THE EFFECT OF 2, 4-DICHLORPHENOXYACETIC ACID ON THE UPTAKE AND DISTRIBUTION OF CALCIUM AND PHOSPHORUS IN BARLEY

Thesis for the Degree of Ph. D.
MICHIGAN STATE UNIVERSITY
Lawrence H. Smith
1959

This is to certify that the

thesis entitled

THE EFFECT OF 2,4-DICHLORPHENOXYACETIC ACID ON THE UPTAKE AND DISTRIBUTION OF CALCIUM AND PHOSPHORUS IN BARLEY

presented by

LAWRENCE H. SMITH

has been accepted towards fulfillment
of the requirements for

Ph. D. degree in Farm Crops

C. M. Harrison
Major professor

Date 6/26/59

O-169

EFFECT OF 2,4-DICHLOROPHENOXYACETIC ACID ON SEEDLING DEVELOPMENT AND UPTAKE AND DISTRIBUTION OF CALCIUM AND PHOSPHORUS IN BARLEY

By

LAWRENCE H. Hubert SMITH

A THESIS

Submitted to the School for Advanced Graduate Studies of
Michigan State University of Agriculture and
Applied Science in partial fulfillment of
the requirements for the degree of

DOCTOR OF PHILOSOPHY

Department of Farm Crops

Year 1959

LAWRENCE H. SMITH

A study was made on the effect of 2,4-D applied as a pre-emergence spray and at the three- and five-leaf stages of barley upon the uptake and distribution of calcium and phosphorus.

It was found that the 2,4-D applied as pre-emergence spray significantly reduced the dry weight, and ash, calcium and phosphorus content. Radiograms indicated a failure of the plants treated with 2,4-D to translocate calcium^{45} and phosphorus^{32} to the above ground portion of the plant. However, there was no discernible effect of 2,4-D upon the distribution of these two elements within the plant.

Histological sections of root tissue revealed extensive proliferation of cells and lateral root formation initiating from the pericycle in all plants treated with 2,4-D.

Microradiograms disclosed the fact that both phosphorus^{32} and calcium^{45} were concentrated in the proliferated lateral root areas of those plants treated with 2,4-D.

Two,4-D applied to barley at the three-leaf stage also effected a significant reduction in dry weight and ash, calcium and phosphorus content of the treated plant, as compared to the check. However the dry weight, ash, phosphorus and calcium content of the plants treated with 2,4-D, expressed as percentage of the check plants, was greater than that found to occur in the plants treated with a pre-emergence spray.

A failure to translocate phosphorus^{32} and calcium^{45} from the roots to the above ground portions was also observed in those plants treated at the three-leaf stage.

Barley treated with 2,4-D at the five-leaf stage showed no significant effects of the 2,4-D upon the dry weight, ash, calcium and phosphorus content or distribution of calcium^{45} and phosphorus^{32} within the treated plants.

It was found that the greatest effect of 2,4-D upon the calcium and phosphorus content in barley occurred in the pre-emergence application with the least effect at the five-leaf stage with the three-leaf application being intermediate in response.

It is suggested that 2,4-D directly affects the uptake and translocation mechanism in barley.

C. M. Harrison

ACKNOWLEDGMENT

The author wishes to express his sincere appreciation to Drs. C. M. Harrison and R. S. Bandurski for their advice and counsel during the course of this investigation.

He is especially indebted to Dr. Bandurski for the procurement of radioisotopes and the use of his laboratory facilities and equipment.

Grateful acknowledgment is also due to Dr. E. J. Benne and staff for their assistance and counsel in the chemical assays required for this study.

INTRODUCTION

The advent of chemical weed control has greatly eased man's endless struggle to eliminate competition by undesirable plants with those species which he immediately wishes to cultivate.

Of the many chemicals available to the producer, none is used more often or more carelessly than 2,4-dichlorophenoxyacetic acid (2,4-D). Its selectivity in destroying broad-leaved weeds in preference to many narrow-leaved species renders it especially valuable in the production of small grains. However, little is known of its mode of action or influence upon the metabolism of the small grain plant.

Recent experiments studying the effect of 2,4-D upon nutrient uptake of broad-leaved plants have indicated that there is a decided influence upon nutrient uptake and specifically upon certain ions in preference to others.

It is the purpose of this study to determine the influence of 2,4-D on the uptake and distribution of calcium and phosphorus in barley treated at various stages of plant growth.

REVIEW OF LITERATURE

Fang and Butts[4], utilizing carboxyl (C^{14}) labeled 2,4-D, studied the absorption and translocation of this compound in corn and wheat. Using bean plants for comparison, it was found that 2,4-D was absorbed by the monocotyledons, but at a slower rate than that exhibited by the bean plants.

The translocation of C^{14} to the apical regions of corn and wheat plants was very slow. There appeared to be a block in young plants at the intercalary meristem of the monocot stems and leaves.

Freiberg and Clark[5] found that when soybean plants were treated with 2,4-D the absorbing capacity of the roots was apparently changed, as indicated by the failure of treated plants to increase in total nitrogen.

Stahler and Whitehead[12] reported that when sugar beet leaves were erroneously sprayed with a solution of 2,4-D, the nitrate content increased.

In studies on the effect of 2,4-D on phosphorus content in cranberry bean plants by Hamner, Rebstock and Sill[8], the total amount of phosphorus in the leaves of the treated plants was lower than that of the non-treated plants. In stem tissues, however, the results were strikingly different.

The total phosphorus content of the stem tissue from plants treated with 2,4-D was higher than that of the non-treated plants. The phosphorus content in the roots of the treated plants was not appreciably different from that found in the non-treated plants.

Fang and Butts[3], with bean plants, found that in all cases, the phosphorus^{32} activity of leaves from plants treated with 2,4-D was much less when compared to leaves of control plants, while the activities in either the stem or root from those two groups of plants showed no significant differences. The distribution of phosphorus^{32} in plants treated with growth regulator differed from that found in control plants.

Loustalot, Moris, Garcia and Pagan[7] have evidence that 2,4-D promoted phosphate accumulation in the roots, leaves and stems of white beans after forty-eight hours. However, one week after treatment, the phosphorus content in roots had increased significantly and this coincided with a sharp decline of phosphorus in the leaves, indicating that it may have been translocated from leaves to roots.

Excised roots of four day old wheat seedlings showed a marked inhibition of nitrate absorption by 2,4-D within three hours after treatment as described by Nance[10]. Further experiments with potassium chloride indicated that 2,4-D inhibition of ion uptake was not a specific nitrate effect.

Weldon, Hamner and Bass[15], working with tobacco seedlings, reported that the tops of treated plants contained a lesser percentage of potassium, sodium and phosphorus and a greater percentage of boron and iron than did untreated plants. Roots of treated plants accumulated more calcium and less copper than roots of untreated plants. There was little or no difference in the accumulation of calcium, copper, magnesium and zinc in the tops of the plants, nor in the accumulation of boron, potassium, manganese, magnesium and phosphorus in the roots of treated and untreated plants.

Plant roots contained greater percentages of boron, copper, iron, magnesium, manganese, sodium and potassium than tops, whether treated or untreated. Calcium accumulated to a greater amount in roots than in the tops of treated plants, although no differences were noted in untreated plants. Potassium accumulated to a greater extent in tops than in roots of untreated plants, while no differences were noted in treated plants. The content of phosphorus in the tops of the treated plants was much less than that of untreated plants.

MATERIALS AND PROCEDURE

In order to evaluate the effect of 2,4-D on the uptake and distribution of calcium and phosphorus in the plant, the following techniques were employed:

(1) Chemical analysis for calcium and phosphorus of above and below ground parts of the plants to determine the relative amounts and proportion of calcium and phosphorus within these two segments of the plant.

(2) Histological sections to determine the effect, if any, of 2,4-D on various plant cells and tissues which might in some way affect uptake and/or distribution of calcium and phosphorus within the plant.

(3) Radioactive isotopes, eg. calcium^{45} and phosphorus^{32}, to determine the effect of 2,4-D upon the distribution of these elements within the plant and to evaluate the uptake of these ions over a short period of time.

(4) Microradiograms, utilizing nuclear track plates, to trace the distribution of calcium^{45} and phosphorus^{32} at the cellular level.

The experiment consisted of five rates of application of 2,4-D; zero, one-quarter, one-half, one and two pounds of the sodium salt of 2,4-D per acre at three stages of growth: pre-emergence, three-leaf stage, and five-leaf stage, with four replications.

In order to avoid the normal fluctuation of temperature, length of day, light intensity, humidity and etc., prevailing under general greenhouse conditions--all of which directly and indirectly affect the

metabolism of plants and thus their mineral nutrition--an attempt at controlled or at least of reproducible conditions was made.

A double unit seed germinator was selected as the basic unit for a controlled chamber, and a bank of twelve daylight florescent tubes and six incandescent bulbs provided the necessary light. These lights provided an average of nine hundred foot candles of light, measured four inches from the false floor of the unit. Day length was varied with a time clock and after preliminary investigations, a sixteen hour day was decided upon. To gain some control of temperature and humidity, cold tap water was continually circulated through the water jacket enclosing the germinator. This water averaged 63 degrees Farenheit throughout the duration of the experiment. The walls of the germinator were colder than the rest of the area and a fan was added to recirculate the air and thus maintain a satisfactory uniform temperature throughout the germinator, which varied from 78 degrees Farenheit plus or minus 2 degrees with the lights on to 68 degrees Farenheit plus or minus 2 degrees with the lights off. A false floor consisting of one-quarter inch mesh hardware cloth over a wooden frame raised the plants one inch above the germinator floor, thereby allowing for pot drainage and circulation of air. The chamber is depicted in Figure 1.

Figure 1: Growth Chamber

Seed of Kindred barley, a malting type spring barley, was obtained from foundation seed-stock. Seeds were selected on the basis of uniformity, apparent freedom from disease, blemishes and mechanical injury.

Plastic containers with a capacity of one and one-half pints were used as "pots" and # 7 Wausan* silica sand as the growth medium. The rate of 2,4-D used with each culture was inscribed on the containers and these were used for similar treatments in repeated experiments. The pots were cleaned after each experiment by a thorough detergent and scouring powder brushing, followed by a nitric acid rinse and three distilled

*American graded Sand Company, Chicago, Illinois

water rinses. Initially the presence of 2,4-D remaining in the pots was checked by the application of the final distilled water rinse to tomato seedlings; however, since there was no herbicide present, as measured by the tomato seedling response, it was felt that the cleaning treatment was effective in removing the 2,4-D and the seedling tests were discontinued.

Holes were cut through the bottom of the plastic pots with a cork borer and glass wool was placed over the opening to allow for drainage.

Six seeds were planted at a depth of one-half inch and on germination were thinned to four plants per culture. The planting was staggered so that all three stages of growth were treated with 2,4-D at the same time. The plants were watered at two day intervals with a modified Hoagland's nutrient solution applied through a sprinkler head and thoroughly flushed with distilled water twice a week.

Prior to the application of the 2,4-D, rubber stoppers were placed in the drainage holes of the pots to make a sealed container. The plants were then ranked according to vigor and general condition within each growth stage. The 2,4-D was applied as an aqueous spray to the tops at forty pounds per square inch, utilizing the pressurized tank and gun in Figure 2.

Figure 2: Spray Application Equipment

Immediately after treatment the plants were watered with nutrient solution and again thereafter at two day intervals.

Fourteen days after treatment the plants, in the three-leaf and five-leaf treatments, were harvested and the tops and roots separated. The plants from the pre-emergence treatments, however, were retained intact. The entire experiment was repeated four times in order to secure adequate material for chemical analysis. Chemical analysis for calcium and phosphorus were run, utilizing standard A.O.A.C. methods[1], as modified by the Department of Agricultural Chemistry, Michigan State University.

In the tracer experiments, the plants were handled in the usual manner with twenty μcuries of the tracer being added directly to the culture at the end of the fourteen day treatment period, separate plantings being required for both calcium^{45} and phosphorus^{32}. Plants utilized for radiograms were harvested in the normal manner, pressed, dried and placed in contact with no-screen x-ray film for a period of seven days. The film was then developed. Sections of plant tissue for microradiograms were taken at harvesttime and immediately frozen by placing in containers which had been previously stored upon dry ice. The sections were then frozen dry and embedded directly into paraffin in the apparatus pictured in Figure 3.

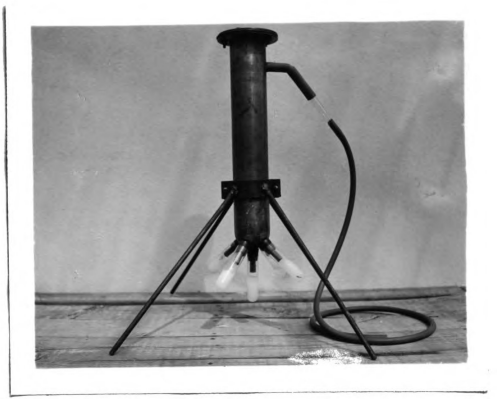


Figure 3: Freeze-Drying Equipment

The tissue was sectioned, placed on slides, the paraffin removed in xylene and the preparation coated with celloidan. The slide was then taped to a one by three nuclear track plate for seven days, after which the nuclear track plate was developed.

The remaining plants were then ground, ashed, and dissolved in concentrated hydrochloric acid and made up to a valume of twenty-five milliliters. Two millilliter aliquots were then counted to determine the radioactivity present. All counts obtained were extrapolated back to zero time to avoid decay corrections.

EXPERIMENTAL RESULTS

Experiment I - Pre-emergence Treatment with 2,4-D

External appearance. The general condition of the check plants were excellent and, in general, two new leaves had been produced during the fourteen day treatment period. However, in the plants treated with 2,4-D, a slight chlorosis was evident as well as definite stunting of growth. As the rate of application of 2,4-D was increased, the severity of stunting increased. At the two pound rate, the plants had made no new growth during the treatment period; whereas at the one-quarter and one-half pound levels, one new leaf had been produced. At the one pound level a new leaf was in the process of being produced, but was still in the rolled stage.

The roots of the check plants were normal in development, whereas the roots from plants treated with 2,4-D were short and thick with numerous protuberances along the root. This reaction to 2,4-D increased in severity with increasing amounts of 2,4-D until at the two pound level the roots were, on the average, from one-half to one inch long with numerous protuberances. From histological sections, it was determined that these protuberances were lateral roots which had been stimulated by 2,4-D.

Dry weight. The stunting effect of 2,4-D upon barley plants when applied as a pre-emergence application was very evident from its influence upon the dry weight of the plants as indicated in Table I.

TABLE I

INFLUENCE OF 2,4-D UPON DRY WEIGHT (IN MILLIGRAMS) OF BARLEY

Replication	Pounds of 2,4-D Applied per Acre				
	0.00	0.25	0.50	1.00	2.00
I	1156.4	730.3	730.7	615.4	541.4
II	1403.7	670.4	668.2	637.9	631.4
III	1325.4	704.6	646.0	648.3	612.6
IV	1274.3	736.8	671.0	701.6	636.2
Mean	1290.0	710.5	679.0	650.8	605.4

R.E. (5% = 8.57 (1% = 12.14 P = 5 C.V. = 7.2%

The data indicates that upon the application of 2,4-D, the dry weight decreased significantly from the control. Within the 2,4-D treatments, the two pound application significantly lowered the dry weight when compared to the one-quarter pound rate. Although a trend of decreasing dry weight with increasing rate of application of 2,4-D was evident, there was no significant difference between the one-half, one and two pound rates of 2,4-D and its effect upon dry weight.

Ash. The data in Table II indicates that the ash content, as well as dry weight, decreased upon the application of 2,4-D.

TABLE II

THE EFFECT OF 2,4-D UPON ASH CONTENT OF BARLEY

Replication	Pounds of 2,4-D Applied per Acre									
	0.00		0.25		0.50		1.00		2.00	
	mg.	%	mg.	%	mg.	%	mg.	%	mg.	%
I	187.7	16.2	78.3	10.7	54.0	7.4	58.9	9.6	50.6	9.4
II	238.2	17.0	68.9	10.2	48.0	7.2	63.3	9.9	49.0	7.8
III	205.8	15.5	59.2	8.4	58.6	9.1	77.8	12.0	46.4	7.6
IV	198.6	15.6	103.4	14.0	66.6	9.9	70.2	10.0	48.9	7.7
Mean	207.6	16.1	77.5	10.9	56.8	8.4	67.6	10.4	48.7	8.1

R.E. (5% = 24.35 (1% = 34.49 P = 5 C.V. = 5.36%

The ash content, calculated as percentage of dry weight and milligrams of total ash, decreased upon application of the 2,4-D when compared to the check except at the one pound rate where a non-significant increase in both percentage and total milligrams was evident. As in the dry weight, the decreases in ash content of plants caused by the 2,4-D treatments were significantly different from that of the control. The ash content of plants treated with the one-quarter pound rate was significantly higher than the two pound rate, with a non-significant trend of decreasing ash content with increasing rate of 2,4-D over the whole experiment.

Calcium. The data presented in Table III indicates the influence of 2,4-D upon the calcium content of barley when applied as a pre-emergence treatment.

TABLE III

THE EFFECT OF 2,4-D UPON CALCIUM CONTENT OF BARLEY

Replication	Pounds of 2,4-D Applied per Acre									
	0.00		0.25		0.50		1.00		2.00	
	mg.	%	mg.	%	mg.	%	mg.	%	mg.	%
I	5.3	.40	2.4	.36	2.2	.30	2.6	.41	1.8	.34
II	4.7	.33	3.2	.44	2.0	.32	2.0	.31	1.8	.29
III	4.2	.36	2.3	.33	1.8	.28	1.9	.29	1.9	.31
IV	4.7	.37	2.4	.33	2.6	.38	2.3	.33	1.6	.25
Mean	4.7	.37	2.6	.37	2.2	.32	2.2	.29	1.8	.30

R.E. (5% = .557 (1% = .79 P = 5 C.V. = 5.5%

The calcium content of barley expressed as milligrams of total calcium was significantly reduced by treatment with 2,4-D; the two pound rate of the growth regulator being significantly lower than the one-quarter pound rate, with the one-half and one pound rates being somewhat lower than the one-quarter pound rate.

Calcium^{45} uptake. The influence of 2,4-D upon the uptake of calcium^{45} over a two hour period is expressed in Table IV.

TABLE IV

UPTAKE OF CALCIUM^{45} BY BARLEY EXPRESSED AS COUNTS PER MINUTE

Replication	Pounds of 2,4-D Applied per Acre				
	0.00	0.25	0.50	1.00	2.00
I	1225	688	512	438	250
II	1275	750	462	413	250
III	1125	725	613	503	288
IV	1375	675	513	413	313
Mean	1250	710	525	442	275

R.E. (5% = 99.05
(1% = 140.32

P = 5

C.V. = 4.6%

Distribution of calcium^{45}. Radiograms of plants treated with calcium^{45} were used for the check and similar plants treated with calcium^{45} and the two pound rate of 2,4-D are depicted in Figure 4. The calcium^{45} in the plant treated with 2,4-D was absorbed by the roots, but not translocated upward as evidenced by the greater exposure of the negative by the roots. The protuberances caused by 2,4-D and the stunted thickened condition of the roots is readily apparent in the plant treated with 2,4-D when compared to the control.

The distribution of calcium^{45} in the tops of the plants showed little or no difference.

The effect of the one-quarter, one-half and one pound rates of 2,4-D upon the distribution of calcium^{45} in the roots of the barley plants was similar to that of the two pound rate.

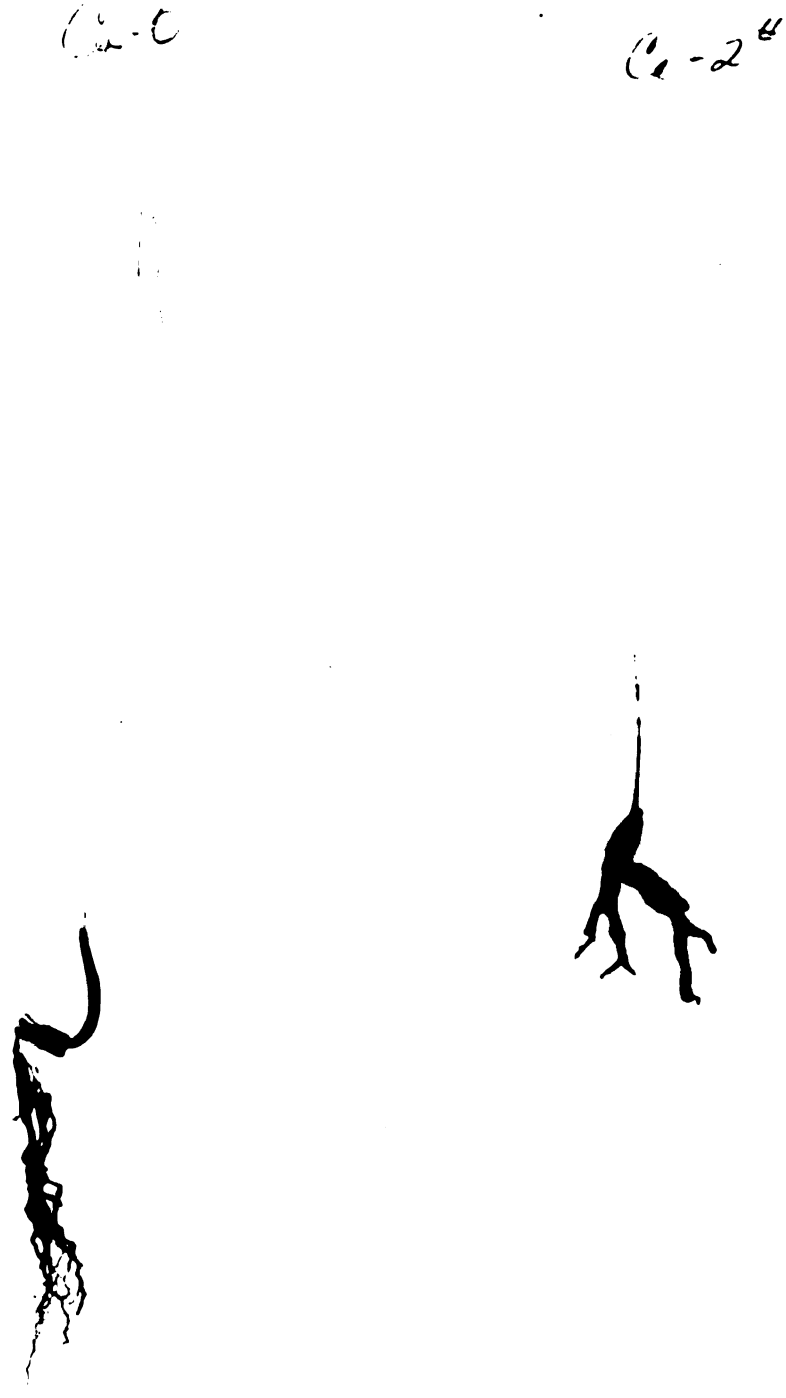


Figure 4: Radiograms of Calcium^{45} in Pre-emergence Treated Barley.

Microradiograms of calcium^{45} are shown in Figure 5. It was found that calcium^{45} accumulated in the lateral root proliferations from the pericycle of the plants treated with 2,4-D, whereas in the non-treated plants the calcium^{45} was present primarily in the cell walls.

Phosphorus. The influence of 2,4-D upon the phosphorus content of barley plants, when applied as a pre-emergence treatment, is expressed in Table V.

TABLE V

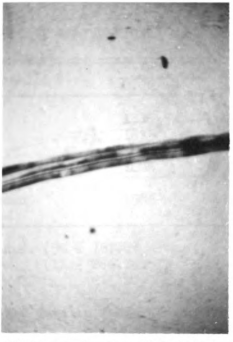
THE EFFECT OF 2,4-D UPON PHOSPHORUS CONTENT OF BARLEY

Replication	Pounds of 2,4-D Applied per Acre									
	0.00		0.25		0.50		1.00		2.00	
	mg.	%	mg.	%	mg.	%	mg.	%	mg.	%
I	18.7	1.28	6.1	.83	6.1	.83	4.6	.76	4.5	.84
II	17.4	1.24	6.2	.93	5.7	.85	5.4	.85	5.1	.81
III	15.0	1.13	6.2	.89	5.7	.65	5.7	.88	4.9	.79
IV	15.1	1.19	7.2	.98	5.5	.82	5.9	.84	4.8	.75
Mean	16.6	1.21	6.4	.91	5.7	.79	5.4	.83	4.8	.80

R.E. (5% = 1.62 (1% = 2.30 P = 5 C.V. = 6.02%

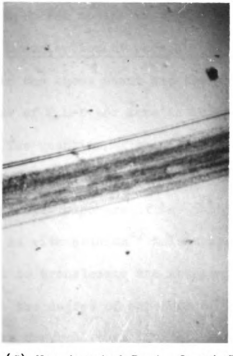
The data in Table V indicates that the phosphorus content, both total weight and percentage of dry weight was significantly decreased by the application of 2,4-D when compared with the non-treated plants. Within the treated plants, the phosphorus content decreased significantly between the one-quarter pound rate and the two pound rate with a decreasing, non-significant trend at the one-half and one pound rates.

Phosphorus^{32}. The uptake of phosphorus^{32} over a two hour uptake period, as influenced by 2,4-D, is expressed in Table VI.

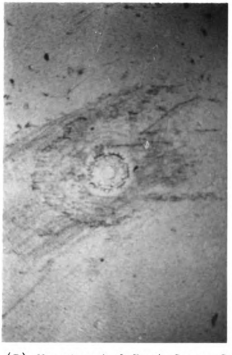


(A) Treated Root, Longi-Section.

(B) Treated Root, Cross-Section.



(C) Non-treated Root, Longi-Section.



(D) Non-treated Root, Cross-Section.

Figure 5: Microradiograms of Calcium^{45} in Pre-emergence Treated Barley.

TABLE VI

UPTAKE OF PHOSPHORUS^{32} BY BARLEY EXPRESSED AS COUNTS PER MINUTE

Replication	Pounds of 2,4-D Applied per Acre				
	0.00	0.25	0.50	1.00	2.00
I	1315	865	675	405	245
II	1875	745	515	465	235
III	1469	825	565	415	215
IV	1685	805	605	525	225
Mean	1586	810	590	453	230

R.E. (5% = 187.89 (1% = 266.18 P = 5 C.V. = 7.62%

The data in Table VI shows that 2,4-D decreases phosphorus uptake when subjected to one-quarter pound or more of 2,4-D applied as a pre-emergence treatment, in that the counts per minute of phosphorus^{32} absorbed was significantly decreased by pre-emergence treatment with 2,4-D when compared to the untreated check.

Distribution of phosphorus^{32}. The distribution of phosphorus^{32} within the check plant and the plant receiving phosphorus^{32} and two pounds of 2,4-D per acre is illustrated in Figure 6.

The quantity of phosphorus^{32} in the top of the plant treated with 2,4-D was less than that found in the untreated plant as evidenced by the greater exposure of the photographic plate (darkness) of the control.

As with calcium^{45} this appears due to the failure of the treated plant to translocate the absorbed phosphorus to the leaves and stems, since the degree of exposure of the x-ray film is at least equal to that of the control, thus giving a rough estimation of the amount of phosphorus^{32} present.

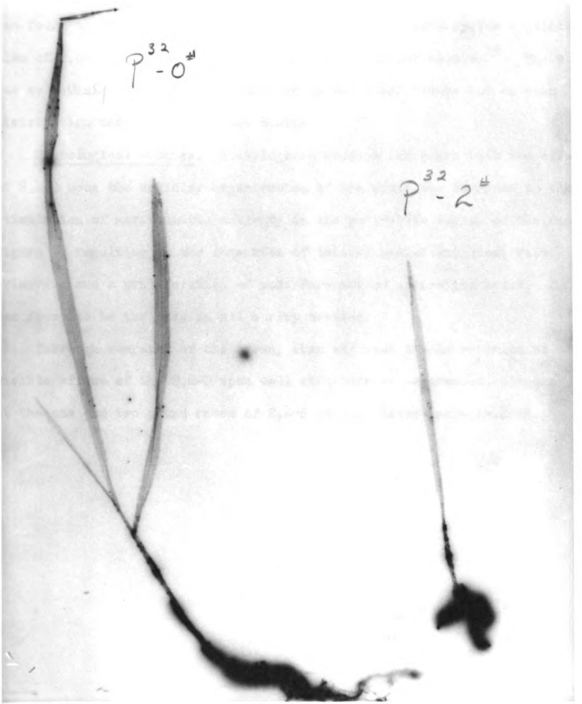
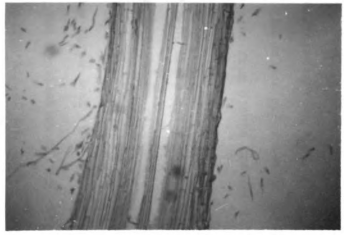


Figure 6: Radiograms of Phosphorus^{32} in Pre-emergence Treated Barley.

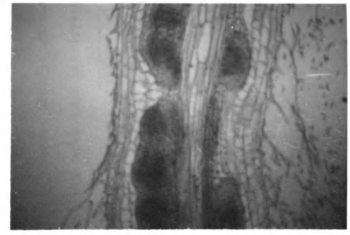
Distribution of phosphorus^{32} at cellular level. The phosphorus^{32} was found to concentrate in the lateral roots stimulated by the application of 2,4-D in a manner analogous to that found for calcium^{45}. There was no definite cellular concentration in the check plants but an even distribution throughout the root tissue.

Histological studies. Histological studies indicated that the effect of 2,4-D upon the cellular organization of the plant was confined to the stimulation of meristematic activity in the pericyclic region of the root, Figure 7, resulting in the formation of lateral and adventitious root primordia and a proliferation of undifferentiated parenchyma cells. This was found to be the case in all barley treated.

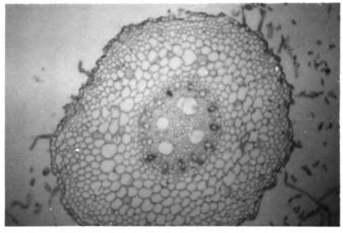
Thorough searches of the crown, stem and leaf tissue revealed no visible effect of the 2,4-D upon cell structure or maturation, although at the one and two pound rates of 2,4-D tubular leaves were evident.



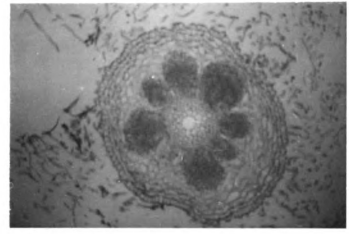
(A) Non-treated Root, Longi-Section.



(B) Treated Root, Longi-Section.



(C) Non-treated Root, Cross-Section.



(D) Treated Root, Cross-Section.

Figure 7: Root Histological Sections.

Experiment II - Three-leaf Stage

General description. The check plants appeared in good condition with no evidence of disease or insect damage. During the two week treatment period, the check plants had, in general, produced two new fully developed leaves. The one-quarter and one-half pound rates each resulted in one new leaf, while in the one and two pound rates, there was no visible evidence of new leaf growth at the end of the fourteen day period.

Dry weight. The plants treated with 2,4-D at the three-leaf stage of development were separated, at harvest, into tops and roots and the dry weight of each taken, Tables VII and VIII, whereas Table IX shows the totals of both roots and tops.

The data in Table VII indicates that the 2,4-D treatment significantly reduced the dry weight of the barley when compared to the check. There was no significant difference between the dry weights obtained at the different rates of 2,4-D.

The values presented in Table VIII indicate that the dry weight of the roots from plants treated with 2,4-D was significantly decreased from that of the check. In a comparison of the effects of increasing rates of 2,4-D, there was a non-significant downward trend in dry weight as the amount of growth regulator was increased.

Ash. The influence of 2,4-D upon ash content of barley when applied at the three-leaf stage is presented in Tables X, XI, and XII.

The application of the herbicide served to decrease both percentage and milligrams of ash in the roots of treated plants, Table XI, as compared

Experiment II - Three-leaf Stage

General description. The check plants appeared in good condition with no evidence of disease or insect damage. During the two week treatment period, the check plants had, in general, produced two new fully developed leaves. The one-quarter and one-half pound rates each resulted in one new leaf, while in the one and two pound rates, there was no visible evidence of new leaf growth at the end of the fourteen day period.

Dry weight. The plants treated with 2,4-D at the three-leaf stage of development were separated, at harvest, into tops and roots and the dry weight of each taken, Tables VII and VIII, whereas Table IX shows the totals of both roots and tops.

The data in Table VII indicates that the 2,4-D treatment significantly reduced the dry weight of the barley when compared to the check. There was no significant difference between the dry weights obtained at the different rates of 2,4-D.

The values presented in Table VIII indicate that the dry weight of the roots from plants treated with 2,4-D was significantly decreased from that of the check. In a comparison of the effects of increasing rates of 2,4-D, there was a non-significant downward trend in dry weight as the amount of growth regulator was increased.

Ash. The influence of 2,4-D upon ash content of barley when applied at the three-leaf stage is presented in Tables X, XI, and XII.

The application of the herbicide served to decrease both percentage and milligrams of ash in the roots of treated plants, Table XI, as compared

TABLE VII

EFFECT OF 2,4-D UPON DRY WEIGHT (IN MILLIGRAMS) OF ABOVE GROUND PORTION OF BARLEY PLANTS WHEN APPLIED AT THREE-LEAF STAGE

Replication	Pounds of 2,4-D Applied per Acre				
	0.00	0.25	0.50	1.00	2.00
I	1380.0	907.8	732.4	795.6	978.2
II	1143.7	782.8	514.5	600.6	700.0
III	1263.8	1087.3	1037.4	856.2	767.3
IV	1327.3	644.4	639.5	814.9	620.8
Mean	1278.7	855.6	731.0	766.8	768.9

R.E. (5% = 183.1
(1% = 259.4

P = 5

C.V. = 6.19%

TABLE VIII

EFFECT OF 2,4-D UPON THE DRY WEIGHT (IN MILLIGRAMS) OF ROOTS OF BARLEY PLANTS WHEN APPLIED AT THREE-LEAF STAGE

Replication	Pounds of 2,4-D Applied per Acre				
	0.00	0.25	0.50	1.00	2.00
I	290.0	158.2	155.5	124.2	77.7
II	341.9	143.1	134.0	126.5	74.3
III	231.2	179.4	151.0	114.4	88.6
IV	199.6	146.1	124.3	121.1	86.1
Mean	265.7	156.7	141.2	121.6	81.7

R.E. (5% = 45.8
(1% = 64.9

P = 5

C.V. = 8.89%

TABLE IX

INFLUENCE OF 2,4-D APPLIED AT THREE-LEAF STAGE UPON DRY WEIGHT (IN MILLIGRAMS) OF BARLEY

Replication	Pounds of 2,4-D Applied per Acre				
	0.00	0.25	0.50	1.00	2.00
I	1670.0	1066.0	887.9	919.8	1055.9
II	1485.6	925.9	648.5	727.1	774.4
III	1495.0	1266.7	1188.4	970.6	855.9
IV	1526.9	790.5	763.8	936.0	706.9
Mean	1544.4	1012.3	872.2	888.4	848.3

R.E. (5% = 188.3
(1% = 266.7

P = 5

C.V. = 5.42%

TABLE X

THE EFFECT OF 2,4-D APPLIED AT THREE-LEAF STAGE
UPON ASH CONTENT OF BARLEY TOPS

	Pounds of 2,4-D Applied per Acre									
	0.00		0.25		0.50		1.00		2.00	
Replication	mg.	%	mg.	%	mg.	%	mg.	%	mg.	%
I	262.5	19.02	149.2	16.44	119.7	16.34	130.4	16.39	151.3	15.47
II	206.0	18.01	121.8	15.56	80.6	15.67	90.1	15.00	112.2	16.03
III	249.9	19.77	182.6	16.79	184.9	17.82	145.0	16.94	124.7	16.25
IV	254.3	19.16	128.7	19.97	103.5	16.19	126.7	15.55	85.8	13.82
Mean	243.2	18.99	145.6	17.19	122.2	16.51	123.1	15.97	118.5	15.39

R.E. (5% = 30.9
(1% = 43.8

P = 5

C.V. = 6.12%

TABLE XI

THE EFFECT OF 2,4-D APPLIED AT THREE-LEAF STAGE
UPON ASH CONTENT OF ROOTS OF BARLEY

	Pounds of 2,4-D Applied per Acre									
	0.00		0.25		0.50		1.00		2.00	
Replication	mg.	%	mg.	%	mg.	%	mg.	%	mg.	%
I	76.3	26.34	40.5	25.61	34.8	22.41	27.2	21.88	15.2	19.47
II	96.1	28.15	35.3	24.72	30.8	23.00	24.8	19.59	13.4	17.98
III	61.7	26.72	34.8	19.39	27.3	18.14	20.0	17.48	9.7	10.95
IV	54.3	27.22	34.3	23.41	26.8	21.69	23.9	19.75	16.0	18.60
Mean	72.1	27.08	36.2	23.28	29.9	21.31	24.0	19.68	13.6	16.75

R.E. (5% = 10.9
(1% = 15.4

P = 5

C.V. = 11.34%

TABLE XII

THE EFFECT OF 2,4-D APPLIED AT THREE-LEAF STAGE
UPON ASH CONTENT OF TOTAL PLANT OF BARLEY

	Pounds of 2,4-D Applied per Acre									
	0.00		0.25		0.50		1.00		2.00	
Replication	mg.	%	mg.	%	mg.	%	mg.	%	mg.	%
I	378.8	22.68	224.2	21.03	172.1	19.38	176.0	19.14	184.5	17.47
II	342.1	23.03	186.5	20.14	125.4	19.34	125.8	17.30	131.7	17.01
III	347.6	23.25	229.2	18.09	213.7	17.98	167.0	17.21	116.4	13.60
IV	354.1	23.19	171.5	21.69	144.7	18.94	165.2	17.65	114.6	16.21
Mean	355.7	23.04	202.9	20.24	164.0	18.91	158.5	17.83	136.8	16.07

R.E. (5% = 36.6
(1% = 51.9

P = 5

C.V. = 5.35%

to the untreated check. In addition, as the rate of application of 2,4-D was increased, the percentage and milligrams of ash also increased.

The ash content of the barley tops, Table X, responded similarly to that of the roots, although less strikingly so. However, the application of 2,4-D served to significantly depress the percentage and milligrams of total ash in the above ground portion of barley.

Within the treatments of growth regulator, Table XII, there was a significant decrease in ash content between the one-half, one and two pound rates when compared to the one-quarter pound rate, and a significant decrease at the two pound rate from the one-half and one pound rates. However, at the one-half and one pound rates, the differences in ash contents were statistically non-significant.

Calcium. Tables XIII, XIV, and XV represent the calcium content of the roots, tops and total barley plant as affected by 2,4-D applied at the three-leaf stage.

The 2,4-D treatment significantly decreased the calcium content of roots, both in percentage and total milligrams, when compared to the untreated roots. A decrease in calcium was correlated with increasing rates of application of 2,4-D.

The calcium content in barley tops was similar to that for roots. It is to be noted that there is a highly significant decrease in milligrams of calcium between the check and treated plants. However, the percentage of calcium is relatively unaffected by the 2,4-D treatment, although there is a slight decreasing trend.

A highly significant decrease in calcium content was found in the 2,4-D treated plants, Table XV, when compared to the non-treated plants.

TABLE XIII

THE EFFECT OF 2,4-D APPLIED AT THREE-LEAF STAGE UPON THE CALCIUM CONTENT OF BARLEY TOPS

	Pounds of 2,4-D Applied per Acre									
	0.00		0.25		0.50		1.00		2.00	
Replication	mg.	%	mg.	%	mg.	%	mg.	%	mg.	%
I	8.8	.64	5.6	.62	4.3	.59	4.6	.58	6.0	.61
II	7.2	.63	4.8	.61	2.8	.55	3.1	.52	3.6	.51
III	7.5	.59	5.8	.53	5.3	.51	4.5	.52	3.9	.51
IV	8.4	.63	4.1	.63	3.8	.60	3.8	.46	2.6	.42
Mean	8.0	.62	5.1	.60	4.1	.56	4.0	.52	4.0	.51

R.E. (5% = 1.1 (1% = 1.6 P = 5 C.V. = 6.50%

TABLE XIV

THE EFFECT OF 2,4-D APPLIED AT THREE-LEAF STAGE UPON CALCIUM CONTENT OF BARLEY ROOTS

	Pounds of 2,4-D Applied per Acre									
	0.00		0.25		0.50		1.00		2.00	
Replication	mg.	%	mg.	%	mg.	%	mg.	%	mg.	%
I	9.3	3.20	4.2	2.67	2.7	1.76	1.5	1.24	.8	.99
II	11.8	3.46	3.7	2.60	2.3	1.69	1.9	1.48	.5	.67
III	5.7	2.48	4.3	2.40	3.7	2.43	1.9	1.67	.5	.58
IV	7.1	3.53	3.1	2.14	2.1	1.68	1.9	1.56	.7	.79
Mean	8.5	3.17	3.8	2.45	2.7	1.89	1.8	1.49	.6	.76

R.E. (5% = 2.2 (1% = 3.1 P = 5 C.V. = 8.76%

TABLE XV

THE EFFECT OF 2,4-D APPLIED AT THREE-LEAF STAGE UPON THE CALCIUM CONTENT OF COMPLETE BARLEY PLANT

	Pounds of 2,4-D Applied per Acre									
	0.00		0.25		0.50		1.00		2.00	
Replication	mg.	%	mg.	%	mg.	%	mg.	%	mg.	%
I	32.1	1.92	17.6	1.65	10.5	1.18	8.4	.91	8.5	.80
II	30.5	2.05	14.9	1.61	7.3	1.12	7.3	1.00	4.6	.59
III	23.0	1.54	18.6	1.47	17.5	1.47	10.7	1.10	4.7	.55
IV	31.8	2.08	11.0	1.39	8.7	1.14	9.5	1.01	4.3	.61
Mean	29.4	1.90	15.5	1.53	11.0	1.23	9.0	1.01	5.5	.64

R.E. (5% = 5.2 P = 5 C.V. = 11.05%

Within the 2,4-D treatments, there was a continued decrease in both percentage content and total milligrams of calcium as the rates of application of 2,4-D were increased.

Calcium^{45}. Calcium^{45} uptake by barley roots, tops and the complete plant, in counts per minute is shown in Tables XVI, XVII and XVIII.

The roots of treated plants show a significant decrease in counts per minute of calcium^{45} at the one-half, one and two pound rates when compared to the one-quarter pound rate. The two pound rate significantly depressed calcium^{45} uptake from all other treatments.

The data in Table XVI shows the calcium^{45} translocated from roots to above ground parts and a significant decrease in counts per minute of calcium^{45} between the treated and non-treated plants. Within the treated plants there is a statistically significant difference between the two pound rate and all other treatments, with no significant difference between the one-quarter, one-half and one pound rates of 2,4-D.

A significant decrease in calcium^{45} uptake was evident between the 2,4-D treated and non-treated plants with the greatest difference occurring at the highest rate of 2,4-D. Within the treated plants, there was a decrease in counts per minute of calcium^{45} as the rate of 2,4-D increased.

Distribution of calcium^{45}. Radiograms of all five treatments are presented in Figures 8, 9, 10, 11 and 12 to indicate the effect of increasing rates of 2,4-D upon the uptake of calcium^{45}. The uptake was measured by the degree of exposure of the x-ray film, and indicates its subsequent translocation and distribution from the roots.

It is to be noted that the growth regulator has less effect upon calcium^{45} uptake and distribution applied as a spray on the plant at the

TABLE XVI

UPTAKE OF CALCIUM^{45} BY ABOVE GROUND PORTIONS OF BARLEY AT THE THREE-LEAF STAGE EXPRESSED AS COUNTS PER MINUTE

	Pounds of 2,4-D Applied per Acre				
Replication	0.00	0.25	0.50	1.00	2.00
I	2144	945	700	749	554
II	3085	1067	825	966	671
III	1777	758	681	512	438
IV	1982	995	745	774	598
Mean	2247	941	738	750	565

R.E. (5% = 370
(1% = 525

P = 5

C.V. = 10.52%

TABLE XVII

UPTAKE OF CALCIUM^{45} BY BARLEY ROOTS AT THE THREE-LEAF STAGE EXPRESSED AS COUNTS PER MINUTE

	Pounds of 2,4-D Applied per Acre				
Replication	0.00	0.25	0.50	1.00	2.00
I	7643	3246	2876	2745	1823
II	8209	4574	3149	2832	1463
III	7125	3122	2200	2085	1626
IV	7543	3742	3009	1823	1700
Mean	7630	3671	2809	2371	1653

R.E. (5% = 774
(1% = 1097

P = 5

C.V. = 6.35%

TABLE XVIII

UPTAKE OF CALCIUM^{45} BY BARLEY AT THE THREE-LEAF STAGE EXPRESSED AS COUNTS PER MINUTE

	Pounds of 2,4-D Applied per Acre				
Replication	0.00	0.25	0.50	1.00	2.00
I	9787	4191	3576	3494	2377
II	11294	5641	3974	3798	2134
III	8902	3880	2881	2597	2064
IV	9525	4737	3754	2597	2298
Mean	9877	4612	3546	3122	2218

R.E. (5% = 759
(1% = 1075

P = 5

C.V. = 4.83%

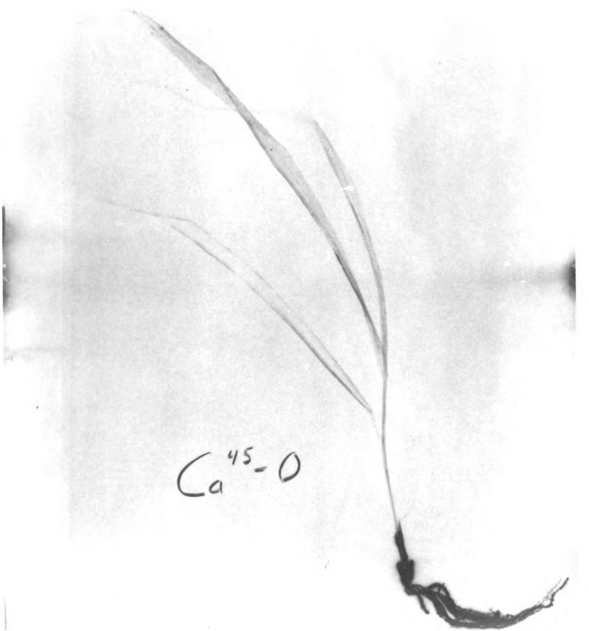


Figure 8: Radiogram of Calcium^{45} in Non-treated Barley.

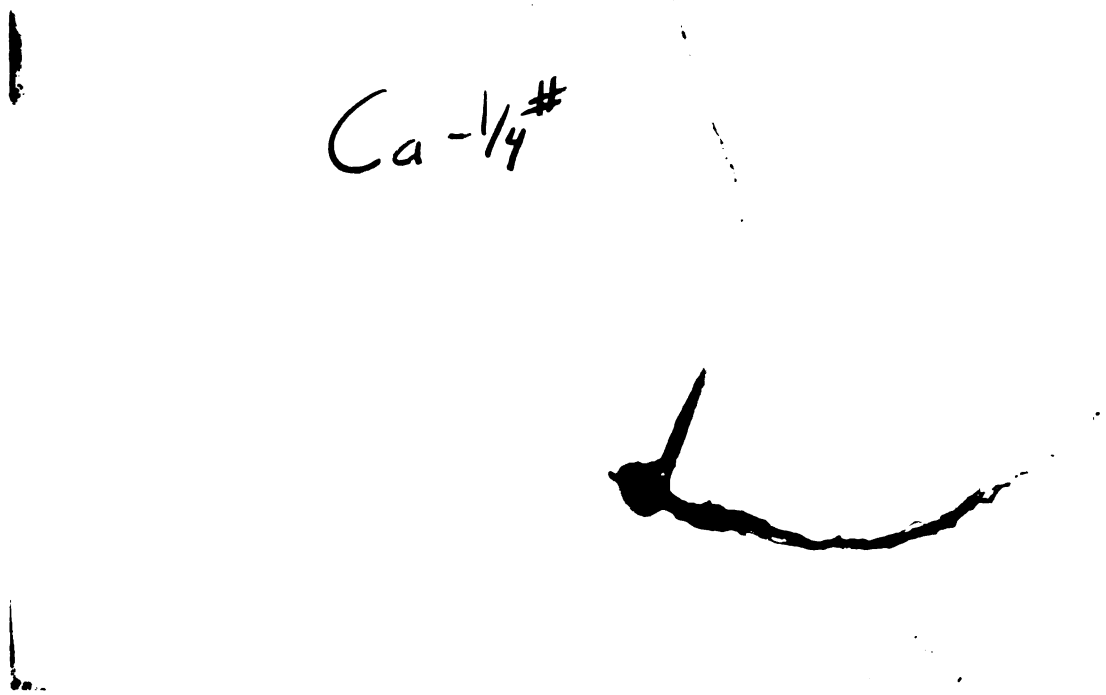


Figure 9: Radiogram of Calcium^{45} in Barley Treated with One-quarter Pound of 2,4-D per Acre at Three-leaf Stage.

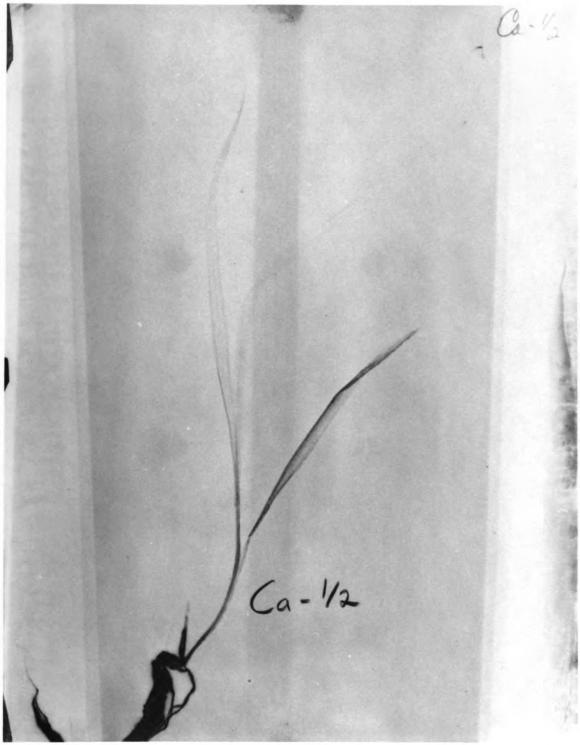


Figure 10: Radiogram of Calcium^{45} in Barley Treated with One-half Pound of 2,4-D per Acre at Three-leaf Stage.

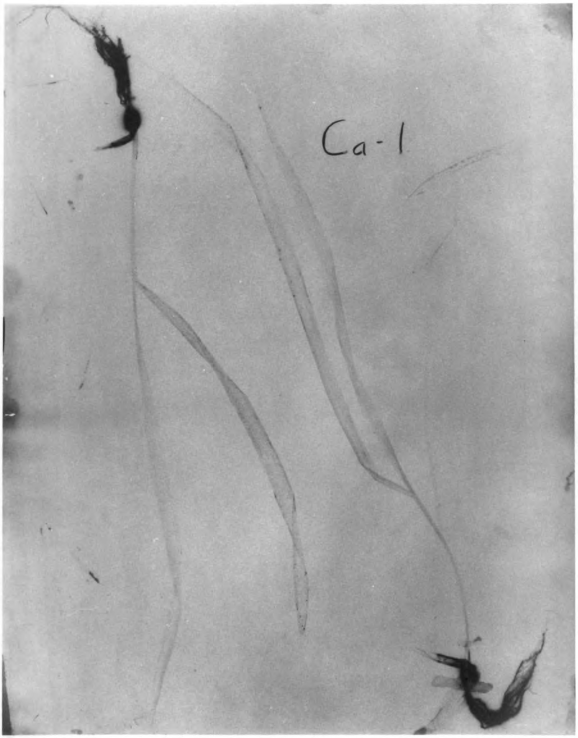


Figure 11: Radiogram of Calcium[45] in Barley Treated with One Pound of 2,4-D per Acre at Three-leaf Stage.

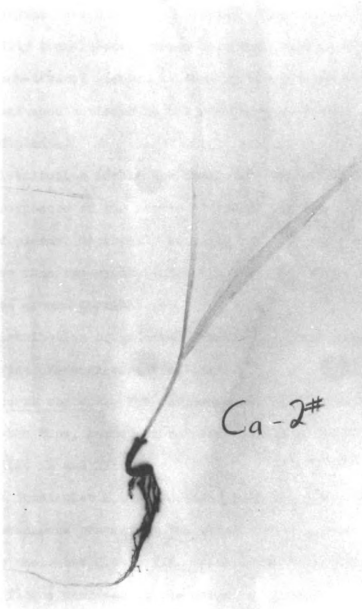


Figure 12: Radiogram of Calcium^{45} in Barley Treated with Two Pounds of 2,4-D per Acre at Three-leaf Stage.

three-leaf stage than was apparent in the pre-emergence application. The figures reveal that the translocation of calcium^{45} to the older leaves decreased as the rate of 2,4-D increased. The calcium^{45}, moreover, was not as readily translocated upward from the roots in the treated plants as in the non-treated plants, as seen by the greater exposure of lower stem and leaf sheath tissue in the treated plants when compared to the non-treated plants.

The distribution within the roots was impossible to determine due to the overexposure of the roots. However, the degree of exposure of the treated plants, especially in areas of root proliferation was somewhat greater than the check, indicating failure of the calcium^{45} to move to the above ground portions.

The distribution of calcium^{45} at the cellular level was identical to that of the pre-emergence treatments.

Phosphorus content. The influence of 2,4-D upon the phosphorus content of the tops, roots and complete plants of barley is presented in Tables XIX, XX and XXI.

It was found that 2,4-D decreased both the percentage and milligrams of phosphorus present in the above ground portion of barley when compared to the check, Table XIX. Within the 2,4-D treatments, there was a significant decrease in the weight of phosphorus at the two pound when compared to the one-quarter pound rate.

The influence of 2,4-D upon the phosphorus content of roots, Table XX, was not as striking as in the tops. There was a non-significant decrease in milligrams of phosphorus between the untreated and the one-quarter pound rate of 2,4-D with a significant difference between the

TABLE XIX

THE EFFECT OF 2,4-D APPLIED AT THREE-LEAF STAGE UPON PHOSPHORUS CONTENT OF BARLEY TOPS

	Pounds of 2,4-D Applied per Acre									
	0.00		0.25		0.50		1.00		2.00	
Replication	mg.	%	mg.	%	mg.	%	mg.	%	mg.	%
I	12.7	.92	8.4	.92	6.4	.87	6.7	.84	7.5	.77
II	11.9	1.04	7.5	.96	4.8	.93	5.1	.85	6.0	.85
III	13.0	1.03	10.3	.95	9.5	.92	7.7	.90	6.5	.85
IV	13.1	.99	6.1	.95	5.6	.87	7.1	.87	5.2	.84
Mean	12.7	1.00	8.1	.95	6.6	.90	6.7	.87	6.3	.83

R.E. (5% = 1.6
(1% = 2.2

P = 5

C.V. = 5.82%

TABLE XX

THE EFFECT OF 2,4-D APPLIED AT THREE-LEAF STAGE UPON PHOSPHORUS CONTENT OF BARLEY ROOTS

	Pounds of 2,4-D Applied per Acre									
	0.00		0.25		0.50		1.00		2.00	
Replication	mg.	%	mg.	%	mg.	%	mg.	%	mg.	%
I	5.6	1.93	2.9	1.85	4.0	2.60	3.7	2.98	2.7	3.48
II	6.8	2.00	5.3	3.73	3.5	2.62	4.3	3.38	2.8	3.79
III	4.4	1.89	5.0	2.81	2.9	1.90	2.9	2.49	2.0	2.26
IV	3.7	1.84	3.2	2.20	3.2	2.56	3.4	2.84	1.7	2.01
Mean	5.1	1.92	4.1	2.65	3.4	2.42	3.6	2.92	2.3	2.89

R.E. (5% = 1.1
(1% = 1.6

P = 5

C.V. = 8.92%

TABLE XXI

THE EFFECT OF 2,4-D APPLIED AT THREE-LEAF STAGE UPON PHOSPHORUS CONTENT OF BARLEY

	Pounds of 2,4-D Applied per Acre									
	0.00		0.25		0.50		1.00		2.00	
Replication	mg.	%	mg.	%	mg.	%	mg.	%	mg.	%
I	23.9	1.43	14.8	1.39	15.4	1.74	17.6	1.91	22.5	2.13
II	22.6	1.52	21.8	2.35	11.5	1.78	15.4	2.12	18.0	2.32
III	21.8	1.46	23.8	1.88	16.8	1.41	16.5	1.70	13.4	1.56
IV	21.7	1.42	12.5	1.58	13.1	1.72	17.4	1.86	10.1	1.43
Mean	22.5	1.46	18.2	1.80	14.2	1.66	16.7	1.90	16.0	1.86

R.E. (5% = 5.35
(1% - 7.59

P = 5

C.V. = 9.09%

one-half, one and two pound rates and the check. However, when the percentage of phosphorus is observed, it is evident that the 2,4-D treatment resulted in an increase in percentage of phosphorus with all 2,4-D treatments being significantly higher than that of the control.

The data in Table XXI indicates that the application of 2,4-D at the three-leaf stage resulted in a significant decrease in phosphorus content of the treated roots and tops when compared to the control, whereas the effect upon the complete plant was statistically insignificant. There was, however, a non-significant decrease in milligrams of phosphorus in treated plants as compared to the non-treated plants. The 2,4-D also appears to have effected a non-significant increase in percentage of phosphorus in the treated plants over the check.

Phosphorus^{32}. The information obtained in the study of the effect of 2,4-D upon the uptake of phosphorus^{32} by barley is presented in Tables XXII, XXIII, and XXIV.

The values obtained reveal a striking effect of 2,4-D upon the uptake of phosphorus^{32} by roots, Table XXIII, there being a highly significant difference in counts per minute between the control and the 2,4-D treated plants. Within the treated plants, there is a significantly lower difference between the two pound and the one-quarter, one-half and one pound rates.

A comparison of the values obtained for the effect of 2,4-D on phosphorus^{32} uptake by barley expressed as root, top and complete plant values reveal that much of the phosphorus^{32} taken up by the roots, in both the treated and non-treated plants, was not translocated upward as evidenced by the reduction of activity by the tops, Table XXII.

TABLE XXII

UPTAKE OF PHOSPHORUS^{32} BY ABOVE GROUND PORTION OF BARLEY AT THE THREE-LEAF STAGE EXPRESSED AS COUNTS PER MINUTE

	Pounds of 2,4-D Applied per Acre				
Replication	0.00	0.25	0.50	1.00	2.00
I	1524	1465	1408	1353	1299
II	1432	1398	1307	1246	1212
III	1634	1469	1317	1240	1202
IV	1625	1407	1375	1276	1272
Mean	1554	1435	1352	1279	1246

R.E. (5% = 61
(1% = 87

P = 5

C.V. = 1.33%

TABLE XXIII

UPTAKE OF PHOSPHORUS^{32} BY BARLEY ROOTS AT THE THREE-LEAF STAGE EXPRESSED AS COUNTS PER MINUTE

	Pounds of 2,4-D Applied per Acre				
Replication	0.00	0.25	0.50	1.00	2.00
I	10928	4982	4170	4120	3147
II	11198	3635	3554	3533	2546
III	11314	3729	3570	2600	2500
IV	12089	3579	3901	3821	2687
Mean	11382	3981	3800	3769	2720

R.E. (5% = 381
(1% = 541

P = 5

C.V. = 2.21%

TABLE XXIV

UPTAKE OF PHOSPHORUS^{32} BY BARLEY AT THE THREE-LEAF STAGE EXPRESSED AS COUNTS PER MINUTE

	Pounds of 2,4-D Applied per Acre				
Replication	0.00	0.25	0.50	1.00	2.00
I	12452	6447	5578	5473	4446
II	12630	5033	4861	4779	3758
III	12948	5198	4887	4840	3702
IV	13714	4986	5276	5097	3959
Mean	12936	5416	5150	5047	3966

R.E. (5% = 615
(1% = 871

P = 5

C.V. = 2.81%

However, there is a statistically significant difference between values obtained for the control and those obtained from the plants treated with 2,4-D. As the rate of 2,4-D was increased, the quantity of phosphorus^{32} translocated from the roots to the tops decreased.

The application of the growth regulator significantly decreased the uptake of phosphorus^{32} over a two hour period when compared to the non-treated plants. Within the treated plants, there was a significant decrease at the two pound rate of 2,4-D when compared to the one-quarter, one-half and one pound rates. There appears to be a non-significant decrease in phosphorus^{32} uptake from the one-quarter to the one pound level, with an overall decrease in phosphorus^{32} uptake initiated by the application of 2,4-D and further decreasing with increasing rates of 2,4-D.

Distribution of phosphorus^{32}. Figures 13, 14, 15, 16 and 17 depict the influence of 2,4-D upon the uptake and translocation of 2,4-D with barley treated at the three-leaf stage.

The most obvious effect of the 2,4-D, upon comparing the treated to the non-treated plants, is that of a reduction in the quantity of phosphorus^{32} absorbed. The tops of the check plants are much darker, due to greater photographic exposure, than are those of the treated plants, with the difference in exposure increasing as the rate of the herbicide application was increased. As in the treatments with calcium^{45}, the amount of isotope translocated to the older leaves decreased as the 2,4-D increased.

The degree of exposure due to the absorbed phosphorus^{32} in the roots of the treated plants again indicated that the phosphorus^{32} apparently was not being translocated upward. The proliferated root areas are

easily distinguishable in the treated plants and it was found, by means of microradiograms, there was an accumulation of phosphorus^{32} in these lateral roots.

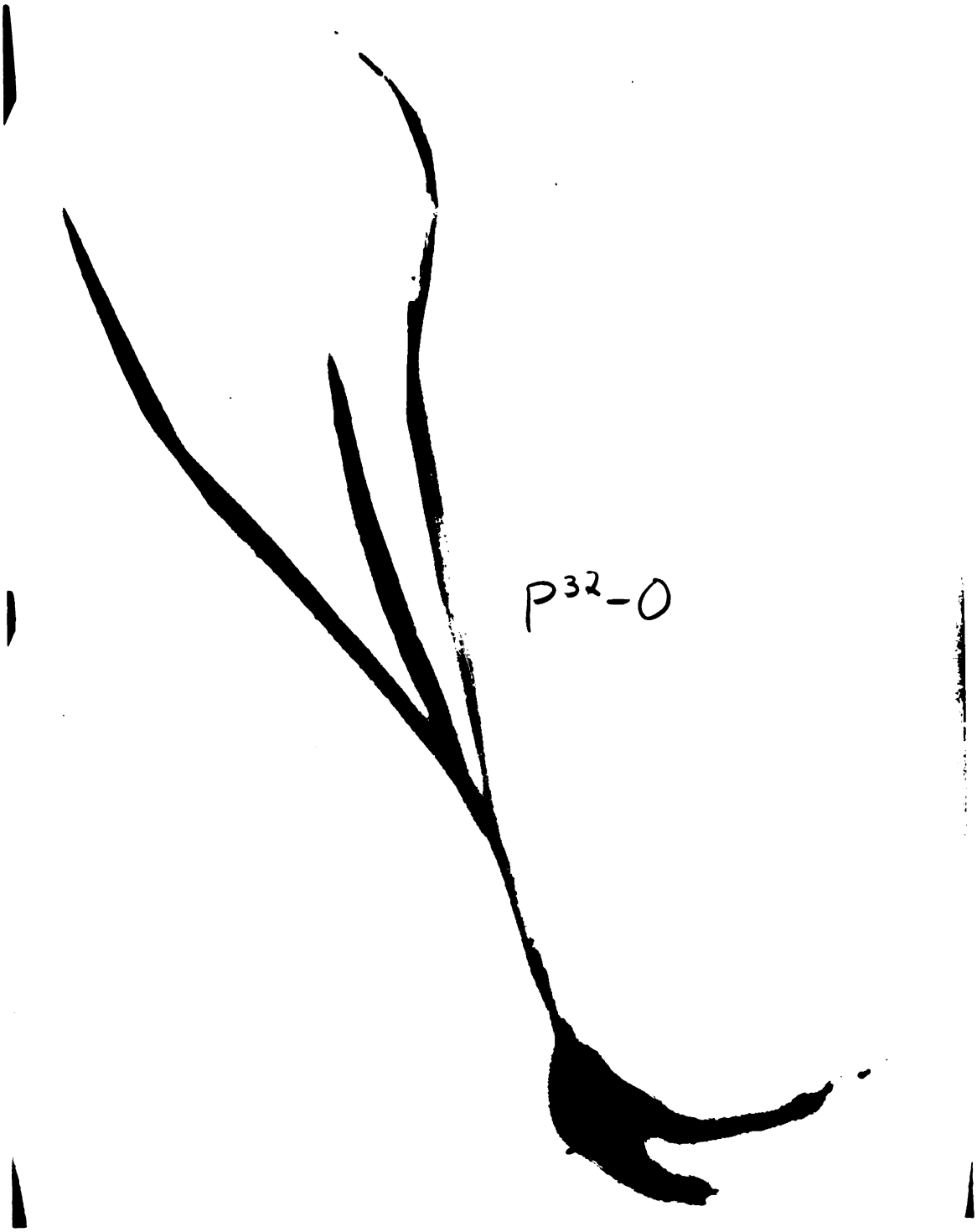


Figure 13: Radiogram of Phosphorus^{32} in Non-treated Barley

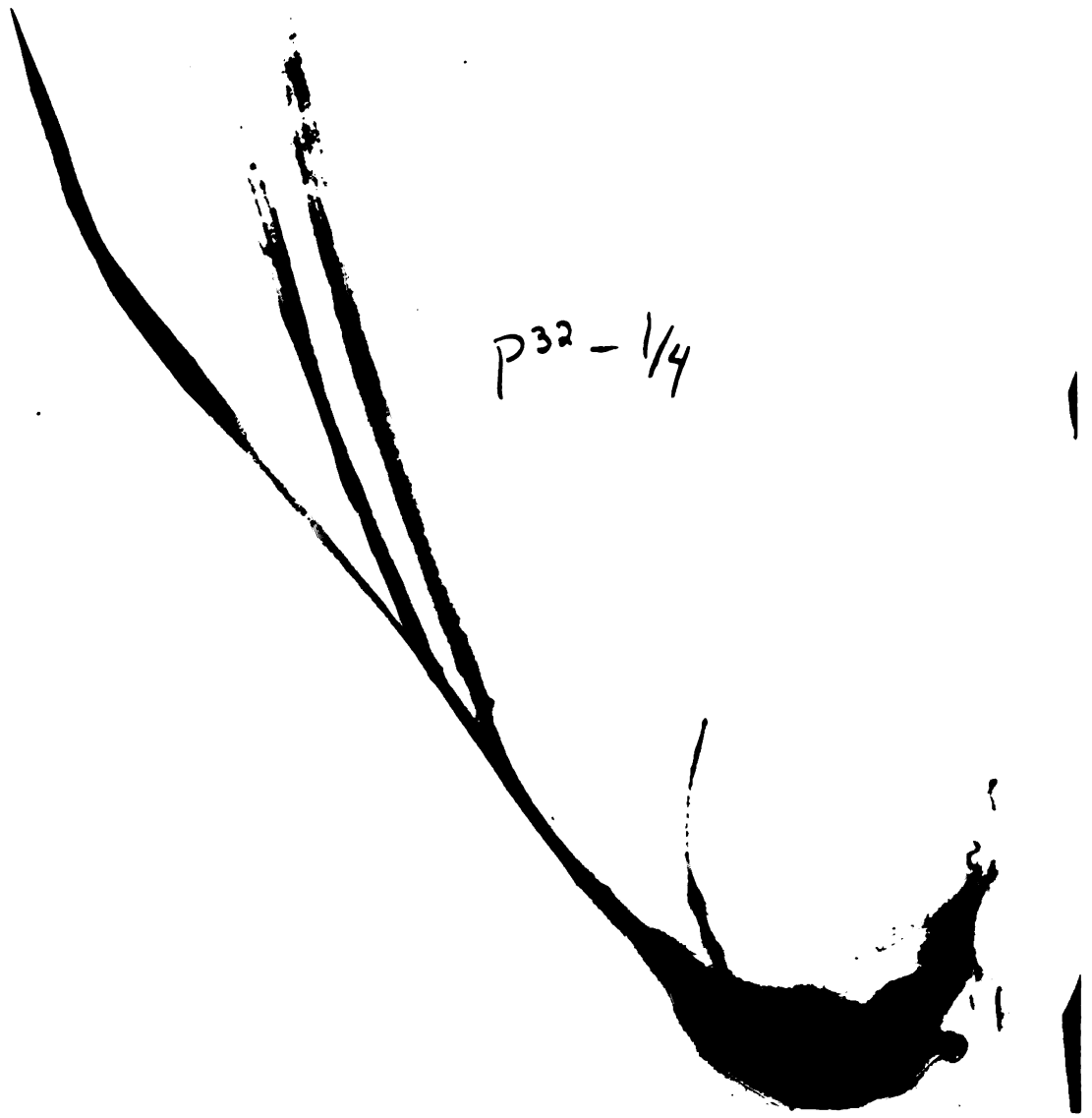


Figure 14: Radiogram of Phosphorus^{32} in Barley Treated with One-quarter Pound of 2,4-D per Acre at Three-leaf Stage.

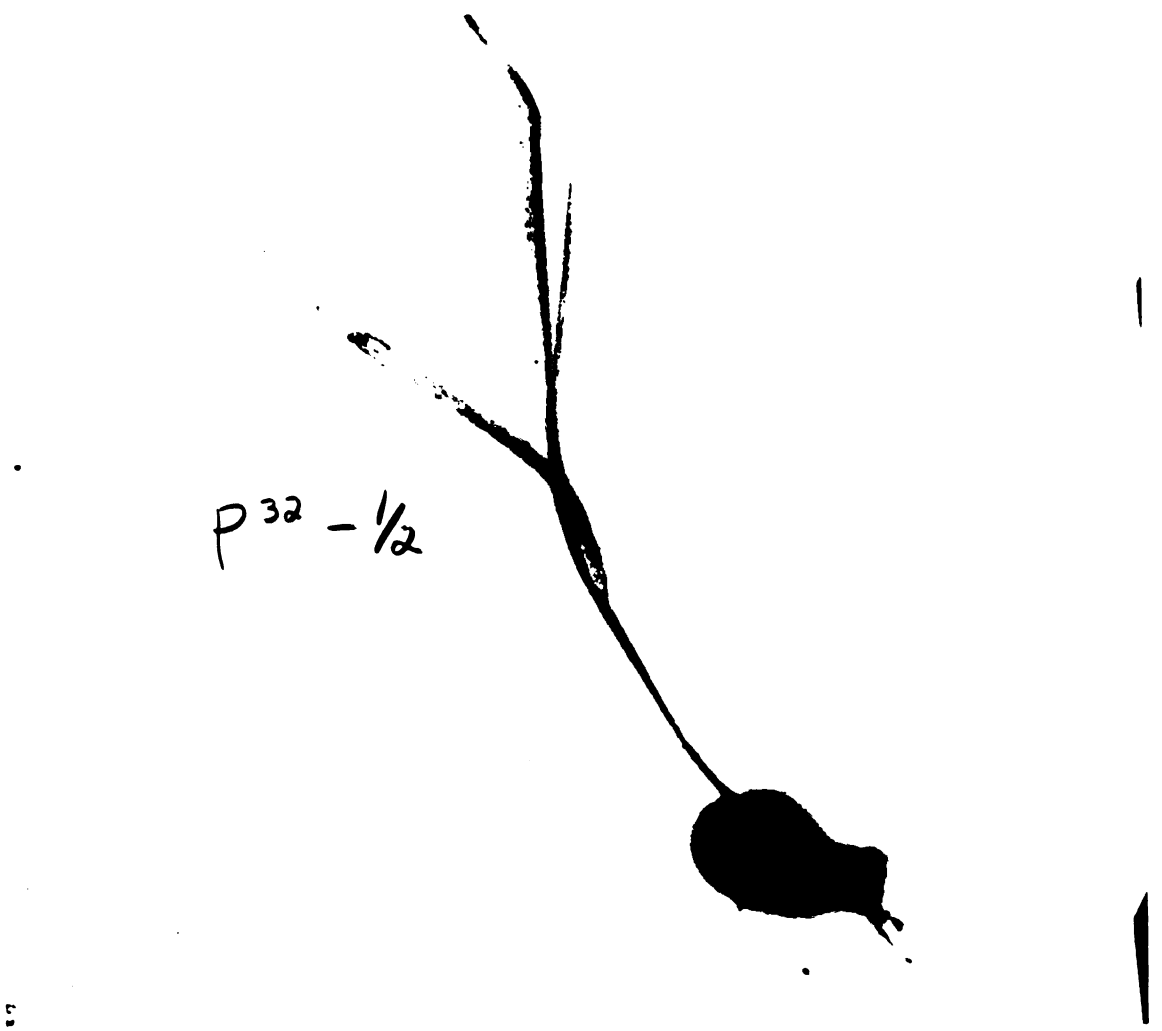


Figure 15: Radiograms of Phosphorus^{32} in Barley Treated with One-half Pound of 2,4-D per Acre at Three-leaf Stage.

Figure 16: Radiogram of Phosphorus^{32} in Barley Treated with One Pound of 2,4-D per Acre at Three-leaf Stage.

Figure 17: Radiogram of Phosphorus^{32} in Barley Treated with Two Pounds of 2,4-D per Acre at Three-leaf Stage.

Figure 17: Radiogram of Phosphorus^{32} in Barley Treated with Two Pounds of 2,4-D per Acre at Three-leaf Stage.

Experiment III - Five-leaf Stage

General appearance. The non-treated plants were free of visible insect or disease damage at the time of treatment and at the end of the treatment period, two new leaves had been produced. Frequently, however, the oldest leaf or the two oldest leaves would be dead or dying. This was also observed in the treated plants. The plants treated with 2,4-D were also free of insect and disease damage at the time of treatment, and, in general, the one-quarter and one-half pound rates had produced two new leaves during the treatment period; whereas the one and two pound rates had produced only one new leaf.

The greatest observable effect of the 2,4-D treatment was the stunting of the roots. With increasing rates of 2,4-D, the greater was the amount of stunting, thickening and reduction in branching of the roots.

Dry weight - roots. The data in Table XXVI shows that there was a significant decrease in dry weight of roots between the control and the 2,4-D treated plants. Within the 2,4-D treatments, there was a decline in dry weight of roots with increasing quantities of the herbicide.

Dry weight - tops. There was less influence of 2,4-D upon the dry weight of the above ground portion of barley, Table XXV, than that observed in roots. The decrease in dry weight occurring at the one-quarter, one-half and two pound rates, as well as the increase in dry weight at the one pound level, are not statistically different from that of the control.

Dry weight - complete plant. The data presented in Table XXVII summarizes the dry weight of the complete plants as affected by 2,4-D

TABLE XXV

EFFECT OF 2,4-D UPON THE DRY WEIGHT (IN MILLIGRAMS) OF ABOVE GROUND PORTION OF BARLEY PLANTS WHEN APPLIED AT FIVE-LEAF STAGE

Replication	Pounds of 2,4-D Applied per Acre				
	0.00	0.25	0.50	1.00	2.00
I	1882.8	1602.7	1876.0	1646.3	904.6
II	1985.1	1875.6	2297.2	2289.3	1434.2
III	2167.6	2031.1	1935.6	1853.3	2081.9
IV	1817.9	2147.7	1603.0	2663.3	1645.9
Mean	1963.4	1914.3	1928	2113	1517

R.E. (5% = 474
(1% = 672

P = 5

C.V. = 7.48%

TABLE XXVI

EFFECT OF 2,4-D UPON THE DRY WEIGHT (IN MILLIGRAMS) OF ROOTS OF BARLEY PLANTS WHEN APPLIED AT FIVE-LEAF STAGE

Replication	Pounds of 2,4-D Applied per Acre				
	0.00	0.25	0.50	1.00	2.00
I	673.0	506.1	494.0	490.0	446.5
II	538.9	487.2	458.5	451.0	444.7
III	686.6	581.1	593.6	545.5	522.1
IV	633.6	615.9	552.2	499.4	475.7
Mean	633.0	547.6	524.6	496.5	472.3

R.E. (5% = 44.6
(1% = 63.2

P = 5

C.V. = 2.48%

TABLE XXVII

EFFECT OF 2,4-D UPON THE DRY WEIGHT (IN MILLIGRAMS) OF BARLEY PLANTS WHEN APPLIED AT FIVE-LEAF STAGE

Replication	Pounds of 2,4-D Applied per Acre				
	0.00	0.25	0.50	1.00	2.00
I	2555.8	2108.8	2370.0	2136.3	1351.1
II	2524.0	2462.8	2755.7	2740.3	1878.9
III	2854.2	2612.2	2529.2	2398.8	2604.0
IV	2451.5	2763.6	2155.2	3162.7	2121.6
Mean	2596.4	2486.9	2452.5	2609.5	1988.9

R.E. (5% = 529.8
(1% = 750.4

P = 5

C.V. = 5.82%

when applied at the five-leaf stage. It is to be noted that the one pound rate of 2,4-D increased the total dry weight of the plant above that of the control, although the increase was not significantly different. The one-quarter, one-half and two pound rates resulted in a slight, non-significant decrease in dry weight when compared to the check.

Ash - roots. The application of the herbicide, 2,4-D, at the five-leaf stage resulted in a significant decrease in total milligrams of ash between the control and the treated plants, Table XXIX, with no significant effects of 2,4-D upon the percentage content.

Within the treated plants, however, there was no discernible pattern of increase or decrease in either percentage or total weight of ash.

Ash - tops. It is to be observed, Table XXIII, that the growth regulator had no consistent or significant effect either on the percentage content or total milligrams of ash. However, in general, there was a slight reduction in weight of ash with 2,4-D application, with the exception of the one pound level which actually appears to stimulate increased ion uptake resulting in a higher ash content. The percentage content of ash remained essentially constant in both the treated and non-treated plants.

Ash - complete plant. The data presented in Table XXX indicates that the application of 2,4-D to barley at the five-leaf stage had no significant effect, either upon the percentage or the total milligrams of ash.

Calcium - roots. Two,4-D applied as a spray at the five-leaf stage of barley plants effected a significant decrease in milligrams of calcium in the roots when compared to the non-treated plants, Table XXXII.

TABLE XXVIII

EFFECT OF 2,4-D APPLIED AT FIVE-LEAF STAGE UPON ASH CONTENT OF ABOVE GROUND PORTION OF BARLEY

	Pounds of 2,4-D Applied per Acre									
	0.00		0.25		0.50		1.00		2.00	
Replication	mg.	%	mg.	%	mg.	%	mg.	%	mg.	%
I	384.1	20.4	318.9	19.9	373.3	19.9	334.2	20.3	191.8	21.2
II	418.9	21.1	393.9	21.0	445.7	19.4	480.8	21.0	286.8	20.0
III	466.0	21.5	406.2	20.0	373.6	19.3	367.0	19.8	450.0	21.6
IV	367.2	20.2	451.0	21.0	333.4	20.8	522.0	19.6	330.8	20.1
Mean	409.1	20.8	392.5	20.5	381.5	19.9	426.0	20.2	314.9	20.7

R.E. (5% = 31.2
(1% = 44.2

P = 5

C.V. = 2.42%

TABLE XXIX

EFFECT OF 2,4-D APPLIED AT FIVE-LEAF STAGE UPON ASH CONTENT OF ROOTS OF BARLEY

	Pounds of 2,4-D Applied per Acre									
	0.00		0.25		0.50		1.00		2.00	
Replication	mg.	%	mg.	%	mg.	%	mg.	%	mg.	%
I	118.5	17.6	87.0	17.2	90.4	18.3	82.3	16.8	90.2	20.2
II	97.0	18.0	80.4	16.5	85.3	18.6	72.6	16.1	80.5	18.1
III	127.7	18.6	101.1	17.4	106.8	18.0	94.4	17.3	101.8	19.5
IV	107.7	17.0	103.5	16.8	106.0	19.2	82.4	16.5	95.1	20.0
Mean	112.7	18.0	93.0	17.0	97.1	18.5	82.9	16.7	91.9	19.5

R.E. (5% = 7.7
(1% = 10.9

P = 5

C.V. = 2.39%

TABLE XXX

EFFECT OF 2,4-D APPLIED AT FIVE-LEAF STAGE UPON ASH CONTENT OF BARLEY

	Pounds of 2,4-D Applied per Acre									
	0.00		0.25		0.50		1.00		2.00	
Replication	mg.	%	mg.	%	mg.	%	mg.	%	mg.	%
I	485.6	19.0	392.2	18.6	452.7	19.1	397.4	18.6	279.7	20.7
II	494.7	19.6	463.0	18.8	523.6	19.0	509.7	18.6	358.9	19.1
III	573.7	20.1	488.5	18.7	473.0	18.7	446.2	18.6	536.4	20.6
IV	456.0	18.6	522.3	18.9	431.0	20.0	613.6	19.4	426.4	20.1
Mean	502.5	19.3	466.5	18.8	470.1	19.2	491.7	18.8	400.4	20.1

R.E. (5% = 99.2
(1% = 140.5

P = 5

C.V. = 6.33%

TABLE XXXI

THE EFFECT OF 2,4-D APPLIED AT FIVE-LEAF STAGE UPON THE CALCIUM CONTENT OF ABOVE GROUND PORTIONS OF BARLEY

	Pounds of 2,4-D Applied per Acre									
	0.00		0.25		0.50		1.00		2.00	
Replication	mg.	%	mg.	%	mg.	%	mg.	%	mg.	%
I	14.1	.75	11.2	.70	13.5	.72	11.7	.71	7.3	.81
II	14.1	.71	11.4	.61	17.9	.78	17.4	.76	9.9	.69
III	13.9	.64	17.5	.86	14.5	.75	13.3	.72	13.5	.65
IV	12.7	.74	15.9	.74	11.9	.74	20.0	.75	12.2	.74
Mean	13.7	.71	14.0	.73	14.5	.75	15.6	.74	10.7	.72

R.E. (5% = 4.0
(1% = 5.6

P = 5

C.V. = 8.71%

TABLE XXXII

THE EFFECT OF 2,4-D APPLIED AT FIVE-LEAF STAGE UPON CALCIUM CONTENT OF ROOTS OF BARLEY

	Pounds of 2,4-D Applied per Acre									
	0.00		0.25		0.50		1.00		2.00	
Replication	mg.	%	mg.	%	mg.	%	mg.	%	mg.	%
I	9.8	1.85	7.0	1.39	6.5	1.31	5.0	1.02	4.4	.99
II	9.5	1.76	7.3	1.49	6.3	1.38	5.3	1.18	5.2	1.17
III	12.7	1.85	8.3	1.43	8.5	1.43	7.3	1.34	6.4	1.23
IV	9.5	1.50	8.9	1.44	7.6	1.38	6.5	1.31	6.1	1.28
Mean	10.4	1.64	7.9	1.44	7.2	1.38	6.0	1.21	5.5	1.17

R.E. (5% = .9
(1% = 1.3

P = 5

C.V. = 3.67%

TABLE XXXIII

THE EFFECT OF 2,4-D APPLIED AT FIVE-LEAF STAGE UPON THE CALCIUM CONTENT OF BARLEY

	Pounds of 2,4-D Applied per Acre									
	0.00		0.25		0.50		1.00		2.00	
Replication	mg.	%	mg.	%	mg.	%	mg.	%	mg.	%
I	28.1	1.10	22.1	1.05	24.2	1.02	18.6	.87	12.2	.90
II	31.3	1.24	25.9	1.05	30.0	1.08	26.6	.97	17.5	.93
III	35.7	1.25	30.0	1.15	27.6	1.09	24.7	1.03	24.5	.94
IV	27.5	1.12	30.1	1.09	22.9	1.06	32.6	1.03	21.4	1.01
Mean	30.7	1.18	27.0	1.09	26.2	1.06	25.6	.98	18.9	.95

R.E. (5% = 5.8
(1% = 8.3

P = 5

C.V. = 6.77%

Within the 2,4-D treatments, the calcium content at the one and two pound rates were significantly lower than those at the one-quarter and one-half pound rates. The overall effect of increasing rates of 2,4-D was that of decreasing calcium content with increasing rate of 2,4-D.

Calcium - tops. In contrast to the effect of 2,4-D upon the calcium content of roots, the data in Table XXXI indicates that there is no significant effect of the growth regulator upon the calcium content of the above ground portions of barley.

Calcium - complete plant. In Table XXXIII it is to be noted that the calcium content of the complete plant was significantly decreased by the application of two pounds of 2,4-D per acre when compared to the non-treated plants. There was no significant effect upon the percentage of calcium in the total plant.

Calcium^{45} - roots. The uptake of calcium^{45} over a two hour uptake period, treated at the five-leaf stage with 2,4-D, was significantly decreased from the control, as evidenced by the data in Table XXXV.

Within the roots of the treated plants, the counts per minute of calcium^{45} was significantly decreased by increasing rates of 2,4-D, with significant differences occurring between all treatments.

Calcium^{45} - tops. The data in Table XXXIV indicates that the content of calcium^{45} in the above ground portion of the treated barley was significantly decreased by 2,4-D from that of the control.

Within the plants treated with 2,4-D, there was a definite decrease in counts per minute of calcium^{45} with increasing rates of application of 2,4-D. The two pound rate resulted in significantly lower counts per minute than all other treatments.

TABLE XXXIV

UPTAKE OF CALCIUM^{45} BY ABOVE GROUND PORTIONS OF BARLEY AT THE FIVE-LEAF STAGE EXPRESSED AS COUNTS PER MINUTE

	Pounds of 2,4-D Applied per Acre				
Replication	0.00	0.25	0.50	1.00	2.00
I	1092	901	729	579	451
II	1198	821	670	728	365
III	1314	799	687	672	324
IV	1120	806	854	709	543
Mean	1181	832	735	672	421

R.E. (5% = 144
(1% = 204

P = 5

C.V. = 5.59%

TABLE XXXV

UPTAKE OF CALCIUM^{45} BY BARLEY ROOTS AT THE FIVE-LEAF STAGE EXPRESSED AS COUNTS PER MINUTE

	Pounds of 2,4-D Applied per Acre				
Replication	0.00	0.25	0.50	1.00	2.00
I	2832	1769	1495	1110	610
II	2745	1635	1546	1359	503
III	2045	1510	1623	1304	626
IV	2823	1700	1346	1328	463
Mean	2611	1654	1503	1275	551

R.E. (5% = 342
(1% = 485

P = 5

C.V. = 6.70%

TABLE XXXVI

UPTAKE OF CALCIUM^{45} BY BARLEY AT THE FIVE-LEAF STAGE EXPRESSED AS COUNTS PER MINUTE

	Pounds of 2,4-D Applied per Acre				
Replication	0.00	0.25	0.50	1.00	2.00
I	3924	2670	2224	1689	1061
II	3943	2456	2216	2087	868
III	3359	2309	2310	1976	950
IV	3943	2506	2200	2037	1006
Mean	3792	2485	2238	1947	971

R.E. (5% = 292
(1% = 414

P = 5

C.V. = 3.83%

Calcium^{45} - complete plant. The effect of 2,4-D upon the uptake of calcium^{45} by barley treated at the five-leaf stage is presented in Table XXXVI.

From the data, Table XXXVI, treatment with 2,4-D caused a significant decrease in calcium^{45} uptake, as compared to the check. Within the treated plants, the uptake of calcium^{45} was decreased by increasing rates of 2,4-D, with all rates of 2,4-D being significantly different from each other.

Calcium^{45} distribution. The effect of 2,4-D upon the uptake and translocation of calcium^{45} is presented in Figures 18 and 19. The tops of the treated plants were found to contain much less calcium^{45} than the check, as evidenced by the degree of exposure of the x-ray film. There appeared to be an influence of 2,4-D upon the number of tillers produced, especially in plants treated at the five-leaf stage. Figure 19 indicates that calcium^{45} was concentrated in the intercalary meristem of the developing tiller.

The lack of translocation of the calcium^{45} from the roots to the tops of barley is evident from the differences in degree of exposure present in the tops of the treated and non-treated plants while the root exposures are relatively comparable.

Calcium^{45} distribution at cellular level. The distribution in the five-leaved treatments were comparable to those of the pre-emergence treatments.

Phosphorus - roots. The data, Table XXXVIII, shows that 2,4-D has no significant effect upon either the percentage composition or the total quantity of phosphorus present in the roots. However, there is a

Figure 18: Radiogram of Calcium^{45} in Non-treated Barley.

Figure 19: Radiogram of Calcium[45] in Barley Treated with Two Pounds of 2,4-D per Acre at Five-leaf Stage.

TABLE XXXVII

THE EFFECT OF 2,4-D APPLIED AT FIVE-LEAF STAGE UPON PHOSPHORUS CONTENT OF BARLEY TOPS

	Pounds of 2,4-D Applied per Acre									
	0.00		0.25		0.50		1.00		2.00	
Replication	mg.	%	mg.	%	mg.	%	mg.	%	mg.	%
I	17.9	.95	17.2	1.07	20.1	1.07	16.6	1.01	9.7	1.07
II	22.4	1.13	21.4	1.14	23.7	1.03	23.1	1.01	15.5	1.08
III	23.4	1.08	21.5	1.06	21.3	1.10	18.7	1.01	23.3	1.07
IV	19.6	1.08	22.3	1.04	14.6	.91	27.7	1.04	17.6	1.07
Mean	20.8	1.06	20.6	1.08	19.9	1.03	21.5	1.02	16.5	1.07

R.E. (5% = 1.9
(1% = 2.7

P = 5

C.V. = 2.82%

TABLE XXXVIII

THE EFFECT OF 2,4-D APPLIED AT FIVE-LEAF STAGE UPON PHOSPHORUS CONTENT OF ROOTS OF BARLEY

	Pounds of 2,4-D Applied per Acre									
	0.00		0.25		0.50		1.00		2.00	
Replication	mg.	%	mg.	%	mg.	%	mg.	%	mg.	%
I	12.3	1.83	8.9	1.76	10.0	2.02	9.8	2.00	8.4	1.89
II	9.8	1.81	8.8	1.80	7.8	1.71	8.5	1.88	7.5	1.69
III	9.9	1.44	8.4	1.44	11.2	1.89	9.8	1.79	10.3	1.98
IV	12.2	1.93	9.6	1.56	11.2	2.03	6.8	1.37	8.6	1.80
Mean	11.1	1.75	8.9	1.64	10.1	1.91	8.7	1.76	8.7	1.84

R.E. (5% = 1.8
(1% = 2.6

P = 5

C.V. = 5.67%

TABLE XXXIX

THE EFFECT OF 2,4-D APPLIED AT FIVE-LEAF STAGE UPON PHOSPHORUS CONTENT OF BARLEY

	Pounds of 2,4-D Applied per Acre									
	0.00		0.25		0.50		1.00		2.00	
Replication	mg.	%	mg.	%	mg.	%	mg.	%	mg.	%
I	35.5	1.39	29.9	1.42	36.7	1.55	32.3	1.51	20.8	1.48
II	37.1	1.47	36.2	1.47	37.8	1.37	40.0	1.46	26.1	1.39
III	36.0	1.26	32.7	1.25	37.9	1.50	33.6	1.40	39.8	1.53
IV	37.0	1.51	35.9	1.30	31.7	1.47	38.3	1.21	30.6	1.44
Mean	36.4	1.40	33.7	1.36	36.0	1.47	36.1	1.40	29.3	1.46

R.E. (5% = 6.4
(1% = 9.0

P = 5

C.V. = 5.51%

TABLE XXXVII

THE EFFECT OF 2,4-D APPLIED AT FIVE-LEAF STAGE UPON PHOSPHORUS CONTENT OF BARLEY TOPS

	Pounds of 2,4-D Applied per Acre									
	0.00		0.25		0.50		1.00		2.00	
Replication	mg.	%	mg.	%	mg.	%	mg.	%	mg.	%
I	17.9	.95	17.2	1.07	20.1	1.07	16.6	1.01	9.7	1.07
II	22.4	1.13	21.4	1.14	23.7	1.03	23.1	1.01	15.5	1.08
III	23.4	1.08	21.5	1.06	21.3	1.10	18.7	1.01	23.3	1.07
IV	19.6	1.08	22.3	1.04	14.6	.91	27.7	1.04	17.6	1.07
Mean	20.8	1.06	20.6	1.08	19.9	1.03	21.5	1.02	16.5	1.07

R.E. (5% = 1.9 (1% = 2.7 P = 5 C.V. = 2.82%

TABLE XXXVIII

THE EFFECT OF 2,4-D APPLIED AT FIVE-LEAF STAGE UPON PHOSPHORUS CONTENT OF ROOTS OF BARLEY

	Pounds of 2,4-D Applied per Acre									
	0.00		0.25		0.50		1.00		2.00	
Replication	mg.	%	mg.	%	mg.	%	mg.	%	mg.	%
I	12.3	1.83	8.9	1.76	10.0	2.02	9.8	2.00	8.4	1.89
II	9.8	1.81	8.8	1.80	7.8	1.71	8.5	1.88	7.5	1.69
III	9.9	1.44	8.4	1.44	11.2	1.89	9.8	1.79	10.3	1.98
IV	12.2	1.93	9.6	1.56	11.2	2.03	6.8	1.37	8.6	1.80
Mean	11.1	1.75	8.9	1.64	10.1	1.91	8.7	1.76	8.7	1.84

R.E. (5% = 1.8 (1% = 2.6 P = 5 C.V. = 5.67%

TABLE XXXIX

THE EFFECT OF 2,4-D APPLIED AT FIVE-LEAF STAGE UPON PHOSPHORUS CONTENT OF BARLEY

	Pounds of 2,4-D Applied per Acre									
	0.00		0.25		0.50		1.00		2.00	
Replication	mg.	%	mg.	%	mg.	%	mg.	%	mg.	%
I	35.5	1.39	29.9	1.42	36.7	1.55	32.3	1.51	20.8	1.48
II	37.1	1.47	36.2	1.47	37.8	1.37	40.0	1.46	26.1	1.39
III	36.0	1.26	32.7	1.25	37.9	1.50	33.6	1.40	39.8	1.53
IV	37.0	1.51	35.9	1.30	31.7	1.47	38.3	1.21	30.6	1.44
Mean	36.4	1.40	33.7	1.36	36.0	1.47	36.1	1.40	29.3	1.46

R.E. (5% = 6.4 (1% = 9.0 P = 5 C.V. = 5.51%

TABLE XXXVII

THE EFFECT OF 2,4-D APPLIED AT FIVE-LEAF STAGE UPON PHOSPHORUS CONTENT OF BARLEY TOPS

	Pounds of 2,4-D Applied per Acre									
	0.00		0.25		0.50		1.00		2.00	
Replication	mg.	%	mg.	%	mg.	%	mg.	%	mg.	%
I	17.9	.95	17.2	1.07	20.1	1.07	16.6	1.01	9.7	1.07
II	22.4	1.13	21.4	1.14	23.7	1.03	23.1	1.01	15.5	1.08
III	23.4	1.08	21.5	1.06	21.3	1.10	18.7	1.01	23.3	1.07
IV	19.6	1.08	22.3	1.04	14.6	.91	27.7	1.04	17.6	1.07
Mean	20.8	1.06	20.6	1.08	19.9	1.03	21.5	1.02	16.5	1.07

R.E. (5% = 1.9 (1% = 2.7 P = 5 C.V. = 2.82%

TABLE XXXVIII

THE EFFECT OF 2,4-D APPLIED AT FIVE-LEAF STAGE UPON PHOSPHORUS CONTENT OF ROOTS OF BARLEY

	Pounds of 2,4-D Applied per Acre									
	0.00		0.25		0.50		1.00		2.00	
Replication	mg.	%	mg.	%	mg.	%	mg.	%	mg.	%
I	12.3	1.83	8.9	1.76	10.0	2.02	9.8	2.00	8.4	1.89
II	9.8	1.81	8.8	1.80	7.8	1.71	8.5	1.88	7.5	1.69
III	9.9	1.44	8.4	1.44	11.2	1.89	9.8	1.79	10.3	1.98
IV	12.2	1.93	9.6	1.56	11.2	2.03	6.8	1.37	8.6	1.80
Mean	11.1	1.75	8.9	1.64	10.1	1.91	8.7	1.76	8.7	1.84

R.E. (5% = 1.8 (1% = 2.6 P = 5 C.V. = 5.67%

TABLE XXXIX

THE EFFECT OF 2,4-D APPLIED AT FIVE-LEAF STAGE UPON PHOSPHORUS CONTENT OF BARLEY

	Pounds of 2,4-D Applied per Acre									
	0.00		0.25		0.50		1.00		2.00	
Replication	mg.	%	mg.	%	mg.	%	mg.	%	mg.	%
I	35.5	1.39	29.9	1.42	36.7	1.55	32.3	1.51	20.8	1.48
II	37.1	1.47	36.2	1.47	37.8	1.37	40.0	1.46	26.1	1.39
III	36.0	1.26	32.7	1.25	37.9	1.50	33.6	1.40	39.8	1.53
IV	37.0	1.51	35.9	1.30	31.7	1.47	38.3	1.21	30.6	1.44
Mean	36.4	1.40	33.7	1.36	36.0	1.47	36.1	1.40	29.3	1.46

R.E. (5% = 6.4 (1% = 9.0 P = 5 C.V. = 5.51%

decrease in total phosphorus at all levels of 2,4-D when compared to the check, with the greatest difference occurring at the one and two pound rates. There is a slight increase in phosphorus content, both in percentage and total milligrams, at the one-half pound level of 2,4-D when compared to the one-quarter, one and two pound rates.

Phosphorus - tops. The effect of 2,4-D upon the phosphorus content of the above ground portions, Table XXXVII, is very similar to that found in the roots. However, in the case of the tops, the total quantity of phosphorus remains almost constant, except for a slight increase at the one pound rate of 2,4-D and a rather decided decrease at the two pound rate.

The percentage composition of phosphorus in the check and treated plants shows little variation over the entire experiment.

Phosphorus - complete plants. The data, Table XXXIX, indicates that 2,4-D had no significant effect upon either the percentage composition or the total quantity of phosphorus in barley.

Phosphorus^{32} - roots. Phosphorus^{32} uptake by roots, as influenced by 2,4-D treatments, was significantly decreased when compared to the check, Table XXXXI.

There was a decreasing trend in phosphorus^{32} uptake as the rate of 2,4-D was increased. These differences were statistically significant at the one and two pound rates when compared to the one-quarter pound rate, while the phosphorus^{32} content at the two pound level was significantly different from all other treatments.

Phosphorus^{32} - tops. The effect of 2,4-D upon the phosphorus^{32} content in the above ground portions, Table XXXX, was considerably less than

TABLE XXXX

UPTAKE OF PHOSPHORUS^{32} BY ABOVE GROUND PORTION OF BARLEY AT THE FIVE-LEAF STAGE EXPRESSED AS COUNTS PER MINUTE

Replication	Pounds of 2,4-D Applied per Acre				
	0.00	0.25	0.50	1.00	2.00
I	5348	4537	5243	7244	1329
II	4261	5270	5982	6671	715
III	5602	5671	4288	5493	1191
IV	5715	3438	2309	4472	1280
Mean	5232	4729	4456	5970	1129

R.E. (5% = 1643
(1% = 2328

P = 5

C.V. = 11.36%

TABLE XXXXI

UPTAKE OF PHOSPHORUS^{32} BY BARLEY ROOTS AT THE FIVE-LEAF STAGE EXPRESSED AS COUNTS PER MINUTE

Replication	Pounds of 2,4-D Applied per Acre				
	0.00	0.25	0.50	1.00	2.00
I	13619	11079	10186	9562	8350
II	14158	10186	9011	8982	6298
III	16533	14228	12874	10324	7339
IV	14226	10929	9382	9579	6896
Mean	14634	11605	10363	9612	7196

R.E. (5% = 1262
(1% = 1788

P = 5

C.V. = 3.52%

TABLE XXXXII

UPTAKE OF PHOSPHORUS^{32} BY BARLEY AT THE FIVE-LEAF STAGE EXPRESSED AS COUNTS PER MINUTE

Replication	Pounds of 2,4-D Applied per Acre				
	0.00	0.25	0.50	1.00	2.00
I	18967	15616	15429	16806	9579
II	18409	15456	14993	15653	7013
III	22135	19899	17162	15817	8530
IV	19941	14367	11691	14051	8176
Mean	19863	16335	14819	15582	8325

R.E. (5% = 2291
(1% = 3245

P = 5

C.V. = 4.55%

that of roots, as indicated by the lack of a significant difference between the treated and check plants except at the two pound rate. There was a general decreasing trend in phosphorus^{32} content as the rate of 2,4-D was increased, except at the one pound level where a non-significant increase occurred.

Phosphorus^{32} - complete plant. There was a significant decrease in phosphorus^{32} uptake, Table XXXXII, in the treated plants as compared to those non-treated. Within the 2,4-D treatments, the two pound rate resulted in a significantly lower uptake than the one-quarter, one-half and one pound rates.

Phosphorus^{32} distribution. Figures 20 and 21 depict the influence of 2,4-D upon the uptake and translocation of phosphorus^{32}. The plants treated with 2,4-D were lower in phosphorus^{32} content than those of the check as evidenced by the difference in degree of exposure. The figures also indicate that less phosphorus^{32} was translocated to the older leaves in the treated than in the non-treated plants. There appears to be a greater concentration of phosphorus^{32} in the nodes of the treated plants than of the non-treated plants.

The concentration of phosphorus^{32} in the roots of the treated plants was less than that of the non-treated plants; however, the differences are not as great as those of the top portions, indicating that the phosphorus^{32} was not translocated from the roots to the tops in those plants treated with 2,4-D.

Phosphorus^{32} distribution at cellular level. The phosphorus^{32} distribution at the cellular level was analogous to that of the pre-emergence treatments.

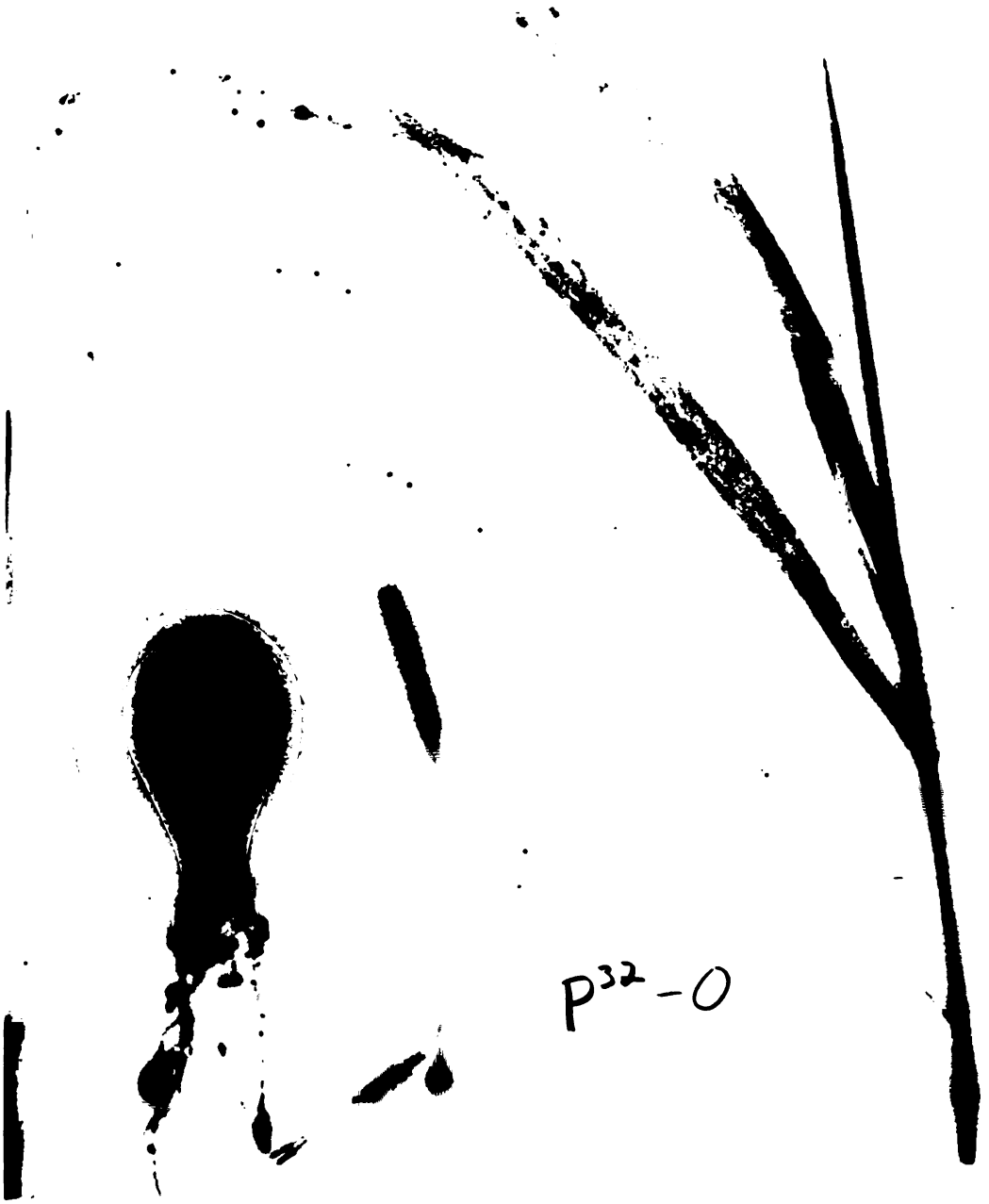


Figure 20: Radiogram of Phosphorus^{32} in Non-treated Barley.

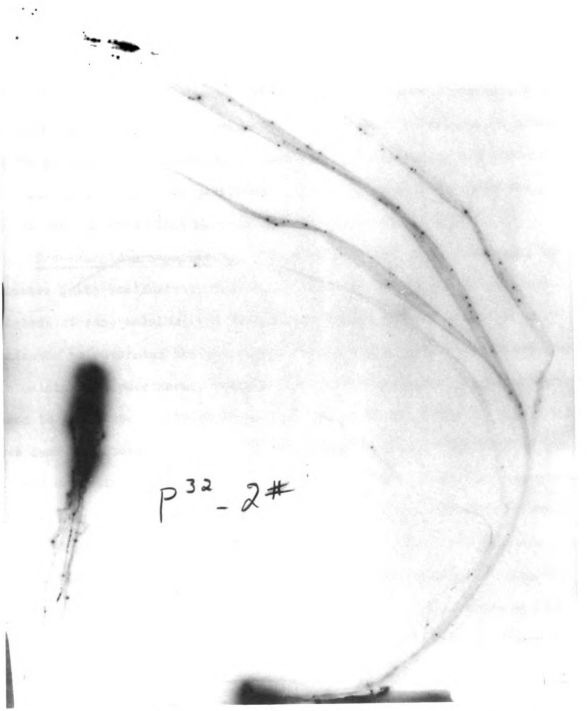


Figure 21: Radiogram of Phosphorus^{32} in Barley Treated with Two Pounds of 2,4-D per Acre at Five-leaf Stage.

DISCUSSION

This experiment was conducted under rather constant conditions of temperature; although there was a distinct diurnal effect due to presence or absence of light, duration and intensity of radiation, and humidity present in the converted germinator. These reproducible conditions produced very uniform plant material for the experiment.

Pre-emergence experiments. The data resulting from this study indicates quite conclusively that 2,4-D does affect the dry weight, and content of ash, calcium, and phosphorus present in the plant. In an endeavor to determine the reasons for these highly significant decreases in calcium and phosphorus, radioisotopes and histological sections were used to check the uptake of these ions over a short period of time. This was done to determine whether the effect was a direct inhibition of ion uptake or indirectly through a reduction in total root area of the plant (absorbing capacity) and alteration of the conducting and translocating tissue present in the plant. Radiograms of the gross plant indicate some inhibition of translocation of both calcium^{45} and phosphorus^{32} from the root to the above ground portions of the plant. However, there was no discernible effect by 2,4-D upon the distribution of these two elements within the plant.

Histological sections of stem and leaf tissue revealed no effect of the 2,4-D treatment upon either cellular differentiation or distribution. This is somewhat at odds with the effect of 2,4-D upon beans as stated

by Swanson[13]. He found that proliferation of cells from cambium, parenchyma cells, ray cells and pericycle can be stimulated by the application of 2,4-D, provided there are cells in the stem which are immature enough to undergo meristematic activity. However, root sections revealed extensive proliferation of cells and lateral root formation initiating from the pericycle in those plants treated with 2,4-D. The phloem, xylem and other tissues appeared normal and unaffected by treatment with the growth regulator, thus eliminating the effect of a failure to translocate the ions, thereby reducing ion uptake.

Microradiograms disclose the fact that both phosphorus^{32} and calcium^{45} were concentrated in the proliferated lateral root areas of those plants treated with 2,4-D; whereas in the check plants there was no discernible pattern or area of ion accumulation. This ion accumulation in areas of rapid meristematic activity, such as found in these areas of proliferation, is to be considered normal. Thus, the reduced ion uptake experienced either by cellular obstructions or through a change in distribution cannot be accounted for, since chemical assay of the treated roots indicate they are lower in total phosphorus and calcium than the check. Hence the depression of ion uptake is not simply a matter of failure of the roots to translocate the ions to the leaves.

Swanson, in a comparison of the effects of 2,4-D upon the anatomical response of the roots of dicots and monocots, concluded that dicots generally made a greater anatomical response to 2,4-D than did monocots, although the responses were similar.

Mitchell[9] observed that tissues which respond most easily to such substances as 2,4-D are those that possess a high level of oxidation-

reduction activity--phloem, endodermis, cambium, pericycle, xylem parenchyma. If auxin was applied, the character of the response of these tissues resulted in cell division and the organization of these cells into tissues and finally their orientation into organs such as roots.

The proposals of Lundegardh[14] that energy transfer for ion absorption was concerned particularly with the terminal oxidase system of roots, especially the cytochrome-cytochrome oxidase system, coupled with the fact that the chief effects of 2,4-D are confined to those tissues which possess a high level of oxidation-reduction activity, indirectly provides a somewhat plausible explanation for depression of ion uptake by 2,4-D. Thus, this growth regulator may affect ion uptake through its influence upon those cells high in this capacity. This concept is further substantiated by Smith[11] reporting on a study of the effect of 2,4-D upon rice and barley where it was found that oxygen uptake by barley was depressed by the application of 2,4-D. It was suggested that the aerobic phase of respiration was most sensitive to 2,4-D. This is in agreement with the fact that plant growth is, in general, an aerobic process depending upon the cytochrome oxidase system for its ultimate end.

Studies of the effect of auxins on enzymes in a semi-purified state almost invariably demonstrated that auxins inhibit these enzymes. (Bonner and Bandurski[2].) This further suggests that the effects of auxin on ion uptake may be due to its influence upon those enzymes involved in the oxidation-reduction phenomenon in plants.

It must be stated, however, that although the effects of auxins on the above phenomena are in many instances quite well documented, their

influence upon the ion uptake mechanism is by no means known and the above inferences can only be considered as possible plausible explanations for the results observed.

It is entirely possible that the reduction in ion uptake observed is simply that of a reflection of the reduced absorbing capacity induced by the root stunting, proliferating action of the 2,4-D. Here again the question arises as to how 2,4-D affects this retardation of root growth; the answer to which lies far beyond the scope of this paper.

However, one of the current theories of the mechanism of auxin action is that proposed by Bonner and Bandurski, in which they cite a considerable amount of indirect evidence that the auxin may serve in some manner to couple the respiratory with the growth processes. It is suggested that the participation of auxin in phosphorylation and energy transfer reactions mediates the availability of energy formed in the respiration process for use in growth processes. Thus, any influence of 2,4-D upon phosphorus uptake and metabolism would directly affect the growth of the plant.

The influence of 2,4-D upon barley treated at the three-leaf stage was less striking than that found in the pre-emergence treated plants, Table XXXXIII.

TABLE XXXXIII

DRY WEIGHT, CONTENT OF ASH, PHOSPHORUS AND CALCIUM AFFECTED BY VARIOUS LEVELS OF 2,4-D APPLIED AT THREE STAGES OF GROWTH EXPRESSED AS PERCENTAGE OF CHECK

Stage of Growth	Plant Contents	Pounds of 2,4-D Applied per Acre			
		0.25	0.50	1.00	2.00
Pre-emergence	Dry weight	55.08	52.63	50.44	46.94
	Ash	37.33	27.36	32.56	23.45
	Phosphorus	38.55	34.33	32.53	28.91
	Calcium	55.31	46.80	46.80	38.29
Three-leaf	Dry weight	65.54	56.47	57.52	54.92
	Ash	67.04	46.10	44.56	38.45
	Phosphorus	80.84	63.15	74.30	71.23
	Calcium	52.72	37.41	30.61	18.70
Five-leaf	Dry weight	95.75	94.45	100.50	76.60
	Ash	92.83	98.55	97.85	79.68
	Phosphorus	92.58	98.90	99.17	80.49
	Calcium	87.90	85.45	83.45	61.60

However, the application of the growth regulator served to significantly decrease the dry weight and content of ash, phosphorus and calcium in the roots, tops and complete plant. Tracer uptake studies, utilizing calcium^{45} and phosphorus^{32}, resulted in a significantly depressed uptake of calcium and phosphorus following an application of 2,4-D, thus supporting the results obtained by chemical analysis. The use of tracers, although supporting the chemical assay data, still fails to settle the cause and effect question; in that the depression may be due to a direct effect upon ion uptake as postulated above or merely an indirect effect due to reduction in total root absorbing capacity.

However, it is readily apparent from the data in Table XXXXIII that the susceptibility of barley to inhibition of ion uptake by 2,4-D is

greatest at the pre-emergence stage, least at the five-leaf stage, with the three-leaf stage occupying a position intermediate in sensitivity.

This fact is in agreement with the results obtained by Friesen and Olson[6] who found that there were two critical periods of 2,4-D susceptibility in barley. The first period was a seedling period coinciding with the incidence of leaf initials and the differentiation of the spike in the growing period. This differentiation was found to be completed at the five-leaf stage of the barley, at which time the barley entered a more resistant period which lasted until the boot stage, then the second susceptible period was initiated.

Table XXXXIII provides evidence of the above statements in that the least effect of 2,4-D upon ion uptake was experienced at the five-leaf stage. The data secured from the five-leaf treatments indicates that there was no statistical significant difference effected by 2,4-D between the dry weight, ash content, calcium and phosphorus content in the complete plant and the controls. However, the uptake of phosphorus^{32} and calcium^{45} were significantly decreased by the application of 2,4-D. This may be explained in part by the fact that the bulk of the calcium and phosphorus required for the life of the plant is accumulated early. Thus, the chemical assay may have indicated what was taken up at an earlier period by the plant. Little phosphorus or calcium was taken up after the five-leaf stage, thus there was no evidence of any effect of the growth regulator upon the uptake of calcium and phosphorus.

Another, perhaps more logical postulate, is that of the plant maturing its escape mechanisms, such as detoxification or the incorporation of

the harmful molecule into non-harmful substances, etc. Thus, as the plant matures these protective mechanisms, the result is a decrease in sensitivity and loss of reactivity to 2,4-D by the plant.

CONCLUSION

The data obtained in the study of the effect of 2,4-D upon the uptake and distribution of calcium and phosphorus in barley at the three stages of growth support the following conclusions:

(1) There is a significant decrease in the uptake of calcium a nd phosphorus treated at the pre-emergence stage.

(2) There is a significant decrease in the content of calcium and phosphorus in roots, tops and complete plants in barley treated at the three-leaf stage.

(3) There is no significant influence upon the calcium and phosphorus content in barley treated at the five-leaf stage.

(4) Uptake of calcium^{45} and phosphorus^{32} was significantly depressed by the application of 2,4-D at all stages of growth.

(5) Radiograms and microradiograms revealed little or no effect of 2,4-D upon the distribution of calcium^{45} and phosphorus^{32} within the plant.

(6) Histological sections revealed that 2,4-D stimulated the initiation of lateral roots from the pericycle which ultimately degenerated into a mass of proliferated tissue. There were no other anatomical effects observed.

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