ABSTRACT OF THESIS

SOME EFFECTS OF SEVERAL SALTS AND ESTERS OF 2,4-DICHLOROPHENOXYACETIC ACID ON GROWTH AND RESPIRATION OF BARLEY

Submitted by

Mary Eleanor Durrell

In partial fulfillment of the requirements

for the Degree of Master of Science

Colorado

Agricultural and Mechanical College

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INTRODUCTION

Growth-regulating substances such as 2,4-D (2,4-dichlorophenoxyacetic acid) are now being used widely for the selective control of broad-leaved weeds growing in small grains. There is but little information on the possible effects of these substances on small grains harvested from fields sprayed for weed control. It is the purpose of this study to describe and measure some of the effects of several 2,4-D herbicides on barley seed from fields sprayed for weed control.

The problem

What are the effects of some of the common salts and esters of 2,4-D on barley seed from fields sprayed for weed control.

Problem analysis.--Will they influence seed quality?

(a) Weight of seed
(b) Size of seed
(c) Color of seed

2. Will they affect seed germination?

(a) Rate of germination
(b) Total percentage viable seed
(c) Dormancy

3. Will they affect seed respiration?

4. Will they affect the subsequent crop grown from seed of treated plants?

Delimitation.--This investigation has been limited to the study of the effects of an isopropyl ester, a butyl ester, a triethanolamine salt, and a sodium salt of 2,4-D applied at rates of 1 and 1½ pounds per acre, acid equivalent, on seed from plots of Trebi barley sprayed for weed control in the summer of 1947, and on the growth and respiration of barley grown from this seed.

The background of the study

In the summer of 1947 Mr. B. J. Thornton of the Colorado Experiment Station conducted some experiments for the control of weeds in a field of Trebi barley. Four types of 2,4-D compounds, an isopropyl ester, a butyl ester, a triethanolamine salt, and a sodium salt were sprayed in strips 24 feet wide and 762 feet long across a barley field near Fort Collins. The spraying was done with a 24-foot boom on a John Deere tractor delivering 5 gallons per minute at 60 pounds pressure, and at the rate of 50 gallons per acre. The barley was sprayed in the late-boot stage using 1 and 1½ pounds of 2,4-D acid equivalent per acre.

Material for the tests in the following experiments was taken from these plots. The seed from meter quadrats was threshed in the Experiment Station sample threshing machine made available through the courtesy of Dr. D. W. Robertson. The seed samples were bagged and weighed.

Soon after spraying in 1947, the strips treated with the butyl ester at the rates of 1 and 1½ pounds of 2,4-D acid equivalent per acre, and the strips treated with the isopropyl ester, became lodged. The strips

treated with the sodium salt, at rates of 1 and 1½ pounds of 2,4-D acid equivalent per acre, did not lodge. Examination of the lodged plants showed excessive growth at the stem nodes. There were no apparent structural differences in the heads of the treated plants as compared with those from untreated plants. However, spikelets in the heads from treated plants appeared heavier and darker.

Seed from the treated plants appeared darker in color than those from untreated plants. There was also a difference in seed size. The seeds from treated plants were plumper than those from untreated plants, but were the same in length.

These observations suggested that there might be differences in seed quality and germination.

METHODS AND MATERIALS

Germination studies

In all germination tests made to determine the percentage of viable seed, standard seed laboratory procedures of the Colorado Experiment Station Seed Laboratory were used. For freshly harvested dormant seed, this involved germination in blotters at 15° C. for a period of 8 days. Nondormant seed was germinated at an alternating temperature of 20° C. to 30° C. for 7 days. This procedure is in accord with a recent report of the official seed analysts.

Greenhouse studies

Seed from each of the treated plots and from the check plots (see background of the study) was grown to maturity under greenhouse conditions to determine whether "carry-over" effects were present.

The seed was planted January 15, 1948 in field soil in a greenhouse bench. The planting was planned so that the position of each treatment and control was altered throughout the bench. Twenty-four seeds for each treatment and the control were planted to a row, with one inch between seed and six inches between rows. Each treatment and the control were replicated six times. On the sixth and seventh days after the planting date, measurements were made of seedling growth. The rows were then thinned to 12 plants 2 inches apart. These plants were grown to maturity and their seed harvested on April 7, 1948. During the experiment the bench was lighted an additional 2 hours each day from 12:00 p.m. to 2:00 a.m. by two 150-watt lights hung 3 feet above the bench and controlled by a clock device to turn on and off at the designated times.

Respiration studies

The amount of CO_2 evolved from the germinating barley seed was measured by suspending a given number of seed of known weight over a CO_2-free, saturated barium hydroxide solution. The CO_2 evolved by the seed over a certain period of time was precipitated by the barium hydroxide as barium carbonate. The precipitate was then dissolved by HCl.

The number of cubic centimeters required to dissolve the barium carbonate was converted into terms of milligrams of CO_2.

The seeds used for the respiration study were selected at random from the 1947-season seed samples from plots sprayed with the butyl ester of 2,4-D at the rate of 1½ pounds acid equivalent per acre and from the control plots. They were divided into lots of 10 seeds each, with 20 replicates for each treatment. Each lot was weighed on an analytical balance, the weights recorded, and labeled. The different lots were then placed in individual bags of uniform size made from cheese cloth dipped in paraffin. The paraffin made the bags impervious to water that otherwise would have absorbed CO_2 volume for volume. It also stiffened the cheese cloth preventing the loss of seed through the mesh, and at the same time provided adequate gas exchange around the seed. The seed lots were then soaked in a suspension of 0.3 percent Spergon for 12 hours, as suggested by Denny, who, in a similar study, showed that the Spergon inhibited the growth of surface-borne organisms without affecting seed respiration. Previous experiments had shown that during a soaking period of 12 hours, water was absorbed by the barley seed in sufficient amounts to support seed germination with uniform increases in CO_2 evolution and in seedling growth for a period of 36 hours.

The CO_2-free barium hydroxide used in the respiration experiments was held in a reservoir. Five cc. of barium hydroxide were measured into sterilized Erlenmeyer flasks (125 ml.) from the burette F. The flasks were

then stoppered immediately to prevent the atmospheric CO_2 from reacting with the barium hydroxide. After the barley seed had soaked for 12 hours, a single cheese-cloth bag with ten seeds was suspended in each Erlenmeyer flask above the N/2.5 $Ba(OH)_2$ solution and held firmly in place by a tight-fitting rubber stopper.

Control flasks containing only barium hydroxide were subjected to all of the above manipulations. The amount of CO_2 from the atmosphere of the room, combining with the barium hydroxide, was found to be negligible when measured by the titration method used.

From the exploratory tests it was found that there was less variation between replicates within a treatment when the flasks were agitated during the experiment than when they were not. This agitation broke the layer of barium carbonate that formed on the surface of the barium hydroxide. For this purpose a rack was devised for shaking all of the samples simultaneously for 4 minutes every hour during the experiment.

At the end of each test period the bags of seed were removed from the flasks, the flasks stoppered immediately, and the amount of CO_2 given off by the seed determined.

During the respiration experiment the seeds were germinated in a dark room at a constant temperature of 21° C. $\pm$ 1° C.

Technique of titration

First the excess barium hydroxide was neutralized by adding N/4 HCl with thymol blue as an indicator. Then a given amount of N/10 HCl

was measured into the flask. With brom-phenol blue as an indicator, the solution was then titrated against N/10 NaOH. The amount of N/4 HCl needed to dissolve the precipitated barium carbonate was determined by subtracting from the given amount of HCl measured into the flask the amount of NaOH needed to neutralize the solution. This amount was then expressed in terms of milligrams of CO_2.

SUMMARY OF DATA

Sprays of a triethanolamine salt and a sodium salt of 2,4-D applied at rates of 1 and 1½ pounds acid equivalent per acre to Trebi barley in the late-boot stage produced no obvious changes in the mature plants. A butyl ester at the rate of 1½ pounds per acre, applied under the same conditions, caused elongation of the nodes and lodging. An isopropyl ester at the rate of 1½ pounds per acre caused some lodging. None of these treatments produced a measurable effect on total germination. All produced changes in seed quality as regards seed weight, size and color. Seeds from treated plants were heavier, larger and darker in color. However, when these were planted and grown to maturity in the greenhouse, no carry-over effects could be measured or observed.

Detailed studies of the seed from Trebi barley treated with the butyl ester spray of 2,4-D at a rate of 1½ pounds acid equivalent per acre, showed no significant differences in percentage of viable seed or in the amount of CO_2 respired by seedlings 12 to 48 hours old. It was also

found that seed from the treated plants was significantly heavier, had a higher specific gravity and a greater volume. Laboratory and greenhouse tests showed that the rate of germination and the rates of growth of coleoptiles, primary roots and primary leaves were depressed significantly by treatment.

These data were used in support of a hypothesis of the action of 2,4-D, i.e., that the primary effect on plant growth is on some phase of metabolism not directly concerned in aerobic respiration.

T H E S I S

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WE HEREBY RECOMMEND THAT THE THESIS PREPARED UNDER OUR SUPERVISION BY MARY ELEANOR DURRELL

ENTITLED SOME EFFECTS OF SEVERAL SALTS AND ESTERS OF 2,4-DICHLOROPHENOXYACETIC ACID ON GROWTH AND RESPIRATION OF BARLEY

BE ACCEPTED AS FULFILLING THIS PART OF THE REQUIREMENTS FOR THE DEGREE OF MASTER OF SCIENCE.

CREDITS 8

Committee on Graduate Work

Jas L. Dults — Major Professor

W D Thomas Jr — Minor Professor

L H Dewell — Head of Department

L H Dewell — Dean of Division

Committee on Final Examination

Examination Satisfactory

W D Thomas Jr

L H Dewell

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David W Robertson

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Miss Mary Durrell has demonstrated her ability to read and understand scientific German usually met with in well-edited periodicals and other publications.

Bruno Klinger

Bruno Klinger
Assoc. Professor of Botany
and Plant Pathology

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Chapter I

INTRODUCTION

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Seed from the treated plants appeared darker in color than those from untreated plants. There was also a difference in seed size (Figure 3). The seeds from treated plants were plumper than those from untreated plants, but were the same in length.

These observations suggested that there might be differences in seed quality and germination.

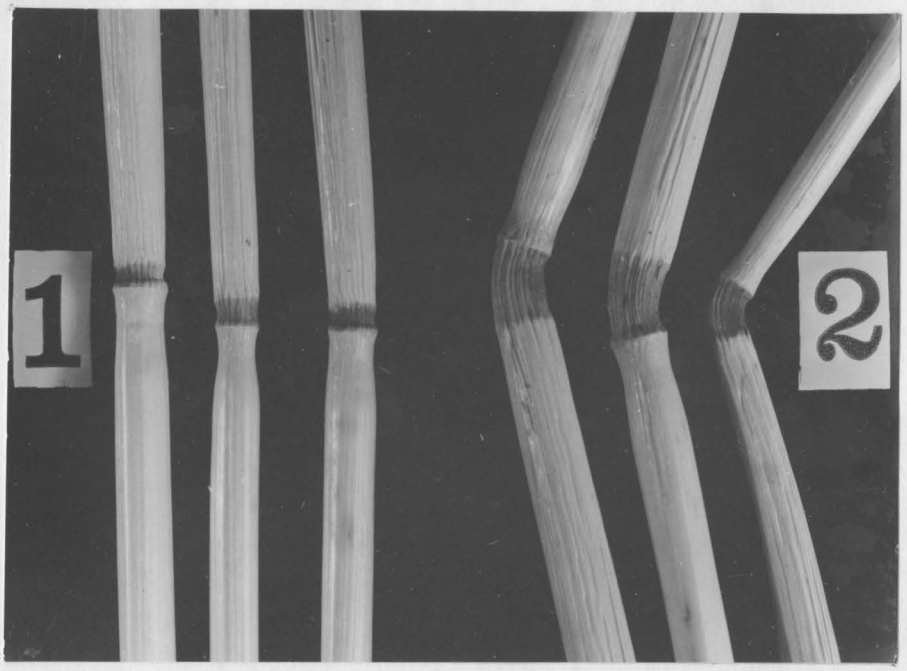

Fig. 1.--Barley nodes from control and treated plants.
1. Nodes from untreated barley.
2. Nodes from lodged plants treated with the butyl ester spray of 2,4-D applied at the rate of 1½ pounds acid equivalent per acre.

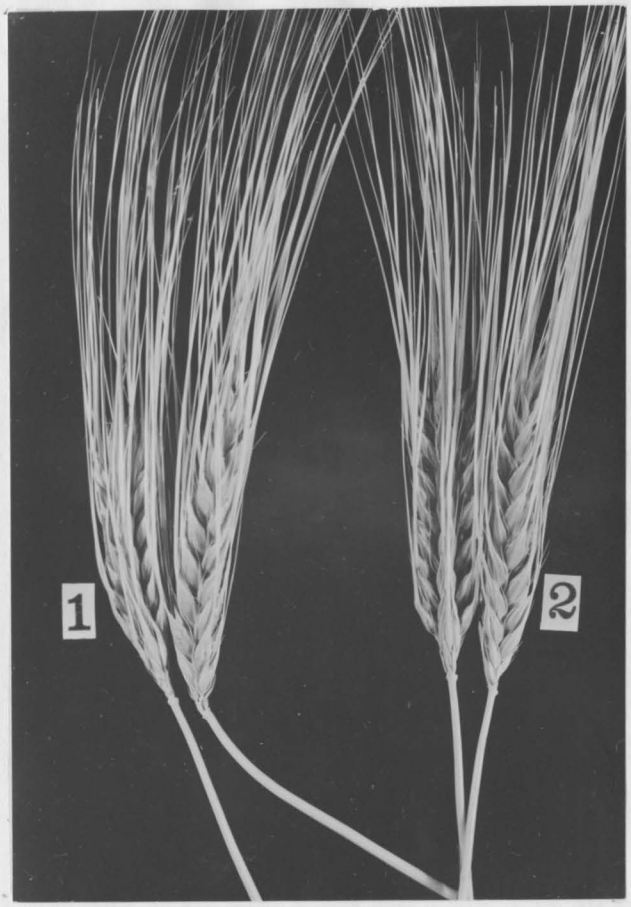

Fig. 2.--Barley heads from control and treated plants.
1. Heads from untreated barley.
2. Heads from barley treated with the butyl ester spray of 2,4-D applied at the rate of 1½ pounds acid equivalent per acre.

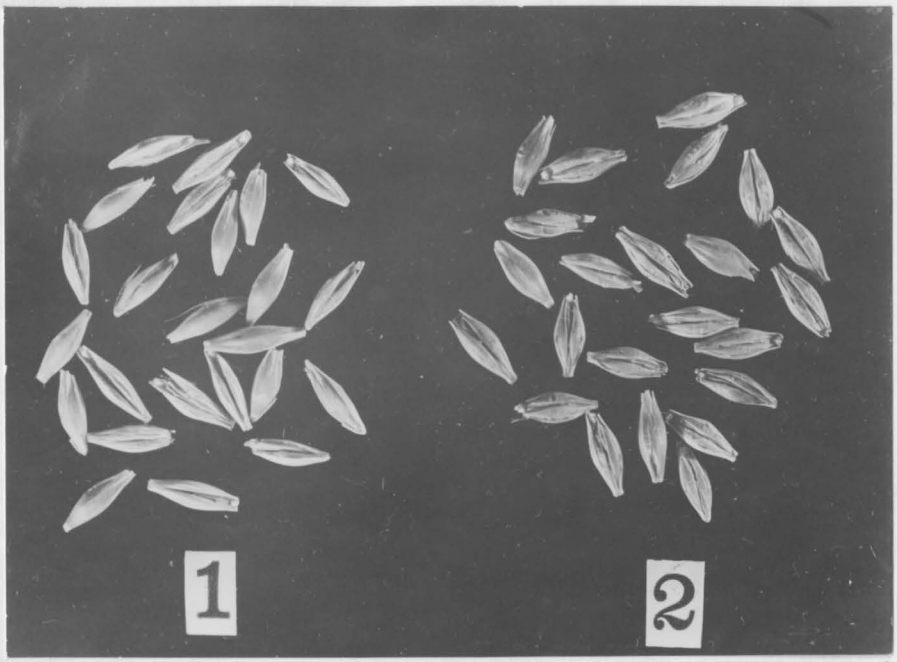

Fig. 3.--Barley seed from control and treated plants.
1. Seed from untreated barley.
2. Seed from barley sprayed with a butyl ester of 2,4-D applied at the rate of 1½ pounds acid equivalent per acre.

Chapter II

REVIEW OF LITERATURE

Effects of 2,4-D sprays on the growth of barley

Buckholtz (4) in 1947, in studying the effect of the sodium salt and the butyl ester of 2,4-D applied at rates of 1, 2, and 4 pounds acid equivalent per acre to Oderbrucker barley, found that lodging was reduced significantly in several instances, that kernel weight was increased significantly by treatments applied at the 4- and 8-inch growth stages, and that there was no significant difference in either kernel weight or lodging reaction between the different treatments.

Klingman (8) in 1947 mentioned the response of barley to 2,4-D sprays. He found severe lodging after spraying with 0.05 percent, 0.10 percent, and 0.15 percent of 2,4-D acid (emulsified with carbowax 1500) emulsions. Plants were treated in the late-boot stage. The plants never fully recovered and showed a high percentage of sterility in the heads.

Leggett (9) in 1948 applied a sodium salt, a triethanol-amine salt, and a butyl ester of 2,4-D at rates of 1/8, 1/4, 1/2, 3/4 and 1 pound per acre to Olli barley at the 2, 5 to 6 leaf, and

late shot-blade stages of growth. He found that the deformed type of head occurred more often in plots sprayed at the 3-leaf stage.

Sexsmith (12) in 1948 applied a butyl ester of 2,4-D at rates of 2, 4, 8, and 12 ounces per acre and a triethanolamine salt of 2,4-D at rates of 4, 8, and 12 ounces per acre to fields of Glacier, Olli, Titan, Trebi, Compana, and Montcalm barley. All rates of both chemicals, except the triethanolamine salt at 4 ounces per acre, adversely affected the weight of the grain produced. It was suggested that the reduction in seed weight was the result of an increase in the number of sterile florets resulting from 2,4-D treatment.

Effects of 2,4-D sprays on the total germination of barley

Buckholtz (4) in 1947 found that 2,4-D sprays of a sodium salt and a butyl ester applied at rates of 1, 2, and 4 pounds acid equivalent per acre to Oderbrucker barley, had no effect upon the total germination of barley seed from treated plots compared with that from untreated plots.

Effects of treating barley seed with 2,4-D solutions on subsequent germination, growth, and respiration

Hseuh and Lou (7) in 1947, in a study of the effect of 2,4-D on barley, found that seed germination was influenced by soaking the seed in different concentrations of 2,4-D solutions.

Low concentrations of 0.007 percent to 0.0035 percent increased the rate of germination over that of the control, while concentrations of 0.014 percent checked germination, and 0.07 percent inhibited all germination. By means of manometric tests, they found that the metabolic activity of seedlings was depressed or inhibited when seed was treated with the higher concentrations (0.1 percent) of 2,4-D. The treated seed, in comparison with control seed, exhibited a low oxygen uptake and a high carbon dioxide evolution, giving a high R.Q. They attributed the difference in respiratory intensity between treated and untreated barley to inability of treated seed to utilize fully the oxygen of the air during germination. The reduction in oxygen available for seed respiration is compensated for by the fermentative activity of germinating seed, and results in high carbon dioxide evolution and a high R.Q.

Related studies

Allard, De Rose, and Swanson (1) in 1946 tested seed of 22 different cereal and broadleaf crop plants. A retardation of the rate of germination and a decrease in the amount of germination resulted upon treating the seed with 2,4-D concentrations of 1 p.p.m. A 15 to 20 percent reduction in the length of roots of wheat seedlings was caused by concentrations as low as 0.01 p.p.m. Concentrations of 1 p.p.m. and greater caused swelling of the seedling roots, and inhibition of lateral root development.

Taylor (14) in 1947 treated wheat seedlings 18 to 24 hours or more old with 2,4-D concentrations up to 10 p.p.m. Manometric measurements of the rate of CO_2 evolution and of O_2 uptake were made at various intervals from the first through the twenty-fourth hour after treatment. He found that the main responses were a reduction in the rate of CO_2 evolution and of O_2 uptake. An apparent reduction in activity in proportion to the concentration of 2,4-D was measured during the first hour after initiation of treatment, and increased with the duration of treatment.

Marth, Toole, and Toole (11) in 1948, in studies of the effect of 2,4-D on seed development and germination in certain cereal and grass crops, reported that the total germination of seed of some cereals was in no way affected by 2,4-D applied at various stages of plant development at the rate of 1.7 pounds 2,4-D acid equivalent per acre.

Audus (3) in 1948, in studies on the phytostatic action of 2,4-D and coumarin on roots of Pisum sativum and Lipidium sativum, found that applications of 2,4-D (0.1-10 p.p.m.) inhibited longitudinal root growth of seedlings. He found that this primary inhibition is completely reversible. The period of inhibition is independent of the concentrations of 2,4-D used and speed of removal of the inhibitor, and seems dependent upon the restoration of the

normal dynamic growth equilibrium. Secondary irreversible inhibitions are dependent on the dislocation of the normal dynamic growth equilibrium, and the possible accumulation of metabolites to toxic concentrations. The extent of growth recovery is determined mainly by the extent of these secondary inhibitions.

Chapter III

METHODS AND MATERIALS

Germination studies

In all germination tests made to determine the percentage of viable seed, standard seed laboratory procedures of the Colorado Experiment Station Seed Laboratory were used. For freshly harvested dormant seed, this involved germination in blotters at 15° C. for a period of 8 days. Nondormant seed was germinated at an alternating temperature of 20° C. to 30° C. for 7 days. This procedure is in accord with a recent report of the official seed analysts (2).

Greenhouse studies

Seed from each of the treated plots and from the check plots (see Background of the study) was grown to maturity under greenhouse conditions to determine whether "carry-over" effects were present.

The seed was planted January 15, 1948 in field soil in a greenhouse bench. The planting was planned so that the position of each treatment and control was altered throughout the bench. Twenty-four seeds for each treatment and the control were planted to a row, with one inch between seed and six inches between rows.

Each treatment and the control were replicated six times. On the sixth and seventh days after the planting date, measurements were made of seedling growth. The rows were then thinned to 12 plants 2 inches apart. These plants were grown to maturity and their seed harvested on April 7, 1948. During the experiment the bench was lighted an additional 2 hours each day from 12:00 p.m. to 2:00 a.m. by two 150-watt lights hung 3 feet above the bench and controlled by a clock device to turn on and off at the designated times.

Respiration studies

The amount of CO_2 evolved from the germinating barley seed was measured by suspending a given number of seed of known weight over a CO_2-free, saturated barium hydroxide solution. The CO_2 evolved by the seed over a certain period of time was precipitated by the barium hydroxide as barium carbonate. The precipitate was then dissolved by HCl. The number of cubic centimeters required to dissolve the barium carbonate was converted into terms of milligrams of CO_2.

The seeds used for the respiration study were selected at random from the 1947-season seed samples from plots sprayed with the butyl ester of 2,4-D at the rate of $1\frac{1}{2}$ pounds acid equivalent per acre and from the control plots. They were divided into lots of 10 seeds each, with 20 replicates for each treatment. Each lot was

weighed on an analytical balance, the weights recorded and labeled. The different lots were then placed in individual bags of uniform size made from cheese cloth dipped in paraffin. The paraffin made the bags impervious to water that otherwise would have absorbed CO_2 volume for volume. It also stiffened the cheese cloth preventing the loss of seed through the mesh, and at the same time provided adequate gas exchange around the seed. The seed lots were then soaked in a suspension of 0.3 percent Spergon for 12 hours, as suggested by Denny (5), who, in a similar study, showed that the Spergon inhibited the growth of surface-borne organisms without affecting seed respiration. Previous experiments had shown that during a soaking period of 12 hours, water was absorbed by the barley seed in sufficient amounts to support seed germination with uniform increases in CO_2 evolution and in seedling growth for a period of 36 hours.

The CO_2-free barium hydroxide used in the respiration experiments was held in a reservoir pictured in Figure 4. Five cc. of barium hydroxide were measured into sterilized Erlenmeyer flasks (125 ml.) from the burette F. The flasks were then stoppered immediately to prevent the atmospheric CO_2 from reacting with the barium hydroxide. After the barley seed had soaked for 12 hours, a single cheese-cloth bag with ten seeds was suspended in each

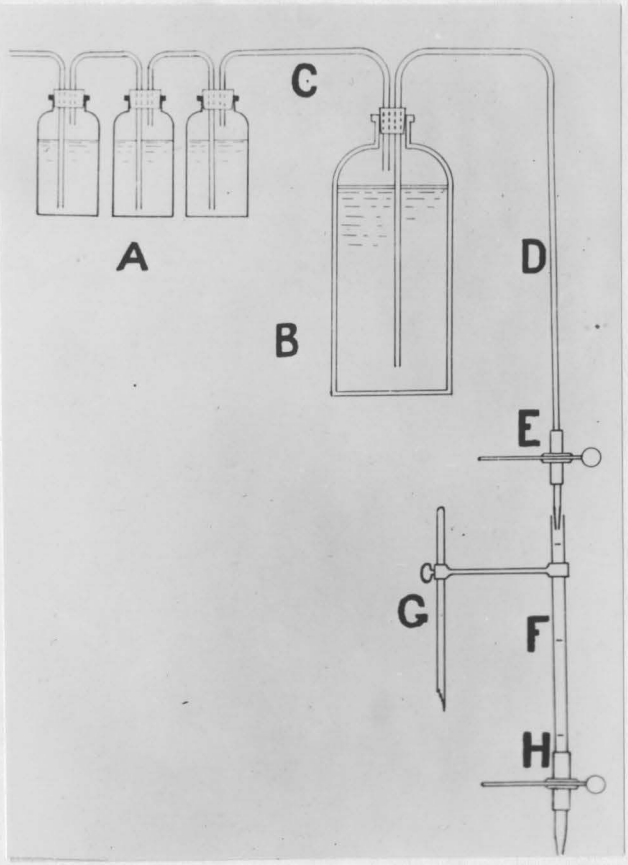

Fig. 4.--Barium hydroxide reservoir.

A. Wash bottles of sodium hydroxide for removing CO_2 from the air.
B. Reservoir for CO_2-free barium hydroxide.
C. Connective tubing.
D. Connective tubing.
E. Rubber tube and clamp for filling measuring burette.
F. Measuring burette.
G. Supporting ring stand.
H. Clamp and rubber tube for draining burette.

Erlenmeyer flask above the N/2.5 $Ba(OH)_2$ solution and held firmly in place by a tight-fitting rubber stopper.

Control flasks containing only barium hydroxide were subjected to all of the above manipulations. The amount of CO_2 from the atmosphere of the room, combining with the barium hydroxide, was found to be negligible when measured by the titration method used.

From the exploratory tests it was found that there was less variation between replicates within a treatment when the flasks were agitated during the experiment than when they were not. This agitation broke up the layer of barium carbonate that formed on the surface of the barium hydroxide. For this purpose a rack was devised for shaking all of the samples simultaneously for 4 minutes every hour during the experiment. The shaker which held 40 flasks is shown in Figure 5. The rack was moved on rollers by an acentric arm geared to a motor. The motor was controlled by an electric timer shown in Figure 6.

At the end of each test period the bags of seed were removed from the flasks, the flasks stoppered immediately, and the amount of CO_2 given off by the seed determined.

During the respiration experiment the seeds were germinated in a dark room at a constant temperature of 20° C. $\pm$ 1° C.

Fig. 5.--Shaker used in respiration studies.

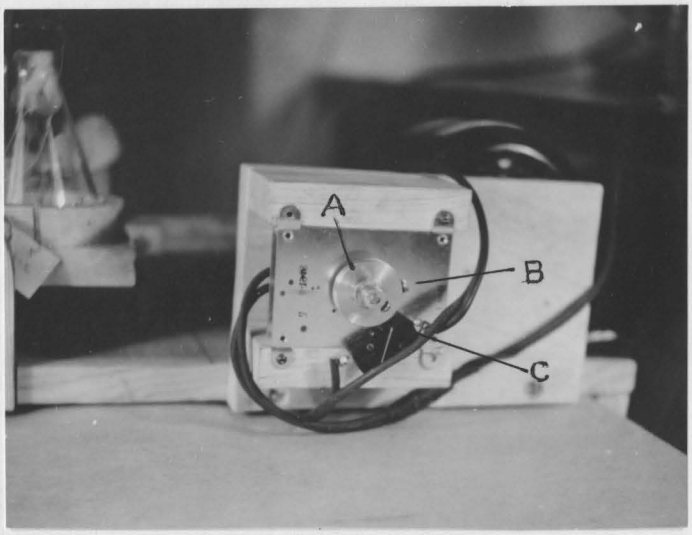

Fig. 6.--Electric timer for regulating shaker:
A rotates once every hour.
B, when in contact with switch
C, causes the motor to run 4 minutes.

Technique of titration

First the excess barium hydroxide was neutralized by adding N/4 HCl with thymol blue as an indicator. Then a given amount of N/10 HCl was measured into the flask. With brom-phenol blue as an indicator, the solution was then titrated against N/10 NaOH. The amount of N/4 HCl needed to dissolve the precipitated barium carbonate was determined by subtracting from the given amount of HCl measured into the flask the amount of NaOH needed to neutralize the solution. This amount was then expressed in terms of milligrams of CO_2. Since one cubic centimeter of N/10 HCl is equivalent to 2.2 mg. CO_2, multiplying the cubic centimeters of HCl by the factor 2.2 gave the amount of CO_2 evolved in milligrams (10).

Chapter IV

ANALYSIS OF DATA

In the study of the effects of several 2,4-D herbicides on barley, tests were made with seed from treated and control plants to determine seed weight, specific gravity of the seed, relative seed dormancy, percentage germination, rate of germination, and seed respiration. Seed from sprayed and control barley was grown to maturity to determine whether "carry-over" effects were present.

Seed weight

Five hundred seeds from each of the eight treated plots and from seven control plots of the 1947 crop were weighed on a torsion balance. Table 1 gives the weight of each lot in grams, with three samples for each of the treated plots.

A detailed study of seed weight was made using data on dry seed weight from the respiration studies. Twenty lots of 10 seeds each from both the control plants and plants receiving the butyl ester spray of 2,4-D, applied at the rate of $1\frac{1}{3}$ pounds of 2,4-D acid equivalent per acre, were weighed on an analytical balance. Table 10 shows the variance analysis of seed weights.

Table 1.--SEED WEIGHT IN GRAMS: 500 SEEDS FROM BARLEY HARVESTED IN 1947 FROM EIGHT 2,4-D TREATED PLOTS AND SEVEN CONTROL PLOTS.

Herbicide	Acid equivalent per acre	Seed weight in grams		
		Sample 1	Sample 2	Sample 3
Tufor 40	1 pound	23.5	22.2	21.8
Esteron 44	1 pound	21.9	22.9	22.2
Weed-No-More	1 pound	23.0	23.2	21.6
2,4-Dow W. K. 70%	1 pound	24.0	22.3	22.0
Tufor 40	1½ pounds	23.3	22.6	22.6
Esteron 44	1½ pounds	23.3	23.1	21.7
Weed-No-More	1½ pounds	22.5	24.2	21.4
Wedanol 70%	1½ pounds	22.7	21.4	21.4
Control 1		15.8		
Control 2		17.3		
Control 3		18.2		
Control 4		14.9		
Control 5		16.6		
Control 6		17.8		
Control 7		18.1		

Specific gravity of seed

Tests were made to determine the specific gravity of seed from barley plants receiving the butyl ester treatment and of seed from control plants. In each test 10 seeds were selected at random from each sample and weighed. The 10 seeds were then placed in a pycnometer which was filled with kerosene with a specific gravity of 0.80 and weighed at 25° C. Kerosene was used in place of water because it was not easily absorbed by the seeds, seeds did not swell in it, and because it readily displaced the air about the crevices of the lemma and palea. The specific gravity was then determined on the basis of the amount of kerosene displaced. Table 2 summarizes these data.

As a measure of seed volume, the volume of water equivalent to the amount of kerosene displaced by the seed was determined. A variance analysis of these data is given in Table 3.

Percentage germination

Germination tests were made on freshly harvested seed to determine if the 2,4-D treatment affected the percentage of viable seed. Two hundred seeds from each of the treated and check plots were planted between moistened blotters and placed in a germinator held at 15° C. constant temperature. A preliminary count of germinating seed was made 6 days after the planting date, and a final count

Table 2.--VARIANCE ANALYSIS OF THE SPECIFIC GRAVITY OF SEED FROM TREATED AND UNTREATED PLANTS.

Variability due to:	D/F	Sum of squares	Mean square	Observed F	Required F .05	Required F .01
Totals	19	0.686,514,6				
Between treatments	1	0.394,524,1	0.394,524,1	24.32	4.41	8.28
Within treatments	18	0.291,990,5	0.016,221,694,44			

Minimum difference required for significance at the .05% level ± 0.119

Minimum difference required for significance at the .01% level ± 0.164

$\bar{X}$ for specific gravity of treated seed = 49.911

$\bar{X}$ for specific gravity of control seed = 47.102

Table 3.--VARIANCE ANALYSIS OF THE VOLUME OF H_2O DISPLACED BY SEED FROM TREATED AND UNTREATED PLANTS.

Variability due to:	D/F	Sum of squares	Mean square	Observed F	Required F .05	Required F .01
Totals	19	0.020,631,925				
Between treatments	1	0.018,395,880	0.018,395,880	14.81	4.41	8.28
Within treatments	18	0.002,236,045	0.000,124,224,722			

Minimum difference required for significance at the .05% level ± 0.010

Minimum difference required for significance at the .01% level ± 0.014

$\overline{X}$ for treated seed = 2.045 cc.

$\overline{X}$ for control seed = 1.438 cc.

made 2 days later. It was found from these tests that seed from both the treated plants and from the control plants germinated uniformly with a high percentage of viable seed. In all of the tests made throughout the period of experimentation, the percentage germination was between 92 percent and 97 percent with no obvious difference between seed samples.

Rate of germination and seedling growth

During the germination tests, it was observed at the time of the final count that the coleoptiles of seed from the sprayed barley plants appeared to be shorter than those from untreated plants. This suggested that there might be a difference in the rate of germination and the rate of seedling growth. Seed from plants treated with the butyl ester and seed from control plants were germinated between moistened blotters. On the third day after the planting date, the primary roots emerged. Root measurements were taken from 50 seedlings selected at random from blotters of 100 seedlings. After measurements were taken the seedlings were discarded and other blotters of seedlings measured for the next age group. Root measurements were made in this manner every 24 hours for 3 days. By the end of this period, the roots had become entangled and could not be removed from the blotters for measuring. On the fourth day after planting, coleoptile measurements were made

in the same way for 5 consecutive days. An analysis of variance of these data is given in Tables 4 and 5.

Germination tests in greenhouse soil

Plants grown to maturity from barley seed under greenhouse conditions as described in the chapter on Methods and Materials, were examined for differences that might have been due to 2,4-D treatment of the parent plants. On the sixth and seventh days after planting date, the distance from the coleoptile tip to the tip of the first leaf was measured. An analysis of variance of this measure of rate of seedling growth is given in Tables 6 and 7.

Germination tests of first-generation seed

Seed was harvested from the mature plants grown in the greenhouse. There were no differences in seed weight or seed size. The seed was germinated at 15° C. constant temperature for seven days. There was no difference in either the percentage of viable seed or in the rate of germination. Freshly harvested seed placed between moist blotters in a 20° C. germinator failed to germinate. Seed from all of the lots tested remained dormant at 20° C. as is characteristic of newly harvested barley.

Respiration studies

A significant difference in the rate of germination of seed from treated plants compared with that of seed from untreated

Table 4.--VARIANCE ANALYSIS OF SEEDLING ROOT MEASUREMENTS FROM SEED OF TREATED AND UNTREATED PLANTS.

Variability due to:	D/F	Sum of squares	Mean square	Obs. F	Req. F .05	Req. F .01
Totals	299	139,250.95				
Between ages	2	104,715.45	52,357.72			
Within ages	(297)	(30,535.50)	102.81			
Between treat. within ages	3	3,261.54	1,087.18	11.72	2.64	3.86
Within treat. within ages	294	27,273.96	92.76			

Minimum difference required for significance at the .05% level ± 3.778
Minimum difference required for significance at the .01% level ± 4.976

Age	$\bar{X}$ for treated	$\bar{X}$ for control
1st day*	13.68 mm.	20.06 mm.
2nd day*	34.48 mm.	41.52 mm.
3rd day*	59.42 mm.	65.76 mm.

* Indicates significant difference between means at the .05% and .01% levels.

Table 5.--THE VARIANCE ANALYSIS OF SEEDLING COLEOPTILE MEASUREMENTS FROM SEED OF TREATED AND UNTREATED PLANTS.

Variability due to:	D/F	Sum of squares	Mean square	Obs. F	Req. F .05	Req. F .01
Totals	499	444,533.24				
Between ages	4	367,650.41	91,912.60			
Within ages	(495)	(76,882.83	155.31			
Between treat. within ages	5	18,225.19	3,645.03	30.45	2.23	3.06
Within treat. within ages	490	58,657.64	119.70			

Minimum difference required for significance at the .05% level ± 4.284
Minimum difference required for significance at the .01% level ± 5.598

Age	$\bar{X}$ for treated	$\bar{X}$ for control
1st day	1.46 mm.	2.38 mm.
2nd day*	13.76 mm.	20.46 mm.
3rd day*	35.24 mm.	45.52 mm.
4th day*	49.90 mm.	66.50 mm.
5th day*	69.26 mm.	86.48 mm.

*Indicates significant difference between means at the .05% and .01% levels.

Table 6.--ANALYSIS OF VARIANCE OF LEAF MEASUREMENTS OF SEEDLINGS FROM CONTROL BARLEY AND BARLEY SPRAYED WITH THE BUTYL ESTER OF 2,4-D. SEEDLINGS WERE GROWN IN THE GREENHOUSE, MEASUREMENTS WERE MADE 6 DAYS AFTER THE PLANTING DATE.

Variability due to:	D/F	Sum of squares	Mean square	Observed F	Required F .05	Required F .01
Totals	287	268.947,778				
Between plots	5	55.325,278				
Within plots	(282)	(213.622,500)				
Between treat. within plots	6	12.385,416	2.064,236,000	2.83	2.13	2.86
Within treat. within plots	276	201.237,084	0.729,119,869			

Table 7.--ANALYSIS OF VARIANCE OF LEAF MEASUREMENTS OF SEEDLINGS FROM CONTROL BARLEY AND BARLEY SPRAYED WITH THE BUTYL ESTER OF 2,4-D; SEEDLINGS WERE GROWN IN THE GREENHOUSE; MEASUREMENTS WERE MADE 7 DAYS AFTER THE PLANTING DATE.

Variability due to:	D/F	Sum of squares	Mean square	Observed F	Required F .05	Required F .01
Totals	287	916.587,19				
Between plots	5	111.899,06				
Within plots	(282)	(804.688,13)				
Between treat. within plots	6	103.770,21	17.295,035,000	6.81	2.13	2.86
Within treat. within plots	276	700.917,92	2.539,557,681			

plants suggested that there might be differences in their rates of respiration. The measure of CO_2 evolution from germinating seed was used to study seed respiration. The seed used for these tests was from plants grown in 1947 receiving the butyl ester spray, and from control plants. Respiration tests were conducted during November, 1948.

According to the description under Methods and Materials, seed lots of 10 seeds each, from treated and control plants with 20 replicates to a treatment, were soaked for 12 hours. Each lot was then placed in a stoppered flask above a saturated solution of barium hydroxide. At 12-hour intervals for 48 hours, the seed was removed and transferred to flasks containing a fresh supply of barium hydroxide.

A covariance analysis was made of each of the four 12-hour periods with the X factor representing the seed weights in grams, and the Y factor representing the CO_2 evolved in milligrams per 10 seedlings. Results of these studies are shown in Tables 8 and 9, and an analysis of covariance of the data in Table 10.

Statistical procedures

All statistical analyses were made using methods for analysis of biological data described by Snedecor (13). Mrs. Landblom, Experiment Station Statistician, recommended the use of

Table 8.--RESPIRATION MEASUREMENTS OF BARLEY SEED HARVESTED FROM PLOTS TREATED WITH 1½ POUNDS PER ACRE, ACID EQUIVALENT, OF THE BUTYL ESTER OF 2,4-D.

Seed lot	Seed weight in grams (10 seeds)	CO_2 evolution in milligrams/10 seedlings			
		1st 12 hrs.	2nd 12 hrs.	3rd 12 hrs.	4th 12 hrs.
1	0.4178	0	1.584	2.376	1.672
2	0.4064	0.264	1.320	1.672	1.628
3	0.4468	0	2.156	2.090	1.650
4	0.4058	0	4.070	1.848	1.452
5	0.3616	0.374	1.584	2.178	1.430
6	0.4382	0.704	1.804	2.706	1.452
7	0.3958	0.352	1.452	2.442	1.628
8	0.3806	0.440	1.848	2.200	1.342
9	0.4439	0.044	1.650	2.794	1.320
10	0.4735	0.242	1.034	3.502	1.694
11	0.4347	0.308	1.694	2.552	1.650
12	0.3732	0	1.804	2.134	1.474
13	0.4288	0.440	1.848	2.442	1.848
14	0.4438	0.440	0.660	2.134	2.024
15	0.4120	0.330	1.320	2.354	1.628
16	0.3904	0.462	1.452	1.056	1.694
17	0.4191	0	1.320	0.660	2.134
18	0.4484	0	1.650	1.540	1.254
19	0.4634	0	1.342	1.078	1.782
20	0.4630	0	1.122	1.914	1.958
Avg.	0.42236	0.220	1.6357	2.0836	1.6357

Table 9.--RESPIRATION MEASUREMENTS OF BARLEY SEED HARVESTED FROM CONTROL PLOTS.

Seed lot	Seed weight in grams (10 seeds)	CO_2 evolution in milligrams/10 seedlings			
		1st 12 hrs.	2nd 12 hrs.	3rd 12 hrs.	4th 12 hrs.
1	0.2953	0.484	1.188	1.914	1.914
2	0.3506	0.484	2.442	3.146	2.002
3	0.3075	0.440	1.694	2.398	1.210
4	0.2973	0.660	1.496	2.376	1.540
5	0.2956	0.638	1.782	2.266	2.222
6	0.3319	0.704	2.156	3.256	1.870
7	0.3020	0	1.716	2.376	1.474
8	0.2950	0.440	1.848	2.552	1.386
9	0.3097	0.814	1.760	2.002	1.276
10	0.2757	0.264	1.166	2.090	1.936
11	0.3134	0.484	1.320	3.630	2.266
12	0.3272	0.550	2.024	2.002	1.496
13	0.3185	0.242	1.870	2.222	0.550
14	0.3088	0	1.364	2.640	1.144
15	0.3047	0	1.386	2.816	1.760
16	0.2969	0.440	2.024	2.706	2.200
17	0.3272	0	1.430	1.650	1.804
18	0.3115	0.418	1.540	0.880	1.870
19	0.3023	0	1.452	2.156	2.156
20	0.3326	0.088	1.232	2.310	2.024
Avg.	0.310185	0.3575	1.6445	2.3694	1.7050

Table 10.--ANALYSIS OF COVARIANCE OF BARLEY SEED WEIGHT IN GRAMS (X) AND CO_2 EVOLUTION IN MILLIGRAMS (Y).

1st 12-hour period

Variability due to:	D/F	Sums of squares and products			1/ X			2/ Y
		x^2	xy	y^2	Obs. F	Req. F .05	Req. F .01	Obs. F
Totals	39	0.150,299,14	-0.184,495,85	2.450,189.5				
Between treatment	1	0.125,821,09	-0.154,233,75	0.239,062,5	195.33	4.10	7.35	0.04
Within treatment	38	0.024,478,05	-0.030,262,10	2.211,027,0				

Variability due to:	D/F	Adjusted sums of squares		Obs. F	Req. F .05	Req. F .01	r
		S.S.	M.S.				
Totals	38	2.223,646,356					-.304
Between treatment	1	0.050,165,622	0.050,165,622	<1	4.11	7.37	——
Within treatment	37	2.173,480,736	0.058,742,722				-.130

1/ Observed F of variance analysis for seed weight in grams (X)
2/ Observed F of variance analysis for CO_2 evolutions in milligrams (Y)

(Continued on following page)

Table 10.--ANALYSIS OF COVARIANCE OF BARLEY SEED WEIGHT IN GRAMS (X) AND CO_2 EVOLUTION IN MILLIGRAMS (Y). (Continued)

2nd 12-hour period

Variability due to:	D/F	Sums of squares and products			1/ X	Req. F		2/ Y
		x^2	xy	y^2	Obs. F	.05	.01	Obs. F
Totals	39	0.150,299,14	-0.055,133,10	10.676,023,6				
Between treatment	1	0.125,821,09	-0.009,870,96	0.000,774,4	195.33	4.10	7.35	0.00027
Within treatment	38	0.024,478,05	-0.046,262,14	10.675,249,2				

Variability due to:	D/F	Adjusted sums of squares		Obs. F	Req. F		r
		S.S.	M.S.		.05	.01	
Totals	38	10.655,799,540,73					.043
Between treatment	1	0.064,244,118,53	0.064,244,118,53	<1	4.11	7.37	___
Within treatment	37	10.591,555,422,20	0.286,258,254				.088

1/ Observed F of variance analysis for seed weight in grams (X)
2/ Observed F of variance analysis for CO_2 evolutions in milligrams (Y)

(Continued on following page)

Table 10.--ANALYSIS OF COVARIANCE OF BARLEY SEED WEIGHT IN GRAMS (X) AND CO_2 EVOLUTION IN MILLIGRAMS (Y). (Continued)

3rd 12-hour period

Variability due to:	D/F	Sums of squares and products			1/ X	Req. F		2/ Y
		x^2	xy	y^2	Obs. F	.05	.01	Obs. F
Totals	39	0.150,299,14	-0.211,610,80	15.843,727,9				
Between treatment	1	0.125,821,09	-0.319,572,28	0.811,680,1	195.33	4.10	7.35	2.05
Within treatment	38	0.024,478,05	-0.107,961,48	15.032,047,8				

Variability due to:	D/F	Adjusted sums of squares		Obs. F	Req. F		r
		S.S.	M.S.		.05	.01	
Totals	38	15.545,794,520,8					-.137
Between treatment	1	0.518,926,113,7	0.518,926,113,7	1.28	4.11	7.37	____
Within treatment	37	15.026,868,407,1	0.406,131,578,0				.040

1/ Observed F of variance analysis for seed weight in grams (X)
2/ Observed F of variance analysis for CO_2 evolutions in milligrams (Y)

(Continued on following page)

Table 10.--ANALYSIS OF COVARIANCE OF BARLEY SEED WEIGHT IN GRAMS (X) AND CO_2 EVOLUTION IN MILLIGRAMS (Y). (Continued)

4th 12-hour period

Variability due to:	D/F	Sums of squares and products			1/ X			2/ Y
		x^2	xy	y^2	Obs. F	Req. F .05	Req. F .01	Obs. F
Totals	39	0.150,299,14	-0.037,157,45	4.782,295,1				
Between treatment	1	0.125,821,09	-0.077,783,81	0.048,024,9	195.33	4.10	7.35	0.04
Within treatment	38	0.024,478,05	-0.046,263,32	4.734,270,2				

Variability due to:	D/F	Adjusted sums of squares		Obs. F	Req. F .05	Req. F .01	r
		S.S.	M.S.				
Totals	38	4.773,108,912,45					-.044
Between treatment	1	0.126,276,019,50	0.126,276,019,5	1.01	4.11	7.37	____
Within treatment	37	4.646,832,892,95	0.125,590,078,1				-.137

1/ Observed F of variance analysis for seed weight in grams (X)
2/ Observed F of variance analysis for CO_2 evolutions in milligrams (Y)

analysis of variance in comparing seed weights, seed volume, specific gravity of seed, and CO_2 evolution. A complex variance analysis was used in measuring root, coleoptile, and leaf growth of seedlings; and an analysis of covariance in measuring CO_2 evolution on the basis of seed weight.

Summary of data presented

In the study of the comparison of seed from barley sprayed with 2,4-D herbicides with that from control plants, data have been presented on seed weights, specific gravity of seed, percentage of viable seed, rate of germination and seedling growth, germination tests in greenhouse soil, germination tests of first-generation seed, and respiration studies.

The original data on which this study is based are on file in the office of the Botany Department, Colorado A & M College.

Chapter V

DISCUSSION

Field observations of the barley used in the foregoing data were in agreement with those of Klingman (8) in that the butyl ester spray of 2,4-D applied to the barley in the late-boot stage caused severe lodging. However, a high percentage of sterility of the heads was not observed here. Also, abnormal heads resulting from spraying, as mentioned by Leggett (9), were not present.

From the analysis of the data, it may be seen that seed from plants treated with 2,4-D was significantly heavier than that from control plants. This is indicated in Table 10. The variance analysis of seed weight shows significant differences at the 0.05 percent and the 0.01 percent levels. This is in agreement with the work of Buckholtz (4), who reported that kernel weight was increased by the sodium salt and butyl ester applications of 2,4-D.

Sexsmith (12) reported that seed weight was affected adversely by the 2,4-D sprays used in his experiment and attributed this to the increase in number of sterile florets. On analyzing his report, it seemed apparent that he came to this conclusion on the basis of yield rather than on the basis of individual seed weights.

In order to check this matter more accurately, tests of specific gravity were made using seed from treated and control plants. Statistical analyses of these data, shown in Tables 2 and 3, indicate that there is a difference in the specific gravity and seed volume. Seed from treated plants had a significantly greater specific gravity and a greater volume.

One of the items discussed by several authors is the effect of 2,4-D on total seed germination. Buckholtz (4) found in his experiments that 2,4-D had no effect upon total germination of barley seed. In a related study Marth, Toole, and Toole (11) found that seed development and total germination of certain cereals were in no way affected by 2,4-D applied at various stages in the development of the parent plant. The germination studies in this thesis are in agreement with these studies. There was no difference in the percentage germination of seed from treated barley compared with control barley.

As far as can be determined from the present literature (4) (11), all germination results were determined on the basis of total percentage germination only. During the germination experiments reported in this paper, it was noticed that there was a difference in the rate of germination. In order to substantiate this observation, measurements were made of root and coleoptile growth of

the 1947 seed germinated in blotters. These results were presented in Tables 4 and 5. A variance analysis of these data indicates that the rate of germination of treated seed and the rate of primary root and coleoptile growth of seed from treated plants was significantly retarded and that this difference was present during the entire course of the experiment.

In Tables 6 and 7 variance analyses of leaf measurements of seedlings from the 1947 lots of seed grown under greenhouse conditions show that there was a significant difference in primary leaf growth at the 0.05 percent level on the sixth day after the planting date, and at the 0.05 percent and 0.01 percent levels on the seventh day. These soil germination and growth results agree with those obtained for the same seed tested in blotters in the laboratory. They suggest that whatever the basis of growth inhibition by the butyl ester of 2,4-D is, it was not overcome by photosynthesis.

Whether the above mentioned effects carried on beyond the first generation into the succeeding crop was the next question. Seed from the 1947 samples was planted and the resulting plants grown to maturity in the greenhouse between December 1947 and March 1948. No differences in growth habit, character of the nodes, seed weight, size and color, or rate of maturity were found in the

succeeding crop. Seed from the plants showed no differences either in total germination or growth rates of primary roots and coleoptiles. It would appear from this evidence that there was no "carry-over" effect of the 1947 treatment into the succeeding crop. This might indicate that the inhibition of roots and coleoptile growth and of leaf growth observed in the early stages of seedling development were comparable to the primary reversible inhibitions described by Audus (3). This is further evidence that the action of 2,4-D is a reversible reaction, providing it is present in low enough concentrations.

An index frequently used for determining the rate of respiration of germinating seed is the measurement of the rate of evolution of CO_2. For measuring CO_2 evolution a technique originally designed by Harrington and Crocker (6), and used successfully by Lyon (10), was adopted. To facilitate respiration measurements a special apparatus was devised and assembled as described in Methods and Materials. Preliminary tests were run to correct errors in the method.

Data obtained from the respiration studies was presented in Tables 8 and 9 and summarized in Table 10. From the variance analyses of CO_2 evolution for each period of 12 hours as shown in Table 10, it can be seen that there was no significant difference

between seedlings from treated and control plants in the amount of CO_2 evolved. A covariance analysis of seed weights and CO_2 evolution indicates that there was no difference in CO_2 evolution on the basis of seed weight. Also it can be seen from these data that seed weight and CO_2 evolution are not correlated.

Results obtained from these studies on the rate of seedling growth from seed of 2,4-D treated barley plants agrees with tests made by Allard, et al, (1) with wheat seed treated with 2,4-D. However, the results from the respiration tests do not agree with either the studies of Taylor (14) who found that wheat seedlings from seed treated with 2,4-D (0.25-10 p.p.m.) showed a reduction in the rate of CO_2 evolution or with the tests of Hseuh and Lou(7) who found that 2,4-D applied to barley seed in concentrations that inhibited total germination resulted in a high rate of CO_2 evolution by subsequent seedlings. It may be that our results are not directly comparable to those of Taylor (14), who worked with wheat, or with those of Hseuh and Lou (7), because of differences in the technique of 2,4-D application.

The results of our studies indicate that the initial effect of 2,4-D on the growth of barley seedlings grown in the dark may be concerned in some phase of metabolism other than aerobic respiration. This is supported by the fact that we secured

significant differences in the growth rates of both coleoptiles and primary roots, but could not show significant differences either in milligrams of CO_2 production per seedling, or per gram of seed weight. Our results are in agreement with the trend of thought of Audus (3), who has suggested that the primary growth inhibition of 2,4-D is a reversible reaction, and that the secondary effects are due to accumulations of toxic metabolic by-products.

Suggestions for further study

During the study of the effects of 2,4-D on barley, the following problems for further investigation were suggested:

1. How does 2,4-D affect the anatomy of the nodes of lodged barley?
2. Does 2,4-D applied as a spray to barley affect the anatomy of the seed?
3. Would retarded growth of seedlings caused by spraying the parent plants with 2,4-D affect the susceptibility of seedlings to soil-borne organisms?
4. Does light influence the rate at which a seedling is able to recover from the primary phytostatic action of 2,4-D?
5. Do other varieties of barley respond to 2,4-D sprays in the same manner as Trebi barley used in these studies?

Chapter VI

SUMMARY

Some common salts and ester sprays of 2,4-D were used in fields of Trebi barley in 1947 for the control of broad-leaved weeds. Tests with barley from these fields were made to determine the effects of the herbicides on seed quality, seed germination, seedling growth, seed respiration, and effects on the subsequent crop. Data were collected on seed weights, specific gravity of seed, the percentage of viable seed, rates of germination and seedling growth, germination tests in greenhouse soil, germination of first-generation seed, and seedling respiration.

Sprays of an isopropyl ester, a triethanolamine salt, and a sodium salt of 2,4-D applied at rates of 1 and 1½ pounds acid equivalent per acre to Trebi barley in the late-boot stage produced no obvious changes in the mature plants. A butyl ester at the rate of 1½ pounds per acre, applied under the same conditions, caused elongation of the nodes and lodging. An isopropyl ester at the rate of 1½ pounds per acre caused some lodging. None of these treatments produced a measurable effect on total germination. All produced changes in seed quality as regards seed weight, size and color.

Seeds from treated plants were heavier, larger and darker in color. However, when these were planted and grown to maturity in the greenhouse, no carry-over effects could be measured or observed.

Detailed studies of the seed from Trebi barley treated with the butyl ester spray of 2,4-D at a rate of 1½ pounds acid equivalent per acre, showed no significant differences in percentage of viable seed or in the amount of CO_2 respired by seedlings 12 to 48 hours old. It was also found that seed from the treated plants was significantly heavier, had a higher specific gravity and a greater volume. Laboratory and greenhouse tests showed that the rate of germination and the rates of growth of coleoptiles, primary roots and primary leaves were depressed significantly by treatment.

These data were used in support of a hypothesis of the action of 2,4-D, i.e., that the primary effect on plant growth is on some phase of metabolism not directly concerned in aerobic respiration.

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