MORPHOLOGICAL AND PHYSIOLOGICAL RESPONSES OF CARNATION AND TOMATO TO ORGANIC PHOSPHORUS INSECTICIDES AND INORGANIC SOIL PHOSPHORUS [1]

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(WITH FOUR FIGURES)

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During the last few years considerable attention has been focused upon the use of the organic phosphorus compounds hexaethyl tetraphosphate and tetraethyl pyrophosphate as the active components of certain commercial insecticides. These substances are known to be toxic not only to insects but to other organisms in general as well. As the names of hexaethyl tetraphosphate and tetraethyl pyrophosphate are generally abbreviated HETP and TEPP, respectively, they will be referred to as such in this paper. HALL and JACOBSEN (**11**) concluded that HETP does not possess a definite structure by demonstrating that it is essentially a mixture of esters containing ethyl metaphosphate, triethyl orthophosphate, and tetraethyl pyrophosphate. These workers believe that the biological potency of the so-called HETP is actually due to the TEPP that it contains, although in a later publication, HALL (**10**) implied that HETP contains other toxic principles in addition to TEPP.

These compounds become of interest to the plant scientist through the formative effects that they elicit in particular sensitive species and this paper reports some of the fundamental effects that these compounds, or their degradation products, have on plant metabolism. The formative effects produced in plants by these compounds so strikingly resemble, in most cases, those of the hormone-type phytocides that a number of workers have attributed the symptoms to 2,4-D contamination of either the insecticides or spray equipment. Carefully controlled experiments have demonstrated, however, that these characteristic symptoms can be initiated consistently by HETP and TEPP in carnation, tomato, and sunflower. This has been the case generally when slightly higher concentrations than recommended for insect control were employed. Other reports (**24, 26, 27, 34**) have appeared confirming this result for the same and other species. SMITH *et al.* (**26, 27**) have noted that tomatoes, roses, chrysanthemums and carnations are susceptible to HETP and TEPP and they cite many grower reports of formative effects appearing in greenhouse ornamentals treated with these compounds. ZIMMERMAN and HARTZELL (**34**) have noted that epinasty was produced in more than 20 species when exposed to HETP and TEPP. The report of MCILRATH (**24**), as well as many Texas grower reports of 2,4-D-

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like symptoms in their cotton fields following the use of organic phosphorus insecticide sprays, adds cotton to the list of susceptible species.

Although carnation was used in the early studies with HETP, tomato was employed in most of the experimental work presented in this paper because of its unusual sensitivity to both HETP and TEPP. The similarity between the formative effects produced by the organic phosphorus compounds and the 2,4-D-type hormone suggests a common mode of action in plant metabolism and indicates a possible explanation of the biological link interrelating physiological function of hormones to the morphological growth response that they effect.

Preliminary experiments with carnation

BACKGROUND OF THE PROBLEM.—The symptoms, subsequently found to be caused by HETP, were first observed in 1948 in a commercial greenhouse at Lexington, Kentucky.

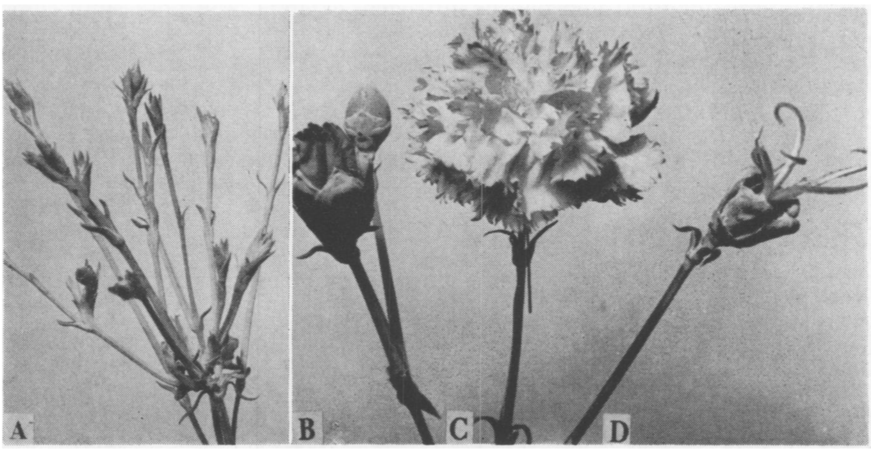


FIG. 1. Aberrated and normal carnation flowers. Left to right: A. Distorted, branched, flowering shoot. B. Normal bud. C. Normal flower. D. Distorted over-developed flower and lack of petals.

The abnormalities were confined to the flower or flowering shoot and consisted primarily of two general distortion patterns. In one case (fig. 1 A) the flowering stalk became repeatedly branched so that the flowers were borne in a cluster. Each flowering stalk developed numerous, as many as 10 to 15, small commercially inferior flowers. The other extreme (fig. 1D) was characterized by having the stamens and pistil excessively over-developed and protruding from the calyx tube one and a half inches or more. The sepals were unusually large with a thick leathery appearance, whereas, the petals did not differentiate or in some cases appeared to have differentiated into stamens. The ovary was also prominent and contained mature seeds long before the vegetative parts of the flower had developed or emerged. The contrast with the arrangement and the developmental pattern of a normal bud and flower (fig. 1 B, C) is brought out by the photograph and by reference to Bailey's treatment of the carnation (**2**).

Investigation revealed that the carnation benches had been fertilized yearly and received especially heavy applications of superphosphate. The grower also reported that the carnation houses had been sprayed twice with a commercial insecticide formulation containing HETP. The last spraying occurred about the time the floral buds were differentiating and appearing.

OBSERVATIONS AND RESULTS.—Several soil samples from each bench were collected, their pH measured electrometrically and the soluble mineral content determined colorimetrically or turbidimetrically for the elements calcium, phosphorus, nitrogen, and potassium by the methods of WOLF and ICHISAKA (**33**). Triplicate representative samples of shoots of normal and distorted plants were collected, their fresh and dry weights determined, and the dried material ground to pass an 80-mesh screen. Samples were then wet ashed with sulphuric acid–salicylic acid and 30% hydrogen peroxide and analyzed for total phosphorus, nitrogen, calcium, and potassium colorimetrically (**33**).

TABLE I

FRESH AND DRY WEIGHT OF CARNATION SHOOTS IN GRAMS, PER PLANT, PERCENTAGE DRY WEIGHT, TOTAL P, TOTAL N, TOTAL K, TOTAL Ca AS PERCENTAGE OF DRY WEIGHT PRODUCTION.

Sample fractions	Normal flowering control	Distorted flower, over-developed type	Distorted, branched, flowering-shoot type
Fresh weight	39.00	34.00	30.00
Dry weight	6.36	6.29	5.79
Percentage dry weight	16.30	18.50	19.30
Total P	0.31	0.46	0.35
Total N	3.01	1.55	1.42
Total K	2.73	4.23	2.26
Total Ca	0.40	0.56	0.41

In all cases the soil reaction of the benches was close to neutrality or slightly acid. Invariably the lower pH values of the soil were correlated with the highest amounts of soluble phosphorus and the frequency and degree of the flowering distortions (fig. 1). The amounts of soluble calcium, nitrogen, and potassium in the bench soil were found to conform to the recommended levels for optimum greenhouse culture of carnations.

It was noted (table I) that the distorted plants had a slightly higher total phosphorus content than the normal flowering plants. The concentration of calcium in the plants in general followed the phosphorus trend as shown by a gradual increase with increasing phosphorus. As has been noted previously (**4, 33**), the phosphorus and nitrogen fractions displayed a reciprocal relationship in that as phosphorus increased the total nitrogen decreased (table I). The level of potassium varied considerably among the three samples but tended to increase somewhat proportionately with phosphorus particularly in the high phosphorus plants (table I, fig. 1 D). The normal flowering controls produced a greater fresh and dry weight than did the distorted plants (table I). The percentage dry weight, as well as observation at the time of sampling, indicated that the abnormal plants were more woody than the normal flowering plants.

REPRODUCTION OF THE SYMPTOMS BY EXPERIMENTATION.—The preliminary observations and analyses (table I) indicated that the factors most likely causing the symptoms, either singly or collectively, were the high soil phosphorus or HETP spray. Therefore formal experiments were performed to determine the role of these factors in inducing the symptoms.

Carnation plants were propagated from cuttings taken from mature healthy plants grown in a commercial greenhouse at Lexington, Kentucky. When vigorously rooted, 20 plants each of the varieties Ovilette, Netta, and Northland were transplanted to 3-inch pots containing rich greenhouse soil. One month later the plants were transplanted to 8-inch pots, in which they were grown to maturity. At the second transplanting each variety was divided into four experimental lots of five plants each, making a total of 15 plants per treatment. Lot 1 was retained in ordinary greenhouse soil containing 175 p.p.m. of soluble P. Lot 2 was grown in soil composed of 10 parts of ordinary greenhouse soil (lot 1 soil) to 1 part of 20% superphosphate. Lot 3 received the same cultural treatment as lot 1 except they were sprayed with aqueous HETP at the preflowerbud stage. Lot 4 was identical to lot 2 except they also received the HETP spray treatment concurrently with lot 3. During and after the spray application lots 3 and 4 were segregated from lots 1 and 2 in the greenhouse. The HETP was applied to runoff with a hand atomizer sprayer at the rate of 600 p.p.m. in water. Two milliliters of Grasselli spreader was added to each liter of HETP solution to facilitate absorption. The plants were then left under normal greenhouse conditions and observed for a 2-months period. By that time neither the control plants nor the high phosphorus plants without spray (lots 1 and 2) had displayed any formative responses. Essentially the same distorted patterns shown in figure 1 were produced, however, by the HETP spray in lots 3 and 4, and the frequency and degree of the symptoms was approximately the same in either treatment. It is believed that the branched flowering-stalk pattern (fig. 1 A) was the result of excessive accumulation of HETP between the leaves and buds of the growing point, when applied at the early stages of flower development, and from which the abnormal growth developed. The overdeveloped floral pattern (fig. 1 D) no doubt resulted from HETP when applied to more mature flower buds later in their reproductive cycle.

Experiments with tomato

METHODS AND PROCEDURE

EXPERIMENTAL PLAN.—The investigation was designed to be conducted in four phases as follows: (1) To study and record the formative, structural and compositional effects of TEPP on tomato over a fairly wide range of concentrations, applied both as an aqueous spray to the foliage and when added as a water solution to the soil (800 p.p.m.). (2) To determine the effects of high phosphorus soil fertilization on the growth and composition of tomato and to determine if extremely high soil applications of inorganic phosphorus would induce formative effects. (3) To determine the effect and possible interrelation of soil applications of inorganic phosphorus (phase 2) and TEPP on plant composition and respiration 48 hours after application. (4) To study and compare the active fractions in technical TEPP (40%) and relatively pure TEPP (98.1%) and to elucidate, if possible, the basic mechanism of their responses in plants.

Preliminary work disclosed that the range of 150 to 4,000 p.p.m. of TEPP covered all the practical degrees of response in tomato. The response produced was greatly modified, however, by the time of year. The above range of concentration was employed, using 40% technical TEPP, to achieve the objectives of phases 1, 2, and 3 of the study. The aqueous spray was applied with a hand atomizer sprayer until the foliage was wet. On the average wetting the foliage required about 5 ml. of solution for young plants and about 10 ml. for mature plants. Grasselli's spreader (tested and proven to be inactive in inducing the TEPP response) was added to the spray to facilitate absorption. In the fourth phase relatively pure TEPP at 400 p.p.m. was used, while in the respiratory measurements 400 and 800 p.p.m. concentrations were employed.

CULTURE OF PLANTS.—Certified seeds of tomato, *Lycopersicon esculentum* Mill, var. Marglobe, were germinated in trays of vermiculite under greenhouse conditions. When about two to three inches in height the seedlings were transplanted to 8-inch pots and grown to the desired experimental stage in a soil compost mixture of Houston black clay, Lufkin fine sandy loam, decomposed manure, and oat hulls. For the first phase of the work young plants were sprayed at the macroscopic flower bud stage. To minimize seasonal variations in response, the TEPP was applied during the months of December, February, and June and the data for this phase is the average total of 15 plants per treatment.

To determine the effects of extremely high levels of inorganic phosphorus nutrition, singly and in combination with spray applications of TEPP at 400 p.p.m., plants were cultured in a mixture of the above soil compost and 20% P_2O_5 in the ratios of 40:1, 20:1, and 10:1 (phases 2 and 3). As a matter of convenience these levels are designated as low phosphorus, medium phosphorus, and high phosphorus. Twelve plants were grown at each level of inorganic phosphorus, four from each level were sampled at the first flower cluster stage and four were harvested at the mature fruit stage. The remaining four plants in each treatment were employed for studying the combined effects of TEPP spray and inorganic phosphorus nutrition (phase 3) and were sprayed at the mature fruit stage and harvested 48 hours after spray application. Eight control plants were cultured in the soil compost without added phosphorus, four being sampled at the first flower cluster stage and four at the mature fruit stage. The unsprayed plants of the three phosphorus levels at the second sampling served also as the controls for the 48-hour TEPP treatment of phase 3. Leaves used in the respiratory measurements were taken from healthy young vegetative plants grown in the compost mixture.

In the fourth phase, both young vegetative and mature plants grown in the soil compost were employed.

SAMPLING, ANALYTICAL, RESPIRATORY, AND ETHYLENE DETECTION METHODS.— Except for the experiment of the first phase to determine the effect on flowering (which ran for 90 days) and the 48-hour experiments the plants were sampled one month after TEPP application. Entire shoots of the plants of the first phase were harvested, all plants of the same treatment combined, and fresh weights determined. The composite samples were then dried in a forced draft oven at 80° C. The samples of the second and third phases were composed only of leaves. After drying to constant weight, dry weights were determined gravimetrically and all samples ground to pass an 80-mesh screen.

The analytical methods used for the labile carbohydrate determination are given in detail by EATON and RIGLER (**6**). Briefly, the sugars were extracted from the oven-dried samples in a Soxhlet apparatus with 80% ethanol and determined by the semi-micro method of WILDMAN and HANSEN (**32**). Sucrose was determined by hydrolyzing an aliquot of the extracted sample with concentrated HCl and computing in the usual manner. Starch contained in the Soxhlet residue (from the sugar extraction) was determined by combined diastatic and acid hydrolysis. The starch-free residue was hydrolyzed by autoclaving with HCl and the reducing values determined expressed as hydrolyzable residue.

Samples for total nitrogen, phosphorus, potassium, and calcium analyses were digested with combined sulphuric-salicylic acid and 30% hydrogen peroxide and their respective values determined colorimetrically (**33**) in a Fisher electrophotometer. Soluble phosphorus was extracted from oven-dried material with 70% ethanol and the extract analyzed colorimetrically for this fraction by the above method. Residual, or 70% alcohol-insoluble phosphorus, was calculated as the difference between total and soluble phosphorus. Determinations for potassium and calcium were checked in a Beckman D. U. Spectrophotometer and found to be in close agreement with the measurements made by the Fisher electrophotometer.

The respiratory rate of tomato leaves was measured, before and after spraying with TEPP, in a standard Warburg apparatus by the modified Warburg respirometer leaf-disc method reported by KLINKER (**18**). Oxygen uptake was measured with two-tenth ml. of 20% KOH in the center well to absorb carbon dioxide. Carbon dioxide was determined by the "direct" method of WARBURG (**29**). All measurements were made in a darkened room at 28° C and with a shaker rate of 120 strokes per minute. The equipment was allowed to equilibrate 15 minutes before starting the measurements. The data presented are the averages of six replications all in close statistical agreement. The plants were sprayed with TEPP two hours prior to. cutting the leaf discs used in the Warburg measurements.

To detect ethylene evolution the " triple response " of etiolated Alaska pea seedlings was employed. This variety is known to respond to as little as 0.05 p.p.m. of ethylene in air. The seed was germinated and cultured according to the technique of PRATT and BIALE (**25**). When about 4 cm. high, one Petri dish each of the seedlings was enclosed individually in sealed desiccators with young tomato plants previously sprayed with 400 and 800 p.p.m. TEPP. To test the possibility that TEPP gives rise to ethylene by degradation, a beaker containing 400 p.p.m. of TEPP in water was placed in another desiccator with the etiolated seedlings. In addition, controls of two types were used, unsprayed tomato plants with the etiolated seedlings and the etiolated seedlings alone.

To determine if the effects of adenosine triphosphate were comparable to TEPP, eight young pre-flowering tomato plants were sprayed to run-off with 250 p.p.m. of the Di Barium salt. Eight comparable plants received the same amount of ATP except by soil application and eight untreated plants served as controls.

Results, phase 1

GENERAL OBSERVATIONS

Plant symptoms and formative effects produced in tomato by TEPP (or its degradation products) can be roughly classified into three types: lethal, inhibitive, and stimulatory. In most cases, concentrations above 2000 p.p.m. were lethal; 800 to 2000 p.p.m. generally produced temporary inhibition, however, in time stimulation of growth resulted. This fact is in agreement with the known effects of growth regulators of the auxin type. The third type of almost immediate stimulation was produced by concentrations from 100 to 400 p.p.m. Further dilution was essentially without effect. The demarcation between these arbitrary types and TEPP concentration was not sharp, but they often merged into one another, and were subject to a factor which at present is called the " seasonal " effect (different responses produced by the same concentrations according to the season of the year applied). This seasonal effect has been noted by others (**24, 26, 27**). Immediate responses photographed five days after application are shown for the various concentrations (fig. 2).

Plants became severely wilted within two hours after application of solutions above 2000 p.p.m., the foliage dried to a gray-brown color and appeared dead within 24 hours after spraying. Some of the first recognizable symptoms were epinasty, almost immediate chlorosis of foliage (fig. 3 B), and wilting and drying of the growing point. The long term effects of TEPP on leaves, stems, and reproductive organs, which markedly resemble those induced by 2,4-D, are shown in figures 3 and 4. One difference, however, seems to be a greater effect of TEPP on the terminal meristem. In most cases, concentrations of 800 p.p.m. or above produced either death or complete inhibition of the terminal growing point. This resulted in the breaking of apical dominance and the forcing of the lateral branches (compare fig. 3 F and fig. 4 A, B, C). This TEPP response in tomato is somewhat similar to that reported for maleic hydrazide (**9**). In accord with

FIG. 2. Effects of TEPP at various concentrations on tomato five days after application. A. Control. B. 2000 p.p.m.—lethal dosage. C. 800 p.p.m.—inhibitive range (note effect on terminal meristem and young branches). D. 400 p.p.m.—stimulatory range. E. 300 p.p.m. (note effect on growing point). F. 200 p.p.m. (arrow points to twisted, distorted growing point).

SMITH *et al.* (**26, 27**), it was also noted that young plants were much more severely affected by TEPP than mature plants.

EFFECTS ON GROWTH AND METABOLISM

STEM ELONGATION.—Young tomato plants at the flower-bud stage of development were selected for uniformity of size and the stem heights measured just prior to spray application of TEPP and again one month later (table II). The data showed that 400 p.p.m. marked the dividing line between stimulation and inhibition of stem elongation.

FLOWERING.—An experiment extending over a period of 90 days was performed to check for stimulatory effects on flowering (table II). The results indicate that days to flowering was shortened by the concentrations at and below 400 p.p.m. SMITH *et al.* (**26, 27**) observed that roses showed increases as high as 30% in flower production following an aerosol HETP application in the summer months. The effects of TEPP on flowering apparently deserve more careful investigation and further work is contemplated.

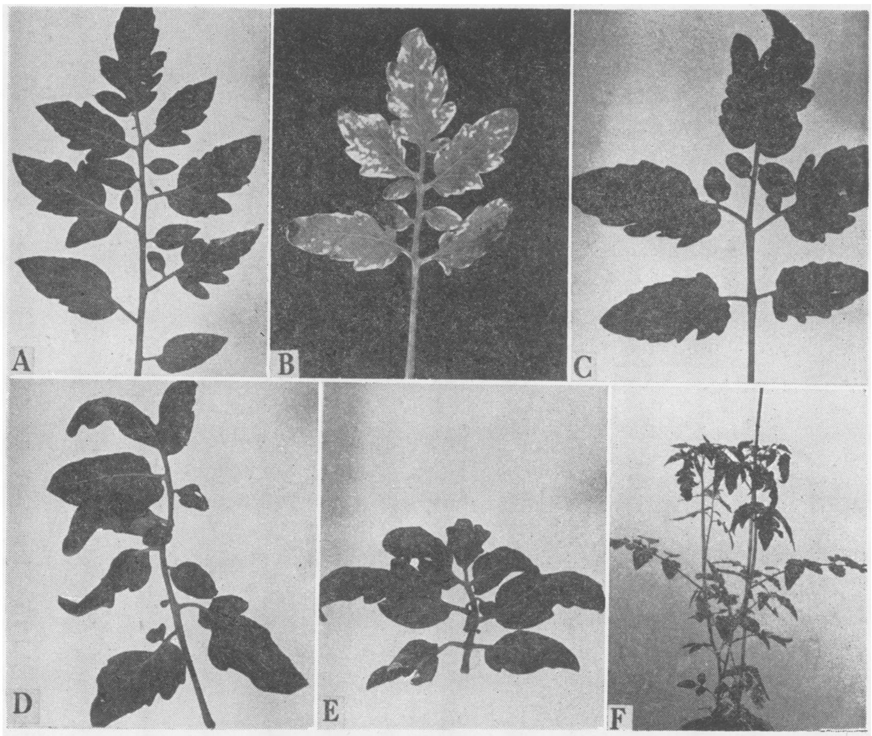


FIG. 3. Long term TEPP effects on foliage of tomato, morphological habit, and similarity of 2,4-D symptoms. A. Control leaf. B. Treated leaf showing mottled chlorosis. C. Slight formative effect appearing at 200 p.p.m. concentration. D. Increasing severity of foliage symptoms with increasing TEPP concentration (800 p.p.m.). E. Extreme leaf formative effect induced at 2000 p.p.m. F. Effect of TEPP on plant habit, resulting in breaking of apical dominance and forcing of laterals to produce twin-shoot type.

WEIGHT PRODUCTION.—The data (table II) show no evidence of increased dry weight from the application of TEPP. The general decrease in the dry weight of the sprayed plants suggests either accelerated plant catabolism or decreased anabolism by TEPP, at least over a 30-day period.

CARBOHYDRATE COMPOSITION.—Carbohydrate analyses (table III) disclosed that, in general, the treated plants at the higher concentrations decreased in carbohydrate content. The large decrease in sucrose and reducing sugars at 2000 p.p.m. suggests statistical significance. In most cases, TEPP caused accelerated hydrolysis of reserve carbohydrates to the soluble forms and apparently increased respiration. For this reason, and because of the results of the growth measurements, the Warburg measurements were made. These are presented in a later section.

ELEMENTAL COMPOSITION.—Analyses showed that total phosphorus increased in sprayed plants somewhat in proportion to TEPP concentrations (table III). Additional fresh samples were washed in running tap water for

FIG. 4. Effects of TEPP and active fractions on vegetative and reproductive structures of tomato. A. Control plant. B. Branching induced by TEPP, arrow points to approximate height at time of application and slight swelling on the stem. C. TEPP-sprayed plant showing malformed, parthenocarpic fruit. D. Young plant self-rejuvenated from old plant killed by 4000 p.p.m. application and still manifesting slight formative effects. E. Mature plants sprayed with tetraethyl pyrophosphate (pure TEPP); diethyl hydrogen phosphate (DEHP) sprayed plant; and triethyl phosphate (TEP) treated plant. F. Young plant sprayed with DEHP and grown to maturity (note extreme 2,4-D-like symptoms).

three hours before oven drying but subsequent analyses revealed no difference between washed and unwashed samples indicating that there was no surface residue. The concentration of nitrogen increased in treated plants up to the 4000 p.p.m. level. Potassium increased only above 400 p.p.m. which was the limit of increased shoot height. Total calcium followed the phosphorus trend and increased throughout with increasing TEPP concentration. Except for converse results in the nitrogen trend the analyses of table III agree in general with those found for carnations (table I).

TABLE II

GROWTH AND FLOWERING RESPONSES OF GREENHOUSE, SOIL-GROWN TOMATO PLANTS OF PHASE 1. EFFECTS OF TEPP ON STEM ELONGATION AND WEIGHT PRODUCTION OF SHOOTS (30 DAYS AFTER APPLICATION) AND FLOWERING. EACH TREATMENT REPRESENTS THE AVERAGE OF 15 PLANTS.

TEPP treatment in p.p.m.	Stem elongation			Weight production			Flowering	
	Initial height	Final height	Change	Fresh wt. per plant	Dry wt. per plant	Per cent. dry wt.	Age at first flowers	Average fls. per plant to 90 days
	cm.	*cm.*	*cm.*	*gms.*	*gms.*	*%*	*days*	*no.*
Control	34	63	29	135.8	16.4	12.1	65	24
150	31	72	41	120.9	14.2	11.7	55	26
200	34	80	46	140.4	16.7	11.9	54	28
300	35	80	45	107.9	13.6	12.6	51	25
400	30	65	35	93.9	11.6	12.4	60	25
800	32	57	26	103.4	12.0	11.6	70	20
2000	31	50	19	54.3	6.4	11.8	82	12
4000	30	Lethal	Lethal	9.8	3.1	31.7	Lethal	Lethal
800 Soil	30	61	31	92.8	11.9	12.9	58	26

TABLE III

COMPOSITIONAL EFFECTS OF TEPP ON CARBOHYDRATE AND ELEMENTAL FRACTIONS OF GREENHOUSE SOIL GROWN TOMATO SHOOTS OF PHASE 1, 30 DAYS AFTER APPLICATION. EACH TREATMENT IS THE COMPOSITE OF 15 PLANTS.

TEPP treatment in p.p.m.	Carbohydrates					Elemental composition			
	Reducing sugars	Sucrose	Starch	Hydrolyzable residue	Total	Total N	Total P	Total K	Total Ca
Control	2.93	1.54	4.02	9.31	17.80	1.30	0.25	3.00	2.56
150	2.15	2.29	3.90	8.94	17.28	1.75	0.26	3.00	2.49
200	3.01	2.05	4.42	8.35	17.83	2.00	0.26	2.80	2.58
300	2.15	2.43	4.23	9.55	18.36	1.50	0.27	2.95	2.66
400	2.49	1.46	3.62	8.43	16.00	1.55	0.27	2.90	2.68
800	2.50	1.28	3.01	10.05	16.84	1.60	0.32	3.20	2.70
2000	0.09	0.04	2.91	9.03	12.07	1.65	0.38	3.50	2.77
4000*	2.58	0.02	6.46	8.56	17.62	0.95	0.40	3.40	2.56
800 Soil	2.45	1.86	4.03	9.51	17.85	1.40	0.25	2.85	2.50

* Lethal dosage but dead plants sampled.

Results, phase 2

General observations

Very little external differences were exhibited among the low 40:1, medium 20:1, and high 10:1 phosphorus plants, except perhaps for a slight increase in stem diameter with increasing phosphorus supply. All plants were extremely green and luxuriant and displayed no apparent phosphorus injury or formative effects. The controls were lighter green and much smaller in size than the low phosphate plants. The most abundant flowering occurred at the low (40:1) phosphorus level and all phosphate-treated plants flowered earlier than the controls.

Weight production.—The plants grown in the phosphorus-enriched soil produced considerably more fresh and dry matter than controls at both sampling dates (table IV). Dry weight increased proportionately with phosphorus fertilization at the first analysis, but the medium level plants had elaborated the most dry weight at the final sampling (table IV).

Carbohydrate composition.—At the first harvest, as phosphorus supply increased, all carbohydrate fractions decreased (table IV). The controls produced a greater total amount of carbohydrates than the high phosphorus plants, which were particularly low in reserve carbohydrates.

At the final harvest the medium 20:1 level plants elaborated significantly higher amounts of carbohydrates, but in the high phosphorus 10:1 plants, carbohydrates decreased sharply, although still above the controls. These trends in carbohydrate production are in general agreement with Eaton's work with sunflower and soybean (**7, 8**) but the levels of phosphorus employed in Eaton's work and the present study actually are too divergent for valid comparison of data.

Elemental composition.—Total phosphorus and nitrogen increased in plants somewhat proportionately with the level of phosphorus fertilization at the early analysis (table IV). Potassium, however, increased with increasing phosphorus application only up to the medium level. Calcium varied erratically and exhibited little correlation with any phosphorus levels. At the mature stage, analyses revealed that phosphorus still increased with added soil phosphorus but the percentage content of the other elements varied considerably, although the high phosphorus 10:1 plants in general contained the highest amounts (table IV).

Results, phase 3

Carbohydrate composition.—When TEPP was applied to the aerial organs of mature plants and the experiment terminated 48 hours after the spray treatment, the subsequent analysis (table V) gave support to the idea of accelerated hydrolysis and respiration suggested by the data of phase 1. At all levels the treated plants showed decreased carbohydrate reserves (hydrolysis) and increased soluble carbohydrates compared to untreated controls (table V). In addition to accelerating enzymatic hydrolysis the

TABLE IV

EFFECT OF INORGANIC PHOSPHORUS LEVEL ON WEIGHT PRODUCTION IN GRAMS, CARBOHYDRATES AND ELEMENTAL COMPOSITION AS PERCENTAGE OF DRY WEIGHT PRODUCTION OF TOMATO LEAVES OF PHASE 2 AT THE TWO DATES INDICATED. EACH TREATMENT REPRESENTS THE AVERAGE OF FOUR PLANTS.

Inorganic phosphorus level	Weight production		Carbohydrates					Elemental composition			
	Fresh wt. per plant	Dry wt. per plant	Reducing sugars	Sucrose	Starch	Hydrolyzable residue	Total	Total N	Total P	Total K	Total Ca
First sampling—first flower cluster stage											
Control	53.5	6.4	2.52	1.30	4.35	4.60	12.77	1.25	0.22	2.95	2.52
Low P	73.6	8.7	3.01	2.59	7.18	6.13	18.91	0.95	0.40	4.00	1.40
Medium P	94.4	11.3	2.93	2.09	5.67	5.47	16.16	2.10	0.42	4.50	1.95
High P	92.8	14.1	2.72	1.34	3.69	3.53	11.28	2.15	0.59	4.40	1.10
Second sampling—mature fruit stage											
Control	94.8	13.3	1.53	1.00	6.05	5.52	14.10	1.14	0.25	2.84	2.40
Low P	116.9	17.2	1.72	1.08	12.13	7.10	22.03	1.20	0.33	3.60	1.35
Medium P	119.0	17.3	3.94	1.63	12.77	8.18	26.52	1.10	0.45	3.25	1.50
High P	130.5	16.1	2.05	0.77	6.93	6.70	16.45	1.65	0.60	3.70	1.80

total amount of carbohydrates of the treated plants compared to their controls diminished about 1% at the high 10:1 phosphorus level and over 4.5% at the medium 20:1 phosphorus level. This would indicate either transformation to other plant constituents, translocation from the plant, or utilization as respiratory substrates.

Elemental composition.—Without exception total phosphorus increased in the treated plants compared to the controls and the amount of increase was more than could be accounted for by the TEPP spray (table V). This increase suggests that primary absorption was influenced. Furthermore, table V showed that phosphorolysis increased in all of the sprayed plants, at all phosphorus levels, when the total phosphorus content was fractionated into the soluble and insoluble forms. Total nitrogen decreased in sprayed plants of the low and high phosphorus supply levels but total potassium increased in all TEPP-treated variables (table V). Values for total calcium increased in treated plants of the low 40:1 and medium phosphorus nutrition levels, but decreased at the high 10:1 phosphorus application. Without exception the medium 20:1 phosphorus plants, sprayed with TEPP, exhibited an increase in all fractions compared to untreated controls (table V).

Respiratory measurements.—Young tomato plants were sprayed with TEPP at 400 and 800 p.p.m. concentrations. These concentrations were employed because growth effects and other measurements indicated that they marked the dividing line between the stimulatory and the inhibitory levels. The average value for oxygen uptake of the unsprayed controls (table VI) is slightly higher than that noted by Klinker (**18**) for six tomato varieties, but it is believed that he used older plants in his study and hence a slower rate of respiration. Oxygen utilization expressed as percentage of the controls definitely confirmed that the 800 p.p.m. concentration inhibited respiration and that the 400 p.p.m. level greatly stimulated oxygen uptake (table VI). Although respiratory measurements were not determined for the concentrations of TEPP used below 400 p.p.m., other evidence indicates that they would have had even a greater accelerating effect upon the respiratory rate. Carbon dioxide evolution followed the same trend as for oxygen uptake at these concentrations; whereas the ratio between the two (respiratory quotient) suggests some interesting features regarding metabolism under the experimental circumstances and deserves further investigation.

Results, phase 4

Active fractions in TEPP.—All commercial formulations of TEPP contain active and inert ingredients. These are tetraethyl pyrophosphate, which is hydrolyzed by water, the rate depending upon temperature; triethyl orthophosphate which is stable and remains in the spray unchanged; ethyl methaphosphate and the higher ethyl polyphosphates which rapidly hydrolyze to diethyl hydrogen phosphate within a few minutes (**28**). Hall and Jacobson (**11**) present the following degradation scheme for pure TEPP:

TABLE V

RELATIONSHIP BETWEEN INORGANIC SOIL PHOSPHORUS LEVEL AND TEPP ON CARBOHYDRATES AND ELEMENTAL COMPOSITION OF TOMATO LEAVES OF PHASE 3 WHEN EXPRESSED AS PERCENTAGE OF THE DRY WEIGHT PRODUCTION. EACH TREATMENT REPRESENTS THE AVERAGE OF FOUR PLANTS SAMPLED 48 HOURS AFTER TEPP APPLICATION.

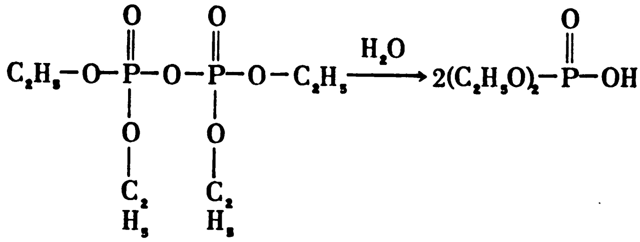
Inorganic phosphorus level	Carbohydrates					Elemental composition					
	Reducing sugars	Sucrose	Starch	Hydrolyzable residue	Total	Total N	Total K	Total Ca	Alcohol soluble P	Alcohol insoluble P	Total P
C*—Low P	1.72	1.08	12.13	7.10	22.03	1.20	3.60	1.35	0.16	0.17	0.33
T**—Low P	2.15	1.75	9.78	6.45	20.13	1.15	3.75	1.40	0.22	0.13	0.35
C—Medium P	3.94	1.63	12.77	8.18	26.52	1.10	3.25	1.50	0.15	0.30	0.45
T—Medium P	4.03	2.80	8.04	7.10	21.97	1.20	3.35	1.75	0.30	0.20	0.50
C—High P	2.05	0.77	6.93	6.70	16.45	1.65	3.70	1.80	0.30	0.30	0.60
T—High P	2.94	2.37	4.59	5.53	15.43	1.55	3.80	1.45	0.48	0.24	0.72

* Control.
** Treated.

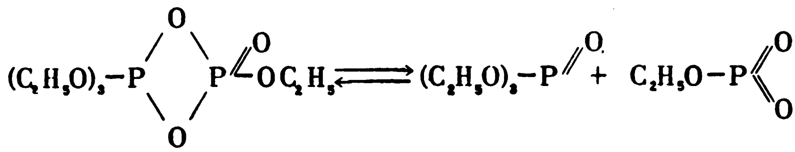
TABLE VI

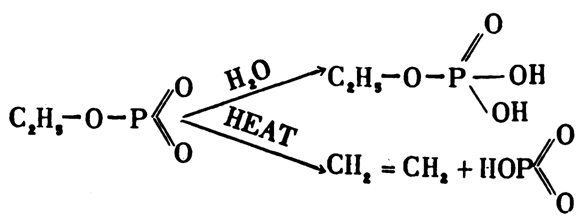
EFFECT OF TEPP ON GAS EXCHANGE IN TOMATO LEAF DISCS (O_2 UPTAKE AND CO_2 PRODUCTION). MEASUREMENTS MADE TWO HOURS AFTER SPRAY APPLICATION.

TEPP treatment in p.p.m.	Oxygen uptake—microliters/mg./hr.				Carbon dioxide output—microliters/mg./hr.				
	First hour	Second hour	Average	Per cent. control	First hour	Second hour	Average	Per cent. control	R. Q.
Control	3.13	3.00	3.06	....	2.98	3.16	3.07	....	1.00
800	2.72	2.00	2.36	77.12	2.51	2.95	2.73	88.95	1.19
400	4.78	5.15	4.97	162.41	3.49	3.06	3.28	106.84	0.66



and also show the scheme of BALAREV (**3**) who believed that TEPP had the structure and underwent degradation as listed below:

1. 

2. 

An experiment was set up to test some of these fractions in which young and mature tomato plants were sprayed with relatively pure TEPP at 4000 p.p.m., triethyl orthophosphate at 4000 p.p.m. and diethyl hydrogen phosphate. The diethyl hydrogen phosphate was obtained by hydrolyzing pure TEPP with 20 times its volume of water for one week at 30° C.

Within two hours or less the TEPP-sprayed plants were manifesting a number of effects: severe epinasty was apparent, and leaves had become chlorotic, and the growing points were twisted and dried. In four hours' time the young plants appeared to be dead while the old plants were exhibiting severe symptoms. At the end of 24 hours, all TEPP-treated plants were dead. The plants treated with the diethyl-hydrogen-phosphate fraction were not immediately affected except to show slight epinasty and the usual growing point symptoms. These effects, at the time, were thought to be due to incomplete hydrolysis of the original TEPP. Observations of the young DEHP-sprayed plants to maturity (fig. 4 F), however, showed that this was not the entire explanation, for these plants displayed the severe formative symptoms, typical of 2,4-D injury, over a longer period of time. The triethyl phosphate-treated series exhibited little effect except burning and chlorosis of the foliage. The responses of the mature plants of this experiment are illustrated in figure 4 E.

COMBINED EFFECTS OF TEPP AND 2,4-D.—To determine if the effects of

TEPP and 2,4-D were additive, four lots of young vegetative tomato plants consisting of four plants to each lot were sprayed with 800 p.p.m. TEPP, 10 p.p.m. 2,4-D, 10 p.p.m. 2,4-D plus 800 p.p.m. TEPP, and 100 p.p.m. 2,4-D plus 800 p.p.m. TEPP, respectively. Within two hours after application, the plants treated with 10 p.p.m. 2,4-D alone were only slightly epinastic, whereas in the plants receiving 800 p.p.m. TEPP alone as well as in both combined levels of 2,4-D plus TEPP treated plants, the usual epinastic and mottled chloroses were apparent. Five days later the 10 p.p.m. 2,4-D plants had mostly recovered from the earlier symptoms but in the 800 p.p.m. TEPP plants death of the growing points was noted. The combined 10 p.p.m. 2,4-D and 800 p.p.m. TEPP-treated plants externally appeared to be dead. The 100 p.p.m. 2,4-D plus 800 p.p.m. TEPP-treated plants were definitely dead and extremely malformed and twisted.

TESTS FOR ETHYLENE EVOLUTION.—Enclosure of the TEPP-sprayed plants in desiccators with etiolated pea seedlings up to 72 hours resulted in some indication that ethylene was produced. Although not conclusive, it is possible that some of the ethylene inhibition symptoms exhibited by the seedlings when enclosed with the sprayed plants were due to normal metabolism of plants and not to TEPP degradation. However, inhibition of the seedlings enclosed with the unsprayed tomato plants was much less severe than it was when the seedlings were enclosed with TEPP-sprayed plants. The treated tomato plants themselves, however, were severely epinastic, chlorotic, and apparently dead at the higher concentration of TEPP. The pea seedlings confined with a hydrolyzing sample of TEPP were comparable to the controls and confirms that ethylene is not a direct hydrolytic product to TEPP.

RESULTS WITH ATP.—Thirty days after the young tomato plants had been sprayed with adenosine triphosphate only slight indications of formative effects were apparent. These symptoms when present largely resembled those elicited by 200 p.p.m. TEPP (fig. 3 C). Some burning and chlorosis of foliage also followed spraying. Compared to the controls the ATP-sprayed plants were slightly stimulated in growth as shown by somewhat heavier foliage and greater shoot length. The plants receiving the soil application of ATP however were essentially the same as the controls.

Discussion

The fact that all degrees of formative response, ranging from stimulation to complete inhibition and death was obtained, depending upon the concentration of TEPP employed, indicated that TEPP or its degradation products possessed definite growth-regulating properties. The data for stem elongation and flowering (table II) showed that approximately the same concentrations of TEPP stimulated or inhibited these divergent processes. This is not surprising as LEOPOLD and THIMANN (**21**) have shown that concentrations of indoleacetic acid that favored stem growth also favored flowering. SMITH *et al.* (**26, 27**) have reported that in roses increased vigor, as shown by a deeper green color, larger leaves, longer and heavier stems, more and larger flowers, followed the use of HETP. Their data showed that the average stem length of cut roses increased three to six inches and flower production by 10 to 30% compared to untreated plants and led these workers to suggest that stimulatory action, beyond that explained by the elimination of spider mites, resulted from the use of HETP.

The lowered dry weight and carbohydrate content of the TEPP-treated plants, in addition to accelerated hydrolysis (or phosphorolysis) revealed that the primary effects of TEPP on plant metabolism was the promotion of enzymatic and respiratory action at the stimulatory concentrations but inhibition of these processes at the retarding levels. The stimulation and inhibition effects of TEPP on respiration were further confirmed by the Warburg determinations (table VI). Therefore, it would seem that the most logical explanation of the mode of action of TEPP would be found in its effects on the enzymatic system of the plant. HALL (**10**) implied that the toxicity of these compounds on animals and insects was manifested through the inhibition of the acetyl choline esterase system. ADLER *et al.* (**1**) believed that in animals the addition of pyrophosphates inhibited isocitric dehydrogenase by the removal of Mg^{++} or Mn^{++} which are required for the activity of coenzyme II. This interpretation, however, is questioned by KREBS (**19**). It has been observed that the energy liberated by the action of succinic dehydrogenase can be utilized for the synthesis of energy-rich phosphate bonds (**5**). The inhibition of succinic dehydrogenase, known to function both in the respiration of plant and animal cells, was accomplished in two steps (**20**). HCN inhibited only the ability to utilize oxygen and not the ability to decolorize methylene blue, but the last step was inhibited by selenite, arsenite, malonic acid, and pyrophosphate (**20**). The inhibition of yeast aldolase but not muscle aldolase by pyrophosphate has also been reported (**31**).

It is also known that ethylene, both at the concentration of 1 p.p.m. and 1000 p.p.m. causes the simple soluble substances of plants to increase at the expense of the higher soluble and insoluble forms (**13, 14, 15, 16**). Ethylene, 1 p.p.m. in air, increased the respiration of potatoes in storage (**16**) and HANSEN (**12**) has confirmed this effect of ethylene on fleshy fruits. However, the pea test, to date, has not demonstrated conclusively that ethylene originated from degradation of the organic phosphorus compounds, even though it is possible for it to arise from thermal breakdown of ethyl metaphosphate at temperatures above 140° C (**11**). A personal communication from Dr. S. A. Hall, of the Bureau of Entomology and Quarantine, Beltsville, Maryland, also stated that they have never been able to demonstrate ethylene evolution from HETP or TEPP, even when sprayed on hot steam pipes in the greenhouse. The fact that the TEPP (or degradation products) stimulus persists in the soil, and that plants show formative effects, at least two months after its addition to the soil, still remains to be satisfactorily explained. Perhaps, it is possible by some unknown mechanism, for ethylene to be evolved from TEPP-degraded products as diethyl hydrogen phosphate or diethyl metaphosphate. ZIMMERMAN and HARTZELL (**34**) arrived at approximately this conclusion in their study.

The formative responses and metabolic effects of TEPP on plants closely coincide with those induced by 2,4-D. KELLEY and AVERY (**17**) have shown that 2,4-D has both stimulatory and inhibitory effects on the respiration of pea and oat tissues. A concentration of 1 gram per liter (1000 p.p.m.) was inhibitory; concentrations of 100 to 200 mg. per liter (100 and 200 p.p.m.) and lower, stimulated oxygen uptake in oat tissue, and 10 mg. per liter and lower were stimulatory to pea tissue. These workers reported that the maximum stimulation of respiration approximated 50%, whereas the maximum stimulation in the present study for the concentrations of TEPP employed was 62%. MCILRATH (**24**) reported that in cotton, using formative effects as the index, low carbohydrate plants are most susceptible to TEPP and HETP. His report was in accord with KELLEY and AVERY's work (**17**) showing that 2,4-D brought about greater stimulation of respiration in starved than unstarved oat tissue.

The elemental composition of plants, both in the first experimental phase conducted for 30 days and the 48-hour experiments showed increased tissue phosphorus in the TEPP-sprayed plants. In some cases the amount was more than could be accounted for by the phosphorus from the TEPP application. The general increase of the elements potassium and calcium in the treated plants, even during the relatively short 48-hour experiment (table V), and the correlation with the respiratory stimulation obtained at 400 p.p.m. level (table VI) suggested increased root absorption and additional support for the hypothesis that respiratory energy is utilized in primary absorption.

The similar, if not identical, formative effects produced by TEPP and 2,4-D and their mutual effect on respiration suggests a common mode of action of the two compounds on plant metabolism, possibly through the effect on aerobic respiration. LIPMANN (**22**) reported that the one known mechanism for the utilization of energy liberated by the metabolism of foodstuff is through the formation of labile high-energy phosphate bonds. LIPMANN (**22**) has estimated that the free-energy decrease of these compounds upon hydrolysis is of the order of magnitude of 10,000 to 12,000 calories per mole. One of the high-energy phosphate linkages known in biological systems is the bond between phosphate and other phosphate groups as in the general type —P—O$\sim$P—. This type of linkage exists in tetraethyl pyrophosphate (see structure on page 517). However, little work apparently has been done on the nature of the energy relations of the phosphorus-oxygen bonds of TEPP. HALL and JACOBSON (**11**) did calculate the Arrhenius heat of activation, which should be a measure of the energy to break the P—O—P linkage by hydrolysis, to be 10,300 calories per mole. This value agrees with the energy necessary for the high-energy phosphorus-bond type of compound given by LIPMANN (**22**). The rapidity of hydrolysis also indicates that TEPP possesses properties of this type of compound. In his explanation of the mode of action of the 2,4-D hormone-type phytocide, VAN OVERBEEK (**30**) postulated that, after combining with suitable proteins, 2,4-D stimulated the liberation of organic phosphate from phosphorylated compounds. The lethal effect of 2,4-D, according to MCELROY (**23**) possibly might be due to greatly stimulated glycolysis and that the oxidative processes concerned in synthesis may not be able to compete with the available hydrogen acceptors and are consequently inhibited.

The data of this study suggest that TEPP functions in reverse to the above hypothesis for 2,4-D, serving either directly as a high-energy phosphorus substrate for transphosphorylation or indirectly in stimulating the natural synthesis of high-energy phosphorus bond compounds by the plant with subsequent liberation of energy for growth. The latter perhaps appears more logical in light of the results obtained with ATP spray. Thus both 2,4-D and TEPP may have the same net result on plant metabolism although effecting this response by different biological pathways.

The increased effect of TEPP plus 2,4-D, contrasted to either compound alone, lends support to this hypothesis. In addition, phosphorolysis was accelerated in the TEPP-treated plants as evidenced by the accumulation of inorganic phosphorus in these plants. If further research substantiates this explanation of hormone action and respiration, some of our present theories must be re-evaluated accordingly.

Summary

1. Morphological, physiological, and metabolic effects produced by the organic phosphorus insecticides, hexaethyl tetraphosphate and tetraethyl pyrophosphate were studied singly and in combination with inorganic soil P in carnation and tomato.

2. The abnormal flowering symptoms first observed in carnation were reproduced in this species by aqueous spray applications of hexaethyl tetraphosphate containing the active constituent tetraethyl pyrophosphate and the symptoms are described and illustrated.

3. Stimulatory, inhibitive, and lethal responses of tomato to TEPP were noted and the concentration ranges of TEPP necessary to produce these were reported. Formative effects, even at extremely high soil levels of superphosphate, were not produced by inorganic phosphorus applications.

4. Of the concentrations tested 400 p.p.m. of TEPP marked the upper limits of stimulation for stem elongation and time of flowering in tomato. The difference in the number of flowers produced was not considered to be significant, however, in this study.

5. Weight production and carbohydrate content decreased in treated plants. Hydrolysis of reserve carbohydrates and the accumulation of soluble sugars were accelerated by TEPP.

6. Respiratory measurements showed that 400 p.p.m. of TEPP greatly accelerated oxygen uptake (62% above the controls), but 800 p.p.m. inhibited oxygen uptake (27% of controls).

7. Phosphorus fractionation disclosed that TEPP increased soluble phos-

phorus at the expense of the insoluble fraction, and indicated accelerated phosphorolysis in the treated plants.

8. The elements K, P, and Ca increased in plants treated with 400 p.p.m. TEPP. These changes are interpreted to mean a stimulation of primary absorption occurred due to the increased respiratory energy resulting from treatment.

9. The similarity in morphological and metabolic responses of plants to 2,4-D and TEPP has been noted and a common mode of action of the two compounds discussed.

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