APPLICATION OF HERBICIDES TO CUT STUMPS OF A WOODY TROPICAL WEED[1]

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Introduction

The use of herbicides in the killing of woody weeds has been reported in a number of papers (**6, 12, 13, 14, 16**); standard spraying methods, essentially as in the treatment of herbaceous plants, were used. Shrubs and trees, however, present a number of special problems. STEWART and GAMMON (**11**) used a fog method of application for tall plants of wild grape. Also, the application of herbicides to the stumps of woody plants after cutting has sometimes been advocated, especially for the more resistant types. HAMNER and TUKEY (**6**) obtained apparent kills of a number of shrubs and young trees by applying 2,4-dichlorophenoxyacetic acid (2,4-D) as a salve to the cut surfaces of stumps.

The tropical weed *Dichrostachys nutans* Benth., referred to locally in Cuba as *aroma* or *marabú*, is a woody leguminous perennial. Its response to 2,4-D applied as a spray has already been reported on (**7, 13, 14**). The plant has remarkable powers of regeneration, ascribable in great part to horizontal underground roots which may extend for many feet, and from which may arise new ratoon growths. It has been found by EAMES (**4**) and is shown below that the roots are filled with stored starch, especially in the inner bark region and around the vessels of the xylem. Whereas individual clumps may be killed by the use of 2,4-D, the roots sometimes have been found to survive and establish new growth some months after the herbicidal treatment. In the further study of the problem of killing this woody plant, it was hoped that stump treatment would offer a new approach. The following report, therefore, deals with treatment of cut stumps and also with numerous experiments designed to throw some light on the entry and transport of the herbicide and the response of the plant.

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Experimentation

TREATMENT OF CUT STUMPS

METHODS.—Areas of adult *aroma* plants, 4–6 feet in height, were marked out in plots approximately 20 × 22 feet (1/100th acre), and the plants were topped to the desired stump height with the use of pruning shears and machetes. Plots as nearly comparable as possible were used, averaging sixty to eighty stumps per plot with individual stumps of about 1–2 inches in diameter at the cut surface.

In the stump treatments emulsions were made in relatively high concentrations and painted on the cut surface of each stump, using a 1-inch paint brush. Approximately 30 ml. of emulsion were used per plot of sixty plants. In some cases application of the emulsion was made some time after the plants had been cut to stumps. In no case was this found to be critical so long as the time elapsed did not exceed 1 week. Painted applications were made between 9:00 and 11:00 A.M. in full sun, generally at about 92° F. (air temperature), so that treatment was followed usually by 6 hours or more of full sun. Grass growing between stumps was cut back periodi-

cally so as to leave the stumps clear and unshaded. Spray applications to the leaves of newly arising basal shoots ("ratoons") were made with a 4-gallon knapsack sprayer, at a rate of about 20 gallons of emulsion per acre.

Readings were made every 2 weeks for a period of 3 months. The following categories were established as a means of expressing the degree of effective treatment over extended periods of time. The number of plants in each category was expressed as the percentage of the total number, established by actual count in every case.

% Old Green: Percentage of total number of plants which retain any green foliage which was present on foliar branches at the time of initial treatment. This corresponds to the sum of the classifications "apparently intact" and "somewhat damaged" of THIMANN (14).

% New Green: Percentage of total number of plants which, following defoliation, showed new young green shoots or leaves.

% No Green: Percentage of total number of plants which showed no foliage whatsoever. This corresponds to the previous classifications "apparently killed" and "seriously injured or dying" (14).

CHOICE OF HERBICIDE.—Earlier work had established that 2,4-D in various forms is an effective agent when applied as a spray to the leaves of the adult *aroma* plant or young ratoons. Subsequent extensive comparisons of the different forms established that the esters were definitely more effective than the salts (7). In the present experiments on stump treatment the butyl ester of 2,4-D was used, being applied in varying concentrations as noted below. For comparison, the butyl ester of 2,4,5-trichlorophenoxyacetic acid (2,4,5-T) was utilized in a series of concentrations. Sodium arsenite, reported by GRAY (5) as an effective herbicide against morning glory and later shown to be effective against deep-rooted perennial weeds by CRAFTS (2), was also tested in stump treatments. It was ineffective against *aroma* as a spray, causing rapid defoliation followed by vigorous growth of apparently normal shoots. Gas oil, applied to the ringed trunk bases, has been utilized as a local expedient in Cuba in an attempt to control *aroma*. Gas oil also has recently been found effective in emulsion mixtures with 2,4-D in the spray treatment of certain tropical weeds (3). Gas oil was therefore tested alone and with 2,4-D as a herbicide, using the stump treatment.

The results of a series of tests of different preparations applied to the stumps of *aroma* are shown in figure 1. The percentages of the total number of plants showing No Green were recorded over a period of weeks, beginning after the second treatment. The rapid appearance of new foliage within 4 weeks after the arsenite treatment (38% solution) is very striking. More dilute solutions were, as expected, even less effective. An aqueous emulsion of the butyl ester of 2,4-D at optimum concentration was established as the most effective herbicide, surpassing all others tried.

It will be noted that development of new shoots was definitely more delayed in the "short" stumps, cut almost to ground level, than in the stumps 2 feet long. Although quite to be expected, this point is of practical value in treatments of this sort.

EFFECT OF CONCENTRATION.—Since only very small volumes can be applied to the cut surface, it was evident that high concentrations would be necessary. Mixtures containing 10, 20, 30, and 40% of the butyl esters of 2,4-D and 2,4,5-T in aqueous emulsion were therefore made up. In each case, two treatments were made 1 week apart. The value of the second treatment is established below. From the data (table 1) it is clear that the 20%

concentration of the butyl ester of 2,4-D is the optimum for this herbicide. 2,4,5-T was consistently less effective than the same concentration of 2,4-D; in the readings it is evident that after 8–9 weeks the extent of new growth was very much greater from the plants treated with 2,4,5-T.

mixtures were made utilizing gas oil instead of water as the diluent, and the stumps were treated with these mixtures in the same way as with the aqueous preparations. The oil used was a light diesel oil (Standard Oil of Cuba), viscosity (100° F.) 35–45, flash-point minimum 150. The results (table 3) indicate

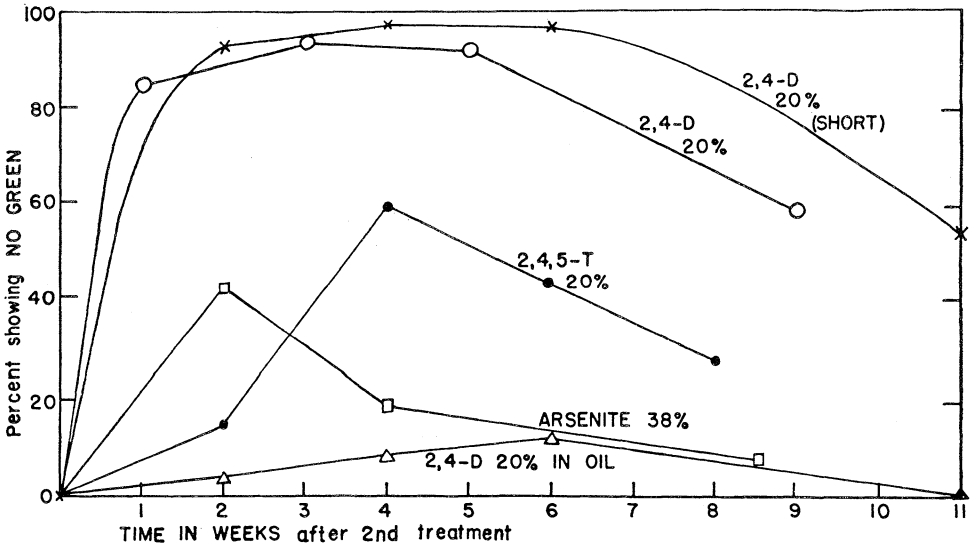

FIG. 1.—Stump treatments of *aroma marabú*. Each application was made with brush on cut surface and repeated 2 weeks later. Number of plants counted to determine each point varied from 50 to 200. All stumps were about 2 feet high except those marked "short," which were only 2–3 inches above ground level. The legends *2,4-D* and *2,4,5-T* refer to butyl esters of these acids, which were in aqueous emulsion except for one series in oil.

EFFECT OF REPEATED TREATMENT.—To determine whether one treatment is sufficient, half of each plot in one experiment was treated a second time 1 week after the initial treatment, using the same concentrations and means of application. The results of such experiments with 2,4-D ester emulsions (table 2) indicate that at the lower concentrations two treatments are considerably more effective than one. With 30 and 40% emulsions, however, the second treatment makes little difference.

EFFECT OF GAS OIL IN APPLICATION MIXTURE.—Using the 2,4-D butyl ester, that oil markedly retards the rate of action of 2,4-D. The oil apparently blocks the entrance of the ester into the stump, allowing only local action. This blocking effect was manifested macroscopically by a ring of callus tissue in the circumference of each stump treated with the oil—2,4-D mixture at 1–2 inches below the surface of application. Above this callus ring the trunk was dry and discolored; below the ring and in the callus itself the trunk was green and living (fig. 2*C*). A similar callus was noted in many of the stumps treated with the aqueous emulsion of 2,4,5-T ester (fig.

2*B*), although of less extent. In no case was such callus formation observed in the control plants or in those treated with the aqueous emulsions of 2,4-D (fig. 2*A*). MINSHALL and HELSON (8) and ROHRBAUGH (10) found that diesel oil, applied to leaves, entered the intercellular spaces of the plant tissue and not the cells themselves. It is possible that distribution of the oil in a similar manner from the cut surface of the stump blocks the transport of 2,4-D, by preventing the normal oxidative processes on which transport probably depends. In any event, sprouting took place vigorously from the lower parts of the stumps treated in this way, as shown by the data plotted in figure 1.[2]

It seems possible that this relative ineffectiveness of oil mixtures is a phenomenon peculiar to the plant studied, since results recently reported by BEATTY (1) indicate satisfactory killing of northern trees with similar treatments.

TABLE 1

COMPARISON OF HERBICIDAL EFFECTS OF BUTYL ESTERS OF DI- AND TRI-CHLOROPHENOXYACETIC ACID IN AQUEOUS EMULSION, AND EFFECT OF CONCENTRATION. ALL PLANTS GIVEN TWO TREATMENTS ON STUMPS. RESULTS EXPRESSED AS "% NO GREEN" (SEE TEXT)

PER CENT	WEEKS AFTER SECOND TREATMENT		
	3	5	9
Concentration of 2,4-D:			
10	42.0	60.3	40.3
20	93.8	91.0	55.9
30	77.4	64.8	47.5
40	71.5	65.6	30.2
	WEEKS AFTER SECOND TREATMENT		
	4	6	8
Concentration of 2,4,5-T:			
10	27.9	20.9	10.9
20	59.3	45.0	27.6
40	86.9	51.8	25.7

ENTRANCE OF 2,4-D INTO PLANT

In order to shed light on the ability of buds to develop from the base or roots of plants whose tops were "apparently killed," an attempt was made to study the penetration of the herbicide into the plant. For this purpose use was made of the slit pea-stem test (15, 17) and of the cucumber-root inhibition test (9). For the extraction, representative plants

[2] In later observations, development of shoots on the oil-treated plants was less active than after treatment with aqueous emulsion, so that after 8 months there was little difference between oil and water. Oil treatment, however, showed no advantage. With 10% 2,4-D the "% No Green" after 8 months was: in water 13, in oil 14; with 20% 2,4-D: in water 27, in oil 30.

TABLE 2

COMPARISON OF SINGLE AND DOUBLE TREATMENT OF STUMPS EXPRESSED AS "% NO GREEN"

	10% 2,4-D		20% 2,4-D		30% 2,4-D		40% 2,4-D	
	Weeks after second treatment							
	5	9	5	9	5	9	5	9
One treatment	49.1	40.6	60.6	29.0	64.7	45.6	58.0	32.5
Two treatments (1 week apart)	60.3	40.3	91.0	55.9	64.8	47.5	65.6	30.2

were selected, and the desired tissue was cut into small pieces, including all parts of the particular plant structure to be analyzed. Five-gm. samples were extracted at room temperature for 12 hours, using 25 ml. of ethyl ether freshly distilled over $FeSO_4$. The ether extract was poured off and the tissue rinsed with 5 ml. of fresh ether. This crude extract, when evaporated down and tested, without further purification, in the cucumber-root inhibition test, was found to inhibit root elongation markedly, whether the plant extracted had been treated with 2,4-D or not. For this reason all subsequent extracts were purified in the following way. To the ether extract was added half its volume of 1% $NaHCO_3$, and the mixture shaken gently. The ether was discarded, and the carbonate solution was acidified with 1 *N* HCl to about pH 2. Then the acidified aqueous solution was extracted twice with equal volumes of ether, the ether evaporated down, and the residue taken up in 10 ml. of water. From this was made a logarithmic dilution series. This purification procedure removed the inhibiting effect of extracts from control plants. The effect is doubtless due to the nonacidic growth-inhibiting substances often reported in plant extracts.

TABLE 3

EFFECT OF BUTYL ESTER OF 2,4-D IN WATER AND IN GAS OIL; FIVE WEEKS AFTER SECOND TREATMENT, EXPRESSED AS "% NO GREEN" (SEE TEXT)

% 2,4-D	PER CENT	
	In water	In gas oil
10	60.3	8.6
20	91.0	15.2

In the slit pea-stem test, stem sections, cut from the second to third nodes of 8-day-old Alaska pea seedlings grown in wet sand in the dark at about 29° C., were slit longitudinally for 3 cm. at the apical end, washed for 1 hour in water, then placed, six sections per dish, in Petri dishes containing 20 ml. of solution. After 24 hours of continued darkness the curvatures were measured with a protractor, and the average angle of curvature determined for each solution. Satisfactory concentration curves were readily established with known concentrations of 2,4-D butyl ester. By comparing these concentration curves with curves determined from the extracts, the probable 2,4-D content of the tissue was de-

FIG. 2.—Long stumps after two applications of herbicide to cut surface. *A*, 20% 2,4-D aqueous emulsion; 10 weeks after second treatment. No swelling. *B*, 20% 2,4,5-T aqueous emulsion; 7 weeks after second treatment. Swollen ring 3–5 cm. below cut surface. *C*, 20% 2,4-D emulsion in oil; 6 weeks after second treatment. Marked swollen ring 3–5 cm. below cut surface.

duced. The results of these extraction experiments (table 4) are expressed as concentration in molarity of 2,4-D, deduced from concentration curves. Repetition of the experiments showed essentially the same results, namely, that no 2,4-D whatever could be found in the roots, although the test was easily capable of detecting minute amounts.

Because of the difficulty in growing uniform pea seedlings under hot tropical conditions, a parallel series of extracts was tested for cucumber-root inhibition.

Results (table 4) confirm the evidence obtained in the pea test. With one possible exception, there was no evidence of 2,4-D in any of the root extracts, whether the plants had been treated only as short a time as 4 days, when defoliation was imminent, or over longer periods up to $2\frac{1}{2}$ weeks when defoliation was complete. Evidence points to the entrance of the growth substance into the ratoon stems and trunk but not into the root itself. In the one exception the apparent concentration was on the border line of sensitivity

TABLE 4

DEDUCED CONCENTRATIONS OF 2,4-D IN TISSUES EXTRACTED AFTER 2,4-D TREATMENTS

MATERIAL EXTRACTED	DAYS AFTER CUTTING	DAYS AFTER 2,4-D ESTER SPRAY AT 1.0%	DEDUCED CONCENTRATION (IN MOLS)	
			Pea test	Cucumber test
Root of topped stump	27	17	0	.....
Root of topped stump	27	13	.....	0
Root of topped stump	26	Untreated	0	0
Root of topped stump	53	6	0	.....
Root of ratoon of sprayed plant	Uncut	6	0	.....
Root of ratoon of sprayed plant	Uncut	4	.....	(0.1×10^{-5})
Trunk of ratoon of sprayed plant	Uncut	6	2.0×10^{-5}	.....
Trunk of ratoon of sprayed plant	Uncut	9	.....	13×10^{-5}
Stems of ratoon of sprayed plant	Uncut	9	.....	45×10^{-5}

The test is reportedly sensitive to 2,4-D in about the same range as the pea test, namely, 5.0–0.005 p.p.m. (**9**). Extractions were made as described above, and serial dilutions using 15 ml. per Petri dish were prepared. Fifteen cucumber seeds (variety Early Fortune)[3] were placed on filter paper in each dish, and the length of the primary root was measured at the end of 96 hours' germination in the dark at 29° C. Root lengths obtained in the unknown extracts were compared with those in known concentrations of the 2,4-D butyl ester, and the concentration of 2,4-D in the unknown deduced therefrom.

[3] Kindly supplied by Dr. A. G. NORMAN, to whom we wish to express our thanks.

of the test under the conditions prevailing. Data on extracts of stems and trunks recorded in table 4 did not wholly exclude surface residues of the herbicide and should not necessarily be interpreted as showing internal concentration alone. It should be added that the fact that this test depends on an inhibition and not on a growth promotion makes it possible that the apparent activity was not solely due to 2,4-D in spite of the preliminary purification of the extracts.

EFFECTS OF 2,4-D TREATMENT ON STARCH CONTENT

It had been noted that the roots of *aroma* were filled with starch, which could be demonstrated readily by stain-

ing fresh sections with an aqueous solution of 1% iodine in 1% KI. Using 400 × magnification, numerical counts were made of the grains in an entire field, including representative areas either in the phloem or bark region or in the xylem.

Starch counts in the roots of 2,4-D-treated ratoons (table 5) show clearly that the starch content was markedly reduced following treatment with 2,4-D spray and that this reduction was correlated closely with the onset of defoliation.

TABLE 5

STARCH COUNTS IN ROOT TISSUE OF RATOONS OF PLANTS SPRAYED WITH 1.0% 2,4-D

No. of days after spraying	Appearance of plant	No. of starch granules per microscopic field (400×)	
		Xylem	Phloem
0	Foliage green	384	*ca* 1000
3	Foliage yellowing	278	700
4	Foliage browning	304	105
6	Defoliated	214	68
6	Defoliated	103 *	46 †
6	Defoliated	154	108

* Mean 157. † Mean 74.

TABLE 6

STARCH COUNTS IN ROOT TISSUE OF 2,4-D-TREATED AND UNTREATED STUMPS OF "AROMA MARABÚ"

Root no.	2,4-D treatment	No. of days		Final appearance of plant	No. of granules per microscopic field (400×)	
		After cutting	After 2,4-D treatment		Xylem	Phloem
1	None	36	.....	1–2-ft. ratoons	85	0
2	None	69	.....	2–3-ft. ratoons	184	352
3	1% spray	47	10	2-ft. ratoons, defoliated	65	3
4	0.5% spray	24	11	1–2-ft. ratoons, defoliated	61	0
5	20% painted twice	50	50	No ratoons	214	632
6	20% painted twice	71	71	No ratoons	138	195

Roots to be tested were dug up and hand-sectioned with a razor blade, stained for 10 seconds in the iodine solution, washed in distilled water, and mounted under a glass cover slip. By use of this technique, observations were made periodically on the roots of treated plants in order to determine the effect, if any, of 2,4-D treatment on the starch content. Both intact and topped plants were observed.

The effect on starch counts of cutting adult plants to stumps and then treating subsequently with 2,4-D is shown in table 6. It is apparent that the removal of foliar tissue which results from topping, and which is followed by ratooning from the base or from main roots near the base, causes at first a reduction in the starch content of the root (root no. 1). Later, when the ratoons provide photo-

synthetic materials above the immediate needs of the plants, these are stored in the roots as starch (root no. 2). Spraying the young ratoons with 2,4-D acts together with the stump-cutting to maintain a very low starch content in the roots (roots nos. 3 and 4) as a result of removing the source of new carbohydrate. The counts in these roots are much lower than in those of table 5. It is to be noted that starch deposits in the xylem are considerably more stable than in the phloem and show a lag in every case compared with the starch content of the phloem in the same root. This indicates that, as would be expected, the starch in the phloem is much more directly dependent on supply from the leaves than that in the xylem. Painting the stumps with 2,4-D solutions prevents the formation of new ratoons by a prolonged bud inhibition, but over this long period there is apparently a replenishment of the storage starch in the root (nos. 5 and 6). In view of the very extensive root systems of these plants, it seems likely that carbohydrate may be supplied from interconnected, untreated plants at a distance. This may, of course, likewise occur without bud inhibition, as in root no. 2. It might be predicted that plants showing low starch content after such a prolonged period as 70 days will show little or no ratooning, whereas high-starch content would indicate probable regrowth. Removal of starch from the roots of *aroma* plants may indeed be a prerequisite to ultimate killing.

Conclusions

The data of these experiments concerning the transport of 2,4-D and its effect on the plant point to the following conclusions. There is no evidence that 2,4-D, applied either as a spray or in paint treatments, enters into the roots of the *aroma* plant (within 17 days) in concentrations demonstrable by the tests utilized, although it does penetrate into the trunks. 2,4-D solutions, when applied to the above-ground parts of the plants, act to reduce the carbohydrate stores present in the roots in the form of starch. Starch content of the roots reflects the condition of the plant and can be lowered by repeated defoliation, either as a result of herbicidal treatment or by topping. It can apparently also be replenished, perhaps from plants with interconnected roots at some distance away. The early stages of regrowth cause a reduction in starch content, and spraying at the appropriate time after growth has begun can therefore be very valuable in bringing about an exhaustion of the plant's reserves. It appears essential to treat all plants in one area at the same time, so as to allow no source for replenishment of carbohydrate to the plants. Topping and 2,4-D treatment act in the same direction toward the removal of storage starch in the roots.

Summary

1. The effectiveness of several herbicides when applied in high concentration by painting on the cut stumps of *aroma* or *marabú* (*Dichrostachys nutans*) has been determined.

2. A 20% aqueous emulsion of the butyl ester of 2,4-dichlorophenoxyacetic acid (2,4-D), applied twice, was the most effective of the preparations tried in repressing new shoot growth. Higher or lower concentrations of this emulsion, the butyl ester of 2,4,5-trichlorophenoxyacetic acid (2,4,5-T), and concentrated sodium arsenite solutions were all less effective.

3. The addition of gas oil to the 2,4-D ester greatly retarded the latter's ef-

fectiveness, and there was evidence of inhibition of its transport in the stump.

4. Extraction and bio-assay of the 2,4-D in portions of treated plants showed no evidence of its entry into the roots, within $2\frac{1}{2}$ weeks, though it was detected in stems.

5. Removal of the tops of the plants causes a decrease in starch content of the roots, but over a period of several weeks the starch content may rise again, possibly as a result of contributions from other plants on the same root system.

6. Defoliation by treatment with 2,4-D also brings about great reduction in starch content, but unhindered development of leafy shoots increases it. It is suggested, therefore, that repeated defoliation may be necessary for killing the underground parts of the plant.

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