AN ABSTRACT OF THE THESIS OF

James Raleigh McKinley (Name) for the M.S. (Degree) in Farm Crops (Major)

Date thesis is presented May 13, 1965

Title FACTORS INFLUENCING THE HERBICIDAL ACTIVITY OF DIMETHYL 2,3,5,6-TETRACHLOROTEREPHTHALATE

Abstract approved Redacted for privacy
(Major professor)

Studies were conducted to determine the site of DCPA toxicity on annual ryegrass. This was done by means of a technique which used pyrex tubing and a vapor barrier to separate exposure of coleoptile and roots of emerging grass seedlings to various concentrations of DCPA. Coleoptiles that emerged through treated soil were completely killed, but when only roots were exposed to treated soil, little shoot reduction was attained.

The toxicity exerted through the roots was investigated by exposing only roots to DCPA-treated soil at concentrations ranging from 0 to 128 ppm. There was no significant shoot reduction when roots were exposed to concentrations as high as 128 ppm. Root growth, however, was greatly reduced when roots grew in soils treated with DCPA at concentrations of 4 ppm and greater.

There was a direct correlation between phytotoxicity and the depth of treated soil through which the coleoptile emerged, which

might indicate greater efficiency would result from shallow incorporation under field conditions. There was also a direct relationship between the extent of phytotoxicity and the concentration of DCPA in the soil through which the coleoptile emerged. These two correlations indicate that the toxicity exerted was directly related to a given quantity of DCPA absorbed. The lethal dosage could be accumulated either by long exposure to low DCPA concentrations or short exposure to high concentrations.

DCPA seems to be an inhibitor of normal mitotic cell division. Enlargements occurred in all areas of meristematic tissue. The swollen area appeared to be due to excessive cell proliferation. DCPA at sub-lethal doses served as a growth stimulator causing increased growth in both roots and shoots. Optimum growth stimulation occurred at a 72°F. temperature. The influence of soil temperature on DCPA toxicity to annual ryegrass was investigated by means of a temperature-controlled water bath in the greenhouse. DCPA was slightly more toxic at 84°F. and 60°F. temperature levels as compared to a medium temperature of 72°F. which would indicate a variation in plant-chemical response rather than chemical property differences. Plants growing in warm soil treated at 2 parts per million were able to overcome early evidence of toxicity better than those growing in cooler soils.

Studies conducted on DCPA degradation in the soil indicated that DCPA was broken down faster at 90°F. than at 70°F. There was no detectable breakdown at the 50°F. temperature level. The half-life of DCPA at 90°F. was 105 days, and 155 days at 70°F.

FACTORS INFLUENCING THE HERBICIDAL ACTIVITY OF DIMETHYL 2,3,5,6-TETRACHLOROTEREPHTHALATE

by

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A THESIS

submitted to

OREGON STATE UNIVERSITY

in partial fulfillment of
the requirements for the
degree of

MASTER OF SCIENCE

June 1965

APPROVED:

Redacted for privacy

Professor of Farm Crops

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Date thesis is presented May 13, 1965

Typed by Gloria M. Foster

ACKNOWLEDGEMENTS

To the following persons I wish to express my sincere appreciation and thanks:

Dr. William R. Furtick, my Major Professor, for his guidance and encouragement throughout my graduate program, as well as for his critical reading and aid during the preparation of the manuscript.

Dr. Arnold P. Appleby for his assistance and guidance in planning the research and for his critical reading and correction of the manuscript.

Dr. William W. Chilcote, who served on my graduate advisory committee.

Mr. D. E. Stallard and Dr. L. Gordon Utter of the Diamond Alkali Company for their cooperation and assistance in DCPA residue analysis.

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FACTORS INFLUENCING THE HERBICIDAL PROPERTIES OF DIMETHYL 2,3,5,6-TETRACHLOROTEREPHTHALATE

INTRODUCTION

In recent years, considerable research has been conducted on the use of pre-emergence herbicides for the selective control of weeds in crops. As a result, numerous recommendations for herbicide uses have been established. However, failure to adequately control weeds often occurs, even though well established recommendations are followed. The reasons for these failures can seldom be defined. A broad, basic knowledge of factors influencing herbicidal activity could lead to specific principles and practices which might bring about more consistent results or at least an intelligent understanding of the reasons for inadequate control.

The herbicide, DCPA (dimethyl 2,3,5,6-tetrachloroterephthalate), is a relatively new chemical which has provided outstanding selective control of many important weed species in a wide range of crops. Basic research on DCPA has not been sufficient to explain certain erratic results obtained in field use and in greenhouse research.

It is apparent that environmental factors are responsible for some of these variations in results, but it is not known which, or to what extent, individual factors are involved.

Trevett, Murphy, and Gardner (31, p. 203), among others, noted

the importance of high moisture conditions for maximum DCPA activity. The Boyce-Thompson Institute for Plant Research, Inc. (4, p. 4) found that DCPA loss was greatly accelerated at high temperatures. Their tests suggest that some of the loss was due to co-distillation with water, although this was believed to be quite small in contrast to the loss by chemical deterioration in the soil. Stallard, Skinner, and Priddle (27, p. 1) found that the apparent "half-life" of a field treatment of DCPA was approximately 100 days, but they did not specify environmental conditions existing at the time of the study. It was observed by the author in preliminary studies that ryegrass seeded in DCPA-treated soil escaped injury when planted at shallow depths. This may have been due to distinct differences in the site of uptake between plant parts.

There are many questions not answered in previous research. The objective of this study was to answer some of the basic questions on the factors important for the uniform performance of DCPA. These factors, once known, may lead to improved cultural practices that would decrease the failures from the use of this compound. Areas of study included (1) site of uptake and phytotoxic effect of DCPA on various plant parts, (2) temperature effect on herbicidal activity of DCPA, (3) DCPA degradation under constant temperatures of 50°F., 70°F., and 90°F., and (4) breakdown under field conditions.

LITERATURE REVIEW

The Herbicidal Properties of DCPA

Discovery of the herbicide DCPA (dimethyl-2,3,5,6-tetrachloro-terephthalate) resulted from cooperative research between Diamond Alkali Chemical Company and Boyce Thompson Institute for Plant Research, Inc. (4, p. 1). Pure DCPA is a white crystalline substance, essentially odorless, with a low vapor pressure and a melting point of 150°C. The solubility in water is less than 0.5 ppm; however, this material is partially soluble in benzene, acetone, xylene and petroleum-derived solvents.

DCPA is a herbicide which has shown considerable promise as a pre-emergence compound. Brown and Furtick (5, p. 35-36) obtained 100 percent control of dodder (Cuscuta campestris) in alfalfa with two pounds active DCPA per acre.

Dawson (8, p. 37) received essentially 100 percent control of dodder in greenhouse studies when using rates of 10 pounds DCPA per acre. In field studies, where 6, 10, and 14 pounds DCPA per acre rates were used, adequate, but not perfect, weed control was obtained. Hoffman and Bayer (20, p. 36-37) conducted a trial to determine the most effective time to apply DCPA on established alfalfa for dodder control. Their results indicated that 7.5 pounds applied in March provided satisfactory season-long control. December applications did not give satisfactory control throughout the entire season; by mid-summer there was no evidence of control.

Hargon, Bayer, and Cialone (16, p. 81-88) found DCPA to be a

selective herbicide for several cucurbit crops. Noll (22, p. 108-109; 23, p. 91), as well as Dallyn and Sawyer (7, p. 110-113) reported that DCPA gave excellent selective weed control in onions on mineral soils. Peters, Yokum, and Stevens (24, p. 521) obtained 100 percent control of crabgrass at a 6.5 pound per acre rate. DiDario (11, p. 161) found DCPA to give excellent weed control in ornamentals at 5, 10, and 15 pounds of active material per acre. Harris, Schuldt, and Limpel (17, p. 2), as well as Trevett, Murphy, and Gardner (31, p. 203), noted the importance of high moisture requirements for herbicidal activity. Trevett and his co-workers obtained 70 percent control of annual ryegrass at a rate of six pounds per acre in 1959 under moist soil conditions. With the same rate in 1960, annual grass control was only 34 percent under low moisture conditions. It was reported by Trevett and Gardner (30, p. 3) that incorporation of DCPA into the top one inch of soil reduced its effectiveness. All indications are that DCPA is only very slightly leached in the soil. Boyce-Thompson Institute for Plant Research, Inc. (3, p. 5) reported that the ester of dimethyltetrachloroterephthalic acid is found in the top 1/4 inch of the soil. The degradation products can be found deeper in the soil, but these are herbicidally inactive. Upchurch and Pierce (34, p. 330; 35, p. 27) found that there are at least two processes that determine the leachability of herbicides: the entrance of the chemical into solution and adsorption on soil colloids. It was reported that within a family of herbicides, there appears to be some relationship between adsorption and solubility. Wolfe, et al. (36, p. 7)

found the extent of movement of four substituted ureas to be related to the order of solubility; greater solubility resulted in less adsorption and thus more movement.

Experiments were conducted by the Boyce-Thompson Institute for Plant Research, Inc. to determine the adsorptive capacity of three clays and charcoal. It was found that adsorption capacity of the clays were quite small, if any. Adsorption by or on charcoal, on the other hand, was quite significant in reducing the activity of DCPA (4, p. 7). Sweet, Cialone, and Hargon (29, p. 173-177) tested DCPA on muck soils and found its herbicidal activity greatly reduced, as compared to results obtained on upland mineral soils. Furtick (15, p. 3) obtained 40 percent control of annual grass using 30 pounds active DCPA per acre in Chillan, Chile, in a clay-loam soil of six percent organic matter. Little or no control was obtained at the 30 pound per acre rate in Temuco, Chile, under volcanic ash soils with a 15 percent organic matter content. Upchurch and Mason (33, p. 9) found that organic matter and cation exchange capacity were significantly correlated with the adsorption capacity of the soil for herbicides.

Longevity of DCPA in the Soil

Stallard, Skinner, and Priddle (27, p. 1) reported that DCPA undergoes a two-step hydrolysis under field conditions; from dimethyl 2,3,5,6-tetrachloroterephthalate to a mono-methyl 2,3,5,6-tetrachloroterephthalate, then to a 2,3,5,6-tetrachloroterephthalic acid. The acid is then ultimately converted to tetrachlorobenzene

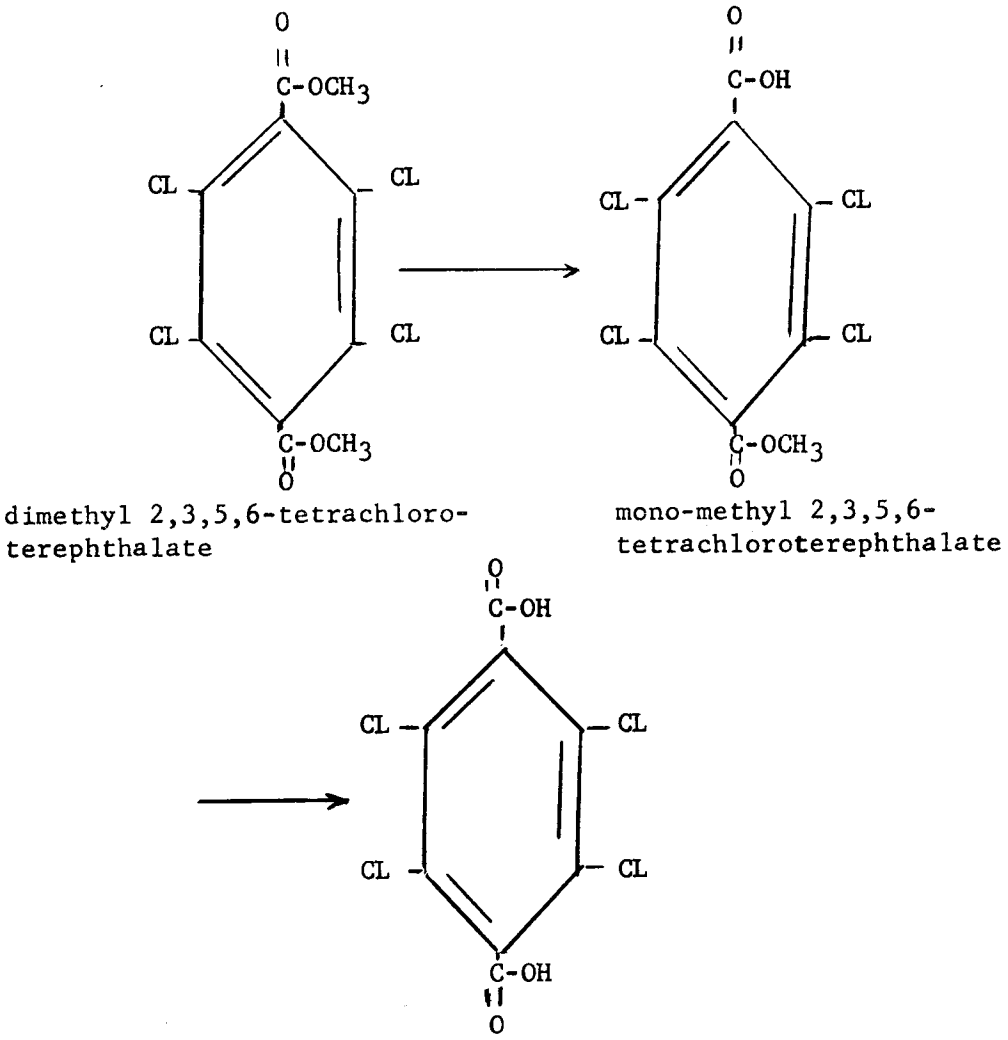


Figure 1. DCPA degradation

(Figure 1). The apparent "half-life" of a field treatment of DCPA herbicide is approximately 100 days.

Troll, Zak, and Waddington (32, p. 484-485), in 1960, obtained 99 percent control of crabgrass with DCPA at the rate recommended by the manufacturer. When no further applications were made to the plots in 1961, only 16 percent control of crabgrass was obtained. This indicated rather rapid breakdown of DCPA in the soil, or loss of activity due to some other reason. Ahren,

Lukens, and Olson (1, p. 517), on the other hand, received 99.9 percent control of crabgrass with a 10 pound per acre treatment in the year of application and 71 percent control the following year, indicating considerable residual activity. In experiments conducted by Limpel, Schultz, and Lamont (21, p. 125), eight pounds active DCPA per acre gave 97 percent control of susceptible weed species 21 days after application, 77 percent control after 34 days, and 54 percent 60 days after treatment.

The Boyce-Thompson Institute for Plant Research, Inc. (4, p. 5) conducted tests with two Texas soils which showed that DCPA loss was greatly accelerated at high temperatures. At the same time, the tests suggested that some of the loss might be due to co-distillation with water from the surface. The amount of loss in this manner was, however, believed to be quite small in contrast to that lost through chemical degradation within the soil. At soil temperatures below 90°F., loss was mainly by chemical deterioration, while above this temperature, loss occurred through a chemical deterioration and co-distillation.

Site of Uptake and Phytotoxicity Effect for DCPA

Apparently there has been no published research conducted on determining the site of uptake in relation to the phytotoxicity effect of DCPA. The majority of available evidence for many soil-applied herbicides would indicate the roots are the important path of entry. Herbicides of this nature, when applied to the soil surface and kept there by strong adsorption or lack of overhead

moisture to leach them into the root zone, usually have little phytotoxicity. The phenyl ureas and triazines used for soil sterilization are frequently applied early in the spring so that rains have a chance to leach some of the herbicide into the root zone before germination occurs (20, p. 140-141). Experiments have been conducted to determine the site of uptake for some of the carbamate herbicides.

Luther Fitch of the Malheur Branch Experiment Station in Oregon (12) noted root and shoot growth reduction of barnyardgrass (Echinochloa crusgalli) and other sensitive species when exposed to DCPA-treated soil. It has been reported by the Boyce-Thompson Institute for Plant Research (4, p. 2-3) that DCPA performed quite poorly when weeds germinated at lower depths and emerged through the dry soil surface containing the herbicide. They also stated that it was therefore essential that DCPA be in contact with the germinating seed and in a soil-water suspension to be most effective. Frieson, Banting, and Walker (14, p. 91-103) demonstrated that diallate (Avadex), 2,3-dichloroallyl diisopropylthiolcarbamate, is effective only when the coleoptile of the wild oat (Avena fatua) comes in contact with the treated soil. No toxic effect was noted in studies where only roots were in contact with the treated soil. Their method consisted of placing the wild oat seed under, over, and into treated soil. Injury was noted only when the coleoptile grew through the treated soil. Frieson, Banting, and Walker (14, p. 91) also noted that the longer the coleoptile was exposed to the treated soil in relation to the depth of planting, the greater

the resulting injury.

Dawson (8, p. 37-39) determined the site of uptake for EPTC (ethyl-N,N-di-n-propylthiolcarbamate) in barnyardgrass (Echinochloa crusgalli) by using a polyethylene vapor barrier and cone to separate coleoptile from root uptake. This technique forced either the coleoptile or the root to grow into treated soil. He was able to provide conclusive evidence that EPTC was taken up primarily through the emerging coleoptile. Appleby (2, p. 31-32) verified work done by Frieson on diallate and work done by Dawson on EPTC and also found the site of uptake for IPC (isopropyl N-phenylcarbamate). The use of an envelope technique and a polyethylene barrier was used separating treated from untreated soil. Oat seeds were allowed to germinate in a small polyethylene envelope, which in turn was placed in a slot in the polyethylene barrier separating the two soil layers. The four treatments were: (1) untreated control, (2) untreated soil above the barrier, IPC-treated soil below the barrier, (3) IPC-treated soil above the barrier and untreated soil below the barrier, and (4) IPC-treated soil above and below the vapor barrier. Roots and coleoptiles were then exposed to different treatments as they emerged through the soil layers. Observations from this experiment indicated that when shoots emerged through the treated soil, the bioassay plant was injured. However, when only roots were exposed to the treated soil, little or no injury was noted.

Temperature Effect on Herbicide Activity

Harris and Warren (18, p. 123-124) have studied the effect of temperature on availability of herbicide molecules in the soil under various temperatures. They showed that an increase in temperature would reduce the amount of herbicide adsorbed and increase the amount in solution available for plant uptake. This illustrates that adsorption processes are exothermic while desorption processes are endothermic in nature. The adsorption of simazine, atrazine, and monuron by bentonite was greater at 0°C. than at 50°C. This, however, does not hold true with all chemicals. Diquat, which is a very strong cation, is completely adsorbed at both temperatures, pointing out that the strong cationic effect of diquat overcame any effect temperature had on the adsorption-desorption processes. Temperature may exert an indirect influence on adsorption through the effect on solubility. In general, solubility and temperature work together to affect adsorption; that is, both greater solubility and higher temperature lead to decreases in adsorption (18, p. 120-126). There have been reports, however, of certain exceptions to this general rule. Freed (13, p. 26) found that temperature effect on solubility of EPTC (ethyl n,n-di-n-propylthiol carbamate) was of greater importance than the effect on adsorption *per se*. Greater adsorption occurred at the higher temperatures. Solubility of the compound increased with decreasing temperature. Since adsorption is interrelated with both temperature and bioactivity, the bio-activity of a herbicide might be expected to be different at various

temperatures. Burnside and Behrens (6, p. 145-156) cited evidence that this may be the case. They found an increase in soil temperature from 59°F. to 86°F. increased the toxicity of simazine to corn. Sheets (26, p. 1-13) reported similar results which indicated simazine was taken up and translocated to a greater extent at 37°C. than at 27°C. Appleby (**2**, p. 39-45) observed the influence of soil temperature on EPTC toxicity. The least amount of toxicity was exhibited at 50°F. and the greatest toxicity was noted at 85°F. with 70°F. being intermediate. Rumburg, Engel, and Meggitt (25, p. 583-586) studied the effect of temperature on the phytotoxicity of DMA (di-sodium methylarsonate) in control chambers using crabgrass as the test plant. The phytotoxicity of DMA to crabgrass increased with increasing temperatures from 60°F. to 85°F.

METHODS, MATERIALS AND RESULTS

The work reported covers eight greenhouse studies and one field experiment. Greenhouse studies were designed to determine the relative sensitivity of plant parts to DCPA. Experiments were also designed to determine the effect of temperature on the phytotoxicity of DCPA. DCPA degradation studies were conducted in the greenhouse and in the field.

Much of the research with DCPA involved certain techniques and methods, identical for all greenhouse experiments. Rather than discuss these methods in each experiment, a general description of methods and materials will be given. Equipment and techniques unique to a specific experiment will be described under the heading of the individual experiments.

General Materials and Methods

All greenhouse studies entailed the use of soil-incorporated DCPA. DCPA was mixed thoroughly with the soil by means of a cement mixer. A desired amount of soil (oven-dry weight basis) was placed in the cement mixer and as the soil tumbled and mixed, the correct amount of commercial DCPA was sprayed on the soil in a water suspension. By adjusting the spray to a relatively fine mist and allowing the soil to mix well as the DCPA suspension was being applied, a uniform concentration of the desired parts per million was obtained. Soil moisture was brought to approximately field capacity at the time of mixing. The soil used in all experiments was a fine

sandy loam soil provided for greenhouse research. The results of a soil analysis are given in Table 1.

Table 1. Chemical and mechanical analysis of greenhouse soil

Chemical analysis					
Soil pH	CEC me/100 g	OM	K me/100 g	Ca me/100 g	Mg me/100 g
6.3	13.9	0.65	0.27	5.8	3.3

The bioassay plant used in all of the research, other than the DCPA breakdown studies, was annual ryegrass (Lolium multiflorum). This plant was selected because of sensitivity to DCPA and ability to grow under a wide soil temperature range. The fact that the plant is a cross-pollinated heterozgous species was a disadvantage as a bioassay species. Therefore, individual differences in responses would be expected.

All experiments conducted in the greenhouse received 12 hours of additional artificial light each day. Alternating temperatures were kept at 75°F. during the day and 70°F. at night. Sub-irrigation was used to keep the soil moist throughout the experiment. Deviations from the normal watering practice will be discussed in the individual experiments.

Plant samples were harvested by cutting plants off at the soil level, putting them into small weighing bottles, and placing them in an oven to dry for 30 hours at 100°C. Weights of the dry plant samples were determined on a micro-balance. All results from experiments were statistically analyzed. Duncan's multiple range

test was used to determine statistical differences between treatment means.

Greenhouse studies on soil placement and concentration in relation to phytotoxicity

It was noticed in preliminary greenhouse studies that annual ryegrass, when planted in a 4 part per million concentration of DCPA, was essentially completely controlled. However, a few plants escaped noticeable injury. Close examination of the escaped plants indicated they either germinated on or near the soil surface or had emerged along the edge of the can. This led to the hypothesis that toxicity was exerted primarily through the emerging coleoptile. The following five experiments were designed to determine the toxicity of DCPA to various plant parts.

I. Greenhouse studies on placement of DCPA in soil

The objective of this experiment was to prove the hypothesis that DCPA was taken up through the emerging coleoptile. An experiment was designed to determine the toxicity of DCPA to various plant parts.

Materials and Methods. The technique used was similar to that reported by Appleby (2, p. 13-15), which permitted use of separate soil layers in which the roots and shoots of germinating seeds were exposed. However, due to the small size of annual ryegrass seed, it was impossible to use the envelope technique for differential root and seed exposure devised by Appleby. The

technique used in this experiment involved the use of small pyrex tubing 3 mm in diameter, which was cut into 1.3 cm lengths. Annual ryegrass seeds were soaked in tap water for three hours. One soaked seed was placed in each small pyrex tube with the embryo end pointing downward (Figure 2). Each end of the tubing was dipped into a wet clay soil. A small clay plug formed in each end of the tubing. The lead from an eversharp pencil was used to push the small plug up into the tubing until it reached the seed (Figure 3). This left a small air space between the two plugs and the ends of the tubing. The air space helped to eliminate DCPA movement through the plug and into the germinating seed.

Eight pyrex tubes, each containing a soaked seed and clay plugs, were forced through a .004 inch thick and 10 inch square sheet of polyethylene. The polyethylene barrier served the function of separating the two soil layers in which the plants grew. This permitted the coleoptile to emerge from the tubing and grow into one soil layer while the primary root emerged and grew into the soil layer below (Figure 3). Such a technique allowed the two soil layers to be treated differently to determine the effect of various concentrations of DCPA on the coleoptile, as compared to the primary root.

Three treatments were used in the experiment: (1) DCPA-treated soil above the barrier with untreated soil below, (2) DCPA-treated soil below the barrier with untreated soil above, and (3) untreated soil both above and below the barrier.

DCPA at the rate of 16 parts per million was mixed with the

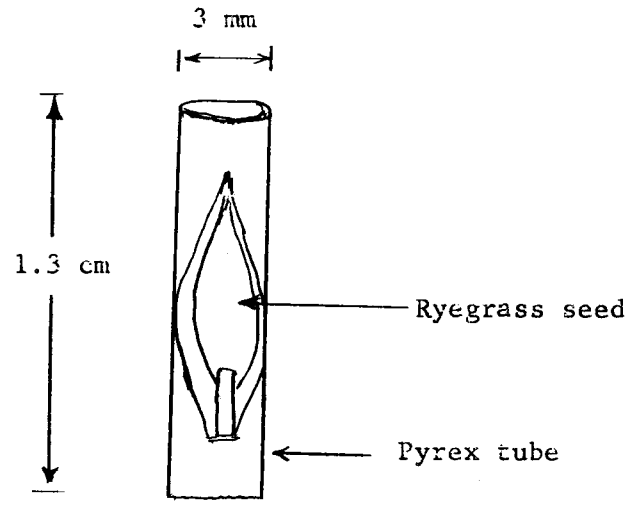


Figure 2. Apparatus used to separate root from shoot uptake

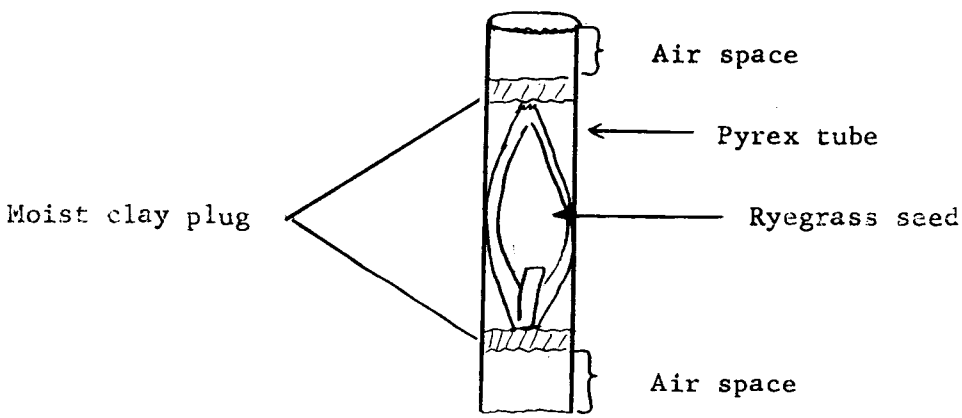


Figure 3. Apparatus showing clay plug and air space

soil. One-gallon cans were filled to within 1-1/2 inch of the top with soil. The polyethylene sheet containing the eight pyrex tubes and their seeds was placed on top of the soil and pressed firmly in place. Four hundred and eighty grams (oven-dry equivalent) representing approximately one inch of soil, was placed over the seeds and polyethylene barrier. Soil above the barrier was watered when necessary with a hose sprinkler, while soil below the barrier was kept moist by placing the can in a pan of water and allowing the

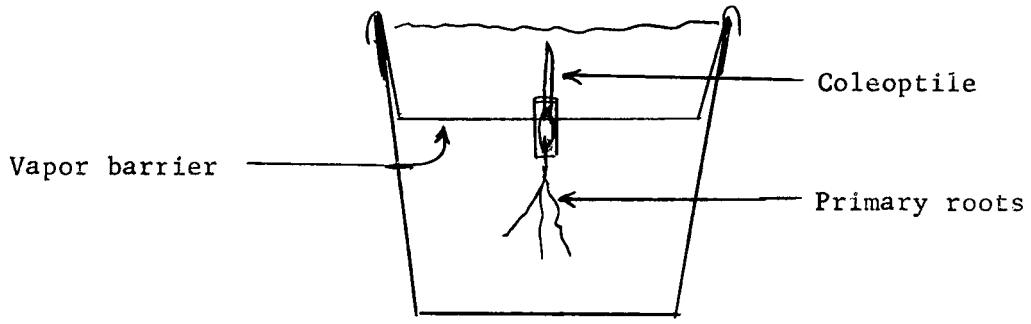


Figure 4. Polyethylene vapor barrier and pyrex tube technique used to separate root from shoot uptake.

moisture to move upward. Five replications were arranged in a randomized block design. The number of plants that emerged and survived in each pot were recorded. Plants were harvested three weeks after the planting date.

Plate I

From left to right:

1. Untreated soil above the barrier, DCPA-treated soil below.
2. DCPA-treated soil above the barrier, untreated soil below.
3. Untreated control.

Results. The results of this experiment are given in Table 2 and further illustrated in Plate I. Annual ryegrass was essentially killed when the coleoptile emerged through the treated soil. Only one plant in a total of 40 emerged. The single plant exhibited considerable injury and did not attain normal growth. When the roots were the only part exposed to the treated soil, only minor injury could be noted.

Table 2. Effect of DCPA placement in relation to phytotoxicity

Area exposed to DCPA	No. emergence	No. survived	Average dry wt/plant[1] (mgs)	Percent of check
1. Roots	34	34	6.85	66.1
2. Coleoptile	1	1	.04	.38
3. Check	32	32	10.34	100

[1]Significant at the 1 percent level

II. DCPA toxicity to the primary root

In the previous study, it was demonstrated that DCPA toxicity was exerted primarily through the emerging coleoptile. When only the coleoptiles were exposed to DCPA at the rate of 16 parts per million, essentially complete control was obtained. The primary root appeared to be able to tolerate a much higher concentration of DCPA than the emerging coleoptile. The purpose of this experiment was to determine how high a concentration of DCPA the primary root could tolerate.

Materials and Methods. DCPA rates of 0, 32, 64, 94, and 128 parts per million were used in the experiment. One-quart size cans were filled within 1-1/2 to 2 inches of the top with treated soil. A polyethylene vapor barrier and the pyrex tube technique already described was used.

The experiment was conducted as a randomized block design with four replications and five DCPA rates. The above-ground portion of the plants was harvested three weeks after the time of planting. At the time of harvest, the number of plants present per pot was recorded. The weight per plant was calculated and results were expressed as a percentage of the check.

Results. The weights of the dried shoots for the respective treatments are given in Table 3. It can be seen from the results that roots exposed to a dosage of 32 parts per million resulted in a decreased total shoot weight of 32.4 percent of the check. However, a four-fold increase in dosage, or 128 parts per million, decreased the yield only 39.2 percent (Plate II). Plants whose primary roots had been exposed to DCPA looked quite normal, except for a reduction in shoot growth. No other symptoms were evident. Treatment means differed significantly from the check but did not differ from one another. The highest rate of DCPA which annual ryegrass primary roots tolerated was not determined. However, it was found that the primary roots could withstand a concentration of 128 parts per million, which is nearly 65-fold above the amount tolerated by the coleoptiles. Further examination will be needed

Plate II

A. Untreated control.
B. 32 ppm DCPA below the barrier, untreated soil above.
C. 64 ppm DCPA below the barrier, untreated soil above.
D. 94 ppm DCPA below the barrier, untreated soil above.
E. 128 ppm DCPA below the barrier, untreated soil above.

Table 3. Average dry weight of annual ryegrass when primary roots are in contact with DCPA

DCPA concentration	No. emergence	No. survived	Average dry wt/plant (mgs)	Percent of check
Check	23	23	17.6[a1]	100
32	24	24	5.7[b]	32.4
64	26	26	5.7[b]	32.4
94	27	27	7.3[b]	41.5
128	23	23	6.9[b]	39.2

[1] Means with the same letter are not significantly different at the 1 percent level.

using even higher rates to determine the lethal rate for primary roots. However, the practical use of any rates higher than 32 parts per million would be extremely limited.

III. DCPA toxicity exerted through primary and secondary roots

The technique used in the preceding experiments worked very satisfactorily for determining DCPA toxicity through contact with the emerging coleoptiles and the primary roots. The technique, however, failed to determine the concentration which subsequently-formed secondary roots could tolerate In the preceding technique, the primary roots protruded through the pyrex tubing into the treated soil layer below (Figure 5). The role of the primary root was, however, partially replaced by the latter-forming secondary roots which, when developed, were exposed to the untreated soil layer above the barrier. Therefore, an experiment was designed to determine the concentration at which toxicity was exerted on the entire root system, including secondary roots.

Materials and Methods. The rates of DCPA used in this experiment were 0, 2, 4, 8, 16, 32, 64, and 128 parts per million. The treated soil was placed into quart-size juice cans. Over the surface of the treated soil was sifted 1/16 inch of untreated soil. One hundred ryegrass seeds were planted on the fine layer of untreated soil and then covered with another 1/16 inch of untreated

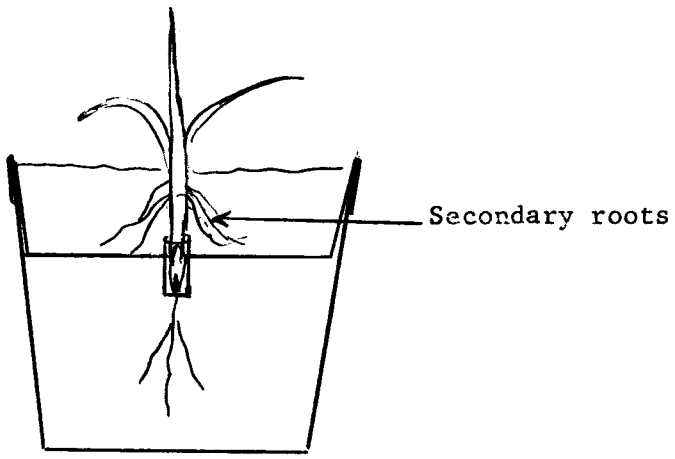


Figure 5. Area of secondary root formation

soil to prevent excessive drying. The fine layer of untreated soil was placed between the treated soil and seeds to prevent the coleoptiles from coming in direct contact with treated soil as they protruded out and upward. The primary and subsequently-formed secondary roots grew downward into the treated soil. This layering technique made it possible to separate root uptake from shoot uptake.

The experiment was conducted in a randomized block design with

four replications and eight treatments. Plant shoots and roots were harvested separately three weeks after planting. Plant roots were washed free of soil by using a garden hose and a fine screen to collect the roots.

Results. The results of this experiment are recorded in Tables 4 and 5 and further illustrated in Plate III. A large experimental error limited statistical detection of differences between treatment means unless differences were large. The 4, 8, 16, and 32 part per million treatments gave a significant stimulation effect to shoot production. No significant stimulation occurred at the 16, 32, and 4 part per million treatment rates. There were no statistical differences in shoot production at the 128 part per million treatment as compared to the check. Some of the results from this experiment are not easily explained. For example, a 2 part per million treatment produced an inhibiting effect on shoot production while the 8, 16, and 32 part per million rates promoted a stimulating effect.

Table 4. Effect of shoot production when roots were exposed to varying concentrations of DCPA

DCPA concentration	Average shoot dry wt/pot (mgs)	Statistical evaluation	Shoot wt. in percent of check
8 ppm	325.7	a[1]	121.5
16 ppm	311.5	a b	116.0
32 ppm	309.3	a b	115.0
4 ppm	274.7	a b c	102.6
0 ppm(check)	268.5	b c	100.0
64 ppm	242.5	c	90.5
2 ppm	237.0	c	88.4
128 ppm	227.1	c	84.5

[1]Means with the same letters are not significantly different at the 5 percent level.

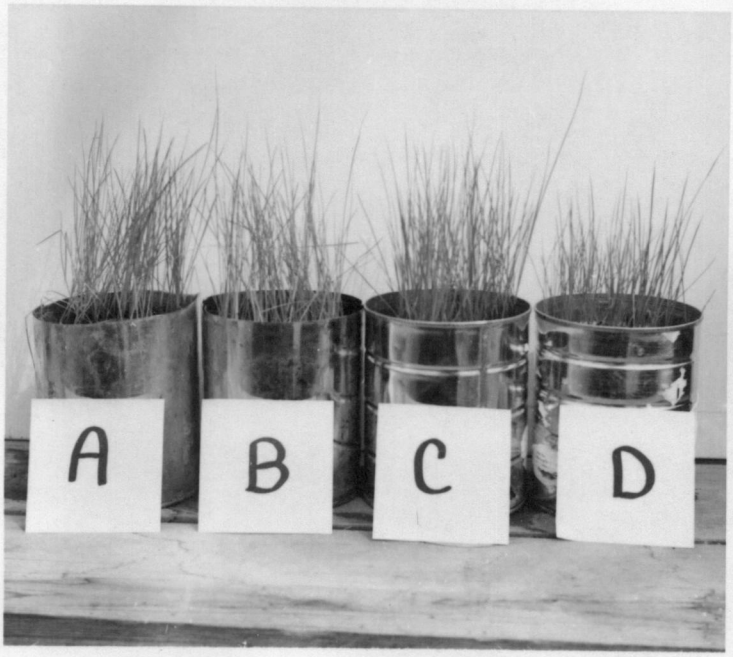


Plate III

Only roots exposed to:

A. Check
B. 8 ppm DCPA-treated soil
C. 64 ppm DCPA-treated soil
D. 128 ppm DCPA-treated soil

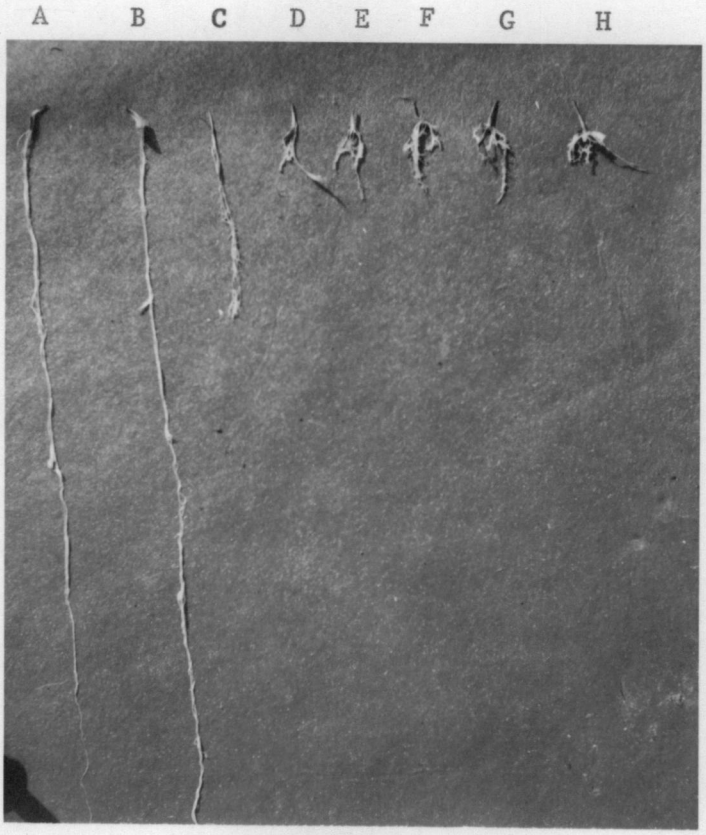


Plate IV

Root growth reduction when grown in soil treated with DCPA at various rates.

A. Check
B. 2 ppm DCPA-treated soil
C. 4 ppm DCPA-treated soil
D. 8 ppm DCPA-treated soil
E. 16 ppm DCPA-treated soil
F. 32 ppm DCPA-treated soil
G. 64 ppm DCPA-treated soil
H. 128 ppm DCPA-treated soil

Table 5. Effect on root growth when roots were exposed to varying concentrations of DCPA

DCPA treatment	Average root length (cms)	Average root dry wt/pot (mgs)	Root wt. in percent of check
0 ppm (check)	28	375.0 a[1]	100
2 ppm	24	255.5 b	68.1
4 ppm	7	234 c	62.4
16 ppm	4	227 c	60.5
8 ppm	2.5	225 c	60
64 ppm	2.5	187.7 d	50.1
128 ppm	2.5	181.7 d	48.5
32 ppm	2.5	140.3 e	37.4

[1]Means with the same letter are not significantly different at the 5 percent level.

The average root weight per treatment is recorded in Table 5 and illustrated in Plant IV. There was no stimulation in root growth from any rates of DCPA used as was demonstrated with shoot growth. All treatments gave a substantial decrease in total root growth. In most cases there was a gradual decrease in root weight as the DCPA concentration increased. The one exception was the 32 part per million treatment, which had the lowest yield. One replication of the 32 part per million treatment yielded much lower than the other three replications which reduced the means.

There was considerable ryegrass root reduction when plants grew in treated soil, but this did not seem to have any reflection on shoot production. This experiment did not lend itself to close statistical evaluation. However, very useful information was obtained. Even at the higher DCPA concentrations, the total shoot weight reduction was never greater than 16 percent, demonstrating the importance of coleoptile contact with the treated soil.

IV. Depth of coleoptile emergence through treated soil in relation to phytotoxicity

Since DCPA is toxic mainly to the coleoptile, it is conceivable that susceptible weeds germinating on or near the soil surface might escape injury. Therefore, it is important to understand the relative injury exerted by DCPA when grasses germinate and emerge from various depths within the treated soil. This experiment was designed to correlate phytotoxicity with depths from which annual ryegrass emerges.

Materials and Methods. DCPA at the rate of 0, 2, 4, and 8 parts per million was thoroughly incorporated into sandy loam soil. The soil was placed in quart-size cans. One hundred annual ryegrass seeds were planted in each container at 0, 1/4, and 1/2 inch depths. This was accomplished by covering the seeds with 100 and 200 grams (oven-dry weight basis) of treated soil to obtain the desired depth of planting. A fine layer of untreated soil was sifted over the treated soil for the 0 inch planting depth prior to planting. This was done to prevent the coleoptiles from coming in direct contact with the treated soil as they emerged. A fine layer of untreated soil was applied over the seeds to prevent them from drying. Untreated checks were established for the three depths of planting in the same manner.

The experiment was conducted in a randomized block design with four replications and six treatments. Plant shoots were harvested three weeks after planting. The results of the experiment are

expressed in percent of the check at the respective depth of planting.

Results. Average plant shoot dry weights are recorded in Table 6. Annual ryegrass, which germinated on the soil surface with only roots exposed to the treated soil, had no apparent shoot injury, regardless of the DCPA concentration. Plants which emerged from the 1/4 and 1/2 inch depths in treated soil were injured. The extent of injury for a given depth of planting was directly related to the concentration of DCPA in the soil through which the coleoptile emerged. As an illustration, at the 1/4 inch planting depth, the 2 part per million treatment yielded 59.5 percent of the check, as compared to 10.6 percent for the 4 part per million treatment and complete control for the 8 part per million treatment. There was also a direct correlation in extent of injury between a given DCPA concentration and depth of treated soil through which the coleoptile emerged. This is illustrated in Plate VI. As an example, annual ryegrass seeded 1/4 inch deep in soil treated with 2 parts per million of DCPA yielded 59.5 percent of the check, whereas this same concentration, when seeded at the 1/2 inch depth, produced 20.1 percent of the check. Increasing the depth from which the seeds were forced to emerge, greatly reduced the concentration of chemical required to kill annual ryegrass. This is clearly illustrated in Plate V.

Close visual observations on injured plants growing at the 1/4 and 1/2 inch depths at 2, 4, and 8 ppm rates, revealed an enlargement at the basal node (Plate VII). The enlarged areas appeared to have resulted from extensive cell proliferation. The leaf sheath

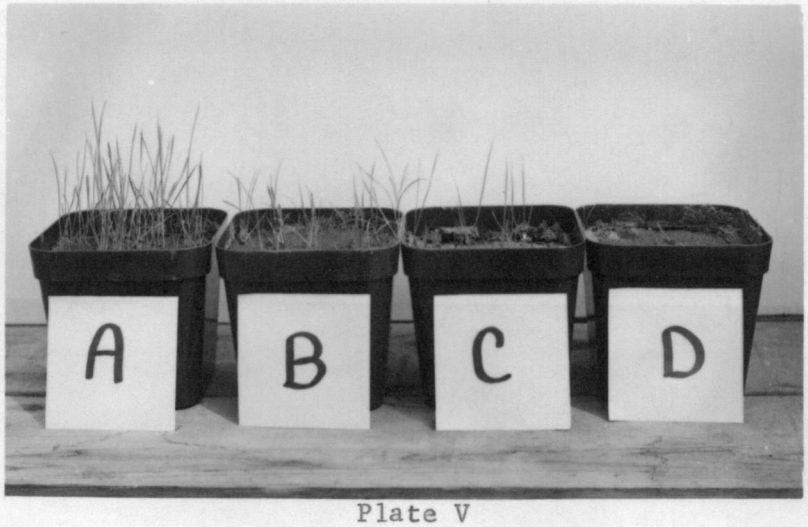


Plate V

A. 2 ppm, seed planted at one-fourth inch depth.
B. 2 ppm, seed planted at one-half inch depth.
C. 4 ppm, seed planted at one-fourth inch depth.
D. 4 ppm, seed planted at one-half inch depth.

Plate VI

A. 2 ppm, seed planted on soil surface
B. 2 ppm, seed planted at one-fourth inch depth.
C. 2 ppm, seed planted at one-half inch depth.

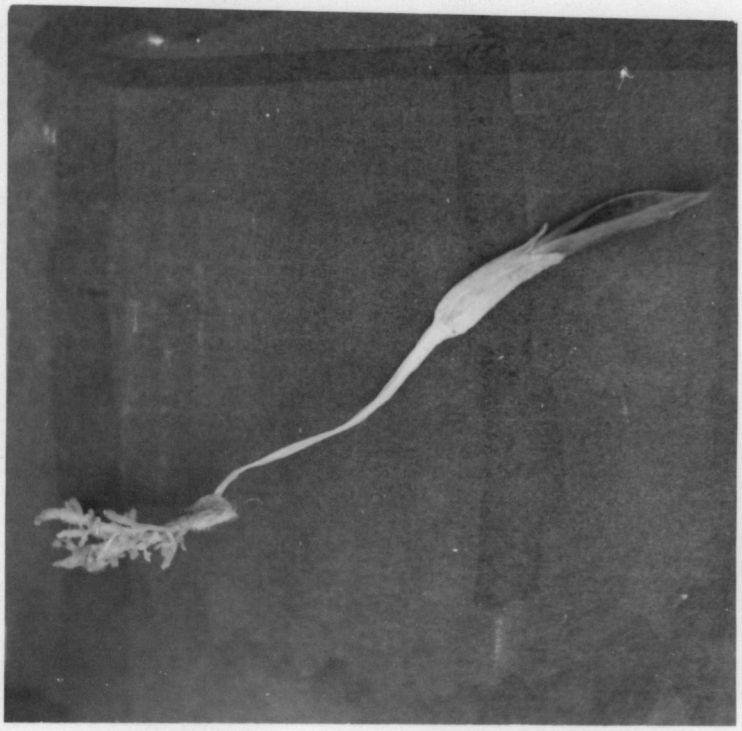


Plate VII

DCPA injury to shoot. Enlargement occurs at the shoot meristematic area. Ruptured shoot is also visible.

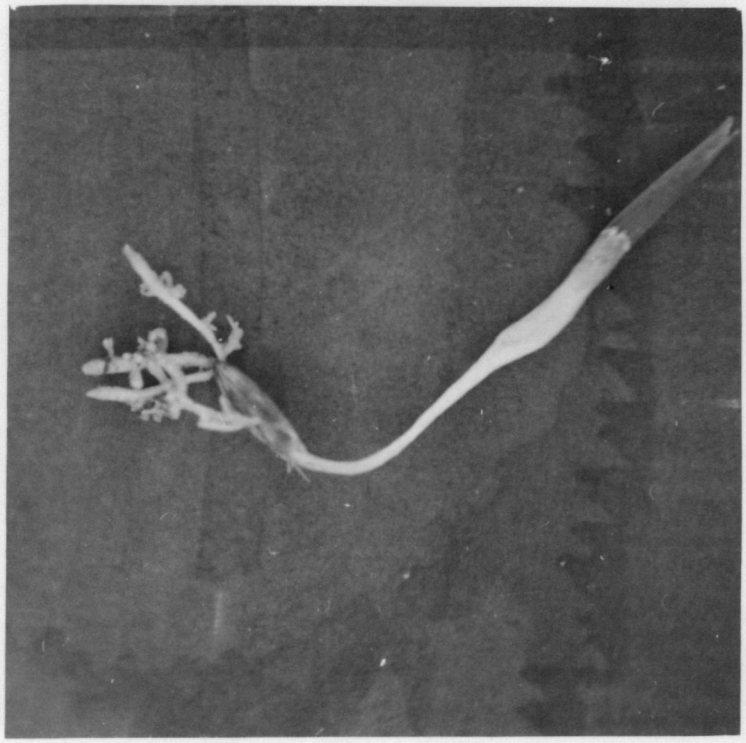


Plate VIII

DCPA injury to roots. Enlargements occur at the root apical meristematic area.

Table 6. Effect of planting depth on ryegrass injury in soil containing varying concentrations of DCPA

Planting depth	Average shoot weight at respective planting depths with varying DCPA concentrations			
	0 ppm	2 ppm	4 ppm	8 ppm
0"	268.50 b[1]	237.00 c	274.75 b	325.75 a
1/4"	228.50 c	136.00 d	24.25 f	0 g
1/2"	227.75 c	64.00 e	0 g	0 g

	Average shoot weight expressed as a percent of check at the respective planting depth			
	0 ppm	2 ppm	4 ppm	8 ppm
0"	100	88.2	102.2	121.3
1/4"	100	59.5	10.6	0
1/2"	100	29.1	0	0

[1]Means with the same letters are not significantly different at the 5 percent level.

of many injured plants had been ruptured, displaying the internal proliferated area. Roots exposed to the 8 part per million DCPA treatment also displayed considerable stunting (Plate VIII). The roots, rather than being long and slender as in the check, were extremely short and enlarged. The greatest amount of enlargement appeared at the apical root.

V. DCPA toxicity to germinating seeds

The four preceding experiments have demonstrated that DCPA is far more toxic to annual ryegrass when the coleoptile is exposed to treated soil as compared to the primary and secondary roots. In all previous experiments, seeds were allowed to initiate germination in a DCPA-free soil or in pyrex tubing. This experiment was designed to determine whether DCPA is toxic during the early processes of seed germination.

Materials and Methods. Soil was treated with 2 ppm of DCPA and placed in four-gallon cans. Several hundred annual ryegrass seeds were planted in each of the cans. The seeds were allowed to imbibe and germinate for various periods of time while in contact with the treated soil or with untreated soil. After 24, 48, 72, and 96 hours, seeds were removed from the soil and washed carefully. Twenty-five seeds were planted in quart-size juice cans at 1/2 inch depths and allowed to continue germination and growth. The experiment was designed as a randomized block using four replications and four treatments. Plants were harvested three weeks after planting. Results are expressed as a percentage of the check for the respective transplanting dates.

Results. The amount of toxicity exerted by DCPA during various stages of seed germination is summarized in Table 7. Duncan's multiple range test was used to test differences among treatment means. There was no significant difference between the 24 and 48 hour exposure to DCPA-treated soil. However, injury did occur when germinating seeds were exposed to treated soil for 72 and 96 hours. Toxicity is believed to have occurred in the longer exposed treatments as a result of DCPA uptake by the emerged coleoptile. The coleoptiles were approximately 1/4 inch in length at 96 hours after planting.

Table 7. DCPA toxicity to annual ryegrass seeds during germination at the 2 ppm rate

Hours in contact with treated seed	Average dry wt/pot	Statistical evaluation	Percent of check
Untreated 24 hours	210.5	a[1]	
2 ppm 24 hours	209.2	a	99.4
Untreated 48 hours	209.2	a	
2 ppm 48 hours	191.0	a	91.4
Untreated 72 hours	178.0	a	
2 ppm 72 hours	109.2	b	61.2
Untreated 96 hours	186.7	a	
2 ppm 96 hours	60.5	c	32.4

[1]Means with same letter are not significantly different at the 5 percent level.

Greenhouse studies on the influence of temperature on DCPA toxicity

Simazine, EPTC, and disodium methyl arsonate have been found to possess greater herbicidal properties at higher temperatures. The purpose of the next two experiments was to determine the influence temperature may have on DCPA activity.

I. Influence of soil temperature on DCPA activity

Materials and Methods. One-gallon cans were filled three-fourths full with treated soil in which 100 annual ryegrass seeds were planted. One inch of soil (600 grams oven-dry weight basis) was placed over the seeds to obtain a uniform planting depth. The cans were covered with a fine polyethylene layer which was held tightly in place with a rubber band. The polyethylene prevented co-distillation of the chemical and also helped maintain a uniform soil

temperature. The cans were then placed in a water bath where the soil temperature could be held at a constant level. Six separate water tanks were used with three treatment levels, 84°F., 72°F., and 60°F. The experiment was set up as a split-plot design with four replications, seven DCPA levels, and three temperature levels. The main plots were temperature levels and the sub-plots were DCPA rates. Two replications were placed in each tank. The treatments were re-randomized within each replication every three days. The water bath in which the cans were placed was maintained at 60°F., 72°F., and 84°F., ± 2°F. The treatments were observed daily. The polyethylene was removed from each can upon appearance of the emerging coleoptile. The date of emergence for each DCPA rate and temperature was recorded. The plant shoots and roots were harvested three weeks after planting. Roots were collected by placing the contents of the gallon can into a fine screen funnel. Water from a hose was used to wash the soil from the roots. Root and shoot weights based on percent of the check were plotted and a dosage-response curve was obtained for each temperature.

Results. The number of days between planting and coleoptile emergence is recorded in Table 9. Coleoptile emergence was delayed as the DCPA increased. The delay of emergence from DCPA was greater at the 84°F. than at the 72°F. or 60°F. temperature levels.

Table 9. Emergence rates of annual ryegrass with various levels of DCPA at three temperatures

DCPA concentration (ppm)	Number of days between planting and emergence		
	84°	72°	60°
Check	4	6	8
1	4	6	8
1.2	4	6	8
1.4	4	6	8
1.6	5	6	8
1.8	7	7	9
2.0	9	8	10

The coleoptiles at lower DCPA concentrations appeared much earlier at the 84°F. temperature than at the 60°F., with 72°F. being intermediate. The highest DCPA concentrations, on the other hand, emerged only one day apart at the 84°F. and 60°F. temperature levels.

Apparently there was more inhibition of shoot emergence at the higher temperatures due to the presence of DCPA. As an illustration, the 2 ppm rate took 2.5 times longer to emerge than the check at the 84°F. temperature level, whereas this same rate took only 1.25 times as long compared to the check at the 60°F. temperature level.

Recorded in Table 10 are the average shoot dry weights per plot and the percent control obtained for each treatment based on the percent of the check at each temperature level. The same data are further illustrated in Figure 6, where the treatments are plotted as a percent of the check at the respective temperature levels. At the 1.0, 1.4, and 1.6 ppm concentrations, there is a noticeable

decrease in shoot dry weight. A sharp decline in total shoot weight was indicated at the 1.8 and 2.0 ppm treatments. There was approximately 50 percent decrease in total shoot weight from 1.6 ppm to a 1.8 ppm treatment at all temperature levels.

Table 10. Effect of temperature on activity of DCPA

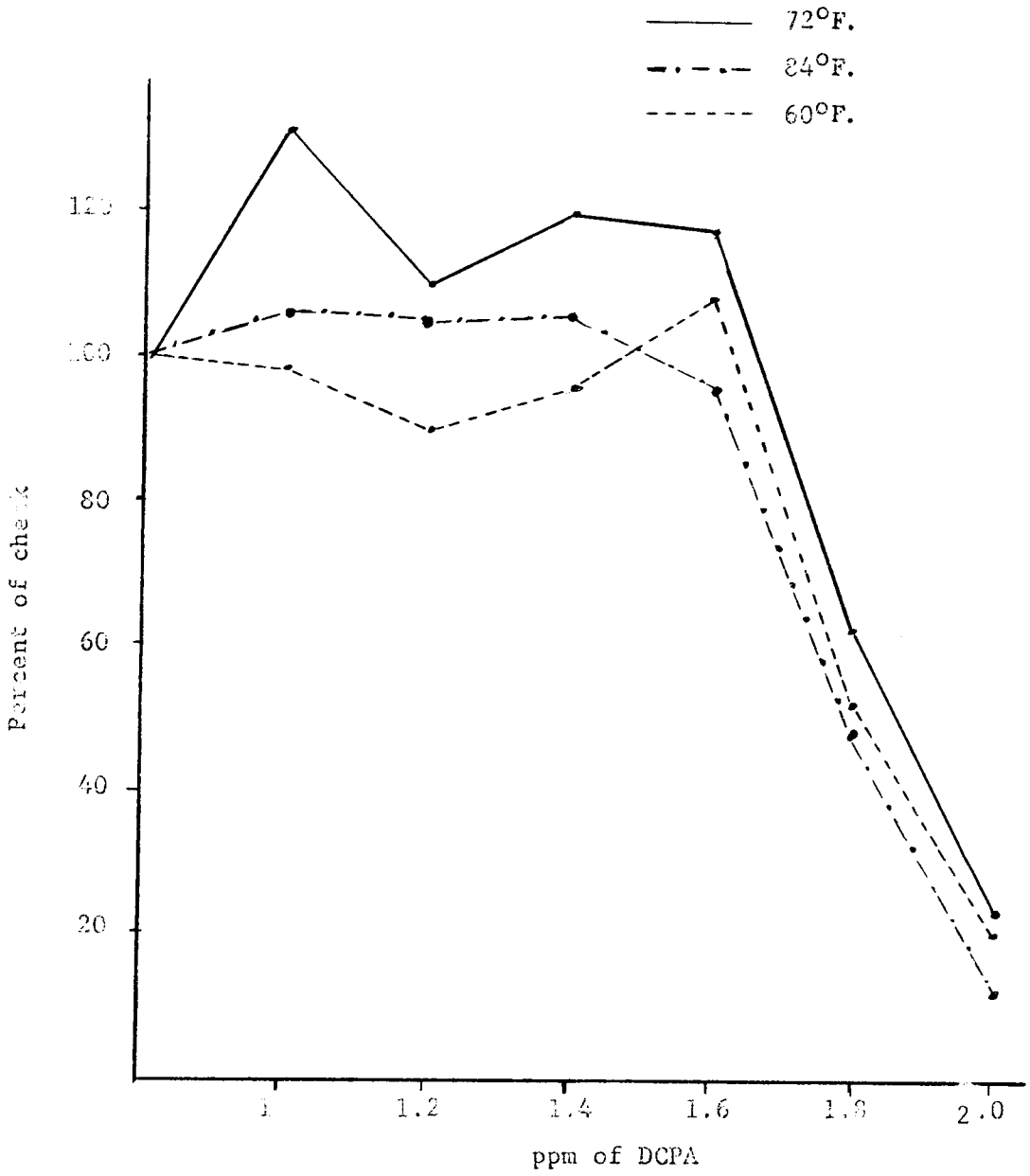
DCPA concentration	Average shoot wt/plot (mgs) Temperatures 84°F.	72°F.	60°F.
Check	326.50 cde[1]	328.75 cde	278.75 e
1.0 ppm	349.50 bcd	437.00 a	273.50 e
1.2 ppm	345.75 bcd	362.25 bcd	249.25 fg
1.4 ppm	348.75 bcd	407.75 ab	264.75 eg
1.6 ppm	314.00 de	388.50 abc	302.75 def
1.8 ppm	164.75 h	205.75 gh	149.00 h
2.0 ppm	39.50 i	76.75 i	57.00 i

	Average shoot wt/plot expressed as a percent of the check Temperature 84°F.	72°F.	60°F.
Check	100	100	100
1.0 ppm	106.1	132.8	98.9
1.2 ppm	105.8	110.0	90.2
1.4 ppm	107.0	120.0	107
1.6 ppm	96.0	118.0	109.8
1.8 ppm	47.7	62.6	53.9
2.0 ppm	12.2	23.4	20.7

[1]Average shoot weights with the same letters behind them are not significantly different at the 5 percent level.

Apparently, the low sub-lethal concentration of DCPA caused a stimulation in growth of annual ryegrass at the 74°F. temperature level. The highest stimulation effect was at 1.0 ppm, where shoots were increased to 132.8 percent of the check. There was slightly more overall DCPA toxicity at the 84°F. and 60°F. temperature levels with the least noticed at the intermediate 74°F. level.

Figure 6. Shoot dry weight expressed as a percent of the check



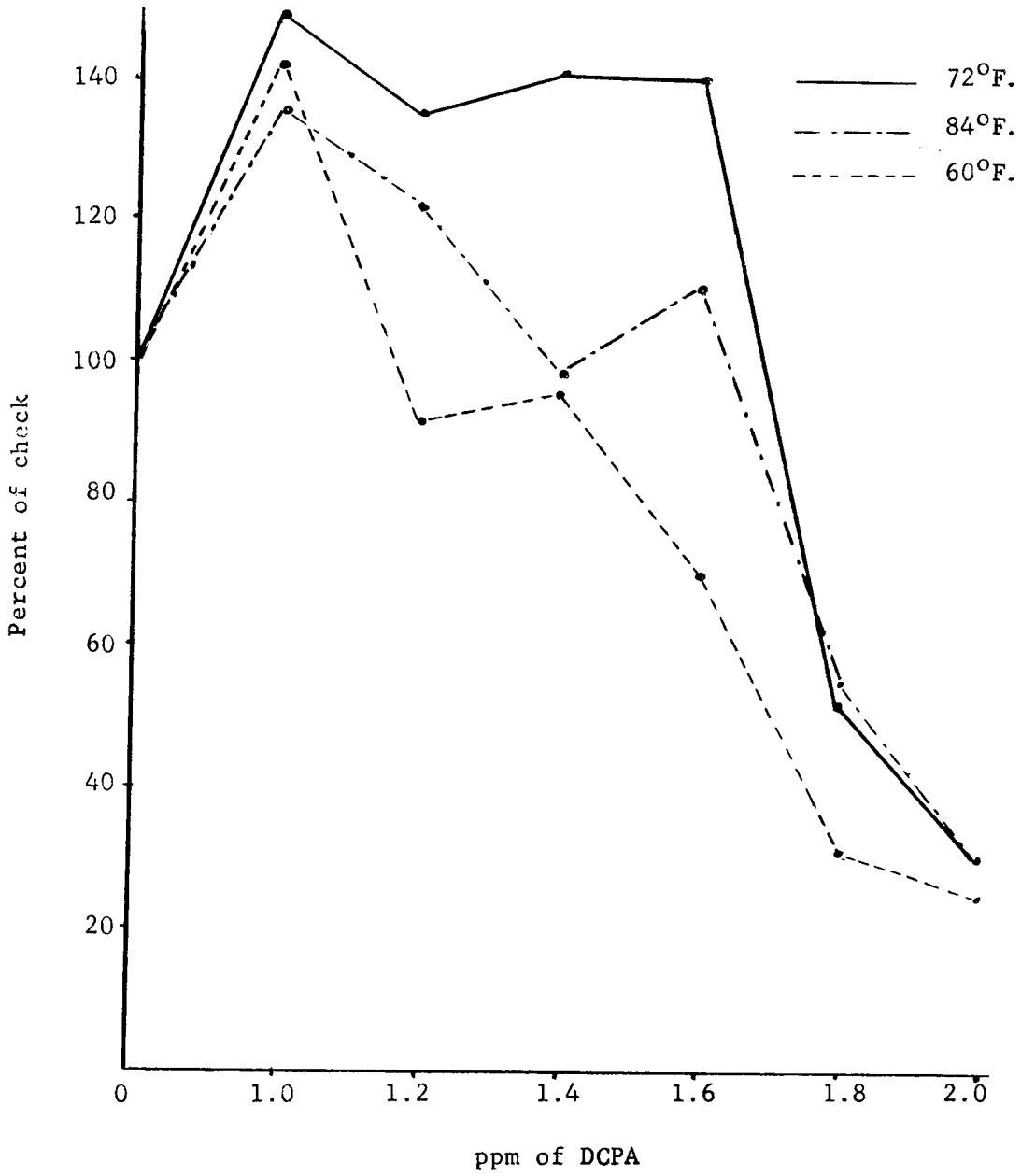
The results of root growth at three temperatures and under various concentrations can be observed in Table 11. It can be seen that there was a significant stimulation effect at the 1 ppm concentration at all temperature levels with the greatest increase received at the 72°F. temperature. There was a stimulation at the 72°F. level in the 1.0, 1.2, 1.4, and the 1.6 ppm concentration with the highest yield at the 1.0 ppm level. These results are illustrated as a percent of the check at their respective temperature levels in Figure 7. Stimulation seems to appear from use of DCPA at sub-lethal dosage and at soil temperatures nearly optimum for the plant.

Table 11. Effect of temperature on DCPA activity on roots

DCPA concentration	Average root wt/plot (mgs) Temperature 84°F.	72°F.	60°F.
Check	171.5 c	208.3 b	211.5 bc
1.0 ppm	233.0 b	309.5 a	301.0 a
1.2 ppm	208.3 bc	281.0 a	199.0 bc
1.4 ppm	194.8 bc	297.0 a	202.5 bc
1.6 ppm	190.0 bc	292.0 a	211.3 bc
1.8 ppm	94.5 de	108.0 d	98.9 de
2.0 ppm	57.3 e	68.8 de	76.5 de

	Average root wt/plot expressed as a percent of the check Temperature 84°F.	72°F.	60°F.
Check	100	100	100
1.0 ppm	135.9	148.6	142.3
1.2 ppm	121.3	134.9	94.1
1.4 ppm	113.6	142.4	95.7
1.6 ppm	110.8	140.2	99.9
1.8 ppm	55.1	51.8	46.7
2.0 ppm	33.2	33.0	36.2

Figure 7. Root dry weight expressed as a percent of the check



II. Effect of three temperature levels on herbicidal activity of DCPA when considering three harvest dates.

In the preceding experiment, at the 2 parts per million treatment, the surviving plants appeared as though they may have been able to overcome the toxic effect exerted by DCPA if not harvested. The objective of this experiment was to determine whether the injured plants at the 2 parts per million rate could overcome the stunting received from DCPA.

Materials and Methods. Soil treated with 2 ppm of DCPA and untreated soil was placed in one-gallon cans to within 1-1/2 to 2 inches of the top. A fine polyethylene layer was placed over the cans to prevent DCPA co-distillation and moisture loss. Twelve one-gallon cans containing treated soil and twelve with untreated soil were placed in each of the three temperature levels. The experiment was designed as a split plot with three temperatures, two chemical rates, and four replications. The temperatures were the main plots with DCPA rates as sub-plots. The water bath temperatures were maintained at 84°F., 72°F., and 60°F. ($\pm$ 2°F.). The polyethylene was removed from the cans when the first coleoptile appeared. The soil was kept moist throughout the experiment by frequent overhead irrigation.

The first harvest was made three weeks after planting, at which time shoots from the treated and untreated plots were harvested at each temperature. The second set of plots was harvested five weeks after planting and the third set was harvested seven weeks after planting.

Results. The results of the temperature studies during three harvest dates are shown in Table 11. Duncan's multiple range test was used to test differences between temperatures in the 2 parts per million treatments. All treatment means across harvest dates were significantly different, and therefore were not designated by the appropriate letters.

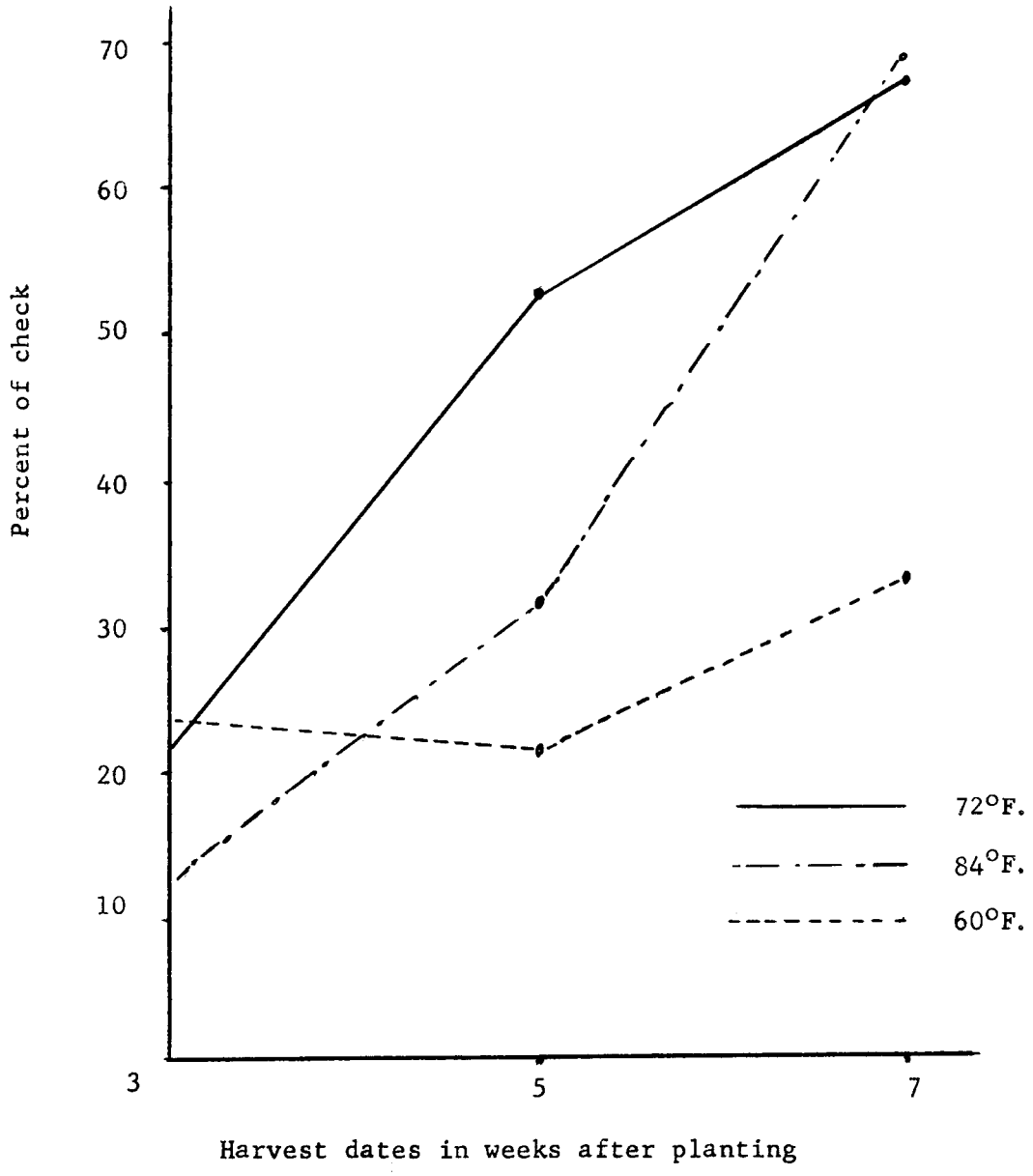
Table 11. Effect of three temperature levels and three harvest dates on the herbicidal activity of DCPA

Rate and harvest date	Ave. shoot dry wt/plot (mgs) 84° F.	% of check	Ave. shoot dry wt/plot (mgs) 72° F.	% of check	Ave. shoot dry wt/plot (mgs) 60° F.	% of check
3rd week harvest date						
Check	287		321		279	
2 ppm	36 b[1]	12.5	70 a	21.1	64.5 a	23.1
5th week harvest date						
Check	638		528		584	
2 ppm	199.7 a	31.3	312.5 b	53.1	123 c	21.1
7th week harvest date						
Check	1,088		925		779.5	
2 ppm	751 a	69.0	624 b	67.4	259 c	33.2

[1]The 2 part per million treatment means with the same letters are not significantly different at the 1 percent level. Comparisons were only made within harvest date across temperature.

The 2 part per million rate at the 84°F. temperature produced a significantly greater herbicidal effect than did the other two temperatures at the first harvest date. The 2 part per million rate produced only 12.5 percent of the check at the 84°F. temperature level, as compared to 21.1 and 23.1 for the 72°F. and 64°F. temperature levels, respectively. By the fifth week an entirely different result was obtained. The DCPA-treated plot at the 84°F. level had increased from 12.5 percent of the check to 31.1 percent of the check, which would indicate an ability to overcome the earlier exerted toxic effect. The yield from treated plots increased from 21.1 to 53.1 at the 72°F. temperature level. In comparison to the 84°F. and 72°F. temperature level, shoot weight increased in treated plots at the 60°F. temperature level. By the seventh week the DCPA-treated plots yielded 69 percent of the check at the 84°F. temperature level, while the treated plots at the 60°F. level increased only up to 33.2 percent. This would indicate that plants growing in warmer soils were better able to overcome the early toxic effects than those growing in cooler soils. If DCPA were toxic through the root system, there would have been a gradual decrease in total shoot weight in treated plots. DCPA was taken up by the coleoptile and once toxicity was exerted via the coleoptile, little to no further injury was obtained, at least not at the rates tested. The summary of these observations is illustrated in Figure 8.

Figure 8. Shoot dry weight expressed as a percent of the check for three temperatures and three harvest dates



DCPA decomposition in the soil

Stallard and Skinner (27, p. 1) pointed out that the half-life of DCPA is around one hundred days. However, no known work has been done to determine how temperature may influence the rate of DCPA loss in the soil. This experiment was designed to determine the effect temperature may have on degradation of DCPA.

I. DCPA decomposition under three temperatures

Materials and Methods. DCPA was thoroughly mixed with soil at the rate of 16 parts per million. Four samples of treated and untreated soil were taken immediately after mixing, dried at room temperature, and placed in plastic bags for storage and later analysis. The remaining soil was placed into one-gallon cans. Each can was covered with polyethylene to prevent DCPA co-distillation and moisture loss. The gallon cans were submerged to within 2 inches of the top, in water, controlled at constant temperatures of 50°F., 70°F., and 90°F. ($\pm$ 2°F.). Every thirty days the gallon cans were removed from the temperature tank, the content of each can was poured separately in a dishpan and thoroughly mixed. Two hundred and fifty gram samples were taken from each can. The soil was then placed back in the can, the polyethylene replaced, and cans were placed back in the temperature tanks. The 250-gram samples were allowed to dry at room temperature and stored in plastic bags for later chemical analysis. The process was repeated three times representing 90 days that degradation was allowed to occur.

This experiment was conducted in the greenhouse in a split plot design with three temperature levels, two DCPA rates, and four replications.

Soil samples from the three month period were subjected to a solvent extraction process to remove dimethyl 2,3,5,6-tetrachloroterephthalate, as well as the inactive degradation products, monomethyl 2,3,5,6-tetrachloroterephthalate and 2,3,5,6-tetrachloroterephthalic acid. This method of extraction was devised by Stallard and Skinner of Diamond Alkali Company herbicide research division (for detailed procedures see Appendix).

Due to lack of facilities required for chemical analysis of the DCPA residue and the degradation products, the residue samples were sent to Diamond Alkali Herbicide Research Center at Painsville, Ohio for chemical analysis. DCPA and the degradation products were detected with the use of a micro-coulometric gas chromatograph.

The DCPA and the degradation products are expressed in parts per million. The extent of degradation that occurred is expressed as a percent of initial concentration.

Results. The results of the soil breakdown studies are recorded in Table 12. The original samples taken immediately after the herbicide was incorporated, are recorded as the 0 date of the decomposition period. There was an average of 11.31 parts per million detected out of the 16 parts per million initially added. The lack of complete recovery of the original concentration could indicate incomplete extraction of DCPA adsorbed to the soil, loss

of the herbicide in the solvent extraction process, or a number of other possibilities. This, however, is not of great importance, since all samples were treated the same and any decrease from the 11.31 parts per million level can be attributed to breakdown of the herbicide.

Table 12. Effect of three temperatures on the rate of breakdown of DCPA

Days between treatment and sampling	Average amount of residue (ppm)		
	DCPA (ester)	1/2 acid	Acid
	90°F.		
0	11.31 a[1]	ND	ND
30	9.08 b	ND	.436
60	8.04 bc	.685	1.103
90	6.51 c	.551	1.310
	70°F.		
0	11.31 a	ND	ND
30	11.53 a	ND	.06
60	8.14 bc	ND	.937
90	8.42 bc	ND	1.64
	50°F.		
0	11.31 a	ND	ND
30	12.30 a	.432	.083
60	12.83 a	.475	ND
90	11.48 a	.287	.141

[1]Numbers with same letters are not significantly different at the 5 percent level.

There was a significant decrease in total DCPA concentration from 0 days to 90 days of incubation time at the 90°F. temperature level. As a general rule, the amount of 2,3,5,6-tetrachloroterephthalic acid (acid) increased as the amount of DCPA (ester) decreased, which is to be expected since it is the degradation

product of DCPA.

There was less overall DCPA breakdown at the 70°F. level as compared to the 90°F. temperature level. No noticeable breakdown had occurred after 30 days. DCPA breakdown was, however, detected after 60 and 90 days of degradation time. There was no significant breakdown at the 50°F. temperature level.

These results indicate that temperature does play an important role in the degradation process of DCPA. The data indicate that the half-life of DCPA at 90°F. is 105 days. The half-life for 70°F. was considerably longer or 155 days.

It may be interesting to note the presence of the half-acid and little to no acid in the samples at the 50°F. level, while the reverse is true at the 70° level.

II. DCPA loss from soil under field conditions

Materials and Methods. The objective of this experiment was to determine the extent of DCPA degradation under field conditions. In order to obtain the information desired, an experiment was established at a location near Corvallis, Oregon, on a Woodburn clay loam soil. The experiment was set up in a randomized block design with one rate of DCPA, two spray dates, and four replications. Each individual plot was 10' x 15'. Sixteen pounds of DCPA were applied with the use of a small one-wheeled plot sprayer. The herbicide was applied in a water suspension. The total volume of water was 36 gallons per acre. The first plots were sprayed on October 1, 1964, and the second application date was November 1 of

the same year. Soil samples were taken from the sprayed lots immediately after application.

Three samples at various locations within each plot were taken with a golf course plug remover. Each core was four inches in diameter and one inch thick. Only the top one inch of soil surface was represented in the sample. The three samples were composited to make a single overall representative sample. The soil samples were dried at room temperature and later ground and placed in plastic bags for storage. The sampling procedure was repeated at monthly intervals during a three-month period. The plots sprayed on November 1 were not sampled immediately after spraying. The initial concentration for both spray dates will be represented by the October 1 samples.

The soil samples were taken to the laboratory where each was subjected to a solvent extraction process. For details see the Appendix.

The residue which was the end product of the extraction process, was placed in small bottles and sent to the T. R. Evans Research Center of the Diamond Alkali Company for chemical analysis. The DCPA and degradation products were detected with the use of a micro-coulometric gas chromatograph.

Results. The results of the field breakdown studies are shown in Tables 13 and 14. The unit of measurement is expressed in micrograms per sample. The greatest amount of DCPA loss from the soil on the first spray date was between October 1 and November 1. At

this time, 25 percent of the DCPA total was lost. Soil temperature for this month was still quite warm. Some of the loss may have resulted from co-distillation or volatilization. There were no detectible degradation products in samples taken from the first spray date. This may indicate that the half-acid and acid had been lost by leaching or some other process. There was a gradual decrease in the total amount of DCPA in the samples from the November sample date until the January 1 sample date.

Table 13. DCPA decomposition in the soil under field conditions from October 1, 1964 to February 1, 1965.

Date of sampling	Average microgram residue per 100 gm sample		
	DCPA	1/2 acid	acid
Oct. 1	389.5 a[1]	12.2	ND
Nov. 1	292.2 b	ND[2]	ND
Dec. 1	265.0 bc	ND	ND
Jan. 1	232.2 c	ND	ND

[1]Numbers with same letters are not significantly different at the 5 percent level.
[2]ND refers to non-detectable.

Plots sprayed on November 1 did not show any detectable breakdown for the first three month period. There was, however, a significant amount of breakdown from January 1 to February 1. It may be of interest to note that all the samples from plots sprayed on November 1 had a detectable amount of the mono-methyl 2,3,5,6-tetrachloroterephthalate (1/2 acid). No explanation is evident for the lack of the 1/2 acid in the October 1 or initial spray date samples, other than that in the initial sample which theoretically should not have contained any. In field studies from November 1 to

January 1, the half-life of DCPA was found to be 100 days.

Table 14. DCPA decomposition in the soil under field conditions from November 1, 1964 to March 1, 1965.

Date of sampling	Average micrograms of residue per 100 gm sample		
	DCPA	1/2 acid	acid
Initial sample	389.5 a[1]	12.2	ND[2]
Dec. 1 sample	313.7 abc	15.50	ND
Jan. 1, 1965	366.2 ab	13.87	ND
Feb. 1, 1965	219.7 c	6.53	ND

[1]Numbers with same letters are not significantly different at the 5 percent level.
[2]ND refers to non-detectable.

DISCUSSION AND CONCLUSIONS

Greenhouse studies on toxicity of DCPA to various plant parts

Results from the experiments pertaining to DCPA toxicity to various plant parts indicated that DCPA exerted its primary phytotoxic effect when absorbed through the coleoptile of emerging annual ryegrass seedlings. Roots which grew through soil treated with DCPA at concentrations sufficient to kill emerging coleoptiles, suffered only slight injury. The pyrex tube and vapor barrier technique used to differentiate between the site of uptake of seed, coleoptile, and primary roots, proved quite satisfactory. The pyrex tube and vapor method prevented any undesirable contact between treated soil and a particular part of the plant. This technique permitted treatment of the roots or coleoptile without exposing the seed to treated soil. The pyrex tube and vapor barrier technique may prove very useful in determining the site of uptake for the large number of new chemicals being placed on the market. The pyrex tubing can be obtained in various diameters to fit the seeds of the plants being tested.

Contrary to speculations made by Boyce Thompson Institute for Plant Research, Inc., there was no detrimental effect of DCPA to annual ryegrass seed germination, at least not at concentrations sufficient to injure emerging coleoptiles. There was, however, injury to germinating seeds after the coleoptile had emerged from the seed and was in contact with the treated soil.

When only primary and secondary roots grew into treated soil at concentrations up to 128 parts per million, there was no significant shoot weight reduction. However, DCPA concentration at 2 parts per million and greater, significantly reduced total root length and weight. The average length of roots grown in an 8 parts per million concentration of DCPA were only 4 centimeters in length as compared to the check with an average root length of 28 centimeters. Root length and weight reduction, however, did not appreciably decrease shoot weight.

These results could have many ramifications. Seeds of weedy grasses frequently germinate on the soil surface when environmental conditions are favorable. It is conceivable that susceptible weedy grasses under such conditions could escape injury, even though relatively high rates of DCPA were applied to the soil surface. The lack of weed control, when only roots and germinating seeds are exposed to DCPA-treated soil, may partially explain the occasional inadequate weed control received.

Where the soil surface is allowed to dry out, a higher degree of control would no doubt be obtained. Control would probably result because of an inadequate root system to obtain moisture from lower depths, rather than the direct effect of the chemical per se.

Roots of crops grown on DCPA-treated soil may be seriously reduced in the soil layers where DCPA is concentrated. This may increase the drought susceptibility of the crop. Since DCPA is not readily moved in the soil, this would not be a problem in well-established, deep-rooted plants. Future research should include

experiments designed to determine the extent of root reduction of crops grown on DCPA-treated soil.

In experiments conducted on depth of planting, it was found that there was a direct relationship between depth from which the coleoptile emerged through treated soil, and the extent of phytotoxicity obtained. There was also a direct relationship between DCPA concentration in the soil through which the coleoptile emerged and injury obtained. This would indicate that a given level of toxicity exerted was directly related to a given quantity of DCPA absorbed. The lethal dosage could be accumulated either by long exposure of the coleoptile to low DCPA concentrations or short exposure to high concentrations. These results suggest the possibility of more effective grass control by shallow incorporation. Ashton and Dunster (3, p. 313) found that incorporation of EPTC to a depth of one inch, when the weed seed was at half-inch depth, appeared to be the most effective position. The incorporation of EPTC to greater depths reduced its effectiveness because of the dilution in a greater volume of soil.

DCPA thoroughly incorporated into the top one-half to one inch of the soil would increase the time the deeper emerging coleoptiles are exposed to DCPA as compared to surface applications. Deeper incorporation would reduce the DCPA concentration in a given soil area, thus reduce the effectiveness of the herbicide.

The symptoms of DCPA injury to annual ryegrass was an enlargement of the basal node of the shoot as well as short enlarged roots.

The roots were larger in diameter than normal. However, the most distinguishing characteristic was a large clump at the end of each short root. The enlargement appeared in the area of the apical root meristem. Since enlargements occurred at both the root and shoot meristem areas, it would appear that DCPA inhibited normal cell division. Cell proliferation was quite pronounced in both root and shoot meristem areas.

Cancerous growth was so pronounced that the cell leaf sheath had been ruptured in many plants exposing the internal proliferated area. The characteristics of DCPA toxicity symptoms are very similar to those observed from excess uses of growth-regulatory compounds such as IAA (3-indoleacetic acid).

Effect of soil temperature on DCPA activity

The results obtained from the experiment conducted on the effect of soil temperature on DCPA activity indicated that DCPA was slightly more toxic at 84°F. and 60°F. as compared to 72°F. This would indicate that annual ryegrass is more susceptible to DCPA at extreme temperatures, as compared to temperatures where it is more adapted, suggesting an interrelationship between plant and chemical rather than a chemical property difference. Higher rates, comparable to those used in field applications, may show no significant differences in weed control when considering different soil temperatures. From results obtained, it appears that temperature is not an important factor influencing weed control.

DCPA at sub-lethal levels definitely stimulated total shoot

and root growth. The stimulation effect on shoots was more noticeable at the 72°F. level and at the 1.0, 1.4, and 1.6 ppm concentration. The roots obtained maximum stimulation at a concentration of 1 part per million. This was true for all temperature levels. The maximum stimulation was obtained again at the 72°F. temperature level where root growth reached 149 percent of the check. In all cases, a definite sharp decrease in shoot and root weight from 1.6 to the 1.8 part per million concentration. This would indicate there is a very fine line between sub-lethal and lethal concentrations.

The growth-regulatory capacities of DCPA are extremely interesting. The discovery of growth regulatory potential opens a whole new field for future research. The uses of growth-regulatory compounds of this nature could be numerous. For example, DCPA applied to the soil at sub-lethal concentrations may cause an overall increase in tuber and root growth in crops such as potatoes, carrots, radishes, and turnips. Foliar and soil application at sub-lethal concentrations may also have an advantageous stimulation effect on shoot growth of numerous crops. DCPA may be effective in formation of proliferated tissue necessary for root and shoot. Future research should definitely be conducted in this area.

At higher temperatures annual ryegrass was able to overcome much of the toxicity exerted on it by DCPA at higher rates. Three weeks after planting, annual ryegrass yielded only 12 percent of the check at the 2 parts per million rate, while seven weeks after planting the same concentration had increased up to 69 percent of

the check as compared to 34 percent at the 60°F. level.

Weedy grasses which escaped the initial toxicity of DCPA may be able to overcome much of the toxicity effect as the soil temperature increases in late spring and early summer. Recovery under field conditions, however, may depend on the concentration of DCPA remaining in the soil. If the DCPA concentration was at levels inhibitory to root growth, little recovery would be expected.

DCPA degradation under controlled soil temperature and under fall and winter soil conditions in the field

Results from a study conducted on DCPA degradation under controlled temperatures indicate that DCPA was broken down faster under 90°F. soil temperature as compared to 70°F. The half-life of DCPA was 105 days at the 90°F. level and 155 days for the 70°F. temperature level. There was no detectable DCPA breakdown at 50°F. There was no means of determining whether degradation was due to microbial or chemical means. Both means of breakdown would be expected to be greater at 90°F. soil temperatures than at the 70°F. temperature levels. These results would indicate a considerably shorter residual life under field conditions where the soil is quite warm. This is believed to be a partial explanation for inadequate mid-summer weed control from early spring applications of DCPA.

The greatest amount of DCPA loss in the field experiments occurred during the months of October and January. These were the clearest and warmest months during the breakdown study, which may explain the increased amount of loss during these two months.

There was no significant herbicidal loss during the months of November or December. The soil during these months was quite wet and frozen much of the time, which would explain the lack of herbicide loss during this period.

Future experiments should be designed to determine the magnitude of herbicide loss through volatility, microbial, and chemical means.

SUMMARY

Studies were conducted to determine DCPA toxicity to various plant parts. The influence of soil temperature on DCPA toxicity to annual ryegrass was investigated. DCPA degradation under greenhouse controlled temperatures and under field conditions was studied. The following results were obtained:

1. DCPA was more phytotoxic to annual ryegrass when the coleoptiles emerged through treated soil than when roots penetrated into treated soil.

2. There was a direct correlation between extent of injury and depth of treated soil through which the coleoptile emerged, suggesting more efficient control by shallow incorporation. There was also a direct relation between injury and the concentration of DCPA in the soil through which the coleoptile emerged.

3. When only primary and secondary roots were exposed to the treated soil, there was no significant shoot reduction at rates up to 128 ppm. Root growth was drastically reduced when they grew in treated soil without any apparent shoot injury. Plants whose roots were drastically reduced from DCPA injury would be more susceptible to drought.

4. No phytotoxic effect resulted when seeds germinated in DCPA concentrations sufficient to injure coleoptiles; injury, however, did occur once the coleoptile had emerged from the seed.

5. There was a significant stimulation effect on shoot growth at 72°F. in plots treated with DCPA at 1.0 ppm. Root stimulation

occurred in all temperatures at the 1 ppm level. The greatest overall stimulation was received at the 72°F. temperature level. At several rates DCPA was more toxic to annual ryegrass at the 84°F. and 60°F. temperatures than at the 72°F. temperature. This indicates a greater susceptibility at temperatures higher or lower than optimum for ryegrass growth.

6. Annual ryegrass was able to overcome the DCPA toxicity more readily at 84°F. than at 60°F. with 72°F. being intermediate.

7. DCPA in the greenhouse was degraded faster at 90°F. than at 70°F. with no detectable breakdown at 50°F. DCPA loss in the field was believed to have occurred from surface washing and leaching during the rainy season, volatilization, and herbicide degradation.

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APPENDIX

APPENDIX

The detailed solvent extraction process for removal of DCPA and degradation products from soil are presented in the following section.

Method for extracting DCPA
and degradation products from soil samples

Procedure

1. Pour 100 grams of soil into a 500 ml Erlenmeyer flask.
2. Add 250 mls of reagent grade acetone plus 10 mls of a .5 normal H_2SO_4.
3. Place a rubber stopper in the Erlenmeyer flask and place the flask on a shaker for about two hours.
4. Remove the flask and allow the soil to settle.
5. Decant 50 or 100 mls of the aliquot. The amount decanted should be recorded.
6. Evaporate the aliquot to near dryness with the aid of a steam bath.
7. Add 50 mls of distilled water and pour into a separatory funnel.
8. Add equal volumes of ethyl ether and shake well and decant. Add another 50 mls of ethyl ether to the water phase and repeat the process and discard the water phase.
9. Pass the ether phase through a crucible stopped with glass wool, containing anhydrous sodium sulfate (granular reagent grade).
10. Evaporate to dryness. The residue remaining contains dimethyl 2,3,5,6-tetrachloroterephthalate (1/2 acid) and 2,3,5,6-tetrachloroterephthalic acid (acid).