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Effect of 2,4-D on the Growth, Seed Production, and Seed Viability of Rose Clover[1]

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SYNOPSIS. Amine and ester formulations of 2,4-D did not reduce the germination of seed of rose clover, *Trifolium hirtum* All., produced after applications made during the rosette and early bud stages. However, seed and forage yields were reduced by both formulations, the ester formulation being more toxic than the amine. Both formulations drastically reduced seed production and germination when applied during the bloom stage. Neither form caused significant damage if applied when seed was ripe.

ROSE clover is the most extensively used legume for seeding cleared brushland and dryland pastures in California (3, 10). It is a winter annual that germinates with the first substantial fall rain and grows slowly as a rosette of leaves until late winter. Then as temperatures rise, it grows rapidly into an upright, much-branched plant. It blooms and sets seed in May. The plants then become dry and cast their seeds, which remain dormant until the fall rains begin. This pattern of development and reseeding makes rose clover particularly well adapted for range use in a climate of mild, moist winters and hot, rainless summers.

Some 27,000,000 acres of land in California are infested by brush and trees of little economic value. Much of this land is potential forage-producing rangeland and may also be improved for watershed and wildlife use if cleared and seeded to superior forage species. About 1,450,000 acres have been cleared by controlled burning since 1945, and 474,000 acres have been seeded. However, most of the undesirable woody species produce sprouts and/or seedlings, following initial clearance, which must be controlled to prevent rapid reversion. The most promising method of control is the application of phenoxyalkylcarboxylic acid type herbicides during the winter and spring after clearance (2). By far the best time to seed cleared areas in most of California is in the fall before the rainy season, to take advantage of the reduced competition during the first growing season after clearance. Legumes included in the seed mixtures have, in many instances, been eliminated by follow-up brush control sprays.

An increasingly popular practice is the establishment of reseeding clovers to improve the quality of the stubble feed on grainland and the volunteer forage in the years not planted to cereals. Again there is a conflict between legume stand survival and herbicidal applications for broadleaved weed control in the grain. Similar weed control problems in rose clover seed fields need solution.

The effect of alkanolamine-salt and propylene-glycol-butyl-ether-ester formulations of 2,4-dichlorophenoxyacetic acid (2,4-D) on the forage production, seed production, and seed viability of rose clover, *Trifolium hirtum* All., was studied in two field experiments.

LITERATURE REVIEW

The tolerance of several perennial species of *Trifolium* to compounds related to 2,4-D applied at various stages of growth has been studied in relation to their effect on forage production. In this country Butler and Aldrich (1) and Schreiber (8) found considerable variation in the tolerance of various species. Results of Orchiltree (7) and Scragg (9) in Britain were in general agreement with those of the above investigators to the effect that *Trifolium repens* and its various strains and subspecies are more tolerant to 2,4-D compounds than other common perennial clovers.

Less information has been reported on the tolerance of the annual reseeding clovers. Meadley and Pearce (5, 6), in Australia, reported that 2,4-D amine and 2,4-D ester applied at the 3- to 4-leaf stage affected seed production of *Trifolium subterraneum* adversely at rates from ¼ to 2 pounds per acre, but germination was essentially unaffected. Maclean et al. (4), in New Zealand, reported that various 2,4-D compounds applied at rates up to one pound per acre to *T. subterraneum* at the seedling, submature, and mature stages had no apparent adverse effect on reseeding ability.

MATERIALS AND METHODS

First experiment—Inoculated rose clover seed was planted in rows 3 feet apart on Yolo loam at Davis, California, in late Octo-

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ber, 1955. An excellent stand was obtained after rain fell on November 14. A factorial set of treatments involving 2,4-D materials, rates, and dates of application was replicated 5 times in a randomized block design with 2 control plots included in each replication. Alkanolamine-salt and propylene-glycol-butyl-ether-ester formulations of 2,4-D were used in all experiments.[3] Both formulations of 2,4-D were applied at rates of 0.75, 1.5, and 3.0 pounds acid equivalent per acre in water to make 22 gallons of spray per acre. One gallon of diesel oil per acre was included in the 2,4-D ester spray as is common practice to enhance the penetration of the herbicide where used for woody plant control (2). Applications were made at the five stages of growth described below:

Date	Stage	Description of plants
Feb. 11, 1956	Rosette	Rosettes averaging 2 inches in diameter and 10 leaves per plant.
Apr. 20, 1956	Early bud	Plants averaging 6 inches high and 6 inches in diameter.
May 5, 1956	Early bloom	Plants averaging 9 inches high and 12 inches in diameter.
May 29, 1956	Late bloom	20% of heads still in bloom, plants averaging 12 inches high and 20 inches in diameter.
June 11, 1956	Early ripe	90% of seed ripe, no change in plant height.

The sprays were applied at 25 to 30 psi with a knapsack sprayer through a boom with 3 tips spaced 9 inches apart. This equipment delivered the desired 22 gallons per acre when operated at a forward speed of 2.0 mph. The rows were hoed frequently to prevent an interaction between the clover response and a treatment-induced variation in weed population. The experiment was irrigated once, on April 2, 1956, when the plants were beginning to show signs of stress from a prolonged drouth. Each plot consisted of 4 rows 20 feet long. Sixteen feet of 1 center row in each plot was harvested for dry matter and seed production during the period June 12 to 21, 1956. Harvesting was by hand clipping between daybreak and 9 a.m. while the humidity was high. This minimized the shattering of seed and leaves. Seed was threshed in a McCormick Deering No. 6 hammermill at 460 rpm through a 1/8-inch round-hole screen. Mechanically damaged seed averaged 3.8%, and this percentage was not affected significantly by the treatments. Germination tests were performed on filter paper in Petri dishes at 16° C. for 8 days. The most favorable temperature for germination was determined in a preliminary test.

Second experiment—In 1956 an additional field experiment was established in an adjacent area. Similar experimental design and methods were used except as follows: The rates of 2,4-D applied were 0.75 and 3.0 pounds per acre, and the plot size was 3 rows 3 feet apart and 25 feet long. Applications were made at the following stages: full bloom, May 15, 1957; late bloom, May 29; early ripe, June 11; and ripe, July 11. Eight feet of the center row was harvested for seed and forage yield on July 12 and 13. A second harvest of seed was made on August 30 and 31, 1957.

RESULTS

First Experiment

Forage production—Both 2,4-D amine and 2,4-D ester caused significant reductions in forage production when applied to rose clover before full bloom (table 1). The amine formulation resulted in forage yields of 55, 57, and 57% of control when applied in the rosette, early bud, and early bloom stages, respectively (averaged for the 3 rates of application). The ester formulation resulted in forage yields of 11, 34, and 56% of control, respectively. Thus, the ester was more damaging than the amine when applied in the rosette and early bud stages, but about the same in the early bloom. Neither of the materials at any rate differed significantly from the control in forage production when applied at either the late bloom or early ripe stages of growth.

Table 1—Effect of 2,4-D materials applied at various stages of growth on the forage and seed production of rose clover, first experiment.

2,4-D material	2,4-D rate, lb./A.	Control (LSD, 1%)	Rosette, Feb. 11	Early bud, Apr. 20	Early bloom, May 5	Late bloom, May 29	Early ripe, June 11
		Forage production (dry, lb./A.)*					
Control	0.00	3,020					
Amine	0.75		2,000	2,100	1,770	2,750	2,820
	1.5		1,760	1,640	1,770	3,340	2,860
	3.0		1,240	1,400	1,620	3,230	2,860
Ester + oil	0.75		660	1,130	1,670	3,460	3,170
	1.5		240	1,030	1,640	2,880	2,700
	3.0		130	900	1,720	3,040	2,800
LSD, 1%		(600)					
		Seed production (lb./A.)					
Control	0.00	541					
Amine	0.75		325	155	6	329	475
	1.5		283	103	7	478	517
	3.0		232	59	2	464	481
Ester + oil	0.75		91	46	6	487	488
	1.5		40	26	1	334	458
	3.0		21	10	2	296	361
LSD, 1%		(165)					
		Seed as % of total dry matter produced					
Control	0.00	17.9					
Amine	0.75		15.5	7.7	0.3	12.0	16.9
	1.5		15.5	6.4	0.6	14.4	18.5
	3.0		19.2	4.2	0.1	14.2	16.8
Ester + oil	0.75		15.2	3.8	0.3	14.1	15.7
	1.5		17.1	2.5	0.1	11.4	16.6
	3.0		15.8	1.2	0.1	9.7	13.2
LSD, 1%		(5.1)					

* Includes seed.

Visual estimates of stand indicated that the maximum reduction in stand for any of the rates or dates of application of 2,4-D amine was 20%. The 2-4-D ester reduced the stand by 70 to 95% when applied at the rosette stage and 15 to 70% at the early bud stage, the greater reductions resulting from the highest rate. Applications of either material at later stages of growth did not affect stand appreciably.

The plants that survived the seedling sprays developed good vigor by the onset of bloom. Plants that survived the early bud stage sprays had fair vigor except those receiving the two high rates of ester, which exhibited only slight recovery by bloom time. Plants sprayed at the early bloom stopped growing and blooming at all rates of both materials. Sprays at the late bloom and early ripe stages had little visually observable effect on the plants, except for a slight hastening of maturity from the late bloom applications.

Seed production—The effects on seed production are of even greater importance than the forage response, since the stand survival of a reseeding type annual depends on the production of sufficient numbers of viable seed. The amine formulation progressively reduced seed production from 52% of control (averaged for all rates) when applied at the rosette stage to 20% of control at early bud, and to practically nil at early bloom. The ester resulted in seed production 9% of control from applications in rosette stage and 5% of control in the early bud stage. The early bloom applications almost completely suppressed seed production. For the amine treatments at late bloom and early ripe stages, only the decrease resulting from the 0.75 pound per acre rate at late bloom exceeded the least significant

[3] Experimental samples of the herbicides were supplied by the Dow Chemical Co., Midland, Michigan.

difference (1%). This result does not follow the trend of the remainder of the data and apparently is a spurious difference. With this exception the data indicate that the amine applications at these stages did not interfere significantly with seed production. For the ester applications the depression in yield from the 1.5 and 3.0 pounds per acre rate at the late bloom and 3.0 pound rate at the early ripe stage exceeded the least significant difference (1%). However, the disturbance of seed production by the ester applied at these 2 late stages of growth was not nearly as drastic as when applied at 3 earlier stages.

The seed percentage data indicate that the plants which survived the rosette applications of either material produced a normal amount of seed relative to total plant growth (table 1). This was not true of the early bud and early bloom stage applications since the seed percentage declined progressively to practically zero at the latter stage and then rose again to near normal levels for the early ripe stage applications.

Seed viability—Normal germination of seed produced by plants sprayed with either 2,4-D amine or ester at the rosette and the early bud stages was unaffected (table 2) even though forage and seed production were markedly restricted at all rates. When either material was applied at the early bloom stage, normal germination was severely reduced. The reduction was least but still severe at the lowest rate for each material. Application of either material at all rates at the early bloom stage almost completely inhibited normal germination, even though substantial amounts of seed were produced. When the clover was sprayed at the early ripe stage, normal germination was reduced moderately at the 0.75 pound per acre rate, more so at the 1.5 pounds per acre rate, and severely at the 3.0 pounds per acre rate of both materials.

A large number of seedlings in the germination tests of some treatments consistently had abnormal symptoms. The

Table 2—Effect of 2,4-D materials applied at various stages of growth on the germination of seed produced by rose clover, first experiment.

2,4-D material	2,4-D rate, lb./A.	Control (LSD, 1%)	Rosette, Feb. 11	Early bud, Apr. 20	Early bloom*, May 5	Late bloom, May 29	Early ripe, June 11
			Normal germination (%)				
Control	0.00	64.2					
Amine	0.75		65.4	65.6	15.4	0.8	45.0
	1.5		65.8	61.8	6.0	0.4	14.0
	3.0		57.2	61.4	2.0	0.4	2.8
Ester + oil	0.75		63.2	62.7	30.0	1.6	35.4
	1.5		62.2	62.2	2.7	0.8	19.6
	3.0		74.2	54.2	-	1.0	8.4
LSD, 1%		(15.0)					
			Abnormal germination (%)				
Control	0.00	0.2					
Amine	0.75		2.0	0.6	45.4	57.8	22.2
	1.5		1.8	4.2	51.3	52.8	58.0
	3.0		2.6	3.0	70.0	55.8	68.8
Ester + oil	0.75		3.6	7.7	36.5	57.2	28.8
	1.5		10.0	6.0	65.3	47.4	49.6
	3.0		3.2	14.4	-	51.2	59.6
LSD, 1%		(16.9)					
			Hard seed (%)				
Control	0.00	27.4					
Amine	0.75		26.4	27.2	20.6	27.0	27.2
	1.5		23.2	22.2	29.3	30.2	18.6
	3.0		29.2	22.4	21.0	31.2	23.0
Ester + oil	0.75		22.8	22.0	11.5	19.6	28.2
	1.5		17.0	24.0	21.0	40.6	21.4
	3.0		8.2	18.8	-	31.8	24.2
LSD, 1%		(13.4)					

* LSD does not apply to early bloom treatments as insufficient seed was produced on many of the plots for germination tests. Means for these treatments are based on at least two plots.

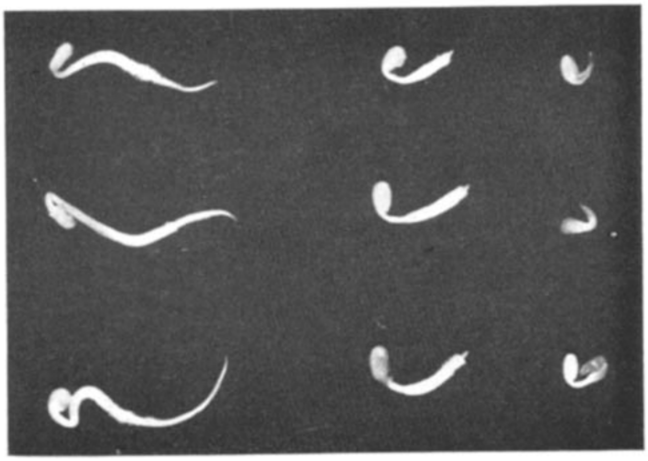

Figure 1—Left, normal seedlings from control plots. Center, moderately damaged seedlings, and right, severely damaged seedlings from 2,4-D sprayed plots. (Actual size.)

radicle emerged slowly, and the meristem constricted to a discolored point with the tissue immediately above being club shaped (figure 1). Seedlings having these symptoms were transplanted to greenhouse flats and were observed to have a very low rate of survival, whereas normal seedlings continued normal growth under the same conditions. Thus, these abnormal seedlings would not be considered "germinated" in the accepted sense of the word. The data for abnormal germination, in general, exhibit an inverse relationship with the normal germination results (table 2). There was relatively little abnormal germination in seed produced by plants sprayed in the rosette and early bud stages, and normal germination was correspondingly high. Applications at the three later stages all produced large numbers of abnormal seedlings, and normal germination was low.

Several of the treatments produced hard seed percentages that deviated significantly from the control (table 2). Worthy of note is the reduction in hard seed resulting from the high rate of 2,4-D ester applied at the seedling stage, which was accompanied by a nonsignificant increase in normal germination. Perhaps this was the result of a delay in maturity caused by the treatment.

Second Experiment

The results of the first test stimulated interest in the responses to a still later spray application and also the effects of weathering of the seed produced on sprayed plants. So a second experiment was set up to include a ripe stage spray application and two harvest dates.

Forage and seed production—Forage production was reduced significantly only by applications at the earliest stage, full bloom, (table 3) fitting in well with the results of the first experiment. Seed production was depressed strongly by spray applications at the full bloom stage. The ester depressed seed yield more than the amine, and the high rate of application of each depressed seed yield more than the low rate when applied at the full bloom stage. The seed yields resulting from the full bloom sprays in this experiment were intermediate in magnitude between those for the early bloom and late bloom sprays of the first experiment. Seed yields from sprays at the late bloom stage in the second experiment were significantly poorer than the control for the high rate of amine and both rates of ester.

Table 3—Effect of 2,4-D materials applied at various stages of growth on the forage and seed production of rose clover, second experiment.

2,4-D material	2,4-D rate, lb./A.	Control (LSD, 1%)	Full bloom, May 15	Late bloom, May 29	Early ripe, June 11	Ripe, July 11
		Forage production (dry,lb./A.)*				
Control	0.00	4,700				
Amine	0.75		4,120	4,830	4,100	4,000
	3.0		3,740	4,010	4,390	4,320
Ester + oil	0.75		3,650	4,030	4,280	3,960
	3.0		3,030	3,940	4,370	4,480
LSD, 1%		(850)				
		Seed production (lb./A.)				
Control	0.00	804				
Amine	0.75		228	688	676	696
	3.0		96	468	736	728
Ester + oil	0.75		112	388	756	720
	3.0		60	292	756	796
LSD, 1%		(175)				
		Seed as % of total dry matter produced				
Control	0.00	17.2				
Amine	0.75		5.5	14.2	16.5	17.3
	3.0		2.6	11.7	16.8	16.9
Ester + oil	0.75		3.1	9.6	17.7	18.4
	3.0		2.0	7.4	17.3	17.8
LSD		(2.2)				

* Includes seed.

Sprays at the early ripe and ripe stages did not affect seed yield significantly. The seed percentage data in this experiment are largely a direct reflection of seed production, since so few of the treatments affected forage production.

Seed viability—Normal germination of seed produced following sprays at the full and late bloom stages was very low for both materials at both rates (table 4). Normal germination increased to a high level although still significantly less than the control for all treatments at the early ripe stage. Some additional improvement was observed from delaying sprays until all seed was fully ripe (July 11, 1958) although the amounts are not entirely consistent between the two harvest dates. If the change in hard seed content between harvest dates is taken into account, the germination of the treated plots relative to the controls did not differ appreciably with harvest date. This indicates that weathering over the period of about 7 weeks involved in this comparison had no appreciable effect on the damaging potential of the 2,4-D materials. The abnormal germination data show a close inverse relationship to the normal germination results. Hard seed content increased from 21.7 to 40.5% in the control plots between the two harvest dates. At the second harvest hard seed was increased significantly by all full bloom sprays and by all late bloom sprays except the low rate of amine. The early ripe and ripe sprays did not affect hard seed content.

DISCUSSION

In seeding an area cleared of brush there is a conflict of aims between control of brush sprouts and seedlings and the survival of seeded legumes. The seeding of the introduced species must necessarily be timed so as to take advantage of the reduced competition during the first growing season after clearance and of the seedbed afforded by the clearance operation. However, control of brush sprouts and seedlings is best obtained by applications of brush-killing sprays also during the first growing season after clearance. A similar conflict arises from weed sprays used in grain-grazing rotations where a reseeding legume is desired. The main purpose of this study was to determine what potential a reseeding annual legume has for perpetuation after being sprayed with 2,4-D materials. This depends on its ability to produce viable seed after such treatment. The main factors involved are (1) plant growth, (2) seed production, and (3) viability of the seed. These factors are interrelated, but are affected quite differently by the timing of the spray. Figure 2 contains graphs of the above factors plotted as averages of the results for the rates of each material against its time of application. Forage production was maintained at a level of 55 to 57% of the control as the amine sprays were delayed successively from the rosette to the bud and early bloom stages. When spraying was delayed beyond the early bloom stage, production rose to values approaching the control. The production curve for the ester progressed similarly at the later stages of application but was considerably lower at the earlier two stages. Seed production declined to a minimum as spraying with either material was delayed until early bloom. Then it progressively increased as spraying was delayed until the

Table 4—Effect of 2,4-D materials applied at various stages of growth on the germination of seed harvested from rose clover on two dates, second experiment.

2,4-D		First seed harvest, July 12-14, 1957					Second seed harvest, August 30-31, 1957				
Material	Rate, lb./A.	Control (LSD, 1%)	Full bloom, May 15	Late bloom, May 29	Early ripe, June 11	Ripe, July 11	Control (LSD, 1%)	Full bloom, May 15	Late bloom, May 29	Early ripe, June 11	Ripe, July 11
					Normal germination (%)						
Control	0.00	72.1					56.5				
Amine	0.75		4.2	11.0	58.2	72.8		4.0	9.8	42.2	52.6
	3.0		2.0	3.8	57.0	63.0		1.8	3.8	43.4	52.8
Ester + oil	0.75		2.4	6.4	53.4	74.6		1.6	7.8	47.2	49.4
	3.0		2.0	4.0	51.6	69.8		0.2	3.8	32.2	52.4
LSD, 1%		(10.6)					(10.5)				
					Abnormal germination (%)						
Control	0.00	1.5					0.2				
Amine	0.75		61.6	60.8	16.6	3.2		37.8	42.6	9.0	2.2
	3.0		59.6	56.0	12.8	12.2		38.2	37.8	13.8	4.4
Ester + oil	0.75		58.6	56.4	21.4	0.4		39.6	36.2	8.2	1.8
	3.0		55.2	59.0	23.4	6.8		37.2	40.0	19.8	4.0
LSD, 1%		(12.8)					(9.5)				
					Hard seed (%)						
Control	0.00	21.7					40.5				
Amine	0.75		24.0	28.2	20.8	20.8		50.8	44.2	45.4	42.4
	3.0		29.8	32.2	27.4	22.6		50.2	52.6	40.0	38.0
Ester + oil	0.75		25.6	29.0	19.4	21.2		50.2	52.8	42.8	43.0
	3.0		28.2	28.2	17.8	22.0		55.4	52.2	45.2	40.6
LSD, 1%		(11.0)					(12.1)				

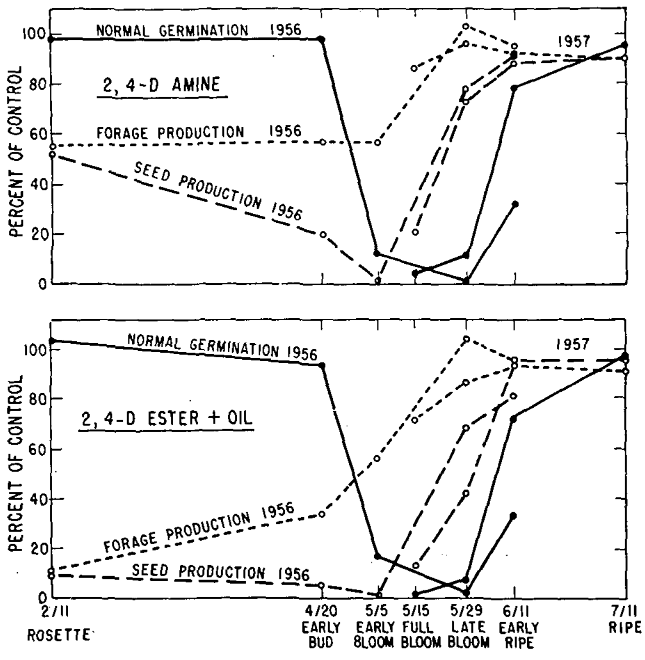

Figure 2—Effect of 2,4-D amine and 2,4-D ester plus diesel oil applied at various stages of growth on forage and seed production and normal germination pércentage of rose clover. Curves are based on averages for the rates used. Results for 1956 and 1957 experiments are indicated by separate curves.

full bloom, late bloom, and early ripe stages. Thus, seed production responded quite independently of total plant growth. In particular, spraying with either material in the early bloom stage reduced seed production to almost nil while forage production was maintained at 56 to 57% of the control.

Germination (normal) followed yet another distinct pattern with the curves for both materials paralleling each other closely. Germination resulting from the seedling and early bud applications was essentially the same as for the control. Then it declined to a very low level for all bloom stage applications. Germination recovered progressively as spraying was delayed until the ripe stage. A discrepancy is apparent between the germination data for the 2 years resulting from the early ripe stage applications of both formulations of 2,4-D. It is suggested that the April irrigation applied in the first experiment retarded the maturation of the clover seed slightly. Thus, the upswing of the germination curves during the ripening period of the first experiment (1956) was delayed relative to that of the second experiment (1957), which was not irrigated.

The points of interest in comparing the germination curves to the forage and seed production curves are (a) rosette and early bud stage sprays of both materials depressed growth and seed production markedly, but had essentially no effect on the germination of seed subsequently produced; (b) both seed production and germination were reduced to very low levels by both materials applied at early and full bloom stages; (c) seed production was substantial when either material was applied at late bloom, but germination was very low; and (d) applications at the ripe stage had little effect on any of the three factors.

No direct measurements have been made as of this writing on the translocation of the 2,4-D materials into the seeds, but a hypothesis may be inferred from the plant responses. It is suggested that although the clover plants' ability to produce seed was interfered with by applications of 2,4-D made up to the early bud stage, amounts carried into the seed were not sufficient to interfere with germination. Translocation into the seed apparently occurred readily from applications during the bloom period. There seems to be a rather definite time relative to the development of seed primordia when 2,4-D application will prevent the seed from forming. Applications made at some later time will permit seed formation but will interfere with germination. It is likely that this latter time is after considerable development of the seed. It may be presumed that the 2,4-D responsible for this injury is also translocated into the seed. The lack of damage from ripe stage applications adds credence to this hypothesis since translocation would be essentially nil at that time. It is also suggested, based on the results of applications at the ripe stage, that 2,4-D which gains entrance by movement through the surrounding dead calyx or pod, and/or from contaminated dust accumulating on the seed during threshing and cleaning operations, is so slight as to be nondamaging to germination.

SUMMARY

A study was conducted to determine the effects of amine and ester formulations of 2,4-D on the growth, seed production, and seed viability of rose clover, a legume used widely for seeding range in California. The practical conclusions are (1) for brush sprout and seedling control in areas cleared of brush and seeded to rose clover, 2,4-D sprays must be confined to the ripe stage or, second-best, to the vegetative stage of rose clover for good likelihood of stand reestablishment from first year rose clover stands, and (2) for control of weedy forbs in rose clover seeded grain fields or in rose clover seed fields, 2,4-D sprays should be applied before the bud stage to avoid damage to seed germination with the realization that seed and forage production very likely will be reduced.

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