SOME PHYSIOLOGIC AND MORPHOLOGIC RESPONSES OF CERTAIN FIELD CROPS TO 2,4-DICHLOROPHENOXYACETIC ACID (2,4-D)

by

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SOME PHYSIOLOGIC AND MORPHOLOGIC RESPONSES OF CERTAIN FIELD CROPS TO 2,4-DICHLOROPHENOXYACETIC ACID (2,4-D)

INTRODUCTION

Early research involving 2,4-dichlorophenoxyacetic acid was directed toward a study of the toxicity of the chemical to various weeds and the effectiveness of 2,4-D in controlling broad-leaved weeds in small grains. In these tests injury to the crop by the chemical may have been overlooked due to the compensating effect of reduced weed competition in the sprayed plots. To overcome this defect the experiments reported herewith were designed to study the effect of the herbicide upon several field crops grown on weed-free soil. The results of such experiments should provide information as to the maximum safe limits of application of the chemical for control of weeds growing in these crops.

REVIEW OF LITERATURE

1. The Effect of Post-emergence Applications of 2,4-D

Hamner and Tukey (19) in 1944 found that 2,4-D exerted no detrimental effect upon a series of grass species tested.

Blackman (5) made recommendations for specific annual weeds in cereal crops. The rates recommended were 1.0, 1.5, and 2.0 pounds per acre. However, Viehmeyer (37), reviewing the abstracts and reports presented at the North Central Weed Control Conference held at Topeka on December 10-12, 1947, stated that the dosages that had been generally recommended and used for the control of annual weeds in crop plants were too high for safety and a downward revision of those dosages was necessary if the maximum and most economical use of the herbicide was to be made.

In a review on 2,4-D and its applications the U.S.D.A. (36) advised that, in the main, it provides a weapon against weeds in drilled and broadcast crops where cultivation is impractical or impossible. Flax, however, was considered as susceptible to injury by 2,4-D as the annual weeds. Therefore, the chemical was not recommended for use in flax.

The influence of climatic factors such as temperature and rainfall upon the herbicidal effects of 2,4-D were observed by Marth and Davis (27) who found that treatment at higher temperatures resulted in more rapid killing. Weaver *et al.* (39) obtained decreased plant response to 2,4-D when application of an aqueous solution was followed by rainfall.

In the greenhouse when rain was simulated six hours or more after application of 2,4-D, plant responses were not decreased, but in a field trial, rainfall within twenty-four hours of application reduced the effect of the herbicide. No diminution in response to the herbicide occurred when an oil solution was used, suggesting that an oil carrier might be useful in regions of heavy rainfall.

One of the first experiments reported on weed-free plots was a study of chemical control of weeds in rice, by Ryker (32). Response to treatment varied with stage of growth at time of application. Plants sprayed at flowering showed considerable blasting. There was normal germination from harvested seeds except where 2,4-D had come in direct contact with the grain itself. Ryker observed that dusting was as effective as spraying for the control of some weeds and more effective for others.

Klingman (24) concluded from his experiments that treating cereals, when weed-free, with 2,4-D reduced yields. He suggested that as much as 3.0 pounds of 2,4-D per acre might be applied for weed control in wheat and barley. Spraying wheat and barley in the boot stage resulted in many sterile heads and significantly depressed yields if the application was made at, or before, jointing, or after heading. Greatest head abnormalities (other than sterility) of wheat and barley occurred in plants sprayed at, or before, the jointing stage.

Applications of an ester and a salt type of 2,4-D at rates of 0.5, 1.0, 1.5, and 2.0 pounds per acre were made to winter wheat in the spring at the jointing stage and in the pre-head stage by Elder (13). He also applied it in the fall at the tillering stage. It appeared that this early application was more severe on wheat yields than the spring applications. Applied in the spring the 0.5 pound per acre rate gave only a slight decrease in yield.

Kusch (25) found that the 1.0, and especially the 2.0 pound rate of butyl ester resulted in measurable differences in response between varieties of the same crop. Rescue wheat was affected most, Apex somewhat less, while Thatcher and Redman were least affected. The latter three varieties reacted with larger kernels and higher bushel weights. Rescue was adversely affected in this respect. In oats, Exeter was most affected, Victory next, then Garry, with Ajax appearing to receive a stimulus. Titan and Velvon barley were adversely affected. Vantage and Plush showed only slight damage. In both oats and barley treatment resulted in increased kernel and bushel weights of all varieties.

Sodium salt, amine, and butyl ester formulations of 2,4-D were used by Leggett (26). The rates were 1.0, 2.0, 3.0, 4.0, and 5.0 pounds per acre applied at three stages of growth. The 3.0, 4.0, and 5.0 pound rates of all formulations, but especially of the amine and ester, resulted in sterile wheat florets. All wheat plots treated in the three-leaf stage resulted in distorted heads. This distortion was absent from plots treated at the two later stages. Except for distorted heads, early spraying resulted in less damage than late sprayings. Leggett found that barley and wheat were similar in their response, while oats was the least resistant of these three cereals. The percentage of sterile florets increased with formulation (sodium salt, to amine, to butyl ester), concentration (check to 5.0 pound rate), and stage of growth (three-leaf, early shot blade to pre-heading). Redwing flax was "wiped out" on all plots at the 5.0 pound rate. The plots receiving the 4.0 pound rate of any formulation and the 3.0 pound rate of either the amine or ester formulations produced no seed. Stage of growth at time of treatment did not affect the result. Compared with the check all plots were delayed in maturity.

Ficht (16) used the same formulations, but the rates were 0.25 and 0.5 pound per acre. Rescue wheat, sprayed when three inches high, developed deformed heads and a noticeably uneven stand at both rates with all formulations. Wheat sprayed before emergence and after the plants were five inches high did not develop deformed heads. Ajax oats and Titan barley sprayed when three inches high showed unevenness of stand, and maturity was delayed three to six days. Spraying Royal flax at early four-leaf stage resulted in a severe reduction in stand from the amine at 0.5 pound per acre and the ester at both 0.25 and 0.5 pound per acre. Flax sprayed in the bud stage with 0.5 pound amine branched more profusely and filled with seed, but was delayed in maturity by about two weeks.

The sodium salt and butyl ester forms of 2,4-D were used by Buchholtz (7). In 1946 he used rates of 1.0, 2.0, and 4.0 pounds per acre, acid equivalent, and in 1947 these were reduced to 0.5, 1.0, and 2.0 pounds per acre. The applications were made at three stages of growth (four, eight, and fourteen inches) to Oderbrucker barley and Vicland oats grown on essentially weed-free plots. The yield of barley was not affected significantly by 2,4-D in either year. Germination of seed from treated plots was fully as good as from untreated checks. Kernel weight of the barley was increased by treatment at the four and eight inch stages of growth. In several instances lodging was reduced by 2,4-D. The higher rates of application, especially at the four and eight inch growth stages depressed oat yields. Neither kernel weight nor lodging reaction was influenced, but germination was reduced by the heavier rates at the four and eight inch growth stages. The sodium salt was less toxic than the butyl ester.

Elder (14) applied three formulations of 2,4-D at 0.5, 1.0, 1.5, and 2.0 pounds per acre to Kanota oats when the plants were three to four inches high and at the pre-heading stage. All the rates at both dates reduced yield. The ester type proved more toxic than the sodium salt or amine types.

Viehmeyer and Wolfe (38) applied three formulations of 2,4-D at dosages of 0.25, 0.5, 1.0, and 1.5 pounds per acre to four varieties of oats and barley at heading time. In both barley and oats the greatest reduction in yield resulted from butyl ester treatments and was in proportion to the dosage. In barley, lodging occurred within forty-eight hours following treatment, and the plots remained lodged. Lodging was due to bending of the second and third nodes following treatment.

The same formulations were used by Derscheid (11), all being applied at 1.0 pound per acre, acid equivalent. Irrespective of barley variety, or date of treatment, the plots treated with ester yielded significantly lower than the checks or the plots treated with an amine or sodium salt. Regardless of variety or formulation, the plots treated in the tillering stage yielded significantly more than plots treated at the seedling, boot, or bloom stage.

Bakke (3) concluded from preliminary experiments that a dilute solution of butyl ester could be used to control certain weeds in flax.

2. <u>Residual Effect of 2,4-D</u>

In 1944 Hamner and Tukey (19) tested the residual effect of 2,4-D in the soil after its application as a herbicide. Twelve species of cereal, lawn and pasture plants were sown on an area sprayed two months earlier. The seeds germinated well and young plants showed no curvatures or formative defects. They suggested that light surface applications prior to seeding might destroy some weeds and not affect germination of the crop sown later.

Cates (9), reviewing work on 2,4-D prior to 1945, stated that there was no evidence of it sterilizing or damaging the soil for subsequent crops. However, Blackman (5) stated that seeding after the use of 2,4-D was out of the question because residues toxic to germinating seeds remain in the soil for at least six weeks. Some of the findings of Hamner *et al.* (18) substantiate this view. In their experiment, concentrations of 2,4-D acid as low as 1 part per million (p.p.m.) applied to mineral or muck soil affected the germination and growth of many seeds.

Marth and Mitchell (28) obtained effective weed control in experimental plots sprayed with concentrations of 500 or 1,000 p.p.m. of 2,4-D per acre.

Carlyle and Thorpe (8) used low concentrations of amine and sodium salts in soil solution to determine the residual effect of 2,4-D. The results revealed that 0.5 p.p.m. (0.21 pounds per acre) of 2,4-D in the soil solution would seriously restrict germination, limit growth, and practically inhibit nodulation of beans, peas, red clover and alfalfa. The growth of rhizobia was not inhibited until a concentration of 0.03% (corresponding to 200 pounds per acre) was reached. The deleterious effect on symbiotic relationship, as indicated by a reduction or inhibition of nodulation, was caused largely by action of the herbicide through the medium of the plant. It was suggested that field applications of 2,4-D would probably be harmful to legumes.

Payne and Fults (31) carried out their study in the greenhouse. When the 2,4-D was mixed with the soil before planting it prevented nodulation in the common bean at a concentration as low as 0.075 pound per acre. At concentrations lower than this nodules formed, but a disintegration of host tissue and a change in the bacterial gram-stain reaction were observed.

Dunham and Robinson (12) treated soil on May 22 with an amine salt of 2,4-D at rates of 1.0 and 2.0 pounds per acre. On May 24 several crops were planted on the treated soil. The first planting of red clover was injured and the flax stand slightly reduced. No injury was observed on oats, corn, soybeans, and sugar beets; nor on the second plantings (June 10) of flax and red clover.

Wheat, corn, peas, and flax were sown one, eighteen, thirty-two, and forty-nine days after application of 1.0 and 2.0 pounds per acre of 2,4-D, in a test reported by Bisal (4). Corn appeared to be entirely immune to toxic effects of 2,4-D. Wheat sown at the first two dates showed some injury, but there were no harmful effects in the later seedings. Flax was only slightly more susceptible than wheat. Peas grew poorly when seeded thirty-two days after application of 2,4-D at 2.0 pounds per acre, but no appreciable effect was evident from the 1.0 pound rate. The residual effect of the 2,4-D did not exceed a forty-nine day period.

Bordeleau (6) applied a sodium salt of 2,4-D at concentrations of 500, 1,000 and 2,000 p.p.m. on June 17. Oats, white clover, corn, flax, and timothy were sown on the treated plots and on checks on each of June 17, July 5, July 21 and August 2. The oats germinated 100 per cent at all dates, but about 50 per cent of the plants from the first two sowings died. There was no residual effect on oats after thirty days. The first sowing of white clover was practically destroyed, but no injurious effects were observable after fifteen days. Flax results were similar to those of white clover. Corn was not affected by 2,4-D. The timothy germinated 100 per cent at all dates, but it died on all plots including the checks.

Using rates of 1.0 and 2.0 pounds per acre of an amine salt of 2,4-D on sandy soil, Harrison (21) found that the detrimental effect of the 2,4-D on the seed and on plant development had altogether disappeared two weeks after application. The plants used were set onions, beets, carrots, snap beans, sweet corn, tomato and raddish seed.

Timmons (34) made soil applications of an ester, a sodium salt, and an amine salt of 2,4-D, and methoxone, on April 18 at 2.0 pounds per acre. The sodium salt was also applied at rates of 1.0, 3.0, and 4.0 pounds per acre. Plantings of alfalfa, buffalo grass, corn, midland milo, and Sudan grass were made April 18, May 2, May 22, and June 2 across treated and untreated plots. Stands of all crops from the first two plantings were reduced by all treatments, the reduction for the second planting being greatest in most cases. Normal stands of buffalo grass, corn, milo, and Sudan grass were obtained on nearly all plots from plantings made May 22 and June 2, and alfalfa planted June 2 produced nearly normal stands. No significant differences were apparent in the effects of different 2,4-D formulations at equivalent rates. Crop yields on treated plots, expressed as a percentage of yields on untreated control plots, were relatively greater than stands in nearly all cases.

In another experiment Timmons (34) made soil applications of the same formulations of 2,4-D at 2.0 pounds per acre on August 30. Plantings of several crops, including alfalfa, were made August 30, September 13, September 27, and October 11 across treated and untreated plots. All treatments prevented nearly all emergence of alfalfa from the first two plantings but did not greatly reduce the stands from later seedings.

Mitchell and Marth (29) made the following observations: 2,4-D in air-dry soil was slowly inactivated, but 1.0 to 14.0 milligrams per pound of soil reduced emergence of mustard seeds after the soil was stored for eighteen months. All rates retarded emergence of barley when the soil was stored air-dry for twelve days, but after one month only the 14.0 milligrams per pound rate caused significant retardation. However, when mixed in warm, moist soil, 2,4-D was rapidly inactivated. Twenty milligrams per pound in soil high in organic matter, stored under warm, moist conditions for a period of two weeks or more, did not significantly reduce the emergence of mustard plants.

Hamner and Tukey (19) in 1944 reported that germinating seed of sweet clover emerging 3 to 7 days after the surface had been sprayed were completely killed.

Anderson and Wolf (2) tried pre-emergence control of weeds in corn with 2,4-D. The corn was planted May 31 and the 2,4-D applied June 7, two days before the corn emerged. They applied 2.7 pounds per acre as dust, while the 5.5 and 9.1 pound per acre rates were sprayed on to the soil. The results indicated that 2.7 pounds per acre of 2,4-D effectively controlled weeds and had no detrimental effect on the corn.

No injury resulted to corn from pre-emergence treatments in an experiment reported by Willard and Homer (40). All treatments of 2 pounds per acre or more of 2,4-D acid controlled annual weeds to the extent that no further cultivation was necessary.

Two separate experiments were conducted by Hernandez _et al_. (22).

In each experiment, the sodium salt of 2,4-D was applied as a pre-planting treatment and as a surface application immediately after planting. There was practically no difference between the dates of application, but the results of the two experiments varied. In one there was no reduction in stand, maturity, or yield of corn; in the other, all treatments caused a delay in maturity and a reduction in yield of approximately 12 and 30% for the 2.0 and 4.0 pound rates respectively.

Spangelo and Anderson (33) found that 1.0 pound per acre of sodium salt or the ester form of 2,4-D gave effective control of annual weeds without any injury to the corn plants.

Hamner _et al_. (20) applied the sodium salt form of 2,4-D at 5.0 pounds per acre as a pre-emergence spray to corn. Adequate weed control was obtained without any reduction in the yield of corn. They found that on account of root stimulation the corn plants on treated plots withstood a severe windstorm which blew over all the corn on the control plots.

The amine and ester at 2.0 pounds per acre and the sodium salt at 4.0 pounds per acre gave effective weed control in a pre-emergence experiment reported by Timmons (35).

Alban and Kierns (1) applied four concentrations of the butyl ester form of 2,4-D within twenty-four hours after planting of 25 vegetable crops. In general the rates of 0.13 and 0.33 pound per acre were not effective herbicides for any appreciable length of time. The 0.66 pound per acre gave adequate control of all weeds present for a three-week period and the 1.32 pound rate gave adequate control for about six weeks. Weed species present were crabgrass, pigweed and purslane.

The period of effective weed control was found to be affected by the amount of moisture present in the soil and by temperature, as mentioned in reviewing the observations of Mitchell and Marth (29).

MATERIALS AND METHODS

The experiments involved in this study are outlined in the following three sections:

1. The effect of 2,4-D applied to cereals and flax.
2. The effect on crop plants of residual 2,4-D in the soil.
3. The effect of 2,4-D on soil micro-organisms and on soil nitrates.

1. The Effect of 2,4-D Applied to Cereals and Flax

Two separate experiments were conducted--one involving cereal crops; the other, flax. The varieties were Redman wheat, Garry oats, Montcalm barley and Royal flax.

The sodium salt and butyl ester formulations were compared. The rates* of 0.5, 1.0, and 2.0 pounds per acre were applied to wheat, oats, and barley, and 0.25, 0.5, and 1.0 pound to flax. The applications were made on June 25, when the plants were approximately eight to ten inches tall, and on July 8, when the cereals were just beginning to head, and the flax was in bloom.

A split plot design was used in both experiments. For the one involving cereals, wheat, oats, and barley were placed in the main blocks. The dates were assigned to sub-blocks and the formulations and rates were randomized on the plots within these. In the flax experiment each replicate was divided into two date blocks. The formulations and rates were randomized within these. Each experiment contained four replicates.

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* The rates of 2,4-D applied are reported on the basis of pure acid. It was sprayed in water solution at a volume of eighty gallons per acre.

Six-row plots eighteen and one-half feet long were used. Spacing between rows was nine inches, and between plots, eighteen inches. The middle two rows of each plot to be treated were sprayed by means of a knapsack sprayer. To increase the accuracy of the method of application an auxiliary tank, similar to the one described by Davis (10), was attached. Prior to harvest one foot was cut off each end of the plots. Yield determinations were based on the two middle rod rows.

Data on weight per measured bushel, and germination, were obtained in addition to the yield data.

Through frequent cultivation all plots were kept essentially weed-free.

Field notes were taken and abnormal plants were tagged. Seeds from the abnormal plants were sown in the greenhouse, and the progeny observed to determine whether any of the abnormalities were inherited.

Twenty-five spikes of each of wheat and barley, and twenty-five oat panicles were collected at harvest time from each plot in three replicates. Fifty seed bolls were taken from each flax plot. All the samples were threshed and seed counts were made to determine whether there was any effect of treatments on sterility.

2. The Effect on Crop Plants of Residual 2,4-D in the Soil

Two experiments were involved in this section—one devoted to alfalfa, barley, flax and millet; the other, to corn. The varieties were Macsel alfalfa, Montcalm barley, Royal flax, Siberian millet, and Hybrid 85 x 15 corn.

The sodium salt and butyl ester forms of 2,4-D were applied to the soil at 1.0 pound per acre on May 21. The crops were sown on treated and untreated soil on May 17, June 3, June 16, July 2 and July 17.

A split plot design was used. Treatments were placed in the main blocks. Crops were in sub-blocks, and the dates of seeding were assigned to the small plots.

All plots consisted of three rows. The corn was planted in rows of twelve hills each. Spacing was three feet each way. For the other crops spacing between rows was nine inches, and between crops, eighteen inches. The rows were eighteen and one-half feet long.

Yield of dry matter in pounds was the criterion of treatment effect. In the case of corn, yield was based on the ten inside hills of the middle row. One foot was cut off each end of the other plots and the middle rod row was harvested for yield.

All trials were made in four replicates, three of which were kept weed-free and used for yield data. Field observations were made on all plots including those in the unweeded replicate.

An attempt was made to determine the effect of the residual 2,4-D on nodulation of alfalfa plants. On September 3, ten plants in each of the treatments, in two replicates, were dug from plots seeded May 17, June 3 and June 16. The roots were washed and the nodules counted.

3. <u>The Effect of 2,4-D on Soil Micro-organisms and on Soil Nitrates</u>

These tests were carried out on soil from plots treated for another experiment (30). It consisted of three replicates with three plots each. The plots were three feet by thirty-six feet with an intervening three foot space.

An untreated check and treatments of 2.0 and 4.0 pounds per acre of butyl ester were compared. The 2,4-D was applied on June 2.

Soil samples for bacteria and fungi plate counts were collected on June 12 and again on July 29. The dry, loose, top soil was discarded and about half an ounce of moist soil from just below the surface was dug with a tablespoon. Six spoons of soil taken at random from a plot were bulked to make up one sample. Two such samples were procured from each plot. Estimates of the numbers of soil bacteria and fungi were secured. The technique used was as described by James and Sutherland (23).

On October 3, soil samples for nitrate determinations were collected at three depths (0-6 inches, 6-12 inches and 1-2 feet) on each plot. By means of an auger six holes were drilled at random on each plot and a composite sample obtained for each depth. Water extracts for the determination of nitrates were made according to the method described by Emerson (15). Determinations were made colorometrically with a Coleman spectrophotometer.

For all experiments involved in this study, the methods of statistical analysis employed were as described by Goulden (17).

Through the courtesy of the Meteorological Division of Air Services, Department of Transport, Canada, a meteorological summary for the Winnipeg Area was obtained. The daily summary for the period May 21 to August 31 is given in Table I.

TABLE I

METEOROLOGICAL SUMMARY

WINNIPEG, MANITOBA

MAY 1947

	Date	Temperature Mean (max. to min.)	Hours of Bright Sunshine	Precipitation (inches)
	21	50.0	7.9	.01
	22	49.0	10.5	
	23	51.3	7.3	.08
	24	47.6	13.6	.09
	25	45.6	2.8	.02
	26	38.0	13.6	T
	27	33.3	7.5	.07
	28	33.0	2.6	T
	29	36.2	2.1	.06
	30	49.6	7.5	
	31	48.5	10.8	
Total			234.7	.85
May Mean		46.2		
Normal*		52.0	241.5	2.21

JUNE 1947

	Date	Temperature Mean (max. to min.)	Hours of Bright Sunshine	Precipitation (inches)
	1	45.0	12.5	
	2	54.6	4.3	
	3	60.0	4.5	.71
	4	55.8	0.0	.29
	5	57.2	2.1	
	6	53.9	1.7	
	7	60.2	4.9	
	8	65.5	7.6	.12
	9	52.1	0.0	.03
	10	47.0	T	
	11	50.0	14.2	

(Continued)

* Based on seventy-three year average at Winnipeg.
T - Trace

TABLE I (Continued)

METEOROLOGICAL SUMMARY

WINNIPEG, MANITOBA

JUNE 1947

	Date	Temperature Mean (max. to min.)	Hours of Bright Sunshine	Precipitation (inches)
	12	59.4	12.8	
	13	61.4	13.5	
	14	59.7	13.6	
	15	65.1	6.0	T
	16	59.4	0.0	.19
	17	65.3	14.5	
	18	65.4	12.4	
	19	67.7	12.9	
	20	68.6	7.1	
	21	70.8	0.3	.22
	22	65.0	0.0	1.15
	23	59.6	1.7	.04
	24	60.2	5.0	.12
	25	61.6	10.4	.06
	26	67.5	9.8	.02
	27	62.0	3.6	.46
	28	62.5	7.8	.23
	29	62.6	13.8	T
	30	63.2	8.2	.04
Total			205.2	3.68
Mean		60.3		
Normal		62.0	247.7	3.07

JULY 1947

	Date	Temperature Mean (max. to min.)	Hours of Bright Sunshine	Precipitation (inches)
	1	61.8	14.8	
	2	69.3	6.5	
	3	74.4	5.9	.38
	4	61.4	7.1	
	5	61.6	13.7	.03
	6	70.0	14.6	
	7	73.3	14.0	
	8	71.2	6.3	T
	9	76.6	12.5	T

(Continued)

TABLE I (Continued)

METEOROLOGICAL SUMMARY

WINNIPEG, MANITOBA

JULY 1947

	Date	Temperature Mean (max. to min.)	Hours of Bright Sunshine	Precipitation (inches)
	10	75.4	9.3	T
	11	75.2	12.8	
	12	76.3	4.1	.32
	13	74.8	7.9	T
	14	76.0	13.5	
	15	75.0	14.7	
	16	79.6	11.6	
	17	70.5	8.5	T
	18	60.5	12.5	T
	19	64.6	10.5	T
	20	54.6	11.7	T
	21	59.6	12.3	
	22	66.7	10.9	.32
	23	72.4	13.6	
	24	70.4	6.7	1.77
	25	70.3	4.6	.04
	26	68.8	6.0	
	27	75.6	13.2	
	28	79.8	12.8	.08
	29	75.0	10.0	T
	30	63.8	6.1	
	31	61.7	12.8	
Total			321.5	2.94
Mean		69.9		
Normal		67.0	300.6	2.95

AUGUST 1947

	Date	Temperature Mean (max. to min.)	Hours of Bright Sunshine	Precipitation (inches)
	1	70.0	9.7	.39
	2	74.7	13.1	
	3	79.8	13.7	T
	4	80.4	9.8	.09
	5	69.7	7.0	.42
	6	69.7	8.3	T

(Continued)

TABLE I (Concluded)

METEOROLOGICAL SUMMARY

WINNIPEG, MANITOBA

AUGUST 1947

	Date	Temperature Mean (max. to min.)	Hours of Bright Sunshine	Precipitation (inches)
	7	60.2	6.3	.52
	8	69.6	5.3	
	9	74.9	8.8	.12
	10	72.0	0.0	.98
	11	64.7	5.4	.33
	12	64.0	12.2	.06
	13	63.2	2.2	.59
	14	63.7	12.4	.06
	15	68.3	6.6	.10
	16	69.2	1.1	.27
	17	65.8	9.4	T
	18	66.9	12.2	
	19	72.4	12.9	
	20	74.4	8.4	
	21	76.3	12.6	
	22	86.1	11.9	
	23	74.9	8.4	
	24	61.6	8.7	T
	25	57.2	11.0	.23
	26	64.8	10.2	
	27	51.6	8.5	
	28	54.5	10.2	.27
	29	63.6	9.6	T
	30	63.4	10.6	
	31	62.4	3.6	.10
Total			270.1	4.53
Mean		68.1		
Normal		64.2	262.6	2.49

RESULTS AND DISCUSSION

The crops included in these experiments will be discussed separately in their respective sections.

1. The Effect of 2,4-D Applied to Cereals and Flax

Wheat

The day following treatment, all plots to which the ester had been applied were lodged. This occurred at both dates of treatment. The degree of lodging increased with the rate of application. A week after treatment the plants had recovered their normal stature, but some crinkling of leaf margins was noticed on plants which had received the 1.0 and 2.0 pound rates. No injury was observed on plots sprayed with the sodium salt formulation.

The 2.0 pound rate of ester, at both dates, resulted in visible sterility, and many culms were six inches shorter than normal. The same effect, to a lesser degree, was noted with the 1.0 pound rate. At the first date, the 1.0 and 2.0 pound rates of ester caused a number of abnormal spikes to be produced. The abnormalities consisted of twisting at the base of the rachis, spikelets borne opposite rather than alternate on the rachis, as well as twin spikelets (Figure 1). Seeds from these were grown in the greenhouse, and the resultant plants appeared normal.

Table II shows the effect of 2,4-D on yield. As an average of both dates the 1.0 and 2.0 pound rates of ester reduced yields significantly. The reduction at the 2.0 pound rate was more severe, but the difference between these two was not significant.

The sodium salt did not cause significant reductions in yield at any rate. The differences in yield, due to date of application of the

a b c d e f

Figure 1. Abnormal wheat spikes due to application of 2,4-D compared with a normal spike.

a - normal
b - twisting at base of rachis and basal sterility
c and d - spikelets near middle of spike opposite rather than alternate
e - twin spikelets and apical sterility
f - twisting at base of rachis and apical sterility

TABLE II

EFFECT OF AN ESTER AND OF A SODIUM SALT OF 2,4-D ON THE AVERAGE YIELD OF WHEAT IN BUSHELS PER ACRE

	Rate 2,4-D Pounds Per Acre	Date of Application June 25	Date of Application July 8	Mean
Ester	0.00	24.9	25.6	25.3
	0.50	22.6	24.6	23.6
	1.00	25.5	17.5	21.4
	2.00	19.4	17.2	18.3
Date Mean (Ester)		23.1	21.2	22.1
Sodium Salt	0.00	27.4	26.0	26.7
	0.50	22.1	25.9	24.0
	1.00	21.4	23.1	22.2
	2.00	25.6	24.6	25.1
Date Mean (Sodium Salt)		24.1	24.9	24.5
Date Mean (Ester and Sodium Salt)		23.6	23.0	

F VALUES

	Calculated	5 Per Cent Point	1 Per Cent Point
Dates	0.19	10.13	
Formulations	4.62	4.07	7.27
Rates	3.33	2.83	4.29
Formulations x Rates	1.83	2.83	
Formulations x Dates	1.48	4.07	
Dates x Rates	1.39	2.83	
Formulations x Rates x Dates	1.32	2.83	

2,4-D, were not significant at any rate for either formulation.

It may be seen from Table III that there was a fairly consistent reduction in the number of kernels per spike as the amount of ester applied increased; but no appreciable reduction for any rate of sodium salt. Date of application had little effect on sterility.

Comparing Tables II and III, a positive relationship between yield and the number of kernels per spike is evident. This is substantiated by the significant positive correlation coefficient obtained.

	Calculated	5 Per Cent Point	1 Per Cent Point
Correlation coefficient	0.471	0.288	0.372

This relationship is shown graphically in Figure 2.

Oats

Considerable blasting was observed on plots treated, at either date, with the 1.0 and 2.0 pound rates of ester. This was not noticed for the 0.5 pound rate of ester, nor for any rate of the sodium salt.

Table IV shows that as an average of dates, a progressive reduction in yield occurred as the concentration of the ester applied increased. The 1.0 and 2.0 pound rates caused significant reductions. The difference between these two was not significant. At the 2.0 pound rate the yield was only 42 per cent of the check.

The sodium salt formulation did not affect yield significantly at any rate. The 0.5 and 1.0 pound rates each yielded 19 per cent more than the check (not significant statistically), while the 2.0 pound rate

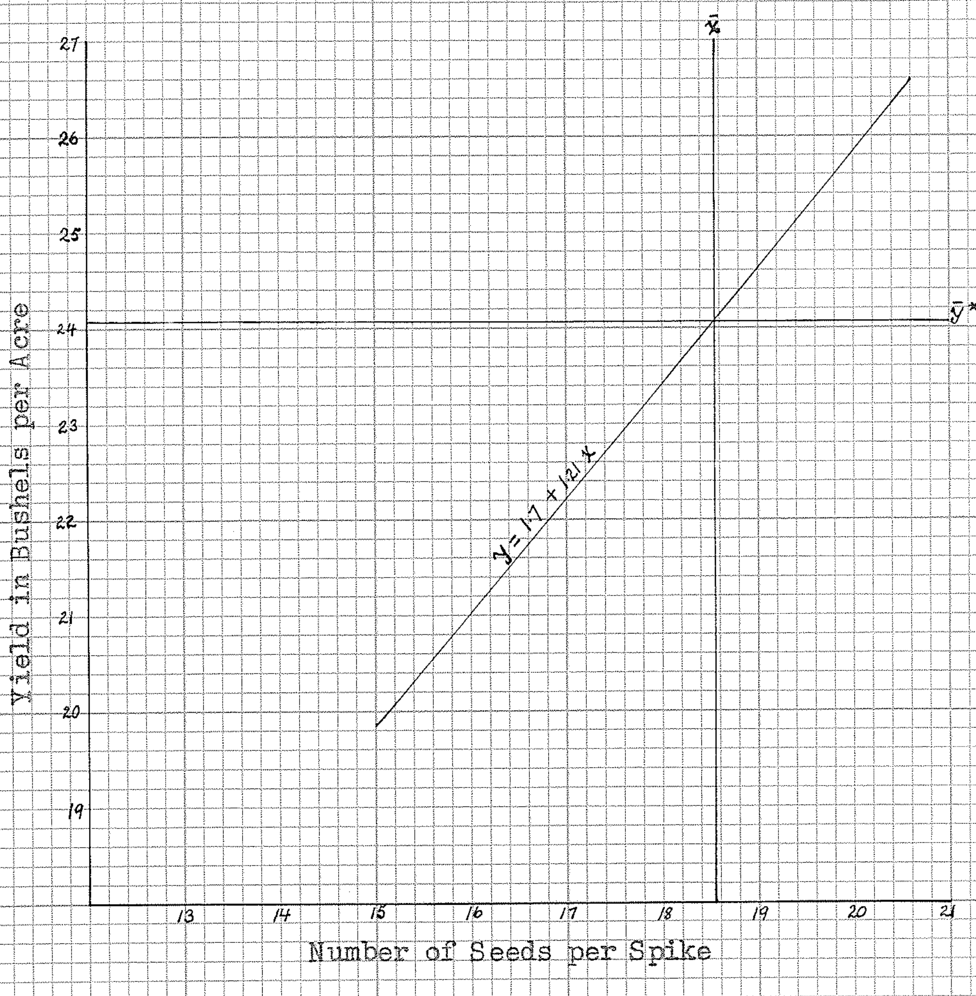
TABLE III

EFFECT OF AN ESTER AND OF A SODIUM SALT OF 2,4-D ON THE AVERAGE NUMBER OF KERNELS PER SPIKE OF WHEAT

	Rate 2,4-D Pounds Per Acre	Date of Application June 25	July 8	Means
Ester	0.00	20.3	20.6	20.4
	0.50	17.6	19.1	18.4
	1.00	19.0	16.1	17.6
	2.00	15.0	15.7	15.4
Date Mean (Ester)		18.0	17.9	17.9
Sodium Salt	0.00	18.6	19.1	18.8
	0.50	19.7	18.7	19.2
	1.00	19.9	19.4	19.6
	2.00	19.5	17.6	18.6
Date Mean (Sodium Salt)		19.4	18.7	19.0
Date Mean (Ester and Sodium Salt)		18.7	18.3	

F VALUES AND NECESSARY DIFFERENCE

	Calculated	5 Per Cent Point	1 Per Cent Point	Necessary Difference at 5 Per Cent Point
Dates	0.68	18.51		
Formulations	9.78	4.20	7.64	
Rates	9.84	2.95	4.57	
Formulations x Rates	8.45	2.95	4.57	1.46
Formulations x Dates	0.79	4.20		
Dates x Rates	1.86	2.95		
Formulations x Rates x Dates	2.74	2.95		



*mean yield of first three replicates

Figure 2. Simple regression graph for yield of wheat in bushels per acre on number of kernels per spike.

TABLE IV

EFFECT OF AN ESTER AND OF A SODIUM SALT OF 2,4-D ON THE AVERAGE YIELD OF OATS IN BUSHELS PER ACRE

	Rate 2,4-D Pounds Per Acre	Date of Application		
		June 25	July 8	Mean
Ester	0.00	31.8	41.2	36.5
	0.50	28.5	29.3	28.7
	1.00	19.9	15.9	17.9
	2.00	11.8	18.8	15.3
Date Mean (Ester)		22.9	26.3	24.6
Sodium Salt	0.00	30.2	32.6	31.3
	0.50	35.8	39.2	37.4
	1.00	36.9	37.8	37.4
	2.00	32.7	25.7	29.2
Date Mean (Sodium Salt)		33.9	33.8	33.8
Date Mean (Ester and Sodium Salt)		28.4	30.0	

F VALUES AND NECESSARY DIFFERENCE

	Calculated	5 Per Cent Point	1 Per Cent Point	Necessary Difference at 5 Per Cent Point
Dates	0.19	10.13		
Formulations	19.42	4.07	7.27	
Rates	6.62	2.83	4.29	
Formulations x Rates	6.30	2.83	4.29	8.48
Formulations x Dates	0.70	4.07		
Dates x Rates	0.58	2.83		
Formulations x Rates x Dates	1.07	2.83		

caused no appreciable reduction in yield.

The differences in yield due to date of application of 2,4-D were not significant.

Table V shows that there was a progressive increase in sterility as the rate of ester applied increased. The 1.0 and 2.0 pound rates caused significant reductions in the number of kernels per panicle. The sodium salt did not cause a significant increase in sterility at any rate. Differences in sterility due to date of application of 2,4-D were not significant.

The correlation coefficient between yield and the number of kernels per panicle was positive and significant.

	Calculated	5 Per Cent Point	1 Per Cent Point
Correlation coefficient	0.560	0.288	0.372

Graphic representation of this relationship is shown in Figure 3.

Barley

The day after treatment, all plots to which the ester had been applied were lodged. This was noted at both dates of treatment. The degree of lodging increased with the rate of application. A week following the first date of treatment the plants had recovered their stature reasonably well. Plots treated at the second date remained lodged. This condition was most severe at the 2.0 pound rate. The basal nodes of plants, in plots which remained lodged, were about three times the normal size. No injury was observed on plots sprayed with the sodium salt.

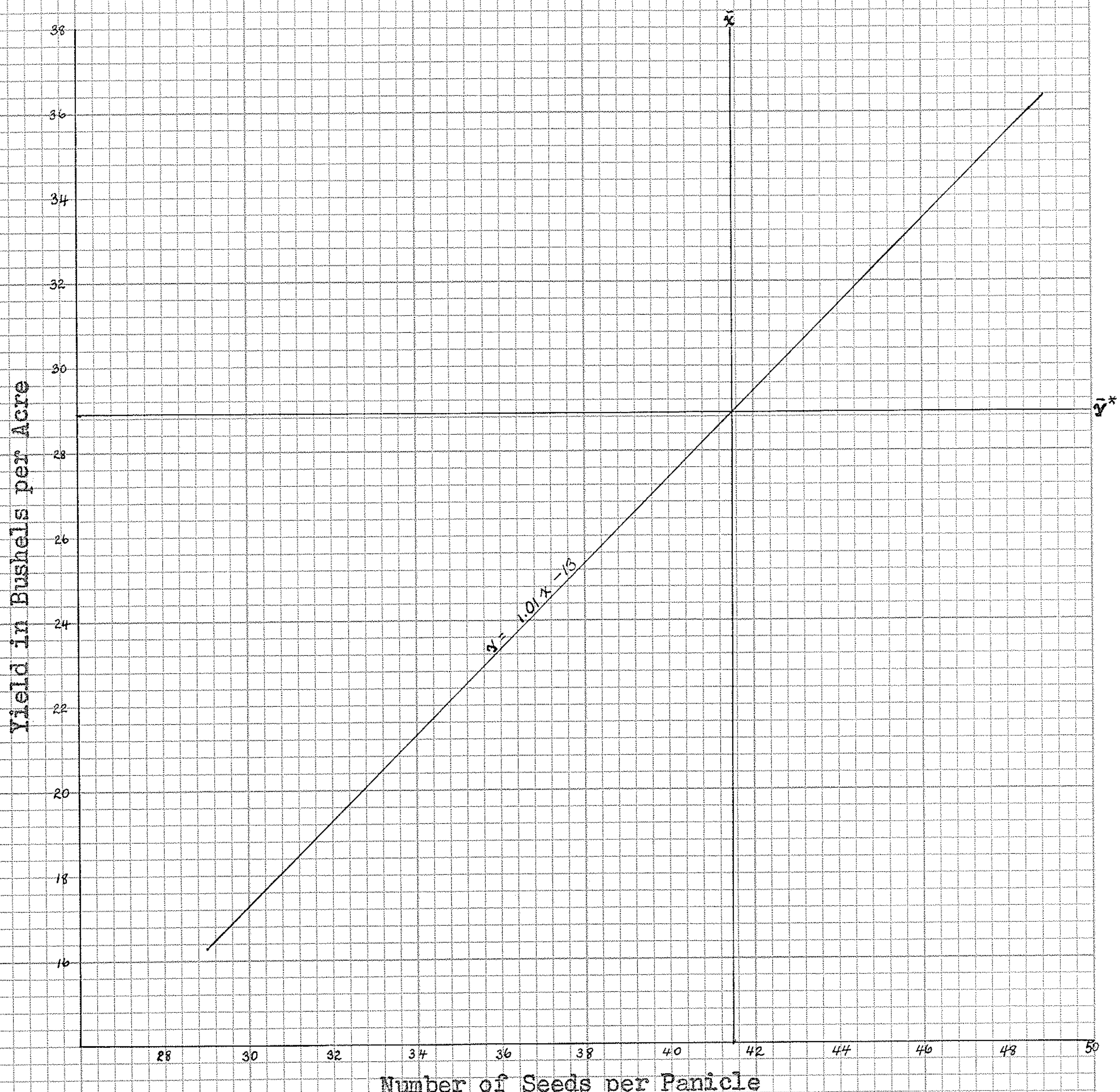
TABLE V

EFFECT OF AN ESTER AND OF A SODIUM SALT OF 2,4-D ON THE AVERAGE NUMBER OF KERNELS PER PANICLE OF OATS

	Rate 2,4-D Pounds Per Acre	Date of Application		Mean
		June 25	July 8	
Ester	0.00	46.8	46.4	46.6
	0.50	41.4	42.9	42.2
	1.00	32.3	41.4	36.8
	2.00	29.0	30.5	29.8
Date Mean (Ester)		37.4	40.3	38.8
Sodium Salt	0.00	48.9	42.4	45.7
	0.50	42.5	43.2	42.8
	1.00	45.4	45.8	45.6
	2.00	42.5	42.3	42.4
Date Mean (Sodium Salt)		44.8	43.4	44.1
Date Mean (Ester and Sodium Salt)		41.1	41.9	

F VALUES AND NECESSARY DIFFERENCE

	Calculated	5 Per Cent Point	1 Per Cent Point	Necessary Difference at 5 Per Cent Point
Dates	0.15	18.51		
Formulations	28.01	4.07	7.27	
Rates	17.46	2.83	4.29	
Formulations x Rates	10.59	2.83	4.29	4.08
Formulations x Dates	4.66	4.07	7.27	
Dates x Rates	2.85	2.83	4.29	
Formulations x Rates x Dates	0.88	2.83		



* mean yield of first three replicates

Figure 3. Simple regression graph for yield of oats in bushels per acre on number of kernels per panicle.

Table VI shows, that as an average of dates, there was a progressive reduction in yield as the rate of ester applied increased. There was a pronounced effect even at the half pound rate. The plots treated at the second date yielded significantly less than those from the first date. The data show that at the second date there was a sharp progressive decrease in yield with increased rates of application. Plots treated with the 2.0 pound rate yielded only 35 per cent of the check.

The sodium salt formulation showed practically no effect on yield, irrespective of rate or date of application. The marked difference in response of the ester and sodium salt formulations at different rates and dates is reflected in the high F values obtained for formulations, and for the simple and triple interactions involving formulations.

The effect of 2,4-D on the number of kernels per spike of barley are shown in Table VII. The ester formulation resulted in a marked decrease in fertility. As an average of the rates of ester applied the damage was more severe at the second than at the first date, and reduced fertility to 62 per cent of the check at the 2.0 pound rate.

The sodium salt showed practically no effect at either date for any rate.

Comparing Tables VI and VII, a close relationship between yield and the average number of kernels per spike is evident. The highly significant positive correlation between yield and the number of kernels per spike substantiates this observation.

	Calculated	5 Per Cent Point	1 Per Cent Point
Correlation coefficient	0.647	0.288	0.372

TABLE VI

EFFECT OF AN ESTER AND OF A SODIUM SALT OF 2,4-D ON THE AVERAGE YIELD OF BARLEY IN BUSHELS PER ACRE

	Rate 2,4-D Pounds Per Acre	Date of Application June 25	July 8	Mean
Ester	0.00	52.3	57.2	54.7
	0.50	45.2	34.4	39.7
	1.00	46.7	26.4	36.5
	2.00	38.4	19.9	29.2
Date Mean (Ester)		45.6	34.5	40.0
Sodium Salt	0.00	51.3	52.5	51.9
	0.50	54.2	53.8	54.0
	1.00	50.9	49.8	50.0
	2.00	45.1	52.9	49.0
Date Mean (Sodium Salt)		50.4	52.1	51.2
Date Mean (Ester and Sodium Salt)		48.0	43.3	

F VALUES AND NECESSARY DIFFERENCE

	Calculated	5 Per Cent Point	1 Per Cent Point	Necessary Difference at 5 Per Cent Point
Dates	2.62	10.13		
Formulations	87.53	4.07	7.27	
Rates	25.26	2.83	4.29	
Formulations x Rates	16.63	2.83	4.29	4.84
Formulations x Dates	28.66	4.07	7.27	
Dates x Rates	5.87	2.83	4.29	
Formulations x Rates x Dates	7.10	2.83	4.29	

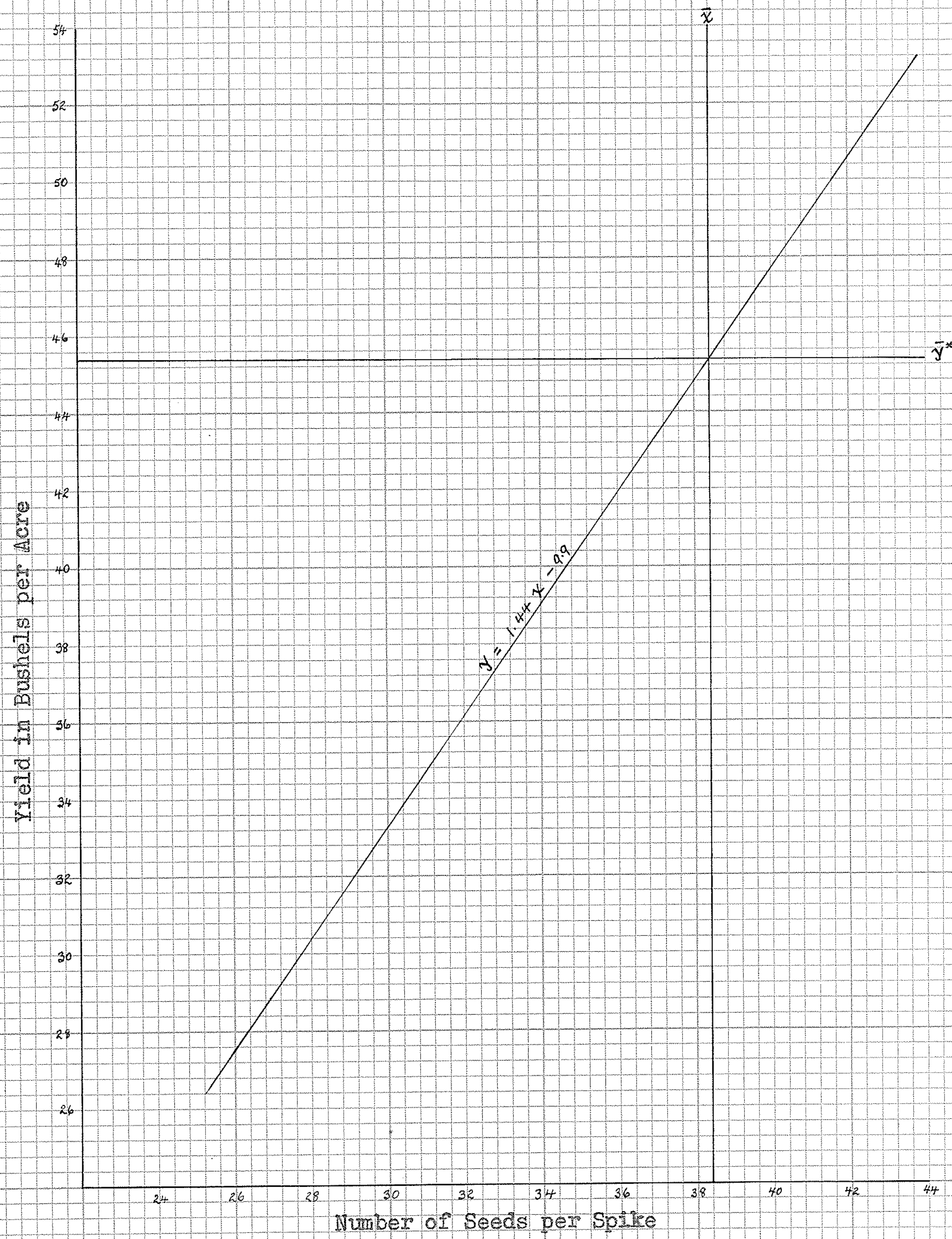
TABLE VII

EFFECT OF AN ESTER AND OF A SODIUM SALT OF 2,4-D ON THE AVERAGE NUMBER OF KERNELS PER SPIKE OF BARLEY

	Rate 2,4-D Pounds Per Acre	Date of Application June 25	July 8	Mean
Ester	0.00	41.6	40.4	41.0
	0.50	38.2	32.4	35.3
	1.00	35.5	35.7	35.6
	2.00	38.1	25.2	31.6
Date Mean (Ester)		38.3	33.4	35.9
Sodium Salt	0.00	43.8	42.9	43.4
	0.50	39.2	41.1	40.2
	1.00	40.5	39.5	40.0
	2.00	41.2	39.9	40.5
Date Mean (Sodium Salt)		41.2	40.8	41.0
Date Mean (Ester and Sodium Salt)		39.8	37.1	

F VALUES

	Calculated	5 Per Cent Point	1 Per Cent Point
Dates	3.89	18.51	
Formulations	41.72	4.20	7.64
Rates	10.82	2.95	4.57
Formulations x Rates	2.92	2.95	
Formulations x Dates	8.38	4.20	7.64
Dates x Rates	3.64	2.95	4.57
Formulations x Rates x Dates	3.57	2.95	4.57



*mean yield of first three replicates

Figure 4. Simple regression graph for yield of barley in bushels per acre on number of kernels per spike.

This relationship is demonstrated graphically in Figure 4.

Flax

At both dates of application all the rates of ester and the 1.0 pound rate of sodium salt caused drooping of the plants. Plots receiving the 1.0 pound rate of sodium salt recovered their normal stature. The 0.25 pound rate of ester resulted in slight twisting of plants, whereas, plants receiving the 0.5 and 1.0 pound rates of ester remained lodged or tangled, and matured ten days to two weeks later than the check plots. Numerous abnormalities resulted from the two higher rates of ester. There was considerable fasciation of branches or stems, and in some cases complete absence of floral organs (Figure 5). A branch with a pair of twin bolls was found, and there were many bolls with less, or more than the normal number of sepals. These are illustrated in Figure 6. Seeds from these were sown in the greenhouse. The resultant plants appeared normal.

Table VIII shows that there was a sharp progressive decrease in yield accompanying increases in the rate of application of the ester. The average yield of the 1.0 pound rate of ester was only 56 per cent of the check.

The sodium salt formulation showed practically no effect on yield at any rate. The differences in yield, due to date of application of the 2,4-D, were not significant at any rate for either formulation.

As an average of dates, all rates of the ester caused significant reductions in the number of seeds per boll, whereas, the sodium salt had practically no effect at any of the rates. From Table IX it may be noted that, as an average of formulations and rates, the first date of application of 2,4-D caused significantly more sterility than the second.

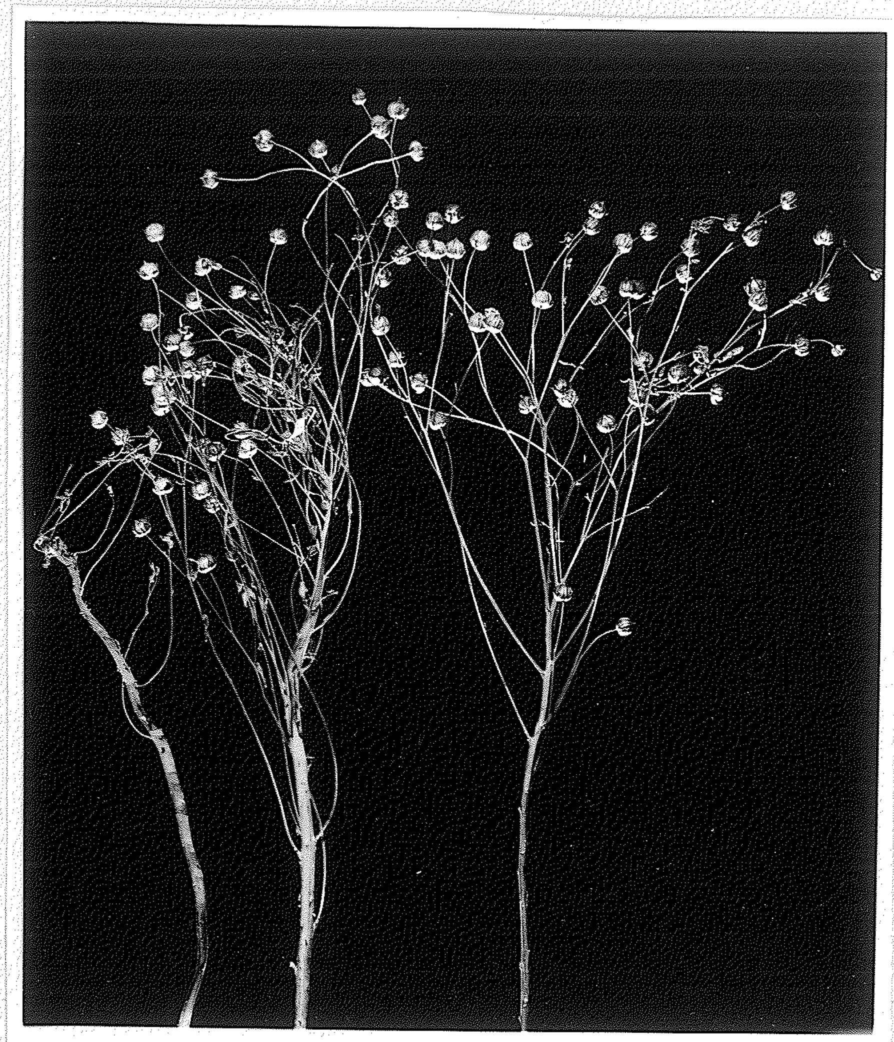
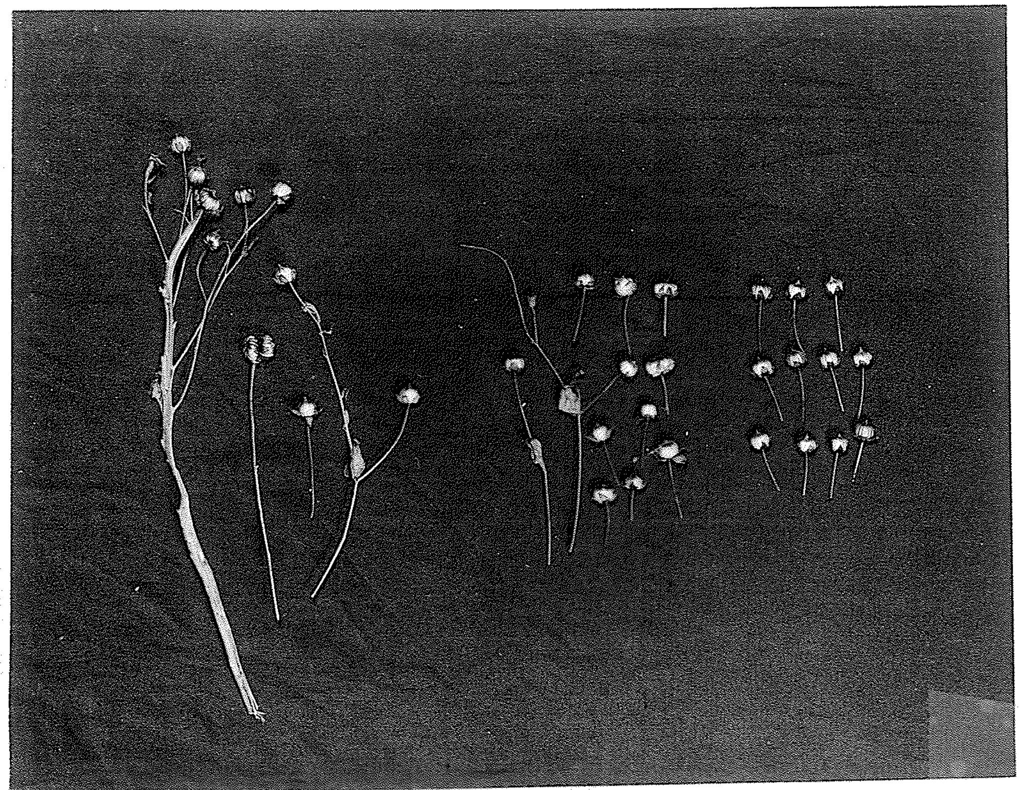


Figure 5. Fasciation of flax stems due to application of 2,4-D; normal plant at right.



a b c d e

Figure 6. Abnormalities produced as a result of 2,4-D application; normal seed bolls at right.

a - fasciated stem terminating in a deformed seed boll
b - twin seed bolls
c - four sepals
d - abnormal leaf
e - abnormal leaves, a deformed boll and seed bolls with two, four and six sepals.

TABLE VIII

EFFECT OF AN ESTER AND OF A SODIUM SALT OF 2,4-D ON THE AVERAGE YIELD OF FLAX IN BUSHELS PER ACRE

	Rate 2,4-D Pounds Per Acre	Date of Application June 25	July 8	Mean
Ester	0.00	28.2	30.6	29.4
	0.25	24.0	25.2	24.6
	0.50	19.1	21.0	20.0
	1.00	16.0	17.1	16.5
Date Mean (Ester)		21.8	23.5	22.6
Sodium Salt	0.00	29.1	30.0	29.5
	0.25	29.0	33.2	31.1
	0.50	27.9	31.1	29.5
	1.00	26.8	26.4	26.6
Date Mean (Sodium Salt)		28.2	30.2	29.2
Date Mean (Ester and Sodium Salt)		25.0	26.8	

F VALUES AND NECESSARY DIFFERENCE

	Calculated	5 Per Cent Point	1 Per Cent Point	Necessary Difference at 5 Per Cent Point
Dates	2.65	10.13		
Formulations	56.44	4.07	7.27	
Rates	16.06	2.83	4.29	
Formulations x Rates	6.77	2.83	4.29	3.52
Formulations x Dates	0.03	4.07		
Dates x Rates	0.40	2.83		
Formulations x Rates x Dates	0.40	2.83		

TABLE IX

EFFECT OF AN ESTER AND OF A SODIUM SALT OF 2,4-D ON THE AVERAGE NUMBER OF SEEDS PER BOLL OF FLAX

	Rate 2,4-D Pounds Per Acre	Date of Application		Mean
		June 25	July 8	
Ester	0.00	7.28	7.82	7.55
	0.25	6.00	7.28	6.64
	0.50	6.32	5.52	5.93
	1.00	6.12	6.52	6.33
Date Mean (Ester)		6.43	6.79	6.61
Sodium Salt	0.00	6.88	7.68	7.28
	0.25	7.38	7.15	7.26
	0.50	6.78	7.62	7.20
	1.00	6.98	7.38	7.18
Date Mean (Sodium Salt)		7.00	7.46	7.23
Date Mean (Ester and Sodium Salt)		6.72	7.12	

F VALUES AND NECESSARY DIFFERENCE

	Calculated	5 Per Cent Point	1 Per Cent Point	Necessary Difference at 5 Per Cent Point
Dates	10.15	10.13	34.12	
Formulations	17.48	4.07	7.27	
Rates	6.08	2.83	4.29	
Formulations x Rates	4.88	2.83	4.29	0.60
Formulations x Dates	0.14	4.07		
Dates x Rates	0.88	2.83		
Formulations x Rates x Dates	7.26	2.83	4.29	

The correlation coefficient between yield and number of seeds per boll was positive and significant.

	Calculated	5 Per Cent Point	1 Per Cent Point
Correlation coefficient	0.536	0.250	0.325

This relationship is shown graphically in Figure 7.

Treatment had no effect upon weight per measured bushel of wheat, oats, barley and flax. This is obvious from reference to Table X. Germination likewise was not affected by treatment, as indicated in Table XI.

2. The Effect on Crop Plants of Residual 2,4-D in the Soil

Although the plan was to keep the crops involved in this section weed-free, it was not practically possible to accomplish this during the earlier stages of the experiment.

As was indicated in materials and methods, one replicate in this series was not weeded. In this replicate treatment with the 1.0 pound rate of both the ester and sodium salt formulations gave effective weed control for a period of almost two months. Weed growth on the check plots was very profuse and consisted mainly of stinkweed (*Thlaspi arvense* L.), and Russian pigweed (*Axyris amarantoides* L.).

For alfalfa, flax, and millet, seeding at the second and third dates resulted in visible reductions in stand on the treated plots.

Due to shading, aphids, and other causes, the July 17 seeding of all crops, except corn, died.

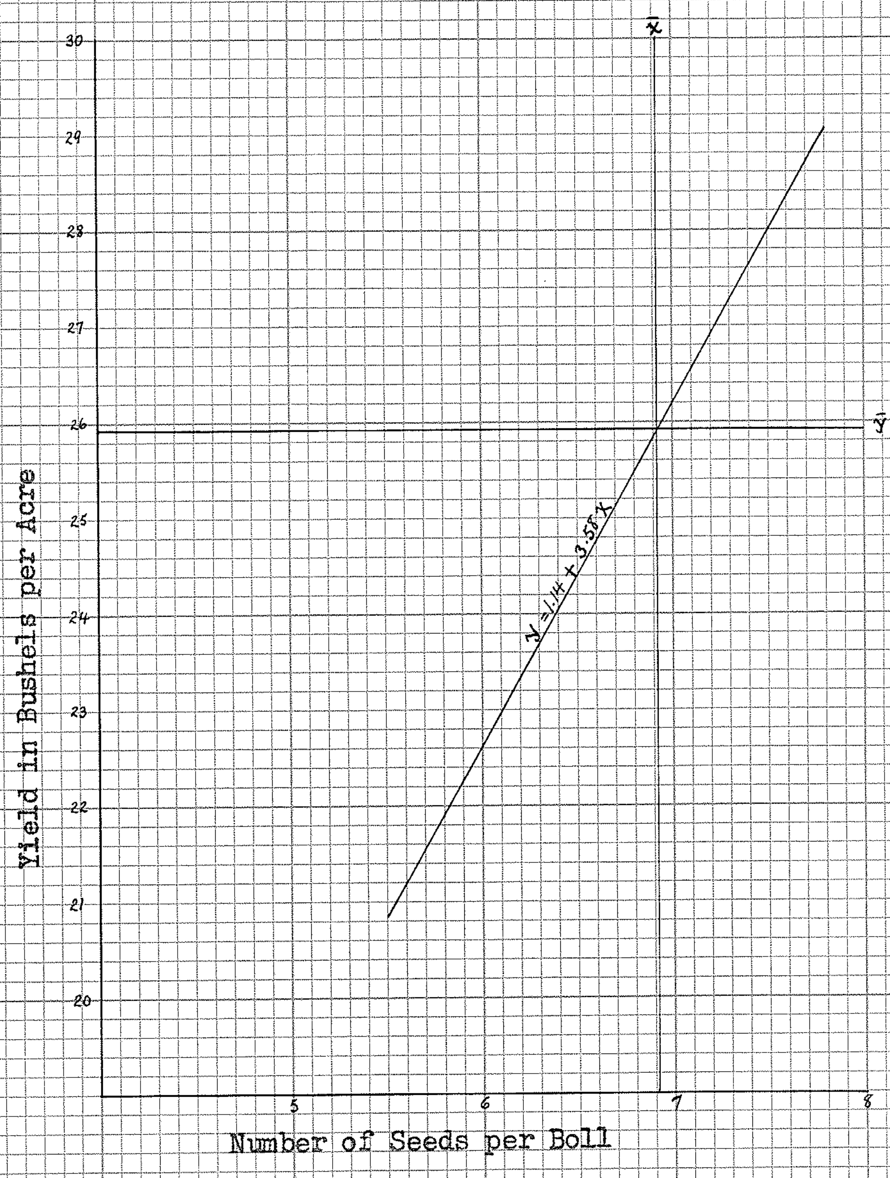


Figure 7. Simple regression graph for yield of flax in bushels per acre on number of seeds per boll.

TABLE X

EFFECT OF 2,4-D ON THE WEIGHT PER BUSHEL IN POUNDS FOR WHEAT, OATS, BARLEY AND FLAX

		Ester			Sodium Salt		
Crop	Rate	Date 1	Date 2	Mean	Date 1	Date 2	Mean
Wheat	0.00	58.5	58.0	58.2	58.0	58.0	58.0
	0.50	58.0	58.0	58.0	58.5	58.0	58.2
	1.00	59.0	58.5	58.8	58.0	58.0	58.0
	2.00	58.5	58.0	58.2	58.5	58.0	58.2
Mean		58.5	58.1	58.3	58.2	58.0	58.1
Oats	0.00	35.5	35.0	35.2	35.0	36.0	35.5
	0.50	35.0	36.0	35.5	35.5	35.5	35.5
	1.00	35.0	36.5	35.8	36.0	35.5	35.8
	2.00	35.5	35.0	35.2	35.0	36.0	35.5
Mean		35.2	35.6	35.4	35.4	35.8	35.6
Barley	0.00	42.5	43.0	42.8	41.5	41.5	41.5
	0.50	42.5	43.5	43.0	42.5	42.5	42.5
	1.00	41.5	43.0	42.2	42.5	43.5	43.0
	2.00	42.0	42.0	42.0	42.0	43.0	42.5
Mean		42.1	42.9	42.5	42.1	42.6	42.4
Flax	0.00	54.0	55.5	54.5	55.0	55.0	55.0
	0.25	54.0	53.5	53.8	54.5	54.5	54.5
	0.50	54.5	53.5	54.0	54.0	55.0	54.5
	1.00	53.5	54.5	54.0	55.0	54.0	54.5
Mean		54.0	54.2	54.1	54.6	54.6	54.6

TABLE XI

EFFECT OF 2,4-D APPLIED TO WHEAT, OATS, BARLEY AND FLAX ON PER CENT GERMINATION OF THE HARVESTED GRAIN

		Ester			Sodium Salt		
Crop	Rate	Date 1	Date 2	Mean	Date 1	Date 2	Mean
Wheat	0.00	78.0	82.0	80.0	78.0	83.0	80.5
	0.50	78.0	80.0	79.0	80.0	76.0	78.0
	1.00	79.0	78.0	78.5	76.0	80.0	78.0
	2.00	77.0	81.0	79.0	82.0	81.0	81.5
Mean		78.0	80.2	79.1	79.0	80.0	79.5
Oats	0.00	96.0	96.0	96.0	94.0	98.0	96.0
	0.50	95.0	94.0	94.5	93.0	96.0	94.5
	1.00	98.0	97.0	97.5	96.0	94.0	95.0
	2.00	100.0	95.0	97.5	94.0	96.0	95.0
Mean		97.2	95.5	96.4	94.2	96.0	95.1
Barley	0.00	94.0	93.0	93.5	98.0	93.0	95.5
	0.50	96.0	94.0	95.0	94.0	96.0	95.0
	1.00	95.0	95.0	95.0	93.0	94.0	93.5
	2.00	94.0	97.0	95.5	97.0	96.0	96.5
Mean		94.8	94.8	94.8	95.5	94.8	95.1
Flax	0.00	58.0	62.0	60.0	65.0	64.0	64.5
	0.25	59.0	63.0	61.0	66.0	65.0	65.5
	0.50	57.0	60.0	58.5	61.0	61.5	61.2
	1.00	61.0	65.0	63.0	63.0	63.0	63.0
Mean		58.8	62.5	60.6	63.8	63.4	63.6

Alfalfa

The effect of residual 2,4-D on the yield of alfalfa is presented in Table XII. The May 17 seeding of alfalfa on plots treated with the ester did not yield significantly lower than the check. The same date of seeding on plots treated with sodium salt showed a significant increase in yield. Significant reductions in yield occurred at both the second and third dates of seeding for both formulations. The reduction was greatest at the second date. The July 2 date of seeding, on plots treated with either formulation, yielded considerably more than the check. This increase in yield might be attributed to more efficient weed control on the treated plots, during the early stages of growth, and to the absence of any residual toxic effect on alfalfa forty-two days after treatment.

Table XIII reveals that, compared with the check, neither of the formulations caused any significant reduction in the number of nodules per plant.

Barley

The yield of barley from 2,4-D treated plots was not significantly lower than the yield from untreated plots. This indicates that there was no residual toxic effect of 2,4-D on barley at any time after the 2,4-D was applied.

TABLE XII

EFFECT OF RESIDUAL 2,4-D IN THE SOIL ON THE AVERAGE YIELD OF ALFALFA IN POUNDS OF DRY MATTER PER ACRE

	Rate 2,4-D Pounds Per Acre			
Date of Seeding	0.00	1.00 (Ester)	1.00 (Sodium Salt)	Mean
May 17	4186	3856	5158	4400
June 3	3709	1173	892	1924
June 16	2380	1455	951	1595
July 2	1044	1450	1662	1385
Treatment Mean	2830	1983	2166	

F VALUES AND NECESSARY DIFFERENCE

	Calculated	5 Per Cent Point	1 Per Cent Point	Necessary Difference at 5 Per Cent Point
Treatments	3.06	6.94		
Dates	68.43	3.16	5.09	
Dates x Treatments	10.45	2.66	4.01	8.71

TABLE XIII

EFFECT OF RESIDUAL 2,4-D IN THE SOIL ON THE AVERAGE NUMBER OF NODULES PER PLANT OF ALFALFA

	Rate 2,4-D Pounds Per Acre			
Date of Seeding	0.00	1.00 (Ester)	1.00 (Sodium Salt)	Mean
May 17	6.3	4.7	14.9	8.6
June 3	7.2	2.7	5.0	5.0
June 16	7.1	9.6	9.0	8.6
Treatment Mean	6.9	5.6	9.6	

F VALUES

	Calculated	5 Per Cent Point
Treatments	3.53	19.00
Dates	3.71	5.14
Dates x Treatments	3.43	4.53

TABLE XIV

EFFECT OF RESIDUAL 2,4-D IN THE SOIL ON THE AVERAGE YIELD OF BARLEY IN POUNDS OF DRY MATTER PER ACRE

Date of Seeding	Rate 2,4-D Pounds Per Acre			Mean
	0.00	1.00 (Ester)	1.00(Sodium Salt)	
May 17	7029	7549	7184	7254
June 3	7453	7030	5846	6776
June 16	3331	3742	4207	3760
July 2	912	1264	669	948
Treatment Mean	4681	4896	4476	

F VALUES

	Calculated	5 Per Cent Point	1 Per Cent Point
Treatments	0.42	6.94	
Dates	20.14	3.16	5.09
Dates x Treatments	0.23	2.66	

FLAX

At the first date of seeding, plots treated with the ester and sodium salt formulations yielded more than the check. Both formulations reduced yields significantly at the second date. A considerable, though not significant, reduction in yield resulted from both formulations at the June 16 date of seeding. As with alfalfa, there was no residual toxic effect upon flax forty-two days after treatment (Table XV).

TABLE XV

EFFECT OF RESIDUAL 2,4-D IN THE SOIL ON THE AVERAGE YIELD OF FLAX IN POUNDS OF DRY MATTER PER ACRE

Date of Seeding	Rate 2,4-D Pounds Per Acre 0.00	1.00 (Ester)	1.00 (Sodium Salt)	Mean
May 17	5608	5704	6986	6099
June 3	5869	3137	2029	3678
June 16	4231	3566	3360	3719
July 2	1290	2034	1814	1713
Treatment Mean	4249	3610	3547	

F VALUES AND NECESSARY DIFFERENCE

	Calculated	5 Per Cent Point	1 Per Cent Point	Necessary Difference at 5 Per Cent Point
Treatments	2.52	6.94		
Dates	43.60	3.16	5.09	
Dates x Treatments	6.39	2.66	4.01	1398

Millet

From Table XVI, it may be seen that the differences in yield between the checks and the treated plots were not significant. However, at the second and third dates of seeding the yields of the treated plots were less than those of the check in the case of both formulations, indicating that there was some toxic effect of the 2,4-D. The marked increases in yield for both formulations at the July 2 date of seeding may have been due to the two factors; more effective weed control on the treated plots, and absence of any residual toxic effects to the millet.

Corn

Table XVII shows that the 2,4-D treatments caused increases in the yield of corn at each date. These increases may have been due largely to more effective weed control on the treated than on the untreated plots.

After application of the 2,4-D on May 21, there was no heavy rain until June 3 (Table 1). The rain may have carried the 2,4-D into the soil, where germinating seeds could be affected more readily. This consideration might account for the fact that toxic effects of the 2,4-D became apparent on the susceptible crops at the second and third dates of seeding, but not at the first.

TABLE XVI

EFFECT OF RESIDUAL 2,4-D IN THE SOIL ON THE AVERAGE YIELD OF MILLET IN POUNDS OF DRY MATTER PER ACRE

	Rate 2,4-D Pounds Per Acre			
Date of Seeding	0.00	1.00 (Ester)	1.00 (Sodium Salt)	Mean
May 17	11224	12144	10524	11297
June 3	10829	10131	7647	9536
June 16	5934	4915	5280	5376
July 2	1703	3101	3680	2828
Treatment of Mean	7428	7573	6783	

F VALUES

	Calculated	5 Per Cent Point	1 Per Cent Point
Treatments	1.69	6.94	
Dates	55.95	3.16	5.09
Dates x Treatments	1.60	2.66	

TABLE XVII

EFFECT OF RESIDUAL 2,4-D IN THE SOIL ON THE AVERAGE YIELD OF CORN IN POUNDS OF DRY MATTER PER ACRE

	Rate 2,4-D Pounds Per Acre			
Date of Seeding	0.00	1.00 (Ester)	1.00 (Sodium Salt)	Mean
May 17	4280	6486	6114	5626
June 3	5667	6377	6340	6128
June 16	3252	5355	5558	4721
July 2	2565	3685	3933	3394
July 17	748	1086	1371	1068
Treatment Mean	3325	4598	4663	

F VALUES AND NECESSARY DIFFERENCE

	Calculated	5 Per Cent Point	1 Per Cent Point	Necessary Difference at 5 Per Cent Point
Treatments	21.35	6.94	18.00	650
Dates	80.36	2.78	4.22	
Dates x Treatments	1.41	2.36		

3. The Effect of 2,4-D on Soil Micro-organisms and on Soil Nitrates

As indicated in Tables XVIII and XIX, 2,4-D soil treatments had no significant effect on the numbers of bacteria and fungi in the soil.

From Table XX it is evident that the nitrate content of the soil was not influenced significantly by 2,4-D.

TABLE XVIII

EFFECT OF 2,4-D SOIL TREATMENT ON AVERAGE PLATE COUNTS OF BACTERIA

	Rate 2,4-D Pounds Per Acre	Date of Sampling June 12	July 29	Mean
Ester	0.00	41.92*	13.92	27.92
	2.00	36.17	12.67	24.42
	4.00	38.12	8.83	23.48
Date Mean		38.73	11.81	

* x 500,000 equals number of bacteria per gram of soil

F VALUES

	Calculated	5 Per Cent Point	1 Per Cent Point
Treatments	0.21	6.94	
Dates	24.16	5.99	13.74
Dates x Treatments	0.01	5.14	

TABLE XIX

EFFECT OF 2,4-D SOIL TREATMENT ON AVERAGE PLATE COUNTS OF FUNGI

	Rate 2,4-D Pounds Per Acre	Date of Sampling June 12	July 29	Mean
Ester	0.00	10.75*	5.54	8.14
	2.00	9.50	8.33	8.92
	4.00	11.12	7.71	9.42
Date Mean		10.46	7.19	

* x 5,000 equals number of fungi per gram of soil

F VALUES

	Calculated	5 Per Cent Point	1 Per Cent Point
Treatments	5.07	6.94	
Dates	23.67	5.99	13.74
Dates x Treatments	3.04	5.14	

TABLE XX

EFFECT OF 2,4-D SOIL TREATMENT ON NITRATE CONTENT OF SOIL

EXPRESSED AS MILLIGRAMS NITRATE PER 100 GRAMS OF OVEN DRY SOIL

Rate 2,4-D Pounds Per Acre		Depth			Mean
		0-6 inches	6-12 inches	1-2 feet	
Ester	0.00	2.74	1.94	1.08	1.92
	2.00	3.37	2.53	1.40	2.43
	4.00	3.09	1.37	0.87	1.78
Depth Mean		3.07	1.95	1.12	

F VALUES

	Calculated	5 Per Cent Point	1 Per Cent Point
Treatments	2.52	6.94	
Depths	5.46	3.88	6.93
Depths x Treatments	0.14	3.26	

SUMMARY

The experiments involved in this study were outlined in three sections:

1. The effect of 2,4-D applied to cereals and flax.
2. The effect on crop plants of residual 2,4-D in the soil.
3. The effect of 2,4-D on soil micro-organisms and on soil nitrates.

The butyl ester and sodium salt formulations of 2,4-D were compared in two of these. In the first, the applications were made at rates of 0.5, 1.0 and 2.0 pounds per acre to wheat, oats, and barley, and at 0.25, 0.5, and 1.0 pound to flax. The plants were eight to ten inches tall at the first date of application. At the second date, the cereals were just beginning to head and the flax was in bloom.

As an average of dates there were progressive decreases in the yield of all crops, accompanying increases in rate of application of the ester. All rates caused significant reductions in yield of barley and flax. Only the 1.0 and 2.0 pound rates reduced the yield of wheat and oats significantly. The effect of the ester on barley was more severe at the second than at the first date. For the other crops the response at the two dates was similar. Some abnormal wheat spikes were collected from plots treated at the first date with the 1.0 and 2.0 pound rates of ester. Flax plots which received the 0.5 and 1.0 pound rate at either date had many abnormal seed bolls. The progeny from seed of these abnormal wheat spikes and flax bolls were normal in appearance. The ester formulation reduced the fertility in all four crops and highly significant positive correlations between fertility and yield were obtained. Sodium salt did not affect significantly the yield of any of the crops at any rate or

date. The weight per measured bushel and germination of harvested grain were not affected adversely by 2,4-D.

The crops involved in the second section were alfalfa, barley, flax, millet and corn. Applications of the ester and sodium salt were made on May 21 at the rate of 1.0 pound per acre. The crops were sown on the treated soil and on untreated checks on five dates, May 17, June 3, June 16, July 2 and July 17.

The response was similar for both formulations. They did not reduce significantly the yields of any of the crops sown on the first date. Alfalfa, flax and millet showed reductions at the second and third dates of seeding. However, no residual toxic effect of 2,4-D was evident when the crops were sown forty-two days after treatment. Barley and corn were not adversely affected at any date. Nodulation of alfalfa was not inhibited or reduced.

Soil treatments did not influence the numbers of soil bacteria and fungi, and the amount of nitrates in the soil.

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