Patterns of absorption and initial translocation of CI\textsuperscript{36}- and C\textsuperscript{14}-labeled 2,2-dichloropropionic acid (dalapon) in both tolerant and susceptible species were described earlier (8). Of equal or perhaps more importance in understanding the physiological action of growth regulators are the long-term translocation, re-distribution, accumulation, and metabolic fate of the compound in association with plant tissues. The recent emphasis on pesticide residues in food and feed products has given the latter considerations an additional order of importance. Despite the foregoing, perhaps our greatest ignorance of herbicides today concerns their ultimate fate in plants. The tendency of a herbicide to persist in an active form or to be detoxified rapidly is likely to be of primary importance, whatever the precise mechanism(s) of herbicidal action.

All of a given herbicide that enters a plant, e.g. via the leaves, is not necessarily available for translocation and/or herbicidal action. Possible mechanisms accounting for the immobilization, inactivation, or disappearance of the compound are adsorption on colloidal surfaces, true accumulation by living cells, metabolic degradation followed by loss or incorporation of breakdown products, and reduced movement due to phase distribution effects as on a chromatogram (6, 29, 30, 31). The direct loss of various substances from roots or other plant parts may also occur (4, 5, 8, 14, 16, 26).

Considerable research has been conducted on the loss or persistence of 2,4-dichlorophenoxyacetic acid (2,4-D) in both active and dormant tissues (17, 18, 19, 22, 29, 31). Several papers are summarized in a recent review (33). Radioactive 2,4-D is known to be metabolized to varying degrees in plant tissues, with the resultant incorporation of C\textsuperscript{14}-labeled compounds and the evolution of C\textsuperscript{14}O\textsubscript{2}. Strong evidence exists also for relatively long persistence of 2,4-D in plants, which leads to symptomatic expression in succeeding seasons (perennials) or succeeding generations (annuals) by transmission through dormant buds or seeds. This latter phenomenon has been observed following both foliar and root applications. 2,4-D is known to persist in plants for longer periods than exogenous indoleacetic acid.

Prior to the present series of experiments (6, 8), none of the foregoing topics had been investigated with respect to dalapon. Herein are reported the results of several studies on the distributional and metabolic fate of dalapon in association with plant tissues. Finally, these results, as well as experimental findings from other sources, will be discussed in relation to possible mechanisms of herbicidal selectivity.

**Materials & Methods**

The general techniques and materials employed in these studies have been described (8, 9). Radiography, counting, extraction and fractionation, and paper partition chromatography were combined to yield both qualitative and quantitative results.

As in the studies reported earlier (8), the following radiolabeled chemicals were used in treating plants or plant material: A: purified 2,2-dichloropropionic acid-Cl\textsuperscript{36} in acetone (12.78 \( \mu \)c/nmole converted to the sodium salt in aqueous solution before application to plants; B: 96% Na-2,2-dichloropropionate-2-C\textsuperscript{14} (0.98 mc/mm); 78% Na-2,2-dichloropropionate-2-C\textsuperscript{14} (0.98 mc/mm). In addition, Na-2,2-dichloropropionate-2-C\textsuperscript{14} (containing on a radioactivity basis over half LiCl\textsuperscript{36}), monochloropropionate-2-C\textsuperscript{14}, and 2,2,3-trichloropropionate-2-C\textsuperscript{14} were used as standards in chromatography.

Extracts were spotted approximately one centimeter diameter on Whatman No. 1 filter paper by use of a micropipette. After equilibration, the chromatograms were developed one-dimensionally overnight using n-butanol and 1.5 n ammonium hydroxide (6, 9, 25).

In one phase of the study, dried, ground fruits of cotton were extracted and fractionated according to the scheme shown in figure 1.

Further procedural details are given in the Results section.

**Results & Discussion**

**Degradation by Micro-Organisms:** Herbicidal activity may be diminished in soils by volatilization, leaching, fixation by adsorption to soil colloids, chemical or photochemical decomposition, and microbiological breakdown. The last of these is perhaps of greatest importance in the disappearance of most organic herbicides tested thus far. Other effects such
FOY—2,2-DICHLOROPROPIONIC ACID & PHYTOTOXICITY

D R Y P L A N T M A T E R I A L
(COTTON)

ETHER
EXTRACT
3x

LIPIDS,
PIGMENTS,
ORGANIC ACIDS

WASH, 10%
Na₂CO₃

AQ U E O S
ETHER

LIPIDS,
PIGMENTS
(CHLOROPHYLLS, CAROTENES,
XANTHOPHYLLS, ETC.)

EVAP. ETHER
TO SMALL VOL.
ADD PET. ETHER
WASHINGTON
80%
EtOH

H₂O
PET. ETHER

PET. ETHER
CARR. COTENES, ETC.

1st
SEPARATION

ANION RESIN
DUOLITE A-2

ELUTE
NH₄OH (IN)
PH 10-11

RESIN

ANIONIC ACIDS
(METABOLIC ACIDS,
PHOSPHORYLATED
CPDS, ETC. - ALSO
DALAPON)

HCl
pH 1.5
CONTINUOUS
ETHER EXTRACT
8 HRS.

AQUEOUS

10%
Na₂CO₃
AND
DE-IONIZED
H₂O

ETHER

AQ U E O S
(DALAPON)

RESIDUE

EXTRACT
80% E10H
2X, 60°C, 3 HRS.

SUGARS,
ORGANIC ACIDS,
AMINO ACIDS, ETC.

CONC.
IN VACUO
55 - 65°C
6 HRS.

FILTRATE

SEPARATE
SUCCESSIVELY
ON ION
EXCHANGE
RESINS

PRECIPITATE
SUPERNATANT

RESIDUE

H₂O
EXTRACT

SOLUBLE PROTEINS, ETC.

RESIDUE

ENZYMATIC
DIGESTION WITH
AMYLASES AND
PROTEINASES TO
TEST FOR ACTIVITY
IN STARCH, INSOLUBLE
PROTEINS AND
RESIDUAL
CARBOHYDRATES

Fig. 1. Analytical scheme followed in categorizing radioactive chemical constituents from the fruits and leaves of cotton 10 weeks after treatment with 96% Na 2,2-dichloropropionate-2-C¹⁴.
as temperature, moisture, organic matter content, nutrient levels, pH, etc. appear to be principally indirect manifestations of the level of activity of soil-borne micro-organisms.

Dalapon has little residual effect under conditions favorable to leaching and/or high micro-biologic activity (13, 27), but it is known to persist in the soil for long periods under less favorable conditions.

During the present studies, one of the first aliquots of pure dalapon-Cl\textsuperscript{36} became contaminated by micro-organisms despite storage in a refrigerator. The changes which occurred in pure stock solution offered positive proof that some species of fungi, possibly Alternaria sp., are capable of using dalapon as a sole substrate to synthesize new substances. The number of Cl\textsuperscript{36}-labeled metabolites formed was somewhat surprising. Average R\textsubscript{f} × 100 values and tentative identification of some of the spots were as follows: 8 (inorganic Cl\textsuperscript{36}), 19, 34 (monochloropropionate), 42, 51 (dalapon), and 62. No special attempt was made to identify the other three substances, but these results were confirmed many times. Dissolving the compound in alcohol rather than water prevented breakdown, but since alcohol is not a normal component of herbicidal sprays, and might exert some unknown effects upon permeability, its routine use was discounted. It was virtually impossible to use aseptic techniques in making numerous applications in the greenhouse from the same stock solution vials and it was not feasible to re-purify repeatedly. This serious technique difficulty was overcome by keeping all stock solutions frozen except during transfers. In order to avoid errors due to volume changes, the tubes were warmed each time to approximately 25°C, the temperature at which they were prepared.

Metabolism by Higher Plants: 1. Preliminary observations. The primary interests in these studies were A: to determine whether dalapon is translocated and accumulated as the intact molecule or is degraded rapidly in tolerant and/or resistant species; B: to determine what metabolic changes occurred (if any), and C: to relate such information to its herbicidal properties. In all short term experiments (3 days or less) in which expressed plant sap was chromatographed directly, only dalapon (R\textsubscript{f} 0.4-0.6) or dalapon plus impurities present in the treatment solution were found. Also, by the liquid extraction of corn leaf disks with water or ethanol 3 hours after treatment (6, 9) no new compounds were detected on chromatograms of the extracts and the ratios of dalapon to radioactive impurities already present were not altered by any (or all) of the following treatments: A: freezing overnight and thawing; B: extracting in an oven (24 hr at 50°C); C: adding 0.1% Vatsol OT to the extracting medium. The R\textsubscript{f} values of dalapon and contaminants (acetate, pyruvate, monochloropropionate, & 2,2,3-trichloropropionate) were lower, in general, than those obtained by Smith (25), using ascending chromatography.

The foregoing, plus results reported earlier (8), indicated that dalapon was metabolized very slowly, if at all, in cotton, sorghum, and corn; however, other tests using more refined techniques were considered necessary.

2. Reaction with tissue homogenates. Either dry or fresh whole cotton or sorghum plant material was homogenized in a ground glass tissue homogenizer at room temperature. Weights were computed to be equivalent to 0.2 g dry wt/10 ml H\textsubscript{2}O. Then 1 ml aliquots, comparable on a density of dry matter per milliliter base, were placed in unstopped bacteriological culture tubes with 10 ml 96% dalapon-2-C\textsuperscript{14} for 3 hours at 25°C. Some aliquots were boiled and re-cooled before adding dalapon; others were frozen and thawed; still others were added directly to fresh or dry tissue. After 3 hours, the contents of all tubes were frozen rapidly, then thawed one by one, centrifuged, 200 ml of the supernatant chromatographed and another 200 ml aliquot plancheted and counted. The test was designed to determine whether or not rapid and obvious enzymatically-controlled changes occur in the dalapon molecule while in association with tissue homogenates from higher plants. Also, the counts obtained from an aliquot (multiplied by a factor and subtracted from the total activity added) yielded the amount of dalapon held in or on plant material. Furthermore, any that remained with the plant residue after exhaustive washing with several hundred volumes of water was presumed to be metabolically incorporated in a non-diffusible constituent.

No new compounds appeared in any treatment. If dalapon was transformed into other compounds, the concentrations were too low for detection by the method used. The lowest possible limits of detectability by the radioautographic method were not established, however, levels of activity corresponding to 13.3 μg dalapon or 2.8 μg Cl\textsuperscript{36} were discernible as images on the X-ray film and by counting (scanning chromatograms). Therefore the sensitivity of the method was as great or greater than these levels. Approximately 30 to 40% of the dalapon was loosely adsorbed on plant material, but no appreciable differences were noticeable among treatments or between species, and all of the activity was easily removed by washing with water, thus indicating that no metabolic incorporation into insoluble macromolecules occurred. The experiment was repeated using dalapon-Cl\textsuperscript{36}, and the same general results were obtained.

3. Chromatography of extracts of various plant parts. Cotton and sorghum plants were treated as in previous tests with 100 ml of either dalapon-2-C\textsuperscript{14} (96%) or dalapon-Cl\textsuperscript{36}. The plants were grown in a light cabinet (500-600 ft-c) for 7 days, washed free of surface radioactivity and sectioned as follows: Cotton, 1) cotyledons (treated); 2) epicotyl (less stem below 1st leaf); 3) stem (epicotyl & hypocotyl), and 4) roots; Sorghum, 1) second and third leaves (treated), 2), first, fourth, fifth, and sixth leaves (including sheaths), 3) younger leaves and stems, and 4) roots. The plant sections were homogenized as in the preceding test and aliquots of these aqueous
extracts and of the nutrient solutions were chromatographed. See (9) for detailed explanation of techniques. Rf values of the radioactive compounds extracted from all plant parts of both species were identical with those of authentic dalapon. Concentrations of activity were greatest in the treated sections and in the epicotyls (of cotton). This is in agreement with other tests. Although the spots on the chromatograms were sometimes displaced by high solute concentrations in the drop, it was clear that dalapon and no other prominent radioactive species was recovered from all plant parts of cotton and sorghum, and from both of the nutrient solutions. Representative cochromatograms are shown in figure 2. There was some indication of a slight increase in the inorganic chloride spot (a trace was already present as an impurity in this aliquot). Although not quantitative ly convincing, this may have some importance in interpreting the initial breakdown steps of dalapon in plants. In comparison with the preceding test it is of interest to notice that a small amount of radioactivity, highest in the treated sections and the epicotyl (of cotton), but present in all plant sections, was not removable. Thus it appears that the equivalent of 3 to 300 cpm/sections was metabolically incorporated into insoluble constituents after 1 week in cotton or sorghum. In the case of C14-labeled dalapon, this would correspond to approximately 0.002 μc (1.1 μg dalapon) per sorghum plant, and 0.01 μc (5.3 μg dalapon) per cotton plant when the applied dose was 1.0 μc (530 μg dalapon) in each case.

To complement these experiments, dried plants or parts of plants known to contain high activity were selected from experiments reported earlier (8), and were extracted and chromatographed in a similar manner. Essentially the same results were obtained as follows: A: dalapon was recovered repeatedly; B: there was a suggestion of an increase in the Cl85 spot after 2 weeks, but again this was inconclusive; C: after 6 to 8 days, a small amount of radioactivity was not removable from the plant residue by homogenizing and washing exhaustively. These results all show that essentially the only radioactive product extractable with water after treatment periods of 8 days or less was dalapon; however, a very small amount of activity seems to be chemically bound thus indicating a very slow incorporation of the labeled atom(s) into insoluble compounds.

4. Distributational and metabolic fate of dalapon in flowering cotton plants. With the foregoing results in mind, it was considered desirable to conduct longer term studies along similar lines. This experiment was outlined with two objectives as follows: A: to determine distribution and accumulation patterns of dalapon in flowering and fruiting cotton plants, which could be correlated with field investigations (some already reported, and others to be mentioned in this section); B: to determine whether or not dalapon undergoes measurable change after long periods (9–10 weeks) in cotton, and if any is carried non-metabolized into the fruits. Cotton was grown in the greenhouse to the early stages of reproductive growth and treated by the cut petiole method already described (8). This insured the rapid introduction of a relatively large amount of pure radioactive compound into the xylem stream, simulating uptake through the soil, but without the complications of soil dilution, adsorption, etc. Also, this technique eliminated the possibility of micro-biological breakdown outside the plant and subsequent absorption of the degradation products, which is known to occur from nutrient solutions after several weeks. The gross distribution of dalapon in the vegetative plant body followed the patterns already established. In this case, there were additional sinks or regions of utilization of food materials into which dalapon was also transported and held. These organs, the reproductive structures, are of most interest in this study (See fig 3 A through D).

It was clear from the autographs that radioactivity was present in some reproductive structures, both those already formed at the time of treatment and those developed after treatment. This observation, plus the fact that the amounts of activity present appeared to be correlated with the movement of assimilates into organs of different ages and relative rates of growth, indicate that accumulation in young flowers, fruits, and seeds was principally by re-distribution in the phloem with the inward movement of food materials. This position is further strengthened

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**Fig. 2.** Radioautograms of representative co-chromatograms. A: 5 μl 96% dalapon-2-C14 stock solution. B: 5 μl impure 2,2,3-trichloropropionate-2-C14 (TCP). C: Dalapon plus TCP, 5 μl each. D: Aqueous extract from roots of dalapon-treated cotton plants. E: Extract in D plus dalapon. F: Aqueous drop (from ether extract) of nutrient solution in which dalapon-treated cotton was grown. G: Extract in F plus dalapon. H: Aqueous extract from dalapon-treated leaves of sorghum. I: Extract in H plus dalapon. See text for more complete explanation.
by the fact that although the entire vegetative tops of the plants are known to be rather uniformly flooded with dalapon via the transpiration stream within 6 hours (8), none of the so-called squares (i.e., both pre-bloom ovaries & immature fruit stages) which abscised within 7 days after treatment contained radioactivity. (The special term "square" refers to the angular appearance created by the leaf-like bracts subtending the flower or fruit.) Little or no movement into a mature leaf below the treated node occurred. Radioactivity was present in all flower parts, including bracts, calyx, corolla, and especially the stigma, staminal column, and individual anthers. Young fertilized ovaries and immature fruits were also high in activity. In the case of older fruits (fig 3 C) it is also clear that activity is widely distributed,
e.g., in the outer fruit walls, carpellary partitions, and seeds, and there was a slight indication of activity even in the fiber. It is especially noteworthy that within the mature or nearly mature seeds, radioactivity is highly concentrated in the embryo, with only traces in the ovule coat. This agrees well with the deep-seated nature of an observed delay in germination (6) and the recovery and identification of actual dalapon from cotton seeds following field application (7).

In the second phase of this study, whole treated fruits were pooled, dried, ground, extracted, and fractionated according to the scheme shown in figure 1. Similar samples of older leaf tissue and from non-treated plants were included for comparison. Since activity was too low in the mature leaf samples for valid appraisal (i.e., considerably less than in the fruits on a dry weight basis), and since no activity was detected in non-treated plants, only the results with the fruit samples are presented. Since these samples were not accurately corrected for self-absorption loss, the amounts of radioactivity recovered in the various fractions as shown in table I are only relative (not absolute values). Nevertheless, some trends were obvious. Most of the activity followed the route which dalapon would take in the extraction and fractionation scheme, i.e., into the ethanol extract, then into the anionic fraction. This fraction contained only one prominent radioactive species which corresponded in Rf to dalapon. Also activity in the distillate and in the neutral fraction is considered to be dalapon, at least in part. Chromatograms of the neutral fraction gave somewhat anomalous results due to the high concentration of solutes in relation to the activity required to produce an image. Although not positively identified, there were at least two spots, one of which appeared to be dalapon. (This could be attributed to less than quantitative removal of dalapon on the anion exchange resin.) Although perhaps 85 to 90% of the radioactivity recovered was still represented as dalapon, the following results point to the existence of other C14-containing compounds; therefore a slight amount of metabolic decomposition must have occurred within the cotton plant. A: Radioactivity was present in the ether soluble fraction. This activity resisted removal upon re-solubilizing in ether and washing with Na2CO3. This step should have removed any organic acids or dalapon, yet no

<table>
<thead>
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<th>cpm</th>
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<tr>
<td>Ether extract</td>
<td>38</td>
</tr>
<tr>
<td>Na2CO3 Wash of ether extract</td>
<td>0</td>
</tr>
<tr>
<td>(After separation of the ether extract</td>
<td></td>
</tr>
<tr>
<td>between 95% EtOH and petroleum ether</td>
<td></td>
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<tr>
<td>activity was about equal in the two</td>
<td></td>
</tr>
<tr>
<td>fractions.)</td>
<td></td>
</tr>
<tr>
<td>Ethanol extract</td>
<td></td>
</tr>
<tr>
<td>Distillate (from concentrating)</td>
<td>127</td>
</tr>
<tr>
<td>Insoluble oily residue (upon concentration)</td>
<td>93</td>
</tr>
<tr>
<td>Anionic fraction</td>
<td>2524</td>
</tr>
<tr>
<td>Cationic fraction</td>
<td>21</td>
</tr>
<tr>
<td>Neutral fraction</td>
<td>448</td>
</tr>
<tr>
<td>Plant Residue (after ether &amp; ethanol extraction)</td>
<td>29</td>
</tr>
<tr>
<td>Water wash of residue</td>
<td>0</td>
</tr>
</tbody>
</table>

* Dried ground cotton fruits were extracted and fractionated according to figure 1. All fractions which contained radioactivity are shown. Data are relative only (not absolute values).

![Fig. 3D. Flower at lower right in (A & A') enlarged and rotated 90°. Radioactivity is light against dark background in this photograph only.](image)
activity was obtained. Upon separation of the ether-soluble portion between 95% ethanol and petroleum ether, activity was about equally distributed in the two fractions. This fact points to the association of small amounts of C\(^{14}\) with both lipids and pigments. B: Spot(s) other than dalapon, albeit faint, were produced by the neutral fraction. C: The cationic fraction showed slight activity. D: A small amount of C\(^{14}\) (approximately 1% of the total recovered) was retained by the plant residue, despite exhaustive extraction. This agrees with earlier observations following 6- to 8-day exposures of cotton and sorghum to dalapon-2-C\(^{14}\) and -Cl\(^{36}\).

Due to the very low radioactivity in the plant residues, enzymatic digestion with amylases and proteases proved unfruitful.

Although not presented in detail, the following observations on monocotyledons should be included in this discussion. Preceding experiments have shown conclusively that dalapon, non-metabolized for the most part, is accumulated in the seeds as well as other fruit parts of cotton, and the capacity to retard germination may persist through years of storage (6, 7). No growth regulator symptoms were produced that were definitely ascribable to dalapon. It is also evident (from other studies) that the characteristic dalapon symptoms, i.e., the active stimulus, may persist over long periods in dormant or quiescent organs of grasses. Examples are A: rhizome buds of Johnson grass (10), B: seeds of wild oat (1), and C: wheat and barley (to be described). In these instances, it was not established whether the delayed or protracted responses are due to dalapon itself or to a biologically active transformation product. In the absence of such information, the fact that typical symptoms develop in grasses and not in cotton, could lead to interesting speculation (see discussion). The actual time interval over which dalapon (or the stimulus) persists is probably not too significant per se, since it may represent a period in which the tissues being sampled are in a relatively inactive or dormant state. Furthermore, measuring effects of persistence in vegetative tissues may be complicated by the reproductive phase (as shown in cotton) because fruiting results in reduced vegetative growth. With a curtailment of activity in vegetative meristems and a concurrent shift in loci of high metabolism to the reproductive structures, dalapon would move into these organs. If, after having accumulated a high concentration of dalapon, the seeds (or buds) mature or become dormant and all metabolic activity is drastically reduced it is logical that the chemical would not be dissipated rapidly, but would be present when growth and more active metabolism were resumed. The following carry-over of the dalapon stimulus through the seeds is believed to illustrate this phenomenon. At rates as low as 4 lb/acre, applied as a pre-plant treatment in the winter (under conditions unfavorable either for leaching or breakdown by micro-organisms), wheat and barley were severely inhibited as a result of absorption of dalapon through the roots (see Acknowledgments). Figure 4 shows carry-over of the stimulus through the seeds into the second and third generations of wheat. Notice that the spikes produced on the first culms were most severely affected in the second generation, thus indicating that re-translocation (from 1st generation seed) and accumulation in the second generation was on a first come, first served basis. The young developing grains that had first access to the re-translocated dalapon (or stimulus) accumulated the greatest portion, thereby leaving only small amounts to be translocated into succeeding spikes. Abnormalities also developed in the third generation seedlings grown from spikes of plants which showed most severe symptoms in the second generation. Some seedlings were simply retarded in growth to varying degrees but otherwise appeared normal; others grew normal coleoptiles but produced no roots; still others produced long roots but no top growth. Although it has been experimentally proven only in cotton, the carry-over effect through the seeds of both

![Fig. 4. Carry-over effect of characteristic dalapon symptoms in A (left) second generation, and B (right) third generation wheat following inhibition of first generation plants by pre-planting application of dalapon at 4 lb/acre. Groups of heads in A are arranged left to right in order of occurrence on the same plant. In B the seedling at left is normal and others show inhibition or other anomalies.](image-url)
dicotyledons and grasses is presumed to be due, principally, to non-metabolized dalapon. This statement should be experimentally confirmed or refuted with respect to seeds and dormant or quiescent buds of grasses as well.

Mechanisms of Dalapon Toxicity: This section is aimed primarily toward integrating the experimental findings (including those of other workers) into a discussion of selective dalapon phytotoxicity.

I. Non-selective versus selective toxicity. Dalapon is capable of producing both an acute (contact) burn and delayed systemic growth regulatory responses following translocation. The relative degree of expression of each type of injury is apparently dependent upon the concentration of dalapon in the cells penetrated and the inherent biochemical susceptibility to the growth regulating action of dalapon at lower concentrations. Wilkinson (32) observed that as the rate, concentration, or time of exposure (each indicative of levels of dalapon in the tissues) is increased the sequence of dalapon responses in plants is successively formative—toxic—lethal. Lower concentrations are thus required to produce formative effects than to cause the death of a single cell, tissue, or plant. The two effects intergrade but are separately recognizable at the extreme levels of each. Several physico-chemical properties of dalapon have been reviewed in relation to their probable contribution toward phytotoxicity (6). The acute toxic effect is believed to be due to the action of the herbicide as a strong acid and as a protein precipitant (21, 23). Membrane destruction undoubtedly accounts for the water-soaked appearance of tissues following leakage of the cellular contents, in a manner suggestive of oil toxicity. High concentrations of H ions or toxic surfactants may also contribute to acute toxicity (6). In cotton, acute toxicity has occurred in regions remote to the site of foliar application as well as following root uptake. Presumably the mechanism is the same, i.e., transport of amounts non-toxic to the tissues traversed, followed by concentration to toxic levels in certain other tissues. On cursory examination, the end result of this non-selective effect (e.g., cupping of leaves, death of terminal buds, and malformation of auxiliary buds) may be misconstrued to be the same growth regulating action seen in susceptible species. Irregular growth patterns resulted, however, from necrosis in certain areas (e.g., sections of the main vascular tissues) and stresses resulting from continued growth in adjacent areas (smaller veins and intercostal regions). The veins were normal near the junction of the petiole and lamina; therefore substances were still able to traverse the affected region. Although the effects described are noticeably different from the proliferation of tissues, etc., seen in grasses, it should be mentioned again that selectivity of action is most frequently not absolute, but relative.

II. Selective herbicidal action of dalapon. The second response, apparently resulting from lower concentrations (in meristem tissues only), appears to exemplify true biochemical selectivity among species. Acute toxicity can reduce or prevent the expression of the systemic growth regulator type of herbicidal effect by obstruction of the translocation process; this response is dependent upon translocation into regions of presently or potentially high metabolic activity.

Initially (6, 8), four factors were considered capable of determining or quantitatively regulating selective herbicidal action among species: A: permeability, including stomatal and cuticular absorption and penetration of membranes; B: translocation, including restriction enroute, and distributional and accumulation patterns; C: metabolic decomposition or inactivation of the herbicide; D: selective biochemical interference of the toxicant with normal metabolic processes.

A. Permeability. Initial absorption into leaves has been discussed in some detail (6, 8). In these studies no unexplained differences were noticed in the penetrability of susceptible and tolerant species by foliar or root applications of dalapon. [Pre-emergence selectivity, however, may be partially explained by differences in permeability of seed coats and the penetrating abilities of various formulations of herbicides (20).]

B. Translocation. The principal difference detected between tolerant and susceptible species was in the degree of retention of dalapon along the transport route. Several workers have attached significance to the apparent restriction of 2,4-D in grass leaves in determining species selectivity (see literature cited in 6, 8). Critical work by Weintraub et al. (30) has shown consistently higher amounts of C14 (labeled 2,4-D) exported out of treated leaves of susceptible plants than tolerant plants. It is reasoned that since the exported 2,4-D brings about the morphological responses on the basis of which plants are judged resistant or susceptible, insufficient amounts may be exported out of the treated leaves of resistant plants to produce a systemic toxic action. Wilkinson (32), on the other hand, attempted to use the same reasoning, in reverse, to explain the increased toxicity of dalapon to grasses following root absorption, by trapping dalapon or hindering its movement out of the leaves. In the present investigation (6, 8) it was quantitatively demonstrated that the translocation of dalapon (a grass-selective herbicide) is restricted in the basal sections of (particularly immature) grass leaves in much the same manner as 2,4-D. In attempting to reconcile these views it must be recognized that the secondary actions of both 2,4-D and dalapon are expressed in the same metabolically active tissues, and it is still the herbicide exported out of the mature leaves, principally, which is responsible for systemic herbicidal action. Retention of dalaron in treated leaves would seem to be particularly disadvantageous (from the standpoint of obtaining rapid systemic action) in the case of perennial grasses, where transport not only to the shoot apical meristem of the treated plant, but into the rhizome.
buds, is desired. That herbicidal effectiveness is reduced by disk ing, mowing, and burning [reviewed in (6)], all of which remove top growth, suggests the slow buildup of dalapon in the rhizomes. Separation from the mother plant too soon by either of these means removes not only the apical dominance effect, but also a large portion of the dalapon which had penetrated but not yet reached the rhizome buds in toxic concentrations. Accumulation occurs in association with the transport of food materials into regions of high metabolic activity. Penetration and translocation can also be greatly protracted, providing no acute toxicity occurs (8). Accumulation does not occur appreciably in dormant or quiescent organs (mature seeds, dormant buds, etc.), but dalapon is found there after it has moved in during periods of active assimilation. Therefore the treated tops, if allowed to remain undisturbed, still are able to exert an apical dominance effect and continue to serve as a reservoir for the release of additional dalapon, should high metabolic activity begin or be resumed. This is in agreement with the observation (2) that a continued exposure to TCA was essential for continued dormancy and probably for subsequent death. The process described probably accounts for the superiority of deep plowing in the field control of perennial grasses. Because of its spectacular nature, other factors may be involved in the case of recovery from dalapon-induced dormancy after burning. Restriction of movement in grass leaves is not likely a blockage of herbicide transport, peculiarly, but a normal consequence of the compound’s movement into these regions of high metabolic activity (assimilate utilization) with the flow of food materials and retention therein. Thus any of a number of solutes would be expected to follow a similar pattern, to a greater or lesser degree, depending upon their adsorptive properties, solubilities, tendency toward metabolic decomposition, etc.

C. Metabolic decomposition or inactivation of dalapon. Dalapon was not rapidly metabolized in either tolerant or susceptible plants; therefore this possibility would not seem of much importance in determining selectivity. In relation to the small amount of breakdown which does apparently occur, however, several points may be considered. 1: Some radioactive species, freely re-translocatable in the xylem, were discovered in the guttate from hyathodes of sorghum following leaf drop treatment with dalapon-Cl36, but not with dalapon-2-C14. 2: Cl36 is readily removed metabolically, at least by microorganisms. 3: There was a slight indication of the presence of inorganic Cl36 on chromatograms of extracts from higher plants 6 to 8 days after treatment. 4: All of these studies employed low tracer levels of dalapon, i.e., perhaps subtle metabolic conversions would occur more noticeably at higher levels. 5: “Propionate inhibits growth by competing with β-alanine for attachment within the yeast cell, thereby preventing the coupling of the pantothentic acid moieties” (15). 6: Pantothentic acid synthesis is one of the most sensitive metabolic processes known to be affected by dalapon and related compounds (12). The ability of the molecule (propionic acid) to compete with β-alanine is decreased by successive increases in chlorination. Conversely, the toxicity of dalapon increased (i.e., approached that of propionic acid) through aging on the shelf for 3 years (presumably undergoing decomposition). (The increase in inhibitory action upon aging is suggestive of the behavior of oils which form organic acids that are highly toxic in the non-dissociated state.)

From the foregoing observations, it is postulated that the delayed growth-regulatory effect manifested after translocation into meristematic tissues of high metabolic activity, may be correlated with the initial steps in decomposition (i.e., de-halogenation) of the dalapon molecule. Thus, instead of constituting a detoxification mechanism, the first (?) steps of dalapon breakdown would tend to increase its potentialities as a competitive inhibitor in pantothentic acid synthesis. After disturbance of the normal functioning of pantothentic acid and coenzyme A, it might then be further metabolized by the common or perhaps by a modified pathway of propionate oxidation. If dalapon-2-C14 were thus degraded, radioactivity might logically appear in almost any fraction of compounds where carbon would normally go. This probably occurred for a small percentage of the applied dalapon after long exposures (9–10 weeks) in cotton. Further investigation is required to establish, with certainty, whether this hypothesis has real significance in relation to either mechanisms of action or selectivity. That such differences are not obvious from herbicidal responses after foliar applications of propionate, mono-, di-, and trichloropropionate, does not detract from the credibility of the hypothesis. Wide differences in penetration and translocation to the cellular site of action may exist within the series of compounds. Species resistant to TCA commonly contain higher amounts of the herbicide in their cell sap than susceptible species (2, 3, 28) indicating that the latter use TCA in their metabolism. That growth reduction and morphological responses are correlated with metabolism of TCA in the tissues seems pertinent to the present discussion.

D. Biochemical interference with normal metabolic processes. Disturbance of energy metabolism could logically lead to the observed herbicidal effects of growth regulators. All anatomical and morphological changes in plants are preceded by biochemical changes. As inferred earlier, no clear cut relationship exists between formative effects and lethality. Attempts to explain mechanisms of action of a substance which produces formative effects must take cognizance of the influence of such compounds upon the normal processes of growth and differentiation, which are themselves incompletely understood. The specific point of attack of a toxicant might be on the production of a substrate, on the enzymes which attack a substrate with the release of energy, and/or on the enzymes involved in the utilization of the
energy produced. The final toxic result, however, may actually be produced by a complex series of sequential and consequential reactions. This may account for the fact that the mechanism(s) of action is (are) known for so few compounds, if indeed any are known with certainty.

The most plausible explanation of the mode of action of dalapon as a growth regulator seems to revolve around pyruvate metabolism, which occupies a key position in relation to other metabolic crossroads. Both competitive and non-competitive inhibition of enzymatic reactions have been attributed to dalapon, and Redemann and Meikle (24) concluded that they may occur simultaneously. The first was thought to result from competition between pyruvate and dalapon for attachment to pyruvate-attacking enzymes, whereas the second (which became noticeable at concentrations of inhibitor of $3.5 \times 10^{-4}$ molar) is probably due to its action as a protein precipitant, perhaps precipitating the pyruvate-enzyme complex. Based on calculations using expected pyruvate concentrations in plant tissues, these workers concluded that if such an inhibition is not responsible for the herbicidal action of dalapon, it certainly could contribute to the stunting effect following translocation.

Perhaps an equally (or more) important primary site of action, still involving pyruvate indirectly, is the competitive inhibition of pantothenic acid synthesis (12). First indications were that dalapon competed with $\beta$-alanine, but later results with pure enzyme preparations have suggested that competition is with pantoin acid rather than $\beta$-alanine. In either case, the synthesis of pantothenic acid, and therefore the production of functional coenzyme A would be impaired. Most all the pantothenic acid in animals and micro-organisms is reportedly present as CoA, although it is also found in nature in other combined forms such as pantetheine and pantetheine. Less is known of its activity and occurrence in higher plants, however several edible reproductive structures are known sources of the vitamin. It is probably synthesized in the leaves and transported into these storage organs during periods of rapid development. The specific effects of pantothenic acid or CoA upon cell differentiation are little known.

The improper functioning of CoA in plants could lead to drastic changes in plant growth and development. For example, some of the important processes believed to be mediated by CoA are A: pyruvate oxidation, B: citrate synthesis, C: $\alpha$-keto-glutarate oxidation in the citric acid cycle, D: fatty acid and steroid synthesis and breakdown, and perhaps E: auxin action—auxins may act through an ester with CoA (auxinyl CoA). Hence CoA is a key compound in plant metabolism and growth through its control of the energy in the linking ester bond. Interference of dalapon with the citric acid cycle in any way, e.g., by competing with pyruvate for enzyme attachment or with $\beta$-alanine for attachment to the other moiety of pantothenic acid could indirectly cause disturbances in ancillary processes, such as nitrogen metabolism.

(The dark green appearance of dalapon-treated plants, delayed maturation, and prolongation of vegetative growth are characteristic of plants having high levels of available nitrogen.)

It is entirely probable that dalapon exhibits more than one primary site of action. Note that $\beta$-alanine yields pyruvic acid on deamination. It is possible that dalapon and related compounds (perhaps increasingly with decreased chlorination) are able to compete with several metabolites that are structurally similar, e.g., $\beta$-alanine, pantionic acid, pyruvic acid—perhaps variably among plant species.

The low response in roots cannot always be attributed to a lack of accumulation of dalapon, as was shown in this study. It seems unlikely that the principal action is in competition with pyruvate, because this substance certainly occurs, albeit fleetingly, in all regions of high respiratory activity which would include root tips as well as shoot apical meristems. If the principal mechanism is the interference with pantothetic acid synthesis, one possible explanation is that synthesis occurs in the shoots, requiring the products of photosynthesis, and if the process should be altered the meristematic areas of the shoots, (by virtue of their closer proximity) would show the deficiency most readily. Abnormalities can occur in roots under certain conditions, as shown by the work of Grigsby et al. (11). This suggests the indirect involvement of light, which is consistent with other observations. Also, it seems safe to assume that light (and elevated temperatures) may indirectly exert an effect by causing an increase in accumulation of dalapon in the tops from soil or nutrient solution through an increase in transpiration.

Differences in susceptibility to dalapon among species and among tissues of a given plant are seemingly dependent upon the presence or absence of key enzymes or enzyme precursors. Further experimentation into the mechanisms of herbicidal selectivity should emphasize biochemical distinctions between susceptible and resistant species.

**Summary & Conclusions**

I. Dalapon-2-C$^{14}$ and -Cl$^{18}$ were further employed in tracer and metabolic studies on cotton, sorghum, and wheat to determine the distribution and metabolic fate of the herbicide in relation to phytotoxicity. Radioautography and counting yielded both qualitative and quantitative data. Extraction, fractionation, and paper chromatographic procedures were used to detect dalapon and metabolic degradation products.

II. Pure dalapon-Cl$^{18}$ in aqueous stock solution was metabolized over a period of several months in the refrigerator by some species of micro-organism(s), possibly Alternaria sp., which produced five new Cl$^{18}$-labeled substances. Two of the substances were tentatively identified as inorganic Cl$^{18}$ and monochloropropionate-Cl$^{18}$. Similar conversions are probably operative in soils, accounting for the disap-
pearance of dalapon toxicity under favorable conditions.

III. Ready absorption and translocation of dalapon, including redistribution and accumulation (e.g., in vegetative meristems, flowers, fruits, & seeds) in response to shifts in loci of high metabolic activity were confirmed.

IV. Dalapon was absorbed, translocated, re-distributed, and accumulated in higher plants, principally as the intact molecule or dissociable salt thereof, and remained essentially non-metabolized for long periods especially in dormant or quiescent tissues.

V. Seven days after foliar treatment of cotton and sorghum, for example, dalapon, and no other prominent radioactive substance, was recoverable with water or ethanol from all plant parts and from the nutrient solutions in which the plants were grown.

VI. Ten weeks after treatment (through severed petioles) approximately 85 to 90% was recoverable as dalapon from cotton fruits of various ages. The remainder of the radioactivity was associated with the ether-soluble portion, the neutral and cationic fractions of an ethanol extract, and the insoluble plant residue.

VII. Some slow metabolic decomposition resulted in the release of C14O (from dalapon-C14O) or the incorporation of C14 (from dalapon-2-C14) into other compounds. Eventual breakdown possibly involves an initial dehalogenation followed by normal or modified propionate oxidation.

VIII. Dalapon stimulus was traced to the third generation in wheat; it was transmitted in the seeds after exposure of first generation seedlings to pre-plant applications of dalapon (4 lb/acre) in the field.

IX. Two physiologically distinct types of action (acute toxicity and delayed growth regulatory responses) were confirmed. The former is believed to be due to the action of the herbicide as a fairly strong acid and protein precipitant, which non-selectively causes disruption of the plasma membranes and destruction of cellular constituents. The second response resulting from lower concentrations in meristematic tissues exemplifies true biochemical selectivity among species.

X. Experiments indicated that neither penetrability, translocatability, nor metabolic inactivation of dalapon is preeminently responsible for herbicidal specificity. The key to the moderately selective growth regulating activity of dalapon still seems unquestionably to reside in the protoplasm. Results are not at variance with hypotheses that dalapon inhibits pantothenic acid synthesis and disturbs coenzyme A and pyruvate metabolism.

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Literature Cited


