Transport of the Auxin, Picloram, Through Petioles of Bean and Coleus and Stem Sections of Pea

R. F. Horton and R. A. Fletcher[1]
Department of Botany, University of Guelph, Guelph, Ontario, Canada

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Abstract. **The transport of the synthetic auxin, picloram (4-amino-3,5,6-trichloropicolinic acid) was investigated in sections of petioles of *Phaseolus vulgaris* L. and *Coleus blumei* Benth. and stems of *Pisum sativum* L. Transport of ^{14}C-picloram was basipolar in all tissues, although the degree of polarity was dependant on age. The velocity of picloram movement was calculated at between 0.75 and 1.11 mm/hr. The amount moved in a given time, the flux, was dependant on the concentration applied and the length of the sections used. Picloram did not appear to be metabolized by the tissues during the transport experiments. When compared to the movement of other growth regulators, picloram transport bears marked similarities to that of 2,4-dichlorophenoxyacetic acid.**

Picloram (4-amino-3,5,6-trichloropicolinic acid), a synthetic growth regulator with a unique chemical structure (3) acts as a potent auxin in a variety of test systems when used at low concentrations (7). Picloram, which has been used extensively as a herbicide in recent years, appears to be absorbed and translocated readily after foliar application (3, 4). In the present study the transport of picloram was investigated for 2 specific reasons. Extensive investigations of the phytotoxic effects of high concentrations of picloram have established that this compound is not metabolized rapidly, if at all, by plant tissue. One of the problems encountered in physiological studies of growth regulator transport is the occurrence of varying degrees of degradation of the transported molecule during uptake and within the transport system itself (2, 10, 11). Picloram, because of its apparent chemical stability within the tissue, affords the possibility of studying auxin transport in a more simplified system. It is also important to determine whether the capacity of a substance to be transported in plants in a polar manner is a fundamental property of any molecule which exhibits auxin activity. Kefford and Caso (7) have pointed out that picloram possesses a chemical structure which is markedly different from that of any other natural or synthetic auxin.

It is desirable that synthetic growth regulators with a phytotoxic action which in part is dependant upon their chemical stability within plants, are also investigated in terms of their usefulness as tools in a further understanding of a basic physiological phenomena. The technique of applying an isotopically labeled growth regulator in agar blocks to either end of a tissue section and measuring the subsequent transport into plain agar receiver blocks placed at the opposite end allows an accurate determination of the course of transport over short time periods, together with an estimation of the amount of uptake from donor blocks (1, 2, 8, 10, 11, 12). McCready and Jacobs (11) have pointed out that most of the information concerning the movement of the native auxin, IAA, comes from such experiments, whilst ideas on the movement of phytotoxic growth regulators are derived largely from experiments using more nearly intact plants.

In the present study, several initial experiments, which established the polarity of picloram movement in plant tissues, were carried out with sections excised from *Coleus* and pea plants. The basipolar movement of isotopically labeled picloram was then investigated more fully in sections cut from the petioles of primary leaves of beans.

Materials and Methods

Tissue sections were excised from the expanding petioles of 6 to 8 day old plants of *Phaseolus vulgaris* L. Contender. In experiments to investigate the transport of picloram in petioles of different ages, 4 to 26 day old plants were used. In some experiments, sections were prepared from petioles of *Coleus blumei* Benth and from just below the apex of 8 day old plants of *Pisum sativum* L. Little Marvel. All the plants used were grown in a greenhouse.

Gels of 1.5 % agar containing the potassium salt of ^{14}C-carboxyl-labeled picloram (specific activity 4.25 μc/mg) were sectioned into cylindrical blocks of 20 μl volume. A donor block containing ^{14}C-picloram was applied to one end of a tissue section.

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A plain agar receiver block was placed at the other end. The sections were laid across the 2 mm gap between 2 glass slides resting on a layer of 3 % agar in a 9 cm petri dish and kept in the dark at 25°. After the transport period the donor and receiver blocks from 10 sections were pooled and assayed for radioactivity by the methods described by McCready (9). Two groups of 10 blocks of each sample of active agar were assayed to determine the amount of radioactivity applied initially to the sections. The self-absorbance of the radioactive samples of dried agar was assumed to be uniform.

In investigations of the transport of isotopically-labeled growth regulators it is essential to determine whether the radioactivity remains in the form of the applied chemical throughout the experiment. After 48 hr of transport from agar blocks containing 5 mg/l ^{14}C-picloram, 20 tissue sections and the 4 corresponding groups of agar blocks were repeatedly eluted with ether and the eluate cochromatographed with picloram on silica gel thin layer plates in phenol-water (5:1 v/v). The developed plates were subdivided into 10 equal bands between the origin and the solvent front. The gel from these bands was removed and suspended in 15 ml of scintillation fluid (5 g 2,5-diphenyloxazole in 1 liter toluene). Radioactivity was determined in a Unilux II liquid scintillation counter.

Results and Discussion

The transport of picloram was shown to be basipolar in sections from bean and *Coleus* petioles and stems of peas (table I). In all 3 tissues the amount of radioactivity in the basipetal receiver blocks was at least 3 times greater than that in acropetal receiver blocks. Furthermore, polarity was also exhibited in the uptake of ^{14}C-picloram from blocks placed at the physiologically apical or basal ends of the sections.

The time course of transport of picloram applied to bean petiole sections at 3 different initial concentrations is shown in figure 1. For comparative purposes the amounts of radioactivity in the receiver blocks have been plotted on scales proportional to the applied picloram concentrations. After an initial lag period, the basipetal transport of picloram continued at a fairly steady rate which was maintained for the 12 hr duration of the experiment. Although polarity of movement occurred at each concentration at each time of determination, there are significant differences in the uptake and subsequent transport of picloram after application at different initial concentrations. While 12.7 % of the picloram applied at 1 mg/l moved through the tissue in 12 hr, only 8.7 % appeared in the receiver blocks after application at 5 mg/l. This difference may be due in part to the limited ability of the tissue to absorb picloram at the cut surface, or to the limited capacity of the transport system itself. As uptake has obviously occurred at a changing rate from blocks containing changing concentrations of picloram (fig 2), a detailed analysis of the data is difficult. However, it was clear that more than half the applied picloram had left the basipetal donor blocks during the 12 hr period. Uptake was again polar; more picloram had left the basipetal donors than the acropetal donors.

The degree of polarity of picloram transport decreased with increasing tissue age in both bean and *Coleus* petioles (table II). Sections which showed marked polarity of transport also exhibited stimulated elongation during the 24 hr treatment

Table I. *Basipetal and Acropetal Movement of ^{14}C-picloram in Petole Sections of Bean and* Coleus *and Stem Sections of Pea*

The total radioactivity supplied was 2896 cpm. The sections were 3.2 mm in length. The transport period was 24 hr.

	Transport	Radioactivity in block: Donor blocks	Receiver blocks
		cpm	*cpm*
Phaseolus vulgaris (petioles)	Basipetal	1164	763
	Acropetal	1935	55
Coleus blumei (petioles)	Basipetal	2052	435
	Acropetal	2362	16
Pisum sativum (stem)	Basipetal	1680	523
	Acropetal	2195	151

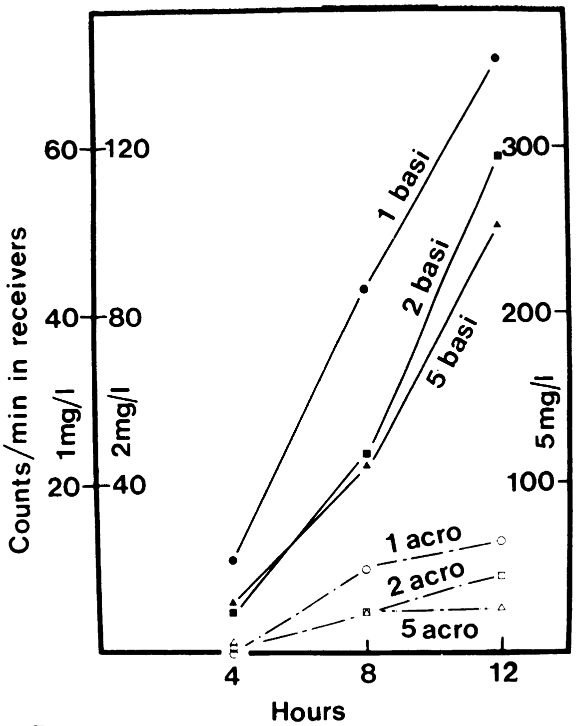

FIG. 1. Time course of acropetal (acro) and basipetal (basi) movement of ^{14}C-picloram into receiver blocks. Picloram supplied at 1 mg/l (552 cpm), 2 mg/l (1167 cpm) and 5 mg/l (2896 cpm) to sections 3.2 mm in length cut from petioles of primary leaves of *Phaseolus*.

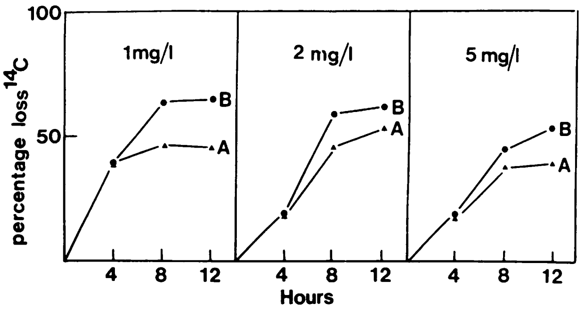

FIG. 2. Time course of the loss of ^{14}C-picloram from basipetal and acropetal donor blocks placed on 3.2 mm bean petiole sections. Data from same experiment as figure 1. A) Acropetal donors, B) basipetal donors.

with picloram. Because of the progressive increase with age in the diameter of the tissue sections particular care must be taken in interpreting the data. It appears that the decreased polarity of picloram in older bean petioles is due to an increase in acropetal transport. In contrast, the decreasing polarity in *Coleus* is associated with a decline in basipetal transport. Therefore, the pattern of transport of picloram in these tissues is similar to that of IAA and 2,4-D in that the overall degree of polarity is dependant on the age of the tissue and its correlated ability to elongate under the influence of applied auxin (5). Furthermore, the physiological reasons for the decline in the degree of polarity in older bean petioles is due to a different set of factors from those regulating the decline in *Coleus* petioles (12).

Values for the mean velocity and flux of picloram movement can be derived from the data for the course of transport. To ensure the validity of the derivations it was first necessary to examine the nature of the lag period occurring between application of the donor blocks and the establishment of a steady rate of accumulation of radioactivity in the receiver blocks. There are at least 2 possible reasons for this lag period (10). Firstly, processes occurring at the ends of the sections, such as recovery from injury or saturation time of immobilization sites, and, secondly, the time taken for picloram to be transported through the length of the sections. In order to distinguish between these 2 possibilities the course of transport through sections of different lengths was investigated (fig 3). The line of best fit of the experimental data can be calculated by the method of least squares. The intersect of the derived line and the time axis will indicate the average time taken for molecules of picloram to be transported through the sections. The calculated times for transport through the 3 sections are 2.67, 5.70, and 7.69 hr; these times are roughly in the same proportion as the initial section lengths. On this evidence it was concluded that the time taken for transport through the sections, rather than immobilization at the cut surface, was the major reason for the delay in detectable radioactivity appearing in the receiver blocks. The data shows that the subsequent rate of picloram transport into basipetal receiver blocks decreased with increasing lengths of the tissue sections.

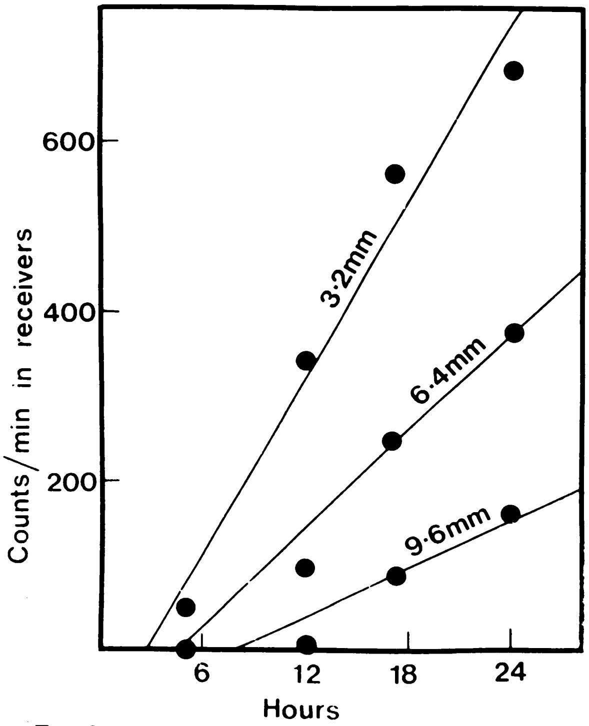

FIG. 3. Time course of basipetal movement of ^{14}C-picloram into receiver blocks. Picloram supplied at initial concentrations of 5 mg/l to *Phaseolus* petiole sections of different lengths.

Table II. *Basipetal and Acropetal Movement of* ^{14}*C-picloram Through Petiole Sections of Different Ages*

The total radioactivity supplied was 2896 cpm. The sections were 3.2 mm in length. The transport period was 24 hr.

Age of petioles (days)	*Phaseolus vulgaris* 4	8	14	26	*Coleus blumei* Young	Old
Mean length of petioles (mm)	12.9	37.3	56.2	64.1	8-16	29-51
Counts/min in receivers						
Basipetal	687	708	996	827	397	223
Acropetal	13	56	531	641	16	101
Polarity (A/B × 100)	1.9	7.9	53.3	77.5	4.0	45.3
% Elongation after picloram application at apex	18.8	12.5	3.1	0	28.1	3.1
% Elongation after picloram application at base	18.8	6.3	3.1	0	15.6	0

The velocity of basipetal transport in bean petioles derived from the line of best fit of the data, remained relatively constant at between 0.75 and 1.11 mm/hr in bean petioles for a range of section lengths and initial concentrations of applied picloram (table III). 2,4-D is transported at a velocity of 0.6 to 1.0 mm/hr in bean petioles, while IAA moves at a velocity of up to 6 mm/hr (10, 11).

Table III. *Velocity and Flux of Basipetal Movement of* ^{14}*C-picloram in Petiole Sections of Bean*

Data from same experiments as figure 1 and figure 3.

Sampling times (hrs)	4,8,12			5,12,17,24		
^{14}C-picloram (mg/l)	1	2	5	5	5	5
Section length (mm)	3.2	3.2	3.2	3.2	6.4	9.6
Velocity (mm/hr)	0.75	1.11	1.02	0.82	0.89	0.80
Flux (cpm/mm/hr)	7.4	13.3	27.6	35.4	20.6	9.1

The slope of the line of best fit calculated from the data gives an estimation of the flux of picloram movement. The flux was dependant on both the concentration of picloram applied and the length of the tissue sections. The relationship between flux and section length has been noted for 2,4-D transport (1, 8, 10). The course of picloram transport in bean petioles bears a marked similarity to that of 2,4-D with a correspondingly low velocity when compared to that of IAA. The acropetal velocity of picloram movement appears to be very similar to the basipetal velocity although the flux is considerably lower. Picloram, like 2,4-D (10), continues to move into basipetal receiver blocks after 24 hr of transport, whilst IAA transport (11) reaches a maximum after only 8 to 12 hr.

Chromatographic analysis of extracts of tissue sections and agar blocks after an extended transport period showed detectable radioactivity only at R_F 0.5 which corresponded to the R_F of synthetic picloram in the system employed. Recent experiments by Hamill (personal communication) using ^{14}C-picloram at phytotoxic concentrations on bean plants show that radioactivity is not lost from the tissue as CO_2. It was concluded that the radioactivity remains in the form of ^{14}C-picloram throughout the experiment. The apparent difference in the transport of synthetic and natural auxin may, in part, be due to the more rapid breakdown of IAA in the tissues, and subsequent immobilization of radioactive products. Some radioactivity from applied ^{14}C-2,4-D may also become immobilized in the tissue (10). The inability of the tissue to metabolize picloram may explain the fact that a very high proportion—up to 30 %—of the applied picloram can be transported into basipetal receiver blocks within 24 hr.

Jacobs (5, 6) has pointed out that the crucial factor in the difference between IAA and 2,4-D transport in *Coleus* petioles may lie in the preferred path of movement. More 2,4-D moves basipetally through cambial and vascular tissues, whilst IAA is transported through pith parenchyma. The prerequisite of active cambial proliferation for 2,4-D transport may explain the ineffectiveness of the compound as a herbicide for control of monocotyledonous plants. The high flux of the polar transport of picloram may be the result of such vascular transport accompanied by a low degree of breakdown and immobilization.

Acknowledgment

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Literature Cited

1. De La Fuente, R. F. and A. C. Leopold. 1966. Kinetics of polar auxin transport. Plant Physiol. 41: 1481–84.
2. Goldsmith, M. H. M. and K. V. Thimann. 1962. Some characteristics of movement of IAA in coleoptiles of *Avena*. 1. Uptake, destruction, immobilization, and distribution of IAA during basipetal translocation. Plant Physiol. 37: 492–505.
3. Hamaker, J. W., H. Johnston, R. T. Martin, and C. Redemann. 1963. A picolinic acid derivative: a plant growth regulator. Science 141: 363.
4. Merkle, M. G. and F. S. Davis. 1967. Effect of moisture stress on absorption and movement of picloram and 2,4,5-T in beans. Weeds 15: 10–12.
5. Jacobs, W. P. 1967. Comparison of the movement and vascular differentiation effects of the endogenous auxin and of phenoxyacetic acid weedkillers in stems and petioles of *Coleus* and *Phaseolus*. Ann. N. Y. Acad. Sci. 155: 102–17.
6. Jacobs, W. P. and C. C. McCready. 1967. Polar transport of growth-regulators in pith and vascular tissues of *Coleus* stems. Am. J. Botany 54: 1035–40.
7. Kefford, N. P. and O. H. Caso. 1966. A potent auxin with unique chemical structure—4 amino-3,5, 6-trichloropicolinic acid. Botan. Gaz. 127: 159–63.
8. Leopold, A. C. and R. K. De La Fuente. 1967. The polarity of auxin transport. Ann. N. Y. Acad. Sci. 144: 94–101.
9. McCready, C. C. 1958. A direct-plating method for the precise assay of carbon-14 in small liquid samples. Nature 181: 1406.
10. McCready, C. C. 1963. Movement of growth regulators in plants. 1. Polar transport of 2,4-dichlorophenoxyacetic acid in segments from the petioles of *Phaseolus vulgaris*. New Phytologist 62: 3–18.
11. McCready, C. C. and W. P. Jacobs. 1963. Movement of growth regulators in plants. II. Polar transport of radioactivity from indoleacetic acid-(^{14}C) and 2,4-dichlorophenoxyacetic acid-(^{14}C) in petioles of *Phaseolus vulgaris*. New Phytologist 62: 19–34
12. McCready, C. C. and W. P. Jacobs. 1963. Movement of growth regulators in plants. IV. Relationships between age, growth and polar transport in petioles of *Phaseolus vulgaris*. New Phytologist 62: 360–66.
13. Schrank, A. R. 1968. Growth and geotropic responses of *Avena* coleoptiles to 4-amino-3,5,6-trichloropicolinic acid. Physiol. Plantarum 21: 314–22.