The effects of rates of 2,4-D, volumes and types of carrier and stage of growth on sterility injury in spring wheat
by Robert LWarden

A THESIS Submitted to the Graduate Faculty in partial fulfillment of the requirements for the degree of Master of Science in Agronony
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This injury is the abnormally high incidence of sterile florets, which under field conditions has occurred only when the 2,4-D application was. made in volumes of carrier of less than three gallons, per acre. The" actual occurrence of this damage, is probably less than one percent of the total acreage treated with 2,4-D in low volumes, but losses to individuals are large.

The author personally investigated a number of commercial field damage cases. In addition to the above statement on volumes, it was determined that most damage cases occurred from applications made at "stages of growth normally considered to be the least injurious to wheat. No definite differences in varietal tolerance were noted in spite of the fact that many people believe that the variety, Rescue, is more susceptible" than other varieties. Damage has occurred under field conditions where both fuel oil and water have been used as carriers, although oil caused injury under experimental conditions while water did not. Many of the cases have occurred when application was made within several days following a minimum temperature of below 40° F and within several days of a rain.

Experimental work comparing volumes of carrier, type of carrier, rates of 2,4-D and dates of application was conducted for three years, 1949-1951. These factors may contribute to the occurrence of the damage, but none appear to be directly responsible.

It appears that some factor in the environment predisposes the spring wheat plant to injury. Low temperatures preceeding applications and soil conditions may influence the predisposition.

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ABSTRACT

Injury to hard red spring wheat caused by 2,4-D in 1948 indicated the need for studies to determine its cause.

This injury is the abnormally high incidence of sterile florets, which under field conditions has occurred only when the 2,4-D application was made in volumes of carrier of less than three gallons per acre. The actual occurrence of this damage is probably less than one percent of the total acreage treated with 2,4-D in low volumes, but losses to individuals are large.

The author personally investigated a number of commercial field damage cases. In addition to the above statement on volumes, it was determined that most damage cases occurred from applications made at stages of growth normally considered to be the least injurious to wheat. No definite differences in varietal tolerance were noted in spite of the fact that many people believe that the variety, Rescue, is more susceptible than other varieties. Damage has occurred under field conditions where both fuel oil and water have been used as carriers, although oil caused injury under experimental conditions while water did not. Many of the cases have occurred when application was made within several days following a minimum temperature of below 40° F and within several days of a rain.

Experimental work comparing volumes of carrier, type of carrier, rates of 2,4-D and dates of application was conducted for three years, 1949-1951. These factors may contribute to the occurrence of the damage, but none appear to be directly responsible.

It appears that some factor in the environment predisposes the spring wheat plant to injury. Low temperatures preceeding applications and soil conditions may influence the predisposition.

INTRODUCTION

With the occurrence of severe damage to spring wheat as an apparent result of 2,4-D applications in 1948, it was obvious that some detrimental and previously unreported response to the herbicide was occurring. This damage is the result of an injury which causes a high percentage of the florets to be nonproductive or sterile.

Such sterility has been caused by 2,4-D applications at the stages of growth when sexual parts were nearing maturity. The stages of growth at the time of application were investigated in commercial fields, and it was found that most applications had been made at, what were considered, safe stages of growth.

This damage has been reported only where the 2,4-D applications were made in low volumes of carrier.

While most 2,4-D applications in experimental work have been made in relatively high volumes of carrier per acre, most commercial applications within the state are made in volumes of ten gallons or less per acre. Where damage has occurred, volumes of three gallons or less per acre have been used. Low volumes of carrier might be one of the major causes for the observed damage, and the use of high volumes, is perhaps, a serious error on the part of investigators in determining the responses of cereals to 2,4-D.

This thesis presents information dealing with the response of hard red spring wheat to 2,4-D applications made at several rates in low volumes of oil and water carriers at two stages of growth.

REVIEW OF LITERATURE

Damage in the form of floret sterility to spring wheat by the application of 2,4-D at stages of growth considered to be safe, is a phenomenom which has not been reported to any extent in the literature to date. However, the few available reports are reviewed herein along with certain work which refers to several responses of cereal grains to 2,4-D.

Warden (9, 10) has reported on the occurrence of the injury in Montana where it has apparently occurred more consistantly than in other areas.

J. J. Sexsmith of the Experimental Station, Lethbridge, Alberta, Canada described sterility and its occurrence in southern Alberta in 1952 (7). His report states that a fairly large number of damage cases were observed at scattered points.

The first report received by Mr. Sexsmith was about a field that had been sprayed ten days earlier, in which the wheat appeared to be stunted and burned as a result of the 2,4-D application. This field later developed typical sterility symptoms. All known cases of the damage were observed where applications were made in a fuel oil by aircraft.

At least six varieties of spring wheat were involved in the Alberta damage. Damage varied between fields from 10 to 15 percent damaged heads to as high as 80 percent containing less than half of the normal number of kernels.

R. B. Widdifield (11) has reported similar damage in North Dakota. Most North Dakota cases seemed to occur where fuel oil was used as a carrier for 2,4-D applied by aircraft.

The reponse of spring wheat and other small grains to 2,4-D applications has been of interest to a number of investigators.

It is possible to induce injury in the form of sterile florets, which is very similar to the damage described herein, by application during the stages of growth when the flower parts are nearing sexual maturity, according to many authors, including Derscheid (2), Derscheid et al. (3), Krall (5) and Olson (6). These workers have not induced sterility at the so called safe stages of growth, which follow stooling and precede the boot stage.

Anderson (1) in an experiment where he planted barley at several dates and sprayed on different dates when the barley was in the three leaf stage, found that the number of abnormal kernels was greatly increased when applications were made two days before and two days after a frost. In another similar experiment an application made the morning after a frost a very high incidence of abnormal kernels was produced.

The number of seed per head of barley as affected by 2,4-D has been studied by Derscheid et al. (3) who found that yield reductions were closely associated with numbers of seed per spike. Applications at early stages of growth also resulted in a reduced number of spikes per unit area.

K. Holly (4) has published an interesting paper on the effects of volume application on the activity of 2,4-D and MCP. His study considered the amount of spray and toxicant retained per plant, and the effects of volumes on yields of small grains and flax, and on the control of weeds.

Volumes used ranged from 14 to 175 gallons per acre. It is assumed that the gallonage mentioned is in terms of imperial gallons.

Yields of weed free wheat, barley and oats were reduced only slightly by the lower volumes. In detailed greenhouse studies with flax in some instances no differences due to volumes were obtained, while in other cases toxicity was increased at low volumes.

Greenhouse trials with wild mustard, Brassica alba, in the four leaf stage and seedling barley in the two leaf stage were used to study the effect of volumes on retention. With no shielding between plants, it was determined that volumes above 33 gallons per acre did not greatly increase the amount of spray retained by wild mustard. This was probably because the leaf surface was completely wet at 33 gallons per acre, with the result that additional spray could not be retained. With barley, volumes of spray retained per plant were reduced proportionally with volume reductions from 175 to 43 gallons per acre. The amount retained, between 10 and 43 gallons per acre, was constant. Holly explains this by stating that all large droplets roll off the plant, and that droplets below a certain mass will be retained on moderately inclined surfaces. The proportion of small droplets probably increased as volumes decreased, since he reduced the nozzle size used with the lower volumes.

With a constant amount of a herbicide being applied per acre, the decreasing volumes increased the actual amount of toxicant retained per plant. At 10 gallons per acre the amount of toxicant retained per barley plant was 3.5 times as great as at 43 gallons per acre.

The variations in the amount of toxicant retained on both wild mustard and barley under greenhouse conditions was much greater than variations in toxicity measured under field conditions. Holly believes that spray retention and penetration are variable independent factors, which upon occasion may affect results. A case of high retention, plus high penetration will greatly alter the actual amount of toxicant absorbed by a crop plant in contrast to a case of low retention and penetration.

MATERIALS AND METHODS

Thatcher, a hard red spring wheat variety was used as the test plant in the experimental portion of this study. Thatcher was selected because it is one of the most widely grown varieties in the state, and one on which the observed damage had occurred.

Plantings in all three years (1949-1951) were made with a grain drill, which was adjusted to seed four rows spaced one foot apart. Each drill strip was spaced four feet apart to provide uniform spacing for each plot. After the wheat had germinated and emerged, individual plots ten feet long were marked off by cutting out a strip about eight inches wide across the drill strip. Thus individual plots were four feet wide and ten feet long, and each contained four rows of wheat. For yield determinations, eight feet of each of the two center rows were harvested. This resulted in sixteen square feet being used for the yield sample area.

Three replications were used in all of the test work.

The plot design used in 1949 was a modified split plot, in which blocks within replications were treated at each of two dates. Volumes of carrier and rates of 2,4-D were randomized within date blocks. In 1950 and 1951 dates were again the main blocks, but rates became sub-blocks with volumes randomized within. Because of this change in design, no attempt is made to combine the three years data into one analysis.

The design of the experiments in 1950 and 1951 resulted in the need for two analyses. One included the carrier comparison, and is termed Test 1, while the date comparison is termed Test 2. The form used for these analyses is presented in Tables I and II. A split plot analysis of variance as described by Snedecor (8) was used.

Table I. The analysis of variance form used in Test 1 for all characters studied.

Source of Variation	Degrees of Freedom
Replications	2
Rates	3
Error (a)	6
Subtotal	11
Volumes	3
Volumes x Rates	9
Error (b)	24
Subtotal	47
Carriers	1
Carriers x Rates	3
Carriers x Volumes	3
Carriers x Volumes x Rates	9
Error (c)	32
TOTAL	95

The treatments used in Test 1 during the two year period consisted of four rates of a 2,4-D ester in all possible combinations with four volumes of two carriers. Test 2 differed only in that two dates of application

Table II. The analysis of variance form used in Test 2 for all characters studied.

Source of Variation	Degrees of Freedom
Replications	2
Dates	1
Error (a)	2
Subtotal	5
Rates	3
Rates x Dates	3
Error (b)	12
Subtotal	33
Volumes	3
Volumes x Dates	3
Volumes x Rates	9
Volumes x Dates x Rates	9
Error (c)	48
TOTAL	95

were substituted for the two carriers. These growth stages were the early tiller stage in all three years, the late tiller stage in 1949, and the early boot stage in 1950 and 1951.

Carriers used were water and a number two diesel fuel. Volumes of carrier used were 1, 2, 4 and 8 gallons per acre. Rates of 2,4-D used were 0, .2, .4 and .8 pounds 2,4-D acid equivalent per acre. In 1949 a butyl ester formulation was used, and in 1950 and 1951 an isopropyl ester formulation was used.

The inclusion of water at the early date was accomplished by doubling the size of the early date blocks, and pairing the carrier plots within each rate sub-block.

Application was made by a direct pressure spray unit which discharged the spray material through a two nozzle boom. The nozzles were placed twenty-four inches apart, so that their discharge points were centered between the first and second and between the third and fourth rows of each plot. The application was made by turning the boom into a tray at the covered end of the frame, and then drawing it the length of the plot where the exposure period was ended by directing the spray into a trough at the open end of the frame.

To prevent drift and help insure the deposition of the spray material on the plot, a frame four feet wide, ten feet long and four feet high with the two sides and one end covered with a light cloth was used.

To obtain a satisfactory pattern with the nozzles used, it was necessary to have them pointing at an angle of about twenty degrees above the horizontal plane. This arrangement apparently gave the droplets the time necessary to spread out sufficiently to produce an even distribution.

The liquid required to obtain the necessary volumes was small with 3.47 milliliters per plot being the volume equivalent to one gallon per acre. This volume was discharged in one second by the two nozzles (Tee Jet #690039) at a pressure of thirteen pounds per square inch, measured at the beginning of the fifty feet of $\frac{1}{4}$ inch hose used as a pressure line from the compression tank to the spray unit.

The one second exposure period for the one gallon application required the applicator to make one fast step and a jump over the front trough. This short exposure period is subject to an extreme amount of error, and accordingly care was taken to prevent such errors from occurring.

The higher volumes were obtained by increasing the exposure period. On this basis, exposure times for the higher volumes were 2, 4 and 8 seconds.

For the purposes of the experiment, weeds were controlled mechanically by means of wheel and hand hoes. The plots were weeded about three times during the season to prevent the few weeds present from having an effect on yield.

Yield samples were threshed with a Vogel nursery thresher. After threshing, each sample was cleaned and weighed in grams.

In 1950 and 1951 samples of ten heads were harvested from each plot. These ten head samples were subjected to a number of determinations to obtain information on how the treatments affected yield components. These were analyzed in the same manner as the yield data.

The ten head samples were taken from the yield rows at random by gathering a handful of standing culms at several places in each plot, and collecting the spike on every fourth culm. In the several cases where either nine or eleven heads were picked in error, the total sample was used and adjusted to a ten head basis.

Determinations made by direct units of measurements included weight per hundred seed, weight of seed in ten heads, total number of spikelets in ten heads and number of sterile spikelets in ten heads. From these, number of seed in ten heads, number of fertile spikelets in ten heads, number of seed per fertile spikelet and number of heads per plot were calculated.

The results of observations reported herein were obtained by personal observation of damaged fields, conversations with producers of damaged fields, and custom applicators directly concerned with damage cases. In other instances information was obtained from people having knowledge of the damage.

In 1950 a number of farmers who suffered damage were contacted directly and asked a number of specific questions regarding the damage which they suffered. These data are presented in condensed form. In a few of these cases the applicator was also contacted to supply certain information on which the farmer was not certain. However, in spite of all possible checking, some of the data is likely to be in error.

Where actual application dates were obtained for specific places, weather data for a period before and after spraying were taken from weather records at the station closest to the area where the specific damage case occurred.

EXPERIMENTAL RESULTS

Experimental results for the three years are presented by variables rather than by years for the characters studied. As mentioned in Materials and Methods, data obtained in 1950 and 1951 resulted in the necessity of having two statistical analyses for each year.

For purposes of simplicity the analysis containing the carrier comparison is called Test 1, and the analysis containing the date comparison is referred to as Test 2.

Rates of 2,4-D

Rates of 2,4-D used were 0, .2, .4 and .8 pounds 2,4-D per acre. The butyl ester was used in 1949 and the isopropyl ester formulation in 1950 and 1951. "F" values and data on rates for all characters studied are presented in Tables III. and IV.

Increasing rates of 2,4-D generally reduced yields. The only exception being for Test 1 in 1950, in which the "F" test narrowly missed significance.

The observed effect of rates of 2,4-D on yields was normal, except that yields particularly in 1951 were reduced to a somewhat greater extent than would be expected on the basis of unpublished studies by the Montaña Agricultural Experiment Station.

In 1951 large yield reductions were produced by increasing rates of 2,4-D. This injury resulted from the application made with oil as a carrier at the early date of application. This indicates that rates of 2,4-D do influence the amount of injury.

The weight of seed in ten heads resulted in differences which were quite similar to the yield data, except that "F" values were larger in all cases, and that differences in Test 1 in 1950 resulted in significance at the 5 percent level. The mean weight of seed in ten heads at the .8 pounds rate was 84 percent of the zero rate, while the actual yield at the

.8 pound rate was 81 percent of the check. Reduced variability is apparently the reason why the weight of seed in ten heads resulted in significance, while the actual plot yield did not.

Table III. Values of "F" for rates of 2,4-D for the listed spring wheat characters studied in Tests 1 and 2 for all or part of the three year period, 1949-1951. Rates per acre used were 0, .2, .4 and .8 lbs. 2,4-D acid equivalent as an ester.

	Test 1		Test 2		
Year	1950	1951	1949	1950	1951
Measurement			"F" Values		
Yield, Bushels per acre	2.67	27.37**	2.81*	7.48**	19.98**
Wt. of seed in 10 hds, Gms.	6.77*	82.14**		8.47**	115.77**
Heads per plot, No.	.21	1.50		.28	.32
Wt. per 100 seed, Grams	3.41	6.97*		.68	.77
Seed in 10 heads, No.	8.06*	31.54**		6.59**	32.75**
Spikelets in 10 heads, No.	.80	.91		.63	.41
Spikelets sterile, Percent	2.36	39.13**		7.10**	43.31**
Seed/fertile spikelet, No.	6.21*	30.74**		4.45*	33.89**
* Necessary F, for P=.05	4.76	4.76	2.76	3.49	3.49
** Necessary F, for P=.01	9.78	9.78	4.13	5.95	5.95

From this it would appear that a random sampling of a relatively few known number of heads could be used to obtain an indication of actual yield data under certain conditions.

The number of heads per plot was determined by dividing the weight of seed in ten heads into the actual plot yield. While this was not an independent estimate, it is felt that it does have some value as an estimate of tillering. No significant differences due to rates were found. This would seem to be logical since the tillers on the plants presumably were formed before the herbicidal applications were made.

The weight of 100 seed was influenced significantly by rates of 2,4-D only in Test 1 in 1951. In this determination seed weight was reduced by

Table IV. The influence of four rates of 2,4-D on the mean values for the listed spring wheat characters studied in Tests 1 and 2 for all or part of the three year period, 1949-1951.

	Test 1*							
Year	1950				1951			
2,4-D rate, lbs./acre	0	.2	.4	.8	0	.2	.4	.8
Measurement								
Yield, Bushels per acre	48.9	43.4	43.5	42.8	36.9	33.5	28.9	24.0
Wt. of seed in 10 hds,Gms.	9.01	7.95	7.54	7.59	7.59	6.26	5.80	4.65
Heads per plot, No.	545	555	580	568	593	529	504	539
Wt. per 100 seed, Grams	3.56	3.47	3.44	3.42	3.00	2.93	2.88	2.73
Seed in 10 heads, No.	252	226	219	224	253	222	200	180
Spikelets in 10 heads,No.	144	141	143	144	138	136	137	136
Spikelets sterile,Percent	14.5	17.3	18.1	17.9	12.9	17.8	21.7	31.6
Seed/fertile spikelet,No.	2.05	1.95	1.87	1.90	2.10	1.92	1.85	1.76

	Test 2**			
Year	1949			
2,4-D rate, lbs./acre	0	.2	.4	.8
Measurement				
Yield, Bushels per acre	39.8	41.6	36.9	36.1

	Test 2**							
Year	1950				1951			
2,4-D rate, lbs./acre	0	.2	.4	.8	0	.2	.4	.8
Measurement								
Yield, Bushels per acre	50.1	42.5	40.4	37.0	36.2	33.1	30.6	23.9
Wt.of seed in 10 hds,Gms.	9.25	8.28	7.65	7.30	7.15	6.26	5.91	4.70
Heads per plot, No.	542	521	522	507	511	524	524	535
Wt. per 100 seed, Grams	3.56	3.52	3.51	3.49	2.91	2.90	2.93	2.83
Seed in 10 heads, No.	259	234	220	215	244	221	200	174
Spikelets in 10 heads,No.	146	144	142	145	138	135	137	137
Spikelets sterile,Percent	14.5	17.7	19.0	20.9	13.2	19.4	19.6	28.3
Seed/fertile spikelet,No.	2.08	1.97	1.90	1.86	2.05	1.93	1.85	1.68

* For $\bar{x}$, n=24; 2 carriers, 4 volumes and 3 replications.
** For $\bar{x}$, n=24; 2 dates, 4 volumes and 3 replications.

increasing rates of 2,4-D. This finding is in accord with producers' statements which have indicated that seed produced in damaged areas is shrivelled.

The number of spikelets, or rachis nodes, per plot was not influenced by rates of 2,4-D. This agrees with studies on the floral development of the wheat spike in which it was found that the number of rachis nodes is determined at an early stage of growth.

The percentage of spikelets sterile or producing no seed, indicated that one of the yield reducing effects of 2,4-D is an increase in the number of spikelets producing no seed. Most of the spikelets in which sterility was induced were apical in spike position, although in severe cases some basal sterility was also observed. Increases in sterility due to increasing rates were obtained for percent spikelets sterile in 1950 and 1951. The mean number of seed produced in each fertile spikelet was reduced by increasing rates of 2,4-D.

On the basis of the data obtained from the ten head samples, the yield reducing effects of 2,4-D rates on Thatcher wheat, when applications are made at normal stages of growth, are due to lighter seed, a higher percentage of sterile spikelets, and more sterile florets in fertile spikelets. Tillering and spikelet formation were not factors in reducing yield.

Volumes of Carrier

Volumes of carrier used were 1, 2, 4 and 8 gallons of total volume per acre.

Volume comparisons have been included as a major factor in the experimental work because all of the injury cases which have come to the attention of the author have been where carriers have been used in low volumes. In no case has damage under field conditions been reported where volumes of more than three gallons per acre have been used.

Table V. Values of "F" for volumes of carrier for the listed spring wheat characters studied in Tests 1 and 2 for all or part of the three year period, 1949-1951. Volumes of carrier used were 1, 2, 4 and 8 gallons per acre.

	Test 1		Test 2		
Year	1950	1951	1949	1950	1951
Measurement			"F" Values		
Yield, Bushels per acre	1.04	1.16	3.41*	.36	1.76
Wt. of seed in 10 hds, Gms.	1.95	9.25**		.46	.91
Heads per plot, No.	1.01	.90		.18	1.78
Wt. per 100 seed, Grams	1.40	1.85		.48	.69
Seed in 10 heads, No.	1.06	1.70		.44	2.04
Spikelets in 10 heads, No.	3.43*	.44		1.40	.37
Spikelets sterile, Percent	1.53	.11		1.34	.50
Seed/fertile spikelet, No.	.19	.97		.37	.39
* Necessary F, for P=.05	3.01	3.01	2.76	2.80	2.80
** Necessary F, for P=.01	4.72	4.72	4.13	4.22	4.22

The effect of volumes on the characters studied have not been significant in most cases, Table V. In some cases trends seem to be apparent, but the differences in relation to population variation are not great enough to be significant, Table VI.

Yields of grain were increased where increasing volumes of carrier were used in 1949. In 1950 and 1951 yields at volumes greater than one gallon were generally slightly higher than the yields at the one gallon volume with the exception of Test 2 in 1950, where one bushel per acre was the greatest difference.

Weight of seed in ten heads for 1950 and 1951 indicated about the same trend as yields. An exception was the difference obtained for Test 2

Table VI. The influence of four volumes of carrier containing 2,4-D on the mean values for the listed spring wheat characters studied in Tests 1 and 2 for all or part of the three year period, 1949-1951.

Test 1*

Year	1950				1951			
Volume of carrier	1	2	4	8	1	2	4	8
Measurement								
Yield, Bushels per acre	43.9	43.9	45.6	44.1	29.0	31.6	31.3	31.4
Wt. of seed in 10 hds, Gms.	7.62	8.00	8.15	8.35	5.91	6.05	6.45	5.90
Heads per plot, No.	583	558	563	544	597	538	505	523
Wt. per 100 seed, Grams	3.43	3.49	3.47	3.50	2.89	2.88	2.92	2.84
Seed in 10 heads, No.	223	229	235	235	209	207	218	222
Spikelets in 10 heads, No.	142	141	145	145	136	136	137	136
Spikelets sterile, Percent	18.1	16.8	16.4	16.4	21.2	21.1	20.4	21.3
Seed/fertile spikelet, No.	1.93	1.95	1.96	1.93	1.88	1.88	1.97	1.90

Test 2**

Year	1949			
Volume of carrier	1	2	4	8
Measurement				
Yield, Bushels per acre	34.6	39.0	39.8	41.1

Test 2**

Year	1950				1951			
Volume of carrier	1	2	4	8	1	2	4	8
Measurement								
Yield, Bushels per acre	43.0	42.0	42.9	42.9	29.7	32.1	31.2	30.8
Wt. of seed in 10 hds, Gms.	7.90	8.13	8.26	8.18	5.84	5.85	6.22	6.11
Heads per plot, No.	532	524	514	522	509	561	515	509
Wt. per 100 seed, Grams	3.51	3.54	3.51	3.53	2.91	2.86	2.92	2.89
Seed in 10 heads, No.	227	231	238	232	204	203	210	222
Spikelets in 10 heads, No.	144	143	147	144	136	136	137	137
Spikelets sterile, Percent	19.3	17.7	17.8	17.3	21.8	20.6	20.6	20.1
Seed/fertile spikelet, No.	1.95	1.96	1.98	1.92	1.87	1.85	1.90	1.89

* For $\bar{x}$, n=24; 2 carriers, 4 rates and 3 replications.
** For $\bar{x}$, n=24; 2 dates, 4 rates and 3 replications.

in 1951. In this case the weight of the seeds increased progressively up to the four gallon volume and dropped in the eight gallon volume to the approximate level of the one gallon rate.

The "F" values for this character were larger than those for yields in three out of the four comparisons.

The number of heads per plot was not affected by volume to any significant degree, and no pattern or trend was apparent.

Weight per 100 seed was not affected by volumes since differences were generally very small. In field damage where injury has occurred in low volume application the reduced number of seed has been shrivelled.

The number of seed in ten heads was not affected significantly, although in all tests the mean number increased as volume increased.

The total number of spikelets in ten heads was influenced to a significant degree in Test 1 in 1950. In this case the four and eight gallon volumes produced greater spikelet numbers than did the one and two gallon volumes. In the other tests no differences were evident.

The percent spikelets sterile was not influenced by volumes. It would appear that if low volumes are the primary factor in the damage that the number of spikelets producing seed would be lower for the low volumes.

The number of seed per spikelet and number of seed per fertile spikelet likewise did not respond significantly to volume changes, although the two low volumes had a slightly lower number of fertile spikelets in both tests than did the high volumes.

On the basis of the data obtained in these tests, it would appear that volumes of carrier varying from one to eight gallons have little consistent effect on yield. Indications are that higher volumes will result in slightly higher yields. In 1950 and 1951 the yield reductions at low volumes may be accounted for by slightly increased numbers of sterile florets.

Carriers

Carriers are defined as the materials used to dilute the herbicide so that it can be applied efficiently.

Two carriers, water and No. 2 diesel fuel were used in the experimental comparison. This comparison was made at the early date only and consequently all carrier comparisons are found in Test 1. Carriers were compared in 1950 and 1951.

As will be observed in Table VII., the influence of carriers was very different for each of the two years. In 1950 one of the eight characters measured showed a highly significant difference, while in 1951 seven of the eight were significant with six reaching the one percent level of significance.

Such a difference between years would indicate that environmental influences can have a tremendous effect on the response of wheat to 2,4-D carrier. Most field cases of damage have occurred where oil has been used as a carrier.

Yield data in 1950 indicated no difference due to carriers, while in 1951 oil reduced yields.

The weight of seed in ten heads was not different in 1950, and was reduced in 1951 when oil was used as the carrier. The number of heads per plot was reduced by the oil carrier in 1951 but not in 1950.

Weight per 100 seed was reduced in 1951 by visible shrivelling, but not in 1950. In addition to the loss of weight per seed in 1951, the

Table VII. Values of "F" for, and the influence of, two carriers on the mean values for the listed spring wheat characters studied in Test 1 in 1950 and 1951.

Year	1950	1951	1950		1951	
Carrier			Water	Oil	Water	Oil
Measurement	"F" Values		Data Means***			
Yield, Bushels per acre	.51	80.04**	44.8	44.5	34.2	27.4
Wt. of seed in 10 hds, Gms.	.42	65.74**	8.10	7.95	7.01	5.14
Heads per plot, No.	.02	6.28*	563	560	493	539
Wt. per 100 seed, Grams	.58	50.62**	3.48	3.47	2.97	2.79
Seed in 10 heads, No.	.11	46.93**	231	229	237	191
Spikelets in 10 heads, No.	.02	2.26	143	143	136	137
Spikelets sterile, Percent	9.72**	104.38**	15.9	18.0	15.8	26.2
Seed/fertile spikelet, No.	.48	45.66**	1.93	1.96	2.03	1.79

* Necessary F, for P = .05 4.15 4.15
** Necessary F, for P = .01 7.50 7.50
*** For $\bar{x}$, n = 48; 4 rates, 4 volumes and 3 replications.

number of seed in ten heads was also reduced. The cumulative effect of these two characters would seem to account for the 1951 reduction in yield when the negative effect of number of heads per plot is considered.

The number of spikelets in ten heads was not affected significantly. The percent of spikelets sterile was affected in 1950 and 1951 with oil increasing the degree of sterility.

Number of seed per fertile spikelet was reduced by the oil carrier in 1951 but not in 1950.

Date Comparisons

Applications of four rates of 2,4-D in the four volumes of the No. 2 diesel oil carrier were accomplished at two dates for each of the two years. The stages of growth between years were not exact, but were reasonably close. Data means and "F" values are presented in Table VIII.

Table VIII. Values of "F" for, and the influence of dates of application on mean values*** for, the listed spring wheat characters studied in Test 2 for the three year period, 1949-1951.

Year Measurement	1949	1950	1951
		"F" Values	
Yield, Bushels per acre	.22	1.04	27.86*
Wt. of seed in 10 hds, Gms.		2.75	49.47*
Heads per plot, No.		32.33*	1.50
Wt. per 100 seed, Grams		31.64*	12.10
Seed in 10 heads, No.		1.46	9.57
Spikelets in 10 heads, No.		.78	2.33
Spikelets sterile, Percent		.01	39.13*
Seed/fertile spikelet, No.		.03	502.93**
* Necessary F, for P=.05	18.51	18.51	18.51
** Necessary F, for P=.01	98.49	98.49	98.49

Data Means

Year Time of Application Measurement	1949 Early	1949 Late	1950 Early	1950 Late	1951 Early	1951 Late
Yield, Bushels per acre	38.4	38.9	44.5	40.5	27.4	34.5
Wt. of seed in 10 hds,Gms.			7.95	8.29	5.14	6.87
Heads per plot, No.			560	486	539	508
Wt. per 100 seed, Grams			3.47	3.57	2.79	3.00
Seed in 10 heads, No.			229	235	1.91	229
Spikelets in 10 heads, No.			143	145	136	137
Spikelets sterile, Percent			18.0	18.0	26.2	15.4
Seed/fertile spikelet, No.			1.96	1.93	1.79	1.97

*** For $\bar{x}$, n=48; 4 rates, 4 volumes and 3 replications.

The date comparisons are at a disadvantage in the analysis because of the experimental design. The design carried the two dates as main plots within replications. This resulted in one degree of freedom for dates and two degrees for error.

Yield data indicated no differences in 1949 and 1950 and a difference at the five percent level in 1951. This difference was in favor of the late application date. The difference in yield due to dates is principally the result of injury in the form of sterile florets which occurred when 2,4-D was applied at the early stage of growth.

Weight of seed in ten heads was also reduced by the early date application in 1951, but not in 1950.

Number of heads per plot and weight per 100 seed were significantly higher at the early date in 1950 with an opposite but nonsignificant difference in 1951.

The number of seed in ten heads was not affected in either year. The number of spikelets in ten heads was not different in either year. The percent of spikelets sterile differed between the two dates in 1951 with an increase being shown at the early date.

Number of seed per spikelet and per fertile spikelet were reduced in 1951, with the latter difference being highly significant. The differences obtained for these comparisons in 1950 were very small.

Interactions of Rates and Volumes

The "rate x volume" interactions were part of each of the two tests. Values of "F" are presented in Table IX and mean data in Table X for Test 1 and Table XI for Test 2.

In general few differences of a significant nature occurred in this interaction with only four being noted in seventeen comparisons.

Weight of seed in ten heads differed at the five percent level of significance in Test 1 in 1951. This difference appears to be the result of a smaller decrease at the eight gallon rate due to increasing rates of 2,4-D.

Table IX. Values of "F" for the "rate x volume" interactions for the listed spring wheat characters studied in Tests 1 and 2 for all or part of the three year period, 1949-1951. In Test 1 two carriers were used, while in Test 2 applications were made at two dates.

	Test 1		Test 2		
Year	1950	1951	1949	1950	1951
Measurement			"F" Values		
Yield, Bushels per acre	1.27	1.38	.36	.77	2.02
Wt. of seed in 10 hds, Gms.	.77	3.03*		1.87	1.72
Heads per plot, No.	.23	.96		.68	.85
Wt. per 100 seed, Grams	1.63	3.83**		1.16	1.69
Seed in 10 heads, No.	.47	3.61**		.70	2.43*
Spikelets in 10 heads, No.	1.14	.41		.58	.60
Spikelets sterile, Percent	1.41	1.98		1.04	2.02
Seed/fertile spikelet, No.	1.30	1.16		.98	1.20
* Necessary F, for P .05	2.30	2.30	2.04	2.08	2.08
** Necessary F, for P .01	3.25	3.25	2.72	2.80	2.80

Weight per 100 seed was affected in Test 1 in 1951. Here the discrepancy in main effects seemed to be less of a decrease at the two and four gallon volumes at the .2 and .4 pound rates of 2, 4-D than occurred at the one and eight gallon volumes.

The number of seed in ten heads was affected significantly in the interaction in 1950 and 1951 according to the "F" fest. The relatively low number of seed in the two and four gallon volumes of carrier at the .8 lb. rate of 2,4-D appears to be responsible for the difference.

The other significant interaction occurred in Test 2 in 1952, in which the rate x volume interaction for the number of seed in ten heads resulted in a significant difference. In this case, considerable variation at the one gallon volume existed between rates in relation to the

Table X. The influence of rates of 2,4-D in volumes of carrier on mean values* for the listed spring wheat characters studied in Test 1 in 1950 and 1951.

Volume, Gallons per acre	1950 2,4-D rate, lbs. per acre				1951 2,4-D rate, lbs. per acre			
	0	.2	.4	.8	0	.2	.4	.8
	Yield, Bushels per acre							
1	48.4	42.2	44.0	41.0	37.6	30.7	23.8	23.8
2	49.7	43.2	42.3	40.2	36.1	37.2	31.6	21.3
4	47.7	46.8	43.9	44.0	36.5	34.8	30.0	24.0
8	49.8	41.5	43.4	45.9	37.6	31.0	30.2	26.8
	Wt. of Seed in 10 Heads, Grams							
1	8.35	7.33	7.36	7.46	7.83	6.21	4.81	4.77
2	9.45	7.80	7.59	7.15	7.52	6.44	6.15	4.08
4	9.02	8.55	7.62	7.42	7.92	6.69	6.68	4.51
8	9.23	8.12	7.61	8.34	7.10	5.70	5.57	5.25
	Heads Per Plot, No.							
1	581	578	603	569	431	501	494	513
2	538	570	557	568	487	590	516	561
4	532	550	589	580	472	523	450	567
8	531	521	569	554	532	486	558	516
	Wt. Per 100 Seed, Grams							
1	3.47	3.45	3.45	3.48	3.01	2.95	2.77	2.82
2	3.64	3.53	3.40	3.39	2.97	2.97	2.96	2.63
4	3.62	3.42	3.44	3.38	3.08	2.93	3.00	2.68
8	3.52	3.48	3.50	3.51	2.93	2.87	2.79	2.79
	Seed in 10 Heads, No.							
1	244	212	213	221	260	211	173	192
2	258	224	224	210	253	217	208	152
4	252	239	221	228	256	228	223	163
8	255	229	218	237	242	232	198	215

Table X. (Continued)

Volume, Gallons per acre	1950 2,4-D rate, lbs. per acre 0	.2	.4	.8	1951 2,4-D rate, lbs. per acre 0	.2	.4	.8
				Spikelets in 10 Heads, No.				
1	143	138	146	141	137	134	136	137
2	140	139	139	145	136	137	138	138
4	144	143	146	146	139	135	138	134
8	150	143	142	146	139	136	138	134
				Spikelets Sterile, Percent				
1	16.9	19.4	17.3	18.6	11.3	18.8	24.8	29.7
2	12.4	18.0	17.8	19.1	12.1	15.5	19.8	37.0
4	13.7	15.4	19.0	17.6	12.3	17.0	19.9	32.6
8	15.0	16.2	18.4	16.4	15.8	19.8	22.4	27.2
				Seed Per Fertile Spikelet, No.				
1	2.07	1.92	1.79	1.93	2.13	1.95	1.67	1.76
2	2.10	1.97	1.96	1.95	2.11	1.87	1.87	1.68
4	2.03	1.98	1.85	1.96	2.09	2.01	2.01	1.75
8	2.01	1.93	1.87	1.93	2.06	1.87	1.84	1.85

* For $\overline{x}$, n=6; 2 carriers and 3 replications.

Table XI. The influence of rates of 2,4-D in volumes of carrier on mean values* for the listed spring wheat characters studied in Test 2 for the three year period, 1949-1951.

Volume, Gallons per acre	1949 2,4-D rate, lbs./A 0	.2	.4	.8	1950 2,4-D rate, lbs./A 0	.2	.4	.8	1951 2,4-D rate, lbs./A 0	.2	.4	.8
						Yield, Bushels Per Acre						
1	34.0	37.4	32.6	34.6	50.9	41.4	41.6	35.0	37.0	31.6	26.2	24.0
2	41.2	42.0	38.6	34.0	50.0	41.9	38.4	37.6	34.9	35.7	35.9	24.1
4	41.2	43.2	39.3	35.6	48.6	44.5	40.8	37.8	37.2	34.3	30.0	23.3
8	42.9	43.9	37.4	40.3	51.1	42.4	40.7	37.6	35.8	30.8	32.4	24.2

Table XI. (Continued)

Volume, Gallons per acre	1950 2,4-D rate, lbs. per acre 0	.2	.4	.8	1951 2,4-D rate, lbs. per acre 0	.2	.4	.8
			Wt. of Seed in 10 Heads, Grams					
1	8.79	7.30	7.71	7.81	7.21	6.27	4.74	5.13
2	9.71	8.41	7.23	7.19	6.68	6.31	6.22	4.21
4	9.17	8.78	7.84	7.26	7.56	6.32	6.57	4.44
8	9.36	8.63	7.80	6.95	7.17	6.15	6.11	5.01
			Heads Per Plot, No.					
1	580	551	546	451	518	487	543	488
2	533	514	525	524	525	579	542	599
4	521	509	519	507	498	551	454	559
8	534	512	498	545	505	480	558	493
			Wt. Per 100 Seed, Grams					
1	3.52	3.46	3.54	3.53	2.96	2.96	2.81	2.92
2	3.63	3.59	3.42	3.51	2.81	2.91	2.94	2.78
4	3.55	3.47	3.55	3.46	3.00	2.90	3.03	2.75
8	3.55	3.59	3.53	3.45	2.89	2.84	2.93	2.90
			Seed in 10 Heads, No.					
1	255	211	218	226	243	211	167	196
2	267	237	217	205	235	218	212	146
4	258	249	224	219	252	218	216	156
8	255	239	222	212	248	237	204	199
			Spikelets in 10 Heads, No.					
1	145	142	145	145	137	134	135	138
2	144	142	139	145	137	136	138	135
4	148	146	146	147	139	138	139	134
8	147	146	139	144	138	136	135	139
			Spikelets Sterile, Percent					
1	16.6	20.8	18.5	21.3	13.9	19.0	27.3	27.1
2	12.3	17.6	20.1	21.1	13.1	16.8	18.7	34.0
4	14.3	16.2	20.4	20.2	13.7	16.7	20.4	31.5
8	14.7	16.5	17.1	20.9	13.2	19.4	19.6	28.3
			Seed Per Fertile Spikelet, No.					
1	2.12	1.89	1.84	1.97	2.06	2.00	1.68	1.75
2	2.13	2.02	1.91	1.77	1.97	1.91	1.89	1.62
4	2.05	2.03	1.92	1.92	2.10	1.89	1.96	1.66
8	2.03	1.93	1.92	1.79	2.06	1.94	1.85	1.70

* For $\bar{x}$, n=6; 2 carriers and 3 replications.

to the pattern established by the other volumes and the mean of rates. The .4 pound rate was considerably lower for the one gallon than for any other volume, while at the .8 pound rate the number of seed was consider- higher than the mean of the other three rates.

Interactions of Dates and Rates

"Date x rate" interactions are given in Table XII. Data means are presented in Table XIII.

Table XII. Values of "F" for "date x rate" interactions for all listed spring wheat characters studied in Test 2. Rates of 2,4-D used were 0, .2, .4 and .8 lbs. acid equivalent per acre applied at two dates.

Year Measurement	1949	1950 "F" Values	1951
Yield, Bushels per acre	3.76*	2.00	7.53**
Wt. of seed in 10 hds. Gms.		.49	44.02**
Heads per plot, No.		1.13	1.39
Wt. per 100 seed, Grams		.77	6.63**
Seed in 10 heads, No.		.47	5.53*
Spikelets in 10 heads, No.		1.98	2.03
Spikelets sterile, Percent		1.16	22.76**
Seed/fertile spikelet, No.		.35	3.15
* Necessary F, for P=.05	2.76	3.49	3.49
** Necessary F, for P=.01	4.13	5.95	5.95

In this series of interactions in 1951 the analysis for all characters except number of heads per plot and number of spikelets in ten heads resulted in significant or highly significant interactions. In all cases the greatest differences occurred relative to the check at the early date. This is in contrast to the 1950 results when no significant differences occurred.

Table XIII. The influence of rates of 2,4-D applied on two dates on the mean values* for the listed spring wheat characters studied in Test 2 for the three year period, 1949-1951.

Year / Measurement	Rate 2,4-D lbs/acre	1949 Early	1949 Late	1950 Early	1950 Late	1951 Early	1951 Late
Yield, Bushels/Acre	0	43.9	35.7	50.1	50.1	36.3	36.1
	.2	40.1	43.2	41.6	43.5	31.1	35.0
	.4	34.4	39.5	44.7	36.0	25.1	36.1
	.8	35.1	37.1	41.6	32.4	17.0	30.8
Wt. of seed in 10 heads, Gms.	0			9.12	9.39	7.05	7.25
	.2			7.81	8.74	5.55	6.98
	.4			7.60	7.70	4.89	6.94
	.8			7.29	7.32	3.09	6.30
Heads per plot, No.	0			548	536	519	503
	.2			539	504	540	508
	.4			591	453	519	529
	.8			563	450	578	492
Wt. per 100 seed, Grams	0			3.55	3.57	2.94	2.89
	.2			3.44	3.61	2.85	2.95
	.4			3.46	3.56	2.80	3.06
	.8			3.41	3.56	2.58	3.09
Seed in 10 heads, No.	0			255	263	240	249
	.2			225	242	205	237
	.4			220	221	174	226
	.8			217	213	145	204
Spikelets in 10 heads, No.	0			146	145	138	137
	.2			139	149	133	139
	.4			144	141	136	137
	.8			144	146	137	135
Spikelets sterile, Percent	0			15.2	13.8	13.9	13.0
	.2			18.5	17.0	21.5	14.4
	.4			19.0	19.0	26.5	16.5
	.8			19.4	22.3	42.8	17.7
Seed/fertile spikelet, No.	0			2.06	2.10	2.01	2.09
	.2			1.99	1.95	1.88	1.99
	.4			1.88	1.91	1.72	1.97
	.8			1.90	1.83	1.54	1.82

* For $\bar{x}$, n=12; 4 volumes and 3 replications.

In the 1949 yield data a significant interaction occurred. This interaction is because the zero rate yielded highest at the early date and the lowest at the late date.

Interactions of Carriers and Rates

"Carrier x rate" interactions exhibited about the same response in both years as the "date x rate" interaction. In 1950 no significant interactions were obtained, while in 1951 four of eight comparisons produced highly significant differences, Tables XIV and XV.

Table XIV. Values of "F" for "carrier x rate" interactions for all listed spring wheat characters in Test 1. Carriers compared were diesel fuel and water. Rates were 0, .2, .4 and .8 lbs. 2,4-D acid equivalent per acre.

Year	1950	1951
Measurement	"F" Values	
Yield, Bushels per acre	1.71	10.04**
Wt. of seed in 10 hds.,Gms.	.77	3.78*
Heads per plot, No.	.40	.33
Wt. per 100 seed, Grams	.97	2.42
Seed in 10 heads, No.	.43	2.22
Spikelets in 10 heads, No.	.79	1.63
Spikelets sterile, Percent	.25	18.02**
Seed per fertile spikelet, No.	.20	4.34*
* Necessary F, for P=.05	2.90	2.90
** Necessary F, for P=.01	4.46	4.46

Yield, weight of seed in ten heads, percent spikelets sterile and number of seed per fertile spikelet were affected to the greatest degree by the early application. It appears that all of the significant interactions are due to a difference of degree in response rather than to a difference in direction of the response.

Table XV. The influence of rates of 2,4-D in two carriers on the mean values* for the listed spring wheat characters studied in Test 1 for 1950 and 1951.

Year Measurement	Rate 2,4-D lbs/acre	1950 Water	 Oil	1951 Water	 Oil
Yield, Bushels per acre	0	47.7	50.1	37.6	36.3
	.2	45.3	41.6	35.8	31.1
	.4	42.2	44.7	32.7	25.1
	.8	44.0	41.6	30.9	17.0
Wt. of seed in 10 hds, Gms.	0	8.90	9.12	8.13	7.05
	.2	8.09	7.81	6.97	5.55
	.4	7.49	7.60	6.72	4.89
	.8	7.90	7.29	6.22	3.09
Heads per plot, No.	0	542	548	467	519
	.2	570	539	514	540
	.4	568	591	489	519
	.8	572	563	500	578
Wt. per 100 seed, Grams	0	3.57	3.55	3.06	2.94
	.2	3.50	3.44	3.00	2.85
	.4	3.45	3.46	2.96	2.80
	.8	3.42	3.41	2.88	2.58
Seed in 10 heads, No.	0	250	255	266	240
	.2	227	225	239	205
	.4	218	220	227	174
	.8	231	217	216	145
Spikelets in 10 heads	0	142	146	138	138
	.2	143	139	138	133
	.4	143	144	138	136
	.8	144	144	137	137
Spikelets sterile, Percent	0	13.8	15.2	11.9	13.9
	.2	16.1	18.5	14.0	21.5
	.4	17.2	19.0	16.9	26.5
	.8	16.4	19.4	20.4	42.8
Seed per fertile spikelet, No.	0	2.04	2.06	2.18	2.01
	.2	1.91	1.99	1.97	1.88
	.4	1.85	1.88	1.97	1.72
	.8	1.91	1.90	1.98	1.54

* For $\bar{x}$, n=12; 4 volumes and 3 replications.

Interaction of Dates and Volumes

"Date x volume" interactions resulted in no significant interactions in the seventeen comparisons, Tables XVI and XVII. These results indicate that dates had little or no influence on injury due to volumes of carrier used.

Table XVI. Values of "F" for "volume x date" interactions for all spring wheat characters studied. Applications were made in four volumes at two dates.

Year	1949	1950	1951
Measurement		"F" Values*	
Yield, Bushels per acre	1.01	.26	.85
Wt. of seed in 10 hds, Gms.		1.87	2.18
Heads per plot, No.		.59	.85
Wt. per 100 seed, Grams		1.16	2.02
Spikelets in 10 heads, No.		.71	.46
Seed in 10 heads, No.		1.40	.70
Spikelets sterile, Percent		1.89	.73
Seed/fertile spikelet, No.		.15	.31
* Necessary F, for P=.05		2.80	2.80

Table XVII. The influence of volumes of carrier on dates of application on mean values* for the listed spring wheat characters as studied in Test 2 for all or part of the three year period, 1949-1951.

Year Measurement	Volume, Gallons/A	1949 Early	1949 Late	1950 Early	1950 Late	1951 Early	1951 Late
Yield, Bushels/acre	1	34.0	35.3	43.8	40.6	25.5	33.9
	2	39.9	38.0	44.5	39.4	29.4	34.8
	4	37.6	42.7	45.0	40.8	27.2	35.2
	8	42.1	40.2	44.7	41.2	27.5	34.1
Wt. of seed in 10 heads, Gms.	1			7.38	8.43	4.96	6.72
	2			8.16	8.11	5.38	6.33
	4			7.95	8.57	5.32	7.12
	8			8.33	8.04	4.92	7.30
Heads per plot, No.	1			580	484	507	511
	2			550	493	568	554
	4			563	465	534	497
	8			543	501	547	471
Wt. per 100 seed, Grams	1			3.44	3.59	2.80	3.02
	2			3.50	3.57	2.82	2.90
	4			3.44	3.58	2.81	3.03
	8			3.49	3.56	2.74	3.04
Seed in 10 heads, No.	1			218	237	1.87	2.22
	2			234	229	1.88	2.18
	4			234	241	1.86	2.35
	8			232	231	2.03	2.41
Spikelets in 10 heads, No.	1			141	147	136	136
	2			141	144	137	135
	4			147	147	137	138
	8			145	143	135	138
Spikelets sterile, Percent	1			20.4	18.2	27.1	16.6
	2			16.7	18.8	25.0	16.3
	4			18.4	17.1	26.1	15.1
	8			16.6	17.9	26.6	13.6
Seed per fertile spikelet, No.	1			1.94	1.97	1.79	1.95
	2			1.98	1.93	1.78	1.91
	4			1.98	1.98	1.79	2.01
	8			1.92	1.92	1.79	1.99

* For $\bar{x}$, n=12; 4 rates and 3 replications.

Interactions of Carriers and Volumes

This interaction produced one significant difference in the sixteen comparisons, Tables XVIII. and XIX. This difference occurred in 1950 for the measurements comparing the percent of spikelets sterile. In this case the percentage of sterile spikelets decreased somewhat with increasing volumes of the oil, while the percentage sterile spikelets with the water carrier remained fairly constant. The other "F" values did not approach significance.

Table XVIII. Values of "F" for "volume x carrier" interactions for all listed spring wheat characters in Test 1. Diesel fuel and water were used as carriers and each was applied in volumes of 1, 2, 4 and 8 gallons per acre

Year	1950	1951
Measurement	"F" Values	
Yield, Bushels per acre	.10	1.39
Wt. of seed in 10 heads, Gms.	.77	.71
Heads per plot, No.	.01	.17
Wt. per 100 seed, Grams	1.12	.74
Seed in 10 heads, No.	.47	.85
Spikelets in 10 heads, No.	.33	1.74
Spikelets sterile, Percent	3.26*	.77
Seed/fertile spikelet, No.	.27	1.25
* Necessary F, for P=.05	2.90	2.90

Table XIX. The influence of type of carrier on volumes of carrier on mean values* for the listed spring wheat characters studied in Test 2 in 1950 and 1951.

Year / Measurement	Volume Gallons/A	1950 Water	1950 Oil	1951 Water	1951 Oil
Yield, Bushels per acre	1	43.9	43.8	32.5	25.5
	2	43.4	44.5	33.7	29.4
	4	46.2	45.0	35.5	25.5
	8	45.6	44.7	35.3	27.5
Wt. of seed in 10 hds, Gms.	1	7.87	7.38	6.85	4.96
	2	7.83	8.16	6.72	5.38
	4	8.36	7.95	7.58	5.32
	8	8.32	8.33	6.89	4.92
Heads per plot, No.	1	585	580	488	507
	2	561	555	508	568
	4	562	563	476	534
	8	544	543	499	547
Wt. per 100 seed, Grams	1	3.44	3.44	2.97	2.80
	2	3.47	3.50	2.95	2.82
	4	3.49	3.44	3.03	2.81
	8	3.51	3.49	2.95	2.74
Seed in 10 heads, No.	1	228	218	231	187
	2	224	234	227	188
	4	236	234	250	186
	8	237	232	240	203
Spikelets in 10 heads, No.	1	142	141	136	136
	2	141	141	138	137
	4	143	147	136	137
	8	146	145	140	135
Spikelets sterile, Percent	1	15.8	20.4	15.3	27.1
	2	17.0	16.7	17.2	25.0
	4	14.4	18.4	14.8	26.1
	8	16.3	16.6	16.0	26.6
Seed per fertile spikelet, No.	1	1.91	1.94	1.96	1.79
	2	1.92	1.98	1.98	1.78
	4	1.93	1.98	2.14	1.79
	8	1.95	1.92	2.02	1.79

* For $\bar{x}$=12; 4 rates and 3 replications.

Rate, Carrier, Volume and Rate, Date, Volume Interactions

"Rate x carrier x volume" and "rate x date x volume" interactions produced no significant differences in the sixteen and seventeen comparisons, respectively, for Tests 1 and 2 over the three year period, Tables XX. and XXI. Data are not presented because of the lack of differences and the difficulty of interpretation.

Table XX. Values of "F" for "rate x carrier x volume" interactions for the listed spring wheat characters in Test 1. The four rates were applied in two carriers, each of which were used in four volumes.

Year	1950	1951
Measurement	"F" Values*	
Yield, Bushels per acre	.49	.54
Wt. of seed in 10 hds, Gms.	.78	1.33
Heads per plot, No.	.21	.58
Wt. per 100 seed, Grams	1.50	1.03
Seed in 10 heads, No.	.70	.79
Spikelets in 10 heads, No.	.63	.26
Spikelets sterile, Percent	.72	1.13
Seed/fertile spikelet, No.	.14	.79
* Necessary F, for P=.05	2.19	2.19

Table XXI. Values of "F" for the "rate x date x volume" interactions for the listed spring wheat characters in Test 2. The four rates were applied at each of two stages of growth in four volumes.

Year	1949	1950	1951
Measurement		"F" Values*	
Yield, Bushels per acre	1.14	.83	1.00
Wt. of seed in 10 hds, Gms.		.59	.99
Heads per plot, No.		.40	.65
Wt. per 100 seed, Grams		1.57	1.03
Seed in 10 heads, No.		.45	1.42
Spikelets in 10 heads		1.62	.30
Spikelets sterile, Percent		.44	1.45
Seed/fertile spikelet, No.		.54	1.20
* Necessary F, for P=.05	2.04	2.08	2.08

OBSERVATIONAL RESULTS AND DISCUSSION

Following the first known occurrence of the sterility injury or damage in 1948, additional cases have been reported in each subsequent year through 1952.

The damage to the wheat was observed to be an excessive amount of floret sterility in which the ovule did not develop into a mature seed. This sterility was accompanied by almost normal development of the other flower parts. The ovule, stigma and anthers in sterile florets appeared to be normal for the stage of growth at the time of flowering. The degree of sterility within damaged areas remained fairly constant, except that occasional tillers seemed to escape.

This damage remained constant throughout the variation of conditions which will be found in most fields. There was one exception to this observation where the wheat appeared to exhibit less sterility in the low spots.

All damaged fields contained normally fertile and sterile streaks or strips of wheat running through the fields in relatively straight lines. These streaks are explained by the fact that the applicators were not obtaining an even distribution of the spray material, Table XXII.

These streaks were easily visible in mature wheat because the sterile heads were much more erect and were darker colored than the normally fertile heads. This darker coloration also included the straw to such an extent that the stubble in sterile streaks could be identified easily.

Table XXII. Summary of the information obtained from ten farmers whose wheat was sprayed by eight different flight operators in in 1950. In all cases the wheat exhibited a high percentage of sterile florets. Numbers following the measurement represent the number of cases in which the particular response occurred.

Variety
Rescue-3, Marquis-5, Ceres-2, Thatcher-1, Mida-1.
Relative seeding date
Early-3, average-6, late-1.
Previous cropping
Fallow-9, wheat-1.
Growing conditions
Good-9, average-1.
Date sprayed
June 8-1, June 17-1, June 23 to 29-7, unknown-1.
Stage of growth
Pre-boot-8, boot-1, heading-1.
Formulation of 2,4-D
Isopropyl ester-2, butyl ester-6, unknown-2.
Rate of 2,4-D, pounds per acre
.2 to .3-6, .3 to .4-1, unknown-3.
Carrier
Water-3, oil-7.
Volume of carrier, gallons per acre
Water: 1.5-2, 3-1.
Oil: 75-1, 1-5, unknown-1.
Nature of injury
Sterility-10.
Acres damaged
Less than 100-3, 100 to 200-4, 200 and above-3. Total, 2532 acres
Estimated yield reduction, percent
20 to 30-3, 30 to 40-3, 40 to 50-2, more than 50-2.
Damage in streaks
Yes-10.
Differential weed kill noted between damaged and undamaged areas
Yes-4, No-6.
How applied
Aircraft-10.

Where Russian thistle occurred in the fields, control was good in damaged strips and poor in undamaged areas. Where only 2,4-D susceptible mustard species occurred, control was good in both damaged and undamaged areas.

The injury for the most part has been confined to somewhat specific areas each year. In 1948 and 1951 most of the reported damage was confined to the northeastern part of the state, while sterility in 1949, 1950 and 1952 occurred predominately in the triangle area of northcentral Montana.

This injury has also been reported from North and South Dakota in 1948 and 1951, and a considerable number of cases were reported in southern Alberta in 1952 (7).

The actual acreage so damaged is not known, but it is not large when considered as a percent of the acreage treated by low volume methods. However, where this damage has occurred considerable loss has been suffered by individual operators. Several fields have not been harvested because of the damage. The largest single loss reported to date was an estimated 16,000 bushel loss from an 800 acre job where damage occurred.

Certain characteristics of damaged fields were constant throughout. These characteristics are described in the following paragraphs.

The fact that reported cases of injury have more or less been confined to a certain area in a single year would seem to indicate that a certain combination of environmental conditions is necessary for the expression of the injury.

Of the environmental conditions which do vary between years and areas, the most obvious are temperature and rainfall. These two factors in turn will influence the growth and the development of wheat in many ways. In a number of damage cases maximum and minimum temperatures were obtained from the weather station nearest the injured field. Of fifteen cases where dates of application were known, eleven fields were treated within two days following a minimum temperature of below 40° F. Temperatures preceeding the six dates of experimental applications, by two days or less, at Bozeman indicated that temperatures of below 40° F occurred on three of the six application dates. Injury to experimental plots was evident on one of the three dates.

This would seem to indicate that temperatures below 40° F preceeding application may help predispose spring wheat to the injury. This is supported to some extent by a flight operator's observation that the injury cases, which his organization was involved in resulted when applications were made shortly after a period of cold, cloudy weather.

However, it should be noted that many fields were treated in the same general areas on dates when injury occurred. Thus it would appear that some other factor is also involved in the injury.

The only crop which has been affected is hard red spring wheat. Why winter wheat, oats and barley, with the possible exception of one field of barley, have not been affected is not known.

Since a number of varieties of spring wheat have been injured it would appear that there is little difference in the tolerance of varieties commonly grown in the state. In field injury cases Rescue, Thatcher, Ceres, Mida and Marquis have suffered injury. Sexsmith (7) reported injury to Saunders, Red Bobs, Thatcher, Marquis, Redman and Rescue. One damaged field of Marquis was surrounded with a Rescue border. In this field there was no visible difference in the degree of sterility between varieties. The variety, Rescue, has been considered by some to be more susceptible than others, but this does not seem to be true in relation to the cases of injury observed by the writer.

In all cases of damage which have come to the author's attention, the 2,4-D application has been made in volumes of three gallons per acre or less. The low volumes were applied by aircraft and turbine type sprayers.

However, since this injury has been noted only on a small percentage of the spring wheat treated with 2,4-D in low volumes of carrier, it is difficult to say that low volume is responsible, or to condemn the use of low volumes in 2,4-D application. The experimental results seem to justify this theory in that decreasing volumes decreased yields significantly in only one year out of three, Tables V. and VI. Holly's (4) hypothesis that differential deposition and absorption will occur may be an explanation for the variability between years.

In 1951 when typical injury symptoms were induced in the experimental plots, volumes up to eight gallons per acre did not produce differences. In addition, the interactions of volumes with dates, carriers and rates were not significant in most cases.

In conclusion it would appear that low volumes of carrier for 2,4-D application are not in themselves directly responsible for the injury.

Improved weed control in damaged areas in some fields is evidence that the damaged strips received a higher rate of 2,4-D than did the undamaged. Rates of 2,4-D, therefore, are important in the expression of the damage. The rates of 2,4-D in general use in the area approximate .3 pounds. Visible injury was apparent in the .2 pound rate in the experimental injury case. Thus it seems certain that the injury can be induced at 2,4-D rates of less than .5 pounds per acre. This rate, according to unpublished Montana Agricultural Experiment Station data, could not be responsible for the serious yield reductions unless some additional factor or factors which increase the susceptibility of the wheat plant to 2,4-D applications were present.

The effect of increasing rates on the experimental yields in all three years appeared to be somewhat larger than the expected effect of the rates used. This was particularly true in 1951 when injury was obtained. The effect of increasing rates on yields in the experimental injury case was severe in that the highest rate reduced yields to about fifty percent of the check.

Since 2,4-D is necessary to induce the injury and high rates increase the degree of injury, it appears that rates are important in the injury, and that much of the damage has occurred at rates well below those sometimes used in field applications.

Carriers commonly used in low volume application are No. 2 diesel fuel, stove oil and water. Injury has resulted under field conditions when these carriers have been used. Most injury cases have resulted where an oil has been used as the carrier. However, since most applications have also been made with one of the fuel oils as a carrier, it is impossible to theorize on the relative occurrence of injury with the different types of carriers in Montana. Sexsmith's (7) report indicated that all injury cases which he studied occurred where an oil was used as the carrier.

In the experimental comparison of oil and water as carriers no differences were observed in 1950, Table VI. In 1951, injury resulted where oil was used as a carrier, while no injury occurred as a result of the application with water as a carrier when applications were made on the same date, in the same volumes and with the same rates of 2,4-D. This is a clear cut difference due to the carrier. Why injury was induced by the use of the oil carrier is not understood, but it would appear that differential herbicide deposition and/or entry into the plant is involved. It is entirely possible that the better wetting ability of the oil solution of 2,4-D over the water emulsion caused the difference.

Oil applications at 1, 2, 4 and 8 gallons per acre were made without 2,4-D (0 rate) in each of three years. Volumes as great as 8 gallons per acre caused severe burn in some cases, but did not reduce yields, Tables X and XI. This indicates that the experimental injury which occurred where oil was used as a low volume carrier is not due to the toxic effects of the oil itself.

Under field conditions many cases of leaf tip burn of wheat leaves have resulted where applications have been made in oil. A few of these have developed into injury cases, but the majority have not.

This evidence leads to the belief that injury is somewhat more likely to occur where an oil carrier is used, but since injury has also occurred where water has been used as a carrier it would seem that an oil carrier is not a necessary prerequisite for the induction of the injury.

Since it is known that extensive sterility can result from 2,4-D applications at the stages of growth when the flower parts are nearing maturity, one of the objectives of the observations of commercial field injury cases was to determine the stage of growth of the wheat when the 2,4-D application was made. In cases where information on stage of growth and date appeared to be reliable, it was determined that, in all but one of the cases observed, 2,4-D was applied within the tiller-boot interim of wheat plant development. No cases of spikelet malformation or other abnormalities were observed in any field, so apparently all applications were made at stages of growth after tillering was complete.

The injury which was sustained by the experimental plots in 1951 occurred with an application made on June 20 to tillered spring wheat about seven inches tall. Sexsmith (7) induced typical injury in 1949 in his nursery plots at nearly the same stage of growth with a 2,4-D rate of .5 pounds per acre. A considerable percentage of the field injury cases has resulted when applications were made two to three weeks before heading. This stage is later than the growth stage in the experimental injury case. Sexsmith also reports that most damage cases occurred where 2,4-D applications were made within the recommended rates of 2,4-D at stages of growth normally considered to be safe.

It is concluded that this particular injury can be induced at a number of growth stages.

The establishment of the thesis that injury to spring wheat can result from 2,4-D applications at normal rates and at stages of growth considered to be safe is important in making recommendations for the use of 2,4-D. In addition, injury resulting in sterility at tolerant growth stages is evidence that either the susceptibility of spring wheat can be greatly affected under certain conditions or that certain mechanical factors governing selectivity vary widely.

In conclusion it appears that the injury to wheat is the result of the application of 2,4-D in low volumes of carrier in the presence of a certain set of environmental factors which are not definitely known. It does appear that temperatures below 40° F may be one predisposing factor. However, certain additional factors, environmental or otherwise, seem to be necessary to fully explain the occasional severe yield reduction in spring wheat which results from the 2,4-D injury.

SUMMARY

The yields of hard red spring wheat in a number of commercial fields have been reduced greatly in the state in each year since 1948 due to treatment with 2,4-D.

These severe yield reductions have been the direct result of an abnormally high incidence of florets which produced no seed. Within fields the injury has also occurred in strips or streaks, which were due to uneven distribution of the spray material.

The only crop on which injury has been reported is spring wheat. Rescue has been considered by some as being the most susceptible variety, but proof is lacking.

All known cases of injury within the state have occurred where 2,4-D was applied in low volumes of carrier. However, since the damaged acreage is small in relation to the acreage treated by low volume methods, it is apparent that low volumes in themselves are not responsible for the injury. The effects of 2,4-D in volumes of carrier ranging from 1 to 8 gallons per acre were not consistant in reducing yields in experimental trials.

Rates of 2,4-D as low as .2 pounds acid equivalent per acre can induce the injury and higher rates will increase its severity. Most cases of injury under field conditions have resulted when 2,4-D rates approximating .3 pounds per acre were applied.

Most injury cases have occurred where a fuel oil was used as a carrier but several cases have also been reported where water was employed as the carrier. In the one experimental trial when the injury was induced, injury

was limited to the use of oil as the carrier. Water applied under the same conditions did not induce injury. As a result it would appear that the use of oil as a carrier is somewhat more likely to induce injury than is water.

Detailed measurements on the effects of the injury in the experimental plots were recorded and observed to be associated with a high percentage of sterile spikelets, more sterile florets within fertile spikelets and reduced weight per 100 seed. The injury was not observed to be associated with number of heads per plot.

Experimental applications were made at two dates in each of the three years. With the exception of the injury case at the early stage of growth in 1951 no differences due to dates were apparent. It appears that this injury can occur at a number of stages of growth when spring wheat is usually the most tolerant to 2,4-D applications.

Apparently this injury is the result of a complex of factors, some of which are unknown, involving the application of 2,4-D in low volumes of carrier. Temperatures below 40° F preceeding application and certain other environmental conditions probably assist in predisposing spring wheat to the injury.

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