# THE EFFECT OF 2,4-DICHLOROPHENOXYACETIC ACID (2,4-D) ON THE DEVELOPMENTAL PROCESS IN OATS AND BARLEY

bу

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# THE EFFECT OF 2,4-DICHLOROPHENOXYACETIC ACID (2,4-D) ON THE DEVELOPMENTAL PROCESS IN OATS AND BARLEY

#### INTRODUCTION

Spraying of grain crops with 2,4-dichlorophenoxyacetic acid (2,4-D) for the control of weeds has become a widespread practice. In such spray operations the aim is to kill most or all of the weed species without seriously damaging the grain crop. While the cereal grains are generally considered resistant to 2,4-D, the resistance is relative, and consequently under certain conditions the cereal may be more or less affected. It has been recognized for some time that one of the most important factors determining the reaction of the cereal is the stage of growth of the crop at the time of 2,4-D application.

Previous research by a large number of workers has shown that barley is highly susceptible to damage from 2,4-D during two periods in its development. The first is an early seedling period extending from shortly after emergence until the plants are about five inches tall (general field level) and are in the early 3-leaf stage. The second susceptible period begins when the plants are well advanced into the boot stage, 12 to 14 days in advance of heading, and continues until spike emergence. Between these

two periods and following the last one, barley is generally highly resistant.

In oats the picture has been somewhat different. In general, the most susceptible stages have occurred from about the 3-leaf stage when the plants are six inches tall, and continuing until the early boot stage. However, the results with oats have been somewhat inconsistent. For this reason field experiments with oats were included in this investigation.

Considerable research has been done on establishing these vulnerable stages. The object has been to furnish a basis for recommending periods during which 2,4-D may be safely applied to the growing crop.

This investigation was undertaken in an effort to obtain fundamental information about these vulnerable stages, and possibly associate the resulting damage with the developmental process in barley and oats. In these experiments the stages of growth have been described both by means of the external morphological development of the plants and by dissection and micro-study of the growing point.

#### REVIEW OF LITERATURE

At the North Central Weed Control Conference held in Des Moines, Iowa, December, 1946, the necessity for information on the effects of 2,4-D on crop plants was stressed, and it was decided to include such investigations in the 1947 research program. As a result many investigators since 1947 have studied the toxic effect of the herbicide on growing crops. Some workers have stressed rates and formulation of 2,4-D in their experiments while others laid emphasis on studying the sensitivity of different varieties of cereals. Other workers have emphasized the stage of growth of the growing crop at time of treatment and compared the sensitivity of various stages. It soon became apparent that within rates recommended for economical weed control the limiting factor for cereal reaction was stage of growth of the crop at the time of 2,4-D application.

An extensive experiment involving the influence of stage of growth on the sensitivity of barley and wheat to 2,4-D was done by Olson, Zalik, Breakey, and Brown (24). They applied the ester formulation of 2,4-D at rates of 8 and 12 ounces per acre (aqueous solution) at 15 to 23 stages of growth starting before emergence of the crop and continuing at approximately three day intervals until after heading. In both crops there were two rather widely separated periods during which severe damage was done as measured by reduced yields. The first was an early seedling period when the plants ranged from  $\frac{5}{4}$  inch to 5 inches in height. The second

was a much later period, shortly before heading in barley and about 11 to 12 days before heading in wheat. Between these two widely separated periods little or no damage was done in either crop. Conspicuous morphological abnormalities were induced by the early stage treatments. These results were obtained at three widely separated stations in Manitoba and were in good agreement with each other.

Friesen (9, 10, 11, 12, 13, 14) applied different formulations of 2,4-D at varying rates up to 1.0 pound acid per acre to oats and barley at different stages of growth. His most significant results were obtained in experiments wherein the applications were made at 15 to 20 stages of growth starting before emergence and continuing at approximately three day intervals. His results agreed closely with those of Olson et al in showing two highly vulnerable periods in barley, one embracing seedling stages up to about six inches plant height, and a later period in an advanced boot to early pre-heading stage. In addition to yield reduction, leaf and spike abnormalities characterized the early period, and sterility the second or later period. Lodging and delayed maturity were further characteristic effects. Oats showed damage over periods corresponding to those in barley, and also at stages between them. In one year in particular the most severe yield reduction was caused by treatment applied during this intermediate period. Oats showed greater damage than barlev.

Another extensive piece of work involving the influence of stages of growth has been done by Andersen and Hermansen They applied a sodium salt of 2,4-D (also a sodium salt of 4K-2M) to barley and oats, twice a week to barley and once a week to oats. The rates of application were 1.0, 4.0 and 16.0 kg. per hectare (approximately 0.9, 3.5 and 14.0 pounds per acre). The treatments began in April when barley and oats were in the 2-leaf stage, and continued until shortly before heading. They found that barley showed the highest number of abnormalities and oats the lowest yield of grain. greatest number of culm abnormalities (tubular leaf, mainly) followed treatment applied immediately after sprouting. Spike abnormalities appeared in greatest numbers following treatment applied 8 days later in barley and 17 days later in oats, when the plants had respectively 3 and 4 to 5 leaves and the height was respectively 5 and 8 inches. The dates of greatest number of abnormalities and lowest yield were identical under the 2,4-D treatments. Oats and barley also showed a large decrease in yield when sprayed about a fortnight before heading.

Derscheid (8) treated an early and a late variety of barley with one pound acid equivalent of 2,4-D ester per acre at 9 stages of growth. The chemical was applied at 3-day intervals beginning at the 4 to 5-leaf stage, and growing points were dissected to determine the exact stage of growth at the time of treatment. The most susceptible period occurred before the 5-leaf stage, when the differentiation of tiller buds was inhibited. The number of tillers was reduced, the

number of spikes decreased and yield greatly depressed. A relatively tolerant period extended from the 5-leaf to the early boot stage. A relatively susceptible second period occurred between the pre-heading and late heading stages. Yield reductions were due to a decreased number of seeds, which the author states may have been the result of an inhibition of embryo sac and gamete development, or an increased vegetative competition as the result of stunting of the central culm. The most resistant period occurred in the postheading stage when relatively heavy application of 2,4-D did not decrease the yield. He noted that vegetative abnormalities occurred during the first susceptible period, while the arrangement of florets in the spike was affected at stages immediately following this. Blasted florets were caused by treatments in the pre-heading stages.

Leggett (21, 22) treated oats and barley at 3-day intervals with a butyl ester of 2,4-D at 8 ounces acid per acre. In barley serious yield reductions resulted from spraying at the 3-leaf, very late shot-blade, flowering, and early milk stages. In oats yield reductions took place when spraying had been done 21 to 36 days after seeding (4-leaf stage to early shot-blade stages).

Foster (15, 16, 17, 18) treated oats and barley at 64 stages of growth with 3 formulations of 2,4-D. Damage to barley was most severe during the early seedling stage when heading was delayed up to 13 days, deformed heads were common, and yields reduced. Spraying in the shot-blade stage delayed

heading from 1 to 5 days. Oats was reported damaged over a somewhat longer period. In 1949 treatments applied within 33 days after emergence caused a reduction in yield, delayed heading, and many sterile florets. The damage from the ester was emphasized. In 1950, 2,4-D had no noticeable effect on date of heading, deformities, or amount of blast present.

Shaw and Willard (28, 29) and Bernard and Willard (2) treated oats at 9 stages of growth with 2 formulations of 2,4-D. In 1948 no significant reduction in yield took place with rates up to 1.0 pound acid per acre, but in 1949 and 1950, highly significant injury was obtained at the 2-leaf stage (26 days after planting), the jointing stage, and the boot stage from all rates above 0.25 pounds acid per acre. The least injury was recorded at the late milk stage.

Robinson, Dunham, and Shulstad (25) treated Mindo oats with 2,4-D at two day intervals. Yields were reduced from every spraying through the full boot-stage, a period of 26 days, with reductions as high as 60 per cent at the fully tillered stage. Similar results were reported by Shafer (26, 27).

Carder (5) in 1950 applied two formulations of 2,4-D to Olli barley at 16 stages of crop growth. Yields were reduced with all rates up to 8 ounces acid per acre when treatments were made at emergence and again from the shot-blade to the early heading stage.

Davidson (6) in 1951 treated 5 varieties of oats with 2,4-D at 21 stages of growth, beginning before emergence and

continuing at 3 and 4 day intervals until 10 days before the grain had ripened. The results showed a gradual decrease in oat yields from shortly after emergence to early shot-blade stage together with deformed leaves prior to and including the 3-leaf stage; less straw strength from the 3-leaf to the early shot-blade stage; deformed panicles and uneven ripening between the 2- and 4-leaf stage; and blasting from the early shot-blade to the early heading stage. A similar experiment was carried out by Olson and Brown (23) in 1951, who recorded sharp yield reductions over a period of 15 to 18 days when the plants were advancing from 6 or 7 inches (4 to 5 leaves) to 13 or 14 inches (shot-blade stage).

Unrau and Corns (31) showed that meiotic irregularities occur in cereals if treated with 2,4-D at certain stages of growth. They applied 12 ounces acid equivalent per acre of 2,4-D ester at 3 day intervals to Stewart and Thatcher wheat and Olli barley. Treatments were begun at emergence and continued until heading time. Extreme meiotic irregularities were present in material sprayed on dates 4, 5, and 6, and some were also present in material from dates 9, 10, and later. Aberrations were of many types and the percentage of abnormal metaphases and anaphases ranged from 40.4 to 31.9 in the plants examined.

#### MATERIALS AND METHODS

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

- 1. The effect of 2,4-D on Montcalm barley applied at different stages of growth in the green-house.
- 2. The reaction of Ajax oats in the field to 2,4-D applied at different stages of growth.
- 3. The effect of 2,4-D on Ajax oats applied at different stages of growth in the greenhouse.

# 1. The Effect of 2,4-D on Montcalm Barley Applied at Different Stages of Growth in the Greenhouse

This experiment was conducted in the greenhouse during the winter of 1951.

Montcalm barley was sprayed with an isopropyl ester of 2,4-D at 32 stages of growth, beginning before emergence and continuing at one or two day intervals until well past the full heading stage. The first application was made on January 13, three days after seeding and one day before emergence; the last treatment on March 10, sixteen days after heading. The rate of 2,4-D applied was 12 ounces acid equivalent per acre in 120 gallons of water. Spraying was done with a small hand sprayer.

The stage of development of the main culm was recorded at each spraying date; both as to the external morphological development (height in inches, number of leaves, relation to heading, etc.), and by examination of the growing point.

The latter was done by taking representative plants at each spraying date and observing the stage of development of the growing point. Dissections and interpretation of stage of development were made as outlined by Bonnett (4). Photomicrographs were made of the various stages. This portion of the study was carried out on the main culms only.

At harvest time individual plants were studied carefully with regard to the incidence of various types of abnormalities following treatment at the different stages. Observations were made separately on the main culms and the later tillers and photographs were made of all the abnormalities studied.

Yields were determined separately for the main culms and tillers. Each plant, for the most part, had two tillers that produced a mature spike. The main culms and the tillers were harvested on April 6 and April 20 respectively.

Per cent sterility was determined by counting the sterile and fertile florets in each spike. This was also done separately for the main culms and tillers.

Observations on height of plants at maturity, number of days to heading, and number of days to maturity were made on the main culms only.

The experiment contained 32 treatments and 8 checks, randomized in each of 6 replicates. The plants were grown in 6 inch pots, and there were 3 plants in each pot. The soil used was a mixture of 4 parts Red River Clay (sterilized) to 1 part of sand.

Temperature was controlled in this experiment and kept near 75 degrees during the daytime and 60 degrees at night. Humidity, although not definitely controlled, remained constant near 65 per cent. Natural sunlight was supplemented with artificial light for 11 hours a day by means of four 100-watt bulbs placed four feet above the grain. The crop was watered when necessary.

# 2. The Reaction of Ajax Oats in the Field to 2,4-D Applied at Different Stages of Growth

Two separate experiments were conducted, one in 1950 at Headingly, the other in 1951 at Winnipeg. Both were carried out under field conditions.

The object of this investigation was to apply 2,4-D to Ajax oats at 20 stages of growth beginning between seeding and emergence and continuing at short intervals until past the heading stage. Accordingly, in 1950, 20 treatments of a commercially formulated isopropyl ester of 2,4-D was applied to Ajax oats at the rate of 8 ounces pure acid equivalent per acre. The first application was made on June 6, five days after seeding and six days before emergence; the last on August 11, eleven days after heading.

In 1951 the experiment was repeated using a commercially formulated butyl ester of 2,4-D, again at the rate of 8 ounces pure acid equivalent per acre. The first application was made on May 14, five days after seeding and four days before emergence; the last on July 19, nine days after heading. The interval between treatments was again 3 days (with variations

due to the weather).

A quadruple lattice design was used in both experiments, and the methods of statistical analysis employed were as described by Goulden (19).

Three-row plots eighteen and one-half feet long were used. Spacings between rows and plots was nine inches. Prior to harvest one foot was cut off each end of the plots. Yield determinations were based on the centre rod row. All three rows in each plot were treated and the chemical was applied with a knapsack sprayer. To increase the accuracy of the method of application an auxiliary tank, similar to the one described by Davis (7), was attached. The applications were in the form of a water solution at a volume of 60 gallons per acre under 30 pounds of pressure.

Data on weight per measured bushel, 1000-kernel weight, and number of days to maturity were obtained in addition to the yield data.

Through frequent cultivation all plots were kept essentially weed free. Field notes were taken as required.

# 3. The Effect of 2,4-D on Ajax Oats Applied at Different Stages of Growth in the Greenhouse

This experiment was conducted in the greenhouse during the winter of 1951-1952, and was similar to the study on barley referred to as section 1, page 9.

Ajax oats was sprayed with a butyl ester of 2,4-D at 30 stages of growth, beginning before emergence and con-

tinuing at two day intervals until well past the full heading stage. The first application was made on November 10, three days after seeding and two days before emergence; the last treatment on January 19, sixteen days after heading. The rate of chemical applied and the method of application was similar to the corresponding study on barley.

Studies on the development of the growing point, the incidence of abnormalities, yields, amount of tillering, maturity, and plant height, were carried out as described under the barley experiment (section 1, page 9) and by Bonnett (3). Again separate studies of the main culms and later tillers were made whenever possible. Per cent sterility for each treatment date was not calculated due to the difficulty encountered in distinguishing between sterile and fertile florets in oats. The main culms and tillers were harvested on January 29 and February 18 respectively.

The experiment contained 30 treatments and 6 check plots, randomized in each of 6 replicates. The plants were grown in 6 inch pots and there were 3 plants in each pot. Each plant for the most part had 3 tillers that produced a mature panicle. The soil used was a mixture 8 parts Red River Clay (sterilized), 2 parts of sand, and 1 part of peat.

Temperature was again controlled at 75 degrees in the daytime and 60 degrees at night. Humidity averaged 60 per cent over the duration of the experiment. Natural sunlight

was supplemented with artificial light for 11 hours a day by means of four 100-watt bulbs placed 4 feet above the grain.

For all experiments involved in this study and the corresponding study on barley, the methods of statistical analysis employed were as described by Goulden (19).

#### RESULTS AND DISCUSSION

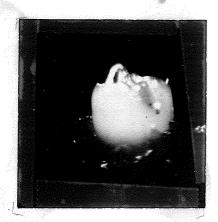
The experiments included in this investigation will be discussed separately in their respective sections.

## 1. The Effect of 2,4-D on Montcalm Barley Applied at Different Stages of Growth in the Greenhouse

Plates 1, 2, 3, 4, 5, and 6 show the stages of development of the growing point and spike at each spraying date.

The morphological stage of development (height in inches, number of leaves, relation to emergence and heading, etc.) of the barley crop is also given.

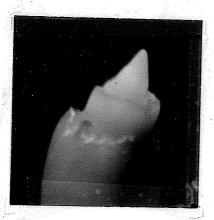
The development of the growing point as observed in this study could be divided into two phases. phase consisted of two stages; first, the growing point produced only leaf initials, and secondly, the undifferentiated portion of the growing point elongated in preparation for spike development. The first stage commenced with germination and continued until 4 days after emergence when the plants were  $5\frac{1}{2}$  inches tall and had 2 leaves (treatment date 5, Plate 2). The second stage continued until 8 days after emergence when the plants were 7 inches tall and had 3 leaves (treatment date 8, Plate 2). The beginning of the second phase was marked by the appearance of double ridges on the growing point (see treatment date 9, Plate 3), followed by the differentiation and development of the spike and its parts. The order of differentiation of the various parts of the spike as far as could be seen in this study



X24

TREATMENT DATE 1
3 days after planting, 1 day before emergence. Growing point showing production of leaf initials.

Results: Plants normal in all respects.



X24

TREATMENT DATE 3

2 days after emergence.

Plants 3 inches tall, 1-leaf stage. Growing point showing production of leaf initials.

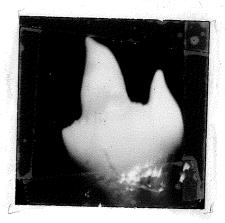
Results: Leaf deformities in the 7th leaf of the main culms. Tillers all normal.



X24

TREATMENT DATE 2

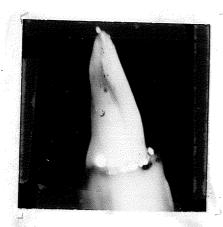
1 day after emergence.
Plants 1½ inches tall,
1-leaf stage. Growing
point showing production
of leaf initials.
Results: Leaf deformities
in the 6th leaf of the main
culm. Tillers all normal.



X24

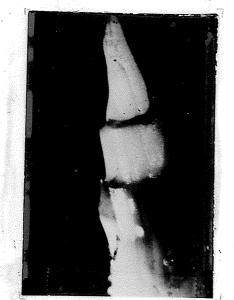
TREATMENT DATE 4

3 days after emergence.
Plants 4½ inches tall, 1-leaf stage. Growing point showing production of leaf initials.
Tiller primordia at right.
Results: Leaf deformities in 7th leaf and a few spike deformities in the main culms.
Tillers all normal.



X28

TREATMENT DATE 5
4 days after emergence.
Plants 5½ inches tall, 2leaf stage. Growing point
elongated.
Results: Some leaf deformities in the 8th leaf (check
plots only had 7 leaves) of
the main culms; also many
spike deformities in the main
culms. Only trace of damage
in tillers.



X28

TREATMENT DATE 7
6 days after emergence. Plants 6½ inches tall, 2-leaf stage. Elongated growing point shows slight depression near the tip. Results: All the main culms have spike deformities. The tillers have numerous leaf deformities.



X28

TREATMENT DATE 6
5 days after emergence. Plants 6 inches tall, 2-leaf stage. Growing point elongated but smooth in outline.

Results: All of the main culms showing spike deformities.

About one-half of the tillers showing leaf deformities.

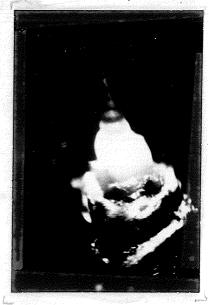


X40

TREATMENT DATE 8
8 days after emergence. Plants
7 inches tall, 2-leaf stage.
Growing point shows marked depression; upper part becomes the spike. Results: All the main culms have spike deformities. Many tillers have leaf deformities.



X12



X40

TREATMENT DATE 9 9 days after emergence. Plants 8 inches tall, 3-leaf stage. Spikelet differentiation visible. Results: All the main culms showed spike deformities. Numerous leaf deformities and some spike deformities in the tillers.

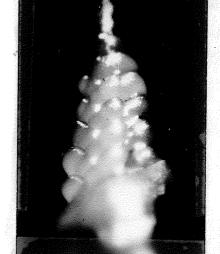
X40

TREATMENT DATE 10 11 days after emergence. Plants 11 inches tall, 3-leaf stage. Spikelet differentiation becoming more clear. Results: 75% of the spikes in the main culms abnormal. Few leaf deformities and many spike deformities in tillers.



TREATMENT DATE 11

13 days after emergence. Plants 12 inches tall, 3-4 leaf stage. Floret differentiation beginning. Results: A few spike deformities in the main culms. Spike deformities very numerous in the tillers.



X40

TREATMENT DATE 12

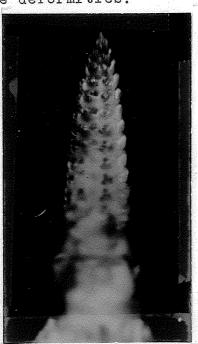
15 days after emergence. Plants 13 inches tall, 4-leaf stage. Floret differentiation quite Results: All the main clear. culms normal. All the tillers showed spike deformities.

X40

X24



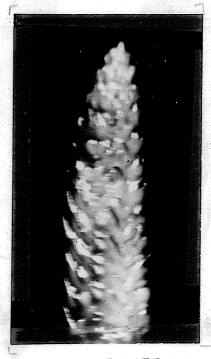
TREATMENT DATE 13
17 days after emergence.
Plants 15 inches tall, 4leaf stage. Young spike
moving upward in sheath.
Results: Main culms normal.
Tillers still showing many
spike deformities.



TREATMENT DATE 15

18 days before heading. Plants
17 inches tall, 5-leaf stage.

Lemma and palea clearly visible
in young florets. Results: Both
main culms and tillers normal
in all respects.



X40

TREATMENT DATE 14

19 days after emergence. Plants
16 inches tall, 5-leaf stage.

Young spike now ½ inch above ground level.

Results: Main culms normal.

Some tillers still showed spike deformities.



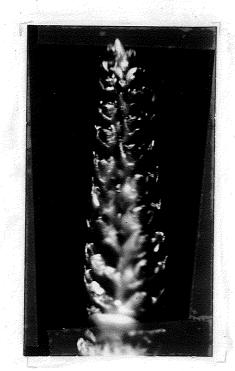
X24

TREATMENT DATE 16

16 days before heading. Plants
18 inches tall, 6-leaf stage.

Awns beginning to show.

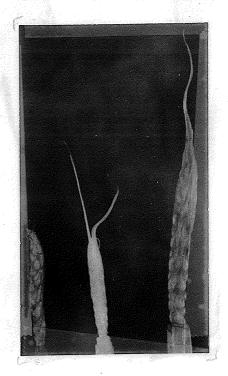
Results: Main culms and tillers
normal.



X16

TREATMENT DATE 17
14 days before heading. Plants
20 inches tall, 6-7 leaf stage.
Reproductive organs beginning
to form in the young florets.
Results: Considerable sterility in the main culms.
Tillers normal.





XA

(Early flag-leaf stage).
Left to right:
Treatment date 18.

12 days before heading. Treatment date 19.

11 days before heading. Treatment date 20.

9 days before heading.
Results: Main culms nearly completely sterile. Tillers appear normal in all respects.

At left: (flag-leaf stage). Treatment date 21.

7 days before heading. Treatment date 22.

6 days before heading. Treatment date 23.

4 days before heading.

Results: Main culms nearly completely sterile in all cases.

Tillers normal in all respects.



Left to right: Treatment date 24. 2 days before heading. Treatment date 25. Heading stage. Treatment date 26. 2 days after heading. Treatment date 27. 4 days after heading. Treatment date 28. 6 days after heading.

Results:

Main culms normal in all respects. A high degree of sterility in the tillers.

X.5



Left to right:

Treatment date 29.

8 days after heading.

Treatment date 30.

11 days after heading. Treatment date 31.

13 days after heading. Treatment date 32.

16 days after heading.

Results:

Main culms normal in all respects. The tillers still showed a high degree of sterility.

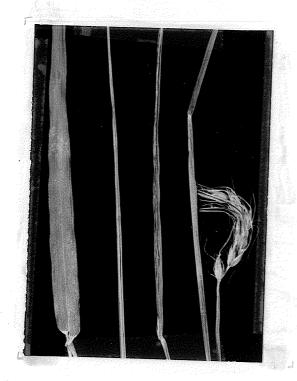
X.5

were: spikelet initials, glumes, lemma and palea, awns, and reproductive parts.

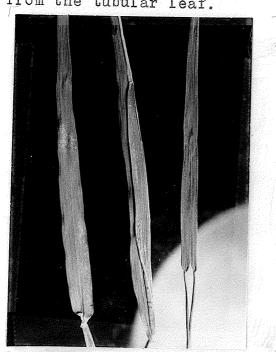
#### Effect of the Treatments on Abnormalities

The pots that were treated just before emergence produced culms and tillers that were normal in all respects. Those that were treated 1, 2, 3, and 4 days after emergence showed leaf deformities on all or nearly all of the plants. The most conspicuous of these were tubular or onion-like leaves; others showed fusion of two leaves, leaves with double midribs and supernumerary leaves. The spikes emerged with difficulty from many of these leaves, and many were completely sterile. The tillers produced in these pots were quite normal in all respects. The growing point during this period was showing tiller primordia. Plates 7 and 8 show the various types of leaf or vegetative deformities studied.

Beginning with the pots treated 4 days after emergence, spike abnormalities began to appear on the main culms. They appeared in greatly increased numbers following the next treatment, and in those treated 5, 6 and 8 days after emergence, all the main culms showed spike abnormalities. These consisted of branched spikes, supernumerary spikelets, tweaking (elongated internodes and opposite spikelets) twisted and twinned kernels, etc. The last of these deformities appeared in the pots treated 11 days after emergence. The first visible evidence of spike differ-



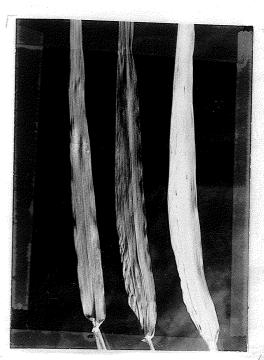
Leaf deformities, left to right:
Normal leaf.
Onion-like, or tubular leaf.
Onion-like leaf.
Incomplete heading resulting from the tubular leaf.



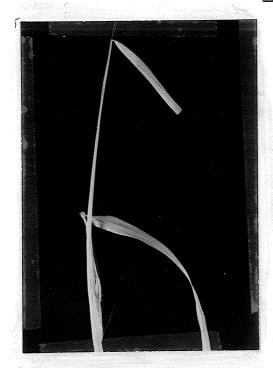
Leaf deformities, left to right:
Normal leaf.
Two leaves fused.
Two leaves fused.



Leaf deformities: left to right Incomplete heading due to onion-like leaf, spike fertile. Incomplete heading due to onion-like leaf, spike sterile.



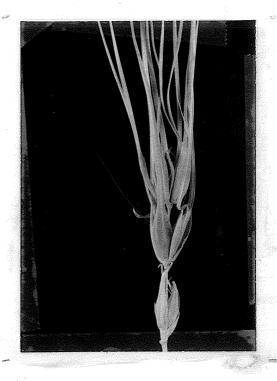
Leaf deformities, left to right: Normal leaf. Leaf showing double midrib. Leaf showing double midrib.



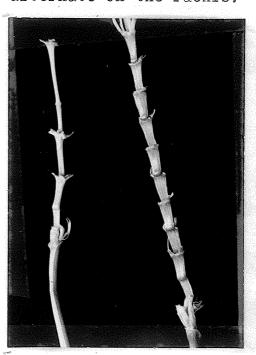
Leaf deformity:
Plant having a supernumerary leaf. Spike failed to emerge.



Spike deformity:
Spike of barley showing elongated rachis internode and spikelets opposite instead of alternate on the rachis.



Spike deformity:
Spike of barley showing elongated rachis internodes and spikelets opposite instead of alternate on the rachis.



Spike deformity:

At left is the rachis of the spike illustrated above showing the elongation of the rachis internodes. Normal rachis at right.

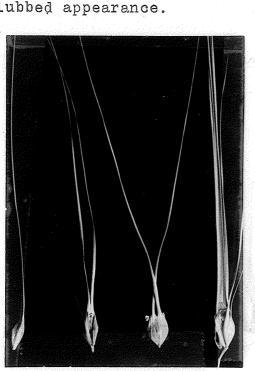
entiation appeared in the pots treated 8 days after emergence. From here on spike differentiation became quite clear. In the meantime leaf deformities began to appear on the tillers. Their appearance on the tillers, therefore, coincided with their disappearance on the main culms. The tillers began to show spike deformities in the pots treated 8 days after emergence. Plates 8, 9, 10, and 11 show the various types of spike deformities studied.

After the ninth treatment date, the interval between treatments was extended to two days instead of one. The pots receiving the eleventh, twelfth, thirteenth, fourteenth, and fifteenth treatments produced main culms that were normal in all respects. During this period the plants advanced from a height of 12 inches (stretched) to 18 inches. By this time the spike was one inch above the ground level and lemmae and anthers were beginning to form. The tillers on the pots treated at this stage still showed some spike deformities.

Beginning with the sixteenth treatment date some sterility was induced in the main culms. This increased, becoming complete or practically so in the pots receiving the eighteenth treatment and continuing through the twenty-third treatment which was applied 4 days before heading. This corresponds to early stages of development in floral parts, anthers, ovary, style and stigma and continuing until fertilization has taken place. The tillers at this time remained normal. From the twenty-fourth treatment, applied 2 days before full heading through the final or thirty-second



Spike deformity:
Spike of barley showing supernumerary spikelets at each node resulting in a clubbed appearance.



Spike deformity: left to right:
Normal barley kernel and awn.
Barley kernel with two awns.
Fusion of two lemmas.
Barley kernel with many awns,
some partly fused.



Spike deformity:
Spike of barley showing supernumerary spikelets at one node only.

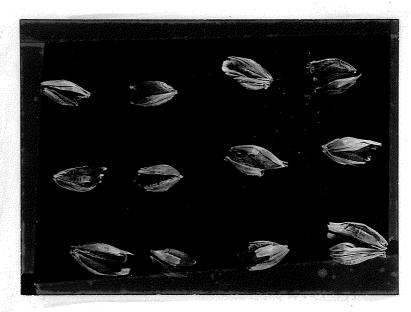


Spike deformity:

Barley spikes showing twisting of the necks. Spike at
left appears normal; at right,
is badly deformed.

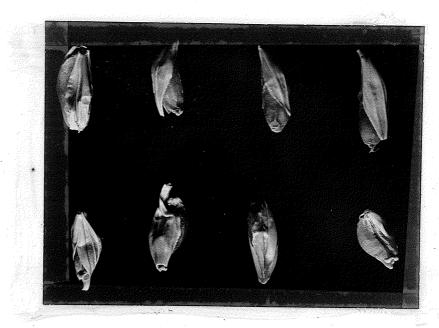


Spike deformity, left to right:
Supernumerary spikelets.
Branched spike.
Triple spike.



Spike deformity:

Twinned or double kernels
resulting from fused lemmas and
other spike deformities.



Spike deformity:
Twisted kernels resulting from or caused by spike deformities.



Spike deformity:
Dehulled or naked kernels resulting from 2,4-D treatment.

treatment no sterility was induced in the main culms. During this period the treatments caused a high degree of sterility in the tillers. Plate 12 shows two types of sterility caused by 2,4-D treatment at these stages.

Figure 1 shows the per cent of main culms and tillers exhibiting the various types of abnormalities studied.

Figure 2 shows per cent sterility induced by the 2,4-D treatments.

These results agree well with those of Derscheid (8), who reported that vegetative abnormalities of constricted sheath and incomplete heading were prevalent on plants treated before floral initiation occurred; whereas, spike abnormalities consisting of tweaked spike, double spike, and multiple spikelets were most prevalent on plants treated during the period of floral initiation and development. Blasted florets were most common on plants treated near heading time due to an inhibition of embryo sac and gamete development.

### Effect of Treatment on Yield

In order to relate the yields to the internal development of the growing point at the time of treatment and to the visible abnormalities and sterility induced by the treatments, separate harvests were made of the main culms and tillers.

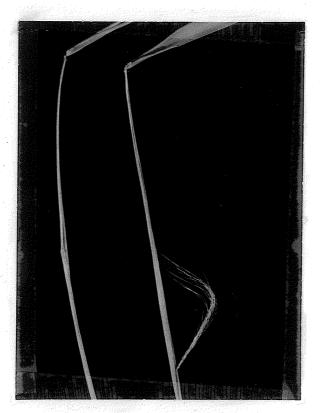
Sharp yield reductions in the main culms took place as a result of the treatments applied during about a 4 day

#### COMPLETE STERILITY

Left to right:
Normal spike of barley.

Spike of barley morphologically normal but completely sterile due to 2,4-D treatment.





## COMPLETE STERILITY

Spikes that are completely sterile and not emerged due to 2,4-D treatment. Samples taken at maturity. Spike at right partially removed to show its position relative to the flag-leaf.

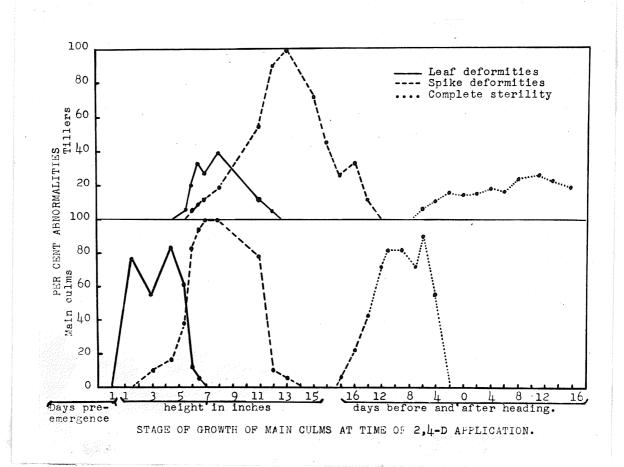


Figure 1. Graphs showing the percentage of abnormalities in barley treated with 2,4-D at 32 stages of growth with 12 ounces (isopropyl ester) pure acid equivalent per acre (Greenhouse, 1951).

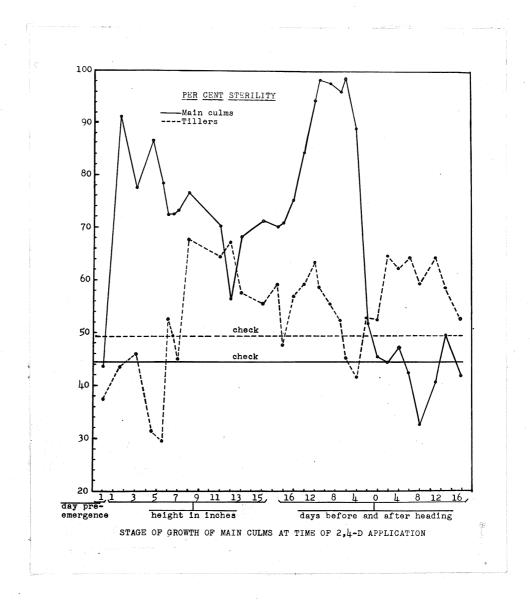


Figure 2. Graphs showing the percentage of sterility in barley treated with 2,4-D (isopropyl ester) at 32 stages of growth with 12 ounces pure acid equivalent per acre (Greenhouse, 1951).

period after emergence. This was during the single leaf stage and corresponded to the period when leaf abnormalities were being caused. Difficulty in spike emergence resulted from these treatments and much sterility was apparent in these spikes. Some reduction also took place following the treatments applied during the fifth to the ninth days after emergence, extending over the two into the three leaf stage, a period during which spike abnormalities were being induced. The greatest yield reduction in the tillers took place in the pots treated during this latter period. Evidently the tillers were in the same stage of development during this period as the main culms were during the preceding one.

The greatest yield reduction in the main culms took place in the pots treated 14 to 4 days before heading.

This was the period during which virtually complete sterility was induced in the spikes produced on the main culm. Reduction in yield of tillers occurred over a period of several days immediately following, corresponding to a very high degree of sterility in the tillers. Actually the reduction in yield of tiller produced grain was slight, even though the percentage of sterility was high. For some reason the spikes produced on the tillers were longer than those on the untreated checks, so that the net number of kernels produced was as great or greater than in the case of the checks.

When the combined yields of main culms and tillers were determined it was found that the greatest reduction took place in the pots treated from the fifth to ninth days after emergence, corresponding exactly with the period when spike deformities were induced in the main culms and leaf deformities in the tillers. The reduction at the late preheading stages was somewhat less in degree, and corresponded with the period of greatest reduction in the main culm yields. Table 1 shows the yields for the main culms, tillers, and total yield. The same data are presented graphically in Figure 3. The stage of growth on the abscissa refers to the main culm only.

Applying these results to field conditions the degree of yield reduction, and the range of the periods of yield reduction would depend upon the extent to which environment influenced tiller production. If conditions were unfavourable for tiller development, so that most plants consisted of single main culms, sharp yield reductions would be expected as a result of treatment soon after emergence, and some reduction by later treatments during the two-leaf and early three leaf stages. If, on the other hand, conditions favoured tiller development, there might be little or no reduction resulting from the earlier treatments in the single leaf stage, because the tiller yields would offset the damage caused to the main culm. There would be a sharp yield reduction from treatments applied in the two- and early

TABLE 1

Yields in bushels per acre of the main culms, tillers, and total yield in barley treated with 2,4-D (isopropyl ester) at 32 stages of growth with 12 ounces pure acid equivalent per acre. (Greenhouse experiment, 1951).

Treatment date	Main culms	Tillers	Total
1 2 3 4 5 6 7 8	25.1 3.9* 9.4* 5.8* 10.3* 14.5* 14.6* 13.8*	27.0 29.2 30.2 33.9 32.8 13.0 16.7 17.8	52.1 33.1 39.6 39.7 43.2 27.5* 31.3*
9 10 11 12 13 14 15 16	11.3* 14.2* 20.4 14.4* 12.5* 13.3* 12.4* 11.1*	12.4 19.3 15.5 26.1 23.9 24.2 21.9	23.7* 33.5 35.9 40.5 36.3 37.5 34.3 36.8
17 18 19 20 21 22 23	6.8* 2.5* 0.7* 1.0* 1.9* 0.6* 5.4* 20.9	27.7 25.6 31.8 33.2 28.7 31.7 33.9 18.5	34.5 28.1* 32.5* 34.2 30.6* 32.3* 45.3 39.4
25 26 27 28 29 30 31 32	23.5 26.8 20.9 25.1 29.9 26.8 19.5 24.3	24.0 19.4 20.2 21.2 26.1 24.6 20.7 18.1	47.5 46.2 41.1 46.3 56.0 51.4 40.1 42.3
Mean of check: L.S.D. 5% level	23.8 6.6	20.4	44.2 11.7

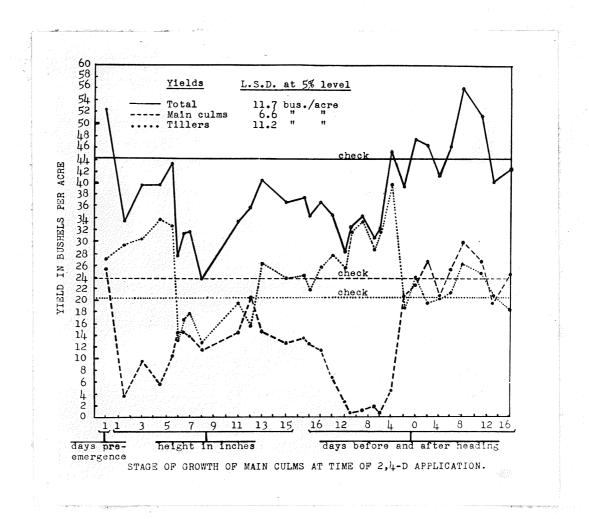


Figure 3. Graphs showing the yields of the main culms, the tillers, and the total yield in barley treated with 2,4-D (isopropyl ester) at 32 stages of growth with 12 ounces pure acid equivalent per acre (Greenhouse, 1951).

three-leaf stages because the damage here would be a combination of the lesser main culm damage and the severe tiller damage.

The reduction in yield resulting from treatment in the pre-heading stages would be very sharp if there were little or no tiller development, and would probably be greater than the seedling stage reduction, because of the very high degree of sterility induced by treatments at these stages. Favourable conditions for tillering would reduce the apparent damage during this period and would extend its range.

Actually, damage in the field has usually taken place during late pre-heading stages.

Figure 4 presents graphically the data on days to heading, days to maturity and height of crop at time of harvest. The straight lines represent the performance of the controls for the respective factors. These data refer to the main culms only. The graph shows two widely separated periods at which some delay in heading and maturity was induced by 2,4-D treatment and these correspond to the stages at which low yields were recorded in the main culms. Heading was delayed by 2 to 5 days when treatment was made within 11 days after emergence and 3 to 8 days when treatment was made over a period from 21 to 4 days in advance of heading. The maximum delay in heading occurred when treatment was made 9 days in advance of heading. Maturity was delayed at essentially the same stages but the maximum of 6 days

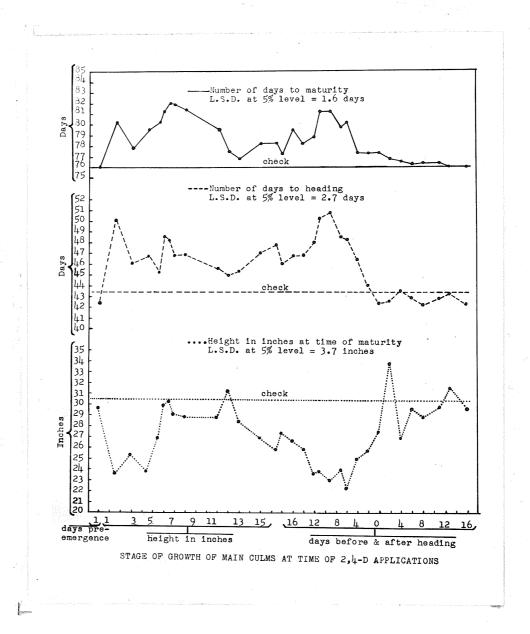


Figure 4. Graphs showing the number of days to heading, the number of days to maturity, and the height at maturity of barley treated at 32 stages of growth with 2,4-D (isopropyl ester) at 12 ounces pure acid equivalent per acre (Greenhouse, 1951).

was reached when treatment was made 6 days after emergence. Shorter straw resulted from treatments at 1 to 3 days after emergence, and again from 12 to 4 days in advance of heading. This also corresponds to the stages of lowest yields in the main culms.

Andersen and Hermansen (1) determined the length of the developing spikelet at each spraying date. On May 4, their second spraying date with barley, when the height of the plant was 13 cm., the length of the spike was 0.48 mm.

Treatment on this date caused the greatest number of spike abnormalities under the 4-kg application of 2,4-D, and also the greatest reduction in yield. Shortly before heading there was another susceptible period which Andersen and Hermansen considered to be the time when sex cells began to develop. Commenting on these results the authors state, "The fact that the occurrence of abnormalities depends on the stage of development of the plant seems to indicate that the differentiation of cells forming the first rudiment of an organ may be profoundly affected by hormone derivatives given at a certain early date."

The greenhouse study reported herewith fully supports this theory. When leaf initials were undergoing development in the growing point treatment with 2,4-D resulted in a high degree of damage to the leaves. When the growing point had begun to elongate in preparation for spike differentiation and continuing until spike differentiation had become quite

clear, treatment with 2,4-D resulted in extensive damage to the spikes. Damage in the pre-heading stages occurred during the differentiation of anthers and stigma and early growth of the floral parts. A high degree of sterility was associated with the damage that took place at this time. This indicates that when the different plant organs are in their meristematic condition they can be profoundly affected by 2,4-D treatment.

Derscheid (8), (whose work was published after the present investigation was begun), treated barley at 9 stages of growth. He found damage at the early stages to be associated with the laying down of floral primordia either in the main culms or some of the tillers. at the stages just prior to heading resulted during antheses. As the tillers developed somewhat later it was found that damage here resulted somewhat later which corresponded to the stages of growth in the main culms at which damage occurred, namely while floral primordia were laid down and again during antheses. The work of Derscheid emphasizes the importance of stage of development in relation to 2,4-D injury. It seems quite evident from his work that differentiating flower primordia are particularly susceptible to 2,4-D, and that 2,4-D has some detrimental effect on fertilization during antheses. He states that during the post-heading period there is no differentiation of meristematic tissue and the application of 2,4-D does not reduce yields.

# 2. The Reaction of Ajax Oats in the Field to 2,4-D Applied at Different Stages of Growth

The pertinent data obtained are presented in Tables 2, 3, 4, and 5. The yields for both years are plotted as a function of stage of growth in Figure 5, with the points on the abscissa adjusted so that any one point represents the same stage of morphological development for both curves.

In both years highly significant yield reductions occurred as a result of treatment at the 7 and 9 inch stage. In 1950, treatment at the  $4\frac{1}{2}$  inch stage also depressed the yield below the level of significance. results were highly consistent for the 2 years (see Graph, Figure 5). The most vulnerable stage occupied a period of only 11 days in 1950 and 6 days in 1951. Damage was done at stages other than that emphasized above. In both years the 3 spraying dates following the 9 inch stage showed decreased yields. The degree of reduction, however, was relatively small. Serious lodging was associated with these treatment dates. The overall yield reductions, therefore, resulted from spraying when the plants were advancing from the 4 to 5 leaf stage to the early boot stage. This corresponds to the first period of resistance in barley.



#### TABLE 2

Effect of spray applications of 2,4-D (isopropyl ester) on oats. Treatments made in 1950 at 3-day intervals (beginning at pre-emergence and continuing past the heading stage). Chemical applied at the rate of 8 ounces acid equivalent per acre.

Date of applica-tion	Stage of growth	Abnormalities	Yield bu, per acre
June 6	pre-emergence/	Normal.	41.3
10	pre-emergence <sup>≁</sup>	Trace of onion-like leaves.	46.9
13	2 inches	Many onion-like leaves. Erect growth.	37.0
16	$2\frac{1}{2}$ inches	Many onion-like leaves. Very erect growth.	36.9
20	3 inches	Many onion-like leaves. Erect.	44.6
24	4 inches	Many onion-like leaves. Few panicle deformities.**	41.8
27	4½ inches	Few onion-like leaves. Many panicle deformities.	34.8*
30	5 inches	Trace of onion-like leaves. Many panicle deformities, severe lodging.	39.3
July 4	7 inches	Severe lodging. Panicle deformities.	35.2*
8	9 inches	Severe lodging. Few panicle deformities.	30.2*
- 11	12 inches	Severe lodging.	40.0
14	13 inches	Only slight lodging.	44.6
18	16 inches	Normal.	40.7
21	10 days b.h***	Normal.	46.6
25	7 days b.h***	Normal.	48.3
29	3 days b.h***	Normal.	46.7
Aug. 1	heading	Normal.	46.0
5	4 days a.h****	Normal.	45.5
8	7 days a.h****	Normal.	42.4
11	10 days a.h ****	Normal.	47.8
Averag	e yield of check	plots	46.3
L.S.D.	at 5 per cent le	vel	11.08

Five and nine days after seeding.Difference significant at 5% level.

<sup>\*\*</sup> Clustering of the panicle, non-split glumes, blasting, basal sterility.

<sup>\*\*\*</sup> Days before heading.
\*\*\* Days after heading.

# TABLE 3

Effect of spray application of 2,4-D (butyl ester) on oats. Treatments made in 1951 at 3-day intervals (beginning at pre-emergence and continuing past the heading stage). Chemical applied at the rate of 8 ounces acid equivalent per acre).

	te of ication	Stage of growth	Abnormalities	Yield bu. per acre
May	14 17 21	pre-emergence/ pre-emergence/ l inch,1 leaf	Normal Normal Few onion-like leaves	130.8 121.8 119.8
	25	3 inches, 2 leaves	Onion-like leaves. Erect growth.	130.0
May	30	4 inches, 3 leaves	Onion-like leaves. Erect growth.	119.4
June	2	5 inches, 3 leaves	Onion-like leaves. A few panicle de- formities.**	118.8
	5	6 inches, 4 leaves	Onion-like leaves. Panicle deformities.	117.6
	9	7 inches, 4 leaves	Panicle deformities. Badly lodged.	88.8*
June	12	9 inches, 4-5 leaves	Panicle deformities. Lodged badly.	85.0*
	15	10 inches, 5-6 leaves	Badly lodged.	109.0
	19	13 inches, 6 leaves	Slightly lodged.	103.4
	22	15 inches, 6-7 leaves	Normal.	107.7
June	27	13 days before heading	Normal.	1.23,9
	30	10 days before heading	Normal.	131.8
July	3	7 days before heading	Normal.	112.3
	6	4 days before heading	Normal.	113.2

# TABLE 3 (continued)

Effect of spray application of 2,4-D (butyl ester) on oats. Treatments made in 1951 at 3-day intervals (beginning at pre-emergence and continuing past the heading stage). Chemical applied at the rate of 8 ounces acid equivalent per acre).

Date of application	Stage of growth	Abnormalities	Yield bu. per acre
July 10	heading	Normal.	130.1
13	3 days after heading	Normal.	117.6
16	6 days after heading	Normal.	118.5
19	9 days after heading	Normal.	118.8
Average yie	eld of check plots		124.0
ш.Б.D. at 5	per cent level		21.52

f 4 and 7 days after seeding.

<sup>\*</sup> Difference significant at 5% level.

<sup>\*\*</sup> Clustering of the panicle, non-split glumes, blasting, basal sterility.

### TABLE 4

Effect of spray application of 2,4-D (isopropyl ester) on the bushel weight, 1000-kernel weight, and number of days to maturity of oats. Treatments made in 1950 at 3-day intervals. Chemical applied at the rate of 8 ounces acid equivalent per acre.

Date applica		Bushel weight	1000-kernel weight grams	Seeding to maturity days
June	6	35.5	27.75*	95
	10	36.5	26.00	95
	13	34.1*	26.40	95
	16	32.5*	26.29	95
June	20	34.3*	25.29	97*
	24	33.4*	26.56	9 <b>7*</b>
	27	31.4*	26.11	99*
	30	33.6*	25.75	99*
July	4	32.5*	25.54	99*
	8	33.0*	25.78	97*
	11	34.8*	25.25*	97*
	14	37.3	26.25	97*
July	18	36.6	26.25	95
	21	36.6	26.60	95
	25	37.0	27.00	95
	29	36.5	25.25*	95
Aug.	1	37.0	26.00	95
	5	36.8	26.25	95
	8	35.6	25.00*	95
	11	36.5	26.78	95
Ave	rage of	checks 36.9	26.32	95
L.S	.D. at	5% level .70	1.05	0.0

 $<sup>\</sup>neq$  For stage of growth see Table 2.

<sup>\*</sup> Significantly different from checks at 5 per cent level.

# TABLE 5

Effect of spray application of 2,4-D (butyl ester) on the bushel weight, 1000-kernel weight, and number of days to maturity of oats. Treatments made in 1951 at 3-day intervals. Chemical applied at the rate of 8 ounces acid equivalent per acre.

	te of Buication	ushel weight	1000-kernel weight	Seeding to maturity
-		pounds	grams	days
May	14 17 21 25	35.9 36.1 37.5 36.3	25.13 26.50 25.83 25.45	96.5 97.0 97.5 98.0*
May June	30 2 5 9	34.6* 34.5* 36.1 33.8*	26.44 27.50 25.95 25.48	98.5* 99.5* 99.5* 100.5*
June	12 15 19 22	35.6 34.9* 35.0* 37.9	26.13 24.96 26.94 26.38	97.5* 96.5 98.0* 97.0
June July	27 30 3 6	38.1 37.5 37.6 37.5	26.24 26.53 26.78 26.31	97.0 97.0 97.0 97.0
July	10 13 16 19	36.4 36.3 36.0 37.1	26.31 23.80 25.84 26.55	98.0* 96.5 96.5 96.5
Aver	age of check	s 36.7	25.74	96.5
L.S.I	). at 5% leve	1.68	·	1.12

 $<sup>\</sup>neq$  For stage of growth see Table 3.

<sup>\*</sup> Significantly different from checks at 5 per cent level.

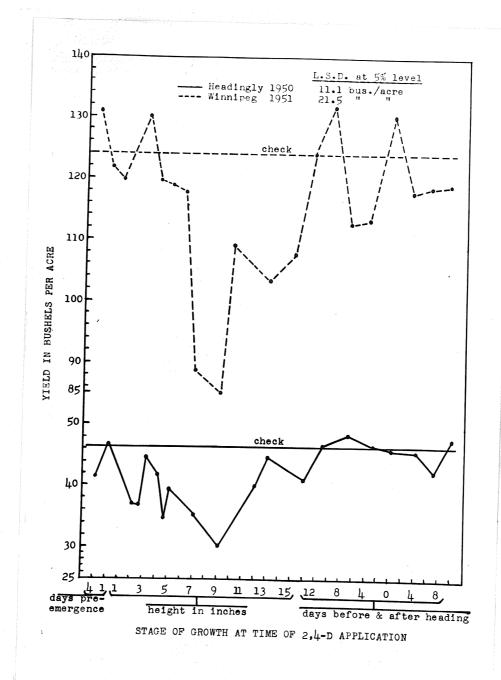


Figure 5. Graphs showing the yield of oats under treatment with 2,4-D applied at 20 stages of growth. Chemical applied at 8 ounces pure acid equivalent per acre of isopropyl ester and butyl ester in 1950 and 1951 respectively (Field experiments).

The results reported by Andersen and Hermansen (1), Friesen (12, 14), Leggett (22), Robinson, Dunham, and Shulstad (25), Olson and Brown (23), Shaw and Willard (29), and Davidson (6), reference to which has already been made in the literature review, agree closely with those reported here.

The occurrence of abnormalities in oats paralleled closely those in barley with the exception that the onionlike leaves appeared over a longer period of time. In oats the onion-like leaves resulted from treatment within 18 days after emergence of the crop while in barley no onion-like leaves were observed when treated 9 days after emergence. Following the onion-like leaves, abnormalities of the panicle were produced. Clustering of the panicle, non-split glumes, blasting, and sterility were common. The plots showing panicle deformities were also severely lodged in most cases. The period in which the panicles were deformed and severe lodging occurred coincided with the period of lowest yields. The decreases in yield, as previously reported, therefore appear to be due to the combined effect on the panicle and on the weakening of the straw. The abnormalities noted in oats are listed and described briefly for the respective stages in Tables 2 and 3.

Andersen and Hermansen (1) observed tubular leaves in oats resulting from treatment up to 17 days after

sprouting when the plants had 4 to 5 leaves and were 8 inches in height. Following this period panicle abnormalities occurred. Similar observations are reported by Friesen (12, 14), Davidson (6), Robinson, Dunham, and Shulstad (25), and Leggett (22). Slife and Fuelleman (30) suggest that the lodging resulting in oats is due to a decrease in the size of the root system caused by 2,4-D treatment.

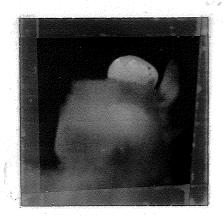
The bushel weight, 1000-kernel weight, and number of days to maturity are given in Tables 4 and 5. In 1950 bushel weight was significantly decreased by all treatments from emergence to the 12 inch stage (treatment dates June 13 to July 11). In 1951 all treatments applied from the 4 inch stage (May 30) to the 13 inch stage (June 19), with the exception of treatment dates June 5 and June 12, caused a significant decrease in bushel weight. In 1950, 1000-kernel weight was significantly increased at treatment date June 6 (treated 5 days after seeding) and significantly decreased at treatment dates July 11. July 29. and August 8 (12 inch stage, 3 days before heading, and 7 days after heading respectively). In 1951 there was no significant difference in the 1000-kernel weight at any of the treatment dates. Maturity was delayed from 2 to 4 days in 1950 at treatment dates June 20 to July 14 inclusive (3 inch stage to 13 inch stage), and from 1 to 4 days in 1951 at treatment dates May 25 to June 19 inclusive

(3 inch stage to 13 inch stage). The results regarding maturity are therefore nearly identical for the two years.

# 3. The Effect of 2,4-D on Ajax Oats Applied at Different Stages of Growth in the Greenhouse

Plates 13, 14, 15, 16, 17, and 18 show the stages of development of the growing point and panicle at each spraying date. The morphological stage of development (height in inches, number of leaves, relation to emergence and heading, etc.) of the oat crop is also given.

The growing point of oats, like that of barley, passes through two stages in its development. In the first stage the growing point remains short, the leaf initials differentiate, leaves grow, and tiller buds develop in the axils of the leaves at the base of the stem. Treatment dates 1 to 9 inclusive (Plate 13, 14, and 15), show this first stage. During the second stage the internodes of the stem elongate, and the branches, spikelets, and flower parts differentiate and develop. Previous to the differentiation of the branches of the panicle, the growing point elongates (see treatment date 11, Plate 15), but the elongation is not as marked as in barley. Panicle formation is first indicated by the appearance of single, lateral, alternate projections arising beneath the apex of the growing point (see treatment date 16, Plate 16). Spikelets differentiate from the tips of the branch primordia, and the empty glumes are the first of the spikelet parts to differentiate. Flower parts differentiate in the following order: 1emma, anthers, palea, lodicules, and pistil.

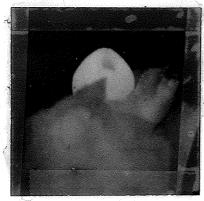


X32

X32

TREATMENT DATE 1
3 days after planting.
2 days before emergence.
Growing point showing production of leaf initials.
Results: Main culms and
tillers normal in all respects.

TREATMENT DATE 2
Coleoptile just emerging.
Growing point showing production of leaf initials.
Results: Numerous leaf deformities in main culms.
Tillers normal in all respects.



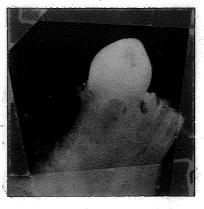
X32

TREATMENT DATE 3

2 days after emergence.

Plants 1½ inches tall, 1leaf stage. Growing point showing production of leaf initials. Results: All the main culms showing leaf deformities. Tillers

all normal.



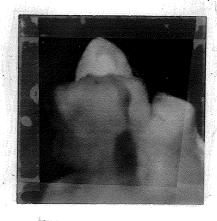
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TREATMENT DATE 4

3 days after emergence.
Plants 2½ inches tall, 1leaf stage. Growing point
showing production of leaf
initials. Results: Nearly
all of the main culms showing leaf deformities. A
trace of leaf deformities
in the tillers.

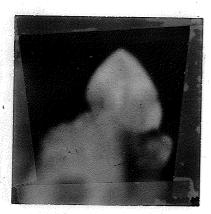


TREATMENT DATE 5
5 days after emergence.
Plants 3½ inches tall, 12 leaf stage. Growing
point showing production
of leaf initials and tiller primordia. Results: All
of the main culms showed
leaf deformities. A few
leaf deformities in the
tillers.



X32

TREATMENT DATE 6
7 days after emergence.
Plants 5 inches tall, 2-leaf stage. Growing point showing production of leaf initials and tiller primordia.
Results: All the main culms and about one-half of the tillers showed leaf deformities.



X32

TREATMENT DATE 7

9 days after emergence.

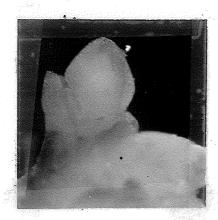
Plants 5½ inches tall, 2—
leaf stage. Growing point
showing production of leaf
initials. Results: All the
main culms and two-thirds
of the tillers showing leaf
deformities.



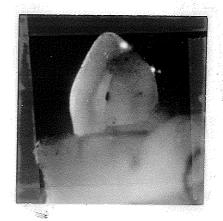
X32

TREATMENT DATE 8

11 days after emergence.
Plants 6 inches tall, 2-leaf stage. Growing point showing leaf initials and tiller primordia. Results: Nearly all of the main culms and tillers showing leaf deformities.



X32



X32

TREATMENT DATE 9
14 days after emergence.
Plants 8 inches tall, 3-leaf stage. Growing point showing production of leaf initials.
Results: Nearly all of the main culms and tillers showing leaf deformities. The more advanced main culms showed panicle deformities.

TREATMENT DATE 10
15 days after emergence.
Plants 9 inches tall, 3-leaf stage. Growing point beginning to elongate.
Results: A few leaf deformities and many panicle deformities in the main culms. Many tillers showing leaf deformities.



X32



X32

TREATMENT DATE 11
17 days after emergence.
Plants 10 inches tall, 3-leaf stage. Growing point elongated. Tiller primordia still visible. Results: Many panicle - and a few leaf - deformities in the main culms. Many leaf- and a few panicle- deformities in the tillers.

TREATMENT DATE 12

19 days after emergence.
Plants 11 inches tall, 3-leaf stage. Small depressions evident on the growing point.
Results: Many panicle - but no leaf deformities in the main culms. Many leaf and panicle deformities in the tillers.



X32



X32

TREATMENT DATE 13
21 days after emergence.
Plants 12 inches tall, 4-leaf
stage. Growing point elongated.
Small depressions visible.
Results: Only a trace of panicle deformities in the main culms. Many leaf and panicle deformities in the tillers.

TREATMENT DATE 14
23 days after emergence.
Plants 13½ inches tall, 4leaf stage. Panicle differentiation quite evident.
Results: Main culms normal
in all respects. Many panicle
deformities in the tillers,
also a few leaf deformities
in the tillers.



X32



X32

TREATMENT DATE 15
26 days after emergence.
Plants 16 inches tall, 4-leaf stage. Panicle differentiation quite evident.
Results: Main culms normal in all respects. Tillers showing some leaf- and many panicle deformities.

TREATMENT DATE 16
29 days after emergence.
Plants 17 inches tall, 4-leaf stage. Branching of the panicle beginning as single, lateral, alternate projections.
Results: Main culms normal in all respects. Tillers showing a trace of leaf- and many panicle- deformities.



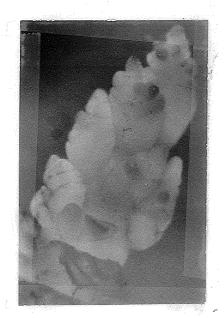
X32



X32

TREATMENT DATE 17
21 days before heading.
Plants 18 inches tall, 5-leaf stage. Branching of the panicle quite evident. Empty glumes becoming visible.
Results: Main culms normal in all respects. Many panicle deformities in the tillers.

TREATMENT DATE 18
19 days before heading.
Plants 21 inches tall, 5leaf stage. Branching of the second order beginning.
Results: Considerable sterility in the main culms. Some panicle deformities in the tillers.

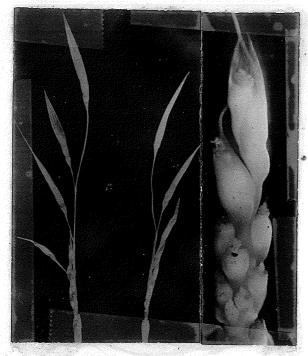


X32

X32

TREATMENT DATE 19
16 days before heading.
6-leaf stage. Spikelet formation quite evident.
Results: High degree of sterility in the main culms.
Trace of panicle deformities in the tillers.

TREATMENT DATE 20
13 days before heading.
7-leaf stage.
Results: A high degree of sterility in the main culms. Also some sterility in the tillers.



**X**8

# Right to left:

Treatment date 21.

10 days before heading.
7-8 leaf stage.
Results: High degree of sterility in the main culms.
Some sterility in tillers.
Treatment date 22.

7 days before heading.
Early flag-leaf stage.
Results: Some sterility in both the main culms and tillers.
Treatment date 23.

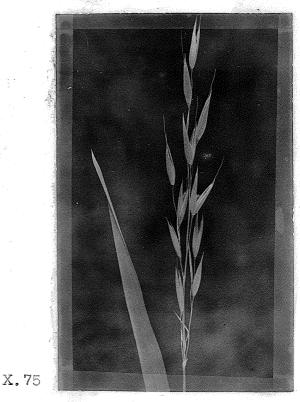
5 days before heading. Flag-leaf stage.
Results: A high degree of sterility in the tillers.
Main culms normal.



X2

Right to left:
Treatment date 24.

3 days before heading.
Treatment date 25.
Heading stage.
Results: Main culms normal.
Some sterility in tillers.



Treatment dates:
26 to 30 inclusive, 4 to
16 days past heading.
Results: Main culms and
tillers normal in all
respects.

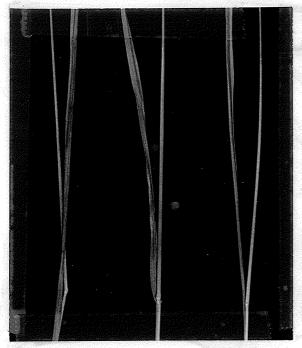
X.5

#### Effect of the Treatments on Abnormalities

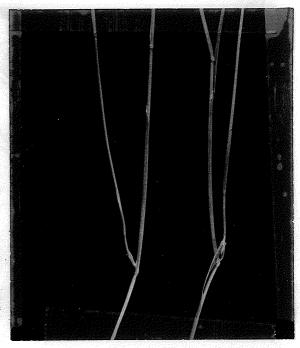
The pots that were treated before emergence produced culms and tillers that were normal in all respects. Those that were treated 1 to 15 days after emergence (treatment dates 2 to 10 inclusive) showed leaf or vegetative deformities on all or nearly all of the plants. The most conspicuous of these were tubular or onion-like leaves; others showed aerial tillering, twisted stems, and supernumerary leaves. The panicles emerged with difficulty from many of these leaves, and many were completely sterile. The growing point during this period was showing only leaf initials and tiller primordia were evident on plants 3 days after emergence. The tillers produced these deformities in pots sprayed 5 to 26 days after emergence of the main culm. Plate 19 shows the various types of leaf or vegetative deformities studied.

Beginning with the pots treated 14 days after emergence, panicle abnormalities began to appear on the main culms. They appeared in greatly increased numbers following the next 2 treatment dates. These consisted of basal and central sterility, non-split glumes, and branching at the uppermost node. The last of these deformities appeared in the pots treated 21 days after emergence. The first visible evidence of panicle differentiation appeared in the pots treated 17 days after emergence, and from here

# Vegetative Abnormalities Induced by 2,4-D Treatment



Tubular or onionlike leaves.



Incomplete heading due to the onion-like leaves, (normal panicle at right)



Twisted stem caused by the onion-like leaves.



Aerial tillering.

on panicle differentiation became quite clear. The tillers began to show panicle deformities in the pots treated
17 days after emergence, and their appearance continued
until the nineteenth treatment date (treated 36 days after
emergence). Plate 20 shows some of the panicle abnormalities studied.

The pots receiving the fourteenth, fifteenth, and sixteenth treatments produced main culms that were normal in all respects. During this period the plants advanced from a height of  $13\frac{1}{2}$  inches (stretched) to 17 inches and had 4 to 5 leaves. At this time the panicle was one inch above the ground level and the empty glumes were beginning to form. Except for a few panicle deformities, the tillers were normal in pots receiving the eighteenth and nineteenth treatment.

Beginning with the seventeenth treatment date some plants showed complete sterility in the main culms. This increased, and in pots receiving the twentieth and twenty-first treatment, over 50 per cent of the panicles on the main culms were completely sterile. This period corresponds to early stages in the development of the floral parts, and continues until fertilization has taken place. From the twenty-fourth treatment date, applied 3 days before heading, through the final or thirtieth treatment date no sterility was induced in the main culms. Following treatment dates 20 to 26 inclusive (13 days before head-

# Panicle Abnormalities Induced by 2,4-D Treatment



Basal Sterility. (normal panicle at right)



Central Sterility. (normal panicle at right)



Multiple spikelets



Complete sterility. (normal panicle at right)

ing to 4 days after heading) a high degree of sterility was recorded for the tillers.

Table 6 shows the percentage of plants exhibiting the various types of abnormalities in both the main culms and tillers. It can be seen that no treatment date resulted in both main culms and tillers being normal in all respects.

# Effect of the Treatments on Yield

In order to relate the yields to the development of the growing point at the time of treatment and to the visible abnormalities induced, separate harvests were made of the main culms and tillers as was done in the case of barley.

In the main culms significant yield reductions took place as a result of all treatments applied between emergence and heading. However, two periods stand out as being particularly low in yield. The first extends from emergence to 14 days after emergence (seedling leaf stage to the early 3-leaf stage) and corresponds to the period when leaf abnormalities were being caused. Difficulty in panicle emergence with the resulting high degree of sterility account for the exceptionally low yields during this period. The second period includes treatment dates 16 to 21 (late 4-leaf stage to early flag-leaf stage) and corresponds to the early stages in the development of the floral parts, at which time many panicles showed complete sterility.

# TABLE 6

The percentage of oat plants exhibiting various types of abnormalities due to 2,4-D treatment. Chemical applied at the rate of 12 ounces pure acid per acre, at 30 different stages of growth.

Treat-	Leaf	đe:	formities	Panicle	deformities	Complete	sterility
ment date	Ma: cu	in Ims	Tillers	Main culms	Tillers	Main culms	Tillers
1	•				*	-	
2		6.7	Name .		<del></del>	•	
3	100	0.0	economic (			-	****
4		4.4	4.3	-	_		-
5		0,0	17.6	-			
6		0.0	46.9		***	. <del>-</del>	
7		0.0	64.1	page.			
8 .		4.4	69.0	_	****	Name	
9		2.2	75.7	22.2	_	_	_
10		3.9	55.3	72.2	****		_
11	22	2.2	53.6	77.8	10.7	appiere	
12		-	44.7	38.9	26.3	-	_
13			44.4	11.1	28.9		
14		-	16.7		57.1		_
15	-	-	26.7	<del></del>	50.0	and and	21
16	-	-	10.0		52.5		
17	-		5.3	****	55.3	5.6	
18	***	-			20.9	27.8	
19	-	-	****	dom:	10.2	38.9	4.1
20	-	-	-		7.5	61.1	22.5
21		-	-	*****	4.8	55.6	23.8
22	-	-		-	· auto	33.3	22.6
23	-	-	- HELPAN		_	5.6	42.0
24	-	-		-	·	·	18.9
25	-			_	سناه	-	4.9
26	-	-			com	-	4.7
27	_	-		-		-	1.6
28	-	•		****	all the second		
29	-	-		<del>-</del>		-	
30	-	-		-			<del></del>

Significant reductions in tiller yields occurred as the result of treatment from emergence to 4 days past heading. Again two periods showed somewhat lower yields than the immediately preceding and immediately following periods. The first was represented by treatment dates 5 to 11 inclusive, and corresponds to the period when leaf deformities were being caused in the tillers. The second period included treatment dates 20 to 25 inclusive, and corresponds to the period when many panicles showed complete sterility in the tillers.

When the combined yields of main culms and tillers were determined it was found that again highly significant yield reductions took place in the pots treated from emergence to 4 days past heading. Again the greatest reductions occurred at two widely separated periods. first period included treatment dates 5 to 8 inclusive (plants  $3\frac{1}{2}$  to 6 inches tall) and corresponds to the period when both the main culms and tillers showed low yields due to leaf abnormalities. The second period included treatment dates 20 and 21 (13 and 10 days before heading, and prior to the shot-blade stage) and corresponds to a period when both the main culms and the tillers showed exceptionally low yields because of extensive sterility. Table 7 shows the yields for the main culms, tillers, and total yield for each treatment date. The stage of growth at time of treatment date is also given.

# TABLE 7

Effect of spray application of 2,4-D (butyl ester) on the yield of oats. Treatments made at 30 stages of growth at the rate of 12 ounces pure acid equivalent per acre.

Treatment	Stage of	Yields		
date	growth	Main culms	Tillers	Total
1	pre-emergence	12.1	38.6	50.7
2	\frac{1}{2} \text{ inch, 1 leaf} \\ \frac{1}{12} \text{ ", 1 leaf} \\ \frac{1}{2} \text{ ", 1 leaf} \\ \frac{1}{2} \text{ ", 1 leaf} \\ \frac{3}{12} \text{ ", 1-2 leaves} \end{array}	2.8*	23.5*	26.3*
3	$1\frac{1}{2}$ ", $1$ leaf	0.5*	17.7*	18.2*
4	$2\frac{1}{2}$ ", l leaf	0.2*	20.1*	20.4*
5	$3\frac{1}{2}$ ", 1-2 leaves	0.7*	11.7*	12.4*
6	5 ", 2 leaves	0.2*	12.0*	12.2*
7	5를 " , 2 leaves	3.3*	14.8*	18.0*
8	6 ", 2 leaves	2.7*	10.7*	13.4*
9	8 ", 3 leaves	3.8*	16.2*	%O.0S
10	9 ", 3 leaves	6.Q*	12.7*	18.7*
11	10 " , 3 leaves	5.4*	13.4*	18.7*
12	11 " , 3 leaves	6.6*	18.4*	25.0*
13	12 " , 4 leaves	6.3*	18.7*	25.0*
14	$13\frac{1}{2}$ ", 4 leaves	6.5*	22.9*	29.4*
15	16 ", 4 leaves	5.2*	16.4*	21.5*
16	17 " , 4 leaves	2.6*	18.0*	20.6*
17	18 " , 5 leaves	3.3*	17.5*	20.8*
18	21 ", 5 leaves /	1.7*	19.7*	21.4*
19	6 leaf, 16 days b.h.	4.3*	20.3*	24.6*
20	7 ", 13 " "	3.6*	13.2*	16.8*
21	7-8 " , 10 " "	1.6*	15.3*	16.9*
22	8 11, 7 11 11	3.8*	20.2*	24.0*
23	8 ", 5 " "	6.1*	20.6*	26.7*
24	8 ", 3 " "	7.6*	18.2*	25.8*
25	heading stage	11.6	15.7*	27.1*
26	4 days past heading	10.8	22.0*	32.9*
27	8 11 11 11	13.9	32.3	46.2
28	11 " " "	11.3	28.7	40.0
29	13 " " "	9.2	38.5	47.7
30	16 " " "	9.6	38.1	47.7
Average	e yield of check pots	11.8	<b>3</b> 3.3	45.1
L.S.D.	at 5% level	3,35	8.98	9.32

<sup>/</sup> days before heading.

<sup>\*</sup> Significantly different from checks at 5% level.

# Effect of the Treatments on Heading, Maturity, and Height of Crop

Table 8 shows the data on the number of days to heading and maturity, and the height of the crop at maturity. The data refer to the main culms only. Heading was delayed from 1 to 12 days at treatment dates 2 to 17 inclusive (emergence to 18 inch stage). Maturity was delayed from 3 to 7 days over essentially the same period. The length of straw was significantly shortened at treatment dates 2 to 12 inclusive, 18, 21, and 22. The stage of growth of the crop at each of these treatment dates is given in Table 7 and Plates 13 to 18.

# Effect of the Treatments on Tillering

Table 8 also gives data on the average number of tillers per pot. Each pot contained 3 plants and in the check pots each plant, for the most part, had 3 tillers that produced a mature panicle (average number of tillers per check pot - 9.97). The number of tillers produced per pot was significantly reduced by treatment dates 5 to 26 inclusive, with the exception of treatment dates 19, 22, and 23. This would account, to a great extent, for the highly significant reductions in yield over such a wide range of growth stages.

It can be readily seen that the results obtained in the greenhouse do not agree in all details with those

# TABLE 8

Effect of spray applications of 2,4-D (butyl ester) on heading, maturity, height of crop, and amount of tillering in oats. Treatments made at 30 stages of growth at the rate of 12 ounces pure acid equivalent per acre.

Treatment date	Seeding to heading	Seeding to maturity	Height of crop at maturity	Average number of tillers per pot
	days	days	inches	
1 23 4 5 6 7 8 9 0 1 1 1 2 1 3 1 4 5 6 7 8 9 9 0 1 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2	57.4 58.5** 69.0** 70.0** 69.5** 63.5** 63.5** 59.5** 59.5** 59.5** 57.7.0000000000000000000000000000000000	79.0 82.0* 85.5* 85.5* 84.5* 83.0* 82.0* 82.5* 82.5* 82.5* 79.5 79.5 79.5 79.5 79.5 79.5 79.5 79.5	36.0 24.29.0***********************************	10.67 10.67 8.33 7.83 5.67* 5.33* 6.50* 4.83* 6.17* 6.33* 7.50* 7.00* 6.67* 6.33* 7.17* 8.17 6.67* 7.00* 8.83 8.33 6.17* 6.83* 7.17* 10.17 9.50 10.33 10.50
Check pots	57.0	78.5	34.7	9.97
L.S.D. at 5% level	1.30	2.02	3.45	2.32

obtained in the field. Regarding abnormalities the results were nearly identical for the two investigations. In the field and in the greenhouse onion-like leaves resulted from spraying within 18 and 17 days after emergence respectively. This was followed in both cases by a short period when panicle deformities occurred. However the yield pattern obtained in the greenhouse does not agree with the field results. The greenhouse results showed highly significant yield reductions when treated from emergence to heading, with two separate periods being particularly susceptible. In the field only one short period showed highly significant yield reductions, and this was an intermediate period in comparison to the greenhouse results. In the field, as previously mentioned, the greatest reductions in yield were associated with serious lodging of the crop. This would not be of importance in the greenhouse. Perhaps the heavy application of 2,4-D in the greenhouse (12 ounces acid per acre) had some effect on the plants which was not evident under the lighter rate used in the field. This was undoubtedly the case in the early seedling stages where the tubular leaves caused serious yield reductions in the greenhouse but failed to cause any decrease in the field.

This greenhouse experiment with oats fully supports the view of Leggett (20) and Andersen and Hermansen (1) who state that oats is the most susceptible of all cereal crops to 2,4-D.

#### SUMMARY

Barley and oats were sprayed with an aqueous solution of 2,4-D at 32 and 30 stages of growth respectively, beginning before emergence and continuing at 1 or 2 day intervals until after heading. The experiments were conducted in the greenhouse during the winters of 1951 and 1952. The rate of chemical applied was 12 ounces acid equivalent per acre. The isopropyl ester formulation of 2,4-D was used on barley and the butyl ester formulation on oats. Photomicrographs were made at each spraying date to determine the stage of development of the growing point. addition, oats was treated in the field with an isopropyl ester and butyl ester of 2,4-D in 1950 and 1951 respectively, at 20 stages of growth, beginning before emergence and continuing at 3 day intervals until after heading. The chemical was applied in the form of an aqueous spray at the rate of 8 ounces acid equivalent per acre.

In barley there were two critical periods when severe damage resulted from 2,4-D treatment. The first was an early seedling period extending from emergence to the early 3-leaf stage. This damage took place during the period when leaf initials were being produced and the tiny spike was differentiating in the growing point. Conspicuous leaf deformities characterized the early part of this period and spike or spikelet deformities the latter part. The second susceptible period began

when the plants were well advanced into the boot stage, 12 to 14 days before heading and continued until just before spike emergence. A high degree of sterility was associated with the damage that took place at this time and occurred during the differentiation of anthers and stigma and early growth of the floral parts.

The results with oats were somewhat inconsistent. In the greenhouse highly significant yield reductions occurred when treated at all stages from emergence to heading. However, there were two periods when particularly severe damage resulted. The first was an early seedling period extending from the 3½ inch stage to the 6 inch stage. This corresponded to the laying down of leaf initials in the growing points of both the main culms and tillers, with a high degree of leaf deformities resulting. The second period took place when treatment was made 13 and 10 days before heading, just prior to the shot-blade stage of the main culm. period corresponded to the early stages in the development of floral parts and a high degree of sterility was recorded. Between the two above-mentioned periods panicle deformities occurred in either the main culms or some of the tillers, and considerable decreases in yield were recorded. In the field the most highly significant yield reductions were recorded during this intermediate period, with the results being remarkably consistent for the two years. Besides panicle deformities, serious lodging was associated with 2,4-D treatment at this time in the field. Other than the lodging
factor and the possible effects of the extremely heavy
rate of 2,4-D used in the greenhouse experiment, the
author can offer no explanation for the discrepancy
between the greenhouse and field results regarding
yields. In most other factors the results were similar
for the greenhouse and field experiments with oats.

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