RESISTANCE MECHANISMS OF *OXALIS PES-CAPRAE* (SOURSOB) TO 2,4-DICHLOROPHENOXYACETIC ACID

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Summary

The uptake, translocation, and metabolism by *O. pes-caprae* plants of carboxyl- and methyl-labelled [^{14}C]-2,4-dichlorophenoxyacetic acid (2,4-D) were studied by chromatographic, radioautographic, and scintillation-counting techniques. In young *O. pes-caprae* plants some translocation of ^{14}C out of the treated leaves occurred while in older plants it was severely restricted. Qualitative and quantitative chromatography of the acidic fractions of methanolic extracts soluble in ether or ethyl acetate or both revealed the presence of at least 12 radioactive compounds, often within 1 day from treatment. The bulk of the activity (90–95%), was present in a spot chromatographically identical with 2,4-D regardless of the region of the plant or the position of ^{14}C in the molecule. Evidence was obtained that about half of the "presumed" uptake was not accounted for even after $^{14}CO_2$ evolution, leakage from roots, and residual activity in extracted material are taken into account. The observed instability of the various radioactive products upon rechromatography underlines the caution that must be exercised in interpreting the physiological significance of interconversions. It is concluded that restricted translocation and metabolic attack of the 2,4-D molecule, with the formation of unstable intermediates, some of which may be volatile, are contributing factors in the high resistance of *O. pes-caprae* to the herbicidal action of 2,4-D.

I. Introduction

Many approaches have been used to study the mechanism of resistance of various species to auxin-type herbicides but no unifying hypothesis has emerged. In some resistant species the uptake or translocation or both of the applied herbicide to critical regions of the plant, such as meristems, is restricted (Gallup and Gustafson 1952; Fang and Butts 1954; Weintraub, Reinhart, and Scherff 1956; Petersen 1958; Ashton 1962). Although this mechanism may explain some cases of resistance it does not account for those situations where a substance is known to reach a critical region of the plant but fails to cause damage. In such cases metabolic detoxification mechanisms have been inferred since it is generally believed, although unequivocal evidence is lacking, that the unaltered, free 2,4-D molecule is the physiologically active agent (Butts and Fang 1956; Hay and Thimann 1956*a*; Andreae and Good 1957; Luckwill and Lloyd-Jones 1960*a*, 1960*b*; Bach 1961; Slife *et al.* 1962).

The translocation and fate of radioactive 2,4-dichlorophenoxyacetic acid (2,4-D) in a resistant species, *Oxalis pes-caprae* (soursob), have been studied to obtain information on the problem of resistance, in general, and the mechanism of soursob's high tolerance towards 2,4-D in particular.

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II. Methods

(a) *Plant Material*

The life cycle of *O. pes-caprae* has been described previously in detail (Ducellier 1914; Thoday 1926; Thoday and Davey 1932) but a brief account here will serve to underline the problems involved. After sprouting of the bulb and emergence of the shoot above ground, one of the roots originating at the base of the parent bulb enlarges transversely (stage 1: young plants). This root, which at full maturity may attain a diameter of about 1 cm and a length of 20–30 cm, eventually contracts longitudinally by the progressive collapse of well-defined transverse plates of cells (stage 2: mature plants). *O. pes-caprae* plants were used at these two developmental stages. The retraction of the contractile root is accompanied by the elongation of a rhizome connecting the root with the aerial parts of the plant and bearing buds that enlarge and develop into the next generation of bulbs. By the end of the season the thick root contracts completely, tops and roots wither and die, and the daughter bulbs remaining enter a period of dormancy lasting 4–5 months.

(b) *Treatment of Plants*

[^{14}C]2,4-D, labelled in either the carboxyl or methyl position, was obtained from the Radiochemical Centre, Amersham, England, and repurified chromatographically. Solutions of radioactive and non-radioactive 2,4-D were mixed to give the desired concentrations of 2,4-D and amount of radioactivity in terms of counts per second. In all experiments each plant was treated with 250–500 μg of 2,4-D (activity of the order of 0·05 μc). Each treatment involved five plants growing in the same pot and the solution was applied to 2–5 young, fully expanded leaves on each plant.

(c) *Harvesting and Extraction Procedures*

The plants were separated into the following parts: treated leaves including petioles (region A), untreated leaves (region B), apical crown, rhizome, and fibrous roots (region C), and contractile root (region D).

The treated leaves (region A) were washed for 30 sec in 20 ml 50% ethanol, drained thoroughly against the sides of the beaker, rinsed, blotted, weighed, and frozen. Aliquots of the leaf washings were counted to determine total activity remaining on the leaf surface. Regions B, C, and D were weighed and frozen without the above washing procedure.

The frozen material was extracted with 90% methanol at 4°C for 24 hr. After washing with two changes of methanol the material was air dried and stored, while the methanolic extract was evaporated to dryness at 40°C under an air stream. The residue was taken up in 0·5M $NaHCO_3$ and if any pigments were present (regions A, B, and C) they were removed by shaking with toluene (no radioactivity passed into the toluene phase under neutral conditions). The water phase was extracted with three lots of ether; this neutral ether fraction was non-radioactive in all cases. The water phase was then acidified to pH 3·5 and extracted with ether to yield the acidic ether-soluble fraction. Following this, the water phase was further extracted

with three lots of ethyl acetate at either pH 3·5 or 2·3 to yield the acidic ethyl acetate-soluble fraction. The water phase was kept for determining residual radioactivity. Aliquots of the various fractions were counted to keep a quantitative check on each step of the extraction. Next, the various fractions were evaporated to dryness, taken up in a known volume of methanol, and aliquots were counted or used for chromatography.

(*d*) *Chromatography and Quantitative Assay of Active Spots*

After equilibration for 3 hr, chromatograms were developed to a distance of 20–22 cm on Whatman No. 1 paper by the ascending method in isopropanol–ammonia–water (10 : 1 : 1 v/v). The distribution of radioactivity was determined by eluting each spot (located radioautographically) in 10 ml of absolute methanol, evaporating the eluate to dryness, taking up the residue in a known volume of methanol, and counting an aliquot.

(*e*) *Hydrolysable Conjugates of 2,4-D*

Aliquots of eluted spots were refluxed for 1 hr in 1·0N HCl or 1·0N KOH. After cooling the pH was adjusted to 3·5 and the hydrolysate extracted with ether to separate any free 2,4-D produced during hydrolysis. The ether phase was reduced in volume and used for chromatography and subsequent radioautography.

(*f*) *Evolution of Radioactive CO_2*

Plants were placed in transparent plastic chambers provided with inlet and outlet tubes. Supplementary illumination (fluorescent light) was provided between 9 a.m. and 5 p.m. and the CO_2 that had accumulated was collected at these two times by flushing out the chambers and absorbing the CO_2 in NaOH. After precipitation with $BaCl_2$ the resulting $BaCO_3$ was dried, weighed, and aliquots counted.

(*g*) *Scintillation Counting*

(i) *Equipment.*—An Ecko N612 counter and N530 scaler were used at 1150 volts, discriminating bias 10, and linear amplifier gain 100. Counting was carried out at room temperature and under these conditions background was 1 count per second.

(ii) *Counting of Solutions.*—By using 5 ml of Diotol scintillator (Herberg 1960) 0·2 ml of methanol containing ^{14}C could be counted satisfactorily (efficiency 85%) but only 0·1 ml could be used if the radioactivity was present in water or bicarbonate (efficiency 65%). In the toluene used to remove pigments from the extracts counting efficiency was further reduced, but even so it is possible to conclude that the toluene phase contained only traces of radioactivity.

(iii) *Counting of $^{14}CO_2$ as $BaCO_3$.*—Weighed aliquots of $BaCO_3$, about 100 mg, were suspended by shaking in a thixotropic gel prepared by adding Thixcin R (Baker Castor Oil Co.) to a solution of toluene 2,5-diphenyloxazole (PPO) and *p*-bis-2,5-phenyloxazylbenzene (POPOP) (Helf 1958). The efficiency of this system was about 38%.

TABLE 1

BALANCE SHEET OF RADIOACTIVITY

Carboxyl-labelled 2,4-D was applied to leaves of young (expt. 1) or mature (expt. 2) plants as indicated after painting the leaves with wetting agent (0·1% Comprox solution) and allowing to dry

Conditions of Application of Labelled 2,4-D	Time (days)	Applied Activity (counts/sec) [A]	Activity in Leaf Washings: (counts/sec) [B]	Activity in Leaf Washings: (as % of applied activity) [C]	Decrease in Activity of Leaf Washings after 5 Days (%) [D]	Presumed Uptake (counts/sec) [E=A − B]	Total Activity Extracted (counts/sec) [F]	Activity Unaccounted for (as % of activity presumably taken up) $\left[G=\frac{(E-F)}{E}\times 100\right]$
Experiment 1 (young plants)								
In water	1	17950	15950	89	—	2000	870	56
	5	17950	14700	82	7	3250	1160	64
	12	17950	13100	73	—	4850	1660	66
Experiment 2 (mature plants)								
In water, pH 7·0	1	6990	4240	61	—	2750	850	79
	5	6990	3600	52	9	3390	1570	54
In water, pH 4·5	1	6980	3880	56	—	3100	1140	63
	5	6980	3680	53	3	3300	1070	68
In 2% sucrose, pH 7·0	1	6840	4480	66	—	2360	930	60
	5	6840	2800	41	25	4040	1780	56
In 2% sucrose, pH 4·5	1	6810	3880	57	—	2930	960	67
	5	6810	2320	34	23	4490	2380	47
In 0·1% orthophosphate, pH 7·0	1	6760	4000	59	—	2760	1290	53
	5	6760	3240	48	11	3520	1720	51
In 0·1% orthophosphate, pH 4·5	1	6840	4640	68	—	2200	840	62
	5	6840	2640	39	29	4200	2160	49

(iv) *Counting of Residual Activity in Methanol-extracted Tissue.*—Of various methods tested the most reliable involved adding weighed aliquots of the dried material to 5 ml of "Hyamine hydroxide" (Nelson, personal communication), leaving for 24 hr at room temperature, and then counting aliquots. Initially spurious counts were obtained, e.g. background was 15 counts/sec, but after 3 hr the normal background of 1 count/sec was reached and counting remained stable. Therefore, all counting was done 3 hr after introducing the aliquot into the scintillator.

III. Results

(*a*) *Amount of Radioactivity Recovered from the Leaf Surface*

The data in Table 1 (columns A, B, C, and D) indicate certain consistent trends regardless of the method of application of 2,4-D and the stage of plant development. For example, the amount of radioactivity recovered from the leaf surface decreases with time (column D) and at least part of this decreased radioactivity is reflected in an increase of that extractable from the plant (column F). However, the specific conditions of application may affect the extent of the reduction of activity in the washings between the first and fifth days (column D) as in the case of sucrose additive compared with water. The pH of the applied solution is also a contributing factor under certain conditions as in the case of addition of orthophosphate.

(*b*) *Unaccounted Activity*

It is commonly believed that radioactivity recovered in leaf washings is in the form of unchanged 2,4-D and that the difference between the amount applied and that recovered has been taken up by the plant. This may not always be so. Chromatography of leaf washings revealed spots other than 2,4-D, indicating instability when in contact with the leaf surface (no changes were detected when dried aliquots of the active solutions were kept in test tubes under environmental conditions identical with the plants). Further, a large and reasonably constant amount of the radioactivity presumably taken up was unaccounted for in all experiments (Table 1, columns E, F, and G). In view of this, the following possibilities have been considered:

(1) *Loss of Activity during Recovery of Leaf Washings.*—The method used was checked by determining the *total* activity in extracts from unwashed leaves; in this case also the magnitude of the unaccounted activity was comparable with that shown in Table 1.

(2) *Respiratory Loss of* $^{14}CO_2$.—Both carboxyl- and methyl-labelled 2,4-D gave identical results, with the total $^{14}CO_2$ recovered over 6 days amounting to about 4% of the missing activity.

(3) *Leakage of Activity from the Roots.*—Only about 0·5% of the missing activity was found in the ambient solution of plants grown in water culture.

(4) *Radioactivity of Plant Material Insoluble in Cold Methanol.*—After extraction in cold methanol the plant material was refluxed for 1 hr in 80% methanol, placed for 24 hr at 20°C in "Hyamine hydroxide", and then for 30 min at 60°C in "Hyamine hydroxide", but only traces of activity were recovered at each of these steps.

The possibility remains that radioactivity is incorporated into volatile substances other than CO_2 and lost during the entry, translocation, and metabolism of 2,4-D in the plant, and during processing of the material. There is as yet no clue as to the nature of these postulated "volatiles"; clearly a detailed study would be desirable. As a first step the direct counting of radioactivity in the gaseous environment of the plant, and a similar check for radioactivity emitted during processing of the extracts, could be made by means of gas-counting equipment. However, this equipment was not available for these studies to be made. Because of these uncertainties, the term "presumed uptake" has been used here.

(c) *Translocation of Radioactivity*

Typical distribution patterns of ^{14}C in *O. pes-caprae* plants are shown in Table 2 and it is clear that the developmental stage of the plant at treatment determines this distribution to some extent.

TABLE 2

DISTRIBUTION OF EXTRACTABLE RADIOACTIVITY IN PLANTS AT TWO DEVELOPMENTAL STAGES

Conditions of Application of Labelled 2,4-D	Time (days)	Total Activity (counts/sec) Recovered from:			
		Treated Leaves	Untreated Leaves	Apical Crown	Contractile Root
Experiment 1 (young plants)					
In water	1	795 (91·4)*	10 (1·1)	65 (7·5)	0 (0)
	5	540 (46·5)	145 (12·5)	420 (36·2)	55 (4·7)
	12	950 (57·2)	270 (16·3)	230 (13·9)	210 (12·6)
Experiment 2 (mature plants)					
In water, pH 7·0	5	1500	40	20	10
In water, pH 4·5	5	1020	30	20	10
In 2% sucrose, pH 7·0	5	1700	40	30	10
In 2% sucrose, pH 4·5	5	2350	10	10	10
In 0·1% orthophosphate, pH 7·0	5	1680	20	10	10
In 0·1% orthophosphate, pH 4·5	5	2100	30	20	10
Mean percentage distribution (experiment 2 only)		(96·9)	(1·6)	(1·0)	(0·4)

* Percentage distribution given in parenthesis.

In experiment 1 (young plants) the percentage distribution of activity is calculated for each sampling time to demonstrate the gradual translocation of ^{14}C with time. In experiment 2 (mature plants), where only traces of activity were exported from the treated leaves, only the data for the fifth day are given, and these are pooled for a given plant region (since distribution is similar under all conditions of application of 2,4-D) to give a mean percentage. Comparison of the percentages for the fifth day shows that in mature plants about 95% of the activity recovered was found in the treated leaves while in young plants a significant part of the activity moved out of the treated leaves and some reached the developing contractile root.

(*d*) *Distribution of Radioactivity in the Various Fractions*

The distribution of activity followed the same general pattern in all experiments. Table 3 was compiled from the data in experiment 1 where young plants were used and translocation to the rest of the plant was appreciable (see also Table 2). On the fifth and twelfth days active fractions were obtained from all regions of the plant and the results show that the acidic ether-soluble fraction contained the bulk

TABLE 3

DISTRIBUTION OF RADIOACTIVITY IN FOUR REGIONS OF THE PLANT AMONGST THREE ACTIVE FRACTIONS OF THE METHANOLIC EXTRACTS OF EXPERIMENT 1

The neutral ether fraction was inactive

Plant Region	Time (days)	Radioactivity (counts/sec) in:		
		Ether-soluble Fraction (pH 3·5)	Ethyl Acetate-soluble Fraction (pH 3·5)	Residual Water Phase
Treated leaves	1	735	20	40
Untreated leaves		10	0	0
Apical crown		65	0	0
Contractile root		0	0	0
Total (counts/sec)		810	20	40
Total (%)		93·1	2·3	4·6
Treated leaves	5	460	40	40
Untreated leaves		105	20	10
Apical crown		390	30	10
Contractile root		55	0	0
Total (counts/sec)		1010	90	60
Total (%)		87·0	7·8	5·2
Treated leaves	12	460	280	210
Untreated leaves		190	60	20
Apical crown		190	30	10
Contractile root		190	10	10
Total (counts/sec)		1030	380	250
Total (%)		62·0	22·9	15·1

of the activity. The remainder partitioned about equally between the ethyl acetate and the water phase at pH 3·5 (in other experiments at pH 2·3 all the activity entered the ethyl acetate phase). It is noteworthy that the activity of the acidic ether-soluble fraction declines with time while that of the ethyl acetate phase tends to increase.

(*e*) *Chromatographic Analysis of the Extracts*

Since analysis of regions B, C, and D was hindered in most cases by low levels of radioactivity, especially in the ethyl acetate fraction, the work described in this

and subsequent sections involved material from treated leaves. However, in cases where considerable activity was present in other parts of the plant, e.g. experiment 1, and analysis was practicable, no differences were evident. The results in Figure 1 were compiled from experiment 2 to illustrate the differences in the qualitative composition of the acidic ether-soluble and ethyl acetate-soluble fractions and the relative activities of the various compounds.

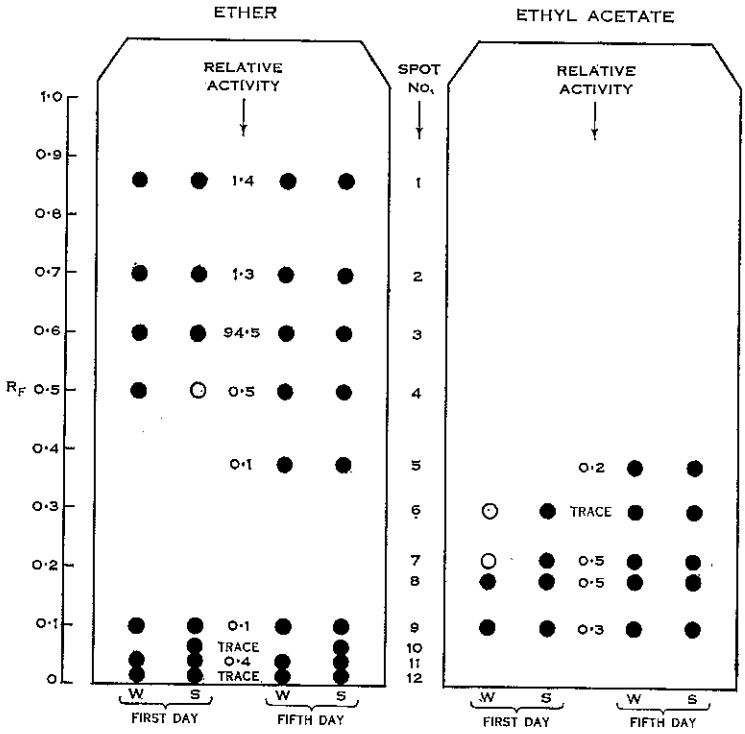


Fig. 1.—Chromatographic analysis of radioactive compounds in the acidic ether-soluble and ethyl acetate-soluble fractions of methanolic extracts from the treated leaves in experiment 2. The relative activity of all spots at a given position is calculated as the percentage of total activity recovered in the eluates of both fractions. Data from the pH 7·0 series are shown but results were essentially the same for pH 4·5. *W*, labelled 2,4-D applied in water; *S*, labelled 2,4-D applied in 2% sucrose. The results with orthophosphate were identical with those in water. ● Radioactive spot definitely present. ○ Presence uncertain due to extremely low activity.

The following conclusions may be drawn: spots 1, 2, 3, and 4 are found exclusively in the ether fraction under all conditions of application and on both days. It is to be noted that spots 1, 2, and 4 were also found in old solutions of labelled 2,4-D, apparently arising spontaneously. Spots 5 and 9 partition in both ether and ethyl acetate but spot 5 seems to be a slowly forming compound since it appears only on the fifth day. Spots 6, 7, and 8 are primarily compounds soluble in ethyl acetate. Spot 10, which partitions only into the ether fraction, appeared, at least in this experiment, only when 2,4-D was applied in the presence of sucrose, while spots 11 and 12 partitioned into the ether fraction and were present on both dates.

The relative radioactivities of the various spots have been expressed as a percentage of the total radioactivity recovered from both fractions. About 95% of the activity is found in spot 3 (the 2,4-D position). Carboxyl- and methyl-labelled 2,4-D gave identical results.

(*f*) *Fate of 2,4-D in Excised Tissues*

Treatment of intact plants does not give unequivocal information on the fate of the herbicide in regions remote from the site of application because of possible translocation of byproducts. To elucidate the fate of carboxyl- and methyl-labelled 2,4-D in the apical crown and contractile root, slices of these organs, 1 mm thick, were incubated for 24 hr at room temperature in the appropriate solutions. The tissues were then extracted with methanol and after reduction in volume the total extract was chromatographed. Aliquots of the ambient solution were also chromatographed.

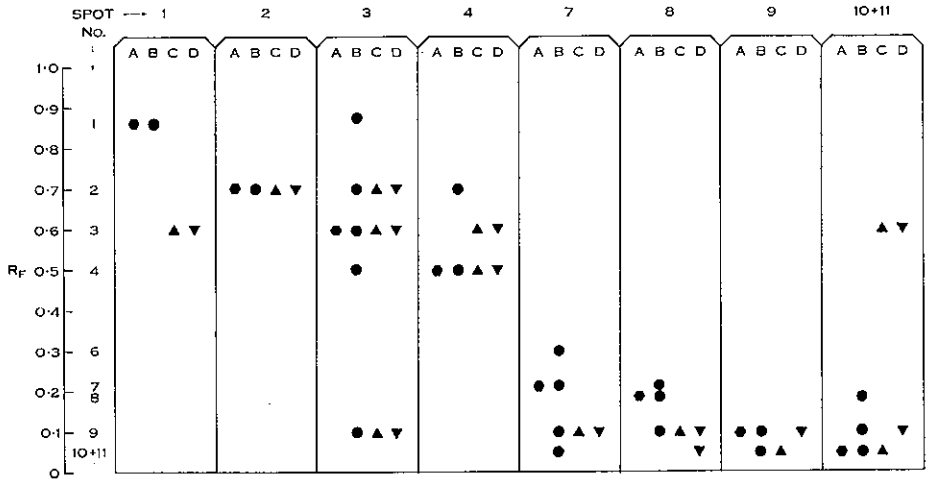


Fig. 2.—Behaviour of radioactive compounds (column A), extracted from treated leaves on the fifth day of experiment 2, upon rechromatography (column B), acid hydrolysis (column C), or alkaline hydrolysis (column D). Spot number 3 is chromatographically identical with authentic 2,4–D. Spots 10 and 11 were not easy to elute separately and hence were combined.

The compositions of the apical crown and the root extracts were essentially the same when carboxyl-labelled 2,4-D was supplied. In the case of the methyl-labelled compound the root extracts did not contain spot 7 although the total radioactivity of the extract was higher than that of the roots exposed to the carboxyl label. This leads to the suggestion that in the roots the mechanisms responsible for changes in the 2,4-D molecule involving the methyl carbon are less efficient. Supporting evidence was provided by the chromatographic analysis of the ambient solutions where spot 7 was present in carboxyl-labelled 2,4-D but absent in methyl-labelled 2,4-D. Spots 9 and 12 were also found in the ambient solutions but in extremely low amounts.

(*g*) *Characterization of the more Active Compounds*

Figure 2 shows the results of hydrolytic tests: acid and alkaline hydrolysis of spots 1, 4, and 10+11 yielded a compound with an R_F typical of authentic 2,4-D, indicating that these active compounds are probably conjugates; acid and alkaline hydrolysis of spot 3 yielded the additional spots 2 and 9; interconversions of spots 7, 8, 9, and 10+11 had as a common feature the formation of spot 9 after alkaline hydrolysis. However, even more informative than these hydrolytic tests is the behaviour of the various spots upon elution and rechromatography (column B, Fig. 2). It is clear that, at least under the specified experimental conditions, a number of compounds appeared spontaneously.

IV. Discussion

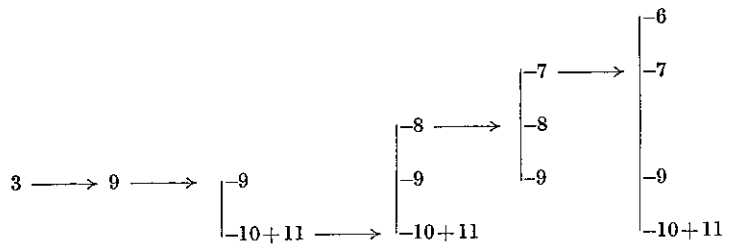
Evidence has been produced (Gallup and Gustafson 1952; Fang and Butts 1954) that in resistant monocotyledons the uptake of auxin herbicides is smaller than in susceptible dicotyledons. Other work (Butts and Fang 1956; Weintraub, Reinhart, and Scherff 1956; Ashton 1962; Morgan and Hall 1962; Slife *et al.* 1962) has shown that differential absorption is not always correlated with resistance or susceptibility. That the degree of absorption from the leaf surface cannot be taken as a basis for interpreting inherent resistance or susceptibility is strengthened by the evidence that absorption is influenced by external conditions like temperature and humidity (Barrier and Loomis 1957; Pallas 1960) and the pH of the solution (Crafts 1956), and that widely variable absorptions can be obtained for a given species under identical conditions (Holley, Boyle, and Hand 1950). Nevertheless, the amount taken up reflects at least part of the fate of the applied substance. But, in the present experiments the large proportion of radioactivity that was unaccounteed for precluded the estimation of actual uptake, and the value of "presumed uptake" is questionable.

Restricted translocation is considered to be the primary mechanism of resistance in certain monocotyledons (Gallup and Gustafson 1952; Fang and Butts 1954; Weintraub, Reinhart, and Scherff 1956; Petersen 1958; Ashton 1962). In *O. pes-caprae* the extent of ^{14}C (applied as 2,4-D-1-^{14}C) translocation was related to the developmental stage of the plant, being considerably greater in the younger plants (Table 2, cf. distribution percentages on the fifth day in experiments 1 and 2). It has been shown that the translocation of 2,4-D from leaves is dependent on the concurrent export of carbohydrate. For example, the simultaneous application of 2,4-D and sucrose greatly enhances the translocation of the weedicide from carbohydrate-starved leaves (Mitchell and Brown 1946; Lindner, Brown, and Mitchell 1949; Crafts 1956; Hay and Thimann 1956*b*; Leonard and Crafts 1956; Barrier and Loomis 1957; Leonard 1958; Clor 1961). But, if the plant is in a state of development when no "food" is being exported from the leaves then translocation of applied 2,4-D would be normally restricted and small amounts of sugar added to the leaf along with the 2,4-D would not alter the translocation pattern (Crafts 1956). This seems to be the case in the older plants where the presence of sucrose did not increase the negligible translocation of 2,4-D. The approximate amounts of free 2,4-D that moved into the apical crown when 500 μg of 2,4-D was applied per plant have been

calculated for the fifth day from treatment: young plants, 10 μg; mature plants, 1 μg. These calculations also illustrate the small extent of translocation of 2,4-D in *O. pes-caprae* since only 0·2–2·0% of the applied material reached the apical crown.

Qualitative and quantitative chromatographic analysis of methanolic extracts (Tables 2 and 3; Fig. 1) indicated the presence of a number of radioactive compounds although the bulk of extractable activity in all regions of the plant was found in a position corresponding to that of authentic 2,4-D. Whether this substance is identical with 2,4-D is uncertain in view of the recent work of Bach (1961) who recovered a substance having the same R_F as 2,4-D but differing from the authentic molecule in a number of qualitative tests. The balance of the activity extracted from *O. pes-caprae* was found in 11 additional positions. Of these spots 7 and 8 are interesting in that they are insoluble in ether but soluble in water and ethyl acetate, and possibly correspond to substances with similar properties reported previously (Holley 1952; Jaworski and Butts 1952; Fang and Butts 1954; Weintraub *et al.* 1954; Audus and Symonds 1955; Jaworski, Fang, and Freed 1955; Butts and Fang 1956; Andreae and Good 1957; Luckwill and Lloyd-Jones 1960*a*, 1960*b*; Bach 1961; Bach and Fellig 1961; Slife *et al.* 1962).

The spontaneous changes that take place upon rechromatography of the various compounds extracted from *O. pes-caprae* plants following application of 2,4-D draw attention to the difficulties of assessing the significance of these products in terms of the physiological response of the plant. For example, a series of spontaneous changes can be proposed on the basis of Figure 2 that will result in the formation of all slow-moving compounds from spot 9 which, in turn, arises from a compound recovered from the plant and chromatographically identical with 2,4-D:



These results underline the strong possibility that the whole spectrum of radioactive compounds detected in the extracts arises during processing. It must be noted that these spontaneous changes did not occur in 2,4-D solutions (except spots 1, 2, and 4, which do not enter this scheme) but they were only observed when spot 3 (the 2,4-D position) was isolated from extracts. This may be indirect evidence supporting Bach's (1961) more direct observations that the radioactive compound with the same R_F as authentic 2,4-D is a changed molecule susceptible to spontaneous interconversion. A similar instability of radioactive products has been reported by Weintraub *et al.* (1952) who noted that radioactivity was lost from chromatograms upon storage, indicating the presence of volatile products. This finding may be relevant to an explanation of the large proportion of radioactivity that could not be accounted for by normal extractions or by determinations of $^{14}CO_2$ evolution, residual activity in the tissue, and leakage from the roots. All of

these facts lead to the conclusion that the radioactivity was probably lost in some volatile form involving both carbons of the side chain.

Volatilization may be a detoxification mechanism and, coupled with restricted translocation, may account for the high resistance of *O. pes-caprae* to 2,4-D.

V. Acknowledgments

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