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Influence of 2,3,5-Triiodobenzoic Acid and 1-N-Naphthylphthalamic Acid on Indoleacetic Acid Transport in Carnation Cuttings: Relationship with Rooting

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Abstract. ^{3}H-IAA transport in excised sections of carnation cuttings was studied by using two receiver systems for recovery of transported radioactivity: agar blocks (A) and wells containing a buffer solution (B). When receivers were periodically renewed, transport continued for up to 8 h and ceased before 24 h. If receivers were not renewed, IAA transport decreased drastically due to immobilization in the base of the sections. TIBA was as effective as NPA in inhibiting the basipetal transport irrespective of the application site (the basal or the apical side of sections). The polarity of IAA transport was determined by measuring the polar ratio (basipetal/acropetal) and the inhibition caused by TIBA or NPA. The polar ratio varied with receiver, whereas the inhibition by TIBA or NPA was similar. Distribution of immobilized radioactivity along the sections after a transport period of 24 h showed that the application of TIBA to the apical side or NPA to the basal side of sections, increased the radioactivity in zones further from the application site, which agrees with a basipetal and acropetal movement of TIBA and NPA, respectively. The existence of a slow acropetal movement of the inhibitor was confirmed by using ^{3}H-NPA. From the results obtained, a methodological approach is proposed to measure the variations in polar auxin transport. This method was used to investigate whether the variations in rooting observed during the cold storage of cuttings might be related to changes in polar auxin transport. As the storage period increased, a decrease in intensity and polarity of auxin transport occurred, which was accompanied by a delay in the formation and growth of adventitious roots, confirming the involvement of polar auxin transport in supplying the auxin for rooting.

Key Words. *Dianthus caryophyllus*—Indoleacetic acid—Naphthylphthalamic acid—Polar auxin transport—rooting of cuttings—Triiodobenzoic acid

Introduction

In stems, the endogenous auxin, indoleacetic acid (IAA), as well as synthetic analogues move in a polar manner, from apex to base. To explain this polar auxin transport (PAT), Rubery and Sheldrake (1974) and Raven (1975) proposed a chemiosmotic hypothesis based on the existence of specific efflux carriers located on the basal side of transporting cells. Most evidence suggests that PAT occurs in vascular cambium and its partially differentiated products (Morris and Thomas 1978), although polar transport in other cells has also been reported (see review by Lomax et al. 1995). PAT is assumed to play a decisive role in regulating different processes of plant development such as vascular differentiation (Aloni 1988), adventitious root formation (Katsumi et al. 1969; Liu and Reid 1992) or stem growth (Sánchez-Bravo et al. 1991, 1992). A recent review (Estelle 1998) emphasized that the spatial and temporal regulation of auxin transport might be a key aspect in many growth processes. The use of chemicals capable of inhibiting polar transport became a valuable and powerful tool in demonstrating the involvement of PAT in such processes. Two of the most frequently used inhibitors are 2,3,5-triiodobenzoic acid (TIBA) and the phytotropin, 1-N-naphthylphthalamic acid (NPA). These compounds specifically bond to sites other than the IAA binding site in the efflux carrier but unlike NPA, TIBA itself can be polarly transported (see review of Lomax et al. 1995).

Abbreviations: IAA, Indoleacetic acid; I_T and v, Intensity and velocity of IAA transport; NPA, 1-N-naphthylphthalamic acid; PAT, polar auxin transport; TIBA, 2,3,5-triiodobenzoic acid.

We dedicate this paper to the memory of Prof. F. Sabater García, a wonderful scientist and human being, who died November 1997.

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Different procedures have been proposed to investigate PAT by using sections isolated from different plant organs (shoots, roots, petioles, flower stalks etc.). Most of the experimental approaches are modifications of the well-established method of Van der Weij (1932). In this method, sections are placed upright or inverted (to study basipetal or acropetal auxin transport, respectively) on a receiver system (agar blocks or wells containing a buffer solution); radioactive auxin is applied to the upper end and radioactivity is recovered in the receiver and measured. Some studies monitor the time course of transport over a period whereas other studies measure the IAA transported over a fixed time. There is also divergence as to the application of inhibitors, and both application to the apical and basal side of the sections has been recommended. Parameters such as the polar ratio (the ratio between the basipetal and the acropetal transport) and the inhibition produced by TIBA or NPA have been used to determine the overall polarity of auxin transport. In spite of all this diversity, no systematic study has determined the efficiency of the different methods or evaluated the utility of parameters as qualitative and/or quantitative indexes of the polarity of auxin transport. Comparative studies showing the differences in the action of TIBA and NPA are also scarce (Thomson et al. 1973).

In the present work we have used different procedures to study the polar transport of IAA and the action of TIBA and NPA in sections from carnation cuttings. Most studies on PAT used seedlings and primary shoots, although cuttings from secondary shoots are used in commercial production of rooted plants such as carnations. A previous study (Garrido et al. 1996) suggested that the variations in rooting observed in carnation cuttings might be mediated by changes in auxin transport. In the present work we discuss the ability of different procedures and parameters to qualitatively and quantitatively measure PAT and to describe some variations in auxin transport that might improve the rooting process in commercial carnation production.

Materials and Methods

Plants

Carnation (*Dianthus caryophyllus* L. cv Virginie) cuttings (Barberet & Blanc, Puerto Lumbreras, Murcia, Spain) were pinched from mother plants and stored for a week in a cold chamber as described by Garrido et al. (1996). Cuttings from 9- and 5-month-old mother plants were used. Ten-mm long sections extending from 8 mm below to 2 mm above the oldest node of the cuttings (see inset in Fig. 2) were used to study the IAA transport.

Transport of IAA

IAA transport was studied following the method of Van der Weij (1932) as described previously (Sánchez-Bravo et al. 1992) with some modifications. Labeled IAA (5 μL aqueous solutions 0.14 μM (5-^{3}H)-IAA) was applied to the upper cut surface of excised sections. Preliminary experiments showed that application of IAA through a donor agar block or as a solution deposited on the cut surface had a similar effect on IAA transport. Individual sections were introduced into conical plastic tubes to ensure their vertical position and then placed on a receiver system to recover transported radioactivity at different time periods. Two receiver systems were used: solid agar blocks (A) and wells containing a buffer solution (B). In (A), the lower end of the section was placed on a cylindrical (8.5 mm diameter, 5.0 mm thickness) block of agar (1% in water), that was replaced at hourly intervals for up to 6 h; the transport from 6 to 8 h and from 8 to 24 h was also measured. In (B), the section was immersed to a depth of 1 mm in a well containing 250 μL phosphate buffer pH 6.5; the receiver (well with buffer) was replaced at the same time intervals as in (A). The same section was used for the different transport periods. At a given time period (t), the sum total of the radioactivity in the replaced receivers (obtained from the same section) from 0 to t h (i.e. Σ_0^tBq) was plotted to obtain the corresponding exponentially shaped transport curves (Figs. 1 and 3). Each point on the curves corresponded to the mean value ± SE of 4–5 sections. The linear parts of these curves were fitted by the least squares method. From the linear regression equations ($y = mx + b$), the intercept with the × axis ($t_o = -b/m$) served to determine the transport velocity (v in mm h^{-1}): v = length of sections/t_o. The slope (m) of the equations represents the transport intensity (I_T in Bq h^{-1}). IAA transport during a 6 h period with no renewal of the receiver system (well with buffer) was also studied. Basipetal and acropetal IAA transport was studied by placing the section in normal (upright) and inverted position, respectively. The influence of TIBA and NPA on IAA transport was studied as follows: in (A), 5 μL of a solution 100 μM in TIBA or NPA were added to the upper end of the section 10 min before IAA application; in (B), inhibitors were added to the buffer, the final concentration being 2 μM. Cuttings used in the experiments in

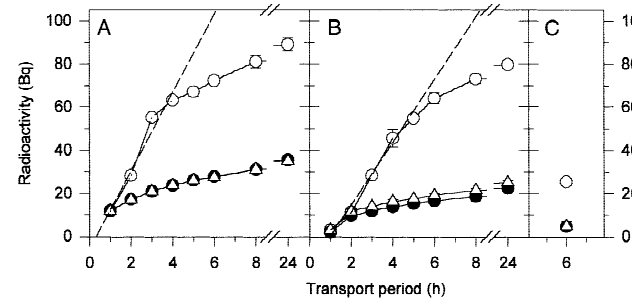

Fig. 1. Basipetal and acropetal transport of IAA and influence of NPA. Sections from carnation cuttings pinched from 9-month-old mother plants were used. Upright (for basipetal) or inverted (for acropetal transport) sections were put on agar blocks (A) or immersed to a depth of 1 mm in buffer contained in a well (B and C). ^{3}H-IAA was applied to the upper end of the sections. The radioactivity recovered in the receiver (agar blocks or wells with buffer) was measured at different time periods. The receiver was periodically renewed (A and B) or maintained with no renewal (C). NPA was applied to the upper end of the sections 10 min before IAA application in A; in B and C the inhibitor was applied to the lower end by adding to the buffer. The radioactivity measured in the receiver at the different time periods was totaled and plotted to obtain the transport curves. Each point corresponds to the mean values±SE of 4–5 sections. Basipetal transport in the absence (○) or presence (△) of NPA; acropetal transport (●). The linear parts of the transport curves were fitted by the least-squares method (discontinuous lines).

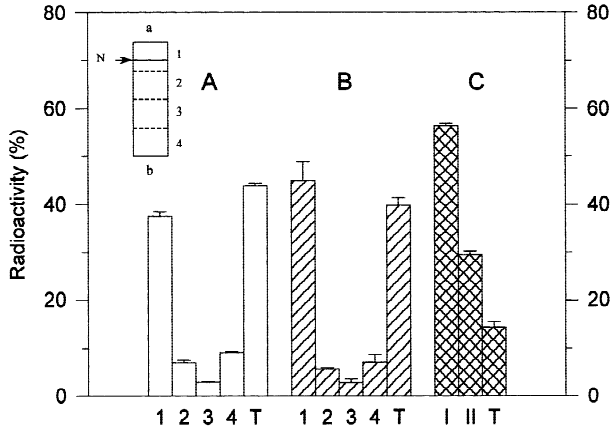

Fig. 2. Longitudinal distribution of radioactivity immobilized after basipetal transport. Data correspond to the assays A (white), B (diagonal hatching) and C (diagonal crosshatching) presented in Figures 1A, B, and C, respectively. At the end of the transport period (24 h in A and B, 6 h in C) the sections were divided into equal segments. Four 2.5 mm zones designed as 1, 2, 3, and 4 starting from the apical end were used in A and B, and two 5 mm segments (I and II, equivalents to zones 1+2 and 3+4, respectively) in C. Individual segments were immersed in scintillation cocktail. The radioactivity present in each zone was measured and expressed as percentage of the total recovered in the section. The percentage of radioactivity transported at 24 h (T) is also presented. Mean values ± SE of 4-5 sections. Inset: schematic drawing of a carnation cutting section; a, apical end; b, basal end; N, node; dotted lines delimit the zones 1, 2, 3 and 4 into which the sections were divided.

Figs. 1 and 3 were obtained from 9- and 5-month-old mother plants, respectively.

At the end of the transport period (24 h in most experiments), the sections were cut in four 2.5 mm segments designated as zones 1, 2, 3, and 4, starting from the apical end (see inset in Fig. 2) to determine the longitudinal distribution of the radioactivity not transported. Since negligible radioactivity was still being transported at 24 h (see Results), the remaining radioactivity at this time must have been trapped by the section tissues. Bearing this in mind, the term immobilization refers to the radioactivity trapped at any point along the sections when transport had stopped.

Transport of NPA

The transport of NPA was studied following the procedure described for IAA transport. Labeled NPA (5 μL aqueous solution 0.07 μM 2, 3, 4, 5 (n) ^{3}H-NPA, 330 Bq) was applied to the upper end of the section (in assay A) or added to the buffer in the well (in assay B). Transport was studied in upright and inverted sections. Receivers were periodically renewed and their radioactivity measured. At the end of the transport period (24 h), the sections were divided into four segments as described above and the radioactivity in each segment was measured.

Rooting of Cuttings

Cuttings from 9-month-old mother plants were planted in a substrate of peat-perlite (70:30, v/v) in the greenhouse under conditions described by Garrido et al. (1996). Eleven days after planting, the percentage of rooting and of rooted plants exhibiting commercial quality were calculated. Commercial quality in rooting was characterized by the presence of 30–40 well developed roots of 1–9 cm in length (Garrido et al. 1996). Data correspond to the mean value ± SD of three replicates (20 plants per sample). There were no roots or root primordia in the cuttings before planting.

Measurement of Radioactivity

The receivers (agar blocks or wells with buffer) as well as the section segments were immersed in separate vials containing 10 mL of cocktail (Biogreen 102, Scharlau, F.E.R.O.S.A, Barcelona, Spain) and stored in darkness at room temperature for 24 h before measuring the activity of ^{3}H in a Rack Beta, model 1211, liquid scintillation counter (LKB, Turku, Finland), as described previously (Sánchez-Bravo et al. 1988).

Chemicals and Radiochemical

Radioactive IAA, (5-^{3}H)IAA, SA 926 GBq mmol^{-1}, was obtained from Amersham International (Amersham, Buckinghamshire, UK). Radioactive NPA, 2, 3, 4, 5 (n)-^{3}H-NPA, SA 2,146 GBq mmol^{-1}, was from American Radiolabeled Chemical, Inc, St Louis, MO, USA. Aqueous solutions were prepared as described by Sánchez-Bravo et al. (1988). The radiochemical purity was checked periodically by TLC and only unaltered solutions were used. TIBA was from Calbiochem and NPA from Uniroyal Chemical. Other reagents and solvents were of analytical grade.

Statistical Analysis

Experiments were repeated at least twice. Fisher's least significant difference (LSD) multiple range test was used to analyze the variance. Data showing significant differences at the 95% confidence level were taken into account in the interpretation of the results.

Results

Transport and Immobilization of IAA in the Sections

Data in Figures 1A and B show that the amount of IAA basipetally transported increased with time up to 8 h when the receiver system (agar blocks in A, wells with buffer in B) was periodically renewed. Transported radioactivity from 8 to 24 h was less than 10% of that transported during the first 8 h period. This fact suggests that IAA transport in these assays was extremely low after 8 h and had presumably stopped by 24 h. Although the values of I_T showed no significant difference (Table 1), the amounts of radioactivity basipetally transported after 4 h were higher in assay A than in B (Fig. 1), which suggests that in the present assay conditions, the agar blocks were more efficient receivers of transported radioactivity than the buffer in wells. The radioactivity transported at 6 h when the receiver (well with buffer) was not renewed (25.4 Bq according to Fig. 1C) was clearly lower than that obtained during the same time period when the receiver was renewed at hourly intervals

Table 1. Transport of IAA and inhibition by NPA.

Assay	I_T (Bq · h^{-1})	V (mm · h^{-1})	Polar ratio	Inhibition (%)	Immobilization (%)		
				NPA	Basipetal	Acropetal	NPA
A	18.1 ± 2.5^{a}	31.2 ± 5.5^{a}	2.5 ± 0.1^{a}	61.8 ± 1.3^{a}	56.2 ± 0.7^{a}	81.2 ± 0.4^{d}	81.6 ± 1.4^{d}
B	14.5 ± 1.6^{a}	10.2 ± 3.5^{b}	3.4 ± 0.1^{b}	67.9 ± 2.1^{a}	60.3 ± 1.6^{b}	$88.2 \pm 1.2^{c,e}$	$87.3 \pm 1.1^{c,e}$
C	4.2 ± 0.03^{b}	—	5.6 ± 0.4^{c}	80.6 ± 5.5^{b}	85.6 ± 0.9^{c}	97.4 ± 0.1^{f}	97.6 ± 0.5^{f}

Data of assays A, B, and C were obtained from the curves presented in Figs. 1A, B, and C, respectively. The values of I_T and v were obtained from the regression line of the linear phase of the basipetal transport curves (assays A and B). I_T in assay C was obtained by dividing the basipetal transport by 6 h. The polar ratio was calculated by dividing the amount of IAA transported in a basipetal direction by that in an acropetal direction. The inhibition of basipetal transport by NPA is expressed as the percentage of reduction caused by NPA on the basipetal transport measured in absence of the inhibitor. Data for the calculation of polar transport and NPA inhibition correspond to those obtained after a transport period of 24 h (assays A and B) or 6 h (assay C). At the end of the transport period, the sections were immersed in scintillation cocktail and the radioactivity immobilized by individual sections was measured and expressed as the percentage of the total recovered (immobilized + transported). Mean values ± SE of 4–5 sections. Different superscripts in each column (and also across files in the immobilization data) denote significant differences ($P < 0.05$) between the means.

(65.0 Bq according to Fig. 1B). The transport in inverted sections (acropetal transport) was clearly lower than the basipetal transport in all the assays (Fig. 1A–C). The basipetal/acropetal ratio, which is frequently used as an index of the polarity of auxin transport, was ≥ 2.5, the highest value being in assay C, in which the receiver was not renewed (Table 1). NPA did not alter the acropetal transport (data not shown) but strongly reduced the basipetal transport of IAA in all the assays (Fig. 1). The inhibition produced by NPA applied to the apical side (assay A) was close to that obtained after its application to the basal side of the sections (assay B) (Table 1). The greatest NPA inhibition was obtained when the receiver was not renewed (assay C, Table 1).

Data in Table 1 show that immobilized radioactivity increased as IAA transport decreased. Thus, higher immobilization was observed after acropetal than after basipetal transport and NPA increased immobilization compared with the basipetal control. The highest immobilization values were obtained in assay C (Table 1), which exhibited the lowest values of IAA transport (Fig. 1C). The distribution of immobilized radioactivity along the section after basipetal IAA transport (Fig. 2) indicated that most remained near the IAA application site that is, in the first apical 2.5 mm (zone 1) of the section in assays A and B. Of the total radioactivity recovered (transported + immobilized), the transported radioactivity in assay B and C was 39.6 and 14.4%, respectively (Fig. 2). This means that 25% of transportable radioactivity remained immobilized in the sections when the receiver was not renewed (assay C). Most of this radioactivity was recovered from the lowest half of the sections, which immobilized 29.9 % in assay C whereas the same region in assay B (zones 3 and 4) contained 9.8% (Fig. 2).

Comparative Study of the Effects Produced by TIBA and NPA

The transport curves obtained for sections from 5-month-old mother plant cuttings (Fig. 3) confirmed some features of IAA transport observed in Figure 1. Thus, time course curves showed a similar shape and transport was higher in assay A than in B. In addition, I_T was higher and the polar ratio was lower in assay A than in assay B, whereas NPA strongly inhibited basipetal transport irrespective of the application site of the compound (Table 2). Immobilized radioactivity in sections after a 24 h transport period increased as transport decreased (Table 2). All the above confirm the data obtained in the experiment presented in Table 1. In addition, Table 2 shows that TIBA was as effective as NPA in inhibition of basipetal transport, irrespective of the application site (see also Figs. 3A and B).

Longitudinal distribution of immobilized radioactivity after basipetal transport in assay A was similar to that in assay B (Fig. 4). In both assays, the highest values appeared near the site of IAA application (zone 1); below this zone immobilization decreased in zones 2 and 3, whereas the amount in the basal end (zone 4) was higher than in zones 2 and 3. The same distribution pattern was observed in carnation cutting sections pinched from 9-month-old mother plants (Fig. 2). In inverted sections used for acropetal transport, most of the immobilized radioactivity (84–90%) occurred at the site of IAA application (zone 4), successive zones showed a progressive decrease (Fig. 4A, B). The presence of transport inhibitors modified the profile of basipetal control in a manner dependent on the site of inhibitor application. In apically treated sections (assay A), immobilization of IAA at its application site (zone 1) was considerably

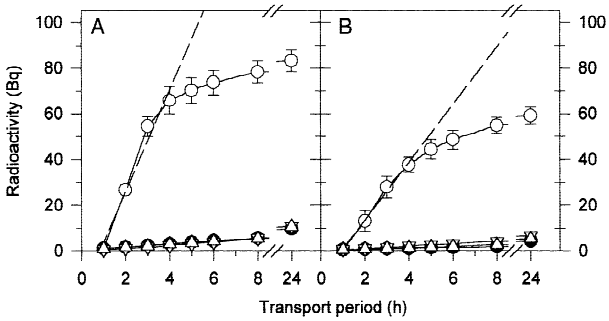

Fig. 3. Basipetal and acropetal transport of IAA and influence of TIBA and NPA. Sections from carnation cuttings pinched from 5-month-old mother plants were used. Assays A and B and application of TIBA and NPA were carried out as described in Figure 1. Basipetal transport in the absence (○) or the presence of TIBA (▽) or NPA (△); acropetal transport (●).The linear parts of the transport curves were fitted by the least-squares method (discontinuous lines).

higher (33.6% in TIBA- and 48.6% in NPA-treated sections), but lower in the base of sections (11 and 20% for TIBA and NPA, respectively in zone 4) compared with corresponding values of the basipetal control (Fig. 4A). When TIBA or NPA was applied to the basal side (assay B) no significant changes in the percentage of immobilized radioactivity were observed in zone 1 compared to the basipetal control. On the other hand, TIBA produced a drastic increase in zone 4, whereas NPA increased the values in zones 2 and 3 and reduced the value in zone 4 compared with the basipetal control. These results suggest that the effects of NPA move in an acropetal direction.

Transport of NPA in Sections from Carnation Cuttings

In view of the effect produced by basal application of NPA on the distribution of immobilized radioactivity along the sections (Fig. 4), experiments were performed to investigate the movement of labeled NPA in the sections. Figure 5 shows that 24 h after application of ^{3}H-NPA to the basal side of upright (Fig. 5C) or inverted (Fig. 5B) sections, radioactivity was detected in zones distant from the application site. Since no radioactivity was detected in the receivers during this 24 h period, the velocity of the NPA movement was clearly lower than that of IAA. Application of ^{3}H-NPA to the apical side of the sections (Fig. 5A) resulted in little movement of NPA because the radioactivity present in zones 2, 3, and 4, located above the application site (zone 1), was clearly lower than that obtained after the application of ^{3}H-NPA to the basal side of sections (compare Figs. 5A and B). Considering the amount of radioactivity present in zones other than the application zone, a polar ratio (basipetal/acropetal) of 2.3 was obtained for NPA transport from data in Figures 5A and B. These results indicate that NPA moved slowly in the sections and mainly in an acropetal direction. The presence of IAA and a high concentration of unlabeled NPA did not affect this ^{3}H-NPA movement (compare Figs. 5B and C).

Influence of PAT on the Rooting of Carnation Cuttings

Cold storage of cuttings prior to planting for rooting is needed to match production of rooted plants with demand. Previous studies (Garrido et al. 1996) showed that rooting of carnation cuttings was strongly influenced by storage duration. To study whether such variations in the rooting process were related with changes in PAT, several parameters of auxin transport were determined in sections from cuttings cold stored for different times. The rooting process of cuttings was also evaluated. PAT parameters were measured following the transport assay described in Figures 1A and 2A (agar blocks) using TIBA as inhibitor. Cuttings stored for 4 weeks showed lower values of I_T and polarity (TIBA inhibition and polar ratio) compared with cuttings stored for 2 weeks (Table 3). Rooting parameters (percentage of rooting and rooted plants of commercial quality) showed lower values in 4- than in 2-week stored cuttings (Table 3). No significant differences in the PAT or rooting parameters were observed when the storage period increased from 4 to 8 weeks.

Discussion

Time course curves of IAA transport in carnation cutting sections (Figs. 1 and 3) showed a linear phase followed by a decline until cessation of transport between 8 and 24 h after IAA application. Curves for basipetal transport such as those presented in Figures 1 and 3 have been reported in stem sections of many species (see reviews of Goldsmith 1977 and Kaldeway 1984). From these curves, parameters for IAA transport such as I_T and v can be obtained for comparative purposes (Sánchez-Bravo et al. 1992). To obtain such curves the receiver must be periodically renewed. In the present work we have studied the efficiency of two different receiver systems: the most frequently used (agar blocks) and that of wells containing buffer solution, which have also been used in some studies (Cambridge and Morris 1996). Basipetal transport (Figs. 1 and 3) and the value of I_T (Table 2) was higher when agar blocks were used, which might be related to differences in IAA diffusion in a solid phase (agar) compared to a liquid phase (buffer solution) rather than to other factors such as the volume of receiver or

Table 2. Transport of IAA and inhibition by TIBA and NPA.

				Inhibition (%)		Immobilization (%)			
Assay	I_T (Bq · h^{-1})	V (mm · h^{-1})	Polar ratio	TIBA	NPA	Basipetal	Acropetal	TIBA	NPA
A	23.1 ± 1.1[a]	11.5 ± 4.6[a]	8.8 ± 0.8[a]	86.8 ± 2.0[a]	86.9 ± 1.3[a]	66.6 ± 2.3[a]	95.3 ± 0.5[c]	95.5 ± 0.5[c]	95.3 ± 0.4[c]
B	12.6 ± 0.7[b]	10.8 ± 1.5[a]	13.6 ± 0.6[b]	88.5 ± 1.1[a]	90.8 ± 0.6[a]	74.8 ± 1.9[b]	97.9 ± 0.2[c]	97.0 ± 0.2[c]	97.6 ± 0.1[c]

The data of the assays A and B were obtained from the curves presented in Figs. 3A and B, respectively, as described in Table 1. After 24h of transport, the sections were immersed in scintillation cocktail and the radioactivity immobilized by individual sections was measured and expressed as a percentage of the total recovered (immobilized + transported). Mean values ± SE of 4–5 sections. Different superscripts in each column (and also across files in the inhibition and immobilization data) denote significant differences ($P < 0.05$) between the means.

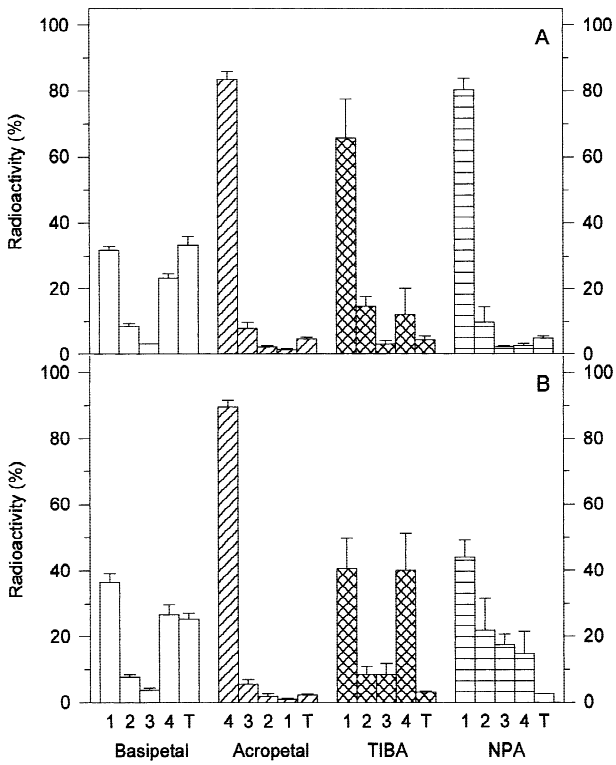

Fig. 4. Longitudinal distribution of radioactivity immobilized after transport of ^{3}H-IAA. Sections used for transport in assays A and B in Figure 3 were divided into four segments (zones 1, 2, 3 and 4, see inset in Fig. 2). Individual segments were immersed in scintillation cocktail. The radioactivity immobilized in each zone was measured and expressed as percentage on the total recovered (immobilized + transported) in the section. The percentage of radioactivity transported at 24 h (T) is also presented. Basipetal transport in the absence (white) or the presence of TIBA (diagonal crosshatching) or NPA (horizontal lines); acropetal transport (diagonal hatching).

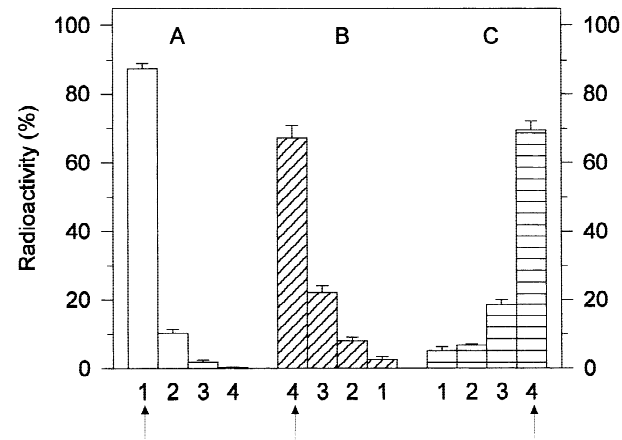

Fig. 5. Longitudinal distribution of radioactivity after application of ^{3}H-NPA to the sections. Radioactive NPA (5 μl, 0.07 μM) was applied to the upper end of upright (A, white) or inverted (B, diagonal hatching) sections placed on agar blocks. In C (horizontal lines), unlabeled IAA (5 μl, 0.14 μM) was applied to the upper end of upright sections and labeled (5 μl, 0.07 μM) plus unlabeled NPA (5 μl, 100 μM) was added to the buffer. Thus, assay C was identical to that presented in Figure 4B. 24 h after the application of ^{3}H-NPA, the sections were divided into four equal segments (zones 1, 2, 3, and 4; see inset in Fig.2), which were separately immersed in scintillation cocktail. The radioactivity present in each zone is expressed as percentage of the total measured in the four zones. Mean values±SE of 4–5 sections are presented. Arrows denote the site of ^{3}H-NPA application.

pH, both of which were almost identical in the two systems.

In some studies of IAA transport, the receiver is not renewed and recovered radioactivity (Cambridge and Morris 1996) or the distribution of radioactivity along the section (Okada et al. 1991) after a long (4–18 h) transport period is used to study polar transport. These methods seem inadequate for determining I_T because the amount of transported radioactivity is substantially lower than that obtained when the receiver is renewed (compare Figs. 1B and 1C). This reduction in transport could be the result of IAA being diffused back from the receiver to the section, where it may be immobilized. The high values of immobilized radioactivity after 6 h of transport (Table 1) and the noticeable increase of untransported radioactivity recorded in the lower half of the section (Fig. 2) support this interpretation.

Two parameters used in evaluating the polarity of auxin transport are polar ratio (see Kaldeway 1984, Diaz-Sala et al. 1996) and, more frequently, the inhibitory effect produced by compounds such as TIBA or NPA on basipetal auxin transport. Table 1 shows that the highest

Table 3. Influence of cold storage on PAT and rooting.

Storage period (weeks)	I_T (Bq · h^{-1})	TIBA inhibition (%)	Polar ratio	Rooting (%)	Commercial quality (%)
2	20.7 ± 1.8^a	62.6 ± 1.3^a	2.6 ± 0.1^a	91.5 ± 2.8^a	23.2 ± 2.5^a
4	15.0 ± 1.0^b	46.9 ± 1.7^b	1.8 ± 0.1^b	70.1 ± 6.5^b	6.0 ± 4.0^b
8	15.7 ± 1.2^b	50.0 ± 2.0^b	1.9 ± 0.2^b	72.4 ± 2.1^b	0

Carnation cuttings from 9-month-old mother plants were stored for different periods in a cold chamber. At the end of the storage period, PAT was studied in cutting sections following the procedure described in Figures 1A and 2A and the values of I_T, TIBA inhibition and polar ratio were obtained from the transport curves. Cuttings stored for different periods were planted for rooting in the greenhouse. Eleven days after planting, the rooting status of each plant was observed and the percentage of rooting and that of plants exhibiting commercial quality were calculated. Different superscripts in each column denote significant differences ($P < 0.05$) between the means.

polar ratio and NPA inhibition values were obtained when the receiver was not renewed (assay C). Such high values were probably an artifact because basipetal transport with or without NPA was strongly reduced in these conditions (compare Figs. 1B and 1C) for the reasons cited above. When the receivers were periodically renewed, the polar ratio was higher when buffer in wells was used as opposed to agar blocks, although the values of NPA inhibition were similar in both receiver systems (Tables 1 and 2).This discrepancy between the polar ratio and the NPA inhibition was probably due to the fact that acropetal transport was not polar (insensitive to TIBA and NPA) while much, but not all, of the basipetal transport was polar (the inhibition produced by TIBA or NPA was 62-68% in Table 1 and 87-91% in Table 2). Based on these results, and considering the specificity of TIBA and NPA for the carriers involved in PAT, it seems that inhibition of basipetal transport by TIBA or NPA is a better quantitative index of polarity than the polar ratio. This conclusion is supported by data in Tables 1 and 2, which show that i) TIBA was as effective as NPA in inhibiting basipetal transport, and ii) the efficiency of both inhibitors was independent of the application site (apical or basal side of sections).

Though the above suggests that TIBA and NPA affect IAA transport equally, some interesting differences can be deduced from the distribution of radioactivity immobilized after application of both inhibitors. Figure 4 shows that the inhibitory effect of TIBA was stronger near its application site because the radioactivity measured in zone 1 after apical application (assay A) and zone 4 after basal application (assay B) was much higher than that recorded in the basipetal control. Radioactivity in zone 2 also increased after apical application of TIBA (Fig. 4A), which agrees with the idea that TIBA itself may be polarly transported in a basipetal direction, as was demonstrated by Thomson et al. (1973) and observed by Botía et al. (1992). However, apical application of NPA resulted in higher immobilization in zone 1 and no variation in zone 2 compared to the basipetal control (Fig. 4A), suggesting the existence of basipetal NPA transport, unlike in the case of TIBA. Basal application of NPA, however, did not increase radioactivity near the application site (zone 4), but produced a substantial increase in zones 2 and 3 compared to the basipetal control (Fig. 4B). In other words, the effect of NPA was detected above its application site, which suggests an acropetal migration of this inhibitor. Such movement of NPA has been proposed by Sundberg et al. (1994) in pine shoots although no movement of ^{3}H-NPA could be detected in corn coleoptiles (Thomson et al. 1973). By using labeled NPA, we demonstrated for the first time that this inhibitor slowly moved in an acropetal direction in sections from carnation cuttings (Fig. 5). The presence of radioactivity in zones 2 and 3 after basal application of ^{3}H-NPA (Fig. 5C) would explain the high immobilization of ^{3}H-IAA observed in these zones when basipetal IAA transport was inhibited by basal application of NPA (Fig. 4B).

Some features of IAA transport were not dependent on the age of mother plants from which cuttings were obtained. Thus, the transport curves showed a similar shape (Figs. 1 and 3); NPA inhibited PAT irrespective of the application site in the sections (the apical, assay A, or the basal side, assay B, Tables 1 and 2); immobilization increased as transport diminished (Tables 1 and 2). This similarity must be considered an inherent feature of PAT in carnation cuttings. Despite similarities, several differences can be observed in Tables 1 and 2 between IAA transport in 5-month- and 9-month-old cuttings: I_T decreased and v increased with age in assay A; the polar ratio, NPA inhibition and the percentage of immobilized radioactivity in the basipetal control decreased with age. These differences might be attributed to age differences of mother plants. Therefore the methodological approach proposed in the present work seems useful to evaluate variations of PAT and was used to investigate whether the variations in PAT might modify rooting of carnation cuttings. Based on the inhibition of rooting produced after removal of the endogenous auxin source (Haissig, 1970; Eliasson and Areblad, 1984) or after application of PAT inhibitors (Katsumi et al. 1969; Liu and Reid,

1992), the involvement of PAT in the rooting process has been proposed. Formation of adventitious roots in carnation cuttings occurs in the basal end and depends on several factors. One of these, the cold storage period prior to planting, has been previously studied (Garrido et al. 1996). The data in Table 3 show that the values of I_T and the polarity of auxin transport decreased when the storage period increased from 2 to 4 weeks, no further variations being observed at 8 weeks. These changes in PAT parameters during cold storage were accompanied by a delay in rooting, as deduced from the percentage of rooting and of rooted plants of commercial quality. In addition, the application of NPA to the basal internode of carnation cuttings exhibiting a high polarity in auxin transport produced a full inhibition of the rooting process (data not shown). All the above confirm the involvement of PAT in rooting and shows that a factor that modified PAT parameters produced a parallel variation in the rooting process. The results in Table 3 suggest that PAT might play a decisive role in regulating the auxin supply needed for formation and growth of adventitious roots in carnation cuttings.

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