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The Effect of 2,4-Dichlorophenoxyacetic Acid on Certain Weed and Crop Seeds

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The Effect of 2,4-Dichlorophenoxyacetic Acid on Certain Weed and Crop Seeds[1]

L. E. Everson[2] and R. S. Dunham[3]

ANNUAL AND BIENNIAL weeds, which cause a large portion of our crop losses, depend almost entirely on the production of seeds for survival. Seeds of these plants are produced in enormous numbers, disseminated, and stored in the soil until weather conditions are favorable for their germination and growth. The soil acts as a reservoir for the seeds, thus assuring the survival of the weed species.

Reduction of the weed seed population of the soil can be accomplished by cultural methods but these are expensive, incomplete, and slow, largely because of varying periods of seed dormancy. The use of chemicals has been considered a possible method, but the many disadvantages of the inorganic herbicides discouraged extensive use. With the introduction of 2,4-D many attempts were made to kill weeds by pre-emergence spraying. Results were very erratic.

Investigators who studied the pre-emergence method confined their observations to the presence or absence of emerged seedlings. The direct effect of the growth regulator on seeds and seedlings has seldom been noted. It has been generally assumed that when soil was treated with 2,4-D it was necessary for the seeds to germinate before the chemical became effective. If ungerminated weed seeds are affected by growth regulators, the possibility of using these herbicides to kill weed seeds should be investigated thoroughly.

The objective of this study was primarily to determine the effect of aqueous solutions of 2,4-D on the germination of certain crop and weed seeds, to measure the persistence of 2,4-D in seeds, and to discover the extent of 2,4-D penetration into seeds; secondarily the objective of this study was to determine the effect of volatile 2,4-D on the germination of weed and crop seeds.

[1] From a thesis submitted by the senior author to the University of Minnesota in partial fulfillment of the requirements for the degree of Doctor of Philosophy. The senior author expresses his appreciation to the Department of Botany and Plant Pathology of Iowa State College for the use of material and the privilege of working out the problem at the college. The authors are indebted to Dr. H. K. Hayes and Dr. H. L. Thomas of the Division of Agronomy and Plant Genetics, University of Minnesota, for critical reading of the manuscript and assistance in statistical analysis of the data, to Dr. D. X. Isely and J. G. Darroch of the Division of Botany and Plant Pathology of Iowa State College for similar assistance, and to Dr. G. L. McNew, formerly of Iowa State College, for helpful technical suggestions.

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To attain these objectives five experiments were carried out under controlled conditions in the laboratory. The studies included (1) the effect of 2,4-D on radicle growth of nondormant seeds, including wild mustard (*Brassica kaber*), pennycress (*Thlaspi arvense*), curly dock (*Rumex crispus*), wheat (*Triticum vulgare*), and corn (*Zea mays*); (2) the effect of 2,4-D on radicle growth of embryo-dormant and impermeable seeds; (3) the persistence of 2,4-D, as measured by radicle growth, in seeds in wet storage at 2° C., in dry storage at 30° C., and in storage in moist peat soil at 30° C.; (4) the penetration of 2,4-D through seed coats of nondormant and dormant seeds; and (5) the effect of volatile 2,4-D on the radicle growth of seeds of corn and wild mustard.

LITERATURE REVIEW

AKAMINE (*1*)[4] and Heal and Thompson (*15*) have reviewed the literature on 2,4-D through 1949 in a general and comprehensive manner. The brief review which follows discusses research that suggested the present study or is directly related to it.

The use of 2,4-D for the practical control of weeds by killing seeds or seedlings was suggested by Hamner *et al.* (*14*), Mitchell and Marth (*24*), and Allard *et al.* (*6*), among others. Their studies indicated that the action of 2,4-D applied to the soil is selective, that seedlings are more responsive to 2,4-D than are older plants, and that in some cases the germination of weed seeds may be inhibited by this herbicide. Warren *et al.* (*31*) agreed with the general idea but questioned the effect on germination, believing that only germinated seeds are killed.

The feasibility of this concept was explored by a large number of investigators, who employed the pre-emergence method of applying 2,4-D to the soil after the crop had been sown or planted and before it emerged. Successful results were reported by many workers (*4, 5, 7, 13, 17, 18, 20, 26, 27, 28, 30, 32, 33,* and *34*), but others had poor results or failures (*9, 10, 11,* and *12*).

The variable outcome of these trials indicated that factors other than the sensitivity of weed and crop plants were influencing the results. Differences in soil type, moisture, and organic matter content were listed by Wolf (*34*), Dunham (*10*), and Fuelleman (*12*) as probable causes of the erratic results.

Heggeness and Herbert (*16*) used flax as a test plant in soil treated with 2,4-D in the greenhouse, sowing it at different periods after the soil was treated. Best emergence and growth of seedlings occurred when the treated soil was relatively high in organic matter and low in pH and colloids.

Arakeri and Dunham (*8*) studied environmental factors affecting the pre-emergence treatment of corn. Factors included were the following: application of water to simulate rainfall after treatment, depth of planting the crop, dosage of the chemical, time of application, and type, pH, and organic matter content of the soil.

Most important factors were found to be water, pH, and organic matter content of the soil; less important was time of application; and least important were depths of planting and dosages. In this experiment good control of both grass and broadleaf weeds was obtained without injuring the corn crop when the pH or organic matter of the soil was high or when no rain fell between planting and emergence of the crop on soil of low pH and low organic matter.

Certain investigators became interested in determining whether 2,4-D affected the viability of seeds or whether its action was restricted to the seedlings. Hamner *et al.* (*14*) found that the germination of many seeds was affected by soaking in low concentrations of 2,4-D. Hsueh and Lou (*19*) studied the effects of 2,4-D on seed germination with the Warburg respirometer. Their results showed that 2,4-D at 100 parts per million (p.p.m.) promoted germination, but at 1,000 p.p.m., aerobic respiration and germination were inhibited.

Warren *et al.* (*31*) observed that treatments seemed to affect only germinating seeds, since new weeds started to grow in undisturbed soil when the toxicity of the 2,4-D had disappeared.

Mitchell and Brown (*22*) reported that some seeds will eventually germinate even though the manure or soil in which they occur is treated with sufficient 2,4-D to be lethal for a period of several weeks. These authors suggest that seeds of some species may be relatively insensitive to 2,4-D while dormant, and that seeds remaining dormant in soil or manure until the chemical is inactivated may not be affected.

In further studies by these workers with southern giant curled mustard (*Brassica juncea*), selected seeds in different stages of germination were allowed to imbibe a 500 p.p.m. solution of the salt of 2,4-D for 30 seconds and then were rinsed. Using subsequent root growth as a criterion, Mitchell and Brown found that resting seeds were relatively insensitive to the salt but became more sensitive as they absorbed water and ruptured their seed coats. Emerging radicles were extremely sensitive to the salt solution. In another experiment mustard seed in the resting stage was treated in a solution of 500 p.p.m. of ammonium salt of 2,4-D for 10 minutes, and root growth was reduced 70 per cent. A one-second treatment of mustard seedlings completely checked radicle elongation.

These same workers checked the sensitivity of subterranean clover (*Trifolium subterraneum*) by placing seeds fully imbibed with water in soil mixed with 2,4-D at four pounds per acre. Seeds were placed in boxes of this treated soil and also in boxes of untreated soil. Half of the boxes of untreated and treated soil were maintained at 20° C. and half maintained at 30° C. Seeds placed in untreated soil at 20° C. germinated and grew vigorously. Some of the seeds in the treated soil at 20° C. germinated and grew above the surface of the soil, but these plants were deformed and stunted.

All seeds at 30° C. remained dormant. After 19 days the 2,4-D was completely inactivated, and all boxes of seeds were then transferred to 20° C. The clover seeds in both treated and untreated soil germinated and grew vigorously. No abnormalities were observed.

From these results Mitchell and Brown concluded that this species of clover was relatively resistant to 2,4-D while dormant, but became extremely sensitive to the acid after the seed coats were broken and the seeds began to germinate.

Akamine (*2*) reports that 2,4-D had no effect on impermeable seeds of kao haole (*Leucaena glauca*) until the seeds imbibed moisture. One grass species imbibed water (and presumably 2,4-D) under any conditions but germinated in only a limited temperature range. In spite of previous absorption of 2,4-D at an unfavorable germination temperature, such seeds germinated normally when transferred to a medium without 2,4-D at a favorable germination temperature. On the other hand, the germination of nondormant seeds was affected by the chemical immediately.

[4] Numbers in parentheses refer to Literature Cited, page 23.

MATERIALS AND METHODS

FOR CONVENIENCE in the discussion of results each of the five experiments listed in the introduction will be outlined separately. Before describing the detailed studies, the general methods, materials, and terminology will be described.

The crop seeds included in this study were tendergreen beans (*Phaseolus vulgaris*), Iowa W. F. 9x38-11 field corn (*Zea mays*), Hubam sweet clover (*Melilotus albus*), subterranean clover (*Trifolium subterraneum*), and hard red spring wheat (*Triticum vulgare*). The weed seeds used were butterprint (*Abutilon theophrasti*), night flowering catchfly (*Silene noctiflora*), curly dock (*Rumex crispus*), pennycress (*Thlaspi arvense*), giant ragweed (*Ambrosia trifida*), Pennsylvania smartweed (*Polygonum pennsylvanicum*), pale smartweed (*Polygonum lapathifolium*), and wild mustard (*Brassica kaber*). All seed lots were hand harvested during the 1948 season, with the exception of wheat, which represented commercial seed. All seed lots germinated 90 per cent or better.

Triethanolamine salt of 2,4-D, a 95 per cent pure product which contains 60 per cent active 2,4-D acid, was used for aqueous solutions of 2,4-D. The volatile 2,4-D products used were a commercial butyl ester containing 40 per cent active 2,4-D acid, and an allyl ester of 2,4-D.

For convenience the words "seeds" and "seed coats" are used in the commonly accepted functional rather than morphological sense in these studies. In the study of penetration of 2,4-D through seed coats, the structure removed and referred to as "seed coat" is as follows:

Corn—pericarp
Beans—true seed coat (integuments)
Giant ragweed—involucre and pericarp
Butterprint—true seed coat (integuments)

The germinators used were equipped with automatic temperature controls, which limited fluctuation of temperature to 1° C. When alternating temperatures were used the high temperature was maintained for eight hours, and the low temperature for 16 hours. The seeds were exposed to fluorescent lights during the high temperature period.

The treatment consisted of soaking the seeds in aqueous solutions of 2,4-D from one to 48 hours. Duration of treatment was always less than the time required for the embryo to break the seed coat but usually long enough to permit maximum imbibition. Controls received identical treatment except that tap water was used in place of 2,4-D solutions.

After rinsing, as subsequently explained, the seeds were placed on a blue-gray blotting paper substrate in nine-cm. petri dishes for studies of germination and radicle growth.

All seed lots except those treated with volatile 2,4-D were rinsed for four hours in running water after treatment. The purpose of rinsing was to remove 2,4-D from the outside of the seed coats so that the cause of subsequent injury or stimulation to the seedling would be limited to 2,4-D which had penetrated the ungerminated seeds.

In rinsing, tap water was run into a glass jar, then siphoned by glass tubes into large-mouth bottles (120-ml. size for large seeds or 60-ml. size for small seeds) containing the treated seeds. Small seeds such as curly dock and night flowering catchfly were tied loosely in one thickness of cheesecloth during rinsing. The use of siphon tubes resulted in a gentle lifting movement of the water and seeds.

The technique used in rinsing is discussed in order to determine how thoroughly the 2,4-D was removed. The rinsing procedure tested whether the aqueous 2,4-D solution penetrated the seed coats, or whether uncombined 2,4-D still capable of affecting emerging seedlings was present on the outside of the seed coats after rinsing. The procedure was as follows:

Corn seeds were soaked in a 10,000 p.p.m. solution of 2,4-D for 16 hours, rinsed in running water four hours, and the rinse water tested for 2,4-D. The amount of 2,4-D present was determined by a bio-assay in which cucumber seedlings (variety, National Pickling) were used as test plants (25). Cucumber seeds were placed between moistened seed germination blotters at 30° C., and after 30 hours seedlings 5 to 12 mm. long were selected for study. Tap water and 2,4-D solutions of 0.01 or 0.1 p.p.m. were used as the comparative moistening agents.

Ten replicates of 10 seedlings each were then set up in a randomized block design. Cucumber radicles were measured, then dipped in tap water, in rinse water, or in a 2,4-D solution, and the seedlings placed in petri dishes. Twelve cc. of tap water, rinse water, or 2,4-D solution were used to moisten the substrate, providing an excess of moisture for the seedlings. At the end of 24 hours radicle lengths were again determined, the original measurements subtracted, and the average difference of the radicle lengths obtained.

Table 1. Average Difference in Radicle Lengths of National Pickling Cucumber Seedlings Before and After Dipping in Water, Rinse Water, and 2,4-D Solutions

	millimeters
Water	23.26
Rinse water from treated corn seed	21.88
2,4-D solutions	
0.01 p.p.m.	19.12*
0.1 p.p.m.	8.72*
L.S.D. at 5 per cent level	1.92

* $P < 0.05$

The data in table 1 indicate that the rinsing was effective, since seedling growth after treatment with the rinse water was not significantly reduced. Therefore, no appreciable amount of 2,4-D acid was present on the outside of the seed coats of corn after rinsing.

Nondormant seeds or seeds which had been specially handled to break dormancy were germinated at alternating temperatures of 20° and 30° C. In experiments using dormant seeds it was necessary to break dormancy prior to germination. The seeds of sweet clover and butterprint used were dormant because they were impermeable to water.

Seed coat impermeability of the sweet clover was broken by rubbing the seeds between sheets of "00" sandpaper. Impermeability of butterprint was broken by placing the seeds on a moist substrate for two days at 2° C., then transferring to 35° C. for one day before germinating at alternating temperatures of 20° and 30° C.

Giant ragweed, pale smartweed, and Pennsylvania smartweed have an embryo dormancy.

Dormancy of giant ragweed was broken by placing the seeds on a moist substrate at 2° C. for two months before transferring the seeds for germination. The dormancy of pale smartweed was broken by a daily alternation from 10° to 35° C. for 10 days. Seeds were kept in cheese boxes filled with an inch of water. At the end of this period the seeds were transferred to a moist quartz sand substrate and germinated.

The method of breaking dormancy for Pennsylvania smartweed was similar to that used for pale smartweed except that a daily alternation from 2° to 35° C. was used for a two-week period before the transfer.

The sample of wild mustard (which often has embryo dormancy) had only about four per cent dormant seeds, so the entire sample was handled with nondormant seeds.

Newly harvested subterranean clover seeds imbibe water and swell when placed on moist blotting paper and kept

at 30° C., but they do not germinate. When these seeds are transferred to 20° C. they germinate readily. Seeds of the subterranean clover sample used in these studies remained dormant in moist peat at 30° C. for three weeks. This information was used in the study to determine the effect of 2,4-D on subterranean clover seeds during this period of dormancy.

The measure of 2,4-D effect was based on radicle growth after treatment as compared to radicle growth of nontreated seeds. Measurements were taken when radicles of the controls were from 10 to 40 mm. long.

An outline of the materials and methods pertaining to specific experiments follows:

The Effect of 2,4-D on Radicle Growth of Nondormant Seeds

This experiment was designed to study the radicle growth of seeds soaked in aqueous 2,4-D solutions in comparison with those soaked in tap water. Three kinds of weed seeds—wild mustard, pennycress, and curly dock—and two kinds of crop seeds—wheat and corn—were included in the study. Seeds of these species were treated, rinsed, germinated, and the radicles measured. The treatment was identical for each species, except for the duration of corn seed treatment as noted in the outline below. The study of each species was carried out separately from the others.

A split plot design was used, consisting of duration of treatment as main plots with the concentration of 2,4-D as subplots.

Number of replicates	Five of 50 seeds each
Concentrations	Tap water and 0.1, 1, 10, 100, and 1,000 p.p.m. 2,4-D solutions
Duration of treatment	2, 4, 8, and 16 hours (1, 3, 9, and 27 hours for corn)

The Effect of 2,4-D on Radicle Growth of Dormant Seeds

Prior to the study a preliminary check using 2,4-D on embryo-dormant and impermeable seeds was made to determine if 2,4-D would cause the seeds to germinate under normally favorable germination temperatures. Seeds of Pennsylvania smartweed, pale smartweed, giant ragweed, butterprint, and sweet clover were soaked in tap water or in a 10 or 1,000 p.p.m. 2,4-D solution for 12 hours.

The seeds were then placed under germinating conditions without rinsing. After two months this part of the experiment was discontinued because the seeds failed to germinate.

The study was divided into two parts using seeds with embryo dormancy and seeds impermeable to water. After soaking the seeds in aqueous 2,4-D solutions, rinsing in water, and breaking impermeability or dormancy, the seeds were germinated and the radicles measured. Details of the design of the experiment, replication, concentration, and length of treatment are given in the following paragraphs.

Pennsylvania smartweed, pale smartweed, and giant ragweed were used as examples of plants with embryo-dormant seeds.

Plot design	Unpaired comparisons
Number of replicates	Five of 50 seeds each
Concentrations	Tap water and a 10,000 p.p.m. 2,4-D solution
Duration of treatment	48 hours

The impermeable seeds used were butterprint and sweet clover. Seeds that did not gain weight after soaking in water were called impermeable. Parallel tests were run treating the seeds before and after breaking impermeability.

Plot design	Randomized block
Number of replicates	Five of 50 seeds each
Concentrations	Tap water and 10 and 1,000 p.p.m. 2,4-D solutions
Duration of treatment	12 hours

The Persistence of 2,4-D in Seeds under Storage, as Measured by Radicle Growth

This study was divided into three parts with seeds stored wet on blotters at 2° C., stored dry in envelopes at 30° C., or stored in moist peat soil at 30° C. All seeds were soaked in aqueous 2,4-D solutions, rinsed in water, and placed in storage (see following paragraphs). Subsequent to storage the seeds were germinated and the radicles measured.

Seeds of night flowering catchfly, curly dock, and giant ragweed were chosen for the study of the effect of wet storage on 2,4-D treated seeds. The seeds were stored on moist blotter paper in petri dishes at 2° C. for three months. A rapid decrease of viability under these cool wet storage conditions made longer storage undesirable. A complete series of five replicates was checked each month.

Plot design	Unpaired comparisons
Number of replicates	Five of 50 seeds each
Concentrations	Tap water and a 1,000 p.p.m. 2,4-D solution
Duration of treatment	12 hours

Seeds of wild mustard, night flowering catchfly, curly dock, and giant ragweed were used for the study of the effect of dry storage on 2,4-D treated seeds. After treating and rinsing, the seeds were dried at 30° C. and stored in envelopes. Once a month seeds of each species were removed from storage, a complete series of five replicates germinated, and the radicles measured.

Plot design	Unpaired comparisons
Number of replicates	Five of 50 seeds each
Concentrations	Tap water and a 1,000 p.p.m. 2,4-D solution
Duration of treatment	12 hours

The seeds stored in moist peat soil were under normally favorable germinating conditions; therefore, dormant seeds had to be used. Five kinds of dormant seeds—sweet clover, butterprint, Pennsylvania smartweed, giant ragweed, and subterranean clover—were used to compare the persistence of triethanolamine salt of 2,4-D in impermeable and permeable seeds when stored in warm moist peat soil at 30° C.

It was first necessary to determine the length of time required for 2,4-D inactivation in peat soil under the conditions present in the germinator. Peat containing 76 per cent moisture, based on air-dry weight at 105° C., was used. Triethanolamine salt of 2,4-D was thoroughly mixed with the peat soil at the rate of 8.2 mg. of 2,4-D acid per pound of soil.

One-half pound of this treated soil was placed in each of eight cottage cheese boxes. One hundred cc. of water were added to each box immediately, and 70 cc. weekly thereafter. The boxes were placed in a germinator set at 30° C. and an approximate relative humidity of 85 per cent.

Methods similar to those of Mitchell and Brown (22) were used in sowing the mustard seeds. These seeds were sown in the soil at weekly intervals, and the growth of the seedlings was compared to the growth of those sown in untreated peat soil. At the end of three weeks no indication of 2,4-D remained. In testing persistence of 2,4-D in seeds, therefore, the seeds should be stored in warm moist peat soil for at least three weeks.

The seeds of sweet clover, butterprint, Pennsylvania smartweed, and giant ragweed were treated, rinsed, and mixed in untreated peat soil. They were then stored in cheese boxes as described above. After seven weeks the seeds were sifted from the soil and the dormancy broken. The seeds were then germinated and the radicles measured.

Plot design	Unpaired comparisons
Number of replicates	Sweet clover and butterprint, five of 50 seeds each; smartweed and ragweed, five of 10 seeds each
Duration of treatment	12 hours

Subterranean clover seeds were handled somewhat differently. With these seeds three treatments were set up as follows in five boxes each: 1. seeds soaked in tap water, rinsed, and stored in untreated peat soil; 2. seeds soaked in a 10,000 p.p.m. 2,4-D solution, rinsed, and stored in untreated peat soil; and 3. seeds soaked in tap water, rinsed, and stored in peat soil mixed with 2,4-D (method described on page 7).

The moisture level was kept constant by the use of cloth wicks inserted into each box from the bottom. The ends of the wicks were placed in pans of water beneath the boxes, thus maintaining a constant moisture level near soil saturation. After three weeks the seeds were sifted from the soil and the results noted.

Plot design	Randomized block
Number of replicates	Five boxes of 200 seeds each
Concentrations	Tap water and a 10,000 p.p.m. 2,4-D solution
Duration of treatment	12 hours

The Penetration of 2,4-D Through Seed Coats of Nondormant and Dormant Seeds

This study was planned to obtain information concerning the extent of penetration into nondormant, impermeable, and embryo-dormant seeds. Included were seeds of giant ragweed (embryo-dormant), butterprint (impermeable), and beans and corn (nondormant). They were soaked in an aqueous 2,4-D solution and rinsed. Parallel tests were then made by comparing two groups: seeds with coats removed and seeds with coats intact after rinsing. Special treatment was necessary in all cases for breaking the dormancy of butterprint and giant ragweed.

Seed coats were removed from beans after rinsing by slitting them with a dissecting needle along the line of intersection between the two cotyledons and then slipping off the seed coat. The seed coats of corn were removed by tearing them off at the tip or embryo end and slipping off the rest of the coat.

Seed coats of butterprint were removed by chipping the edges of the seed coats with a razor blade, placing a dissecting needle between the seed coats and cotyledons, and prying the coats off. Seed coats of giant ragweed were removed by clipping the edges and forcing out the seeds. In all cases the seed and the hands of the experimenter were rinsed in running water after the removal of each seed coat. Methods of seed coat removal are illustrated in figure 1.

After removal of seed coats the seeds were germinated and the radicles measured.

Plot design	Unpaired comparisons
Number of replicates	Ten of 10 seeds each
Concentrations	Tap water and a 1,000 p.p.m. 2,4-D solution for beans and corn. Tap water and a 10,000 p.p.m. 2,4-D solution for butterprint and ragweed
Duration of treatment	12 hours

The Effect of Volatile 2,4-D on the Radicle Growth of Corn and Wild Mustard Seeds

The objective of this study was to determine whether seeds placed in the vicinity of volatile 2,4-D would be injured by the fumes. Dry seeds were exposed to volatile 2,4-D for periods of 16 or 48 hours and imbibed seeds (that is, seeds soaked in water for 24 hours) were placed in a 2,4-D atmosphere for 16 hours. A corresponding 48-hour treatment of imbibed seeds was not possible because of the initiation of germination after 24 hours of soaking.

Seeds were exposed by placing them under a bell jar around an open petri

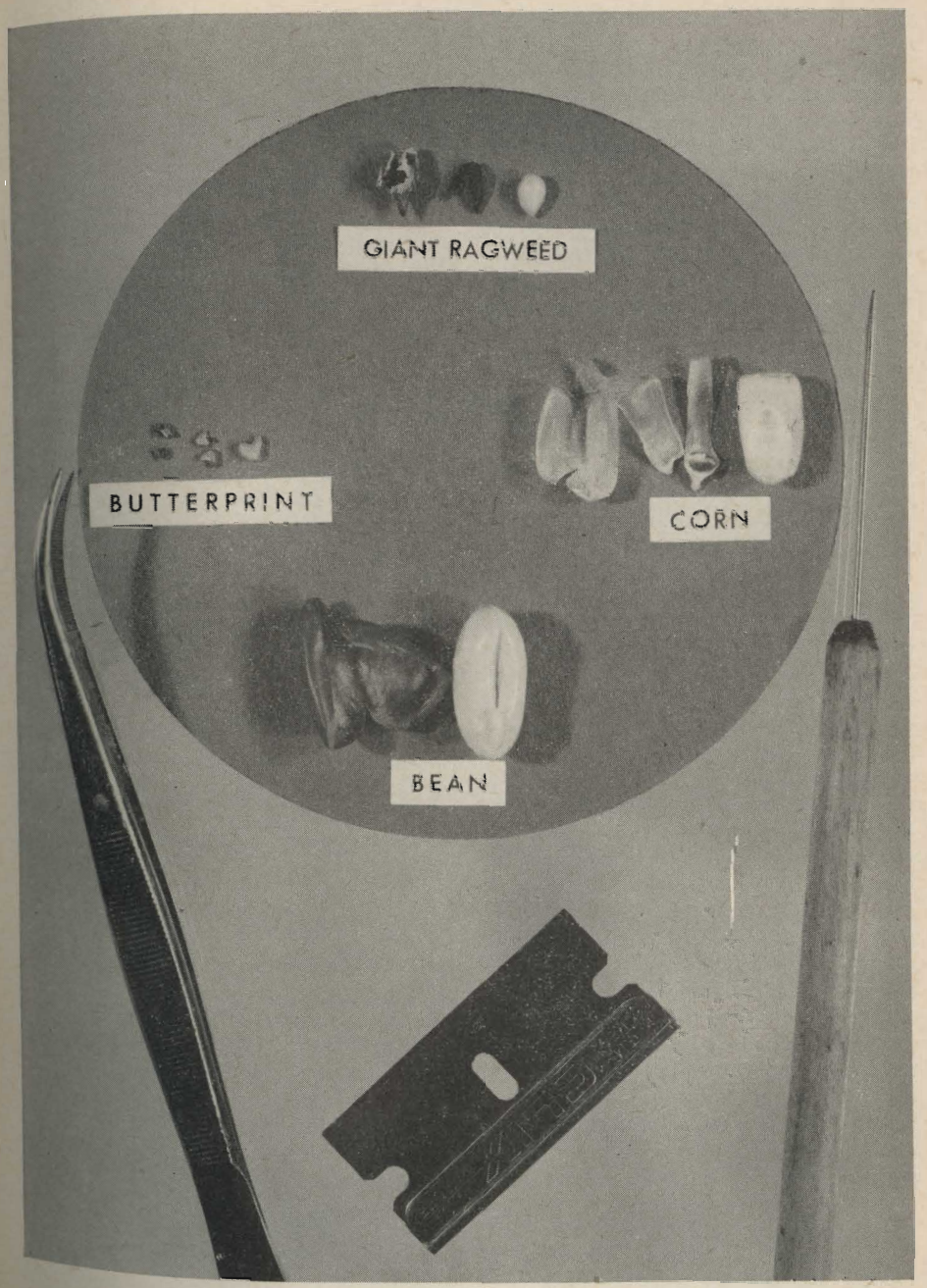

Fig. 1. Methods of removing seed coats and materials used.

dish containing the reagent in absorbent cotton. The treatment was carried out at 28° C. in a relative humidity of approximately 80 per cent. After treatment the seeds were germinated without rinsing and the radicles were measured.

In the case of dry corn the usual number of seeds and replicates was not used. There was a trend of inhibition of radicle growth after the 48-hour treatment using 10 seeds in 10 replicates, but the differences were not significant; therefore, to be certain whether there was inhibition the 10 dishes were considered one replication, which was repeated three times.

Plot design	Randomized block
Number of replicates	Corn, three of 100 seeds each; wild mustard, five of 50 seeds each
Formulation	Volatile ester
Duration of treatment	Dry seeds, 16 or 48 hours; imbibed seeds, 16 hours

EXPERIMENTAL RESULTS

The Effect of 2,4-D on Radicle Growth of Nondormant Seeds

THE EFFECTS that five concentrations of 2,4-D, in comparison with the control, had on radicle growth were studied separately for each species. Seeds of each species were divided into four lots, and each lot was soaked for two, four, eight, or 16 hours. The data from each species were tabulated for length of radicle and analyzed by means of an analysis of variance. Highly significant F values were obtained in all cases for duration (i.e., the four periods of soaking) with the exception of wheat; for concentration; and for the interaction of concentration × duration.

Since the environmental conditions through all periods of soaking were not strictly comparable the interpretation of the results was made primarily in relation to the concentrations of 2,4-D that were studied. As the statistical studies showed a highly significant quadratic effect of concentrations it is apparent that the effect of various concentrations is curvilinear. The least significant differences were calculated at the five per cent point among concentrations.

The important results can be seen easily by a diagrammatic summary presented in figures 2 through 6. The figures at the bottom of each graph, i.e., −1, 0, 1, 2, and 3 are the log of 0.1, 1, 10, 100, and 1,000 p.p.m. 2,4-D concentrations respectively. The logs were used for convenience in plotting and

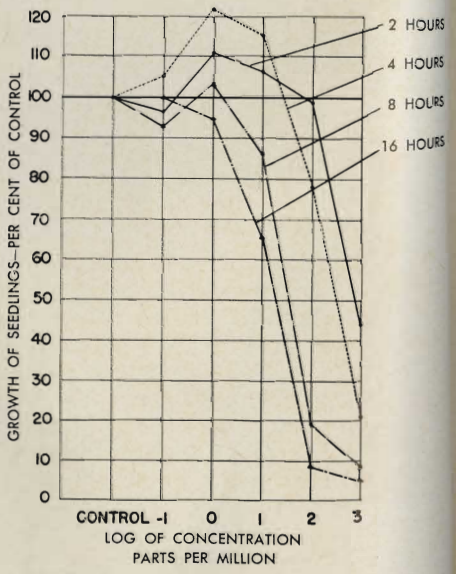

Fig. 2. The effects of 2,4-D concentration and duration of treatment on wild mustard seeds—seeds rinsed four hours after treatment.

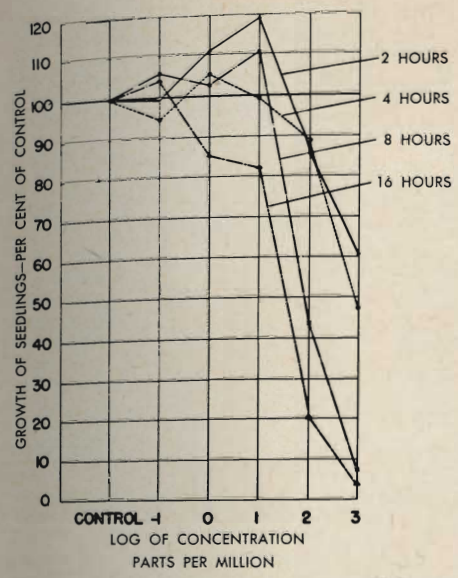

Fig. 3. The effects of 2,4-D concentration and duration of treatment on pennycress seeds—seeds rinsed four hours after treatment.

handling the data, and the L.S.D. values as calculated were used in interpreting differences. These values are directly applicable to differences in radicle growth in the diagrammatic summary, which presents the effect of 2,4-D in comparison with the control calculated as 100.

WEED SPECIES

The responses of wild mustard and pennycress were similar, as seen in figures 2 and 3. There was a general tendency for radicles soaked in 0.1 or 1 p.p.m. concentrations for two, four, or eight hours to be longer than the radicles of the control. This tendency indicates a possible growth stimulation but was not statistically significant in any of the comparisons. A larger number of replications would be needed to determine whether this tendency was due to random sampling or to stimulation of radicle growth as a result of the treatment.

Inhibition, resulting in a significantly shorter growth of the radicle, began (for both species) with the 10 p.p.m. concentration and the 16-hour soaking. Greater inhibition followed from heavier concentrations or longer soaking with the same concentration.

Curly dock showed more tolerance to high concentrations of 2,4-D (see figure 4) than wild mustard or pennycress. There was a general tendency for concentrations of 0.1 and 1 p.p.m of 2,4-D to stimulate radicle growth of the curly dock, as was noted with wild mustard and pennycress. The increased length of radicle in the 1 p.p.m. concentration with the eight-hour soaking was the only statistically significant increase in growth found in the entire study. The first statistically significant reduction in radicle length occurred with the 100 p.p.m. concentration and 16-hour soaking. The relative tolerance of the three species of weeds may be noted by a comparison of figures 2, 3, and 4.

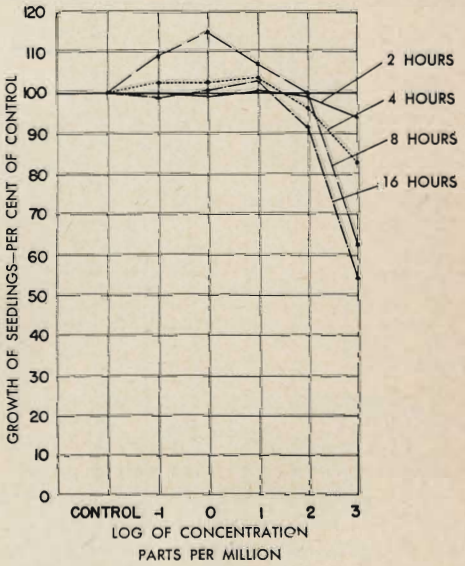

Fig. 4. The effects of 2,4-D concentration and duration of treatment on curly dock seeds—seeds rinsed four hours after treatment.

CROP SPECIES

The degrees of response of wheat and corn, as given in figures 5 and 6, were similar to each other and were intermediate between those of mustard and pennycress and that of curly dock. Inhibition of growth of the radicle was statistically significant with corn at the 100 p.p.m. concentration and one-hour period of treatment and with wheat at the same concentration for a two-hour period. Longer periods of treatment or increased concentrations gave a progressively greater inhibition of growth. There was an apparent stimulation at low concentrations, but the increases in radicle length were not statistically significant for any comparison.

Maximum effects on the radicle growth of nondormant seeds were found in mustard, pennycress, and wheat, for radicle length was less than five per cent of the control at the 1,000 p.p.m. concentration and the 16-hour period for these three species. Minimum effect occurred in curly dock, while results for corn approached those for wheat, mustard, and pennycress. It is clear from this experiment that soaking nondormant seeds in solutions of 2,4-D results in inhibited germination as measured by radicle growth.

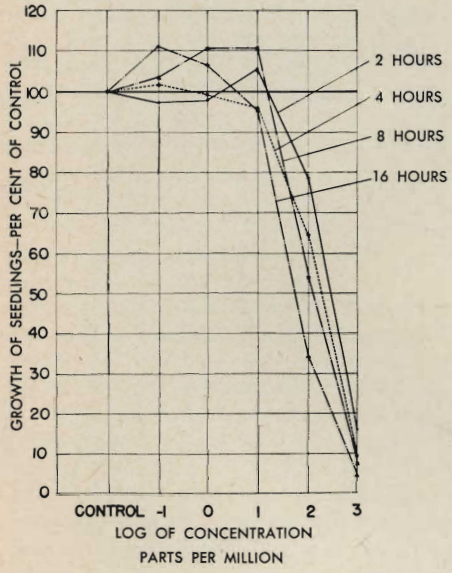

Fig. 5. The effects of 2,4-D concentration and duration of treatment on wheat seeds—seeds rinsed four hours after treatment.

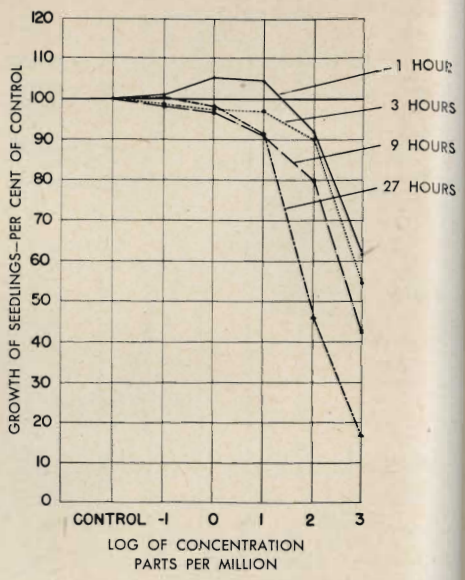

Fig. 6. The effects of 2,4-D concentration and duration of treatment on corn seeds—seeds rinsed four hours after treatment.

The Effect of 2,4-D on Radicle Growth of Dormant Seeds

Since radicle growth of nondormant seeds was found to be inhibited by a strong solution of 2,4-D, this experiment was planned to determine if dormant seeds would also be affected by 2,4-D solutions. The effects of 2,4-D on embryo-dormant and impermeable seeds were studied separately and are reported in tables 2 and 3 respectively.

The data in table 2 indicate that embryo-dormant seeds of pale and Pennsylvania smartweed that had been soaked in a 10,000 p.p.m. solution of 2,4-D for 48 hours and then rinsed contained sufficient 2,4-D to reduce radicle growth. Giant ragweed seeds did not germinate at all after this treatment.

Table 2. The Effect of 48 Hours Soaking in 2,4-D Solution on Radicle Growth of Weed Seeds with Embryo Dormancy (Dormancy Broken after Treating and Rinsing)

	Control	Soaked in 2,4-D at 10,000 p.p.m.	Mean diff.	t value
	Average radicle length in mm.			
Pale smartweed	19.32	9.44	—9.88	11.91*
Pennsylvania smartweed	27.50	2.78	—24.72	9.80*
Giant ragweed	29.20	No growth	.....	.....

* $P < 0.05$

The effect of 2,4-D on impermeable seeds (table 3) was similar to that on nondormant and embryo-dormant seeds. Radicle growth was inhibited by high 2,4-D concentrations. Seeds soaked after their impermeability was broken were more severely inhibited than those soaked while impermeable.

Figures 7 and 8 are photographs of seedlings from seeds of butterprint and sweet clover that had been soaked in a 2,4-D solution, compared with untreated seedlings of the same species.

Table 3. The Effect of 12 Hours Soaking in 2,4-D Solution on Radicle Growth of Impermeable Seeds of Butterprint and Sweet Clover

	Control	Soaked in 2,4-D at 10 p.p.m.	Soaked in 2,4-D at 1,000 p.p.m.	L.S.D. at 5% level
	Average radicle length in mm.			
Impermeable seed				
Butterprint	27.7	18.5*	7.3*	3.16
Sweet clover	17.6	17.5	4.4*	1.94
Permeable seed				
Butterprint	27.6	4.3*	1.3*	1.64
Sweet clover	24.8	2.0*	1.0*	1.86

* $P < 0.05$

The Persistence of 2,4-D in Seeds under Storage, as Measured by Radicle Growth

Studies were made on the persistence of 2,4-D in seeds by measuring the radicle growth of germinating seedlings after storage under three conditions. The results will be discussed separately for the three types of storage: on moist blotters at 2° C., under dry conditions at 30° C., and in moist peat soil. As in the other experiments the seeds were rinsed in water after the 2,4-D treatment but before storage.

Table 4. The Persistence of 2,4-D in Weed Seeds Stored Wet at 2° C. After Soaking in 2,4-D Solution 12 Hours

	Control	Soaked in 2,4-D at 1,000 p.p.m.	Mean diff.	t value
	Average radicle length in mm.			
Night flowering catchfly				
Stored two months	15.00	5.96	—9.04	12.26†
Stored three months	19.36	13.24	—6.12	1.05
Curly dock				
Stored one month	13.00	7.92	—5.08	4.32†
Stored two months	16.30	14.64	—1.66	2.24
Giant ragweed				
Stored three months	21.60	No growth	.....	.....

* $P < 0.05$
† $P < 0.01$

WET STORAGE ON BLOTTERS AT 2° C.

Data on the radicle growth of night flowering catchfly and curly dock after wet storage are given in table 4. The last month in which 2,4-D produced a statistically significant radicle inhibition and the first month in which the effect of 2,4-D was not statistically sig-

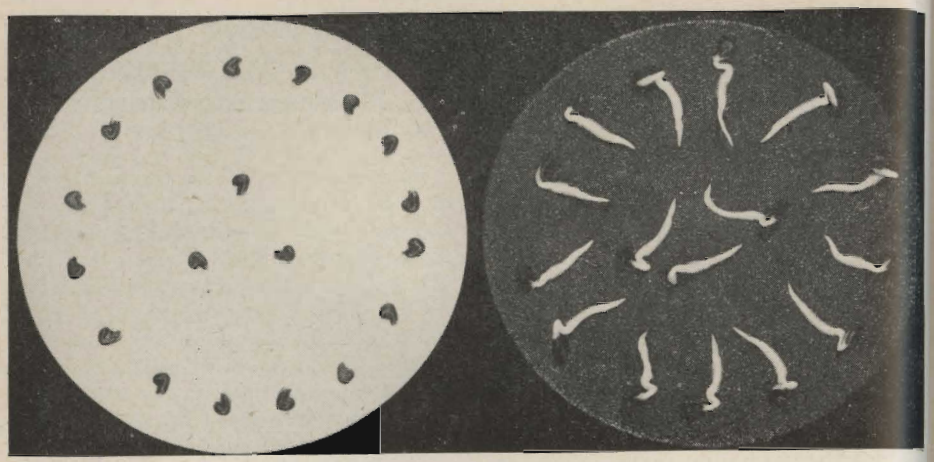

Fig. 7. The effect of 12 hours' soaking in 2,4-D on impermeable seeds of butterprint—seeds rinsed and impermeability broken after treatment. Left, seeds soaked in 1,000 p.p.m. solution; right, seeds soaked in tap water.

nificant are shown. These periods of storage were determined by studies made at the end of each month. Data for giant ragweed are shown in the table only after three months of storage, since treated seeds of giant ragweed were decayed at three months and storage was discontinued.

The "t" test was used for a comparison of radicle growth of control seeds with those treated with 2,4-D. Table 4 shows that when seeds were stored on a wet substrate at 2° C. after treatment, results differed with weed species. Night flowering catchfly radicles from treated seeds were significantly inhibited after two months' storage, but after three months they were not significantly shorter than the control. After one month's storage radicles from treated curly dock seeds were significantly shorter than the controls, but not after two months' storage. As pointed out, treated giant ragweed seeds had decayed after three months. Seeds of both curly dock and night flowering catchfly are quite tolerant to 2,4-D—possibly because they do not imbibe much solution or because they are inherently resistant.

The radicle growth of seeds of all four species was studied at the end of each month for a period of six months. The results in table 5 are those obtained at the close of the six-month period.

Table 5. The Persistence of 2,4-D in Weed Seeds Stored Dry for Six Months After Soaking in 2,4-D Solution 12 Hours

	Control	Soaked in 2,4-D at 1,000 p.p.m.	Mean diff.	t value
	Average radicle length in mm.			
Wild mustard	18.56	2.78	−15.78	8.41*
Night flowering catchfly	15.14	3.44	−11.70	14.50*
Curly dock	12.44	10.04	− 2.40	3.33†
Giant ragweed	20.50	No growth	.....	.....

* $P < 0.01$
† $P < 0.05$

The effects of 2,4-D were evident on wild mustard, night flowering catchfly, curly dock, and giant ragweed after six months of dry storage following treatment. The "t" value was highly significant for the first two species and significant at the five per cent point for

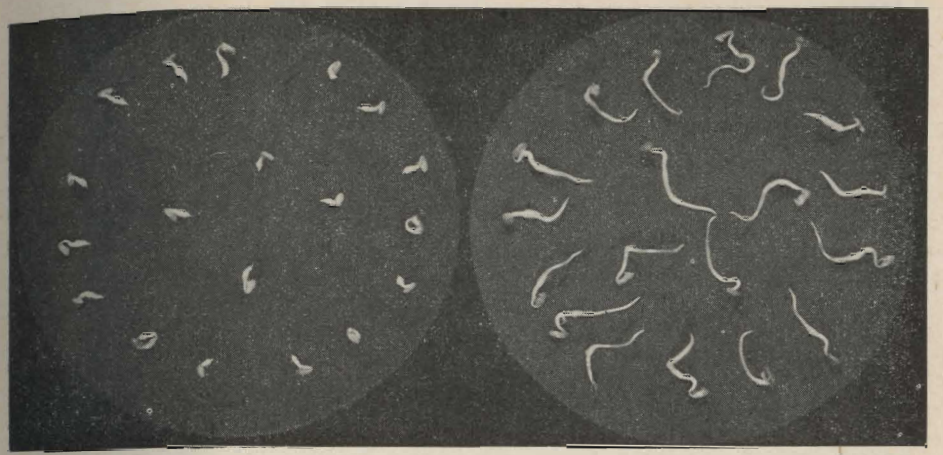

Fig. 8. The effect of 12 hours' soaking in 2,4-D on impermeable seeds of sweet clover—seeds rinsed and impermeability broken after treatment. Left, seeds soaked in 1,000 p.p.m. solution; right, seeds soaked in tap water.

curly dock. Giant ragweed failed to germinate. The data show that 2,4-D persisted longer in seeds stored dry than stored wet, but factors other than moisture were involved.

STORAGE IN MOIST PEAT SOIL AT 30° C.

It is apparent that 2,4-D persists in seeds for a considerable time under controlled conditions in the laboratory, but from a practical point of view it is important to know if it persists in seeds stored in moist soil. The study of this possibility was made by storing dormant seeds for seven weeks in moist peat soil at a temperature of 30° C. after treatment with 2,4-D. After storage the seeds were removed from the soil, their dormancy broken as described in the section on Materials and Methods, and the effect of 2,4-D on radicle growth then determined.

Radicle lengths of impermeable seeds of sweet clover and butterprint are reported in table 6, and the seedlings of these plants are shown in figure 9. These radicle lengths and seedlings indicate that 2,4-D carried by the seeds was no longer present after seven weeks' storage in warm peat soil in amounts large enough to affect the seedlings. The disappearance of the 2,4-D is attributed to decomposition brought about by the soil.

The 2,4-D present only on the seed coats was apparently readily accessible to soil organisms and chemicals. The dormant seeds with permeable seed coats, such as Pennsylvania smartweed and giant ragweed, still showed the effects of the 2,4-D even after the seven

Table 6. The Persistence of 2,4-D in Dormant Seeds When Stored in Moist Peat Soil Seven Weeks Before Breaking Dormancy

	Control	Soaked in 2,4-D at 10,000 p.p.m.	Mean diff.	t value
	Average radicle length in mm.			
Seeds with impermeable seed coats				
Sweet clover	13.16	12.98	−.18	.266
Butterprint	11.33	12.18	+.85	1.55
Seeds with permeable seed coats				
Pennsylvania smartweed	58.98	4.74	−54.24	5.93*
Giant ragweed	25.50	No growth	.....	.....

* $P < 0.01$

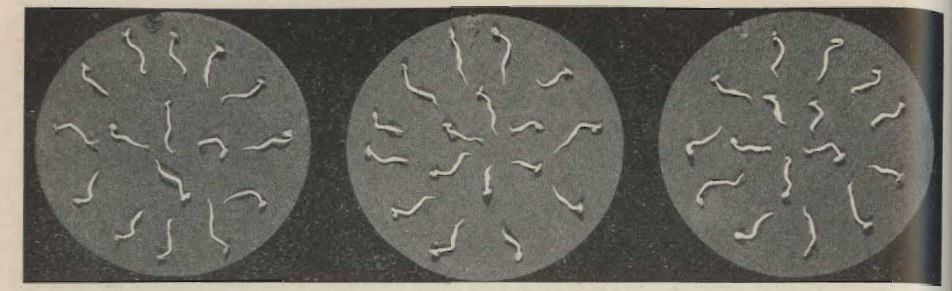

Fig. 9. The persistence of 2,4-D in impermeable seeds of sweet clover after seven weeks' storage in moist peat soil. Left, untreated seeds placed in soil treated with 8.2 mg. of 2,4-D acid per pound of soil; center, seeds soaked in 10,000 p.p.m. solution for 12 hours and placed in untreated soil; right, untreated seeds placed in untreated soil.

weeks' storage in warm moist peat soil. Treated seeds of giant ragweed did not germinate after transfer to alternating temperatures of 20° and 30° C., and when seed coats were removed it was found that they were decayed.

A somewhat similar study was carried out with subterranean clover. However, in this case an added method was used, which consisted of soaking the seeds in tap water in exactly the same manner as for the control and then placing them at 30° C. in moist peat soil to which 2,4-D had been added.

It was planned to store the seed of subterranean clover in peat soil also for seven weeks. At the end of three weeks it was found that (1) 40 per cent of the seeds soaked in tap water and stored in untreated soil had started to sprout, 54 per cent had imbibed water but had not germinated, and six per cent of the seeds remained hard; (2) 92 per cent of the seeds soaked in a 10,000 p.p.m. 2,4-D solution and stored in untreated soil at 30° C. had died (decayed with no indication of having sprouted) and eight per cent remained hard; (3) two per cent of the seeds soaked in tap water and stored in treated soil had abnormal sprouts, 95 per cent were decayed, and three per cent stayed hard.

These studies of seed storage in moist peat soil show that the radicle growth of sweet clover and butterprint seeds with impermeable seed coats was not inhibited by soaking in 10,000 p.p.m. of 2,4-D, followed by rinsing to remove the 2,4-D from the outside of the seeds. They are examples of seeds which 2,4-D does not penetrate beyond the seed coat. It seems probable that the 2,4-D was sufficiently accessible to soil organisms and chemicals so that it was decomposed during storage in the peat.

The results were very different with Pennsylvania smartweed, giant ragweed, and subterranean clover, which have permeable seed coats. Apparently penetration of 2,4-D extended beyond the seed coats in these species, and radicle growth was inhibited even after storage in the soil.

The Penetration of 2,4-D Through Seed Coats of Nondormant and Dormant Seeds

Results of storing treated seeds in moist peat soil indicated that a 2,4-D solution did not penetrate seed coats impermeable to water but did penetrate permeable seed coats. This experiment gives more information concerning this penetration.

The experiment with tendergreen beans and field corn seeds, which are representatives of nondormant seeds, consisted of a comparative study of the growth of the radicles after one group

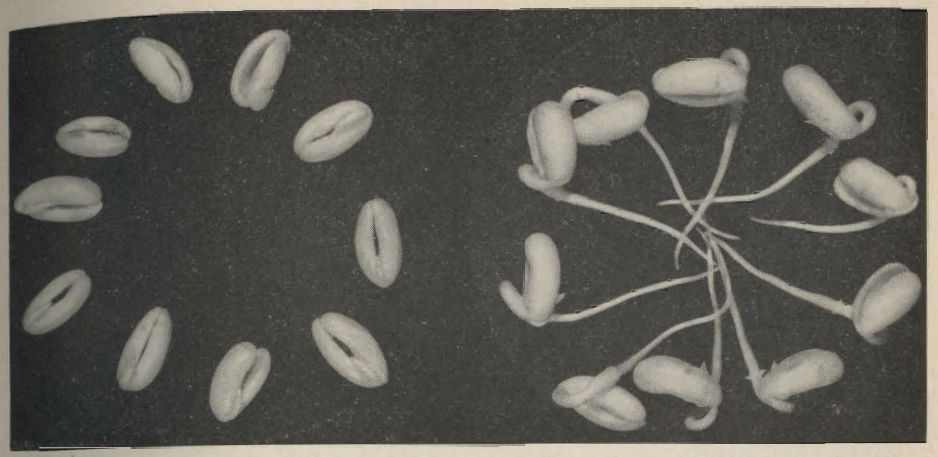

Fig. 10. The penetration of 2,4-D through water-permeable seed coats of beans—seeds soaked 12 hours, rinsed, and coats removed. Left, seeds soaked in 1,000 p.p.m solution; right, seeds soaked in tap water.

of seeds was treated with 1,000 p.p.m. of 2,4-D and another group was untreated. The study was made both with and without the seed coats removed after treatment and rinsing. The primary purpose of these studies was to learn whether there was 2,4-D penetration beyond the seed coat. The results are presented in tabular form in table 7 and also in figures 10 and 11.

The results indicate that 2,4-D did penetrate the permeable seed coats of beans and corn. Inhibition of germination was so severe in beans that the seedlings did not grow appreciably. Although initial inhibition of the corn was severe, the seedlings from seeds with or without the seed coats removed recovered from the initial effect and continued to grow.

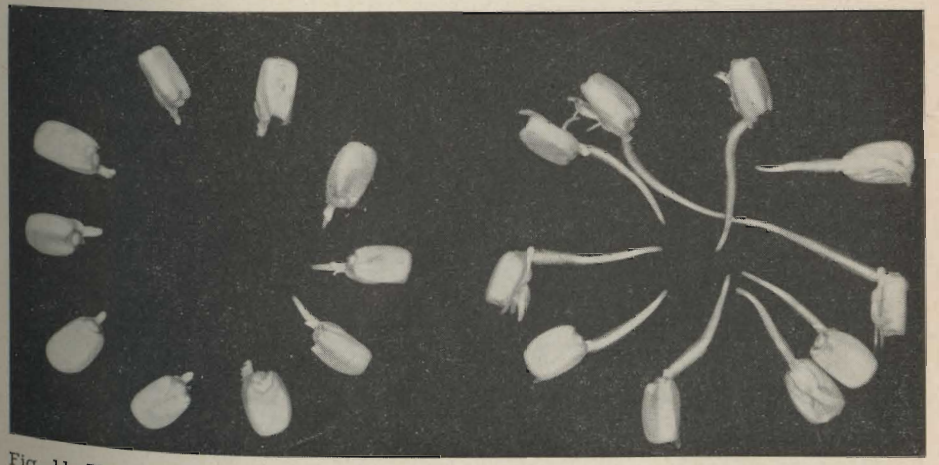

Fig. 11. The penetration of 2,4-D through water-permeable seed coats of corn—seeds soaked 12 hours, rinsed, and coats removed. Left, seeds soaked in 1,000 p.p.m. solution; right, seeds soaked in tap water.

Similar studies with dormant seeds of giant ragweed and butterprint are summarized in table 8 and illustrated in figures 12 and 13. The results indicate that 2,4-D did penetrate the permeable yet embryo-dormant seeds of giant ragweed, because there was no germination of seeds with seed coats either removed or left intact (figure 12).

The 2,4-D did not penetrate the seed coats of butterprint, which are impermeable to water. This is shown by the fact that radicle growth of seeds from which the seed coats had been removed

Table 7. The Penetration of 2,4-D Through Water-Permeable Seed Coats of Nondormant Bean and Corn Seeds

	Control	Soaked in 2,4-D at 1,000 p.p.m.	Mean diff.	t value
	Average radicle length in mm.			
Seed coats removed after rinsing				
Beans	23.48	No growth	.....	.....
Corn	15.15	1.90	13.25	9.14*
Seed coats not removed after rinsing				
Beans	18.29	No growth	.....	.....
Corn	23.89	5.43	18.46	12.65*

* $P < 0.01$

Table 8. The Penetration of 2,4-D Through Seed Coats of Dormant Giant Ragweed and Butterprint Seeds

	Control	Soaked in 2,4-D at 10,000 p.p.m.	Mean diff.	t value
	Average radicle length in mm.			
Seed coats removed after rinsing				
Giant ragweed	15.75	No growth	.....	.....
Butterprint	30.62	31.90	+1.18	.535
Seed coats not removed after rinsing				
Giant ragweed	29.00	No growth	.....	.....
Butterprint	25.60	No growth	.....	.....

was normal, as may be seen in figure 13. When the seed coats were not removed, however, no growth occurred—indicating that sufficient 2,4-D was retained in the seed coat to cause severe inhibition.

The Effect of Volatile 2,4-D on the Radicle Growth of Corn and Wild Mustard Seeds

Seeds of certain plants are affected by aqueous solutions of 2,4-D; however, under dry soil conditions it might be

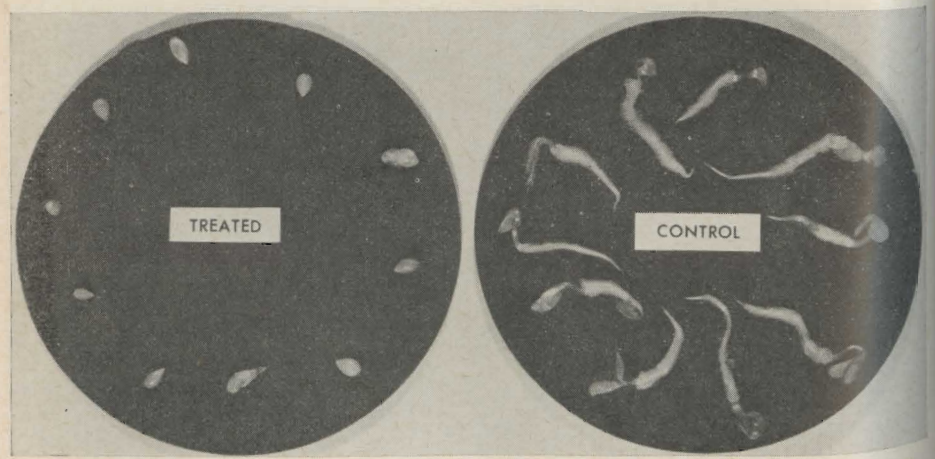

Fig. 12. The penetration of 2,4-D through seed coats of dormant giant ragweed seeds—seeds soaked 12 hours, rinsed, coats removed, and dormancy broken. Left, seeds soaked in 10,000 p.p.m. solution; right, seeds soaked in tap water.

Table 9. The Effect of Volatile 2,4-D on Dry Seeds of Corn and Wild Mustard

	Control	Length of treatment 16 hrs.	48 hrs.	L.S.D. at 5% level
	Average radicle length in mm.			
Corn	32.83	33.80	29.10*	2.88
Wild mustard	11.79	8.14*	7.12*	1.31

* $P < 0.05$

necessary to use a chemical which is toxic to seeds but which does not depend on an aqueous solution for penetration. The study with volatile 2,4-D was made to determine its effect on both dry and wet seeds of corn and wild mustard.

Results with dry seeds are given in table 9. The differences between the control and corn treated for 48 hours and between the control and wild mustard treated for either 16 or 48 hours were significant at the five per cent point. L.S.D. values were calculated at the five per cent level. While volatile 2,4-D significantly affected radicle growth of both corn and wild mustard seeds the inhibition was not very great.

There was no significant reduction in length of corn or mustard radicles, however, when the seeds were soaked in water before they were placed in a 2,4-D atmosphere for 16 hours. Results with imbibed seeds are given in table 10.

An attempt was made to study the effect of 48-hour exposure to volatile 2,4-D on the imbibed seeds, but this proved impossible. Growth started about 24 hours after the seeds were removed from water; hence 2,4-D would have acted on seedlings, not seeds.

Table 10. The Effect of 16 Hours Exposure to Volatile 2,4-D on Corn and Wild Mustard Seeds Soaked in Water Four Hours Prior to Treatment

	Control	Allyl ester	Butyl ester	L.S.D. at 5% level
	Average radicle length in mm.			
Corn	47.37	47.15	48.71	F value not significant
Wild mustard	35.90	30.50	32.30	F value not significant

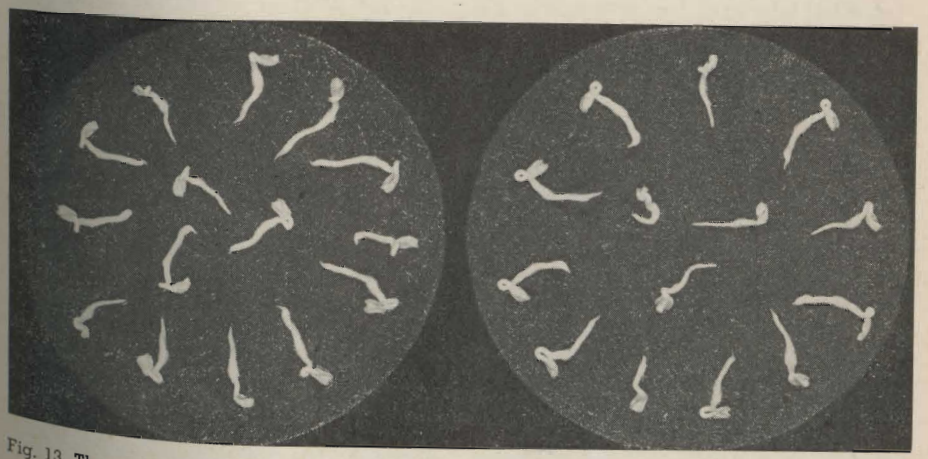

Fig. 13. The penetration of 2,4-D solution through impermeable seed coats of butterprint seeds—seeds soaked 12 hours, rinsed, and coats removed. Left, seeds soaked in 10,000 p.p.m. solution; right, seeds soaked in tap water.

DISCUSSION

THE FACT that radicle growth was inhibited in seeds used in the first two experiments of this study shows that 2,4-D may affect seeds before there are visible signs of germination except swelling as a result of imbibition. The emerging radicles could not have been affected by uncombined 2,4-D remaining on the outside of the seed coat, since all seeds were thoroughly rinsed after treatment.

Both nondormant and embryo-dormant seeds were affected by 2,4-D and even seeds with impermeable seed coats showed an inhibition of radicle growth. However, the effect was greater when the impermeability was broken before soaking the seeds in 2,4-D.

Curly dock exhibited more tolerance to 2,4-D than the other species used, and this tolerance may be explained by the fact that both its rate of imbibition and its total imbibition were less than those of other species. If rates and amounts of imbibition vary rather widely among species and if they are related to tolerance, there is a possibility that there could be selective injury to the viability of mixed seeds. Further studies might result in methods that could be used to kill weed seeds or other mixtures in seed lots.

There was an indication that radicle growth was stimulated in concentrations of 0.1 and 1 p.p.m. of 2,4-D. This apparent tendency was not studied adequately to determine whether the effect was due to the treatment or was the result of random sampling. The work of Hsueh and Lou (*19*) indicates, however, that stimulation is possible.

The studies indicated that 2,4-D persisted longer in seeds stored dry than wet, but the results with wet seeds were not as reliable as those with dry seeds. This was due to a decrease in viability under wet storage because of unfavorable conditions which made interpretation of the data difficult.

It is evident from the study of seeds stored in moist peat soil that impermeable seeds retain 2,4-D only in the seed coats and that this 2,4-D may be inactivated if exposed to soil organisms and chemicals. This confirms observations by Akamine (*2*) that hard seeds are not affected by 2,4-D until the seeds imbibe moisture.

Mitchell and Brown (*22*), working with subterranean clover seeds stored in soil at temperatures which kept the seeds dormant, found that these seeds were relatively resistant to 2,4-D. In this study the reactions of seeds treated before storage in soil and of untreated seeds placed in treated soil gave evidence that 2,4-D did affect them while dormant. The conditions of the two experiments were not identical. The penetration of 2,4-D may be influenced by such factors as seed lot, moisture in seed at time of storage, soil, and 2,4-D concentration.

The studies in this bulletin showed that the effects of 2,4-D were also evident on radicles of dormant permeable seeds of giant ragweed and Pennsylvania smartweed after treated seeds had been stored in moist peat for seven weeks. Other evidence that dormancy at the time of treatment did not influence the susceptibility of seeds to 2,4-D injury is found in the study of penetration. When seed coats of giant ragweed and Pennsylvania smartweed were removed after treatment, subsequent radicle growth showed that 2,4-D had penetrated the seed coats of permeable seeds whether dormant or not.

Akamine (*2*) reported that a species of grass with which he worked was not affected by 2,4-D as long as seeds were dormant. His criterion of normal germination, however, was emergence of seedlings from the soil, as contrasted to measurement in our studies of radicle growth after treatment. Akamine assumed that once a grass seedling had emerged it would grow normally. This criterion of emergence would undoubtedly be practical in field work, but it does not measure the initial effect of 2,4-D on dormant grass seeds. It was observed in our studies and has been reported by several investigators (*14, 21, 23, 29, 35*) that grass species are relatively tolerant to 2,4-D and usually recover from any initial effects.

The trial of a volatile 2,4-D indicated that the use of this form of the chemical is not very practical. Wet seeds of corn and mustard were not affected and dry seeds only to a slight degree. The difficulty of applying volatile 2,4-D so that it would be retained for several hours and the hazards of using this formulation outside seem to preclude its use in the field. The slight inhibition of radicle growth after 16 hours of exposure indicates that volatile 2,4-D would not be very satisfactory for killing seeds in bins or sacks. However, the results do emphasize the importance of not storing 2,4-D in the near vicinity of seeds.

Although it is not known whether it is possible to treat soil under field conditions and obtain the same results on seeds shown in these studies, some information gained from these experiments is of practical importance for preplanting treatments. It seems obvious that even high concentrations of triethanolamine salt of 2,4-D will not kill or injure seeds of all weed species present in the soil. If adequate amounts are applied to moist soil, however, it is expected that permeable seeds would be affected.

Further testing of chemicals for killing weed seeds in the soil seems worth while. Although the agricultural value of any herbicide must finally be verified in field trials, laboratory testing can be of material assistance both in screening a large number of chemicals and in determining factors that influence results. Chemicals that do not kill seeds in the laboratory would probably be unsatisfactory in the field. It is difficult, too, to separate factors influencing the effect of 2,4-D on seeds from factors influencing the effect on seedlings by field tests.

Field tests, however, are necessary to show whether a chemical which is toxic to weed seeds in the laboratory will also kill these seeds in the field, whether such a chemical is readily inactivated, and whether the cost will make field application practical.

SUMMARY

IN A STUDY of the effect of 2,4-D on seeds as measured by radicle growth the following facts were found:

1. Crop and weed seeds were affected by aqueous solutions of the triethanolamine salt of 2,4-D. This effect was inhibitory at concentrations of 100 and 1,000 p.p.m. of 2,4-D, and it was surmised that these or higher concentrations could be lethal to some species. Sixteen hours of soaking in 2,4-D solutions caused inhibition of radicle growth of most species at lower concentrations than did treatments of shorter duration.

2. Dry corn and mustard seeds were subject to slight injury by exposure to volatile 2,4-D; wet seeds were not affected.

3. The 2,4-D or its effects persisted in seeds or seed coats at least six months when stored dry at 30° C. Dormant, water-permeable seeds showed the inhibitory effects of 2,4-D treatments after they had been stored in warm moist peat soil for seven weeks. The

2,4-D on or in the seed coats of impermeable seeds was inactivated when the seeds were stored in warm moist peat soil.

4. When the seed coats of dormant or nondormant water-permeable seeds were removed after treatment with 2,4-D, subsequent seedling growth was markedly less than that of the controls. When the seed coats of treated impermeable seeds were removed, the treated seeds responded like the controls.

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