SOME EFFECTS OF VARIOUS CHEMICAL TREATMENTS ON LEGUMES AND CERTAIN OTHER CROPS

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SOME EFFECTS OF VARIOUS CHEMICAL TREATMENTS ON LEGUMES AND CERTAIN OTHER CROPS

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ABSTRACT

This thesis reports on the effects of various growth regulating substances applied at various rates and in various ways to certain crop plants including potatoes, spring wheat, winter wheat, flax, red clover, sweet clover, alsike clover and alfalfa.

Soaking of potato eyepieces in solutions of various chemicals before planting failed to accelerate emergence or increase yield. Emergence was decreased and delayed and tuber yields reduced by as much as 72 per cent by certain chemical treatments.

Spraying of winter wheat and spring wheat with growth substances was without effect. Variation in susceptibility of flax to damage from similar concentrations of various chemicals was recognized.

Application of herbicidal sprays to sweet clover and alfalfa showed that sweet clover could not be successfully weeded from alfalfa by 2,4-D derivatives. Top growth of alfalfa and sweet clover was very susceptible to 2,4-dichlorophenoxyacetic acid and 2,4,5-trichlorophenoxyacetic acid, but their roots possessed marked resistance.

Soil applications of phenoxy compounds at time of planting showed that very small quantities of these chemicals in the soil would decrease emergence of alfalfa, sweet clover, red clover and alsike clover. Emergence

stimulation was apparent at certain concentrations but was too greatly affected by environmental conditions to be consistently predictable.

Certain low rates of phenoxy compounds sprayed on alfalfa throughout the flowering period significantly increased seed set but the margin of safety between rates causing increase or decrease was narrow.

Possible practical applications of the various results are discussed.

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INTRODUCTION

The increase in the practical applications of plant growth regulators within the last decade has opened a vast field of research. Investigations mostly confined to selective weed control and weed eradication have resulted in the present multi-million dollar business in synthetic hormone herbicides of which 2,4-dichlorophenoxy-acetic acid is a major product. Although 2,4-D has been widely advertised and used as a herbicide there are other important uses of this and similar chemicals. Already many specialized uses have been found for these substances in horticultural work. Methods of chemical growth control have been effective in connection with rooting of cuttings, induction of dormancy in tubers and nursery stock in storage, the breaking of dormancy in tubers, production of flower clusters in place of leafy branches, stimulation of bud-growth, blossom thinning, control of flowering, influence on fruit set and control of preharvest drop of fruits. Since horticultural crops can be affected in these ways, it would seem worthwhile to investigate various possibilities in connection with growth control of field crops.

In Alberta, as in other parts of the world, agronomists strive to secure or develop high yielding, high

quality crop plants well adapted to the environment. As knowledge of growth regulating substances accumulates and the mechanism of action becomes more clearly understood it seems probable that plant characteristics such as flowering time, flowering abundance, seed setting and maturity date as well as plant composition, such as protein and carbohydrate content, may be regulated to make plants more adaptable to a specific environment and for a specific use.

The writer's investigations reported in this paper were undertaken at the University of Alberta to determine the effects on legumes and certain other crops of various growth regulating chemicals applied at herbicidal and non-herbicidal rates.

LITERATURE REVIEW

It is well known that plants bend toward the light. The history of plant hormones or growth regulators commenced in 1880 when Charles Darwin became interested in this phenomenon. The advancement in knowledge pertaining to these substances is recorded by Boysen Jensen et al (5). Depending on concentration of chemical used, hormonal treatments of plants can cause a range of reactions extending from growth stimulation to inhibition or to death of certain organs or of whole plants.

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The Treatment of Seed, Seedlings and Growing Plants With Growth Substance.

In 1933 it was shown that hormones govern, to some extent, the growth of buds (35). One practical application, which has resulted from these findings, is the use of growth substances to induce dormancy and to break dormancy in potatoes. This work is reviewed by Avery et al (4). The potassium salts of naphthaleneacetic acid (K.N.A.) and the methyl ester of naphthaleneacetic acid (MeNA) are capable of preventing formation of sprouts on potato tubers in storage while ethylene chlorohydrin and several other chemicals are capable of breaking either natural or induced dormancy. Smith et al (32) sprayed potato plants in the field with Me NA, sodium 2,4-dichlorophenoxyacetate, a-naphthaleneacetic acid, indoleacetic acid and 2,4,5 - trichlorophenoxyacetic acid and found that sprout growth was significantly reduced in the stored tubers.

Many attempts have been made to influence the subsequent growth of plants by applications of growth regulators to seeds. There have been both positive and negative results. Thimann and Lane (36) treated oat and wheat seeds with indoleacetic acid and found that, the general vegetative growth of the shoot was accelerated, the dry weight of the plant was often increased more than 50 per cent and a slight hastening of flowering occurred.

On the other hand Stewart and Hamner (34) soaked seeds of various vegetable and field crop plants in a number of different hormone solutions comprising a wide range of concentrations and obtained no significant increase in growth in either field or greenhouse trials. Pearse (27) records that Amlong and Naundorf obtained increased germination and increased plant size in radishes and sugar beets while Walrand using the same chemical on sugar beet seeds reported negative results.

Many controversial results have been obtained by using growth substances on growing plants. Grace (16) reported that the growth of young tomato plants was greatly increased if the plants were watered daily with nutrient solutions containing growth hormones. An optimum concentration was found and the extent of stimulation was greatest in young plants. Pearse (27) states that Amlong and Naundorf reported acceleration of vegetative growth and flowering by foliar applications of indoleacetic acid. Hamner (18) on the other hand could find no particular benefit from adding small quantities of growth regulators to the soil around the growing plants. Pearse (27) concludes by mentioning that Zimmerman in 1947 considered there was no established proof that any synthetic growth substances stimulate the growth of the whole plant.

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Kerns (10) found that naphthaleneacetic acid and other naphthalene compounds sprayed on the foliage of pineapples at low concentrations advanced the flowering time by at least two months. Conversely, flowering could be retarded by heavier applications. Van Overbeek (37) has been successful in fully controlling flowering time in pineapple with naphthaleneacetic acid and 2,4-D. Leopold and Thimann (21) shortened by 35% the time to flowering of barley treated with naphthaleneacetic acid. Zimmerman and Hitchcock (42) working with tomato plants stimulated the production of flower clusters on leafy branches by using triiodobenzoic acid.

In reviewing the uses of plant growth regulators Weintraub and Norman (41) state that experiments aimed at prolonging the blossoming period of decorative fruit trees have given promising results. Gustafson (17) has shown that the retention of the flower on tobacco is a hormone phenomenon. Application of hormones resulted in retention of the flowers, but many of these flowers did not produce fruits.

There are few indications that seed set can be increased by hormone treatments. Ennis et al (12) noticed that potato plants sprayed with 2,4-D had set a considerable number of seed balls whereas the control plants failed to set any fruit. Conceivably, seed set might be increased by use of chemicals to induce flowers to remain on the plant

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Growth regulators have been used on various plants in attempts to alter the chemical composition of the plant parts. Erickson <u>et al</u> (13) showed conclusively that the protein content of wheat increased in direct relation to the amount of 2,4-D applied in heavy herbicidal rates. Other workers, however, have shown that such protein increases are accompanied by a decrease in yield of grain apparently comparable to that which occurs under drought conditions. Warden and Bullette (38) sprayed wheat with 2,4-D ester and obtained a highly significant increase in protein content of grain. There was a positive correlation between concentration of 2,4-D and protein content and a negative correlation between protein content and yield. High protein content was associated with severe stunting of the wheat plants. Friesen (14) used similar treatments in 1947 and reports that milling and baking qualities of the wheat were not affected. Pearse (27) reviewing the work of Amlong and Naundorf, noted that the sugar content of sugar beets was increased 123 per cent by soaking the seeds in a solution of indoleacetic acid before planting.

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<u>The Response of Plants to Growth Substances Used as Herbicides.</u>

Marth and Mitchell (22) in describing the action of 2,4-D applied in lethal concentrations to susceptible growing plants, pointed out that the growth substance does not cause rapid superficial burning like other toxic weed killers do but that it brings about morphological and physiological responses at the point of application or in parts of the plant at some distance from the actual point of contact by active translocation within the tissues. Growth responses are characterized by distorted stem and leaf growth, inhibition of bud growth, especially the terminal buds, and sometimes by the production of galls on the thickened roots and main stems. Later the cells and tissues of the plant disintegrate and the plant dies. Weaver and De Rose (39) found that great stunting of bean plants occurred when 2,4-D was applied to the youngest parts of the plants. The growth substance readily passed downward through the plant and the velocity of movement was apparently greatly influenced by the photosynthetic activity and the age of tissue. Mitchell and Brown (26) concluded that when 2,4-D was applied to the leaves of bean plants the translocation

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Selective weeding of one species from another with plant growth regulators, as herbicides, is possible because different species show varying degrees of susceptibility to the same application. Pearse (27) compiled a list of 193 weed plants arranging them according to their sensitivity to 2,4-D. There were three divisions namely: Species readily killed; Species relatively resistant although growth retarded; and Very resistant species. The corresponding classification of weeds in western Canada include Susceptible, Intermediate and Resistant categories. (28). Akamine (1) in reviewing selectivity of growth regulators concluded that 2,4-D has relatively little effect on most of the grasses if treated at or before jointing or after heading. Legumes are generally considered to be very susceptible to herbicides but there are variations according to species and chemical as shown by Miller (24), Kratochvil et al (19), Carder (7) Buchholtz (6), Churchill and Grigsby (9) and Shaw and Willard (31). The margin of safety between dosages of 2,4-D that injure most broad leaf crop plants and the dosages which effectively control weeds is usually narrow. Weed control is often confined to a certain stage of growth.

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The presence of selectivity within a species has been shown. Albrecht (2) found that the tolerance of bent-grass to 2,4-D varied considerably among strains. In 27 strains these variations extended from no injury to some strains, to almost complete destruction of others.

Derivatives of the same chemical compound show varied effects on certain species. Ennis et al (12) showed that 2,4,5-trichlorophenoxyacetic acid had an extremely injurious effect on the Irish potato but such agents as 2,4-D and 2-methyl-4-chlorophenoxyacetic acid when applied at the same rate in aqueous sprays produced little inhibition of either vegetative growth or tuber yield.

The age of plant has an important bearing on the susceptibility of the plant to herbicides. Robinson (29), summarizing trials with flax, generalized that flax is susceptible in the cotyledon stage, moderately resistant from the early rosette stage or first leaf stage to about two weeks before full bloom, very susceptible in bud and bloom stages and resistant after boll formation. These data indicate that developing floral and ovarial structures are as severely affected by the herbicide as young vegetative plant parts are. Klingman (20) working with oats and barley also expressed this view.

Most manufacturers of 2,4-D and other hormone herbicides recommend the use of these substances on clear warm days for best results. Marth and Davis (23) found that

plants are killed more readily by 2,4-D under temperature conditions favorable for growth.

Rainfall occurring shortly after a spray application of 2,4-D will result in a decreased plant response if the growth regulator is applied in aqueous spray. This was shown to be true by Weaver et al (40). If the growth substance was applied in oil solution there was no diminution in the response to the herbicide. In another investigation Weaver and De Rose (39) determined that the leaves of young soybean plants absorbed maximum amounts of growth regulator within six hours from the time of application.

Pre-emergence soil treatment is another method of plant growth control. Akamine (1) concluded from his review of the literature that since the emergence of most plants has been shown to be adversely affected or entirely prevented when selective growth regulators are applied to the soil the difference in the degree of susceptibility of the species is minimized. Mitchell and Brown (25) agreed with Allard et al (3) nevertheless that broadleaved species generally seemed to be more sensitive to 2,4-D and 2-methyl-4-chlorophenoxyacetic acid than are grass species when planted in soil treated with these compounds.

The foregoing review, though not an exhaustive account of research on plant growth regulators, has been selected to represent and provide access to the literature having a bearing on the projects discussed in the ensuing pages.

PART I.

PRELIMINARY INVESTIGATIONS WITH ANNUAL CROPS

Introduction

The foregoing literature review has indicated that various effects on plants have been obtained by the use of growth regulators at various concentrations, applied in various ways and at different stages of plant growth. Apparently effects extend from growth inhibition to stimulation depending on such factors as chemical concentration and time of application.

This project was planned to investigate effects of various chemicals on perennial legume crops under field conditions. While suitable plots of alfalfa, sweet clover and red clover were being established during the first year preliminary investigations were carried out on annual crops so that techniques could be developed and resulting effects on growth and yield observed. In this way it was hoped to learn which of a large number of available chemicals might have most promise for the ensuing investigations.

Certain effects which have been reported as a result of soaking seeds and potato tubers before planting have been indecisive. In view of this fact the studies reported in Section I were undertaken to investigate further

the effects of this type of treatment on morphological and phenological responses, yield and quality of spring wheat, winter wheat, flax, corn, peas, soybeans, and potato eye-pieces at Edmonton. Similarly the works reported in Section II was undertaken to study the effects of spray treatments with various chemicals on spring wheat, fall wheat and flax.

PART I.

PRELIMINARY INVESTIGATIONS WITH ANNUAL CROPS

Section I. Preplanting Treatments With Various Chemicals.

Materials and Methods

The following chemicals and combinations of substances were used in various tests:

	Chemical	Abbreviations Used
(1)	2,4-dichlorophenoxyacetic acid in 0.5% carbowax 1500.	2,4-D
(2)	2,4,5-trichlorophenoxyacetic acid (commercial isopropyl ester) Dow Chemical Co.	2,4,5-T
(3)	D.D.T. (commercial 50% wettable powder) Canadian Industries Limited.	D.D.T.
(4)	Naphthaleneacetic acid in 0.5% carbowax 1500. Dow Chemical Co.	N.A.A.
(5)	Parachlorophenoxyacetic acid in 0.5% carbowax. Dow Chemical Co.	P.C.P.
(6)	A-o-chlorophenoxyproprionic acid (commercial). Dow Chemical Co.	A.O.C.P.
(7)	Composite mixture containing chemicals 1-6.	Comp.
(8)	Sugar solution 1% hexose and 1% sucrose.	Sugar
(9)	4-chloro-toloxyacetic acid, 20 p.p.m. with 0.5% carbowax 1500.	C.T.A.
(10)	Pressed juice of flowering dandelions.	Dan.

Chemical	Abbreviations Used
(11) Trichlorobenzene, 20 p.p.m. with Triton X emulsifier.	T.C.B.
(12) Chlordane, 20 p.p.m. with Triton X emulsifier.	Chlor.

Test 1 (Soaked Seeds), 1949.

Counted separate lots of seeds of Red Bobs spring wheat, Kharkov winter wheat, Cheyenne winter wheat, Redwing flax, Altagold corn, Homesteader peas and Kabott soybeans were soaked for 6 hours in concentrations of 5, 20 and 50 p.p.m. of chemicals 1 - 7 and in concentrations of preparations of 8 - 12 as indicated above. Seeds for check plots were soaked for the same period in distilled water. After soaking, the seeds were dried for 16 hours. Two replicates of randomized field plots were seeded in late May. Rows each 6 feet long were spaced 2 feet apart. Peas, corn and soybeans were planted 50 seeds per row and the other species at 150 seeds per row. The soil at seeding time was very dry. Emergence counts were taken when the seedlings were approximately 2 inches tall.

Test 2 (Potatoes), 1949.

Netted Gem potato eyepieces of uniform size were cut from the central portion of the tubers, and then dried for 16 hours. Counted separate lots were soaked for

6 hours in concentrations of 5,10,20,50 and 200 p.p.m. of chemicals 1 - 7 inclusive, and in concentrations of chemicals 8 - 12 inclusive as noted above. Eyepieces for check plots were soaked in water. Triplicate randomized field plots, in factorial design, were planted in mid-May. In the design each block was assigned to a particular chemical and included treatments at 5 concentrations as well as a check, with each treatment represented by a row containing 8 eyepieces. Block number 8 was assigned the name "Group". It contained chemical treatments 8 - 12 above and a check row. Rows were 12 feet long and spaced 3 feet apart. Rows were planted along the margins of the plots to eliminate border effect. At planting time the soil was very dry.

Emergence counts were recorded June 7th, 11th, 18th, 24th and July 4th and 16th. The tubers were dug by hand in mid-September. Those from each row were bagged separately and placed in storage preparatory to counting and weighing. The resulting data were analyzed statistically.

Test 3 (Potatoes), 1950.

Tubers of uniform size from each row in Test 2 were retained in storage. In the spring eyepieces from these were planted in the same design as Test 2 with the exclusion of the D.D.T. and "Group" blocks. Emergence was recorded in the first 2 weeks of June and tubers were dug in mid-September. Yields were so constant in all rows

that only the material from rows of the 200 p.p.m. treatments and from corresponding checks were bagged separately prior to storage, weighing of the tubers, and statistical analysis of the data.

Test 4 (Potatoes), 1951.

In reviewing the results of Test 2 it was noted that T.C.B. had in all replicates caused plants to emerge a few days earlier than the check. Although the difference was not quite statistically significant, further investigation was considered advisable. Twenty-five potatoes of uniform size were selected from check material of Test 3. Three eyepieces were cut from the central region of each potato to eliminate variations due to bud dominance, insofar as possible. The 3 eyepieces from each potato were identified and soaked for 6 hours in 20 p.p.m., 40 p.p.m. of T.C.B. and water respectively. They were then planted at a uniform depth in the same pot and identified by stakes. The 25 pots were placed on the greenhouse bench and watered as required. Emergence was recorded at regular intervals until one month after planting. No stimulation was evident with the concentrations used.

Experimental Results

Test 1 (Soaked Seeds).

Very dry weather after planting, in combination with the dry condition of the soil at time of planting, prevented conclusive observations regarding time and percentage of emergence of seedlings. Counts were made twice but the behavior of the checks was so erratic that further observations were considered inadvisable. In general however, the highest rate of chemical application (50 p.p.m.) retarded emergence. The winter wheat remained vegetative throughout the summer.

Test 2 (Potatoes) 1949.

The potatoes were not affected by drought to the same extent as were the seeds in Test 1. The variance analysis of the row emergence data appears in Table 1. The F. value 18.57 indicates that the differences between the treatment means were highly significant. The means of the 200 p.p.m. treatments for 2,4-D and A.O.C.P. were just significantly lower than the means of their checks while the means of the 200 p.p.m. treatments for 2,4,5-T, N.A.A., P.C.P. and Comp. were considerably lower than the means of their controls. This is shown in Column 5, Table 2. These data indicate that soaking of Netted Gem potato eye-pieces for 6 hours, in aqueous solution of 200 p.p.m. of

TABLE 1

Variance Analysis of the Data for Days to Emergence of Plants from Potato Eye-pieces Soaked in Various Chemical Solutions

Source of Variation	Degrees of Freedom	Sum of Squares	Mean Squares	F. Value
Chemicals	6	442.4	73.7	1.75
Replicates	2	407.6	203.8	4.84*
Error (1)	12	504.5	42.0	
Treatments	5	4484.7	896.9	18.57**
Chemical x treatments	30	629.4	20.9	0.43
Error (2)	70	3380.7	48.3	
Total	125			

* Significant at the 5 per cent level
** Significant at the 1 per cent level
M.S.D. for treatments = 4.6 days per row.

TABLE 2

Summary of Effects of Chemical Treatments on Row Emergence, Survival, Yield per Plant, and Top Growth of Netted Gem Potatoes

Col. 1	2.	3.	4.	5.	6.
Chemical	Rate in p.p.m.	Total No. of Plants (3 replicates)	Mean Yield in ozs. per Plant	Mean No. of days for complete row emergence	Mature size of tops in % of Check
N.A.A.	200	8	23	60	50% or less
P.C.P.	200	24	7	56	50% or less
2,4,5-T	200	23	18	60	50% or less
2,4-D	200	23	18	45	50% or less
A.O.C.P.	200	24	17	47	100%
Comp.	200	23	16	52	75%
Check		24	25	33	100%

2,4,5-T, N.A.A., P.C.P., 2,4-D, A.O.C.P. and Comp. before planting, significantly delayed emergence.

Column 6, Table 2 summarizes the visible effects of the various chemicals and concentrations on top size of mature plants. Of the above mentioned chemicals and concentrations those tried but not listed did not visibly affect top growth. During the early part of the season 200 p.p.m. of 2,4-D and P.C.P. produced marked abnormalities in the leaves and stems. Some of the 200 p.p.m. treatments had a lethal effect on the eyepieces. This is shown in Column 3, Table 2.

The variance analysis of the data for tuber yield per row appears in Table 3. The F value 8.71 indicates a highly significant difference between tuber yields due to the effects of the different chemical treatments. Table 4 compares the chemical concentrations which significantly decreased tuber yield and delayed emergence. Column 4, Table 2, gives the mean yield per plant for treatments which, either affected top growth or which significantly reduced tuber yield.

Test 3 (Potatoes) 1950.

Potato emergence in 1950 was normal and no effects on top growth were noted throughout the season. This would indicate that the effects of the chemical treatments were not carried over in the progeny of the treated material. As a further check on this observation it was decided to

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Test 3 ('Katahdin' 1950).

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TABLE 3

Variance Analysis of the 1949 Yield Data for the Test with Potato Eyepieces Soaked in Various Chemical Solutions Prior to Planting

Source of Variation	Degrees of Freedom	Sum of Squares	Mean Square	F. Value
Chemicals	6	10150.2	1691.7	0.50
Replicates	2	8686.2	4343.1	1.29
Error (1)	12	40468.8	3372.4	
Treatments	5	109551.6	21910.3	8.71**
Chemicals x Treatments	30	75441.0	2514.7	2.30**
Error (2)	70	76682.4	1095.5	
Total	125			

** Significant at the 1 per cent level.
M.S.D. for treatments = 54 ounces per row.

TABLE 4

Summary of Treatments that Significantly Decreased Yield and Delayed Emergence of Netted Gem Potatoes

Chemical	Rates (p.p.m.) decreasing yield significantly	Rates (p.p.m.) delaying emergence significantly
A.O.C.P.	-	200
~~N.D.T.~~	-	-
P.C.P.	200	200
2,4-D	200	200
N.A.A.	200	200
2,4,5-T	10, 20 200	200
Comp.	-	200

compare the yields following the 200 p.p.m. treatments with those of the checks. Table 5 summarizes these results. The data were analyzed statistically but no significant differences were found.

TABLE 5

Summary of 1950 Tuber Yields from the Progeny of Material Treated in 1949

	Mean Tuber Yield in Pounds per Row					
Chemical	2,4,5-T	2,4-D	N.A.A.	A.O.C.P.	P.C.P.	Comp.
Check	22.8	23.0	21.3	20.1	22.8	20.5
200 p.p.m.	19.8	21.6	18.5	17.8	22.5	19.8

Test 4 (Potatoes), 1951.

The first plants emerged 18 days after the eye-pieces were planted. Emergence was recorded at 3 day intervals till one month after planting. Table 6 shows the percentage emergence at these various dates.

TABLE 6

Percentage Emergence of Netted Gem Potatoes After Soaking Eyepieces in Trichlorobenzene Solutions

Days after Planting	Check	T.C.B. 20 p.p.m.	T.C.B. 40 p.p.m.
	Percentage Emergence		
18	68	44	16
21	84	68	24
24	84	68	40
27	96	72	40
30	96	72	40

Discussion

It is highly probable that, if moisture conditions had been normal in the spring of 1949, results very different from those of Tests 1 and 2 would have been obtained. More pronounced effects were expected in the potato eyepiece test but since the mean time to emergence of the checks was 33 days it is possible that the effect of the chemicals diminished with the elapsing of such a period of time in the soil.

Considering the results obtained, it is evident that the various chemicals at rates of 200 p.p.m. had varied effects on the eyepieces and also on the subsequent growth of

the plants. N.A.A. at 200 p.p.m. was drastic in its action on the eyepieces as it killed 66 per cent of them. The lethal effect of N.A.A. on bud growth of trees has been shown by other workers (4). From results obtained it is evident that the 200 p.p.m. treatments retarded the growth of sprouts from the eyepieces in the following order of decreasing effects: N.A.A. and 2,4,5-T equal, P.C.P., Comp., 2,4-D and A.O.C.P.

Although N.A.A. at 200 p.p.m. was lethal to some eyepieces it did not have an appreciable residual effect upon the growth of those which survived since their yield was almost equivalent to the checks. The tops, however, were smaller than those of the checks. P.C.P. was outstanding in its effects on both top and tuber yield. The 200 p.p.m. P.C.P. treatment reduced top size by 50 per cent or more and reduced yield 72 per cent. There evidently was an extreme residual effect attributable to only a small amount of this particular chemical in the plant. Top growth was retarded equally by 2,4,5-T and 2,4-D at 200 p.p.m. and yield was also reduced approximately 30 per cent. 2,4,5-T however delayed emergence considerably more than did 2,4-D.

Since none of the treatments increased tuber yield, they appear to have no value from a practical standpoint apart from their possible use to prevent sprouting of non dormant potatoes in storage. N.A.A., P.C.P., 2,4,5-T and 2,4-D would appear to be most promising in this regard.

the plants. T.C.A. at 800 p.p.m. was drastic in its action on the eyepieces as it killed 60 per cent of them. The lethal effect of T.C.A. on the growth of grass has been shown by other workers (4). From results obtained it is evident that the 200 p.p.m. treatments retarded the growth of sprouts from the eyepieces in the following order of decreasing effects: T.C.A. and 2,4,5-T equal, P.C.P., Comp., 2,4-D and [illegible].

Although T.C.A. at 800 p.p.m. was lethal to some eyepieces it did not have an appreciable residual effect upon the growth of those which survived since their yield was almost equivalent to the checks. The tops, however, were smaller than those of the checks. P.C.P. was outstanding in its effects on both top and tuber yield. The 800 p.p.m. P.C.P. treatment reduced top size by 80 per cent or more and reduced yield 75 per cent. There evidently was an extreme residual effect attributable to only a small amount of this particular chemical in the plant. Top growth was retarded equally by 2,4,5-T and 2,4-D at 200 p.p.m. and yield was also reduced approximately 60 per cent. 2,4,5-T however delayed emergence considerably more than did 2,4-D.

Since none of the treatments increased tuber yield, they appear to have no value from a practical standpoint apart from their possible use by growers for treating of non dormant potatoes in storage. T.C.A., P.C.P., 2,4,5-T and 2,4-D would appear to be most promising in this respect.

N.A.A., 2,4,5-T and 2,4-D have already been used successfully by other workers (32) for this purpose. It would seem inadvisable to use tubers previously treated in this way as seed potatoes since sprout growth would likely be inhibited, as with N.A.A., or tuber yield reduced, as with P.C.P. Counter treatments with other chemicals might be used to break the imposed dormancy. Ethylene chlorohydrin has been employed successfully in this regard (4). Apparently 2,4,5-T was the most toxic of all the chemicals used on Netted Gem potatoes as it reduced yield significantly following applications as low as 10 p.p.m. These results agree with those of Ennis _et al_ (12) who worked with Irish potatoes.

In the second year, eyepieces which were cut from the progeny of the previous test emerged and grew normally. Although the yields of the 200 p.p.m. rows in most cases were slightly below those of the checks statistical analysis proved these differences to be insignificant. From these results it is apparent that carryover of chemical, within the tubers from soaked eyepieces, would not be sufficient to affect yields in the second year. Whether or not this would be true following vine spraying remains to be determined.

Although 20 p.p.m. of trichlorobenzene appeared to stimulate slightly the sprouting of potato eyepieces under field conditions it definitely inhibited sprouting

M.H.A., 2,4,5-T and 2,4-D have already been used successfully by other workers (6,) for this purpose. It would seem inadvisable to use tubers previously treated in this way as seed potatoes since normal growth would likely be inhibited, as with M.H.A., or lower yield produced, as with T.C.A. Further studies with other chemicals might be used to break the apparent dormancy. Ethylene chlorohydrin has been employed successfully in this regard (4). Apparently 2,4,5-T was the most toxic of all the chemicals used on Netted Gem potatoes as it reduced yield significantly following applications as low as 10 p.p.m. These results agree with those of Smith et al (12) who worked with Irish potatoes.

In the second year, specimens which were cut from the progeny of the previous ones emerged and grew normally. Although the yields of the 500 p.p.m. used in most cases were slightly below those of the checks statistical analysis proved these differences to be insignificant. From these results it is apparent that carryover of chemical, within the tubers from treated specimens, would not be sufficient to affect yields in the second year. Whether or not this would be true following vine spraying remains to be determined.

Although 50 p.p.m. of [illegible] appeared to stimulate slightly the sprouting of [illegible] under field conditions it [illegible] under greenhouse conditions. One month from the time of planting, emergence of the treated and untreated material was at a maximum. Following the 20 and 40 p.p.m. treatments 28 and 60 per cent of the eyepieces from respective treatments failed to emerge as compared with a failure of growth of 4 per cent of the check material. From these results it is apparent that T.C.B., which is normally used as an insecticide, may have some possibilities as a plant growth inhibitor.

Summary

1. Significant delays in emergence and subsequent decrease in tuber yield of Netted Gem potatoes were caused by soaking eyepieces for 6 hours in 200 p.p.m. solutions of 2,4-dichlorophenoxyacetic acid, 2,4,5-trichlorophenoxyacetic acid, naphthaleneacetic acid and parachlorophenoxyproprionic acid immediately before planting. Other concentrations namely 5, 10, 20 and 50 p.p.m. of these chemicals had no significant effect on emergence or on yield except for 2,4,5-T. Applications as low as 10 p.p.m. of 2,4,5-T significantly reduced yield of tubers.

2. Sixty six per cent of the eyepieces soaked in a 200 p.p.m. solution of N.A.A. failed to emerge. The tuber yield per surviving plant was not decreased however.

3. Tuber yield of plants from eyepieces soaked in 200 p.p.m. of P.C.P. was reduced by 72 per cent.

4. Eyepieces from the progeny of the soaked eyepieces emerged normally and their tuber yields did not vary significantly from check yields. However there was a marked trend towards a yield reduction from eyepieces from treated material.

5. In these tests 2,4,5-T showed the greatest toxicity to Netted Gem potatoes.

6. A practical use for some of the chemicals has been discussed.

7. At certain rates trichlorobenzene, an insecticide, had a definite inhibiting effect upon sprouting of potato eyepieces in soil under greenhouse conditions.

Section II. Chemical Spray Treatments of Growing Plants

Materials and Methods

Test 1 (Spring wheat).

Red Bobs spring wheat was seeded at the rate of 1 1/2 bushels per acre in the middle of May, 1949. After emergence, randomized plots were arranged in factorial design with 3 replicates. Each block contained 6 plots, each 5 rows wide and 18.5 feet long. The chemicals and concentrations used were the same as in Test 2 Section I. Solutions were applied from hand sprayers at the rate of 100 cc. per 37 square feet or approximately 26 gallons per acre. Sprayer hoods and wind screens were used to eliminate drift. Spraying was commenced early in June and continued at weekly intervals until the sixth and final treatment was applied when the wheat was headed out. The plots were harvested in early September and the threshed samples were weighed and retained for protein analysis by the Kjeldahl method using mercuric oxide as the catalyst. The yield data were analyzed statistically.

Test 2 (Flax).

In this test with flax, chemicals and methods were the same as in Test 1. Flax was sown at 1/4 bushel per acre. The first spraying was on June 15th during the 4 leaf stage

and the sixth was applied during the bud stage. These plots were not harvested owing to irregular germination and uneven growth resulting from drought conditions.

Test 3 (Flax).

Plots of flax were sprayed with 2,4-D, 2,4,5-T, D.D.T., P.C.P., N.A.A., and Comp. at rates of 500, 1000, and 2000 p.p.m. during the bud stage. Solutions were applied with hand sprayers at approximately 26 gallons per acre. The results were recorded July 16th.

Test 4 (Winter wheat).

Kharkov winter wheat was seeded in the middle of May 1949. Chemicals and methods used were the same as in Test 1.

Test 5 (Winter wheat).

Duplicated plots of Kharkov winter wheat which had remained vegetative throughout the season were sprayed on August 15th, with solutions of 2,4-D amine, 2,4,5-T ester and a mixture of both. Application was at the rate of 50 gallons per acre with rates of 1, 2, 3, 4 and 5 pounds of acid equivalent per acre.

Experimental Results

Test 1 (Spring wheat).

Careful observation of the spring wheat revealed no visible effects of spray treatments on growth, heading or ripening. Table 7 shows the variance analysis of the yield data. Apparently Red Bobs spring wheat was not significantly increased or decreased in yield by the chemical applications. Protein data for the various treatments varied so slightly from those of the checks that statistical analysis was considered not to be worthwhile.

Test 2 (Flax).

In this test flax germination was so uneven that, at the time of the first spray application, some seedlings were in the 4 leaf stage while others had just emerged. At the time of the fourth application it was noted that the 200 p.p.m. 2,4,5-T rate had caused chlorosis and stunting. Three successive applications of 200 p.p.m. thus had a serious effect upon flax plants in the stage of active growth. A similar but less pronounced effect was observed in the plots treated with 50 p.p.m. of 2,4,5-T. By early September no plants remained alive in the 2,4,5-T 200 p.p.m. plots while those in the 50 p.p.m. plots were alive but

TABLE 7

Variance Analysis of the Yield Data from Chemically Sprayed Spring Wheat.

Source of Variation	Degrees of Freedom	Sum of Squares	Mean Square	F. Value
Chemicals	6	22833	3805.5	0.92
Replicates	2	31147	15573.5	3.76
Error (1)	12	49663	4138.6	
Treatments	5	5730	1146.0	0.83
Chemicals x Treatments	30	41584	1386.1	2.13**
Error (2)	70	45493	649.9	
Total	125			

** Significant at the 1 per cent level.

seriously stunted. No other effects due to treatment were observed. Growth was so irregular that plots were not harvested.

Test 3 (Flax).

As noted above, flax plots were treated at bud stage in mid-July with herbicidal rates of various chemicals in order to determine their relative toxicity to this crop. A summary of the effects of the treatments appear in Table 8. Under the conditions of the test, flax showed responses to the applied growth regulators in the following order of

TABLE 8

Responses of Budding Red Wing Flax to
Herbicidal Rates of Chemicals

Chemical	Rates P.P.M.	Visible effects on topgrowth: 5 Days after treatment	Visible effects on topgrowth: 2 1/2 Months after treatment
2,4,5-T	2000	severe twisting, partial chlorosis	All plants dead.
	1000	severe twisting, partial chlorosis	30% of plants alive, growth retarded
	500	severe twisting, partial chlorosis	30% of plants alive, growth retarded
2,4-D	2000	severe twisting	50% of plants alive, growth retarded
	1000	severe twisting	100% of plants alive, growth retarded
	500	slight twisting	100% of plants alive, growth retarded
P.C.P.	2000	severe twisting, partial chlorosis	60% of plants alive, growth retarded
	1000	slight twisting	100% of plants alive, growth normal
	500	No visible effect	100% of plants alive, growth normal
N.A.A.	2000	No visible effect	100% of plants alive, growth normal
	1000	No visible effect	100% of plants alive, growth normal
	500	No visible effect	100% of plants alive, growth normal
Comp.	2000	severe twisting, partial chlorosis	50% of plants alive, growth retarded
	1000	severe twisting	100% of plants alive, growth normal
	500	slight twisting	100% of plants alive, growth normal

decreasing susceptibility: 2,4,5-T, 2,4-D, Comp., P.C.P., and N.A.A. At 2000 p.p.m. 2,4,5-T was lethal while N.A.A. had no visible effects on the flax plants.

Test 4 (Winter Wheat).

The spring planted Kharkov winter wheat grew very well. After 6 weekly sprays no visible effects were observed. By the middle of August the vigorous vegetative growth was about 2 feet tall.

Test 5 (Winter wheat).

Ten days after the herbicidal rates of the chemicals were applied to Kharkov winter wheat there was evidence of burning caused by all rates except 2,4-D at 1 and 2 pounds per acre. Burning became progressively worse with increase in concentration of chemicals. Three pounds per acre of 2,4,5-T caused damage equivalent to that from 5 pounds of 2,4-D. Because all the wheat winter killed it was not possible to observe later effects of these treatments such as may have occurred the following spring.

Discussion

The results obtained from the chemical spray treatments of spring and winter wheat were of no practical value. Winter wheat remained vegetative and spring wheat was not

affected either in growth, yield or protein content. The treatments applied were considerably below the herbicidal rates known to affect yield and protein content (13).

The spray treatments gave an indication of the extent of damage produced on flax by the various chemicals, applied during the bud stage of this crop. As shown earlier, certain chemicals did not affect the flax plants while others killed the complete stand.

Summary

1. The yield and protein content of Red Bobs spring wheat was not significantly affected by any of the chemical treatments outlined in Section I.

2. Spring planted Kharkov winter wheat remained vegetative after the various chemical treatments.

3. Flax plants at the bud stage responded to aqueous chemical sprays in the following order of increasing susceptibility: Naphthaleneacetic acid, parachlorophenoxyproprionic acid, composite mixture, 2,4-dichlorophenoxyacetic acid and 2,4,5-trichlorophenoxyacetic acid. At the 2000 p.p.m. rate 2,4,5-T was lethal while N.A.A. caused no visible response.

PART II.

LEGUME CROP INVESTIGATIONS

Introduction

Since it has been shown that growth regulators possess both growth inhibitory and growth stimulatory powers, investigation of the extent and importance of such responses in legume crops seemed desirable.

In the experiments dealt with in Section I an effort was made to investigate the possibility of selectively weeding sweet clover from alfalfa at three stages of growth and also to determine possibilities of using synthetic hormone herbicides for weed control purposes within these crops.

Section II was undertaken to explore possible favorable or unfavorable effects of phenoxy compounds in the soil on germination and growth of legume seedlings.

The objectives of the work reported in Section III were to determine if hormone sprays applied at relatively low rates at intervals throughout the flowering season would significantly affect seed set of alfalfa and red clover.

PART II.

LEGUME CROP INVESTIGATIONS

Section I. Herbicidal Applications of Synthetic Growth - Regulators to Alfalfa and Sweet Clover

Materials and Methods

Synthetic hormone herbicides that were readily available as commercial formulations were used in these investigations. These chemicals were:

Chemical	Abbreviations Used
1. 2,4-dichlorophenoxyacetic acid (amine) Naugatuck.	2,4-D amine
2. 2,4-dichlorophenoxyacetic acid (ester) Green Cross.	2,4-D ester
3. 2,4,5-trichlorophenoxyacetic acid (ester) Naugatuck.	2,4,5-T ester

In the following tests the solutions were applied with a Dobbins tank-type pressure sprayer.

Test 1 (Alfalfa).

When plants were 4 inches tall, randomized plots in two replicates, in a one year old stand of Grimm alfalfa

were sprayed with 2,4-D amine and 2,4,5-T ester at rates of 2, 4, 8 and 16 ounces acid equivalent in 45 gallons of water per acre. Plot area was approximately 1/2000 acre. Observations were recorded in June, July and August.

Test 2 (Alfalfa).

During the early flowering stage randomized and replicated plots, in a one year old stand of alfalfa, were sprayed with chemicals 1, 2 and 3 above at rates of 1/2, 1, 2, 4, 8 and 16 ounces per acre. Plot size and gallonage was the same as in Test 1. Observations were recorded at intervals throughout the summer.

Test 3 (Alfalfa).

The remnants of the alfalfa field, which were not under test, were mown July 31st. When the second growth was 6 to 8 inches tall on August 10th, randomized and replicated plots were sprayed with eleven rates of chemicals 1, 2 and 3 above ranging from 1 ounce to 4 pounds per acre. Plot size and gallonage was the same as in Test 1. Effects of the treatments were recorded on September 2nd and October 5th.

Test 4 (Sweet Clover).

Sweet clover 4 inches tall was treated in the same way as the plots in Test 1. Observations were recorded in June, July and August.

Test 5 (Sweet clover).

Sweet clover at the early flowering stage was given the same treatments as the plots in Test 2, and observations were recorded at the same times.

Test 6 (Sweet clover).

Regrowth of sweet clover in the 6 - 8 inch stage was treated with the same chemicals and concentrations in the same way as Test 3. Observations were recorded September 2nd and October 5th.

Experimental Results

Test 1 (Alfalfa).

As a result of the spray treatments on alfalfa at the early growth stage, only 2,4-D amine at 2 ounces per acre was without visible effect. All higher rates of amine and all rates of 2,4,5-T ester caused root brittleness, leaf chlorosis, stem twisting and stunting of the plant. Vigorous regrowth occurred from the crowns and from the nodes of the prostrate stems in all plots treated with 4 ounces per acre or more. The 1 pound per acre rate of 2,4-D caused death to 15 per cent of the plants while 2,4,5-T at 1 pound per acre killed 30 per cent of the plants. Flowering was delayed from 2 to 3 weeks by rates of 2,4-D higher than

2 ounces and by all rates of 2,4,5-T. One pound of 2,4,5-T per acre delayed flowering about 6 weeks. Toward the end of the season the regrowth in the plots treated with 1 pound of amine or ester per acre was as dense as the check plots and much taller despite the earlier death of some of the plants in these plots.

Test 2 (Alfalfa).

Treatments with the esters of 2,4-D and 2,4,5-T at rates of 1 ounce acid equivalent or less, and with the amine at 2 ounces or less per acre, applied to alfalfa during the early flowering stage, had no lasting effect on the appearance of the plants. The 2 ounce rate of the esters and 4 ounce rate of the amine checked the growth of stems. This retardation led to regrowth from the nodes and crowns. The terminal buds were all killed. Flowering was thus delayed about 1 month. All rates above 2 ounces of ester and above 4 ounces of amine caused leaf chlorosis and inhibition of stem growth to such an extent that regrowth was almost entirely from the crowns and not from the lateral buds on the stems. Seed pods were not present in these plots at harvesting time. All topgrowth and some roots of plants treated with 1 pound of the ester per acre died within 1 month after the spray application. By the end of the second month regrowth commenced from about 30 per cent of the plants.

Test 3 (Alfalfa).

All rates except 1 ounce of amine caused visible distortion of alfalfa regrowth, the severity increasing with the rate. Observations recorded 5 weeks after treatment showed that growth was retarded by 2 ounces or less of amine and by 1 ounce of the esters per acre. Rates greater than these caused growth inhibition, chlorosis and stem and leaf necrosis. Increase in severity was proportional to increase in rates. Although 1 pound per acre or higher rates of chemical appeared to have killed 80 to 100 per cent of the alfalfa roots a final assessment cannot be made until the next growing season arrives.

Test 4 (Sweet Clover).

As regards the spray treatments of sweet clover at the early growth stage, 2 ounces of amine per acre caused slight stem distortion while all higher rates of amine and all ester rates prostrated the stems. Within two weeks after treatment, anthocyanin pigmentation became more evident in all treated stems and depth of color paralleled treatment rates. Lesions on roots and stems appeared following all treatments of 8 ounces or more of amine and 4 ounces or more of esters. Regrowth from the crowns and also from the lateral buds on the stems was vigorous in plots treated with 1 pound or less of amine per acre and 8 ounces or less of

the esters per acre. Regrowth from terminal buds occurred only in plots treated with less than 4 ounces of amine per acre and with less than 2 ounces of the esters per acre. Thus of the treatments used here, it is assumed that terminal buds were killed by rates higher than 2 ounces of amine and 1 ounce of the esters per acre, and that lateral buds were killed by rates higher than 8 ounces of the esters per acre. Eighty per cent of the plants in the plots treated with 1 pound of the esters per acre were killed, the remainder produced some new growth in late August. Flowering was delayed 2 to 3 weeks by 2 and 4 ounces of the esters and by 4 ounces of amine per acre, 4 weeks by 1 pound of amine and 8 ounces of the esters per acre, and 8 weeks by 1 pound of the esters per acre. Almost without exception the results of this test show that 2,4-D or 2,4,5-T esters had a comparable effect on sweet clover. Rate for rate these esters were much more toxic to legume vegetation than was 2,4-D amine. In general twice as much 2,4-D amine as ester was required to produce a comparable effect on sweet clover. In the following discussion it can therefore be assumed that amine at double the ester rate produced a similar effect on the plants.

<u>Test 5 (Sweet clover).</u>

Treatments of sweet clover with 1 ounce or less of ester per acre applied during the early flowering stage the esters per acre. Regrowth from terminal buds occurred only in plots treated with less than 2 ounces of amine per acre and with less than 1 ounce of the esters per acre. Thus of the treatments used here, it is assumed that terminal buds were killed by rates higher than 2 ounces of amine and 1 ounce of the esters per acre, and that lateral buds were killed by rates higher than 2 ounces of the esters per acre. Eighty per cent of the plants in the plots treated with 1 pound of the esters per acre were killed, the remainder produced some new growth in late August. Flowering was delayed 2 to 3 weeks by 2 and 4 ounces of the esters and by 4 ounces of amine per acre, 4 weeks by 8 ounces of amine and 8 ounces of the esters per acre, and 6 weeks by 1 pound of the esters per acre. Almost without exception the results of this test show that 2,4-D or 2,4,5-T esters had a comparable effect on sweet clover. Also for rate these esters were much more toxic to legume vegetation than was 2,4-D amine. In general twice as much 2,4-D amine as ester was required to produce a comparable effect on sweet clover. In the following discussion it can therefore be assumed that amine at double the ester rate produced a similar effect on the plants.

Test 5 (Sweet clover).

Treatments of sweet clover with 1 ounce or less of ester per acre applied during the early flowering stage had no visible lasting effect on the plants. The rates of 2 ounces or greater checked growth and caused severe twisting of stems but regrowth was vigorous from crowns and from terminal and lateral buds of plants treated with 4 ounces or less. Plants treated with 8 ounces of ester per acre sent forth regrowth from the crown and lower nodes of the stem, hence it is assumed that this concentration killed the terminal and upper lateral buds. The 1 pound rate killed 60 per cent of the plants but regrowth from the crown and lower nodes of the remainder of the plants appeared at the end of July. Anthocyanin pigmentation was more noticeable in the plants treated with 8 ounces and 1 pound per acre. Flower abundance decreased as concentration of chemical increased from 1 ounce up to 1 pound. The latter rate killed all inflorescenses on the plants.

Test 6 (Sweet clover).

All treatments except 1 ounce of amine per acre applied when sweet clover regrowth was 6 to 8 inches tall caused visible distortion of the plants. Observations recorded during the first week in October, 5 weeks after the treatment, showed that growth was retarded by 2 ounces or more of ester per acre. The 4 ounce rate prostrated the stems and killed 50 per cent of the leaves while concentrations of 8 ounces or greater prostrated all stems and killed all the leaves. There was no regrowth from these plants had no visible lasting effect on the plants. The rates of 2 ounces or greater checked growth and caused severe twisting of stems and regrowth was vigorous from crowns and from terminal and lateral buds of plants treated with 4 ounces or less. Plants treated with 8 ounces of ester per acre sent forth regrowth from the crown and lower nodes of the stem, hence it is assumed that this concentration killed the terminal and upper lateral buds. The 1 pound rate killed 50 per cent of the plants but regrowth from the crown and lower nodes of the remainder of the plants appeared at the end of July. Anthocyanin pigmentation was more noticeable in the plants treated with 8 ounces and 1 pound per acre. Flower abundance decreased as concentration of chemical increased from 1 ounce up to 1 pound. The latter rate killed all inflorescences on the plants.

Test 6 (Sweet clover).

All treatments except 1 ounce of ester per acre applied when sweet clover regrowth was 3 to 4 inches tall caused visible distortion of the plants. Observations recorded during the first week in October, 6 weeks after the treatment, showed that growth was retarded by 2 ounces or more of ester per acre. The 4 ounce rate prostrated the stems and killed 50 per cent of the leaves while concentrations of 8 ounces or greater prostrated all stems and killed all the leaves. There was no regrowth from these plants before winter but this should not be taken as final indication that all the plants were killed. Observations should be made in the spring and a further test might be advisable to study the effects of chemical on regrowth from a stand mowed earlier in the season than August 1st.

Discussion

The results from herbicidal treatments of alfalfa and sweet clover indicated that these plants were very susceptible to phenoxy compounds insofar as damage to top growth was concerned. Rates ordinarily used for weed control purposes damaged these plants at any stage of growth to such an extent that regrowth occurred only from the crown and lower nodes of the stem. Despite the fact that top growth was susceptible there was no evidence that the roots were easily killed. Applications of 1 pound per acre of 2,4,5-T ester killed about 30 per cent of the alfalfa plants and 80 per cent of the sweet clover plants in the early growth stage. Similarly 80 per cent of both alfalfa and sweet clover was killed by the same application at flowering time. Applications of 1/2 pound per acre of 2,4-D and 2,4,5-T esters and of 1 pound per acre of 2,4-D amine, applied to 6 to 8 inch regrowth after mowing, appeared to kill all the plants, but owing to the lateness of the season

confirmation remained for future observation.

In all tests 2,4-D and 2,4,5-T esters caused comparable effects while 2,4-D amine was about one half as toxic at the same rate. Sweet clover was slightly more susceptible to damage than was alfalfa at the early growth stage, but at other stages appeared equally sensitive. These findings indicate that selective weeding of sweet clover from alfalfa stands by the use of these chemicals is not feasible.

The results suggest, however, that chemical control of weeds in alfalfa and sweet clover during the early growth stage might be possible. Under the conditions of the test in 1950, applications of 1 pound of 2,4-D amine or 8 ounces of the esters retarded the growth of all plants and killed some of them, but by the end of August the potential forage yield in the plots appeared to be even greater than that of the checks. The fact that forage yield appeared to increase even after a percentage of the plants were killed suggests the possibility of using these chemicals as sprays to thin out alfalfa stands to improve later seed production.

Legume seed growers find second year stands of alfalfa are often too weedy to cut for seed, and hence mow the fields for hay. Because of the weed content the quality of the hay is often impaired. The results obtained in the above experiments suggest that weedy fields could be sprayed with 8 ounces of 2,4-D amine or 4 ounces of 2,4,5-T

ester at the early growth stage. This application would seriously distort the alfalfa plants and probably delay flowering approximately 3 weeks, but if the weed growth was controlled the second growth would likely be more vigorous than the first, and therefore the quantity and quality of the hay would be improved. Moreover in view of the fact that there were seed pods on the plants in the experimental plots treated in this way there might, in a long season, be a possibility of harvesting a crop of seed. It would be necessary to investigate this method under field conditions before practical recommendations could be made.

Unpublished investigations at the University of Alberta have indicated that certain contact herbicides can be used successfully to remove annual weeds from alfalfa stands without damage to the crop. Such treatments would probably be more desirable to the seed grower but additional testing involving comparison of both types of herbicides would seem to be worthwhile.

Summary

1. The difference in the susceptibility of alfalfa and sweet clover to 2,4-dichlorophenoxyacetic acid or to 2,4,5-trichlorophenoxyacetic acid was insufficient to permit selective weeding of sweet clover from alfalfa.

2. In all tests the esters of 2,4-D and 2,4,5-T caused comparable root injury, stem and leaf distortion and damage to reproductive organs, but it was necessary to apply the amine formulation of 2,4-D at twice the rate of ester application to produce an equal toxic effect.

3. Alfalfa and sweet clover tops were very susceptible to the phenoxyacetic acid derivatives, but the roots could be considered quite resistant since concentrations greater than 1 pound of ester per acre were required to kill them.

4. Possible practical applications of the results of these tests have been discussed.

Section II. Effects of Chemical Soil Treatments on Emergence of Legume Seedlings.

Materials and Methods

This investigation involved the planting of 264 flats of soil with approximately 100,000 legume seeds. The tests were begun on a small scale in the greenhouse early in 1950 to determine the concentration of 2,4-D required in the soil solution to prevent emergence of seeds of alfalfa, sweet clover, alsike and red clover, and to determine possible variations in species susceptibility to the chemical.

As the test progressed indications of a stimulative effect of certain concentrations of 2,4-D became evident. The test was then moved outdoors where the number of replicates could be increased to improve accuracy and where environmental conditions would be more natural than in the greenhouse. Subsequently esters of 2,4,5-T and 2,4-D were included in the experiment to compare their effects with those of 2,4-D amine at the same concentrations.

Eight successive tests were carried out. The general procedure was the same for all experiments except that varying concentrations were used from time to time as shown below:

Test number	Test period	Chemicals	No. of replicates	Concentrations used p.p.m.
1.	Feb.15--Mar.15	2,4-D	2	1000, 50
2.	Mar.15--Apr.15	2,4-D	2	10, 0.5
3.	May 15--June15	2,4-D,2,4,5-T	4	0.01, 0.001
4.	June 8--June30	2,4-D, 2,4-D ester 2,4,5-T	4	0.005, 0.0005
5.	July 1--Aug.15	2,4-D, 2,4-D ester 2,4,5-T	4	0.0001, 0.00005
6.	Aug.15--Sept.5	2,4-D, 2,4-D ester 2,4,5-T	4	0.05, 0.1
7.	Dec. 1--Dec.30	2,4,5-T.	3	10, 1, 0.1, 0.01, 0.0001
8.	Jan. 1--Jan.30	2,4,5-T	3	2, 3, 4, 5, 6, 7, 8.

Flats (22.5 x 14.5 inches) of 3 to 1 soil sand mixture were each planted with 100 seeds of each species viz: alfalfa, alsike clover, sweet clover and red clover. Each flat contained 8 randomized rows of 50 seeds per row. Immediately after planting, 3 litres of water was applied to each check flat by means of a spray type watering can and the flats for hormone treatment watered with equal amounts of chemical solution. This moistening was usually sufficient to permit germination and emergence to take place before additional watering was required. As soon as the seedlings in the check flats reached the 3 leaf stage emergence was recorded and

comparisons expressed as difference between percentages of emergence of treated and untreated material.

Experimental Results

Tests 1 - 8 (alfalfa, alsike clover, red clover and sweet clover).

Test 1.

Within 9 days no seedlings emerged in the flats treated under greenhouse conditions with 50 and 1000 p.p.m. of 2,4-D amine. Emergence in the check flats was at a maximum at that time. One month from the time of treatment seedlings had not emerged in the treated flats.

Test 2.

The emergence was reduced by treatments of both 10 p.p.m. and 0.5 p.p.m. of 2,4-D amine under greenhouse conditions as shown in Figure 1. This test also supplied data on the relative susceptibility of the different species to 2,4-D under the conditions of soil treatment. Table 9 illustrates these observations. From these data it is evident that when 10 p.p.m. was used the increasing order of species susceptibility was: Red clover, alsike and alfalfa approximately equal while sweet clover was most susceptible.

comparisons expressed as difference between percentages of emergence of treated and untreated material.

Experimental Results

Tests 1 - 8 (alfalfa, white clover, red clover and sweet clover).

Test 1.

Within 7 days no seedlings emerged in the flats treated under greenhouse conditions with 50 and 1000 p.p.m. of 2,4-D amine. Emergence in the check flats was at a maximum at that time. Two weeks from the time of treatment seedlings had not emerged in the treated flats.

Test 2.

The emergence was reduced by treatments of both 10 p.p.m. and 0.5 p.p.m. of 2,4-D amine under greenhouse conditions as shown in Figure 1. This test also supplied data on the relative susceptibility of the different species to 2,4-D under the conditions of soil treatment. Table 9 illustrates these observations. From these data it is evident that when 10 p.p.m. was used the increasing order of species susceptibility was: Red clover, alfalfa and white approximately equal while sweet clover was most susceptible.

FIGURE 1

Effect of Soil Applications of 2,4-D amine on the Emergence of Legume Seedlings

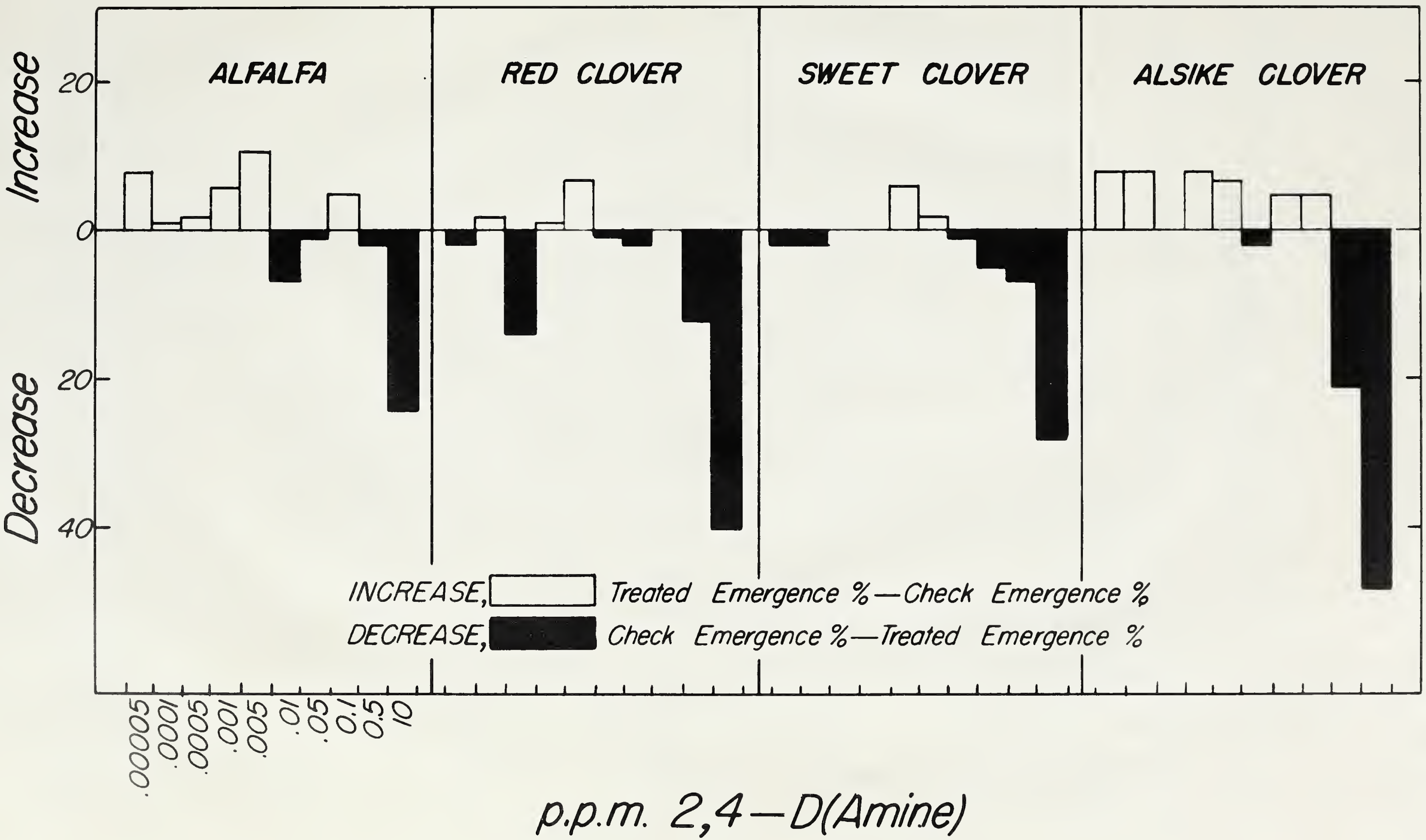


TABLE 9

Effects of 2,4-D amine on Emergence of Legume Seedlings

	Alfalfa	Sweet Clover	Red Clover	Alsike
Check mean emergence	30	28	56	61
10 p.p.m. mean emergence	6	0	16	13
Decrease in emergence	24	28	40	48
% decrease	80	100	71	79

Test 3.

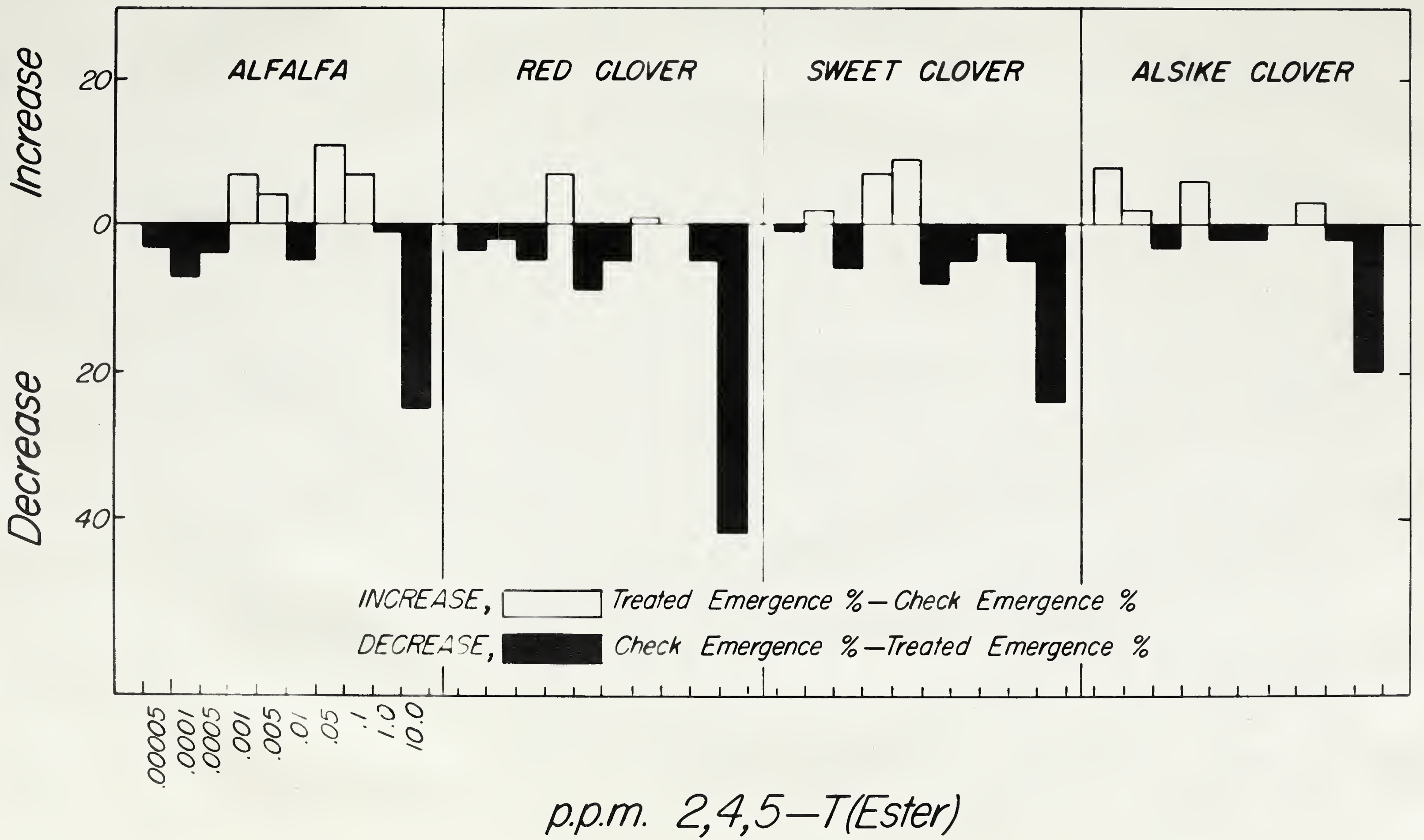
This test was performed outdoors with 4 replicates and 2 chemicals at concentrations of 0.01 and 0.001 p.p.m. The mean percentages of emergence for all species and chemicals following 0.001 p.p.m. applications were equal to or greater than the corresponding check means. These results are shown in Figures 1 and 2. Under the conditions of the trial an emergence stimulation range apparently existed in the vicinity of 0.001 p.p.m. for both 2,4-D amine and 2,4,5-T ester.

Test 4.

In order to more fully investigate the stimulation range concentrations of 0.005 and 0.0005 p.p.m. of 2,4-D amine,

FIGURE 2

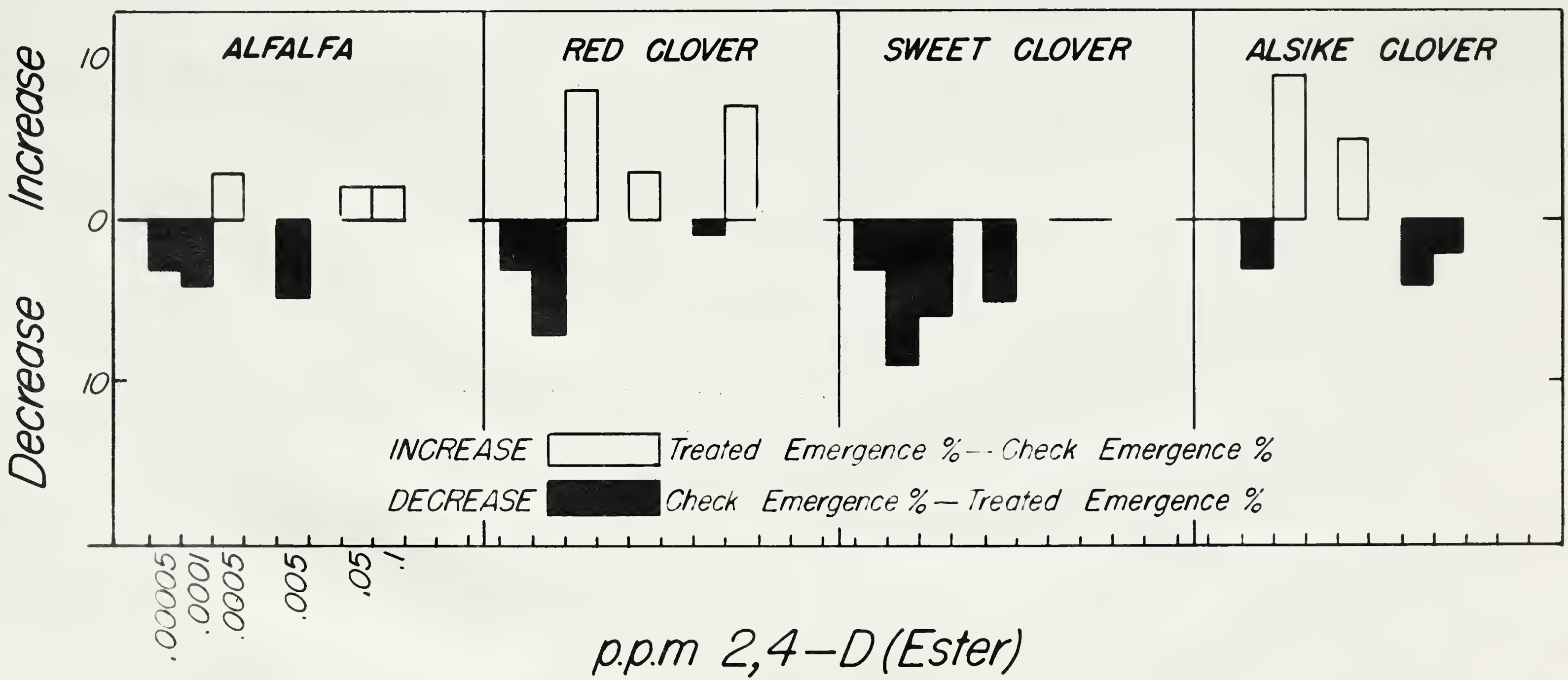
Effects of Soil Applications of 2,4,5-T ester on the Emergence of Legume Seedlings



2,4-D ester and 2,4,5-T ester were used. Concentrations of 0.005 p.p.m. of 2,4-D increased emergence of all species more than did 0.001 p.p.m. On the other hand, 0.0005 p.p.m. of 2,4-D had no effect on alsike and sweet clover but increased the emergence of alfalfa and decreased the emergence of red clover. Application of 0.005 p.p.m. 2,4,5-T ester increased emergence of alfalfa and sweet clover but decreased emergence of red clover and alsike while an application of 0.0005 p.p.m. 2,4,5-T decreased emergence of all species. A concentration of 0.0005 p.p.m. of 2,4-D ester increased emergence of alfalfa, red clover and alsike but decreased emergence of sweet clover. A concentration of 0.005 p.p.m. of 2,4-D ester slightly increased emergence in red clover and alsike, but decreased it in alfalfa and sweet clover. These data for 2,4-D ester suggest that if a rate of 0.001 p.p.m.had been applied the species may have responded in a similar manner as they did to 2,4,5-T ester at that concentration. Figure 3 shows the increases and decreases in mean emergences above or below the check mean resulting from applications of various concentrations of 2,4-D ester. Certain concentrations which were not included in the tests are necessarily omitted from the table, but corresponding spaces are left in the graph to avoid confusion when making inter-chemical comparisons of comparable rates of treatment.

FIGURE 3

Effects of Soil Applications of 2,4-D ester on the Emergence of Legume Seedlings



Test 5.

This test was first carried out in mid-July but heavy rains immediately after treatment necessitated covering the flats with tarpaulins. The seedlings became etiolated and died when they were exposed to the sun. The test was then repeated but rain interfered again, and the cover had to be left over the flats on the day following application of solutions. This confinement had a forcing effect on the seedlings and they began to emerge the next morning. Consequently, it is probable that the results obtained from this test are not comparable with those from previous tests. The results are included in Figures 1, 2 and 3.

Test 6.

This test was carried out in August using concentrations of 0.1 and 0.05 p.p.m. of all 3 chemicals. Sweet clover reacted normally in that emergence was not increased by either concentration of either growth regulator. The other species showed increases and decreases which did not seem to conform to the pattern indicated by the previous tests. Evidence of this erratic behavior is apparent in Figures 2 and 3. It was hoped that tests could be performed which would include all the concentrations of one chemical at one time thus eliminating the time factor. Owing to

Test 5.

This test was first carried out in mid-July but heavy rains immediately after treatment necessitated covering the flats with tarpaulins. The seedlings became etiolated and died when they were exposed to the sun. The test was then repeated but rain interfered again, and the cover had to be left over the flats on the day following application of solutions. This confinement had a forcing effect on the seedlings and they began to emerge the next morning. Consequently, it is probable that the results obtained from this test are not comparable with those from previous tests. The results are included in Figures 1, 2 and 3.

Test 6.

This test was carried out in August using concentrations of 0.1 and 0.03 p.p.m. of all 3 chemicals. Sweet clover reacted normally in that emergence was not increased by either concentration of either growth regulator. The other species showed increases and decreases which did not seem to conform to the pattern indicated by the previous tests. Evidence of this erratic behavior is apparent in Figures 2 and 3. It was hoped that tests could be performed which would include all the concentrations of one chemical at one time thus eliminating the time factor. Owing to early frosts, however, these tests could not be performed outdoors where they should have been done in order to justify comparisons with the results of other outdoor tests.

Test 7.

Lack of bench space in the greenhouse necessitated carrying out this test with only 3 replicates and 6 concentrations (0.0001, 0.001, 0.01, 0.1, 1 and 10 p.p.m.) of 2,4,5-T ester. Substantial increases over emergence of checks were found only with alsike treated with 0.001, 0.01, 0.1 p.p.m. as shown in Figure 4.

Since the effects of 10 p.p.m. of 2,4,5-T in Test 7 and 10 p.p.m. of 2,4-D amine in Test 2 were both investigated under greenhouse conditions it seemed justifiable to compare the effects of the 10 p.p.m. treatments on the emergence of the various species. Table 10 shows the effects of 10 p.p.m. of 2,4,5-T on the emergence of these species, and Table 11 summarizes the effects of 10 p.p.m. 2,4-D amine and 2,4,5-T ester thus showing the relative susceptibility of the 4 species to the 2 chemicals under the conditions of the tests. It is apparent that sweet clover was most susceptible to both chemicals and that alfalfa and alsike clover responded similarly to either chemical. Red clover on the other hand was most resistant of all species to 2,4-D but moderately susceptible to 2,4,5-T.

Effects of Soil Applications of 2,4,5-T ester on the Emergence of Legume Seedlings

TABLE 10

Effects of 2,4,5-T ester on Emergence of Legume Seedlings.

	Alfalfa	Sweet clover	Red clover	alsike
Check mean emergence	51	40	80	61
10 p.p.m. mean emergence	26	16	38	31
Decrease in emergence	25	24	42	30
% decrease	49	60	53	49

TABLE 11

Summary of Susceptibility of Legume Species to Soil Applications of 2,4-D amine and 2,4,5-T ester

Chemical and Concentration	% Decrease in Emergence			
	Alfalfa	Alsike	Red Clover	Sweet Clover
10 p.p.m. 2,4,5-T ester	49	49	53	60
10 p.p.m. 2,4-D amine	80	79	71	100

Test 8.

This test was planned to investigate the effects of concentrations of 2,4,5-T ester between 1 and 10 p.p.m. under greenhouse conditions as in Test 7. Figure 4 combines the results of Tests 7 and 8.

Discussion

The first two tests to study the effects of various concentrations of 2,4-D amine on the emergence of legume seedlings, indicated that concentrations of 0.5 p.p.m. or greater applied to the soil at planting time reduced the emergence of seedlings under greenhouse conditions. Emergence decreased with increasing concentration of chemical and a variation in susceptibility according to species was indicated. The greenhouse tests carried out the following winter with 2,4,5-T ester showed responses generally the same as from 2,4-D amine, but when concentrations between 1 and 10 p.p.m. were tested increases of 2 to 8 per cent in emergence were apparent about midway in this range while below and above this point emergence was generally reduced. These results are not explicable on the basis of the earlier observation that susceptibility increased with increasing concentration. Variation in the environment may have been partly responsible for the differences since

Test 8.

This test was planned to investigate the effects of concentrations of 2,4,5-T ester between 1 and 10 p.p.m. under greenhouse conditions as in Test 7. Figure 4 compares the results of Tests 7 and 8.

Discussion

The first two tests to study the effects of various concentrations of 2,4-D amine on the emergence of lettuce seedlings, indicated that concentrations of 0.2 p.p.m. or greater applied to the soil at planting time reduced the emergence of seedlings under greenhouse conditions. Emergence decreased with increasing concentration of chemical and a variation in susceptibility according to species was indicated. The greenhouse tests carried out the following winter with 2,4,5-T ester showed responses generally the same as from 2,4-D amine, but when concentrations between 1 and 10 p.p.m. were tested increases of 2 to 5 per cent in emergence were apparent about midway in this range while below and above this point emergence was generally reduced. These results are not explicable on the basis of the earlier observation that susceptibility increases with increasing concentration. Variation in the environment may have been partly responsible for the differences since the latter tests were carried out in December and January when growing conditions were greatly different from those in the previous March and April. It was very difficult to maintain suitable temperature in the greenhouse during the very cold weather in December and January. Growth was slow under the cold conditions, and the response to growth regulators likewise is different than when weather conditions are favorable for active growth (23). This might also partially explain why the lower concentrations (0.0001 to 1 p.p.m.) failed to stimulate emergence in all species except alsike clover. Alsike is naturally adapted to a moist, cool environment.

The results of the remaining tests, performed outdoors under more natural conditions, at first seemed to conform to a definite pattern. Generally concentrations of 0.01 p.p.m. of 2,4-D amine and 2,4,5-T ester depressed emergence while concentrations of 0.005 p.p.m. and 0.001 p.p.m. caused an increase in percentage emergence. Concentrations of 0.0005 p.p.m. were responsible for still less stimulation or in some cases a slight decline in percentage emergence. The weather throughout these two tests in May and June was hot, dry and sunny. Test 5 was carried out in July and August, but cannot be compared with the others as the seedlings were forced by abnormal conditions prevailing under the tarpaulin covers. The results of this test were included with the results of the other tests to demonstrate the latter tests were carried out in December and January when growing conditions were greatly different from those in the previous March and April. It was very difficult to maintain suitable temperatures in the greenhouse during the very cold weather in December and January. Growth was slow under the cold conditions, and the response to growth regulators likewise is different than when weather conditions are favorable for active growth (20). This might also partially explain why the lower concentrations (0.0001 to 1 p.p.m.) failed to stimulate emergence in all species except alsike clover. Alsike is naturally adapted to a moist, cool environment.

The results of the remaining tests, performed outdoors under more natural conditions, at first seemed to conform to a definite pattern. Generally concentrations of 0.01 p.p.m. of 2,4-D amine and 2,4,5-T ester decreased emergence while concentrations of 0.005 p.p.m. and 0.001 p.p.m. caused an increase in percentage emergence. Concentrations of 0.0005 p.p.m. were responsible for still less stimulation or in some cases a slight decline in percentage emergence. The weather throughout these two tests in May and June was hot, dry and sunny. Test 5 was carried out in July and August, but cannot be compared with the others as the seedlings were formed by abnormal conditions prevailing under the burlap covers. The results of this test were included with the results of the other tests to demonstrate more effectively the extent to which results are modified by environment. During Test 6 in late August and early September the nights were cooler, and the days not so hot as those during earlier experiments. In this test the behavior of the species was very erratic involving increases and decreases in emergence for which no explanatory generalization appeared possible.

The results have shown that a variation in susceptibility of the various species to the chemicals exists and also that environmental effects probably modify chemical effects to a large extent.

The tests also indicate that soil treatments intended to stimulate growth or increase emergence of alfalfa, sweet clover, alsike clover and red clover would serve no useful purpose on a field scale because the observed increases in emergence were so small and because there is, as yet, no possible way of being sure of stimulation from a particular concentration of chemical under varying environmental conditions.

Summary

1. Applications of 7 p.p.m. or greater of the amine formulation of 2,4-dichlorophenoxyacetic acid and of the butyl ester of 2,4,5-trichlorophenoxyacetic acid

definitely decreased emergence of alfalfa, alsike, red clover and sweet clover seedlings when applied as a pre-emergence soil treatment under greenhouse conditions.

2. The legume species varied in their responses to 2,4-D and 2,4,5-T. The order of increasing susceptibility to injury from 2,4-D at 10 p.p.m. was: red clover, alsike and alfalfa equal, and sweet clover most susceptible, whereas the order of susceptibility to 2,4,5-T at 10 p.p.m. was alfalfa and alsike equal, red clover next and sweet clover most susceptible.

3. The evidence of damage by applications of 0.5 p.p.m. or greater of 2,4-D and 2,4,5-T suggests inadvisability of post planting, pre-emergence treatments of small weeds which happen to emerge before the legume seedlings in newly planted fields.

SECTION III. Effects of Chemical Blossom - Sprays on Seed Set of Legumes

Materials and Methods

The following hormone herbicides were used in these investigations:

Chemical	Abbreviations Used
1. 2,4-dichlorophenoxyacetic acid (amine) Naugatuck.	2,4-D amine
2. 2,4-dichlorophenoxyacetic acid (ester) Green Cross.	2,4-D ester
3. 2,4,5-trichlorophenoxyacetic acid (ester) Naugatuck.	2,4,5-T ester
4. a-o-chlorophenoxyproprionic acid (formulation A 900) Dow.	A.O.C.P.

A Dobbins tank-type pressure sprayer was used in the following tests.

Test 1 (Red Clover). 1949.

In a 3 year old stand of Altaswede red clover, on the farm of H. McLaughlan at Spruce Grove, west of Edmonton, plots 2 1/2 feet by 9 1/2 feet were arranged in factorial design with 3 replicates. During the late flowering stage

the plots were sprayed at the rate of 50 gals. per acre with concentrations of 5, 15 and 50 p.p.m. of 2,4-D amine, 2,4,5-T ester and a mixture of both. Notes were taken 1 week after treatment and plots were harvested late in September. Yield data on threshed and cleaned seed samples were analyzed statistically.

Test 2 (Alfalfa), 1950.

Prior to the flowering stage 84 plots were arranged in factorial design, with 3 replicates, in a fairly uniform 1 year old stand of Grimm alfalfa at the University of Alberta. Plots were approximately 22.5 square feet in area. In each replicate chemicals 1, 2, 3 and 4 were applied, 2,4-D amine and A.O.C.P. at 1/16, 1/8, 1/4, 1/2, 1 and 2 ounces, and 2,4-D ester and 2,4,5-T ester at 1/32, 1/16, 1/8, 1/4, 1/2 and 1 ounce acid equivalent in 45 gallons of water per acre. A check plot was provided for each chemical in each replicate. Plots were sprayed five times at approximately weekly intervals throughout the flowering season. (July 3 - August 1st). Applications were made only on clear warm days. Visual affects of treatments were observed at intervals throughout the season.

At harvest time a rectangular iron quadrat 4 feet 4 inches by 5 feet or approximately 1/2000 of an acre was used to mark off the sampling area. After cutting, the alfalfa was dried in the field for 2 weeks, then threshed,

and weighed.

A study of the yield data showed that 5 plots on one side of the test had extremely high yields in comparison to the others. This was attributable to the fact that during the season the border on that portion of the test was accidentally removed during cultivation of the roadways. The yield values for these 5 plots were discarded and new ones calculated using the missing plot technique (15). Statistical analysis of the data was then carried out.

Test 3, 1950.

A duplicate of Test 2 was laid out in a 3 year old stand of Grimm alfalfa on the farm of K. McKenzie at Tiger Lily west of Barrhead. Due to a combination of circumstances these plots were sprayed only twice, during the flowering stage, with a ten day intervening period between sprays. The plots were harvested in early September, and the samples dried indoors at the University of Alberta. The samples were subsequently threshed, cleaned, and weighed, and the yield data analyzed statistically.

Experimental Results

Test 1 (Red Clover), 1949.

Three days after the spray treatments of red clover twisting of plants was general throughout the treated plots

Using extent of twisting as a criterion 2,4,5-T was most effective and 2,4-D amine least injurious. At 50 p.p.m. 2,4-D amine appeared to have an effect equal to that of 2,4,5-T at 15 p.p.m. The variance analysis of the yield data for test 1 is found in Table 12. It shows that the treatments had no significant effect on seed yield. This result however, may have been partially attributable to an early frost in the fall of 1949 which caused damage to immature red clover seeds. A duplicate experiment was planned for 1950 but much of the red clover winter killed and no fields suitable for experimental purposes were located.

TABLE 12

Variance Analysis of the Seed Yield Data for Red Clover

Source of Variation	Degrees of freedom	Sum of Squares	Mean Squares	F. Value
Chemicals	2	112.2	56.10	0.54
Replicates	2	247.4	123.70	1.18
Error (1)	4	418.7	104.68	
Treatments (rates)	3	42.2	14.07	0.92
Chemicals x Treatments	6	128.6	21.43	1.40
Error (2)	17	263.8	14.66	
Total	34			

Tests 2 and 3 (Alfalfa), 1950.

Alfalfa plots at the University of Alberta were compared at the time of the fourth treatment. Table 13 summarizes observations concerning flowering behavior and pod production of the plants. At the time the notes were recorded it was quite evident that both esters had approximately the same effect at equal concentrations, and also that twice as great a concentration of amine was required to produce an effect equal to that of the ester. Apparently sprays containing greater than 1/8 ounce 2,4-D amine or 1/16 ounce of 2,4-D ester and 2,4,5-T ester delayed abscission of flowers. Later in the season these flowers, after withering, dropped from the plants. Such behavior was especially noticeable in the plots treated with 1 and 2 ounces of acid equivalent per acre.

The effects of the chemical treatments on the yields of these plots are shown in Figures 5 and 6. The difference between the mean yields of treated and check plots was calculated as per cent increase or decrease over the control. The bar graphs compare the per cent increase or decrease for each concentration at both Edmonton and Tiger Lily.

The variance analysis of the alfalfa yield data for the Edmonton plots is shown in Table 14 and that for the Tiger Lily plots in Table 15. The F values indicated

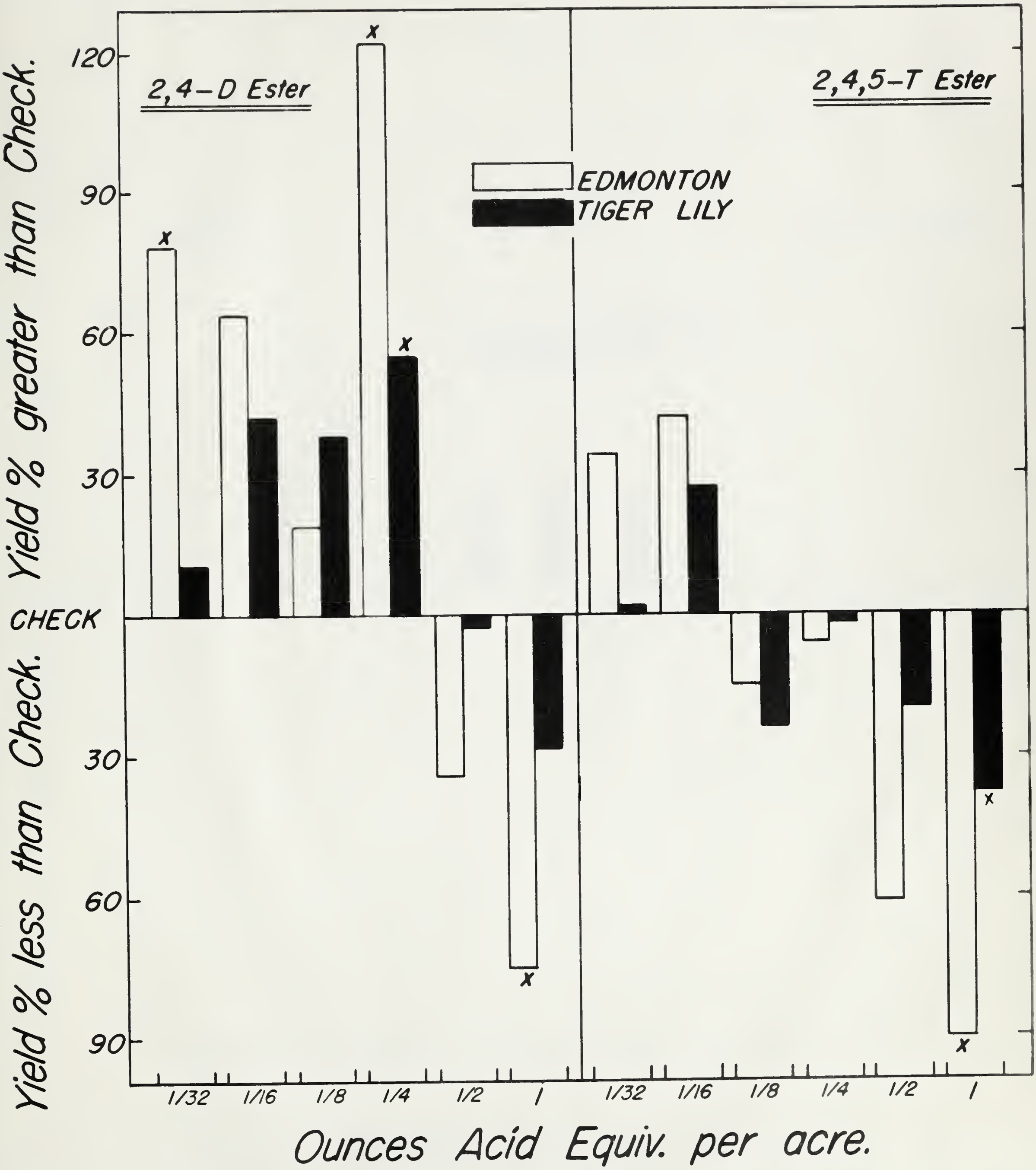
TABLE 13

Summary of Effects of Blossom Sprays on Topgrowth of Alfalfa

Treatment	Rate ounces/acre	Leaf chlorosis	Stem distortion	Fresh flowers	Withered flowers	Pod set
Untreated		-	-	numerous	few	good
2,4,5-T ester) 2,4-D ester) 2,4-D amine	1) (2)	partial chlor- osis	twisting & thickening	trace	numerous	poor
2,4,5-T ester) 2,4-D ester) 2,4-D amine	1/2) (1)	nil	twisting & thickening	few	many	fair
2,4,5-T ester) 2,4-D ester) 2,4-D amine	1/4) (1/2)	nil	nil	few	many	good
2,4,5-T ester) 2,4-D ester) 2,4-D amine	1/8) (1/4)	nil	nil	many	many	good
2,4,5-T ester) 2,4-D ester) 2,4-D amine	1/16 & 1/32) (1/8 & 1/16)	nil	nil	numerous	few	good
A.O.C.P.	2	nil	nil	many	many	good
A.O.C.P.	1, 1/2, 1/4, 1/8, 1/16	nil	nil	numerous	few	good

FIGURE 5

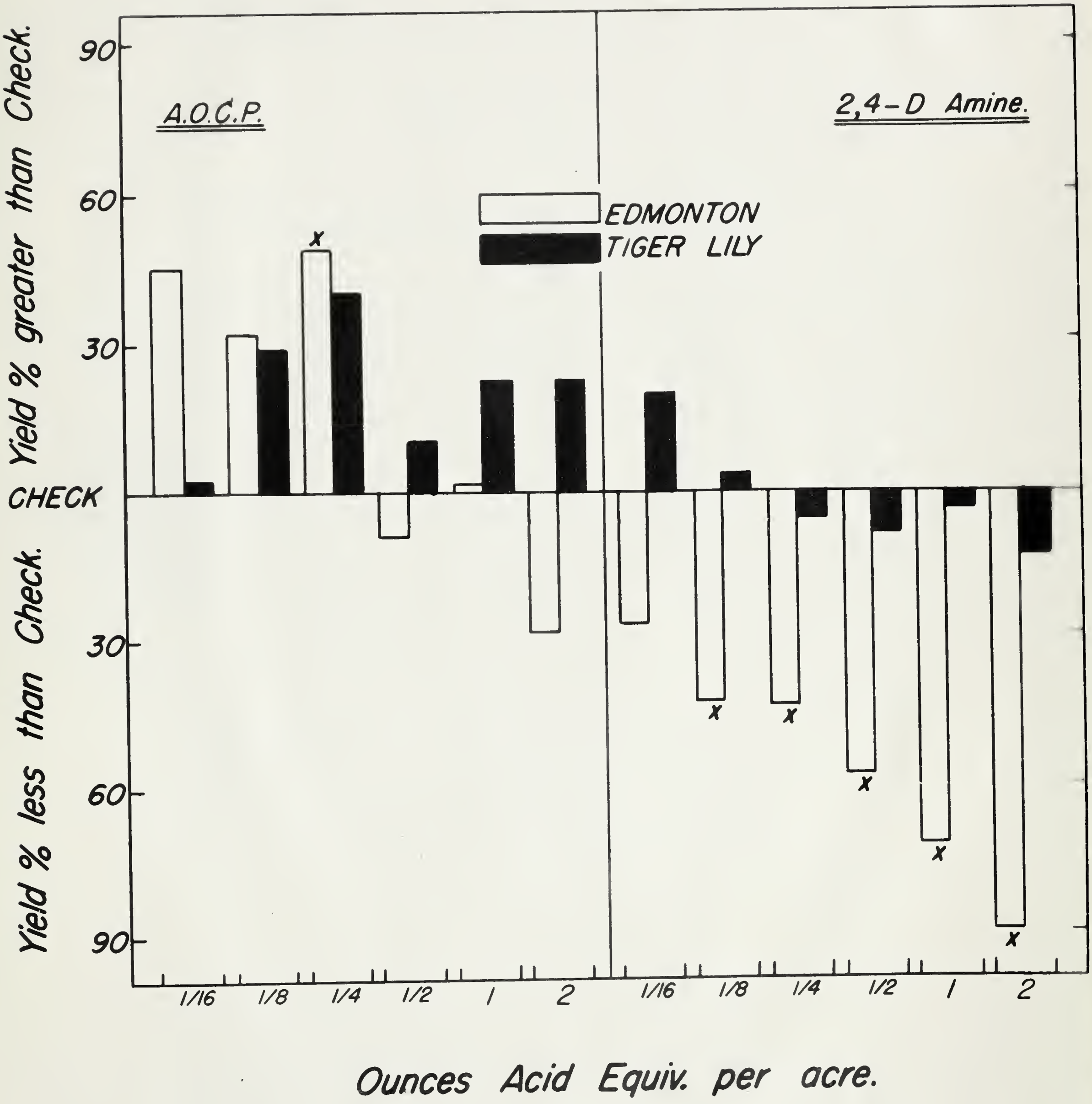
Effects of Blossom Sprays on Alfalfa Seed Yield



* Yields significantly different from controls.

FIGURE 6

Effect of Blossom Sprays on Alfalfa Seed Yield



* Yield significantly different from controls.

that mean yield differences due to treatments were significant to the 1 per cent level at Edmonton, and to the 5 per cent level at Tiger Lily.

TABLE 14

Variance Analysis of Alfalfa Seed Yield
Data for Edmonton Plots

Source of Variation	Degree of freedom	Sum of Squares	Mean Squares	F. Value
Chemicals	3	648.1	216.0	2.9
Replicates	2	1118.7	559.4	7.5*
Error (1)	6	448.0	74.7	
Treatments (rates)	6	1448.6	248.7	7.0**
Chemicals x Treatments	18	643.0	35.7	2.5**
Error (2)	43	624.4	14.5	
Total	78			

* Significant at the 5 per cent level.

** Significant at the 1 per cent level.

M.S.D. for treatments = 6.2 grams per plot.

TABLE 15

Variance Analysis of Alfalfa Seed Yield
Data for Tiger Lily Plots

Source of Variation	Degree of freedom	Sum of Squares	Mean Squares	F. Value
Chemicals	3	669.4	223.1	3.2
Replicates	2	1831.3	915.7	13.3**
Error (1)	6	412.6	68.8	
Treatments (rates)	6	1925.5	320.9	3.1*
Chemicals x Treatments	18	2988.9	166.1	1.6
Error (2)	48	4916.8	102.4	
Total	83			

* Significant at the 5 per cent level.

** Significant at the 1 per cent level.

M.S.D. for treatments = 16.5 grams per plot.

Table 16 shows a comparison of the significant results obtained from these analyses. Apparently 1 ounce per acre of 2,4,5-T, whether applied twice or five times to the alfalfa plants, was sufficient to cause significant decreases in yield and similarly applications of 1/4 ounce of 2,4-D ester per acre when applied either twice or five times increased yield significantly. In certain cases where significant increases or decreases were obtained only at Edmonton and not at Tiger Lily there must have been a cumulative effect from the 5 treatments.

TABLE 16

Summary of Rates of Chemical Applications Significantly Affecting Yield of Alfalfa at Edmonton and Tiger Lily.

	EDMONTON		TIGER LILY	
	Rates (ounces/ acre) causing significant yield decrease	Rates (ounces/ acre) causing significant yield increase	Rates (ounces/ acre) causing significant yield decrease	Rates (ounces/ acre) causing significant yield increase
2,4-D amine	1/8,1/4,1/2,1,2	nil	nil	nil
2,4-D ester	1	1/32, 1/4	nil	1/4
2,4,5-T ester	1	nil	1	nil
A.O.C.P.	nil	1/4	nil	nil

Discussion

Since the chemical was applied late in the flowering stage of Altaswede red clover and the first killing frost was 3 weeks later, it is probable that the seeds produced by the flowers, fresh at the time of the treatment, were immature when the frost occurred, and hence damaged by it. Shrivelled seeds were noted during cleaning of the samples so it is quite possible that any potential effect of the chemical treatments on the seed yields was masked by the frost. Sokoljskaja (33) reported increases in seed yield following sprays of 0.001 per cent of 2,4-D, but the amount of application was not stated. Further tests using various rates at various gallonages applied at various times throughout the flowering stage must be carried out before final decisions regarding effects of such chemicals on seed set of red clover can be made. Such tests would have been undertaken in 1950 at the University of Alberta had the red clover not been winter killed.

The tests carried out in 1950 using successive spray applications of 2 ounces or less of 2,4-D amine and ester, 2,4,5-T and A.O.C.P. per acre produced various results with alfalfa at University of Alberta. Where twisting and thickening of stems was noted in the 2,4-D and 2,4,5-T plots it was found later that the same applications had caused considerable reductions in seed yield. This was

Discussion

Since the chemical was applied late in the flowering stage of alsike and red clover and the first killing frost was 3 weeks later, it is probable that the seeds produced by the flowers, fresh at the time of the treatment, were immature when the frost occurred, and hence damaged by it. Shrivelled seeds were noted during cleaning of the samples so it is quite possible that any potential effect of the chemical treatments on the seed yields was masked by the frost. Rokitnicka (53) reported increases in seed yield following sprays of 0.001 per cent of 2,4-D, but the amount of application was not stated. Further tests using various rates at various chemicals applied at various times throughout the flowering stage must be carried out before final decisions regarding effects of such chemicals on seed set of red clover can be made. Such tests would have been undertaken in 1960 at the University of Alberta had the red clover not been winter killed.

The tests carried out in 1960 using successive spray applications of 1 ounce or less of 2,4-D amine and ester, 2,4,5-T and M.C.P. per acre produced various results with alfalfa at University of Alberta. Where bending and thickening of stems was noted in the 2,4-D and 2,4,5-T plots it was found later that the same applications had caused considerable reductions in seed yield. This was attributable to a lethal effect of the chemical on many of the flower buds.

The graph for 2,4-D at Edmonton, Figure 6, appears to give an abnormal illustration of the effect of this chemical on yield. The mean yield for the check plots in the 2,4-D block was unusually high in comparison with the other check means. If there had been more replicates in this test the mean check yield would probably have been smaller, and hence more in line with that of comparable untreated material. In this event the graph would have resembled that for Tiger Lily plot yields.

It seems highly probable that, since retention of flowers on tobacco has been shown to be a hormone phenomenon (17), hormones applied at the right concentration might cause the retention of the flowers on alfalfa. In the observations at Edmonton it was apparent that faded flowers were retained on the treated plants for a much longer period than on the checks. These flowers might have been killed and their abscission process thus destroyed or there may have been a hormonal effect, due to the applied chemical, preventing the formation of the abscission layer. If the latter was the case it seems possible that the flowers which were retained were in a condition receptive to pollen for a longer period than normal and hence their opportunity for pollination increased as compared with that of the untreated flowers. As a result increased seed set could be expected. Since 1/4 ounce of 2,4-D ester per acre increased seed yield significantly attributable to a lethal effect of the chemical on some of the flower buds.

The graph for 2,4-D at Edmonton, Figure 6, appears to give an abnormal illustration of the effect of this chemical on yield. The mean yield for the check plots in the 2,4-D block was unusually high in comparison with the other check means. If there had been more replicates in this test the mean check yield would probably have been smaller, and hence more in line with that of comparable untreated material. In this event the graph would have resembled that for Vigor Lily plot yields.

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A.O.C.P. seems to show promise of being able to increase seed yield of alfalfa. At Tiger Lily yield was increased by all rates of this chemical applied only twice during flowering, while at Edmonton, where plots received 5 applications, yield was increased by the lower rates and decreased following the higher rates of application. One of the increases at Edmonton, a rise of 49 per cent, was statistically significant. Possibly 3 or even 4 applications instead of the 5 actually applied might have been sufficient to give increases more significant than those observed.

More work will have to be done before definite conclusions can be drawn. Plot size and number of replications should be increased to eliminate variation which is common in perennial crops. If it is possible to consistently increase seed set by these treatments, the minimum number of treatments necessary for optimum increases should be ascertained so that as little as possible mechanical damage would occur to the crop as a result of the operation of standard field spray equipment.

Field spray equipment should be used in further experiments because coverage by such sprayers is not as thorough as with hand sprayers. Use of a fog type spray might possibly be more effective than a high gallonage spray for this type of work. Probably large droplets from regular spray equipment contain more than enough solution to cause the flowers to remain on the plant, and may because of this possible excess damage the flower. A fog spray might provide better coverage of a greater number of flowers by volumes just sufficient to have the desired hormonal effect on the flower. It is known that pollen contains a hormone and also an enzyme system which can release a hormone from its bound form in the tissue of the pistil as the pollen tubes grow. This hormone initiates growth in the ovary, and since some of it migrates to the base of the pistil it is believed that part of it moves into the pedicel of the flower to prevent the formation of the abscission layer (17). This layer ordinarily forms when flowers are not fertilized and have outlived their natural life on the plant. The amount of hormone released by or stimulated into action by the pollen would be relatively small, and hence it is felt that a fog type application of synthetic hormone, intended to produce a comparable effect, might be most suitable for this type of work.

Future investigations should also include caged plots or portions of plots from which insects would be excluded to make possible determinations as to whether or not

any increases in seed yield of chemically treated plots are due to selfing or to crossing. If increases in seed yield were found to be due to selfing then progeny tests would have to be carried out to determine whether or not there was a loss of vigor due to chemically induced self pollination. If there was a loss of vigor it would not be in the interests of agriculture to perfect a method of increasing seed set by these means.

If there was no loss of vigor or if increases were due to cross pollination, and if techniques could be developed satisfactorily costs would appear not to be the limiting factor. Under conditions in Alberta an increase in seed yield of only 20 pounds per acre would be sufficient to meet the cost of operation for 4 successive sprays with 2,4-D at rates that produced increases in the foregoing tests. Work of this type would have to be very accurate since, on the basis of available results, the margin between rates which increase and those which decrease yield appears to be very narrow. For example; 1/4 of an ounce of 2,4-D ester per acre gave a significant increase in yield, but 1/2 ounce per acre reduced yield and 1 ounce per acre significantly decreased yield of alfalfa in the tests at Edmonton.

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Summary

1. Alfalfa plants treated during flowering stage with successive applications of the esters of 2,4-dichlorophenoxyacetic acid and of 2,4,5-trichlorophenoxyacetic acid at rates of 1/8 to 1 ounce of acid equivalent in 45 gallons of water per acre or with 2,4-D amine at 1/4 to 2 ounces per acre retained their flowers for longer times than did those which were untreated.

2. Successive sprays of 1/4 ounce acid equivalent of 2,4-D ester in 45 gallons of water per acre applied during flowering stage of alfalfa increased seed yield significantly both at Edmonton and at Tiger Lily. The increases were 122 per cent and 55 per cent respectively.

3. Successive sprays of 1 ounce acid equivalent of 2,4-D ester in 45 gallons of water per acre applied during flowering stage decreased seed yield of alfalfa significantly both at Edmonton and at Tiger Lily. The decreases were 75 per cent and 28 per cent respectively.

4. At Tiger Lily all rates from 1/16 to 2 ounces acid equivalent of a-o-chlorophenoxyproprionic acid in 45 gallons of water per acre increased seed yield whereas

at Edmonton only rates from 1/16 to 1/4 ounce increased seed yield. Of these increases only 1/4 ounce per acre at Edmonton caused a statistically significant increase in yield.

5. Practical applications of the results have been discussed.

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