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The tolerance of subterranean clover (Trfolium subterranean L.) to chlorinated phenoxyacetic derivatives

G. R. W. Meadly

G. A. Pearce

The Tolerance of Subterranean Clover (Trifolium subterraneum L.) to Chlorinated Phenoxyacetic Derivatives

By G. R. W. MEADLY, M.Sc., Officer in Charge, Weeds and Seeds Branch, and G. A. PEARCE, B.Sc. (Agric.), Botanist

THE selective phytocidal properties of certain growth-regulating substances were confirmed when Slade, Templeman and Sexton (1945) found in 1940 that applications of 25 lb. naphthylacetic acid per acre to oats weedy with charlock (Brassica sinapis) killed the weed without causing permanent injury to the crop. Within two years of this work investigators in both England and America had recognised the strong growth-regulatory and herbicidal effects of chlorinated phenoxyacetic derivatives.

Many chemicals were screened for selective herbicidal properties and development occurred along two main lines. In America the derivatives of 2,4-dichlorophenoxyacetic acid (2,4-D) were favoured while in England, where exploratory work commenced somewhat earlier, the chemical selected for development was 2-methyl-4 chlorophenoxyacetic acid (M.C.P.A.). This was due in part at least to the greater availability in England of chloro-cresol as opposed to chloro-phenol.

In general, monocotyledons including grasses are resistant to these chemicals and broad-leaved herbaceous dicotyledons are susceptible. This is a generality with many exceptions, however, and much work in recent years has been undertaken to define the tolerance of crop and pasture species as well as the dosage required for specific weeds. In Western Australia, the main cultivated species involved are cereals and subterranean clover.

A considerable amount of research has been undertaken relative to safe levels of treatment for cereals and although some variations in results have been reported the practical position is relatively clear. Susceptible growth stages are known and it is generally recognised that oats is more likely to be affected than wheat with barley intermediate. Trials undertaken by Meadly (in press) at the Merredin Research Station involved as variables different varieties of cereals, formulations of growth regulators and rates and times of application. With the relatively low amounts of herbicides applied to weeds in cereal crops in this State, usually less than 8 oz. acid equivalent per acre, providing the growth stage of the crop is taken into consideration no significant damage to the cereal is likely.

In a country so dependent on subterranean clover as a component of pastures and a soil improver it is important that information should be available concerning its reactions to the various formulations, particularly during the season of establishment. A considerable amount of work dealing with a number of species of *Trifolium* has been published in England, America and New Zealand. Much of this pertains to perennial species such as white clover (*T. repens*) and red clover (*T. pratense*). It is not intended to review all this literature but to make reference to conclusions drawn by a few of the workers with a view to summarising the findings.

Following field trials Scragg (1953) concluded that the establishment of red clover was not seriously impaired by spraying with sodium or amine salts of M.C.P.A. at 1 lb. acid equivalent per acre, either in an undersown crop or direct reseed. The vigour of plants was reduced but not their numbers. The amine of 2,4-D had a more severe effect on red

clover than both formulations of M.C.P.A. White clover, on the whole, was not greatly reduced nor was there any marked difference between the herbicides. Amine M.C.P.A. appeared to be most and amine 2,4-D least harmful to this species. Greenhouse tests showed that alsike clover and trefoil are very susceptible to herbicides and confirmed that 2,4-D is more harmful than M.C.P.A. to red clover but no more toxic to white clover.

Pot experiments undertaken by Holly (1953) indicated that the resistance of all grasses, even in the very early seedling stages is so much greater than that of legumes that the effects on the latter must always be the primary consideration in spraying newly sown seed mixtures. Appreciable mortality of S100 white clover can be caused by doses as low as ½ lb. acid equivalent per acre of M.C.P.A. (sodium) and 2,4-D (triethanolamine) while 4 oz. of either compound can cause reduction in growth. In the case of S123 red clover growth reduction occurred with ½ lb. 2,4-D (triethanolamine) or 1 lb. of M.C.P.A. (sodium) per acre. Both grasses and clovers showed increased resistance to the two compounds with increasing age of the seedlings. As with Scragg's trials M.C.P.A. (sodium) appeared to be slightly less toxic than 2,4-D (triethanolamine) with red clover but the reverse applied with white clover. Holly could not detect differences in reaction of strains of either clover. He drew attention to the fact that commercial M.C.P.A. (sodium) formulations containing a variety of additional compounds can be more toxic than the 2,4 isomer alone when applied in low volume to S123 red clover.

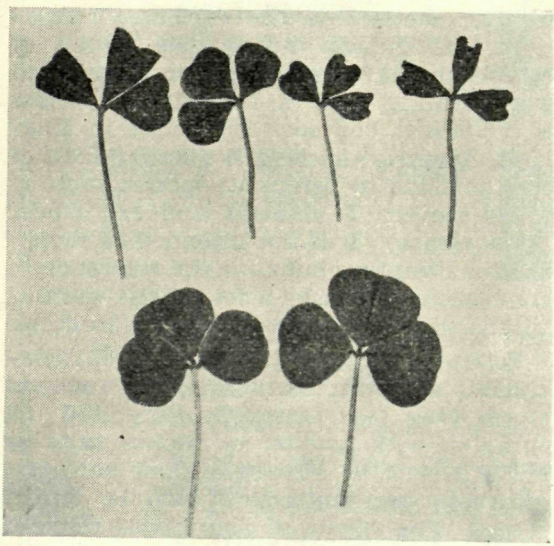

Fig. 1.—Normal leaves of subterranean clover (below), compared with those affected by 2,4-D

Experiments reported by Ochiltree (1954) largely support the previous findings. He concluded that broad and late flowering red clovers are much more susceptible to damage at all stages of growth from the application of 2,4-D derivatives than from the M.C.P.A. derivatives tested at equivalent rates of application. Seedlings of white clover are more susceptible to M.C.P.A. than to 2,4-D derivatives but the difference is less marked than the reverse experience with red clover.

Clarke (1953), following work in British Columbia, summed up that generally, in the seedling year legumes are more tolerant to M.C.P.A. than to 2,4-D formulations. Several instances of damage with M.C.P.A. ester have been reported, however, and many have shown the sodium salt of M.C.P.A. to be relatively ineffective for weed control. It would appear, therefore, that the amine of M.C.P.A. will find a wide use and that amine of 2,4-D will continue to be employed, particularly where very light rates are permissable. During the last two years the amine of M.C.P.A. has gained favour in America and Canada when cereals undersown to legumes are involved. Marshall and Van Geluwe (1953) reported greater tolerance of legumes generally to the diethanolamine salt of M.C.P.A. compared with the sodium salt of M.C.P.A. and the triethanolamine salt of 2,4-D. The National Weeds Committee of Canada (Western Section) (1955) in its recommendations for 1956, states that grain crops undersown with alfalfa, red and alsike clover can be treated to kill susceptible weeds with M.C.P.A. amine at 2-4 oz. acid equivalent per acre or with the sodium salt at 3-6 oz. per acre.

Since the advent of pasture spraying for weed control in New Zealand, detailed work has been carried out on the tolerance of clover species to herbicides including the hormone-like chemicals. Fitzgerald (1953) reported that preparations

Fig. 2.—Showing the equipment used for spraying in the clover tolerance trials at Narrogin (July 20, 1955)

of M.C.P.A. appeared to be less severe on clovers than those of 2,4-D and that at the seedling stage subterranean clover was more tolerant than white clover with red clover intermediate. All species became more tolerant with increasing age.

Following further work Lynch (1954) supported Fitzgerald's observations and indicated increasing severity in the order of metallic salts, amine salts and esters. Unfortunately the water-based polyethylene glycol esters and esters of M.C.P.A. caused as much damage as the more frequently used ethyl, butyl and butoxyethanol esters. White clover alone seemed more susceptible to M.C.P.A in the young stages but once past, say six months growth, white clover falls into line with the other clover species and M.C.P.A. has less effect on it than 2,4-D. New Zealand trials and experience are reported fully by Maclean (1955) and Maclean, Matthews and Fitzgerald (1955), their conclusions being similar to those of Fitzgerald and Lynch.

Following trials at Walpeup, Longerenong and Werribee, Hore (1954) reported on the tolerance of cereals and barrel medic (*Medicago tribuloides*) to various formulations. The population of barrel medic sown with a wheat crop was significantly reduced by the application per acre of 2 oz. acid equivalent ester 2,4-D, 4 oz. sodium M.C.P.A. or 4 oz. amine 2,4-D. With trials under pasture conditions Johnston, Russell, Preston and Done (1954) found that the reaction of subterranean clover varied according to the time of spraying, the formulation used and the strain.

Applications of up to 16 oz. acid equivalent per acre of the sodium salt and amine of M.C.P.A. did not noticeably affect clover density, leaf size or flowering at any rate or at any time of spraying. All formulations of 2,4-D at all rates including 4 oz. acid equivalent per acre affected subterranean clover. It was considered that only at the 1 lb. rates reduction in the number of clover plants occurred.

Meadly and Pearce (1955) noted while undertaking some exploratory work in 1952 on a pasture dominated by the Dwalganup strain of subterranean clover that plants, except at the small seedling stage, were not readily killed by growth-regulating herbicides. Many plants persisted following the application of 2 lb. acid equivalent per acre of sodium M.C.P.A. and 1½ lb. amine 2,4-D. It was apparent, however, that even light applications as low as 4 oz. acid equivalent per acre retarded the date of flowering. At Toodyay in 1954 a crop of wheat undersown with Dwalganup subterranean clover was sprayed at rates of 4, 8, 12, 16, 24 and 32 oz. acid equivalent per acre of sodium M.C.P.A. amine 2,4-D and ethyl ester 2,4-D. With M.C.P.A. the yield of seed per subterranean clover plant was significantly depressed for all treatments above the 16 oz. level. In the case of amine 2,4-D the depression occurred with all treatments above the

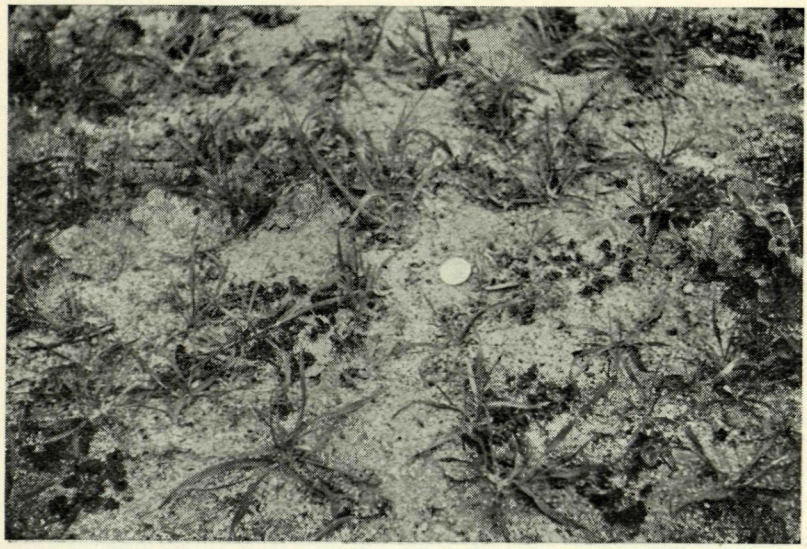

Fig. 3.—Growth stage of plants at Narrogin when sprayed on July 27. The coin is a florin

4 oz. level while with the ester 2,4-D all treatments caused a significant reduction in seed yield.

FURTHER TRIALS

A practice often followed for the establishment of subterranean clover, particularly the Dwalganup strain, is to sow along with a cereal. Frequently, weed growth in the crop warrants spraying but any appreciable effect on seed formation of the clover in the first year can affect seriously the rate of establishment. Unlike when dealing with old clover land few, if any, dormant seeds from the initial sowing are present in the soil at the commencement of the second season when regeneration is dependent upon seed formed in the season of sowing. Most overseas investigations have dealt with white and red clovers and trials in which subterranean clover has been included have pertained practically entirely to pasture conditions. When subterranean clover has received attention, the main considerations have been the lethal dosage at different growth stages and also the effect on yield. Although these aspects are important to Western Australian agriculture, the main question to be answered is the reaction to various formulations of clover sown with a cereal crop. Experience has shown that weeds are usually more susceptible to growth-regulating herbicides when growing with cereals than under pasture conditions. It is reasonable to assume that subterranean clover would react in a similar manner. When the clover plants are beyond the young seedling stage the rates usually applied are unlikely to cause a high proportion of mortality but the effect on floral development and time of flowering can be a major factor influencing seed setting and consequently re-establishment the following season. In the lower rainfall districts the short growing season is barely adequate for the early Dwalganup strain and any delay in flowering must be viewed with concern.

Tentative recommendations were possible following the Toodyay trial already referred to, but bearing in mind the importance of any decision regarding spraying, it was decided to extend the scope of the trials and undertake them at four different centres— Geraldton, Bolgart, Toodyay and Narrogin. By widely spacing the trial centres and undertaking the work in successive seasons it was considered that most of the difficult variables associated with such research could be eliminated. Owing to rain falling shortly after application of the chemicals it was necessary to discard the results obtained from Bolgart.

The treatments and layout were the same at each centre. The chemicals applied were 4, 8, 12, 16, 24 and 32 oz. acid equivalent per acre of sodium M.C.P.A. triethanolamine 2,4-D and ethyl ester 2,4-D. For convenience these will be referred to as M.C.P.A., amine and ester and

references to ounces will imply acid equivalent per acre. No check was made of the 2-4 isomer content of the M.C.P.A. Each plot was 12ft. wide and three chains long and each treatment was replicated three times. Spraying was carried out with a 12ft. boom, seven gallons of solution per acre being applied. The centre 6ft. of each plot was used for assessing results. At each site the chemical was applied at the growth stage of weeds and crop considered most advantageous for the control of the weeds, at the same time presenting minimum risk of crop damage. The wheat was six to eight inches high and stooling but there was some difference in the size of the clover plants at the different centres. By sowing the clover at 8 lb. per acre an adequate emergence was obtained for assessment of results.

Observations and recordings were made with a view to defining effects on the growth and population of the clover plants, number and periodicity of flowers, seed formation, size of seed and germination. The most satisfactory way of obtaining and recording this information presented some difficulties, particularly with reference to flowering periods and numbers of flowers produced. Establishment counts of clover seedlings were made at the time of spraying, 3 x 4 square links being used. With three replications, plants on 36 square links were recorded for each treatment. Survival counts were made later, on the same quadrats which were also used subsequently for the seed production estimations. With a strain having the spreading habit of Dwalganup, burrs arising from plants within the quadrats were formed beyond their confines but at the same time intrusions from plants outside also occurred. It was found impracticable to trace all burrs arising from the recorded plants and in view of the reciprocal interchange of runners, all burrs within the quadrats were harvested. Following separation of the burrs from the soil, the seeds were removed by means of a small motor driven thresher, specially constructed for handling experimental quantities.

The same quadrats were also used for the flower counts. The first was made as soon as flowers appeared and the counts were repeated at approximately weekly intervals until flowering was virtually completed. There was no way of ensuring that the same flower heads were not recorded at successive counts but, as the

Table 1—TOODYAY.

Treatments.	Initial Plant Count.	Final Plant Count.	Total Flower Count.	Total Seed Count.	Average Seeds per Initial Plant.	Total Seed Weight gms.	Germination per per cent.
4 ozs. Sodium MCPA	153	107	505	5,917	36·9	63·52	97
8 " " "	153	96	551	6,417	41·9	60·83	92
12 " " "	153	111	363	4,625	30·2	56·90	92
16 " " "	143	93	313	4,385	30·7	56·74	92
24 " " "	149	124	292	4,095	27·5	54·25	94
32 " " "	156	116	252	2,664	17·1	43·16	92
4 ozs. Amine 2, 4-D	149	112	412	4,513	30·3	50·74	90
8 " " "	126	120	318	3,439	27·3	60·01	93
12 " " "	134	98	255	3,353	25·0	60·32	92
16 " " "	144	121	313	3,707	25·7	55·44	94
24 " " "	139	106	219	2,221	16·0	40·48	90
32 " " "	151	119	150	1,098	7·4	43·48	89
4 ozs. Ester 2, 4-D	151	115	395	4,099	27·1	66·82	91
8 " " "	133	105	340	3,462	26·0	49·44	90
12 " " "	136	117	341	2,838	20·9	55·61	92
16 " " "	135	110	214	2,469	14·9	48·74	91
24 " " "	162	132	254	2,039	12·6	45·82	93
32 " " "	150	114	223	1,425	11·9	43·00	89
CONTROL 1	144	106	927	3,986	27·7	63·81	91
" 2	138	98	905	5,343	38·7	54·86	93
" 3	157	101	927	4,586	29·2	55·85	96

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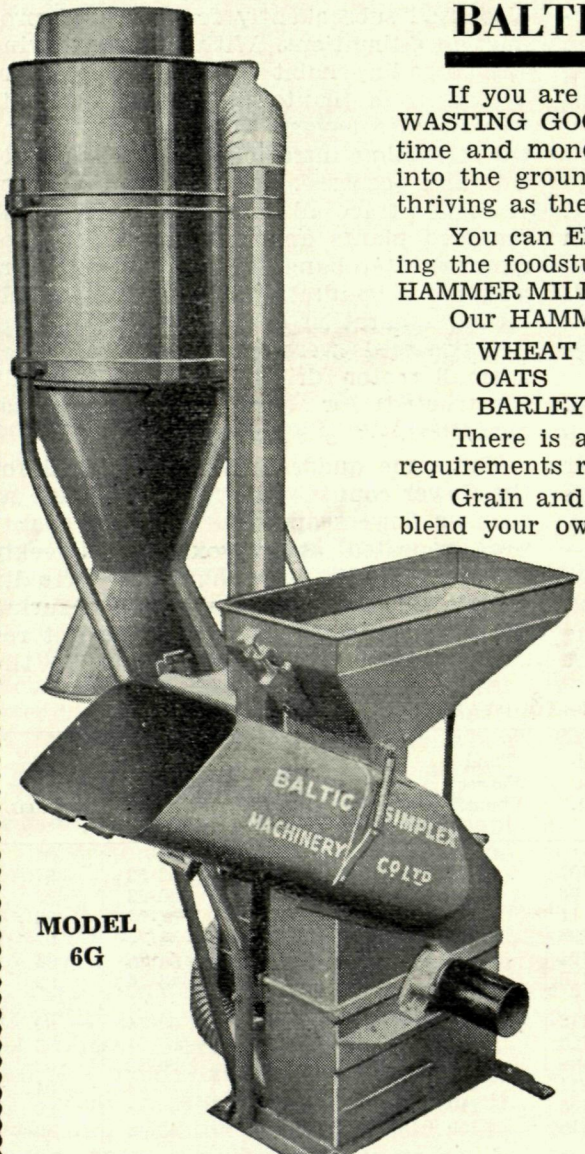

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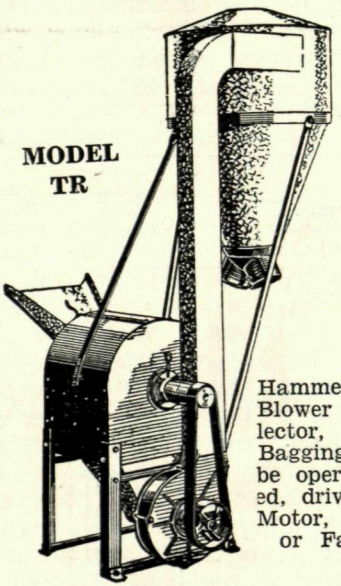

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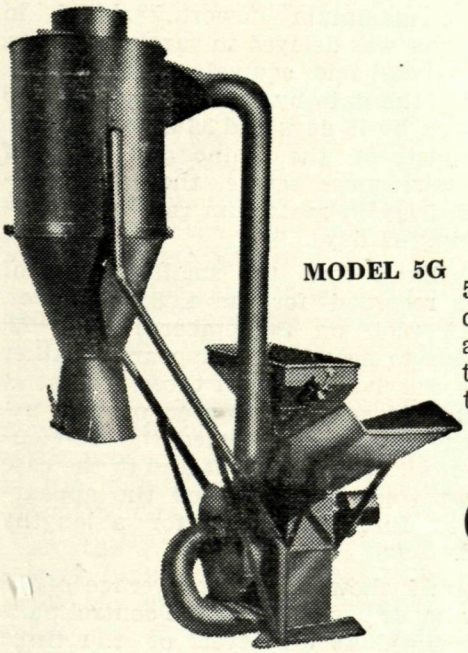

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Table 2—TOODYAY.

Treatments.	Plants (36 sq. links).	Flower Heads.							Total effective flower heads.	Effective flower heads per plant.
	2-9-55.	2-9-55.	12-9-55.	22-9-55.	29-9-55.	10-10-55.	21-10-55.	3-11-55.		
4 ozs. Sodium MCPA	153	93	19	28	**195**	164	86	13	505	3·3
8 „ „ „	153	79	18	24	158	**233**	86	22	541	3·5
12 „ „ „	153	86	2	3	67	**169**	89	33	363	2·4
16 „ „ „	143	86	....	....	34	**166**	94	19	313	2·2
24 „ „ „	149	90	....	....	11	**132**	109	40	292	2·0
32 „ „ „	156	95	....	....	1	**83**	**118**	50	252	1·6
4 ozs. Amine 2, 4-D	149	84	....	....	96	**175**	101	40	412	2·8
8 „ „ „	126	80	....	....	28	**154**	101	35	318	2·5
12 „ „ „	134	80	....	....	3	90	**111**	51	255	1·9
16 „ „ „	144	97	....	....	8	142	**139**	32	321	2·2
24 „ „ „	139	79	....	....	....	68	**97**	54	219	1·6
32 „ „ „	151	89	....	....	....	15	54	**81**	150	1·0
4 ozs. Ester 2, 4-D	151	101	....	....	28	**207**	109	51	395	2·6
8 „ „ „	133	81	....	....	....	**151**	109	80	340	2·5
12 „ „ „	136	91	....	....	....	113	**154**	74	341	2·5
16 „ „ „	135	71	....	....	....	73	**94**	47	214	1·6
24 „ „ „	162	106	....	....	....	44	**143**	67	254	1·5
32 „ „ „	150	93	....	....	....	44	**95**	84	223	1·5
CONTROL 1	144	93	217	**226**	175	95	78	43	927	6·4
„ 2	138	95	181	**199**	190	118	81	41	905	6·5
„ 3	157	105	196	**225**	180	107	88	26	927	5·9

same procedure was followed with all treatments, the figures obtained enabled comparisons to be made between flower population and time of flowering for the various treatments.

Figures relative to each centre have been tabulated and are given as totals for the 36 square links of each treatment utilised. The method found most convenient for presenting the effect of treatment on seed yield was to graph ounces acid equivalent per acre of each chemical against the average of log $(x + 1)$ with x being the number of seeds per four square links.

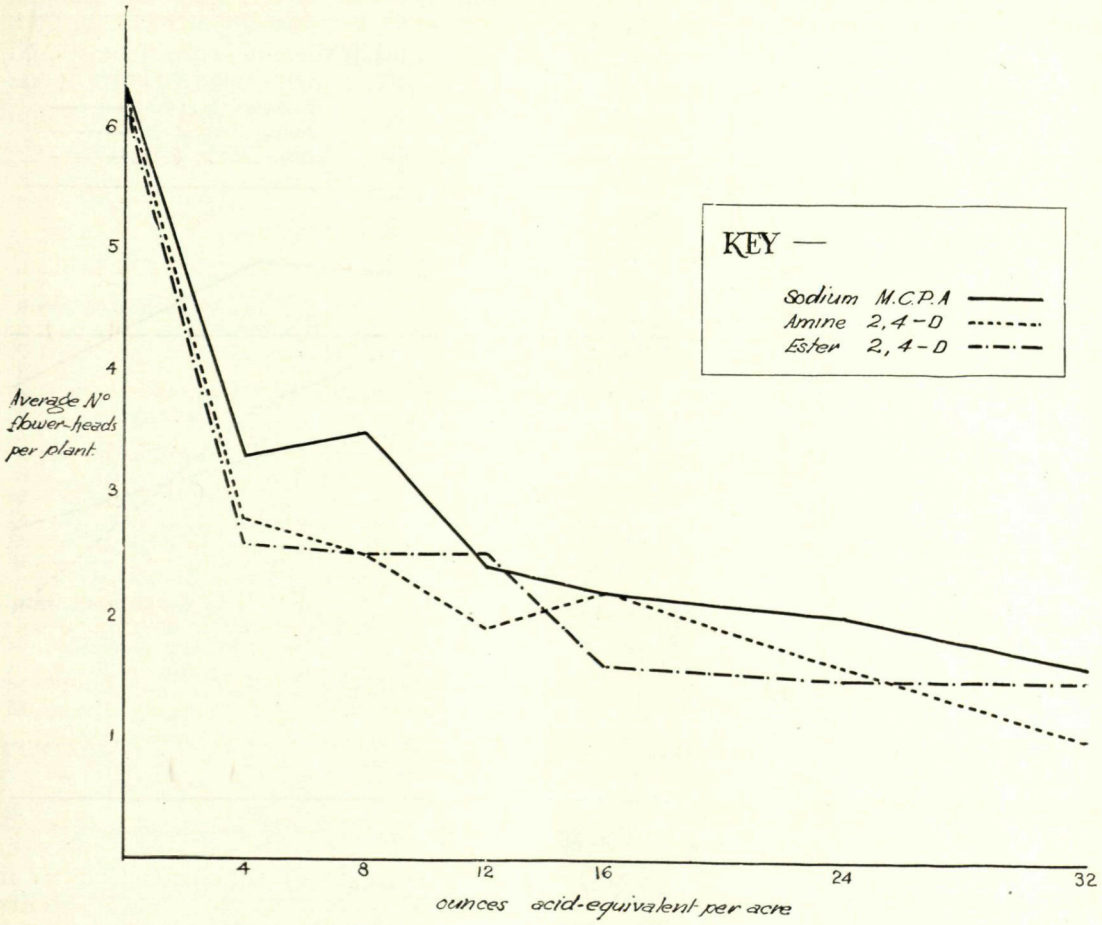
TOODYAY

The site consisted of a loamy sand previously growing York Gum (*Eucalyptus foecunda* var. *loxophloeba*), Wandoo (*E. redunca* var. *elata)* and Jam (*Acacia acuminata*). A previous cereal crop was grown in 1954 immediately following clearing.

Spraying was carried out at Toodyay on September 2, a little later than planned due to boggy conditions. The clover plants were more advanced than at other centres being up to six inches across, and flowering had commenced. Flowering was depressed and retarded by all treatments as shown by Table 2 which records the numbers of flowers present on the various plots at approximately weekly intervals from the date the chemicals were applied. Besides the reduction in total number of flowers compared with the controls the date of maximum flowering shown in heavy type was delayed to varying extents. Four ounces acid equivalent of M.C.P.A. retarded the date by seven days; 8, 12, 16 and 24 oz. by 18 days and 32 oz. by 29 days. The effects of the amine and ester of 2,4-D were more severe, the largest recorded delay in maximum rate of flowering being 42 days.

It is likely that the small number of flowers recorded for the 4, 8 and 12 oz. M.C.P.A. plots on September 12 and 22, were due to new flowers appearing rather than to survival of any of those present at the time of spraying. Based on this assumption, spraying arrested the development of all flowers present and in the case of most treatments delayed the appearance of further flowers for a lengthy period.

Graph 1 shows that the average of 6.3 flower heads per plant on the control plots was reduced at all levels of the three formulations used. The tendency was for



— GRAPH 1 —

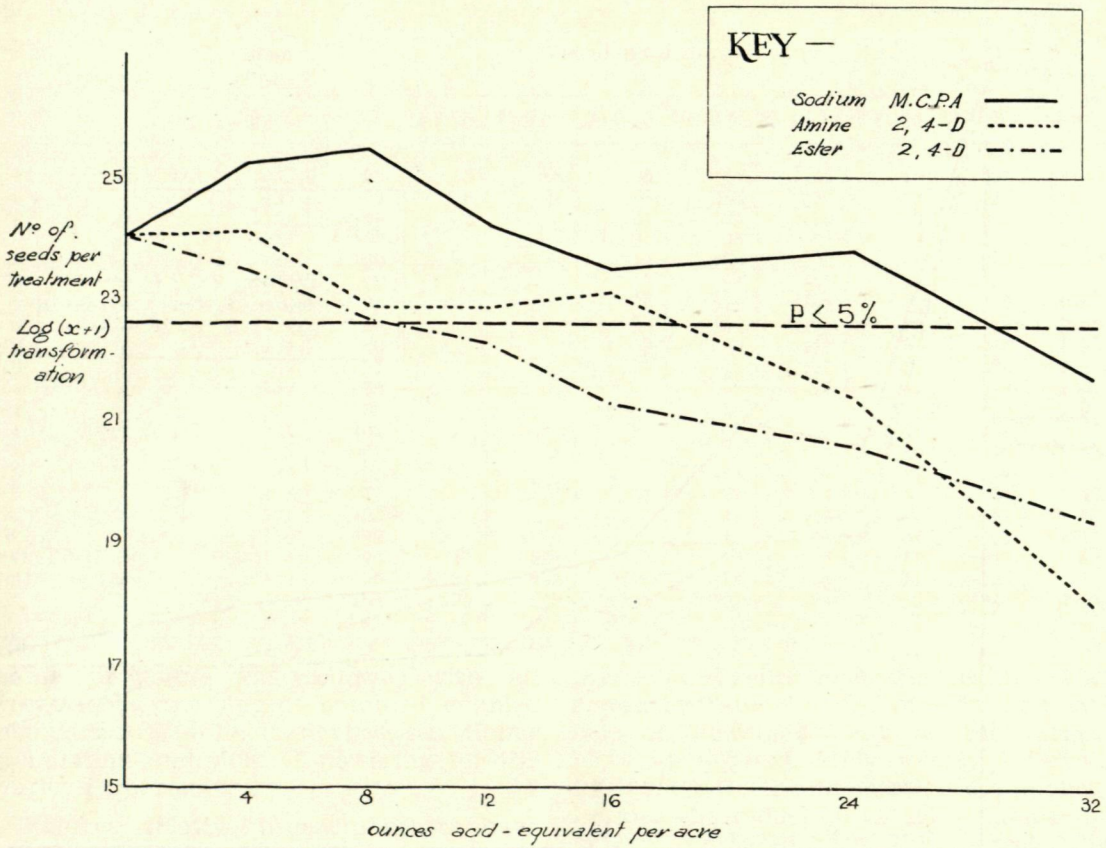
Showing the effects of three formulations at different levels on the average number of flower-heads per plant (Toodyay)

greater reduction with higher rates of application but the three formulations followed a similar pattern.

The initial and final plant counts showed that there was no greater reduction with any of the treatments than with the controls. A proportion of seedling wastage can always be expected as indicated by Meadly (1946). The number of seeds produced was reduced significantly at the higher levels of all treatments as shown by Graph II.

As the plant numbers remained relatively uniform the average number of seeds per plant showed a corresponding reduction. At the $P < 5$ per cent. level a significant reduction in seed numbers occurred above 8 oz. acid equivalent ester 2,4-D, 16 oz. amine 2,4-D and 24 oz. sodium M.C.P.A. Viability of the seed was not affected by any treatment, the germination figures listed in Table I being uniform and high.

The effect on seed formation must be interpreted in relation to the season and unfortunately, from the viewpoint of this experiment, the long growing season of 1955 was inopportune. Based on Prescott's formula of effective rainfall being equivalent to rainfall $0.54 \times (\text{evaporation})^{0.7}$, the average date of closure of the growing season at Toodyay is October 9, and in 1955 the season was extended by four weeks. From Table 2 it can be seen that maximum flowering of untreated plants occurred on September 22, M.C.P.A. treat-



— GRAPH 11 —

seed produced in relation to chemical applied (Toodyay)

ments up to 24 oz. acid equivalent per acre and ester and amine of 2,4-D up to 8 oz. had reached this stage by October 10. Other treatments were appreciably later. In 1955, October 10 was still four weeks in advance of the estimated conclusion of the growing season. There is no doubt that the effects of treatments would have been much more severe in an "average" growing season having a closing date of October 9. With nine of the treatments the flowering peak was reached after October 10 and in 11 cases more flowers were recorded after than before that date. This contention is supported by the earlier work of Meadly and Pearce (1955) at Toodyay in which the yields of subterranean clover seed were significantly depressed by sodium M.C.P.A. treatments above the 16 oz. acid equivalent per acre level, by all treatments of amine 2,4-D above 4 oz. and by all ester 2,4-D treatments including 4 oz. These trials were undertaken in 1954 when the effective growing season closed on September 30.

An increase in seed production with 4 oz. and 8 oz. sodium M.C.P.A. would at first appear to be an anomaly. There was a similar trend at Narrogin, however, and the slight delay in flowering could have resulted in more favourable conditions for seed formation, temperature and light period being possible factors. Again, the stimulation of crops by low rates of growth regulators has been postulated and this possibility cannot be excluded.

NARROGIN

The area used for the trial comprised a gritty sand overlying clay and, during the winter, water remained on the surface for

lengthy periods. The land, which carried mainly sheoak (*Casuarina Huegeliana*) had been cleared for some time but had not previously been sown to clover. Spraying was carried out on July 27, when the clover plants were two to three inches across and mainly at the four trifoliate leaf stage.

Table 3—NARROGIN.

Treatments.	Initial plant counts.	Final plant counts.	Total flower count.	Total seed count.	Average seeds per initial plant.	Germination per cent.
4 ozs. Sodium MCPA	232	232	1,096	18,300	78·9	94
8 " " "	266	259	1,318	14,440	55·5	97
12 " " "	224	213	899	12,935	57·7	97
16 " " "	193	193	669	12,097	62·7	96
24 " " "	209	209	771	10,886	52·1	94
32 " " "	259	241	478	8,890	34·3	93
4 ozs. Amine 2, 4-D	239	239	1,090	10,582	44·3	96
8 " " "	213	212	929	10,503	49·3	92
12 " " "	235	233	469	9,386	39·9	93
16 " " "	182	179	454	9,762	53·6	92
24 " " "	267	247	431	8,432	31·6	92
32 " " "	213	151	224	6,587	30·9	92
4ozs. Ester 2, 4-D	250	250	678	10,128	40·5	93
8 " " "	217	205	224	8,670	39·9	90
12 " " "	204	195	226	7,744	24·0	96
16 " " "	251	147	151	4,756	18·9	91
24 " " "	268	152	131	3,979	14·9	93
32 " " "	234	64	51	1,190	5·1	93
CONTROL 1	182	182	1,044	10,953	60·2	97
" 2	205	205	1,088	11,350	55·4	95
" 3	264	264	978	15,770	59·7	99

Table 4—NARROGIN.

Treatments.	Plants.	Flower heads.							Total effective flower heads.	Effective flower heads per plant.
	20-7-55.	2-9-55.	3-9-55.	16-9-55.	23-9-55.	30-9-55.	7-10-55.	14-10-55.	20-10-55.	
4 ozs. Sodium MCPA	232	66	140	195	**304**	170	169	38	14	1,096	4·7
8 " " "	266	111	209	348	**376**	129	126	16	3	1,318	3·9
12 " " "	224	21	83	186	**306**	125	142	29	7	899	4·0
16 " " "	193	12	49	164	**214**	97	117	11	5	669	3·5
24 " " "	209	19	58	166	**196**	131	163	19	19	771	1·6
32 " " "	259	3	18	125	**147**	54	114	15	2	478	1·8
4 ozs. Amine 2, 4-D	239	50	169	251	**323**	126	154	14	3	1,090	4·5
8 " " "	213	84	163	229	**238**	98	88	23	6	929	4·4
12 " " "	235	21	50	71	**165**	47	96	15	4	469	2·0
16 " " "	182	10	72	75	**152**	44	84	11	6	454	2·5
24 " " "	267	....	28	56	111	76	**121**	25	14	431	1·6
32 " " "	213	....	18	28	47	29	**79**	17	6	224	1·0
4 ozs. Ester 2, 4-D	250	14	98	162	**209**	51	132	9	3	678	2·7
8 " " "	217	....	10	24	56	25	**91**	10	8	224	1·0
12 " " "	204	....	5	17	45	28	**104**	21	6	226	1·1
16 " " "	251	....	....	5	12	25	**78**	22	9	151	0·6
24 " " "	268	....	....	3	4	10	**90**	17	7	131	0·5
32 " " "	234	....	....	2	1	5	**38**	2	3	51	0·2
CONTROL 1	182	72	154	264	**297**	115	114	19	4	1,039	5·7
" 2	205	67	171	283	**310**	104	118	11	1	1,065	5·2
" 3	264	74	165	252	**319**	73	108	14	1	1,006	3·8

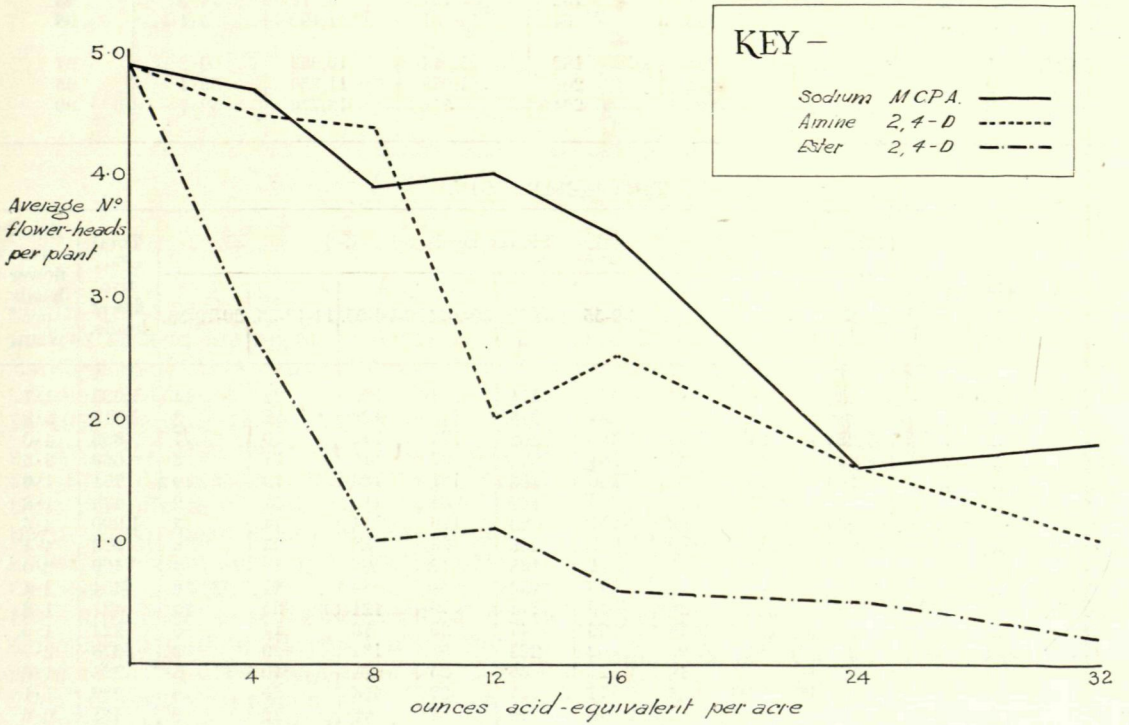
Flowering commenced on September 2, and flower counts were then continued at weekly intervals until October 20. Table 4 shows that the controls along with all sodium M.C.P.A. treatments reached the flowering peak on September 23. This also applied with 4, 8, 12 and 16 oz. amine 2,4-D and 4 oz. ester 2,4-D. The corresponding date for the two highest amine treatments was retarded by 14 days to October 7, which also applied with all ester treatments above the 4 oz. level.

Graph III shows a steep reduction in flower heads per plant with all ester treatments. There was no decided decrease with 8 oz. amine 2,4-D or 16 oz. sodium M.C.P.A. but above these levels there was an obvious reduction in the number of flower heads per plant. This arbitrary figure was based on the intial plant counts and therefore plant mortality accentuated the reduction.

The initial and final plant counts (Table 3) show no wastage in the controls and also a number of the treatments. This is unusual and reflects the very favourable conditions including freedom from weeds and absence of insect pests. The only appreciable reduction in plant members occurred with 32 oz. amine 2,4-D and 16, 24 and 32 oz. ester 2,4-D.

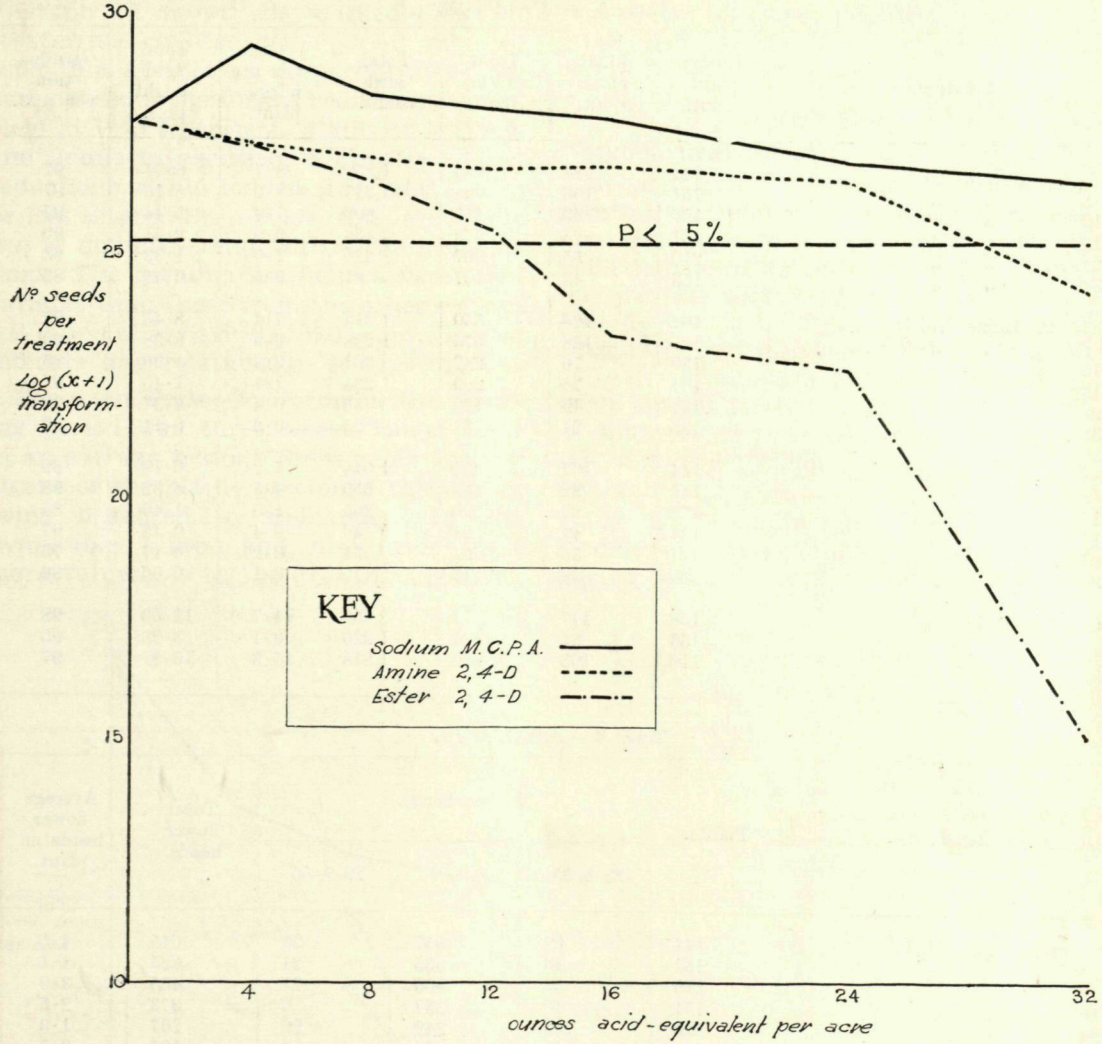
The effect of the treatments on seed setting is shown in Graph IV. At $P < 5$ per cent. sodium M.C.P.A. caused no significant reduction which was also the case with amine 2,4-D except for the highest rate—32 oz. acid equivalent per acre. With ester 2,4-D, however, all treatments above 12 oz. gave significant reductions and 12 oz. level itself was marginal.

As already mentioned, the interesting trend of increased seed production with the lower levels of M.C.P.A. experienced at Toodyay was repeated at Narrogin. The



— GRAPH III —

Showing the effects of three formulations at different levels on the average number of flower-heads per plant (Narrogin)



—GRAPH IV—

Seed produced in relation to chemical applied (Narrogin)

germination of the seed was not affected by any treatment and was uniformly high as shown in Table 3.

The growing season at Narrogin was estimated to have been extended by nine days in 1955. It was apparent, throughout the season, however, that a relatively impervious layer near the surface, retained the moisture and the experimental site was boggy for extended periods. This would have created a longer growing season than that estimated climatically.

GERALDTON

The experimental area 20 miles north-east of Geraldton was comprised of a sandy soil previously growing low sand plain scrub. The land was newly cleared. The plots were sprayed on July 6, when the somewhat unthrifty clover plants were about two inches across with three to four trifoliate leaves.

Plant wastage occurred on all plots including the controls but was most manifest in the case of the higher levels, particularly of amine and ester 2,4-D.

Table 5—GERALDTON.

Treatments.	Initial plant count.	Final plant count.	Total flower count.	Total seed count.	Average seeds per initial plant.	Total seed weight gms.	Germination per cent.
4 ozs. Sodium MCPA	144	84	645	1,257	8·7	8·74	97
8 „ „ „	151	100	625	1,237	8·2	8·15	97
12 „ „ „	116	69	455	809	7·0	5·44	97
16 „ „ „	151	75	373	438	2·9	2·51	90
24 „ „ „	210	55	267	220	1·0	1·33	93
32 „ „ „	153	43	107	81	0·5	0·45	83
4 ozs. Amine 2, 4-D	164	108	550	1,214	7·4	8·43	97
8 „ „ „	187	128	675	1,298	6·9	9·38	93
12 „ „ „	139	76	247	307	2·2	1·96	85
16 „ „ „	164	76	269	234	1·4	1·45	92
24 „ „ „	152	46	120	55	0·4	0·37	93
32 „ „ „	135	21	31	25	0·2	0·14	90
4 ozs. Ester 2, 4-D	144	107	401	1,044	7·2	6·13	89
8 „ „ „	146	88	451	650	4·5	4·32	98
12 „ „ „	175	81	220	265	1·5	1·40	85
16 „ „ „	124	41	98	49	0·4	0·45	97
24 „ „ „	127	35	55	31	0·2	0·17	75
32 „ „ „	157	22	15	10	0·1	0·06	86
CONTROL 1	158	117	771	2,330	14·7	12·63	98
„ 2	133	82	851	1,220	9·1	8·28	95
„ 3	119	103	860	1,818	15·3	16·81	97

Table 6—GERALDTON.

Treatments.	Plants.	Flower heads.			Total flower heads.	Average flower heads on plant.
		25–8–55.	9–9–55.	29–9–55.		
4 ozs. Sodium MCPA	144	67	**552**	26	645	4·5
8 „ „ „	151	46	**555**	24	625	4·1
12 „ „ „	116	34	**400**	21	455	3·9
16 „ „ „	151	10	**357**	6	373	2·3
24 „ „ „	210	5	**242**	20	267	1·3
32 „ „ „	153	1	**98**	8	107	0·7
4 ozs. Amine 2, 4-D	164	24	**491**	35	550	3·4
8 „ „ „	187	41	**607**	27	675	3·6
12 „ „ „	139	6	**225**	16	247	1·8
16 „ „ „	164	15	**247**	7	269	1·6
24 „ „ „	152	3	**106**	11	120	0·8
32 „ „ „	135	....	**31**	....	31	0·2
4 ozs. Ester 2, 4-D	144	28	**355**	18	401	2·8
8 „ „ „	146	7	**406**	38	451	3·0
12 „ „ „	175	4	**267**	49	320	1·8
16 „ „ „	124	....	**85**	13	98	0·8
24 „ „ „	127	....	**50**	5	55	0·4
32 „ „ „	157	....	**15**	....	15	0·1
CONTROL 1	158	74	736	50	860	5·4
„ 2	133	92	719	40	851	6·4
„ 3	119	64	660	47	771	6·5

It was only possible to make three flower counts between commencement of flowering on August 25, and virtual completion on September 29. These figures did not permit conclusions regarding effect of treatment on date of maximum flowering but the average number of flower heads per plant was reduced considerably by the higher rates, particularly of ester 2,4-D. The trend is shown in Graph V.

Graph VI shows the marked effect of treatments on seed setting. All rates of ester, including 4 oz. acid equivalent per acre caused a significant reduction in seed yield at $P < 5$ per cent. Rates of M.C.P.A. and amine in excess of 8 oz. also caused reductions with a tendency for M.C.P.A. to be less severe. Even below this level there was a definite trend with both formulations. The germination figures were more variable than those from the other centres but were all relatively high and did not indicate any relationship to treatment.

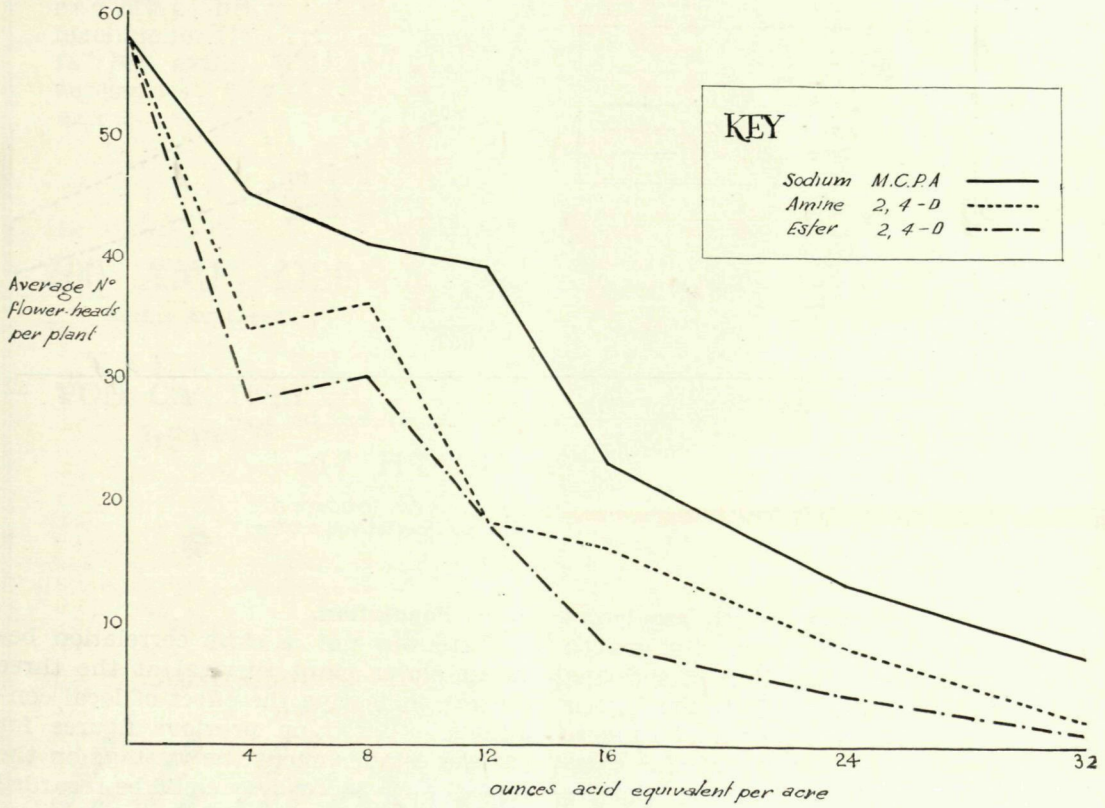
Based on Prescott's formula, the growing period was three weeks longer than in an average season. Presumably the low vigour of the plants precluded them from being benefited to any extent by the favourable season and also made them more vulnerable to the growth regulators. The effects on clover population and seed production are likely to have been more severe in an adverse season.

SUMMARY

Morphological Effects.

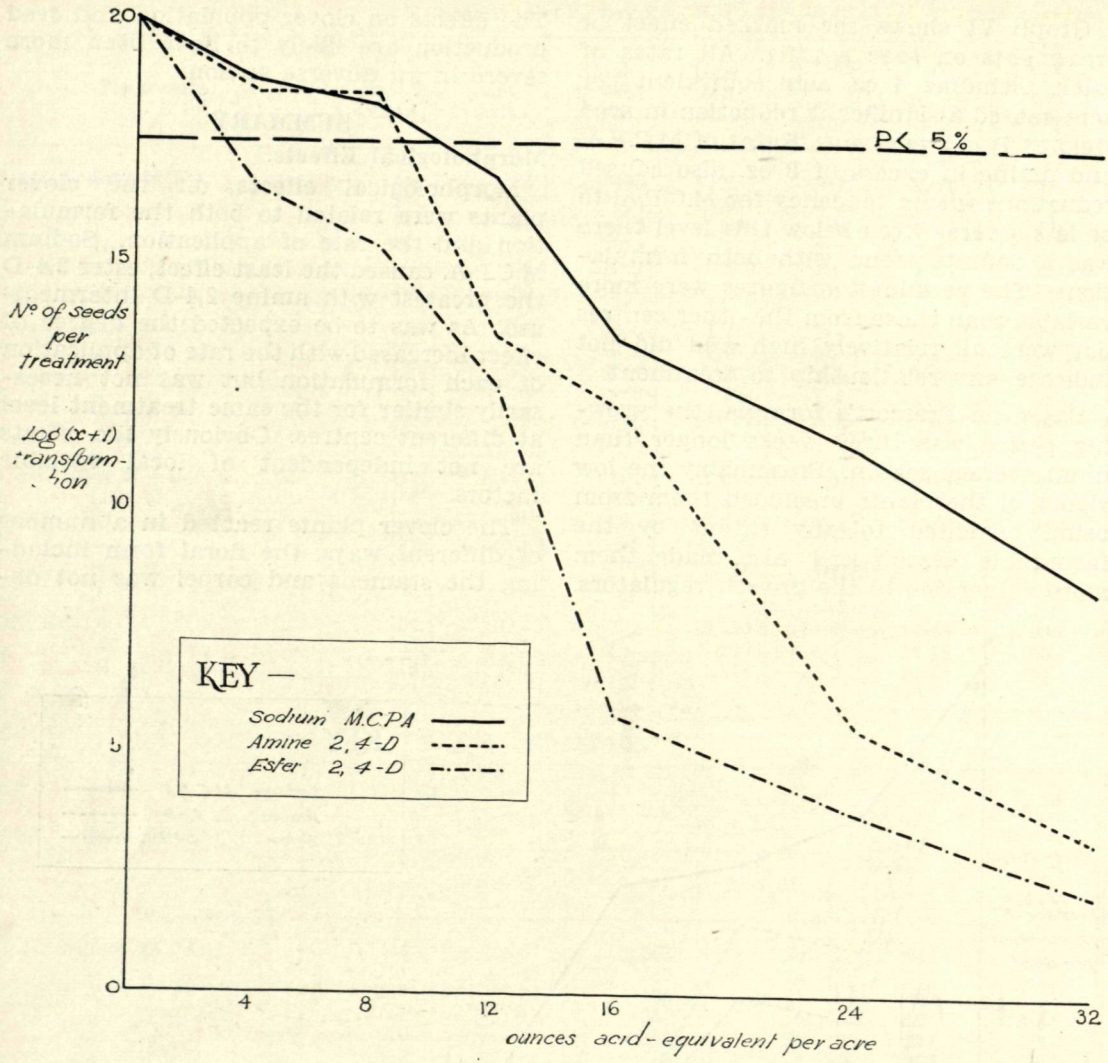
Morphological effects on the clover plants were related to both the formulation and the rate of application. Sodium M.C.P.A. caused the least effect, ester 2,4-D the greatest with amine 2,4-D intermediate. As was to be expected the degree of effect increased with the rate of application of each formulation but was not necessarily similar for the same treatment level at different centres. Obviously the effects are not independent of local variable factors.

The clover plants reacted in a number of different ways, the floral form including the stamens and carpel was not ob-



— GRAPH V —

Showing the effects of three formulations at different levels on the average number of flower-heads per plant (Geraldton)



— GRAPH VI —

Seed produced in relation to chemical applied (Geraldton)

viously altered by the growth regulators applied prior to flowering but the growth of flowers present at the time of spraying was generally arrested. With the higher rates, reduction in the length of runners caused a dwarfing which in extreme cases could be described as a rosetting. Besides being smaller, the leaves became narrow, wedge-shaped and a clearing of the veins was apparent. Severely affected plants were not unlike those showing a zinc deficiency.

Plant Population.

There was not a close correlation between clover plant survival at the three centres, indicating the effect of local conditions. Based on previous figures for pasture establishment the wastage on the control plots at Toodyay could be regarded as average and there was no greater reduction with any of the treatments. At Narrogin there was little or no difference between the initial and final counts with the controls and also a number of the

treatments. This is somewhat unusual and no doubt was due to very favourable conditions relative to moisture and freedom from weeds and insect pests.

The only appreciable reduction in plant numbers occurred with 32 oz. amine 2,4-D and 16, 24 and 32 oz. ester 2,4-D. Plant wastage during the season occurred on all plots at Geraldton, including the controls. It was most evident, however, in the case of the higher levels, particularly of amine and ester 2,4-D. The figures indicate that at Toodyay no reduction of plant numbers was caused by any treatment, at Narrogin 32 oz. amine 2,4-D and 16 oz. ester 2,4-D had effect, while at Geraldton the corresponding figures were 16 oz. sodium M.C.P.A., 12 oz. amine 2,4-D and 8 oz. ester 2,4-D. Although there was some difference in the size of the clover plants at the three centres, at the time of spraying all crops were at a favourable growth stage for treatment for weed control. It may be concluded that at the rates used in Western Australia for the control of cruciferous weeds there is little risk of causing a substantial reduction in the number of clover plants in a first year stand.

Flower Numbers and Periodicity.

The arbitrary method used in recording floral development has already been described. An accurate average figure for flowers produced per plant cannot be estimated because of the possibility of duplication with successive counts and direct comparisons cannot be made between the figures from the different centres because the number of counts made was not uniform. The procedure followed was considered to be the most practicable and supplied the information desired viz.: the effect of treatment on time of flowering and total flower production.

The reaction to treatment at Toodyay where the plants were flowering at the time of spraying was more marked than at the other sites where spraying preceded the initial flowering. All treatments caused a substantial reduction in the number of flowers formed. The higher rates caused the greater reduction but the formulations followed a similar pattern. The date of maximum flowering was delayed to varying extents and was influenced by both the formulation and rate of application. Four ounces acid equivalent M.C.P.A. per acre retarded the date by seven days; 8, 12, 16 and 24 oz. by 18 days and 32 oz. by 29 days. The effects of the amine and ester of 2,4-D were even more severe, the largest recorded delay in maximum rate of flowering being 42 days in the case of 32 oz. of ester.

At Narrogin there was a steep reduction in flower formation with all ester treatments. There was no decided decrease with 8 oz. of amine or 16 oz. M.C.P.A. but above these levels there was an obvious reduction. The flowering peak was not delayed by any of the M.C.P.A. treatments but was retarded 14 days by 24 and 32 oz. of amine and all ester rates with the exception of 4 oz. The restricted number of counts at Geraldton did not permit conclusions regarding effect of treatment on date of maximum flowering but the average number of flower heads per plant was reduced considerably by the higher rates, particularly of ester 2,4-D.

Seed Formation.

At Toodyay the number of seeds produced per plot was reduced at the higher levels of all treatments with a corresponding reduction in the average number of seeds per plant. At the $P < 5$ per cent. level, significant decreases occurred above 8 oz. acid equivalent ester, 16 oz. amine and 24 oz. M.C.P.A. An increase in seed production with 4 and 8 oz. M.C.P.A. could at first appear to be an anomaly. There was a similar trend at Narrogin, however, and the slight delay in flowering could have resulted in more favourable conditions for seed formation, temperature and light period being possible factors. Again the possibility of stimulation by small quantities of growth regulators cannot be excluded.

M.C.P.A. caused no significant reduction in seed setting at Narrogin which was also the case with amine 2,4-D except for the 32 oz. level. With the ester, however, all treatments above 12 oz. were significant and the 12 oz. level itself was marginal. The most marked reaction was recorded at Geraldton. All rates of 2,4-D, including 4 oz., caused a significant reduction in seed yield. M.C.P.A. and amine in excess

of 8 oz. also caused reductions with a tendency for M.C.P.A. to be less severe. Even below this level there was a trend with both formulations. With a number of the treatments the seeds produced were fewer than the plants on the plot at the time of the initial count.

The germination of seed from all plots at Toodyay and Narrogin was uniformly high. Figures for Geraldton were more variable but there was no big reduction in viability or relationship to treatment.

DISCUSSION AND CONCLUSIONS

Growth regulators applied to Dwalganup subterranean clover can cause several manifestations of agricultural significance without causing the death of the plants, in fact the lethal dosage is relatively high. The shape and size of the leaves, and length of the runners, may be affected, along with the floral development and subsequent seed setting. In the season of establishment the quantity of herbage for grazing is not an important consideration but the production of seed to ensure a satisfactory stand of clover the following year is vital. A reduction in the population of clover plants only occurred at rates in excess of those used for the control of such weeds as wild radish and wild turnip but the same could not be said regarding seed formation. With the three formulations used, the effects were related directly to the quantity applied, but ester 2,4-D was consistently more severe, with sodium M.C.P.A. least and amine 2,4-D intermediate.

When interpreting the results of the trials recorded, it is necessary to consider not only the tolerance of the clover but also the relative effectiveness of the treatments for the control of weeds. Most of the spraying of cereal crops in Western Australia is directed against wild turnip (*Brassica Tournefortii),* wild radish (*Raphanus raphanistrum*) and mustard (*Sisymbrium spp*). Wild turnip is highly susceptible and 4 oz. acid equivalent per acre of all formulations have given consistently good results. Wild radish and mustard, however, are more difficult to control chemically. Trials with wild radish by Meadly and Pearce (1954) showed that 4 oz. acid equivalent of ester 2,4-D per acre under favourable conditions gave a satisfactory degree of control. At the 6 oz. level the ester was completely effective. On the other hand 4 oz. of M.C.P.A. and amine 2,4-D affected a proportion of the wild radish plants but were not satisfactory. The 6 oz. rate of both gave reasonable control although some plants recovered and set seed and the treatments were less effective than 4 oz. of ester 2,4-D. For wild radish control, 4 oz. of ester approximates in effectiveness 8 oz. of M.C.P.A. or amine 2,4-D. With this evaluation the active isomer content of the M.C.P.A. was not taken into account, the rates being based on the active content as stated by the manufacterer.

Another important consideration when drawing conclusions and deciding practical recommendations is the influence of the very favourable 1955 growing season on the results. Virtually all treatments affect floral formation if applied after flowering commences but spraying is usually carried out before this growth stage has been reached. The main influence on seed setting and the capacity to produce a satisfactory stand of clover the following year, is the delay in flowering and consequently seed setting, due to treatment. As already outlined, the effective growth period was extended at all centres, the estimated increase being four weeks at Toodyay, three weeks at Geraldtion and nine days at Narrogin where a clay subsoil also assisted in retaining moisture. There is no doubt that in a less favourable season, the retarding of flower development would have a much greater influence on seed formation. The extended growing season at Geraldton was no doubt counterbalanced, in part at least, by the unthrifty condition of the young plants on a low fertility soil. The results at this centre probably gave the best guide to reactions that can be expected in an "average" season.

With due consideration to the variables already outlined the following recommendations are made:—

(1) Wild turnip in cereal crops can be controlled by 4 oz. acid equivalent per acre of amine 2,4-D and sodium M.C.P.A. When this is the only weed concerned and clover

plants are present one of these formulations should be used. It is scarcely necessary to add that 4 oz. ester 2,4-D is equally effective and would be quite appropriate to use for wild turnip control in the absence of clovers.

(2) With a first-year stand of clover grown with a cereal the use of ester, 2,4-D for the control of any weed is not favoured. Under such conditions 6-8 oz. acid equivalent per acre of amine 2,4-D or sodium M.C.P.A. should be used for wild radish and mustard. There is slightly less risk of affecting the clover with the M.C.P.A. but the amine gives somewhat better weed control at comparable rates. If the clover plants are unthrifty M.C.P.A. should be used. When the weeds are two to four inches across and growing actively, 6 oz. will give practical control but 8 oz. acid equivalent per acre is preferable, if conditions tend to make the weeds more resistant. Susceptibility decreases as the weeds become larger and also if growth is slow due to such factors as low temperatures or lack of moisture.

(3) In the case of a crop sown on old clover land, the procedure recommended for wild radish and turnip with a first year stand of clover would also be applicable. Where a substantial regeneration from dormant seeds can be expected, however, the stand is unlikely to be affected appreciably by 4 oz. acid equivalent ester 2,4-D per acre and this more active treatment could well be used when a crop is badly weed infested or conditions are not favourable for weed control with growth regulators.

(4) The above recommendations apply when spraying is undertaken at the optimum growth stage for weed control and during the period of minimum risk to the cereal. At this stage when the cereal is six to eight inches high and stooling, the clover plants are usually two to four inches across with four or more trifoliate leaves. Small clover seedlings may be killed by treatments that are quite safe at the later stage, while spraying some time after flowering has commenced can be very detrimental to seed setting.

REFERENCES

Clarke, M. F., 1953. Progress Report on Weed Control in Forage Legumes. Proc. Seventh West Canadian Weed Cont. Conf. 40-47.

Fitzgerald, J. N., 1953. Tolerance of Clovers to Weed Killers Proc. Sixth New Zeal. Weed Cont. Conf. 89-91.

Holly, K., 1953. The Effect of Growth Regulator Herbicides on Seedling Clovers and Grasses. Proc. Brit. Weed Cont. Conf. 116-124.

Hore, H. L., 1954. Crop Tolerance to Hormone-type Herbicidal Sprays and Their Effect on Undersown Medics. Rep. Aust. Weed Cont. Conf.

Johnston, A. N., Russell, K. W., Preston, S. M., and Done G. S., 1954. The Tolerance of Subterranean Clover to Chemical Weed Control in Improved Pastures. Rep. Aust. Weed Cont. Conf.

Lynch, P. B., 1954. Control of Pasture Weeds in Relation to Clover Tolerance. Proc. Seventh New Zeal. Weed Cont. Conf. 28-31.

MacLean, S. M., 1955. Clover Tolerance. Proc. Eight New Zeal. Weed Cont. Conf. 28-34.

Maclean, S. M., Mathews, L. J., and Fitzgerald, J. W., 1955. Tolerance of Clovers to Weed Killers. New Zeal. of Agric., 90: 450-457.

Marshall, E. R., and Van Geluwe, J., 1953. Three Years Results with the use of M.C.P. and 2,4-D for Weed Control in Seeded Grains.

Meadly, G. R. W., 1956. Investigations into the Stalling of Subterranean Clover in the Lower Rainfall Pasture Belt of Western Australia. J. Dept. Agric., W. Aust. (Second Series) XXIII—85-123.

Meadly, G. R. W. (in press) The Reaction of Plants to Growth Regulators with Particular Reference to Weed Control. J. Roy Soc., W. Aust.

Meadly, G. R. W., and Pearce, G. A., 1954. The Chemical Control of Wild Radish. J. Dept. Agric., W. Aust. (Third Series) 3: 309-315.

Meadly, G. R. W., and Pearce, G. A. 1955. The Effect of Hormone-like Herbicides on Dwalganup Subterranean Clover. J. Dept. Agric. W. Aust. (Third Series) 4: 457-459.

Ochiltree, W., 1954. The Effect of Growth Regulator Weedkillers on Clover—Report on 1954 Experiments. Proc. Brit. Weed Cont. Conf. 125-141.

Scragg, E. B., 1953. The Phytocidal Effects of Hormone Type Herbicides on Clover Species and Strains with Reference to the Spraying of Undersown Crops and Directly Reseeded Grassland. Proc. Brit. Weed Conf. 107-116.