THE RELATIVE TOXICITY OF CERTAIN PHENOLIC DERIVATIVES TO THE ROOTS OF MAJOR CROP AND WEED PLANTS

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Introduction and Literature Review

The application of residual dosages of herbicides to soil after planting but before crop emergence to prevent the development of many kinds of weed seedlings has been the subject of much field experimentation during recent years. This type of application is called residual pre-emergence weed control. There have been a number of reports of experiments on the use of 2,4-dichlorophenoxy-acetic acid for residual pre-emergence weed control in corn. Also of current interest among weed-control research workers is the use of residual dosages of certain phenolic compounds for pre-emergence control of many small-seeded weeds in certain large-seeded crops including cotton, corn, beans, the cucurbits, and crops grown from roots, tubers, corms and other vegetative organs. (1)(3). Pentachloro-phenol (hereafter called PCP) and 4,6-dinitro-o-sec.butyl phenol (hereafter called DNOSBP) and their salts are the phenolic compounds that have been used extensively for pre-emergence experimentation because of their known high degree of foliage toxicity. (6) (7).

In Hawaii the sodium salt of PCP has been used as a ground spray between rows of established sugar cane to prevent weed seedlings from emerging. The phenolic compounds have shown promise as post-emergence residual ground sprays between rows of a number of growing crops including cotton, corn and nursery stock.

Field observations (1) have indicated that the large-seeded crops which have been successfully treated with residual pre-emergence dosages are protected in part by depth of planting. Roots of weeds germinating near the surface appear to be markedly affected while the deeper crop plant roots are generally below the level of toxic soil. The primary shoot apparently does not absorb toxic quantities as it pushes up through soil containing the toxicant. Weeds that do emerge with the crop have been observed to frequently come from a depth similar to that of the crop.

Although depth protection is believed to be an important factor in selectivity, field observationsby the writer in 1948 indicated clearly the occurrence of specificity of reaction between the different phenolic compounds and certain plants. Thus lamb's quarters (Chenopodium album) appeared relatively susceptible to DNOSBP and pigweed (Amaranthus retroflexus) relatively susceptible to PCP. Cotton has been reported to be less subject to injury by weed-control dosages of DNOSBP than by equally effective dosages of PCP. (5)

Exploratory tests with seeds grown in petri dishes with varying concentrations of the ammonium salt of DNOSBP (hereafter called NH_4DNOSBP) and the sodium salt of PCP (hereafter called NaPCP) showed wide species differences in reaction. Thus the field observation that diversity of physiological response to the different chemicals is a probable factor in selectivity was further confirmed.

The experiments herein reported were conducted to determine the relative toxicity of several phenolic compounds to the roots and seeds of crop and weed species under conditions divorced from the variables inherent in field tests. These toxicity data were considered desirable for the purpose of determining with which crops the different compounds would be relatively safe for residual pre-emergence weed control if depth protection under a given set of soil conditions was not adequate for selective action. It was also considered desirable to determine whether any small-seeded crops that must be planted at a relatively shallow depth possess enough tolerance to the phenolic compounds to permit use for residual preemergence treatment.

Small dosages of these chemicals in general contact

formulations have been used as contact pre-emergence sprays to kill tiny weed seedlings that emerge before the crop. Although the necessity for very accurate timing makes this technique of limited use it is very promising for certain slow-emerging crops that will not tolerate larger residual dosages. Even the minimum amounts required for contact killing of weed seedlings may sometimes leave enough residue to injure certain crops. Accurate toxicity data were needed as a basis for determining the relative safety of the different phenolic compounds for contact pre-emergence spraying of a given crop.

In addition to DNOSBP and PCP and their salts several other phenolic derivatives were included to determine their possible value for residual soil treatment. To facilitate a better understanding of how these compounds prevent weed seed emergence when used as residual pre-emergence herbicides observations were made on the nature of their toxicity to roots and seeds.

Experimental Technique

The foliage toxicity of various phenolic compounds has been compared in numerous tests but no experiments on root and seed toxicity other than pre-emergence tests in soil have come to the writer's attention. A laboratory

technique for determining relative toxicity had to be developed.

Exploratory tests in which seeds were planted in dry quartz sand and the containers flooded with the toxic solutions did not prove satisfactory. Many seedlings emerged even though the roots were badly stunted and emergence counts alone did not reveal the degree of toxicity. Removal from sand to examine roots proved difficult.

The work of Swanson (12) and Ready and Grant (10) suggested in vitro tests using solutions varying in concentrations. After preliminary trials petri dish culture was decided upon as best suited to a rapid screening program. This technique made possible the frequent and critical examination of roots, and space and labor requirements were kept at a minimum.

Several trials with lettuce, tomato, bean and corn as test species indicated that solutions would have to be replenished to insure uniform contact of the developing roots with the toxicant. Otherwise some roots grew into the air above the moist filter paper with little evidence of toxicity. By flooding the seed with the test solution, removing the excess after a few hours and then re-flooding and draining at intervals of 30 to 40 hours thereafter, roots were kept in contact with the toxicant. Variations due to

uneven drying were also minimized.

After numerous measurements with several species of length and weight of both root and top growth, root length was chosen as the best criterion of toxicity. Recording individual root measurements on a calculating machine and entering only the total and mean length for each dish on record sheet was found convenient.

Sample Size.

Root lengths for three replicates of fifty lettuce seedlings each were recorded individually for the purpose of obtaining a statistical estimation of the size of samples and number of replicates needed for a reasonable degree of accuracy. The formula used for this determination and its application has been discussed by Henry, Down and Baten. (8)

For the data analyzed 3 dishes containing 14 seeds each or 4 dishes containing 11 seeds each were found to give a degree of accuracy within 10 percent of the general mean x the Standard Error of the individual means.

Populations of other species or even other seed samples of the same species could not be assumed to exhibit the same degree of uniformity, and the results

of this analysis were considered only as a guide pending experience with additional material. Four plates of ten plants each were decided upon for further work until Standard Errors of various treatments for a number of species could be calculated in order to determine the range in variability one might expect. Measuring a total of 40 roots per treatment was not considered too time consuming.

Pre-germination of Seed.

A high degree of varability between plants in the control of some populations with respect to time of germination suggested selection of samples grown in a germinating dish until radicles emerged as a method of decreasing variability. Comparisons were made with lettuce, corn and onion of the method used heretofore involving the treatment of dry seed vs. pre-sprouting and the selection of uniform samples with short radicles for subsequent treatment. Quadruplicate 10-seed samples were treated with distilled water using each of the two methods. In all cases small or dis-colored seeds were eliminated. The data are presented in Table 1.

Table 1. Mean Root Length and S. E. values for Four Ten-Seed dishes for Each of Three Crops Treated Dry and After Pre-germination.

	Treated Dry	Pre-germinated
Corn	92.6 ± 16.1	98.0 ± 6.3
Lettuce	19.4 ± 4.6	22.1 ± 1.0
Onion	22.5 ± 8.5	24.3 ± .6

The Standard Error values in Table 1 show that variability was decreased with all three species when pregerminated seeds were used. The greater mean root length for the pregerminated treatments is due largely to the complete lack of seeds showing no germination or slow germination. Such seeds were present in many of the dishes in which dry seeds were treated. All three samples germinated 90 percent or better according to germination test data supplied by the seedsman from whom they were procured. Even a greater decrease in variability might be expected from the pre-germination technique if samples of lower vitality were used.

Effect of Temperature.

In order to determine whether temperature uniformity was required to obtain comparable results for different tests a comparison was made of Great Lakes lettuce at two temperatures with NaPCP and at four temperatures with NH_4DNOSBE

In addition Red Kidney beans were grown at two temperatures following treatment with NaPCP. The temperatures used covered the range through which the species involved will germinate and grow. Root length was expressed as a percentage of the control for each temperature in order to compare relative degrees of inhibition at different temperatures.

Although differences were noted between root length means for different temperatures at a single concentration, consistent trends were suggested only for the lowest temperature for lettuce and the highest temperature for beans treated with NH_4DNOSBP. As these differences were not great and the temperatures involved were extreme, the conclusion was drawn that the fluctuations in temperature ordinarily encountered under room conditions are unlikely to introduce an appreciable error in conducting root toxicity tests with phenolic compounds according to the technique outlined above. A constant temperature chamber used for holding petri dishes in some of the later tests proved desirable from the standpoint of leveling the time required for growth of the untreated roots to a given length.

Effect of Light.

In view of the findings of Weintraub (13) that the inhibitory effect of several substances on the growth of lettuce was lessoned in the presence of light it was consi-

dered desirable to compare results with cultures grown in light and in dark at a constant temperature. The Great Lakes variety of lettuce was used and the lighted cultures exposed to a 100-watt incandescent lamp at a distance of five feet. The unlighted cultures were placed on the same shelf in a temperature control chamber which varied from 65° to 68°F. and covered with black paper. The light was sufficient to induce marked chlorophyl development in the cotyledons while those of unlighted cultures were yellow.

There were no differences of significance between the lighted and unlighted cultures with either NaPCP or NH_4DNOSBP. This test does not preclude the possibility that at greater intensity a light effect might occur. In view of these results it seems unlikely that variations in light under room conditions would introduce an appreciable error.

Lettuce is among the more light sensitive species with respect to germination and was therefore oonsidered a good plant for this test. The possibility that light might affect the sensitivity of other species to these compounds was investigated only by cursory observation of bean, corn, and Kentucky blue grass treated with various concentrations of NaPCP. No influence of light on root inhibition was indicated.

The Effect of Seed Vitality

Considerable variation in time of germination occurred with most seed lots when being pre-germinated. The question arose as to the relative toxicity of the phenolic compounds under investigation to the early vs. the late germinating seeds within a given sample. Error could conceivably be introduced if seeds sprouting quickly and those somewhat delayed in germination were divergent in their degree of sensitivity.

In order to elucidate this point a test was made of onions taken from a germinating dish at intervals. The first lot was drawn when the seeds sprouting first had radicles 2 to 5 mm. long. A second lot consisting of seeds in a similar stage of development was drawn 30 hours later and a third lot 72 hours after the first. Treatments were made with NaPCP with the concentrations indicated in Table 2. The petri dishes were placed in a 65°-68°F. temperature control room and each lot was removed and measured after it had been held 120 hours after the first treatment. Thus each lot had the same opportunity for root elongation. Table 2 presents the results of this test.

Table 2. Root Length in mm. of Three Lots of Brigham Yellow Globe Onion Varying in Time of Sprouting After Growing for 120 Hours at 60-63°F. Following Treatment with NaPCP. Each Value is the Mean of Three 10-Seed Samples

Concentration (PCP equivalent)	1st. Seeds to Sprout	Seeds Sprouting 30 Hours Later	Seeds Sprouting 72 Hours After First
Control	29 ± 1.0	28 ± 1.6	24 ± 2.3
1 ppm.	22 ± .8	23 ± 1.0	18 ± 1.6
2 ppm.	16 ± .9	15 ± .8	12 ± 1.1
4 ppm.	14 ± 1.0	13 ± .2	11 ± 1.6
8 ppm.	7 ± .6	9 ± .4	9 ± .8

A variance analysis was run to determine the significance of differences between the three lots drawn at successive intervals from the germinating dish. The analysis dataare presented as follows:

Source of Variation	D.F.	Sums of Squares	Variance	F
Total	14	694		
Treatments	4	650		
Sprouting Time	2	27	13.5	6.4
Error	8	17	2.1	

Tabular F value for 1% level = 5.28

Differences between the first and second lots were obviously of no significance. In view of the highly significant F value differences between lot three drawn 72 hours after the first radicle emergence must differ significantly from the other lots at the 1% level. It is of further interest that the Standard Errors of the means of the 72

hour samples were consistently higher indicating greater variability. Based on this test it was decided to draw samples from the germinating dishes no longer than 24 hours after the first radicles emerged.

Conceivably seed vitality factors unrelated to speed of sprouting could cause variation in sensitivity to phenolic compounds. The question arose as to the likelihood of error resulting from the use of a weak lot of seed having limited capacity for root growth. High and low germinating lots of seed were obtained for the same variety of beans and onions and subjected to a replicated test. No differences of significance between means resulted in tests with high and low germinating samples of the same species. However, Standard Error values for the low-germinating samples tended to be greater than for the high-germinating seed indicating greater variability. Care was therefore taken to procure seed samples having a high germination percentage for subsequent tests.

Summary of Technique.

Based on experience gained in preliminary tests with several species and on the experiments discussed above the following method was used in subsequent work.

1. A seed sample of good vitality was germinated on moist blotters in covered dishes held at a temperature suitable for germination of the species involved. When required for the prevention of mold sodium hypochlorite solution was used for surface sterilization of the seed.

2. Within 24 hours after the first seeds showed a radicle, uniform samples were transferred to petri dishes fitted with a filter paper over which had been poured the solutions to be tested. Radicle length varied from two to ten millimeters depending on the species. Excess liquid was drained from the dishes within one-half hour after transfer. Seeds were placed so all root tips were touching the moist filter paper.

3. Except as noted ten sprouting seeds were placed in each dish. Three or more replications of each treatment were made according to the circumstances as discussed for each experiment.

4. Preliminary tests to establish the toxicity range for the species involved were made as required. A geometric progression of concentrations beginning with one-half part per million was used. Concentrations were calculated on a parent phenolic compound equivalent basis in all instances. Each set of treatments included a control to

which only distilled water was applied.

5. At intervals of 30 to 40 hours following transfer the solutions were replenished with enough to cover the bottom of the dish. All roots were then wet by tipping the dish from side to side. After 15 to 30 minutes the excess liquid was drained off and the roots brought in contact with the moist paper in so far as possible. The liquid changes were usually made twice between the time of transfer and measuring roots.

6. The dishes were held at room temperature on a table. Measurements were taken after considerable growth of the control had occurred and after an apparent deceleration of growth indicated that food reserves were approaching depletion. For the most part, roots were measured from five to seven days after transfer.

7. Root measurements were made by removing one seedling at a time and stretching the root along a millimeter scale. Each root length was entered on an adding machine and the means of the ten plant samples rounded off to the nearest unit were recorded. Accuracy of measurement was estimated to be within ten percent for the species with short roots but undoubtedly increased with root length.

8. Variability was calculated as the Standard Error of the mean and expressed to the first decimal place. When S. E. values indicated excessive variability within a treatment another replicate for that treatment and the control were grown and measurements made after the same interval used in the original test.

9. In order to compare relative toxicities of a compound to different species and to compare tests on the same species that might vary somewhat in the root length of the control root length of the various treatment means was expressed as a percentage of the control. The Standard Error of the means were calculated wherever a measure of variability was needed.

Toxicity Curves for Some Representative Species

A suitable index of toxicity was needed to facilitate comparisons between chemicals on a given species and between species with a given chemical. To explore the possibility of establishing such an index, tests were made on eight species with both NaPCP and NH_4DNOSBP. Based on field results and preliminary laboratory tests these eight species were believed to vary in their degree of sensitivity to the chemicals. Three members of the Gramineae family having different seed sizes were chosen because of the postulated importance of seed size to selectivity under field conditions. Each of the remaining five species represented a different plant family.

Preliminary trials established the toxicity range for each species. A geometric progression of concentrations ranging from a point near the threshold to one near complete inhibition was then employed. The technique described earlier was used throughout. Four replicates were run and the variation expressed as the Standard Error of the mean. Root measurement data for each of the eight species are expressed as a percentage of the control in table 3 and are presented graphically in figure 1.

Table 3. Root length data expressed as a percentage of the control.

Compound and conc.	crop species: Kentucky Blue Grass	Barley	Corn	Lettuce	Alfalfa	Onion	Cucumber	Radish
Control	100±3.3	100±3.3	100±5.5	100±5.1	100±5.2	100±2.9	100±4.5	100±2.2
Na PCP (PCP equiv.)								
1 ppm	78±2.8	-	-	86±.4	-	71±4.6	-	-
2 ppm	61±2.2	100±5.2	-	73±1.8	81±6.2	58±2.5	92±3.7	94±2.0
4 ppm	50±5.0	71±4.2	88±3.4	41±2.7	66±5.2	50±3.3	84±.9	74±4.0
8 ppm	22±1.7	54±5.4	64±4.0	27±1.8	52±2.4	29±3.7	40±2.2	35±2.7
16 ppm	11±1.7	43±3.8	43±2.0	14±2.3	38±1.9	13±2.5	29±1.2	19±1.3
32 ppm	-	22±1.3	23±1.3	-	14±2.4	-	16±1.1	14±1.4
64 ppm	-	12±1.4	14±.8	-	-	-	-	-
128 ppm	-	-	10±1.5	-	-	-	-	-
NH_4DNOSBP (DNOSBP equiv.)								
1/2 ppm	-	-	-	82±2.3	-	-	-	-
1 ppm	89±3.9	103±3.8	-	50±2.7	100±5.2	75±2.5	-	74±2.7
2 ppm	72±6.1	74±5.4	95±3.6	32±3.6	62±5.2	75±7.0	95±1.7	45±4.1
4 ppm	33±2.2	63±4.7	77±2.9	18±1.8	24±2.9	54±2.5	81±5.5	21±3.9
8 ppm	22±3.3	35±2.8	52±3.1	9±1.3	19±2.9	29±2.5	59±1.8	13±3.9
16 ppm	17±2.7	18±1.7	23±1.6	-	14±2.4	13±.4	40±1.3	6±3.2
32 ppm	-	13±1.8	16±.6	-	-	-	21±1.6	-
64 ppm	-	-	9±1.3	-	-	-	-	

The actual threshold was not experimentally determined, but by projection of the curves an approximation may be visualized. Because roots were allowed to begin growth before treatment the point of complete inhibition was not zero but fell between five and ten percent of the control. Where the highest concentration did not cause complete inhibition extrapolation at the lower end of the curve will provide an estimation of this extreme toxicity level.

It will be seen from figure 1 that all regressions tend to be curvilinear with the slope decreasing with an increase in concentration. The equation of the regression appears to follow that for the exponential growth curve:

$$y = ax^b$$

When the data were plotted on logarithmic paper it was found that the regression tended to be linear, thus further indicating that the root toxicity curves for phenolic derivatives probably fit the exponential equation.

There is similarity between the curves presented in figure 1 for root toxicity to those presented by Blackman, Holly and Roberts (4) for foliage toxicity from dinitro phenolic compounds. Ivens and Blackman (9) have obtained similar curves by plotting root growth of corn against concentration of pentachlorophenol.

Among possible indices of toxicity the concentration in ppm. required for a given reduction in root length was considered most desirable because the sensitivity of different species to a given chemical could be readily compared as well as the toxicity of different chemicals to a given species.

Root inhibition equivalent to 25 percent of the control was first considered desirable because this degree of inhibition was generally lethal. However, interpolation at this level was found to be less accurate than at lower concentrations because of the decreasing slope of the curve. Furthermore, greater variability at higher concentrations, as indicated by higher Standard Error values, increased the liklihood of error in the curves at their lower end. Root inhibition equivalent to 50 percent of the control was finally chosen as a suitable index of toxicity.

Thus, the Toxicity Index for a phenolic derivative on a given species equals the concentration, in parts per million, required to inhibit root length to 50 percent of the control as determined by the technique outlined above. This concentration was determined by graphic interpolation from curves fitted to the data. The toxicity index was estimated to the closest unit for values above 8 ppm. and to the closest five-tenths unit for values below 8 ppm.

The Toxicity Indices obtained by interpolation of the graphs in figure 1 for the two compounds are presented in table 4.

In order to determine the reproducibility of these tests, solutions equal in concentration to the Toxicity Indices were used on each of the eight species and the actual reduction in root length determined. These data are presented in table 4.

Table 4. Root length expressed as a percent of the control for eight species treated with concentrations of NaPCP and NH_4DNOSBP equivalent to the Toxicity Index.

Crop Species	NaPCP		NH_4DNOSBP	
	tox. index	root length	tox. index	root leng
Kentucky blue grass	3.5	55±3.0	2.5	58±30
Barley	9	56±2.3	5	49±2.9
Corn	13	58±3.8	8	56±2.5
Lettuce	3	42±1.1	1	45±1.8
Alfalfa	9	42±2.3	2.5	69±8.1
Onion	3.5	41±5.3	4.5	53±5.9
Cucumber	7	40±2.7	10	46±2.0
Radish	6	48±3.2	2	57±3.7

It will be seen from Table 4 that the root inhibition obtained varied from the expected value of 50 percent by as much as 19 percent in the case of alfalfa treated with NH_4DNOSBP. Some measure of reliability was obviously needed. Except at higher concentrations the results of which did not materially effect the interpolated value the Standard Errors in table 3 seldom exceeded ten percent of the mean. The interpolated value of 50 percent was assigned an arbitary S. E. error value of 10 percent on the assumption that this interpolated value should be no more variable than the experimental values.

Using the conventional test for significance the values in Table 4 differing significantly from +50 $\pm$ 5 and the pairs differing significantly from each other were determined.

It was found that the only value differing significantly from 50 $\pm$ 5 at the 5 percent level was that of alfalfa treated with NH_4DNOSBP. The only pair differing significantly from each other at this level was the alfalfa treatments. It was, therefore, concluded that the results presented in Table 3 and in figure 1 were repeated within a reasonable degree of accuracy for seven species but not for alfalfa.

A further test was made with alfalfa using four concentrations for each chemical. Results did not agree with the first test and new curves were drawn as presented in figure 2 Using concentrations equal to the Toxicity Indices of 5.5 for NaPCP and 3.5 for NH_4DNOSBP treatments were made for the purpose of checking the accuracy of the new curves. Root length values expressed as a percentage of the comparative control were obtained as follows: NaPCP 48 $\pm$ 2.4, NH_4DNOSBP 52 $\pm$ 1.6. Thus the second curves were found to be more accurate and the Toxicity Indices of 5.5 for NaPCP and 3.5 for NH_4DNOSBP should be considered reliable rather than those given in Table 3. No explanation for the obvious error in the first alfalfa test is offered.

Toxicity Indices for Crop and Weed Species

The Toxicity Indices for a number of crop and weed species were determined by interpolation from curves fitted to data for three or more concentrations of each chemical.

Description of Experiments

The concentrations used were chosen after a trial run and in each case, bracketed the 50 percent degree of inhibition. Three 10-seed replicates were made for the control and each of the three concentrations except in a few instances as noted. Spinach was grown in 20-seed lots because of extreme variability. Sugar beet which was also quite variable was grown in 20-seedball lots and the twenty longest seedlings in each dish were measured. Because of germination difficulties data for chickweed is based on 5-seed samples replicated three times and for crabgrass on 5-seed samples replicated five times. Table 5 presents the Toxicity Indices for the species tested for NH_4DNOSBP and NaPCP.

The species marked with an asterisk were subsequently tested with solutions equal to the Toxicity Indices in order to check the results of the first test. Root inhibition from these concentrations did not vary significantly from 50 $\pm$ 5 nor did they vary significantly from each other except in the case of tomato and carrot. An entirely new

test of these two was made and curves modified to fit the new data. The Toxicity Index given in Table 5 for these two represents the second test.Toxicity Indices for the eight species discussed earlier are included.

To facilitate comparing the relative sensitivity of different species to the two compounds the quotient of the Toxicity Index for NaPCP divided by the Toxicity Index for NH_4DNOSBP was calculated for each species. These quotients are presented in the right hand column of Table 5 and will hereafter be designated as the PCP/DNOSBP quotients.

Table 5. Toxicity indices for NaPCP and NH_4DNOSBP on several crops and weeds arranged according to family with the PCP/DNOSBP quotient.

Species	Common name	Variety	Toxicity Index NaPCP	Toxicity Index NH_4DNOSBP	PCP/DNOSBP quotient
Gramineae					
Digitaria sanguinalis	Crabgrass		3	2.5	1.2
Panicum ramosum	Millet		8	4.5	1.8
Setaria lutescens	Yellow Foxtail		5.5	4	1.4
*Poa pratensis	Kentucky Blue Grass		3.5	2.5	1.4
*Lolium sp.	Domestic Rye Grass		7	5.5	1.3
Avena sativa	Oat	Kent	14	7	2.0
*Triticum vulgare	Wheat	Yorkwin	5.5	3	1.8
Triticum vulgare	Wheat		7	3.5	2
Secale cereale	Rye	Rosen	7.5	3.5	2.1
*Hordeum vulgare	Barley	Bay	9	5	1.8
*Zea mays	Corn	Ohio M15	13	8	1.6
Zea mays	Corn	Iona	14	8	1.7
Amaranthaceae					
Amaranthus sp.	Tampala		6.5	2.5	2.6
Amaranthus retroflexus	Red Root Pigweed		8	6	1.3
Alsinaceae					
Stellaria media	Common Chickweed		7	3	2.3
Liliaceae					
Allium cepa	Onion	Brigham Yellow Globe	4.5	3.5	1.3
Allium cepa	Onion	White Portugal	3	3	1.0
Chenopodiaceae					
*Beta vulgaris	Sugar beet		4.5	3	1.5
Spinacia oleracea	Spinach	Giant Thick Leaved	3	2	1.5
Chenopodium album	Lambsquarters		10	4	2.5
Umbelliferae					
*Daucus carota	Carrot	Streamliner	5	5	1.0
Cruciferae					
Raphanus sativus	Radish	Early Scarlet Globe	6	2.5	2.4
Raphanus sativus	Radish	Crimson Giant	6	2	3.0
Brassica rapa	Turnip	Purple Top White Globe	6	2	3.0
*Brassica oleracea	Cabbage	Golden Acre	10	3	3.3
Brassica oleracea	Cauliflower	Early Snowball	8	2.5	3.2
Brassica Kaber	Wild Mustard		8	3	2.7
Linaceae					
Linum usitatissimum	Flax	Dakota	6	10	.6
Linum usitatissimum	Flax	Crystal	3	4	.7

Table 5 continued.

Leguminosae					
*Trifolium repens	White Clover	Common	10	16	.6
Trifolium pratense	Red Clover	Mammoth	11	9	1.2
*Trifolium pratense	Red Clover	Medium	12	15	.8
Trifolium hybridium	Alsike Clover		8	4	2.0
*Medicago sativa	Alfalfa	Grimm	5.5	3.5	1.6
Lespedeza japonica	Lespedeza	Kobe	10	14	.7
*Lespedeza stipulacea	Lespedeza	Korean	16	20	.8
Vicia villosa	Vetch	Hairy	20	19	1.0
Pisum sativum	Pea	Thomas Laxton	18	8	2.3
Phaseolus vulgaris	Bean	Michelite	21	12	1.7
*Phaseolus vulgaris	Bean	Stringless Green Pod	16	11	1.5
Vigna sinensis	Cow pea	Clay	9	6	1.5
Glycine soja	Soy bean	Manchu	13	9	1.4
Phaseolus lunatus	Lima Bean	Henderson Bush	13	11	1.2
Solanaceae					
Lycopersicum esculentum	Tomato	Garden State	7	5	1.4
*Capsicum frutescens	Pepper	California Wonder	8	7	1.1
Solanum melongena	Eggplant	Black Beauty	11	11	1.0
Polygonaceae					
*Fagopyrum sagittatum	Buckwheat		2	3.5	.6
*Polygonum persicaria	Lady's Thumb		2	4	.5
Malvaceae					
Gossypium hirsutum	Cotton	Delta Pine 15	22	17	1.3
Gossypium hirsutum	Cotton	Stoneville 2B	25	18	1.4
Hibiscus esculentus	Okra	Clemson Spineless	12	12	1.0
Cucurbitaceae					
Cucumis sativus	Cucumber	Straight 8	8	10	.8
*Cucumis sativus	Cucumber	White Spine	7	10	.7
Citrullus vulgaris	Watermelon	Dixie Queen	15	16	.9
Cucurbita maxima	Squash	Hubbard	12	12	1.0
*Cucumis melo	Muskmelon	Honey Rock	8	12	.7
Compositae					
Lactuca sativa	Lettuce	Grand Rapids	4	1.5	2.7
Lactuca sativa	Lettuce	Great Lakes	3	1	3.0
Cichorium intybus	Wild Chicory		5	3	1.7
Ambrosiaceae					
Ambrosia elatior	Ragweed		3.5	2	1.7

Discussion of data in table 5

In general there is a trend toward higher Toxicity Indices for the larger seeded crops, however there are enough exceptions to preclude generalizations. Buckwheat for example has a fairly large seed yet shows a low toxicity index particularly toward NaPCP. Among the small seeded crops white and red clover and the lespedezas show remarkable resistance to both compounds in contrast to other small seeded legumes.

The trend toward greater physiologic resistance on the part of large seeded species can hardly account for the marked selectivity noted in field tests. Depth protection of the roots coupled with lack of absorption by top growth as it pushes through the toxic surface layer must still be considered as important factors.

Those small seeded crops showing a high Toxicity Index to one or the other of the compounds should be given residual preemergence treatments on an experimental basis. Planting at as great a depth as possible would undoubtedly be desirable. Larger seeded crops not having a high Toxicity Index may still be adaptable to residual pre-emergence treatment but the risk would be greater under conditions of shallow planting, porous soil or heavy rainfall.

Probably the most significant information gained from these experiments concerns the marked species differences in response to the different chemicals as indicated by the varying PCP/DNOSBP quotients. Theoretically when this quotient is similar for a given crop and a given weed there would be no advantage of one material over another with respect to physiologic selectivity. If the quotient for the crop is lower than for a given weed NH_4DNOSBP should show the greatest selectivity. If the crop quotient is greater than that for the weed NaPCP should show the greatest selectivity.

Because weeds vary in their relative tolerance to these chemicals and many weed species are often present this line of reasoning has practical value only when the crop quotient is extreme. The cucurbits, onion, flax, okra, white clover, the lespedezas, carrot, egg plant, vetch and buckwheat all have quotients of 1 or less indicating a high relative tolerance to NH_4DNOSBP. Radish, turnip, cabbage, cauliflower, and lettuce have quotients of 3.0 or more. As several common weeds have lower quotients NaPCP should have greater selectivit for these crops with the type of weed complex often encountered Where the PCP/DNOSBP quotient for the crop varies from about 1.5 to 2.5 there is probably little choice of chemicals for pre-emergence treatments unless the dominant weed species is more sensitive to one material than the other. For example if the most prevalent weed is lady's thumb or similar polygonace

weeds, PCP should be used because of the extreme sensitivity of this group to this compound.

A crop quotient vs. weed quotient favorable to the use of a given chemical does not mean that a residual treatment has any chance of success. The actual crop tolerance and possibilities of deep planting must also be considered. It appears unlikely that any small-seeded crops that must be planted within an inch of the surface will prove adaptable to residual pre-emergence methods, however, field tests with the more resistant should be made. These data do suggest that for contact pre-emergence sprays PCP in oil or in emulsions may prove safer on crops with a high PCP/DNOSBP quotient. On the other hand crops with a low quotient should be less likely to show injury from the very small dosages used in contact pre-emergence applications from the application of DNOSBP in oil or in emulsion form.

Family trends in the PCP/DNOSBP quotient are rather consistent except for the legumes as will be noted in Table 5. No marked differences between varieties of a given species were found with respect to the PCP/DNOSBP quotient. The two varieties of flax appeared to differ markedly in their toleranc This difference may in part be due to differences in seed vitality as the Crystal variety was slow in germinating.

Screening of other phenolic compounds at concentrations equal to the Toxicity Index of NH_4DNOSBP or NaPCP as a standard for purposes of comparison suggests itself as a quick way to determine which compounds approach or exceed them in activity. The possibility of variation in tolerance between varieties or seed lots suggests the desirability of determining the Toxicity Index of the seed at hand for the standard compound before using this seed for routine screening of other compounds or derivatives.

Combinations of NaPCP and NH_4DNOSBP

Because of the wide species differences in response to the two compounds it was evident that a mixture might be of value as a residual pre-emergence spray for a weed complex composed of species of divergent sensitivity. In order to determine whether the two compounds are additive or possibly synergistic in their effect tests were conducted with eight species. Triplicated treatments were made with a solution composed of a concentration of each compound equal to one-half its Toxicity Index as listed in Table 5. The results of this experiment are presented in Table 6.

Table 6. Root length, expressed as a percentage of the comparative control, from treatments with a concentration equal to one-half the Toxicity Index for NaPCP plus one-half the Toxicity Index for NH_4DNOSBP.

Species	Root length expressed as percentage of control
Kentucky blue grass	55 ± 3.5
Barley	60 ± 2.9
Corn	58 ± 3.0
Lettuce	39 ± 3.2
Alfalfa	52 ± 1.6
Onion	53 ± 7.0
Cucumber	46 ± 4.0
Radish	57 ± 2.7

None of the values in Table 6 deviated significantly from 50 ± 5 at the 5 percent level. It was concluded, therefore, that the effects of the two compounds are additive.

Toxicity of Parent Phenolic Compounds vs. their Salts

Favorable field results with formulations of parent PCP and DNOSBP suggested the desirability of comparing the parent compounds with their salts in petri dish tests.

Because of its very weak acid properties PCP is released from solutions of NaPCP upon absorption of CO_2 from the air. With CO_2 being liberated from germinating seeds it appears likely that only the parent PCP is present shortly after solutions are poured on the dishes. Solubility of PCP in water is low but the limits were not exceeded except at the highest concentrations used.

Comparisons were made of fresh NaPCP solutions vs. a similar solution through which an excess of CO_2 had been bubbled. Comparisons were also made between NH_4DNOSBP solutions and solutions of the parent DNOSBP formed by the addition of just enough dilute H_2SO_4 to the salt solution to cause the yellow color to disappear indicating that the free DNOSBP had been liberated by the displacement of the ammonium ion. Triplicate petri dish tests were made on six species at concentrations equivalent to the Toxicity Indices for the respective compounds. These data are presente in table 7. It will be seen that no differences of significance were found between either parent compound and its salt.

Table 7. Mean root length in millimeters for a three petri dish test of parent phenols vs. their salts on six species each at a concentration equivalent to the toxicity index.

	PCP			DNOSBP		
Species	Concentration	Root length for sodium salt	Root length for parent PCP	Concentration	Root length for ammonium salt	Root length for parent DNOSBP
Buckwheat	2	33± 1.3	30 ± 2.6	4	40 ± 3.6	36 ± 2.1
Radish	6	37 ± 1.9	37 ± 2.4	2	42 ± 2.3	40 ± .9
Cucumber	7.5	32 ± 1.5	36 ± 2.7	10	41 ± 1.6	38 ± 2.0
Red Clover	11	14 ± .6	13 ± 1.0	9	19 ± .3	18 ± 1.0
Lettuce	3	11 ± .3	12 ± 0	1	15 ± .5	17 ± .6
Wheat	5	49 ± 1.6	43 ± 2.8	2.5	56 ± 2.0	58 ± 3.1

Comparison of NH_4DNOSBP with its para isomer.

Crafts (6) reported markedly greater foliage toxicity from NH_4DNOSBP than from its para isomer (ammonium salt of 2,6, dinitro 4 sec. butyl phenol referred to in Figure 3 as DNPSBP). A comparison of the root toxicity of the two isomers was made on four species using the same technique heretofore employed. These data are expressed graphically in figure 3.

The markedly greater root toxicity of the salt of the ortho isomer (DNOSBP) is evident with a glance at the figure. It is of interest to note that the curve for the salt of the para isomer (DNPSBP) slopes more gradually than that of the ortho isomer. Thus there is a greater percentage difference between the two isomers at higher than at lower concentrations.

Root Toxicity of Alkanol Amine Salts of DNOSBP

Pre-emergence field tests with the triethanol amine salt of DNOSBP in 1949 indicated that this derivative is equally effective as the ammonium salt. Because of certain advantages from the formulation standpoint alkanol amine salts are considered the most suitable DNOSBP derivatives for pre-emergence weed control. A commercial formulation placed on the market in 1950 by the Dow Chemical Company under the proprietory name Premerge contains three pounds of DNOSBP per gallon as alkanol amine salts of the ethanol and isopropanol series.

Root toxicity studies were made of several alkanol amine salts in comparison with the ammonium salt using the technique earlier developed. The concentration used for each species was its DNOSBP Toxicity Index. The results are presented in Table 8. The mixtures indicated in the table were commercially available alkanol amines. It will be seen that no differences of conceivable significance were found between the ammonium salt and any of the alkanol amine salts.

Table 8. Root Length of Four Species Grown in Solutions of Several Alkanol Amine Salts at Concentrations Equivalent to the Toxicity Index of DNOSBP. Root Length is Expressed as a Percentage of the Control.

DNOSBP Amine Salt	Radish 2 ppm.	Wheat 2,5 ppm.	Cucumber 10 ppm	Lettuce 1.5 ppm.
diethanol	51 ± 2.1	56 ± 3.6	47 ± 3.0	51 ± 2.6
triethanol	54 ± 1.0	52 ± 1.3	49 ± 2.1	46 ± 4.1
mixed diethanol and monoisopropanol	51 ± 3.6	58 ± .8	45 ± 6.2	50 ± 5.8
mixed mono ethanol and di-isopropanol	47 ± 4.1	60 ± 1.6	41 ± 3.1	48 ± 2.6
ammonium	52 ± 2.6	55 ± 4.2	48 ± 1.1	55 ± 5.2

Root Toxicity of the Sodium Salt of Dinitro-o-cresol

Crafts (6) found that dinitro-o-cresol was the least toxic to foliage of a series of homologous alkyl dinitrophenolic compounds. In the same tests DNOSBP was the most toxic. It was of interest to learn whether a similar difference between the two compounds exists with respect to root toxicity.

Tests were made with salts of DNOSBP and dinitro-o-cresol on four species at a concentration equal to the Toxicity Index for NH_4DNOSBP for each lot of seed used. The results are presented in table 9.

Table 9. Root Length of Four Species Grown in Solutions of the Sodium Salt of Dinitro-o-cresol and NH_4DNOSBP at the Toxicity Index of the Latter. Root Length Expressed as a Percentage of the Control.

Compound	Radish 2 ppm.	Wheat 3.5 ppm.	Cucumber 10 ppm.	Lettuce 1.5 ppm.
Na Dinitro-o-cresol	49 ± 1.9	86 ± 2.4	48 ± 2.5	115 ± 0.7
NH_4DNOSBP	53 ± 3.6	52 ± 1.5	59 ± 0.5	55 ± 2.5

It will be seen from table 9 that the salt of DNOSBP inhibited root growth of wheat, cucumber and lettuce more than did the salt of Dinitro-o-cresol. With radish there was slightly greater inhibition from the salt of dinitro-o-cresol however the difference was not significant. It is of interest to note an apparent stimulation to roots of lettuce from the concentration used of the salt of dinitro-o-cresol. This great diversity in relative response of roots of different species to the two homologous compounds is greater than has been noted for foliage reaction; however Barrons and Grigsby (2) and Blackman, Holly and Roberts (4) have reported specific differences in foliage reaction to the two compounds.

Root Toxicity of Tri-and Tetrachlorophenols

The sodium salts of 2,4,5-trichlorophenol and tetrachlorophenol were compared with NaPCP for root toxicity on 4 species. A concentration equal to the NaPCP Toxicity Index for each species was employed for all 3 compounds. The data are presented in Table 10. It will be seen that there tends to be a progressive increase in root toxicity with increasing chlorination which is in agreement with observations on foliage toxicity.

Table 10. Root Length Expressed as a Percent of the Control for 4 Species Treated with Sodium Salt Solutions of 3 Chlorophenols all at the NaPCP Toxicity Index for the Species Involved.

Compound	Radish 6 ppm.	Wheat 5 ppm.	Cucumber 7.5 ppm.	Lettuce 3 ppm.
2,4,5-trichlorophenol sodium, salt	72 ± 4.3	82 ± 6.1	78 ± 2.9	72 ± 3.9
tetrachlorophenol, sodium salt	54 ± 3.6	68 ± 5.0	60 ± 5.3	60 ± 5.6
NaPCP	46 ± 5.0	53 ± 4.6	52 ± 2.8	46 ± 5.0

Notes on the Nature of the Phytotoxicity of Phenolic Derivatives

Several observations were made during the course of these experiments on the apparent nature of the reaction of seeds and seedlings to the phenolic compounds. Although further experiments would be required to permit firm conclusions on the nature of the toxicity of phenolic compounds to roots, these observations are recorded here in the event that they will be of interest to those working with phenolic herbicides.

The herbicidal action of these compounds when used as foliar sprays is generally recognized as being of a contact nature. Little movement within the plant beyond the creeping action of the oil used as a solvent is believed to occur. Aqueous solutions of the salts of phenolic compounds used as selective sprays apparently kill tissue only beneath the surface that is actually wet with the spray. A few large droplets result in "burning" of holes in the leaf which may otherwise appear healthy.

In these petri dish tests top growth showed few signs of necrosis indicating that at the concentrations used there was little absorption through the cuticle. As discussed earlier in this report reduction in top growth at sublethal

concentrations was far below the reduction in root growth. This lack of apparent top effect on plants whose roots were markedly reduced in growth indicates that translocation from roots to tops is of little importance. With carrots it was noted that top growth showed signs of a toxic effect after the cotyledons had unfolded and the hypocotyl was two to three centemeters long. Whether this resulted from delayed absorption through the cuticle or translocation through the roots is not known. Lettuce and chicory showed some necrotic lesions on cotyledons at higher concentrations indicating cuticular absorgtion. The lack of top effect except in these instances agrees with field observations on the emergence of healthy seedlings of large seeded crops through a toxified surface layer of soil.

NH_4DNOSBP consistently caused chlorosis of the cotyledons of the Cruciferae even at dosages no greater than the equivalent of the Toxicity Index. NaPCP did not induce chlorosis on the same species even at concentrations causing an almost complete inhibition of root growth. With no other species was a similar chlorotic effect observed.

Concentrations up to those equivalent to the Toxicity Index seldom caused necrosis of roots during the first few days. Rate of root growth was retarded from the time treat-

ment was first made but roots generally had a healthy appearance, except for the lack of root hairs. Death of root tips was evident at higher concentrations within two days after treatment and at sub-lethal concentrations after a longer period. High concentrations completely inhibited further growth of sprouted seeds once they were treated with the solution. When dry seeds were placed directly in toxic solutions in the first exploratory tests growth was inhibited to about the same extent as when sprouted seeds were treated.

As noted earlier, roots had to be wet at intervals and kept in contact with the moist filter paper to insure uniformity of results. It was observed that sprouting seeds placed on the paper with the radicle pointing upward produced a root that appeared healthy as indicated by the presence of root hairs and lack of any dis-coloration. Apparently the toxicant was not absorbed through the contents of the seed in contact with the paper but enough water was absorbed for some growth. It was further observed that when only a part of the roots of the branching system of wheat and barley were in contact with the paper those suspended had a healthy appearance and developed root hairs.

To study further the localization of the effect of

phenolic compounds on root growth sprouting wheat seeds with roots approximately 1 cm. in length were so placed that a portion of the roots of each seedling grew into water and a portion into an 8 ppm. solution of NH_4DNOSBP. After 4 days the roots in the toxic solution had not elongated and were dis-colored. The roots growing in water had elongated three-to four-fold and showed no signs of injury. Top growth appeared to be in no way affected. See Figure 8.

The conclusion was drawn from these observations that root toxicity from these compounds is largely of a contact nature. This conclusion was substantiated by the fact that several crops with large seeds produced lateral roots rather freely if not re-wet with the solution even though the primary root had been completely inhibited. This recovery power of the larger-seeded species may be a factor in their ability to survive field treatment.

Suggested Further Research

Although these *In vitro* experiments cannot be used to predict field results with certainty they do indicate probable toxicities in soil. It is noteworthy that lady's thumb was observed to be hypersensitive to NaPCP in 1948 field plots. This observation was verified in petri dish

tests. Unless the two compounds are differentially adsorbed on soil colloids there appears to be no reason why the PCP/DNOSBP quotients reported above should not pertain in soil. Leaching studies to determine possible differences in downward movement in soil should prove fruitful.

Favorable results with lettuce at low temperatures indicate that practical weed control may be obtained from early spring applications thus confirming 1948 field observations. Further field work on temperature relationships is needed.

The diverse PCP/DNOSBP quotients obtained suggest the possible desirability of mixtures of the two compounds for field tests with different weed complexes. For general use a residual pre-emergence herbicide must prove satisfactory for a wide variety of weed species.

Field work designed to test the results obtained in these experiments should be conducted with particular reference to crops with extreme PCP/DNOSBP quotients. Small-seeded crops showing outstanding tolerance should be subjected to experiments with residual pre-emergence sprays.

In the course of the tests of parent compounds vs. their salts (Table 7) it was observed that a slight excess of acid caused a marked increase in the toxicity of a given

solution. Subsequent tests with lettuce and alfalfa confirmed these observations.

Simon and Blackman (11) have pointed out the effects of varying pH on the toxicity of phenolic compounds to fungi. Activity was increased with lower pH and in some experiments relative toxicities were greatly altered by pH changes. The relationship of soil pH to crop tolerance and weed control from residual pre-emergence treatment was beyond the scope of this research but should be investigated further.

Summary and Conclusions

1. A technique was developed for determining the toxicity of phenolic compounds to roots.

2. The relative toxicity to many species of the ammonium salt of 4,6-dinitro-O-sec. butyl phenol (NH_4DNOSBP) and of the sodium salt of pentachlorophenol (NaPCP) is presented.

3. A wide variation in species reaction to each of these chemicals was found to exist.

4. Relative toxicity to the two compounds varied from species to species.

5. Combinations of the two compounds gave only an additive effect.

6. The parent phenolic compounds and their salts were equal in root effect. No differences were found in root inhibition between a number of alkanol amine salts and the ammonium salt of DNOSBP.

7. NH_4DNOSBP was found to be considerably more toxic to roots than its para isomer which is in agreement with observations on foliage reaction.

8. The sodium salt of Dinitro-o-cresol was found to be considerably less toxic to roots of three out of the four species tested than NH_4DNOSBP.

9. Chlorophenols increased in root toxicity from tri- through tetra- through penta-substitutions.

10. The nature of toxicity to roots of the phenolic compounds was observed to be essentially of a contact type with little evidence of translocation.

LITERATURE CITED

1. Barrons, Keith C. Pre-emergence weed control. Down to Earth 4:(No. 3) 2-4 1948.

2. Barrons, Keith C, and Grigsby B.H. The control of weeds in canning peas with chemical sprays. Michigan Agricultural Experiment Station Quarterly Bulletin 28: 145-156, 1945.

3. Barrons, Keith C., Mullison, Wendell R., Fitzgerald, C.D., and Coulter, L. L. Certain phenolic compounds for residual pre-emergence weed control in horticultural crops. Proceedings North Central Weed Control Conference, Springfield, Ill. 5:34-39 1948.

4. Blackman, G.E., Holly, K. and Roberts, H.A., The comparative toxicity of phytocidal substances. Symposia of the Society for Experimental Biology 3:283-317 1949.

5. Cowart, L.E., Stamper, E.R., and Creasy, L.E. Studies on weed control in cotton. Proceedings of the Southern Weed Conference, Baton Rouge, La. 2:66-67 1949.

6. Crafts, A.S., A new herbicide 2,4-dinitro 6 secondary butyl phenol. Science 10-417-418 1945.

7. Crafts, A. S. General Contact Weed Killers, California Agricultural Extension Service Circular 137, Revised 1949.

8. Henry, G.F., Down, E.E., and Baten, W.D. An adequate sample of corn plots with reference to moisture and shelling percentages. Journal American Society of Agronomy 34:777-781 1942.

9. Ivens, G. W., and Blackman, G. E., The effects of Phenyl carbamates on the growth of higher plants. Symposia of the Society for Experimental Biology 3: 266-282 1949.

10. Ready, Daniel, and Grant, Virginia Q. A rapid sensitive method for determination of low concentrations of 2,4-dichlorophenoxyacetic acid in aqueous solution. Botanical Gazette 109: 39-44 1947.

11. Simon, E.W., and Blackman, G.E. The significance of hydrogen-ion concentration in the study of toxicity. Symposia of the Society for Experimental Biology 3:253-265 1949.

12. Swanson, Carl P., A simple bio-assay method for the determination of low concentrations of 2,4-Dichlorophenoxyacetic acid in aqueous solutions. Botanical Gazette 107;507-509 1946.

13. Weintraub, R.L. Influence of light on chemical inhibition of lettuce seed germination. Smithsonian Miscellaneous Collection 107:1-8 1948.

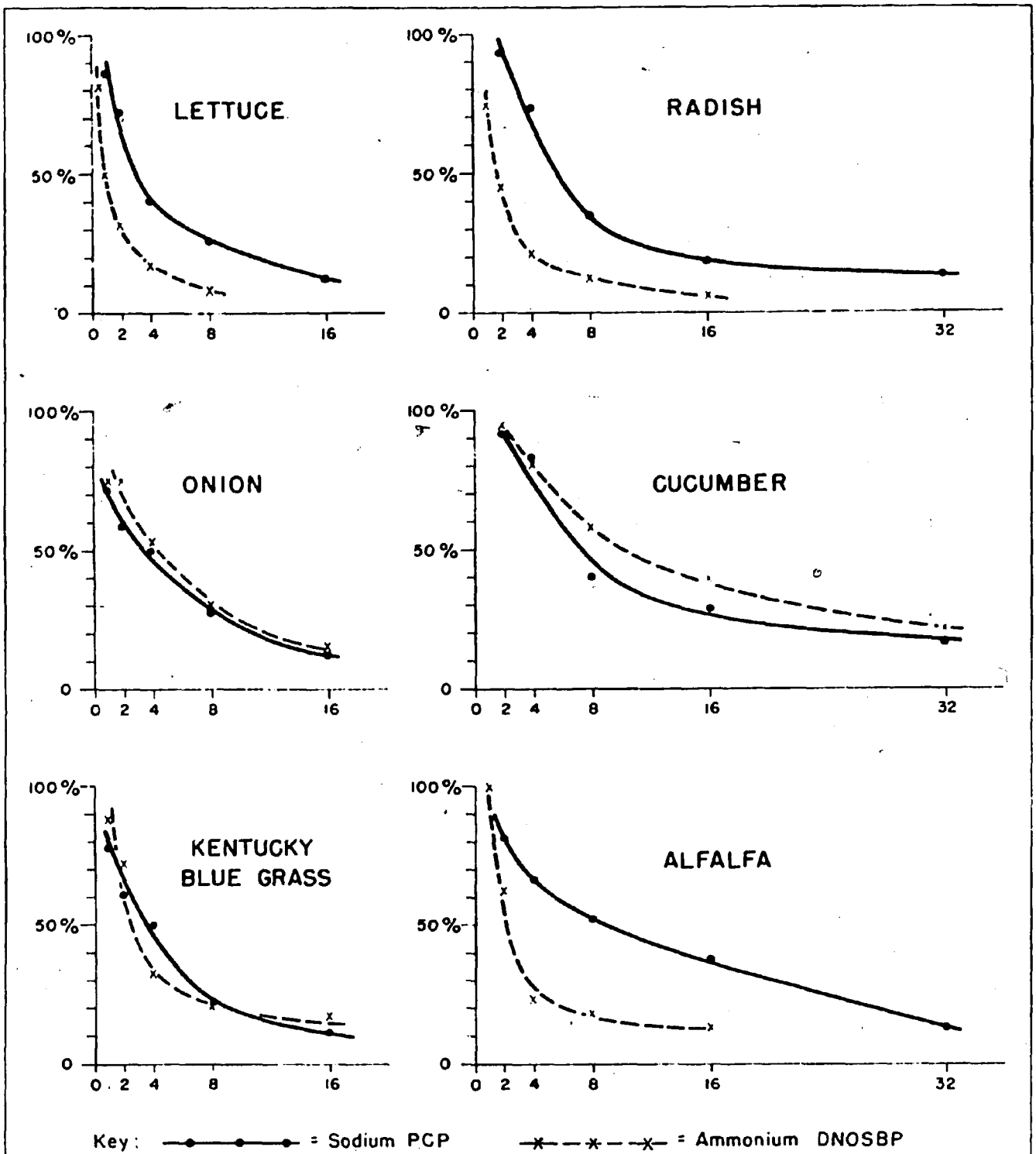

Figure 1 Toxicity curves for eight representative species

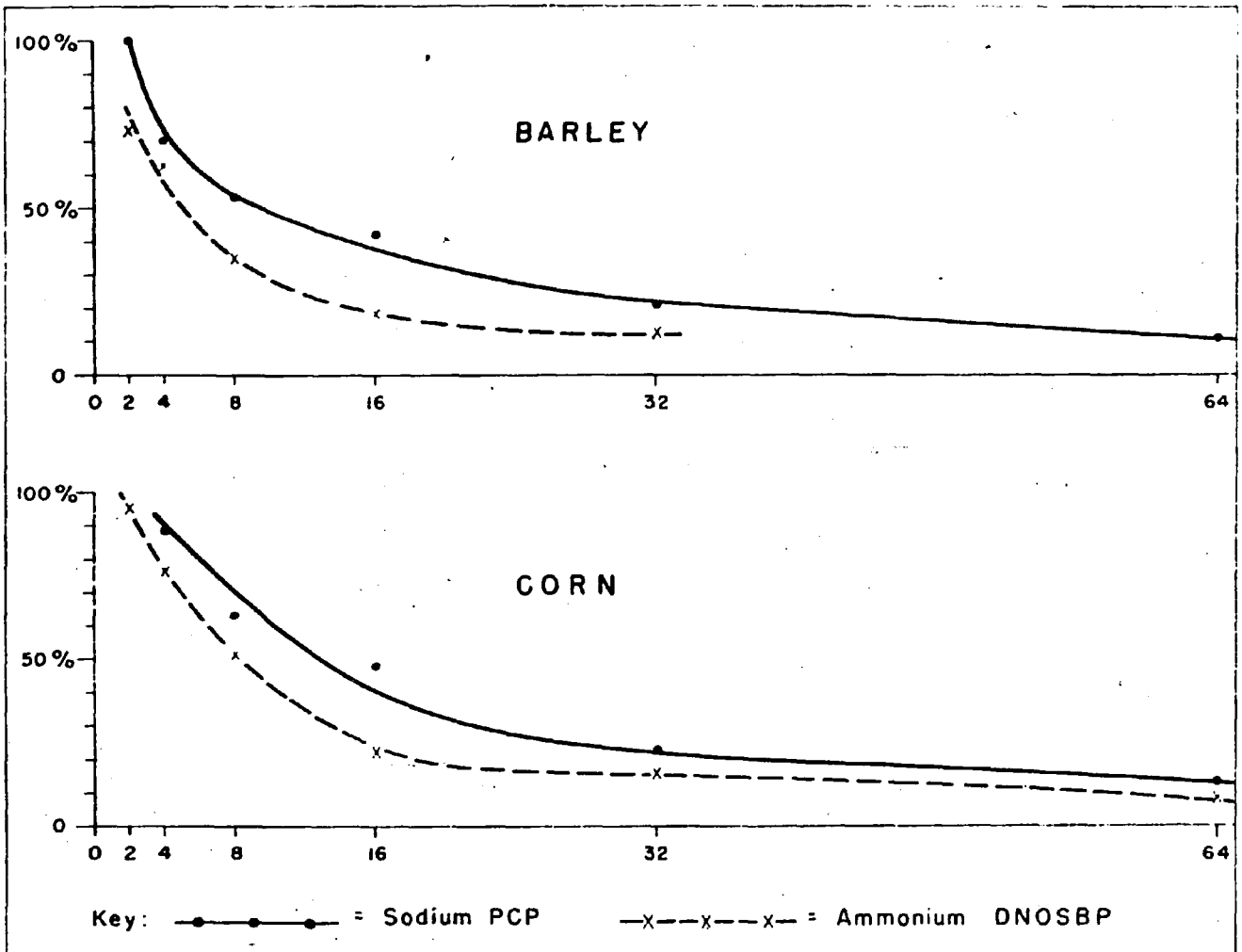

Figure 1 continued.

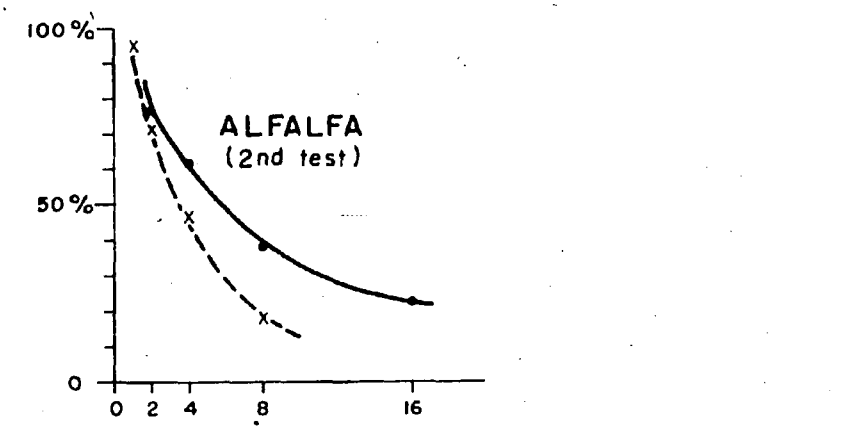

Figure 2 Revised curve for alfalfa based on second test.

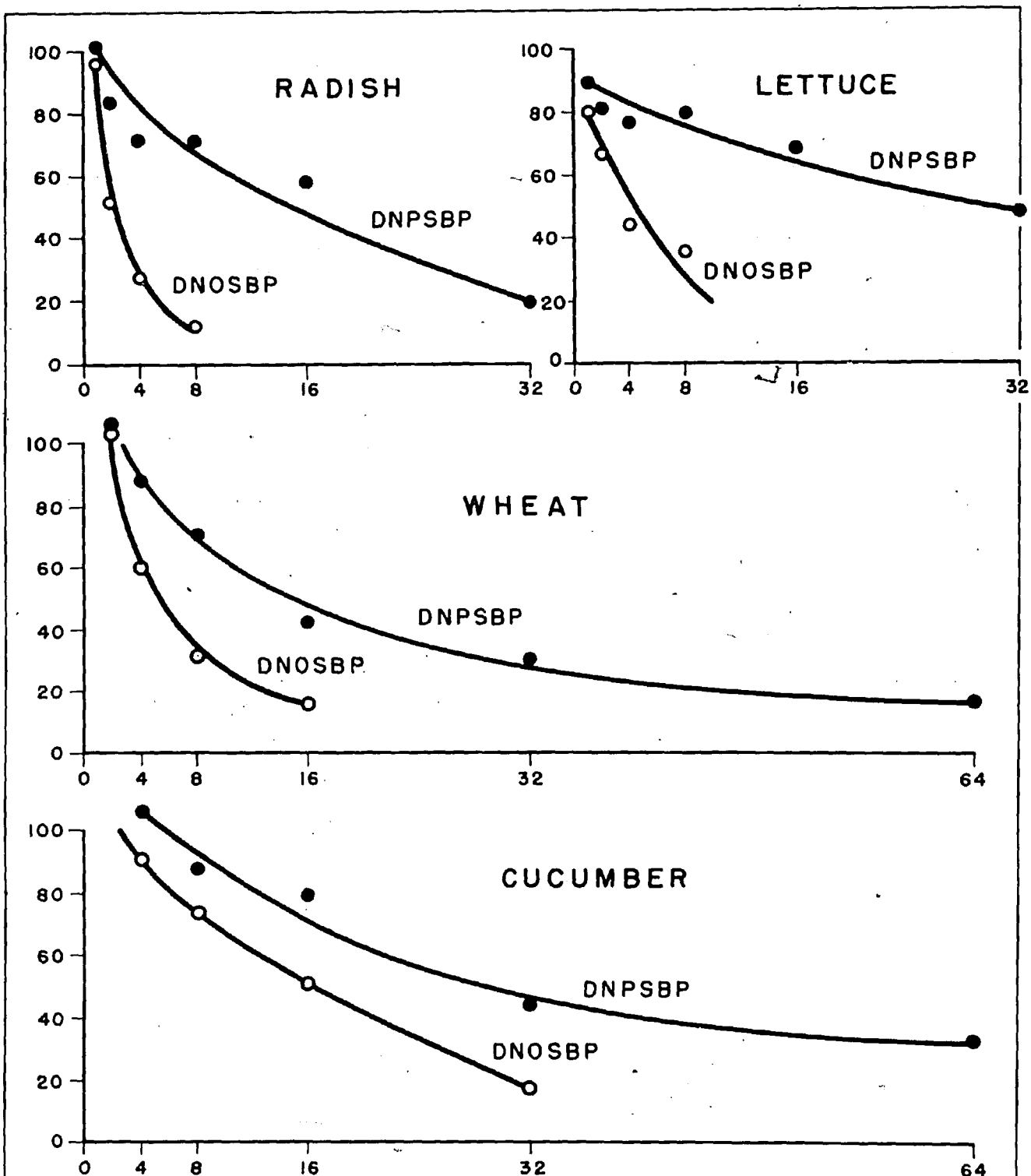

Figure 3. Root toxicity curves for ortho and para dinitrosecondarybutyl phenol. The vertical scale represents root length expressed as a percentage of the control. The horizontal scale represents the concentration in ppm. of DNOSBP and DNPSBP equivalent.

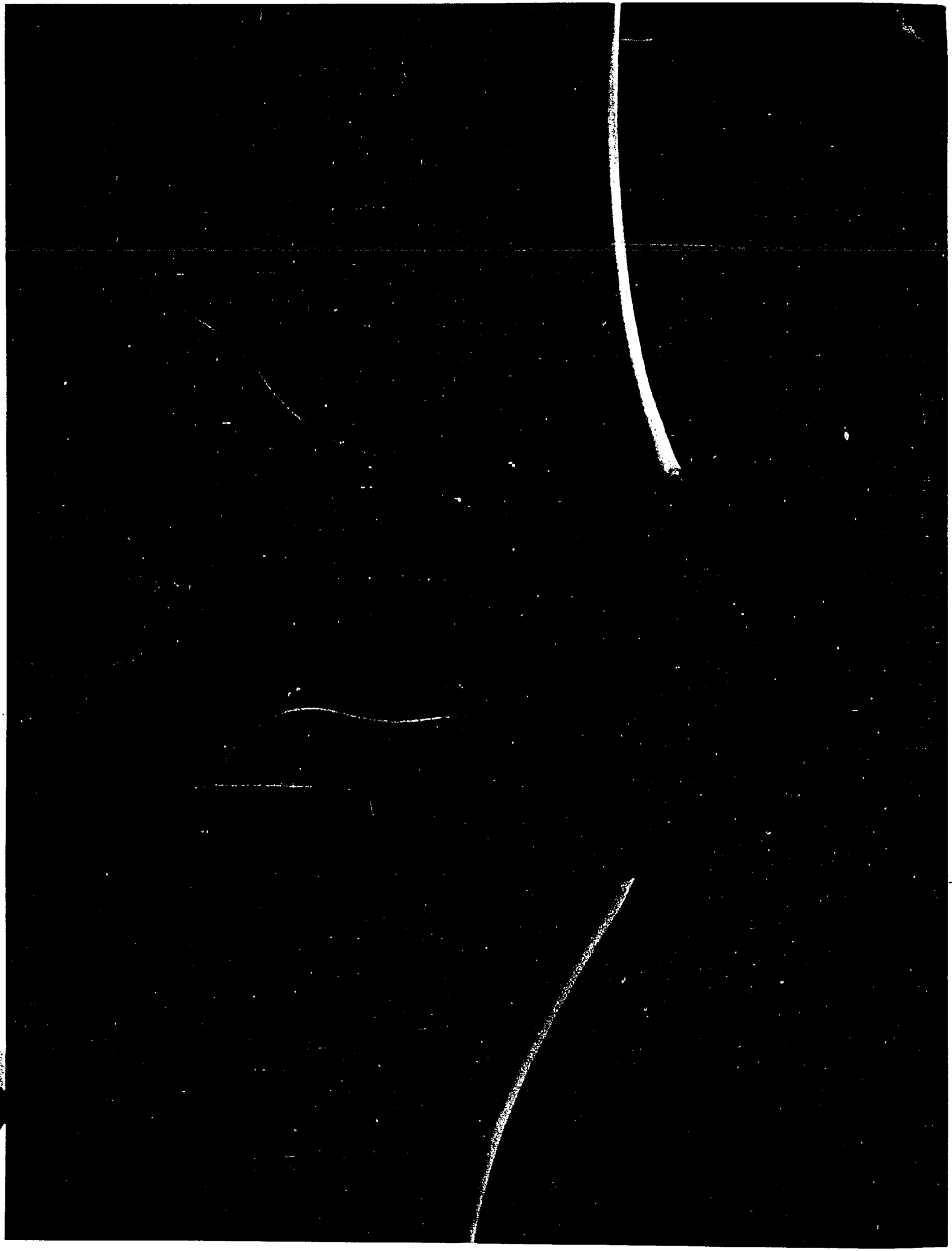

Figure 8 - Wheat seedlings which were grown with the left hand roots in water and the right hand roots in an 8 ppm. solution of NH_4DNOSBP.

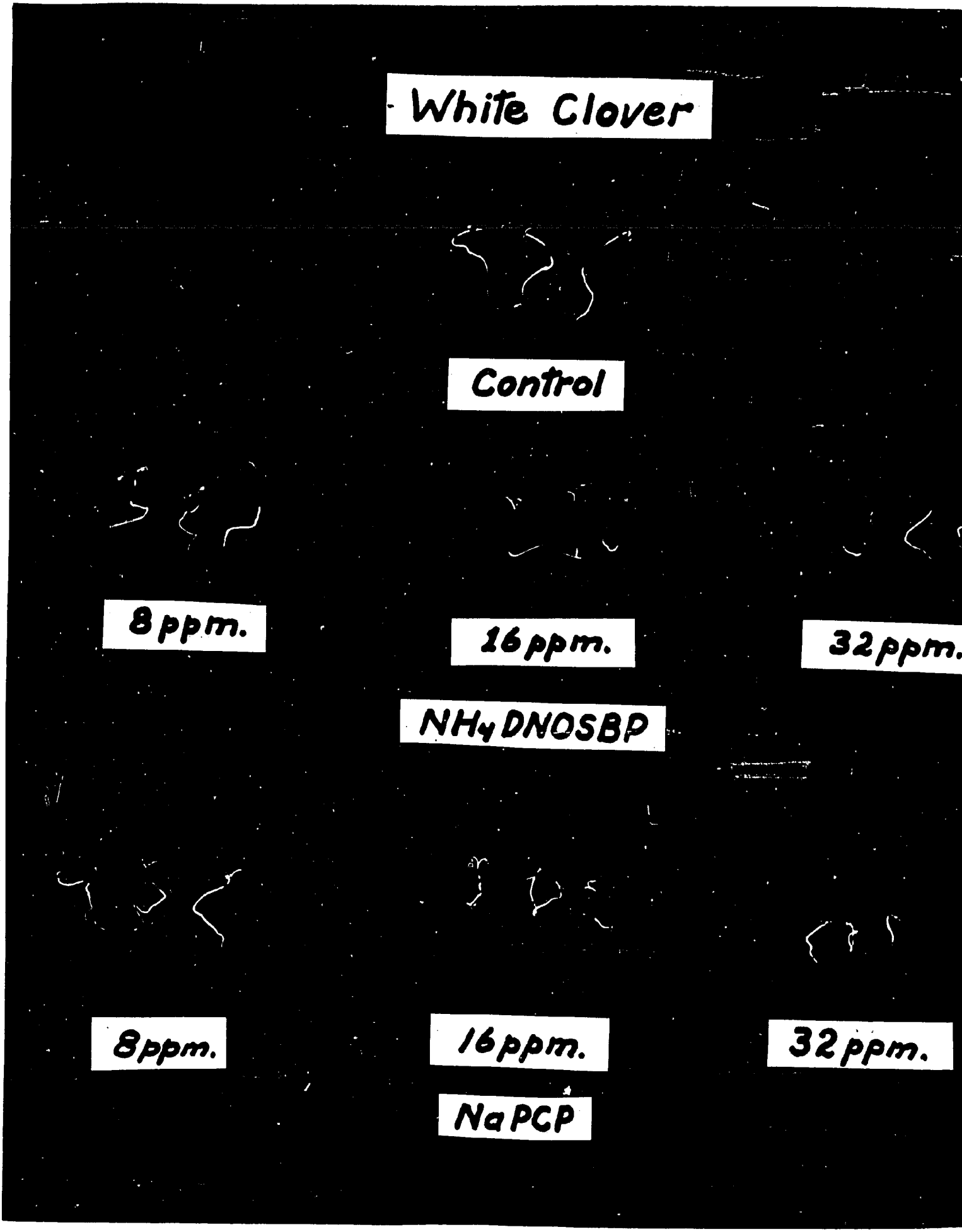
White Clover
Control
8 ppm.
16 ppm.
32 ppm.
NH4 DNOSBP
8 ppm.
16 ppm.
32 ppm.
Na PCP

Figure 7 - Representative white clover seedlings at end of growth period showing marked resistance to NH_4DNOSBP and slightly greater susceptibility to NaPCP.

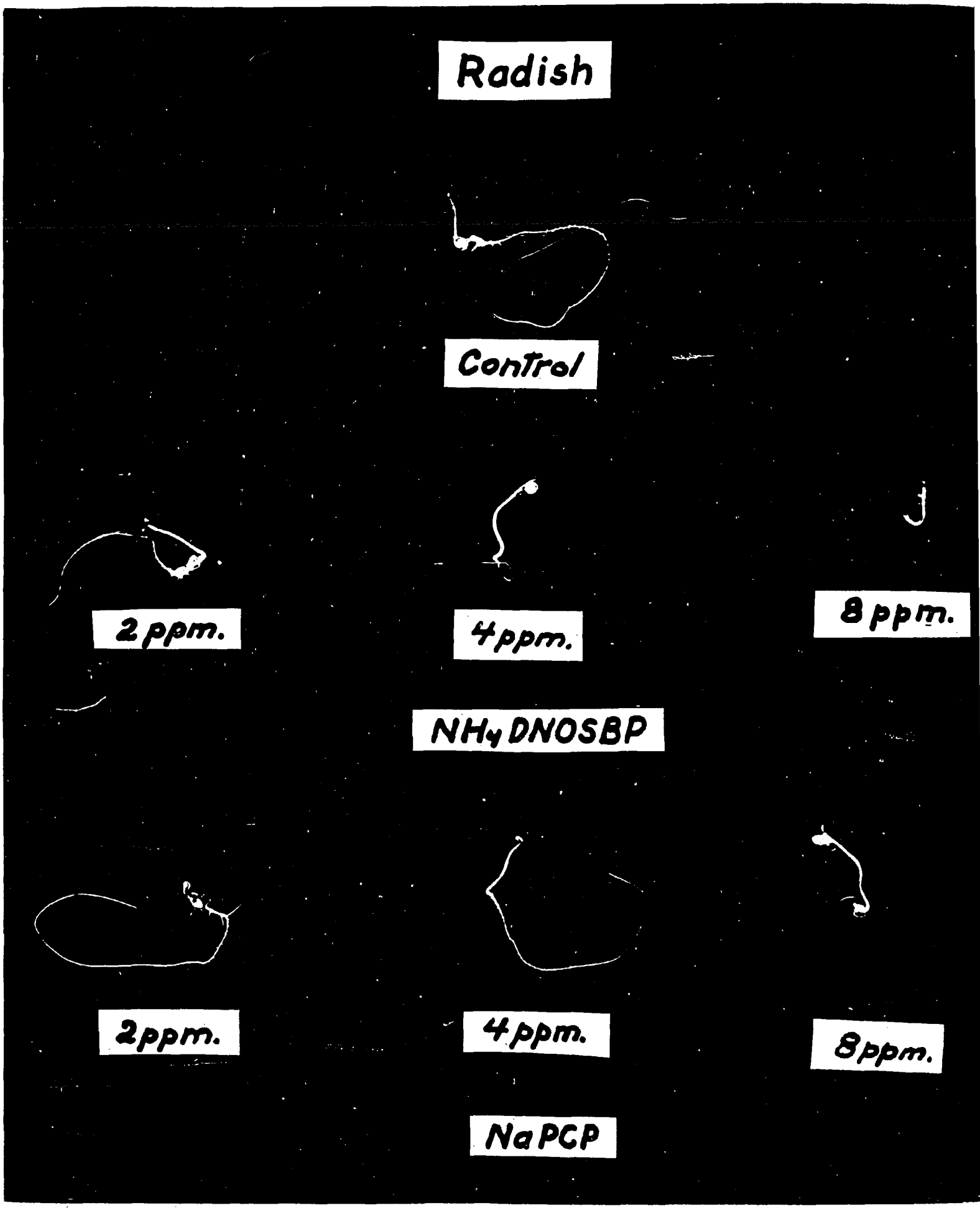
Radish
Control
2 ppm.
4 ppm.
8 ppm.
NH4 DNOSBP
2 ppm.
4 ppm.
8 ppm.
Na PCP

Figure 6 - Representative Radish seedlings at end of growth period showing relative high degree of susceptibility to NH_4DNOSBP and slightly lower susceptibility to NaPCP.

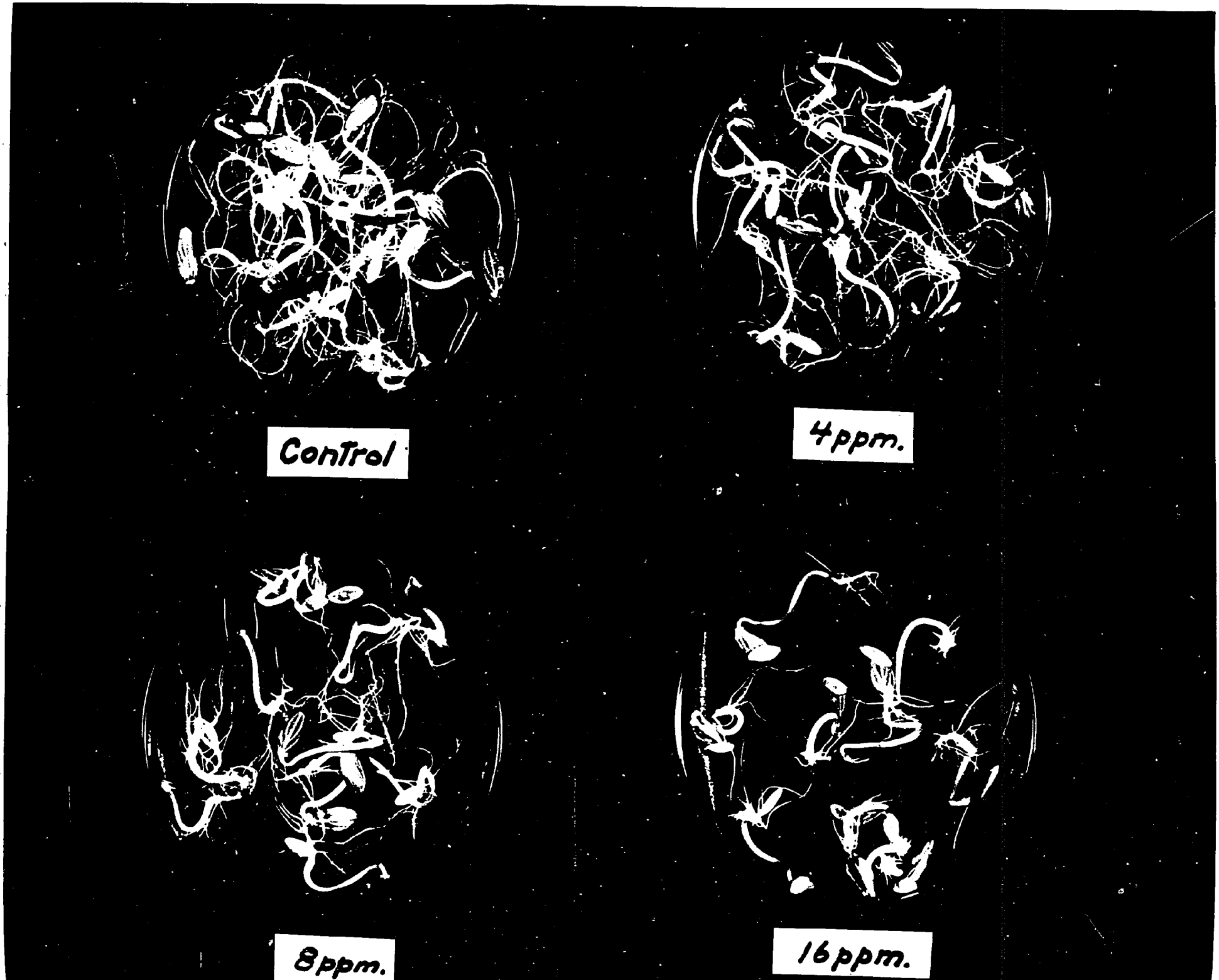
Control
4ppm.
8ppm.
16ppm.

Figure 5- Cucumber seedlings grown in varying concentrations of NH_4DNOSBP. Filter papers were replaced with black paper to facilitate photography.

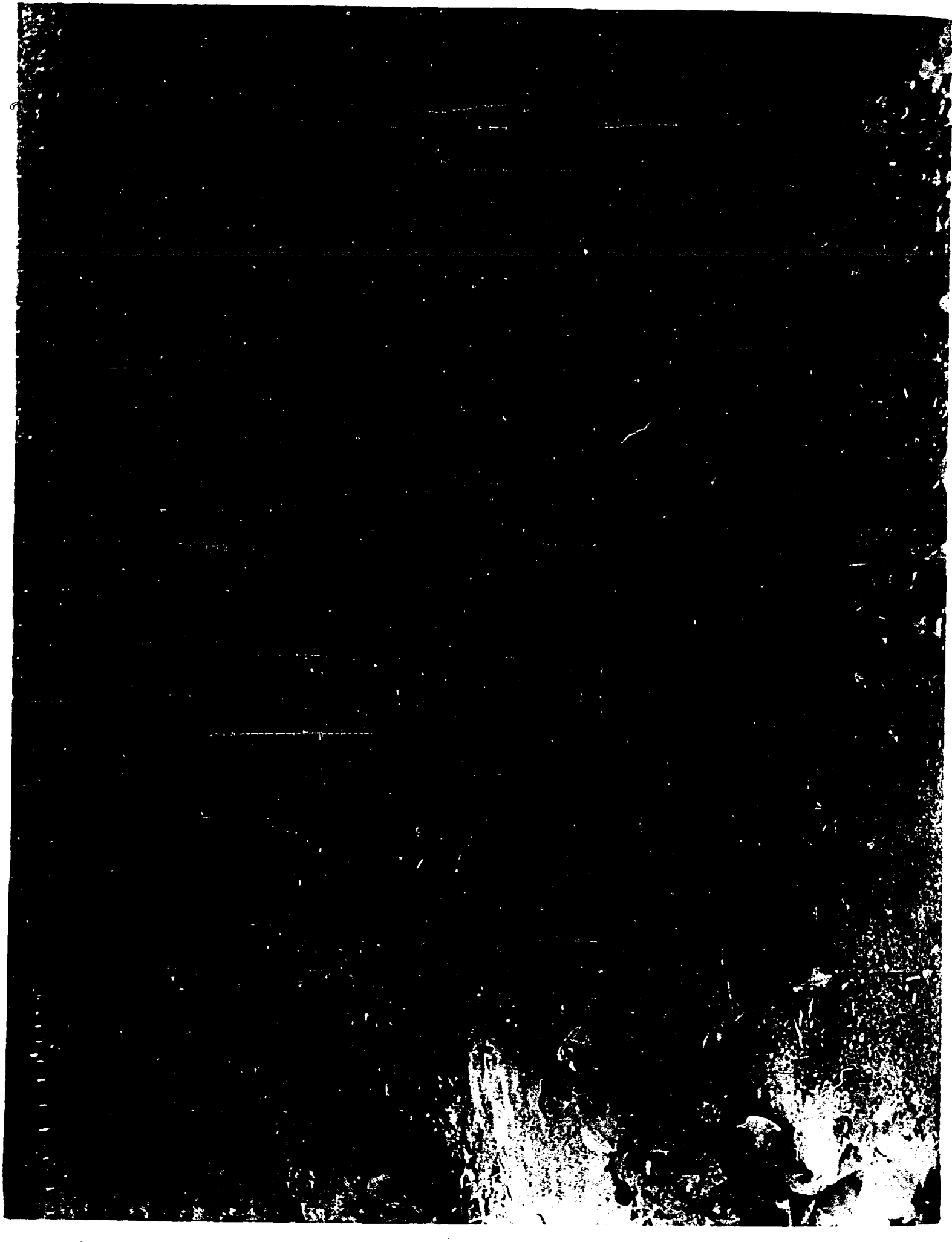

Figure 4 - Snapbeans, left sprayed with 9 pounds per acre NH_4DNOSBP and right unsprayed control. Weeds are primarily lambsquarters and red root pigweed.