THE RETENTION AND EFFECT OF 2,4-DICHLOROPHENOXYACETIC ACID
(2,4-D) SPRAYS ON WINTER WHEAT

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INTRODUCTION

When chemicals were first used to control weeds is not known. However, nonselective materials, such as salt, ashes, and various industrial by-products, have been used for centuries to control and eliminate vegetation in fence rows, pathways, and along roadsides. The use of chemical sprays for the control of weeds in cereal crops was started in the latter part of the nineteenth century with the discovery of the selective herbicidal action of such inorganic chemicals as iron sulfate, copper sulfate, sodium arsenite, and sulfuric acid. The discovery of the selectivity of these compounds is believed to have been made accidentally in trials of copper salts on cereal crops for the control of fungus diseases. The copper salts killed or seriously injured the broadleaf weeds with little or no injury to the cereal crops. These chemicals were used successfully for a period of about 50 years to control such broadleaf weeds as mustard, cockle bur, ragweed, dandelions, daisies, and wild lettuce.

The new and present-day era of selective weed control with the growth regulating chemicals started about 1944 when 2,4-dichlorophenoxyacetic acid (2,4-D) came into prominence for its weed killing properties. Far greater selectivity is possible with these new organic compounds than with the old inorganic salts used prior to 1944. However, even with the plant-growth regulators, the interception and retention of

herbicidal sprays is still a factor of prime importance but one which has received very limited study.

This study was conducted to determine the amount of a 2,4-D spray which was retained by a cereal crop under field conditions. The scope of this investigation included a study of the factors affecting the retention of 2,4-D sprays by winter wheat and the effect of these sprays upon the growth and yield of the plants.

REVIEW OF LITERATURE

The Development of a Spray Program

Contact Herbicides: The discovery of selective herbicides probably was made by Bonnet, a Frenchman, in 1896. He noticed that many weeds in cereals were killed or severely injured by the application of copper salts which did not injure the grain. He further observed that plants with broad rough leaf surfaces and exposed growing points were readily killed by certain chemical sprays, whereas other plants with narrow smooth leaves and well protected growing points were only temporarily injured. In 1898 this same worker, cooperating with Brandin and Ducos, employed dilute sulfuric acid successfully as a selective herbicide. Rebate (77) continued this work in France with sulfuric acid and in 1911 made recommendations for the spraying of both winter and spring cereals to control broadleaf weeds.

Selective spraying of wheat in North Dakota was started in 1896 by Bolley (12). This was probably the first use of selective herbicides in this country. The chemicals commonly used by Bolley

were iron sulfate, copper sulfate, corrosive sublimate, and sodium arsenite. The recommended rates per acre were 75 to 100 pounds of iron sulfate, 12 to 14 pounds of copper sulfate, or $1\frac{1}{2}$ pounds of sodium arsenite at a volume of 52 gallons per acre. These rates were sufficient to destroy mustard and many other annual weeds in cereals without doing any serious damage to the grain. These treatments gave substantial increases in yield when used on severely infested grain fields.

Some of the other early investigators who worked with selective herbicides in this country were Olive (68) of South Dakota and Moore and Stone (65) of Wisconsin. These men worked chiefly with iron sulfate and recommended its use as a 20 per cent solution at a volume of 52 gallons per acre applied when the wheat was 6 to 12 inches high and the mustard plants were still small.

Cereal crops seemed to be the ideal ones for the use of selective herbicides. Many other workers became interested in chemical weed control during the period 1896 to 1912. Practically all of the investigators reported success in their experiments. However, after about 1912 interest in the control of annual weeds in cereals lapsed in America. The factors contributing to this declining interest were the lag in production of adequate spray machinery, the frequent lack of success because of low humidity, the large volume of water required in spraying, and the immense scale upon which grain farms were operated, thus precluding the possibility of completing a successful spray program within the short time during which both weeds and

cereals were in the proper stages for treatment. It was not until 20 to 30 years later that any real interest was shown in selective weed control in the United States.

However, in Europe, where the agriculture was of a more intensive nature, the interest in selective weed control in grain fields grew steadily. Extensive investigations were carried out in Italy, France, and Norway. In 1927, Aslander (4) reported the results of a greenhouse study on the effects of environmental factors on plants sprayed with herbicides. His results merit mention because the factors he studied are important with our present-day herbicides. He studied the effects of soil moisture, atmospheric humidity, temperature, and artificial rain on field mustard (Brassica arvensis) and Cornelian oat (Avena sativa) plants treated with iron sulfate and sulfuric acid. Aslander further determined that mustard plants grown in dry soil had a much more compact anatomical structure than those grown in medium moist or wet soil. He believed that this characteristic accounted for their greater resistance to herbicidal sprays. Iron sulfate sprays were found to be most effective in an atmosphere at about 100 per cent relative humidity while sulfuric acid gave the best results in dry air. Temperature also had a marked influence upon the effect of sprays. At 30° C. a 2 per cent solution of sulfuric acid killed the mustard plants in one hour while at 6° C. the same effect was obtained only after five hours. A 15 per cent solution of iron sulfate was more effective when sprayed at a lower temperature, as the evaporation of the water and crystallization of

the sulfate was very slow, thus giving the solution a longer time to act. Artificial rain, produced by sprinkling, applied to the plants one hour after they had been sprayed with a 2 per cent solution of sulfuric acid failed to decrease the effect of the spray. Plants grown in moist soil sprayed with a 15 per cent solution of iron sulfate were but slightly harmed when "rain" was applied six hours after spraying. Plants grown in dry soil were unharmed. The oats remained undamaged in the above experiments.

Research in selective weed control in the United States lagged far behind that being carried on in England and France from 1912 to 1940. However, a slight and temporary revival of interest occurred in this country in the decade 1920 to 1930. In this period Brown and Streets (15) recommended the use of sulfuric acid for the control of broadleaf weeds in grain fields of Arizona. During the same period Bissey and Butler (7) in New Hampshire ran experiments on the control of mustard, which was the most common weed in grain fields. In an effort to find a chemical with a high degree of toxicity per unit weight and also relatively non-corrosive, a group of ten of the inorganic chemicals commonly employed for weed control at that time was tested under controlled conditions.

Blackman (8) carried out replicated experiments in 1932 and 1933 to determine the efficiency of dilute sulfuric acid and copper sulfate for destroying annual weeds in cereal crops in England. He reported good results from either 9.2 per cent sulfuric acid or 5.0 per cent copper sulfate applied at a rate of 100 gallons per acre

when the weeds were sprayed in the seedling stage. Results of his work showed that the control of weeds resulted in increases in yields up to 75 per cent, depending to a great extent on the amount of infestation. This work was continued in England during 1934 and 1935 by Blackman and Templeman (11). In addition to sulfuric acid, they tested nitric acid, ammonium hydrogen sulfate, sodium hydrogen sulfate, ammonium thiocyanate, as well as the effect of a wetting agent in the spray. The only compound to compare favorably with sulfuric acid was nitric acid and the addition of a wetting agent (Agral 1 at 0.1 per cent) did little or nothing to increase the efficiency of either acid as a herbicide.

The use of inorganic chemicals for selective weed control in cereals reached its peak in the decade 1930 to 1940 in France and England. In France 500,000 acres of cereals were sprayed with sulfuric acid in 1933; in England in 1934 over 20,000 acres of grain were sprayed. The lack of suitable equipment for applying dilute sulfuric acid was one of the factors which retarded the use of that chemical as a herbicide in the United States during the same period. Ball and French (5), working in California, developed what they considered to be a successful sprayer for this purpose in 1935.

The first really new selective herbicide to appear on the market was Sinox (sodium salt of 4,6-dinitro-0-cresol). It was released by French workers about 1938. Sinox was capable of killing many broad-leaf plants on contact without injuring the grasses. The discovery of this new herbicide rekindled an interest in selective chemical

weeding, and it was soon used in this country for weed control in winter and spring grains, flax, and some vegetable crops. Harris and Hyslop (42) conducted extensive experiments in Oregon with Sinox and a mixture of ammonium sulfate and Sinox in equal parts. They found the spraying of weed infested fields of wheat and other grain crops to be highly successful in controlling such annual weeds as fireweed or fiddleneck (Amsinckia intermedia) and common mustard (Brassica arvensis) and in increasing grain yields.

Growth Regulator Herbicides: About 1939 organic chemists, working with plant physiologists, began to study a new group of organic compounds called plant hormones or growth regulators. Some of the early uses of these compounds were to prevent pre-harvest drop of apples, to encourage rooting of cuttings, and to aid in the ripening and coloring of fruit. It was not until about 1942 that these new compounds were given serious consideration as herbicides. The outstanding difference between these new herbicides and the old contact herbicides is that in many plants the newer ones are translocated readily without the plant cells being killed. An extensive research program for the development of these materials as herbicides was started simultaneously in the United States and England in 1943. However, due to security regulations connected with World War II the results of these investigations were not published until the end of that War.

The first and by far the most important of these compounds to attract attention was 2,4-D. It has been the most widely used and is

still the most important selective herbicide. Some of the other compounds to receive attention were 2-methyl-4-chlorophenoxyacetic acid (MCP), 2,4,5-trichlorophenoxyacetic acid (2,4,5-T), and isopropyl-N-phenylcarbamate (IPC). Thousands of these growth regulator substances have been synthesized and their properties studied, but none has replaced the original ones as general herbicides. This is especially true of 2,4-D as a selective herbicide to control broadleaf weeds in grasses.

The experimental use of plant-growth regulators as herbicides in England was started in 1943. In that year the experiments were confined to weed control in oats. The experiments were expanded in 1944 to include both oats and barley, and in 1945 extensive studies were made on wheat, oats, and barley. Templeman and Halliday (87) used both MCP and 2,4-D in their investigations in England. They applied the sodium salts of these two compounds as both sprays and dusts at rates of 1,2,4, and 8 pounds per acre in aqueous sprays at 100 gallons per acre or in calcium carbonate dust at 2 cwt. per acre. The time of application varied from pre-emergence to a height of 16 to 24 inches for the cereal plants. Some of their general conclusions were as follows: (1) Spraying gave generally better results than dusting; (2) MCP was generally more effective than 2,4-D; (3) Pre-emergence applications were little affected by the weather on the day of application, but when the weeds were already through the soil heavy rainfall on the day of treatment seemed to reduce the degree of control; (4) There was no damage to the plots by any of the treatments as shown by the grain yields of the plots at time of harvest; and (5) Two pounds

per acre of MCP gave good control of most of the annual weeds found in the grain fields.

Between 1943 and 1947 Blackman and Roberts (9,10), also working in England, made simultaneous comparisons as to the relative effectiveness of sulfuric acid, cupric chloride, dinitro-O-cresol, and the two growth regulating substances, MCP and 2,4-D, for the destruction of annual weeds in winter wheat. As a result of weed eradication, increases in grain yield ranging from 6 to 113 per cent were recorded, and for 12 experiments the average increase for the most appropriate treatment was 23 per cent. The results of two experiments with the growth regulating substances indicated that the greatest selectivity was obtained when the rate did not exceed 2 or 3 pounds per acre.

Other than the work carried out at Camp Detrick and Beltsville, Maryland, the use of growth regulators for large scale field experiments was not started in the United States until about 1946. Most of the herbicidal work in this country has been done with 2,4-D, while little use has been made of MCP.

To develop a sound spray program from which recommendations for effective weed control without danger of serious injury to the grain crop has required several years of extensive investigations. The growth regulators were new and relatively little was known of the physiology of their mechanism of entry, translocation, or action on plants. Research on all phases of the program has made good progress, but there is still a need for much study on many of the fundamental aspects in order to clarify many puzzling questions now arising from the widespread use of growth regulator herbicides.

The solubility of pure 2,4-D in water is too low to allow for its use as a spray material. As it began to come into general use as a herbicide three types of formulations became available on the market. These were salts, amines, and esters with several formulations in each group. It soon became apparent that gross differences could result from the application of equal rates (calculated on an acid equivalent basis) of the various formulations and some smaller differences could occur between the different salts, amines, or esters. Greater damage to the grain crop from the esters was reported by Helgeson (43), Helgeson, et al. (45), Olson (69), Overland and Rasmussen (72), Templeman and Wright (89), Friesen (41), Longchamp and Gautheret (55), and Murphy (66). Most of the same authors also reported the amines to be intermediate between the salts and esters in their herbicidal activity. Dunham, et al. (28), Phillips (73), Staniforth and Atkins (84), Belzile (6), Carpenter and Dickson (18), and Longchamp, et al. (56) reported equal weed control or crop injury from using the esters at only one half of the rate used for the salts and amines. As a result of the work reported above and large amounts of unpublished data, the esters are now considered to possess more potent herbicidal properties and are generally recommended at about one-half the normal rate for salts or amines.

When 2,4-D was released and placed on the market for general use as a selective herbicide in 1946, many of the spray applications were made in large volumes of water similar to those previously used for the inorganic contact herbicides. For example, Klingman (49)

conducted a series of experiments on spring wheat, oats, and barley in 1946 in which he applied, 1, 2, and 3 pounds per acre of 2,4-D in 250 gallons of water. This large amount of water required for spraying had always been one of the serious objections to a successful spraying operation for large acreages. However, it was not long until investigators began to study the use of low volume sprays. New types of sprayers had to be developed in order to apply these low volume sprays evenly and successfully. The results of spray applications of 5 to 10 gallons per acre generally gave excellent results and volumes of this magnitude were quickly adopted for commercial spraying. However, there is an almost complete absence of published experimental data to either prove or disprove their value as compared to high volume sprays. In some recent work Holly (46) found that approximately 3.5 times the amount of agent was deposited on barley at 10 gallons per acre as at 175 gallons per acre, however, the phytotoxicity was similar for the two volumes. The fact that good weed control can be obtained with only 5 or 10 gallons of water per acre, accounts for the extreme usefulness of growth-regulator herbicides for spraying large acreages.

One of the first points to come to the attention of investigators was the relative tolerance of cereal crops to 2,4-D at different stages of growth. A number of extensive studies have been conducted in order to determine the effects of 2,4-D application at all stages of growth from pre-emergence to the dough stage. Pre-emergence and fall applications on winter cereals have not been generally satisfactory and serious yield reductions have been reported by Willard (96),

Fraser (37), Phillips (74,75), Slife, et al. (82), and Chabrolin (19). Applications at very early stages of growth on spring cereals have been equally unsatisfactory as shown by the work of Derscheid (24), Friesen (41), Friesen and Olson (40), Olson, et al. (71), Andersen (2), and Foster (36). Not all of the investigations are in complete agreement as to the so-called susceptible or resistant stages in the growth of cereal plants. However, investigators, in general, agree that cereal plants are susceptible to 2,4-D from the time of emergence to early tiller stage. A second susceptible period soon became evident about the late joint, boot, and early head stages of growth. Drastic yield reductions have been reported at these stages even from the use of very low rates. This general period of susceptibility has been reported by Andersen (2), Klingman (49), Helgeson (43,44), Helgeson, et al. (45), Derscheid (23,24), Fraser (37), Friesen (41), Friesen and Olson (40), Olson (70), Olson, et al. (71), Phillips (75), Slife, et al. (82), and Warden (93). There is general agreement among investigators that there are also two more or less resistant periods in the development of cereal plants. The first of these periods is between the two susceptible ones just previously described. It is usually considered to begin when the tillering stage is reached and to last until early boot stage. The other resistant period is a post-fertilization one which lasts from the milk stage until harvest time. Some damage can be done by heavy applications of ester formulations during the first resistant period but there is little, if any, danger of doing appreciable damage to the crop during the post-fertilization period.

The establishment of rates of application has received as much attention and has been as difficult to establish definitely as any other phase of the program. It has been difficult to make general recommendations that will be suitable in all cases because of the effect of environmental factors, variations in formulations, varietal differences, weed population, etc. Rates varying all the way from 1/8 to 3 pounds acid equivalent per acre have been tested. As a rule, rates of $\frac{1}{4}$ to $\frac{3}{4}$ pounds acid equivalent per acre, depending upon the formulation which is used and the weed species present, have given satisfactory weed control with little or no damage to the cereal. Results of this kind have been reported by Belzile (6), Bowman and Talley (13), Clydesdale (21), Dunham, et al. (28), Elder (30), Freed, et al. (38), Lindsey (54), Murphy (66), Overland and Rasmussen (72), Phillips (73,75), Slife, et al. (82), and Zadow (99). The above rates are for cereals not seeded to legumes and may do serious damage to legumes in cereals as reported by Leefe (52), Olson (69) and Slife, et al. (82). However, some recent tests with MCP on cereals seeded to legumes by Marshall (57), Marshall and Van Geluwe (58), and Van Geluwe and Marshall (92) have given hopeful results since legumes appear to be more tolerant to MCP than to 2,4-D.

Substantial yield increases can be expected from proper treatments with 2,4-D where broadleaf weeds are a serious problem. Carpenter and Dickson (18), Elder (30), Templeman and Halliday (88), and Tingey (91) have reported yield increases of 10 to 25 per cent and net returns of as much as \$8 per acre from spray applications of 2,4-D.

This seems in line with the work of Wolcott and Carlson (98) who reported a 30 per cent reduction in the yield of spring cereals from either a 28 per cent infestation of quack grass or a 53 per cent infestation of Canada thistle. Of course, the increase that may be expected as a result of weed control is dependent upon the initial degree of weed infestation, on the supply of moisture and plant nutrients in the soil, and on other local factors.

With widespread use of 2,4-D, it was observed that some varieties appeared to be more susceptible than others. Andersen (2), Derscheid, et al. (26,27), Foster (36), Friesen (41), Krall (50), Middleton, et al. (64), Olson (69), Phillips (73), Staniforth and Atkins (84), and Warden (93) have reported differential responses in cereal varieties. However, Helgeson, et al. (45), Slife and Fuelleman (81), and Woestemeyer (97) reported that the yield response to treatment was the same for all varieties studied. A close study of all the reports indicates that the stage of growth at time of treatment, rate of application, and temperature may be factors which largely determine whether or not varietal differences can be observed.

With the exception of the recent work of Holly (46) there is no report in the literature of a study of the amount of retention of herbicidal sprays by cereal plants. It is not unreasonable to believe that an extensive investigation of this factor might give some help in explaining the frequent variation in results obtained from a given amount of 2,4-D.

The work of Fogg (35) showed that distinct and sometimes considerable variations in the degree of wetting of leaves by water may occur

not only between species or varieties but also from place to place on the same plant and within a short period time on the same leaf. Further, he determined that the magnitude of the contact angle is one of the major factors affecting the amount of water retained on a leaf since it determines the form of the droplets and hence their behavior. When the contact angle is high, the droplet is nearly spherical and consequently is easily displaced. The squat drop produced when the contact angle is low has a large area of contact and a center of gravity situated very close to the leaf surface making it more stable and, as a result, more water is retained on the leaf. This area of contact between a leaf surface and water or a solution deposited on it is an important factor in determining the amount of dissolved substances entering or leaving the leaf.

Ennis (31) suggested that the varying effectiveness of herbicides in different carriers might be assignable in part to unlike retention of the sprays by the foliage. In a later study with soybeans, Ennis, et al. (33) showed that spray retention differences attributable to leaf type, carrier, and leaf angle were highly significant. The pubescent leaves retained more droplets than the glabrous leaves. More Carbowax 1500 spray than wholly aqueous spray was retained by leaves of both types, and leaves in a horizontal position retained more spray than those at a 45° angle from the horizontal.

Holly (46) found that with barley there was a directly proportional reduction in volume of spray retained per plant as the volume-rate of application was reduced from 175 to 43 gallons per acre. Further reduction in volume to as little as 10 gallons did not appreciably

reduce the volume of spray retained by the plant. When a fixed amount of toxicant was applied, the amount retained per plant did not vary significantly at volumes between 175 and 43 gallons per acre, owing to the proportion of spray retained remaining unchanged. However, below 43 gallons per acre, this proportion increased; in consequence, the amount of toxicant deposited on the plant increased considerably until at 10 gallons per acre it was approximately $3\frac{1}{2}$ times the amount deposited at 175 gallons per acre.

There are several reports in the literature of the addition of surface-active or wetting agents to aqueous sprays of 2,4-D and without exception their herbicidal effectiveness has been increased by these substances. Ennis and Boyd (32) reported the addition of Carbowax to aqueous-spray solutions of 2,4-D enhanced its activity in inhibiting growth of kidney-bean plants. Thimann (90) found that the addition of Carbowax definitely increased the toxicity of aqueous 2,4-D sprays on some woody plants. Laning and Aldrich (51) and Staniforth and Loomis (85) also reported increased effectiveness of herbicidal sprays from addition of wetting agents. Ennis (31) and Ennis, et al. (33) reported increased retention, and Rice (78), Mitchell and Linder (63), and Bryan, et al. (17) found increased absorption by the leaves of aqueous sprays due to the addition of surface-active agents.

According to the work of McNeal (59), Derscheid (24), Derscheid, et al. (26,27), lower seed weight was not associated with reduced yields of cereals. However, Shafer (79) reported that yield decrease from 2,4-D treatment was due in part to depressed seed weight.

Erickson, et al. (34), Helgeson, et al. (45), and Warden (93) have reported increased protein content as a result of 2,4-D treatments regardless of a decrease or increase in grain yield. This is not in complete agreement with the work of Phillips (73,74), Phillips and Shellenberger (76), and Shellenberger, et al. (80) who reported that an increase in protein content was always accompanied by a reduction in yield and that in no case was there an increase in total protein per acre. Overland and Rasmussen (72) reported that the application of 2,4-D had no effect on the nitrogen content of the two wheat varieties studied.

The effect of 2,4-D on the milling and baking qualities of wheat has been studied by Friesen (41), Phillips (73,74), Phillips and Shellenberger (76), and Shellenberger, et al. (80). None of these workers found any serious effect on the quality of wheat from 2,4-D applications.

It is known from the work of Allard, et al. (1) and Hsueh and Lou (47) that 2,4-D will check germination and prevent normal seedling development of the cereal crops. Lower germination of wheat treated in late joint, boot or bloom stages was reported by Helgeson, et al. (45) and Phillips (74). These results suggested that perhaps sufficient 2,4-D might be translocated to the seed to prevent normal germination. However, Phillips (73), Templeman and Halliday (88), Derscheid (24), Derscheid, et al. (26,27), and Derscheid and Kratochvil (25) found that the germination of wheat from plots treated with 2,4-D was not impaired even by a heavy rate of application. Likewise, there were no indications that any of the 2,4-D treatments had any effect upon the percentage of

normal seedlings or the yield of grain when seed from treated plots was planted the following year.

Most herbicides, especially the growth-regulator ones, must gain entry into the plant in order to be effective. Norman, et al. (67) suggested that many of the cases of specificity, both as to herbicide and plant species, relate to the factor of entry into either the foliage or the root. Crafts (22) has suggested that nonpolar compounds can pass through the cuticle of leaves readily, whereas polar compounds enter with difficulty. The data indicate that nonpolar phenols in water, or in oil, are more toxic than their salts in water. Early testing confirmed this mechanism for 2,4-D compounds; that is, the esters were more toxic than the ammonium salts, which in turn were more toxic than the sodium salt. He gave a valuable generalization concerning absorption of herbicides which was: for penetration of the cuticle, and absorption by foliage, nonpolar compounds should be used; however, materials are absorbed by roots principally as ions from an aqueous environment so that in soil applications the water soluble and ionizable polar compounds might be expected to be more effective than relatively insoluble and nonpolar compounds.

The usual method of entry for 2,4-D is described by Norman, et al. (67) as penetration through the epidermal surface by (a) diffusion as a vapor through the stomates, (b) mass movement as a liquid through the stomates, or (c) diffusion through the cuticle and epidermal walls. Stomatal entry might account for the superiority of volatile esters of 2,4-D over the non-volatile acid and salts. However, diffusion through the cuticle is probably the most important means of entry.

According to Fogg (35) the amount of a solution which enters a leaf is a function of the time and area of contact; both factors are related to the degree of wetting of the surface and the contact angles of discrete droplets, if present. He also noted positional and species differences in wettability of leaves, but these have not been correlated with studies on herbicidal entry. The role of leaf hairs in entry may be important as indicated by Ennis and Boyd (32) and Ennis, et al. (33). The response to 2,4-D has been reported to be greater at low pH than at high pH by Clark and Freiberg (20), and Mitchell, et al. (60). It seems possible that the greater response at a low pH might be brought about by an increase in the amount of entry into the plants. The work of Bryan, et al. (17) suggested that some type of chemical or enzymatic reaction may limit the absorption of 2,4-D by leaves.

The 2,4-D which gains access to superficial cells rarely, if ever, remains entirely restricted to these cells but generally becomes widely distributed. The mechanism for its translocation depends upon factors such as point of application, stage of development of the plant, and environmental conditions. All water soluble substances will spread to some extent by diffusion but the velocity of this process is so low that it probably plays only a very minor role in the movement of 2,4-D. Weaver and DeRose (94) have shown that 2,4-D passes upward but not downward through dead segments of stems, and Mitchell and Brown (62) found when 2,4-D was applied to the root system it was translocated through non-living cells of the stem, which indicated that it probably traveled upward in the transpiration stream of the Xylem. The work of

Weintraub and Brown (95) showed that 2,4-D introduced into the transpiration stream may be carried through non-living regions of stem or petiole and is independent of movement of sugars, but when the 2,4-D is applied to the surface of the leaves it is conducted via the phloem by the same mechanism which governs translocation of endogenous metabolites. Export of the 2,4-D from the leaf in this fashion appears to depend on concurrent movement of foodstuffs and is hence most rapid under conditions favoring photosynthesis according to the work of Linder, et al. (53), Mitchell and Brown (62), Rice (78), and Weaver and DeRose (94). However, Weintraub and Brown (95), using bean seedlings devoid of translocatable carbohydrates in which the 2,4-D would not move from the leaf, were able to bring about its movement by supplying sugar to the leaves externally. Thus, the translocation of growth regulators seems to require only that the normal mechanism of transport of organic materials be set in operation by whatever means.

The effect of 2,4-D on many plant processes including photosynthesis, respiration, stomatal behavior, water relationships, and nutritive ion absorption and accumulation has been shown. Freeland (39) found that apparent photosynthesis was decreased in bean plants when sprayed with 100 ppm of 2,4-D. He stated that the effect upon the rate of apparent photosynthesis in beans cannot be accounted for entirely by its effect on respiration. The effect of 2,4-D on respiration has been studied by Brown (16), Freeland (39), Hsueh and Lou (47), Kelly and Avery (48), Mitchell, et al. (60), and Taylor (36). The findings of these investigators show that very low concentrations cause a transitory increase in carbon dioxide evolution;

intermediate concentrations produce a permanent increase in respiratory rate which may continue until the plant dies; and high concentrations may bring about a markedly subnormal rate of carbon dioxide evolution which is sometimes preceded by an initial stimulation. Bradbury and Ennis (14) found that top or soil treatments of kidney bean plants with 2,4-D were followed by partial stomatal closure and that this early stomatal closure in treated leaves was a direct effect of the growth-regulator on the leaves rather than an indirect effect on the water-absorbing mechanism of the roots. Brown (16) has shown that the total amount of water absorbed and transpired by sprayed bean plants during 5 days immediately following treatment was less than that for comparable untreated ones, but the treated plants had a higher percentage of moisture than the untreated ones which showed that transpiration was restricted more than absorption. Tiller differentiation in wheat was reduced relatively more than the uptake of nitrogen according to the work of Asana, et al. (3).

Carbohydrate metabolism is frequently affected by 2,4-D treatments. Mitchell and Brown (61) found an increase in sugars and a decrease in starch and dextrin immediately after treatment followed by a diminishing of all three fractions. They suggested that depletion of carbohydrates is a factor resulting in the death of plants which have been treated with 2,4-D, but this view has been challenged by Smith (83) on the grounds that abundant carbohydrate is still present when death occurs. Smith suggested interference with normal phloem function and food transport as the proximal cause of death.

Eames (29) states that the effect of 2,4-D is upon the maturing tissues with the injury becoming evident as the tissues continue development.

EXPERIMENTAL PROCEDURE

The objectives of this investigation were to determine the amount of a 2,4-D spray which was retained by a cereal crop under field conditions, the effect of various factors on retention, and the effect of these sprays upon the growth and yield of the plants. In order to carry out an investigation of this type, it was necessary to devise some means of accurately determining how much of a particular spray was intercepted and retained by the crop plants. It was considered impractical to attempt to measure the actual amount of 2,4-D remaining on the leaves because it has been determined that a part of it moves into the leaves soon after application. The technique finally decided upon was to incorporate into the sprays a water soluble dye which does not penetrate intact plants and one which at the same time can be washed off completely from the plant surface. The dye which proved best for this purpose was duPont Anthraquinone Blue. All spray solutions had 0.4 per cent of this dye, an amount which approaches its limit of solubility, incorporated into them.

The investigations were conducted in 1951 and 1952 at Camp Detrick, Frederick, Maryland. Plot size in 1951 was 4 drill rows with 7 inch spacing and 18 feet long, an area of 42 square feet, and in 1952, 5 drill rows, or $52\frac{1}{2}$ square feet per plot. Plots were protected at time of spraying by a movable spray chamber (figure 1) to prevent

FIGURE 1. - Movable spray chamber for protecting plots at time of spraying

drift of spray material to adjacent plots. The spray chamber was $4\frac{1}{2}$ feet wide, 5 feet high, and 20 feet long, and constructed of a light metal frame covered with a wind-resistant cloth. The spray applications were made with a modified DeVilbiss type MBC spray gun having a Decorator's combination spray head (MBC-231) and veiling cap, and using compressed nitrogen as a source of pressure. A pressure of 10 pounds per square inch was used which produced droplets chiefly in the size range of $\frac{1}{2}$ to 2 mm in diameter. All plots were covered 4 to 6 times in the spraying operation to insure uniform coverage over the entire area.

Two samples, each 1 foot of a drill row selected at random, were cut off at ground level from each plot approximately 10 minutes after it was sprayed. This allowed the spray to dry on the leaves but did not allow the sun to fade the dye. The samples were taken to the laboratory where they were washed in a measured amount of distilled water and an aliquot of the resulting wash solution was used to determine the amount of dye present. The concentration of dye in the solutions was determined with an Evelyn photoelectric colorimeter equipped with a 540 M filter. After these determinations were made, the per cent of dye present in each solution was read from a standard density curve (figure 2) which was prepared by using solutions of known concentrations of the dye. The amount of dye retained by the plants was determined from the per cent of dye in the wash solutions and this was used to obtain the per cent of retention for the dye. The per cent of retention for 2,4-D was assumed to be the same as it was

FIGURE 2. STANDARD DENSITY CURVE FOR DU PONT ANTHRA-
QUINONE BLUE DYE USING AN EVELYN PHOTOELECTRIC
COLORIMETER WITH A 540 M FILTER

for the dye. By this means the amount of 2,4-D retained was calculated in grams per plot. The average of the two samples was used for these determinations.

In 1951 two rows $16\frac{1}{2}$ feet long and in 1952 three rows $16\frac{1}{2}$ feet long were harvested and yield data obtained for each plot. The wheat was cut with a hand sickle and threshed in a small Turner thresher. The grain was put in paper bags and placed in a drying oven at 100° F. for 7 days. After the grain was removed from the oven it was cleaned with a Vac-A-Way seed cleaner, weighed on a Fisher spring balance and the weight recorded in grams per plot. A 100-grain sample was taken from each of the 1952 plots for the 1000-kernel weight determinations and germination tests.

RESULTS AND DISCUSSION

Retention Studies

Effect of Stages of Development, Volumes of Spray, and Rates of 2,4-D on Retention: The first factors to be investigated were stages of development, volumes of spray, and rates of 2,4-D. Stage of development was considered to be of prime importance because of the enormous differences in the effect of herbicidal sprays on yields at various stages in the development of wheat plants. Volume of spray seemed to stand out as one of the first factors which should be investigated in any study on retention. A randomized block design experiment with 4 replications, 9 stages of development, 3 volumes, and 3 rates was used to study the effect of these factors. Rates of $\frac{3}{4}$, $1\frac{1}{2}$, and 3 pounds

acid equivalent per acre of the triethanolamine salt of 2,4-D were applied in volumes of 3, 6, and 12 gallons per acre of aqueous spray to Thorne wheat at the following stages of development:

- Stage Ia. March 21, 1951, wheat $3\frac{1}{2}$ to $4\frac{1}{2}$ inches high and in the 4 leaf stage.
- Stage Ib. April 11, 1951, wheat $6\frac{1}{2}$ to $8\frac{1}{2}$ inches high and in the 5 leaf stage.
- Stage Ic. April 18, 1951, wheat 9 to 11 inches high and completely tillered.
- Stage Id. April 28, 1951, wheat 15 to 21 inches high in early jointing stage with one joint and the tip of the head $1\frac{1}{2}$ to 3 inches above ground level.
- Stage Ie. May 7, 1951, wheat 21 to 24 inches high with 2 joints and the tip of the head 7 to 12 inches above ground level.
- Stage If. May 12, 1951, wheat 24 to 30 inches high in the boot stage with 3 joints, the head 2 inches in length and 12 to 19 inches above ground level.
- Stage Ig. May 19, 1951, wheat 30 to 36 inches high in the late boot stage with head $2\frac{1}{2}$ inches in length and 24 to 28 inches above ground level.
- Stage Ih. May 24, 1951, wheat mostly in the early head stage with approximately 10 per cent of the heads still in the boot.
- Stage Ii. May 31, 1951, wheat in the post-fertilization or pre-milk stage.

The amount of 2,4-D retained was calculated in grams for each plot by the method described previously. The plot size used for these determinations was 2 rows $16\frac{1}{2}$ feet long. These data are summarized in tables 1 and 2. The average per cent of retention for the 3 volumes at the 9 stages of development is shown in figure 3 and the analysis of variance of the data is given in table 3. From a study of this analysis it is obvious that stage of development, volume of spray, and rate of 2,4-D each had a highly significant effect upon the amount of 2,4-D retained by the plants.

The amount of retention increased at each successive stage of development until the plants reached stage Ig (table 1 and figure 3). The amount of retention for stages Ih and Ii was slightly lower than that of stage Ig but higher than at any of the earlier ones. This coincides with the leaf area of the wheat plants; that is, the leaf area increases until late boot or early head stage and then decreases as the plant matures. At stage Ia approximately 55 per cent of the 2,4-D was retained which appears to be rather high for this early stage of development but can be explained by the fact that the winter had been very mild and the wheat had continued to grow during the entire winter season. For this reason the plants were very heavily tillered with a considerable amount of top growth and growing rapidly at that time. The retention of approximately 95 per cent of the 2,4-D at stage Ig was not surprising when one considered the very thick stand of wheat. There was a highly significant effect of stage upon retention regardless of volume or rate.

TABLE 1. - Retention of 2,4-D in grams per plot by Thorne wheat

Rate lb/A	Volume gal/A	Stages of development									
		Ia	Ib	Ic	Id	Ie	If	Ig	Ih	Ii	
0.75	3	0.198	0.255	0.265	0.306	0.306	0.307	0.309	0.298	0.304	
	6	0.185	0.203	0.278	0.281	0.272	0.301	0.301	0.276	0.304	
	12	0.146	0.161	0.215	0.257	0.243	0.309	0.298	0.290	0.283	
Average		0.176	0.206	0.252	0.281	0.273	0.306	0.303	0.288	0.297	
1.50	3	0.376	0.518	0.563	0.585	0.600	0.611	0.634	0.608	0.641	
	6	0.365	0.509	0.570	0.589	0.618	0.621	0.647	0.608	0.620	
	12	0.362	0.361	0.392	0.524	0.488	0.502	0.620	0.586	0.570	
Average		0.368	0.463	0.509	0.566	0.569	0.578	0.634	0.600	0.611	
3.00	3	0.805	0.954	1.088	1.192	1.208	1.230	1.184	1.266	1.230	
	6	0.712	1.073	1.095	1.212	1.115	1.133	1.297	1.230	1.234	
	12	0.646	0.863	0.964	0.993	1.163	1.221	1.242	1.156	1.154	
Average		0.721	0.963	1.049	1.132	1.162	1.194	1.241	1.217	1.206	
Stage means		0.422	0.544	0.604	0.660	0.668	0.693	0.726	0.702	0.704	

*Each figure represents the average of 4 replicates

Least significant differences:
 (a) for body of table
 (b) for rate averages
 (c) for stage means

5% level
 0.137
 0.079
 0.046

1% level
 0.180
 0.104
 0.060

TABLE 2. - Means for retention of 2,4-D in grams per plot by Thorne wheat

Rate lb/A	Volume, gal/A			Rate means
	3	6	12	
0.75	0.283	0.267	0.245	0.265
1.50	0.571	0.572	0.490	0.544
3.00	1.129	1.122	1.044	1.098
Volume means	0.661	0.654	0.593	0.636

Least significant differences:	5% level	1% level
(a) for body of table	0.046	0.060
(b) for rate means	0.026	0.035
(c) for volume means	0.026	0.035

TABLE 3. - Analysis of variance for retention of 2,4-D by Thorne wheat

Source	d.f.	Mean square	F
Replications	3	0.157145	16.18**
Stages	8	0.348076	35.84**
Volumes	2	0.150158	15.46**
Rates	2	19.440064	2001.45**
Stages x Volumes	16	0.009078	0.94
Stages x Rates	16	0.048546	5.00**
Volumes x Rates	4	0.011168	1.15
Error	272	0.009713	

** Significant at 1% level of probability.

FIGURE 3. AVERAGE PER CENT OF 2,4-D APPLIED IN DIFFERENT AMOUNTS OF SPRAY RETAINED BY THORNE WHEAT.

The highly significant effect of volumes upon retention was due almost entirely to the 12 gallon volume. The average per cent of retention for the 3, 6, and 12 gallon volumes was 86, 85, and 76 per cent respectively. From a study of figure 3, it is obvious that there is no real difference between the per cent of retention for the 3 and 6 gallon volumes. The differences between them were quite small and inconsistent for the various stages. However, the 12 gallon volume gave a lower retention at every stage than either the 3 or 6 gallon ones. This consistent difference appears to be a real one as shown by the analysis of variance (table 3). The greatest differences were in the first 5 stages when the leaf area of the plants was small and the run-off from the high volume was greatest. None of the variation in retention can be attributed to the surface tension of the spray solutions because tests failed to show differences between them. These results indicate that there will be little, if any, increase in the amount of retention from reducing the volume below approximately 6 gallons per acre.

These results appear to be in agreement with the work of Holly (46) on barley. He did not find a significant variation in the toxicant retained when a fixed amount was applied in volumes between 175 and 43 gallons per acre. However, between 43 and 10 gallons per acre, which was the lowest volume used in his investigations, he found the amount of toxicant retained by the plants increased considerably, until at 10 gallons per acre it was approximately $3\frac{1}{2}$ times the amount deposited at the 175 gallons per acre volume. There was no indication of leveling off at the 10 gallon volume.

There was a highly significant overall effect of rate upon retention. From the averages for rates in table 1, it was calculated that at the $3/4$ pound rate the amount of chemical retained increased an average of 0.0134 grams per stage; at the $1\frac{1}{2}$ pound rate, an increase of 0.0270 grams per stage; and at the 3 pound rate, an average of 0.0539 grams per stage. As might be expected, when the rate was doubled and redoubled (i.e., from $3/4$ to $1\frac{1}{2}$ to 3 pounds) and other factors remained constant, the increase in the amount retained approximately doubled and redoubled (i.e., from 0.0134 to 0.0270 to 0.0539 grams).

Rate by stage was the only significant interaction. This highly significant interaction indicates that there were real variations in the rate effect upon the amount of retention from one stage to another. Increasing the rate by a factor of 4 (i.e., from $3/4$ to 3 pounds) increased the retention by 0.545 grams at stage Ia (i.e., from 0.176 to 0.721, see table 1); by 0.757 grams at stage Ib; by 0.797 grams at stage Ic; etc. until at stages Ig, Ih, and Ii the retention increased by approximately 0.0920 grams.

Effect of Formulations on Retention: The next factor studied was types of 2,4-D formulations. Thorne wheat was used for this study in a split plot design experiment, split between rates, with 4 replications, 6 formulations, 4 stages of development, and 2 rates. A volume of 5 gallons per acre was used in this experiment. One and 3 pounds of acid equivalent of 2,4-D of each of the following formulations were applied:

1. Triethanolamine salt with the spray solution at pH 7.75.
2. Triethanolamine salt with 1 per cent of Tween 20 (a wetting agent).
3. Triethanolamine salt with 1 per cent Tergitol 7 (a wetting agent).
4. Butyl ester, commercial product of Bartels and Shores, 40 per cent ester, 32 per cent acid equivalent.
5. Isopropyl ester, commercial product Dow Esteron 44, 44 per cent ester, 37 per cent acid equivalent.
6. Triethanolamine salt with the spray solution adjusted to pH 4.45 with dilute hydrochloric acid.

Spray applications were made at the following stages of development:

- Stage IIa. April 8, 1952, wheat in the late tillering stage, mostly 4 to 5 tillers and 5 to 6 inches high.
- Stage IIb. April 30, 1952, wheat in the early jointing stage, mostly 1 joint and 10 to 14 inches high.
- Stage IIc. May 14, 1952, wheat in the boot stage, 24 to 30 inches high, head $1\frac{1}{2}$ to 2 inches long and 16 to 18 inches above ground level.
- Stage IId. May 27, 1952, wheat in the early head stage, 28 to 34 inches high, bottom of head mostly 1 inch above the top of the boot, approximately 10 per cent of the heads not completely emerged from the boot.

The plot size for retention determinations was 3 rows $16\frac{1}{2}$ feet long. The amount of 2,4-D retained was calculated for each plot in grams and the data are summarized in tables 4 and 5. An analysis of

TABLE 4. - Retention in grams per plot for 2,4-D formulations*

Formulation	Rate lb/A	Stages of development			
		IIa	IIb	IIc	IIId
1	1	0.112	0.248	0.410	0.402
	3	0.570	0.748	1.078	1.261
Average		0.341	0.498	0.744	0.832
2	1	0.168	0.306	0.321	0.414
	3	0.437	0.869	0.891	1.261
Average		0.303	0.588	0.606	0.838
3	1	0.194	0.287	0.478	0.459
	3	0.396	0.642	1.314	1.364
Average		0.295	0.465	0.896	0.912
4	1	0.136	0.229	0.416	0.426
	3	0.446	0.730	1.105	1.212
Average		0.291	0.480	0.761	0.819
5	1	0.157	0.263	0.482	0.465
	3	0.753	0.869	1.489	1.547
Average		0.455	0.566	0.986	1.006
6	1	0.126	0.222	0.364	0.429
	3	0.370	0.891	1.110	1.181
Average		0.248	0.557	0.737	0.805
Stage means		0.322	0.526	0.788	0.869

*Each figure represents the average of 4 replicates

Least significant differences:	5% level	1% level
(a) for body of table	0.228	0.300
(b) for formulation averages	0.161	0.212
(c) for stage means	0.066	0.087

variance for the data is given in table 6 and the average per cent of retention for the 6 formulations at the 4 stages of development is shown in figure 4.

TABLE 5. - Means in grams per plot for retention of 2,4-D formulations

Formulation	Rate, lb/A		Formulation means
	1	3	
1	0.293	0.914	0.604
2	0.302	0.865	0.584
3	0.355	0.929	0.642
4	0.302	0.873	0.588
5	0.342	1.164	0.753
6	0.285	0.888	0.587
Rate means	0.313	0.939	0.626

Least significant differences: 5% level 1% level

(a) for body of table	0.114	0.150
(b) for formulation means	0.080	0.109
(c) for rate means	0.046	0.061

TABLE 6. - Analysis of variance for retention of 2,4-D formulations

Source	d.f.	Mean square	F
Replications	3	0.079794	8.42
Rates	1	18.798781	1985.09**
Error (a)	3	0.009470	-
Formulations	5	0.139263	5.26**
Stages	3	3.001775	113.42**
Formulations x Stages	15	0.037471	1.42
Rates x Formulations	5	0.078186	2.95*
Rates x Stages	3	0.652821	24.67**
Error (b)	153	0.026466	-

* Significant at the 5% level of probability

** Significant at the 1% level of probability

It can be observed from the analysis of variance in table 6 that formulations had a highly significant effect upon the amount of 2,4-D retained by the wheat. It is obvious from a study of table 5 and figure 4 that the formulation having the highest retention was 5 (isopropyl ester from Dow Esteron 44) and the next highest one was 3 (triethanolamine salt with 1 per cent Tergitol 7). The differences among the other 4 formulations were small and non-significant. Therefore, almost the entire effect of formulations is due to numbers 5 and 3 which gave the highest and second highest retention respectively at 3 of the 4 stages of development. Observations made in the field after the sprays had dried yielded interesting information regarding the formulation containing Tergitol 7. Leaves sprayed with this formulation had an even coat of dye over their entire surface; whereas leaves sprayed with any other formulation had heavy spots of dye surrounded by an area without any dye. Undoubtedly this played an important part in the greater retention of this formulation. There was no visual evidence to explain the greater retention of the isopropyl ester formulation, but probably the wetting agents present in it were responsible since the butyl ester formulation did not show any increase in retention over the triethanolamine salt formulations. Density determinations of the spray solutions failed to show differences between any of the 6 formulations.

The increase in retention obtained with the use of a wetting agent in this study is in agreement with the work of Ennis, et al. (33) who reported an increase in the amount of retention on soybean leaves from the addition of wetting agents to aqueous sprays. The effect

FIGURE 4. AVERAGE PER CENT OF 2,4-D FORMULATIONS
RETAINED BY THORNE WHEAT.

of the wetting agents appears to result mainly from a decrease in the surface tension of the spray solution. With this in mind, it is not surprising that some wetting agents are more effective than others as was the case in this investigation.

The effect of stage was highly significant (table 6) and followed the same general pattern as in the previous experiment; that is a rapid increase in retention during the early stages of development with a leveling off as the plants approached the heading stage. The difference between stages IIc and IId was significant at the 5 per cent level of probability while the difference between any other two stages was significant at the 1 per cent level.

This experiment was not designed especially for a comparison of rates but it is interesting to note that the effect was highly significant and a 3-fold increase in the rate (i.e., 1 to 3 pounds per acre) resulted in exactly a 3-fold increase in the amount retained (0.313 to 0.939 grams per plot) as shown by the rate means in table 5. The rate by stage interaction was also highly significant (table 6) as was shown in the first experiment (table 3). The rate by formulation interaction was significant at the 5 per cent level of probability. This interaction indicates that there were variations in the rate effect upon retention from one formulation to another. This can be observed by studying table 5 and noting that for formulation 5 increasing the rate from 1 to 3 pounds increased the retention by 0.822 grams, while the average increase obtained for the 6 formulations by this same increase in rate was only 0.626 grams.

Effect of Varieties on Retention: There are numerous reports in the literature on the reaction of wheat varieties to 2,4-D treatments. The majority of these reports agree that varietal differences do exist but fail to give any explanation to account for them. It seemed possible that at least a part of this variation in response might be due to a difference in the amount of 2,4-D retained by the plants. With this in mind, it was decided to determine the effect of varieties on retention. A total of 288 wheat varieties were screened in greenhouse tests for their vegetative response to 2,4-D in an effort to select varieties which would differ in their reaction under field conditions. On the basis of these greenhouse tests the varieties were separated into three groups: susceptible, tolerant, and resistant. Six soft red winter wheats commonly grown in this area were selected (3 from the susceptible and 3 from the resistant group) for testing in the field, as follows:

Butler - susceptible
Royal - resistant
Vigo - susceptible
Clarkan - resistant
Fairfield - susceptible
Cornell 595 - resistant

The six varieties were planted in a split plot design experiment, split between rates, with 4 replications, 4 stages, and 2 rates. The triethanolamine salt formulation was used at rates of 1 and 3 pounds acid equivalent in a volume of 5 gallons per acre at the following stages:

Stage IIIa. April 17, 1952, fully tillered stage

	<u>Height (inches)</u>	<u>No. of tillers</u>
Butler	5 - 6	4 - 5
Royal	4 - 5	5 - 6
Vigo	5 - 6	4 - 5
Clarkan	6 - 7	5 - 6
Fairfield	6 - 7	4 - 5
Cornell 595	6 - 7	5 - 6

Stage IIIb. May 9, 1952, jointing stage

	<u>Height (inches)</u>	<u>No. of joints</u>	<u>Height to tip of head (inches)</u>
Butler	17 - 19	3	6 - 7
Royal	16 - 18	2	4 - 5
Vigo	19 - 21	3	7 - 8
Clarkan	18 - 20	3	6 - 7
Fairfield	15 - 17	2	5 - 6
Cornell 595	16 - 18	2	4 - 5

Stage IIIc. May 22, 1952, boot stage

	<u>Height (inches)</u>	<u>Height to tip of head (inches)</u>	<u>Length of head (inches)</u>
Butler	24 - 26	16 - 17	$2\frac{1}{2}$ - 3
Royal	22 - 24	16 - 17	$2\frac{1}{2}$ - 3
Vigo	23 - 26	16 - 17	$2\frac{1}{2}$ - 3
Clarkan	23 - 25	17 - 18	3 - $3\frac{1}{2}$
Fairfield	24 - 28	17 - 19	$2\frac{1}{2}$ - 3
Cornell 595	22 - 24	15 - 16	$2\frac{1}{2}$ - 3

Stage IIId. May 28, 1952, early head stage

	<u>Height (inches)</u>	<u>% of heads emerged from boot</u>	<u>Length of head (inches)</u>	<u>Distance from top of boot to bottom of head (inches)</u>
Butler	30 - 34	90	$2\frac{1}{2}$ - 3	$\frac{1}{2}$
Royal	26 - 30	85	$2\frac{1}{2}$ - 3	0
Vigo	28 - 32	90	3 - $3\frac{1}{2}$	$\frac{1}{2}$
Clarkan	29 - 33	95	3 - $3\frac{1}{2}$	$\frac{1}{2}$
Fairfield	28 - 32	90	$2\frac{1}{2}$ - 3	$\frac{1}{2}$
Cornell 595	26 - 30	90	$2\frac{1}{2}$ - 3	$\frac{1}{2}$

The plot size for retention determinations was 3 rows $16\frac{1}{2}$ feet long. The amount of 2,4-D retained was calculated in grams for each plot and the data are summarized in tables 7 and 8. Analysis of variance of the data is given in table 9 and the average per cent of retention for the 6 varieties at the 4 stages of development is shown in figure 5.

The analysis of variance of the data failed to show a statistically significant difference among varieties in the amount of retention (table 9). However, the amount of retention was greater for each of the susceptible varieties than for any of the resistant ones. When the 3 susceptible varieties were considered as a group and the 3 resistant ones as another, there was a significant difference between the two groups with the susceptible one having the higher retention. The average per cent of retention for the susceptible varieties ranged from 63 to 67 per cent, while for the resistant ones it ranged from 57 to 60 per cent (figure 5).

This indicates that there is an effect of varieties on retention, but the magnitude of this effect is somewhat less than for the factors reported previously. It is unlikely that differences in the amount of retention of 2,4-D can be the factor directly responsible for varietal differences in wheat, but may be associated with it.

Rate, stage of development, and the rate by stage interaction had a highly significant effect upon the amount of retention (table 9). The effect of these factors in this experiment was very similar to that reported in the two previous ones.

TABLE 7. - Retention of 2,4-D in grams per plot by wheat varieties*

Variety	Rate lb/A	Stages of development			
		IIIa	IIIb	IIIc	IIId
Butler	1	0.156	0.346	0.356	0.389
	3	0.740	1.306	1.297	1.168
	Average	0.448	0.826	0.827	0.779
Royal	1	0.156	0.337	0.350	0.346
	3	0.534	1.199	1.244	1.221
	Average	0.345	0.768	0.797	0.784
Vigo	1	0.142	0.294	0.318	0.373
	3	0.682	1.392	1.400	1.453
	Average	0.412	0.843	0.859	0.913
Clarkan	1	0.168	0.276	0.304	0.330
	3	0.584	1.163	1.288	1.252
	Average	0.376	0.720	0.796	0.791
Fairfield	1	0.241	0.430	0.416	0.419
	3	0.610	1.239	1.288	1.163
	Average	0.426	0.835	0.852	0.791
Cornell 595	1	0.171	0.291	0.391	0.400
	3	0.709	1.118	1.230	1.132
	Average	0.440	0.705	0.811	0.766
Stage means		0.408	0.783	0.824	0.804

*Each figure represents the average of 4 replicates

Least significant differences:	5% level	1% level
(a) for body of table	0.278	0.367
(b) for variety averages	0.197	0.259
(c) for stage means	0.080	0.106

TABLE 8. - Means in grams per plot for retention of 2,4-D by wheat varieties

Varieties	Rate, lb/A		Variety means
	1	3	
Butler	0.312	1.128	0.720
Royal	0.297	1.050	0.674
Vigo	0.282	1.232	0.757
Clarkan	0.270	1.072	0.671
Fairfield	0.376	1.075	0.726
Cornell 595	0.313	1.047	0.680
Rate means	0.308	1.101	0.705

Least significant differences:	5% level	1% level
(a) for body of table	0.139	0.183
(b) for variety means	0.098	0.130
(c) for rate means	0.057	0.075

TABLE 9. - Analysis of variance for retention of 2,4-D by wheat varieties

Source	d.f.	Mean square	F
Replications	3	0.585253	2.71
Rates	1	30.031896	139.11**
Error (a)	3	0.215890	-
Varieties	5	0.040551	1.03
Susceptible	2	0.012590	0.32
Resistant	2	0.001334	0.03
Susceptible vs Resistant	1	0.174906	4.43*
Stages	3	1.882124	47.62**
Varieties x Stages	15	0.010796	0.27
Rates x Varieties	5	0.062512	1.58
Rates x Stages	3	0.558516	14.13**
Error (b)	153	0.039523	-

* Significant at the 5% level of probability

** Significant at the 1% level of probability

FIGURE 5. AVERAGE PER CENT OF 2,4-D RETAINED BY WHEAT VARIETIES.

Yield Studies

Effect of Stage of Development, Volume of Spray, and Rate of 2,4-D on Yield of Grain: Yield data were taken for all plots of the experiment used to investigate the effect of these factors on retention (page 26). The center $16\frac{1}{2}$ feet of the 2 center rows were harvested and used for yield determinations. The data are summarized in tables 10 and 11, an analysis of variance is given in table 12, and the yield expressed as per cent of controls for the 3 rates at the 9 stages of development is shown in figure 6.

The stage of development at which application was made was found to have a highly significant effect on yield (table 12). The yield productions from treatments at stages If and Ih were significantly larger than those at the other stages (table 10). These yield reductions were due primarily to the susceptibility of the plant at those stages and not to differences in the amount of chemical retained because the highest retention was at stage Ig (table 1 and figure 3).

The effect of rate on yield was highly significant, an increase in rate caused a greater yield reduction at susceptible stages. This increase in yield reduction can be accounted for by an increase in the amount of chemical retained (table 2). There were real variations in the rate effect from one stage to another which accounts for the highly significant stage by rate interaction (table 12). At stages Ib, Ic, Id, and Ii increasing the rate resulted in little or no reduction of yield, but at stages Ia, Ie, If, Ig, and Ih, there was a significant effect from the higher rates (table 10 and figure 6).

TABLE 10. - Yield in grams per plot from Thorne wheat sprayed with 2,4-D*

Rate lb/A	Volume gal/A	Stages of development									
		Ia	Ib	Ic	Id	Ie	If	Ig	Ih	Ii	
0.75	3	514	506	481	494	426	402	450	281	476	
	6	463	499	500	485	432	446	451	297	472	
	12	532	445	489	485	463	431	460	340	452	
Average		503	483	490	488	440	426	454	306	467	
1.50	3	422	489	470	473	459	327	431	221	487	
	6	415	452	434	492	474	333	416	203	422	
	12	489	514	521	432	449	366	447	244	503	
Average		442	485	475	466	461	342	431	223	471	
3.00	3	432	435	492	491	373	305	382	161	514	
	6	377	477	429	470	469	254	365	144	467	
	12	414	524	442	466	403	221	391	125	435	
Average		408	479	454	476	415	260	379	143	472	
Stage means		451	482	473	477	439	343	421	224	470	

*Each figure represents the average of 4 replicates

Least significant differences:
 (a) for body of table
 (b) for rate averages
 (c) for stage means

5% level	1% level
76	100
44	57
25	33

TABLE 11. - Means for yield of Thorne wheat sprayed with 2,4-D

Rate lb/A	Volume, gal/A			Rate means
	3	6	12	
0.75	448	449	455	451
1.50	420	405	441	422
3.00	398	384	380	387
Volume means	422	413	425	420
Least significant differences:				
(a) for body of table			5% level	1% level
(b) for rate means			25	33
(c) for volume means			15	19

TABLE 12. - Analysis of variance for yield of Thorne wheat sprayed with 2,4-D

Source	d.f.	Mean square	F
Replications	3	111,725	37.85**
Stages	8	261,038	88.43**
Volumes	2	4,696	1.59
Rates	2	108,952	36.91**
Stages x Volumes	16	3,148	1.07
Stages x Rates	16	13,825	4.68**
Volumes x Rates	4	5,474	1.85
Error	272	2,952	-

** Significant at the 1% level of probability

FIGURE 6. YIELD OF THORNE WHEAT SHOWN AS PER CENT OF CONTROLS

Effect of Formulations on Yield: The same experiment which was used to study the effect of formulations on retention (page 33) was used to determine their effect on yield. The center $16\frac{1}{2}$ feet of the 3 center rows were harvested and used for yield determinations. The data are summarized in tables 13 and 14, an analysis of variance is presented in table 15, and the yield expressed as per cent of controls for the 6 formulations at the 4 stages of development is shown in figure 7.

Formulations of 2,4-D were found to have a highly significant effect on yield (table 15). The yield reductions from formulation 3 (see page 34 for description of formulations) were significantly greater than those for formulations 1, 2, 5, or 6 and formulation 4 gave significantly greater reductions than formulations 1, 2, or 6 (table 14 and figure 7). Formulation 5 actually gave a greater yield reduction than formulations 1, 2, or 6, but the difference was non-significant. Any greater yield reduction from formulation 5 over others could be accounted for on the basis of the amount of chemical retained because it had a significantly higher retention than any other formulation (table 5). Higher retention may account in part for the greater yield reductions from formulation 3 since it had the second highest retention. However, since the retention for formulation 4 was no greater than for 1, 2, or 6 and the retention of formulations 3 and 4 was less than for 5, there must be other factors involved which are largely responsible for the greater yield reductions from these formulations. The factor which appears most likely to account

TABLE 13. - Yield in grams per plot for Thorne wheat treated with 6 formulations of 2,4-D*

Formu- lation	Rate lb/A	Stages of development			
		IIa	IIb	IIc	IIId
1	1	458	494	402	340
	3	412	438	314	212
Average		435	466	358	276
2	1	486	466	342	355
	3	446	429	279	209
Average		466	448	311	282
3	1	521	441	314	215
	3	386	397	178	87
Average		454	419	246	151
4	1	399	450	370	267
	3	448	382	217	186
Average		424	416	294	227
5	1	372	412	411	328
	3	479	429	258	199
Average		426	421	335	264
6	1	454	438	402	321
	3	404	440	341	186
Average		429	439	372	254
Stage means		439	435	319	242

*Each figure represents the average of 4 replicates

Least significant differences:	5% level	1% level
(a) for body of table	78	102
(b) for formulation averages	55	72
(c) for stage means	22	30

TABLE 14. - Mean yields in grams per plot for Thorne wheat treated with 6 formulations of 2,4-D

Formulation	Rate, lb/A		Formulation means
	1	3	
1	424	344	384
2	412	341	377
3	373	262	318
4	372	308	340
5	381	341	361
6	404	343	374
Rate means	394	323	359

Least significant differences:	5% level	1% level
(a) for body of table	39	51
(b) for formulation means	27	36
(c) for rate means	16	21

TABLE 15. - Analysis of variance of yield of Thorne wheat treated with 6 formulations of 2,4-D

Source	d.f.	Mean square	F
Replications	3	43,949	3.36
Rates	1	241,543	18.49**
Error (a)	3	13,064	-
Formulations	5	20,534	6.67**
Stages	3	437,431	142.02**
Stages x Formulations	15	6,933	2.27**
Rates x Formulations	5	4,533	1.47
Rates x Stages	3	34,175	11.10**
Rates x Formulations x Stages	15	5,642	1.83*
Error (b)	138	3,080	-

* Significant at the 1% level of probability

** Significant at the 5% level of probability

FIGURE 7. YIELD OF THORNE WHEAT SHOWN AS PER CENT OF CONTROLS FOLLOWING TREATMENT WITH 2,4-D FORMULATIONS

for the greater activity of formulations 3 and 4 is penetration of the chemical into the leaf tissue. As mentioned under the retention study, formulation 3 left an even coat of material over the entire leaf surface, while all the other formulations left heavy spots surrounded by areas without any material. It appears very likely that this would allow a greater amount of the chemical to enter the leaf. Formulation 4 was the butyl ester of 2,4-D and its high volatility could account for a greater penetration of this chemical.

The stage of development at which application was made was found to have a highly significant effect upon yield (table 15). Stages IIa and IIb out-yielded the controls, due to the control of bindweed present in the wheat (figure 7), but stages IIc and IId showed significant yield reductions. The stage by formulation interaction was highly significant (table 15) indicating that the formulations did not act alike at all stages. This can be observed from figure 7; formulations 3 and 4 produced much greater yield reductions at stages IIc and IId than did other formulations, but at stages IIa and IIb there were only small differences among formulations.

There was a significant difference between rates with the high rate giving greater yield reductions (table 14). The differences between rates at stages IIa and IIb were small, but at stages IIc and IId they were much larger which accounts for the highly significant stage by rate interaction. The second order interaction of rate by formulation by stage was also significant.

Effect of 2,4-D on Yield of Wheat Varieties: The center $16\frac{1}{2}$

feet of the 3 center rows of the plots in the experiment used to study the effect of wheat varieties on retention (page 39) was harvested to determine the effect of 2,4-D on the yield of 6 varieties of wheat. In order to do this, it was necessary to determine if the untreated controls for the varieties yielded differently. The control plots which had been placed in the experiment as a control stage were analyzed to see if a difference existed between varieties. It is obvious from a study of tables 16 and 17 that there were no significant differences among the varieties studied. On the basis of this test the 6 varieties were considered to have the same yielding ability and any differences in yield of treated plots were considered to be the result of treatments. The data are summarized in tables 18 and 19, an analysis of variance is given in table 20, and the yield of the 6 varieties expressed as per cent of controls for each variety at the 4 stages of development is shown in figure 8.

TABLE 16. - Yield in grams per plot for untreated controls of wheat varieties*

Variety	Rate, lb/A		Variety means
	1	3	
Butler	375	403	389
Royal	383	385	384
Vigo	363	394	379
Clarkan	347	390	369
Fairfield	352	382	367
Cornell 595	379	425	402

* Each figure represents the average of 4 replicates.

Least significant differences:	5% level	1% level
(a) for body of table	81	108
(b) for variety means	57	77

TABLE 17. - Analysis of variance for untreated controls of 6 wheat varieties

Source	d.f.	Mean square	F
Replications	3	51,980	6.61
Rates	1	10,740	1.36
Error (a)	1	7,866	-
Varieties	5	1,402	0.44
Error (b)	35	3,181	-

After receiving spray applications of 2,4-D, varieties had a significant effect on yield. This was true at every stage as was shown by separate analyses made for each stage. The effect of varieties was about the same for the first 3 stages but at stage III_d it was 3 times as great as at any of the earlier stages, which accounts for the highly significant variety by stage interaction.

There can be little doubt that wheat varieties do respond differently to applications of 2,4-D. However, the whole matter of varietal differences appears very complicated. Butler, Vigo, and Fairfield had been selected as susceptible varieties and Royal, Clarkan, and Cornell 595 as resistant ones on the basis of their seedling vegetative response in greenhouse tests. It is obvious from a study of table 19 that not all of the varieties responded in the same manner in the field as they had in the greenhouse tests. It can be observed from the averages in figure 8 that Clarkan, which had been selected as resistant, was one of the most susceptible and Fairfield, which had been selected as susceptible, was one of the most resistant. A study of the variety averages in table 18 reveals that the varieties responded differently

TABLE 18. - Yield in grams per plot for wheat varieties treated with 2,4-D*

Variety	Rate lb/A	Stages of development			
		IIIa	IIIb	IIIc	IIId
Butler	1	423	358	379	249
	3	407	362	382	134
	Average	415	360	381	192
Royal	1	374	426	309	284
	3	372	329	304	358
	Average	373	378	307	321
Vigo	1	343	359	350	279
	3	382	415	342	227
	Average	363	387	346	253
Clarkan	1	368	348	336	277
	3	367	322	304	205
	Average	368	335	320	241
Fairfield	1	360	431	385	363
	3	330	421	364	261
	Average	345	426	375	312
Cornell 595	1	403	398	389	378
	3	507	429	414	253
	Average	455	414	402	316
Stage means		387	383	355	273

*Each figure represents the average of 4 replicates

Least significant differences:	5% level	1% level
(a) for body of table	73	97
(b) for variety averages	52	68
(c) for stage means	21	28

TABLE 19. - Mean yields in grams per plot for wheat varieties treated with 2,4-D

Variety	Rate, lb/A		Variety means
	1	3	
Butler	352	321	337
Royal	348	341	345
Vigo	333	342	338
Clarkan	332	300	316
Fairfield	385	344	365
Cornell 595	392	401	397
Rate means	357	342	350

Least significant differences:	5% level	1% level
(a) for body of table	37	48
(b) for variety means	26	34
(c) for rate means	15	20

TABLE 20. - Analysis of variance of yield of 6 wheat varieties treated with 2,4-D

Source	d.f.	Mean square	F
Replications	3	136,584	9.15
Rates	1	11,797	0.79
Error (a)	3	14,921	-
Varieties	5	24,808	9.06**
Stages	3	135,523	49.50**
Varieties x Stages	15	10,092	3.69**
Rates x Varieties	5	3,824	1.40
Stages x Rates	3	14,414	5.26**
Rates x Varieties x Stages	15	6,035	2.20**
Error (b)	138	2,738	-

** Significant at the 1% level of probability

FIGURE 8. YIELD OF 6 VARIETIES OF WHEAT AS PER CENT OF CONTROLS FOR EACH AFTER TREATMENT WITH 2,4-D.

at different stages of development; that is, the susceptible varieties were not the same ones for each stage. For example, Fairfield was the most susceptible variety at stage IIIa and the most resistant one at stage IIIb. The varieties which were relatively susceptible for each stage (figure 8) are as follows:

Stage IIIa. - Royal, Vigo, Clarkan, and Fairfield.

Stage IIIb. - Butler and Clarkan.

Stage IIIc. - Royal, Vigo, and Clarkan.

Stage IIId. - Butler, Vigo, and Clarkan.

Averages - Butler, Vigo, and Clarkan.

Cornell 595 stood out as being the only variety not to appear in the susceptible group at any stage, and Fairfield was susceptible only at stage IIIa. The yield differences can not be attributed to difference in retention because Butler, Vigo, and Fairfield consistently retained the highest amounts of chemical.

Stage of development at which application was made stands out as being a very important factor just as it did in the other experiments. The overall effect of rate was nonsignificant, but in a separate analysis of stage IIId (table 21) it was significant. The rate by variety interaction was also significant at stage IIId. The stage by rate interaction was highly significant and can be explained by the large difference between rates at stage IIId which was not evident at other stages. The rate by variety by stage interaction was also highly significant.

TABLE 21. - Analysis of variance of yield of 6 wheat varieties at stage IIId

Source	d.f.	Mean square	F
Replications	3	30,147	7.70
Rates	1	51,025	13.03*
Error (a)	3	3,917	-
Varieties	5	21,822	14.02**
Rates x Varieties	5	10,734	6.89**
Error (b)	30	1,557	-

* Significant at the 5% level of probability

** Significant at the 1% level of probability

Kernel Weight and Seed Viability Studies

Effect of 2,4-D on 1000-Kernel Weight of Thorne Wheat: The effect on 1000-kernel weight of 6 formulations of 2,4-D applied at 4 stages of development and at 2 rates was studied from samples of grain harvested from plots used to determine the effect of 2,4-D formulations on spray retention (page 33). The results of the 1000-weight determinations are summarized in tables 22 and 23 and the analysis of variance for the data is given in table 24.

The 1000-kernel weight of Thorne wheat was reduced significantly by 2,4-D applications. The factor having the greatest effect on 1000-kernel weight was stage of development. At stages IIa, IIb, and IIc the 1000-kernel weight was significantly lower than at stage IIId, and at stage IIa it was even significantly lower than at stages IIb and IIc (table 22). Thus, stage of development at which application was made appears to have an opposite effect on 1000-kernel weight as compared to its effect on yield. A comparison of the stage means in

TABLE 22. - The 1000-kernel weights in grams for Thorne wheat treated with 2,4-D*

Formulation	Rate lb/A	Stages of development			
		IIa	IIb	IIc	IId
1	1	37.0	38.5	39.3	36.9
	3	37.5	37.3	38.5	38.3
	Average	37.3	37.9	38.9	37.6
2	1	38.1	37.8	38.0	39.6
	3	35.2	37.5	36.2	38.8
	Average	36.7	37.7	37.1	39.2
3	1	37.0	37.6	37.3	38.3
	3	36.2	36.6	35.2	39.1
	Average	36.6	37.1	36.8	38.7
4	1	36.0	37.4	35.9	38.8
	3	36.4	37.0	37.0	38.3
	Average	36.2	37.2	36.5	38.6
5	1	36.2	36.6	36.2	37.7
	3	35.7	36.9	36.8	39.2
	Average	36.0	36.8	36.5	38.5
6	1	37.2	37.7	39.2	39.1
	3	35.9	37.6	37.3	39.2
	Average	36.6	37.7	38.8	39.2
Stage means		36.6	37.4	37.4	38.6

*Each figure represents the average of 4 replicates

Least significant differences:	5% level	1% level
(a) for body of table	1.9	2.5
(b) for formulation averages	1.3	1.8
(c) for stage means	0.5	0.7

TABLE 23. - Mean 1000-kernel weights in grams for Thorne wheat treated with 2,4-D

Formulation	Rate, lb/A		Formulation means
	1	3	
1	37.9	37.9	37.9
2	38.4	36.9	37.7
3	37.6	36.8	37.2
4	37.0	37.2	37.1
5	36.7	37.2	37.0
6	38.3	37.5	37.9
Rate means	37.7	37.3	37.5
Least significant differences:			
		5% level	1% level
(a) for body of table		0.9	1.2
(b) for formulation means		0.7	0.9
(c) for rate means		0.4	0.5

TABLE 24. - Analysis of variance of 1000-kernel weights of Thorne wheat treated with 2,4-D

Source	d.f.	Mean square	F
Replications	3	16.34	13.39*
Rates	1	8.59	7.04
Error (a)	3	1.22	-
Formulations	5	6.16	3.37**
Stages	3	35.60	19.45**
Formulations x Stages	15	3.11	1.70
Rates x Formulations	5	4.23	2.31*
Rates x Stages	3	3.97	2.17
Error (b)	153	1.83	-

* Significant at 5% level of probability

** Significant at 1% level of probability

tables 13 and 22 shows that the lowest kernel weight was at stage IIa when the yield was the highest and the highest kernel weight was at stage IIId when the yield was the lowest. However, the 1000-kernel weight for stage IIId was 38.6 grams which compares very closely with that of 38.4 grams for the controls. This indicates that 2,4-D

applications at heading stage which reduce the yield as much as 50 per cent may have little or no effect on seed weight. Therefore, the yield reduction at this late stage must be the result of a decrease in the number of kernels produced. On the other hand, early applications may tend to reduce the overall vigor of the plants without a decrease in the number of kernels formed.

Formulations had a significant effect on 1000-kernel weight. It was reduced significantly by formulations 3, 4, and 5 (see page 34 for description of formulations) but was reduced slightly, if at all, by 1, 2, and 6. The reduction in seed weight appears to be associated with reduced yields because the same formulations which caused the greatest reduction in seed weight also produced the greatest yield reductions (figure 7).

The difference between rates was not statistically significant in the analysis of variance (table 24). However, the F value was relatively large (7.04) and only 1 and 3 degrees of freedom were available for testing its significance, possibly, if more degrees of freedom had been available, the effect would have been significant. Actually, by a test for the least significant difference between means in table 23, the 3-pound rate was significantly lower than the 1-pound rate. The effect of rate is complicated somewhat by the fact that formulations 4 and 5 caused a lower seed weight at the 1-pound rate while other formulations caused a lower weight at the 3-pound rate. This accounts for the significant rate by formulation interaction.

Effect of 2,4-D on 1000-Kernel Weight of 6 Wheat Varieties:

The experiment used to study the effect of varieties on retention (page 39) was used to study the effect of 2,4-D on seed weight of wheat varieties. In order to do this, it was necessary to determine if the untreated controls of the varieties differed in their 1000-kernel weights. A study of table 26 shows that there is a significant difference among the 1000-kernel weights of the 6 varieties. From table 25, it is evident that this difference is due primarily to the high kernel weight of Butler, which was significantly higher than Royal, Clarkan, or Fairfield.

TABLE 25. - The 1000-kernel weights in grams for untreated controls of 6 wheat varieties*

Variety	Rate, lb/A		Variety means
	1	3	
Butler	35.0	35.3	35.2
Royal	32.5	34.2	33.4
Vigo	34.1	34.4	34.3
Clarkan	33.8	33.8	33.8
Fairfield	33.7	33.6	33.7
Cornell 595	34.5	34.5	34.5

* Each figure represents the average of 4 replicates.

Least significant differences:	5% level	1% level
(a) for body of table	1.6	2.2
(b) for variety means	1.2	1.5

The data for the 6 varieties of wheat treated at 4 stages of development with 2 rates of 2,4-D are summarized in tables 27 and 28. One can observe from the analysis of variance given in table 29 that variety did have a highly significant effect on 1000-kernel weight. However, it must be remembered that in the untreated controls Butler

TABLE 26. - Analysis of variance of 1000-kernel weights for untreated controls of 6 wheat varieties

Source	d.f.	Mean square	F
Replications	3	4.23	1.45
Rates	1	1.58	0.53
Error (a)	3	2.97	-
Varieties	5	3.27	2.55*
Rates x Varieties	5	1.72	1.34
Error (b)	30	1.28	-

* Significant at the 5% level of probability

had a significantly higher weight than Royal, Clarkan, or Fairfield. With this in mind, there can be little, if any, significance attached to the fact that in the treated plots Butler had a significantly higher 1000-kernel weight than Royal, Vigo, Clarkan, or Cornell 595 (table 28). The real difference is with Fairfield which had the second lowest seed weight for the control plots, but for the treated ones, it was significantly higher than any of the other varieties. From a comparison of tables 25 and 28, it is obvious that there is no real difference between the control and the treated plots for any variety except Fairfield which actually showed an increase in 1000-kernel weight from 33.7 to 36.0 grams as a result of 2,4-D treatment. Undoubtedly, the kernel weight of this variety was an important factor in its reaction in the yield study. There it had the lowest yield for the control plots and the second highest yield for the treated plots.

The effect of rate of 2,4-D was not statistically significant in this experiment but the heavier rate generally gave slightly lower 1000-kernel weights. However, there were two exceptions to this;

TABLE 27. - The 1000-kernel weights in grams for 6 varieties of wheat treated with 2,4-D*

Variety	Rate lb/A	Stages of development			
		IIIa	IIIb	IIIc	IIId
Butler	1	35.7	34.9	35.5	36.2
	3	34.1	35.1	36.5	34.3
	Average	34.9	35.0	36.0	35.3
Royal	1	32.5	33.4	33.9	33.5
	3	33.2	32.3	34.4	32.8
	Average	32.9	32.9	34.2	33.2
Vigo	1	34.8	34.0	34.8	33.0
	3	33.1	34.6	34.9	32.0
	Average	34.0	34.3	34.9	32.5
Clarkan	1	35.9	33.5	34.1	34.1
	3	32.8	32.5	33.2	33.0
	Average	34.4	33.0	33.7	33.6
Fairfield	1	34.2	34.9	37.1	36.4
	3	33.9	35.5	38.9	36.5
	Average	34.1	35.2	38.0	36.5
Cornell 595	1	34.3	33.7	34.5	34.1
	3	36.7	33.4	34.9	33.2
	Average	35.5	33.6	34.7	33.7
Stage means		34.3	34.0	35.3	34.1

*Each figure represents the average of 4 replicates

Least significant differences:	5% level	1% level
(a) for body of table	1.9	2.5
(b) for variety averages	1.3	1.8
(c) for stage means	0.5	0.7

TABLE 28. - Mean 1000-Kernel weights in grams for 6 varieties of wheat treated with 2,4-D

Variety	Rate, lb/A		Variety means
	1	3	
Butler	35.6	35.0	35.3
Royal	33.3	33.2	33.3
Vigo	34.2	33.7	34.0
Clarkan	34.4	32.9	33.7
Fairfield	35.7	36.2	36.0
Cornell 595	34.2	34.6	34.4
Rate means	34.6	34.3	34.4
Least significant differences:			
		5% level	1% level
(a) for body of table		0.9	1.2
(b) for variety means		0.7	0.9
(c) for rate means		0.4	0.5

TABLE 29. - Analysis of variance of 1000-kernel weights of 6 wheat varieties treated with 2,4-D

Source	d.f.	Mean square	F
Replications	3	4.46	3.38
Rates	1	3.15	2.39
Error (a)	3	1.32	-
Varieties	5	35.51	19.40**
Stages	3	13.48	7.37**
Varieties x Stages	15	5.89	3.22**
Rates x Varieties	5	4.70	2.57*
Rates x Stages	3	5.23	2.86*
Error (b)	153	1.83	-

* Significant at the 5% level of probability

** Significant at the 1% level of probability

Fairfield and Cornell 595 each had higher seed weights for the 3-pound rate. This accounts for the significant rate by variety interaction. With the exception of applications at late stages of development which produced sterility, any applications of 2,4-D which reduced the yield usually reduced the seed weight to some extent.

However, most of the seed weight reductions were small and were not necessarily accompanied by decreased yield. In other words the number of kernels per head may have increased due to weed control from 2,4-D applications. This would prevent the reduced seed weight from being reflected in the yields.

The effect of stage of development at which application was made was highly significant in this experiment with stage IIIc being significantly higher than the other stages. Stages IIIa, IIIb, and IIId were almost identical with each other and with the average for the controls. In stage IIIc each variety showed an increase over the average for the other stages, but the increase for Fairfield was more than twice as great as for any other variety. This appears to have been largely responsible for the highly significant variety by stage interaction. The significant rate by stage interaction can be explained by comparing the effect of rate at stage IIIc with its effect at other stages. From a study of table 27 it can be observed that at stage IIIc the 1000-kernel weights were higher for the 3-pound than for the 1-pound rate while for the other stages the 3-pound rate gave lower weights. It has been demonstrated in the 2 experiments on 1000-kernel weights that 2,4-D does have an effect on seed weight. Its effect is not a clear-cut one but is influenced by 2,4-D formulations, stages of development, rates of 2,4-D, and varieties of wheat used. It appears definite that a reduction in yield from 2,4-D does not always mean a lower seed weight. In both experiments there was no effect on 1000-kernel weights at the stages having the greatest yield reductions. However, seed weight was affected significantly at

other stages. Thus, a reduction in yield at earlier stages of development is apparently accompanied by a decrease in seed weight. Reduction in yield with treatment at later stages was sometimes due to reduced number of kernels because there was no decrease in kernel weight. Treatments near anthesis produced a high per cent of sterility which was the factor largely responsible for the drastic yield reductions from these treatments.

Effect of 2,4-D on Germination of Thorne Wheat: The effect on germination of 6 formulations of 2,4-D applied at 4 stages of development and at 2 rates was studied from samples of grain harvested from plots used to determine the effect of 2,4-D formulations on spray retention (page 33). The results of the germination tests, single determinations of 100 kernels, are summarized in tables 30 and 31 and the analysis of variance for the data is given in table 32.

The effect of formulations on germination was not statistically significant as shown by the analysis of variance and the test for least significant differences between means. The effect of stages is not very clear-cut. Judging from the analysis of variance, stages at which applications were made did not have a significant effect on germination, but with the test for least significant differences between means, stage IIId was significantly lower than stage IIb. Since the slightly lower germination at stage IIId was rather consistent for all formulations and rates a small, but real, reduction in germination may have occurred at this stage.

TABLE 30. - Germination in per cent for Thorne wheat
treated with 2,4-D*

Formu- lation	Rate lb/A	Stages of development			
		IIa	IIb	IIc	IIId
1	1	92	90	95	91
	3	88	93	87	89
	Average	90	92	91	90
2	1	90	93	89	86
	3	84	91	90	87
	Average	87	92	90	87
3	1	89	91	90	88
	3	94	90	83	90
	Average	92	91	87	89
4	1	88	94	95	95
	3	87	89	90	88
	Average	88	92	93	92
5	1	89	87	94	87
	3	88	90	85	89
	Average	89	89	90	88
6	1	91	95	91	92
	3	91	88	86	85
	Average	91	92	89	89
Stage means		90	91	90	89

*Each figure represents the average of 4 replicates

Least significant differences:	5% level	1% level
(a) for body of table	7	10
(b) for formulation averages	5	7
(c) for stage means	2	3

TABLE 31. - Mean germination in per cent for Thorne wheat treated with 2,4-D

Formulation	Rate, lb/A		Formulation means
	1	3	
1	92	89	91
2	90	88	89
3	90	89	90
4	93	89	91
5	89	88	89
6	92	88	90
Rate means	91	89	90

Least significant differences:	5% level	1% level
(a) for body of table	4	5
(b) for formulation means	3	4
(c) for rate means	1	2

TABLE 32. - Analysis of variance for germination of Thorne wheat treated with 2,4-D

Source	d.f.	Mean square	F
Replications	3	239	14.06*
Rates	1	305	17.94*
Error (a)	3	17	-
Formulations	5	21	0.78
Stages	3	43	1.59
Formulations x Stages	15	24	0.89
Rates x Formulations	5	26	0.96
Rates x Stages	3	47	1.74
Error (b)	153	27	-

* Significant at the 5% level of probability

The 3-pound rate of every formulation had a lower germination than the corresponding 1-pound rate (table 31). This effect of rate was significant in the analysis of variance and highly significant in the test for least significant differences between means. From these results there can be little doubt that the 3-pound rate did

significantly reduce the germination of Thorne wheat. There were no significant interactions between any of the factors involved.

Effect of 2,4-D on Germination of 6 Wheat Varieties: The experiment used to study the effect of varieties on retention (page 39) was used to study the effect of 2,4-D on germination of wheat varieties. The germination of the untreated controls for the 6 varieties was tested and the results are given in tables 33 and 34. It is obvious from a study of these tables that there were no significant differences in germination among varieties.

TABLE 33. - Germination in per cent for untreated controls of 6 wheat varieties*

Variety	Rate, lb/A		Variety means
	1	3	
Butler	92	95	94
Royal	91	96	94
Vigo	92	93	93
Clarkan	95	94	95
Fairfield	91	94	93
Cornell 595	91	90	91

* Each figure represents the average of 4 replicates

Least significant differences:	5% level	1% level
(a) for body of table	7	9
(b) for variety means	5	7

TABLE 34. - Analysis of variance for germination of untreated controls of 6 wheat varieties

Source	d.f.	Mean Square	F
Replications	3	60	1.02
Rates	1	44	0.75
Error (a)	3	59	-
Varieties	5	19	0.76
Error (b)	35	25	-

The data for the 6 varieties of wheat treated at 4 stages of development with 2 rates of 2,4-D are summarized in tables 35 and 36. Neither varieties, stages, nor rates had a significant effect on germination as shown by the analysis of variance in table 37. However, the F value for stages at which applications were made was just short of the 5 per cent level of probability, and when the test for least significant differences was used, stage IIId was significantly lower than stage IIIa. This is in line with the previous experiment on germination studies which showed the germination to be significantly lower at stage IIId. Thus, it appears that germination may be reduced slightly by applications of 2,4-D applied at this late stage of development which was an early head stage in both experiments.

A study of the means in table 36 reveals that the germination for Royal was significantly lower than the other varieties and that the 1-pound rate was lower than the 3-pound rate. However, it can be observed in table 37 that the rate by variety interaction was significant but neither of the factors gave an F value that even approached significance. When the figures in the body of table 36 are studied, it can be seen that the lower germination for Royal resulted from the low germination at the 1-pound rate and not from an overall effect of the variety. Likewise, the lower germination for the 1-pound rate is the result of the low germination for Royal and Clarkan at that rate and not due to an overall effect of the rate. No logical explanation appears evident for the reduced germination of these 2 varieties at the lower rate of 2,4-D,

TABLE 35. - Germination in per cent for 6 varieties of wheat treated with 2,4-D*

Variety	Rate lb/A	Stages of development			
		IIIa	IIIb	IIIc	IIId
Butler	1	97	93	92	89
	3	96	92	94	89
	Average	97	93	93	89
Royal	1	91	91	87	88
	3	95	92	94	92
	Average	93	92	91	90
Vigo	1	92	96	90	94
	3	94	90	94	92
	Average	93	93	92	93
Clarkan	1	90	91	93	91
	3	96	94	95	96
	Average	93	93	94	94
Fairfield	1	95	95	91	93
	3	96	94	92	95
	Average	96	95	92	94
Cornell 595	1	91	91	91	93
	3	94	94	94	94
	Average	93	93	93	94
Stage means		94	93	93	92

*Each figure represents the average of 4 replicates

Least significant differences:	5% level	1% level
(a) for body of table	5	7
(b) for variety averages	4	5
(c) for stage means	2	2

therefore, it must be considered within the realm of experimental error.

TABLE 36. - Mean germination in per cent for 6 varieties of wheat treated with 2,4-D

Variety	Rate, lb/A		Variety means
	1	3	
Butler	93	93	93
Royal	89	93	91
Vigo	93	93	93
Clarkan	91	95	93
Fairfield	94	94	94
Cornell 595	92	94	93
Rate means	92	94	93

Least significant differences:	5% level	1% level
(a) for body of table	3	4
(b) for variety means	2	3
(c) for rate means	1	2

TABLE 37. - Analysis of variance for germination of 6 wheat varieties treated with 2,4-D

Source	d.f.	Mean square	F
Replications	3	42.0	0.59
Rates	1	133.0	1.87
Error (a)	3	71.0	-
Varieties	5	19.0	1.37
Stages	3	34.0	2.45
Varieties x Stages	15	16.1	1.16
Rates x Varieties	5	34.2	2.46*
Rates x Stages	3	18.7	1.34
Error (b)	153	13.9	-

* Significant at the 5% level of probability

SUMMARY AND CONCLUSIONS

Field studies were conducted to determine the amount of 2,4-D sprays retained by winter wheat. The average retention of 2,4-D sprays varied from approximately 25 to 95 per cent depending upon the following factors: stage of development, volume of spray, rate of 2,4-D, formulation of 2,4-D, and variety of wheat. The effect of the above factors on yield, seed weight, and germination of the grain was determined.

Stages of development had a highly significant effect on retention in all experiments. The amount of retention increased at each successive stage of development until the plants reached the late boot stage. At early head and post-fertilization stages the amount of retention was slightly less than at the late boot stage.

The volumes used in this study (3, 6, and 12 gallons per acre) had a highly significant effect on retention. There was no real difference between the retention of the 3 and 6 gallon volumes, but the 12 gallon volume gave a lower retention at every stage than either the 3 or 6 gallon volumes. The greatest difference occurred during early stages when the leaf area of the plants was small and the run-off from the high volume was greatest. These results indicate that there will be little, if any, increase in the amount of retention obtained by reducing the volume below approximately 6 gallons per acre.

Rates of 2,4-D had a highly significant effect upon the amount of herbicide retained. As the rate was increased (i.e., from 3/4 to

1½ to 3 pounds) and other factors remained constant, the amount of 2,4-D retained increased approximately proportionally (i.e., from 0.265 to 0.544 to 1.098 grams).

There was a highly significant effect of 2,4-D formulations on the amount of 2,4-D retained by the wheat. The isopropyl ester from Dow Esteron 44 and the triethanolamine salt with 1 per cent Tergitol 7 (a wetting agent) gave the highest and second highest retention respectively. Wetting agents probably played an important role in the higher retention of these formulations. There were only very slight differences in retention among the other 4 formulations.

The effect of varieties on retention was not as great as the other factors which were investigated. The differences among varieties were not statistically significant, but when the 3 varieties selected for susceptibility to 2,4-D were placed in one group and the 3 varieties with the highest resistance in another, there was a statistically significant difference between the two groups.

The factor having the greatest effect on yield was the stage of development at which 2,4-D was applied. This factor had a highly significant effect in each experiment. The greatest yield reductions occurred when applications were made at the early head stage (when anthesis was taking place). Smaller but significant yield reductions occurred from treatments approximately 2 weeks before heading when the wheat was in the boot stage.

Rates of $3/4$, 1, $1\frac{1}{2}$, and 3 pounds of the acid equivalent of 2,4-D were used in these investigations. All rates produced significant yield reductions when applied at susceptible stages, but no significant reductions occurred from any rate at resistant stages. The most resistant period was between the fully tillered and the jointing stage.

The effect of volumes of 3, 6, and 12 gallons per acre on yield was not statistically significant except between the 6 and 12 gallon volumes at the $1\frac{1}{2}$ pound rate. This suggests that there may be other factors such as coverage or absorption into the leaf which prevents the highly significant effects of volume on retention from being manifested in the yields.

Formulations had highly significant effects on yields, but chiefly when applied at susceptible stages. The triethanolamine salt with 1 per cent of Tergitol 7 produced greater yield reductions than the butyl or isopropyl esters (without wetting agents).

Wheat varieties were found to differ significantly in their response to applications of 2,4-D. However, the differences among varieties are not very clear-cut since the same varieties were not the most susceptible at all stages. The varietal yield differences can not be attributed to differences in retention.

The 1000-kernel weight of Thorne wheat was not reduced by 2,4-D applications at the early head stage which was the stage at which the greatest yield reduction occurred. However, the kernel

weight was reduced significantly by treatments at earlier stages with the greatest reduction at the first stage. The yields were the highest when 2,4-D was applied at the first stage.

The effect of 2,4-D formulations on 1000-kernel weight was highly significant. The 3 formulations which reduced yields the most also reduced the kernel weight significantly, but the 3 other formulations had little, if any, effect on it.

There was no clear difference in effect on 1000-kernel weight of Thorne wheat when rates of 1 or 3 pounds of 2,4-D was applied. However, indications were that the 3-pound rate may have reduced the seed weight slightly more.

The only differential effect of 2,4-D on the 1000-kernel weight of the 6 wheat varieties was a significant increase in the weight for Fairfield, due to treatment. Fairfield was one of the varieties which showed the least effect of 2,4-D on yield.

The germination of seed from plots treated with 2,4-D at the early head stage was reduced slightly. This reduction was too small to be considered as serious.

All formulations had essentially the same effect on germination. The differences between rates of application were highly significant, with the 3-pound rate of every formulation reducing the germination below that of the 1-pound rate. The reductions were small but generally consistent at all stages for all formulations.

There were no consistent differences between varieties in the effect of 2,4-D on germination.

In general, the greatest retention of 2,4-D sprays occurred at late stages of development from low volume applications (3 to 6 gallons per acre) of formulations containing effective wetting agents. Rates of $3/4$ to 3 pounds of acid equivalent did not produce serious yield reductions when applied at resistant stages, but even the lowest rate reduced yields as much as 40 per cent when applied at the most susceptible stages. Fully tillered wheat was most resistant and the crop was most susceptible at the early heading stage. Effective wetting agents approximately doubled the toxicity of triethanolamine salt formulations, making them more toxic than ester formulations without wetting agents. Wheat varieties showed fairly large differences in their resistance to spray applications of 2,4-D. Kernel weight was reduced slightly by heavy rates of the more toxic formulations, but this reduction did not always occur when the crop was treated at the more susceptible stages. Heavy rates of 2,4-D applied at the more susceptible stages reduced seed viability slightly.

Rates of 2,4-D sufficient for the control of most broadleaf weeds ($1/4$ to $3/4$ pounds per acre) can safely be applied to winter wheat at the fully tillered stage without danger of any serious effects on yield, kernel weight, or seed viability.

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AUTOBIOGRAPHY

I, Harvey Darrell Woofter, was born in Glenville, West Virginia, January 31, 1923. I received my secondary school education in the public schools of Gilmer County, West Virginia. The first two years of my undergraduate training were obtained at Glenville State Teachers College. The final two years of my undergraduate training were obtained at West Virginia University, from which I received the degree Bachelor of Science in 1943. I received an appointment as County Agricultural Agent with the West Virginia Agricultural Extension Service in 1943 and served in this capacity until 1948 except for two years which were spent in the Army of the United States. From The Ohio State University, I received the degree Master of Science in 1949. While in residence at The Ohio State University, I acted in the capacity of assistant to Dr. C. A. Lamb of the Agronomy Department during the year 1948-49. In 1949 I received an appointment as Research Agronomist with the Department of the Army and held this position for two years. In 1951, I returned to the Agronomy Department of The Ohio State University to complete the requirements for the degree Doctor of Philosophy.