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TRANSLOCATION AND METABOLISM OF 2-METHOXY-3,6-DICHLOROBENZOIC ACID IN PLANTS

by

© FA-YAN CHANG

A THESIS

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The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies for acceptance, a thesis entitled "Translocation and Metabolism of 2-Methoxy-3,6-dichlorobenzoic Acid in Plants", submitted by Fa-Yan Chang in partial fulfilment for the degree of Doctor of Philosophy.

Supervisor

External Examiner

Date Oct. 30, 1969

ABSTRACT

Translocation and metabolism of C^{14}-carboxyl-labeled 2-methoxy-3,6-dichlorobenzoic acid (dicamba-C^{14}) in Tartary buckwheat (Fagopyrum tataricum (L.) Gaertn.), wild mustard (Sinapis arvensis L.), barley (Hordeum vulgare L.), and wheat (Triticum vulgare L.) were studied using autoradiography, liquid scintillation counting, and paper and thin-layer chromatography.

Uptake of dicamba took place through both the foliage and the roots of all four plant species. Foliar absorption was more complete by Tartary buckwheat and wild mustard than by barley and wheat. The radioactivity from dicamba-C^{14} was strongly accumulated in the shoot meristems in the two weedy species whereas in the two cereal crops it was more or less evenly distributed in the plants following uptake by the roots, and tended to remain on or in the treated leaf after foliage application.

Transport of dicamba in petiole segments of Tartary buckwheat and bean (Phaseolus vulgaris L.) and in coleoptile sections of corn (Zea mays L.), was basipetally polar. The radioactivity accumulating in the receiving agar blocks in contact with tissue sections of all species was unaltered dicamba. The velocity of dicamba transport in Tartary buckwheat petiole segments was calculated as 0.8 mm/hr. The basipetal transport of dicamba was dependent on its concentration in the donor blocks of agar, the length of segments, and the temperature; it was promoted by added

ATP or sucrose but inhibited by DNP. Acropetal movement of dicamba was less affected by the additives, but it did show some temperature dependence. Exposing the buckwheat plants from which the petiole segments were taken to light for 24 hours before the experiment increased the subsequent uptake of dicamba from donor blocks and promoted retention of the chemical by the tissue; the flux of transport was reduced by the light treatment. Movement of sucrose in the tissue segments was non-polar while IAA showed higher polarity in transport than dicamba.

Chromatography of ethanol extracts of dicamba-treated plants revealed that metabolism of the herbicide occurred in all plant species studied. The metabolites were conjugated with plant constituents, probably largely with sugars. After hydrolysis of the conjugated products a common major metabolite was identified as 5-hydroxy-2-methoxy-3,6-dichlorobenzoic acid (5-OH dicamba). A minor metabolite in barley and wheat plants was identified as 3,6-dichlorosalicylic acid. A small amount of the parent compound, dicamba, was released upon hydrolysis of the conjugates from wild mustard, barley, and wheat plants but not from Tartary buckwheat plants. The rate of dicamba metabolism was high in wheat and barley, low in wild mustard, and very low in Tartary buckwheat.

Under the experimental conditions in the greenhouse, the four species tolerated dicamba treatment in the order wheat, barley, wild mustard, and Tartary buckwheat. This ranking corresponds with the

ability of the plants to degrade dicamba and is inversely related to the amount of dicamba absorption and translocation in them.

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INTRODUCTION

Dicamba (2-methoxy-3,6-dichlorobenzoic acid) has been used as a herbicide to control many broadleaved weeds in cereal crops. Tartary buckwheat (Fagopyrum tataricum (L.) Gaertn.), a prohibited noxious weed in Alberta and other parts of the Prairie Provinces of Canada, is one of the species which can be effectively controlled in wheat and barley crops by this herbicide. This species is very sensitive to dicamba application and the effect of treatment usually is rapid and persistent.

Bearing in mind the above considerations, the present investigation was designed to study the uptake, translocation, and metabolism of dicamba in Tartary buckwheat plants. Since the control of this weed in wheat and barley is selective, it was of interest to evaluate the role of selective uptake, translocation, and degradation of dicamba by Tartary buckwheat and the cereal species in determining their susceptibility to the herbicide. For comparison, a moderately susceptible species, wild mustard, was also included in the study.

Auxins such as indoleacetic acid (IAA) and 2,4-dichlorophenoxyacetic acid (2,4-D) are known to be transported in a polar fashion in plant tissue segments. Dicamba is a synthetic auxin with many characteristics similar to those of IAA and 2,4-D and, therefore, it could be expected to show the character of polar transport. In an attempt to explore the nature of this polar

transport, experiments on dicamba movement in tissue segments were carried out with Tartary buckwheat seedlings and other plants. The results were compared to the transport of IAA and sucrose in the same tissue.

LITERATURE REVIEW

Translocation in plants

When a compound is applied to leaves or roots of a plant, it may be absorbed by the plant tissue or left as residue on the leaf surface or in the soil. Once it has entered the plant it may be immobilized in the tissue near the point of entry or translocated into other parts of the plant, depending upon the characteristics of the compound and the nature of the plant itself.

There are two systems in the plant which may be involved in translocation, the symplast and the apoplast. These terms were introduced by Münch in 1930, according to Leonard (57), with symplast designating the sum total of the interconnected living protoplasm of the plant and the apoplast the non-living cell wall phase around the symplast. The symplast is connected from cell to cell by plasmodesmata connections, or tubules. The sieve tubes are considered a part of the symplast, while the tracheids, xylem vessels, cell walls and intercellular spaces are regarded as making up the apoplast.

The upward transport through the xylem in the transpiration stream is considered to be due mainly to the reduced pressure resulting from the evaporation of water from the mesophyll (16). Translocation in the symplast is more complicated. Although several theories have been proposed to explain the mechanism of translocation in this living conduit, no one of them has ever been generally

accepted (101). However, there is evidence that the translocation of assimilates follows a source-to-sink pattern, from the regions of synthesis of foods to regions of their utilization (e.g., 1, 3, 51). After many years of work with radioactive herbicides, Crafts et al. (19) concluded that systemic distribution of foliage-applied phloem-mobile compounds follows the way of assimilate translocation. The consistent bypassing of mature leaves, the high concentrations in young growing shoot tips, root tips, and intercalary meristems, and the reversibility of flow brought about by proper manipulation, are interpreted as indication of a mass-flow type of mechanism (19).

Herbicides can be divided into several categories on the basis of translocation characteristics (57): (1) those that move slightly if at all, e.g., oil and oil-soluble esters of 2,4-D and 2,4,5-T, diquat; (2) those that are translocated in the symplast primarily, e.g., the acid and salt forms of 2,4-D and 2,4,5-T; (3) those that are translocated in the apoplast only, e.g., fenuron and other substituted ureas and the symmetrical triazines; (4) those that move in both the symplast and apoplast, e.g., amitrole, maleic hydrazide. Symplastic movement is of greatest importance following leaf application, while apoplastic movement is of greatest importance following root application. Compounds possessing the ability to move in both the symplast and the apoplast have the greatest potential as herbicides. Some compounds may leak from roots into the culture medium, examples being 2,4-D, MCPA, 2,3,6-TBA, dicamba, and picloram (10, 18, 21, 45, 66, 67, 68, 77).

2-Methoxy-3,6-dichlorobenzoic acid, with the common name dicamba and the commercial name 'Banvel D', was first introduced for field testing during 1961 (17). The acid is only slightly soluble in water; the dimethylamine salt formulation, however, is readily water-soluble. It has been used with success for the control of Canada thistle and has effectively controlled several other broadleaved weeds such as green smartweed, wild buckwheat, and Tartary buckwheat in cereal grains (e.g., 17, 23, 52, 103).

Limited available information suggests that dicamba is translocated readily in plants. Hodgson (41) reported that, after spray application, dicamba or some derivative was translocated over far greater distances in bracken plants than he had observed for any other herbicide. In grape cuttings dicamba-C^{14} moved readily from the roots to the shoots; it became especially concentrated in the margins of young leaves (58). With foliar application the label also appeared in the root. Following foliar application to bean plants, a detectable amount of dicamba was exuded from the root into the surrounding medium (45, 68). The root exudate was chromatographically identical with the applied compound. Studies of Foy and Hurtt (21) involving nutritional status, degree of aeration and addition of inhibitors suggested an active component in the root excretion phenomenon.

Cain (9) reported that Polygonum pennsylvanicum plants released dicamba from the roots into a nutrient solution but did not

readily move the chemical from the roots into shoots of the plants. Dicamba applied to a single leaf was rapidly translocated into the stem but not into the leaves. In maize, little movement of dicamba into the roots was evident following treatment of a single leaf, though dicamba was readily taken up through the roots (9).

In purple nutsedge (Cyperus rotundus L.) foliarly applied dicamba moved both acropetally and basipetally, and the herbicide became widely distributed throughout the aerial parts of the plant and accumulated in the regions of meristematic activity (70). Root-applied dicamba generally became distributed throughout the plant except within the tubers and the tips of the leaves. Similar results were obtained by Ray and Wilcox (87) with the same plant species.

Canada thistle foliage and roots readily absorbed dicamba, after which it was translocated by both phloem and xylem (10). Dicamba tended to accumulate in young, growing leaves following both foliar and root uptake. After foliage application, small amounts of dicamba were exuded by the roots into the surrounding soil, and it also moved readily through connecting creeping roots from one shoot to another.

Polar transport of auxins

A remarkable feature of auxin transport in plants is its basipetal polarity, i.e., the movement of auxin is preferentially or even exclusively from the apex toward the base of the plant.

The polarity of auxin transport was first noted by Went in 1928 (109). In his classic experiments he found a very strict polarity of movement of auxin in oat coleoptiles. This strictly polar manner of auxin movement was confirmed by the intensive investigations of van der Weij (104, 105) and others (e.g., 29, 33, 46, 64, 95, 113, 117). In fact, the general view of the early workers on auxin transport was, as summarized by Went and Thimann (110), that auxin moved only from the apex toward the base, and that there was no acropetal movement even when an external supply of auxin was added to the basal end of the isolated section. It is now known that acropetal transport of auxin also takes place in a variety of plant tissues (e.g., 48, 49, 74, 78), but the transport is always predominantly basipetal. In flowering and fruiting stems the polarity is generally weak and considerable amounts of auxin have been reported to be translocated acropetally (62, 78, 89).

The polar orientation of auxin transport in roots apparently is less well defined than that in aerial portions, and the evidence is sometimes conflicting. Hertel and Leopold (39) reported that in roots of etiolated seedlings of Zea mays, acropetal movement (towards the root tip) was less than half the basipetal movement. However, some recent contributions on this subject describe predominantly acropetal movement in roots of other species. After applying IAA-C^{14} to the apical or the basal ends of root segments of Vicia faba, the distribution of radioactivity in the segments clearly indicated preferential movement towards the apex (119). In Lens root segments,

twice as much ^{14}C from the applied IAA-^{14}C was accumulated in receivers at the apical ends of the segments as in receivers at their base (82). In Convolvulus root segments acropetal transport of IAA was about seven times the basipetal transport (5). The velocity and the time course of IAA transport in root sections of Lens and Phaseolus were similar to those in shoots, but the strongly polar movement was acropetal in roots, rather than basipetal as in shoots (55). Strongly acropetal transport of IAA was also reported for roots of Zea mays, Avena, Triticum, and Helianthus (91, 114, 115).

Polar transport of auxin occurs not only in isolated segments of tissue, but also in intact plants (15, 47, 80, 84, 96, 108, 110). Auxin is produced in the apical growing regions of the shoot and is translocated away from the tip in a basipetal direction. This basipetal movement of auxin forms the basis of a wide variety of orientation or correlation effects such as apical dominance, tropistic movements of plant parts, and the basal orientation of root formation (59, 60, 110).

IAA is not the only substance which can move in the polar transport system. Polarity of movement has also been demonstrated for 1-naphthaleneacetic acid (NAA), indolebutyric acid (34, 64), 2,4-D (37, 38, 71, 74), 2,4,5-trichlorophenoxyacetic acid (2,4,5-T) (50), and 4-amino-3,5,6-trichloropicolinic acid (picloram) (43). However, many auxin analogues including some phenoxyacetic acids, benzoic acid, phthalamic acids, and others are incapable of being translocated in the polar

transport system (cf. 60).

The velocity of auxin movement in plant segments differs from one substance to another (4, 64, 71, 74, 111). Leopold and Lam (64), for example, found a velocity of about 8 mm/hr for transport of IAA, 7 mm/hr for NAA, and 3 mm/hr for indolebutyric acid, through sunflower sections. McCready (71) reported a velocity for 2,4-D of about 1 mm/hr through bean petiole segments. Horton and Fletcher (43) reported a velocity for picloram in bean petioles at between 0.75 and 1.11 mm/hr. Velocities vary not only between auxins, but also between tissues. For example, the velocities of transport of IAA ranged from 14 mm/hr through corn coleoptiles down to 6 mm/hr through corn roots, with intermediate velocities for oat coleoptiles and sunflower stem sections (60).

The basipetal transport of auxin appears to be an active process, on the basis of many characteristics of the transport. As clearly established by van der Weij (104), the velocity of auxin transport is far greater than that of simple diffusion. Polar transport is restricted to compounds with auxin activity such as IAA, 2,4-D and a few others. Organic acids chemically related to auxins but lacking the auxin's ability to stimulate growth, such as tryptophane (112) and benzoic acid (37), are not transported polarly. 1-NAA promotes growth and is readily transported while 2-NAA is inactive and does not move in a polar and active way (37). Thus the polar transport system is selective in nature. The auxin can be

moved against a concentration gradient (71, 104). The polar transport system is sensitive to metabolic inhibitors (12, 13, 36, 38, 39, 54, 60, 79, 83, 122). It is dependent upon oxygen (27, 28, 29, 30, 31, 113).

The evidence for active acropetal transport remains equivocal (cf. 32). The dependence upon donor concentration (71) and the relative insensitivity to temperature (73), inhibitors (27, 73), and oxygen concentration (27, 28, 31, 113) suggest that acropetal movement may be predominantly by passive diffusion. However, de la Fuente and Leopold (20) and Keitt and Baker (53) reported an active acropetal transport of auxin, the evidence being the roughly similar velocities in opposing directions and the inhibition of a fraction of the acropetal movement by TIBA (2,3,5-triiodobenzoic acid). The general finding that the polarity of transport increases with the length of segments (20, 71, 73) was interpreted differently by authors holding different views. In the view of McCready (71, 73), for example, the increased polarity of movement in long segments was accounted for by the decreased acropetal flux due to diffusion, which would be inversely proportional to the length of path. Leopold and co-workers (20, 61), on the other hand, concluded that this characteristic of polar transport was compatible with a different hypothesis on the nature of movement of auxin (cf. next paragraph).

The real mechanism of polar transport is not yet clear. Leopold and his colleagues (12, 13, 20, 38, 39, 60, 61, 63) have developed the view that the polarity of auxin transport is a consequence of a secretive function and not of an uptake function. The

polarity of transport is said to be achieved by a more effective secretion of auxin out of the basal end of each cell. The polar difference between the apical and basal ends of one cell may be very small, but it could be amplified exponentially in passing through a file of similar cells. Thus the extent of polarity observed can be related to the number of cross-walls traversed, i.e., polarity increases with length of the section. The evidence supporting this hypothesis is the observation that transport into the receiver is more effectively reduced if an inhibitor like TIBA is applied to the basal than to the apical end of the section (12, 13), and that uptake by both the section and the cells is less sensitive to inhibition than release from the section (12, 13, 39). However, the possibility of an alternative explanation for the observed inhibition of export was pointed out by Goldsmith (27, 32). TIBA might increase auxin immobilization, resulting in a decrease of exportable material. An increase in the amount of auxin immobilized in tissue, following TIBA treatment, has been shown (116, 117).

Recently a mobilization hypothesis has been suggested by Zaerr and Mitchell (120), who found that IAA accelerated cell division and the formation of root primordia, particularly at the basal ends of hypocotyl segments of bean. They proposed that the polar movement of auxin in isolated segments is associated with the mobilization and utilization of plant constituents at growth centers; the polarity of auxin movement is the result of a polar distribution

of growth. There was a direct correlation of IAA transport with the ability to initiate roots as well as with the degree of ^{14}C-accumulation in the morphological base of the segments.

Herbicide metabolism in plants

Since the early work of the investigators at the Boyce-Thompson Institute with ethylene chlorohydrin (75), it has been known that plants have the ability to detoxify certain compounds through metabolic activity. It now has been demonstrated that decomposition of herbicides by metabolic processes is of importance in the mode of action of these chemicals and the amount of residue that may be left on crops harvested for human utilization (22).

The fate of herbicides in plants has been reviewed a number of times in recent years (6, 22, 40, 92, 100). In general, four major pathways are involved in herbicide metabolism, i.e., oxidation, reduction, hydrolysis, and conjugation. As indicated by Freed and Montgomery (22), "while a given metabolic path may predominate for a particular compound, it is quite common for any one or more of the detoxication mechanisms to be simultaneously operative. Thus, while a compound may undergo oxidative metabolism principally, it may be found that a portion of the administered drug will simultaneously be undergoing conjugation, reduction or hydrolysis."

In most instances, the metabolism of herbicides in plants is considered to be an inactivation or detoxication process. There are some chemicals, however, which are initially inactive but are rendered

phytotoxic by the metabolic action of the treated plant. The beta-oxidation of 4-(2,4-dichlorophenoxy)-butyric acid to 2,4-D is a classical example. Another example is the production of toxic free radicals by the action of an initial metabolic step in the plant on the bipyridilium quaternary salts known as diquat and paraquat (6).

Compared to other herbicides such as the phenoxy-acids, the triazines, the triazoles, and the substituted carbamates, considerably less is known about the metabolism of dicamba and other benzoic acid derivatives used as herbicides. Minarik et al. (76) reported that benzoic acid derivatives were more persistent in plants than were phenoxy derivatives. In Canada thistle plants, dicamba was slowly degraded to an unknown metabolite; radioactive CO_2 was also released from the treated plants (10). Following chromatography of water extracts from various parts of wheat and wild buckwheat plants treated with dicamba, Quimby and Nalewaja (85) concluded that the compound was conjugated or metabolized in the plants. The metabolism of dicamba was more rapid and more extensive in the main culms of wheat than in wild buckwheat meristems.

At least two metabolites of dicamba were detected in Johnsongrass although 50% of the ^{14}C remained as the unaltered herbicide five days after application (44). On acid hydrolysis, dicamba was released from one metabolite. In bean, only dicamba was recovered five days after treatment. Broadhurst et al. (7) found a rapid

degradation of dicamba in wheat and bluegrass. Two dicamba derivatives were detected in the extracts of the treated plants. After hydrolysis, the major metabolite was identified as 5-hydroxy-2-methoxy-3,6-dichlorobenzoic acid (5-OH dicamba). The minor derivative was 3,6-dichlorosalicylic acid.

Magalhaes et al. (70) found no labeled metabolites of dicamba-C^{14} in purple nutsedge extracts regardless of the age or part of the plant analyzed. Ray and Wilcox (87) also failed to reveal any dicamba metabolites in treated plants of the same species. In excised roots of corn and barley, however, Ray (86) reported some degradation of dicamba; 5-OH dicamba was the major metabolite and DCSA was the minor one in both species. An additional metabolite, 3,6-dichlorogentisic acid, was found in extracts of barley shoots and roots.

MATERIALS AND METHODS

Materials

The radioactive dicamba (dicamba-C^{14}) used throughout the experiments was labeled with carbon-14 at the carboxyl group with a specific activity of 1.89 mc/mmole. The guaranteed radiochemical purity of this compound was greater than 99%; when analyzed chromatographically, it was found to be 99.5% pure. 3,6-Dichlorosalicylic acid-carboxyl-C^{14} (DCSA-C^{14}) had a specific activity of 3.54 mc/mmole. These two chemicals were obtained from New England Nuclear Corporation through the courtesy of Velsicol Chemical Corporation. Indoleacetic acid-2-C^{14} (IAA-C^{14}) with a specific activity of 9.2 mc/mmole and sucrose-U-C^{14} (sucrose-C^{14}) with a specific activity of 11.8 mc/mmole were purchased from The Radiochemical Center.

The main plant used was Tartary buckwheat (Fagopyrum tataricum (L.) Gaertn.). Other plants used were wild mustard (Sinapis arvensis L.), barley (Hordeum vulgare L. var. Jubilee), wheat (Triticum vulgare L. var. Thatcher), bean (Phaseolus vulgaris L. var. Dutch brown), and corn (Zea mays var. Golden Early Market).

Except where specified otherwise, seedlings of the plants were grown in soil in pots or flats, in a growth chamber at 24°C under a 16-hour light regime with a light intensity of 1500 ft-c at plant level; these conditions are at or near the optimum for Tartary buckwheat (24). At the required stage of growth the plants

were transferred to a greenhouse or a dark room for treatment. In the greenhouse supplementary lighting was given during the winter months to keep the day-length at 16 hours.

Autoradiography

Autoradiographic studies of dicamba translocation in plants following foliar or root application were carried out by the methods described by Crafts et al. (19, 81, 118).

Tartary buckwheat and wild mustard plants with three to four mature leaves and barley and wheat plants at the three-leaf stage were treated with the herbicide by placing 10 µl of dicamba-C^{14} solution in 50% ethanol on the upper surface of the first or second leaf. The total dose of dicamba was 0.1 µc (approximately 12 µg). The treatment was applied in the greenhouse and the treated plants were kept there until they were harvested, 0.5 hour to 20 days later. At the end of the treatment periods the treated spots were covered with a piece of masking tape to prevent radiocontamination. Duplicate plants were harvested, freeze-killed with crushed dry ice, and freeze-dried. The drying process was completed in about two weeks. The dried plant materials were humidified, mounted and flattened, and then autoradiographed using Ansco Non-Screen Safety X-ray film. The exposure time was four days for wheat and barley plants and four weeks for Tartary buckwheat and wild mustard plants. To check possible artifacts, untreated plants were exposed to X-ray films in a similar way. Films were developed in Ansco Liquadol developer

for four minutes and fixed for ten minutes or longer in Kodak fixer.

When translocation of the chemical following root absorption was studied, the plants were grown in soil and transferred four days before treatment to one-half strength Hoagland's nutrient solution in glass containers wrapped with aluminum foil. For treatment the plants were further transferred to 150-ml beakers containing 100 ml nutrient solution with 1.0 μc of dicamba-C^{14} (concentration 1.2 μg/ml). After one day's uptake of the treatment solution, the plants were transferred back to the glass containers containing non-radioactive nutrient solution. The plants were then harvested at different time intervals following treatment. At harvest the roots were rinsed under running tap water for three minutes. Freeze-killing, drying, and autoradiography were carried out following the same procedures as in foliage treatment, except that the exposure time was four weeks for all the plants.

Preparation of plant extracts

Tartary buckwheat, wild mustard, barley and wheat plants were foliarly treated with dicamba-C^{14} in the same way as mentioned above. Four (Tartary buckwheat and wild mustard) or ten (barley and wheat) plants grown in one pot made up one replicate and two replicates were used in each treatment. At harvest the residue of dicamba-C^{14} on the surface of the treated leaf was washed off with 20 ml of 50% ethanol. The plants were cut into several parts and the separated plant parts were stored at -20°C until they were extracted.

The plant parts were ground in 95% ethanol in a Waring blender. The ground materials were kept at room temperature for twelve hours or longer, and then filtered using a Buchner funnel and Whatman No. 1 filter paper. The residues were extracted again in ethanol overnight. The first and second extracts were combined and concentrated under reduced pressure at 40-45°C until nearly dry and then brought up to 10 ml with 95% ethanol. These extracts were used for quantitative determination of dicamba distribution in the plants and for the degradation studies.

After ethanol extraction, the radioactivity left in the plant residue was determined by counting (see page 22) a small portion of the residue or the extract of the residue after acid hydrolysis. It was found that only a small fraction of the total radioactivity was associated with the residue. At 20 days from treatment, for example, the radioactivity in plant residue of all the species tested was 1.5 to 2.0% of the total dose applied. These radioactivities were not analyzed further.

The concentrated plant extracts frequently were too sticky for chromatographic analysis, especially for thin-layer chromatography. The crude extracts were purified, therefore, on a florisil column (1.5 x 15 cm). The extract was evaporated to a small volume, rinsed into the column with chloroform, washed with 100 ml chloroform and then 200 ml diethyl ether, and dried by drawing air through the column (7). The radioactivity then was eluted from the column with 400 ml 95% ethanol. Most of the green pigments were in the chloroform and

the ether washing, the brownish coloured material at the top of the florisil column, and more than 97% of the radioactivity in the ethanol eluate. The ethanol eluate was concentrated again by evaporating, and part of it was used for chromatographic analysis.

Since benzoic acid and dicamba or their metabolites have been reported to form conjugates with plant constituents (7, 56), the purified extract was hydrolyzed before identification of the metabolites was carried out. The alcohol in the extract was evaporated and the residue was hydrolyzed with 4 N hydrochloric acid or sodium hydroxide for four hours on a steam bath (7). After hydrolysis, water was added to the solution so that the final pH value was 1 to 2, and the solution was then extracted with chloroform until there was no detectable radioactivity in the water layer. The chloroform was evaporated to dryness and the radioactive compounds in the evaporating flask were taken up in a convenient volume of 95% ethanol for further analysis by paper and thin-layer chromatography.

Chromatography

The metabolic fate of dicamba-C^{14} in the plants was studied by paper and thin-layer chromatography. In paper chromatography, Whatman No. 2, 3MM, 4, and 20 papers were used. For thin-layer chromatography, instant thin-layer plates, plastic films pre-coated with silica gel G, and glass plates coated with silica gel G, silica gel GF, aluminum oxide, or cellulose, were used. The solvents used were (a) isopropanol:ammonium hydroxide (28%):water (8:1:1), (b) butanol:ammonium hydroxide:water (8:1:1), (c) benzene:dioxane:

acetic acid (90:25:4), (d) benzene:acetic acid:water (2:2:1), (e) 2,6-lutidine:water (65:35), and (f) butanol:ethanol:water (2:1:1). Among these chromatography media and solvents, Whatman No. 1 paper in combination with the solvent system (a) and glass plates coated with silica gel GF in combination with the solvents (a) or (c) gave the best separation and, therefore, were used in all of the experiments unless otherwise stated.

In paper chromatography a small amount of the extract, containing a known amount of radioactivity, was applied as a band on a 4-cm strip of chromatography paper and dried by a stream of warm air. The chromatograms were placed in the chromatography tank for three hours' saturation and then were developed descendingly. The solvent was allowed to run 30 cm. Radioactive regions of chromatograms were detected using a Nuclear Chicago Actigraph III Radiochromatograph Scanner. Autoradiograms were also made on Ansco Non-Screen X-ray film. Exposure time varied with activity level. To obtain more accurate quantitative information, radioactive zones on the chromatograms, located by reference to the autoradiograms or the scanning results, were cut out and the activity on the paper pieces was assayed in a liquid scintillation counter. In the case of identification of derivatives, standards of dicamba-C^{14}, DCSA-C^{14}, non-radioactive 5-OH dicamba and 3,6-dichlorogentisic acid (di-OH dicamba) were also chromatographed at the same time. Visualization of the non-radioactive compounds on the chromatograms was done under UV light, and by means of a spray of 1.7 g silver nitrate

in 10 ml water and 5 ml concentrated ammonium hydroxide, diluted to 200 ml with acetone (suggested by P.B. Polen; Velsicol Chemical Corporation).

For thin-layer chromatography, 0.25-mm layers of silica gel GF were prepared on 20-cm square glass plates with the Desaga coating apparatus, as described by Stahl (97). In order to obtain uniform coating, the glass plates were thoroughly scrubbed with a scouring powder such as 'Ajax', and then thoroughly brushed under running water, rinsed clean with distilled water and dried. For five plates, 25 g silica gel was mixed with 50 ml distilled water by vigorous shaking for 30 to 45 seconds in a stoppered conical flask (200 to 250 ml). It was then transferred immediately to the coating apparatus for coating the plates. The plates were air-dried overnight, activated in an oven at 110°C for 30 minutes and stored in a desiccated plate cabinet until used. Plant extracts, 10 to 20 µl depending on the radioactivity, were spotted on the plates with a capillary micro-pipette, and dried with a warm air flow from a hair drier. Samples of reference compounds were also spotted on the plates. The plates were developed ascendingly for 10 to 12 cm from the origin in a Desaga developing chamber pre-saturated with solvent, and then dried in air. Localization of dicamba-C^{14} and its metabolites and the non-radioactive reference compounds on the thin-layer chromatograms was done in the same way as on paper chromatograms.

Liquid scintillation counting

Unless otherwise stated, all of the quantitative determination of radioactivity was done in a Nuclear Chicago Mark I liquid scintillation spectrometer. The scintillation solution used was prepared by dissolving 120 g naphthalene, 6 g 2,5-diphenyloxazol (PPO), and 0.5 g 1,4-bis-2-(5-phenyloxazolyl)-benzene (POPOP) in dioxane to make up one litre. The samples usually were counted in 15 ml of this solution at 8°C.

In liquid scintillation systems, energy often is lost before it reaches the photomultiplier tubes, with a consequent reduction of detection efficiency. This phenomenon is known as quenching and the extent of quenching varies considerably with the nature and amount of the quenchers — colored materials, water, oxygen, halogenated compounds and polar compounds (107). In order to compare samples with dissimilar degrees of quenching, some method of determining the counting efficiency for each sample must be used.

In this investigation, the counting efficiency was determined by the external standard method. Standard quenching curves were prepared by counting a series of differently quenched samples, containing a known amount of C^{14}, in two channels set to monitor different portions of the energy spectrum. The channels ratio of the external standard counts then was plotted against the observed counting efficiency to get the quenching curve. One such curve is shown in Figure 1. The quenching agents used in the standard curve preparation were extracts of untreated plants, tissue homogenate,

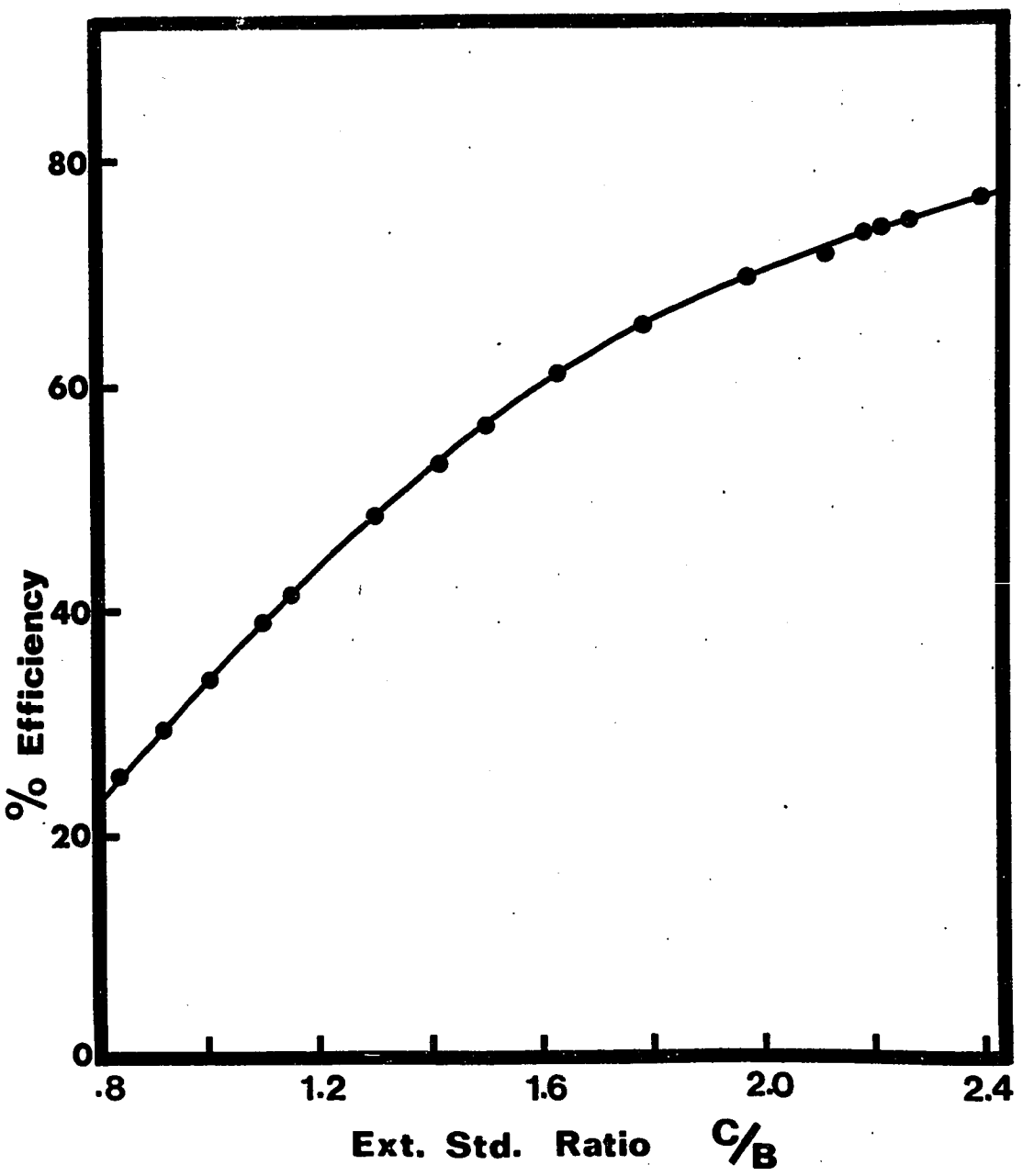


Figure 1. One of the quenching curves used for carbon-14 assay in Nuclear-Chicago Mark I liquid scintillation counter. Samples quenched with ethanol extract of untreated Tartary buckwheat plants. Efficiencies determined using C^{14}-$NaHCO_3$ standard. Counting time 20 minutes; temperature 8°C. The settings: L - U, L = 0.0; U = 9.9. Attenuation, channel B = A640; channel C = D912.

ethanol or water, depending on the nature of the samples to be counted. From the appropriate standard curve, the counting efficiency for each sample could be determined and the observed radioactivity in counts per minute (cpm) then was converted to disintegrations per minute (dpm).

Polarity of dicamba transport

Studies on dicamba transport through tissue segments were carried out using methods similar to those described by McCready (71). Petiole segments of Tartary buckwheat seedlings were used as the main material throughout the investigation. In some experiments which established the polarity of dicamba transport, petiole segments of Dutch brown bean and coleoptile sections of corn seedlings were also used.

Tartary buckwheat was grown in flats in the growth chamber. Plants for experiments were taken about 12 days after planting, when the petioles of the first leaves were 2-3 cm in length and approximately 1.3 mm in diameter. Twenty hours before treatment the seedlings were transferred from the growth chamber to a dark room where the temperature was about the same as in the growth chamber, i.e., 24°C. Two segments of 3.25 mm long each, unless otherwise stated, were cut from the middle of the petiole of the first leaf with a triple-bladed cutter. One of the segments was used for basipetal and another for acropetal transport. Equal numbers of distal and proximal segments were used for transport in the two directions.

Dutch brown bean seedlings were also grown in flats in the growth chamber and transferred to the dark room 20 hours before use. Seven-day old seedlings with petioles of the primary leaves 4-5 cm long were chosen for the experiment. A segment 3.25 or 6.5 mm long was cut from each of the two primary petioles of the seedlings. One of the paired segments from a plant was used for basipetal transport and the other for acropetal transport.

Corn seeds were soaked in water for 24 hours and then sown in moist vermiculite. They were grown in the dark room for six days, until the coleoptiles were 2-2.5 cm in length. Segments 3.25 and 6.50 mm in length were excised approximately 1 mm below the apex of the coleoptiles, and the leaf tissue inside the coleoptile sections, if any, was carefully extruded from the section with a needle.

Agar (Bacto-Agar, 'DIFCO' Standardized) was dissolved in water to give a 1.5% w/v sol by heating in a boiling water bath. For agar blocks containing additives, such as dicamba-C^{14} or IAA-C^{14}, the measured solution and the agar sol were mixed in a glass vial kept in a water bath at 45-55°C using a previously warmed plastic tube connected to a glass syringe; the final agar concentration for all the blocks was always kept at 1.5% by adjusting the concentration of the agar sol before mixing. The sol, with or without additives, was drawn into a glass tube of bore diameter 3.30 mm and the tube was then sealed at both ends with parafilm. When the agar had solidified at room temperature, a cylinder of agar gel was extruded from the tube on a glass plate by gravity or by air pressure from a rubber

pipette bulb. This cylinder was then divided into blocks, each 2.73 mm long, by means of a multiple cutter consisting of eleven stainless safety-razor blades spaced with plexiglass. The volume of the agar blocks was 23.4 mm^3.

All the manipulations of the excision of the segments and the setting up of the transport system were carried out in a glass humid chamber under a green safelight[112] in the dark room. In order to prevent damage to the tissue, the plant segments were handled with forceps on the points of which were fastened extensions of thin cardboard[112].

For basipetal transport, a donor block (containing dicamba-C^{14} or IAA-C^{14}) was applied to the apical cut surface of the segment, and a receiver block (agar only) to the basal cut surface. To measure acropetal movement, the donor block was applied to the basal end of the segment and a receiver block to its apical end. Each segment, with its blocks retained in position by surface tension, was laid horizontally on a glass plate. Ten segments constituted a treatment and two treatments were arranged in separate rows on a plate; one treatment for acropetal and another for basipetal transport. Each plate was placed in a 15-cm covered petridish which contained a wet filter paper to maintain high humidity. The petridishes containing the transport systems were kept in a moist atmosphere in a dark incubator at a constant temperature (normally 25°C) for the duration of the experiment.

At the end of the transport period, each set of ten agar blocks was pooled for radioactivity assay in a scintillation counter. The ten blocks were transferred to a counting vial containing 12 ml of the scintillation liquid, and the radioactivity was determined twelve hours after transfer. To determine the radioactivity remaining in the tissue, the segments were ground in acetone in a tissue homogenizer, and the homogenate was transferred into a counting vial. After the acetone was evaporated in an air stream, 0.5 ml of 95% ethanol was added to each vial and shaken well. Scintillation solution was added then and the samples were counted.

Detection of $C^{14}O_2$

Decarboxylation of dicamba-C^{14} in Tartary buckwheat plants was studied by determining the rate of $C^{14}O_2$ liberation from the treated plants by the method described by Chow et al. (11). Four plants in a pot, each treated with 0.1 μc of dicamba-C^{14} by foliar application, were placed in a bell-jar wrapped with aluminum foil and containing two strips of filter paper (2.5 x 4 cm) impregnated with p-(diisobutylcresoxyethoxyethyl)-dimethylbenzylammonium hydroxide (hydroxide of Hyamine 10-X). The bottom of the jar was sealed to a plastic plate with vaseline. In order to minimize artifact effects from starvation of the plants, after each two-day period the plants were replaced with a new set (the used plants were never used again). The $C^{14}O_2$ collected on each paper strip was counted in a liquid scintillation counter in a vial containing 18 ml of the dioxane solution system.

Comparison of plant susceptibility to dicamba

The sensitivity to dicamba of the four test species was determined by foliar application of the dimethylamine salt of the herbicide. Pot-planted Tartary buckwheat and wild mustard plants at the four-leaf stage and barley and wheat plants at the three-leaf stage were sprayed with different concentrations of dicamba with a cabinet sprayer. The rates of application varied from 0.125 to 8 oz/A (ounces per acre) for Tartary buckwheat, 0.125 to 32 oz/A for wild mustard, and 4 to 64 oz/A for barley and wheat. All the spray treatments were applied in 10 gal/A of water. Subsequently, the plants were kept in the greenhouse for observation. Two weeks after treatment the above-ground parts of the plants were harvested, oven-dried and weighed.

RESULTS

SECTION A: TRANSLOCATION

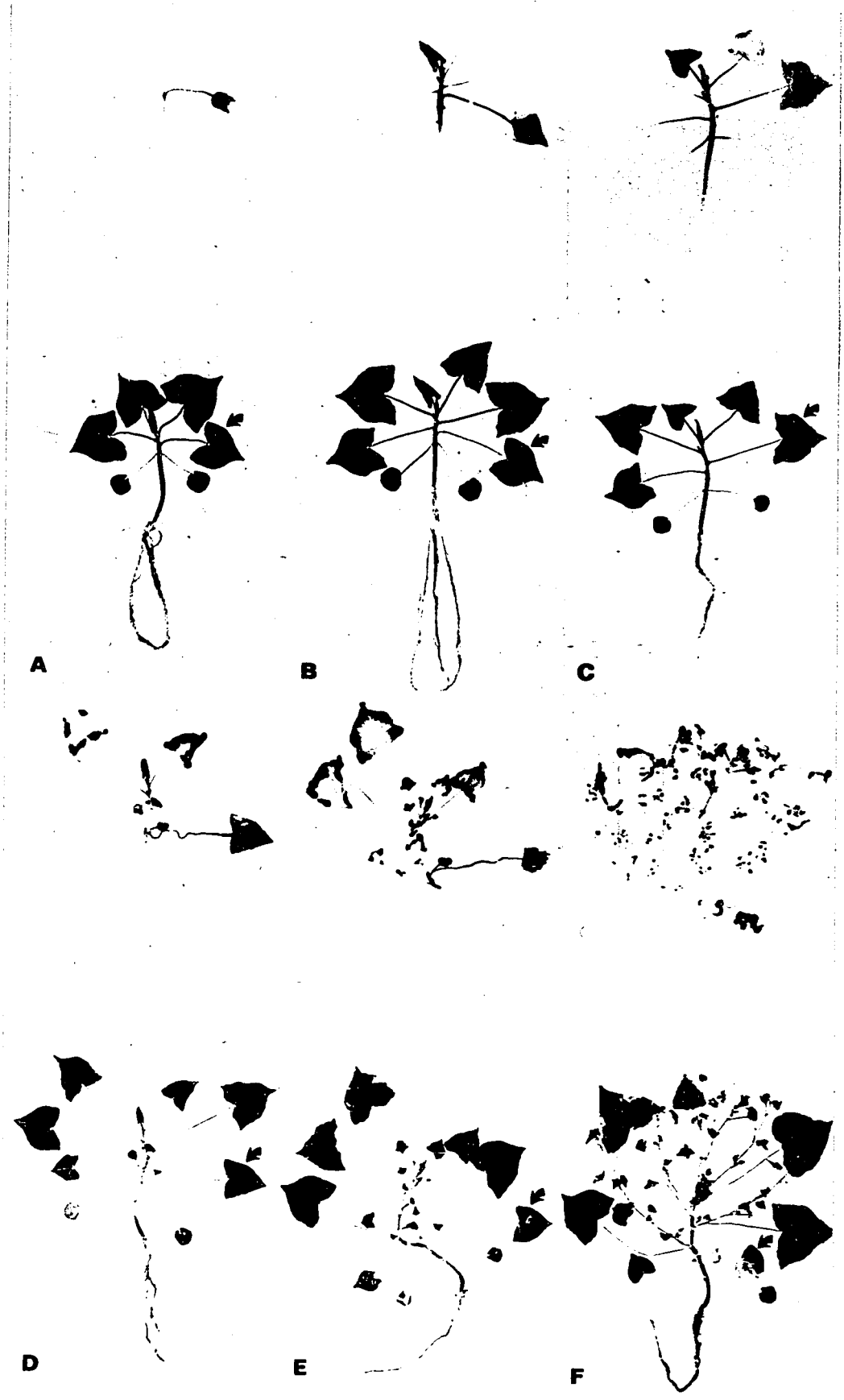
Translocation in Tartary buckwheat

(1) Autoradiographic studies

Plant mounts and autoradiograms in Figure 2 show the time course of translocation of radioactivity in Tartary buckwheat plants following application of 0.1 μc dicamba-C^{14} in a droplet on a single leaf. The radioactive material that appeared in the plant parts was chiefly in the form of unaltered dicamba-C^{14} as determined by chromatographic analysis of the plant extracts (see Metabolism section). Therefore, the movement of the label in the plant represented mainly the translocation of the applied herbicide.

Dicamba moved out of the treated leaf and was translocated both downward and upward in the stem within one hour following application. Four hours after treatment an appreciable amount of the label had accumulated in the rapidly developing young leaves at the growing tip. Some of the radioactivity appeared in the mature leaves too, but it was much less than that in the growing apex. Four days after application, new buds formed in the leaf axils and dicamba appeared in these new tissues also. With time, more and more new buds formed and dicamba was retranslocated from the older leaves to these new tissues (Figure 2E & F). When the young leaves became mature gradually, dicamba tended to accumulate at the leaf

Figure 2. Time-course of translocation of dicamba-C^{14} in Tartary buckwheat plants treated with 0.1 μc of dicamba-C^{14} (1.89 mc/mmole) in a 10 μl droplet on a single leaf. The lettered photographs represent the plant mounts, the corresponding photographs above them the autoradiograms. Arrows indicate the treated leaves. (A) 1 hr., (B) 4 hrs., (C) 1 day, (D) 4 days, (E) 10 days, (F) 20 days.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B
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margins causing local death of the tissue, and only the dead tissues at the leaf margins retained the radioactivity.

While intensive accumulation of dicamba occurred in growing tips of the above-ground parts of the treated plants, no translocation to the root was observed from the autoradiograms in Figure 2. It might be that the radioactivity had accumulated in root tips which were broken off during the washing procedure at harvest. To examine this possibility, filter paper culture was used as described by Crafts and Yamaguchi (19). Seedlings of Tartary buckwheat at the cotyledon stage were transferred to glass-backed filter paper (Whatman No. 3) in a glass jar covered with aluminum foil. One-half strength Hoagland's nutrient solution was supplied from the bottom of the jar by capillary action of the filter paper. Four days after transplanting, 2 µl of the treatment solution containing 0.02 µc of dicamba-C^{14} was applied to one of the cotyledonary leaves of the plant. Three days after treatment, the plants were harvested and processed for autoradiography. The results indicated that an appreciable amount of dicamba was translocated to the roots and had accumulated in the root tips (Figure 3).

Autoradiograms of Tartary buckwheat plants treated in nutrient solution (Figure 4) indicate a rapid upward movement of dicamba following root uptake. Within one day, the label was distributed throughout the whole plant with a tendency to accumulate in the young leaves at the shoot apex (Figure 4A). As compared to the distribution pattern of the foliarly applied herbicide, more of the

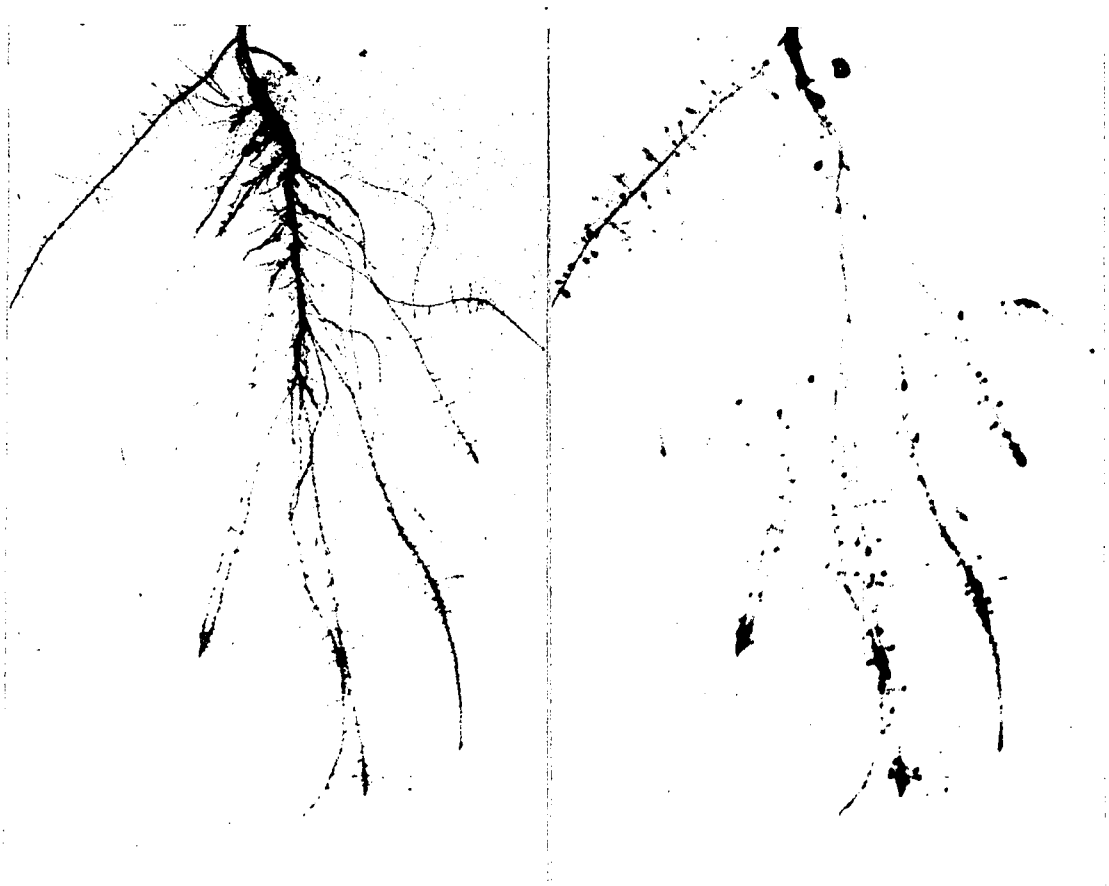
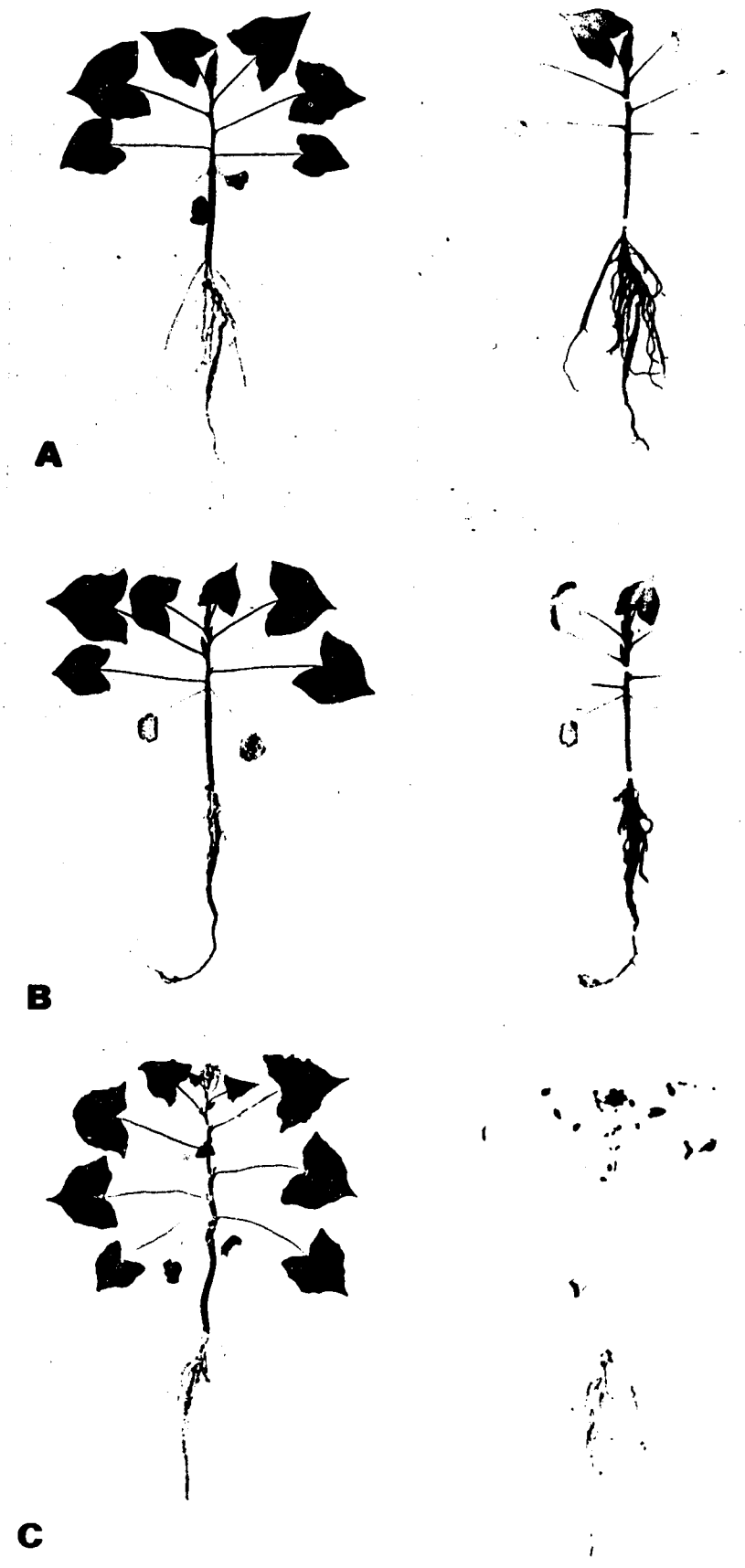


Figure 3. Mount (left) and autoradiogram (right) of roots of a Tartary buckwheat plant cultured on filter paper showing accumulation of dicamba-C^{14} in root tips three days after application of 0.02 μc of the labeled herbicide to one cotyledonary leaf.

Figure 4. Time-course of translocation of dicamba in Tartary buckwheat plants following root uptake from nutrient solution containing 1.0 μc dicamba-C^{14} (1.89 mc/mmole) in 100 ml. The lettered photographs represent the plant mounts, the corresponding photographs to the right of them the autoradiograms. Periods after one-day's uptake: (A) 0 day; (B) 3 days; (C) 9 days.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radioactivity was in the mature leaves after root absorption than following leaf application. However, when time proceeded, dicamba was again retranslocated to the actively growing parts of the plants, including the young leaves and the axillary buds and the flower buds at the shoot apex (Figure 4C). A substantial amount of radioactivity was retained in the roots as long as nine days after the treatment solution has been removed. It is interesting to note that radioactivity was present in one of the cotyledonary leaves but not in other mature leaves in the three- and nine-day treatments (Figure 4B & C). It likely resulted because dicamba entered these cotyledonary leaves with the transpiration stream during the uptake period, but could not move out again because the tissues were injured and were no longer active in photosynthesis; without export of photosynthates, the herbicide would not be able to be translocated from these tissues. The opposite cotyledonary leaves (Figure 4B & C) were dead at the time of treatment, and no radioactivity entered them.

(2) Quantitative determination

To obtain quantitative information on the distribution pattern of dicamba, radioactivity present in the extracts from different parts of the plants following foliar application was determined; the results are presented in Figures 5 and 6.

The amount of radioactivity recovered as residue on the surface of the treated leaf dropped sharply during the first day (Figure 5), indicating rapid uptake of the herbicide by the leaf tissue. Further absorption took place much more slowly. Radioactivity

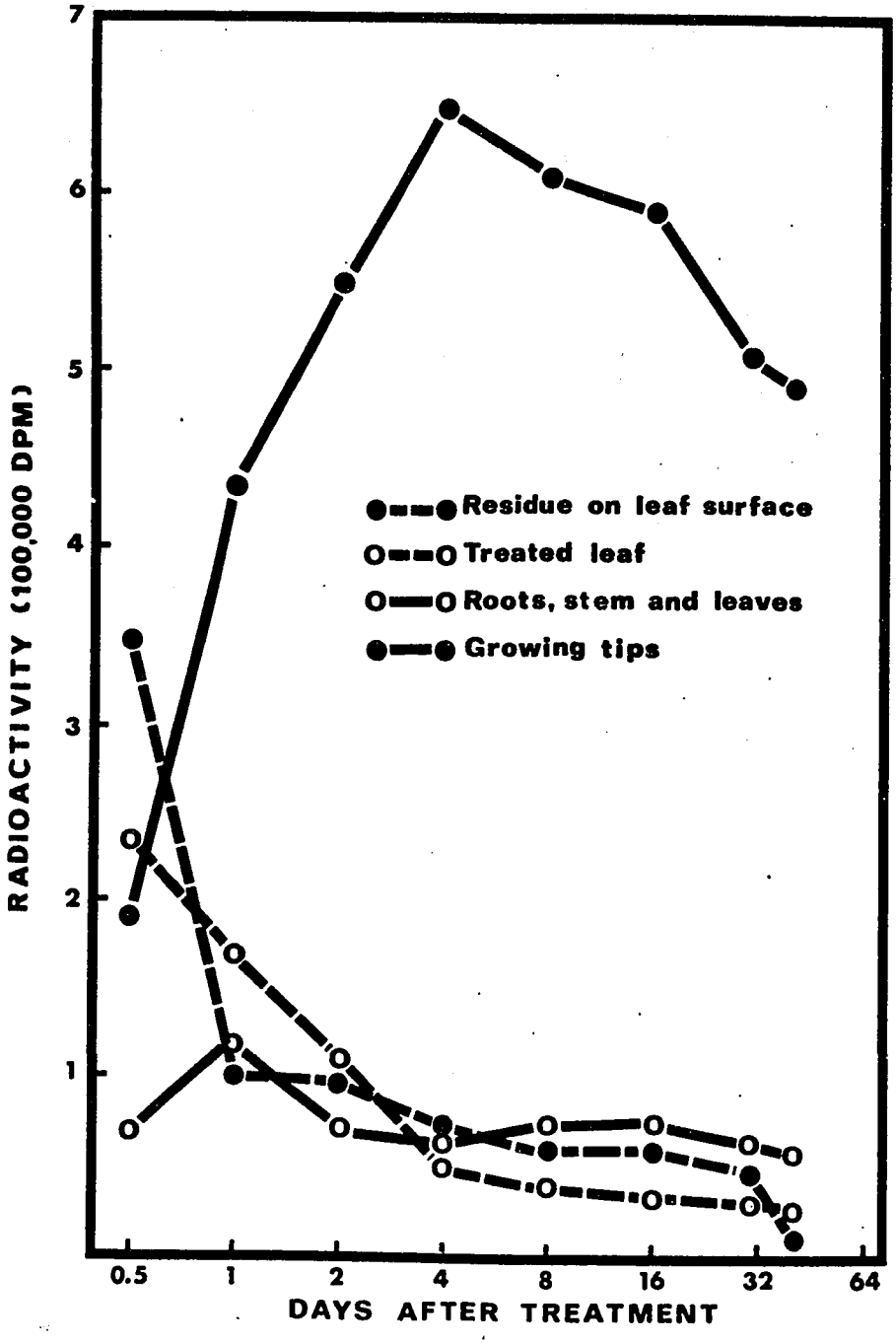


Figure 5. Distribution of radioactivity in different parts of plants at various times following application of 0.1 μc of dicamba-C^{14} (1.89 mc/mmole) to a single leaf of Tartary buckwheat plants. Data plotted are means for two replicates of four plants each.

in the treated leaf also declined rapidly, and this would indicate the rapid translocation of the herbicide out of the treated leaf. In the stem, root, and mature leaves, radioactivity never accumulated to a high level. In the growing tips, on the other hand, including the buds and the rapidly developing young leaves at the shoot apex and in the leaf axils, radioactivity increased very rapidly up to four days, then declined slowly. Twelve hours after treatment, the total activity in plant parts other than the treated leaf was already higher than that in the treated leaf, i.e., more than one-half of the dicamba absorbed during the first half day was exported from the treated leaf during that same period. From one day on, most of the recovered radioactivity was present in the growing tips.

The tendency of dicamba to accumulate in young tissue is shown better by the specific activity in the plant parts (Figure 6). In the treated leaf the specific activity declined rapidly during one to four days following application. The specific activity in the roots, stems and mature leaves remained very low. The growing tips had the highest specific activity, starting twelve hours after treatment. Four days after treatment, the specific activity in the growing tips was 14 times as high as in the treated leaf and more than fifty times as high as that in other parts of the plants including the roots, stems and mature leaves. After four days, the specific activity in the growing tips declined, presumably because of the rapid development of more new tissue in the leaf

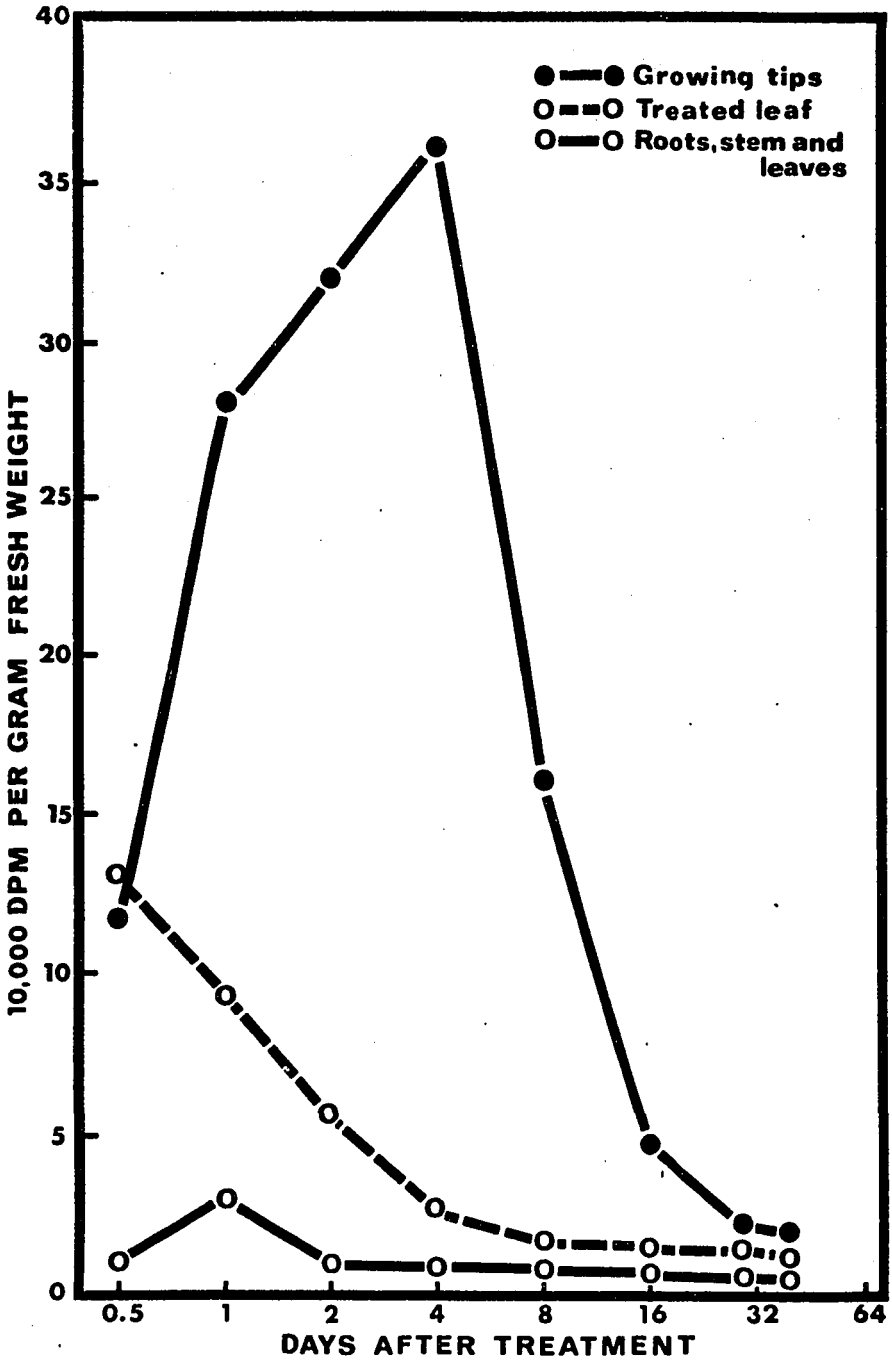


Figure 6. Distribution of radioactivity in Tartary buckwheat plant parts following application of 0.1 μc of the herbicide to a single leaf. Data obtained from the same experiment as in Figure 5, but presented as dpm/g fresh weight.

axils to which part of the dicamba was retranslocated.

The total radioactivity recovered from the plants decreased with time; about 33% of the applied dose was lost in forty days. There are a number of possibilities of accounting for the loss of dicamba activity from the plants, e.g., decarboxylation, root exudation, and volatilization. Gentner (25) found that vapor of dicamba applied to soil caused injury to Pinto beans in a closed system. Burnside and Lavy (8) reported that about 50% of the dicamba activity was lost from a bare planchet during 11 weeks. To investigate the extent of volatilization loss under the conditions used for the translocation studies, dicamba-C^{14} on planchets, each containing 0.1 μc of the isotope, was exposed to sunlight in the greenhouse and the activity was determined periodically in a Nuclear-Chicago gas flow counter. Samples stored in the dark were also counted for comparison. About 50% of the original activity was lost during forty days in the light; the reduction of activity in the dark was 32% during the same period (Figure 7). It is understandable then that volatilization could be responsible for part of the loss of dicamba activity from the plants, at least from the surface of the treated leaf. Decarboxylation and root exudation losses are considered separately in other sections (pages 64 and 38, respectively).

(3) Root exudation

Tartary buckwheat plants were grown in soil in 15-cm pots, with some plants seeded one week earlier than others. After

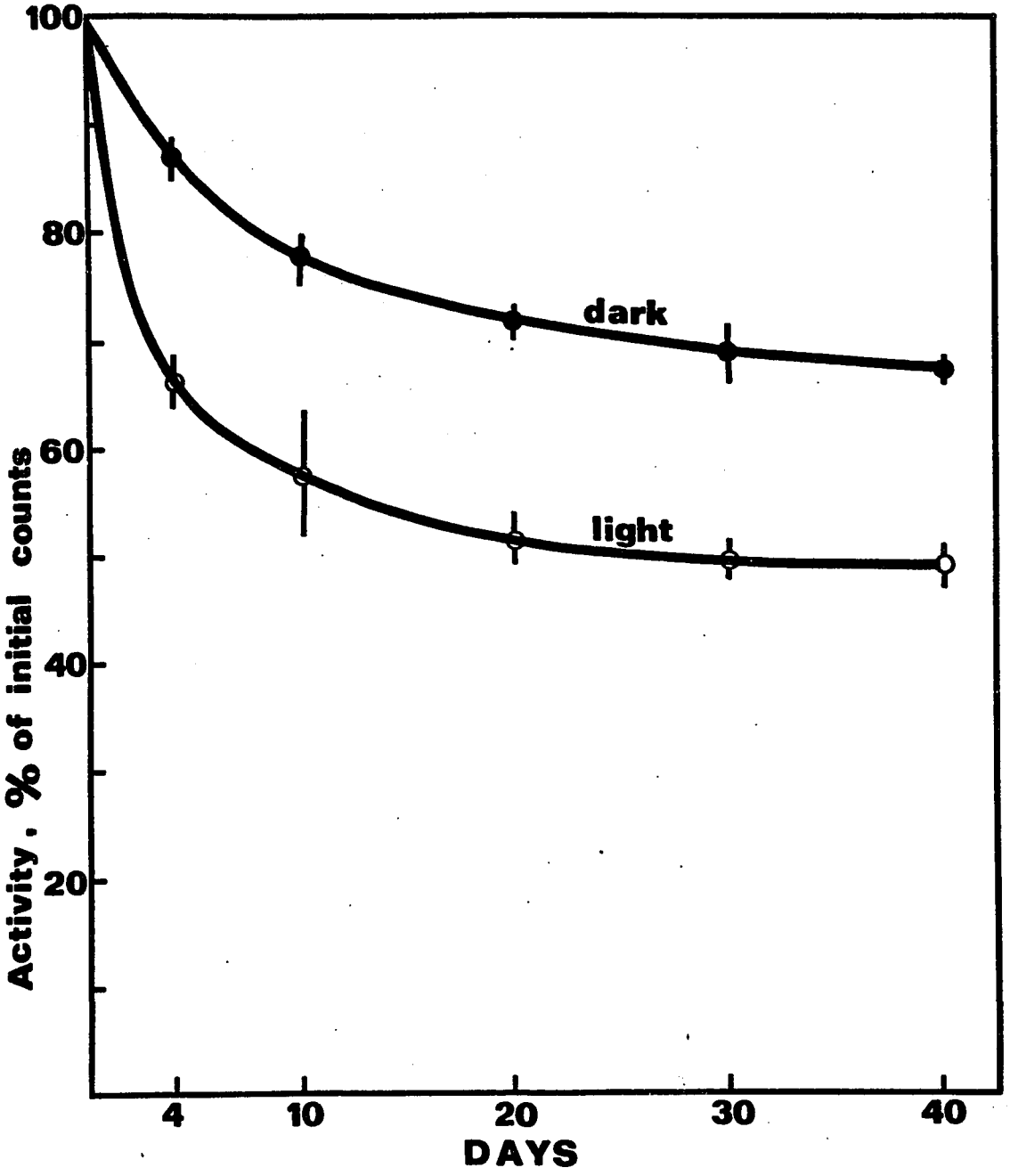


Figure 7. Loss of radioactivity from bare planchets each containing 0.1 μc of dicamba-C^{14}, in sunlight or in the dark. Each symbol represents the average of eight replicate samples. Vertical lines in the graph represent standard errors of means.

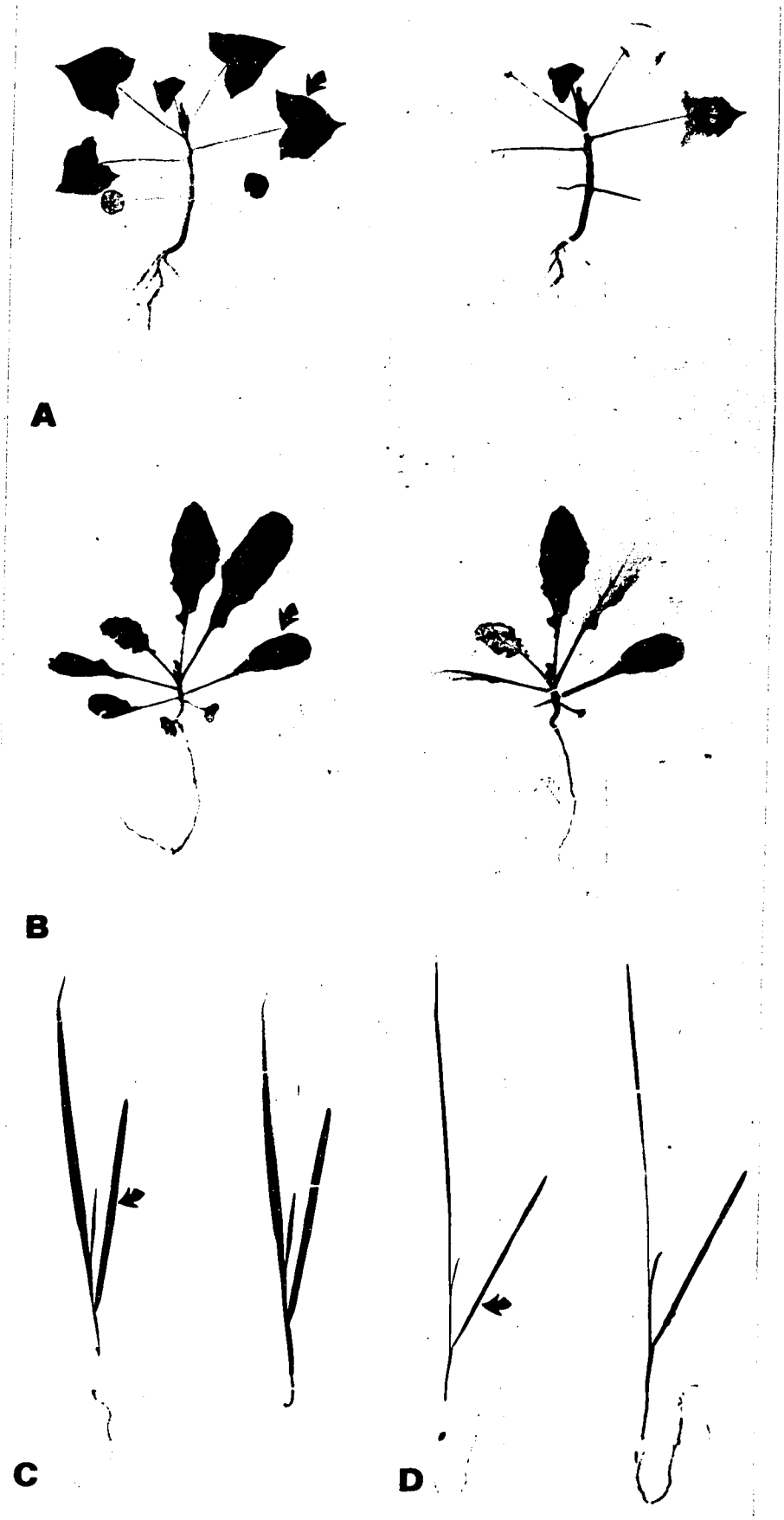
germination, two older plants and two younger seedlings were selected for each pot. The two older plants, at the three-leaf stage, were treated by applying 12 μg of dicamba to a single mature leaf of each plant, taking precautions to avoid contamination of the soil or the untreated plants. Injury symptoms were observed on the untreated young plants, indicating that they had absorbed a phytotoxic amount of the herbicide from the soil. This herbicide must have been exuded from the roots of the treated plants.

To obtain more quantitative results, Tartary buckwheat plants grown in nutrient solution were treated with 0.1 μc of dicamba-C^{14} by foliar application. The nutrient solution was replaced at different time intervals. The liquid was evaporated to dryness, the residue was dissolved in 95% ethanol, and the amount of radioactivity was determined in the liquid scintillation counter. Results from eight plants indicated that an average of 9% of the applied activity was recovered from the liquid medium in the first five days after application, and another 8% in the following ten days. All of the radioactivity present in the nutrient solution was chromatographically identical to the parent compound, dicamba-C^{14}.

Translocation in wild mustard, barley and wheat

Figure 8 shows the distribution of dicamba-C^{14} or its derivatives in Tartary buckwheat, wild mustard, barley and wheat one day following application of 0.1 μc of the labeled herbicide in a 10 ul droplet on a single leaf. The radioactivity showed a strong tendency

Figure 8. Distribution of radioactivity from dicamba-C^{14} in Tartary buckwheat (A), wild mustard (B), barley (C) and wheat (D), one day following application of 0.1 μc of the herbicide to a single leaf as indicated by the arrows. The lettered photographs represent the plants, the corresponding photographs to the right of them the autoradiograms.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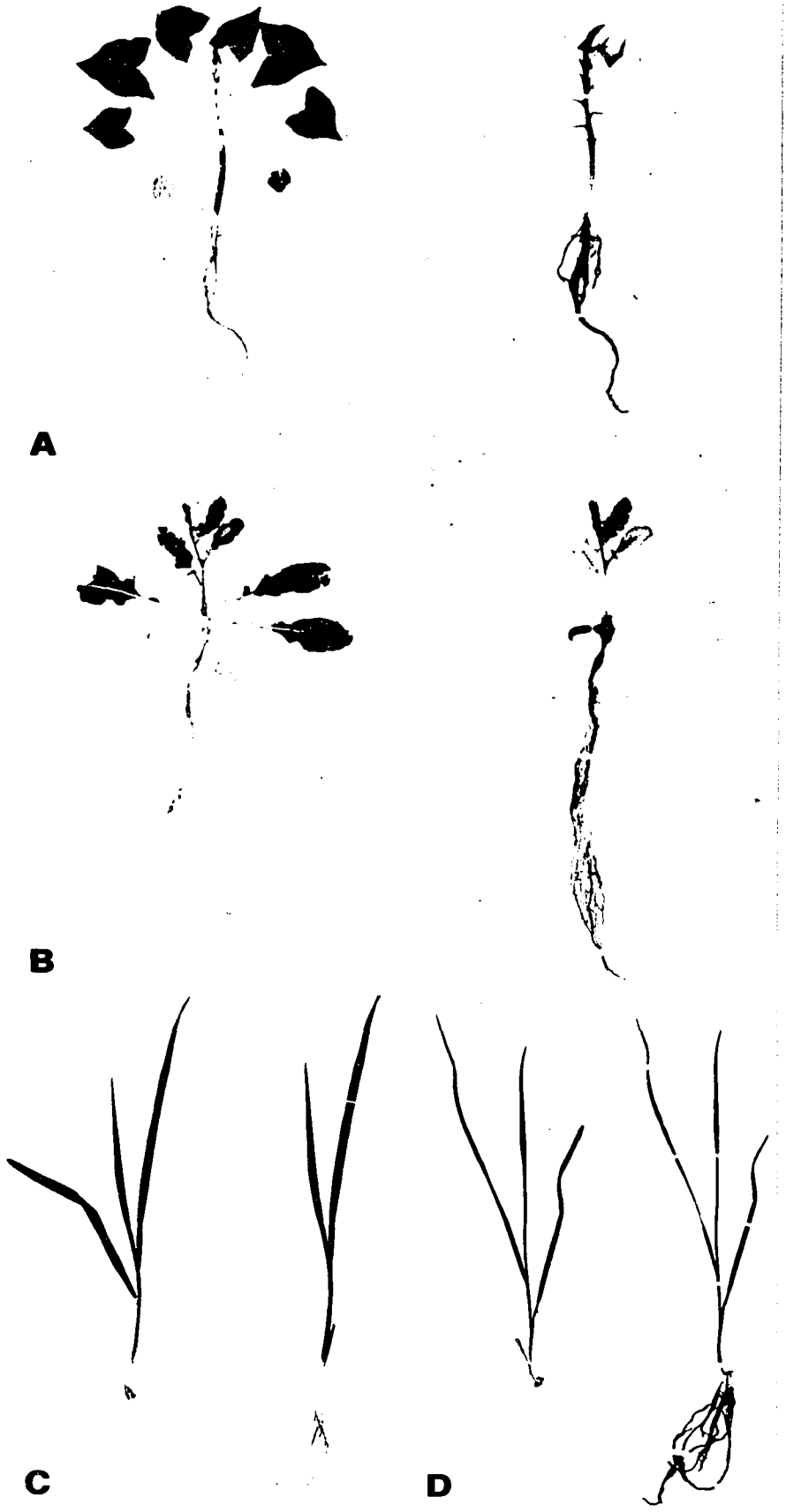
to accumulate in young leaves at the shoot apex of Tartary buckwheat and wild mustard plants, whereas in barley and wheat plants the label was more or less evenly distributed throughout the plants with some tendency to accumulate in tips of the wheat leaf. Also, more of the total radioactivity appeared to remain in the treated leaf of wheat and barley than in Tartary buckwheat and wild mustard plants.

The distribution patterns of dicamba-C^{14} or its derivatives in these four species after root absorption (Figure 9) were similar to those following leaf application, except that there was more activity present in the roots after application via the nutrient solution.

Quantitative data on the absorption and translocation of dicamba-C^{14} in the four species after foliar application were obtained at time intervals ranging from one to twenty days (Figure 10). Absorption of dicamba by leaf tissues of wild mustard and Tartary buckwheat was much faster and more complete than that by the two grass species. The amount of radioactivity translocated out of the treated leaf was in the order of: Tartary buckwheat, wild mustard, barley, wheat. Thus, more dicamba was retained in the treated leaf of wheat than in the other species, and the treated leaf of Tartary buckwheat retained the least. The results are in agreement with those of the autoradiographic studies.

Figure 9. Distribution of radioactivity from dicamba-C^{14} in Tartary buckwheat (A), wild mustard (B), barley (C), and wheat (D) one day following uptake from nutrient solution containing 1.0 μc of the labeled herbicide in 100 ml. The lettered photographs represent the plants, the corresponding photographs to the right of them the autoradiograms.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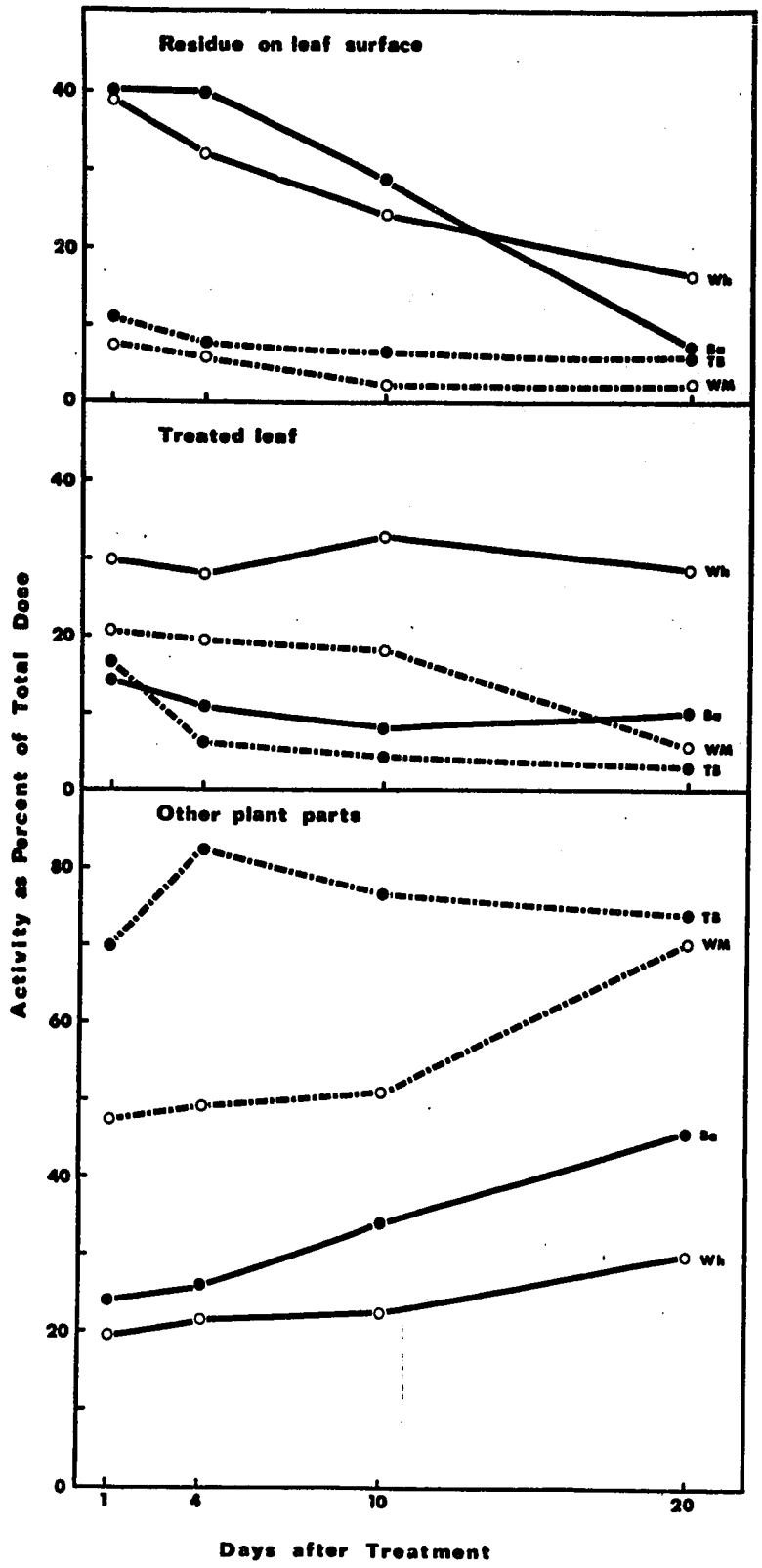


Figure 10. Time-course of translocation of dicamba-C^{14} in Tartary buckwheat (TB), wild mustard (WM), barley (Ba), and wheat (Wh) after application of 0.1 μc of the labeled herbicide in 10 μl solution to a single leaf. Radioactivity presented as percentage of total dose applied.

SECTION B: TRANSPORT IN PLANT SEGMENTS

Polarity of dicamba transport

The experimental results of transport of dicamba-C^{14} in petiole segments of Tartary buckwheat and bean, and in coleoptile sections of corn, are shown in Table 1. The ratio of radioactivity in the basipetal receivers to that in the acropetal receivers was adopted as a convenient measure of the polarity of transport. It is clear from these results that the transport of dicamba was basipetally polar in all tissues tested though the degree of polarity varied. The amount of radioactivity in basipetal receiver blocks was 160 times as high as that in acropetal receiver blocks in experiments with 6.5 mm segments of corn coleoptiles. The polarity of transport in Tartary buckwheat petiole was not as high as in bean petioles or corn coleoptile tissue, but basipetal transport was consistently greater than acropetal transport.

To check whether the radioactivity transported through the tissue segments was associated with the chemical applied or with its degradation products, the radioactive material in receiver blocks (Tartary buckwheat, bean, corn) and in tissue segments (Tartary buckwheat only) was repeatedly extracted with ethanol and water and the extracts were chromatographed with dicamba-C^{14} on Whatman No. 1 paper and silica gel thin-layer plates in isopropanol-ammonia-water (8:1:1). The results showed that only a single labeled compound with the same Rf value as dicamba was present in the extracts.

Table 1. Polarity of movement of dicamba-C^{14} in petiole segments of Tartary buckwheat and bean and coleoptile segments of corn. Dicamba-C^{14} was supplied at an initial concentration of 5 mg/L in donors. The transport period was 24 hours. Radioactivity in donors and receivers is shown as percentage of total radioactivity supplied.[a]

Plant segments	Transport[b]	% Radioactivity in Donors	% Radioactivity in Receivers	Polarity[c]
3.25 mm				
Tartary buckwheat	A	75.2	6.1	1.3*
(petiole)	B	74.8	7.7	
Dutch brown bean	A	73.2	7.3	2.6**
(petiole)	B	59.9	18.8	
Corn	A	64.0	1.6	20.9**
(coleoptile)	B	24.4	33.4	
6.50 mm				
Tartary buckwheat	A	75.7	1.0	2.2**
(petiole)	B	73.9	2.2	
Dutch brown bean	A	73.5	0.5	10.2**
(petiole)	B	62.5	5.1	
Corn	A	57.8	0.1	160.0**
(coleoptile)	B	17.0	16.0	

a Data for transport in Tartary buckwheat and bean petioles are from one experiment while those in corn are from another set of experiments. Each figure in the table represents the average of three replications.

b A = acropetal transport; B = basipetal transport.

c Polarity = radioactivity in basipetal receiver/radioactivity in acropetal receiver.

* and ** indicate significance at the 5% and 1% probability level, respectively, as determined by Student's t test.

Time-course of transport of dicamba and IAA

The time-course of movement of dicamba-C^{14} supplied at an initial concentration of 5 mg/L in donor blocks, in 3.25 mm petiole segments of Tartary buckwheat, was compared to that of IAA-C^{14}. The results are shown in Table 2 and Figures 11, 12, and 13.

Radioactivity in donor blocks decreased with time after the experiment was set up. Since no radioactivity disappeared from donors until they were in contact with tissue segments, it is assumed that the radioactivity lost from the donors was taken up by the tissue segments. Uptake of dicamba-C^{14} and IAA-C^{14} (Figure 11) was slow during the first four hours, more rapid during the next twenty hours, and then slowed down again. In 24 hours the net loss of IAA-C^{14} or dicamba-C^{14} from basipetal donors was only slightly greater than that from acropetal donors. Uptake of IAA-C^{14} was much greater than that of dicamba-C^{14} - about 70 per cent of the IAA-C^{14} supplied was lost from donors set up for basipetal transport in a 24-hour period, whereas the loss of dicamba-C^{14} was only 25 per cent during the same period.

A substantial part of the radioactivity that was lost from the donors was retained by the tissue segments (Figure 12). After 24 hours, for example, three-fourths of the activity lost from the donors was recovered from the tissue segments.

Radioactivity of IAA-C^{14} appeared in the receivers in basipetal transport after a short lag period, and increased with time up to 12

Table 2. Time-course of the movement of IAA-C^{14} and dicamba-C^{14} in 3.25 mm segments of Tartary buckwheat petioles. Both chemicals were supplied at an initial concentration of 5 mg/L in donors.

Compound and Time (hr)	Radioactivity (as % of original donor)[a]								
	Acropetal				Basipetal				
	Donor	Tissue	Receiver	Total	Donor	Tissue	Receiver	Total	Polarity[b]
IAA									
2	96.5	2.2	0.25	99.0	95.4	2.6	1.05	99.1	4.2*
4	94.3	3.4	0.33	98.0	91.7	4.3	1.93	97.9	5.8*
8	85.0	11.4	0.67	97.1	81.5	12.8	2.62	96.9	3.9*
12	71.4	20.5	1.06	93.0	68.0	22.0	3.00	93.0	2.8*
24	31.9	49.1	2.00	83.0	30.8	51.2	3.10	85.1	1.6*
48	12.4	69.2	2.50	84.1	12.5	68.2	3.31	84.0	1.3
Dicamba									
2	98.3	1.4	0.09	99.8	98.0	1.5	0.12	99.6	1.3*
4	97.0	2.2	0.38	99.7	96.4	2.1	0.50	99.0	1.3*
8	94.5	4.1	0.97	99.6	94.3	3.7	1.32	98.3	1.4*
12	89.1	8.0	2.02	99.1	88.6	7.4	2.95	99.0	1.5*
24	76.0	18.7	5.86	100.6	74.6	17.5	7.65	99.7	1.3*
48	60.3	21.3	19.39	101.0	61.4	18.1	19.74	99.2	1.0

[a] Data represent the average of four replications from two experiments of two replicates each.

[b] Polarity = radioactivity in basipetal receiver/radioactivity in acropetal receiver.

* Significant at the 5% probability level as determined by Student's t test.

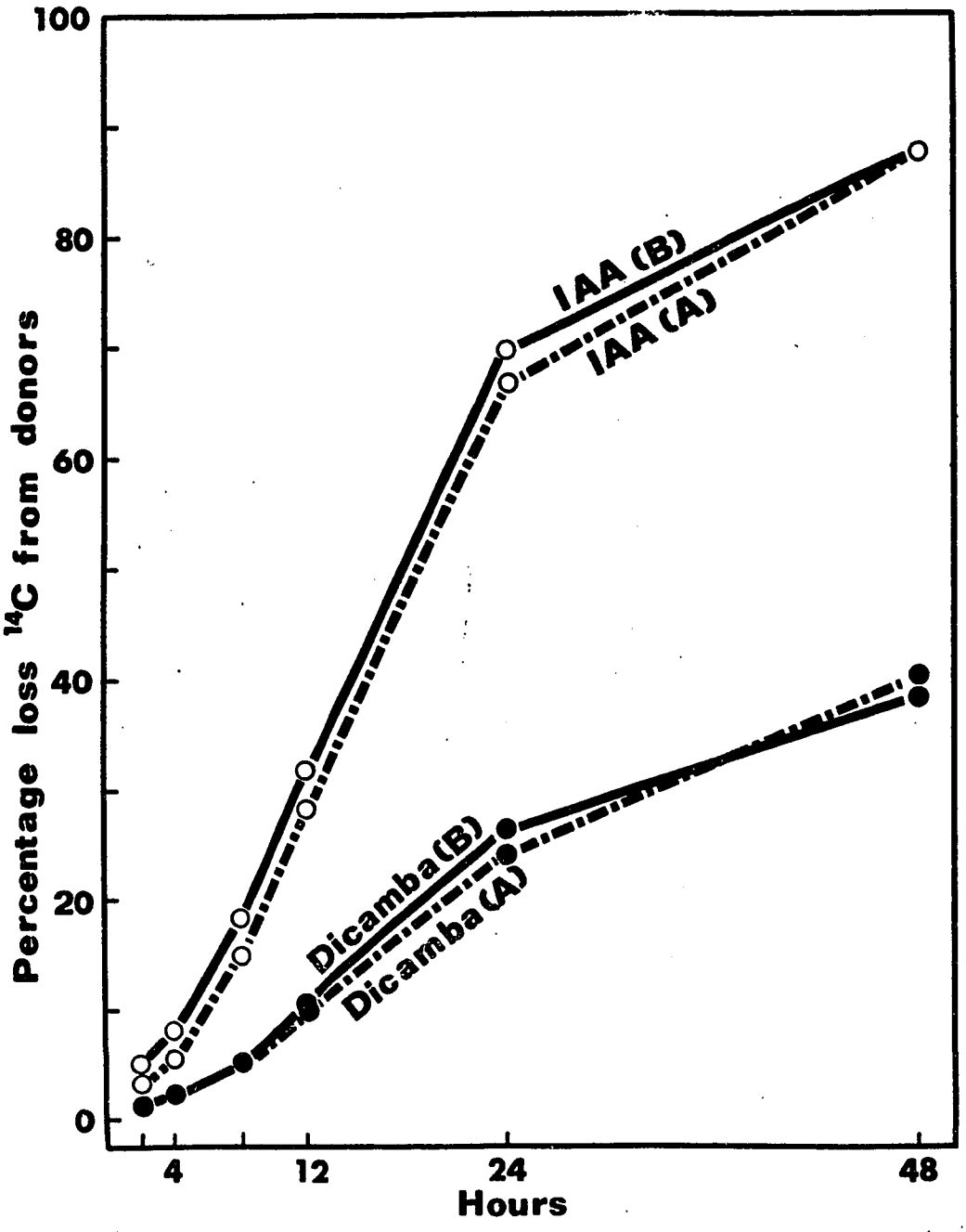


Figure 11. Time-course of the loss of radioactivity of dicamba-C^{14} and IAA-C^{14} from basipetal (B) and acropetal (A) donors placed on 3.25 mm segments of Tartary buckwheat petioles. The initial concentration of both chemicals was 5 mg/L in the donor blocks. Plotted from data in Table 2.

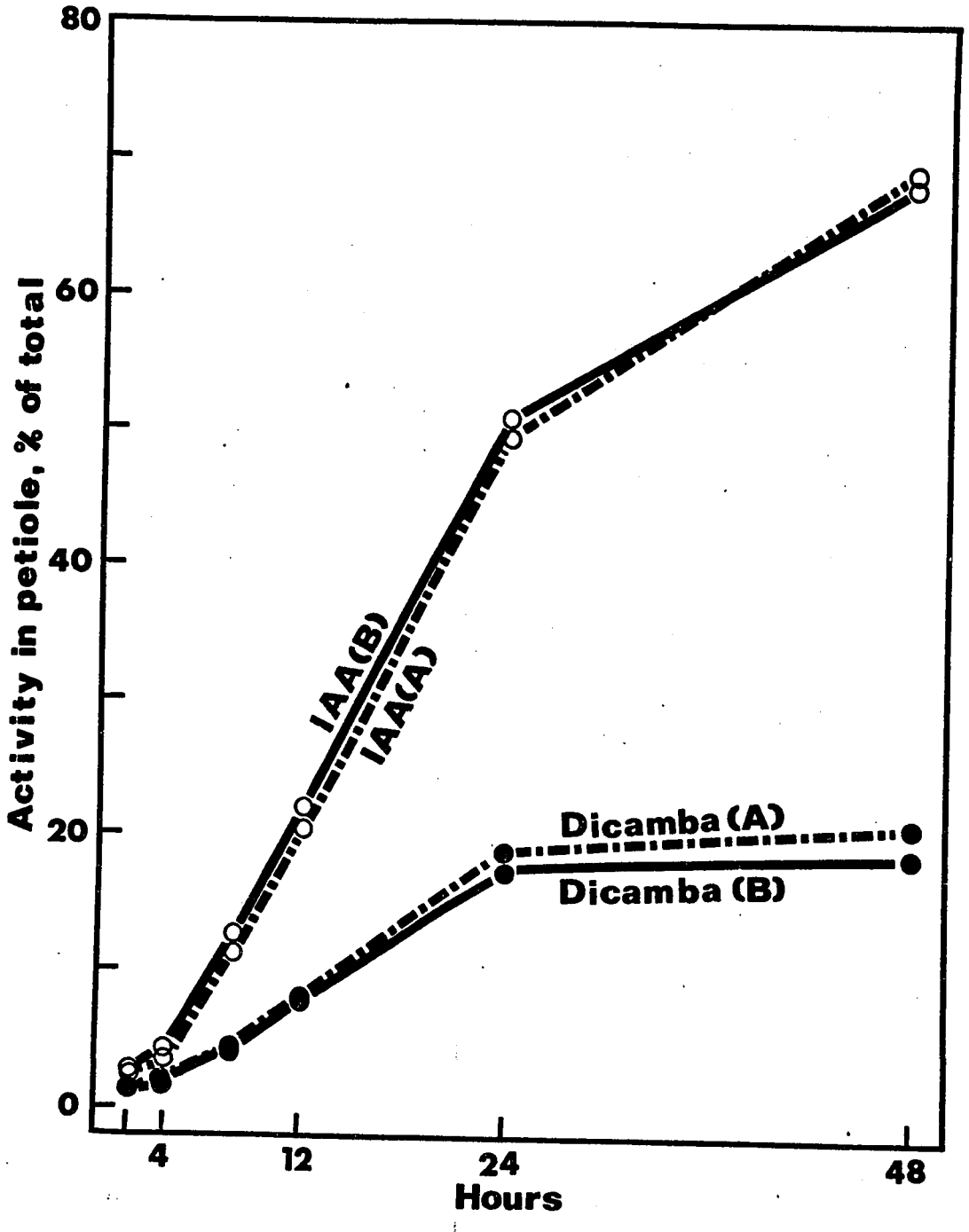


Figure 12. Percentage of total radioactivity from dicamba-C^{14} and IAA-C^{14} retained in 3.25 mm segments of Tartary buckwheat petioles. The initial concentration of both chemicals in the donors was 5 mg/L. (A) acropetal transport; (B) basipetal transport.

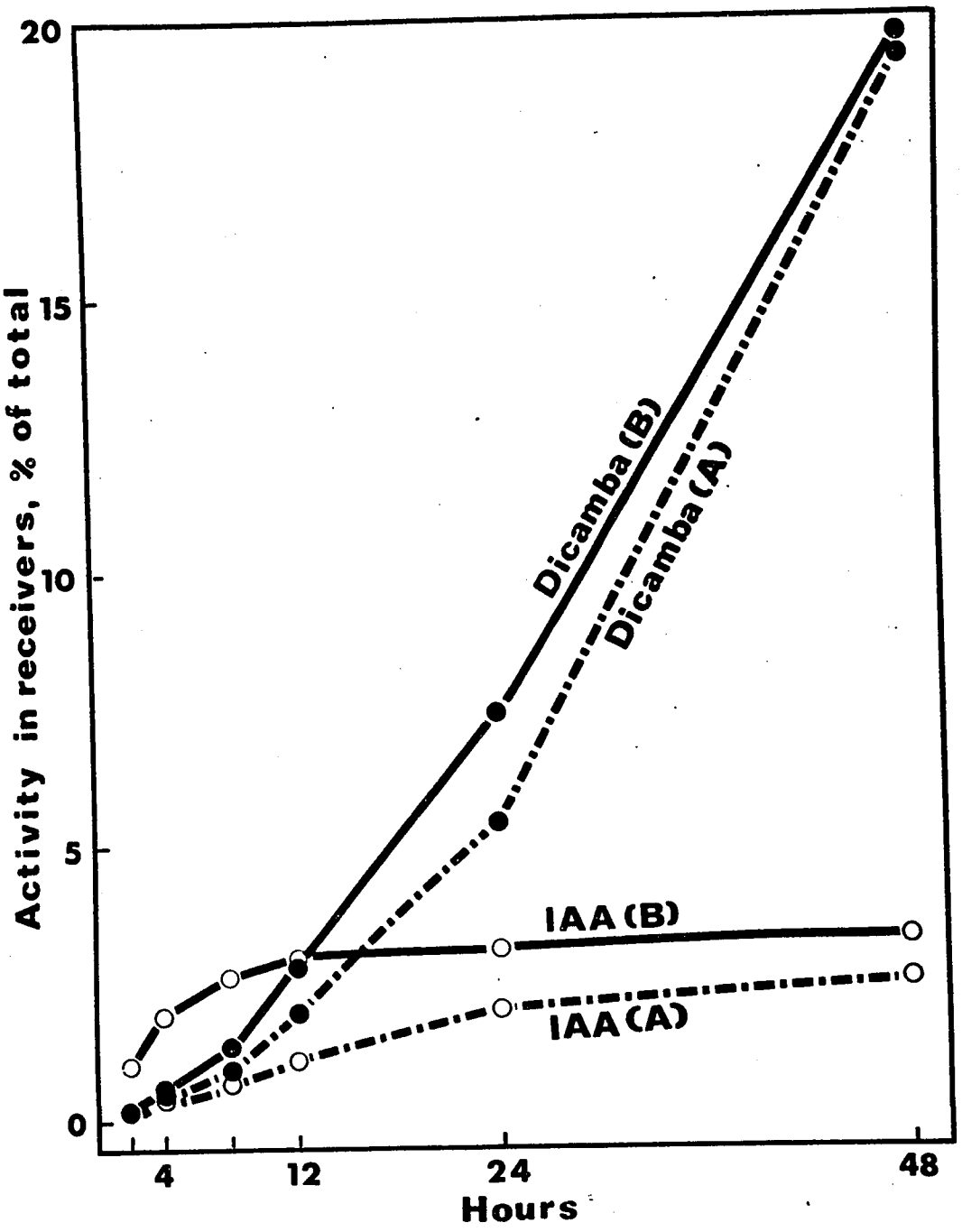


Figure 13. Time-course of acropetal (A) and basipetal (B) movement of dicamba-C^{14} and IAA-C^{14} into receiver blocks through 3.25 mm petiole segments of Tartary buckwheat. Both chemicals were supplied at an initial concentration of 5 mg/L in the donors, and the radioactivity in the receivers is presented as a percentage of the total radioactivity supplied.

hours, after which it leveled off (Figure 13). The radioactivity in the acropetal receivers also increased with time, and the increase continued over a longer period, but the activity in the acropetal receivers was always much lower than that in the basipetal receivers. In the case of dicamba-C^{14}, the activity appearing in basipetal receivers was low at first, as compared to IAA-C^{14}, but then a rapid increase occurred which was maintained up to 48 hours.

The degree of polarity varied from time to time, with a maximum at four hours for IAA and at twelve hours for dicamba; at 48 hours the differences between basipetal and acropetal transport of both the chemicals were no longer significant. The polarity of transport in these segments was consistently greater for IAA than for dicamba.

The total activity of IAA-C^{14} recovered from the donors, the receivers, and the tissue segments was less than the initial activity supplied, indicating that some activity of IAA-C^{14} was lost from the transport assembly during the experimental period. In a 24-hour period, for example, about 15 per cent of the initial activity of IAA-C^{14} disappeared from the transport system. Since no activity disappeared from the donors unless they were in contact with tissue segments, the activity of IAA-C^{14} must be lost from the tissue. Since IAA-2-C^{14} was used, the loss of radioactivity could not be a result of simple decarboxylation of the compound. It is possible that IAA was further degraded in the tissue and the C^{14} was then lost to the air. No such losses occurred from the dicamba-C^{14}

transport assembly (Table 2).

Movement of sucrose-C^{14}

In order to compare the movement of dicamba with that of sugars, C^{14}- uniformly labelled sucrose (sucrose-C^{14}, 11.8 mc/mmole) was used in the transport studies using the same plant material, petiole segments of Tartary buckwheat. The results are shown in Table 3.

Table 3. Movement of sucrose-C^{14} through petiole segments of Tartary buckwheat plants in 24 hrs. The initial concentration of sucrose-C^{14} in the donors was 2.9 mg/L.

Segment lengths	Transport	Radioactivity (dpm)*in. Donors	Tissue	Receivers
3.25 mm	Acropetal	25,884	9,284	534
	Basipetal	25,731	9,430	546
6.50 mm	Acropetal	24,992	10,650	64
	Basipetal	25,188	10,452	68

* Data represent the average of three replicates of ten segments each.

It is clear from Table 3 that the amount of radioactivity recovered from the basipetal receivers was approximately the same as that from the receivers arranged for acropetal transport, regardless of the length of the segments used. Therefore, the movement of

sucrose in the tissue segments was not polar. In addition, the amount of radioactivity moved into receivers in proportion to the supplied dose was much smaller for sucrose than for dicamba. It appears that the movement of sucrose in the tissue sections is simply by passive diffusion.

Effect of segment length on transport

The rate of dicamba transport through Tartary buckwheat petiole segments into receivers decreased with increasing segment length, especially in acropetal transport (Table 4). As a consequence, the polarity of transport increased with increasing length of the tissue sections. At 24 hours, for example, the ratios of basipetal to acropetal movement through segments of 3.25 mm, 6.50 mm, and 9.75 mm were approximately 1.2, 2.2, and 3.3, respectively.

Table 4. Effect of length of petiole segments of Tartary buckwheat on the movement of dicamba-C^{14} supplied at an initial concentration of 5 mg/L in donors. (Standard = 29,950 dpm)

Transport time (hours)	Radioactivity (dpm) in receivers *					
	3.25 mm		6.50 mm		9.75 mm	
	A	B	A	B	A	B
4	99	130	-	-	-	-
8	293	380	33	51	-	-
12	638	650	69	121	6	12
16	1,114	1,358	195	310	9	33
20	1,590	1,860	240	480	19	63
24	2,028	2,345	314	694	32	107

* A = acropetal receivers; B = Basipetal receivers.

Straight lines of best fit of the experimental data were plotted by the method of least squares (Figure 14). The intercepts on the time axis by the straight lines were used to estimate the average time taken for dicamba molecules to traverse the segments (104). The estimated times for transport through the segments are 4.1 hours for 3.25 mm, 8.0 hours for 6.50 mm, and 11.9 hours for 9.75 mm; these times are roughly proportional to the length of the tissue sections. The average velocity of dicamba transport is thus calculated as 0.8 mm/hr. This velocity is only one-tenth of that of IAA, which is calculated as 8 mm/hr.

Effect of concentration on transport

Transport data of dicamba-C^{14} applied at different concentrations to 3.25 mm petiole segments of Tartary buckwheat are shown in Table 5. Both the absolute amount and the relative amount (expressed as percentage of the total dose applied) of dicamba appearing in the receivers increased with increasing concentration of the chemical in the donor blocks up to 50 mg/L. The increase was more obvious for acropetal than for basipetal movement and, in consequence, the polarity of transport was abolished gradually at higher concentrations. As the concentration was increased to 100 mg/L, the percentage of dicamba in the receivers dropped slightly though the absolute amount was still increasing.

The amount of dicamba retained by the tissue sections also increased with increasing donor concentration, but not in proportion

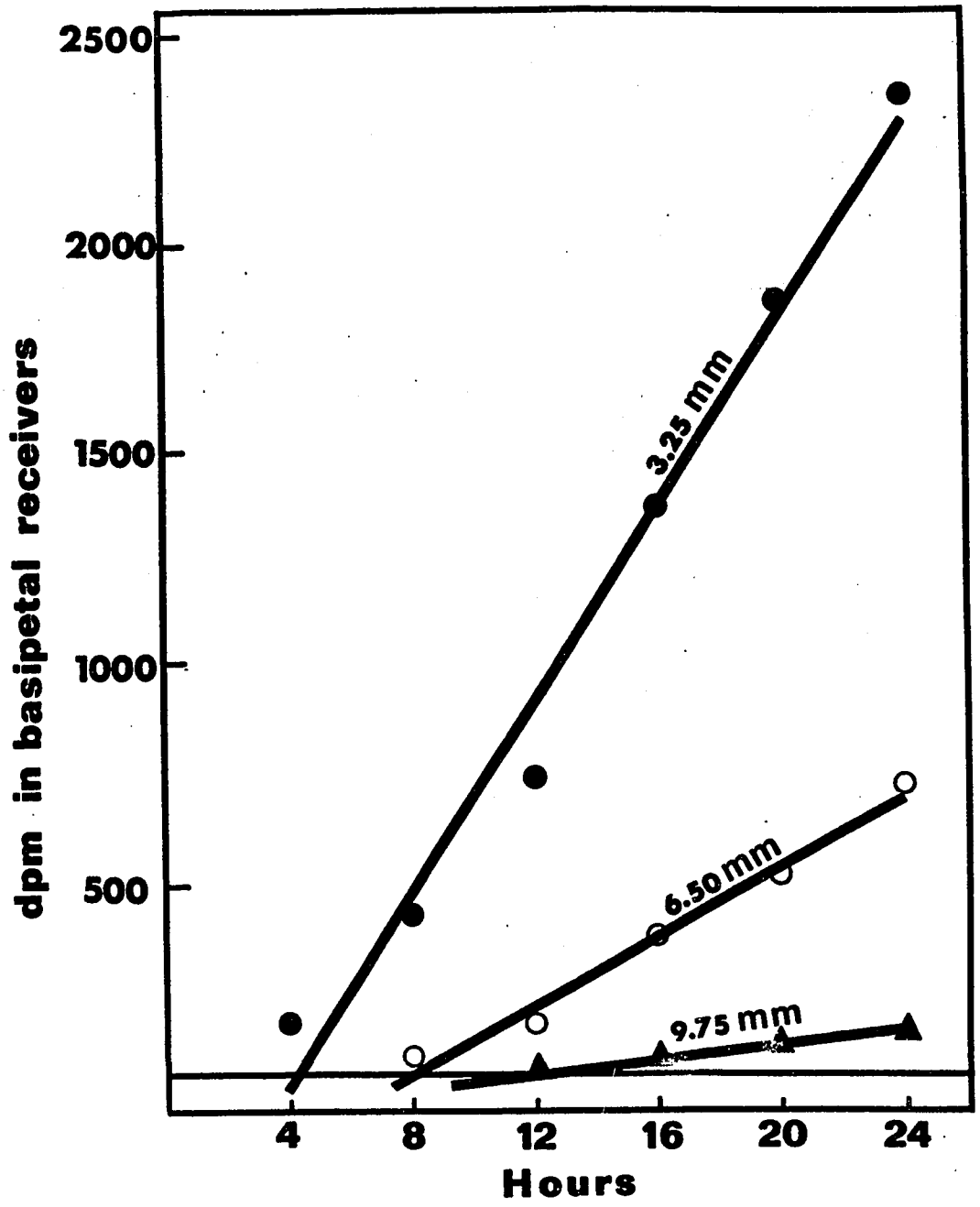


Figure 14. Time-course of basipetal transport into receivers of dicamba-C^{14} supplied at an initial concentration of 5 mg/L (standard = 29,950 dpm in donors) to Tartary buckwheat petiole segments of different lengths.

Regression equations:

3.25 mm, y = 115.50x - 486;
6.50 mm, y = 41.25x - 330;
9.75 mm, y = 8.03x - 90.

Table 5. Effect of concentration on the movement of dicamba-C^{14} into petiole segments of Tartary buckwheat and receivers in 24 hrs. The segments were 3.25 mm in length. Dicamba-C^{14} was supplied at initial concentrations of 1, 10, 20, 50 and 100 mg/L. Radioactivity in petioles and receivers is shown as percentage of initial activity in donors.

Concentration (mg/L)	% radioactivity			
	Acropetal		Basipetal	
	Petioles	Receivers	Petioles	Receivers
1	34.3±0.2	4.0±0.2	35.3±0.9	5.3±0.1
10	17.7±0.8	4.8±0.1	18.1±0.7	5.3±0.1
20	12.0±0.1	5.3±0.2	12.4±0.1	6.4±0.3
50	9.1±0.9	6.9±0.8	8.9±0.4	6.7±0.2
100	8.6±0.3	5.8±0.3	8.2±0.4	5.6±0.3

Data represent means of three replicates ± one standard error.

to the dose applied, resulting in a great decrease in its percentage. The relative amount of uptake of dicamba (activity in petioles and in receivers) also decreased as the donor concentration was increased from 1 to 100 mg/L.

Effect of temperature on transport

The rate of transport of dicamba-C^{14} through 3.25 mm and 6.50 mm petiole segments of Tartary buckwheat plants was measured at 5°, 15°, and 25°C (Figure 15). The amount of dicamba that reached the receivers was small at low temperature, and the rate of transport increased with increasing temperature from 5° to 25°C. In this temperature range, the Q_{10} values calculated for acropetal transport

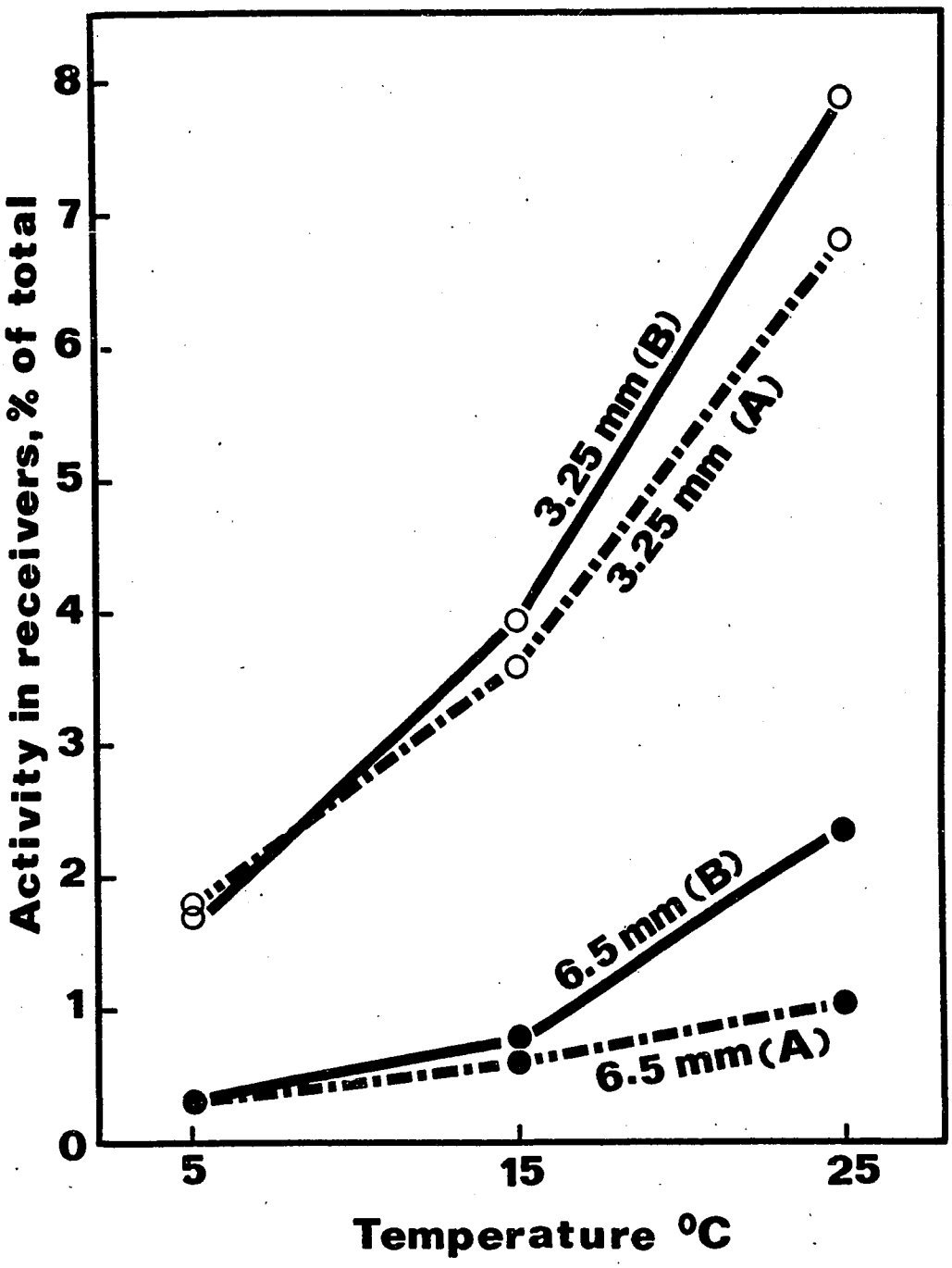


Figure 15. Transport of dicamba-C^{14} into receivers through 3.25 mm and 6.50 mm petiole segments of Tartary buckwheat plants at temperatures of 5°, 15°, and 25°C. The concentration of dicamba-C^{14} in donors was 5 mg/L, and the transport period was 24 hrs. A = acropetal receivers; B = basipetal receivers.

were 1.4 in 3.25 mm segments and 1.3 in 6.50 mm segments; for basipetal transport the Q_{10} values were 1.8 and 3.4 in 3.25 mm and 6.50 mm segments, respectively. Thus, the response to temperature was greater for basipetal than for acropetal movement. Consequently, the polarity of transport also increased with increasing temperature in the range studied, particularly with 6.50 mm segments.

Effect of light and sucrose on transport

Uneven irradiation of the petiole while still on the plants resulted in curvature of the tissue segments during the transport period. Therefore, the plants were moved into darkness twenty hours before treatment in most instances. Since this dark treatment of the plants might affect the transport system, however, transport of dicamba-C^{14} in petiole segments of Tartary buckwheat plants pre-treated for one day in the dark (dark plants) was compared to that with plants grown under 16-hour daylength in the growth chamber (light plants).

Results from preliminary experiments indicated that transport was increased slightly in the dark plants, especially for acropetal movement, but dark treatment resulted in a decrease of the total uptake of dicamba by the tissue segments. Segments from the light plants (light segments) retained more dicamba than the segments from the dark plants (dark segments).

The differences between results obtained with light and dark plants could be explained in terms of the energy reserves of the

tissues. Light-grown plants with rapid photosynthesis should have more food reserves than dark plants which would have exhausted a large portion of their energy reserves during the one-day dark period. With a greater energy supply the light segments could absorb and retain more of the chemical in the tissue than the dark segments, provided that uptake and retention are energy-requiring processes.

If an energy relation was indeed involved in the uptake and retention of dicamba by the tissue, the addition of sucrose to the agar blocks, thus supplying energy to the tissue, should also affect the transport. Results of experiments with and without 2% sucrose in both the donor and receiver blocks applied to 3.25 mm segments from dark and light plants are shown in Figure 16. Segments from light plants took up about three times as much dicamba as did segments from dark plants. The amount of dicamba retained by the light segments was about five times as much as that in the dark segments. The amount of dicamba transported into the receivers, on the other hand, was decreased by the light treatment. The effect of light treatment was even more obvious when the amount of dicamba entering receivers was expressed as a percentage of total uptake. In petiole segments from dark plants 68 per cent of the activity taken up by the tissue was in the basipetal receivers and 63 per cent in acropetal receiver blocks. In segments from light plants, however, only 23 per cent and 19 per cent of the total uptake was recovered from the basipetal and acropetal receivers, respectively. Addition of sucrose to the agar blocks completely abolished the effect of dark

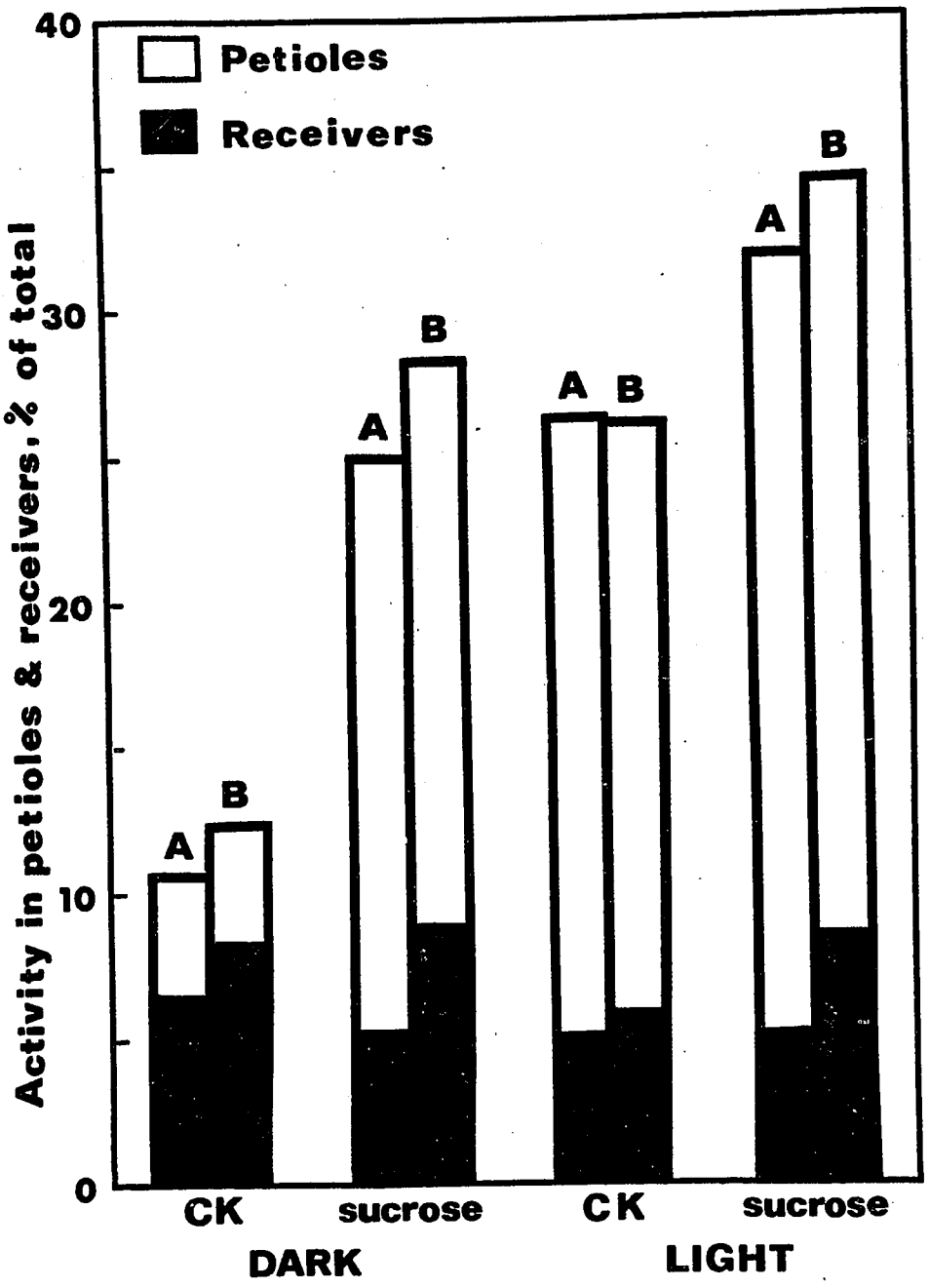


Figure 16. Transport of dicamba-C^{14} through 3:25 mm petiole segments cut from Tartary buckwheat plants grown in a 16-hr daylength (light plants) or pretreated in the dark for one day (dark plants). Agar blocks (both donors and receivers) contained 2% sucrose or no sucrose (CK). Initial concentration of dicamba-C^{14} in donor blocks was 5 mg/L. The transport period was 24 hrs.

treatment of the plants. The sucrose supply also increased basipetal transport while acropetal movement was little affected. Polarity was increased substantially, therefore, in the presence of added sucrose.

Effect of ATP and DNP on transport

The effect of ATP (adenosine triphosphate), a high-energy compound, and DNP (dinitrophenol), a metabolic inhibitor, on dicamba transport was studied using 6.5 mm petiole segments from Tartary buckwheat plants kept in the dark one day before use. ATP or DNP was incorporated into agar blocks (both donor and receiver) at concentrations of 2×10^{-3}M and 10^{-5}M, respectively. The transport results are shown in Figure 17.

The addition of ATP in agar blocks increased both the amount of dicamba uptake by the tissue and its subsequent transport into the receivers. The effect of DNP was the opposite -- it reduced both uptake and transport, especially the former.

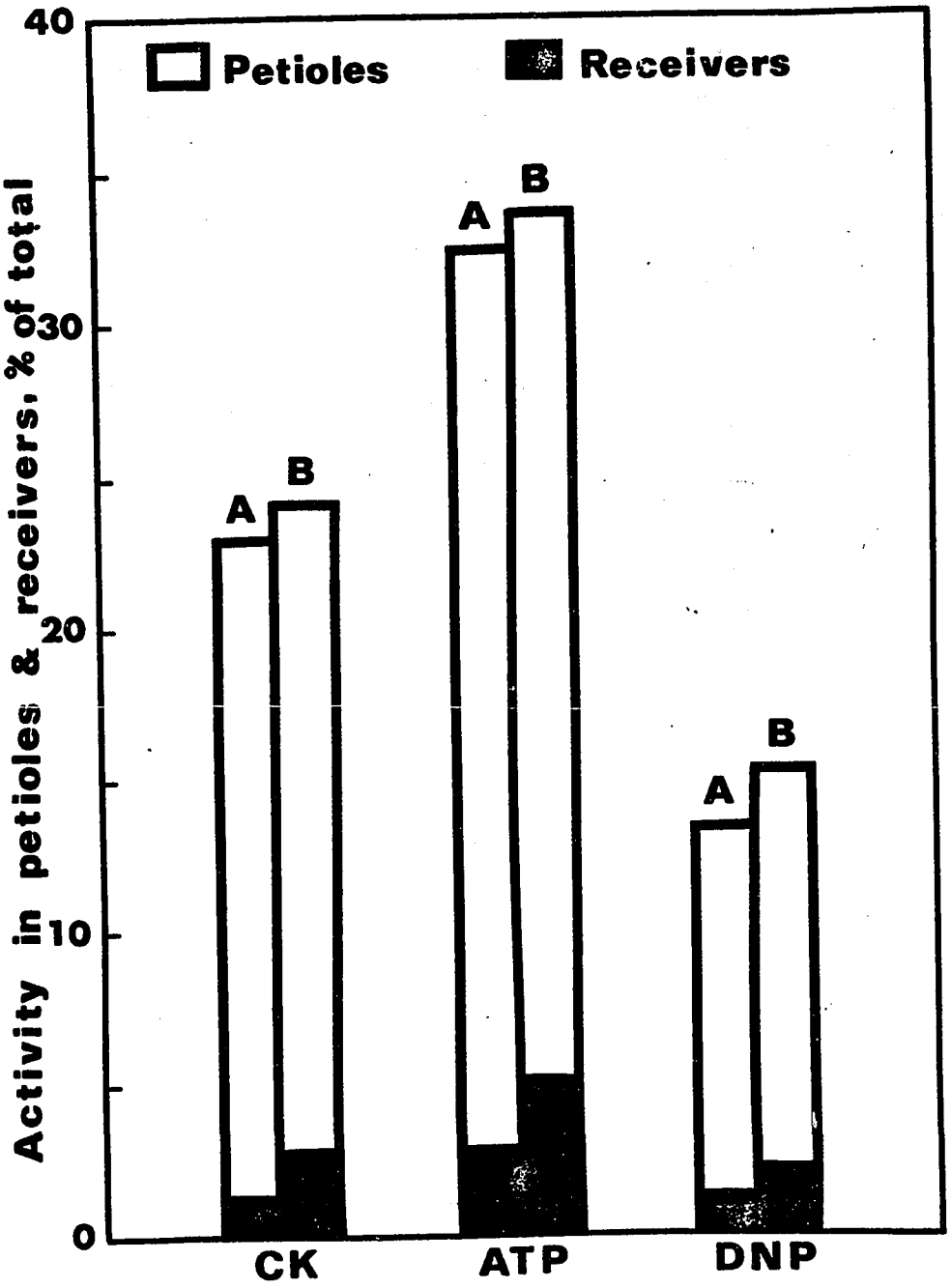


Figure 17. Effect of ATP (2×10^{-3}M) and DNP (10^{-5}M) in agar blocks (both donors and receivers) on the transport of dicamba-C^{14} through 6.50 mm petiole segments of Tartary buckwheat in 24 hrs. The concentration of dicamba-C^{14} in donor blocks was 5 mg/L. A = acropetal transport; B = basipetal transport; CK: without ATP or DNP.

SECTION C: METABOLISM

Dicamba metabolism in Tartary buckwheat

(1) Decarboxylation

Tartary buckwheat plants were treated with 0.1 μc of dicamba-C^{14} on a single leaf. The radioactivity released by the plants in the form of $C^{14}O_2$ was collected and counted. Figure 18 shows the cumulative total radioactivity collected from four plants which received a total amount of labeled herbicide equivalent to 880,000 dpm. It is apparent that some decarboxylation of dicamba-C^{14} occurred in Tartary buckwheat plants, but it occurred extremely slowly; during a period of 40 days, the total radioactivity so collected was less than 1 per cent of the total dose applied.

(2) Metabolites in plant extracts

The metabolic fate of dicamba in Tartary buckwheat was studied using paper and thin-layer chromatographic techniques. Scans of the chromatograms showed radioactivity from the treatment solution of dicamba-C^{14} as a single peak at a mean Rf 0.67 with Whatman No. 1 paper developed descendingly in the solvent system isopropanol-ammonia-water (8:1:1) (Figure 9, upper). Radioactive materials in the plant extract ran as several peaks. In addition to the main peak at the dicamba position, small amounts of activity were present at mean Rf values of 0.05 and 0.28 (Figure 19, middle).

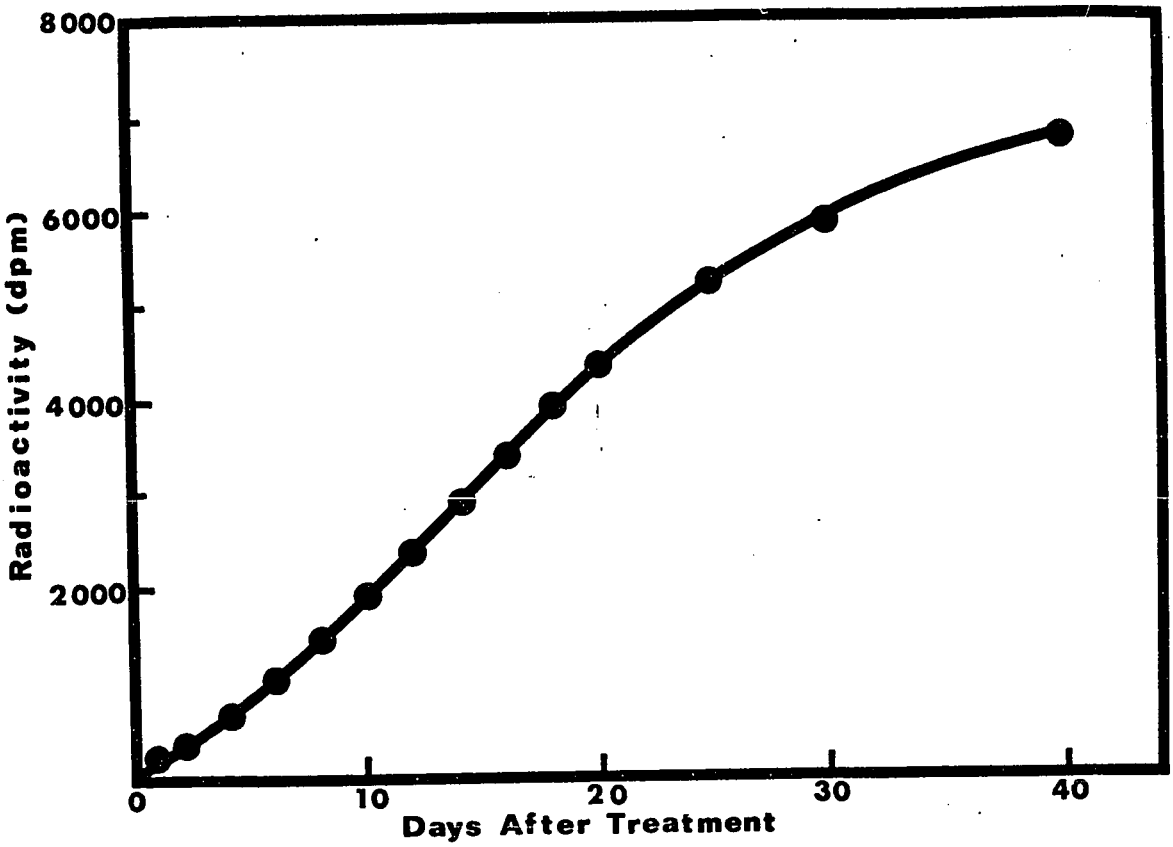


Figure 18. Radioactivity as $C^{14}O_2$ collected (cumulative total) from four Tartary buckwheat plants which received a total dose of 880,000 dpm of dicamba-C^{14} by foliage application. Data represent the average of duplicate treatments.

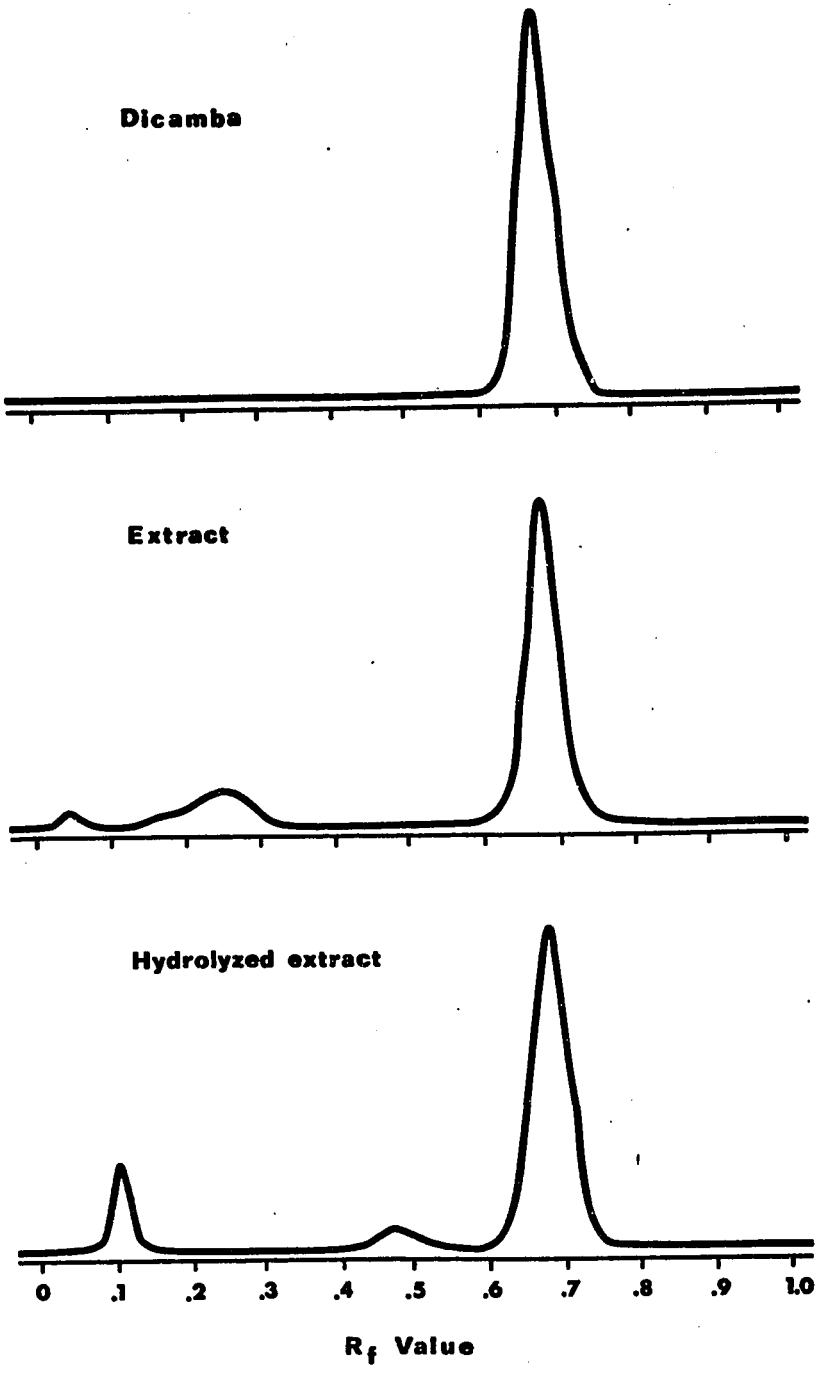


Figure 19. Distribution of radioactivity along chromatograms of dicamba-C^{14}, and ethanol extracts of Tartary buckwheat plants 40 days following application of 0.1 μc dicamba-C^{14} to a single leaf. Chromatographic solvent: isopropanol-ammonia-water, 8:1:1.

It has been reported (7) that dicamba or its metabolites may form conjugates with plant constituents. Thus, the additional peaks might represent metabolites of dicamba or conjugated products of dicamba or its metabolites. If they were conjugates, acid or alkaline hydrolysis should release the compounds from the plant constituent moiety. Therefore, the plant extracts were hydrolyzed with acid or base and then chromatographed again. Scans of the chromatograms of the hydrolyzed extracts indeed show a different feature; the main peak remained in the same position but the peaks at Rf 0.05 and 0.28 were replaced by two new peaks at Rf 0.09 and 0.45 (Figure 19, bottom).

To identify the radioactive derivatives, plant extracts after hydrolysis were co-chromatographed, on paper and thin-layer plates, with dicamba-C^{14} and the predictable degradation products 5-OH dicamba, DCSA-C^{14} and di-OH dicamba (3,6-dichlorogentisic acid) (Figure 20). By paper chromatography the compound at Rf 0.09 was identified as 5-OH dicamba and the one at Rf 0.45 as DCSA. The identification was confirmed by thin-layer chromatography with different solvent systems (Figures 21 and 22).

Because there was more than one peak on the chromatograms of the plant extracts both before and after hydrolysis, it was necessary to determine which peak gave rise to which compound on hydrolysis. Radioactive compounds at the different Rf values were eluted from the paper chromatogram with ethanol and then water, on a shaker overnight. The eluates were hydrolyzed with acid and then

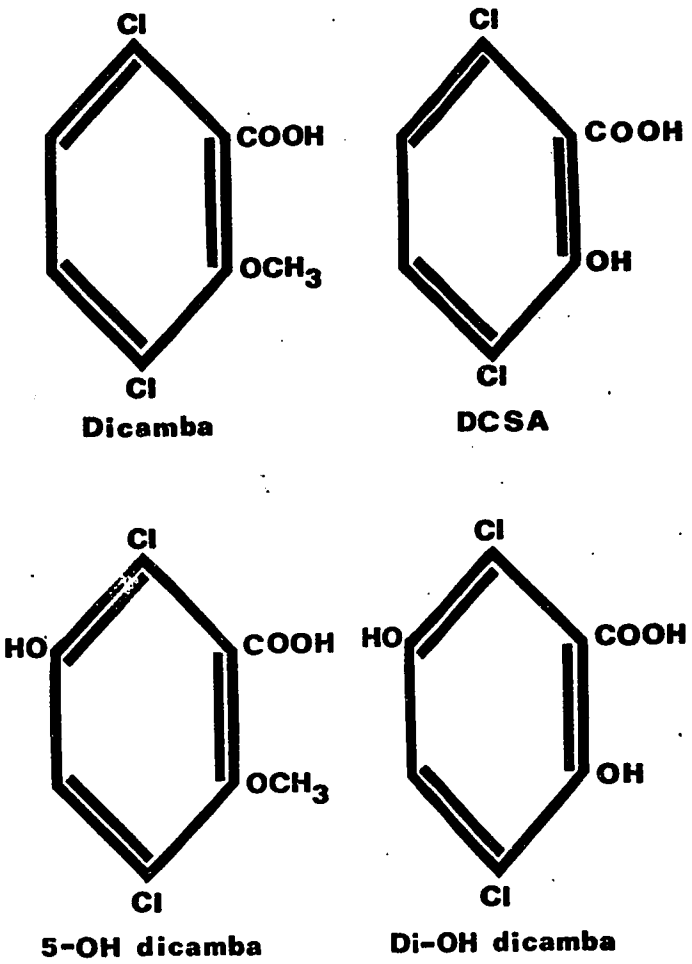


Figure 20. Structural formulas of dicamba and some of its derivatives, 3,6-dichlorosalicylic acid (DCSA), 5-hydroxy-2-methoxy-3,6-dichlorobenzoic acid (5-OH dicamba), and 3,6-dichlorogentisic acid (di-OH dicamba).

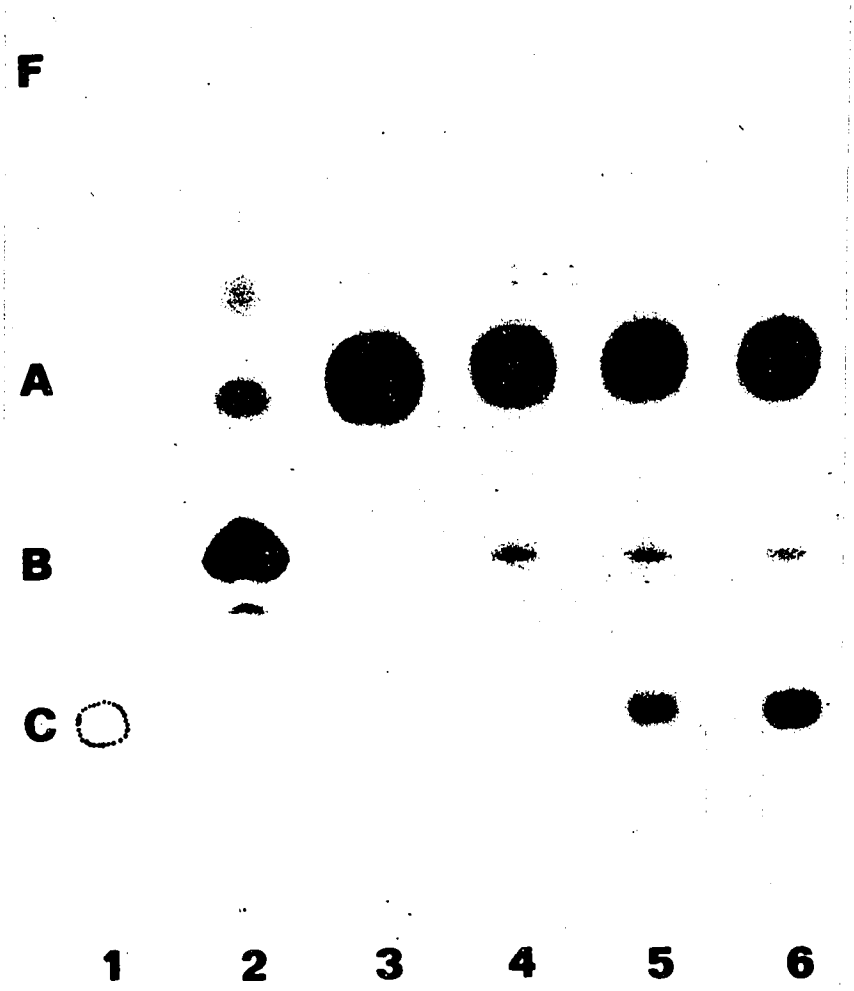


Figure 21. Autoradiogram of thin-layer chromatogram of dicamba-C^{14} and its derivatives, and hydrolyzed extracts of Tartary buckwheat plants at different times after treatment with 0.1 μc of C^{14}-labeled dicamba to a single leaf. Thin-layer of 0.25 mm silica gel GF on glass plate, developed in isopropanol-ammonia-water (8:1:1). (1) 5-OH dicamba; (2) DCSA and impurities; (3) dicamba; and extracts of plants (4) 1 day, (5) 10 days, and (6) 40 days after treatment.

Spots: (A) dicamba; (B) DCSA; (C) 5-OH dicamba.

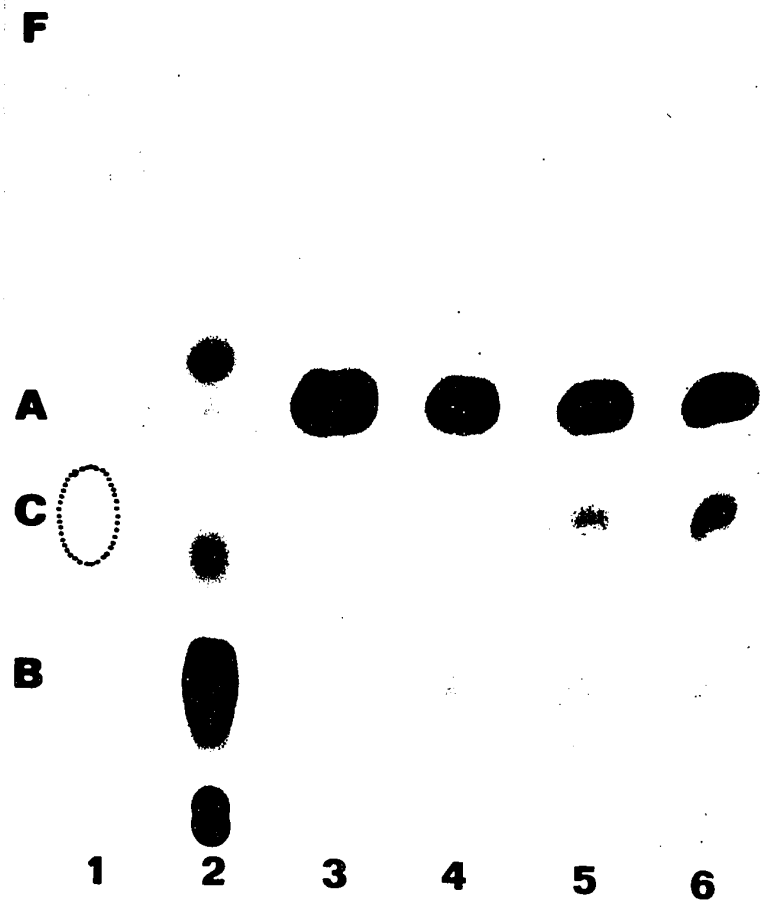


Figure 22. Autoradiogram of thin-layer chromatogram of dicamba-C^{14} and its derivatives and hydrolyzed extracts of Tartary buckwheat plants at different times after treatment with 0.1 μc of dicamba-C^{14} to a single leaf. Thin-layer of 0.25 mm silica gel GF on glass plate was developed in benzene-dioxane-acetic acid (90:25:4).

(1) 5-OH dicamba; (2) DCSA and impurities; (3) dicamba; extracts of plants (4) 1 day, (5) 10 days, and (6) 40 days after treatment.

Spots: (A) dicamba; (B) DCSA; (C) 5-OH dicamba.

chromatographed separately. It was found that hydrolysis of the eluate from the chromatogram at Rf 0.05 yielded 5-OH dicamba. Hydrolysis of the eluate from Rf 0.28 yielded the same product, 5-OH dicamba. Neither of them produced DCSA. When the main peak at the dicamba position was eluted and hydrolyzed, it yielded dicamba and approximately 2 per cent of DCSA. It might be that a conjugated product of DCSA had the same Rf value as dicamba and that upon hydrolysis DCSA was released from the plant constituent. However, when pure dicamba was hydrolyzed, the same amount of DCSA was produced. It appears then that the DCSA obtained from the hydrolysis of the ethanol extracts was not a metabolite of dicamba in Tartary buckwheat plants, but a breakdown product of this herbicide during the hydrolysis process.

As shown in the chromatograms (Figures 19, 21, 22), the derivatives of dicamba had a lower Rf value than the parent compound in the solvent systems used. The change in Rf values upon hydrolysis suggests that the metabolite was conjugated with plant constituents in the plant tissue, and that it was released from the conjugation by hydrolysis. The conjugated products ran as two or more peaks on the chromatogram, indicating conjugation with more than one compound. The conjugates were more soluble in water than dicamba. The major conjugate was not very stable; its Rf value on the paper chromatogram varied in the range of 0.16 to 0.28. In most cases it gave a peak of mean Rf 0.28 with very broad shoulders. Upon heating to 80°C the peak of a higher Rf (0.28) shifted to a peak of lower

Rf (0.16). The conjugate gave a positive reaction for sugar with benzidine and a negative reaction for amino acid with ninhydrin. It appears that the metabolite was conjugated with sugar(s). Upon heating, partial hydrolysis of the sugar molecules might occur, resulting in a shift of the position on the chromatogram. By acid or base treatment the conjugate was completely hydrolyzed and the stable compound 5-OH dicamba was released.

The conjugation product was further characterized by hydrolysis with a specific enzyme. Beta-glucosidase (Almond emulsion, activity 1200 units/mg, obtained from Calbiochem, Los Angeles, Calif.) was dissolved in water (1 mg/ml); 0.5 ml of the enzyme solution was mixed with 0.5 ml of the substrate (the major conjugate at a mean Rf 0.28) and 0.3 ml of sodium-acetate buffer (0.1 M, pH 5). The mixture was incubated at 37°C for three hours and then chromatographed. The results indicated that 5-OH dicamba was produced by enzymatic hydrolysis from the conjugated product. This would indicate that the metabolite was conjugated as glucoside, and also suggests that the acid or base used in hydrolysis did not alter the metabolite.

To examine the phytotoxicity of the metabolite and its conjugation product, these compounds were eluted from the chromatograms and applied to the foliage of young Tartary buckwheat seedlings. Dicamba and 5-OH dicamba were also used for comparison. A complication here was the probable presence of plant materials in the

eluates, which might injure the leaf tissue if applied in a high concentration. Thus the test was compromised by using small doses, 1 µg or less. At these low doses, no injury symptoms were observed on plants treated with the metabolite or its conjugated product, but obvious injury occurred on plants treated with dicamba even at a dose as low as 0.05 µg. No symptoms occurred on plants treated with the authentic 5-OH dicamba at doses up to 10 µg/plant.

The metabolite and its conjugated product were absorbed by the leaf tissue and translocated in the plant of Tartary buckwheat, as evidenced by extracting and counting of plants treated on the foliage with these materials (Table 6).

Table 6. Distribution of dicamba-C^{14} or its derivatives in Tartary buckwheat seedlings 19 days after application to a single leaf (4,100 dpm/plant). Data presented as percentage of total radioactivity recovered.*

Compound	Radioactivity, %		
	Residue on leaf surface	Treated leaf	Other parts of plant
Dicamba	10.3	4.3	85.4
Metabolite	23.3	25.2	51.5
Conjugated metabolite	24.6	32.3	43.1

* Recovery varied from 50 to 70 per cent.

As determined by chromatographic separation and counting, the rate of metabolism of dicamba in Tartary buckwheat plants was

very low (Table 7). Only 13 per cent of the total recovered radioactivity was present as compounds other than dicamba-C^{14} 40 days after foliar application of this herbicide.

Table 7. Extent of metabolism of dicamba-C^{14} in Tartary buckwheat plants after application of 0.1 μc of dicamba-C^{14} (1.89 mc/mmole) in a 10 μl droplet on a single leaf. Data presented as percentage of total radioactivity recovered from plant extracts before hydrolysis.

Days after treatment	Metabolites, %		Dicamba, %
	Rf 0.05	Rf 0.28	Rf 0.67
0	0	0	100
1	0.2	0.5	99.3
4	0.3	1.3	98.4
10	0.6	4.6	94.8
20	1.0	9.0	90.0
40	1.5	12.0	86.5

The rate of metabolism of dicamba in different parts of Tartary buckwheat plants varied somewhat. In the treated leaf it was two to three times as high as in other parts of the plants (Table 8). This observation suggested that mature leaves could metabolize dicamba more rapidly than could other parts of the plants. Thus, samples were taken from untreated mature leaves (containing very low radioactivity) and other parts of treated

Table 8. Extent of dicamba metabolism in different parts of plants after application of 0.1 μc dicamba-C^{14} to a single mature leaf. Data presented as percentage of total radioactivity recovered.

Days after treatment	Metabolites, %	
	Treated leaf	Other parts
1	1.6	0.6
4	6.2	1.3
10	12.0	4.9
20	18.0	9.7

plants, and analyzed quantitatively. No differences in the extent of dicamba metabolism were found between these samples, however.

Since the conversion of dicamba to 5-OH dicamba has been found to occur in sunlight (Chirchirillo, 1966, in 121), it is possible that photodecomposition of dicamba occurred on the surface of the treated leaf and that the degradation product then penetrated into the leaf tissue and accumulated there. Thus, the dicamba residue on the leaf surface was chromatographed. In addition, dicamba-C^{14} in sterile water or in its dry form was exposed to sunlight or placed in the dark, and analyzed chromatographically at different time intervals up to 40 days. No degradation product of dicamba was detected in samples collected from the leaf surface, from dry dicamba in the dark or in the light, or from dicamba solution in the dark. An unidentified radioactive product (at an

Rf value between those of 5-OH dicamba and dicamba in isopropanol-ammonia-water, 8:1:1) was detected in the dicamba solution exposed to sunlight. This photochemical alteration was definite but very slow; only 1.5 per cent of the total radioactivity was in the altered form after 40 days exposure to sunlight in a covered petri-dish in the greenhouse. This conversion was much slower than the rate of dicamba metabolism in the treated leaf.

(3) Metabolism in detached leaves

Uniform mature leaves with petioles 7-8 cm long were detached from Tartary buckwheat plants with a pair of sharp scissors. The cut end of the petiole was immersed into water immediately after cutting. Two to three hours later, leaves of good turgidity were selected for treatment. For treatment, 0.02 μc of dicamba-C^{14} in 0.2 ml water in a small glass vial was supplied to each leaf. The treatment solution was taken up from the cut end of the petiole in one to one and one-half hours. In order to get complete uptake of the herbicide, a small amount of water was added to the container after the treatment solution was absorbed. Each treatment consisted of 10 leaves and three replicates were used. After treatment, the leaves were cultured in water in a growth cabinet at 22°C and a 16-hr daylength for one to ten days (the leaves remained green and turgid for at least ten days under the experimental conditions). At the end of the treatment periods, the leaves were separated into petioles and blades, and ground and extracted in 95% ethanol. The

extracts were subjected to radioactivity assay and chromatographic analysis. The radioactivity in the water was also determined.

As shown in Figure 23, dicamba-C^{14} was translocated into the leaf blades after being taken up by the petioles. An approximately equal amount of activity was retained in the petiole, however. The water medium also contained some radioactivity, and it increased with time up to six days after treatment. It appears that a large portion of the dicamba that entered the leaf tissue was exported back to the water again during the culture period.

Results of chromatographic analysis indicated that the radioactivity recovered from the water was in the form of unaltered dicamba, but metabolism of the herbicide did occur in the leaf blades and petioles. The degradation product of dicamba in detached leaves was the same as that found in intact plants, i.e., 5-OH dicamba. The rate of metabolism in leaf blades was approximately twice as high as that in the petioles (Table 9). The percentage of dicamba present in an altered form in the blades of these detached leaves after ten days corresponds with that present in treated leaves of intact plants after ten days (Table 8, page 75).

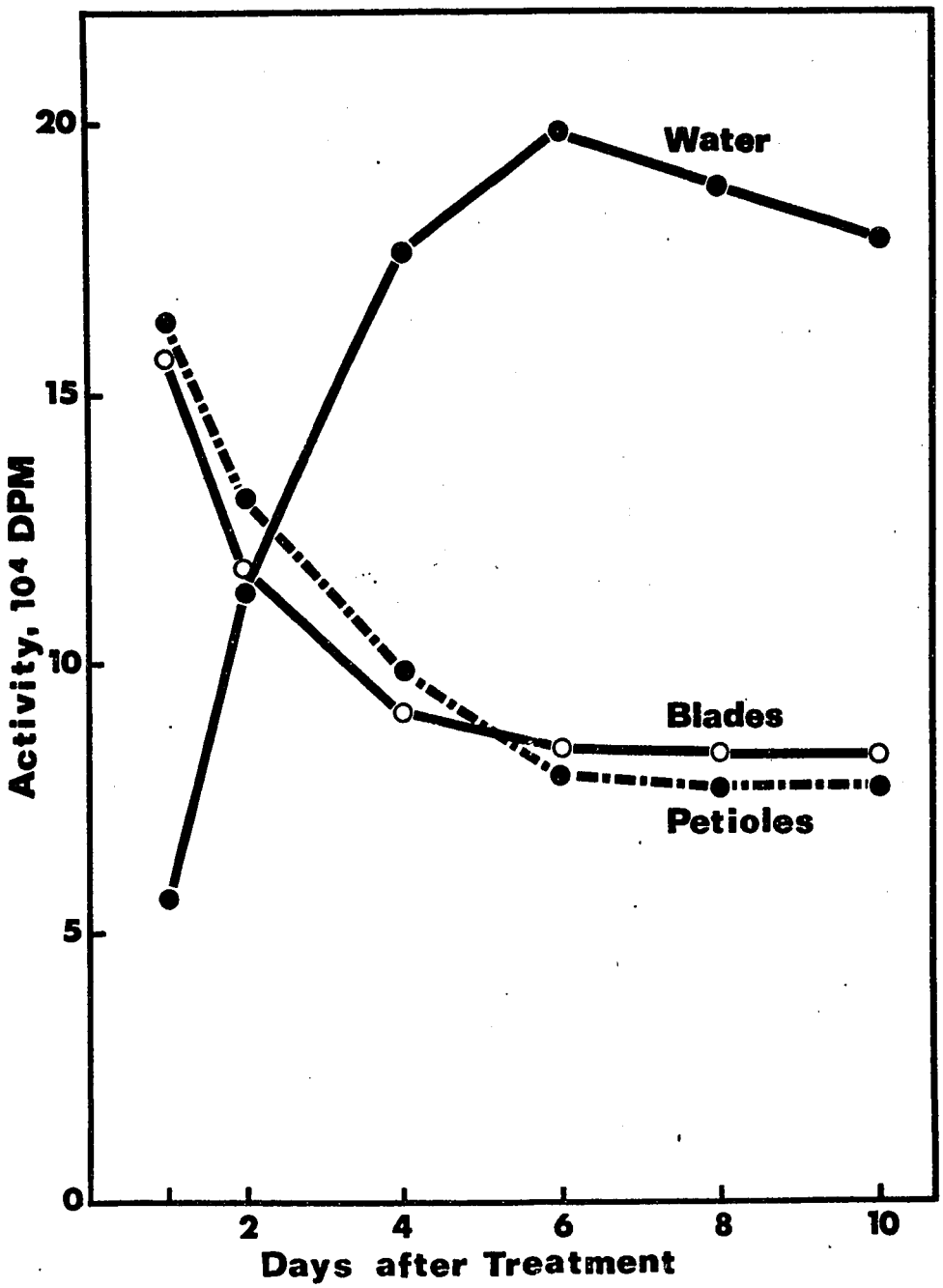


Figure 23. Distribution of radioactivity in petiole, leaf blade, and water medium after absorption of 0.02 μc dicamba-C^{14} in 0.2 ml water solution from the cut end of the petiole of detached Tartary buckwheat leaves. After treatment, the leaves were cultured in water until harvest. Each symbol in the graph represents the total radioactivity from 10 leaves which received a total of 0.2 μc of the isotope, equivalent to 440,000 dpm.

Table 9. Extent of metabolism of dicamba-C^{14} in detached leaves of Tartary buckwheat plants following uptake of 0.02 μc of dicamba-C^{14} in 0.2 ml water solution from the cut end of the petiole. The amount of metabolite is expressed as a percentage of the total radioactivity recovered in ethanol extract.

Days after treatment	Metabolites, %	
	Leaf blades	Petioles
1	1.9	0.6
2	3.2	1.0
4	5.5	2.0
6	7.9	4.8
8	9.4	4.5
10	12.3	6.2

Dicamba metabolism and plant susceptibility

(1) Susceptibility to dicamba of Tartary buckwheat, wild mustard barley and wheat

Tartary buckwheat, wild mustard, barley and wheat plants sprayed with the dimethylamine salt of dicamba showed different degrees of injury. Tartary buckwheat plants were seriously affected by treatment with 0.125 oz/A of dicamba and all plants died within two weeks after treatment with 1 or 4 oz/A (Figure 24). Wild mustard plants were also injured severely at these doses but few plants died during the two-week experiment period. The rates of application which caused death of Tartary buckwheat plants and seriously affected wild mustard caused no significant injury symptoms

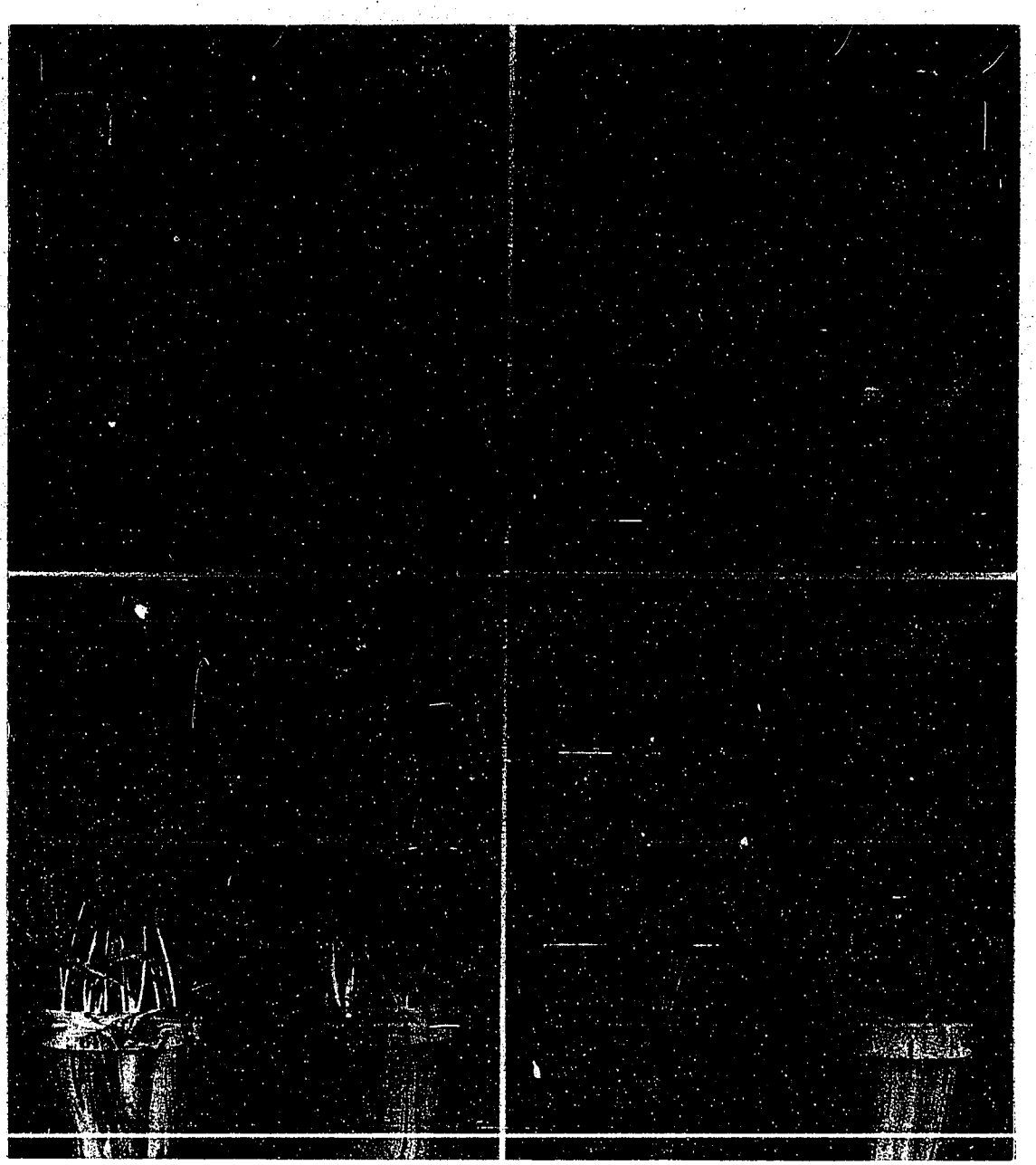


Figure 24. Tartary buckwheat (upper left), wild mustard (upper right), barley (lower left), and wheat (lower right) plants two weeks after foliar spray with 4 oz/A of dicamba. Left: untreated, right: treated.

to barley or wheat; no barley or wheat plants died in the two-week period even at a dose as high as 64 oz/A.

The effect of dicamba application on the growth of these four species is shown in Figure 25, in which the dry weight of the plants, expressed as a percentage of the untreated control, is plotted against the rates of application of the herbicide. From the graph, ED_{50} values (equivalent dose of the chemical required to reduce growth by half) for these species were estimated. The ED_{50} of dicamba was about 0.5 oz/A for Tartary buckwheat and wild mustard, 20 oz/A for barley, and 30 oz/A for wheat, under the experimental conditions in the greenhouse. These results confirm that Tartary buckwheat is very sensitive to dicamba, wild mustard is somewhat less susceptible, barley is resistant, and wheat is the most resistant.

(2) Comparison of metabolism of dicamba in Tartary buckwheat, wild mustard, barley and wheat

To investigate the metabolic fate of dicamba in plants of different susceptibility to this herbicide, 0.1 μc of dicamba-C^{14} was applied to a single leaf of each plant. At different time intervals after application, the plants were harvested and ground and extracted in ethanol, and the extracts were analyzed chromatographically.

Figure 26 shows an autoradiogram of thin-layer chromatograms of dicamba-C^{14} and the ethanol extracts of the four species 20 days following treatment. The extracts were chromatographed before

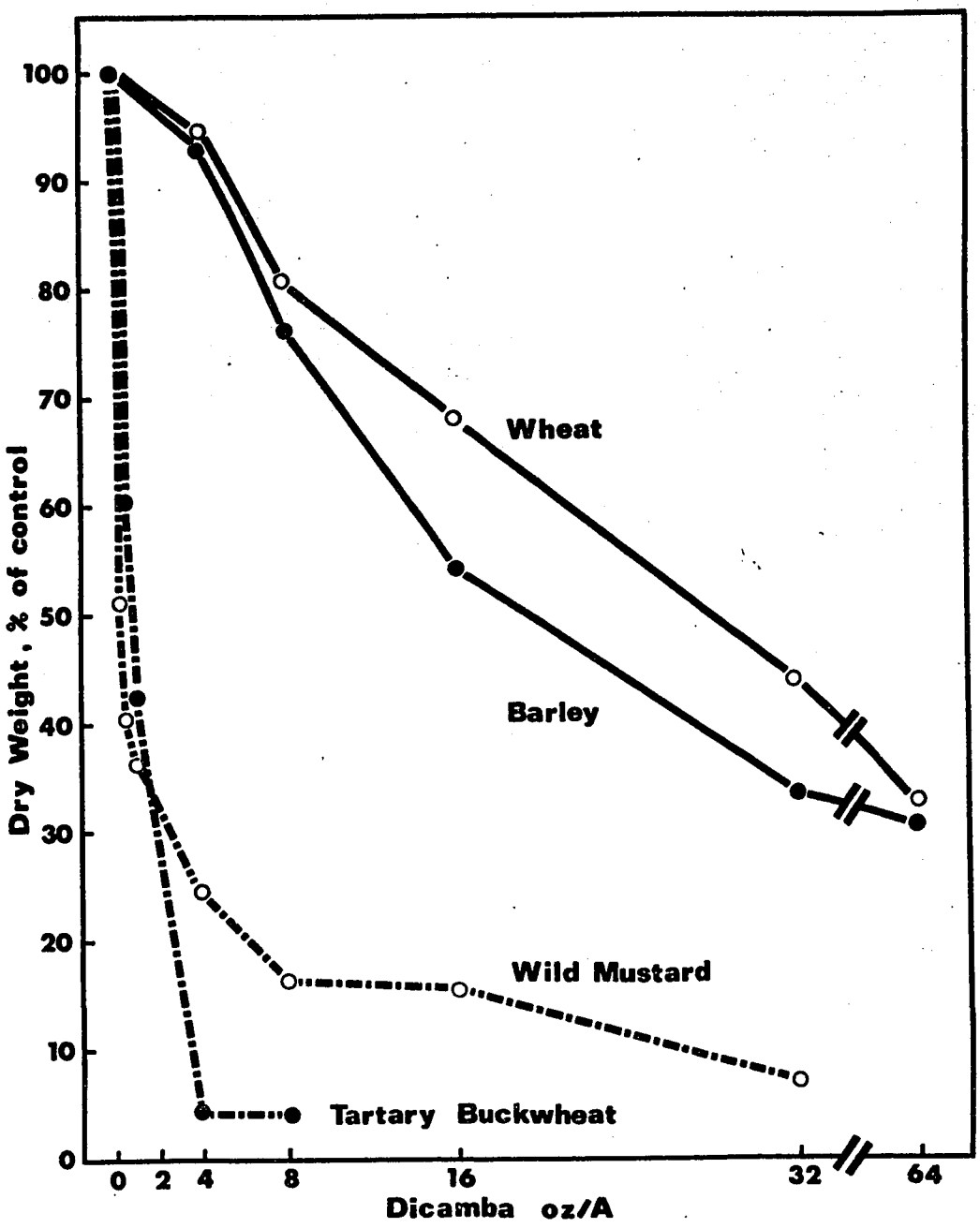


Figure 25. Effect of foliar spray of dicamba on the growth of Tartary buckwheat, wild mustard, barley, and wheat plants. The plants were grown in the greenhouse and harvested two weeks after treatment.

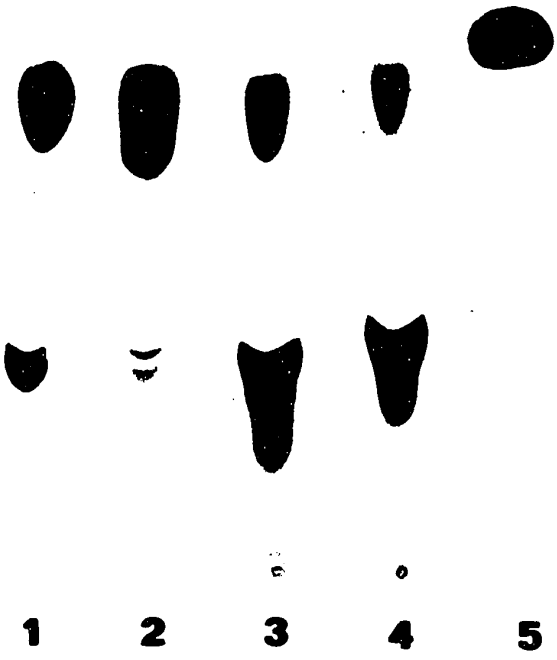


Figure 26. Autoradiogram of thin-layer chromatograms of the ethanol extracts of Tartary buckwheat (1), wild mustard (2), barley (3), and wheat (4), 20 days following foliar application of 0.1 μc of C^{14}-labeled dicamba, and the authentic sample of dicamba-C^{14} (5).

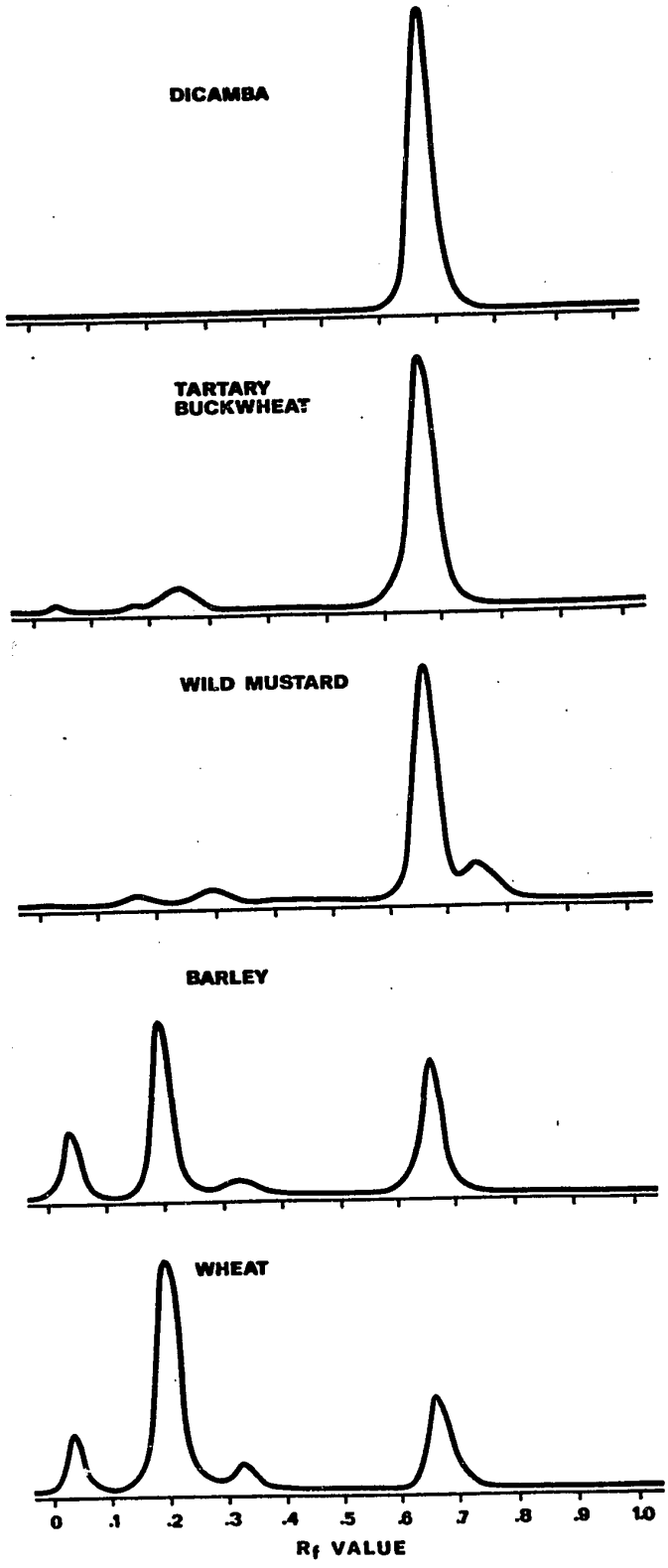
Thin-layer of 0.25 mm silica gel GF on glass plate was developed in isopropanol-ammonia-water (8:1:1).

hydrolysis. The dark spots at the highest Rf value are the unaltered dicamba-C^{14}; the spots of lower Rf values are the degradation products of the herbicide. Dicamba in plant extracts ran a little slower than pure dicamba, probably due to the presence in the extracts of sticky substances derived from the plant tissue. The long spot of the wild mustard extract (Figure 26, No. 2) consisted of two components - the unaltered dicamba-C^{14} and a derivative of it; this was demonstrated by paper chromatography (cf. Figure 27).

Scans of the paper chromatograms of the plant extracts (before hydrolysis) of the four species 20 days following treatment are shown in Figure 27. As previously, extracts from Tartary buckwheat plants ran as a major peak at the dicamba position and several small peaks at lower Rf values. Radioactive compounds in extracts from wild mustard gave a main peak at the Rf of dicamba, two minor peaks at lower Rf values (0.16 and 0.30), and a small peak at a mean Rf of 0.77 which is higher than the Rf of dicamba (0.67). The main peak of activity in the plant extracts from barley and wheat was not at the position of dicamba, but at Rf 0.20; two small peaks occurred at Rf 0.03 and 0.33.

The radioactive materials on the chromatograms at Rf values other than dicamba were eluted, hydrolyzed, and co-chromatographed with authentic samples for identification. The radioactive material with the mean Rf 0.16 from the extract of wild mustard plants yielded 5-OH dicamba, and those at Rf values 0.30 and 0.77 all

Figure 27. Distribution of radioactivity along chromatograms of dicamba-C^{14} and ethanol extracts of Tartary buckwheat, wild mustard, barley, and wheat plants 20 days after foliar application of 0.1 μc of dicamba-C^{14} (1.89 mc/mmole). Chromatographic solvent: isopropanol-ammonia-water (8:1:1).


DICAMBA
TARTARY BUCKWHEAT
WILD MUSTARD
BARLEY
WHEAT
0
.1
.2
.3
.4
.5
.6
.7
.8
.9
1.0
Rf VALUE

produced dicamba, upon hydrolysis. In the extracts from barley and wheat, the main peak at Rf 0.20 and the small peak at Rf 0.03 produced 5-OH dicamba upon hydrolysis whereas the one at Rf 0.33 yielded DCSA and dicamba in about equal proportion.

Here also, the extent of metabolism of dicamba varied between the treated leaf and other plant parts in wild mustard, barley and wheat (Table 10). The extent of metabolism was consistently greater in the treated leaf than in other parts of the plant.

Table 10. Extent of metabolism of dicamba in different parts of the plants at various times following application of 0.1 μc of dicamba-C^{14} (1.89 mc/mmole) to a single leaf of each plant. Data represent the radioactivity in forms other than unaltered dicamba-C^{14} in ethanol extracts of the plant parts as a percentage of the total radioactivity in the extracts.

Plants and parts	% metabolites		
	1 day	4 days	10 days
Wheat:			
Treated leaves	51.4	72.2	95.0
Other parts	18.0	42.2	54.0
Barley:			
Treated leaves	15.0	42.9	73.3
Other parts	4.9	25.3	55.5
Wild mustard:			
Treated leaves	2.7	4.6	9.6
Other parts	1.0	2.5	3.6

The time-course of metabolism of dicamba-C^{14} in the four species following foliar application of 0.1 μc of the chemical to each plant is shown in Figure 28. Clearly the metabolism of dicamba in wheat plants was very rapid; nearly one-half of the herbicide was metabolized in one day after treatment and 93 per cent of it was in forms other than dicamba 20 days following application. Metabolism of dicamba in Tartary buckwheat plants, on the contrary, was very slow; no detectable metabolism occurred in one day and only 10 per cent of the herbicide was degraded 20 days after treatment. The rates of metabolism of this herbicide in wild mustard and barley plants were intermediate with the rate in barley being close to that in wheat and the rate in wild mustard close to that in Tartary buckwheat.

It is evident that the rates of dicamba detoxification in the species studied correlated well with the degree of resistance to dicamba of each species. The more susceptible the species, the slower the rate of detoxification of the herbicide.

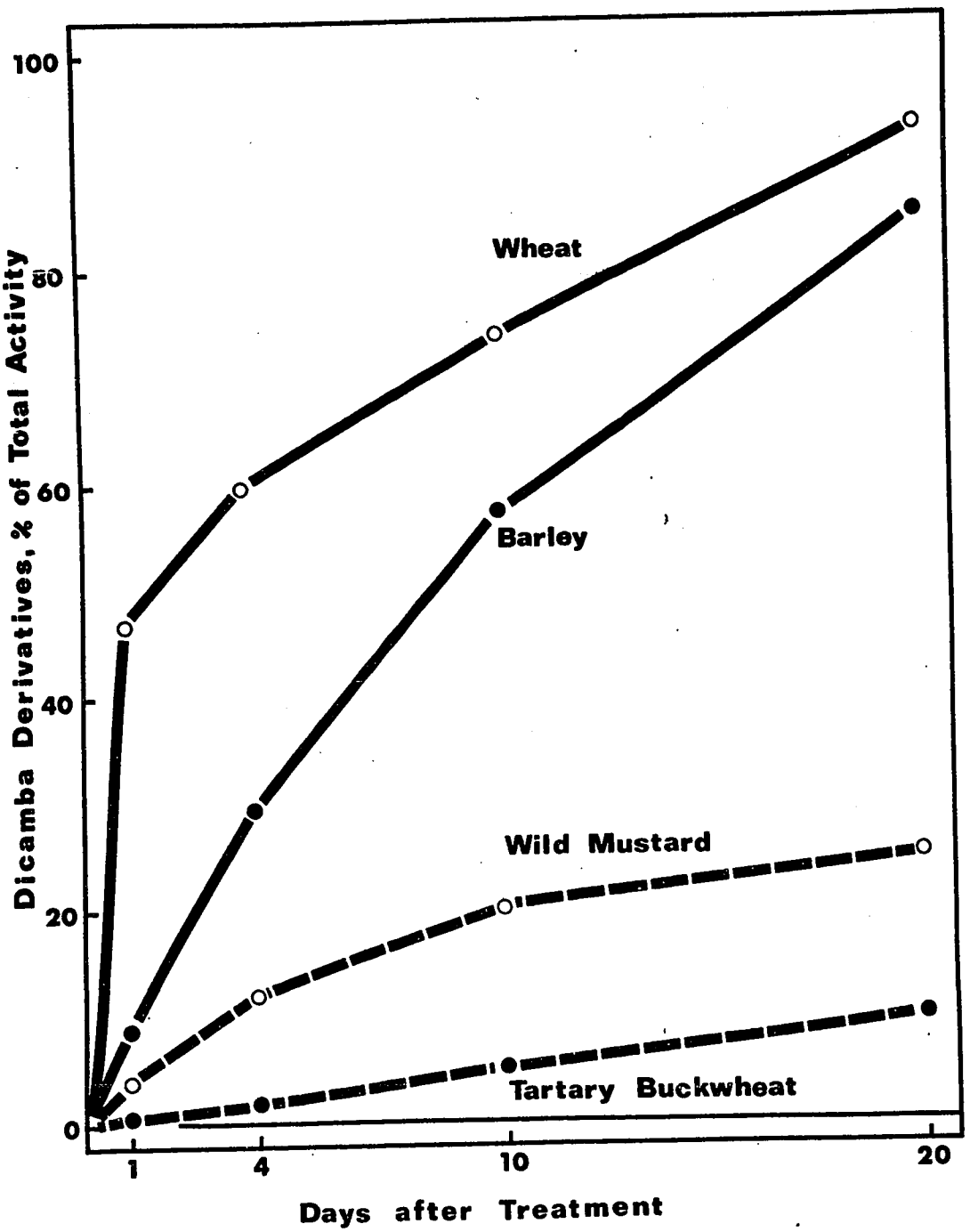


Figure 28. Time-course of the metabolism of dicamba-C^{14} in selected species following application of 0.1 μc of the labeled herbicide to a single leaf of each plant. The rate of metabolism is presented as the radioactivity of dicamba derivatives in percent of the total radioactivity recovered from the ethanol extracts of the plants.

DISCUSSION AND CONCLUSION

Translocation of dicamba in Tartary buckwheat

Translocation of dicamba in Tartary buckwheat plants was very rapid. One hour after foliar application a detectable amount of it was translocated out of the treated leaf and moved both upward and downward in the stem. The herbicide was then accumulated in the rapidly developing tissues at the shoot apex and root tips. When the young leaves matured, the chemical was retranslocated to the newly forming tissues including the young buds in the leaf axils, with only a small part of the activity being left in the injured tissue at leaf margins. The rapid redistribution of dicamba in the plants indicates a high mobility of the chemical in this species. The translocation seems to follow a source-to-sink pattern of distribution of photosynthates, which fits well with the mass-flow mechanism of phloem transport.

Since a little bit of the activity of dicamba also appeared in mature leaves, it appears that the compound migrated from phloem to xylem and then moved to the mature leaves in the transpiration stream. According to Crafts et al. (19), the ability of a herbicide to leak from phloem to xylem relates to its ability to be exuded by the roots. In cases where leakage into the transpiration stream occurs, leakage into the external medium of the roots also takes place. Root exudation of dicamba in Tartary buckwheat plants was observed in both soil and water cultures.

Distribution of dicamba in the plants following root uptake was similar to that after foliar application, except that more activity appeared in the mature leaves following root absorption than following leaf treatment. This is because translocation of root-absorbed compounds follows the way of water distribution through the xylem whereas translocation of foliar applied phloem-mobile chemicals is associated with the movement of food materials. Theoretically, root-absorbed radioactivity should be evenly distributed in the plant, if it is freely mobile. However, the highest concentration of dicamba was accumulated in the rapidly developing leaves at the shoot apex after uptake by the roots. This type of accumulation following root uptake would indicate a rapid redistribution of the herbicide with the assimilate flow in the plant.

Polarity of dicamba transport in tissue segments

After application of dicamba-C^{14} in agar blocks to one end of the tissue segments from petioles of Tartary buckwheat and bean, and coleoptiles of corn, radioactivity was recovered from the receiver blocks attached to the other end of the section in the form of unaltered dicamba. The amount of radioactivity recovered from the receivers at the base of the segments was consistently higher than that from the receivers at the apical end, i.e., the transport of dicamba in the segments was basipetally polar. The results confirm the observation of Zaerr and Mitchell (120) who, in research concurrent with the studies conducted here, found that dicamba was translocated in a polar fashion in hypocotyl segments

of Pinto bean seedlings. The polarity of transport in petiole segments of Tartary buckwheat was lower than that in bean petiole and corn coleoptile; this is perhaps partly the nature of the plant species but it may also be due to the use of relatively old petiole tissue. Results from some experiments, which are not cited in the text, showed that younger tissues gave higher polarity, but it was very difficult to work with the younger plants.because their petioles were too thin.

The degree of polarity of dicamba transport also varied with the length of the segments. In petiole segments from Tartary buckwheat, the rate of dicamba transport into receivers decreased with increasing length of the segments. The decrease with length was more obvious for acropetal than for basipetal transport and, therefore, the polarity of transport increased with increasing length of the tissue sections. This observation is in harmony with the suggestion of Leopold and his colleagues (20, 61, 63) that a very small polar difference in each cell can be amplified into large polar differences in passing through a file of similar cells, thus increasing the polarity in an exponential manner with increasing distance of travel of the auxin through the tissue.

The velocity of basipetal transport of dicamba through petiole segments of Tartary buckwheat plants was independent of segment length, and was calculated as 0.8 mm/hr. This velocity is approximately the same as the reported values of 0.6 - 1.0 mm/hr for 2,4-D (71) and 0.75 - 1.11 mm/hr for picloram (43) in petiole segments of bean seedlings.

It has been reported that transport of auxins through tissue sections increased with increasing donor concentrations over a limited range, and that further increase of the donor concentration caused a decrease of the relative transport rate (26, 33, 39, 90, 104, 106). The present results with Tartary buckwheat petioles showed a marked increase in the rate of dicamba transport when the concentration of this growth-regulator in donors was increased from 1 mg/L to 50 mg/L. Further increase of the concentration decreased the relative amount of transport, but the absolute amount of transport still increased. The increased transport with increasing concentration could not be a hyperbolic function of the uptake, because the relative amount of dicamba taken up by the tissue actually decreased with increasing donor concentrations. It is also possible that the increased rate of transport is simply the result of reduced retention by the tissue, because the relative amount of dicamba that remained in the tissue decreased sharply with increasing donor concentrations. Several authors (26, 33, 60) have indicated the existence of a static auxin pool in plant tissues. As auxin is taken up by tissues, only a portion of it is effectively transported, with the rest remaining in a static or non-transported pool. A saturation hypothesis has also been proposed to explain the limited transport of auxin in higher concentrations. The results of dicamba transport are compatible with both of these hypotheses. At low concentrations the dicamba absorbed by the tissue is retained largely in the static pool, but when the concentration is increased, the binding pool is gradually saturated and more dicamba is available for transport. As the concentration is increased still further,

the transport pool also becomes saturated, and a decrease in transport results.

Basipetal transport of dicamba through segments of Tartary buckwheat petioles was increased by the addition of sucrose or ATP to the agar blocks, and decreased by the addition of DNP or by lowering the temperature. The evidence suggests that basipetal movement of dicamba in this tissue is dependent on metabolic energy, and thus is active transport. Acropetal movement of dicamba was less affected by the additives and the temperature change, suggesting that a diffusion component was involved in this process. However, the Q_{10} values (1.3 - 1.4) indicate that acropetal movement might not be entirely passive diffusion, though the active component of transport here was much less important than in basipetal transport.

A 24-hour dark treatment of Tartary buckwheat plants prior to tissue excision (dark plants) greatly decreased total uptake of dicamba by the tissue segments compared to that of plants grown in a normal 16-hour daylength (light plants). The effect of the dark treatment on the capacity of the tissue to retain the chemical was even more obvious. About 77 per cent of the absorbed dicamba was retained in the segments from the light plants arranged for basipetal transport while the tissue segments from dark plants retained only 32 per cent of the absorbed activity. The results are in harmony with the finding of Thimann and Wardlaw (102) who reported that light of high intensity greatly promoted the accumulation

of IAA in segments from green stems of pea. The uptake and retention of dicamba by the tissue sections appear to be related to the energy reserves of the tissues. The suggestion is supported by the finding that addition of sucrose or ATP to the agar blocks increased the amount of total uptake and retention whereas the addition of DNP decreased uptake and subsequent accumulation in the tissue.

While light treatment of the plants decreased transport in both directions, acropetal movement was affected more than basipetal transport in most of the experiments. This finding is in general agreement with the results of Harel (35) who observed that continuous exposure of intact bean plants to white light for 48 hours reduced subsequent acropetal transport of 2,4-D in petiole sections from these plants, as compared to results with plants kept in the dark for the same length of time. Harel accounts for the decreased acropetal transport by the possibility that light destroys endogenous auxin and reduces the auxin level of the plants. IAA has been shown to stimulate its own transport (e.g., 65, 88) or the transport of 2,4-D (38, 88), but it is difficult to see how the endogenous auxin can control particularly the acropetal movement but not the basipetal transport. I think an energy relationship is at least partly involved in the light treatment and the transport of the auxins, if the dark or light treatment is as long as one or two days and light of high intensity is used. Both retention and basipetal transport of the auxin are dependent on metabolic energy. A higher level of energy reserves in the light plant would cause

more retention of the compound in the tissue, but it should also enhance the capacity of the basipetal transport system. Acropetal movement, on the other hand, is less dependent on metabolic energy. Thus the acropetal transport is reduced in the tissues from the light plants with a higher level of energy reserves.

The movement of sucrose in Tartary buckwheat petiole segments was non-polar. This is not surprising, because sucrose is not a growth regulator, and polar transport seems to be restricted to auxins which show a typical growth-stimulating action (e.g., 37). Comparing the transport of dicamba to IAA, it was found that the polarity of transport was consistently greater for IAA than for dicamba. Moreover, IAA was transported at a velocity of 8 mm/hr while dicamba at only 0.8 mm/hr. The rate of uptake of IAA by the tissue segments was much greater than that of dicamba, but more dicamba than IAA was transported to the receivers. Activity of IAA in the basipetal receivers stopped increasing after a 12-hour transport, while activity of dicamba was still increasing in the receivers even at the end of the 48-hour experiment period. This prolonged transport of dicamba and the higher flux of its transport during the later stage may be explained by the stability of the compound in the tissue. The lower amount of IAA in the receivers may be a result of its high degree of immobilization and decomposition in the plant segments. Similarly, the slowdown or cessation of IAA transport into basipetal receivers after 12 hours, while its uptake was still continuing, may be accounted for by the possibility

that the radioactivity of IAA taken up by the tissue at the later stage was all immobilized in the tissue or lost to the air after degradation. But why it was not so or to a lesser extent in the acropetal transport system is hard to explain.

Transport of dicamba in the tissue segments was much slower than its translocation in intact plants. This is probably due to the involvement of different paths and mechanisms for these transports. The transport in tissue sections is basipetally polar whereas in intact plants it appears to follow the way of assimilate distribution in the phloem or water flow in the xylem. Transport in segments is believed to take place in all living tissues. Polarity of transport of dicamba may also exist in intact plants, but it is hard to distinguish from the gross transport by the present studies.

Dicamba metabolism in Tartary buckwheat plants

Dicamba was metabolized in Tartary buckwheat plants, but the rate of metabolism was so slow that only 13 per cent of the recovered activity was in forms other than dicamba 40 days after application of a sub-lethal dose to a single leaf.

There was evidence that the derivatives of dicamba in the plant extracts were conjugates of one metabolite. The conjugation was as glucoside(s). After hydrolysis, the metabolite was identified chromatographically as 5-OH dicamba, a major metabolic product of dicamba found in wheat and bluegrass (7) and in excised root tissues of barley and corn (86). The compound 3,6-dichlorosalicylic acid

(DCSA) which has been reported as a minor metabolite in the above mentioned plants (7, 86) was not detected in Tartary buckwheat plants treated with dicamba. However, DCSA was found to be a degradation product of dicamba during acid or alkaline hydrolysis. Since both 5-OH dicamba and its conjugated product are non-phytotoxic, the conversion of dicamba to the metabolite can be considered a detoxication process in the plants. However, such a slow rate of metabolism apparently is not enough to protect the plants from the herbicide.

The rate of dicamba metabolism in the treated leaf of Tartary buckwheat plants, and also in treated leaves of barley, wheat and wild mustard, was two to three times as fast as in other parts of the plants. This phenomenon also has been reported for Canada thistle (10). Since detached mature leaves metabolized dicamba at about the same rate as did the treated leaf of intact plants, it was thought that mature leaves might have a higher capacity to degrade this compound than young leaves or stem tissues. This possibility was ruled out, however, by the finding that the proportion of metabolite in extracts from old leaves was the same as in extracts from young leaves. There was no evidence of degradation of dicamba on the leaf surface of the treated leaf, which could complicate the distribution pattern, and, furthermore, the metabolite of dicamba isolated from the plant extract was also translocated in the plants. Differential rates of decarboxylation in different parts of the plants also cannot account for the differences between old and young

leaves, because of the extremely low over-all rate of decarboxylation. It is possible that dicamba was compartmented in the plant tissues in such a way that more of it was exposed to enzyme degradation in the treated leaf than in other parts of the plants. Compartmentation of organic acids has been found in many plant tissues (e.g., 69).

Dicamba translocation, metabolism and selectivity

Selective uptake, translocation and metabolism have been shown to be responsible for selective action of a number of herbicides (e.g., 3, 14, 42, 93, 94, 98, 99). Broadhurst et al. (7) reported a very rapid metabolism of dicamba in two resistant species, wheat and bluegrass. In the susceptible species, nutsedge, on the contrary, no metabolism of this herbicide was detected by Magalhaes et al. (70) or by Ray and Wilcox (87). In Canada thistle (10), which is also a susceptible species, dicamba was translocated rapidly in the plant and accumulated in the meristematic tissues, but metabolism of the herbicide occurred only very slowly. Quimby and Nalewaja (85) found that, after foliar application, the highest concentration of dicamba accumulated in the meristems of wild buckwheat, a susceptible species, and in the tips of treated leaves of wheat. Dicamba was conjugated or metabolized in wheat main culms much quicker and to a greater extent than in wild buckwheat. All these results suggest a relationship between the resistance of the plants and their ability to translocate and degrade this herbicide. Under the conditions of the present study, the susceptibility of four test species to dicamba was in the order of: Tartary buckwheat > wild mustard >> barley >

wheat. The rates of absorption and translocation of dicamba following foliar application in the four species ranked in the same order whereas a ranking according to the rate of metabolism of dicamba followed the reverse order. Activity from dicamba-C^{14} was strongly accumulated in the meristematic tissues of Tartary buckwheat and wild mustard plants, while in the two cereal plants it tended to accumulate at the tips of the treated leaves.

It is concluded that the selective uptake, translocation and metabolism of dicamba by wheat, barley, wild mustard and Tartary buckwheat all play an important role in their susceptibility or resistance to this herbicide.

LITERATURE CITED

1. Aronoff, S. 1955. Translocation from soybean leaves. II. Plant Physiol. 30: 184-185.

2. Baker, R.S. and G.F. Warren. 1962. Selective herbicidal action of amiben on cucumber and squash. Weeds 10: 219-224.

3. Biddulph, O. and R. Cory. 1965. Translocation of C^{14} metabolites in the phloem of bean plant. Plant Physiol. 40: 119-129.

4. Black, M.K. and D.J. Osborne. 1965. Polarity of transport of benzyladenine, adenine and indole-3-acetic acid in petiole segments of Phaseolus vulgaris. Plant Physiol. 40: 676-680.

5. Bonnett, H.T., Jr., and J.G. Torrey. 1965. Auxin transport in Convolvulus roots cultured in vitro. Plant Physiol. 40: 813-818.

6. Brian, R.C. 1964. The metabolism of herbicides. Weed Res. 4: 105-117.

7. Broadhurst, N.A., M.L. Montgomery and V.H. Freed. 1966. Metabolism of 2-methoxy-3,6-dichlorobenzoic acid (dicamba) by wheat and bluegrass plants. J. Agr. Chem. 14: 585-588.

8. Burnside, O.C. and T.L. Lavy. 1966. Dissipation of dicamba. Weeds 14: 211-214.

9. Cain, P.S. 1966. An investigation of the herbicidal activity of 2-methoxy-3,6-dichlorobenzoic acid. Ph.D. Thesis, Univ. Illinois, 1966. Diss. Abstr. 27: 3757-3758-B.

10. Chang, F.Y. and W.H. Vanden Born. 1968. Translocation of dicamba in Canada thistle. Weed Science 16: 176-181.

11. Chow, P.N., O.C. Burnside, T.L. Lavy, and H.W. Knoche. 1966. Absorption, translocation, and metabolism of silvex in prickly pear. Weeds 14: 38-41.

12. Christie, A.E. and A.C. Leopold. 1965. On the manner of triiodobenzoic acid inhibition of auxin transport. Plant and Cell Physiol. 6: 337-345.

13. Christie, A.E. and A.C. Leopold. 1965. Entry and exit of indoleacetic acid in corn coleoptiles. Plant and Cell Physiol. 6: 453-465.

14. Colby, S.R. 1966. The mechanism of selectivity of amiben. Weeds 14: 197-201.

15. Cumming, B.G. 1959. The control of growth and development in red clover (Trifolium pratense L.). III. Endogenous diffusible auxin. Can. J. Bot. 37: 1049-1062.

16. Crafts, A.S. 1961. Translocation in Plants. Holt, Rinehart and Winston. New York, N.Y. 182 pp.

17. Crafts, A.S. and W.W. Robbins. 1962. Weed Control. 3rd ed. McGraw-Hill Book Co., Inc. 660 pp.

18. Crafts, A.S. and S. Yamaguchi. 1958. Comparative tests on the uptake and distribution of labeled herbicides by Zebrina pendula and Tradescantia fluminensis. Hilgardia 27: 421-454.

19. Crafts, A.S. and S. Yamaguchi. 1964. The Autoradiography of Plant Materials. Univ. of Calif. Agric. Publ., Manual 35. Berkeley, Calif. 143 pp.

20. De la Fuente, R.K. and A.C. Leopold. 1966. Kinetics of polar auxin transport. Plant Physiol. 41: 1481-1484.

21. Foy, C.L. and W. Hurtt. 1967. Further studies on root exudation of exogenous growth regulators in Phaseolus vulgaris. Abstr. 1967 Mtg. Weed Soc. Am., p. 40.

22. Freed, V.H. and M.L. Montgomery. 1963. The metabolism of herbicides by plants and soils. Residue Reviews 3: 1-18.

23. Friesen, H.A. 1962. Green smartweed, Polygonum scabrum, control in Eagle oats with various herbicides. Res. Report Nat'l. Weed committee (Western Section) 9: 98-99.

24. Friesen, H.A. and D.A. Dew. 1966. The influence of temperature and soil moisture on the phytotoxicity of dicamba, picloram, bromoxynil and 2,4-D ester. Can. J. Plant Sci. 46: 653-660.

25. Gentner, W.A. 1964. Herbicidal activity of vapors of 4-amino-3,5,6-trichloropicolinic acid. Weeds 12: 239-240.

26. Gillespie, B. and K.V. Thimann. 1963. Transport and distribution of auxin during tropistic response. I. The lateral migration of auxin in geotropism. Plant Physiol. 38: 214-225.

27. Goldsmith, M.H.M. 1966. Movement of indoleacetic acid in coleoptiles of Avena sativa L. II. Suspension of polarity by total inhibition of the basipetal transport. Plant Physiol. 41: 15-27.

28. Goldsmith, M.H.M. 1966. Maintenance of polarity of auxin movement by basipetal transport. Plant Physiol. 41: 749-754.

29. Goldsmith, M.H.M. 1967. Movement of pulses of labeled auxin in corn coleoptiles. Plant Physiol. 42: 258-263.

30. Goldsmith, M.H.M. 1967. Separation of transit of auxin from uptake: Average velocity and reversible inhibition by anaerobic conditions. Science 156: 661-663.

31. Goldsmith, M.H.M. 1967. Comparison of aerobic and anaerobic movement of 3-indoleacetic acid in coleoptiles of oats and corn. In: Biochemistry and Physiology of Plant Growth Substances. pp. 1037-1050. Runge Press, Ottawa, 1968. (Proc. 6th Intern. Conf. Plant Growth Subst., Ottawa, 1967)

32. Goldsmith, M.H.M. 1968. The transport of auxin. Ann. Rev. Plant Physiol. 19: 347-360.

33. Goldsmith, M.H.M. and K.V. Thimann. 1962. Some characteristics of movement of indoleacetic acid in coleoptiles of Avena. I. Uptake, destruction, immobilization, and distribution of IAA during basipetal translocation. Plant Physiol. 37: 492-505.

34. Gorter, Chr. J. and H. Veen. 1966. Auxin transport in explants of Coleus. Plant Physiol. 41: 83-86.

35. Harel, S. 1969. Modification of 2,4-dichlorophenoxyacetic acid movement in bean petioles by light. Plant Physiol. 44: 615-617.

36. Hay. J.R. 1956. The effect of 2,4-dichlorophenoxyacetic acid and 2,3,5-triiodobenzoic acid on the transport of indoleacetic acid. Plant Physiol. 31: 118-120.

37. Hertel, R., M.L. Evans, A.C. Leopold and H.M. Sell. 1969. The specificity of the auxin transport system. Planta 85: 238-240.

38. Hertel, R. and R. Flory. 1968. Auxin movement in corn coleoptiles. Planta 82: 123-144.

39. Hertel, R. and A.C. Leopold. 1963. Versuche zur Analyse der Auxintransports in der Koleoptlie von Zea mays L. Planta 59: 535-562.

40. Hilton, J.L., L.L. Jansen and H.M. Hull. 1963. Mechanism of herbicide action. Ann. Rev. Plant Physiol. 14: 353-384.

41. Hodgson, G.L. 1964. Sodium-3,6-dichloro-2-methoxybenzoate for the control of bracken (Pteridium aquilinum L. Kuhn): results of preliminary trials. Weed Res. 4: 167-168.

42. Hogue, E.J. and G.F. Warren. 1968. Selectivity of linuron on tomato and parsnip. Weed Sci. 16: 51-54.

43. Horton, R.F. and R.A. Fletcher. 1968. Transport of the auxin, picloram, through petioles of bean and Coleus and stem sections of pea. Plant Physiol. 43: 2045-2048.

44. Hull, R.J. and M.R. Weisenberg. 1967. Translocation and metabolism of dicamba in Sorghum halepense and Phaseolus vulgaris. Plant Physiol. 42: S40.

45. Hurtt, W. and C.L. Foy. 1965. Some factors influencing the excretion of foliarly-applied dicamba and picloram from roots of Black Valentine beans. Plant Physiol. Supp. 40: 48.

46. Jacobs, W.P. 1950. Auxin transport in the hypocotyl of Phaseolus vulgaris L. Am. J. Bot. 37: 248-254.

47. Jacobs, W.P. 1952. The role of auxin in differentiation of xylem around a wound. Am. J. Bot. 39: 301-309.

48. Jacobs, W.P. 1954. Acropetal transport and xylem regeneration, a quantitative study. Am. Naturalist 88: 327-337.

49. Jacobs, W.P. 1961. The polar movement of auxin in the shoots of higher plants: Its occurrence and physiological significance. In: Plant Growth Regulators. pp. 397-409. Iowa State Univ. Press, 1961. (proc. 4th Intern. Conf. Plant Growth Regulation, New York, 1959).

50. Jacobs, W.P. 1968. Hormonal regulation of leaf abscission. Plant Physiol. 43: 1480-1495.

51. Jones, H., R.V. Martin and H.K. Porter. 1959. Translocation of ^{14}carbon in tobacco following assimilation of ^{14}carbon dioxide by a single leaf. Ann. Botany 23: 493-508.

52. Keys, C.H. 1962. Comparison of Banvel compounds for control of wild buckwheat, Polygonum convulvulus. Res. Report, Nat'l Weed Committee (Western Section) 9: 80-81.

53. Keitt, G.W., Jr. and R.A. Baker. 1967. Acropetal movement of auxin: dependence on temperature. Science 156: 1380-1381.

54. Keitt, G.W., Jr. and F. Skoog. 1957. Effect of some substituted benzoic acids and related compounds on the distribution of callus growth in tobacco stem explants. Plant Physiol. 34: 117-122.

55. Kirk, S.C. and W.P. Jacobs. 1968. Polar movement of indole-3-acetic acid-^{14}C in roots of Lens and Phaseolus. Plant Physiol. 43: 675-682.

56. Klämbt, H.D. 1962. Conversion in plants of benzoic acid to salicylic acid and its β-glucoside. Nature 196: 491.

57. Leonard, O.A. 1963. Translocation of herbicides in woody plants. Proc. Soc. Amer. Foresters. Boston, Mass. pp. 99-103.

58. Leonard, O.A., L.A. Lider, and R.K. Glenn. 1966. Absorption and translocation of herbicides by Thompson seedless (Sultanina) grape, Vitis vinifera L. Weed Res. 6: 37-49.

59. Leopold, A.C. 1961. The transport of auxin. In: Encyclopedia of Plant Physiology. W. Ruhland, Ed. Springer-Verlag, Berlin. 14: 671-682.

60. Leopold, A.C. 1963. The polarity of auxin transport. In: Meristems and Differentiation. Brookhaven Symp. Biol. 16: 218-234.

61. Leopold, A.C. and R.K. de la Fuente. 1967. The polarity of auxin transport. Ann. New York Acad. Sci. 144: 94-101.

62. Leopold, A.C. and S.F. Guernsey. 1953. Auxin polarity in the Coleus plant. Bot. Gaz. 115: 147-54.

63. Leopold, A.C. and O.F. Hall. 1966. Mathematical model of polar auxin transport. Plant Physiol. 41: 1476-1480

64. Leopold, A.C. and S.L. Lam. 1961. Polar transport of three auxins. In: Plant Growth Regulators. pp. 411-418. (4th Intern. Conf. Plant Growth Regulation, New York, 1959). Iowa State Univ. Press.

65. Leopold, A.C. and S.L. Lam. 1962. The auxin transport gradient. Physiol. Plant. 15: 631-638.

66. Linder, P.J., J.C. Craig, Jr., F.E. Cooper and J.W. Mitchell. 1958. Translocation of growth regulators: Movement of 2,3,6-trichlorobenzoic acid from one plant to another through their root system. J. Agr. Food Chem. 6: 356-357.

67. Linder, P.J., J.C. Craig, Jr. and T.R. Walton. 1957. Movement of C^{14}-tagged alpha-methoxyphenylacetic acid out of roots. Plant Physiol. 32: 572-575.

68. Linder, P.J., J.W. Mitchell, and G.D. Freeman. 1964. Persistence and translocation of exogenous regulating compounds that exude from roots. J. Agr. Food Chem. 12: 437-438.

69. Maclennan, D.H., H. Beevers, and J.L. Harley. 1963. Compartmentation of acids in plant tissues. Biochem. J. 89: 316-327.

70. Magalhaes, A.C., F.M. Ashton, and C.L. Foy. 1968. Translocation and fate of dicamba in purple nutsedge. Weed Science 16: 240-245.

71. McCready, C.C. 1963. Movement of growth regulators in plants. I. Polar transport of 2,4-dichlorophenoxyacetic acid in segments from the petioles of Phaseolus vulgaris. The New Phytologist 62: 3-18.

72. McCready, C.C. 1966. Translocation of growth regulators. Ann. Rev. Plant Physiol. 17: 283-294.

73. McCready, C.C. 1968. The polarity of auxin movement in segments excised from petioles of Phaseolus vulgaris L. In: Biochemistry and Physiology of Plant Growth Substances. pp. 1005-1023. Runge Press, Ottawa, 1968. (Proc. 6th Intern. Conf. Plant Growth Subs. 1967, Ottawa)

74. McCready, C.C. and W.P. Jacobs. 1963. Movement of growth regulators in plants. II. Polar transport of radioactivity from indoleacetic acid-^{14}C in petioles of Phaseolus vulgaris. New Phytologist 62: 19-34.

75. Miller, L.P. 1937. Decomposition of ethylene chlorohydrin in potato tubers. Contrib. Boyce. Thompson Inst. 8: 479-492.

76. Minarik, C.E., D. Ready, A.G. Norman, H.E. Thompson and J. Fred Owings, Jr. 1951. New growth regulating compounds. II. Substituted benzoic acids. Bot. Gaz. 113: 135-142.

77. Mitchell, J.W., P.J. Linder, and M.B. Robinson. 1961. Mechanism of root exudation of alpha-methoxyphenylacetic acid in the bean plant. Bot. Gaz. 123: 134-137.

78. Naqvi, S.M. and S.A. Gordon. 1965. Auxin transport in flowering and vegetative shoots of Coleus blumei Benth. Plant Physiol. 40: 116-118.

79. Niedergang-Kamien, E. and A.C. Leopold. 1957. Inhibitors of polar auxin transport. Physiol. Plant. 10: 29-38.

80. Oserkowsky, J. 1942. Polar and apolar transport of auxin in woody stems. Am. J. Bot. 29: 858-866.

81. Pallas, J.E.,Jr. and A.S. Crafts. 1957. Critical preparation of plant material for autoradiography. Science 125: 192-193.

82. Pilet, P.E. 1964. Auxin transport in Lens roots. Nature 204: 561-562.

83. Pilet, P.E. 1965. Polar transport of radioactivity from ^{14}C-labeled-β-indolylacetic acid in stems of Lens culinaris. Physiol. Plant. 18: 687-702.

84. Pilet, P.E. 1967. In vitro and in vivo auxin and cytokinin translocation. In: Biochemistry and Physiology of Plant Growth Substances. pp. 993-1004. The Runge Press, Ottawa, 1968. (Proc. 6th Intern. Conf. Plant Growth Substances, Ottawa, 1967)

85. Quimby, P.C.,Jr. and J.D. Nalewaja. 1968. Translocation and fate of dicamba-C^{14} in wheat and wild buckwheat. Abstr. 1968. Mtg. Weed Sci. Soc. Am. p. 36.

86. Ray, B.R. 1967. Chemical control of nutsedge (Cyperus rotundus L.) and the metabolism of 3,6-dichloro-o-anisic acid (dicamba). Ph. D. Thesis, Univ. Florida, 1967. Diss. Abstr. 29: 20-B.

87. Ray, B.R. and M. Wilcox. 1969. Translocation of the herbicide dicamba in purple nutsedge, Cyperus rotundus. Physiol. Plant. 22: 503-505.

88. Rayle, D.L., R. Ouitrakul and R. Hertel. 1969. Effect of auxins on the auxin transport system in coleoptiles. Planta 87: 49-53.

89. Sastry, K.S.K. and R.M. Muir. 1965. Transport of indoleacetic acid in pedicels of tomato and its relation to fruit growth. Bot. Gaz. 126: 13-19.

90. Scott, T.K. and W.P. Jacobs. 1963. Auxin in Coleus stems: limitation of transport at higher concentrations. Science 139: 589-590.

91. Scott, T.K. and M.B. Wilkins. 1968. Auxin transport in roots. II. Polar flux of IAA in Zea roots. Planta 83: 323-334.

92. Shaw, W.L., J.L. Hilton, D.E. Moreland, and L.L. Jansen. 1960. Herbicides in plants. In: The Nature and Fate of Chemicals Applied to Soils, Plants, and Animals. U.S. Agr. Res. Serv. ARS 20-9, pp. 119-33.

93. Shimabukuro, R.H. 1967. Atrazine metabolism and herbicidal selectivity. Plant Physiol. 42: 1269-1276.

94. Sikka, H.C. and D.E. Davis. 1968. Absorption, translocation, and metabolism of prometryne in cotton and soybean. Weed Sci. 16: 474-477.

95. Skoog, F. 1937. A deseeded Avena test method for small amounts of auxin and auxin precursors. J. Gen. Physiol. 20: 311-334.

96. Skoog, F. 1938. Absorption and translocation of auxin. Am. J. Bot. 25: 361-372.

97. Stahl, E. 1965. Thin-layer Chromatography; a Laboratory Handbook. Berlin, Springer-Verlag. 553 pp.

98. Stephenson, G.R. and S.K. Ries. 1967. The movement and metabolism of pyrazon in tolerant and susceptible species. Weed Res. 7: 51-60.

99. Stoller, E.W. 1969. The kinetics of amiben absorption and metabolism as related to species sensitivity. Plant Physiol. 44: 854-860.

100. Swanson, C.R. 1965. Metabolic fate of herbicides in plants. U.S. Agr. Res. Serv. ARS 34-66, 36 pp.

101. Thaine, R. 1964. Long distance transport in plants. World Rev. Pest Control 3: 175-186.

102. Thimann, K.V. and I.F. Wardlaw. 1963. The effect of light on the uptake and transport of indoleacetic acid in the green stem of the pea. Physiol. Plant. 16: 368-377.

103. Vanden Born, W.H. 1962. Comparison of the effect of Banvel D and T on Tartary buckwheat growing in wheat, when applied at different stages of growth. Abstr. Research Rept. Nat'l Weed Comm. (Western Section) 9: 89-90.

104. Van der Weij, H.G. 1932. Der Mechanismus des Wuchsstofftransportes. Rec. Trav. Bot. Neerl. 29: 379-496.

105. Van der Weij, H.G. 1934. Der Mechanismus des Wuchsstofftransportes II. Rec. Trav. Bot. Neerl. 31: 810-857.

106. Veen, H. 1967. On the relation between auxin transport and auxin metabolism in explants of Coleus. Planta 73: 281-295.

107. Wang, C.H. and D.L. Willis. 1965. Radiotracer Methodology in Biological Science. Prentice-Hull, Inc., New Jersey. 382 pp.

108. Wareing, P.F., C.E.A. Hanney and J. Digby. 1964. The role of endogenous hormones in cambial activity and xylem differentiation. In: The Formation of Wood in Forest Trees. pp. 323-344. Academic Press, New York.

109. Went, F.W. 1928. Wuchsstoff und Wachstum. Rec. Trav. Bot. Neerl. 25: 1-116.

110. Went, F.W. and K.V. Thimann. 1937. Phytohormones. The Macmillan Co., New York. 294 pp.

111. Went, F.W. and R. White. 1939. Experiments on the transport of auxin. Bot. Gaz. 100: 465-484.

112. Whitehouse, R.L. and S. Zalik. 1967. Translocation of indole-3-acetic acid-1'-^{14}C and tryptophan-1-^{14}C in seedlings of Phaseolus coccineus L. and Zea mays L. Plant Physiol. 42: 1363-1372.

113. Wilkins, M.B. and M. Martin. 1967. Dependence of basipetal polar transport of auxin upon aerobic metabolism. Plant Physiol. 42: 831-839.

114. Wilkins, M.B. and T.K. Scott. 1968. Auxin transport in roots. Nature 219: 1388-1389.

115. Wilkins, M.B. and T.K. Scott. 1968. Auxin transport in roots. III. Dependence of the polar flux of IAA in Zea roots upon metabolism. Planta 83: 335-346.

116. Winter, A. 1967. The promotion of the immobilization of auxin in Avena coleoptiles by triiodobenzoic acid. Physiol. Plant. 20: 330-336.

117. Winter, A. 1967. 2,3,5-Triiodobenzoic acid and the transport of 3-indoleacetic acid. In: Biochemistry and Physiology of Plant Growth Substances. pp. 1063-1076. The Runge Press, Ottawa, 1968. (Proc. 6th Intern. Conf. Growth Subs., Ottawa, 1967)

118. Yamaguchi, S. and A.S. Crafts. 1958. Autoradiographic method for studying absorption and translocation of herbicides using C^{14}-labeled compounds. Hilgardia 28: 161-191.

119. Yeomans, L.M. and L.J. Audus. 1964. Auxin transport in roots - Vicia faba. Nature 204: 559-562.

120. Zaerr, J.B. and J.W. Mitchell. 1967. Polar transport related to mobilization of plant constituents. Plant Physiol. 42: 863-874.

121. Zick, W.H. and T.R. Castro. 1966. Dicamba -- dissipation in and on living plants. Proc. 8th Brit. Weed Control Conf. p. 265.

122. Zwar, J.A. and A.H.G.C. Rijven. 1956. Inhibition of transport of indole-3-acetic acid in the etiolated hypocotyl of Phaseolus vulgaris L. Austr. J. Biol. Sci. 9: 528-538.