Hormones and *Cuscuta* Development: IAA Uptake Transport and Metabolism in Relation to Growth in the Absence and Presence of Applied Cytokinin

G. Paliyath,[1] I. Rajagopal,[2] P. O. Unnikrishnan, and S. Mahadevan

Department of Biochemistry, Indian Institute of Science, Bangalore 560 012, India

Abstract. Transport of 1-^{14}C-IAA in successive stem segments of *Cuscuta* was strictly basipetal in growing and non growing regions of the vine with a flux velocity of 10–12 mm/h (intercept method). This transport showed a distinct peaked profile, increasing from a low value at 10 mm from the apex to a maximum between 50 and 90 mm before declining to a low value again around 160 mm at which elongation growth ceased. The IAA transport profile paralleled the in vivo growth rate profile, though the latter peaked ahead of transport. A better correlation was observed between the profile of growth responsiveness of the vine to exogenous IAA application and the profile of IAA transport. Growth responsiveness was determined as the differential in growth rate of stem segments in vitro in the absence and presence of growth optimal concentration of IAA (10 μm). Retention of exogenous IAA in the stem was maximal where transport decreased, and this coincided with the region of maximal conjugation of applied 1-^{14}C-IAA to aspartic acid to form indoleacetylaspartate (IAAsp). In addition to aspartate, IAA was conjugated to a small extent to an unidentified compound. IAA destruction by decarboxylation was greatest where transport was low, particularly in the nongrowing region, where lignification oc-

Abbreviations: BA, benzyl adenine; GA, gibberellic acid (GA$_3$); HPLC, high-pressure liquid chromatography; IAA, indole-3-acetic acid; 1-^{14}C-IAA, carboxyl-labeled IAA; IAAsp, indole-3-acetyl-aspartic acid; 1-^{14}C-NAA, 1-^{14}C-α-napthaleneacetic acid; POPOP, (1,4-bis(5-phenyl-2 oxazolyl)benzene; PPO, diphenyloxazole; PUR, pulse uptake and retention; TLC, thin-layer chromatography.

curred (i.e., beyond 180 mm). At concentrations up to 20 μM, a pulse of 1-^{14}C-IAA chased by "cold" IAA moved as a peak (with a peak displacement velocity of 12–18 mm/h) in the "growth" region of the vine, but became diffusionlike where growth either fell off steeply or ceased. At a higher (50 μM) IAA concentration, though uptake was not saturated, transport in the growth region became diffusionlike, indicating saturation of the system. Reduced IAA flux in the region where growth responsiveness to IAA declined coincided with the region of increased IAA conjugation. However, it cannot be concluded whether increased IAA conjugation was the cause or effect of decreased IAA flux. Application of benzyladenine to the vines in vivo, a treatment that elicited haustoria formation by 72 h, resulted in the inhibition of both IAA transport and elongation growth rate in the subapical region. In vitro treatment of vine segments with BA similarly increased IAA retention and decreased IAA transport. IAA loss was suppressed, and conjugation to IAAsp was enhanced.

The axial organization of higher plants arises from a polarity that is established early in development (Coleman and Thorpe 1985). The factors responsible for the establishment of polarity are poorly understood, but hormone gradients appear to be important, and the transport of auxin, which occurs in a polar, predominantly basipetal manner, takes on a special significance. Indeed, polar auxin transport is believed to play a major regulatory role throughout growth and development of plants, with evidence suggesting it to be "a controlling factor in elongation, tropisms, cambial division and vascular differentiation, apical dominance, senescence and abscission" (Goldsmith 1977). Rubery (1980) has suggested that by producing pH and electrical gradients in the tissue through which it moves, auxin may also set up polar movements of other substances such as gibberellic acid and abscisic acid. Thus, besides the effects of auxin per se on growth and differentiation, its polar movement may play a special role in development.

In several plants, comparison of in vivo elongation rate and basipetal auxin flux down the length of the shoot has shown that the former peaks ahead of the latter. This has led to the comment that "while maximal polarity or basipetal movement of auxin is likely to be the stage with maximum elongation, concomitant elongation is not required for strongly polar basipetal movement of auxin" (Jacob 1979). Moreover, cytokinins, known to inhibit shoot elongation in some plants (Zeroni and Hall 1980) and cause lateral cell expansion, possibly through their effects on ethylene formation (Fuchs and Lieberman 1968), are believed to exert their action by influencing auxin transport, retention, and metabolism (Jacob 1972, Lau and Yang 1973).

Free-hanging *Cuscuta* vine is an ideal system for the study of auxin transport and its relation to growth and development because of its simple, cylindrical morphology, the absence of secondary sites of auxin synthesis such as leaves and a long zone of elongation. Besides, in vivo or in vitro application of a cytokinin like BA precipitated the complete syndrome needed for the survival of this parasite, namely, promotion of subapical coiling growth to twine around and the induction of haustoria to draw nutrients from a host (Paliyath et al. 1978; Rajagopal et al. 1988, Ramasubramanian et al. 1988). These cytokinin-in-

duced effects appeared to be initiated by low endogenous auxin, since applied high auxin inhibited them. This paper describes some features of auxin (IAA) uptake, transport, retention, and metabolism in *Cuscuta* in relation to its growth, in the absence and presence of applied cytokinin.

Methods and Materials

Plant materials, vine marking, in vivo and in vitro growth measurements, and incubation following BA treatment were as described in previous papers (Rajagopal et al. 1988, Ramasubramanian et al. 1988). Segments were treated with cycloheximide (10 μg/ml) and chilled immediately after the growth period, to arrest further growth during length measurement in a microcomparator. In vivo application of BA in lanolin was as described by Paliyath et al. (1978).

Auxin Transport

Was measured by a modification of the classical donor-stem-receiver setup (Went and Thimann 1937).

Donor

Labeled auxin (1-^{14}C-IAA) normally 10–20 μM, in melted buffered agar (1 mM K_2HPO_4-citrate buffer, pH 5, containing 1.5% agar), was drawn into surgical-quality polythene tubing (i.d. 2 mm). After cooling, the tubing was cut into 2-mm cylinders with a sharp razor blade. Measurement of radioactivity in several individual blocks indicated a deviation to be normally within 5% of the mean, which was taken as the donor-initial (Di) value for a particular preparation. Di counts were usually between 8000 and 16,000 cpm.

Stem

These were 6.4-mm segments excised from the desired 10-mm regions of the vine using a two-bladed cutter after marking the apical end with indelible ink. Trimming segments at both ends to 6.4 mm eliminated the increased cut-end IAA-decarboxylating activity that developed in isolated 10-mm segments following prolonged incubation. The apical 0–10 mm of a vine was always discarded because of numerous lateral buds.

The diameter of the cut surface of the stem bit facing the apical end of the transport segment was measured with a microcomparator at $\times 15$ magnification (Rajagopal et al. 1988) to calculate cross-sectional area.

Receiver

Discs (6 mm) of Whatman No. 1 filter paper dipped in melted buffered agar (see donor) and placed on individual 1-cm^2 polythene sheets served as re-

ceivers. A 5-µl drop of the same buffer applied to the receiver improved contact with the stem during transport.

Transport was allowed to proceed at 27 ± 1°C in a humid atmosphere. At the end of the transport period (normally 3 h), donor, stem, and receiver were transferred to individual vials containing 5 ml of scintillation fluid and counted after overnight extraction, to obtain the following parameters: transport (T) = cpm in receiver; retention (R) = cpm in stem after transport; loss (L) of radioactivity of applied 1-^{14}C-IAA = Di − (Df + T + R), where Di = initial cpm in the donor, Df = final cpm in the donor; uptake (U) = (Di − Df) = T + R + L.

Pulse-Chase of 1-^{14}C-IAA

The procedure of Goldsmith and Thimann (1962) was employed. A 32-mm segment from the desired region, following treatment, if any, was trimmed at both ends to 30 mm and set up for transport in a holder. Radiolabeled donor was placed at the apical end for 15 min and replaced by a "cold" donor containing unlabeled IAA at the same concentration for the desired time (30–90 min). The segment was then cut into 10 equal segments from the apex (S1 to S10) and individually counted. Radiolabeled and cold donor blocks were also separately counted to obtain Df and to determine if any back diffusion of counts occurred from the stem cut surface into cold donor. Total c.p.m. in all segments (S1 to S10) was taken as pulse uptake and retention (PUR) and represented pulse uptake minus any loss due to back diffusion into cold donor and decarboxylation.

Radioactivity Measurement

The scintillation fluid (Naqvi and Gordon 1965) contained 5 g PPO and 250 mg POPOP in 1000 ml of a mixture of toluene and freshly distilled ethanol (3:1, v/v). Counting of samples (1 or 2 × 1 min) was done in a Beckman LS-100 or LKB-Rackbeta counter.

Chromatography of IAA Metabolites

Following 3 h of IAA transport, stem segments (16 or 40) of each region were extracted overnight with 10 ml of methanol (analytical grade) at −20°C. The segments were reextracted twice with 5 ml cold methanol and 5µg carrier IAA added to the pooled extracts which were then flash-evaporated at 30°C. Aliquots of the residue were redissolved in methanol and counted or chromatographed on silica gel precoated plastic TLC sheets (Eastman Chromogram No. 6061) with authentic IAA and IAAsp as reference markers. Solvent systems for TLC were (1) chloroform:methanol:acetic acid (75:20:5, v/v); (2) Benzene:n-butanol:acetic acid:water (95:5:5:1, v/v); (3) isopropanol:ammonia

(28%):water (8:1:1, v/v). After chromatography and drying, lanes representing each sample were divided into sixteen 1-cm segments and individually counted. Reference indoles were visualized by spraying with p-dimethylamino-cinnamaldehyde (PDAC) reagent (Mahadevan and Stowe 1972).

Labeled IAA and IAAsp in aliquots of stem extracts were confirmed by reversed-phase HPLC on a Micropak MCH-10 column (Varian) using the Varian 5000 HPLC instrument. Using a flow rate of 1 ml/min and a 20–60% gradient of methanol in distilled water containing 0.033 M acetic acid over 20 min, the elution of reference IAAsp (retention time 5.6 min) and IAA (retention time 10.6 min) was monitored at 290 nm with a Varichrom UV-Vis monitor. One-milliliter fractions of the HPLC eluate were collected, and radioactivity was determined in the IAA and IAAsp elution regions.

Chemicals

3-Indolyl-1-^{14}C-acetic acid (52 or 59 mCi/mmol) was obtained from Amersham International, Buckinghamshire, England. Hormones, PPO, and POPOP were purchased from Sigma Chemical Co., USA. IAAsp was chemically synthesized by the procedure of Mollan et al. (1972) and stored as a solution in acetonitrile at −20°C. Other chemicals were reagent or analytical-grade.

Results

Pattern of Auxin Transport, Retention, and Destruction Along the Vine

Transport of IAA was strictly basipetal in all regions of the vine tested up to 600 mm from the apex. Figure 1A shows the extent of IAA transported by freshly excised segments in various regions of vines up to 300 mm. Transport increased from low value in the 10- to 20-mm region to a maximum between 50 and 90 mm before declining to a low value again around 160 mm, where growth ceased (inset, Fig. 1A). Transport thereafter remained more or less the same up to 300 mm. The region of maximal transport varied in individual vines and normally occurred between 50 and 90 mm. Typically, during a 3-h experiment with 25 ng of IAA in donor block, the amount of IAA transported into the receiver was about 5 ng where transport was maximal and about 1.5 ng where it was low. The velocity of transport was about 10–12 mm/h in all regions (data not given) as determined by the intercept method of Van der Weij (Went and Thimann 1937).

Retention of radioactivity in the stem segments from the 10 to 300 mm region during IAA transport (Fig. 1B) also showed a pattern of increase and decrease. Maximum retention occurred around 120–140 mm.

Depending on the region, about 20–40% of the applied label of 1-^{14}C-IAA was lost, i.e., not recovered in donor, stem, or reciever, after 3 h of transport (Fig. 1C). In similar experiments using 1-^{14}C-α-naphthaleneacetic aicd (1-^{14}C-NAA), which was transported like IAA but not decarboxylated, 90–95% of the applied radioactivity could be recovered in donor, stem, and receiver after 3 h
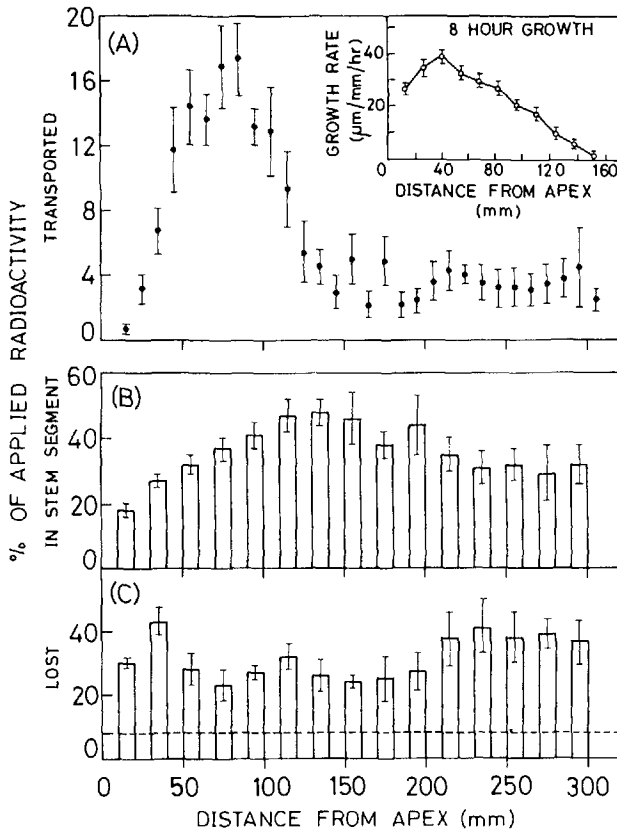
Fig. 1. (A) Patterns of 1-^{14}C-IAA transport in *Cuscuta* vine segments (10–300 mm). Each data point is the mean percent ± SE of applied IAA transported in 3 h by segments from six vines. Transport was measured in 6.4-mm segments excised from the desired regions of the vine as described in Methods and Materials. Inset: Pattern of *in vivo* growth rate along the vine during 8 h of growth. (From Fig. 1 of Rajagopal et al. 1988.) (B) Pattern of radioactivity retention in alternate *Cuscuta* vine segments (10–300 mm) following 3 h of 1-^{14}C-IAA transport: Each data point is the mean percent ± SE of applied radioactivity retained in stem from four vines. (C) Pattern of loss of radioactivity in alternate *Cuscuta* vine segments (10–300 mm) following 3 h of 1-^{14}C-IAA transport. Each data point is the mean percent ± SE of applied radioactivity lost during transport in segments from four vines. (Dashed line indicates nonspecific loss. For details see text.) Data are pooled from experiments done on different days at different times of the year. Intervening alternate segments of B and C were used to generate data such as those given in Fig. 5.

of transport irrespective of the region of the vine (data not given), indicating an average 8% nonspecific loss (including enhanced quenching when label is distributed in stem and receiver). The loss of label of 1-^{14}C-IAA above this value of nonspecific loss (dashed line in Fig. 1C) was taken as region-specific oxidative decarboxylation of IAA, occurring at the cut ends and possibly within the tissue. Decarboxylation loss was low (10–15%) in the regions of maximum transport or of stem retention and conjugation (i.e., 50–180 mm) and increased to about 30–40% in the later, nongrowing region (i.e., >180 mm). Loss appeared to be somewhat greater in the apical (10–40 mm) region also.
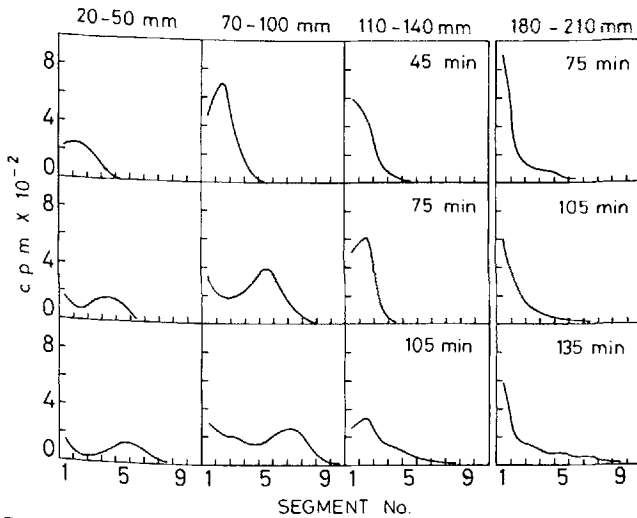
Fig. 2. Pattern of distribution of radioactivity following a pulse (15 min) of 1-¹⁴C-IAA application and chase with cold IAA (at the same concentration) for various time intervals in segments from growing (20–50, 70–100, or 110–140 mm) and nongrowing (180–210 mm) regions of *Cuscuta* vines. The chase time intervals were 30, 45, and 90 min for the growing regions and 60, 90, and 120 min for the nongrowing region. Di = 11,000 cpm. At each time point, different regions of the same vine were used for determining the distribution of radioactivity. (For other details see Methods and Materials.)

At growth-optimal concentration of IAA (10–15 μM) in donor block, about 50–85% entered the stem during 3 h of transport. Depending on the region and donor concentration, 10–50% of this uptake was exported into receivers.

Patterns of Transport of a Pulse of 1-¹⁴C-IAA

The patterns of distribution of radioactivity in 30-mm segments from 20–50, 70–100, 110–140, and 180–210 mm regions of the vine following application of a pulse of 1-¹⁴C-IAA for 15 min and a chase with cold IAA for 30, 60, or 90 min is given in Fig. 2. At the donor concentration used (about 15 μM), IAA moved as a distinct peak in the 20–50 and 70–100 mm regions, but transport appeared progressively more diffusionlike in the latter two regions. Capacity for uptake in the latter regions was apparently greater than their capacity to transport at this concentration. The velocity of the transport in the first two regions, calculated from peak displacement, was 12–18 mm/h, which was somewhat greater than that obtained by the intercept procedure.

Pulse uptake and retention (PUR) and movement of IAA in the actively growing regions (20–50 and 55–85 mm) of the same vines were compared, using a range of IAA concentration in the donor block (about 10–50 μM) (Table 1; Fig. 3). PUR of IAA by the 55- to 85-mm region was about 70% more than in the 20- to 50-mm region. However, the increased uptake and retention of IAA by segments from the later (55–85 mm) region were not solely due to

Table 1. Uptake and retention of a 15-min pulse of 1-¹⁴C-IAA by segments from two regions of the same vines.

Parameter	Region (mm)	
	20–50	55–85
Percent PUR[a]	7.9 ± 0.5 (100)	13.5 ± 0.7 (171)**
Area of uptake[b]	1.13 ± 0.11 (100)	1.40 ± 0.14 (124)
	correlation coeff., r	
Donor concentration[c] vs. PUR	0.94	0.93

[a] PUR as percent of applied counts (± SE) following a 15-min pulse of 1-¹⁴C-IAA and a 90-min chase with cold IAA. For experimental details see Methods.
[b] Cross-sectional area of segments at the apical ends: (mm² ± SE). Parentheses: % of 20- to 50-mm values.
[c] 1-¹⁴C-IAA concentrations in donors were (a) 9–13 μM (8 replicates); (b) 22 μM (2 replicates); (c) 49 μM (4 replicates). Cold IAA donor for chase were for (a) 10 μM, for (b) 20 μM, and for (c) 50 μM.
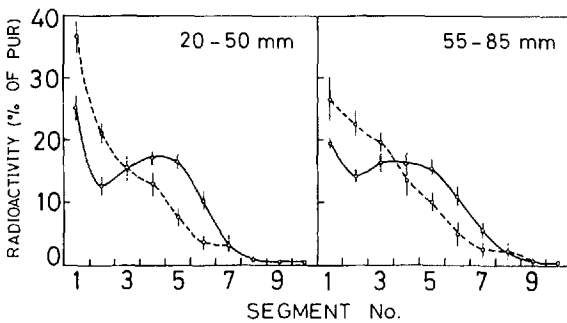** Significance 1% of 20- to 50-mm region value.

Fig. 3. Movement of a pulse of 1-¹⁴C-IAA in 20- to 50-mm and 55- to 85-mm regions of the same *Cuscuta* vines at low (transport-nonsaturating) and high (transport-saturating) IAA concentrations. Nonsaturating (up to 20 μM IAA): O———O + SE (10 replicates). Saturating (50 μM IAA): O– – – – –O + SE (4 replicates). Pulse was with 1-¹⁴C-IAA for 15 min, and chase was for 90 min with about the same concentration of "cold" IAA in donor block. (Distribution of radioactivity in the vine segments (S1 to S10) is expressed as percent of PUR. For other details, see Methods and Materials and Table 1).

its greater cross-sectional area, as this had increased by only 24% (Table 1). PUR was correlated with donor concentration in both regions (r' values 0.94 and 0.93), indicating nonsaturation of uptake in this concentration range of about 10–50 μM (Table 1). Transport of IAA was not saturated up to 20 μM donor concentration where a pulse moved as a peak, but it was saturated in both regions when concentration was raised to 50 μM, and the pattern became diffusionlike (Fig. 3).

Comparison of Growth Rate and Auxin Transport Patterns

The general region of increase and decrease of IAA transport coincided with
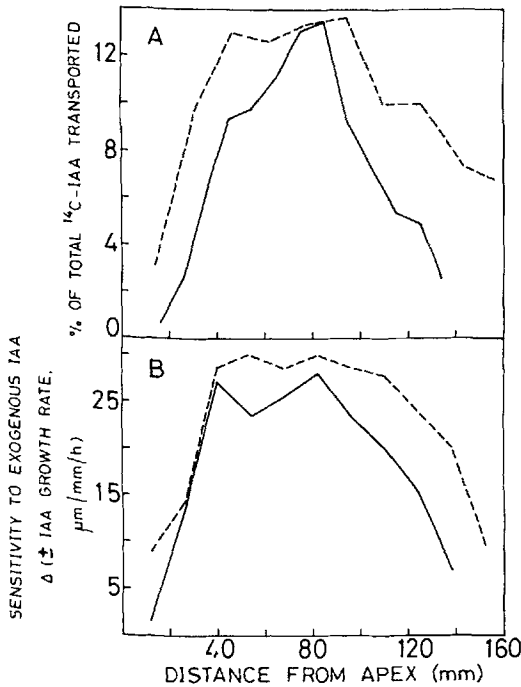
Fig. 4. The pattern of 1-¹⁴C-IAA transport and the pattern of sensitivity of vine segments to growth-optimal concentrations of exogenous IAA along the length (10–160 mm) of *Cuscuta* vines. (A) 1-¹⁴C-IAA transport (as percent total radioactivity transported) in freshly excised (————) segments or segments floated in water for 2 h (— — — — — —) prior to transport. The transport period was 3 h. (B) Sensitivity to exogenous IAA is expressed as the difference in the in vitro growth rate (μm/mm/h) in the presence and absence of 10 μM IAA. Growth rate was measured after 3 h (————) or 8 h (— — — — — —) of growth. Twenty replicates were used for determining growth rate in each region. For experimental details see Methods and Materials.

the in vivo growth rate profile (Fig. 1A, inset); however, the peak growth rate (40 mm) clearly occurred in younger segments than the peak of transport (50–100 mm). Figure 4 compares the pattern of the difference in in vitro growth rate of segments from various regions of the vine in the presence or absence of growth optimal (10 μM) IAA during either 3-h or 8-h growth with the pattern of auxin transport in similar segments, either freshly excised or floated in water for 2 h before transport. It may be seen that segments from regions capable of increased growth in the presence of applied IAA also transported IAA better, and the in vitro growth rate fell off sharply where transport also declined.

Retention and Auxin Metabolism

Following 3 h of 1-¹⁴C-IAA transport in segments from the 10-to 200-mm region of a vine, alternate segments were immediately counted, and the remaining segments were counted after incubation in a Petri dish in a moist at-
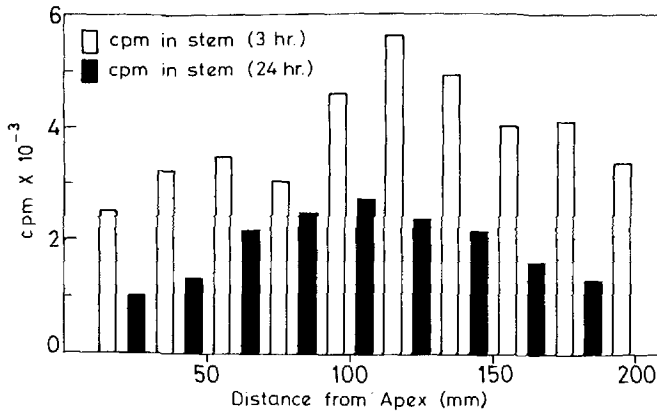
Fig. 5. Pattern of retention of radioactivity in *Cuscuta* segments along the vine following basipetal transport of 1-^{14}C-IAA. Segments (6.4 mm) were excised from successive 10-mm regions of a vine (10–210 mm) and set up for basipetal 1-^{14}C-IAA transport as described in Material and Methods. Following 3-h transport, alternate stem segments were either immediately transferred to vials with scintillation cocktail and counted (open bars) or following incubation for 24 h at 27 ± 1°C in the dark (solid bars). During incubation, segments were placed horizontally in a Petri dish lined with filter paper moistened with buffer (see Table 3).

mosphere for 24 h. Figure 5 shows that radioactivity declined by 35–50% during 24 h of incubation, probably being lost as $^{14}CO_2$ following decarboxylation of the carboxyl-labeled IAA. The pattern of residual radioactivity showed a peak around 100 mm. This residual activity was resistant to loss during further incubation and was predominantly ^{14}C-IAAsp, identified by comparison with synthetic IAAsp by TLC (R_f 0.33, 0.03, 0.29 in solvent systems A, B, and C) and by retention time during HPLC (see Methods). A small amount of radioactivity was also found in an unidentified metabolite, X.

The relative abundance of IAA, IAAsp, and compound X following 3 h of transport in segments from regions 40–50, 80–90, 130–140, and 200–210 mm in two experiments (using two solvents systems) is given in Table 2. Conjugation of IAA to IAAsp was highest in the 130- to 140-mm region in terms of actual counts as well as percent of total counts in the extracts chromatographed. The pattern of metabolism of IAA to IAAsp in the four regions was (130–140) > (80–90) > (40–50) ≥ (200–210) mm, which paralleled stem retention of radioactivity during transport.

Effect of BA on In Vivo Growth and IAA Transport

Application of 0.5% BA in lanolin to vines in vivo inhibited overall elongation, caused loose coiling growth, and induced haustoria production by 72 h (Paliyath et al. 1978). In 8-h in vitro growth experiments, BA increased growth rate and curvature of segments from the subapical 5- to 40-mm region and exhibited synergistic interaction with IAA (Rajagopal et al. 1988). Auxin transport and retention following in vivo BA application was therefore examined.

Figure 6 compares the pattern of in vivo elongation growth in the three re-

Table 2. TLC separation[a] and distribution of labeled IAA, IAAsp, and compound X following 3 h of 1-^{14}C-IAA transport in segments from growing and nongrowing regions.

Exp. No.	Compound	R_f	Regions (mm) (cpm)[b]			
			40–50	80–90	130–140	200–210
1	IAA	0.94	5556	7038	7529	8256
	IAAsp	0.33	2068	4276	5288	1836
	(%)[c]		(14)	(23)	(29)	(12)
	X	0.03	1627	983	212	276
2	IAA	0.78	5633	4752	6555	5021
	IAAsp	0.03	3087	4232	7887	2896
	(%)[c]		(18)	(23)	(33)	(18)
	X	0.5	439	495	640	400

[a] TLC solvent system (see Methods and Materials): Exp. 1, solvent A; Exp. 2, solvent B.
[b] cpm, Equivalent of eight segments, loaded and (% recovered in IAA, IAAsp, and X) for the four regions were:
   Exp. 1: 14,331 (65); 18,493 (66); 18,118 (72); 14,937 (69).
   Exp. 2: 17,034 (54); 18,364 (52); 23,888 (63); 16,200 (51).
[c] Percent of counts loaded in IAAsp.

gions I, II, and III of vines marked initially at 20, 50, and 100 mm from the apex following application of lanolin (control) or lanolin containing 0.5% BA. Though BA-treated vines showed loose coiling, these could be gently straightened for growth measurements. Vines coiled on themselves were discarded. Overall inhibition of growth by BA treatment after 48 h was about 28%, inhibition of first-day growth being 11% and that of second-day growth 41%. Inhibition of second day growth in BA-treated vines was maximal (67%) in region II (i.e., the initial 20- to 50-mm region), which after 24 h of growth extended from 37 to 93 mm from the apex (Fig. 6A). This region roughly coincides with the 40- to 100-mm region of normal vines, segments from which zone were maximally growth-responsive to exogenous IAA (see Fig. 4).

Transport and retention of 1-^{14}C-IAA was measured after 48 h in the 20-to 90-mm region of the control and BA-treated vines. This length overlapped the zone of maximal growth inhibition in BA-treated vines at that time. The results (Fig. 6B) showed that in BA-treated vines, IAA transport was drastically inhibited, whereas its retention in the stem increased in this region when compared to the controls. On average, IAA transport was inhibited by about 85%, and its retention increased by about 70%, in the central (30–70 mm) part of this region. The zones of growth inhibition, decreased auxin transport, and increased auxin retention more or less overlapped, and this was the region where the maximum number of haustoria were produced 72 h after BA treatment.

Effect of In Vitro BA Treatment on IAA Transport

Segments of the 30-to 50-mm region were most responsive to BA in in vitro induction of haustoria (Ramasubramanian et al. 1988). Auxin transport (3 h) in trimmed segments from the 30-to 40-mm region was therefore measured immediately, or 24, 48, and 72 h after BA (50 μM) treatment for 1 h and incubation

under conditions optimal for haustoria production (Ramasubramanian et al. 1988).

The tendency for reduced auxin transport by about 30% was noticeable even after 1 h of BA treatment, as shown in Table 3. However, variability in this region of increasing auxin transport (Fig. 1) could mask significance unless many replicates were used. At 24 h after BA treatment, transport in controls has fallen sharply, but BA induced a further significant drop. At 48 and 72 h, transport was low in both control and BA-treated segments. The most significant difference was in enhanced stem retention of radioactivity, which was 152–183% of the control values. Enhanced stem retention at any point following BA application was apparently not primarily due to enhanced uptake but rather due to decreased loss of radioactivity possibly owing to decreased decarboxylation of the 1-^{14}C-IAA.

Conjugation of IAA in BA-Treated Segments

Segments treated with or without BA were trimmed and allowed to take up 1-^{14}C-IAA during 3 h of transport. Segments were extracted with methanol, and extracts were analyzed by TLC. The results (Table 4) indicated that about two times the amount of label was extracted from segments treated with BA. IAA and IAAsp in BA-treated vine were 2 and 2.8 times those in untreated controls, respectively. BA-treated segments therefore showed increased retention of IAA, enhanced accumulation of 1-^{14}C-IAA, and a lowered loss of radioactivity of 1-^{14}C-IAA taken up.

Discussion

The strict basipetal polarity of auxin in both growing and nongrowing regions of *Cuscuta* vine perhaps reflects the unidirectional development of this parasite where all materials move from haustoria to the shoot tip and where excised vines exhibit the "growing in front while dying behind" syndrome so essential for its survival. The absence of a bidirectional flow of material at any stage of development may be a reason for the absence of acropetal auxin transport, characteristically seen in some aging stems and petioles (Jacob 1979), which support such a bidirectional flow, spatially or temporally.

Along the vine, IAA flux increases from a low value near the apex to a maximum before declining again to low value around 160 mm, where growth also ceases. Though this region overlaps the region of growth, in vivo growth rate peaks ahead of auxin flux along the vine, as seen in other plants (Jacob 1979). However, a comparison of the profile of growth responsiveness of segments to exogenous IAA with the IAA transport profile indicated that they more or less superimpose. The region nearest the apex (5–20 mm) exhibiting greatest endogenous growth rate in vitro was also the region most growth-responsive to added cytokinin (in the presence or absence of IAA) but least responsive to added IAA alone, whereas the region maximally responsive to exogenous IAA (40 mm to 100 mm) and where auxin flux peaked showed no added growth response to any other hormone (Rajagopal et al. 1988). The re-
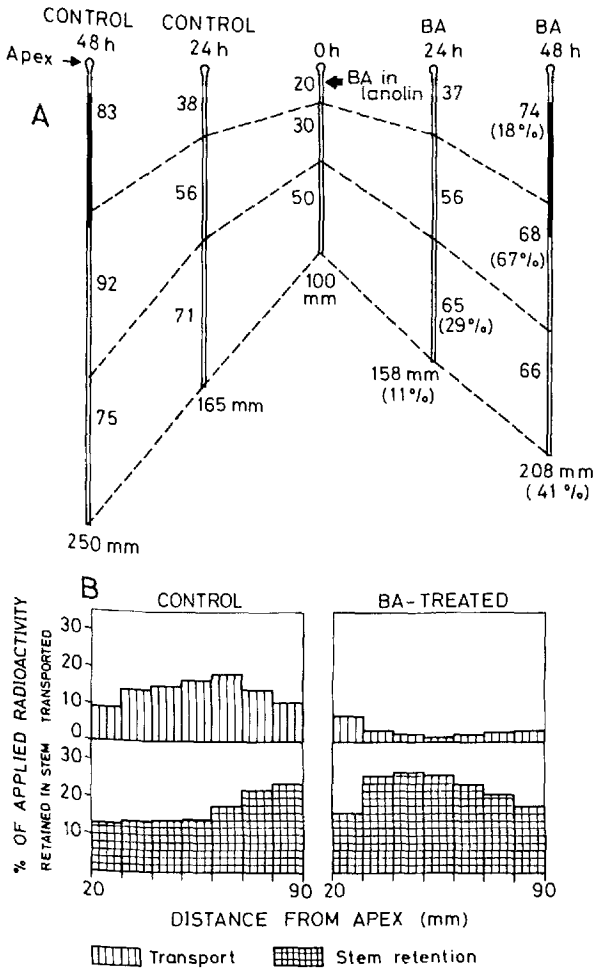
Fig. 6. Effect of BA application on in vivo elongation growth and 1-¹⁴C-IAA transport and retention in *Cuscuta* vines. (A) *Cuscuta* vines were marked at 20, 50, and 100 mm from the apex to delineate regions I, II, and III and treated with either plain lanolin (control) or 0.5% BA in lanolin (0 h; center). Dashed lines indicate relative position of markings after 24 and 48 h of growth in control (left) or BA-treated (right) vines. Numbers alongside vines indicate length (mm) of each region at 24 and 48 h; numbers below indicate total length. Numbers in parentheses indicate percent inhibition of growth following BA treatment during the previous 24 h as compared to equivalent region of control. Values are the average of seven (BA-treated) or eight (control) vines. After 48 h, the 20-to 90-mm region of the vines (shaded region) was used for IAA transport. (B) Transport (T) and stem retention (R) of 1-¹⁴C-IAA by segments from the 20-to-90-mm region of control (left) or BA-treated (right) vines after 48 h growth. T (top) and R (bottom) are expressed as percent of applied radioactivity. Segments (6.4 mm) were excised from successive 10-mm portions of the 20-to 90-mm region of either six control or BA-treated vines to determine IAA transport or retention. Transport period was 3 h. Di = 14,800.

gion with the capacity for maximal auxin flux therefore, exhibited maximum sensitivity toward applied IAA for growth, irrespective of whether this growth was realized in vivo.

Table 3. 1-^{14}C-IAA uptake, retention, transport, and destruction in 30- to 40-mm region segments following BA treatment.

Treatment[1]	Incubation (h)	Uptake	Retention	Transport	Loss
			(cpm ± SE)		
None	0	5523 ± 179	1387 ± 42	1785 ± 77	2351 ± 168
BA	0	5689 ± 202	1426 ± 53	1281 ± 74*	2982 ± 190
None	24	5654 ± 141	1816 ± 68	186 ± 25	3653 ± 177
BA[a]	24	5877 ± 245	2760 ± 92*	32 ± 5*	3085 ± 268
None	48	6220 ± 169	1649 ± 183	57 ± 8	4515 ± 250
BA[a]	48	6196 ± 235	3000 ± 119*	46 ± 9	3150 ± 272*
None	72	7071 ± 205	2404 ± 130	53 ± 9	4614 ± 286
BA[b]	72	7202 ± 353	4110 ± 218*	47 ± 7	3050 ± 366*

[1] Segments were treated with or without 50 μM BA for 1 h prior to incubation under haustoria-inducing conditions (see Ramasubramanian et al. 1988). Di = 8900, 9000, 9200, and 11,000 cpm at the four time points.
[a,b] Replicates: a = 13; b = 15; others 16.
* Significance 1% of respective control value; others not significant.

Table 4. Distribution of label in IAA and IAAsp following 3 h of 1-^{14}C-IAA transport by segments from the 30- to 40-mm region incubated for 72 h following treatment with or without BA.[a]

Treatment	Radioactivity[b]	
	IAA (cpm)	IAAsp (cpm)
None	49,800	29,500 (29)[c]
BA (50 μM)	102,400	82,250 (38)

Total cpm extracted from 40 segments were control—102,800 cpm; BA-treated—214,900 cpm.
[a] BA treatment as in Table 3. Ends of segments were trimmed prior to IAA transport.
[b] Total cpm in IAA or IAAsp following TLC using solvent A (see Methods and Materials).
[c] Parentheses: radioactivity in IAAsp as percent of extracted counts.

A comparison of the amount and distribution or radioactivity during the transport of a pulse of 1-^{14}C-IAA in segments on the upslope of the transport profile suggested that both uptake and secretion of IAA by cells in this region had increased. These two processes are believed to occur by the cooperation of two elements in the plasma membrane, namely transmembrane diffusion of undissociated IAA and/or evenly distributed saturable, specific H^+/IAA^- symport for uptake and asymmetrtically distributed, specific saturable, possibly electrogenic, auxin carrier for secretion (Rubery 1980, Goldsmith 1977, 1982, Hertel et al. 1983). Both of these elements could be increasing in segments in the ascending limb of the transport profile, with the necessary pH gradient for enhanced IAA uptake being provided in a self-reinforcing manner (Rubery 1980) by the increased proton efflux caused by IAA-induced growth in this region of increasing sensitivity to exogenous IAA.

The capacity for IAA uptake and transport clearly increased along the length of the vine from 20 to 100 mm when tested at the growth-optimal concentration range (10–20 μM). At a higher concentration (50 μM), uptake was not satu-

rated, but transport was. Thus, transport of IAA appeared to occur without saturation of the secretion or uptake sites when growth optimal concentration of IAA was applied to any region in the ascending limb of the transport profile. Significantly, this concentration of applied IAA induced straight growth in segments from the subapical 5-to 40-mm region, at which concentration it could override the coiling growth induced by BA (Rajagopal et al. 1988). A lower auxin concentration (0.1 µM), on the other hand, enhanced BA-induced coiling growth, and it was argued that such interactions could occur in vivo to produce the interconvertible straight and coiling modes of growth seen in nature (Rajagopal et al. 1988). IAA transport capacity in this region therefore matches its uptake capacity at a concentration where straight growth is possible. Extending the argument, a fall in the production of auxin at the apex with a concomitant decrease in its availability for transport should be interpreted as curved or coiling growth in vivo.

Beyond 110 mm, both uptake and transport capacities declined, the latter more than the former, leading to its saturation as seen by a change in the appearance of the pattern in the transport of a pulse and by the increased retention of label in the stem during conventional transport. Increased retention of IAA, characteristic of the descending limb of the transport profile, may arise by any of the following reasons: decrease in auxin carrier sites, buildup of an endogenous transport inhibitor, increased sequestering of IAA in the vacuole due to increase in its pH, and chemical conjugation.

Although no evidence exists for the first two, a comment can be made on the third. Maximal transport through existing carrier sites would be favored if cytoplasmic auxin concentration is kept high by maintaining cytoplasmic pH 1–2 units above wall and vacuolar pH, a condition achieved when IAA-induced proton efflux into these compartments occurs as a prelude to growth (Rubery 1980, Sze 1985). As the cell expands and the vacuole becomes the largest compartment of the cell, more IAA would be present in the vacuole than in the cytoplasm, though its concentration in the later compartment would be higher. With a decrease of proton efflux and growth, any increase of vacuolar pH would cause more IAA to move into the vacuole from the cytoplasm with an eventual decrease in its availability for transport. A rise in vacuolar pH with age of the cell has been reported (Guern et al. 1982).

Conjugation of IAA to IAAsp increased steadily and reached a maximum where transport fell off. Though induction of IAAsp-forming enzymes in other plants has been shown to occur in 2 h (Sembdner et al. 1980), it is unlikely that the pattern of conjugation seen in *Cuscuta* vine arises exclusively by the de novo induction of the conjugating enzymes during the 3-h transport period, for this explains neither lower conjugation in the nongrowing region nor the greater conjugation in the BA-treated segments. The pattern rather suggests the distribution of activity of the enzymes already present in vivo, a viewpoint that may be resolved by in vitro assay of the conjugating ability along the length of the vine or following BA treatment.

Much of the loss of label of applied 1-^{14}C-IAA appeared to be due to enzymatic decarboxylation of IAA, as results of other experiments not given here showed that the label of 1-^{14}C-IAA was not similarly lost. The loss was greater in the nongrowing (180–300 mm) region, where the capacity for IAA conjuga-

tion also fell off. This region also coincided with the zone of lignification of the secondary xylem (Nagaiah et al. 1977).

The inhibition of the auxin transport system following BA application, manifested in vivo by a reduction in growth rate below the point of BA application and in vitro by a reduction in auxin flux after BA treatment, reinforces the argument for a low auxin status needed to initiate cytokinin-promoted subapical coiling growth or haustoria initiation (Rajagopal et al. 1988, Ramasubramanian et al. 1988). In the opposite direction, GA application, while promoting elongation growth of isolated stem apices in culture, also enhanced auxin transport (Maheshwari et al. 1980). Significantly, GA also inhibited BA-induced haustoria formation in vitro (Ramasubramanian et al. 1988).

With a fall in auxin transport, auxin retention in vine tissue increased following BA application, whether applied in vivo or in vitro.The increased IAA retention, associated with decreased IAA loss (i.e., decreased IAA decarboxylation), apparently results in its increased conjugation to IAAsp. Increased auxin in the tissue 24 h after BA application, however, has no inhibitory effect on haustoria development once induced (Ramasubramanian et al. 1988), and auxin may even be a requirement for the cell division activity associated with this process.

In *Cuscuta*, therefore, the several general effects of cytokinins, namely, lowered shoot elongation growth and concomitant enhanced lateral expansion as a possible consequence of altered auxin transport, retention, and metabolism, have been combined with a rather unique ability to form haustoria, enabling it to lead the life it does.

References

Coleman WK, Thorpe TA (1985) Polarity. In: Pharis RP, Reid DM (eds) Hormonal regulation of development. III. Encyclopedia of plant physiology, New Series 11. Springer, New York, pp 116–132

Fuchs Y, Lieberman M (1968) Effect of kinetin, IAA and gibberellic acid on ethylene production and their interactions in growth of seedlings. Plant Physiol 43:2029–2036

Goldsmith MHM (1977) The polar transport of auxin. Annu Rev Plant Physiol 28:439–478

Goldsmith MHM (1982) A saturable site responsible for polar transport of indole-3-acetic acid in sections of maize coleoptile. Planta 155:68–75

Goldsmith MHM, Thimann KV (1962) Some characteristics of movement of indole acetic acid in coleoptiles of *Avena*. I. Uptake, destruction, immobilization and distribution of IAA during basipetal translocation. Plant Physiol 37:492–505

Guern J, Kurkdjian A, Mathieu Y (1982) Hormonal regulation of intracellular pH: Hypotheses versus facts. In: Wareing PF (ed) Plant growth substances 1982. Academic Press, New York, pp 427–437

Hertel R, Lomax TL, Brigs WR (1983) Auxin transport in membrane vesicles from *Cucurbita pepo* L. Planta 157:193–201

Jacob WP (1972) The movement of plant hormones: Auxin, gibberellins and cytokinins. In: Carr DJ (ed) Plant growth substances 1970. Springer, New York, pp 701–709

Jacob WP (1979) Plant hormones and plant development. Cambridge University Press, London

Lau OL, Yang SF (1973) Mechanism of a synergistic effect of kinetin on auxin-induced ethylene production; suppression of auxin conjugation. Plant Physiol 51:1011–1014

Mahadevan S, Stowe BB (1972) An intermediate in the synthesis of glucobrassicins from 3-indoleacetaldoxime by woad leaves. Plant Physiol 50:43–50

Maheshwari R, Shailini C, Veluthambi K, Mahadevan S (1980) Interaction of gibberellic acid and indole-3-acetic acid in the growth of excised *Cuscuta* shoot tips *in vitro*. Plant Physiol 65:186–192

Mollan RC, Donnelly DMX, Harmey MA (1972) Synthesis of indole-3-acetylaspartic acid. Phytochemistry 11:1485–1488

Nagaiah K, Kumar SA, Mahadevan S (1977) L-phenylalanine ammonia-lyase from the lignified and non-lignified regions of *Cuscuta chinensis*. Phytochemistry 16:667–671

Naqvi SM, Gordon SA (1965) Auxin transport in flowering and vegetative shoots of *Coleus blumei* Benth. Plant Physiol 40:116–118

Paliyath G, Maheshwari R, Mahadevan S (1978) Initiation of haustoria in *Cuscuta* by cytokinin application. Curr Sci 47:427–429

Rajagopal I, Ramasubramanian TS, Paliyath G, Mahadevan S (1988) Hormones and *Cuscuta* development. Interaction of cytokinin and indole-3-acetic acid in the growth and curvature of subapical stem segments. J Plant Growth Regul 7:121–132

Ramasubramanian TS, Paliyath G, Rajagopal I, Maheshwari R, Mahadevan S (1988) Hormones and *Cuscuta* development. In vitro induction of haustoria by cytokinin and its inhibition by other hormones. J Plant Growth Regul 7:133–144

Rubery PH (1980) The mechanism of transmembrane auxin transport and its relation to the chemiosmotic hypothesis of the polar transport of auxin. In: Skoog F (ed) Plant growth substances 1979. Springer, New York, pp 50–60

Sembdner G, Gross D, Liebisch HW, Schneider G (1980) Biosynthesis and metabolism of plant hormones. In: MacMillan J (ed) Hormonal regulation of development. I. Encyclopedia of plant physiology, New Series 9. Springer, New York, p 364

Sze H (1985) H+-translocating ATPases: Advances using membrane vesicles. Annu Rev Plant Physiol 36:175–208

Went FW, Thimann KV (1937) Phytohormones. Macmillan, New York, p 93

Zeroni M, Hall MA (1980) Molecular effects of hormone treatment on tissue. In: MacMillan J (ed) Hormonal regulation of development. I. Encyclopedia of plant physiology, New Series 9. Springer, New York, p 530