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Transport of the Auxin 2,4-Dichlorophenoxyacetic Acid Through Abscission Zones, Pulvini, and Petioles of Phaseolus vulgaris

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Summary. Measurements were made of the transport of 2,4-dichlorophenoxyacetic acid-^{14}C (2,4-D) through segments cut from the region of the distal abscission zone in young and old primary leaves of *Phaseolus vulgaris* L. When old leaves were used basipetal transport of 2,4-D in segments including pulvinar tissue, abscission zone, and petiolar tissue was much less than in wholly petiolar segments. In both young and old plants, segments consisting entirely of pulvinar tissue transported 2,4-D basipetally at a velocity about half that in petiolar tissue. At both ages the flux of 2,4-D through pulvinar tissue was less than that through petiolar tissue. In segments from old leaves the flux through pulvinar tissue was much less than in young plants; the flux through petiolar tissue changed little with age. There was no change with age in the velocity of basipetal transport. The distribution of ^{14}C along segments including the abscission zone showed no marked discontinuity. It was concluded that the pulvinus limited the basipetal movement of 2,4-D through segments from old leaves which included both pulvinar and petiolar tissue, but there was no evidence that the abscission zone itself was a barrier to auxin transport.

In studies of the hormonal factors that regulate abscission many experiments have been described using segments cut from the petioles of leaves of *Phaseolus vulgaris* (1, 7). These segments, which consist of the distal pulvinus and part of the petiole proximal to it, are by convention termed "explants" (2). Growth regulators are generally applied to one or other of the cut ends. Biggs and Leopold (3) have pointed out that when explants are treated in this way transport of the growth regulator from the point of application to the separation zone is probably necessary for it to be effective. However, little information is so far available either on the movement of applied growth regulators within explants, or on possible differences in such movement between explants from petioles at different stages of development. It is known that the amount of auxin transported by some tissues changes during aging (6, 9, 13, 14).

In experiments described by Rubinstein and Leopold (16), naphthaleneacetic acid-^{14}C (NAA) was applied to one or other end of a bean explant for 10 minutes to 20 hours and radioactivity was later determined in 2 mm sections of either pulvinar or adjacent petiolar tissue from the 2 sides of the abscission zone. After both distal and proximal applications made to segments undergoing a variety of experimental treatments it was always found that radioactivity was higher in the section which was nearer to the point of application of the NAA. However, the relative radioactivity on the 2 sides of the abscission zone varied considerably with the different treatments, and the overall distribution of ^{14}C within the explants was not described.

Various observations suggest that a significant physiological barrier may exist between the adjacent tissues of the pulvinus and the petiole in bean leaves. They differ strikingly in anatomical structure, as shown for instance in figure 2 in (5) and figures 8 and 9 in (4). Before abscission occurs the activity of pectin methylesterase may be as much as 3 times higher in pulvinar than in petiolar tissue (15). When the tissues are about to separate the pulvinus is usually yellowing while the petiole is still green, and this visible difference must indicate a physiological discontinuity.

The need for information on the transport of auxin in these anatomically and physiologically different tissues in relation to abscission has been emphasized elsewhere (7). It is not known whether pulvinus and petiole tissues transport auxin to the same extent, nor whether the cells of the abscission zone effect any regulation of either acropetal or basipetal movement of auxin. In an endeavor to study

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these questions we have investigated the movement of 1 auxin, 2,4-dichlorophenoxyacetic acid (2,4-D), both in pulvinus and petiole tissues and across the abscission zone in leaves of *Phaseolus vulgaris*. A brief account of this work was given at the 1962 International Horticultural Congress (8).

Materials and Methods

Seedlings of *P. vulgaris* L. var. Canadian Wonder were grown in a greenhouse. Segments were cut from the distal ends of the petioles of the primary leaves by means of a double-bladed cutter. In some experiments pulvinus-petiole segments 8 mm long, including pulvinar tissue, abscission zone, and petiolar tissue, were compared with sections of the same length cut from wholly petiolar tissue just proximal to the abscission zone, as shown in figure 1A. (Proximal and distal refer throughout the paper to position relative to the stem.) In other experiments, segments 2.8 or 3.3 mm long, cut from the distal pulvinus, were compared with segments of the same length cut from wholly petiolar tissue just proximal to the abscission zone, figure 1B. When these short segments were cut directly from leaves, the cut surfaces of the pulvinar tissues sometimes became sufficiently distorted to impair contact with the agar blocks applied to them. To minimize this source of error, in experiment 13 (table II and fig 3) longer pieces were first excised from the leaves so as to include a small part of the lamina and about 5 mm of the petiole. After 1.5 hours, a 2.8-mm segment was cut from the pulvinar region of each longer piece; subsequent distortion of the pulvinus was negligible.

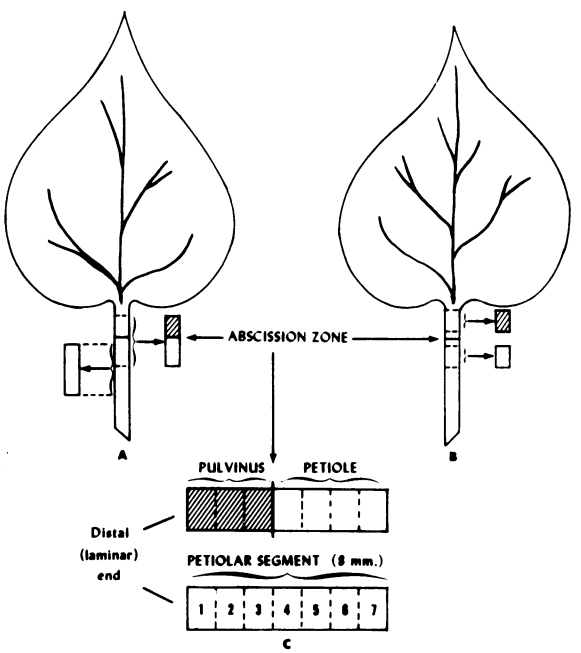

FIG. 1. Positions from which A) 8-mm segments with or without abscission zone, and B) 3-mm segments of pulvinar or petiolar tissue only, were excised from primary leaves of *Phaseolus vulgaris;* C) Diagram of method of dividing segments into 7 sections for determination of the distribution of ^{14}C in the tissue.

Potassium 2,4-dichlorophenoxyacetate-carboxyl-^{14}C was applied at a concentration of 20 μM (4.4 mg/liter) in a donor block of 1.5 % aqueous agar gel to 1 end of each segment. The blocks were usually 22.4 μl in volume; for sections from older leaves, larger blocks were sometimes used (33.4 μl). A receiver block of plain agar gel of the same size was applied to the other end of the segment. To measure basipetal movement a donor block was applied to the distal end of each segment and a receiver to the proximal end. In some experiments paired segments from the opposite petioles of each plant were used to compare basipetal and acropetal movement, with the arrangement of the blocks reversed. Sixteen replicate segments were used for each treatment. After the agar blocks had been applied the segments were kept in a humid atmosphere in darkness at 25°. At the end of an experiment each of the sets of 16 pooled agar blocks (basipetal donors, basipetal receivers, acropetal donors or acropetal receivers) was assayed for ^{14}C with an end-window Geiger-counter. For fuller details of the methods see (11). Estimates of the net uptake of 2,4-D into the tissue were obtained by subtracting the counts on donor blocks at the end of an experiment from counts on sets of 16 fresh donor blocks which had not been in contact with tissue. In experiments with wholly petiolar segments McCready (11) found that all the ^{14}C recovered in receiver blocks had the chromatographic properties of the original 2,4-D.

At the end of some experiments on 8-mm segments, the distribution of ^{14}C along them was determined as follows. With a razor blade each segment was subdivided into 7 sections of approximately equal length, as shown in figure 1C. For the pulvinus-petiole segments the separation between sections 3 and 4 was exactly at the abscission zone. Each set of 16 sections was rapidly weighed on a torsion balance and transferred to a glass homogenizer. One ml of water was added and the tissue was dispersed by hand grinding. An 0.8 ml portion of the suspension was pipetted on to an etched, tared aluminum planchet and dried under an infrared lamp. After reweighing, the planchets were assayed for ^{14}C with an end-window counter. The results were corrected for self-absorption by referring the dry weights of the samples to a calibration curve derived from a control experiment in which different amounts of nonradioactive tissue were ground in 1 ml aliquots of a solution of radioactive 2,4-D.

Each experiment was repeated 2 or more times with essentially the same results.

Results

Table I shows the basipetal and acropetal movement of 2,4-D from agar block to agar block through

Table I. *Basipetal and Acropetal Movement of 2,4-D-^{14}C through 8-mm Segments with or without Abscission Zones from Petioles of Young or Old Leaves*

Segments of 2 types were used: A) Pulvinus-petiole, including pulvinar tissue, abscission zone and petiolar tissue; B) wholly petiolar, consisting of petiolar tissue only, excised immediately proximal to the abscission zone. Duration of transport was 24 hours. Each result represents the corrected counts per minute from 16 agar blocks, given as means of duplicate treatments ± standard errors. Counts from the 2 experiments are not directly comparable.

Age of leaves:		Young (Expt 10)			Older (Expt 12)*		
Petiole length:		40–50 mm			66–100 mm		
Original donors:		23,253 cpm			32,831 cpm		
Block size:		22.4 µl			33.4 µl		
Direction of transport	Type of segment	Donors	Uptake	Receivers	Donors	Uptake	Receivers
Basipetal	Pulvinus-petiole	10,904 ± 28	12,349 ± 28	290 ± 12	25,560 ± 828	7,271 ± 828	16 ± 1
Basipetal	Wholly petiolar	5,079 ± 559	18,174 ± 559	290 ± 15	14,246 ± 203	18,585 ± 203	194 ± 9
Acropetal	Pulvinus-petiole	9,980 ± 370	13,273 ± 370	6 ± 4	16,634 ± 70	16,197 ± 70	25 ± 7
Acropetal	Wholly petiolar	9,798 ± 276	13,455 ± 276	6 ± 2	17,516 ± 76	15,315 ± 76	66 ± 8

* During this experiment the temperature accidentally fell from 25° to 21°.

8-mm segments cut either with or without including the abscission zones. Results are shown for 2 experiments in which plants at different stages of development were used, as indicated by the overall lengths of their petioles. As smaller agar blocks were used for the younger plants the data from the 2 experiments are not precisely comparable.

For both ages of leaves, uptake from distal donors into the pulvinar tissue of pulvinus-petiole segments including the abscission zone was less than such uptake into the petiolar tissue of wholly petiolar segments without an abscission zone; this was especially so in the segments from older plants. Uptake from proximal donors, which always took place into petiolar tissue, was unaffected by the inclusion of pulvinar tissue in the segments. In wholly petiolar segments, uptake from distal donors exceeded uptake from proximal donors, which could be explained at least in part by the considerably greater transport of 2,4-D in the basipetal direction, in accord with previous observations on this tissue (11).

In young plants basipetal transport through pulvinus-petiole segments equalled that through wholly petiolar segments, but in segments from older plants basipetal transport through pulvinus-petiole segments was reduced to about 8 % of that through wholly petiolar segments. This striking change with age, which was fully confirmed in other experiments, was much too large to be accounted for by the minor differences in procedure between the 2 experiments. Acropetal movement, like basipetal movement, was the same in the 2 types of segment from young plants, but in older plants acropetal movement was less in the presence of the abscission zone than in its absence, suggesting that the movement of auxin might be restricted either by the pulvinar tissue or at the abscission zone itself.

At the end of a similar experiment with plants at 2 stages of development the distribution of ^{14}C along both types of segment was determined and the results are shown in figure 2. Earlier experiments by McCready (11) showed that when 2,4-D at 5 mg per liter was supplied to bean petiolar segments 5.44 mm long, after 24 hours transport was still proceeding at nearly its maximum rate. It can therefore be assumed that figure 2 represents the distribution of ^{14}C in segments which are actively transporting 2,4-D-^{14}C. The generally lower level of ^{14}C in the pulvinus-petiole segments supplied at their distal ends is consistent with the lower distal uptake into pulvinar tissue shown in table I.

The graphs reveal no major discontinuity in the distribution of ^{14}C at the abscission zone. The curve for the younger segments supplied with proximal donors does show a slightly increased gradient at the abscission zone, but there is no reason to suppose that this is of any greater significance than comparable minor irregularities in other parts of the curves.

Table II and figures 3 and 4 give the results of 2 experiments on the progress of basipetal transport of 2,4-D. In each experiment short segments of wholly pulvinar and wholly petiolar tissue were compared. Segments for the 2 experiments were taken from plants at different stages of development. The lengths of the segments and the volumes of the agar blocks applied were smaller for the younger plants, so that again comparisons between the young and old plants can be made only in general terms.

Three aspects of these results may be considered in relation to possible differences in auxin transport between the 2 tissues. Firstly, uptake of 2,4-D from the donor blocks was greater into petiolar than into pulvinar segments at both stages of development, as was shown in table I. With both tissues uptake into young segments continued throughout the experiment, but uptake into older segments soon slowed down. However, in every instance the amount of 2,4-D entering the segments greatly exceeded the amount transported through them into the receiver blocks, so that there was no reason to suppose that transport was limited by uptake.

Secondly, the velocity of 2,4-D transport through

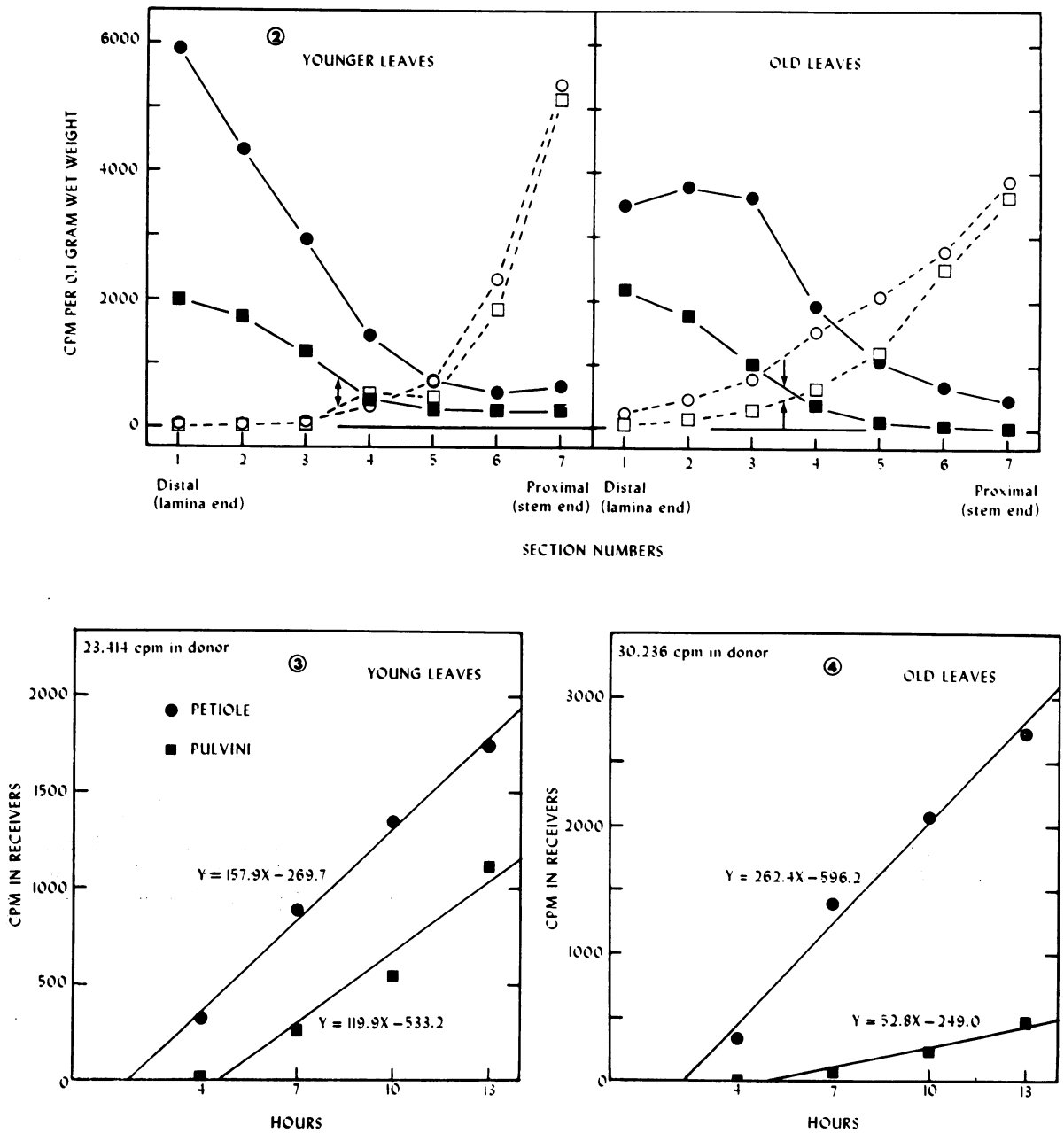

FIG. 2. Distribution of ^{14}C in 8-mm segments treated for 24 hours with 2,4-D-^{14}C at distal end (solid symbols) or proximal end (open symbols and dashed line). The circles represent data from wholly petiolar segments. The squares represent data from segments composed of pulvinar tissue (sections 1–3), abscission zone (location shown by arrows), and petiolar tissue (sections 4–7).

FIG. 3,4. Time course of accumulation of 2,4-D-^{14}C in receivers in contact with pulvinar or petiolar segments cut from young (fig 3) or old (fig 4) leaves. The calculated regression lines and equations are shown. For details see table II.

Table II. *Time Course of Basipetal Transport of 2,4-D-^{14}C through Pulvinar or Petiolar Segments from Young or Old Leaves*

Each result represents the corrected cpm from 16 agar blocks. Counts from the 2 experiments are not directly comparable.

Age of Leaves:	Young (Expt 13)			Older (Expt 11)		
Original donors:	23,414 cpm			30,236 cpm		
Block size:	22.4 μl			33.4 μl		
Segment length:	2.8 mm			3.3 mm		
Hours	Donors	Uptake	Receivers	Donors	Uptake	Receivers
			Pulvinus			
4	20,342	3072	18	26,036	4200	6
7	19,159	4255	266	26,036	4200	71
10	18,424	4990	540	25,688	4548	249
13	17,289	6125	1120	26,036	4200	472
			Petiole			
4	16,142	7272	329	21,722	8514	343
7	12,176	11,238	873	19,548	10,688	1394
10	10,122	13,292	1344	19,939	10,297	2070
13	6370	17,044	1744	18,974	11,262	2730

each tissue can be estimated following the principle of van der Weij (17); for details see (11), page 16. In figures 3 and 4 the straight lines are those of the closest fit obtained by the method of least squares. The calculated intercepts of these lines on the abscissae show that 2,4-D moved through young and old pulvini with velocities of 0.6 and 0.7 mm per hour respectively. The segments from corresponding young and old petioles gave velocities of 1.6 and 1.5 mm per hour respectively. Apparently, petioles transported 2,4-D at a higher velocity than pulvini, and within each type of organ there was no change with age in the velocity of basipetal transport.

Thirdly, the slope of each fitted line is proportional to the average net flux of 2,4-D entering the receivers from the segments. At both ages the flux through petiolar segments exceeded that through pulvinar segments. Comparing old with young plants, the flux through petiolar tissue showed an increase with age rather more than proportional to the increased supply of 2,4-D in the larger donors. In marked contrast, the flux of 2,4-D through old pulvini was less than half that through young ones, despite the larger amount supplied. In young plants the flux through pulvini was 76 % of that through petioles; in old plants this ratio fell to 20 %.

Discussion

When old leaves were used the basipetal movement of 2,4-D-^{14}C through 8-mm pulvinus-petiole segments was much less than that through wholly petiolar segments, so that either the pulvinar tissue or the abscission zone seemingly limited transport. As the distribution of ^{14}C along pulvinus-petiole segments showed no marked discontinuity at the abscission zone, transport appeared to be limited not by the abscission zone itself but rather by the whole of the pulvinar tissue. When young leaves were used basipetal movement through the 2 types of 8-mm segment was the same, and the development of a difference in the older leaves was presumably due to a decrease in the ability of the pulvinar tissue to transport 2,4-D.

These deductions from the longer segments about transport through the pulvinus are open to 2 possible criticisms. In the first place they are based on measurements of the amounts of 2,4-D transported during an arbitrarily fixed time. Any difference in the amount transported might be due solely to a difference in the velocity of transport, and the flows of auxin through the 2 tissues after a steady state had been reached might be equal. In the second place it was difficult to ensure that pulvinus-petiole segments from young and old plants included exactly the same proportion of pulvinar tissue, and if the latter did limit transport, an apparent change with age might be due merely to a change in the relative lengths of pulvinar and petiolar tissue in the segments.

These objections do not apply to the more direct comparisons of transport through the 2 regions using short segments cut wholly from pulvinar or wholly from petiolar tissue. A time study of the movement of 2,4-D through these segments enabled velocity and flux to be separately compared. Both were less in pulvinar tissue; flux, but not velocity, decreased in pulvinar segments from the older leaves. These observations confirmed that the difference in transport between the longer pulvinus-petiole and wholly petiolar segments from old leaves could be accounted for by differences in transport through the pulvinar tissue. It is unnecessary to suppose that the abscission zone itself is a barrier to the transport of 2,4-D.

The present investigation has shown that when auxins are applied to the cut ends of explants of the kind used in experiments on abscission the amount of auxin transported through the tissue and its concentration in various regions of the segment will de-

pend in part on the age of the tissue. This is particularly relevant to experiments in which a growth regulator is applied to an explant 18 hours after cutting (16), when some of the biochemical and physiological changes associated with senescence may already have taken place.

In considering the possible bearing of these findings on auxin movement in the intact plant, it must be remembered, firstly, that the slow polar movement observed in segments excised from petioles is not the only way in which auxin leaves the lamina. Little and Blackman (10) have shown that IAA applied to the laminae of entire seedlings of *Phaseolus* was transported to the hypocotyl at a much higher velocity, 20 to 24 cm per hour, suggesting movement by a mechanism different from that found in segments excised from *Phaseolus* petioles, in which IAA moves with a velocity of about 6 mm per hour (12). Secondly, although in both these transport systems the movement of 2,4-D resembles in several ways that of IAA (10, 12) it cannot be assumed that the behaviour recorded here for 2,4-D would necessarily be the same for IAA. Nevertheless it seems reasonable to suppose that changes in auxin transport through different tissues such as have been demonstrated in the present experiments must affect the distribution of endogenous auxin in and around abscission zones.

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