THE ABSORPTION AND TRANSLOCATION OF 2,4-DICHLOROPHENOXYACETIC ACID BY BEAN PLANTS

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(WITH SEVEN FIGURES)

Received September 4, 1951

Introduction

The compound 2,4-dichlorophenoxyacetic acid (2,4-D) and similar compounds applied locally to aerial parts of plants may cause responses in plant parts distant from the place of treatment. Such responses have been used by several investigators as a measure of the translocation of 2,4-D. These studies have produced an important body of evidence on growth-regulator translocation.

It has been reported (**2**, **5**) that when 2,4-D was absorbed by roots it passed upward through dead segments of stems, but when absorbed by the leaves it did not readily move downward through killed portions of stems and petioles. MITCHELL and BROWN (**2**) found that 2,4-D was not translocated from the leaves of young bean plants that had been depleted of carbohydrate in the dark. When such bean plants were treated on one leaf, at various times after exposure to light, 2,4-D was translocated to the epicotyls, where it caused curvature in amounts approximately proportional to the sugar content of the leaves. Leaves deprived of CO_2 in the light were shown not to export 2,4-D. It has been demonstrated (**4**, **6**) that carbohydrate-depleted bean plants can be induced to move 2,4-D from the leaves in the dark by applying sucrose, glucose, fructose, maltose, and galactose to the leaves. WEAVER and DEROSE (**5**) have shown that partial clipping of leaves, defoliation, and reduction of light intensity serve as inhibitors of downward translocation. These data support the view that 2,4-D may move upward in the xylem and downward in the phloem, and that phloem transport from the leaves is directly correlated with the transport of food produced by photosynthesis.

The present paper reports several experiments in which the curvature of the epicotyl of seedling beans was used as a measure of the quantity and velocity of translocation of 2,4-D.

Methods

Red kidney bean seedlings were grown individually in four-inch pots. The plants were used when the primary leaves were almost fully expanded and before appreciable development of terminal buds above the second nodes had begun. An aqueous solution of 500 p.p.m. 2,4-D was made by

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adding 1.9% alcohol as cosolvent. A droplet of 10 microliters of this solution, containing 5 μg. 2,4-D, applied to the upper surface of one leaf along the midrib by means of a micropipette, constituted the usual treatment. Epicotyl curvatures were measured with a protractor.

Experimental results

THE GENERAL NATURE OF THE CURVATURE REACTION

EXPERIMENT 1.—The type of response obtained under greenhouse conditions is shown in figure 1. Each curve in this figure was plotted from the

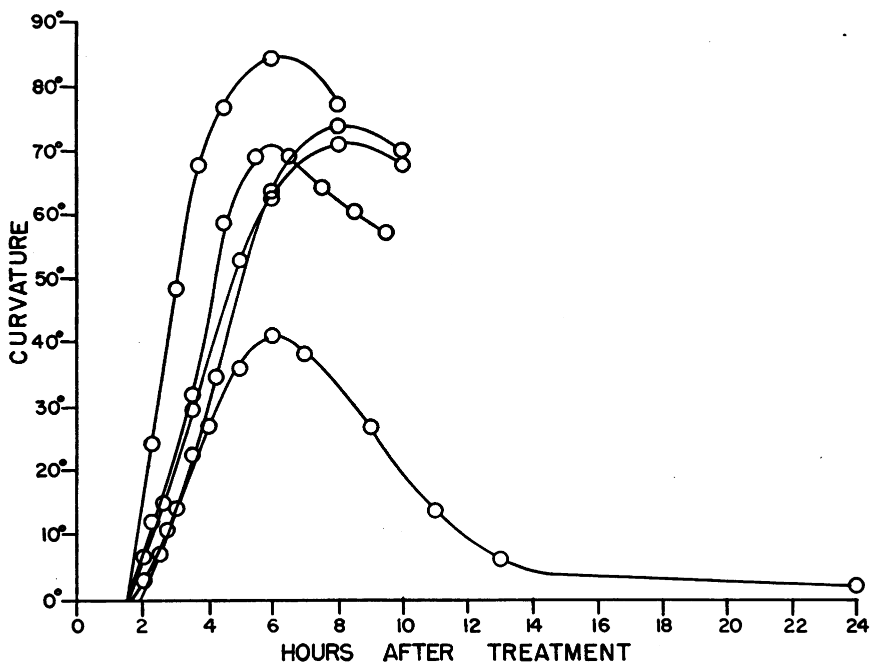

FIG. 1. Curvature response of samples of bean seedlings to applications of 5 μg. 2,4-D on one primary leaf, showing day to day variation in response.

average curvature of a group of 6 to 15 plants. Identical treatments of 5 μg. 2,4-D were applied between 9 A.M. and 10 A.M. on the upper surface of the midrib on different days to groups of plants that to all outward appearances were alike. The difference in response shown here is typical day to day fluctuation which may be due to minor variations in environment during or prior to the test. Curvature typically begins somewhat less than two hours after treatment and increases for three to five hours reaching a maximum five to eight hours after application of 2,4-D. The stem then begins to return to vertical and may recover to a condition of more or less random curvature in 24 hours.

In the cases reported here, the point of treatment was about 5 cm. from the place on the epicotyl where the reaction occurred. The 2,4-D or some stimulus resulting from it must have moved at a speed of at least 2.5 cm. per hour if no allowance of time is made for the initiation of curvature after the 2,4-D had been translocated.

Effect of dose on curvature

Experiment 2.—Samples of eight plants each were treated on the upper surface of the leaves, on the midrib, 1 cm. from the base of the leaf blades, with 2.5, 5, 10, and 20 μg. of 2,4-D. Curvatures are plotted against time after treatment in figure 2. The time from treatment to the beginning of curvature appears to be independent of the concentration applied. The initial rate of curvature increases with concentration. It is probable that under any given conditions the maximum curvature attained is a measure of the amount of 2,4-D absorbed and translocated, and the initial slope of the curve is likewise an indicator of the concentration of 2,4-D applied.

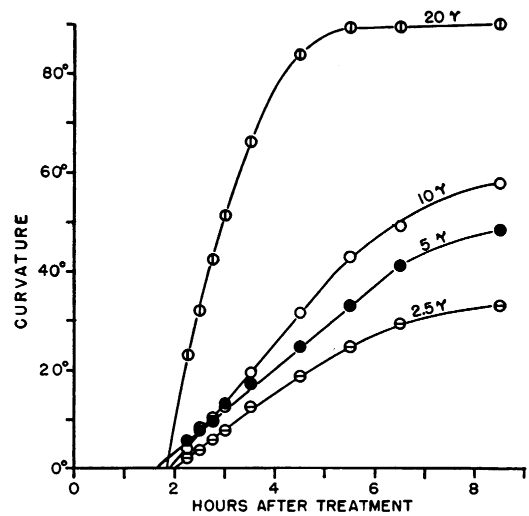

Fig. 2. Curvature response of kidney bean seedlings to different doses of 2,4-D.

Time factors in the absorption and translocation of 2,4-D

Experiment 3.—If large numbers of plants are treated with 2,4-D at a given point on their leaves and the treated leaves are removed from various groups of these plants at successively longer times after treatment, it should be possible to determine the minimum time required for 2,4-D to move through the leaf and enter the stem.

One hundred and five bean plants in the primary leaf stage were selected for uniformity from a larger group and randomly arranged in seven groups of 15 plants each. One primary leaf on each plant was treated on the upper surface along the midrib, 3 cm. from the base of the blade. The average length of the petioles was 4.05 cm., making the total distance of translocation from the point of treatment to the node approximately 7 cm. Plants of six groups were defoliated by removing the treated leaf adjacent to the node one and one half, one and three fourths, two, two and one half, three, and three and one half hours respectively after treatment. Plants of the remaining group were not defoliated. Stem curvatures were measured at hourly intervals from two and one half to nine and one half hours. Values for the series are graphed in figure 3.

It can be seen that after removal of the leaf, curvature continues unaffected for about the same time in all cases and then abruptly begins to decrease. The time at which this change in slope occurred is estimated, by inspection of figure 3, to be about one hour; this indicates that there was a time lag of about one hour between interruption of the 2,4-D supply and the beginning of recovery.

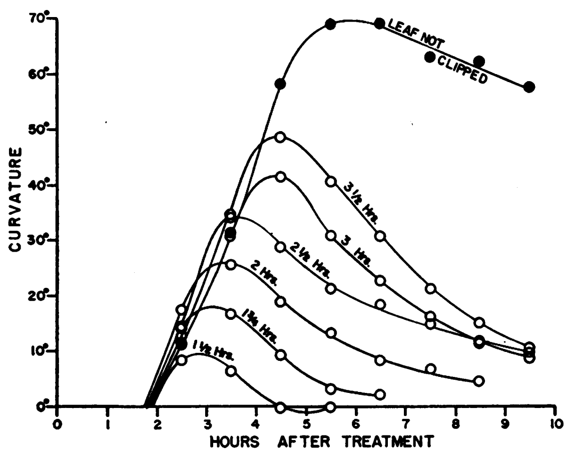

FIG. 3. Curvature of six 15 plant samples of bean seedlings having the 2,4-D-treated primary leaf of the plants removed adjacent to the node one and one half to three and one half hours after treatment compared with curvature of nondefoliated plant sample.

Maximum curvature of the control group was attained about five and one half hours after treatment. The fact that recovery did not begin as abruptly as in plants from which leaves were removed and that the negative slope of recovery was not so steep, indicates that the supply of 2,4-D from the leaf was gradually exhausted rather than abruptly terminated. Subtracting the one hour time-lag, estimated above, from the time of maximum curvature, it appears that translocation of 2,4-D into the stem had been essentially completed four and one half hours after treatment. This is in agreement with values of five hours (5) and four hours (3) obtained by more direct methods.

In figure 4 maximum curvature is plotted as a function of the time from treatment to removal of the leaf. When the curve is extrapolated back to zero degrees, we obtain an estimate of the longest time that the treated leaf could have been allowed to remain on the plant and still cause curvature.

This value of 82 minutes probably corresponds to the time at which the 2,4-D first emerged from the petiole and entered the stem. The control curve (fig. 3) shows that first curvature began 125 minutes after treatment. Subtraction of the time required for absorption and translocation (82 minutes) gives a value of 43 minutes for the time required to initiate the curvature reaction after 2,4-D has entered the vascular system of the stem.

It is concluded: 1. 2,4-D entered the leaf and moved the 7 cm. to the stem in about 82 minutes; 2. bending of the stem began about 43 minutes later (this time factor was measured as 45 and 36 minutes, respectively, on two other occasions); 3. when the treated leaf was removed, about 60 minutes elapsed before the curvature response was altered; 4. absorption of the 2,4-D by the leaf and its translocation into the stem was completed in about four and one half hours.

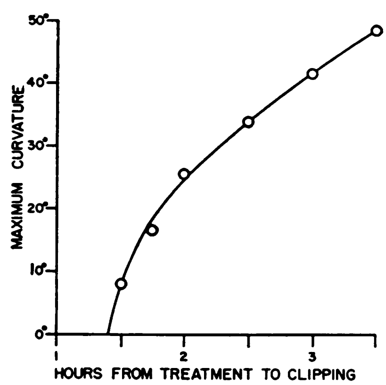

FIG. 4. Maximum curvature attained by groups of bean plants plotted as a function of the time treated leaves were allowed to remain attached (from data of fig. 3). The intercept on the time axis is 82 minutes.

EXPERIMENT 4.—Treatments applied to the midrib of the leaf more distant from the place of curvature on the epicotyl should cause curvature to begin later than treatments applied close to the epicotyl. The difference in time divided by the difference in distance of travel should give the velocity of movement of 2,4-D through the vascular system. This principle is employed in the present experiment.

Seven groups of six plants each were treated by applying 2,4-D on the upper surface of the midrib 5 cm. from the base of the blade of one leaf, and the opposite leaf was treated 1 cm. from its base. The difference in distance to be moved was thus 4 cm. Curvature due to the more distant treatment was called tip, and curvature due to treatment at the base of the leaf was called base. If both treatments were applied at the same time, one would expect base curvature to occur first because of the shorter distance of translocation. If time were allowed to elapse between treatment of the tip and treatment of the base of successive groups of plants, we should expect to approach a time when 2,4-D arrives simultaneously from the two different sources, resulting in zero or random curvature values. For this reason, treatment at the base of the leaf was applied to the plants of seven groups 0, 5, 10, 15, 20, 25, and 30 minutes, respectively, after application of the tip treatment to the opposite leaf.

Curvatures were measured two hours after treatment of the tip. This initial curvature was in the plane of the two petioles; subsequent curvature was frequently in other directions and accordingly difficult to interpret. Results are given in figure 5. The curve shown in this figure is the parabola of best fit as determined by the least-squares method. The point of zero curvature, which is 5.28 ± 1.68 minutes, indicates a 4-cm. movement of 2,4-D in that period, corresponding to an average velocity of 45.4 cm. per hour.

EXPERIMENT 5.—It was demonstrated in experiment 3 that the total time for the absorption of 2,4-D and its translocation from the leaves could be

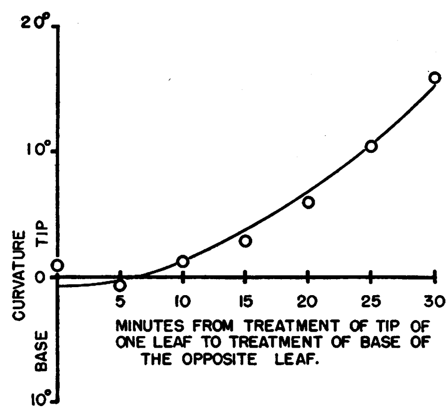

FIG. 5. Curvatures resulting from 2,4-D treatment of opposite leaves at different distances from the node. The intercept on the horizontal axis of the best-fitting parabola is 5.28 minutes, the estimated time required for movement of 2,4-D over the path difference between the two leaves of 4 cm.

measured by removing the leaves at various times after treatment. This principle was utilized to measure the velocity of movement of the initial increment of 2,4-D in the phloem by applying the dose on the leaf at two different distances from the stem and measuring the increased time required for the 2,4-D to arrive at the stem from the greater distance.

Each plant of six groups of 15 was treated on the upper surface of one primary leaf on the midrib 1 cm. from the base of the blade, and the plants of six additional groups were similarly treated 5 cm. from the base of the blade. The distances to be translocated were respectively 5 and 9 cm. from the node. The treated leaves were removed from one group of each series one and one half, one and three fourths, two, two and one half, three, and three and one half hours after treatment.

The maximum curvature was plotted against the time the treated leaf was removed (fig. 6), and a line was fitted to each series by the method of least-squares. When extrapolated back to zero curvature, values of 61.5 ± 3.9 minutes and 79.4 ± 7.6 minutes were obtained, respectively, for the treatments 5 cm. and 9 cm. from the node. These figures represent time elapsed from treatment of the leaves to entry of the 2,4-D into the epicotyl. Translocation over a path 4 cm. longer required 17.9 ± 8.5 minutes indicating a velocity of 13.5 cm. per hour. Two other determinations made by a variation of this technique yielded velocity estimates of 49 and 106 cm. per hour.

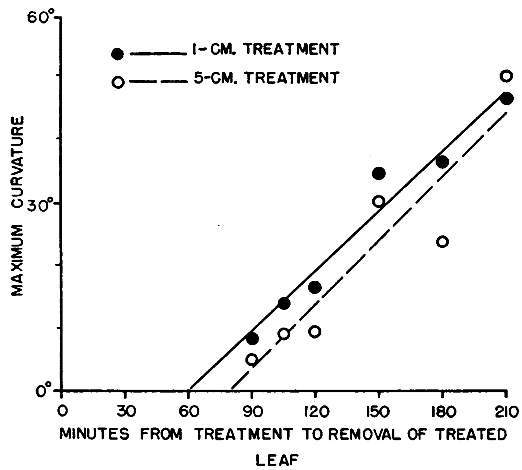

FIG. 6. Time required for 2,4-D to move to the stem from a position on the midrib 1 cm. from the base of the leaf blade is compared with time required for translocation from a point 5 cm. from the base of the blade. The difference in intercepts on the time axis is an estimate of the time required for 2,4-D to move the path difference of 4 cm.

ABSORPTION OF 2,4-D THROUGH THE CUTICLE AND TRANSLOCATION INTO THE PHLOEM

EXPERIMENT 6.—The time required for movement of 2,4-D from a droplet on the surface of a leaf into the phloem of the leaf veins was estimated on leaves of seven groups of eight bean seedlings each. Applications of 2,4-D were made to one primary leaf, on the upper surface along the midrib, about midway between the base and tip of the lamina and at a total distance of 6 cm. from the node. The treated areas of the leaves in one group were punched out with a corkborer 1 cm. in diameter one hour after application of the solution; leaves of other groups were punched at quarter-hour intervals up to two hours, and at two and one half and three hours after treatment. To determine whether punching the leaf prevented further translocation of 2,4-D that had already moved outside the punched area, treatment was applied 5 mm. below freshly punched 1-cm. holes in leaves of six plants. A similarly treated group of plants having leaves that had not been punched served as a control. The response of punched leaves did not differ significantly from the response of the control.

Some bending resulted in all groups of plants in which the treated portion of the leaf was left in place for more than an hour. The maximum curvatures attained are plotted in figure 7 as a function of the time of removal of the leaf punches. The zero curvature point of 63 minutes is based upon the intercept of a line calculated on the basis of all points except the one hour figure which resulted in no curvature. This should represent the time required for absorption and movement, across the boundary of the area to be punched, of the least amount of 2,4-D that would cause curvature. The time required for translocation of 2,4-D through the 5 mm. of phloem to the edge of the area punched would, on the basis of the rates reported above, be on the order of one to three minutes. The remaining hour must

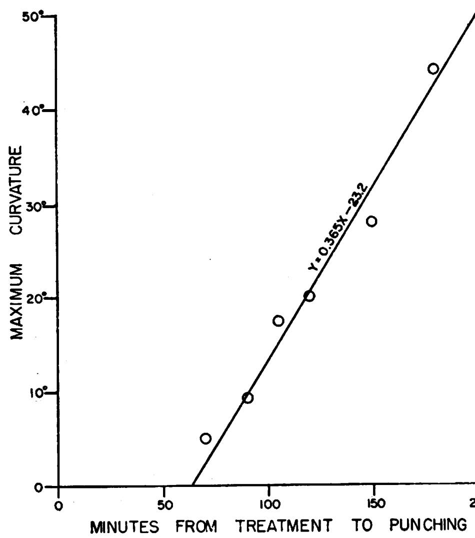

FIG. 7. The points shown are average maximum curvatures of groups of plants having the 2,4-D-treated areas of primary leaves punched out at increasing intervals of time.

have been occupied in movement of the growth substance through the cuticle, epidermis, and mesophyll into the phloem. Similar experiments produced values of 60, 58, and 46 minutes for the transport of 2,4-D from the exterior of leaves into the phloem.

Discussion

Presumably in the movement of 2,4-D from the leaf surface to the tissues of the epicotyl, penetration of the cuticle is followed by movement through the epidermis and mesophyll into the phloem, which serves as a path for transport from the leaf into the stem. It is probable that the stomata are not concerned in the entry of 2,4-D from water solutions (**5**). The recovery from bending could be due to movement of 2,4-D out of the affected section of the stem, the gradual equalization of its concentration on all sides of the epicotyl eliminating any differential effect of growth stimulation, or the breakdown or detoxification of the 2,4-D. In any case, recovery should be attributed to the normal mechanism of geotropism.

The entire process of translocation from leaf surface to epicotyl is accomplished in about 40 minutes less time than is necessary for the appearance of the first response in the epicotyl. This total time for translocation, amounting to about 70 to 80 minutes, is independent of the dose of 2,4-D applied to the leaf, and is correspondingly independent of the 2,4-D concentration gradient. Increased dose is expressed in greater amplitude of curvature. Higher rates of application apparently result in the absorption and movement of greater quantities of 2,4-D without increasing the velocity of translocation.

The leaf tissue underlying a droplet of 2,4-D solution contains numerous veinlets. According to DOUTT (**1**) the shortest path to the phloem from the upper epidermis of the bean leaf involves at least one palisade parenchyma cell and one or more cells of the bundle sheath. The minimum distance from the upper surface of the leaf to the phloem is about 25 to 30 μ. The distance that the 2,4-D must travel varies in practice, as not every part of the applied dose is directly over a vascular bundle. Rate experiments are interpreted as determining response from the first increment of 2,4-D moved into the phloem, this presumably representing movement over the shortest path. From the values of 63, 60, 58, and 46 minutes for movement from the droplet into the phloem, velocities of 25 to 35 μ per hour for translocation in nonvascular tissue may be assumed.

In the seedling bean, the leaf traces to the first true leaves merge in the pulvinus at the enlarged base of the petiole, and materials leaving the leaf pass through a common vascular bundle at that point (**1**). There are direct vascular connections at the second node from the common vascular bundle of the pulvinus to the opposite side of the epicotyl. Although no studies of translocation through individual vascular bundles were made, the consistent direction of curvature away from the treated side makes it apparent that transport was predominantly along direct rather than circuitous routes.

The velocity measurements of 13, 45, 106, and 49 cm. per hour indicate that rates of 50 or more centimeters per hour might be considered typical under the conditions studied. Speed of transport in the phloem is thus on the order of 5,000 to 25,000 times faster than movement in the mesophyll.

Summary

The curvature reaction of kidney bean seedlings was used as a measure of the translocation of 2,4-D from the leaves. When a small quantity of 2,4-D was applied to one leaf of a bean seedling under greenhouse conditions, curvature began in approximately two hours, and reached a maximum in about six hours, at which time recovery began. The time from application of 2,4-D to the beginning of curvature was independent of the amount of 2,4-D applied. The maximum curvature attained was a measure of the

amount of 2,4-D absorbed and translocated, and the rate of bending, once it began, was likewise determined by the dose.

It was found that the 2,4-D moved through the cuticle, epidermis, and mesophyll to the phloem with a velocity of approximately 30 μ per hour. Once inside the phloem it was translocated the several centimeters to the epicotyl at a velocity varying through a range of 10 to 100 cm. per hour. After the 2,4-D reached the epicotyl, about 40 minutes elapsed before curvature began, and when the supply of 2,4-D to the epicotyl was interrupted, an hour elapsed before recovery began. Under the conditions of these tests, the maximum amount of 2,4-D was absorbed by leaves in the first four and one half hours after treatment.

The writer is indebted to Dr. Alden S. Crafts for aid and counsel, to Dr. Herbert B. Currier for advice, and to Harold R. Drever for help with the experiments.

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