RESPONSES OF RUMEX OBTUSIFOLIUS L.

TO SEVERAL

'HORMONE' HERBICIDES.

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CHAPTER I

INTRODUCTION:

Weeds have been a problem to man ever since he began to till the soil. Their presence is a factor lowering yield and increasing the cost of production of almost every economic crop. Weed eradication and control measures therefore, are bound to loom large in the management of crops at various stages throughout their growing period.

Following upon such discoveries as those of Slade et al (1) and Mitchell and Hamner (2) that synthetic growth-regulating substances possessed properties capable of causing violent and often fatal disturbances to plant growth, new and effective methods of destroying undesirable species were developed. In fact, it can be said that the whole thought and practice of weed control was revolutionized by the promotion of certain practical aspects proceeding from the rapidly accumulating knowledge of plant-growth substances.

Research soon indicated, however, that weed control problems did not suddenly cease to exist, for many species were shown to possess a moderate to high degree of resistance to the 'hormone' materials. Among such species were included docks (Rumex spp.); perennials, characterized by a long tapering or fanged storage root system, and a strong capacity to produce adventitious buds from the short portion of underground stem.

Preferring medium to heavy damp soil conditions, these weeds are serious invaders of pastures on rich dairying land while heavy infestations in annual crops can lead to greatly reduced yields. It was but a natural development therefore, that the possibility of control of these plants by the new 'hormone' herbicides was investigated in some of the initial experiments.

One of the earliest observations on the susceptibility of Rumex spp. to 2,4-dichlorophenoxy-acetic acid (2,4-D) was made by Marth and Mitchell (3). The first large scale trials however, were made in South England, where, during the 1945-46 season, 177 acres of heavily dock-infested pasture and crop land were either dusted or sprayed with the sodium salt of 2-methyl-4-chlorophenoxyacetic acid (MCPA) at rates of 2 lb. acid equivalent (A.E.) per acre as a dust, or 1 lb. A.E. per acre as a spray (4). Moderate susceptibility was indicated, as was shown by the spectacular epinastic responses and frequent death of the leaves and petioles, as well as by the decreased infestation of the treated fields when examined some weeks later. The extent to which the roots had been killed, however, remained undecided. Templeman (5) reporting on MCPA applied at 8 lb. A.E. per acre likewise was unable to reach a conclusion as to the extent of root kill, but Tincker (6) made a claim for a 50% eradication of Rumex crispus with 'Methoxone' - an MCPA dust preparation.

By using the sodium salt of 2,4-D as a powder dispensed in a dose of 5% in bentonite, Scarponi (7) claimed that it was possible to destroy Rumex crispus. No confirmation of this report has appeared but Pellegrini found that a formulation containing the sodium salt of 2,4-D in solution was an improvement (8). Gysel (9) and Wurgler (10) have reported varying successes with 2,4-D preparations similar to that of Pellegrini, sprayed on to individual plants. In a Western Australian experiment a 2,4-D compound at 4 lb. A.E. per acre reduced the prevalence of dock in a pasture from an 80% to a 50% infestation; the post-treatment analysis being made 6 months after spraying (11).

The results reported by Halliday and Templeman (12) and Holmes (13) for the sodium salts of MCPA and 2,4-D respectively, indicate that the differential responses shown by dock plants are not so much due to the kind of herbicide employed as to the stage of maturity at the time of treatment.

In the trials conducted by the New Zealand Department of Agriculture, several preparations, in addition to the materials already discussed, have been studied for their herbicidal effects on Rumex spp. (14,15). These include both oil-based, and the water soluble polyethylene glycol-based esters of 2,4-D, the triethanolamine salt of 2,4-D and 2,4,5-trichlorophenoxyacetic acid (2,4,5-T) compounds. It was found that in addition to stage of development, variation in response couldalso be attributed to the herbicidal preparation used. Thus, the oil-based ethyl ester of 2,4-D was more effective than the sodium salt of either MCPA or 2,4-D. Also the results obtained with the polyethyleneglycol-based esters of 2,4-D were very promising.

It is seen from the above review of work done, that much information pertaining to the control of docks by 'hormone' substances has been collected, but the data has been principally of an observational or empirical nature rather than of a quantitative kind. The preliminary observational trials are now largely completed. For further progress in the development of effective treatment, it is now important that more accurate assessments of the toxicities of the various 'hormone' herbicides be undertaken from the point of view of the percentage mortalities achieved at varying dosage rates. That is, more accurate evaluations in terms of killing capacity of herbicides used at different concentrations under diverse environmental conditions are required.

By the use of techniques involving quantal responses, assessments of some herbicides on several weed species other than Rumex, have been made.

Willis (16), for example, compared the relative effectiveness of two 'hormone' herbicides as agents in buttercup control. He used the factors of plant numbers, flower-stalk numbers and weight of foliage per unit area, before and after treatment, as criteria for comparison. Plant numbers per unit area was the only factor used in a similar experiment reported by Woodford for dinitrobutylphenol preparations as toxic agents to Stellaria media in peas (17).

It is data of this kind that is urgently required for all weed species that are regularly the object of spraying programmes. Rumex species come within this category and one of the aims of the present study has been the assessment of the relative toxicity of several hormone herbicides on Rumex obtusifolius L. growing in a ryegrass-clover pasture. Stage of growth has been recognized as having an important influence on the efficacy of weed control treatments. In the present investigation therefore, stage of growth has been studied as a variable modifying the assessments.

Germinating seeds are readily damaged by contact with high concentrations of growth regulating substances (18,19,20). Mullison and Hummer (21) showed that exposure of seeds of many species to the vapours of several of these chemicals resulted in malformations of the seedlings and reduced germination percentages. But evidence for these 'hormone' materials causing damage to ripened seeds, attached to the parent plant at the time of spraying, is meagre. Studies made by Marth et al (22) on the effect of 2,4-D acid on maturing grass and cereal seeds showed that neither the germination capacity of the sound seed nor the vigour of the seedlings was detrimentally affected. McIlrath et al (23) however, found that cotton seeds, present at the time of spraying with 2,4-D, were severely damaged, as evidenced by the reduced viability of the seeds and the reduced survival rate of the seedlings.

In an endeavour to add further information on this aspect of the action of growth-regulating substances, an examination was made during the present investigations of the abnormal effects produced by various 'hormone' herbicides on very immature and nearly ripened seeds present on <u>Rumex obtusifolius</u> plants at the time of spraying.

Measures which tend to exhaust the readily-available energy reserves of plants have long been known to weaken their ability to recover from damage and so the possibility of eradication is enhanced. Hence, in order to facilitate more effective control of many notorious weeds, changes in the levels of carbohydrate materials in root and other storage organs have been determined at various stages of development for several plants including <u>Convolvulus arvensis</u> (24, 25, 26), <u>Agropyron repens</u> (27) and <u>Cirsium arvense</u> (27,28).

From the work of several investigators, it became evident that the 'hormone' herbicides cause a loss in Dry Weight of plant parts (29,30). Experiments with Ipomoea lacunosa (29), Convolvulus arvensis (31), Taraxacum officinale (32) and Fagopyrum esculentum (33) have all indicated that this loss is due to a steady decline in the weight of starch and starch-like substances. Recent work by Rhodes (34) on the influence of an MCPA preparation on the metabolism of tomatoes, strongly suggests that the effectiveness of the toxic action resides in the capacity to deplete storage materials, especially in the roots.

Although death of a plant following 'hormone' treatment might not be the direct outcome of carbohydrate depletion, it seems logical to conclude that damage would be more severe at a time when energy reserves are at a low ebb. Work was undertaken therefore, to determine the level of readily-available carbohydrate reserves of dock roots at several stages of plant development.

Order of Presentation of Material.

The experimental procedures and results are presented in three chapters. The contents of each chapter are as follows:-

CHAPTER II: Part 1 - a description of the experimental procedure followed for the assessment of toxicity of several 'hormone' herbicides on plants of *Rumex obtusifolius*.

Part 2 - the results of the experiments described in Part 1.

Part 3 - an account of the several kinds of damage exhibited by the subterranean parts of the treated plants.

CHAPTER III: Part 1 - a study of the effects of several 'hormone' herbicides on the viability of *Rumex obtusifolius* seeds.

Part 2 - an investigation of the malformations shown by seedlings germinating from the above lines of seed.

Part 3 - descriptions of the abnormal plants developed from the malformed seedlings.

CHAPTER IV: The determinations of the readily-available carbohydrate reserves in dock roots taken from plants in various stages of development.

C H A P T E R I I

EVALUATION OF HERBICIDES.

Part 1. Experimental Details.

The evaluation of the several herbicides to be tested on docks required that a determination be made of the number of these plants growing in the plots before and after treatment. Either of two methods could have been used. One was the quadrat method which involves counting the number of plants falling within a quadrat thrown at random on to each plot a set number of times. Although easy to apply, the method provides an estimation only of plant density. Furthermore, in studies involving perennial species, for which often many months are required for the completed response to be given, there is the problem of distinguishing treated, but recovered, plants from others which have become established subsequent to spraying. Small shoots, regenerating from the roots of severely damaged plants, are likely to be very readily confused with newly emerged seedlings.

The second method involves having some means of accurately identifying all plants treated, so that their response can be readily determined at succeeding analyses. This method was adopted for the experiment to be discussed.

(i) Layout of Experiment. The field chosen for the experiment was a heavily dock-infested dairy pasture on the Massey Agricultural College Farm. It had been sown to certified H.I. and perennial ryegrass, and red and white clover about 18 months previously and had been regularly topdressed. Utilization during the second season had been by grazing only. The sward at the time just prior to this experiment was thick where the docks had not become dominant, and was tending towards rankness. Docks were very abundant and vigorous and were to be found in every stage of development from seedlings to flowering and seeding.

Two blocks, each consisting of thirteen plots, were laid across one end of the field. Down the centre of each plot, which measured 11 yd. x 4 yd., a strip 12" wide and 11 yd. long was marked out. A distance of at least 5 yd. was kept between these adjacent central strips (see Fig. I).

The 11 yd. x 1 ft. strips within the larger plots were surveyed in detail for the number and relative position of all dock plants growing within them, while the 11 yd. x 4 yd. areas were designed in order to obtain a more even spray distribution over the narrow strips through the need to apply larger quantities of material to the bigger area.

(ii) <u>Plant Recording</u>. The following procedure was adopted for the identification of every dock plant growing within the centre strips. Each 33 ft. x 1 ft. area was examined progressively in a

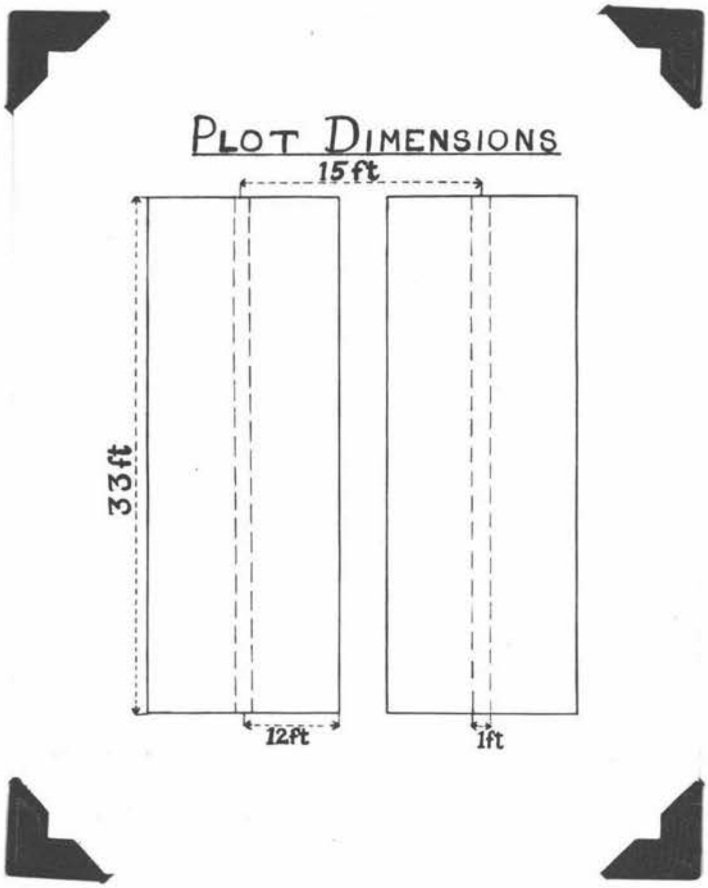


Fig.1 - Diagram showing the measurements used within and between the plots.

1 ft.square sections with the aid of a tape measure and several cords. Small scale charts showing the number and relative position of all Rumex obtusifolius plants found growing in each of the thirty-three sections of all plots, were drawn. Since the foliage of a dock plant grows almost directly above the root and does not spread by lateral stems, little difficulty was experienced in distinguishing individual plants. The number of plants in each square foot averaged about four.

In addition to recording their presence, the plants were classified according to stage of development. In a field experiment of this nature, it was impossible to group with any high degree of accuracy. Three rather broad divisions were adopted, therefore, into which the plants could be assigned with reasonable facility, and, at the same time, be representative of three important developmental stages. The three categories chosen were designated 'young', 'mature', and 'seed' and were distinguished as follows:-

(a) 'Young':- small plants with leaves not exceeding 4 inches in length and no stem development above the ground level. Frequently the number of leaves per plant was two only.

(b) 'Mature':- included all plants not identified as 'young' but from which there was no stem growth above the ground level. The representatives of of this group were generally vigorously growing plants approaching vegetative maturity. The leaves, which were more numerous than on young plants, were large and often reached a length of 12 inches.

(c) 'Seed':- in this category were included both those plants with an actively growing stem, and those which were already flowering or ripening seed. There were few radical leaves but small cauline leaves were distributed along the stem.

(iii) Spraying. The spray mixtures were applied on January 25th, 1952, five days after the plant counts had been completed. Six commercial 'hormone' herbicides, of which five were derivatives of phenoxyacetic acid, were tested. The formulation of each was as follows:-

(a) Ethyl ester of 2,4-D containing 3.6 lb. A.E. per gallon.

(b) Butoxyethanol ester of 2,4-D " 3.6 lb. A.E. per gallon.

(c) Sodium 4-chloro-orthotoloxy acetate* containing 3.4 lb. A.E. per gallon.

(d) Butyl ester of 2,4,5-T containing 3.6 lb. A.E. per gallon.

(e) Alkalolamine salt of 2,4-D " 3.6 lb. A.E. per gallon.

(f) Ethyl ester of 2,4-D based in polyethylene glycol& containing 3.6 lb. A.E. per gallon.

*Closely related to 4-chloro-2-methylphenoxyacetic acid (MCPA).

Each material was applied at two concentrations; the weaker being at the rate of 1 lb. A.E. per acre at a dilution of 1 in a 100, and the stronger at the rate of 2 lb. A.E. per acre at a 1 in 50 dilution. Thus there was a total of twelve different spray mixtures under test. All treatments and a control were included and randomly distributed once within each block, so that the whole experiment was in duplicate. Fig. 2 shows the layout of the plots within each of the two blocks and the treatment that each plot was given.

All applications were made with a knapsack sprayer, which was thoroughly rinsed in clean water between each different material or dilution used. In an endeavour to get uniform dosages on each plot, actions, such as the speed of walking, the pumping and the height that the boom was maintained above ground level, were all kept as constant as possible. In determining the application rate to each plot, the volume of material which was invariably left in the bottom of the spray tank after spraying was measured and subtracted from the quantity which had been measured in. The difference represented the total volume applied.

The weather on the day of spraying was warm and overcast. The Grassland's Division weather station recorded a maximum temperature of 66·0°F. and 60·6°F. at 9 a.m. Rain was nil, bright sunshine less than an hour, and the wind was from the west with a force a

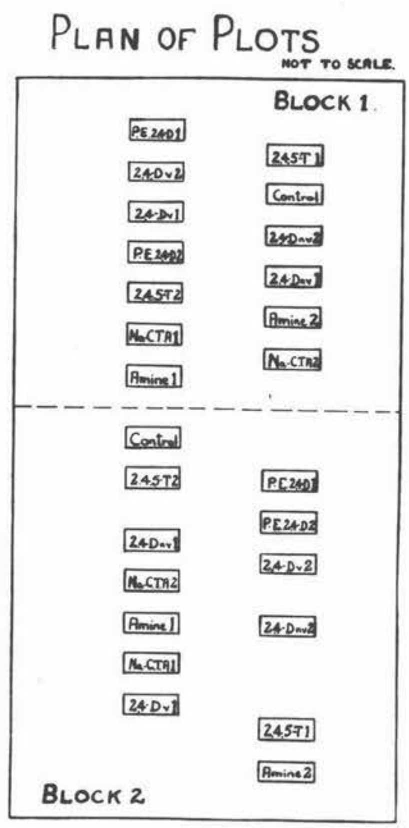


Fig. 2 - Diagram showing the distribution of the various plot treatments within the two blocks.

of 3. The next day (January 26th) was clear and sunny with a maximum temperature of 72.1 °F.

Observations of an empirical kind only, were made fairly regularly during the fortnight immediately following spraying.

The first detailed observation to determine the nature and extent of damage to the individual plants was made between February 24th and March 1st, about five weeks after spraying. Each plant which had been recorded on the charts, was re-identified and a note made as to whether it possessed, or was completely lacking, visible foliage growth. Those without such growth were ones which had been seriously injured by the herbicide and thus included those which might be eventually destroyed. Plants growing new shoots however were obviously in the process of recovery. Only a few 'young' plants had actually begun to rot. Most of the defoliated crowns remained intact.

The absence of foliage did not hamper the process of identification, as the defoliated stem-base region was found either projecting slightly above ground level, or, if subterranean, surmounted by a small hole left in the soil surface by the decaying petioles.

A second observation was made after a further five weeks, between March 30th and April 4th. The procedure followed was similar to that on the first occasion, and once more a note was made of the condition of each plant. Inspection after fifteen weeks indicated no substantial change - it then being late autumn.

The final examination was made thirty weeks after spraying, between August 23rd and 25th. The spring growth had begun, and both the recovered and the apparently unaffected dock plants were growing strongly. Plants which had recovered since the autumn were readily re-identified, but in cases where no foliage growth had appeared, it was now necessary to probe for the root. In nearly every such case the root was found to be in an advanced state of decay, but in a few instances where decay had not progressed far, young shoots, still below the ground surface, were found to be developing. In these circumstances the plant was recorded as recovered.

From the final observation, the percentage mortality obtained with the sprays on each of the three developmental stages recognized for the experiment, was calculated.

During the course of the collection of quantitative data, the plants were examined also for the purpose of ascertaining the manner in which the defoliated crowns were damaged. Material for study was dug from plot areas outside the centre experimental strips eleven weeks after spraying and photographs were taken. Results of this section of the work are presented later in this chapter.

Part 2. Results.

(i) Injury to Foliage.

The immediate reaction of the plants was very similar for the first week irrespective of the treatment. Leaves curled due to curvature of the main rib and petioles became very twisted. Stems, if in an actively growing condition, also showed similar responses, especially in the apical region.

The leaf mesophyll tissue however, tended to be affected in one of three ways, depending on the spray treatment. These were -

(a) A slow yellowing, appearance of moribund areas and eventual death of the leaf. This type of response occurred frequently with ethyl ester of 2,4-D, butoxyethanol ester of 2,4-D, polyethylene glycol 2,4-D, and amine 2,4-D at both concentrations, but chiefly at the higher in the case of the latter two.

(b) The appearance over a limited area on the leaf of brown necrotic patches. The damaged leaf was not destroyed however. This effect was most obvious with the 2 lb. rate of both 2,4,5-T and sodium 4-chloro-orthotoloxy acetate (Na-CTA).

(c) Recovery from vein curvature without any sign of necrotic tissue was a characteristic response to both Na-CTA and 2,4,5-T at the lower rate of application.

From these observations, it was possible within a fortnight, to distinguish the herbicides which were proving relatively ineffective from those which were causing a more profound disruption to growth. Plants in the 2,4,5-T and Na-CTA plots showed general recovery and growth of new foliage. Even the twisted stems became less curved and continued growth. Only in the lighter dosages of the others was there any appreciable recovery at this time.

(ii) Effect on Pasture:

The value of a herbicide does not depend wholly on its potency as a weedkiller, but also on its relative innocuousness to the crops in which weeds to be controlled are growing. Hence in the present study, the extent to which the pasture was detrimentally affected by the sprays was noted.

Clover, generally, showed considerable susceptibility, as was evidenced by the appearance of brown, scorch-like lesions on the leaflets and the disintegration of much stem tissue into a soft rot condition.

Damage was very apparent in the cases of 2,4,5-T at 1 lb. and 2 lb., amine at 2 lb., and the ethyl and butoxyethanol esters of 2,4-D at 2 lb. Mild responses only occurred in the 1 lb. plots of amine-2,4-D and polyethylene glycol-2,4-D, while Na-CTA was almost without effect. After about five weeks however, recovery was fairly general, but in the 2,4,5-T and the 2 lb. amine-2,4-D plots the clover was very much thinner. In no instance was the ryegrass noticeably reduced in vigour.

In presenting the quantal results leading to the determination of the value of the several herbicides, the data will be examined from two aspects:-

(a) the effectiveness of each hormone weedkiller as a herbicide on <u>Rumex obtusifolius</u> at three stages of development.

(b) the rate of plant recovery according to the stage of growth and the material used - that is, the time factor.

(iii) <u>Effectiveness of Materials Tested</u>.

The percentage mortalities obtained at the three stages of development with the varying treatments, are set out Table 1. Detailed results are contained in Appendix I.

TABLE I.

THE PERCENTAGE MORTALITY IN PLANTS OF RUMEX OBTUSIFOLIUS ACCORDING TO HERBICIDE TREATMENT AND STAGE OF DEVELOPMENT.

Treatment*	Stage of Development		
	'Seed'	'Mature'	'Young'
2,4,5-T 1	0	3.5	11.3
2,4,5-T 2	1.1	1.6	5.4
Na-CTA 1	0	8.6	4.1
Na-CTA 2	0	8.6	16.7
Amine 1	0	10.2	26.1
Amine 2	2.9	55.8	51.3
2,4-D v 1	1.9	16.9	24.6
2,4-D v 2	0	41.3	46.1
2,4-D nv 1	0	12.7	25.0
2,4-D nv 2	0	14.3	27.3
P.E. 2,4-D 1	2.7	17.5	30.0
P.E. 2,4-D 2	0	20.0	26.1

*The following abbreviations for the treatments studied, will be employed from hereon.

2,4,5-T 1	= 1 lb. A.E. per acre 2,4,5-T.
2,4,5-T 2	= 2 lb. A.E. " " 2,4,5-T.
Na-CTA 1	= 1 lb. A.E. " " Na-CTA.
Na-CTA 2	= 2 lb. A.E. " " Na-CTA.
Amine 1	= 1 lb. A.E. " " Amine 2,4-D
Amine 2	= 2 lb. A.E. " " Amine 2,4-D
2,4-D v 1	= 1 lb. A.E. " " ethyl ester of 2,4-D (volatile).
2,4-D v 2	= 2 lb. A.E. " " ethyl ester of 2,4-D (volatile).
2,4-D nv 1	= 1 lb. A.E. " " butoxyethanol ester of 2,4-D (non-volatile).
2,4-D nv 2	= 2 lb. A.E. per acre butoxyethanol ester of 2,4-D (non-volatile).
P.E. 2,4- D 1	= 1 lb. A.E. per acre polyethylene glycol 2,4-D.
P.E.2,4-D 2	= 2 lb. A.E. " " polyethylene glycol 2,4-D.

From the above figures several points are immediately outstanding. These can be listed as follows:-

(i) The almost complete resistance of plants in an advanced stage of development - the 'seed' plants. In eight of the twelve treatments, no mortality occurred, whilst in the remaining four, the numbers killed did not exceed 3% even in the best instance. In fact, of the 1054 plants classified as 'seed', a total of only 7 from all the plots was destroyed.

(ii) No material proved to be really effective. Apart from the 2 lb. rate of amine and 2,4-D volatile, which reduced infestation by about 50% and 40% respectively, the effectiveness of the other materials, whether measured on the 'young' or 'mature' plants, was in most cases considerably less. Few managed to control 30% of the plants and several did not give even a 10% eradication.

(iii) Results with 2,4,5-T and Na-CTA were the poorest. The former, apart from an 11.3% control of young plants at the 1 lb. dosage rate, gave percentages ranging from 1.6% to 5.4%. Figures for the latter were between 4.1% and 16.7% with the best control being given by the 2 lb. A.E. per acre dosage on 'young' plants.

(iv) Of all the treatments, amine gave the best results. On 'mature' plants it eradicated 55.8%, whilst on 'young' plants 51.3% were killed. With kills of 41.3% and 46.1% respectively, 2,4-D volatile was the second most effective herbicide.

(v) In all but two cases, the 'young' plants were more readily killed than the 'mature' plants. Severity of the sprays was about twice as great on the former as on the latter group.

(vi) Doubling the application rate of any material gave results which varied with the material. Thus 2,4-D nv 1 and 2,4-D nv 2 gave a 12.7% and 14.3% control respectively on 'mature' plants. Comparable figures for amine-1 and amine-2 were 10.2% and 55.8%. That is, 2,4-D non-volatile differed from amine by not effecting a greater control at the higher application rate. A similar result to that of 2,4-D non-volatile was recorded for 2,4,5-T, Na-CTA and P.E.2,4-D.

To enable valid conclusions to be reached from the data presented in Table I, the percentage figures for the 'mature' and 'young' plants were treated statistically. With the number of plants under test in each plot approximately the same, it was found possible to use unweighted, instead of weighted, percentage mortalities without impairing the results to any extent. To ensure homogeneity of variance in the data, all figures were transformed to angles (angle = arc $\sin\sqrt{\%}$) using the table published by Snedecor (35).

Table II contains the transformed data arranged in an ascending numerical order according to the effectiveness of the 'hormone' herbicide.

TABLE II.

ANGLES OF THE PERCENTAGE MORTALITY DATA FOR THE VARIOUS TREATMENTS AND TWO STAGES OF DEVELOPMENT.

Treatment	'Mature'	Treatment	'Young'
2,4,5-T 2	4.91	Na-CTA 1	11.67
2,4,5-T 1	10.91	2,4,5-T 2	13.88
Na-CTA 1	16.78	2,4,5-T 1	19.68
Na-CTA 2	17.17	Na-CTA 2	26.56
Amine 1	19.66	2,4-D nv 2	29.50
2,4-D nv 1	20.13	2,4-D nv 1	30.03
2,4-D nv 2	22.01	Amine 1	31.44
2,4-D v 1	24.07	P.E.2,4-D 2	31.96
P.E.2,4-D 1	25.00	2,4-D v 1	32.38
P.E.2,4-D 2	25.85	P.E.2,4-D 1	32.92
2,4-D v 2	37.79	2,4-D v 2	42.96
Amine 2	48.24	Amine 2	46.67

The results of the analysis of variance of the angles are presented in Table III.

TABLE III

ANALYSIS OF VARIANCE OF ANGLES FROM TABLE II.

Source	Sums of Squares	Degrees of Freedom	Mean Square	F value	Result
Replications	27.0	1	27.0	.45	-
Treatments	4945.2	11	448.7	7.50	* *
Stage of Development	496.0	1	496.0	8.29	* *
Interaction	266.9	11	24.3	.41	-
Error	1376.3	23	59.8		
Total	7111.4	47			

** highly significant

The presence of highly significant differences in the data according to both treatment and stage of development is indicated. The "t" value for the 5% level of significance within the treatments was calculated as 11.3 by the formula $d_{.05} = t_E \sqrt{\frac{2(EMS)}{r}}$

where t_E = value of t for error degrees of freedom,

EMS = error mean square,

and r = replications.

Using this figure as a measuring rod in Table II, it can be seen that on mature plants, amine 2 was superior to all other sprays except 2,4-D v 2, and even in this case the difference came very close to the

5% significance level. It follows therefore, that amine 2 was much better than amine 1. Comparing 2,4-D v 2 with other treatments, the results are seen to be similar in nature to those of amine 2, but of course, not of the same magnitude. On 'young' plants amine 2 was significantly more effective than all the other treatments except one and showed up better than 2,4-D v 2, which, in this case, excelled only four at the 5% level.

The applications of 2,4-D non-volatile and P.E.2,4-D at both dosages, and 2,4-D volatile and amine 2,4-D at the 1 lb. rate, each gave kills which were intermediate in magnitude between those of amine 2 and 2,4-D v 2 on the one hand and the Na-CTA and 2,4,5-T sprays on the other. With the intermediate group itself however, the results were all very similar. This was most marked with the young plants, for which the angle transformations were all within the range 20.50 to 32.92. Although between the percentage mortalities of the intermediate group and the superior amine 2 and 2,4-D v 2 treatments, there tended to be a distinct division, there was a very definite gradation of results down to the less effective Na-CTA and 2,4,5-T treatments. That is, 2,4,5-T 1, Na-CTA 1 and Na-CTA 2 were not inferior to all other herbicides.

Use of the "t" value over the range of results for the herbicides concerned, provides the basis for these conclusions.

From Table III, it is seen that stage of development of the docks was an important factor in determining the response to any of the hormone herbicides. The differences in percentage mortalities (expressed as angles) brought about by the various materials for the 'young' and 'mature' plants are presented in Table IV. The figures were determined by subtraction of the 'mature' value from that of the 'young'.

TABLE IV.

DIFFERENCES IN PERCENTAGE KILLS ACCORDING TO STAGE OF DEVELOPMENT. (DATA IN ANGLES.)

Treatment	Difference (Young-mature)	Treatment	Difference (Young-mature)
2,4,5-T 1	8.77	2,4-D v 1	8.31
2,4,5-T 2	8.97	2,4-D v 2	5.17
Na-CTA 1	5.11	2,4-D nv 1	9.90
Na-CTA 2	9.39	2,4-D nv 2	7.49
Amine 1	11.78	P.E.2,4-D 1	7.92
Amine 2	-1.57	P.E.2,4-D 2	6.11

With a "t" value at the 5% significance level of 4.6, it is at once apparent that all sprays but one were more lethal on the lesser developed growth. In other words, the plants classified as 'young' exhibited a much greater sensitivity to the sprays than the 'mature' ones.

Experimental error is the only explanation which can be offered for the low result given by amine 2 on 'young' docks, as only in one of the plots was the atypical response given.(see Appendix I).

(iv) The Rate of Plant Reoovery

From the data collected at five, ten and thirty weeks after spraying, it was possible to get some indication of the importance of time in the recovery of *Rumex obtusifolius* to various 'hormone' herbicides.

Table V shows the percentage of plants remaining either defoliated or actually destroyed at the three intervals after spraying for the 'mature' and 'young' stages of development. The figures for the thirtieth week represent the final kill. Calculations are based on the data contained in Appendix I.

TABLE V.

PERCENTAGES OF 'MATURE' AND 'YOUNG' PLANTS KILLED, OR REMAINING DEFOLIATED, AT THREE INTERVALS AFTER SPRAYING.

Treatment	Time (weeks)	'Mature'	'Young'
2,4,5-T 1	5 10 30	14 7 4	17 11 11
2,4,5-T 2	5 10 30	2 2 2	14 7 5
Na-CTA 1	5 10 30	12 10 9	11 6 4
Na-CTA 2	5 10 30	13 13 9	26 20 17
Amine 1	5 10 30	66 53 10	87 50 26
Amine 2	5 10 30	86 79 56	72 62 51
2,4-D v 1	5 10 30	39 31 17	52 37 25
2,4-D v 2	5 10 30	80 80 41	77 69 46
2,4-D nv 1	5 10 30	57 44 13	56 50 25
2,4-D nv 2	5 10 30	55 52 14	64 49 27
P.E.2,4-D 1	5 10 30	43 40 18	60 46 30
P.E.2,4-D 2	5 10 30	57 52 20	58 45 26

It is seen from these results that the numbers of plants which remained defoliated at the fifth week did not prove to be very reliable measures of the true lethal effects sustained. Thus, in no less than seven treatments in the 'young' group and six in the 'mature', more than 50% of the plants were in a defoliated state. Figures ranged as high as 85% (Amine 2) and 87% (Amine 1) and several others exceeded 60%. At the end of ten weeks there were still three applications which were apparently giving more than a 50% control of 'matures', whilst in the 'young', there remained six in the same category. By thirty weeks however, at which time the true lethality was determinable, only those 'young' and 'mature' plants which had been sprayed with amine 2 were found to have been destroyed in numbers exceeding 50% of the total treated.

Since a winter season came between the tenth and thirtieth weeks, docks receiving non-toxic quantities of herbicide might well have taken a somewhat longer time to recover than if such a period had not intervened. Therefore, it would be of little value to compare the rates of recovery during this period. A comparison was made however for the interval between the fifth and tenth weeks, over which there was no general cessation of growth. Table VI contains the data necessary for this comparison. Figures express as a percentage, the number of plants which, although defoliated at five weeks, had begun to grow again by ten weeks.

TABLE VI.

PERCENTAGE RECOVERY BETWEEN THE FIFTH AND TENTH WEEKS AFTER SPRAYING FOR THE 'MATURE' AND 'YOUNG' PLANTS.

Treatment	'Mature'	'Young'
2,4,5-T 1	50	34
2,4,5-T 2	0	54
Na-CTA 1	15	50
Na-CTA 2	0	23
Amine 1	21	43
Amine 2	8	15
2,4-D v 1	20	30
2,4-D v 2	0	10
2,4-D nv 1	22	10
2,4-D nv 2	4	23
P.E.2,4-D 1	6	23
P.E.2,4-D 2	8	23

Two features are noteworthy. Firstly, docks defoliated by the 1 lb. A.E. per acre rate of herbicide recovered much faster than those treated at the rate of 2 lb. A.E. per acre. Disregarding the figures for the 2,4,5-T and Na-CTA formulations due to the small number of plants involved, the recovery rate for the 1 lb. treatments averaged about 20%, while for the 2 lb. ones it was about 10%. The greatest regeneration occurred with Amine 1 (21%, 43%) and

2,4-D v 1 (20%, 30%) while Amine 2 (8%, 15%) and 2,4-D v 2 (0 %, 10%) caused far more profound effects. Other materials tended to be intermediate in their toxicity and hence in their degree of disruption to the plant.

Secondly, regardless of 'hormone' or concentration, recovery rate varied with the stage of development of the docks. Again omitting the unreliable results for Na-CTA and 2,4,5-T, it is seen that 'young' plants recovered more rapidly than the 'mature'. Regeneration of the 'young' was about twice as great as that of the 'mature' in the Amine 1 (43%, 21%) and Amine 2 (15%, 8%) plots, and up to six times greater in the P.E.2,4-D plots. An apparently atypical result was shown by 2,4-D mv 1 (22%, 10%). Experimental error, together with natural variability in plant resistance, could be the cause of such a discrepancy, as there is no basis for believing that 2,4-D mv 1 has a greater toxicity on 'mature' than 'young' growth.

PART 3.

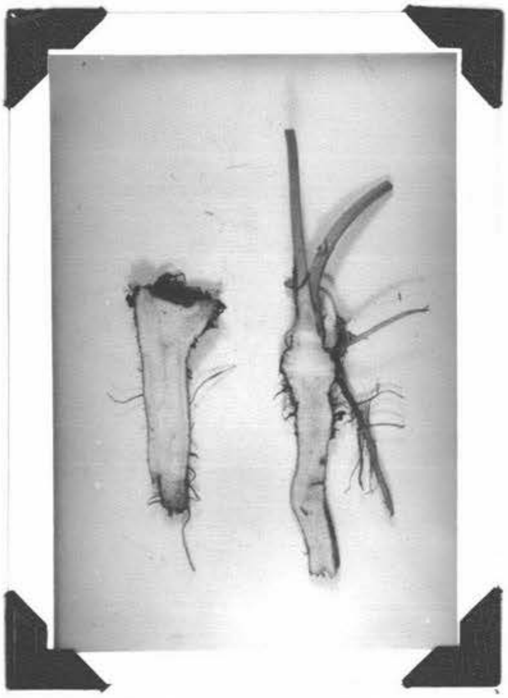
The Nature of the Damage Sustained by the Stem-base Region of the Plants.

Although in most cases the leaves and petioles had long since decayed, the subterranean stem and root were found to be either intact but modified in form, or, in more severely damaged plants, the distal part of the stem was necrosing. All 'seed' plants were, of course, exceptions in that they did not show any injury of the underground parts (see Fig. 3.). The responses shown by the 'mature' and 'young' plants were able to be classified into four fairly distinct groups which, together with photographic illustrations of typical representatives, were as follows:-

Group A. (See Figs. 4,5b and 11c.) This group was distinguished by a small ledge which girdled the stem usually about midway along its length. Not found at all on unsprayed specimens, this ledge seldom extended very deeply into the stem tissues (Fig. 11c shows an exception). There was no necrosis and foliage was invariably growing from the apical region of the stem. This type of deformation was found in Na-CTA-treated plants only, and, compared to the other responses, it would appear to be the outcome of very mild injury only.

Fig.3 - A sprayed 'seed' plant showing no sign of injury.

Fig.4 - Injury in the form of a small ledge girdling the stem.



(a) (b)

Fig.5 - Longitudinal section of roots showing
(a) Group C-type damage;
(b) Group A-type damage.

Fig.6 - Group B-type damage as indicated by the extensive proliferation.

Group B. (See Fig. 6.) Although similar to Group A in that at least a portion of the distal part of the stem remained, specimens of this class had large masses of proliferating tissue about the stem base. There was no active terminal growth from the stem. Plants from Na-CTA, 2,4-D non-volatile and P.E. 2,4-D plots showed this form of injury most commonly.

Group C. (See Fig. 7). This type of deformation was in the form of a severe crown damage and was found in all plots treated with a 2,4-D-based material. The greater portion of the stem was rotted away so that only a small portion remained adjacent to the root. Frequently the necrosis was observed to be penetrated further into the medulla than into the vascular region (see Fig. 5a). In some specimens, callus tissue developing from the vascular tissues had formed a distinct ridge about the medulla.

Group D. (See Fig. 8). Plants of this group were characterized by a large proliferating mass of tissue that extended completely over the distal end of the stem. In not all cases had the callus completely grown over the medulla as is illustrated in Fig. 8b. There was no remaining stem tip as in Group B. Figure 8a shows a variation of the response in which the proliferation developed on one side of the stem only. Group D was recorded

(a) (b)

Fig. 7 - Roots showing severe injury of the Group C type. Note young shoot in (a).

(a) (b)

Fig. 8 - Callus growth partly (a) and completely (b) overlying the distal end of the underground stem.

Fig. 9 - Untreated 'mature' plant.

from all plots receiving a 2,4-D-based herbicide treatment and occasionally from Na-CTA 2 plots and differed from Group C in that there was a much greater development of callus tissue.

Figure 9 shows a control plant with which to compare the several malformations discussed above.

Many injured plants were examined on which new shoots and buds were found to have developed always from the stem region basal to the proliferations and in no case were they found developing from the callus tissue. The proliferations were frequently observed to be in a partly moribund condition.

Figure 7a shows a defoliated plant in which young growth has developed from a part of the stem, basal to the region of abnormal activity. Figure 10 shows a similar example but the growth is much more advanced and vigorous. Note that there is no growth from the apex of the main stem. That small plants react in a fashion similar to the larger ones is illustrated by Figures 11a and 11b. Adventitious buds are seen to have originated basal to the proliferations.

The character of the new foliage on recovered plants was variable. Whereas in treatments which caused but slight injury, the shape of the leaves and vigour of the growth was comparable to controls, plants that recovered from more severe damage almost

Fig.10 - New growth on a spray-injured plant. Note destruction of the original stem apex.

(a) (b) (c)

Fig.11 - Recovery in small plants Note girdling ledge along stem of (c).

invariably had much smaller and narrower leaves than normal in the initial stages of regeneration. That is, the growth was much less vigorous.

Plants which succumbed to herbicide failed to produce any new shoot growth. Their remains indicated that the visible external injury was of a severe Group C or Group D type. In the latter event, the proliferation had not developed, evidently to any purpose.

CHAPTER III

HERBICIDE DAMAGE TO SEEDS AND KINDS OF SEEDLING MALFORMATION.

PART 1.

Effects of the Herbicides on Viability of Rumex obtusifolius Seeds.*

(i) Choice of Material and its Collection.

The plots laid out for the purposes of the experiment described in Chapter II had many docks at all stages of development at the time of spraying on January 25th. Therefore, the same plots were able to be used for the investigation into the effects of several 'hormone' herbicides on the viability of *Rumex obtusifolius* seeds.

In order to have a sufficient number of plants to ensure a reasonable validity of the results, the reaction of two stages of seed development only was able to be studied. These were at mid-flowering and advanced seed-ripening. Representatives for the former group (hereafter called 'soft') were limited to those plants in which flowering, as evidenced by the prominence of the anthers, was in progress at least in the middle portion of the raceme. Usually

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* Technically the seed of *Rumex obtusifolius* is a fruit; as the pericarp, although not fused to the testa, is closely associated with it. In this paper, however, the fruit will be referred to as a seed, for the studies are concerned with the true seed.

the uppermost sections carried unopened buds, while the proximal part of the raceme had completed flowering and the seeds were soft and green. Plants chosen for the latter group (hereafter called 'hard') had completely finished flowering, the tepals were changing in colour from green to brown and the fruit pericarps, which varied from a golden to a light brown colour, were hard.

Ten plants, to represent each of the above two groups, were tagged in duplicate plots for those treatments that were to be studied. The following herbicide formulations were represented:-

2,4,5-T 1; 2,4,5-T 2; Na-CTA 1; Na-CTA 2; Amine 1; Amine 2; 2,4-D nv 1; 2,4-D nv 2; P.E.2,4-D 1; P.E.2,4-D 2.

The last two were investigated on the 'hard' seeds only. Controls were also tagged.

It became necessary a fortnight after spraying to gather all tagged material, as the College milking herd was to begin grazing the field containing the plots. This was unfortunate, for, although the 'hard' seeds were well ripened, the racemes carrying the 'soft' seeds were still green.

In the laboratory the harvested material was dried for three weeks, after which the seeds, together with enclosing tepals, were pulled from the stems and stored in packets.

(ii) Some Preliminary Investigations.

(a) General.

Before the sprayed seed samples were tested for viability, a preliminary investigation was made on some untreated seed. It was necessary (a) to determine the germinative capacity of fresh seed, and (b) to compare the germination capacity of seeds freed of tepals with that of seeds remaining in the tepals. The need to study the former aspect was felt in view of the reports in the literature bearing on dock seed germination. Gill (36) observed an 84% and 88% germination for freshly harvested and milk-ripe seed of Rumex crispus respectively. Fluctuating temperature and light conditions were required up to the third month, but thereafter germination occurred readily at constant temperature. Gardner (37) reported a low germination capacity of fresh Rumex obtusifolius seeds but others have had germinations of 65% and more (38,39).

Data on the influence of tepals on seed germination was needed in order that the most satisfactory way by which to test the viability of the treated seed samples, could be determined.

(b) Experimental.

One hundred each of tepal-free and tepal-enclosed seeds from the same sample were counted on to moist filter papers and germinated at room temperature.

The results, included in Appendix II, are summarised in the graphs of Figure 12.

High germination percentages were obtained. For the tepal-free seeds, 67% germinated between the fourth and sixth day, and by the ninth day the total was 90%. Emergence from the tepal-enclosed seeds was much slower and irregular. By the ninth day only 20% had germinated; the 50% level was reached on the fourteenth day, and 90% by the twentieth day. The decreased rate of emergence of the tepal-enclosed seeds was probably due to the poorer light conditions, for light is a necessary requirement for germination of Rumex species (37).

The conclusions reached from this preliminary experiment therefore, were that freshly-harvested, ripe, dock seed germinates readily, and that quicker and more regular germinations are given by tepal-free seed. Hence the seed samples collected from the various plots were rubbed vigorously in a calico bag to remove the seeds from the enclosing tepals.

(iii) Techniques used in testing germination of treated seed.

The first experiment was put down on March 12th. The seeds were counted on to absorbent pads (100 to each) placed on 3" wide glass strips held about 1½" above a water bath. Pieces of filter paper connected the pads to the water. The bath was put on the laboratory bench next to a window and covered with

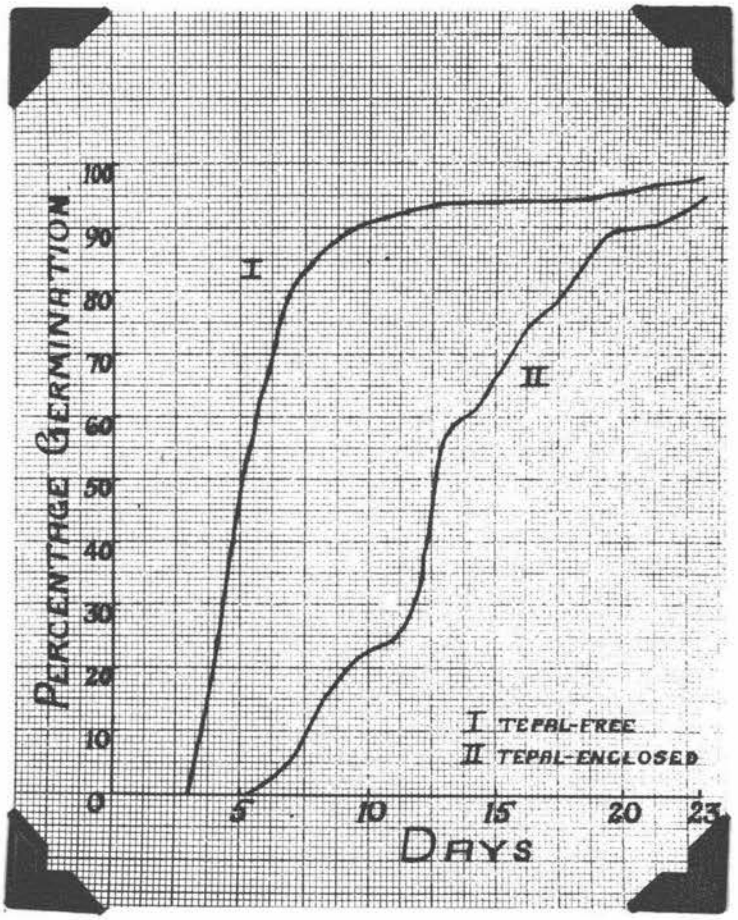


Fig.12.- Comparison of germination behaviour of tepal-free and tepal-enclosed untreated dock seeds.

two large sheets of glass. Germination proceeded under fluctuating room temperatures. Each different spray formulation was represented by 400 seeds and each plot by 200 seeds. Results were recorded each day (see Appendix III).

Although the test indicated that there was a wide variation in viability between samples, results were not able to be satisfactorily collected after about ten to twelve days. This limitation was imposed by the heavy fungous contamination which became evident about this time. It is probable that the invasion came from the air when the glass covers were removed at counting. A point noted from the trial however, was that the reproducibility of results between the two pads, each containing a hundred seeds, prepared for each sample, was such that the results from one pad adequately reflected the sample response.

Using a modified method a second test, involving one hundred seeds from each sample, was started on May 12th. The pads with the seeds were put on a layer of absorbent cotton wool in Petri dishes. Distilled water was added, and the covered dishes were put in a germinating cabinet open to daylight and maintained at 20°-22°C. The experiment was terminated after three weeks.

On August 4th a third germination test using the same method was started. The period for collection of data however, was extended from three weeks to sixteen weeks (112 days). Seedlings were removed from the pads as they freed themselves from the protective coats. Results for the first three weeks were similar to those of the earlier test. An analysis to compare the data of the second and third tests showed, that the results, as a whole, were essentially the same. Data from the third test only, therefore, is discussed. In order to compare the germination capacity of the different samples, the results, as at the twenty-first day (i.e. three weeks) were taken. By this time, control samples had ceased activity. Appendices IV and V respectively contain the data recorded for the second and third experiment.

(iv) Results: The results are presented in two sections as follows:- (a) for the three week period;(b) for the sixteen week period.

(a) For the three weekperiod.

Germinations were recorded on most pads containing 'hard' seeds by the fourth day. On no less than thirteen, the first seedlings emerged on the third day. The 'soft' seed samples were slower to show growth. The first germination was on the fourth day on a pad containing amine 1-treated seed. Other samples did not show life for periods up to fifteen days. Germinations on most pads, except those with control seeds, continued sporadically over the whole four months. No further activity was shown by controls however, after about the eighteenth day.

It was noted that seeds with a withered or pinched appearance and which were frequently encountered in treated samples, did not germinate as well as those which appeared to be normal.

Table VII contains the germination percentages for the 'hard' seed samples as determined at 21 days. The percentages given are each the average of the duplicate plots within any one treatment.

TABLE VII.

PERCENTAGES OF 'HARD' SEEDS TREATED WITH VARIOUS HERBICIDES, GERMINATED WITHIN THREE WEEKS, EXPRESSED TO NEAREST WHOLE NUMBER AND IN ANGLES.

Treatment	% Germination	Angle Transformation
2,4,5-T 1	48	43.5
2,4,5-T 2	44	41.3
Na-CTA 1	53	46.8
Na-CTA 2	28	31.3
Amine 1	53	43.2
Amine 2	46	45.9
2,4-D nv 1	70	56.5
2,4-D nv 2	45	42.1
P.E. 2,4-D 1	58	49.3
P.E. 2,4-D 2	47	43.0
Control	92	73.1

It is seen that all treatments had a depressing effect on germination. Thus whereas control gave an average germination of 92%, the highest result given by a treated sample was 70%. This figure occurred with 2,4-D nv 1. Most of the others varied between 40% and 60%. Na-CTA 2 - treated seed was reduced to a 28% viability and so was the most drastically affected.

The analysis of variance for the data in Table VII was calculated and the result is presented in Table VIII. The computation was made with transformed data (see Table VII, column 3).

TABLE VIII.

ANALYSIS OF VARIANCE OF ANGLES REPRESENTING GERMINATION PERCENTAGES OF 'HARD' SEEDS SPRAYED WITH HERBICIDE MATERIAL.

Source	Sums of Squares	Degrees of Freedom	Mean Square	F Value	Result
Blocks	52.7	1	52.7		
Treatments	2243.5	10	224.4	5.26	* *
Error	426.9	10	42.7		
Total	2723.1	21			

* * highly significant.

Highly significant differences between the treatments are indicated.

The distribution of the real variations was determined by use of the "t" value of 14.6, calculated for the 5% level of significance (for formula, see page 23), over the angles included in Table VII.

The following are the conclusions to be drawn from the analysis.

(a) Seeds from all treatments were significantly depressed in germination as compared to control.

(b) Most of the variations between treatments were significant.

(c) There was a marked tendency for applications of 2,4-D nv 1 to depress germination less than in the case of others, but the divergence did not come up to the 5% significance level except between 2,4,5-T 2 and Na-CTA 2.

(d) Na-CTA 2-treated seeds showed a germination reduction which was significantly lower than that of Na-CTA 1, 2,4-D nv 1 and P.E. 2,4-D 1.

The results for the 'soft' seeds are presented in Table IX.

TABLE IX.

PERCENTAGES OF 'SOFT' SEEDS TREATED WITH VARIOUS HERBICIDES GERMINATED WITHIN THREE WEEKS.
All figures to the nearest whole number.

Treatment	% Germination	Treatment	% Germination
2,4,5-T 1	15	Amine 1	2
2,4,5-T 2	0	Amine 2	2
Na-CTA 1	18	2,4-D nv 1	14
Na-CTA 2	1	2,4-D nv 2	2
Control	36		

All samples including control had a low viability. The author considers that gathering the material from the field when still far from ripe was largely responsible for the low figures and the control germination of 36 % would seem to be in support of this contention. Nevertheless, the treatments definitely depressed results still further. In several cases (Na-CTA 2, Amine 1, Amine 2 and 2,4-D nv 2) germination was almost completely inhibited, and with 2,4,5-T 2 it was totally so. Apart from Amine 1 and Amine 2, the 2 lb. A.E. per acre dosage reduced germination much more than the 1 lb. A.E. per acre dosage. Thus, whereas with 2,4,5-T 1, Na-CTA 1 and 2,4-D nv 1 the percentages were 15, 18 and 14 respectively, those of the higher application rate were 0%, 1% and 2%. Both amine formulations were severe. The percentage was two in each case. All herbicides, except Amine 1, gave similar results at equivalent concentrations.

(b) <u>For the sixteen week period.</u>

Germination data collected for the 'hard' seeds over the 112 days is included in Appendix VI and summarised in Figs. 13, 14 and 15.

It is seen that over the period of observation, seeds from all the samples continued to germinate but in relatively decreasing numbers. The same relative effects of the herbicides, as obtained at the end of three weeks, was maintained however. Additional

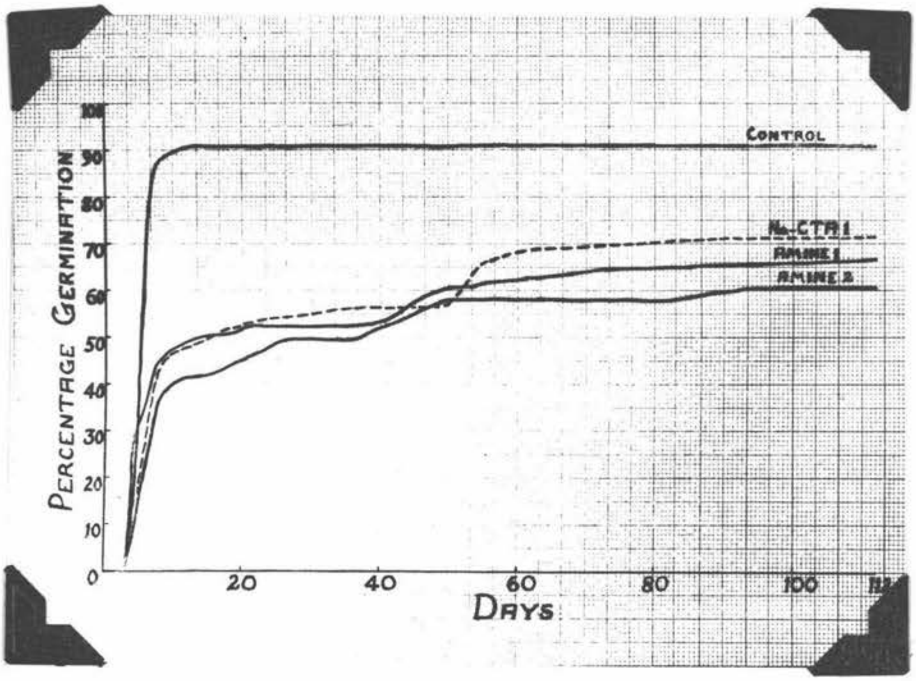


Fig.13 - Progress of germination in control and amine 1, amine 2 and Na-CTA 1 contaminated seeds over 112 days.

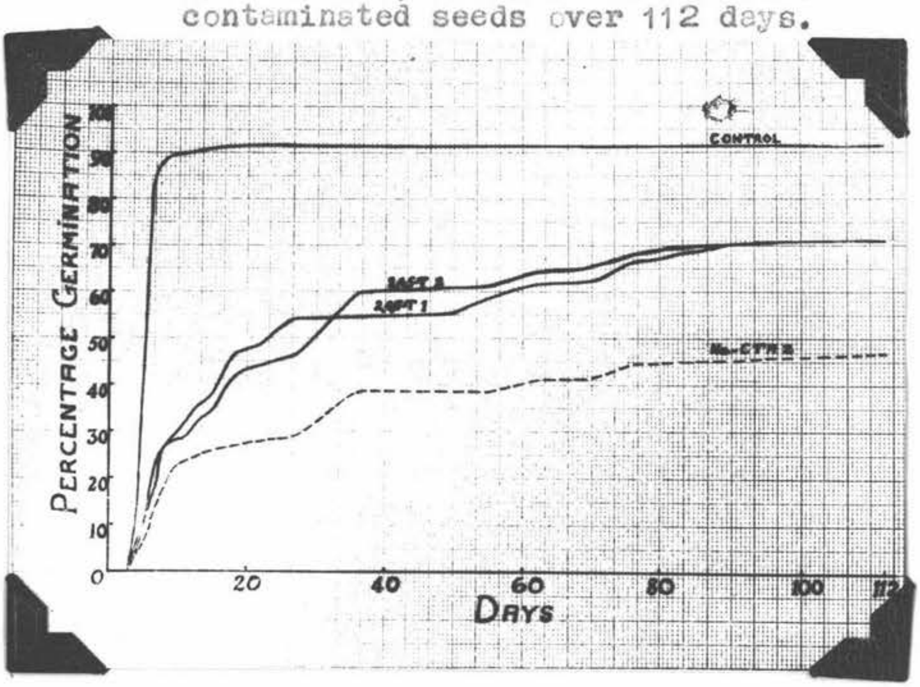


Fig.14 - Progress of germination in control and 2,4,5-T 1, 2,4,5-T 2 and Na-CTA 2 contaminated seeds over 112 days.

b

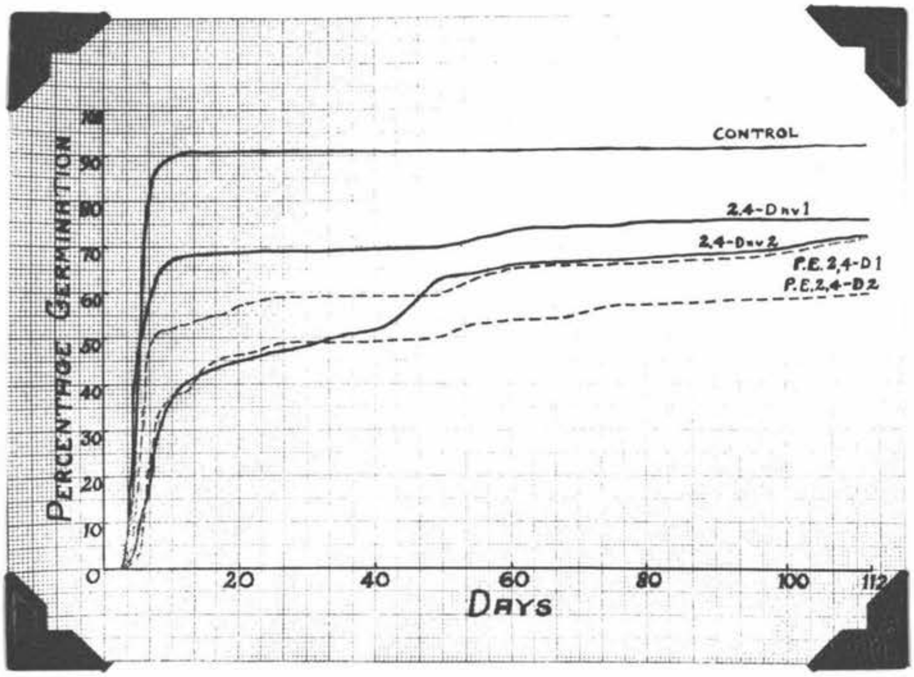


Fig.15 - Progress of germination in control and 2,4-D nv 1, 2,4-D nv 2, P.E.2,4-D 1 and P.E. 2,4-D 2 contaminated seed over 112 days.

germinations were less numerous in the 2,4-D nv 1 lots than in the others, while the greatest changes occurred with seeds treated with 2,4,5-T 2, Na-CTA 2 and 2,4-D nv 2. Although the germination total of most of the samples increased but little from week to week, in several there was at least one large rise between weekly totals. For example, there was an increase from 46% to 60% between the fourth and fifth week with the 2,4,5-T 2 lot and the total for 2,4-D nv 2 sample rose from 52% to 63% between the sixth and seventh week.

In nearly every case the number of seedlings emerging after approximately 75 days was negligible.

Results with the 'soft' seeds were similar to those of the 'hard' in that there were sporadic emergences up to about the 75th day and thereafter, there was only a negligible change. Germination percentage totals as at 21 days and 75 days are given in Table X.

TABLE X.

GERMINATION TOTAL (AS PERCENTAGES) FOR 'SOFT' SEEDS AFTER 21 AND 75 DAYS ON MOIST PADS.

Treatment	21 days	75 days
2,4,5-T 1	15	23
2,4,5-T 2	0	0
Na-CTA 1	18	44
Na-CTA 2	1	3
Amine 1	2	7
Amine 2	2	7
2,4-D nv 1	14	19
2,4-D nv 2	2	8
Control	36	36

In only one case was the change in total over the extended period greater than 8% - a fact which further emphasises the low viability of the samples.

Control, as was to be expected, did not vary, nor did 2,4,5-T 2 - treated seeds, which were totally inhibited. A considerable increase occurred with the Na-CTA 1-treated samples, and although the maximum attained of 44% was 8% higher than that of control, it is very unlikely that the treatment had any stimulating effect. The atypical result does indicate however, the unreliability of the values obtained for the 'soft' seed samples; this probably being due to the early harvest.

Part 2.

Injury Shown by the Seedlings.

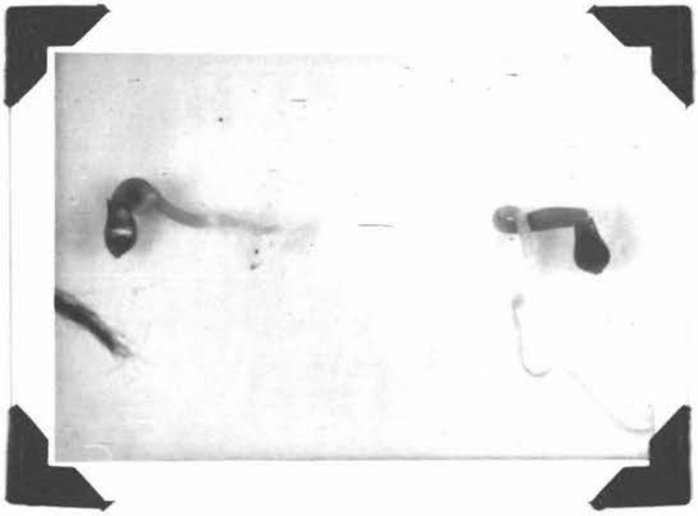
Several authors have shown that, in addition to retarded rate of germination and loss of viability in seeds coming in contact with 2,4-D preparations, morphological aberrations appear in the seedlings (20, 21, 23, 40, 41). Typical responses noted have included inability to form lateral roots, swellings of the hypocotyl, general retardation of growth and modifications in the first leaves.

This section describes the kinds of malformation observed in the seedlings growing on the moist pads during the germination studies. Similar responses were found in both the 'hard' and 'soft' samples, but the quantitative data presented has been taken entirely from the former, as the number of seedlings involved in this group was greater.

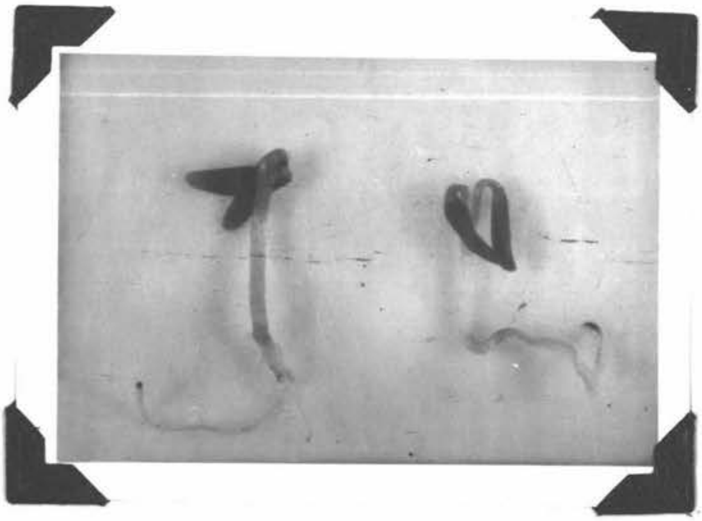
(1) Malformations Shown.

Malformations were found to be of three main types. These were:-

(a) (see Figs. 16 and 17). A complete and tight twist in the distal section of hypocotyl or actually embracing the cotyledons. Although very apparent in the early stages of growth, this deviation from the normal seedling form disappeared soon after the outer coats were lost. The figures illustrating this type show several phases of the condition.



<u>Fig.16</u>.- Seedlings with a complete twist in the upper part of the hypocotyl.



<u>Fig.17</u> - More advanced seedlings in which there is a return to a normal appearance by loss of the twist condition.

(b) (see Figure 18). A slight to severe epinasty in one or more sections of the axis, but there was no twist as in type (a). In seedlings exhibiting only a slight epinasty it was the root that was mainly involved, but in the severely affected ones, the epinasty occurred in the hypocotyl as well. Figure 18 shows an example of three seedlings in which gross epinastic curvatures brought about a complete loss of organised growth in the longitudial direction, with the result that a tangled mass was produced.

(c) Two variants of this type, which was characterized by the failure of the root to elongate, were recognized:-

(i) in which the non-developing root was the only sign of an irregularity. (See Fig. 19.)

(ii) in which the base of the hypocotyl swelled into a bulb-like growth which often developed a red pigmentation. From the swelling there grew out many fine adventitious roots, one of which became the new main root. There was little elongation of the hypocotyl. (See Figs. 20 & 21.)

Growth of the root into the air with the cotyledons lying on the germination pad was another irregularity observed in a few seedlings immediately after emergence.

For comparisons, a normal seedling is shown in Fig. 21A.

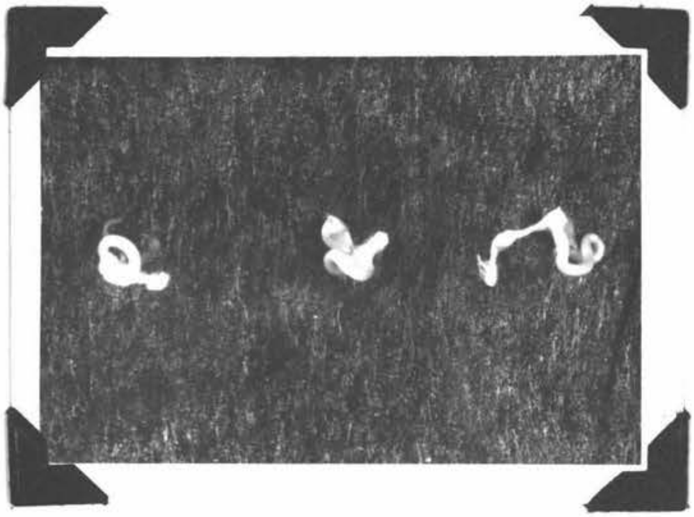


Fig. 18 - Seedlings showing severe epinasty in both the roots and hypocotyl.

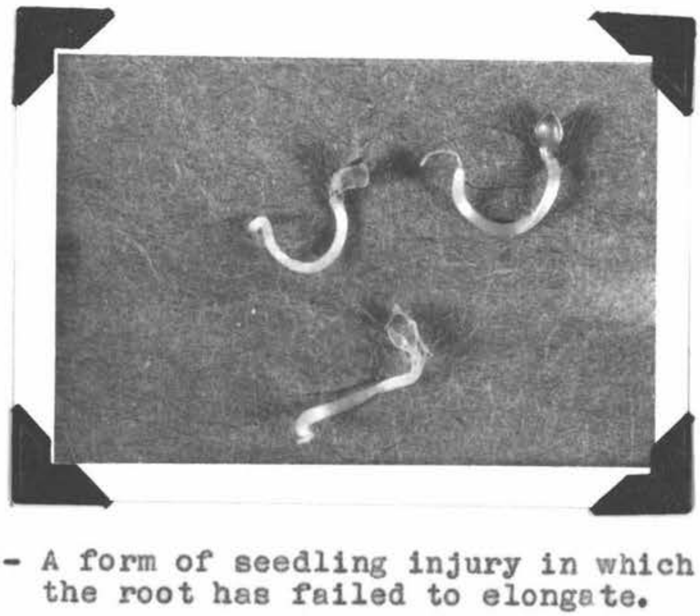
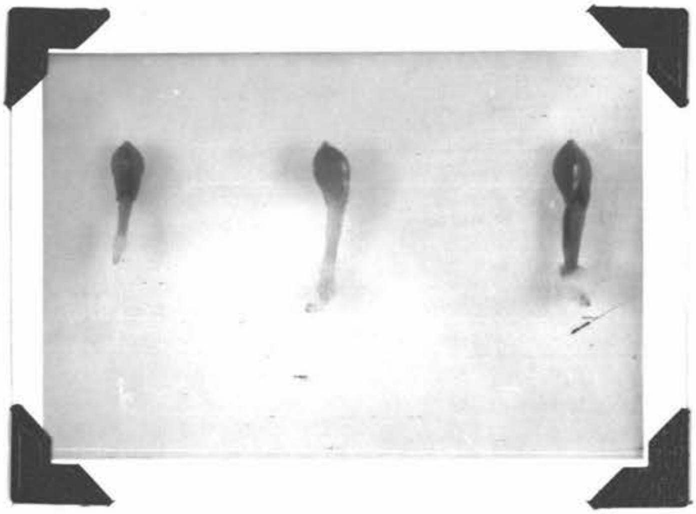
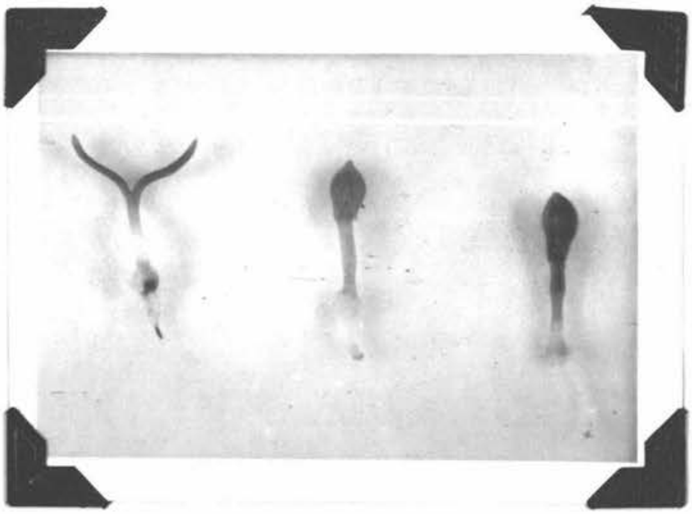


Fig. 19 - A form of seedling injury in which the root has failed to elongate.

b





Figs. 20 and 21.- Seedlings with inhibited root elongation and in which the base of the hypocotyl has swelled into a bulb-like growth. Note the retention of the testa - a characteristic of these seedlings.

Fig. 21A - A normally developed dock seedling.

(ii) Relation Between Kind of Malformation and Seed Treatment.

The data in Table XI shows the distribution of the various kinds of malformation in relation to the 'hormone' herbicide responsible for the abnormality. The figures express the percentages of seedlings, germinated by the 21st day, coming within each category. Those listed as undetermined were just germinating at the end of the period and were therefore not able to be classified. For the detailed experimental data see Appendix VII.

TABLE XI.

PERCENTAGE DISTRIBUTION OF SEEDLING ABNORMALITIES ACCORDING TO SEED TREATMENT.

Treatment	Abnormality						
	Normal	Twist	Epinasty	Short Root (i)	Short Root (ii)	Short Root Total	Undetermined
2,4,5-T 1	38	18	28	6	8	14	2
2,4,5-T 2	37	12	33	10	2	12	6
Na-CTA 1	25	30	29	15	0	15	1
Na-CTA 2	27	13	24	27	4	31	5
Amine 1	42	25	15	5	12	17	1
Amine 2	36	13	6	14	28	42	3
2,4-D nv 1	44	19	11	11	12	23	3
2,4-D nv 2	40	8	16	18	18	36	0
P.E.2,4-D 1	47	20	19	3	8	11	3
P.E.2,4-D 2	34	14	27	7	17	24	1
Control	37	47	16	0	0	0	0

Although normal seedlings predominated with most treatments, they did not account for 50% of the total in any instance. The average occurrence was 37% and the range 25% to 47%. The control plot too, with 37% of such seedlings, also came within this range. In the control, a much larger proportion amounting to no less than 47% of the total, showed the twist phenomenon, but this cannot be regarded as a normally occurring characteristic, for studies made with other non-treated seeds failed to reveal any similar response. The fact that 16% also showed a slight degree of epinasty would seem to provide evidence that some contamination had taken place. It is concluded therefore that the controls had been contaminated somewhat by spray drift from other plots. The incidence of the twist in other samples fluctuated widely. Amine 1 and Na-CTA 1 induced the condition most commonly; there being a 25% and 30% incidence respectively. The next most frequent occurrence of twist was with the 1 lb. dosage rate of the other herbicides. The abnormality was not common in the 2 lb. dosage samples and the range was from 12% (in the case of 2,4-D mv 2) to 25% (Na-CTA 2). In general, the incidence of twist was about twice as great in the 1 lb. treatments as in the 2 lb. ones.

The percentage of seedlings showing epinastic responses varied from 6 % with Amine 2 to 33% with 2,4,5-T 2. There was not a close association between incidence and dosage rate. Thus, although 2,4,5-T, 2,4-D non-volatile and P.E. 2,4-D all caused more epinasty when used at the rate of 2 lb. A.E. per acre than at 1 lb. A.E. per acre, Amine and Na-CTA had the opposite effect. The three herbicides 2,4,5-T, Na-CTA and P.E. 2,4-D, induced epinasty rather more frequently than the formulations of Amine and 2,4-D non-volatile and the 2 lb. dosage rate of each of these three caused epinasty in at least 25% of seedlings, while the highest value for either Amine or 2,4-D non-volatile was 16%.

The malformation characterized by the lack of root elongation was far more prevalent in those samples treated at the rate of 2 lb. A.E. per acre than 1 lb. A.E. per acre. Incidence under herbicides applied at the lesser rate varied from 24% to 42%, except in the case of 2,4,5-T, while the range for the other category was 11% to 23%. Incidence under 2,4,5-T 2 was only 12% and so was comparable in effect with the lesser dosage rate group.

An examination of the data for the two variants of this type of malformation showed that inhibition of root growth without accompanying swellings in the hypocotyl was definitely characteristic in the Na-CTA treatment. Na-CTA 1 and Na-CTA 2 dosages gave 15% and 27% incidence respectively, while inhibition of root elongation accompanied by hypocotyl enlargement occurred to the extent of only 4% of the seedlings from Na-CTA 2-treated seeds and was entirely absent with the Na-CTA 1 ones.

In contrast to Na-CTA, Amine revealed a marked capacity to cause hypocotyl swelling along with the lack of root growth. The respective figures for the variants (i) and (ii) were 5% and 12% in the case of Amine 1 and 14% and 28% in the case of Amine 2. That is, variant (ii) was twice as prevalent as variant (i). Seedlings from P.E. 2,4-D-treated seeds exhibited variant (ii) malformation rather more frequently than variant (i), but there was almost an even distribution among the two forms in seedlings from the 2,4-D non-volatile-treated samples. With 2,4,5-T, variant (i) tended to be more common than variant (ii) but the difference was not great.

PART 3.

Further Development of the Malformed Seedlings.

Further study was made on the malformed seedlings in order to determine the way in which subsequent growth was modified, and to relate this to the nature of the damage found in the seedling.

Fourteen days after the commencement of the first germination test, seedlings, representing the normal and the several abnormal growth forms, were selected at random and transferred from the germination pads to small flower pots filled with Shannon peat. The selection included samples from all treatments. They were not planted in the soil but merely propped upright on the moist surface with soil particles. Care was taken to ensure that the roots actually made contact with the soil. The pots were kept on the glasshouse bench and observations were taken at regular intervals over the succeeding four months.

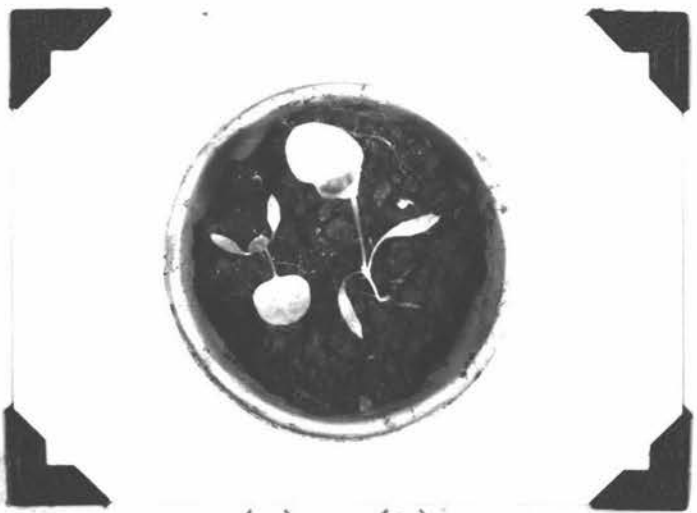
(i) Abnormalities Shown.

In addition to the normal course of development (see Fig. 22a), five other types could be readily distinguished. These were:-

1. Slow Growth: (Fig. 23a.) As the name suggests, plants in this group showed a slower rate of growth, as estimated by the number of leaves emerged at any one time, than the normal. Thus for example, when normally-growing plants had the fourth leaf fully opened, the slower-growing plants

(a) (b)

Fig.22 - (a) A healthy normally developed plant.
(b) A plant in which a large "cup" structure has developed in place of the usual first leaf.

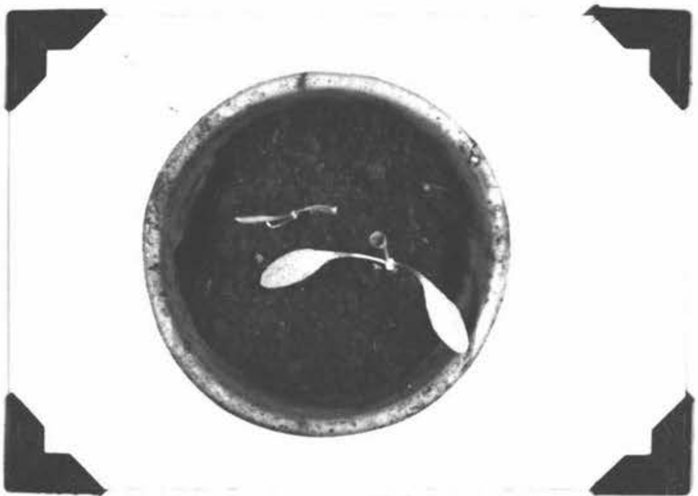


(a) (b)

Fig.23 - (a) A slowly-growing plant. Note the short but wide leaf.
(b) A plant with a less-perfectly formed "cup" structure.

still remained in the second leaf stage. The appearance of the plant was more or less typical of the species, but the leaf dimensions were usually smaller. Sometimes also, the leaves were slightly atypical in shape by virtue of a reduction in the length / breadth ratio. That is, they were short and wide.

2. <u>Presence of "Cups"</u>: (Figs. 22b,23b,24b.) There were numerous examples in which the first leaf failed to develop normally. Instead, the lateral margins were fused to a greater or lesser extent along their length, so that a "cup-like" structure was formed. The "cup" was usually borne on a long petiole. Later-developing leaves were never fused, but were always abnormally shaped in that they were small and almost round. Dimensions of the "cups" varied greatly between plants. In the more vigorously growing ones, the "cup" diameter was 1 cm. to 2.5 cm., the depth 1 c.m. to 1.5 c.m. and the petiole length about 2.5 c.m. to 4 c.m. Figures 22b and 23b show typical representatives. "Cups" on less vigorous plants were much smaller. Typical measurements were, diameter 2 to 4 mm., depth 1 to 2 mm. and petiole length about 10 mm. Fig. 24b illustrates the type. A feature of all small plants bearing a "cup" was the abnormally large ochrea about the base of the first petiole. (See Fig. 25.)



(b)

Fig. 24 - On a much less vigorous plant than in Fig. 22b, a small "cup" has been developed.

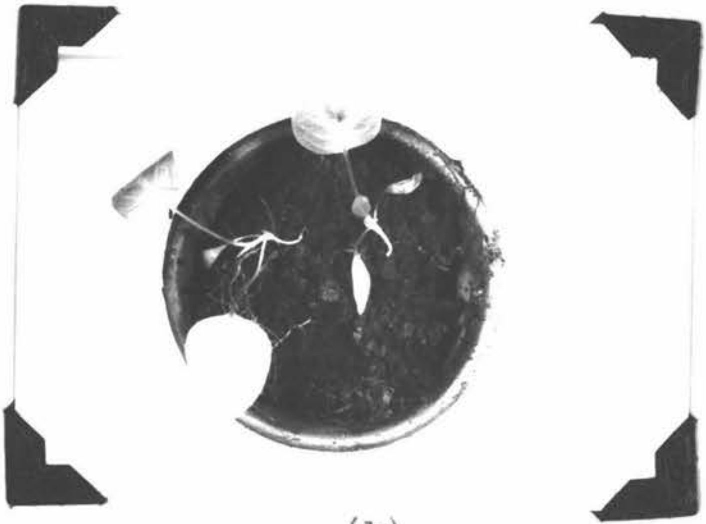
Fig. 25 - A close view of a poorly growing plant with an imperfectly formed "cup" to show the abnormally large ochrea.

3. <u>Fused Cotyledons</u>: Three examples occurred in which the cotyledons were fused along the proximal part of their lateral margins. The resulting structures were about 3 to 4 mm. long from the tips of the cotyledons to the rather swollen base. It was two months before any leaves appeared on these plants. The first leaf was typically small and round, about 1 to 2 mm. in length, and it emerged from the base of the fused cotyledons. Succeeding leaves appeared at more frequent intervals.

4. <u>Forked Lamina</u>: (see Fig. 26b). The first leaf of one plant developed a fork in the distal end of the lamina. Succeeding leaves, however, were normal. Fig. 27 shows a plant with the lamina of a cotyledon forked in a similar way.

5. Several seedlings were so completely disorganised that they made little growth and eventually died. Two were a tangled mass due to gross curvatures of the root and hypocotyl (see Fig. 28). In the others, a small mass of green tissue grew from the apical meristem, but it did not differentiate definitely into leaf and petiole (See Fig. 24a.)

With the exception of these few plants which had no capacity for organised growth, all, regardless of abnormality, eventually began to produce leaves which were typical of the species. Also there was an increase in growth rate as the plant reverted to normal development.



(b)

Fig.26 - A plant with a fork in the distal end of the first formed lamina.

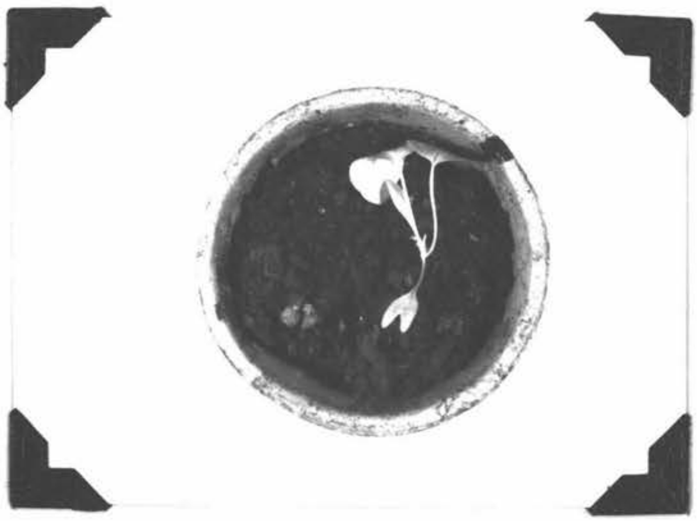


Fig.27 - A plant with a fork in the distal end of a cotyledon.

Fig.28 - A grossly twisted seedling with no capacity for organised growth. Nearly all specimens of this kind died.

(ii) Course of Growth in Relation to Seedling Malformation.

An investigation was made of the relationship between seedling malformation and the kind of plant abnormality shown after about four weeks of growth. The result is shown in Table XII.

TABLE XII

THE RELATIONSHIP BETWEEN SEEDLING MALFORMATION AND THE SUBSEQUENT PLANT ABNORMALITY.

Figures represent plant numbers.

Seedling Malformation	Plant Abnormality				
	Normal	Slow Growth	"Cups"	Fused Cotyledons	Disorganised Growth
Normal	12	2	2	-	-
Twist	4	4	-	1	-
Epinasty	-	6	3	2	1
Short Root (ii)*	-	-	12	-	-
Root Upwards	-	-	1	-	4

* Inhibited root growth accompanied by swelling of the hypocotyl.

The outstanding feature shown in the above Table is that all seedlings which had inhibited root growth, accompanied by enlargement of the basal part of the hypocotyl, developed into plants bearing a "cup" in place of the normal first leaf. "Cups" were formed from other seedling types as well, including two from apparently normal seedlings. Normal seedlings, however, grew mainly into healthy plants typical of the species.

The few seedlings found with the root growing up into the air were all transferred to pots for further observation. In all cases but one there was no further development and death followed. The one exception grew into a "cup"-bearing plant.

A range of plant abnormalities occurred with seedlings showing the 'twist' and epinastic malformations. Plants from the 'twist' seedlings were mostly either normal or slow growers. Those from the 'epinastic' seedlings were not normal but developed according to any one of the classified abnormalities. Half of them grew slowly and three of the twelve produced "cups".

It was from 'twist' and epinastic seedlings that the occasional fused-cotyledon condition arose.

CHAPTER IV

ESTIMATION OF READILY-AVAILABLE CARBOHYDRATE CONTENT.

An analysis was made of the level of readily-available carbohydrate constituents for one time of the year, in roots, of Rumex obtusifolius plants at various stages of development. By this procedure, an estimation was obtained of the probable nature and extent of root reserves in the plants which were sprayed with 'hormone' herbicide at about the same period.

(i) Stages of Development Studied.

Roots from plants representing five different stages of development were collected for analysis. The stages included, with a description of each, were as follows:-

(1) 'Young' - small plants with leaves not exceeding a length of 4" and the greatest diameter of the roots was not more than ½". These small plants were equivalent to the 'young' ones distinguished in the plots used for the spraying trials (see page 11).

(2) 'Mature'- vigorous plants with large leaves always exceeding a length of 4" and frequently reaching a length of 12". There was no stem growth above the ground level. This class of plants was equivalent to the one designated

similarly for the purposes of the spraying trials (see page 11).

(3) '<u>Intermediate</u>' - also represented by plants with abundant and well developed foliage, but differing from the mature in the possession of a stem on which none of the flower buds were opened.

(4) '<u>Flowering</u>' - included those plants in which the florets midway along the raceme were in full bloom. Usually distal florets were unopened, while the proximal ones had passed anthesis.

(5) '<u>Seeding</u>' - plants of this group were finished flowering and fruits were ripening. Fresh foliage growth was developing at the base of the dying stem.

(ii) <u>Collection of Material</u>:

An area of ground measuring 4 yd. x 4 yd., and on which docks of all stages of development were abundant, was chosen for the source of material. The area used was kept small so as to eliminate, as far as was possible, variations in soil fertility which might introduce serious error into the results and so make comparisons invalid.

All selected plants were dug down to a depth of 12", freed of adhering soil, and the stem and leaves were cut off. The parts remaining, consisting of the root and a short piece of stem, were wrapped in newspaper and placed on a damp cloth in a bucket which was kept covered with a wet towel. This procedure, necessary to prevent loss of moisture from the roots, was essentially the same as that observed by Bakke et al (25) in their study of the root reserves of Convolvulus arvensis. In the laboratory, the roots were carefully washed free of all adhering matter, dried of excess moisture, and weighed. The material was then subjected to live steam in an autoclave for ten minutes after which it was dessicated at 110°C for 2½ days. The oven-dry roots were weighed again for the purpose of calculating dry-weight data and then stored for future analysis.

Ten roots from each of the five stages of development under investigation were taken for the chemical analysis. Every root was determined separately for starch, non-reducing sugar and reducing sugar content.

(iii) Methods of Chemical Analysis (adapted from Loomis & Schull (41)).

Each root was cut into small pieces, weighed and plunged into about 100 ml. of boiling 80% alcohol and refluxed for one hour, after which the mixture was filtered and washed. The filtrate, containing both the reducing and non-reducing sugars, was evaporated to dryness, taken up with distilled water, cleared with a saturated basic lead acetate solution, deleaded with a saturated sodium oxalate solution, and made up to either 250 ml. or 500 ml. This was Solution A.

The residue from the filtered mixture, containing the starch, was dried at 105°C for 24 hours, and 1 gm. of this weighed, crushed, dry material was treated with 200 ml. of 10% alcohol for 3 hours to remove the dextrins. The extracted residue was dried to remove alcohol, transferred to a beaker containing 100 ml. of distilled water, and heated on a boiling water bath for 30 minutes to gelatinise the starch. On cooling to 30-38°C, the material, with a pinch of diastase added, was left overnight in an oven regulated to 35°C. It was then filtered into a 250 ml. flask, cleared and deleaded, as was Solution A, and made up to volume. Two hundred of the 250 ml. of solution were then hydrolysed in the cold with 10 ml. of concentrated hydrochloric acid for three hours, neutralized with sodium hydroxide, and made up to 250 ml. with distilled water. This was Solution B. By this method the starch was converted to reducing sugar and determined as such.

In preparing Solution C for the determination of total sugars, a 20 ml. aliquot of Solution A was hydrolysed for sixteen minutes on a boiling water bath with 1.5 ml. of 2 normal sulphuric acid. The cooled solution was neutralized with sodium hydroxide and made up to 100 ml. with distilled water.

(iv) The Estimation of Starch and Sugars.

The glucose content of Solution A,B and C was determined by the iodometric method described by Somogyi (42) (see Appendix VIII for details). This involved titrating excess iodine, quantitatively released from an alkaline copper sulphate - iodate and glucose solution, against a .005 normal sodium thiosulphate solution.

(v) Results:

The percentage content, on a fresh weight basis, of reducing sugar, non-reducing sugar, starch, and total readily available carbohydrate, all expressed in glucose equivalents, for the fifty roots analysed is given in Appendix IX. It should be noted that total readily-available carbohydrate, as estimated in this experiment, does not include a dextrin fraction and therefore is a different quantity from that sometimes expressed by this term. A summary of the results is made in Table XIII.

TABLE XIII.

LEVELS OF SEVERAL CARBOHYDRATE MATERIALS IN DOCK ROOTS TAKEN FROM PLANTS AT DIFFERENT STAGES OF DEVELOPMENT.

Figures express average percentages calculated on a fresh weight basis.

Stages of Development	Average Percentages			
	Reducing Sugar	Non-Reducing Sugar	Starch	Total Carbohydrate
Young	0.310	0.675	4.27	5.26
Mature	0.346	0.470	1.55	2.36
Intermed-iate	0.533	0.428	1.20	2.16
Flowering	0.413	0.367	0.92	1.70
Seeding	0.365	0.235	1.16	1.76

The results show that there were considerable variations in the sugar and starch content between the several stages of development. Reducing sugar was highest in the 'intermediate', followed by 'flowering', 'seeding', 'mature' and 'young' in that order. The last three gave similar values (0.31% to 0.365%), all of which were somewhat bigger than 50% of the highest result obtained.

Non-reducing sugar was present in greatest amount in the 'young' roots and progressively decreased as the stage of development advanced to ripening of the seeds. The range of 0.235% to 0.675% indicated that the content in the older roots averaged only about one third of that of the young roots. Compared with reducing sugar the content of non-reducing sugar was greater in the first two groups but smaller in the remaining three.

Starch occurred in much higher amounts than did either of the sugars. The percentage found in the stages from 'mature' to 'seeding' fluctuated between 0.92% for the 'flowering' samples to 1.55% for the 'mature' ones. 'Young' roots averaged the very much higher content of 4.27%.

From these figures the total readily-available carbohydrate was calculated. It is seen that the highest content was in the roots of the 'young' plants. The percentage content recorded of 5.26 was at least double that of roots coming from 'mature' and 'intermediate' plants and about three times greater than that found in roots taken from 'flowering' and 'seeding' plants.

The percentage ratios of starch / total carbohydrate, non-reducing sugar / total carbohydrate and reducing sugar / total carbohydrate were calculated and are presented in Table XIV.

TABLE XIV.

THE PERCENTAGE RATIOS EACH OF STARCH, NON-REDUCING SUGAR AND REDUCING SUGAR TO TOTAL CARBOHYDRATE IN DOCK ROOTS AT FIVE DIFFERENT STAGES OF DEVELOPMENT.

Figures calculated on a Fresh Weight basis.

Stage of Development	% Ratios to total carbohydrate		
	Reducing Sugar	Non-Reducing Sugar	Starch
'Young'	5.9	12.9	81.2
'Mature'	14.5	19.9	65.6
'Intermed-iate'	24.6	19.8	55.6
'Flowering'	24.3	21.6	54.1
'Seeding'	20.7	13.4	65.9

It is seen that starch was by far the most important component of total carbohydrate. In all groups of roots analysed, it accounted for more than 50% of the total, while in roots from 'young' plants the percentage ratio reached 81%. The sugars, with ratios of about 20%, would appear therefore to be of much lesser importance than starch in possibly inducing a fluctuation of the total carbohydrate quantity. The diagram in Fig. 29 shows this relationship more clearly.

To determine the significance of the results for total carbohydrate, an analysis of variance was calculated. A preliminary examination of the data for homogeneity of variance in all groups however,

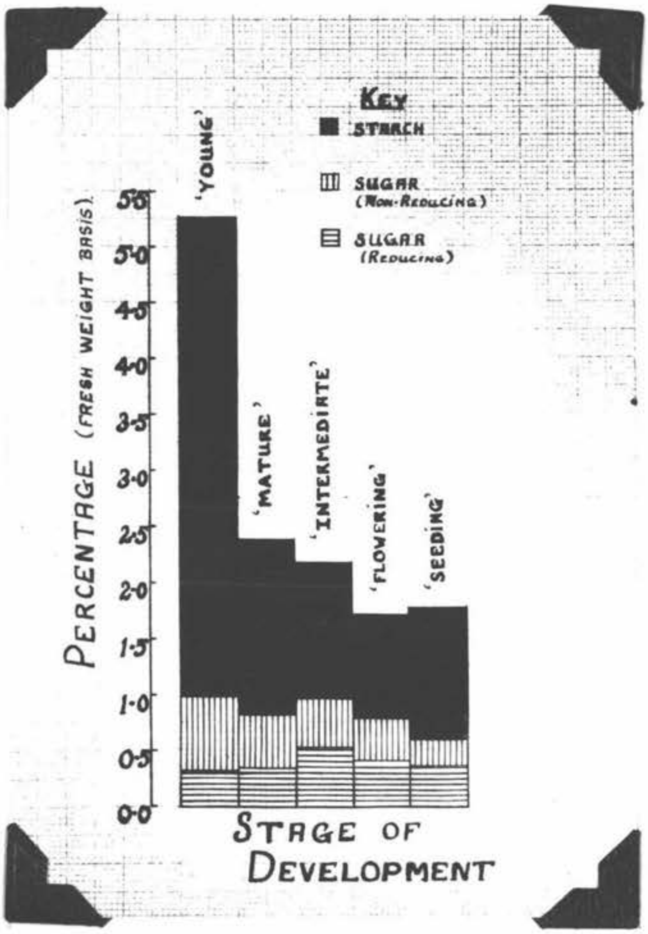


Fig.29 - Diagram to show the relative importance of starch, non-reducing sugar and reducing sugar in dock roots of plants at five different stages of development.

revealed that the variance within the results for the 'young' roots was not homogeneous. An angle transformation did not eliminate the irregularity. Hence the analysis was confined to the groups other than 'young'. The result is given in Table XV.

TABLE XV.

ANALYSIS OF VARIANCE FOR TOTAL CARBOHYDRATE IN DOCK ROOTS AT FOUR STAGES OF DEVELOPMENT.

Source	Sums of Squares	Degrees of Freedom	Mean Square	F Value	Result
Stage of Development	3.05	3	1.02	3.30	*
Residue	11.14	36	.309		
Total	14.19	39			

*significant

By means of a "t" test it was found that the total carbohydrate content of the 'mature' roots was significantly different from that of both the 'flowering' and 'seeding' roots at the 5% level. That is, 2.36% was significantly greater than either 1.70% or 1.76%. From this result, it would seem that the level of total carbohydrate in the 'young' roots was also very different from that in the other groups.

(vi) The heterogeneity of variance in the 'young' roots.

An investigation was made into the heterogeneity of variance in the 'young' roots. The total carbohydrate determined for each 'young' root analysed is presented in Table XVI. The fresh weight for each is also shown.

TABLE XVI.

FRESH WEIGHT IN GMS. AND TOTAL CARBOHYDRATE CONTENT (AS A PERCENTAGE) FOR EACH 'YOUNG' ROOT.

Data in ascending order of root weight.

Root Weight	% Total Carbohydrate.
1.19	6.32
1.45	9.36
1.46	8.25
1.87	3.73
1.89	5.09
1.92	3.11
2.28	2.46
2.34	5.14
2.40	4.53
2.42	4.61

An inspection of the data suggested that a linear relationship might exist between fresh weight of the roots and the amount of total carbohydrate.

The result of a test for linear regression of total carbohydrate content on fresh root weight is given in Table XVII.

TABLE XVII.

ANALYSIS FOR LINEAR REGRESSION OF TOTAL CARBOHYDRATE CONTENT ON FRESH WEIGHT IN 'YOUNG' DOCK ROOTS.

Source	Sums of Squares	Degrees of Freedom	Mean Square	F Value	Result
Due to Regression	19.89	1	19.89	6.98	*
Error	22.79	8	2.85		
Total	42.68	9			

*significant

From the significant result, a linear dependence between the two variables concerned, was therefore established. The regression coefficient was negative. Thus, as the root weight increased, its total readily-available carbohydrate content decreased.

In Fig. 30 has been plotted the data contained in Table XVI. The regression line was fitted to it by use of the formula: $y = \bar{y} + b(x-\bar{x})$

Where y = total carbohydrate in mgms/gm. root weight

x = weight of root

and b = regression coefficient (-3.278).

It is seen that the regression line does not fit the data very closely, but this could not be expected,

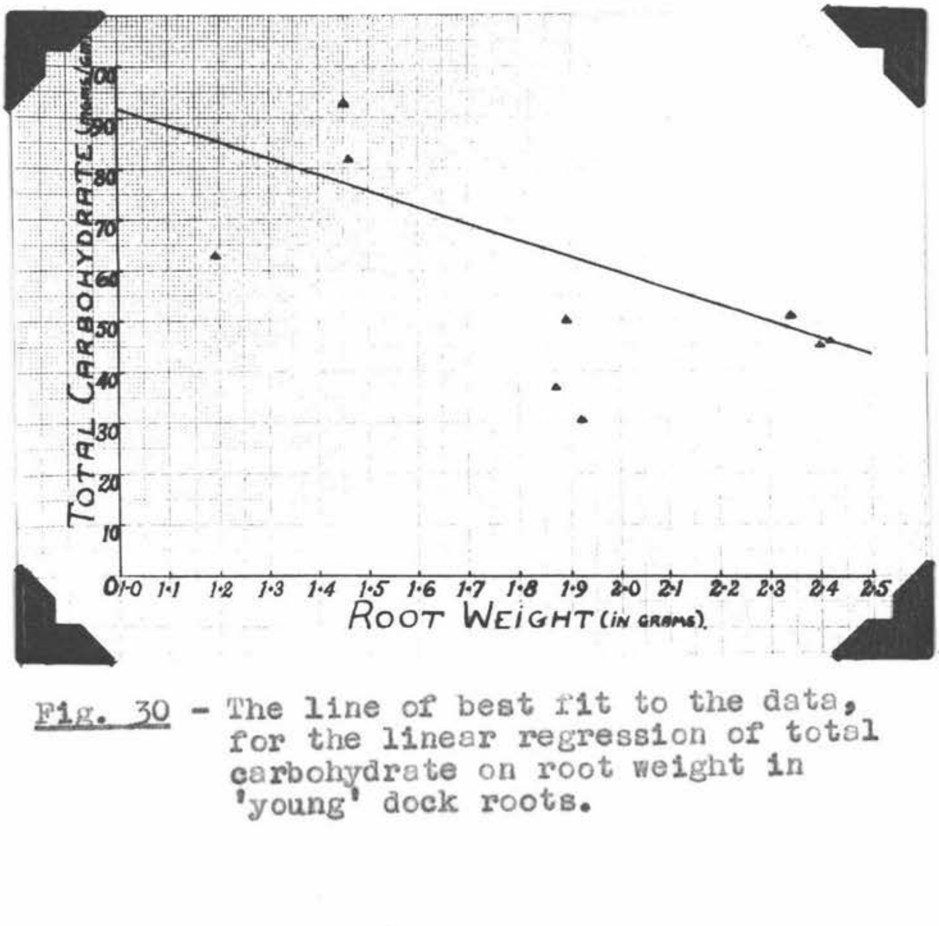


Fig. 30 - The line of best fit to the data, for the linear regression of total carbohydrate on root weight in 'young' dock roots.

as the number of roots samples was small and also it must be remembered, that living material does not conform closely to any pattern but only tends to do so. The result, however, adequately accounts for the irregularity found in the data.

DISCUSSION.

In evaluation of the relative toxicities of herbicides on perennial weeds, it is essential to have an accurate method by which to determine the number of plants killed by the treatments. The method used for the evaluations described in the present study involved marking the relative positions of all dock plants growing in the experimental strips on suitably prepared charts. The method proved to be satisfactory. Identifications of all treated plants, whether recovered or in an advanced state of decay, were easily made throughout the experimental period of 30 weeks. In addition, new plants which appeared during this time were able to be distinguished readily from those which had been sprayed. That time was an important consideration in this evaluation was emphasised by the fact, that the docks were slow in completing their response to the herbicide treatments. Of the plants which were in a state of defoliation at 10 weeks after spraying, many still retained the capacity to regenerate, as was shown by the large number which recovered at some time between the tenth and thirtieth weeks. Therefore, if the final assessment had been determined on defoliated plants at say, the tenth week, the results would have been erroneous. It was not possible, due to the intervention of a winter period between the tenth and thirtieth weeks, to make any conclusions as to the approximate time that would be taken by docks to give a completed response to herbicides in an environment allowing continual growth. The indications, however, are that up to at least three months would be necessary. The actual length of time involved probably varies with the stage of development of the plants at the time of treatment. The results of this experiment have not provided any accurate information pertaining to this suggestion, but it was found that the percentage of young plants recovering between the fifth and tenth weeks was considerably in excess of that observed for mature plants. That the recovery rates also varied with the treatments was no doubt a reflection of the intensity of injury sustained. The point is supported by the fact that the treatments which proved to be most toxic, were those with which the rate of recovery of both 'mature' and 'young' plants was low between the fifth and tenth weeks.

Using the criterion of number of plants killed, the results of the evaluation showed, that the relative toxicity of the several hormone herbicides investigated varied according to the concentration of the herbicide, the stage of development of the plants, and the nature of the herbicide material. The higher level of control achieved with herbicides used at a rate of 2 lb. A.E. per acre as compared with 1 lb. A.E. per acre was probably due to the greater uptake of active material into the cells. A greater disruption of cellular processes would result from the increased uptake, and hence there would be a net gain in percentage mortality.

The importance of stage of development was indicated by the highly significant increase in number of 'young' plants killed as compared to the mortality which occurred in the other more advanced stages of development. This difference would appear to gain an even greater significance from the fact, that the limits of the criteria by which the plants to be treated were classified into stages of development were set fairly wide, thus allowing considerable variation to exist within each class. In addition, there were not infrequent examples of plants which tended to overlap between the classes, and in such cases a largely subjective decision had to be made.

Very little is known as to the reasons for varying susceptibility with stage of development, but greater metabolic activity and a different course of metabolism in the plant cells may be involved. In those plants which were flowering or seeding at the time of spraying, the almost complete resistance could well be due to the small amount of herbicide that could be absorbed by the comparatively small leaf area which is possessed by plants at this stage.

From the present meagre knowledge of the mode of action of growth regulators at high concentrations in plant cells, it is not possible to interpret the variations in effectiveness found between the various 'hormone' herbicides. As an exception, however, it is possible that in the case of Na-CTA, the low phytotoxicity was the result of an insufficient uptake of active material into the plant tissues. This sodium salt formulation, characterised by the possession of polar properties, might have been less readily absorbed through the cuticular layer covering the leaves. Evidence for this suggestion came from the frequent observation that the foliage of most plants treated with Na-CTA was not often destroyed but usually remained intact, and recovered from the initial curvatures induced in the vein tissues.

Little foliage damage and a low phytotoxic capacity was also shwon by 2,4,5-T, but as this material was in the butyl ester form, and therefore non-polar in character, it is suggested that poor penetration was not a prime factor limiting efficient action of this herbicide. As there is little evidence to suggest that 2,4,5-T compounds lack toxicity at the cell level, it may be that this material was not translocated at a rate sufficient to allow accumulation of toxic amounts in the plant tissues.

From the point of view of successful eradication of Rumex obtusifolius, the evaluation of herbicides, as determined under the prevailing environmental conditions of this experiment, showed that no material was really effective. Amine 2, which was the best of those tested, did not kill more than about 50% of the plants at either the 'young' or 'mature' stage at the first application. There is the possibility, however, although it was not pursued in this study, that a second spray application on the recovered growth would have increased the percentage kill to a level somewhat nearer to complete control.

One would suspect that root and stem tissues recovering from the first application would be in a weakened, and therefore more susceptible, condition due to the food reserves being depleted by basic metabolic processes and the apparent capacity of 'hormone' herbicides to stimulate the utilisation of carbohydrate materials. Also, if small amounts of herbicide persisted in the tissues for some time after the initial recovery, this weakness might be intensified. There is the limitation, however, that the success of any second application would depend on the amount of leaf area exposed at the treatment, for the leaf area, which has been shown to be relatively small in plants in the early stages of recovery, determines the quantity of toxic material which can be intercepted and absorbed.

Further investigation is required to determine whether there are changes in the degree of susceptibility in plants during recovery from the first treatment. Only then could the value of a second spraying be ascertained.

The writer believes that the generally high degree of resistance of Rumex obtusifolius to 'hormone' herbicides, (apart from Na-CTA and 2,4,5-T), is in part due to poor translocation and distribution of the toxic agent through the subterranean stem regions. From the work of Roberts and Hughes (43), it has been shown that docks are able to grow shoot buds only from stem or hypocotyl tissue. The root has no capacity for shoot bud formation. Therefore it follows, that in order to eradicate a dock plant, all stem and hypocotyl tissues must be incapable of producing new growths. From this fact, it can be concluded that the high rate of recovery with 'hormone' herbicides is due to an incomplete destruction of stem and hypocotyl tissues.

From the results of the studies on the extent of the damage occurring in the subterranean parts of the injured plants, it was clear, that although the distal portion of the stem in many cases was totally destroyed, the proximal portion remained intact. That some necrosis did occur would seem to indicate that resistance was not necessarily due to an inherent physiological condition of the cells, which made them immune to the action of 'hormone' materials. In other words, factors operated to prevent an adequate accumulation of these materials in the cells of those stem tissues which were not killed. The frequent occurrence of proliferation tissue in that part of the stem proximal to the necrosed areas would seem to indicate that some hormone accumulated there. If the theory of Thimann (44), that auxins stimulate growth by protection of growth enzymes against naturally-occurring inhibitors, can be extended to mean that growth can be retarded or prevented by over-protection of these enzymes, then the occurrence of such proliferations could be ascribed to stimulation by relatively small quantities of hormone substance. This could occur if there was a restriction in the distribution of the hormone herbicide material within the stem tissues. This analysis is speculative, but the suggestion is offered that future work relating to the problem of resistance in docks, could well include comprehensive studies on the translocation and distribution of synthetic growth regulators within the stem.

Investigation into the germination capacity of seeds attached to plants at the time of spraying showed, that there was a significant reduction in the viability of all samples, regardless of the herbicide applied.

The loss was much greater in unripened than almost ripe seeds. In view of the fact that those plants which were in the reproductive phase were almost completely resistant to any toxic action of 'hormone' herbicides, the immediate question posed concerns the means by which the seed embryos became infected. There are two possibilities. The first is, that hormone material entering the plant was translocated directly into the seeds along with other solutes. This could have occurred especially in those plants bearing very immature seeds, for in such, there would be an active movement of food supplies to the reproductive parts, thus enhancing the chances of a fairly ready translocation of herbicide in the same direction. The high percentage of aborted seeds collected from these plants would seem to support the argument. This theory is not very attractive however for those plants bearing ripening seeds, due to the fact that it is difficult to perceive how the material would gain entry into the plant tissues in quantities at all significant and then be effectively translocated.

The second possibility is that herbicide material contaminated the pericarp and during germination, moved into the embryonic tissue with the imbibed water. The close investment of the seed with perianth members, however, would be expected to provide protection against such direct contamination. But external contamination could be the result of vapours of the

This change in relative toxicity is probably due to different environmental conditions operating to change both the amount of material absorbed, and the reaction of the material in the two respective types of tissue.

Many seedlings, germinating from sprayed seed samples, were malformed, but no specificity was found between the type of malformation and the 'hormone' causing it. There were definite indications however, that certain injuries were more prevalent with some treatments. To the writer's knowledge, there have been no reports in the literature bearing on this aspect of the types of malformation induced by different but closely-related growth regulators. Until more is known of the nature of growth processes and the ways in which growth regulators can modify and change these, it will not be possible to explain any of these variations in response.

Except for one case, there was no evidence that a particular form of seedling malformation was followed by a specific abnormality with the further growth of the plant. The one exception occurred with those seedlings in which there was an inhibition of root elongation and the development of swellings in the hypocotyl. Every one developed a "cup" in place of the normal first leaf. This would seem to indicate that not only the root meristem, but also the shoot meristem, was in some way disorganised.

In almost all plants, regardless of the type of abnormality shown, there was eventually a complete recovery. That is, in some way the hormone stimulus was exhausted with additional growth and development. Until a more intimate knowledge is possessed of the way in which growth regulators upset the normal physiological balance of cells and tissues however, it will not be possible to understand this reaction. But there is the thought, that research directed specifically towards the differences in formative effects produced by closely-related growth-regulating substances, would give interesting results.

The chemical analyses for the readily-available carbohydrates in roots showed that there were significant variations in level at different stages of development of the plant. Therefore, if, as Rhodes suggests (34), the hormone herbicides are effective through their capacity to deplete root reserve materials, then those plants with the higher reserves should have a potentially greater resistance. But the results of the analysis showed that 'young' plants had significantly higher levels than 'mature' plants, despite the fact that it has been clearly shown that resistance to 'hormone' herbicides is greater in the 'mature' plants. Furthermore, in the 'young' roots, it was found that the level of total readily-available carbohydrate, relative to other constituents, decreased as root weight increased. It would seem therefore, that some other factor, or factors, was important in modifying the reaction at the different growth stages. As mentioned previously, a study of the pattern of conduction and distribution of materials in roots of various ages and stages of development, might yield valuable information.

With so little known, it is not easy to predict the influence of the low total readily-available carbohydrate level of dock roots, as found at the flowering and seeding stage of development, on the susceptibility to 'hormone' sprays of the succeeding vegetative phase of the plant.

It is experiment alone that will provide the answer.

SUMMARY.

1. A study has been made to compare some of the responses shown by plants of Rumex obtusifolius at several stages of development, to sprays of the following 'hormone' herbicides;- ethyl ester of 2,4-D, butoxyethanol ester of 2,4-D, sodium 4-chloro-orthotoloxyacetate, butyl ester of 2,4,5-T, alkalolamine salt of 2,4-D and ethyl ester of 2,4-D, based in polyethylene glycol. Each material was applied at the rate of both 1 lb. and 2 lb. acid equivalent per acre.

2. In the assessment of the herbicides for their capacity to kill docks under the environmental conditions of the experiment, none of the materials were found to be very effective. The alkalolamine salt of 2,4-D was significantly more toxic than the others on vegetatively mature plants and it gave the best eradication of young plants. The ethyl ester of 2,4-D was the second most successful herbicide on both these stages while both 2,4,5-T and Na-CTA gave poor control.

3. The toxicity of most of the sprays was about twice as great on young as on more mature plants. Plants which were in flower or seed however, were almost completely resistant. With non-lethal doses of 'hormone', it was found that the time elapsing before the appearance of regeneration growth was much less with plants receiving a 1 lb. than a 2 lb.

treatment. In addition, the time varied with stage of development of the plants at spraying.

4. From an examination of the stem base region of many herbicide-defoliated plants, four distinct and commonly occurring manifestations of injury were recorded.

5. All the herbicides had a marked depressing effect on the germination of seeds attached to parent-plant at the time of spraying irrespective of whether they were green or nearly ripe. Loss in viability however, was far greater in green seeds. The relative effect of each herbicide material was also noted.

6. A description was made of the various malformations occurring in seedlings germinated from sprayed seed and of the abnormalities which appeared with their further growth. Some correlation between malformation and herbicide was found.

7. An analysis of dock roots showed that the level of total readily-available carbohydrate decreased with the progressive development of the plants up to the seeding stage.

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APPENDIX I.

DETAILED PLOT RESULTS SHOWING NUMBER OF DOCK PLANTS TREATED AND NUMBER DEFOLIATED AND/OR KILLED WITH THE VARIOUS HERBICIDE SPRAYS.

Stages of development. Y = 'Young'; M = 'Mature' S = 'Seed'

Block	Treatment	Stage of Development	No. of Plants	No. defoliated at 5 wks.	No. defoliated at 10 wks.	No. killed (30 wks.)
I	2,4,5-T 1	Y M S	24 24 37	5 7 2	4 3 0	4 1 0
II	2,4,5-T 1	Y M S	29 33 43	4 1 0	2 1 0	2 1 0
I	2,4,5-T 2	Y M S	29 27 52	4 0 1	2 0 1	2 0 1
II	2,4,5-T 2	Y M S	64 34 43	9 1 0	4 1 0	3 1 0
I	Na-CTA 1	Y M S	39 33 40	5 2 0	2 1 0	1 1 0
II	Na-CTA 1	Y M S	34 25 28	3 5 0	2 5 0	2 4 0
I	Na-CTA 2	Y M S	21 30 42	13 5 1	9 5 0	8 3 0
II	Na-CTA 2	Y M S	45 40 39	4 4 0	4 4 0	3 3 0
I	Amine 1	Y M S	16 19 37	13 13 2	7 6 1	5 3 0
II	Amine 1	Y M S	30 40 30	27 26 0	16 25 0	7 3 0
I	Amine 2	Y M S	44 31 37	30 26 2	25 23 2	19 17 1

APPENDIX I (Contd.)

Block	Treatment	Stage of Development	No. of Plants	No. defoliated at 5 wks.	No. defoliated at 10 wks.	No. killed (30 wks.)
II	Amine 2	Y M S	32 46 32	25 40 4	22 38 4	20 26 1
I	2,4-D v 1	Y M S	23 26 68	13 11 3	7 7 2	5 4 1
II	2,4-D v 1	Y M S	42 39 37	21 14 4	17 13 3	11 7 1
I	2,4-D v 2	Y M S	28 19 27	22 14 2	19 14 1	12 4 0
II	2,4-D v 2	Y M S	24 27 28	18 23 1	17 23 1	12 15 0
I	2,4-D nv 1	Y M S	25 33 26	17 17 0	16 15 0	7 6 0
II	2,4-D nv 1	Y M S	27 30 42	12 19 2	10 13 1	6 2 0
I	2,4-D nv 2	Y M S	17 15 55	12 8 0	10 8 0	3 2 0
II	2,4-D nv 2	Y M S	38 27 40	23 15 0	17 14 0	12 4 0
I	P.E. 2,4-D 1	Y M S	32 17 31	19 10 6	14 10 4	10 5 2

APPENDIX I (Contd.)

Block	Treatment	Stage of Develop-ment	No. of Plants	No. defoliated at		No killed (30 wks.)
				5 wks.	10 wks.	
II	P.E. 2,4-D1	Y M S	18 23 42	11 7 0	9 6 0	5 2 0
I	P.E. 2,4-D2	Y M S	46 36 71	28 18 4	20 15 2	10 2 0
II	P.E. 2,4-D2	Y M S	23 29 42	12 19 0	11 19 0	8 11 0
I	Control	Y M S	40 39 52	0 0 0	0 0 0	0 0 0
II	Control	Y M S	37 32 33	0 0 0	0 0 0	0 0 0

APPENDIX II.

GERMINATION OF TEPAL-FREE and TEPAL-ENCLOSED SEEDS.
EACH SAMPLE CONTAINED 100 SEEDS.

Days	No. Tepal-free Germinating	Progressive % Germination	Days	No. tepal-Enclosed Germinating	Progress-ive % Germination
3	0	0	3	0	0
4	21	21	4	0	0
5	29	50	5	0	0
6	17	67	6	2	2
7	15	82	7	3	5
8	3	85	8	8	13
9	5	90	9	7	20
10	1	91	10	3	23
11	1	92	11	1	24
12	1	93	12	8	32
13	1	94	13	26	58
14	0	94	14	2	60
16	0	94	16	14	74
17	0	94	17	3	77
18	0	94	18	5	82
19	1	95	19	7	89
20	1	96	20	1	90
21	1	97	21	0	90
22	0	97	22	2	92
23	1	98	23	3	95

APPENDIX III.

PROGRESSIVE GERMINATION PERCENTAGES FOR SPRAY-CONTAMINATED 'HARD' SEED. TEST SET DOWN ON MARCH 12th, 1952.

Treatment	Replication	Mar. 16	Mar. 17	Mar. 18	Mar. 19	Mar. 20	Mar. 21	Mar. 22	Mar. 23	Mar. 24
2,4,5-T 1	Ia	-	1	7	15	21	25	28	31	34
	Ib	1	3	12	17	22	27	34	35	36
	IIa	4	13	31	40	52	59	65	65	66
	IIb	2	13	40	48	52	55	58	59	61
2,4,5-T 2	Ia	-	4	10	16	24	31	32	32	32
	Ib	-	3	5	9	18	23	26	27	27
	IIa	5	8	14	17	17	26	33	34	36
	IIb	5	15	20	22	23	27	34	38	38
Na-CTA 1	Ia	-	5	10	12	15	22	23	25	25
	Ib	3	9	14	14	15	18	22	24	30
	IIa	1	13	17	35	43	48	50	54	55
	IIb	1	7	14	33	43	48	51	55	55
Na-CTA 2	Ia	-	5	7	12	13	14	15	17	17
	Ib	1	5	6	6	8	9	11	13	13
	IIa	3	13	35	41	45	50	50	52	52
	IIb	5	11	41	46	56	61	64	67	69
Amine 1	Ia	20	39	54	62	65	67	69	70	72
	Ib	15	28	50	54	57	61	62	62	62
	IIa	6	14	19	23	25	28	28	29	30
	IIb	9	15	18	22	22	24	26	28	29
Amine 2	Ia	2	6	15	20	24	26	28	28	29
	Ib	-	4	10	18	21	26	29	33	38
	IIa	1	8	14	26	28	30	36	38	40
	IIb	-	12	19	27	32	34	38	39	40
2,4-D nv1	Ia	4	28	47	55	57	59	62	62	63
	Ib	5	35	43	45	54	68	68	70	73
	IIa	6	24	30	51	54	58	62	64	66
	IIb	4	24	36	49	54	62	68	70	70

APPENDIX III. (Contd.)

Treatment	Replication	Mar. 16	Mar. 17	Mar. 18	Mar. 19	Mar. 20	Mar. 21	Mar. 22	Mar. 23	Mar. 24
2,4-D nv 2	Ia	-	9	13	24	27	32	32	32	37
	Ib	2	8	11	28	30	33	35	44	44
	IIa	-	5	8	10	16	17	26	34	36
	IIb	-	3	7	15	20	20	26	32	40
P.E. 2,4-D 1	I	1	13	29	42	47	50	50	53	53
	II	2	17	28	38	47	50	51	53	54
P.E. 2,4-D 2	I	3	9	18	28	37	41	44	46	49
	II	3	7	17	39	50	50	50	50	51
Control	Ia	5	23	51	73	81	83	86	86	86
	Ib	5	18	27	58	68	75	79	79	80
	IIa	3	11	47	67	77	87	90	92	92
	IIb	4	18	69	81	88	88	88	88	88

APPENDIX IV.

PROGRESSIVE GERMINATION PERCENTAGES FOR SPRAY-CONTAMINATED SEED. TEST SET DOWN ON MAY 12th, 1952.

A. 'HARD' SEED.

Treatment	Block	No. of days from start of test.											
		3	4	5	6	7	8	9	10	12	15	18	21
2,4,5-T 1	I	-	1	6	13	22	26	27	29	31	33	35	35
	II	-	7	24	29	38	40	40	41	45	48	49	51
2,4,5-T 2	I	-	3	10	20	24	25	25	25	25	29	30	30
	II	-	7	18	22	26	30	30	31	35	35	36	37
Na-CTA 1	I	-	3	12	16	20	22	22	23	25	28	28	28
	II	1	6	23	47	55	60	63	67	68	69	70	70
Na-CTA 2	I	-	2	4	7	12	13	14	15	16	18	19	19
	II	-	1	14	27	32	36	40	40	43	44	44	45
Amine 1	I	-	12	31	40	40	42	42	42	43	43	43	44
	II	1	5	12	18	19	20	21	22	26	26	26	26
Amine 2	I	-	3	3	7	13	14	15	15	17	18	18	18
	II	2	16	32	45	54	54	54	54	57	59	61	61
2,4-D nv 1	I	-	23	50	64	65	68	68	68	68	68	68	70
	II	1	12	32	46	56	56	57	57	57	59	60	60
2,4-D nv 2	I	-	3	13	25	38	42	44	47	49	51	55	56
	II	-	5	14	18	25	29	30	30	33	36	37	40
P.E.2,4-D 1	I	-	5	25	40	44	50	51	53	57	58	60	60
	II	-	1	20	36	44	48	49	51	54	54	55	55
P.E.2,4-D 2	I	-	2	16	27	32	38	39	41	43	47	47	49
	II	-	5	19	25	29	35	36	36	41	43	45	45
Control	I	1	19	35	56	70	78	83	85	86	86	87	88
	II	1	14	33	66	78	88	88	88	89	89	89	89

APPENDIX IV. (CONTD.)

B. 'SOFT' SEED.

Treatment	No. of days from start of test											
	3	4	5	6	7	8	9	10	12	15	18	21
2,4,5-T 1	-	-	1	2	3	4	8	9	9	11	12	14
2,4,5-T 2	-	-	-	-	-	-	-	-	-	1	1	1
Na-CTA 1	-	1	4	6	16	20	21	23	24	30	30	32
Na-CTA 2	-	-	-	-	-	-	-	-	-	-	-	-
Amine 1	-	3	3	3	3	3	3	3	4	5	5	6
Amine 2	-	-	-	-	2	2	2	2	3	3	4	5
2,4-D nv 1	-	-	4	7	7	10	11	11	14	15	15	16
2,4-D nv 2	-	-	-	-	-	-	-	-	-	-	1	3
Control	-	1	13	26	36	41	43	44	44	45	45	45

APPENDIX V.

PROGRESSIVE GERMINATION PERCENTAGES OF SPRAY-CONTAMINATED SEEDS. TEST SET DOWN ON AUGUST 4th, 1952.

A. 'HARD' SEED.

Treatment	Block	No. of days from start of test.												
		3	4	5	6	7	8	9	10	11	12	15	18	21
2,4,5-T 1	I	-	2	3	5	8	14	14	17	18	21	21	37	37
	II	-	3	17	24	30	40	43	43	46	48	52	56	58
2,4,5-T 2	I	1	4	5	17	19	23	25	25	25	25	31	44	46
	II	2	8	15	19	28	31	31	32	32	36	37	40	41
Na-CTA 1	I	1	7	16	20	30	33	34	34	34	36	36	41	41
	II	-	9	23	38	53	58	60	60	60	60	63	64	65
Na-CTA 2	I	2	6	8	8	11	12	15	15	15	15	16	17	18
	II	-	2	5	11	18	24	29	30	32	33	35	36	37
Amine 1	I	2	24	39	45	52	55	55	55	55	55	59	59	62
	II	6	11	25	29	35	37	39	41	42	42	42	43	43
Amine 2	I	1	2	5	11	15	25	28	28	28	28	28	29	32
	II	1	9	23	37	45	48	50	53	53	55	56	58	60
2,4-D nv 1	I	9	34	55	61	65	69	69	70	71	71	72	72	72
	II	1	15	33	47	55	62	64	65	65	65	65	67	67
2,4,-D nv 2	I	-	5	15	22	34	37	39	41	42	42	42	44	44
	II	-	-	1	6	16	22	31	33	36	39	43	44	46
P.E. 2,4-D 1	I	1	4	18	39	48	50	50	50	51	51	52	52	53
	II	-	8	28	45	50	54	54	54	55	55	57	58	62
P.E. 2,4-D 2	I	-	1	3	13	18	28	31	35	35	35	42	43	43
	II	-	1	2	20	34	40	40	41	41	41	45	49	50
Control	I	1	13	57	72	86	89	90	91	91	91	92	92	92
	II	1	17	53	78	88	89	89	89	90	91	91	91	91

APPENDIX V. (CONTD.)

B. 'SOFT' SEED.

Treatment	No. of days from start of test.												
	3	4	5	6	7	8	9	10	11	12	15	18	21
2,4,5-T 1	-	-	2	7	9	10	11	12	12	13	13	14	15
2,4,5-T 2	-	-	-	-	-	-	-	-	-	-	-	-	-
Na-CTA 1	-	-	1	3	3	7	8	11	12	13	16	17	18
Na-CTA 2	-	-	-	-	-	-	-	-	1	1	1	1	1
Amine 1	1	1	1	1	1	1	1	1	2	2	2	2	2
Amine 2	-	-	-	-	-	1	1	2	2	2	2	2	2
2,4-D nv 1	-	-	2	7	7	7	7	8	10	10	12	13	14
2,4-D nv 2	-	-	-	-	-	-	-	-	-	-	1	2	2
Control	-	2	14	28	29	31	31	33	34	34	34	34	36

APPENDIX VI.

ADDITIONAL GERMINATIONS IN 'HARD' SEED SAMPLES FROM 21 to 112 DAYS FROM START OF TEST.

Treatment	Block	% Germ. at 21 days	No. of days from start of test											% Germ. at 112 days
			27	36	41	48	55	62	69	76	83	97	112	
2,4,5-T 1	I	37	5	-	-	-	4	6	-	7	2	5	1	67
	II	58	8	1	-	1	3	-	-	3	1	-	-	75
2,4,5-T 2	I	46	5	15	-	-	1	-	-	5	3	1	1	78
	II	41	-	13	1	-	-	7	1	1	-	1	-	65
Na-CTA 1	I	41	2	4	-	1	7	3	-	-	-	2	-	60
	II	65	1	-	-	-	11	3	-	1	1	-	1	83
Na-CTA 2	I	18	1	8	-	-	-	2	1	4	1	2	-	37
	II	37	1	12	-	-	-	3	-	2	-	-	2	57
Amine 1	I	62	-	-	2	8	2	-	1	-	-	1	1	77
	II	43	-	-	-	6	-	3	-	2	1	-	1	56
Amine 2	I	32	3	-	6	11	-	1	-	-	-	2	-	55
	II	60	4	-	-	-	-	-	-	-	-	2	-	66
2,4-D nv 1	I	72	-	1	-	-	4	1	-	-	2	-	-	80
	II	67	-	1	-	-	-	3	-	1	-	-	-	72
2,4-D nv 2	I	44	1	4	-	9	-	2	-	-	1	1	5	67
	II	46	3	4	2	13	2	1	-	2	1	1	-	75
P.E.2,4-D1	I	53	1	-	-	-	6	1	-	2	-	-	7	70
	II	62	2	1	-	-	-	4	1	-	1	-	1	72
P.E.2,4-D 2	I	43	1	-	-	2	4	2	-	3	-	1	-	56
	II	50	4	-	-	-	2	-	1	2	1	-	2	62
Control	I	92	-	-	-	-	-	-	-	-	-	-	-	92
	II	91	-	-	-	-	-	-	-	-	-	-	-	91

APPENDIX VII.

CLASSIFICATION OF THE SEEDLINGS EMERGED BY THE 21st DAY IN THE THIRD GERMINATION TEST OF 'HARD' SEEDS ACCORDING TO TYPE OF ABNORMALITY SHOWN.

Treatment	No. of Seed-lings	No. of Normal Seed-lings	Nos. of Abnormal Types				Un-deter-mined
					Short Root		
			Twist	Epinasty	Un-swollen	Swollen	
2,4,5-T 1	95	36	17	26	6	8	2
2,4,5-T 2	92	34	11	30	9	2	6
Na-CTA 1	104	26	31	30	16	0	1
Na-CTA 2	55	15	7	13	15	2	3
Amine 1	105	44	26	16	5	13	1
Amine 2	90	32	12	6	13	25	2
2,4-D nv 1	139	61	26	16	15	16	5
2,4-D nv 2	90	36	7	15	16	16	0
P.E.2,4-D 1	115	54	23	22	3	9	4
P.E.2,4-D 2	93	32	13	25	6	16	1
Control	183	68	86	29	0	0	0

APPENDIX VIII.

IODOMETRIC TECHNIQUE USED FOR DETERMINATION OF GLUCOSE CONTENT.

From SOMOGYI, M. Jn. Biol. Chem. 160: 61-68. 1945.

Composition of Reagent:

The alkaline copper-tartrate reagent was constituted as follows:- 1 litre of the solution contained 28 gm. of anhydrous disodium phosphate, 100 ml. of normal sodium hydroxide, 40 gm. of Rochelle salt, 8 gm. of cupric sulphate (crystalline), and 180 gm. of anhydrous sodium sulphate.

Preparation:

The phosphate and tartrate were dissolved in about 700 ml. of water, the sodium hydroxide was added, and then, with stirring, 80 ml. of a 10% copper sulphate solution were introduced. Finally the sodium sulphate was added and, when dissolved, the solution was diluted to 1 litre and allowed to stand for a day or two before use.

Iodometric Technique:

The analytical procedure was carried out as follows:- 5 ml. of the above reagent and 5 ml. of a glucose solution were added to a 25 x 200 mm. Pyrex test-tube, covered with a glass bulb, and heated for 16 mins. by immersion in a vigorously boiling water bath. After cooling in running water, 2 ml. of an approximately 2.5% solution of potassium iodide was added by running it carefully from a pipette down the wall of the test-tube without causing agitation of the contents. Next, 2 ml. of approximately 2-normal sulphuric acid were added - the acid being rapidly dropped so that the entire contents of the tube were mixed and acidified at once. The mixture was then titrated against .005 -normal sodium thiosulphate solution using starch to detect the end point.

Each ml. of the titration value was equivalent to 0.135 mg. of glucose.

APPENDIX IX.

CARBOHYDRATE ANALYSIS OF 'YOUNG' ROOTS. CONTENTS, ALL ON FRESH WEIGHT BASIS, EXPRESSED AS GLUCOSE (mgms. per gm.)

Root No.	Fresh Wgt. (Gms.)	% D.W.	Reducing Sugar	Non-reducing Sugar	Starch	Total
1	2.28	35.4	3.96	6.02	14.63	24.61
2	1.92	34.8	2.98	3.84	24.26	31.08
3	2.42	40.5	3.99	8.33	33.76	46.08
4	1.87	38.2	3.11	5.97	28.24	37.32
5	2.40	33.9	1.39	3.64	40.26	45.29
6	2.34	40.7	3.34	5.71	42.32	51.37
7	1.46	45.7	2.63	10.74	69.10	82.47
8	1.89	35.9	3.31	7.60	39.96	50.87
9	1.19	37.6	2.79	7.79	52.66	63.24
10	1.45	35.0	3.54	8.36	81.70	93.60

APPENDIX IX (CONTD.)

CARBOHYDRATE ANALYSIS OF 'MATURE' ROOTS. CONTENTS, ALL ON FRESH WEIGHT BASIS, EXPRESSED AS GLUCOSE (mgms. per gm.)

Root No.	Fresh Wgt. (Gms.)	% D.W.	Reducing Sugar	Non-reducing sugar	Starch	Total
1	9.79	37.4	2.97	6.54	17.26	26.77
2	9.23	37.5	3.33	4.18	18.85	26.36
3	11.36	31.7	2.04	2.42	21.94	26.40
4	10.47	35.8	3.51	4.76	7.15	15.42
5	10.44	39.4	2.99	5.87	17.18	26.04
6	8.14	33.7	3.95	4.81	17.81	26.57
7	11.89	32.4	4.44	2.88	8.87	16.19
8	10.73	39.3	3.86	6.28	25.79	35.93
9	12.61	37.0	4.07	4.90	12.24	21.21
10	8.49	34.7	3.42	4.35	7.60	15.37

APPENDIX IX (CONTD.)

CARBOHYDRATE ANALYSIS OF 'INTERMEDIATE' ROOTS.
CONTENTS, ALL ON FRESH WEIGHT BASIS, EXPRESSED AS GLUCOSE (mgms. per gm.)

Root No.	Fresh Wgt. (Gms.)	% D.W.	Reducing Sugar	Non-reducing Sugar	Starch	Total
1	5.97	35.9	4.01	4.36	7.41	15.78
2	10.83	36.3	4.79	3.80	8.92	17.51
3	7.22	33.5	4.79	1.52	6.36	12.61
4	5.30	38.0	7.85	4.38	8.58	20.81
5	9.30	34.9	5.40	5.49	6.79	17.68
6	12.84	32.6	4.81	1.43	17.10	23.34
7	6.30	39.0	6.50	3.02	14.49	24.01
8	11.50	43.2	4.66	4.29	20.81	29.76
9	4.91	41.0	5.23	8.20	11.27	24.70
10	12.71	38.3	5.22	6.28	18.65	30.15

APPENDIX IX (CONTD.)

CARBOHYDRATE ANALYSIS OF 'FLOWERING' ROOTS. CONTENTS, ALL ON FRESH WEIGHT BASIS, EXPRESSED AS GLUCOSE (mgms. per gm.)

Root No.	Fresh Wgt. (Gms.)	% D.W.	Reducing Sugar	Non-reducing Sugar	Starch	Total
1	17.16	28.9	1.68	1.49	4.25	7.42
2	19.15	33.3	3.73	2.69	5.42	11.84
3	12.43	37.8	3.05	4.54	12.97	20.56
4	6.30	35.1	2.28	3.91	7.09	13.28
5	7.97	40.1	9.04	7.84	10.53	27.41
6	5.85	34.2	5.24	3.57	11.41	20.22
7	8.54	35.6	3.09	2.60	8.55	14.24
8	10.93	36.7	6.61	3.77	7.69	18.07
9	5.35	35.3	2.72	3.28	15.23	21.23
10	19.35	38.7	3.81	3.05	9.02	15.88

APPENDIX IX (CONTD.)

CARBOHYDRATE ANALYSIS OF 'SEEDING' ROOTS. CONTENTS, ALL ON FRESH WEIGHT BASIS, EXPRESSED AS GLUCOSE (mgms. per gm.)

Root No.	Fresh Wgt. (Gms.)	% D.W.	Reducing Sugar	Non-reducing Sugar	Starch	Total
1	11.87	36.4	4.42	3.20	4.85	12.47
2	12.56	38.0	2.16	3.62	15.34	21.12
3	8.99	36.2	4.20	2.68	8.74	15.62
4	13.40	35.1	3.75	1.17	15.33	20.25
5	9.05	34.7	6.66	4.55	9.53	20.74
6	14.10	37.0	3.55	0.32	18.44	22.31
7	7.94	34.9	2.10	1.90	16.05	20.05
8	9.15	33.9	3.19	1.99	10.07	15.25
9	9.51	32.0	3.79	2.39	3.83	10.01
10	13.06	31.4	2.66	1.65	13.73	18.04