Analysis of 2,4-D Transport

Shogo Yamaguchi
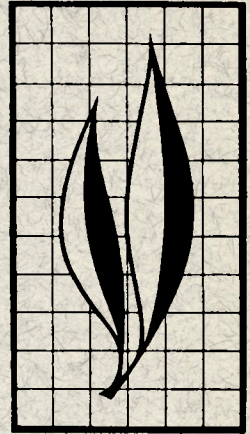
Dinitrophenol, anoxia, and starvation reduced the absorption and retention of 2,4-D in roots and leaves of bean, cotton, barley and soybean plants. Phloem translocation of the compound was like-wise reduced. Apoplast mobility of the compound was, however, increased.

In 6-hour pH series tests, absorption of 2,4-D by roots of intact bean plants was many times greater at pH 3 than at pH 11. At pH 3, roots had absorbed 25 per cent of its supplied 2,4-D applied in 10^{-6} M concentration in 100 ml of nutrient solution. One third (loosely adsorbed) of this absorbed amount was leached out by tap water in 2 hours; the residual two-thirds was considered metabolic absorption. The amount of this metabolic absorption seemed directly related to the degree of loose adsorption of 2,4-D.

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INTRODUCTION

IN STUDIES on translocation it has always been observed that 2,4-D distribution in any plant is more limited than that of amitrole or maleic hydrazide. Concentration in and around the translocating vascular tissues near the point of application is commonly high but steeply declines with increasing distance from the application point. Often translocation of 2,4-D is limited; it may not reach the buds or root tips. The gradient is usually steep under conditions attending a slow rate of growth (Crafts and Yamaguchi, 1958).

In the field, it is recognized that the period of successful control of field bindweed with 2,4-D is very short, a few weeks or less, in spite of a long period of growth (Crafts, 1956). This short time of concomitant maximum downward translocation corresponds with the interval of early flowering, but not the spring flush of vegetative growth. In an autoradiographic test with radiolabeled 2,4-D, amitrole, and maleic hydrazide applied to zebrina plants, growing very slowly because of low nutrition, the extent of 2,4-D translocation was no more than one internode while that of the other two compounds was many internodes (Crafts and Yamaguchi, 1958). With the more mobile compounds such as amitrole or maleic hydrazide, rate, distance or quantity of phloem translocation may correlate directly with the rate of growth or storage. 2,4-D however has proven an exception; a pro-nounced longitudinal translocation in zebrina coincided only with a very high rate of growth.

The view that active absorption by the vascular and cortical parenchyma depletes the 2,4-D being carried by the sieve tubes (Crafts and Yamaguchi, 1958), agrees with the frequent observation that 2,4-D applied to the culture solution in concentrations of 10^{-6} M or less does not move upward into the shoot of the plant, except in extremely small concentrations. It is retained in the root. As long as 2,4-D is absorbed by the symplast and excluded from the apoplast, there can be no 2,4-D in the transpiration stream. Any translocatory activity of the phloem would presumably carry root-applied 2,4-D toward the root tips (where growth is occurring).

Absorption, retention and the release of 2,4-D in leaves and roots have been investigated in several ways including darkening of the plant, use of metabolic poisons, pretreating with 2,4-D in a test for translocation of amitrole, and extraction and chromatography.

MATERIALS AND METHODS

Materials used include *Hordeum vulgare* Linn. cv. White California Mariout, *Gossypium hirsutum* Linn. cv. Acala, *Glycine Max* cv. Harosoy, and *Phaseolus vulgaris* Linn. cv. Red Kidney. The plants were germinated in sand, transplanted as soon as possible, with the utmost care to avoid plant in-

jury, to half-strength Hoagland's nu-
trient solution contained in pint jars
wrapped with aluminum foil. Bean
plants were used at the primary leaf
stage; barley plants at the 4-leaf stage;
soybean plants at 3-leaf stage; cotton
plants at 2-, 4-, and 6-leaf stages. For
any one experiment, plants were se-
lected for uniformity of size and growth
stage.

Dinitrophenol (DNP) was pipetted
into the culture solution before, at, or
during the treatment time. It readily
penetrated the roots and was distrib-
uted throughout the barley and soybean
plants, as evidenced by the appearing
of yellow color of DNP in the plant. In
cotton plants which wilted during the
first day but recovered the second, DNP
was taken up to a lesser extent as evi-
denced by the lesser amount of the
yellow color of DNP showing in the
plant. With barley and soybean plants,
10^{-4} M DNP was used; 10^{-5} M was in-
effective and 10^{-3} M lethal in a few days.
With larger cotton plants 10^{-4} M was
ineffective, and 10^{-3} was used. Plants
did not die from treatment with DNP
at 10^{-4} M, but usually exhibited some
degree of flaccidity of the roots and
some wilting of the leaves. The concen-
trations of DNP used reduced cellular
retention of 2,4-D and permitted its up-
ward transport.

Other metabolic inhibitors were
tested for reduction of cellular reten-
tion of 2,4-D in the roots. They were
iodoacetamide, sodium fluoride, sodium
arsenate, sodium arsenite and sodium
azide. Fluoride was ineffective on bean
plants even at 10^{-2} M, but it is an in-
hibitor of catalase and enolase, two en-
zymes not directly involved in respira-
tion. Arsenate and arsenite were tested
on cotton plants and found to be effec-
tive at 10^{-4} M; they were used, although
they caused some wilting. Azide at 10^{-4}
M not only reduced cellular retention,
but also permitted some upward move-
ment of 2,4-D, in bean plants.

To minimize reactions within the
plant-2,4-D system, such as complexing

and decarboxylation of the 2,4-D (Ja-
worski et al., 1955, Luckwill and Lloyd-
Jones, 1960), and bending and alter-
ation of growth pattern on the part of
the plants, 2,4-D* (asterisk designates
radiolabeled 2,4-D) of high specific ac-
tivity was used in combination with
short-treatment time. The amount of
culture solution was either 100 or 50 ml
at the start of the treatment time. The
concentration of 2,4-D* was 10^{-6} M in
all cases of root treatment. At this con-
centration root growth was somewhat
modified, but tissue remained turgid.
Care was taken to maintain the upper
third of the roots out of the treatment
solution to keep the solution well below
the stem and to aid root aeration.

Darkening of plants was accom-
plished by confinement in a pasteboard
box covered with black cloth and kept
under the greenhouse bench. During
treatment the plants were momentarily
exposed to light.

At harvest the roots were rinsed
under running tap water. Freeze-drying
and autoradiography were carried out
according to methods described in
Yamaguchi and Crafts, 1958. Film ex-
posure time was 10 days; all leaf treat-
ments and some root treatments had
2,4-D* of 1.24 mc/mmole; other root
treatments had 2,4-D* of 12.3 mc/mmole.
2,4-D* dosage was $\frac{1}{10}$ μmole in 10 μliters
of solution with $\frac{1}{10}$ per cent Tween 20
(polyoxyethylene sorbitan monolau-
rate) for spot application to the leaf;
for root treatment the same quantity
was added to 100 ml of culture solution.
When only 50 ml of culture solution
was used, $\frac{1}{20}$ μmole was used to keep
the concentration in the culture solution
the same, at 10^{-6} M.

The labeled 2,4-D used was labeled
in the carboxyl carbon in both cases. It
had a trace of labeled impurity which
moved at the front of the chromato-
graph solvent system of isopropyl alco-
hol-20 per cent ammonium acetate in
water-glacial acetic acid (79:20:1)
while 2,4-D* moved at rf of 78. In the
evaluation of a faint autoradiographic

image, where a fair amount of high specific activity 2,4-D was used in the culture solution, as in figure 5 left, there may be a question as to the presence of 2,4-D*. No point is made of such faint images presented; only sharp images are considered.

RESULTS

Dinitrophenol at 10^{-4} M, included along with unlabeled 2,4-D at 10^{-5} M in 100 ml of culture solution per soybean plant, permitted upward movement of 2,4-D, causing stem swelling and yellowing of the leaves after several days (figure 1, right). Compared with this, the other plant (figure 1, left) which had 2,4-D, but no DNP, was inhibited in growth, but there were no formative effects of 2,4-D in the stem or leaves. Boyd (private communication), however, has shown that 2,4-D*, applied in the culture solution to Red

Kidney bean plants without DNP, does move up if the concentration is 10^{-4} M or greater, causing drastic symptoms in the stem and leaves.

Preliminary 1- and 4-day tests with DNP and sodium azide and with low specific activity 2,4-D. Autoradiographs of a preliminary 4-day test with soybean plants, involving 10^{-4} M DNP and 10^{-6} M 2,4-D*, 1.24 mc/mmole, in 100 ml culture solution showed an effect of plant age; in the plant about a week older there was the usual retention of 2,4-D* in the roots, with only a trace up in the leaves (figure 2, left). In the younger plant the retention in the roots was very small and the image of 2,4-D* in the leaves was somewhat darker than in the older plant.

In a 4-day test on barley plants the same 2,4-D*, but two levels of DNP were used. At 10^{-4} M DNP in the nutrient solution, 2,4-D* absorption and
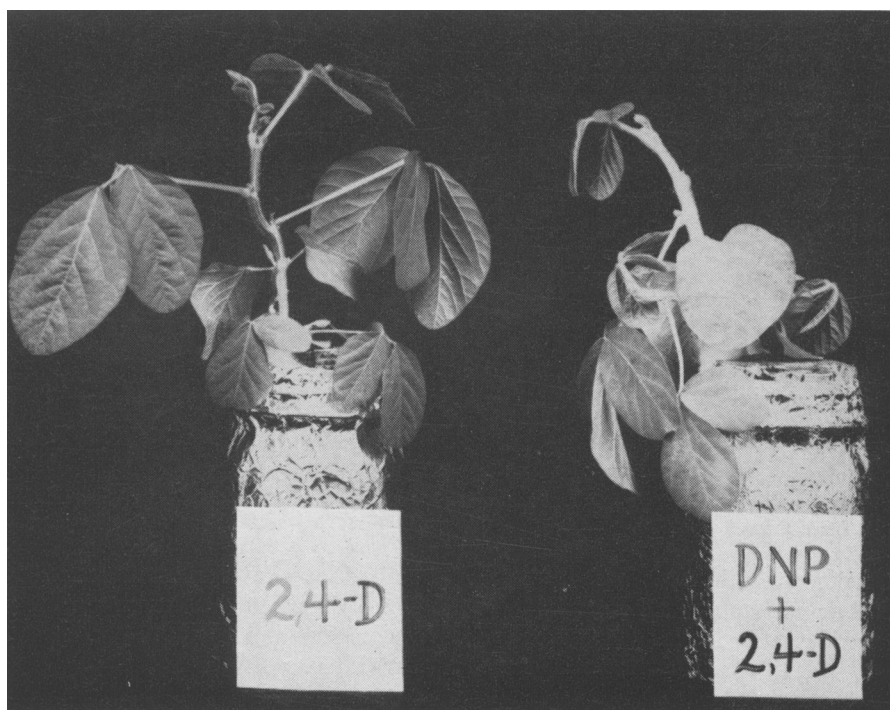

Fig. 1. Soybean plants showing the effect of 2,4-D and DNP. The plant at the right is exhibiting 2,4-D symptoms of stem-swelling and general yellowing. The plant at left, treated with same concentration of 2,4-D, 10^{-5} M, but without DNP, shows no such symptoms of 2,4-D.
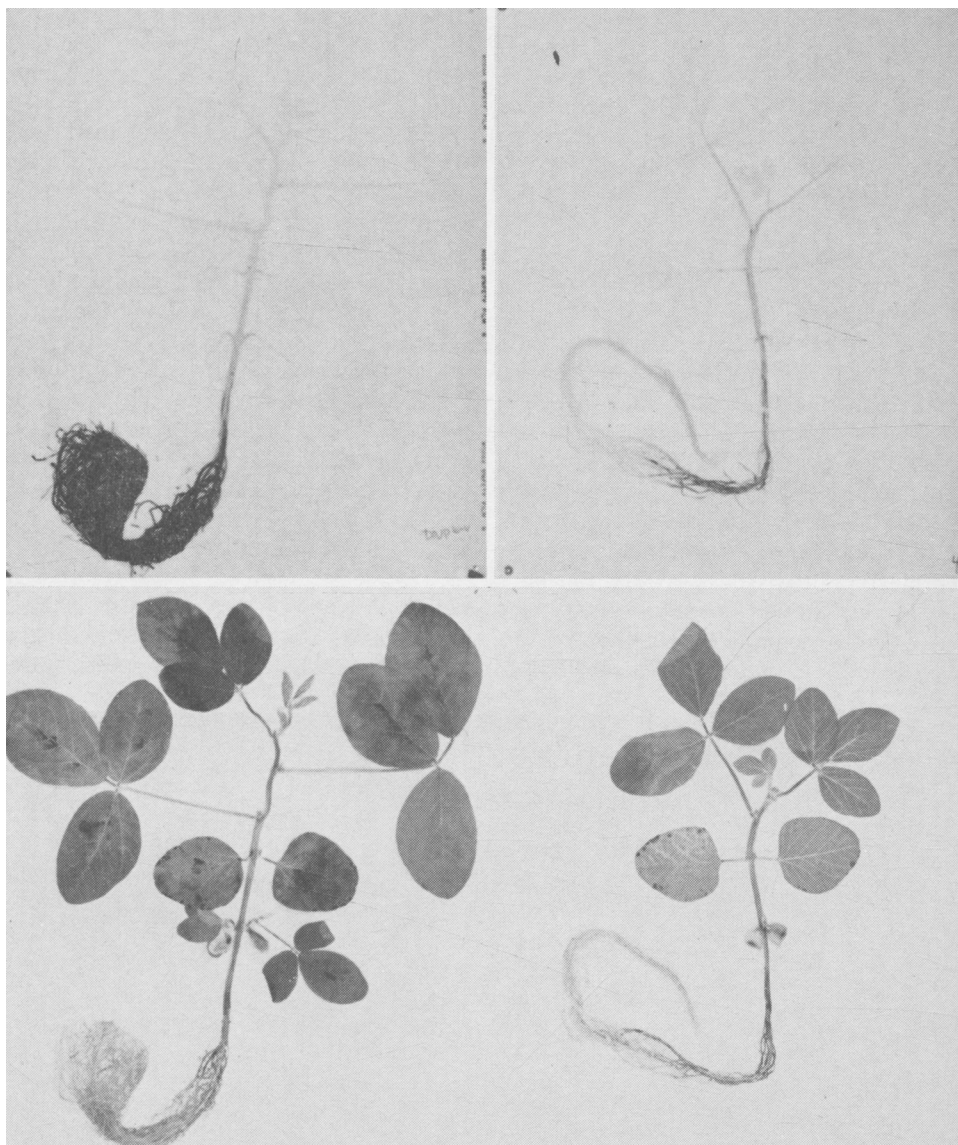
Fig. 2. Differences of upward movement of 2,4-D due to plant age difference in soybean. Plant at right was about 5 days younger. DNP was 10^{-4} M concentration; 2,4-D, 1.24 mc/mmole, was 10^{-6} M. Treatment 4 days.

retention in the roots was somewhat less than without the DNP; little 2,4-D* moved into the leaves (figure 3, left). At 10^{-3} M, DNP itself produced regions of necrosis in the leaves; a substantial amount of 2,4-D* was carried into the leaves, (figure 3, right); retention in the roots was nearly obliterated. At this toxic dosage of DNP all absorption of

2,4-D* appeared nonmetabolic; retention by the root was minimal, the plant was sickly and the roots had lost their turgidity.

Several other metabolic inhibitors were tested. Sodium fluoride, up to 10^{-2} M, in the culture solution for 4 days, did not seem to increase upward movement of 2,4-D in the stem or leaves—
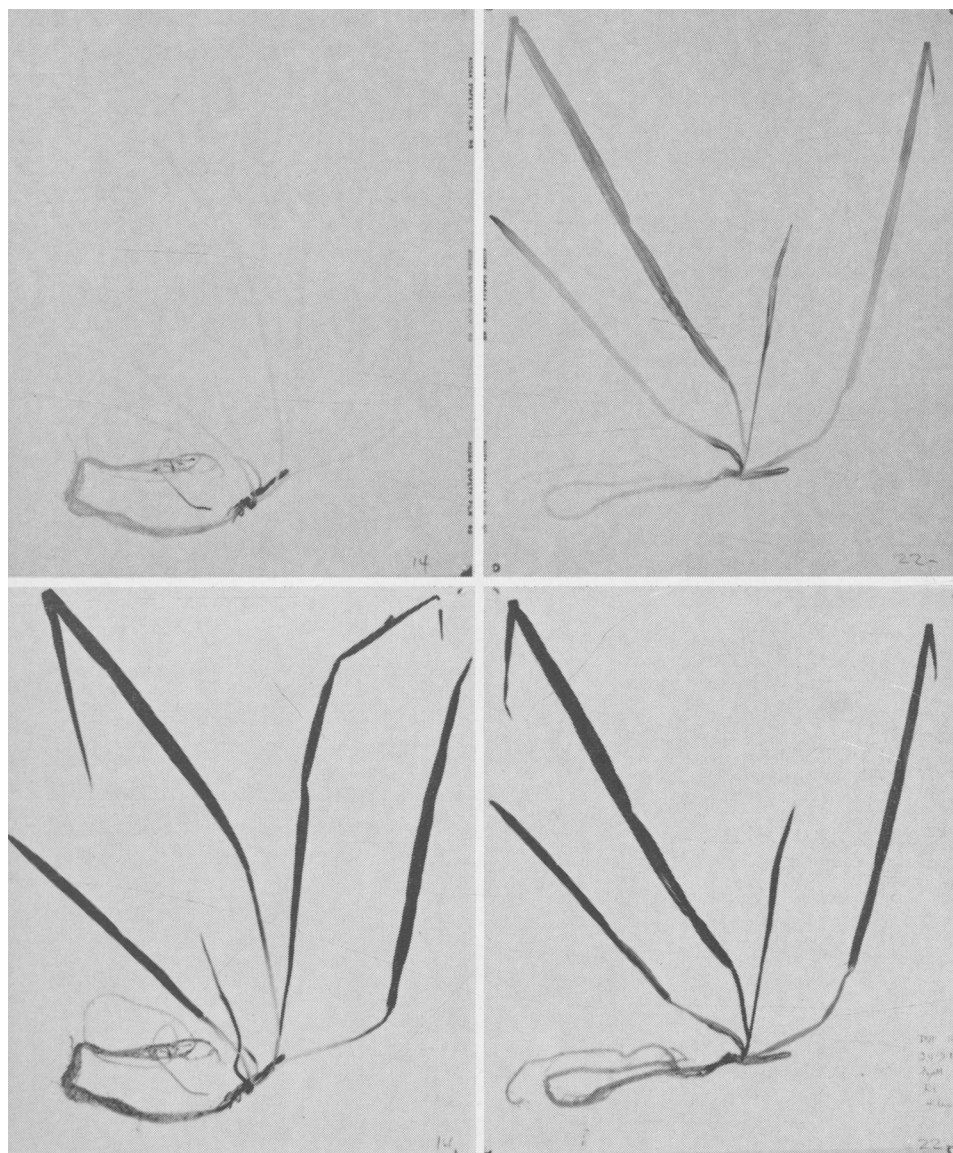
Fig. 3. Barley plants showing reduced root absorption and retention, with increased distribution in the leaves of 2,4-D* by DNP, 10^{-4} M (*left*), 10^{-3} M (*right*). 2,4-D, 1.24 mc/mmole, at 10^{-6} M in 100 ml half-strength Hoagland's solution. Treatment 4 days.

no symptoms developed. Sodium azide at 10^{-4} M, included simultaneously with 2,4-D* at 10^{-6} M in 100 ml culture solution inhibited absorption and retention of 2,4-D* in the roots and resulted in a higher concentration of 2,4-D* in the hypocotyl (figure 4, middle). Pretreatment with 10^{-3} M azide for one day very effectively inhibited 2,4-D* absorption and retention in the roots of Red Kidney bean and permitted upward movement of 2,4-D* (figure 4, right). This was a 1-day experiment with the lower specific activity 2,4-D*, 1.24 mc/mmole. 2,4-D* distribution in the cortical tissues of the stem and the time involved in the short distance of upward movement, indicated movement of 2,4-D* up-
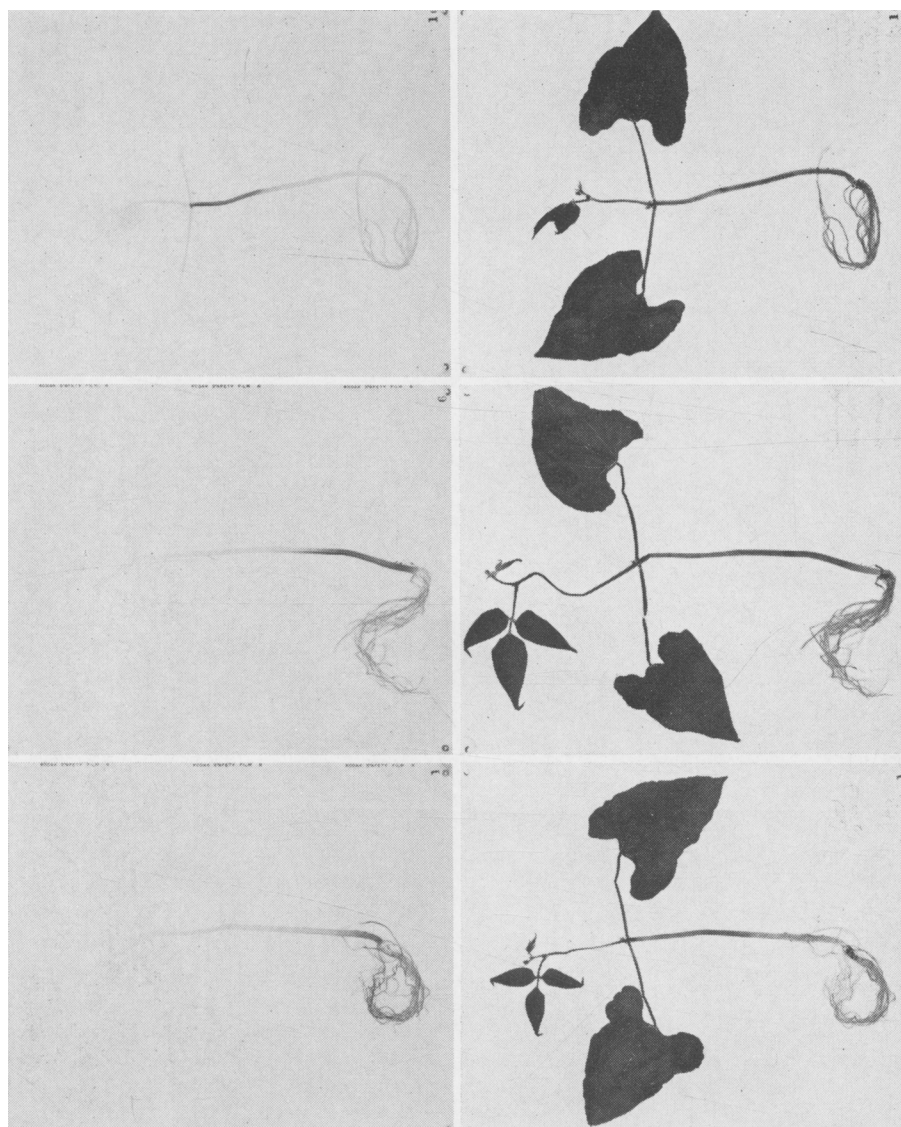
Fig. 4. Bean plants showing upward movement of 2,4-D* as a result of azide inclusion in the culture solution. *Left* shows effect of 2,4-D* alone; *center*, 2,4-D* and azide (10^{-4} M) applied simultaneously; *right*, azide (10^{-3} M) applied one day ahead of 2,4-D*. 2,4-D*, 1.24 mc/mmole, at 10^{-6} M; 1 day treatment.

ward through the cortical tissues rather than by xylem. The plants did not wilt, but the roots were not fully turgid.

Soybean. In soybean plants DNP at 10^{-4} M effectively inhibited absorption and retention of 2,4-D* in the roots and permitted its upward movement in younger plants, with three mature leaves or fewer. With older soybean plants, 10^{-3} M DNP was more effective, but caused the roots to be flaccid and eventually produced necrotic regions in the leaves. Therefore younger plants and 10^{-4} M DNP were chosen. Use of a high specific activity 2,4-D, 12.3 mc/mM, and 6-hour treatment produced
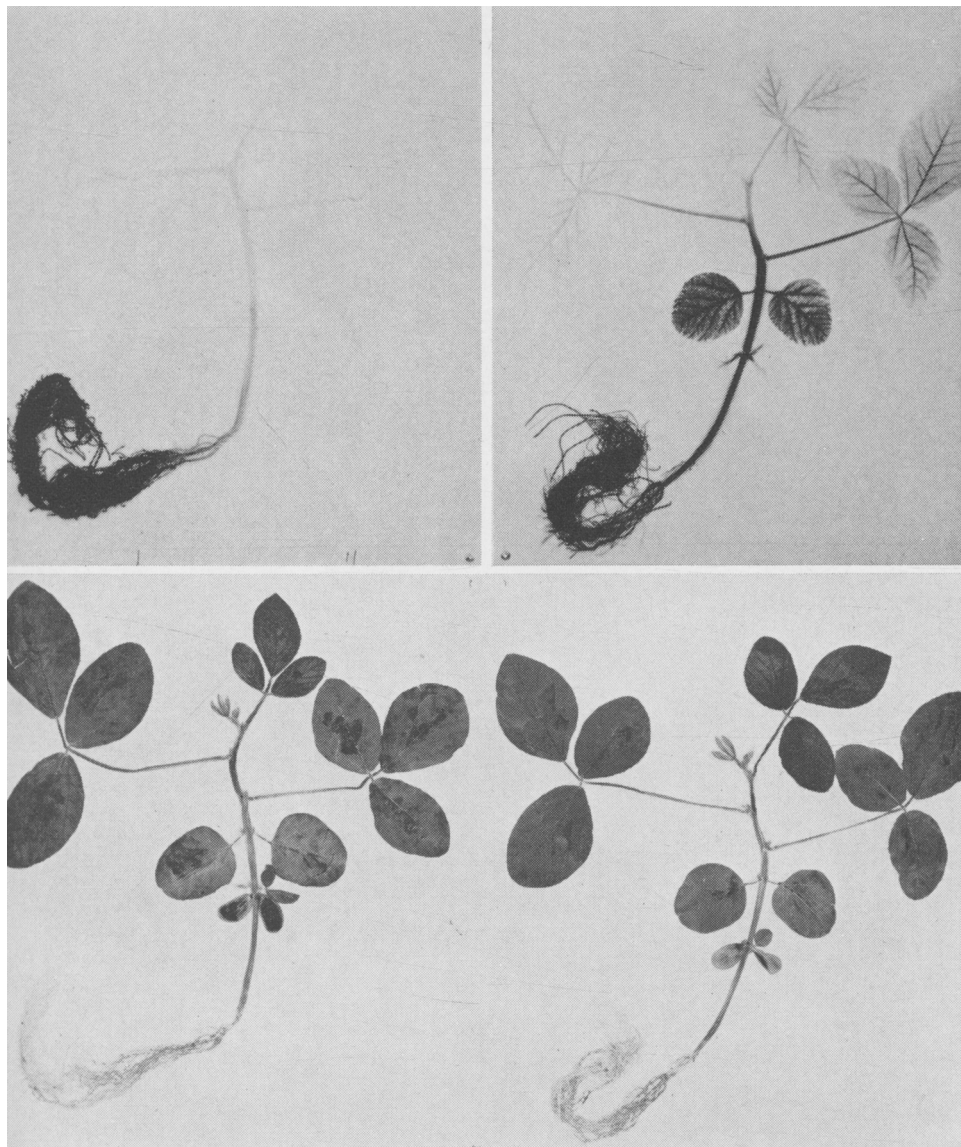

Fig. 5. Soybean plants showing upward distribution of 2,4-D* in a 6-hour treatment with 2,4-D*, 12.3 mc/mmole, at 10^{-6} M (*left*); and with admixture of DNP at 10^{-4}. The high specific activity 2,4-D permitted detection of a light image in the upper regions of the stem and the petiole of the control plant (left) which is not possible with the low specific activity 2,4-D.
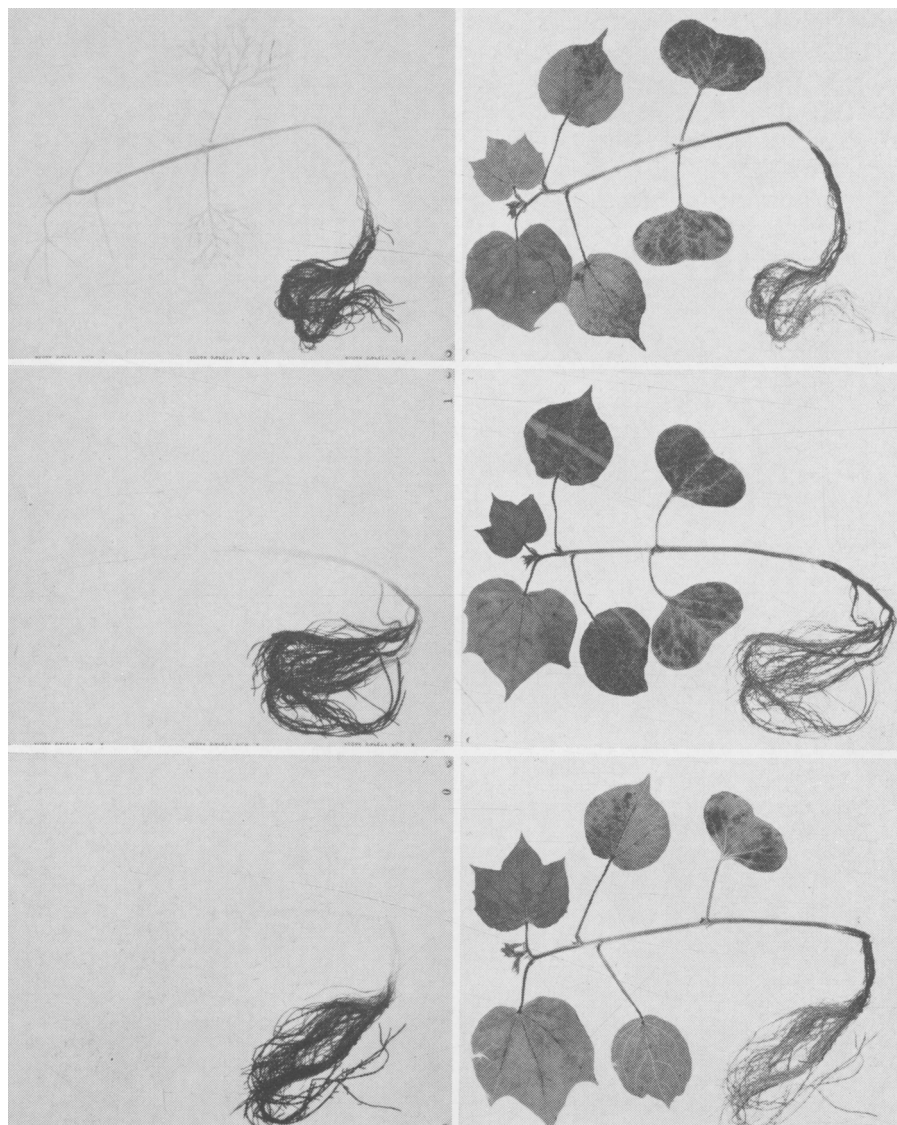
Fig. 6. Cotton, several weeks old, showing upward movement of 2,4-D* with DNP at 10^{-3} M. *Left*, 2,4-D* alone; *middle*, 2,4-D* and DNP at 10^{-4}; *right*, 2,4-D* and DNP at 10^{-3}. 2,4-D*, 12.3 mc/mmole, at 10^{-6} M per 50 ml culture solution; treatment 6 hours.

results before any observable injury to the plants. Film exposure time was kept the same. Figure 5, left, shows the distribution pattern of the high specific activity 2,4-D* at the end of the 6-hour experiment. The concentration of 2,4-D* was 10^{-6} M. At the right is the enhanced upward distribution of the 2,4-D* as a result of DNP inclusion at 10^{-4} M. The amount of 2,4-D* in the stem is 10 to 20 times greater than in the stem of the control, by image density comparisons; the amount in the leaf blades shows the image of the three leaves while there is no image at all in the leaf blades of the control.

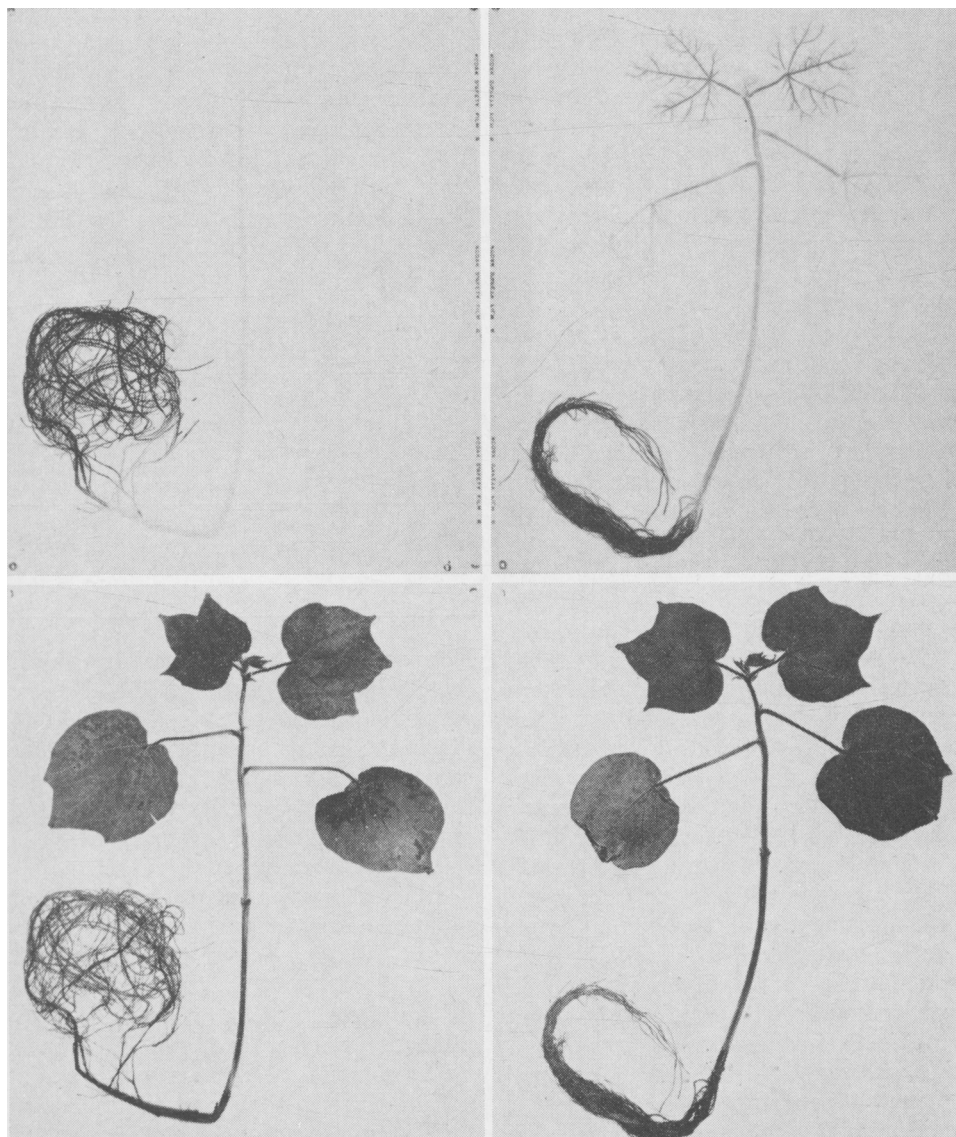**Large retention of 2,4-D by cotton roots.** Cotton plants about 10 inches

Fig. 7. Cotton plants showing the effect on upward movement of 2,4-D* caused by the application of the DNP 2 days ahead of the 2,4-D*. *Right,* with DNP; *left,* 2,4-D* alone. 2,4-D*, 12.3 mc/mmole, at 10^{-6} M per 50 ml culture solution, DNP at 10^{-4}. Treatment 6 hours.

high, treated with $\frac{1}{20}$ μmole of 2,4-D*, 12.3 mc/mmole, per 50 ml culture solution, and harvested after 6 hours showed no movement up from the roots (figure 6, left). Inclusion of DNP at 10^{-3} M permitted movement of some labeled 2,4-D all the way up the stem and into some of the mature leaves (figure 6, right); inclusion of DNP at 10^{-4} M was less effective (figure 6, middle). However, autoradiographs did not show reduced 2,4-D* retention in the roots. The cotton plants seemed not to absorb DNP as evidenced by absence of the yellow color of DNP in the leaves and stems. Other metabolic inhibitors, iodoacetamide, arsenate, arsenite, and azide at 10^{-3} M generally reduced absorption and retention of 2,4-D* in the roots, but did not permit upward movement of 2,4-D* in cotton.

To study the nature of 2,4-D accumulation in roots, exchange tests were run. Cotton plants 10 inches high were pretreated with unlabeled 2,4-D at 10^{-6} M in 100 ml of culture solution for 6 hours. Then the culture solution was replaced with one containing labeled 2,4-D at 10^{-6} M and DNP at 10^{-3} M and continued for 6 hours. The autoradiographic image of the roots was just as intense as those without the unlabeled 2,4-D pretreatment; undoubtedly there was exchange of some sort. Also there was some movement of 2,4-D* up the stem. When DNP was applied at 10^{-4} M one or two days before the application of 2,4-D* to the roots, the upward movement of 2,4-D* was greater than in a simultaneous treatment (figure 7, right; compare with figure 6, center). 2,4-D treatment alone is shown in figure 7, left. To study release of 2,4-D* from roots, four sets of two cotton plants each were treated for 6 hours in half-strength Hoagland's nutrient solution containing the same 10^{-6} M 2,4-D*, 12.3 mc/mmole, but containing also 0, 10^{-5}, 10^{-4}, and 10^{-3} M DNP. One plant of each set was washed under running tap water for one minute and the other for 60

minutes; figure 8 shows the autoradiographic results for the 0 and 10^{-3} M concentrations; the others were intermediate in image density. Image density in the autoradiographs indicated that more than half of the activity was eluted by the 60-minute wash as compared with that for one minute. The eluted activity does not speak for metabolic retention, although counting and calculations imply total concentrations greater than external, of more than threefold. Contrary to expectation it was also found that there was greater absorption and retention with greater DNP concentration. By grinding and counting the roots of individual plants the following net counts per minute were obtained (table 1):

TABLE 1

RESIDUAL WITH COTTON ROOTS
AFTER WASHING

.	pH of the treatment solution	Counts per minute residual per root	
		one-minute wash	60-minute wash
no DNP........	5.1	6,770	2,410
10^{-5} DNP.......	4.9	7,110	2,870
10^{-4} DNP.......	4.3	11,818	2,660
10^{-3} DNP.......	3.7	13,350	3,270

Net residual activity in the jars ranged from 165,000 to 185,000 CPM: the dry weight of the roots ranged from 92 to 111 mg. Chromatography of an 80 per cent ethyl alcohol extract of the roots showed that the activity was associated entirely with 2,4-D*, even in the roots washed for 60 minutes.

In another experiment similar cotton plants were given various 6-hour treatments. When a 6-hour treatment with 10^{-6} M 2,4-D*, of 12.3 mc/mmole, in 50 ml nutrient solution was followed by a 6-hour period with 50 ml of fresh nutrient solution, the activity was redistributed in the roots and an unusually high accumulation of activity occurred at the root tips (figure 9, right). The redistribution was seemingly a result
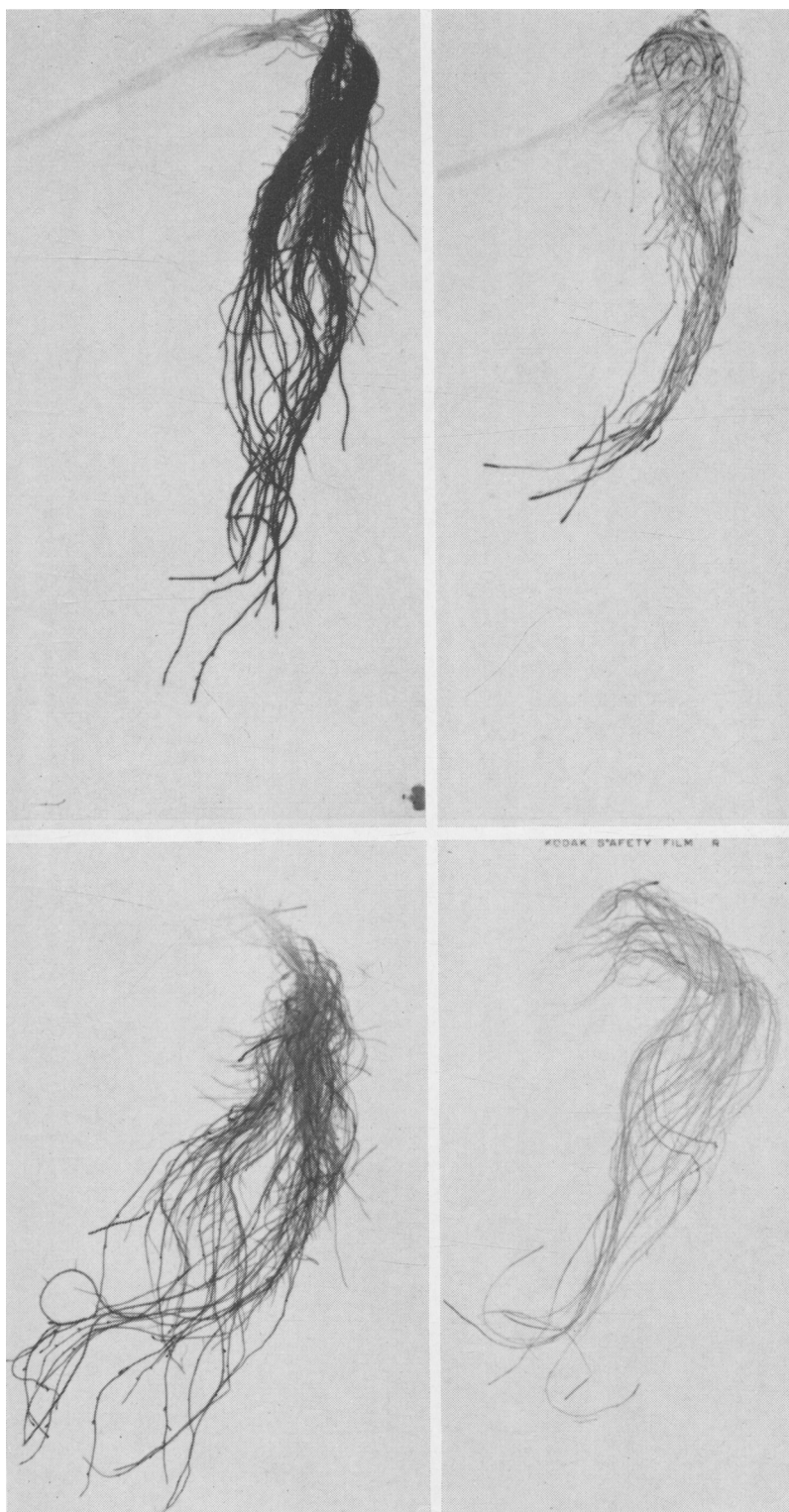
Fig. 8. Autoradiographs of 2,4-D* treated cotton roots washed for one minute, (upper two), and for 60 minutes, (lower two). The right pair was treated in a culture solution containing 2,4-D* at 10^{-6} M and DNP at 10^{-3} M; the left pair was treated with 2,4-D* at 10^{-6} M alone.
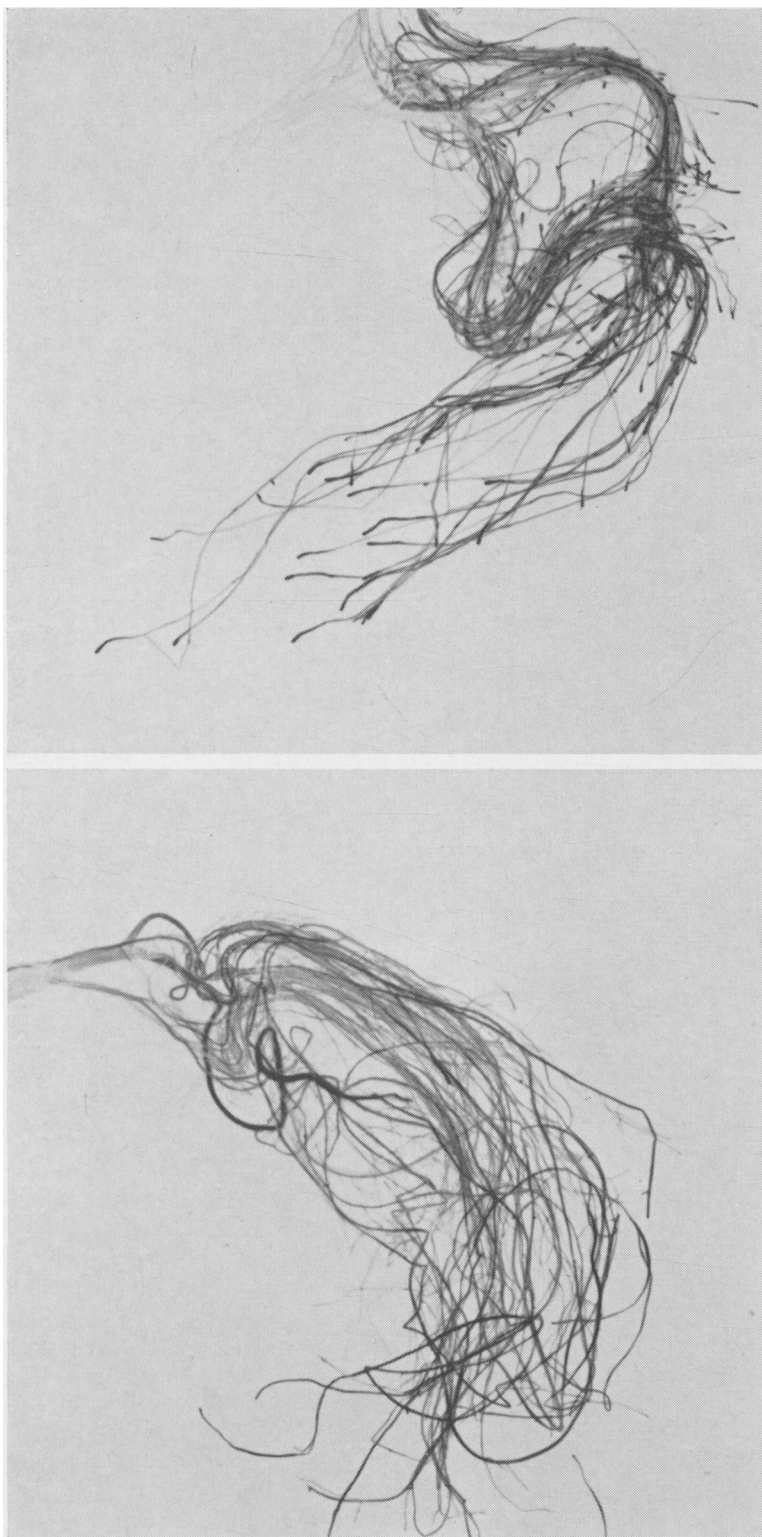
Fig. 9. Unusually high accumulation of 2,4-D at the root tips in cotton by transfer to fresh solution. Both roots were treated 6 hours with 2,4-D*, 12.3 mc/mmole, at 10^{-6} M in 50 ml culture solution. Left root was transferred to a fresh solution with 10^{-4} M DNP and no 2,4-D*, and retained 6 hours. Root at right was transferred to a fresh culture solution and held 6 hours.
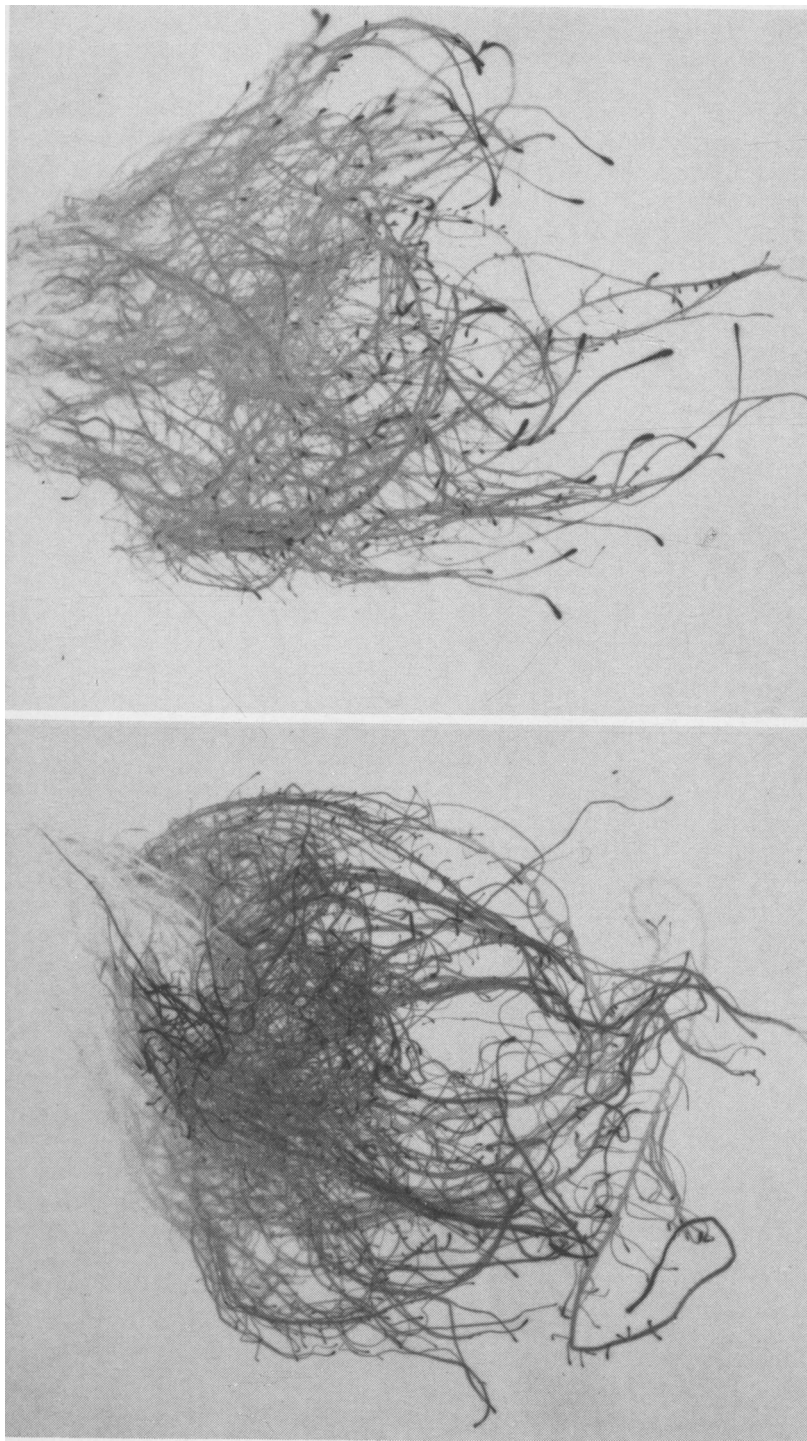
Fig. 10. Unusually high accumulation of 2,4-D* at root tips by the use of arsenate in the culture solution, in Red Kidney bean. Both roots were treated 6 hours with 2,4-D* 12.3 mc/mmole at 10^{-6} M in 50 ml culture solution. The root at right received, in addition, sodium arsenate at 10^{-4} M.

of accumulation in the more mature region of the root, followed by a resumption of the normal phloem translocation in the second 6-hour period when the 2,4-D was omitted from the nutrient solution. Being phloem-mobile, 2,4-D* would be translocated to the root tips if its supply concentration were not at the toxic level. Such high accumulation at the root tips was not apparent when the 2,4-D* was retained during the second 6-hour period, or when DNP, 10^{-4} M, treatment followed (figure 9, left).

The fact that 2,4-D* was not released from the roots when roots containing 2,4-D* were transferred to a fresh solution containing DNP at 10^{-4} M (figure 9, left) was contrary to expectations on the basis of DNP being a metabolic inhibitor. This information is further evidence for the assumption that DNP affects the movement of 2,4-D by other than direct metabolic means, the first evidence being that of the association of root flaccidity with the upward movement of 2,4-D.

Sodium arsenate permits 2,4-D accumulation at root tips. In an experiment with Red Kidney bean plants, the same sort of accumulation at root tips was obtained by the use of sodium arsenate at 10^{-4} M in 50 ml culture solution, simultaneously with 2,4-D*, 12.3 mc/ mmole, at 10^{-6} M, for 6 hours (figure 10, right). The contrasting distribution obtained by the use of 2,4-D* alone is shown in figure 10, left. As with the cotton plants treated with DNP, the arsenate somehow permitted greater phloem movement of 2,4-D*, but not greater absorption. In this treatment there was no enhanced upward movement of 2,4-D*. With some wilting which attended the arsenate treatment, upward water movement in the xylem may have been lower than with 2,4-D* alone. The pH of the solution was 6.1.

The pH effect on 2,4-D absorption by bean roots. The results of the arsenate experiment were compared with those of a pH experiment in which a value of

pH 2.9 and a value of pH 10.0 were compared. Adjustment of the pH was obtained by addition of sulfuric acid and potassium hydroxide. Red Kidney bean plants were used. Treatment time was 6 hours. 2,4-D*, 12.3 mc/mmole, was used at 10^{-6} M in 100 ml of culture solution. Note in figure 11, left, the image density due to 2,4-D* absorption from a culture solution at pH 2.9. Absorption was much greater here than at the normal pH of Hoagland's nutrient solution, which varied from 4.7 to 5.4, without the DNP. Absorption by the roots was followed by a massive migration up the hypocotyl. Judged from image density 2,4-D* is in the cortical tissues. If it were in the xylem or had moved up in the xylem, 6 hours would have been more than ample time for movement up into the leaves. 2,4-D* concentration at 10^{-6} M at the usual pH of Hoagland's nutrient solution has not permitted such absorption or such upward movement in Red Kidney bean plants, even with longer time; compare figure 11 left with figure 12 left, an autoradiograph of the usual 2,4-D* absorption (19 hours) from Hoagland's nutrient solution.

At the pH of 10, 2,4-D* absorption was still less than at the normal pH of Hoagland's nutrient solution. Moreover, the roots showed a fairly normal phloem translocation of 2,4-D*, with accumulation at root tips. Compare figure 11 right with figure 12 left. Perhaps the concentration entering the root tissues was at a subtoxic level at this high pH.

Because of the seeming effect of pH on 2,4-D absorption by roots, the pH levels of the solutions containing DNP were tested. With increasing DNP concentration, pH value of the solution decreased correspondingly (table 1).

To examine the pH effect more fully, low pH and high pH ranges were tested. The intermediate range was excluded because it allows an intermediate degree of absorption of 2,4-D. Sulfuric acid and sodium hydroxide were added for pH adjustments to 1, 2, 3, 9, 10, 11, and 12. Uniform two-week old Red Kidney
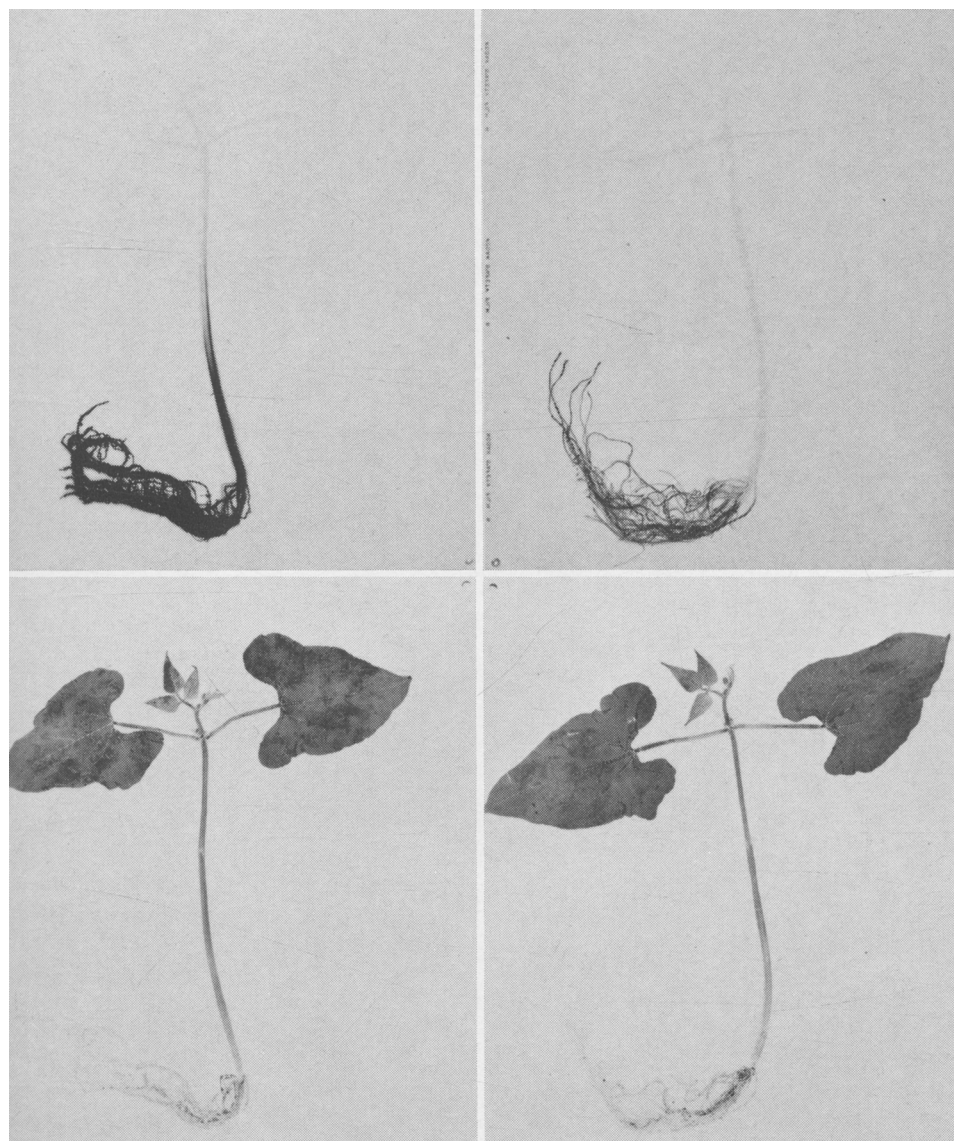
Fig. 11. The pH effect on 2,4-D* absorption and retention by bean roots. *Left,* culture solution pH at 2.9; *right,* culture solution pH at 10.0. 2,4-D, 12.3 mc/mmole, 10^{-6} M/100 ml. Treatment 6 hours.

bean plants were used. Two groups of plants were placed in pH adjusted solutions for 2 hours and then transferred to the pH adjusted solutions containing the 2,4-D* ("equilibrated"). Two other groups of plants were placed directly from the culture solution to the pH adjusted solutions containing the 2,4-D* ("unequilibrated"). At the end of 6

hours the roots of all plants were rinsed for a moment under running tap water. For autoradiography, a group from each of the two treatments was freeze-dried. For leaching, plants from the remaining group from each of the two treatments were placed individually in 100 ml of tap water, pH 7.8, for 1 hour; leaching was continued in a second 100
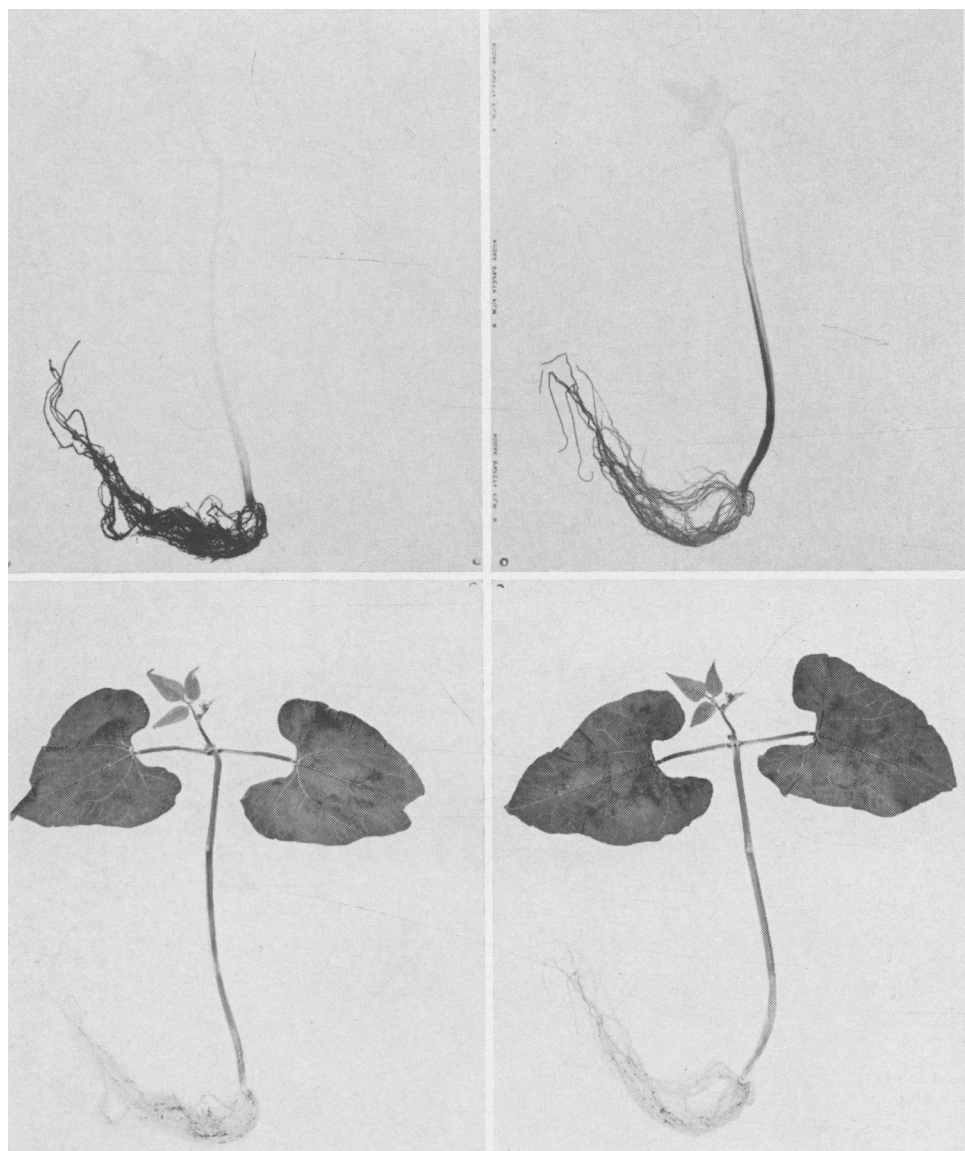
Fig. 12. The upward distribution of 2,4-D as effected by oxygen exclusion, in Red Kidney bean. *Left:* distribution under aerobic conditions; *right:* distribution with nitrogen. Treatment 19 hours. 2,4-D, 12.3 mc/mmole, 10^{-6} M, 100 ml.

ml of tap water for a second hour. The leached 2,4-D* was adsorbed on 10 mg of decolorizing charcoal, Norit A, and filtered out on glass fiber filter paper; the radioactivity was counted, adjusted for background and self-absorption (43 per cent) and tabulated, table 2. Autoradiographs of the unleached group are presented in figure 13.

The pH effect: nonmetabolically absorbed 2,4-D. With respect to the group which was unequilibrated for pH, a comparison of the leached activities shows that ten times greater amount was leached from roots of pH 3 treatment than from those of pH 11 treatment. At the pH of 1, 2, and 12, the roots lost their turgidity in less than 2

TABLE 2

THE RELEASE OF RADIOACTIVITY FROM BEAN ROOTS HELD 6 HOURS IN
HALF-STRENGTH HOAGLAND'S NUTRIENT SOLUTION ADJUSTED TO THE
VARIOUS pH's AND CONTAINING LABELED 2,4–D 10^{-6} M; 219,000 CPM/100 ML.

Unequilibrated Series

pH	1	2	3	9	10	11	12
CPM/1st hour leach	10,600	12,200	12,400	1,400	1,300	887	1,090
CPM/2d hour leach	2,260	1,400	1,850	517	431	335	91
Total CPM leached	12,900	13,600	14,200	1,920	1,740	1,220	1,180
Applied CPM recovered in the two leaches, per cent	5.9	6.2	6.5	0.88	0.79	.55	.54
Residual CPM in roots	1,160	5,220	43,400	7,850	8,270	2,660	−32
Applied CPM residual with roots, per cent	0.53	2.38	19.80	3.58	3.77	1.21	0
Dry weight of roots, grams	0.071	.073	.097	0.088	.144	.158	.100
Estimated fresh weight of roots, grams	.95	.97	1.3	1.1	1.9	2.1	1.3

Equilibrated Series

pH	1	2	3	9	10	11	12
CPM/1st hours leach	7,670	7,240	9,210	1,440	1,400	409	1,340
CPM/2d hour leach	1,090	949	2,030	515	513	218	160
Total CPM leached	8,760	8,190	11,200	1,950	1,910	627	1,500
Applied CPM recovered in the two leaches, per cent	4.0	3.7	5.1	0.89	0.87	0.28	0.68
Residual CPM in roots	1,000	2,890	39,000	5,810	4,540	609	−21
Applied CPM residual with roots, per cent	0.45	1.3	17.8	2.65	2.07	0.27	0
Dry weight, roots, grams	0.063	.060	.114	.112	.095	.071	.071
Estimated fresh weight of roots, grams	.84	.80	1.5	1.5	1.2	.95	.95

hours; there was no recovery in these roots during the leaching period of 2 hours. Yet the roots subjected to the lower injurious pH's of 1 and 2 had absorbed large amounts of activity; there was little absorption from pH 12. The leached activity in all cases is, therefore, most likely a nonmetabolic component of the total absorbed activity over the entire pH range tested, including the toxic extremes. In the unequilibrated series of table 2 the quantity of activity in the leachate alone from root treatments at pH 1, 2 and 3 was approximately 6 per cent of the total applied to 100 ml of solution. The roots weighed from .95 to 1.3 grams and accumulation amounts to approximately 6 times the external concentration.

The pH effect: Metabolically absorbed 2,4-D. The leached roots were freeze-dried and autoradiographed, fig-ure 14. The activity residual with the roots should, therefore, be the metabolic component of absorption—it was withheld after two 1-hour leachings in 100 ml of water; it was extractible with 80 per cent ethyl alcohol. The roots subjected to pH of 12 showed no residual activity autoradiographically after the leaching. The pH of 11 permitted a very small residual activity which most clearly showed accumulation at the root tips. From the pH of 11 to pH of 3 there was more than fifteenfold greater retention. At pH of 3, the lowest pH at which the root tissues were uninjured, the residual activity was the greatest, table 2 and figure 14. It amounted to 19.8 per cent of the applied, metabolically absorbed in 1.3 grams of roots, an accumulation of 15 times against gradient. But this gradient is only apparent because the real gradient is against the 2,4-D*
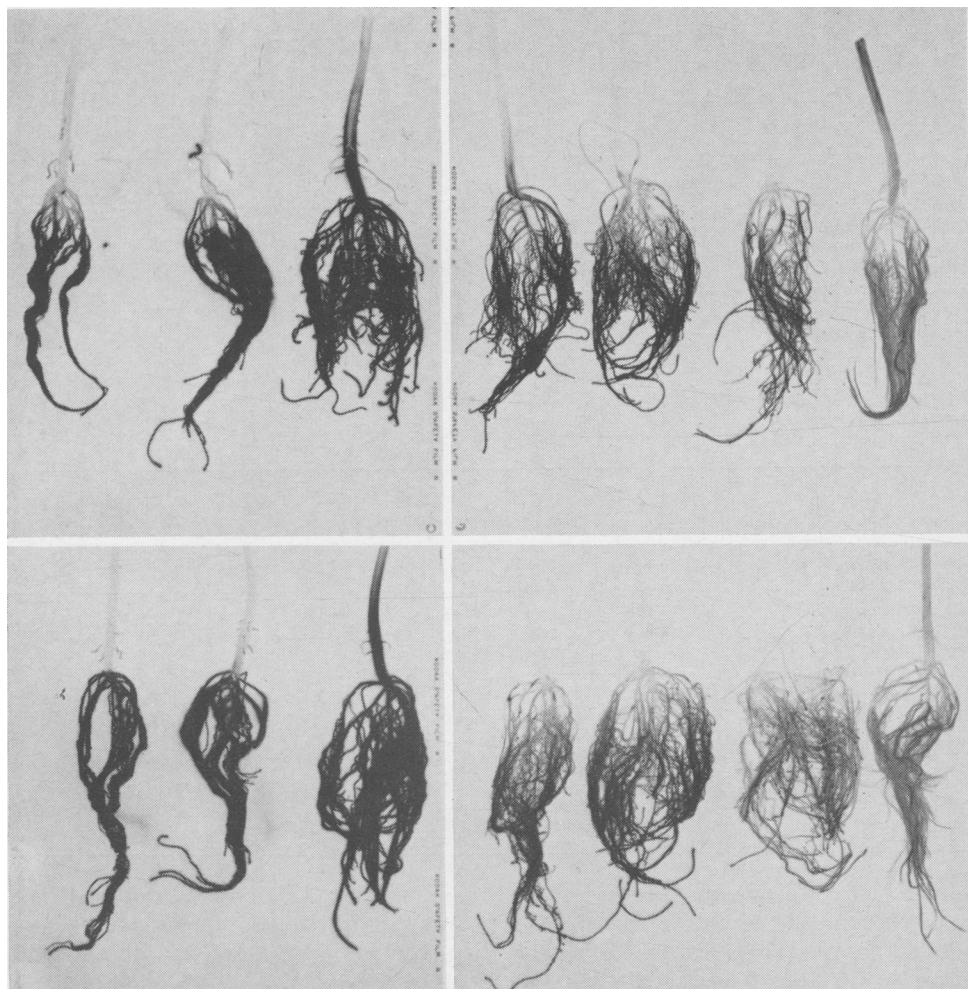
Fig. 13. Effect of culture solution pH on the absorption and retention of 2,4-D* by bean roots. *Top row,* directly transferred from Hoagland's nutrient solution to pH adjusted Hoagland's solution containing 2,4-D*; *bottom row,* pH equilibrated for 2 hours prior to transfer to treatment solution. From *left* to *right,* pH 1, 2, 3, 9, 10, 11 and 12. 2,4-D 12.3 mc/mmole, 10^{-6} M/100 ml solution. Treatment 6 hours.

loosely adsorbed on cell membrane, cellulosic and other surfaces, which evidently act as the source of 2,4-D* for metabolic absorption. Therefore, if the residual activity in the pH 3 treatment is compared with the loosely adsorbed 2,4-D*, which was leached out, the accumulation is really 2.5 times against gradient—though the term "accumulation against gradient" is poorly applicable here because adsorption, a surface phenomenon, is involved. Over the pH range 11 to 3 this accumula-

tion amounted to 2 to 4 times that of the adsorbed 2,4-D* (that which was leached out; see table 2).

This point of view is based on the observation that the 2,4-D* physically accumulated on the surfaces is readily removed. It is supposed that this adsorbed 2,4-D* is in equilibrium with the 2,4-D* in the nutrient solution and acts as a source for metabolic absorption.

At pH of 1 and 2 the roots seemed dead. After leaching, there were a few strands with high activity in the pH 2
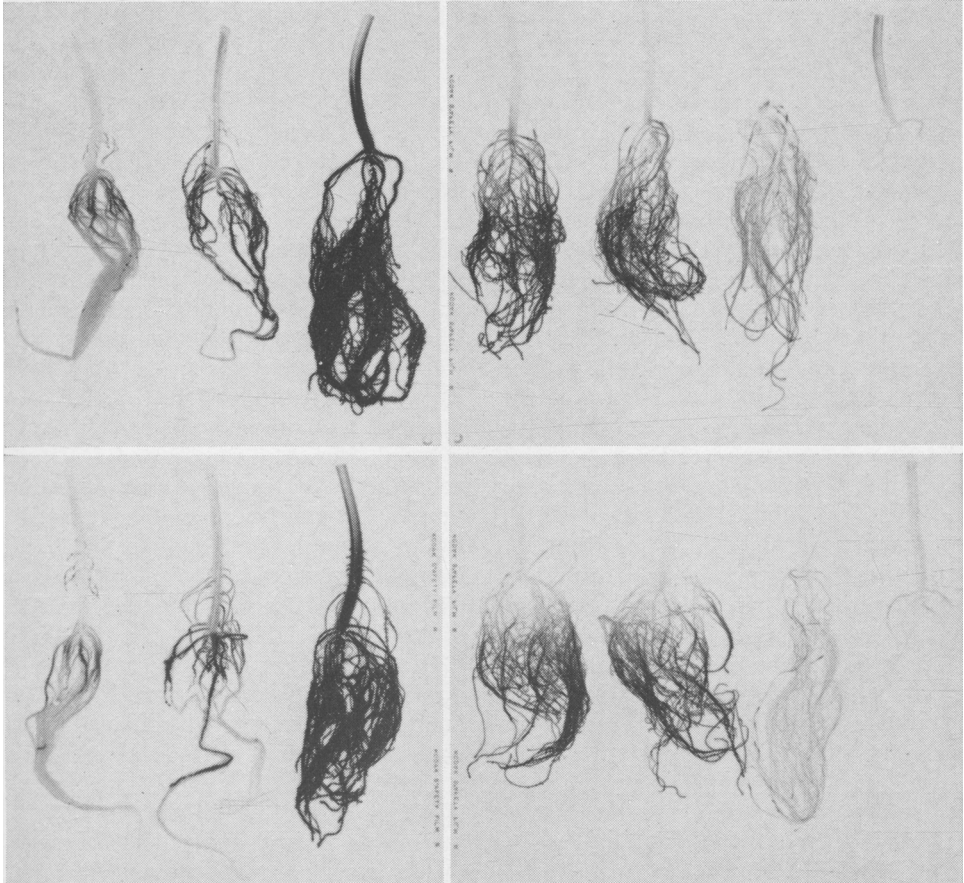
Fig. 14. Bean roots similarly treated as in figure 13, but leached for 2 hours and then freeze-dried and autoradiographed. The leached activities tabulated in table 2 came from these roots, which were later ground and assayed for the residual activities tabulated in table 2.

treated roots. Otherwise, residual activity was very low; compare figure 14 and table 2.

The effect of two-hour equilibration in the pH adjusted solutions was expressed as a depression of metabolic absorption (residual CPM) at all pH's tested, table 2. The autoradiographic image of the leached roots is lighter (figure 14) as compared with the unequilibrated series. This is a reflection of damage to the mechanism by which the metabolically absorbed 2,4-D is retained against leaching.

The results of the pH experiments permit the assumption that (1) absorption of 2,4-D is partly a loose phyisical binding and that (2) metabolic absorp-

tion of 2,4-D is much greater at the lower pH's of the nutrient solution because of this loose physical binding which permits a high accumulation of 2,4-D at surfaces which, in turn, enhances metabolic uptake. Presumably, the 2,4-D molecule, only as the un-ionized uncharged particle (as in a solution of pH of 3) is able to approach the negatively charged cellulosic and cell membrane surfaces.

Anoxia with nitrogen bubbling. The metabolic aspect of 2,4-D absorption by the roots was investigated further by continuous bubbling of the culture solution with nitrogen, a means of exclusion of oxygen from the roots and the lower regions of the stem. Red Kidney bean

plants were started in sand, transferred to solution culture in the very early primary-leaf stage, without injuring the roots, and grown a few days before treatment. The culture jars were equipped with drawn glass tubing bubblers, with a polyethylene guard in each jar to protect the bean stem from solution splatter. A polyethylene cover was also used across the mouth of each jar. The nitrogen was passed through a mixture of activated charcoal and Sponge Rok and then to the bubblers. Upper parts of the plants, to the level of the cotyledonary nodes, were exposed to the greenhouse atmosphere. Each jar with a plant and with 100 ml of culture solution was prebubbled for 2 hours. One micromole of unlabeled 2,4-D (10^{-5} M) was added to the solution through a small puncture in the polyethylene cover. Nitrogen bubbling was continued. In 24 hours a slight curvature of the stem was observed. 2,4-D at 10^{-5} M under normal culture has always strongly inhibited top growth in the bean, but has never moved up the stem in sufficient amounts to cause stem curvature. In a more critical experiment labeled 2,4-D at 10^{-6} M (slightly inhibitory to top growth) was used and the plants were freeze-dried and autoradiographed. Figure 12 shows a comparison of the control (left) and the effect of oxygen exclusion from the roots at 19 hours (right). There was a slow, but large movement up the stem, as with pH 3 treatment (figure 11, left), except that retention in the roots was considerably less. The upward movement of 2,4-D by the exclusion of oxygen from the roots is another evidence for the involvement of a metabolic factor in the normal lack of upward movement of 2,4-D from the roots. The metabolic factor would normally absorb and retain 2,4-D in the root tissues and tend to translocate it toward root tips.

Starvation by dark confinement.
Another approach to the problem was to darken the plants for several days, thereby depleting food reserves so that metabolic factors would be at a minimum; and then to observe the distribution pattern of 2,4-D* from root application. Bean plants kept in darkness for 5 days, with a 1-day 2,4-D* treatment to roots (at 10^{-6} M) on the fifth day, did not absorb as much 2,4-D* into the roots as those in the light; they still accumulated some 2,4-D* at root tips; however, upward movement was not enhanced by the dark confinement. The roots retained their turgidity. The lack of upward movement of 2,4-D* here was not understood; 2,4-D* absorption and retention were greatly reduced. Possibly anoxia and DNP do not inhibit the same process of retention; DNP may increase the bypass of 2,4-D through the endodermis, and into the xylem; at any rate the roots do lose turgidity with the effective concentration of DNP.

In the same experiment the leaves of duplicate bean plants were given 24-hour spot treatments with labeled 2,4-D after various dark periods (figure 15). Up to 42 hours, the longest time of dark confinement before 2,4-D* treatment, there was decreasing phloem translocation from the treated leaves. The fact that phloem translocation was very limited reflects the low level of mobile food reserves. 2,4-D* dispersed over a larger area within the treated leaf, with longer dark confinement before treatment with 2,4-D*, figure 15. This dispersal of 2,4-D in the treated leaf is understandable on the assumption that metabolic energy was low and cellular absorption and retention of 2,4-D was low, thereby permitting a larger degree of diffusional and transpirational movement of 2,4-D than possible under normal conditions of culture.

Movement of foliar applied 2,4-D*.
The increased apoplastic movement of 2,4-D* was expressed in other ways. When the usual $\frac{1}{10}$ μmole per 10 μliter droplets of the labeled 2,4-D were applied to soybean leaves and 40 μliter of 2×10^{-3} M DNP were added to some of these droplets and equal volumes of
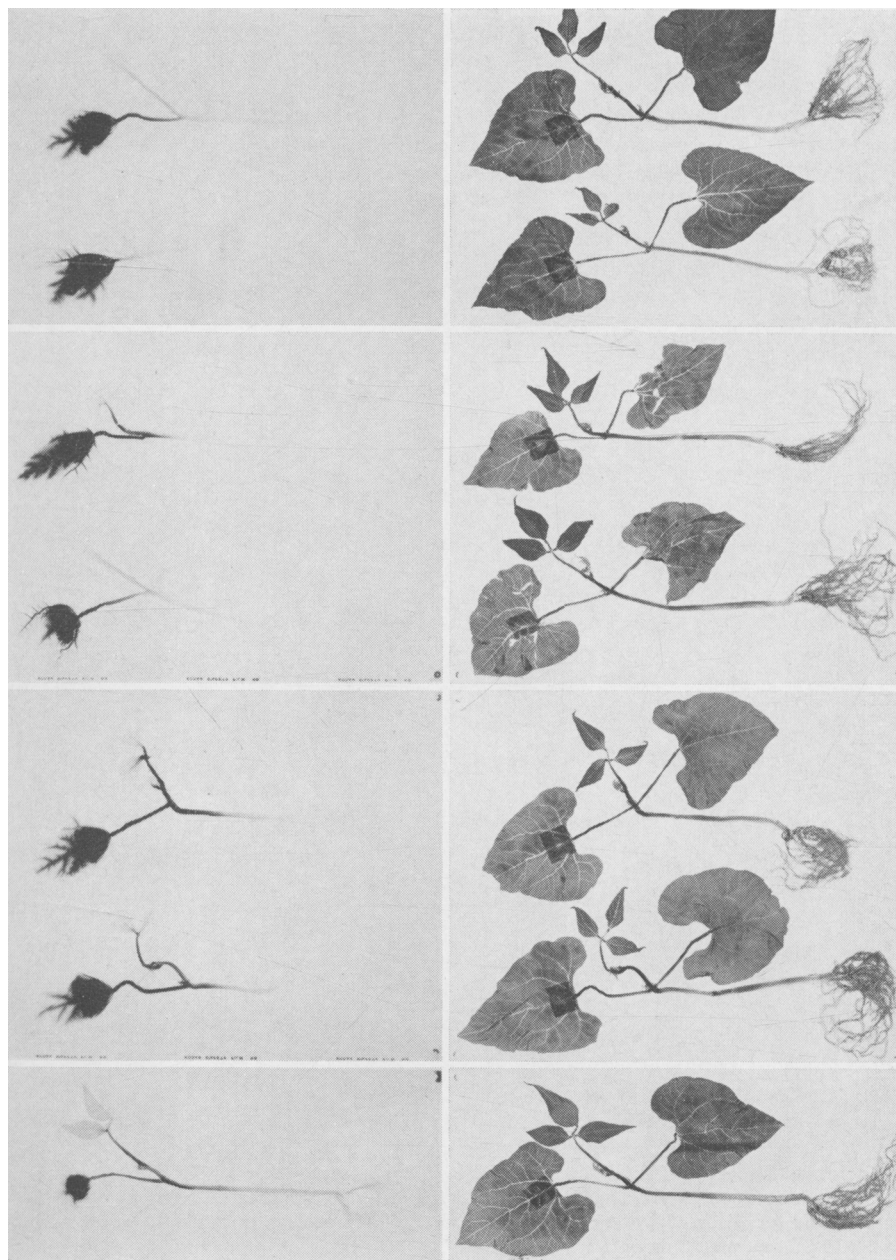
Fig. 15. Dispersal of 2,4-D* in 24-hour treated bean leaves as affected by dark periods of various duration. All plants were placed in the dark and treated at various hours; 6 hours (*first on left*), 18 hours (*second and third*), 30 hours (*fourth and fifth*), and 42 hours (*sixth and seventh*). 2,4-D* at 1.24 mc/mmole, 1/10 μmole in 10 μliter of solution was applied to the leaves and plants held in the dark for a 1-day treatment time.
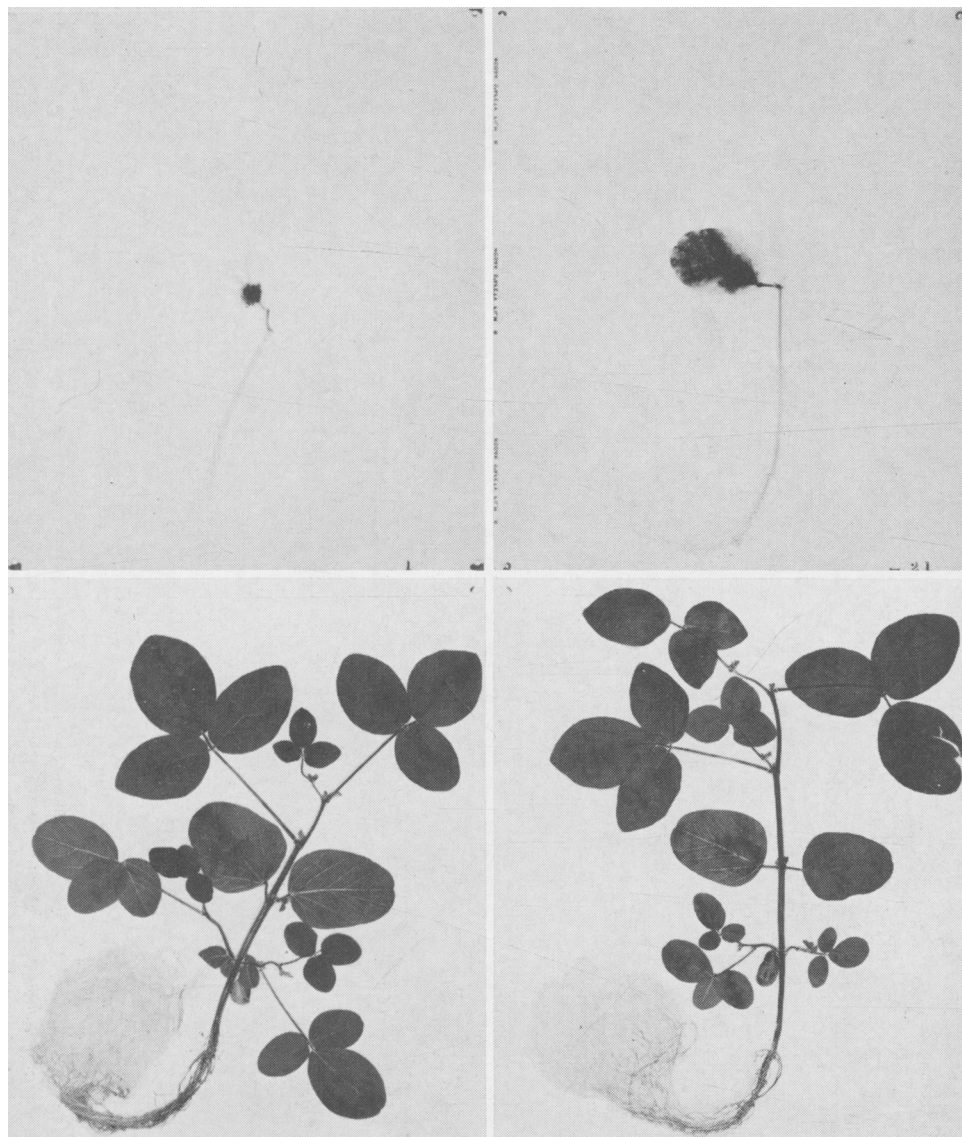
Fig. 16. Increased dispersal of 2,4-D in a treated leaf, by incorporation of DNP in the treatment solution, with soybean. *Left:* Leaf treated with 10 μliter of the treated solution of 2,4-D 1.24 mc/mmole and 40 μliter of water; *right:* treated similarly, except that the 40 μliter were of DNP at 2×10^{-3}. Treatment 3 days.

water to others, the results clearly showed that DNP greatly increased apoplastic dispersal of the 2,4-D* in the treated leaves (figure 16). Here phloem translocation of 2,4-D* to the roots was also increased. When cotton cotyledons were treated in like manner, apoplastic dispersal of 2,4-D* in the cotyledons was increased, but phloem translocation was somewhat less than without the DNP (figure 17, right pair versus the center pair). The left pair shows the usual 2,4-D* application of $\frac{1}{10}$ μmole per 10 μliter in 50 per cent ethyl alcohol and $\frac{1}{10}$ per cent Tween 20. At this concentration the 2,4-D* dis-
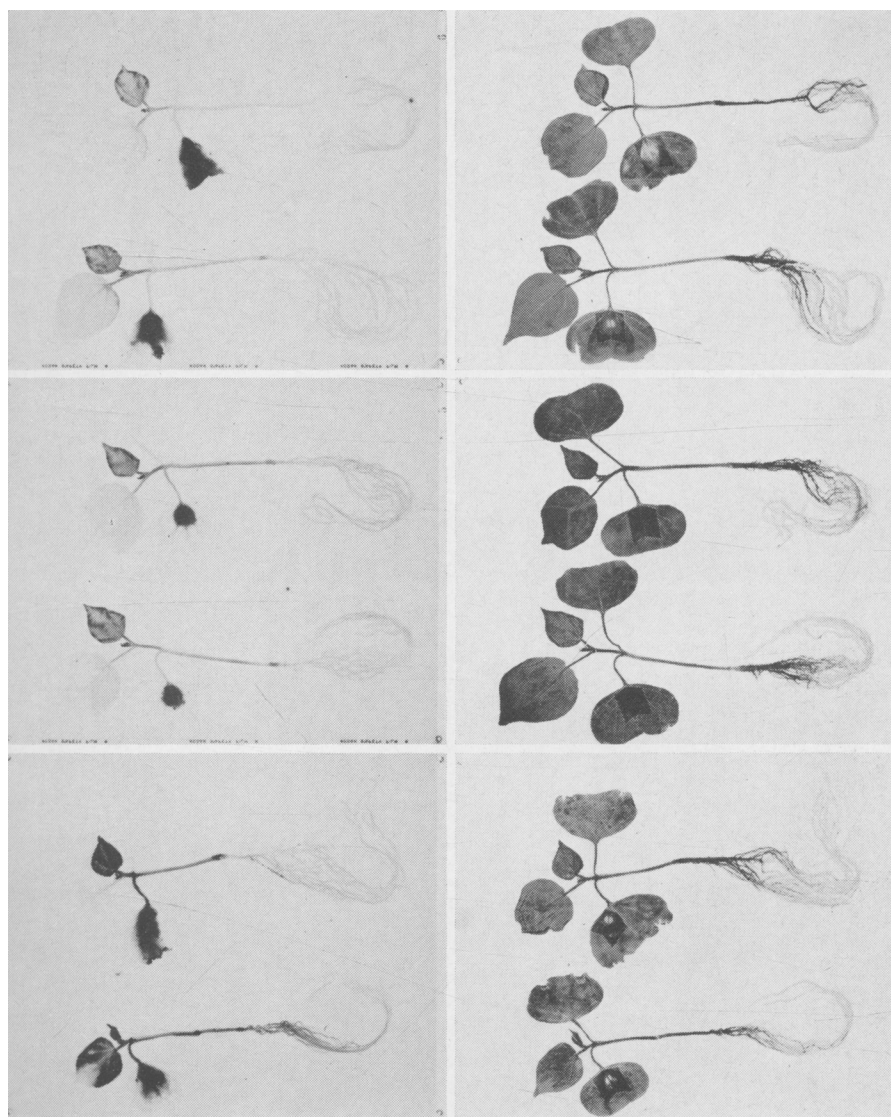
Fig. 17. Greater dispersal of 2,4-D in treated cotyledons of cotton, with DNP. The dosage of 2,4-D* 1.24 mc/mmole was uniformly 1/10 μmole. For the left pair of plants this 2,4-D was contained in the usual treatment solution, 10 μliter or 50 per cent ethyl alcohol and 1/10 per cent Tween 20. For the middle pair 40 μliter of water were applied with the usual treatment solution. For the pair at right, the 40 μliter were of aqueous DNP at 2×10⁻³ M.
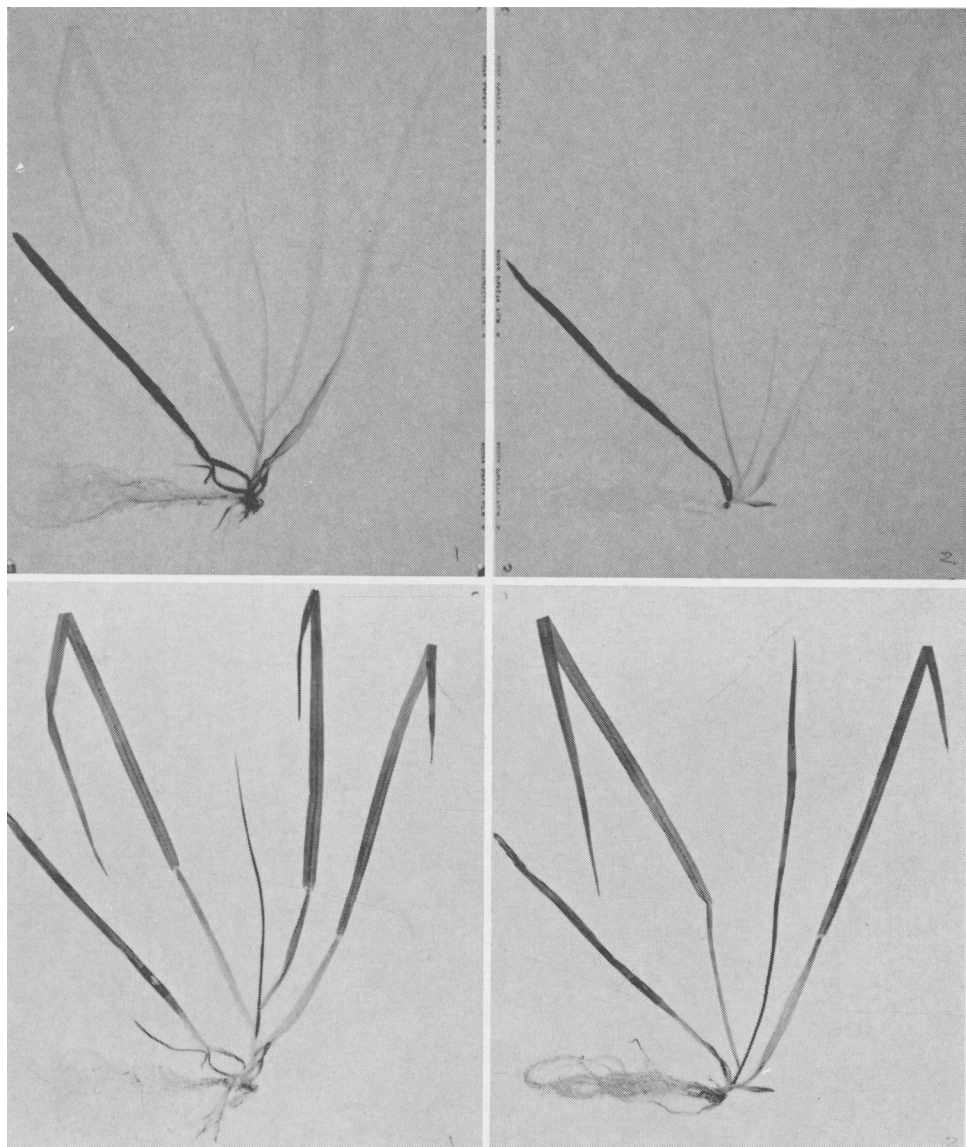
Fig. 18. Reduced phloem translocation of 2,4-D, by DNP feeding through the roots, in barley. *Left*: Usual 2,4-D distribution to roots and leaves, with 1-day treatment, in a barley plant. *Right*: With DNP added to the culture solution a day prior to 2,4-D leaf treatment, root shows greatly reduced 2,4-D translocation out from the treated leaf. 2,4-D, 1.24 mc/mmole, 1/10 μmole in 10 μliter of solution.

perses fairly freely in the treated cotyledons of the cotton plant.

As a variation of the above, the usual labeled 2,4-D solution was applied to the leaf, but the DNP was fed through the roots. Figure 18 shows in barley the limited phloem translocation of 2,4-D* after 1-day root pretreatment with DNP at 10^{-4} M (right), as compared with no DNP pretreatment (left). Apoplastic dispersal of 2,4-D* in the treated leaves cannot be considered because 2,4-D* usually disperses freely in the treated barley leaf. Figure 19 shows the limited 2,4-D* distribution in Red Kidney bean, after 1-day root pretreatment
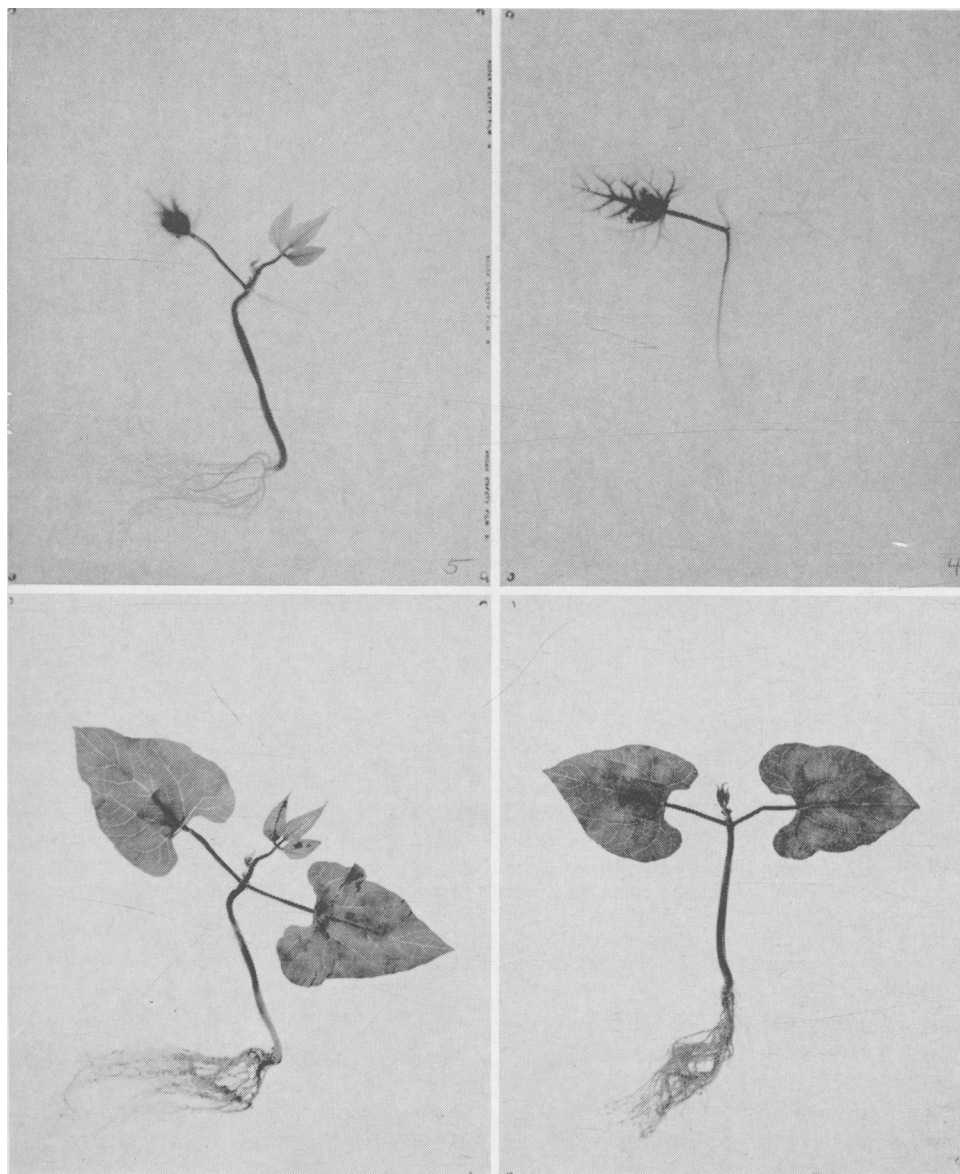
Fig. 19. Reduced phloem translocation as well as increased dispersal of 2,4-D shown in the treated leaves of the bean as a result of DNP feeding through the roots. *Left:* Usual distribution pattern of 2,4-D 1.24 mc/mmole, 1/10 μmole, applied to a primary leaf, with 1-day treatment. *Right:* Effect of DNP applied through the roots 1 day ahead of the 2,4-D application.

with DNP at 10^{-4} M (right), compared with the control (left). Dispersal in the treated leaf was greatly increased; movement into the petiole was increased; but further translocation to stem, bud, and roots was greatly reduced. The labeling of the opposite leaf was somewhat more intense than in the control; this is considered as evidence for apoplastic movement (Pallas and Crafts, 1957). The limited phloem translocation and distribution of 2,4-D* in these experiments may be a result of greater apoplastic mobility whereby
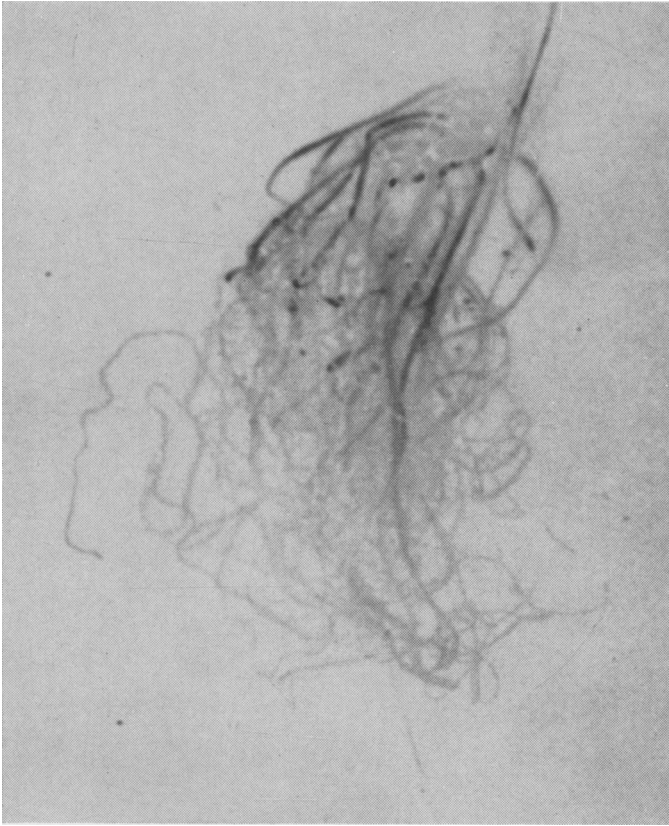
Fig. 20. Essentially complete inhibition of 2,4-D* absorption and
retention in the roots as a result of application of 1/10 μmole of
2,4-D to both primary leaves of a bean plant 2 days ahead of the
application of the labeled 2,4-D* 1.24 mc/mmole, at 10^{-6} M concen-
tration, via the culture solution. Labeled 2,4-D treatment 1 day.

the transpiration stream tends to carry the 2,4-D* toward the transpirational areas of the leaves; also it may result from reduced normal sugar translocation caused by disorganization of the phloem (Eames, 1950; Muni, 1959).

Complete freedom from metabolic absorption and retention was never obtained by the use of DNP at 10^{-4} M or by dark confinement, intended for exhaustion of food reserves. Exclusion of oxygen by the use of nitrogen was effective, without such injury as that caused by DNP. Another method was a 2-day pretreatment of each primary leaf of the Red Kidney bean plant with $\frac{1}{10}$ μmole of unlabeled 2,4-D, followed by a root treatment with 10^{-6} M labeled 2,4-D. Absorption and retention of 2,4-D* by the roots was nearly completely inhibited (figure 20). This probably results from a condition of starvation caused by complete lack of food transport to the roots. From Muni's work (1959) complete disruption of the vacular system might be anticipated as a result of the 2,4-D pretreatment. This is corroborative evidence for metabolic uptake and retention.

DISCUSSION

The rapid or greater entrance of 2,4-D into plant leaves from an acidic solution has been attributed to the undissociated molecules of 2,4-D as being more able to penetrate the plant apoplast, such as the cuticle and the cell wall which are negatively charged (Orgell and Weintraub, 1957; Crafts, 1956). The results on 2,4-D root uptake from a pH series of solutions correlate well with these observations. The results show further that there occurs a physical accumulation, an adsorption, supposedly on cell membrane, on cellulosic, and other surfaces of root tissues, which is a loose binding and probably in a steady state with the 2,4-D concentration in the bathing solution. This 2,4-D is entirely removed by leaching with tap water, table 2 and figures 13 and 14.

This surface accumulation seems to hold some real relationship with metabolic accumulation. Over the pH range, not immediately injurious (pH 11 through 3), the metabolic accumulation was roughly two to four times over the surface accumulation, in a 6-hour treatment with 2,4-D* concentration at 10^{-6} M. At solution pH of 11 the physical and the metabolic uptake of 2,4-D* were both very low and the sum of the two roughly equaled the 2,4-D* concentration of the bathing solution; yet, the autoradiograph of the leached roots, figure 14, clearly shows the accumulation at the root tips so typical of movement by phloem transport. At solution pH of 3 the physical and metabolic absorption were more than 10 times as great as at pH of 11. It seems likely that such a relationship could have existed in the work of Wedding *et al.* (1954), in which the inhibition of photosynthesis in chlorella by 2,4-D was shown to be related to the concentration of undissociated 2,4-D acid molecules in the solution used for treatment. The upward movement of 2,4-D into the tops of bean plants from a concentration of 10^{-4} M or

greater in the nutrient solution (Boyd, private communication) may have involved a lowering of the pH of the Hoagland's nutrient solution: a large physical and a large metabolic accumulation appears to be followed by slow but large upward movement. The rate of this movement was approximately 10 cm in 6 hours, figures 11 and 12. With DNP included in the nutrient solution the upward movement of 2,4-D occurred without such initial accumulation on and in the roots; the rate of upward movement was twice as fast; the lower stem was often without intense labeling and the tracer showed up in the veins of the leaves, figures 2, 5, 6, and 7. The mode or the path of upward movement of 2,4-D from a solution containing DNP is certainly different from one having a low pH value.

2,4-D absorption from foliar application would seemingly involve the same principles of absorption as from nutrient solution. Quite often the limiting factor in 2,4-D translocation is the movement through internal tissues down to the phloem. A high degree of absorption and retention by the cells in the region of application is the common phenomenon, as well demonstrated by the potato tuber tissue method (Crafts, 1961). This internal movement in the treated leaf was enhanced considerably by DNP applied simultaneously with 2,4-D* to the leaf surface (figures 16 and 17) and by DNP distribution in the tops from root application (figure 19) and by dark treatment starvation (figure 15), but internal movement enhanced by these means usually led to reduced phloem translocation from these leaves because of reduced metabolism. Seemingly there is a certain form of toxicity associated with the larger dosages and concentrations of 2,4-D, which limits the cell to cell movement as well as phloem translocation of the compound. For example, the optimal

dosage to a primary leaf of a young bean plant for general distribution is .2 to .4 μmole (Crafts and Yamaguchi, 1964) and higher dosages are much too toxic. Means used to reduce cellular absorption and retention of 2,4-D also reduced phloem translocation of 2,4-D. Nevertheless, a herbicidal dosage of 2,4-D applied to bean plants has been made somewhat more effective by the inclusion of Tween 20 in the spray solution of 2,4-D; it was thought that the extra effectiveness came from reduced injury to photosynthate source leaves (Leonard, 1958).

One cause of limited phloem translocation of 2,4-D is the rapid loss of sieve tube membrane semipermeability, as well as the large absorption and retention by parenchyma of the treated region. For example, note the difference in image intensity of the petiole of plant in figure 15, left, as compared with those of plants second and third in the same figure. For another example, the limited phloem translocation of 2,4-D from seemingly mature, but later-formed leaves of barley (Petersen, 1958) and translocation "block" in the intercalary meristem of the stems and leaves of young monocotyledonous plants (Fang and Butts, 1954) may well be partly related to high permeability of the cell membrane—our autoradiographs of 2,4-D* treated barley leaves show extensive internal dispersal which is not found in treated leaves of bean plants—and

partly related to absorption and retention associated with growth (Crafts and Yamaguchi, 1958). For another example, in plants which were pretreated one day with 2,4-D, the distribution pattern of labeled amitrole showed considerable loss into the parenchyma along the path of translocation, with corresponding decrease of general distribution; this was observed even in simultaneous treatments of unlabeled 2,4-D and labeled amitrole (with zebrina and bean plants, author's unpublished data). Further evidence of loss of sieve tube membrane semipermeability comes from anatomical studies in bean plants which show that sieve tubes are crushed by the eighth day after a spray treatment with 125 ppm 2,4-D in carbowax (Eames, 1950). Is it possible that the effect of DNP in increasing the mobility of 2,4-D in the treated leaf and roots and the effect of starvation and of anoxia in increasing the mobility of 2,4-D roots, are not all expressions of altered membrane permeability, the alterations seemingly mediated through the depression of metabolism? In those cases where root turgidity was irreversibly lost, further irreversible alterations of permeability may be concerned.

Upward movement and distribution into the tops, of minute concentrations of 2,4-D from root uptake, as in the case of cotton (Clor, 1951; Crafts, 1961), may be examples related to long exposure periods.

SUMMARY

By autoradiographic method 2,4-D absorption and retention by roots and leaves was studied. Metabolic inhibitors, anoxia and starvation, as well as pH differences were employed. Some of the autoradiographic root absorption experiments were further analyzed by GM counting. Intact plants of Red Kidney bean, Acala cotton, White California Mariout barley and Harosoy soybean were used. Treatment was mostly with high-activity 2,4-D, 12.3 millicuries per

mmole, spot applied to the leaf at high concentration or applied through the nutrient solution at 10^{-6} M, with a treatment time of mostly 6 hours.

The results indicated that metabolic absorption and retention of 2,4-D was reduced by DNP. This reduction of metabolic absorption and retention resulted in increased apoplast mobility and reduced phloem mobility of 2,4-D. Similar results were obtained by starvation and anoxia.

The pH effect of the nutrient solution, accounting for greater root absorption of 2,4-D at the lower pH values was found to be composed (1) of loose physical binding or adsorption and (2) of metabolic absorption. The physical adsorption moiety, leached out into tap water by two 1-hour soakings, was 10 times greater at pH 3 than at pH 11. Adsorption at pH 1 and 2 (the roots were irreversibly injured) was much like that at pH 3; at pH 12 (the roots were irreversibly injured) it was much like that at pH 11. The metabolic absorption (the moiety not leached out by two 1-hour soakings) was also 10 times greater at pH 3 than at pH 11. Over this pH range the metabolic moiety was two to four times greater than the adsorption moiety. Total absorption by 1.3 grams of roots in 100 ml of solution was as much as 25 per cent of the labeled 2,4-D applied at 10^{-6} M. At pH 12 and at 1 and 2, where the roots were irreversibly injured, metabolic absorption was nil or minimal. Seemingly, metabolic absorption of 2,4-D under conditions of more favorable pH was directly related to the initial adsorptive accumulation of this compound. Along with the large absorption of 2,4-D from a nutrient solution at pH 3, there was a large amount moving up in the cortical tissues of the bean hypocotyl.

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