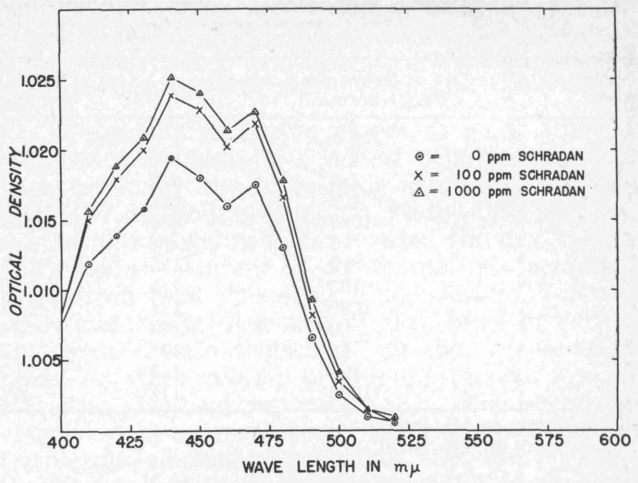


Figure 6. Absorption spectra of the carotenoids (carotene and xanthophylls) extracted from plants treated with schradan.

in the section on Persistence and Distribution of Schradan.

The carbohydrate data (Table 17) show that total sugars in the leaves and stems increase as the treatment levels increase, except at the highest concentration of schradan, where a decided reduction is apparent. It appears noteworthy that the control roots contained more total sugars than the roots of the 10 p.p.m. plants, but less than those harvested from the 100 p.p.m. schradan series. The roots of the 1,000 p.p.m. plants contained the least amount of total sugars of any treatment.

The highest starch value in the leaves was found in plants grown in 10 and 100 p.p.m. of schradan, being followed in decreasing order by 1,000 p.p.m. and the controls. There was a decrease in starch content of the stems as the treatment level increased, except at the highest concentration of the insecticide. The starch content of the roots followed the same order of accumulation as the total sugars in respect to treatment, except a high accumulation of starch was evident at the 1,000 p.p.m. level.

TABLE 17. CARBOHYDRATE FRACTIONS OF COTTON PLANTS GROWN IN SOLUTION CULTURE CONTAINING SCHRADAN

Plant part	Schradan treatment	Carbohydrate fractions		
		Total sugars	Starch	Total
	P.p.m.	—Values as percent of dry weight—		
Leaves	0	1.70	7.08	8.78
	10	1.83	9.56	11.39
	100	1.84	9.44	11.28
	1000	1.16[2]	7.77	8.93
Stems	0	2.98	4.00	6.98
	10	3.27	3.57	6.84
	100	3.84[1]	2.91	6.75
	1000	1.50[2]	5.13	6.63
Roots	0	3.30	2.12	5.42
	10	2.75	1.20[1]	3.95
	100	3.66	4.67[2]	8.33
	1000	0.73[2]	4.18[2]	4.91

[1] Significant at 5% level.
[2] Significant at 1% level.

TABLE 18. NITROGEN FRACTIONS, TOTAL PHOSPHORUS AND SCHRADAN CONTENTS OF COTTON PLANTS GROWN IN SOLUTION CULTURE CONTAINING SCHRADAN

Plant part	Schradan treatment	NO_3-N	Soluble nitrogen	Insoluble nitrogen	Total nitrogen	Total phosphorus	Total schradan
	P.p.m.	— — — — — — — —		Values as percent of dry weight		— — — — — — — —	
	0	0.020	0.890	3.568	4.458	0.862	0.000
Leaves	10	.019	.875	3.570	4.445	.902	.009
	100	.018	.956	3.566	4.522	.940	.097
	1000	.006[1]	1.337[2]	3.392	4.729	1.121[1]	.873
	0	0.183	1.414	2.086	3.500	0.567	0.000
Stems	10	.195	1.564	1.924	3.488	.580	.003
	100	.175	1.637	1.685	3.322	.554	.008
	1000	.188	1.635	1.911	3.546	.570	.108
	0	0.134	1.146	2.026	3.172	0.870	0.000
Roots	10	.131	1.125[1]	2.562	3.687	.935	.004
	100	.129	1.028[1]	2.593	3.621	.872	.018
	1000	.112[1]	.976[2]	2.592	3.568	1.013[1]	.459

[1] Significant at 5% level.
[2] Significant at 1% level.

Table 18 shows that the soluble and total nitrogen fractions in the leaves increased as the treatment level increased, but no difference was found in the insoluble nitrogen fraction, except at the 1,000 p.p.m. level, where a decline was noted. Nitrate nitrogen in the leaves and roots was inversely correlated with treatment, while the total phosphorus in the leaves and roots increased as the treatment level increased. The accumulation of schradan in the leaves, stems and roots was found to be correlated with the supply of schradan in the substrate.

DISCUSSION

Cotton plants, when grown to maturity in solution cultures containing a gradient of schradan content for varying absorption periods, accumulated approximately 80 percent of the insecticide in the leaves. Metcalf and March (24) reported similar findings for the lemon plant. Of the total schradan detectable in the cotton plant, very small quantities were found in the roots and less in the stems and petioles. Only 1 to 3 percent of the total schradan was found in the young bolls and flowers, indicating that either small amounts are translocated to the fruiting structures, or that the insecticide is rapidly metabolized at reproductive sites. If other systemic insecticides accumulate in plant parts in the same proportions as shown for schradan, the control of leaf-feeding insects is very promising. Those that feed in other plant portions, such as fruiting structures, may be difficult to control by these insecticides.

Although no translocation studies were conducted on schradan in this series of investigations, it appears that the xylem is the chief avenue of acropetal movement. The basipetal transport should perhaps be investigated further, even though reports (19, 31) have shown that the downward movement results quite readily, possibly in the phloem.

In no case were the seed, which were harvested from plants sprayed weekly with either demeton or schradan, found to contain measurable amounts of the insecticides. Chemical determinations for schradan, and insect bioassay on the seed extracts or of the seedlings, were found to be negative for both insecticides. Even seed obtained from the plants grown in solution cultures containing continuous supplies of schradan were found to have a maximum of 10 p.p.m. on the highest level (1,000 p.p.m.). No seed were found to produce seedlings which were toxic to mites or aphids. Metcalf (25), however, showed that cotton plants sprayed with P^{32} schradan at the rate of 1 pound per acre accumulated 167 p.p.m. of this insecticide in the oil-free residue 43 days after application. The raw oil was found to contain 107 p.p.m. but this amount was reduced to 0.02 p.p.m. after the refining process.

Casida *et al.* (3) found that schradan absorbed by pea seed is not completely metabolized during germination. Similarly, Ivy (19) noted that cotton seed soaked for 2 hours in solutions containing as low at 0.5 percent schradan produced seedlings which were also toxic to aphids and mites. The present investigation established that 10 p.p.m. schradan, when expressed on a fresh weight basis, are more than adequate to kill these insects in leaf tissue. In light of Metcalf's work (25), one wonders why none of the seedlings produced from the seed obtained from plants treated in these experiments contained enough residual insecticide to kill mites and aphids. It is believed from the work performed in this series of experiments that very little technical schradan ever reaches the seed in the original form.

It was shown (19) that cotton plants may be toxic to mites and aphids for 6 weeks or longer when seed treatments employing schradan were used. In the persistence study performed in the present investigation, the length of time required to render the schradan inactive was not proportional to the concentration in the plant. Although a tenfold increase in schradan was found between successive treatments, the length of persistence did not increase proportionally. Table 19 summarizes the insect bioassay study and shows that the maximum time for insect protection was less than 3 weeks, even though 100 times the amount usually applied for insect control was used.

TABLE 19. SUMMARY OF TABLES 4 AND 5 SHOWING PERSISTENCE TIME OF SCHRADAN IN COTTON AS MEASURED BY COTTON APHID AND SPIDER MITE BIOASSAY

Schradan	Schradan per plant	Time required for aphid population to increase	Time required for spider mite population to increase
P.p.m.	Mg.	Days	Days
0	0.00	—	—
10	0.16	5-6	4
100	1.47	12	7
1000	11.32	18	14

Several explanations may be offered for the apparent reduction in persistence time found in this experiment. Probably one of the most important was the rapid dilution of the systemic in the actively growing plant. Metabolic decomposition of the insecticide probably took place rapidly since these plants were growing vigorously during the experiment. Although not investigated, one wonders how much, if any, of the systemic was lost through transpiration and guttation. Heath *et al.* (17) reported that the loss of schradan from plants by evaporation, root excretion and washing out of leaves by rain was negligible. The same authors also state that the rate of loss is remarkably independent of plant species and the main reason for the disappearance of the insecticide is metabolic decomposition.

It has been mentioned previously that pure schradan is relatively non-toxic to cholinesterase activity; however, once it is metabolized by plants (3, 12, 23) and animals (8), drastic inhibition results, being more severe in the latter. Hartley (15) discussed the intensification of schradan and proposed that the metabolic intensification of this insecticide in plants is of little or no importance in both insects and mammalin toxicology. Casida *et al.* (3), however, suggested that insects require the intermediary action of the plant in converting this systemic to an active metabolite which is then ingested by the feeding insect where it ultimately combines with cholinesterase. It was shown (30) that when insect bioassays were used to measure toxicity, the Valentine bean was not instrumental in increasing the toxic properties of schradan. Although some of the insecticide was converted to a more reactive form in the plant, it was suggested by the same authors that the unmetabolized schradan was converted to the active form after ingestion by the insect.

As shown in the section on Metabolic Intoxication of Schradan, the quantitative determination of residual schradan in young cotton plants was made by using cholinesterase activity measurements. Cholinesterase inhibition of schradan after passage through the plant was 5 to 12 times more effective in inhibiting cholinesterase activity than before it passed through the plant. These values compare favorably with those obtained by Casida *et al.* (3) who report 18 fold enhancement of schradan by the pea plant.

Attempts to determine the presence of schradan and demeton in young bolls by similar cholinesterase activity measurements were not successful because of the presence of natural inhibitors. Gossypol, a constituent of the boll, was an effective inhibitor of cholinesterase.

The results of the development study, though more detailed, are not the first report of growth stimulation by the insecticide schradan. Casida *et al.* (3), without citing data, stated that low concentrations of schradan increased the dry weight and moisture content of pea plants. Cotton seedlings from seed soaked in dilute solutions of the insecticide were observed by Tsi (36) to be larger than the controls. A similar response of plants to other related organophosphorus insecticides has also been reported (13). The beneficial effect has been ascribed to (a) the available nutritive phosphorus of schradan (36, 40), (b) the bond energy of the P-O-P linkage (29) or (c) the effect of the insecticide on some enzyme such as phosphatase (3).

Data from the section on Nutritive Value of Schradan Phosphorus show that small amounts of phosporus liberated from the metabolized schradan were used in growth; its effects being reflected in the increase in dry weight over phosphorus deficient plants. It was apparent, however, that the phosphorus supplied from schradan could not replace an equivalent amount of inorganic phosphate, although the phosphate released from the insecticide was used to some extent in the growth of the plant. The role of schradan as a supplemental source of phosphorus must be minor, because when sufficient quantities of phosphorus are available in the nutrient solution, the additional amounts as supplied by minute quantities of schradan in the 10 p.p.m. treatments are not sufficient to account for the observed stimulation in growth. Neither was there any significant difference in the total phosphorus content of the plants of the two treatments (0 and 10 p.p.m.).

Figure 7 shows plants grown in complete nutrient solution and others grown in complete nu-

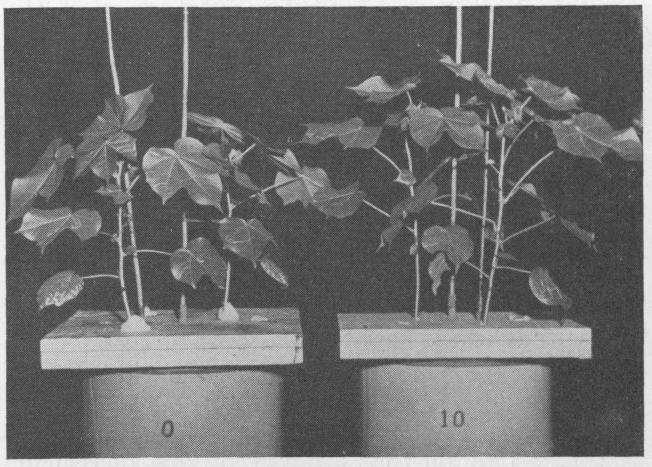


Figure 7. Cotton plants at 35 days in complete nutrient solutions without 0 and with 10 p.p.m. schradan. Photographed 15 days after addition of schradan.

trient solution with 10 p.p.m. schradan added. The treated plants are obviously larger than those grown on nutrient solution alone.

Data of the current study suggest the chloroplast pigments as a possible factor in explaining the stimulatory growth responses of some plants to organophosphorus insecticides. For, in the absence of other inhibitory or limiting factors, it logically follows that one result of an increase in these important pigments would be accelerated photosynthesis and a corresponding increase in carbohydrates; which in turn could result in increased growth (dry weight). This was indicated in cotton by the increase in the dry weights and photosynthetic pigments at the 10 and 100 p.p.m. concentrations of schradan over the control. That a still greater increase in these pigments at the 1,000 p.p.m. concentration was accompanied by a reduction instead of an increase in dry weight indicates, of course, that some other equally vital reaction of photosynthesis was at the same time greatly inhibited, or that carbohydrate oxidation was accelerated without accompanying growth. Although little is known about the mechanism of the phytotoxic action of schradan, there is evidence that in high concentrations it hinders the activity of the leaf phosphatase (3). Since the enzyme acts to hydrolyze the phosphate linkage of both high energy and ester phosphate compounds, inhibition of its activity would reduce carbohydrate utilization in cellular synthesis, regardless of a high photosynthetic potential, to a level too low to sustain normal growth.

The noted beneficial effects of schradan on vegetative development and photosynthetic pigments did not extend to the reproductive activities of cotton. All concentrations reduced the yield of seed cotton to less than that of the control through either a reduction in boll size alone (10 p.p.m.) or a combination of this effect with (a) a reduction in number of flowers and (b) increase in boll shedding (100 and 1,000 p.p.m.).

Eaton (10) considers the number of bolls per gm. of fresh leaves and stems (relative fruitfulness) to be a more critical measurement of the tendency of the cotton plant toward reproductive versus vegetative growth than is the percentage of flowers that develop into mature bolls. Following this criterion, Table 8 shows that the relative fruitfulness values decrease stepwise with each corresponding increase in concentration of schradan. Although a part of a consistent trend, relative fruitfulness was reduced by the 10 p.p.m. concentration of schradan and further by the 100 and 1,000 p.p.m. concentrations. Our data are not extensive enough to support a conclusion that significance is attached to the reduction at the 10 p.p.m. level in weight of seed cotton per plant, or weight of seed cotton per boll, Table 9.

In laboratory tests with cotton seedlings, Ivy *et al.* (18) found that 6 p.p.m. of this insecticide in nutrient solution gave complete kill of spider mites and cotton aphids. Plants of the present study were continusously supplied with schradan and, in all probability, they accumulated the insecticide in concentrations above those that are required for adequate insect control. It has been regarded essential, notwithstanding, that information be developed which permits conclusions on the phytotoxic activity of this important insecticide.

As the schradan in the nutrient solutions increased up to 1,000 p.p.m., there was a proportional increase in the schradan content of the cotton plant. On the other hand, if the nitrogen level in the solutions was varied from low to high, and the schradan supply held constant, the absorption of the insecticide decreased with each increase in nitrogen level. Casida *et al.* (3) had reported that increasing quantities of phosphorus in the substrate caused a reduction in the amount of schradan absorbed by the pea plant. Within the range of phosphorus concentrations supplied to plants in the present investigation, no significant difference could be found in the amount of schradan absorbed at the high (31 p.p.m.) and low (2 p.p.m.) phosphorus levels. The data presented by Casida *et al.* (3) also show no significant difference in the schradan absorbed by pea plants grown in 5 and 40 p.p.m. phosphorus levels, which are comparable with those of this study. Their treatment containing 320 p.p.m. phosphorus definitely reduced the uptake of the insecticide, being significantly different from the 40 p.p.m. series. The external supply of potassium apparently had no influence on the absorption of schradan in this investigation.

In light of the data presented by Casida *et al.* (3) on the influence of phosphorus on the absorption of schradan and the information offered in the present study concerning nitrogen, it may be that the absorption of this insecticide is reciprocal with the supply of anions in the substrate. Additional data, however, are required to substantiate this view.

Although numerous chemical determinations were made on young cotton plants and on seed obtained from treated plants, only minor differences for the most part were found. It is noteworthy that schradan caused an increase in the seed protein over the controls, while a simultaneous reduction in oil resulted. Demeton, however, produced a reversal of this trend. The explanation for the opposite effects of these two materials is not clear, since one would anticipate that these two organic phosphorus insecticides would influence like biochemical systems in the plant in a similar manner.

The leaves of plants grown in solution cultures which contained 10 and 100 p.p.m. schradan had slightly higher total carbohydrates than the leaves of control plants; however, this difference was not statistically significant. The total sugars were reduced at the highest schradan level (100 p.p.m), while starch was increased. The plants

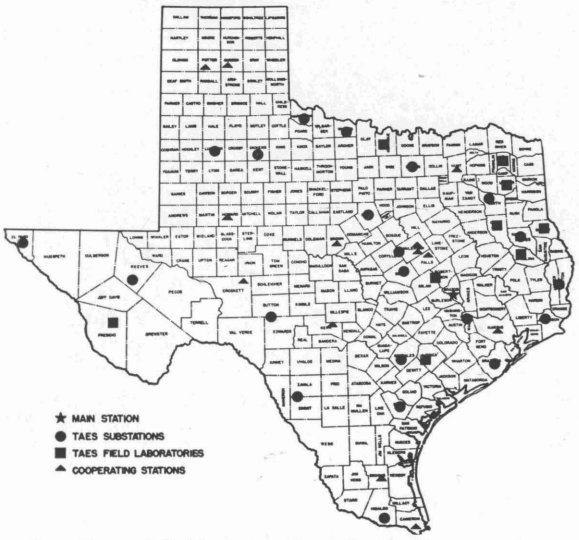
maintained on this level exhibited considerable toxicity and the growth of the plants was drastically reduced over that of other treatments. Additional work correlating respiration with carbohydrate content in the plant is required to interpret data of this nature adequately.

Soluble nitrogen accumulated in the leaves of plants grown on the two highest levels of schradan. The amount of nitrogen detected was more than that present in the schradan fraction, indicating that additional soluble forms from cellular synthesis were accumulating. Protein nitrogen, however, was not affected.

LITERATURE CITED

1. Bond, J. A. B. Trunk absorption of a systemic chemical. Bull. Ent. Res. 44: 97-99. 1953.
2. Casida, J. E., Allen, T. C. and Stahmann, M. A. Reaction of certain octamethylpyrophosphoramide derivatives with chymotrypsin. J.A.C.S. 74: 5548. 1952.
3. Casida, J. E., Chapman, R. K. and Allen, T. C. Relation of absorption and metabolism of OMPA by pea plants to available phosphorus. Jour. Econ. Ent. 45: 568-578. 1952.
4. Casida, J. E. and Stahmann, M. A. Metabolism and mode of action of schradan. Agri. and Food Chem. 1: 883-888. 1953.
5. Casida, J. E., Chapman, R. K., Stahmann, M. A. and Allen, T. C. Metabolism of schradan by plants and insects to a toxic phosphoramide oxide. Jour. Econ. Ent. 47: 64-71. 1954.
6. Craighead, F. C. and St. George, R. A. Experimental work with the introduction of chemicals in the sap stream of trees for the control of insects. Jour. Forestry 36: 26-34. 1938.
7. David, W. A. L. Insecticidal action studies with bis-dimethylamino-phosphonous anhydride containing 32 phosphorus. Ann. Appl. Biol. 38: 508-524. 1951.
8. DuBois, K. P., Doull, J. and Coon, J. M. Studies on the toxicity and pharmacological action of octamethylphosphoramide (OMPA, Pestox III). Jour. Pharmacol. Expt. Therap. 99: 376-393. 1950.
9. Eaton, F. M. and Rigler, N. E. Effect of light intensity, nitrogen supply, and fruiting on carbohydrate utilization by the cotton plant. Plant Physiol. 20: 380-411. 1945.
10. Eaton, F. M. Influence of growth "hormones" on boll retention by cotton plants. Bot. Gaz. 111: 313-316. 1950.
11. Guthrie, J. D., Hoffpauir, C. L., Steiner, E. T. and Stansbury, M. F. Survey of the chemical composition of cotton fibers, cottonseed, peanuts, and sweet potatoes. U.S.D.A., Agri. Res. Adm., Bureau of Agri. and Ind. Chem. 1944.
12. Hall, S. A., Stohlman, J. W. III and Schechter, M. S. Colorimetric determinations of octamethylpyrophosphoramide. Anal. Chem. 23: 1866-1868. 1951.
13. Hall, Wayne C. Morphological and physiological responses of carnation and tomato to organic phosphorus insecticides and inorganic soil phosphorus. Plant Physiol. 26: 502-524. 1951.
14. Hall, W.C. An outline of methods and procedures commonly used in plant physiological research. Texas A. and M. College System. Mimeo. 1954.
15. Hartley, G. S. Organo-phosphorus insecticides. The behaviour of schradan in the plant. Chem. and Ind. pp. 529-532. May, 1954.
16. Hatcher, John T. and Wilcox, L. V. Colorimetric determination of boron using carmins. Anal. Chem. 22: 567-569. 1950.
17. Heath, D. F., Lane, D. W. and Llewellyn, M. Studies on commercial octamethylpyrophosphoramide. "Octamethylpyrophosphoramide in the living plant." Jour. Sci. Food and Agri. 2: 60-73. 1952.
18. Ivy, E. E., Iglinski, W. Jr. and Rainwater, C. F. Translocation of octamethyl pyrophosphoramide by the cotton plant and toxicity of treated plants to cotton insects. Jour. Econ. Ent. 43: 620-626. 1950.
19. Ivy, E. E. Control of insects and spider mites by translocated compounds. Ph. D. Dissertation. Texas A. and M. College. 1951.
20. Ivy, E. E. Chemical characteristics of phosphorus compounds to kill aphids and spider mites by systemic action. Agri. Chem. 137: 47-51. 1953.
21. Jeppson, L. R., Josser, M. J. and Comolin, J. O. Tree-trunk application as a possible method of using systemic insecticides on citrus. Jour. Econ. Ent. 45: 669-671. 1952.
22. Loomis, W. E. and Schull, C. A. Methods in plant physiology. 1st Ed. McGraw-Hill Book Co., Inc., New York and London. 1937.
23. Metcalf, R. L. and March, R. B. Studies of the mode of action of parathion and its derivatives and their toxicity to insects. Jour. Econ. Ent. 42: 721-728. 1949.
24. Metcalf, R. L. and March, R. B. Behavior of octamethyl pyrophosphoramide in citrus plants. Jour. Econ. Ent. 45: 988-997. 1953.
25. Metcalf, R. L. Radiotracers in study of systemic insecticides. Agri. Chem. 9: 33-35. 1954.
26. Mitchell, J. W., Dugger, Jr., W. M., and Gauch, H. G. Increased translocation of plant-growth modifying substances due to application of boron. Science 118: 354-355. 1953.
27. Neiswander, C. R. and Norris, V. H. Introduction of selenium into plant tissue as a toxicant for insects and mites. Jour. Econ. Ent. 33: 517-525. 1940.
28. O'Brien, R. D. and Spencer, E. Y. Metabolism of octamethylpyro-phosphoramide by insects. Agri. Food Chem. 1: 946-950. 1953.
29. Oesper, P. Sources of the high energy content in energy-rich phosphates. Arch. Biochem. 27: 255-270. 1950.
30. de Pietri-Tonelli, Pietro and March, R. B. Relation of the activation of schradan in plant tissues to its toxicity to insects and mites. Jour. Econ. Ent. 47: 902-908. 1954.
31. Reynolds, H. T. Entomological aspects of systemic pesticides. Agri. Chem. 9: 28-31. 1954.
32. Ripper, W. E. Systemic insecticides. Paper read to the Third International Congress of Crop Protection on Sept. 17, 1952 at Sorbonne, Paris. Pest Control Ltd., Cambridge, England.
33. Schrader, G. The development of systemic insecticides based on organic phosphorus compounds. Hofchen-Briefe, Bayer Pflanzen-Schutz-Nachrichten. (English edition) 4: 161-170. 1952.
34. Shertz, F. M. The quantitative determination of chlorophyll. Plant Physiol. 3: 323-334. 1928.
35. Shertz, F. M. The extraction and separation of chlorophyll (a and b), carotin and xanthophyll, preliminary to their quantitative determination. Plant Physiol. 3: 211-216. 1928.
36. Tsi, Chao-Seng. Protection against aphids by seed treatment. Nature 166: 909-910. 1950.
37. Wedding, R. T. and Metcalf, R. L. Translocation of radioactive octamethyl pyrophosphoramide in black valentine bean plants. Bot. Gaz. 114: 180-189. 1952.
38. Wedding, R. T. Plant physiological aspects of the use of systemic insecticides. Agri. and Food Chem. 1: 832-835. 1953.
39. Willard, H. H., Merritt, L. L. and Dean, J. A. Instrumental methods of analysis. 2nd Ed. D. Von Nostrand Co., Inc., Toronto, New York, and London. 1951.
40. Wolfenbarger, D. O. Nutritional value of phosphatic insecticides. Jour. Econ. Ent. 41: 818-819. 1948.
41. Zeid, M. M. I. and Cutkomp, L. K. Effects associated with toxicity and plant translocation of three phosphate insecticides. Jour. Econ. Ent. 44: 898-905. 1951.

[Blank Page in Original Bulletin]



Location of field research units in Texas maintained by the Texas Agricultural Experiment Station and cooperating agencies

State-wide Research

★

The Texas Agricultural Experiment Station is the public agricultural research agency of the State of Texas, and is one of nine parts of the Texas A&M College System

IN THE MAIN STATION, with headquarters at College Station, are 16 subject-matter departments, 2 service departments, 3 regulatory services and the administrative staff. Located out in the major agricultural areas of Texas are 21 substations and 9 field laboratories. In addition, there are 14 cooperating stations owned by other agencies, including the Texas Forest Service, the Game and Fish Commission of Texas, the U. S. Department of Agriculture, University of Texas, Texas Technological College and the King Ranch. Some experiments are conducted on farms and ranches and in rural homes.

RESEARCH BY THE TEXAS STATION is organized by programs and projects. A program of research represents a coordinated effort to solve the many problems relating to a common objective or situation. A research project represents the procedures for attacking a specific problem within a program.

THE TEXAS STATION is conducting about 350 active research projects, grouped in 25 programs which include all phases of agriculture in Texas. Among these are: conservation and improvement of soils; conservation and use of water in agriculture; grasses and legumes for pastures, ranges, hay, conservation and improvement of soils; grain crops; cotton and other fiber crops; vegetable crops; citrus and other subtropical fruits; fruits and nuts; oil seed crops—other than cotton; ornamental plants—including turf; brush and weeds; insects; plant diseases; beef cattle; dairy cattle; sheep and goats; swine; chickens and turkeys; animal diseases and parasites; fish and game on farms and ranches; farm and ranch engineering; farm and ranch business; marketing agricultural products; rural home economics; and rural agricultural economics. Two additional programs are maintenance and upkeep, and central services.

RESEARCH RESULTS are carried to Texas farm and ranch owners and homemakers by specialists and county agents of the Texas Agricultural Extension Service.

Texas Agricultural Experiment Station, R. D. Lewis, Director, College Station, Texas