CLIMATIC FACTORS AFFECTING HERBICIDAL ACTIVITY OF SODIUM TRICHLORACETATE IN DIFFERENT SOILS

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A thesis submitted to The University of New Zealand in partial fulfillment of the requirements for the Degree of Master of Agricultural Science (Horticulture).

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INTRODUCTION

Trichloracetic acid (T C A) has long been known as a protein precipitant, but it was not until 1947 that research workers in the U.S.A. found it to be an effecive grass killer. Immediately it was subjected to a considerable amount of experimentation, but it is only since 1950 that any trials have been conducted with this herbicide in New Zealand.

Most of this early research was of an observational or emperical nature, and the results obtained were often inconsistent. However, it was soon determined that there was little downward translocation of T C A when foliar applications were made, and that for maximum kill it was essential for the herbicide to come into contact with the grass roots. Best control of couch (*Agropyron repens* L.), for example, has been obtained when the T C A was sprayed on the upturned sod and light rain fell within a few days after application.

Before commerical usage of any newly developed herbicide is recommended on agricultural land it is desirable to know the fate of that herbicide when applied to the soil, whether it will persist and be cumulative so that subsequent crops will be effected, if a short period of residual activity can be expected, or if the compound is rapidly dissipated. To this writer's knowledge, no attempt had been made in New Zealand to undertake a quantitative study of the effects soil type, temperature and rainfall have on the rate of inactivation and distribution of T C A when applied to the soil. Such an investigation therefore seemed pertinent, and more especially because results of similar studies overseas were not in full agreement.

The published reports showed that both chemical and biological tests had been employed to determine the concentrations, or relative amounts of T C A in the soil, but in no instance had the two methods been employed for the one experiment. It was therefore considered that in a future investigation some useful purpose would be served by a comparison of results obtained by both tests.

REVIEW OF LITERATURE

The first published report of the herbicidal potential of T C A was by Ryker (43) in 1947 at the Fourth Annual North Central (U.S.A.) Weed Control Conference. He claimed that T C A had given encouraging results when applied as a pre-emergence treatment for the control of grass weeds in broadleaf crops.

According to McCall and Zahnley (22), the grass killing properties of T C A were first discovered by E.I. du Pont de Nemours and Company, Inc. Their tests had shown that ammonium trichloracetate was useless as a general contact herbicide for it produced only superficial damage to some of the broad-leaved weeds on which it was applied, but it completely killed all grass plants against which it was tested, including barley, wheat, corn, couch (_Agropyron repens_ L.) and Bermuda grass (_Cynodon dactylon_ (L.) Pers.).

Residual Activity

McCall and Zahnley (22) concluded from their experiments that the control of perennial and annual grasses could be obtained with acre rates of 80 to 150 lb. and 20 to 60 lb. T C A respectively. They found that soil sterility was severe but temporary, toxicity disappearing within 30 to 90 days depending upon the amount of rainfall. Similar figures were quoted by Lynch (21). The North Central (U.S.A.) Weed Control Conference Research Committee of 1948 (27) reported that the residual toxicity of T C A persists for 30 days or more, depending upon precipitation, while Barrons (2) suggests 60 to 90 days as the maximum period. Much longer residual action was reported by Arakeri and Dunham (1). Under the conditions of their experiments T C A persisted in lethal amounts to soybeans for about 120 days and it remained in smaller quantities for at least another 90 days. Normal growth of spring planted crops on land treated with T C A the previous autumn, to control perennial grasses, was demonstrated by the Dow Chemical Company (11) but Carder (8) claims that in Western Canada the effects of 50 lb. or more of T C A per acre applied in the autumn may last well into the following spring.

As the result of some Australian work, Green (17) considers that T C A breaks down rapidly in the soil, but it is advisable to wait 60 to 90 days before replanting, and a full year's weathering may be necessary before extremely sensitive plants could be planted.

Turnquist (47) observed no differences in growth and yields of spring sown sweet corn, onions, beans, peas, cabbage, cauliflower and tomatoes, on land that had received 38 to 114 lb. T C A per acre the previous autumn. Summer applications of up to 200 lb. T C A were found by Pavlychenko (33) not to impair the germination and normal development of spring sown cereals.

Extreme residual effect has been reported by Robinson and Dunham (42). T C A applications ranging from 11 to 176 lb. per acre were made by them to an old sod of Agropyron repens in September, 1948. Soybeans planted in February, 1949 were killed or severely injured by all rates of application. A second planting in 1950 was materially reduced in yields on the 48 to 176 lb. plots. It was not until the following year that the latter plots produced symptomless crops.

Temperature Effects

The shortest period of residual activity of T C A has been reported by Loustalot and Ferrer (20). They found that soil treated at the rate of 30 lb. per acre and subsequently stored at 45°C was devoid of toxicity at the end of 2 weeks, whereas toxicity persisted for at least 2 months when the soil was similarly treated and stored at 10°C. Ogle and Warren (29) also demonstrated that breakdown of T C A was proportional to the temperature and concluded that long residual control could not be expected in humid regions.

Ebell (14) has found TCA most effective under West Canadian conditions when applied in the late autumn. Cold weather and frozen soil does not appear to permit appreciable decomposition or leaching of TCA during the winter months yet the chemical penetrates to seedling root depth where it is most effective against early spring weed growth. Because of the slow rate of breakdown under those conditions, Ebell states that TCA must be used in the summerfallow year to allow ample time for residual effect to disappear before a crop is planted.

For similar reasons the North Central (U.S.A.) Weed Control Conference Research Committee of 1953 (28) consider that in sub-humid regions the residual effect of the late summer or autumn applications of TCA can be expected to extend into the growing season following treatment.

Soil Type Effects

Loustalot and Ferrer (20) observed that TCA persisted longer in clay soil than in sandy soil or a sandy-clay mixture. Jary (18) reported that there was a tendency for the control of *Agropyron repens* from any particular dosage to be better on heavy soils than on light ones, while McCall and Zahnley (22), and research workers of the Dow Chemical Company (11) found the reverse to be so. Matthews (24) considers that the efficiency of TCA is impaired on heavy soils. Lynch (21) emphasises the importance of soil type on the action of TCA and states that heavy soils require heavier rates of this herbicide to give a similar result to lighter rates in sandy soils.

Ogle and Warren (29) demonstrated that the rate of breakdown of TCA increased progressively from sandy soil to silt loam to muck soil.* Because of this slow breakdown in mineral soils they contended that a long residual effect might be expected in arid and semi-arid regions. Barrons and Hummer (3) likewise demonstrated that the inactivation

*- Muck soil is American equivalent for peat.

of TCA was greater in a soil high in organic matter than one of low organic content, but Rai and Hamner (38) found the reverse to obtain. The latter observed that in sandy and clay loams dissipation of TCA, as shown by wheat yields, was complete after 64 days whereas injury occurred even after 108 days in muck soil.

Parker (31) in his review of chemical weed control in sugar beet states that the activity of salts of TCA is greatest in the least organic soils. Blough and Fults (6) experienced some damage to sugar beets grown in sandy soil treated with TCA at rates as low as 5 lb. per acre but detected no injury to this crop grown in a loam treated at 15 lb. per acre. More recently Blough and Fults (7) have reported that in the same geographical and climatic areas the selective action of TCA is variable between soil types. In support of these field observations they have demonstrated in the glasshouse that on a medium textured loam relatively high in organic matter, selective control of annual grasses without crop injury was obtained with TCA at 15 lb. per acre, whereas non-selective phytotoxicity resulted at this and lesser rates when applied to a loamy fine sand and a silty clay loam, both of which were deficient in organic nitrogen. They suggest as one possible explanation that a substantial portion of the herbicide may be adsorbed by the organic colloids in the case of the loam. Rai and Hamner (38) support this theory, but Peters (37) reports that there is no evidence of TCA fixation by soil colloids.

Moisture Effects

Loustalot and Ferrer (20) found that TCA persisted for 2 months in soil with moderate moisture, a greater period than that in saturated soil, and that toxicity did not decrease with time as long as the soil remained air dry. Lynch (21) observed that TCA did not give satisfactory control of grasses on land subject to frequent flooding.

It is generally agreed that the moisture content of the soil has an important influence upon the effectiveness of soil applications of TCA, best control of grass weeds having been obtained when the soil is moderately moist. (2, 4, 5, 12, 16, 17, 22, 24, 50). Ten to twenty pounds of TCA per acre is usually sufficient to control seedling grasses, but Barrons and others (4) stress the importance of adequate soil moisture for control at these rates. They point out that if TCA is applied to soil so dry that the surface does not become moist at night through capillary movement, much of the herbicide will remain ineffective on the surface and though subsequent rains may dissolve it, the grass weeds may meanwhile have reached appreciable size and require much greater rates for control.

Moore and Myers (25) considered that the moisture condition of the soil prior to treatment with TCA did not influence the control of deep rooted Johnson grass (*Sorghum halepense* (L.) Pers.)

Rainfall Effects

Arakeri and Dunham (1) concluded that of the factors they studied, rain had the most important influence over results with TCA. Soybeans they sowed in plots given a preemergence spray of TCA, germinated and grew normally when protected from rain, but immediately they were exposed to rain plant injury occurred, and increased in intensity as the season progressed and more rain fell. Where water was added after TCA application the crop was a complete failure.

Moore and Myers (25) also demonstrated conclusively the importance of adequate rain shortly after TCA applications for effective control of *Sorghum halepense*. They obtained excellent control with 200 lb. per acre when rain in excess of one inch fell within 14 days after treatment, whereas three times this dosage was ineffective when no rain exceeding 0.10 inches fell until 55 days after treatment.

Many other workers (2, 4, 11, 12, 16, 17, 30, 37) have reported superior control of grass weeds when rain fell within a few days of TCA application, but that excessive rains following treatment has resulted in inferior control, especially of shallow rooted species such as *Agropyron repens*, for much of the herbicide is leached below the zone of root growth, particularly on porous soils. One report (11) states that in light sandy soil a prolonged heavy rain can almost completely leach TCA from the soil. Barrons et al (4) and other (21, 22) consider that the adverse effects of soil moisture extremes are less likely on heavy to medium textured soils than they are on light sandy soils.

It is the opinion of Lynch (21) that a considerable fall of rain is essential subsequent to TCA applications for effective herbicidal activity. Peters (37) studied rainfall data in connection with his experimental control of *Agropyron repens* with TCA and concluded that 1.5 to 3 inches of rain followed by several days of little or no rainfall resulted in good control. Matthews (24) considers that on heavy soils up to 1 inch of rain is required for activation of TCA.

Arakeri and Dunham (1) found that small quantities of TCA did not disappear more rapidly in 2 inches of soil leached with 4 inches of water than in unleached soil. Loustalot and Ferrer (20) determined that with no rain TCA remained in the upper 2 inches of soil, $\frac{1}{4}$ inch rain moved it down into the fourth inch of soil and $\frac{1}{2}$ inch or 1 inch caused it to move to at least the eight inch depth.

Ogle and Warren (29) applied a small amount of TCA to the surface of different soils and determined at which depth the greatest concentration of the herbicide occurred after leaching with 2 inches of rain. These were found to be 2, 4 and 6 inches for silt loam, muck soil and sandy soils respectively. Their leaching studies showed that in sandy soils most of the TCA moved out with 2 inches and all with 4 inches of water. Likewise in the muck soil all the toxicity disappeared with 4 surface inches of water, but slightly more was required for complete leaching of the TCA from the silt loam. They concluded that soil type had little effect on the movement of TCA, but the writer considers their results show a definite soil type effect.

Action of TCA

Barrons (2) observed that more TCA was required for a given result on very fertile soil, and with the knowledge that TCA is utilized as a protein precipitant, postulated that its action may be related to nitrogen absorption and metabolism. However, as pointed out by Woodford (51), the concentration at which trichloracetic acid affects plant tissue growth is well below the concentration required for the precipitation of plant proteins. Woodford suggested that TCA had a much more subtle effect on plant metabolism, and was probably associated with auxin controlled growth processes.

Tibbits and Holm (46) were unable to determine whether the tolerance of many dicotyledons to TCA is associated with an ability of those plants to prevent absorption and translocation of the chemical to meristematic tissues, or whether the lack of injury may be due to an early metabolism of the compound.

The exact nature of the action of TCA is still unknown.

Effect of TCA on Soil Micro-organisms

Kratochvil (19) treated small samples of silt loam with eight herbicides and determined the effect on soil micro-organisms by measuring the reduction in evolved gas. TCA caused the greatest depression in microbial activity. Data is not given in respect of the toxicity period.

Use of TCA

The differences in susceptibility to TCA between certain crop plants and grass weeds has enabled the weeding of several crops with this herbicide (4). *Agropyron repens* has been controlled in areas planted with asparagus, cabbage and cauliflower; annual grasses have been controlled in linen flax and established lucerne. Pre-emergence applications of TCA have been used to control annual grasses in sugar and red beets, gladiolus, asparagus, potatoes and cruciferous crops, (4, 21, 36).

MATERIALS AND METHODS

Soil of three distinct types, namely, Makarua peaty loam, Manawatu silt loam, and Foxton loamy sand, was obtained from cultivated fields in the Manawatu District for the experiments.

Tilled soil was selected because numerous experiments had shown that cultivation in conjunction with TCA applications enhanced the herbicidal action by rapidly bringing the chemical into close contact with the grass roots. Furthermore, it is more often on arable, and horticultural land in particular, that grass weeds are a serious problem and measures directed at their control are adopted.

All soils were rubbed through a sieve of ¼ inch mesh which was sufficient to eliminate clods without pulverising them too much. Thorough mixing of each soil was then effected to ensure homogeneity.

Sodium trichloracetate ($CCl_3.COONa$) was employed for all the experiments since this salt has been found the most satisfactory formulation of trichloracetic acid for herbicidal purposes, and is available commercially. It is highly soluble in water, (152 gms/100 gms. H_2O), and therefore is usually applied as an aqueous solution. It has the disadvantage of being hygroscopic and is slightly corrosive to iron, zinc and aluminium. The rates of application mentioned are not calculated as the acid equivalents but refer to the technical grade product used which had an active ingredient of 90% sodium trichloracetate.

Hereafter the abbreviation "TCA" refers to sodium trichloracetate.

Methods of Determination of TCA Concentration in Soil

In the literature reviewed, Barrons and Humner (6) were the only investigators to employ a chemical test for quantitative determinations of trichloracetic acid in the soil. All others used plant indicator methods which have the advantage of possibly detecting any effects of breakdown products. It therefore seemed pertinent to utilize both techniques and assess their merits for future work.

Selection of Plant Indicator

Preliminary research in New Zealand had shown that in heavy soils upwards of 80 lb. TCA per acre was required for good control of *Agropyron repens*, while 60 lb. had achieved the same degree of control in lighter soils. Most seedling grasses were readily killed with as little as 10 lb. TCA per acre (27).

It was thus apparent that the acre rates at which TCA was likely to be used commercially extended over a wide range and it was considered that the abovementioned limits should be exceeded in any experimental work undertaken.

The problem then was to select a plant which would give an even gradation of growth when sown in soils treated with TCA at rates varying from 5 to 100 lb. per acre. The extent of the experiments contemplated required further that the test plant be one that germinated and grew rapidly with minimum attention. The common cereals fulfilled these requirements and their erect habit of growth commended their use.

Barrons and Hummer (3) had classified wheat and 14 other cereals as susceptible to TCA. Oat was the only cereal in their tests that showed some tolerance to this herbicide. Robinson and Dunham (42) grew the undermentioned crops in soils containing residues of TCA and found their tolerance to be in the following descending order - flax, oats, corn, barley, wheat and soybean.

Pavlychencho (32, 35) treated plots of spring sown cereals with pre-emergence applications of TCA and obtained the results shown in Table 1.

Cereal \ TCA (lb./acre)		5	10	15	20	25
Wild Oats (<u>Avena fatua</u>)	1950	73	-	66	-	46
Oats (<u>Avena sativa</u>)	1950	72	-	82	-	69
	1951	100	62	60	35	-
Barley (<u>Hordenm vulgare</u>)	1950	96	-	17	-	2
	1951	0	16	0	0	-
Wheat (<u>Triticum vulgare</u>)	1950	4	-	0	-	0
	1951	0	0	0	0	0
Spring Rye (<u>Secale cereale</u>)	1950	94	-	58	-	34

Table 1. Plant dry weights expressed as percentages of controls.

Foster (15) obtained 25, 50 and 91% control of wild oats in flax treated with 10, 20 and 40 lb. TCA respectively per acre.

Clearly, wheat and barley were unsuitable as the experimental plant indicator and the choice therefore lay between oats and rye. Seed of wild oats was not available and the growth habit of rye ruled it out.

<u>Oat Varieties Tested</u>

(a) <u>Tolerance to TCA</u>

Samples of nine varieties of oats were obtained and their tolerance to several rates of TCA determined. Six seed trays, 13" x 10" x 5", were filled with a good loam which had been sieved and thoroughly mixed to ensure homogeneity. Ten seeds of each oat variety were sown in rows 1 inch apart in each tray with two border rows of Garton oats either end. The trays were then adequately watered and subsequently sprayed with an aqueous solution of TCA, one each at the following rates —— 5, 10, 15, 30, 50 lb. per acre. The sixth was left untreated as the control.

After a growth period of 14 days the height of each

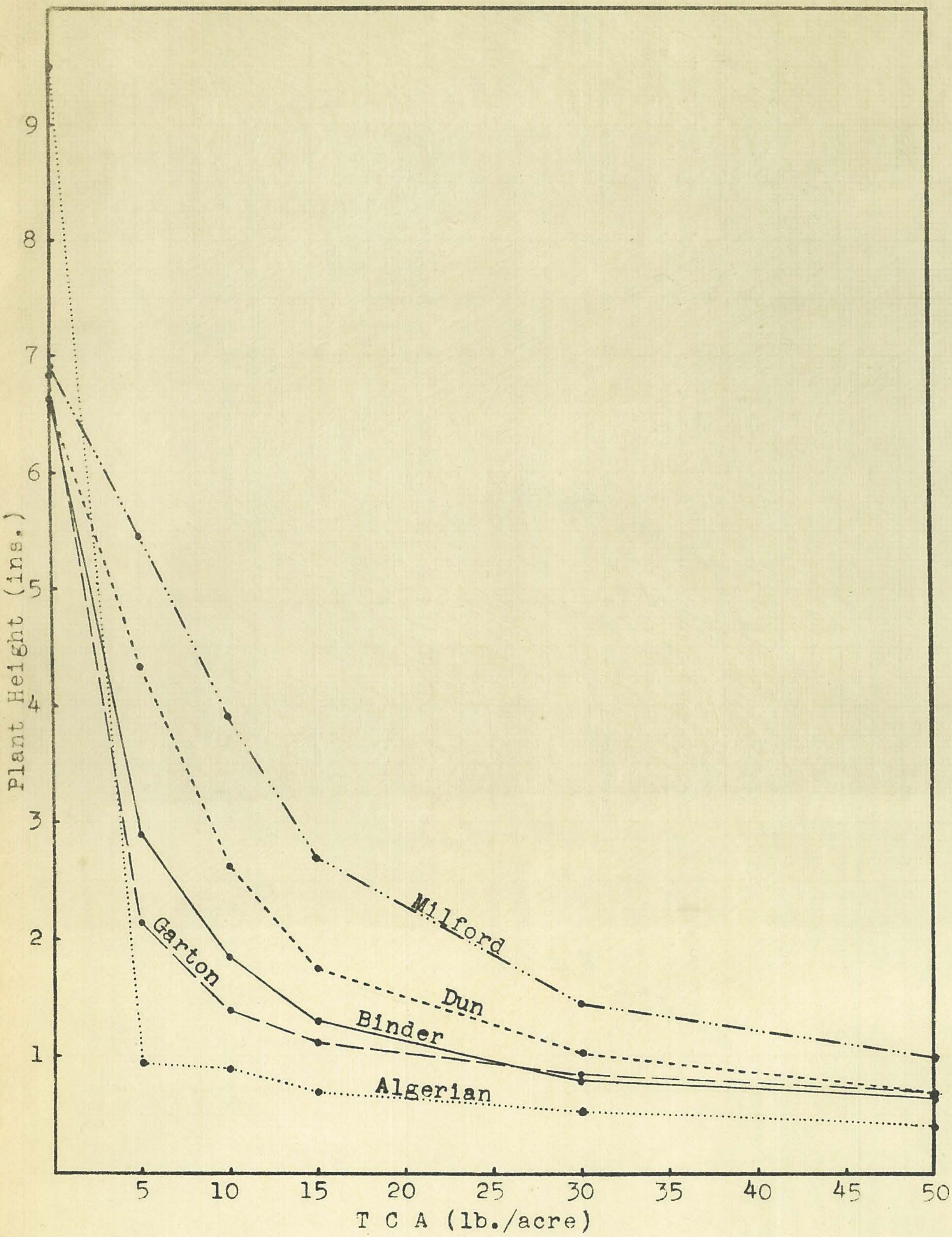


Fig.1. Growth tolerance curves of five oat varieties treated with a range of rates of T C A.

oat plant was measured and the total weight of fresh tops of each variety for each rate of TCA recorded. A summary of results is given in Table A1 of the Appendix. The gradation of tolerance of 5 varieties is illustrated in Fig. 1.

(b) Seed Viability and Evenness of Growth

Concurrently with the tolerance test, 130 seeds of each oat variety was sown separately in trays prepared as above, but no TCA treatment was given. Germination counts were made after 5 and 8 days respectively and the evenness of growth of each variety assessed visually. Results are given in Table A2 of the Appendix.

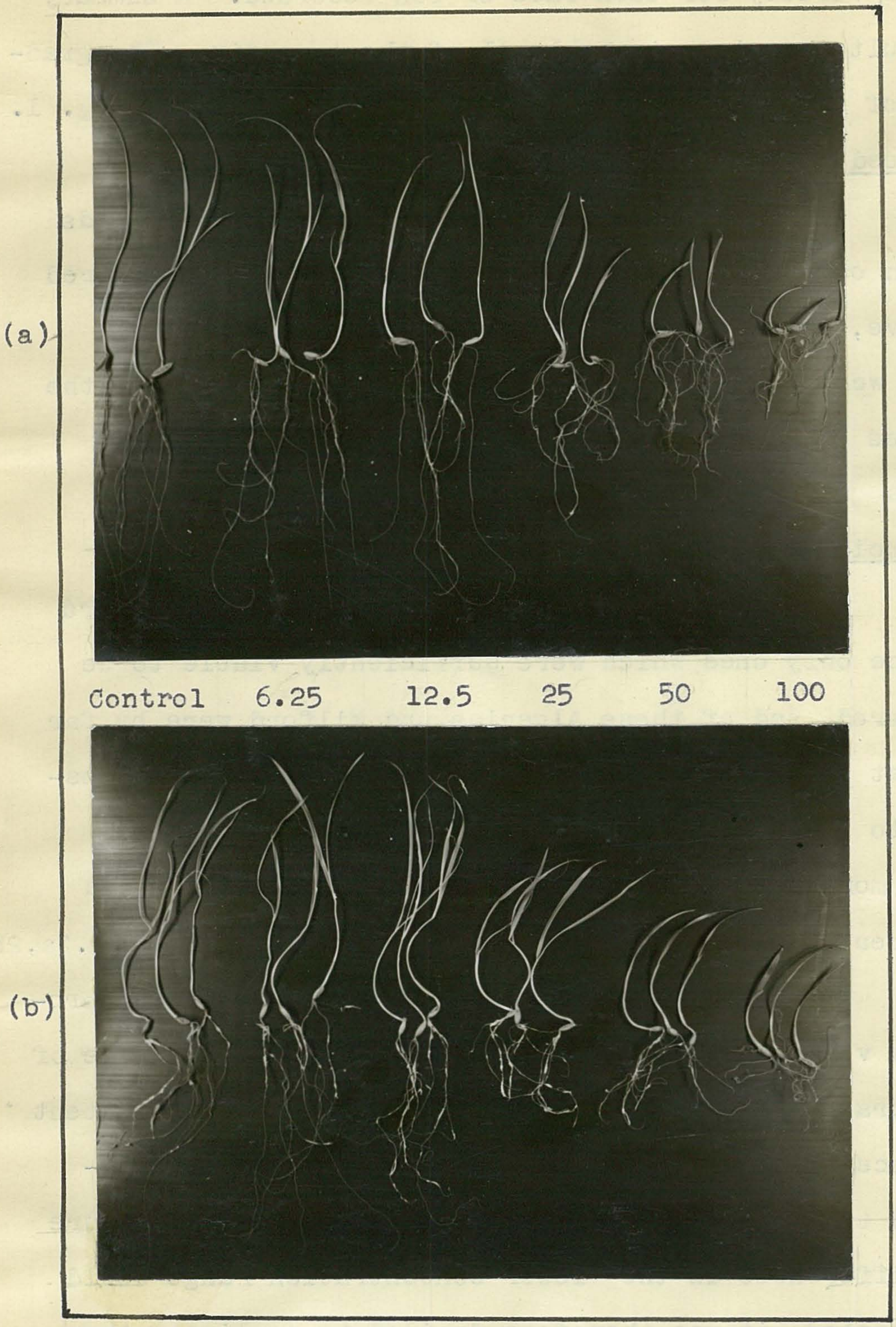
(c) Choice of Variety

The varieties Algerian, Dun, Garton and Milford were the only ones which were sufficiently viable to be considered, and of these Algerian and Milford were by far the most even in growth height. Algerian is highly sensitive to small quantities of TCA while Milford shows a marked morphological response only in the presence of a much greater concentration of the herbicide. (See Figs.2a,2b.)

Milford appeared the logical choice since it produced a very even gradation of growth through the range of concentrations tested, but it was not known to what extent the concentration of TCA would be reduced by the superimposed treatments of the experiments planned. To ensure that differences in the lower concentration range would be detected, it was ~~therefore~~ decided to replicate the first experiment using both Algerian and Milford as the plant indicators.

(d) Coefficients of Variation

A seed tray was prepared and 130 Milford oats sown as in the seed viability test. After 14 days' growth detailed measurements were made and the coefficients of variation calculated as given in Table 2.



Figs. 2a, 2b. Algerian (a) and Milford (b) oats, 12 days after germination in petrie dishes, showing increased phytotoxicity as the concentration of TCA is increased. The germination pads were impregnated with 10 ml. of TCA solution of the concentration (p.p.m.) indicated.

	Height of First Leaf	Fresh Weight of Tops	Oven-dry Weight of Tops
Mean	7.85 ins.	0.299 gms.	0.0219 gms.
Std. Deviation	0.92 ins.	0.048 gms.	0.0036 gms.
Coeff. of Variation	11.73%	16.20%	16.65%

Table 2. Summary of coefficients of variation for Milford oats

The height of the first leaf was the measure of least variation and therefore it was used throughout the experiments.

(e) Minimum Plant Number per Treatment

The formula $d=\frac{3}{\sqrt{n}}C$ was employed to determine the minimum number of plants required per treatment, where -

d = detectable difference at 5% level

c = coefficient of variation

n = number of plants per treatment

d was arbitrarily taken to be about 8% of the mean as it was anticipated that in the projected experiments the individual treatment responses would support one another because of the range of levels intended for each variable.

Substituting in the formula "the height of the first leaf" values from Table 2:— $0.08 \times 7.85 = \frac{3 \times 11.73}{\sqrt{n}}$

n = 32 approximately

Biological Test

The two oat varieties Algerian and Milford were used as plant indicators in the first experiment, but as the result of information gained therefrom, only Milford was employed for the second experiment.

Equal weights of soil for each treatment and particular soil type were placed in 3 inch clay pots and 16 oat seeds inserted vertically at equal spacing around the perimeter of each. It was found that this number of oats could be grown per pot without undue crowding while the above method of planting meant each seedling received, as near as possible, equal light and moisture and the etiolation of any plants was precluded. Two pots were required for each treatment to provide the minimum number of plants. The seeds were covered with ¼ inch of coarse sand. Sufficient moisture for germination

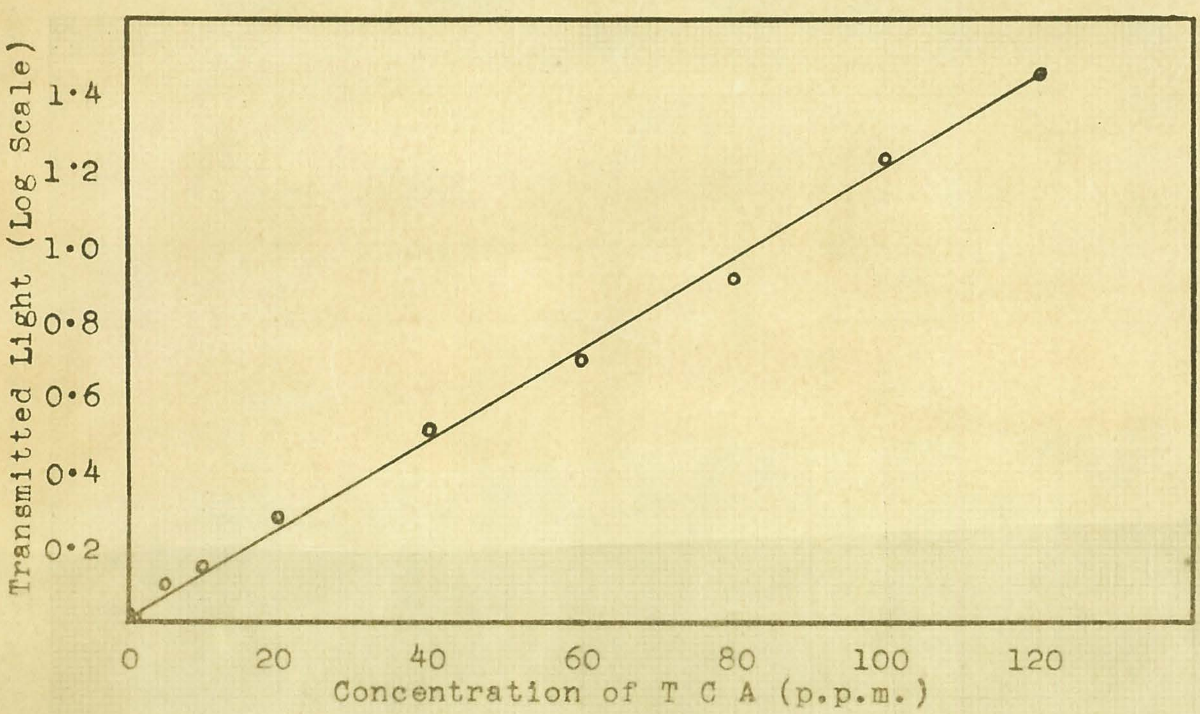


Fig.3.Standard graph for pyridine test for T C A.

and growth, but not an excessive amount to cause leaching, was applied by means of a fine sprinkler. At the conclusion of 14 days' growth the coarse sand was readily tipped off and the heights of the first leaves measured in millimeters from the grain apexes.

The heights of plants in treated soil relative to plant height in untreated soil was regarded as a measure of TCA concentration.

Chemical Test

The colormetric test described by Barrons and Hummer was employed, with only slight modification, throughout this investigation. The basic principle of the test is the breakdown of trichloracetic acid in the presence of sodium hydroxide to yield chloroform which when boiled with pyridine develops a magenta colour. The intensity of colour, determined by means of a photometer, is a measure of the concentration of trichloracetic acid.

A standard solution was prepared by dissolving 1 gm. TCA in 100 ml. distilled water (i.e. 10,000 p.p.m.) Aliquots were diluted to obtain a range of concentrations from 5 to 120 p.p.m., and the standard graph (Fig. 3.) prepared.

The Zeiss photometer employed was equipped with a logarithmic scale and thus the light transmitted was read direct.

For quantitative detection of TCA in the soil samples, 20 gms. soil was vigorously shaken with 20 ml. water, filtered, and the colour developed by 0.5 ml. of the filtrate determined and the concentration of TCA read off the standard curve. These quantities were pre-determined as those which in the leaching experiment (Experiment 1), eliminated the need to use the extreme limits of the photometer scale where accurate readings are difficult.

For the second experiment, 50 gms. of soil was shaken with 25 ml. water and 0.5 ml. samples tested. A greater volume of test solution caused turbidity in the pyridine layer which made accurate colour matching difficult. The silt loam extract was worst in this respect but the addition of a few drops of barium hydroxide caused floculation of the soil particles and a clear sample was readily obtained on filtering.

Greater sensitivity appeared to result from adding the pyridine ($7\frac{1}{2}$ ml.) to the soil extract (0.5 ml.) and the sodium hydroxide (5 ml. of 1/20 N) last than by other sequences. Because of the extreme sensitivity of the test, it was essential to run blanks on all reagents including the distilled water since appreciable colour may be obtained. The addition of a few drops of barium hydroxide was not found to interfere with the test.

Barrons and Hummer (6) reported that in their tests the magenta colour developed was stable for a period of several hours but that was not found to be so in these experiments and therefore all readings were made 2 minutes after the test material had been boiled for 5 minutes.

A chemical test performed on samples of each soil type within two to three hours after TCA treatment, showed that the procedure adopted obtained approximately 95 and 90% recovery of the herbicide from the loamy sand and the peaty and silt loams respectively.

EXPERIMENTAL

Two main experiments were conducted to determine in three different soils :-

(1) The effect of rain on the movement of TCA;

(2) The effect of temperature on the rate of breakdown of TCA.

Experiment 1

Columns of soil were treated with varying rates of TCA, subjected to fixed amounts of simulated rain distributed evenly over a period of one month, and then the distribution of the TCA determined.

(a) Preparation of Soil Columns

To facilitate the rapid and accurate sampling of soil columns at various depths, a modified technique of Robbins, Craft and Raynor (40) was employed. It was calculated that from each one inch horizon, approximately 40 cubic inches of soil would be required for the various tests. To provide this volume, cylinders 9 inches high and 7.1/3 inches in diameter were therefore constructed from polyethylene tubing, bases being fused into position by means of heat.

In the centre of each base a small incision was made in the form of a cross and over this a few large pieces of crock placed to enable rapid drainage.

Makarua peaty loam was lightly packed into one cylinder to within ½ inch of its top. An equal weight of soil was then placed in a further 24 cylinders and each compacted to the same height. The same procedure was adopted for the other two soil types thus ensuring a uniform degree of consolidation of each cylinder of the same soil type.

The filled cylinders were then watered uniformly with a fine sprinkler and left to stand for 24 hours for any excess moisture to drain out.

(b) Application of TCA

Four cylinders of each soil type were treated with TCA at the rates of 6.25, 12.5, 25 and 50 lb. per acre and five at the 100 lb. rate, and four left untreated as controls. Rather than attempt to accurately treat each cylinder in turn all those to receive the same dosage were randomised within a block 6 feet x 9 feet and the entire block treated as uniformly as possible by means of a hand pump which delivered a fine spray.

A stock solution of TCA was prepared and aliquots taken for the various treatment rates. In each instance

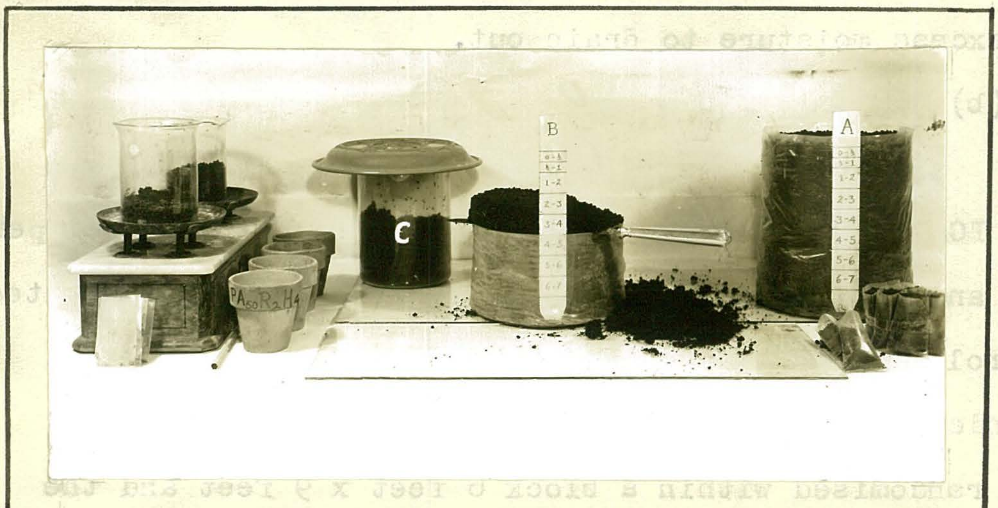


Fig. 4. Technique for dissection of soil columns.

(See text of paragraph opposite.)

the volume of spray was made up to 3 pints which is equivalent to 300 gals. per acre. This volume is at least three times that which would normally be used but it enabled more even dispersion of the spray.

(c) Rain Treatment

The cylinders were then grouped in four blocks in preparation for rain treatment. One cylinder of each soil type for each rate of application was included in each block. The extra three cylinders treated at the 100 lb. rate were stood aside and received no rain treatment. To ensure that at the highest rainfall rates water did not stagnate in the cylinders they were spanned across wooden slats such that the bases were convex and any water which leached completely through the columns readily drained out.

Simulated rain equivalent to $\frac{1}{2}$, 1, 2 and 4 inches was applied, by means of a watering can fitted with a fine rose, to blocks A, B, C and D respectively. One quarter of the rain was applied 3 days after the TCA application and the remainder in three equal amounts at weekly intervals. In other experiments, all the rain has been applied in one application 24 hours after the spraying, but it was considered that spreading the rain over a period of one month, as in this experiment, the highest rates more closely parallelled normal rainfall for the Manawatu District where the average monthly rainfall approximates 3 inches.

After the final rain treatment, the cylinders of soil were left to stand for 3 days, when it was considered all leaching would have ceased, before sampling commenced.

(d) Dissection of Soil Columns

A measuring rod was first inserted between the plastic cylinder and soil and then the top of cylinder turned outwards and pulled down to expose in turn each one inch horizon of soil which was sliced off with a carving knife (See Fig. 4. at left). Only the soil from the four

Fig. 5. Layout of Block A, Milford oats, in the glasshouse. Blocks B,C, and D were similarly arranged.

levels, 0 to 1, 1 to 2, 3 to 4, and 5 to 6 inches was retained for analysis. As each of these horizons was sliced off it was thoroughly mixed in a beaker and then an equal weight of soil, for each particular soil type, placed in four 3 inch clay pots for the biological test. A small sample was also placed in a plastic tube for the chemical test. Because of the volume of work at this juncture the four tubes of soil from each cylinder were bound together and placed in a refrigerator set at -5°C in order to arrest any breakdown of the TCA by soil micro-organisms,* before the soils could be subjected to the chemical test described on page 15.

(e) <u>Layout for Biological Test</u>

The pots were placed in trays and set out in 4 blocks using the split plot technique. Each tray contained 18 pots -

All 3 soil types x all 6 rates of TCA, for a particular rain, horizon combination.

Included also in 12 trays within each block was one pot from the cylinders receiving no rain after treatment with TCA at the 100 lb. per acre rate - i.e. 3 soil types x 4 horizons. Milford oats were sown in the blocks A and B and Algerian in blocks C and D, - i.e. two replications for each variety. (Fig.5.)

The trays were supported on raised platforms on the glasshouse benches so that the tops of the pots were above the level of the concrete wall and thus shading effects greatly minimised. The pots were randomised within the trays, and the trays within the blocks, the latter being changed daily and each time the trays turned end for end. By this procedure it was considered that the total light received by each pot was almost equal without the need for complete randomisation of all pots within each block daily.

Plant heights were recorded after 14 days' growth.

* Based on findings of Loustalot and Ferrer(20)and others(14,2[illegible]

Experiment 2

Blocks of soil were treated with TCA, maintained at different temperatures for two months and at intervals during this period samples tested for TCA content whence the rate of breakdown of this herbicide was determined.

(a) Treatment of Soils with TCA

The remainder of the three bulk lots of Foxton loamy sand, Manawatu silt loam, and Makarua peaty loam, which had been sieved and mixed in preparation for Experiment 1, were thoroughly wetted and left to drain for 48 hours when each was again well mixed.

A wooden grid of three units, each 51" x 51" x 4" was constructed on a concrete floor and each of the units filled uniformly with one of the soil types.

A stock solution of TCA was prepared and aliquots taken for the two treatment rates. In each instance, the volume of spray was made up to 3 pints and applied, by means of a hand pump which delivered a fine spray, as uniformly as possible within the perimeter of the grid. This spray volume is equivalent to 300 gals. per acre.

The first filling of the grid was used as the control and therefore was sprayed with water only. The second and third fillings received TCA treatment at the rates of 50 and 100 lb. per acre respectively.

It was considered preferable for this experiment to have an even distribution of the TCA throughout the soil mass, therefore after the treatment, the soil in each of the grid units was, in turn, thoroughly mixed by repeatedly turning it per shovel on the adjoining concrete floor.

(b) Preparation of Temperature Controlled Propagating Pits

Three propagating pits in the greenhouse were utilised for this experiment. Two were equipped with electrical (hot wire) soil warming units and the temperatures in each thermostatically controlled. One was set initially at 62°F and the other at 74°F while the third was left unheated, the ambient temperature being approximately 50°F.

A grid of nine units, each 17 x 14 inches, was constructed on the sand base in each pit. Three units were filled to a depth of 4 inches with Makarua peaty loam, one untreated and the other two treated with TCA at 50 and 100 lb. per acre respectively. Similarly treated soil of the other two types were placed in the remaining six units.

(c) Moisture and Temperature Maintenance

Throughout the duration of the experiment each pit was regularly lightly damped down to maintain a moisture level sufficient for plant growth and for the normal activity of soil microorganisms. The small volume of water for this purpose was delivered through a fine rose and it was considered that negligible leaching occurred.

Twice daily, at 9 a.m. and 3 p.m., the temperature in each pit was recorded and in the initial stages the thermostats regulating the temperatures of the two heated pits adjusted slightly as required to maintain the temperatures as near as possible to the pre-determined figures.

(d) Tests for TCA Content (See also pages 14 to 16)

From each of the 27 units in the 3 pits, samples were taken 6, 18, 39 and 58 days after treatment, and tested immediately for residual TCA content. Several samples were taken at random from each unit by plunging an open sharp-edged metal cylinder of 1" diameter into the soils to a depth of 3 inches and withdrawing the contained volume of soil. The samples from each unit were bulked and thoroughly mixed and any soil surplus to the requirements below returned to that unit from which it was taken.

Two 3 inch clay pots were filled with soil from each unit making a total of 54. These were placed at random in 3 trays, and as in Experiment 1, the trays raised on a platform so that the pots were not shaded by the greenhouse walls. In each pot 16 Milford oats were grown and at the conclusion of an arbitrary growth period, the heights of the first leaves measured.

The environmental conditions in the unheated greenhouse varied slightly for the 4 biological tests and thus for the second, and subsequent tests, the growth period was extended or shortened by a few days such that the height of the oats in the control pots approximated the height the controls attained in the first test (i.e. that conducted 6 days after T C A treatment).

Immediately the pots were set up a chemical test was performed on each of the 27 soil samples.

<u>No True Replication</u>

Basically neither experiment was replicated. It was not physically possible with the facilities available and within the time allotted to have extended the experimental work undertaken. Though replication is desirable it is doubtful if such would have materially affected the results obtained.

In respect of Experiment 1 the number of rain or T C A treatment rates could have been reduced to permit replication but it was considered preferable to obtain information on a wide range or rates in the belief that confidence in the reality of effects would be derived from an internal consistency of trends between levels of the various factors.

The second experiment was limited by the availability of only three propagation pits. Again confidence in the results depended on obtaining a consistency of effects of various factors.

Though in both experiments there was no basic replication, the minimum plant requirement per treatment was divided between two pots, and in the first experiment this was replicated in that there were two pots for each variety.

RESULTS

Experiment 1

The results of the biological and chemical tests, classified by soils, amount of simulated rain, amount of T C A and depth of soil, with soil and rain as primary classes, are presented in Tables A3 and A4 of the Appendix*. In the chemical test, a trace of colour was developed in all control samples. It was known that no T C A had been applied to these soils prior to their being taken from the field and therefore the slight colour developed by the pyridine test must have been due to impurities. For clarity, the control readings have been adjusted to zero and the concentration of T C A in the treated columns reduced accordingly by the amount of "apparent T C A" in the respective control.

From inspection of the data one would anticipate interactions between most, if not all, of the factors involved. It was considered that the most intelligible approach to the analysis of the three sets of results would be to plot the biological data, separately for each oat variety, against the chemical data to see if the oats were responding to any effects not disclosed by the chemical tests. Should any such effects exist, the particular data could then be subjected to statistical analysis.

The simplest combination of factors, levels of T C A and depths within each soil, are shown graphically in Figs. 6a, 6b, while Figs. 7a, 7b show the combination of levels of T C A and rainfall applied to each soil. In no instance can any consistent trend be discerned other than that due to factors which affect the concentration of T C A. It is clear that the heights of the oat plants are directly related to the active concentrations of T C A, as determined by the chemical analyses, and that breakdown products are not significantly affecting these results. Soil levels and precipitation are not significant other than to change the amount of T C A.

* Hereafter for tables prefixed by A see Appendix.

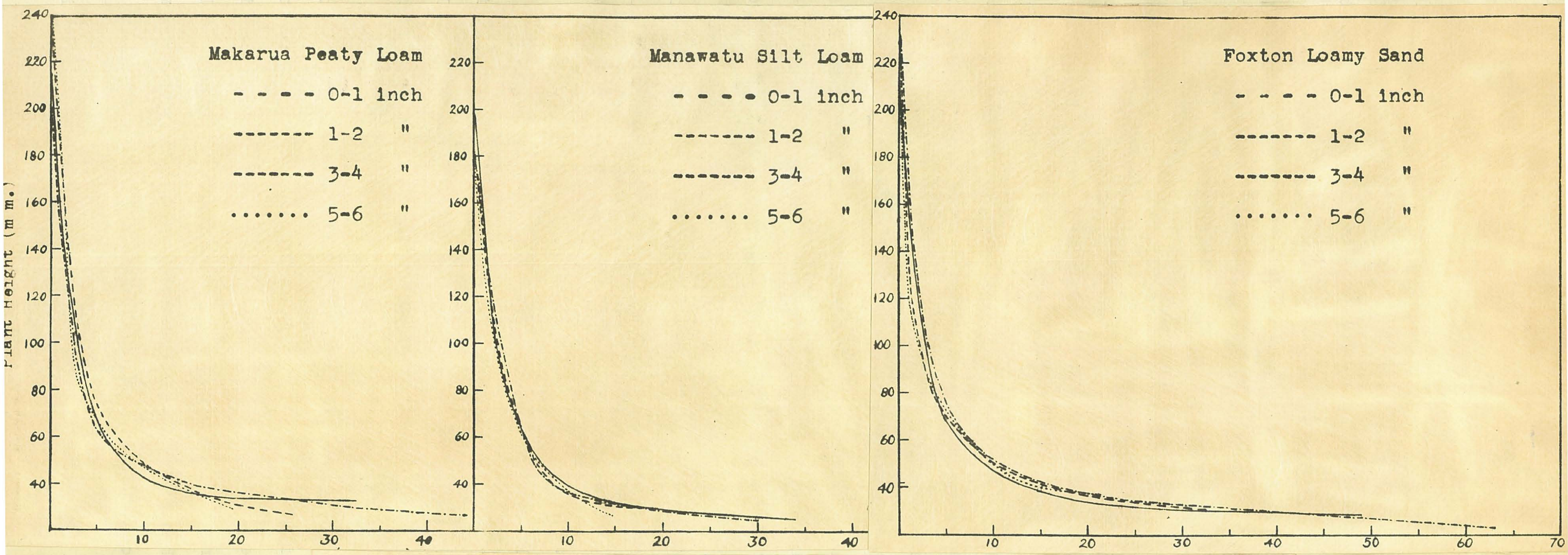


Fig.6a. Plot of Algerian oat height against residual concentraion of T C A at various soil depths.

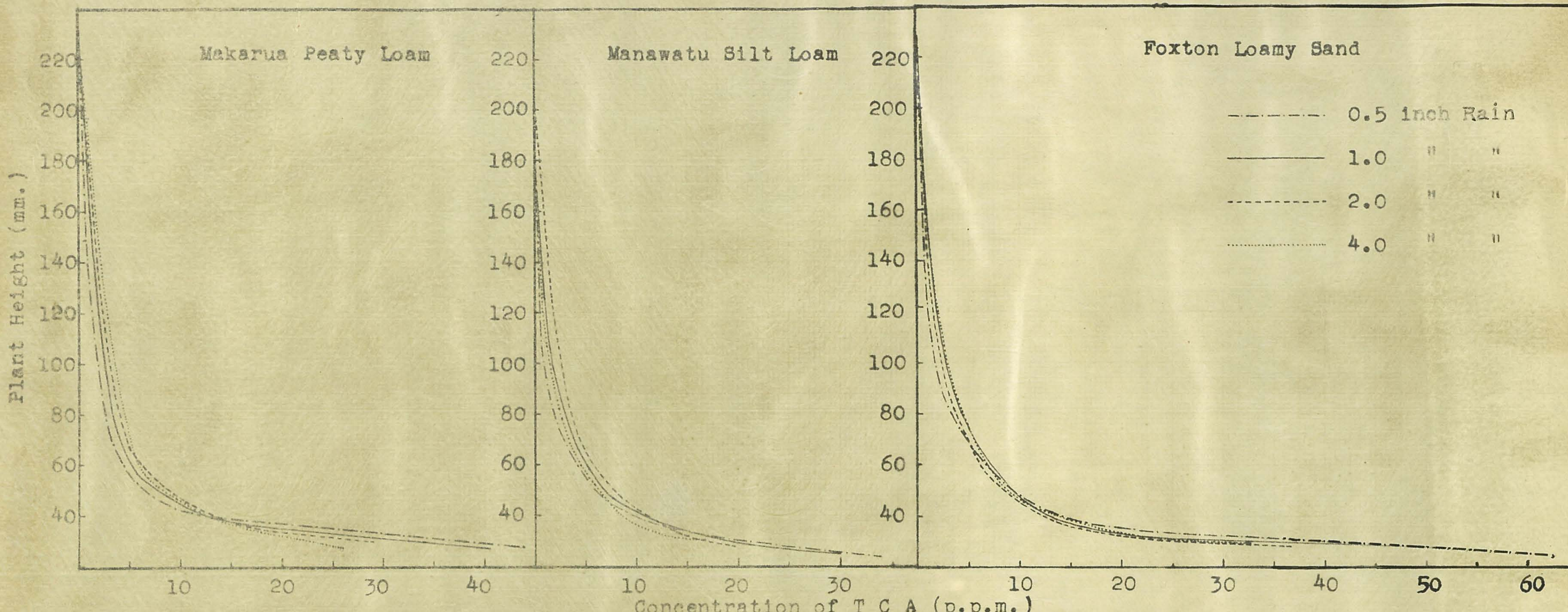


Fig.7a. Plot of Algerian oat height against residual concentration of T C A after rain treatment.

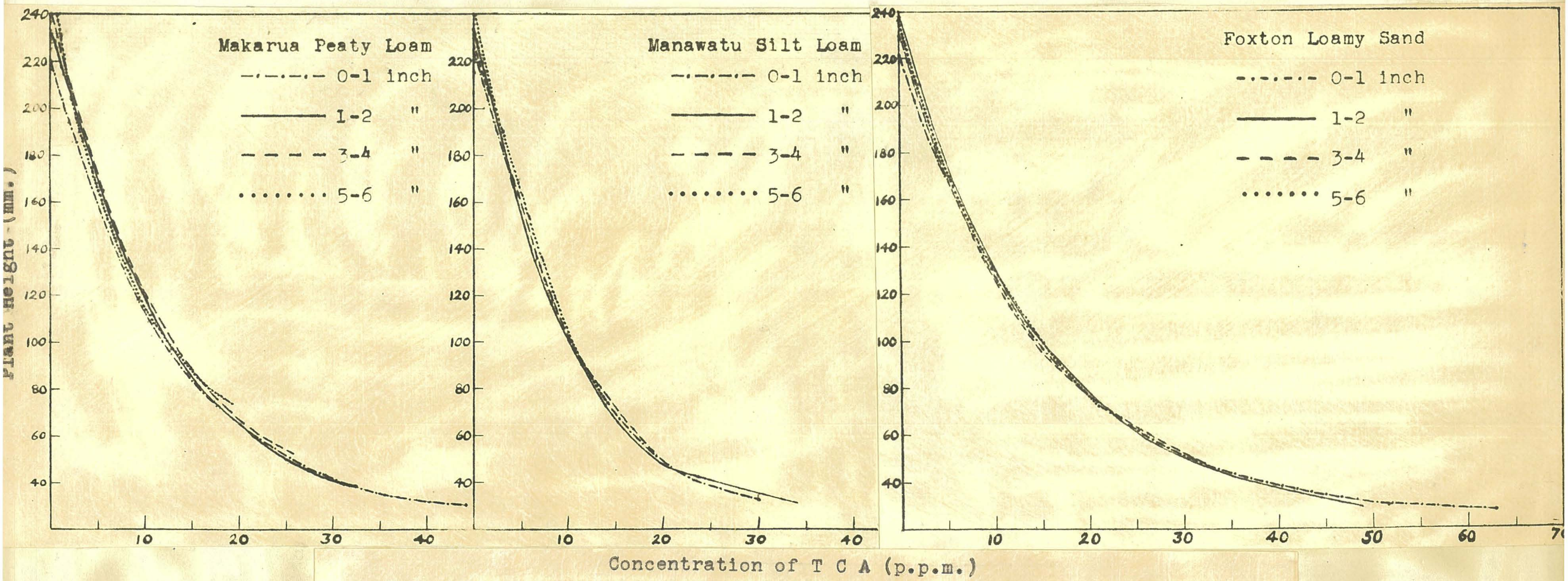


Fig.6b. Plot of Milford oat height against residual concentration of T C A at various soil depths.

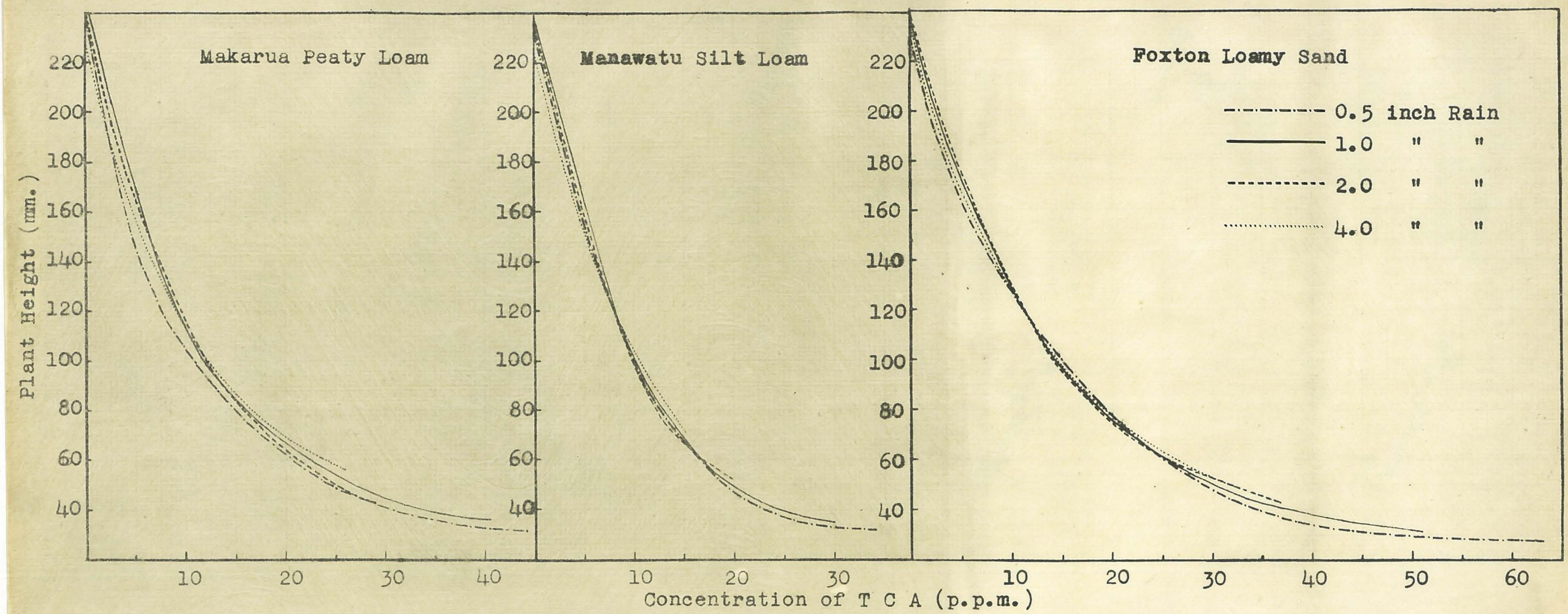


Fig. 7b. Plot of Milford oat height against residual concentration of T C A after rain treatment.

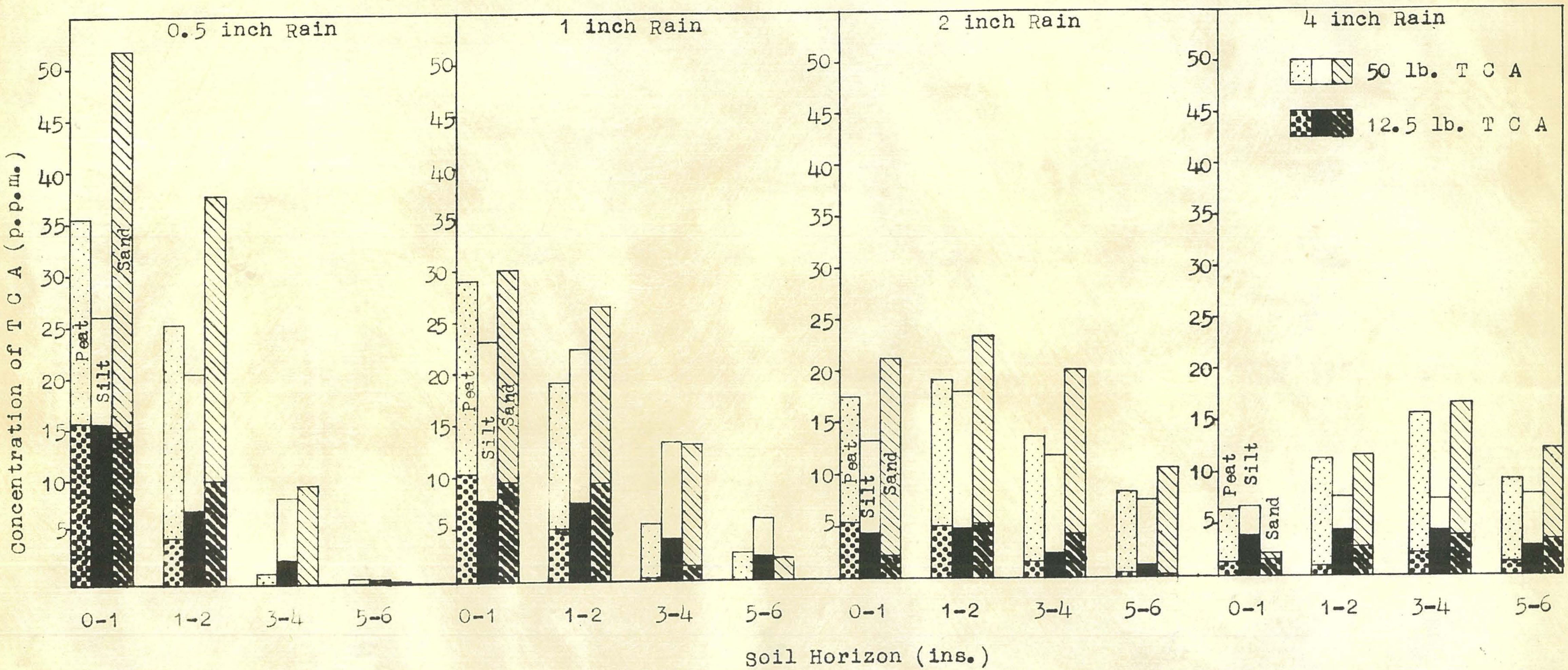


Fig.8. Effect of leaching on movement of T C A applied to the soil surface at 12.5 and 50 lb. per acre.

There is such a close correspondence between the biological and chemical tests that it was redundant to consider them separately. The tabulated results (Tables A3, A4) and the graphical presentation of the residual concentration of T C A in those columns treated at the 12.5 and 50 lb. per acre rates (Fig. 8), shows clearly that the residual concentration of T C A in each soil and at each depth is dependent on the joint effect of amount of rain and rates of T C A applied.

The experiment as designed has no true replication since there was only a single column prepared for each factorial combination of soil, T C A level and amount of rain. The partitioning of each column into four pots, two for each of two varieties of oats, made possible the estimation of a biological assay error which however understates, to degree unknown, the true error for comparison of treatments. A statistical analysis, subject to this limitation, was made of the oat data and this confirmed the visual impression that all main effects, first order and higher order interactions were significant; that is the pattern of yield for levels of T C A x depth changes with level of rain and with soil. The essential details of the primary analyses within soils and levels of rain are given in Tables A5a,A5b, and the full analyses involving the interaction between soils is given in Tables A6a,A 6b.

It must be kept in mind that the moisture content of the soil columns was adjusted to approximately field capacity prior to their treatment with T C A. Although the volume of herbicidal spray applied was equivalent to only 0.013 inches of rain, it was considered that this amount may have transported some of the T C A below the surface inch because of the moisture condition of the soil columns at the time of treatment.

In order to find out the initial distribution of T C A, the three soil columns which received no rain after

Fig. 9. Distribution of TCA applied to the soil surface at the rate of 100 lb. per acre as an aqueous spray equivalent in volume to 300 gals. per acre (equals 0.013 surface inches of water), No rain was applied subsequently. From left to right—— Milford oats grown in soil horizons 0-1", 1-2", 3-4", 5-6", and Algerian oats similarly. Top row—— Manawatu silt loam; Middle row——Makarua peaty loam; Bottom row——Foxton loamy sand.

the application of 100 lb. T C A per acre were dissected and the horizons tested for T C A content. TableA7. incorporates the results of both the chemical and biological tests, and the latter is also illustrated in Fig. 9. The growth of Algerian oats indicated that a little T C A had leached to the 3 to 4 inch horizon in the silt loam and loamy sand, but only a trace was indicated at this depth in the peaty loam. Milford oats grown in the same soils from this region showed no depression of growth indicating that the T C A concentration was of a low order which was confirmed by the chemical test, being 0.5, 2.3 and 2.8 p.p.m. respectively for the peaty loam, silt loam and loamy sand. An appreciable amount of T C A was found in the 1 to 2 inch horizon of all soils, but again the peaty loam contained the lowest concentration indicating that the herbicide is less mobile in the organic soil. In all soils, the amount of T C A in the surface inch was greater than that found after 0.5 inch of rain treatment. Neither test detected T C A in the 5 to 6 inch horizon of any of the soils. It could therefore be assumed that none of the applied herbicide had been leached from the soil columns, and since each received the same amount of T C A initially, different rates of loss must have been due to the different physical properties and biological activity of the three soils. The sums of the concentrations for the four horizons tested in each soil column present a striking difference, being 102.3, 70.8 and 66.8 p.p.m. for loamy sand, peaty loam and silt loam respectively. The rate of breakdown or inactivation of the T C A in the peaty loam and silt loam is obviously very similar but much faster than in the sandy loam.

An inspection of Table A4 similarly reveals that the overall recovery of T C A from the peaty loam and silt loam was approximately equal. Much greater recovery was obtained from the loamy sand.

Referring again to Fig. 8 and also **Fig. 10, (overleaf)**, it can be seen that the general pattern of leaching of T C A is similar in the three soils, but that there was a

S.

TCA 6¼ lb./ac. P.

Sd.

Algerian Oats

S.

TCA 12½ lb./ac. P.

Sd.

S.

TCA 25 lb./ac. P.

Sd.

Milford Oats

S.

TCA 50 lb./ac. P.

Sd.

Soil Horizons 0-1, 1-2, 3-4, 5-6; 0-1, 1-2, 3-4, 5-6; 0-1, 1-2, 3-4, 5-6; 0-1, 1-2, 3-4, 5-6;"

greater herbicide movement in the loamy sand than in the other two soil types. Even after the application of 0.5 or 1 inch of rain the greatest concentration of T C A was still in the surface inch of soil of all three types, but as would be expected, the concentration was reduced with each increment of rain. With the application of 2 inches of rain the residual T C A was fairly evenly distributed throughout the columns to a depth of 4 inches, and an appreciable amount leached to the 5 to 6 inch horizon, and no doubt deeper, but no attempt was made to analyse the leachates from the columns. In the peaty loam and loamy sand the greatest concentration of T C A was found in the 3 to 4 inch horizon after 4 inches of rain treatment. Approximately three-quarters of this amount was found in both the 1-2 and 5-6 inch horizons with a little less in the surface inch of the peaty loam and appreciably less in the loamy sand. In the silt loam receiving 4 inches of rain, the residual T C A was uniformly distributed throughout the entire columns with the exception of the one treated at the 100 lb. rate in which, possibly by chance, the concentration in the surface inch was only half that in the lower levels. It is obvious that when the simulated rain is applied evenly over a period of one month to each soil treated initially with 12.5 lb. or more of T C A per acre, an amount in excess of 4 inches is required to reduce the herbicide concentration to insignificant levels in the surface inch and lower horizons to a depth of 6 inches.

Experiment 2

The results of the chemical and biological tests are presented in Tables A8 and A9 respectively. The logarithmic values are given since these were used for the statistical analyses. The chemical data has been adjusted, as for Experiment 1, in respect of "apparent T C A" in the controls. Because of the slightly different environmental conditions in the glasshouse for each of the biological tests it has been necessary to reduce the average recorded heights of all control plants to 100 and express the heights of plants grown in treated soil as a percentage of the respective control thereby enabling a comparison of relative heights. The values given in Table A9. for the 50 and 100 lb. rates are the sums of the two percentages calculated for the two pots of oats for each treatment.

The temperature in the unheated pit reached an average daily reading of approximately 60°F on 25th September when the last test was made. This rise in the ambient temperature had little effect on the heated pits until the final three weeks when a maximum rise of 3.5°F was recorded in both pits giving final readings of 65.5° and 77.5°F respectively. In the statistical analyses described below, these temperature rises have been ignored.

The different drying rates of the soils in the three pits required different watering rates to maintain them at an approximately uniform moisture condition. This water was delivered as a fine spray from a hose during the routine damping down of the glasshouse and therefore no record of the total volumes applied was possible.

(1) Chemical Test

The chemical data was analysed for each soil separately using logarithms of active concentrations of T C A. By making an analysis of relative effects it was hoped that the interaction of concentration with the other factors would be more simply expressed and that there was some prospect that higher order interactions would not be substantially in excess of true replicate error, had the experiment been replicated in fact.

The separate and the combined analyses for the three soils are given in Table A10. It is clear from the separate analyses, that the effect of period of treatment, concentration and temperature and all first order interactions except temperature x concentration are significantly greater than the second order interaction in each instance.

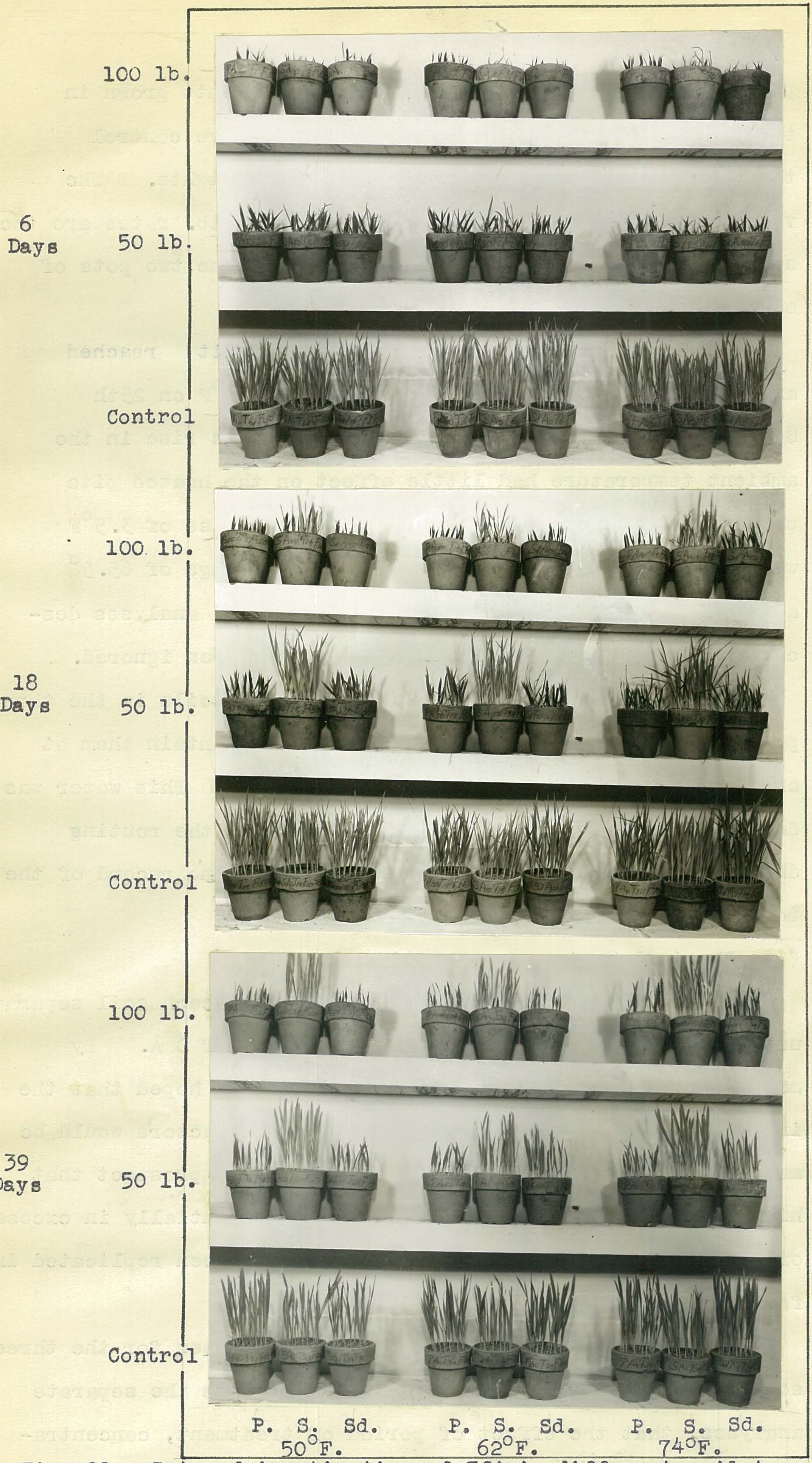


Fig. 11 Rate of inactivation of TCA in different soil types.

The mean square for temperature x concentration is consistently less than the second order interaction, but this is likely to be a chance effect. The indications are that the temperature x concentration and higher interactions with days and soils are homogeneous and may be taken as pooled estimate of error. This gives a mean square of 0.00093, based on 24 degrees of freedom, which is used for the error term for both the separate and combined analyses.

Relative to this estimate of replicate variance the combined analysis indicates a significant difference between soils and an interaction of soils with days, temperatures and concentrations of which the most important is soils x days. The main contribution to this interaction comes from substantially faster rate of decline in the Manawatu silt loam than in the other two soils. The temperature effect is also greater in the Manawatu silt loam than in the Foxton loamy sand and even more so than is Makarua peaty loam.

Manawatu silt loam differs in its pattern from the other soils in that the difference between the logarithms of residual T C A for both 50 and 100 lb. per acre treatment rates does not change with time, whereas with the peaty loam and loamy sand the difference becomes smaller. With the latter two soil types the effect of period of treatment on the higher concentration is relatively greater than on the lower concentration.

In the interaction of days x temperature all soils show the same pattern of a greater rate of herbicide dissipation with time at the highest temperature and this is most apparent with the silt loam. The component of interaction which is most affected by this feature is the difference in linear trend for 50°F and 74°F. The difference in all cases is significant.

(2) Biological Test

The logarithms of the heights of the oat plants (Table A9) give a pattern of responses which closely parallel the results of the chemical assay. (See also Fig. 11) An analysis of variance of the biological data is presented in Table A11.

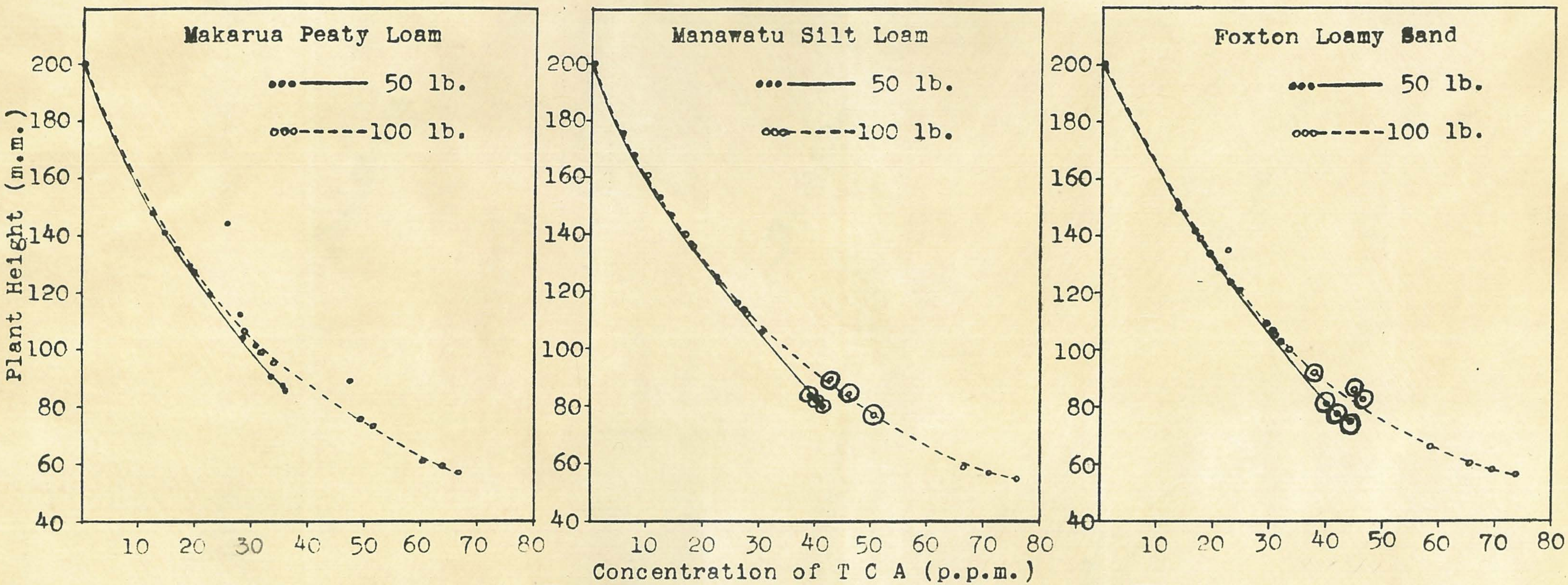


Fig. 12 Plot of Milford oat height against residual concentration of T C A. (See Table A12 for covariance analyses of encircled points)

Because of the non-linear relation between oat height and residual T C A one would expect some difference in trends in the interaction tables. The plot of oat height against the residual concentration of T C A for each of the soils (Fig. 12), suggests that the same residual concentration of T C A in soils treated initially at different rates give different yields. For example there is a very strong indication that the height of oats sown in the Manawatu silt loam 6 and 18 days after treatment with T C A at 50 and 100 lb. per acre respectively, when the residual herbicide concentration was in each instance approximately 40 to 50 p.p.m., was slightly greater in the soils treated initially at the 100 lb. rate.

The indications are that the slopes of the plant growth curves against active concentrations for the two rates of T C A are different in these regions. However if one ignores this, a covariance analysis (Table A12) restricted to the extreme values* of the curve for the 50 lb. application rate and the corresponding nearest on the 100 lb. curve indicates that the differences in the oat heights at a common concentration of active material are real for both the loamy sand and silt loam. The separation of the two curves for the Makarua peaty loam was less marked but otherwise very similar. In the latter instance, however, the values for 39 days on the 100 lb. curve are those which correspond more nearly to the extreme values on the 50 lb. curve.

* Encircled points of Fig. 12

DISCUSSION OF RESULTS

The experimental results presented in the preceding pages have confirmed the findings of workers in other countries that the movement of T C A in the soil is dependent on the soil type and rainfall, and that these factors, together with temperature, govern the rate of loss of the herbicide from the soil. However the degree to which these factors affect the herbicidal activity of T C A was here demonstrated to be very different from the reported results of some other similar experiments.

The soils used in this investigation were Makarua peaty loam, Manawatu silt loam, and Foxton loamy sand. The observation of Arakeri and Dunham (1) that rainfall most significantly affected the persistence of T C A in the soil was confirmed by the results of the present study. The pattern of leaching of T C A was found to be similar in the three soil types but there was a greater herbicide movement in the loamy sand than in the other two soil types. Since T C A is highly soluble in water it would be expected that the herbicide would be carried freely with the gravitational movements of the soil water. Such movements would be greatest in the loamy sand through the relatively large interstices between sand particles. There would be few macropores between the fine soil particles of the peaty loam and silt loam, and imbition of water by the colloidal matter of these soil types tends to clog the connecting pores and restrict gravitational water flow.

The very poor control of <u>Agropyron repens</u> infesting certain porous soils in the Auckland Province can be simply accounted for by the moderately high rainfall rapidly leaching the T C A beneath the root zone.

If it was desired to use T C A as a pre-emergence spray for the control of seedling annual grasses in a crop intolerant to this herbicide, the results obtained in this investigation suggest that deep planting would afford the crop little protection from the toxic effects of T C A in any of the soil types studied for even without subsequent rain an appreciable quantity of the herbicide was found at a deptch of 2 inches in all soils. The applied spray volume was equivalent to 0.013 surface inches of rain. In each of the three soil types some T C A was found to leach to a depth of 4 inches after the application of $\frac{1}{4}$ inch of rain which confirms the findings of Loustalot and Ferrer (20). However, if the soil is not tilled after T C A applications, between 1 and 2 inches of rain would be required in the month following treatment to carry a lethal amount of the herbicide to the deepest roots of rhizomatous grasses, such as Agropyron repens, in all three soils studied. The normal monthly rainfall for the Manawatu District should preclude any failure to control the above species due to insufficient of the applied T C A coming into contact with the roots.

Whereas Ogle and Warren (29) found that 2 inches of rain resulted in the greatest concentration of T C A being found at a depth of 2, 4 and 6 inches respectively in silt loam, peat loam and sandy loam, in the current investigation the greatest concentration, after 2 inches of rain, was found at the 1 to 2 inch level in each of the three soils studied which were similar to those above. The conflicting results can possibly be accounted for by the fact that in the former experiment all the artificial rain was applied at the one time, while in the latter it was given in four equal amounts at weekly intervals. It would be reasonable to predict that the large volume of water applied to the soil shortly after T C A treatment would have the greatest leaching effect, and this would be most evident in sandy soils.

The growth of oats, used as plant indicators, showed that more than 4 inches of rain is required to reduce to innocuous levels, even in the surface inch of the loamy sand, the concentration of T C A applied at the 6 lb. per acre rate and logically there was a greater residual toxicity in the surface of those soils which received greater amounts of T C A.

It would appear that Digitaria sanguinalis is much more tolerant to T C A than were the oats employed in this investigation for Ogle and Warren (29) using D. sanguinalis as a plant indicator, reported that in sandy and silt loams treated with 8 lb. T C A per acre most of the herbicide leached out with 2 inches of rain and all with 4 inches, and that while 2 inches removed little T C A from the surface inch of peaty loam all leached out with 4 inches of rain. Though the latter workers applied all the rain at the one time and the soil types may have been very different from those employed in the current studies, the need for careful interpretation of results is indicated when plant indicators are used for detecting T C A residues in the soil.

Blough and Fults (7) suggested as a result of their experiments that a substantial portion of applied T C A may be adsorbed by organic colloids. The results of the present investigation would suggest that the inorganic colloids similarly adsorb T C A which theory supports the opinion of Rai and Hammer (38). Adsorption of T C A by inorganic colloids would account in the chemical tests for less recovery of T C A from the silt loam than the sandy loam which was deficient in both forms of colloidal material. If this theory is correct, the biological test suggests that the adsorbed T C A is held in a form not readily available to plants and that they show a morphological response indicative of only the free material. The same view is held by Ogle and Warren (29). The colloidal adsorption of T C A would account for the findings of McCall and Zahnley (22), Lynch (21) and others, that higher rates of this herbicide are required for control of Agropyron repens in clay loams than in lighter soil types.

In the studies reported here, an increase in the temperature produced a small but significant increase in the rate of breakdown of T C A in the soil. Though Kratochvil (19 demonstrated that small amounts of T C A reduced the activity of soil micro-organisms, it is probable that some of the breakdown of T C A can be attributed to microbial activity. A small rise in the soil temperature would be expected to increase biological activity and result in a more rapid dissipation of the herbicide. However, the period of time for the breakdown of T C A was shown to be more significant than temperature in these studies where the lowest temperature was 50° and the highest 74°F. Eight weeks after the application of T C A the residual herbicide concentration in the soil treated at 50 and 100 lb. per acre respectively was found in both the peaty loam and loamy sand, at all temperatures, to be approximately 35 and 27% that amount detected 6 days after the herbicide application. Dissipation of T C A in the silt loam was different in that the rate of breakdown was similar for both treatment rates but after 8 weeks the percentage residual T C A was shown to be approximately 15 and 28% of that at the sixth day test in the pits at 50°F. and 74°F respectively. No satisfactory explanation can be suggested to account for the dissipation of T C A in the silt loam not following the pattern of the other two soil types. Ogle and Warren (29) found that peaty loam treated with 8 lb. T C A per acre and stored at temperatures between 46° and 95°F. required 12 weeks for complete breakdown of the herbicide. Silt loam and fine sand treated similarly required more than 12 weeks for complete dissipation of the herbicide, but at the 12th week a greater amount of T C A was found in the sand than in the silt loam which compares with the tests at the 8th week in the current investigation.

The pattern of curves of oat height plotted against T C A concentration (Fig. 12.) in the temperature study may possibly be explained by the knowledge that soils taken from the field and placed in pots in the glasshouse may accumulate very large amounts of nitrogen. The nitrogen accumulation after 18 days incubation would be expected to be more than that after only 6 days. Though the warmer temperature would be partly responsible for the shorter growth period required for control plants to attain equal heights in the second and subsequent biological tests, the very small rise in temperature at the second test would suggest that a greater supply of nitrogen may have been partly responsible for the more rapid growth. If this explanation is the correct one, then it would appear that the rate of nitrogen accumulation after the second test date was very much slower than initially. This would account for the curves converging as the nitrogen level approximated its maximum.

It cannot be argued that the result obtained was due to beneficial breakdown products of T C A for, if such was the case, the curves would rather diverge because with the passage of time there would be a greater amount of breakdown product from the highest treatment rate.

The very close parallelism of results obtained by the chemical and biological tests shows that there is little to choose between them for the detection of T C A in soil. However, for any future analyses of soils for the presence of this herbicide the chemical test would be strongly favoured because it is very quick and the active concentration is determined, whereas, with the biological test, one can only compare yields of the test plants as an indication of the relative amounts of T C A. Differences in nutrient levels can seriously mask the true effects in biological tests as can also the environmental conditions during the test growth period. Though a growth period of 14 days was found sufficient for oats, other test plants may require longer to produce adequate growth for comparison of treatment effects, and, where a herbicide breaks down rapidly in the soil, the concentration at the commencement of a biological test may be very different from that at the conclusion. Such was not the case with T C A under the conditions studied.

Indicator plants may be superior to chemical analyses for the estimation of residual amounts of other herbicides when there is a significant effect due to breakdown products.

For full information, the simultaneous analysis by chemical and biological methods may be desirable for other herbicides, but for T C A the chemical test alone provides adequate information and is superior to a biological test because of the many variables with the latter.

An observation from the preliminary work to the main study which deserves emphasis is that a particular concentration of T C A is not equally phototoxic to all varieties of oats grown under identical conditions. Other workers (3, 32, 42) have tested the tolerance of many crops to different rates of T C A, and have grouped them according to whether they were tolerant, susceptible or of intermediate tolerance. Such broad groupings serve as a general guide, but varieties have seldom been specified and it is probable that in each instance the tolerance rating of a species has been based on the reaction of a single variety. It is unlikely that the difference in varietal tolerance detected in this investigation is peculiar to oats treated with T C A, and it is therefore concluded that recommendations of herbicide rates for selective weed control on a commercial scale should not be based on the tolerance assessment of only one variety of the crop in question.

SUMMARY

The effect of rainfall and temperature on the distribution of herbicidal activity of sodium trichloracetate (T C A) applied to three different soil types are presented.

Preliminary investigations showed nine varieties of Avena sativa to respond differently to equal concentrations of T C A and two were selected, for the main investigation, as being suitable indicators for the biological assay of residual T C A in the soil. Conjointly with each biological test for T C A a pyridine colour test was made and the results compared.

1. In the glasshouse T C A was applied at five rates (6.25, 12.5, 25, 50 and 100 lb. per acre) to columns of peaty loam, silt loam and loamy sand. These were then leached with four rain rates (0.5, 1, 2 and 4 inches), given in four equal amounts at weekly intervals, and the residual quantity of herbicide at various depths (0 - 1, 1 - 2, 3 - 4 and 5 - 6 inches) determined.

The retention of T C A in the loamy sand was less than in the other soil types, but the general pattern of leaching was similar in each. Rainfall more significantly affected the movement of T C A than did soil type. All main effects, first and higher order interactions were shown to be significant.

2. The above three soils were treated with T C A at 50 and 100 lb. per acre and stored in propogating pits at 50°, 62° and 74°F. After 6, 18, 39 and 58 days the residual amount of herbicide was determined by both biological and chemical tests.

An increase in temperature was found to significantly affect the rate of breakdown of T C A but the period of time for inactivation was demonstrated to be far more important. The greatest effect of temperature was in the silt loam.

The very close parallelism of results by the chemical and biological tests in both experiments indicates

that there is no effect due to breakdown products of T C A. Since the chemical test enables the rapid determination of the herbicidal concentration it is preferred to the biological test which is laborious, subject to many variables and provides information of only comparative amounts of T C A.

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APPENDIX

Tabulated results of the experiments, and statistical analyses presented in the following pages.

Oat Variety	Rate of T C A (lb./acre)											
	0		5		10		15		30		50	
	Height (ins.)	Weight (gms.)	Height (ins.)	Weight (gms.)	Height (ins.)	Weight (gms.)	Height (ins.)	Weight (gms.)	Height (ins.)	Weight (gms.)	Height (ins.)	Weight (gms.)
Abundance	5.93	0.1935	2.42	0.0715	1.31	0.0499	1.23	0.0659	0.60	0.0236	0.58	0.0247
Algerian	9.50	0.2942	0.94	0.0279	0.90	0.0342	0.69	0.0247	0.52	0.0148	0.40	0.0095
Binder	6.62	0.2113	2.89	0.1300	1.86	0.0813	1.30	0.0553	0.79	0.0280	0.63	0.0273
Dun	6.57	0.1620	4.32	0.1793	2.63	0.0965	1.75	0.0608	1.02	0.0337	0.68	0.0161
Garton	6.84	0.2450	2.15	0.1185	1.40	0.0796	1.11	0.0575	0.84	0.0426	0.67	0.0285
Milford	6.91	0.2582	5.45	0.2396	3.89	0.2020	2.69	0.1251	1.45	0.0644	0.98	0.0393
Onward	7.34	0.2717	2.09	0.0941	1.57	0.0756	1.26	0.0674	0.94	0.0561	0.80	0.0358
Russet	5.44	0.1899	2.10	0.1036	1.22	0.0538	0.88	0.0334	1.02	0.0445	0.41	0.0151
Spitfire	5.74	0.1683	2.13	0.0751	0.93	0.0469	1.20	0.0499	0.78	0.0288	0.48	0.0131

Table A1. Average height and fresh weight of tops of nine varieties of oats grown in loam treated with 5 rates of TCA. Milford is the most tolerant and Algerian the least tolerant of the herbicide.(See Fig.1 for graphical presentation of height data of 5 varieties.)

Oat Variety	Percentage Germination Interim 5 Days	Percentage Germination Final 8 Days	* Visual Assessment of Height Variations
Abundance	52	57	2
Algerian	97	98	5
Binder	69	71	2
Dun	84	99	2
Garton	96	97	4
Milford	99	100	5
Onward	89	94	3
Russet	89	90.	3
Spitfire	43	49	1

* 5 = little variation; 1 = much variation.

Table A 2. Oat seed vitality and growth variation. Algerian and Milford obviously the most satisfactory lines for use as plant indicators.

Source	D.F.	Rain (inches) 0.5 M.S.	1 M.S.	2 M.S.	4 M.S.		
Blocks	1	193.2	22.0	70.7	0.3		
Horizon (H)	3	17043.1 xxx	4426.6 xxx	1308.1 xx	669.1 x		
H x Blocks	3	307.4	115.5	36.2	62.0	Pooled Error (12 D.F.)	130.3
TCA (T)	5	4200.7 xxx	8258.4 xxx	11044.5 xxx	8305.0 xxx		
T x H	15	427.6 xxx	716.1 xxx	555.0 xxx	162.4		
(T, T x H) x Blocks	20	131.4	135.4	33.4	71.7	Pooled Error (80 D.F.)	93.0

Manawatu Silt Loam

Source	D.F.	Rain (inches) 0.5 M.S.	1 M.S.	2 M.S.	4 M.S.		
Blocks	1	54.1	47.1	34.4	295.9		
Horizon (H)	3	4238.7 xxx	957.4 xx	391.4	58.4		
H x Blocks	3	98.0	318.1	45.3	39.9	Pooled Error (12 D.F.)	125.3
TCA (T)	5	4701.7 xxx	3834.5 xxx	6651.8 xxx	3877.7 xxx		
T x H	15	396.7 xxx	223.7 xxx	157.2 x	268.2 xxx		
(T, T x H) x Blocks	20	77.7	82.0	39.9	94.1	Pooled Error (80 D.F.)	73.4

Foxton Loamy Sand

Source	D.F.	Rain (inches) 0.5 M.S.	1 M.S.	2 M.S.	4 M.S.		
Blocks	1	15.4	120.0	85.0	0.1		
Horizon (H)	3	12647.0 xxx	5100.7 xxx	1017.8 x	1499.1 x		
H x Blocks	3	255.8	431.2	339.3	133.4	Pooled Error (12 D.F.)	289.9
TCA (T)	5	4026.9 xxx	6926.0 xxx	10141.9 xxx	6382.2 xxx		
T x H	15	904.1 xxx	435.0 xxx	271.8 xx	295.4 xxx		
(T, T x H) x Blocks	20	86.3	35.1	118.2	139.1	Pooled Error (80 D.F.)	94.7

Table A5a. Analyses of variance in Algerian oat height. (x $P<0.05$; xx $P<0.01$; xxx $P<0.001$.)

(See page 24 for details.)

Source	D.F.	Rain (inches) 0.5 M.S.	1 M.S.	2 M.S.	4 M.S.		
Blocks	1	5.2	3.9	15.7	96.3 x		
Horizon (H)	3	14921.7 xxx	8582.3 xxx	1878.0 xxx	160.6 xxx		
H x Blocks	3	25.8	24.3	8.0	5.0	Pooled Error (12 D.F.)	15.8
TCA (T)	5	3991.5 xxx	6888.3 xxx	10439.8 xxx	7326.4 xxx		
T x H	15	996.3 xxx	723.8 xxx	224.2 xxx	118.5 xxx		
(T, T x H) x Blocks	20	14.2	12.6	20.8	36.0	Pooled Error (80 D.F.)	20.9

Manawatu Silt Loam

Source	D.F.	Rain (inches) 0.5 M.S.	1 M.S.	2 M.S.	4 M.S.		
Blocks	1	23.4	39.5	16.5	0.0		
Horizon (H)	3	8754.9 xxx	4587.3 xxx	727.5 xxx	141.5 x		
H x Blocks	3	9.2	17.1	104.4	15.0	Pooled Error (12 D.F.)	36.4
TCA (T)	5	5853.8 xxx	8494.7 xxx	9630.1 xxx	4487.7 xxx		
T x H	15	612.6 xxx	232.2 xxx	191.1 xxx	140.3 xx		
(T, T x H) x Blocks	20	28.3	23.9	35.7	101.6	Pooled Error (80 D.F.)	47.4

Foxton Loamy Sand

Source	D.F.	Rain (inches) 0.5 M.S.	1 M.S.	2 M.S.	4 M.S.		
Blocks	1	125.7	5.3	12.7	40.2		
Horizon (H)	3	13127.5 xxx	8697.8 xxx	1368.2 xxx	469.6 xxx		
H x Blocks	3	19.5	18.0	38.4	56.3	Pooled Error (12 D.F.)	33.1
TCA (T)	5	5283.5 xxx	6632.9 xxx	10126.4 xxx	6586.1 xxx		
T x H	15	1005.8 xxx	993.7 xxx	243.2 xxx	229.3 xxx		
(T, T x H) x Blocks	20	25.9	35.5	47.7	101.4	Pooled Error (80 D.F.)	52.6

Table A5b. Analyses of Variance in Milford oat height. (x $P<0.05$; xx $P<0.01$; xxx $P<0.001$.)

(See page 24 for details.)

(a)

Source	D.F.	Makarua Peaty Loam M.S.	Manawatu Silt Loam M.S.	Foxton Loamy Sand M.S.	Pooled Main Effects M.S.	Interaction with Soils Source	D.F.	M.S.
Blocks	1	82.3	347.5	142.0	523.5	Soil (S)	2	18433.1 xxx
Horizons (H)	3	13069.6 xxx	3208.5 xxx	9078.9 xxx	22584.7 xx	S x H	6	1368.0 xxx
Rain (R)	3	2133.5 xxx	592.9 xxx	536.9	2512.1 xx	S x R	6	377.2 x
H x R	9	3459.1 xxx	812.5 xxx	3728.6 xxx	6822.3 xx	S x H x R	18	588.9 xxx
Error	15	117.8	105.8	237.2	242.6	Pooled Error	272	89.0
TCA (T)	5	28549.0 xxx	8150.9 xxx	25704.2 xxx	70698.7 xxx	S x T	10	852.7 xxx
T x H	15	970.4 xxx	442.9 xxx	802.6 xxx	1794.2 xxx	S x T x H	30	210.8 xxx
T x R	15	1086.5 xxx	304.9 xxx	590.9 xxx	1226.7 xxx	S x T x R	30	377.8 xxx
T x H x R	45	296.9 xxx	201.0 xxx	367.9 xxx	559.5 xxx	S x T x H x R	90	153.1 xxx
Error	80	93.0	73.4	94.7	(89.0)	Pooled Error	272	89.0

(b)

Source	D.F.	Makarua Peaty Loam M.S.	Manawatu Silt Loam M.S.	Foxton Loamy Sand M.S.	Pooled Main Effects M.S.	Interaction with Soils Source	D.F.	M.S.
Blocks	1	81.1	7.7	88.3	81.4	Soils (S)	2	3929.8 xxx
Horizons (H)	3	15941.0 xxx	8841.4 xxx	13025.8 xxx	36075.2 xxx	S x H	6	866.5 xxx
Rain (R)	3	786.2 xxx	822.6 xxx	62.1	651.4 xxx	S x R	6	511.6 xxx
H x R	9	3200.5 xxx	1789.9 xxx	3545.8 xxx	8028.0 xxx	S x H x R	18	254.1 xxx
Error	15	15.3	33.9	32.8	42.0	Pooled Error	272	36.3
TCA (T)	5	26722.6 xxx	27449.4 xxx	27630.3 xxx	81347.1 xxx	S x T	10	227.6 xxx
T x H	15	1007.1 xxx	665.7 xxx	1224.3 xxx	2525.5 xxx	S x T x H	30	185.8 xxx
T x R	15	641.2 xxx	338.9 xxx	332.9 xxx	977.4 xxx	S x T x R	30	167.8 xxx
T x H x R	45	351.9 xxx	170.2 xxx	415.9 xxx	691.5 xxx	S x T x H x R	90	123.2 xxx
Error	80	20.9	47.4	46.3	(36.3)	Pooled Error	272	36.3

Table A6. Analysis of variance in oat height. (a) Algerian (b) Milford. (x $P<0.05$; xx $P<0.01$; xxx $P<0.001$.)

(See page 24 for details.)

Soil Type	Horizon (ins.)	TCA Concn. (p.p.m.)	Avg.Plant Height* (mm.) Milford	Algerian
Makarua Peaty Loam	0 - 1	57.6	30.1	27.2
	1 - 2	12.7	119.5	43.4
	3 - 4	0.5	206.4	154.6
	5 - 6	0.0	214.6	192.3
	All Horizons	70.8	570.6	417.5
Manawatu Silt Loam	0 - 1	46.1	26.9	25.8
	1 - 2	18.4	81.1	35.1
	3 - 4	2.3	186.4	115.1
	5 - 6	0.0	172.6	180.9
	All Horizons	66.8	467.0	356.9
Foxton Loamy Sand	0 - 1	68.0	30.2	24.9
	1 - 2	31.5	51.5	34.9
	3 - 4	2.8	219.9	111.9
	5 - 6	0.0	204.8	206.3
	All Horizons	102.3	506.4	378.0

* Sum of two replicates

Table A7. Distribution of T C A applied to the soil surface at the rate of 100 lb.per acre in an aqueous solution equivalent to 300 gals.per acre (equals 0.013 surface inches of water), and subjected to no rain treatment.

Period of Heat Treatment		6 Days			18 Days			39 Days			58 Days		
Soil Type	Temperature F \ T C A lb./ac.	50	100	Total	50	100	Total	50	100	Total	50	100	Total
Makarua Peaty Loam	50	1.53	1.83	3.36	1.47	1.71	3.18	1.41	1.53	2.94	1.16	1.28	2.44
	62	1.55	1.78	3.33	1.45	1.67	3.12	1.36	1.50	2.86	1.08	1.30	2.38
	74	1.55	1.81	3.36	1.49	1.69	3.18	1.29	1.46	2.75	1.09	1.23	2.32
	All Temps.	4.63	5.42	10.05	4.41	5.07	9.48	4.06	4.49	8.55	3.33	3.81	7.14
Manawatu Silt Loam	50	1.62	1.82	3.44	1.48	1.70	3.18	1.35	1.58	2.93	1.09	1.25	2.34
	62	1.59	1.88	3.47	1.43	1.63	3.06	1.25	1.42	2.67	0.88	1.23	2.11
	74	1.60	1.85	3.45	1.49	1.66	3.15	1.15	1.36	2.51	0.76	1.00	1.76
	All Temps.	4.81	5.55	10.36	4.40	4.99	9.39	3.75	4.36	8.11	2.73	3.48	6.21
Foxton Loamy Sand	50	1.62	1.84	3.46	1.50	1.77	3.27	1.48	1.58	3.06	1.28	1.32	2.60
	62	1.65	1.87	3.52	1.48	1.67	3.15	1.39	1.53	2.92	1.22	1.35	2.57
	74	1.60	1.82	3.42	1.47	1.65	3.12	1.36	1.48	2.84	1.13	1.24	2.37
	All Temps.	4.87	5.53	10.40	4.45	5.09	9.54	4.23	4.59	8.82	3.63	3.91	7.54
All Soils	50	4.77	5.49	10.26	4.45	5.18	9.63	4.24	4.69	8.93	3.53	3.85	7.38
	62	4.79	5.53	10.32	4.36	4.97	9.33	4.00	4.45	8.45	3.18	3.88	7.06
	74	4.75	5.48	10.23	4.45	5.00	9.45	3.80	4.30	8.10	2.98	3.47	6.45
	All Temps.	14.31	16.50	30.81	13.26	15.15	28.41	12.04	13.44	25.48	9.69	11.20	20.89

Table A8. Effect of temperature on the rate of inactivation of T C A in soils. Figures presented are the logarithms

(p.p.m.) of T C A determined photometrically.

Period of Heat Treatment		6 Days			18 Days			39 Days			58 Days		
Soil Type	Temperature F / T C A lb./ac.	50	100	Total	50	100	Total	50	100	Total	50	100	Total
Makarua Peaty Loam	50	1.95	1.76	3.71	2.02	1.86	3.88	2.16	1.98	4.14	2.15	2.11	4.26
	62	1.94	1.79	3.73	2.05	1.95	4.00	2.08	2.00	4.08	2.17	2.10	4.27
	74	1.93	1.77	3.70	2.00	1.88	3.88	2.11	2.03	4.14	2.17	2.13	4.30
	All Temps.	5.82	5.32	11.14	6.07	5.69	11.76	6.35	6.01	12.36	6.49	6.34	12.83
Manawatu Silt Loam	50	1.90	1.77	3.67	2.03	1.89	3.92	2.10	2.05	4.15	2.18	2.14	4.32
	62	1.92	1.74	3.66	2.06	1.95	4.01	2.14	2.06	4.20	2.23	2.15	4.38
	74	1.91	1.76	3.67	2.02	1.92	3.94	2.17	2.09	4.26	2.25	2.21	4.46
	All Temps.	5.73	5.27	11.00	6.11	5.76	11.87	6.41	6.20	12.61	6.66	6.50	13.16
Foxton Loamy Sand	50	1.89	1.76	3.65	2.01	1.82	3.83	2.03	1.96	3.99	2.13	2.11	4.24
	62	1.88	1.75	3.63	2.03	1.92	3.95	2.08	2.00	4.08	2.15	2.13	4.28
	74	1.91	1.78	3.69	2.04	1.93	3.97	2.09	2.03	4.12	2.18	2.14	4.32
	All Temps.	5.68	5.29	10.97	6.08	5.67	11.75	6.20	5.99	12.19	6.46	6.38	12.84
All Soils	50	5.74	5.29	11.03	6.06	5.57	11.63	6.29	5.99	12.28	6.46	6.36	12.82
	62	5.74	5.28	11.02	6.14	5.82	11.96	6.30	6.06	12.36	6.55	6.38	12.93
	74	5.75	5.31	11.06	6.06	5.73	11.79	6.37	6.15	12.52	6.60	6.48	13.08
	All Temps.	17.23	15.88	33.11	18.26	17.12	35.38	18.96	18.20	37.16	19.61	19.22	38.83

Table A9. Effect of temperature on the rate of inactivation of T C A in different soils. Figures presented are the log average heights, expressed as percentages of controls, of two replicates of 16 Milford oats.

T C A lb./ac.	Active Constituent (x)	Oat Yields R_1	Oat Yields R_2	Total (y)	Active Constituent (x)	Oat Yields R_1	Oat Yields R_2	Total (y)
50	41.4	40.44	39.59	80.03	42.1	40.75	37.25	78.00
	39.0	43.75	40.43	84.18	44.5	38.45	36.45	74.90
	40.2	42.50	39.35	81.85	40.1	37.70	43.25	81.95
	120.6			246.06	126.7			234.85
100	50.4	36.10	40.73	76.83	46.7	39.40	44.05	83.45
	42.7	46.04	42.79	88.83	45.0	46.25	39.65	85.90
	45.8	41.83	42.19	84.02	37.7	48.10	43.85	91.95
	138.9			249.68	129.4			261.30
Total	259.5			495.74	256.1			496.15

Analysis of Covariance

	x^2	xy	y^2	Residue	D.F.	M.S.	F	Signif.
Treatments	55.81	10.61	0.53	62.91	1	62.91	10.50	P=.01
Residual Error	32.90	51.78	81.60	0.11	3	0.03		
Treatments plus Error	88.71	41.17	82.13	63.02				
Comparable Replicate Error				53.80	6	8.97		
Pooled Error				53.91	9	5.99		

Analysis of Covariance

	x^2	xy	y^2	Residue	D.F.	M.S.	F	Signif.
Treatments	1.21	11.90	99.18	121.91	1	121.91	7.21	P<.05
Residual Error	55.44	57.07	80.68	21.93	3	7.31		
Treatments plus Error	56.65	45.17	179.86	143.84				
Comparable Replicate Error				130.29	6	21.72		
Pooled Error				152.22	9	16.91		

TableA12Comparison of encircled points of Fig.12;i.e. the extreme values of the curve for the 50 lb. application rate of TCA and the corresponding nearest on the 100 lb. curve. The analyses of covariance indicates that the differences in oat height at an approximately equal concentration of TCA is real for both the silt loam and loamy sand. This has been attributed to nitrogen accumulation and not an